Trop J Nat Prod Res, October 9(10): 4899 - 4908

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In silico Study of the Inhibitory Effects of Sanguinarine and its Proposed Derivative on Phosphoinositide 3-kinase (PI3K) Isoforms

Niran A. Al-Ogaili[1]*and Noor M. Mohammed[2]

[1]*Department of Pharmacognosy, College of Pharmacy, Al-Farabi University, Baghdad, 1011, Iraq*
[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, Al-Farabi University, Baghdad, 1011, Iraq*

ARTICLE INFO

Article history:
Received 22 August 2025
Revised 07 October 2025
Accepted 13 October 2025
Published online 01 November 2025

ABSTRACT

Breast cancer (BC) remains the most commonly diagnosed cancer in women worldwide, with its incidence continuing to rise and often experiences resistance to anticancer drugs. Sanguinarine, a prominent benzophenanthridine alkaloid found in many plants, has garnered attention for its multifaceted antitumor activities, through different mechanisms. This study aims to explore the potential inhibitory effect of Sanguinarine alkaloid (SG) and its proposed structurally modified derivative (SGD), using *in silico* methods, against the PI3K catalytic subunits (p110α, p110β, p110γ, p110δ). Molecular docking was performed to assess the binding affinities of the studied drugs with various PI3K isoforms, while their drug-likeness and pharmacokinetic (ADMET) properties were predicted using *in silico* approaches. All analyses were performed *in silico*, and no laboratory synthesis or biological assays were conducted. Preliminary *in silico* analyses indicated non-significant interactions between the Sanguinarine alkaloid and all PI3K isoforms, accompanied by unfavorable pharmacokinetic and toxicity predictions in comparison with the reference drug, Alpelisib. However, the derived compound exhibited a pan PI3K inhibitory activity with highly significant ($p < 0.05$) binding affinities with all PI3K isoforms (p110α, p110β, p110γ, p110δ) and possessed an acceptable pharmacokinetic and toxicity profile compared to the parent alkaloid. These findings concluded that the proposed derivative (SGD) could be a potential candidate for the development of a novel antibreast cancer drug, obtained by targeting PI3K catalytic subunits. Our future objectives include synthesizing the new molecule, characterizing it by NMR, MS, and IR, and conducting *in vitro* and *in vivo* investigations

Keywords: Sanguinarine, Sanguinarine Derivative, Pan phosphatidylinositol 3-kinase inhibitors, Pan phosphatidylinositol 3-kinase isoform, Docking, Breast Cancer

Introduction

Breast cancer (BC) remains the most commonly diagnosed cancer in women worldwide, with its incidence continuing to rise — particularly in localized hormone receptor-positive (HR+) disease — despite improvements in mortality from earlier detection and systemic therapy.[1] Within the HR+/HER2– tumors, activating mutations in PIK3CA (encoding the class I catalytic subunit p110α of phosphoinositide 3-kinases, PI3Ks) drive the oncogenic Phosphoinositide 3-kinase/Protein Kinase B/Mammalian Target of Rapamycin (PI3K/AKT/mTOR) signaling, and contribute to the resistance to endocrine therapy.[2] Targeted inhibition of this pathway has therefore become a cornerstone of precision oncology in HR+ advanced BC.[2,3] The clinical advancements of PI3K inhibitors has progressed rapidly in recent years. In 2024, inavolisib a next-generation mutant-selective PI3Kα inhibitor, showed significantly improved progression-free survival in the phase 3 INAVO-120 trial and subsequently gained FDA approval in combination with palbociclib and fulvestrant for treating endocrine-resistant, PIK3CA-mutant HR+/HER2– advanced breast cancer.[2,3]

*Corresponding author. E mail: neran.abdulhusain@alfarabiuc.edu.iq
Tel: +9647808439163

Citation: Al-Ogaili NA and Mohammed NM. *In silico* Study of the Inhibitory Effects of Sanguinarine and its Proposed Derivative on Phosphoinositide 3-kinase (PI3K) Isoforms. Trop J Nat Prod Res. 2025; 9(10): 4899 – 4908 https://doi.org/10.26538/tjnpr/v9i10.29

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Although these advances are clinically meaningful, important challenges persist, such as: Dose-limiting toxicities (notably hyperglycemia), variable durability of response due to pathway cross-talk, and adaptive or compensatory signaling across PI3K isoforms, which can limit long-term effectiveness.[4,5] For example, real-world and modeling analyses confirm that clinically significant Alpelisib-related hyperglycemia remains frequent and requires proactive mitigation strategies that may constrain dose intensity and adherence.[4]

Mechanistically, compensatory activation of alternative class I PI3K isoforms and tumor–immune ecosystem effects are increasingly recognized as resistance mechanisms to isoform-selective PI3Kα blockade.[5] Recent preclinical studies show that PI3Kβ mediates immune evasion in PTEN-deficient breast tumors, providing a rationale for broader or combinatorial isoform targeting to counteract immune-suppressive rewiring.[5] These insights have renewed interest in pan-PI3K inhibition — that is, simultaneous inhibition of p110α/β/γ/δ — to suppress tumor-intrinsic signaling, while modulating the tumor microenvironment and enhancing antitumor immunity.[6] Early exemplars, such as KTC1101, demonstrate that rationally designed pan-PI3K inhibitors can inhibit tumor growth and synergize with Programmed cell death protein 1 (PD-1) blockade in multiple models, supporting this therapeutic concept.[6] Yet, achieving pan-isoform potency together with acceptable pharmacokinetics and safety, has proven to be difficult, underscoring the need for new chemical matter that balances the breadth of target engagement with drug-likeness.

Natural products remain a fertile source of anticancer scaffolds due to privileged chemotypes that engage in diverse biological targets. Sanguinarine (SG), a benzophenanthridine alkaloid derived from plants in the Papaveraceae family, has shown multi-modal antitumor activity –including apoptosis induction, ferroptosis, and invasion/angiogenesis suppression– across several tumor types.[7] Notably, recent mechanistic

studies in breast cancer cells have identified the PI3K/AKT axis as one of Sanguinarine's key targets, suggesting that this scaffold may interact with and modulate this signaling pathway.[8] However, native SG is limited by suboptimal selectivity, potential off-target toxicity, and pharmacokinetic liabilities that complicate development.[7] This motivates targeted derivatization to enhance polarity, hydrogen-bonding capacity, and oral drug-likeness, while preserving or amplifying the PI3K-pathway engagement.

Modern *in silico* pipelines enable a rapid triage of such derivatives by integrating structure-based docking with predictive absorption–distribution–metabolism–excretion–toxicity (ADMET) profiling, thereby reducing the cost and de-risking early discovery.[9,10] Contemporary online platforms (e.g., SwissADME and pkCSM) and curated evaluations of free academic tools have markedly improved accuracy and coverage of key developability parameters (e.g., solubility, permeability, metabolic liabilities, cardiotoxicity), facilitating medicinal chemistry iteration prior to synthesis.[10,11] In parallel, contextual clinical literature on PI3K-pathway therapeutics continues to refine the target product profiles for next-generation inhibitors, including the need to mitigate on-target metabolic toxicities and to blunt compensatory isoform signaling.[2,4]

Given the clinical impact and limitations of the current isoform-selective PI3Kα inhibitors, [2,4] evidence that other isoforms (e.g., PI3Kβ) sustain immune evasion and adaptive signaling,[5] and given the multifaceted antitumor potential of SG with putative PI3K/AKT involvement,[7,8] we posited that a rational modification of the SG scaffold could yield a derivative with pan-PI3K engagement and a more favorable ADMET profile. Conceptually, introducing a cysteine-like thioether or analogous polar functionality could strengthen H-bonding within ATP-site pockets across isoforms, improve aqueous solubility, and reduce lipophilicity-driven toxicity features consistent with oral drug-likeness and broader therapeutic index.[10,11]

This study compares the docking-predicted binding affinities and interaction networks of SG with a designed derivative across p110α, p110β, p110γ, p110δ; evaluates drug-likeness and ADMET properties using validated, up-to-date web platforms; and contextualizes whether a pan-isoform profile can mitigate compensatory signaling and immune-evasion mechanisms, relative to isoform-selective inhibition.

To our knowledge, this is the first integrated *in silico* investigation that has designed a cysteine-adduct–inspired Sanguinarine derivative (SGD), explicitly optimized for pan-PI3K binding, and benchmarks its predicted pharmacology and developability against contemporary clinical standards in the PI3K space (e.g., Alpelisib). By uniting pathway biology, natural-product derivatization, and modern ADMET analytics, we aim to nominate a tractable lead for subsequent synthesis and biological validation in PI3K-driven breast cancer.

Materials and Methods

Docking Procedure

The *in silico* docking process predicts the binding interaction between the protein targets and the drug. For this prediction, the GOLD (Genetic Optimization for Ligand Docking) Suite Version (2024.3.0) software (Cambridge Crystallographic Data Centre, UK, 2024) was employed. The X-ray Crystallographic structure of the PI3K lipid kinase isoforms p110α (PDB: 4JPS – alpha), p110β (PDB: 2Y3A – beta), p110δ (PDB: 4XE0 - delta), and p110γ (PDB: 5G2N – gamma) proteins were downloaded from the Protein Data Bank (PDB). The water molecules involved in binding site interaction were conserved, and those that were not involved in the binding interaction with the reference drug were removed. Also hydrogen atoms were added to the amino acid residues, to achieve proper ionization and tautomeric states, which were downloaded from the PDB. Chem 3D (v. 23.1.1) was utilized to apply the Molecular Mechanics (MM2) force field to decrease the energy of the ligands. Hermes visualizer (v. 2024.3.0 CCDC) program in the GOLD Suite was used to set up the PI3K isoform–binding sites for the docking process. The downloaded proteins were docked to SG, SGD, and reference drug Alpelisib. All parameters utilized in the docking procedure were left at their default levels, and all the solutions were assessed using the Piecewise Linear Potential (CHEMPLP) fitness function. The interaction between the amino acid residues of the active sites of PI3K isoforms with SG, SGD, and Alpelisib, were evaluated using the docking data, which included the binding mode, docked position, and binding-free energy.[12]

The proposed Structural Modification of the Sanguinarine Alkaloid

It was reported that L-Cysteine served as a nucleophile that could be reacted with an electrophile (SG) to form a Sanguinarine-Cysteine thioether adduct.[13] The structural modification of SG was proposed theoretically and no chemical synthesis or experimental procedures were performed. No laboratory reagents, solvents, or biological assays were involved at this stage of the study. All analyses in this study were carried out *in silico*.

Pharmacokinetic and Toxicity Evaluation

An important step in the drug discovery process is the assessment of drug-likeness, pharmacokinetics, and toxicity profile of the drug suitability. SwissADME online platform (https://www.swissadme.ch/index.ph p)[14] from Swiss Institute of Bioinformatics was used for the preliminary study of the physicochemical properties, such as, lipophilicity, water solubility, pharmacokinetic parameters, bioavailability score, and drug likeness characteristics of the studied compounds and the reference drug. In addition, pkCSM-pharmacokinetic online web-based tools,[15] by the Biosig Lab, were employed for the comparison of the toxicity profiles between the studied drugs.

Statistical analysis

One way analysis of variance (ANOVA) statistical analysis was used to determine the significance between the interactions of the studied drugs, with different PI3K isoforms. A *p*-value less than 0.05 was regarded as statistically significant. Graph Pad prism 8 was employed for this purpose.

.

Results and Discussion

Molecular Docking of the Studied Compounds with PI3K isoforms

In silico programs are beneficial in drug development, as they support the identification of new drug candidates through screening, designing, and prediction of their therapeutic potential.[16] The *in-silico* method is also being used in the prediction of drug toxicity, allowing researchers to identify possible unfavorable effects at the start of the process of development, thus saving time and money.[17] Docking was used to determine the best fit of SG and SGD to the binding site of the PI3K catalytic isoforms (p110α, p110β, p110γ, p110δ), as compared to the reference drug Alpelisib. The current study revealed non-significant interactions (PLP fitness score) of the SG alkaloid with all isoforms, when compared to the reference drug (Table 1).

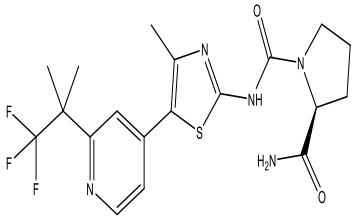
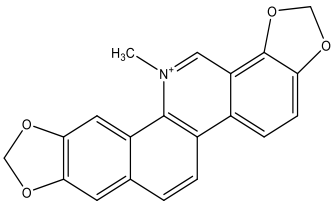
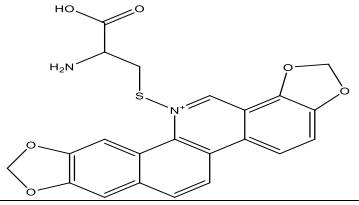
The proposed Structural Modification of the Sanguinarine Alkaloid

Studies show that PI3Kα is frequently mutated in cancers,[18] and in order to increase the binding affinity of SG to this isoform and optimize the binding affinities to the other isoforms, as well as enhance its ADMET profile, a structural modification of the alkaloid has been proposed.

The L-Cysteine amino acid was proposed to modify the SG alkaloid. Here, the iminium bond of the charged SG was considered to be the point of chemical reaction with the nucleophilic sulfur of the thiol group of L-Cysteine.[19] The end product of this reaction yields Sanguinarine-Cysteine thioether Adduct (13-((2-amino-2-carboxyethyl)thio) [1,3]dioxolo[4',5':4,5]benzo[1,2-c][1,3]dioxolo[4,5-i]phenanthridin-13-ium), as illustrated in Figure 1. Additionally, the L-Cysteine was proposed as a proper additive, as it is derived from an organic natural origin, non-toxic, available, and stable.[20] The significant ($p < 0.05$) increase in the binding affinities to all PI3K isoforms and the improvement in the ADMET profile of the SGD compared to SG, were attributed to the added cysteine moiety (Table 1). The Ser919 and Tyr836 residues of the ATP-binding pocket of the p110α formed hydrogen bonds with the OH and NH2 of the added

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: The structures of studied drugs and their average PLP fitness score on PI3K isoforms

Ligand	Chemical Structure	PLP Fitness Score			
		4JPS - Alpha	2Y3A -Beta	5G2N-Gamma	4XE0 -Delta
Alpelisib		76.21664	61.57642	59.57179	62.63305
SG[1]		68.36205	62.50813	72.86986	62.97549
SGD[2]		77.32569	79.03689	86.68814	83.36378

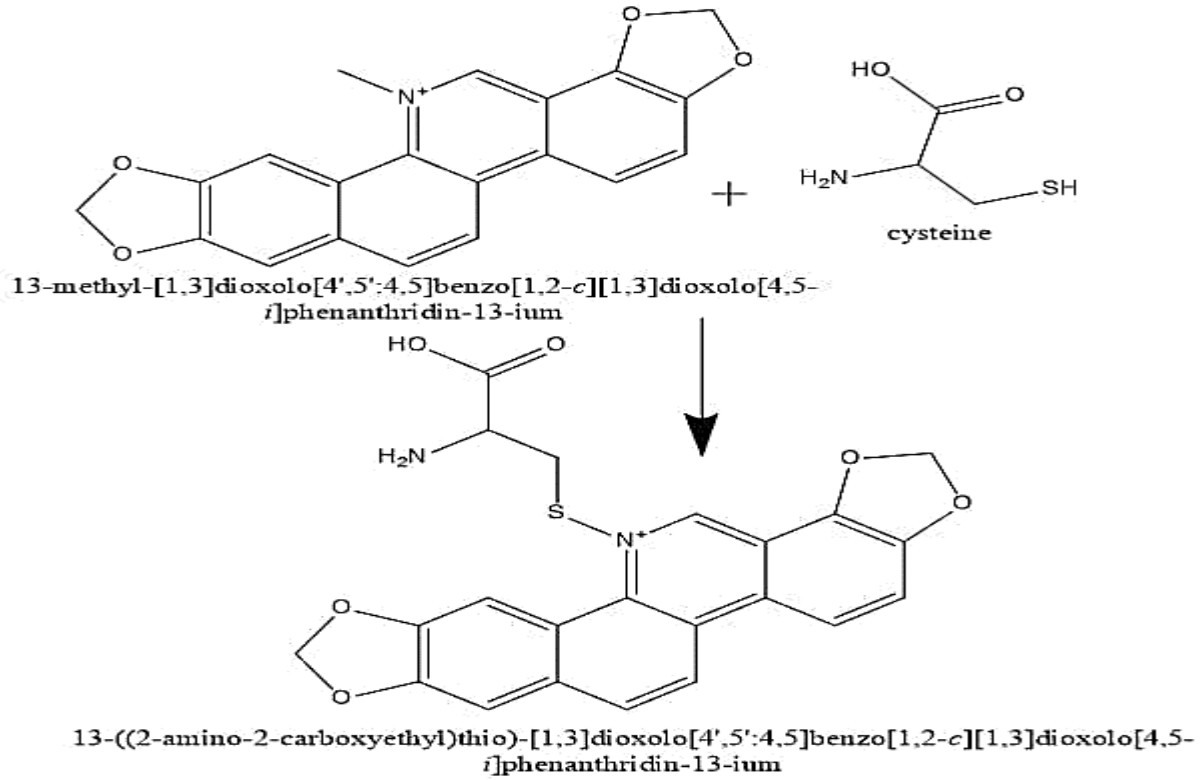


Figure 1: Proposed chemical reaction between Sanguinarine(SG) and L-Cysteine amino acid to produce Sanguinarine Derivative(SGD)

cysteine moiety and with the oxygen of the dioxymethylene bridge of the parent molecule, respectively. In addition, SGD formed hydrophobic interactions with Tyr836, Asp933, Ser919, Thr856, Gln859, Ser854, Trp780, and the Val851 residues, to strengthen its inhibitory mechanism (Figure 2A). The OH and NH2 of SGD formed three hydrogen bonds with the Asp917 and Thr853 residues of the p110β isoform; as also one H-bond was formed between the oxygen of the dioxymethylene bridge and Tyr833. Other hydrophobic interactions involved Ser851, Thr853, Asp917, Tyr833, Ile930, Asp807, Ile845, Glu846, and Asp931 (Figure 2B). In the case of the p110γ isoform, SGD interacted with Asp950, Tyr867, Asp951, Thr887, and Asp964 through hydrogen bonding and with Asn951, Asp950, Asp964, Tyr867, Ile963, Ile879, Glu880, Trp812, Ala885, and Met953 through hydrophobic interactions (Figure 2C).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

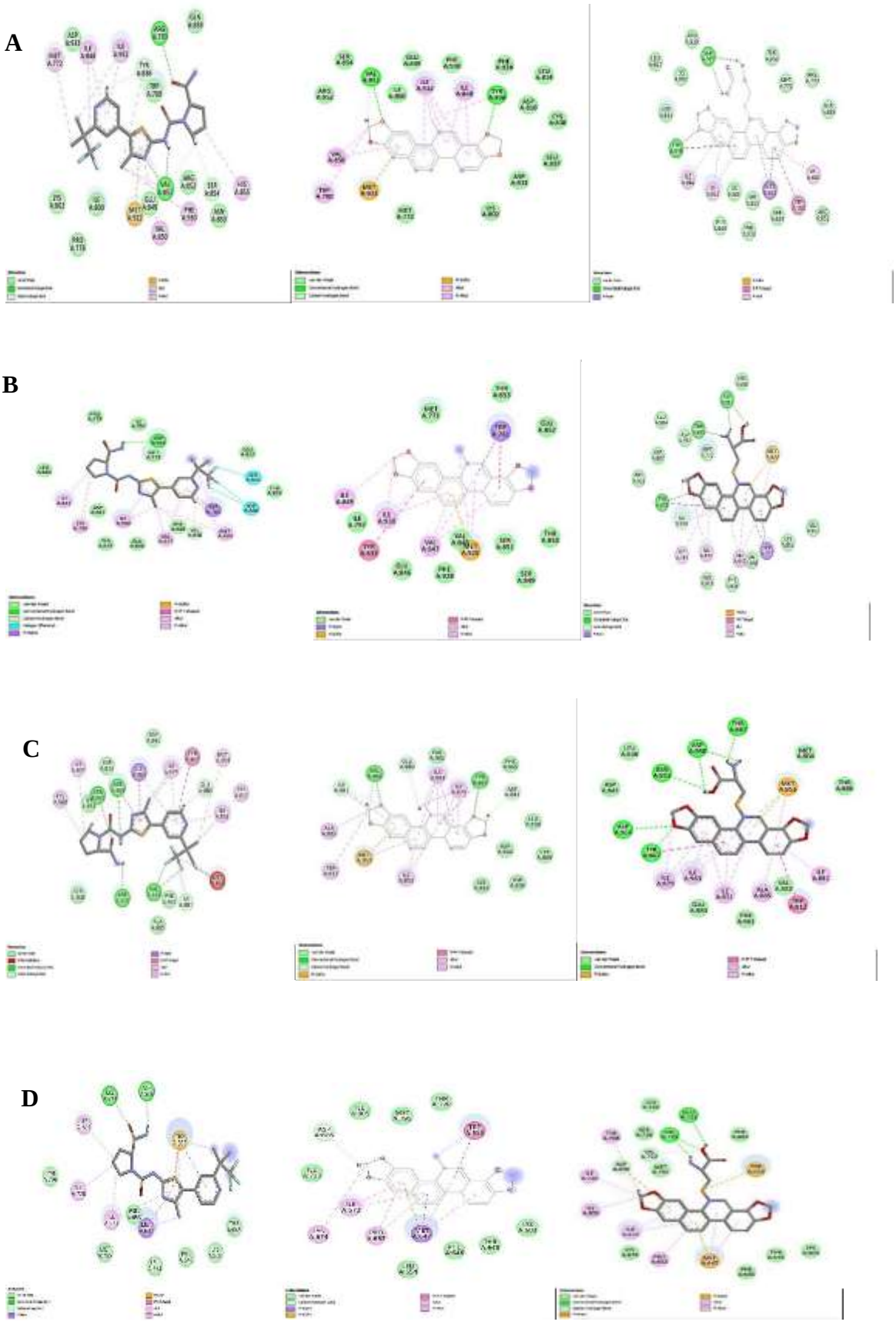


Figure 2: The Docking interaction between p110α (A), p110β (B), p110γ (C), p110δ (D) isoform and Alpelisib (Left), Sanguinarine (Middle), Sanguinarine Derivative (Right)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

It was observed that the PLP score of the Alpelisib interaction with the p110γ isoform was the lowest among the isoforms. This was due to the formation of an unfavorable bump caused by the MET953 residue of the protein that could hinder proper binding.[21] However, the SGD showed significant binding on the Gamma isoform and overcame the steric hindrance caused by MET953 residue (Figure 2C). The interactions between p110δ isoform and SGD formed hydrogen bonds with Val723 and Glu721, besides forming hydrophobic bonds with Val723, Met795, Ile805, Tyr708, Asp806, Ile720, Thr645, Trp655, Val722, and Glu721 (Figure 2D). The differences noticed in the binding affinities between the SG and the four PI3K isoforms compared to its derivative (SGD) may be due to several factors like unsuitable conformation[22] and molecular size,[23] steric hindarnce[23] of SG, or improper hydrogen and hydrophobic bond formation,[24] which have resulted in relatively poor fitting into the binding cavities of proteins.

The docking results showed that SGD bound non-selectively to all isoforms, which indicated a pan-PI3K inhibitory activity. Typically, flat inhibitors were non-selective and this was the case with SG and eventually SGD.[25] Pan inhibitors could be more advantageous than selective inhibitors in several aspects

In general, pan-PI3K inhibitors impede PI3Kα and PI3Kβ directly, to hamper the growth of tumors, and they attack PI3Kδ and PI3Kγ, to improve tumor immunity by immune cell function modulation[26,27]and reshape the tumor microenvironment (TME).[28] It has been reported by Peng *et. al*.,[6] that synthesized novel pan-PI3K inhibitor (KTC1101), significantly hindered cancer cell growth and hampered tumor progression *in vitro* and *in vivo*, respectively. Additionally, TME was modulated due to increase in CD8+ T cells and innate immune cell infiltration. Other studies have shown that p110α and p110β isoforms possessed overlapping roles, so a simultaneous inhibition of these isoforms may be required for optimal antitumor activity.[29] Also, Zhou *et.al*.,[30] who designed B591, a pan-PI3K inhibitor, showed a decrease in cancer stem cells (CSCs) and successfully impeded BC metastasis and retarded tumor relapse after treatment with paclitaxel.

It has been observed that the available PI3K inhibitors often encountered resistance, inadequate bioavailability, possibility of adverse effects due to off-target interactions, narrow therapeutic indices, and acquired resistance.[31] Acquired resistance may develop from a compensatory mechanism that is commonly observed in selective types of PI3K inhibitors.[32] An effective approach applied to get over those compensatory mechanisms is by blocking all isoforms belonging to class I PI3K by pan-PI3K inhibitors. Also, combining of PI3K inhibitors with other BC therapies may overcome resistance. It has been reported that PI3K inhibitors play a role in restoring sensitivity to other therapies when included in combination regimens.[33] Effective combinations with PI3K inhibitors may include receptor tyrosine kinase inhibitors (RTK),[34] endocrine therapy,[35] poly-ADP-ribose polymerase (PARP) inhibitors,[36] and immune checkpoint targeting agents.[37]

Another concerning issue is the adverse effects associated with pan PI3K inhibitors. Mitigating some of the adverse effects and enhancing the anticancer activity of the pan PI3K inhibitors, several therapeutic approaches and strategies have been suggested. These dosing regimens or approaches have been explored to increase the tolerability to PI3K inhibitors, even as they achieve sufficient PI3K pathway inhibition. These include the use of dietary and pharmaceutical strategies,[38] the lowest effective dose,[39] intermittent dosing,[39] pulsatile dosing,[40] and a combination of PI3K inhibitors with other BC therapies.[33] As a result, there is an increasing demand for an upcoming generation of pan-PI3K inhibitors, providing suitable pharmacological and safety profiles.

Pharmacokinetic and Toxicity Evaluation

A preluding study of the ADMET profiles of the investigated compounds was essential to save time, efforts, and cost in drug development using online *in silico* tools.[41] The drug properties are illustrated according to different categories like physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and medicinal chemistry. The predicted pharmacokinetic profile of the proposed SGD showed optimal physicochemical characteristics and fulfilled the requirements of the Lipniski's rule[42-44] (Table 2). The molar refractivity parameter showed that SGD was within the optimal range,[45] whereas, SG showed a relatively lower value (Table 2). This parameter influences molecular interactions, solubility, absorption properties, and eventually bioavailability.[45] In the current study, the reference compound and SGD showed almost similar Topological Polar Surface Area (TPSA) values, while the SG alkaloid showed lower polarity with TPSA 40.80 Å2 (Table 2). The high TPSA of SGD made it difficult to penetrate the blood brain barrier (BBB) and were unlikely to cause harm to the CNS. On the contrary, the SG with lower TPSA may be harmful to CNS, owing to its potential ability to penetrate the BBB.[46] The lipophilicity prediction of all the studied compounds were within the optimal range, however, the logarithm of the n-octanol/water partition coefficient (Log Po/w) values of the reference drug and SG, favored lipophilicity (Table 2).

Table 2: The Physicochemical properties of the Sanguinarine(SG), Sanguinarine Derivative(SGD) compared to Alpelisib obtained by SwissADME

Physicochemical Properties Parameter Optimal Values	Alpelisib	SG	SGD
Formula	$C_{19}H_{22}F_3N_5O_2S$	$C_{20}H_{14}NO_4^+$	$C_{22}H_{17}N_2O_6S^+$
Mwt. (g / mol) < 500 Dalton	441.47	332.3	437.45
Fraction Csp 30.25-1	0.47	0.15	0.18
Number of rotatable bonds 0-9	7	0	4
Number of H-bond acceptors<10	7	4	7
Number of H-bond donors<5	2	0	2
Molar Refractivity 40-130 m^3 \ mol	111.31	94.68	116.36
TPSA[1] (Å2)20-130 Å2	129.45	40.80	129.42
Consensus Log $P_{o/w}$[2]1-3	2.95	2.88	1.12

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

ESOL (Log S)> -4	-4.42	-5.24	-3.60
Pharmacokinetics Parameters	Alpelisib	SG	SGD
GI absorption	Low	High	High
Glycoprotein Pump (P-gp) substrate	Yes	Yes	Yes
(BBB)[3] permeation	No	Yes	No
Lipinski's rule of five	Yes	Yes	Yes
Lead-likeness	NA[4]	1 violation; XLOGP3≥3.5	NA[4]

[1]Topological Polar Surface Area; [2]logarithm of the n-octanol/water partition coefficient; [3]Blood Brain Barrier; [4]Not applicable

These compounds might show an expanded volume of distribution, longer half-life, and longer duration of drug / target interaction that might result in toxic or adverse effects, compared to SGD.[47] The Log S (water solubility) of SGD was consistent with the study of Paul *et.al*.,[48] who showed that a Log S value higher than - 4 ensured good solubility in water, which was important for the solubilization and absorption processes in the GI tract (Table 2). The BOILED-EGG model demonstrated potential passive GI absorption of SGD, as it was located in the white region, while SG was located in the yellow region, indicating CNS permeation, as shown in Table 2 and Figure 3.

The SwissADME tool generated a bioavailability radar having a chart figure that showed six physicochemical properties (Mwt., Log Po/w, Csp3, TPSA, Log S, and a number of rotatable bonds) related to oral bioavailability. This chart gave us a prompt idea of whether the studied drug was within the optimal range for oral bioavailability or not, and if it could undergo drug design and development processes.[49]

Almost all the parameters possessed by the SGD were within the optimal range of the bioavailability radar, compared to the SG alkaloid, as shown in Table 2 and Figure 4.

The SG alkaloid was evaluated for its potential lead-likeness, the predicted results completely aligned with the optimal criteria,[49] as mentioned in Table 3. These properties encouraged the structural modification of SG to obtain SGD, a prospective therapeutic candidate.

The predicted outcome of metabolic enzyme inhibition revealed that SGD perfectly matched the reference anticancer drug-enzyme inhibitory profile, in contrast to SG (Table 4). Thus, SGD could display a lower possibility of interaction with other drugs or food that was simultaneously administered.

Moreover, the predicted SGD toxicity profile was relatively acceptable compared to the reference drug and SG, as shown in Table 5. These findings made SGD a suitable candidate for oral dosage forms that could be convenient for patients with breast cancer, due to its decreased lipophilicity, increased water solubility, and oral bioavailability.

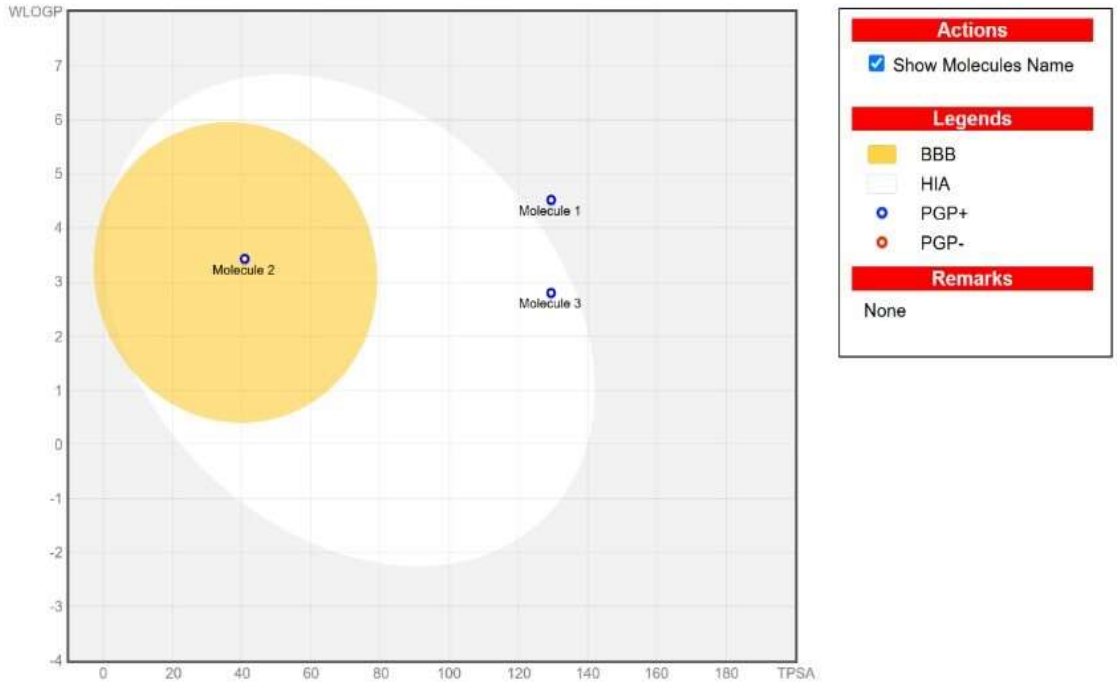


Figure 3: BOILED Egg model of Molecule 1 (Alpesilib), Molecule 2 (Sanguinarine), and Molecule 3 (Sanguinarine Derivative), generated by SwissADME tool

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

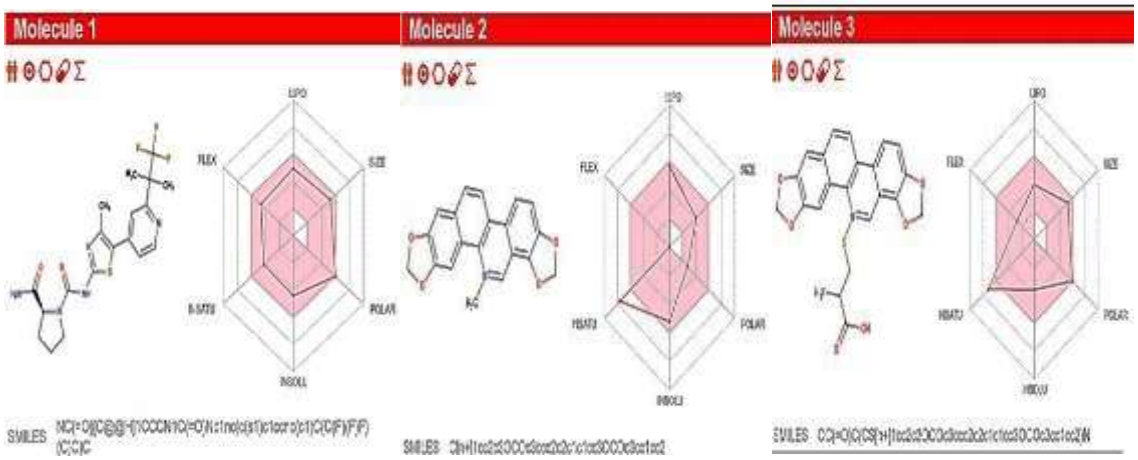


Figure 4: Bioavailability Radar of Molecule 1 (Alpelisib), Molecule 2 (Sanguinarine), and Molecule 3 (Sanguinarine Derivative), obtained by Swiss ADME

Table 3: Potential Lead-likeness values of Sanguinarine (SG) compared to optimal values[49]

Values	Mwt.[1]	Log P	HBA[2]	HBD[3]	RB[4]	TPSA[5]
Optimal	250-350 Da[6]	1-3	≤ 6	≤ 3	≤ 7	≤ 90 A°
SG	332.33 Da	2.88	4	0	0	40.80 A°

[1]Molecular weight; [2]Hydrogen Bond Acceptor; [3]Hydrogen Bond Donor; [4]Rotatable Bond; [5]Topological Polar Surface Area; dalton

Table 4: The potential inhibition of enzymes by the Sanguinarine (SG) and Sanguinarine derivative (SGD) compared to Alpelisib

Enzymes	Alpelisib	SG	SGD
CYP 1A2	No	Yes	No
CYP 2C19	Yes	Yes	Yes
CYP 2C9	No	No	No
CYP 2D6	No	No	No
CYP 3A4	Yes	No	Yes

Table 5: The Studied Compounds' Toxicity Analysis by the pkCSM pharmacokinetics platform

Parameters	Alpelisib	SG	SGD
AMES[1] Toxicity	No	No	No
MTD[2] (log mg/kg/day)	0.022	0.503	0.675
hERG[3] Inhibition I / II	No / No	No / Yes	No / No
LD_{50}	2.563 (mol/kg)	2.685 (mol/ Kg)	2.739 (mol/kg)
Hepatotoxicity	Yes	No	No
Carcinogenicity	No	No	No
Skin Sensitization	No	No	No

[1]Reverse mutation bacterial assay; [2]Maximum Tolerated Dose; [3]Human Ether-à-go-go-Related Gene

Conclusion

Resistance to current PI3K therapies in breast cancer patients necessitates the continuous development of safe and effective PI3K inhibitors. *In silico* procedures play an important role in the preliminary assessment of drug discovery, thus saving time and money. Natural compounds have distinctive structures and biological activities that could be utilized as a starting point for the development of novel therapeutic agents. In the current study, SG, a naturally derived compound was investigated for its potential as a PI3K inhibitor. The preliminary pharmacodynamic study showed non-significant interactions with all isoforms, compared to the reference drug. The proposed structurally modified compound (SGD) showed a highly significant ($p < 0.05$), enhanced interaction with all isoforms, and hence, could be used as a potential pan-PI3K inhibitor in breast cancer. The improvement in the PLP score can be attributed to the incorporation of L-cysteine, which engaged in hydrogen bonding and hydrophobic interactions with the PI3K isoform residues, thereby stabilizing the SG derivative within the receptor binding site and enhancing its ADME and toxicity profiles. Such inhibitors may overcome the resistance observed

with selective PI3K inhibitors and their possible adverse effects may be reduced by different therapeutic strategies or approaches. The obtained data were encouraging, which made the proposed SGD a lead candidate for future synthesis, characterization, and *in vitro* and *in vivo* investigations to combat breast cancer resistance and metastasis, and to improve immunomodulation of the tumor microenvironment.

Conflict of interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors express their appreciation to Prof. Dr. Monther Faisal Mahdi for his kind assistance in conducting the docking procedures on the GOLD program, a component of the CSD-Discovery Suite. Also, we would like to express our sincere gratitude to Dr. Sabah Jawad for his valuable scientific support and remarks.

References

1. Giaquinto AN, Sung H, Newman LA, Freedman RA, Smith RA, Star J, Jemal A, Siegel RL. Breast cancer statistics 2024. CA Cancer J Clin. 2024;74(6):477–495. doi:10.3322/caac.21863.
2. Turner NC, Im S-A, Saura C, Juric D, Loibl S, Kalinsky K, Schmid P, Loi S, Sunpaweravong P, Musolino A, Li H, Zhang Q, Nowecki Z, Leung R, Thanopoulou E, Shankar N, Lei G, Stout TJ, Hutchinson KE, Schutzman JL. Inavolisib-based therapy in PIK3CA-mutated advanced breast cancer. N Engl J Med. 2024;391(17):1584–1596. doi:10.1056/ NEJMoa2404625.
3. U.S. Food and Drug Administration. FDA approves inavolisib with palbociclib and fulvestrant for endocrine-resistant, PIK3CA-mutated, HR-positive, HER2-negative, advanced breast cancer [Internet]. Silver Spring (MD): U.S. Food and Drug Administration; 2024 Oct 10 [cited 2025 Sep 23]. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-inavolisib-palbociclib-and-fulvestrant-endocrine-resistant-pik3ca-mutated-hr-positive.
4. Rodón J, Demanse D, Rugo HS, André F, Janku F, Mayer I, Burris H, Simo R, Farooki A, Hu H, Lorenzo I, Quadt C, Juric D. A risk analysis of alpelisib-induced hyperglycemia in patients with advanced solid tumors and breast cancer. Breast Cancer Res. 2024;26:118. doi:10.1186/s13058-024-01773-1.
5. Bergholz JS, Wang Q, Wang Q, Ramseier M, Prakadan S, Wang W, Fang R, Kabraji S, Zhou Q, Gray GK, Abell-Hart K, Xie S, Guo X, Gu H, Von T, Jiang T, Tang S, Freeman GJ, Kim HJ, Shalek AK, Roberts TM, Zhao JJ. PI3Kβ controls immune evasion in PTEN-deficient breast tumours. Nature. 2023;617(7959):139-146. doi: 10.1038/s41586-023-05940-w.
6. Peng X, Huang X, Lulu TB, Jia W, Zhang S, Cohen L, Huang S, Fan J, Chen X, Liu S, Wang Y, Wang K, Isoyama S, Dan S, Wang F, Zhang Z, Elkabets M, Kong D. A novel pan-PI3K inhibitor KTC1101 synergizes with anti-PD-1 therapy by targeting tumor suppression and immune activation. Mol. Cancer.2024;23:54. https://doi.org/10.1186/s12943-024-01978-0
7. Huang LJ, Lan JX, Wang JH, Huang H, Lu K, Zhou ZN, Xin SY, Zhang ZY, Wang JY, Dai P, Chen XM, Hou W. Bioactivity and mechanism of action of sanguinarine and its derivatives in the past 10 years. Biomed Pharmacother. 2024;173:116406. doi: 10.1016/j.biopha.2024.116406. Epub 2024 Mar 8. PMID: 38460366.
8. Messeha SS, Zarmouh NO, Mendonça P, Alwakwak AA, Soliman KFA. Involvement of AKT/PI3K pathway in sanguinarine's antiproliferative effects in breast cancer cells. CGP. 2023;20(4):323–342. doi:10.21873/cgp.20385.
9. Dulsat J, López-Nieto B, Estrada-Tejedor R, Borrell JI. Evaluation of free online ADMET tools for academic or small biotech environments. Molecules. 2023;28(2):776. doi:10.3390/molecules28020776.
10. Fu L, Shi S, Yi J, Wang N, He Y, Wu Z, Peng J, Deng Y, Wang W, Wu C, Lyu A, Zeng X, Zhao W, Hou T, Cao D. ADMET lab 3.0: an updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. Nucleic Acids Res. 2024;52(W1):W422-W431. doi: 10.1093/nar/gkae236.
11. Hao C, Wei Y, Meng W, Zhang J, Yang X. PI3K/AKT/mTOR inhibitors for hormone receptor-positive advanced breast cancer. Cancer Treat Rev. 2025;132:102861. doi:10.1016/j.ctrv.2024.102861.
12. Kartika IR, Syach AA, Larasati D, Pitaloka SA, Octaviany SN, Kurniadewi F, Dianhar H, Saputra I, Nurjayadi M, Syaadah RS, Namirah I. *In-Silico* Molecular Docking and ADMET Prediction of Natural Compounds In Oncom. Trop JNat Prod Res. 2025; 9(5):2167 -2176. https://doi.org/10.26538/tjnpr/v9i5.42
13. Salih SJ. Synthesis of New Ester Derivatives of Metronidazole as Possible Prodrugs. [Online]. 2007. [Cited 2025 July 1]. Available from: https://www.academia.edu/5040672.
14. Ramos RS, Borges RS, de Souza JSN, Araujo IF, Chaves MH, Santos CBR. Identification of potential antiviral inhibitors from hydroxychloroquine and 1, 2, 4, 5-tetraoxanes analogues and investigation of the mechanism of action in SARS-CoV-2. J. Drug Deliv. Ther. 2019; 9: 366–369. https://doi.org/10.3390/ijms23031781
15. Vijayalakshmi M, Dhanapradeeba V, Kunjiappan S, Sundar K, Pandian SRK. Targeting TLRs with the Derivatives of Mimosa Pudica: An *In Silico* Approach. Biointerface Res. Appl. Chem. 2023;13(3): 237. https://doi.org/10.33263/BRIAC133.237
16. Nimgampalle M, Devanathan V, Saxena A. Importance of *in silico* studies on the design of novel drugs from medicinal plants against 21st-century pandemics: past, present, and future. In: Pandemic Outbreaks in the 21st Century. 2021:211–223. http://dx.doi.org/10.1016/B978-0-323-85662-1.00013-6
17. Roney M, Fasihi MF, Aluwi M. The importance of *in-silico* studies in drug discovery. Intelligent Pharmacy.2024; 2(4):578-579. http://dx.doi.org/10.1016/j.ipha.2024.01.010
18. Gerstung M, Jolly C, Leshchiner I, Dentro SC, Gonzalez S, Rosebrock D, Mitchell TJ, Rubanova Y, Anur P, Yu K, Tarabichi M, Deshwar A, Wintersinger J, Kleinheinz K, Vázquez-García I, Haase K, Jerman L, Sengupta S, Macintyre G, Malikic S, Donmez N, Livitz DG, Cmero M, Demeulemeester J, Schumacher S, Fan Y, Yao X, Lee J, Schlesner M, Boutros PC, Bowtell DD, Zhu H, Getz G, Imielinski M, Beroukhim R, Sahinalp SC, Ji Y, Peifer M, Markowetz F, Mustonen V, Yuan K, Wang W, Morris QD. The evolutionary history of 2,658 cancers. Nature. 2020;578(7793):122-128. doi: 10.1038/s41586-019-1907-7.
19. Vespalec R, Barták P, Šimánek V, Vlčková M. Electrophoretic investigation of interactions of sanguinarine and chelerythrine with molecules containing mercapto group. J. Chromatogr. B. 2003; 797(1–2): 357-366. https://doi.org/10.1016/s1570-0232(03)00308-8
20. Bonifácio VDB, Pereira SA, Serpa J, Vicente JB. Cysteine metabolic circuitries: druggable targets in cancer. Br J Cancer. 2021;124(5):862-879. doi: 10.1038/s41416-020-01156-1.
21. Avashthi H, Srivastava A, Singh DB. Cavity/Binding Site Prediction Approaches and Their Applications. In: Singh, D.B. (Eds.). Computer-Aided Drug Design. Singapore: Springer; 2020. 49-69 p. https://doi.org/10.1007/978-981-15-6815-2_3

22. Maulydia NB, Tallei TE, Ginting B, Idroes R, Nillian D, Faradilla M. Analysis of flavonoid compounds of Orange (Citrus sp.) peel as anti-main protease of SARS-CoV-2: A molecular docking study. ICAGRI. 2021. IOP Conf. Series: Earth and Environmental Science 951 (2022) 012078. doi:10.1088/1755-1315/951/1/012078
23. Yu AC, Lian H, Kong X, Lopez Hernandez H, Qin J, Appel EA. Physical networks from entropy-driven non-covalent interactions. Nat. Commun.2021;12(746). https://doi.org/10.1038/s41467-021-21024-7
24. Danislav S. Spassov. Binding Affinity Determination in Drug Design: Insights from Lock and Key, Induced Fit, Conformational Selection, and Inhibitor Trapping Models. Int. J. Mol. Sci. 2024; 25(13):7124. https://doi.org/10.3390/ijms25137124
25. Furet P , Guagnano V , Fairhurst RA , Imbach-Weese P , Bruce I , Knapp M , Fritsch C , Blasco F , Blanz J , Aichholz R , Hamon J , Fabbro D , Caravatti G. Discovery of NVP-BYL719 a potent and selective phosphatidylinositol-3 kinase alpha inhibitor selected for clinical evaluation. Bioorg Med Chem Lett.2013;23:3741–3748. https://doi.org/10.1016/j.bmcl.2013.05.007
26. Sun P, Zhang X, Wang RJ, Ma QY, Xu L, Wang Y, Liao HP, Wang HL, Hu LD, Kong X, Ding J, Meng LH. PI3Kalpha inhibitor CYH33 triggers antitumor immunity in murine breast cancer by activating CD8(+)T cells and promoting fatty acid metabolism. J Immunother Cancer. 2021;9: e003093. https://doi.org/10.1136/jitc-2021-003093
27. Bergholz JS, Wang Q, Wang Q, Ramseier M, Prakadan S, Wang W, Fang R, Kabraji S, Zhou Q, Gray GK, Abell-Hart K, Xie S, Guo X, Gu H, Von T, Jiang T, Tang S, Freeman GJ, Kim HJ, Shalek AK, Roberts TM, Zhao JJ. PI3Kβ controls immune evasion in PTEN-deficient breast tumours. Nature.2023;617(7959):139-146. doi: 10.1038/s41586-023-05940-w.

35. Dose Scheduling of AZD8835, a Novel Selective Inhibitor of PI3Kalpha and PI3Kdelta, Demonstrates Treatment Strategies for PIK3CA-Dependent Breast Cancers. Mol Cancer Ther.2016;15:877–889. https://doi.org/10.1158/1535-7163.mct-15-0687
36. Glaeske S, Huebner F, Anurin A, Janzer A, Zitzmann-Kolbe S, Juliane P, Glaeske K, Berndt S, Mumberg D, Gorjanacz M, Ziegelbauer K, Kreft B, Liu N. Pulsatile inhibition of PI3K converts immune suppression by Tregs and M2-TAM to anti-tumor immune response in animal models insensitive or resistant to the monotherapies of PI3K and checkpoint inhibitors. Cancer Res.2018;78:LB-123-LB-123. https://doi.org/10.1007/s10238-023-01227-6
37. Massacesi C, Di Tomaso E, Urban P, Hirawa St. PI3K inhibitors as new cancer therapeutics: implications for clinical trial design. Onco Targets Ther.2016;9:203-210. https://doi.org/10.2147/ott.s89967
38. Tao JJ, Castel P, Radosevic-Robin N, Elkabets M, Auricchio N, Aceto N, Weitsman G, Barber P, Vojnovic B, Ellis H, Morse N, Viola-Villegas NT, Bosch A, Juric D, Hazra S, Singh S, Kim P, Bergamaschi A, Maheswaran S, Ng T, Penault-Llorca F, Lewis JS, Carey LA, Perou CM, Baselga J, Scaltriti M. Antagonism of EGFR and HER3 enhances the response to inhibitors of the PI3K-Akt pathway in triple-negative breast cancer. Sci. Signal.2014;7(318):29. https://doi.org/10.1126/scisignal.2005125
39. Schmid P, Pinder SE, Wheatley D, Macaskill J, Zammit C, Hu J, Price R, Bundred N, Hadad S, Shia A, Sarker SJ, Lim L, Gazinska P, Woodman N, Korbie D, Trau M, Mainwaring P, Gendreau S, Lackner MR, Derynck M, Wilson TR, Butler H, Earl G , Parker P, Purushotham A, Thompson A. Phase II randomized preoperative window-of-opportunity study of the PI3K inhibitor pictilisib plus anastrozole compared with anastrozole alone in patients with
28. Collins NB, Al Abosy R, Miller BC, Bi K, Zhao Q, Quigley M, Ishizuka JJ, Yates KB, Pope HW, Manguso RT, Shrestha Y, Wadsworth M, Hughes T, Shalek AK, Boehm JS, Hahn WC, Doench JG, Haining WN. PI3K activation allows immune evasion by promoting an inhibitory myeloid tumor microenvironment. J Immunother Cancer. 2022;10: e003402. https://doi.org/10.1136/jitc-2021-003402
29. Weigelt B, Warne PH, Lambros MB, Reis-Filho JS, Downward J. PI3K pathway dependencies in endometrioid endometrial cancer cell lines. Clin Cancer Res. 2013;19:3533–3544. https://doi.org/10.1158/1078-0432.ccr-12-3815
30. Zhou H, Yu C, Kong L, Xu X, Yan J, Li Y, An T, Gong L, Gong Y, Zhu H, Zhang H, Yang X, Li Y. B591, a novel specific pan-PI3K inhibitor, preferentially targets cancer stem cells. Oncogene.2019;38:3371–3386. https://www.nature.com/articles/s41388-018-0674-5
31. Castel P, Toska E, Engelman JA, Scaltriti M. The present and future of PI3K inhibitors for cancer therapy. Nat Cancer. 2021;2:587–597. https://doi.org/10.1038/s43018-021-00218-4
32. Stanland LJ, Ang HX, Hoj JP, Chu Y, Tan P, Wood KC, Luftig MA. CBF-beta mitigates PI3K-alpha-specific inhibitor killing through PIM1 in PIK3CA mutant gastric cancer. Mol Cancer Res. 2023;21(11):1148–1162. doi: 10.1158/1541-7786.MCR-23-0034
33. Hopkins BD, Pauli C, Du X, Wang DG, Li X, Wu D, Amadiume SC, Goncalves MD, Hodakoski C, Lundquist MR, Bareja R, Ma Y, Harris EM, Sboner A, Beltran H, Rubin MA, Mukherjee S, Cantley LC. Suppression of insulin feedback enhances the efficacy of PI3K inhibitors. Nature. 2020; 560: 499–503. https://doi.org/10.1038/s41586-018-0343-4
34. Hudson K, Hancox UJ, Trigwell C, McEwen R, Polanska UM, Nikolaou M, Gutierrez PM, Avivar-Valderas A, Delpuech O, Dudley P, Hanson L, Ellston R, Jones A, Cumberbatch M, Cosulich SC, Ward L, Cruzalegui F, Green S. Intermittent High-estrogen receptor-positive breast cancer. J Clin Oncol. 2016;34(17):1987–1994. https://doi.org/10.1200/jco.2015.63.919
40. De P, Sun Y, Carlson JH, Friedman LS, Leyland-Jones BR, Dey N. Doubling down on the PI3K-AKT-mTOR pathway enhances the antitumor efficacy of PARP inhibitor in triple negative breast cancer model beyond BRCA-ness. Neoplasia.2014;16(1):43–72. https://doi.org/10.1593/neo.131694
41. Sai J, Owens P, Novitskiy SV, Hawkins OE, Vilgelm AE, Yang J, Sobolik T, Lavender N, Johnson AC, McClain C, Ayers GD, Kelley MC, Sanders M, Mayer IA, Moses HL, Boothby M, Richmond A. PI3K inhibition reduces mammary tumor growth and facilitates antitumor immunity and anti-PD1 responses. Clin Cancer Res.2017;23(13):3371–3384. https://doi.org /10.1158/1078 -0432. ccr-16-2142
42. Jasim SF, Mustafa YF. Synthesis, ADME Study, and antimicrobial evaluation of novel naphthalene-based derivatives. J. Med. Chem. Sci.2022;5(5):793–807. https://doi.org/10.26655/JMCHEMSCI.2022.5.14
43. Younes AH, Mustafa YF. Unveiling the biomedical applications of novel coumarins isolated from Capsicum annuum L. Seeds by a multivariate extraction technique, Chem. Biodivers.2024;21:6. https://doi.org/10.1002/cbdv.202400581
44. Jagannathan R. Characterization of drug-like chemical space for cytotoxic marine metabolites using multivariate methods. ACS Omega.2019;4(3):5402–5411. https://doi.org/10.1021/acsomega.8b01764
45. Pollastri MP. Overview on the rule of five. Curr. Protocol. Pharmacol.2010;49(1). https://doi.org/10.1002/0471141755.ph0912s49
46. Ibrahim ZY, Uzairu A, Shallangwa GA, Abechi SE. Pharmacokinetic predictions and docking studies of substituted aryl amine-based triazolopyrimidine designed inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

(PfDHODH), Future J. Pharmaceut. Sci.2021;7(1):133. http://dx.doi.org/10.1186/s43094-021-00288-2

47. Prasanna S, Doerksen R. Topological polar surface area: a useful descriptor in 2D-QSAR, Curr. Med. Chem.2009;16(1):21–41. https://doi.org/10.2174/092986709787002817
48. Miller RR, Madeira M, Wood HB, Geissler WM, Raab CE, Martin IJ. Integrating the impact of lipophilicity on potency and pharmacokinetic parameters enables the use of diverse chemical space during small molecule drug optimization, J. Med. Chem.2020;63 (21):12156–12170. https://doi.org/10.1021/acs.jmedchem.9b01813
49. Paul D. Leeson PD, Bento AP, Gaulton A, Hersey A, Manners EJ, Radoux CJ, Leach AR. Target-Based Evaluation of "Drug-Like" Properties and Ligand Efficiencies. J. Med. Chem. 2021; 64(11): 7210–7230. https://doi.org/10.1021/acs.jmedchem.1c00416
50. 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):1–13. https://doi.org/10.1038/srep42717