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In Silico Screening and Molecular Dynamics Validation of *Bryophyllum Pinnatum* Flavonoids as Pi3kγ Inhibitors For Rheumatoid Arthritis.

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Success O. Olubode: Writing - Conceptualization, original draft and methodology. Success O. Olubode and Olamide V. Awelewa: Visualization and Validation. Success O. Olubode, Olamide V. Awelewa and Lateef Bello : Writing, Formal analysis – review and editing. Noble E. Olaogbebikan, Samuel A. Oginni and Joy A. Akinnusi: Data curation. Success O. Olubode, Olamide V. Awelewa, and Olufemi A. Akinola: Result analysis. Samuel O. Olubode, Sidiqat A. Shodehinde and Oluwafemi S. Bakare: Supervision and Validation.

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ABSTRACT

Rheumatoid arthritis (RA) is a long-term autoimmune inflammatory disorder that causes progressive joint destruction. This damage is partly due to the unusual activation of the phosphatidylinositol 3 kinase gamma (PI3Kγ) isoform in immune cells and synovial fibroblasts. In this study, a computational method that combined molecular docking, pharmacophore modelling, ADMET prediction, binding free energy calculations, AutoQSAR modelling, and 100 ns molecular dynamics (MD) simulations was used, and aimed to assess the potential of bioactive compounds from *Bryophyllum pinnatum* against PI3Kγ (PDB ID: 2A4Z) as a new target for treating RA. The molecular docking showed that Luteolin, Quercetin and Kaempferol could be potenial lead with docking scores of -11.044, -10.723 and -10.553 respectively. The lead compounds were found to interact with specific amino acid residues that are important for inhibition of PI3Kγ. ADMET profiling indicated favourable drug-like qualities, meeting Lipinski's Rule of Five and having acceptable TPSA and logKp values. The MM-GBSA binding free energies ranged from −45.07 to −44.03 kcal/mol, suggesting stronger and more stable binding than tofacitinib's −21.04 kcal/mol. AutoQSAR predicted pIC_{50} values reinforced the high inhibitory potency, with luteolin showing the greatest predicted activity at 7.053. The MD simulations over 100 ns indicated conformational stability, with RMSD values primarily below 3.6 Å for the protein backbone and maintained key protein-ligand contacts such as hydrogen bonds, hydrophobic interactions, and water bridges throughout the trajectory. These findings suggest that luteolin, quercetin, and kaempferol from Bryophyllum pinnatum could have strong anti-inflammatory effects in RA by selectively inhibiting PI3Kγ-mediated signalling. This presents a promising basis for developing plant-based therapies and encourages further experimental validation in vitro and in vivo.

KEYWORDS: *Bryophyllum Pinnatum,* Rheumatoid arthritis, PI3Kγ, Molecular docking, and MD Simulation.

INTRODUCTION

Rheumatoid arthritis (RA) is a long-term, symmetrical, inflammatory autoimmune condition that primarily impacts small joints, subsequently affecting larger joints and eventually involving the skin, eyes, heart, kidneys, and lungs. Frequently, the joints' bone and cartilage suffer destruction, leading to weakened tendons and ligaments[1]. This joint damage results in deformities and bone loss, which is typically very painful for the affected individual. Typical symptoms of RA consist of morning stiffness in the affected joints lasting more than 30 minutes, as well as fatigue, fever, weight loss, tenderness, swelling, warmth in the joints, and the presence of rheumatoid nodules beneath the skin[2]. The disease typically begins between the ages of 35 and 60, characterized by periods of remission and flare-ups. It can also affect young children, including those under the age of 16, known as juvenile RA (JRA), which shares similarities with RA but does not show the presence of rheumatoid factor[3-5]. Clinically, RA can be distinguished from osteoarthritis (OA) as it primarily impacts the proximal interphalangeal (PIP) and metacarpophalangeal (MP) joints, whereas OA generally involves the distal interphalangeal (DIP) joint. OA, being the most prevalent form of arthritis, results from wear and tear rather than an autoimmune origin[6,7]. It has been estimated that rheumatoid arthritis (RA) impacted 17.6 million individuals globally in 2020, with a worldwide age-adjusted prevalence rate of 0.21%, based on the Global Burden of Disease (GBD) study, marking an increase of 14.1% since 1990[8]. The GBD 2021 RA Collaborators project that the number of RA patients will reach 31.7 million by 2050[9]. Chinese epidemiological research indicates that there are around 5 million individuals with RA in China, resulting in a prevalence rate of 0.42%[10]. Studies on cost-effectiveness have shown that the significant economic burden associated with RA has been routinely underestimated[11,12].

Traditional herbal medicine has continued to inspire countless individuals over recent years because of its beneficial effects on the health of many people in Asia, using specific phytochemicals derived from traditional plants[13]. The integration of various fields such as artificial intelligence, bioinformatics, and computational biology, along with vast amounts of scientific data, has propelled medical research toward diverse target-identification methods through the utilization of a network pharmacology (NP) approach[14].

Bryophyllum pinnatum, commonly referred to as the life plant or Maternity plant, is a perennial herb that grows abundantly and is utilized in traditional medicine across tropical regions of Africa, the Americas, India, China, and Australia. This plant thrives in the southern regions of Nigeria[15] It is

particularly recognized for its wound-healing abilities. Furthermore, it attracts significant interest for its medicinal characteristics in both folk medicine and modern medical practices. *B. pinnatum* contains various phytochemicals, including flavonoids, saponins, tannins, and alkaloids[16]. The leaves are rich in vitamins such as ascorbic acid, riboflavin, thiamine, and niacin, as well as minerals like calcium, zinc, and phosphorus[17]. Studies indicate that the leaves exhibit numerous medicinal properties, including antimicrobial effects, antifungal activity, antiulcer effects, antihypertensive benefits, antidiabetic properties, anti-inflammatory qualities, analgesic effects, and wound healing capabilities[18]. Additionally, there are findings suggesting that the leaves have anti-tumor effects[19] and exhibit sedative and muscle relaxant properties[20]. In Benin City, Nigeria, the leaves are boiled and filtered through a clean white cloth, with the resulting liquid prepared for daily consumption by individuals suffering from asthma. The leaves and bark serve as a bitter tonic, act as an astringent for the bowels, provide pain relief, and have carminative properties, making them helpful for diarrhea and vomiting. They can be used both externally and internally to alleviate various types of pain and inflammation, as well as to treat different bacterial, viral, and fungal infections, including leishmaniasis, earaches, upper respiratory infections, stomach ulcers, flu, and fever[21]. The herb is also utilized to aid in the expulsion of the placenta following the birth of a baby. The juice extracted from the leaves is utilized in the management of coughs, bronchial conditions, blood dysentery, jaundice, and gout. It is effective both externally and internally for relieving diverse pains and inflammations, as well as for treating various bacterial, viral, and fungal infections, flu, and fever[19]. Many herbal practitioners refer to the plant as a master herb or a remedy with comprehensive antimalarial properties[22]. *B. pinnatum* is abundant in alkaloids, triterpenes, glycosides, flavonoids, cardenolides, steroids, bufadienolides, and lipids. The leaves include a specific group of chemicals known as bufadienolides, which are highly active and possess antibacterial, antitumorous, and insecticidal effects[21].

PI3K gamma (PI3Kγ) plays a significant role in the development of rheumatoid arthritis (RA) and is a promising target for therapy. Studies using both genetic deletion and specific pharmacological inhibitors in mouse models of arthritis have shown that blocking PI3Kγ reduces joint inflammation and damage. The identification and development of specific PI3Kγ inhibitors demonstrates that both genetic deletion of PI3Kγ and oral treatment with a selective inhibitor (AS-605240) significantly reduce the severity of arthritis in collagen-induced arthritis (CIA) models. It validates PI3Kγ as a therapeutic target by showing the protective effects correlate with defective neutrophil migration[23].

In rheumatoid arthritis (RA), PI3Kγ, which is mainly found in leukocytes, promotes both innate and adaptive immune responses by enhancing the movement and infiltration of inflammatory cells such as neutrophils and macrophages into synovial tissues. Additionally, its role in the overactive PI3K/AKT/mTOR pathway further exacerbates RA pathology by increasing the production of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6. It also uniquely modulates synovial fibroblasts, leading to their excessive growth, anti-apoptotic survival, and invasive characteristics that damage cartilage and bone. Furthermore, PI3Kγ stimulates abnormal blood vessel formation in the synovium, contributing to sustained chronic inflammation and tissue injury.

Innovative methods, such as molecular docking is carried out to investigate the mechanism of *Bryophyllum pinnatum* in the treatment of RA. The aim of this research is that the outcome of this work plan will pave the way for further investigation and give adequate fundamental principles for developing a specialized treatment against RA.

METHODOLOGY

Protein selection and preparation

The PI3Kγ stucture, identified by the Protein Data Bank (PDB) ID 2A4Z, was sourced from the official PDB website (https://www.rcsb.org/). Before its application in this research, the protein underwent comprehensive pre-processing which consists of several options. They are assigning bond orders, add hydrogen, delete water molecules and fill loops to enhance the assignment of hydrogen bonds and to minimize its structure using the OPLS4[24] force field. The minimized protein obtained was then employed for subsequent analyses and investigations.

Ligand generation and preparation

A library of compounds for *Bryophyllum pinnatum* was created in SDF format by extracting pertinent compounds from the PubChem web database. The obtained compounds were further processed utilizing the LigPrep tool[25]. This tool enabled the transformation of the initially available two-dimensional molecules into three-dimensional forms. The procedure included ionizing the molecules at a pH of 7.0 ± 0.2 and removing any existing salts with the help of Epik. The OPLS4[24] force field was employed to guarantee precise ionization and appropriate representation of the tautomeric states of the compounds[26].

Receptor grid generation

To identify the exact area where the ligand interacts with the protein, a receptor grid was established. This grid was developed using the Receptor Grid Generation tool and concentrated on the protein's binding domain[27.] By choosing the co-crystallized ligand located at the active site of 2A4Z, the binding site was identified. To aid in the following molecular docking process, a grid box was automatically generated for the protein. The dimensions of the grid box were specified as follows: X = 42.92, Y = 16.57, and Z = 33.02.

Pharmacophore modeling and screening

Pharmacophore modeling was used to predict the fundamental attributes that are important for binding to the active site via E-pharmacophore[28]. Also, this hypothesis served as a criterion to filter out compounds that did not possess at least two of the five critical features identified in the pharmacophore analysis.

Molecular docking

The molecular docking process was performed using the Glide tool[29], utilizing Maestro 11.1 software[30]. The compound library, which had already been filtered down to 25 compounds from 30 compounds, was docked into the prepared grid of protein targets. One docking algorithm was employed: Extra Precision (XP) algorithm, which is the most rigorous. The selected compounds (25) were subjected to XP docking and during the docking process, the protein was considered a rigid body, while the ligand’s rotatable bonds were allowed to freely move and adopt different conformations.

Absorption Distribution Metabolism Excretion/Toxicity screening

To evaluate the absorption, distribution, metabolism, and excretion (ADME) properties of the five leading compounds alongside the standard drug, their effectiveness was estimated utilizing the swissADME server. The swissADME server (https://www.swissadme.ch/) provides valuable predictions concerning the ADME characteristics of substances. These characteristics are crucial in the drug development process as they assist in assessing a compound's potential effectiveness as a medication. By examining these features, meaningful information can be obtained about the compound’s ADME, aiding the decision-making process in drug discovery and development.

Binding energy studies

The molecular mechanics-generalized Born surface area (MM-GBSA) is a computational technique employed to determine the energy associated with different elements within a molecular system, which includes optimized free receptors, free ligands, and the complex created when a ligand binds to a receptor. In this research, the Prime rotamer search methods available in Maestro were utilized alongside the OPLS4 force field and the VSGB solvent model. These tools and models were engaged to conduct the required calculations and simulations.

Development of AutoQSAR Model

To investigate the activity of PI3K gamma (PI3Kγ) inhibitors, we collected some inhibitors from the CHEMBL database. The FASTA sequence of the protein, sourced from the PDB, served as a query for the extraction of these compounds. To enable further analysis, the retrieved compounds were converted to the ".SDF" file format using Data Warrior software. Through the AutoQSAR module, a QSAR model was developed with these compounds. Several models were created, and the top-performing model was then utilized to estimate the pIC50 values of the highest-ranked compounds along with a standard compound. This QSAR model facilitated the prediction of the inhibitory activity of these compounds, yielding significant insights into their potential effectiveness as PI3K gamma (PI3Kγ) inhibitors.

MD simulation

To simulate the dynamic properties of the biological environment and water molecules, molecular dynamics (MD) simulation was utilized. By applying Newton's equations, these simulations clarify the movements and interactions of various components, including water, ions, small molecules, macromolecules, and more complex systems[31]. In this research, a 100-nanosecond MD simulation was performed using the Desmond[32] software from Schrödinger. The protein-ligand complexes chosen for the MD simulations were based on previous docking studies, offering a predictive insight. The protein-ligand systems were created using the System Builder tool within Schrödinger suites (version 2021-2). The simulation employed the OPLS4 force field, and the solvent model was set as TIP3P within an orthorhombic box. Counter ions were added as necessary to ensure the model was neutral. NaCl (0.15 M) was included to replicate physiological conditions. The NpT ensemble was selected for a thorough simulation, utilizing the Martyna–Tuckerman–Klein Barostat at a temperature of 300K and standard atmospheric pressure. Before the simulation commenced, the model was subjected to relaxation, and the trajectory data for each run was recorded for analysis at intervals of 100ns. The stability of the

simulations was evaluated by calculating the root mean square deviation (RMSD) of the protein and ligand over time. Furthermore, the root mean square fluctuation (RMSF) and interactions between the protein and ligand were analyzed to gain understanding of the system's behavior. This methodology enabled a comprehensive exploration of the molecular dynamics and interactions within the simulated biological environment.

RESULTS AND DISCUSSION

Before molecular docking, a pharmacophore model was created based on the complex formed by the protein and its co-crystallized ligand. The PHASE module within the Schrödinger suite was used to gather vital information about the molecular orientation of key functional groups that play a significant role in the high-affinity binding of ligands to the target protein. The resulting E-pharmacophore model (Figure 1) included seven hydrophobic groups and ten aromatic rings.

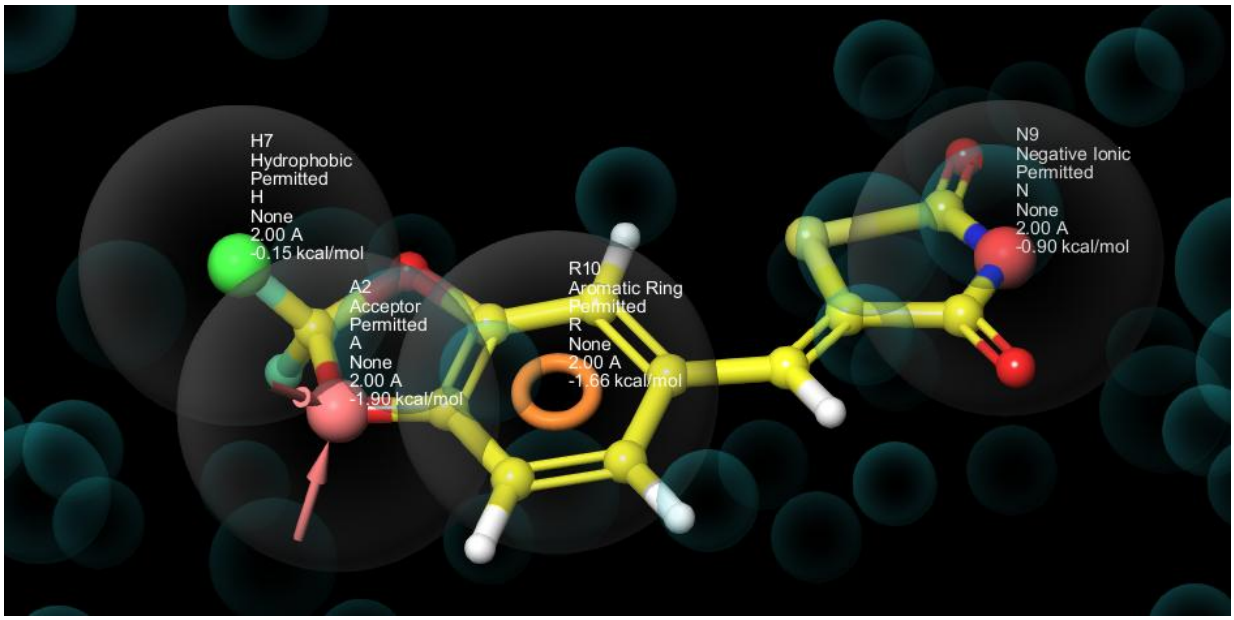


Fig 1. Pharmacophore hypothesis generated.

The pharmacophore hypothesis was employed to filter through the collection of compounds, facilitating the discovery of ligands that share vital characteristics essential for interacting with the target protein.

The molecular docking analysis revealed five compounds from *B. prinnatum* that showed significant inhibitory potential against the target enzyme compared to the co-crystallised ligand, and also exhibiting

docking scores lower than that of the standard compound tofacitibib (Supplemental Figure 1). A more negative docking score suggests a greater inhibitory potential.

The compounds Luteolin, Quercetin, Kaempferol demonstrated outstanding inhibitory activity, with docking scores of -11.044, -10.723 and -10.553 respectively. In comparison to co-crystalized ligand and Tofacitinib, a standard drug for treating RA, has a docking scores of -9.628 and -6.205 kcal/mol (Table 4).

Table 1. *Docking scores of the leading compounds and standard against P13K gamma.*

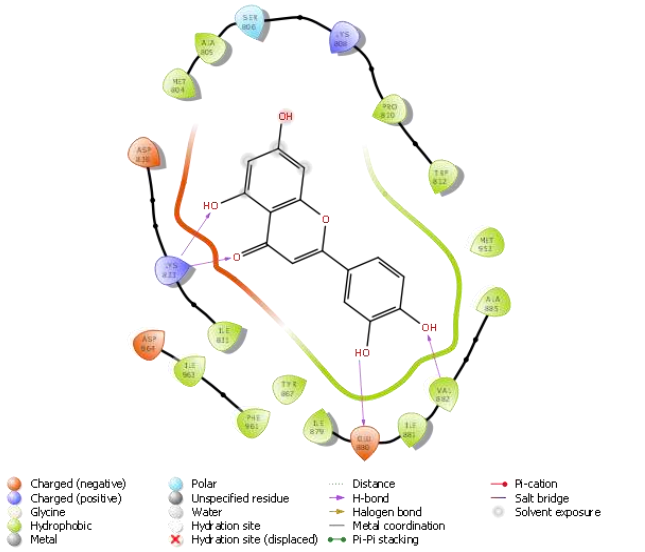
COMPOUND NAME	DOCKING SCORE
Luteolin	-11.044
Quercetin	-10.723
Kaempferol	-10.553
Co-crys	-9.628
Tofacitinib	-6.205

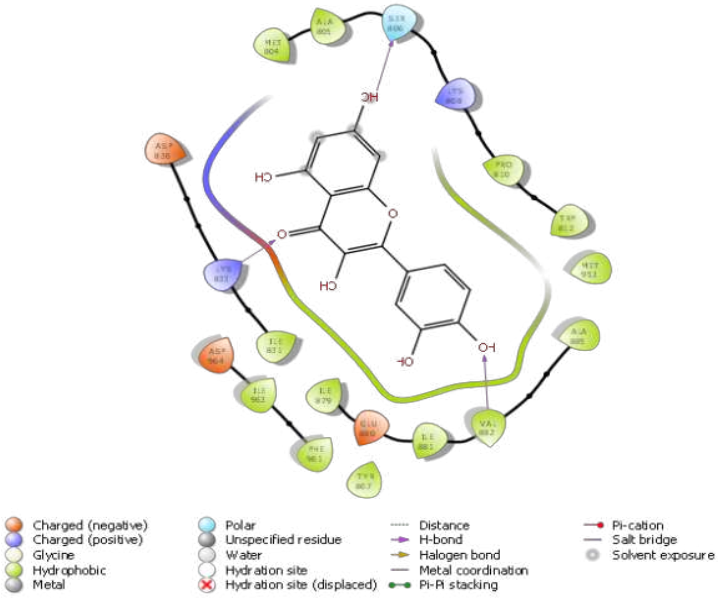
Structural-based drug design emphasizes the relationship between the protein and ligand, since the degree of inhibition is mainly influenced by the ligand's interaction with particular amino acid residues at the active site of the intended enzyme[33]. Table 2 and Figure 2 illustrate the major interactions that significantly contribute to the inhibition of PI3K gamma in this research. Luteolin created three hydrogen bonds with LYS 833, VAL 882 and GLU 880, Quercetin created three hydrogen bonds with SER 806, VAL 882 and LYS 833. Also, Kaempferol generated hydrogen bonds with SER 806, LYS 833, and VAL 882 with a single PI-PI stacking bond with TYR 867. Additionally, the co-crystalized ligand generated one hydrogen bond with VAL 882, and a single PI-PI stacking bond with TYR 867. The standard drug Tofacitinib, showed no interaction with the hydrophobic amino acids. Hydrogen bonds are crucial intermolecular forces that define the physical properties of water, stabilize the 3D structure of proteins and DNA, and influence solubility, melting points, and viscosity while PI-PI stacking is vital for stabilizing molecular structures, molecular recognition, and supramolecular assembly.

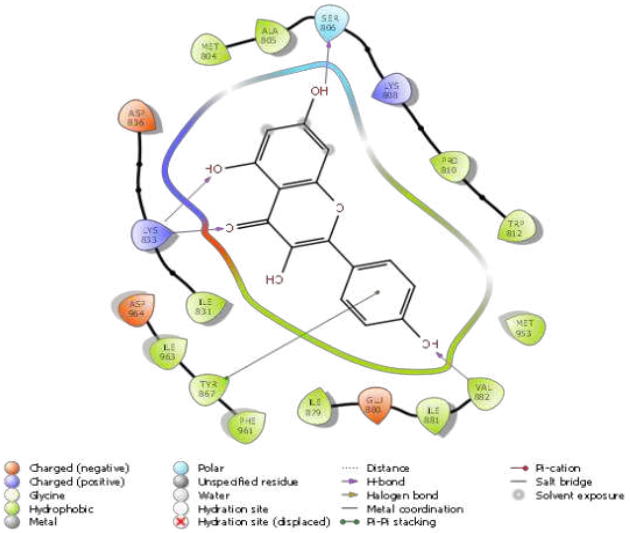
Table 2. *Interaction profile of leading compounds including the standard within the active site of P13K gamma*

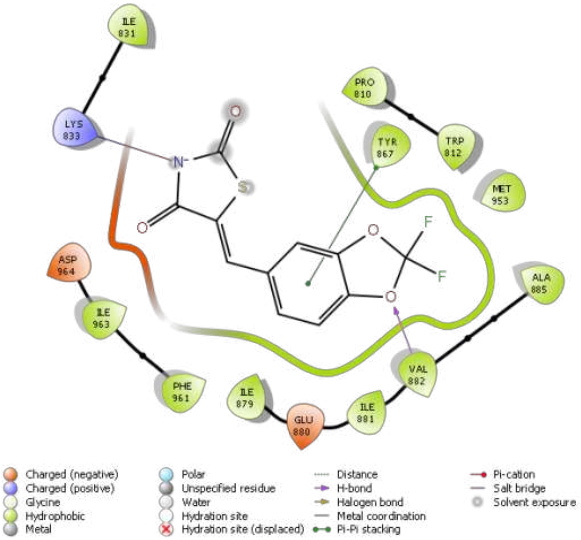
COMPOUND NAME	H-BOND	HYDROPHOBIC AMINO ACID	OTHER INTERACTIONS
Luteolin	LYS 833, VAL 882, GLU 880	TRP 812, PRO 810, LYS 808, SER 806, ALA 805, MET 804, ASP 836, ILE 831, PHE 961, ILE 963, ASP 964, TYR 867, ILE 879, ILE 881, ALA 885, MET 953.	None
Quercetin	SER 806, VAL 882, LYS 833	MET 953, TRP 812, PRO 810, LYS 808, ALA 805, MET 804, ASP 836, 1LE 831, ASP 964, ILE 963, PHE 961, TYR 867 ILE 879, GLU 880, ILE 881, ALA 885.	None
Kaempferol	SER 806, LYS 833, VAL 882	MET 953, TRP 812, PRO 810, LYS 808, ALA 805, MET 804, ASP 836, 1LE 831, ASP 964, ILE 963, PHE 961, ILE 879, GLU 880, ILE 881.	PI-PI stacking: TRY 867
Co-crystalized ligand	VAL 882	MET 885, TRP 812, PRO 810, ILE 831, ASP 964, ILE 963, PHE 961, ILE 879, GLU 880, ILE 881, ALA 885.	PI-PI stacking: TRY 867. Salt bridge: LYS 833.
Tofacitinib	None	MET 804, ALA 805, SER 806, THR 887, ASP 950, ASN 951, MET 953, TYR 867, VAL 882, ILE 881, GLU 880, ILE 879, TRP 812, PRO 810, LYS 833, ILE 831, ASP 964, ILE 831, ASP 964, ILE 963, PHE 961.	None

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Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure


Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
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Charged (negative)
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Glycine
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Charged (negative)
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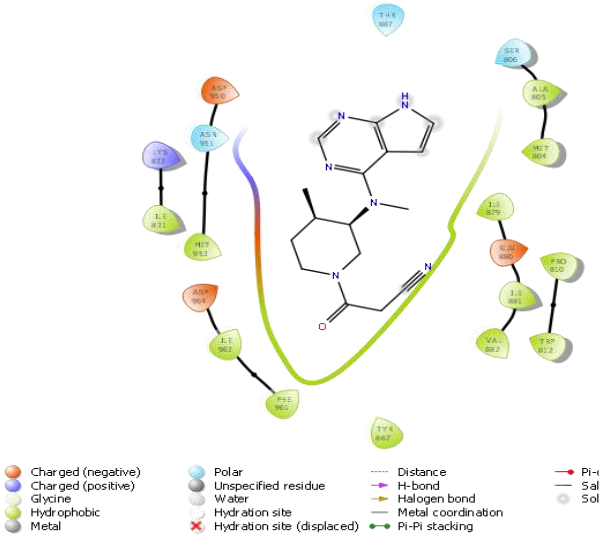

Charged (negative)
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Pi-cation
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Figure 2. The 2D structures of interaction profile of P13K gamma–ligand complexes with luteolin, quercetin, kaempferol, co-crystalized ligand and tofacitinib after molecular docking studies.

ADMET and Drug Likeness

The assessment of ADME is an essential component of the drug development process, offering critical insights into how drugs interact within biological systems[34]. In this research, the swissADME server was used to forecast the drug-like characteristics and possible toxicity of the chosen lead compounds as well as the reference compound. To assess the drug likeness of these compounds, the Lipinski[35] rule, which comprises five fundamental criteria for evaluating oral activity was applied. The findings, shown in Table 3, reveal that all selected compounds display favorable physicochemical properties, such as low molecular weight, a favorable bioavailability score, and adequate solubility. Importantly, these attributes position the lead compounds as leading drug-like candidates according to their physicochemical characteristics. Furthermore, none of the compounds breached more than zero of the Lipinski rule criteria.

Table 3. Drug likeness prediction of the hit compounds and standard.

COMPOUND NAME	MW	HBA	HBD	ILOGP	PSA	LOGKP	ROF
Luteolin	286.24	6	4	1.86	111.13	-6.25	0
Quercetin	302.24	7	5	1.63	131.36	-7.05	0
Kaempferol	286.24	6	4	1.70	111.13	-6.70	0
Co-crys	284.22	7	0	1.76	77.90	-6.06	0
Tofacitinib	312.37	4	1	1.70	88.91	-7.14	0

Abbreviations: iLogP, implicit log P; PSA, polar surface area; MW, Molecular weight; HBA, number of hydrogen bond acceptor; HBD, number of hydrogen bond donor; ROV, rule of five (Lipinski)

The iLogP (implicit log P) value is used to determine the n-octanol/water partition coefficient of compounds, which reflects their lipophilicity. Lipophilicity influences a drug's solubility and its ability

to permeate membranes, with iLogP serving as a predictive tool for these characteristics[36]. All compounds, along with the standard, were predicted to show lipophilic traits, as their values fell below five. According to Veber's rule, compounds with a polar surface area (TPSA) of less than 140 Å2 tend to demonstrate good oral bioavailability. In this analysis, every compound had TPSA values under 140. The predicted logKp values, expressed in cm/s, for the compounds, including the standard, ranged from −3.96 to −7.24. A more negative logKp indicates a reduced capability for the molecule to permeate the skin[35]. Tofacitinib, the standard drug, was estimated to possess the lowest skin permeability score (–7.14 cm/s), also hydrogen bond donor ≤ 5 and hydrogen bond acceptor ≤ 10[37].

MMGBSA

This is a post docking validation which combines molecular mechanics and solvation models to calculate the binding affinity of a ligand-protein docking and strain energies of several compounds and the target protein. The high binding energy calculation efficiency of MMGBSA has been emphasized by numerous publications as one of the most dependable techniques for assessing ligand-protein affinity[38,39]. The MM-GBSA prime tool was used and compounds with high docking score were selected for the post validation which are Luteolin, Quercetin and Kaempferol which are compound from *B. pinnatum* and compare to the reference ligand the co-crystalized and Tofacitinib standard drug used in this study. The binding free energies for the docked complexes of Luteolin, Quercetin and Kaempferol were -45.07, -44.03 and -44.79 kcal/mol respectively. This shows that the hit compounds are stable in the binding pocket of the target protein. The co-crystalized ligand and Tofacitinib the standard drug has binding free energies of -44.57 and -21.04 kcal/mol. The MM-GBSA method improves the precision of virtual screening outcomes and offers important information about the stability of protein-ligand complexes inside the binding pocket. The lead compounds' favorable binding properties are shown by their negative binding free energy values, which further supports their potential as powerful inhibitors.

Table 4. Binding free energy calculation results for lead compounds with target protein using prime MM-GBSA

COMPOUND NAME	ΔGBind	ΔGBind Covalent	ΔGBind H-bond	ΔGBinde Solv GB	ΔGBind vdw
Luteolin	-45.07	3.27	-2.78	23.36	-30.56
Quercetin	-44.03	3.65	-2.89	24.56	-31.37
Kaempferol	-44.79	3.83	-2.73	24.96	-26.52
Co-crys	-44.57	-0.49	-2.09	-24.11	-36.55
Tofacitinib (standard)	-21.04	7.78	-0.52	15.01	-33.17

AUTO QSAR

The developed QSAR model accurately replicates the biological activities of the leading compounds and compares them to the standard PI3Kγ. The AutoQSAR module was used in constructing the QSAR model. The best model (kpls_desc_19) for predicting the pIC50 values of the top compounds has an R-squared value of 0.6646 and a Q-squared value of 0.6625. The root mean square error (RMSE) stands at 0.5626, with a standard deviation (S.D) of 0.5584 (Supplementary Table 1). Figure 3 illustrates scatter plots depicting the model's performance. The dataset's predictive and observed values are comprehensively detailed in Supplementary Table 2. In addition, Table 5 highlights that the top compounds exhibit superior pIC50 values compared to the standard.

Table 5. Pred IC50 of the leading compounds and standard against P13K gamma.

COMPOUND NAME	pIC50
Luteolin	7.053
Quercetin	6.952
Kaempferol	6.627

Co-crys	6.737
Tofacitinib (standard)	6.563

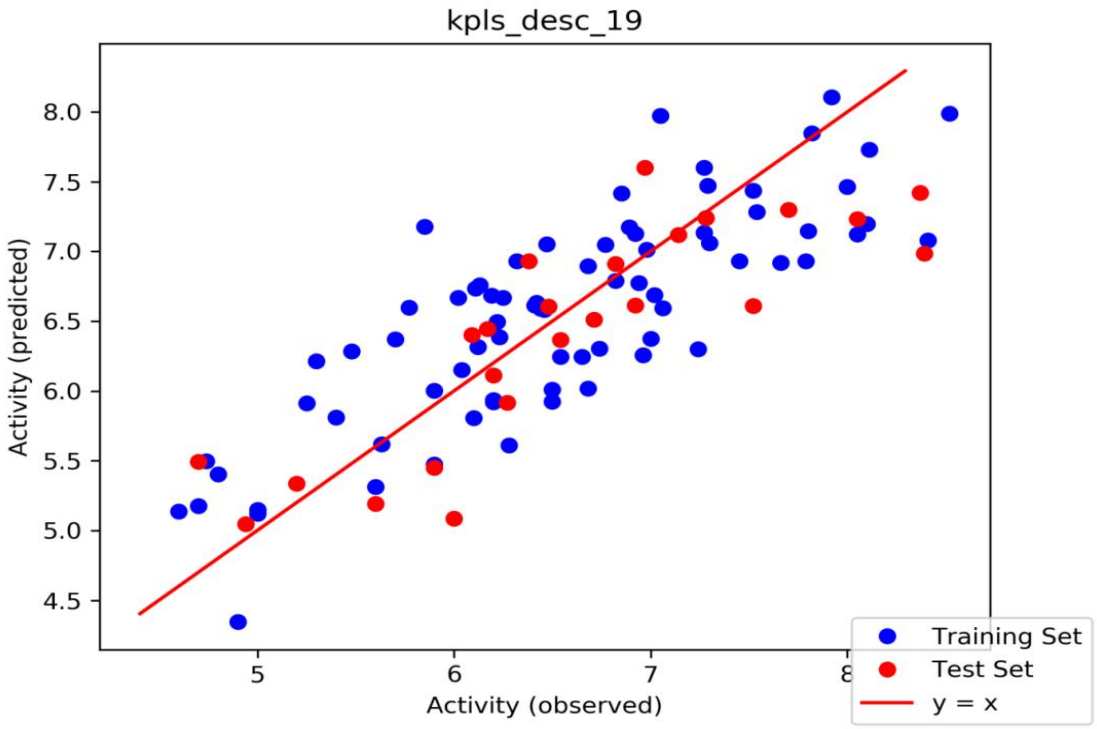


Fig. 3. Analysis of the scatter plot showing the experimental and predicted values using the most accurate model identified by AutoQSAR.

The scatter plot illustrated in Fig. 3 indicates that the model generally aligns with the trend of the observed data; however, there is noticeable deviation between the observed and predicted pIC50 values, especially within both the training and test sets. The performance appears to be slightly improved on the training set (blue points), as more points are situated closer to the diagonal line. Conversely, the test set (red points) exhibits greater deviation, which is common as the model attempts to generalize to new data. All compounds demonstrated high pIC50 values; Luteolin stands out as the most promising compound owing to its elevated pIC50 value (7.053), signifying its exceptional potency in inhibiting the biological target when compared to other compounds. This compound may be prioritized for additional development or testing due to its remarkable activity.

MD SIMULATION

RMSD and RMSF

The MD simulation was performed on the protein–ligand complexes using the Desmond tool from the Maestro Schrodinger suite (version 2021)[32]. The selected complexes displayed strong binding affinity, stable IFD conformations, and favorable pharmacokinetic and pharmacodynamic characteristics. To assess the stability and convergence of PI3K gamma (PI3Kγ), this process (molecular dynamics simulation) studies were carried out. After completing the docking and post-docking evaluations in this study, three compounds were selected, along with the reference ligand and standard drug, as the top ligands to act as PI3K gamma (PI3Kγ) inhibitors. To understand the interactions between the chosen lead compounds and PI3Kγ, molecular dynamics (MD) simulations were conducted alongside docking calculations[40]. A 100-nanosecond MD simulation was carried out on the protein-ligand complexes, and the Root Mean Square Deviation (RMSD) along with the Root Mean Square Fluctuation (RMSF) were studied, and the results are analysed below;

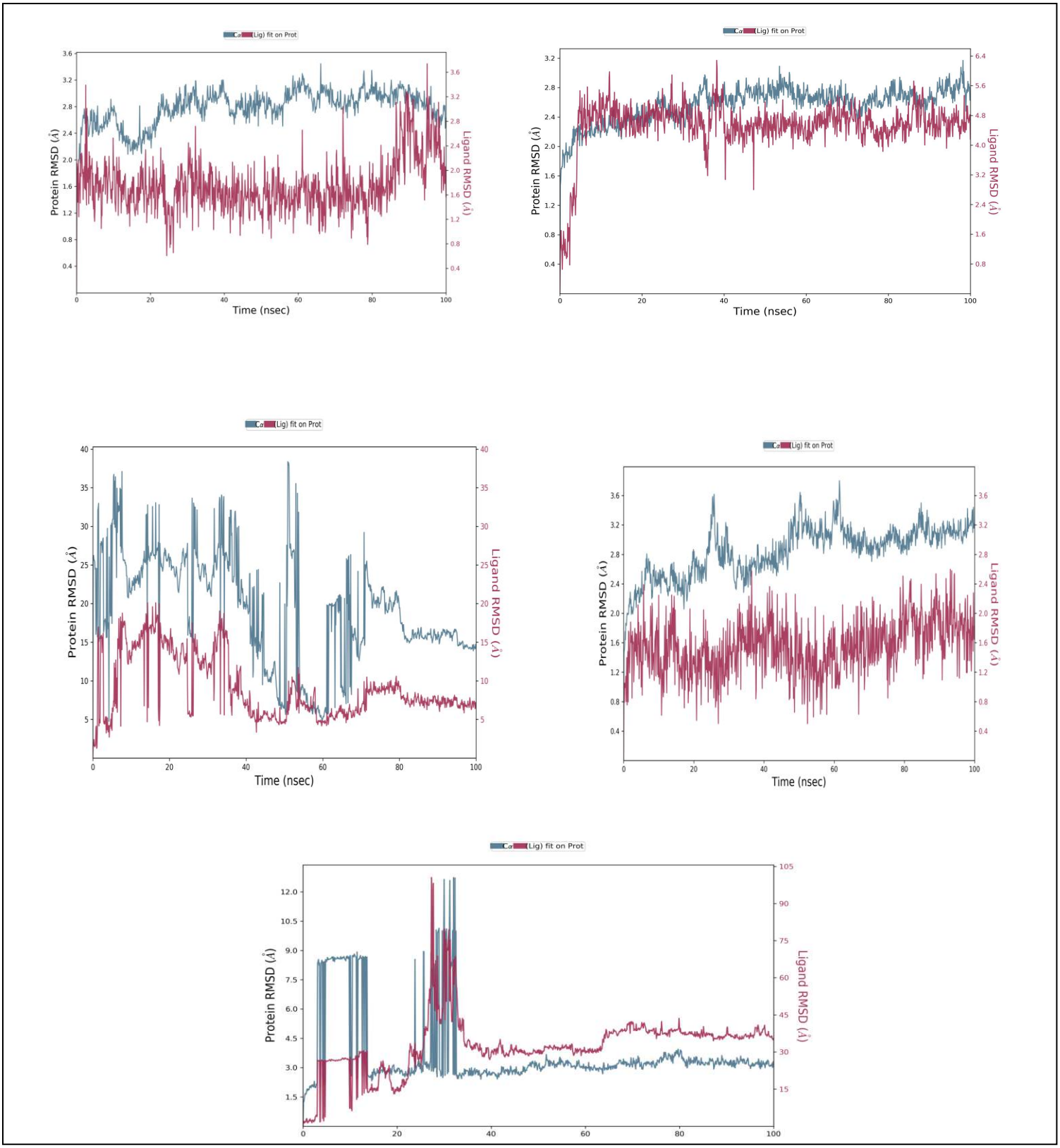


Figure 4. The RMSD of PI3Kγ (black) complexed with Luteolin, Quercetin, Kaempferol, co-crystalized ligand and tofacitinib (red) over a time period of 100ns.

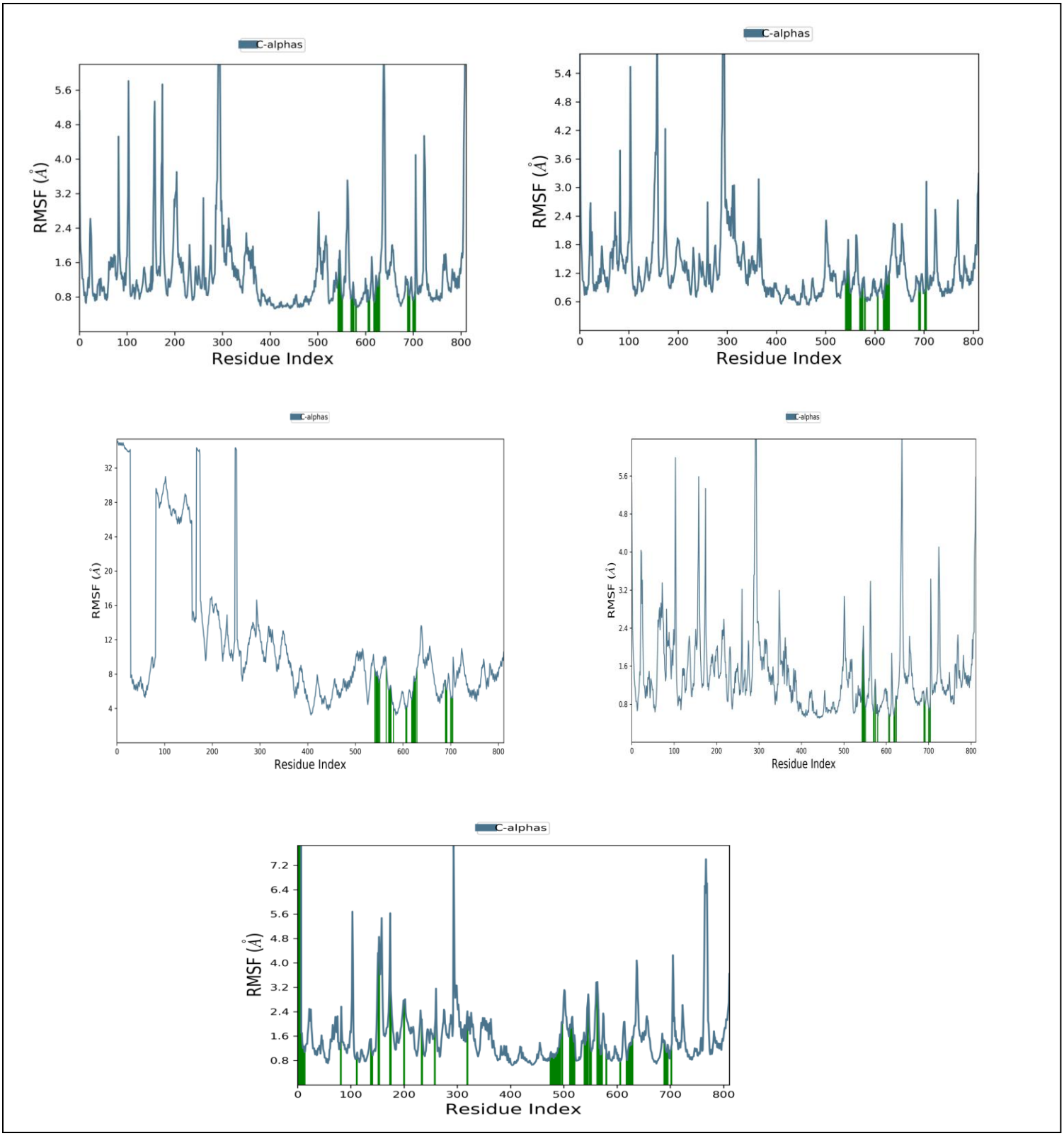


Fig 5. The RMSF of the residues of PI3Kγ over a time period of 100 ns.

Based on the simulation data for Protein-Ligand RMSD shown in figure 4, Luteolin undergoes significant conformational alterations before establishing its optimal binding orientation within PI3Kγ. The relatively stable protein RMSD, which generally remains below 3.6Å, indicates that the protein structure maintains its integrity during the simulation, which is crucial for its biological activity. The obtained results revealed that the protein RMSD displayed elevated values ranging from 2.0-3.2Å, with fluctuations observed throughout the simulation. In contrast, the ligand RMSD showed initial variability before achieving stability. For the quercetin complex, both ligand and protein RMSDs exhibited variations up to 20ns within the range of 0.4-2.8Å, after which they stabilized for the remainder of the simulation (see Fig 4). The Kaempferol complex reached stability between 80-100ns, having undergone fluctuations until around 80ns, where the ligand RMSD demonstrated low values of up to 15Å (Fig 4). Similarly, the reference ligand also displayed initial fluctuations before gradually stabilizing (Fig 4). Tofacitinib (standard drug) complex experienced fluctuations during the initial 30ns, followed by a gradual stabilization from approximately 40-100ns (Fig 4). Overall, the ligand RMSD of the complexes was typically lower than that of the protein RMSD. The variation in RMSF was assessed to evaluate internal fluctuations within the protein and ligands, as illustrated in Figure 5. Each compound exhibited fluctuations at significant residue positions. The RMSF analysis indicated that the binding of the lead compound enhanced the flexibility of the protein's active site, substrate-binding pocket, and other areas. This implies that Luteolin, Quercetin, kaempferol, the co-crystalized ligand, and Tofacitinib (the standard) may alter the enzyme's structure, potentially influencing its catalytic function.

In summary, the MD simulation offered insights into the dynamic behavior and stability of the protein-ligand complexes in physiological conditions, providing valuable data for drug development research.

PROTEIN LIGAND CONTACT

The protein interactions with the ligand were monitored during the simulation. Protein-ligand interactions (or 'contacts') are categorized into four types: Hydrogen Bonds, Hydrophobic, Ionic and Water Bridges. Each interaction type contains more specific subtypes, which can be explored through the 'Simulation Interactions Diagram' panel. These interactions are summarized, as shown in the plots below;

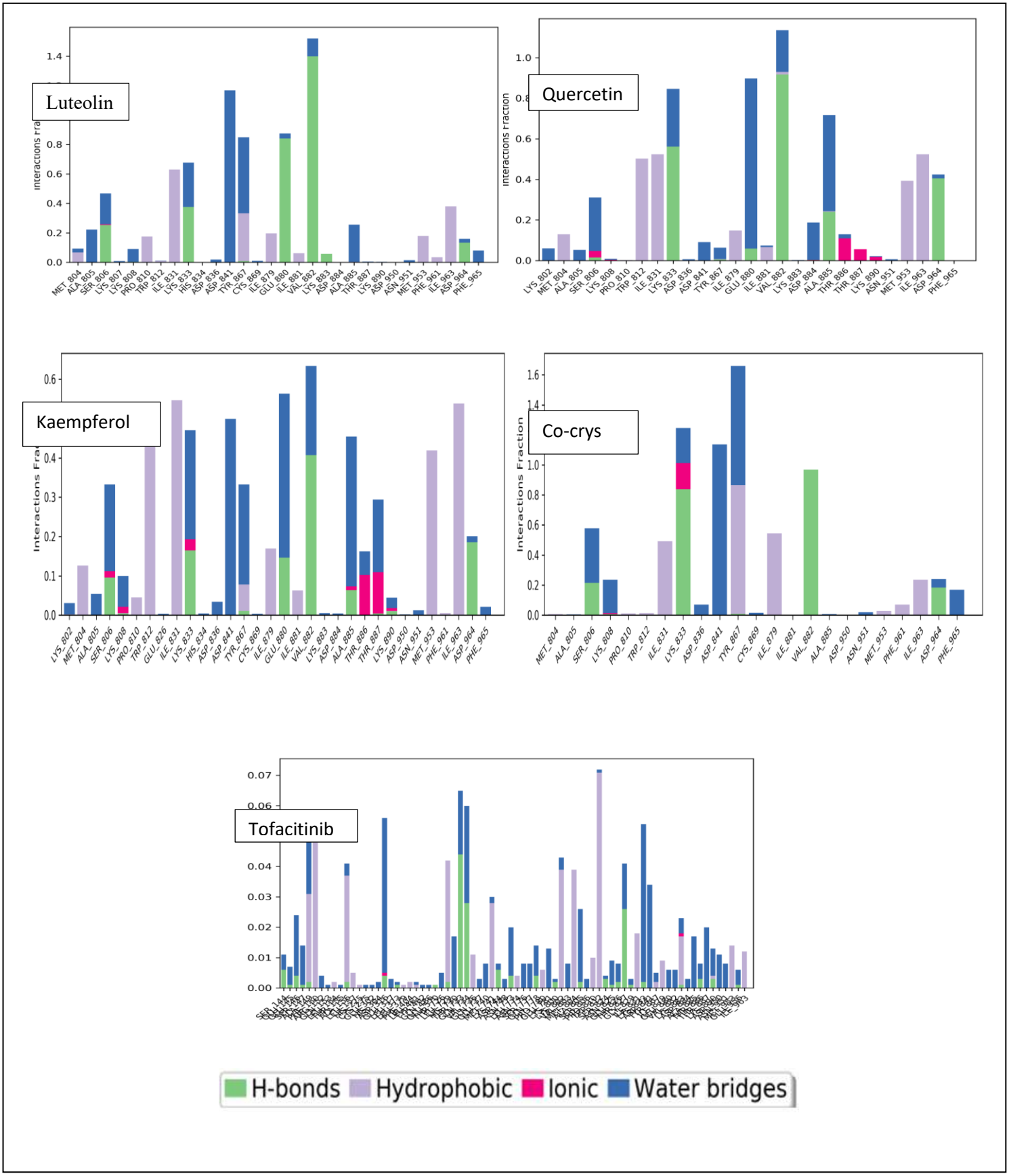


Figure 6: Protein–ligands contacts monitored throughout 100ns simulation run time of PI3Kγ (2A4Z) complex with the lead compounds.

The amino acid residues that formed hydrogen bonds with the ligand included Lys833, Glu880, Val882, Lys883, Asp964, Ala885, Lys808, Tyr867, Thr887, Lys890, Ser144, Glu145, Glu146, Ser147, Ala149, and Glu956 (Figure 6). All five complexes displayed interactions involving water bridges. Additionally, hydrophobic interactions with similar amino acid residues were found to significantly contribute to the stability of the five complexes. Ionic bonds were present in all five complexes as well.

CONCLUSION

This study offers strong evidence that Bryophyllum pinnatum could be a source of new PI3Kγ inhibitors for treating rheumatoid arthritis. Using a multi-step in silico approach that included pharmacophore-based screening, extra-precision molecular docking, ADMET evaluation, MM-GBSA free energy calculations, QSAR-based activity prediction, and extensive 100 ns molecular dynamics simulations, three main flavonoids; luteolin, quercetin, and kaempferol stood out as promising candidates. These compounds showed better binding affinity and favorable interaction profiles with important hydrogen bonds to LYS833, VAL882, GLU880, and SER806, along with hydrophobic and π-stacking contacts. They also had greater predicted inhibitory strength when compared to both the co-crystallized ligand and the approved RA treatment tofacitinib. Additional analyses, with MM-GBSA ΔG_bind values ranging from −45.07 to −44.03 kcal/mol, confirmed stable complex formation and maintained key interactions under simulated physiological conditions. This reinforces their potential to interfere with PI3Kγ-driven inflammation, neutrophil/macrophage infiltration, cytokine overproduction, and synovial issues in RA.

All lead compounds showed acceptable drug-likeness and pharmacokinetic profiles, meeting Lipinski's Rule of Five and displaying favorable solubility and bioavailability predictions. These findings support the well-documented traditional use of B. *pinnatum* in African ethnomedicine for inflammatory conditions and add to existing preclinical evidence of its anti-inflammatory effects. Overall, the data provide a strong scientific reason for the anti-RA properties of Bryophyllum pinnatum constituents through PI3Kγ inhibition. They also lay a solid groundwork for future research. It is highly recommended to validate these findings through in vitro kinase assays, cell-based models of synovial inflammation, and animal models of collagen-induced arthritis. This will help confirm these in silico

predictions and promote the development of safe, effective, and accessible phytopharmaceutical options for treating rheumatoid arthritis.

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