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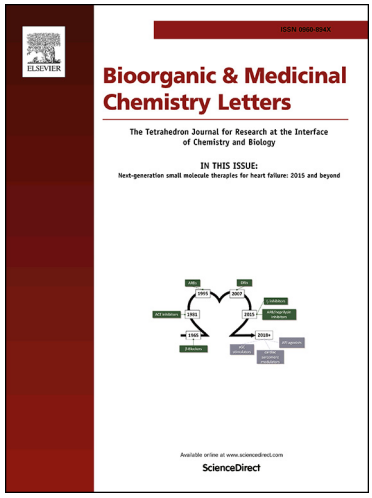

Synthesis and biological evaluation of novel 5,6-dihydropyrimido[4,5-f]quinazoline derivatives as potent CDK2 inhibitors

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Synthesis and biological evaluation of novel 5,6-dihydropyrimido[4,5-f]quinazoline derivatives as potent CDK2 inhibitors

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Abstract

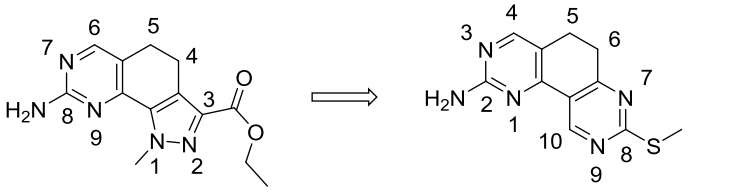
As serine/threonine kinase, the cyclin dependent kinase 2 (CDK2) is a promising target for various diseases such as cerebral hypoxia, cancer, and neurodegenerative diseases. Here we reported the structure-based synthesis and biological evaluation of novel 5,6-dihydropyrimido[4,5-f]quinazoline derivatives as CDK2 inhibitors, which exhibited potent CDK2 inhibitory activities, as well as anticancer activities in low concentration against two human cancer cell lines (MCF-7 and HCT116). In particular, compounds **11a** and **11f** (IC_{50} values of 0.11 and 0.09 μM for CDK2, respectively) have demonstrated significantly inhibitory potency against CDK2 and have showed great inhibitory activities against MCF-7 and HCT116 cell lines.

Keywords: cyclin dependent kinase, anticancer, design and discovery, molecular docking

Cells will progress only through four phases of the cell cycle to divide and replicate, namely, G1, S phase, G2, and M phase [1-2]. Since unrestrained growth is one of the hallmarks of cancer, it is common to disrupt the cell-cycle regulation in malignant cells [3]. Although targeting the pathways that regulate the cell cycle has drawn great attention in oncology for many years [4], the more recent development of selective and potent inhibitors of specific CDKs leads to burgeoning interest in this field.

CDK2 is a member of the CDK family which plays a central role in regulating cell cycle progression. CDK2 is frequently over-expressed in human tumors, and needed for the proliferation of tumor cell [5-6]. The CDK2 inhibitors such as Dinaciclib, Milciclib, BMS-387032, PHA-793887 [7-10] have been studied extensively in clinical research and it has been demonstrated that cell cycle arrest would cause cell apoptosis. Meanwhile, ongoing clinical trials have produced promising data on the potential efficacy of CDK2 inhibitors [11].

Most of the reported CDK2 inhibitors [12-13] interact with Leu83 residue at ATP-site. The tricyclic scaffold of 4,5-dihydro-1*H*-pyrazolo[4,3-h]quinazoline was previously identified as an inhibitor of other cell cycle kinases like Aur-A, PLK1 and CDK2 [14-16]. The pyrimidine portion of the three-membered ring scaffold could form a pair of important donor-acceptor-donor hydrogen bonds with the CDK2 hinge region (Leu83), which was essential. Our team referred to the 4,5-dihydro-1*H*-pyrazolo[4,3-h]quinazoline scaffold [16] to retain the structure of pyrimidine and then altered the portion of pyrazolo ring. Through the previous screening, we discovered the compound 8-(methylthio)-5,6-dihydropyrimido[4,5-f]quinazolin-2-amine (**a**) which displayed weak inhibition activity (IC_{50}=4.72 μM) against CDK2. As a further development of the fused quinazoline ring systems, we identified a novel series of 5,6-dihydropyrimido[4,5-f]quinazoline as CDK2 inhibitors (Fig. 1). We report the synthetic process and the structure-activity relationship that led from the initial hit (**a**) to identification of a potent CDK2 inhibitor.



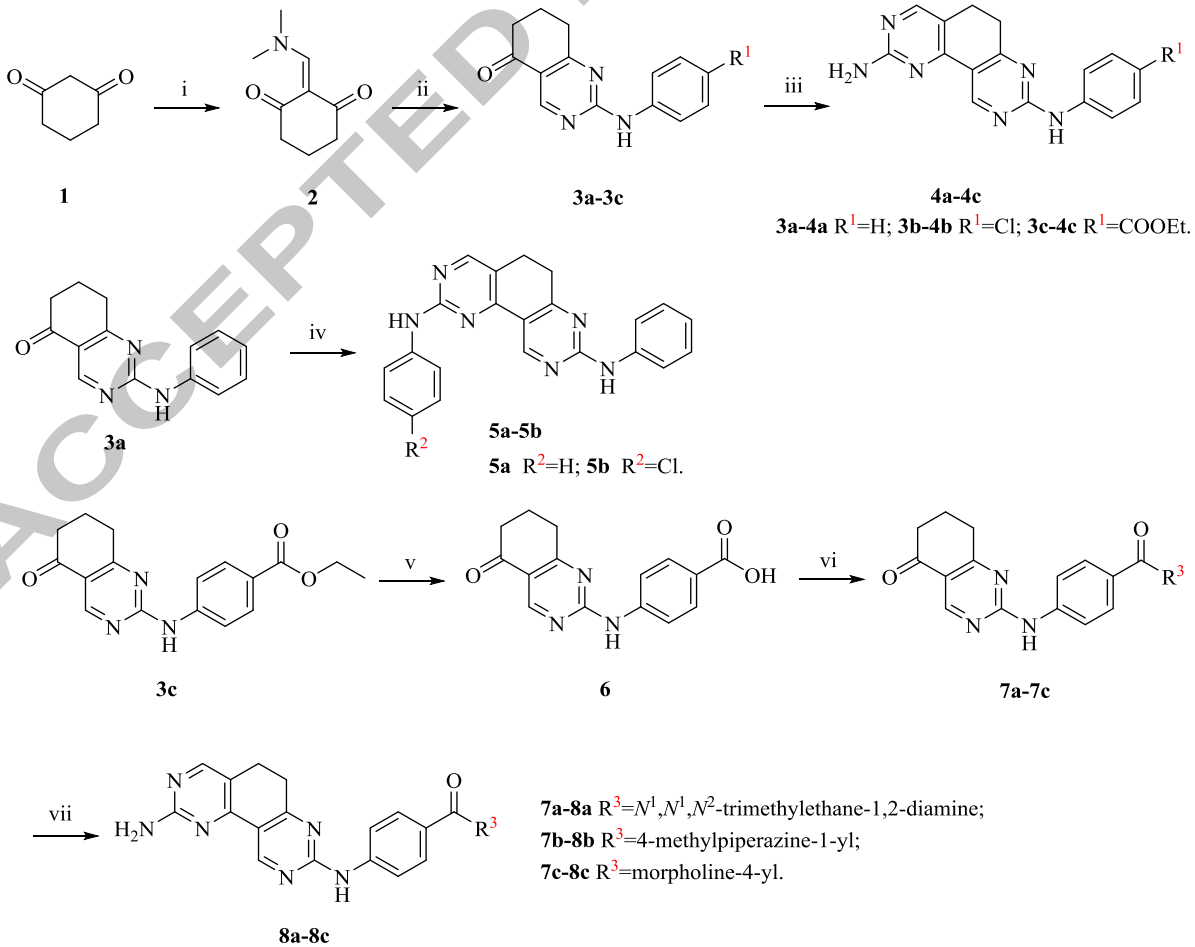
a

CDK2/Cyclin A2 IC_{50}=4.72 μM

4,5-dihydro-1*H*-pyrazolo[4,3-*h*]quinazoline 5,6-dihydropyrimido[4,5-*f*]quinazoline

Figure 1. The structure of 4,5-dihydro-1*H*-pyrazolo[4,3-h]quinazoline and 5,6-dihydropyrimido[4,5-f]quinazoline scaffold

According to the related literatures [17], The synthetic approach to this class of compounds (scheme 1) started from commercially available 1,3-cyclohexanedione. Condensation with *N*,*N*-dimethylformamide dimethyl acetal gave rise to compound **2.** The compound **2** was condensed with guanidine derivatives in DMF to afford the pyrimidine derivatives **3a-3c**, which were converted into the corresponding 5,6-dihydropyrimido[4,5-f]quinazoline analogues **4a-4c** and **5a-5b**. Hydrolysis of carboxylic ester **3c** gave the corresponding acid **6**, which were submitted to the coupling with amines to provide amides **7a-7c**. Then, **7a-7c** reacted with *N*,*N*-dimethylformamide dimethyl acetal and guanidine hydrochloride to afford the fused 5,6-dihydropyrimido[4,5-f]quinazoline derivatives **8a-8c**. The synthetic route to 5,6-dihydropyrimido[4,5-f]quinazoline analogues is shown in Scheme 2. Compound **2** reacted with S-methylisothiourea sulfate to obtain **9** under 80 °C. Pyrimidine ring compounds **10a-10f** were obtained by cyclization of compound **9** and aniline guanidine. Compound **10e** were submitted to the coupling with amines to provide amides **11a-11h**.



1 **2** **3a-3c** **4a-4c**

3a-4a R^1=H; **3b-4b** R^1=Cl; **3c-4c** R^1=COOEt.

3a **5a-5b**

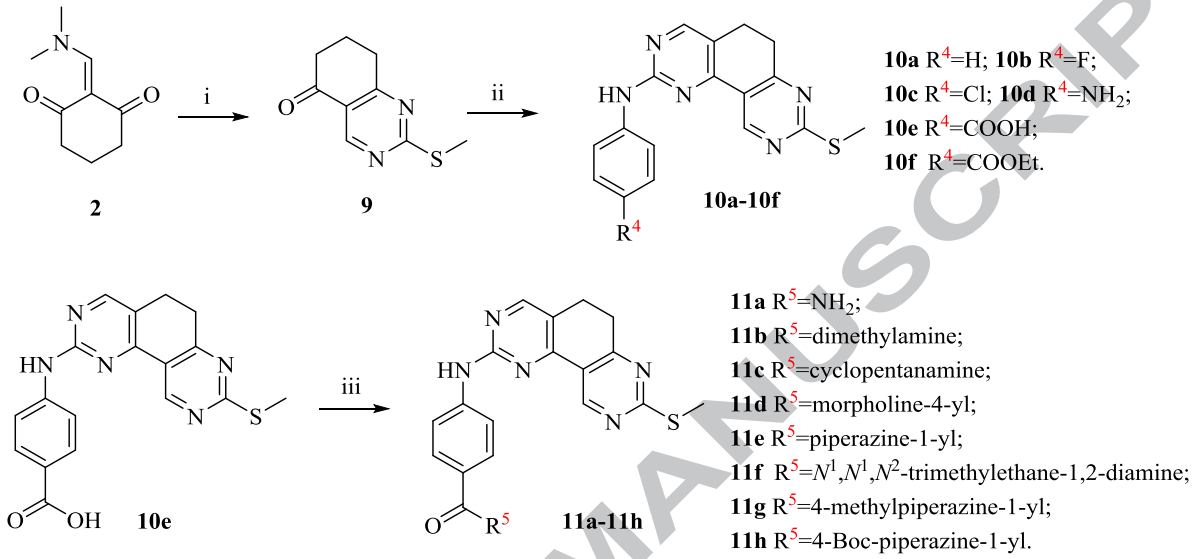
5a R^2=H; **5b** R^2=Cl.

3c **6** **7a-7c**

8a-8c

7a-8a R^3=N^1,N^1,N^2-trimethylethane-1,2-diamine;
7b-8b R^3=4-methylpiperazine-1-yl;
7c-8c R^3=morpholine-4-yl.

Scheme 1. Synthesis of compounds **8a-8c**. Reagents and conditions: (i) *N*,*N*-dimethylformamide dimethyl acetal, r.t., 8 h, 90%; (ii) phenylguanidine derivatives, Na_2CO_3, 1,4-dioxane, reflux, 80-90%; (iii) *N*,*N*-dimethylformamide dimethyl acetal, DMF, 110 °C, 4 h; guanidine hydrochloride, Na_2CO_3, DMF, 110 °C, 50-70%; (iv) *N*,*N*-dimethylformamide dimethyl acetal, DMF, 110 °C, 4 h; phenylguanidine derivatives, Na_2CO_3, DMF, 110 °C, 60-70%; (v)1.5M NaOH in 95% EtOH, 80 °C, 4 h, 85%; (vi)R_3NH_2, HOBT, EDC, DMF, r.t., 8 h, 60-80%; (vii) *N*,*N*-dimethylformamide dimethyl acetal, DMF, 110 °C, 4 h; guanidine hydrochloride, Na_2CO_3, DMF, 110 °C, 40-60%.



Scheme 2. Synthesis of compounds **11a-11h**. Reagents and condition: (i) S-methyllisothiourea sulfate, Na_2CO_3, 1,4-dioxane, reflux, 8 h, 90%; (ii) *N*, *N*-dimethylformamide dimethyl acetal, DMF, 110 °C, 4 h; phenylguanidine derivatives, Na_2CO_3, DMF, 110 °C, 60-80%. (iii) R_5NH_2, HOBT, EDC, DMF, r.t., 8 h, 50-80%.

All synthesized compounds were evaluated for inhibitory activity against CDK2 and anti-proliferative activity against human cell lines MCF-7 and HCT116 taking BMS-387032 as a positive control. Table 1 summarized the structure-activity relationship (SAR) of derivatives modified at the 2-position and 8-position of the hit compound **a**. Firstly, we examined the substituent effect of 8-position of 5,6-dihydropyrimido[4,5-f]quinazoline scaffold. All the compounds, with the exception of **4c**, **8b** and **8c**, showed an increased activity with respect to the initial compound **a** (IC_{50}=4.72 μM) against CDK2. Subsequently work continued on introducing different substitution at para position of benzene ring of compound **4a**, but there was no significant improvement on inhibitory activity. As is showed in the results, the amino carboxylate compounds **8a-8c** demonstrated the reduced inhibitory potency for CDK2, when compared with compound **4a**.

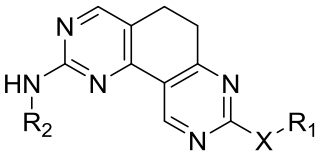
According to the molecular modeling studies reported in the literature [18], we analyzed the structure in detail, the introduction of phenylamine at C-8 resulted in hydrogen bonds forming with Leu83. Likewise, the replacement at C-2 with phenylamine showed the same effect. Then, we defined R^2 as phenyl and X as sulfur atom, we found that compound **10a** (IC_{50}=0.41 μM) showed good inhibitory activity to CDK2. Next, we systematically analyzed substituent effects on 4-position of benzyl ring with various substituent, including hydrogen, methyl amino and so on (Table 1). The introduction of amino and amide on the 4-position of phenyl resulted a significant improvement in activity against CDK2. The compound **10d** (IC_{50}=0.16 μM) exhibited stronger inhibitory activity against CDK2. The halogen compounds (**10b-10c**) and ester compounds (**10e-10f**) showed weaker inhibitory activities compared to **10e**. Compound **11a** (IC_{50}=0.11 μM) exhibited much better inhibitory potencies against CDK2 kinases as compared to **10d** (IC_{50}=0.16 μM).

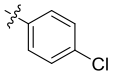
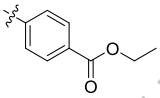
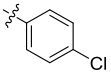
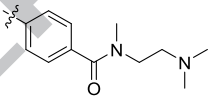
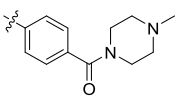
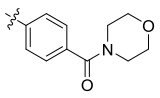
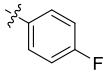
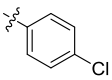
Based on the overall anticancer activity parameter, mean IC_{50}, the potencies of the tested compounds could be generally ranked in the following order: 2-substituted phenylamine compounds (**11a-11h**) >2-substituted phenylamine compounds (**10a-10f**) > 8-substituted phenylamine compounds (**4a-4c, 5a-5b** and **8a-8c**). The results indicated that the phenylamine at

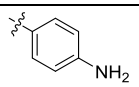
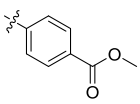
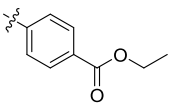
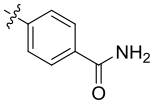
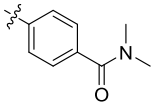
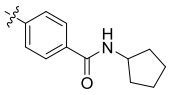
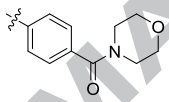
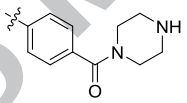
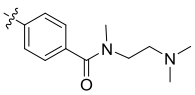
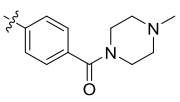
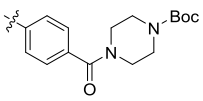
the C-2 position derivatives **10a-10f** and **11a-11h** were again relatively more potent than the phenylamine at the C-8 position derivatives **4a-4c**, **5a-5b** and **8a-8c.** The compounds (**4a-4c**, **5a-5b** and **8a-8c**) showed weak inhibitory activity against CDK2 and anti-proliferative activity against MCF-7 and HCT116 as well. When we defined the C-2 position as phenylamine and the C-8 position as methyl sulfide, compounds (**10a-10f** and **11a-11h**) performed better. Furthermore, compound **11f** with the N^1,N^1,N^2-trimethylethane-1,2-diamine moiety displayed potent inhibitory activity (IC_{50}=0.09 μM) and anti-proliferative activity (MCF-7, IC_{50}=1.1 ± 0.1 μM; HCT116, IC_{50}= 1.4 ± 0.2 μM) of all compounds.

Table 1

Results for inhibition of CDK2 and anti-proliferative activity against MCF-7 and HCT116 by compounds **4a-4c, 5a-5b, 8a-8c, 10a-10f, 11a-11h.**



ID	X	R_1	R_2	IC_{50}[a](μM)	IC_{50}[b] ± SD (μM)	
				CDK2/Cyclin A2	MCF-7	HCT116
4a	**N**		**H**	1.70	27.6 ± 1.1	25.3 ± 2.4
4b	**N**		**H**	1.85	>30	>30
4c	**N**		**H**	NA	>30	>30
5a	**N**			**1.54**	20.6 ± 0.5	18.1 ± 3.7
5b	**N**			2.03	23.5 ± 0.6	26.3 ± 0.7
8a	**N**		**H**	3.2	>30	>30
8b	**N**		**H**	5.9	>30	>30
8c	**N**		**H**	NA	>30	>30
10a	**S**	**Me**		**0.41**	15.6 ± 0.4	12.7 ± 1.0
10b	**S**	**Me**		0.35	8.4 ± 0.3	7.9 ± 0.4
10c	**S**	**Me**		0.38	7.0 ± 0.7	8.3 ± 0.2

10d	**S**	**Me**		**0.16**	1.4 ± 0.3	1.6 ± 0.2
10e	**S**	**Me**		0.98	17.8 ± 0.6	12.8 ± 0.9
10f	**S**	**Me**		1.25	25.4 ± 0.2	14.3 ± 0.4
11a	**S**	**Me**		**0.11**	**1.3 ± 0.2**	**0.9 ± 0.2**
11b	**S**	**Me**		0.53	3.7 ± 0.4	5.3 ± 0.5
11c	**S**	**Me**		1.26	6.5 ± 1.7	9.1 ± 0.2
11d	**S**	**Me**		0.19	2.1 ± 0.5	3.2 ± 1.4
11e	**S**	**Me**		0.16	**1.5 ± 0.3**	**1.3 ± 0.1**
11f	**S**	**Me**		**0.09**	**1.1 ± 0.1**	**1.4 ± 0.2**
11g	**S**	**Me**		0.18	2.5 ± 0.6	3.8 ± 0.5
11h	**S**	**Me**		3.7	19.4 ± 0.4	17.2 ± 0.7
BMS-387032				0.052	1.0 ±0.3	0.7 ±0.2

[a] Values are means of at least two or more experiments. Standard deviations were <10% of the mean. NA means data not available. [b] Values are means of at least three or more experiments.

Kinases selectivity of compound **11f** was further evaluated with other CDK kinase and was summarized in Table 2. Compound **11f** displayed better inhibitory activity against CDK2 and CDK5 (IC_{50} values of 0.09 μM and 0.68 μM) than CDK1 and CDK7 (IC_{50} values of 1.25 μM and 3.60 μM). Compound **11f** showed weak inhibitory activity against CDK4 and CDK6 (IC_{50}>5 μM). Thus far it could be identified as a potential and selective CDK2 inhibitor.

Table 2

Selectivity profile of compound **11f**

CDKs	CDK1	CDK2	CDK4	CDK5	CDK6	CDK7
IC_{50}[a](μM)	1.25	0.09	>5	0.68	>5	3.60

[a] Values are means of at least two or more experiments. Standard deviations were <10% of the mean.

To better understand the mechanism of inhibition, molecular docking was used to analyze the putative binding mode of the designed compound with CDK2 [19]. The crystal structure of CDK2 in complex with an ATP-competitive inhibitor (PDB code 2BPM) was selected for the docking studies. As depicted in docking mode of compound **10d** with CDK2 (Fig. 2), the pyrimidine core of **10d** formed a pair of donor-acceptor-donor hydrogen bonds with the CDK2 hinge region (Leu83). And the amino forms a hydrogen bond to the main-chain of Asp86.

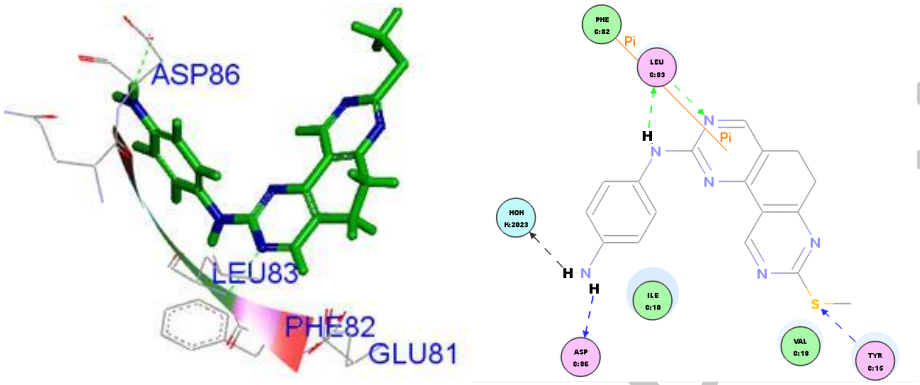


Figure 2. Compound **10d** bound to CDK2. Hydrogen bonds are shown as green dashed lines, residues of CDK2 and are shown with carbon and nitrogen colored green and blue, respectively.

Conclusions

In summary, we have designed and synthesized a series of novel 5,6-dihydropyrimido[4,5-f]quinazoline derivatives as CDK2 inhibitors. The biochemical activity of all compounds against CDK2/A2 kinase and in vitro cell proliferation cytotoxicity on human cancer cell lines MCF-7 and HCT116 were reported in table 1 and table 2. The brief SAR study demonstrated that the phenylamine at the C-2 position had better inhibitory potency in comparison with the phenylamine at the C-8 position derivatives. Similarly, amide derivatives had better inhibitory potency than amino carboxylate derivatives for CDK2 kinase. Derivatives **11a** and **11f** were proved to be the best compounds of the series, showing anti-proliferative activity in the low micro molar range on the MCF-7 and HCT116 cell lines, remarkable activity on CDK2 kinase (IC_{50} values of 0.11 and 0.09 μM for CDK2, respectively). Studies to explore the mechanism of these compounds are needed and now in progress. The evolution of these series of compounds will be reported in due course.

Acknowledgements

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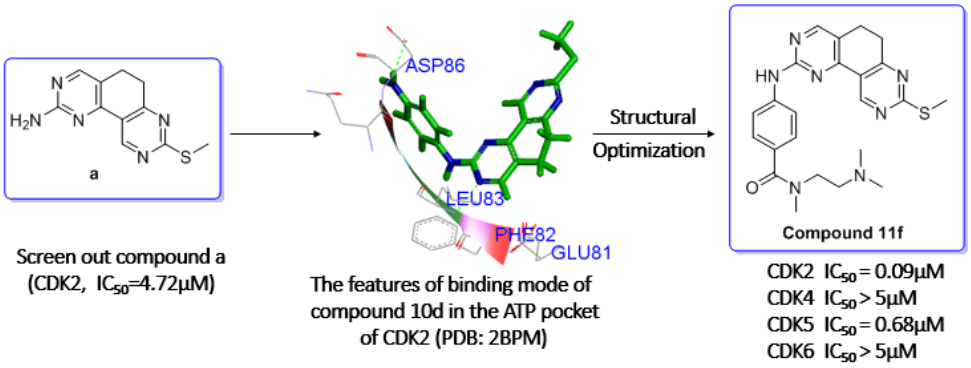
Graphical Abstract



Highlights

- New 5,6-dihydropyrimido[4,5-f]quinazoline derivatives were designed and synthesized
- CDK2 inhibitory activity was evaluated
- Anti-proliferative activity was in vitro evaluated against MCF-7 and HCT116 cells
- Molecular docking in the active site of CDK2 enzyme