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Discovery of (*R*)-5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile, a novel CHK1 inhibitor for hematologic malignancies

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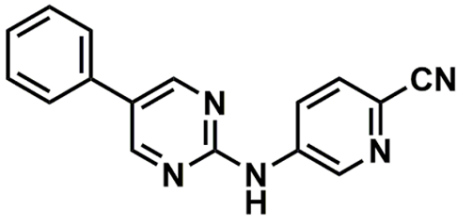
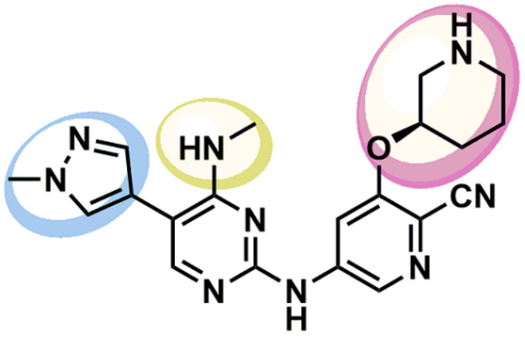


MCL1020

CHK1 IC_{50} = 1.61 μM



(*R*)-17

CHK1 IC_{50} = 0.4 nM
CHK2 IC_{50} = 1729.0 nM
Z-138 IC_{50} = 0.013 μM
TGI: (Z-138, 20 mg/kg I.V.) 90.29%

Discovery of (*R*)-5-((5-(1-Methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile, a Novel CHK1 Inhibitor for Hematologic Malignancies

Lexian Tong[a,1], Pinrao Song[b,1], Kailong Jiang[c,d,1], Lei Xu[c,d,e], Tingting Jin[a], Peipei Wang[c,d], Xiaobei Hu[c,d], Sui Fang[d], Anhui Gao[c,d], Yubo Zhou[c,d], Tao Liu[*,a], Jia Li[*,c,d,e,f], Yongzhou Hu[*,a]

[a]*ZJU-ENS Joint Laboratory of Medicinal Chemistry, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China*

[b]*Shanghai Haini Pharmaceutical Co. Ltd., Yangtze River Pharmaceutical Group, Shanghai 201318, People's Republic of China*

[c]*National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China*

[d]*University of Chinese Academy of Sciences, No. 19A Yuquan Road, Beijing 100049, People's Republic of China*

[e]*ShanghaiTech University, shanghai 201210, People's Republic of China*

[f] *Open Studio for Druggability Research of Marine Natural Products, Pilot National Laboratory for Marine Science and Technology (Qingdao), 1 Wenhai Road, Aoshanwei, Jimo, Qingdao, 266237, China*

**Corresponding authors: Tao Liu, E-mail: Lt601@zju.edu.cn; Jia Li, E-mail: jli@simm.ac.cn; Yongzhou Hu, huyz@zju.edu.cn*

[1]*Equal contribution*

Abstract

Through virtual screening, we identified the lead compound MCL1020, which exhibited modest CHK1 inhibitory activity. Then a series of 5-(pyrimidin-2-ylamino)picolinonitrile derivatives as CHK1 inhibitors were discovered by further rational optimization. One promising molecule, (***R***)-**17**, whose potency was one of the best, had an IC_{50} of 0.4 nM with remarkable selectivity (>4300-fold CHK1 *vs*. CHK2). Compound (***R***)-**17** effectively inhibited the growth of malignant hematopathy cell lines especially Z-138 (IC_{50}: 0.013 μM) and displayed low affinity for hERG (IC_{50} > 40 μM). Moreover, (***R***)-**17** significantly suppressed the tumor growth in Z-138 cell inoculated xenograft model (20mg/kg I.V., TGI = 90.29%) as a single agent with body weight unaffected. Taken together, our data demonstrated compound (***R***)-**17** could be a promising drug candidate for the treatment of hematologic malignancies.

Keywords: CHK1 kinase inhibitor; selectivity; monotherapy; hematologic malignancies

1. Introduction

The genomic integrity within eukaryotic cells is threatened from both endogenous and exogenous sources of damage. In response to DNA damage, a complex series of interactions sense and mediate this process. A central protein involved in the DNA damage response (DDR) is Checkpoint Kinase 1 (CHK1), a serine/threonine kinase [1]. It responds to replication fork abnormalities via ATR-dependent phosphorylation at two sites, Ser-345 and Ser-317, through which it activates an array of downstream events to provoke cells to undergo S or G2/M phase cell-cycle arrest, preserve replication fork viability, activate DNA repair mechanisms, and terminate the checkpoint to resume cell division cycle [2-4].

In general, cancer cells harboring a deficient tumor p53 pathway lack efficient G1 checkpoint and maintaining the G2/M checkpoint fully depend on checkpoint kinases [5, 6]. Therefore, the pharmacological inhibition of checkpoint kinases in combination with the DNA damaging chemotherapy or radiotherapy will selectively sensitize p53 deficient cancer cells to chemotherapeutics and induce mitotic catastrophe and cell death [7-9]. Such combined therapy results in mild side-effects towards healthy cells while efficiently eradicating the cancer cells. Currently, quite a number of diverse CHK1 inhibitors have been disclosed and evaluated in

clinical trials [10-13]. Early CHK1 inhibitors such as AZD7762, XL844, and PF477736, often showed low selectivity [12, 14], and most of them have been halted after early phase clinical trials because of being associated with unfavorable toxicity profile or limited efficacy. In an attempt to overcome these drawbacks, potent and selective CHK1 inhibitors have received significant attentions. The CHK1 inhibitors developed in recent years including SRA-737 (**1**), GDC-0575 (**2**), and LY2880070 (**3**), were likely to benefit from increased selectivity and potency [15, 16].

Recent researches demonstrated the single agent efficacy for CHK1 inhibitors in specific cancer types, which could significantly enhance the clinical benefit of future drugs in this class. Intrinsic DNA damage and the activation of CHK1 signaling resulting from high endogenous replication stress appear to underlie the effectiveness of the inhibitors in many cases [12]. Preclinical data reported suggested that inhibition of CHK1 may result in caspase-dependent apoptosis of MYC-overexpressing cells, so CHK1 inhibitors should be evaluated as potential drugs against MYC-driven malignancies such as certain B-cell lymphoma/leukemia, neuroblastoma, and some breast cancers [17, 18]. Indeed some of CHK1 inhibitors such as SRA-737(**1**) and LY2606368 (**4**), were reported could be as single agents in treating malignancy and now these two compounds are in phase II clinical trials. The benefit of CHK1 inhibition remains to be tested, so the studies on small-molecule CHK1 inhibitors have aroused great concern from a large number of research groups including ours [19-24]. Herein, we described the discovery and development of a novel class of CHK1 inhibitors which contained 2-amino pyrimidine scaffold.

In order to discover novel scaffolds of CHK1 inhibitors, we screened the Chembridge database and our in-house compound library that led to the identification of MCL1020 (Fig. 2) with 2-amino pyrimidine scaffold as a lead. With structure guided efforts toward optimization of a 2-amino pyrimidine class of CHK1 inhibitors, we identified potent and highly selective CHK1 inhibitor (***R***)-**17** which shown strong single agent activity in Z-138 cell inoculated xenograft model.

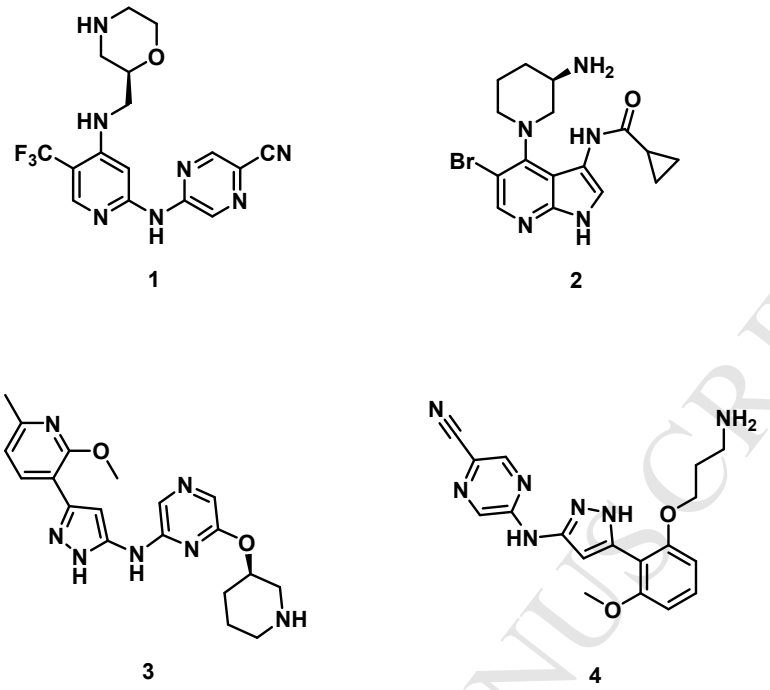


Figure 1. Structures of representative CHK1 inhibitors SRA-737 (**1**), GDC-0575 (**2**), LY2880070 (**3**), LY2606368 (**4**).

2. Results and discussion

2.1. Virtual screening

Several CHK1 crystal structures have recently been published, which allows structure-based drug design to be used in the search for novel classes of CHK1 inhibitors. We explored a compound library from Chembridge database which contains about 60000 small molecules and our in-house compound library containing about 3000 small molecules. These compounds were docked into the active site of CHK1 kinase (PDB code 2YM8) [25], using the Discovery Studio (version 2.1)/Ligandfit suite, in which six scoring functions (LigScore1, LigScore2, -PLP1, -PLP2, Jain, and -PMF) were utilized [26, 27]. Statistical quality of the models was evaluated by enrichment factor (EF) metrics and receiver operating curve (ROC) analysis [28]. The results revealed that LigScore2 outperformed all other scoring functions. Therefore, 10 compounds were finally selected based on balanced scores from LigScore2 and druglike properties (Table S2). Details of the workflows, screening compound numbers and filters used for the virtual screening,

and postprocessing analyses with scoring functions are detailed in the Supporting Information (Section 1).

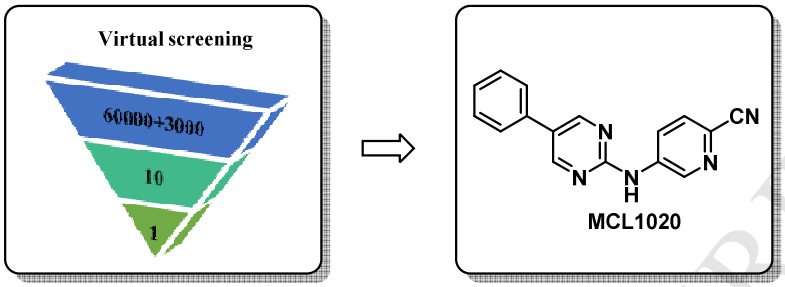
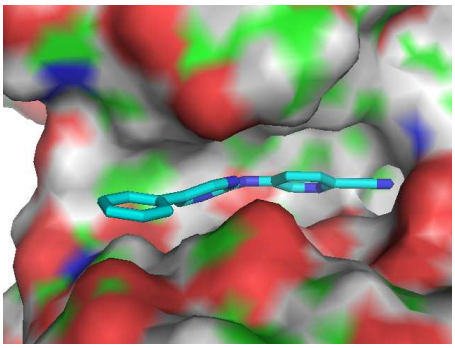


Figure 2. Schematic showing the discovery of new CHK1 inhibitor MCL1020.

2.2. Novel CHK1 inhibitors design strategy

Among these 10 compounds, MCL1020 grabbed our attention because it was the only one derived from our internal compound library harboring novel skeleton. And it was intriguing that MCL1020 could be regarded as scaffold hopping from SRA-737 (**1**)，thus we initially tested the CHK1 inhibitory activity of MCL1020 *in vitro*. Gratifyingly, MCL1020 exhibited modest CHK1 inhibitory activity (IC_{50} = 1.61 μM). To further understand binding characteristics of MCL1020 with CHK1, docking of MCL1020 into CHK1 X-ray crystal structure (PDB code 2YM8) was performed (Fig. 3). The docking result indicated that the MCL1020 properly occupied the ATP pocket by interacting with a diverse range of the sites of the kinase. The kinase hinge-binding 2-amino pyrimidine core was predicted to form two hydrogen-bonded polar contacts with the Cys87 and Glu85 residues. The N of pyridine accepted a hydrogen bond from the nearest of the three waters and 2-cyano of pyridine accepted a hydrogen bond from the protonated catalytic lysine (Lys38). The area around the ribose region was unexplored, and our initial effort concentrated on probing this area. It appears that key residues in ribose pocket including Glu91, Glu134, and Asn135, a polar fragment usually vector toward this pocket [29, 30].

(a)



(b)

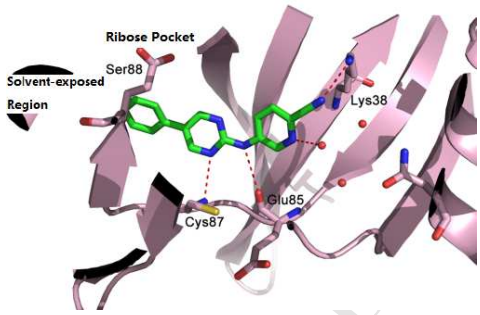


Figure 3. (a) Docking of MCL1020 to CHK1 (PDB code 2YM8, van der Waals surfaces); MCL1020 is shown as sticks colored green for carbon, blue for nitrogen; (b) Docking of MCL1020 to CHK1 (PDB code 2YM8); CHK1 interacting residues are shown as sticks colored pink for carbon, blue for nitrogen, red for oxygen; MCL 1020 is shown as sticks colored green for carbon, blue for nitrogen; Figure prepared with Discovery Studio (version 2.1)

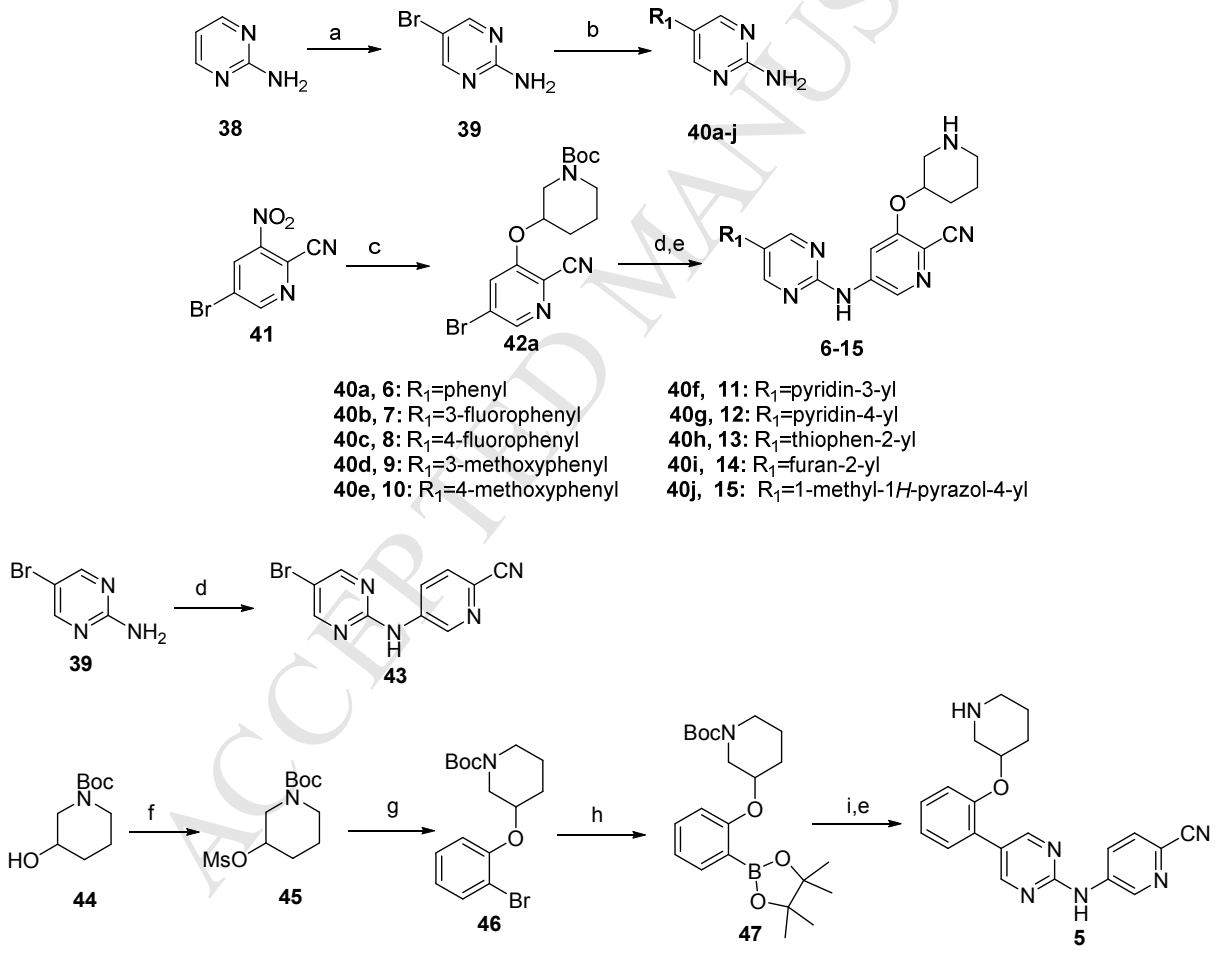
2.3. Chemistry

All target compounds were prepared by routes outlined in Schemes 1−3. The synthesis of 2-amino pyrimidine derivatives **5**−**15** is shown in Scheme 1. The synthesis started from commercially available pyrimidin-2-amine **38**, which was initially brominated with NBS to obtain 5-bromopyrimidin-2-amine **39**, followed by Suzuki−Miyaura coupling to give **40a**−**j**. Commercially available **41** reacted with 1-Boc-3-hydroxypiperidine **44** to provide intermediate **42a** [31]. Buchwald–Hartwig cross-coupling of **42a** with **40a**−**j** and subsequent acidic deprotection afforded **6**−**15** (Scheme 1) [32]. Intermediate **43** was obtained by Buchwald−Hartwig cross-coupling of **39** and 5-bromopicolinonitrile. Compound **44** was transformed into **45** by methyl sulfonylation with MSCl. The nucleophilic substitution of **4**5 with 2-bromophenol was performed in the presence of appropriate base to obtain compound **46**. Intermediate **47** was obtained by Suzuki−Miyaura coupling of **46** and bis(pinacolato)diboron in dry atmosphere. Then compound **5** was obtained by Suzuki−Miyaura coupling and subsequent acidic deprotection.

The synthesis of 2-amino pyrimidine derivatives **16**−**31** is shown in Scheme 2. Commercially available **48** reacted with sodium methylate or methylamine to give **49** and **50**, respectively. Nucleophilic displacement of **49** or **50** with ammonium hydroxide afforded the corresponding 2-

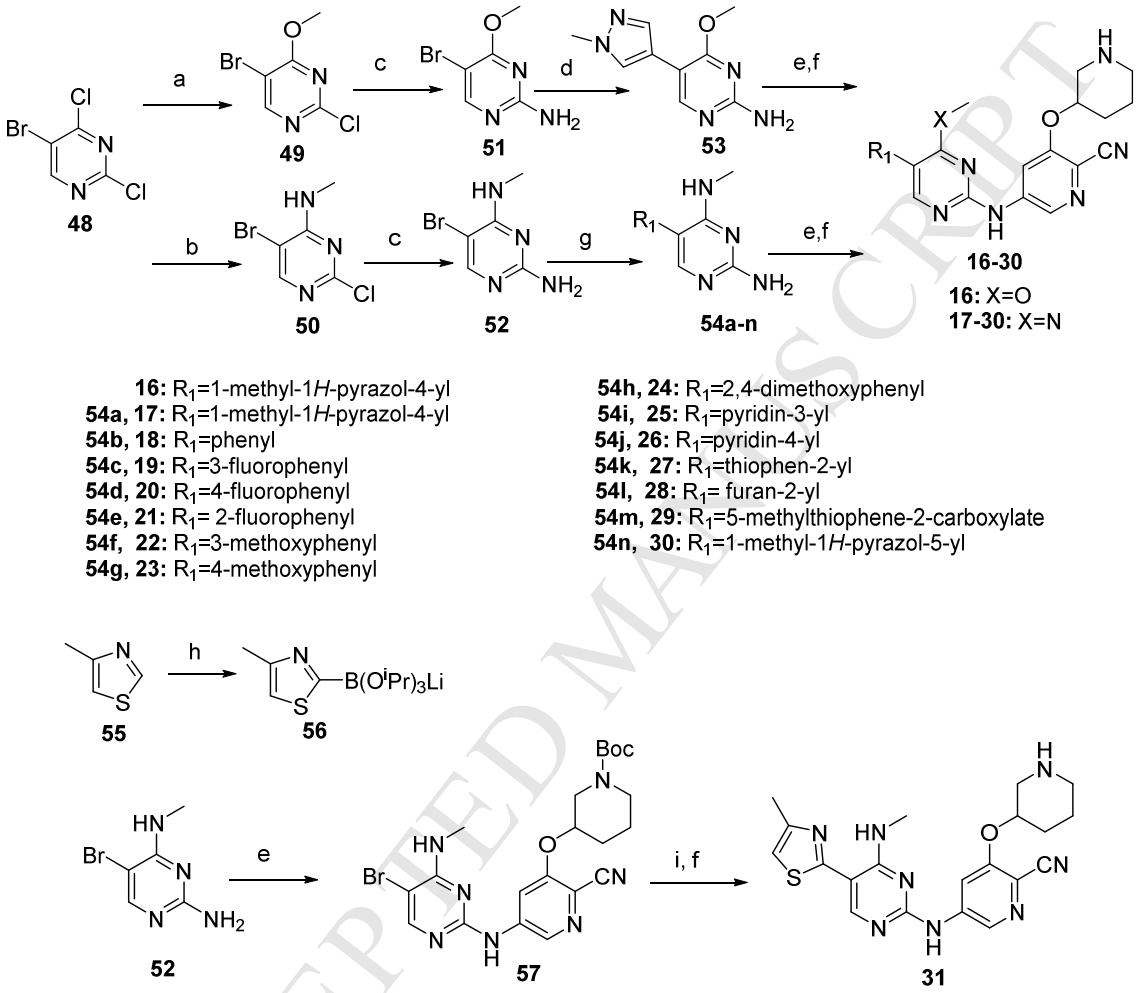
amino pyrimidine derivatives **51** or **52** [33]. Compounds **16**−**30** were obtained by **51** or **52** from sequential Suzuki-Miyaura coupling and Buchwald-Hartwig cross-coupling reactions, followed by acidic deprotection, respectively. Commercially available 4-methylthiazole **55** was converted to lithium-bearing compound **56** through reaction with triisopropyl borate in the presence of appropriate base n-butyllithium**.** Compound **31** was obtained by **52** under reaction of Buchwald–Hartwig coupling then Suzuki−Miyaura coupling, followed by acidic deprotection.

The synthesis of 2-amino pyrimidine derivatives (***R***)-**17**, (***S***)-**17**, **32**−**37** is shown in Scheme 3. Intermediate **42b-j** were obtained in the same method as **42a**. Compound (***R***)-**17**, (***S***)-**17**, **32**−**37** were formed in the same method as **17**.

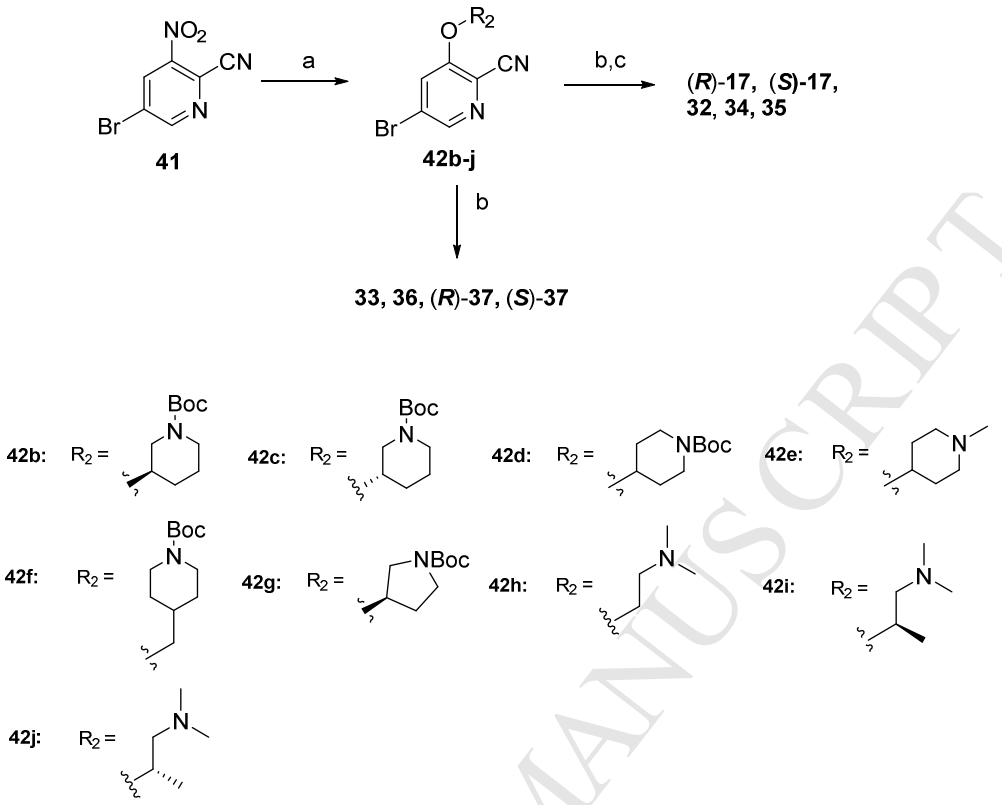


Scheme 1. Synthetic routes of compounds **5**−**15**. Reagents and conditions: (a) NBS, MeCN, rt (97%); (b) R_1-boronic acid/ester, $Pd(dppf)Cl_2$, 1 N Na_2CO_3, DME, reflux (79%−87%); (c) **44**, NaH, THF, 0°C−rt, 3 h (79%); (d) **40a**−**j** for synthesizing **6**−**15**, 5-bromopicolinonitrile for synthesizing **43**, $Pd_2(dba)_3$, Cs_2CO_3, Xantphos,

dioxane, reflux, 5 h, (70%−87%); (e) CF_3COOH, CH_2Cl_2, rt (80%); (f) MsCl, TEA, DMAP, CH_2Cl_2, 0°C, 1 h (78%); (g) 2-bromophenol, Cs_2CO_3, DMF, 70°C, 5 h (43%); (h) Bis(pinacolato)diboron, Pd(dppf)Cl_2, TEA, dioxane, reflux (77%); (i) **43**, Pd(dppf)Cl_2, 1 N Na_2CO_3, DME, reflux (65%).



Scheme 2. Synthetic routes of compounds **16**−**31**. Reagents and conditions: (a) Na, MeOH, rt, 3 h (90%) ; (b) CH_3NH_2, MeOH, rt, 3 h (88%); (c) $NH_3 \cdot H_2O$, dioxane, 160°C, 3 h (78%); (d) 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, Pd(dppf)Cl_2, 1 N Na_2CO_3, DME, reflux (80%); (e) **42a**, $Pd_2(dba)_3$, Cs_2CO_3, Xantphos, dioxane, reflux, 5 h (75% − 84%); (f) CF_3COOH, CH_2Cl_2, rt (80%); (g) R_1-boronic acid/ester, Pd(dppf)Cl_2, 1 N Na_2CO_3, DME, reflux (65%−84%); (h) Triisopropyl borate, n-butyllithium, isopropanol, dry toluene/ THF (v/v = 4:1), −78°−0°C; (i) **56**, Pd(dppf)Cl_2, CuCl, $ZnCl_2$, Cs_2CO_3, DMF, 100°C (70%).



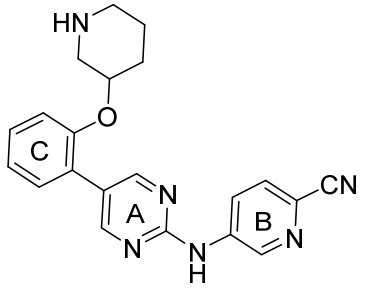
Scheme 3. synthetic routes of compounds (***R***)-**17**, (***S***)-**17**, **32**−**37**. Reagents and conditions: (a) NaH, R_2-OH,THF, 0°C−rt, 3 h (51%−90%); (b) **54a**, $Pd_2(dba)_3$, Cs_2CO_3, Xantphos, dioxane, reflux, 5 h (74%−84%); (c) CF_3COOH, CH_2Cl_2, rt (80%).

2.4. Biological evaluation

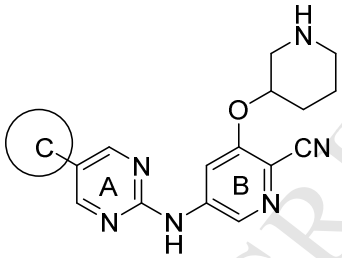
2.4.1. CHK1 inhibition and structure activity relationship (SAR) studies

We hypothesized that introducing a polar fragment directed toward the ribose region could not only enhance affinity for CHK1 by interacting with additional residues near the ribose region but also improve physicochemical property of molecule. Furthermore we analyzed the complex of MCL1020 and CHK1 and found the C-ring (phenyl) directed toward solvent-exposed surface and B-ring (pyridine) substituted a small substituent where could adopt a large group (Fig. 3), so the polar fragment was introduced on these two rings. We were pleased to find that the potency of the optimized compound **6**, the hydrophilic segment located in the B-ring, was significantly improved with IC_{50} 11 nM (Table 1), while the hydrophilic segment located in the C-ring as

shown in table 1, compound **5** had no inhibitory effect on CHK1. Taking compound **6** as our new lead, we conducted further structure modification to explore the structure−activity relationships.



5



6-15

Table 1. SAR exploration of the location of hydrophilic segment and C-ring

Cpd.	C-ring	IC_{50} (nM)[a]
5	-	6053.5±1198.55
6	phenyl	10.95±0.57
7	3-fluorophenyl	14.30±0.85
8	4-fluorophenyl	21.19±1.70
9	3-methoxyphenyl	15.92±2.93
10	4-methoxyphenyl	8.34±0.67
11	pyridin-3-yl	99.58±3.00
12	pyridin-4-yl	58.94±2.87
13	thiophen-2-yl	1.96±0.65
14	furan-2-yl	12.71±0.67
15	1-methyl-1*H*-pyrazol-4-yl	3.75±0.94

[a]Values are means of three experiments.

The modification toward the C-ring (5-phenyl ring) in compound **6** was to install different groups in the para- or meta- positions to give compounds **7**−**10**. Subsequently, aromatic

heterocyclic rings were introduced to replacement of C-ring. The results demonstrated that introduction of electron-withdrawing (**7** and **8**) or donating (**9** and **10**) groups in the either para- or meta- position of phenyl ring had a little effect on the potencies against CHK1. Replace phenyl ring with pyridine ring yielded compound **11** and **12** that demonstrated a relatively modest drop in potencies, while the potencies of five-membered heteroaromatic ring analogues were kept or increased. Thiophen-2-yl **13** and 1-methyl-1*H*-pyrazol-4-yl **15** shown the best potency in this series, regrettably, the poor aqueous solubility of **13** was assumed to be limiting its further development.

Further analysis of ribose region suggests that there is still some space at 4-position of B-ring where can adopt small substituent, thus, we add a small substituent at this position to see whether the potency would be affected. At first, methoxy group or methylamino group was introduced to the 4-position of B-ring of the compound **15** giving two new compounds **16** and **17**. A sharp drop in potency was observed when introducing methoxy group. In contrast, the potency of 4-methylamino pyrimidine analogue **17** increased about 4 times relative to that of compound **15**. Encouragingly, a set of 4-methylamino pyrimidine analogues were synthesized (**18-31**, Table 2). As expected, the potencies of these newly prepared analogues presented in Table 2 were maintained or slightly increased compared with compounds presented in Table 1. Introduction of a substituent in ortho-position of C-ring may cause unfavorable interactions with residues surrounding the ATP-binding pocket and weaken the potency against CHK1 (**20** vs **21**, **23** vs **24**). In this turn, 1-methyl-1*H*-pyrazol-4-yl **17** (IC_{50} = 0.9 nM) shown the best potency, was chosen as the basis for further structural optimization.

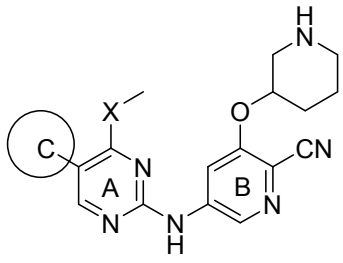


Table 2. SAR exploration of X at 4-position of A-ring and C-ring

Cpd.	C-ring	X	IC_{50} (nM)[a]
16	1-methyl-1*H*-pyrazol-4-yl	O	1406.0±199.40

17	1-methyl-1*H*-pyrazol-4-yl	N	0.89±0.06
18	phenyl	N	2.56±0.55
19	3-fluorophenyl	N	4.49±0.94
20	4-fluorophenyl	N	8.76±0.62
21	2-fluorophenyl	N	29.02±0.29
22	3-methoxyphenyl	N	3.62±0.71
23	4-methoxyphenyl	N	19.43±18.34
24	2,4-dimethoxyphenyl	N	365.25±14.78
25	pyridin-3-yl	N	15.14±1.68
26	pyridin-4-yl	N	4.72±1.51
27	thiophen-2-yl	N	1.43±0.09
28	furan-2-yl	N	2.58±0.34
29	5-methylthiophene-2-carboxylate	N	1.80±0.17
30	1-methyl-1*H*-pyrazol-5-yl	N	517.35±73.19
31	4-methylthiazol-2-yl	N	4.72±1.51

[a] Values are means of three experiments.

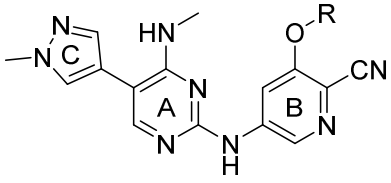
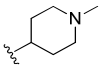
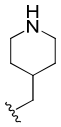


Table 3. SAR exploration of R at 3-position of B-ring

Cpd.	R	IC_{50} (nM)[a]
(***R***)-**17**		0.4±0.0

(***S***)-**17**		8.88±0.45
32		0.5±0.1
33		1.3±0.3
34		6.44±0.23
35		2.43±0.26
36		26.30±8.32
(***R***)-**37**		26.33±6.84
(***S***)-**37**		59.49±11.12
LY2603618	-	10.4±5.2
LY2606368	-	0.7±0.4

[a] Values are means of three experiments.

Having identified 1-methyl-1*H*-pyrazol-4-yl as a preferred C-ring, our efforts focused on exploring the R substituent. Various amine substitutions were introduced on the B-ring (Table 3). As was observed in table 3, two pairs of enantiomers (***R***)-**17** and (***S***)-**17**, (***R***)-**37** and (***S***)-**37** together shown that the potencies of compounds with *R* configuration were superior to *S*. The potency of compound **32** that contained piperidine was retained (IC_{50} = 0.5 nM). When methylation of the piperidine of **32** gave compound **33**, potency reduced by 2.6-fold, indicating that the substituents holding the secondary amine were more suitable than tertiary amine. Subsequent introduction of piperidin-4-ylmethyl led **34**, which was more than 12-fold less potent than **32**. Five-member heterocyclic analogue **35** exhibited a 6-fold decrease in activity compared with six-member heterocyclic analogue (***R***)-**17**. On the other hand, introduction of linear aminoalkylamines giving compounds **36**-**37** resulted in dramatically drop of potency compared

with **33**. In this series, cyclic substituents introduced were more suitable than linear substituents, and compound (***R***)-**17** was deemed as the most potent compound.

2.4.2. Kinase selectivity profile of (***R***)-**17**

Selectivity of inhibition for a particular kinase is a well-known issue, as well as CHK1. The chemical modifications toward compound MCL1020 resulted in one promising molecule (***R***)-**17**. Thus, compound (***R***)-**17** was selected to assess for selectivity against 15 kinases belonged to four kinase subfamilies (CHK1, CHK2, AMPKα2β1γ1, AMPKα1β1γ1, PIM1, PIM3 from CAMK subfamily; CDK1, CDK2 from CMGC subfamily; p70S6k, PKC, PKA from AGC subfamily; FLT3, BLK, LCK, LYN from TK subfamily). The results were summarized in Table 4. Compound (***R***)-**17** displayed excellent selectivity against CHK2 (>4300-fold) and was significantly more selective than that of positive control LY2606368 (58-fold CHK1 *vs*. CHK2). Cyclin-dependent kinases, such as CDK1 and CDK2, important in regulating the cell cycle, were also not significantly inhibited by (***R***)-**17**. Compared with LY2606368, (***R***)-**17** displayed a little bit stronger binding against PIM1 and PIM3 and showed similar kinase inhibition against other selected kinases. These results indicated the (***R***)-**17** had good kinase selectivity profiles and was almost equal to LY2606368.

Table 4. Kinase inhibition profile of compound (***R***)-**17Ac** against selected protein kinases

Kinase	(***R***)-**17Ac**[a] IC_{50}(nM)	LY2606368 IC_{50}(nM)	Kinase	(***R***)-**17 Ac**[a] IC_{50}(nM)	LY2606368 IC_{50}(nM)
CHK1	0.4±0.0	0.56±0.16	p70S6k	625.68±124.89	382.18±17.75
CHK2	1729.0±60.8	32.57±2.13	PKC	＞10 μM	n.t.[b]
AMPKα2β1γ1	91.11±9.59	30.09±5.09	PKA	＞10 μM	n.t.[b]
AMPKα1β1γ1	107.50±12.42	101.62±9.92	FLT3	100.45±9.35	385.98±22.95
PIM1	511.80±73.31	＞10μM	BLK	2713.75±209.85	4409.00±686.79
PIM3	735.53±176.37	＞10μM	LCK	3156.00±334.56	>10μM
CDK1	＞10μM	＞10μM	LYN	4297.50±437.58	>10μM
CDK2	＞10μM	＞10μM			

[a] (***R***)-**17Ac** is acetate of (***R***)-**17**

[b] n.t. indicates not tested.

2.4.3. In vitro antiproliferative activities of (***R***)-**17**

On the basis of potency and kinase selectivity profile, in particular the selectivity over CHK2, (***R***)-**17** was progressed to more detailed studies. Recent evidence suggested that cell lines derived from malignant hematopathy cell lines exhibited greater sensitivity to the CHK1 inhibitor than cell lines derived from solid tumors [34, 35]. So the growth inhibitory potencies of (***R***)-**17** against six malignant hematopathy cell lines were examined, and the results were presented in table 5. Among these six malignant hematopathy cell lines, (***R***)-**17** seemed to be even more selective for Jeko-1, MV4-11 and Z-138 cell lines (IC_{50} <40 nM). In particular, (***R***)-**17** inhibited the proliferation of Z-138 with IC_{50} values of 0.013 μM, which displayed the best activity over all six cell lines. This encouraging cellular assay data led to further profiling of (***R***)-**17**.

Table 5. Cellular activities of the (***R***)-**17**

Cpd.	$IC_{50}(\mu M)$[a]					
	RPMI8226	Mino	Romas	Jeko-1	MV4-11	Z-138
(***R***)-**17**	1.990	0.155	0.095	0.036	0.039	0.013
LY2603618	4.546	0.254	0.533	0.920	0.869	0.364
LY2606368	0.045	1.110	0.004	0.004	0.004	0.033

[a] Values are means of three experiments. For SD values see Table S3.

2.4.4. In vivo PK/PD evaluation

Given the encouraging biological profile, pharmacokinetic (PK) properties of compound (***R***)-**17** was further evaluated. Key PK parameters are summarized in Table 6. After I.V. administration of 15 mg/kg compound (***R***)-**17**, the area under the concentration−time curve ($AUC_{0-\infty}$), the half-life ($T_{1/2}$), peak concentration (C_{max}), and plasma clearance (CL) of (***R***)-**17** were 370 ng/mL·h, 2.23 h, 849 ng/mL, and 135 mL/min/kg, respectively. Compound (***R***)-**17** exhibited reasonable PK properties in mice.

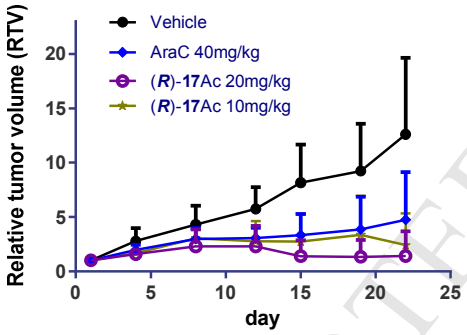
Table 6. PK parameters of (***R***)-**17** in mice

	K_{el} (L/kg)	$T_{1/2}$ (h)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng/mL·h)	$AUC_{(0-\infty)}$ (ng/mL·h)	Vd_{ss} (L/kg)	CL (mL/min/kg)	MRT (h)

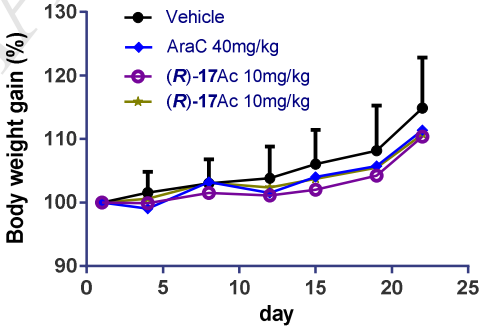
IV(15mg/kg)	0.365	2.23	849	363	370	6.42	135	0.80

The antitumor efficacy of compound (***R***)-**17** as a single agent was tested in Z-138 cell inoculated xenograft mouse model. When tumors had reached an average volume of 100 mm^3, the mice were grouped and treated intravenously with 10 or 20 (mg/kg)/d of compound (***R***)-**17Ac** ((***R***)-**17**Ac is acetate of (***R***)-**17**) , Cytarabine (AraC, as a standard drug) or vehicle (5% Mannitol) 5 times every week for 3 weeks. The results of this study (Fig. 4) showed that compound (***R***)-**17**Ac administered on this schedule led to a dose-dependent inhibition of tumor growth. Tumor growth inhibitions of 78.64% and 90.29% were observed at doses of 10 and 20mg/kg, respectively. Notably, no significant body weight loss and no adverse effects were observed for compound (***R***)-**17**Ac during the *in vivo* studies. All of the studies presented here support (***R***)-**17** as a novel candidate and deserve further research and development.

(a)



(b)



(c)

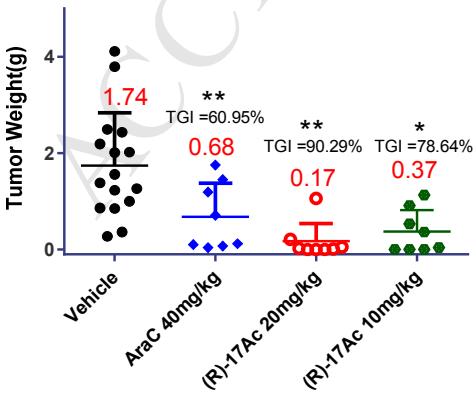
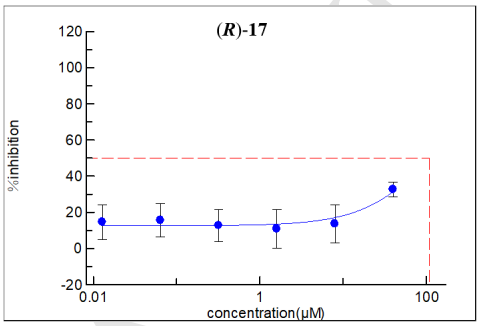


Figure 4. In vivo tumor growth inhibition for (***R***)-**17**Ac. Female nu/nu mice bearing established human Z-138 inoculated xenograft were administered I.V. with either vehicle (5% Mannitol), AraC at 40 mg/kg or (***R***)-**17**Ac at 10 and 20mg/kg body weight 5 times every week for 3 weeks. (a) Relative tumor volume. Initial tumor size was set as 1. (b) Body weight of mice. (c) Tumor weight measurements from Z-138 xenograft mice after 21 days compound administration. Numbers in red indicate the mean tumor weight in each group (* P<0.05，** P<0.01 *vs* vehicle).

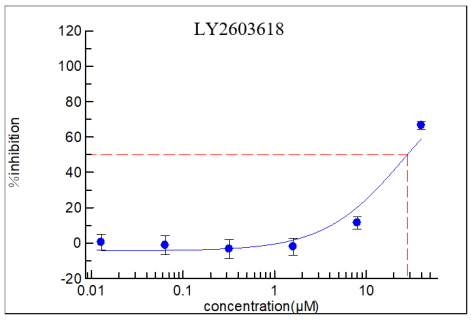
2.4.4. The hERG inhibition of the compound (***R***)-**17**

CHK1 inhibitors XL-844 and AZD7762 halted after early phase clinical trials because of being associated with cardiotoxicity [12], which to some extent incurred by certain affinity for the hERG ion channel, thus hERG inhibition of (***R***)**-17** was investigated initially. As shown in figure 5, compound (***R***)**-17** displayed low affinity for hERG (IC_{50} > 40 μM), which was less potent in inhibiting hERG compared with positive controls LY2603618, LY2606368 (IC_{50} = 28.1 μM, IC_{50} = 20 μM, respectively). The hERG model classified compounds as hERG-inactive if their percentage inhibition was less than 40% @ 10 μM [15], so compound (***R***)**-17** could be seen as hERG-inactive and the wider therapeutic index against hERG of (***R***)**-17** was suitable for preclinical evaluation.

(a)



(b)



(c)

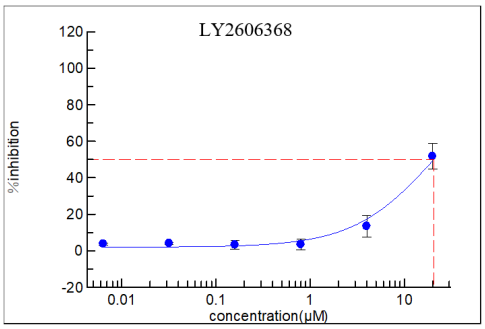


Figure 5. (a) The hERG inhibition of the compound (***R***)-**17**. (b) The hERG inhibition of the positive control compound LY2603618. (c) The hERG inhibition of the positive control compound LY2606368.

2.5. Molecular modeling of compound (***R***)-**17** with CHK1

Compound (***R***)-**17** shows optimal CHK1 inhibitory activities, hence, the binding mode of compound (***R***)-**17** within CHK1 were elucidated using a docking model (Fig. 6). As is shown in Figure 6, compound (***R***)-**17** binds to the ATP-binding site of CHK1 in an orientation similar to that of MCL1020 (Fig. 3b). The 2-amino pyrimidin skeleton of compound (***R***)-**17** forms two conserved hydrogen bonds with the hinge region of CHK1, one between the N1 of pyrimidin and Cys87 and the other between the NH of amino and Glu85. The N of pyridine accepts a hydrogen bond from the nearest of the three waters and 2-cyano of pyridine accepts a hydrogen bond from the protonated catalytic lysine (Lys38), related to selectivity. The piperidine NH forms additional polar interactions with the side chains of Glu134. In general, the docking results further confirm the rationality of our design strategy.

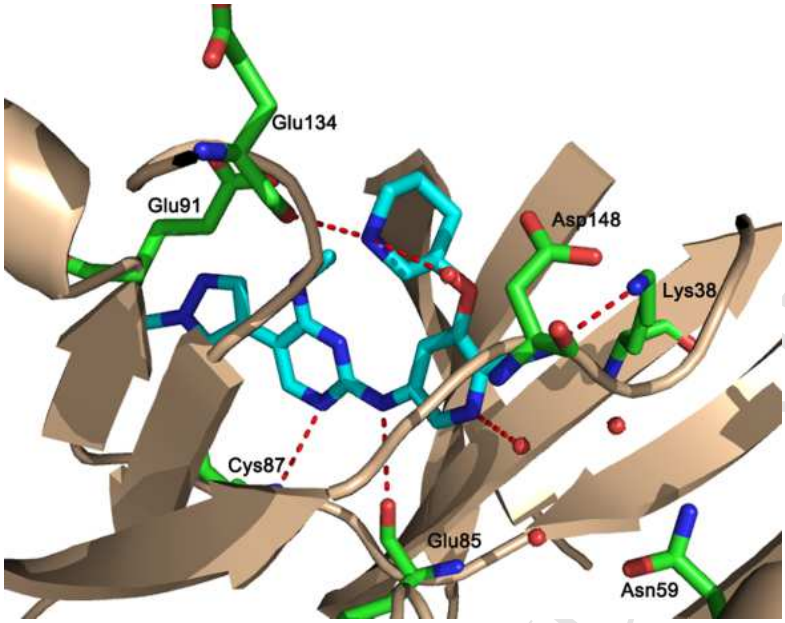


Figure 6. Compound (***R***)-**17** docked into CHK1 kinase (PDB code 2YM8). CHK1 interacting residues are shown as sticks colored green for carbon, blue for nitrogen, red for oxygen; (***R***)-**17** is shown as sticks colored light blue for carbon, dark blue for nitrogen; Figure prepared with Discovery Studio (version 2.1)

3. Conclusions

Through virtual screening and rational drug design, we finally identified (***R***)-**17** bearing 2-amino pyrimidine scaffold as a novel CHK1 inhibitor. Compound (***R***)-**17** is a selective inhibitor, which potently inhibited CHK1 with IC_{50} value of 0.4 nM and exhibited selectivity >4300-fold against CHK2. Additionally, (***R***)-**17** significantly inhibited the growth of malignant hematopathy cell lines (IC_{50} ＜40 nM) such as Z-138, MV4-11, Jeko-1. Ultimately, the antitumor efficacy of compound (***R***)-**17** as a single agent was tested in Z-138 cell inoculated xenograft mouse model. Pleasingly, tumors arising in these animals underwent significant growth inhibition in response to treatment with (***R***)-**17** (20mg/kg I.V., TGI = 90.29%) with mice body weight unaffected. Meanwhile, (***R***)-**17** displayed low affinity for hERG (IC_{50} > 40 μM), could be seen as hERG-inactive and its wider therapeutic index against hERG was suitable for preclinical evaluation. In summary, all these reported results illustrated that CHK1 inhibitor (***R***)-**17** could be as potential drug candidate to treat hematologic malignancies.

4. Experimental section

Reagents and solvents were obtained from commercial suppliers and used without further purification. All reactions were monitored by TLC, using silica gel plates with fluorescence F254 and UV light visualization was used as the visualizing agent. Column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factory, Qingdao, China. Melting points were determined on a Büchi melting point B-540 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III spectrometer at 500 MHz or Bruker AM 400 at 400 MHz. All NMR spectra were calibrated using residual solvents as internal references (for $CDCl_3$: ^{1}H NMR = 7.26, ^{13}C NMR = 77.16; DMSO-d6: ^{1}H NMR = 2.50, ^{13}C NMR = 39.52). Coupling constants (J) are expressed in hertz (Hz). All chemical shifts were reported in parts per million (ppm). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Electrospray ionization mass spectroscopy (ESI-MS) spectra were obtained with a Shimadzu LCMS-2020 mass spectrometer with mobile phases as methanol and water containing 0.1% formic acid. High resolution mass spectra (HRMS) were measured on an Agilent 6224 TOF LC/MS spectrometer using ESI-TOF (electrospray ionization-time of flight). HPLC analysis was performed on Agilent 1260 infinity equipped with Cosmosil 5C18-AR-□ column (4.6 mm × 250 mm), eluting at 1.0 mL/min using a listed gradient: (mobile phase A, 0.1%CF_3COOH in H_2O; mobile phase B, MeCN). Purity of all biologically tested compounds was determined by HPLC to be > 95%.(Supporting Information (Section 4 and 5).).

4.1. 5-bromopyrimidin-2-amine (39). A stirred solution of 2-aminopyridine (2.5 g, 12 mmol) in MeCN (25 ml) cooled in an ice water bath was treated with N-bromosuccinimide (4.6 g, 28 mmol). The resulting solution was stirred in the dark at room temperature overnight. The solvent was evaporated under reduced pressure. Water (100mL) added, and the precipitate filtered and washed with ice water. The crude product was dried in a vacuum desiccator to obtain **39** as an off white powder (4.4 g, 97%). m.p.: 241−243 °C.

4.2. General Procedure A for the Synthesis of Compounds (40a-j). A mixture of appropriate aryl bromide (10 mmol), appropriate boronic acid/ester (12 mmol), $Pd(dppf)Cl_2$ (0.50 mmol), aq Na_2CO_3 (1 M; 20 mL) in DME (80 mL) was heated 85 °C overnight under nitrogen. The cooled reaction mixture was absorbed onto silica gel, and solvent was removed by evaporation. The

residue obtained was purified by column chromatography (eluent gradient CH_2Cl_2: MeOH = 90:1), gave **40a**−**j**.

4.3. 5-phenylpyrimidin-2-amine (40a). Compound **40a** was prepared according to general procedure A. Yield: 86%; m.p.: 159−161 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.57 (s, Ar-H, 2H), 7.62 (d, *J* = 7.6 Hz, Ar-H, 2H), 7.45 (t, *J* = 7.2 Hz, Ar-H, 2H), 7.34 (t, *J* = 7.2 Hz, Ar-H, 1H), 6.77 (s, NH, 2H); ESI-MS: *m/z* = 172 $[M+1]^+$.

4.4. 5-(3-fluorophenyl)pyrimidin-2-amine (40b). Compound **40b** was prepared according to general procedure A. Yield: 83%; m.p.: 168−170 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.62 (s, Ar-H, 2H), 7.53–7.43 (m, Ar-H, 3H), 7.16−7.11 (m, Ar-H, 1H), 6.86 (s, NH_2, 2H); ESI-MS: *m/z* = 190 $[M+1]^+$.

4.5. 5-(4-fluorophenyl)pyrimidin-2-amine (40c). Compound **40c** was prepared according to general procedure A. Yield: 85%; m.p.: 172−174 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.58 (s, Ar-H, 2H), 7.68−7.64 (m, Ar-H, 2H), 7.29 (t, *J* = 8.8 Hz, Ar-H, 2H), 6.78 (s, NH_2, 2H); ESI-MS: m/z = 190 $[M+1]^+$.

4.6. 5-(3-methoxyphenyl)pyrimidin-2-amine (40d). Compound **40d** was prepared according to general procedure A. Yield: 87%; m.p.: 133−135 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.58 (s, Ar-H, 2H), 7.36 (t, *J* = 8.0 Hz, Ar-H, 1H), 7.18 (d, *J* = 7.6 Hz, Ar-H, 2H), 6.90 (dd, *J* = 8.4 Hz, 1.6 Hz, Ar-H, 1H), 6.78 (s, NH_2, 2H), 3.81 (s, CH_3, 3H); ESI-MS: *m/z* = 202 $[M+1]^+$.

4.7. 5-(4-methoxyphenyl)pyrimidin-2-amine (40e). Compound **40e** was prepared according to general procedure A. Yield: 82%; m.p.: 165−167 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.51 (s, Ar-H, 2H), 7.55 (d, *J* = 8.8 Hz, Ar-H, 2H), 7.01 (d, *J* = 8.8 Hz, Ar-H, 2H), 6.67 (s, NH_2, 2H), 3.78 (s, CH_3, 3H); ESI-MS: *m/z* = 202 $[M+1]^+$.

4.8. 5-(pyridin-3-yl)pyrimidin-2-amine (40f). Compound **40f** was prepared according to general procedure A. Yield: 79%; m.p.: 183–185 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.87 (s, Ar-H, 1H), 8.64 (s, Ar-H, 2H), 8.53 (br, Ar-H, 1H), 8.05 (d, *J* = 7.6 Hz, Ar-H, 1H), 7.46 (d, *J* = 4.0 Hz, Ar-H, 1H), 6.89 (s, NH_2, 2H); ESI-MS: *m/z* = 173 $[M+1]^+$.

4.9. 5-(pyridin-4-yl)pyrimidin-2-amine (40g). Compound **40g** was prepared according to general procedure A. Yield: 87%; m.p.: 196–198 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.75 (s, Ar-H, 2H), 8.58 (br, Ar-H, 2H), 7.70 (d, J = 4.8 Hz, Ar-H, 2H), 7.06 (s, NH_2, 2H); ESI-MS: *m/z* = 173 $[M+1]^+$.

4.10. 5-(thiophen-2-yl)pyrimidin-2-amine (40h). Compound **40h** was prepared according to general procedure A. Yield: 84%; m.p.: 156−158 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.53 (s, Ar-H, 2H), 7.49 (d, J = 4.8 Hz, Ar-H, 1H), 7.38 (d, J = 2.8 Hz, Ar-H, 1H), 7.12 (t, J = 4.4 Hz, Ar-H, 1H), 6.87 (s, NH_2, 2H); ESI-MS: *m/z* = 178 $[M+1]^+$.

4.11. 5-(furan-2-yl)pyrimidin-2-amine (40i). Compound **40i** was prepared according to general procedure A. Yield: 813%; m.p.: 156−158 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.57 (s, Ar-H, 2H), 7.69 (s, Ar-H, 1H), 6.88 (s, NH, 2H), 6.78 (d, J = 2.0 Hz, Ar-H, 1H), 6.56 (s, Ar-H, 1H); ESI-MS: *m/z* = 162 $[M+1]^+$.

4.12. 5-(1-methyl-1H-pyrazol-4-yl)pyrimidin-2-amine (40j). Compound **40j** was prepared according to general procedure A. Yield: 85%; m.p.: 174−176 °C; ^{1}HNMR (400 MHz, DMSO-*d*6): δ 8.46 (s, Ar-H, 2H), 8.03 (s, Ar-H, 1H), 7.78 (s, Ar-H, 1H), 6.57 (s, NH_2, 2H), 3.84 (s, CH_3, 3H); ESI-MS: *m/z* = 176 $[M+1]^+$.

4.13. General Procedure B for the Synthesis of Compounds (42a-k). A mixture of sodium hydrogen (7.5 mmol), appropriate alcohol (5.0 mmol) in dry THF (100 mL) was cooled at 0 °C, then the mixture was stirred at room temperature for 30 min. Adding the reaction liquid to the mixture of 5-bromo-3-nitropicolinonitrile (5.0 mmol) in 50 THF (50 mL) dropwise at 0 °C then the mixture was stirred at rt for 3 h. The reaction solution was treated with saturated NH_4Cl and was extracted with EtOAc. The organic layer was collected, washed with brine, and dried over $MgSO_4$, concentrated. The residue obtained was purified by column chromatography (eluent gradient 10−20% petroleum ether in EtOAc), gave **42a**−**k**.

4.14. Tert-butyl 3-((5-bromo-2-cyanopyridin-3-yl)oxy)piperidine-1-carboxylate (42a). Compound **42a** was prepared according to general procedure B. Yield: 79%; as light yellow oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.26 (s, Ar-H, 1H), 7.53 (s, Ar-H, 1H), 4.38 (br, CH, 1H), 3.63

(br, CH, 1H), 3.51 (br, CH, 1H), 3.38 (br, CH, 2H), 1.98−1.96 (m, CH, 1H), 1.91−1.85 (m, CH, 2H), 1.51 (br, CH, 1H), 1.36 (s, CH_3×3, 9H); ESI-MS: *m/z* = 382 $[M+1]^+$.

4.15. Tert-butyl (*R*)-3-((5-bromo-2-cyanopyridin-3-yl)oxy)piperidine-1-carboxylate (42b). Compound **42b** was prepared according to general procedure B. Yield: 76%; as light yellow oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.26 (s, Ar-H, 1H), 7.53 (s, Ar-H, 1H), 4.38 (br, CH, 1H), 3.63 (br, CH, 1H), 3.51 (br, CH, 1H), 3.38 (br, CH, 2H), 1.98−1.96 (m, CH, 1H), 1.91−1.85 (m, CH, 2H), 1.51 (br, CH, 1H), 1.36 (s, CH_3×3, 9H); ESI-MS: *m/z* = 382 $[M+1]^+$.

4.16. Tert-butyl (*S*)-3-((5-bromo-2-cyanopyridin-3-yl)oxy)piperidine-1-carboxylate (42c). Compound **42c** was prepared according to general procedure B. Yield: 78%; as light yellow oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.26 (s, Ar-H, 1H), 7.53 (s, Ar-H, 1H), 4.38 (br, CH, 1H), 3.63 (br, CH, 1H), 3.51 (br, CH, 1H), 3.38 (br, CH, 2H), 1.98−1.96 (m, CH, 1H), 1.91−1.85 (m, CH, 2H), 1.51 (br, CH, 1H), 1.36 (s, CH_3×3, 9H); ESI-MS: *m/z* = 382 $[M+1]^+$.

4.17. Tert-butyl 4-((5-bromo-2-cyanopyridin-3-yl)oxy)piperidine-1-carboxylate (42d). Compound **42d** was prepared according to general procedure B. Yield: 80%; m.p.: 97−99 °C; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.34 (s, Ar-H, 1H), 7.51 (s, Ar-H, 1H), 4.66−4.62 (m, CH, 1H), 3.69−3.63 (m, CH_2, 2H), 3.52−3.46 (m, CH_2, 2H), 1.99−1.92 (m, CH_2, 2H), 1.90−1.82 (m, CH_2, 2H), 1.47 (s, CH_3×3, 9H); ESI-MS: *m/z* = 382 $[M+1]^+$.

4.18. 5-bromo-3-((1-methylpiperidin-4-yl)oxy)picolinonitrile (42e). Compound **42e** was prepared according to general procedure B. Yield: 51%; as light yellow oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.44 (s, Ar-H, 1H), 8.28 (s, Ar-H, 1H), 4.81−4.78 (m, CH, 1H), 2.57 (br, CH_2, 2H), 2.32−2.28 (m, CH_2, 2H), 2.21 (s, CH_3, 3H), 1.96−1.92 (m, CH_2, 2H), 1.75−1.69 (m, CH_2, 2H); ESI-MS: *m/z* = 296 $[M+1]^+$.

4.19. Tert-butyl4-(((5-bromo-2-cyanopyridin-3-yl)oxy)methyl)piperidine-1-carboxylate(42f). Compound **42f** was prepared according to general procedure B. Yield: 80%; as light yellow oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.34 (s, Ar-H, 1H), 7.51 (s, Ar-H, 1H), 4.20 (d, *J* = 11.5 Hz, CH_2, 2H), 3.95 (br, CH_2, 2H), 2.80 (br, CH_2, 2H), 2.14−2.05 (m, CH, 1H), 1.89 (br, CH_2, 2H), 1.47 (s, CH_3×3, 9H), 1.33−1.26 (m, CH_2, 2H)； ESI-MS: *m/z* = 206 $[M+1]^+$.

4.20. Tert-butyl (*R*)-3-((5-bromo-2-cyanopyridin-3-yl)oxy)pyrrolidine-1-carboxylate (42g). Compound **42g** was prepared according to general procedure B. Yield: 75%; as light yellow oil; ^{1}HNMR (500 MHz, DMSO-*d*6): δ 8.48 (d, J = 2.0 Hz, Ar-H, 1H), 8.29 (s, Ar-H, 1H), 5.32 (br, CH, 1H), 3.62−3.55 (m, CH, 1H), 3.49−3.44 (m, CH_2, 2H), 3.39−3.33 (m, CH_2, 1H), 2.21−2.16 (m, CH_2, 1H), 2.13−2.09 (m, CH_2, 1H), 1.41 (s, CH_3×3, 9H); ESI-MS: m/z = 368 $[M+1]^+$.

4.21. 5-bromo-3-(2-(dimethylamino)ethoxy)picolinonitrile (42h). Compound **42h** was prepared according to general procedure B. Yield: 77%; as light yellow oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.27 (s, Ar-H, 1H), 7.49 (s, Ar-H, 1H), 4.16 (t, J = 7.0 Hz, CH_2, 2H), 2.78 (t, J = 7.0 Hz, CH_2, 2H), 2.31 (s, CH_3×2, 6H); ESI-MS: m/z = 270 $[M+1]^+$.

4.22. (*R*)-5-bromo-3-((1-(dimethylamino)propan-2-yl)oxy)picolinonitrile (42i). Compound **42i** was prepared according to general procedure B. Yield: 70%; as light yellow oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.31 (s, Ar-H, 1H), 7.62 (s, Ar-H, 1H), 4.62−4.55 (m, CH, 1H), 2.76−2.71 (m, CH_2, 1H), 2.54−2.49 (m, CH_2, 1H), 2.31 (s, CH_3×2, 6H), 1.40 (d, J = 8.0 Hz, CH_3, 3H); ESI-MS: m/z = 284 $[M+1]^+$.

4.23. (*S*)-5-bromo-3-((1-(dimethylamino)propan-2-yl)oxy)picolinonitrile (42j). Compound **42j** was prepared according to general procedure B. Yield: 74%; as light yellow oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.31 (s, Ar-H, 1H), 7.62 (s, Ar-H, 1H), 4.62−4.55 (m, CH, 1H), 2.76−2.71 (m, CH_2, 1H), 2.54−2.49 (m, CH_2, 1H), 2.31 (s, CH_3×2, 6H), 1.40 (d, J = 8.0 Hz, CH_3, 3H); ESI-MS: m/z = 284 $[M+1]^+$.

4.24. General Procedure C for the Synthesis of Compounds (6-15). A mixture of appropriate aryl bromides (0.50 mmol), appropriate arylamine (0.50 mmol), Pd_2 $(dba)_3$ (0.0050 mmol), Xantphos (0.012 mmol), Cs_2CO_3(1.0 mmol) in dry dioxane (10 mL) was heated 101 °C for 5 h under nitrogen. The cooled reaction mixture was absorbed onto silica gel, and solvent was removed by evaporation. The residue obtained was purified by column chromatography (eluent gradient 4-5% EtOH in CH_2Cl_2), gave intermediate (79%−87%). CF_3COOH (2.2 mmol) mL) was added in a mixture of intermediate (0.36 mmol) in CH_2Cl_2 (2.2 mL). The reaction mixture was stirred at room temperature for 2 h. Then the solvent was removed by evaporation, the residue obtained was purified by column chromatography (eluent CH_2Cl_2: MeOH: TEA =10:1:1), gave **6**−**15**.

4.25. 5-((5-phenylpyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (6). Compound **6** was prepared according to general procedure C. Yield: 75%; m.p.: 84−86 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.55 (s, NH, 1H), 8.99 (s, Ar-H, 2H), 8.65 (d, J = 2.0 Hz, Ar-H, 1H), 8.39 (d, J = 2.0 Hz, Ar-H, 1H), 7.78 (d, J = 7.0 Hz, Ar-H, 2H), 7.52 (t, J = 7.5 Hz, Ar-H, 2H), 7.43 (t, J = 7.0 Hz, Ar-H, 1H), 4.42−4.37 (m, CH, 1H), 3.19 (d, J = 12.0 Hz, 2.0 Hz, CH_2, 1H), 2.81−2.77 (m, CH_2, 1H), 2.66−2.62 (m, CH_2, 1H), 2.56−2.53 (m, CH_2, 1H), 2.14−2.11 (m, CH_2, 1H), 1.76−1.71 (m, CH_2, 1H), 1.65−1.58 (m, CH_2, 1H), 1.54–1.46 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.33, 157.24, 155.92, 141.82, 134.17, 134.03, 129.13, 127.91, 125.97, 116.20, 113.83, 109.24, 74.69, 49.64, 45.28, 29.82, 24.26; ESI-MS: m/z = 373 $[M+1]^+$. ESI-HRMS m/z calcd for $C_{21}H_{21}N_6O^+$ 373.1777, found 373.1779 $[M + H]^+$. HPLC purity 97%.

4.26. 5-((5-(3-fluorophenyl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (7). Compound **7** was prepared according to general procedure C. Yield: 72%; m.p.: 107−109 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.59 (br, NH, 1H), 9.03 (s, Ar-H, 2H), 8.65 (d, J = 1.5 Hz, Ar-H, 1H), 8.37 (s, Ar-H, 1H), 7.70 (d, J = 10.5 Hz, Ar-H, 1H), 7.65 (d, J = 8.0 Hz, Ar-H, 1H), 7.56 (dd, J = 14.0 Hz, 8.0 Hz, Ar-H, 1H), 7.26 (td, J = 8.5 Hz, 2.5 Hz, Ar-H, 1H), 4.42−4.37 (m, CH, 1H), 3.19 (dd, J = 12.0 Hz, 2.0 Hz, CH_2, 1H), 2.82−2.78 (m, CH_2, 1H), 2.67−2.63 (m, CH_2, 1H), 2.57−2.52 (m, CH_2, 1H), 2.14−2.11 (m, CH_2, 1H), 1.77−1.71 (m, CH_2, 1H), 1.66−1.59 (m, CH_2, 1H), 1.54−1.47 (m, CH_2, 1H); ^{13}C NMR (125 MHz, DMSO): δ 163.73, 161.79, 158.62, 157.25, 156.23, 141.75, 136.54, 136.48, 134.24, 131.17, 131.10, 124.71, 122.02, 121.99, 116.20, 114.71, 114.54, 113.97, 112.85, 112.67, 109.39, 74.64, 49.55, 45.24, 29.77, 24.17. ESI-HRMS m/z calcd for $C_{21}H_{20}FN_6O^+$ 391.1683, found 391.1687 $[M + H]^+$. HPLC purity 98%.

4.27. 5-((5-(4-fluorophenyl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (8). Compound **8** was prepared according to general procedure C. Yield: 78%; m.p.: 182−184 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.52 (br, NH, 1H), 8.94 (d, J = 1.0 Hz, Ar-H, 2H), 8.63 (d, J = 2.0 Hz, Ar-H, 1H), 8.35 (d, J = 2.0 Hz, Ar-H, 1H), 7.87 – 7.74 (m, Ar-H, 2H), 7.32 (t, J = 9.0 Hz, Ar-H, 2H), 4.37 (dt, J = 8.5, 4.5 Hz, CH, 1H), 3.17 (ddd, J = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.78 (dt, J = 12.5, 4.5 Hz, CH_2, 1H), 2.63 (dd, J = 12.0, 8.0 Hz, CH_2, 1H), 2.56–2.51 (m, CH_2, 1H), 2.15–2.08 (m, CH_2, 1H), 1.73 (dp, J = 13.0, 4.0 Hz, CH_2, 1H), 1.65–1.55 (m, CH_2, 1H), 1.54–1.43 (m, CH_2, 1H).^{13}C NMR(125 MHz, DMSO-d_6): δ 163.08, 161.13, 158.26, 157.24, 155.89, 141.80, 134.14, 130.58, 130.56, 128.16, 128.10, 125.14, 116.20, 116.08, 115.91, 113.83,

109.20, 74.69, 49.65, 45.28, 29.82, 24.27. ESI-HRMS *m/z* calcd for $C_{21}H_{20}FN_6O^+$ 391.1683, found 391.1684 $[M + H]^+$. HPLC purity 98%.

4.28. 5-((5-(3-methoxyphenyl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (9). Compound **9** was prepared according to general procedure C. Yield: 74%; m.p.: 102−104 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.53 (br, NH, 1H), 8.97 (s, Ar-H, 2H), 8.64 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.35 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.35–7.27 (m, Ar-H, 2H), 6.96 (ddd, *J* = 8. 5, 2.5, 1.0 Hz, Ar-H, 1H), 4.38 (tt, *J* = 8.0, 4.0 Hz, CH, 1H), 3.83 (s, CH_3, 3H), 3.17 (ddd, *J* = 12.0,4.0, 1.5 Hz, CH_2, 1H), 2.78 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.64 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.57–2.51 (m, CH_2, 1H), 2.17–2.06 (m, CH2, 1H), 1.73 (ddt, *J* = 13.5, 9.0, 4.5 Hz, CH2, 1H), 1.68–1.56 (m, CH_2, 1H), 1.55–1.43 (m, CH_2, 1H). ^{13}C NMR(125 MHz, DMSO-d_6): δ 159.91, 158.39, 157.24, 156.02, 141.82, 135.42, 134.17, 130.22, 125.85, 118.18, 116.22, 113.83, 113.64, 111.36, 109.21, 74.66, 55.22, 49.63, 45.29, 29.81, 24.24. ESI-HRMS *m/z* calcd for $C_{22}H_{23}N_6O_2^+$ 403.1882, found 403.1881 $[M + H]^+$. HPLC purity 97%.

4.29. 5-((5-(4-methoxyphenyl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (10). Compound **10** was prepared according to general procedure C. Yield: 75%; m.p.: 218−220 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 10.46 (s, NH, 1H), 8.90 (s, Ar-H, 2H), 8.62 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.35 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.88–7.48 (m, Ar-H, 2H), 7.21–6.88 (m, Ar-H, 2H), 4.37 (dt, *J* = 8.8, 4.4 Hz, CH, 1H), 3.80 (s, CH_3, 3H), 3.24–3.11 (m, CH, 1H), 2.78 (dt, *J* = 12.4, 4.2 Hz, CH, 1H), 2.63 (dd, *J* = 12.2, 8.0 Hz, CH, 1H), 2.57–2.51 (m, CH, 1H), 2.17–2.05 (m, CH, 1H), 1.75–1.70 (m, CH, 1H), 1.65–1.56 (m, CH, 1H), 1.53–1.45 (m, CH, 1H).^{13}C NMR(100 MHz, DMSO-d_6): δ 159.24, 157.85, 157.25, 155.33, 141.91, 134.07, 127.19, 126.29, 125.84, 116.24, 114.60, 113.64, 109.00, 74.69, 55.20, 49.69, 45.31, 29.84, 24.31; ESI-HRMS *m/z* calcd for $C_{22}H_{23}N_6O_2^+$ 403.1882, found 403.1882 $[M + H]^+$. HPLC purity 99%.

4.30. 3-(piperidin-3-yloxy)-5-((5-(pyridin-3-yl)pyrimidin-2-yl)amino)picolinonitrile (11). Compound **11** was prepared according to general procedure C. Yield: 75%; m.p.: 221–223°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.60 (br, NH, 1H), 9.05 (s, Ar-H, 2H), 8.99 (dd, *J* = 2.5, 1.0 Hz, Ar-H, 1H), 8.66 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.61 (dd, *J* = 4.5, 1.5 Hz, Ar-H, 1H), 8.36 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.22–8.16 (m, Ar-H, 1H), 7.52 (ddd, *J* = 8.0, 5.0, 1.0 Hz, Ar-H, 1H), 4.40 (tt, *J* = 8.0, 4.0 Hz, CH, 1H), 3.18 (ddd, *J* = 12.0, 4.0, 1.5 Hz, CH, 1H), 2.79 (dt, *J* = 12.5, 4.5 Hz, CH, 1H), 2.66 (dd, *J* = 12.0, 8.0 Hz, CH, 1H), 2.55 (ddd, *J* = 12.5, 9.5, 3.0 Hz, CH, 1H), 2.17–

2.08 (m, CH, 1H), 1.77–1.71 (m, CH, 1H), 1.66–1.58 (m, CH, 1H), 1.55–1.45 (m, CH, 1H). ^{13}C NMR (125 MHz, DMSO) δ 158.73, 157.28, 156.31, 148.93, 147.03, 141.76, 134.29, 133.51, 130.00, 124.00, 123.30, 116.23, 114.03, 109.47, 74.69, 49.61, 45.29, 29.82, 24.23. ESI-HRMS *m/z* calcd for $C_{20}H_{20}N_7O^+$ 374.1729, found 403.1882 [M + H] $^+$. HPLC purity 95%.

4.31. 3-(piperidin-3-yloxy)-5-((5-(pyridin-4-yl)pyrimidin-2-yl)amino)picolinonitrile (12). Compound **12** was prepared according to general procedure C. Yield: 71%; m.p.: 232–234°C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 10.67 (br, NH, 1H), 9.12 (s, Ar-H, 2H), 8.67 (d, *J* = 4.4 Hz, Ar-H, 2H), 8.65 (s, Ar-H, 1H), 8.35 (s, Ar-H, 1H), 7.84 (d, *J* = 6.0 Hz, Ar-H, 2H), 4.41-4.37 (m, CH, 1H), 3.20 (d, *J* = 12.0 Hz, CH_2, 1H), 3.82-2.79 (m, CH_2, 1H), 2.68-2.63 (m, CH_2, 1H), 2.57-2.52 (m, CH_2, 1H), 2.14-2.12 (m, CH_2, 1H), 1.76-1.73 (m, CH_2, 1H), 1.67-1.58 (m, CH_2, 1H), 1.55-1.46 (m, CH_2, 1H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (br, NH, 1H), 9.10 (s, Ar-H, 2H), 8.80–8.54 (m, Ar-H, 3H), 8.33 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.91–7.74 (m, Ar-H_2, H), 4.38 (dq, *J* = 8.4, 4.2 Hz, CH, 1H), 3.24–3.10 (m, CH, 1H), 2.79 (dt, *J* = 12.4, 4.4 Hz, CH, 1H), 2.64 (dd, *J* = 12.2, 8.0 Hz, CH, 1H), 2.59–2.52 (m, CH, 1H), 2.11 (dd, *J* = 12.4, 5.2 Hz, CH, 1H), 1.76–1.70 (m, CH, 1H), 1.65–1.56 (m, CH, 1H), 1.54–1.45 (m, CH, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 159.23, 157.17, 156.46, 150.27, 141.50, 141.31, 134.32, 122.96, 120.08, 116.10, 114.25, 109.67, 74.71, 49.63, 45.28, 29.80, 24.24; ESI-HRMS *m/z* calcd for $C_{20}H_{20}N_7O^+$ 374.1729, found 374.1728 [M + H] $^+$. HPLC purity 97%.

4.32. 3-(piperidin-3-yloxy)-5-((5-(thiophen-2-yl)pyrimidin-2-yl)amino)picolinonitrile (13). Compound **13** was prepared according to general procedure C. Yield: 74%; m.p.: 212–214 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.57 (br, NH, 1H), 8.92 (s, Ar-H, 2H), 8.59 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.34 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.71–7.50 (m, Ar-H, 2H), 7.18 (dd, *J* = 5.0, 3.5 Hz, Ar-H, 1H), 4.38 (tt, *J* = 8.0, 4.0 Hz, CH, 1H), 3.16 (ddd, *J* = 12.0, 4.0, 1.5 Hz, CH, 1H), 2.78 (dt, *J* = 12.5, 4.5 Hz, CH, 1H), 2.64 (dd, *J* = 12.0, 8.0 Hz, CH, 1H), 2.54 (td, *J* = 9.5, 5.0 Hz, CH, 1H), 2.15–2.08 (m, CH, 1H), 1.77–1.68 (m, CH, 1H), 1.67–1.56 (m, CH, 1H), 1.55–1.44 (m, CH, 1H); ^{13}C NMR(125 MHz, DMSO-d_6): δ 157.99, 157.24, 154.62, 141.66, 136.45, 134.17, 128.54, 126.14, 124.27, 121.12, 116.20, 113.90, 109.23, 74.64, 56.01, 49.62, 45.30, 29.81, 24.26; ESI-HRMS *m/z* calcd for $C_{19}H_{19}N_6OS^+$ 379.1341, found 379.1342 [M + H] $^+$. HPLC purity 98%.

4.33. 5-((5-(furan-2-yl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (14). Compound **14** was prepared according to general procedure C. Yield: 70%; m.p.: 200–202 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 10.56 (br, NH, 1H), 8.93 (s, Ar-H, 2H), 8.57 (d, *J* = 2.0 Hz,

Ar-H, 1H), 8.33 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.79 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.03 (d, *J* = 3.6 Hz, Ar-H, 1H), 6.63 (dd, *J* = 3.6, 2.0 Hz, Ar-H, 1H), 4.37 (td, *J* = 8.0, 4.0 Hz, CH, 1H), 3.16 (dt, *J* = 12.0, 2.8 Hz, CH, 1H), 2.78 (dt, *J* = 12.4, 4.4 Hz, CH, 1H), 2.63 (dd, *J* = 12.4, 8.0 Hz, CH, 1H), 2.58–2.51 (m, CH, 1H), 2.11 (dd, *J* = 12.4, 5.2 Hz, CH, 1H), 1.75–1.70 (m, CH, 1H), 1.65–1.56 (m, CH, 1H), 1.53–1.44 (m, CH, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.75, 157.22, 153.17, 148.22, 143.35, 141.61, 134.16, 117.89, 116.18, 113.88, 112.07, 109.23, 106.18, 74.66, 49.63, 45.30, 29.81, 24.27; ESI-HRMS *m/z* calcd for $C_{19}H_{19}N_6O_2^+$ 363.1569, found 363.1569 [M + H] $^+$. HPLC purity 97%.

4.34. 5-((5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (15). Compound **15** was prepared according to general procedure C. Yield: 74%; m.p.: 227–229 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.43 (s, NH, 1H), 8.87 (s, Ar-H, 2H), 8.58 (d, *J* = 2.0 Hz, Ar-H,1H), 8.35 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.23 (s, Ar-H, 1H), 7.97 (d, *J* = 1.0 Hz, Ar-H, 1H), 4.37 (tt, J = 8.0, 4.0 Hz, Ar-H, 1H), 3.88 (s, CH_3, 3H), 3.17 (ddd, J = 12.0, 4.0, 1.5 Hz, CH , 1H), 2.79 (dt, J = 12.5, 4.5 Hz, CH, 1H), 2.64 (dd, J = 12.0, 8.0 Hz, CH, 1H), 2.55 (dd, J = 9.5, 3.0 Hz, CH, 1H), 2.13 (dt, J = 11.0, 5.0 Hz, CH, 1H), 1.74 (dp, J = 13.0, 4.5, 4.0 Hz, CH, 1H), 1.61 (dtd, J = 12.5, 9.5, 9.0, 4.0 Hz, CH, 1H), 1.50 (dddd, J = 16.5, 13.0, 8.5, 5.0 Hz, CH, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 157.30, 154.28, 142.00, 135.77, 134.01, 127.60, 119.79, 116.31, 115.48, 113.43, 108.73, 74.63, 49.71, 45.33, 38.77, 29.85, 24.30. ESI-HRMS *m/z* calcd for $C_{19}H_{21}N_8O^+$ 377.1838, found 377.1837 [M + H] $^+$. HPLC purity 99%.

4.35. 5-((5-bromopyrimidin-2-yl)amino)picolinonitrile (43). Compound **43** was prepared according to general procedure C. Yield: 70%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.64 (s, NH, 1H), 8.98 (s, Ar-H, 1H), 8.76 (s, Ar-H, 2H), 8.43 (d, *J* = 11.0 Hz, Ar-H, 1H), 7.98 (d, *J* = 10.5 Hz, Ar-H, 1H); ESI-MS: *m/z* = 276 $[M+1]^+$.

4.36. Tert-butyl 3-((methylsulfonyl)oxy)piperidine-1-carboxylate (45). 1-N-tert-butyloxycarbonyl-3-hydroxypiperidine (1.4 g, 7.0 mmol), methanesulfonyl chloride (1.2 g, 10.5 mmol) and DMAP (171 mg, 1.4 mol) with triethylamine (1.8 g, 1.8 mol) suspend in 14 mL CH_2Cl_2. The reaction mixture was stirred at 0 °C for 1 h, then the mixture was partitioned between aqueous citric acid (5%, 20 mL) and CH_2Cl_2 three times. The combined organic layers

were washed with brine, dried, filtered, and concentrated, gave light yellow oil (1.52 g, 78%). This product was used in the next step without purification.

4.37. Tert-butyl 3-(2-bromophenoxy)piperidine-1-carboxylate (46). A solution of 2-bromophenol (451 mg, 2.6 mmol) and tert-butyl 3-((methylsulfonyl)oxy)piperidine-1-carboxylate (800 mg, 2.9 mmol) in DMF (10 mL) was added Cs_2CO_3 (2.1 g, 6.5 mmol). The reaction mixture was stirred at 70°C for 5 h under nitrogen then cooled to room temperature. The mixture was partitioned between saturated aqueous water and EtOAc three times. The combined organic layers were washed with water, brine, dried, filtered, and concentrated, gave light yellow oil (398 mg, 43%). This product was used in the next step without purification; ESI-MS: *m/z* = 356 $[M+1]^+$.

4.38. Tert-butyl 3-(2-(4, 4, 5, 5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)piperidine-1-carboxylate (47). A mixture of **46** (310 mg, 0.87 mmol), bis(pinacolato)diboron (254 mg, 2.2 mmol), Pd(dppf)Cl_2 (51 mg, 0.070 mmol), Cs_2CO_3 (710 mg, 2.2 mmol) in DME (80 mL) was heated at 85 °C overnight under nitrogen. Upon cooling, the insoluble material was filtered off and washed with DME The filtrate was concentrated, gave **47** (271 mg, 77%) and product was used in the next step without purification; ESI-MS: *m/z* = 404 $[M+1]^+$.

4.39. 5-((5-(2-(piperidin-3-yloxy)phenyl)pyrimidin-2-yl)amino)picolinonitrile (5). A mixture of **47** (271 mg, 0.67 mmol), **43** (155 mg, 0.56 mmol), Pd(dppf)Cl_2 (31 mg, 0.050 mmol), aq Na_2CO_3 (1 M; 0.56 mL) in DME (2 mL) was heated at 85 °C overnight under nitrogen. The cooled reaction mixture was absorbed onto silica gel, and solvent was removed by evaporation. The residue obtained was purified by column chromatography (eluent gradient 50-90% petroleum ether in EtOAc), gave tert-butyl 3-(2-(2-((6-cyanopyridin-3-yl)amino)pyrimidin-5-yl)phenoxy)piperidine-1-carboxylate (172 mg, 65%). CF_3COOH (2.2 mmol) mL) was added in a mixture of tert-butyl-3-(2-(2-((6-cyanopyridin-3-yl)amino)pyrimidin-5-yl)phenoxy)piperidine-1-carboxylate (0.36 mmol) in CH_2Cl_2 (2.2 mL). The reaction mixture was stirred at room temperature for 2 h. Then the solvent was removed by evaporation, the residue obtained was purified by column chromatography (eluent CH_2Cl_2:MeOH:TEA =10:1:1), gave **5** (108 mg, 80%); m.p.: 151–154 °C; ^{1}HNMR (500 MHz, DMSO-*d*6): δ 10.57 (s, NH, 1H), 9.03 (s, Ar-H, 1H), 8.85 (s, Ar-H, 2H), 8.53 (d, *J* = 8.5 Hz, Ar-H, 1H), 7.96 (d, *J* = 8.5 Hz, Ar-H, 1H), 7.57 –

7.25 (m, Ar-H, 2H), 7.25–6.91 (m, Ar-H, 2H), 4.35 (s, CH, 1H), 3.00 (d, J = 12.5 Hz, CH_2, 1H), 2.70 -2.58(m, CH_2, 2H), 2.06–1.86 (m, NH, 1H), 1.71–1.30 (m, CH_2, 3H), 1.22–1.16 (m, CH_2, 1H), 0.82 (s, CH_2, 1H). ^{13}CNMR (125 MHz, DMSO): δ 157.80, 154.05, 141.47, 140.54, 129.90, 129.55, 129.41, 124.32, 124.08, 123.88, 123.27, 121.08, 118.18, 114.12, 72.70, 49.53, 45.48, 29.95, 23.87. ESI-HRMS *m/z* calcd for $C_{21}H_{22}N_6O^+$ 373.1777, found 377.1839 $[M + H]^+$. HPLC purity 98%.

4.40. 5-bromo-2-chloro-4-methoxypyrimidine (49). A solution of 5-bromo-2, 4-dichloropyrimidine (456 mg, 2.0mmol) in *Abs* MeOH (44mL) was added sodium methylate (Na (56 mg, 2.4 mmol) in 1.8 mL) dropwise at 0°C. The reaction mixture was stirred at room temperature for 3 h. Then the solvent was removed by evaporation, the residue obtained was purified by column chromatography (eluent gradient 20−25% petroleum ether in EtOAc), gave **49** (402 mg, 90%) as a white powder; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.44 (s, Ar-H, 1H), 4.11 (s, CH_3, 3H); ESI-MS: *m/z* = 223 $[M+1]^+$.

4.41. 5-bromo-2-chloro-N-methylpyrimidin-4-amine (50). A solution of 5-bromo-2, 4-dichloropyrimidine (5.0 g, 22 mmol) in MeOH (44 mL) was added methylamine (33% in EtOH, 55 mmol) dropwise at 0°C. The reaction mixture was stirred at room temperature for 3 h. The precipitate was collected by filtration, washed with 40°C water, and dried, to yield 48 as a white powder (4.3g, 88%); m.p.: 139–141 °C; ^{1}HNMR (500 MHz, DMSO-*d*6): δ 8.85 (s, Ar-H, 1H), 7.75 (br, NH, 1H), 2.85 (d, J = 4.0 Hz, CH_3, 3H); ESI-MS: *m/z* = 222 $[M+1]^+$.

4.42. General Procedure D for the Synthesis of Compounds (51, 53). To a solution of appropriate arylamine (5.0 mmol) in dioxane (5mL) was added NH3•H2O (5mL) in sealed tube. The reaction mixture was stirred at 160 °C for 3 h. Then the solvent was removed by evaporation, the residue obtained was purified by column chromatography (eluent gradient 20−30% petroleum ether in EtOAc), gave **51, 53**.

4.43. 5-bromo-4-methoxypyrimidin-2-amine (51). Compound **51** was prepared according to general procedure D. Yield: 75%; ^{1}HNMR (500 MHz, $CDCl_3$): δ 8.13 (s, Ar-H, 1H), 5.41 (s, NH_2, 2H), 3.91 (s, CH_3, 3H); ESI-MS: *m/z* = 205 $[M+1]^+$.

4.44. 5-bromo-N^4-methylpyrimidine-2,4-diamine (53). Compound **53** was prepared according to general procedure D. Yield: 78%; ^{1}HNMR (500 MHz, $CDCl_3$): δ7.86 (s, Ar-H, 1H), 5.22 (br, NH, 1H), 4.85 (br, NH_2, 2H), 2.99 (d, J = 6.0 Hz, CH_3, 3H); ESI-MS: m/z = 204 $[M+1]^+$.

4.45. N^4-methyl-5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidine-2,4-diamine (54a). Compound **54a** was prepared according to general procedure A. Yield: 75%; m.p.: 226−228°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 7.88 (s, Ar-H, 1H), 7.49 (s, Ar-H, 1H), 7.35 (s, Ar-H, 1H), 6.22 (br, NH_2, 2H), 5.96 (q, J = 4.5 Hz, NH, 1H), 3.51 (s, CH_3, 3H), 2.86 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 205 $[M+1]^+$.

4.46. N^4-methyl-5-phenylpyrimidine-2,4-diamine (54b). Compound **54b** was prepared according to general procedure A. Yield: 82%; ^{1}HNMR (500 MHz, $CDCl_3$): δ 7.69 (br, Ar-H, 1H), 7.49 (d, J = 5.5 Hz, Ar-H, 2H), 7.13–7.09 (m, Ar-H, 3H), 6.43 (q, J = 4.5 Hz, NH, 1H), 6.25 (br, NH_2, 2H), 2.85 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 201 $[M+1]^+$.

4.47. 5-(3-fluorophenyl)-N^4-methylpyrimidine-2,4-diamine (54c). Compound **54c** was prepared according to general procedure A. Yield: 84%; ^{1}HNMR (500 MHz, $CDCl_3$): δ 7.60 (br, Ar-H, 1H), 7.47–7.43 (m, Ar-H, 1H), 7.16–7.11 (m, Ar-H, 3H), 6.36 (q, J = 4.5 Hz, NH, 1H), 6.21 (br, NH_2, 2H), 2.79 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 219 $[M+1]^+$.

4.49. 5-(4-fluorophenyl)-N^4-methylpyrimidine-2,4-diamine (54d). Compound **54d** was prepared according to general procedure A. Yield: 80%; m.p.: 218−220 °C; ^{1}HNMR (500 MHz, $CDCl_3$): δ 7.57 (s, Ar-H, 1H), 7.31 (d, J = 8.5 Hz, Ar-H, 2H), 7.07 (d, J = 8.5 Hz, Ar-H, 2H), 6.17 (q, J = 4.5 Hz, NH, 1H), 6.11 (br, NH_2, 2H), 2.85 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 219 $[M+1]^+$.

4.50. 5-(2-fluorophenyl)-N^4-methylpyrimidine-2,4-diamine (54e). Compound **54e** was prepared according to general procedure A. Yield: 76%; m.p.: 117−119 °C; ^{1}HNMR (500 MHz, $CDCl_3$): δ 7.49 (s, Ar-H, 1H), 7.41–7.37 (m, Ar-H, 1H), 7.30–7.27 (m, Ar-H, 1H), 7.25–7.22 (m, Ar-H, 2H), 6.08 (br, NH_2, 2H), 6.04 (q, J = 4.5 Hz, NH), 2.75 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 219 $[M+1]^+$.

4.51. 5-(3-methoxyphenyl)-N^4-methylpyrimidine-2,4-diamine (54f). Compound **54f** was prepared according to general procedure A. Yield: 77%; m.p.: 171−173°C; ^{1}HNMR (500 MHz,

DMSO-d_6): δ 7.55 (s, Ar-H, 1H), 7.34–7.30 (m, Ar-H, 1H), 6.88–6.86 (m, Ar-H, 2H), 6.84 (s, Ar-H, 1H), 6.15 (q, J = 4.5 Hz, NH), 6.01 (br, NH_2, 2H), 3.78 (s, CH_3, 3H), 2.77 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 231 $[M+1]^+$.

4.52. 5-(4-methoxyphenyl)-N^4-methylpyrimidine-2,4-diamine (54g). Compound **54g** was prepared according to general procedure A. Yield: 80%; m.p.: 186−188°C; ^{1}HNMR (500 MHz, $CDCl_3$): δ 7.47 (s, Ar-H, 1H), 7.22 (d, J = 8.5 Hz, Ar-H, 2H), 6.99 (d, J = 8.5 Hz, Ar-H, 2H), 6.09 (q, J = 4.5 Hz, NH, 1H), 6.03 (br, NH_2, 2H), 3.77 (s, CH_3, 3H), 2.76 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 231 $[M+1]^+$.

4.53. 5-(2, 4-dimethoxyphenyl)-N^4-methylpyrimidine-2,4-diamine (54h). Compound **54h** was prepared according to general procedure A. Yield: 75%; m.p.: 162−164°C; ^{1}HNMR (500 MHz, $CDCl_3$): δ 7.35 (br, Ar-H, 1H), 6.99 (d, J = 8.0 Hz, Ar-H, 1H), 6.61 (s, Ar-H, 1H), 6.57 (d, J = 8.0 Hz, Ar-H, 1H), 5.87 (br, NH_2, 2H), 5.62 (br, NH, 1H), 3.79 (s, CH_3, 3H), 3.71 (s, CH_3, 3H), 2.72 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 261 $[M+1]^+$.

4.54. N^4-methyl-5-(pyridin-3-yl)pyrimidine-2,4-diamine (54i). Compound **54i** was prepared according to general procedure A. Yield: 78%; m.p.: 183−185°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.50 (br, Ar-H, 2H), 7.72 (d, J = 8.0 Hz, Ar-H, 1H), 7.56 (s, Ar-H,1H), 7.43–7.40 (m, Ar-H, 1H), 6.35 (q, J = 4.5 Hz, NH, 1H), 6.14 (br, NH_2, 2H), 2.76 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 202 $[M+1]^+$.

4.55. N^4-methyl-5-(pyridin-4-yl)pyrimidine-2,4-diamine (54j). Compound **54j** was prepared according to general procedure A. Yield: 73%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 8.43 (br, Ar-H, 1H), 7.31 (d, J = 8.5 Hz, Ar-H, 2H), 7.08 (d, J = 8.5 Hz, Ar-H, 2H), 6.18 (q, J = 4.5 Hz, NH, 1H), 6.17 (br, NH_2, 2H), 2.82 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 202 $[M+1]^+$.

4.56. N^4-methyl-5-(thiophen-2-yl)pyrimidine-2,4-diamine (54k). Compound **54k** was prepared according to general procedure A. Yield: 82%; m.p.: 147−149°C; ^{1}HNMR (500 MHz, $CDCl_3$): δ 7.66 (s, Ar-H, 1H), 7.51 (d, J = 4.5 Hz, Ar-H, 1H), 7.13–7.11 (m, Ar-H, 1H), 7.06 (d, J = 2.5 Hz, Ar-H, 1H), 6.30 (q, J = 4.5 Hz, NH, 1H), 6.21 (br, NH_2, 2H), 2.80 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 207 $[M+1]^+$.

4.57. 5-(furan-2-yl)-N^4-methylpyrimidine-2,4-diamine (54l). Compound **54l** was prepared according to general procedure A. Yield: 81%; m.p.: 99−101°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 7.64 (s, Ar-H, 1H), 7.57−7.48 (m, Ar-H, 1H), 6.54 (br, Ar-H, 2H), 6.44 (q, J = 3.5 Hz, NH, 1H), 6.22 (br, NH_2, 2H), 2.86 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 191[M+1]$^+$.

4.58. Methyl 5-(2-amino-4-(methylamino)pyrimidin-5-yl)thiophene-2-carboxylate (54m). Compound **54m** was prepared according to general procedure A. Yield: 75%; m.p.: 213−215°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 7.78 (d, J = 4.0 Hz, Ar-H, 1H), 7.77 (s, Ar-H, 1H), 7.16 (d, J = 4.0 Hz, Ar-H, 1H), 6.56 (q, J = 4.5 Hz, NH, 1H), 6.40 (br, NH_2, 2H), 3.82 (s, CH_3, 3H), 2.80 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 265 [M+1]$^+$.

4.59. N^4-methyl-5-(1-methyl-1*H*-pyrazol-5-yl)pyrimidine-2,4-diamine (54n). Compound **54n** was prepared according to general procedure A. Yield: 65%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 7.54 (s, Ar-H, 1H), 7.47 (d, J = 1.5 Hz, Ar-H, 1H), 6.24 (br, NH_2, 2H), 6.20 (d, J = 1.5 Hz, Ar-H, 1H), 6.07 (q, J = 4.5 Hz, NH, 1H), 3.61 (s, CH_3, 3H), 2.75 (d, J = 4.5 Hz, CH_3, 3H); ESI-MS: m/z = 205 [M+1]$^+$.

4.60. 5-((4-methoxy-5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (16). Compound **16** was prepared according to general procedure C. Yield: 79%; m.p.: 227−229 °C; ^{1}H NMR (500 MHz, DMSO-*d*6): δ 8.95 (br, NH, 1H), 8.69 (s, Ar-H, 1H), 8.23 (s, Ar-H, 1H), 8.17 (s, Ar-H, 1H), 8.03 (s, Ar-H, 1H), 7.69 (s, Ar-H, 1H), 4.48 – 4.22 (m, CH, 1H), 3.91 (s, CH_3, 3H), 3.90 (s, CH_3, 3H), 3.13 (d, J = 12.0 Hz, CH_2, 1H), 2.77 (d, J = 11.5 Hz, CH_2, 1H), 2.61 (dd, J = 12.0, 8.0 Hz, CH_2, 1H), 2.14–2.03 (m, CH_2, 1H), 1.74−1.69 (m, CH_2, 1H), 1.62−1.55 (m, CH_2, 1H), 1.49–1.36 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-*d*6): δ 163.23, 158.00, 157.73, 156.80, 141.09, 137.99, 136.35, 130.10, 115.95, 114.93, 112.60, 112.32, 107.74, 74.56, 54.34, 49.65, 45.24, 38.65, 29.80, 24.17. ESI-HRMS m/z calcd for $C_{20}H_{23}N_8O_2^+$407.1944, found 407.1943 [M + H] $^+$. HPLC purity 100%.

4.61. 5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (17). Compound **17** was prepared according to general procedure C. Yield: 79%; m.p.: 207−209 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ9.91 (s, NH, 1H), 8.50 (s, Ar-H, 2H), 7.90 (s, Ar-H, 1H), 7.89 (s, Ar-H, 1H), 7.61 (s, Ar-H,1H), 6.71 (t, J = 4.5 Hz, NH, 1H), 4.37 (tt, J = 8.0, 4.0 Hz, CH, 1H), 3.88 (s, CH_3, 3H), 3.13 (ddd, J = 12.0, 4.0, 1.5Hz, CH_2,1H),

2.93 (d, *J* = 4.5 Hz, CH_3, 3H), 2.76 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.60 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.52 (d, *J* = 8.5 Hz, CH_2, 1H), 2.08 (dd, *J* = 12.0, 5.0 Hz, CH_2, 1H), 1.74–1.68 (m, CH_2, 1H), 1.62–1.54 (m, CH_2, 1H), 1.45–1.37 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.26, 157.74, 157.37, 153.08, 142.73, 137.65, 134.11, 129.43, 116.49, 113.76, 112.67, 107.99, 104.51, 74.25, 49.83, 45.31, 38.66, 29.92, 28.31, 24.25. ESI-HRMS *m/z* calcd for $C_{20}H_{24}N_9O^+$406.2104, found 406.2103 $[M + H]^+$. HPLC purity 95%.

4.62. 5-((4-(methylamino)-5-phenylpyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (18). Compound **18** was prepared according to general procedure C. Yield: 75%; m.p.: 158−160 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.95 (br, NH, 1H), 8.53 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.52 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.81 (s, Ar-H, 1H), 7.47 (dd, *J* = 8.0, 7.0 Hz, Ar-H, 2H), 7.42–7.36 (m, Ar-H, 3H), 6.75 (q, *J* = 4.5 Hz, NH, 1H), 4.37 (tt, *J* = 8.0, 4.0 Hz, CH, 1H), 3.14 (ddd, *J* = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.92 (d, *J* = 4.5 Hz, CH_3, 3H), 2.76 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.54–2.50 (m, CH_2, 1H), 2.13–2.04 (m, CH_2, 1H), 1.74–1.68 (m, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.46–1.35 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.19, 158.16, 157.34, 153.92, 142.66, 134.65, 134.18, 129.01, 128.77, 127.45, 116.42, 112.84, 112.76, 108.19, 74.25, 49.78, 45.27, 29.89, 28.28, 24.20; ESI-HRMS *m/z* calcd for $C_{22}H_{24}N_7O^+$ 402.2042, found 402.2040 $[M + H]^+$. HPLC purity 99%.

4.63. 5-((5-(3-fluorophenyl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (19). Compound 19 was prepared according to general procedure C. Yield: 78%; m.p.: 156–158 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.02 (s, Ar-H, 1H), 8.59–8.43 (m, Ar-H, 2H), 7.86 (s, Ar-H, 1H), 7.51 (q, *J* = 7.5 Hz, Ar-H, 1H), 7.23 (qd, *J* = 8.5, 8.0, 4.0 Hz, Ar-H, 3H), 6.91 (q, *J* = 4.5 Hz, NH, 1H), 4.38 (dq, *J* = 8.5, 4.5 Hz, CH, 1H), 3.14 (dd, *J* = 12.5, 3.5 Hz, CH_2, 1H), 2.91 (d, *J* = 4.5 Hz, CH_3, 3H), 2.77 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.63 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.54 (d, *J* = 12.5 Hz, CH_2, 1H), 2.08 (d, *J* = 8.5 Hz, CH_2, 1H), 1.75–1.68 (m, CH_2, 1H), 1.63–1.56 (m, CH_2, 1H), 1.46–1.37 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.38, 161.44, 160.04, 158.35, 157.28, 154.16, 142.58, 137.12, 137.06, 134.24, 130.94, 130.87, 124.97, 116.39, 115.74, 115.56, 114.33, 114.16, 112.98, 111.61, 108.31, 74.07, 49.50, 45.13, 29.71, 28.26, 23.88; ESI-HRMS *m/z* calcd for $C_{22}H_{23}FN_7O^+$ 420.1948, found 420.1944 $[M + H]^+$. HPLC purity 99%.

4.64. 5-((5-(4-fluorophenyl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-

yloxy)picolinonitrile (20). Compound **20** was prepared according to general procedure C. Yield: 77%; m.p.: 168–170°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.95 (s, NH, 1H), 8.53 (d, J = 2.0 Hz, Ar-H, 1H), 8.51 (d, J = 2.0 Hz, Ar-H, 1H), 7.80 (s, Ar-H, 1H), 7.51–7.36 (m, Ar-H, 2H), 7.36–7.21 (m, Ar-H, 2H), 6.75 (d, J = 4.5 Hz, NH, 1H), 4.37 (tt, J = 8.0, 4.0 Hz, CH, 1H), 3.13 (ddd, J = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.91 (d, J = 4.5 Hz, CH_3, 3H), 2.76 (dt, J = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, J = 12.0, 8.0 Hz, CH_2, 1H), 2.54–2.50 (m, CH_2, 1H), 2.13–2.04 (m, CH_2, 1H), 1.74–1.67 (m, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.45–1.36 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.62, 160.68, 160.30, 158.23, 157.33, 153.91, 142.63, 134.17, 131.07, 131.01, 116.40, 115.88, 115.71, 112.86, 111.89, 108.21, 74.28, 49.80, 45.28, 29.89, 28.22, 24.21; ESI-HRMS *m/z* calcd for $C_{22}H_{23}FN_7O^+$ 420.1948, found 420.1951 $[M + H]^+$. HPLC purity 98%.

4.65. 5-((5-(2-fluorophenyl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (21). Compound **21** was prepared according to general procedure C. Yield: 74%; m.p.: 145–147°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.98 (s, NH, 1H), 8.54 (d, J = 2.0 Hz, Ar-H, 1H), 8.52 (d, J = 2.0 Hz, Ar-H, 1H), 7.81 (s, Ar-H, 1H), 7.46 (dtd, J = 9.5, 5.5, 2.5 Hz, Ar-H, 1H), 7.38 (td, J = 7.5, 2.0 Hz, Ar-H, 1H), 7.34–7.26 (m, Ar-H, 2H), 6.72 (q, J = 4.5 Hz, NH, 1H), 4.38 (tt, J = 8.0, 4.0 Hz, CH, 1H), 3.14 (ddd, J = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.91 (d, J = 4.5 Hz, CH_3, 3H), 2.75 (dt, J = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, J = 12.0, 8.0 Hz, CH_2, 1H), 2.52 (d, J = 12.5 Hz, CH_2, 1H), 2.13–2.04 (m, CH_2, 1H), 1.74–1.68 (m, CH_2, 1H), 1.63–1.56 (m, CH_2, 1H), 1.45-1.37 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.90, 160.28, 158.96, 158.68, 157.32, 154.76, 142.57, 134.22, 132.08, 132.05, 130.09, 130.02, 124.92, 124.89, 122.02, 121.89, 116.37, 116.07, 115.90, 112.98, 108.40, 106.77, 74.30, 49.79, 45.28, 29.88, 28.15, 24.21. ESI-HRMS *m/z* calcd for $C_{22}H_{23}FN_7O^+$ 420.1948, found 420.1947 $[M + H]^+$. HPLC purity 99%.

4.66. 5-((5-(3-methoxyphenyl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (22). Compound **22** was prepared according to general procedure C. Yield: 78%; m.p.: 206–208°C; ^{1}HNMR (500 MHz, DMSO-d_6): 9.95 (s, NH, 1H), 8.53 (d, Ar-H, J = 2.0 Hz, 1H), 8.52 (d, J = 2.0 Hz, Ar-H, 1H), 7.83 (s, Ar-H, 1H), 7.49–7.30 (m, Ar-H, 1H), 7.10–6.88 (m, Ar-H, 3H), 6.77 (d, J = 4.5 Hz, NH, 1H), 4.37 (tt, J = 8.0, 4.0 Hz, CH, 1H), 3.80 (s, CH_3, 3H), 3.13 (ddd, J = 12.0,4.0, 1.5 Hz, 1H), 2.92 (d, J = 4.5 Hz, CH_3, 3H), 2.76 (dt, J = 12.5, 4.5 Hz, 1H), 2.61 (dd, J = 12.0, 8.0 Hz, CH_2, 1H), 2.54–2.50 (m, CH_2, 1H), 2.12–2.03 (m, CH_2, 1H), 1.74–1.68 (m, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.45–1.37 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.14, 159.60, 158.16, 157.34, 153.81, 142.65, 135.97, 134.17, 130.06, 120.92,

116.41, 114.06, 113.33, 112.85, 112.66, 108.19, 74.29, 55.01, 49.82, 45.29, 29.91, 28.26, 24.23; ESI-HRMS *m/z* calcd for $C_{23}H_{26}N_7O_2^+$ 432.2148, found 432.2143 $[M + H]^+$. HPLC purity 100%.

4.67. 5-((5-(4-methoxyphenyl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (23). Compound **23** was prepared according to general procedure C. Yield: 74%; m.p.: 131-134°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.91 (s, NH, 1H), 8.52 (t, J = 1.5 Hz, Ar-H, 2H), 7.76 (s, Ar-H, 1H), 7.36–7.25 (m, Ar-H, 2H), 7.09–6.97 (m, Ar-H, 2H), 6.66 (q, J = 4.5 Hz, NH, 1H), 4.37 (tt, J = 8.0, 4.0 Hz, CH, 1H), 3.80 (s, CH_3, 3H), 3.13 (ddd, J = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.91 (d, J = 4.5 Hz, CH_3, 3H), 2.76 (dt, J = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, J = 12.0, 8.0 Hz, CH_2, 1H), 2.54–2.50 (m, CH_2, 1H), 2.08 (dd, J = 12.0, 5.5 Hz, CH_2, 1H), 1.74–1.68 (m, CH_2, 1H), 1.64–1.53 (m, CH_2, 1H), 1.48–1.35 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.43, 158.73, 157.96, 157.34, 153.55, 142.71, 134.14, 130.06, 126.63, 116.43, 114.47, 112.73, 112.58, 108.08, 74.27, 55.15, 49.82, 45.29, 29.90, 28.25, 24.23; ESI-HRMS *m/z* calcd for $C_{23}H_{26}N_7O_2^+$ 432.2148, found 432.2150 $[M + H]^+$. HPLC purity 97%.

4.68. 5-((5-(2,4-dimethoxyphenyl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (24). Compound **24** was prepared according to general procedure C. Yield: 75%; m.p.: 141–143°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.87 (s, NH, 1H), 8.54 (d, J = 2.0 Hz, Ar-H,1H), 8.52 (d, J = 2.0 Hz, Ar-H, 1H), 7.65 (s, Ar-H,1H), 7.07 (d, J = 8.5 Hz, Ar-H, 1H), 6.66 (d, J = 2.5 Hz, Ar-H, 1H), 6.61 (dd, J = 8.5, 2.5 Hz, Ar-H, 1H), 6.31 (q, J = 4.5 Hz, NH, 1H), 4.38 (tt, J = 8.0, 4.0 Hz, CH, 1H), 3.81 (s, CH_3, 3H), 3.74 (s, CH_3, 3H), 3.14 (ddd, J = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.89 (d, J = 4.5 Hz, CH_3, 3H), 2.76 (dt, J = 12.5, 4.5 Hz, CH_2, 1H), 2.62 (dd, J = 12.0, 8.0 Hz, CH_2, 1H), 2.55–2.51 (m, CH_2, 1H), 2.15–2.03 (m, CH_2, 1H), 1.74–1.68 (m, CH_2, 1H), 1.65–1.54 (m, CH_2, 1H), 1.49–1.34 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.74, 160.71, 158.25, 158.11, 157.37, 154.11, 142.85, 134.14, 131.90, 116.48, 115.27, 112.61, 109.92, 108.01, 105.31, 98.90, 74.20, 55.36, 55.27, 49.76, 45.25, 29.87, 28.16, 24.15; ESI-HRMS *m/z* calcd for $C_{24}H_{28}N_7O_3^+$ 462.2254, found 462.2257 $[M + H]^+$. HPLC purity 98%.

4.69. 5-((4-(methylamino)-5-(pyridin-3-yl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (25). Compound **25** was prepared according to general procedure C. Yield: 72%; m.p.: 221–222°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.99 (s, NH, 1H), 8.61–8.56 (m, Ar-H, 2H), 8.54 (d, J = 2.0 Hz, Ar-H, 1H), 8.51 (d, J = 2.0 Hz, Ar-H, 1H), 7.85 (s, Ar-H, 1H), 7.81 (ddd, J = 8.0, 2.5, 1.5 Hz, Ar-H, 1H), 7.48 (ddd, J = 8.0, 5.0,1.0 Hz, Ar-H, 1H), 6.96 (q, J = 4.5 Hz, NH, 1H), 4.37 (tt, J = 8.0, 4.0 Hz, CH, 1H), 3.13 (ddd, J = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.91

(d, *J* = 4.5 Hz, CH_3, 3H), 2.76 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.54–2.50 (m, CH_2, 1H), 2.13–2.04 (m, CH_2, 1H), 1.75–1.65 (m, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.47–1.35 (m, CH_2, 1H). ^{13}C NMR(125 MHz, DMSO-d_6): δ 160.37, 158.58, 157.32, 154.50, 149.46, 148.44, 142.54, 136.55, 134.22, 130.70, 123.84, 116.37, 113.01, 109.51, 108.36, 74.31, 49.81, 45.29, 29.90, 28.20, 24.22; ESI-HRMS *m/z* calcd for $C_{24}H_{28}N_7O_3^+$ 403.1995, found 403.1991 $[M + H]^+$. HPLC purity 98%.

4.70. 5-((4-(methylamino)-5-(pyridin-4-yl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (26). Compound **26** was prepared according to general procedure C. Yield: 76%; m.p.: 227–228°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ10.05 (s, NH, 1H), 8.66–8.58 (m, Ar-H, 2H), 8.54 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.49 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.93 (s, Ar-H, 1H), 7.48–7.41 (m, Ar-H, 2H), 7.05 (d, *J* = 4.5 Hz, NH, 1H), 4.37 (tt, *J* = 8.0, 4.0 Hz, CH, 1H), 3.13 (ddd, *J* = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.92 (d, *J* = 4.5 Hz, CH_3, 3H), 2.76 (dt, *J* = 12.5, 4.5 Hz, CH_2,1H), 2.61 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.55–2.50 (m, CH_2,1H), 2.08 (dq, *J* = 13.5, 4.5 Hz, CH_2, 1H), 1.75–1.65 (m, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.47–1.35 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 159.80, 158.69, 157.30, 154.66, 150.09, 142.63, 142.41, 134.27, 123.39, 116.34, 113.18, 110.09, 108.50, 74.29, 49.76, 45.27, 29.88, 28.27, 24.18; ESI-HRMS *m/z* calcd for $C_{21}H_{23}N_8O^+$ 403.1995, found 403.1998 $[M + H]^+$. HPLC purity 98%.

4.71. 5-((4-(methylamino)-5-(thiophen-2-yl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (27). Compound **27** was prepared according to general procedure C. Yield: 80%; m.p.: 176–178°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.04 (s, NH, 1H), 8.51 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.49 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.94 (s, Ar-H, 1H), 7.61 (dd, *J* = 5.0, 1.5 Hz, Ar-H, 1H), 7.26–7.13 (m, Ar-H, 2H), 6.94 (q, *J* = 4.5 Hz, NH, 1H), 4.37 (dq, *J* = 8.5, 4.0 Hz, CH, 1H), 3.13 (ddd, *J* = 12.5,4.0, 1.5 Hz, CH_2, 1H), 2.95 (d, *J* = 4.5 Hz, CH_3, 3H), 2.75 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.60 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.54–2.51 (m, CH_2, 1H), 2.08 (dd, *J* = 12.0, 5.5 Hz, CH_2,1H), 1.75–1.65 (m, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.44–1.36 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.11, 158.31, 157.33, 154.45, 142.43, 135.45, 134.24, 128.10, 126.61, 126.01, 116.39, 113.08, 108.39, 105.77, 74.29, 49.82, 45.32, 29.93, 28.40, 24.26; ESI-HRMS *m/z* calcd for $C_{20}H_{22}N_7OS^+$ 408.1607, found 408.1609 $[M + H]^+$. HPLC purity 99%.

4.72. 5-((5-(furan-2-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (28). Compound **28** was prepared according to general procedure C. Yield: 77%; m.p.: 203–205°C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.06 (s, NH, 1H), 8.51 (d, *J* = 2.0

Hz, Ar-H, 1H), 8.47 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.24 (s, Ar-H, 1H), 7.81–7.66 (m, Ar-H, 1H), 7.04 (q, *J* = 4.5 Hz, Ar-H, 1H), 6.75 (dd, *J* = 3.5, 1.0 Hz, Ar-H, 1H), 6.61 (dd, *J* = 3.5, 2.0 Hz, NH, 1H), 4.37 (tt, *J* = 8.0, 4.0 Hz, CH, 1H), 3.13 (ddd, *J* = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 3.01 (d, *J* = 4.5 Hz, CH_3, 3H), 2.76 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.56–2.51 (m, CH_2, 1H), 2.08 (td, *J* = 9.5, 8.0,4.0 Hz, CH_2, 1H), 1.75–1.65 (m, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.44–1.36 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO): *δ* 158.43, 157.91, 157.31, 152.89, 148.47, 142.32, 142.26, 134.26, 116.37, 113.15, 111.52, 108.42, 106.40, 102.68, 74.28, 49.80, 45.31, 29.90, 28.40, 24.21; ESI-HRMS *m/z* calcd for $C_{20}H_{22}N_7O_2^+$392.1835, found 392.1838 $[M + H]^+$. HPLC purity 96%.

4.73. Methyl-5-(2-((6-cyano-5-(piperidin-3-yloxy)pyridin-3-yl)amino)-4-(methylamino)pyrimidin-5-yl)thiophene-2-carboxylate (29). Compound **29** was prepared according to general procedure C. Yield: 75%; m.p.: 230-233°C; ^{1}HNMR (500 MHz, DMSO-d_6): *δ* 10.08 (s, NH, 1H), 8.50 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.43 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.00 (d, *J* = 1.5 Hz, Ar-H, 1H), 7.80 (d, *J* = 4.0 Hz, Ar-H, 1H), 7.26 (d, *J* = 4.0 Hz, Ar-H, 1H), 7.08 (t, *J* = 4.5 Hz, NH, 1H), 4.35 (dt, *J* = 8.5, 4.5 Hz, CH, 1H), 3.83 (s, CH_3, 3H), 3.19–3.09 (m, CH_2, 2H), 2.94 (d, *J* = 4.5 Hz, CH_3, 3H), 2.76 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.56–2.51 (m, CH_2, 1H), 2.15–2.02 (m, CH_2, 1H), 1.75–1.65 (m, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.44–1.36 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): *δ* 161.66, 159.82, 158.69, 157.25, 155.01, 143.50, 142.19, 134.39, 131.31, 127.39, 116.33, 113.37, 108.60, 104.82, 74.24, 52.23, 49.76, 45.30, 29.88, 28.41, 24.17; ESI-HRMS *m/z* calcd for $C_{22}H_{24}N_7O_3S^+$466.1661, found 466.1663 $[M + H]^+$. HPLC purity 95%.

4.74. 5-((5-(1-methyl-1*H*-pyrazol-5-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (30). Compound **30** was prepared according to general procedure C. Yield: 74%; m.p.: 229-230 °C; ^{1}HNMR (500 MHz, DMSO-d_6): *δ* 10.06 (br, NH, 1H), 8.54 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.50 (s, Ar-H, 1H), 7.86 (s, Ar-H, 1H), 7.53 (d, *J* = 2.0 Hz, Ar-H, 1H), 6.76 (q, *J* = 4.5 Hz, NH, 1H), 6.31 (d, *J* = 2.0 Hz, Ar-H, 1H), 4.37 (tt, *J* = 8.0, 4.0 Hz, CH, 1H), 3.66 (s, CH_3, 3H), 3.13 (ddd, *J* = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.91 (d, *J* = 4.5 Hz, CH_3, 3H), 2.76 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.54–2.50 (m, CH_2, 1H), 2.12–2.04 (m, CH_2, 1H), 1.76–1.65 (m, CH_2, 1H), 1.64–1.52 (m, CH_2, 1H), 1.44–1.36 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): *δ* 160.54, 159.14, 157.29, 155.61, 142.40, 138.22, 135.66, 134.31, 116.33, 113.21, 108.60, 107.38, 101.52, 74.30, 49.78, 45.27, 36.50, 29.89, 28.14, 24.20.

ESI-HRMS *m/z* calcd for $C_{20}H_{24}N_9O^+$406.2104, found 406.2105 $[M + H]^+$. HPLC purity 99%.

4.75. Lithium triisopropoxy(4-methylthiazol-2-yl)borate (56). A mixture of 4-methylthiazole (1.0 g, 10 mmol), triisopropyl borate (2.4 mL, 10 mmol) in dry toluene/ THF (v/v=4:1) at –78°C was added n-butyllithium (3.8 mL, 2.5 M n-hexane solution, 9.6 mmol) dropwise for 85 min and stirred for 135 minutes. Then the mixture was slowly warming to 0 °C for 1 h, added isopropanol (2.9 mL) and stirred overnight. The reaction mixture was evaporated under reduced pressure and added dry acetone (15 mL), then evaporated under reduced pressure until homogeneous. The precipitate was collected by filtration under nitrogen, washed with 50°C MeCN, and dried, to yield **56** as a white powder. This product was used in the next step without purification.

4.76. Tert-butyl-3-((5-((5-bromo-4-(methylamino)pyrimidin-2-yl)amino)-2-cyanopyridin-3-yl)oxy)piperidine-1-carboxylate (57). A mixture of **42a** (0.50 mmol), **53** (0.50 mmol), $Pd_2(dba)_3$ (0.0050 mmol), Xantphos (0.012 mmol), Cs_2CO_3(1.0 mmol) in dry dioxane (10 mL) was heated at 101 °C for 5 h under nitrogen. The cooled reaction mixture was absorbed onto silica gel, and solvent was removed by evaporation. The residue obtained was purified by column chromatography (eluent gradient 4-5% EtOH in CH_2Cl_2), gave **57** (70%) as a white powder; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.05 (s, NH, 1H), 8.46 (s, Ar-H, 1H), 8.41 (s, Ar-H, 1H), 8.13 (s, Ar-H, 1H), 7.32 (q, J = 4.5 Hz, NH, 1H), 4.59 (br, CH, 1H), 3.96–3.93 (m, CH_2, 1H), 3.77–3.70 (m, CH_2, 1H), 3.54–3.43 (m, CH_2, 1H), 3.07–2.96 (m, CH_2, 1H), 2.93 (d, J = 4.5 Hz, CH_3, 3H), 1.94 (br, CH_2, 2H), 1.85–1.80 (m, CH_2, 1H), 1.48–1.46 (m, CH_2, 1H), 1.38 (s, CH_3×3, 9H); ESI-MS: *m/z* = 504 $[M+1]^+$.

4.77. 5-((4-(methylamino)-5-(4-methylthiazol-2-yl)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (31). A mixture of **57** (106 mg, 0.21 mmol), **56** (124 mg, 0.42 mmol), Pd(dppf)Cl_2 (8 mg, 0.011 mmol), CuCl (2 mg, 0.021 mmol), $ZuCl_2$(29 mg, 0.21 mmol), Cs_2CO_3 (138 mg, 0.42 mmol) in dry DMF (10 mL) was heated at 90 °C overnight under nitrogen. Upon cooling, the insoluble material was filtered off and washed with DMF. The filtrate was concentrated, and the residue obtained was purified by column chromatography (eluent gradient 2-3% EtOH in CH_2Cl_2), gave intermediate (79 mg, 72%) as a white solid; CF_3COOH (1.0 mmol) mL) was added in a mixture of intermediate in CH_2CH_2 (1 mL). Then the solvent was removed by evaporation, the residue obtained was purified by column chromatography (eluent CH_2Cl_2:MeOH:TEA =10:1:1), gave **31** as a yellow solid (51 mg, 80%); ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.28 (br, NH, 1H), 9.36 (q, J = 3.5 Hz, NH, 1H), 8.54–8.53 (m, Ar-H, 2H), 8.49

(s, Ar-H, 1H), 7.21 (d, *J* = 1.0 Hz, Ar-H, 1H), 4.43–4.38 (m, CH, 1H), 3.47–3.42 (m, CH_2, 1H), 3.16–3.15 (m, CH_2, 1H), 3.14 (d, *J* = 3.6 Hz, CH_3, 3H), 2.80–2.75 (m, CH_2, 1H), 2.65–2.61 (m, CH_2, 1H), 2.44 (s, CH_3, 3H), 2.13–2.07 (m, CH_2, 1H), 1.76–1.69 (m, CH_2, 1H), 1.63–1.58 (m, CH_2, 1H), 1.46–1.41 (m, CH_2, 1H); ESI-HRMS *m/z* calcd for $C_{20}H_{23}N_8OS^+$423.1716, found 423.1713 $[M + H]^+$. HPLC purity 77%.

4.78. (*R*)-5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (*R*-17). Compound (***R***)**-17** was prepared according to general procedure C. m.p.: 182–184 °C; Yield: 76%; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.90 (s, NH, 1H), 8.51 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.49 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.90 (d, *J* = 1.0 Hz, Ar-H, 1H), 7.89 (s, Ar-H, 1H), 7.60 (d, *J* = 1.0 Hz, Ar-H, 1H), 6.70 (q, *J* = 4.5 Hz, NH, 1H), 4.37 (tt, *J* = 8.0, 4.0 Hz, CH, 1H), 3.88 (s, CH_3, 3H), 3.13 (ddd, *J* = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.93 (d, *J* = 4.5 Hz, CH_3, 3H), 2.76 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.55–2.50 (m, CH_2, 1H), 2.12–2.04 (m, CH_2, 1H), 1.74–1.68 (m, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.46–1.34 (m, CH_2, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.26, 157.73, 157.36, 153.07, 142.72, 137.64, 134.12, 129.41, 116.47, 113.77, 112.69, 108.02, 104.51, 74.24, 49.80, 45.30, 38.65, 29.90, 28.29, 24.20. ESI-HRMS *m/z* calcd for $C_{20}H_{24}N_9O^+$406.2104, found 406.2102 $[M + H]^+$. HPLC purity 99%.

4.79. (*S*)-5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-3-yloxy)picolinonitrile (*S*-17). Compound (***S***)**-17** was prepared according to general procedure C. Yield: 80%; m.p.: 181–183 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 9.90 (s, NH, 1H), 8.51 (d, *J* = 2.0 Hz, Ar-H, 1H), 8.49 (d, *J* = 2.0 Hz, Ar-H, 1H), 7.90 (d, *J* = 1.0 Hz, Ar-H, 1H), 7.89 (s, Ar-H, 1H), 7.60 (d, *J* = 1.0 Hz, Ar-H, 1H), 6.70 (d, *J* = 4.5 Hz, NH, 1H), 4.37 (tt, *J* = 8.0, 4.0 Hz, CH, 1H), 3.88 (s, CH_3, 3H), 3.13 (ddd, *J* = 12.0, 4.0, 1.5 Hz, CH_2, 1H), 2.93 (d, *J* = 4.5 Hz, CH_3, 3H), 2.76 (dt, *J* = 12.5, 4.5 Hz, CH_2, 1H), 2.61 (dd, *J* = 12.0, 8.0 Hz, CH_2, 1H), 2.55–2.50 (m, CH_2, 1H), 2.13–2.03 (m, CH_2, 1H), 1.71 (ddt, *J* = 13.0, 8.5, 4.0 Hz, CH_2, 1H), 1.62–1.55 (m, CH_2, 1H), 1.47–1.35 (m, CH_2, 1H).^{13}C NMR (125 MHz, DMSO-d_6): δ 160.26, 157.73, 157.36, 153.07, 142.72, 137.64, 134.12, 129.41, 116.47, 113.77, 112.69, 108.02, 104.51, 74.24, 49.80, 45.30, 38.65, 29.90, 28.29, 24.20. ESI-HRMS *m/z* calcd for $C_{20}H_{24}N_9O^+$406.2104, found 406.2103 $[M + H]^+$. HPLC purity 100%.

4.80. 5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-4-yloxy)picolinonitrile (32). Compound **32** was prepared according to general procedure C. Yield: 75%; m.p.: 141–143 °C; ^{1}H NMR (500 MHz, DMSO-d6): δ 9.91 (br, NH, 1H), 8.52 (d, J = 2.0 Hz, Ar-H, 1H), 8.46 (d, J = 2.0 Hz, Ar-H, 1H), 7.90 (s, Ar-H, 1H), 7.89 (s, Ar-H, 1H), 7.60 (d, J = 1.0 Hz, Ar-H, 1H), 6.73 (br, NH, 1H), 4.57 (s, CH, 1H), 3.88 (s, CH_3, 3H), 3.12–2.96 (m, CH_2, 2H), 2.92 (d, J =4.5 Hz, CH_3, 3H), 2.67–2.53 (m, CH_2, 2H), 2.02–1.88 (m, CH_2, 2H), 1.67–1.52 (m, CH_2, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.26, 157.74, 157.15, 153.10, 142.72, 137.67, 134.15, 129.45, 116.54, 113.78, 112.78, 108.16, 104.54, 74.67, 42.94, 38.69, 31.31, 28.25. ESI-HRMS *m/z* calcd for $C_{20}H_{24}N_9O^+$406.2104, found 406.2101 [M + H] $^+$. HPLC purity 99%.

4.81. 5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-((1-methylpiperidin-4-yl)oxy)picolinonitrile (33). Compound **33** was prepared according to general procedure C. Yield: 72%; m.p.: 186–189 °C; ^{1}HNMR (500 MHz, DMSO-d_6): ^{1}H NMR (500 MHz, DMSO-d6): δ 9.91 (br, NH, 1H), 8.52 (d, J = 2.0 Hz, Ar-H, 1H), 8.46 (d, J = 2.0 Hz, Ar-H, 1H), 7.90 (s, Ar-H, 1H), 7.89 (s, Ar-H, 1H), 7.60 (d, J = 1.0 Hz, Ar-H, 1H), 6.72 (d, J = 4.5 Hz, NH, 1H), 4.53 (dq, J = 8.5, 4.5, 4.0 Hz, CH, 1H), 3.88 (s, CH_3, 3H), 2.92 (d, J = 4.5 Hz, CH_3, 3H), 2.59 (s, CH_2, 2H), 2.24 (s, CH_2, 2H), 2.20 (s, CH_3, 3H), 2.02–1.90 (m, CH_2, 2H), 1.79-1.72 (m, CH_2, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.25, 157.73, 157.14, 153.07, 142.72, 137.66, 134.14, 129.44, 116.50, 113.77, 112.75, 108.13, 104.54, 73.22, 51.72, 45.74, 38.68, 30.02, 28.27. ESI-HRMS *m/z* calcd for $C_{21}H_{26}N_9O^+$420.2260, found 420.2258 [M + H] $^+$. HPLC purity 98%.

4.82. 5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(piperidin-4-ylmethoxy)picolinonitrile (34). Compound **34** was prepared according to general procedure C. Yield: 74%; m.p.: 180–183 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 9.94 (br, NH, 1H), 8.59 (d, J = 2.0 Hz, Ar-H, 1H), 8.41 (d, J = 2.0 Hz, Ar-H, 1H), 7.89 (s, Ar-H, 1H), 7.88 (s, Ar-H, 1H), 7.60 (s, Ar-H, 1H), 6.69 (q, J = 4.4 Hz, NH, 1H), 3.92 (d, J = 6.4 Hz, CH_2, 2H), 3.88 (s, CH_3, 3H), 2.96 (d, J = 12.0 Hz, CH_2, 2H), 2.92 (d, J = 4.4 Hz, CH_3, 3H), 2.50–2.40 (m, CH_2, 2H), 1.74–1.63 (m, CH_2, 2H), 1.21–1.10 (m, CH_2, 2H). ^{13}C NMR (125 MHz, DMSO): δ 160.22, 158.46, 157.75, 153.16, 142.91, 137.64, 133.95, 129.44, 116.36, 113.75, 111.52, 107.04, 104.55, 71.98, 52.02, 42.67, 38.68, 32.92, 28.22. ESI-HRMS *m/z* calcd for $C_{21}H_{26}N_9O^+$420.2260, found 420.2258 [M

+ H] $^{+}$. HPLC purity 98%.

4.83. (*R*)-5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)-3-(pyrrolidin-3-yloxy)picolinonitrile 35.Compound **35** was prepared according to general procedure C. Yield: 77%; m.p.: 222–223 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 9.91 (br, NH, 1H), 8.59 (d, J = 2.0 Hz, Ar-H, 1H), 8.37 (d, J = 2.0 Hz, Ar-H, 1H), 7.90 (d, J = 1.6 Hz, Ar-H, 2H), 7.61 (s, Ar-H, 1H), 6.81–6.62 (m, NH, 1H), 4.94 (dd, J = 7.6, 4.0 Hz, CH, 1H), 3.88 (s, CH_3, 3H), 3.13 (dd, J = 12.4, 5.2 Hz, CH_2, 1H), 2.92 (s, CH_3, 3H), 2.91 (s, CH_2, 2H), 2.85–2.78 (m, CH_2, 1H), 2.09–2.00 (m, CH_2, 1H), 1.87–1.81(dt, CH_2, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.27, 157.71, 157.57, 152.96, 142.65, 137.64, 133.78, 129.42, 116.48, 113.75, 112.40, 107.90, 104.63, 80.01, 52.80, 45.48, 38.66, 33.25, 28.16. ESI-HRMS *m/z* calcd for $C_{19}H_{22}N_9O^{+}$392.1947, found 392.1949 [M + H] $^{+}$. HPLC purity 97%.

4.84. 3-(2-(dimethylamino)ethoxy)-5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)picolinonitrile (36). Compound **36** was prepared according to general procedure C. Yield: 80%; m.p.: 182–184 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 9.95 (s, NH, 1H), 8.56 (d, J = 2.0 Hz, Ar-H, 1H), 8.47 (d, J = 2.0 Hz, Ar-H, 1H), 7.90 (d, J = 0.8 Hz, Ar-H, 1H), 7.90 (s, Ar-H, 1H), 7.61 (d, J = 0.8 Hz, Ar-H, 1H), 6.71 (d, J = 4.4 Hz, NH, 1H), 4.22 (t, J = 5.6 Hz, CH_2, 2H), 3.88 (s, CH_3, 3H), 2.93 (d, J = 4.4 Hz, CH_3, 3H), 2.70 (t, J = 5.6 Hz, CH_2, 2H), 2.24 (s, CH_3×2, 6H); ^{13}C NMR(125 MHz, DMSO-d_6): δ 160.20, 158.42, 157.71, 153.04, 142.81, 137.59, 133.82, 129.36, 116.41, 113.76, 111.71, 106.91, 104.52, 66.98, 57.06, 45.54, 45.49, 38.63, 28.17; ESI-HRMS *m/z* calcd for $C_{19}H_{24}N_9O^{+}$394.2104, found 394.2103 [M + H] $^{+}$. HPLC purity 96%.

4.85. (*R*)-3-((1-(dimethylamino)propan-2-yl)oxy)-5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)picolinonitrile (*R*-37). Compound (***R***)-**37** was prepared according to general procedure C. Yield: 77%; m.p.: 103–105 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 9.94 (s, NH, 1H), 8.53 (s, Ar-H, 2H), 7.92 (s, Ar-H, 1H), 7.90 (s, Ar-H, 1H), 7.62 (s, Ar-H, 1H), 6.72 (q, J = 4.4 Hz, NH, 1H), 4.66 (q, J = 6.0 Hz, CH, 1H), 3.90 (s, CH_3, 3H), 2.94 (d, J = 4.4 Hz, CH_3, 3H), 2.69–2.55 (m, CH_2, 1H), 2.51–2.47 (m, CH_2, 1H), 2.23 (s, CH_3×2, 6H), 1.34 (d, J = 6.0 Hz, CH_3, 3H); ^{13}C NMR (100 MHz, DMSO): δ 160.29, 157.77, 152.98, 142.76, 137.66, 134.02, 129.44, 116.54, 113.77, 112.62, 107.83, 104.61, 74.03, 63.54, 45.94, 45.82, 38.66, 28.12, 17.89; ESI-HRMS *m/z* calcd for $C_{20}H_{26}N_9O^{+}$408.2260, found 408.2260 [M + H] $^{+}$. HPLC purity 98%.

4.86. (*S*)-3-((1-(dimethylamino)propan-2-yl)oxy)-5-((5-(1-methyl-1*H*-pyrazol-4-yl)-4-(methylamino)pyrimidin-2-yl)amino)picolinonitrile (*S*-37). Compound (***S*)-37** was prepared according to general procedure C. Yield: 74%; m.p.: 147–149 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 9.92 (s, NH, 1H), 8.51 (s, Ar-H, 2H), 7.90 (s, Ar-H, 1H), 7.88 (s, Ar-H, 1H), 7.61 (s, Ar-H, 1H), 6.71 (q, J = 4.4 Hz, NH, 1H), 4.65 (q, J = 6.0 Hz, CH, 1H), 3.88 (s, CH_3, 3H), 2.93 (d, J = 4.4 Hz, CH_3, 3H), 2.60–2.55 (m, CH_2, 1H), 2.49–2.44 (m, CH_2, 1H), 2.21 (s, CH_3×2, 6H), 1.32 (d, J = 6.4 Hz, CH_3, 3H); ^{13}C NMR (100 MHz, DMSO): δ 160.28, 157.76, 152.96, 142.75, 137.65, 134.01, 129.43, 116.54, 113.77, 112.61, 107.82, 104.61, 74.01, 63.53, 45.93, 38.65, 28.11, 17.88; ESI-HRMS *m/z* calcd for $C_{20}H_{26}N_9O^+$408.2260, found 408.2260 $[M + H]^+$. HPLC purity 98%.

(**33, 36,** (***R*)-37,** (***S*)-37**) was prepared according to general procedure C but were not treated with CF_3COOH.)

Notes

The authors declare no competing financial interest.

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Abbreviations

$(Boc)_2O$, ditertbutyl decarbonate; NBS, N-bromosuccinimide; MSCl, methanesulfonyl chloride; SAR, structure-activity relationship; DMAP, 4-dimethylaminopyridine; TFA, triethylamine; TLC, thin-layer chromatography; THF, tetrahydrofuran; DMF, N,N-dimethylformamide; Boc, t-

butyloxy carbonyl; TFA, trifluoroacetic acid; DME, 1,2-dimethoxyethane. PD, pharmacodynamic; PK, Pharmacokinetic; AUC, area under the curve; hERG, human ether-a-go-go related gene product.

Appendix A. Supplementary data

The following is the Supplementary data to this article:

More detailed computational methods about virtual screening workflows; Mean and SD values of enzyme-inhibitory and antiproliferative activities of representative compounds; Biology assay; ESI-HRMS spectra and HPLC purity analysis of selected compounds **6**, (***R***)-**17**, **32**, **33**, and **35**; ^{1}H and ^{13}C spectra of compounds **5**–**37** (PDF); Molecular formula strings (CSV).

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Highlights

• Novel potent and highly selective CHK1 inhibitors.

• 36 novel 5-(pyrimidin-2-ylamino) picolinonitrile derivatives as CHK1 inhibitors were discovered.

• The compound (***R***)-**17** significantly suppressed growth of Z138-xenografted tumors as a single agent.