Computational Design, Molecular Docking and Molecular Dynamics Evaluation of Curcumin-Derived C-Src Kinase Inhibitors For Targeted Cancer Therapy

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Abstract:
Src kinase is one of the important targets for cancer therapy due to its involvement in tumor progression and metastasis. This paper presents the design and evaluation of a series of (2E,5E)-2,5-bis((1,3-diphenyl-1H-pyrazol-4-yl)methylene)cyclopentanone derivatives intended to be c-Src kinase inhibitors. Molecular docking studies pinpointed five lead compounds with acceptable binding affinities. Subsequent MD simulations corroborated these findings while additionally elucidating their stability as well as interaction profiles. Conformational analysis through RMSD and RMSF revealed that Cpen04 and Cpen13 were likely to conform to the most stable binding configurations, preserving rather strong hydrogen bond as well as hydrophobic interactions. It was further established by the ligand-protein contact analysis that they were indeed exhibiting good interaction modes. From these results, the designed compounds will be a potent c-Src kinase inhibitor; hence future work will involve experimental validation to confirm their therapeutic applicability.

Keywords: Curcumin derivatives, c-Src kinase, Cyclopentanone derivatives, Monoketone, Anticancer drug design

1. Introduction:

One of the most important global health problems is cancer, which is characterized by unrestrained proliferation of cells, invasion and metastasis. The disease results from a combination of genetic mutations epigenetic alterations and dysregulated signaling pathways. Of the several important drivers Src family kinases SFKs are one of the most significant forces that regulate a plethora of cellular functions ranging from proliferation to survival angiogenesis as well as metastasis [1]. Non-receptor tyrosine kinase c-Src is both overexpressed and hyperactivated in several cancers including breast lung colorectal and pancreatic malignancies. Misregulation of c-Src has been linked with adverse prognosis chemotherapy resistance as well as increased metastatic potential [2]. Despite the promise of many c-Src inhibitors such as dasatinib and bosutinib clinical experience has not been encouraging because of problems such as off-target effects toxicity and acquired resistance. All these have shown need for cutting-edge, selective yet potent c-Src kinase inhibitors with improved efficacy together with safety profiles [3].

Curcumin is a bioactive compound originating from *Curcuma longa* that has been studied widely for its diverse pharmacological properties, anticancer, anti-inflammatory and antioxidant activities being just a few [4]. A lot of research has been carried out to establish that curcumin exerts its anticancer effects by interacting with several signaling pathways which include NF-κB, PI3K/Akt, and MAPK besides inhibiting kinases c-Src [5]. Though it poses promising therapeutic potential, the clinical use of curcumin has been adversely affected by its aqueous solubility, systemic bioavailability and metabolic degradation. Fastened these issues in the hands of structural modification of curcumin intended to enhance pharmacokinetic and pharmacodynamic properties [6].

A series of (2E,5E)-2,5-bis((1,3-diphenyl-1H-pyrazol-4-yl)methylene)cyclopentanone derivatives was designed in the context of this study as prospective c-Src kinase inhibitors [7]. A novel scaffold was deliberately created to optimize drug-like properties of curcumin while keeping essential pharmacophoric attribute [8]. The structural rigidity and enhanced metabolic stability of cyclopentanone core shape are introduced; on the other hand, the pyrazole moiety enhances hydrogen bonding as well as π-π stacking interactions with the ATP-binding site of c-Src kinase. All these modifications are expected to improve binding affinity, selectivity and inhibitory activity toward c-Src kinase instead of merely swinging down its activity for an anticancer purpose [9].

Computer-aided drug design (CADD) revolutionized the field of drug discovery by allowing rational identification and optimization of lead compounds through computational modeling. As a result, the time and cost associated with the drug discovery process have dramatically been reduced [10]: CADD techniques include molecular docking, molecular dynamics (MD) simulations, and free energy calculations. While molecular docking predicts the binding mode and interactions of small molecules at the active site of target proteins, MD simulations give information regarding the stability and conformational dynamics of ligand-protein complexes under physiological conditions. In silico methods permit a more profound insight into molecular interactions, thereby facilitating the selection of promising candidates for further experimental validation [11]. Based on this the designed derivatives of (2E,5E)-2,5-bis((1,3-diphenyl-1H-pyrazol-4-)methylene)cyclopentanone will possess remarkable c-Src kinase inhibiting activity due to effective interacting forces at the ATP-binding site [7] . Through molecular docking and dynamics simulations, the study aim to evaluate their binding affinity, stability and structural integrity, thereby identifying novel lead compounds for targeted cancer therapy [12].

2. Materials and Methods

2.1 Molecular Docking

Drug Design

Based on the literature review 25 derivatives of (3*E*,5*E*)-3,5-bis((3-(2/3/4-substitutedphenyl)-1-phenyl-1*H*-pyrazol-4-yl) methylene) piperidin-4-one was designed as shown in figure 1 and the substituents are shown in table 1.

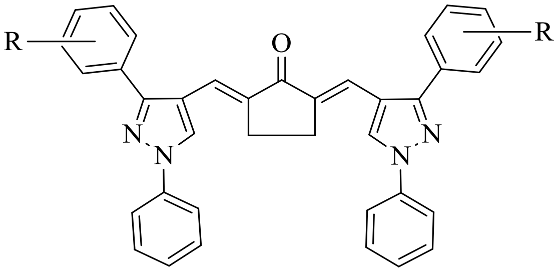

Figure 1. General Structure of compounds

Table 1: Designed compounds with substituents

Sl. no	**Codes**	**R**
1	Cpen01	**H**
2	Cpen02	**2-OCH_3**
3	Cpen03	**3-OCH_3**
4	Cpen04	**4-OCH_3**
5	Cpen05	**2-CH_3**
6	Cpen06	**3-CH_3**
7	Cpen07	**4-CH_3**
8	Cpen08	**2-NO_2**
9	Cpen09	**3-NO_2**
10	Cpen10	**4-NO_2**
11	Cpen11	**2-Cl**
12	Cpen12	**3-Cl**
13	Cpen13	**4-Cl**
14	Cpen14	**2-F**
15	Cpen15	**3-F**
16	Cpen16	**4-F**
17	Cpen17	**2-Br**
18	Cpen18	**3-Br**
19	Cpen19	**4-Br**
20	Cpen20	**2-OH**
21	Cpen21	**3-OH**
22	Cpen22	**4-OH**
23	Cpen23	**2-NH_2**
24	Cpen24	**3-NH_2**
25	Cpen25	**4-NH_2**

Protein Preparation

The X-ray crystallographic structure of c-Src kinase, a breast cancer targeted inhibitor, with PDB ID 2OIQ, was retrieved from the RCSB Protein Data Bank (PDB: http://www.rcsb.org/pdb). Missing amino acids were added by Charm-GUI for the selected proteins and also optimization of the protein

Ligand Preparation

Designed compounds were drawn using Chemsketch tool. Ligand energy was minimized, and an H-bond was added using the Avogadro tool (https://avogadro.cc/ accessed 15 April 2023) [13].

Docking Studies

The molecular docking study was performed using the Auto Dock tool. The docking scores obtained were compared to that of the standard chemotherapeutic drug and Discovery Studio Visualizer was also used to visualize the interaction of each complex obtained so as to get 2D and 3D images of the interactions [14].

2.2 Molecular Dynamics Simulation

Only the selected protein-ligand complexes were further screened through MD simulations using the OPLS 2005 force field within the DESMOND v6.3 module of the Schrödinger suite [15]. This process is documented in minimized and relaxed solvated systems for complexes, where a system builder tool was placed cubic box around the protein-ligand complex with TIP3P water as a solvent model. The protein-ligand complex was further optimized and re-scored to add more counterions for proper equilibration of the system's charges. In order to achieve physiological-like salt concentrations, NaCl at 0.15 M was added to the system at a distance of 20 Å from the ligand. Minimized and relaxed solvated systems for complexes were prepared following DESMOND's standard procedure, as previously described for minimized and relaxed solvated systems for complexes [16]. Two minimization tasks and four short simulations precede production time; these constitute what is known as the equilibrium phase. Molecular dynamics simulations were performed on top-scored ligand-receptor complexes for run times of 100 ns. Simulations used the OPLS 2005 force field [17] and NPT ensemble at temperature 300 K and pressure1.01325 bar. The Complex RMSD (Root-Mean-Square Deviation), RMSF-Root mean square fluctuations, Protein-Ligand interactions, and interaction with other amino acid residues were evaluated through Simulation Event Analysis provided by DESMOND module [18].

3. Results and Discussion

3.1 Molecular Docking Results

The molecular docking was implemented to determine the strength of binding for the newly designed compound of (2E,5E)-2,5-bis((1,3-diphenyl-1H-pyrazol-4-yl)methylene)cyclopentanone derivatives (that is from Cpen series) in the c-Src kinase active site. The docking scores are shown in table 2 and table 3 shows the best five candidates along with their key interactions shown in figure 2. Among the screened compounds, Cpen04 showed the best docking score of -9.69 kcal/mol, followed by Cpen10 with a score of -9.49 kcal/mol, Cpen13 with -9.44 kcal/mol, Cpen07 with -9.31 kcal/mol, and Cpen01 with -8.87 kcal/mol. Such values indicate a strong binding affinity with c-Src kinase which suggests potential inhibitory activity.

The binding interactions of the top five compounds were analyzed to understand how these compounds stabilize their mechanisms in the active site of c-Src kinase. For instance, Cpen04 had a critical hydrogen bond interaction with ASN568, which would presumably be the reason for its strong affinity. Hydrogen bonding is molecular recognition as well as target specificity, thereby inhibiting potential. The Cpen10 derivative had several hydrogen bond interactions with GLU531, GLU571, and ARG570; all these interact strongly within the kinase pocket. Such polar interactions would enhance selectivity for the c-Src binding site. Compound Cpen13 was found to have a pi-cation interaction with LYS514, ligands can be strengthened by electrostatic stabilization. Pi-cation interactions are a hallmark among kinase inhibitors and play an important role in target selectivity. Cpen07 is very similar to Cpen13 and has also formed a pi-cation interaction with LYS514. In contrast to all other top-ranked compounds, Cpen01 exhibited only hydrophobic interactions inside the active site. Although she lacks direct hydrogen bonding or electrostatic interactions with it, its high docking score indicates that she is well suited within the binding pocket, perhaps due to shape complementarity and van der Waals forces.

The docking scores for the best compounds are similar to or better than those of the known c-Src inhibitors, dasatinib and bosutinib, which have docking scores of about -8 to -10 kcal/mol. These strong hydrogen bonds and also electrostatic interactions in the designed derivatives indicate that these derivatives might possess better selectivity and potency against c-Src kinase.

The molecular docking study identified five lead compounds with potential binding affinities toward c-Src kinase. The detected hydrogen bonds, pi-cation interactions, and hydrophobic contacts in the molecules highlight an encouraging binding profile for kinase inhibition.

Table 2: Docking scores of Top twenty-five compounds of Cpen with c-Src Kinase

Codes	**Names**	**Docking score**
Cpen01	(2*E*,5*E*)-2,5-bis((1,3-diphenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-8.97
Cpen02	(2*E*,5*E*)-2,5-bis((3-(2-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-8.12
Cpen03	(2*E*,5*E*)-2,5-bis((3-(3-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-8.05
Cpen04	(2*E*,5*E*)-2,5-bis((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-9.69
Cpen05	(2*E*,5*E*)-2,5-bis((1-phenyl-3-(o-tolyl)-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-7.54
Cpen06	(2*E*,5*E*)-2,5-bis((1-phenyl-3-(m-tolyl)-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-7.27
Cpen07	(2*E*,5*E*)-2,5-bis((1-phenyl-3-(p-tolyl)-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-9.31
Cpen08	(2*E*,5*E*)-2,5-bis((3-(2-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-7.72
Cpen09	(2*E*,5*E*)-2,5-bis((3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-6.47
Cpen10	(2*E*,5*E*)-2,5-bis((3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-9.49
Cpen11	(2*E*,5*E*)-2,5-bis((3-(2-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-8.24
Cpen12	(2*E*,5*E*)-2,5-bis((3-(3-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-8.46
Cpen13	(2*E*,5*E*)-2,5-bis((3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-9.44
Cpen14	(2*E*,5*E*)-2,5-bis((3-(2-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-7.32
Cpen15	(2*E*,5*E*)-2,5-bis((3-(3-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-7.14
Cpen16	(2*E*,5*E*)-2,5-bis((3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-8.26
Cpen17	(2*E*,5*E*)-2,5-bis((3-(2-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-6.89
Cpen18	(2*E*,5*E*)-2,5-bis((3-(3-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-8.61
Cpen19	(2*E*,5*E*)-2,5-bis((3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-6.61
Cpen20	(2*E*,5*E*)-2,5-bis((3-(2-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-7.6
Cpen21	(2*E*,5*E*)-2,5-bis((3-(3-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-6.51
Cpen22	(2*E*,5*E*)-2,5-bis((3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-8.99
Cpen23	(2*E*,5*E*)-2,5-bis((3-(2-aminophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-7.67
Cpen24	(2*E*,5*E*)-2,5-bis((3-(3-aminophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-7.01
Cpen25	(2*E*,5*E*)-2,5-bis((3-(4-aminophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)cyclopentanone	-6.22

Table 3: Docking scores of top five compounds of Cpen with c-Src Kinase

Sl. No	Codes	Docking score (kcal/mol)	Key Interactions	Type of interactions
1	Cpen01	-8.87	No	Hydrophobic interactions
2	Cpen04	-9.69	ASN568	Hydrogen-bond
3	Cpen07	-9.31	LYS514	Pi-cation
4	Cpen10	-9.49	GLU531, GLU571, ARG570	Hydrogen-bond
5	Cpen13	-9.44	LYS514	Pi-cation

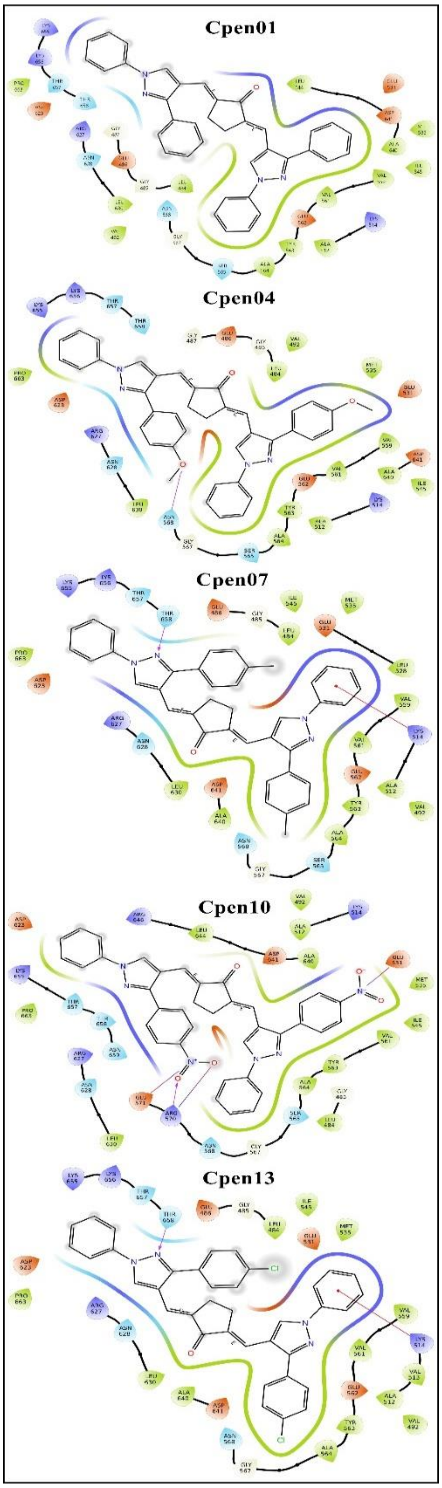

Figure 2: 2D interactions of Cpen01, Cpen04, Cpen10, Cpen13 and Cpen07 with c-Src Kinase

3.2 Molecular Dynamics Simulation Results

The RMSD analysis gives an idea about the structural stability of the protein-ligand complexes during the 10 ns simulation. For all the five complexes, Cpen01, Cpen04, Cpen07, Cpen10, and Cpen13, the RMSD values remained stabilized within the range of 1-5 Å that means the protein was stable in its structure throughout the simulation, shown in figure 3. Of all the ligands studied, Cpen04 showed least deviation (~1.5 Å) which implies that a good convergence of the system was achieved whereas Cpen10 exhibited slightly higher fluctuations (~2.8 Å) that could be considered as evidence for minor conformational change. For ligand RMSD, the most stable binding conformations were shown by the compounds Cpen04 and Cpen13 with RMSD values remaining below 2 Å in relation to the protein which indicated that the ligands stayed in the binding pocket without significant displacement. On the other hand, Cpen10 showed an increase in RMSD (~3.2 Å) which suggested slight repositioning or flexibility inside the pocket.

RMSF analysis was performed to report the flexibility of individual amino acid residues. As expected, the loop regions showed the highest fluctuations ~3-5 Å and were most pronounced at the N- and C-terminals, shown in figure 4. The residues that are critical for ligand interaction, ASN568, LYS514, and GLU531, were found to be stable with RMSF values below 2 Å, indicating that there are no significant disturbances introduced by ligand binding. Of the ligands, Cpen04 had the least fluctuation (~1.2 Å), which could further establish its stable binding; on the other hand, Cpen10 exhibited the highest RMSF (~2.9 Å) and hence presumed to possess more flexible functional groups. All these factors are quite well correlated with their respective ligand RMSD results.

The molecular dynamics simulation probably showed that Cpen04 and Cpen13 had the most stable interactions with c-Src kinase, evidenced by lower RMSD, reduced ligand fluctuations, and higher contact frequencies between protein and ligand. The presence of hydrogen bonds, pi-cation interactions, and hydrophobic interactions contributed significantly to their stability.

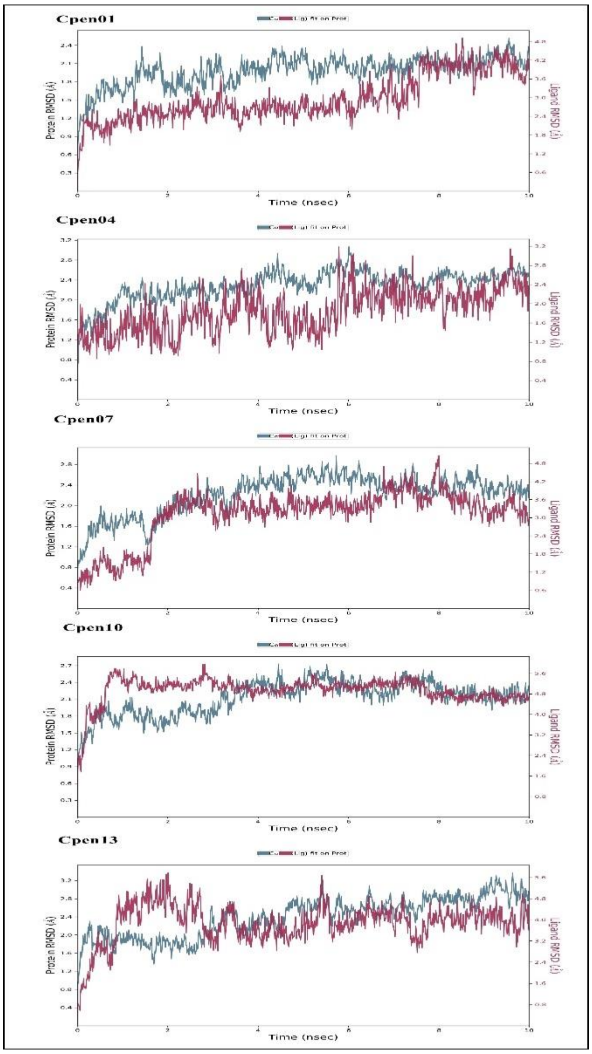

Figure 3: RMSD graphs of (a) Cpen01 (b) Cpen04 (c) Cpen10 (d) Cpen13 and (e) Cpen07 with c-Src Kinase

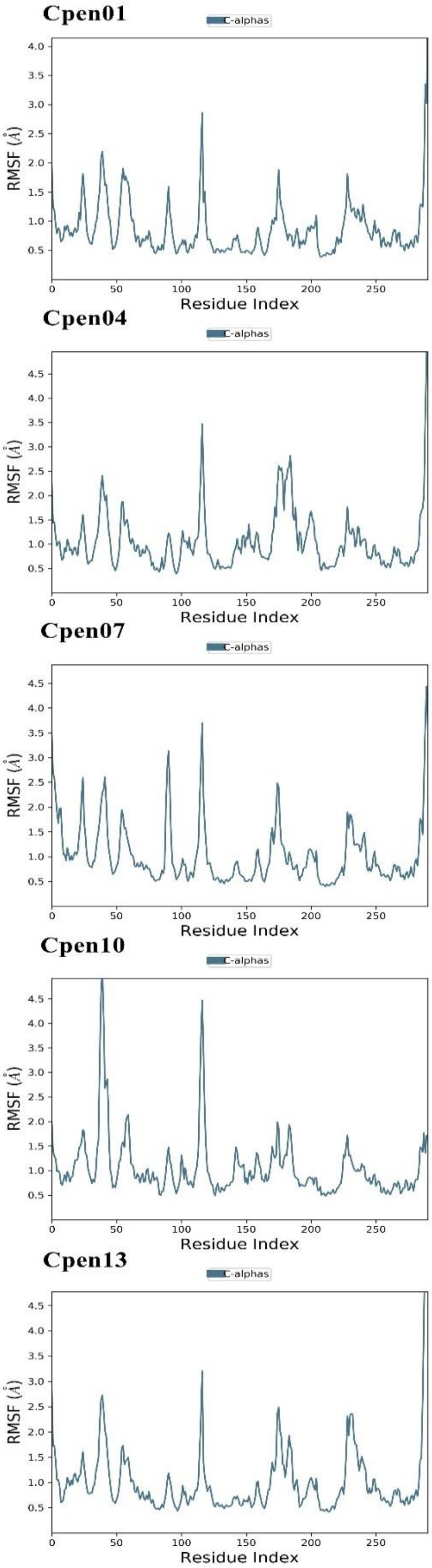

Figure 4: RMSF graphs of (a) Cpen01 (b) Cpen04 (c) Cpen10 (d) Cpen13 and (e) Cpen07 with c-Src Kinase

Conclusion:

This research integrates the use of computational methods in drug discovery to find and evaluate new inhibitors of c-Src kinase. Promising candidates were identified through molecular docking and subsequently verified by

MD simulations. The structural stability of Cpen04 and Cpen13 was confirmed from the RMSD and RMSF analyses, whereas significant and persistent hydrogen bonding along with hydrophobic interactions was highlighted in the ligands-protein interaction profile.Of the five compounds tested, Cpen04 was the most promising and stable candidate with a high interaction frequency and low fluctuation plus a strong binding affinity. Cpen13 showed considerable stability as well; thus, it could be another viable candidate to pursue. Future work will involve in synthesis, *in vitro* and *in vivo* validation will confirm the biological activity of the lead compounds. These findings further support the development of selective c-Src kinase inhibitors for potential therapeutic applications in cancer management.

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Conflict of interest

The authors declare that they have no conflict of interest.

Consent for Publication

The authors give the consent for publication.

Data Availability

All data generated or analysed during this study are included in this manuscript.

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