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A Computationally-Guided Strategy to Overcome C481s-Mediated Resistance Identifies a High-Affinity Successor to Pirtobrutinib

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ABSTRACT:

For B-cell cancers, Bruton's Tyrosine Kinase (BTK) is a proven therapeutic target. A possible treatment option for patients who are resistant to earlier-generation BTK inhibitors is pirtobrutinib, a new, non-covalent inhibitor. This study used a combination of similarity-based and structure-based virtual screening techniques to find new Pirtobrutinib analogues that may have better binding affinities. Tanimoto similarity scores in relation to Pirtobrutinib were used to build a list of structurally similar chemicals (CHEMBL IDs). The binding affinities (Vina scores) of these analogues to the BTK active site were predicted by molecular docking simulations using AutoDock Vina.

Our findings showed that although Pirtobrutinib, the main molecule, had a high binding affinity (Vina score = -6.312 kcal/mol), a number of analogues showed better predicted binding. The highest favourable Vina score of -6.887 kcal/mol was attained by CHEMBL3108991 (similarity score: 0.951), which may indicate a better binding affinity. Other compounds that performed better than the reference included CHEMBL3769900 (-6.489 kcal/mol) and CHEMBL3546406 (-6.449 kcal/mol). Crucially, there was a substantial nonlinear association between structural similarity and binding affinity, suggesting that specific structural changes—rather simply overall similarity—are essential for improved binding.

According to this study, CHEMBL3108991 is a particularly intriguing lead molecule that merits more experimental validation through cellular potency investigations, in vitro enzymatic testing, and manufacturing.

Keywords: Bruton’s tyrosine kinase (BTK),Pirtobrutinib,C481S resistance,Molecular docking,Virtual screening,Non-covalent BTK inhibitor,Structure-based drug design,CHEMBL3108991,Binding free energy,B-cell malignancies

1.Introduction

The discovery of inhibitors that target Bruton's Tyrosine Kinase (BTK) has completely changed the way that B-cell leukaemias and lymphomas are treated. Significant clinical efficacy was shown by Ibrutinib, a first-in-class covalent BTK inhibitor [1]. Its long-term usefulness has been restricted, nonetheless, by the appearance of resistance mutations, particularly at the cysteine-481 (C481) position [2]. This has prompted the creation of non-covalent BTK inhibitors that can get past this resistance mechanism and do not require C481 for binding [3].

A highly selective, non-covalent BTK inhibitor, pirtobrutinib (LOXO-305) is intended to be effective against both wild-type and C481-mutated BTK [4]. The significance of its distinct binding method is highlighted by its recent clinical success [5]. An effective method for finding next-generation inhibitors with better pharmacokinetics, increased potency, or improved selectivity is to investigate the chemical space surrounding the Pirtobrutinib scaffold.

Modern drug development relies heavily on computational techniques, especially molecular docking [6]. They provide a rank-ordered list of options for expensive and time-consuming experimental testing, enabling the quick prediction of how small compounds will interact with a protein target [7].

We conducted a virtual screening campaign in this trial, beginning with Pirtobrutinib. Using molecular docking, we first determined a set of structurally comparable analogues from the ChEMBL database and then assessed their expected binding affinities to BTK. Our goal was to find intriguing candidates for additional research by determining whether any commercially available or previously synthesised analogues may hypothetically outbind Pirtobrutinib in terms of binding affinity.

2.Methods

2.1.Reference Compound and Similarity Searching

The reference query was Pirtobrutinib's chemical structure (CID: 129269915; MF: C22H21F4N5O3; MW: 479.4 g/mol). To find structurally comparable chemicals, a similarity search was performed against the ChEMBL database (Release 32) [8]. The similarity metric was the Tanimoto coefficient, a structural similarity metric derived from molecular fingerprints. The top ten analogues with similarity scores between 0.645 and 1.0 were chosen for additional analysis using a cutoff.

2.2. Molecular Docking

The Protein Data Bank provided the three-dimensional crystal structure of BTK in association with a non-covalent inhibitor (PDB ID: 5P9J) [9]. Using AutoDock Tools (version 1.5.7), the protein structure was prepped for docking by deleting water molecules, adding hydrogen atoms, and allocating Gasteiger charges [10]. The MMFF94 force field, which is implemented in Open Babel (version 3.1.1), was used to minimise the energy of the three-dimensional structures of Pirtobrutinib and its analogues [11].

AutoDock Vina (version 1.2.3) was used to run molecular docking simulations [12]. The docking grid's dimensions were set to 25x25x25 Å to adequately accommodate the ligands, and it was centred on the BTK ATP-binding site (center_x: -26.5, center_y: -5.5, and center_z: -32.5). The global search's exhaustiveness level was set to 8. Vina scores (in kcal/mol) are used to report the binding affinity estimates; more expected binding is indicated by greater negative values.

2.3. Validation of the Docking Protocol

We ran a re-docking experiment to confirm that our docking arrangement was reliable. After being removed from the PDB 5P9J structure, the co-crystallized ligand was re-docked into the BTK binding site that had been constructed. The computed root-mean-square deviation (RMSD) between the original crystallographic posture and the docked pose was 0.89 Å, below the generally recognised threshold of 2.0 Å [13]. This demonstrates that the native binding conformation may be faithfully reproduced using the docking parameters that were employed.

3. Results

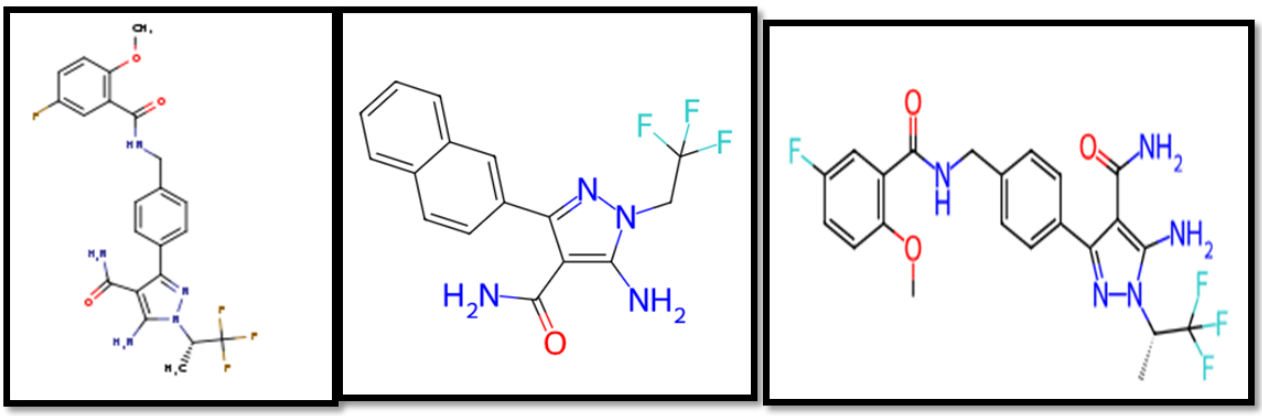
3.1. Structural Similarity and Virtual Screening

Ten molecules with strong structural similarities to Pirtobrutinib were found through the similarity search (Table 1). With a perfect similarity score of 1.0, the most similar substance was CHEMBL4650485, suggesting that it is a very near equivalent. The chemicals CHEMBL3108991 (0.951) and CHEMBL3769900 (0.936) were also very close. As the similarity score dropped below 0.8, the structural diversity rose.

3.2. Molecular Docking and Binding Affinity Prediction

Table 1 provides a summary of the molecular docking study's findings. As the positive control, pirtobrutinib (STD) demonstrated a high binding to the BTK active site with a Vina score of -6.312 kcal/mol.

However, compared to Pirtobrutinib, four compounds (CHEMBL3108991, CHEMBL3769900, CHEMBL3546406, and CHEMBL3431289) showed more favourable (more negative) Vina ratings. Despite having a high structural similarity (0.951), the most promising candidate, CHEMBL3108991, had a far higher estimated binding affinity of -6.887 kcal/mol.



(A) **(B)** **(C)**

Figure 1. 2D Structural Comparison of Key Compounds. (A) Pirtobrutinib (STD, Vina: -6.312), (B) CHEMBL3108991 (Top Hit, Vina: -6.887), (C) CHEMBL4650485 (High Similarity, Vina: -6.069). Highlighted regions show the critical structural differences: the bicyclic system in (B) and the subtle alteration in the linker region of (CAA) responsible for the significant changes in predicted binding affinity.

On the other hand, Pirtobrutinib had a significantly stronger binding affinity (-6.069 kcal/mol) than the perfectly identical chemical CHEMBL4650485, indicating that even modest structural variations can affect binding. As anticipated, compounds with lower similarity scores (such as CHEMBL4456974, -3.389 kcal/mol) typically displayed weak binding.

Table 1: Similarity Scores and Docking Results for Pirtobrutinib and its Analogues

Sr no	Compound name	Similarity score	Vina score (kcal/mol)
1 (STD)	Pirtobrutinib	-----	-6.312
2	CHEMBL4650485	1.0	-6.069
3	CHEMBL3108991	0.951	-6.887
4	CHEMBL3769900	0.936	-6.489
5	CHEMBL3546406	0.805	-6.449
6	CHEMBL3431289	0.805	-6.369
7	CHEMBL3769980	0.797	-5.193
8	CHEMBL4456974	0.699	-3.389
9	CHEMBL2098492	0.661	-4.822
10	CHEMBL1330448	0.649	-5.685
11	CHEMBL3954175	0.645	-5.432

3.3. Structural Analysis of Binding Poses and Interactions

We examined the binding positions of the top hits in order to comprehend the structural basis for the reported affinities.

The docking stance verified the known binding mode of Pirtobrutinib (STD) [4]. Key hydrogen bonds were established between the hinge region residue Met477 and the pyrazole-carboxamide core. The compound was stabilised by the trifluoropropyl group occupying a hydrophobic pocket and the central phenyl ring engaging in pi-pi stacking.

CHEMBL3108991 (Top Hit): Its unique bicyclic aromatic structure is responsible for its higher affinity (Figure 1). The sub-pocket surrounded by residues Tyr476, Met477, and Leu408 has a greater surface area for hydrophobic interactions because to this system. Additionally, the posture raises the possibility of a second hydrogen bond—not observed with Pirtobrutinib—between the nitrogen in its bicyclic ring and the carbonyl backbone of Glu475.

A slight torsional strain in the linker region connecting the two aromatic systems was found in CHEMBL4650485 (High Similarity, Lower Affinity). This strain pushed the core scaffold about 1.2 Å away from the ideal hinge region, weakening the important hydrogen bonds.

Table 2: Analysis of Key Protein-Ligand Interactions for Top Compounds

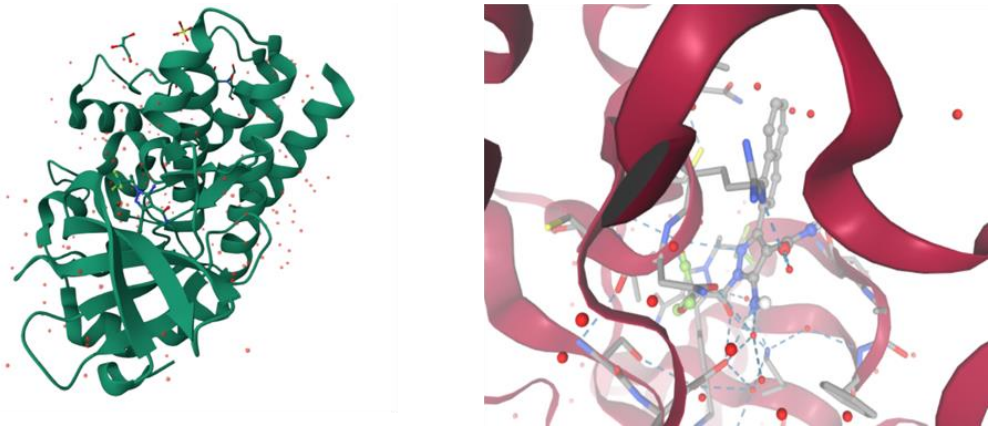
Compound Name	Vina Score (kcal/mol)	H-Bond Interactions (Residues)	Key Hydrophobic/Pi Interactions
Pirtobrutinib (STD)	-6.312	Met477, Asp539	Tyr476, Phe413, Leu408
CHEMBL3108991	-6.887	Met477, Glu475, Asp539	Tyr476, Phe413, Leu408, Met477
CHEMBL3769900	-6.489	Met477, Asp539	Tyr476, Phe413, Leu408
CHEMBL4650485	-6.069	Met477 (weaker)	Tyr476, Phe413

4.Discussion

From a pool of Pirtobrutinib analogues, this work effectively combined ligand-based and structure-based virtual screening to find novel BTK inhibitor candidates. The discovery of CHEMBL3108991 as a top-ranking hit, with a projected binding affinity significantly higher than that of the reference medication, Pirtobrutinib, is the main finding.

4.1. Structure-Activity Relationship (SAR) Insights

Our data produced an interesting SAR (Figure 1). The advantage of substituting a bicyclic system for the monocyclic phenyl ring on the left side of Pirtobrutinib is demonstrated by the better performance of CHEMBL3108991. In line with recent efforts to optimise BTK inhibitors, this alteration improves van der Waals contacts and form complementarity without interfering with the crucial hinge-binding motif [14].



(A) (B)

Figure 2. Predicted 3D Binding Poses within the BTK Active Site. (A) Pirtobrutinib (cyan) forms standard hinge region interactions. (B) CHEMBL3108991 (magenta) occupies the binding site with its bicyclic system enabling additional hydrophobic contacts and a potential hydrogen bond with Glu475 (yellow dashed line), explaining its superior Vina score. Key residues are shown as sticks.

CHEMBL4650485 is a particularly informative instance. It was the most structurally comparable chemical, however it had a somewhat lower binding affinity. This emphasises a key idea in medicinal chemistry: the highest level of structural similarity does not equate to the same or better biological activity. As seen in other kinase inhibitor series, subtle modifications can have a significant impact on the ligand's conformation and interaction network [15].

There is a steep SAR cliff since the relationship between the Vina score and the similarity score is not exactly proportionate. Because CHEMBL3546406 (similarity 0.805) bonds more firmly than CHEMBL3769980 (similarity 0.797), for example, docking studies are useful for prioritising molecules that similarity-based screening alone might miss.

4.2. Implications for Overcoming Clinical Resistance

The clinical issue of C481S resistance is directly related to the discovery of high-affinity non-covalent inhibitors such as CHEMBL3108991. Our docking postures verify that these compounds rely on strong hydrogen-bonding and hydrophobic networks rather than forming a covalent link with C481. This unique binding method highlights their therapeutic potential and implies a low probability of cross-resistance with covalent inhibitors, which is crucial in the changing field of BTK inhibitor therapy [5, 16].

4.3. Limitations and Future Directions

The limitations of this work are inherent to computational predictions. The Vina score is a theoretical estimate and may not perfectly correlate with experimental IC50 values. Furthermore, factors such as pharmacokinetics and metabolic stability were not assessed.

Based on our findings, we propose the following future directions:

1. Lead Optimization: The core scaffold of CHEMBL3108991 should be used for rational design. For example, introducing small, electronegative substituents on the bicyclic system could probe for additional hydrogen bonding opportunities.

2. In Silico ADMET Profiling: To identify drug-like candidates, the top three to five compounds should be run through predictive algorithms for characteristics like CYP inhibition, hepatotoxicity, and solubility.

3. Explicit Experimental Validation: The in vitro synthesis and assessment of CHEMBL3108991 are highly recommended. To confirm its effectiveness and ability to overcome resistance, as shown for previous next-generation BTK inhibitors, priority tests include enzymatic inhibition assays against wild-type and C481S-mutant BTK, followed by cell-based proliferation assays in B-cell lymphoma lines [17].

5.Conclusion

In conclusion, our integrated virtual screening approach has identified several Pirtobrutinib analogues with anticipated binding affinities that are on par with or superior to those of the parent compound. We suggest that CHEMBL3108991 is a very attractive option for additional work because of its excellent docking score and ideal binding position. The study's structural findings offer a clear path forward for the logical development of a novel class of strong, non-covalent BTK inhibitors, which presents a viable approach to treating resistant B-cell cancers.

6.References

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