Design, Synthesis, and Biological Evaluation of Orally Bioavailable CHK1 Inhibitors Active against Acute Myeloid Leukemia

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Checkpoint kinase 1 (CHK1) is a central component in DNA damage response and has emerged as a target for antitumor therapeutics. Herein, we describe the design, synthesis, and biological evaluation of a novel series of potent diaminopyrimidine CHK1 inhibitors. The compounds exhibited moderate to potent CHK1 inhibition and could suppress the proliferation of malignant hematological cell lines. The optimized compound **13** had a CHK1 IC_{50} value of 7.73 ± 0.74 nM, and MV-4-11 cells were sensitive to it ($IC_{50} = 0.035 \pm 0.007$ μM). Furthermore, compound **13** was metabolically stable in mouse liver microsomes *in vitro* and displayed moderate oral bioavailability *in vivo*. Moreover, treatment of MV-4-11 cells with compound **13** for 2 h led to robust inhibition of CHK1 autophosphorylation on serine 296. Based on these biochemical results, we consider compound **13** to be a promising CHK1 inhibitor and potential anticancer therapeutic agent.

Introduction

Checkpoint kinase 1 (CHK1) is a serine/threonine kinase that plays an important role in the DNA damage response (DDR).[1] Upon damage by genotoxic agents, ionic irradiation or faults in the DNA replication process, CHK1 is activated by upstream kinase ataxia telangiectasia and Rad3-related (ATR), and in turn phosphorylates downstream protein CDC25 resulting in cell-cycle arrest in the S or G2/M phase.[2–4] This complex signaling network ensures that DNA repair pathways are in response to DNA damage, avoiding mitotic catastrophe and resultant cell death or apoptosis.[5,6] Whilst normal cells arrest at S, G1/S and G2/M checkpoints for DNA repair, cancer cells harbor functional defects in p53 and are dependent upon S and G2/M checkpoints to repair DNA damage. Previous studies have shown that the inhibition of CHK1 can abrogate S and G2/M checkpoints, promoting cancer cell death.[7–9] CHK1 inhibitors therefore hold promise as a promising class of anticancer therapeutic agents.

Considerable efforts have been made to identify CHK1 inhibitors and several molecules have reached early clinical trials (Figure 1), but only prexasertib (LY2606368) and SRA737 (CCT245737) have entered phase II clinical trials. First-generation CHK1 inhibitors such as AZD-7762, PF00477736 and SCH900776 show low selectivity, limited efficacy and unfavorable toxicity, further clinical developments of these molecules have been halted.[10–12] Moreover, they are administered intravenously. To overcome these limitations, second-generation

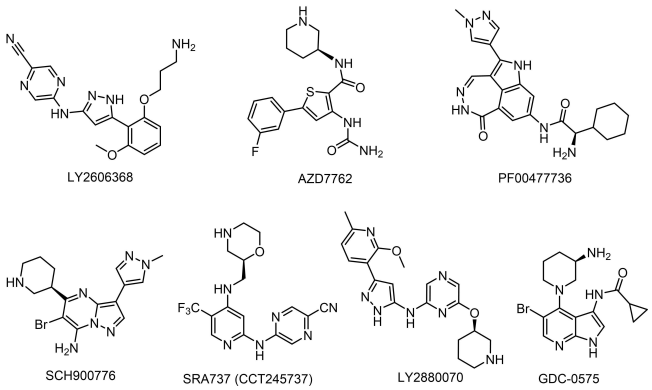

Figure 1. Structures of the clinical candidate checkpoint kinase inhibitors.

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CHK1 inhibitors have been synthesized. SRA737, LY2880070 and GDC-0575 show an increased selectivity, potency and safety, and all are orally bioavailable, which would provide more flexible administration.[13,14] In addition, a great amount of CHK1 inhibitors in combination with various DNA-damaging agents have been evaluated of their therapeutic effect against different types of solid tumors in early clinical trials, including non-small-cell lung cancer (NCT01139775), head and neck cancer (NCT02555644), melanoma (NCT00779584) and others.[15–17] Until recently, few evidences suggested that CHK1 inhibitors exhibited single-agent activity in inducing cell death in human leukemia and lymphoma cells, including B-cell lymphoma, chronic lymphocytic leukemia, and acute myeloid leukemia.[18–20] Thus, there still remains a strong need for novel CHK1 inhibitors with oral bioavailability and inhibitory activity against leukemia and lymphomas.

Our group has been continuously developing anticancer drugs especially small molecule protein kinase inhibitors and established an in-house compound library. In our previous work[21] lead compound MCL-1020 (**1**) with moderate CHK1 inhibition (IC_{50} = 1.61 μM) was found through virtual screening in-house compound library. Further SAR exploration at 3-position of pyridine led to the identification of a series of 2-amino pyrimidine derivatives, these compounds showed potent CHK1 inhibition. However, the most promising compound suffered from poor pharmacokinetic profile and was administered intravenously. Oral compounds provided an advantage of flexibility in administration. To obtain oral compounds, we refocused on our initial screening lead compound MCL-1020. From potential binding mode of MCL-1020 with CHK1 (Figure 2), we found that they could form hydrogen bonds in the hinge region and specificity pocket. However, MCL-1020 lacked interactions with polar residues as Glu91, Glu134 and Asn135 in the ribose region of CHK1, which had been proved important for CHK1 inhibition. We explored whether amine substituents on the C-4 of pyrimidine are located in this region and make interactions with CHK1. Introduction of a piperidin-4-amine at the C-4 position of pyrimidine led to compound **2**. We are encouraged that compound **2** showed an increased CHK1 inhibitory potency. Considering phenyl group at the C-5 position of pyrimidine of MCL-1020 was easily hydroxylated to result in poor pharmacokinetic profile, we planned to modify the C-5 substituents through replacement phenyl group with five-membered heterocycles which were more metabolically stable. Based on SAR exploration (Figure 3), we expanded the library of diaminopyrimidine derivatives and assayed their inhibitory activity against CHK1. We further evaluated the anti-proliferative activity of the compounds against a range of malignant hematological cell lines, and explored their metabolic stability *in vitro* and pharmacokinetics *in vivo*. Moreover, we evaluated the effects of representative compounds on the inhibition of CHK1 signaling.

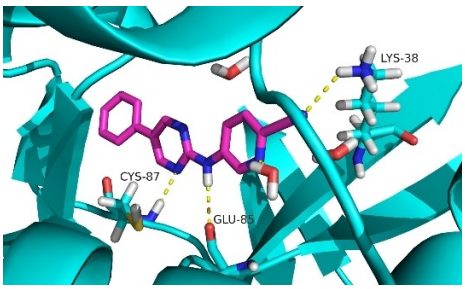


Figure 2. Binding characteristics of MCL-1020 (**1**).

Results and Discussion

Chemistry

The synthesis of the compounds **2**–**9** are outlined in Scheme 1. Initially, the commercially available 5-bromo-2, 4-dichloropyrimidine **17** was treated with *tert*-butyl 4-aminopiperidine-1-carboxylate in acetonitrile to produce **18**, which was subsequently aminated to afford **19**. Suzuki cross-coupling of compound **19** was then performed with various boronic acids/esters to provide **20**–**25**, which subsequently underwent Buchwald-Hartwig reactions with 5-bromo-2-cyanopyridine followed by TFA deprotection to give the final products **2**–**7**. Commercially available 5-bromo-2-cyanopyridine was installed via a Buchwald–Hartwig reaction with **19** to afford **26**. The Boc group was then removed through treatment with TFA to yield the final product **8**. Suzuki cross-coupling of **26** with lithium triisopropoxy(4-methylthiazol-2-yl)borate was performed and followed by TFA deprotection to give the final product **9**.

The synthesis of compounds **10**–**16** are shown in Scheme 2. Treatment of compound **17** with various commercially available amines gave **27**–**32**, which were subsequently aminated to produce the corresponding intermediates **33**–**38**. These intermediates were then subjected to Suzuki cross-coupling with 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole to produce intermediates **39**–**44**. The Buchwald–Hartwig reaction of **39**–**43** followed by TFA deprotection afforded the final products **10**–**14**. Buchwald-Hartwig reaction of **44** directly provided the final compounds **15** and **16**.

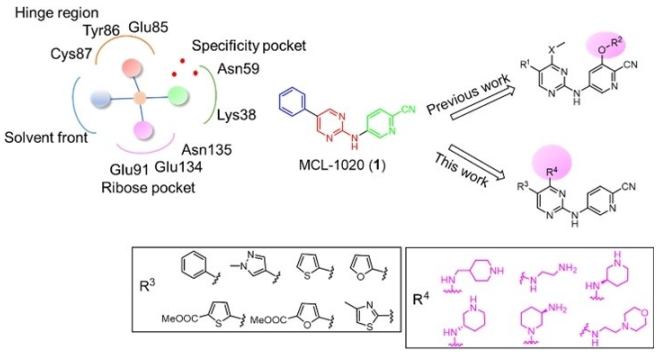
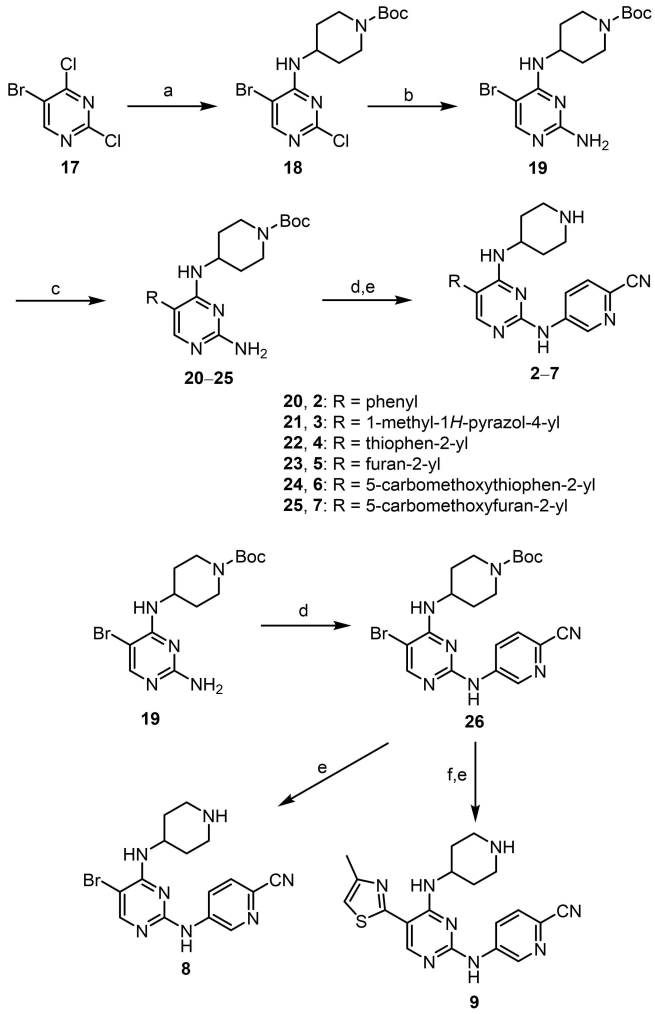
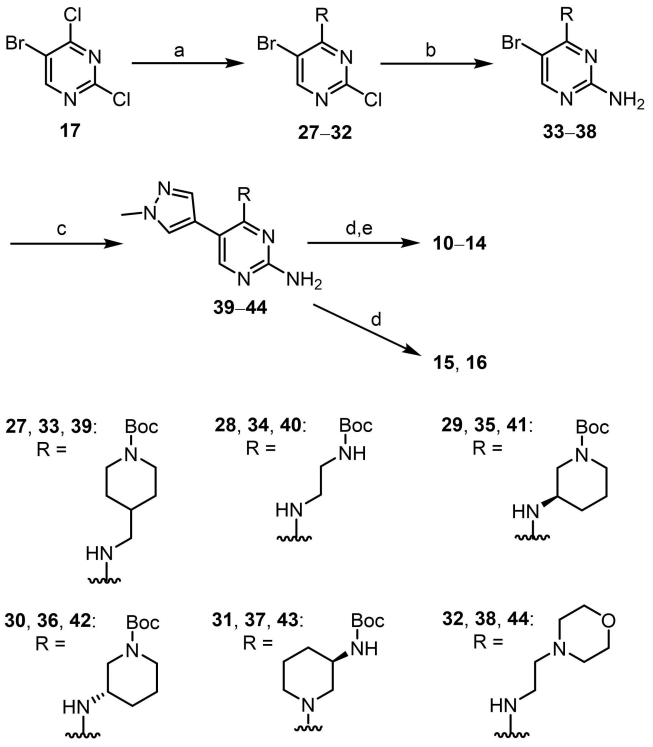


Figure 3. Design of novel diaminopyrimidine CHK1 inhibitors.



Scheme 1. Synthetic route of compounds **2**–**9**. a) *tert*-Butyl 4-aminopiperidine-1-carboxylate, ACN, RT, 82%; b) $NH_3 \cdot H_2O$, ethanol, 100 °C, 80%; c) corresponding boronic acid/ester, $Pd(dppf)Cl_2$, 1N Na_2CO_3, DME, reflux, 77–90%; d) 5-bromo-2-cyanopyridine, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, dioxane, reflux, 70–85%; e) TFA, CH_2Cl_2, 65–80%; f) lithium triisopropoxy(4-methylthiazol-2-yl)borate, $Pd(dppf)Cl_2$, CuCl, $ZnCl_2$, Cs_2CO_3, DMF, 100 °C, 45%.

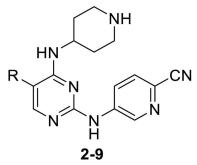


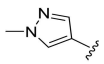
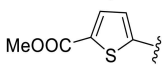
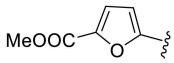
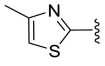
Scheme 2. Synthetic route of compounds **10**–**16**. a) corresponding amine, ACN, RT, 82–95%; b) $NH_3 \cdot H_2O$, ethanol, 100 °C, 78–85%; c) 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, $Pd(dppf)Cl_2$, 1N Na_2CO_3, DME, reflux, 77–84%; d) 5-bromo-2-cyanopyridine or 5-bromopyrimidine-2-carbonitrile, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, dioxane, reflux, 70–85%; e) TFA, CH_2Cl_2, 65–80%.

In vitro structure-activity relationships of CHK1 inhibition

Newly synthesized compounds **2**–**16** were assessed in ADP-Glo™ Kinase Assays to investigate their inhibitory activity against CHK1. Compound **2** was synthesized by introduction of a piperidin-4-amine at the C-4 position of pyrimidine. It was encouraging to find that compound **2** displayed increased CHK1 inhibition ($IC_{50} = 139.40 \pm 22.63$ nM) compared to **1**. This indicated that C-4 substituent of pyrimidine probably made interactions with CHK1. Whereas, phenyl group was easily hydroxylated, we further explored C-5 substituents of pyrimidine through replacement phenyl group with five-membered heterocycles. As shown in Table 1, the five-membered heterocycles (**3**, **4**, **5** and **9**) showed excellent CHK1 inhibitory potency. Interestingly, whilst ester substitutions on the thiophene or furan ring (compounds **6** and **7**) resulted in a loss of CHK1

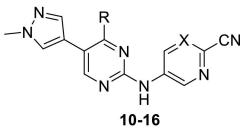
Table 1. CHK1 inhibitory activity of compounds **2**–**9**.



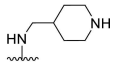
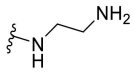
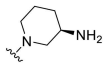
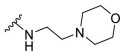
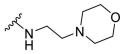
Cpd	R	CHK1 IC_{50} [nM][a]
2		139.40 ± 22.63
3		19.59 ± 10.68
4		43.22 ± 9.10
5		7.81 ± 1.10
6		89.07 ± 21.26
7		32.99 ± 5.15
8		45.32 ± 8.27
9		3.31 ± 1.22

[a] IC_{50} values are the mean of three independent experiments.

Table 2. CHK1 inhibitory activity of compounds **10**–**16**.



10-16

Cpd	R	X	CHK1 IC_{50} [nM][a]
10		C	35.32 ± 8.26
11		C	46.70 ± 0.81
12		C	169.20 ± 14.57
13		C	7.73 ± 0.74
14		C	315.00 ± 252.58
15		C	241.25 ± 19.87
16		N	358.00 ± 125.72
staurosporine			1.20 ± 0.05

[a] IC_{50} values are the mean of three independent experiments.

inhibition (compare **6** vs. **4**; **7** vs. **5**). Bromo-derivative **8** and five-membered heterocycle **4** showed similar CHK1 inhibition.

Though compounds **5** and **9** showed potent inhibition of CHK1 activity ($IC_{50} < 10$ nM), both had lower aqueous solubility compared to **3** (see the Supporting Information). To obtain a broader range of compounds, several other amines were added to the C-4 position whilst 1-methylpyrazole substitution of compound **3** was kept constant at the C-5 position (Table 2). As was observed upon inserting a methylene between the amine and piperidine (**10**), decreased potency was observed compared to **3**. Analogs **11**–**14** were synthesized by reducing the distance of the two nitrogen atoms on substitutions at the C-4 position. Compounds **10** and **11** showed similar CHK1 inhibition. The pair of enantiomers **12** and **13** demonstrated that the *S* configuration was better than *R*, whilst compound **13** was 20 times more potent than **12**. It illustrated that chirality was significantly important to anticancer activities. Surprisingly, the (*R*)-piperidin-3-amine (**14**) showed a pronounced loss of activity, suggesting that a secondary amine next to the pyrimidine is required. The replacement of amino (**11**) by morpholine (**15**) resulted in an approximately 5-fold loss of potency. Finally, replacement of the pyridine with pyrimidine showed a similar CHK1 potency, suggesting that no additional contacts with the CHK1 protein were made (**16** vs. **15**).

In vitro cytotoxic activity

Based on their highly potent CHK1 inhibitory activity, compounds **5**, **9**, and **13** were selected for assessment of their growth inhibitory activity against five malignant hematological cell lines (RPMI8226, Mino, Ramos, Jeko-1, and MV-4-11) and peripheral blood mononuclear cells (PBMC) from normal donors. As shown in Table 3, compounds **5**, **9** and **13** displayed low inhibitory activity against RPMI8226 cells with IC_{50} values > 1 μM. For Mino and Jeko-1 cells, three compounds showed moderate inhibitory potency. And for Ramos cells, compounds **5** and **13** showed moderate inhibitory potency. Excitingly, all three compounds strongly inhibited MV-4-11 cells with IC_{50} values of 0.044 ± 0.001 μM, 0.035 ± 0.006 μM and 0.035 ± 0.007 μM, respectively. Meanwhile, all three compounds showed low inhibitory potency on the PBMC. Compounds **5** and **9** showed excellent inhibitory potency but modestly lower aqueous solubility compared to **13** (see the Supporting Information). Based on the potency and solubility data, compound **13** was selected for further studies.

Pharmacokinetic parameters of compound 13

As compound **13** showed potent CHK1 inhibition and strongly inhibited the growth of MV-4-11 cells, it was selected for further pharmacokinetic evaluation in rats. After oral dosing of 20 mg/kg, plasma samples were collected at defined time points (0.083, 0.25, 0.5, 1, 2, 4, 6, 24, 30 h). As shown in Table 4, compound **13** showed moderate absorption and exposure ($t_{1/2} = 5.3$ h, $C_{max} = 195.31$ ng/mL, $AUC_{0\text{-}t} = 1372.75$ h ng/mL). In addition, metabolic stability of compound **13** was evaluated in mouse liver microsomes *in vitro*. Compound **13** showed high levels of stability in mouse liver microsomes. These data suggested that compound **13** was worth of further development.

Table 3. *In vitro* growth inhibition of hematological cell lines with **5**, **9**, and **13**.

Cpd	IC_{50}[μM][b] RPMI8226	Mino	Ramos	Jeko-1	MV-4-11	PBMC
5	3.339 ± 0.316	0.708 ± 0.156	0.536 ± 0.074	0.342 ± 0.015	0.044 ± 0.001	3.551 ± 0.832
9	1.149 ± 0.031	1.954 ± 0.294	NT[a]	0.269 ± 0.008	0.035 ± 0.006	1.148 ± 0.187
13	3.597 ± 0.287	0.608 ± 0.082	0.401 ± 0.040	0.253 ± 0.050	0.035 ± 0.007	7.874 ± 1.015
AZD7762	0.409 ± 0.035	0.185 ± 0.032	0.137 ± 0.025	0.048 ± 0.013	0.036 ± 0.005	> 10

[a] NT indicates not-tested. [b] IC_{50} values are the mean of three independent experiments.

Table 4. Pharmacokinetic profiles of compound **13**.

Parameters	In rat (20 mg/kg, i.g.)
tmax (h)	2.17
t1/2 (h)	5.30
Cmax (ng/mL)	195.31
AUC0-t (h ng/mL)	1372.75
AUC0-∞ (h ng/mL)	1411.60
MRT0-∞ (h)	6.15
liver microsome stability (%, remaining percent @ 0.5 h) [mouse]	93

Effects of compound 13 on the inhibition of CHK1 signaling

To fully understand the mechanism through which compound **13** inhibited the proliferation of MV-4-11 cells, we explored the phosphorylation status of CHK1 through western blot analysis, including CHK1 autophosphorylation on Ser296, and DNA damage-mediated CHK1 phosphorylation on Ser345 by its upstream kinase ataxia telangiectasia and Rad3-related (ATR).

The phosphorylation of serine 296 (pSer296) of CHK1 is the autophosphorylation site of CHK1,[22] and its level can well indicate the strength of different CHK1 inhibitors.[19,23–26] The phosphorylation of serine 345 (pSer345) of CHK1 is the key site of CHK1 phosphorylation found in the C-terminal regulatory domain of CHK1. Different from pSer296, CHK1 inhibitors induce and enhance the level of pSer345 CHK1.[27–29] As shown in Figure 4, in the absence of compound **13**, the autophosphorylation of CHK1 was evident in MV-4-11 cells. Following treatment of MV-4-11 cells with increasing concentrations of compound **13** for 2 h, CHK1 autophosphorylation on Ser296 was strongly inhibited in a concentration-dependent manner, particularly at 160 nM. Moreover, DNA damage-mediated ATR-CHK1 signaling was also activated by compound **13** in a concentration-dependent manner.

Molecular modeling of compound 13 with CHK1

Since compound **13** displayed potent CHK1 inhibition and suppressed the growth of cancer cell lines, it was docked into the ATP-binding site of CHK1 to analyze its potential binding mode. As shown in Figure 5, the 2-aminopyrimidine core

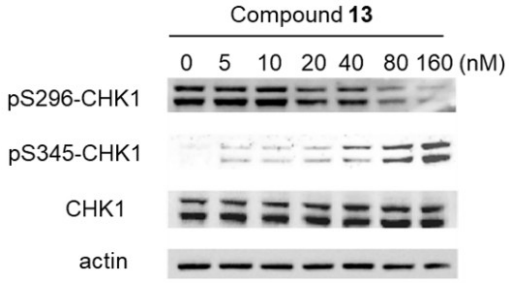


Figure 4. Inhibition of CHK1 signaling in MV-4-11 cells treated with the indicated concentrations of compound **13** for 2 h.

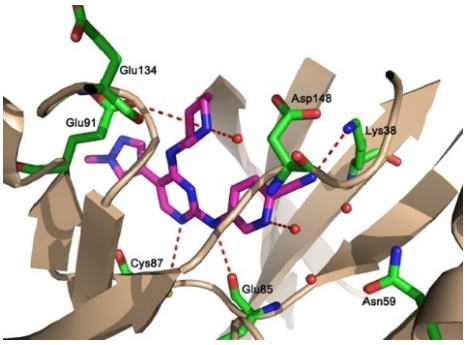


Figure 5. Binding mode of compound **13** with CHK1 kinase (PDB ID: 2YM8). Residues of CHK1 (green) that interact with **13** (magenta) are highlighted. Images were prepared using Discovery Studio (version 2.1).

formed two hydrogen bonds with Glu85 and Cys87 in the hinge region of CHK1 which significantly contributed to its interaction. The N of 2-cyano of pyridine formed a hydrogen bond with Lys38 whilst the NH of pyridine formed a hydrogen bond with three conservative H_2O molecules. Moreover, the NH of piperidine formed hydrogen bonds with Glu91 and a water. This docking highlighted the feasibility of design strategy.

Conclusion

In this study, our efforts toward development of new diaminopyrimidine derivatives of CHKI inhibitors with oral bioavailability for the treatment of leukemia led to the compound **13**. Compound **13** exhibited potent CHK1 inhibitory activity with IC_{50} value of 7.73 ± 0.74 nM. Meanwhile, **13** showed excellent cellular growth inhibitory activity, most notably in MV-4-11 cells ($IC_{50} = 0.035 \pm 0.007$ μM). Moreover, compound **13** was metabolically stable in mouse liver microsomes *in vitro* and showed moderate oral bioavailability ($AUC_{0\text{-}t} = 1372.75$ h ng/mL) *in vivo*. Treatment of MV-4-11 cells with compound **13** for 2 h also strongly inhibited the autophosphorylation of CHK1 on S296. These biochemical data demonstrate that compound **13** represents a promising CHK1 inhibitor and is now worthy of further exploration as an anticancer therapeutic agent.

Experimental Section

Chemistry

Reagents and solvents were purchased from commercial sources and used without further purification. Column chromatography was performed on silica gel (200–300 mesh). Melting points were determined on Büchi Melting Point B-540 apparatus without correction. The mass spectrometer was operated in Shimadzu LCMS-2020 mass spectrometer with methanol and water containing 0.1% formic acid as mobile phases. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance III spectrometer 500 MHz or Bruker AM 400 MHz with $CDCl_3$ or $[D_6]$DMSO as the solvent. Chemical shifts were reported in parts per million (ppm) relative to internal TMS. Coupling constants (*J* values) were reported in Hertz

(Hz) and proton coupling patterns were designated as single (s), doublet (d), double doublet (dd), triplet (t), quartet (q) and multiplet (m). All assayed compounds were determined by high performance liquid chromatography on an Agilent 1260 infinity equipped with a COSMOSIL 5C18-AR-II column (4.6ID×250 mm). Absorbance values were measured at 254 nm with a UV detector. A flow rate of 1.0 mL/min was used with a mobile phase A, 0.1% CH_3COONH_4, 0.1% HCOOH in H_2O and mobile phase B, CH_3OH. The specific rotation data were determined by SGW®-533 at 589 nm ($[\alpha]_D$).

tert-Butyl 4-((5-bromo-2-chloropyrimidin-4-yl)amino) piperidine-1-carboxylate (18)

A mixture of 5-bromo-2,4-dichloropyrimidine (0.5 mmol), *tert*-butyl 4-aminopiperidine-1-carboxylate (0.55 mmol), and trimethylamine (0.10 mL) in acetonitrile (11 mL) was stirred at room temperature for 2 h. Completion of the reaction was monitored by thin-layer chromatography (TLC) on silica gel. The reaction mixture was evaporated under reduced pressure. The residue was then purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 4:1–3:1) to give compound **18**. Yield: 82%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.26 (s, 1H), 7.38 (d, J = 10.0 Hz, 1H), 4.17–4.08 (m, 1H), 3.98–3.95 (m, 2H), 2.82–2.79 (m, 2H), 1.76–1.72 (m, 2H), 1.61–1.51 (m, 2H), 1.41 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{14}H_{20}BrClN_4O_2$: 391.05 $[M+H]^+$; found: 391.06.

tert-Butyl 4-((2-amino-5-bromopyrimidin-4-yl)amino) piperidine-1-carboxylate (19)

Compound **18** (1.23 mmol) was dissolved in ethanol (5 mL) in a sealed tube. The reaction mixture was then added $NH_3{\cdot}H_2O$ (5 mL) and heated at 100 °C for 12 h. The completion of the reaction was monitored by TLC on silica gel and the reaction mixture was evaporated under reduced pressure. After cooling to room temperature, the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 2:1) to give compound **19**. Yield: 80%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.78 (s, 1H), 6.22 (d, J = 10.5 Hz, 1H), 6.19 (s, 2H), 4.15–4.05 (m, 1H), 3.97–3.94 (m, 2H), 2.88–2.75 (m, 2H), 1.77–1.73 (m, 2H), 1.53–1.43 (m, 2H), 1.41 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{14}H_{22}BrN_5O_2$: 372.10 $[M+H]^+$; found: 372.09.

General procedure A for the synthesis of compounds 20–25

A mixture of compound **19** (1.43 mmol), corresponding boronic acid/ester (1.72 mmol), Pd(dppf)Cl$_2$ (0.07 mmol), and 1N Na_2CO_3 (2.8 mL) in DME (14 mL) were heated to reflux overnight. The completion of the reaction was monitored by TLC on silica gel. After cooling to room temperature, the residue was purified by column chromatography on silica gel (CH_2Cl_2/ethanol = 25:1) to give the product.

tert-Butyl 4-((2-amino-5-phenylpyrimidin-4-yl)amino) piperidine-1-carboxylate (20)

General procedure A. Yield: 80%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.09 (s, 1H), 7.52 (s, 2H), 7.51 (s,2H), 7.40 (s, 1H), 5.51 (s, 1H), 4.76 (s, 2H), 4.18–4.09 (m, 1H), 4.02 (m, 2H), 2.93 (m, 2H), 1.99–1.97 (m, 2H), 1.45 (s, 9H), 1.32–1.27 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{20}H_{27}N_5O_2$: 370.22 $[M+H]^+$; found: 370.22.

tert-Butyl 4-((2-amino-5-(1-methyl-1*H*-pyrazol-4-yl) pyrimidin-4-yl)amino)piperidine-1-carboxylate (21)

General procedure A. Yield: 81%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.66 (s, 1H), 7.49 (s, 1H), 7.37 (s, 1H), 4.89 (s, 2H), 4.83 (d, J = 8.0 Hz, 1H), 4.15–4.08 (m, 1H), 4.04 (br, 2H), 3.96 (s, 3H), 2.93 (t, J = 12.5 Hz, 2H), 1.99–1.97 (m, 2H), 1.46 (s, 9H), 1.33–1.25 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{18}H_{27}N_7O_2$: 374.22 $[M+H]^+$; found: 374.23.

tert-Butyl 4-((2-amino-5-(thiophen-2-yl)pyrimidin-4-yl)amino) piperidine-1-carboxylate (22)

General procedure A. Yield: 80%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.73 (s, 1H), 7.51 (d, J = 6.0 Hz, 1H), 7.13–7.11 (m, 1H), 7.08 (s, 1H), 6.22 (br, 2H), 5.91 (d, J = 10.0 Hz, 1H), 4.15–4.09 (m, 1H), 3.91–3.88 (m, 2H), 2.82 (m, 2H), 1.80–1.78 (m, 2H), 1.45–1.39 (m, 2H), 1.38 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{18}H_{25}N_5O_2S$: 376.17 $[M+H]^+$; found: 376.18.

tert-Butyl 4-((2-amino-5-(furan-2-yl)pyrimidin-4-yl)amino) piperidine-1-carboxylate (23)

General procedure A. Yield: 80%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.98 (s, 1H), 7.66 (s, 1H), 6.54 (m, 2H), 6.28 (s, 2H), 6.12 (d, J = 10.0 Hz, 1H), 4.22–4.15 (m, 1H), 3.93–3.91 (m, 2H), 2.84 (s, 2H), 1.86–1.83 (m, 2H), 1.49–1.40 (m, 2H), 1.40 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{18}H_{25}N_5O_3$: 360.20 $[M+H]^+$; found: 360.22.

tert-Butyl 4-((2-amino-5-(5-(methoxycarbonyl)thiophen-2-yl) pyrimidin-4-yl)amino)piperidine-1-carboxylate (24)

General procedure A. Yield: 82%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.80 (s, 1H), 7.77 (d, J = 2.8 Hz, 1H), 7.16 (d, J = 2.8 Hz, 1H), 6.39 (s, 2H), 6.22 (d, J = 6.4 Hz, 1H), 4.20–4.12 (m, 1H), 3.93–3.91 (m, 2H), 3.82 (s, 3H), 2.80 (m, 2H), 1.80–1.78 (m, 2H), 1.49–1.41 (m, 2H), 1.39 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{20}H_{27}N_5O_4S$: 434.18 $[M+H]^+$; found: 434.20.

tert-Butyl 4-((2-amino-5-(5-(methoxycarbonyl)furan-2-yl) pyrimidin-4-yl)amino)piperidine-1-carboxylate (25)

General procedure A. Yield: 80%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.21 (s, 1H), 7.39 (d, J = 3.2 Hz, 1H), 6.82 (d, J = 2.8 Hz, 1H), 6.55 (s, 2H), 6.51 (d, J = 6.0 Hz, 1H), 4.27–4.20 (m, 1H), 3.83 (m, 2H), 3.81 (s, 3H), 3.02 (m, 2H), 1.90–1.87 (m, 2H), 1.47–1.44 (m, 2H), 1.41 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{20}H_{27}N_5O_5$: 418.20 $[M+H]^+$; found: 418.19.

General procedure B for the synthesis of compounds 2–7

A mixture of the appropriate arylamines (0.986 mmol), 5-bromo-2-cyanopyridine (0.986 mmol), $Pd_2(dba)_3$ (0.00986 mmol), Xantphos (0.02 mmol), and Cs_2CO_3 (1.48 mmol) in 1,4-dioxane (6 mL) were heated to reflux for 5 h. The completion of the reaction was monitored by TLC on silica gel. The reaction mixture was then evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2/ethanol = 30:1) to give intermediates. The intermediates were then dissolved in 10 mL CH_2Cl_2 and cooled at 0 °C. The mixture was then added TFA (5.0 mL) at room temperature for 3 h and the reaction mixture was evaporated under reduced pressure. The residue was then purified by column chromatography on silica gel (CH_2Cl_2/ethanol (NH_3) = 100:3) to give the product.

5-((5-Phenyl-4-(piperidin-4-ylamino)pyrimidin-2-yl)amino) picolinonitrile (2)

General procedure B. Yield: 78%; m.p. 239–242 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.01 (s, 1H), 9.08 (d, J = 2.5 Hz, 1H), 8.43 (dd, J = 8.5, 2.5 Hz, 1H), 7.92 (d, J = 8.5 Hz, 1H), 7.88 (s, 1H), 7.50–7.47 (m, 2H), 7.27–7.23 (m, 2H), 7.22–7.17 (m, 1H), 6.61 (d, J = 7.5 Hz, 1H), 4.14–4.04 (m, 1H), 3.16–3.07 (m, 2H), 2.81–2.68 (m, 2H), 1.96–1.87 (m, 2H), 1.60–1.54 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{21}H_{21}N_7$: 372.19 $[M+H]^+$; found: 372.20.

5-((5-(1-Methyl-1*H*-pyrazol-4-yl)-4-(piperidin-4-ylamino) pyrimidin-2-yl)amino)picolinonitrile (3)

General procedure B. Yield: 82%; m.p. 220–222 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.00 (s, 1H), 9.05 (d, J = 2.5 Hz, 1H), 8.42 (dd, J = 8.5, 2.5 Hz, 1H), 7.93 (s, 2H), 7.91 (s, 1H), 7.61 (s, 1H), 6.52 (d, J = 7.0 Hz, 1H), 4.18–4.07 (m, 1H), 3.88 (s, 3H), 3.32–3.29 (m, 2H), 3.06–2.95 (m, 2H), 2.11–2.01 (m, 2H), 1.95–1.85 (m, 2H). ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.32, 157.97, 154.20, 141.75, 141.70, 137.98, 129.80, 129.68, 123.84, 122.89, 118.82, 114.28, 104.93, 48.99, 45.60, 39.14, 32.60; LC-MS (ESI): *m/z* calcd for $C_{19}H_{21}N_9$: 376.19 $[M+H]^+$; found: 376.19.

5-((4-(Piperidin-4-ylamino)-5-(thiophen-2-yl)pyrimidin-2-yl) amino)picolinonitrile (4)

General procedure B. Yield: 80%; m.p. 160–162 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.05 (s, 1H), 9.07 (d, J = 2.5 Hz, 1H), 8.43 (dd, J = 8.5, 2.5 Hz, 1H), 7.98 (s, 1H), 7.89 (d, J = 8.5 Hz, 1H), 7.60 (t, J = 4.5 Hz, 1H), 7.22 (d, J = 3.0 Hz, 1H), 7.19–7.16 (m, 1H), 6.36 (d, J = 8.0 Hz, 1H), 4.10–3.96 (m, 1H), 2.95–2.93 (m, 2H), 2.60–2.55 (m, 2H), 1.88–1.80 (m, 2H), 1.49–1.41 (m, 2H). ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 158.60, 158.05, 154.99, 141.41, 141.01, 135.61, 129.19, 128.19, 126.38, 126.01, 123.74, 122.82, 118.27, 105.78, 48.82, 45.22, 32.38; LC-MS (ESI): *m/z* calcd for $C_{19}H_{19}N_7S$: 378.14 $[M+H]^+$; found: 378.15.

5-((5-(Furan-2-yl)-4-(piperidin-4-ylamino)pyrimidin-2-yl) amino)picolinonitrile (5)

General procedure B. Yield: 77%; m.p. 188–190 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.07 (s, 1H), 9.06 (d, J = 2.4 Hz, 1H), 8.42 (dd, J = 8.4, 2.4 Hz, 1H), 8.27 (s, 1H), 7.88 (d, J = 11.0 Hz, 1H), 7.77 (s, 1H), 6.76 (d, J = 3.0 Hz, 1H), 6.66–6.60 (m, 1H), 6.55 (d, J = 7.4 Hz, 1H), 4.14–3.98 (m, 1H), 3.03–2.94 (m, 2H), 2.68–2.55 (m, 2H), 1.96–1.85 (m, 2H), 1.57–1.41 (m, 2H). ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 157.72, 157.00, 153.52, 148.82, 142.35, 141.41, 140.89, 129.16, 123.75, 122.88, 118.24, 111.55, 106.22, 102.45, 48.68, 45.16, 32.57; LC-MS (ESI): *m/z* calcd for $C_{19}H_{19}N_7O$: 362.17 $[M+H]^+$; found: 362.18.

Methyl 5-(2-((6-cyanopyridin-3-yl) amino)-4-(piperidin-4-ylamino)pyrimidin-5-yl) thiophene-2-carboxylate (6)

General procedure B. Yield: 71%; m.p. 183–185 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.12 (s, 1H), 9.08 (s, 1H), 8.42 (d, J = 8.8 Hz, 1H), 8.05 (s, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.83 (d, J = 4.0 Hz, 1H), 7.29 (d, J = 3.6 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 4.10–3.95 (m, 1H), 3.84 (s, 3H), 3.05–2.90 (m, 2H), 2.63–2.50 (m, 2H), 1.87–1.78 (m, 2H), 1.52–1.42 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{21}H_{21}N_7O_2S$: 436.15 $[M+H]^+$; found: 436.16.

Methyl 5-(2-((6-cyanopyridin-3-yl) amino)-4-(piperidin-4-ylamino)pyrimidin-5-yl) furan-2-carboxylate (7)

General procedure B. Yield: 74%; m.p. 226–228 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.22 (s, 1H), 9.07 (d, J = 2.0 Hz, 1H), 8.46 (s, 1H), 8.41 (dd, J = 8.5, 2.5 Hz, 1H), 7.93 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 3.5 Hz, 1H), 7.01 (d, J = 3.5 Hz, 1H), 6.91 (d, J = 7.0 Hz, 1H), 4.15–4.03 (m, 1H), 3.84 (s, 3H), 3.05–2.95 (m, 2H), 2.70–2.58 (m, 2H), 1.98–1.89 (m, 2H), 1.49–1.37 (m, 3H). ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 158.28, 158.13, 156.91, 155.06, 153.65, 141.89, 141.60, 140.61, 129.18, 124.14, 123.28, 119.96, 118.16, 107.48, 100.85, 51.76, 48.34, 44.62, 32.35; LC-MS (ESI): *m/z* calcd for $C_{21}H_{21}N_7O_3$: 420.17 $[M+H]^+$; found: 420.17.

tert-Butyl 4-((5-bromo-2-((6-cyanopyridin-3-yl)amino) pyrimidin-4-yl)amino)piperidine-1-carboxylate (26)

A mixture of compound **19** (1.0 mmol), 5-bromo-2-cyanopyridine (1.0 mmol), $Pd_2(dba)_3$ (0.01 mmol), Xantphos (0.02 mmol) and Cs_2CO_3 (1.50 mmol) in 1,4-dioxane (8 mL) were heated to reflux for 5 h. The completion of the reaction was monitored by TLC on silica gel. The reaction mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/ethanol = 30:1) to give compound **26**. Yield: 77%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.06 (s, 1H), 8.95 (d, J = 2.5 Hz, 1H), 8.42 (dd, J = 9.0 Hz, 2.5 Hz, 1H), 8.16 (s, 1H), 7.97 (d, J = 9.0 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 4.17–4.09 (m, 1H), 4.03 (s, 2H), 2.83 (s, 2H), 1.85–1.82 (m, 2H), 1.61–1.52 (m, 2H), 1.43 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{20}H_{24}BrN_7O_2$: 474.12 $[M+H]^+$; found: 474.13.

5-((5-Bromo-4-(piperidin-4-ylamino)pyrimidin-2-yl)amino) picolinonitrile (8)

Compound **26** (0.5 mol) was dissolved in 4 mL CH_2Cl_2 and cooled at 0 °C. The mixture was then added TFA (2 mL) at room temperature for 3 h. The reaction mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/ethanol (NH_3) = 100:3) to give compound **8**. Yield: 70%; m.p. 228–230 °C;^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.01 (s, 1H), 9.01 (d, J = 2.0 Hz, 1H), 8.35 (dd, J = 8.5, 3.0 Hz, 1H), 8.13 (s, 1H), 7.90 (d, J = 9.0 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 4.06–3.91 (m, 1H), 3.01–2.96 (m, 2H), 2.58–2.51 (m, 2H), 1.83–1.74 (m, 2H), 1.57–1.47 (m, 2H). ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 157.54, 157.34, 155.99, 141.41, 140.83, 129.16, 123.81, 122.95, 118.20, 94.71, 49.24, 45.43, 32.38; LC-MS (ESI): *m/z* calcd for $C_{15}H_{16}BrN_7$: 374.07 $[M+H]^+$; found: 374.08.

5-((5-(4-Methylthiazol-2-yl)-4-(piperidin-4-ylamino) pyrimidin-2-yl)amino)picolinonitrile (9)

A mixture of compound **26** (0.211 mmol), lithium triisopropyl (4-methylthiazol-2-yl)borate (0.422 mmol), Pd(dppf)Cl_2 (0.011 mmol), 1 N Cs_2CO_3 (0.422 mmol), CuCl (0.021 mmol), and $ZnCl_2$ (0.211 mmol) in DMF (10 mL) was heated to 100 °C overnight. The completion of the reaction was monitored by TLC on silica gel. The reaction mixture was treated with water and CH_2Cl_2. The organic layer was collected, washed with brine, and dried over Na_2SO_4, evaporated under reduced pressure. The residue was dissolved in 3 mL CH_2Cl_2 and cooled at 0 °C. The mixture was added TFA (1.5 mL) at room temperature for 3 h. The reaction mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/ethanol (NH_3) = 100:3) to give compound **9**. Yield: 56%; m.p. > 250 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.28 (s, 1H), 9.61 (d, J = 7.5 Hz, 1H), 9.08

(d, $J=2.5$ Hz, 1H), 8.56 (s, 1H), 8.44 (dd, $J=8.5$, 2.5 Hz, 1H), 7.93 (d, $J=9.0$ Hz, 1H), 7.23 (d, $J=1.0$ Hz, 1H), 4.17–4.08 (m, 1H), 3.01–2.95 (m, 2H), 2.71–2.63 (m, 2H), 2.42 (s,3H), 2.02–1.95 (m, 2H), 1.49–1.41 (m, 2H). ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=163.95$, 158.31, 156.81, 155.36, 151.02, 141.66, 140.61, 129.24, 124.25, 123.36, 118.18, 111.58, 103.55, 47.74, 44.42, 32.57, 16.64; LC-MS (ESI): *m/z* calcd for $C_{19}H_{20}N_8S$: 393.15 $[M+H]^+$; found: 393.16.

General procedure C for the synthesis of compounds 27–32

A mixture of 5-bromo-2,4-dichloropyrimidine (0.44 mmol), corresponding amines (0.48 mmol), and trimethylamine (0.08 mL) in acetonitrile (10 mL) were stirred at room temperature for 2 h. The completion of the reaction was monitored by TLC on silica gel. The reaction mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 4:1–3:1) to give the product.

tert-Butyl 4-(((5-bromo-2-chloropyrimidin-4-yl)amino)methyl) piperidine-1-carboxylate (27)

General procedure C. Yield: 92%; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=8.12$ (s, 1H), 5.64 (t, $J=11.0$ Hz, 1H), 4.14–4.10 (m, 2H), 3.46 (t, $J=6.0$ Hz, 2H), 2.74 (t, $J=11.0$ Hz, 2H), 1.85–1.76 (m, 1H), 1.73–1.71 (m, 2H), 1.46 (s, 9H), 1.25–1.16 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{15}H_{22}BrClN_4O_2$: 405.06 $[M+H]^+$; found: 405.08.

tert-Butyl (2-((5-bromo-2-chloropyrimidin-4-yl)amino)ethyl) carbamate (28)

General procedure C. Yield: 94%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=$ 8.24 (s, 1H), 7.68 (t, $J=5.0$ Hz, 1H), 6.96 (t, $J=5.5$ Hz, 1H), 3.42–3.38 (m, 2H), 3.16–3.13 (m, 2H), 1.37 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{11}H_{16}BrClN_4O_2$: 351.01 $[M+H]^+$; found: 351.03.

tert-Butyl (*R*)-3-((5-bromo-2-chloropyrimidin-4-yl)amino) piperidine-1-carboxylate (29)

General procedure C. Yield: 85%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=$ 8.24 (s, 1H), 7.35 (d, $J=10.0$ Hz, 1H), 4.13–4.06 (m, 1H), 3.94–3.91 (m, 2H), 2.81–2.75 (m, 2H), 1.73–1.69 (m, 2H), 1.60–1.49 (m, 2H), 1.39 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{14}H_{20}BrClN_4O_2$: 391.05 $[M+H]^+$; found: 391.06.

tert-Butyl (*S*)-3-((5-bromo-2-chloropyrimidin-4-yl)amino) piperidine-1-carboxylate 30

General procedure C. Yield: 82%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=$ 8.24 (s, 1H), 7.35 (d, $J=10.0$ Hz, 1H), 4.13–4.06 (m, 1H), 3.94–3.91 (m, 2H), 2.81–2.75 (m, 2H), 1.73–1.69 (m, 2H), 1.60–1.49 (m, 2H), 1.39 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{14}H_{20}BrClN_4O_2$: 391.05 $[M+H]^+$; found: 391.06.

tert-Butyl *(R)*-(1-(5-bromo-2-chloropyrimidin-4-yl) piperidin-3-yl)carbamate (31)

General procedure C. Yield: 81%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=$ 8.01 (s, 1H), 3.60–2.56 (m, 1H), 3.31–3.06 (m, 2H), 2.98–2.94 (m, 2H), 1.85–1.60 (m, 2H), 1.53–1.43 (m, 2H), 1.38 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{14}H_{20}BrClN_4O_2$: 391.05 $[M+H]^+$; found: 391.05.

5-Bromo-2-chloro-N-(2-morpholinoethyl)pyrimidin-4-amine (32)

General procedure C. Yield: 95%; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=8.12$ (s, 1H), 6.46 (s, 1H), 3.76 (s, 4H), 3.58 (s, 2H), 2.66 (s, 2H), 2.55 (s, 4H); LC-MS (ESI): *m/z* calcd for $C_{10}H_{14}BrClN_4O$: 321.00 $[M+H]^+$; found: 321.03.

General procedure D for the synthesis of compounds 33–38

Appropriate aryl chlorides (1.23 mmol) were dissolved in ethanol (5 mL) in sealed tube. The reaction mixture was added $NH_3{\cdot}H_2O$ (5 mL) and was heated at 100 °C for 12 h. The completion of the reaction was monitored by TLC on silica gel. The reaction mixture was evaporated under reduced pressure. After cooling to room temperature, the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 2:1) to give the product.

tert-Butyl 4-(((2-amino-5-bromopyrimidin-4-yl)amino)methyl) piperidine-1-carboxylate (33)

General procedure D. Yield: 85%; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.87$ (s, 1H), 5.27 (t, $J=11.0$ Hz, 1H), 4.81 (s, 2H), 4.13 (s, 2H), 3.35–3.33 (m, 2H), 2.70 (s, 2H), 1.78–1.74 (m, 1H), 1.72–1.69 (m, 2H), 1.46 (s, 9H), 1.20–1.14 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{15}H_{24}BrN_5O_2$: 386.11 $[M+H]^+$; found: 386.12.

tert-Butyl (2-((2-amino-5-bromopyrimidin-4-yl)amino)ethyl) carbamate (34)

General procedure D. Yield: 81%; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.78$ (s, 1H), 6.94 (t, $J=5.5$ Hz, 1H), 6.57 (t, $J=5.5$ Hz, 1H), 6.22 (s, 2H), 3.37 (dd, $J=11.5$ Hz, 6.0 Hz, 2H), 3.14 (dd, $J=12.0$ Hz, 6.0 Hz, 2H), 1.38 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{11}H_{18}BrN_5O_2$: 332.06 $[M+H]^+$; found: 332.08.

tert-Butyl (*R*)-3-((2-amino-5-bromopyrimidin-4-yl)amino) piperidine-1-carboxylate (35)

General procedure D. Yield: 79%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=$ 7.82 (s, 1H), 6.24 (s, 2H), 5.99 (br, 1H), 3.99 (m, 1H), 3.62–3.57 (m, 2H), 3.00 (m, 2H), 1.83–1.80 (m, 1H), 1.68–1.63 (m, 2H), 1.41–1.37 (m, 1H), 1.37 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{14}H_{22}BrN_5O_2$: 372.10 $[M+H]^+$; found: 372.10.

tert-Butyl (*S*)-3-((2-amino-5-bromopyrimidin-4-yl)amino) piperidine-1-carboxylate (36)

General procedure D. Yield: 75%; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=$ 7.82 (s, 1H), 6.24 (s, 2H), 5.99 (br, 1H), 3.99 (m, 1H), 3.62–3.57 (m, 2H), 3.00 (m, 2H), 1.83–1.80 (m, 1H), 1.68–1.63 (m, 2H), 1.41–1.37 (m, 1H), 1.37 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{14}H_{22}BrN_5O_2$: 372.10 $[M+H]^+$; found: 372.10.

tert-Butyl (*R*)-(1-(2-amino-5-bromopyrimidin-4-yl) piperidin-3-yl)carbamate (37)

General procedure D. Yield: 72%; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.97$ (s, 1H), 6.89 (d, $J=8.0$ Hz, 1H), 6.49 (s, 2H), 3.95–3.88 (m, 2H), 2.77–2.67 (m, 2H), 1.87–1.84 (m, 1H), 1.76–1.73 (m, 1H), 1.61–1.52 (m, 1H), 1.39 (s, 9H), 1.38–1.34 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{14}H_{22}BrN_5O_2$: 372.10 $[M+H]^+$; found: 372.12.

5-Bromo-N^4-(2-morpholinoethyl)pyrimidine-2,4-diamine (38)

General procedure D. Yield: 78%; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.87$ (s, 1H), 5.96 (br, 1H), 4.79 (s, 2H), 3.75 (t, $J = 4.5$ Hz, 4H), 3.50 (dd, $J = 11.0$ Hz, 6.0 Hz, 2H), 2.62 (t, $J = 6.0$ Hz, 2H), 2.52–2.51 (m, 4H); LC-MS (ESI): *m/z* calcd for $C_{10}H_{16}BrN_5O$: 302.05 $[M+H]^+$; found: 302.06.

General procedure E for the synthesis of compounds 39–44

A mixture of appropriate aryl bromides (1.50 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1.72 mmol), Pd(dppf)Cl_2 (0.075 mmol), and 1N Na_2CO_3 (3.0 mL) in DME (15 mL) were heated to reflux overnight. The completion of the reaction was monitored by TLC on silica gel. After cooling to room temperature, the residue was then purified by column chromatography on silica gel (CH_2Cl_2/ethanol = 25:1) to give the product.

***tert*-Butyl 4-(((2-amino-5-(1-methyl-1*H*-pyrazol-4-yl) pyrimidin-4-yl)amino)methyl)piperidine-1-carboxylate (39)**

General procedure E. Yield: 70%; m.p. 174–176 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.67$ (s, 1H), 7.50 (s, 1H), 7.38 (s, 1H), 5.01 (t, $J = 5.5$ Hz, 1H), 4.81 (s, 2H), 4.10 (s, 2H), 3.96 (s, 3H), 3.31 (t, $J = 6.0$ Hz, 2H), 2.68 (m, 2H), 1.76–1.69 (m, 1H), 1.67–1.64 (m, 2H), 1.45 (s, 9H), 1.17–1.09 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{19}H_{29}N_7O_2$: 388.24 $[M+H]^+$; found: 388.25.

***tert*-Butyl (2-((2-amino-5-(1-methyl-1*H*-pyrazol-4-yl) pyrimidin-4-yl)amino)ethyl)carbamate (40)**

General procedure E. Yield: 80%; ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta =$ 7.78 (s, 1H), 7.60 (s, 1H), 7.50 (s, 1H), 6.92 (t, $J = 4.5$ Hz, 1H), 6.05 (t, $J = 4.5$ Hz, 1H), 5.97 (s, 2H), 3.85 (s, 3H), 3.36–3.35 (m, 2H), 3.13 (m, 2H), 1.35 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{15}H_{23}N_7O_2$: 334.19 $[M+H]^+$; found: 334.20.

***tert*-Butyl (*R*)-3-((2-amino-5-(1-methyl-1*H*-pyrazol-4-yl) pyrimidin-4-yl)amino)piperidine-1-carboxylate (41)**

General procedure E. Yield: 81%; ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta =$ 7.75 (s, 1H), 7.62 (s, 1H), 7.47 (s, 1H), 5.98 (s, 2H), 5.49 (m, 1H), 4.05–3.99 (m, 1H), 3.71–3.43 (m, 2H), 3.03 (m, 2H), 1.81–1.78 (m, 1H), 1.59–1.56 (m, 2H), 1.42–1.33 (m, 1H), 1.33 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{18}H_{27}N_7O_2$: 374.22 $[M+H]^+$; found: 374.20.

***tert*-Butyl (*S*)-3-((2-amino-5-(1-methyl-1*H*-pyrazol-4-yl) pyrimidin-4-yl)amino)piperidine-1-carboxylate (42)**

General procedure E. Yield: 78%; ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta =$ 7.75 (s, 1H), 7.62 (s, 1H), 7.47 (s, 1H), 5.98 (s, 2H), 5.49 (m, 1H), 4.05–3.99 (m, 1H), 3.71–3.43 (m, 2H), 3.03 (m, 2H), 1.81–1.78 (m, 1H), 1.59–1.56 (m, 2H), 1.42–1.33 (m, 1H), 1.33 (s, 9H); LC-MS (ESI): *m/z* calcd for $C_{18}H_{27}N_7O_2$: 374.22 $[M+H]^+$; found: 374.21.

***tert*-Butyl (*R*)-(1-(2-amino-5-(1-methyl-1*H*-pyrazol-4-yl) pyrimidin-4-yl)piperidin-3-yl)carbamate (43)**

General procedure E. Yield: 68%; ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta =$ 7.75 (s, 1H), 7.62 (s, 1H), 7.56 (s, 1H), 6.93 (d, $J = 8.0$ Hz, 1H), 3.88 (s, 3H), 3.64–3.61 (m, 1H), 3.44–3.41 (m, 1H), 2.71–2.69 (m, 1H), 2.62–2.60 (m, 1H), 1.81–1.79 (m, 1H), 1.63–1.61 (m, 1H), 1.57–1.54 (m, 1H), 1.39 (s, 9H), 1.32–1.21 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{18}H_{27}N_7O_2$: 374.22 $[M+H]^+$; found: 374.22.

5-(1-Methyl-1*H*-pyrazol-4-yl)-N^4-(2-morpholinoethyl) pyrimidine-2,4-diamine (44)

General procedure E. Yield: 76%; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.70$ (s, 1H), 7.56 (s, 1H), 7.42 (s, 1H), 5.75 (m, 1H), 4.91 (s, 2H), 3.97 (s, 3H), 3.68 (t, $J = 4.0$ Hz, 4H), 3.49 (t, $J = 6.0$ Hz, 2H), 2.57 (t, $J = 6.0$ Hz, 2H), 2.46 (s, 4H); LC-MS (ESI): *m/z* calcd for $C_{14}H_{21}N_7O$: 304.18 $[M+H]^+$; found: 304.20.

General procedure F for the synthesis of compounds 10–14

A mixture of the appropriate arylamines (1.0 mmol), 5-bromo-2-cyanopyridine (1.0 mmol), $Pd_2(dba)_3$ (0.01 mmol), Xantphos (0.02 mmol) and Cs_2CO_3 (1.50 mmol) in 1,4-dioxane (8 mL) were heated to reflux for 5 h. The completion of the reaction was monitored by TLC on silica gel. The reaction mixture was then evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2/ethanol = 30:1) to give intermediates. Then the intermediates were dissolved in 10 mL CH_2Cl_2 and cooled at 0 °C. The mixture was then added to TFA (5 mL) at room temperature for 3 h and the reaction mixture was evaporated under reduced pressure. The residue was then purified by column chromatography on silica gel (CH_2Cl_2/ethanol (NH_3) = 100:3) to give the product.

5-((5-(1-Methyl-1*H*-pyrazol-4-yl)-4-((piperidin-4-ylmethyl) amino)pyrimidin-2-yl)amino)picolinonitrile (10)

General procedure F. Yield: 79%; m.p. 231–233 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta = 9.93$ (s, 1H), 9.03 (d, $J = 2.5$ Hz, 1H), 8.46 (dd, $J = 8.5$, 2.5 Hz, 1H), 7.93–7.91 (m, 2H), 7.89 (s, 1H), 7.61 (s, 1H), 6.81 (t, $J = 6.0$ Hz, 1H), 3.89 (s, 3H), 3.42–3.38 (m, 2H), 3.24 (d, $J = 12.5$ Hz, 2H), 2.82–2.72 (m, 2H), 2.03–1.93 (m, 1H), 1.84–1.76 (m, 2H), 1.46–1.35 (m, 2H); LC-MS (ESI): *m/z* calcd for $C_{20}H_{23}N_9$: 390.21 $[M+H]^+$; found: 390.22.

5-((4-((2-Aminoethyl)amino)-5-(1-methyl-1*H*-pyrazol-4-yl) pyrimidin-2-yl)amino)picolinonitrile (11)

General procedure F. Yield: 80%; m.p. 175–177 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta = 9.93$ (s, 1H), 9.03 (d, $J = 2.5$ Hz, 1H), 8.49 (dd, $J = 9.0$, 2.5 Hz, 1H), 7.93 (s, 1H), 7.92–7.89 (m, 2H), 7.63 (s, 1H), 6.63 (t, $J = 5.0$ Hz, 1H), 3.88 (s, 3H), 3.39 (dd, $J = 12.0$, 6.5 Hz, 2H), 2.76 (t, $J = 6.5$ Hz, 2H). ^{13}C NMR (125 MHz, [D_6]DMSO): $\delta = 159.78$, 157.58, 153.24, 141.31, 141.19, 137.52, 129.28, 123.30, 122.28, 118.35, 113.90, 104.54, 43.67, 40.50, 38.65. LC-MS (ESI): *m/z* calcd for $C_{16}H_{17}N_9$: 336.16 $[M+H]^+$; found: 336.20.

(*R*)-5-((5-(1-Methyl-1*H*-pyrazol-4-yl)-4-(piperidin-3-ylamino) pyrimidin-2-yl)amino)picolinonitrile (12)

General procedure F. Yield: 78%; m.p. 145–148 °C; $[\alpha]_D^{20} = +58.00$ ($c = 0.5$ in CH_3OH); ^{1}H NMR (500 MHz, [D_6]DMSO): $\delta = 9.91$ (s, 1H), 9.04 (d, $J = 2.5$ Hz, 1H), 8.47 (dd, $J = 8.5$, 2.5 Hz, 1H), 7.91 (s, 1H), 7.90 (s, 1H), 7.88 (d, $J = 8.5$ Hz, 1H), 7.62 (s, 1H), 6.16 (d, $J = 8.0$ Hz, 1H), 4.09–4.02 (m, 1H), 3.89 (s, 3H), 2.95 (dd, $J = 11.5$, 3.0 Hz, 1H), 2.75–2.66 (m, 1H), 2.62–2.52 (m, 2H), 1.84–1.74 (m, 1H), 1.69–1.62 (m, 1H), 1.61–1.54 (m, 1H), 1.48–1.41 (m, 1H). LC-MS (ESI): *m/z* calcd for $C_{19}H_{21}N_9$: 376.19 $[M+H]^+$; found: 376.20.

(*S*)-5-((5-(1-Methyl-1*H*-pyrazol-4-yl)-4-(piperidin-3-ylamino) pyrimidin-2-yl)amino)picolinonitrile (13)

General procedure F. Yield: 73%; m.p. 206–208 °C; $[\alpha]_D^{20} = -59.00$ ($c = 0.5$ in CH_3OH); ^1H NMR (500 MHz, [D_6]DMSO): $\delta = 9.90$ (s, 1H), 9.03 (d, $J = 2.0$ Hz, 1H), 8.46 (dd, $J = 9.0$, 2.5 Hz, 1H), 7.90 (s, 2H), 7.87 (d, $J = 8.5$ Hz, 1H), 7.63 (s, 1H), 6.15 (d, $J = 8.0$ Hz, 1H), 4.09–4.04 (m, 1H), 3.89 (s, 3H), 2.74–2.67 (m, 1H), 2.62–2.53 (m, 2H), 1.83–1.76 (m, 1H), 1.69–1.62 (m, 1H), 1.58 (m, 1H), 1.49–1.40 (m, 1H). ^{13}C NMR (125 MHz, [D_6]DMSO): $\delta = 158.84$, 157.62, 153.49, 141.27, 141.18, 137.31, 129.23, 129.16, 123.31, 122.35, 118.32, 113.91, 104.43, 50.81, 47.07, 45.98, 38.69, 29.50, 24.13. LC-MS (ESI): *m/z* calcd for $C_{19}H_{21}N_9$: 376.19 $[M+H]^+$; found: 376.20.

(*R*)-5-((4-(3-Aminopiperidin-1-yl)-5-(1-methyl-1*H*-pyrazol-4-yl) pyrimidin-2-yl)amino)picolinonitrile (14)

General procedure F. Yield: 70%; m.p. 182–184 °C; ^1H NMR (500 MHz, [D_6]DMSO): $\delta = 10.09$ (s, 1H), 9.03 (d, $J = 2.5$ Hz, 1H), 8.44 (dd, $J = 8.5$, 2.5 Hz, 1H), 8.11 (s, 1H), 7.93–7.89 (m, 2H), 7.63 (s, 1H), 3.87 (s, 3H), 3.79–3.74 (m, 1H), 3.68–3.63 (m, 1H), 2.76–2.67 (m, 2H), 1.87–1.80 (m, 1H), 1.66–1.57 (m, 2H), 1.51–1.42 (m, 1H), 1.20–1.11 (m, 1H). ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 162.88$, 157.45, 156.83, 141.23, 141.05, 136.68, 129.32, 128.15, 123.47, 122.57, 118.28, 116.91, 107.29, 55.62, 47.46, 47.36, 38.58, 33.66, 23.15. LC-MS (ESI): *m/z* calcd for $C_{19}H_{21}N_9$: 376.19 $[M+H]^+$; found: 376.21.

5-((5-(1-Methyl-1*H*-pyrazol-4-yl)-4-((2-morpholinoethyl)amino) pyrimidin-2-yl)amino)picolinonitrile (15)

A mixture of compound **44** (1.0 mmol), 5-bromo-2-cyanopyridine (1.0 mmol), $Pd_2(dba)_3$ (0.01 mmol), Xantphos (0.02 mmol), and Cs_2CO_3 (1.50 mmol) in 1,4-dioxane (8 mL) were heated to reflux for 5 h. The completion of the reaction was monitored by TLC on silica gel. The reaction mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/ethanol = 30:1) to give compound **15** Yield: 84%; m.p. 189–191 °C; ^1H NMR (500 MHz, [D_6]DMSO): $\delta = 9.90$ (s, 1H), 9.05 (d, $J =$ 2.5 Hz, 1H), 8.47 (dd, $J = 9.0$, 2.5 Hz, 1H), 7.92 (s, 1H), 7.91 (s, 1H), 7.86 (d, $J = 9.0$ Hz, 1H), 7.63 (d, $J = 1.0$ Hz, 1H), 6.55 (t, $J = 5.0$ Hz, 1H), 3.89 (s, 3H), 3.60–3.55 (m, 4H), 3.52 (dd, $J = 12.0$, 6.5 Hz, 2H), 2.53 (t, $J = 6.5$ Hz, 2H), 2.45–2.38 (m, 4H). ^{13}C NMR (125 MHz, [D_6]DMSO): $\delta = 159.50$, 157.61, 153.19, 141.24, 137.40, 129.17, 129.09, 123.39, 122.37, 118.34, 113.91, 104.45, 66.32, 56.33, 53.07, 38.67, 37.37; LC-MS (ESI): *m/z* calcd for $C_{20}H_{23}N_9O$: 406.20 $[M+H]^+$; found: 406.21.

5-((5-(1-Methyl-1*H*-pyrazol-4-yl)-4-((2-morpholinoethyl)amino) pyrimidin-2-yl)amino)pyrimidine-2-carbonitrile (16)

A mixture of compound **44** (1.0 mmol), 5-bromopyrimidine-2-carbonitrile (1.0 mmol), $Pd_2(dba)_3$ (0.01 mmol), Xantphos (0.02 mmol), and Cs_2CO_3 (1.5 mmol) in 1,4-dioxane (8 mL) were heated to reflux for 5 h. The completion of the reaction was monitored by TLC on silica gel. The reaction mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/ethanol = 30:1) to give compounds **16**. Yield: 80%; m.p. 170–172 °C; ^1H NMR (500 MHz, [D_6]DMSO): $\delta = 10.10$ (s, 1H), 9.34 (s, 2H), 7.95 (s, 1H), 7.92 (s, 1H), 7.64 (d, $J = 0.6$ Hz, 1H), 6.63 (t, $J = 5.3$ Hz, 1H), 3.90 (s, 3H), 3.60–3.55 (m, 4H), 3.51 (dd, $J = 12.3$, 6.5 Hz, 2H), 2.54 (t, $J = 6.7$ Hz, 2H), 2.42 (s, 4H). ^{13}C NMR (125 MHz, [D_6]DMSO): $\delta = 160.00$, 157.59, 153.62, 146.23, 139.23, 137.89, 134.43, 129.63, 117.18, 114.21, 105.40, 66.78, 56.72, 53.54, 39.16, 37.93; LC-MS (ESI): *m/z* calcd for $C_{19}H_{22}N_{10}O$: 407.20 $[M+H]^+$; found: 407.21.

Determination of compound potency (IC_{50} values)

Human recombinant CHK1 protein, which was fused with GST (aa1-476) was purchased from Sino Biological Inc. The enzymic activity was detected by the HTRF® KinEASE™ STK S1(Cisbio). The CHK1 protein and peptide substrates were diluted in HTRF kinase buffer (1X Kinase buffer, 5 mM $MgCl_2$, 1 mM DTT), and then compounds of different concentrations, CHK1 protein and peptide substrates were added to the 384 plates (ProxiplateTM-384 Plus, PerkinElmer). The final volume of the reactions is 10 μl (2% DMSO, 0.075 ng/μl CHK1, 1 μM STK1, 50 μM ATP). The antibody was added after the kinase reactions incubated at room temperature for 1 h. The phosphorylation activity of CHK1 can be determined by detecting the ratio of fluorescence signals at 665 and 620 nm. The IC_{50} value of the compound was calculated using the software GraphPad Prism 5, and the equation "sigmoidal dose-response (variable slope)" was chosen for curve fitting.

In vitro cytotoxic activities

A 90 μL of cells were plated into 96-well microculture plates and then added with 10 μL of different concentrations of compounds for 72 h at 37 °C in 5% CO_2. Then, 20 μL of MTS was added to incubate for 3 h at 37 °C and then recording absorbance at 490 nm (background subtraction at 690 nM)with SpectraMAX340. The inhibition rate of the compound on the growth of cancer cells was calculated as follows. Growth inhibitory ratio = $(A_{control} - A_{sample})/A_{control} \times 100\%$ The IC_{50} value of the compound was calculated using the software GraphPad Prism 5.

Metabolic stability *in vitro* assays

Mouse liver microsome was purchased from Research Institute for Liver Diseases (Shanghai) Co., Ltd. Mouse liver microsome (1.0 mg) was diluted in PBS buffer (1 mL), and followed mixed with 1 mM NADPH solution. Microsome-NADPH solution was prewarmed at 37 °C for 5 min. 10 μL of a 100 μg/mL compound was then added to Microsome-NADPH solution and then divided into 3 aliquots. The mixture was kept at 37 °C and 10 μL aliquots from each aliquot were taken at 30 min. The reaction was quenched with 490 μL of methanol containing 1 μg/mL internal standard compound. After quenching, the mixtures were vortexed and centrifuged. 10 μL of the supernatant was injected into an LC/MS system to test peak areas of compound **13**.

In vivo PK evaluation

This study was performed in strict accordance with the Laboratory Animal Management Regulations (State Scientific and Technological Commission Publication No. 8–27 Rev. 2017) and was approved by Shanghai Institute of Materia Medica (Shanghai, Chian). SD rats were purchased from the JOINN Laboratories (Suzhou). After oral dosing of 20 mg/kg of compound **13** (5% DMSO, 5% Solutol, 90% (0.5%MC), Blood samples were collected at nine time points (0.083, 0.25, 0.5, 1, 2, 4, 6, 24, 30 h). Plasma was separated by centrifugation at 5000 rpm for 10 min at 4 °C and analyzed by LC-MS/MS (Waters XEVO TQ–S). Pharmacokinetic parameters were analyzed by Masslynx® V4.1.

Western blotting

The primary antibodies Chk1 (catalog number: sc-8408) was from Santa Cruz Biotechnology, Beta-actin (catalog number: AM1021B) was from Abgent, P-Chk1(Ser345) (catalog number: 2341 s) and P-Chk1(Ser296) (catalog number: 2349 s) were from Cell Signaling

(Cell Signaling Technology, Inc). Cells treated as indicated were lysed, then separated on SDS-PAGE after electrophoresis. Proteins were transferred to Nitrocellulose Blotting Membrane (GE Healthcare). The membranes were placed in primary antibody for 12 h at 4 °C. Primary antibody was detected using horseradish peroxidase linked secondary antibodies (Jackson ImmunoResearch Laboratories, Inc). Immunoreactive bands were detected by Clarity Western ECL Substrate (BIO-RAD; for details see the Supporting Information).

Molecular docking

The molecular docking was performed using Discovery Studio (version 2.1) with the default option. The X-ray crystal structure of CHKI protein (PDB ID: 2YM8) was applied as the docking template. The CHK1 protein was prepared by removing crystallographic waters and ligands, adding missing hydrogen bond in CHARMM force field. The 3D conformation of compound **13** was prepared by Discovery Studio 2.1/Diverse conformation generation, then whose energy minimized by DS 2.1/Minimize molecule. For the docking calculation of compound **13**, Discovery Studio 2.1/C-DOCKER was used to generate the conformations. The graphical image was analyzed by PyMOL v0.99.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: CHK1 inhibitor · diaminopyrimidine · DNA damage response · oral bioavailability

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