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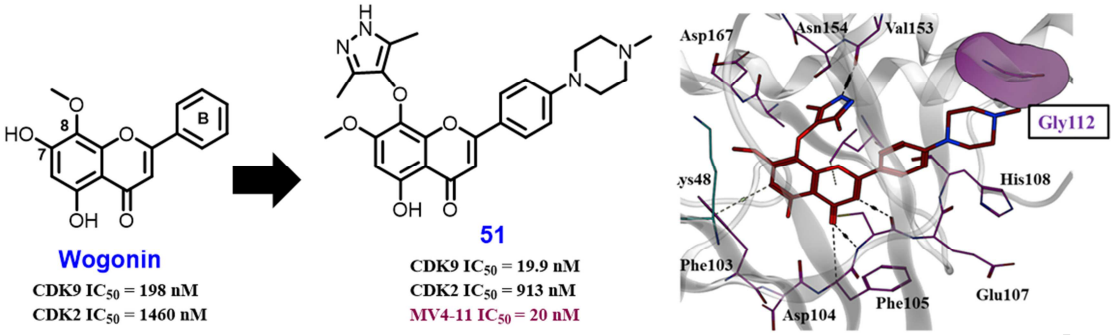

Design of wogonin-inspired selective cyclin-dependent kinase 9 (CDK9) inhibitors with potent *in vitro* and *in vivo* antitumor activity

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Abstract:

Wogonin, a natural product isolated from the plant *Scutellaria baicalensis*, has been shown to be a potent and selective inhibitor of CDK9. With the purpose of investigating the activity and selectivity of this chemical scaffold, several series of wogonin derivatives were prepared and screened for CDK9 inhibition and cellular antiproliferative activity. Among these compounds, the drug-like compound **51** showed potent activity against CDK9 (IC_{50} = 19.9 nM) and MV4-11 cell growth (IC_{50} = 20 nM). In addition, compound **51** showed much improved physicochemical properties, such as water solubility, compared with the parent compound wogonin. The follow-up studies showed that the compound **51** is selective toward CDK9-overexpressing cancer cells over normal cells. Preliminary mechanism studies on the anticancer effect indicated that **51** inhibited the proliferation of MV4-11 cells via caspase-dependent apoptosis. In addition, highlighted compound **51** showed significant antitumor activity in mouse acute myeloid leukemia (AML) models without producing apparent toxic effects *in vivo*, which gave us a new tool for further investigation of CDK9-targeted inhibitor as a potential antitumor drug especially for AML.

Keywords: Wogonin, CDK9, AML, flavonoid, antitumor

1. Introduction

Cyclin-dependent kinases (CDKs) are members of the serine/threonine kinase family, and act as heterodimeric complexes including a catalytic subunit and a regulatory subunit called cyclin. CDKs are generally divided into cell-cycle CDKs and transcriptional CDKs, depending on their specific roles [1-2]. Given their central roles in cell-cycle progression, transcription and many other biological processes, CDKs together with their corresponding cyclins have been pursued as logical targets for anticancer therapy for more than a decade, and a large number of CDK inhibitors have been developed as potential anticancer drugs [1-3]. The first generation of CDK inhibitors, such as flavopiridol, *R*-Roscovitine and SCH 727965 (Fig. 1), were almost non-selective with marked inhibitory activity and able to inhibit CDKs at nanomolar concentrations [4]. However, early efforts to find pan-CDKs inhibitors led to a narrow therapeutic window and serious side effects, which limits their clinical advantages [5].

Numerous studies have found that tumor cells lacking CDK1/2/4/6 can still proliferate normally and CDK2/4/6 knockout mice remain viable, which suggests a highly functional redundancy and compensatory mechanisms among CDK family members, especially cell-cycle CDKs [6–8]. Accordingly, inhibition of transcriptional CDKs has gained increasing attention in recent years following the observation that most pan-CDKs inhibitors exert their antitumor activity through the CDK9-mediated down-regulation of transcription of anti-apoptotic proteins such as MCL-1 [9–12]. The overexpression of CDK9 can promote the expression of MCL-1, which is involved in the pathogenesis of acute myeloid leukemia (AML), mixed-lineage leukemia (MLL) and adult T-cell leukemia/lymphoma cells (ATL) [13–16]. These findings suggest that CDK9 could be a potential target for cancer therapy.

Recently, several selective CDK9 inhibitors with potent *in vitro* and *in vivo* antitumor activity have been reported [17]. **12u**, LY2857785, and LDC000067 with CDK9 IC_{50} values of 14 nM, 11 nM, and 44 nM, respectively, are in preclinical studies [18–21]. BAY-1143572, the first selective CDK9 inhibitor that entered clinical research in 2016, showed a CDK9 IC_{50} value of 13 nM which is 100-fold more potent than that against CDK2 (IC_{50} = 1300 nM) [22].

Wogonin (Fig. 1), one of the bioactive flavones isolated from the Chinese herbal Huang-Qin (*Scutellaria baicalensis* Georgi, commonly known as Chinese Skullcap), has been shown to have various pharmacological activities including neuroprotective, antioxidative, anti-inflammatory, and especially antitumor [23–25]. Importantly, wogonin has shown virtually no toxicity for normal cells at concentrations that are lethal to tumor cells, indicating its potential for clinical application [23,26]. Recent studies have shown that wogonin can inhibit CDK9 and lead to rapid downregulation of MCL-1 in cancer cells [23]. Here, we report the design, synthesis and biological evaluation of a series of

wogonin derivatives as potent and selective CDK9 inhibitors.

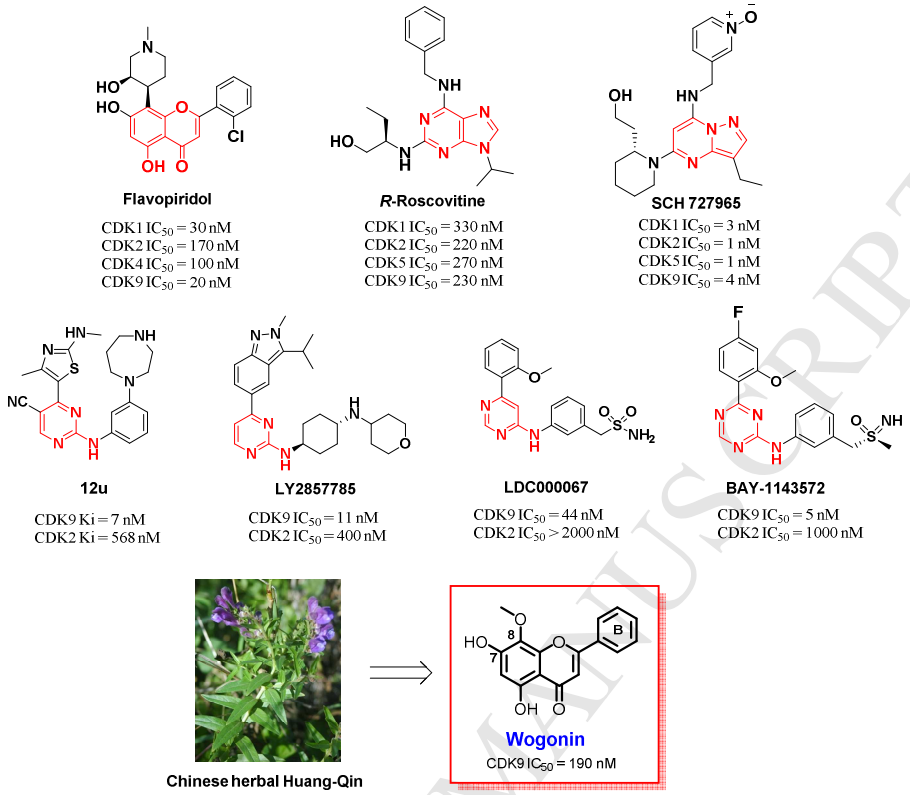


Fig. 1. Structures of some representative non-selective CDK inhibitors and selective CDK9 inhibitors.

2. Chemistry

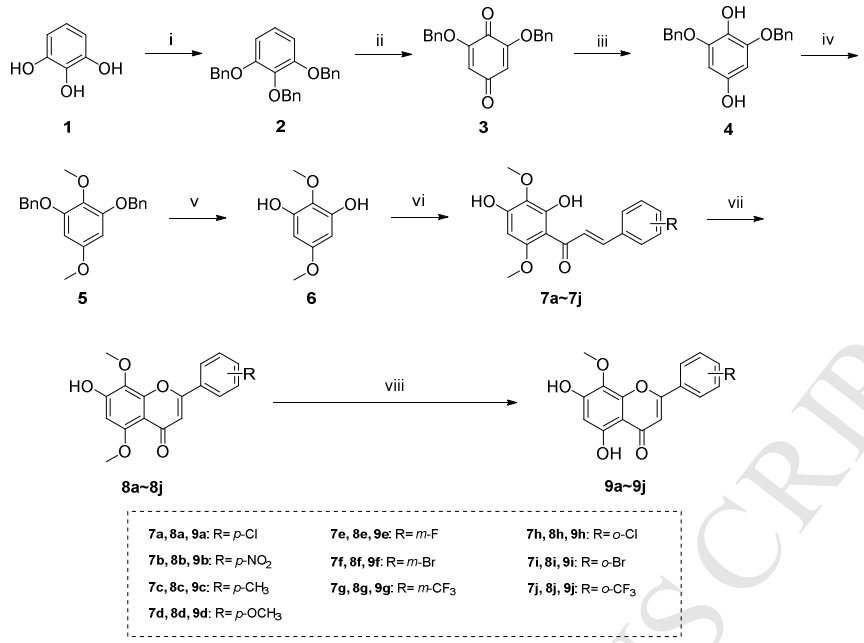
The general synthesis of wogonin derivatives **9a**−**9j** in Scheme 1 was adapted from a previously described method in the literature [26-27]. In brief, pyrogallic acid **1** was first treated with benzyl bromide to give intermediate **2**, followed by oxidation with 65% HNO_3 to get the corresponding quinone **3**. Then, **3** was reduced by sodium thiosulfate to obtain the phenol **4**. Subsequently, methylation of **4** was performed to obtain intermediate **5**, which was debenzylated to give intermediate **6**. After Friedel-Crafts acylation with different cinnamoyl chloride derivatives, the corresponding flavones **8a**−**8j** were formed through cyclization. The final compounds **9a**−**9j** were prepared by selective demethylation of intermediates **8a**−**8j** (Scheme 1).

The chemistry for the synthesis of wogonin derivatives **20a**−**20d** and **24a**−**24l** is outlined in Scheme 2. Starting from phloroglucinol **10**, the Hoesch reaction was first carried out to form intermediate **11**. After selective methylation and aldol condensation with *m*-bromobenzaldehyde and *p*-fluorobenzaldehyde, intermediates **13a**−**13b** were obtained. Subsequently cyclization and deprotection of two methyl groups with 48% HBr produced intermediates **15a**−**15b**. Then, sulfate compounds **16a**−**16b** were produced through the Elbs oxidation reaction with potassium persulfate in

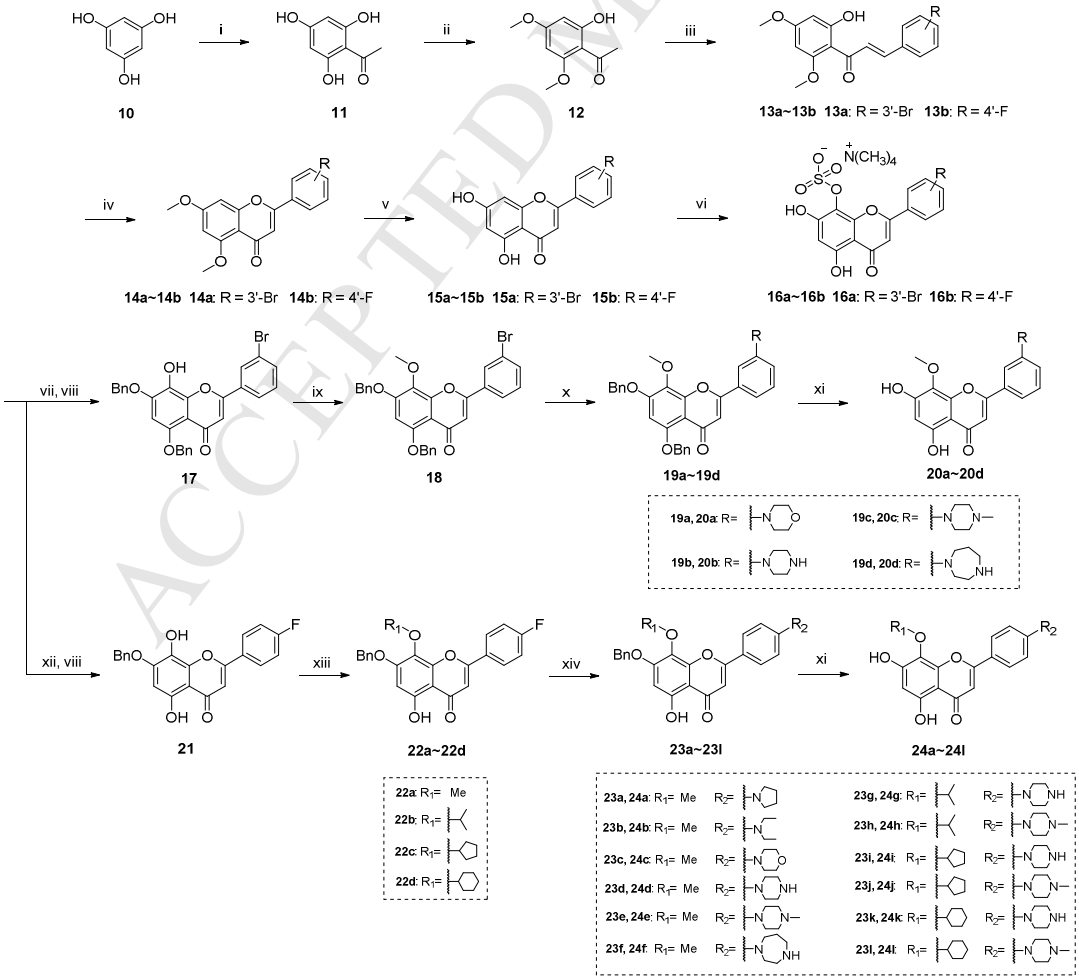
water. Subsequently, O5-benzylation and O7-benzylation of the sulfate compound **16a** followed by hydrolysis and O8-methylation generated intermediate **18**, while selective O7-benzylation of the sulfate compound **16b** followed by hydrolysis and O8-alkylation generated intermediates **22a**−**22d**. Intermediates **19a**−**19d** were obtained from **18** by treatment with different amines *via* the Buchwald-Hartwig coupling reaction, and the desired compounds **20a**−**20d** were formed by debenzylation. Intermediates **23a**−**23l** can be easily prepared from **22a**−**22d** by treatment with different amines through nucleophilic aromatic substitution, and the desired compounds **24a**−**24l** were formed by debenzylation.

Scheme 3 illustrates the synthesis of derivatives **30a**−**30b**. The preparation of intermediate **28** started from the treatment of intermediate **21** with **27**, which can be easily obtained from pyrrolidin-3-ol **25**. The target compounds **30a**−**30b** were produced after nucleophilic aromatic substitution and deprotection of benzyl and carboxybenzyl groups. Scheme 4 shows the synthesis of derivatives **36a**−**36b**. The 7- and 5-hydroxyl of intermediate **16b** were first protected with benzyl group and *p*-toluenesulfonyl group, respectively, followed by hydrolysis and the Mitsunobu reaction to obtain intermediate **33**. Intermediates **35a**−**35b** were obtained from **33** by selective deprotection of the *p*-toluenesulfonyl group and nucleophilic aromatic substitution. The final compounds **36a**−**36b** were prepared by deprotection of the benzyl group. The synthesis of compounds **39a-39b** are shown in Scheme 5, the 2-chloromethylfuran was reacted with **21** to obtain compound **37**, which was further treated with piperazine or methylpiperazine to obtain **38a-38b** in good yield (85%-90%). Next, the deprotection of benzyl generated target compounds **39a-39b** (Scheme 5).

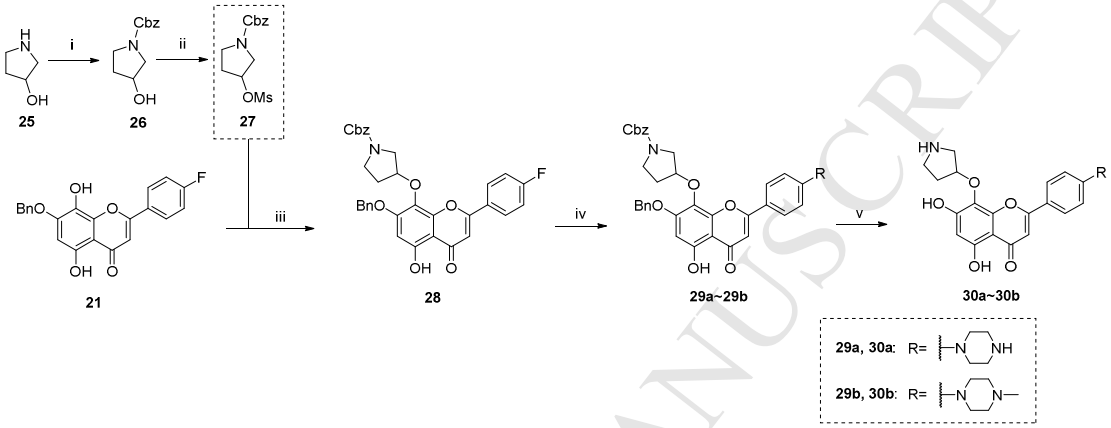
Compounds **43a-43b** were synthesized as shown in Scheme 6. Briefly, compound **21** was also used as a starting materal and was treated with 3-chloro-2,4-pentanedione under basic conditions to produce compound **40** followed by a condensation reaction with hydrazinium hydroxide to generate the key intermediate **41**. Then, piperazine or methylpiperazine was introduced to obtain compounds **42a-42b**. Ultimately, the target compounds **43a-43b** were obtained by deprotection of the benzyl group (Scheme 6). Compound **48** was synthesized in a similar way as compound **39b**, except for using ethyl formate instead of 3-chloro-2,4-pentanedione to obtain intermediate **44** (Scheme 7). Starting form compound **41**, the target compound **51** was obtained through debenzylation, methylation and substituted with methylpiperazin (Scheme 8). As depicted in Scheme 1-8, a total of 35 derivatives of wogonin were synthesized and characterized based on spectral properties.



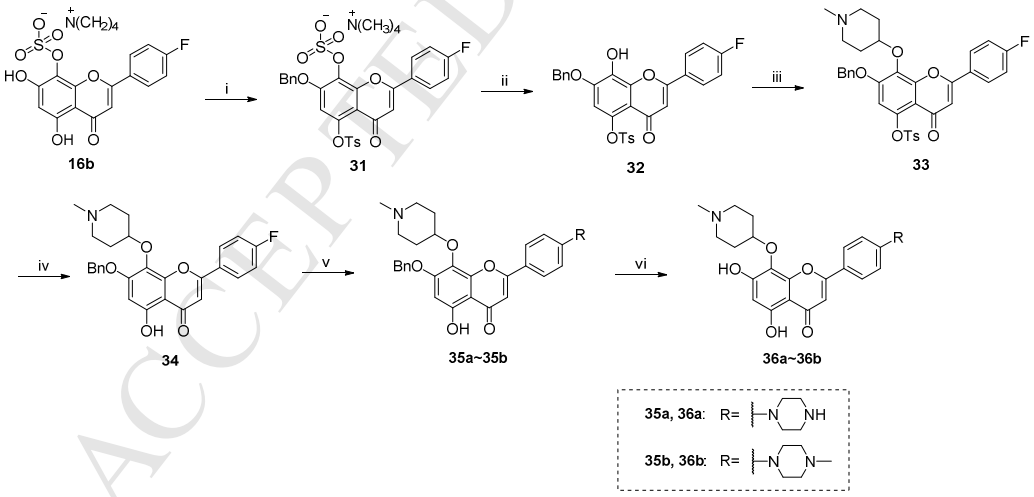
Scheme 1. Reagents and conditions: (i) $PhCH_2Br$, K_2CO_3, acetone, reflux, 48 h, 87%; (ii) AcOH, 65% HNO_3, 40 °C, 4 h, 45%; (iii) $Na_2S_2O_4$, $CH_3COOC_2H_5/H_2O$, r.t., 1 h, 37%; (iv) Me_2SO_4, aq NaOH, C_2H_5OH, r.t., 3 h, 85%; (v) H_2, Pd/C, MeOH, r.t., 8 h, 90%; (vi) Cinnamoyl chloride derivatives, BF_3-Et_2O, CH_2Cl_2, reflux, 1.5 h~6 h, 35%~80%; (vii) I_2, DMSO, 120 °C, 5 h~8 h, 30%~40%; (viii) $AlCl_3$, CH_3CN, reflux, 8 h, 65%~85%.



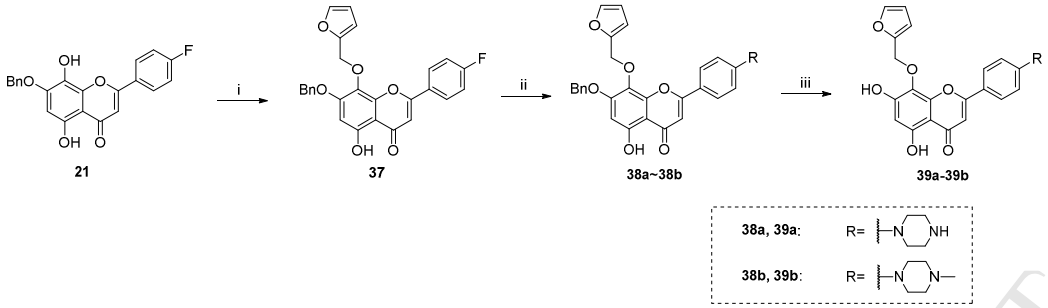
Scheme 2. Reagents and conditions: (i) CH_3CN, $ZnCl_2$, HCl (g), Et_2O, 0 ºC, 40 h, 71%; (ii) Me_2SO_4, K_2CO_3, acetone, reflux, 4 h, 83%; (iii) *p*-fluorobenzaldehyde or *m*-bromobenzaldehyde, aq KOH, C_2H_5OH, 40 ºC, 10 h~12 h, 85%~90%; (iv) I_2, DMSO, 130 ºC, 5 h~8 h, 95%~97%; (v) HBr, reflux, 60 h~72 h, 82%; (vi) $K_2S_2O_8$, Me_4NOH, H_2O, 30 ºC, 3 h~4 h, 60%; (vii) $PhCH_2Br$, Cs_2CO_3, DMF, 90 ºC, 6 h, ; (viii) 6M HCl, r.t., 10 h, 29%~39%; (ix) Me_2SO_4, K_2CO_3, acetone, reflux, 3 h, 90%; (x) appropriate amine, $Pd_2(dba)_3$, BINAP, t-BuOK, toluene, reflux, 5 h, 40%~70%; (xi) H_2, Pd/C, THF/MeOH, 30 ºC~45 ºC, 6 h~12 h, 30%~70%; (xii) $PhCH_2Br$, K_2CO_3, DMF, 50 ºC, 5 h; (xiii) Me_2SO_4, appropriate bromoalkane or cyclohexyl methanesulfonate, K_2CO_3 or Cs_2CO_3, DMF, 55 ºC~85 ºC, 4 h~9 h, 50%~90%; (xiv) appropriate amine, DIPEA, DMSO, 80 ºC~120 ºC, 8 h~20 h, 44%~80%.



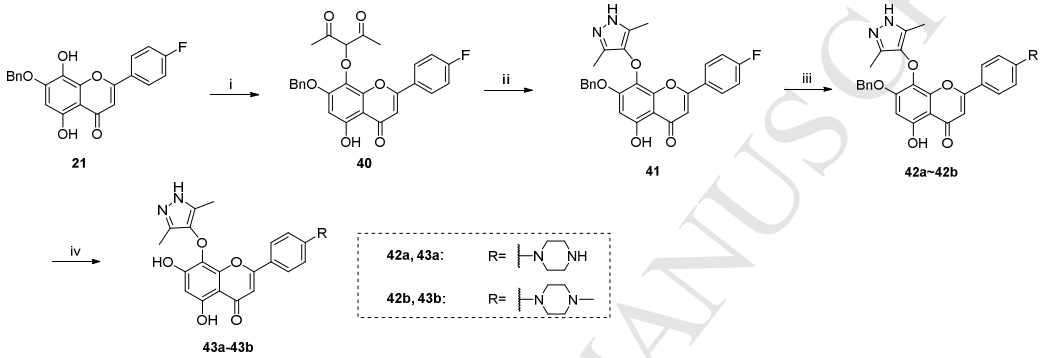
Scheme 3. Reagents and conditions: (i) Cbz-Cl, K_2CO_3, CH_2Cl_2/H_2O, r.t., 5 h, 80%; (ii) $MeSO_2Cl$, TEA, CH_2Cl_2, 0 ºC, 4 h, 80%; (iii) K_2CO_3, DMF, 80 ºC, 8 h, 50%; (iv) appropriate amine, DIEA, DMSO, 80 ºC~120 ºC, 8 h~20 h, 44%~80%; (ii) H_2, Pd/C, THF/MeOH, 30 ºC~45 ºC, 6 h~12 h, 30%~70%.



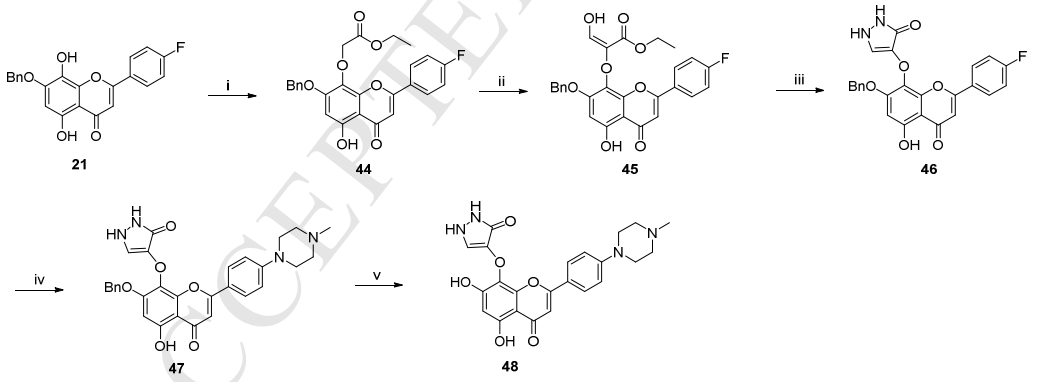
Scheme 4. Reagents and conditions: (i) (a) $PhCH_2Br$, K_2CO_3, DMF, 50 ºC, 5 h, (b) TsCl, DMAP, Cs_2CO_3, DMF, 50 ºC, 4 h; (ii) 6M HCl, r.t., 10 h, 20%; (iii) 1-methylpiperidin-4-ol, DEAD, PPh_3, THF, r.t., 1 h, 75%; (iv) KOH, MeOH, reflux, 90%; (v) appropriate amine, DIEA, DMSO, 80 ºC~120 ºC, 8 h~20 h, 44%~80%; (ii) H_2, Pd/C, THF/MeOH, 30 ºC~45 ºC, 6 h~12 h, 30%~70%.



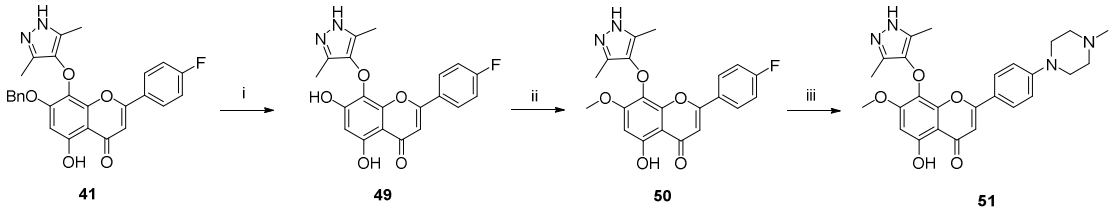
Scheme 5. Reagents and conditions: (i) 2-(chloromethyl)furan, K_2CO_3, CH_3CN, reflux, 2 h, 60%; (ii) appropriate amine, DIEA, DMSO, 80 ºC~120 ºC, 8 h~20 h, 44%~80%; (iii) H_2, Pd/C, THF/MeOH, 15 ºC, 6 h~12 h, 30%~70%.



Scheme 6. Reagents and conditions: (i) 3-chloro-2,4-pentanedione, K_2CO_3, acetone, reflux, 3 h, 50%; (ii) hydrazinium hydroxide, EtOH, CH_3COOH, r.t., 90%; (iii) appropriate amine, DIEA, DMSO, 80 ºC~120 ºC, 8 h~20 h, 44%~80%; (iv) H_2, Pd/C, THF/MeOH, 30 ºC~45 ºC, 6 h~12 h, 30%~70%.



Scheme 7. Reagents and conditions: (i) Ethyl chloroacetate, K_2CO_3, acetone, reflux, 4 h, 60%; (ii) Ethyl formate, NaH, THF, 45 ºC, 4 h, 55%; (iii) hydrazinium hydroxide, MeOH, r.t., 70%; (iv) 1-methylpiperazine, DIEA, DMSO, 85 ºC, 8 h, 45%; (v) H_2, Pd/C, THF/MeOH, 30 ºC, 8 h, 40%.



Scheme 8. Reagents and conditions: (i) H_2, Pd/C, THF/MeOH, 30 ºC, 6 h; (ii) Me_2SO_4, K_2CO_3, acetone, 50 ºC, 3 h,

65%. (iii) methylpiperazine, DIEA, DMSO, 60 to 80 ºC, 8 h, 68.5%.

3. Results and discussion

3.1. Rational drug design

Due to the evolutionary conservation of the ATP binding site, the development of highly selective small molecule ATP-antagonistic CDK inhibitors is not an easy task [20, 28]. CDK9 specificity is not easy to achieve due to its cross-reaction with other cell cycle CDKs, in particular CDK2. By analyzing the crystal structures of CDK2 and CDK9, we found that the ATP binding pocket of CDK9 is more flexible than that of CDK2, indicating that the CDK9 ATP pocket can accommodate larger ligands (Fig. 2). Therefore, in this study, a series of derivatives with substituents at the *para* or *meta* position of the B ring of wogonin were designed and synthesized to obtain selective CDK9 inhibitors.

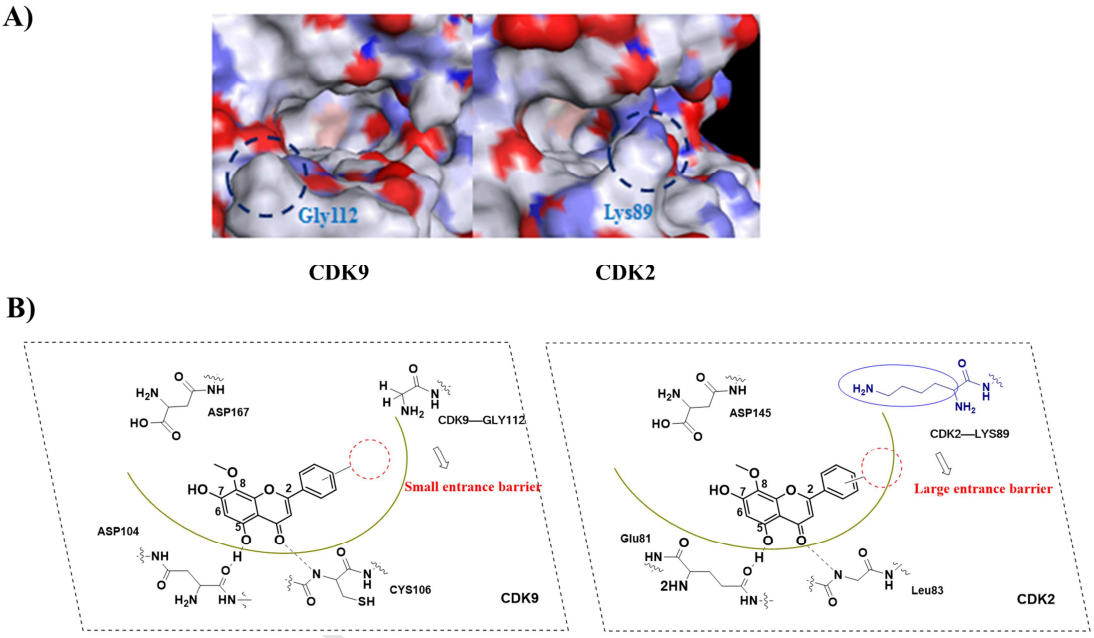


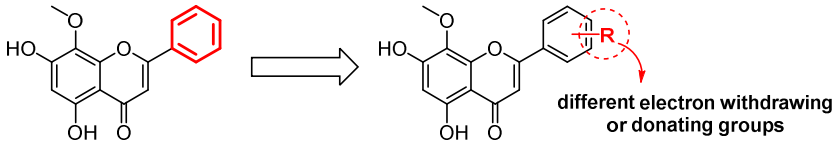
Fig. 2. (A) The comparison of the ATP binding pocket in CDK9 and CDK2. (B) The binding mode of wogonin in CDK9 and CDK2.

3.2. Structure-activity relationship analysis

We first designed and synthesized several derivatives with different electron withdrawing or donating groups on the B ring of wogonin to explore the effect on its potency and selectivity for CDK9. CDK2 was used as a control since it has a high sequence identity with other CDKs. All compounds were tested against CDK9 and CDK2 (at a concentration of 1 μM), as well as the hepatoma cells cell line HepG2. The results are summarized in **Table 1**, together with the potencies of the inhibitory effects of wogonin as reference. It was found that introduction of electron withdrawing or donating substituents at different positions of the B ring of wogonin offer neither obvious enhancement in the kinase activity nor in the anticancer activity. In particular, electron donating groups, such as methyl or methoxyl (compound **9c** and **9d**), have detrimental effects on the inhibitory activity.

Table 1

In vitro activity of wogonin derivatives with B-ring modification



Comp.	R	Kinase inhibition (%)[a]		Cytotoxicity
		CDK9/T1	CDK2/A	IC_{50}/μM[b], HepG2
wogonin	-	67.8	40.7	17.6
9a	*p*-Cl	46.4	ND	34.2
9b	*p*-NO_2	32.5	ND	>100
9c	*p*-CH_3	19.2	ND	24.9
9d	*p*-OCH_3	27.6	ND	42.4
9e	*m*-F	56.2	ND	59.4
9f	*m*-Br	42.6	ND	38.2
9g	*m*-CF_3	52.3	ND	>100
9h	*o*-Cl	51.4	ND	>100
9i	*o*-Br	56.5	18.9	33.5
9j	*o*-CF_3	54.6	ND	>100

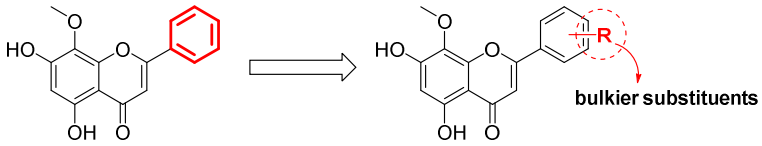
[a] Percent inhibition at 1 μM, values are the averages derived from at least three replicates.

[b] Anti-proliferative activity by MTT-72 h assay, values are the averages from at least five independent dose response curves.

Then, a series of derivatives with bulkier substituents at the *para* or *meta* position of the B ring of wogonin were additionally designed and synthesized. According to the data presented in **Table 2**, compounds **20a**−**20d** and **24a**−**24f** exhibited enhanced selectivity for CDK9 compared to wogonin. Generally, the CDK9 inhibitory activity of the *para*-substituted compounds **24a**−**24f** was significantly higher than that of the *meta*-substituted compounds **20a**−**20d**. In addition, among these compounds, the piperazine and methylpiperazine substituted analogues **20b**, **20c**, **24d** and **24e** exhibited potent proliferstion inhibitory activity in the HepG2 cell line, while the morpholine and pyrrolidine substituted analogues **20a**, **24a** and **24c** showed dramatically reduced antiproliferative activity. Compound **24e** was the most potent and selective CDK9 inhibitor in this series with a HepG2 IC_{50} value of 6.6 μM.

Table 2

In vitro activity of wogonin derivatives with further B-ring modification



Comp.	R	Kinase inhibition (%)[a]		Cytotoxicity
		CDK9/T1	CDK2/A	IC_{50}/μM[b], HepG2
20a	*m*-morpholino	45.4	NA	>100
20b	*m*-piperazin-1-yl	46.4	NA	9.9
20c	*m*-methylpiperazin-1-yl	46.5	12.2	13.1
20d	*m*-1,4-diazepan-1-yl	60.9	11.9	21.7
24a	*p*-pyrrolidin-1-yl	33.9	NA	>100
24b	*p*-diethylamino	58.0	29.2	6.4
24c	*p*-morpholino	58.0	NA	78.3
24d	*p*-piperazin-1-yl	72.1	NA	17.9
24e	*p*-methylpiperazin-1-yl	81.2	NA	6.6
24f	*p*-1,4-diazepan-1-yl	65.4	12.7	18.2

[a] Percent inhibition at 1μM, values are the averages derived from at least three replicates.

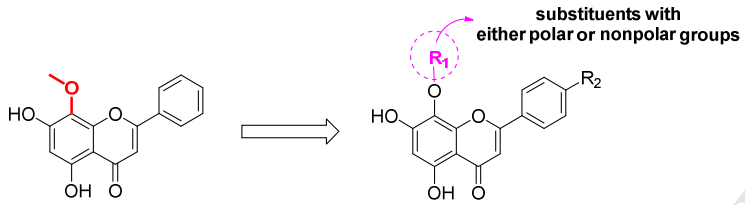
[b] Anti-proliferative activity by MTT-72 h assay, values are the averages from at least five independent dose response curves.

In order to improve the kinase inhibitory activity and cellular antiproliferative potency, we further modified the 8-methoxy group of wogonin based on activity data collected from previous analogues. Different polar and nonpolar groups were introduced to the O8 of wogonin, while piperazine and methylpiperazine were introduced to the *para*-position of the B ring. *In vitro* enzyme activity assay showed that this series of compounds had a similar CDK9 selectivity profile, but their inhibitory activity varied widely (**Table 3**). The O8-isopropyl substituted analogues were more potent than the corresponding O8-cyclopentyl, O8-cyclohexyl and furylmethyl substituted analogues, as indicated by compounds **24g**, **24i**, **24l** and **42a**. This finding suggests that it is difficult to accommodate large-sized hydrophobic groups in the cavity where the wogonin O8 is located. Modification of hydrophilic groups like pyrrolidinyl, methylpiperidinyl, and dimethylpyrazolyl at the O8-position resulted in compounds **30a**−**30b**, **36a**−**36b** and **43a-43b**, which showed comparable selectivity and inhibitory activity for CDK9, but their cellular potency increased dramatically. Further determination of the CDK9 and CDK2 IC_{50} values of the representative compounds **24g**, **24h**, **43a** and **43b** showed that their CDK9 inhibitory activities were significantly improved compared to wogonin (**Table 4**). In particular,

compound **43a** was the most potent CDK9 inhibitor with an IC_{50} value of 10.8 nM, which was ten-fold higher than that of wogonin. On the other hand, they had weak inhibitory activity against CDK2.

Table 3

In vitro activity of wogonin derivatives with 8-O-modification



Comp.	R_1	R_2	Kinase inhibition (%)[a]		cytotoxicity
			CDK9/T1	CDK2/A	IC_{50}/μM[b], HepG2
24g		*p*-piperazin-1-yl	88.6	31.4	18.0
24h		*p*-methylpiperazin-1-yl	82.1	24.0	14.8
24i		*p*-piperazin-1-yl	14.1	ND[c]	5.3
24j		*p*-methylpiperazin-1-yl	30.7	ND	15.8
24k		*p*-piperazin-1-yl	52.1	ND	22.8
24l		*p*-methylpiperazin-1-yl	56.5	ND	27.8
30a		*p*-piperazin-1-yl	74.0	NA[d]	>100
30b		*p*-methylpiperazin-1-yl	57.0	NA	>100
36a		*p*--piperazin-1-yl	74.6	NA	39.4
36b		*p*-methylpiperazin-1-yl	71.8	NA	6.7
39a		*p*-piperazin-1-yl	40.2	NA	8.3
39b		*p*-methylpiperazin-1-yl	52.6	NA	7.0
43a		*p*-piperazin-1-yl	97.1	32.2	8.5

43b		*p*-methylpiperazin-1-yl	98.1	51.1	6.3
48		*p*-methylpiperazin-1-yl	34.0	ND	8.1

[a] Percent inhibition at 1 μM, values are the averages derived from at least three replicates.

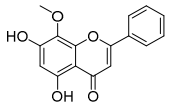
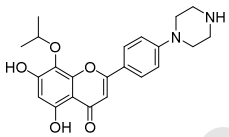
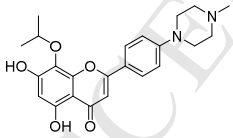
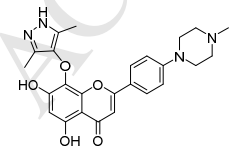
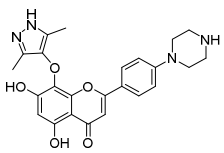
[b] Anti-proliferative activity by MTT-72 h assay, values are the averages from at least five independent dose response curves.

[c] ND represents not determine.

[d] NA represents no activity.

Table 4

Inhibition (IC_{50}) of representative compounds **24g-24h**, **43a-43b** and **wogonin** against CDK9, CDK2 and cancer cells HepG2, A549, HCT116 and MV4-11.

Comp.	**Structure**	**Kinase inhibition IC_{50} (nM)[a]**		**HepG2**	**A549**	**HCT116**	**MV4-11**
		CDK9/T1	**CDK2/A**				
wogonin		198	1460	17.6	42.3	27.3	30.7
24g		94.1	NA	17.9	5.5	3.4	6.1
24h		179	NA	17.7	4.1	12.8	6.3
43a		10.8	1040	8.6	5.5	4.8	4.4
43b		12.7	830	6.3	2.5	3.3	2.2

[a] Values are the averages from three-independent experiments.

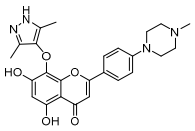
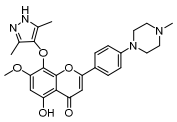
3.3. Cellular antiproliferative activity towards a panel of cancer cell lines

The cellular antiproliferative activity of representative compounds was further evaluated in a panel of human cancer cell lines including MV4-11 (leukemia), A549 (lung), HCT116 (colon) and MCF-7 (breast) using the tetrazolium dye (MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Table 4). Wogonin was selected as the control and the inhibitory activity of wogonin toward these cancer cells was similar to that reported by others [29-31]. Most of the representative compounds showed potent inhibitory activity toward all four cell lines. Especially compounds **43a** and **43b**, which were active against all four cell lines, and showed much improved inhibitory activity toward these cell lines compared with wogonin. It is worth noting that compound **43a** and **43b** exhibited the strongest inhibitory effect against CDK9 with an IC_{50} value of 10.8 and 12.7 nM, respectively.

3.4. Drug-like properties-inspired optimization

By combining the selectivity for CDK9 and inhibitory activity against cancer cells, we intended to select compound **43a** for further *in vivo* evaluation. However, this compound showed poor physicochemical properties, especially low solubility in water (1.0 μg/mL), which may limit its application in further *in vivo* studies. Therefore, for further *in vivo* evaluation studies, it was necessary to improve the solubility of this compound. By analyzing its structure, it was suspected that the presence of the two hydroxyls may limit its drug-like property. The hydroxyl at the 5-position formed an important hydrogen bond with CDK9, while the C-7 hydroxyl did not show a crucial role in the binding with CDK9. Thus, a methyl group was introduced at the C-7 hydroxyl position to obtain compound **51**. The solubility and Log $D_{7.4}$ were determined for compounds **43a** and **51**. Wogonin was selected as the control. Two methods were used to determine the solubility. Their intrinsic aqueous solubility was determined on a Gemini Profiler instrument (*p*ION Inc., Woburn, MA, USA) using the 'goldstandard' Avdeef-Bucher potentiometric titration method. Compound **51** (28 μg/mL) showed a 28-fold higher solubility than compound **43a** without methyl at C-7. Another method was used to determine their solubility under acidic condition, compound **51** showed dramatically increased solubility (solubility was determined in diluted methanesulfonic acid buffer at pH = 4.5). The hydrophobicity parameter Log $D_{7.4}$ of compounds **51** was also found to be more acceptable than that of compound **43a**. (Table 5). In addition, compound **51** showed comparable inhibitory activity against CDK9 and selectivity towards CDK2. Thus, compound **51**, which showed improved physicochemical properties and potent CDK9 inhibitory activity, was selected for further pharmacological evaluation both *in vitro* and *in vivo*.

Table 5
Physicochemical properties and inhibition against CDKs of compounds **43a**, **51** and **Wogonin**.

Comp.	**Structure**	Intrinsic solubility（μg/mL）	Solubility (pH = 4.5)（μg/mL）	Log *D* (pH = 7.4)	CDK9 IC_{50} (nM)	CDK2 IC_{50} (nM)
43a		1.0	30	2.91	10.8	1040
51		28	4540	2.01	19.9	913
Wogonin	-	13	149	1.18	198	1460

3.5. Molecular docking

The molecular docking studies were performed to understand the interaction pattern of the representative compound **51** in the active site of CDK9 (PDB ID: 3BLR). The overlay of **51** with flavopiridol in the binding site is shown in Fig. 3A. The key interactions of **51** with the key residues in the active site are shown in Fig. 3B and 3C. As depicted in Fig. 3B, compound **51** could well embed in the ATP binding pocket of CDK9. The carbonyl group of compound **51** forms a hydrogen bond with Cys106 in the hinge region. In addition, compound **51** forms another important H-bonds with Ala153. Besides these H-bond interactions, two other important H-π interactions, specifically with Phe103 and Leu156, were also observed. The benzomethylpiperazine moiety could easily reach the solvent area. When docking the compound into the CDK2 ATP binding site (PDB ID: 4BCP, Fig. 3D), the steric hindrance of Lys89 at the inlet of the ATP binding pocket makes it difficult for compound **51** to form a stable conformation and thus cannot bind tightly to CDK2. The corresponding amino acid in the CDK9 active site is Gly112, which is more favorable for accommodating the piperazinyl of compound **51**. This difference in binding conformations may be the reason for the high potency and selectivity of compound **51** toward CDK9.

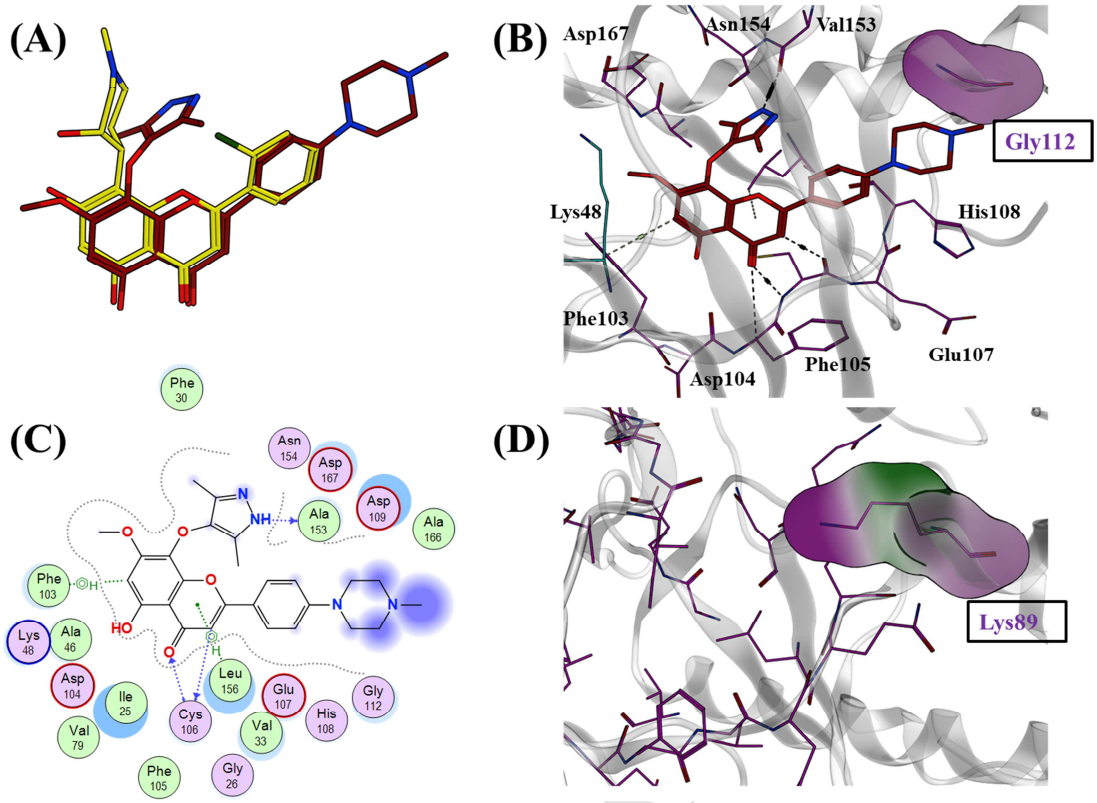


Fig. 3. (A) Overlay of flavopiridol (yellow) with compound **51** (brown) in the active site of CDK9. (B) The docking model of compound **51** in a complex with CDK9. The hydrogen bonds ares depicted by black dotted lines. (C) Two-dimensional view of the key interactions of **51** with the active site residues. (D) The active site of CDK2. The steric hindrance of Lys89 is shown as surface.

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Table 7
Antiproliferative activity of compound **51** against a panel of human tumor cell lines

Human cell line		cytotoxicity IC_{50} (μM)[a]	Human cell line		cytotoxicity IC_{50} (μM)[a]
Origin	**Designation**		**Origin**	**Designation**	
Leukemia	HL60	5.21	Colon carcinoma	HT29	1.80
Leukemia	MV4-11	0.02	Colon carcinoma	SW480	1.86
Leukemia	CEM	1.24	Lung carcinoma	A549	1.81
Leukemia	THP-1	0.79	Gastric carcinoma	BGC803	3.12
Breast carcinoma	MCF-7	0.48	Cervical carcinoma	Hela	2.32
Breast carcinoma	BT-549	1.85	Myeloma	RPMI8226	0.82
Hepatic carcinoma	HepG2	2.67	Renal (normal)	HEK293	22.1

Colon carcinoma	HCT-116	0.39	Liver (normal)	L02	15.1

[a] Anti-proliferative activity by MTT-72 h assay, values are the averages from at least five independent dose response curves.

*3.6. Evaluation of the cellular inhibitory effect and mechanism of action of **51***

The cellular antiproliferative activity of compound **51** was analyzed in a panel of 14 cancer cell lines (CDK9-expressing) and 2 normal cell lines (Table 7). The analysis revealed that compound **51** had potent cellular antiproliferative activity against these cancer cell lines, especially against the leukemia cancer cell line MV4-11, with an IC_{50} value of 20 nM. When compound **51** was used to treat the normal cell lines (HEK293 and L02), it exhibited low toxicity (IC_{50} values of 15 and 22 μM). It is worth noting that the introduction of methyl in the 7-position of compound **51**, it significantly improved the antiproliferative activity against certain types of tumor cell lines, including MV4-11 and HCT116, compared with **43a**. The evaluation of the inhibition of cellular showed that compound **51** has a good cellular therapeutic window, with a superior profile to that of flavopiridol. Compared with flavonoid derivatives previously reported by our collaborative group, compound **51** exhibited the best *in vitro* antitumor activity profile [32-33].

Based on the results of the analysis of the *in vitro* antitumor activity, the MV4-11 cell line was selected to examine the effects of compound **51** on the CDK9-mediated signaling pathways (Fig. 4). The effect of the treatment with compound **51** on the distribution of cells in the cell cycle was first assessed by propidium iodide (PI) staining. Synchronized cells were r left untreated as control or treated with compound **51**. Compound **51** (at 0.25 or 1.25 μM) induced an obvious increase in the number of cells in the G1 phase with a decrease was detected in the S phase at 24 h, which may be due to the higher selectivity of compound **51** toward the inhibition of CDK9 (a transcriptional regulator, not a cell cycle regulator).

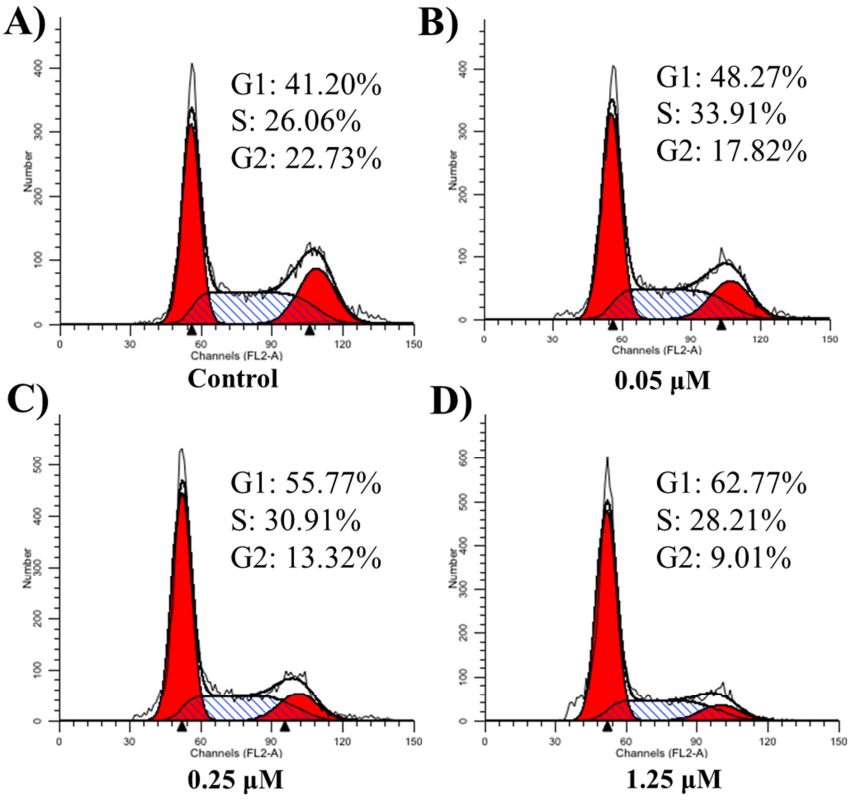

Fig. 4. Effects of compound **51** on cell cycle progression. (A) the control. (B) **51** (0.05 μM). (C) **51** (0.25 μM). (D) **51** (1.25 μM).

We also evaluated apoptosis in MV4-11 cell line after treatment with the indicated concentrations of **51** for 24 h. The nuclei changes in MV4-11 cells were observed by confocal microscopy, after staining with DAPI. As shown in Fig. 5A, untreated MV4-11 cells were stained uniformly with blue fluorescence, while compound **51**-treated cells emitted bright fluorescence, which indicates the early phenomena of apoptosis. In addition, the nuclei showed a dose-dependent and time-dependent chromatin condensation and characteristic apoptotic morphological changes when treated with **51** (Fig. 5B). To further confirm the compound **51**-induced apoptosis, the Annexin V/PI staining assay was employed. After cells were treated with 0.05, 0.25 and 1.25 μM of compound **51** for 24 h, the percentage of total apoptotic cells was 17.95, 28.54 and 35.48%, respectively, indicating the induction of apoptosis in a concentration-dependent manner (Fig. 5C). Together, these results demonstrated that compound **51** inhibited proliferation of MV4-11 cells through a concentration- and time-dependent apoptotic mechanism.

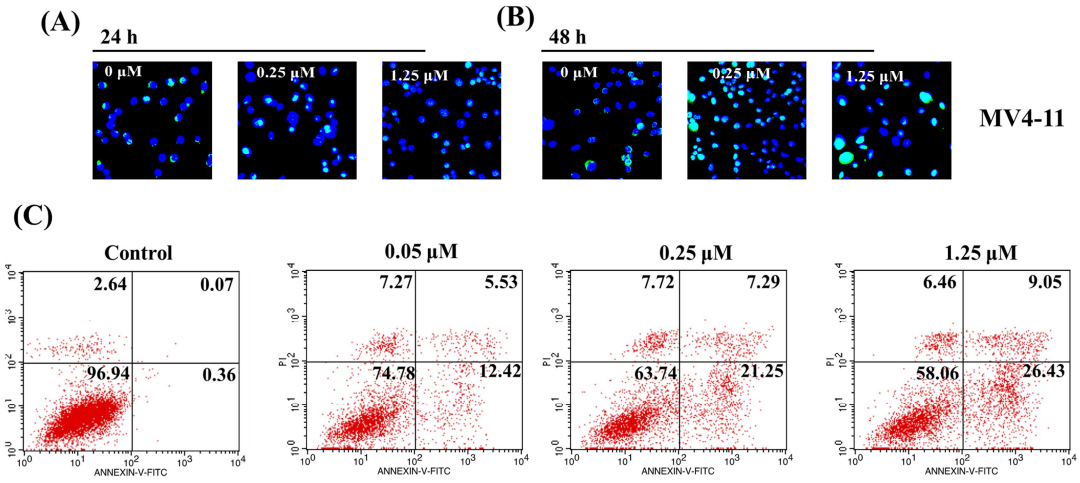

Fig. 5. Effect of compound **51** on apoptosis in MV4-11 cells by microscopy with DAPI staining after treatment with compound **51** for 24 h (A) or 48 h (B), and by flow cytometry (C).

Western blot analysis was performed to study the cell proliferation inhibitory mechanism. As expected, upon 4 h treatment with compound **51**, the direct phosphorylation site in the carboxy-terminal domain of phosphorylated-RNA Pol II Ser2 was dose-dependently inhibited. In addition, the expression level of proteins, such MCL-1 and c-MYC were dose-dependently decreased. MCL-1 and c-MYC have been reported to be important antiapoptotic proteins, which could hinder apoptosis in cancer cells (Fig. 6). In contrast, the antiapoptotic protein BCL-2 was not affected when treated with compound **51**. Furthermore, we also analyzed the effects of compound **51** on important apoptosis markers. After treatment for 24 h with compound **51**, a dose-dependent increase in the protein levels of apoptosis proteins cleaved PARP and caspase-3 was observed (Fig. 6). Together, all the results suggested that in MV4-11 cells compound **51** can induce apoptosis by targeting CDK9.

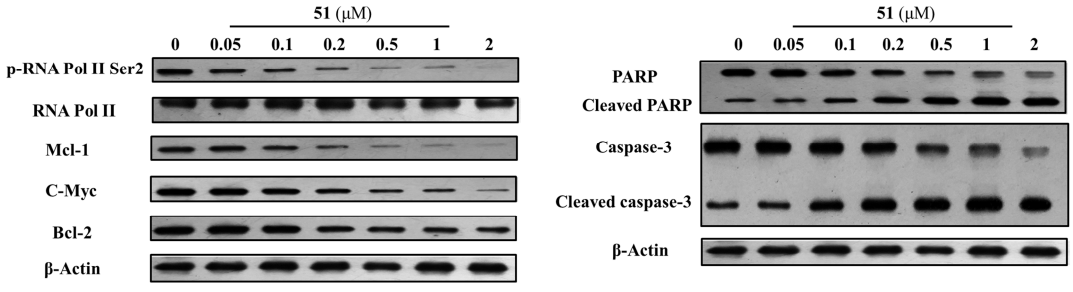

Fig. 6. Cellular effects of compound **51** on CDK9-mediated signaling pathways and apoptosis in MV4-11 cells.

*3.7. In vivo evaluation of compound **51**.*

We also evaluated the *in vivo* antitumor activity of compound **51** in the subcutaneous MV4-11 AML xenograft murine model. The nude mice bearing MV4-11 tumor xenografts were randomly

injected with vehicle, the positive control drug doxorubicin (5 mg/kg) and compound **51** (15, 30 and 45 mg/kg) every other day for three weels. The treatment with compound **51** resulted in the marked inhibition of tumor growth (Fig. 7A, B). On day 21 of treatment with compound **51** (15, 30 and 45 mg/kg), the tumor growth inhibition (TGI) was 22.9, 42.6 and 53.0%, respectively, without cansing any mortality. In addition, there was no significant change in the body weight after treatment with **51** at various concentrations. Thus, the *in vivo* study results further confirmed the antitumor potential of compound **51**.

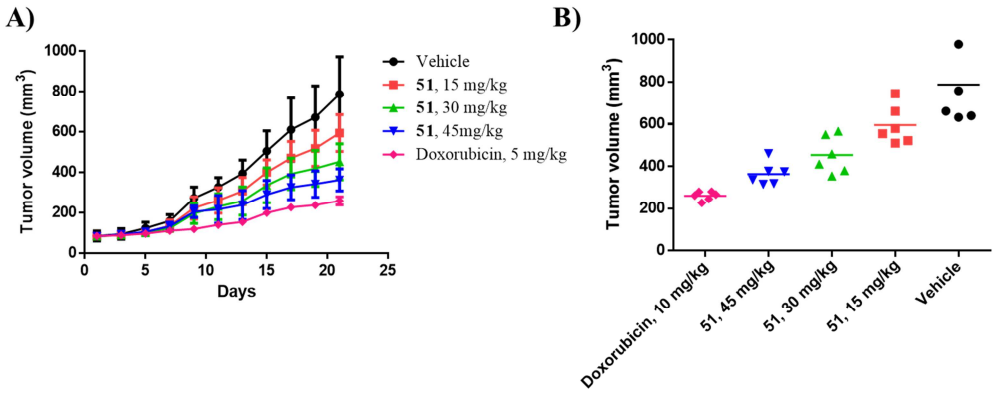

Fig. 7. In vivo anticancer efficacy of compound **51**. (A) compound **51** and the positive control doxorubicin suppressed the tumor growth *in vivo* in MV4-11 tumor xenografts nude model. (B) Tumor volumes of each mouse on day 21.

4. Conclusion

In this study, inspired by the selective CDK9 flavonoid antitumor compound Wogonin, a series of derivatives were designed and synthesized. Among them, a promising compound, namely compound **51** (named as **LBJ-23**) emerged as the most specific inhibitor of CDK9 with an IC_{50} value of 19.9 nM and exhibited excellent selectivity toward other CDKs. The preliminary docking studies may explain the selectivity toward CDK9. Additionally, it also shows an acceptable drug-like properties with good solubility both in aqueous and weak acid environment. Compound **51** exhibited strong antiproliferative activity against a panel of established cell lines including leukemia cell lines and solid tumor cell lines by inducing apoptotic cell death. Furthermore, the highlighted compound **51** showed significant antitumor efficacy without obvious toxic effects *in vivo*, which provide us a new chemical tool for further investigate research CDK9-targeted inhibitors as a potential antitumor drug, especially for AML.

5. Experimental procedures

5.1. Chemistry

General experimental methods. All chemicals and reagents and were purchased from commercial sources. Organic solutions were concentrated in a rotary evaporator (Büchi Rotavapor) below 55 ^{o}C under reduced pressure. Silica gel thin-layer chromatography was performed on precoated plates GF-254 and visualized under UV light. Melting points were determined with a Melt-Temp II apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-300 instrument using deuterated solvents with tetramethylsilane (TMS) as internal standard. Chemical shifts are given in ppm (δ). The multiplicities are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. IR spectra were recorded on a Nicolet iS10 Avatar FT-IR spectrometer using KBr film. ESI-mass and high resolution mass spectra (HRMS) were recorded on a Water Q-Tofmicro mass spectrometer. All tested compounds exhibited greater than 95% purity unless otherwise noted. The synthesis routes of **9a-9j** were shown in previos works [26-27].

*5.1.1. 2, 4, 6-Trihydroxyacetophenone hydrate (**11**).*

Pyrogallol (**10**) (63 g, 0.5 mol) was dissolved in anhydrous ether (300 mL), then anhydrous $ZnCl_2$ (13.4 g, 0.1 mol) and anhydrous acetonitrile (50 g, 1.25 mol) were added and then the reaction mixture was stirred in the presence of dried HCl gas at 0 ^{o}C for 40 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was raised to room temperature and filtered. The filter cake was dried to constant weight and dissolved in water (200 mL). Then the mixture was refluxed for 6 h and a large amount of solid was formed. The mixture was cooled to room temperature, filtered, and the cake was dried to give a white solid (60 g, 71% yield). mp 119-121 ^{o}C.

*5.1.2. 4, 6-Dimethoxy-2-hydroxyacetophenone (**12**).*

Compound **11** (33.6 g, 0.2 mol) was dissolved in anhydrous acetone (350 mL) and K_2CO_3 (63.48 g, 0.46 mol) was added. Then dimethyl sulphate was added dropwise at room temperature and the mixture was heated to 45 ^{o}C for 4 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature, filtered and the filtrate was poured into 5 volumes of water and adjusted to pH = 3 - 4 with 1 M HCl to precipitate a large amount of solid. The mixture was filtered and dried to give a white solid (32.5 g, 83% yield). mp 80-82 ^{o}C.

5.1.3. (E)-3-(3-Bromophenyl)-1-(2-hydroxy-4, 6-dimethoxyphenyl)prop-2-en-1-one (***13a***).

Compound **12** (30 g, 0.15 mol) was dissolved in ethanol (450 mL) and *p*-fluorobenzaldehyde (31 g, 0.17 mol) was added. Then 7% KOH aqueous solution (280 mL, 0.38 mol) was added dropwise and the mixture was kept below 5 ^{o}C and then heated to 40 ^{o}C for 12 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature and poured into 10 volumes

of water and adjusted to pH = 3 - 4 with 6 M HCl to precipitate a large amount of solid. The mixture was filtered and dried to give a yellow solid (50.1 g, 90% yield). mp 190-193 °C.

5.1.4. (E)-3-(4-Fluorophenyl)-1-(2-hydroxy-4, 6-dimethoxyphenyl)prop-2-en-1-one (***13b***).

Compound **12** (16.36 g, 84 mmol) was dissolved in ethanol (250 mL) and *p*-fluorobenzaldehyde (11.39 g, 92 mmol) was added. Then 7% KOH aqueous solution (150 mL, 0.21 mol) was added dropwise and the mixture was kept below 5 °C and then heated to 40 °C for 12 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature and poured into 10 volumes of water and adjusted to pH = 3-4 with 6 M HCl to precipitate a large amount of solid. The mixture was filtered and dried to give a yellow solid (21.33 g, 84.6% yield). mp 200-202 °C.

5.1.5. 2-(3-Bromophenyl)-5, 7-dimethoxy-4H-chromen-4-one (***14a***).

Compound **13a** (16.7 g, 46 mmol) was dissolved in DMSO (40 mL) at the temperature of 60 °C and I_2 (cat., 0.32 g, 1.2 mmol) was added. Then the mixture was heated to 130 °C for 8 h. The reaction was monitored by TLC. After the end of the reaction, the hot mixture was poured into 10 volumes of a saturated aqueous solution of sodium thiosulfate to precipitate a large amount of solid. The mixture was filtered and dried to give a yellow solid (16.2 g, 97% yield). mp 278-280 °C.

5.1.6. 2-(4-Fluorophenyl)-5, 7-dimethoxy-4H-chromen-4-one (***14b***).

Compound **13b** (17.33 g, 57 mmol) was dissolved in DMSO (43 mL) at the temperature of 60 °C and I_2 (cat., 0.36 g, 1.4 mmol) was added. Then the mixture was heated to 130 °C for 5 h. The reaction was monitored by TLC. After the end of the reaction, the hot mixture was poured into 10 volumes of a saturated aqueous solution of sodium thiosulfate to precipitate a large amount of solid. The mixture was filtered and dried to give a yellow solid (16.3 g, 95% yield). mp 291-295 °C.

5.1.7. 2-(3-Bromophenyl)-5, 7-dihydroxy-4H-chromen-4-one (***15a***).

Compound **14a** (20 g, 55 mmol) and 48% hydrobromide (250 mL) were refluxed for 72 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature and poured into 10 volumes of ice water to precipitate a large amount of solid. The mixture was filtered and the cake was washed with water until the pH was neutral, dried to give a yellow solid (15.1 g, 82% yield). mp 192-196 °C.

5.1.8. 2-(4-Fluorophenyl)-5, 7-dihydroxy-4H-chromen-4-one ***(15b).***

Compound **14b** (21.36 g, 71 mmol) and 48% hydrobromide (300 mL) were refluxed for 60 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature and poured into 10 volumes of ice water to precipitate a large amount of solid. The mixture was filtered and the cake was washed with water until the pH was neutral, dried to give a crude product (20.9 g). The solid was purified by polyamide column chromatography to give a yellow solid (16.2 g,

83% yield). mp 206-210 °C.

5.1.9. Tetramethylammonium-5, 7-dihydroxy-4-oxo-2-(3-bromophenyl)-4H-chromen-8-yl sulfate (***16a***).

Compound **15a** (21.2 g, 63.7 mmol) and tetramethylammonium hydroxide (69.19 g, 38.2 mmol) were dissolved in water (530 mL) and potassium persulfate (42.93 g, 169 mmol) was added eight times every 20 minutes. The mixture was kept below 30 °C for 3.5 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was filtered and the filtrate was adjusted to pH = 6 - 7 with potassium dihydrogen phosphate (about 30 g) to precipitate solid. Then sodium chloride (45 g) was added three times every 5 minutes and the mixture was stirred at room temperature overnight. The mixture was filtered and the cake was washed with methanol (20 mL) and dried to give a reddish brown solid (19.2 g, 60.1% yield).

5.1.10. Tetramethylammonium-5, 7-dihydroxy-4-oxo-2-(4-fluorophenyl)-4H-chromen-8-yl sulfate (***16b***).

Compound **15b** (18 g, 71 mmol) and tetramethylammonium hydroxide (71.89 g, 40 mmol) were dissolved in water (450 mL) and potassium persulfate (35.7 g, 130 mmol) was added eight times every 20 minutes. The mixture was kept below 30 °C for 3 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was filtered and the filtrate was adjusted to pH = 6 - 7 with potassium dihydrogen phosphate (about 30 g) to precipitate solid. Then sodium chloride (45 g) was added three times every 5 minutes and the mixture was stirred at room temperature overnight. The mixture was filtered and the cake was washed with methanol (20 mL) and dried to give a yellowish solid (17 g, 60.8% yield).

5.1.11. 5, 7-Bis(benzyloxy)-2-(3-bromophenyl)-8-hydroxy-4H-chromen-4-one (***17***).

Compound **16a** (5 g, 10 mmol) was dissolved in DMF (35 mL) and Cs_2CO_3 (19.4 g, 60 mmol) was added. Benzyl bromide (7 mL, 60 mmol) was added dropwise and then the mixture was heated to 90 °C for 6 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature, filtered, and the cake was washed with methanol (20 mL). The filtrate was adjusted to pH = 1 - 2 with 6 M HCl and the mixture was stirred at room temperature overnight to precipitate a large amount of solid. Then the mixture was filtered and the cake was washed with methanol (10 mL) and dried to give a yellow solid (1.5 g, 28.5% yield). mp 290-292 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.10 (s, 1H, 8-OH), 8.35 (s, 1H, Ar-H), 7.90 (m, 3H, Ar-H), 7.50 (m, 10H, Ar-H), 6.94 (s, 1H, CHCO), 6.86 (s, 1H, Ar-H), 5.34 (s, 2H, OCH_2-Ar), 5.17 (s, 2H, OCH_2-Ar) ppm.

*5.1.12. 5, 7-Bis(benzyloxy)-2-(3-bromophenyl)-8-methoxy-4H-chromen-4-one (**18**).*

Compound **17** (200 mg, 0.38 mmol) was dissolved in acetone (40 mL) and K_2CO_3 (104.3 mg, 0.76 mmol) and dimethyl sulfate (71.5 mg, 0.57 mmol) were added. The mixture was refluxed for 3 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature, filtered, and the cake was washed with dichloromethane (10 mL). The filtrate was

evaporated under reduced pressure. The crude products were recrystallized from mixed solvent of hexane and dichloromethane to give a yellow solid (185 mg, 90% yield). mp 230-233 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.12 (s, 1H, Ar-H), 7.42 (m, 13H, Ar-H), 6.95 (s, 1H, CHCO), 6.83 (s, 1H, Ar-H), 5.32 (s, 2H, OCH_2-Ar), 5.20 (s, 2H, OCH_2-Ar), 3.85 (s, 3H, $ArOCH_3$) ppm.

*5.1.13. 5, 7-Bis(benzyloxy)-8-methoxy-2-(3-morpholinophenyl)-4H-chromen-4-one (**19a**).*

$Pd_2(dba)_3$ (21.1 mg, 0.023 mmol) and BINAP (28.65 mg, 0.046 mmol) were added in anhydrous toluene (30 mL). The mixture was stirred at room temperature for 15 minutes under N_2 protection, then compound **18** (250 mg, 0.46 mmol), morpholine (80.16 mg, 0.92 mmol) and potassium *t*-butoxide (103.25 mg, 0.92 mmol) were added. The mixture was refluxed for 5 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature and evaporated under reduced pressure. The crude products were purified over silica gel column chromatography (# 100-200) to give a yellow solid (185 mg, 73.2% yield). mp 230-233 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.21 (s, 1H, Ar-H), 7.53 (m, 13H, Ar-H), 7.01 (s, 1H, CHCO), 6.89 (s, 1H, Ar-H), 5.32 (s, 2H, OCH_2-Ar), 5.24 (s, 2H, OCH_2-Ar), 3.83 (s, 3H, $ArOCH_3$), 3.74 (s, 4H, $N(CH_2C\underline{H}_2)_2O$), 3.28 (s, 4H, $N(C\underline{H}_2CH_2)_2O$) ppm.

5.1.14. 5, 7-Bis(benzyloxy)-8-methoxy-2-(3-(piperazin-1-yl)phenyl)-4H-chromen-4-one (***19b***).

Yellow solid (100 mg, 57% yield). mp 225~227 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.21 (s, 1H, Ar-H), 7.55 (m, 13H, Ar-H), 6.98 (s, 1H, CHCO), 6.87 (s, 1H, Ar-H), 5.32 (s, 2H, OCH_2-Ar), 5.25 (s, 2H, OCH_2-Ar), 3.83 (s, 3H, ArOCH3), 3.20 (s, 4H, $N(C\underline{H}_2CH_2)_2NH$), 2.91 (s, 4H, $N(CH_2C\underline{H}_2)_2NH$) ppm.

5.1.15. 5, 7-Bis(benzyloxy)-8-methoxy-2-(3-(4-methylpiperazin-1-yl)phenyl)-4H-chromen-4-one (***19c***).

Yellow solid (135 mg, 65% yield). mp 230~233 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.21 (s, 1H, Ar-H), 7.60 (m, 13H, Ar-H), 7.00 (s, 1H, CHCO), 6.89 (s, 1H, Ar-H), 5.32 (s, 2H, OCH_2-Ar), 5.26 (s, 2H, OCH_2-Ar), 3.84 (s, 3H, ArOCH3), 3.36 (s, 4H, Ar-$N(C\underline{H}_2CH_2)_2N$), 3.00 (s, 3H, NCH_3), 2.75 (s, 4H, Ar-$N(CH_2C\underline{H}_2)_2N$) ppm.

*5.1.16. 5, 7-Bis(benzyloxy)-2-(3-(1, 4-diazepan-1-yl)phenyl)-8-methoxy-4H-chromen-4-one (**19d**).*

Yellow solid (110 mg, 37% yield). mp 235~237 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.10 (s, 1H, Ar-H), 7.53 (m, 13H, Ar-H), 7.00 (s, 1H, CHCO), 6.87 (s, 1H, Ar-H), 5.28 (s, 2H, OCH_2-Ar), 5.19 (s, 2H, OCH_2-Ar), 3.86 (s, 3H, ArOCH3), 3.62 (s, 4H, Ar-$N(C\underline{H}_2)_2$), 2.95 (s, 2H, $NHC\underline{H}_2$), 2.70 (s, 2H, $NHC\underline{H}_2$), 1.84 (m, 2H, $NCH_2C\underline{H}_2CH_2NH$) ppm.

*5.1.17. 5, 7-Dihydroxy-8-methoxy-2-(3-morpholinophenyl)-4H-chromen-4-one (**20a**).*

Compound **19a** (185 mg, 0.27 mmol), Pd/C (60 mg) and tetrahydrofuran (10 mL) were added in methanol (10 mL). The mixture was stirred in the presence of H_2 and heated to 35 °C for 6 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room

temperature and filtered. The filtrate was evaporated under reduced pressure and purified over silica gel column chromatography (# 100-200) to give a yellow solid (78 mg, 77.3% yield). mp 204~206 ^{o}C. HRMS (ESI^{+}) calcd for $C_{20}H_{20}NO_6$ [M + H]$^{+}$: 370.1291, found 370.1271. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.53 (s, 1H, 5-OH), 10.81 (s, 1H, 7-OH), 7.53-7.41 (m, 3H, Ar-H), 7.20 (d, 1H, *J*= 7.50 Hz, Ar-H), 7.05 (s, 1H, CHCO), 6.30 (s, 1H, Ar-H), 3.85 (s, 3H, $ArOCH_3$), 3.77 (s, 4H, $N(CH_2C\underline{H}_2)_2O$), 3.23 (s, 4H, $N(C\underline{H}_2CH_2)_2O$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.1, 163.4, 157.3, 156.2, 151.5, 149.6, 131.6, 129.8, 127.6, 118.7, 116.7, 112.2, 105.0, 103.7, 99.0, 66.0, 61.0 (2 × C), 48.0 (2 × C) ppm. IR (KBr) ν 3245, 2829, 1657, 1586, 1507, 1444, 1356, 1251, 1163, 1023, 1010, 863, 837, 699, 671 cm^{-1}.

5.1.18. 5, 7-Dihydroxy-8-methoxy-2-(3-(piperazin-1-yl)phenyl)-4H-chromen-4-one (***20b***).

Yellow solid (30 mg, 44.7% yield). mp 230~232 ^{o}C. HRMS (ESI^{+}) calcd for $C_{20}H_{21}N_2O_5$ [M + H]$^{+}$: 369.1450, found 369.1439. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.51 (s, 1H, 5-OH), 7.45 (m, 3H, Ar-H), 7.18 (d, 1H, *J*= 7.20 Hz, Ar-H), 7.00 (s, 1H, CHCO), 6.26 (s, 1H, Ar-H), 3.84 (s, 3H, $ArOCH_3$), 3.20 (s, 4H, $N(C\underline{H}_2CH_2)_2NH$), 2.90 (s, 4H, $N(CH_2C\underline{H}_2)_2NH$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 181.8, 163.3, 158.5, 156.2, 151.8, 149.6, 131.6, 129.8, 127.8, 118.8, 116.3, 112.3, 104.8, 103.1, 99.3, 60.8, 48.6 (2 × C), 45.2 (2 × C) ppm. IR (KBr) ν 3494, 2842, 1654, 1605, 1583, 1491, 1452, 1353, 1255, 1179, 1107, 1026, 992, 799, 670, 549 cm^{-1}.

5.1.19. 5, 7-Dihydroxy-8-methoxy-2-(3-(4-methylpiperazin-1-yl)phenyl)-4H-chromen-4-one (***20c***).

Yellow solid (55 mg, 58% yield). mp 265~268 ^{o}C. HRMS (ESI^{+}) calcd for $C_{21}H_{23}N_2O_5$ [M + H]$^{+}$: 383.1607, found 383.1599. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.52 (s, 1H, 5-OH), 10.89 (s, 1H, 7-OH), 7.52 (m, 3H, Ar-H), 7.27 (d, 1H, *J*= 7.50 Hz, Ar-H), 7.11 (s, 1H, CHCO), 6.34 (s, 1H, Ar-H), 3.85 (s, 3H, $ArOCH_3$), 3.35 (s, 4H, $Ar\text{-}N(C\underline{H}_2CH_2)_2N$), 3.01 (s, 3H, NCH_3), 2.73 (s, 4H, $Ar\text{-}N(CH_2C\underline{H}_2)_2N$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.1, 163.2, 157.4, 156.1, 150.1, 149.6, 131.8, 130.1, 127.7, 119.4, 117.3, 113.1, 105.1, 103.7, 99.1, 61.0, 52.1 (2 × C), 45.5 (2 × C), 42.2 ppm. IR (KBr) ν 3433, 2944, 2679, 2606, 1658, 1592, 1522, 1434, 1364, 1275, 1257, 1026, 921, 837, 780, 692, 553 cm^{-1}.

5.1.20. 2-(3-(1, 4-Diazepan-1-yl)phenyl)-5, 7-dihydroxy-8-methoxy-4H-chromen-4-one (***20d***).

Yellow solid (35 mg, 46.8% yield). mp 208~210 ^{o}C. HRMS (ESI^{+}) calcd for $C_{21}H_{22}N_2O_5$ [M + H]$^{+}$: 383.1529, found 383.1599.^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.55 (s, 1H, 5-OH), 7.31 (m, 3H, Ar-H), 6.97 (s, 1H, Ar-H), 6.93 (s, 1H, CHCO), 6.23 (s, 1H, Ar-H), 3.83 (s, 3H, $ArOCH_3$), 3.61 (s, 4H, $Ar\text{-}N(C\underline{H}_2)_2$), 2.95 (s, 2H, $NHC\underline{H}_2$), 2.72 (s, 2H, $NHC\underline{H}_2$), 1.86 (m, 2H, $NCH_2C\underline{H}_2CH_2NH$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 181.7, 163.5, 159.0, 156.3, 149.5, 148.5, 131.8, 130.0, 127.9, 114.9, 113.1, 108.4, 104.6, 102.9, 99.4, 60.7, 50.9, 47.3, 47.2, 46.9, 27.9 ppm. IR (KBr) ν 3410, 2927, 1652, 1598, 1499, 1368, 1175, 1107, 1026, 838, 772, 690, 561 cm^{-1}.

5.1.21. 7-Benzyloxy-2-(4-fluorophenyl)-5, 8-dihydroxy-4H-chromen-4-one (***21***).

Compound **16b** (7.8 g, 17.7 mmol) was dissolved in DMF (55 mL) and K_2CO_3 (4.9 g, 35.5 mmol) was added. Benzyl bromide (2.8 mL, 23 mmol) was added dropwise and then the mixture was heated to 60 °C for 5 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature, filtered, and the cake was washed with methanol (20 mL). The filtrate was adjusted to pH = 1 - 2 with 6 M HCl and the mixture was stirred at room temperature overnight to precipitate a large amount of solid. Then the mixture was filtered and the cake was washed with methanol (10 mL) and dried to give an Orange yellow solid (2.6 g, 38.8% yield). mp 290-292 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.69 (s, 1H, 5-OH), 9.13 (s, 1H, 8-OH), 7.92 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.45 (m, 5H, Ar-H), 7.12 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.93 (s, 1H, CHCO), 6.79 (s, 1H, Ar-H), 5.34 (s, 2H, OCH_2-Ar) ppm.

*5.1.22. 7-Benzyloxy-2-(4-fluorophenyl)-5-hydroxy-8-methoxy-4H-chromen-4-one (**22a**).*

Compound **21** (720 mg, 1.9 mmol) was dissolved in acetonitrile (60 mL) and K_2CO_3 (660 mg, 4.8 mmol) and dimethyl sulfate (240 mg, 2.1mmol) were added. The mixture was refluxed for 4.5 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature, filtered, and the cake was washed with dichloromethane (20 mL). The filtrate was evaporated under reduced pressure. The crude products were recrystallized from mixed solvent of n-hexane and methylene chloride to give a yellow solid (685 mg, 92% yield). mp 210-212 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.70 (s, 1H, 5-OH), 7.93 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.46 (m, 5H, Ar-H), 7.10 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.94 (s, 1H, CHCO), 6.80 (s, 1H, Ar-H), 5.33 (s, 2H, OCH_2-Ar), 3.85 (s, 3H, $ArOCH_3$) ppm.

5.1.23. 7-Benzyloxy-2-(4-fluorophenyl)-5-hydroxy-8-isopropoxy-4H-chromen-4-one (***22b***).

Compound **21** (1 g, 2.65 mmol) was dissolved in DMF (15 mL) and K_2CO_3 (550 mg, 4.0 mmol) and bromoisopropane (490 mg, 4.0 mmol) were added. The mixture was heated to 80 °C for 5 h and the reaction was monitored by TLC. After the end of the reaction, the hot mixture was poured into 10 volumes of ice water to precipitate a large amount of solid. The mixture was filtered and the cake was recrystallized from mixed solvent of *n*-hexane and methanol to give a yellow solid (580 mg, 52.3% yield). mp 215-216 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.89 (s, 1H, 5-OH), 7.88 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.52 (m, 5H, Ar-H), 7.21 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.94 (s, 1H, CHCO), 6.80 (s, 1H, Ar-H), 5.33 (s, 2H, OCH_2-Ar), 4.45 (m, 1H, $C\underline{H}(CH_3)_2$), 1.29 (d, 6H, *J*= 6.00 Hz, $CH(C\underline{H}_3)_2$) ppm.

5.1.24. 7-Benzyloxy-8-cyclopentyloxy-2-(4-fluorophenyl)-5-hydroxy-4H-chromen-4-one (***22c***).

Compound **21** (1 g, 2.65 mmol) was dissolved in DMF (15 mL) and K_2CO_3 (550 mg, 4.0 mmol) and bromoisopropane (490 mg, 4.0 mmol) were added in the presence of N_2. The mixture was heated to 90 °C for 10 h and the reaction was monitored by TLC. After the end of the reaction, the hot mixture

was poured into 10 volumes of ice water to precipitate a large amount of solid. The mixture was filtered and the cake was purified by polyamide column chromatography to give a yellow solid (850 mg, 72% yield). mp 230-233 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ =12.60 (s, 1H, 5-OH), 7.65 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.49 (m, 5H, Ar-H), 7.19 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.98 (s, 1H, CHCO), 6.36 (s, 1H, Ar-H), 5.32 (s, 2H, OCH_2-Ar), 4.86 (m, 1H, $C\underline{H}(CH_2)_4$), 1.67 (m, 8H, $CH(C\underline{H}_2)_4$) ppm.

5.1.25. 7-Benzyloxy-8-cyclohexyloxy-2-(4-fluorophenyl)-5-hydroxy-4H-chromen-4-one (***22d***).

Cyclohexanol (5 mL, 48 mmol) and triethylamine (13.3 mL, 96 mmol) were added in dichloromethane (30 mL). Methanesulfonyl chloride (5.5 mL, 72 mmol, diluted with 10 mL of dichloromethane) was added dropwise below 5 °C and then the mixture was heated to room temperature for 5 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was filtered and the filtrate was wash with 1 M HCl (20 mL), Saturated sodium bicarbonate solution (20 mL) and Saturated saline solution (20 mL) and then dried by anhydrous sodium sulfate. The mixture was filtered and the filtrate was evaporated under reduced pressure to give the oily compound of methanesulfonyl cyclohexanol (1.06 g).

Compound **21** (1.5 g, 3.97 mmol) was dissolved in DMF (20 mL) and K_2CO_3 (1.37 g, 10 mmol) and methanesulfonyl cyclohexanol (1.06 g) were added in the presence of N_2. The mixture was heated to 80 °C for 8 h and the reaction was monitored by TLC. After the end of the reaction, the hot mixture was poured into 10 volumes of ice water and adjusted to pH = 4 - 5 with 1 M HCl to precipitate a large amount of solid. The mixture was filtered and the cake was purified by polyamide column chromatography to give a yellow solid (1.1 g, 63.2% yield). mp 235-238 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.91 (s, 1H, 5-OH), 7.78 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.50 (m, 5H, Ar-H), 7.26 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.94 (s, 1H, CHCO), 6.77 (s, 1H, Ar-H), 5.30 (s, 2H, OCH_2-Ar), 4.00 (m, 1H, $C\underline{H}(CH_2)_5$), 1.90 (m, 4H, $CH(C\underline{H}_2)_5$), 1.53 (m, 2H, $CH(C\underline{H}_2)_5$), 1.21 (m, 4H, $CH(C\underline{H}_2)_5$) ppm.

The synthesis methods of **23a**-**23l** were shown in supporting information.

*5.1.26. 5, 7-Dihydroxy-8-methoxy-2-(4-(pyrrolidin-1-yl)phenyl)-4H-chromen-4-one (**24a**).*

Compound **23a** (168 mg, 0.38 mmol), Pd/C (40 mg) and tetrahydrofuran (10 mL) were added in methanol (10 mL) in the presence of H_2. The mixture was heated to 35 °C for 7 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature, filtered and the filtrate was evaporated under reduced pressure. The crude product was purified over silica gel column chromatography (# 100-200) to give a yellow solid (100 mg, 74.4% yield). mp 185-188 °C. HRMS (ESI$^+$) calcd for $C_{20}H_{20}NO_5$ $[M + H]^+$: 354.1341, found 354.1274. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.77 (s, 1H, 5-OH), 7.86 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.97 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.55 (s, 1H, CHCO), 6.46 (s, 1H, Ar-H), 4.00 (s, 3H, $ArOCH_3$), 3.42 (s, 4H, $N(C\underline{H}_2CH_2)_2$), 1.70 (s, 4H, $N(CH_2C\underline{H}_2)_2$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 187.0, 168.8, 152.2, 150.9, 143.7, 140.4, 127.6,

127.1 (2 × C), 124.4, 113.0 (2 × C), 105.6, 98.6, 94.9, 56.6, 53.9 (2 × C), 23.1 (2 × C) ppm. IR (KBr) ν 3651, 2854, 1532, 1562, 1523, 1294, 1165, 1125, 1023, 1010, 863, 837, 701, 671 cm^{-1}.

3.1.27. 2-(4-(Diethylamino)phenyl)-5, 7-dihydroxy-8-methoxy-4H-chromen-4-one (***24b***).

Compound **23b** (180 mg). Yellow solid (75 mg, 53% yield). mp 165-170 °C. HRMS (ESI^+) calcd for $C_{20}H_{22}NO_5$ $[M + H]^+$: 356.1498, found 356.1480. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.81 (s, 1H, 5-OH), 10.63 (s, 1H, 7-OH), 7.88 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.81 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.70 (s, 1H, CHCO), 6.25 (s, 1H, Ar-H), 3.84 (s, 3H, $ArOCH_3$), 3.44 (q, 4H, *J*= 6.00 Hz, $N(C\underline{H}_2CH_3)_2$), 1.14 (t, 6H, *J*= 6.00 Hz, $N(CH_2C\underline{H}_3)_2$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.0, 164.7, 157.2, 156.7, 150.7, 149.8, 128.5 (2 × C), 128.0, 116.2, 111.7 (2 × C), 103.8, 101.1, 99.2, 61.5, 44.4 (2 × C), 12.9 (2 × C) ppm. IR (KBr) ν 3183, 2970, 1564, 1503, 1414, 1386, 1350, 1198, 1156, 1022, 944, 820, 712 cm^{-1}.

5.1.28. 5, 7-Dihydroxy-8-methoxy-2-(4-morpholinophenyl)-4H-chromen-4-one (***24c***).

Compound **23c** (145 mg). Yellow solid (55 mg, 47.4% yield). mp 190-195 °C. HRMS (ESI^+) calcd for $C_{20}H_{20}NO_6$ $[M + H]^+$: 370.1291, found 370.1271. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.77 (s, 1H, 5-OH), 10.68 (s, 1H, 7-OH), 7.85 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.87 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.54 (s, 1H, CHCO), 6.46 (s, 1H, Ar-H), 3.98 (s, 3H, $ArOCH_3$), 3.77 (s, 4H, $N(CH_2C\underline{H}_2)_2O$), 3.23 (s, 4H, $N(C\underline{H}_2CH_2)_2O$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.0, 163.9, 157.1, 156.2, 151.5, 145.0, 127.6 (2 × C), 118.7, 116.1, 113.2 (2 × C), 105.0, 103.7, 99.0, 66.0, 61.0 (2 × C), 48.0 (2 × C) ppm. IR (KBr) ν 3245, 2829, 1562, 1524, 1493, 1327, 1265, 1212, 1123, 1023, 852, 814, 752, 663 cm^{-1}.

5.1.41. 5, 7-Dihydroxy-8-methoxy-2-(4-(piperazin-1-yl)phenyl)-4H-chromen-4-one (***24d***).

Compound **23d** (178 mg). Yellow solid (60 mg, 42% yield). mp 190-195 °C. HRMS (ESI^+) calcd for $C_{20}H_{21}N_2O_5$ $[M + H]^+$: 369.1450, found 369.1439. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.56 (s, 1H, 5-OH), 7.89 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.14 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.82 (s, 1H, CHCO), 6.35 (s, 1H, Ar-H), 3.98 (s, 3H, $ArOCH_3$), 3.20 (s, 4H, $N(C\underline{H}_2CH_2)_2NH$), 2.90 (s, 4H, $N(CH_2C\underline{H}_2)_2NH$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.1, 163.3, 158.5, 156.2, 151.8, 149.6, 127.8 (2 × C), 118.8, 116.3, 113.3 (2 × C), 104.8, 103.1, 99.3, 60.8, 48.6 (2 × C), 45.2 (2 × C) ppm. IR (KBr) ν 3537, 2953, 1643, 1605, 1575, 1482, 1443, 1386, 1215, 1186, 1124, 992, 837, 670, 553 cm^{-1}.

5.1.29. 5, 7-Dihydroxy-8-methoxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-4H-chromen-4-one (***24e***).

Compound **23e** (165 mg). Yellow solid (50 mg, 37.4% yield). mp 190-195 °C. HRMS (ESI^+) calcd for $C_{21}H_{23}N_2O_5$ $[M + H]^+$: 383.1607, found 383.1640. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.70 (s, 1H, 5-OH), 7.82 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.99 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.57 (s, 1H, CHCO), 6.43 (s, 1H, Ar-H), 4.05 (s, 3H, $ArOCH_3$), 3.44 (t, *J*= 6.00 Hz, 4H, Ar-$N(C\underline{H}_2CH_2)_2N$), 3.01 (t, *J*= 6.00 Hz, 4H, Ar-$N(CH_2C\underline{H}_2)_2N$), 2.43 (s, 3H, NCH_3) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.3, 167.8, 157.5, 156.1, 150.9, 149.6, 127.3 (2 × C), 118.5, 116.4, 113.0 (2 × C), 105.1, 103.7, 99.1, 61.0, 52.1 (2

× C), 45.5 (2 × C), 42.2 ppm. IR (KBr) ν 3430, 2854, 2648, 1653, 1454, 1367, 1228, 1210, 1026, 921, 837, 780, 692, 583 cm^{-1}.

5.1.30. 2-(4-(1, 4-Diazepan-1-yl)phenyl)-5, 7-dihydroxy-8-methoxy-4H-chromen-4-one (***24f***).

Compound **23f** (165 mg). Yellow solid (50 mg, 37.4% yield). mp 202-205ºC. HRMS (ESI^+) calcd for $C_{21}H_{23}N_2O_5$ $[M + H]^+$: 383.1607, found 383.1599. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.80 (s, 1H, 5-OH), 7.88 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.88 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.72 (s, 1H, CHCO), 6.27 (s, 1H, Ar-H), 3.84 (s, 3H, $ArOCH_3$), 3.63 (s, 4H, Ar-N($C\underline{H}_2$)$_2$), 2.98 (s, 2H, $NHC\underline{H}_2$), 2.73 (s, 2H, $NHC\underline{H}_2$), 1.86 (m, 2H, $NCH_2C\underline{H}_2CH_2NH$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 181.5, 163.6, 157.5, 156.3, 153.4, 149.3, 130.6, 127.9, 125.0, 119.7, 111.7, 111.2, 103.1, 100.2, 98.8, 60.2, 58.2, 51.6, 48.2, 47.0, 28.9 ppm. IR (KBr) ν 3131, 2854, 1655, 1578, 1400, 1358, 1101, 872, 775, 690, 550 cm^{-1}.

5.1.31. 5, 7-Dihydroxy-8-isopropoxy-2-(4-(piperazin-1-yl)phenyl)-4H-chromen-4-one (***24g***).

Compound **23g** (180 mg). Yellow solid (80 mg, 54.6% yield). mp 210-213 ºC. HRMS (ESI^+) calcd for $C_{22}H_{25}N_2O_5$ $[M + H]^+$: 397.1763, found 397.1710. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.75 (s, 1H, 5-OH), 7.90 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.08 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.76 (s, 1H, CHCO), 6.27 (s, 1H, Ar-H), 4.38 (m, 1H, $C\underline{H}(CH_3)_2$), 3.28 (s, 4H, $N(C\underline{H}_2CH_2)_2NH$), 2.84 (s, 4H, $N(CH_2C\underline{H}_2)_2NH$), 1.30 (d, 6H, *J*= 6.09 Hz, $CH(C\underline{H}_3)_2$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.0, 164.4, 156.3, 152.1, 151.1, 149.8, 127.6 (2 × C), 125.1, 119.0, 113.1 (2 × C), 103.4, 101.7, 98.8, 73.8, 50.7 (2 × C), 46.6 (2 × C), 22.3 (2 × C) ppm. IR (KBr) ν 3379, 2813, 1616, 1525, 1506, 1368, 1262, 1237, 1119, 1026, 821, 663, 595 cm^{-1}.

*5.1.32. 5, 7-Dihydroxy-8-isopropoxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-4H-chromen-4-one (**24h**).*

Compound **23h** (155 mg). Yellow solid (65 mg, 51.2% yield). mp 240-242 ºC. HRMS (ESI^+) calcd for $C_{23}H_{27}N_2O_5$ $[M + H]^+$: 411.1920, found 411.1890. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.76 (s, 1H, 5-OH), 10.59 (s, 1H, 7-OH), 7.92 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.11 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.79 (s, 1H, CHCO), 6.28 (s, 1H, Ar-H), 4.37 (m, 1H, $C\underline{H}(CH_3)_2$), 3.34 (s, 4H, Ar-N($C\underline{H}_2CH_2)_2N$), 3.34 (s, 3H, NCH_3), 2.45 (s, 4H, Ar-N($CH_2C\underline{H}_2)_2N$), 1.30 (d, 6H, *J*= 6.09 Hz, $CH(C\underline{H}_3)_2$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 181.0, 167.8, 157.5, 156.1, 150.9, 149.6, 127.3 (2 × C), 118.5, 116.4, 113.0 (2 × C), 105.1, 103.7, 99.1, 74.0, 52.1 (2 × C), 45.5 (2 × C), 42.2, 22.1 (2 × C) ppm. IR (KBr) ν 3430, 2854, 2648, 1653, 1454, 1367, 1228, 1210, 1026, 921, 837, 780, 692, 583 cm^{-1}.

5.1.33. 5, 7-Dihydroxy-8-cyclopentyloxy-2-(4-(piperazin-1-yl)phenyl)-4H-chromen-4-one (***24i***).

Compound **23i** (160 mg). Yellow solid (65 mg, 49.3% yield). mp 255-258 ºC. HRMS (ESI^+) calcd for $C_{24}H_{27}N_2O_5$ $[M + H]^+$: 423.1920, found 423.1913. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.75 (s, 1H, 5-OH), 7.88 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.04 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.76 (s, 1H, CHCO), 6.26 (s, 1H, Ar-H), 4.81 (m, 1H, $C\underline{H}(CH_2)_4$), 3.40 (s, 4H, $N(C\underline{H}_2CH_2)_2NH$), 2.83 (s, 4H, $N(CH_2C\underline{H}_2)_2NH$), 1.50 (m, 8H, $CH(C\underline{H}_2)_4$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.0, 164.6, 155.8, 152.1, 151.1,

149.8, 127.6 (2 × C), 125.1, 119.0, 113.1 (2 × C), 103.4, 101.7, 99.1, 83.7, 48.6 (2 × C), 45.1 (2 × C), 32.3 (2 × C), 23.3 (2 × C) ppm. IR (KBr) ν 3434, 2954, 1651, 1603, 1446, 1366, 1251, 1193, 1104, 999, 839, 788, 693, 559 cm^{-1}.

5.1.34. 5, 7-Dihydroxy-8-cyclopentyloxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-4H-chromen-4-one (***24j***).

Compound **23j** (160 mg). Yellow solid (70 mg, 52.8% yield). mp 202-204 °C. HRMS (ESI^+) calcd for $C_{25}H_{29}N_2O_5$ $[M + H]^+$: 437.2076, found 437.2065. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.72 (s, 1H, 5-OH), 10.60 (s, 1H, 7-OH), 7.84 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.02 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.56 (s, 1H, CHCO), 6.46 (s, 1H, Ar-H), 4.98 (m, 1H, C<u>H</u>$(CH_2)_4$), 3.47 (s, 4H, N($C\underline{H}_2CH_2)_2$NH), 2.78 (s, 4H, N($CH_2C\underline{H}_2)_2$NH), 2.52 (s, 3H, NCH_3), 1.88 (m, 8H, CH($C\underline{H}_2)_4$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.0, 163.4, 157.8, 155.8, 151.4, 150.0, 127.8 (2 × C), 125.1, 119.0, 113.1 (2 × C), 104.9, 103.7, 99.0, 84.0, 54.4 (2 × C), 47.7 (2 × C), 45.6, 32.3 (2 × C), 23.1 (2 × C) ppm. IR (KBr) ν 3435, 2952, 1651, 1597, 1496, 1432, 1375, 1328, 1260, 1006, 930, 862, 788, 566 cm^{-1}.

5.1.35. 5, 7-Dihydroxy-8-cyclohexyloxy-2-(4-(piperazin-1-yl)phenyl)-4H-chromen-4-one (***24k***).

Compound **23k** (178 mg). Yellow solid (60 mg, 40.8% yield). mp 195-200 °C. HRMS (ESI^+) calcd for $C_{25}H_{29}N_2O_5$ $[M + H]^+$: 437.2076, found 437.2089. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.80 (s, 1H, 5-OH), 7.90 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.07 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.75 (s, 1H, CHCO), 6.25 (s, 1H, Ar-H), 4.09-4.07 (m, 1H, C<u>H</u>$(CH_2)_5$), 3.20 (s, 4H, N($C\underline{H}_2CH_2)_2$NH), 2.83 (s, 4H, N($CH_2C\underline{H}_2)_2$NH), 1.98-1.93 (m, 2H, CH($C\underline{H}_2)_5$), 1.76 (m, 2H, CH($C\underline{H}_2)_5$), 1.51 (m, 2H, CH($C\underline{H}_2)_5$), 1.23 (m, 4H, CH($C\underline{H}_2)_5$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 181.6, 163.4, 158.2, 155.8, 153.5, 149.7, 127.5 (2 × C), 125.1, 119.0, 113.0 (2 × C), 109.0, 103.0, 98.9, 60.1, 52.4 (2 × C), 47.5 (2 × C), 45.1, 31.9 (2 × C), 25.1 (2 × C), 23.6 ppm. IR (KBr) ν 3131, 1648, 1604, 1577, 1513, 1400, 1278, 1237, 1200, 1105, 820, 545 cm^{-1}.

5.1.36. 5, 7-Dihydroxy-8-cyclohexyloxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-4H-chromen-4-one (***24l***).

Compound **23l** (165 mg). Yellow solid (70 mg, 51% yield). mp 200-205 °C. HRMS (ESI^+) calcd for $C_{26}H_{31}N_2O_5$ $[M + H]^+$: 451.2233, found 451.2243. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.70 (s, 1H, 5-OH), 10.60 (s, 1H, 7-OH), 7.87 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.06 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.75 (s, 1H, CHCO), 6.26 (s, 1H, Ar-H), 4.03 (m, 1H, C<u>H</u>$(CH_2)_5$), 3.34 (s, 4H, N($C\underline{H}_2CH_2)_2$NH), 2.48 (s, 4H, N($CH_2C\underline{H}_2)_2$NH), 2.20 (s, 3H, NCH_3), 1.89 (m, 2H, CH($C\underline{H}_2)_5$), 1.74 (m, 2H, CH($C\underline{H}_2)_5$), 1.49 (m, 2H, CH($C\underline{H}_2)_5$), 1.20 (m, 4H, CH($C\underline{H}_2)_5$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 181.7, 163.6, 157.4, 155.8, 153.0, 149.7, 127.6 (2 × C), 125.1, 119.0, 113.1 (2 × C), 104.9, 103.7, 99.0, 80.2, 54.1 (2 × C), 47.5 (2 × C), 45.5, 32.2 (2 × C), 25.1, 23.5 (2 × C) ppm. IR (KBr) ν 3131, 1654, 1512, 1400, 1114, 859, 616, 545 cm^{-1}.

5.1.37. Benzyl 3-hydroxypyrrolidine-1-carboxylate (***26***).

3-Hydroxypyrrolidine hydrochloride (**25**) (3 g, 14.8 mmol) and K_2CO_3 (10.2 g, 73.9 mmol) were added in dichloromethane (30 mL) and water (30 mL). Benzyl chloroformate (2.5 g, 14.8 mmol) was added dropwise and the mixture was kept below 5 °C and then heated to 25 °C for 5 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was separated and the aqueous layer was extracted with dichloromethane (30 mL). The organic layer was washed with saturated aqueous solution of sodium bicarbonate (20 mL) and brine (20 mL).Then the mixture was dried by anhydrous sodium sulfate and filtered. The filtrate was evaporated under reduced pressure to give an oily compound (2.6 g).

5.1.38. Benzyl 3-((methylsulfonyl)oxy)pyrrolidine-1-carboxylate (***27***).

Compound **26** (2.6 g, 11.8 mmol) and triethylamine (3.3 mL, 17.6 mmol) were added in dichloromethane (30 ml). Methanesulfonyl chloride (1.4 mL, 17.6 mmol) was added dropwise and the mixture was kept below 5 °C and then heated to 25 °C for 3.5 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was filtered and the filtrate was washed with 1 M HCl (20 mL), saturated aqueous solution of sodium bicarbonate (20 mL) and brine (20 mL). Then the mixture was dried by anhydrous sodium sulfate and filtered. The filtrate was evaporated under reduced pressure to give an oily compound (3.1 g, 80% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.35 (m, 5H, Ar-H), 5.22 (s, 2H, OCH_2-Ar), 5.09 (m, 1H, $OC\underline{H}(CH_2)_2$), 3.78 (m, 2H, $CHC\underline{H}_2N$), 3.56 (m, 2H, $CHCH_2C\underline{H}_2N$), 3.09 (s, 3H, SO_2CH_3), 2.05 (m, 2H, $CHC\underline{H}_2CH_2N$) ppm.

5.1.39.Benzyl-3-((7-benzyloxy-2-(4-fluorophenyl)-5-hydroxy-4-oxo-4H-chromen-8-yl)oxy)pyrrolidine-1-carboxylate (***28***).

Compound **21** (0.32 g, 0.85 mmol) was dissolved in DMF (10 mL) and K_2CO_3 (290 mg, 2.15 mmol) and compound **27** (280 mg, 1.02 mmol) were added. The mixture was heated to °C for 10 h and the reaction was monitored by TLC. After the end of the reaction, the hot mixture was poured into 10 volumes of ice water and adjusted to pH = 4 - 5 with 1 M HCl to precipitate a large amount of solid. The mixture was filtered and the cake was purified by polyamide column chromatography to give a yellow solid (300 mg, 56.8% yield). mp 200-205° C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.91 (s, 1H, 5-OH), 7.78 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.50 (m, 5H, Ar-H), 7.35 (m, 5H, Ar-H), 7.26 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.94 (s, 1H, CHCO), 6.77 (s, 1H, Ar-H), 5.30 (s, 2H, OCH_2-Ar), 5.22 (s, 2H, OCH_2-Ar), 5.09 (m, 1H, $OC\underline{H}(CH_2)_2$), 3.78 (m, 2H, $CHC\underline{H}_2N$), 3.56 (m, 2H, $CHCH_2C\underline{H}_2N$), 2.05 (m, 2H, $CHC\underline{H}_2CH_2N$) ppm.

5.1.40.

Benzyl-3-((7-benzyloxy-2-(4-(piperazin-1-yl)phenyl)-5-hydroxy-4-oxo-4H-chromen-8-yl)oxy)pyrrolidine-1-carboxylate (***29a***).

Compound **28** (300 mg, 0.5 mmol) was added in anhydrous DMSO (8 mL) and heated to 60 °C.

Then piperazine (220 mg, 2.5 mmol) and DIEA (130 mg, 1 mmol) were added in the presence of N_2 and heated to 80 °C for 14 h. The reaction was monitored by TLC. After the end of the reaction, the hot mixture was poured into saturated saline (10 mL). The mixture was filtered and the cake was washed with water (10 mL) and dried. Then the crude products were recrystallized from mixed solvent of methanol and dichloromethane to give a yellow solid (230 mg, 68.9% yield). mp 165-170 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.91 (s, 1H, 5-OH), 7.78 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.50 (m, 5H, Ar-H), 7.35 (m, 5H, Ar-H), 7.26 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.94 (s, 1H, CHCO), 6.77 (s, 1H, Ar-H), 5.30 (s, 2H, OCH_2-Ar), 5.22 (s, 2H, OCH_2-Ar), 4.00 (m, 1H, $OCH(CH_2)_2$), 3.78 (m, 2H, $CHCH_2N$), 3.56 (m, 2H, $CHCH_2CH_2N$), 3.19 (s, 4H, $N(CH_2CH_2)_2NH$), 2.90 (s, 4H, $N(CH_2CH_2)_2NH$), 2.05 (m, 2H, $CHCH_2CH_2N$) ppm.

5.1.41.

Benzyl-3-((7-benzyloxy-2-((4-methylpiperazin-1-yl)phenyl)-5-hydroxy-4-oxo-4H-chromen-8-yl)oxy)pyrrolidine-1-carboxylate (***29b***).

Compound **28** (100 mg). Yellow solid (72 mg, 60% yield). mp 182-188 °C. HRMS (ESI$^+$) calcd for $C_{24}H_{28}N_3O_5$ $[M + H]^+$: 438.2023, found 451.2263. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.91 (s, 1H, 5-OH), 7.78 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.50 (m, 5H, Ar-H), 7.35 (m, 5H, Ar-H), 7.26 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.94 (s, 1H, CHCO), 6.77 (s, 1H, Ar-H), 5.30 (s, 2H, OCH_2-Ar), 5.22 (s, 2H, OCH_2-Ar), 4.00 (m, 1H, $OCH(CH_2)_2$), 3.78 (m, 2H, $CHCH_2N$), 3.56 (m, 2H, $CHCH_2CH_2N$), 3.36 (s, 4H, Ar-$N(CH_2CH_2)_2N$), 2.51 (s, 4H, Ar-$N(CH_2CH_2)_2N$), 2.25 (s, 3H, NCH_3), 2.05 (m, 2H, $CHCH_2CH_2N$) ppm.

5.1.42. 5, 7-Dihydroxy-2-(4-(piperazin-1-yl)phenyl)-8-(pyrrolidin-3-yloxy)-4H-chromen-4-one (***30a***).

Compound **29a** (230 mg, 0.35 mmol), Pd/C (70 mg) and a drop of acetic acid were added in tetrahydrofuran (10 mL) and methanol (10 mL) in the presence of H_2.The mixture was heated to 40 °C for 12 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature and filtered. The filtrate was evaporated under reduced pressure and purified over silica gel column chromatography (# 100-200) to give a yellow solid (95 mg, 49% yield). mp 200-205 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.75 (s, 1H, 5-OH), 7.92 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.07 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.76 (s, 1H, CHCO), 6.30 (s, 1H, Ar-H), 4.87 (m, 1H, $OCH(CH_2)_2$), 3.42 (m, 4H, $CHCH_2N$ & $CHCH_2CH_2N$), 3.20 (s, 4H, $N(CH_2CH_2)_2NH$), 3.07 (s, 4H, $N(CH_2CH_2)_2NH$), 2.10 (m, 2H, $CHCH_2CH_2N$). ^{13}C NMR (75 MHz, DMSO-d_6): 182.0, 163.1, 159.0, 156.0, 151.9, 150.0, 131.6, 129.8, 125.2, 118.8, 116.4, 112.2, 104.7, 103.2, 99.3, 74.3, 48.8 (2 × C), 45.2 (2 × C), 22.3 (2 × C) ppm. IR (KBr) ν 3132, 2970, 1654, 1603, 1512, 1073, 547 cm^{-1}.

5.1.43. 5, 7-Dihydroxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-8-(pyrrolidin-3-yloxy)-4H-chromen-4-one (***30b***).

Compound **29b** (300 mg). Yellow solid (110 mg, 50.1% yield). mp 195-198 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.89 (s, 1H, 5-OH), 7.89 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.20 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.61 (s, 1H, CHCO), 5.96 (s, 1H, Ar-H), 4.88 (m, 1H, OC<u>H</u>$(CH_2)_2$), 3.68 (m, 2H, CHC<u>H</u>$_2$N), 3.56 (m, 2H, $CHCH_2C\underline{H}_2N$), 3.36 (s, 4H, Ar-N$(C\underline{H}_2CH_2)_2$N), 2.51 (s, 4H, Ar-N$(CH_2C\underline{H}_2)_2$N), 2.25 (s, 3H, NCH_3), 2.06 (m, 2H, $CHC\underline{H}_2CH_2N$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): 182.1, 163.3, 157.9, 156.0, 151.3, 150.0, 131.5, 129.8, 125.0, 118.9, 116.5, 112.2, 104.8, 103.7, 99.0, 74.6, 54.4 (2 × C), 47.8 (2 × C), 45.6, 22.2 (2 × C) ppm. IR (KBr) ν 3131, 2851, 1648, 1400, 1365, 1323, 1113, 1003, 859, 850, 546 cm^{-1}.

5.1.44. 7-Benzyloxy-2-(4-fluorophenyl)-8-hydroxy-4-oxo-4H-chromen-5-yl-4-methylben-zenesulfonate (***32***).

Compound **16b** (6 g, 13.6 mmol) and K_2CO_3 were added in DMF (59 mL). Benzyl bromide (2.45 g, 14.3 mmol) was added dropwise and then the mixture was heated to 55 °C for 2 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature and filtered. Cs_2CO_3 (13.3 g, 40.8 mmol) and *p*-toluenesulfonyl chloride (2.8 g, 15.7 mmol) were added in the filtrate. The mixture was heated to 50 °C for 2 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was filtered and the cake was washed with methanol (50 mL). The filtrate was adjusted to pH = 1-2 with 6 M HCl and stirred overnight at room temperature to precipitate a large amount of solid. The mixture was filtered and the cake was washed with methanol (10 mL) and dried to give a yellow solid (1.9 g, 26.2% yield). mp 168-172 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.99 (s, 1H, 8-OH), 8.17 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.65 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.40 (m, 9H, Ar-H), 6.79 (s, 1H, CHCO), 6.78 (s, 1H, Ar-H), 5.19 (s, 2H, OCH_2-Ar), 2.38 (s, 3H, Ar-CH_3) ppm.

5.1.45.

7-Benzyloxy-2-(4-fluorophenyl)-8-((1-methylpiperidin-4-yl)oxy)-4-oxo-4H-chromen-5-yl-4-methylbenze nesulfonate (***33***). Compound **32** (400 mg, 0.75 mmol), 4-methylpiperidinol (129 mg, 1.12 mmol) and Ph_3P (493 mg, 1.88 mmol) were added in anhydrous tetrahydrofuran (100 mL). The mixture was stirred for 10 minutes and DEAD (327 mg, 1.88 mmol) was added dropwise in the presence of N_2 and low temperature. Then the mixture was heated to 25 °C for 1 h. The reaction was monitored by TLC. After the end of the reaction, the mixture was evaporated under reduced pressure and purified over silica gel column chromatography (# 100-200) to give a white solid (360 mg, 75% yield). mp 177-180 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.08 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.76 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.45 (m, 9H, Ar-H), 6.89 (s, 1H, CHCO), 6.83 (s, 1H, Ar-H), 5.16 (s, 2H, OCH_2-Ar), 4.25 (m, 1H, Ar-OCH), 2.65 (t, 2H, *J*= 6.00 Hz, $C\underline{H}_2NCH_3$), 2.39 (s, 3H, $CH_2NC\underline{H}_3$), 2.10 (s, 3H, Ar-CH_3), 1.96 (t, 2H, *J*= 6.00 Hz, $C\underline{H}_2NCH_3$), 1.83 (t, 2H, *J*= 6.00 Hz, OCH$(C\underline{H}_2)_2$), 1.74 (t, 2H, *J*= 6.00 Hz, OCH$(C\underline{H}_2)_2$) ppm.

5.1.46. 7-Benzyloxy-2-(4-fluorophenyl)-5-hydroxy-8-((1-methylpiperidin-4-yl)oxy)-4H-chromen-4-one

(**34**).

Compound **33** (200 mg, 0.31 mmol) and KOH (35 mg, 0.62 mmol) were added in methanol (20 mL). The mixture was refluxed for 1.5 h and the reaction was monitored by TLC. After the end of the reaction, water (20 mL) was added and the mixture was adjusted to pH = 7 with 1 M HCl to precipitate a large amount of solid. The mixture was filtered and the cake was dried and recrystallized from mixed solvent of ethanol and ether to give a yellow solid (140 mg, 90% yield). mp 212-215 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.63 (s, 1H, 5-OH), 8.12 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.76 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.45 (m, 5H, Ar-H), 7.02 (s, 1H, CHCO), 6.72 (s, 1H, Ar-H), 5.25 (s, 2H, OCH_2-Ar), 4.04 (m, 1H, Ar-OCH), 2.63 (t, 2H, *J*= 6.00 Hz, $C\underline{H}_2NCH_3$), 2.08 (s, 3H, $CH_2NC\underline{H}_3$), 1.96 (t, 2H, *J*= 6.00 Hz, $C\underline{H}_2NCH_3$), 1.72 (m, 4H, $OCH(C\underline{H}_2)_2$) ppm.

5.1.47.

7-Benzyloxy-5-hydroxy-8-((1-methylpiperidin-4-yl)oxy)-2-(4-(piperazin-1-yl)phenyl)-4H-chromen-4-on e (***35a***). Compound **34** (400 mg, 0.84 mmol) was added in anhydrous DMSO (10 mL) and heated to 60 ^{o}C. Then piperazine (724 mg, 8.4 mmol) and DIEA (500 mg, 4.2 mmol) were added in the presence of N_2 and heated to 80 ^{o}C for 8 h. The reaction was monitored by TLC. After the end of the reaction, the hot mixture was poured into saturated saline (10 mL). The mixture was filtered and the cake was washed with water (10 mL) and dried to give a yellow solid (400 mg, 89% yield). mp 190-193 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.93 (s, 1H, 5-OH), 7.90 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.46 (m, 5H, Ar-H), 7.06 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.80 (s, 1H, CHCO), 6.65 (s, 1H, Ar-H), 5.24 (s, 2H, OCH_2-Ar), 4.02 (m, 1H, Ar-OCH), 3.25 (s, 4H, $N(C\underline{H}_2CH_2)_2NH$), 2.80 (s, 4H, $N(CH_2C\underline{H}_2)_2NH$), 2.64 (t, 2H, *J*= 6.00 Hz, $C\underline{H}_2NCH_3$), 2.09 (s, 3H, $CH_2NC\underline{H}_3$), 1.97 (t, 2H, *J*= 6.00 Hz, $C\underline{H}_2NCH_3$), 1.73 (m, 4H, $OCH(C\underline{H}_2)_2$) ppm.

5.1.48.

7-Benzyloxy-5-hydroxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-8-((1-methylpiperidin-4-yl)oxy)-4H-chro men-4-one (***35b***).

Compound **34** (200 mg, 0.42 mmol) was added in anhydrous DMSO (10mL) and heated to 60 ^{o}C. Then methylpiperazine (420 mg, 4.2 mmol) and DIEA (270 mg, 2.1 mmol) were added in the presence of N_2 and heated to 80 ^{o}C for 15 h. The reaction was monitored by TLC. After the end of the reaction, the hot mixture was poured into saturated saline (10 mL). The mixture was filtered and the cake was washed with water (10 mL) and dried to give a yellow solid (180 mg, 77% yield). mp 205-210 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.86 (s, 1H, 5-OH), 7.84 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.47 (m, 5H, Ar-H), 7.08 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.68 (s, 1H, CHCO), 6.65 (s, 1H, Ar-H), 5.20 (s, 2H, OCH_2-Ar), 4.06 (m, 1H, Ar-OCH), 3.38 (s, 4H, Ar-$N(C\underline{H}_2CH_2)_2N$), 2.86 (s, 4H, Ar-$N(CH_2C\underline{H}_2)_2N$), 2.64 (t, 2H, *J*= 6.00 Hz, $C\underline{H}_2NCH_3$), 2.37 (s, 3H, NCH_3), 2.09 (s, 3H, $CH_2NC\underline{H}_3$), 1.97 (t, 2H, *J*= 6.00 Hz, $C\underline{H}_2NCH_3$),

1.76 (m, 4H, OCH(C<u>H</u>$_2$)$_2$) ppm.

5.1.49. 5, 7-Dihydroxy-8-((1-methylpiperidin-4-yl)oxy)-2-(4-(piperazin-1-yl)phenyl)-4H-chromen-4-one (***36a***).

Compound **35a** (400 mg, 0.76 mmol), Pd/C (100 mg) and a drop of acetic acid were added in tetrahydrofuran (20 mL) and methanol (20 mL) in the presence of H_2.The mixture was heated to 40 ^oC for 12 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature and filtered. The filtrate was evaporated under reduced pressure and purified over silica gel column chromatography (# 100-200) to give a yellow solid (155 mg, 49% yield). mp 210-212 ^oC. HRMS (ESI^+) calcd for $C_{25}H_{30}N_3O_5$ $[M + H]^+$: 452.2185, found 452.2135. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.93 (s, 1H, 5-OH), 7.91 (d, 2H, *J*= 9.00 Hz, Ar-H), 7.08 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.42 (s, 1H, CHCO), 6.05 (s, 1H, Ar-H), 4.36 (m, 1H, Ar-OCH), 3.36 (s, 4H, N(C<u>H</u>$_2$CH$_2$)$_2$NH), 3.00 (s, 4H, N(CH$_2$C<u>H</u>$_2$)$_2$NH), 2.89 (t, 2H, *J*= 6.00 Hz, C<u>H</u>$_2$NCH$_3$), 2.26 (s, 3H, CH$_2$NC<u>H</u>$_3$), 2.20 (t, 2H, *J*= 6.00 Hz, C<u>H</u>$_2$NCH$_3$), 1.90 (m, 4H, OCH(C<u>H</u>$_2$)$_2$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.46, 164.46, 158.58, 157.46, 153.77, 149.76, 127.58, 126.17, 120.36, 114.42, 104.88, 102.76, 95.46, 77.24, 52.92, 48.51, 45.86, 45.75, 31.7 ppm. IR (KBr) ν 3217, 1654, 1516, 1400, 1105, 859, 546 cm^{-1}.

5.1.50. 5, 7-Dihydroxy-2-(4-(4-methylpiperazin-1-yl)phenyl)-8-((1-methylpiperidin-4-yl)oxy)-4H-chromen-4-one (***36b*****).**

Compound **35b** (100 mg, 0.18 mmol) and Pd/C (30 mg) were added in tetrahydrofuran (10 mL) and methanol (10 mL) in the presence of H_2. The mixture was heated to 40 ^oC for 12 h and the reaction was monitored by TLC. After the end of the reaction, the mixture was cooled to room temperature and filtered. The filtrate was evaporated under reduced pressure and purified over silica gel column chromatography (# 100-200) to give a yellow solid (54 mg, 64.4% yield). mp 250-252 ^oC. HRMS (ESI^+) calcd for $C_{26}H_{32}N_3O_5$ $[M + H]^+$: 466.2342, found 466.2335. ^1H NMR (300 MHz, DMSO-d_6): δ = 12.70 (s, 1H, 5-OH), 7.77 (d, 2H, *J*= 9.00 Hz, Ar-H), 3.96 (d, 2H, *J*= 9.00 Hz, Ar-H), 6.52 (s, 1H, CHCO), 6.28 (s, 1H, Ar-H), 4.29 (m, 1H, Ar-OCH), 3.38 (s, 4H, Ar-N(C<u>H</u>$_2$CH$_2$)$_2$N), 2.97 (s, 4H, Ar-N(CH$_2$C<u>H</u>$_2$)$_2$N), 2.59 (t, 2H, *J*= 6.00 Hz, C<u>H</u>$_2$NCH$_3$), 2.37 (s, 3H, NCH$_3$), 2.09 (s, 3H, CH$_2$NC<u>H</u>$_3$), 2.60 (t, 2H, *J*= 6.00 Hz, C<u>H</u>$_2$NCH$_3$), 1.76 (m, 4H, OCH(C<u>H</u>$_2$)$_2$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): δ = 182.28, 163.82, 157.90, 157.49, 153.27, 149.77, 127.58, 124.75, 120.52, 114.46, 104.48, 103.04, 99.46, 77.24, 54.69, 52.63, 47.30, 46.11, 45.45, 30.75 ppm. IR (KBr) ν 3441, 2952, 1597, 1496, 1432, 1375, 1328, 1260, 1006, 930, 862, 788, 699 cm^{-1}.

The synthesis of compounds **39a-39b**, **32a-43b**, **48** and **51** were shown in Supporting Information.

5.2. Biological experiments.

5.2.1. In vitro kinase assay.

All assays were carried out using a radioactive (^{33}P-ATP) filter-binding assay at Reaction Biology Corp, USA. The general protocol for CDK2 is as follows: CDK2/cyclin A or CDK9/cyclin T (3 ng/μL, 4.5 μL) was assayed against Histone H1 (1 mg/mL) or substrate peptide (YSPTSPSYSPTSPSYSPTSPKKK) (0.3 mM), and 0.05 mM [^{33}P-γ-ATP] (50−1000 cpm/pmole) and incubated for 2 h at room temperature. Assays were stopped by the addition of 5 μL of 0.5 M (3%) ortho-phosphoric acid and then harvested onto P81 Unifilter plates with a wash buffer of 50 mM ortho-phosphoric acid.

5.2.2. Cell lines and culture

These cell lines were obtained from Cell Bank of Shanghai, Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. A549, HepG2, HCT116, MV4-11, L02 cells were cultured in DEME (GiBco, USA) supplemented with 10% FBS, 1% penicillin/streptomycin. HL60, HT29, SW480, THP-1, BGC803, MCF-7, Hela, BT-549, RPMI8226 and HEK293 were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supported with 10% FBS and 1% penicillin/streptomycin. Adherent cells were grown in tissue culture flasks until they were 80-90% confluent prior to use. For suspension cells, cells were collected by spin down at 1000 rpm/min for 5 min before use.

5.2.3. Cell viability inhibition assay.

Cells were seed in 96-well culture plates (3000-5000 cells/well) and cultured overnight in a humidified atmosphere of 5% CO_2 at 37 ^{o}C. The cells were treated with various concentrations of compounds and equivalent amounts of DMSO used as negative control. After 72 h incubation, 20 μL of MTT (Sigma-Aldrich, St. Louis, MO) solution (5 mg/mL) was added into each well. After 4 h incubation at 37 ^{o}C, the supernatants were removed and 100 μL DMSO were added into each well. Absorbance was measured at 490 nm with Multiskan sky (Themo scientific). Data were normalized to control groups and represented by the means of five measurements. IC_{50} was taken as the concentration that caused 50% cells death and was calculated by Prism 7.0 (GraphPad Software, San Diego, CA)

5.2.4. Apoptosis assays.

DAPI staining. MV4-11 cells were plated into 6-well plates and were incubated various concentrations of **51** for 24 h or 48 h in a humidified atmosphere of 5% CO_2 at 37 ^{o}C, followed by addition of DAPI (4'-6-diamidino-2-phenylindole dihydrochoride, Sigma). After 20 min incubation, the cells were visualized and photographed using an Olympus confocal microscope (AiryScan LSM800).

Annexin V/PI double staining assay. The apoptosis-mediated cell death was evaluated using an Annexin V-FITC/PI Apoptosis Detection kit (Biovision, CA) according to the manufacturer's instructions. Cellular fluorescence was measured by flow cytometric analysis after supravital staining. Then data acquisition and analysis were performed in a FACSCalibur flow cytometer using CellQuest

software (BD Biosciences, Franklin Lakes, NJ). The normal cells were in the lower left section (Annexin V-, PI-), cells in the early and median stage of apoptosis were in the lower right section (Annexin V+, PI-), whereas cells in the late stage of apoptosis were in the upper right section (Annexin V+, PI+).

5.2.5. Western-blot analysis.

Compound **51** treated-MV4-11 cells were harvested, washed with cold PBS before lysed in RIPA for 1h. Whole-cell protein lysates were prepared and insoluble material was pelleted at 12000 rpm for 20 min at 4 ^{o}C. The supernatant was collected and the protein concentration was measured using BCA protein assay. SDS-PAGE loading buffer (Beyotime) was combined with equivalent quantity of protein in boiled water for 5 min. Proteins were fractionated on SDS-PAGE and then transferred onto PVDF membranes (PerkinElmer, Northwalk, CT, USA). Membranes were blocked with 5% BSA for at 25 ^{o}C for 1 h and then incubated overnight at 4 ^{o}C with specific primary antibodies, followed by DyLight 800 labled secondary antibody at 37 ^{o}C for 1 h in the dark. Finally, the detection was performed by the Odyssey infrared imaging System (LO-COR, Lincoln, Nebraska, USA). The antibodies were purchased from Cell Signaling Technology and the dilutions of the antibodies were according to instruction from it.

5.2.6. Molecular docking.

CDK9 and CDK2 complex was obtained from the protein data bank (PDB ID: 3BLR, 4BCP) [20,34] and the structures were editor according to provide a monomer of the protein and protonated use GOLD 5.1. The ligand was then removed to leave the receptor complex. Compound **51** was energy minimized using MOE2019.0101 with Amber10:EHT forcefield. For docking, GOLD 5.1 software was employed and the active site was defined as being any volume with 8 Å of the native ligand in its crystal pose in 3BLR. The number of genetic algorithm (GA) run was set to 10. Each GOLD run was saved and the strongest scoring binding pose of each ligand (subject to a rmsd default distance threshold of 1.5 Å) was compared to that of the reference ligand position observed in the crystal structure. The best output poses of the ligands generated were analyzed on the basis of ChemScore, feasibility of hydride transfer process, and H-bonding to the enzyme. The best poses were visualized with MOE 2019.0101.

5.2.7. In vivo antitumor activity.

Female nude (nu/nu) BALB/c mice (5-6 weeks) were used for the determination of in vivo anticancer activity. 5×10^6 MV4-11 cells in PBS were injected in to the subsutaneous space on the hind flanks. The cells were suspended in a 1:1 mixture of RPMI-160 and Matrigel. Tumors were allowed to develop and when it approached almost 100 mm^3, the mice were divided into 5 experimental groups with 6 mice in each group. Injections of PBS, **51** (15 mg/kg, 30 mg/kg and 45 mg/kg) and doxorubicin

(5 mg/kg) as a positive control. Compound **51** was dissolved in methanesulfonic acid buffer with molar concentration ratio of 1:1. All agents were administered every other day for 21 days through the tail vein (iv), and tumor growth was monitored and measured every day. After three weeks, mice were euthanized and the average tumor wet weights were calculated. The tumor volume was calculated using the formula ($L \times B^2/2$) mm^3 (L indicates length; B indicates width) and a vernier caliper.

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Highlights

- Several novel wogonin derivatives were discovered as potent CDK9 inhibitors through SAR.
- The drug-like compound **51** showed potent activity toward CDK9 (IC_{50} = 19.9 nM) and MV4-11 cell growth (IC_{50} = 20 nM)
- Preliminary mechanism studies on the anticancer effect indicated that **51** inhibited the cell growth via caspase-dependent apoptosis.
- Compound **51** was shown to kill cancer cells efficiently in *in vivo* model.