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RESEARCH ARTICLE

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Design, synthesis, and primary activity assays of baicalein derivatives as cyclin-dependent kinase 1 inhibitors

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Abstract

Malignant tumor is a disease with high mortality. Traditional treatment methods have many disadvantages, such as side-effects, drug resistance. Because cyclin-dependent kinase 1 (CDK1) plays an indispensable role in cell cycle regulation, it became an attractive target in rational anti-cancer drug discovery. Herein, we reported a series of baicalein derivatives, which remarkably repressed the proliferation of MCF-7 tumor cells and the activity of CDK1/cyclin B kinase. Among them, compound **4a** displayed better inhibition rate than flavopiridol against MCF-7 proliferation at the concentration of 50 μg/ml, comparable to compound CGP74514A, while compound **3o** possessed the best activity against CDK1/cyclin B kinase (IC_{50} = 1.26 μM). The inhibitory activities toward the kinase well correlated with anti-proliferative activities. Molecular docking results suggested that compound **3o** can interact with the key amino acid residues, E81, L83, and D146, of CDK1 through hydrogen bond just like flavopiridol does. And it can also form an extra hydrogen bond with D146 by its introduced 7-acrylate group, which flavopiridol does not have. These findings proved that baicalein derivatives can be used as CDK1 inhibitors fighting against cancer.

KEYWORDS

baicalein derivatives, cyclin-dependent kinase 1, inhibitor

1 | INTRODUCTION

Malignant tumor is a kind of disease caused by multiple gene mutations under the concurrent action of genetic and environmental factors, which leads to uncontrolled cell cycle regulation and unlimited cell proliferation (Lapenna & Giordano, 2009). In recent years, the incidence of tumor is increasing, which always posts a threat to human health (Fouad & Aanei, 2017; Hanahan & Weinberg, 2011). Traditional chemotherapy has several disadvantages, side-effects, drug resistance, and so on (Bradner et al., 2017; Solaki & Ewald, 2018). Therefore, there is an urgent need to seek new targets and anti-cancer drugs with high efficiency, selectivity, and low toxicity.

Since unraveling the mechanism of cell cycle regulation in the early 1970s, cell cycle checkpoint has become an important target for anti-cancer drug development (Sánchez-Martínez et al., 2015). The cell cycle is a series of events that lead to cell division and duplication (Lapenna & Giordano, 2009), which can be divided into four phases: G1 (presynthetic growth), S (DNA synthesis), G2 (premitotic growth), and M (mitotic) phases. This process is regulated by cyclin-dependent kinases (CDKs) and their cognate cyclins, along with their endogenous inhibitors (CDKIs; Malumbre & Barbacid, 2005, 2009). Deregulation of the cell cycle occurs in the pathogenesis of various human diseases, especially cancers, where CDKs, cyclins, and endogenous CDKIs are deeply involved. CDKs play key roles in the regulation of the cell cycle (Denicourt & Dowdy, 2004; Deshpande et al., 2005). Particularly, CDKs were found to play an important part in tumorigenesis directly or through signaling cascades indirectly (Uziel et al., 2006). Hence, CDKs have become promising anti-cancer targets and it is believed that the inhibition of CDKs could

effectively suppress tumor growth (Asghar et al., 2015; Jorda et al., 2018; Roskoski, 2016, 2019). All CDKs have two-lobed structures: N-terminal lobe rich in β sheets and C-terminal lobe rich in α helices. These two lobes are joined together by a hinge polypeptide strand. The cleft between the two lobes is the binding site of ATP. Most inhibitors developed nowadays belong to ATP-competitive inhibitors (Pavletich, 1999). Because of the similarity of ATP-binding sites between different CDKs (Węsierska-Gądek et al., 2009), most potent compounds entered clinical trials in the early stage; for example, alvocidib (flavopiridol), milciclib (PHA-848125), roniciclib (BAY-1000394), AT7519, and TG02 (SB-1317; Figure 1) are pan-CDK inhibitors (Mou et al., 2020). Owing to the complex mechanisms of pan-CDK inhibitors, they usually caused side-effects including hepatic dysfunction, nausea, vomiting, and fatigue. So development of selective small molecular CDK inhibitors is necessary and urgent (Huwe et al., 2003).

CDK1 controls the entry from G2 phase to M phase in mammalian cells (Shapiro, 2006). It is also reported that CDK1 can drive all the events that are required in cell cycle in the absence of interphase CDKs (CDK2, 3, 4 and 6; Santamaría et al., 2007; Vassilev et al., 2006). Hence, CDK1 became a novel target for exploitation of selective CDK inhibitors as targeted anti-cancer drugs.

The co-crystal structures of CDK1/cyclin B-Cks2 with several CDK inhibitors were resolved by Noble and Martin *et al*. (Wood et al., 2019). In the co-crystal complex of CDK1/cyclin B-Cks2 with flavopiridol (PDB: 6GU2), the chromone core of flavopiridol is sandwiched between A31 and L135, forms two hydrogen bonds with the main-chain amide of L83 and carbonyl of E81 in the hinge region, and forms hydrophobic interactions with the gatekeeper residue F80 of CDK1, while the piperidinol moiety forms network of interactions with K33 and D146. Finally, the chlorophenyl group of flavopiridol forms hydrophobic interactions with V18 and I10 (Wood et al., 2019). The binding mode of flavopiridol with CDK1/cyclin B-Cks2 provides the fundament for the design of CDK1 inhibitors with flavonoid scaffold.

Baicalein (Figure 1) is a natural flavonoid isolated from the root of *Scutellaria baicalensis* Georgi or from baicalin by hydrolysis (Shen et al., 2003). It can induce cell apoptosis and cell cycle arrest by downregulating CDK1, CDK2, cyclin D2, and cyclin A and upregulating CDKIs in G1 and G2 phases, and also by downregulating the expression of CDK4/cyclins B and D (Eichhorn & Efferth, 2012). Two compounds with flavonoid scaffold, flavopiridol and P276-00 (Figure 1), derived from the natural product rohitukine (Mahajan et al., 2015; Figure 1) showed very strong CDK inhibitory activities (Blachly et al., 2016; Cassaday et al., 2015) and are currently used in the clinical trials for tumor treatment. So we selected baicalein as lead compound and docked it to the ATP-binding site of CDK1/cyclin B-Cks2 (PDB: 6GU2; Figure 2) to determine its structural modification protocol as a CDK1 inhibitor. The results showed that the chromone core of baicalein can be accommodated to the hinge region of CDK1 with the help of two hydrogen bonds with E81 and L83. And it also forms hydrophobic interaction with V18, A31 and L135. The phenyl group forms hydrophobic interaction with I10. But it does not form any interaction with the

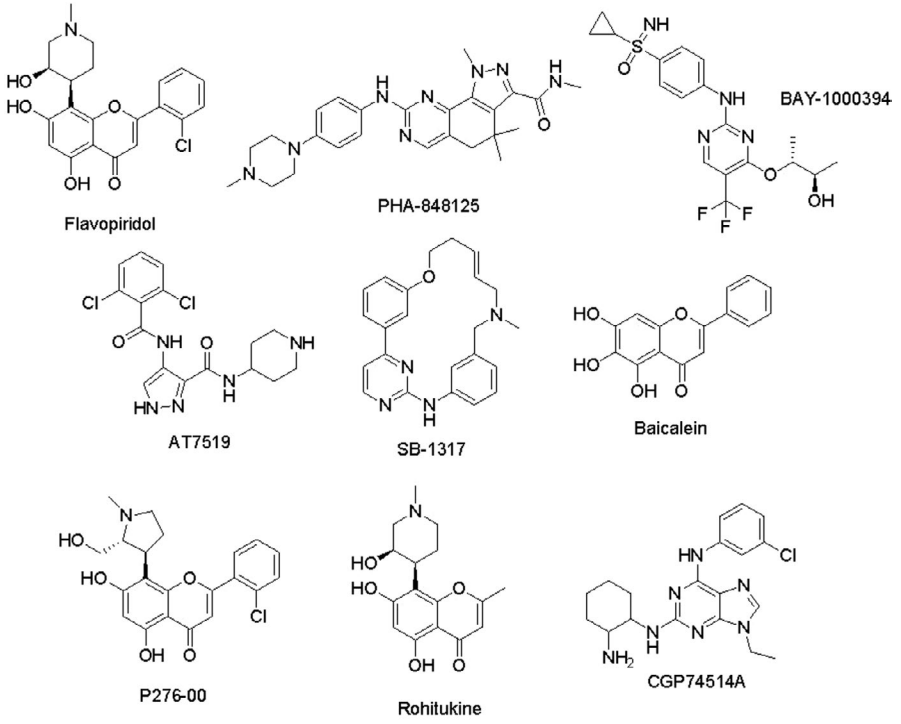


FIGURE 1 FIGURE Selected chemical structures of CDK inhibitors

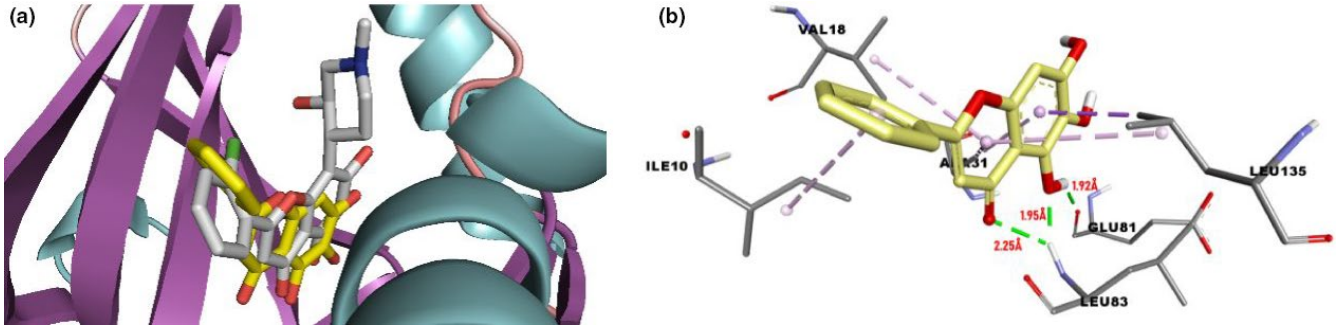


FIGURE 2 (a) Binding mode of baicalein with CDK1 (baicalein in yellow, while flavopiridol in pale). (b) Interactions between baicalein and CDK1's binding site

important D146 of CDK1 compared with flavopiridol. The main reason is that there are no other hydrogen bond donors or acceptors with large volume on baicalein's A ring except for hydroxyl groups.

Accordingly, in our subsequent structural modification process, the key chromone core and phenyl group of baicalein were kept, and hydrophobic groups to position-6 or position-7 and amine methylenes to position-8 were introduced to baicalein's A ring (Figure 3). It is expected that after modification, the introduced substituent groups of baicalein could form hydrogen bonds with D146 and hydrophobic interactions with other amino acid residues of CDK1, so as to improve baicalein derivatives' inhibitory activities toward CDK1.

In this paper, we choose CDK1 as the target and baicalein as the lead compound to modify the A ring of baicalein based on the docking results (Figure 2). And then, we found a new kind of CDK1 inhibitors with flavonoid as scaffold through biological activity evaluations and structure–activity relationship (SAR) assessment.

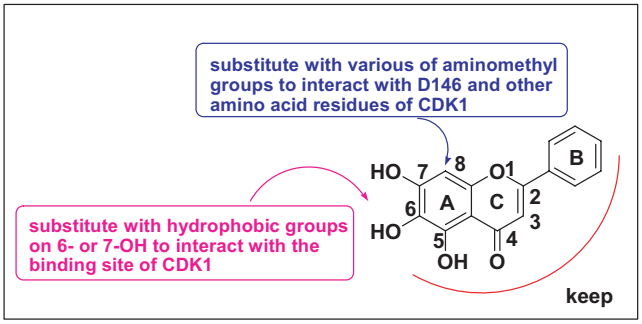


FIGURE 3 Modification strategy of baicalein

2 | EXPERIMENTAL SECTION

2.1 | Chemistry

2.1.1 | Reagents and instruments

All solvents were of analytical reagents, commercially available, and used without further purification. Thin-layer chromatography (TLC) with silica gel precoated glass and fluorescent indicator was used to monitor the reactions. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AV-III-600 instrument. High-resolution mass spectral (MS) data were determined on an Agilent 6540 UHD accurate mass Q-TOF/MS instrument in a low-resonance electrospray mode (ESI). CCK-8 was purchased from Dojindo Molecular Technologies, Inc., Kumamoto, Japan. MCF-7 cells were purchased from Lanmeng Biomedical Technology Co., Ltd, Hebei, China. CDK1/cyclin B kinase was provided by Chundu Biomedical Technology Co., Ltd, Wuhan, China. Molecular docking was conducted on Discovery Studio, version 5.

2.1.2 | Synthesis procedures

Synthesis of compounds 1

To a solution of baicalein (4 mmol) in 30 ml methanol was added 37% formaldehyde solution (6 mmol), one kind of secondary amine (4.8 mmol) in turn (Zhang et al., 2008). After that, the mixture was stirred at 30–70°C. The progress of the reaction was monitored by TLC until the reaction is completed and a large amount of yellow precipitate appeared. The yellow precipitate was filtered under vacuum, washed with a small amount of methanol, and dried in a vacuum oven to obtain the product.

*5,6,7-Trihydroxy-8-(morpholinomethyl)-2-phenyl-4H-chromen-4-one (***1a***).* Yellow powder, yield 93.5%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 8.08–8.10 (m, 2H, H-2′,6′), 7.58–7.63 (m, 3H, H-3′,4′,5′), 6.96 (s, 1H, H-3), 3.95 (s, 2H, H-9), 3.62 (brs, 4H, H-11,12), 2.61 (brs, 4H, H-10,13). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.7 (C-4), 163.0 (C-2), 154.7 (C-7), 148.5 (C-8a), 146.4 (C-5), 132.3 (C-4′), 131.6 (C-1′), 129.7 (C-3′,5′), 129.4 (C-6), 126.7 (C-2′,6′), 104.9 (C-4a), 104.1 (C-3), 100.6 (C-8), 66.5 (C-11,12), 53.1 (C-10,13), 52.0 (C-9).

*5,6,7-Trihydroxy-2-phenyl-8-(thiomorpholinomethyl)-4H-chromen-4-one (**1b**).* Yellow powder, yield 88.7%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 12.78 (s, 1H, 5-OH), 8.08–8.09 (m, 2H, H-2′,6′), 7.59–7.63 (m, 3H, H-3′,4′,5′), 6.95 (s, 1H, H-3), 3.97 (s, 2H, H-9), 2.87 (t, J = 5.0 Hz, 4H, H-10,13), 2.67 (t, J = 5.0 Hz, 4H, H-11,12). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.7 (C-4), 163.0 (C-2), 154.9 (C-7), 148.5 (C-8a), 146.4 (C-5), 132.3 (C-4′), 131.6 (C-1′), 129.7 (C-6), 129.4 (C-3′,5′), 126.8 (C-2′,6′), 104.9 (C-4a), 104.1 (C-3), 100.7 (C-8), 54.4 (C-10,13), 52.6 (C-9), 27.6 (C-11,12).

*5,6,7-Trihydroxy-8-((4-methylpiperazin-1-yl)methyl)-2-phenyl-4H-chromen-4-one (**1c**).* Yellow powder, yield 89.6%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 8.07–8.08 (m, 2H, H-2′,6′), 7.57–7.61 (m, 3H, H-3′,4′,5′), 6.93 (s, 1H, H-3), 4.02 (s, 2H, H-9), 2.69 (brs, 4H, H-10,13), 2.51 (brs, 4H, H-11,12), 2.20 (s, 3H, H-14). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.4 (C-4), 162.6 (C-2), 156.2 (C-7), 148.4 (C-8a), 146.0 (C-5), 132.2 (C-4′), 131.6 (C-1′), 131.4 (C-6), 129.6 (C-3′,5′), 126.7 (C-2′,6′), 104.9 (C-4a), 103.7 (C-3), 100.0 (C-8), 54.7 (C-10,13), 52.2 (C-11,12), 45.9 (C-9), 40.0 (C-14).

*2-Phenyl-8-((pyrrolidin-1-yl) methyl)-4H-chromene-5,6,7-triol (**1d**).* Yellow powder, yield 92.5%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 8.05–8.06 (m, 2H, H-2′,6′), 7.57–7.58 (m, 3H, H-3′,4′,5′), 6.81 (s, 1H, H-3), 4.35 (s, 2H, H-9), 3.12 (brs, 4H, H-10,13), 1.90 (brs, 4H, H-11,12). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 180.9 (C-4), 163.8 (C-2), 161.0 (C-7), 150.1 (C-8a), 143.7 (C-5), 132.0 (C-4′), 131.7 (C-1′), 130.7 (C-6), 129.6 (C-3′,5′), 126.5 (C-2′,6′), 104.9 (C-4a), 101.0 (C-3), 97.2 (C-8), 53.2 (C-10,13), 49.7 (C-9), 23.4 (C-11,12).

*8-((2H-Pyrrol-1(5H)-yl) methyl)-5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one (**1e**).* Yellow powder, yield 91.8%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 8.05–8.07 (m, 2H, H-2′,6′), 7.56–7.61 (m, 3H, H-3′,4′,5′), 6.88 (s, 1H, H-3), 5.89 (brs, 2H, H-11,12), 4.38 (s, 2H, H-9), 3.80 (brs, 4H, H-10,13). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.8 (C-4), 161.9 (C-2), 159.7 (C-7), 149.0 (C-8a), 145.1 (C-5), 132.0 (C-4′), 131.8 (C-1′), 130.2 (C-6), 129.6 (C-3′,5′), 127.0 (C-2′,6′), 126.6 (C-11,12), 104.9 (C-4a), 102.5 (C-3), 99.1 (C-8), 59.4 (C-10,13), 50.3 (C-9).

*8-((Dimethylamino) methyl)-5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one (**1f**).* Yellow powder, yield 89.8%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 8.05–8.07 (m, 2H, H-2′,6′), 7.56–7.59 (m, 3H, H-3′,4′,5′), 6.82 (s, 1H, H-3), 4.21 (s, 2H, H-9), 2.61 (s, 6H, H-10,11). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.2 (C-4), 162.6 (C-2), 161.3 (C-7), 149.8 (C-8a), 144.2 (C-5), 131.9 (C-4′), 131.8 (C-1′), 130.5 (C-6), 129.6 (C-3′,5′), 126.5 (C-2′,6′), 104.9 (C-4a), 101.5 (C-3), 97.0 (C-8), 53.5 (C-9), 43.2 (C-10,11).

*8-((Dipropylamino) methyl)-5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one (**1g**).* Yellow powder, yield 92.4%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 12.57 (s, 1H, 5-OH), 8.04–8.06 (m, 2H, H-2′,6′), 7.56–7.61 (m, 3H, H-3′,4′,5′), 6.85 (s, 1H, H-3), 4.27 (s, 2H, H-9), 2.77 (t, J = 6.0 Hz, 4H, H-10,13), 1.64 (m, 4H, H-11,14), 0.88 (t, J = 6.0 Hz, 6H, H-12,15). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.7 (C-4), 161.8 (C-2), 160.7 (C-7), 148.8 (C-8a), 144.9 (C-5), 132.0 (C-4′), 131.8 (C-1′), 130.3 (C-6), 129.6 (C-3′,5), 126.6 (C-2′,6′), 105.0 (C-4a), 102.3 (C-3), 97.7 (C-8), 54.8 (C-10,13), 50.4 (C-9), 18.7 (C-11,14), 11.8 (C-12,15).

Synthesis of compound 2

To a solution of baicalein (4 mmol) in 30 ml dimethylformamide (DMF) were added bromobenzyl (5.2 mmol, 0.62 ml), potassium carbonate (12 mmol), and potassium iodide (12 mmol) successively (Gao et al., 2015). The mixture was stirred for 7 hr under nitrogen atmosphere at 60–70°C. After that, the mixture was filtered under vacuum and a few drops of formic acid were added. The filtrate was then evaporated under vacuum and dispersed in cold water to form a suspension. The suspension was neutralized and filtered to obtain the crude product. The crude product was purified by silica gel column chromatography and recrystallization.

*7-(Benzyloxy)-5,6-dihydroxy-2-phenyl-4H-chromen-4-one (**2**).* Yellow crystal, yield 65%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 12.57 (s, 1H, 5-OH), 8.82 (s, 1H, 6-OH), 8.05–8.07 (m, 2H, H-2′,6′), 7.54–7.62 (m, 5H, H-3′,4′,5′,3″,5″), 7.42–7.45 (m, 2H, H-2″,6″), 7.35–7.38 (m, 1H, H-4″), 7.04 (s, 1H, H-8), 6.97 (s, 1H, H-3), 5.30 (s, 2H, H-9). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.2 (C-4), 163.1 (C-2), 153.5 (C-7), 149.6 (C-8a), 146.4 (C-5), 136.2 (C-1″), 131.8 (C-4′), 130.8 (C-1′), 130.4 (C-6), 129.0 (C-3′,5′), 128.4 (C-3″,5″), 127.9 (C-4″), 127.7 (C-2″,6″), 126.2 (C-2′,6′), 105.3 (C-4a), 104.6 (C-3), 92.4 (C-8), 70.2 (C-9). HRMS (ESI) m/z $[M + H]^+$ Calcd for $C_{22}H_{17}O_5$ 361.1076 found 361.1077.

Synthesis of compounds 3

To a solution of different carboxylic acid (2.2 mmol) in 30 ml anhydrous THF was added HOBt (2.6 mmol) under −5°C (Mou et al., 2009). DCC (2.6 mmol) dissolved in 20 ml of anhydrous THF was dropped into the above reaction solution. The mixture was stirred at −5°C for 12 hr to get the active ester and then filtered under vacuum. The filtrate would be used in the next step.

To a solution of one of compounds **1** (2.2 mmol) in 50 ml anhydrous THF were added dimethylaminopyridine (DMAP, 0.43 mmol) and triethylamine (0.8 ml) in turn under the stirring condition. Then, the above active ester filtrate was dropped into the reaction solution slowly. After completion (monitored by TLC), the organic solvent was removed and the residue was purified by silica gel column chromatography

to get the corresponding compound **3**. (The eluant was dichloromethane: methanol; sometimes, further recrystallization was needed.)

*5,6-Dihydroxy-8-(morpholinomethyl)-4-oxo-2-phenyl-4H-chromen-7-yl benzoate (**3a**).* Yellow powder, yield 21.2%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.17 (s, 1H, 5-OH), 8.12–8.13 (m, 4H, H-2′,6′,2″,6″), 7.72–7.79 (m, 1H, H-4″), 7.57–7.65 (m, 5H, H-3′,4′,5′,3″,5″), 7.01 (s, 1H, H-3), 4.17 (s, 2H, H-9), 3.68 (brs, 4H, H-12,13), 2.81 (brs, 4H, H-11,14). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.3 (C-4), 164.1 (C-10), 163.2 (C-2), 160.7 (C-7), 152.8 (C-8a), 152.1 (C-5), 134.4 (C-4″), 132.5 (C-4′), 131.4 (C-1′), 130.3 (C-2″,6″), 129.7 (C-3′,5′), 129.4 (C-3″,5″), 129.2 (C-1″), 126.9 (C-2′,6′), 123.1 (C-6), 105.2 (C-4a), 102.7 (C-3), 99.5 (C-8), 65.8 (C-12,13), 52.7 (C-9), 52.4 (C-11,14). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{27}H_{24}NO_7$ 474.1553 found 474.1549.

*5,6-Dihydroxy-8-(morpholinomethyl)-4-oxo-2-phenyl-4H-chromen-7-yl 2-bromobenzoate (**3b**).* Yellow crystal, yield 59.4%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.20 (s, 1H, 5-OH), 8.12–8.15 (m, 3H, H-2′,6′,6″), 7.85–7.86 (m, 1H, H-3″), 7.57–7.65 (m, 5H, H-3′,4′,5′,4″,5″), 7.01 (s, 1H, H-3), 4.21 (s, 2H, H-9), 3.70 (brs, 4H, H-12,13), 2.87 (brs, 4H, H-11,14). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.2 (C-4), 163.1 (C-2,10), 161.1 (C-7), 153.1 (C-8a), 152.2 (C-5), 135.1 (C-4″), 134.6 (C-1″), 132.6 (C-3″), 132.4 (C-6″), 131.4 (C-4′), 130.7 (C-1′), 129.7 (C-3′,5′), 128.4 (C-6), 126.9 (C-2′,6′), 123.0 (C-5″), 122.0 (C-2″), 105.2 (C-4a), 102.5 (C-3), 99.3 (C-8), 65.7 (C-12,13), 52.6 (C-9), 52.3 (C-11,14). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{20}H_{20}NO_6$ 370.1291 found 370.1284.

*5,6-Dihydroxy-8-(morpholinomethyl)-4-oxo-2-phenyl-4H-chromen-7-yl 4-methoxybenzoate (**3c**).* Yellow crystal, yield 70.3%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.07 (s, 1H, 5-OH), 8.12–8.13 (m, 2H, H-2′,6′), 8.06 (d, J = 12.0 Hz, 2H, H-2″,6″), 7.60–7.65 (m, 3H, H-3′,4′,5′), 7.09 (d, J = 12.0 Hz, 2H, H-3″,5″), 7.02 (s, 1H, H-3), 4.17 (s, 2H, H-9), 3.86 (s, 3H, H-15), 3.67 (brs, 4H, H-12,13), 2.81 (brs, 4H, H-11,14). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.3 (C-4), 164.1 (C-10), 163.7 (C-4″), 163.2 (C-2), 160.7 (C-7), 152.7 (C-8a), 152.2 (C-5), 132.5 (C-1″,2″,6″), 131.4 (C-6), 129.7 (C-3′,5′), 126.9 (C-2′,6′), 123.2 (C-4′), 121.3 (C-1′), 114.6 (C-3″,5″), 105.2 (C-4a), 102.8 (C-3), 99.5 (C-8), 65.8 (C-15), 56.1 (C-12,13), 52.7 (C-9), 52.4 (C-11,14). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{28}H_{26}NO_8$ 504.1658 found 504.1692.

*(2E)-5,6-Dihydroxy-8-(morpholinomethyl)-4-oxo-2-phenyl-4H-chromen-7-yl cinnamate (**3d**).* Yellow powder, yield 50.2%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.15 (s, 1H, 5-OH), 8.11–8.12 (m, 2H, H-2′,6′), 7.86 (d, J = 16.1 Hz, 1H, H-16), 7.80–7.82 (m, 2H, H-2″,6″), 7.59–7.64 (m, 3H, H-3′,4′,5′), 7.44–7.47 (m, 3H, H-3″,4″,5″), 7.01 (s, 1H, H-3), 6.92 (d, J = 16.1 Hz, 1H, H-15), 4.16 (s, 2H, H-9), 3.69 (brs, 4H, H-12,13), 2.80 (brs, 4H, H-11,14). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.4 (C-4), 164.4 (C-10), 163.3 (C-2), 160.2 (C-7), 152.7 (C-8a), 152.1 (C-5), 146.8 (C-16), 134.4 (C-1″), 132.5 (C-4′), 131.3 (C-1′), 131.0 (C-4″), 129.7 (C-3′,5′), 129.5 (C-3″,5″), 129.1 (C-2″,6″), 126.9 (C-2′,6′), 122.9 (C-6), 117.4 (C-15), 105.2 (C-4a), 103.0 (C-3), 99.5 (C-8), 65.9 (C-12,13), 52.7 (C-9), 52.4 (C-11,14). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{20}H_{20}NO_6$ 370.1291 found 370.1286.

*5,6-Dihydroxy-8-(morpholinomethyl)-4-oxo-2-phenyl-4H-chromen-7-yl methacrylate (**3e**).* Yellow powder, yield 19.5%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 8.10–8.11 (m, 2H, H-2′,6′), 7.59–7.64 (m, 3H, H-3′,4′,5′), 6.99 (s, 1H, H-3), 6.29 (s, 1H, H-16*E*), 5.90 (s, 1H, H-16*Z*), 4.14 (s, 2H, H-9), 3.69 (brs, 4H, H-12,13), 2.79 (brs, 4H, H-11,14), 2.01 (s, 3H, H-17). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.3 (C-4), 164.7 (C-10), 163.2 (C-2), 160.2 (C-7), 152.6 (C-8a), 152.0 (C-5), 135.4 (C-15), 132.5 (C-4′), 131.3 (C-1′), 129.7 (C-3′,5′), 128.2 (C-6), 126.9 (C-2′,6′), 122.9 (C-16), 105.2 (C-4a), 102.9 (C-3), 99.5 (C-8), 65.9 (C-12,13), 52.7 (C-9), 52.4 (C-11,14), 18.6 (C-17). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{20}H_{20}NO_6$ 370.1291 found 370.1294.

*5,6-Dihydroxy-4-oxo-2-phenyl-8-(thiomorpholinomethyl)-4H-chromen-7-yl benzoate (**3f**).* Yellow crystal, yield 70.1%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.14 (s, 1H, 5-OH), 8.12–8.14 (m, 4H, H-2″,6″,2′,6′), 7.75–7.77 (m, 1H, H-4″), 7.60–7.64 (m, 5H, H-3′,4′,5′,3″,5″), 7.01 (s, 1H, H-3), 4.20 (s, 2H, H-9), 3.06 (t, J = 5.0 Hz, 4H, H-11,14), 2.75 (t, J = 5.0 Hz, 4H, H-12,13). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.7 (C-4), 163.5 (C-10), 162.6 (C-2), 160.6 (C-7), 152.3 (C-8a), 151.5 (C-5), 133.8 (C-4″), 131.9 (C-4′), 130.8 (C-1′), 129.7 (C-2″,6″), 129.1 (C-3′,5′), 128.8 (C-3″,5″), 128.6 (C-1″), 126.4 (C-2′,6′), 122.5 (C-6), 104.7 (C-4a), 102.0 (C-3), 98.8 (C-8), 54.8 (C-11,14), 53.3 (C-9), 26.2 (C-12,13). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{27}H_{24}NO_6S$ 490.1324 found 490.1346.

*5,6-Dihydroxy-4-oxo-2-phenyl-8-(thiomorpholinomethyl)-4H-chromen-7-yl 2-bromobenzoate (**3g**).* Light yellow powder, yield 66.5%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.18 (s, 1H, 5-OH), 8.12–8.15 (m, 3H, H-2′,6′,6″), 7.83–7.86 (m, 1H, H-3″), 7.57–7.64 (m, 5H, H-3′,4′,5′,4″,5″), 7.00 (s, 1H, H-3), 4.23 (s, 2H, H-9), 3.11 (brs, 4H, H-11,14), 2.78 (brs, 4H, H-12,13). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.1 (C-4), 163.1 (C-10), 163.0 (C-2), 161.6 (C-7), 153.1 (C-8a), 152.2 (C-5), 135.0 (C-4″), 134.5 (C-1″), 132.6 (C-3″), 132.4 (C-6″), 131.4 (C-4′), 130.7 (C-1′), 129.7 (C-3′,5′), 128.4 (C-6), 126.9 (C-2′,6′), 123.1 (C-5″), 121.9 (C-2″), 105.2 (C-4a), 102.3 (C-3), 99.1 (C-8), 53.8 (C-11,14), 53.2 (C-9), 26.6 (C-12,13). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{27}H_{23}BrNO_6S$ 568.0429 found 568.0315.

*5,6-Dihydroxy-4-oxo-2-phenyl-8-(thiomorpholinomethyl)-4H-chromen-7-yl 4-methoxybenzoate (**3h**).* Light yellow powder, yield 68.3%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.09 (s, 1H, 5-OH), 8.13 (d, J = 12.0 Hz, 2H, H-2″,6″), 8.03–8.04 (m, 2H, H-2′,6′), 7.60–7.65 (m, 3H, H-3′,4′,5′), 7.06 (d, J = 12.0 Hz, 2H, H-3″,5″), 7.02 (s, 1H, H-3), 4.19 (s, 2H, H-9), 3.84 (s, 3H, H-15), 3.08 (brs, 4H, H-11,14), 2.74 (brs, 4H, H-12,13). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.2 (C-4), 164.0 (C-10), 163.7 (C-4″), 163.0 (C-2), 161.4 (C-7), 152.8 (C-8a), 152.2 (C-5), 132.4 (C-2″,6″,4′), 131.4 (C-1′), 129.7 (C-3′,5′), 126.9 (C-2′,6′), 123.3 (C-6), 121.3 (C-1″), 114.6 (C-3″,5″), 105.2 (C-4a), 102.6 (C-3), 99.4 (C-8), 56.1 (C-15), 53.9 (C-11,14), 53.3 (C-9), 26.7 (C-12,13). HRMS (ESI) *m/z* [M + H]$^+$ Calcd for $C_{28}H_{26}NO_7S$ 520.1430 found 520.1440.

*(2E)-5,6-Dihydroxy-4-oxo-2-phenyl-8-(thiomorpholinomethyl)-4H-chromen-7-yl cinnamate (**3i**).* Light yellow crystal, yield 69.5%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.11 (s, 1H, 5-OH), 8.11–8.13 (m, 2H, H-2′,6′), 7.86 (d, J = 16.1 Hz, 1H, H-16), 7.80–7.82 (m, 2H, H-2″,6″), 7.59–7.65 (m, 3H, H-3′,4′,5′), 7.45–7.48 (m, 3H, H-3″,4″,5″), 7.01 (s, 1H, H-3), 6.91 (d, J = 16.1 Hz, 1H, H-15), 4.18 (s, 2H, H-9), 3.04 (brs, 4H, H-11,14), 2.76 (brs, 4H, H-12,13). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.3 (C-4), 164.4 (C-10), 163.2 (C-2), 160.6 (C-7), 152.7 (C-8a), 152.1 (C-5), 146.8 (C-16), 134.4 (C-1″), 132.5 (C-4′), 131.3 (C-1′), 131.3 (C-4″), 129.7 (C-3′,5′), 129.5 (C-3″,5″), 129.1 (C-2″,6″), 126.9 (C-2′,6′), 123.0 (C-6), 117.4 (C-15), 105.2 (C-4a), 102.8 (C-3), 99.4 (C-8), 53.9 (C-11,14), 53.3 (C-9), 26.8 (C-12,13). HRMS (ESI) *m/z* [M + H]$^+$ Calcd for $C_{29}H_{26}NO_6S$ 516.1481 found 516.1505.

*5,6-Dihydroxy-4-oxo-2-phenyl-8-(thiomorpholinomethyl)-4H-chromen-7-yl methacrylate (**3j**).* Light yellow crystal, yield 59.8%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.11 (s, 1H, 5-OH), 8.10–8.11 (m, 2H, H-2′,6′), 7.58–7.64 (m, 3H, H-3′,4′,5′), 6.99 (s, 1H, H-3), 6.23 (s, 1H, H-16*E*), 5.89 (s, 1H, H-16*Z*), 4.16 (s, 2H, H-9), 3.03 (brs, 4H, H-11,14), 2.76 (brs, 4H, H-12,13), 2.01 (s, 3H, H-17). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.3 (C-4), 164.7 (C-10), 163.2 (C-2), 160.6 (C-7), 152.7 (C-8a), 152.0 (C-5), 135.4 (C-15), 132.5 (C-4′), 131.3 (C-1′), 129.7 (C-3′,5′), 128.2 (C-6), 126.9 (C-2′,6′), 123.0 (C-16), 105.2 (C-4a), 102.7 (C-3), 99.4 (C-8), 53.9 (C-11,14), 53.3 (C-9), 26.8 (C-12,13), 18.6 (C-17). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{20}H_{20}NO_5S$ 386.1062 found 386.1068.

*5,6-Dihydroxy-8-((4-methylpiperazin-1-yl)methyl)-4-oxo-2-phenyl-4H-chromen-7-yl benzoate (**3k**).* Yellow powder, yield 18.9%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.16 (s, 1H, 5-OH), 8.11–8.13 (m, 4H, H-2′,6′,2″,6″), 7.71–7.77 (m, 1H, H-4″), 7.58–7.62 (m, 5H, H-3′,4′,5′,3″,5″), 6.95 (s, 1H, H-3), 4.22 (s, 2H, H-9), 2.92 (brs, 4H, H-11,13), 2.51 (brs, 4H, H-12,13), 2.23 (s, 3H, H-15). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.7 (C-4), 164.1 (C-10), 163.5 (C-2), 162.6 (C-7), 153.1 (C-8a), 152.1 (C-5), 134.3 (C-4″), 132.3 (C-4′), 131.5 (C-1′), 130.2 (C-2″,6″), 129.6 (C-3′,5′), 129.4 (C-3″,5″), 129.4 (C-1″), 126.8 (C-2′,6′), 123.7 (C-6), 106.5 (C-4a), 105.1 (C-3), 101.4 (C-8), 53.5 (C-11,14), 51.4 (C-9), 50.2 (C-12,13), 45.4 (C-15). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{21}H_{23}N_2O_5$ 383.1607 found 383.1622.

*5,6-Dihydroxy-8-((4-methylpiperazin-1-yl)methyl)-4-oxo-2-phenyl-4H-chromen-7-yl 2-bromobenzoate (**3l**).* Light yellow powder, yield 32.8%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.23 (s, 1H, 5-OH), 8.08–8.11 (m, 3H, H-2′,6′,6″), 7.83–7.84 (m, 1H, H-3″), 7.54–7.63 (m, 5H, H-3′,4′,5′,4″,5″), 6.94 (s, 1H, H-3), 4.25 (s, 2H, H-9), 2.97 (brs, 4H, H-11,14), 2.51 (brs, 4H, H-12,13), 2.24 (s, 3H, H-15). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.6 (C-4), 164.2 (C-10), 163.2 (C-2), 162.4 (C-7), 153.4 (C-8a), 152.1 (C-5), 135.0 (C-4″), 134.4 (C-1″), 132.5 (C-3″), 132.2 (C-6″), 131.5 (C-4′), 131.1 (C-1′), 129.6 (C-3′,5′), 128.4 (C-6), 126.8 (C-2′,6′), 123.7 (C-5″), 121.8 (C-2″), 105.1 (C-4a), 101.0 (C-3), 98.6 (C-8), 53.3 (C-11,14), 52.1 (C-9), 51.4 (C-12,13), 45.4 (C-15). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{21}H_{23}N_2O_5$ 383.1607 found 383.1694.

*5,6-Dihydroxy-8-((4-methylpiperazin-1-yl)methyl)-4-oxo-2-phenyl-4H-chromen-7-yl 4-methoxybenzoate (**3m**).* Yellow crystal, yield 50.9%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ:13.08 (s, 1H, 5-OH), 8.11–8.12 (m, 2H, H-2′,6′), 8.02 (d, J = 12.0 Hz, 2H, H-2″,6″), 7.58–7.64 (m, 3H, H-3′,4′,5′), 7.04 (d, J = 12.0 Hz, 2H, H-3″,5″), 6.97 (s, 1H, H-3), 4.22 (s, 2H, H-9), 3.83 (s, 3H, H-16), 2.92 (brs, 4H, H-12,13), 2.51 (brs, 4H, H-11,14), 2.20 (s, 3H, H-15). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.7 (C-4), 163.9 (C-10), 163.7 (C-4″), 163.6 (C-2), 162.5 (C-7), 153.0 (C-8a), 152.2 (C-5), 132.3 (C-1″,2″,6″), 131.5 (C-6), 129.7 (C-3′,5′), 126.8 (C-2′,6′), 123.8 (C-4′), 121.6 (C-1′), 114.6 (C-3″,5″), 105.1 (C-4a), 101.5 (C-3), 98.8 (C-8), 56.0 (C-16), 53.5 (C-11,14), 52.3 (C-9), 51.6 (C-12,13), 45.6 (C-15). HRMS (ESI) *m/z* [M + H]$^+$ Calcd for $C_{29}H_{29}N_2O_7$ 517.1975 found 517.1980.

*(2E)-5,6-Dihydroxy-8-((4-methylpiperazin-1-yl)methyl)-4-oxo-2-phenyl-4H-chromen-7-yl cinnamate (**3n**).* Yellow powder, yield 52.9%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.13 (s, 1H, 5-OH), 8.09–8.11 (m, 2H, H-2′,6′), 7.83 (d, J = 16.1 Hz, 1H, H-17), 7.78–7.80 (m, 2H, H-2″,6″), 7.58–7.64 (m, 3H, H-3′,4′,5′), 7.46–7.47 (m, 3H, H-3″,4″,5″), 6.95 (s, 1H, H-3), 6.88 (d, J = 16.1 Hz, 1H, H-16), 4.20 (s, 2H, H-9), 2.90 (brs, 4H, H-11,14), 2.51 (brs, 4H, H-12,13), 2.24 (s, 3H, H-15). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.9 (C-4), 164.4 (C-10), 162.7 (C-2), 152.9 (C-7), 152.1 (C-8a), 146.5 (C-5), 134.4 (C-17), 132.3 (C-1″), 131.5 (C-4′), 131.2 (C-1′), 129.7 (C-3′,5′), 129.5 (C-3″,4″,5″), 129.0 (C-2″,6″), 126.8 (C-

2′,6′), 123.4 (C-6), 117.6 (C-16), 105.1 (C-4a), 101.8 (C-3), 98.9 (C-8), 53.7 (C-11,14), 52.3 (C-9), 51.5 (C-12,13), 45.5 (C-15). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{30}H_{29}N_2O_6$ 513.2026 found 513.2063.

*5,6-Dihydroxy-8-((4-methylpiperazin-1-yl)methyl)-4-oxo-2-phenyl-4H-chromen-7-yl methacrylate (***3o***).* Light yellow powder, yield 40.3%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.06 (s, 1H, 5-OH), 7.08–7.10 (m, 2H, H-2′,6′), 7.57–7.63 (m, 3H, H-3′,4′,5′), 6.93 (s, 1H, H-3), 6.26 (s, 1H, H-17*E*), 5.87 (s, 1H, H-17*Z*), 4.20 (s, 2H, H-9), 2.89 (brs, 4H, H-11,14), 2.51 (brs, 4H, H-12,13), 2.23 (s, 3H, H-15), 2.00 (s, H, H-18). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.8 (C-4), 164.8 (C-10), 163.0 (C-2), 162.6 (C-7), 152.9 (C-8a), 152.0 (C-5), 135.6 (C-16), 132.3 (C-4′), 131.5 (C-1′), 129.6 (C-3′,5′), 127.9 (C-6), 126.8 (C-2′,6′), 123.5 (C-17), 105.1 (C-4a), 101.6 (C-3), 98.7 (C-8), 53.7 (C-11,14), 52.4 (C-9), 51.5 (C-12,13), 45.6 (C-15), 18.7 (C-18). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{21}H_{23}N_2O_5$ 383.1607 found 383.1607.

*5,6-Dihydroxy-4-oxo-2-phenyl-8-((pyrrolidin-1-yl)methyl)-4H-chromen-7-yl benzoate (***3p***).* Yellow crystal, yield 24.8%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.20 (s, 1H, 5-OH), 8.08–8.13 (m, 4H, H-2″,6″,2′,6′), 7.69–7.75 (m, 1H, H-4″), 7.58 (m, 5H, H-3′,4′,5′,3″,5″), 6.81 (s, 1H, H-3), 4.38 (s, 2H, H-9), 3.24 (brs, 4H, H-11,14), 1.93 (brs, 4H, H-12,13). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 180.4 (C-4), 169.0 (C-10), 164.3 (C-2), 161.1 (C-7), 154.3 (C-8a), 152.3 (C-5), 133.9 (C-4″), 131.9 (C-4′), 131.8 (C-1′), 130.1 (C-1″), 130.1 (C-2″,6″), 129.6 (C-3′,5′), 129.2 (C-3″,5″), 126.6 (C-2′,6′), 125.3 (C-6), 104.9 (C-4a), 98.3 (C-3), 98.0 (C-8), 53.2 (C-11,14), 40.4 (C-9), 23.3 (C-12,13). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{20}H_{20}NO_5$ 354.1341 found 354.1341.

*5,6-Dihydroxy-4-oxo-2-phenyl-8-((pyrrolidin-1-yl)methyl)-4H-chromen-7-yl 4-methoxybenzoate (***3q***).* Yellow powder, yield 17.9%. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 12.89 (s, 1H, 5-OH), 8.24 (d, J = 12.0 Hz, 2H, H-2″,6″), 7.82–7.83 (m, 2H, H-2′,6′), 7.53–7.54 (m, 3H, H-3′,4′,5′), 6.97 (d, J = 12.0 Hz, 2H, H-3″,5″), 6.63 (s, 1H, H-3), 4.28 (s, 2H, H-9), 3.88 (s, 3H, H-15), 2.87 (brs, 4H, H-11,14), 1.92 (brs, 4H, H-12,13). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 182.4 (C-4), 164.2 (C-10), 163.8 (C-4″), 162.9 (C-2), 160.5 (C-7), 152.8 (C-8a), 151.5 (C-5), 132.7 (C-2″,6″), 131.7 (C-4′), 131.6 (C-1′), 129.2 (C-3′,5′), 126.1 (C-2′,6′), 123.1 (C-6), 121.4 (C-1″), 113.7 (C-3″,5″), 105.6 (C-4a), 103.7 (C-3), 98.8 (C-8), 55.5 (C-15), 53.6 (C-11,14), 51.9 (C-9), 23.7 (C-12,13). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{28}H_{26}NO_7$ 488.1709 found 488.1741.

*8-((2H-Pyrrol-1(5H)-yl)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl benzoate (***3r***).* Light yellow powder, yield 29.7%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.16 (s, 1H, 5-OH), 8.10–8.13 (m, 2H, H-2″,6″), 8.06–8.07 (m, 2H, H-2′,6′), 7.72–7.74 (m, 1H, H-4″), 7.57–7.61 (m, 5H, H-3′,4′,5′,3″,5″), 6.85 (s, 1H, H-3), 5.91 (brs, 2H, H-12,13), 4.48 (s, 2H, H-9), 4.00 (brs, 4H, H-11,14). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 180.9 (C-4), 167.4 (C-10), 164.3 (C-2), 161.6 (C-7), 153.9 (C-8a), 152.3 (C-5), 134.0 (C-4″), 132.0 (C-4′), 131.8 (C-1′), 130.1 (C-2″,6″), 129.9 (C-1″), 129.6 (C-3′,5′), 129.3 (C-3″,5″), 126.7 (C-2′,6′), 125.9 (C-12,13), 124.8 (C-6), 105.0 (C-4a), 99.3 (C-3), 98.1 (C-8), 59.4 (C-11,14), 51.0 (C-9). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{27}H_{22}NO_6$ 456.1447 found 456.1469.

*8-((2H-Pyrrol-1(5H)-yl)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl 4-methoxybenzoate (***3s***).* Light yellow powder, yield 52.6%. ^{1}H-NMR (DMSO-d_6,600 MHz) δ: 13.13 (s, 1H, 5-OH), 8.04–8.07 (m, 4H, H-2″,6″,2′,6′), 7.56–7.60 (m, 3H, H-3′,4′,5′), 7.10–7.11 (m, 2H, H-3″,5″), 6.86 (s, 1H, H-3), 5.97 (brs, 2H, H-12,13), 4.47 (s, 2H, H-9), 3.98 (brs, 4H, H-11,14), 3.87 (s, 3H, H-15). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 180.9 (C-4), 167.1 (C-10), 163.9 (C-2), 163.8 (C-4″), 161.7 (C-7), 153.7 (C-8a), 152.3 (C-5), 132.3 (C-2″,6″), 132.0 (C-4′), 131.7 (C-1′), 129.6 (C-2′,6′), 126.7 (C-3′,5′), 126.0 (C-12,13), 124.7 (C-6), 122.0 (C-1″), 114.5 (C-3″,5″), 105.0 (C-4a), 99.6 (C-3), 98.1 (C-8), 59.4 (C-15), 56.0 (C-11,14), 51.1 (C-9). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{28}H_{24}NO_7$ 486.1553 found 486.1619.

*8-((2H-Pyrrol-1(5H)-yl)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl methacrylate (***3t***).* Light yellow powder, yield 63.7%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.10 (s, 1H, 5-OH), 8.04–8.06 (m, 2H, H-2′,6′), 7.56–7.60 (m, 3H, H-3′,4′,5′), 6.84 (s, 1H, H-3), 6.23 (s, 1H, H-16*E*), 5.92 (brs, 2H, H-12,13), 5.83 (s, 1H, H-16*Z*), 4.45 (s, 2H, H-9), 3.97 (brs, 4H, H-11,14), 1.99 (s, 3H, H-17). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 180.9 (C-4), 167.0 (C-10), 164.9 (C-2), 161.7 (C-7), 153.6 (C-8a), 152.1 (C-5), 135.9 (C-15), 132.0 (C-4′), 131.7 (C-1′), 129.6 (C-3′,5′), 127.3 (C-6), 126.7 (C-2′,6′), 126.0 (C-12,13), 124.6 (C-16), 105.0 (C-4a), 99.5 (C-3), 98.0 (C-8), 59.4 (C-11,14), 51.1 (C-9), 18.7 (C-17). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{24}H_{22}NO_6$ 420.1447 found 420.1554.

*8-((Dimethylamino)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl benzoate (***3u***).* Yellow crystal, yield 43.8%. ^{1}H-NMR (DMSO-d_6, 600MHz) δ: 8.28–8.29 (m, 2H, H-2″,6″), 7.83–7.84 (m, 2H, H-2′,6′), 7.49–7.63 (m, 6H, H-3′,4′,5′,3″,4″,5″), 6.65 (s, 1H, H-3), 4.09 (s, 2H, H-9), 2.50 (s, 6H, H-11,12). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.5 (C-4), 164.4 (C-10), 163.2 (C-2), 159.7 (C-7), 152.9 (C-8a), 151.9 (C-5), 133.5 (C-4″), 131.8 (C-4′), 131.6 (C-1′), 130.5 (C-2″,6″), 129.2 (C-3′,5′), 129.0 (C-1″), 128.5 (C-3″,5″), 126.1 (C-2′,6′), 123.0 (C-6), 105.7 (C-4a), 104.1 (C-3), 98.5 (C-8),

55.5 (C-9), 44.3 (C-11,12). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{18}H_{18}NO_5$ 328.1185 found 328.1200.

*8-((Dimethylamino)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl 2-bromobenzoate (**3v**).* Yellow powder, yield 16.0%. ^{1}H-NMR (DMSO-d_6, 600MHz) δ: 8.24 (d, J = 7.4 Hz, 1H, H-6″), 7.83–7.84 (m, 2H, H-2′,6′), 7.72 (d, J = 7.7 Hz, 1H, H-3″), 7.54–7.58 (m, 3H, H-3′,4′,5′), 7.37–7.44 (m, 2H, H-4″,5″), 6.65 (s, 1H, H-3), 4.11 (s, 2H, H-9), 2.52 (s, 6H, H-11,12). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 182.4 (C-4), 163.3 (C-10), 163.2 (C-2), 159.9 (C-7), 153.0 (C-8a), 152.1 (C-5), 134.6 (C-4″), 133.2 (C-1″), 132.6 (C-3″), 131.8 (C-6″), 131.5 (C-4′), 130.6 (C-1′), 129.2 (C-3′,5′), 127.2 (C-6), 126.1 (C-2′,6′), 122.8 (C-5″), 122.8 (C-2″), 105.7 (C-4a), 103.9 (C-3), 98.5 (C-8), 55.3 (C-9), 44.2 (C-11,12). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{18}H_{18}NO_5$ 328.1185 found 328.1170.

*8-((Dimethylamino)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl methacrylate (**3w**).* Yellow powder, yield 17.5%. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.82–7.83 (m, 2H, H-2′,6′), 7.54–7.57 (m, 3H, H-3′,4′,5′), 6.64 (s, 1H, H-3), 6.47 (s, 1H, H-14*E*), 5.80 (s, 1H, H-14*Z*), 4.07 (s, 2H, H-9), 2.50 (s, 6H, H-11,12), 2.11 (s, 3H, H-15). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 182.5 (C-4), 165.1 (C-10), 163.2 (C-2), 159.6 (C-7), 152.8 (C-8a), 151.8 (C-5), 135.2 (C-13), 131.8 (C-4′), 131.6 (C-1′), 129.2 (C-3′,5′), 127.7 (C-6), 126.1 (C-2′,6′), 123.0 (C-14), 105.7 (C-4a), 104.0 (C-3), 98.5 (C-8), 55.4 (C-9), 44.3 (C-11,12), 18.5 (C-15). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{18}H_{18}NO_5$ 328.1185 found 328.1194.

*8-((Dipropylamino)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl benzoate (**3x**).* Yellow powder, yield 29.8%. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 13.18 (s, 1H, 5-OH), 8.07–8.13 (m, 4H, H-2″,6″,2′,6′), 7.72–7.77 (m, 1H, H-4″), 7.58–7.61 (m, 5H, H-3′,4′,5′,3″,5″), 6.85 (s, 1H, H-3), 4.38 (s, 2H, H-9), 2.94 (t, J = 6.0 Hz, 4H, H-11,14), 1.66–1.70 (m, 4H, H-12,15), 0.86 (t, J = 6.0 Hz, 6H, H-13,16). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 181.0 (C-4), 167.7 (C-10), 164.2 (C-2), 161.8 (C-7), 153.5 (C-8a), 152.2 (C-5), 134.1 (C-4″), 132.0 (C-4′), 131.7 (C-1′), 130.2 (C-2″,6″), 129.8 (C-1″), 129.6 (C-3′,5′), 129.3 (C-3″,5″), 126.7 (C-2′,6′), 124.5 (C-6), 105.0 (C-4a), 99.6 (C-3), 97.2 (C-8), 54.4 (C-11,14), 50.1 (C-9), 17.9 (C-12,15), 11.6 (C-11,16). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{18}H_{18}NO_5$ 384.1811 found 384.1833.

*8-((Dipropylamino)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl 2-bromobenzoate (**3y**).* Yellow powder, yield 53.9%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.23 (s, 1H, 5-OH), 8.07–8.10 (m, 3H, H-2′,6′,6″), 7.82–7.84 (m, 1H, H-3″), 7.55–7.61 (m, 5H, H-3′,4′,5′,4″,5″), 6.85 (s, 1H, H-3), 4.40 (s, 2H, H-9), 2.96 (t, J = 7.8 Hz, 4H, H-11,14), 1.67–1.70 (m, 4H, H-12,15), 0.87 (t, J = 7.4 Hz, 6H, H-13,16). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 180.9 (C-4), 167.9 (C-10), 163.4 (C-2), 161.7 (C-7), 153.7 (C-8a), 152.3 (C-5), 134.9 (C-4″), 134.2 (C-1″), 132.4 (C-3″), 132.0 (C-6″), 131.7 (C-4′), 131.6 (C-1′), 129.6 (C-3′,5′), 128.4 (C-6), 126.7 (C-2′,6′), 124.5 (C-5″), 121.6 (C-2″), 105.0 (C-4a), 99.3 (C-3), 97.2 (C-8), 54.3 (C-11,14), 50.0 (C-9), 17.8 (C-12,15), 11.5 (C-13,16). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{18}H_{18}NO_5$ 384.1811 found 384.1815.

*8-((Dipropylamino)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl 4-methoxybenzoate (**3z**).* Yellow powder, yield 29.4%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.12 (s, 1H, 5-OH), 8.04–8.08 (m, 4H, H-2′,6′,2″,6″), 7.57–7.62 (m, 3H, H-3′,4′,5′), 7.10–7.12 (m, 2H, H-3″,5″), 6.85 (s, 1H, H-3), 4.37 (s, 2H, H-9), 3.87 (s, 3H, H-17), 2.92 (t, J = 6.0 Hz, 4H, H-11,14), 1.65–1.71 (m, 4H, H-12,15), 0.86 (t, J = 6.0 Hz, 6H, H-13,16). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.1 (C-4), 167.5 (C-10), 163.8 (C-2,4″), 161.8(C-7), 153.3 (C-8a), 152.2 (C-5), 132.3 (C-2″,6″), 132.0 (C-4′), 131.7 (C-1′), 129.6 (C-3′,5′), 126.7 (C-2′,6′), 124.5 (C-6), 122.0 (C-1″), 114.6 (C-3″,5″), 105.0 (C-4a), 99.7 (C-3), 97.3 (C-8), 56.0 (C-17), 54.4 (C-11,14), 50.1 (C-9), 17.9 (C-12,15), 11.6 (C-13,16). HRMS (ESI) *m/z* [M + H]$^+$ Calcd for $C_{30}H_{32}NO_7$ 518.2179 found 518.2181.

*8-((Dipropylamino)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl 2-(2-chlorophenyl)acetate (**3A**).* Yellow powder, yield 59.7%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.12 (s, 1H, 5-OH), 8.05–8.06 (m, 2H, H-2′,6′), 7.56–7.60 (m, 4H, H-3′,4′,5′,6″), 7.48–7.49 (m, 1H, H-3″), 7.33–7.35 (m, 2H, H-4″,5″), 6.83 (s, 1H, H-3), 4.35 (s, 2H, H-9), 4.05 (s, 2H, H-17), 2.91 (t, J = 7.8 Hz, 4H, H-11,14), 1.65–1.71 (m, 4H, H-12,15), 0.87 (t, J = 6.0 Hz, 6H, H-13,16). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.1 (C-4), 168.4 (C-10), 167.3 (C-2), 161.8 (C-7), 153.3 (C-8a), 152.1 (C-5), 134.3 (C-4″), 132.9 (C-1″), 132.6 (C-3″), 132.3 (C-6″), 132.0 (C-4′), 131.7 (C-1′), 129.6 (C-3′,5′), 127.6 (C-6), 126.7 (C-2′,6′), 126.4 (C-5″), 124.4 (C-2″), 105.0 (C-4a), 99.6 (C-3), 97.3 (C-8), 54.4 (C-11,14), 48.8 (C-9), 38.3 (C-17), 18.0 (C-12,15), 11.6 (C-13,16). HRMS (ESI) *m/z* [M + H]$^+$ Calcd for $C_{30}H_{31}ClNO_6$ 536.1840 found 536.1971.

*(2E)-8-((Dipropylamino)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl cinnamate (**3B**).* Yellow powder, yield 62.3%. ^{1}H-NMR (DMSO-d_6,600 MHz) δ: 13.13 (s, 1H, 5-OH), 8.07–8.08 (m, 2H, H-2′,6′), 7.80 (d, J = 16.1 Hz, 1H, H-18), 7.79–7.82 (m, 2H, H-2″,6″), 7.57–7.62 (m, 3H, H-3′,4′,5′), 7.46–7.47 (m, 3H, H-3″,4″,5″), 6.89 (d, J = 16.1 Hz, 1H, H-17), 6.85 (s, 1H, H-3), 4.37 (s, 2H, H-9), 2.92 (t, J = 7.7 Hz, 4H, H-11,14), 1.65–1.69 (m, 4H, H-12,15), 0.87 (t, J = 7.4 Hz, 6H, H-13,16). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.1 (C-4), 167.3 (C-10), 164.5 (C-2), 161.8 (C-7), 153.2 (C-8a), 152.2 (C-5), 146.0 (C-18), 134.5 (C-1″),

132.1 (C-4′), 131.7 (C-1′), 131.1 (C-4″), 129.6 (C-3′,5′), 129.4 (C-3″,5″), 129.0 (C-2″,6″), 126.7 (C-2′,6′), 124.3 (C-6), 118.1 (C-17), 105.0 (C-4a), 99.8 (C-3), 97.3 (C-8), 54.5 (C-11,14), 50.3 (C-9), 18.1 (C-12,15), 11.6 (C-13,16). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{31}H_{32}NO_6$ 514.2230 found 514.2253.

*8-((Dipropylamino)methyl)-5,6-dihydroxy-4-oxo-2-phenyl-4H-chromen-7-yl methacrylate (***3C***).* Light yellow powder, yield 52.5%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.10 (s, 1H, 5-OH), 8.05–8.07 (m, 2H, H-2′,6′), 7.56–7.62 (m, 3H, H-3′,4′,5′), 6.83 (s, 1H, H-3), 6.23 (s, 1H, H-18*E*), 5.84 (s, 1H, H-18*Z*), 4.35 (s, 2H, H-9), 2.92 (t, J = 6.0 Hz, 4H, H-11,14), 1.99 (s, 3H, H-19), 1.66–1.71 (m, 4H, H-12,15), 0.87 (t, J = 6.0 Hz, 6H, H-13,16). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.0 (C-4), 167.4 (C-10), 164.8 (C-2), 161.8 (C-7), 153.3 (C-8a), 152.1 (C-5), 135.9 (C-17), 132.0 (C-4′), 131.7 (C-1′), 129.6 (C-3′,5′), 127.4 (C-6), 126.7 (C-2′,6′), 124.3 (C-18), 105.0 (C-4a), 99.6 (C-3), 97.2 (C-8), 54.4 (C-11,14), 50.1 (C-9), 18.7 (C-19), 17.9 (C-12,15), 11.6 (C-13,16). HRMS (ESI) *m/z* [(M-acid) + H]$^+$ Calcd for $C_{18}H_{18}NO_5$ 384.1811 found 384.1790.

Synthesis of compounds 4 and 5

Compounds **4** and **5** were prepared by the method mentioned above for the preparation of compound **2**, employing compounds **1a, b** as starting materials and 1, 4-dibromobutane instead of bromobenzyl.

*2,3,4,5-Tetrahydro-12-hydroxy-7-(morpholinomethyl)-9-phenyl-[1,4]dioxocino [2,3-g] chromen-11-one (***4a***).* Yellow crystal, yield 9.7%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 12.94 (s, 1H, 5-OH), 8.10–8.11 (m, 2H, H-2′,6′), 7.59–7.64 (m, 3H, H-3′,4′,5′), 7.04 (s, 1H, H-3), 4.64 (t, J = 5.6 Hz, 2H, H-10), 4.15 (t, J = 5.1 Hz, 2H, H-13), 3.71 (s, 2H, H-9), 3.53 (brs, 4H, H-15,16), 2.46–2.51 (m, 4H, H-14,17), 1.95–1.97 (m, 2H, H-11), 1.73–1.74 (m, 2H, H-12). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 183.1 (C-4), 163.9 (C-2), 158.0 (C-7), 153.6 (C-8a), 151.2 (C-5), 132.6 (C-6), 131.4 (C-4′), 131.1 (C-1′), 129.7 (C-3′,5′), 126.9 (C-2′,6′), 106.9 (C-4a), 106.1 (C-3), 105.0 (C-8), 73.5 (C-10), 72.2 (C-13), 66.8 (C-15,16), 53.6 (C-14,17), 50.2 (C-9), 28.7 (C-11), 24.7 (C-12). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{24}H_{26}NO_6$ 424.1760 found 424.1820.

*2,3,4,5-Tetrahydro-12-hydroxy-9-phenyl-7-(thiomorpholinomethyl)-[1,4]dioxocino[2,3-g]chromen-11-one (***4b***).* Yellow crystal, yield 12.0%. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 12.93 (s, 1H, 5-OH), 8.09–8.10 (m, 2H, H-2′,6′), 7.59–7.64 (m, 3H, H-3′,4′,5′), 7.02 (s, 1H, H-3),4.62 (t, J = 5.6 Hz, 2H, H-10), 4.15 (t, J = 5.6 Hz, 2H, H-13), 3.71 (s, 2H, H-9), 2.73 (brs, 4H, H-14,17), 2.51–2.57 (m, 4H, H-15,16), 1.95 (m, 2H, H-11), 1.74 (m, 2H, H-12). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 183.1 (C-4), 163.9 (C-2), 158.0 (C-7), 153.6 (C-8a), 151.2 (C-5), 132.6 (C-6), 131.4 (C-4′), 131.1 (C-1′), 129.7 (C-3′,5′), 126.9 (C-2′,6′), 107.2 (C-4a), 106.1 (C-3), 105.0 (C-8), 73.5 (C-10), 72.2 (C-13), 54.8 (C-14,17), 50.6 (C-9), 28.7 (C-11), 27.7 (C-15,16), 24.7 (C-12). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{24}H_{26}NO_5S$ 440.1532 found 440.1603.

*2,3,4,5-Tetrahydro-7-((3,4,5,11-tetrahydro-12-hydroxy-11-oxo-9-phenyl-2H-[1,4]dioxocino[2,3-g]chromen-7-yl)methyl)-12-hydroxy-9-phenyl-[1,4]dioxocino[2,3-g]chromen-11-one (***5***).* Yellow powder, yield 10.7%. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 12.85 (s, 2H, 2 × 5-OH), 7.92–7.93 (m, 4H, 2 × H-2′,6′), 7.50–7.55 (m, 6H, 2 × H-3′,4′,5′), 6.69 (s, 2H, 2 × H-3), 4.43 (s, 2H, H-9), 4.28 (brs, 4H, 2 × H-13), 4.19 (brs, 4H, 2 × H-10), 1.65 (brs, 8H, 2 × H-11,12). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 183.1 (2 × C-4), 163.9 (2 × C-2), 157.3 (2 × C-7), 153.0 (2 × C-8a), 150.3 (2 × C-5), 131.9 (2 × C-6), 131.6 (2 × C-4′), 130.6 (2 × C-1′), 129.2 (2 × C-3′,5′), 126.2 (2 × C-2′,6′), 108.6 (2 × C-4a), 105.6 (2 × C-3), 104.8 (2 × C-8), 73.5 (2 × C-13), 70.3 (2 × C-10), 28.9 (2 × C-12), 23.9 (2 × C-1), 18.3 (C-9). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{39}H_{33}O_{10}$ 661.2074 found 661.2093.

2.2 | Activity assays

2.2.1 | Anti-MCF-7 tumor cell proliferative experiment

All the target compounds and controls were dissolved in dimethyl sulfoxide (DMSO) to prepare 5 mg/ml stock solutions (Meegan et al., 2001). The stock solution was filtered by 0.2 μM filter and diluted into 5 concentrations (50, 10, 2, 0.4, and 0.08 μg/ml) with starving medium.

MCF-7 cells were diluted to 100,000/ml. 0.1 ml of cell suspension was inoculated into 96-well plate. After 24 hr of culture, the primary medium was discarded and replaced with starving medium (1640 medium +2% fetal bovine serum) for 12 hr. Then, the medium was discarded and replaced with compounds with different concentrations in eight multiple wells. After 72 hr of culture, 10 μl of CCK-8 reagent was added into each well and the 96-well plates were incubated for 1 hr at 37°C. The OD value was measured at the wavelength of 450 nm. The inhibition rates on 50 μg/ml were recorded. The IC_{50} values were also obtained from the plot of activity versus inhibitor concentration by using GraphPad Prism 5 software.

2.2.2 | Anti-CDK1/cyclin B kinase activity experiment

In vitro CDK1/cyclin B assay was performed as described by the manufacturer (Ha et al., 2016). All the test compounds

and positive controls were dissolved in DMSO to prepare 10 mmol/L stock solutions. The stock solutions were diluted 20 times by double distilled water and then 10 times by buffer to 50 μmol/L working concentration (two times of the test concentration) and stored in refrigerator at −20°C. CDK1/cyclin B kinase was aliquoted into the buffer and incubated with test compounds at various concentrations (25, 5, 1, 0.2, and 0.04 μmol/L) at 20°C for 30 min. The 10 μl mixture was incubated with buffer containing Reagent C (130 μl), Reagent D (20 μl), Reagent E (20 μl), and Reagent *F* (20 μl) at room temperature for 3 min, and then, the final mixture was immediately put into 30°C spectrophotometer (340-nm wavelength) for detection. The value of every minute was recorded until 90 min. The D-value between the 0-min value and the 90-min value is used to calculate the IC_{50} value.

2.2.3 | Molecular docking study

Molecular docking study was finished by CDOCKER program of Discovery Studio to explore the predicted binding mode of baicalein and compound **3o** with CDK1 (Yan et al., 2020). The co-crystal structure of CDK1/cyclin B-Cks2 with flavopiridol (PDB: 6GU2) was obtained from the PDB database. The edge water molecules were removed, and hydrogen atoms were added to the protein by clean protein module. And then, the corresponding amino acids were protonated and energy minimization was performed. The structures of baicalein and compound **3o** were introduced. Hydrogen atoms and CHARMm field were added. According to the binding position of flavopiridol in CDK1, a sphere with a radius of 9.0×10^{-10} m was set, and then, flavopiridol was extracted from the complex. Flavopiridol was re-docked to the original protein by CDOCKER program. The RMSD value of the docking conformation and crystal conformation was calculated to determine the credibility of docking results. According to the above conditions, baicalein and compound **3o** were docked to the flavopiridol binding site of CDK1. Other docking parameters in the program were kept default. The docking simulation using CDOCKER was prepared according to the user guidance. The binding pose figure was prepared by PyMOL.

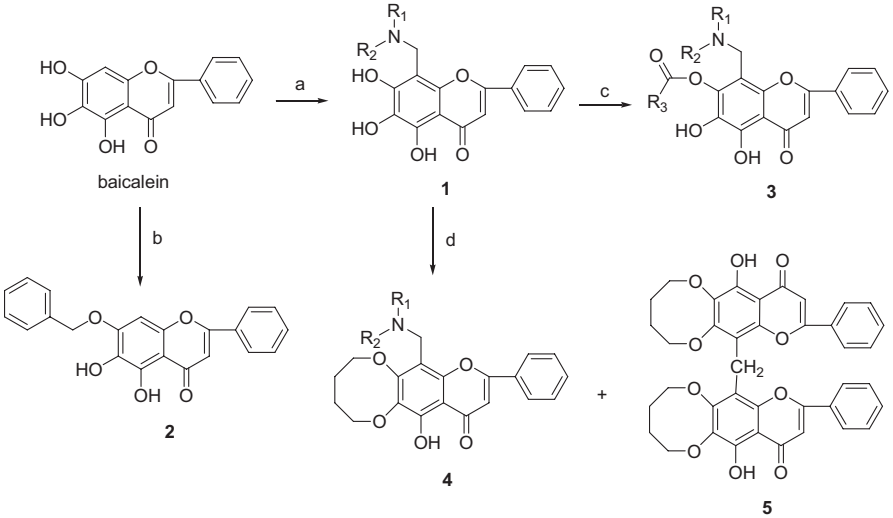
3 | RESULTS AND DISCUSSION

3.1 | Chemistry

The synthetic route of all target compounds is outlined in Scheme 1 with baicalein as the starting material. Compounds **1a–g** were synthesized by Mannich reaction of baicalein with formaldehyde and various secondary amines in methanol. Compound **2** was synthesized by etherification of baicalein with bromobenzyl under the catalyst of K_2CO_3 and KI in *N*, *N*-DMF. Compounds **3a-z** and **3A-C** were obtained by esterification of compounds **1a-g** with various carboxylic acids in the presence of *N*, *N′*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as catalysts in tetrahydrofuran (THF). Compounds **4a, b** and compound **5** were produced by etherification of compounds **1a, b** with 1,4-dibromobutane in a method similar to the synthesis of compound **2**.

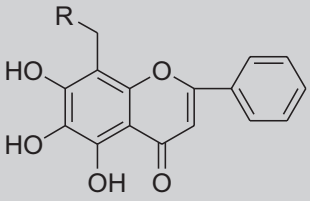
Compounds **1** (Table 1) were characterized by ^{1}H, ^{13}C-NMR techniques. Compounds **3** were characterized by ^{1}H, ^{13}C-NMR, and ESI-MS techniques and chemical method (Table 2). Compounds **2**, **4** (Table 3), and **5** were characterized by ^{1}H, ^{13}C-NMR, and ESI-MS techniques.

From ^{1}H-NMR, ^{13}C-NMR, and ESI-MS data, it can be confirmed that compounds **3** were generated by esterification of only one equivalent carboxylic acid with compounds **1**, which were single-substituted products. The single peak at δ12.9 ppm or so in the ^{1}H-NMR spectrum is the signal of 5-phenolic



SCHEME 1 Synthesis of baicalein derivatives. Reagents and conditions: (a) HCHO, NHR_1R_2/MeOH; (b) BnBr, K_2CO_3, KI/DMF; (c) carboxylic acid, DCC, HOBt/anhydrous THF; (d) $BrCH_2CH_2CH_2CH_2Br$, K_2CO_3, KI/DMF

TABLE 1 Substituent groups of compounds **1**



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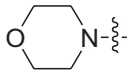
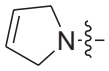
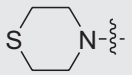
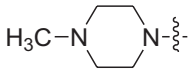
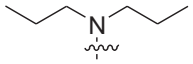
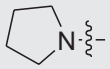
1a		**1e**	
1b		**1f**	
1c		**1g**	
1d			

hydroxyl. So esterification took place on 6- or 7-phenolic hydroxyl group. As there is no hydrogen atom on the ester bond, the 2D-NMR spectrum cannot determine whether the acylation is on the 6- or 7-phenolic hydroxyl group.

In our research, the position of acylation was confirmed by the chemical method. Because the flavonoids with *O*-diphenol hydroxyl groups in structure can form green to brown or even black precipitate (Scheme 2) with strontium chloride ($SrCl_2$) in ammonia methanol solution. By this method, it can be determined whether there are *O*-diphenol hydroxyl groups in compounds **3**, and then determined the position of substitution.

From the combination of ^{1}H, ^{13}C-NMR and ESI-MS techniques and chemical method, it can be confirmed that the accurate structures of compounds **3** were as in Scheme 1.

3.2 | Results of activity assays

3.2.1 | Anti-proliferative activity toward MCF-7 tumor cells

Cell counting kit-8 (CCK-8) assay was carried out for evaluating the anti-proliferative activities of the target compounds coupled with three positive controls. The IC_{50} value of flavopiridol is 0.08 μM, a little higher than the IC_{50} of 0.03 μM reported in the literature (Ahn et al., 2007). At the concentration of 50 μg/ml, the inhibition rates of the test compounds on MCF-7 tumor cells are shown in Table 4.

The results of inhibition rates at the concentration of 50 μg/ml showed that 20 target compounds had higher activities than that of baicalein (63.9%). Among them, compound **4a** exhibited the best inhibition rate (96.7%), comparable to CGP74514A (96.7%; Figure 1), which is a selective CDK1 inhibitor (Imbach et al., 1999), and better than flavopiridol (90.0%), while compounds **3C**, **3o**, and **3c** exhibited comparable activity with flavopiridol (87.8%, 86.9%, and 86.0%, respectively).

The substituent groups at position-7 and position-8 determined the activities of the target compounds.

For position-8, the 8-aminomethylene substitution is very important to inhibit the growth of tumor cells. Compound **2**, with a benzyloxy group at position-7 and no substituent group at position-8, showed no inhibitory activity toward MCF-7 tumor cell proliferation, suggesting the importance of the nitrogen-containing functional group in position-8. The reason may be that the nitrogen atom in the nitrogen-containing functional group can form hydrogen bond with the target, thus enhancing the activity of the compound. The results also demonstrated that compounds with position-8 linked with *N*-methylpiperazine methylene or thiomorpholine methylene have better activities. For example, the inhibition rate of **3o** is up to 86.9%, and **1b** is up to 84.8%. The reason may be due to the extra hydrogen bond interaction with target by the second nitrogen atom in *N*-methylpiperazine and the sulfur atom in thiomorpholine.

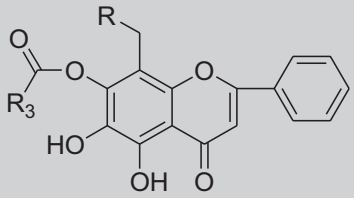
For position-7, cinnamoyloxy- and α-methacryloyloxy-substituted compounds have excellent activities. The inhibition rate of compound **3d** is 79.4%, and **3C** is 87.8%. The reason may be that compared with the aryl acyloxy group, cinnamoyloxy and α-methacryloyloxy groups have longer chains and better flexibility, can stretch into the active site of the target to form hydrophobic interactions.

In addition, 6, 7-bietherification products have better activities than those of 7-esterification products. For example, **4a** is the most active compound, with inhibition rate up to 96.7%. The reason may be that the hydrocarbon part of the octad dioxane can form hydrophobic interaction with the target. On the other hand, the oxygen atom of the ether bond is hydrophilic, while the hydrocarbon part is lipophilic, so it is easy to pass the membrane of tumor cells.

3.2.2 | Anti-CDK1/cyclin B kinase activity

In anti-MCF-7 tumor cell proliferative assay, a half of the target compounds showed good activities. In order to elucidate the CDK1/cyclin B kinase inhibition activities of the target compounds, the compounds with desirable anti-MCF-7 activities were chosen to conduct the anti-CDK1/cyclin B kinase activity assay. CGP74514A was used as positive control. The IC_{50} value of CGP74514A is 0.20 μM, comparable to the IC_{50} of 0.11 μM reported in the literature (Imbach et al., 1999). The results are shown in Table 5.

TABLE 2 Substituent groups of compounds **3**



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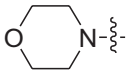
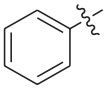
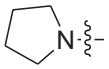
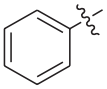
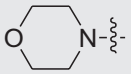
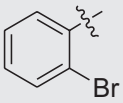
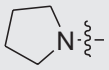
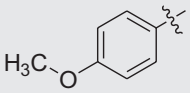
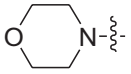
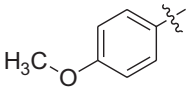
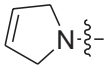
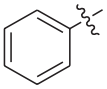
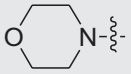
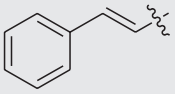
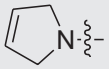
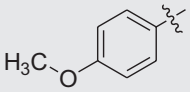
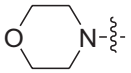
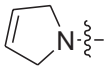
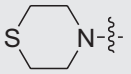
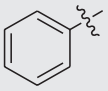
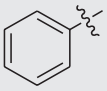
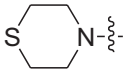
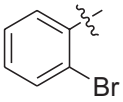
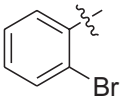
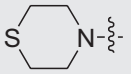
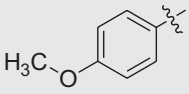
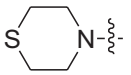
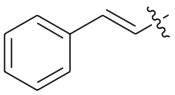
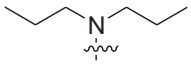
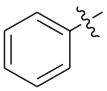
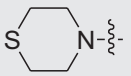
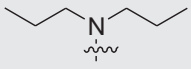
3a			**3p**		
3b			**3q**		
3c			**3r**		
3d			**3s**		
3e			**3t**		
3f			**3u**		
3g			**3v**		
3h			**3w**		
3i			**3x**		
3j			**3y**		
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3l			**3A**		
3m			**3B**		

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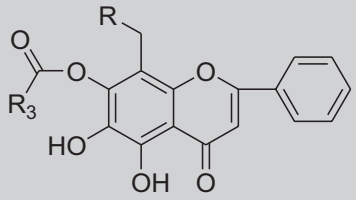
TABLE 2 (Continued)



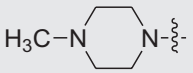
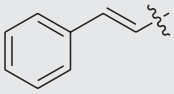
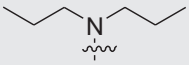
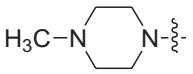
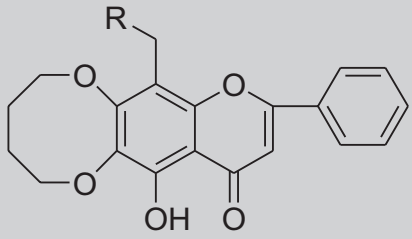
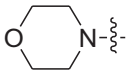
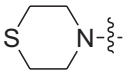
Cpds.	R	R3	Cpds.	R	R3
3n			3C		
3o					

TABLE 3 Substituent groups of compounds **4**



Cpds.	R	Cpds.	R
4a		4b	

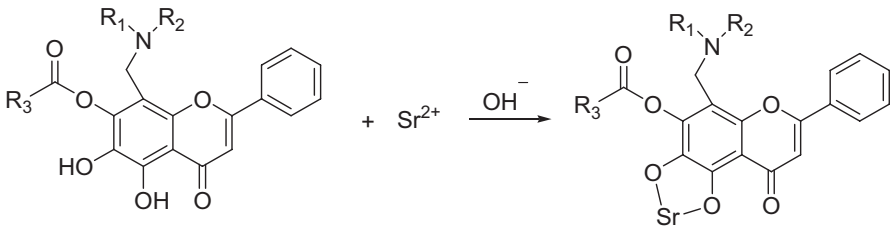
The results showed that most of the test compounds possessed good inhibitory activities against CDK1/cyclin B with the IC_{50} value distributing between 1.26 and 30.17 μM, which is, however, weaker than that of the positive control (IC_{50} = 0.20 μM). Compound **3o** showed the best anti-CDK1/cyclin B activity (IC_{50} = 1.26 μM), and compounds **3f**, **1b**, and **3C** had considerable activities (2.36, 2.43, and 2.72 μM, respectively).

Compounds **3o** and **3C** with 7-α-methacryloyloxy groups possessed excellent inhibitory activities against both MCF-7 tumor cells and CDK1/cyclin B kinase, which fully verified the importance of the 7-α-methacryloyloxy group in the target compounds, while compounds **4a** and **3c** only performed well in anti-MCF-7 tumor cell proliferative activity assay, which can be attributed to the ether groups in their structures. The better histocompatibility of ether bond makes it more permeable through the membrane of tumor cells, but it did not show any advantage in kinase activity assay. Compounds **1b** and **3f** with 8-thiomorpholine methylene groups showed desirable activities in anti-CDK1/cyclin B kinase activity assay. That coincides with their anti-MCF-7 tumor cell proliferative activity assay results.

In order to analyze the correlation between cell activity and kinase inhibition activity, the Pearson correlation coefficient analysis was performed using SPSS 19. From the analysis results, it can be seen that p value equals to .006 (<.01), indicating that there is a linear correlation between the two variables, and r value equals to .714, indicating that there is a strong correlation between the two variables. They were positively correlated with each other (Figure 4).

3.3 | Docking study of compound 3o with CDK1/cyclin B

Compound **3o** showed the best activity in anti-CDK1/cyclin B kinase activity assay. With the aim of exploring the interaction mode, molecular simulation work of compound **3o** binding with CDK1/cyclin B-Cks2-flavopiridol co-crystal complex (PDB: 6GU2) was carried out. Random conformation search was employed to identify predicted ligand–protein binding conformation that are closer to the crystal ones. The RMSD value of flavopiridol docking conformation



SCHEME 2 Reaction of *O*-diphenol hydroxyl with strontium chloride

TABLE 4 Inhibition rates of the test compounds (50 μg/ml) on MCF-7 tumor cells

Cpds.	Inhibition rate (%)	Cpds.	Inhibition rate (%)
1a	77.4	**3o**	86.9
1b	84.8	**3p**	16.3
1c	81.5	**3q**	53.5
1d	52.9	**3r**	62.2
1e	58.2	**3s**	7.7
1f	64.2	**3t**	54.8
1g	74.2	**3u**	30.1
2	0.0	**3v**	0.0
3a	0.0	**3w**	65.8
3b	11.9	**3x**	74.1
3c	86.0	**3y**	19.9
3d	79.4	**3z**	34.0
3e	78.4	**3A**	47.4
3f	76.8	**3B**	64.8
3g	56.7	**3C**	87.8
3h	45.2	**4a**	96.7
3i	79.6	**4b**	ND
3j	79.1	**5**	65.9
3k	62.0	Baicalein	63.9
3l	30.1	Flavopiridol	90.0
3m	14.8	CGP74514A	96.7
3n	78.9		

Note: ND means "not determined."

compared with its crystal conformation was 1.3209. So, the binding results are creditable. In the compound **3o**-CDK1 binding results, it can be seen that compound **3o** binds to CDK1's active site in a resembling stretching conformation with flavopiridol (Figure 5a). The chromone core of compound **3o** can occupy hinge region of CDK1 and forms hydrogen bonds with E81 and L83 and hydrophobic interactions with F80, V18, and V64. The phenyl group forms hydrophobic interactions with I10 and V18. The introduced 8-nitromethyl piperazine methylene moiety interacts with D146 just as flavopiridol does, while the introduced 7-acrylate group also interacts with D146 through hydrogen bond and V64 through hydrophobic interaction (Figure 5b). The fact that compound **3o** can form an extra interaction with D146 through its 7-acrylate group may be the reason for its best activity. It could be concluded that position-7 substitution is crucial to CDK1 binding. It is worth to mention that molecular docking has certain guiding significance for the structural modification, but it is not completely consistent with the actual situation.

TABLE 5 Activities of chosen target compounds

Cpds.	MCF-7 IC_{50} (μM)	CDK1/cyclin B IC_{50} (μM)
CGP74514A	7.74	0.20
1b	24.33	2.43
3c	24.29	16.72
3d	27.34	17.36
3e	28.48	18.21
3f	25.41	2.36
3i	30.14	NA
3j	28.23	NA
3n	29.46	19.27
3o	24.10	1.26
3w	26.16	5.16
3x	25.86	22.79
3B	32.11	30.17
3C	15.39	2.72
4a	20.18	NA
5	32.48	30.11

Note: NA means "no activity."

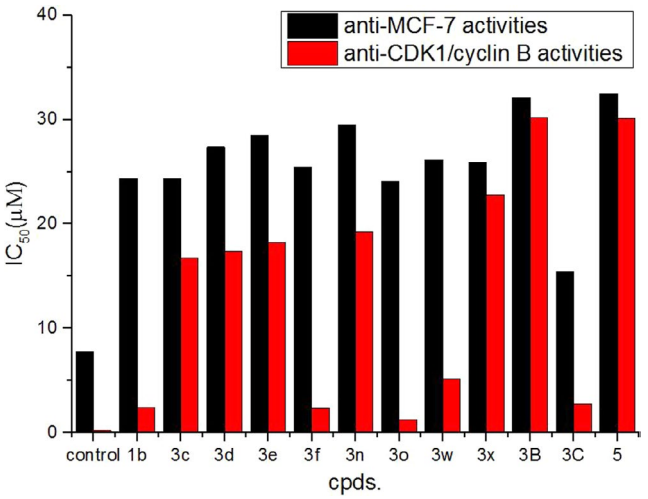


FIGURE 4 Bar chart of the IC_{50} of test compounds against MCF-7 tumor cells and CDK1/cyclin B

4 | CONCLUSIONS

A series of baicalein derivatives were designed and synthesized with CDK1 as the target. Anti-MCF-7 tumor cell proliferative assay results showed that compound **4a** possessed higher inhibition rate than flavopiridol and was comparable to CGP74514A at the concentration of 50 μg/ml. Half of the target compounds

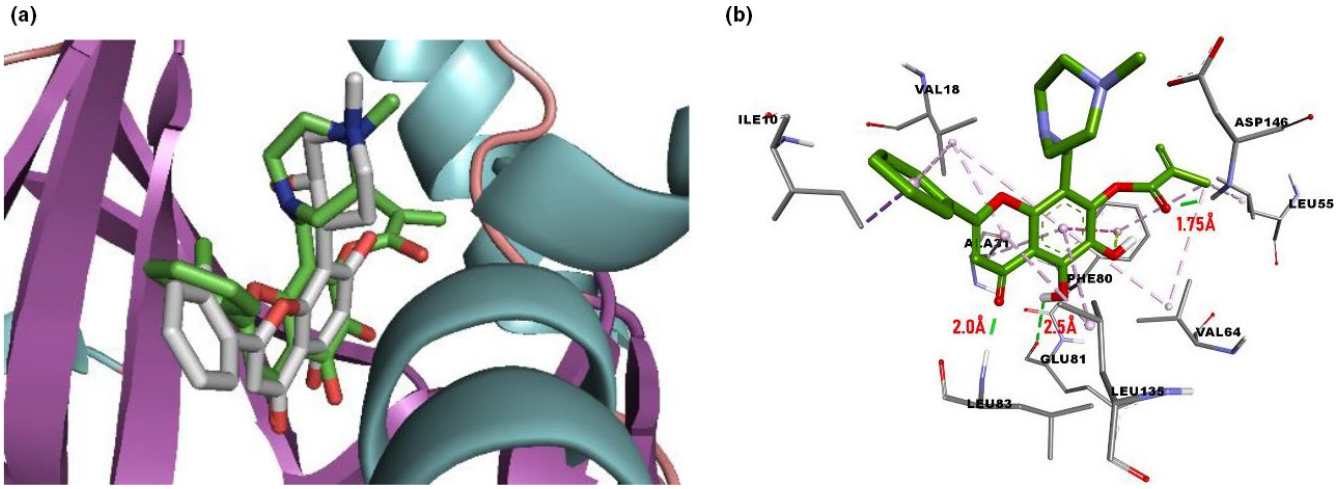


FIGURE 5 (a) Compound **3o** and flavopiridol bind to the hinge region of CDK1 with a similar stretching conformation (compound **3o** in green, while flavopiridol in pale). (b) Binding mode of compound **3o** with CDK1

exhibited better activities against MCF-7 proliferation than the lead compound baicalein. The nitrogen atom in position-8 can easily form hydrogen bond with the target, so 8-aminomethylene substitution, especially 8-*N*-methylpiperazine methylene or thiomorpholine methylene substitution, shows excellent activity toward tumor cells. Position-8 substitution is very important to baicalein derivatives' inhibition activity. Flexible substituent groups in position-7, for example, cinnamoyloxy and α-methacryloyloxy groups, could adapt to the active site of the target through hydrogen bond and hydrophobic interactions, the corresponding compounds showed considerable activities. 6, 7-Bietherification products have better activities than those of 7-esterification products. Anti-CDK1/ cyclin B kinase results of the test target compounds showed that compound **3o** has the best IC_{50}. The molecular docking results showed that compound **3o** can interact with the important amino acid residues E81, L83, and D146 of CDK1 through hydrogen bond just like flavopiridol does. And it can also form an extra hydrogen bond with D146 by its introduced 7-acrylate group, which flavopiridol does not have. So, position-7 substitution is also beneficial to CDK1 binding. Correlation analysis results demonstrated that there is a correlation between the inhibition of MCF-7 tumor cell proliferation and the inhibition of kinase activity.

Based on the above results, in our future work, fragment with two heteroatoms will be introduced to position-8 and more flexible hydrophobic groups will be introduced to positon-7 in baicalein. More 6, 7-bietherification products will further be synthesized to verify our conjecture. In addition, the quantitative SAR study should be further conducted with more compounds in the following research.

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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