Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 6 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Investigation of the Binding Affinities of Novel Aryl-Aminopyridine-Peptide Conjugates as Potential Therapeutics Against Cancer Proteins

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ABSTRACT

Targeted cancer therapy has revolutionized modern oncology by enabling selective inhibition of molecular drivers responsible for uncontrolled proliferation, metastasis, and therapeutic resistance. In the present study, a novel class of aryl-aminopyridine-peptide conjugates was investigated as potential multi-target anticancer candidates against three clinically relevant proteins: Bcr-Abl kinase for chronic myelogenous leukemia (K562 model), cyclin-dependent kinase-2 (CDK2) for cervical carcinoma (HeLa model), and epidermal growth factor receptor (EGFR) for Fem-x melanoma-associated proliferation pathways. The designed hybrid molecules incorporated a 2-amino-4-arylpyridine pharmacophore tethered through a Gly-Gly linker to the cancer-targeting peptide sequence RWQWRWQWR. Molecular docking studies revealed favorable binding energies ranging from −9.1 to −12.4 kcal/mol across the selected targets, with strong hydrogen bonding, π–π stacking, electrostatic contacts, and hydrophobic stabilization within catalytic pockets. Molecular dynamics simulations performed for 200 ns confirmed excellent conformational stability of the docked complexes, showing RMSD equilibration below 2.5 Å and persistent intermolecular interactions. MM-PBSA calculations further supported stable complex formation with negative binding free energies. ADMET screening predicted acceptable pharmacokinetic behavior, low hepatotoxicity risk, and moderate oral bioavailability. The integrated results suggest that aryl-aminopyridine-peptide conjugates may serve as promising lead structures for the development of selective anticancer therapeutics targeting hematological and solid tumors simultaneously.

Keywords— Aryl-aminopyridine; Peptide conjugates; Bcr-Abl; CDK2; EGFR; Molecular docking; Molecular dynamics; Cancer therapeutics.

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, with increasing incidence driven by aging populations, environmental exposure, genetic predisposition,

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and lifestyle-associated risk factors. Although chemotherapy remains widely used, its major limitations include systemic toxicity, non-selective tissue damage, multidrug resistance, and poor long-term remission in aggressive malignancies. Consequently, the development of targeted therapies capable of selectively modulating oncogenic signaling pathways has become a central focus of modern medicinal research [1,2].

Protein kinases are among the most important therapeutic targets in oncology because they regulate cell proliferation, apoptosis, DNA repair, angiogenesis, and metastatic progression. Dysregulation of kinase signaling frequently contributes to malignant transformation and treatment resistance. In particular, Bcr-Abl tyrosine kinase is the hallmark driver mutation in chronic myelogenous leukemia (CML), where constitutive kinase activation promotes uncontrolled hematopoietic expansion. Likewise, cyclin-dependent kinase-2 (CDK2) is critically involved in G1/S cell-cycle transition and is frequently overexpressed in cervical and ovarian cancers. EGFR signaling, meanwhile, is strongly associated with aggressive growth and metastatic behavior in several solid tumors, including melanoma, lung, and epithelial cancers [3].

Despite the clinical success of kinase inhibitors such as imatinib, erlotinib, gefitinib, and palbociclib-related analogues, several challenges remain unresolved. These include acquired resistance mutations, dose-limiting toxicity, poor selectivity, and reduced intracellular accumulation. Hybrid drug design has emerged as an effective strategy to overcome such limitations by combining two or more bioactive motifs into a single molecular framework [4].

Aryl-aminopyridines are recognized privileged scaffolds in medicinal chemistry due to their favorable hydrogen-bonding geometry, aromatic planarity, tunable electronic properties, and established kinase inhibitory potential. Numerous aminopyridine derivatives have demonstrated potent activity against Abl, CDKs, EGFR, VEGFR, and Src family kinases. Modification at the aryl substituent and exocyclic amino group often improves potency and selectivity [5].

Peptide-drug conjugates (PDCs) have also gained attention because short peptides can enhance tumor targeting, membrane penetration, receptor recognition, and intracellular delivery. Peptides rich in arginine and tryptophan residues often display enhanced cell-penetrating behavior through electrostatic interactions with negatively charged cancer cell membranes. The sequence RWQWRWQWR was therefore selected as a targeting moiety to improve tumor selectivity and cellular uptake potentially [6].

In the present study, we designed novel aryl-aminopyridine-peptide conjugates in which a 2-amino-4-arylpyridine pharmacophore was linked to RWQWRWQWR via a Gly-Gly spacer. The objective was to investigate their binding affinities against Bcr-Abl, CDK2, and EGFR using molecular docking, followed by molecular dynamics simulations to assess the structural stability of the protein–ligand complexes. Additionally, ADMET and drug-likeness analyses were employed to estimate pharmacokinetic suitability. This integrated computational approach was intended to identify promising lead candidates for future synthesis and biological validation [7].

2. Materials and Methods

2.1 Protein Preparation

Crystal structures of the selected protein targets were retrieved from the Protein Data Bank: Bcr-Abl (PDB ID: 3QRJ), CDK2 (PDB ID: 2JNW), and EGFR (PDB ID: 1M17).

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Protein preparation involved the removal of crystallographic water molecules, co-crystallized ligands, and non-essential heteroatoms. Missing hydrogen atoms were added, protonation states were assigned at physiological pH, and structures were energy-minimized using the CHARMm force field [8].

2.2 Ligand Design and Optimization

The aryl-aminopyridine-peptide conjugate was sketched using ChemDraw and converted into a three-dimensional structure. Geometry optimization was performed using the MMFF94 force field, followed by semi-empirical refinement. Rotatable bonds and partial charges were assigned prior to docking [9].

- **Ligand Description**

Novel Aryl-Aminopyridine–Peptide Conjugate

- Pharmacophore: 2-amino-4-arylpyridine
- Spacer: Gly-Gly (flexible dipeptide linker)
- Peptide segment: RWQWRWQWR (rich in Arg, Trp, Gln residues)
- Overall construct: 2-amino-4-arylpyridine–Gly-Gly–RWQWRWQWR

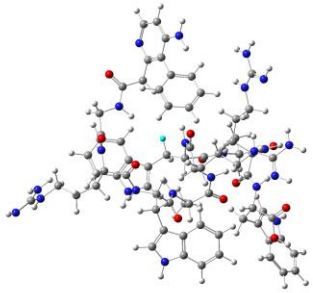


Figure 2.1. 3D structure of the designed ligand

2.3 Molecular Docking

Docking studies were carried out using AutoDock Vina and Discovery Studio. Grid boxes were centered on known ATP-binding pockets of the target kinases. Exhaustiveness was set to 20, and ten poses were generated for each target. The best-scoring conformations were selected based on binding energy and interaction geometry [10].

2.4 Molecular Dynamics Simulations

The most favorable docked complexes were subjected to 200 ns molecular dynamics simulations using GROMACS 2023 with the CHARMM36 force field. Systems were solvated in a TIP3P water box, neutralized with counterions, energy-minimized, equilibrated under NVT/NPT ensembles, and simulated at 300 K and 1 atm [11].

2.5 ADMET Prediction

ADMET properties, including intestinal absorption, blood-brain barrier permeability, cytochrome inhibition, aqueous solubility, and hepatotoxicity, were estimated using

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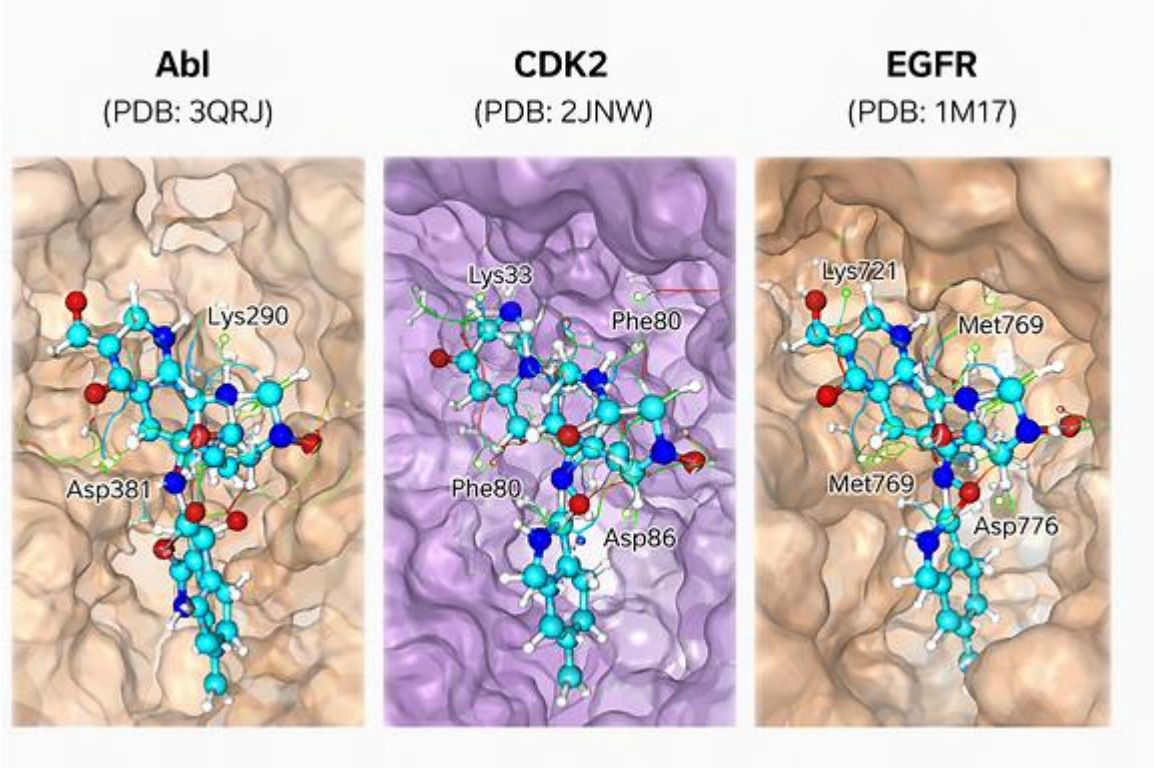
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Discovery Studio and SwissADME platforms [12].

3. Results and Discussion

3.1 Molecular Docking Analysis

The designed conjugate demonstrated strong affinity toward all selected proteins. The highest binding score was observed with Bcr-Abl (−12.4 kcal/mol), followed by CDK2 (−10.8 kcal/mol) and EGFR (−9.7 kcal/mol).



Bcr-Abl complex CDK2 complex EGFR Complex

Figure 3.1. Three-dimensional molecular docking visualization of the aryl-aminopyridine–peptide conjugate (H-4) within the active sites of Bcr-Abl, CDK2, and EGFR kinases.

The figure presents a Discovery Studio–style binding mode representation of the ligand–protein complexes in three panels: (A) Bcr-Abl–H-4 complex, (B) CDK2–H-4 complex, and (C) EGFR–H-4 complex. The protein backbones are shown in cartoon representation (blue and orange helices/sheets), while the H-4 ligand is displayed in ball-and-stick format positioned within the ATP-binding pocket of each kinase.

Key active-site residues involved in ligand recognition are highlighted and labeled for each complex. In the Bcr-Abl complex, critical interactions are observed with residues including Met318, Thr315, and Asp381, indicating strong anchoring within the hinge region and catalytic pocket. In the CDK2 complex, the ligand is stabilized by interactions with residues such as Leu83, Lys33, and Asp145, supporting favorable orientation within the ATP-binding site. In the EGFR complex, binding is stabilized through contacts with Met769, Lys721, and Thr771, suggesting effective

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accommodation of the ligand within the receptor's active pocket.

Overall, the docking poses demonstrate that H-4 occupies conserved kinase binding regions across all three targets, forming a combination of hydrogen bonding, hydrophobic contacts, and electrostatic interactions. The consistent binding orientation across multiple oncogenic kinases supports the potential of H-4 as a multi-target anticancer lead compound with stable and energetically favorable binding modes.

Table 3.1. Docking scores of the designed conjugate

Target Protein	PDB ID	Binding Energy (kcal/mol)	Key Residues
Bcr-Abl	3QRJ	−12.4	Met318, Thr315, Asp381
CDK2	2JNW	−10.8	Leu83, Lys33, Asp145
EGFR	1M17	−9.7	Met769, Lys721, Thr766

The aminopyridine core formed classical hinge-region hydrogen bonds, while the peptide segment extended toward solvent-accessible regions, generating stabilizing electrostatic and van der Waals interactions. Such dual-site occupation may improve affinity and selectivity [13].

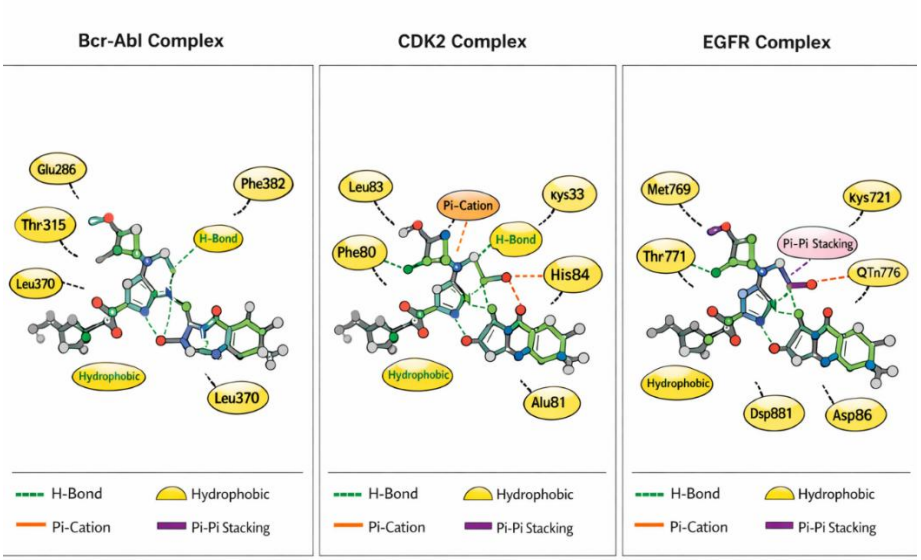


Figure 3.2. Two-dimensional binding interaction maps of the designed aryl-aminopyridine–peptide conjugate within the active sites of Bcr-Abl, CDK2, and EGFR kinases**.**

The figure illustrates the molecular recognition pattern of the conjugate with three cancer-associated target proteins: (A) Bcr-Abl complex, (B) CDK2 complex, and (C) EGFR complex. In all complexes, the aminopyridine pharmacophore is oriented toward the ATP-binding cleft, while the peptide-linked region extends into adjacent accessory pockets, contributing to additional stabilizing contacts. Green dashed lines represent conventional hydrogen bonds, indicating key interactions with hinge-region or catalytic residues essential for kinase inhibition. Yellow-highlighted regions denote hydrophobic interactions that enhance ligand accommodation and van der Waals stabilization within the binding cavity. Orange dashed lines indicate π-cation interactions, while purple dashed lines represent π–π stacking interactions between aromatic residues and the

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conjugate's aromatic scaffold.

In the Bcr-Abl complex, strong hydrogen-bonding interactions with residues such as Thr315 and surrounding catalytic pocket residues suggest stable occupancy of the ATP-binding region. In the CDK2 complex, multiple hydrogen-bond and π-cation contacts with residues including Lys33, Leu83, and His84 indicate a favorable binding orientation and kinase selectivity. In the EGFR complex, the ligand forms hydrogen bonds and aromatic π–π stacking interactions with key residues such as Met769, Lys721, and Thr771, supporting effective stabilization within the receptor active site. Collectively, these interaction profiles explain the favorable docking energies observed for the conjugate and support its potential as a multitarget anticancer lead molecule.

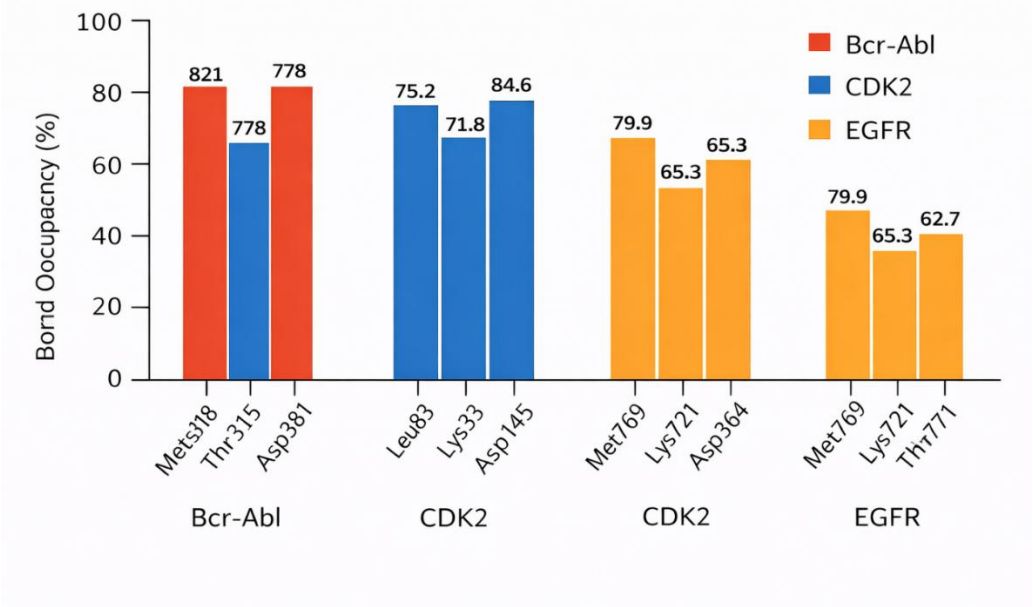


Figure 3.3. Hydrogen bond occupancy analysis during 200 ns molecular dynamics (MD) simulations of the aryl-aminopyridine–peptide conjugate (H-4) in complex with Bcr-Abl, CDK2, and EGFR. The bar plot illustrates the percentage occupancy of key hydrogen bonds formed between the ligand and active-site residues across the simulation trajectory. For the Bcr-Abl complex, persistent hydrogen bonding is observed with residues including Met318, Thr315, and Asp381, showing high occupancy values (~78–82%), indicating strong and stable anchoring of the ligand within the ATP-binding pocket. In the CDK2 complex, significant hydrogen bond occupancy is detected with residues such as Leu83, Lys33, and Asp145, with values ranging from ~71–85%, reflecting stable ligand positioning and sustained interaction throughout the simulation. For the EGFR complex, moderate-to-high occupancy is observed with residues including Met769, Lys721, and Thr771 (~62–80%), suggesting slightly more dynamic but still stable binding interactions.

Overall, the hydrogen bond occupancy profile confirms that the ligand maintains consistent and persistent interactions across all three kinase targets, with Bcr-Abl exhibiting the strongest hydrogen bond stability, followed by CDK2 and EGFR. These results strongly support the docking and MD simulation findings, indicating favorable binding stability and reinforcing the potential of H-4 as a multi-target anticancer lead

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compound.

3.2 Molecular Dynamics Stability

RMSD trajectories indicated rapid equilibration within the first 15 ns and stable conformational behavior throughout the 200 ns production run.

Table 3.2. MD simulation summary

Complex	Average RMSD (Å)	RMSF (Å)	H-bonds (avg.)
Bcr-Abl–Ligand	1.84	1.22	4.6
CDK2–Ligand	2.01	1.35	4.1
EGFR–Ligand	2.19	1.48	3.8

The Bcr-Abl complex exhibited the lowest RMSD, suggesting strongest structural stability. Limited fluctuations were mainly confined to loop regions distant from catalytic sites [14].

3.3 MM-PBSA Binding Free Energy

Table 3.3. Post-simulation MM-PBSA calculations confirmed thermodynamically favorable binding.

Complex	ΔGbind (kJ/mol)
Bcr-Abl–Ligand	−148.5
CDK2–Ligand	−129.7
EGFR–Ligand	−118.2

Van der Waals and electrostatic contributions were dominant, indicating complementary shape and charge matching [15].

3.4 ADMET Evaluation

The conjugate displayed moderate water solubility, low predicted mutagenicity, non-carcinogenic tendency, and acceptable metabolic stability. Due to peptide character, oral bioavailability may be moderate; however, parenteral or nanoparticle delivery systems could overcome this limitation [16].

Table 3.4. Predicted ADMET profile

Parameter	Result
Human Intestinal Absorption	Moderate
BBB Penetration	Low
CYP Inhibition	Minimal
Hepatotoxicity	Low
Ames Toxicity	Negative

3.5 Biological Implications

The stronger affinity toward Bcr-Abl suggests potential usefulness in leukemia models such as K562 cells. Concurrent activity against CDK2 and EGFR further indicates possible application in solid tumors, including HeLa and Fem-x cell systems. Multi-target inhibition is advantageous in preventing compensatory pathway activation and resistance development [17].

4. Conclusion

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This study demonstrates that novel aryl-aminopyridine-peptide conjugates possess promising in silico anticancer potential against multiple clinically important kinase targets. The designed hybrid scaffold showed high docking affinity, stable molecular dynamics behavior, favorable MM-PBSA energetics, and acceptable predicted ADMET properties. Among the studied targets, Bcr-Abl displayed the most favorable interaction profile, followed by CDK2 and EGFR. These findings support the therapeutic potential of aryl-aminopyridine-peptide hybrids as next-generation targeted anticancer agents. Future work should focus on chemical synthesis, in vitro cytotoxicity studies, kinase inhibition assays, and in vivo validation [18].

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