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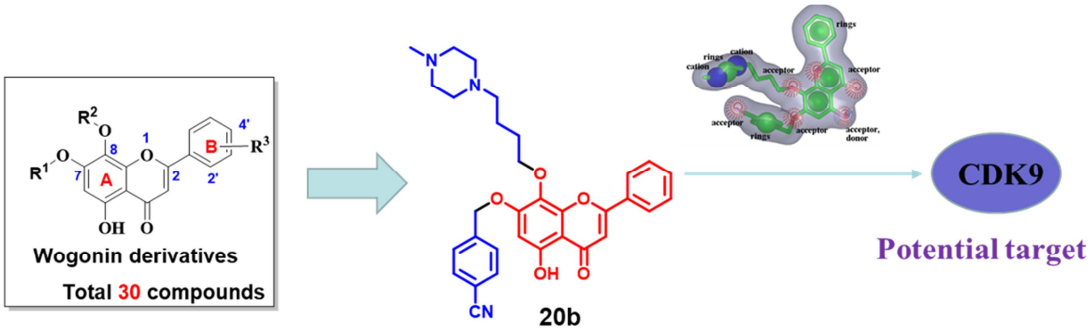


$IC_{50}$ = 1.72 μM (LNCaP) $IC_{50}$ = 2.42 μM (MCF-7)
$IC_{50}$ = 2.65 μM (Hela) $IC_{50}$ = 0.59 μM (HepG-2)
$IC_{50}$ = 2.14 μM (A549) $IC_{50}$ = 3.70 μM (B16)

# Discovery and synthesis of novel Wogonin derivatives with potent antitumor activity *in vitro*

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## Abstract

Phenotypic screening of high quality compound library is one of the most effective strategy to obtain novel bioactive compounds. Recently, our group have constructed a Wogonin-scaffold library with substituents diversity and successfully obtained a series of potent compounds. Herein, we reported the synthesis of these compounds and evaluated the *in vitro* antitumor activity against a panel of human tumor cell lines. Most of them showed good activity with a broad spectrum and preliminary structure-activity relationship for the substitutions were obtained. Further biological assays showed that the most potent compounds **18n** and **20b** could significantly enhance the intracellular ROS level and induce the cell apoptosis at low micromole level. Through similarity searching, CDK9 was identified as the potential target for **20b**, which could be a start point for next structure-based drug design.

**Keywords:** Wogonin, antitumor, ROS, Phenotypic screening, target fishing.

## 1. Introduction

Cancer remains the leading cause of death worldwide though much important progress has been achieved recently. It is expected that annual cancer cases will rise to 22 million in the next two decades [1]. Treatments for cancers include surgery, radiation therapy, and chemotherapy. However, surgery is usually the treatments of choice for early stage cancers, for patients with unrespectable disease, local control can be achieved with radiation therapy combined with chemotherapy, molecular-targeted drugs, or some combination [2,3]. Currently, chemotherapy is still the main type of treatment for cancer, and phytochemicals have been a crucial part of antineoplastic drugs [4]. Indeed, more than half of anticancer drugs approved are either natural products or developed based on the knowledge gained form natural products. Moreover, about 75 percent of plant-derived drugs used today in the clinic come from traditional medicines [4].

The dry root of Scutellaria, also named HuangQin in China, is one of the most popular and multi-purpose herb used in China. In the traditional Chinese medicines (TCM), extracts from the Scutellaria radix are widely used for clinical treatment of hyperlipemia, atherosclerosis, hypertension, dysentery, common cold and inflammatory diseases. In addition, recently, Scutellaria alone or in combination with other herbs has been shown to possess cytostatic effect on several cancer cells *in vitro* [5,6] and also *in vivo* in mouse tumor models [7,8]. To identify the antitumor components of Scutellaria, many researches have been focused on separating the effective components. It has been proved that the antitumor constituents of Scutellaria are flavonoids. Among these flavonoids, Wogonin (**1**) has been reported to selectively kill cancer with little to no toxicity to normal cells at concentrations that are lethal to tumor cells.[5] And it has been approved by the China Food and Drug Administration (CFDA) for phase I/II clinical studies since 2014 [9]. However, **1** and its simple derivatives have often been reported at pharmacologically nonachievable high micromolar concentrations (20 to 200 μM) against cancer cells [10]. Hence, medicinal chemistry strategies are necessary to optimize the drug-like profiles of these compounds to ensure that the full clinical applications for flavonoids are realized.

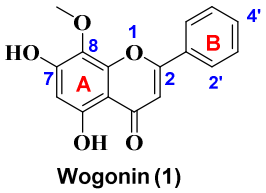



**Fig. 1**. The structure of Wogonin (**1**).

Phenotypic screening is an important approach in drug discovery and attracts great interests recently [11]. As an alternative method to target-based screening, phenotypic screening discovers drug hits or leads based on assessing a molecule's effects on the whole-cell level. Thus, phenotypic screening is one of the drug discovery strategy for identifying initial starting points that are pharmaceutically relevant and potentially available for medicinal chemistry optimization [12]. Analysis of the origins of new antitumor drugs approved by the US Food and Drug Administration (FDA) in recent years, a significant number were identified from phenotypic screening approaches [13-14]. Thus, our group planned to employ the phenotypic screening method to discover efficient derivatives of **1**. The success and efficiency of phenotypic screening is largely dependent on high quality compounds libraries [15]. In order to develop high quality compound library for phenotypic screening, our laboratory recently synthesized plenty of Wogonin derivatives to construct a library contains more than two hundred compounds. Interestingly, preliminary *in vitro* antitumor assay identified a series of compounds which showed efficient antitumor activity for several types of human cancer cell lines [16]. In this work, we selected and reported the most potent compounds in the library and all of the selected compounds were further screened using four different types of cancer cells to clarify the influence of the substitutions at different positions on the antitumor activity. Among them, the most two potent compound **18n** and **20b** showed good antitumor activity against all tested cancer cell lines and could effectively induce the apoptosis through ROS mediated pathways in HepG2 cancer cells. Through similarity searching, CDK9 was identified as the potential target for **20b**, which could be a start point for next structure-based drug design of Wogonin derivatives.

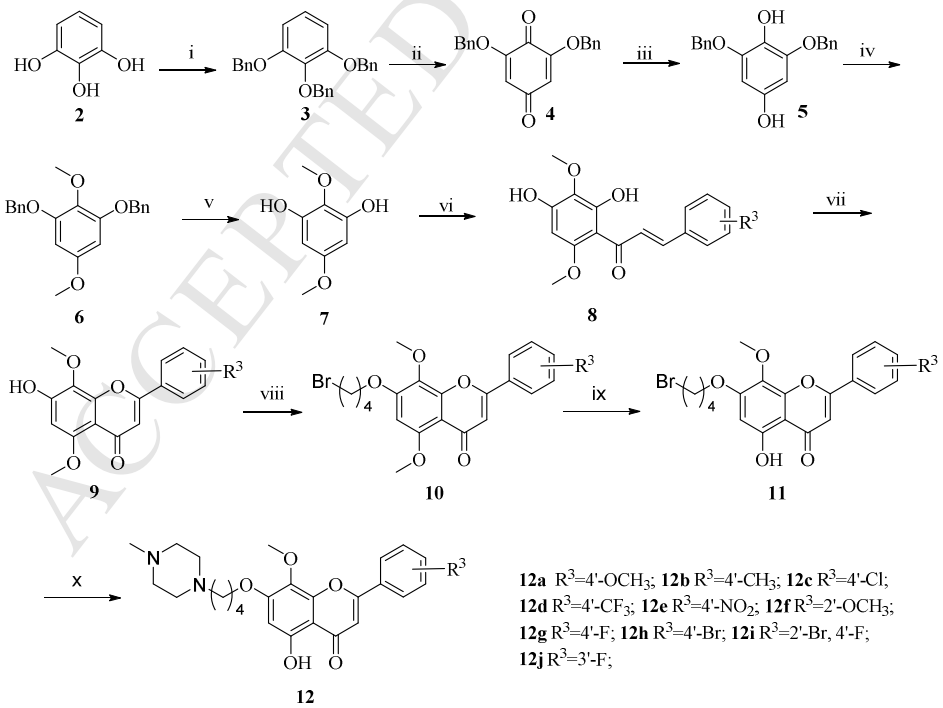
## 2. Chemistry

The common synthetic routes of the target compounds were outlined in Scheme 1-4. Compounds **12a-12j** were synthesized as shown in Scheme 1. Benzylation of **2** was conducted by reaction with benzyl bromide in $K_2CO_3$ to afford intermediate **3**. Oxidation of **3** using 65% $HNO_3$ provided the quinone mediate **4**, which was further reduced by sodium thiosulfate to the phenol **5**. Methylation of **4** with dimethylsulfate obtained intermediate **6**, which then carried the debenzylation reaction to give compound **7** using hydrogen/palladium-carbon system. Followed by Friedel-Crafts acylation with cinnamoyl chloride derivatives using $BF_3$-$Et_2O$ as catalyst provided the intermediate **8**. Subsequently, the intermediate **8** was cyclized to the flavones **9** mediated by iodine at 120 $^oC$. And the flavone **9** was then introduced a four-carbon linker to 7-OH to form the key intermediate **10**. Compound **10**

was obtained through demethylation of intermediate **11**. Finally, the target compounds **12a-12j** were obtained from **11** with *N*-methylpiperazine by reflux in acetonitrile.

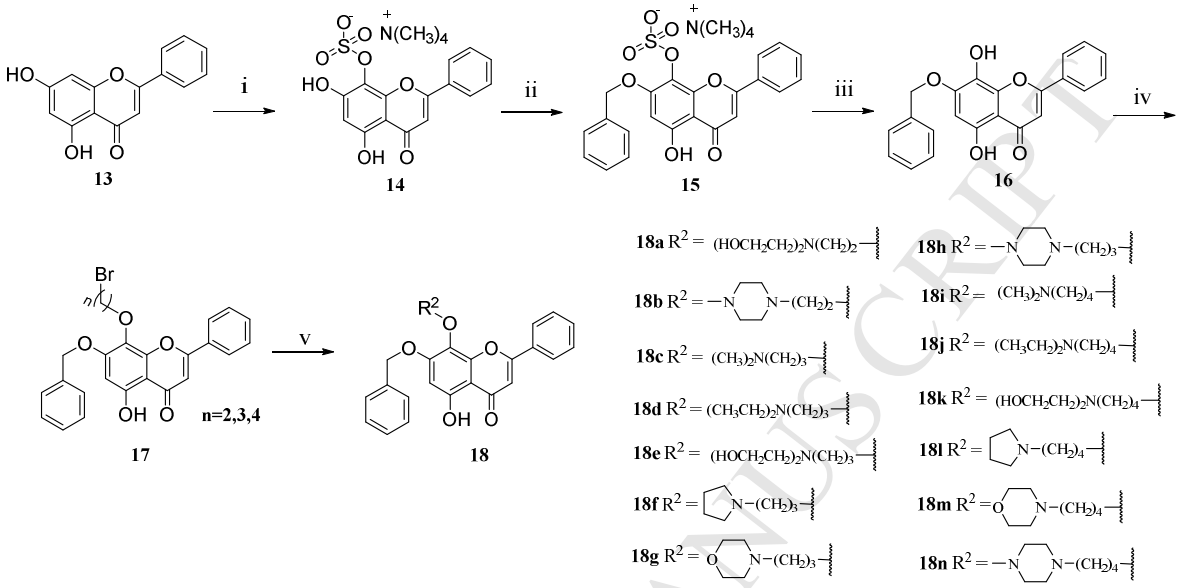
Scheme 2 described the synthesis of target compounds **18a-18n**. These compounds were synthesized in five steps from chrysin (**13**). Tetramethylammonium hydroxide reacted with **13** resulted in the intermediate **14** in 30% yield. The benzylation of **14** was conducted by reaction with benzyl bromide in the presence of potassium carbonate to afford intermediate **15**. Then compound **16** was obtained by acidified **15** in the presence of hydrochloric acid. Followed by a two to four-carbon linker to 8-OH to form intermediate **17**, which then reacted with various amines to give the target compounds **18a-18n**.

Based on the compound **18n**, compounds **20a-20f** were obtained as outlined in Scheme 3. Debenzylation of **18n** give rise to the compound **19**, which was subsequently reacted with corresponding amines to provide target compounds **20a-20f** in 30-60% yield (Scheme 3). The structures of all compounds were confirmed by $^1$H NMR and electrospray ionization mass spectrometry (ESI-MS). Before these compounds were used in biological experiments, they were purified by silica gel column chromatography and HPLC was used to determine their purity (all > 95%).

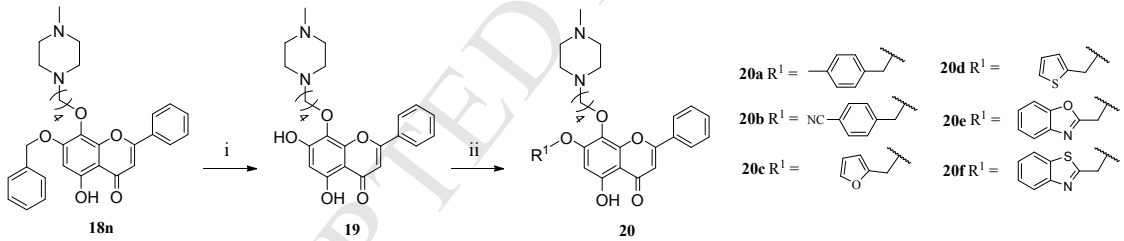



**Scheme 1.** Synthesis of compounds **12a-12j**. Reagents and conditions: (i) $PhCH_2Br$, $K_2CO_3$, acetone, reflux, 48 h, 87%; (ii) AcOH, 65% $HNO_3$, 40 °C, 4 h, 45%; (iii) $Na_2S_2O_4$, $CH_3COOC_2H_5/H_2O$, r.t., 1 h, 37%; (iv) $(CH_3)_2SO_4$,

NaOH, $C_2H_5OH$, r.t., 3 h, 85%; (v) $H_2$, Pd/C, MeOH, r.t., 8 h, 90%; (vi) Cinnamoyl chloride derivatives, $BF_3$-$Et_2O$, $CHCl_3$, reflux, 1.5-6 h, 35-80%; (vii) $I_2$, DMSO, 120 °C, 5-8 h, 30-40%; (viii) $AlCl_3$, $CH_3CN$, reflux, 8 h, 65-85%. (viii) $Br(CH_2)_4Br$, $K_2CO_3$, acetone, reflux, 8 h, 60-75%; (ix) $AlCl_3$, $CH_3CN$, reflux, 6 h, 70-90%; (x) *N*-methylpiperazine, dry $CH_3CN$, reflux, 5 h, 50-64%.




**Scheme 2**. Synthesis of compounds **18a-18n**. Reagents and conditions: (i) $K_2S_2O_8$, $(CH_3)_4NOH$, $H_2O$, r.t., 7 h, 30%; (ii) $PhCH_2Br$, $K_2CO_3$, DMF, 50 °C, 6 h; (iii) 6 M HCl, r.t., 8 h, 40%; (iv) $Br(CH_2)_nBr$, $K_2CO_3$, acetone, reflux, 8 h, 75%; (v) substituted amino derivatives, $CH_3CN$, reflux, 1–5 h, 30–60%.




**Scheme 3**. Synthesis of compounds **20a**-**20f**. Reagents and conditions: (i) Pd/C, $H_2$, THF, r.t., 4 h, 42%; (ii) R-Cl, base, $CH_3CN$, reflux, 1–5 h, 30–60%.

## 3. Results and Discussion

### *3.1. In vitro antitumor activity.*

The results of *in vitro* antitumor activity of all compounds against four cancer cell lines were summarized in Table 1. Notably, most compounds showed good antitumor activity against all the four cancer cell lines. Namely, these compounds exhibited a broad spectrum of inhibition on human cancer cells, with relatively low $IC_{50}$ values ranging from 0.6 μM to 10 μM. In addition, they were generally more active than the control **1**, carboplatin and 5-fluorouracil (5-Fu). As for the first series,

compounds **12a-12j** with substituents at B-ring, showed the similar activity toward all cancer cells. Among compounds **18a-18n**, **18n** showed the most potent activity, especially for HepG2. Based on the structure of **18n**, we synthesized compounds **20a-20f**, and the activity of these compounds against the four cancer cells were in the same level with **18n**. In addition, it was worth mentioning that nearly all the compounds possessed significant better selectivity against HepG2 cancer cell line than other three cancer cell lines, indicating the good potency of these structures for the treatment of human hepatocarcinoma. Among these compounds, two most promising compounds **18n** and **20b** exhibited the highest antitumor activity against A549, MCF-7, B16 and HepG2 with $IC_{50}$ values of 2.91, 2.14 μM (A549), 2.93, 2.42 μM (MCF-7), 2.37, 3.70 μM (B16), and 1.50, 0.59 μM (HepG2), respectively. These two compounds showed approximately 2-20-fold more potent than carboplatin and 5-Fu in this biological assay.

*3.2. Selective killing effect of representative compounds **20b** in cancer cells.*

In an effort to obtain antitumor drugs with efficacy and safety, representative compound **20b** was further assessed versus other cancer cells and normal cells L02. As shown in Fig. 2A, the compound treatment markedly induced cell death in all cancer cells with concentration-dependent trend. And this compound showed antitumor activity against all cancer cells with $IC_{50}$ about 1 μM, shifting the selectivity ratio 4.3-fold to 27-fold (the fold is the ratio of the $IC_{50}$ of normal cell L02 to the $IC_{50}$ of cancer cells). Among these cancer cell lines, HepG2 was the most sensitive cancer cell line for **20b**, with the selectivity ratio 27-fold. When the normal cells L02 were incubated with this compound for 48 h, there were little apparent reduction in cell viability under the concentration of 15 μM. However, the inhibitory curve was jumped at the concentration of 30 μM, indicated that this compound would be toxic at high concentrations towards normal cells. The results represented that compound **20b** could show an exposure time window but with a relatively narrow concentration range *in vitro*. However, compared with the control Wogonin, this compound had a cancer-cell-selective killing property, which showed a more potent profile toward cancer cells to that of control.

**Table 1**
$IC_{50}$ values for Wogonin derivatives against A549, MCF-7, B16 and HepG2 cancer cell lines.

| Entry | No. | A549 | MCF-7 | B16 | HepG2 | Mean |
|---|---|---|---|---|---|---|
| | | $IC_{50}$ (μM) | | | | |
| 1 | **12a** | 2.32 | 3.98 | 4.10 | 4.20 | 3.65 |
| 2 | **12b** | 3.81 | 4.02 | 5.43 | 1.00 | 3.57 |
| 3 | **12c** | 1.43 | 3.21 | 2.09 | 3.75 | 2.62 |
| 4 | **12d** | 2.89 | 2.98 | 4.99 | 2.40 | 3.32 |
| 5 | **12e** | 3.21 | 4.09 | 2.89 | 2.62 | 3.20 |
| 6 | **12f** | 6.79 | 5.43 | 9.30 | 2.33 | 5.96 |
| 7 | **12g** | 2.22 | 3.86 | 4.32 | 2.81 | 3.30 |
| 8 | **12h** | 2.13 | 3.21 | 4.33 | 2.32 | 3.00 |
| 9 | **12i** | 2.98 | 3.09 | 3.44 | 2.13 | 2.91 |
| 10 | **12j** | 2.09 | 2.89 | 4.52 | 2.58 | 3.02 |
| 11 | **18a** | 6.40 | 6.07 | 3.69 | 3.17 | 4.83 |
| 12 | **18b** | 4.41 | 4.43 | 4.37 | 2.89 | 4.02 |
| 13 | **18c** | 2.91 | 3.21 | 5.42 | 4.95 | 4.12 |
| 14 | **18d** | 3.62 | 3.21 | 4.90 | 3.01 | 3.69 |
| 15 | **18e** | 3.15 | 4.23 | 6.89 | 3.55 | 4.46 |
| 16 | **18f** | 4.72 | 2.23 | 7.98 | 2.66 | 4.40 |
| 17 | **18g** | 8.91 | 8.72 | 10.0 | 2.43 | 7.52 |
| 18 | **18h** | 3.94 | 1.02 | 3.76 | 3.11 | 2.96 |
| 19 | **18i** | 1.09 | 3.21 | 3.22 | 2.09 | 2.40 |
| 20 | **18j** | 3.91 | 1.92 | 4.88 | 1.65 | 3.09 |
| 21 | **18k** | 4.35 | 1.09 | 3.76 | 3.14 | 3.09 |
| 22 | **18l** | 1.10 | 1.89 | 3.21 | 4.66 | 2.72 |
| 23 | **18m** | 6.60 | 9.90 | 5.41 | 3.29 | 6.30 |
| 24 | **18n** | 2.91 | 2.93 | 2.37 | 1.50 | 2.42 |
| 25 | **20a** | 3.20 | 2.22 | 3.29 | 5.09 | 3.45 |
| 26 | **20b** | 2.14 | 2.42 | 3.70 | 0.59 | 2.21 |
| 27 | **20c** | 3.60 | 2.77 | 3.53 | 4.11 | 3.50 |
| 28 | **20d** | 2.10 | 3.18 | 2.12 | 4.56 | 2.99 |
| 29 | **20e** | 3.87 | 4.98 | 2.08 | 4.11 | 3.76 |
| 30 | **20f** | 1.06 | 4.85 | 2.78 | 5.44 | 3.53 |
| 31 | **1** | 20.1 | 28.0 | 26.4 | 19.5 | 23.5 |
| 32 | carboplatin | 11.1 | 12.2 | 25.1 | 25.7 | 18.5 |
| 33 | 5-Fu | 8.9 | 14.7 | 1.19 | 10.4 | 8.80 |

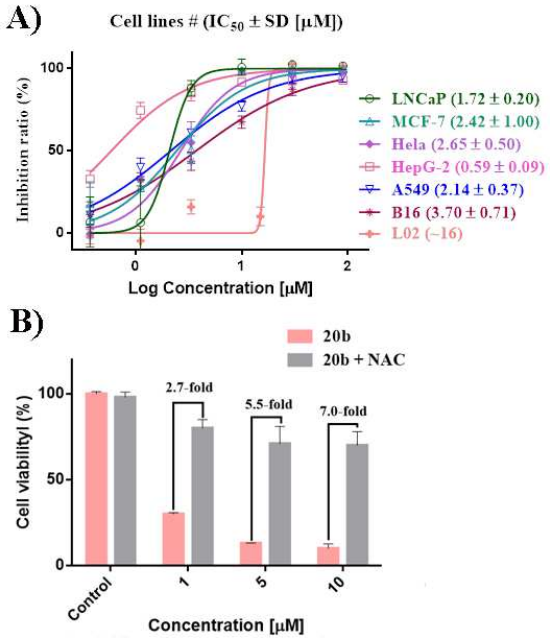



**Fig. 2.** (A) Cell death of cancer cells (LNCap, MCF-7, Hela, HepG-2, A549 and B16) and normal hepatic cells (L02) treated with compound **20b**. (B) Cell viability with different concentrations (1, 5, 10 μM) of compound **20b** or **20b** with NAC. The selectivity ratio of cell viability between **20b** treated with NAC and **20b** were calculated as

2.7-fold (1 μM), 5.5-fold (5 μM) and 7.0-fold (10 μM).

*3.3. Induction of ROS accumulation in HepG2 cells by **18n** and **20b**.*

Several Wogonin derivatives have been reported to exert their antitumor activity by accumulating ROS in cancer cells [17], we next determined the effect of the representative compounds **18n** and **20b** on cellular ROS level in HepG2 cancer cells by flow cytometry. To demonstrate the effects of **18n** and **20b** on ROS induction, we determined the production of ROS with a probe 2',7'-dichlorfluorescein diacetate (DCFH-DA), which is converted to a green fluorescent product via oxidation. After being uptaken by cells, DCFH-DA is hydrolyzed by cellular esterases to dichlorodihydrofluorescein (DCFH), which is trapped within the cell. The DCFH is then oxidized to fluorescent dichlorofluorescent by action of cellular ROS. As shown in Fig.3, a rapid production of ROS occurred after the exposure of HepG2 cells to compounds **18n** and **20b**. After treating with these two compounds for 24 h, a marked increase in ROS levels was caused with increasing concentration of each compound (2 μM, 4 μM or 6 μM). The results indicated that these two compounds induced ROS production in dose-dependent manners in cancer cells. In addition, we examined whether ROS made effect on the cancer cell viability. HepG2 was incubated with these two compounds or compounds with 5 mM N-acetylcysteine (NAC) for 48 h, respectively. As expected, the NAC treated group got higher cell viability (the selectivity ratio between the NAC treated group and without NAC treated group was shown in Fig. 2), which indicated that these compounds **20b** might enhance the intracellular ROS level and induce the cell apoptosis (Fig. 2B).

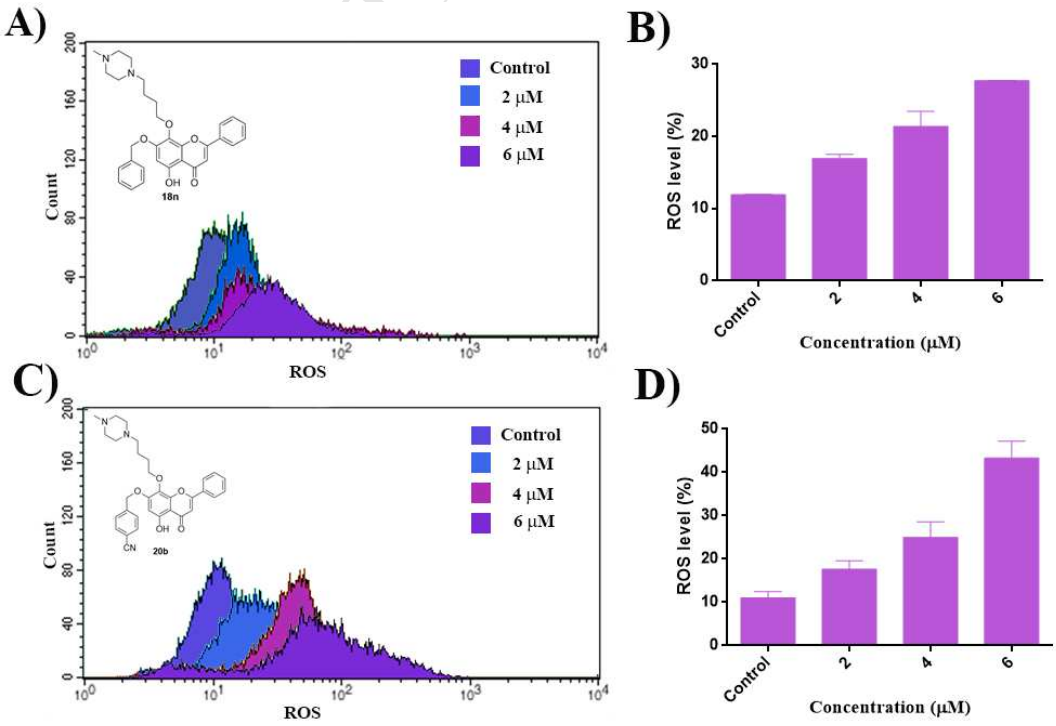


**Fig. 3**. Effects **of 18n** (A,B) and **20b** (C,D) on the ROS of of HepG2 cells. The level of intracellular reactive oxygen species (ROS) in HepG2 cells was measured by flow cytometry/ Data were shown as mean ± SEM (n = 3).

### *3.4. Apoptosis induced by compounds **18n** and **20b** in HepG2 cells.*

It has been previously reported that Wogonin and its several derivatives exerted antitumor activity of by inducing a mitochondrial-mediated apoptosis [18]. The representative compounds **18n** and **20b** were employed to investigate the apoptosis effect. The apoptotic cell counts were measured using AnnexinV-FITC/PI by flow cytometry. HepG2 cells were treated with 2 μM, 4 μM and 6 μM of compounds **18n** and **20b** for 24 h. The DMSO (0.1%) was used as vehicle control. As shown in Fig.4, exposure to 6 μM of compounds **18n** and **20b** resulted in 20.7% and 30.7% of cell apoptosis respectively, compared with 3.4% and 2.6% of DMSO-treated group, while lower doses treatment showed weaker effect. These data indicated that this series of compounds could induce HepG2 apoptosis in a dose-dependent manner.

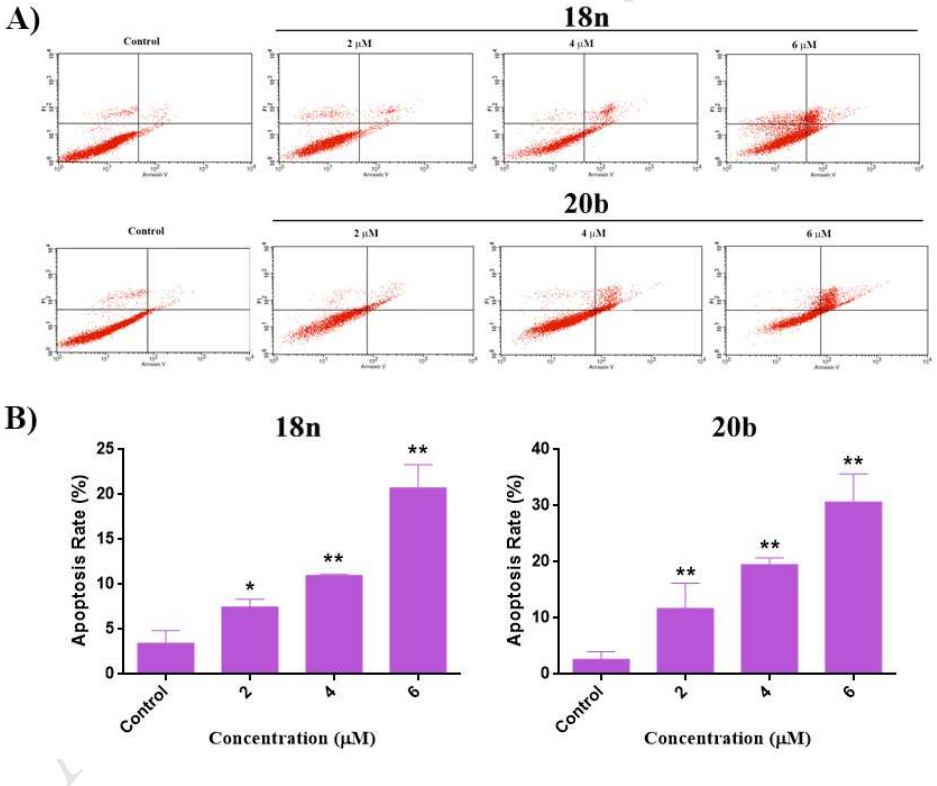



**Fig. 4**. (A) Apoptosis effect of compounds **18n** (2, 4, 6 μM) and **20b** (2, 4, 6 μM) in HepG2 cells by Annexin V-FITC/PI assay for 24 h. (B) Quantification of apoptosis rate of compound **18n** and **20b**.

### *3.5. Ligand similarity search for exploring the potential target of **20b**.*

According to the preliminary *in vitro* biological results, compounds **18n** and **20b** could be promising anticancer candidates. It has been shown that these two compounds induced apoptosis through increasing cellular ROS level. However, to further design compounds with better drug-like properties and pharmacological activity based on the scaffold of them, identifying the specific protein which could be a potential target for this series of compounds is especially urgent to be study. In silico target fishing is an emerging field that aims at predicting biological targets of molecules based on their chemical structure [19]. These in silico method complement much more expensive biological experimental approaches to drug design and have been integrated into virtually all modern drug-discovery programs. Among the in silico methods, similarity searching is an efficient method to discover the target. The fundamental idea underlying ligand-based approaches is that two similar ligands are possible to possess similar target-binding profiles. Target information on a unique collection of 267 anticancer compounds was extracted from the Cambridge cancer compound library in Selleck Co. For 27 molecules, no target information could be obtained which were excluded from further analysis. The remaining 240 molecules were assigned to 220 targets that were reviewed manually. **Fig. 5** showed the distribution of the compounds for these 220 targets. Remarkably, to 203 targets only one drug is assigned in the database, indicating the target richness of these compounds.

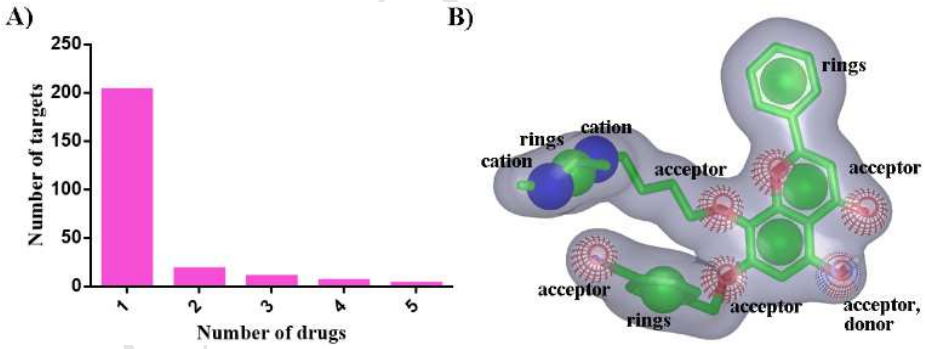
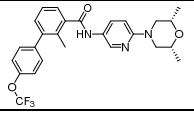
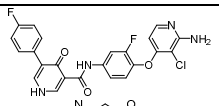
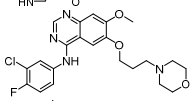
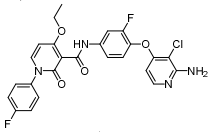
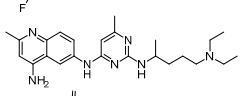
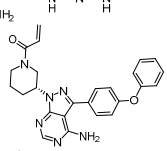
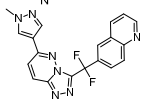
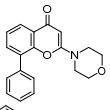
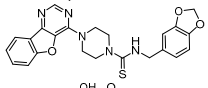
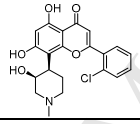



**Fig. 5.** (A) The number of drugs belong to different to number of targets. Most of these compounds belong to the specific target. (B) The ROCs model developed based on the scaffold of **20b**.

## Table 2

The screened compounds and potential targets based on the method of similarity search.

| No. | Compound | Structure | ShapeTanimoto Score | Target |
|---|---|---|---|---|
| **1** | LDE225 (NVP-LDE225, Erismodegib) |  | 0.58 | Smoothened |

| | | | | |
|---|---|---|---|---|
| **2** | BMS 794833 |  | 0.57 | c-Met |
| **3** | Gefitinib (Iressa) |  | 0.55 | EGFR |
| **4** | BMS 777607 |  | 0.55 | c-Met |
| **5** | NSC 23766 |  | 0.54 | Rac |
| **6** | PCI-32765 (Ibrutinib) |  | 0.52 | Src |
| **7** | JNJ-38877605 |  | 0.49 | c-Met |
| **8** | LY294002 |  | 0.46 | PI3K |
| **9** | Amuvatinib (MP-470) |  | 0.45 | c-Met |
| **10** | Flavopiridol (Alvocidib) |  | 0.45 | CDK |

Based on the representative scaffold of **20b**, a shape query using ROCs was developed to screen the database. Compounds with top ten ShapeTanimoto score were selected, and these compounds were assigned to 7 targets. All of the selected compounds were localized in the similarity range of 0.45-0.58 (Table 2), which means that the selected compounds had certain diversity with the reference ligands. Notably, among the top ten compounds, four of them targeting c-Met which may be a potential target for these series of compounds. Then, we decided to determine the biological functions of the representative compound **20b** targeting the screened enzyme. Contrary to expectations, the *in vitro* enzymatic assays showed that the compound **20b** was not a good inhibitor of c-Met with $IC_{50}$ at 35 μM. However, it was a potential selective inhibitor of CDKs with $IC_{50}$ at 0.87 μM against CDK9, while $IC_{50}$ at 6.7 μM against CDK2. And the compound did not show inhibiting activity against other screened enzymes. We also docked the compound **20b** into the crystal structure of CDK9 (PDB ID: 3BLR), it binds to CDK9 at the ATP-binding pocket in a similar manner to the binding of the native ligand flavopiridol. There are hydrogen bonds form the **20b** O4 oxygen and O5 hydroxyl to the residues Cys106 and Phe105. The scaffold also formed the H…π stacking interactions with Asp167 and Phe103. The long side chain at 8-position towards the solvent

area. The docking study further proved that this series of compounds were potential CDK9 inhibitors. Thus, according to the results, our group further designed another series of compounds which targeting CDK9 base on compound **20b** through the structure-based drug design. And several compounds selectively inhibited CDK9 with $IC_{50}$ at nanomolar concentration were discovered and the detailed results would be reported in the near future. In addition, the relationship between the inhibitory of CDK9 enzyme and the enhancement of the intracellular ROS level are also studying in our lab.

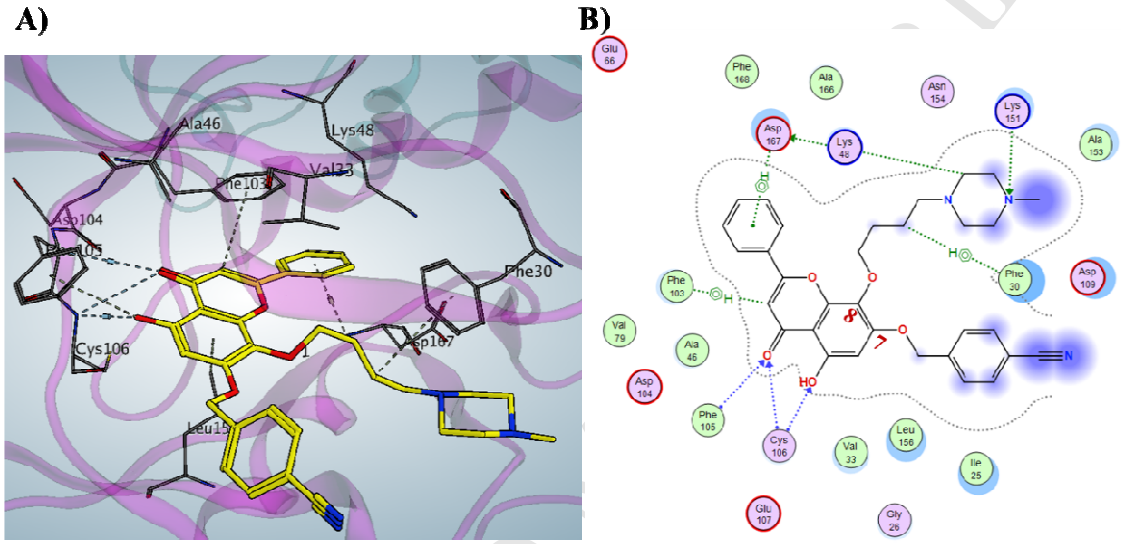



**Fig. 6.** (A) Docked conformation of compound **20b** into active site of CDK9. The interaction mode was obtained through molecular docking (PDB ID: 3BLR) and depicted using MOE 2016.09. The carbon atoms of the compounds and the key residues in the active site of CDK9 were colored in yellow and gray, respectively. The H-bonds were shown as dot lines. (B) The 2D ligand interactions of **20b** with key residues in the active site of CDK9.

## 4. Conclusions

In summary, inspired by the flavonoid antitumor scaffold, a series of derivatives were synthesized and screened for antitumor activity. Among them, the potent thirty novel flavonoid derivatives were selected and reported in this paper. Most of the selected compounds possessed good *in vitro* antitumor activity with a broad spectrum. Within this series of compounds, **18n** and **20b** exhibited the highest antitumor activity against A549, MCF-7, B16 and HepG2 with $IC_{50}$ values of 2.91, 2.14 μM (A549), 2.93, 2.42 μM (MCF-7), 2.37, 3.70 μM (B16), and 1.50, 0.59 μM (HepG2), respectively. Further biological assays showed that HepG2 was the most sensitive cancer cell line for **20b**. Compounds **18n** and **20b** could significantly enhance the intracellular ROS level and induce the cell apoptosis at 6 μM. Further through similarity searching, CDK9 was identified as the potential target for **20b**, which could be a start point for next structure-based drug design. Taken together, the findings disclosed herein endorse the therapeutic potential of these novel Wogonin derivatives and deserve further studies to explore their potential utility as antitumor agents.

## 5. Experimental Section

### *5.1. Chemistry*

#### *5.1.1. General Experimental Methods*

Reactions were monitored by thin-layer chromatography on silica gel plates (60F-254) visualized under UV light. Melting points were determined on a Mel-TEMP II melting point apparatus without correction. $^{1}$HNMR and $^{13}$CNMR spectra were recorded in $CDCl_3$ on a Bruker Avance 300 MHz spectrometer at 300 MHz and 75 MHz, respectively. Chemical shifts ($\delta$) are reported in parts per million (ppm) from tetramethylsilane (TMS) using the residual solvent resonance ($CDCl_3$: 7.26 ppm for $^{1}$H NMR, 77.16 ppm for $^{13}$C NMR. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). IR spectra were recorded on a Nicolet iS10 Avatar FT-IR spectrometer using KBr film. MS spectra were recorded on a LC/MSD TOF HR- MS Spectrum. Flash column chromatography was performed with 100-200 mesh silica gel and yields refer to chromatographically and spectroscopically pure compounds.

All chemicals purchased from commercial suppliers were used as received unless otherwise stated. Reactions and chromatography fractions were monitored by Merck silica gel 60 F-254 glass TLC plates.

*5.1.2. Synthesis*

**1,2,3-Tribenzyloxybene (3).** To a solution of gallic acid (100 g, 0.8 mol) in acetone (1000 mL), were added KI (26.7 g, 0.16 mol), $K_2CO_3$ (220 g, 0.16 mol) and benzyl bromide (265 mL, 2.4 mol) successively. The reaction mixture was heated to reflux. After the end of the reaction (monitored by TLC (PE : EA = 10 : 1)), the mixture was filtered and the cake was washed with dichloromethane (100 mL × 4). The filtrate was evaporated under vacuum to afford the crude product, which was purified through recrystallization with ethanol (1000 mL) to give **3** as a white solid (275 g, 87.5%). m.p. 68-70 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 7.30-7.27 (m, 15H, ArH), 6.92 (t, 1H, $J$ = 8.4 Hz, ArH), 6.64 (d, 2H, $J$ = 8.4 Hz, ArH), 5.1 (s, 4H, -$OCH_2$Ph), 5.07 (s, 2H, -$OCH_2$Ph) ppm. EI-MS (m/z): 91, 350, 396 $[M]^+$.

**2,6-Dibenzyloxy-*p*-benzoqunine (5). 3** (60 g, 0.126 mol) was dissolved in acetic acid (100 mL), and the mixture was stirred at 40 $^{o}$C for 30 min. Then, 65% $HNO_3$ (3 mL) was added dropwise and the mixture was stirred for an 4 h. After that, the mixture was filtered, and 10 mL $HNO_3$ was added to the filtrate to stir for another 4 h. The mixture was filtered, and crude **4** (40 g) was obtained as the cake without further purification. Then**,** to a solution of **4** (30 g) in EA (260 mL) was added excess sodium hydrosulphite portionwise and the mixture was stirred for 40 minutes. Then the mixture was filtered and the organic layer was separated, washed with dilute hydrochloric acid (100 mL × 2) and saturated sodium chloride solution (100 mL × 2), successively and dried over $Na_2SO_4$. The mixture was filtered, concentrated under reduced pressure to afford the crude 5, which was recrystallized with toluene to give **5** as a pale-white solid (14 g, 34%). m.p. 115-117 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 7.43-7.26 (m, 10H, Ar-H), 6.15 (s, 2H, ArH), 5.16 (s, 1H, ArOH), 5.10 (s, 4H, -$OCH_2$Ph), 4.36 (s, 1H, ArOH) ppm. MS-EI: 323 $(M+H)^+$, 322 $(M)^+$.

**1,4-dimethoxy-2,6-dibenzyloxybenzene (6).** To a stirred solution of 2,6-dibenzyloxy-*p*-benzoqunine (**5**) (27g, 0.084 mol) in ethanol (214 mL) was added dimethyl sulfate (20 mL) and then 28% NaOH was added dropwise to adjust pH to 8-9. The reaction mixture was stirred for 2 h at room temperature. After cooling, the suspension was filtered. The precipitate was washed with water to neutral, and dried to obtain **6** as a white solid (25.1 g, 85.5%). $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 7.46-7.26 (m, 10H, ArH), 6.19 (s, 2H, ArH), 5.12 (s, 4H, -$OCH_2$Ph), 3.84 (s, 3H, -$OCH_3$), 3.68 (s, 3H, -$OCH_3$) ppm.

**2,6-dimethoxy-benzene-1,4-diol** (**7**). A mixture of **6** (10 g, 0.029 mol) and Pd/C (10%, 0.43 g) in MeOH (70 mL) was pumped with $H_2$ for 8 h. After the end of the reaction, the solution was filtered through a pad Celite. The filtrate was concentrated to afford **7** as a yellow oil (4.86 g, 99%). Formula: $C_8H_{10}O_4$ MS(EI) m/z:127,155,170$[M]^+$.

**(*E*)-1-(2,4-dihydroxy-3,6-dimethoxyphenyl)-3-(4-methoxyph enyl)prop-2-en-1-one (8a)**. To a stirred solution of *p*-methoxyl ethyl cinnamyl chloride (3.89 g, 0.02 mol) and (**7**) (3.37 g, 0.02 mol) in dry diethyl ether (10 mL) was added $BF_3$•$Et_2O$ (5.1 mL) and the reaction mixture was stirred for 1.5 h at 40 $^oC$. After cooling, the mixture was filtered and the precipitate was washed with methanol (15 mL × 3). Then **8a** was obtained as a red solid (3.2 g, 51%). $^1$H-NMR (300MHz, DMSO-$d_6$): $\delta$ 8.33 (d, 1H, *J* = 15.3 Hz, -CH=CH-CO), 7.91 (d, 1H, *J* = 15.3 Hz, -CH=CH-CO), 7.65 (d, 2H, *J* = 8.7 Hz, Ar-H), 6.98 (d, 2H, *J* = 8.7 Hz, Ar-H), 6.10 (s, 1H, Ar-H), 4.00 (s, 3H, -$OCH_3$), 3.98 (s, 3H, -$OCH_3$), 3.89 (s, 3H, -$OCH_3$) ppm. IR (KBr): 3420 (OH), 2943($CH_3$), 1597(C=O), 1511, 1383, 1242, 1176, 1131, 1018 $cm^{-1}$. MS-EI (m/z) (m/z):331$[M+1]^+$.

**7-Hydroxy-5,8-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (9a).** To a stirred solution of Compound **8** (6.45 g, 0.0195 mol) in dry DMSO (18 mL) was added catalytic amount of $I_2$, and the reaction mixture was heated to 140 $^oC$ for 5 h. Then the mixture was cooled to room temperature, filtered and the precipitate was washed with methanol (8 mL × 3). Then **9a** was obtained as a yellow solid (2.75 g, 35%). m.p. 242-245 $^oC$; $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$7.87 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.04 (d, 2H, *J* = 8.7 Hz, Ar-H), 6.67 (s, 1H, Ar-H), 6.53 (s, 1H, -C=CH), 4.04 (s, 3H, -$OCH_3$), 3.94 (s, 3H, -$OCH_3$ ), 3.89 (s, 3H, -$OCH_3$ ) ppm. IR (KBr): 3441(OH), 3111(C-H, Aromatic), 1647(C=O), 1595(C=C), 1576, 1356, 1253, 1114, 1044, 746 $cm^{-1}$; MS-EI (m/z):285, 313, 328$[M]^+$.

**7-(4-Bromobutoxy)-5,8-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (10a)**. A mixture of **9a** (143 mg, 0.44 mmol), $K_2CO_3$ (361 mg, 2.61 mmol) and 1,4-dibromobutane (0.21 mL, 1.74 mmol) in acetone (71 mL) was refluxed for 8 h. Then the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure, and the residual obtained was slurried in PE (20 mL) to obtain **10a** as a white solid (140 mg, 69.7%). m.p.124-126 $^oC$; $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 7.88 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.02 (d, 2H, *J* = 8.7 Hz, Ar-H),

6.60 (s, 1H, Ar-H), 6.42 (s, 1H, -C=CH), 4.20 (t, 2H, *J* = 5.8 Hz, $BrCH_2$-), 3.97 (s, 3H, -$OCH_3$ ), 3.95 (s, 3H, -$OCH_3$ ), 3.89 (s, 3H, -$OCH_3$ ), 3.55 (t, 2H, *J* = 6.1 Hz, -$CH_2O$-), 2.11 (m, 4H, -$CH_2CH_2CH_2CH_2$-) ppm. IR (KBr): 3441(OH), 2948($CH_3$), 2843($CH_2$), 1649(C=O), 1599(C=C), 1510, 1341, 1258, 1185, 1114, 1046, 831 $cm^{-1}$; MS-EI (m/z): 463$[M-1]^+$, 465$[M+1]^+$;

**7-(4-Bromobutoxy)-5-hydroxy-8-methoxy-2-(4-methoxphen yl )-4H-chromen-4-one (11a)**. To a solution of **10a** (107 mg, 0.23 mmol) in dry acetonitrile (53 mL) was added $AlCl_3$ (245 g, 1.84 mmol). The rection mixture was refluxed for 6 h. After cooling, duilted HCl (5.7 mL, 4 mol/L) was added and the mixture was extracted with DCM (20 mL × 3). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to afford the crude 11a as a yellow solid (100 mg, 97.1%). m.p.185-187 $^oC$; $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 7.92 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.04 (d, 2H, *J* = 8.9 Hz, Ar-H), 6.58 (s, 1H, Ar-H), 6.39 (s, 1H, -C=CH), 4.13 (t, 2H, *J* = 5.9 Hz, $BrCH_2$-), 3.93(s,3H, -$OCH_3$ ), 3.90 (s, 3H, -$OCH_3$ ), 3.53 (t, 2H, *J* = 6.1 Hz, -$CH_2O$-), 2.10 (m, 4H, -$CH_2CH_2CH_2CH_2$-) ppm. MS-EI (m/z): 449$[M]^+$;

**7-(4-(4-Methylpiperazin-1-yl)butoxy)-2-(4-methoxyphenyl)-5-hydroxy-8-methoxy-4H-chromen-4-one (12a).** To a stirred solution of **11a** (120 mg, 0.26 mmol) in anhydrous acetonitrile (60 mL) was added *N*-methylpiperazine (0.15 mL, 0.13 mmol), and the reaction mixture was heated to reflux. After the end of the reaction (monitored by TLC (PE: EA: Triethylamine = 1: 1: 0.01)), the mixture was concentrated under reduced pressure, and purified by chromatography to give 12a as a yellow solid (80 mg, 64 %). m.p. 145 - 147 $^oC$. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.63 (s, 1H, 5-Ar-OH), 7.90 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.04 (d, 2H, *J* = 8.8 Hz, Ar-H), 6.58 (s, 1H, Ar-H), 6.44 (s, 1H, -C = CH), 4.13 (t, 2H, *J* = 6.4, -O-$CH_2$-), 3.92 (s, 3H, -$OCH_3$), 3.90 (s, 3H, -$OCH_3$), 2.80 (m, 10H, -N(($CH_2$)$_2$)$_2$)N-$CH_2$-), 2.53 (s, 3H, -$NCH_3$), 1.92 (m, 2H, -$OCH_2$-$CH_2$-$CH_2$-), 1.78 (t, 2H, -$OCH_2CH_2CH_2CH_2$N-) ppm. IR (KBr): 3427(OH), 2938($CH_2$), 2784, 2678, 2595, 1656(C=O), 1603(C=C), 1507, 1426, 1377, 1263, 1182, 1120, 1029, 835 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{26}H_{33}N_2O_6$ $[M + H]^+$ 469.2333, found 469.2330.

**7-(4-(4-Methylpiperazin-1-yl)butoxy)-2-(4-methylphenyl)-5-hydroxy-8-methoxy-4H-chromen-4-one (12b).** The mixture of **11b** (100 mg, 0.23 mol), anhydrous acetonitrile (50 mL) and *N*-methylpiperazine (100 mg, 0.23 mmol) was heated to reflux under stirring. After the end of the

reaction (monitored by TLC (PE: EA: Triethylamine = 1: 1: 0.01)), the mixture was concentrated under reduced pressure, and purified by chromatography to give **12b** as a yellow solid (50 mg, 48 %). m.p.145-147 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.58 (s, 1H, 5-Ar-OH), 7.84 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.33 (d, 2H, *J* = 8.1 Hz, Ar-H), 6.63 (s, 1H, Ar-H), 6.42 (s, 1H, -C=CH), 4.11 (t, 2H, *J* = 6.2 Hz, -O-$CH_2$-), 3.92 (s, 3H, -$OCH_3$), 2.41-2.44 (m, 13H, N($CH_2CH_2$)$_2$ and Ar$CH_3$), 2.30 (s, 3H, N$CH_3$), 1.90 (m, 2H, N$CH_2CH_2CH_2CH_2$O), 1.71 (m, 2H, N$CH_2CH_2CH_2CH_2$O) ppm. IR (KBr): 3410 (OH), 2932($CH_2$), 2789, 1661(C=O), 1608(C=C), 1586, 1436, 1337, 1277, 1191, 1037, 820 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{26}H_{33}N_2O_5$ $[M + H]^+$ 453.2384, found 453.2387.

**7-(4-(4-Methylpiperazin-1-yl)butoxy)-2-(4-chlorophenyl)-5-hydroxy-8-methoxy-4H-chrome n-4-one (12c).** The mixture of **11c** (102 mg, 0.22 mmol), anhydrous acetonitrile (50 mL) and *N*-methylpiperazine (0.12 mL, 1.1 mmol) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC (PE: EA: Triethylamine = 1: 1: 0.01)), the mixture was concentrated under reduced pressure, and purified by chromatography to give **12c** as a yellow solid (60 mg, 56.6 %). m.p. 195-198 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.49 (s, 1H, ArOH),7.88 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.52 (d, 2H, *J* = 8.7 Hz, Ar-H), 6.64 (s, 1H, Ar-H), 6.46 (s, 1H, -C=CH), 4.14 (t, 2H, *J* = 5.7 Hz, $BrCH_2$-), 3.92 (s, 3H, -$OCH_3$), 2.85 (m, 8H, N($CH_2CH_2$)$_2$N), 2.61 (m, 2H, -$OCH_2CH_2CH_2CH_2$N-), 1.92 (m, 2H, -$OCH_2CH_2CH_2CH_2$N-), 1.79 (m, 2H, -$OCH_2CH_2CH_2CH_2$N-) ppm. IR (KBr): 3441 (OH), 2938 ($CH_2$), 2677, 1658 (C=O), 1612 (C=C), 1586, 1416, 1376, 1337, 1276, 1033, 830 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{25}H_{30}ClN_2O_5$ $[M + H]^+$ 473.1838, found 473.1837.

**7-(4-(4-Methylpiperazin-1-yl)butoxy)-2-(4-(trifluoromethyl)phenyl) -5-hydroxy-8-methoxy-4H-chromen-4-one (12d).** The mixture of **11d** (118 mg, 0.24 mmol), anhydrous acetonitrile (59 mL) and *N*-methylpiperazine (0.13 mL, 1.2 mmol) was heated to reflux under stirring. After the end of the reaction (monitored by TLC (PE: EA: Triethylamine = 1: 1: 0.01)), the mixture was concentrated under reduced pressure, and purified by chromatography to give **12d** as a yellow solid (100 mg, 82.6 %). m.p. 81-83 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.47 (s, 1H, 5-Ar-OH), 8.05 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.80 (d, 2H, *J* = 8.1 Hz, Ar-H), 6.78 (s, 1H, Ar-H), 6.45 (s, 1H, -C=CH), 4.13 (t, 2H, *J* = 6.8 Hz, -O-$CH_2$-), 3.99 (s, 3H, -$OCH_3$), 2.53 (m, 8H, N($CH_2CH_2$)$_2$N), 2.38 (s, 3H, N$CH_3$), 1.90 (m, 2H, N$CH_2CH_2CH_2CH_2$O), 1.71 (m, 4H,

$NCH_2CH_2CH_2CH_2O$) ppm. IR (KBr): 3442 (OH), 2935 ($CH_2$), 2790, 1661 (C=O), 1593 (C=C), 1422, 1289, 1170, 1119, 1032, 839, 806 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{26}H_{30}F_3N_2O_5$ $[M + H]^+$ 507.2101, found 507.2097.

**7-(4-(4-Methylpiperazin-1-yl)butoxy)-2-(4-nitrophenyl)-5-hydroxy-8-methoxy-4H-chromen-4-one (12e)**. The mixture of **11e** (130 mg, 0.28 mmol), anhydrous acetonitrile (65 mL) and *N*-methylpiperazine (0.15 mL, 1.4 mmol) was heated to reflux under stirring. After the end of the reaction (monitored by TLC (PE: EA: Triethylamine = 1: 1: 0.01)), the mixture was concentrated under reduced pressure, and purified by chromatography to give **12e** as a yellow solid (70 mg, 51.8 %). m.p. 80–84 $^oC$. $^1H$-NMR (300 MHz, $CDCl_3$): $\delta$ 12.33 (s, 1H, 5-Ar-OH), 8.41 (d, 2H, $J$ = 8.4 Hz, Ar-H), 8.12 (d, 2H, $J$ = 8.4 Hz, Ar-H), 6.76 (s, 1H, Ar-H), 6.46 (s, 1H, -C=CH), 4.13 (t, 2H, $J$ = 6.4 Hz, -O-$CH_2$-), 3.93 (s, 3H, -$OCH_3$), 2.50 (m, 10H, N($CH_2CH_2$)$_2$$NCH_2$-), 2.34 (s, 3H, $NCH_3$), 1.92 (m, 2H, $NCH_2CH_2CH_2CH_2O$), 1.75 (m, 2H, $NCH_2CH_2CH_2CH_2O$) ppm. IR (KBr): 3426 (OH), 2934 ($CH_2$), 2795, 1655 (C=O), 1604 (C=C), 1523 (N=O), 1345 (N=O), 1114, 1030, 848, 689 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{25}H_{30}N_3O_7$ $[M + H]^+$ 484.2078, found 484.2076.

**7-(4-(4-Methylpiperazin-1-yl)butoxy)-2-(2-methoxyphenyl)-5-hydroxy-8-methoxy-4H-chromen-4-on (12f)**. The mixture of **11f** (70 mg, 0.15 mmol), anhydrous acetonitrile (35 mL) and *N*-methylpiperazine (0.08 mL, 0.75 mmol) was heated to reflux under stirring. After the end of the reaction (monitored by TLC (PE: EA: Triethylamine = 1: 1: 0.01)) , the mixture was concentrated under reduced pressure, and purified by chromatography to give **12f** as a yellow solid (45 mg, 61.7 %). m.p. 82-83 $^oC$. $^1H$-NMR (300 MHz, $CDCl_3$): $\delta$ 12.65 (s, 1H, Ar-H), 7.98 (dd, 1H, $J_1$ = 1.5 Hz; $J_2$ = 7.9 Hz, Ar-H), 7.50 (td, $J_1$ = 1.5 GHz; $J_2$ = 8.0 Hz, 1H, Ar-H), 7.13 (t, 1H, $J$ = 7.5 Hz, Ar-H), 7.07 (m, 2H, Ar-H), 6.41 (s, 1H, -C=CH), 4.11 (t, 2H, $J$ = 6.4, -O-$CH_2$-), 3.95 (s, 3H, -$OCH_3$ ), 3.90 (s, 3H, -$OCH_3$), 2.44 (m, 10H, -N(($CH_2$)$_2$)$_2$)N-$CH_2$-), 2.29 (s, 3H, -$NCH_3$), 1.90 (m, 2H, -$OCH_2$-$CH_2$-$CH_2$-), 1.71 (m, 2H, -$OCH_2$-$CH_2$-$CH_2$-$CH_2$N-) ppm. IR(KBr): 3434 (OH), 2932 ($CH_2$), 2794, 2755, 1657 (C=O), 1609 (C=C), 1583, 1507, 1400, 1376, 1256, 1165, 1131, 1018, 856, 821, 753 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{26}H_{33}N_2O_6$ $[M + H]^+$ 469.2333, found 469.2334.

**7-(4-(4-Methylpiperazin-1-yl)butoxy)-2-(2-fluorophenyl)-5-hydroxy-8-methoxy-4H-chromen-4-on (12g)**.

The mixture of **11g** (1.1 g, 2.5 mmol), anhydrous acetonitrile (100 mL) and *N*-methylpiperazine (1.3 g, 12.6 mmol) was heated to reflux under stirring. After the end of the reaction (monitored by TLC(PE: EA = 2 : 1)), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure, and purified by chromatography to give **12g** as a yellow solid (680 mg, 61.8%). m.p. 195-197 $^{o}$C. $^{1}$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 12.62 (s, 1H, ArOH), 8.15 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.44 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.04 (s, 1H, Ar-H), 6.61 (s, 1H, -C=CH), 4.18-4.15 (m,2H,-$CH_2$-O-Ar), 3.85 (s, 3H, -$OCH_3$), 3.36 (s, 2H, -$OCH_2CH_2CH_2CH_2N$-), 2.35-2.06 (m, 8H, -$N(CH_2CH_2)_2N$-), 2.19-2.13 (m, 3H, -$NCH_3$), 1.91-1.80 (m, 2H, -$OCH_2CH_2CH_2CH_2N$-), 1.61-1.56 (m, 2H, -$OCH_2CH_2CH_2CH_2N$-) ppm. $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 182.2 (-CO-), 165.9 (-O-CH=), 162.6, 158.0, 156.6, 148.8, 129.1, 129.0, 128.6, 127.4, 127.3, 120.3, 104.9, 103.9 (R-O-$C_6$H-OH), 96.7 (=CH-CO-), 68.8 (-O-$CH_2$), 67.2 (-O-$CH_3$), 61.1, 59.3, 56.8, 54.1 (-$N(CH_2CH_2)_2N$-), 51.9, 45.0 (-N-$CH_3$), 26.3, 22.4 (-O-$C_4H_8$-N) ppm. IR(KBr): 3448 (OH), 3128 (C-H, aromatic), 2941 ($CH_2$), 2341, 1661 (C=O), 1637(C=C), 1618(C=C), 1598 (C=C), 1508, 1421, 1400, 1336, 1285, 1235, 1115, 1035, 836, 811, 595 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{25}H_{30}FN_2O_5$ $[M + H]^+$ 457.2133, found 469.2135.

**7-[4-(4-Methylpiperazin-1-yl)butoxy]-2-(4-bromophenyl)-5-hydroxy-8-methoxy-4H-chromen-4-one (12h).** To a stirred solution of **11h** (1.97 g, 4 mmol) in anhydrous acetonitrile (100 mL) was added *N*–methylpiperazine (1.90 g, 20 mmol) with nitrogen passing through the mixture. Then the reaction mixture was heated to reflux. After the end of the reaction (monitored by TLC (PE: EA: = 2: 1)), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure, and purified by chromatography to give **12h** as a yellow solid (1.18 g, 59.0 %). m.p.191-192 $^{o}$C. $^{1}$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 12.57 (s, 1H, ArOH), 8.01 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.83 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.07 (s, 1H, Ar-H), 6.61 (s, 1H, -C=CH), 4.16 (t, 2H, *J* = 6.3 Hz, -$CH_2$-O-Ar), 3.85 (s, 3H, -$OCH_3$), 2.38 (m, 10H, -$N(CH_2CH_2)_2N$-, -$OCH_2CH_2CH_2CH_2N$-), 2.20 (s, 3H, -N-$CH_3$), 1.79 (t, 2H, *J* = 6.6 Hz, -$OCH_2CH_2CH_2CH_2N$-), 1.60 (t, 2H, *J* = 6.8 Hz, -$OCH_2CH_2CH_2CH_2N$-) ppm. $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 182.6 (-CO-), 162.7 (-O-CH=), 158.5, 157.1, 149.3, 132.8, 130.5, 128.7, 126.4(-$C_6H_4$-Br), 105.8, 104.5 (R-O-$C_6$H-OH), 97.3 (=CH-CO-), 69.3(-O-$CH_2$), 61.6 (-O-$CH_3$), 57.5 (-$N(CH_2CH_2)_2N$-), 54.9, 52.7, 45.8 (-N-$CH_3$), 26.8, 22.9 (-O-$C_4H_8$-N) ppm. IR (KBr): 3448 (OH), 3128 (C-H, aromatic), 2933 ($CH_2$), 2361, 1660

(C=O), 1638 (C=C), 1617 (C=C), 1583 (C=C), 1534, 1421, 1400, 1376, 1274, 1224, 1167, 1033, 1010, 825, 810, 583 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{25}H_{30}BrN_2O_5$ $[M + H]^+$ 517.1333, found 524.1123.

**7-[4-(4-Methylpiperazin-1-yl)butoxy]-2-(4-fluoro-2-bromophenyl)-5-hydroxy-8-methoxy-4 H-chromen-4-one (12i).** To a stirred solution of **11i** (1.15 g, 11.1 mol) in anhydrous acetonitrile (60 mL) was added *N*–methylpiperazine (1.07 g, 55.5mmol) with nitrogen passing through the mixture. Then the reaction mixture was heated to reflux. After the end of the reaction (Monitored by TLC (PE: EA: = 2: 1)), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure, and purified by chromatography to give **12i** as a yellow solid (0.73 g, 61.3 %). m.p.173–176 $^oC$. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.86 (s, 2H, Ar-H), 7.52 (s, 1H, Ar-H), 6.66 (s, 1H, Ar-H), 6.58 (s, 1H, -C=CH), 4.17 (s, 2H, -$CH_2$-O-Ar), 3.76 (s, 3H, -$OCH_3$), 2.41 (s, 3H, -N-$CH_3$), 2.29 (s, 10H, -$OCH_2CH_2CH_2CH_2$N-, -N($CH_2CH_2$)$_2$N-), 1.79 (t, 2H, *J* = 6.6 Hz, -$OCH_2CH_2CH_2CH_2$N-), 1.60 (t, 2H, *J* = 6.8 Hz, -$OCH_2CH_2CH_2CH_2$N-) ppm. $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 181.9 (-CO-), 163.6 (-O-CH=), 158.2, 156.7, 149.2, 133.5, 129.9, 128.6, 122.2, 121.0, 115.7 (-$C_6H_4$-Br), 110.5, 103.9, 96.9 (=CH-CO-), 68.8 (-O-$CH_2$), 61.2 (-O-$CH_3$), 56.7, 54.0 (-N($CH_2CH_2$)$_2$N-), 51.3, 45.9 (-N-$CH_3$), 26.2, 22.3 (-O-$C_4H_8$-N) ppm. IR (KBr): 3448 (OH), 3134 (C-H, aromatic), 2724, 2361, 1655 (C=O), 1617 (C=C), 1460, 1400, 1371, 1278, 1224, 1179, 1111, 1002, 853, 553 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{25}H_{29}BrFN_2O_5$ $[M + H]^+$ 535.1238, found 535.1246.

**7-[4-(4-Methylpiperazin-1-yl)butoxy]-2-(3-fluorophenyl)-5-hydroxy-8-methoxy-4H-chrome n-4-one (12j).** To a stirred solution of **11j** (0.93 g, 0.0025 mol) in anhydrous acetonitrile (50 mL) was added *N*–methylpiperazine (1.07 g, 0.0106 mol) with nitrogen passing through the mixture. Then the reaction mixture was heated to reflux. After the end of the reaction (monitored by TLC (PE: EA: = 2: 1)), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure, and purified by chromatography to give **12j** as a yellow solid (0.48 g, 49.5%). m.p.194–195 $^oC$. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 12.54 (s, 1H, ArOH), 7.92 (t, 2H, *J* = 7.6 Hz, Ar-H), 7.67 (t, 1H, *J* = 6.2 Hz, Ar-H), 7.50 (t, 1H, *J* = 6.9 Hz, Ar-H), 7.12 (s, 1H, Ar-H), 6.62 (s, 1H, -C=CH), 4.16 (t, 2H, *J* = 6 Hz, -$CH_2$-O-Ar), 3.86 (s, 3H, -$OCH_3$), 2.45 (m, 10H, -$OCH_2CH_2CH_2CH_2$N-, -N($CH_2CH_2$)$_2$N-), 2.24 (s, 3H, -N-$CH_3$), 1.79 (t, 2H, *J* = 6.6Hz, -$OCH_2CH_2CH_2CH_2$N-), 1.60 (t, 2H,

*J* = 6.8 Hz, -$OCH_2CH_2CH_2CH_2N$-) ppm. $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 181.8 (-CO-), 164.7 (-O-CH=), 161.3, 158.3, 156.7, 149.2, 133.4, 129.9, 122.2, 121.0, 115.6 (-$C_6H_4$-Br), 110.5, 103.9), 96.9 (=CH-CO-), 69.0 (-O-$CH_2$), 61.2 (-O-$CH_3$), 56.6, 54.3 (-$N(CH_2CH_2)_2N$-), 45.9 (-N-$CH_3$), 51.0, 26.3, 23,3 (-O-$C_4H_8$-N) ppm. IR (KBr): 3448 (OH), 3128 (C-H, aromatic), 2935 ($CH_2$), 2787, 2363, 1659 (C=O), 1593 (C=C), 1508, 1400, 1337, 1264, 1227, 1119, 1034, 812, 697 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{25}H_{30}BrFN_2O_5$ $[M + H]^+$ 457.2133, found 457.2127.

**7-(Benzyloxy)-8-(2-(bis(2-hydroxyethyl)amino)ethoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (18a)**. The mixture of **17a** (0.5 g, 1.1 mmol), diethanolamine (1 g), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. The mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 20: 1) to give 18a as a yellow solid (0.25 g, 37.46 %). m.p. 94-96 $^o$C. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.5 (s, 1H, 5-OH), 7.93 (m, 2H, , Ph-H), 7.55 (m, 3H, *J* = 1.65 Hz,Ph-H), 7.44 (m, 5H, Ph-H), 6.65 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 5.22 (s, 2H, $ArOCH_2$ ), 4.12 (t, 2H, *J* = 5.1 Hz,O-$CH_2$), 3.66 (t, 4H, *J* = 5.2 Hz, 2 × $\underline{CH_2}OH$), 3.04 (t, 2H, *J* = 5.1 Hz, N-$CH_2$), 2.77 (t, 4H, *J* = 5.2 Hz ,2 × N-$CH_2$) ppm. IR (KBr): 3395 (OH), 3061 (C-H, aromatic), 3026, 2943 ($CH_2$), 2861 ($CH_2$), 2814, 1660 (C=O), 1610 (C=C), 1586 (C=C), 1508, 1451, 1419, 1374, 1339, 1276, 1226, 1210, 1191, 1123, 1072, 1030, 846, 766, 700 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{28}H_{30}NO_7$ $[M + H]^+$ 492.2017, found 492.2025.

**7-(Bnzyloxy)-5-hydroxy-8-(2-(4-methylpiperazin-1-yl)ethoxy)-2-phenyl-4H-chromen-4-one (18b).** The mixture of **17b** (0.5 g, 1.1 mmol), mthylpiperazine (216 mg, 2.2 mmol), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. The mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 50: 1) to give **18b** as a yellow solid (0. 3 g, 59.76 %).

m.p. 142-144 $^{o}$C; $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$12.55 (s, 1H, 5-OH), 7.95 (m, 2H, Ph-H), 7.55 (m, 3H, Ph-H), 7.43 (m, 5H, Ph-H), 6.67 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 5.17 (s, 2H, $ArOCH_2$), 4.16 (t, 2H, $J$ = 5.7 Hz, O-$CH_2$), 2.84 (t, 2H, $J$ = 5.7 Hz, N-$CH_2$), 2.80 - 2.37 (m, 8H, 4 × N-$CH_2$), 2.2 (s, 3H, N-$CH_3$) ppm. IR: 3464 (OH), 3115 (C-H, aromatic), 2973 ($CH_3$), 2932 ($CH_2$), 2792, 2684, 1655 (C=O), 1613 (C=C), 1585 (C=C), 1503, 1449, 1435, 1372, 1337, 1272, 1201, 1115, 1030, 1009, 816, 695 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{29}H_{31}N_2O_5$ $[M + H]^+$ 487.2227, found 487.2231.

**7-(Benzyloxy)-8-(3-(dimethylamino)propoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (18c).** The mixture of **17c** (0.53 g, 1.05 mmol), dimethylamine hydrochloride (1 g), $K_2CO_3$ (0.6 g, 4.4 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure and purified by chromatography (EA: MeOH = 10: 1) to give 1**8c** as a yellow solid (0. 25 g, 47.40 %). M.p. 208–210 $^{o}$C; $^{1}$H-NMR (300 MHz, DMSO): $\delta$12.65 (s, 1H, 5-OH), 8.09 (m, 2H, Ph-H), 7.83 (m, 3H, Ph-H), 7.53 (m, 5H, Ph-H), 7.05 (s, 1H, Ar-H), 6.82 (s, 1H, Ar-H), 5.30 (s, 2H, $ArOCH_2$ ), 4.23 (t, 2H, $J$ = 5.82 Hz, O-$CH_2$), 3.06 (t, 2H, $J$ = 5.82 Hz, N-$CH_2$), 2.56 (s, 6H, 2×O-$CH_3$) ppm. IR: 3461 (OH), 3124 (C-H, aromatic), 2943 ($CH_2$), 2677, 1663 (C=O), 1610 (C=C), 1509, 1451, 1397, 1374, 1339, 1273, 1227, 1118, 832, 688 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{27}H_{28}NO_5$ $[M + H]^+$ 446.1962, found 446.1969.

**7-(Benzyloxy)-8-(3-(diethylamino)propoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (18d)**. The mixture of **17d** (0.53 g, 1.1 mmol), diethylamine (0.15 g, 2.2 mmol), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure and purified by chromatography (EA: MeOH = 20: 1) to give **18d** as a yellow solid (0. 25 g, 52.32 %). m.p. 172–173 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$12.6 (s, 1H, 5-OH), 7.89 (m, 2H, Ph-H), 7.58 (m, 3H, Ph-H), 7.46 (m, 5H, Ph-H), 6.67 (s, 1H, Ar-H), 6.54 (s, 1H, Ar-H), 5.11 (s, 2H, $ArOCH_2$), 4.14 (t, 2H, $J$ = 5.01 Hz, O-$CH_2$), 3.13 (m, 6H, 3 × N-$CH_2$), 2.38 (m, 2H, O-$CH_2$-$\underline{CH_2}$), 1.45 (t, 6H, $J$ = 2.67 Hz, 2 × $CH_2$-$\underline{CH_3}$) ppm. IR (KBr): 3416 (OH), 3221, 3061 (C-H, aromatic), 2976 ($CH_3$), 2943 ($CH_2$), 2737, 2652, 2489, 1656 (C=O), 1616 (C=C), 1506, 1450, 1399, 1374, 1340, 1272, 1221, 1121, 1044, 1010, 809, 770, 694, 640 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{29}H_{32}NO_5$ $[M + H]^+$ 474.2275, found 474.2287.

**7-(Benzyloxy)-8-(3-(bis(2-hydroxyethyl)amino)propoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (18e).** The mixture of **17e** (0.53 g, 1.1 mmol), diethanolamine (1 g), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. Then the mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 20: 1) to give **18e** as a yellow solid (0.3 g, 55.22 %). m.p. 125–126 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.55 (s, 1H, 5-OH), 7.93 (m, 2H, Ph-H), 7.56 (m, 3H, Ph-H), 7.54 (m, 5H, Ph-H), 6.61 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 5.19 (s, 2H, $ArOCH_2$), 4.12 (t, 2H, $J$ = 6.00 Hz, O-$CH_2$), 3.39 (t, 4H, $J$ = 6.6 Hz, 2 × HO-$\underline{CH_2}$), 2.01 (m, 6H, 3 × N-$\underline{CH_2}$) ppm. IR (KBr): 3469 (OH), 3162, 3073 (C-H, aromatic), 3038, 2958 ($CH_2$), 2873 ($CH_2$), 2779, 1660 (C=O), 1613 (C=C), 1587, 1508, 1450, 1717, 1389, 1374, 1333, 1226, 1208, 1187, 1118, 1030, 1017, 1006, 839, 807, 698, 684 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{29}H_{32}NO_7$ $[M + H]^+$ 506.2173, found 506.2172.

**7-(Benzyloxy)-5-hydroxy-2-phenyl-8-(3-(pyrrolidin-1-yl)propoxy)-4H-chromen-4-one (18f).** The mixture of **17f** (0.53 g, 1.1 mmol), pyrrolidine (150 mg, 2.2 mmol), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100 mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. The mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 30: 1) to give **18f** as a yellow solid (0.43 g, 81.22 %). m.p. 186–188 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.54 (s, 1H, 5-OH), 7.95 (m, 2H, Ph-H), 7.55 (m, 3H, Ph-H), 7.45 (m, 5H, Ph-H), 6.67 (s, 1H, Ar-H), 6.4 (s, 1H, Ar-H), 5.19 (s, 2H, $ArOCH_2$), 4.13 (t, 2H, $J$ = 6.3 Hz, O-$CH_2$), 2.71 (t, 2H, $J$ = 6.3 Hz, N-$CH_2$), 2.47 (br, 4H, 2 × N-$\underline{CH_2}$), 2.09 (m, 2H, O-$CH_2$-$\underline{CH_2}$-$CH_2$), 1.76 (br, 4H, N-$CH_2$-$\underline{CH_2}$-$\underline{CH_2}$) ppm. IR (KBr): 3469 (OH), 3162, 3073 (C-H, aromatic), 3038, 2958 ($CH_2$), 2873 ($CH_2$), 2779, 1660 (C=O), 1613 (C=C), 1587, 1508, 1450, 1389, 1374, 1272, 1226, 1208, 1187, 1118, 1030, 1017, 1006, 839, 807, 765, 755 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{29}H_{30}NO_5$ $[M + H]^+$ 472.2118, found 472.2125.

**7-(Benzyloxy)-5-hydroxy-8-(3-morpholinopropoxy)-2-phenyl-4H-chromen-4-one (18g).** The mixture of **17g** (0.53 g, 1.1 mmol), morpholine (186 mg, 2.2 mmol), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. Then the mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 20: 1) to give **18g** as a yellow solid (0. 46 g, 83.42 %). m.p. 144–146 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.54 (s, 1H, 5-OH), 7.94 (m, 2H, Ph-H), 7.55 (m, 3H, Ph-H), 7.47 (m, 5H, Ph-H), 6.72 (s, 1H, Ar-H), 6.67 (s, 1H, Ar-H), 5.17 (s, 2H, $ArOCH_2$), 4.12 (t, 2H, *J* = 6.1 Hz, O-$CH_2$), 3.68 (t, 4H, *J* = 6.3 Hz, 2 × O-$CH_2$), 2.58 (t, 2H, N-$\underline{CH_2}$, *J* = 10.1 Hz), 2.39 (br, 4H, 2 × N-$\underline{CH_2}$), 2.04 (m, 2H, O-$CH_2$-$\underline{CH_2}$-$\underline{CH_2}$) ppm. IR (KBr): 3461 (OH), 3115(C-H, aromatic), 2954 ($CH_2$), 2879, 2867 ($CH_2$), 2813, 2761, 1661 (C=O), 1613 (C=C), 1587, 1451, 1419, 1374, 1274, 1230, 1210, 1189, 1117, 1029, 1006, 999, 766, 757 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{29}H_{30}NO_6$ $[M + H]^+$ 488.2068, found 488.2068.

**7-(Benzyloxy)-5-hydroxy-8-(3-(4-methylpiperazin-1-yl)propoxy)-2-phenyl-4H-chromen-4-one (18h).** The mixture of **17h** (0.53 g, 1.1 mmol), ethylpiperazine (216 mg, 2.2 mmol), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. Then the mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 20: 1) to give **18h** as a yellow solid (0.40 g, 69.33 %). m.p. 161–163 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.47 (s, 1H, 5-OH), 7.94 (m, 2H, Ph-H), 7.55 (m, 3H, Ph-H), 7.44 (m, 5H, Ph-H), 6.67 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 5.18 (s, 2H, $ArOCH_2$), 4.13 (t, 2H, *J* = 6.3 Hz, O-$CH_2$), 2.62 (t, 2H, *J* = 7.2 Hz, N-$CH_2$), 2.57 (br, 6H, *J* = 10.1 Hz, 3 × N-$\underline{CH_2}$), 2.30 (s, 3H, N-$\underline{CH_3}$), 2.04 (m, 2H, O-$CH_2$-$\underline{CH_2}$-$\underline{CH_2}$) ppm. IR (KBr): 3472 (OH), 3116 (C-H, aromatic), 2933 ($CH_2$), 2785, 1663 (C=O), 1614 (C=C), 1587, 1508, 1451, 1375, 1336, 1274, 1228,

1187, 1166, 1031, 1008, 998, 764, 638 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{30}H_{33}N_2O_5$ $[M + H]^+$ 501.2384, found 501.2390.

**7-(Benzyloxy)-8-(4-(dimethylamino)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (18i).** The mixture of **17i** (0.55 g, 1.1 mmol), dimethylamine hydrochloride (1 g), $K_2CO_3$ (0.6 g, 4.4 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure and purified by chromatography (EA: MeOH = 10: 1) to give **18i** as a yellow solid (0. 29 g, 56.22 %). m.p. 138–140 $^oC$. $^1H$-NMR (300 MHz, $CDCl_3$): $\delta$12.54 (s, 1H, 5-OH), 7.93 (m, 2H, Ph-H), 7.56 (m, 3H, Ph-H), 7.46 (m, 5H, Ph-H), 6.67 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 5.17 (s, 2H, $ArOCH_2$), 4.08 (t, 2H, $J$ = 5.4 Hz, O-$CH_2$), 2.50 (m, 2H, N-$CH_2$), 2.27 (s, 6H, 2 × N-$CH_3$), 1.83 (m, 4H, O-$CH_2$-$\underline{CH_2}$-$\underline{CH_2}$) ppm. IR (KBr): 3453 (OH), 3061 (C-H, aromatic), 2943 ($CH_2$), 2861 ($CH_2$), 2808, 2755, 1661 (C=O), 1613 (C=C), 1587, 1507, 1450, 1419, 1374, 1335, 1273, 1226, 1209, 1188, 1119, 1031, 1008, 998, 807, 765, 699, 685 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{30}H_{33}N_2O_5$ $[M + H]^+$ 460.2118, found 460.2123.

**8-(4-(Dimethylamino)butoxy)-5,7-dihydroxy-2-phenyl-4H-chromen-4-one (18j).** The mixture of **17j** (0.55 g, 1.1 mmol), diethylamine (0.15 g, 2.2 mmol), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure and purified by chromatography (EA: MeOH = 20: 1) to give **18j** as a yellow solid (0.3 g, 56.86 %). m.p. 125-126 $^oC$. $^1H$-NMR (300 MHz, $CDCl_3$): $\delta$12.52 (s, 1H, 5-OH), 7.95 (m, 2H, Ph-H), 7.55 (m, 3H, Ph-H), 7.46 (m, 5H, Ph-H), 6.67 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 5.19 (s, 2H, $ArOCH_2$ ), 4.08 (t, 2H, $J$ = 5.43 Hz, O-$CH_2$), 2.50 (m, 6H, 3 × N-$CH_2$), 1.85 (m, 2H, O-$CH_2$-$\underline{CH_2}$-$CH_2$), 1.65 (m, 4H, O-$CH_2$-$CH_2$-$\underline{CH_2}$) ppm. IR: 3455 (OH), 3109 (C-H, aromatic), 2966 ($CH_3$), 2941 ($CH_2$), 2867 ($CH_2$), 2790, 1662 (C=O), 1613 (C=C), 1587, 1507, 1450, 1374, 1336, 1274, 1229, 1211, 1188, 1118, 1030, 1008, 999, 837, 807, 765, 700 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{30}H_{34}NO_5$ $[M + H]^+$ 488.2431, found 488.2436.

**7-(Benzyloxy)-8-(4-(bis(2-hydroxyethyl)amino)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (18k)**. The mixture of **17k** (0.55 g, 1.1 mmol), diethanolamine (1 g), $K_2CO_3$ (0.3 g, 2.2 mmol)

and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. Then the mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 10: 1) to give **18k** as a yellow solid (0. 36 g, 63.42 %). m.p. 111–113 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.52 (s, 1H, 5-OH), 7.92 (m, 2H, Ph-H), 7.56 (m, 3H, Ph-H), 7.46 (m, 5H, Ph-H), 6.67 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 5.19 (s, 2H, $ArOCH_2$ ), 4.08 (t, 2H, *J* = 5.8 Hz, O-$CH_2$), 3.61 (t, 4H, *J* = 5.3 Hz, 2 × $\underline{CH_2}$-OH), 2.61 (m, 6H, 3 × N-$CH_2$), 2.13 (br, 2H, 2 × $CH_2$-$\underline{OH}$), 1.82 (m, 2H, O-$CH_2$-$\underline{CH_2}$-$CH_2$), 1.73 (m, 2H, O-$CH_2$-$CH_2$-$\underline{CH_2}$) ppm. IR (KBr): 3143 (C-H, aromatic), 2958 ($CH_2$), 1654 (C=O), 1579, 1400, 1275, 1191, 1101, 1010, 841, 767, 674, 558 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{30}H_{34}NO_7$ $[M + H]^+$ 520.2330, found 520.2336.

**7-(Benzyloxy)-5-hydroxy-2-phenyl-8-(4-(pyrrolidin-1-yl)butoxy)-4H-chromen-4-one (18l)**. The mixture of **17l** (0.55 g, 1.1 mmol), pyrrolidine (150 mg, 2.2 mmol), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. The mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 10: 1) to give **18l** as a yellow solid (0. 43 g, 81.63 %). m.p. 132–134 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.53 (s, 1H, 5-OH), 8.22 (m, 2H, Ph-H), 7.93 (m, 3H, Ph-H), 7.46 (m, 5H, Ph-H), 6.67 (s, 1H, Ar-H), 6.48 (s, 1H, Ar-H), 5.19 (s, 2H, $ArOCH_2$), 4.09 (t, 2H, *J* = 6.4 Hz, O-$CH_2$), 2.46 (m, 6H, 3 × N-$CH_2$), 1.89 (m, 2H, $CH_2$-$\underline{CH_2}$), 1.75 (br, 6H, 3 × $CH_2$- $\underline{CH_2}$) ppm. IR (KBr): 3469 (OH), 3162, 3097 (C-H, aromatic), 3032, 2946 ($CH_2$), 2873 ($CH_2$), 2772, 2678, 1662 (C=O), 1613 (C=C), 1587, 1507, 1451, 1420, 1374, 1335, 1273, 1228, 1211, 1188, 1030, 1008, 998, 837, 808, 765, 699 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{30}H_{32}NO_5$ $[M + H]^+$ 486.2275, found 486.2285.

**7-(Benzyloxy)-5-hydroxy-8-(4-morpholinobutoxy)-2-phenyl-4H-chromen-4-one (18m)**. The mixture of **17m** (0.55 g, 1.1 mmol), morpholine (186 mg, 2.2 mmol), $K_2CO_3$ (0.3 g, 2.2 mmol) and

anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. The mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 10: 1) to give **18m** as a yellow solid (0.45 g, 82.56 %). m.p. 136–138 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.53 (s, 1H, 5-OH), 7.94 (m, 2H, *J* = 5.8 Hz, Ph-H), 7.53 (m, 3H, *J* = 5.8 Hz, Ph-H), 7.46 (m, 5H, *J* = 1.7 Hz, Ph-H), 6.67 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 5.19 (s, 2H, $ArOCH_2$), 4.09 (t, 2H, *J* = 6.1 Hz, O-$CH_2$), 3.67 (t, 4H, *J* = 4.4 Hz, 2 × O-$CH_2$), 2.35 (m, 6H, 3 × N-$CH_2$), 1.87 (m, 2H, O-$CH_2$-$\underline{CH_2}$-$CH_2$), 1.75 (m, 2H, *J* = 5.8 Hz, O-$CH_2$ -$CH_2$-$\underline{CH_2}$) ppm. IR (KBr): 3463 (OH), 3097 (C-H, aromatic), 3026, 2946 ($CH_2$), 2867 ($CH_2$), 2802, 2761, 1661 (C=O), 1613 (C=C), 1587, 1507, 1450, 1419, 1373, 1335, 1274, 1230, 1214, 1187, 1116, 1029, 1007, 999, 865, 806, 765, 701, 685 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{30}H_{32}NO_6$ $[M + H]^+$ 502.2224, found 502.2225.

**7-(Benzyloxy)-5-hydroxy-8-(4-(4-methylpiperazin-1-yl)butoxy)-2-phenyl-4H-chromen-4-one (18n).** The mixture of **17n** (0.55 g, 1.1 mmol), methylpiperazine (216 mg, 2.2 mmol), $K_2CO_3$ (0.3 g, 2.2 mmol) and anhydrous acetonitrile (25 mL) was heated to reflux under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately, and the filtrate was concentrated under reduced pressure. The residual obtained was dissolved in DCM (100mL), washed by water (50 mL × 3) and saturated sodium chloride solution (50 mL × 3), successively, and dried over anhydrous sodium sulfate. The mixture was filtered, concentrated under reduced pressure and purified by chromatography (EA: MeOH = 20: 1) to give **18n** as a yellow solid (0. 41 g, 72.75 %). m.p. 166–168 $^{o}$C. $^{1}$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.53 (s, 1H, 5-OH), 7.94 (m, 2H, Ph-H), 7.53 (m, 3H, Ph-H), 7.43 (m, 5H, Ph-H), 6.67 (s, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 5.19 (s, 2H, $ArOCH_2$), 4.09 (t, 2H, *J* = 6.2 Hz, O-$CH_2$), 2.42 (m, 8H, 4 × N-$CH_2$), 2.28 (s, 3H, N-$CH_3$), 1.87 (m, 2H, O-$CH_2$-$\underline{CH_2}$-$CH_2$), 1.75 (m, 2H, O-$CH_2$-$CH_2$-$\underline{CH_2}$) ppm. IR (KBr): 3451 (OH), 3097 (C-H, aromatic), 3056, 3032, 2935 ($CH_2$), 2861 ($CH_2$), 2789, 1661 (C=O), 1613 (C=C), 1587, 1507, 1450, 1374, 1333, 1273, 1225, 1187, 1165, 1119, 1008, 997, 837, 805, 764, 701, 684 cm$^{-1}$. ESI-HRMS m/z calculated for $C_{30}H_{35}N_2O_5$ $[M + H]^+$ 515.2540, found 515.2544.

**7-(4-Methyl-benzyloxy)-8-(4-(4-methylpiperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (20a).** The mixture of **19a** (0.21 g, 0.5 mmol), 2-chloromethylfuran (0.08 g, 0.6 mmol), $K_2CO_3$ (0.17 g, 1.3 mmol) and anhydrous acetonitrile (22 mL) was heated to 60 $^oC$ under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately to remove $K_2CO_3$, and the filtrate was concentrated under reduced pressure and purified by chromatography (DCM: MeOH = 30: 1) to give **20a** as a yellow solid (0.1 g, 20 %). m.p. 104 - 106 $^oC$. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.54 (s, 1H, Ar-OH), 7.93 (q, 2H, *J* = 2.4 Hz, Ph-H), 7.57 (t, 2H, *J* = 2.4 Hz, Ph-H), 7.33 (d, 2H, Ar-H), 7.24 (t, 2H, Ar-H), 6.68 (s, 1H, -C=CH), 6.50(s, 1H, -C=CH), 5.15 (s, 2H, O-CH2-Ar), 4.08 (t, 2H, O-$CH_2$-R), 2.60 (s, 8H, N-$(CH_2CH_2)_2$-N), 2.39 (s, 6H, $CH_3$-N/$CH_3$-Ar) ppm. $^{13}$C-NMR (75 MHz, $CDCl_3$): $\delta$ 182.1 (-CO-), 163.4 (-O-C=), 156.8, 138.0, 131.4, 128.9, 127.6, 127.1, 126.9, 125.8, 104.9, 96.7 (=CH-CO-), 80.5, 76.9, 76.5, 76.0 (N-$(CH_2CH_2)_2$-N), 73.4, 70.5, 57.4, 46.1 (N-$(CH_2)_4$-O) ppm. IR (KBr): 3414 (OH), 3075 (C-H, aromatic), 2931 ($CH_2$), 2750, 1655 (C=O), 1617 (C=C), 1452, 1374, 1333, 1274, 1181, 1107, 1014, 811, 692, 579. ESI-HRMS m/z calculated for $C_{32}H_{37}N_2O_5$ $[M + H]^+$ 529.2697, found 529.2718.

**7-(4-Cyano-benzyloxy)-8-(4-(4-methylpiperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (20b).** The mixture of **19b** (0.21 g, 0.5 mmol), 4-nitrobenzyl bromide (0.21 g, 0.6 mmol), $K_2CO_3$ (0.29 g, 1.3 mmol) and anhydrous acetonitrile (22 mL) was heated to 55 $^oC$ under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately to remove $K_2CO_3$, and the filtrate was concentrated under reduced pressure and purified by chromatography (DCM: MeOH = 30: 1) to give **20b** as a yellow solid (0.13 g, 43 %). m.p. 116–118 $^oC$. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.56 (s, 1H, Ar-OH), 7.94 (q, 2H, *J* = 2.9 Hz, Ph-H), 7.55 (t, 3H, *J* = 2.9 Hz, Ph-H), 7.48 (d, 1H, Ar-H), 6.69 (s, 1H, -C=CH), 6.57 (s, 1H, -C=CH), 6.51 (d, 1H, Ar-H), 6.41 (q, 1H, Ar-H), 5.15 (s, 2H, O-$CH_2$), 4.05 (t, 2H, N-$CH_2$), 2.34 (s, 3H, N-$CH_3$) ppm. $^{13}$C-NMR (75 MHz, $CDCl_3$): $\delta$ 182.2 (-CO-), 163.4 (-O-C=), 156.9, 156.6, 142.9, 131.4, 130.9, 128.6, 125.8, 110.2, 110.1, 104.9, 96.8 (=CH-CO-), 76.9, 76.5, 76.1, 73.6 (N-$(CH_2CH_2)_2$-N), 62.7 (O-$CH_2$-Ar), 57.6, 54.4, 45.8 (-N-$CH_3$), 27.7, 22.8 (N-$(CH_2)_4$-O) ppm. IR (KBr): 3568, 3432 (OH), 3118 (C-H, aromatic), 2937 ($CH_2$), 2792, 1654 (C=O), 1617 (C=C), 1589, 1451, 1400, 1374, 1334, 1109, 1004, 918, 683, 601, 568 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{32}H_{34}N_3O_5$ $[M + H]^+$ 540.2493, found 540.2508.

**7-(Furan-2-ylmethoxy)-8-(4-(4-methylpiperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (20c).** The mixture of **19c** (0.42 g, 1.0 mmol), 2-chloromethylfuran (0.11 g, 1.3 mmol), $K_2CO_3$ (0.29 g, 3.0 mmol) and anhydrous acetonitrile (25 mL) was heated to 60 $^oC$ under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately to remove $K_2CO_3$, and the filtrate was concentrated under reduced pressure and purified by chromatography (DCM: MeOH = 30: 1) to give **20c** as a yellow solid (0.1 g, 20 %). m.p.165–167 $^oC$. $^1H$-NMR (300 MHz, $CDCl_3$): $\delta$ 12.56 (s, 1H, Ar-OH), 7.94 (q, 2H, *J* = 2.9 Hz, Ph-H), 7.55 (t, 3H, *J* = 2.9 Hz, Ph-H), 7.48 (d, 1H, Ar-H), 6.69 (s, 1H, -C=CH), 6.57 (s, 1H, -C=CH), 6.51 (d, 1H, Ar-H), 6.41 (q, 1H, Ar-H), 5.15 (s, 2H, O-$CH_2$), 4.05 (t, 2H, N-$CH_2$), 2.34 (s, 3H, N-$CH_3$) ppm. $^{13}C$-NMR (75 MHz, $CDCl_3$): $\delta$182.2 (-CO-), 163.4 (-O-C=), 156.9, 156.6, 142.9, 131.4, 130.9, 128.6, 125.8, 110.2, 110.1 (Ar-C), 104.9 (-$C_6H_5$), 96.8 (=CH-CO-), 76.9, 76.5, 76.1, 73.6 (N-($CH_2CH_2$)$_2$-N), 62.7, 57.6, 54.4, 45.8 (-N-$CH_3$), 27.7, 22.8 (N-$(CH_2)_4$-O) ppm. IR (KBr): 3568, 3432 (OH), 3118 (C-H, aromatic), 2937 ($CH_2$), 2792, 1654 (C=O), 1617 (C=C), 1589, 1451, 1400, 1374, 1334, 1109, 1004, 918, 683, 601, 568 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{29}H_{33}N_2O_6$ $[M + H]^+$ 505.2333, found: 505.2347.

**7-(Thiophen-2-ylmethoxy)-8-(4-(4-methylpiperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (20d).** The mixture of **19d** (0.13 g, 1.0 mmol), 2-chloromethyl thiophene (0.13 g, 1.0 mmol), $K_2CO_3$ (0.29 g, 3.0 mmol) and anhydrous acetonitrile (20 mL) was heated to 50 $^oC$ under stirring. After the end of the reaction (monitored by TLC), the mixture was filtered immediately to remove $K_2CO_3$, and the filtrate was concentrated under reduced pressure and purified by chromatography (DCM: MeOH = 30: 1) to give **20d** as a yellow solid (0.22 g, 25 %). m.p. 171 – 173 $^oC$. $^1H$-NMR (300 MHz, $CDCl_3$): $\delta$ 12.54 (s, 1H, Ar-OH), 7.92 (m, 2H, Ph-H), 7.54 (m, 3H, Ph-H), 7.35 (q, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 7.02 (q, 1H, Ar-H), 6.67 (s, 1H, -C=CH), 6.52 (s, 1H, -C=CH), 5.34 (s, 2H, O-$CH_2$-Ar), 4.06 (t, 2H, O-$CH_2$), 2.53 (d, 8H, -N-($CH_2CH_2$)$_2$-N-), 2.46 (d, 2H, N-$CH_2$ ), 2.34 (s, 3H, N-$CH_3$), 1.83 (t, 2H, N-$(CH_2)_4$-O), 1.76 (t, 2H, N-$(CH_2)_4$-O) ppm. $^{13}C$-NMR (75 MHz, $CDCl_3$): $\delta$ 182.7 (-CO-), 164.0 (-O-C=), 157.3, 157.2, 138.0, 131.9, 131.4, 129.1 (R-O-C6H-OH), 127.3, 126.7, 126.3 (($CH_2CH_2$)$_2$-S), 105.4 (-$C_6H_5$), 97.2 (=CH-CO-), 77.4, 77.0, 76.5, 74.0 (N-($CH_2CH_2$)$_2$-N), 65.9 (O-$CH_2$-Ar), 58.0, 54.6, 45.7 (-N-$CH_3$), 28.2 (N-$(CH_2)_4$-O) ppm. IR (KBr): 3423 (OH), 3133 (C-H, aromatic), 1663 (C=O), 1617 (C=C) $cm^{-1}$. ESI-HRMS m/z calculated for $C_{29}H_{33}N_2O_5S$ $[M + H]^+$ 521.2105, found: 521.2139.

**7-(Benzo[d]oxazol-2-ylmethoxy)-8-(4-(4-methylpiperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (20e).** The mixture of **19e** (0.41 g, 1.0 mmol), 2-chloromethylbenzoxazole (0.18 g, 1.1 mmol), $K_2CO_3$ (0.29 g, 3.0 mmol) and anhydrous acetonitrile (20 mL) was heated to 50 $^oC$ under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately to remove $K_2CO_3$, and the filtrate was concentrated under reduced pressure and purified by chromatography (DCM: MeOH = 30: 1) to give **20e** as a yellow solid (0.21 g, 20 %). m.p. 178–180 $^oC$. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.54 (s, 1H, Ar-OH), 7.92 (m, 2H, Ph-H), 7.77 (m, 1H, Ar-H), 7.56 (q, 3H, *J* = 5.9 Hz, Ph-H), 7.39 (m, 2H, Ar-H), 6.68 (s, 1H, -C=CH), 6.60 (s, 1H, -C=CH), 5.44 (s, 2H, O-$CH_2$-Ar), 4.12 (t, *J* = 5.8 Hz, 2H, O-$CH_2$), 2.42 (t, *J* = 5.8 Hz, 8H, N-$(CH_2CH_2)_2$-N), 2.37 (s, 2H, N-$CH_2$), 2.30 (s, 3H, N-$CH_3$), 1.85 (t, 4H, N$(CH_2)_4$-O), 1.73 (t, 4H, N$(CH_2)_4$-O) ppm. $^{13}$C-NMR (75 MHz, $CDCl_3$): $\delta$ 182.7 (-CO-), 164.1 (-O-C=), 160.1, 157.2, 156.9, 150.9, 149.9, 140.7, 132.0, 131.3, 126.3, 124.8, 120.6 (Ar-C), 106.0 (-$C_6H_5$), 97.3(=CH-CO-), 77.4, 77.0, 76.6, 74.4 (N-$(CH_2CH_2)_2$-N), 63.6 (O-$CH_2$-Ar), 58.1, 54.8, 45.8 (-N-$CH_3$), 28.2, 23.3 (N-$(CH_2)$4-O) ppm. IR (KBr): 3432 (OH), 3134 (C-H, aromatic), 2941 ($CH_2$), 2790, 1659 (C=O), 1617 (C=C), 1588, 1451, 1399, 1382, 1336, 1275, 1125, 1115 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{32}H_{34}N_3O_6$ $[M + H]^+$ 555.2442, found: 556.2458.

**7-(Benzo[d]thiazol-2-ylmethoxy)-8-(4-(4-methylpiperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (20f).** The mixture of **19f** (0.41 g, 1.0 mmol), 2-chloromethylbenzothiazole (0.19 g, 1.1 mmol), $K_2CO_3$ (0.29 g, 3.0 mmol) and anhydrous acetonitrile (20 mL) was heated to 50 $^oC$ under stirring. After the end of the reaction (Monitored by TLC), the mixture was filtered immediately to remove $K_2CO_3$, and the filtrate was concentrated under reduced pressure and purified by chromatography (DCM: MeOH = 30: 1) to give **20f** as a yellow solid (0.22 g, 21 %). m.p. 181–183 $^oC$. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 12.55 (s, 1H, Ar-OH), 8.05 (d, 1H, *J* = 8.3 Hz, Ar-H), 7.94 (m, 2H, Ph-H), 7.54 (m, 3H, Ph-H), 7.49 (m, 1H, Ar-H), 6.69 (s, 1H, -C=CH), 6.54 (s, 1H, -C=CH), 5.59 (s, 2H, O-$CH_2$-Ar), 4.16 (t, 2H, O-$CH_2$), 2.46 (d, 8H, N-$(CH_2CH_2)_2$-N), 2.31 (s, 3H, N-$CH_3$) ppm. $^{13}$C-NMR (75 MHz, $CDCl_3$): $\delta$ 182.7 (-CO-), 166.5 (-O-C=), 164.1, 157.3, 156.7, 152.8, 132.0, 131.3, 129.2, 126.4, 126.3, 125.5, 123.3, 121.8, 105.9, 105.6 (Ar-C), 97.2 (=CH-CO-), 77.4, 77.0, 76.5, 74.4 (N-$(CH_2CH_2)_2$-N), 68.6, 58.2, 54.8, 45.8 (-N-$CH_3$), 28.3, 23.4 (N-$(CH_2)_4$-O)

ppm; IR (KBr): 3433 (OH), 3134 (C-H, aromatic), 1657 (C=O), 1617 (C=C), 1450, 1400, 1377, 1335, 1124 $cm^{-1}$. ESI-HRMS m/z calculated for $C_{32}H_{34}N_3O_5S$ $[M + H]^+$ 572.2214, found: 572.2234.

*5.2. Biological experiments.*

*5.2.1. In vitro antitumor assays.*

Cell lines of different histological origins were cultivated according to the manufacturer's instructions. The cells were assayed with compounds using different concentrations. The plates were incubated at 37 $^oC$ in 5% $CO_2$ for 24 h. After that, compounds exposure for different cells at 72 h. Cell viability was determined based on mitochondrial conversion of MTT to formazan. The absorbance (A) was determined at 570 nm. $IC_{50}$ was taken as the concentration that caused 50% inhibition of cell viability and calculated by the Logit method.

The activity of the compounds in tumoral cells was classified according to $IC_{50}$ as: High (< 5 μM), Good (5-10 μM), moderate (11-25 μM), Low (> 26 μM).

*5.2.2. Measurement of intracellular ROS levels.*

Intracellular ROS was analyzed from the transformation of nonfluorescent DCFH-DA to its fluorescent derivative. HepG2 cells were seeded at $6 \times 10^4$ cells/well in 12-well plates and cultured for 24 h, followed by incubation with compounds **18n** and **20b** (2, 4, 6 μM) for 24 h, the cells were washed with PBS and serum-free medium and then incubated with serum-free DCFH-DA solution (Sigma) at 37 $^oC$ for 30 min. After harvest, the cell was washed with cold serum-free meiym for two times, added 200 mL serum-free medium, transferred into black with clear bottom 96-well plates (Corning 3603) and measured using flow cytometry (FACSCalibur, Becton Dickinson), and was analyzed by the software Modfit and CellQuest (BD Biosciences, Franklin Lakes, NJ) with settings at excitation and emission equal to 488/525nm.

*5.2.3. Apoptosis assays.*

Apoptosis-mediated cell death of tumor cell was examined using a double staining method with FITC-labelled Annexin V/PI Apoptosis Detection kit (Biovision, CA) according to the manufacturer's instructions. Flow cytometric analysis was performed 10-15 min after supravital staining. Data acquisition and analysis were performed in a Becton Dickinson FACSCalibur flow

cytometer using CellQuest software (BD Biosciences, Franklin Lakes, NJ). The lower left section of fluorocytogram (Annexin V-, PI-) represents the normal cells, lower right section of fluorocytogram (Annexin V+, PI-) represents early and median apoptosis cells, upper right section of fluorocytogram (An+, PI+) represents late apoptosis cells.

*5.2.4. Kinase Inhibition Assays.*

Kinase inhibition profiles were determined using KinaseProfiler services provided by Kaiji Corp., and ATP concentrations used are the Km of corresponding kinases.

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## Highlights

- A series of novel flavonoids were synthesized and evaluated as potent antitumor compounds.
- Compounds **18n** and **20b** exhibited potent antitumor activity against several cancer cells.
- Compounds **18n** and **20b** could significantly enhance the intracellular ROS level and induce the cell apoptosis.
- Through similarity searching, CDK9 was identified as the potential target for **20b**, which could be a start point for next drug design.