Docking of Potent Anticancer Agents; 4-(Pyrazol-4yl)-Pyrimidine Derivatives as Selective Cyclin-Dependent Kinase 4/6 Inhibitors

Akrinmah, Daryono Hadi Tjahyono, and Amir Musadad

ABSTRACT—Cyclin-dependent kinases (CDKs) are regulatory protein kinases which involved in cell cycle control. Many CDK inhibitors have been studied for anticancer potential. Here we conducted a docking study of 4-(pyrazol-4-yl)-pyrimidine derivatives as CDK1/2 and CDK4/6 inhibitors. Selectivity is an important aspect regarding the anticancer effect. In this computational research, we analyzed the interaction of 4-(pyrazol-4-yl)-pyrimidine derivatives with their receptors, CDK4/6 and CDK2. We compared the docking result of the parent compound, the most selective, and the least selective compound. Docking of the three compounds was performed using software Arguslab CDK 4.0.1 to assess the interaction with the receptors. Three docking parameters were analyzed; Gibbs free energy (∆G), atoms and residue of receptor involved in hydrogen bonding, and the bonding length. All three compounds had value of ∆G < 0, indicating that the interaction between the ligand and receptor was spontaneous. However, none of these parameters and descriptors values could explain the selectivity order of the three compounds.

Index Terms—Anticancer, CDK inhibitor, computational chemistry, docking, pyrazolo pyrimidine derivatives.

I. INTRODUCTION

Abnormal proliferation and out of control cell cycle is the main characteristic of cancer cells. Cyclin-dependent kinase (CDK) is a protein kinase which involved in controlling cell cycle. It regulates transitions of one phase to another. CDKs activity is controlled by several complex mechanisms. CDK activation requires certain cyclin binding and phosphorylation of a conserved threonine by the CDK-activating kinase (CAK) [1]. CDK’s important role in cell cycle leads them to become a potential anticancer compounds [2]. Their inhibition mechanism commonly involve a competition with ATP for binding in the kinase ATP-binding site [3]. CDK inhibitors form hydrogen bonding with the certain residue at ATP binding pocket of the CDKs. This bonding is affected by the polarity of the substituents that interact with the ATP binding pocket [4].

Young Shin Cho et al. synthesized 4-(pyrazol-4-yl)-pyrimidines derivatives as CDK1, 2 and CDK4/6 inhibitors. Results showed that inhibition of CDK4/6 kinase activity stopped the tumor cells progression in various in vivo and in vitro models, while CDK1 inhibition leads to apoptosis of all cell systems investigated. It indicates that selective CDK4/6 inhibitors potentially have a larger therapeutic window compared with pan-CDK inhibitors [5]. Selectivity of the CDK-inhibitors is important for their anticancer pharmacological activity [3].

Quantitative structure activity relationship (QSAR) is one of the most effective methods in new drug development, since it makes the process more efficient, less expensive and time consuming. It is used particularly in optimizing lead compounds and designing new chemical entities [6].

QSAR study of 4-(pyrazol-4yl)-pyrimidines derivatives and molecular design of CDK1/2 and CDK 4/6 inhibitors as potent anticancer agents were already executed before. This research used several softwares, the modeling and the geometry structures optimization of the 4-(pyrazol-4-yl)-pyrimidine derivatives molecules were done by Gaussian software. Calculation of descriptors value and optimization of the CDK inhibitors were done using software Arguslab CDK 4.0.1. The QSAR analysis were performed by MOE 2009.10 and SPSS Statistics 17.0, respectively. The results were then validated by LOO (Leave One Out) method to obtain the QSAR equation with the highest q². The QSAR equations are shown in Table I [7].

The substituents at the parent compound which replaced are chlorine (R₂) and isopropyl (R₁) at the pyrazole ring. Substitution at R₁ position affects its hydrophobic interaction toward CDKs and substitution at R₂ can affect its CDK4 inhibition activity [5].

The substituents for new drug design were; –CH₃, –NH₂, –F, –Cl, –C₃H₇₆, –N(CH₃)₂, –NO₂, and –CF₃. Modification were performed in combinatorial at R₁ and R₂ position of the pyrazole ring, shown in Fig. 1. The predicted IC₅₀ of the new compounds were calculated with the established QSAR equations, the highly selective CDK4 inhibitors indicated by

---

TABLE I: QSAR EQUATIONS OF CDK2 AND CDK4 INHIBITORS

<table>
<thead>
<tr>
<th>Activity</th>
<th>QSAR Equation</th>
<th>Statistic Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDK2 Inhibition</td>
<td>Log IC₅₀CDK2 = 1.893(±0.982) – 3.359 x10⁵ (±6.652 x10⁴)AM1_E +0.01391 ASA_H – 0.2242 polarizability</td>
<td>r = 0.940, ( r^2 = 0.884, F = 7.7557, q^2 = 0.81142, n = 15 )</td>
</tr>
<tr>
<td>CDK4 Inhibition</td>
<td>LogIC₅₀CDK4 = 0.5346(±0.9034) + 1.713x10⁻⁵ (±0.0652x10⁴)AM1_E +0.01391 ASA_H – 0.2242 polarizability</td>
<td>r = 0.960, ( r^2 = 0.921, F = 13.4045, q^2 = 0.88718, n = 16 )</td>
</tr>
</tbody>
</table>

---

Manuscript received June 14, 2013, revised September 18, 2013.

Akrinmah is with the Pharmacy Department, Sriwijaya University, South Sumatra, Indonesia (e-mail: akrinmah@gmail.com).

Hadi Tjahyono and Amir Musadad are with the School of Pharmacy, Bandung Institute of Technology, West Java, Indonesia.
the low IC₅₀ for CDK4 and high IC₅₀ for CDK2. The highest selective CDK4 inhibitor was parent compound substituted by CF₃ at both R₁ and R₂, which has the biggest molecular weight of all substituents. The last two selective CDK4 inhibitors were compounds with substituent CH₃ and substituent NH₂ at both R₁ and R₂ position, and its selectivity value were 21-fold and 18-fold, respectively [7].

In this computational chemistry research, we conduct docking study on a series of 4-(pyrazol-4-yl)-pyrimidine derivatives towards its receptors, CDK4 and CDK2. We compared the docking result between three compounds; the parent compound (a), the most selective (b) and the least selective (c) compound. The molecular structure of the three compounds and their anticancer activities are shown in Fig. 2.

II. METHODS

Structures of the three compounds were optimized by Hartree-Fock method to verify the most stable conformation with minimum energy. Software used for this optimization was Gaussian 03W. The descriptors values AM1E, ASA_H, mr and polarizability were calculated by MOE 2009.10.

Docking program used in this study was Arguslab 4.0.1. The molecular structure of CDK2 protein can be downloaded from the website of protein data bank (PDB), www.rcsb.org. The molecular structure of CDK4 was not yet available. However, the structure of the CDK4 and CDK6 is homologous with the amino acid residue sequence similarity of 68% and 81% similarity in the ATP binding pocket region [4]. Therefore, the structure of CDK6 was used in the docking process.

The method used for docking validation was pose selection. In this method, a compound with a known conformation and orientation from a cocrystal structure is redocked into the target’s active site [11]. Natural ligand of the receptor molecule CDK is ATP. At CDK PDB file downloaded, ATP ligand is only found in CDK2. Thus in the validation process, molecule of ATP was redocked into CDK2.

The main parameters to be compared of the three compounds were (i) Gibbs free energy, ∆G, (ii) atoms and residue of receptor involved in hydrogen bonding, and (iii) bonding length.

The CDK ATP binding pocket consists of four regions, the third region (residues 78-90) is the residue involved in the formation of hydrogen bonds with ATP or CDK inhibitor compounds [4]. Therefore, residue number 78 to 90 is a binding site that used in the process of docking the inhibitor compounds into CDK2 and CDK6 receptor.

III. RESULTS AND DISCUSSION

In docking validation, ATP was redocked into CDK2. The rmsd value was 2.77 Å at grid resolution 0.15. Docking programs are preferred to predict experimental poses with an averaged deviation from 1.5 to 2 Å rmsd [12], [13]. However, this has been an issue for the available docking programs. In some reports, rmsd in range 1.5-3.5 Å is still acceptable [14], [8].

The docking results of the three compounds to CDK2 and CDK6 are shown in Fig. 3 and Fig. 4. It shows the interaction position between the ligand and the receptor. If there was no interaction, the ligand and the receptor would be far from each other. The numbers in red are the bonding length, it can be seen more clearly in the tables below.
Ligand docking compound parameters for each receptor are shown in Table II and Table III. Free energy change ($\Delta G$) is a parameter that indicates the affinity and stability of the interaction between the ligand to the receptor. $\Delta G$ value $<$0 indicates the interaction takes place spontaneously, and the value of $\Delta G$ $>$0 indicates no interaction can take place spontaneously [15].

**TABLE II: DOCKING PARAMETERS FOR LIGAND-CDK2 INTERACTION**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$\Delta G$ (kCal/mol)</th>
<th>Atoms and residue of receptor involved in hydrogen bonding</th>
<th>Bonding length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent Compound</td>
<td>-8.1046</td>
<td>N 84 HIS, N 20 LYS, O 84 HIS</td>
<td>2.9491</td>
</tr>
<tr>
<td>The most selective compound</td>
<td>-7.5512</td>
<td>N 84 HIS, N 20 LYS, O 8 GLN</td>
<td>2.7610</td>
</tr>
<tr>
<td>The least selective compound</td>
<td>-7.4101</td>
<td>N 84 HIS, N 20 LYS, O 85 GLN</td>
<td>2.9900</td>
</tr>
</tbody>
</table>

**TABLE III: DOCKING PARAMETERS FOR LIGAND-CDK6 INTERACTION**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$\Delta G$ (kCal/mol)</th>
<th>Atoms and residue of receptor involved in hydrogen bonding</th>
<th>Bonding length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent compound</td>
<td>-7.8877</td>
<td>O 95 THR, O 10 ASP, O 95 THR</td>
<td>2.426</td>
</tr>
<tr>
<td>The most selective compound</td>
<td>-6.5808</td>
<td>N 583 ARG</td>
<td>2.3901</td>
</tr>
<tr>
<td>The least selective compound</td>
<td>-6.6075</td>
<td>O 99 GLU, O 13 TYR</td>
<td>2.9991</td>
</tr>
</tbody>
</table>

The docking result of receptors CDK2 and CDK6 shows that all three compounds had value of $\Delta G$ $<$0 which indicates that the interaction between the ligand and receptor was spontaneous. There is no particular order of these parameters that can be associated with their selectivity order.

However, there is a distinction of the residue of the receptor CDK2 that involved in hydrogen bonding. Three of them formed hydrogen bonding to 84 HIS and 20 LYS residue with different length. The order of the bonding length at 84 HIS residue reflects the selectivity of the compounds, the more selective compound has the shorter bonding length. However, since there is no other supporting data, the exact correlation of this parameter can not be concluded.

Both QSAR equations for CDK2 and CDK4/6 included AM1_E (total energy), and ASA_H (hydrophobic surface area) descriptors. The distinction between the two QSAR equations was that CDK2 equation contained descriptor mr, while CDK4 contained polarizability.

Therefore, it was predicted that the molar refractivity and
polarizability distinction in both equations indicated that the steric and polarizability parameter could possibly determine the selectivity of CDK2 and CDK4/6 inhibitors [7]. Molar refractivity is a measure of the steric bulk of a molecule. The value is proportional to the molecular weight of the compound. While polarizability indicates atomic or molecular charge distribution of a molecule [6].

The descriptors values of the three compounds are shown in Table IV.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Activity</th>
<th>Descriptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent compound</td>
<td>IC50 CDK2: 1.435µM IC50 CDK4: 0.011µM Selectivity: 130.455</td>
<td>AM1_E 669.156 10.797 42.84</td>
</tr>
<tr>
<td>The most selective compound</td>
<td>IC50 CDK2: 94.174µM IC50 CDK4: 0.010µM Selectivity: 9035.232</td>
<td>-163476.45 641.294 10.009 38.53</td>
</tr>
<tr>
<td>The least selective compound</td>
<td>IC50 CDK2: 1.012 µM IC50 CDK4: 0.056 µM Selectivity: 18.059</td>
<td>-101238.65 610.426 9.392 38.11</td>
</tr>
</tbody>
</table>

Since there was no docking parameters and descriptor value can explain the selectivity order of the three compounds, this study is expected to be verified with another docking software in another experiment.

REFERENCES


Akrimah was born in Pekanbaru, 4th July 1985. Graduated from School of Pharmacy, Bandung Institute of Technology, West Java, Indonesia, major in pharmacy technology for bachelor degree (2007) and medicinal chemistry for master degree (2012). She has been a lecturer in Pharmacy Department Sriwijaya University, South Sumatra, Indonesia, since May 2012. Before, she was an executive editor at local doctors magazine, Micro Plus, in Palembang, South Sumatra, Indonesia (2008-2010).