Design, synthesis, and molecular docking study of 3H-imidazole[4,5-c]pyridine derivatives as CDK2 inhibitors

Yi-Zhe Wu | Hua-Zhou Ying | Lei Xu | Gang Cheng | Jing Chen | Yong-Zhou Hu | Tao Liu | Xiao-Wu Dong

1 ZJU-ENS Joint Laboratory of Medicinal Chemistry, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, Hangzhou Institute of Innovative Medicine, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, P. R. China
2 School of Life Science and Technology, ShanghaiTech University, Shanghai, P. R. China
3 State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, P. R. China
4 College of Pharmaceutical Science, Zhejiang Chinese Medical University, Hangzhou, P. R. China

Abstract

A novel series of imidazo[4,5-c]pyridine-based CDK2 inhibitors were designed from the structure of CYC202 via scaffold hopping strategy. These compounds were synthesized and biologically evaluated for their CDK2 inhibitory and in vitro anti-proliferation potential against cancer cell lines. Several compounds exhibited potent CDK2 inhibition with IC₅₀ values of less than 1 µM. The most potent compound 5b showed excellent CDK2 inhibitory (IC₅₀ = 21 nM) and in vitro anti-proliferation activity against three different cell lines (HL60, A549, and HCT116). The molecular docking and dynamic studies portrayed the potential binding mechanism between 5b and CDK2, and several key interactions between them were observed, which would be the reason for its potent CDK2 inhibitory and anti-proliferation activities. Therefore, the pyridin-3-ylmethyl moiety would serve as an excellent pharmacophore for the development of novel CDK2 inhibitors for targeted anti-cancer therapy.

Keywords

cancer, CDK2 inhibitor, imidazo[4,5-c]pyridine, targeted therapy

1 | INTRODUCTION

Different types of cancers are often characterized by abnormal regulation of the cell cycle, generally triggered by dysregulation of different cell cycle regulators.⁹⁻¹¹ Cyclin dependent kinases (CDKs) are a family of crucial enzymes which activate the cell cycle transitions.⁴ CDK2 complexes with cyclin A/E in G1-S phase and regulates the cell cycle through the phosphorylation of Rb or other replication factors.⁵⁻⁷ The overexpression of CDK2 was noticed in many forms of cancer such as lung carcinoma, melanoma, osteosarcoma, and ovarian carcinoma.⁸⁻¹¹ The inhibition of CDK2 can induce apoptosis in the cancer cells, whereas the normal cells would undergo merely a reversible cell cycle arrest.¹²,¹³ Hence, the scientists consider CDK2 as an attractive target for developing novel cancer therapies. Many chemically diverse CDK2 inhibitors have entered in clinical trials during the last decade; most significant of which are ATP competitive inhibitors, the small molecule inhibitors which occupy the adenosine-binding region of the ATP binding pocket to disrupt the interaction of ATP and CDK2.¹⁴,¹⁵ Purine analogs are widely used as the scaffold of CDK2 inhibitors due to their ability to mimic the adenosine part of ATP. Many potent purine-derived CDK2...
inhibitors (Figure 1) like the first purine-derived CDK2 inhibitor olomoucine have been developed recently. Some researchers utilized 3H-imidazole[4,5-c]pyridine, a bioisostere of purine, as a scaffold for developing several drug molecules, except CDK2 inhibitors (Figure 2). In this study, we have designed and synthesized a new series of 3H-imidazole[4,5-c]pyridine derivatives following bioisosteric and scaffold hopping strategies, and biologically evaluated those compounds (Figure 2) for CDK2 inhibitory activities and in vitro anti-proliferation potential against cancer cell lines. The compound 5b with most potent CDK2 inhibitory potential was selected for further molecular docking and dynamic studies to understand the binding mechanisms between 5b and CDK2.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Scheme 1 depicts the synthetic route for preparing the 3H-imidazole[4,5-c]pyridine. The nitration of 4-hydroxypyridine yielded the di-nitro compound 1. The compound 1 was treated with SOCl2 to produce an unstable intermediate, which reacted with ethylamine to generate 2. Two nitro groups of 2 were reduced and the resultant product was chlorinated in a single step, and the resultant product was further treated with triethyl orthoformate to yield compound 3. The amino group of 3 was oxidized to nitro group by treating with H2O2 to produce 4. Two side chains were introduced to the 3H-imidazole[4,5-c]pyridine core to afford the first series of targeted molecules (5a–j). The nitro groups of the selected compounds were further reduced to amino to obtain the second series of the targeted molecules (6b–f).

2.2 | CDK2 inhibition and anti-proliferation activities

The anti-proliferation potentials of the synthesized compounds were evaluated against three different cancer cell lines, and in vitro CDK2 inhibitory activities of synthesized compounds were examined during this study. CYC202 was employed as a positive control. The results are shown in the Table 1. Compounds 5b and 5g–j, with 2-aminobutanol group in R2 position displayed significantly better CDK2 inhibition and anti-proliferation than other compounds; therefore, the 2-aminobutanol group in R2 position is crucial both for the CDK2 inhibitory activity and anti-proliferation potential. Compound 5b exhibited the most potent CDK2 inhibitory activity with the IC50 value of 21 nM, as well as potent anti-proliferation efficacy against three different cancer cell lines with the IC50 values at micromolar range (IC50 values of 3.71, 3.85, and 1.56 for HL60, A549, and HCT116, respectively). The reduction of the nitro group to amino significantly attenuated the anti-proliferation potential. The replacement of pyridine of 5b with benzene (5g) or substituted benzene (5h, 5i) significantly diminished the CDK2 inhibitory activity with a little depletion of anti-proliferation potential.
2.3 | Molecular docking and dynamic study

The compound 5b was docked into ATP-binding pocket of CDK2 using LigandFit program embedded in Discovery Studio 2.5. Molecular dynamics (MD) simulations and interaction decomposition analyses were performed to evaluate the quantitative energy contributions per-residue to the binding affinity of the inhibitor 5b. The RMSD value of CDK2 reached equilibrium and fluctuated around an average value of 200 ps simulation time. The RMSD value of the protein backbone was calculated. After 500 ps production simulation, distance between the inhibitor (5b) and the key amino acids GLU12, LEU83, ASP86, and LYS89 of CDK2 converged, as the system reached equilibrium (see Figure 3a). The protein/ligand interaction was analyzed from the stable conformation obtained from MD simulation. As shown in the Figures 3b and 3c, the inhibitor 5b could form several H-bonds with the residues of CDK2 protein in the hinge area. Apart from the preserved one of purine nitrogen with backbone NH group of LEU83, another three bonds were contributed by pyridinyl group with LYS89, hydroxyl group with ASP86, and amino group with GLU12, respectively. In accordance with the interaction energy decomposition, the contributions from the residues GLU12, LEU83, ASP86, and LYS89 were $-5.98$, $-2.03$, $-7.23665$, and $-26.2796$ kcal/mol, respectively (Figure 3d). From the binding pattern and chemical structure of 5b, we speculate that aminopyrimidine moiety may be an important pharmacophore for these CDK2 inhibitors.

3 | CONCLUSION

In this study, a series of 3H-imidazole[4,5-c]pyridine derivatives were designed, synthesized, and biologically evaluated for CDK2 inhibitory activity based on bioisosteric and scaffold hopping strategies. Among these compounds, compound 5b displayed excellent CDK2 inhibition activities (IC_{50} = 21 nM) and anti-proliferation potential against three different cancer cell lines. The molecular docking and dynamic simulations were utilized for identifying the binding mechanisms between compound 5b and CDK2, and demonstrated that the pyridin-3-ylmethyl skeleton would be an excellent pharmacophore for developing the CDK2 inhibitors for targeted anti-cancer therapy.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Melting points were determined with a Büchi Melting Point B-450 apparatus (Büchi Labortechnik, Flawil, Switzerland). The $^1$H NMR spectra were recorded on a Bruker Avance DMX 500 at 500 MHz (chemical shifts are expressed as d values relative to TMS as internal standard). ESI (positive) was recorded on an Esquire-LC-00075 spectrometer. All reactions were monitored by thin-layer chromatography (TLC). All reagents were obtained from commercial sources and used without further purification unless stated.

The HR-MS spectra, HPLC profiles, and NMR spectra of the investigated compounds are provided as Supporting Information. The InChI codes of the investigated compounds together with some biological activity data are also provided as Supporting Information.

4.1.2 | Procedure for the synthesis of 3,5-dinitropyridin-4-ol (1)

To a stirred mixture of concentrated H$_2$SO$_4$ (500 mL) and HNO$_3$ (140 mL) was added 40 g of 4-hydroxypyridine portion-wise and heated to 140°C. After 12 h, the mixture was poured into ice water and a lot of solid formed. The precipitated solid was filtered and washed with water to afford 66 g of product. Yield: 85%. $^1$H NMR (δ, DMSO): 8.95 (s, 2H). ESI-MS: m/z = 186.01 [M+H]$^+$.  

4.1.3 | Procedure for the synthesis of N-ethyl-3,5-dinitropyridin-4-amine (2)

Of 1, 10 g was suspended in SOCl$_2$, drops of DMF were added. After refluxing overnight, the SOCl$_2$ was removed under vacuum. The
TABLE 1 The CDK2 inhibition activity and anti-proliferation activity of 5a–j and 6b–f against HL60, A549, HCT116 cell lines

<table>
<thead>
<tr>
<th>Cpd</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HL60      A549      HCT116    CDK2</td>
</tr>
<tr>
<td>5a</td>
<td><img src="5a.png" alt="Chemical Structure" /></td>
<td><img src="5a.png" alt="Chemical Structure" /></td>
<td>&gt;50       18.07      8.79       88.68</td>
</tr>
<tr>
<td>5b</td>
<td><img src="5b.png" alt="Chemical Structure" /></td>
<td><img src="5b.png" alt="Chemical Structure" /></td>
<td>3.71      3.85       1.56       0.021</td>
</tr>
<tr>
<td>5c</td>
<td><img src="5c.png" alt="Chemical Structure" /></td>
<td><img src="5c.png" alt="Chemical Structure" /></td>
<td>12.60     &gt;50        13.85       8.55</td>
</tr>
<tr>
<td>5d</td>
<td><img src="5d.png" alt="Chemical Structure" /></td>
<td><img src="5d.png" alt="Chemical Structure" /></td>
<td>&gt;50       &gt;50        30.58       15.47</td>
</tr>
<tr>
<td>5e</td>
<td><img src="5e.png" alt="Chemical Structure" /></td>
<td><img src="5e.png" alt="Chemical Structure" /></td>
<td>&gt;50       14.94       18.41       6.91</td>
</tr>
<tr>
<td>5f</td>
<td><img src="5f.png" alt="Chemical Structure" /></td>
<td><img src="5f.png" alt="Chemical Structure" /></td>
<td>&gt;50       8.41        5.38        N.T.</td>
</tr>
<tr>
<td>5g</td>
<td><img src="5g.png" alt="Chemical Structure" /></td>
<td><img src="5g.png" alt="Chemical Structure" /></td>
<td>&gt;50       7.99        16.34       0.235</td>
</tr>
<tr>
<td>5h</td>
<td><img src="5h.png" alt="Chemical Structure" /></td>
<td><img src="5h.png" alt="Chemical Structure" /></td>
<td>2.01      4.92        7.56        0.366</td>
</tr>
<tr>
<td>5i</td>
<td><img src="5i.png" alt="Chemical Structure" /></td>
<td><img src="5i.png" alt="Chemical Structure" /></td>
<td>2.38      21.57       20.20       0.415</td>
</tr>
<tr>
<td>5j</td>
<td><img src="5j.png" alt="Chemical Structure" /></td>
<td><img src="5j.png" alt="Chemical Structure" /></td>
<td>4.34      21.17       19.73       1.98</td>
</tr>
<tr>
<td>6b</td>
<td><img src="6b.png" alt="Chemical Structure" /></td>
<td><img src="6b.png" alt="Chemical Structure" /></td>
<td>&gt;50       &gt;50        &gt;50         N.T.</td>
</tr>
<tr>
<td>6c</td>
<td><img src="6c.png" alt="Chemical Structure" /></td>
<td><img src="6c.png" alt="Chemical Structure" /></td>
<td>2.67      15.41       16.18       14.46</td>
</tr>
<tr>
<td>6d</td>
<td><img src="6d.png" alt="Chemical Structure" /></td>
<td><img src="6d.png" alt="Chemical Structure" /></td>
<td>&gt;50       &gt;50        &gt;50         N.T.</td>
</tr>
<tr>
<td>6e</td>
<td><img src="6e.png" alt="Chemical Structure" /></td>
<td><img src="6e.png" alt="Chemical Structure" /></td>
<td>&gt;50       N.T         &gt;50         N.T.</td>
</tr>
<tr>
<td>6f</td>
<td><img src="6f.png" alt="Chemical Structure" /></td>
<td><img src="6f.png" alt="Chemical Structure" /></td>
<td>32.9      &gt;50        &gt;50         N.T.</td>
</tr>
<tr>
<td>CYC202</td>
<td><img src="CYC202.png" alt="Chemical Structure" /></td>
<td><img src="CYC202.png" alt="Chemical Structure" /></td>
<td>17.90     13.54       6.65        N.T.</td>
</tr>
</tbody>
</table>
resulting residue was dissolved in dry THF and cooled to 0°C. The solution of ethyl amine in THF was added portion-wise and the pH was monitored by pH paper. When the pH paper indicated the pH value of solution is above 10, stopped adding the ethyl amine solution. The mixture kept stirring at 0°C for another 1 h. The reaction mixture was diluted with ethyl acetate and washed with NaHCO3 solution. The organic layer was dried with Na2SO4, filtered and concentrated under vacuum to give a crude product which was purified via chromatography to give 8 g of yellow solid. Yield: 70%. 1H NMR (δ, d6-DMSO): 9.07 (s, 2H), 8.55 (s, 1H), 3.08 (q, 2H), 1.24 (t, 3H). 13C NMR (δ, d6-DMSO): 151.1, 142.3, 134.8, 41.5, 14.8. ESI-MS: m/z = 213.1 [M+H]+.

4.1.4 | Procedure for the synthesis of 4,6-dichloro-1-ethyl-1H-imidazo[4,5-c]pyridin-7-amine (3)

To a stirred solution of 2 (10 g) in concentrated HCl (50 mL) at 60°C was added 1.5 eq of SnCl2 portion-wise and kept stirring at this temperature for 1 h. Then the temperature was warmed to 90°C and another 0.5 eq of SnCl2 was added. After stirring at 90°C for 2 h, the reaction mixture was poured into ice water. The pH was adjusted to 8 by ammonia and a lot of solid precipitated. The precipitated solid was filtered off and the filtrate was extracted with ethyl acetate. The organic layer was dried with Na2SO4, filtered and concentrated under vacuum to give a crude product which was purified via chromatography to give 8 g of yellow solid. Yield: 70%. 1H NMR (δ, d6-DMSO): 7.27 (s, 1H), 3.14 (q, 2H), 1.23 (t, 3H). ESI-MS: m/z = 231.0 [M+H]+.

4.1.5 | Procedure for the synthesis of 4,6-dichloro-1-ethyl-7-nitro-1H-imidazo[4,5-c]pyridine (4)

Of 3, 2.5 g was dissolved in 20 mL of concentrated H2SO4 and cooled to −5°C. The mixture of 30% H2O2 (35 mL) and concentrated H2SO4 (25 mL) was added dropwise. The mixture was stirred at −5°C for 3 h and quenched with ice water. The aqueous layer was extracted with ethyl acetate. The organic layer was dried with Na2SO4, filtered and concentrated under vacuum to give a crude product, which was purified via chromatography to give 600 mg of yellow solid. Yield: 20%. 1H NMR (δ, d6-DMSO): 8.80 (s, 1H), 4.15 (q, 2H), 1.34 (t, 3H). ESI-MS: m/z = 260.9 [M+H]+.

4.1.6 | General procedure for the synthesis of 5a-j

To a stirred solution of 4 (100 mg) in 3 mL of NMP was added 1.05 eq of corresponding amine (R1NH2). The reaction mixture was stirred at 80°C. The reaction was monitored by TLC. When the TLC indicated the 4 was consumed, 1.5 eq of the other amine (R2NH2) was added. The mixture was warmed to 100°C and stirred for 3–10 h. Then the reaction mixture was poured into 20 mL of water and extracted with ethyl acetate. The organic layer was washed with brine, dried with Na2SO4, filtered and concentrated under vacuum to give a crude product which was purified via chromatography to give the nitro containing product.

2-(1-(1-Ethyl-7-nitro-4-((pyridin-3-ylmethyl)amino)-1H-imidazo-[4,5-c]pyridin-6-yl)piperidin-2-yl)ethan-1-ol (5a)

Yield: 57% for two steps. 1H NMR (δ, CDCl3): 8.74 (s, 1H), 8.57 (d, 1, J = 4.5 Hz), 7.81 (d, 1H, J = 8 Hz), 7.60 (s, 1H), 7.38 (dd, 1H, J = 4, 8 Hz),
imidazo[4,5-1]pyridine-6-yl)amino)butan-1-ol (5e)
Yield: 61% for two steps. 

1HNMR (δ, CDCl3): 9.76 (d, 1H, J = 7.6 Hz), 8.82 (s, 1H), 8.60 (d, 1H, J = 4.5 Hz), 7.85 (d, 1H, J = 8 Hz), 7.60 (s, 1H), 7.41 (dd, 1H, J = 4.5, 8 Hz), 6.95 (s, 1H), 4.87 (m, 2H), 4.52 (q, 2H), 4.35 (m, 1H), 3.74 (m, 1H), 3.68 (m, 1H), 1.82 (m, 1H), 1.79 (m, 1H), 1.38 (t, 3H), 1.01 (t, 3H). HPLC purity: 100%. ESI-MS: m/z = 386.2 [M+H]+. HRMS (ESI) m/z = 386.1941 [M+H]+.

2-((1-Ethyl-7-nitro-4-((pyridin-3-ylmethyl)amino)-1H-imidazo[4,5-c]pyridine-6-yl)amino)butan-1-ol (5f)
Yield: 48% for two steps. 

1HNMR (δ, CDCl3): 9.70 (d, 1H, J = 8 Hz), 9.06 (t, 1H), 8.04 (s, 1H), 7.40 (m, 2H), 7.15 (t, 2H), 4.91 (t, 1H), 4.66 (m, 2H), 4.47 (q, 2H), 4.18 (m, 1H), 3.51 (m, 2H), 1.65 (m, 1H), 1.51 (m, 1H), 1.19 (t, 3H), 0.82 (t, 3H). 13C NMR (δ, d6-DMSO): 162.8, 160.4, 154.2, 143.4, 136.1, 132.3, 129.6, 123.9, 115.3, 110.1, 61.9, 54.7, 44.5, 43.8, 24.3, 16.9, 10.8. HPLC purity: 100%. ESI-MS: m/z = 403.2 [M+H]+. HRMS (ESI) m/z = 403.1894 [M+H]+.

2-((1-Ethyl-7-nitro-4-((4-methoxybenzyl)amino)-1H-imidazo[4,5-c]pyridine-6-yl)amino)butan-1-ol (5i)
Yield: 34% for two steps. 

1HNMR (δ, d6-DMSO): 9.71 (d, 1H, J = 8 Hz), 9.01 (t, 1H), 8.03 (s, 1H), 7.25 (d, 2H), 7.12 (d, 2H), 4.91 (t, 1H), 4.64 (m, 2H), 4.45 (q, 2H), 4.20 (m, 1H), 3.57 (m, 2H), 2.27 (s, 3H), 1.66 (m, 2H), 1.53 (m, 1H), 1.18 (t, 3H), 0.83 (t, 3H). ESI-MS: m/z = 415.2 [M+H]+.

2-((1-Ethyl-7-nitro-4-((1-phenylethyl)amino)-1H-imidazo[4,5-c]pyridine-6-yl)amino)butan-1-ol (5j)
Yield: 53% for two steps. 

1HNMR (δ, CDCl3): 9.65 (d, 1H, J = 8 Hz), 8.90 (d, 1H, J = 8 Hz), 8.06 (s, 1H), 7.45 (d, 2H, J = 7.6 Hz), 7.32 (t, 2H, J = 7.6 Hz), 7.22 (t, 1H, J = 7.2 Hz), 5.43 (m, 1H), 4.95 (t, 1H), 4.44 (q, 2H), 4.21 (m, 1H), 3.65 (m, 1H), 3.54 (m, 1H), 1.57 (d, 3H), 1.45 (m, 1H), 1.37 (m, 1H), 1.17 (t, 3H), 0.73 (t, 3H). HPLC purity: 96.03%. ESI-MS: m/z = 399.2 [M+H]+. HRMS (ESI) m/z = 399.2145 [M+H]+.

4.1.7 General procedure for the synthesis of 6b–f

To a stirred solution of corresponding nitro containing compound in MeOH was added 2 eq of SnCl2. After stirring at 60°C for 2 h, the reaction mixture was poured into ice water. The pH was adjusted to 8 by ammonia and a lot of solid precipitated. The precipitated solid was filtered off and the filtrate was extracted with ethyl acetate. The combined organic layer was dried with Na2SO4, filtered and concentrated under vacuum to give a crude product which was purified via chromatography.

2-((4-Benzylamino)-1-ethyl-7-nitro-1H-imidazo[4,5-c]pyridin-6-yl)amino)butan-1-ol (5g)

Yield: 64% for two steps. 1H NMR (δ, d6-DMSO): 7.78 (s, 1H), 7.37 (d, 2H, J = 7.6 Hz), 7.28 (t, 2H, J = 7.2 Hz), 7.19 (t, 2H, J = 7.6 Hz), 7.04 (t, 1H), 5.97 (s, 1H), 4.61 (d, 2H), 4.37 (m, 1H), 4.08 (q, 2H), 3.46 (m, 2H), 1.67 (br, 2H), 1.34 (m, 6H). ESI-MS: m/z = 385.1 [M+H]+.
1-Ethyl-6-(pyridin-1-yl)-N^4-(pyridin-3-ylmethyl)-1H-imidazo-[4,5-c]pyridine-4,7-diamine (6c) Yield: 32%. ^1H NMR (δ, d6-DMSO): 8.56 (s, 1H), 8.37 (d, 1H J = 4 Hz), 7.79 (s, 1H), 7.73 (d, 1H J = 7.8 Hz), 7.27 (dd, 1H J = 4.8, 7.6 Hz), 7.19 (t, 3H), 5.96 (s, 1H), 4.58 (d, 2H), 4.04 (q, 2H), 3.32 (br, 4H), 1.49 (m, 6H), 1.31 (t, 3H). ESI-MS: m/z = 352.2 [M+H]^+

2-(1-(7-Amino-1-ethyl-4-((pyridin-3-ylmethyl)amino)-1H-imidazo[4,5-c]pyridin-6-yl)piperidin-4-yl)ethan-1-ol (6d) Yield: 45%. ^1H NMR (δ, CDCl3): 8.64 (s, 1H), 8.45 (d, 1H J = 4 Hz), 7.73 (d, 1H J = 7.5 Hz), 7.52 (s, 1H), 7.20 (dd, 1H J = 4.7, 7.5 Hz), 5.84 (br, 2H), 4.78 (d, 2H), 4.15 (m, 2H), 4.04 (q, 2H), 3.72 (t, 2H), 2.68 (m, 2H), 1.77 (m, 2H), 1.54 (q, 2H), 1.40 (t, 3H), 1.33 (m, 3H). ESI-MS: m/z = 396.2 [M+H]^+.

2,2′-((7-Amino-1-ethyl-4-((pyridin-3-ylmethyl)amino)-1H-imidazo[4,5-c]pyridin-6-yl)azanediyl)bis(ethan-1-ol) (6e) Yield: 34%. ^1H NMR (δ, CDCl3): 8.69 (s, 1H), 8.49 (d, 1H J = 3.5 Hz), 7.81 (d, 1H J = 8 Hz), 7.59 (s, 1H), 7.25 (m, 1H), 5.74 (s, 1H), 5.00 (d, 2H J = 5.5 Hz), 4.01 (q, 2H), 3.84 (t, 4H), 3.65 (t, 4H), 1.41 (t, 3H). ESI-MS: m/z = 372.2 [M+H]^+.

4.2 Biological assays

4.2.1 CDK2 inhibition assay

The recombinant CDK2 was purchased from Carna Biosciences, Inc. It was co-expressed with cyclinA2 using baculovirus expression system and was purified by using glutathione sepharose chromatography. Kinase assay was carried out with the Z-LYTE Kinase Assay kit-Ser/Thr12 Peptide (Invitrogen, Carlsbad, CA). The enzyme activity was carried out in 10 µL system containing 4 nM enzyme, 25 µM ATP, and 2 μM substrate peptide in 50 mM HEPES, pH 7.5, 0.01% Brij-35, 10 mM MgCl2, 1 mM EGTA. After 1 h reaction, the development reagent was added, and the reaction was terminated after 1 h. All reactions were carried out in triplicate. IC_{50} values (concentration at which a 50% of enzyme inhibition is shown) were derived from a nonlinear regression model (curvefit) based on sigmoidal dose response curve (variable slope) and computed using GraphPad Prism version 5 (GraphPad Software, La Jolla, CA, USA).

4.2.2 Anti-proliferation activities assay

The antiproliferative activity in vitro against several cancer cell lines was measured by MTT assay. These cancer cell lines included HL60, A549, and HCT116. All tested compounds were dissolved in DMSO at concentrations of 10.0 mg/mL and diluted to appropriate concentrations. Cells were plated in 96-well plates for 24 h and subsequently treated with different concentrations of all tested compounds for 72 h. Viable cells were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay kit (MTT, Sigma) according to operation instructions provided by the manufacturer. The concentration of drug causing 50% inhibition in absorbance compared with control cells (IC_{50}) was calculated using the software of dose-effect analysis with microcomputers.

4.3 Molecular docking

The docking studies of compound 5b with CDK2 were performed using LigandFit module from Discovery Studio 2.5. Protein Data Bank (PDB) entry 3DQ was selected as the CDK2 protein. For preparing receptors, the solvent molecules and original ligands from the crystals were removed and polar hydrogen atoms were added, and then subjected to CHARMM force field. The binding pocket was defined via the “Find Sites as Volume of Selected Ligand” tool. For preparing ligands, the 3D-structure of ligands was initially constructed. Polar hydrogen atoms were added and subjected to CHARMM force field and Minimization.

4.3.1 Molecular dynamic (MD) simulation

The docked structures of inhibitor 5b complexed with CDK2 were used as the initial structures for MD calculations. A CHARMM force field was applied to the complex and the resulting system was subjected to double-fold minimization (10000 cycles of steepest descent minimization and 10000 cycles of conjugate gradient minimization). The system was gradually heated from 50 to 300 K over a period of 100 ps and subsequently equilibrated for 20 ps. Starting from the last frame of the equilibration, a production simulation was performed for 700 ps using the NPT ensemble under a constant temperature of 300 K and pressure of 1 atm. Other parameters of MD simulation were maintained at the default Discovery Studio configuration.

4.3.2 Interaction energy analysis

In order to explore the inhibitor–protein interaction mode and highlight the key residues responsible for the binding affinity, interaction energies for each inhibitor were further decomposed into individual residue contributions using the “Calculate Interaction Energy” protocol. The stable coordinates extracted in the last 700 ps MD simulation were employed here for the interaction energy decomposition analysis.

ACKNOWLEDGMENTS

This study was financially supported by Natural Science Foundation of China (81673294), Science and Technology Planning Project of Zhejiang Province (2016C33067), and Key New Drug Creation and Manufacturing Program (2018ZX09711002-011-022).
CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ORCID

Gang Cheng https://orcid.org/0000-0002-1760-9539  
Tao Liu https://orcid.org/0000-0001-8762-3339  
Xiao-Wu Dong https://orcid.org/0000-0002-2178-4372

REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，
提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。
图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具