Molecular Basis of Differential Selectivity of Cyclobutyl-Substituted Imidazole Inhibitors against CDKs: Insights for Rational Drug Design

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Abstract
Cyclin-dependent kinases (CDKs) belong to the CMGC subfamily of protein kinases and play crucial roles in eukaryotic cell division cycle. At least seven different CDKs have been reported to be implicated in the cell cycle regulation in vertebrates. These CDKs are highly homologous and contain a conserved catalytic core. This makes the design of inhibitors specific for a particular CDK difficult. There is, however, growing need for CDK5 specific inhibitors to treat various neurodegenerative diseases. Recently, cis-substituted cyclobutyl-4-aminoimidazole inhibitors have been identified as potent CDK5 inhibitors that gave up to 30-fold selectivity over CDK2. Available IC50 values also indicate a higher potency of this class of inhibitors over commercially available drugs, such as roscovitine. To understand the molecular basis of higher potency and selectivity of these inhibitors, here, we present molecular dynamics simulation results of CDK5/p25 and CDK2/CyclinE complexed with a series of cyclobutyl-substituted imidazole inhibitors and roscovitine. The atomic details of the stereospecificity and selectivity of these inhibitors are obtained from energetics and binding characteristics to the CDK binding pocket. The study not only complements the experimental findings, but also provides a wealth of detailed information that could help the structure-based drug designing processes.

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Introduction

Cyclin-dependent kinases (CDKs) play crucial roles in eukaryotic cell division cycle. They belong to the CMGC subfamily of protein kinases and assist the γ-phosphate transfer from ATP to peptide substrates [1], [2]. At least seven different CDKs have been reported to be implicated in the cell cycle regulation in vertebrates. Among these, CDK2 functions during the progression of cell cycle from the G1 to S phase [3], [4]. CDK2, like most of the other CDKs, follows a two-step process to become fully functional: (i) the association with the regulatory subunit – cyclin A or cyclin E, (ii) phosphorylation of residue Thr160 located in the so-called activation loop [5], [6]. However, certain CDKs, e.g. CDK5 do not follow this mode of activation. The activity of CDK5 is restricted to nervous system by the localization of its activators p25/p35/p39, the binding of which makes CDK5 fully active without the subsequent requirement of phosphorylation of the activation loop residue [7], [8]. While aberrant activity of CDK2 has been identified in a number of diseases including cancer, embryonic lethality, male sterility etc., the deregulation of CDK5 causes serious neurodegenerative disorders, e.g. Alzheimer’s disease, lateral sclerosis, stroke etc [9–11].

CDKs are highly homologous and contain a conserved catalytic core. For example, CDK2 and CDK5 share a sequence homology of 60%, with the substrate binding pocket alone showing nearly 93% sequence similarity [8], [12]. The 3D structures of CDKs are mainly composed of two domains, the N and the C-terminal domains (Figure 1) [13], [14]. The catalytic cleft that binds ATP is located at the interface of these two domains. A glycine rich loop, commonly known as G-loop, lies above the ATP binding pocket and is conserved in many kinases. The primary function of this loop is to align the substrate and ATP correctly, for a smooth transfer of the γ-phosphate [15–17]. The N-terminal domain is primarily composed of a β-sheet, containing five antiparallel β-strands, and one α-helix. This helix with the “P5xA5xRE” motif is a signature of this class of proteins and constitutes the main point of interaction with activator proteins. The loop which precedes the PsxAsxRE helix, known as the 40s loop, also interacts with the activator protein. The C-terminal domain is predominantly α-helical and contains the so-called T-loop, the residue Thr160 of which becomes phosphorylated by CAK for CDK2 activation [13–18]. However, CAK does not phosphorylate CDK5 on the analogous Ser159 [8], [18]. The catalytic pockets of CDK2 and CDK5 are primarily comprised of 20 residues, three of which differ from CDK2 to CDK5 as follows: Lys83 to Cys83, His84 to Asp84 and Asp145 to Asn144 [12]. The respective partner proteins, Cyclin E and p25, though have less sequence homology, are structurally similar with both possessing the typical cyclin box fold.

Due to their key regulatory roles, CDKs have become important pharmaceutical targets for inhibitor design [9], [19].
There is a particular demand for CDK5 specific inhibitors to treat various neurodegenerative diseases [20]. However, it is difficult to design the inhibitor specific to a particular CDK due to the structural homology among number of CDKs [4]. Very recently, Helal et al. have identified novel cis-substituted cyclobutyl-4-aminoimidazole inhibitors that gave improved enzyme and cellular potency against CDK5/p25 with up to 30-fold selectivity over CDK2/Cyclin E [21]. To understand the molecular basis of higher potency of these inhibitors, here we carry out all-atom molecular dynamics simulations of active CDK5/p25 and CDK2/CyclinE bound to a series of cyclobutyl-substituted imidazole inhibitors. The atomic details of the stereospecificity and selectivity of these inhibitors are obtained from energetics and binding characteristics to the CDKs.

### Materials and Methods

#### Simulation Details

The initial structures of inhibitor-bound CDK2/Cyclin E and CDK5/p25 complexes were obtained by docking the inhibitors in the available crystal structures of active CDK2 (PDB ID: 1W98) and CDK5 (PDB ID: 3O0G) [22], [23]. We considered three different imidazole inhibitors in this study: N-\{1-(cis-3-hydroxycyclobutyl)-1H-imidazol-4-yl\}-2-(4-methoxyphenyl)acetamide, N-\{1-(trans-3-hydroxy cyclobutyl)-1H-imidazol-4-yl\}-2-(4-methoxyphenyl)acetamide, and N-\{1-(cis-3-(acetylamino)cyclobutyl)-1H-imidazol-4-yl\}-2-(4-methoxyphenyl)acetamide. Hereafter these molecules are abbreviated as cis-OH, trans-OH, and cis-N-acetyl, respectively, and their chemical structures are included in Fig. 1. In vivo and in vitro studies have shown distinctly different inhibitory effects of these molecules on CDK2 and CDK5 [21]. Table 1 lists the experimentally determined IC50 values of these inhibitors.

In this study, we examined the interactions of the inhibitor bound active CDK2/Cyclin E and CDK5/p25 complexes with the activators, cyclin E and p25 by superposing the inhibitors without changing the inhibitor positions. For this purpose, the crystal structure coordinates of cis-OH and cis-N-acetyl were extracted from their bound complex with CDK2 (PDB ID: 3IGG and 3IG7, respectively, [21]) and were docked manually to CDK2/Cyclin E and CDK5/p25 complexes by superposing the CDK structures without changing the inhibitor coordinates. A similar docking protocol has been adopted earlier to study the protein-ligand interactions and was validated by comparing with the available crystal structures [24–26]. The corresponding trans-isomers were created and the structure were optimized by using Gaussian 03 program using B3LYP functional and 6–311+G* basis set, before docking to the CDKs.

### Table 1. Reported IC50 values of the selected inhibitors in nM.

| Inhibitor | CDK2/CyclinE | CDK5/p25 |
|-----------|--------------|----------|
| cis-OH    | 66.5         | 93       |
| trans-OH  | 763          | 1090     |
| cis-N-acetyl | 63        | 9        |
| roscovitine | 700         | 160      |

Data are collected from Refs. 21,42.

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CDKs [27]. The atom-centered RESP charges for all inhibitors were determined via fits to the electrostatic potentials obtained from the calculated wave functions. The missing interaction parameters in the inhibitors were generated using antechamber tools in Amber [28]. As controls, the crystal structures of roscovitine-bound active CDK2 and CDK5 complexes were also simulated (respective PDB IDs are: 3DDQ, 1UNL) [29], [30].

For simulations, the hydrogens for heavy atoms were added by leap program in Amber 11.0 package [28]. Added hydrogens were energy minimized for 1000 steps using the conjugate gradient and another 1000 steps using the steepest descent algorithm. The protonation states of histidines - HID or HIE - were determined by another 1000 steps using the steepest descent algorithm. The converged structures were further simulated to generate the 50 ns production data. The two variants CDK2:L83C and CDK2:H104D were also simulated for 50 ns following the same protocol. For control roscovitine-bound CDK simulations, the production data was generated for 20 ns each. Thus a total of ten simulations were performed in the study (Table S1). The long-range electrostatic interactions were treated by using Particle-Mesh Ewald sum [33] and SHAKE was used to constrain all bonds involving hydrogen atoms. Amber11 molecular dynamics simulation package with Amber ff99SB force field was used for all simulations [34].

Free Energy Calculations

Binding free energies ($\Delta G_{\text{bind}}$) of the inhibitors were calculated by Molecular Mechanics Poisson Boltzmann Surface Area (MMPBSA) approach [35]. For every system, the block averaged $\Delta G$ values were calculated from five independent windows of 2 ns (i.e. last 10 ns trajectory). The binding free energy of an inhibitor is obtained by taking the difference between the free energies of the protein-inhibitor complex ($G_{\text{complex}}$), the unbound protein ($G_{\text{receptor}}$), and the inhibitor ($G_{\text{ligand}}$):

$$\Delta G_{\text{bind}} = G_{\text{complex}} - G_{\text{receptor}} - G_{\text{ligand}}$$

The $\Delta G_{\text{bind}}$ values were computed using the scripts available with AMBER 11 programme [28], where $\Delta G_{\text{bind}}$ is calculated from the changes in the molecular mechanical gas phase energy ($\Delta E_{\text{MM}}$), entropic contribution, and solvation free energy due to the binding of ligand to receptor for the formation of complex:

$$\Delta G_{\text{bind}} \approx \Delta E_{\text{MM}} + \Delta G_{\text{solv}}$$

The MM gas phase energy term ($\Delta E_{\text{MM}}$) takes care of the electrostatic and van der Waal’s interactions between protein and ligand. The $\Delta G_{\text{solv}}$ is estimated by solving the linearised Poisson Boltzmann equation for each of the three states ($\Delta G_{\text{polar}}$) and adding an empirical term for hydrophobic contributions to it ($\Delta G_{\text{nonpolar}}$). The hydrophobic contribution is calculated from the solvent accessible surface area. It is customary to neglect the entropic contribution ($\Delta S$), as the calculations involve binding of similar type of ligands to the receptor. Hence, the computed values will be termed as the relative binding free energies. The experimental free energy of binding values ($\Delta G_{\text{exp}}$) was determined from the IC$_{50}$ values by using the equation: $\Delta G = -RT\ln IC_{50}$ [36], [37].

Results and Discussion

Binding of cis- and trans-OH to Active CDK2 and CDK5

To test the stability of the systems, we monitored the root mean squared deviations (RMSD) of the inhibitor-bound CDK complexes from the starting structures. The convergence of RMSD values at approximately 5 ns of the simulation time indicates that the systems were well equilibrated and have attained stability (Fig. S1). Interestingly, the cis-OH bound CDK complexes were found to exhibit significantly lower RMSDs than the corresponding trans complexes. RMSDs of the inhibitors alone in the complexes also
show a similar trend (Fig. S2), implying a better binding of cis-OH inhibitor to both CDK2 and CDK5 binding pockets than the trans-OH inhibitor.

The analyses of local fluctuations of the CDK residues also suggest a stronger protein-inhibitor interaction in cis-OH, as exemplified by the lower B-factor values of the functionally relevant loops and helices (Fig. 2). For example, the G-loop and αD helix that are known to play crucial roles in ligand binding, show considerably reduced fluctuations in cis-OH-CDK complexes. Most of the other important regions of CDK, such as 40s loop, PSTAIRE helix, T-loop, and residues around substrate binding pocket also show reduced fluctuations in cis-OH-CDK2 complex. A similar trend was noticed for cis-OH-CDK5 complex. The modulated fluctuations of PSTAIRE/PSLAARE helix and 70s loop, which lie at CDK-cyclin/p25 interfaces, imply that the binding of inhibitors to substrate-binding pocket can also affect the binding of CDKs to the activators, allosterically [38]. Interestingly, all the inhibitor-bound complexes displayed high fluctuations around the conserved CMGC kinase domain.

To obtain a better understanding of the interactions, we compared the average structures of the cis- and trans-OH bound CDK2 and CDK5 complexes. This is shown in Fig. 3. For clarity,
shown. Color scheme is similar to Fig. 3.

Figure 5. Average structures of the cis-N-acetyl bound CDK complexes. For clarity, only the inhibitors and the adjacent protein residues are shown. (A) cis-N-acetyl bound CDK2, (B) cis-N-acetyl bound CDK5. Possible modes of interactions are indicated by dotted lines with average distance s

Table 2. Free energy of binding of cis- and trans-OH inhibitors to CDKs from MMPBSA calculations.

| Complex     | ΔG | ΔAGcis-trans | ΔAGcis-trans (expt) |
|-------------|----|--------------|---------------------|
| cis-OH-CDK2 | −20.21 ± 1.05 | — | — |
| trans-OH-CDK2 | −18.26 ± 1.03 | −1.95 | −1.46 |
| cis-OH-CDK5 | −20.97 ± 2.6 | — | — |
| trans-OH-CDK5 | −19.63 ± 1.67 | −1.34 | −1.45 |

All energy values are in kcal/mol and ΔAGcis-trans = ΔGcis − ΔGtrans

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The binding of inhibitors to CDKs was further amplified by calculating their average interaction energies over the final 10 ns simulation trajectory. The total interaction energy of cis-OH was found to be much greater than trans-OH in both CDK2 and CDK5 complexes (Fig. 4). Individual interactions of the protein residues with inhibitor moieties can explain such a difference. For example, the hinge region residues Leu83 in CDK2 and Cys83 in CDK5 interact stronger with imidazole ring of cis-OH than that of the trans-OH inhibitor. Adjacent residues His144 bound to both complexes (Fig. 4). Individual interactions of the protein residues with inhibitor moieties can explain such a difference. For example, the hinge region residues Leu83 in CDK2 and Cys83 in CDK5 interact stronger with imidazole ring of cis-OH than that of the trans-OH inhibitor. Adjacent residues His144 in CDK2 and F82, D86 and K89 in CDK5 also show larger interaction energies with cis-OH. The diminished hydrophobic interaction of trans-OH with F80 is also reflected in the lower interaction energy values. For CDK2-inhibitor complex, the most significant difference in energy was observed due to Asp145, which lay deep inside the substrate binding pocket (−13.08 kcal/mol in cis-OH vs. −3.01 kcal/mol in trans-OH). The neighbouring A144 also displayed considerable lowering in interaction with trans-OH. Leu83 also contributes differently by about 2 kcal/mol in the two complexes (−9.91 kcal/mol in cis- versus −8.13 kcal/mol in trans-OH). The interaction of hydrophobic Phe80 is also found to be more favourable with cis-OH. The contribution of polar Lys33 is repulsive for both the inhibitors, while bound to CDK2. In case of CDK5, however, Lys33 involves in favourable interactions with both the inhibitors. But, it interacts very differently with cis- and trans-OH (−6.88 kcal/mol in cis- and −2.13 kcal/mol in trans-OH) and contributes most significantly toward the difference in total interaction energy in CDK5. Residue Asn144, the analogue of Asp145 in CDK2, contributes negligibly toward inhibitor binding in CDK5. The residues Phe80, Glu81, Phe82 and Cys83 located in the hinge region also showed increased interaction energy with cis-OH. In brief, the analysis suggests that the interaction of cis-OH inhibitor is stronger than trans-OH in both CDK2 and CDK5 and the main contribution toward inhibitor binding comes from Asp145 in CDK2 and Lys33 in CDK5. Time evolutions of the interaction distances also show that the dynamics of these systems differ significantly and the interactions persist longer for cis-OH than the trans-OH inhibitor (Fig. S4, S5).

To get a quantitative comparison of the binding strengths, we computed the free energy of binding of the inhibitors to CDK2 and CDK5 from the simulation-generated trajectories via MMPBSA method. Table 2 lists the binding free energies of cis-
OH and trans-OH, complexed with active CDKs. The binding of cis-OH was found to be stronger in both CDK2/cyclin E and CDK5/p25 complexes and irrespective of the method of calculation. The computed $\Delta G_{\text{binding}}$ are in very good agreement with experimental data [21].

Binding of cis-N-acetyl to Active CDK2 and CDK5

The N-acetyl analogue of cis-OH, cis-N-acetyl has shown a ten-fold improved potency over cis-OH against CDK5/p25 in vitro (IC$_{50}$ values: 9 vs. 93 nM; Table 1). Moreover, it showed a seven-fold better selectivity for CDK5 over CDK2 (IC$_{50}$ values: 9 vs. 63 nM). To understand these differences, we carried out comparative studies of cis-OH and cis-N-acetyl bound active CDK2 and CDK5 complexes. The N-acetyl bound CDK complexes were simulated for 50 ns and the stability were assured from the convergence of energy components and RMSDs from the crystal structures (data not shown). The comparison of local fluctuation of the protein residues implies a stronger protein-inhibitor interaction in cis-N-acetyl bound CDKs, particularly in CDK5 complex (Fig. S6,S7).

To obtain a better understanding of improved potency and selectivity of cis-N-acetyl inhibitor against CDK5/p25 complex, we compared the average structures of the inhibitor bound CDK complexes. This is shown in Fig. 5. For clarity, only the inhibitors and the adjacent protein residues that involve in direct interactions are shown. Most of the interactions present in cis-OH-CDK complexes were seen to be retained in cis-N-acetyl bound CDKs, particularly in CDK5 complex (Fig. S6,S7).

To quantify the interactions, the inhibitor-protein interaction energies are calculated and shown in Figs. 6 and 7. A marginal increase in total interaction was observed for N-acetyl-CDK2 complex compared to the corresponding cis-OH complex ($-252.08$ kcal/mol vs. $-251.11$ kcal/mol). Residue-level analysis shows a marked decrease in interaction of N-acetyl inhibitor with Asp145 with respect to cis-OH inhibitor. The adjacent Ala144 also shows a weaker interaction with N-acetyl inhibitor. However, the repulsive interaction of Lys33 with cis-OH reverts to a favourable interaction with cis-N-acetyl, as shown in Fig. 6a. This along with slightly more favourable...
interactions of Ile10 and hinge region residues Phe80, Glu81 etc. makes cis-N-acetyl as equally potent as cis-OH in inhibiting CDK2. These interactions seem to persist over the entire production phase of the simulations, as shown in the time evolution of a few representative interaction distances (Fig. S10). The cis-N-acetyl bound CDK5 complex, however, shows a large increase in interaction energy by about 10 kcal/mol, compared to the corresponding cis-OH complex (Fig. 6b). Residue-level analysis shows that Lys33 makes almost half of the total difference in energy. The allosteric residue, Lys89 also appears to contribute significantly in the energy difference. Even the hinge region residues, particularly Asp84 and Gln85 contributed more favourably towards the interaction with N-acetyl inhibitor. As Fig. 7 shows, the better selectivity of N-acetyl inhibitor for CDK5 over CDK2 mainly stems from more favourable Lys33 interaction. Additionally, the variant residues Cys83, Asp84 and neighbouring Gln85 help better inhibitor interaction in CDK5. Another variant Asn144 also appears to help inhibitor-CDK5 interactions. Importantly, the interaction of allosteric Lys89 becomes favourable in CDK5 (Fig. S9). In a nutshell, the interaction of residue Lys33 with acetyl group plays the major role in improved potency of cis-N-acetyl inhibitor over cis-OH. The selectivity of cis-N-acetyl for CDK5 presumably comes from the variant residues Cys83, Asp84, Asn144, which modulate the interaction network by subtly restructuring the binding pocket, as a result of which residues Lys33, Lys89 etc. involve in stronger interactions.

To get a better estimate of the binding strengths, we computed the free energy of binding of cis-N-acetyl to CDK2 and CDK5 from the simulation-generated trajectories via MMPBSA method (Table 3). The binding energy values go parallel with the higher potency of cis-N-acetyl inhibitor over cis-OH against CDK5/p25, even though these two inhibitors do not show much difference against CDK2/cyclin E complex. The ΔG\textsubscript{Nacetyl-OH} was −2.0 kcal/mol and −0.31 kcal/mol for CDK5 and CDK2, which match favourably with the experimental data. The selectivity of N-acetyl inhibitor for CDK5 complex is also evident from the table, where ΔG\textsubscript{CDK5-CDK2} was computed to be −2.45 kcal/mol from MMPBSA calculation.

### Effect of Mutations
To elucidate the physical characteristics of the binding pocket, we have also calculated the solvent accessible surface area (SASA) of the pocket (Table 4, Fig. S11) and mapped its electrostatic potential (Fig. 8; SASA is calculated using naccess program [40] and the average SASA values in Table 4 are obtained from its time evolution in Fig. S11. The electrostatic potential map is obtained from the average structures of the cis-N-acetyl bound CDK complexes using DelPhi program [41]. The calculated SASA values indicate that the binding pocket of CDK5 is smaller than CDK2. The electrostatic potential map shows that the pocket is more electropositive in CDK5 complex, particularly deep inside the cavity. This is due to the Asp145/Asn144 variant and inward movement of allosteric Lys89 (see Fig. S8). Recall that the N-acetyl group of the inhibitor contains many electronegative atoms, which thus find a suitable environment to remain stable. This can also explain why cis-OH with a smaller electronegative –OH head-group binds relatively weakly to the pocket than N-acetyl.

To check if the other two CDK2 variants contribute to pocket volume, even though they reside exterior to the binding pocket, we created the mutants, CDK2:L83C and CDK2:H84D. These complexes were also simulated for 50 ns after equilibration. The computed volumes and electrostatic potential map of these mutants are also included in Table 4 and Fig. 8. As evident from the table and potential map, both mutations reduce the pocket volume and induce similar changes to the electrostatic potential as seen in CDK5 complex. Taken together, the inhibitors bind relatively strongly to CDK5 binding pocket due to the smaller volume and electropositive nature of the binding pocket. The atomic-level details on CDK-inhibitor interactions presented here could help the design of more specific CDK inhibitors.

### Binding of Roscovitine to Active CDK2 and CDK5
The binding of N-acetyl inhibitor to CDKs is also compared with the binding of commercially available CDK inhibitor, roscovitine [42]. As table 1 indicates, the inhibitory effect of N-acetyl on active CDK2 and CDK5 is much greater than roscovitine. To understand this differential inhibition, a compar-

### Table 3. Free energy of binding of cis-OH and cis-N-acetyl inhibitors to CDKs from MMPBSA calculations.

| Complex         | ΔG  | ΔΔG\textsubscript{Nacetyl-OH} | ΔΔG\textsubscript{Nacetyl-OH} (expt) |
|-----------------|-----|-----------------------------|-------------------------------------|
| cis-OH-CDK2     | −20.21 ± 1.05 | −0.31 | −0.03 |
| cis-N-acetyl-CDK2 | −20.52 ± 1.07 | 0.31 | −0.03 |
| cis-OH-CDK5     | −20.97 ± 2.6  | 0.31 | 0.03 |
| cis-N-acetyl-CDK5 | −22.97 ± 3.00 | 2.00 | 1.41 |

All energy values are in kcal/mol and ΔΔG\textsubscript{Nacetyl-OH} = ΔG\textsubscript{Nacetyl} − ΔG\textsubscript{OH}. doi:10.1371/journal.pone.0073836.t003

### Table 4. Average solvent accessible surface area (SASA) of the substrate binding pocket of CDKs.

| Protein complex | SASA (Å\(^2\)) | Std. dev. |
|-----------------|-----------------|-----------|
| CDK2 wild type  | 5240.20         | 92.63     |
| CDK5 wild type  | 4754.80         | 170.74    |
| CDK2:L83C variant | 5149.64   | 85.81     |
| CDK2:H84D variant | 4876.07   | 94.72     |

SASA is calculated by removing the cis-N-acetyl inhibitor from the pocket and rolling a probe of radius 1.4 Å across the pocket. doi:10.1371/journal.pone.0073836.t004
ative analysis of their mode of binding to CDKs has been carried
out from the 20 ns simulation trajectory of each roscovitine-bound
complex. Fig. 9 presents the time-averaged structures of N-acetyl
and roscovitine bound CDK complexes, superimposed on each
other. Clearly, the peripheral moieties of both N-acetyl and
roscovitine make similar contacts with CDKs. For example,
Leu83/Cys83 interact with imidazole ring of N-acetyl and purine
ring of roscovitine with equal strength, as exemplified by their
similar H-bonding distances in Fig. 9. The terminal phenyl moiety
involves in hydrophobic interaction with Ile10 in both inhibitor
bound complexes. However, the characteristic interactions of N-
acetyl with Lys33 and Asp145/Asn144 were completely missing
for roscovitine (Fig. 9). The time evolution of such an interaction
distance between Lys33 and the closest inhibitor atom shows that
roscovitine could never reach to the base of the deep binding
cavity of CDKs (Fig. S12). Moreover, the stacking interaction of
cyclobutyl ring with Phe80 was also absent in roscovitine bound
CDK complexes.

The calculation of residue-level interaction energies reflects a
similar trend (Fig. 10). Even though a few neighbouring residues,
such as Ile10, Val18, Glu81 and Asp86 have similar or marginally
higher interaction with roscovitine, most of the other pocket
residues contribute more toward N-acetyl interaction. Major
contributor toward the larger binding strength of N-acetyl was
Lys33, followed by hinge region residues Leu83/Cys83, His84/
Asp84, Gln85. The hydrophobic Phe80 and the CDK2/CDK5
variant residue Asp145/Asn144 also contribute more favourably
toward the N-acetyl inhibitor. Consequently, the total interaction
energy of N-acetyl with CDKs turns out to be much greater than
roscovitine. The decomposition of total energy into electrostatic
and van der Waal components indicates that N-acetyl fared over
roscovitine through the electrostatic interaction (Table 5). The six
fold increase of electrostatic component for the former mainly
stems from the polar interaction of its N-acetyl group with Lys33,
Asp145/Asn144, which reside deep into the CDK binding pocket.
Hence, the future strategy for designing more potent and specific
CDK inhibitors might incorporate polar functional groups that
can reach deep into the CDK binding pocket through a
hydrophobic linker, such as the cyclobutyl ring here.

Conclusions
Cis-substituted cyclobutyl-4-aminoimidazole inhibitors have
been identified as novel CDK5 inhibitors that gave improved
enzyme and cellular potency with many fold selectivity over
CDK2. The molecular basis of higher potency and selectivity of
this class of inhibitors over commercially available drugs is also
unknown. Here we present atomic-level details of the interactions
of some of these CDK-inhibitor complexes to understand these
differences. Results suggest that the aminoimidazole inhibitors
can reach deep into the substrate-binding pocket through the linker
cyclobutyl group. Moreover, they involve in strong electrostatic
interactions with CDK residues Lys33, Asp145/Asn144 that
reside at the base of the cavity. The better selectivity of these
inhibitors for CDK5 mainly stems from the variant residues
Cys83, Asp84, Asn144, which modulate the interaction network
by subtly restructuring the binding pocket and realigning the
allosteric residues, Lys33, Lys89. This turns the CDK5 pocket
more electropositive and smaller in volume for more favourable
interactions with molecules carrying multiple electronegative sites.
The results are validated by comparing the computed free energy of binding of the imidazole inhibitors to CDKs with the available experimental values. Moreover, the mode of binding of the commercially available drug, roscovitine to CDKs in the simulated complexes is also compared to the available crystal structure. An excellent match has been observed in both instances, which tempted us to conclude that the future strategy for designing more potent and specific CDK inhibitors could involve the incorporation of polar functional groups at the tip of the inhibitor molecules, which can go deep into the binding pocket through a hydrophobic linker.

Supporting Information

Figure S1 The Cα root mean squared deviations (RMSD) of CDKs bound to cis- and trans-OH inhibitors. Time evolution is shown for final 35 ns for cis-OH-CDK2 (black), trans-OH-CDK2 (red), cis-OH-CDK5 (green), and trans-OH-CDK5 (blue) complexes.

Figure S2 RMSDs of the inhibitors bound to CDKs. Black: cis-OH bound to CDK2, red: trans-OH bound to CDK2, green: cis-OH bound to CDK5, blue: trans-OH bound to CDK5.

Figure S3 The time evolution of the salt-bridge between Asp145/Asn144 and Lys33 in CDKs. Results are shown for the distances (A) between carboxyl group of Asp145 and the side chain amino group of Lys33 in CDK2 and (B) between amide group of Asn144 and the side chain amino group of Lys33 in CDK5. Color scheme: Red for cis-OH bound and black for trans-OH bound CDK complex. See Fig. 3 for atom notations.

Figure S4 Time evolution of the interaction of cis-/trans-OH inhibitor with (A) Asp145 in CDK2 and (B) Asn144 in CDK5. Interactions are shown in terms of the distance between the hydroxyl group of the inhibitors and the backbone NH of Asp145/Asn144. Color scheme is similar to Fig. S3. See Fig. 3 for atom notations.

Figure S5 Time evolution of the interaction of cis- and trans-OH inhibitors with Lys33 in CDK5. Interactions are shown in terms of the distance between the hydroxyl group of the inhibitors and the side chain N of Lys33. Color scheme is similar to Fig. S3. See Fig. 3 for atom notations.

Table 5. The contribution of electrostatic and van der Waals energy toward the total interactions in inhibitor-CDK5 complexes.

| Complex       | Total Energy | Electrostatic | van der Waals |
|---------------|--------------|---------------|---------------|
| cis-N-acetylCDK5 | −53.53±5.56  | −27.5±6.12    | −26.03±2.17   |
| Roscovitine-CDK5 | −36.28±8.36  | −6.12±2.11    | −31.86±1.5    |

All energies are in kcal/mol.
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Author Contributions

Conceived and designed the experiments: SLR SS. Performed the experiments: SLR. Analyzed the data: SLR SS. Contributed reagents/materials/analysis tools: SS. Wrote the paper: SLR SS.

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