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# Discovery of Coumarin Derivatives as Potent and Selective Cyclin-Dependent Kinase 9 (CDK9) Inhibitors with High Antitumour Activity

Junyu Xu,[†] Xinren Wang,[†] Jianhang Huang,[†] Hongmei Li,[†] Shuwen Li,[†] Chenhe Liu,[†] Ruinan Dong,[†] Gaoyuan Zhu,[†] Chunqi Duan,[†] Fei Jiang,[†] Yanmin Zhang,[†§] Yuqin Zhu,[†] Tianyi Zhang,[†] Yadong Chen,[†‡§*] Weifang Tang[†‡*] and Tao Lu[†‡§*]

[†]School of Sciences, China Pharmaceutical University, 639 Longmian Avenue, Nanjing 211198, China.

[‡]State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China.

[§]Laboratory of Molecular Design and Drug Discovery, School of Science, China Pharmaceutical University, 639 Longmian Avenue, Nanjing 211198, China.

## Abstract

Specific inhibition of CDK9 is considered a promising strategy for developing effective anticancer therapeutics. However, most of the reported CDK9 inhibitors are still at an early stage of development and lack selectivity against other CDKs. Herein, we discovered coumarin derivative **30i** as a potent CDK9 inhibitor with high selectivity (8300-fold over CDK7). Binding mode analysis illustrated that the substituent coumarin moiety is a critical group for CDK9 selectivity by occupying a flexible hinge/αD region, which is sterically hindered in other CDKs. Compound **30i** showed excellent cellular antiproliferative activity, moderate pharmacokinetic property and low hERG inhibition. Moreover, **30i** significantly induced tumour growth inhibition in a dose-dependent manner without causing an obvious loss of body weight in an MV4-11 xenograft mice model. Altogether, these results suggest that **30i** may serve as a potential acute myeloid leukaemia (AML) therapeutics by selectively targeting CDK9.

## Keywords

Coumarin derivatives, CDK9 inhibitor, Selectivity, Structure-activity relationship, Antitumour Activity.

## Highlights

- A series of novel coumarin derivatives were discovered as CDK9 inhibitors.
- Compound **30i** showed high selectivity (160- to 8300-fold) over CDK1/2/3/4/5/6/7/8/19.
- A substituted coumarin located at the hinge/αD region was suggested to improve selectivity and inhibition of CDK9.
- Compound **30i** induced tumour growth inhibition in a dose-dependent manner in an AML mice model.
- Compound **30i** dose-dependently inhibited CDK9-mediated phosphorylation of RNAPII.

## 1. Introduction

Cyclin-dependent kinases (CDKs), a family of serine/threonine protein kinases, play a critical role in the control of cell division cycle progression (e.g., CDK1 − 6) and regulation of gene transcription (e.g., CDK7 − 13 and 19) in eukaryotic cells [1,2]. As a CDK family member, CDK9 combines with its corresponding cyclin partners (cyclin T1, T2a, T2b, or K) to form positive transcription elongation factor b (P-TEFb), phosphorylating residue Ser2 within the C-terminal domain (CTD) of RNA polymerase II (RNAPII) to facilitate gene transcription elongation [3-5]. The prevailing view is that many cancer cells are dependent on the sustained expression of short-lived antiapoptotic proteins (e.g., Mcl-1, Bcl-2 and XIAP); however, CDK9 is a vital regulator that stimulates transcriptional elongation of antiapoptotic proteins [6,7]. Inhibition of CDK9-mediated phosphorylation of RNAPII can rapidly reduce the levels of antiapoptotic proteins, leading to the promotion of apoptosis and inhibition of tumour cell proliferation [8-11]. Deregulated CDK9-related pathways are frequently observed in many human malignancies; thus, CDK9 is considered an attractive cancer therapeutic target [12-19]. The first-generation clinical CDK9 inhibitor candidates, such as flavopiridol (alvocidib) and R-roscovitine (seliciclib), have excellent pharmaceutical properties and *in vivo* antitumour efficacy and are associated with the downregulation of CDK9-mediated antiapoptotic protein transcription [10,11,20-22]. However, CDK9 specificity was not achieved, which might cause dose-limiting toxicity and adverse events, and thus result in restricted clinical applications [23].

Designing selective CDK inhibitors has become more prevalent. Intensive attempts of developing structurally diverse CDK inhibitors have led to approvals of CDK4/6 inhibitors (palbociclib (Pfizer), ribociclib (Novartis) and abemaciclib (Eli Lilly)) [24-26]. So Several selective CDK9 inhibitors have been actively pursued by modifying the scaffold of previous pan-CDK inhibitors [27]; however, most of these pan-CDK inhibitors show cross-reactivity with at least two other CDK family members on the same order of magnitude or show significant effects on other protein kinases [28-34]. The design of highly selective CDK9 inhibitors remains a great challenge at the present stage due to the high sequence homology among the CDK family members [35,36]. Herein, we report our studies to design, synthesize and evaluate a series of coumarin derivatives, leading to the identification of a highly potent and selective CDK9 inhibitor **30i** as a potential antitumour agent.

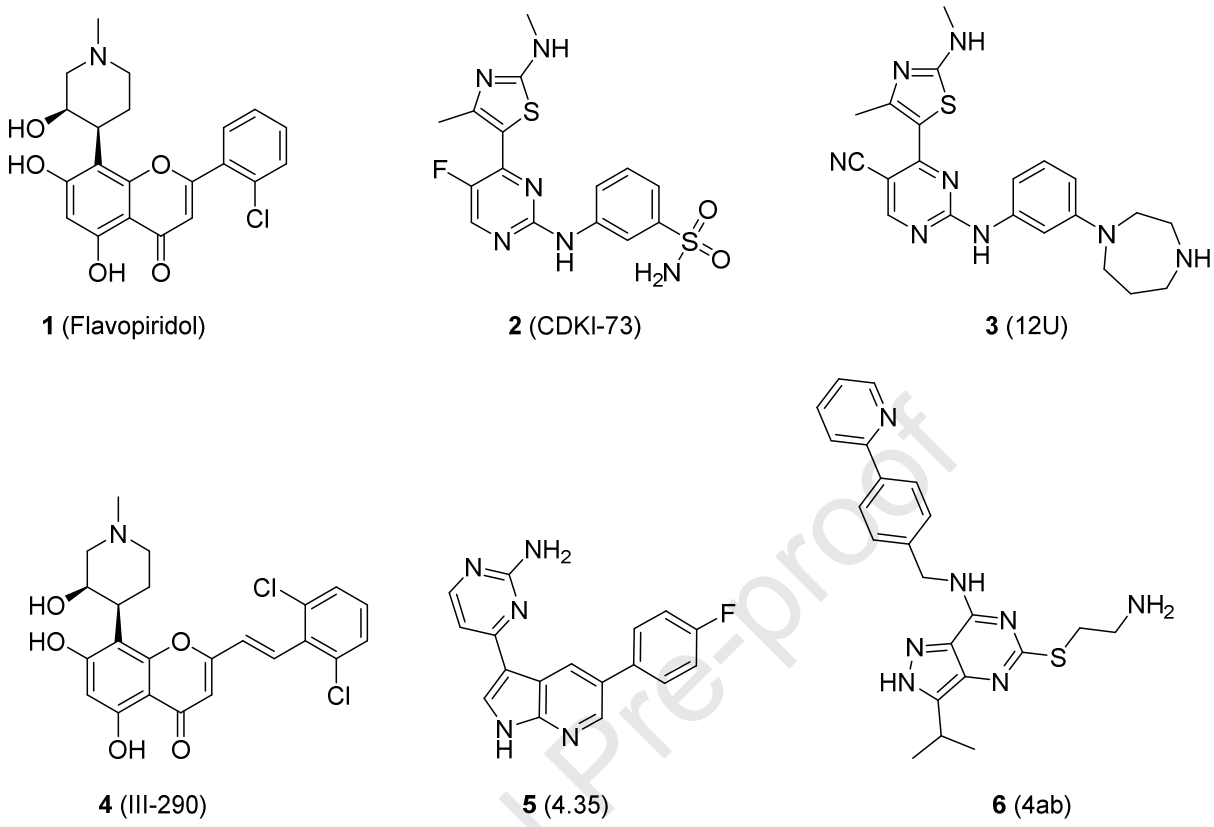



**Figure 1.** Chemical structures of representative CDK9 inhibitors recently.

## 2. Results and Discussion

### 2.1. Discovery and Research of Hit Compound **7**

Inspection of published CDK inhibitors revealed that a majority of these molecules occupy the ATP-binding pocket [27]. Wang *et al.* reported that selective inhibition of CDK9 by **3** (12U) results from the relative malleability of the ATP binding area, and a bulky 1,4-diazepan-1-ylaniline moiety can be accommodated within a less crowded and different electrostatic environment [31,37]. Further comparison of the hinge/αD region sequence alignment of CDK9/2/1/3/4/5/6/7/8 in the ATP-binding area (**Figure 2A/C** and supplementary **Figure S1**/**S2)** showed that the Gly112/Ala111 residues of CDK9 are much smaller than the residues at the same position in other CDKs within this αD-helix, such as the Lys89/Lys88 residues in CDK2, and the Thr102/Arg101 residues in CDK4 [38]. This indicates the Gly112/Ala111 of αD-helix region in CDK9 may accommodate bulkier moieties.

Coumarin scaffolds are rarely reported for the design of CDK inhibitors; however, some naturally occurring

and synthetic compounds that contain the coumarin core have recently been reported to induce apoptosis of tumour cells, resulting from the inhibition of CDK-associated kinase activity [39-42]. Moreover, some structurally related flavanoids including flavopiridol [43], IIIM-290 [29], voruciclib [21], riviciclib [44] and others [20]) have been successfully designed as CDK inhibitors. In addition, coumarin derivatives have similar pharmacological effects to CDK9 inhibitors, including antitumour [45,46], antiviral [47], cardioprotective [48] and other effects [49,50].

Inspired by the above research information, we screened coumarin derivatives from our natural product-like molecule library for the inhibition of CDKs. As shown in **Figure 2D**, we identified a hit compound **7** which had moderate inhibition of CDK9 (CDK9/cyclin T1 $IC_{50}$: 184 nM), and showed selectivity (7-fold) for CDK9 over CDK2. In particular, it inhibits CDK7 in uM range ($IC_{50}$ > 27000 nM) indicating that **7** possessed selectivity for CDK9 not only against cell cycle CDKs but also against transcriptional CDKs. To better understand the selectivity of **7** towards CDK9, we docked compound 7 to a CDK9 crystallographic structure (PDB code: 4BCG). In **Figure 2B**, the C2-carbonyl of coumarin forms a hydrogen bond with the NH of Asp109, and the coumarin moiety is accommodated above the flexible area of CDK9 at the tail of hinge area and αD-helix. In addition, the N1-pyrimidine moiety accepts a hydrogen bond from the NH of Cys106, and the C2-NH of the pyrimidine ring donates a hydrogen bond to the carbonyl of Cys106. In conclusion, molecule **7** is strongly fixed in the ATP-binding cleft through three hydrogen bonds at the hinge area. The nonconserved hydrophobic area and gatekeeper region (Phe103) at the C4- or C5-position of pyrimidine and the solvent accessible region at the C3- or C4-position of coumarin can be exploited for the design of CDK9 inhibitors. Therefore, we carried out a structure-activity relationship study of coumarin derivatives with the aim of identifying novel, potent and selective inhibitors of CDK9 as antitumour agents. The chemical structures and biological activity results are summarized in **Table 1** − **Table 3.**

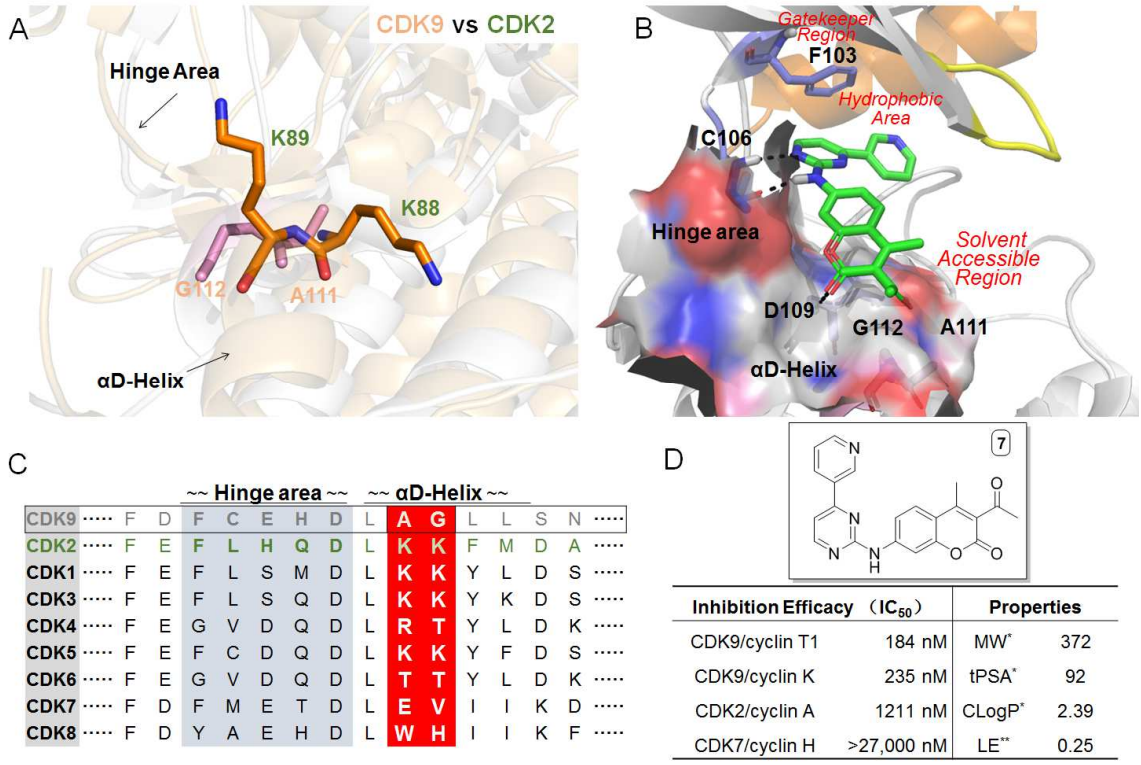


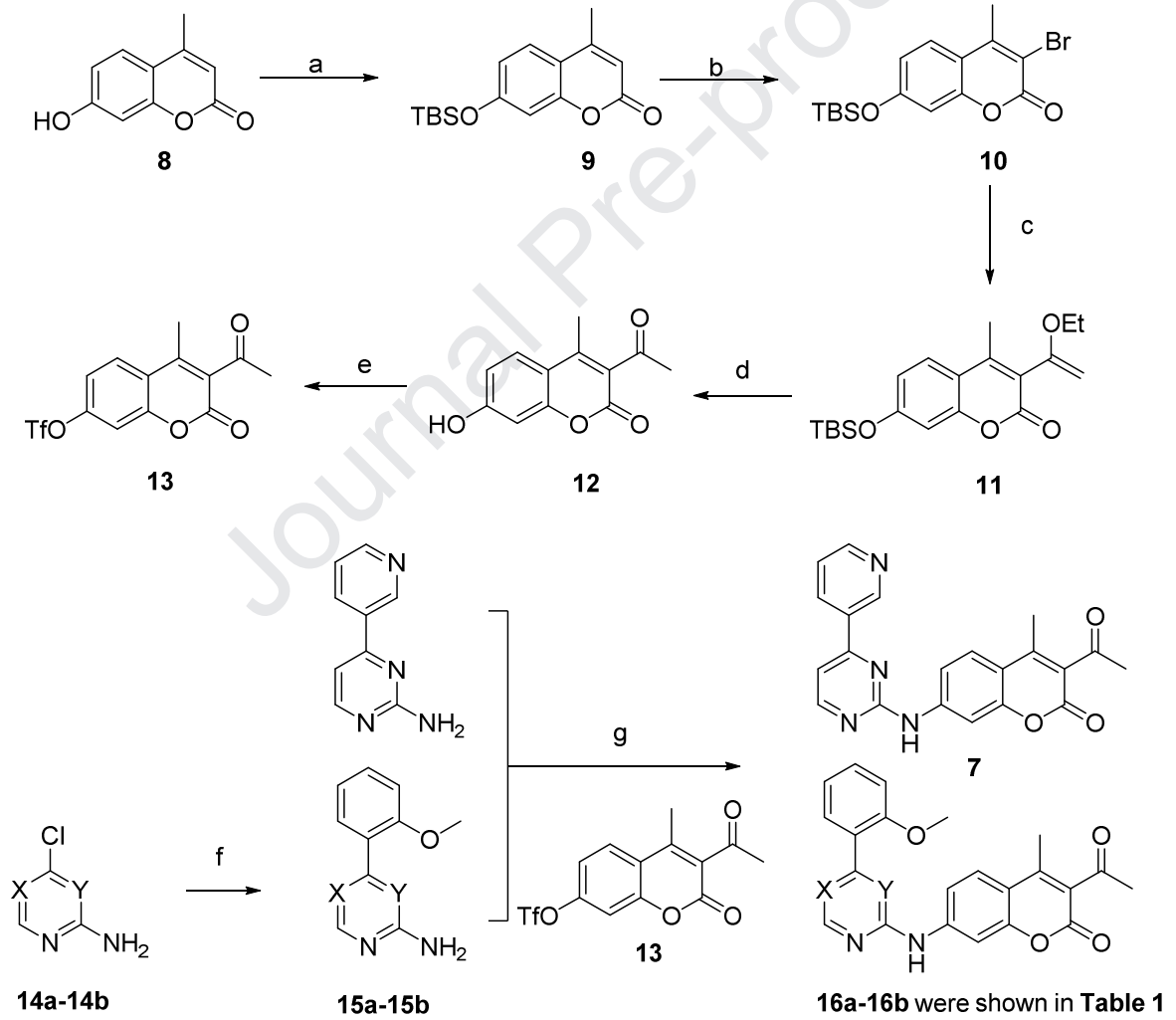

**Figure 2.** (A) Overlapping the key amino acid residues of CDK9 and CDK2 at the αD-helix area. (B) Compound **7** (green stick model) docked into the published crystal complex of CDK9 (PDB code: 4BCG); the key residues between CDK9 are labelled, and the hinge/αD region is shown in surface representation. Hydrogen bonds are depicted by black dotted lines. (C) Hinge/αD region sequence alignment of CDK9/2/1/3/4/5/6/7/8. (D) Chemical structure, inhibition efficacy and properties of compound **7**. The mean $IC_{50}$ values were measured in duplicate. *The properties were calculated by ChemDraw Ultra 2017 software; **ligand Efficiency of CDK9/cyclin T1: LE = -p$IC_{50}$/n, n = heavy atom count (or non-hydrogen atom count).

## 2.2. Chemistry

The desired target compounds **16a-16b** were synthesized as depicted in **Scheme 1**. Commercially available 7-hydroxy-4-methyl-2*H*-chromen-2-one (**8**) was used as the starting material. The hydroxy at the C7-position was found to interfere in subsequent reactions and was therefore masked as the *tert*-butyldimethylsilyl ether **9** in the presence of imidazole as a base. Bromination of **8** with NBS at room temperature resulted in bromide **10**. Preparation of key intermediate **13** started from the Stille coupling reaction of **10** with 1-ethoxyvinyltri-n-butyltin using $PdCl_2(PPh_3)_2$ as the catalyst, followed by treatment with hydrochloric acid in refluxing methanol

to yield **12**, which was then esterified with trifluoromethanesulfonic anhydride in the presence of triethylamine as the base in an ice bath. Compounds **15a-15b** were prepared through the Suzuki-Miyaura coupling reaction of commercial compounds **14a-14b** and (2-methoxyphenyl)boronic acid using $Pd(dppf)Cl_2$ as the catalyst. Target compounds **16a-16b** were synthesized by the Buchwald-Hartwig amination reaction of aminopyrimidine derivatives **15a-15b** and key coumarin intermediate **13** using $Pd(OAc)_2$ as the catalyst. Compound **7** was synthesized under the same conditions with the commercially available 4-(pyridin-3-yl)pyrimidin-2-amine and intermediate **13**.

**Scheme 1:**



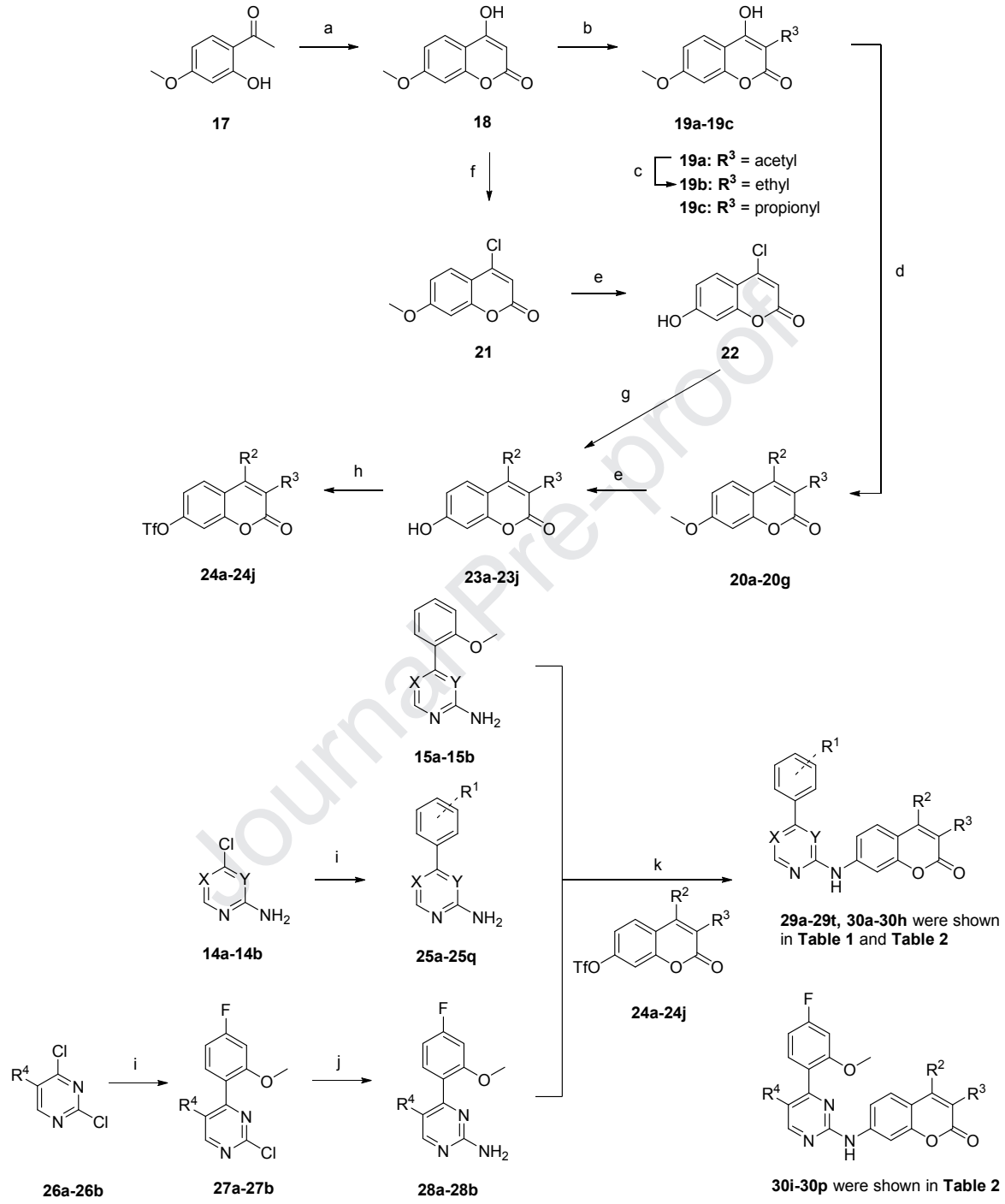

*Reagents and conditions*: (a) TBDMSCl, imidazole, DMF, r.t., 6 h; (b) NBS, $CH_3CN$, r.t., 9 h; (c) $PdCl_2(PPh_3)_2$, 1-ethoxyvinyltri-n-butyltin, DMF, 80 °C, 6 h; (d) HCl (6M, in methanol), reflux, 12 h; (e) $Tf_2O$,

$Et_3N$, DCM, 0 °C, 1 h; (f) (2-methoxyphenyl)boronic acid, $Na_2CO_3$, $Pd(dppf)Cl_2$, 1,4-dioxane : $H_2O$ = 7 : 1, $N_2$, 95 °C, 2−6 h; (g) **13**, $Pd(OAc)_2$, $Cs_2CO_3$, Xantphos, $N_2$, 100 °C, 2−16 h.

The synthesis of target compounds **29a**-**29t** and **30a**-**30p** is depicted in **Scheme 2**. In an effort to further modify the C3- and C4-positions of the coumarin core, commercially available paeonol was reacted with diethyl carbonate in the presence of sodium hydride as the base with heating (100 °C, 4 h) to give compound **18**. Alterations to the C3-position were achieved by a series of modifications to **18**; C3-substituted **19a** or **19c** were obtained by treating **18** with acetic acid or propionic anhydride in the presence of phosphorus oxychloride, respectively. Reduction conveniently converted **19a** to **19b** with sodium cyanoborohydride as the reductant. In order to introduce the hydrophilic fragment, a nucleophilic substitution reaction was utilized to install different heterocyclic substituents at the C4-position by refluxing under basic conditions to give **20a-20g**. Cleavage of the ether at the C7-position with aluminium chloride in refluxing toluene led to **23a-23g**. Moreover, halogenation of **18** formed **21** with the use of phosphorus oxychloride and triethylamine, and then cleavage of the ether at the C7-position was carried out under similar conditions as those for compound **23a**, followed by the introduction of heterocyclic substituents to the C4-position, leading to **23h-23j**. Finally, a series of key intermediates **24a-24j** were obtained by esterification under similar conditions as those for intermediate **13**.

A series of aminopyrimidine compounds with C-C linkages at the C4-position of pyrimidine were formed by palladium-mediated chemistry, and the Suzuki-Miyaura coupling reaction was used to install a series of substituted aromatic groups giving compounds **25a-25q**. In an effort to prepare C5-substituted pyrimidine derivatives, Suzuki-Miyaura coupling reactions of the commercially inexpensive agent **26a** ($R^2$ = F) or **26b** ($R^2$ = Cl) with 4-fluoro-2-methoxyphenylboronic acid were performed with $Pd(dppf)Cl_2$ as the catalyst. However, further amination reactions of **27a-27b** with ammonium hydroxide were performed under heating (85 °C) conditions, giving aminopyrimidine derivatives **28a-28b**. The desired target compounds **29a-29t** and **30a-30p** were synthesized by the Buchwald-Hartwig amination reaction using $Pd(OAc)_2$ as the catalyst between the aminopyrimidine derivatives **15a-15b**/**25a-25q**/**28a-28b** and key coumarin intermediates **24a-24j**. In addition, **29d**-**29e** were obtained by treatment with hydrochloric acid at room temperature for deprotection of the *N*-Boc group.

**Scheme 2:**




*Reagents and conditions*: (a) diethyl carbonate, NaH, toluene, 100 °C, 4 h; (b) $POCl_3$, $R^3OH$ or $(R^3)_2O$, 105 °C, 1 h; (c) $NaBH_3CN$, AcOH, r.t., 0.5 h; (d) $R^2H$, reflux, 2−6 h; (e) $AlCl_3$, toluene, 90 °C, 3−6 h; (f) $POCl_3$, $Et_3N$, 100 °C, 3 h; (g) $R^2H$, $Et_3N$, EtOH, reflux, 3 h; (h) $Tf_2O$, $Et_3N$, DCM, 0 °C, 1 h. (i) phenylboronic

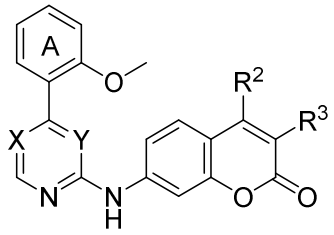
acid derivative, $Na_2CO_3$, $Pd(dppf)Cl_2$, 1,4-dioxane : $H_2O$ = 7 : 1, $N_2$, 95 °C, 2−6 h; (j) $NH_4OH$, isopropanol, 85 $^oC$, 6 h; (k) i. relative intermediate **28a**−**24j**, $Pd(OAc)_2$, $Cs_2CO_3$, Xantphos, $N_2$, 100 °C, 2−16 h; ii. or further deprotection of the *N*-Boc, HCl (4M, in Dioxane), r.t., 0.5 h.

## 2.3. Structure-Activity Relationships (SARs)

Based on the molecular modelling studies of compound **7**, the C7-, C4- and C3-positions of coumarin were modified to explore the hydrophobic area, hinge region and solvent accessible region for potent CDK9 inhibition. All of these coumarin derivatives were screened for their enzymatic activity against CDK9/cyclin T1 or CDK2/cyclin A *in vitro*. The initial screening was verified and duplicated at a single concentration of 1.0 μM, followed by selection of the active compound for determination of the $IC_{50}$ values against CDK9 and CDK2 enzymatic activities and the antiproliferative activities of the MV4-11 human acute myeloid leukaemia (AML) cell line.

With the aim of finding more potent analogues, we preferentially evaluated the 2-methoxyphenyl group at the A position as the hydrophobic region and gatekeeper region (**Table 1**). Analogues **16a** and **16b** showed significant improvement in CDK9 inhibition with an $IC_{50}$ values of 6.5 nM and 37.6 nM but poor toxicity in MV4-11 cells. We then introduced a morpholino as the hydrophilic heterocycle towards the solvent-accessible region in analogue **29a**, potently inhibited CDK9 ($IC_{50}$: 3.1 nM) and improved cellular toxicity 10-fold compared with the toxicity of **16a**. When changing the acetyl group at $R^3$ position in **29a**, analogues **29b** ($R^3$ = Et) and **29c** ($R^3$ = H) showed over 20-fold and 15-fold losses in CDK9 inhibition, respectively, indicating that the acetyl group at the C3-position of coumarin was favourable. Notably, the introduction of heterocyclic piperazin-1-yl (**29d**) or 1,4-diazepan-1-yl (**29e**) at the C4-position of coumarin displayed comparable potency and selectivity for CDK9 and enhanced cellular antiproliferative activity compared to the antiproliferative activity of analogue **16a** ($R^2$= Me).

**Table 1.** Structures and biological evaluations of compounds **7**, **16a - 16b** and **29a** – **29e**.




| Comp. | $R^2$ | $R^3$ | X | Y | Enzymatic $IC_{50}$ /nM [a] (% Enzymatic activity) [b] | | Cell Inhibitory $IC_{50}$ (μM) [a] |
|---|---|---|---|---|---|---|---|
| | | | | | CDK9/ cyclin T1 | CDK2/ cyclin A | MV4-11 |
| **7** [c] | Me | acetyl | CH | N | 184 | 1211 | 12.88 |
| **16a** | Me | acetyl | CH | N | 6.5 | 451.2 | 10.16 |
| **16b** | Me | acetyl | N | CH | 37.6 | (64.5±0.7) | ＞10 |
| **29a** | morpholino | acetyl | CH | N | 3.1 | 1283 | 1.01 |
| **29b** | morpholino | Et | CH | N | 67.3 | 10870 | 4.72 |
| **29c** | morpholino | H | CH | N | 47.2 | (57.9±0.3) | 4.57 |
| **29d** | piperazin-1-yl | H | CH | N | 25.3 | 3664 | 1.34 |
| **29e** | 1,4-diazepan-1-yl | H | CH | N | 11.7 | 2056 | 1.68 |

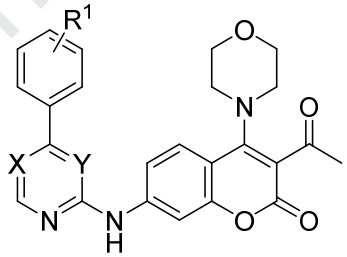
[a] The mean values of $IC_{50}$ were measured in duplicate. [b] % Enzyme activities were measured at 1.0 μM concentration of test compound, n=2. NA: not determined. [c] A moiety was pyridin-3-yl.

To further explore the hydrophobic area and gatekeeper region for affinity improvement, the diversity of the substituents was evaluated to investigate how varying the substituents at the phenyl group will affect the activity against CDK9 (**Table 2**). Replacement of 2-methoxyl with a 3-methoxyl (**29f**), 4-methoxyl (**29g**), 2,4-

dimethoxyl (**29h**) or 2,3-dimethoxyl (**29i**) showed slightly reduced inhibition of CDK9, indicating that an ortho-substituent on the phenyl ring was most favourable. Interestingly, the introduction of 2-trifluoromethyl (**29l**) or 2-nitryl (**29m**) resulted in a significant loss in CDK9 inhibition compared with 2-methyl (**29n**) and 2-ethyl (**29o**), suggesting that an electron-deficient phenyl ring at the C4-position of pyrimidine was not tolerated. This may benefit from the aromatic-aromatic interaction of the electron-rich phenyl group with the Phe103 gatekeeper residue of CDK9. The increased lipophilicity of fluorine (**30a-30c**) at the 2-methoxyphenyl group resulted in an improvement in cellular antiproliferative activity possibly due to an increase in cellular permeability; simultaneously, this modification showed less of an effect on CDK9 inhibitory activity.

To understand the SAR of the pyrimidine of the hinge region, the substituted (C4-pyrimidin-2-yl)amino group was replaced by the substituted (C6-pyrimidin-2-yl)amino group at the C7-position of coumarin. Notably, the CDK9 inhibitory activities of analogues **29p** – **29t** were lost, and this is likely due to the electron density alteration that disrupted the hydrogen-bond interaction with Cys106 in the hinge region. The electron-rich phenyl ring was still favourable in the hydrophobic area when the (C6-pyrimidin-2-yl)amino group was located in the hinge region.

**Table 2.** Structures and biological evaluations of compounds **29a**, **29f** – **29t** and **30a** – **30c**.




| Comp. | $R^1$ | X | Y | Enzymatic $IC_{50}$ /nM [a] (%Enzymatic activity) [b] | | Cell Inhibitory $IC_{50}$ (μM) [a] |
|---|---|---|---|---|---|---|
| | | | | CDK9/ cyclin T1 | CDK2/ cyclin A | MV4-11 |
| **29a** | 2-MeO | CH | N | 3.1 | 1283 | 1.01 |
| **29f** | 3-MeO | CH | N | 18.4 | (54.1±2.3) | 1.49 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **29g** | 4-MeO | CH | N | 81.7 | (21.6±0.4) | 6.49 |
| **29h** | 2,4-2MeO | CH | N | 54.2 | (46.3±4.0) | 0.82 |
| **29i** | 2,3-2MeO | CH | N | (51.7±4.8) | NA | 5.07 |
| **29j** | H | CH | N | 228 | NA | NA |
| **29k** | 2,6-2F | CH | N | 267 | NA | ＞10 |
| **29l** | 2-$CF_3$ | CH | N | 2488 | NA | NA |
| **29m** | 2-$NO_2$ | CH | N | 2335 | NA | ＞10 |
| **29n** | 2-Me | CH | N | 489 | NA | NA |
| **29o** | 2-Et | CH | N | 66.5 | (40.5±1.7) | 9.84 |
| **29p** | 2-MeO | N | CH | 210 | 3415 | ＞10 |
| **29q** | H | N | CH | (103.9±7.7) | NA | NA |
| **29r** | 2-Me | N | CH | (92.2±1.8) | NA | NA |
| **29s** | 2-Et | N | CH | (90.0±0.5) | NA | NA |

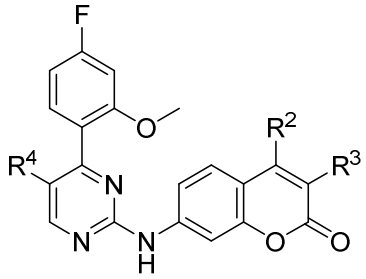
| | | | | | | |
|---|---|---|---|---|---|---|
| **29t** | 2-$CF_3$ | N | CH | (106.3±3.6) | NA | NA |
| **30a** | 4-F, 2-MeO | CH | N | 8.8 | 1328 | 0.25 |
| **30b** | 5-F, 2-MeO | CH | N | 21.8 | (49.6±4.1) | 1.74 |
| **30c** | 6-F, 2-MeO | CH | N | 13.7 | (58.7±1.0) | 0.52 |

[a] The mean values of $IC_{50}$ were measured in duplicate. [b] % Enzyme activities were measured at 1.0 μM concentration of test compound, n=2. NA: not determined.

Although compounds with 4-fluoro-2-methoxyl at the $R^1$ position and pyrimidin-2-ylamino in the hinge region showed good potency against CDK9, there was no evidence to clarify which hydrophilic group would be optimal after considering the cellular toxicity and physical properties. Therefore, hydrophilic heterocycles were investigated using the optimized structure of 4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl as the hydrophobic segment. The details of further optimization are shown in **Table 3**. Regrettably, substitution of the morpholino with 4-methylpiperazin-1-yl (**30d**), 4-ethylpiperazin-1-yl (**30e**) or piperidin-1-yl (**30f**) at the $R^2$ position did not significantly improve the cellular toxicity or CDK9 inhibitory activity. In the context of **30a**, replacement of the 3-acetyl with a 3-propionyl (**30g**) at the $R^3$ position exhibited a similar enzymatic potency and antiproliferative activity profile; however, replacement of the 3-acetyl with a hydrogen (**30h**) showed a significant reduction in cytotoxicity.

To further improve cellular toxicity, we introduced fluorine (**30i** and **30j**) or chlorine (**30o** and **30p**) to the C5-pyrimidine position. This modification had less of an impact on the inhibitory activity against CDK9. However, the cellular antiproliferative activity of **30i** ($R^4$ = F) was better than that of both **30o** ($R^4$ = Cl) and **30a** ($R^4$ = H). In the context of **30i**, the substitution with heterocyclic groups or a 3-propionyl group towards the solvent accessible region was explored. The 4-morpholino and 3-acetyl groups of **30i** turned out to be optimal in terms of improving cellular toxicity.

**Table 3.** Structures and biological evaluations of compounds **30a**, **30d** – **30p**.




| Comp. | $R^2$ | $R^3$ | $R^4$ | Enzymatic $IC_{50}$ /nM [a] (%Enzymatic activity) [b] | | Cell Inhibitory $IC_{50}$ (μM) [a] |
|---|---|---|---|---|---|---|
| | | | | CDK9/ cyclin T1 | CDK2/ cyclin A | MV4-11 |
| **30a** | morpholino | acetyl | H | 8.8 | 1328 | 0.25 |
| **30d** | 4-methylpiperazin-1-yl | acetyl | H | 16.4 | (74.8±1.3) | 0.52 |
| **30e** | 4-ethylpiperazin-1-yl | acetyl | H | 10.5 | (61.8±8.0) | 0.74 |
| **30f** | piperidin-1-yl | acetyl | H | 6.5 | (73.1±9.4) | 0.73 |
| **30g** | morpholino | propionyl | H | 3.6 | 1087 | 0.43 |
| **30h** | morpholino | H | H | 18.2 | 1330 | 2.28 |
| **30i** | morpholino | acetyl | F | 2.0 | 320 | 0.09 |
| **30j** | 4-methylpiperazin-1-yl | acetyl | F | 1.5 | 336 | 0.40 |
| **30k** | 4-ethylpiperazin-1-yl | acetyl | F | 1.2 | 305 | 0.55 |
| **30l** | 1,4-diazepan-1-yl | acetyl | F | 1.6 | 496 | 0.42 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **30m** | morpholino | propionyl | F | 0.7 | 338 | 0.42 |
| **30n** | morpholino | H | F | 1.6 | (22.0±0.1) | 3.11 |
| **30o** | morpholino | acetyl | Cl | 3.6 | 401 | 1.01 |
| **30p** | 4-methylpiperazin-1-yl | acetyl | Cl | 1.7 | 642 | 1.39 |

[a] The mean values of $IC_{50}$ were measured in duplicate. [b] % Enzyme activities were measured at 1.0 μM concentration of test compound, n=2.

## 2.4. Kinase Selectivity Profile

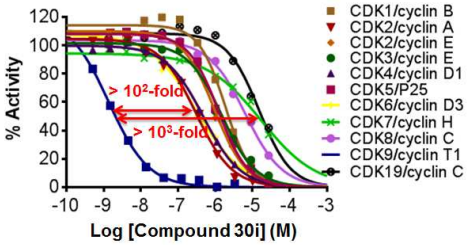
As the coumarin derivatives demonstrated satisfying CDK9 inhibitory activities, further studies of the kinase selectivity were conducted. Representative compound **30i** was screened in a panel of structurally similar CDK kinase isoforms, including CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclin E, CDK3/cyclin E, CDK4/cyclin D1, CDK5/p25, CDK6/cyclin D3, CDK7/cyclin H, CDK8/cyclin C, CDK9/cyclin T1 and CDK19/cyclin C. The CDK family screening results (**Figure 3A**/**B**) showed reasonable CDK9 selectivity. Compound **30i** exhibited the most prominent inhibition of CDK9/cyclin T1 ($IC_{50}$: 2 nM) and presented 160- to 978-fold selectivity over CDK1/2/3/4/5/6 (cell cycle CDKs) and more than 3250-fold selectivity over CDK7/8/19 (transcriptional CDKs), indicating that **30i** is highly selective for the inhibition of CDK9 among the CDK family members.

A

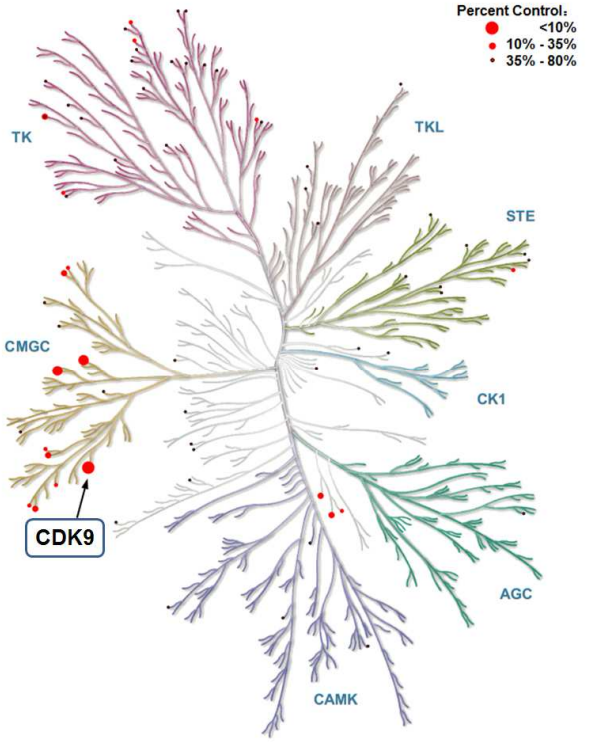
| CDKs | $IC_{50}$ (nM) | CDKs | $IC_{50}$ (nM) |
|---|---|---|---|
| CDK9/cyclin T1 | 2 | CDK5/p25 | 1261 |
| CDK1/cyclin B | 1955 | CDK6/cyclin D3 | 336 |
| CDK2/cyclin A | 320 | CDK7/cyclin H | 16,600 |
| CDK2/cyclin E | 1009 | CDK8/cyclin C | 6500 |
| CDK3/cyclin E | 1506 | CDK19/cyclin C | 15,540 |
| CDK4/cyclin D1 | 471 | | |

* The mean values of $IC_{50}$ are measured in duplicate.

B




C




"Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)"

**Figure 3.** Selectivity profiling of representative compound **30i**. (A) The $IC_{50}$ values and (B) *dose-activity curves* of different CDK family members demonstrated the high selectivity of **30i** for CDK9. (C) Comprehensive kinase selectivity profile of **30i** with 372 wild-type kinases. Determination was carried out in duplicate at a single dose concentration of 1.0 μM of **30i**, and the [γ-$^{33}$P]ATP-based enzymatic assay was performed by Reaction Biology Corporation. The TREEspot image was generated by the online KinMap program (http://kinhub.org/kinmap/) and the red circle codes for kinase activity.

In order to further investigate the kinase selectivity among other protein kinases, **30i** was screened against a comprehensive panel of 372 protein kinases using the kinase assay services at Reaction Biology Corporation, including the kinase families AGC, CAMK, CMGC, CK1, TK, TKL and others (**Figure 3C**; for detailed data, see supplementary **Table S1**). Impressively, **30i** showed the most potent inhibition against CDK9, strong inhibition against GSK-3α/β and MAK (enzymatic activity below 10%), medium inhibition against 8 types of kinases (enzymatic activity ranging from 10% to 35%), weak inhibition against 76 types of kinases (enzymatic activity ranging from 35% to 80%), and hardly any inhibition against 282 types of kinases (enzymatic activity

over 80%). The selectivity screening of CDK family isoforms and protein kinases demonstrates that **30i** is a significantly selective CDK9 inhibitor.

## 2.5. Molecular Modelling of Compound **30i** with CDK9

Given that compound **30i** was highly potent and selective inhibition of CDK9, we tried to understand the binding mechanism of **30i** by molecular modelling. The compound **30i** was docked into the active site of CDK9 (**Figure 4A**), and **30i** adopts the same binding mode as hit compound **7** and is located in a narrow cleft of the ATP binding site between the N-terminal and C-terminal lobes. The 7-(pyrimidin-2-ylamino)-2*H*-chromen-2-one core forms three hydrogen bonds in the hinge region of CDK9 (pyrimidin-2-ylamino 1N···H-N: Cys106, 2C-N-H···O: Cys106, and coumarin 2C=O···H-N Asp109). The pyrimidine ring is sandwiched between the hydrophobic side chains Ala46 and Leu156, forming van der Waals interactions at the adenine area of ATP (**Figure S4**). The phenyl ring of coumarin is contacted from above by Ile25 to enable Van der Waals interactions at the edge of the ATP-binding area. Furthermore, the 4-fluoro-2-methoxyphenyl substituent of **30i** occupies the hydrophobic region and likely induces lowering of the glycine-rich loop (G-Loop) into the ATP binding site, enhancing interactions within the ATP-binding pocket.

The C4-morpholino and C3-acetyl of coumarin are located in the entranceway of the ATP-binding pocket and extend into the solvent-accessible area. The C4-morpholino sticks out of the binding cleft and seemingly does not directly interact with CDK9, which explains the fact that small (i.e., C4-methyl) or bulky (i.e., C4-morpholino or C4-1,4-diazepan) functional groups are found to be favourable and tolerable. As mentioned, CDK9 possesses a more flexible and flatter active site than other CDKs [37]. The 3-acetyl-4-morpholino-2*H*-chromen-2-one moiety of **30i** is available to form a hydrogen bond with Asp109 and occupy the flexible hinge/αD region of CDK9 comfortably, whereas there is steric hindrance in the other CDK family members. Overlapping of the crystal structures of CDK2 (cyan, PDB: 4BCP) and CDK9 (grey, PDB: 4BCG), the steric hindrance from the Lys89 residue of CDK2 obviously hampers the interactions between CDK2 and the 3-acetyl-4-morpholino-2*H*-chromen-2-one moiety of **30i** (**Figure 4C**), which results in a structural reversal to avoid this steric hindrance and creates an unfavourable binding model when **30i** is docked into CDK2 (**Figure S4**). The steric hindrance also exists in other CDKs, which may explain the highly selective inhibition against CDK9.

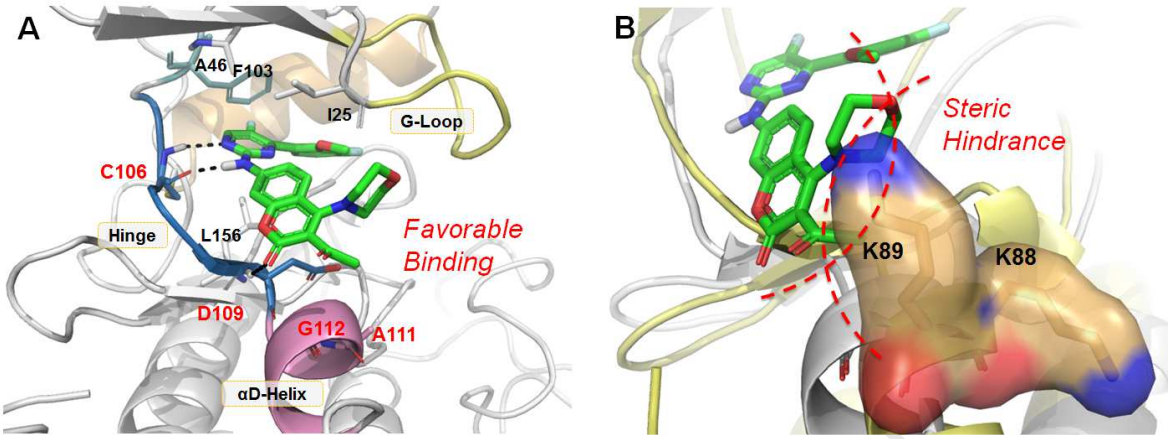



**Figure 4.** Binding mode analysis of compound **30i** docked into CDK9 kinase (grey ribbon representation, PDB code: 4BCG). The docking images were generated by the PyMOL molecular graphics system. The key residues between CDK9 and CDK2 are labelled, and hydrogen bonds in all panels are depicted by black dotted lines. (A) Compound **30i** (green stick model) docked into CDK9. (B) Comparison of the binding mode of **30i** within CDK9 overlaid onto the equivalent region of CDK2. The steric hindrance is shown by red dotted lines.

## 2.6. *In Vitro* Cell Assays

Further cellular antiproliferative activities of **30a** and **30i** were evaluated against a panel of different types of cancer cell lines (e.g., pancreatic cancer, gastric cancer, melanoma, liver cancer, breast cancer, colon cancer and non-small-cell lung cancer and leukaemia). The results are depicted in **Table 4**. As expected, the growth of all cancer cell lines was suppressed. **30i** displayed much higher antiproliferative activities than **30a**. In particular, the $IC_{50}$ values of **30i** inhibition against BxPC-3 (pancreatic cancer), A-375 (melanoma), Hep G2 (liver cancer) and MV-4-11 (leukaemia) cells were less than 0.1 μM, indicating that **30i** might be the most prominent antitumour agent among this series.

**Table 4**. Antiproliferative Activities of **30a** and **30i** against a Panel of Human tumour Cell Lines.

| Panel | Cell line | $IC_{50}$ (μM) [a] | |
|---|---|---|---|
| | | **30a** | **30i** |
| Pancreatic Cancer | BxPC-3 | 0.91 | 0.08 |
| | PANC-1 | 1.88 | 0.30 |
| | MIA PaCa-2 | 1.60 | 0.46 |
| Gastric Cancer | NCI-N87 | 3.58 | 0.60 |
| Melanoma | A-375 | 0.96 | 0.09 |

| | | | |
|---|---|---|---|
| Liver Cancer | Hep G2 | 0.56 | 0.10 |
| Breast Cancer | MDA-MB-231 | 1.58 | 0.23 |
| Colon Cancer | SW620 | 2.70 | 0.43 |
| | HCT-116 | 0.66 | 0.28 |
| Non-Small Cell | A549 | 2.36 | 0.47 |
| Lung Cancer | NCI-H460 | 2.15 | 0.64 |
| Leukaemia | molm-13 | 0.26 | 0.26 |
| | MV-4-11 | 0.25 | 0.09 |

[a] The mean $IC_{50}$ values were measured in duplicate.

## 2.7. Pharmacokinetics of **30a** and **30i** in Liver Microsomes of Multiple Species

Following the enzymatic and cellular activities evaluation, a preliminary metabolic stability study of **30a** and **30i** was assessed by *in vitro* liver microsomes of multiple species, including *Homo sapiens*, monkeys, dogs and rats. The results are shown in **Table 5**. These compounds had moderate terminal half-lives ($T_{1/2}$) with values of at least 0.5 h in human liver microsomes. This series of compounds has an acceptable level of metabolic stability for subsequent *in vivo* evaluations.

**Table 5.** Pharmacokinetics in Liver Microsomes of Multiple Species.

| **Compounds** | **$T_{1/2}$** [a] (min) | **$CL_{int}$** [b] (μL/min/mg protein) | **Species** |
|---|---|---|---|
| **30a** | 33.5 | 41.4 | Human |
| | 22.2 | 62.5 | Monkey |
| | 14.1 | 98.3 | Dog |
| | 9.61 | 144 | Rat |
| **30i** | 35.7 | 38.8 | Human |
| | 24.2 | 57.3 | Monkey |
| | 14.0 | 98.7 | Dog |
| | 2.01 | 689 | Rat |
| Midazolam [c] | 12.1 | 287 | Human |
| | 6.29 | 551 | Monkey |
| | 2.31 | 1499 | Dog |
| | 3.82 | 908 | Rat |

[a] $T_{1/2}$ is half-life. [b] $CL_{int}$ is the intrinsic clearance. [c] Midazolam was used as control compound.

## 2.8. hERG assay

Inhibition of the cardiovascular ion channel hERG is the most important mechanism for drug-induced

prolongation of QT interval [51]. To understand the cardiotoxic effects, we assayed the hERG activity on cardiovascular ion channels before the compound advanced into *in vivo* studies. The assay was based on the competition of a fluorescently labelled tracer binding to a membrane preparation containing hERG. The results are shown in **Figure S5**. Compound **30i** had low hERG inhibitory activity ($IC_{50}$: 10080 nM) and over 5000-fold selectivity for CDK9, indicating that it has low potential for detrimental effects on the heart.

## 2.9. Pharmacokinetic Profile of **30i** *in vivo*

As compound **30i** showed high potency *in vitro*, the pharmacokinetic profile was evaluated in ICR mice through oral gavage (p.o.) and intravenous administration (i.v.). The plasma concentration-time curve is shown in **Figure S6**. After a single 30 mg/kg dose administered by oral gavage, the maximum concentration ($C_{max}$) reached 665 ng/mL, the area under the curve ($AUC_{0-t}$) was 1200 ng/mL·h and the bioavailability (F%) was 14.3%. After intravenous administration of a 2 mg/kg dose, the half-life ($T_{1/2}$) was found to be 0.23 h, the clearance (CL) was 3.56 L/h/kg, and the volume of distribution ($Vd_{ss}$) was 0.67 L/kg. The moderate pharmacokinetic (PK) properties (**Table 6**) suggested that compound **30i** was suitable for intravenous administration to evaluate its antitumour efficacy *in vivo*.

**Table 6**. Pharmacokinetic analysis of **30i** in ICR mice by p.o. and i.v. route.

| | | **30i** | |
|---|---|---|---|
| Parameters [a] | Unit | p.o., 30 mg/kg | i.v., 2 mg/kg |
| $T_{1/2}$ | h | NR | 0.23 |
| $T_{max}$ | h | 0.25 | - |
| $C_0$ | $ng \cdot mL^{-1}$ | - | 3060 |
| $C_{max}$ | $ng \cdot mL^{-1}$ | 665 | - |
| $AUC_{0-t}$ | $ng \cdot h \cdot mL^{-1}$ | 1200 | 560 |
| $AUC_{0-\infty}$ | $ng \cdot h \cdot mL^{-1}$ | NR | 561 |
| CL | $L \cdot kg^{-1} \cdot h^{-1}$ | - | 3.56 |
| $Vd_{ss}$ | $L \cdot kg^{-1}$ | - | 0.67 |
| F | % | 14.3 | - |

[a] ICR mice (n = 9). Parameters were calculated from composite mean plasma concentration-time data. NR, no record.

## 2.10. *In Vivo* Efficacy of Compound **30i** in the MV4-11 Tumour Xenograft Model

Compound **30i** showed the best tumour cell growth inhibition *in vitro*, which facilitated us to further study its potential as an antitumour agent. The antitumour efficacy of **30i** was assessed in an MV4-11 mice xenograft model with intravenous administration for 21 consecutive days. When the tumours grew to an average volume of 180 $mm^3$, the mice were randomly assigned to five groups (6 mice per group). Three experimental groups received 10, 20 or 40 mg/kg (mpk) doses of **30i**, and the other two control groups were treated with vehicle (saline) as a negative control and a 40 mpk dose of cytarabine as a positive control [52].

The antitumour efficacy is shown in **Figure 5**. Compound **30i** demonstrated marked inhibition of tumour growth *in vivo* in a dose-dependent manner. In particular, treatment with 40 mpk doses of **30i** significantly suppressed the tumour progression and tumour growth inhibition (TGI) values up to 100% from days 14 to 32. Compound **30i** was more efficacious than cytarabine at the same dose of 40 mpk. It is worth noting that no obvious body weight loss or mortality occurred in any of the mice groups during the treatment period, indicating that there was no general cytotoxicity below a dose of 40 mg/kg/day and a good safety window for further biological evaluation. The significant reduction in tumour volume was photographed at the end of treatment. The average tumour weight after treatment with **30i** (experimental group of 40 mpk doses) was 0.18 g, which was less than the tumour weights from the positive control group of cytarabine (0.26 g) and negative control (1.00 g) groups.

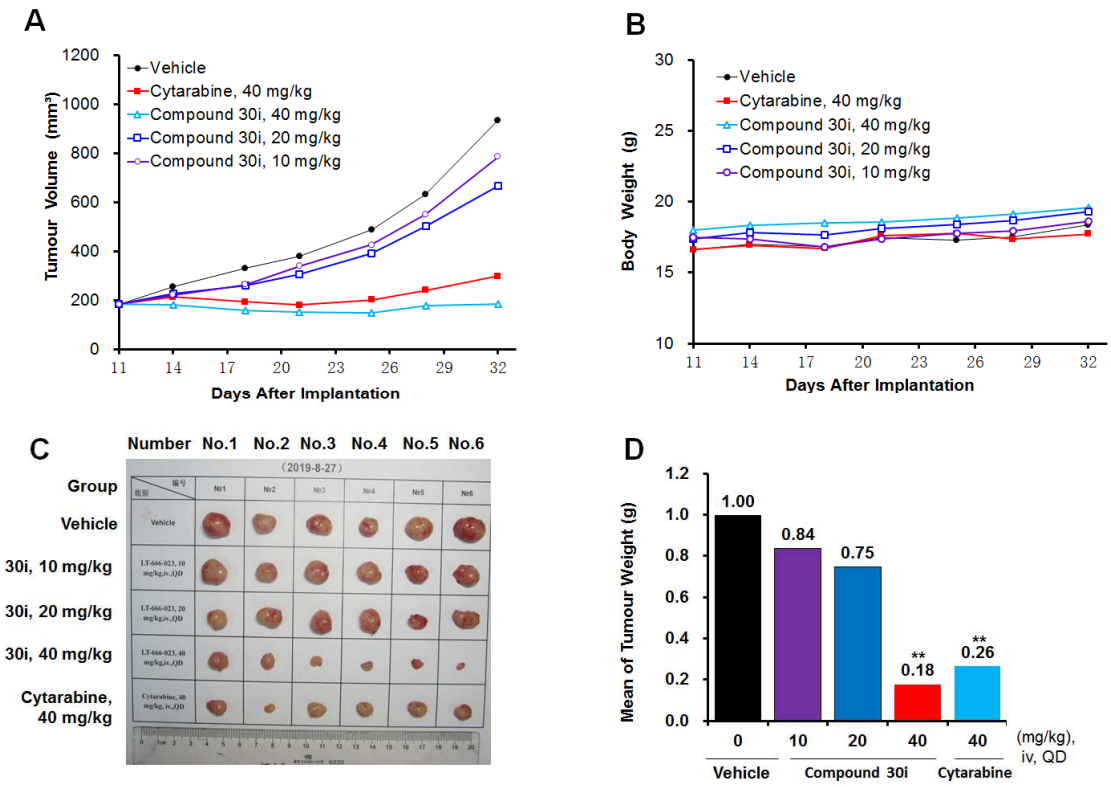



**Figure 5**. The antitumour efficacy of **30i** in the MV4-11 xenograft model. Female BALB/c nude mice bearing MV4-11 tumour xenografts were treated by intravenous injection (i.v., QD) with **30i** at 0 (vehicle), 10, 20, or 40 mpk or with cytarabine at 40 mpk. (A) Tumour volumes and (B) body weights were measured in the MV4-11 xenograft mice after **30i** or cytarabine administration. (C) Representative photographs of the tumours in each group after treatment with 0 (vehicle), 10, 20, or 40 mpk **30i** or 40 mpk cytarabine. (D) Comparison of the final tumour weight in each group after 21 days treatment period. Numbers on the columns indicate the mean tumour weight in each group. (**) $p < 0.01$.

## 2.11. Cellular Mode of Action

The cellular mode of action of **30i** was investigated for CDK9-mediated signalling pathways by western blot analysis. As expected, after treatment of MV4-11 cells for a period of 24 h, a gradual decrease in phosphorylation at Ser2 of the CTD of RNAPII (p-RNAPII (S2), a CDK9-mediated phosphorylation site) was observed in a dose-dependent manner. The expression levels of Mcl-1 and c-Myc were clearly reduced at a concentration of 0.1 μM **30i**, and the complete disappearance of Mcl-1 and c-Myc were observed in the range

of 0.2 μM to 0.8 μM **30i**. Analogous results were also observed with 0.8 μM flavopiridol, such as a decrease in phosphorylation at Ser2 of the CTD of RNAPII and a reduction in Mcl-1 and c-Myc. Thus, cellular CDK9 inhibition with compound **30i** was achieved.

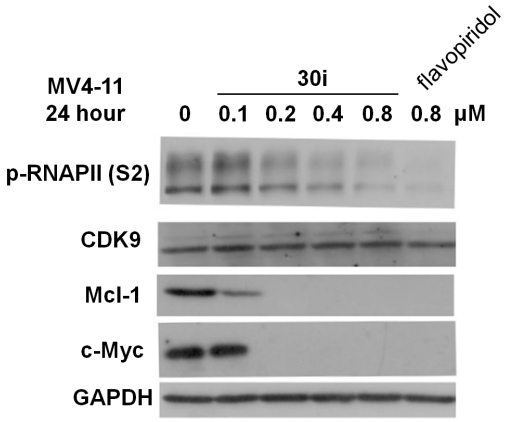



**Figure 6**. Western blot analysis of MV4-11 cells treated with **30i** or Flavopiridol for 24 h. GAPDH antibody was used as an internal control.

## 3. Conclusions

Specific inhibition of CDK9 has been validated as a viable approach for targeted cancer therapy. In this paper, we discovered a coumarin derivative **7** as a hit molecule with selective inhibition against CDK9. Then, a series of derivatives of 7-(pyrimidin-2-ylamino)-2*H*-chromen-2-one and 7-(pyrimidin-4-ylamino)-2*H*-chromen-2-one were designed, synthesized and evaluated as antitumour agents. This effort led to the discovery of a highly promising potent and selective CDK9 inhibitor **30i**, which effectively inhibited CDK9 with an $IC_{50}$ value of 2 nM and presented 160- to 978-fold selectivity versus CDK1/2/3/4/5/6 (cell cycle CDKs) and over 3250-fold selectivity versus CDK7/8/19 (transcriptional CDKs). Further profiles of kinase selectivity showed that **30i** most selectively inhibit CDK9 among 372 kinases. Analysis of the binding mode demonstrated that the 3-acetyl-4-morpholino-2*H*-chromen-2-one moiety occupied the flexible hinge/αD region of CDK9 which shows the steric hindrance in other CDKs, and this may account for its remarkable selectivity. Furthermore, **30i** demonstrated impressive antiproliferative activity in a panel of tumour cell lines, including leukaemia, pancreatic cancer, gastric cancer, melanoma, liver cancer, breast cancer, colon cancer and non-small-cell lung cancer. The moderate metabolic stability in liver microsomes and low hERG inhibition demonstrated

acceptable drug-like properties. *In vivo*, **30i** exhibited a moderate pharmacokinetic profile, and it significantly induced tumour growth inhibition in a dose-dependent manner in an MV4-11 (AML) mice xenograft model without causing mortality or an obvious loss of body weight. The study of cellular mode of action suggests **30i** downregulated Mcl-1 and c-Myc by dose-dependently inhibiting the CDK9-mediated phosphorylation of RNAPII. In summary, this study identified a promising coumarin-based CDK9 inhibitor **30i** with high potency and specificity which may shed light for developing CDK9-mediated cancer therapies.

## 4. Experimental section

4.1. General Methods. All reactions were monitored by analytical thin-layer chromatography (TLC), which was visualized by ultraviolet light (254 nm or 356 nM). All solvents were obtained from commercial sources and were purified according to standard procedures. Purification of the products was accomplished by flash chromatography using silica gel (100-200 mesh). Melting points were determined by X-4 digital display micro-melting point apparatus (Beijing Tech Instrument Co., Ltd.). All NMR spectra were recorded with Bruker AVANCE AV-600 spectrometer at 300 MHz or 400 MHz in DMSO or $CDCl_3$: chemical shifts ($\delta$) are given in ppm, coupling constants ($J$) in Hz and following abbreviations are used to indicate the multiplicity in NMR spectra: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet). The solvent signals were used as references (residual DMSO in DMSO-$d_6$: $\delta_H$ = 2.50 ppm, $\delta_c$ = 39.5 ppm; $CHCl_3$ in $CDCl_3$: $\delta_H$ = 7.26 ppm, $\delta_c$ = 77.0 ppm). Mass spectra were obtained on the Agilent 1100 LC/MSD mass spectrometer (Agilent, USA) and Q-tofmicro MS (micromass company). High resolution mass spectrometry (HRMS) was recorded on TOF perimer for $ES^+$. The purity of biologically evaluated compounds was >95% as determined by HPLC analysis (Agilent C18 column, $CH_3OH$ and $H_2O$ as the mobile phase, monitored by UV absorption at 250 and 365 nm).

4.1.1. *7-((tert-butyldimethylsilyl)oxy)-4-methyl-2H-chromen-2-one* **(9)**. 7-hydroxy-4-methyl-2*H*-chromen-2-one (1.76 g, 10.00 mmol, purchased from BidePharm Company Inc.) and t-butyldimethylsilyl chloride (4.52 g, 30.00 mmol) were dissolved in DMF (50 mL) at room temperature and then treated with 1*H*-imidazole (3.40 g, 50.00 mmol). After 6 hours ethyl ether (50 mL × 3) and water (250 mL) was poured into the solution and the reaction mixture was transferred into a separation funnel. The reaction mixture was washed with brine (25 mL × 3). The organic layer was dried over magnesium sulfate and concentrated to yield the residue, which was chromatographed (ethyl acetate/petroleum, 4%) on silica gel to afford white solid (2.10 g, 72%). ESI-MS *m/z*: 291 $[M+H]^+$.

4.1.2. *3-bromo-7-((tert-butyldimethylsilyl)oxy)-4-methyl-2H-chromen-2-one* **(10)**. N-bromosuccinimide (2.14 g, 12.00 mmol) was added to a solution of **9** (2.90 g, 10.00 mmol) in $CH_3CN$ (50 mL). The mixture was stirred at room temperature for 9 h, then ethyl acetate (50 mL × 3) and water (250 mL) was poured into the solution and the reaction mixture was transferred into a separation funnel. The reaction mixture was washed with brine (25 mL × 3). The organic layer was dried over magnesium sulfate and concentrated to yield the

residue, which was chromatographed (ethyl acetate/petroleum, 1%) on silica gel to afford white solid (2.96 g, 80%). ESI-MS *m/z*: 370 $[M+H]^+$.

4.1.3. *7-((tert-butyldimethylsilyl)oxy)-3-(1-ethoxyvinyl)-4-methyl-2H-chromen-2-one* **(11)**. **10** (1.00 g, 2.70 mmol) was combined with tributyl(1-ethoxyvinyl)stannane (1.82 mL, 5.40 mmol) and $PdCl_2(PPh_3)_2$ (189 mg, 0.27 mmol) in anhydrous DMF (30 mL). The mixture was stirred at 80 °C for 6 h, then ethyl acetate (50 mL × 3) and water (150 ml) was poured into the solution and the reaction mixture was transferred into a separation funnel. The reaction mixture was washed with brine (50 mL × 3). The organic layer was dried over magnesium sulfate and concentrated to yield the crude product (0.73 g, 75%), which was available in the next step without further purification.

4.1.4. *3-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one* (**12**). The crude intermediate of **11** (500 mg, 0.39 mmol) was dissolved in HCl solution (20 mL, 6M in methanol) and was stirred at 80 °C for 12 h. After completion of the reaction as monitored by TLC, the mixture was cooled to room temperature. The pH of solution was adjusted to 7 by NaOH slowly. The solvent was evaporated under reduced pressure. The residue was purified by chromatography (ethyl acetate/hexane, 25%) on silica gel to afford white solid (187 mg, 62%). $^1$H-NMR(300 MHz, DMSO-$d_6$) $\delta$ 10.81 (s, 1H), 7.75 (d, $J$ = 8.8 Hz, 1H), 6.86 (dd, $J$ = 8.8, 2.3 Hz, 1H), 6.75 (d, $J$ = 2.3 Hz, 1H), 2.46 (s, 3H), 2.34 (s, 3H). ESI-MS *m/z*: 241 $[M+Na]^+$.

4.1.5. *3-acetyl-4-methyl-2-oxo-2H-chromen-7-yl trifluoromethanesulfonate* **(13)**. To an oven-dried 25 mL round-bottom flask was charged with **12** (110 mg, 0.50 mmol) and anhydrous triethylamine (78 μL, 0.60 mmol) in dry dichloromethane (10 mL). The resulting mixture was stirred at -10 $^oC$ for 15 mins, then trifluormethanesulfonic anhydride (100 μL, 0.60 mmol) was added dropwise over a period of 15 min. The mixture was further stirred at room temperature for additional 1 h until the completion of the reaction as monitored by TLC. The reaction was quenched with a saturated solution of aqueous $NaHCO_3$ in an ice bath and washed with water. The organic layer was dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was purified by chromatography (ethyl acetate/hexane, 15%) on silica gel to afford white solid (148 mg, 84%). $^1$H-NMR(300 MHz, DMSO-$d_6$) $\delta$ 8.10 (d, $J$ = 8.9 Hz, 1H), 7.83 (d, $J$ = 2.4 Hz, 1H), 7.59 (dd, $J$ = 8.9, 2.4 Hz, 1H), 2.48 (s, 3H), 2.40 (s, 3H). ESI-MS *m/z*: 373 $[M+Na]^+$.

4.1.6.1. *4-(2-methoxyphenyl)pyrimidin-2-amine* **(15a)**. To a round-bottom flask was charged with 4-

chloropyrimidin-2-amine (500 mg, 3.9 mmol), 2-methoxyphenylboronic acid (608 mg, 4.0 mmol) $Pd(dppf)Cl_2$ (0.2 mmol) and base $Na_2CO_3$ (7.8 mmol) under nitrogen atmosphere, then 1,4-dioxane (21 mL) and water (3 mL) were added and the vessel was immediately sealed tightly. The resulting mixture was heated at 95 °C for 2 h until the completion of the reaction as monitored by TLC. The cooled mixture was diluted with water and exhaustively extracted with ethyl acetate (30 mL × 3). The organic phase was washed by brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (678 mg, 86%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.23 (d, *J* = 5.2 Hz, 1H), 7.79 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.38 – 7.49 (m, 1H), 7.14 (d, *J* = 8.1 Hz, 1H), 7.00 – 7.10 (m, 2H), 6.58 (s, 2H), 3.84 (s, 3H). ESI-MS *m/z*: 202 $[M+H]^+$.

4.1.6.2. *6-(2-methoxyphenyl)pyrimidin-4-amine* **(15b)**. The title compound was prepared according to the same procedure of **15a** on 6-chloropyrimidin-4-amine (500 mg, 3.9 mmol) and (2-methoxyphenyl)boronic acid (608 mg, 4.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (661 mg, 87%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.41 (d, *J* = 1.2 Hz, 1H), 7.85 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.44–7.38 (m, 1H), 7.14 (dd, *J* = 8.4, 1.1 Hz, 1H), 6.99–7.08 (m, 2H), 6.83 (s, 2H), 3.85 (s, 3H).ESI-MS *m/z*: 202 $[M+H]^+$.

4.1.7.1. *3-acetyl-7-((4-(2-methoxyphenyl)pyrimidin-2-yl)amino)-4-methyl-2H-chromen-2-one* **(16a).** To a round-bottom flask was charged with the intermediate **15a** (24 mg, 0.12 mmol), **13** (42 mg, 0.12 mmol), $Pd(OAc)_2$ (0.01 mmol), Xantphos (0.01 mmol) and base $Cs_2CO_3$ (78 mg, 0.24 mmol) under nitrogen atmosphere, then anhydrous toluene (5 mL) was added and the vessel was immediately sealed tightly. The resulting mixture was heated at 100 °C for 6 h until the completion of the reaction as monitored by TLC. The cooled mixture was diluted with water and exhaustively extracted with ethyl acetate (10 mL × 3). The organic phase was washed by brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford products (27 mg, 56% yield) as a white solid. mp: 140-142 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 10.38 (s, 1H), 8.62 (d, *J* = 5.2 Hz, 1H), 8.20 (d, *J* = 1.8 Hz, 1H), 7.88 – 7.96 (m, 1H), 7.84 (d, *J* = 8.9 Hz, 1H), 7.74 (dd, *J* = 8.9, 1.9 Hz, 1H), 7.42 – 7.59 (m, 2H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.12-7.17 (m, 1H), 3.90 (s, 3H), 2.48 (s, 3H), 2.37 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 201.32, 163.71, 159.88, 159.27, 158.45, 158.13, 154.08, 151.40, 145.86, 132.39,

130.72, 127.42, 126.17, 123.67, 121.19, 115.82, 114.62, 113.01, 112.73, 104.34, 56.19, 31.45, 15.58. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{23}H_{20}N_3O_4$: 402.1448, found: 402.1456. HPLC retention time 5.85 min, purity 97.5%.

4.1.7.2. *3-acetyl-7-((6-(2-methoxyphenyl)pyrimidin-4-yl)amino)-4-methyl-2H-chromen-2-one* **(16b).** The title compound was prepared according to the same procedure of **16a** on 0.36 mmol scale **15b** and **13**. Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford products (76 mg, 53% yield) as a white solid. mp: 234-236 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 10.29 (s, 1H), 8.87 (s, 1H), 8.19 (d, *J* = 2.0 Hz, 1H), 8.00 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.88 (d, *J* = 8.9 Hz, 1H), 7.65 – 7.56 (m, 2H), 7.54 – 7.45 (m, 1H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.08 – 7.13 (m, 1H), 3.93 (s, 3H), 2.49 (s, 3H), 2.38 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 201.29, 160.64, 160.32, 159.13, 158.19, 158.13, 153.92, 151.16, 145.16, 132.02, 130.76, 127.62, 125.87, 124.17, 121.15, 116.26, 113.63, 112.67, 108.79, 105.36, 56.27, 31.44, 15.60. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{23}H_{20}N_3O_4$ : 402.1448, found: 402.1450. HPLC retention time 4.75 min, purity 99.1 %.

4.1.8. *4-hydroxy-7-methoxy-2H-chromen-2-one* **(18)**. To the solution of paeonol (10.0 g, 60.20 mmol) in anhydrous toluene (100 mL) was added diethyl carbonate (10.7 g, 90.30 mmol), then the suspension of NaH (60% dispersion in mineral oil, 12.0 g, 300.90 mmol) in anhydrous toluene (50 mL) was added dropwise over a period of 30 min and was stirred in an ice bath. The mixture was further stirred at 100 °C for 4 h, then it was cooled to 0 °C. The residual NaH of mixture was quenched with cool water in an ice bath carefully and washed with diethyl ether (50 mL × 3). The aqueous phase was acidified to pH 3 with 2M HCl slowly, then the white precipitation was occurred. The resulting precipitated solid was collected by filtration, washed with water (50 mL × 3) followed by petroleum ether (20 mL × 3), and then dried overnight to afford white solid (10.67 g, 92%). $^1$H-NMR(300 MHz, DMSO-$d_6$) $\delta$ 12.36 (s, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 6.95 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 5.45 (s, 1H), 3.85 (s, 3H). ESI-MS *m/z*: 215 $[M+Na]^+$.

4.1.9.1. *3-acetyl-4-hydroxy-7-methoxy-2H-chromen-2-one* (**19a**). To an oven-dried 100 mL round-bottom flask was charged with **18** (3.00 g, 15.61 mmol), phosphorus oxychloride (5.6 mL) and acetic acid (16 mL), then the resulting mixture was heated at 105 °C for 1 h. After the completion of the reaction as monitored by TLC, the mixture was slowly cooled to room temperature and the white precipitation was occurred. The

resulting precipitated solid was collected by filtration and was crystallized by ethanol to afford white solid (2.65 g, 72%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 17.98 (s, 1H), 7.93 (d, $J$ = 9.5 Hz, 1H), 7.04 – 6.99 (m, 2H), 3.91 (s, 3H), 2.64 (s, 3H). ESI-MS *m/z*: 257 $[M+Na]^+$.

4.1.9.2. *3-ethyl-4-hydroxy-7-methoxy-2H-chromen-2-one* (**19b**). To an oven-dried 25 mL round-bottom flask was charged with **19a** (100 mg, 0.28 mmol), sodium cyanoborohydride (36 mg, 0.57 mmol) and acetic acid (2 mL), then the resulting mixture was stirred at room temperature for 0.5 h. After the completion of the reaction as monitored by TLC, the mixture was slowly cooled to room temperature and the white precipitation was occurred. The resulting precipitated solid was collected by filtration and was crystallized by ethanol to afford white solid (75 mg, 78%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 11.12 (s, 1H), 7.85 – 7.78 (m, 1H), 6.93 (dd, $J$ = 4.4, 2.2 Hz, 2H), 3.84 (s, 3H), 2.47 (d, $J$ = 7.3 Hz, 2H), 1.02 (t, $J$ = 7.3 Hz, 3H).ESI-MS *m/z*: 221 $[M+H]^+$.

4.1.9.3. *4-hydroxy-7-methoxy-3-propionyl-2H-chromen-2-one* (**19c**). To an oven-dried 100 mL round-bottom flask was charged with **18** (3.00 g, 15.61 mmol), phosphorus oxychloride (5.6 mL) and propionic anhydride (31 mL, 31.22 mmol), then the resulting mixture was heated at 105 °C for 1 h. After the completion of the reaction as monitored by TLC, the mixture was slowly cooled to room temperature and the white precipitation was occurred. The resulting precipitated solid was collected by filtration and was crystallized by ethanol to afford white solid (2.68 g, 58%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 17.73 (s, 1H), 7.90 (s, 1H), 7.00 (m, 2H), 3.91 (s, 3H), 3.11 (dd, $J$ = 7.5, 3.8 Hz, 2H), 1.10 (t, $J$ = 7.5 Hz, 3H). ESI-MS *m/z*: 249 $[M+H]^+$.

4.1.10. General Procedure A for Synthesis of Compound **20a-20g**. To an oven-dried round-bottom flask was charged with compound **19a-19c** (1.0 equiv) and the corresponding amine (10.0 equiv), then the resulting mixture was refluxed for a period time (usually 2-6 h) until the completion of the reaction as monitored by TLC. The mixture was slowly cooled to room temperature and was poured into water. Then the resulting mixture exhaustively extracted with dichloromethane (50 mL × 3). The organic phase was washed by brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel using ethyl acetate/petroleum ether as the eluent to afford the products.

4.1.10.1. *3-acetyl-7-methoxy-4-morpholino-2H-chromen-2-one* (**20a**). The title compound was prepared according to general procedure A on **19a** (1.00 g, 4.27 mmol) and morpholine (3.72 mL, 42.70 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford white solid

(677 mg, 52%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 7.92 (d, *J* = 8.8 Hz, 1H), 7.16 (d, *J* = 2.4 Hz, 1H), 7.07 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.90 (s, 3H), 3.72–3.24 (m, 8H), 2.34 (s, 3H). ESI-MS *m/z*: 326 $[M+Na]^+$.

4.1.10.2. *3-ethyl-7-methoxy-4-morpholino-2H-chromen-2-one* (**20b**). The title compound was prepared according to general procedure A on **19b** (100 mg, 0.45 mmol) and morpholine (0.5 mL, 5.7 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford white solid (98 mg, 75%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.71 (d, *J* = 9.5 Hz, 1H), 6.92 (d, *J* = 6.5 Hz, 2H), 3.83 (d, *J* = 5.5 Hz, 3H), 3.79 (d, *J* = 3.9 Hz, 4H), 3.28 – 3.16 (m, 4H), 2.62 – 2.52 (m, 2H), 1.10 (t, *J* = 7.3 Hz, 3H). ESI-MS *m/z*: 290 $[M+H]^+$.

4.1.10.3. *7-methoxy-4-morpholino-3-propionyl-2H-chromen-2-one* (**20c**). The title compound was prepared according to general procedure A on **19c** (100 mg, 0.4 mmol) and morpholine (0.5 mL, 5.7 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford white solid (87 mg, 69%). ESI-MS *m/z*: 318 $[M+H]^+$.

4.1.10.4. *3-acetyl-7-methoxy-4-(4-methylpiperazin-1-yl)-2H-chromen-2-one* (**20d**). The title compound was prepared according to general procedure A on **19a** (3.00 g, 12.8 mmol) and 1-methylpiperazine (14.2 mL, 128.1 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford white solid (1.94 g, 48%). ESI-MS *m/z*: 339 $[M+Na]^+$.

4.1.10.5. *3-acetyl-4-(4-ethylpiperazin-1-yl)-7-methoxy-2H-chromen-2-one* (**20e**). The title compound was prepared according to general procedure A on **19a** (3.00 g, 12.8 mmol) and 1-ethylpiperazine (16.3 mL, 128.1 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford white solid (1.28 g, 30%). ESI-MS *m/z*: 331 $[M+H]^+$.

4.1.10.6. *3-acetyl-7-methoxy-4-(piperidin-1-yl)-2H-chromen-2-one* **(20f)**. The title compound was prepared according to general procedure A on **19a** (1.00 g, 4.27 mmol) and piperidine (3.9 mL, 42.70 mmol). After extraction of the resulting mixture, the solvent was removed under reduced pressure to get the residue without purification.

4.1.10.7. *tert-butyl 4-(3-acetyl-7-methoxy-2-oxo-2H-chromen-4-yl)-1,4-diazepane-1-carboxylate* (**20g**). The title compound was prepared according to general procedure A on **19a** (100 mg, 0.40 mmol) and *tert*-butyl 1,4-diazepane-1-carboxylate (0.8 mL, 4.06 mmol). After extraction of the resulting mixture, the solvent was

removed under reduced pressure to get the residue without purification.

4.1.11. *4-chloro-7-methoxy-2H-chromen-2-one* (**21**). To an oven-dried 250 mL round-bottom flask was charged with **18** (5.00 g, 26.02 mmol) and phosphorus oxychloride (39 mL). Then triethylamine (4.33 mL, 31.22 mmol) was slowly added to the flask in an ice bath and the resulting mixture was heated at 100 °C for 3 h. After the completion of the reaction as monitored by TLC, the mixture was cooled to room temperature and was poured into ice water, stirred, and then exhaustively extracted with ethyl acetate (50 mL × 3). The organic phase was washed by brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was purified by chromatography (ethyl acetate/hexane, 20%) on silica gel to afford white solid (4.30 g, 79%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 7.81 (d, $J$ = 8.8 Hz, 1H), 7.12 (d, $J$ = 2.2 Hz, 1H), 7.08 (dd, $J$ = 8.8, 2.4 Hz, 1H), 6.73 (s, 1H), 3.91 (s, 3H). ESI-MS *m/z*: 233 $[M+Na]^+$.

4.1.12. *4-chloro-7-hydroxy-2H-chromen-2-one* (**22**). To an oven-dried 100 mL round-bottom flask was charged with **21** (1.00 g, 4.75 mmol), aluminium chloride (2.08 g, 15.68 mmol) and anhydrous toluene (30 mL), then the resulting mixture was refluxed for 4 h. After the completion of the reaction as monitored by TLC, the mixture was slowly cooled to room temperature. The solvent was evaporated under reduced pressure, than The residue was purified by chromatography (ethyl acetate/hexane, 30%) on silica gel to afford white solid (769 mg, 82%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 10.95 (s, 1H), 7.71 (d, $J$ = 8.8 Hz, 1H), 6.90 (dd, $J$ = 8.8, 2.2 Hz, 1H), 6.79 (d, $J$ = 2.2 Hz, 1H), 6.62 (s, 1H). ESI-MS *m/z*: 219 $[M+Na]^+$.

4.1.13. General Procedure B for Synthesis of Compound **23a-23g**. To an oven-dried round-bottom flask was charged with compound **20a-20g** (1.0 equiv), aluminium chloride (3.3 equiv) and anhydrous toluene (30 mL), then the resulting mixture was refluxed for a period time (usually 3-6 h) until the completion of the reaction as monitored by TLC. The mixture was slowly cooled to room temperature. The solvent was evaporated under reduced pressure, then the residue was purified by chromatography on silica gel to afford products.

4.1.13.1. *3-acetyl-7-hydroxy-4-morpholino-2H-chromen-2-one* (**23a**). To an oven-dried 100 mL round-bottom flask was charged with **20a** (1.00 g, 3.30 mmol), aluminium chloride (1.45 g, 10.90 mmol) and anhydrous toluene (30 mL), then the resulting mixture was refluxed for 4 h. After the completion of the reaction as monitored by TLC, the mixture was slowly cooled to room temperature. The solvent was evaporated under reduced pressure, then the residue was purified by chromatography (ethyl acetate/petroleum

ether, 65%) on silica gel to afford products white solid (653 mg, 68%). ESI-MS *m/z*: 312 $[M+Na]^+$.

4.1.13.2. *3-ethyl-7-hydroxy-4-morpholino-2H-chromen-2-one* (**23b**). The title compound was prepared according to general procedure B on 0.3 mmol scale **20b**. Purification by column chromatography (ethyl acetate/petroleum ether, 65%) on silica gel to afford white solid (50 mg, 53%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.36 (s, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 6.78 (d, *J* = 8.8 Hz, 1H), 6.66 (s, 1H), 3.78 (s, 4H), 3.23 (s, 4H), 2.61 – 2.52 (m, 2H), 1.09 (t, *J* = 7.2 Hz, 3H). ESI-MS *m/z*: 276 $[M+Na]^+$.

4.1.13.3. *7-hydroxy-4-morpholino-3-propionyl-2H-chromen-2-one* (**23c**). The title compound was prepared according to general procedure B on 0.88 mmol scale **20c**. Purification by column chromatography (ethyl acetate/petroleum ether, 65%) on silica gel to afford white solid (180 mg, 67%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.74 (s, 1H), 7.86 (d, *J* = 8.7 Hz, 1H), 6.91 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.84 (d, *J* = 2.1 Hz, 1H), 3.72 – 3.26 (m, 8H), 2.58 (dd, *J* = 7.5, 3.8 Hz, 2H), 1.24 (t, *J* = 7.5 Hz, 3H). ESI-MS *m/z*: 304 $[M+H]^+$.

4.1.13.4. *3-acetyl-7-hydroxy-4-(4-methylpiperazin-1-yl)-2H-chromen-2-one* (**23d**). The title compound was prepared according to general procedure B on 1.5 mmol scale **20d**. Purification by column chromatography (ethyl acetate/petroleum ether, 70%) on silica gel to afford pale yellow solid (299 mg, 66%). ESI-MS *m/z*: 303 $[M+H]^+$.

4.1.13.5. *3-acetyl-4-(4-ethylpiperazin-1-yl)-7-hydroxy-2H-chromen-2-one* (**23e**). The title compound was prepared according to general procedure B on 1.5 mmol scale **20e**. Purification by column chromatography (ethyl acetate/petroleum ether, 70%) on silica gel to afford pale yellow solid (246 mg, 52%). ESI-MS *m/z*: 317 $[M+H]^+$.

4.1.13.6. *3-acetyl-7-hydroxy-4-(piperidin-1-yl)-2H-chromen-2-one* **(23f)**. The title compound was prepared according to general procedure B on 1.66 mmol scale **20f**. Purification by column chromatography (ethyl acetate/petroleum ether, 70%) on silica gel to afford white solid (236 mg, 50%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.85 (s, 1H), 8.20 (d, *J* = 8.9 Hz, 1H), 8.05 (d, *J* = 2.3 Hz, 1H), 7.62 (dd, *J* = 8.8, 2.3 Hz, 1H), 3.73 – 3.43 (m, 2H), 3.30 – 3.20 (m, 2H), 2.36 (s, 3H), 1.66 – 1.47 (m, 6H).

4.1.13.7. *3-acetyl-4-(1,4-diazepan-1-yl)-7-hydroxy-2H-chromen-2-one* (**23g**). The title compound was prepared according to general procedure B on 0.25 mmol scale **20g**. After the resulting mixture was refluxed for 6 h, the mixture was slowly cooled to room temperature and removed the solvent. The residual was slowly

dissolved in HCl (1 mL, 4M in 1,4-dioxane) and stirred at room temperature for 1 h. The solution was added cool water and was acidified to pH 7 with saturated solution of aqueous $NaHCO_3$ slowly, then the white precipitation was occurred. The resulting precipitated solid was collected by filtration, washed with water (10 mL × 3) followed by petroleum ether (10 mL × 3), and then dried overnight to afford white solid (28 mg, 37%). The solid was used for next reaction without purification.

4.1.14. General Procedure C for Synthesis of Compound **23h-23j**. To an oven-dried round-bottom flask was charged with compound **22** (1.0 equiv), the corresponding amine (1.5 equiv), and absolute ethyl alcohol (10 mL per 1.0 mmol) and anhydrous triethylamine (0.1 equiv), then the resulting mixture was refluxed for a period time (usually 2 h) until the completion of the reaction as monitored by TLC. The mixture was slowly cooled to room temperature and was poured into water. Then the resulting mixture exhaustively extracted with dichloromethane (50 mL × 3). The organic phase was washed by brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel using ethyl acetate/petroleum ether as the eluent to afford the products.

4.1.14.1. *7-hydroxy-4-morpholino-2H-chromen-2-one* (**23h**). The title compound was prepared according to general procedure C on **22** (500 mg, 2.5 mmol) and morpholine (332 mg, 3.8 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford white solid (536 mg, 86%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 10.50 (s, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 6.78 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.69 (d, *J* = 2.4 Hz, 1H), 5.52 (s, 1H), 3.81 (t, *J* = 4.5 Hz, 4H,), 3.21 (t, *J* = 4.6 Hz, 4H,). ESI-MS *m/z*: 270 $[M+Na]^+$.

4.1.14.2. *tert-butyl 4-(7-hydroxy-2-oxo-2H-chromen-4-yl)piperazine-1-carboxylate* (**23i**). The title compound was prepared according to general procedure C on **22** (500 mg, 2.5 mmol) and *tert*-butyl 1-piperazinecarboxylate (710 mg, 3.8 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford white solid (726 mg, 84%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 10.53 (s, 1H), 7.54 (d, *J* = 8.8 Hz, 1H), 6.77 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.68 (d, *J* = 2.3 Hz, 1H), 5.51 (s, 1H), 3.53 (s, 4H)1, 3.17 (s, 4H), 1.43 (s, 9H). ESI-MS *m/z*: 369 $[M+Na]^+$.

4.1.14.3. *tert-butyl 4-(7-hydroxy-2-oxo-2H-chromen-4-yl)-1,4-diazepane-1-carboxylate* (**23j**). The title compound was prepared according to general procedure C on **22** (300 mg, 1.52 mmol) and *tert*-butyl 1,4-diazepane-1-carboxylate (458 mg, 2.29 mmol). Purification by column chromatography (ethyl

acetate/petroleum ether, 50%) on silica gel to afford white solid (430 mg, 80%). ESI-MS *m/z*: 383 $[M+Na]^+$.

4.1.15. General Procedure D for Synthesis of Compound **24a-24j**. To an oven-dried round-bottom flask was charged with compound **23a-23j** (1.0 equiv) and anhydrous triethylamine (1.2 equiv) in dry dichloromethane (10 mL per 1.0 mmol). The resulting mixture was stirred at -10 $^oC$ for 15 mins, then trifluormethanesulfonic anhydride (1.2 equiv) was added dropwise over a period of 15 min. The mixture was further stirred at room temperature for a period time (within 1 h) until the completion of the reaction as monitored by TLC. The reaction was quenched with a saturated solution of aqueous $NaHCO_3$ in an ice bath and washed with water. The organic layer was dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel using ethyl acetate/petroleum ether as the eluent to afford the products.

4.1.15.1. *3-acetyl-4-morpholino-2-oxo-2H-chromen-7-yl trifluoromethanesulfonate* (**24a**). The title compound was prepared according to general procedure D on 1.0 mmol scale **23a**. Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (353 mg, 84%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.20 (d, *J* = 8.9 Hz, 1H), 8.06 (d, *J* = 2.4 Hz, 1H), 7.63 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.26–3.80 (m, 8H,), 2.39 (s, 3H). ESI-MS *m/z*: 444 $[M+Na]^+$.

4.1.15.2. *3-ethyl-4-morpholino-2-oxo-2H-chromen-7-yl trifluoromethanesulfonate* (**24b**). The title compound was prepared according to general procedure D on 0.36 mmol scale **23b**. Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (97 mg, 66%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 7.99 (d, *J* = 8.9 Hz, 1H), 7.70 (d, *J* = 2.0 Hz, 1H), 7.52 – 7.40 (m, 1H), 3.80 (d, *J* = 4.0 Hz, 4H), 3.29 – 3.19 (m, 4H), 2.61 (q, *J* = 7.3 Hz, 2H), 1.12 (t, *J* = 7.3 Hz, 3H). ESI-MS *m/z*: 408 $[M+Na]^+$.

4.1.15.3. *4-morpholino-2-oxo-3-propionyl-2H-chromen-7-yl trifluoromethanesulfonate* (**24c**). The title compound was prepared according to general procedure D on 0.33 mmol scale **23c**. Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (100 mg, 70%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.19 (s, 1H), 8.00 (d, *J* = 2.3 Hz, 1H), 7.60 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.87 – 3.45 (m, 8H), 2.74 – 2.59 (m, 2H), 1.28 (t, *J* = 7.5 Hz, 3H). ESI-MS *m/z*: 436 $[M+H]^+$.

4.1.15.4. *3-acetyl-4-(4-methylpiperazin-1-yl)-2-oxo-2H-chromen-7-yl trifluoromethanesulfonate* (**24d**). The

title compound was prepared according to general procedure D on 1.3 mmol scale **23d**. Purification by column chromatography (ethyl acetate/petroleum ether, 40%) on silica gel to afford products white solid (452 mg, 79%). ESI-MS *m/z*: 457 $[M+Na]^+$.

4.1.15.5. *3-acetyl-4-(4-ethylpiperazin-1-yl)-2-oxo-2H-chromen-7-yl trifluoromethanesulfonate* (**24e**). The title compound was prepared according to general procedure D on 0.32 mmol scale **23e**. Purification by column chromatography (ethyl acetate/petroleum ether, 40%) on silica gel to afford products white solid (97 mg, 68%). ESI-MS *m/z*: 449 $[M+H]^+$.

4.1.15.6. *3-acetyl-2-oxo-4-(piperidin-1-yl)-2H-chromen-7-yl trifluoromethanesulfonate* **(24f)**. The title compound was prepared according to general procedure D on 0.35 mmol scale **23f**. Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (96 mg, 66%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.20 (d, $J$ = 8.9 Hz, 1H), 8.05 (d, $J$ = 2.3 Hz, 1H), 7.62 (dd, $J$ = 8.8, 2.3 Hz, 1H), 3.73 – 3.43 (m, 2H), 3.30 – 3.20 (m, 2H), 2.36 (s, 3H), 1.63 – 1.47 (m, 6H).

4.1.15.7. *3-acetyl-4-(1,4-diazepan-1-yl)-2-oxo-2H-chromen-7-yl trifluoromethanesulfonate* (**24g**). The title compound was prepared according to general procedure D on 0.20 mmol scale **23g**. After extraction of the resulting mixture, the solvent was removed under reduced pressure to get the residue without purification.

4.1.15.8. *4-morpholino-2-oxo-2H-chromen-7-yl trifluoromethanesulfonate* (**24h**). The title compound was prepared according to general procedure D on 1.2 mmol scale **23h**. Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (394 mg, 87%). ESI-MS *m/z*: 402 $[M+Na]^+$.

4.1.15.9. *tert-butyl 4-(2-oxo-7-(((trifluoromethyl)sulfonyl)oxy)-2H-chromen-4-yl)piperazine-1-carboxylate* (**24i**). The title compound was prepared according to general procedure D on 1.2 mmol scale **23i**. Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (476 mg, 83%). ESI-MS *m/z*: 501 $[M+Na]^+$.

4.1.15.10. *tert-butyl 4-(2-oxo-7-(((trifluoromethyl)sulfonyl)oxy)-2H-chromen-4-yl)-1,4-diazepane-1-carboxylate* (**24j**). The title compound was prepared according to general procedure D on 1.1 mmol scale **23j**. Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (461 mg, 83%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 7.99 (t, $J$ = 8.8 Hz, 1H), 7.70 (d, $J$ = 2.4 Hz, 1H),

7.45 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.62 (s, 1H), 3.75–3.39 (m, 8H), 1.95 (s, 2H), 1.30 (s, 9H). ESI-MS *m/z*: 515 $[M+Na]^+$.

4.1.16. General Procedure E for Synthesis of Compound **25a-25q**. To a round-bottom flask was charged with the corresponding aromatic halogen (1.0 equiv), the corresponding boronic acid (1.05 – 1.25 equiv), $Pd(dppf)Cl_2$ (0.05 equiv) and base $Na_2CO_3$ (2.0 equiv) under nitrogen atmosphere, then 1,4-dioxane (14 mL) and water (2 mL) were added and the vessel was immediately sealed tightly. The resulting mixture was heated at 95 °C for a period time (usually 2-6 h) until the completion of the reaction as monitored by TLC. The cooled mixture was diluted with water and exhaustively extracted with ethyl acetate (30 mL × 3). The organic phase was washed by brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel using ethyl acetate/petroleum ether as the eluent to afford the products.

4.1.16.1. *4-(3-methoxyphenyl)pyrimidin-2-amine* **(25a)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and 3-methoxyphenylboronic acid (152 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (112 mg, 72%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.31 (d, *J* = 5.0 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 5.2 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 6.70 (s, 2H), 3.83 (s, 3H). ESI-MS *m/z*: 202 $[M+H]^+$.

4.1.16.2. *4-(4-methoxyphenyl)pyrimidin-2-amine* **(25b)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and 4-methoxyphenylboronic acid (152 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (102 mg, 66%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.25 (d, *J* = 5.2 Hz, 1H), 8.05 (d, *J* = 8.6 Hz, 2H), 7.05 (t, *J* = 7.0 Hz, 3H), 6.59 (s, 2H), 3.82 (s, 3H). ESI-MS *m/z*: 202 $[M+H]^+$.

4.1.16.3. *4-(2,4-dimethoxyphenyl)pyrimidin-2-amine* **(25c)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and (2,4-dimethoxyphenyl)boronic acid (182 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (100 mg, 56%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.17 (d, *J* = 4.9 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.09 (d, *J* = 4.8 Hz, 1H), 6.64 (d, *J* = 7.9 Hz, 2H), 6.35 (s, 2H), 3.85 (s, 3H), 3.83

(s, 3H). ESI-MS *m/z*: 232 $[M+H]^+$.

4.1.16.4. *4-(2,3-dimethoxyphenyl)pyrimidin-2-amine* **(25d)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and (2,3-dimethoxyphenyl)boronic acid (182 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (107 mg, 60%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.17 (d, *J* = 4.9 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.09 (d, *J* = 4.8 Hz, 1H), 6.64 (d, *J* = 7.9 Hz, 2H), 6.35 (s, 2H), 3.85 (s, 3H), 3.83 (s, 3H). ESI-MS *m/z*: 232 $[M+H]^+$.

4.1.16.5. *4-phenylpyrimidin-2-amine* **(25e)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and phenylboronic acid (122 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (98 mg, 74%). ESI-MS *m/z*: 172 $[M+H]^+$.

4.1.16.6. *4-(2,6-difluorophenyl)pyrimidin-2-amine* **(25f)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and (2,6-difluorophenyl)boronic acid (158 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (103 mg, 64%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 9.90 (s, 1H), 8.75 (d, *J* = 5.0 Hz, 1H), 8.08 (d, *J* = 5.6 Hz, 1H), 7.50 (d, *J* = 5.7 Hz, 1H), 7.31 (s, 1H), 6.30 (s, 2H). ESI-MS *m/z*: 208 $[M+H]^+$.

4.1.16.7. *4-(2-(trifluoromethyl)phenyl)pyrimidin-2-amine* **(25g)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and (2-(trifluoromethyl)phenyl)boronic acid (190 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (102 mg, 55%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.34 (d, *J* = 5.1 Hz, 1H), 7.79 (d, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.53 (dd, *J* = 15.5, 7.9 Hz, 2H), 6.83 (d, *J* = 5.0 Hz, 1H), 6.81 (s, 2H). ESI-MS *m/z*: 240 $[M+H]^+$.

4.1.16.8. *4-(2-nitrophenyl)pyrimidin-2-amine* **(25h)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and (2-nitrophenyl)boronic acid (167 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (112 mg, 67%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.36 (d, *J* = 5.0 Hz, 1H), 7.99 (d, *J* =

7.5 Hz, 1H), 7.79 (d, *J* = 7.5 Hz, 1H), 7.71 (t, *J* = 7.0 Hz, 2H), 6.81 (d, *J* = 5.0 Hz, 1H), 6.71 (s, 2H). ESI-MS *m/z*: 217 $[M+H]^+$.

4.1.16.9. *4-(o-tolyl)pyrimidin-2-amine* **(25i)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and *o*-tolylboronic acid (136 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (109 mg, 76%). ESI-MS *m/z*: 186 $[M+H]^+$.

4.1.16.10. *4-(2-ethylphenyl)pyrimidin-2-amine* **(25j)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and (2-ethylphenyl)boronic acid (150 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (98 mg, 69%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.28 (d, *J* = 5.0 Hz, 1H), 7.41 – 7.21 (m, 4H), 6.64 (s, 1H), 6.62 (s, 2H), 2.72 (q, *J* = 7.5 Hz, 2H), 1.07 (t, *J* = 7.5 Hz, 3H). ESI-MS *m/z*: 200 $[M+H]^+$.

4.1.16.11. *6-phenylpyrimidin-4-amine* **(25k)**. The title compound was prepared according to general procedure E on 6-chloropyrimidin-4-amine (100 mg, 0.8 mmol) and phenylboronic acid (122 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (98 mg, 74%). ESI-MS *m/z*: 172 $[M+H]^+$.

4.1.16.12. *6-(o-tolyl)pyrimidin-4-amine* **(25l)**. The title compound was prepared according to general procedure E on 6-chloropyrimidin-4-amine (100 mg, 0.8 mmol) and *o*-tolylboronic acid (136 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (87 mg, 61%). ESI-MS *m/z*: 186 $[M+H]^+$.

4.1.16.13. *6-(2-ethylphenyl)pyrimidin-4-amine* **(25m)**. The title compound was prepared according to general procedure E on 6-chloropyrimidin-4-amine (100 mg, 0.8 mmol) and (2-ethylphenyl)boronic acid (150 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (90 mg, 63%). ESI-MS *m/z*: 200 $[M+H]^+$.

4.1.16.14. *6-(2-(trifluoromethyl)phenyl)pyrimidin-4-amine* **(25n)**. The title compound was prepared according to general procedure E on 6-chloropyrimidin-4-amine (100 mg, 0.8 mmol) and (2-(trifluoromethyl)phenyl)boronic acid (190 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (102 mg, 55%). ESI-MS *m/z*: 240

$[M+H]^+$.

4.1.16.15. *4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine* **(25o)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and (4-fluoro-2-methoxyphenyl)boronic acid (170 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (117 mg, 67%).$^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.23 (d, *J* = 5.2 Hz, 1H), 7.87 (dd, *J* = 8.6, 7.3 Hz, 1H), 7.06 (d, *J* = 3.9 Hz, 1H), 7.03 (d, *J* = 4.6 Hz, 1H), 6.89 (td, *J* = 8.4, 2.4 Hz, 1H), 6.55 (s, 2H), 3.86 (s, 3H). ESI-MS *m/z*: 220 $[M+H]^+$.

4.1.16.16. *4-(5-fluoro-2-methoxyphenyl)pyrimidin-2-amine* **(25p)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and (5-fluoro-2-methoxyphenyl)boronic acid (170 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (112 mg, 66%).$^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.27 (d, *J* = 5.1 Hz, 1H), 7.84 (s, 1H), 7.65 (dd, *J* = 9.8, 3.1 Hz, 1H), 7.18 (d, *J* = 4.3 Hz, 1H), 7.14 (t, *J* = 4.7 Hz, 1H), 6.62 (s, 2H), 3.84 (s, 3H). ESI-MS *m/z*: 220 $[M+H]^+$.

4.1.16.17. *4-(6-fluoro-2-methoxyphenyl)pyrimidin-2-amine* **(25q)**. The title compound was prepared according to general procedure E on 4-chloropyrimidin-2-amine (100 mg, 0.8 mmol) and (6-fluoro-2-methoxyphenyl)boronic acid (170 mg, 1.0 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford products white solid (104 mg, 62%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.26 (d, *J* = 4.9 Hz, 1H), 7.42 (dd, *J* = 15.3, 8.3 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.87 (t, *J* = 8.8 Hz, 1H), 6.63 (s, 2H), 6.57 (d, *J* = 4.9 Hz, 1H), 3.74 (s, 3H). ESI-MS *m/z*: 220 $[M+H]^+$.

4.1.17.1. *2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine* **(27a)**. The title compound was prepared according to general procedure E on 2,4-dichloro-5-fluoropyrimidine (100 mg, 0.6 mmol) and (4-fluoro-2-methoxyphenyl)boronic acid (170 mg, 0.7 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (113 mg, 74%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.91 (d, *J* = 1.8 Hz, 1H), 7.54 (dd, *J* = 8.5, 6.8 Hz, 1H), 7.16 (dd, *J* = 11.4, 2.4 Hz, 1H), 6.98 (td, *J* = 8.4, 2.4 Hz, 1H), 3.84 (s, 3H). ESI-MS *m/z*: 257 $[M+H]^+$.

4.1.17.2. *2,5-dichloro-4-(4-fluoro-2-methoxyphenyl)pyrimidine* **(27b)**. The title compound was prepared according to general procedure E on 2,4,5-trichloropyrimidine (100 mg, 0.6 mmol) and (4-fluoro-2-

methoxyphenyl)boronic acid (170 mg, 0.7 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 20%) on silica gel to afford products white solid (103 mg, 63%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.98 (s, 1H), 7.43 (dd, *J* = 8.5, 6.7 Hz, 1H), 7.15 (dd, *J* = 11.4, 2.3 Hz, 1H), 6.96 (d, *J* = 2.4 Hz, 1H), 3.82 (s, 3H). ESI-MS *m/z*: 273 $[M+H]^+$.

4.1.18.1. *5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine* **(28a)**. To a 15 mL sealed tube was charged with **27a** (307 mg, 1.2 mmol), ammonium hydroxide (2 mL) and isopropanol (2 mL), then the resulting mixture was refluxed for 6 h. After completion of the reaction as monitored by TLC, the cooled mixture was diluted with water and exhaustively extracted with ethyl acetate (20 mL × 3). The organic phase was washed by brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was purified by chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford the white solid (163 mg, 58%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.27 (d, *J* = 2.1 Hz, 1H), 7.40 (dd, *J* = 8.4, 7.0 Hz, 1H), 7.08 (dd, *J* = 11.5, 2.3 Hz, 1H), 6.90 (td, *J* = 8.4, 2.3 Hz, 1H), 6.68 (s, 2H), 3.80 (s, 3H). ESI-MS *m/z*: 238 $[M+H]^+$.

4.1.18.2. *5-chloro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-amine* **(28b)**. To a 15 mL sealed tube was charged with **27b** (163 mg, 0.6 mmol), ammonium hydroxide (1 mL) and isopropanol (1 mL), then the resulting mixture was refluxed for 6 h. After completion of the reaction as monitored by TLC, the cooled mixture was diluted with water and exhaustively extracted with ethyl acetate (20 mL × 3). The organic phase was washed by brine, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was purified by chromatography (ethyl acetate/petroleum ether, 30%) on silica gel to afford the white solid (103 mg, 68%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.98 (s, 1H), 7.43 (dd, *J* = 8.5, 6.7 Hz, 1H), 7.15 (dd, *J* = 11.4, 2.3 Hz, 1H), 6.96 (d, *J* = 2.4 Hz, 1H), 3.82 (s, 3H). ESI-MS *m/z*: 254 $[M+H]^+$.

4.1.19. General Procedure F for Synthesis of Compound **7, 29a-29t** and **30a-30p**

To a round-bottom flask was charged with the intermediate **15a-15b**/**25a-25q**/**28a-28b** (1.0 equiv), **13**/**24a-24j** (1.0 equiv), $Pd(OAc)_2$ (0.05 equiv), Xantphos (0.05 equiv) and base $Cs_2CO_3$ (2.0 equiv) under nitrogen atmosphere, then anhydrous toluene (5 mL) was added and the vessel was immediately sealed tightly. The resulting mixture was heated at 100 °C for a period time (usually 2-16 h) until the completion of the reaction as monitored by TLC. The cooled mixture was diluted with water and exhaustively extracted with ethyl acetate (10 mL × 3). The organic phase was washed by brine, dried over anhydrous $Na_2SO_4$, and evaporated under

reduced pressure. The residue was purified by chromatography on silica gel using ethyl acetate/petroleum ether as the eluent to afford the products.

4.1.19.1. *3-acetyl-4-methyl-7-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)-2H-chromen-2-one* (**7**). The title compound was prepared according to general procedure F on 4-(pyridin-3-yl)pyrimidin-2-amine (67 mg, 0.39 mmol, purchased from BidePharm Company Inc.) and **13** (137 mg, 0.39 mmol). Purification by column chromatography (ethyl acetate/petroleum ether, 50%) on silica gel to afford products (135 mg, 93% yield) as a white solid. mp: 276-278 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.49 (s, 1H), 9.39 (d, *J* = 1.8 Hz, 1H), 8.78 (dd, *J* = 4.7, 1.2 Hz, 1H), 8.75 (d, *J* = 5.2 Hz, 1H), 8.56 – 8.53 (m, 1H), 8.17 (d, *J* = 2.0 Hz, 1H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.79 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.69 (d, *J* = 5.2 Hz, 1H), 7.64 (dd, *J* = 7.9, 4.8 Hz, 1H), 2.49 (s, 3H), 2.39 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 194.85, 162.49, 162.17, 160.07, 159.25, 154.01, 152.25, 151.04, 148.71, 145.54, 135.00, 132.42, 127.56, 124.49, 123.95, 116.00, 113.32, 110.35, 104.66, 31.45, 15.62. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{21}H_{17}N_4O_3$: 373.1295, found: 373.1299. HPLC retention time 2.34 min, purity 98.7%.

4.1.19.2. *3-acetyl-7-((4-(2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(29a).** The title compound was prepared according to general procedure F on 0.24 mmol scale **15a** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (66 mg, 58% yield) as a white solid. mp: 264-266 °C. $^{1}$H-NMR (300 MHz, DMSO-$d_6$) δ 10.43 (s, 1H), 8.64 (d, *J* = 5.2 Hz, 1H), 8.43 (d, *J* = 1.7 Hz, 1H), 7.86 – 7.96 (m, 2H), 7.70 (dd, *J* = 8.9, 1.8 Hz, 1H), 7.45 – 7.59 (m, 2H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.12-7.17 (m, 1H), 3.91 (s, 3H), 3.78 – 3.26 (m, 8H), 2.35 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 163.83, 163.77, 159.86, 158.45, 158.13, 157.20, 146.65, 132.40, 130.82, 126.13, 125.91, 121.15, 119.56, 117.11, 116.27, 114.63, 112.71, 104.69, 67.06, 66.54, 56.17, 47.07, 42.08, 18.99. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}N_4O_5$: 473.1819, found: 473.1823. HPLC retention time 3.70 min, purity 96.0%.

4.1.19.3. *3-ethyl-7-((4-(2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(29b).** The title compound was prepared according to general procedure F on 0.36 mmol scale **15a** and **24b**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (54 mg, 33% yield) as a white solid. mp: 240-242 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.19 (s, 1H), 8.58 (d,

*J* = 5.2 Hz, 1H), 8.12 (s, 1H), 7.90 (d, *J* = 7.6 Hz, 1H), 7.67 (dd, *J* = 21.4, 8.9 Hz, 2H), 7.50-7.55 (m, 1H), 7.42 (d, *J* = 5.2 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 1H), 7.12-7.17 (m, 1H), 3.90 (s, 3H), 3.80 (s, 4H), 3.26 (s, 4H), 2.65 – 2.53 (m, 2H), 1.10 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 163.67, 163.64, 160.06, 158.32, 158.10, 156.13, 153.40, 143.77, 132.27, 130.73, 126.31, 125.83, 121.16, 117.91, 115.16, 114.18, 112.69, 112.64, 104.75, 67.23, 56.16, 51.29, 20.44, 14.52. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{27}N_4O_4$: 459.2027, found: 459.2032. HPLC retention time 6.53 min, purity 100.0%.

4.1.19.4. *7-((4-(2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(29c).** The title compound was prepared according to general procedure F on 0.22 mmol scale **15a** and **24h**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (37 mg, 39% yield) as a white solid. mp: 178-180 °C. $^1$H NMR (300 MHz, DMSO) δ 10.22 (s, 1H), 8.57 (d, *J* = 5.1 Hz, 1H), 8.13 (d, *J* = 3.8 Hz, 1H), 7.89 (d, *J* = 5.9 Hz, 1H), 7.63 (s, 2H), 7.52 (d, *J* = 6.7 Hz, 1H), 7.42 (d, *J* = 5.1 Hz, 1H), 7.27 – 7.19 (m, 1H), 7.13 (t, *J* = 7.0 Hz, 1H), 5.53 (s, 1H), 3.88 (s, 3H), 3.80 (s, 4H), 3.22 (s, 4H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 163.69, 162.10, 161.41, 160.02, 158.36, 158.09, 155.18, 144.50, 132.31, 130.73, 126.27, 126.04, 121.18, 114.77, 114.35, 112.69, 109.06, 105.22, 94.30, 66.22, 56.17, 51.50. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{24}H_{23}N_4O_4$: 431.1714, found: 431.1717. HPLC retention time 2.72 min, purity 98.1%.

4.1.19.5. *7-((4-(2-methoxyphenyl)pyrimidin-2-yl)amino)-4-(piperazin-1-yl)-2H-chromen-2-one* **(29d).** The title compound was prepared according to general procedure F on 0.23 mmol scale **15a** and **24i**. Purification by column chromatography (ethyl acetate/petroleum ether, 70%) on silica gel to afford products (32 mg, 33% yield) as a white solid. mp: 152-153 °C. $^1$H-NMR (300 MHz, DMSO-*d*6) δ 10.26 (s, 1H), 8.64 (d, J = 5.2 Hz, 1H), 8.17 (d, *J* = 1.6 Hz, 1H), 7.89 – 8.00 (m, 1H), 7.53 – 7.74 (m, 3H), 7.48 (d, *J* = 5.2 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.17-7.22 (m, 1H), 5.54 (s, 1H), 3.95 (s, 3H), 3.21 (s, 4H), 2.96 (s, 4H), 1.28 (s, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ163.70, 162.17, 161.82, 160.04, 158.35, 158.10, 155.19, 144.40, 132.30, 130.74, 126.28, 126.02, 121.18, 114.71, 114.32, 112.70, 109.31, 105.25, 93.73, 56.17, 52.45, 45.64. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{24}H_{24}N_5O_3$: 430.1874, found: 430.1878. HPLC retention time 6.46 min, purity 99.7 %.

4.1.19.6. *4-(1,4-diazepan-1-yl)-7-((4-(2-methoxyphenyl)pyrimidin-2-yl)amino)-2H-chromen-2-one* **(29e).** The title compound was prepared according to general procedure F on 0.25 mmol scale **15a** and **24j**. Purification by column chromatography (ethyl acetate/petroleum ether, 70%) on silica gel to afford products

(38 mg, 38% yield) as a white solid. mp: 146-148 °C. $^{1}$H-NMR (300 MHz, DMSO-$d_6$) δ 10.24 (s, 1H), 8.65 (d, *J* = 5.2 Hz, 1H), 8.13 (s, 1H), 7.96 (d, *J* = 6.8 Hz, 1H), 7.78 (d, J = 8.9 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.55-7.60 (m, 1H), 7.48 (d, *J* = 5.2 Hz, 1H), 7.28 (d, *J* = 8.3 Hz, 1H), 7.17-7.22 (m, 1H), 5.32 (s, 1H), 3.96 (s, 3H), 2.63 – 3.69 (m, 4H), 2.88 – 3.02 (m, 4H), 1.92 – 1.94 (m, 2H), 1.28 (s, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 163.70, 162.13, 160.05, 159.74, 158.35, 158.10, 155.33, 144.03, 132.29, 130.74, 126.96, 126.29, 121.17, 114.28, 114.05, 112.69, 109.43, 105.48, 88.23, 56.17, 55.69, 51.48, 48.90, 48.33, 30.43. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{25}H_{26}N_5O_3$: 444.2030, found: 444.2035. HPLC retention time 10.19 min, purity 98.5 %.

4.1.19.7. *3-acetyl-7-((4-(3-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(29f).** The title compound was prepared according to general procedure F on 0.36 mmol scale **25a** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (84 mg, 50% yield) as a white solid. mp > 300 °C. $^{1}$H-NMR (300 MHz, DMSO-$d_6$) δ 10.40 (s, 1H), 8.69 (d, *J* = 5.2 Hz, 1H), 8.48 (d, *J* = 1.9 Hz, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.75 (m, 3H), 7.60 (d, *J* = 5.3 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.15 (dd, *J* = 7.9, 2.2 Hz, 1H), 3.92 (s, 3H), 3.76 – 3.20 (m, 8H), 2.35 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 164.08, 163.85, 163.75, 160.26, 160.00, 159.75, 157.20, 146.51, 138.22, 130.60, 125.96, 119.90, 119.62, 118.01, 117.26, 116.39, 111.88, 110.19, 104.85, 67.06, 66.54, 55.73, 47.08, 42.09, 18.98. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}N_4O_5$: 473.1819, found: 473.1822. HPLC retention time 3.50 min, purity 100%.

4.1.19.8. *3-acetyl-7-((4-(4-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(29g).** The title compound was prepared according to general procedure F on 0.36 mmol scale **25b** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (84 mg, 50% yield) as a white solid. mp: 196-198 °C. $^{1}$H-NMR (300 MHz, DMSO-$d_6$) δ 10.31 (s, 1H), 8.62 (d, *J* = 5.3 Hz, 1H), 8.34 (d, *J* = 1.9 Hz, 1H), 8.22 – 8.13 (m, 2H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.78 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.51 (d, *J* = 5.4 Hz, 1H), 7.13 (m, 2H), 3.86 (s, 3H), 3.75 – 3.29 (m, 8H), 2.37 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.44, 164.06, 163.87, 163.77, 162.27, 159.97, 159.14, 157.15, 146.66, 129.16, 128.99, 125.97, 119.58, 117.17, 116.32, 114.84, 109.28, 104.80, 67.06, 66.55, 55.89, 47.08, 42.09, 19.02. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}N_4O_5$: 473.1819, found: 473.1821. HPLC retention time 3.38 min, purity 100%.

4.1.19.9. *3-acetyl-7-((4-(2,4-dimethoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one*

**(29h).** The title compound was prepared according to general procedure F on 0.30 mmol scale **25c** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (65 mg, 43% yield) as a white solid. mp: 220-222 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.32 (s, 1H), 8.57 (d, *J* = 5.3 Hz, 1H), 8.40 (d, *J* = 1.8 Hz, 1H), 8.04 – 7.96 (m, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.70 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.49 (d, *J* = 5.3 Hz, 1H), 6.72 - 6.74 (m, 2H), 3.93 (s, 3H), 3.87 (s, 3H), 3.77 – 3.20 (m, 8H), 2.35 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 163.81, 163.78, 163.27, 163.13, 159.93, 159.74, 158.27, 157.21, 146.77, 132.00, 125.89, 119.55, 118.49, 117.07, 116.18, 113.94, 106.47, 104.59, 99.24, 67.06, 66.55, 56.26, 55.98, 47.08, 42.08, 18.99. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{27}N_4O_6$: 503.1925, found: 503.1932. HPLC retention time 3.37 min, purity 100%.

4.1.19.10. *3-acetyl-7-((4-(2,3-dimethoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(29i).** The title compound was prepared according to general procedure F on 0.30 mmol scale **25d** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (66 mg, 42% yield) as a white solid. mp: 145-147 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.48 (s, 1H), 8.68 (d, *J* = 5.1 Hz, 1H), 8.39 (s, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 8.9 Hz, 1H), 7.39 – 7.44 (m, 2H), 7.26 (s, 1H), 7.24 (s, 1H), 3.89 (s, 3H), 3.74 (s, 3H), 3.71 – 3.27 (m, 8H), 2.34 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 164.01, 163.85, 163.75, 159.94, 158.68, 157.17, 153.38, 147.87, 146.56, 131.76, 125.96, 124.73, 121.96, 119.58, 117.16, 116.35, 115.30, 114.39, 104.75, 67.06, 66.54, 61.18, 56.44, 47.07, 42.08, 19.00. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{27}N_4O_6$: 503.1925, found: 503.1934. HPLC retention time 2.05 min, purity 100%.

4.1.19.11. *3-acetyl-4-morpholino-7-((4-phenylpyrimidin-2-yl)amino)-2H-chromen-2-one* **(29j).** The title compound was prepared according to general procedure F on 0.42 mmol scale **25e** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (80 mg, 43% yield) as a white solid. mp: 226-228 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.41 (s, 1H), 8.70 (d, *J* = 5.2 Hz, 1H), 8.37 (s, 1H), 8.21 (d, *J* = 3.5 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.59 (d, *J* = 3.4 Hz, 4H), 3.76 – 3.34 (m, 8H), 2.36 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.44, 164.49, 163.89, 163.76, 160.07, 159.60, 157.14, 146.56, 136.78, 131.66, 129.49, 127.51, 126.01, 119.60, 117.21, 116.41, 110.11, 104.89, 67.06, 66.55, 47.08, 42.09, 19.02. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{25}H_{23}N_4O_4$: 443.1714, found:

443.1722. HPLC retention time 2.27 min, purity 100%.

4.1.19.12. *3-acetyl-7-((4-(2,6-difluorophenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(29k).** The title compound was prepared according to general procedure F on 0.35 mmol scale **25f** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (81 mg, 44% yield) as a white solid. mp: 288-290 °C. $^{1}$H-NMR (300 MHz, DMSO-$d_6$) δ 10.61 (s, 1H), 8.79 (d, *J* = 4.9 Hz, 1H), 8.33 (s, 1H), 7.91 (d, *J* = 8.7 Hz, 1H), 7.72 – 7.62 (m, 2H), 7.33 (m, 2H), 7.26 (d, *J* = 4.8 Hz, 1H), 3.76 – 3.34 (m, 8H), 2.35 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 163.93, 163.72, 161.26, 161.20, 159.86, 159.45, 158.77, 158.71, 157.88, 157.11, 146.18, 132.93, 132.83, 125.99, 119.60, 117.22, 116.62, 115.94, 115.72, 112.97, 112.72, 105.00, 67.05, 66.54, 47.06, 42.08, 19.01. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{25}H_{21}F_2N_4O_4$: 479.1525, found: 479.1529. HPLC retention time 3.96 min, purity 96.8 %.

4.1.19.13. *3-acetyl-4-morpholino-7-((4-(2-(trifluoromethyl)phenyl)pyrimidin-2-yl)amino)-2H-chromen-2-one* **(29l).** The title compound was prepared according to general procedure F on 0.39 mmol scale **25g** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (66 mg, 43% yield) as a white solid. mp: 253-254 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.55 (s, 1H), 8.74 (d, *J* = 5.0 Hz, 1H), 8.32 (s, 1H), 8.00 – 7.91 (m, 2H), 7.90 – 7.75 (m, 2H), 7.64 – 7.69 (m, 2H), 7.13 (d, *J* = 5.0 Hz, 1H), 3.68 – 3.44 (m, 8H), 2.32 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.42, 166.44, 163.90, 163.71, 159.38, 159.02, 157.11, 146.20, 137.72, 133.68, 133.14, 131.50, 130.35, 127.11, 127.04, 125.95, 119.60, 117.22, 116.56, 114.02, 104.96, 67.05, 66.54, 47.05, 42.07, 19.00. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{22}F_3N_4O_4$: 511.1588, found: 511.1593. HPLC retention time 2.24 min, purity 97.9 %.

4.1.19.14. *3-acetyl-4-morpholino-7-((4-(2-nitrophenyl)pyrimidin-2-yl)amino)-2H-chromen-2-one* **(29m).** The title compound was prepared according to general procedure F on 0.33 mmol scale **25h** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (84 mg, 51% yield) as a white solid. mp: 154-156 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.48 (s, 1H), 8.80 (d, *J* = 5.0 Hz, 1H), 8.22 – 8.10 (m, 2H), 7.90 (m, 2H), 7.86 – 7.79 (m, 2H), 7.66 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.35 (d, *J* = 5.0 Hz, 1H), 3.75 – 3.29 (m, 8H), 2.37 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.42, 164.06, 163.94, 163.71, 160.03, 159.43, 157.08, 149.01, 146.02, 133.85, 132.91, 131.68, 131.59, 125.93, 125.19, 119.60, 117.22, 116.66, 112.82, 105.04, 67.06, 66.54, 47.07, 42.08, 19.03. HRMS (ESI) *m/z* $[M+H]^+$ calcd for

$C_{25}H_{22}N_5O_6$: 488.1565, found: 488.1573. HPLC retention time 3.33 min, purity 95.3 %.

4.1.19.15. *3-acetyl-4-morpholino-7-((4-(o-tolyl)pyrimidin-2-yl)amino)-2H-chromen-2-one* **(29n).** The title compound was prepared according to general procedure F on 0.39 mmol scale **25i** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (86 mg, 49% yield) as a white solid. mp: 227-229 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.44 (s, 1H), 8.70 (d, *J* = 5.1 Hz, 1H), 8.36 (s, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.9 Hz, 1H), 7.51 (d, *J* = 7.1 Hz, 1H), 7.39 (m, 3H), 7.17 (d, *J* = 5.1 Hz, 1H), 3.71 – 3.25 (m, 8H), 2.44 (s, 3H), 2.33 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.42, 167.84, 163.87, 163.74, 159.67, 158.81, 157.16, 146.49, 138.22, 136.02, 131.45, 129.92, 129.79, 126.57, 125.98, 119.59, 117.18, 116.38, 114.26, 104.76, 67.05, 66.54, 47.06, 42.08, 20.59, 19.00. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}N_4O_4$ : 457.1870, found: 457.1873. HPLC retention time 2.59 min, purity 98.4 %.

4.1.19.16. *3-acetyl-7-((4-(2-ethylphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(29o).** The title compound was prepared according to general procedure F on 0.36 mmol scale **25j** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (84 mg, 49% yield) as a white solid. mp: 216-217 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.48 (s, 1H), 8.71 (dd, *J* = 8.1, 5.0 Hz, 1H), 8.41 – 8.30 (m, 1H), 7.96 – 7.84 (m, 1H), 7.73 – 7.61 (m, 1H), 7.51 – 7.30 (m, 4H), 7.14 (dd, *J* = 8.1, 5.0 Hz, 1H), 3.81 – 3.27 (m, 8H), 2.80 (d, *J* = 7.4 Hz, 2H), 2.33 (s, 3H), 1.10 (t, *J* = 7.6 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.41, 168.31, 163.87, 163.72, 159.63, 158.86, 157.15, 146.44, 141.99, 138.04, 129.97, 129.79, 129.65, 126.47, 125.98, 119.59, 117.17, 116.40, 114.28, 104.76, 67.05, 66.54, 47.06, 42.08, 25.83, 18.99, 16.03. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{27}N_4O_4$ : 471.2027, found: 471.2034. HPLC retention time 2.56 min, purity 100%.

4.1.19.17. *3-acetyl-7-((6-(2-methoxyphenyl)pyrimidin-4-yl)amino)-4-morpholino-2H-chromen-2-one* **(29p).** The title compound was prepared according to general procedure F on 0.36 mmol scale **15b** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (76 mg, 45.0% yield) as a white solid. mp: 222-224 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 10.31 (s, 1H), 8.89 (s, 1H), 8.48 (d, *J* = 1.7 Hz, 1H), 8.03 – 7.92 (m, 2H), 7.61 (s, 1H), 7.55 – 7.43 (m, 2H), 7.22 (d, *J* = 8.3 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 3.93 (s, 3H), 3.58 – 3.20 (m, 8H), 2.37 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.42, 164.05, 163.67, 160.66, 160.37, 158.20, 158.12, 157.06, 145.99, 132.04, 130.76, 126.22, 125.87,

121.16, 119.65, 117.47, 116.80, 112.66, 108.87, 105.79, 67.06, 66.55, 56.27, 47.07, 42.09, 19.00. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}N_4O_5$ : 473.1819, found: 473.1815. HPLC retention time 3.38 min, purity 96.7 %.

4.1.19.18. *3-acetyl-4-morpholino-7-((6-phenylpyrimidin-4-yl)amino)-2H-chromen-2-one* **(29q).** The title compound was prepared according to general procedure F on 0.42 mmol scale **25k** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (65 mg, 35% yield) as a white solid. mp: 202-204 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.36 (s, 1H), 8.91 (s, 1H), 8.48 (s, 1H), 8.11 – 8.03 (m, 2H), 7.97 (d, *J* = 8.7 Hz, 1H), 7.59 – 7.49 (m, 4H), 7.39 (s, 1H), 3.81 – 3.37 (m, 8H), 2.37 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.42, 164.09, 163.67, 162.29, 161.05, 158.67, 157.03, 145.77, 137.02, 131.14, 129.49, 126.99, 126.27, 119.66, 117.52, 116.93, 106.00, 103.99, 67.05, 66.55, 47.07, 42.10, 19.00. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{25}H_{23}N_4O_4$: 443.1714, found: 443.1721. HPLC retention time 2.30 min, purity 100%.

4.1.19.19. *3-acetyl-4-morpholino-7-((6-(o-tolyl)pyrimidin-4-yl)amino)-2H-chromen-2-one* **(29r).** The title compound was prepared according to general procedure F on 0.39 mmol scale **25l** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (64 mg, 36% yield) as a white solid. mp: 242-244 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.33 (s, 1H), 8.91 (s, 1H), 8.50 (d, *J* = 1.7 Hz, 1H), 7.97 (d, *J* = 8.7 Hz, 1H), 7.49 (dd, *J* = 11.9, 4.8 Hz, 2H), 7.45 – 7.27 (m, 3H), 7.03 (s, 1H), 3.77 – 3.34 (m, 8H), 2.39 (s, 3H), 2.37 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 165.66, 164.11, 163.67, 160.44, 158.02, 157.04, 145.73, 138.45, 136.06, 131.41, 129.68, 126.57, 126.27, 119.66, 117.52, 116.94, 108.21, 106.00, 67.05, 66.54, 47.07, 42.10, 20.61, 19.00. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}N_4O_4$: 457.1870, found: 457.1872. HPLC retention time 2.54 min, purity 98.4 %.

4.1.19.20. *3-acetyl-7-((6-(2-ethylphenyl)pyrimidin-4-yl)amino)-4-morpholino-2H-chromen-2-one* **(29s).** The title compound was prepared according to general procedure F on 0.39 mmol scale **25m** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (70 mg, 41.2% yield) as a white solid. mp: 154-156 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.35 (s, 1H), 8.90 (s, 1H), 8.51 (d, *J* = 1.4 Hz, 1H), 7.97 (d, *J* = 8.7 Hz, 1H), 7.50 (dd, *J* = 8.8, 1.5 Hz, 1H), 7.47 – 7.28 (m, 4H), 7.00 (s, 1H), 3.77 – 3.37 (m, 8H), 2.76 (q, *J* = 7.4 Hz, 2H), 2.34 (s, 3H), 1.14 (q, *J* = 7.9 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.42, 166.15, 164.12, 163.66, 160.45, 158.00, 157.04, 145.71, 142.25, 138.27, 129.78, 126.47, 126.29,

119.68, 117.53, 116.97, 108.14, 106.03, 67.05, 66.54, 47.07, 42.10, 26.01, 19.01, 16.29. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{27}N_4O_4$: 471.2027, found: 471.2034. HPLC retention time 2.34 min, purity 100%.

4.1.19.21. *3-acetyl-4-morpholino-7-((6-(2-(trifluoromethyl)phenyl)pyrimidin-4-yl)amino)-2H-chromen-2-one* **(29t).** The title compound was prepared according to general procedure F on 0.39 mmol scale **25n** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (66 mg, 43% yield) as a white solid. mp: 200-202 °C. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.13 (s, 1H), 7.85 – 7.77 (m, 2H), 7.72 – 7.50 (m, 4H), 7.10 (d, *J* = 7.2 Hz, 1H), 6.97 (s, 1H), 3.92 – 3.40 (m, 1H), 3.89 – 3.67 (m, 4H), 3.64 – 3.55 (m, 1H), 3.44 – 3.33 (m, 2H), 2.29 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 164.18, 164.10, 163.63, 160.13, 158.02, 157.00, 145.49, 138.10, 133.10, 131.65, 130.16, 127.24, 127.04, 127.00, 126.94, 126.33, 123.09, 119.70, 117.62, 117.15, 108.13, 106.24, 67.05, 66.54, 47.06, 42.09, 19.01. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{22}F_3N_4O_4$: 511.1588, found: 511.1594. HPLC retention time 2.47 min, purity 98.8 %.

4.1.19.22. *3-acetyl-7-((4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(30a).** The title compound was prepared according to general procedure F on 0.33 mmol scale **25o** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (54 mg, 33% yield) as a white solid. mp: 214-216 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 10.39 (s, 1H), 8.61 (d, *J* = 4.8 Hz, 1H), 8.37 (d, *J* = 1.7 Hz, 1H), 7.96 (t, *J* = 7.5 Hz, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 1H), 7.44 (d, *J* = 4.8 Hz, 1H), 7.12 (d, *J* = 10.8 Hz, 1H), 6.99 (d, *J* = 6.5 Hz, 1H), 3.92 (s, 3H), 3.78 – 3.24 (m, 8H), 2.34 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 166.09, 163.85, 163.77, 163.62, 162.83, 159.90, 159.81, 159.79, 158.62, 157.18, 146.60, 132.54, 132.43, 125.92, 122.58, 122.55, 119.57, 117.11, 116.30, 114.31, 107.97, 107.76, 104.72, 101.06, 100.80, 67.05, 66.55, 56.78, 47.07, 42.08, 18.98. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{24}FN_4O_5$: 491.1725, found: 491.1731. HPLC retention time 2.44 min, purity 100%.

4.1.19.23. *3-acetyl-7-((4-(5-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(30b).** The title compound was prepared according to general procedure F on 0.33 mmol scale **25p** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (52 mg, 32% yield) as a white solid. mp: 220-221 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 10.46 (s, 1H), 8.68 (d, *J* = 5.2 Hz, 1H), 8.42 (d, *J* = 1.9 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.76 (dd, *J* = 9.6, 3.3 Hz, 1H), 7.67

(dd, *J* = 8.9, 2.0 Hz, 1H), 7.54 (d, *J* = 5.2 Hz, 1H), 7.47 – 7.33 (m, 1H), 7.26 (dd, *J* = 9.2, 4.5 Hz, 1H), 3.91 (s, 3H), 3.78 – 3.24 (m, 8H), 2.34 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.42, 163.83, 163.75, 162.27, 159.80, 158.93, 157.91, 157.17, 155.56, 154.72, 146.49, 127.11, 127.04, 125.92, 119.58, 118.65, 118.42, 117.14, 116.86, 116.61, 116.35, 114.46, 114.39, 104.77, 67.05, 66.55, 56.80, 47.07, 42.08, 18.98. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{24}FN_4O_5$: 491.1725, found: 491.1735. HPLC retention time 2.55 min, purity 99.6 %.

4.1.19.24. *3-acetyl-7-((4-(2-fluoro-6-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(30c).** The title compound was prepared according to general procedure F on 0.33 mmol scale **25q** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (52 mg, 32% yield) as a white solid. mp: 218-220 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.49 (s, 1H), 8.70 (d, *J* = 5.0 Hz, 1H), 8.36 (d, *J* = 1.7 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.64 (dd, *J* = 8.8, 1.8 Hz, 1H), 7.52 (dd, *J* = 15.3, 8.4 Hz, 1H), 7.10 (d, *J* = 5.0 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 6.95 – 7.00 (m, 1H), 3.83 (s, 3H), 3.77 – 3.27 (m, 8H), 2.34 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 163.87, 163.75, 161.39, 160.66, 159.78, 158.95, 158.63, 158.29, 158.22, 157.18, 146.40, 132.09, 131.98, 125.94, 119.57, 117.12, 116.44, 116.22, 116.06, 115.89, 108.53, 108.34, 108.32, 104.76, 67.05, 66.54, 56.75, 47.07, 42.08, 18.98. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{24}FN_4O_5$: 491.1725, found: 491.1734. HPLC retention time 1.80 min, purity 98.1 %.

4.1.19.25. *3-acetyl-7-((4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-(4-methylpiperazin-1-yl)-2H-chromen-2-one* **(30d).** The title compound was prepared according to general procedure F on 0.33 mmol scale **25o** and **24d**. Purification by column chromatography (ethyl acetate/petroleum ether, 70%) on silica gel to afford products (80 mg, 48% yield) as a white solid. mp: 166-168 °C. $^1$H NMR (600 MHz, DMSO-$d_6$) δ 10.58 (s, 1H), 8.74 (d, *J* = 1.8 Hz, 1H), 8.30 (d, *J* = 1.9 Hz, 1H), 8.02 – 7.94 (m, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.61 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.58 (dd, *J* = 8.4, 6.9 Hz, 1H), 7.18 (dd, *J* = 11.4, 2.3 Hz, 1H), 6.97 – 7.04 (m, 1H), 3.88 (s, 3H), 3.74 (d, *J* = 16.3 Hz, 1H), 3.50 (d, *J* = 9.2 Hz, 1H), 3.32 (d, *J* = 3.2 Hz, 1H), 3.24 – 3.20 (m, 1H), 2.43 (s, 1H), 2.36 (d, *J* = 3.8 Hz, 1H), 2.32 (s, 3H), 2.27 (d, *J* = 7.7 Hz, 1H), 2.19 (s, 3H), 2.15 (d, *J* = 8.0 Hz, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.46, 166.09, 163.97, 163.70, 163.62, 162.83, 159.90, 159.81, 159.79, 158.62, 157.18, 146.64, 132.53, 132.43, 125.91, 122.57, 122.54, 119.49, 117.14, 116.27, 114.33, 107.97, 107.75, 104.71, 101.06, 100.80, 56.77, 54.72, 54.03, 45.40, 44.82, 19.03. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{27}FN_5O_4$: 504.2042, found: 504.2051. HPLC retention time 3.47 min, purity 100%.

4.1.19.26. *3-acetyl-4-(4-ethylpiperazin-1-yl)-7-((4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-2H-chromen-2-one* **(30e).** The title compound was prepared according to general procedure F on 0.33 mmol scale **25o** and **24e**. Purification by column chromatography (ethyl acetate/petroleum ether, 70%) on silica gel to afford products (85 mg, 50.0% yield) as a white solid. mp: 158-160 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.41 (s, 1H), 8.64 (d, *J* = 5.2 Hz, 1H), 8.39 (d, *J* = 1.6 Hz, 1H), 8.02 – 7.94 (m, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.69 (dd, *J* = 8.9, 1.6 Hz, 1H), 7.46 (d, *J* = 5.2 Hz, 1H), 7.15 (dd, *J* = 11.5, 2.3 Hz, 1H), 6.97 -7.03 (m, 1H), 3.93 (s, 3H), 3.61 (d, 2H), 3.29 – 3.14 (m, 2H), 2.37 (d, *J* = 7.2 Hz, 4H), 2.33 (s, 3H), 2.20 (m, 2H), 1.00 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.40, 166.08, 163.62, 163.47, 163.45, 162.81, 159.89, 159.81, 159.79, 158.60, 157.15, 146.57, 132.54, 132.43, 125.91, 122.56, 122.53, 119.88, 117.09, 116.30, 114.29, 107.96, 107.75, 104.72, 101.04, 100.79, 56.77, 53.32, 52.48, 51.94, 46.61, 41.51, 18.96, 12.31. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{28}H_{29}FN_5O_4$: 518.2198, found: 518.2204. HPLC retention time 7.09 min, purity 100%.

4.1.19.27. *3-acetyl-7-((4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-(piperidin-1-yl)-2H-chromen-2-one* **(30f).** The title compound was prepared according to general procedure F on 0.33 mmol scale **25o** and **24f**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (91 mg, 57% yield) as a white solid. mp: 196-198 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.42 (s, 1H), 8.64 (d, *J* = 5.3 Hz, 1H), 8.39 (d, *J* = 1.9 Hz, 1H), 7.98 (dd, *J* = 8.6, 7.2 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.69 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.46 (d, *J* = 5.2 Hz, 1H), 7.15 (dd, *J* = 11.5, 2.4 Hz, 1H), 6.97 – 7.03 (m, 1H), 3.93 (s, 3H), 3.71 – 3.20 (m, 4H), 2.33 (s, 3H), 1.62 – 1.47 (m, 6H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.43, 166.08, 163.62, 163.28, 163.12, 162.82, 159.89, 159.82, 159.79, 158.61, 157.14, 146.53, 132.54, 132.44, 125.92, 122.58, 122.55, 120.29, 117.07, 116.32, 114.29, 107.97, 107.76, 104.71, 101.05, 100.80, 56.78, 47.62, 42.21, 26.75, 25.84, 24.47, 18.90. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{26}FN_4O_4$: 489.1933, found: 489.1939. HPLC retention time 9.70 min, purity 97.7 %.

4.1.19.28. *7-((4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-3-propionyl-2H-chromen-2-one* **(30g).** The title compound was prepared according to general procedure F on 0.33 mmol scale **25o** and **24c**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (42 mg, 25% yield) as a white solid. mp: 128-130 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.36 (s, 1H),

8.63 (d, *J* = 5.3 Hz, 1H), 8.39 (d, *J* = 1.9 Hz, 1H), 8.00 (dd, *J* = 8.7, 7.1 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.70 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.47 (d, *J* = 5.2 Hz, 1H), 7.14 (dd, *J* = 11.5, 2.4 Hz, 1H), 6.94 - 7.01 (m, 1H), 3.94 (s, 3H), 3.69 – 3.32 (m, 8H), 2.70 – 2.55 (m, 2H), 1.26 (t, J = 7.5 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.63, 167.43, 166.10, 163.68, 163.64, 162.76, 159.94, 159.83, 159.81, 158.66, 157.29, 146.62, 132.53, 132.43, 125.91, 122.54, 122.51, 118.95, 117.18, 116.33, 114.31, 107.91, 107.70, 104.75, 101.08, 100.82, 66.98, 66.55, 56.77, 47.13, 42.07, 26.10, 11.88. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{26}FN_4O_5$: 505.1882, found: 505.1887. HPLC retention time 4.81 min, purity 100%.

4.1.19.29. *7-((4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(30h).** The title compound was prepared according to general procedure F on 0.33 mmol scale **25o** and **24h**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (47 mg, 32% yield) as a white solid. mp: 150-152 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.24 (s, 1H), 8.59 (d, *J* = 5.2 Hz, 1H), 8.10 (s, 1H), 8.05 – 7.90 (m, 1H), 7.64 (s, 2H), 7.42 (d, *J* = 5.2 Hz, 1H), 7.15 (dd, *J* = 11.5, 2.1 Hz, 1H), 6.99 (d, *J* = 2.1 Hz, 1H), 5.55 (s, 1H), 3.92 (s, 3H), 3.81 (s, 4H), 3.23 (s, 4H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 166.01, 163.55, 162.68, 162.07, 161.37, 159.96, 159.83, 159.73, 158.45, 155.16, 144.44, 132.44, 132.34, 125.98, 122.70, 122.67, 114.76, 113.99, 109.08, 107.95, 107.73, 105.24, 100.99, 100.73, 94.33, 66.22, 56.74, 51.50. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{24}H_{22}FN_4O_4$: 449.1620, found: 449.1624. HPLC retention time 8.39 min, purity 98.3 %.

4.1.19.30. *3-acetyl-7-((5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(30i).** The title compound was prepared according to general procedure F on 0.30 mmol scale **28a** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (44 mg, 24% yield) as a white solid. mp: 150-152 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.57 (s, 1H), 8.73 (d, *J* = 1.9 Hz, 1H), 8.30 (d, *J* = 1.9 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.65 – 7.52 (m, 2H), 7.17 (dd, *J* = 11.5, 2.3 Hz, 1H), 6.99 (d, *J* = 2.4 Hz, 1H), 3.87 (s, 3H), 3.71 – 3.25 (m, 8H), 2.33 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.39, 166.16, 163.85, 163.73, 159.16, 159.05, 157.20, 156.14, 152.44, 152.26, 149.92, 146.50, 146.38, 146.25, 132.59, 132.48, 125.98, 119.57, 118.99, 116.89, 116.39, 107.89, 107.67, 104.20, 100.90, 100.64, 67.04, 66.54, 56.82, 47.06, 42.08, 18.96. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{23}F_2N_4O_5$: 509.1631, found: 509.1633. HPLC retention time 4.41 min, purity 99.2%.

4.1.19.31. *3-acetyl-7-((5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-(4-methylpiperazin-1-yl)-2H-chromen-2-one* **(30j).** The title compound was prepared according to general procedure F on 0.30 mmol scale **28a** and **24d**. Purification by column chromatography (ethyl acetate/petroleum ether, 70%) on silica gel to afford products (44 mg, 28% yield) as a white solid. mp: 148-150 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.58 (s, 1H), 8.74 (d, *J* = 1.8 Hz, 1H), 8.30 (d, *J* = 1.9 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.61 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.58 (dd, *J* = 8.4, 6.9 Hz, 1H), 7.18 (dd, *J* = 11.4, 2.3 Hz, 1H), 6.98 - 7.01 (m, 1H), 3.88 (s, 3H), 3.74 (d, *J* = 16.3 Hz, 1H), 3.50 (d, *J* = 9.2 Hz, 1H), 3.32 (d, *J* = 3.2 Hz, 1H), 3.24 – 3.20 (m, 1H), 2.43 (s, 1H), 2.36 (d, *J* = 3.8 Hz, 1H), 2.32 (s, 3H), 2.27 (d, *J* = 7.7 Hz, 1H), 2.19 (s, 3H), 2.15 (d, *J* = 8.0 Hz, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.37, 163.71, 163.50, 159.16, 159.05, 157.19, 156.18, 152.43, 149.93, 146.36, 132.59, 132.48, 125.99, 119.88, 119.00, 116.87, 116.41, 107.90, 107.67, 104.21, 100.90, 100.65, 56.83, 55.47, 54.72, 46.45, 46.04, 41.36, 18.96. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{26}F_2N_5O_4$: 522.1947, found: 522.1952. HPLC retention time 5.52 min, purity 98.8 %.

4.1.19.32. *3-acetyl-4-(4-ethylpiperazin-1-yl)-7-((5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-2H-chromen-2-one* **(30k).** The title compound was prepared according to general procedure F on 0.18 mmol scale **28a** and **24e**. Purification by column chromatography (ethyl acetate/petroleum ether, 70%) on silica gel to afford products (70 mg, 73% yield) as a white solid. mp: 140-142 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.57 (s, 1H), 8.74 (s, 1H), 8.30 (s, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.59 (dd, *J* = 14.2, 7.9 Hz, 2H), 7.17 (d, *J* = 11.3 Hz, 1H), 7.03 – 6.95 (m, 1H), 3.87 (s, 3H), 3.70 (s, 2H), 3.51 (s, 2H), 3.25 (s, 2H), 2.36 (s, 2H), 2.32 (s, 3H), 2.20 (s, 2H), 1.00 (t, *J* = 6.7 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.36, 166.16, 163.70, 163.48, 163.42, 159.16, 159.05, 157.18, 156.18, 156.15, 152.43, 152.27, 149.92, 146.50, 146.36, 146.25, 132.58, 132.48, 125.98, 119.90, 118.99, 116.87, 116.41, 107.89, 107.67, 104.20, 100.90, 100.64, 56.83, 53.32, 52.48, 51.94, 46.59, 41.51, 18.95, 12.31. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{28}H_{28}F_2N_5O_4$: 536.2104, found: 536.2104. HPLC retention time 5.81 min, purity 97.3 %.

4.1.19.33. *3-acetyl-4-(1,4-diazepan-1-yl)-7-((5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-2H-chromen-2-one* **(30l).** The title compound was prepared according to general procedure F on 0.33 mmol scale **28a** and **24g**. Purification by column chromatography (ethyl acetate/petroleum ether, 80%) on silica gel to afford products (39 mg, 23% yield) as a white solid. mp: 156-158 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ

10.58 (d, *J* = 2.9 Hz, 1H), 8.74 (s, 1H), 8.30 (dd, *J* = 3.7, 2.0 Hz, 1H), 7.90 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.61 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.58 (dd, *J* = 8.3, 6.8 Hz, 1H), 7.17 (dd, *J* = 11.4, 2.2 Hz, 1H), 6.98 – 7.01 (m, 1H), 3.87 (s, 3H), 3.59 – 3.50 (m, 2H), 3.38 – 3.32 (m, 4H), 2.89 – 2.73 (m, 4H), 2.34 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.53, 166.16, 164.94, 163.70, 163.30, 163.21, 159.16, 159.05, 157.18, 156.15, 152.42, 149.93, 146.51, 146.35, 146.27, 132.61, 132.49, 126.00, 120.81, 119.01, 116.88, 116.49, 107.90, 107.68, 104.21, 100.90, 100.64, 56.83, 49.92, 48.82, 47.65, 44.39, 29.49, 19.07. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{26}F_2N_5O_4$: 522.1947, found: 522.1950. HPLC retention time 8.68 min, purity 96.6 %.

4.1.19.34. *7-((5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-3-propionyl-2H-chromen-2-one* **(30m).** The title compound was prepared according to general procedure F on 0.41 mmol scale **28a** and **24c**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (130 mg, 61% yield) as a pale yellow solid. mp: 176-178 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.59 (s, 1H), 8.74 (d, *J* = 1.8 Hz, 1H), 8.31 (d, *J* = 1.8 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.68 – 7.52 (m, 2H), 7.17 (dd, *J* = 11.5, 2.2 Hz, 1H), 6.95 – 7.02 (m, 2.3 Hz, 1H), 3.87 (s, 3H), 3.78 –3.28 (m, 8H), 2.61 (dd, *J* = 7.5, 3.8 Hz, 2H), 1.25 (t, *J* = 7.5 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.60, 167.46, 166.17, 163.72, 163.64, 159.16, 159.05, 157.32, 156.14, 152.44, 149.93, 146.43, 132.51, 129.30, 129.10, 125.99, 118.96, 116.94, 116.43, 107.86, 107.64, 104.22, 100.90, 100.63, 66.98, 66.54, 56.82, 47.12, 42.07, 26.10, 11.94. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{25}F_2N_4O_5$: 523.1788, found: 523.1781. HPLC retention time 4.75 min, purity 99.3 %.

4.1.19.35. *7-((5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(30n).** The title compound was prepared according to general procedure F on 0.43 mmol scale **28a** and **24h**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (80 mg, 40% yield) as a white solid. mp: 161-163 °C. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.38 (s, 1H), 8.68 (d, *J* = 1.7 Hz, 1H), 8.01 (d, *J* = 1.8 Hz, 1H), 7.63 (d, *J* = 8.9 Hz, 1H), 7.60 – 7.53 (m, 2H), 7.16 (dd, *J* = 11.4, 2.2 Hz, 1H), 7.08 – 6.93 (m, 1H), 5.54 (s, 1H), 3.86 (s, 3H), 3.80 (s, 4H), 3.22 (s, 4H), 2.51 (s, 2H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 166.14, 163.68, 162.03, 161.35, 159.12, 159.01, 156.35, 156.33, 155.16, 152.24, 152.09, 149.77, 146.45, 146.20, 144.25, 132.47, 132.37, 126.08, 119.21, 114.50, 109.18, 107.94, 107.72, 104.80, 100.84, 100.58, 94.33, 66.21, 56.81, 51.48. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{24}H_{21}F_2N_4O_4$:

467.1525, found: 467.1519. HPLC retention time 5.39 min, purity 99.4 %.

4.1.19.36. *3-acetyl-7-((5-chloro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-morpholino-2H-chromen-2-one* **(30o).** The title compound was prepared according to general procedure F on 0.28 mmol scale **28b** and **24a**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (42 mg, 29% yield) as a white solid. mp: 152-154 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.69 (s, 1H), 8.78 (s, 1H), 8.27 (d, *J* = 1.9 Hz, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.63 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.47 (dd, *J* = 8.4, 6.8 Hz, 1H), 7.16 (dd, *J* = 11.4, 2.3 Hz, 1H), 6.96 - 6.99 (m, 1H), 3.86 (s, 3H), 3.75 – 3.26 (m, 8H), 2.36 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.41, 165.72, 163.94, 163.69, 163.27, 162.70, 158.56, 158.45, 157.97, 157.54, 157.09, 145.96, 131.80, 131.70, 126.05, 121.91, 121.74, 119.63, 117.19, 116.72, 107.60, 107.39, 104.96, 100.76, 100.51, 67.04, 66.54, 56.67, 47.06, 42.09, 18.98. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{26}H_{23}ClFN_4O_5$: 525.1336, found: 525.1346. HPLC retention time 4.69 min, purity 98.6 %.

4.1.19.37. *3-acetyl-7-((5-chloro-4-(4-fluoro-2-methoxyphenyl)pyrimidin-2-yl)amino)-4-(4-methylpiperazin-1-yl)-2H-chromen-2-one* **(30p).** The title compound was prepared according to general procedure F on 0.28 mmol scale **28b** and **24d**. Purification by column chromatography (ethyl acetate/petroleum ether, 60%) on silica gel to afford products (44 mg, 29% yield) as a white solid. mp: 154-156 °C. $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 10.70 (s, 1H), 8.76 (s, 1H), 8.26 (d, *J* = 1.7 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.62 (dd, *J* = 8.9, 1.7 Hz, 1H), 7.50 – 7.40 (m, 1H), 7.15 (dd, *J* = 11.5, 2.2 Hz, 1H), 6.93 - 6.99 (m, 1H), 3.84 (s, 3H), 3.68 (m, 4H), 3.10 (d, *J* = 7.1 Hz, 2H), 2.73 (s, 3H), 2.54 (s, 2H), 2.34 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 173.46, 164.21, 163.65, 163.27, 162.72, 158.54, 158.43, 157.96, 157.57, 157.09, 146.01, 131.80, 131.70, 126.06, 124.30, 121.85, 121.77, 119.44, 117.22, 116.66, 107.62, 107.41, 104.93, 100.77, 100.50, 56.67, 54.50, 53.84, 46.22, 19.07. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{26}ClFN_5O_4$: 538.1652, found: 538.1639. HPLC retention time 3.91 min, purity 97.3 %.

## 4.2. Biology methods

### 4.2.1. Biochemical Kinase Activity Assay

Kinase activity rate and $IC_{50}$ values of CDKs were determined using the HotSpot$^{SM}$ miniaturized radioisotope filter binding assay platform at Reaction Biology Corporation (Malvern, PA, USA). The kinase and corresponding substrate were mixed in reaction buffer (20 mM Hepes pH 7.5, 10 mM $MgCl_2$, 1 mM

EGTA, 0.02% Brij35, 0.02 mg/mL BSA, 0.1 mM $Na_3VO_4$, 2 mM DTT, 1% DMSO) at room temperature. Then DMSO solution of compound (starting at 1/10/27 μM with 3-fold serial dilution) were delivered into the kinase reaction mixture for 20 min at 25 °C. The reaction was initiated by addition of a mixture of ATP (Sigma) and [γ-$^{33}$P] ATP (PerkinElmer) to a final concentration of 10 μM, then the reaction incubated for 120 min at 25 °C. The kinase activities were tested by filter binding method, further $IC_{50}$ values and curve fits were calculated by Prism 8.0 (GraphPad Software, San Diego, CA).

### 4.2.2. Cell Growth Inhibition Assay

The effects of target compounds on cell proliferation were performed by AnoncoBio (Nanjing, China) and Sundia MediTech (Shanghai, China). Experimental procedure: Cell seeding: Spin down suspension cells and resuspend in growth medium, then count with cell counter. Dilute cell suspensions in growth medium to desired density. Seed 100 μL cells into 96-well plate in growth medium according to the plate map. Medium only is used as background control (Min). Incubate at 37 °C, overnight. Treatment of compound: 10 mM compound solution in DMSO was diluted with growth medium to 3x final concentration (50 μM). Add 50 μL 3x diluted compound solution to cells and incubate at 37 °C, 5% $CO_2$ for 72 h. Measurement of luminescent signal: Equilibrate the assay plate to room temperature, then add 40 μL of CellTiter-Glo® Reagent (Promega, USA) into each well. Mix contents for 2 minutes on an orbital shaker to induce cell lysis. Incubate at room temperature for 60 minutes to stabilize luminescent signal. Finally, luminescent signal was recorded on the multilabel reader (Envision, USA). $IC_{50}$ values were calculated using Prism 8.0 (GraphPad Software, San Diego, CA).

### 4.2.3. Metabolic Stability in Liver Microsomes

The metabolic stability of compound in rat (Corning), dog (Corning), monkey (RILD) and human (Corning) liver microsomes were tested following the same procedures by 3D BioOptima Co., Ltd. (Suzhou, China). Compound solutions (**30a** and **30i**) with final concentration of 1.0 μM and liver microsomal were incubated in 37 $^{o}$C constant temperature water bath pot. The whole incubation system included tested compounds (1.0 μM), liver microsomal (0.5 mg/mL), $MgCl_2$ (3 mM), NADPH (1 mM) and Phosphate-buffered saline buffer (PBS, 100 mM, Ph 7.4). Incubation time was 0 min, 5 min, 15 min, 30 min, and 60 min. The mixture reaction was terminated when hatch arrived time point. The rest of the drug concentration in the reaction system was

determined and analysed by the LC-MS/MS. Drug elimination rate constant k ($min^{-1}$), elimination half-life $T_{1/2}$ (min), and *in vitro* intrinsic clearance $CL_{int,\ in\ vitro}$ ($\mu L \cdot min^{-1} \cdot mg^{-1}$ protein) were calculated according to the following equations: k = - slope, $T_{1/2}$ = 0.693/k, $C_{Lint,\ in\ vitro}$ = $k/C_{protein}$, where $C_{protein}$ ($mg \cdot mL^{-1}$) is the microsomal protein concentration in the incubation system.

### 4.2.4. hERG Assay

The hERG assays were based on the competition of fluorescently labelled Tracer binding to the membrane preparation containing hERG and performed at Reaction Biology Corporation (Malvern, PA, USA) as supporting information described.

### 4.2.5. Pharmacokinetic Profile *In Vivo*

The body weights of 18 male ICR mice (Vital River Laboratory Animal Technology Co., Ltd., Zhejiang, China) were 25.1 – 28.8 g. The mice were randomly divided into two groups (each group contained 9 mice) and fasted overnight (12 h). One group of ICR mice was administered **30i** by the oral route at a dose of 30 mg/kg, and the other group of ICR mice was administered **30i** by the intravenous route at a dose of 2.0 mg/kg.

Dispensing method of the injection solution: 2.03 mg of compound, 1.006 mL of DMSO, 1.006 mL of PEG200 and 3.018 mL of saline were stirred for 15 min at 25 °C to generate a 0.4 mg/mL solution of compound. Dispensing method of the oral solution: 15.04 mg of compound and 0.5% CMC-Na (including 0.2% Tween 80) were stirred for 15 min at 25 °C to get a 3 mg/mL solution of compound.

The blood sample collection time were 0 h, 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, and 24 h (after intravenous administration) and 0 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, and 24 h (after oral administration). Approximately 80 μL of venous blood was collected at each set time point in EDTA-K2 tubes after treatment with the compounds followed by centrifugation for 10 min. The upper plasma was collected and stored below -20 °C for testing. An aliquot of 10 μL of sample was added to 200 μL of ACN containing verapamil (5 ng $mL^{-1}$) and glibenclamide (50 ng $mL^{-1}$) for protein precipitation. The mixture was vortexed for 10 min and then centrifuged at 3700 rpm for 8 min. Then, 70 μL of the supernatant was added to 70 μL water and vortexed for 10 min. An aliquot of 10 μL of the mixture was injected into an LC-MS/MS system (API 4000: LC-MS-MS-010) for analysis. An Agilent ZORBAX XDB-C18 column (50×2.1 mm, 3.5 μm) was used for the analysis of ICR mice plasma. The mobile phases were $H_2O$ (0.1% formic acid) and ACN (0.1% formic acid).

The blood drug concentration-time data was analysed by a statistical method using the DAS 2.1.1 program to calculate the pharmacokinetic parameters, including $T_{1/2}$ (elimination half-life), $T_{max}$ (the time to peak of the plasma concentration), $C_0$ (extrapolated concentration at zero time point), $C_{max}$ (maximum observed plasma concentration), $AUC_{0-t}$ (area under the plasma concentration–time curve from 0 to the last measurable time point), $AUC_{0-\infty}$ (area under the plasma concentration–time curve from time zero to infinity), CL (clearance), and $Vd_{ss}$ (volume of distribution). Bioavailability was calculated as follows: F (%) = $AUC_{0-\infty}$ (oral)/$AUC_{0-\infty}$ (iv) × dose (iv)/dose (oral) × 100%.

### 4.2.6. MV4-11 Xenograft Tumour Model

Six-week-old female BALB/c nude mice were obtained from the Vital River Laboratory Technology Co., Ltd, all mice were housed in a specific pathogen-free facility and used according to animal care regulations of Sundia meditech company, LTD. Prior to implantation, cells were harvested during exponential growth. Five million MV4-11 cells in PBS were formulated as a 1:1 mixture with Matrigel (BD Biosciences) and injected into the subcutaneous space on the right flank of BALB/c nude mice. Daily Intravenous injection was initiated when MV4-11 tumours had reached a size of 150−200 $mm^3$. Animals were then randomized into treatment groups of 6 mice each for efficacy studies and dosed with **30i** (0, 10, 20, or 40 mg/kg/day) or cytarabine (40 mg/kg/day). The compounds were dissolved in the solution of dimethylacetamide (20%), polyoxyethylated castor oil (40%) and anhydrous ethanol (40%). Tumour growth was measured twice a week using Vernier calipers for the duration of the treatment (Sundia meditech company, LTD). The volume was calculated as follows: tumour volume ($mm^3$) = [($a \times b^2$) / 2] in which a was the long diameter and b was the short diameter. The percentage of tumour growth inhibition (TGI) was calculated as follows: TGI (%) = 100 × {1 - [(tumour $volume_{final}$ - tumour $volume_{initial}$ for the compound - treated group) / (tumour $volume_{final}$ - tumour $volume_{initial}$ for the vehicle - treated group)]}. Body weight of mice was measured twice or three times per week.

### 4.2.7. Western Blots

Western blotting was performed as described. MV4-11 cells were treated with DMSO, serially diluted compound **30i** and 0.8 μM Flavopiridol for 24 h. Cells were then washed in 1 × PBS and lysed in cell lysis buffer. Antibodies were purchased as follows: p-RNAPIIS2 (Covance), CDK9, Mcl-1, c-Myc, and GAPDH (Cell Signalling Technology). GAPDH antibody was used as an internal control.

### 4.3 Molecular Modelling

All Molecular docking simulations were performed using Schrödinger Suite. Crystal structures of CDK2 (PDB code: 4BCP) and CDK9 (PDB code: 4BCG) were downloaded from the Protein Data Bank (PDB), then protein preparation of the CDK2/9 were carried out with the Protein Preparation Wizard module and Receptor Grid Generation module. Preparation of all the ligands were all initially minimized by the LigPrep module with ionization generated possible states at target pH = 7.0 ± 2.0. s. Force field option was OPLS3 and other parameters were set to the default value. Compounds **30i** was flexibly docked into the ATP-binding sites of CDK2/9 using the Ligand Docking module with standard settings in both standard precision (SP) and extra precision (XP) mode. Only the best pose with good hydrogen bond geometries and low energy conformations were considered for further analysis. Docking structures and figures were presented by the PyMOL molecular graphic system.

## Author information

Corresponding Authors

*(Tao Lu) Tel: +86-25-86185180; Fax: +86-25-86185179; E-mail: lutao@cpu.edu.cn

*(Weifang Tang) Tel: +86-25-86185182; E-mail: twf@cpu.edu.cn

*(Yadong Chen) Tel: +86-25-86185153; Fax: +86-25-861851170; E-mail: ydchen@cpu.edu.cn

Author Contributions

The authors declare no competing financial interest. The manuscript was written through contributions of all the authors. All the authors have given approval to the final version of the manuscript.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at XXXX

## Abbreviations

**CDK**, Cyclin-Dependent Kinase; **pan-CDK inhibitor**, CDK inhibitors frequently inhibit other protein kinases and were not discriminate between different CDKs; **PDB**, Protein Data Bank; **RNAPII**, RNA polymerase II; **AML**, acute myeloid leukaemia; **Mcl-1**, myeloid cell leukaemia-1; **FDA**, Food and Drug Administration; **SAR**, Structure-activity relationship; **PK**, pharmacokinetics; **$IC_{50}$**, Half maximal inhibitory concentration; **$T_{1/2}$**, elimination half-life; **$AUC_{0-t}$**, area under the plasma concentration–time curve from 0 to the last measurable time point; **$AUC_{0-\infty}$**, area under the plasma concentration–time curve from time zero to infinity; **$C_{max}$**, maximum observed plasma concentration; **$C_0$**, extrapolated concentration at zero time point; **CL**, clearance; **Boc**, *tert*-Butoxycarbonyl; **DMSO**, Dimethyl sulfoxide; **NBS**, N-bromosuccinimide; **hERG**, human Ether-a-go-go Related Gene;

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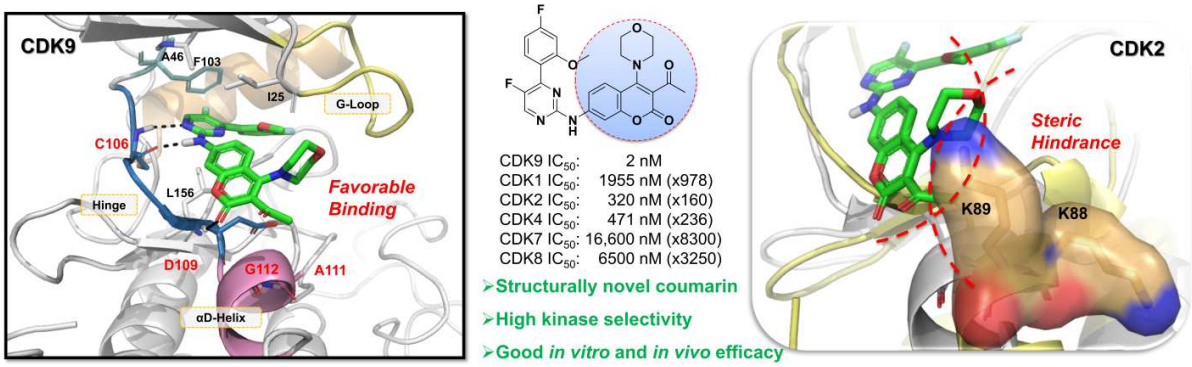
**Table of Contents graphic**



## Highlights

- A series of novel coumarin derivatives were discovered as CDK9 inhibitors.
- Compound **30i** showed high selectivity (160- to 8300-fold) over CDK1/2/3/4/5/6/7/8/19.
- A substituted coumarin located at the hinge/αD region was suggested to improve selectivity and inhibition of CDK9.
- Compound **30i** induced tumour growth inhibition in a dose-dependent manner in an AML mice model.
- Compound **30i** dose-dependently inhibited CDK9-mediated phosphorylation of RNAPII.

**Declaration of interests**

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: