Targeting the cyclin-binding groove site to inhibit the catalytic activity of CDK2/cyclin A complex using p27^{KIP1}-derived peptidomimetic inhibitors

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Abstract Functionally activated cyclin-dependent kinase 2 (CDK2)/cyclin A complex has been validated as an interesting therapeutic target to develop the efficient antineoplastic drug based on the cell cycle arrest. Cyclin A binds to CDK2 and activates the kinases as well as recruits the substrate and inhibitors using a hydrophobic cyclin-binding groove (CBG). Blocking the cyclin substrate recruitment on CBG is an alternative approach to override the specificity hurdle of the currently available ATP site targeting CDK2 inhibitors. Greater understanding of the interaction of CDK2/cyclin A complex with p27 (negative regulator) reveals that the Leu-Phe-Gly (LFG) motif region of p27 binds with the CBG site of cyclin A to arrest the malignant cell proliferation that induces apoptosis. In the present study, Replacement with Partial Ligand Alternatives through Computational Enrichment (REPLACE) drug design strategies have been applied to acquire LFG peptide-derived peptidomimetics library. The peptidomimetics function is equivalent with respect to substrate p27 protein fashion but does not act as an ATP antagonist. The combined approach of molecular docking, molecular dynamics (MD), and molecular electrostatic potential and ADME/T prediction were carried out to evaluate the peptidomimetics. Resultant interaction and electrostatic potential maps suggested that smaller substituent is desirable at

the position of phenyl ring to interact with Trp217, Arg250, and Gln254 residues in the active site. The best docked poses were refined by the MD simulations which resulted in conformational changes. After equilibration, the structure of the peptidomimetic and receptor complex was stable. The results revealed that the various substrate protein-derived peptidomimetics could serve as perfect leads against CDK2 protein.

Keywords Protein–protein interaction · Cyclin-binding groove · Peptidomimetic inhibitor · Molecular docking · MD simulation

Introduction

The active enzyme complex of cyclin-dependent kinase 2 (CDK2) associated with cyclin A is an attractive cancer therapeutic target to regulate the uncontrolled cell division. Through phosphorylation, CDK2 has governed the signaling pathway of G1-S phase transition of cell cycle [47]. Approximately, 20 different chemical classes of ATP-binding site targeting CDK inhibitors are available. They are analogues of purine, pyrimidine, and natural metabolites isolated from microbial strains and their derivatives. The major obstacles of inhibitors that target the ATP-binding site of the kinases are their poor selectivity for a single CDK isoform. The well-known example, R-roscovitine has multiple target inhibition activity on CDK1, CDK2, CDK5, CDK7, and CDK9, which leads to higher toxic side effects [45, 14]. The diverse groups of substrate proteins, classified as tumor suppressors (pRb, p107, and p130), transcription factors (E2F, MybB, ID2, and ID3), and p53, p21, and p27 inhibitor proteins [41, 28], have separate binding sites to interact with CDK2/cyclin A complex for the phosphorylation and activation. Therefore, interruption of protein–protein interaction

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(PPI) between CDK2 complex and regulator proteins leads to the structural modification and affects the enzyme association range. Disrupting the conserved cyclin-binding groove (CBG) of cyclin A is one of the possible ways to specifically inactivate the G1 to S phase transition of CDK2 complex [2]. Designing of peptide-based inhibitors that target the CBG site is a revolutionary approach to block the malignant cellular proliferation [9, 26, 27].

Targeting the PPI site with small molecule inhibitors has been perceived as a complicated task due to bulky and flat hydrophobic surfaces of the active site. Now, the belief has changed and it is comprehended that not all the substrate protein residues are essential for interaction and only few are crucial for binding in the active site of protein [7]. Earlier in vitro and in vivo studies proved that cyclin-dependent kinase inhibitor (CDKI)-derived peptides specifically bind to CBG and induce antitumor effects [23]. The p27 negative regulator protein interferes the CDK2/cyclin A complex phosphorylation with elongation factor (E2F) and sensitizes the cells into apoptosis [39]. The most conserved cyclin groove recognition motif (CRM) of p27 has “Leu-Phe-Gly” (LFG) residues that involve the primary anchoring at Ile213, Leu214, Trp217, Arg250, Leu253, and Gln254 residues of cyclin A [18]. The studies on CDK2 enzyme inhibition illustrated the CRM region-derived acetyl-capped Arg-Lys-Leu-Phe-Gly (penta peptide, RKLFG) sequence which significantly restricts the CDK2 complex activity even at low micromolar range [2]. But, the peptide inhibitors have two main obstacles: (i) the intracellular protein-degrading enzymes easily degrade the peptides before target binding and (ii) they slow down the lipid bilayer penetration of the cell membrane [15]. Hence, unnatural amino acid groups are incorporated into the peptides which develop into peptidomimetics that improve the penetration ability and stability [18].

In the present study, the CBG region of cyclin A targeting peptidomimetic leads was designed. The CBG site recognizing LFG peptide sequence was taken and extended with optimized unnatural amino acid derivatives to achieve the low-molecular-weight peptidomimetics. These peptidomimetic leads are alternative sources of small molecule inhibitors in many strategies especially where specific, less toxic, and noncompetitive inhibition is required [26]. Molecular docking protocols have been applied to validate the right orientation (poses) for maximum interaction and scoring function of the peptidomimetic with the active site. Docking simulations facilitate to identify the molecular interaction of ligands with binding sites that are responsible for significant biological activity. The final observation gives the scheme of specific interactions and further modification of ligands to obtain potent leads [21]. Understanding the electrostatic energies of protein–ligand or protein–protein docking is essential to observe the changes in the protonation state of the complex and improve the potential modification of ligands

[24]. Poor pharmacokinetic and toxicity of lead molecules are the major causes of failures in clinical trials. The screening process of good absorption, distribution, metabolism, excretion (ADME) and minimum toxicity helps to identify leads from large group of databases thus accelerating the drug discovery process successfully [56]. The molecular dynamics (MD) simulation is useful to examine the motion of docked protein complex in one or two quadrillionths of a second to include the solvent water and ionic substances (sodium, potassium, or chloride). The protein flexibility leads to dramatic conformational changes and affects the binding affinity of the ligand. The stability of the protein complex upon the binding of ligand has been calculated using advance computers, suitable algorithm designing, and molecular force fields [12]. All structural modifications, molecular docking, electrostatic potential analysis, biological property prediction, and MD simulations have been made to identify the best CDK2 targeting peptidomimetics. The promising results on prediction of probable peptidomimetics may lead to future leads with enhanced specificity.

Materials and methods

Principle and structural designing of peptidomimetics

The experimental reports suggested that leucine, phenylalanine, and glycine motif containing pentapeptide (RKLFG) potentially inhibited the CDK2/cyclin A complex activation [9, 1]. The docking analysis showed that the binding pocket of cyclin groove mainly concerned with the hydrophobic amino acid residues and LFG motif region plays central role in CBG site recognition and specific interaction (Fig. 1). The suitable modification on the side chains of the LFG residues improves the hydrophobic contacts in the binding site and could enhance the potency when compared to the truncated peptide leads [9]. Thus, LFG motif was taken as template, and N-terminal capped with suitable unnatural amino acid derivatives converts them into effective peptidomimetics. Here, Replacement with Partial Ligand Alternative through Computational Enrichment (REPLACE) approach was implemented to obtain the useful lower molecular weight peptidomimetic leads [1]. This method allows extension of the peptides with pharmacologically acceptable fragment hits. Unnatural amino acid derivatives such as 2-amino-3-phenylpropanoic acid, 2-amino butyric acid, chlorophenyl, and different derivatives were added at the N-terminal end of the LFG residues to mimic the amide bond of the peptide [7]. ChemDraw Ultra 11.0 suite was employed to draw the 2D structure of the LFG sequence and a number of modifications. The modified structures were converted into *mol* file format and given as input file for the structural optimization, toxicity prediction, and further computational simulation analysis.

Computational details of optimization of peptidomimetics

Calculation of the ground state electronic structure energy optimization for peptidomimetic molecules has been carried out using the hybrid density functional theory (DFT) with wave function of Becke, three-parameter, Lee–Yang–Parr (B3LYP) with the basis sets of 6-31+G(d,p) in the Gaussian 03w package. The structure was treated without any symmetry consideration and the default spin during the geometry optimization [4]. The B3LYP/6-31+G(d,p) level of theory gives the reliable electronic configuration of molecules. The standard optimization conditions of maximum force 0.000450, RMS force 0.000300, maximum displacement 0.001800, and RMS displacement 0.001200 have been used for the optimization [46, 55]. The peptidomimetic structure which has the value below this range was identified as a ground state energy structure. The geometry optimization was performed for the penta peptide and LFG derivatives using the initial configuration which was obtained by the drawn structures of LFG motif and 1–18. These configurations were allowed for energy minimization to attain the global minimum energy at B3LYP/6-31+G(d,p) level without any constraints.

Toxicity prediction of peptidomimetic libraries

OSIRIS property explorer web server (<http://www.organic-chemistry.org/prog/peo/>) was used to predict the fragment-based drug likeness of lead compounds. Structurally optimized peptidomimetics were screened through various biological activity parameters like mutagenicity, tumorigenicity, irritating effects, reproductive effects, and drug-related properties of cLogP, LogS (solubility), and drug likeness. The toxicity risk was assessed by color codes of green, yellow, and red which indicate no risk, medium risk, and high risk respectively. Moreover, drug score values are computed from the outcome of all the above toxicity and bioavailability parameters [5]. The drug score has been computed from the following equations,

$$ds = \prod \left(\frac{1}{2} + \frac{1}{2} si \right) \cdot \prod ti \quad (1)$$

$$S = \frac{1}{1 + e^{ap+b}} \quad (2)$$

where ds is the drug score. S_i is the contributions calculated directly from cLogP, LogS, molecular weight, and drug likeness (p_i) via the second equation which describes a spline curve; a and b parameters are (1, −5), (1, 5), (0.012, −6), and (1, 0) for cLogP, LogS, molecular weight, and drug likeness, respectively; and t_i is a measure of the contribution from the tumorigenicity, mutagenicity, and irritant and reproductive effective toxicity risk types. The t_i values are 1.0, 0.8, and

0.6 which denote that a compound has no risk, medium risk, and high risk of toxicity, respectively [5, 6].

A positive drug score value indicates that compound is free from toxicity and helps to exclude the undesired peptidomimetic derivatives for further screening process. The peptidomimetics which meet the preferred drug score value were chosen as desired drug candidates. The prediction was executed by the Registry of Toxic Effects of Chemical Substances (RTECS) toxicological database that includes more than 160,000 non-drug-like (Fluka) compounds classified as tumorigenic and mutagenic chemical groups. The prediction process relies on a precomputed set of structural fragments that give rise to toxicity alerts in case they are encountered in the structures or any derivative fragment was considered a risk factor [5, 6].

Molecular docking environment setup

The binary protein complex structure of CDK2/cyclin A and RKLFG inhibitor (PDB ID: 1URC; resolution factor 2.60 Å) were retrieved from Protein Data Bank (PDB) [38]. The geometric criteria of PDB structures validated that geometry restraints of torsion angles, such as the main-chain ψ , Φ (Ramachandran plot), or side chain, are often set more tightly of even at 3-Å resolution [36]. Therefore, the present resolution factor does not affect the docking and dynamics simulation analysis. Before docking process, protein structure was subjected to preparation and optimization steps with the aid of Schrodinger's Protein Preparation Wizard tool using OPLS-2005 force-field. The water molecules which were farther away than 5 Å from the ligand and not having the bond interaction with protein residues were identified and removed. Subsequently, the restrained minimization of protein structure was continued until the average root-mean-square deviation (ARMSD) of heavy atom reached 0.30 Å [13, 44]. To ensure the accuracy of the docking parameters, the known penta peptide inhibitor was docked into the CBG site of the cyclin A (PDB ID: 1URC) using Grid-Based Ligand Docking with Energetics (Glide) at Extra Precision (XP) level. The co-crystallized peptide inhibitor was removed from the binary CDK2/cyclin A protein complex, and the position of penta peptide was set as active site for docking. The same known peptide was redocked with that active site. The receptor's nonpolar atoms accounted for the van der Waals radii scaling, the default value being defined as 1.00 Å with a partial atomic charge of 0.25. The grid box was generated at the centroid of active site with $X=40.6641$, $Y=23.461$, and $Z=0.16$ as Cartesian coordinates. The grid box restored the van der Waals and Coulomb fields of the active site residues, and each atom of the ligand interacts with these fields. The explicit water technology and descriptors were applied in Glide XP scoring which allows the appropriate screening of peptidomimetic library. In Glide XP docking, crude score values and geometrical

Fig. 1 CDK2/cyclin A/p27-derived RKLFG peptide complex (PDB ID: 1URC). The 2D interaction map shows the residual interaction between cyclin A and RKLFG peptide inhibitor. Leucine, phenylalanine, and

glycine (LFG) motif region acts as template and extends at R1, R2, and R3 position. The unnatural amino acid groups used to derive the peptidomimetics

filters weed out false positives and to provide a better correlation between excellent poses and good scores. A small number of surviving docking solutions can be subjected to a Monte Carlo procedure to try and minimize the energy score. The best docked structure was chosen using a Glides docking energy, Glide energy, and Glide Emodel energy. Glide energy is a modified Coulomb–van der Waals interaction energy and Glide Emodel, which combines glide score, coulombic, van der Waals, and strain energy of the ligand. All these enrichment energy calculations facilitate the process of finding out the best docked peptidomimetics [13, 17, 51].

The 2D structure of peptidomimetics was converted into 3D conformation to generate a series of low-energy conformations and optimization using LigPrep 2.6 [25]. Before the energy minimization process of peptidomimetic structures, the following steps were undertaken: (i) addition of implicit hydrogen atoms, (ii) neutralization of charged groups, (iii) generation of various ionization, and (iv) tautomerization and arriving at chiralities of the ligand molecule. The peptidomimetic structures were arrived at by means of the OPLS-2005 force field. Further, optimization of ground state electronic energy of the synthetic peptidomimetic molecules was carried out using Jaguar 7.9 [20]. The complete geometry optimization was performed using hybrid DFT with B3LYP. Optimization process was performed in an aqueous environment using the Poisson–Boltzmann solvent so as to simulate physiological conditions [42]. All the peptidomimetic inhibitors were minimized by quantum mechanical (QM) methods applied by Jaguar suite.

Binding free energy calculation

The docked complex was subjected to the binding free energy calculation using molecular mechanics generalized Born surface area (MM-GBSA) approach employed by Prime 3.1 [33]. The scoring functions may fail if they do not properly account for solvation, entropy, or polarizability. OPLS-2005 force field and GB/SA continuum solvent model were used to validate the accuracy of the docking score which confirmed the stability of the docking complex. Binding energy was calculated by the following equations [50]:

$$\Delta G_{bind} = \Delta E + \Delta G_{solv} + \Delta G_{SA} \quad (3)$$

$$\Delta E = E_{complex} - E_{protein} - E_{ligand} \quad (4)$$

where $E_{complex}$, $E_{protein}$, and E_{ligand} are the minimized energies of the protein-inhibitor complex, protein, and inhibitor, respectively.

$$\Delta G_{solv} = G_{solv}(complex) - G_{solv}(protein) - G_{solv}(ligand) \quad (5)$$

where ΔG_{solv} is generalized born electrostatic solvation energy. $G_{solv}(complex)$, $G_{solv}(protein)$, and $G_{solv}(ligand)$ are the solvation free energies of complex, protein, and ligand, respectively.

$$\Delta G_{SA} = G_{SA}(complex) - G_{SA}(protein) - G_{SA}(ligand) \quad (6)$$

where ΔG_{SA} is the nonpolar contribution to the solvation energy due to the surface area. $G_{SA(complex)}$, $G_{SA(protein)}$, and $G_{SA(ligand)}$ are the surface energies of complex, protein, and ligand, respectively.

The simulations were carried out using the GBSA continuum model. Prime uses a surface generalized Born (SGB) model employing a Gaussian surface instead of a van der Waals surface for better representation of the solvent-accessible surface area [33].

Molecular electrostatic potential surface analysis

The molecular electrostatic potential (MESP) state analysis could be employed to find the favorable charge distribution of peptidomimetic, which is responsible for the CBG interaction. The polar and charged molecules increase the selectivity of the binding site than less polar and charged ligand environment. Quantum mechanical methods elucidate the charge-based description of the ligand-target site interaction [19]. The specific interaction occurs only if complementary charge is distributed between active site and peptidomimetic or any ligand. The best scored peptidomimetics were again optimized to change the structures in the Gaussian environment. Optimized peptidomimetics were treated without any symmetry consideration with the default spin. The electrostatic equilibrium was analyzed with the Gaussian 03 employing DFT. The wave function of B3LYP and the basis sets of 6-31+G(d,p) were employed [40].

Assessment of drug-like properties of peptidomimetics

Pharmacokinetic parameters were analyzed to examine the drug-like properties of the modified peptide derivatives using QikProp 3.5 [34]. QikProp employs the BOSS program and OPLS-AA force field for simulation of hits on organic solutes in periodic boxes of explicit water molecules. This simulation helps to obtain the configurational average for pharmacological descriptors. Best hits were compared with the predefined acceptable range values [34]. All the peptidomimetics and known peptide conformations were energy minimized before the prediction of pharmacological properties. The descriptors, molecular weight, volume, total solvent accessible surface area (SASA), water/gas partition coefficient (QPlogPw), polarizability in cubic angstroms (QPpolrz), and van der Waals surface area of polar nitrogen and oxygen atoms (PSA) were used to evaluate the drug-like properties of the small molecule like peptidomimetics.

Molecular dynamics simulation

The stability of the minimum energy docking complex was determined by the MD simulation analysis through Desmond 3.1 module [11]. The structurally well-prepared protein–

peptidomimetic complex has to undergo appropriate explicit solvent system setup before the simulation process. The system builder predefined the simulation state with OPLS-2005 force field and transferable intermolecular potential 3 point (TIP3P) water solvent model set up in 0.15 M of salt solution containing Na^+ and Cl^- ions. The number of atoms and volume in known peptide inhibitor and peptidomimetics system was ranged from 71024 to 90689 and 756479 to 1726533 Å, respectively (Table S3). The system was subjected to the local energy minimization until a gradient threshold (25 kcal/mol/Å) was reached using a hybrid method of the steepest decent and the limited-memory Broyden–Fletcher–Goldfarb–Shanno (LBFGS) algorithms. A maximum of 3,000 iteration steps were used. The minimum energy scored complexes of peptidomimetics, 2, 3, 5, and 9, and crystal protein complex were employed for 10-ns simulation. The simulation system was relaxed by constant NPT (number of atoms N, pressure P, temperature T) ensemble condition to generate simulation data for post-simulation analyses [11, 37]. The temperature value was defined as 300 K for the whole simulation process using Nose–Hoover thermostats and stable atmospheric pressure (1 atm) carried out by Martina–Tobias–Klein barostat method [43]. The multi-time step RESPA integrator algorithm was used to investigate the equation of motion in dynamics. The time step consists of 2, 2, and 6 fs for bonded, “near” nonbonded, and “far” nonbonded interactions, respectively. SHAKE algorithm was employed to constrain the atoms which are involved in hydrogen bond interaction. The short range electrostatic and Lennard–Jones interactions were estimated by setting up the cutoff value as 9-Å radius. The long-range electrostatic interactions were evaluated by using particle mesh Ewald (PME) method with the simulation process using periodic boundary conditions (PBC) [50, 37]. Energy and trajectory analysis data were documented at 1.2- and 4.8-ps intervals respectively for statistical analysis. Final results were visualized using Maestro graphical interface.

Results and discussion

Optimization of peptidomimetics

Prediction of least energy peptidomimetic conformation is important for potential bioactive conformation associated with the binding process. The energy calculations express the global minimum energy conformation of peptidomimetics in solution phase. Peptide optimization through QM methods was performed to transform bioactive peptide into drug-like peptidomimetic lead compounds. The quantum mechanical-calculated vibrational frequencies for the stable compounds (compound 1 to

18) are depicted in Fig. S1. All the vibrational frequency modes were present in the positive region of 0 to 4,000 cm^{-1} indicating that modeled compounds were stable and could be possible to experimental synthesis. QM calculations are utilizing these peptidomimetics capabilities to explore the optimization of potency and selectivity while decreasing the size required for activity. The peptides are having many rotatable bonds, and based on the energy level, the peptides are changing its conformations. After the 500 iterations of QM optimization, the peptidomimetics attaining its least energy level and the final conformations are more stable and predominant for docking calculations. The least energy conformer of the peptidomimetic is taken for the whole study. The results of energy parameters of known peptide and LFG derivatives are represented in Table S1. The energy score is provided for determining the energy consumption for the particular peptide or peptidomimetic optimization. The known peptide inhibitor shows $-1,153,532.61$ kcal/mol, while all the peptidomimetics show $>-720,130$ kcal/mol.

Functional group modifications and toxicity prediction of peptidomimetics

In the REPLACE strategy-based designing, more than 200 peptidomimetic inhibitor structures were obtained. The LFG motif extended with phenyl propanoic acid, chloro phenyl, 2-amino butyric acid, methyl group, and various unnatural amino acid substituents. The QM methods of optimization were performed to obtain the global minimum energy conformation of known penta peptide inhibitor and peptidomimetics. Generating the least energy conformation of peptides or peptidomimetics is a very essential task to predict the bioactive conformation, which is associated with the receptor binding mechanism [42]. In terms of optimization, the least energy conformers of peptidomimetics were chosen for preliminary fragment-based toxicity prediction using OSIRIS toxicity tool. Positive values for drug score and drug likeness scores were observed in 18 derivatives as shown in Table 1. It confirms that the derivatives do not possess the risk of tumorigenic, mutagenic, and reproductive defects and can be assessed as drug-like candidates. Incorporation of antagonist-peptide with amino butyric acid strengthens the stability and improves the binding affinity [15]. N-methylation has increased the flexibility of the peptide to interact with the binding site [31, 10]. The N-methylation of hydrophobic amino acid residue decreases the bulkiness of peptides and improves the cell permeability to cross over the lipophilic cell membranes [22, 49]. Halogenation improves the penetration ability of the aromatic substituent [16]. The extension of peptide sequence with appropriate substitution

could induce the resistant mechanism against degradation and enhance the pharmacological properties [57, 58].

Molecular docking and atomic interaction

The peptide was redocked to the binding site of cyclin A, and RMSD between the conformation of peptide and redocked peptide was 0.35 Å. The minimum RMSD value confirmed the accuracy of the docking protocol. Seven docking models were chosen (peptidomimetics 2, 3, 4, 5, 8, 9, and 17) by Glide docking energy, Glide energy, and Glide Emodel energy for further analysis (Table 2). The unnatural amino acid derivatives improved the interaction of hydrophobic CBG region and dramatically increased the binding potency. Interestingly, peptidomimetics, 2,3,5, and 9, showed enhanced docking scores of -8.46 , -8.31 , -10.02 , and -9.02 kcal/mol, respectively, which are similar or better than the known penta peptide inhibitor binding score (-8.55 kcal/mol). The key amino acid interactions of the peptidomimetics exhibited excellent correlation with the reported LFG motif containing penta peptide inhibitor, as shown in Fig. 2.

The N-terminal substituent residues of peptidomimetics formed the crucial interactions with the hydrophobic binding pocket of CBG through the hydrogen bond and π - π stacking. The pentapeptide (RKLFQ) inhibitor formed hydrogen bonds with CBG residues of Gln254, Asp283, and Asp284 (Fig. 1). All the peptidomimetics interact with Gln254 except peptidomimetic 3, even though it significantly interacts with Trp217. In addition, peptidomimetic 2 showed extra significant interaction with Arg250. Moreover, some additional Asp216, Glu220, Gly251, Ile281, Thr282, Thr285, and Tyr286 amino acids are involved in hydrogen bond interactions, which were not seen in the crystallized peptide inhibitor (Fig. 2). Peptidomimetics 3 and 5 also exhibited the π - π stacking with Trp217 amino acid residue (Fig. 3). The details of atomic hydrogen bond interaction, bond distance, description of hydrogen bond donor, and acceptor groups between amino acid and peptidomimetics are shown in Table 3. Peptidomimetics make the H bond and π - π stacking with the CBG region of cyclin A. The significant flexibility of peptidomimetic substituents enhances the possible hydrogen bond interaction with target site amino acids. The nonbonded π - π stacking is one of the essential interactions formed between aromatic amino acids ring atoms [30]. Additionally, the natural p27 inhibitor exploits the H bond with conserved Trp217, Arg250, and Gln254 residues of CBG. Those H bond contacts strengthen the p27-cyclin A interaction [39]; identically, all the peptidomimetics also impersonate the crucial interactions. However, the experimental study also has provided the evidence for the fact that LFG containing synthetic lower molecular weight leads exhibited greater or equivalent selectivity than natural octapeptide inhibitor [9].

Table 1 Different chemical moieties incorporated with template LFG motif structure and best 18 peptidomimetics screened by OSIRIS property predictor tool

Compounds	R1	R2	R3	Drug score	Drug likeness
LFG motif	H	H	H	0.46	1.6
1	Methyl	H	H	0.65	0.31
2	H	2-Amino butyric acid	H	0.83	2.30
3	2-Aminobutyric acid	Methyl	H	0.83	6.53
4	2-Aminobutyric acid	H	H	0.81	5.99
5	Methyl	2-Amino butyric acid	H	0.97	6.05
6	H	Methyl	H	0.04	0.69
7	Ethyl	H	H	0.67	0.69
8	2-Aminobutyric acid	Ethyl	H	0.41	0.51
9	H	Ethyl	H	0.75	0.7
10	Methyl	Phenyl	H	0.68	5.74
11	H	2-Amino-3-phenyl-propanoic acid	H	0.62	3.52
12	2-Amino-3-phenyl-propanoic acid	H	H	0.61	2.23
13	2-Amino-3-phenylpropanoic acid	Methyl	H	0.32	3.66
14	Methyl	2-Amino-3-phenylpropanoic acid	H	0.53	3.23
15	H	Methyl	Phenyl	0.25	3.39
16	Benzyl	H	H	0.15	3.89
17	H	Benzyl	H	0.61	3.56
18	H	<i>O</i> -Chlorophenyl	H	0.35	4.15

MM-GBSA binding energy calculation

In the present study of the assessment of molecular docking with a related post-scoring approach, MM-GBSA is reported for CDK2/cyclin A complex. The results from binding free energy prediction using MM-GBSA are listed in supplementary Table S2. The structural information of CBG site has been the key in driving the design and development of peptidomimetic inhibitors. Crystallography has revealed that the CBG

Table 2 Docking score and binding free energy of best seven peptidomimetic inhibitors

Compounds	Glide docking energy	Glide energy	Glide Emodel energy	ΔG_{bind}^a
RKLFG	−8.54	−65.95	−110.18	−68.41
2	−8.45	−46.64	−93.16	−68.31
3	−8.30	−51.05	−91.42	−67.03
4	−8.09	−57.51	−84.07	−44.44
5	−10.01	−72.73	−146.70	−76.19
8	−7.59	−49.42	−72.67	−61.61
9	−9.01	−52.40	−118.13	−73.84
17	−6.59	−51.75	−65.03	−63.06

^a ΔG_{bind} is the binding free energy between receptor and peptidomimetics calculated using MM/GBSA in Prime module. All the energy values in kilocalories per mole

site of cyclin A can accommodate a number of diverse molecular frameworks, exploiting various sites of interaction. In addition, the LFG-derived peptidomimetics have been identified that could be targeted to increase specificity and potency. These results suggest that it may be possible to design pharmacologically relevant ligands that act as specific and potent inhibitors of CDK2 activity. These structures have shown that a large number of diverse compounds have the necessary ability to satisfy the hydrogen-bonding potential of the CBG site (Gln254 and Trp217) in addition to complementing the shape and chemistry of the cleft. These diverse back bones provide frameworks for the development of future inhibitors through exploitation of subsites. The calculated free energies (ΔG_{bind}) of the active CDK2/cyclin A complex with peptidomimetic inhibitors range from −44.44 to −76.19 kcal/mol. According to the energy components of the binding free energies (Table S2), the major favorable contributors to ligand binding are van der Waals (ΔG_{vdw}) and nonpolar solvation terms (ΔG_{solvSA}). The peptidomimetics 2 and 9 exhibited the ΔG_{solvSA} energy scores of −32.80 and −30.25 kcal/mol, respectively, which are better than the known penta peptide inhibitor. The peptidomimetics 5 and 9 showed the significant ΔG_{vdw} energy scores of −40.04 and −38.64 kcal/mol, respectively. The calculated ΔG_{bind} scores of peptidomimetics 5 and 9 are −76.19 and

Fig. 2 Extra precision Glide docking interaction map. **a** Peptidomimetic-2 interacts with active site of cyclin A. **b** Peptidomimetic-3 interacts with active site of cyclin A. **c** Peptidomimetic-5 interacts with active site of cyclin A. **d** Peptidomimetic-9 interacts with active site of cyclin A

−73.84 kcal/mol, respectively, which are more significant than those of known peptide inhibitors (Table S2). Hence, it is clearly apparent that ΔG_{solvsA} and ΔG_{vdw} are the driving force for ligand binding. Enhanced docking energy score values and MM-GBSA-based binding free energy confirmed hydrogen bond interactions by key residues of Trp217, Arg250, Gln254, and other amino acids. The Gln254 key residue interaction was conserved in all the peptidomimetics and known peptide inhibitor.

More precise methods have been effectively anticipated for binding free energy calculation [29]. Regrettably, these approaches are not realistically available to rank the compounds, as these are computationally expensive. In addition, there are various other approaches, such as linear interaction energy [3] and molecular mechanics Poisson–Boltzmann surface area [48]. Molecular-mechanics-based scoring methods using all atom force fields coupled MM-GBSA to model solvation have seen

an upsurge in popularity. In previous study of remarkable results obtained with this methodology when compared to docking scoring functions, the MM-GBSA procedure provided more superior correlation between calculated binding free energies and biological activity of diverse set of CDK2 inhibitors. The notable results from MM-GBSA rescoring approach could be a more attractive alternative to the free energy perturbation (FEP) and thermodynamic integration (TI) methodologies for rank ordering. It can be as accurate approach to handle more structurally dissimilar ligands and more diverse set of pharmaceutically relevant targets, and structure-based lead optimization against CDK2 at a fraction of the computational cost [52–54]. Along with them, the MM-GBSA method is using a single minimized protein–ligand complex instead of ensembles of snapshots from MD trajectories. Therefore, it became an efficient method to refine and rescore docking screening results [52, 54].

Fig. 3 **a** 2D interaction map of π – π stacking between active site of cyclin A and peptidomimetic-3. **b** 2D interaction map of π – π stacking between active site of cyclin A and peptidomimetic-5

Molecular electrostatic potential properties of the peptidomimetics

RKLFG peptide and peptidomimetics 2, 3, 4, 5, 8, 9, and 17 have shared the same binding pocket, although they showed a difference in shape and electrostatic potential (Fig. 4). The conformational analysis showed that conformational space accessed by these compounds was very different. The best pose from molecular docking study was selected to generate the electrostatic potential maps. The CBG site is able to accommodate the structurally and electrostatically diverse inhibitors by using a critical set of interactions with each ligand. The MESP was applied to interpret and predict the reactive behavior of the electrophilic and nucleophilic reactions. The MESP plays a key role in the initial step of bioactive conformation explaining the interactions between the ligand–receptor. The different values of the electrostatic potential at the surface are represented by different colors; red represents regions of most negative electrostatic potential, blue represents regions of most positive electrostatic potential, and green represents regions of moderate potential. Potential increases in the order red, orange, yellow, green, and blue. Red, green, and blue colors indicate the high accumulation of the negative charge, neutral region, and the positively charge region, respectively [40]. The negative charged region of phenylalanine and the surrounding groups play a key role in CBG binding. Thus, the electrostatic potentials of inhibitors play a significant role in the interaction with CBG and consequently influence the inhibition effect. In all peptidomimetics, the phenyl ring-associated N–H donor group significantly

influences the interaction. The MESP plotted for peptidomimetics has showed the most electronegative potential region (red color) over the oxygen atom in the peptide bonds. The most interesting thing is that the oxygen atom in the peptide bond which shows the higher negative charge was orientated adjacent to the Gln254 and Trp217 to make a strongest hydrogen bond interaction and π – π stacking interaction, respectively. All those interactions are very crucial to inhibit cyclin recruitment.

ADME property analysis

Prediction of pharmacokinetic property is an essential task to determine the pharmacological potential of leads. The predicted pharmacokinetic parameters also sturdily supported that the newly designed peptidomimetics have small molecule drug-like properties in many aspects and predicted values shown in Table 4. The unknown derivatives showed lower molecular weight ranging from 374.57 to 645.80. Volume parameter is explained as total solvent-accessible volume in cubic angstroms and exhibited range from 1,305.51 to 2,060.52. The total SASA ranged from 703.25 to 1,049.03 and QPlogPw coefficient ranges from 20.76 to 33.52, which is within the desired values (footnote of Table 3). QPpolrz and PSA values ranged from 33.45 to 45.83 and from 103.43 to 162.25, respectively. Overall, the predicted pharmacokinetic value falls under the defined range. The peptidomimetics which had the best docking score, free energy binding score, and electrostatic charge distribution also exhibited the desired

Table 3 Description of hydrogen bond formed between the crucial amino acids and best scored peptidomimetics inhibitors

Compounds	H bonds	Atomic interaction	H bond donor	H bond acceptor	Distance (Å)
RKLFG	4	Gln254(O)OE1-H	N-H (Pep) ^a	C=O (Pr) ^b	1.98
		Asp283(O)OD2-H	N-H (Pep)	C=O (Pr)	2.23
		Asp283(O)OD2-H	N-H (Pep)	C=O (Pr)	1.95
		Asp284(O)OD2-H	N-H (Pep)	C=O (Pr)	1.53
2	4	Arg250(O)O-H	N-H (Pep)	C=O (Pr)	2.10
		Gln254(H)H-O	N-H (Pr)	C=O (Pep)	1.96
		Gln254(O)OE1-H	N-H (Pep)	C=O (Pr)	1.65
		Thr282(O)O-H	N-H (Pep)	C=O (Pr)	2.09
3	3	Gly251(O)O-H	N-H (Pep)	C=O (Pr)	1.97
		Asp283(O)OD2-H	N-H (Pep)	C=O (Pr)	2.36
		Tyr286(H)HG1-H	N-H (Pr)	C=O (Pep)	1.76
4	4	Asp216(O)OD2-H	N-H (Pep)	C=O (Pr)	2.00
		Glu220(O)OE2-H	N-H (Pep)	C=O (Pr)	1.66
		Gln254(H)HE22-O	N-H (Pr)	C=O (Pep)	1.90
		Asp283(O)OD2-H	N-H (Pep)	C=O (Pr)	2.04
5	4	Gln254O(O)E1-H	N-H (Pep)	C=O (Pr)	2.27
		Gln254O(O)E1-H Asp283(O)OD2-H	N-H (Pep) N-H (Pep)	C=O (Pr) C=O (Pr)	1.76 1.92
		Asp284(O)OD2-H	N-H (Pep)	C=O (Pr)	1.65
8	2	Gln254O(O)E1-H	N-H (Pep)	C=O (Pr)	2.09
		Gln254(C)CA-O	N-H (Pr)	C=O (L)	1.71
9	4	Asp216(O)D2-H	N-H (Pep)	C=O (Pr)	1.79
		Glu220(O)OE2-H	N-H (Pep)	C=O (Pr)	1.94
		Gln254O(O)E1-H	N-H (Pep)	C=O (Pr)	2.35
		Ile281(O)O-H	N-H (Pep)	C=O (Pr)	1.97
17	4	Gln254(H)HE22-O	N-H (Pr)	C=O (Pep)	2.11
		Gln254(H)HE22-H	N-H (Pep)	C=O (Pr)	2.00
		Glu220(O)OE2-H	N-H (Pep)	C=O (Pr)	1.87
		Asp216(O)D2-H	N-H (Pep)	C=O (Pr)	1.61

^a Pep-hydrogen bond forming atoms of peptidomimetics^b Pr-hydrogen bond forming atoms of protein residues

range of the pharmacokinetic properties than the known peptide inhibitor.

Molecular dynamics simulation analysis

The significant docking poses of peptidomimetic complexes 2, 3, 5, and 9 and known peptide complex were taken for MD simulation analysis. The SHAKE algorithm employing simulation analysis offered a means of confirmation of the above results for the peptidomimetics and protein complex when simulated at 10-ns duration. The duration of MD simulation was limited for 10 ns based on the revelations of a detailed study on refinement of protein structure using MD by Raval et al. [35]. Based on their study, it is suggested that protein structure drifts away from the native structure under long MD simulations and hence limiting time period of simulation. Further, it was observed that 3-ns MD simulation was enough to reach one stable local minima and the structure

corresponding to this time period was able to discriminate binding affinity of homology models of protein structure [32, 50]. Moreover, we observed that within 10 ns, the CDK2/cyclin A-peptidomimetic complex had reached stable local minima by 4-ns simulation.

The ARMED value of crystallized protein complex has been calculated as 2.39 Å. The peptidomimetics 2, 3, 5, and 9 exhibited an ARMED of 2.30, 2.25, 1.79, and 2.37 Å, respectively. The stability of crystal structure complex and peptidomimetics 2, 3, 5, and 9 during 10-ns MD simulation was illustrated in Fig. 5a–e, which shows that the CDK2 complex association could not undergo much fluctuation during the simulation. The peptidomimetic complexes 2, 3, 5, and 9 have been stabilized throughout the simulation with respect to their hydrogen bond interaction. The descriptive statistical analyses of RMSD of the peptidomimetic in 10-ns MD simulation from the initial structures are reported in Table 5. The temperature and pressure exploited by the system were ranged

Fig. 4 The molecular electrostatic potential map of known peptide (RKLFG) inhibitor and chosen seven peptidomimetics (compounds 2, 3, 4, 5, 8, 9, and 17)

Table 4 ADME property evaluation of the small molecule like peptidomimetic inhibitors

Compounds	Molecular weight ^a	SASA ^b	Volume ^c	QPlogPw ^d	QPpolrz ^e	PSA ^f
RKLFG	645.80	1,049.03	2,060.52	33.52	33.45	162.25
2	405.0	820.33	1,473.42	28.09	36.43	151.50
3	419.61	730.55	1,393.34	27.66	35.85	140.74
4	433.63	754.19	1,445.04	26.48	40.94	140.88
5	419.63	749.51	1,440.60	26.28	38.69	142.42
8	374.57	814.49	1,405.54	21.06	35.81	112.02
9	394.98	703.25	1,305.51	21.25	35.43	103.43
17	456.71	858.64	1,603.81	20.76	45.83	108.87

^a Molecular weight (acceptable range 130.0–725.0)

^b Total solvent accessible surface are (SASA) (acceptable range 300.0–1,000.0)

^c Volume (acceptable range 500.0–2,000.0)

^d Predicted water/gas partition coefficient (QPlogPw) (acceptable range 4.0–45.0)

^e Polarizability in cubic angstroms (QPpolrz) (acceptable range 13.0–70.0)

^f van der Waals surface area of polar nitrogen and oxygen atoms (PSA) (acceptable range 7.0–200.00)

Fig. 5 **a** RMSD plot of native peptide inhibitor and chosen peptidomimetics bound with CDK2/cyclin A complex for the timescale of 10-ns simulation. **b** Comparative RMSD plot of native peptide inhibitor and peptidomimetic-2. **c** Comparative RMSD plot of native peptide inhibitor and peptidomimetics-3. **d** Comparative RMSD plot of native peptide inhibitor and peptidomimetics-5. **e** Comparative RMSD plot of native peptide inhibitor and peptidomimetics-9

from 298.61 to 298.83 K and 0.78 to 0.83 respectively. The thermodynamic appliances of temperature and pressure utilized by the system are described in Table S3. The initial energy level and minimized energy level at the end of simulation were ranged from −211.32 to −279.12 and −532.17 to −642.75 kcal/mol, respectively. The peptidomimetic complexes 2 and 5 exhibited the better minimum energy level than known peptide inhibitor. The energy level description of the simulated complex was depicted in Table S3. The minimum standard deviations of peptidomimetics 2, 3, 5, and 9 are 0.251, 0.247, 0.252, and 0.286 with confidence level (95.0 %) 0.003, 0.003, 0.003, and

0.004, respectively. The entire peptidomimetic complex was deviated less during the dynamic simulation than known peptide–protein complex. The active site residual interaction of Gln254 was observed throughout the simulation process; peptidomimetic 2 interacts with Arg250 to a greater extent. Usually, the guanidium group, present in the side chain of arginine residue, forms the hydrogen bond with negatively charged groups and provides stronger binding affinity [8]. All those significant interactions were facilitated to retain the integrity of CDK2/cyclin A-peptidomimetics complex association than known peptide complex. Moreover, the association of

Table 5 Descriptive analyses of RMSD of the peptidomimetics in 10-ns simulation

Complex	Mean	Standard deviation	Standard error	Minimum	Maximum	Confidence level (95 %)
RKLFG	2.42	0.34	0.007	0.99	2.78	0.005
2	2.30	0.25	0.005	1.01	2.58	0.003
3	2.25	0.24	0.005	0.91	2.66	0.003
5	2.39	0.25	0.005	0.92	2.62	0.003
9	2.37	0.28	0.006	1.05	2.70	0.004

peptidomimetics and CDK2 complex can be restored through the electrostatic and other nonbonded interactions.

Conclusion

Functionally activated CDK2/cyclin A complex has been validated as an interesting therapeutic target to develop the efficient antineoplastic drug, based on the cell cycle arrest. The REPLACE methodology has been used to design the combination of both peptide and pharmacologically important unnatural amino acid derivatives to get the functionally active peptidomimetics. The natural p27 inhibitor separately has binding site for both CDK2 and cyclin A. The p27-derived LFG motif containing peptidomimetic targets only the cyclin-binding site not the ATP-binding site of CDK2. These peptidomimetics could enhance the stabilization of CDK2 complex to prevent further phosphorylation and hamper the cell proliferating molecular mechanism. The drug score and drug likeness value prediction revealed that the peptidomimetics do not have the risk of tumorigenicity and mutagenicity effects. The experimental analysis assured that the unnatural amino acid group associated with peptide sequence could possess the promising activity. Further, the screened peptidomimetics were evaluated by means of docking simulation and MM/GBSA calculation and MD simulation analysis of protein complex. The ESP analysis has shown that the phenyl aromatic ring and its surrounded electronegative charged region are essential for hydrogen bonding with CBG residues. In addition, the best scored peptidomimetics exhibited good ADME/T properties. Further, the final peptidomimetics obtained from our results would be helpful in the designing of novel anticancer drugs.

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Conflict of interest No conflict of interest is there to declare.

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