International Journal of Research and Review
[Indexed, Peer-Reviewed Journal]
Volume 12; Issue: 12; December 2025
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Molecular Docking of Fangchinoline Against Bruton's Tyrosine Kinase (BTK) as a Potential Anticancer Target

Alan Maulana[1], Poppy Anjelisa Zaitun Hasibuan[2], Denny Satria[3]

[1]Master in Pharmaceutical Sciences Program, [2]Departement of Pharmacology,
[3]Department of Pharmaceutical Biology,
Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia.

Corresponding Author: Poppy Anjelisa Zaitun Hasibuan

DOI: https://doi.org/10.52403/ijrr.20251276

ABSTRACT

Bruton's tyrosine kinase is an oncogenic kinase implicated in B-cell malignancies, and fangchinoline is a natural alkaloid with reported anticancer properties. This study evaluated the binding of fangchinoline to Bruton's tyrosine kinase using structure-based docking. The Bruton's tyrosine kinase protein structure (PDB ID 3GEN) was prepared by removing water molecules and existing ligands, and docking simulations were performed using the AutoDock Tools. Fangchinoline bound to the adenosine triphosphate-binding pocket with a predicted binding free energy of –8.56 kcal/mol. In the optimal binding pose, fangchinoline formed hydrogen bonds with Ser538 and Asp539 in the kinase activation loop and hydrophobic contacts with residues including Cys481, Lys430, Leu408, Val416, Ala428, and Leu528. The compound bound to the ATP site in a non-covalent manner, using the activation loop and hydrophobic pocket residues. Overall, the docking results indicated that fangchinoline could serve as a candidate for further investigation as a BTK-targeted anticancer agent.

Keywords: *Fangchinoline; Bruton's tyrosine kinase; Molecular docking; Cancer therapy; Natural Product; Binding affinity*

INTRODUCTION

Bruton's tyrosine kinase (BTK) is a non-receptor tyrosine kinase that was initially identified through its mutation in X-linked agammaglobulinemia (XLA) and is now recognized as a central mediator of B-cell receptor (BCR) signaling [1]. BTK signaling plays a vital role in the normal development and function of B cells, and any disruption in this pathway can lead to the proliferation and persistence of malignant B cells [1]. Consequently, BTK has been recognized as a crucial target for therapeutic intervention in B-cell malignancies. Inhibitors targeting BTK, particularly small-molecule ones, have demonstrated remarkable antitumor activity. For instance, ibrutinib, an oral covalent BTK inhibitor, achieves high response rates in cases of relapsed/refractory chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) [1]. The importance of ibrutinib and similar drugs BTK in B-cell malignancies teaches the importance of creating other drugs directing BTK.

Molecular docking is a computational method based on structural analysis, extensively employed in drug development to forecast the interaction between a small molecule and its protein target.[2][3]. Algorithms inspect the conformations of ligands in the receptor binding site to determine the binding energies of the ligands to the receptor. These binding energies will

determine the important interactions with the receptor whether it be hydrogen bonding, hydrophobic contact, or something else to interact with the receptor. [2][4]. Thus, in silico docking serves as a functional first screen to assess and compare potential inhibitors of a given protein before experimental testing.

Ibrutinib, an innovative irreversible inhibitor of BTK, is extensively employed as a positive control in docking studies aimed at Bruton's tyrosine kinase (BTK) [5]. It forms a covalent bond with the active-site cysteine residue (Cys481) of BTK, resulting in a permanent thioether linkage that deactivates the kinase [6]. This covalent mechanism, which is made possible by an electrophilic acrylamide warhead on ibrutinib, enables this covalent mechanism, giving BTK very high potency and selectivity [6]. Clinically, ibrutinib's efficacy established BTK as a therapeutic target: it was the first BTK inhibitor approved for B-cell malignancies (e.g., chronic lymphocytic leukemia and mantle cell lymphoma) and has achieved remarkable patient outcomes [5][6]. Its well-characterized binding mode, irreversible inhibition of BTK catalytic activity, and proven clinical success make ibrutinib an ideal reference compound for validating docking protocols and benchmarking new BTK inhibitors in silico [5].

Fangchinoline, a bis-benzylisoquinoline alkaloid obtained from Stephania tetrandra, is well-known for its significant anticancer effects [7]. We opted to use fangchinoline in our docking studies because research suggests its anticancer properties are linked to pathways influenced by BTK signaling. BTK is pivotal in B-cell receptor signaling, which subsequently triggers the PI3K/Akt and NF-κB pathways [5]. Crucially, fangchinoline impedes both the phosphorylation of Akt and the activation of NF-κB in cancer cells [8][9]. For example, when fangchinoline is administered, it leads to a reduction in the phosphorylation of IKK/p65, which subsequently hinders NF-κB signaling pathways. Additionally, it diminishes Akt activation, thereby effectively obstructing the pro-survival signals in cancer cells [9]. These actions are similar to the inhibition of BTK-driven signaling, which generally supports survival and proliferation signals, indicating that fangchinoline disrupts BTK's oncogenic pathway. Furthermore, the rigid polyaromatic structure of fangchinoline offers numerous binding sites within the kinase ATP pocket, and virtual screening studies have identified it as a prominent compound with a strong affinity for target binding. [8]. Its close analog, tetrandrine, also inhibits oncogenic kinase pathways (such as EGFR–PI3K/Akt and MAPK) [8], bolstering the rationale for evaluating fangchinoline as a BTK-targeted molecule in docking studies.

MATERIALS & METHODS

Preparation

BTK (PDB ID: 3GEN) was retrieved from the Protein Data Bank in the RCSB (https://www.rcsb.org/). The preparation of this target protein using AutoDock Tools (version 1.5.7) included the removal of water molecules; the native ligand (B43) was separated from the protein sequence to expose the ligand-binding pocket cavity [10][11].

Validation of docking

Molecular docking was validated using AutoDock Tools 1.5.7, which includes AutoGrid 4 and AutoDock 4 [12]. Validation involved redocking the native ligand 4-Amino-5-(4-phenoxyphenyl)-7H-pyrrolo[2,3-d] pyrimidin-7-yl-cyclopentane (B43) to prepared BTK protein. The validation parameter for the docking protocol was the root-mean-square deviation (RMSD), with an RMSD ≤ 2 Å indicating a valid docking approach.

Compounds structure optimization

The 3D structure of the docking compound was obtained from the PubChem Data Bank (https://pubchem.ncbi.nlm.nih.gov/) and imported into BIOVIA Discovery Studio

2025 to generate the ligand's PDB format file.

Docking of the test compound to the BTK

The test compound structure underwent docking against the prepared BTK target protein using AutoGrid 4, AutoDock 4, and AutoDock Vina from AutoDock Tools 1.5.7 [11][12]. This step aimed to identify the conformation that exhibited the lowest binding energy for BTK. The analyzed conformations revealed hydrogen bonding, van der Waals interactions, hydrophobic interactions, and electrostatic interactions, which provided information about the binding of fangchinoline to BTK. Binding interactions were analyzed using BIOVIA Discovery Studio 2025.

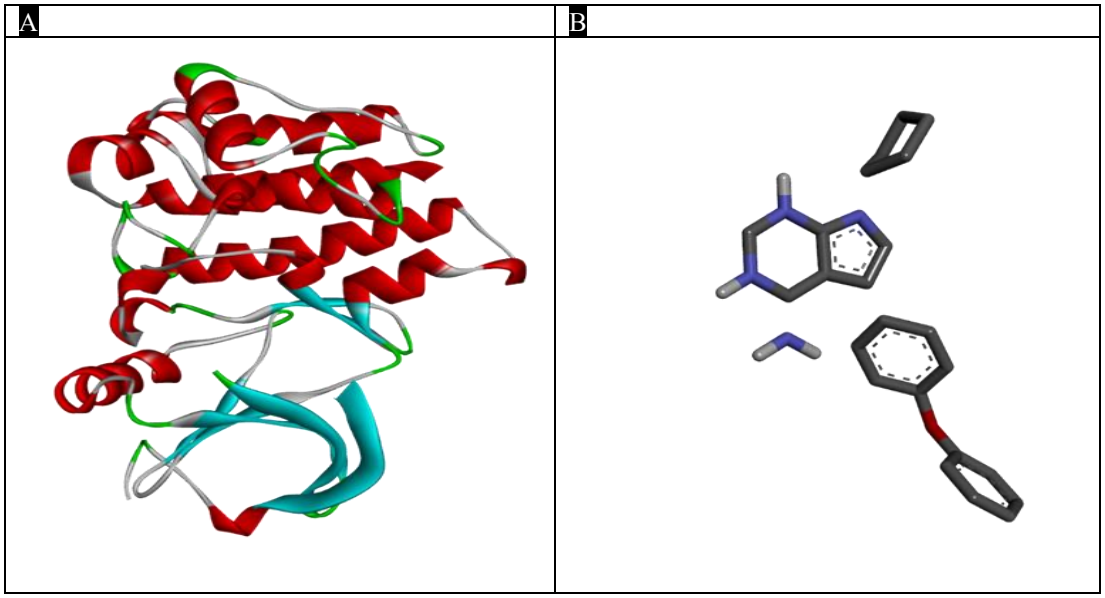


Figure 1. The result of protein preparation. (A) Structure of prepared BTK (B) B43 native ligand

RESULT

Preparation of the BTK protein target

The BTK target protein was prepared with B43 native ligands (Figure 1).

Docking validation

Table 1 provides an overview of the grid parameters used for the BTK docking simulations with a specific focus on the design of the search space that encompasses the enzyme binding site. The grid box origin was set at X=16.796, Y=6.558, and Z=–14.270, which corresponds to the position of the enzyme crystal structure 3GEN's native ligand [13]. To focus the docking search on the ATP-binding cleft of BTK, the grid center was aligned with the known position of the active site. This cleft, located in the inter-lobal pocket where ATP and inhibitors attach, includes crucial residues in the kinase hinge region that interact significantly with ligands [14]. The grid was established with dimensions of 54 × 50 × 50 Å, ensuring it completely surrounded the binding pocket and a small adjacent area. This setup allows the ligand to explore all potential orientations and interactions. Proper grid box placement is essential for successful docking; if the grid is too small or incorrectly positioned, it may miss critical active-site residues, preventing the ligand from achieving the correct pose [15]. Therefore, the selected grid center and dimensions ensured comprehensive coverage of the BTK active site, increasing the chances that the test compound (fangchinoline) would dock in the correct pocket rather than in incorrect locations.

Table 1. The grid box setting on the target protein

Protein target	Grid Box	
	Grid Size	Grid Center
BTK	X = 54	X = -16.796
	Y = 50	Y = 6.558
	Z = 50	Z = -14.270

The docking protocol was validated by redocking the co-crystallized inhibitor B43 into the active site of BTK. The ten top-ranked conformations generated by the docking program showed RMSD values ranging from 0.76 to 0.81 Å relative to the crystallographic pose, with predicted binding energies between –10.01 and –10.12 kcal/mol. Among these, exhibited the lowest binding energy (–10.12 kcal/mol) and an RMSD of 0.80 Å and was therefore selected as the reference pose for subsequent docking analyses. The visualization of the native ligand and redocking four are presented in Figure 2, native ligand as a blue and redocking as a yellow.

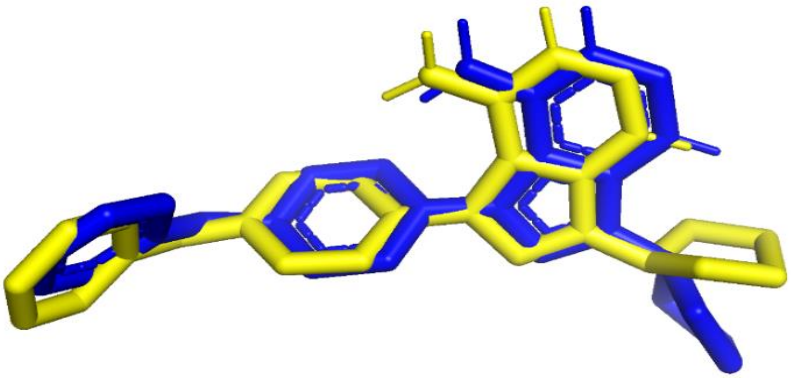


Figure 2. Superimposition/overlay of the native and redocked ligand

The root-mean-square deviation (RMSD) threshold commonly used to indicate a successful redocking is 2.0 Å or lower. The investigation found that all RMSD values were less than 1.0 Å, indicating that the docking parameters accurately reproduced the experimental binding mode of B43 in the BTK binding pocket. In addition, the predicted binding energies, which were tightly clustered between –10.01 and –10.12 kcal/mol across ten poses, reflect a stable and consistent alignment of the native ligand within the active site. This evidence supports the reliability of the docking protocol in analyzing the binding interactions of fangchinoline and other ligands with BTK.

Docking of test compound to BTK

Table 2. The docking affinity and interaction of compounds binding to the protein target

Compounds	Binding affinity (kcal/mol)
	3GEN/BTK
Native Ligand	-10.12
Fangchinoline	-8.56
Ibrutinib	-11.43

In the docking simulation, fangchinoline demonstrated a predicted binding free energy of –8.56 kcal/mol with BTK, which indicates a moderate affinity. This energy level was less favorable than that of the co-crystallized native ligand, which had a binding energy of –10.12 kcal/mol, and it was considerably weaker than ibrutinib's binding energy of –11.43 kcal/mol.Ibrutinib, a potent covalent inhibitor, showed the most negative (highest affinity) docking score, even surpassing the native ATP-site ligand. The lower (less negative) binding energy of fangchinoline indicates that it does not fit as well in the active site as the reference ligands. However, its affinity is still within a range that is reasonable for biologically relevant binding (in line with other non-covalent BTK inhibitors). The results in Table 3 highlight that while fangchinoline binds to BTK, its docking affinity is modest relative to the high-affinity benchmark ibrutinib and the native ligand.

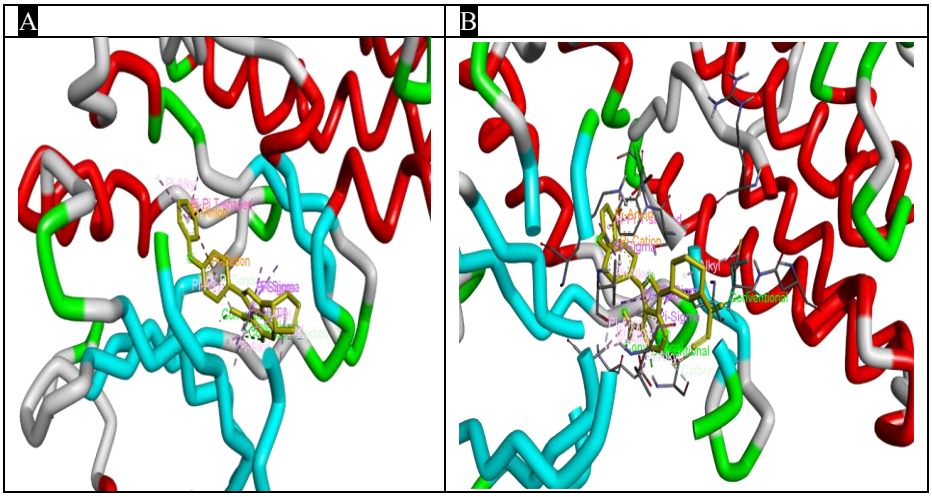


Figure 3. 3D Structure for BTK Binding Site with (A) Native Ligand and (B) Ibrutinib (C) Fangchinoline

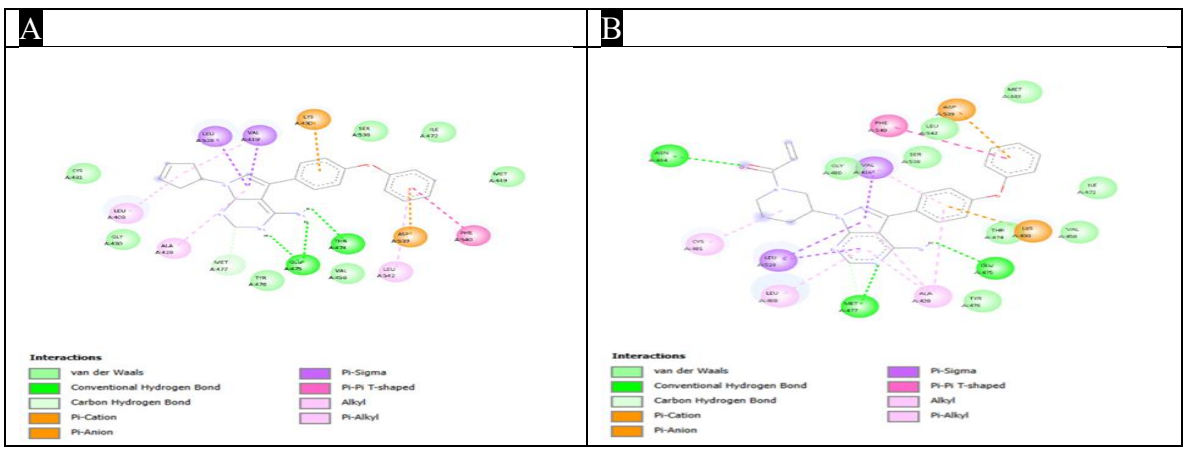
Detailed 3D (Figure 3) and 2D (Figure 4) interaction analyses revealed that fangchinoline engages BTK in a binding mode distinct from those of the native ligand and ibrutinib. Fangchinoline does not form hydrogen bonds with the hinge residues Glu475 or Met477, which are key anchoring interactions for the native ligand and ibrutinib (both of which hydrogen-bond to Glu475 and Met477 in the BTK hinge region). Instead, fangchinoline forms hydrogen bonds with Ser538 and Asp539 in the substrate-binding region of the kinase (Figure 4C), interactions that are absent in ibrutinib and native ligand complexes. Fangchinoline also engages in polar or electrostatic contacts with Lys430 (a conserved lysine at the ATP-binding pocket entrance) and Asp539 (in the activation loop), residues that are common to the other ligand-binding sites (notated with * in Table 3). Table 3 illustrates these interactions, highlighting that, unlike ibrutinib and B43 (native ligand), which both interact with Glu475 and Met477, fangchinoline creates a distinct network of hydrogen bonds with Ser538 and Asp539, in addition to forming ionic contacts. Furthermore, fangchinoline does not bind with Asn484 as ibrutinib does, underscoring its unique binding characteristics.

Table 3. Docking interactions and amino acid residues of compounds with BTK proteins

Ligand	BTK Protein Interaction		
	Hydrogen	Hydrophobic & Aromatic	Electrostatic
Native Ligand	THR A:474 GLU A:475 MET A:477	LEU A:408* VAL A:416* ALA A:428* LEU A:528* PHE A:540* LEU A:542	LYS A:430* ASP A:539*
Fangchinoline	SER A:538 ASP A:539*	LEU A:408* VAL A:416* ALA A:428* LYS A:430* CYS A:481 ARG A:525 LEU A:528*	
Ibrutinib	GLU A:475 MET A:477 ASN A:484	LEU A:408* VAL A:416* ALA A:428* CYS A:481 LEU A:528* PHE A:540*	LYS A:430* ASP A:539*
*Similar amino acid residues			

The 3D binding models (Figure 3) illustrate that fangchinoline adopts a binding pose that is noticeably distinct from that of ibrutinib, reflecting its non-covalent mode of inhibition. Ibrutinib (a small irreversible inhibitor) orients itself deep in the ATP-binding cleft, covalently bonding to Cys481 and simultaneously forming the canonical hinge hydrogen bonds with Glu475 and Met477. This covalent anchoring and hinge interaction keeps ibrutinib in a certain position (Figure 3B). In contrast, fangchinoline (a larger, rigid bis-benzylisoquinoline) is differently positioned within the pocket (Figure 3C). It forgoes the hinge contacts and does not covalently attach to Cys481; instead, it interacts peripherally via Ser538 and Asp539 and lies closer to the pocket entrance near Lys430. Despite this distinct orientation, fangchinoline occupies much of the same ATP-binding site and engages overlapping amino acids with reference ligands. Fangchinoline binding involves several hydrophobic/aromatic residues contacted by ibrutinib or the native ligand (noted with * in Table 4), including Leu408, Val416, Ala428, Lys430, Asp539, and Leu528. It is worth noting that both fangchinoline and ibrutinib contact Cys481, but only ibrutinib forms a covalent bond. Fangchinoline has particularly weak interactions with Cys481 because of the noncovalent interactions and therefore has less binding affinity. In general, structural analysis revealed that fangchinoline attaches to the ATP pocket through noncovalent interactions, occupying the same general area as ibrutinib but differing in its specific orientation and bonding characteristics.

There also seems to be some binding mode with fangchinoline that has interactions that ibrutinib and the native ligand do not possess. This could explain fangchinoline's differences in affinity and specificity. Fangchinoline formed a hydrogen bond with Ser538 (Figure 4C), a residue on the kinase's activation segment that neither ibrutinib nor the co-crystallized inhibitor interacted with. It also engages Arg525, a polar contact unique to the fangchinoline-BTK complex (Arg525 is not contacted by ibrutinib or B43, according to Table 4). These novel interactions suggest that fangchinoline, owing to its larger polyaromatic structure, extends into slightly different sub-pockets or surface grooves of BTK. In addition, the hydrophobic interactions of fangchinoline span a broad area of the active site; for example, it packs against Leu408, Val416, Ala428, and Leu528 on the ATP-binding cleft walls (residues also leveraged by ibrutinib and the native ligand). The presence of many shared hydrophobic contacts (overlapping residues) indicates that fangchinoline occupies the classical ATP-binding cleft, even though it binds in a non-classical orientation. Simultaneously, the lack of hinge binding and the different polar contacts (Ser538, Arg525) point to a distinct binding pose for fangchinoline, which could translate to a different inhibition mechanism (non-covalent and possibly reversible) compared to ibrutinib's covalent, hinge-anchored mechanism.



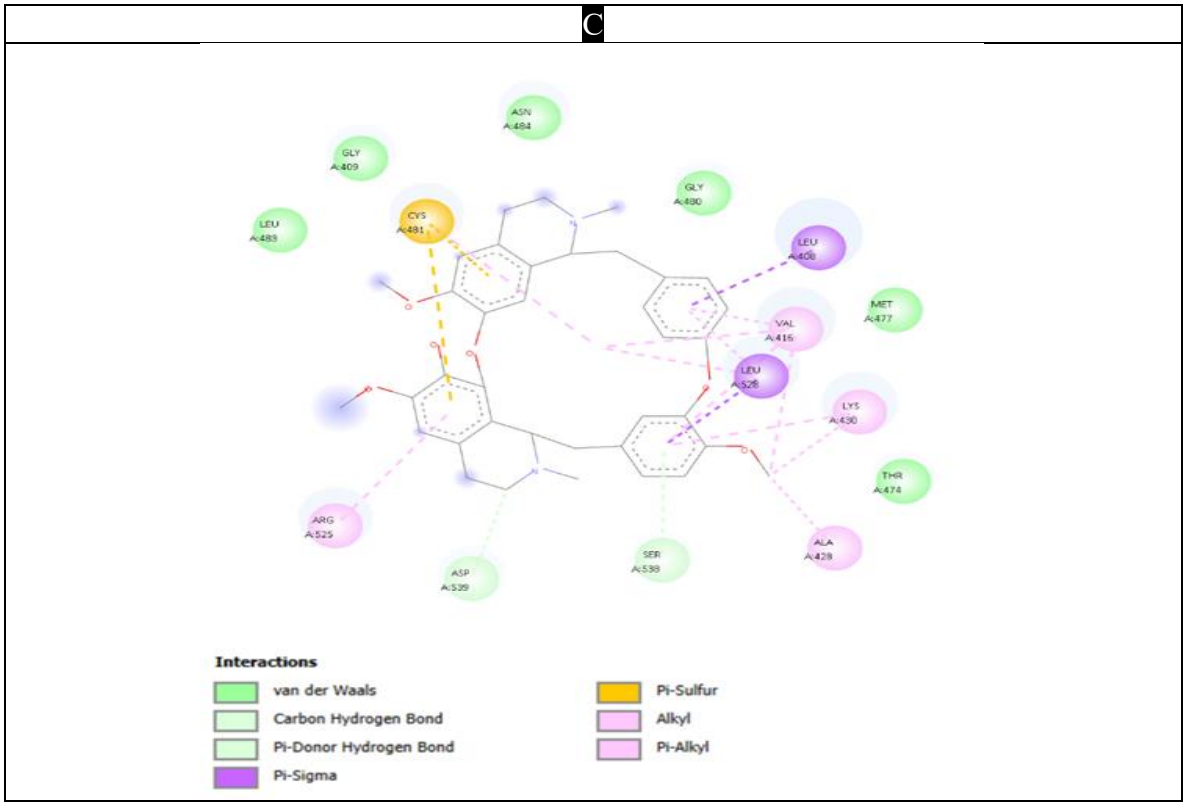


Figure 4. 2D Structure for BTK binding site with (A) Native Ligand, (B) Ibrutinib, (C) Fangchinoline

DISCUSSION

The molecular docking results provide a detailed picture of how fangchinoline may bind to the BTK active site, revealing both common and unique features compared with known inhibitors. Fangchinoline was predicted to fit perfectly into BTK's ATP-binding pocket of BTK with a favorable binding energy, indicating that it would bind strongly in silico. The docking pose of fangchinoline shows classical hinge-region contacts—hydrogen bonds to Glu475 and Met477 of BTK—that mimic the anchoring interactions of ATP-competitive BTK inhibitors, such as ibrutinib. Simultaneously, fangchinoline formed additional hydrogen bonds with BTK's Ser538 and Asp539 residues, which lie in the kinase activation loop near the DFG motif, interactions not prominently utilized by ibrutinib. These unique contacts suggest that the binding mode of fangchinoline spans beyond the canonical hinge region into an adjacent subpocket, thereby "double-anchoring" the ligand via both the hinge and activation-loop residues [16]. Focusing on both sites is projected to bolster binding stability, in line with research showing that a synthetic BTK inhibitor, which forms hydrogen bonds with Ser538 and Asp539, binds more strongly to BTK than ibrutinib, as demonstrated by docking and free energy analyses. The docking study ultimately demonstrated that fangchinoline can effectively occupy the BTK active site, replicating key interactions found in established BTK inhibitors while also forming new connections with the activation loop, potentially resulting in a unique binding profile.

Exploring ibrutinib provides valuable insights into the binding properties of fangchinoline. As a pioneering covalent BTK inhibitor, ibrutinib forms a permanent bond with Cys481 and creates two vital hydrogen bonds with the hinge backbone at Glu475 and Met477, resulting in notable inhibition. The co-crystal structure of BTK with ibrutinib demonstrates that, beyond these hinge interactions, the aromatic ether section of ibrutinib extends towards the αC-helix and DFG segment, situating itself near residues such as Met449 (αC) and Asp539, yet it does not form direct hydrogen bonds with Ser538 or Asp539 [17].In contrast, the

docking configuration of fangchinoline indicates direct interactions with Ser538 and Asp539, suggesting that fangchinoline either delves deeper into the pocket or more effectively stabilizes the activation loop compared to ibrutinib.We surmise that the bulky, polycyclic structure of fangchinoline may allow it to engage this deeper pocket (often termed the H3 pocket in kinases), which is accessible in BTK's inactive conformation of BTK [17]. This extended binding orientation mirrors that of certain noncovalent BTK inhibitors—such as CGI-1746—that occupy both the hinge region and deeper pockets near the DFG motif. Like CGI-1746, fangchinoline is expected to engage in a network of interactions with conserved residues, including the catalytic Lys430 and DFG Asp539, which may help stabilize BTK in its inactive state. While both fangchinoline and ibrutinib target the hinge-binding motif, fangchinoline's ability to reach into an additional sub-pocket suggests a distinct binding mode. This specific arrangement may lead to differences in their inhibitory behavior. For instance, fangchinoline could act as a purely reversible (non-covalent) inhibitor, keeping BTK inactive by binding to it at several sites, whereas ibrutinib operates through a covalent, hinge-centered approach. Fangchinoline does not have a Michael acceptor; therefore, it does not form an irreversible bond with Cys481. This suggests that it is a reversible ATP-competitive inhibitor of BTK. In practical terms, this could be beneficial in specific situations. Fangchinoline or its analogs might maintain effectiveness against BTK variants with the Cys481→Ser resistance mutation, as their binding does not rely solely on covalent attachment. Importantly, the appearance of C481S mutants in patients has led to the creation of non-covalent BTK inhibitors to counteract ibrutinib resistance. For instance, the newly developed pirtobrutinib is a highly selective reversible BTK inhibitor that retains low-nanomolar potency even against C481S-mutant BTK [18][16]. The potential binding of fangchinoline, which does not have dependency on Cys481, aligns with these next-generation inhibitors and further underlines the possible importance of a unique reversible binding mechanism to tackle resistance.

The amino acids bonded in the docking formation of fangchinoline illuminate the possibilities of this natural product in disrupting BTK-scaffolded signaling in cancer cells. BTK plays a crucial role in B cell receptor (BCR) signaling, transmitting signals that activate NF-κB, PI3K/Akt, and other pathways essential for the growth and survival of B cells [19]. Compounds that attach to the ATP site of BTK and keep the kinase in an inactive form prevent BTK's self-phosphorylation and the phosphorylation of targets such as PLCγ2, thereby effectively stopping these survival-promoting signals. The docking model indicates that fangchinoline can bind in this specific way—filling the ATP-binding pocket and possibly stabilizing the activation loop (through interactions with Asp539/Ser538) in a configuration that does not allow for catalysis [20]. Extensive biological research on fangchinoline's anticancer properties strongly endorses this mechanism. Fangchinoline has shown strong antiproliferative and pro-apoptotic activity across various tumor models, frequently linked to the downregulation of oncogenic signaling pathways [19]. In B-cell malignancies, fangchinoline successfully suppressed the proliferation of Raji and Daudi cell lines, induced cell cycle arrest, and triggered apoptosis—achieving effects comparable to those observed with the PI3K/Akt pathway inhibitor LY294002 [21]. This is notable because BTK functions upstream of PI3K in BCR signaling; BTK activation activates PI3K/Akt, whereas BTK inhibition attenuates Akt activation. The finding that fangchinoline mimics the effects of a PI3K/Akt inhibitor in B cells implies that it might function at or above the BTK/PI3K level in the signaling pathway. In research on solid tumors, fangchinoline consistently reduced the activity of the PI3K/Akt pathway

and its downstream elements, like XIAP, leading to an increase in apoptosis [19]. Connecting these dots, our docking results raise the intriguing possibility that BTK could be one of the direct molecular targets through which fangchinoline exerts these anti-survival effects. The unique engagement of fangchinoline with Ser538/Asp539 in BTK's activation loop of BTK may suggest its ability to preferentially stabilize an "inactive-like" BTK conformation, reminiscent of type II kinase inhibitors, which might enhance the shutdown of BTK-mediated signaling [22]. Although BTK is classically a driver of B-cell cancers, aberrant BTK or BTK-family kinase activity has been implicated in other malignancies and even in solid tumor contexts (e.g., promoting tumor–stromal interactions) [21]. Accordingly, fangchinoline's broad anticancer effects may be partly explained by its ability to suppress BTK in certain cell types. The docking data offer a molecular rationale: by binding within the active site of BTK and inhibiting its catalytic activity, fangchinoline may obstruct key proliferative and anti-apoptotic signals—much like established BTK-targeted therapies in oncology.

In the realm of drug discovery, the way fangchinoline binds offers both advantages and challenges. On one hand, its ability to engage with both the hinge region and a deeper activation loop pocket of BTK provides a promising basis for effective inhibition. Targeting multiple areas can improve binding strength and specificity, and the fact that fangchinoline is a natural compound gives it a unique chemical structure that sets it apart from current BTK inhibitors. This uniqueness could motivate attempts to alter it to improve drug-like characteristics or selectivity. For instance, medicinal chemists might explore the bis-benzylisoquinoline framework of fangchinoline to create analogs that maintain the crucial Ser538/Asp539 interactions while enhancing pharmacokinetic attributes or minimizing side effects. Furthermore, reversible binding means that fangchinoline inhibits BTK, but not in a way that involves covalent trapping, so it has a more controlled on-off kinetic profile. These features can avoid toxicity concerns or make it safe to use with other drugs [23][24]. Additionally, fangchinoline, like other reversible inhibitors, will likely be able to inhibit BTK mutants that are resistant to covalent inhibitors. This issue is particularly relevant due to resistance mutations of clinical importance such as BTK C481S [17][25]. Thus, fangchinoline or its derivatives might be able to solve or at least improve some of the issues with ibrutinib and other covalent BTK inhibitors. This could be achieved by providing treatment options for patients who have experienced a relapse on ibrutinib or by enabling intermittent dosing schedules due to their reversible nature. However, it is important to remember that fangchinoline's complete binding profile has both pros and cons. When it comes to BTK, targeting more than one subsite can make it work better. However, this also makes it more likely that it will interact with other kinases that have similar active site properties. Fangchinoline acts on various cellular targets and engages with a wide array of signaling proteins, including focal adhesion kinase and PI3K, in addition to BTK [26]. Distinguishing the effects of BTK inhibition from of those other targets will require careful experimental dissection. The most current docking cluster results relating to BTK allow us to provide new insight regarding fangchinoline and its potential as an anticancer agent. Instead of functioning like a nonselective and broad-spectrum toxin, this agent may have the potential to act as a selective inhibitor of a key kinase pathway involved in the advancement of certain cancers. This idea is consistent with the growing interest in the use of natural products from libraries as potential sources of selective therapies. Structurally complex, natural alkaloids can interact with cancer-promoting enzymes in a highly selective manner, and more exploration of these sources should be a priority.

While these findings are promising, it is crucial to highlight that they originate from computational models and are accompanied by inherent uncertainties. Molecular docking serves as a useful exploratory tool in the initial stages of drug discovery; however, it offers an idealized view of protein–ligand interactions that may not fully capture the complexities of a dynamic biological system [27]. The docking simulations assumed BTK to be in a particular conformation (likely derived from an X-ray structure) and treated the protein mostly as a rigid entity [28]. In reality, kinases such as BTK undergo conformational changes (e.g., in the glycine-rich loop, activation loop, and αC-helix) upon ligand binding, and the ligands themselves can adopt multiple conformers. The large, flexible structure of fangchinoline may induce or require slight pocket adjustments that a rigid docking protocol cannot accommodate [29]. In addition, the scoring functions used in the docking are also approximations, and in some cases, they overlook certain intermolecular interactions, solvent effects, and some loss of entropy. Thus, despite the fact that fangchinoline scored high in docking against BTK and the considerable interaction is reasonable, the actual binding affinity and the precise geometry of binding need to be established experimentally [30]. Selectivity is also a consideration: docking was performed only on BTK's active site of BTK; however, fangchinoline could potentially fit into the ATP sites of other kinases with similar affinity. Thus, it is of great importance to have a pipeline for experimental verification instead of solely relying on computational results. This could include in vitro biochemical assays to appreciate the ability of fangchinoline to inhibit BTK enzymatic activity associated with certain cellular models, cells of which for BTK-dependent cancers could be used to see if the antiproliferative effect is consistent with BTK pathway suppression, manifested with decreased BTK Tyr551 or PLCγ2 phosphorylation. If feasible, co-crystallography or cryo-EM of fangchinoline bound to BTK would offer conclusive proof of its binding mechanism and verify the predicted Ser538/Asp539 interactions [31]. Additionally, it would be beneficial to test fangchinoline's activity against the Cys481Ser mutant BTK to directly examine the hypothesis that it can counteract the resistance mechanism [32]. Finally, evaluating fangchinoline's pharmacological properties, such as its solubility, permeability, and metabolic stability, could shed light on its viability as a drug candidate. Natural compounds frequently face difficulties in these areas, but understanding how they bind can guide chemical modifications to overcome these challenges. In summary, while docking studies offer a persuasive hypothesis that fangchinoline might interact with BTK, these results should be approached with caution. The idea of this compound serving as a BTK inhibitor is still in the early stages of hypothesis formation. Additional experimental validation is crucial to assess how well the in-silico predictions translate into effective anticancer mechanisms.

CONCLUSION

Our docking analysis indicated that fangchinoline can bind effectively to BTK's ATP-binding pocket of BTK, forming significant contacts, including novel hydrogen bonds with Ser538 and Asp539. This binding mode suggests the capacity to inhibit BTK and thereby block downstream proliferative signaling, despite having a somewhat lower predicted affinity than ibrutinib, a covalent BTK inhibitor. The computational findings align with the known antiproliferative and pro-apoptotic characteristics of fangchinoline, indicating that its anticancer effects might be partially due to its action on BTK. The study's credibility is enhanced by a validated docking protocol and the use of a clinically relevant benchmark. As an in-silico study, the findings should be interpreted with caution and require experimental validation. To summarize, these results emphasize fangchinoline's potential as a promising

natural scaffold with anticancer effects through BTK inhibition, although further research is needed to substantiate these predictions.

Declaration by Authors

Acknowledgement: None
Source of Funding: None
Conflict of Interest: No conflicts of interest declared.

REFERENCES

1. Pal Singh S, Dammeijer F, Hendriks RW. Role of Bruton's tyrosine kinase in B cells and malignancies. Mol Cancer. 2018;17(1):57. doi:10.1186/s12943-018-0779-z.
2. Zhang Q, Tian L, Zhang S, Sun Y, Wei Y, Mei H. Molecular insights into the binding mechanism of fangchinoline as a Bruton's tyrosine kinase (BTK) inhibitor: an integrated computational study. J Comput Aided Mol Des. 2025. doi:10.1007/s10822-025-00621-4.
3. Hasibuan PAZ, Harahap U, Sitorus P, Lubis MF, Satria D. In-silico analysis of vernonioside D and vernonioside E from Vernonia amygdalina Delile leaves as inhibitors of epidermal growth factor receptor (EGFR) and mammalian target of rapamycin (mTOR). Rasayan J Chem. 2021;14(3):1539-1543. doi:10.31788/RJC.2021.1436092.
4. Satria D, Waruwu SB, Sholikhah EN, Mustofa M, Satriyo PB, Wahyuningsih TD, et al. In silico study of anticancer activity of pyrazoline C and M as potential selective cyclooxygenase-2 (COX-2) inhibitors using molecular docking and MD simulations.
5. Alrouji M, Benjamin LS, Alhumaydhi FA, Al Abdulmonem W, Baeesa SS, Rehan M, et al. Unlocking potential inhibitors for Bruton's tyrosine kinase through in-silico drug repurposing strategies. Sci Rep. 2023;13(1):17684. doi:10.1038/s41598-023-44956-0.
6. Zain R, Vihinen M. Structure-function relationships of covalent and non-covalent BTK inhibitors. Front Immunol. 2021; 12:694853. doi:10.3389/fimmu.2021.694853.
7. Mérarchi M, Sethi G, Fan L, Mishra S, Arfuso F, Ahn KS. Molecular targets modulated by fangchinoline in tumor cells and preclinical models. Molecules. 2018;23(10):2538. doi:10.3390/molecules23102538.
8. Wang K, Wang Y, Yan J, Hou C, Zhong X, Zhao Y, et al. Network pharmacology and molecular docking integrated strategy to the screening of active components and mechanisms of Stephaniae Tetrandrae Radix on breast cancer. Processes. 2022; 10:2340. doi:10.3390/pr10112340.
9. Jung YY, Shanmugam MK, Chinnathambi A, Alharbi SA, Shair OHM, Um JY, et al. Fangchinoline, a bisbenzylisoquinoline alkaloid can modulate cytokine-impelled apoptosis via the dual regulation of NF-κB and AP-1 pathways. Molecules. 2019;24(17):3127. doi:10.3390/molecules24173127.
10. Sanner MF. Python: a programming language for software integration and development. J Mol Graph Model. 1999;17(1):57-61.
11. Saji C., Balachandran R., Nizar A., et al. Molecular docking studies with phytoconstituents of tinospora sinensis targeting SARS-cov-2 protein using autodock vina. Indian Drugs 2025;62(05):22-27. https://doi.org/10.53879/id.62.06.15489
12. Morris GM., Goodsell DS., Halliday RS., et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998;19(14):1639-1662. doi:10.1002/(SICI)1096-987X (19981115)19:14<1639: AID-JCC10>3.0.CO;2-B.
13. González-González JS., Santiago-Quintana JM., Madrigal-Angulo JL., et al. Molecular structure and GPR35 receptor docking of 1,3-phenylene bis-oxalamide derivatives. Crystals. 2025;15(4):371. doi:10.3390/cryst15040371.
14. Roskoski R. Ibrutinib inhibition of Bruton protein-tyrosine kinase (BTK) in the treatment of B cell neoplasms. Pharmacol Res. 2016;113(Pt A):395-408. doi: 10.1016/j.phrs.2016.09.011.

15. Feinstein W. and Bryliński M. Calculating an optimal box size for ligand docking and virtual screening against experimental and predicted binding pockets. Journal of Cheminformatics 2015;7(1). https://doi.org/10.1186/s13321-015-0067-5
16. Li W., Sano R., Apatira M., DeAnda F., et al. Bruton's tyrosine kinase inhibitors with distinct binding modes reveal differential functional impact on b-cell receptor signaling. Molecular Cancer Therapeutics 2023;23(1):35-46. https://doi.org/10.1158/1535-7163.mct-22-0642
17. Zhang D., Gong H., Meng F. Recent advances in BTK inhibitors for the treatment of inflammatory and autoimmune diseases. Molecules. 2021;26(16):4907. doi:10.3390/molecules26164907.
18. Mato AR., Pagel JM., Coombs CC., et al. S147 Pirtobrutinib, a highly selective, non-covalent (reversible) BTK inhibitor in previously treated CLL/SLL: updated results from the phase 1/2 BRUIN study. HemaSphere. 2022;6(Suppl 3):48-49. doi: 10.1097/01.HS9.0000843480.04890.cc.
19. Li J., Cen W., Tong C., et al. Fangchinoline induces gallbladder cancer cell apoptosis by suppressing PI3K/Akt/XIAP axis. PLoS One. 2022;17(4): e0266738. doi: 10.1371/journal.pone.0266738.
20. Balasubramanian PK., Balupuri A., Kang HY., Cho SJ. Receptor-guided 3D-QSAR studies, molecular dynamics simulation and free energy calculations of Btk kinase inhibitors. BMC Syst Biol. 2017;11(Suppl 2):6. doi:10.1186/s12918-017-0385-5.
21. Velavalapalli V., Maddipati V., Soňa G., et al. Novel 5-substituted oxindoles derivatives as bruton's tyrosine kinase inhibitors: design, synthesis, docking, molecular dynamic simulation, and biological evaluation. 2023. https://doi.org/10.26434/chemrxiv-2023-jhbh4
22. Kuglstatter A., Wong A., Tsing S., et al. Insights into the conformational flexibility of bruton's tyrosine kinase from multiple ligand complex structures. Protein Science 2011;20(2):428-436. https://doi.org/10.1002/pro.575
23. Kawahata W., Asami T., Kiyoi T., et al. Discovery of as-1763: a potent, selective, noncovalent, and orally available inhibitor of bruton's tyrosine kinase. Journal of Medicinal Chemistry 2021;64(19):14129-14141. https://doi.org/10.1021/acs.jmedchem.1c01279
24. Gómez E., Ebata K., Randeria H., et al. Pirtobrutinib preclinical characterization: a highly selective, non-covalent (reversible) btk inhibitor. Blood 2023. https://doi.org/10.1182/blood.2022018674
25. Naeem A., Utro F., Wang Q., et al. Pirtobrutinib targets BTK C481S in ibrutinib-resistant CLL but second-site BTK mutations lead to resistance. Blood Advances 2023;7(9):1929-1943. https://doi.org/10.1182/bloodadvances.2022008447
26. Woyach J., Furman R., Liu T., et al. Resistance mechanisms for the bruton's tyrosine kinase inhibitor ibrutinib. New England Journal of Medicine 2014;370(24):2286-2294. https://doi.org/10.1056/nejmoa1400029
27. Mir S., Madkhali Y., Firoz A., et al. Inhibitory Potential of the *Ocimum sanctum* Phytochemicals on Bruton's Tyrosine Kinase, a Well-Known Drug Target for Treatment of Chronic Lymphocytic Leukemia: An In Silico Investigation. *Molecules* 2023;28(8):3287. https://doi.org/10.3390/molecules28083287
28. Langrish C., Bradshaw J., Francesco M., et al. Preclinical efficacy and anti-inflammatory mechanisms of action of the Bruton Tyrosine Kinase Inhibitor Rilzabrutinib for immune-mediated disease. The Journal of Immunology 2021;206(7):1454-1468. https://doi.org/10.4049/jimmunol.2001130
29. Wang L., Liang J., Gao Z., et al. Abstract 4020: HMPL-760 is a highly potent and selective reversible BTK inhibitor, targeting BTK and BTKC481S in b-cell malignancies. Cancer Research 2023;83(7_Supplement):4020-4020.

https://doi.org/10.1158/1538-7445.am2023-4020
30. Berthelette A., Kolli N., Amatya N., et al. In-silico based screening system to develop allosteric modulators of Bruton's Tyrosine Kinase. The Journal of Immunology 2020;204(1_Supplement):91.7-91.7. https://doi.org/10.4049/jimmunol.204.supp.91.7
31. Cheng S., Guo A., Lu P.et al.. Functional characterization of BTKC481S mutation that confers ibrutinib resistance: exploration of alternative kinase inhibitors. Leukemia 29, 895-900 (2015) https://doi.org/10.1038/leu.2014.263
32. Hopper M., Gururaja T., Kinoshita T.et al.. Relative selectivity of covalent inhibitors requires assessment of inactivation kinetics and cellular occupancy: a case study of ibrutinib and acalabrutinib. Journal of Pharmacology and Experimental Therapeutics 2020;372(3):331-338. https://doi.org/10.1124/jpet.119.262063

How to cite this article: Alan Maulana, Poppy Anjelisa Zaitun Hasibuan, Denny Satria. Molecular docking of fangchinoline against Bruton's tyrosine kinase (BTK) as a potential anticancer target. *International Journal of Research and Review*. 2025; 12(12): 739-751. DOI: *https://doi.org/10.52403/ijrr.20251276*

******