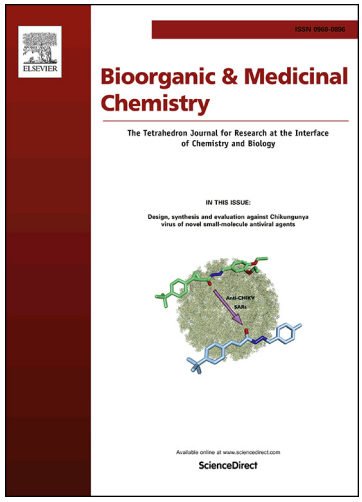
Accepted Manuscript



Synthesis and biological evaluation of pyrimidine derivatives as novel human Pin1 inhibitors

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Synthesis and biological evaluation of pyrimidine derivatives as novel human Pin1 inhibitors

Guonan Cui[a,#], Jing Jin[b,#], Hualong Chen[a], Ran Cao[a], Xiaoguang Chen[b,*], Bailing Xu[a,*]

[a]*Beijing Key Laboratory of Active Substance Discovery and Druggability Evaluation, Institute of Materia Medica, Chinese Academy of Medical Sciences &Peking Union Medical College, Beijing, 100050, China*

[b]*State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences &Peking Union Medical College, Beijing, 100050, China*

Abstract

Pin1 (Protein interacting with NIMA1) is a *cis-trans* isomerase and promotes the amide bond rotation of phosphoSer/Thr-Pro motifs in its substrates. Inhibition of Pin1 might be a novel strategy for developing anticancer agents. Herein, a series of pyrimidine derivatives were synthesized and their Pin1 inhibitory activities were evaluated. Among them, four compounds (**2a, 2f, 2h** and **2l**) displayed potent inhibitory activities against Pin1 with IC_{50} values lower than 3 μM. This series of pyrimidine-based inhibitors presented time-dependent inhibition against Pin1. The structure-activity relationships on the 2-, 4- and 5-positions of the pyrimidine ring were analyzed in details, which would facilitate further exploration of new Pin1 inhibitors.

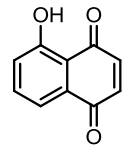
Keywords: Pyrimidine; Pin1; Pin1 inhibitor; PPIase; Anti-cancer agents

1. Introduction

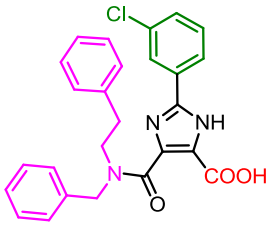
Pin1 (Peptidyl-prolyl *cis-trans* isomerase NIMA-interacting 1) is a member of the Peptidyl-prolyl *cis-trans* isomerase (PPIase) family[1], which is the only known mammalian isomerase that specifically catalyzes the interconversion of *cis-trans* conformations of the amide bond of phosphoSer/Thr-Pro motifs in Pin1 substrates[2]. It has been demonstrated that Pin1 can regulate diverse signaling processes in cell by functioning as a molecular switch and participate in cell cycle progression, apoptosis and immune responses.[3-5] In particular, many research suggested that Pin1 played a critical role in oncogenesis by upregulation of oncogenes and downregulation of tumor suppressors.[6] Therefore, it was speculated that inhibiting Pin1 might be an

# The first two authors contributed equally.
* Corresponding author. Bailing Xu, Tel: +86 10 63166764; Xiaoguang Chen, Tel: +86 10 63166302
E-mail address: xubl@imm.ac.cn (B. Xu); chxg@imm.ac.cn (X. Chen).

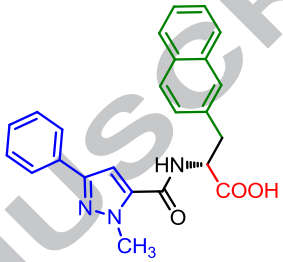
effective way to conquer the aggressive cancers by simultaneously impacting on multiple oncogenic signaling pathways. Furthermore, Pin1 over-expression was observed in a variety of human cancer cells, and its over-expression closely correlated with the occurrence and development of tumors and clinical outcomes.[7,8] Noticeably, cells from Pin1-deficient mice are resistant to the induction of breast cancer by over-expression of oncogene Ras or ErbB2[9] and the depletion of Pin1 on various human cancer cell lines cause mitotic arrest and apoptosis[10]. Hence, Pin1 is a promising target for discovering anti-cancer agents with unique mechanism.



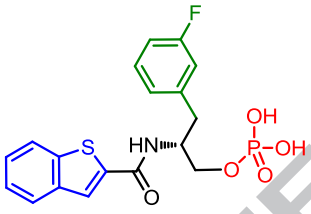
A. Juglone
IC_{50} = 5 μM



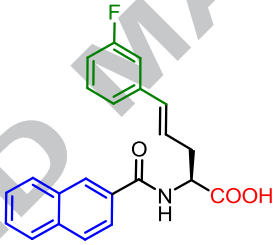
B. IC_{50} = 0.83 μM
GI_{50} = 13 μM (PC3 cells)



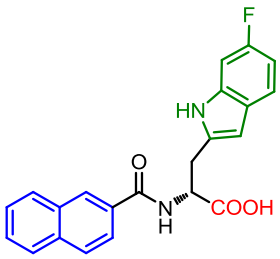
C. IC_{50} = 3.9 μM
GI_{50} = 4.7 μM (PC3 cells)



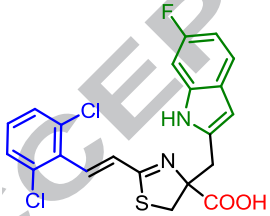
D. K_i = 0.006 μM



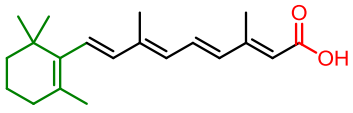
E. K_i = 0.89 μM



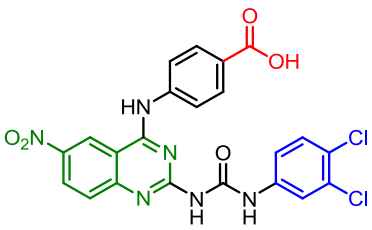
F. K_i = 0.138 μM



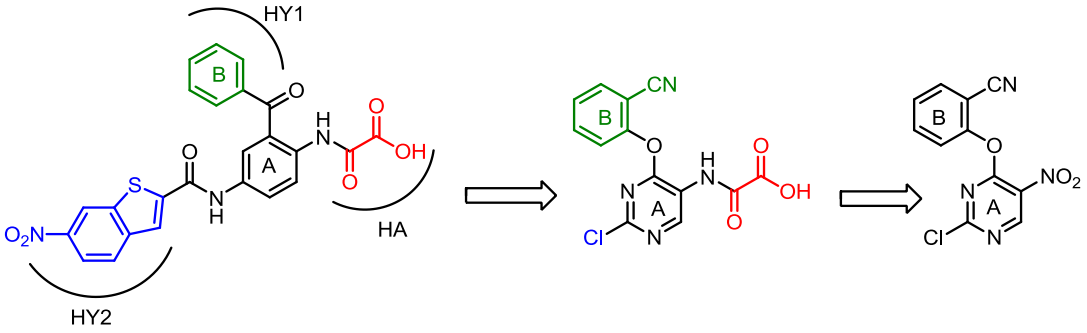
G. K_i = 0.58 μM



H. ATRA IC_{50} = 0.82 μM



I. IC_{50} = 2.9 μM
IC_{50} = 1.9 μM (HT29 cells)



J. IC_{50}=5.99 μM

K. IC_{50}=26.67 μM

L. IC_{50}=9.05 μM

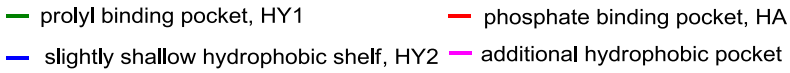


Figure 1. Chemical structures of several reported Pin1 inhibitors and schematic pictures of their binding modes

Research efforts for exploring therapeutically useful Pin1 inhibitors have been made for nearly twenty years since Juglone (A) was identified as an irreversible small molecule inhibitor of Pin1 in 1998.[11] As a result, a number of structurally distinct Pin1 inhibitors have been disclosed (Fig. 1 **A**-**K**).[11-24] Juglone (**A**), discovered by screening a natural product library, is the first irreversible Pin1 inhibitor, which inactivates Pin1 enzymatic activity by covalent binding on Cys113 in the active site.[11,12] However, the detailed binding information of Juglone within the catalytic site has not been resolved. Fragment-based design strategy was exploited by Vernalis and identified phenyl-imidazole and naphthyl-alanine based inhibitors of Pin1, exemplified by compounds **B** and **C**.[13,14] By taking structure-based design approach, Pfizer achieved several potent Pin1 inhibitors, such as compounds **D**, **E**, **F** and **G**.[15-17] Compound **D** is the most potent small molecule inhibitor of Pin1 reported so far. However, due to the poor permeability conferred by the phosphate group, compound **D** failed to show cellular effects.[15] Although tremendous works have been devoted, there are no desirable inhibitors with potent activity in cells reported, due to either poor physicochemical properties or low potency in enzymatic activity. Thus, it is still an enormous challenge to develop highly potent Pin1 inhibitors with drug-like properties. Recently, all-trans retinoic acid (ATRA), a therapeutic agent for acute promyelocytic leukemia, was found to be a potent Pin1 inhibitor, which could inhibit and degrade active Pin1 in cancer cells, suggesting that it was a viable way for using Pin1 inhibitors as anti-cancer agents.[18]

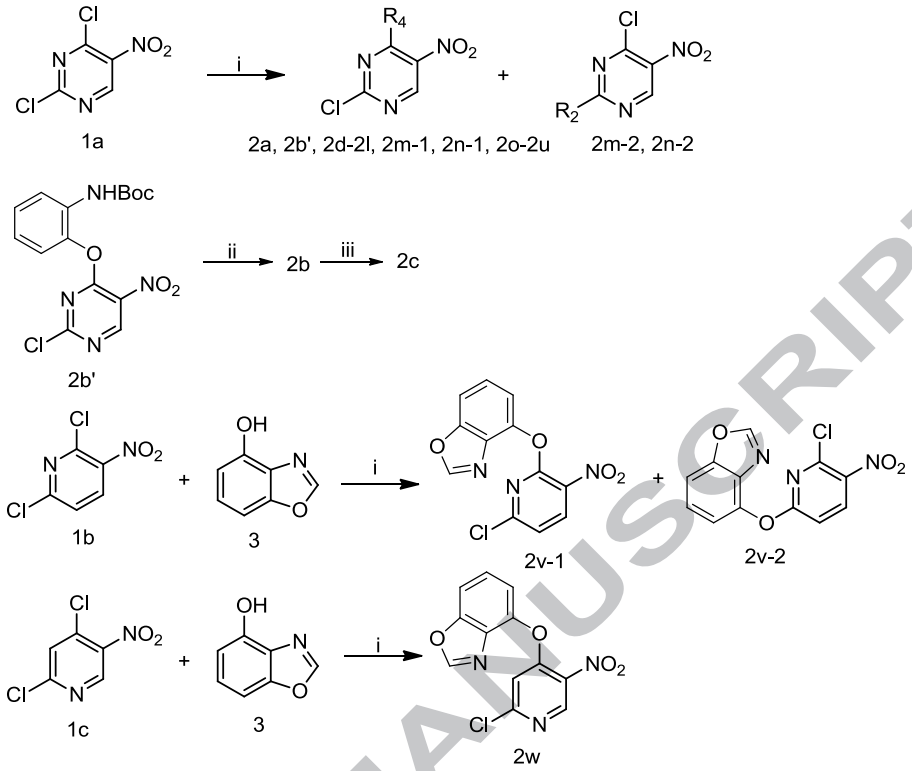
Pin1 poses challenges for drug discovery because of its unique substrate binding site, consisting of a cluster of basic amino acids such as Lys63, Arg68 and Arg69 residues bound to the negatively-charged phosphate ion and less exploitable hydrophobic pocket.[13-19] Generally, the known Pin1 inhibitors occupied the following subpockets, including the prolyl pocket formed by His59, His157, Met130 and Phe134 residues, the slightly shallow hydrophobic shelf lined with His59, Ala118 and Leu22 residues, and the unique phosphate binding pocket. Interestingly, phenyl-imidazole-based Pin1 inhibitors such as compound **B** had a different orientation in terms of the bulky aromatic group, which contacted a hydrophobic region lined with the side chain of Arg68.[13] It has been suggested that it might be an alternative strategy to develop desired Pin1 inhibitors by taking advantage of all of the above mentioned binding regions.

In our previous work, quinazoline-based (**I**, Fig. 1) and benzophenone-based (**J**, Fig. 1) Pin1 inhibitors were discovered by screening our in-house library and further structure modifications.[20,21] Based on the benzophenone-derived human Pin1 inhibitors, a series of novel diarylether derivatives containing an oxalic acid fragment were synthesized and compound **K** (Fig. 1) was identified as a hit with an IC_{50} of 26.67 μM.[22] However, to our surprise, it was found by chance that a key intermediate

(compound **L**, IC_{50} = 9.05 μM) for synthesis of compound **K** showed micromolar potency in the protease-coupled enzyme assay. Due to its low molecular weight and readily accessible structure, we conducted an extensive investigation on structure activity relationships (SAR) on compound **L**. And we found that the pyrimidine derivatives in this work possessed inhibitory effects on Pin1 with covalent binding features. Herein, we reported the chemical synthesis of the designed pyrimidine derivatives and the SAR results of our structure modifications.

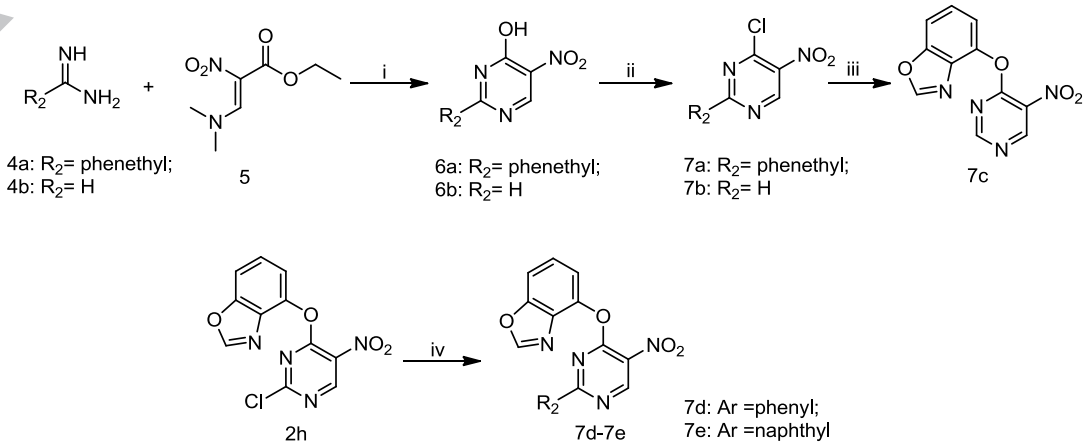
2. Chemistry

The synthesis of a variety of pyrimidine derivatives was presented in Schemes **1-5**, and the chemical structures of target compounds were presented in Tables **1-3**. The aromatic nucleophilic substitution reactions were performed using 2,4-dichloro-5-nitropyrimidine (**1a**) and an array of phenols, alcohols or amines as reactants (Scheme 1). In general, only 4-substituted pyrimidine derivatives were obtained (yields 30.3%~95.9%), and no corresponding 2-substituted regio-isomers were isolated. However, when benzyl alcohol or 2-phenylethanol was reacted with compound **1a,** two regio-isomers generated respectively. Interestingly, in contrast to other reactions, 2-substituted isomers **2m-2** (yield 22.3%) and **2n-2** (yield 35.0%) were obtained as the major products, and 4-substituted isomers **2m-1** (yield 10.9%) and **2n-1** (yield 13.0%) were isolated as minor products in these two cases. In order to confirm the chemical structures of regio-isomers, single crystal X-ray analysis was conducted on some representative compounds. Among the obtained single-isomers, the crystal structures of compounds **2a**, **2d** and **2t** were obtained and shown in Supplementary data (1.1-1.3), which proved to be 4-substituted isomers. As to the chemical structures of isomers **2m-1** and **2m-2**, the structure of compound **2m-1** was confirmed with X-ray diffraction method, and the crystal structure was presented in Supplementary data (1.4). Accordingly, by comparing the ^{1}H-NMR data of compounds **2n-1** and **2n-2** with those of **2m-1** and **2m-2**, we confirmed the structures of these two isomers. The signals of C6-H on pyrimidine ring in 4-substituted isomers **2m-1** and **2n-1** appeared at δ 9.04 and δ 9.02 ppm; while the signals of C6-H in 2-substituted isomers **2m-2** and **2n-2** resonated at δ 9.17 and 9.14 ppm. Compound **2b** was prepared by removing the Boc group of compound **2b'** with TFA. Compound **2b** was further converted into compound **2c** by reaction with methylsulfonyl chloride. The pyridine derivatives **2v-1** and **2w** were constructed through nucleophilic aromatic substitution reaction between compound **1b** or **1c** and 4-hydroxybenzooxazole[25] in 36% and 88% yields, respectively. In fact, 2,6-dichloro-3-nitropyridine **1b** was reacted with 4-hydroxyl benzo[*d*]oxazole giving two isomers under various conditions. The chemical structure of the desired compound **2v-1** was confirmed by X-ray crystallographic analysis (shown in Supplementary data, 1.5). The structure of compound **2w** was confirmed to be 4-substituted isomer according to the literature.[26]



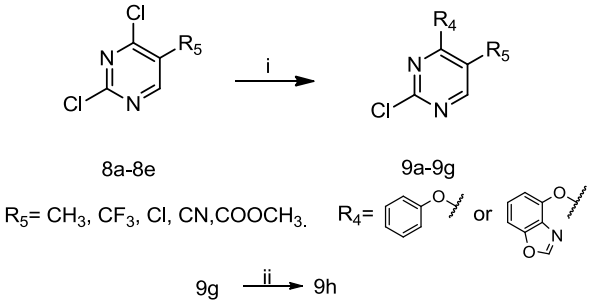
Scheme 1. Reagents and conditions: (i) K_2CO_3 or DIEA, acetone/DMF or DCM or THF, 10.9%~95.9%; (ii) TFA, DCM, 75.2%; (iii) methylsulfonyl chloride, TEA, DCM, 0 °C, 25.5%.

The synthesis of 2-substituted or 2,4-disubstituted pyrimidines **7a-7e** was outlined in Scheme 2. *(Z)*-Ethyl 3-(dimethylamino)-2-nitroacrylate **5** was synthesized by mixing ethyl 2-nitroacetate and 1,1-dimethoxy-*N*,*N*-dimethylmethanamine.[27] Compound **5** was condensed with amidine reagents **4a** or **4b** to construct pyrimidine derivatives **6a** and **6b**, followed by chlorination with $POCl_3$ affording compounds **7a** and **7b**. Compound **7b** was converted into **7c** by substitution reaction with 4-hydroxy benzo[*d*]oxazole (**3**). Compounds **7d** and **7e** could be prepared by Suzuki coupling with compound **2h** and phenyl or naphthyl boric acid, although the yields were somewhat low.

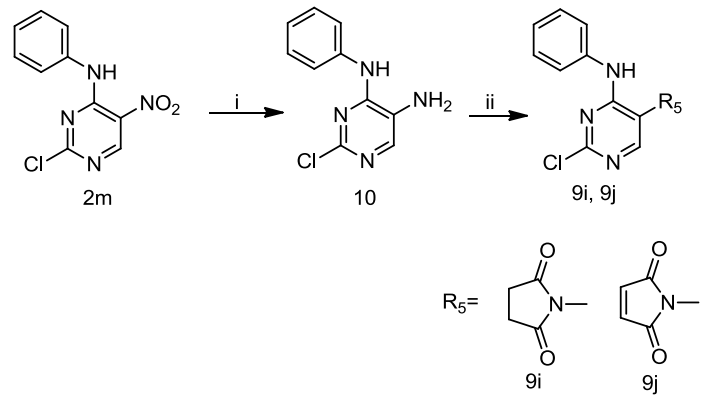


Scheme 2. Reagents and conditions: (i) sodium ethoxide, ethanol, 40 °C, 48.3%~55.8%; (ii) $POCl_3$, DIEA, 80 °C, 14.4%~36.2%; (iii) **3**, DIEA, DCM, 0 °C, 23.8%; (iv) phenyl or 2-naphthyl boric acid, $Pd_2(dppf)_3$, Na_2CO_3, CsF, toluene, DMF, 16.7%~20.4%.

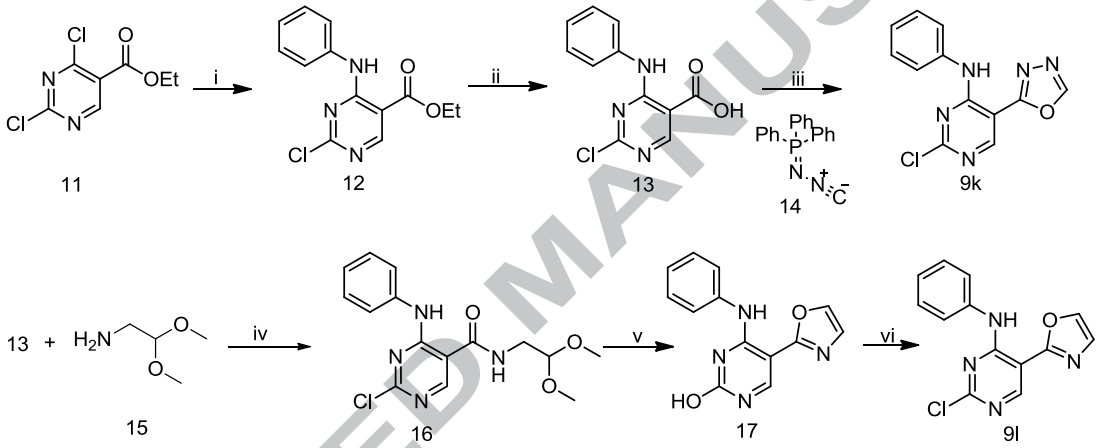
In order to investigate the effects of substituents on the 5-position of pyrimidine ring on the inhibitory activity, a number of 5-substituted pyrimidine derivatives were designed and synthesized, while phenoxyl, benzo[*d*]oxazole oxyl, phenyl amino fragments were chosen as R_4 group according to obtained SAR results. As shown in Scheme 3, compounds **9a-9g** were readily prepared via nucleophilic aromatic substitution reaction, and compound **9h** was obtained by hydrolysis of compound **9g**. Compounds **9i** and **9j** were prepared via condensation reaction between aromatic amine **10** and succinic anhydride or maleic anhydride in 21% and 27% yield, respectively (Scheme 4). According to the synthetic route shown in Scheme 5, the oxadiazole or oxazole substituted derivatives (compounds **9k** and **9l**) were prepared in 3 or 5 steps. The key immediate **13** was synthesized via substitution and hydrolysis reaction starting from ethyl 2,4-dichloropyrimidine-5-carboxylate **11**. Then compound **9k** was synthesized via aza-Witting reaction by mixing compound **13** with (isocyanoimino)triphenylphosphorane **14**[28] for 24 h. The acid **13** was condensed with 2,2-dimethoxyethanamine **15**, followed by cyclization with Eaton's reagent to give compound **17**. Of note, the chlorine atom on the 2-position was converted into a hydroxyl group in the presence of Eaton's reagent[29]. Finally, compound **17** was transformed into the desired compound **9l** by using $POCl_3$ as a chlorinating reagent.



Scheme 3. Reagents and conditions: (i) K_2CO_3, acetone, DMF, 87.7-99%; (ii) NaOH, H_2O, THF, 20.9%.



Scheme 4. Reagents and conditions: (i) Fe, NH_4Cl, ethyl acetate, EtOH, H_2O, 60 °C, 68.9%; (ii) succinic anhydride or maleic anhydride, DMAP, AcOH, 20.6% ~27%.



Scheme 5. Reagents and conditions: (i) DIEA, DCM, 0 °C, 79.6%; (ii) NaOH, THF, H_2O, 96.6%; (iii) DCM, 60.3%; (iv) SO_2Cl_2, TEA, DCM, r.t., 87.0%; (v) Eaton's reagent, argon; (vi) $POCl_3$, DIEA, two-step total yield 12.3%.

3. Biological results and discussion

All the target compounds were screened against Pin1 by a protease-coupled enzyme assay with Suc-Ala-Glu-Pro-Phe-pNA as the substrate.[30,31] The corresponding results are expressed as IC_{50} values and presented in Tables 1-3.

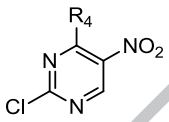
Since compound **L** was identified to be a potent Pin1 inhibitor with IC_{50} value of 9.05 μM in our group, we immediately set to work on SAR studies around the pyrimidine scaffold. Initially, the SAR on 4-substituents of pyrimidine ring was investigated. When phenoxyl or various substituted phenoxyl groups were introduced at the 4-position, all the resulted compounds (**2a-2l**) showed inhibitory activities with IC_{50} values ranging from 1.68 μM to 34.04 μM. Among them, compounds **2a**, **2f**, **2h** and **2l** displayed more potent activities than other diary ether, they had IC_{50} values at low micromolar level (1.68-2.52 μM). It was noticed that compound **2h** was more active than compound **2i** and **2j**, and compound **2l** was more active than compound **2k**, suggesting that heteroaromatic groups were preferred as R_4 group. In addition, when benzyloxy, phenethoxy or phenyl amino moiety was used as R_4 group, the obtained

compounds **2m-1**, **2n-1** and **2q** had inhibitory activities as well. Interestingly, compound **2r** with a phenethylamino group as the 4-substituent showed no inhibition against Pin1, the corresponding phenethoxy substituted analog (compound **2n-1**) had noticeable activity though. Furthermore, the placement of a cycloalkyloxy or cycloalkylamino substituent led to loss of inhibition. Taken together, the results suggested that incorporation of an aromatic group, particularly a heteroaromatic group on the 4-position of pyrimidine ring was beneficial to the potency, presumably due to the positive contributions of the aromatic hydrophobic interactions occurred.

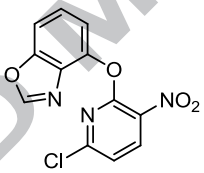
With an aim to probe the contributions of the nitrogen atoms on the pyrimidine ring, two pyridine derivatives (**2v-1** and **2w**) were prepared. None of them produced inhibitory effects on Pin1. This result suggested that both nitrogen atoms exerted strong impacts on the binding affinity. We assumed that the nitrogen atoms probably formed key hydrogen bonds within the pocket, or the electrophilic properties of the pyrimidine ring played a role in the binding. Therefore, we retained the pyrimidine as a scaffold to further vary the substituents on its 2- and 5-positions.

Table 1

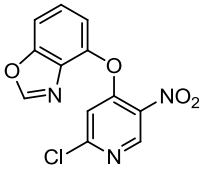
The chemical structures and inhibitory activities against hPin1 of 4-substituted pyrimidine derivatives and substituted pyridine derivatives



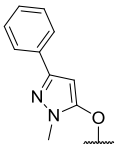
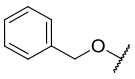
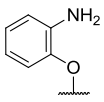
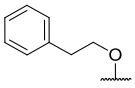
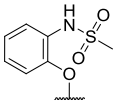
2a-2u

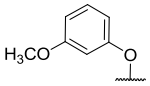
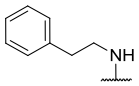
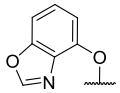
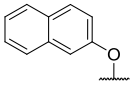
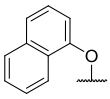
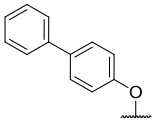


2v-1



2w

Compd.	R_4	IC_{50} (μM)[a]	Compd.	R_4	IC_{50} (μM)
L		9.05±0.06	2l		2.17±0.21
2a		2.24±0.75	2m-1		8.33±2.13
2b		33.5±2.81	2n-1		4.08±0.09
2c		5.20±0.03	2o		>100
2d		4.03±0.83	2p		>100

2e		5.04±0.09	2q		6.66±0.01
2f		2.52±0.27	2r		>100
2g		3.48±0.50	2s		>100
2h		1.68±0.80	2t		>100
2i[b]		16.55±1.54	2u		>100
2j[b]		22.6±2.38	2v-1	-	>100
2k[b]		34.04±2.88	2w	-	>100

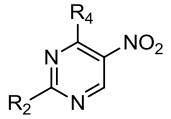
[a] The measured IC_{50} for compound **A** was 5.90 μM; the measured IC_{50} for compound **D** was 0.05 μM; the measured IC_{50} for compound **F** was 2.96 μM; SD, standard deviation of two independent assays.

[b] Compounds **2i**, **2j** and **2k** were obtained in our previous work.[22]

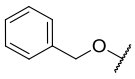
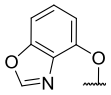
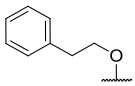
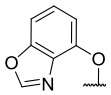
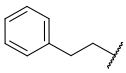
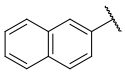
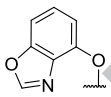
As shown in Table 2, in comparison with compounds **2m-1** and **2n-1**, the corresponding 2-substituted regio-isomers **2m-2** (IC_{50} 7.28 μM) and **2n-2** (IC_{50} 7.27 μM) had micromolar level inhibitory activity as well. Compound **7a** (IC_{50} 13.87 μM) bearing a phenethyl fragment at 2-position showed somewhat lower activity, compared with compound **2m-2**. However, replacement of the chloro atom in compound **2h** (IC_{50} 1.68 μM), the most potent Pin1 inhibitor of this work, with a hydrogen atom, benzene ring or naphthyl group (compounds **7c-7e**) resulted in complete loss of activity. These data suggested that the chloro atom on the pyrimidine core had an important role in the Pin1 inhibitory activity.

Table 2

The chemical structures and inhibitory activities against hPin1 of 2-substituted pyrimidine and 2,4-disubstituted pyrimidine derivatives



Com	R_2	R_4	IC_{50} (μM) [a]	Com	R_2	R_4	IC_{50} (μM) [a]

pd.				pd.			
2m-2		Cl	7.28±0.42	7c	H		>100
2n-2		Cl	7.27±1.14	7d			>100
7a		Cl	13.87±1.51	7e			>100

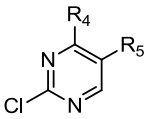
[a] The measured IC_{50} for compound **A** was 5.90 μM; the measured IC_{50} for compound **D** was 0.05 μM; the measured IC_{50} for compound **F** was 2.96 μM; SD, standard deviation of two independent assays.

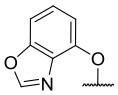
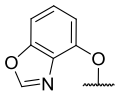
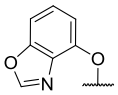
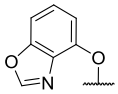
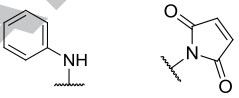
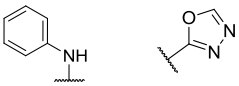
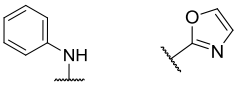
We further explored the SARs on 5-substituents of the 2-chloropyrimidine scaffold by choosing phenoxyl, benzo-oxazole oxyl or phenyl amino as R_4 group. A number of substituents were tentatively selected as R_5 with various electronic parameters. As shown in Table 3, grafting a CH_3 (**9a**, **9b**), CF_3 (**9c**, **9d**), Cl (**9e**) or $COOCH_3$ (**9g**) on the 5-position rendered the inhibitory activity totally lost. While a CN or COOH was presented on the 5-position, the corresponding compounds **9f** (IC_{50} 4.36 μM) and **9h** (IC_{50} 18.9 μM) produced Pin1 inhibitory activity. In particular, compound **9f** had comparable activity with nitro substituted counterpart **2a**. By examining the Hansch's π constant, Hammett's σ constant, and the molar refraction (MR) parameters of these substituents[32], we found out that groups NO_2, CN and COOH had similar properties in terms of hydrophobicity, electronic effects and the molecular volume. They all had less lipophilicity, strong electron withdrawing ability in comparison with CH_3 and Cl groups. Noticeably, the inhibitory activity enhanced along with the increase in electron-withdrawing capability of the substituents (IC_{50} value: **2a** < **9f**, **2h** < **9h**). Interestingly, although the CF_3 and $COOCH_3$ groups had a somewhat strong electron-withdrawing ability, the more bulky size of these groups might cause the loss of activity. These results indicated that a limited space available around 5-position of the pyrimidine ring.

With regard to compounds **9i-9l** bearing a five-membered heterocyclic ring on 5-position, it was delighted that two of them (compounds **9j** and **9l**) exhibited inhibitory activity, especially compound **9l** with an oxazole moiety on 5-position produced a comparable potency with compound **2q**. The oxazole fragment is a good substitute for the NO_2 group with respect to improving the structural novelty and drug-like properties of this class of Pin1 inhibitors. Noticeably, compound **9k** with an oxadiazole as R_5 group gave no inhibition on Pin1, although the oxadiazole is an isosteric molecule of oxazole and it has even stronger electron-withdrawing ability. Together, these results suggested that a strong electron-withdrawing group on 5-position would be beneficial for the potency, and the molecular size and shape of 5-substituents were restricted. The cyano group or oxazole moiety would be the alternative substitute for NO_2 group.

Table 3

The chemical structures and inhibitory activities against hPin1 of compounds **9a-9l**



Compd.	R_4	R_5	IC_{50} (μM) [a]	Compd.	R_4	R_5	IC_{50} (μM) [a]
9a		CH_3	>100	9g		$COOCH_3$	>100
9b		CH_3	>100	9h		COOH	18.9±0.25
9c		CF_3	>100	9i			>100
9d		CF_3	>100	9j			20.2±0.11
9e		Cl	>100	9k			>100
9f		CN	4.36±0.97	9l			9.87±0.95

[a] The measured IC_{50} for compound **A** was 5.90 μM; the measured IC_{50} for compound **D** was 0.05 μM; the measured IC_{50} for compound **F** was 2.96 μM; SD, standard deviation of two independent assays.

The electrophilic property of 2- or 4-chloro-pyrimidine was well recognized, and the obtained SAR results gave us a hint that this series of Pin1 inhibitors possibly interacted with Pin1 through a covalent binding. Thus, the intrinsic time dependent nature of a covalent binding was explored tentatively by choosing four representative inhibitors. As shown in Figure 2, all of the four compounds exhibited time dependent inhibitory effects at different concentrations. At the same concentration of the inhibitor, the inhibitory effects became stronger with the increasing incubation time. The results suggested that these inhibitors might covalent bind with Pin1. The binding features of pyrimidine derivatives deserved to be further investigated, and that would be informative for exploring covalent inhibitors of Pin1.

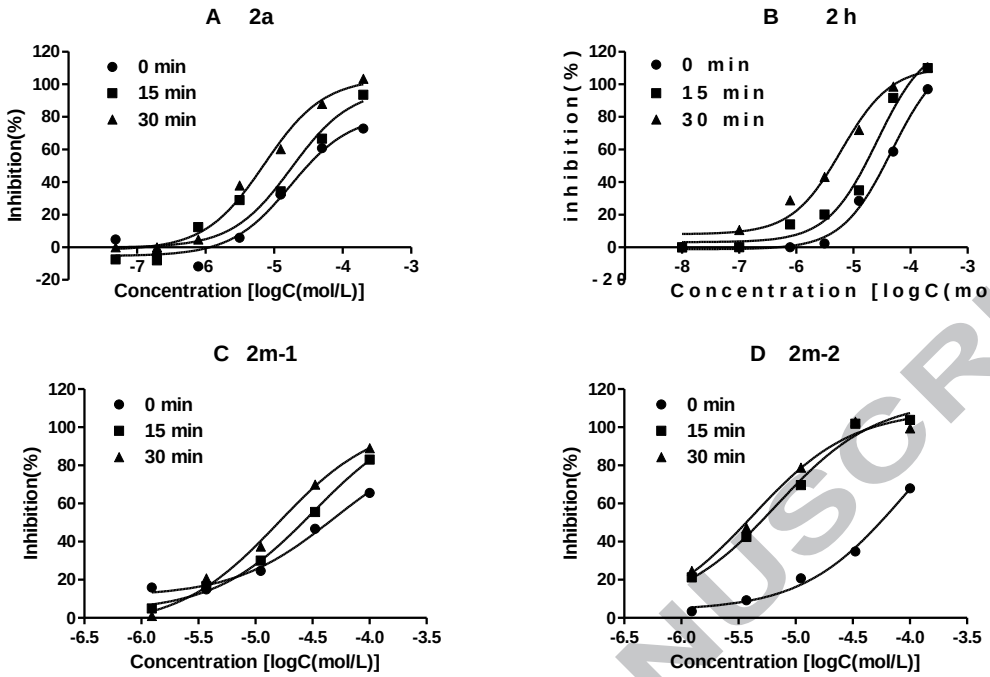


Figure 2. Time-dependent Pin1 PPIase assay of compound **2a** (**A**), **2h** (**B**), **2m-1** (**C**) and **2m-2** (**D**) (●) Pin1 protein and inhibitors (varying concentrations in DMSO) were mixed and tested immediately; (■) Pin1 protein and inhibitors (varying concentrations in DMSO) were incubated for 15 min; (▲) Pin1 protein and inhibitors (varying concentrations in DMSO) were incubated for 30 min.

4. Conclusion

In conclusion, a series of pyrimidine derivatives were disclosed as novel Pin1 inhibitors with IC_{50} values at low micromolar level. The most potent compound **2h** was identified with an IC_{50} value of 1.68 μM. The SAR results and the time-dependent inhibition of this series of pyrimidine derivatives indicated that they possibly inactivate Pin1 by forming a covalent bond within the binding pocket. Except for Juglone, there are no covalent inhibitors against Pin1 reported, although many non-covalent inhibitors have presented. The covalent inhibitor of Pin1 deserved to be explored since it might be an alternative means to address this problematic target.

5. Experimental

5.1. Chemistry

5.1.1. General

^{1}H NMR spectra were recorded with a Varian Mercury 300, 400 or 500 spectrometer using tetramethylsilane (TMS) as the internal standard in Acetone-d_6, DMSO-d_6 or $CDCl_3$. High resolution mass spectra (HRMS) were recorded on an Agilent Technologies LC/MSD TOF spectrometer. Melting points were measured on

a Yanaco micro melting point apparatus. All chemicals and solvents used were of reagent grade without further purification or dried before used. All the reactions were monitored by thin-layer chromatography (TLC) under a UV lamp at 254 nm. Column chromatography separations were performed with silica gel (200–300 mesh).

5.1.2. Chemical synthesis of pyrimidine derivatives

*5.1.2.1 2-Chloro-5-nitro-4-phenoxypyrimidine (**2a**)*

To a stirred solution of 2,4-dichloro-5-nitropyrimidine (**1a**) (970 mg, 5.0 mmol) in DCM (20 mL) at 0 °C was added DIEA (0.87 mL, 5.0 mmol) and phenol (470 mg, 5.0 mmol) dropwise. After stirring at 0 °C for 0.5 h, the reaction mixture was washed with saturated brine (20 mL), water (20 mL×2), and then dried over anhydrous magnesium sulfate. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 40:1) to afford the title compound **2a** as a white solid (1.12 g, 88.8%); mp 119-120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.16 (s, 1H), 7.48 (t, *J* = 8.0 Hz, 2H), 7.36 (t, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 162.97, 161.86, 157.21, 150.92, 129.93, 126.99, 121.21; HRMS (ESI): *m/z*, Calcd. for $C_{10}H_7O_3N_3Cl$ $[M+H]^+$: 252.0171, Found 252.0163.

*5.1.2.2 N_1-(2-chloro-5-nitropyrimidin-4-yl)benzene-1,2-diamine (**2b**)*

To the stirred solution of compound **2b'** (470 mg, 1.28 mmol) in DCM (20 mL) was added TFA (1.96 mL, 25.6 mmol) dropwise, the reaction mixture was then allowed to stir at room temperature overnight. The solvent was washed with ammonium hydroxide and then evaporated under reduced pressure to afford the title compound **2b** as a yellow solid (257 mg, 75.2%); mp 163-166 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.65 (s, 1H), 10.41 (s, 1H), 9.19 (s, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 6.98 (d, *J* = 7.2 Hz, 1H), 6.91 (t, *J* = 7.8 Hz, 1H); HRMS (ESI): *m/z*, Calcd. for $C_{10}H_8O_3N_4Cl$ $[M+H]^+$: 267.0279, Found 267.0274.

*5.1.2.3 N-(2-((2-chloro-5-nitropyrimidin-4-yl)oxy)phenyl)methanesulfonamide (**2c**)*

To a stirred solution of compound **2b** (100 mg, 0.38mmol) in DCM (10 mL) was added TEA (0.21 mL, 1.5 mmol) and methanesulfonyl chloride (0.032 mL, 0.40 mmol) dropwise at 0 °C, after 5 min the reaction mixture was quenched with water (2 mL). The organic layer was separated and washed with water (10 mL×2), then dried over anhydrous magnesium sulfate. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10:1~5:1) to afford the title compound **2c** as a yellow solid (33 mg, 25.5%); mp 204-208 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm) 10.54 (s, 1H), 9.25 (s, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.57-7.42 (m, 3H), 3.45 (s, 3H); HRMS (ESI): *m/z*, Calcd. for $C_{11}H_{10}O_5N_4ClS$ $[M+H]^+$: 345.0055, Found 345.0045.

*5.1.2.4 2-chloro-4-(2-methoxyphenoxy)-5-nitropyrimidine (**2d**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (217 mg, 1.05 mmol), K_2CO_3 (262 mg, 1.90

mmol) and 2-methoxyphenol (118 mg, 0.95 mmol) in DMF (1 mL) and acetone (9 mL) was stirred at -20 °C for 5 h to give the title compound **2d** as a white solid (111 mg, 41.4%); mp 152-153 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm) 9.32 (s, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.29 (d, J = 7.6 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, Acetone-d_6) δ (ppm) 162.72, 162.66, 158.52, 151.59, 140.95, 132.62, 128.69, 122.90, 121.75, 114.00, 56.30; HRMS (ESI): *m/z*, Calcd. for $C_{11}H_9O_4N_3Cl$ $[M+H]^+$: 282.0276, Found 282.0266.

*5.1.2.5 2-Chloro-4-(3-methoxyphenoxy)-5-nitropyrimidine (**2e**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (406 mg, 2.1 mmol), DIEA (0.66 mL, 4.0 mmol) and 3-methoxyphenol (248 mg, 2.0 mmol) in THF (10 mL) was stirred at -40 °C for 3 h to give the title compound **2e** as a white solid (425 mg, 75.4%); mp 89-92 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm) 9.31 (s, 1H), 7.43 (t, J = 7.6 Hz, 1H), 6.96-6.90 (m, 3H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, Acetone-d_6) δ (ppm) 162.95, 162.62, 161.81, 158.42, 153.21, 131.11, 114.11, 113.20, 108.23, 55.82; HRMS (ESI): *m/z*, Calcd. for $C_{11}H_9O_4N_3Cl$ $[M+H]^+$: 282.0276, Found 282.0270.

*5.1.2.6 2-Chloro-4-(2-fluorophenoxy)-5-nitropyrimidine (**2f**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (406 mg, 2.1 mmol), DIEA (0.66 mL, 4.0 mmol) and fluorophenol (224 mg, 2.0 mmol) in THF (10 mL) was stirred at -40 °C for 3 h to give the title compound **2f** as a white solid (490 mg, 82.6%); mp 103-104 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm) 9.39 (s, 1H), 7.49-7.35 (m, 4H); HRMS (ESI): *m/z*, Calcd. for $C_{10}H_6O_3N_3ClF$ $[M+H]^+$: 270.0076, Found 270.0073.

*5.1.2.7 2-Chloro-5-nitro-4-(pyridin-2-yloxy)-pyrimidine (**2g**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (243 mg, 1.25 mmol), K_2CO_3 (328 mg, 2.38 mmol) and 2-hydroxypyridine (114 mg, 1.19 mmol) in DMF (1 mL) and acetone (9 mL) was stirred at 0 °C for 6 h to give the title compound **2g** as a white solid (95 mg, 31.5%); mp 85-87 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.23 (s, 1H), 8.38-8.37 (m, 1H), 7.92 (t, J = 7.2 Hz, 1H), 7.33 (t, J = 6.4 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H); HRMS (ESI): *m/z*, Calcd. for $C_9H_6O_3N_4Cl$ $[M+H]^+$: 253.0123, Found 253.0119.

*5.1.2.8 4-(2-Chloro-5-nitro-pyrimidin-4-yloxy)-benzooxazole (**2h**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (786 mg, 4.07 mmol), K_2CO_3 (1.02 g, 7.40 mmol), 4-hydroxyl benzo[*d*]oxazole (500 mg, 3.70 mmol) in DMF (5 mL) and acetone (45 mL) was stirred at -20 °C for 1 h to give the title compound **2h** as a white solid (886 mg, 71%); mp 120-123 °C; ^{1}H NMR (300 MHz, Acetone-d_6) δ (ppm) 9.43 (s, 1H), 8.54 (s, 1H), 7.77 (dd, J_1 = 8.1 Hz, J_2 = 0.9 Hz, 1H), 7.61 (t, J = 8.1 Hz, 1H), 7.42 (dd, J_1 = 8.1 Hz, J_2 = 0.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 162.98, 161.52,

157.49, 152.82, 151.65, 141.99, 132.47, 131.26, 126.23, 116.99, 110.13; HRMS (ESI): *m/z*, Calcd. for $C_{11}H_6O_4N_4Cl$ $[M+H]^+$: 293.0072, Found 293.0068.

*5.1.2.9 2-Chloro-4-((1-methyl-3-phenyl-1H-pyrazol-5-yl)oxy)-5-nitropyrimidine (**2l**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (130 mg, 0.67 mmol), DIEA (0.09 mL, 0.52 mmol) and 1-methyl-3-phenyl-5-hydroxyl-1*H*-pyrazol[33] (90 mg, 0.52 mmol) in THF (5 mL) was stirred at -40 °C for 0.5 h to give compound **2l** as a yellowish white solid (84 mg, 49.1 %); mp 135-137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.24 (s, 1H), 7.79-7.77 (m, 2H), 7.43-7.39 (m, 2H), 7.33 (tt, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 6.69 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 163.28, 158.44, 157.76, 149.75, 144.44, 132.93, 128.14, 125.24, 91.73, 35.19; HRMS (ESI): *m/z*, Calcd. for $C_{14}H_{11}O_3N_5Cl$ $[M+H]^+$: 332.0545, Found 332.0537.

*5.1.2.10 4-(Benzyloxy)-2-chloro-5-nitropyrimidine (**2m-1**)*
*2-(Benzyloxy)-4-chloro-5-nitropyrimidine (**2m-2**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (194 mg, 1.0 mmol), DIEA (0.18 mL, 1.0 mmol) and benzyl alcohol (93 mg, 1.0 mmol) in DCM (5 mL) was stirred at 0 °C for 5 h to give compound **2m-1** as a white solid (29 mg, 10.9%); mp 127-129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.04 (s, 1H), 7.51-7.49 (m, 2H), 7.43-7.35 (m, 3H), 5.65 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 162.71, 162.02, 156.64, 133.74, 128.93, 128.75, 128.38, 71.21; HRMS (ESI): *m/z*, Calcd. for $C_{11}H_9O_3N_3Cl$ $[M+H]^+$: 266.0327, Found 266.0319; The isomer **2m-2** as a white solid (59 mg, 22.3%); mp 98-99 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.17 (s, 1H), 7.49-7.47 (m, 2H), 7.42-7.34 (m, 3H), 5.55 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.48, 158.15, 156.37, 134.34, 128.84, 128.67, 128.54, 71.71; HRMS (ESI): *m/z*, Calcd. for $C_{11}H_9O_3N_3Cl$ $[M+H]^+$: 266.0327, Found 266.0320.

*5.1.2.11 2-Chloro-5-nitro-4-phenethoxypyrimidine (**2n-1**)*
*4-Chloro-5-nitro-2-phenethoxypyrimidine (**2n-2**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (283 mg, 1.46 mmol), DIEA (0.25 mL, 1.46 mmol) and 2-phenylethanol (178 mg, 1.46 mmol) in DCM (10 mL) was stirred at 0 °C for 20 h to give compound **2n-1** as a white solid (53 mg, 13%); mp >250 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.02 (s, 1H), 7.35-7.23 (m, 5H), 4.78 (t, *J* = 6.8 Hz, 2H), 3.17 (t, *J* = 6.8 Hz, 2H), ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 162.83, 162.25, 156.54, 136.69, 129.16, 128.64, 126.98, 70.73, 34.84; HRMS (ESI): *m/z*, Calcd. for $C_{12}H_{11}O_3N_3Cl$ $[M+H]^+$: 280.0484, Found 280.0481; The isomer **2n-2** as a white solid (144 mg, 35%); mp 215-217 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.14 (s, 1H), 7.34-7.23 (m, 5H), 4.71 (t, *J* = 7.2 Hz, 2H), 3.15 (t, *J* = 7.2 Hz, 2H), ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.62, 158.09, 156.30, 136.84, 128.99, 128.65, 126.87, 70.75,

34.95; HRMS (ESI): *m/z*, Calcd. for $C_{12}H_{11}O_3N_3Cl$ $[M+H]^+$: 280.0484, Found 280.0481.

*5.1.2.12 2-Chloro-4-(cyclopentyloxy)-5-nitropyrimidine (**2o**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (194 mg, 1.0 mmol), K_2CO_3 (138 mg, 1.0 mmol), in cyclopentanol (5 mL) was stirred at 40 °C for 2 d to give compound **2o** as light yellow oil (94 mg, 38.6%); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.00 (s, 1H), 5.76-5.72 (m, 1H), 2.07-2.00 (m, 2H), 1.97-1.81 (m, 4H), 1.73-1.67 (m, 2H).

*5.1.2.13 2-Chloro-4-(cyclohexyloxy)-5-nitropyrimidine (**2p**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (194 mg, 1.0 mmol), K_2CO_3 (138 mg, 1.0 mmol), in cyclohexanol (5 mL) was stirred at 40 °C for 23 h to give compound **2p** as light yellow oil (78 mg, 30.3%); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.00 (s, 1H), 5.46-5.40 (m, 1H), 1.98-1.93 (m, 2H), 1.82-1.68 (m, 4H), 1.52-1.35 (m, 4H); HRMS (ESI): *m/z*, Calcd. for $C_{10}H_{13}O_3N_3Cl$ $[M+H]^+$: 258.0640, Found 258.0628.

*5.1.2.14 2-Chloro-5-nitro-N-phenylpyrimidin-4-amine (**2q**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (250 mg, 1.29 mmol), DIEA (0.22 mL, 1.29 mmol) and aniline (120 mg, 1.29 mmol) in DCM (1 mL) was stirred at 0 °C for 5 min to give the title compound **2q** as a yellow solid (277 mg, 85.8%); mp 171-173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.45 (s, 1H), 9.16 (s, 1H), 7.54 (d, J = 8.0 Hz, 2H), 7.45 (t, J = 7.6 Hz, 2H), 7.29 (t, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.26, 157.60, 153.44, 135.45, 129.30, 126.65, 122.85; HRMS (ESI): *m/z*, Calcd. for $C_{10}H_8O_2N_4Cl$ $[M+H]^+$: 251.0330, Found 251.0329.

*5.1.2.15 2-Chloro-5-nitro-N-phenethylpyrimidin-4-amine (**2r**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (500 mg, 2.59 mmol), DIEA (0.86 mL, 4.92 mmol) and 2-phenylethanamine (299 mg, 2.46 mmol) in THF (15 mL) was stirred at -40 °C for 10 min to give the title compound **2r** as a light yellow solid (383 mg, 55.7%); mp 95-96 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.02 (s, 1H), 8.40 (brs, 1H), 7.35 (t, J = 7.6 Hz, 2H), 7.29-7.24 (m, 3H), 3.95-3.90 (m, 2H), 2.99 (t, J = 7.2 Hz, 2H); HRMS (ESI): *m/z*, Calcd. for $C_{12}H_{12}O_2N_4Cl$ $[M+H]^+$: 279.0643, Found 279.0639.

*5.1.2.16 2-Chloro-N-methyl-5-nitropyrimidin-4-amine (**2s**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (388 mg, 2.0 mmol), DIEA (0.4 mL, 2.4 mmol) and 40% methylamine solution in water (171 mg，2.2 mmol) in DCM (10 mL) was stirred at -60 °C for 3 h to give the title compound **2s** as a yellow solid (251 mg, 66.6%); mp 81-83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.04 (s, 1H), 8.39 (brs,

1H), 3.22 (d, J = 4.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 164.29, 156.94, 156.13, 126.81, 28.32; HRMS (ESI): *m/z*, Calcd. for $C_5H_6O_2N_4Cl$ $[M+H]^+$: 189.0174, Found 189.0169.

*5.1.2.17 2-Chloro-N-cyclopentyl-5-nitropyrimidin-4-amine (**2t**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (500 mg, 2.58 mmol), DIEA (0.51 mL, 3.1 mmol) and cyclopentylamine (171 mg, 2.2 mmol) in DCM (15 mL) was stirred at -40 °C for 1 h to give the title compound **2t** as a yellowish white solid (444 mg, 71.0%); mp 80-81 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.03 (s, 1H), 8.38 (brs, 1H), 4.65-4.57 (m, 1H), 2.21-2.13 (m, 2H), 1.81-1.68 (m, 4H), 1.61-1.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.14, 157.06, 154.95, 126.42, 53.22, 32.98, 23.68; HRMS (ESI): *m/z*, Calcd. for $C_9H_{12}O_2N_4Cl$ $[M+H]^+$: 243.0643, Found 243.0638.

*5.1.2.18 2-chloro-N-cyclohexyl-5-nitropyrimidin-4-amine (**2u**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyrimidine (**1a**) (540 mg, 2.78 mmol), DIEA (0.51 mL, 3.1 mmol) and cyclohexanamine (171 mg, 2.2 mmol) in DCM (15 mL) was stirred at -40 °C for 5 min to give the title compound **2u** as an off-white solid (685 mg, 95.9%); mp 120-122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.03 (s, 1H), 8.34 (brs, 1H), 4.29-4.21 (m, 1H), 2.05-2.02 (m, 2H), 1.81-1.78 (m, 2H), 1.70-1.67 (m, 1H), 1.53-1.25 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.26, 157.25, 154.63, 126.31, 50.19, 32.37, 25.26, 24.40; HRMS (ESI): *m/z*, Calcd. for $C_{10}H_{14}O_2N_4Cl$ $[M+H]^+$: 257.0800, Found 257.0788.

*5.1.2.19 4-(6-Chloro-3-nitro-pyridin-2-yloxy)-benzooxazole (**2v-1**)*
*4-(6-Chloro-5-nitropyridin-2-yloxy)-benzooxazole (**2v-2**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,6-dichloro-3-nitropyridine (**1b**) (212 mg, 1.1 mmol), K_2CO_3 (276 mg, 2.0 mmol) and 4-hydroxyl benzo[*d*]oxazole (135 mg, 1.0 mmol) in acetone (3 mL) and DMF (3 mL) was stirred at room temperature for 3 h to give compound **2v-1** as a white solid (104 mg, 35.7%); mp 178-179 °C; ^{1}H NMR (300 MHz, Acetone-d_6) δ (ppm) 8.67 (d, J = 8.1 Hz, 1H), 8.47 (s, 1H), 7.70 (dd, J_1 = 8.1 Hz, J_2 = 0.9 Hz, 1H), 7.57 (t, J = 8.1 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.37 (dd, J_1 = 8.1 Hz, J_2 = 0.9 Hz, 1H); HRMS (ESI): *m/z*, Calcd. for $C_{12}H_7O_4N_3Cl$ $[M+H]^+$: 292.0120, Found 292.0118; The isomer **2v-2** (72 mg, 24.7%) as a light yellow solid; mp 159-162 °C; ^{1}H NMR (300 MHz, Acetone- d_6) δ (ppm) 8.63 (dd, J_1 = 8.7 Hz, J_2 = 0.9 Hz, 1H), 8.48 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.57 (t, J = 8.4 Hz, 1H), 7.39-7.34 (m, 2H).

*5.1.2.20 4-(2-Chloro-5-nitro-pyridin-4-yloxy)-benzooxazole (**2w**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-nitropyridine (**1c**) (269 mg, 1.39 mmol), K_2CO_3 (384 mg, 2.78 mmol) and 4-hydroxyl benzo[*d*]oxazole (171 mg, 1.27 mmol) in acetone (18 mL) and DMF (2 mL) was stirred at room temperature for 4 h to give the title compound **2w** as a

white solid (324 mg, 87.8%); mp 195-196 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm) 9.07 (s, 1H), 8.54 (s, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.07 (s, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ (ppm) 159.52, 156.42, 154.98, 153.03, 148.02, 144.10, 127.68, 117.43, 113.52, 110.71; HRMS (ESI): *m/z*, Calcd. for $C_{12}H_7O_4N_3Cl$ $[M+H]^+$: 292.0120, Found 292.0109.

*5.1.2.21 4-Chloro-5-nitro-2-phenethylpyrimidine (**7a**)*

To a stirred solution of compound **6a** (1.21 g, 4.90 mmol) dissolved in anhydrous toluene (20 mL) was added phosphorus oxychloride (2.27 g, 14.8 mmol), DIEA (0.85 mL, 4.90 mmol) under argon atmosphere. The reaction was heated to 80 °C and stirred for 2 h. Then the reaction mixture was cooled and quenched with water (10 mL). Ethyl acetate (40 mL) was added and the organic layer was separated and washed with water (30 mL×2), dried over anhydrous magnesium sulfate. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10:1) to afford the title compound **7a** as a white solid (470 mg, 36.2%); mp 102-104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.16 (s, 1H), 7.29-7.22 (m, 5H), 3.39 (t, *J* = 8.0 Hz, 2H), 3.19 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 154.76, 138.85, 138.18, 127.41, 118.25, 118.08, 116.53, 47.90, 42.36; HRMS (ESI): *m/z*, Calcd. for $C_{12}H_{11}O_2N_3Cl[M+H]^+$: 264.0534, Found 264.0526.

*5.1.2.22 4-((5-nitropyrimidin-4-yl)oxy)benzo[d]oxazole (**7c**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 4-chloro-5-nitropyrimidine (**7b**) (50 mg, 0.31 mmol), DIEA (0.05 mL, 0.31 mmol) and 4-hydroxyl benzo[*d*]oxazole (**3**) (47 mg, 0.34 mmol) in DCM (10 mL) was stirred at 0 °C for 0.5 h to give the title compound **7c** as an off-white solid (19 mg, 23.8%); mp 157-159 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.36 (s, 1H), 8.81 (s, 1H), 8.05 (s, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H); HRMS (ESI): *m/z*, Calcd. for $C_{11}H_7O_4N_4$ $[M+H]^+$: 259.0462, Found 259.0459.

*5.1.2.23 4-(5-Nitro-2-phenyl-pyrimidin-4-yloxy)-benzooxazole (**7d**)*

The reaction mixture of **2h** (150 mg, 0.51 mmol), phenylboronic acid (188 mg, 1.54 mmol), sodium carbonate (163 mg, 1.54 mmol), cesium fluoride (77 mg, 0.51 mmol) and Pd(pddf)Cl_2 (78 mg, 0.10 mmol) in toluene (9 mL) and DMF (1 mL) was stirred under argon atmosphere at 80 °C for 1 h. Then the mixture was diluted with ethyl acetate (10 mL) and washed with saturated brine (10 mL), water (10 mL×2), and then dried over anhydrous magnesium sulfate. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 20:1) to afford the title compound **7d** as a white solid (35 mg, 20.4%); mp 164-165 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.59 (s, 1H), 8.77 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.55 (t, *J* = 7.6 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 166.01, 160.84, 157.45, 155.17, 151.49, 142.61, 135.04, 133.33, 132.85, 130.91, 129.48, 129.10, 126.83, 117.91, 110.25; HRMS (ESI): *m/z*, Calcd. for $C_{17}H_{11}O_4N_4$ $[M+H]^+$: 335.0775, Found 335.0763.

*5.1.2.24 4-(2-Naphthalen-2-yl-5-nitro-pyrimidin-4-yloxy)-benzooxazole (**7e**)*

Following the preparation protocol of Section 5.1.2.23, compound **2h** (100 mg, 0.34 mmol), 2-naphthaleneboronic acid (177 mg, 1.03 mmol), sodium carbonate (109 mg, 1.03 mmol), cesium fluoride (52 mg, 0.34 mmol) and Pd(pddf)Cl_2 (52 mg, 0.07 mmol) in toluene (9 mL) and DMF (1 mL) was stirred under argon atmosphere at 80 °C for 3.5 h to give the title compound **7e** as a light yellow solid (27 mg, 16.7%); mp 240-242 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm) 9.59 (s, 1H), 8.70 (s, 1H), 8.50 (s, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 7.93-7.82 (m, 4H), 7.68 (t, *J* = 8.0 Hz, 1H), 7.63-7.50 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 166.01, 160.86, 157.49, 155.20, 151.55, 142.67, 135.31, 132.92, 132.75, 132.47, 130.83, 130.59, 129.81, 129.16, 129.07, 128.17, 127.53, 126.87, 124.73, 117.97, 110.27; HRMS (ESI): *m/z*, Calcd. for $C_{21}H_{13}O_4N_4$ $[M+H]^+$: 385.0931, Found 385.0917.

*5.1.2.25 2-Chloro-5-methyl-4-phenoxypyrimidine (**9a**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-methylpyrimidine (**8a**) (300 mg, 1.84 mmol), K_2CO_3 (508 mg, 1.84 mmol) and phenol (173 mg, 1.84mmol) in acetone (20 mL) was stirred overnight under reflux to give the title compound **9a** as a white solid (390 mg, 96.0%); mp 59-60 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.27 (s, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.27 (t, *J* = 7.6 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 168.38, 159.24, 157.54, 152.01, 129.61, 125.81, 121.40, 117.26, 12.33; HRMS (ESI): *m/z*, Calcd. for $C_{11}H_{10}ON_2Cl$ $[M+H]^+$: 221.0476, Found 221.0475.

*5.1.2.26 4-((2-Chloro-5-methylpyrimidin-4-yl)oxy)benzo[d]oxazole (**9b**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-methylpyrimidine (**8a**) (300 mg, 1.84 mmol), K_2CO_3 (508 mg, 1.84 mmol) and 4-hydroxyl benzo[*d*]oxazole (**3**) (249 mg, 1.84 mmol) in acetone (20 mL) was stirred overnight under reflux to give compound **9b** as a white solid (434 mg, 90.1%); mp 151-153 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.33 (s, 1H), 8.05 (s, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 168.12, 159.62, 157.45, 152.41, 151.62, 143.41, 132.80, 126.00, 117.32, 117.13, 108.96, 12.32; HRMS (ESI): *m/z*, Calcd. for $C_{12}H_9O_2N_3Cl$ $[M+H]^+$: 262.0378, Found 262.0376.

*5.1.2.27 2-Chloro-4-phenoxy-5-(trifluoromethyl)pyrimidine (**9c**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-(trifluoromethyl)pyrimidine (**8b**) (217 mg, 1.0 mmol), K_2CO_3 (276 mg, 2.0 mmol) and phenol (94 mg, 1.0 mmol) in acetone (10 mL) was stirred at 0 °C for 1 h to give compound **9c** as a white solid (263 mg, 96.0%); mp 87-89 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.70 (s, 1H), 7.46 (t, *J* = 8.0 Hz, 2H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 2H); HRMS (ESI): *m/z*, Calcd. for $C_{11}H_7ON_2ClF_3$ $[M+H]^+$: 275.0194, Found 275.0189.

*5.1.2.28 4-((2-Chloro-5-(trifluoromethyl)pyrimidin-4-yl)oxy)benzo[d]oxazole (**9d**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloro-5-(trifluoromethyl)pyrimidine (**8b**) (217 mg, 1.0 mmol), K_2CO_3 (276 mg, 2.0 mmol) and 4-hydroxyl benzo[*d*]oxazole (**3**) (135 mg, 1.0 mmol) in acetone (10 mL) was stirred at 0 °C for 1 h to give compound **9d** as a light pink solid (279 mg, 88.4%); mp 148-150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.69 (s, 1H), 8.05 (s, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 7.2 Hz, 1H); HRMS (ESI): *m/z*, Calcd. for $C_{12}H_6O_2N_3ClF_3$ $[M+H]^+$: 316.0095, Found 316.0092.

*5.1.2.29 2,5-Dichloro-4-phenoxypyrimidine (**9e**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4,5-trichloropyrimidine (**8c**) (367 mg, 2.0 mmol), K_2CO_3 (276 mg, 2.0 mmol) and phenol (188 mg, 2.0 mmol) in acetone (10 mL) was stirred at room temperature for 6 h to give compound **9e** as a white solid (480 mg, 99.0%); mp 82-83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.47 (s, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 165.09, 158.15, 157.46, 151.47, 129.74, 126.40, 121.27, 116.97; HRMS (ESI): *m/z*, Calcd. for $C_{10}H_7ON_2Cl_2$ $[M+H]^+$: 240.9930, Found 240.9928.

*5.1.2.30 2-Chloro-4-phenoxypyrimidine-5-carbonitrile (**9f**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of 2,4-dichloropyrimidine-5-carbonitrile (**8d**) (367 mg, 2.0 mmol), K_2CO_3 (552 mg, 4.0 mmol) and phenol (188 mg, 2.0 mmol) in acetone (10 mL) was stirred at room temperature for 7 h to give compound **9f** as a white solid (408 mg, 88.0%); mp 94-96 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ (ppm) 9.08 (s, 1H), 7.54 (t, *J* = 7.2 Hz, 2H), 7.41-7.35 (m, 3H); HRMS (ESI): *m/z*, Calcd. for $C_{11}H_7ON_3Cl$ $[M+H]^+$: 232.0272, Found 232.0270.

*5.1.2.31 4-(Benzooxazol-4-yloxy)-2-chloro-pyrimidine-5-carboxylic acid methyl ester (**9g**)*

Following the preparation protocol of Section 5.1.2.1, the reaction mixture of methyl 2,4-dichloropyrimidine-5-carboxylate (**8e**) (228 mg, 1.10 mmol), K_2CO_3 (276 mg, 2.0 mmol) and 4-hydroxyl benzo[*d*]oxazole (**3**) (135 mg, 1.0 mmol) in DMF (1 mL) and acetone (9 mL) was stirred at -40 °C for 6 h to give compound **9g** as a white solid (268 mg, 87.7 %); mp 131-133 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.13 (s, 1H), 8.76 (s, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 3.93 (s, 3H); HRMS (ESI): *m/z*, Calcd. for $C_{13}H_9O_4N_3Cl$ $[M+H]^+$: 306.0276, Found 306.0273.

*5.1.2.32 4-(Benzo[d]oxazol-4-yloxy)-2-chloropyrimidine-5-carboxylic acid (**9h**)*

The reaction mixture of compound **9g** (100 mg, 0.36 mmol) and NaOH (72 mg, 1.80 mmol) in water (3 mL) and THF (3 mL) was stirred at room temperature for 3 h. The organic phase was removed by evaporation and the aqueous phase was cooled in

ice-water bath and acidified to pH 2.0 with 2*N* aqueous hydrochloric acid. The resulting precipitate was collected by filtration to give the title compound **9h** as a white solid (20 mg, 20.9 %); mp 118-120°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.10 (s, 1H), 8.76 (s, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 167.77, 164.04, 163.62, 161.37, 155.06 151.50, 142.99, 132.57, 126.85, 117.73, 112.86, 110.05; HRMS (ESI): *m/z*, Calcd. for $C_{12}H_7O_4N_3Cl$ $[M+H]^+$: 292.0120, Found 292.0114.

*5.1.2.33 1-(2-Chloro-4-(phenylamino)pyrimidin-5-yl)pyrrolidine-2,5-dione (**9i**)*

To a stirred solution of 2-chloro-N^4-phenylpyrimidine-4,5-diamine (**10**) (100 mg, 0.45 mmol) and succinic anhydride (90 mg, 0.9 mmol) dissolved in glacial acetic acid (5 mL) was added DMAP (6 mg, 0.05 mmol) under argon atmosphere. After stirred at room temperature for 1 h, the reaction was heated to 90 °C and stirred overnight. Then the reaction mixture was concentrated and dissolved in ethyl acetate (15 mL), washed with saturated aqueous sodium bicarbonate (10 mL×3), water (10 mL×2), and then dried over anhydrous magnesium sulfate. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 3:1) to afford compound **9i** as a white solid (37 mg, 27%); mp 115-116 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.04 (s, 1H), 7.37-7.21 (m, 5H), 6.91 (brs, 1H), 2.68 (s, 4H); HRMS (ESI): *m/z*, Calcd. for $C_{14}H_{12}O_2N_4Cl$ $[M+H]^+$: 303.0643, Found 303.0633.

*5.1.2.34 1-(2-Chloro-4-(phenylamino)pyrimidin-5-yl)-1H-pyrrole-2,5-dione (**9j**)*

Following the preparation protocol of Section 5.1.2.33, the reaction mixture of 2-chloro-N^4-phenylpyrimidine-4,5-diamine (**10**) (200 mg, 0.91 mmol), maleic anhydride (178 mg, 1.81 mmol), DMAP (11 mg, 0.09 mmol) in glacial acetic acid (10 mL) was stirred at 90 °C overnight to give compound **9j** as a yellow solid (56 mg, 20.6%); mp 104-106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.04 (s, 1H), 7.39-7.32 (m, 4H), 7.18 (t, *J* = 7.2 Hz, 1H), 6.89 (brs, 1H), 6.85 (s, 2H); HRMS (ESI): *m/z*, Calcd. for $C_{14}H_{10}O_2N_4Cl$ $[M+H]^+$: 301.0487, Found 301.0478.

*5.1.2.35 2-Chloro-5-(1,3,4-oxadiazol-2-yl)-N-phenylpyrimidin-4-amine (**9k**)*

To a stirred solution of (isocyanoimino)triphenylphosphorane (**14**) (302 mg, 1.0 mmol) dissolved in DCM (8 mL) was added the solution of 2-chloro-4-(phenylamino)pyrimidine-5-carboxylic acid (**13**) (250 mg, 1.0 mmol) in DCM (7 mL) dropwise. After stirred at room temperature for 24 h, the reaction was washed with water (10 mL×3), and then dried over anhydrous magnesium sulfate. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 5:1) to afford compound **9k** as a white solid (132 mg, 60.3%); mp 163-165 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 10.42 (s, 1H), 8.82 (s, 1H), 8.56 (s, 1H), 7.76 (d, *J* = 8.0 Hz, 2H), 7.43 (t, *J* = 8.0 Hz, 2H), 7.23 (t, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 162.73, 161.01, 156.83, 156.66, 151.95, 136.99, 129.09, 125.35, 121.86, 100.10; HRMS (ESI): *m/z*, Calcd. for $C_{12}H_9ON_5Cl$ $[M+H]^+$: 274.0490, Found 274.0483.

*5.1.2.36 2-Chloro-5-(1,3,4-oxadiazol-2-yl)-N-phenylpyrimidin-4-amine (**9l**)*

2-chloro-*N*-(2,2-dimethoxyethyl)-4-(phenylamino)pyrimidine-5-carboxamide (**16**) (574 mg, 1.70 mmol) was dissolved in Eaton's reagent (10 mL) and stirred at 130 °C for 3 h under argon atmosphere. Then the reaction was concentrated and dissolved in ethyl acetate (20 mL) and methanol (4 mL), washed with water (5 mL). The organic phase was dried over anhydrous magnesium sulfate. The crude product 2-hydroxyl-5-(oxazol-2-yl)-4-(phenylamino)pyrimidine (**17**) obtained after concentration as a white solid which was used in the next step without further purification.

Following the preparation protocol of Section 5.1.2.21, the reaction mixture of 2-hydroxyl-5-(oxazol-2-yl)-4-(phenylamino)pyrimidine (**17**), DIEA (0.56 mL, 3.4 mmol) in phosphorus oxychloride（5 mL）was stirred at 90 °C for 5 h to give compound **9l** as a light yellow needle crystal (57 mg, 12.3%); mp 141-142; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 11.10 (s, 1H), 8.84 (s, 1H), 7.79-7.76 (m, 3H), 7.42 (t, *J* = 8.0 Hz, 2H), 7.35 (s, 1H), 7.19 (t, *J* = 7.6 Hz, 1H); HRMS (ESI): *m/z*, Calcd. for $C_{13}H_{10}ON_4Cl$ $[M+H]^+$: 273.0538, Found 273.0530.

5.2. Biological evaluation

5.2.1. Protein expression and purification

The Pet28a-Pin1 plasmid was a gift from Professor Joseph P. Noel (The Salk Institute for Biological Studies, La Jolla, California). The N-terminally His_6-tagged Pin1 was expressed at 22 °C in *E. coli* strain BL21 following induction at an optical density of 0.6 (600 nm) with 0.5 mM IPTG for 20 h in terrific broth. Cells were resuspended in 25 mM Tris-Cl, 500 mM NaCl, 10 mM imidazole, 100 g/mL cocktail, 0.5 mg/mL lysozyme. Following sonication at 4 °C, the soluble supernatant was loaded onto an Ni-NTA (Qiagen) column and washed with 10 bed volumes of washing buffer (50 mM imidazole, 500 mM NaCl, 20 mM Tris-Cl). His_6-Pin1 was eluted with 400 mM imidazole, 500 mM NaCl, 20 mM Tris-Cl and condensed by ultrafiltration (Millipore 5 kDa) with 20 mM Tris-Cl, 100 mM NaCl, 5 mM DTT.

5.2.2. Pin1 PPIase assay and IC_{50} measurements of Pin1 inhibitors

PPIase activities were measured at 6 °C JASCO V-650 spectrophotometer using protease-coupled assay according to Wang et al.[30,31] Suc-Ala-Glu-Pro-Phe-4-nitroanilide in 0.47 M LiCl/trifluoroethanol was used as the substrate. In brief, the assay buffer (855 μL of 35 mM HEPES at pH 7.8), Pin1 (0.5 μL of 850 μg/mL stock solution), and inhibitors (5 μL of varying concentrations in DMSO) were pre-equilibrated in the 1.0 mL quartz cuvette in spectrophotometer for 10 min. Then, 100 μL of ice-cooled chymotrypsin (60 mg/mL in 0.001 M HCl) was added and mixed immediately. Additional 40 μL of substrate (2.5 mM in 0.47 M LiCl/trifluoroethanol) was added to the cuvette and the reaction was monitored by absorbance at 390 nm for 90 s. For each compound, three concentrations (100 μM, 10 μM, 1 μM) were chosen, and the assay was performed in duplicate. The data was analyzed by Graphpad Prism 5.01. The inhibition at each concentration was

calculated according the following equation: Inhibition ratio(%)=[1-(k_X-k_1)/(k_D-k_1)]× 100, where k_X represents the reaction rate in the presence of tested compound, k_D is the reaction rate of DMSO control without the tested compound, k_1 means the reaction rate of blank control without Pin1 protein, it represents the reaction rate of thermal isomerization without any catalysis. The IC_{50} was measured according to the inhibition ratio at each concentration of tested compound.

5.2.3. Time-dependent Pin1 PPIase assay

Parallel PPIase activities were measured with 96-well UV microplate on the BECKMAN COULTER-PARADIGM Detection Platform. The substrate Suc-Ala-Glu-Pro-Phe-4-nitroanilide was dissolved with 0.47 M LiCl/trifluoroethanol. In brief, the assay buffer (280 μL/well, 35 mM HEPES, 100 mM NaCl at pH 7.8), Pin1 protein (1.8~2.5 μL/well of 670 μg/mL stock solution), and inhibitors (1.7 μL/well of varying concentrations in DMSO) were added sequentially in the 96-well UV microplate and incubated for different time (e.g. 0 min, 5 min, 15 min). Then, ice-cooled chymotrypsin (60 mg/mL in stock buffer with 0.001 M HCl and 0.2 mM $CaCl_2$) was added in the 96-well UV microplate (33 μL/well). After that, the whole system was immediately mixed. Finally, the substrate (2.5 mM in 0.47 M LiCl/trifluoroethanol) was added in the system (14 μL/well) and mixed. During the whole process any detectable bubbles should be strictly avoided. The reaction was monitored by detecting the absorbance at 390 nm using kinetic mode (interval 2s, 20~30 cycles) on the BECKMAN COULTER-PARADIGM Detection Platform.

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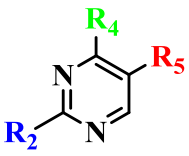
Graphical Abstract:

Synthesis and biological evaluation of pyrimidine derivatives as novel human Pin1 inhibitors

Guonan Cui, Jing Jin, Hualong Chen, Ran Cao, Xiaoguang Chen, Bailing Xu

	**R_2 = H, Cl, Ph, naphthyl, $PhCH_2CH_2$, $PhCH_2O$, $PhCH_2CH_2O$** **R_4 = H, Cl, substituted aryloxy or alkyloxy moieties, substituted arylamino or alkylamino moieties** **R_5 = small size fragments with various electrostatic properties** **IC_{50} = 1.68 – 34.0 μM**