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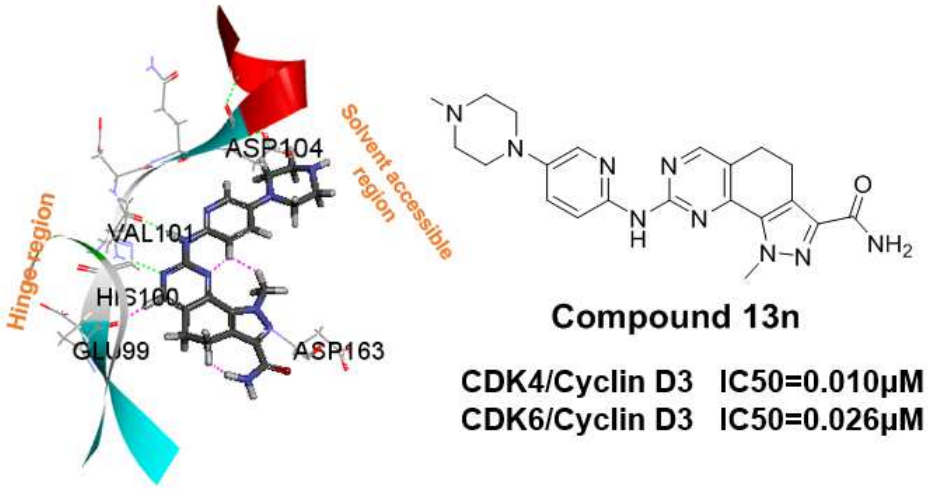
Graphical abstract



Synthesis and SAR of 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline derivatives as potent and selective CDK4/6 inhibitors

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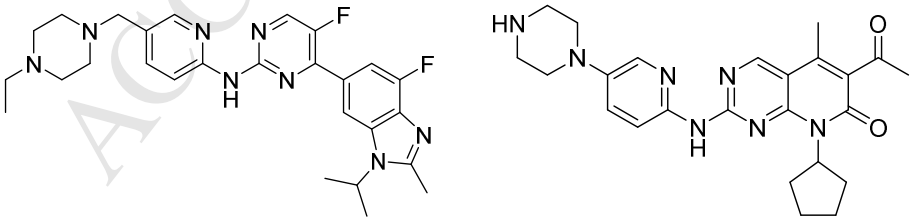
Abstract: CDK4/6 pathway is an attractive target for development of anti-cancer drugs. Herein, we reported the design and synthesis of a series of 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline derivatives as selective CDK4/6 inhibitors. Applied with the optimizing strategy to the initial scaffold, it is found that compound **13n** is able to selectively inhibit CDK4 and CDK6 with IC_{50} values 0.01 and 0.026 μM, respectively. The compound showed good anti-proliferative activity when tested in a panel of tumor cell lines with CDK4/6 related mechanism of action, the results clearly suggest that compound **13n** works much better than Ly2385219 which is a selective CDK4/6 inhibitor. This compound was also found to have favorable pharmacokinetic parameters. Taken together, compound **13n** could be selected for further preclinical evaluation.

Keywords: cyclin dependent kinase, anticancer, cell cycle, kinase selectivity

1. Introduction

CDKs (cyclin dependent kinases) which play a critical role in the process of mitosis are frequently over expressed in human tumors. They are also part of the tumor cell proliferation [1]. There are 21 members in the CDK family, and many of them are popular targets for drug discovery such as CDK1, CDK2, CDK4/6, CDK5, CDK7, CDK8, CDK9. Early efforts to block CDKs with nonselective CDK inhibitors led to little efficacy but with toxicity due to poor selectivity [2]. Result so far indicated that the reason for the clinical failure of pan-CDK inhibitors is too much toxic side effects and the treatment window is too small. Therefore, finding selective CDK inhibitors becomes a direction to reduce the toxic side effects of drugs.

CDK4/6 is an attractive target for development of anti-cancer drugs. Several highly selective CDK4/6 inhibitors such as Ly2385219 [3] and Palbociclib [4] (Fig. 1) have been studied extensively in clinical research. It has been demonstrated that cell cycle arrest would lead to cell apoptosis. Meanwhile, ongoing clinical trials have produced promising data on the potential efficacy of CDK4/6 inhibitors, particularly in treating HR advanced breast cancer, when they improve the response and duration of response to hormonal therapies [5, 6]. It is of great significance to develop novel, oral CDK4/6 inhibitors with good pharmacokinetic properties.



Ly2835219 **Palbociclib**

Figure 1. The structure of Ly2835219 and Palbociclib (CDK4/6 inhibitor)

We had discovered 4,4-dimethyl-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline [7] (Fig. 2) during the literature investigation. The tricyclic system was previously identified as an inhibitor against many kinases such as Aurora A (Aur-A) [8], CDK2 [9] and Polo-like kinase 1 (PLK1) [10]. We used computer-aided drug design software to virtually screen derivatives containing the scaffold. When we introduced 2-aminopyridine fragment contained in

palbociclib at position 8 of 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline (**13l**), we found that Lowest energy quantum mechanical model of compound **13l** and the crystal structures of Palbociclib were well-overlaid (Fig. 2). The similar binding patterns prove that they have similar interaction with CDK4/6. During the course of the study, we used the commercially available Ly2385219 as a control compound to evaluate the anti-tumor effect of our compounds. CDK4/6 is over-expressed in breast cancer MCF-7 and the growth of MCF-7 cells is dependent on CDK4/6. Therefore, we chose MCF-7 to evaluate the inhibitory effect of our compounds on the proliferation of tumor cells. Taken together, We intended to develop a series of 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline derivatives as selective CDK4/6 inhibitors and as anti-tumor agent.

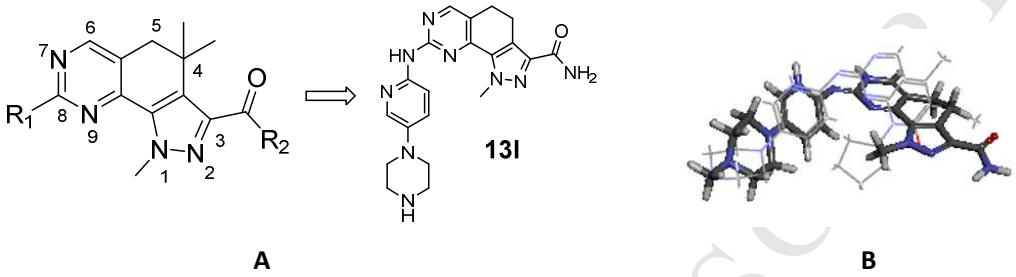
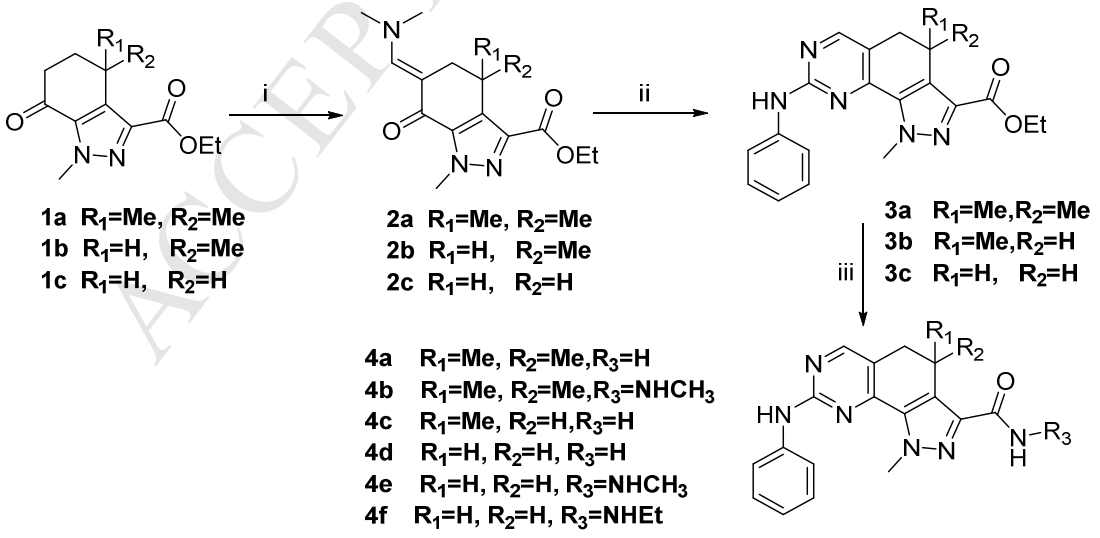


Figure 2. A) The structure of 4,4-dimethyl-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline scaffold. **B**) Lowest energy quantum mechanical model of compound **13l** (thick stick) overlaid with X-ray conformations of Palbociclib (thin stick)

2. Results and Discussion

2.1 Chemistry

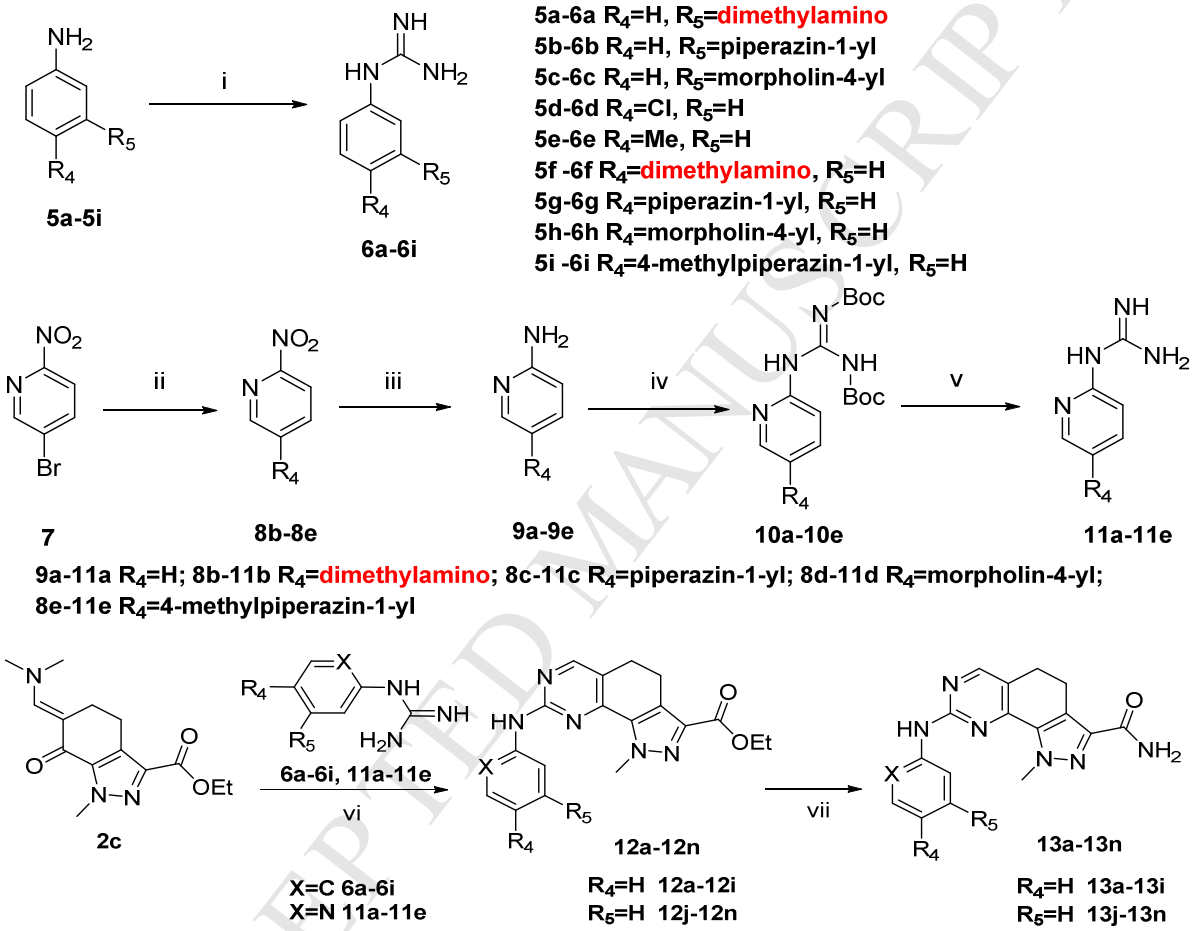
According to the related literatures [11-13], the synthetic route to 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline analogues is shown in Scheme 1. Compounds **1a-1c** reacted with 1,1-dimethoxy-N,N-dimethylmethanamine at 60 □, ethyl 6-[(dimethylamino)methylene]- 7-oxo-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate derivatives **2a-2c** was obtained. The 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline core **3a-3c** was prepared from **2a-2c** and phenylguanidine by cyclization in the presence of dioxane, after hydrolysis and amidation to produce compounds **4a-4f.**



Scheme 1. Synthesis of compound **4a-4f**. Reagents and conditions: (i) N, N-dimethylformamide dimethyl acetal, DMF, 60 □, 4 h, 90%; (ii) Guanidine hydrochloride, K_2CO_3, DMF, 100 □, 80-90%; (iii) 1.5M KOH in 95% EtOH, 70 □; R_3NH_2, HOBT, EDC, DMF, rt, 50-80%.

The synthetic route to different guanidines is shown in Scheme 2. Phenylguanidines **6a-6i** were prepared starting from substituted anilines **5a-5i**, which was reacted with cyanamide. The 5-bromine-2-nitropyridine **7** reacted with 4-methylpiperazine to afford compounds **8a-8e**. Compounds **8a-8e** is reduced to give compounds **9a-9e** by iron powder, and then compounds **9a-9e** is substituted and deprotected to give pyridylguanidines compounds **11a-11e**. Compound **2c** was coupled with various guanidines **6a-6i**, **11a-11e** in the presence of DMF to offer compounds **12a-12n** in 50-70% yield, then treated with the solution of ammonia to produce the target compounds **13a-13n**.

Among the title compounds, fourteen of them **13a-13n** have not been reported.



Scheme 2. Synthesis of compounds **13a-13n**. Reagents and condition: (i) $NCNH_2$, isopropanol, 80 □, 12 h, 60-90%; (ii) DIPEA, acetonitrile, 60 □, 4 h, 50-90%; (iii) Fe, AcOH, DCM, 60 □, 70-90%; (iv) 1,3-di(tert-butyl oxycarbonyl)-2-(trifluoromethyl sulfonyl)guanidine, DCM, room temp, 50-80%. (v) DCM, TFA, room temp, 95%; (vi) dioxane, 90 □, 6 h, 50-90%; (vii) NH_3, EtOH, 80 □, 6 h, 60-90%.

2.2. Biological Activities

Based on the structure of 4,4-dimethyl-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline scaffold, we modified the position 3 and 4 of the 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline at first. Biochemical activity against CDK2/A2, CDK4/D3, CDK6/D3 kinases and in vitro cell proliferation cytotoxicity on MCF-7 cell lines are reported in Table 1. Initial studies showed that both R_1 and R_2 were substituted with H (compound **3c, 4d-4f**) could increase the inhibitory activity to CDK4/6. When both R_1 and R_2 were substituted with methyl, compounds **3a, 4a, 4b** have improved inhibitory activity to CDK2 and no inhibitory activity to CDK4/6. Satisfactorily, it is detrimental not

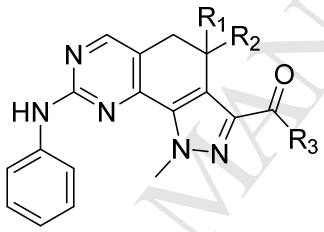
only for CDK2 inhibition (IC_{50} = 0.002 μM) but also for the CDK4/6 (IC_{50} = 0.01/0.06 μM) when R_3 is amino (**4a, 4d**).

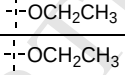
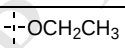
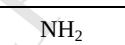
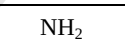
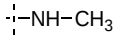
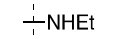
With the R_3 fixed as NH_2, both R_1 and R_2 fixed as H, compound **4d** showing a better activity for CDK4/6 vs CDK2. Next, attention was turned to the phenylamine at the C-8 position of the 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline in an attempt to further improve the potency for CDK4/6.

Activity results reported in Table 2 show that most of modifications at R_5 (**13a-13c**) significantly decreased CDK activity, biochemical activity for the CDK2, 4, 6 kinases and cellular proliferation activity was completely lost. However, encouraging results were obtained by introducing substituent at the position 4 (**13d-13f**). These derivatives (**13d-13f**) not only maintained acceptable CDK2/A2 activity but they also maintained or even improved the selectivity toward CDK4/D3 and CDK6/D3. But the simple dimethyl amide derivative **13f** showed 4-fold loss of activity in the CDK2, 4 biochemical assay and 3-fold reduction in cell proliferation potency compared with compound **13g**. Analysis of the biochemical data reported in Table 2 shows that in a homogeneous series, substitution at positions 4 of the benzene ring was inefficient to increase CDK4/6 activity.

Table 1

Results for inhibition of CDK2, 4, 6 and MCF-7 cell lines by compounds **3a, 3b, 4a-4f**



				IC_{50}[a](μM)			
Compd	R_1	R_2	R_3	CDK2/ Cyclin A2	CDK4/ Cyclin D3	CDK6/ Cyclin D3	MCF-7
3a	Me	Me		0.62	1.0	>10	6.8
3b	Me	H		0.83	>10	>10	NA
3c	H	H		2.00	3.24	7.36	NA
4a	Me	Me	NH_2	0.004	0.010	2.52	NA
4b	Me	Me		0.25	0.450	8.56	6.0
4c	Me	H	NH_2	0.65	5.26	>10	4.3
4d	H	H	NH_2	0.002	0.010	0.058	5.8
4e	H	H		0.037	0.16	1.02	3.1
4f	H	H		0.12	0.45	2.32	NA

[a] Values are means of two or more experiments. NA means data not available.

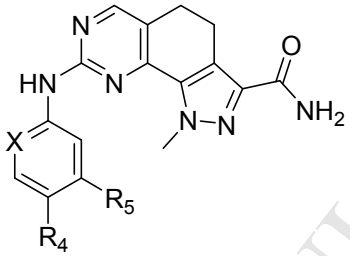
The data confirmed **13g** and **13i** as the most promising compound in terms of cellular activity on MCF-7 cell line, potency against CDK4/D3 (IC_{50} = 0.008/0.003 μM) and CDK6/D3 (IC_{50} = 0.02/0.010 μM). However, there is no significant improvement of selectivity against CDK4/6.

Analysis of reported SAR around palbociclib [14-15] and X-ray structure of palbociclib bound to CDK6 [16], as well as molecular modeling studies, suggested that additional selectivity could be obtained by replacing the aniline with a substituted pyridinylamine. Introduction of the pyridine could enhance selectivity over other kinases via interactions with the side chain of hinge residue His100. Replacing the benzene ring of compounds **4b-4e** with

pyridine motif gave compounds **13k-13n**, which showed increased potency for CDK4/6 as well as improved selectivity over CDK2. It is indeed demonstrated that the selectivity of compound **13l** (selectivity CDK2/CDK4=28.3) is superior to **13g** (selectivity CDK2/CDK4=1.0). The aniline counterpart of **13k-13n** are less selective CDK4/6 inhibitors, as shown with compound **13i**. As shown in table 3, introducing the pyridine group led to the identification of compound **13n** (CDK4 IC_{50}=0.010 μM; CDK6 IC_{50}=0.026 μM), which exhibited a 70-fold selectivity for cdk4 over CDK2. Removal of the piperazine (**13f**) resulted in reduction in potency, selectivity. The piperazine moiety proved to be not only a solubilizing group but also an important selectivity determinant due to deleterious interactions unique to CDK4/6.

Table 2

Results for inhibition of CDK2, 4, 6 and MCF-7 cell lines by compounds **13a-13n**



				IC_{50}[a](μM)			
Compd	X	R_4	R_5	CDK2/ Cyclin A2	CDK4/ Cyclin D3	CDK6/ Cyclin D3	MCF-7
13a	C	H	dimethylamino	1.21	1.85	2.74	NA
13b	C	H	piperazin-1-yl	2.41	5.62	7.76	4.77
13c	C	H	morpholin-4-yl	3.05	3.65	5.79	NA
13d	C	Cl	H	0.04	0.06	0.32	3.72
13e	C	Me	H	0.12	0.25	0.47	NA
13f	C	dimethylamino	H	0.03	0.034	0.045	2.15
13g	C	piperazin-1-yl	H	0.008	0.008	0.02	0.78
13h	C	morpholin-4-yl	H	0.33	0.28	0.35	0.94
13i	C	4-methylpiperazin-1-yl	H	0.001	0.003	0.010	1.05
13j	N	H	dimethylamino	4.20	5.70	>10	NA
13k	N	dimethylamino	H	1.36	0.68	0.55	1.81
13l	N	piperazin-1-yl	H	0.85	0.029	0.036	0.22
13m	N	morpholin-4-yl	H	1.01	0.03	0.04	0.35
13n	N	4-methylpiperazin-1-yl	H	0.70	0.01	0.026	0.19
Ly2835219				0.50	0.008	0.01	0.71

[a] Values are means of two or more experiments. NA means data not available.

Derivatives **13n** and **13m** is proved to be the best compounds of the series, showing anti-proliferative activity in the low micro molar range in the MCF-7 cell line (IC_{50} = 0.19 μM), good activity on CDK4/6 (IC_{50} = 0.010/0.026 μM) and high-level selectivity against CDK2/A3 (IC_{50} = 0.70 μM). Compounds **13n** and **13m** were profiled

against additional cancer cell lines in a 72h proliferation assay (Table 4). The compound resulted active in cells derived from solid tumors showing a cytotoxicity range from 0.19 μM (HCT116) to 2.30 μM (PANC-1). The results clearly suggest that compound **13n** and **13m** works much better than Ly2385219. The compounds were shown good anti-proliferative activity when tested in two tumor cell lines (MCF-7 and HCT116) with CDK4/6 related mechanism of action. Interestingly compounds **13n** and **13m** were also shown anti-proliferative activity in HepG2 and PANC-1 cell lines, which are CDK4/6 unrelated mechanism of action.

Table 3

Results for selective inhibition of CDK2, 4, 6 and MCF-7 cell lines by optimized compounds

Compd	IC_{50}[a](μM)			Selectivity		IC_{50}[a](μM)
	CDK2/ Cyclin A2	CDK4/ Cyclin D3	CDK6/ Cyclin D3	CDK2/CDK4	CDK2/CDK6	MCF-7
4b	0.25	0.45	8.56	0.56	0.03	6.00
4d	0.002	0.01	0.058	0.20	0.03	5.80
4e	0.037	0.16	1.02	0.23	0.04	3.10
13g	0.008	0.008	0.02	1.00	0.80	0.78
13i	0.001	0.003	0.010	0.33	0.10	1.05
13k	1.36	0.68	0.55	2.00	2.50	1.81
13l	0.85	0.029	0.036	28.3	23.6	0.22
13m	1.01	0.03	0.04	33.3	25.2	0.35
13n	0.70	0.01	0.026	70.0	28.1	0.19

[a] Values are means of two or more experiments.

In view of its better overall profile, compounds **13n** and **13m** were selected and further analyzed in pharmacokinetic (PK) experiments. In vivo pharmacokinetic properties were evaluated in mice, following intravenous (iv) and oral (os). Compound **13n** showed pharmacokinetic properties better than **13m** with higher AUC and Cmax (Table 5). Compound **13n** showed a good half-life value and oral bioavailability.

2.3. Molecular docking study

Currently, only the crystal complex structure of CDK6 with its inhibitors have been resolved, and the crystal complex structure of CDK4 with its inhibitors has not been reported [17]. According to related reports [18], CDK6 and CDK4 are high homology, so we used complex structure of CDK6 with Palbociclib (PDB:2EUF) for molecular docking. We used the CDK4/6 inhibitor Palbociclib for molecular docking with CDK6 and found that the docking results were the same as those reported in the literature (docking score is 146). So we used the same method again for our compound **13l** (docking score is 135). The two scores are similar, and the similarity of the binding model prove that they have similar interactions with CDK6.

In the complex structure of CDK6 with **13l** (Fig. 3), as expected, **13l** binds in the ATP-pocket and most of the interactions are similar to these found in the past with a close analog. The pyrimidine core of **13l** formed a pair of donor-acceptor-donor hydrogen bonds with the CDK6 hinge region (Val101), The pyridine could enhance selectivity via interactions with the side chain of hinge residue His100. In addition, the piperazine moiety contributes to the CDK6 activity since it establishes a polar interaction with the side chain of solvent accessible region (Asp104) Compound **13l** occupies the same binding sites. In addition, the oxygen atom of amide forms a hydrogen bond to the main-chain of Asp163.

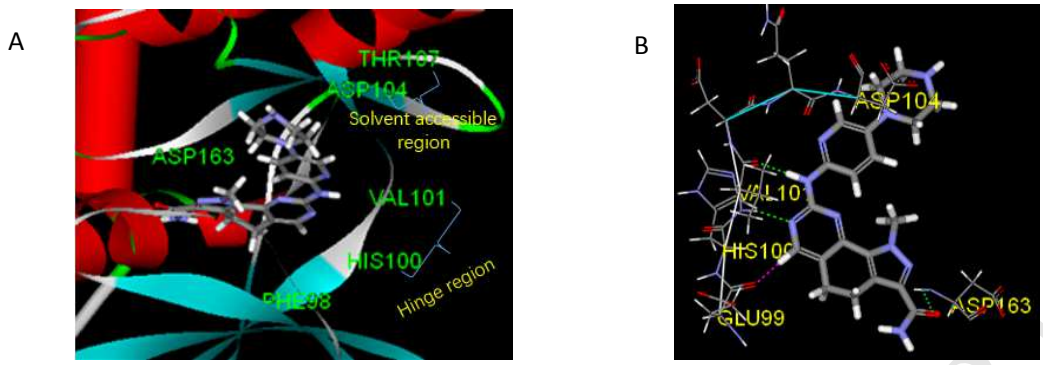


Figure 3. A) the features of binding mode of **13l** in the ATP pocket of CDK6 (PDB:2EUF). B) Compound 13l bound to CDK6. Hydrogen bonds are shown as green dashed lines, residues of CDK6 and compound 13l are shown with carbon, nitrogen and oxygen colored grey, blue and red, respectively.

Table 4

Cell proliferation assay assessing the effects of **13m** and **13n**[a] on different cell lines

compd	Cell proliferation assay, IC_{50} /μM			
	MCF-7	HCT116	HepG2	PANC-1
13m	0.35	0.27	1.58	1.14
13n	0.19	0.13	0.97	2.30
Ly2835219	0.71	0.54	NA	5.94

[a] Values are means of two or more experiments. NA means data not available.

Table 5

PK parameters for compounds **13m** and **13n**[a]

compd	In vivo PK (mouse) 10 mg/kg iv				In vivo PK (mouse) 10 mg/kg os			
	$T_{1/2}$(h)	CL(L/h/kg)	AUC_{∞}(μM h)	V_{ss}	$T_{1/2}$(h)	Cmax	AUC_{∞}(μM h)	*F*(%)
13m	0.94	4.47	5.12	3.60	1.56	0.32	1.14	25.0
13n	1.18	4.18	6.36	4.31	3.97	0.66	2.30	33.2

[a] n=3 animals per study.

3. Conclusion

A series of 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamides were designed and evaluated as CDK4/6 inhibitors. Introduction of a piperazine group on the scaffold through an amide linkage not only improved the solubility but also significantly strengthened the enzymatic inhibitory potency and the cellular activity against MCF-7 cell line. It is now demonstrated that the modification of 4,5-dihydro-1H-pyrazolo[4,3-h]quinazolines including a 2-aminopyridine side chain at the C-8 position could inhibit CDK4/6 with exquisite selectivity. By further optimization, highly potent and selective CDK4/6 inhibitors **13n** was found to have favorable pharmacokinetic parameters, good potency and selectivity profile. These results support further evaluation and development of these compounds for the use as selective CDK4/6 inhibitors for cancer therapy. Studies to explore the mechanism of these compounds are needed and now in progress.

4. Experimental Section

4.1. Chemistry

All chemicals were reagent grade and used as purchased. All reactions were performed under an inert atmosphere of dry argon or nitrogen using distilled dry solvent. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra

were recorded on a Bruker AV□ 400MHz/100MHz spectrometer. The chemical shift values are reported in parts per million (ppm) relative to tetramethylsilane as internal standard in DMSO-*d*6. High-resolution MS data were obtained on an Agilent TOF G6224 mass spectrometer.

*4.1.6. The preparation of compounds **2a-2c***

A mixture of compounds **1a-1c** (2.0 mmol), dimthyl formide dimethylacetal (4.0 mmol) in DMF (10.0 mL) was stirred at 60 □ for 5 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The solvent was evaporated and the residue was purified via column chromatography to afford compounds **2a-2c**.

*4.1.6.1ethyl(Z)-6-((dimethylamino)methylene)-1,4,4-trimethyl-7-oxo-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (**2a**)*. yellow solid. Yield: 65%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.59 (s, 1H, -C=*CH*-), 4.24 (q, J = 6.8 Hz, 2H, -COO*CH*$_2$CH$_3$), 4.24 (s, 3H, -N*CH*$_3$), 3.14 (s, 6H, -N(*CH*$_3$)$_2$), 2.88 (s, 2H, -*CH*$_2$C(CH$_3$)$_2$), 1.43 (s, 6H, -C(*CH*$_3$)$_2$), 1.40 (t, J = 8.0 Hz, 3H, -COOCH$_2$*CH*$_3$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 178.7, 162.6, 150.2, 137.8, 129.2, 103.5, 60.7, 43.8, 39.8.6, 24.5, 21.1, 14.1; HRMS (ESI): calcd for $C_{16}H_{23}N_3O_3$, [(M+H)$^+$], 306.1818, found 306.1802.

*4.1.6.2 ethyl(Z)-6-((dimethylamino)methylene)-1,4-dimethyl-7-oxo-4,5,6,7-tetrahydro-1H-indazole-3-carboxylate (**2b**)*. Yellow solid. Yield: 70%; ^{1}H-NMR($CDCl_3$, 400 MHz) δ: 7.49 (s, 1H, -C=*CH*-), 4.28 (q, J = 6.8 Hz, 2H, -COO*CH*$_2$CH$_3$), 4.24 (s, 3H, -N*CH*$_3$), 3.14 (s, 6H, -N(*CH*$_3$)$_2$), 2.96 (t, J = 6.8 Hz, 1H, -*CH*(CH$_3$)CH$_2$), 2.78 (d, J = 6.8 Hz, 2H, -CH(CH$_3$)*CH*$_2$), 1.42 (d, J = 8.0 Hz, 3H, -CH$_2$CH(*CH*$_3$)-), 1.40-1.44 (m, 3H, -COOCH$_2$*CH*$_3$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 178.7, 162.6, 150.2, 137.8, 129.2, 103.5, 60.7, 43.8, 39.8.6, 24.5, 21.1, 14.1; HRMS (ESI): calcd for $C_{15}H_{21}N_3O_3$, [(M+H)$^+$], 292.1661, found 292.1632.

*4.1.6.3 6-[(dimethylamino)methylene]-1-methyl-7-oxo-4,5,6,7-tetrahydro-1H-pyrazole-3-carboxylate (**2c**)*. Yellow solid. Yield: 68%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.49 (s, 1H, -C=*CH*-N-), 4.28 (q, J = 8.0 Hz, 2H, -COO*CH*$_2$CH$_3$), 4.31 (s, 3H, -N*CH*$_3$), 3.12 (s, 6H, -N(*CH*$_3$)$_2$), 2.90 (t, J = 8.0 Hz, 2H, -C=CCH$_2$*CH*$_2$-), 2.82 (t, J = 8.0 Hz, 2H, -C=C*CH*$_2$CH$_2$-), 1.30 (t, J = 8.0 Hz, 3H, -COOCH$_2$*CH*$_3$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 178.7, 162.6, 150.2, 137.8, 137.4, 129.2, 103.5, 60.8, 43.8, 39.8, 24.5, 21.0, 14.4; HRMS (ESI): calcd for $C_{14}H_{19}N_3O_3$, [(M+H)$^+$], 278.1505, found 278.1502.

*4.1.7. General procedure for the preparation of derivatives **3a-3b**.*

A solution of compounds **1b-2b** (0.24 mmol), phenylguanidine (0.30 mmol) in DMF (2.0 mL) was stirred at 60 ℃ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt, then poured into H_2O (10mL). The aqueous phase was extracted with EtOAc (20.0 mL). The organic phases were then processed in the usual way and chromatographed (5:1 petroleum ether / EtOAc) to yield compounds **3a-3b**.

*4.1.7.1. 1,4,4-trimethyl-8-(phenylamino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxylate (**3a**)*. Yellow solid. Yield: 41%. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.58 (s, 1H, Ar-NH-), 8.40 (s, 1H, -N=CH-), 7.72 (d, J = 8.0 Hz, 2H, H_{Ar}), 7.30 (t, J = 8.0 Hz, 2H, H_{Ar}), 6.97 (t, J = 8.0 Hz, 1H, H_{Ar}), 4.35 (s, 3H, -NCH$_3$), 4.30 (q, J = 8.0 Hz, 2H, -COO*CH*$_2$CH$_3$), 2.71 (s, 2H, -*CH*$_2$C(CH$_3$)$_2$), 1.32 (t, J =8 Hz, 3H, -COOCH$_2$*CH*$_3$), 1.30 (s, 6H, -C(*CH*$_3$)$_2$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 163.9, 159.2, 158.2, 140.9, 140.7, 130.1, 129.0, 121.9, 119.7, 117.3, 32.0, 25.0, 20.6; HRMS (ESI): calcd for $C_{21}H_{23}N_5O_2$, [(M+H)$^+$], 378.1930, found 378.1908.

*4.1.7.2. Ethyl 1,4-dimethyl-8-(phenylamino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxylate (**3b**).* Yellow solid. Yield: 55%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.29 (s, 1H, -N=CH-), 7.57 (d, J = 8.0 Hz, 1H, H_{Ar}), 7.35 (t, J = 8.0 Hz, 2H, H_{Ar}), 7.02 (t, J = 8.0 Hz, 1H, H_{Ar}), 4.46 (q, J = 8.0 Hz, 2H, -COOCH_2CH$_3$), 4.40 (s, 3H, -NCH_3), 3.26 (m, 1H, -CH_2CH(CH_3)-), 2.86 (d, J = 8.6 Hz, 2H, -CH_2CH(CH$_3$)-), 1.43(t, J = 8.6 Hz, 3H, -COOCH_2CH_3), 1.25 (d, J = 8.0 Hz, 3H, -CH(CH_3)-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 163.9, 159.2, 158.2, 140.8, 140.7, 130.1, 128.9, 121.9, 119.7, 117.3, 32.0, 24.9, 20.7; HRMS (ESI): calcd for $C_{20}H_{21}N_5O_2$, [(M+H)$^+$], 364.1773, found 364.1759.

*4.1.8. General procedure for the preparation of derivative **4a**.*

A solution of compound **2a** (0.73g, 2.4 mmol), phenylguanidine (0.41g, 3.0 mmol) in DMF (12.0 mL) was stirred at 90 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt, then poured into H_2O (20mL), filtered. The obtained solid was added to 2M ethanol solution of ammonia (10.0 mL) and the reaction was stirred at 70 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The solvent was evaporated and the residue was purified via column chromatography to afford compound **4a**. Yellow solid. Yield: 45%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.21 (s, 1H, -N=CH-), 7.56 (d, J = 7.8 Hz, 2H, H_{Ar}), 7.35 (t, J = 7.8 Hz, 2H, H_{Ar}), 7.06 (t, J = 7.8 Hz, 1H, H_{Ar}), 4.33 (s, 3H, -NCH_3), 2.72 (s, 2H, -CH_2C(CH_3)$_2$), 1.46 (s, 6H, -CH_2C(CH_3)$_2$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 163.9, 159.2, 158.2, 140.9, 140.7, 130.1, 128.9, 121.9, 119.7, 117.3, 32.0, 25.0, 20.7; HRMS (ESI): calcd for $C_{19}H_{20}N_6O$, [(M+H)$^+$], 349.1777, found 349.1770.

*4.1.9. General procedure for the preparation of derivative **4b**.*

A solution of compound **2a** (73mg, 0.24 mmol), phenylguanidine (41mg, 0.30 mmol) in DMF (2.0 mL) was stirred at 90 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt, then poured into H_2O (10mL), filtered. The obtained solid was added to 2M ethanol solution of methylamine (3.0 mL) and the reaction was stirred at 70 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The solvent was evaporated and the residue was purified via column chromatography (5:1 petroleum ether / EtOAc) to yield compound **4b**. Yellow solid. Yield: 50%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.27 (s, 1H, -N=CH-), 7.57 (d, J = 7.8 Hz, 2H, H_{Ar}), 7.34 (t, J = 7.8 Hz, 2H, H_{Ar}), 7.06 (t, J = 7.8 Hz, 1H, H_{Ar}), 6.91(s, 1H, -CONHCH$_3$), 4.31 (s, 3H, -NCH_3), 2.98 (s, 3H, -CONHCH_3), 2.97(s, 2H, -CH_2C(CH_3)$_2$), 1.46 (s, 6H, -C(CH_3)$_2$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 163.4, 159.3, 157.5, 152.9, 152.5, 142.3, 142.1, 140.8, 135.0, 131.3, 128.9, 121.9, 119.7, 118.2, 41.2, 32.0, 27.4, 26.3; HRMS (ESI): calcd for $C_{20}H_{22}N_6O$, [(M+H)$^+$], 363.1933, found 363.1917.

*4.1.10. General procedure for the preparation of derivative **4c**.*

A solution of compounds **2b** (140mg, 0.24 mmol), phenylguanidine (85mg, 0.30 mmol) in DMF (8.0 mL) was stirred at 90 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt, then poured into H_2O (10mL), filtered. The obtained solid was added to 2M ethanol solution of ammonia (8.0 mL) and the reaction was stirred at 70 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The solvent was evaporated and the residue was purified via column chromatography (5:1 petroleum ether / EtOAc) to afford compound **4c**. Yellow solid. Yield: 48%. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.55 (s, 1H, Ar-NH-), 8.86 (s, 1H, -N=CH-), 8.42 (s, 1H, H_{Ar}), 7.84 (d, J = 8.0 Hz, 2H, H_{Ar}), 7.73 (t, J = 8.0 Hz, 2H, H_{Ar}), 7.30 (t, J = 8.0 Hz, 1H, H_{Ar}), 4.34 (s, 3H, -NCH_3), 3.01 (m, 1H, -CH_2CH(CH_3)-), 2.99 (d, J = 8.6 Hz, 2H, -CH_2CH(CH$_3$)-), 1.25 (d, J = 8.6 Hz, 3H, -CH_2CH(CH_3)-). ^{13}C-NMR (DMSO-d_6, 100MHz): δ 163.9, 159.2, 158.2, 140.9, 140.7, 130.1, 128.9, 121.9,

119.7, 117.3, 37.5, 32.0, 24.9, 20.7; HRMS (ESI): calcd for $C_{18}H_{18}N_6O$, [(M+H)$^+$], 335.1620, found 335.1623.

*4.1.11. General procedure for the preparation of derivative **4d**.*

A solution of compound **2c** (71mg, 0.24 mmol), phenylguanidine (42mg, 0.30 mmol) in DMF (2.0 mL) was stirred at 90 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt, then poured into H_2O (3 mL), filtered. The obtained solid was added to 2M ethanol solution of ammonia (3.0 mL) and the reaction was stirred at 70 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The solvent was evaporated and the residue was purified via column chromatography (5:1 petroleum ether / EtOAc) to afford compound **4d**. Yellow solid. Yield: 60%. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.57 (s, 1H, Ar-NH-), 8.43 (s, 1H, -N=CH-), 7.71 (d, J = 8.0 Hz, 2H, H_{Ar}), 7.30 (d, J = 8.0 Hz, 2H, H_{Ar}), 7.02 (t, J = 8.0 Hz, 1H, H_{Ar}), 4.30 (s, 3H, -NCH_3), 2.99 (t, J = 6.8 Hz, 2H, -N=CHCCH$_2$*CH*$_2$-), 2.89 (t, J = 6.8 Hz, 2H, -N=CHC*CH*$_2$CH$_2$-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 163.4, 159.3, 157.5, 140.8, 134.9, 131.3, 129.0, 121.9, 119.7, 118.2, 32.0, 27.4, 26.3; HRMS (ESI): calcd for $C_{17}H_{16}N_6O$, [(M+H)$^+$], 321.1464, found 321.1460.

*4.1.12. General procedure for the preparation of derivative **4e**.*

A solution of compound **2c** (70mg, 0.24 mmol), phenylguanidine (45mg, 0.30 mmol) in DMF (2.0 mL) was stirred at 90 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt, then poured into H_2O (3 mL), filtered. The obtained solid was added to 2M ethanol solution of methylamine (3.0 mL) and the reaction was stirred at 70 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The solvent was evaporated and the residue was purified via column chromatography (5:1 petroleum ether / EtOAc) to afford compound **4e**. Yellow solid. Yield: 54%. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.43 (s, 1H, Ar-NH-), 8.86 (s, 1H, -N=CH-), 7.74 (d, J = 8.0 Hz, 2H, H_{Ar}), 7.30 (t, J = 8.0 Hz, 2H, H_{Ar}), 7.02 (t, J = 8.0 Hz, 1H, H_{Ar}), 4.30 (s, 3H, -NCH_3), 2.99 (t, J = 6.8 Hz, 2H, -N=CHCCH$_2$*CH*$_2$-), 2.89 (t, J = 6.8 Hz, 2H, -N=CHC*CH*$_2$CH$_2$-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 166.8, 162.7, 161.0, 159.1, 138.9, 138.6, 129.5, 125.3, 125.1, 122.4, 117.8, 116.2, 37.5, 28.3, 26.3, 18.7; HRMS (ESI): calcd for $C_{18}H_{18}N_6O$, [(M+H)$^+$], 335.1620, found 335.1615.

*4.1.13. General procedure for the preparation of derivative **4f**.*

A solution of compound **2c** (0.14g, 0.48 mmol), phenylguanidine (83mg, 0.60 mmol) in DMF (8.0 mL) was stirred at 60 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt, then poured into H_2O (20mL), filtered. The obtained solid was added to 2M ethanol solution of ethylamine (10.0 mL) and the reaction was stirred at 80 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The solvent was evaporated and the residue was purified via column chromatography (5:1 petroleum ether / EtOAc) to afford compound **4f**. Yellow solid. Yield: 55%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.29 (s, 1H, -N=CH-), 7.57 (d, J = 8.0 Hz, 2H, H_{Ar}), 7.35 (t, J = 8.0 Hz, 2H, H_{Ar}), 7.02 (t, J = 8.0 Hz, 1H, H_{Ar}), 4.46 (q, J = 7.4 Hz, 2H, -CONH*CH*$_2$CH$_3$), 4.40 (s, 3H, -NCH_3), 3.10 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$*CH*$_2$-), 2.89 (t, J = 8.0 Hz, 2H, -N=CHC*CH*$_2$CH$_2$-), 1.43 (t, J = 8.0 Hz, 3H, -CONHCH$_2$*CH*$_3$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 166.8, 162.7, 160.7, 159.1, 138.9, 138.6, 129.5, 125.3, 125.1, 122.4, 117.8, 116.2, 37.5, 34.2, 28.3, 18.7, 15.0; HRMS (ESI): calcd for $C_{19}H_{20}N_6O$, [(M+H)$^+$], 349.1777, found 349.1760.

*4.1.1. The preparation of compounds **6a-6i***

A mixture of compounds **5a-5i** (10.0 mmol), concentrated hydrochloric acid (12.2 mmol) and melamine (20.0

mmol) in isopropyl alcohol (10.0 mL) was refluxed for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The reaction mixture was quenched in saturated sodium carbonate solution (20.0 mL) and filtered. The solid was washed with water (50.0 mL). The crude product thus obtained was dried at 50 □ under reduced pressure to get compounds **6a-6i**.

*4.1.1.1 1-[3-(dimethylamino) phenyl] guanidine (**6a**)*. White solid. Yield: 75%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.29 (d, J = 7.9 Hz, 1H, H_{Ar}),7.22 (s, 1H, H_{Ar}), 7.15 (t, J = 8.1 Hz, 1H, H_{Ar}), 6.58 (d, J = 7.8 Hz, 1H, H_{Ar}), 3.02 (s, 6H, Ar-N(CH_3)$_2$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 157.1, 148.9, 134.6, 130.9, 114.4, 109.7, 42.4; HRMS (ESI): calcd for $C_9H_{14}N_4$, [(M+H)$^+$], 179.1297, found 179.1291.

*4.1.1.2 1-[3-(4-methylpiperazin-1-yl) phenyl] guanidine (**6b**)*. White solid. Yield: 50%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 6.83 (t, J = 7.9 Hz, 1H, H_{Ar}), 6.16 – 6.08 (m, 2H, H_{Ar}), 6.03 (d, J = 7.4 Hz, 1H, H_{Ar}), 4.80 (s, 2H, -NH_2), 3.05 – 2.97 (m, 4H, Ar-NCH_2CH$_2$N), 2.44 – 2.37 (m, 4H, Ar-NCH$_2$$CH_2$N), 2.20 (s, 3H, -$NCH_3$). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ152.5, 149.7, 129.6, 106.1, 104.6, 101.9, 55.2, 48.7, 46.2; HRMS (ESI): calcd for $C_{11}H_{17}N_5$, [(M+H)$^+$], 220.1562, found 220.1550.

*4.1.1.3 1-(3-morpholinophenyl)guanidine (**6c**)*. White solid. Yield: 55%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 6.85 (t, J = 7.9 Hz, 1H, H_{Ar}), 6.16 – 6.02 (m, 3H, H_{Ar}), 4.84 (s, 2H, -NH_2), 3.73 – 3.62 (m, 4H, Ar-NCH_2CH$_2$O), 3.03 – 2.92 (m, 4H, Ar-NCH$_2$$CH_2$O). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ 152.6, 149.7, 129.7, 106.4, 104.3, 101.6, 66.7, 49.2; HRMS (ESI): calcd for $C_{11}H_{16}N_4O$, [(M+H)$^+$], 221.1402, found 221.1389.

*4.1.1.4 1-(4-chlorophenyl)guanidine (**6d**)*. White solid. Yield: 80%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.29 (d, J = 7.9 Hz, 1H, H_{Ar}),7.22 (s, 1H, H_{Ar}), 7.15 (t, J = 8.1 Hz, 1H, H_{Ar}), 6.58 (d, J = 7.8 Hz, 1H, H_{Ar}). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 157.1, 149.1, 145.3, 130.4, 105.8, 104.3, 98.0, 54.0, 41.3; HRMS (ESI): calcd for $C_7H_8N_3Cl$, [(M+H)$^+$], 170.0407, found 170.0401.

*4.1.1.5 1-(p-tolyl)guanidine (**6e**)*. White solid. Yield: 80%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: δ 7.07 (d, J = 8.0 Hz, 2H, H_{Ar}), 6.81 (d, J = 7.8 Hz, 2H, H_{Ar}), 2.25 (s, 3H, Ar-CH_3). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 157.1, 149.1, 145.3, 130.4, 105.8, 104.3, 98.0, 54.0, 41.3; HRMS (ESI): calcd for $C_8H_{11}N_3$, [(M+H)$^+$], 150.1031, found 150.1025.

*4.1.1.6 1-[4-(dimethylamino) phenyl] guanidine (**6f**)*. White solid. Yield: 65%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 6.56 (d, J = 8.7 Hz, 2H, H_{Ar}), 6.49 (d, J = 8.7 Hz, 2H, H_{Ar}), 4.38 (s, 2H, -NH_2), 2.68 (s, 6H, -N(CH_3)$_2$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 143.4, 140.7, 115.8, 115.6, 42.3; HRMS (ESI): calcd for $C_9H_{14}N_4$, [(M+H)$^+$], 179.2470, found 179.2465.

*4.1.1.7 1-[4-(piperazin-1-yl) phenyl] guanidine (**6g**)*. White solid. Yield: 45%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.91 (s, 1H, Ar-NH-), 7.82(s, 1H, -*NH*=C-), 6.65(d, J = 8.0 Hz, 2H, H_{Ar}), 6.63(s, 2H, -NH=CNH_2), 6.49(d, J = 8.0 Hz, 2H, H_{Ar}), 3.45 (t, J = 8.0 Hz, 4H, Ar-NCH_2CH$_2$NH), 2.35 (t, J = 8.0 Hz, 4H, Ar-NCH$_2$$CH_2$NH). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ 157.2, 139.6, 128.1, 117.3, 113.5, 57.2, 52.0, 46.6; HRMS (ESI): calcd for $C_{11}H_{17}N_5$, [(M+H)$^+$], 220.1562, found 220.1555.

*4.1.1.8 1-(4-morpholinophenyl)guanidine (**6h**)*. White solid; Yield: 40%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.95 (s, 1H, Ar-NH-), 7.83(s, 1H, -*NH*=C-), 6.65(d, J = 7.8 Hz, 2H, H_{Ar}), 6.63(s, 2H, -NH=CNH_2), 6.49(d, J = 7.8 Hz,

2H, H_{Ar}), 3.44 (t, *J* = 8.0 Hz, 4H, Ar-N*CH*$_2$CH$_2$O), 2.35 (t, *J* = 8.0 Hz, 4H, Ar-NCH$_2$*CH*$_2$O). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 157.1, 139.6, 128.0, 117.2, 113.6, 57.2, 52.0, 46.6; HRMS (ESI): calcd for $C_{11}H_{16}N_4O$, [(M+H)$^+$], 221.1402, found 221.1400.

*4.1.1.9 1- [4-(4-methylpiperazin-1-yl) phenyl] guanidine (**6i**)*. White solid. Yield: 45%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.93 (s, 1H, Ar-NH-), 7.84(s, 1H, -*NH*=C-), 6.65(d, 2H, H_{Ar}), 6.63(s, 2H, -NH=C*NH*$_2$), 6.49(d, 2H, H_{Ar}), 3.44 (t, *J* = 8.0 Hz, 4H, Ar-N*CH*$_2$CH$_2$N), 2.35 (t, *J* = 8.0 Hz, 4H, Ar-NCH$_2$*CH*$_2$O), 2.21 (s, 3H, -NCH$_2$CH$_2$N*CH*$_3$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 157.1, 139.6, 128.0, 117.2, 113.6, 57.2, 52.0, 46.6; HRMS (ESI): calcd for $C_{12}H_{19}N_5$, [(M+H)$^+$], 234.1719, found 234.1712.

*4.1.2. The preparation of compounds **8c-8e***

A mixture of compound **7** (24.7 mmol), morpholine or substituted piperazine (29.2 mmol) and DIPEA (37.1 mmol) in acetonitrile (100 mL) was refluxed for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The solvent was evaporated and the residue was purified via column chromatography to afford compounds **8c**-**8e**.

*4.1.2.1 1-(6-nitropyridin-3-yl)piperazine (**8c**)*. Yellow solid. Yield: 82%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.96 (d, *J* = 9.3 Hz, 1H, Pyr-H), 7.88 (d, *J* = 2.9 Hz, 1H, Pyr-H), 7.26 (dd, *J* = 9.3, 3.0 Hz, 1H, Pyr-H), 3.64 (t, 4H, Pyr-N*CH*$_2$CH$_2$N), 3.32 (t, 4H, Pyr-NCH$_2$*CH*$_2$N). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 151.7, 146.2, 136.6, 125.9, 118.4, 51.3, 45.8; HRMS (ESI): calcd for $C_9H_{12}N_4O_2$, [(M+H)$^+$], 209.1039, found 209.1030.

*4.1.2.2 1-(6-nitropyridin-3-yl)morpholine (**8d**)*. Yellow solid. Yield: 80%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.95 (d, *J* = 9.3 Hz, 1H, Pyr-H), 7.88 (d, *J* = 2.9 Hz, 1H, Pyr-H), 7.25 (dd, *J* = 9.3, 3.0 Hz, 1H, Pyr-H), 3.60 (t, 4H, Pyr-N*CH*$_2$CH$_2$O), 3.32 (t, 4H, Pyr-NCH$_2$*CH*$_2$O). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 151.7, 146.2, 136.6, 125.9, 118.4, 86.3, 53.3; HRMS (ESI): calcd for $C_9H_{11}N_3O_3$, [(M+H)$^+$], 210.0879, found 210.0868.

*4.1.2.3 1-methyl-4-(6-nitropyridin-3-yl)piperazine (**8e**)*. Yellow solid. Yield: 85%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.08 (d, *J* = 12.0, 6.1 Hz, 2H, H_{Ar}), 7.13 (dd, *J* = 9.2, 3.0 Hz, 1H, H_{Ar}), 3.46 (t, 4H, Pyr-N*CH*$_2$CH$_2$N), 2.56 (t, 4H, Pyr-NCH$_2$*CH*$_2$N), 2.40 (s, 3H, -NCH$_3$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 149.9, 133.7, 120.4, 119.7, 52.25, 52.0, 46.7, 11.9; HRMS (ESI): calcd for $C_{10}H_{14}N_4O_2$, [(M+H)$^+$], 223.1195, found 223.1189.

*4.1.3. The preparation of compounds **9c-9e***

A mixture of compounds **8c**-**8e** (26.0 mmol) in acetic acid (10 mL) and methyl alcohol (40 mL) was refluxed, iron (78.0 mmol) was added. The reaction mixture was heated at 60 □ for 2 h. The reaction mixture was poured into saturated aqueous sodium carbonate (100 mL) and extracted with ethyl acetate (3*50 mL), then dried over sodium sulfate, filtered and concentrated. The crude product was purified via column chromatography to afford compounds **9c**-**9e**.

*4.1.3.1 5-(piperazin-1-yl)pyridin-2-amine (**9c**)*. White solid. Yield: 68%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.62 (d, *J* = 2.7 Hz, 1H, Pyr-H), 7.17 (dd, *J* = 8.9, 3.0 Hz, 1H, Pyr-H), 6.40 (d, *J* = 8.8 Hz, 1H, Pyr-H), 5.44 (s, 2H, -NH$_2$), 3.43 (t, *J* = 4.0 Hz, 4H, Pyr-N*CH*$_2$CH$_2$N), 2.85 (t, *J* = 4.0 Hz, 4H, Pyr-NCH$_2$*CH*$_2$N). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 163.0, 162.6, 149.4, 146.9, 133.7, 122.1, 120.3, 43.4, 42.6; HRMS (ESI): calcd for $C_9H_{14}N_4O$, [(M+H)$^+$], 179.1297, found 179.1285.

*4.1.3.2 5-morpholinopyridin-2-amine (**9d**)*. White solid. Yield: 55%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.60 (d, J = 2.7 Hz, 1H, Pyr-H), 7.15 (dd, J = 8.9, 3.0 Hz, 1H, Pyr-H), 6.37 (d, J = 8.8 Hz, 1H, Pyr-H), 5.42 (s, 2H, -NH_2), 3.41 (t, J = 4.0 Hz, 4H, Pyr-N$CH_2$$CH_2$O), 2.83 (t, J = 4.0 Hz, 4H, Pyr-NC$H_2$$CH_2$O). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ 163.0, 162.6, 149.4, 146.9, 133.7, 122.1, 120.3, 43.4, 42.6; HRMS (ESI): calcd for $C_9H_{13}N_3O$, [(M+H)$^+$], 180.1137, found 180.1130.

*4.1.3.3 5-(4-methylpiperazin-1-yl)pyridin-2-amine (**9e**)*. White solid. Yield: 60%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.60 (d, J = 4.0 Hz, 1H, Pyr-H), 7.16 (dd, J_1 = 4.0 Hz, J_2 =4.0 Hz, 1H, Pyr-H), 6.40 (d, J = 8.0 Hz, 1H, Pyr-H), 5.40 (s, 2H, -NH_2), 2.91 (t, J = 4.0 Hz, 4H, Pyr-N$CH_2$$CH_2$N), 2.42 (t, J = 4.0 Hz, 4H, Pyr-NC$H_2$$CH_2$N), 2.20 (s, 3H, -$NCH_3$). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ 153.0, 140.6, 137.0, 128.9, 109.2, 55.1, 50.7, 46.1; HRMS (ESI): calcd for $C_{10}H_{16}N_4$, [(M+H)$^+$], 193.1426, found 193.1453.

*4.1.4. The preparation of compounds **10a-10e***

A mixture of compounds **9a-9e** (10.0 mmol), TEA (15.6 mmol) and 1,3-di-Boc-2-(trifluoromethyl sulfonyl) guanidine 10.0 mmol) in DCM (30.0 mL) was stirred at room temperature for 48 h. The solvent was evaporated and the residue was purified via column chromatography to afford compounds **10a-10e**.

*4.1.4.1 1,3-di(tert-butyl oxycarbonyl) -2-(pyridine-2-amine)formamidine (**10a**)*. White solid. Yield: 55%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.68 (s, 1H, Pyr-NH), 7.86 (s, 1H, Pyr-H), 7.23 (d, J = 7.35 Hz, 1H, Pyr-H), 6.63 (s, 1H, Pyr-H), 6.53 (s, 1H, Pyr-H), 1.42 (s, 18H, -C=N-Boc + NH-Boc). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 158.5, 158.0, 153.7, 148.1, 138.3, 117.9, 109.7, 27.4; HRMS (ESI): calcd for $C_{16}H_{24}N_4O_4$, [(M+H)$^+$], 337.1876, found 337.1868.

*4.1.4.2 1,3-di(tert-butyl oxycarbonyl) -2-[5-(dimethylamino)pyridine-2-amine]formamidine (**10b**)*. White solid. Yield: 75%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.68 (s, 1H, Pyr-NH), 7.95 (s, 1H, Pyr-H), 7.25 (d, J = 7.5 Hz, 1H, Pyr-H), 6.63 (s, 1H, Pyr-H), 2.92 (s, 6H, -$N(CH_3)_2$), 1.48 (s, 18H, -C=N-Boc + -NH-Boc). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 158.9, 157.7, 153.6, 147.7, 134.5, 133.2, 127.7, 110.4, 84.7, 79.8, 41.4, 28.1; HRMS (ESI): calcd for $C_{18}H_{29}N_5O_4$, [(M+H)$^+$], 380.2298, found 380.2290.

*4.1.4.3 1,3-di(tert-butyl oxycarbonyl) -2-[5-(piperazin-1-yl)pyridine-2-amine]formamidine (**10c**)*. White solid. Yield: 65%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.63 (s, 1H, Pyr-NH), 7.95 (s, 1H, Pyr-H), 7.24 (d, J = 7.5 Hz, 1H, Pyr-H), 6.63 (s, 1H, Pyr-H), 3.62 (t, J = 4.8 Hz, 4H, Pyr-N$CH_2$$CH_2$N), 3.04 (t, J = 7.1 Hz, 4H, Pyr-NC$H_2$$CH_2$N), 1.50 (s, 9H, -C=N-Boc), 1.45 (s, 9H, -NH-Boc). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 158.3, 157.5, 153.6, 147.6, 134.5, 133.2, 127.7, 110.4, 84.7, 79.8, 66.4, 53.1, 28.0; HRMS (ESI): calcd for $C_{20}H_{32}N_6O_4$, [(M+H)$^+$], 421.2563, found 421.2555.

*4.1.4.4 1,3-di(tert-butyl oxycarbonyl) -2-[5-(morpholino-1-yl)pyridine-2-amine]formamidine (**10d**)*. White solid. Yield: 78%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.61 (s, 1H, Pyr-NH), 7.98 (s, 1H, Pyr-H), 7.26 (d, J = 7.5 Hz, 1H, Pyr-H), 6.65 (s, 1H, Pyr-H), 3.65 (t, J = 4.8 Hz, 4H, Pyr-N$CH_2$$CH_2$O), 3.08 (t, J = 7.1 Hz, 4H, Pyr-NC$H_2$$CH_2$O), 1.53 (s, 9H, -C=N-Boc), 1.45 (s, 9H, -NH-Boc). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 158.5, 158.0, 153.7, 147.8, 134.5, 133.2, 127.7, 110.8, 84.8, 79.8, 66.5, 53.0, 28.4; HRMS (ESI): calcd for $C_{20}H_{31}N_5O_5$, [(M+H)$^+$], 422.2403, found 422.2389.

*4.1.4.5 1,3-di(tert-butyl oxycarbonyl) -2-[5-(4-methylpiperazin-1-yl)pyridine-2-amine]formamidine (**10e**)*. White solid. Yield: 75%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.21 (s, 1H, Pyr-H), 7.98 (s, 1H, Pyr-H), 7.26 (d, J = 7.5 Hz, 1H,

Pyr-H), 3.18 (t, *J* = 4.8 Hz, 4H, Pyr-N*CH*$_2$CH$_2$N), 2.58 (t, *J* = 7.1 Hz, 4H, Pyr-NCH$_2$*CH*$_2$N), 2.36 (s, 3H, -NCH$_3$), 1.53 (s, 9H, -C=N-Boc), 1.51 (s, 9H, -NH-Boc). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 158.5, 158.0, 153.7, 147.7, 135.3, 134.5, 122.7, 110.8, 84.6, 79.7, 57.2, 52.0, 46.6, 28.4; HRMS (ESI): calcd for $C_{21}H_{34}N_6O_4$, [(M+H)$^+$], 435.2720, found 435.2712.

4.1.5. The preparation of compounds ***11a-11e***

A mixture of compounds **10a-10e** (2.0 mmol), TFA (3 mL) in DCM (10.0 mL) was stirred for 3 h. The solvent was evaporated and the reaction mixture was poured into saturated aqueous sodium carbonate (100 mL) and extracted with ethyl acetate (3*50 mL), then dried over sodium sulfate, filtered and concentrated. The crude product was purified via column chromatography to afford compounds **11a-11e**.

*4.1.5.1 1-(pyridin-2-yl)guanidine (**11a**)*.White solid. Yield: 85%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.06 (d, *J* = 3.7 Hz, 1H, Pyr-H), 7.46 (t, *J* = 7.7 Hz, 1H, Pyr-H), 6.90 (s, 3H, Pyr-H), 6.66 (dd, *J* = 14.7, 7.4 Hz, 2H, -NH$_2$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 144.2, 142.7, 134.5, 129.5, 115.4, 52.8, 46.4, 42.8; HRMS (ESI): calcd for $C_{11}H_{18}N_6$, [(M+H)$^+$], 137.0827, found 137.0816.

*4.1.5.2 1-(5-(dimethylamino)pyridin-2-yl)guanidine (**11b**)*. White solid. Yield: 87%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.01 (d, *J* = 9.0 Hz, 1H, Pyr-H), 7.92 (d, *J* = 3.4 Hz, 1H, Pyr-H), 7.30 (d, *J* = 8.4 Hz, 1H, Pyr-H), 3.12 (s, 6H, -N(CH$_3$)$_2$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 178.7, 162.6, 150.2, 137.8, 137.4, 129.2, 103.5, 60.8, 43.8, 39.8, 24.5, 21.0, 14.4; HRMS (ESI): calcd for $C_{14}H_{19}N_3O_3$, [(M+H)$^+$], 180.1249, found 180.1243.

*4.1.5.3 1-(5-(piperazin-1-yl)pyridin-2-yl)guanidine (**11c**)*. White solid. Yield: 82%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.96 (d, *J* = 9.3 Hz, 1H, Pyr-H), 7.88 (d, *J* = 2.9 Hz, 1H, Pyr-H), 7.26 (dd, *J* = 9.3, 3.0 Hz, 1H, Pyr-H), 3.67 (t, *J* = 5.1 Hz, 4H, Pyr-N*CH*$_2$CH$_2$N), 3.36 (t, *J* = 5.5 Hz, 4H, Pyr-NCH$_2$*CH*$_2$N). ^{13}C-NMR (DMSO-d_6, 100 MHz): 145.3, 142.8, 135.5, 129.3, 117.8, 114.9, 114.6, 46.4, 42.9; HRMS (ESI): calcd for $C_{10}H_{16}N_6$, [(M+H)$^+$], 221.1515, found 221.1510.

*4.1.5.4 1-(5-morpholinopyridin-2-yl)guanidine (**11d**)*. White solid. Yield: 89%; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.95 (d, *J* = 2.6 Hz, 1H, Pyr-H), 7.48 (dd, *J* = 9.0, 2.6 Hz, 1H, Pyr-H), 6.88 (d, *J* = 9.0 Hz, 1H, Pyr-H), 3.80 (t, 4H, Pyr-N*CH*$_2$CH$_2$O), 3.11 (t, 4H, Pyr-NCH$_2$*CH*$_2$O). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 178.7, 162.6, 150.2, 137.8, 137.4, 129.2, 103.5, 60.8, 43.8, 39.8, 24.5, 21.0, 14.4; HRMS (ESI): calcd for $C_{10}H_{15}N_5O$, [(M+H)$^+$], 222.1355, found 222.1302.

*4.1.5.5 1-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)guanidine (**11e**)*. White solid. Yield: 90%; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.91 (d, *J* = 4. 0 Hz, 1H, Pyr-H), 7.84 (dd, J_1 = 4.0 Hz, J_2 = 4.0 Hz, 1H, Pyr-H), 6.92 (d, *J* = 8.0 Hz, 1H, Pyr-H), 3.67 (t, *J* = 12.0 Hz, 4H, Pyr-N*CH*$_2$CH$_2$N), 3.14 (t, *J* = 4.0 Hz, 4H, Pyr-NCH$_2$*CH*$_2$N), 2.82 (s, 3H, -NCH$_3$). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 144.2, 142.7, 134.5, 129.5, 115.4, 52.8, 46.4, 42.8; HRMS (ESI): calcd for $C_{11}H_{18}N_6$, [(M+H)$^+$], 235.1671, found 235.1642.

4.1.14. General procedure for the preparation of derivatives ***13a-13n***.

A solution of compounds **2c** (0.24 mmol), substituted phenylguanidines (**6a-6i**, **11a-11e**) (0.30 mmol) in DMF (2.0 mL) was stirred at 90 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt, then poured into H_2O (10mL). The aqueous phase was extracted with EtOAc (20.0 mL). The organic phases were then processed in the usual way and chromatographed

(5:1 petroleum ether / EtOAc) to yield compounds **12a-12n**. Then compounds **12a-12n** (0.12 mmol) were added to 2M ethanol solution of ammonia (5.0 mL) and the reaction was stirred at 70 □ for 8 h. Progress of reaction was monitored by tlc and after complete conversion of starting material reaction mixture was cooled to rt. The solvent was evaporated and the residue was purified via column chromatography to afford compounds **13a-13n**.

4.1.14.1.8-((3-(dimethylamino)phenyl)amino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide ***(13a)****.* Yellow solid. Yield: 60%. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.19 (s, 1H, Ar-NH-), 8.32 (s, 1H, -N=CH-), 7.45 (d, J = 8.0 Hz, 1H, H_{Ar}), 6.73 (d, J = 8.0 Hz, 1H, H_{Ar}), 6.60 (d, J = 8.0 Hz, 1H, H_{Ar}), 4.30 (s, 3H, -NCH_3), 3.02 (s, 6H, Ar-N(CH_3)$_2$), 2.97 (t, J = 8.6 Hz, 2H, -N=CHCCH_2CH$_2$-), 2.89 (t, J = 8.6 Hz, 2H, -N=CHCCH$_2$$CH_2$-). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ 162.2, 159.6, 146.9, 138.0, 130.6, 126.2, 121.7, 117.8, 113.3, 66.8, 60.7, 24.5, 19.8, 14.7; HRMS (ESI): calcd for $C_{19}H_{21}N_7O$, [(M+H)$^+$], 364.1886, found 364.1882.

4.1.14.2.1-methyl-8-((3-(piperazin-1-yl)phenyl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide ***(13b).*** Yellow solid. Yield: 46%. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.43 (s, 1H, Ar-NH-), 8.86 (s, 1H, -N=CH-), 8.18 (s, 2H, -$CONH_2$), 7.13 (d, J = 8.0 Hz, 1H, H_{Ar}), 6.97 (d, J = 8.0 Hz, 1H, H_{Ar}), 6.60 (d, J = 8.0 Hz, 1H, H_{Ar}), 6.38 (s, 1H, H_{Ar}), 3.95 (s, 3H, -NCH_3), 3.46 (t, J = 8.0 Hz, 4H, Ar-NCH_2CH$_2$N), 2.99 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$$CH_2$-), 2.89 (t, J = 8.0 Hz, 2H, -N=CHCCH_2CH$_2$-), 2.78 (t, J = 8.0 Hz, 4H, Ar-NCH$_2$$CH_2$N). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ 166.8, 162.7, 161.6, 159.1, 149.1, 143.3, 138.6, 130.4, 125.3, 125.1, 116.2, 107.3, 104.3, 100.7, 54.0, 45.8, 37.5, 28.3, 18.7; HRMS (ESI): calcd for $C_{21}H_{24}N_8O$, [(M+H)$^+$], 405.2151, found 405.2142.

4.1.14.3.1-methyl-8-((3-morpholinophenyl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide ***(13c)****.* Yellow solid. Yield: 45%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.40 (s, 1H, Ar-NH-), 8.41 (s, 1H, -N=CH-), 7.28 (d, J = 7.8 Hz, 2H, H_{Ar}), 7.22 (s, 1H, H_{Ar}), 7.15 (d, J = 7.8 Hz, 1H, H_{Ar}), 6.59 (d, J = 8.0 Hz, 1H, H_{Ar}), 4.36 (s, 3H, -NCH_3), 3.75 (t, J = 8.0 Hz, 4H, Ar-NCH_2CH$_2$O), 3.08 (t, J = 8.0 Hz, 4H, Ar-NCH$_2$$CH_2$O), 2.99 (t, J = 4.0 Hz, 2H, -N=CHCCH$_2$$CH_2$-), 2.89 (t, J = 4.0 Hz, 2H, -N=CHCCH_2CH$_2$-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 162.1, 159.2, 157.3, 152.7, 152.0, 141.5, 138.0, 136.5, 129.3, 126.3, 118.8, 111.2, 109.5, 106.8, 66.8, 66.6, 60.6, 49.2; HRMS (ESI): calcd for $C_{21}H_{23}N_7O_2$, [(M+H)$^+$], 406.1992, found 406.1987.

4.1.14.4.8-((4-(dimethylamino)pyridin-2-yl)amino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide ***(13j)****.* Yellow solid. Yield: 58%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.51 (s, 1H, Pyr-NH), 8.41 (s, 2H, -N=CH-), 7.92 (s, 1H, Pyr-H), 7.85 (d, J = 6.8 Hz, 1H, Pyr-H), 7.27 (t, J = 7.4 Hz, 1H, Pyr-H), 7.25 (s, 1H, Pyr-H), 4.36 (s, 3H, -NCH_3), 3.03 (s, 6H, Pyr-N(CH_3)$_2$), 2.99 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$$CH_2$-), 2.89 (t, J = 8.0 Hz, 2H, -N=CHCCH_2CH$_2$-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 162.7, 161.6, 159.1, 153.1, 143.3, 138.6, 130.4, 125.3, 125.1, 116.2, 107.3, 104.3, 100.7, 41.3, 37.5, 28.3, 18.7; HRMS (ESI): calcd for $C_{18}H_{20}N_8O$, [(M+H)$^+$], 365.1838, found 365.1812.

4.1.14.5.1-methyl-8-((4-chlorophenyl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide ***(13d)****.* Yellow solid. Yield: 50%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.72 (s, 1H, Ar-NH-), 8.43 (s, 1H, -N=CH-), 7.75 (d, J = 7.80 Hz, 2H, H_{Ar}), 7.35 (d, J = 7.8 Hz, 2H, H_{Ar}), 4.35 (s, 3H, -NCH_3), 2.97 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$$CH_2$-), 2.85 (t, J = 8.0 Hz, 2H, -N=CHCCH_2CH$_2$-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 162.1, 158.9, 157.4, 152.8, 139.8, 138.0, 136.4, 128.8, 126.5, 125.3, 120.9, 119.3, 60.7, 24.5, 19.6; HRMS (ESI) : calcd for $C_{20}H_{23}N_9O$, [(M+H)$^+$], 406.2104, found 406.2102.

*4.1.14.6.1-methyl-8-(p-tolylamino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (**13e**).* Yellow solid. Yield: 45%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.29 (s, 1H, -N=CH-), 7.65 (d, J = 7.8 Hz, 2H, H_{Ar}), 7.35 (d, J =7.8 Hz, 2H, H_{Ar}), 4.44 (s, 3H, -NCH_3), 3.10 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$*CH*$_2$-), 3.00 (s, 3H, Ar-CH_3), 2.88 (t, J = 8.0 Hz, 2H, -N=CHC*CH*$_2$CH$_2$-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 163.9, 159.2, 158.2, 140.9, 140.7, 130.1, 128.9, 121.9, 119.7, 32.0, 25.0, 20.7; HRMS (ESI): calcd for $C_{20}H_{22}N_8O_2$, [(M+H)$^+$], 407.1944, found 407.1939.

*4.1.14.7.8-((4-(dimethylamino)phenyl)amino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (**13f**).* Yellow solid. Yield: 55%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.19 (s, 1H, Ar-NH-), 8.32 (s, 1H, -N=CH-), 7.45 (d, J = 7.6 Hz, 2H, H_{Ar}), 6.72 (d, J = 7.6 Hz, 2H, H_{Ar}), 4.32 (s, 3H, -NCH_3), 3.02 (s, 6H, -N(CH_3)$_2$), 2.95 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$*CH*$_2$-), 2.80 (t, J = 8.0 Hz, 2H, -N=CHC*CH*$_2$CH$_2$-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 162.2, 159.6, 146.9, 138.0, 130.6, 126.2, 121.7, 117.8, 113.3, 66.8, 60.7, 24.5; HRMS (ESI): calcd for $C_{19}H_{21}N_7O$, [(M+H)$^+$], 364.1886, found 364.1878.

*4.1.14.8.1-methyl-8-((4-(piperazin-1-yl)phenyl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (**13g**).* Yellow solid. Yield: 63%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.30(s, 1H, Ar-NH-), 8.35 (s, 1H, -N=CH-), 7.55 (d, J = 7.8 Hz, 2H, H_{Ar}), 7.00 (d, J = 7.8 Hz, 2H, H_{Ar}), 4.32 (s, 3H, -NCH_3), 3.00 (t, J = 8.0 Hz, 4H, Ar-N*CH*$_2$CH$_2$N), 2.79 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$*CH*$_2$-), 2.89 (t, J = 8.0 Hz, 2H, -N=CHC*CH*$_2$CH$_2$-), 2.78 (t, J = 8.0 Hz, 4H, Ar-NCH$_2$*CH*$_2$N). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 162.2, 159.6, 146.9, 138.0, 130.6, 126.2, 121.7, 117.8, 113.3, 66.8, 60.7, 24.5, 19.8; HRMS (ESI): calcd for $C_{21}H_{24}N_8O$, [(M+H)$^+$], 405.2151, found 405.2148.

*4.1.14.9. 1-methyl-8-((4-morpholinophenyl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (**13h**).* Yellow solid. Yield: 43%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.29 (s, 1H, Ar-NH-), 8.35 (s, 1H, -N=CH-), 7.53 (d, J = 7.8 Hz, 2H, H_{Ar}), 6.90 (d, J = 7.8 Hz, 2H, H_{Ar}), 4.32 (s, 3H, -NCH_3), 3.00 (t, J = 8.0 Hz, 4H, Ar-N*CH*$_2$CH$_2$O), 2.87 (t, J = 8.0 Hz, 4H, Ar-NCH$_2$*CH*$_2$O), 2.99 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$*CH*$_2$-), 2.89 (t, J = 8.0 Hz, 2H, -N=CHC*CH*$_2$CH$_2$-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 166.8, 162.7, 161.6, 159.1, 139.6, 138.6, 128.4, 125.3, 125.1, 118.4, 116.2, 113.6, 66.3, 53.3, 37.5, 28.3; HRMS (ESI): calcd for $C_{21}H_{23}N_7O_2$, [(M+H)$^+$], 406.1992, found 406.1982.

*4.1.14.10 . 8-((5-(dimethylamino)pyridin-2-yl)amino)-1-methyl-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (**13k**).* Yellow solid. Yield: 56%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.51 (s, 1H, Pyr-NH), 8.41 (s, 1H, -N=CH-), 7.87 (s, 1H, Pyr-H), 7.28 (d, J = 7.6 Hz, 1H, Pyr-H), 7.25 (d, J = 7.6 Hz, 1H, Pyr-H), 4.36 (s, 3H, -NCH_3), 3.58 (s, 6H, -N(CH_3)$_2$), 2.98 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$*CH*$_2$-), 2.85 (t, J = 8.0 Hz, 2H, -N=CHC*CH*$_2$CH$_2$-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 162.2, 159.6, 146.9, 138.0, 130.6, 126.2, 121.7, 117.8, 113.3, 66.8, 60.6; HRMS (ESI): calcd for $C_{18}H_{20}N_8O$, [(M+H)$^+$], 365.1838, found 365.1830.

*4.1.14.11.1-methyl-8-((5-(piperazin-1-yl)pyridin-2-yl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (**13l**).* Yellow solid. Yield: 59%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.62 (s, 1H, Pyr-NH), 8.42 (s, 1H, -N=CH-), 7.99 (s, 1H, Pyr-H), 7.97 (d, J = 7.6 Hz, 1H, Pyr-H), 7.41 (d, J = 7.6 Hz, 1H, Pyr-H), 4.36 (s, 3H, -NCH_3), 3.02 (t, J = 4.8 Hz, 4H, Pyr-N*CH*$_2$CH$_2$N), 2.96 (t, J = 8.0 Hz, 2H, -N=CHCCH$_2$*CH*$_2$-), 2.89 (t, J = 4.8 Hz, 2H, -N=CHC*CH*$_2$CH$_2$-), 2.84 (t, J = 4.8 Hz, 4H, Pyr-NCH$_2$*CH*$_2$N). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 157.6, 153.2, 151.8, 148.5, 141.2, 138.3, 133.9, 131.9, 121.9, 121.4, 114.7, 108.2, 56.2, 50.2, 44.9, 41.4, 35.8; HRMS (ESI): calcd for $C_{19}H_{19}N_8O_2$, [(M+H)$^+$], 406.2104, found 406.2100.

*4.1.14.12.1-methyl-8-((5-morpholinopyridin-2-yl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (**13m**)*. Yellow solid. Yield: 50%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.62 (s, 1H, Pyr-NH), 8.42(s, 1H, -N=CH-), 8.03 (d, *J* = 8.0 Hz, 1H, Pyr-H), 7.52 (s, 1H, Pyr-H), 7.44 (d, *J* = 8.0 Hz, 1H, Pyr-H), 4.34 (s, 3H, -NCH_3), 3.77 (t, *J* = 5.8 Hz, 4H, Pyr-N$CH_2$$CH_2$N), 3.11 (t, *J* = 4.8 Hz, 4H, Pyr-N$CH_2$$CH_2$N), 2.99 (t, *J* = 8.0 Hz, 2H, -N=CHCC$H_2$$CH_2$-), 2.81 (t, *J* = 8.0 Hz, 2H, -N=CHC$CH_2$$CH_2$-). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ 162.1, 158.5, 157.2, 152.8, 145.9, 143.7, 138.0, 136.4, 135.8, 126.3, 125.6, 119.1, 114.0, 100.0, 60.6, 50.3, 46.0; HRMS (ESI): calcd for $C_{20}H_{22}N_8O_2$, $[(M+H)^+]$, 407.1944, found 407.1937.

*4.1.14.13.1-methyl-8-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (**13n**)*. Yellow solid. Yield: 55%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.57 (s, 1H, Pyr-NH), 8.53 (s, 1H, -N=CH-), 8.40 (s, 2H, -$CONH_2$), 7.98 (s, 1H, Pyr-H), 6.79 (d, *J* = 8.0 Hz, 1H, Pyr-H), 6.65 (d, *J* = 8.0 Hz, 1H, Pyr-H), 3.95 (s, 3H, -NCH_3), 3.15 (t, *J* = 8.0 Hz, 4H, Pyr-N$CH_2$$CH_2$N), 2.99 (t, *J* = 8.0 Hz, 2H, -N=CHCC$H_2$$CH_2$), 2.89 (t, *J* = 8.0 Hz, 2H, -N=CHC$CH_2$$CH_2$), 2.35 (t, *J* = 8.0 Hz, 4H, Pyr-N$CH_2$$CH_2$N), 2.21 (m, 3H, -$NCH_2CH_2NCH_3$). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ 163.4, 159.3, 157.5, 152.9, 152.5, 142.3, 142.0, 140.8, 134.9, 131.3, 129.0, 122.0, 120.0, 118.2, 41.2, 32.0, 27.4, 26.3; HRMS (ESI): calcd for $C_{21}H_{25}N_9O$, $[(M+H)^+]$, 420.2260, found 420.2252.

*4.1.14.14.1-methyl-8-((4-(4-methylpiperazin-1-yl)phenyl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (**13i**)*. Yellow solid. Yield: 65%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.29 (s, 1H, Ar-NH-), 8.35 (s, 1H, -N=CH-), 7.53 (d, *J* = 8.0 Hz, 2H, H_{Ar}), 6.90 (d, *J* = 8.0 Hz, 2H, H_{Ar}), 4.32 (s, 3H, -NCH_3), 3.00 (t, *J* = 8.0 Hz, 4H, Ar-N$CH_2$$CH_2$N), 2.99 (t, *J* = 7.8 Hz, 2H, -N=CHCC$H_2$$CH_2$), 2.89 (t, *J* = 7.8 Hz, 2H, -N=CHC$CH_2$$CH_2$), 2.48 (t, *J* = 8.0 Hz, 4H, Ar-N$CH_2$$CH_2$N), 2.23 (s, 3H, -$NCH_2CH_2NCH_3$). ^{13}C-NMR (DMSO-$d_6$, 100 MHz): δ 162.2, 159.6, 146.8, 137.9, 130.6, 126.2, 121.7, 117.8, 113.3, 66.8, 60.7, 24.5, 19.8, 14.7; HRMS (ESI): calcd for $C_{22}H_{26}N_8O$, $[(M+H)^+]$, 419.2308, found 419.2305.

4.2. *Biology*

4.2.1. *Kinase assay*

Kinase assay was performed as previously described [19]. The CDK2/CycA2, CDK4/CycD3 and CDK6/CycD3 were from Carna. The Peptide FAM-P8 and Peptide FAM-P18 were from GL Biochem. The ATP and EDTA were from Sigma. The kinase reactions were carried out in 30μL volumes in the reaction solution containing the following: 2μL compound (in 20% DMSO), 18μL kinase in buffer (50mM HEPES, pH 7.5, 5mM $MgCl_2$, 1mM DTT, 0.0015% Brij-35), 10μL of the mixture of FAM-labeled peptide and ATP. The reaction mixture was incubated at 30 □ for 40 min, then add 25μL of stop buffer to stop reaction. Finally, conversion was read from Caliper, then convert conversion values to inhibition values: percent inhibition = (max-conversion)/(max-min) *100. “max” stands for DMSO control, “max” stands for low control. IC_{50} values were fitted by Hill equation with OriginPro 8.

4.2.2. *Cell proliferation assay*

MCF-7 cells were collected using 0.25% trypsin-EDTA and seeded in 96-well plates ($5*10^3$ cells/well) in DMEM medium. HCT116, HepG2, and PANC-1 cells were collected using 0.25% trypsin-EDTA and seeded in 96-well plates ($5*10^3$ cells/well) in PRMI1640 medium, respectively. After 24 h, cells were incubated for 72 h with various concentration of the tested compounds. The cells were stained at 37 □ for 4h with 0.05% MTT dissolved in PBS. The plates were tested by using CellTiter-Glo assay (Promega) and fluorescence was read at 490nm. Then convert OD values to inhibition values: percent inhibition = $(OD_1-OD_2)/OD_1*100\%$, OD_1 stands for blank control, OD_2 stands for drug groups. IC_{50} of proliferation was fitted by Hill equation with OriginPro 8.

4.2.3. *Procedures for mice pharmacokinetic*

In vivo pharmacokinetic properties were evaluated in mice Male ICR mice (20-25 g), following intravenous (iv, 10 mg/kg) and oral (os, 10 mg/kg). All animal experiments were performed in accordance with IACUC protocol or the regulations effective in China. Blood was collected at multiple time points post dose and transferred to an EDTA tube. The blood was centrifuged at 8500 rpm for 15 min, and the plasma was transferred to a polypropylene tube, capped, and stored frozen (-20 ℃) for parent compound analysis. The analysis was conducted by using HPLC separation coupled with mass spectrometric detection. All pharmacokinetic (PK) parameters were derived from concentration-time data by non-compartmental analyses. All pharmacokinetic parameters were calculated with the computer software STATA 12.0.

4.2.4. *Molecular modeling*

The crystal structure of CDK6 complex with palbociclib (PDB code: 2EUF) was downloaded from Protein Data Bank (http://www.rcsb.org/) and used for the docking study. The protein was removed from the structure first. Discovery Studio 3.0 Client was used to analyze the binding sites and force field in ATP package of CDK6. The structure of the compounds **13l** was prepared by using Discovery Studio 3.0 Client.

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Supplementary data

Supplementary data (characterization of compounds) associated with this article can be found, in the online version

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Highlights

Novel 4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline derivatives were designed targeting CDK4/6

Introducing a 2-aminopyridine side chain at the C-8 position could inhibit CDK4/6 with exquisite selectivity over CDK2

Both compounds **13m** and **13n** displayed potent antiproliferative activities against MCF-7 cell

The CDK4/6 inhibitor **13n** exhibited reasonable pharmacokinetic profiles.

Molecular docking in the active site of CDK6