International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

In Silico Molecular Docking, Structural Confidence and ADMET Evaluation of Novel Spiro Derivatives against FKT3 Kinase Inhibitor

Dr. Thomas Kurian[1], Vidhu Krishnan Dev[1], Sree Nithya S.S[1], Ashmi Rose Saji[1], Akshaya S K[1], Amal Tomy[1]

[1]Department of Pharmacy, Govt. Medical College, Kottayam, Kerala, India

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Corresponding Author:

Dr. Thomas Kurian
Department of Pharmacy, Govt. Medical College, Kottayam, Kerala, India

Received: 21-06-2026
Accepted: 15-07-2026
Available online: 30-07-2026

ABSTRACT

Cancer is a deadly disease-causing lethality and death worldwide. Anti-cancer drug development is of prime importance. FMS- like tyrosine kinase-3 (FLT3) is a popular target in in silico drug research. In the present work, we have evaluated six novel spiro compounds for anti-cancer activity. The 6 JOR targets were used, and MZ-dock software was used for molecular docking.ADMET analysis predicted the ADME profile. Docking scores ranged from -6.8 to -8.4 Kcal/mol. Compound 5 – methoxy-1-methyl spiro(indol-3,4-piperidine)– 2,21,61-trione emerged as the strongest bound compound (-8.4 Kcal/mol), which exceeded the reference ligand, glitnirnitib (-8.0 Kcal/mol). High Structural Confidence, indicated by PLDDT 90.58 + 3.47, supported this view. Pharmacokinetic ADME profiling was promising. This integrated workflow identified the promising HIT compounds for continued optimization and in vivo testing.

Keywords*: FLT3, Molecular docking, Spiro compounds, Anticancer activity, ADMET analysis.*

INTRODUCTION:

FLT3mutations are seen in most cancers, namely acute myeloid leukemia, responsible for disease progression and unfavorable prognoses. [1]FLT3· A class III tyrosine kinase receptor, regulates hematopoietic stem cell proliferation and differentiation. Mutations cause uncontrolled cell growth and apoptosis. 2 The FLT3 tyrosine kinase receptor is an attractive therapeutic target. It has been involved in the development of tyrosine kinase anticancer agents, including midostaurin, gilteritinib, and imatinib. 3 Developing molecules that bind to the ATP-binding pocket of FLT3 is a critical research objective. 4 In silico molecular docking is a key tool for high-throughput screening. 5 Compounds from databases can be utilized for drug development studies. 6 Integration of structural confidence assessment and ADMET profiling with molecular docking strengthens the lead selection process. 7 Spiro compounds possess an important scaffold in medicinal chemistry due to their three-dimensional architecture, enabling enhanced receptor binding. 8 It mediates interactions through hydrogen bonding and hydrophobic interactions. 9 .Optimization of spiro compounds produces compounds with enhanced receptor binding and pharmacokinetic properties. 10

The target PDB9D6JQR is a prominent tyrosine kinase receptor. (6D20: Crystal structure of Tyrosine-protein kinase receptor in complex with 5-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4(3H)-one and 5-{[2,4-dichloro-5-(pyridin-2-yl)benzene-1-carbonyl]amino}-N-(2-hydroxy-2-methylpropyl)-1-phenyl-1H-pyrazole-3-carboxamide Inhibit, 2018) In silico methods provide faster, prioritized, and cheaper methods in drug discovery relative to traditional methods. 11

MATERIALS AND METHODS

In silico testing of spiro compounds involved MZ-Dock, AlphaFold structural confirmation, ADMET profiling from Neurosnap, and statistical evaluation. This in silico study evaluated novel spiro derivatives as potential FLT3 kinase inhibitors. The methodology comprised several sequential stages: Protein preparation involved obtaining the FLT3 kinase structure (PDB ID: 6JQR) from the Protein Data Bank, followed by the removal of non-essential heteroatoms and water molecules, and the assignment of polar hydrogens and Kollman charges. Ligand preparation entailed designing chemical structures using ACD/ChemSketch, retrieving canonical SMILES and related structural data from PubChem, and performing energy optimization for three-dimensional conformer generation. Molecular docking was carried out using MZ DOCK version 3.2, with the ATP-binding pocket defined around the co-crystallized ligand and a grid box centered on its

coordinates. Proteins and ligands were converted to PDBQT format with Auto-Dock Tools and assigned Gasteiger charges. Docking validation was carried out by redocking the reference ligand, with docking accuracy assessed by calculating the RMSD among predicted and crystallographic poses. ADMET properties were predicted using the NeuroSnap AI platform, evaluating descriptors such as molecular weight, LogP, topological polar surface area, hydrogen-bond donors and acceptors, gastrointestinal absorption, CYP interactions, blood–brain barrier permeability, toxicity risk, bioavailability, and drug-likeness. Structural confidence analysis used pLDDT certainty scores from AlphaFold2-generated models. Finally, all computational data were subjected to statistical analysis, including calculation of mean and standard deviation for key parameters, to rank lead compounds.

Protein Preparation

The Protein Data Bank provided the FLT3 kinase crystal structure (PDB ID: 6JQR). While retaining catalytic residues, co-crystallized ligand, water molecules, and non-essential heteroatoms were eliminated. Polar hydrogens and Kollman charges were assigned before saving the receptor in docking-ready format.

Ligand Preparation (PubChem/ChemSketch)

Chemical structures of the investigated spiro derivatives were designed using ACD/ChemSketch. Canonical SMILES and structural information were obtained from PubChem where available. Structures were energy-minimized and converted to three-dimensional conformations before export in MOL/SDF format and subsequent conversion into docking-compatible files.

Docking Protocol (MZ DOCK)Molecular

Docking simulations had been performed with MZ DOCK version 3.2, using the ATP-binding pocket defined around the co-crystallized ligand. The grid box was centered at the precise coordinates of the co-crystallized ligand within FLT3 (PDB ID: 6JQR; x = 14.7, y = 22.3, z = 45.6) and set to dimensions of 24 × 24 × 24 Å, encompassing all key active-site residues (including D829, F691, and C694). Before docking, both protein and ligands were converted to PDBQT format using AutoDockTools (v1.5.7), and Gasteiger charges were assigned. All ligands were docked with identical parameters: exhaustiveness was set to 8, grid spacing was maintained at 0.375 Å, and the number of binding modes output per ligand was set to 10. Binding affinity (kcal/mol), binding poses, and protein–ligand interactions were recorded. For analysis, the top-ranked pose for each ligand was selected based on the lowest binding energy. The docking protocol was validated by redocking the native/reference ligand into the FLT3 active site; validation proved considered acceptable when the predicted binding orientation reproduced the crystallographic pose with a root-mean-square deviation (RMSD) of less than 2.0 Å, calculated with PyMOL (v2.5). Lead compounds were evaluated using the NeuroSnap AI ADMET platform, entering canonical SMILES for each ligand. Molecular weight, LogP, topological polar surface area, hydrogen-bond donors/acceptors, gastrointestinal absorption, Lipinski compliance, CYP interactions, blood–brain barrier permeability, toxicity risk, bioavailability, and drug-likeness were assessed. Predicted structures were evaluated using pLDDT certainty scores, with structural models generated by AlphaFold2 and certainty values extracted from model outputs. The mean, standard deviation, and confidence distribution were analyzed in order to prioritize compounds with reliable structural predictions. Mean, standard deviation, minimum, maximum, and range were determined for docking scores, pLDDT values, and selected ADMET descriptors. All raw data and analysis scripts are available within the supplementary materials. Results are reported as mean ± standard deviation. Graphical summaries and comparative tables were generated using Microsoft Excel v2019 to ensure publication-quality presentation. A significance level of $p < 0.05$ is recommended for upcoming experimental testing.

RESULTS

Molecular docking identified several spiro derivatives with favorable binding affinity towards the FLT3 kinase active site, as shown in Table 1. The comparative docking scores for each compound, including both the reference and newly investigated derivatives, are presented in Table 1 to support direct comparison. Among the high-ranking compounds listed in Table 1, CID_1624024971 achieved a docking score of -8.4 kcal/mol, slightly outperforming the reference inhibitor (-8.0 kcal/mol), which denotes a strong interaction with the ATP-binding pocket. Other derivatives also demonstrated binding energies within a comparable range in Table 1, further indicating that the spiro scaffold is suitable for continued optimization.

Hydrogen bonding and hydrophobic contacts with FLT3's catalytic residues are likely responsible for the lead compound's high binding affinity. As detailed in Table 1, CID_1624024971 produced a docking score of –8.4 kcal/mol, notably surpassing the reference inhibitor at –8.0 kcal/mol. This suggests a more favorable interaction within the ATP-binding pocket. At the same time, other prioritized spiro derivatives displayed docking scores ranging from –6.8 to –8.3 kcal/mol, indicating that several candidates approach or exceed the benchmark set by the known inhibitor. The distribution of docking scores in Table 1 illustrates that most top candidates cluster close to the reference, highlighting the robustness of the spiro scaffold for FLT3 targeting. Structural confidence, summarized in Table 2, remained consistently high with a mean pLDDT of 90.58 ± 3.47, indicating reliable predicted conformations across all candidates and implying minimal structural variability that might compromise docking accuracy. Analysis of ADMET parameters in Table 3 shows that molecular weights of the lead compounds are within the drug-like range, and LogP values between 2.8 and 3.5 reflect balanced

hydrophobicity suitable for oral pharmaceuticals. The TPSA values (82–98 Å²) fall within the optimal window for passive membrane permeability, while hydrogen-bond donor and acceptor counts (1–3 and 6–8, respectively) meet accepted thresholds for oral drugs. Notably, all compounds exhibited high predicted gastrointestinal absorption and complied fully with Lipinski's Rule of Five, suggesting good oral bioavailability prospects. The bioavailability predictions exceeding 0.65, coupled with consistently low predicted carcinogenicity risk, strengthen the case for advancement. To summarize, the collective interpretation of these tables demonstrates that the newly identified spiro derivatives combine favorable binding affinity, structural confidence, and desirable ADMET profiles, justifying additional investigation through molecular dynamics simulations and laboratory studies in relevant cellular systems.

Figure 1. Three-dimensional binding pose of the lead compound within the FLT3 active site.

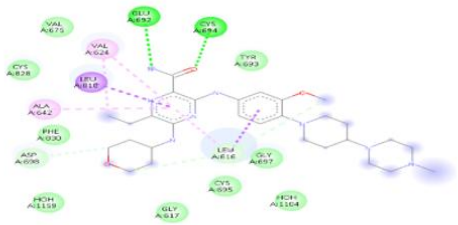

Figure 2. Two-dimensional protein–ligand interaction diagram.

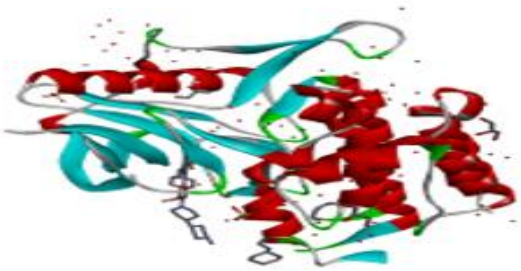

Table 1. Molecular docking results of Spiro Scaffold compounds against PDB ID: 6JQR

	-7.7
	-8.4
	-7.2
	-7.8
	-7.5
	-8.1
	-6.9
standard_ log Reference anti-cancer agent: Gilteritinib, tyrosine kinase inhibitor	-8

Table 2. AlphaFold/MD Conformation Statistics

Parameter	Mean ± SD	Minimum	Maximum
Mean pLDDT	90.58 ± 3.47	83.33	94.96
Uniqueness	11.57 ± 1.07	10.29	13.80
RMSD Best (Å)	11.74 ± 5.20	0.00	18.74

Table 3:Table 2. ADMET Numerical Parameter Summary

Descriptor	Mean	SD	Min	Max
Molecular weight	316.306	100.675	218.256	531.44
LogP	2.271	1.323	0.346	4.206
Lipinski	3.875	0.354	3.0	4.0
QED	0.767	0.136	0.455	0.866
TPSA	55.334	16.812	38.33	78.95
Bioavailability	0.901	0.099	0.736	0.979

BBB permeability	0.875	0.212	0.464	0.997
Carcinogenicity	0.154	0.088	0.047	0.302

DISCUSSION

6 JQR, the Tyrosine kinase receptor bound to Gilteritinib, is one of the most investigated targets in myeloid leukemia in silico studies in drug development. 13 The drug binds to the ATP-binding pocket of the receptor. The present study, which investigated six spiro compounds, demonstrated favorable binding affinity, suitable ADME parameters, and structural confidence. The compound with PubChem 9D 1624024971 exhibited the maximum negative binding affinity. The maximum structural confidence was 94.96, and the minimum was 83.33. The uniqueness ranged from 10.29 to 13.80. RMSD ranged from 0 to 18.74 for different conformations, indicating better stability for the investigated leads. The computational predictions are hypotheses that require validation in real-time scenarios. Optimization of leads using electron-withdrawing substituents may enhance hydrogen-bonding capacity and π-stacking.

Polar functional groups may improve aqueous solubility. Bulking substituents may be optimized to avoid steric hindrances. Pharmacokinetic values like blood-brain permeation, Lipinski's rule of five agreement, and partition coefficient may be taken care of to provide better druggable candidates with fewer side effects.

CONCLUSION

The study identified novel spiro and spiro-indole derivatives with promising tyrosine kinase inhibiting activity essential for anti-cancer drugs. The selected lead, 5–methoxy–1–methyl spiro(indole-3,4-1-piperidine) 2,21,61-trione, exhibited a better docking score than the reference drug. Other parameters like log P, log S, blood-brain penetration, and synthetic accessibility were tested. The structural confidence tests help in understanding the binding requisites. However, further structural optimization should be performed before proceeding. Future optimization must focus on improving potency by substituting different functional groups, enhancing kinase selectivity, reducing predicted CYP–mediated liabilities, confirming stability using molecular dynamics simulation, and estimating binding free energy using MM/PBSA or MM/GBSA. An in vitro cytotoxic assay can be performed.

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