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Synthesis, biological evaluation and molecular modeling study of 2-amino-3, 5-disubstituted-pyrazines as Aurora kinases inhibitors

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ABSTRACT

Serine/threonine protein kinases Aurora A, B, and C play essential roles in cell mitosis and cytokinesis, and a number of Aurora kinase inhibitors have been evaluated in the clinic. Herein we report the synthesis and their antiproliferation of 3,5-disubstituted-2-aminopyrazines as kinases inhibitors. Amongst, 4-((3-amino-6- (3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)-*N*-(3-chlorophenyl) benzamide (**12Aj**) exhibited the strongest antiproliferative activities against U38, HeLa, HepG2 and LoVo cells with IC_{50} values were 11.5 ± 3.2, 1.34 ± 0.23, 7.30 ± 1.56 and 1.64 ± 0.48 μM, as well as inhibited Aurora A and B with the IC_{50} values were 90 and 152 nM, respectively. Molecular docking studies indicated that **12Aj** appeared to form stable hydrogen bonds with either Aurora A or Aurora B. Furthermore, **12Aj** arrested HeLa cell cycle in G2/M phase by regulating protein levels of cyclinB1 and cdc2. In addition, the bioinformatics prediction further revealed that **12Aj** possessed good drug likeness using SwissADME. These results suggested that **12Aj** was worthy of future development of potent anticancer agents as pan-Aurora kinases.

1. Introduction

The Aurora kinase family is a subfamily of serine/threonine kinases that is essential for the regulation of centrosome maturation, mitotic spindle formation, chromosome segregation and cytokinesis during mitosis.[1,2] The family includes three kinases designated as Aurora A, B, and C, which are very closely related in the kinase domain sequence. However, these kinases have quite different and nonoverlapping functions during mitosis.[3] Aurora A plays an important role in centrosome maturation, spindle assembly, meiotic maturation, and metaphase I spindle orientation,[4,5] while Aurora B is a member of the chromosomal passenger complex, critical for chromosome condensation, chromosome orientation on the mitotic spindle and the spindle-assembly checkpoint, as well as the final stages of cytokinesis.[6] Aurora C has similar location as Aurora B and leads to the formation of multinucleated cells.[7]

The potential roles of Aurora kinases in regulating cell mitosis and tumorigenesis make them attractive targets for anticancer therapy.[8,9] Some subtype-selective or pan-Aurora kinase inhibitors have been discovered and moved forward in clinical trials,[10] such as Aurora A inhibitor MLN-8237 (Alisertib)[11] and ENMD-2076,[12] Aurora B inhibitor AZD-1152 (Barasertib)[13] and AT9283,[14] and pan-Aurora inhibitors VX-680 (Tozasertib),[15] AMG900[16] and PHA-739358 (Danusertib)[17] (Figure 1). However, the majority of which focus on hematopoietic cancers. A possible explanation for limited clinical responses in the treatment of solid tumors is the likely need for sustained exposures through a number of cell cycles (for Aurora B inhibitors) or time in mitosis (for Aurora A inhibitors) to elicit a maximum therapeutic response. At such sustained exposures, mechanism-based toxicity may become dose-limiting.[18]

In our previous publications, some 2,4-diaminopyrimidines and 2,4-disubstituted phthalazinones showed Aurora inhibitory activities, and more potent antiproliferations against tumor cell lines compared with the VX-680.[19–22] Pyrazine as a skeletal structure widely applied in protein kinases inhibitors, such as Nek2[23,24], CHK1[25,26], PI3K[27,28] and MNK1/2[29]. Therefore, in continuation of our efforts on scaffold hopping strategy towards Aurora kinase inhibitors, a novel series of 3,5-disubstituted-2-aminopyrazines were designed by replacement of pyrimidine with 2-aminopyrazine fragment (Figure 2). Herein, we report the synthesis of 3,5-disubstituted-2-aminopyrazines, as well as their activities on antiproliferation toward cancer cell lines, Aurora kinase inhibition and cell cycle arrest.

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[1] These authors made equal contributions to this work.

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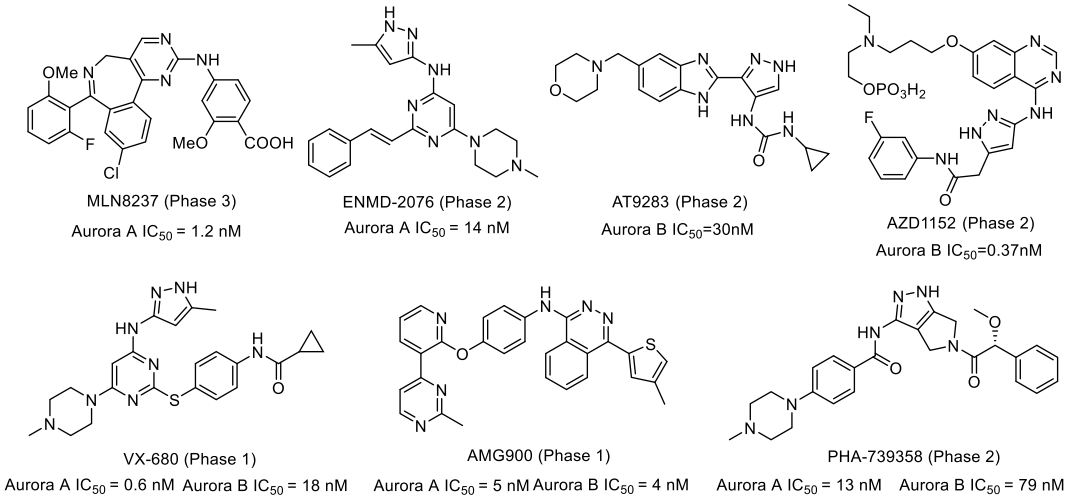


Fig. 1. Representative Aurora kinase inhibitors.

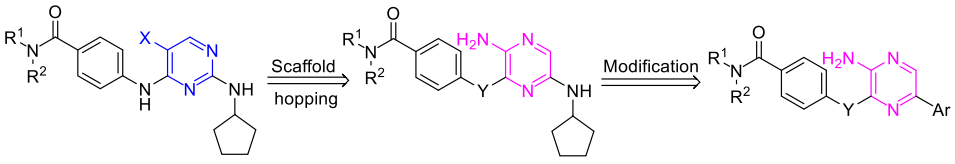


Fig. 2. Design of target compounds.

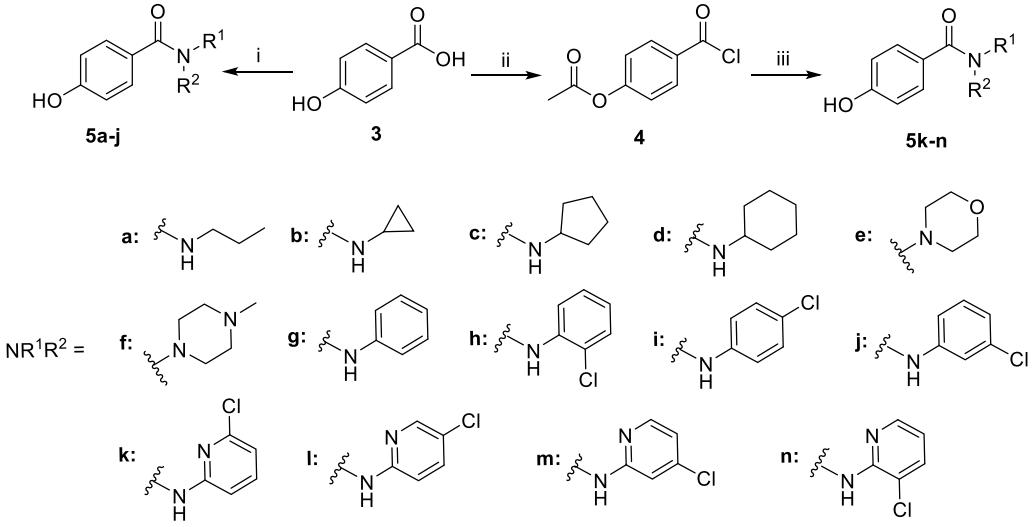
2. Results and discussion

2.1. Chemistry

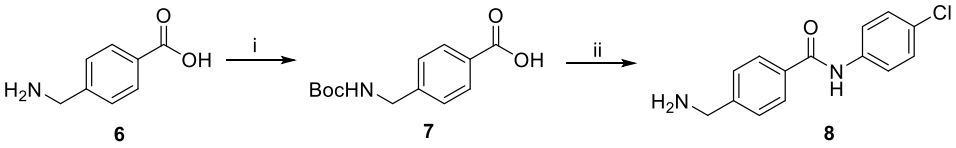
The general synthetic route for intermediates **5a–n** is illustrated in Scheme 1. Specifically, **5a–j** were directly obtained by reacting *p*-hydroxylbenzoic acid (**3**) with various amines in acetone under condensing agent 1-ethyl-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDCI). For intermediates **5k–n**, compound **3** was firstly reacted with acetic anhydride in the presence of 4-methylbenzenesulfonic acid (TsOH), and further treated with thionyl chloride to afford 4-(chlorocarbonyl)phenyl acetate (**4**). After amidation of compound **4** with 3-chloropyridin-2-amine (or 4-chloropyridin-2-amine, or 5-chloropyridin-2-amine, or 6-chloropyridin-2-amine), removal of the protecting group provided *p*-hydroxybenzamides **5k–n** in the methanol solution of potassium hydroxide.

Simultaneously, 4-aminomethylbenzamide (**8**) was prepared from 4-aminomethyl-benzoic acid (**6**) as shown in Scheme 2. Boc protection of the amino group of **6** would give intermediate **7**, which reacted with 4-chloroaniline under tri(dimethylamino)benzotriazol-1-yloxyphos phonium hexafluorophosphate (BOP), and disposed with solution of trifluoroacetic acid in the dichloromethane to yield compound **8**.

The synthesis of derivatives **12Aa–n**, **12Bg–n**, **13A,B** and **16A,B** based on the 2-aminopyrazine scaffolds is shown in Scheme 3. The 3-bromine displacement of 2-amino-3,5-dibromopyrazine (**9**) with intermediates **5a–n** or **8** in *N*-methyl pyrrolidone (NMP) in the presence of K_2CO_3 provided **10a–n** or **11**.[23] Subsequent Suzuki cross-coupling reactions of **10a–n** or **11** with 3,5-dimethylisoxazole-4-boronic acid or 1-methylpyrazole-4-boronic acid pinacol ester under the catalysis of Pd $(dppf)_2Cl_2{\cdot}CH_2Cl_2$ gave the target compounds **12Aa–n**, **12Bg–n** and **13A,B**.[20,23] Besides, compounds **16A,B** were also prepared from **9** by the similar procedures including displacement, amidation and



Scheme 1. Synthesis of compounds **5a–n**. Reagents and conditions: (i) amines, EDCI, acetone, 60 °C, 3 h; (ii) a: Ac_2O, TsOH, 85 °C, 1 h; b: $SOCl_2$, DCM, r.t., 2 h; (iii) a: amines, Et_3N, DCM, r.t., 4 h; b: KOH, MeOH, 75 °C, 2 h.



Scheme 2. Synthesis of compound **8**. Reagents and conditions: (i) Boc_2O, NaOH, H_2O/EtOH (1:1), r.t., 5 h; (ii) a: 4-chloroaniline, BOP, Et_3N, DCM, r.t., overnight; b: CF_3COOH, DCM, r.t., 1 h.

palladium-catalyzed Suzuki cross-coupling reaction. The newly synthesized compounds were characterized by physicochemical and spectral means, and both analytical and spectral data of all the compounds were in full agreement with the proposed structures.

2.2. Biological activity

2.2.1. In vitro antiproliferation activities of target compounds

The *in vitro* antiproliferation activities of target compounds **12Aa–n**, **12Bg–n 13A,B** and **16A,B** were evaluated in a panel of four human tumor cell lines (Human malignant glioblastoma U87, cervical carcinoma HeLa, hepatic carcinoma HepG2 and human colorectal adenocarcinoma LoVo cells) by MTT assay after treatment for 72 h, with VX-680 as a reference compound,[20] and the results are summarized in Table 1. Most target compounds showed moderate to better antiproliferation activities in the four human tumor cell lines, although some of them were less compared with VX-680. Notably, compounds with different substitutions at C-3 position of pyrazine showed various effects in regards to their antiproliferations, compounds **12Aa–n** and **12Bg–n** with *p*-hydroxylbenzoic chains showed more potent antiproliferations than those of compounds **13A,B** and **16A,B** with 4-aminomethylbenzoic or 4-mercaptobenzoic chains at the C-3 position of pyrazine. However, compounds **12Aa–n** and **12Bg–n**, in which the C-5 position of pyrazine were substituted with 3,5-dimethylisoxazole or 1-methylpyrazole, exhibited no obvious differences in their antiproliferations. Furthermore, target compounds with various *p*-hydroxylbenzoic amides (such as cyclopentylamine, morpholine, 1-methylpiperazine, aniline, or chloroanilines) except chloropyridines illustrated potent antiproliferative activities. Among the synthetic target compounds, compound **12Aj** showed the strongest growth-inhibitory activities with the IC_{50} values were 11.5 ± 3.2, 1.34 ± 0.23, 7.30 ± 1.56 and 1.64 ± 0.48 μM for the U87, HeLa, HepG2 and LoVo tumor cell lines, respectively.

2.2.2. Aurora kinases inhibitory activities of the selected compounds

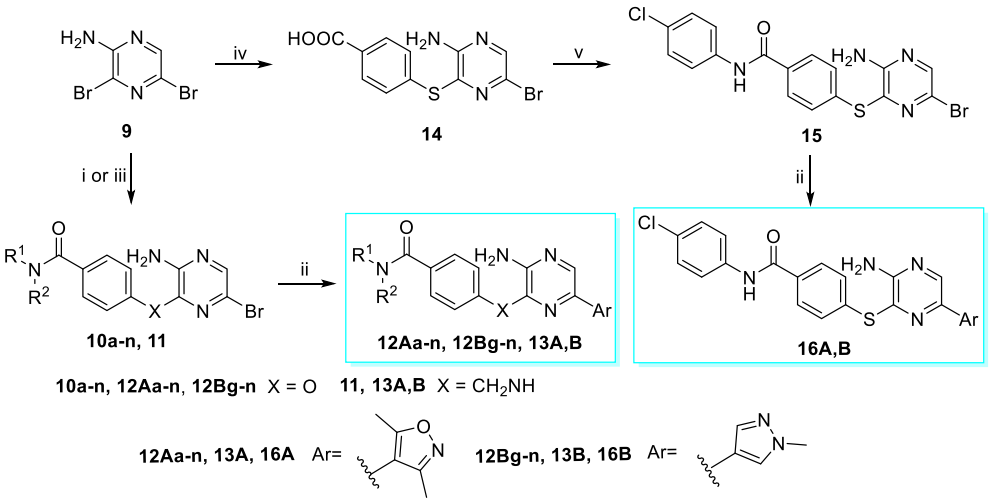
Although pyrazines show various protein kinases inhibitory activities, few 3,5-disubstituted-2-aminopyrazines was reported to inhibit the Aurora kinases. Thus, compounds **12Ad**, **12Ae**, **12Af**, **12Ag**, **12Ai**, **12Aj**, **12Bg**, **12Bj** and **12Bl**, which exhibited better antiproliferative activities toward tumor cells in aforementioned MTT assay, were selected to identify their inhibition activities against Aurora kinases by Kinase-Glo luminescent kinase assay *in vitro*,[30] with VX-680 as reference compound. As showed in Table 2, these compounds showed moderate Aurora inhibitory activities, and less compared with VX-680. Amongst, compound **12Aj** exhibited the most potent inhibition on Aurora A and Aurora B with the IC_{50} values were 90 and 152 nM, respectively. These results suggested that this series of compounds inhibited cancer cell proliferation at least in part by targeting Aurora kinases.

2.2.3. Compound *12Aj* blocks phosphorylation of Aurora kinases in HeLa cells

Aurora A is activated by phosphorylation of Thr288, and Aurora B is activated by phosphorylation of Thr232.[31] To further identification the Aurora kinases were targets of compound **12Aj**, the phosphorylation of Aurora A on Thr288 and Aurora B on Thr232 were investigated in HeLa cells treated with various concentrations of compound **12Aj** (0, 1, 5 and 10 μM) for 24 h by western blot.[32] As shown in Fig. 3, compound **12Aj** obviously decreased the expression level of Aurora A and B, as well as reduced phosphorylation of Aurora A on Thr288 (p-Thr288) and Aurora B on Thr232 (p-Thr232) in a dose-dependent manner. These results suggested that compound **12Aj** is a potential Aurora kinases inhibitor.

2.2.4. Molecular docking of compound *12Aj* binding Aurora kinase

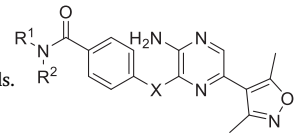
To gain insight into the interaction of compound **12Aj** with Aurora A and Aurora B, docking simulation was performed using Glide module (Glide, version 6.7, Schrödinger, LLC, New York, NY, 2015) of Schrödinger Suite (Maestro, version 10.1, Schrödinger, LLC, New York, NY, 2015).[33] All the figures displaying the docking results were obtained using the scientific software Pymol.[34] The detailed interactions of compound **12Aj** with Aurora A (PDB code: 3H0Z) and Aurora B (PDB code: 4C2V) were shown in Fig. 4. For Aurora A, the ligand **12Aj** appeared to form stable hydrogen bonds with several amino acid residues of Aurora A kinase. Notably, the amino and nitrogen atom on the nuclear formed two stable hydrogen-bond interactions with Glu211 and Ala213 in the hinge region, respectively (Fig. 4A). Besides, the amine group in the side chain established a hydrogen bond with Glu260 and the terminal chlorobenzenecould form π interaction with Tyr219. For Aurora B, the amino and nitrogen atom on the nuclear formed two



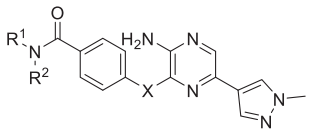
Scheme 3. Synthesis of target compounds **12**, **13** and **16**. Reagents and conditions: (i) **5a–n** (for **10a–n**), K_2CO_3, NMP, 100 °C, 6 h; (ii) 3,5-dimethylisoxazole-4-boronic acid (for **12Aa–n**, **13A** and **16A**) or 1-methylpyrazole-4-boronic acid pinacol ester (for **12Bg–n**, **13B** and **16B**), Pd $(dppf)_2Cl_2{\cdot}CH_2Cl_2$, 2 M Na_2CO_3, dioxane, 100 °C, 4 h; (iii) **8** (for **11**), KOH, dioxane:H_2O = 2:1, 100 °C, 48 h; (iv) 4-mercaptobenzoic acid, KOH, EtOH, 80 °C, 12 h; (v) a: $SOCl_2$, DCM, r.t., 2 h; b: 4-chloroaniline, Et_3N, DCM, r.t., 4 h.

Table 1

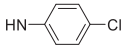
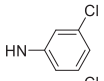
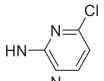
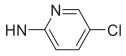
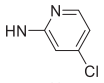
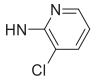
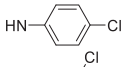
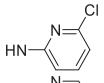
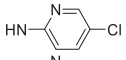
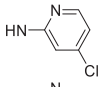
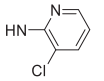
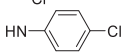
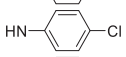
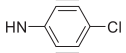
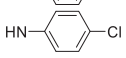
The *in vitro* antiproliferations of target compounds.



12Aa-n, 13A, 16A



12Bg-n,13B, 16B

Compounds	X	NR^1R^2	(IC_{50}, μM)[a,b]			
			U87	HeLa	HepG2	LoVo
12Aa	O		47.8 ± 3.2	32.1 ± 4.3	6.88 ± 1.02	17.5 ± 5.1
12Ab	O		43.3 ± 4.1	17.8 ± 3.5	43.2 ± 4.6	> 100
12Ac	O		57.6 ± 5.2	9.97 ± 2.4	12.8 ± 1.7	25.2 ± 4.2
12Ad	O		40.4 ± 4.7	26.1 ± 3.1	5.42 ± 2.8	21.6 ± 2.6
12Ae	O		27.2 ± 3.3	14.1 ± 2.7	15.4 ± 1.4	10.2 ± 1.6
12Af	O		21.5 ± 2.2	8.03 ± 1.33	17.6 ± 2.5	7.69 ± 1.53
12Ag	O		26.6 ± 2.3	10.5 ± 1.7	11.8 ± 2.1	5.74 ± 1.22
12Ah	O		60.5 ± 5.6	81.3 ± 6.3	> 100	> 100
12Ai	O		15.5 ± 2.1	2.23 ± 0.65	14.4 ± 1.6	1.92 ± 0.12
12Aj	O		11.5 ± 3.2	1.34 ± 0.23	7.30 ± 1.56	1.64 ± 0.48
12Ak	O		64.1 ± 5.4	25.5 ± 2.7	35.7 ± 2.6	39.3 ± 5.1
12Al	O		45.6 ± 3.6	19.2 ± 1.7	35.6 ± 3.4	9.18 ± 1.12
12Am	O		70.8 ± 5.6	37.9 ± 3.8	> 100	> 100
12An	O		45.6 ± 3.5	31.9 ± 3.3	27.2 ± 3.7	14.8 ± 1.5
12Bg	O		17.7 ± 0.8	12.3 ± 1.1	11.6 ± 1.3	9.21 ± 0.76
12Bh	O		44.8 ± 5.2	73.0 ± 6.8	66.9 ± 5.6	41.4 ± 2.4
12Bi	O		35.8 ± 3.3	3.44 ± 0.42	11.9 ± 1.4	3.08 ± 0.43
12Bj	O		21.7 ± 1.2	4.46 ± 0.41	21.2 ± 1.8	24.5 ± 2.2
12Bk	O		54.8 ± 4.6	38.2 ± 3.2	44.2 ± 3.9	48.6 ± 4.7
12Bl	O		22.2 ± 2.1	21.5 ± 1.6	24.2 ± 2.4	6.16 ± 1.12
12Bm	O		47.9 ± 4.7	40.8 ± 3.8	> 100	26.6 ± 2.4
12Bn	O		37.6 ± 2.6	36.1 ± 2.2	21.7 ± 1.8	16.5 ± 1.6
13A	CH_2NH		> 100	> 100	> 100	> 100
13B	CH_2NH		> 100	42.1 ± 3.5	20.1 ± 1.6	43.6 ± 2.6
16A	S		> 100	38.4 ± 2.7	45.2 ± 3.3	49.2 ± 3.6
16B	S		> 100	42.7 ± 4.6	> 100	> 100
VX-680	–	–	14.5 ± 3.4	9.5 ± 1.8	12.8 ± 2.3	13.6 ± 1.6

[a] IC_{50} values are presented as the means ± SD of triplicate experiments.
[b] MTT method drug exposure for 72 h.

Table 2
The *in vitro* kinase inhibitions of selected compounds.

Compounds	(IC_{50}, nM)[a]		Compounds	(IC_{50}, nM)[a]	
	Aurora A	Aurora B		Aurora A	Aurora B
12Ad	230	320	**12Aj**	90	152
12Ae	172	360	**12Bg**	220	164
12Af	143	189	**12Bi**	190	175
12Ag	220	538	**12Bj**	658	272
12Ai	458	260	VX680	1.05	15.7

[a] The IC_{50} values are the means of two experiments.

stable hydrogen-bond interactions with Glu171 and Ala173 in the hinge region, respectively. In addition, the amine group in the side chain established a hydrogen bond with Glu177 and the terminal chlorobenzene formed π interaction with Tyr179 (Fig. 4C). Additionally, the methyl isoxazole of compound **12Aj** could well occupy the hydrophobic pocket of Aurora A and Aurora B (Fig. 4B and D). The model would also be helpful for us to further understand the pyrazine analogues were potent Aurora kinases inhibitors.

2.2.5. Cell cycle arrest of compound *12Aj*

Many studies have shown that Aurora kinase inhibitor VX-680 induces cell cycle arrest in the G2/M phase.[15] Thus, we also investigated the effects of **12Aj** on cell cycle progression using fluorescence-activated cell sorting analysis of HeLa cells stained with propidium iodide.[20] As shown in Figure 5, treatment of HeLa cells with **12Aj** resulted in a dose-dependent accumulation of cells in the G2/M phase with a concomitant decrease in the population of G1 phase cells. The percentages of cells in G2/M phase arrest after treatment with 1, 5 and 10 μM of compound **12Aj** for 24 h were 21.0%, 30.1% and 34.9%, respectively, compared with 10.5% in untreated cultures. These results demonstrated that **12Aj** interfered with cell proliferation by arresting the cell cycle in G2/M.

Cyclin B1 and cdc2 are members of the cell cycle-related protein and regulate the progression of cells into and out of M phase.[35] Therefore, we used western blot to measure protein expression levels of cyclinB1 and cdc2 in HeLa cells following treatment with compound **12Aj** (0, 1, 5 and 10 μM) for 24 h, VX-680 as reference compound.[20] As shown in Fig. 6, treatment of HeLa cells with **12Aj** resulted in an apparent decrease of Cyclin B1 and cdc2, demonstrating that compound **12Aj** can interfere with cell cycle progression. This conclusion is consistent with the cell cycle analysis results.

2.2.6. Drug-likeness of compound *12Aj*

The computational prediction of important physicochemical descriptors related to absorption, distribution, metabolism, and excretion (ADME) properties represents a cost-effective strategy to filter out molecules at early stages of drug-discovery process.[36] Here, we did in silico physicochemical prediction for compound **12Aj** in comparison to VX-680 using SwissADME web service[37], and all results are summarized in Table 3.

Firstly, topological polar surface area (TPSA) used for optimization of drug's ability to permeate cell, molecule with TPSA less than 140 $Å^2$ have good permeating cell membranes.[38] From data depicted in Table 3, compounds **12Aj** and VX-680 had TPSA with 116.16 and 127.37 $Å^2$, respectively, which revealed that either **12Aj** or VX-680 could permeate cell membranes. Simultaneously, consensus lipophilicity ($LogP_{O/W}$) of compounds **12Aj** and VX-680 were 3.56 and 2.95, respectively, that showed similar lipophilicity. Next, predictions of water solubility (LogS) of the compounds revealed that the compounds **12Aj** as well as VX-680 had moderate water solubility. Pharmacokinetics studies indicated that compound **12Aj** and VX-680 had high GI absorption (gastrointestinal absorption) and could not permeate BBB (blood brain barrier). Based on these data, it could be concluded that the compounds were suitable for oral administration. From the skin permeation ($LogK_P$), we infered that compounds **12Aj** possessed similar permeability with VX-680. Finally, the compound **12Aj** obeyed the Lipinski's rule of five. Together, compound **12Aj**, similar to VX-680, follow Lipinski's rule of 5 and possessed moderate to good % human oral absorption.

3. Conclusions

Aurora kinases have been of interest as potential therapeutic targets in oncology. Here we described a series of 3,5-disubstituted-2-aminopyrazine Aurora kinases inhibitors that exerted their antiproliferation activities in human tumor cell lines. We specifically demonstrated that compound **12Aj** has the potential to inhibit Aurora A and Aurora B in HeLa cells. Treatment of HeLa cells with compounds **12Aj** also resulted in G2/M accumulation by regulating the expression of Cyclin B1 and cdc2. Furthermore, the SwissADME prediction showed that compound **12Aj** exhibited good drug likeness. These results suggested that these compounds have potential anticancer activities for further development as Aurora inhibitors.

4. Experiment

4.1. Chemistry

4.1.1. General procedures

All starting materials and reagents were purchased commercially and used without further purified, unless otherwise stated. All reactions were monitored by thin layer chromatograph (TLC) on silica gel GF254 (0.25 mm thick). Column chromatography (CC) was performed on Silica Gel 60 (230–400 mesh, Qingdao Ocean Chemical Ltd., China). HPLC analysis using a UltiMate300 DAD HPLC system equipped with a PU-2089 Plus quaternary gradient pump and a UV-2075 Plus UV–vis detector, using an Alltech Kromasil C18 column with dimensions of 250 mm × 4.6 mm and 5 μm particle size. ^{1}H NMR and ^{13}C NMR spectra were recorded with an Agilent-NMR-Inova 600 spectrometer with TMS as an internal standard, all chemical shift values are reported as ppm. Mass spectra were recorded on an Esquire6000 (ESI-ION TRAP) spectrometer with ESI source as ionization, respectively.

4.1.2. General procedure for synthesis of 4-hydroxybenzamides (*5a–j*)

To a solution of 4-hydroxybenzoic acid (**3**, 690 mg, 5 mmol) in acetone (10 mL) at room temperature was added *n*-propylamine

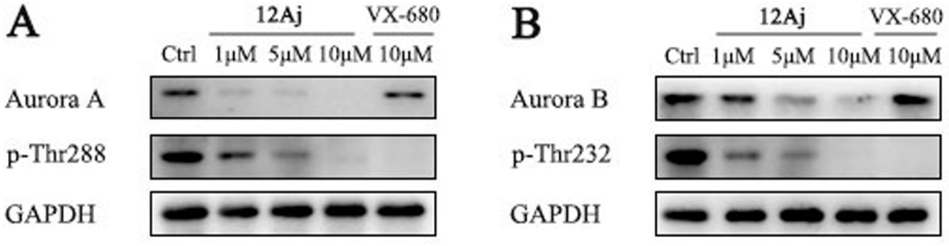


Fig. 3. Western blot for inhibition of p-T288 (A) and p-T232 (B) with various concentrations of compound **12Aj** and VX680 in HeLa cells for 24 h.

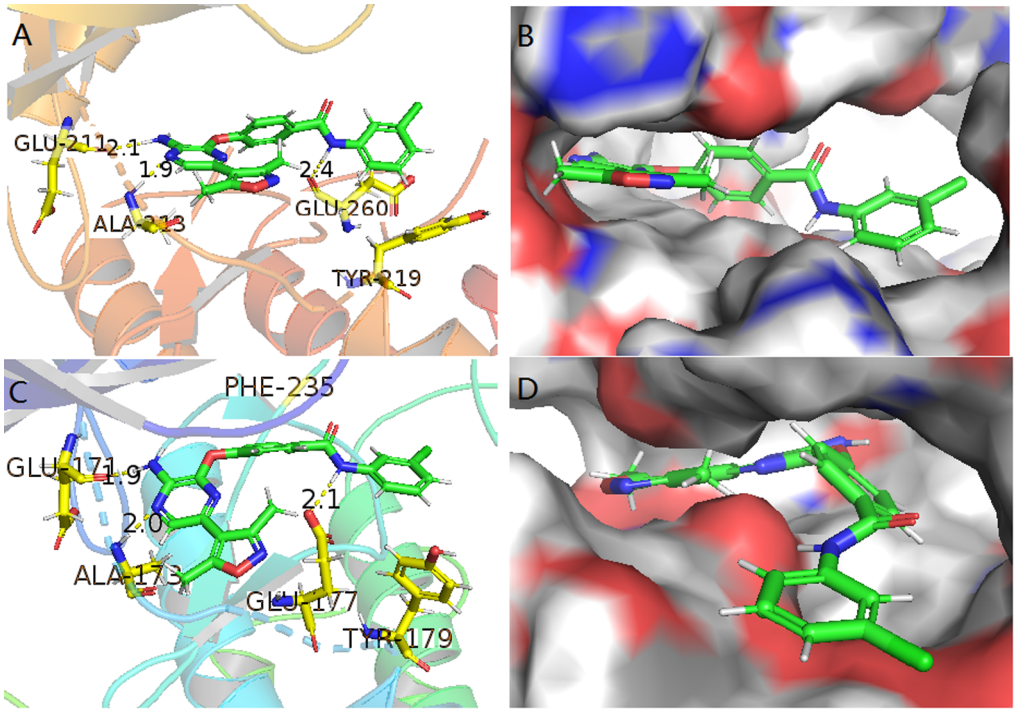


Fig. 4. Molecular binding modes of compound **12Aj** to Aurora A (PDB code: 3H0Z) and Aurora B (PDB code: 4C2V). (A) Predicted binding mode for **12Aj** to Aurora A. (B) Surface of **12Aj** and Aurora A. (C) Predicted binding mode for **12Aj** to Aurora B. (D) Surface of **12Aj** and Aurora B.

followed by EDCI (1.15 g, 6.0 mmol). After stirring the reaction mixture for 12 h at 70 °C, the mixture was concentrated under vacuo, added water (10 mL), extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated under vacuo. The crude product was then purified by column chromatography using dichloromethane/acetone (20:1) to yield the pure **5a**.

Similar procedure as that described for **5a** gave pure **5b–j**.

*4.1.2.1. N-Propyl-4-hydroxybenzamide (**5a**).* Light brown liquid; yield: 90%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.94 (s, 1H), 8.20 (s, 1H), 7.70 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 3.19–3.15 (m, 2H), 1.52–1.48 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H).

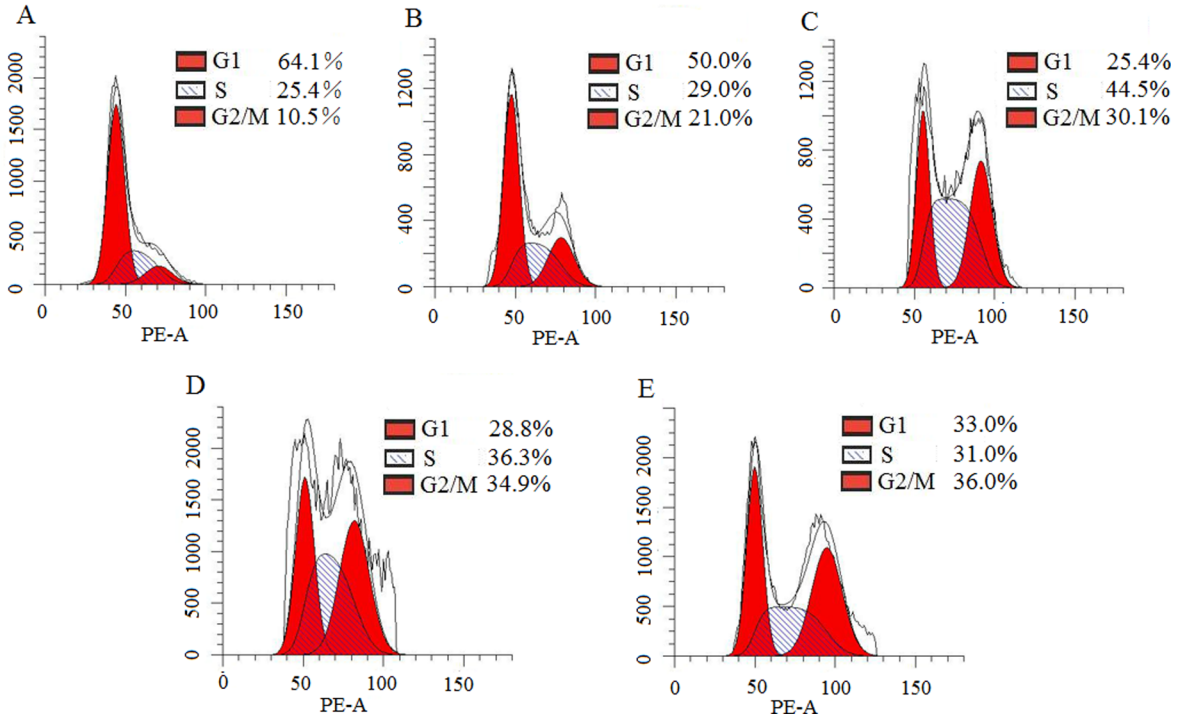


Fig. 5. Effect of **12Aj** and VX-680 on cell cycle progression. (A) Control HeLa cells. (B) HeLa cells treated with 1 μM **12Aj** for 24 h. (C) HeLa cells treated with 5 μM **12Aj** for 24 h. (D) HeLa cells treated with 10 μM **12Aj** for 24 h. (E) HeLa cells treated with 10 μM VX-680 for 24 h.

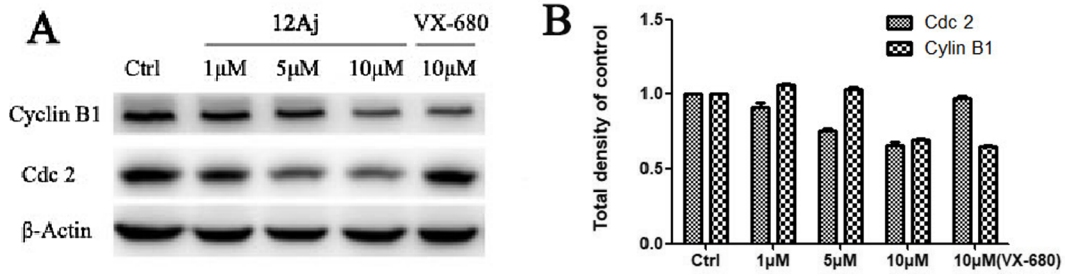


Fig. 6. Western blotting analysis of expression of cyclin B1 and cdc2 in HeLa cells treated with **12Aj** (0, 1, 5 and 10 μM) or VX-680 (10 μM) for 24 h.

Table 3
Prediction of physicochemical properties for "drug-likeness" for compounds **12Aj** and VX-680.

Models	**12Aj**	VX-680
Molecular weight	435.86 g/mol	464.59 g/mol
Num. H-bond acceptors	6	5
Num. H-bond donors	2	3
Num. rotatable bonds	6	8
TPSA (≤140 Å^2)	116.16 Å^2	127.37 Å^2
Consensus Log$P_{O/W}$	3.56	2.95
LogS (ESOL)	−5.01	−4.72
GI absorption	High	High
BBB permeant	No	No
LogK_P (skin permeation)	−6.53 cm/s	−6.71 cm/s
PAINS	0 alert	0 alert
Bioavailability score	0.55	0.55
Lipinski	Yes; 0 violation	Yes; 0 violation

4.1.2.2. N-Cyclopropyl-4-hydroxybenzamide ***(5b)***. Colorless oil liquid; yield: 64%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.12 (s, 1H), 9.95 (s, 1H), 7.87 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 2.85–2.84 (m, 1H), 0.71–0.68 (m, 2H), 0.59–0.56 (m, 2H).

4.1.2.3. N-Cyclopentyl-4-hydroxybenzamide ***(5c)***. White solid; yield: 58%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 9.0 Hz, 2H), 6.74 (d, J = 9.0 Hz, 2H), 4.17–4.14 (m, 1H), 1.84–1.80 (m, 2H), 1.65–1.64 (m, 2H), 1.48–1.45 (m, 4H).

4.1.2.4. N-Cyclohexyl-4-hydroxybenzamide ***(5d)***. White solid; yield: 64%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 8.4 Hz, 2H), 6.74 (d, J = 9.0 Hz, 2H), 3.69–3.68 (m, 1H), 1.76 (t, J = 6.0 Hz, 2H), 1.69 (t, J = 7.8 Hz, 2H), 1.58 (s, 1H), 1.29–1.22 (m, 4H), 1.10–1.07 (m, 1H).

4.1.2.5. (4-Hydroxyphenyl)(morpholino)methanone ***(5e)***. White solid; yield: 80%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 7.27 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 8.4 Hz, 2H), 3.41 (s, 4H), 3.48 (s, 4H).

4.1.2.6. (4-Hydroxyphenyl)(4-methylpiperazin-1-yl)methanone ***(5f)***. White solid; yield: 68%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 7.24 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.4 Hz, 2H), 3.41 (s, 4H), 2.29 (s, 4H), 2.18 (s, 3H).

4.1.2.7. 4-Hydroxy-N-phenylbenzamide ***(5g)***. White solid; yield: 71%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.08 (s, 1H), 9.97 (s, 1H), 7.85 (d, J = 9.0 Hz, 2H), 7.75 (d, J = 7.2 Hz, 2H), 7.32 (t, J = 7.8 Hz, 2H), 7.07–7.06 (m, 1H), 6.86 (d, J = 9.0 Hz, 2H).

4.1.2.8. N-(2-Chlorophenyl)-4-hydroxybenzamide ***(5h)***. Colourless oil liquid; yield: 72%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.12 (s, 1H), 9.74 (s, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.60 (t, J = 8.4 Hz, 1H), 7.54–7.52 (m, 1H), 7.38–7.35 (m, 1H), 7.28–7.25 (m, 1H), 6.87 (d, J = 8.4 Hz, 2H).

4.1.2.9. N-(4-Chlorophenyl)-4-hydroxybenzamide ***(5i)***. Light yellow solid; yield: 76%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.11 (s, 1H), 10.10 (s, 1H), 7.85 (d, J = 9.0 Hz, 2H), 7.80 (d, J = 9.0 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 7.8 Hz, 2H).

4.1.2.10. N-(3-Chlorophenyl)-4-hydroxybenzamide ***(5j)***. Yellow solid; yield: 51%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.15 (s, 1H), 10.14 (s, 1H), 7.96 (s, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.4 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.13 (d, J = 7.8 Hz, 1H), 6.88 (d, J = 9.0 Hz, 2H).

4.1.3. General procedure for synthesis of 4-hydroxybenzamides ***(5k–n)***

A solution of 4-hydroxybenzoic acid (**3**, 1.4 g, 10 mmol) in acetic anhydride (1.6 g, 16.0 mmol) and *p*-methylbenzenesulfonic acid (80 mg, 0.4 mmol) was allowed to stir at 85℃ for 1 h. After completion of the reaction, the reaction mixture was quenched by addition of water and separated in ice-bath. The crude product was then purified by re-crystallization (ethanol-water) to provide 4-acetoxybenzoic acid (1.5 g, yield 83%).

To a solution of 4-acetoxybenzoic acid (1.0 g, 5.6 mmol) in dried DCM (10 mL), thionyl chloride (660 mg, 5.6 mmol) was added dropwise, and the reaction mixture was stirred for 2 h at room temperature. After completion of the reaction, the mixture was concentrated under vacuo to give 4-(chlorocarbonyl)phenyl acetate (**4**), which was used further without purification.

To a solution of 2-amino-6-chloropyridine (2.1 mmol) in dried DCM (10 mL) were added Et_3N (0.4 mL, 3.7 mmol) followed by dropwise addition of a solution of **4** (0.4 g, 2.3 mmol) in dried DCM (5 mL). After stirring the reaction mixture for 4 h at room temperature, the mixture was concentrated under vacuo, and the crude product was purified by column chromatography using DCM/acetone (20:1) to yield pure 4-((6-chloropyridin-2-yl)carbamoyl)phenyl acetate.

To a mixture of 4-((6-chloropyridin-2-yl)carbamoyl)phenyl acetate (2 mmol) in MeOH (10 mL) were added KOH (22 mg, 0.2 mmol), and the reaction mixture was stirred for 2 h at 75℃. After completion of the reaction, 1 M aqueous hydrochloric acid was added dropwise to adjust pH = 3. A precipitate was obtained, and washed with water. The crude product was purified by column chromatography using DCM/MeOH (20:1) to give *N*-(6-chloropyridin-2-yl)-4-hydroxybenzamide (**5k**).

Similar procedure as that described for **5k** gave pure **5l–n**.

4.1.3.1. N-(6-Chloropyridin-2-yl)-4-hydroxybenzamide ***(5k)***. White solid; yield: 75%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.11 (s, 1H), 8.19 (d, J = 8.4 Hz, 1H), 8.08 (d, J = 7.8 Hz, 2H), 7.90 (t, J = 7.8 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 9.0 Hz, 2H).

4.1.3.2. N-(5-Chloropyridin-2-yl)-4-hydroxybenzamide ***(5l)***. White solid; yield: 66%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.99 (s, 1H), 8.42 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 8.04 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 9.0 Hz, 1H), 7.25 (d, J = 9.0 Hz, 2H).

*4.1.3.3. N-(4-Chloropyridin-2-yl)-4-hydroxybenzamide (**5m**)*. White solid; yield: 71%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.36 (d, J = 5.4 Hz, 1H), 8.28 (s, 1H), 7.94 (d, J = 9.0 Hz, 2H), 7.28 (d, J = 4.8 Hz, 1H), 6.86 (d, J = 9.0 Hz, 2H).

*4.1.3.4. N-(3-Chloropyridin-2-yl)-4-hydroxybenzamide (**5n**)*. Colourless oil liquid; yield: 70%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.80 (s, 1H), 8.47 (d, J = 4.8 Hz, 1H), 8.07 (d, J = 3.0 Hz, 1H), 8.05 (d, J = 9.0 Hz, 2H), 7.41 (t, J = 5.4 Hz, 1H), 7.32 (d, J = 9.0 Hz, 2H).

*4.1.4. Synthesis of 4-(aminomethyl)-N-(4-chlorophenyl)benzamide **8***

To a solution of 4-aminomethylbenzoic acid (**6**, 750 mg, 5 mmol) in EtOH (7.5 mL) and water (7.5 mL) were added $(Boc)_2O$ (1.31 g, 6 mmol), following the reaction mixture was stirred for 5 h at room temperature. The reaction mixture was concentrated under vacuo to give **7**, which was used further without purification.

To a solution of **7** (1.04 g, 4 mmol), 4-chloroaniline (0.51 g, 4 mmol) in dry DCM (10 mL) were added BOP (300 mg, 5.0 mmol) and NEt_3 (0.8 mL, 7.5 mmol) following the reaction mixture was stirred for overnight at room temperature. The reaction mixture was added trifluoroacetic acid (0.91 g, 8 mmol), and stirred for another 1 h. The reaction mixture was concentrated under vacuo, and purified by silica gel chromatography using a solution of DCM/acetone (20:1) to yield white solid; yield: 75%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.29 (s, 2H), 8.01 (d, J = 8.4 Hz, 2H), 7.83 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 9.0 Hz, 2H), 4.14 (s, 2H).

*4.1.5. General synthetic procedure of compounds **12Aa–n** and **12Bg–n***

To a solution of 2-amino-3,5-dibromopyrazine (**9**, 253 mg, 1 mmol), 4-hydroxybenzamides (**5a–n**, 1.2 mmol) in NMP (6 mL) was added K_2CO_3 (276 mg, 2.0 mmol). The resulting mixture was refluxed for 6 h at 100 °C under nitrogen gas. After completion of the reaction, the reaction mixture was concentrated under vacuo, and purified by silica gel chromatography using a solution of DCM/acetone (20:1) to yield pure **10a–n**.

To a mixture of 2-amino-5-dibromo-3-sustitutedpyrazines (**10a–n**, 0.5 mmol) and 3,5-dimethylisoxazole-4-boronic acid (or 1-methylpyrazole-4-boronic acid pinacol ester, 0.6 mmol) in dioxane (10 mL) were added $Pd(dppf)_2Cl_2{\cdot}CH_2Cl_2$ (40.8 mg, 0.05 mmol) and 2 M Na_2CO_3 solution (0.05 mL, 0.1 mmol). The resulting mixture was refluxed for 4 h at 100 °C under nitrogen gas. The reaction mixture was concentrated under vacuo, and purified by column chromatography using a solution of DCM/MeOH (20:1) to yield compound **12Aa–n** and **12Bg–n**.

*4.1.5.1. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)- N-propylbenzamide (**12Aa**)*. White solid; Yield: 72%; HPLC Purity: 95% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 7.60 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.44 (t, J = 5.4, 1H), 7.89 (d, J = 7.2 Hz, 2H), 7.79 (s, 1H), 7.31 (d, J = 7.2 Hz , 2H), 6.78 (s, 2H), 3.21–3.18 (m, 2H), 2.29 (s, 3H), 2.07 (s, 3H), 1.53–1.49 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.4, 165.3, 158.0, 155.1, 145.9, 145.2, 134.6, 131.2, 128.6 (2C), 127.8, 121.4 (2C), 112.5, 40.9, 22.3, 11.8, 11.4, 11.0. ESI-MS: m/z 368.2 for [M + H].

*4.1.5.2. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)- N-cyclopropylbenzamide (**12Ab**)*. White solid; Yield: 74%; HPLC Purity: 95% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 7.00 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.44 (d, J = 4.2 Hz, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.81 (s, 1H), 7.32 (d, J = 8.4 Hz, 2H), 6.79 (s, 2H), 2.85–2.84 (m, 1H), 2.29 (s, 3H), 2.07 (s, 3H), 0.71–0.68 (m, 2H), 0.59–0.56 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.6, 165.3, 158.0, 155.1, 145.9, 145.2, 134.7, 130.9, 129.0, 128.6 (2C), 121.3 (2C), 114.6, 23.0, 11.8, 11.0, 5.68 (2C). ESI-MS m/z 366.2 for [M + H].

*4.1.5.3. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)-N-cyclopentylbenzamide (**12Ac**)*. White solid; Yield: 66%; HPLC Purity: 98% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 8.46 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.27 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.78 (s, 1H), 7.30 (d, J = 8.4 Hz, 2H), 6.78 (s, 2H), 4.22–4.18 (m, 1H), 2.29 (s, 3H), 2.07 (s, 3H), 1.87–1.85 (m, 2H), 1.67–1.65 (m, 2H), 1.49–1.45 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.3, 165.0, 158.0, 155.0, 145.9, 145.2, 134.6, 131.3, 128.7 (2C), 127.7, 121.3 (2C), 112.5, 50.9, 32.0 (2C), 23.6 (2C), 11.8, 11.0. ESI-MS m/z 394.2 for [M + H].

*4.1.5.4. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)- N-cyclohexylbenzamide (**12Ad**)*. White solid; Yield: 58%; HPLC Purity: 95% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 9.05 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.18 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.79 (s, 1H), 7.30 (d, J = 9.0 Hz, 2H), 6.77 (s, 2H), 3.74–3.72 (m, 1H), 2.29 (s, 3H), 2.07 (s, 3H), 1.80 (s, 2H), 1.72 (s, 2H), 1.61–1.58 (m, 1H), 1.31–1.28 (m, 4H), 1.13–1.10 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.3, 164.5, 158.0, 155.0, 145.9, 145.2, 134.7, 131.3, 128.7 (2C), 127.7, 121.3 (2C), 112.6, 48.3, 32.4 (2C), 25.2, 24.9 (2C), 11.8, 11.0. ESI-MS m/z 408.2 for [M + H].

*4.1.5.5. (4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy) phenyl) (morpholino)methanone (**12Ae**)*. Light yellow solid; Yield: 48%; HPLC Purity: 97% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 6.72 min); ^{1}H NMR (600 MHz, acetone-d_6) δ 7.82 (s, 1H), 7.54 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 9.0 Hz, 2H), 6.16 (d, J = 6.6 Hz, 1H), 3.67 (s, 4H), 3.58 (s, 4H), 2.34 (s, 3H), 2.14 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.5, 165.3, 157.9, 153.8, 146.1, 145.1, 134.4, 132.1, 128.6 (2C), 127.8, 121.9 (2C), 112.4, 65.9 (2C), 46.1 (2C), 11.8, 11.0. ESI-MS m/z 394.2 for [M − H].

*4.1.5.6. (4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy) phenyl) (4-methylpiperazin-1-yl)methanone (**12Af**)*. Yellow solid; Yield: 55%; HPLC Purity: 98% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 6.88 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.79 (s, 1H), 7.43 (d, J = 9.0 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 6.77 (s, 2H), 3.32 (brs, 8H), 2.27 (s, 3H), 2.18 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.4, 165.3, 157.9, 153.7, 146.1, 145.2, 134.4, 132.5, 128.4 (2C), 127.8, 121.9 (2C), 120.9, 114.8, 112.4, 54.4, 45.4 (2C), 11.8, 11.0. ESI-MS m/z 409.2 for [M + H].

*4.1.5.7. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)- N-phenylbenzamide (**12Ag**)*. White solid; Yield: 72%; HPLC Purity: 95% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 9.29 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.25 (s, 1H), 8.05 (d, J = 9.0 Hz, 2H), 7.83 (s, 1H), 7.78 (d, J = 7.8 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 7.2 Hz, 2H), 7.10 (s, 1H), 6.83 (s, 2H), 2.33 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.3, 164.5, 157.9, 155.5, 145.8, 145.2, 139.0, 134.8, 131.2, 129.1 (2C), 128.4 (2C), 127.8, 123.5, 121.3 (2C), 120.3 (2C), 92.5, 11.7, 10.9. ESI-MS m/z 402.2 for [M + H].

*4.1.5.8. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)- N-(2-chlorophenyl)benzamide (**12Ah**)*. White solid; Yield: 60%; HPLC Purity: 97% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 10.12 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.81 (s, 1H), 7.59 (d, J = 7.2 Hz, 1H), 7.54 (d, J = 7.2 Hz, 1H), 7.40 (d, J = 7.8 Hz, 2H), 7.37 (t, J = 8.4 Hz, 1H), 7.28 (t, J = 7.8 Hz, 1H), 6.79 (s, 2H), 2.31 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.4, 164.6, 158.0, 155.8, 145.8, 145.3, 135.0, 134.9, 130.4, 129.5 (2C), 129.2 (2C), 128.5, 127.8, 127.4 (2C), 121.6 (2C), 112.6, 11.8, 11.0. ESI-MS m/z 436.2 for [M + H].

*4.1.5.9. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)- N-(4-chlorophenyl)benzamide (**12Ai**)*. White solid; Yield: 77%; HPLC Purity: 95% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 12.40 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.05 (d, J = 9.0 Hz, 2H), 7.83 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 8.4 Hz, 4H), 6.80 (s,

2H), 2.33 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.4, 164.7, 158.0, 155.7, 145.8, 145.2, 138.1, 134.8, 131.0, 129.2 (2C), 128.4 (2C), 127.8, 127.2, 121.8 (2C), 121.5 (2C), 112.5, 11.8, 11.0. ESI-MS *m/z* 436.2 for [M + H].

*4.1.5.10. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)- N-(3-chlorophenyl)benzamide (**12Aj**)*. White solid; Yield: 66%; HPLC Purity: 96% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 12.63 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.98 (s, 1H), 7.84 (s, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 9.0 Hz, 2H), 7.39 (t, J = 8.4 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 6.82 (s, 2H), 2.33 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.4, 164.9, 158.0, 155.8, 145.8, 145.2, 140.6, 134.9, 132.8, 130.8, 130.2, 129.3 (2C), 127.8, 123.3, 121.5 (2C), 119.7, 118.6, 112.5, 11.8, 11.0. ESI-MS *m/z* 436.2 for [M + H].

*4.1.5.11. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)- N-(6-chloropyridin-2-yl)benzamide (**12Ak**)*. Yellow solid; Yield: 42%; HPLC Purity: 97% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 7.67 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.16 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 8.12 (d, J = 7.8 Hz, 2H), 7.91 (t, J = 8.4 Hz, 1H), 7.84 (s, 1H), 7.39 (d, J = 9.0 Hz, 2H), 7.28 (d, J = 7.8 Hz, 1H), 6.86 (s, 2H), 2.34 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.4, 158.0, 156.1, 152.3, 147.9, 145.7, 145.3, 141.5, 134.9, 130.0, 129.8 (2C), 127.8, 121.3 (2C), 119.5, 113.3 (2C), 112.5, 11.8, 11.0. ESI-MS *m/z* 437.2 for [M + H].

*4.1.5.12. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)-N-(5-chloropyridin-2-yl)benzamide (**12Al**)*. Yellow solid; Yield: 44%; HPLC Purity: 95% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 10.66 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.03 (s, 1H), 8.45 (d, J = 2.4 Hz, 1H), 8.24 (d, J = 8.4 Hz, 1H), 8.11 (d, J = 9.0 Hz, 2H), 7.97 (dd, J = 9.0, 3.0 Hz, 1H), 7.84 (s, 1H), 7.39 (d, J = 9.0 Hz, 2H), 6.81 (s, 2H), 2.34 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.4, 165.3, 158.0, 156.0, 150.9, 146.2, 145.8, 145.3, 137.8, 134.9, 130.2, 129.8 (2C), 127.8, 125.5, 121.4 (2C), 115.8, 112.5, 11.8, 11.1. ESI-MS *m/z* 437.1 for [M + H].

*4.1.5.13. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy)- N-(4-chloropyridin-2-yl)benzamide (**12Am**)*. Yellow solid; Yield: 46%; HPLC Purity: 96% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 10.16 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.08 (s, 1H), 8.37 (d, J = 4.8 Hz, 1H), 8.28 (d, J = 1.8 Hz, 1H), 8.09 (d, J = 9.0 Hz, 2H), 7.81 (s, 1H), 7.37 (d, J = 9.0 Hz, 2H), 7.29 (dd, J = 5.4, 1.8 Hz, 1H), 6.78 (s, 2H), 2.31 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.6, 165.4, 158.0, 156.1, 153.3, 149.3, 145.7, 145.3, 143.9, 134.9, 130.0, 129.7 (2C), 127.8, 121.4 (2C), 119.7, 114.1, 112.5, 11.8, 11.0. ESI-MS *m/z* 437.1 for [M + H].

*4.1.5.14. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)oxy) -N-(3-chloropyridin-2-yl)benzamide (**12An**)*. Yellow solid; Yield: 37%; HPLC Purity: 97% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 8.42 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.12 (s, 1H), 8.40 (d, J = 5.4 Hz, 1H), 8.31 (d, J = 1.8 Hz, 1H), 8.12 (d, J = 7.8 Hz, 2H), 7.84 (s, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.32 (t, J = 5.4 Hz, 1H), 6.82 (s, 2H), 2.34 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.6, 165.4, 158.0, 156.1, 153.3, 149.3, 145.7, 145.3, 143.8, 134.9, 130.0, 129.7 (2C), 127.8, 121.4 (2C), 119.7, 114.1, 112.5, 11.8, 11.0. ESI-MS *m/z* 437.2 for [M + H].

*4.1.5.15. 4-((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)oxy) -N-phenylbenzamide (**12Bg**)*. White solid; Yield: 76%; HPLC Purity: 99% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 7.81 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.25 (s, 1H), 8.05 (d, J = 9.0 Hz, 2H), 8.04 (s, 1H), 7.84 (s, 1H), 7.79 (d, J = 7.2 Hz, 2H), 7.67 (s, 1H), 7.41 (d, J = 7.8 Hz, 2H), 7.37 (t, J = 7.2 Hz, 2H), 7.10 (s, 1H), 6.54 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.7, 156.0, 145.0, 144.9, 139.1, 135.5, 132.2, 131.1, 130.6, 129.1 (2C), 128.4 (2C), 127.3, 123.5, 120.3 (2C), 120.2 (2C), 119.8, 38.4. ESI-MS *m/z* 387.2 for [M + H].

*4.1.5.16. 4-((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)oxy) -N-(2-chlorophenyl)benzamide (**12Bh**)*. White solid; Yield: 60%; HPLC Purity: 97% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 8.47 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.08 (d, J = 8.4 Hz, 2H), 8.04 (s, 1H), 7.85 (s, 1H), 7.68 (s, 1H), 7.61 (d, J = 6.6 Hz, 1H), 7.56 (t, J = 7.2 Hz, 1H), 7.42 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 7.8 Hz, 1H), 7.31 (t, J = 7.8 Hz, 1H), 6.52 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.6, 156.3, 145.0, 135.5, 135.1, 132.3, 131.1, 129.7, 129.6, 129.5 (2C), 129.3 (2C), 128.4 (2C), 127.4 (2C), 120.3 (2C), 119.8, 38.5. ESI-MS *m/z* 421.2 for [M + H].

*4.1.5.17. 4-((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)oxy)- N-(4-chlorophenyl)benzamide (**12Bi**)*. White solid; Yield: 72%; HPLC Purity: 95% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 10.58 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.05 (d, J = 9.0 Hz, 2H), 8.04 (s, 1H), 7.84 (s, 1H), 7.83 (d, J = 9.0 Hz, 2H), 7.67 (s, 1H), 7.42 (d, J = 7.8 Hz, 4H), 6.53 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.9, 156.2, 145.0, 138.1, 135.5, 131.1, 130.4, 129.7, 129.3, 128.5 (2C), 128.4 (2C), 127.3, 121.8 (2C), 120.3 (2C), 119.8, 114.9, 38.5. ESI-MS *m/z* 421.1 for [M + H].

*4.1.5.18. 4-((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)oxy)- N-(3-chlorophenyl)benzamide (**12Bj**)*. White solid; Yield: 71%; HPLC Purity: 99% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 10.05 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.06 (d, J = 9.6 Hz, 2H), 8.04 (s, 1H), 7.98 (t, J = 1.8 Hz, 1H), 7.84 (s, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.67 (s, 1H), 7.42 (d, J = 9.0 Hz, 2H), 7.39 (t, J = 8.4 Hz, 1H), 7.38 (s, 1H), 7.17 (d, J = 8.4 Hz, 1H), 6.53 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.0, 156.3, 145.0, 140.6, 135.5, 132.9, 132.3, 131.1, 130.3, 130.2, 129.3 (2C), 127.3, 123.2, 120.3 (2C), 119.8, 119.6, 118.6, 114.9, 38.5. ESI-MS *m/z* 421.2 for [M + H].

*4.1.5.19. 4-((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)oxy)- N-(6-chloropyridin-2-yl)benzamide (**12Bk**)*. Yellow solid; Yield: 37%; HPLC Purity: 95% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 9.05 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.13 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 8.12 (d, J = 8.4 Hz, 2H), 8.06 (s, 1H), 7.92 (t, J = 8.4 Hz, 1H), 7.86 (s, 1H) , 7.69 (s, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 7.2 Hz, 1H), 6.57 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.5, 156.6, 152.3, 147.9, 145.1, 144.8, 141.5, 135.5, 132.5, 131.2, 129.8 (2C), 129.3, 127.4, 120.0 (2C), 119.8, 119.4, 113.2, 38.5. ESI-MS *m/z* 422.2 for [M + H].

*4.1.5.20. 4-((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)oxy)-N-(5-chloropyridin-2-yl)benzamide (**12Bl**)*. Yellow solid; Yield: 41%; HPLC Purity: 98% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 9.73 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.99 (s, 1H), 8.45 (d, J = 2.4 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 8.4 Hz, 2H), 8.05 (s, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.85 (s, 1H) , 7.69 (s, 1H), 7.39 (d, J = 9.0 Hz, 2H), 6.53 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.4, 156.6, 150.9, 146.2, 145.1, 144.8, 137.8, 135.5, 132.4, 131.2, 129.8 (2C), 129.5, 127.4, 125.4, 120.1 (2C), 119.8, 115.7, 38.5. ESI-MS *m/z* 422.1 for [M + H].

*4.1.5.21. 4-((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)oxy)- N-(4-chloropyridin-2-yl)benzamide (**12Bm**)*. Yellow solid; Yield: 45%; HPLC Purity: 97% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 6.04 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.05 (s, 1H), 8.37 (d, J = 5.4 Hz, 1H), 8.29 (s, 1H), 8.10 (d, J = 8.4 Hz, 2H), 8.03 (s, 1H), 7.83 (s, 1H) , 7.66 (s, 1H), 7.36 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 5.4 Hz, 1H), 6.50 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.7, 156.7, 153.4, 149.3, 145.1, 144.8, 143.9, 135.6, 132.5, 131.2, 129.8

(2C), 129.3, 127.4, 120.1 (2C), 119.8, 119.7, 114.0, 38.5. ESI-MS *m/z* 422.1 for [M + H].

*4.1.5.22. 4-((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)oxy)- N-(3-chloropyridin-2-yl)benzamide (**12Bn**)*. Yellow solid; Yield: 35%; HPLC Purity: 96% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 9.08 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.09 (s, 1H), 8.40 (d, J = 5.4 Hz, 1H), 8.32 (s, 1H), 8.13 (d, J = 8.4 Hz, 2H), 8.06 (s, 1H), 7.86 (s, 1H) , 7.69 (s, 1H), 7.39 (d, J = 9.0 Hz, 2H), 7.32 (t, J = 3.0 Hz, 1H), 6.53 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.6, 156.7, 153.4, 149.3, 145.1, 144.8, 143.8, 135.5, 132.5, 131.2, 129.8 (2C), 129.3, 127.4, 120.0 (2C), 119.8, 119.7, 114.0, 38.5. ESI-MS *m/z* 422.2 for [M + H].

*4.1.6. General synthetic procedure of compounds **13A,B***

To a solution of 2-amino-3,5-dibromopyrazine (**9**, 253 mg, 1 mmol), 4-aminomethylbenzamide **8** (1.2 mmol) in dioxane (5 mL) and water (5 mL) was added KOH (276 mg, 2.0 mmol). The resulting mixture was refluxed for 48 h at 100℃ under nitrogen gas. After completion of the reaction, the reaction mixture was concentrated under vacuo, and purified by column chromatography using DCM/acetone (20:1) to yield pure **11**.

To a mixture of **11** (0.5 mmol), 3,5-dimethylisoxazole-4-boronic acid (84.6 mg, 0.6 mmol) or 1-methylpyrazole-4-boronic acid pinacol ester (124.8 mg, 0.6 mmol) in dioxane (10 mL) were added Pd(dppf)$_2$Cl$_2$·CH$_2$Cl$_2$ (40.8 mg, 0.05 mmol) and 2 M Na_2CO_3 solution (0.05 mL, 0.1 mmol). The resulting mixture was refluxed for 4 h at 100℃ under nitrogen gas. After completion of the reaction, the reaction mixture was concentrated under vacuo, and purified by column chromatography using DCM/MeOH (20:1) to yield target compounds **13A,B**.

*4.1.6.1. 4-(((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)amino) methyl)- N-(4-chlorophenyl)benzamide (**13A**)*. Yellow solid; Yield: 70%; HPLC Purity: 95% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 12.25 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.27 (s, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.81 (d, J = 7.2 Hz, 2H), 7.46 (d, J = 7.8 Hz, 2H), 7.40 (d, J = 7.2 Hz, 2H), 7.32 (s, 1H), 6.91 (s, 1H), 6.19 (s, 2H), 4.67 (d, J = 6.0 Hz, 2H), 2.35 (s, 3H), 2.15 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.3, 164.8, 158.2, 144.2, 142.8, 141.8, 138.1, 132.9, 129.7, 128.4 (2C), 127.7 (2C), 127.3, 127.1, 126.7 (2C), 121.8 (2C), 113.7, 43.7, 11.9, 11.1. ESI-MS *m/z* 449.2 for [M + H].

*4.1.6.2. 4-(((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)amino) methyl)- N-(4-chlorophenyl)benzamide (**13B**)*. Yellow solid; Yield: 65%; HPLC Purity: 97% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 10.11 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.28 (s, 1H), 7.90 (d, J = 6.6 Hz, 2H), 7.89 (s, 1H), 7.80 (d, J = 8.4 Hz, 2H), 7.69 (s, 1H), 7.55 (d, J = 8.4 Hz, 2H), 7.47 (s, 1H), 7.40 (d, J = 9.0 Hz, 2H), 6.85 (s, 1H), 5.92 (s, 2H), 4.68 (d, J = 6.0 Hz, 2H), 3.81 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.4, 144.5, 142.2, 143.2, 141.7, 138.1, 135.5, 133.0, 132.3, 129.6, 128.4 (2C), 127.6 (2C), 127.5 (2C), 127.0, 121.7 (2C), 121.4, 43.6, 38.5. ESI-MS *m/z* 434.2 for [M + H].

*4.1.7. General synthetic procedure of compounds **16A,B***

To a solution of 2-amino-3,5-dibromopyrazine (**9**, 1.0 g, 4 mmol) and 4-mercaptobenzoic acid (670 mg, 4.4 mmol) in EtOH (10 mL) was added KOH (450 mg, 8 mmol), and the resulting mixture was refluxed for 12 h at 80 ℃. After completion of the reaction, to the reaction mixture was added water and extracted with ethyl acetate, the organic layer was coupled and dried over anhydrous sodium sulfate. The mixture was concentrated under vacuo to give **14**, which was used without further purification.

To a solution of **14** (650 mg, 2 mmol) in dried DCM (10 mL), thionyl chloride (2.2 mmol) was added dropwise, and the reaction continued for 4 h at room temperature. Followed 4-chloroaniline and Et_3N were added into the above mixture, and the reaction mixture was stirred for 4 h at room temperature. After completion of the reaction, the reaction mixture was concentrated under vacuo, and the crude product was purified by silica gel chromatography using a solution of dichloromethane/acetone (20:1) to yield pure **15**.

To a mixture of **15** (0.5 mmol), 3,5-dimethylisoxazole-4-boronic acid (or 1-methylpyrazole-4-boronic acid pinacol ester, 0.6 mmol) in dioxane (10 mL) were added Pd(dppf)$_2$Cl$_2$·CH$_2$Cl$_2$ (40.8 mg, 0.05 mmol) and 2 M Na_2CO_3 solution (0.05 mL, 0.1 mmol). The resulting mixture was refluxed for 4 h at 100 ℃ under nitrogen gas. After completion of the reaction, the reaction mixture was concentrated under vacuo, and the crude product was purified by silica gel chromatography using a solution of DCM/MeOH (20:1) to yield pure compounds **16A,B**.

4.1.7.1. 4-((3-Amino-6-(3,5-dimethylisoxazol-4-yl)pyrazin-2-yl)thio)- N-(4-chlorophenyl)benzamide (16A). Brown solid; Yield: 42%; HPLC Purity: 99% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 6.73 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.44 (s, 1H), 8.00 (s, 1H), 7.98 (d, J = 8.4 Hz 2H), 7.82 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 9.0 Hz, 2H), 6.70 (s, 2H), 2.27 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.8, 164.9, 158.1, 151.5, 138.7, 138.0, 137.1, 134.4, 134.2, 133.4 (2C), 133.1, 128.6 (4C), 127.4, 121.9 (2C), 112.7, 11.8, 10.9. ESI-MS *m/z* 452.1 for [M + H].

*4.1.7.2. 4-((3-Amino-6-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)thio)-N-(4-chlorophenyl)benzamide (**16B**)*. Brown solid; Yield: 37%; HPLC Purity: 97% (MeOH:H_2O = 70:30, 0.5 mL/min, t_R = 6.24 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.33 (s, 1H), 8.28 (s,1H), 7.69 (s, 1H), 7.91 (s, 1H), 7.87 (d, J = 7.8 Hz, 2H), 7.79 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 9.0 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 6.60 (s, 2H), 3.89 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.9, 151.6, 143.8, 141.4, 138.0, 134.7, 133.9, 132.0, 130.5, 128.4 (4C), 127.1 (2C), 127.0, 121.7 (2C), 121.7, 117.9, 39.0. ESI-MS *m/z* 437.1 for [M + H].

4.2. Biology

4.2.1. Antiproliferation assays

Cells were incubated at 37 ℃ in a 5% CO_2 atmosphere. The U38, HeLa, HepG2 and LoVo cells were seeded in 96-well plates at a density of 5 × 10^3 cells/well in 150 μL of the medium with 10% FBS for 24 h. The target compounds **12**, **13**, **16** and VX-680 were dissolved and diluted to various concentrations using culture medium, and then added them to the well and incubated at 37 ℃ for 72 h, then the media was aspirated, and 10 μL of 5 mg/mL MTT solution diluted in serum-free media was added to each well. After 4 h, the supernatant was discarded and 100 μL of DMSO was added to each well. The mixture was shaken on an oscillator and measured at 490 nm using universal microplate reader (Infinite M200 Pro, Tecan Inc.). IC_{50} values were determined from a log plot of percent of control versus concentration. All samples were tested in triplicate.

4.2.2. Kinase-Glo luminescent kinase assay

The synthetic compounds and reference compound were diluted to six concentrations in the PBS and then added 5 μL to the 50 μL reaction mixture (40 mM Tris, pH = 7.4, 10 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml BSA, 10 μM ATP, 0.2 μg/ml Kinase and 100 μM Kemptide acetate salt), and then the kinase reactions were incubated for 30 min at 37 ℃. Then we used Kinase-Glo luminescence kinase assay kit tested luminescent signal of the reaction mixture and calculated the IC_{50} using Prism Graph Pad software.

4.2.3. Western blot analysis

HeLa cells were treated with **12Aj** (0, 1, 5 and 10 μM) or VX-680 (10 μM) for 24 h. Followed the cells were washed and lysed in lysis buffer, total proteins were achieved by centrifuging and the

concentrations were determined by using BCA protein assay kit (Beyotime, Jiangsu, China). For western blot analysis, equal amounts of proteins (20–30 μg) were separated on 12% SDS-PAGE gels and transferred to polyvinylidine difluoride (PVDF) membranes (Millipore Corporation, USA). The blot was blocked in blocking buffer (5% non-fat dry milk in TBST) for 2 h at room temperature, and then incubated with dilute solution (1:500–1:1000) of the antibody against Aurora A and Aurora B (Abcam), phospho-Aurora A (Thr288) and phospho-Aurora B (Thr232) (Cell Signaling Technology), cyclinB1 and cdc2 (BioLegend), and β-actin (ZSGB-BIO) in blocking buffer overnight at 4℃, respectively. The blot was then incubated with appropriate secondary antibody (1:5000–1:10,000 dilution), β-Actin was used as a loading control. The protein bands were visualized using the Gel Imaging System (ChemDoc-It610, UVP, USA).

4.2.4. Analysis of cell cycle by flow cytometry

For cell cycle analysis, we used the cervical carcinoma HeLa cell line grown in RPMI-1640 supplemented with 10% (v/v) heatinactivated fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, and 24 μg/mL gentamicin and incubated at 37℃ in a humidified atmosphere of 5% CO_2 and 95% air. Untreated and drug treated cells ((3–5) × 10^5) were harvested and fixed overnight in 70% ethanol at 4 ˚C. Cells were then washed three times with PBS, incubated for 1 h with 1 mg/mL RNase A and 20 μg/mL propidium iodide at room temperature, and analyzed with a flow cytometer (COULTER EPICS XL, USA) as described in previous publication.[21]

4.3. Molecular docking study

The docking simulation was performed using the Glide module of Schrödinger. Before docking, the ligand **12Aj** was prepared firstly using the LigPrep module in Schrödinger. The X-ray crystal structures of Aurora (http://www.rscb.org./pdb, Aurora A PDB code: 3H0Z and Aurora B PDB code: 4C2V) were derived from the PDB Database and minimized using the Protein Preparation Wizard module in the Schrödinger Suite.[33] The crystal waters were removed and polar hydrogen was added to the two proteins. Then, a grid was generated at the active site of the prepared protein structure using the Grid Generation module of Schrödinger. The Glide docking module of Schrödinger was used to investigate the interactions between the synthesized compounds and Aurora kinases. Each docking process was performed in 250,000 energy evaluation with 10 conformations kept and the most favorable pose of each compound was displayed. And all the figures displaying the docking results were obtained using the scientific software Pymol.

4.4. Bioinformatics prediction

The free SwissADME web (http://www.swissadme.ch) tool available from the Swiss Institute of Bioinformatics (SIB) was used for the calculation of the physicochemical descriptors as well as to predict the ADME parameters and medicinal chemistry of **12Aj** and VX-680.[32]

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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