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Nitrogen-containing flavonoid analogues as CDK1/cyclin B inhibitors: Synthesis, SAR analysis, and biological activity

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ABSTRACT

A series of nitrogen-containing flavonoid analogues were designed and synthesized by Mannich reaction, and screened for the inhibitory activities of cyclin-dependent kinases using a FRET-based biochemical assay method. The results showed that C-8 nitrogen-containing baicalein analogues **3a**–**3f** exhibited potent CDK1/Cyclin B inhibitory activities. 5,6,7-Trihydroxy-8-(dimethylaminomethyl)-2-phenyl-4*H*-chromen-4-one **3a**, 5,6,7-trihydroxy-8-(pyrrolid inylmethyl)-2-phenyl-4*H*-chromen-4-one **3b**, and 5,6,7-trihydroxy-8-(piperidinylmethyl)-2-phenyl-4*H*-chromen-4-one **3c** (IC_{50} 1.05–1.28 μM) were about sixfold more potent than baicalein **2** (IC_{50} 6.53 μM). 5,6,7-Trihydroxy-8-(morpholinomethyl)-2-phenyl-4*H*-chromen-4-one **3d**, 5,6,7-trihydroxy-8-(thiomorpholinomethy)-2-phenyl-4*H*-chrom en-4-one **3e**, and 5,6,7-trihydroxy-8-(4-methylpiperazinylmethyl)-2-phenyl-4*H*-chromen-4-one **3f** (IC_{50} 0.27–0.38 μM) were about 20-fold more potent than baicalein, and were at the same level as flavopiridol (IC_{50} 0.33 μM).

1. Introduction

Protein kinases regulate many critical biological mechanisms, including metabolism and cell growth, proliferation, and differentiation. Aberrations in the activity of the kinases involved in signal transduction have been linked to many human diseases such as cancer, diabetes, and inflammation. The discovery of more than 518 kinases encoded by the human genome has spurred development of rapid screening techniques for potential drugs against these enzymes.[1]

The cyclin-dependent kinases (CDKs) are a prominent family of protein kinases, which play a key role in cell-cycle progression and cellular proliferation.[2] CDKs are multi-subunit enzymes composed of at least a catalytic subunit protein kinases (CDK) and a regulatory subunit (cyclin).[3,4] In many tumors, such as melanomas, pancreatic and esophaegal cancers, endogenous cyclin-dependent kinase inhibitory proteins (CDKIs) are either absent or mutated. Consequently, selective CDK inhibitors may prove to be effective chemotherapeutic agents.[5] CDKs have become attractive therapeutic targets for cancer therapy.[6]

The semisynthetic flavonoid, flavopiridol (Scheme 1), is derived from rohitukine, which is an alkaloid flavonoid present in the Indian plant *Dysoxylum binectariferum*. Flavopiridol could induce cell-cycle arrest at both G1 and G2 phases, and is a potent inhibitor of CDK1, 2, 4, and 6 in a competitive manner with respect to ATP.[7] It is currently in Phase II clinical trials as an antitumor agent.[8]

On other hand, baicalein (5,6,7-trihydorxyflavone), a flavonoid present in the root of *Scutellaria baicalensis* Georgi, has been reported to inhibit cell proliferation in several types of cells. It mediated G1 and G2 growth arrest accompanied by the down-regulation of cyclin D2, cyclin A, CDK1, and CDK2, and up-regulation of p15Ink4B, $p21^{CIP1/Waf1}$, p53, and cyclin E.[9] In addition, baicalein-induced cell-cycle arrest and apoptosis in human lung squamous carcinoma CH27 cells through decreasing the expression and function of CDK4/cyclin B and D.[10] The effects of quercetinon on proliferation and cell-cycle arrest by modulation of CDK1/Cyclin B in prostate cancer cells (PC-3) have been reported. Quercetin led to substantial decrease in the expression of CDK1/Cyclin B1 and phosphorylated pRb while increase in p21. Flowcytometric analysis showed that quercetin blocks G2-M transition, with significant induction of apoptosis.[11] But, two naturally occurring bacailein and quercetin have poor CDK inhibitory activity. Both bacailein and quercetin lack nitrogen-containing ring (D-ring) present in flavopiridol. Therefore, the introduction of nitrogen atom to baicalein and quercetin was suggested to increase their CDKs inhibitory activity. In this paper, sixteen nitrogen-containing baicalein, quercetin, and chalcone analogues were designed and prepared through Mannich reaction. Totally 21 flavonoid analogues were assayed for their CDK1/Cyclin B inhibitory activity using a fluorescence-based, coupled-enzyme format biochemical assay screening procedure from Z′-LYTE™. In addition, the structure–activity relationship (SAR) was summarized herein.

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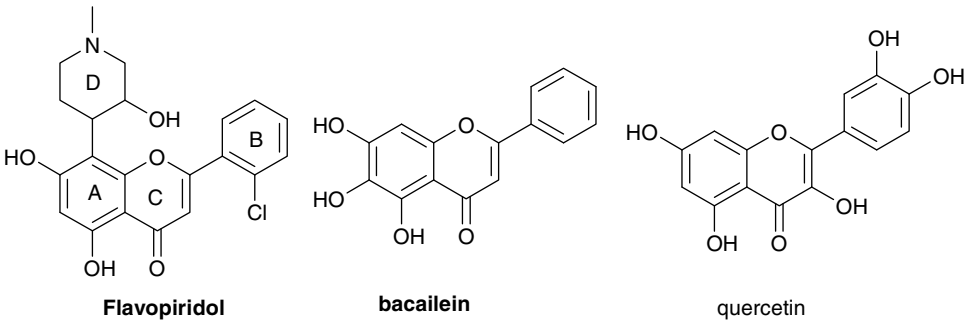
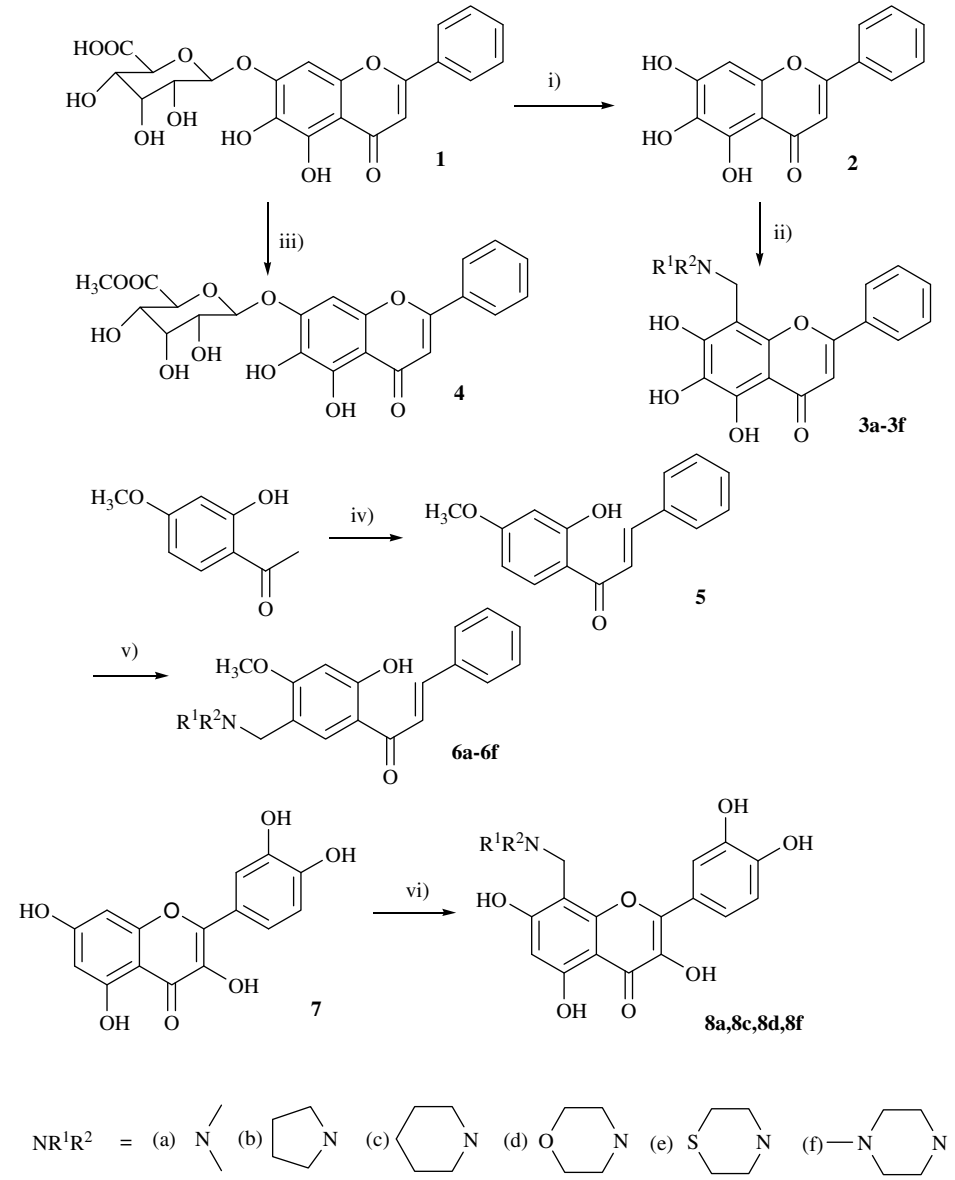


Scheme 1. Chemical structure of flavopiridol, bacailein and quercetin.

2. Chemistry

Nitrogen-containing flavonoid analogues **3a**–**3f**, **6a**–**6f** and **8a**, **8c**, **8d**, **8f** were prepared as shown in Scheme 2. Baicalin **1** was hydrolyzed with 50% H_2SO_4 at 95 °C to provide baicalein **2**. Compound **4** was prepared by treating baicalin **1** with methanol in the presence of catalytic amount of concentrated H_2SO_4. Condensation of 2-hydroxy-4-methoxy-acetophenone with benzaldehyde in 40% KOH, 50% ethanol solution at room temperature for 20 h provided compound **5**. Compounds **3**, **6**, and **8** were prepared by Man-



Scheme 2. Reagents and conditions: Synthesis of the flavonoid analogues. (i) 50% H_2SO_4, 95 °C; (ii) secondary amine, 37% HCHO, CH_3OH; (iii) CH_3OH, H_2SO_4; (iv) benzaldehyde, 40% KOH, EtOH, H_2O; (v) secondary amine, 37% HCHO, isopropanol, HCl; (vi) secondary amine, 37% HCHO, DMSO.

nich reaction using flavonoids, various secondary amines, and formaldehyde solution in ethanol at 20–60 °C yielding the desired analogues.

3. Result and discussion

The flavonoid analogues were assayed for their CDK1/Cyclin B inhibitory activity according to the procedure instruments of Z′-LYTE™ kinase assay kits. The results (ATP 10 μM) for flavonoid analogues **2**–**22** are shown in Table 1. Compounds **3a**–**3c** showed IC_{50} values at 1.05, 1.22, and 1.28 μM, respectively, and were about six-fold more potent than baicalein **2** (IC_{50} 6.53 μM), or 15-fold more potent than baicalin **1** (IC_{50} 14.36 μM). Compounds **3d**–**3f** showed IC_{50} values at 0.28, 0.38, and 0.27 μM, (Figs. 1–3), respectively, and were about 20-fold more potent than baicalein **2**, or about 50-fold more potent than baicalin **1**, at the same level as flavopiridol (IC_{50} 0.33 μM). It was indicated that the presence of the nitrogen on the position C-8 is critical, and introduction of second heteroatom on the position C-8 would significantly increase inhibitory potency. The nitrogen-containing baicalein analogues were analyzed by HPLC-UV (C18 reversed-phase column, the elution system consisted of water/methanol/acetonitrile/formic acid (59:30:10:1, v/v); the flow rate was 1.0 mL/min, and the injection volume was 10 μL); the retention time of **3a**–**3c** which have one heteroatom on the position C-8 was **3c** (21.7 min) > **3b** (18.2 min) > **3a** (13.4 min); the retention time of **3d**–**3f** which have two heteroatoms on the position C-8 was **3e** (17.0 min) > **3d** (12.0 min) > **3f** (10.3 min). The inhibitory potency of **3a**–**3c** was **3a** > **3b** > **3c**, and the inhibitory potency of **3d**–**3f** were **3f** > **3d** > **3e**; it was indicated that the inhibitory potency will increase accompanied with the rise of hydrophilia of the test compounds.

Table 1
CDK1/cyclin B inhibitory activities of flavonoid analogues

No.	Compounds	IC_{50} (μM)	No.	Compounds	IC_{50} (μM)
1	Flavopiridol	0.33	12	**6a**	>20
2	**1**	14.36	13	**6b**	>20
3	**2**	6.53	14	**6c**	>20
4	**3a**	1.05	15	**6d**	>20
5	**3b**	1.22	16	**6e**	>20
6	**3c**	1.28	17	**6f**	>20
7	**3d**	0.28	18	**7**	>20
8	**3e**	0.38	19	**8a**	15.74
9	**3f**	0.27	20	**8c**	18.69
10	**4**	11.64	21	**8d**	>20
11	**5**	>20	22	**8f**	>20

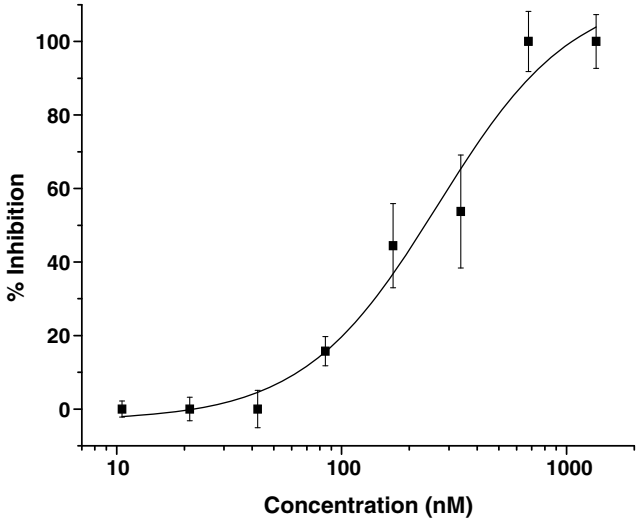


Figure 1. CDK1/cyclin B inhibitory activity of 5,6,7-trihydroxy-8-(morpholinomethyl)-2-phenyl-4*H*-chromen-4-one **3d**.

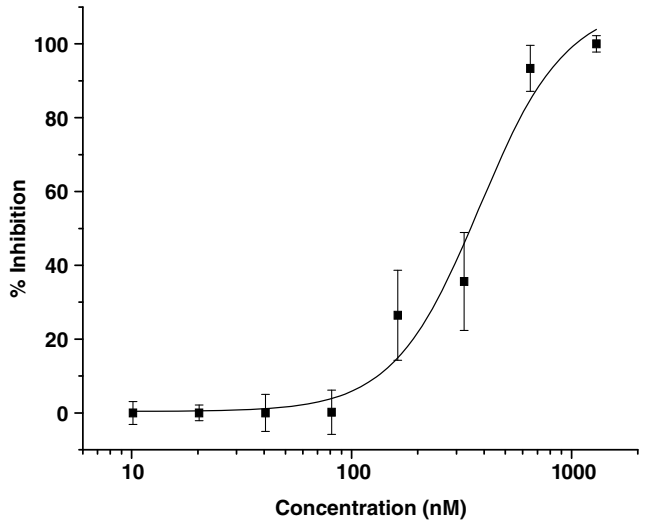


Figure 2. CDK1/cyclin B inhibitory activity of 5,6,7-trihydroxy-8-(thiomorpholinomethyl)-2-phenyl-4*H*-chromen-4-one **3e**.

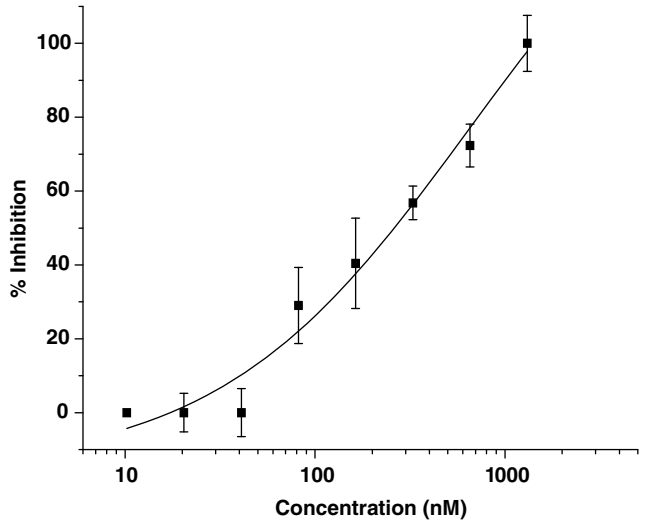


Figure 3. CDK1/cyclin B inhibitory activity of 5,6,7-trihydroxy-8-((4-methylpiperazin-1-yl)methyl)-2-phenyl-4*H*-chromen-4-one **3f**.

According to the X-ray crystal structure of des-chloroavopiridol with CDK2,[12] the piperidine nitrogen, on the position C-8 partially occupies the adenine amidogen binding region, the piperidine nitrogen and Asp 145 interaction seems to be very critical for CDK1/Cyclin B inhibitory activity. Thus, our SAR studies on C-8 were consistent with the literature above. Baicalins **1** and **4** showed IC_{50} values at 14.36, 11.64 μM, respectively, and were twofold less active compared with baicalein, IC_{50} values 6.53 μM; these indicate that the C-7 hydroxyl is meaningful for the inhibition of kinase activity. The X-ray crystal structure of des-chloroavopiridol with CDK2 also indicates that the C-5 hydroxyl and the C-4 carbonyl are involved in critical hydrogen bonds with E-81 and L-83 in the adenine binding pocket. Screening of the commercially available flavonoids showed that at least one hydroxyl group at either C-3 or C-5 of chromone was neces-

sary for inhibitory activities against CDK2.[13] Our results are consistent with the conclusions above, and chalcone analogues **6a–6f** showed no concentration-dependent inhibitory activity, due to the absence of C-2′ and C-5′ hydroxyl. Quercetin analogues almost have no inhibitory activity. These indicate that the presence of hydrophilic substituent groups such as hydroxyl group on the position C-2 would be detrimental to CDK1/Cyclin B inhibitory potency.

4. Conclusions

Our SAR studies on flavonoid analogues for their CDK1/Cyclin B inhibitory activity were suggested as follows: (1) the presence of the nitrogen on position C-8 is critical, and introduction of second heteroatom on position C-8 would increase inhibitory potency. In the same type of substitutes, the inhibitory potency will increase accompanied with the hydrophilicity of the substitute rising because the nitrogen group on position C-8 partially occupies the region which the water-solubility substitutes amidogen group of ATP bind with CDK1/Cyclin B. (2) C-5, C-6, C-7 hydroxyl, and C-4 carbonyl are vital for inhibitory activity since these acidic groups occupy the region of ATP phosphate group bind with CDK1/Cyclin B. (3) The lipophilic substitutes on position C-2 are important, because these lipophilic substitutes occupy lipophilic binding pocket of CDK1/Cyclin B while ATP does not have these lipophilic substitutes. In other words, hydrophilic substitutes would be detrimental to inhibitory potency.

In summary, three compounds with potent CDK1/Cyclin B kinase inhibitory activity were selected from the series of nitrogen-containing flavonoid analogues we designed and prepared. Those compounds were suggested to target the ATP binding site of the kinase as flavopiridol. The CDK1/Cyclin B inhibitory activity of **3d–3f** (IC_{50} 0.27–0.38 μM) was at the same level as flavopiridol (IC_{50} 0.33 μM); compound **3f** was firstly designed and prepared, which was expected to be novel series of CDK1/Cyclin B inhibitors.

5. Experiments

5.1. Instrument

IR spectra were obtained as KBr pellets on a Nicolet 50X FT-IR spectrophotometer. NMR spectra were measured by a Varian INOVA-400 spectrometer, with chemical shifts reported in parts per million (in DMSO, TMS as an internal standard, *J* values were given in Hertz). Mass spectra were obtained on a HP1100LC-MSD spectrometer. HPLC-UV separations were performed on a Shimadzu (Kyoto, Japan) C18 reversed-phase column (150 × 4.6 mm id 5łμm). Melting points are uncorrected.

5.2. Biochemical assay

The CDK1/Cyclin B (Invitrogen Corporation, CA, USA) enzyme activity was measured by a fluorescence kinetic assay using the Z′-LYTE™ Kinase Assay Kits (Invitrogen Corporation, CA, USA). The assay was performed at room temperature (23 °C) in 384-well assay plate. The total volume was 20 μL (contain 2.5 μL 4× test compound, 2.5 μL 4× ATP solution, 5 μL 2× peptide substrate/CDK1/Cylin B mixture, 5 μL development reagent solution, 5 μL stop reagent). The final concentrations of the assay constituents were 240 ng/ml CDK1/Cylin B, 2 μM peptide substrate, and 10 μM ATP. Generally, the compounds were solubilized in 1% DMSO and added to the reaction mixture at eight concentrations (twofold across) with the highest concentration of 20 μM for the flavonoid analogues. The representative compounds **3d–3f** were screened at eight concentrations (twofold across)with the highest concentration of 1 μM to determine and prove IC_{50} values again following the same procedure, respectively. Continuous kinetic monitoring of enzyme activity was performed on Fluoroskan Ascent® FL from thermo scientific (λ_{ex} 400 nm, λ_{em} 445 nm, and λ_{em} 520 nm). The experiment was performed in triplicate and the percent inhibition of enzyme activity was calculated for all the compounds at each concentration.

5.2.1. Baicalein (2)

To a stirred mixture of baicalin (**1**) (from *Scutellaria baicalensis* Geoygi. mp 206 °C) (2 g, 7.41 mmol) in H_2O (10 ml) was added 50% concentrated sulfuric acid (50 ml) at 90 °C, and the mixture was stirred for 10 min and then was poured in ice water (100 ml). The precipitate that formed was collected by filtration, washed, and recrystallized from acetone to give the product as yellow solid (0.36 g, 29.3%). Mp 264–266 °C; MS (API-ES positive) *m*/*z*: 271.1 $[M+H]^+$; IR (KBr, cm^{-1}): 3409, 3093, 1659, 1620, 1586; ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.64 (s, 1H, 5-OH), 10.55 (s, 1H, 7-OH), 8.79 (s, 1H, 6-OH), 8.03–8.05 (t, *J* = 6.4, 1.6 Hz, 2H, Ar-2′, 6′-H), 7.52–7.58 (m, 3H, Ar-3′,4′,5′-H), 6.91 (s, 1H, C═CH), 6.60 (s, 1H, Ar-8-H).

5.2.2. 5,6,7-Trihydroxy-8-(dimethylamino)-2-phenyl-4*H*-chromen-4-one (3a)

To the solution of baicalein (**2**) (0.2 g, 0.74 mmol) in methanol (14 ml) were added dropwise 37% formaldehyde solution (0.0741 g, 0.89 mmol) and 33% dimethylamine solution (0.27 g, 2.00 mmol). The mixture was stirred at room temperature for 2 h. The precipitates were collected, filtered, and washed several times with methanol to get the product as yellow solid (0.41 g, 79.0%). Mp 280–282 °C; MS (API-ES positive) *m*/*z*: 328.1 $[M+H]^+$, 350.1 $[M+Na]^+$, 655.3 $[2M+H]^+$, 677.2 $[2M+Na]^+$; IR (KBr, cm^{-1}): 3403, 3093, 2925, 2861, 1637, 1570, 1509; ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.62 (s, 1H, 5-OH), 8.06–8.05 (m, 2H, Ar-2′, 6′-H), 7.58–7.57 (m, 3H, Ar-3′,4′,5′-H), 6.83 (1H, s, C═CH), 4.23 (s, 2H, CH_2), 2.63 (s, 6H, $N(CH_3)_2$).

5.2.3. 5,6,7-Trihydroxy-8-(pyrrolidin-1-ylmethyl)-2-phenyl-4*H*-chromen-4-one (3b)

From 5,6,7-trihydroxy-8-((dimethylamino)methyl)-2-phenyl-4*H*-chromen-4-one (**3a**), yellow solid (71%) was determined. Mp 213–215 °C; MS (API-ES negative) *m*/*z*: 352.1 $[M-H]^-$; IR (KBr, cm^{-1}): 3407, 3030, 2975, 2869, 1637, 1580, 1521; ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.58 (s, 1H, 5-OH), 8.04–8.03(m, 2H, Ar-2′, 6′-H), 7.57–7.55 (m, 3H, Ar-3′,4′,5′-H), 6.78 (s, 1H, C═CH), 4.34 (s, 2H, CH_2), 3.11 (s, 4H, NCH_2), 1.89 (s, 2H, CH_2).

5.2.4. 5,6,7-Trihydroxy-8-(piperidin-1-ylmethyl)-2-phenyl-4*H*-chromen-4-one (3c)

From 5,6,7-trihydroxy-8-((dimethylamino)methyl)-2-phenyl-4*H*-chromen-4-one (**3a**), yellow solid (71.5%) was obtained. Mp 209–211 °C; MS (API-ES positive) *m*/*z*: 368.1 $[M+H]^+$, 735.3 $[2M+H]^+$, 757.3 $[2M+Na]^+$; IR (KBr, cm^{-1}): 3400, 3050, 2925, 2861, 1637, 1570, 1509; ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.68 (s, 1H, 5-OH), 8.04–8.02 (m, 2H, Ar-3′,4′,5′-H), 7.57 (m, 3H, Ar-3′,4′,5′-H), 6.84 (s, 1H, C═CH),4.16 (s, 2H, CH_2), 2.84 (s, 4H, NCH_2), 1.62 (s, 4H, CH_2),1.48 (s, 2H, CH_2).

5.2.5. 5,6,7-Trihydroxy-8-(morpholinomethyl)-2-phenyl-4*H*-chromen-4-one (3d)

FromP 5,6,7-trihydroxy-8-((dimethylamino)methyl)-2-phenyl-4*H*-chromen-4-one (**3a**), yellow solid (69.0%) was obtained. Mp 275–277 °C; MS (API-ES positive) *m*/*z*: 370.0 $[M+H]^+$; IR (KBr, cm^{-1}): 3402, 3040, 2936, 2861, 1647, 1560, 1502; ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.74 (s, 1H, 5-OH) 8.09–8.08 (m, 2H, Ar-

2′,6′-H), 7.60 (m, 3H, Ar-3′, 4′,5′-H), 6.95 (s, 1H, C=CH), 3.96 (s, 2H, CH_2), 3.61 (s, 4H, OCH_2), 2.62 (s, 4H, NCH_2).

5.2.6. 5,6,7-Trihydroxy-8-(thiomorpholinomethyl)-2-phenyl-4*H*-chromen-4-one (3e)

From 5,6,7-trihydroxy-8-((dimethylamino)methyl)-2-phenyl-4*H*-chromen-4-one (**3a**), yellow solid (65%) was obtained. Mp 242–244 °C; MS (API-ES positive) *m*/*z*: 386.1 $[M+H]^+$; IR (KBr, cm^{-1}): 3378, 3050, 2925, 2861, 1637, 1570, 1509; ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.64 (s, 1H, 5-OH),8.03–8.01 (m, 2H, Ar-2′,6′-H), 7.55–7.53 (m, 3H, Ar-3′, 4′,5′-H), 6.88 (s, 1H, C=CH), 3.91 (s, 2H, CH_2), 2.81–2.80 (m, 4H, SCH_2), 2.62–2.61 (m, 4H, NCH_2).

5.2.7. 5,6,7-trihydroxy-8-((4-methylpiperazin-1-yl)methyl)-2-phenyl-4*H*-chromen-4-one (3f)

From 5,6,7-trihydroxy-8-((dimethylamino)methyl)-2-phenyl-4*H*-chromen-4-one (**3a**), yellow solid (72.2%) was obtained. Mp 236–238 °C; MS (API-ES positive) *m*/*z*: 383.1 $[M+H]^+$, *m*/*z*: 405.1 $[M+Na]^+$, 765.3 $[2M+H]^+$, 787.3 $[2M+Na]^+$; IR (KBr, cm^{-1}): 3402, 3070, 2955, 2841, 1639, 1575, 1503; ^1H NMR (DMSO-d_6, 400 MHz) δ: 12.65 (s, 1H, 5-OH), 8.02–8.01 (m, 2H, Ar-2′, 6′-H), 7.55–7.53 (m, 3H, Ar-3′,4′,5′-H), 6.87 (s, 1H, C=CH), 3.96 (s, 2H, CH_2), 2.62 (s, 8H, NCH_2), 2.13 (s, 3H, NCH_3).

5.2.8. Baicalin methylate (4)

To the solution of baicalin (5 g, 11.66 mmol) in methanol (250 ml) was added catalytic amount of sulfuric acid, and the solution was heated at reflux temperature for 1 h. After filtration, the filtrate solution was cooled at room temperature, and solvent was removed under reduced pressure, to give the compound (**4**) as yellow powder (5.1 g, 95%). Mp 268–270 °C. MS (API-ES positive) *m*/*z*: 459.1 $[M+H]^+$;^1H NMR (DMSO-d_6, 400 MHz) δ: 12.75 (s, 1H, 5-OH), 10.38 (s, 1H, 4′-OH), 8.62 (s, 1H, 6-OH), 8.03–8.05 (m, 2H, Ar-2′,6′-H), 7.52–7.58 (m, 3H, Ar-3′, 4′, 5′-H), 6.91 (s, 1H, C=CH_2), 6.60 (s, 1H, Ar-8-H), 5.26–5.28 (d, 1H, 1″-H), 4.19–4.21 (d, 1H, 5″-H), 3.67 (3H, s, –OCH_3), 3.36–3.45 (m, 3H, 2″,3″,4″-H).

5.2.9. (*E*)-1-(2-Hydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (5)

To the solution of 2-hydroxy-4-methoxy-acetophenone (2.0 g, 12.0 mmol) in ethanol (20 ml) was added 40% KOH solution (30 ml), then was added dropwise benzaldehyde, and the solution was stirred at room temperature for 20 h. The solution was neutralized to pH 7–8 with hydrochloric acid, and the precipitates were collected, filtered, and washed several times with methanol to give a yellow solid (1.79 g, 58%).

5.2.10. (*E*)-1-(5-((dimethylamino)methyl)-2-hydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (6a)

To the solution of (**5**) (0.5 g,1.95 mmol) were added paraformaldehyde (0.088 g, 2. 93 mmol) and 33% dimethylamine solution (0.27 g, 2.00 mmol) in 10 ml isopropanol, in the presence of catalytic amount of hydrochloride, and the mixture was stirred, and fluxed for 6 h, the mixture was cooled and evaporated to dryness. 20 ml of 10% acetic acid was added to the solid residue, and the mixture was filtered and the solution was neutralized to pH 7–8 with ammonia; the precipitates were filtered and washed with water, and the residue was eluted through a silica-gel column with CH_2Cl_2/CH_3OH (20:1) to give (**6a**) as yellow solid (0.254 g, 42.0%). Mp 188–190 °C; MS (API-ES positive) *m*/*z*: 312.1 $[M+H]^+$; IR (KBr, cm^{-1}): 3425, 3020, 2927, 1643, 1570, 1509; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.44 (s, 1H, OH), 8.62 (s, 1H, Ar-2′-H), 8.10–8.14 (d, 1H, *J* = 16 Hz, CH=CH), 7.96–7.93 (m, 2H, Ar-2,6-H), 7.85–7.81 (m, 1H, *J* = 16 Hz, CH=CH), 7.50 (s, 3H, Ar-3,4,5-H), 6.87 (s, 1H, Ar-5′-H), 4.25 (s, 2H, CH_2), 4.00 (s, 3H, CH_3), 2.77 (s, 6H, CH_3).

5.2.11. (*E*)-1-(2-Hydroxy-4-methoxy-5-(pyrrolidin-1-ylmethyl)phenyl)-3-phenylprop-2-en-1-one (6b)

From (*E*)-1-(5-((dimethylamino)methyl)-2-hydroxy-4-methoxyphenyl)-3-phenylprop- 2-en-1-one (**6a**), yellow solid (38.2%) was obtained. Mp 128–130 °C; MS (API-ES positive) *m*/*z*: 338.2 $[M+H]^+$; IR (KBr, cm^{-1}): 3389, 3037, 2925, 1636, 1570, 1498; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.35 (s, 1H, OH), 8.62 (s, 1H, Ar-2′-H), 8.17–8.13 (d, 1H, *J* = 16 Hz, CH=CH), 7.90–7.89 (m, 2H, Ar-2,6-H), 7.88–7.84 (d, 1H, *J* = 16 Hz, CH=CH), 7.43 (s, 3H, Ar-3,4,5-H), 6.64 (s, 1H, Ar-5′-H), 4.26 (s, 2H, CH_2), 3.95 (s, 3H, OCH_3), 2.50 (s, 4H, NCH_2), 1.05–1.03 (m, 4H, CH_2).

5.2.12. (*E*)-1-(2-Hydroxy-4-methoxy-5-(piperidin-1-ylmethyl) phenyl)-3-phenylprop-2-en-1-one (6c)

From (*E*)-1-(5-((dimethylamino)methyl)-2-hydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (**6a**), yellow solid (43.5%) was obtained. Mp 155–157 °C; MS (API-ES positive) *m*/*z*: 352.2 $[M+H]^+$; IR (KBr, cm^{-1}): 3375, 3010, 2974, 1671, 1609, 1474; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.35 (s, 1H, OH), 8.60 (s, 1H, Ar-2′-H), 8.07–8.03 (d, 1H, *J* = 16 Hz, CH=CH), 7.90–7.89 (m, 2H, Ar-2,6-H), 7.82–7.78 (d, 1H, *J* = 16 Hz,CH=CH), 7.43 (s, 3H, Ar-3,4,5-H), 6.64 (s, 1H, Ar-5′-H), 4.17 (s, 2H, CH_2), 3.87 (s, 3H, CH_3), 3.26 (s,4H,CH_2), 2.62–2.66 (m, 4H, CH_2), 0.97–0.98 (m, 2H, CH_2).

5.2.13. (*E*)-1-(2-Hydroxy-4-methoxy-5-(morpholinomethyl)-phenyl)-3-phenylprop-2-en-1-one (6d)

From (*E*)-1-(5-((dimethylamino)methyl)-2-hydroxy-4-methoxy phenyl)-3-phenylprop- 2-en-1-one (**6a**). Yellow solid (41.7%) was obtained. Mp 163–165 °C; MS (API-ES positive) *m*/*z*: 354.2 $[M+H]^+$; IR (KBr, cm^{-1}): 3410, 3057, 2935, 1637, 1570, 1500; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.38 (s, 1H, OH) 8.61 (s, 1H, Ar-2′-H), 8.20–8.16 (d, 1H, *J* = 16 Hz, CH=CH), 7.98–7.97 (m, 2H, Ar-2,6-H), 7.87–7.83 (d, 1H, *J* = 16 Hz, CH=CH), 7.48–7.47 (s, 3H, Ar-3,4,5-H), 6.68 (s, 1H, Ar-5′-H), 4.28 (s, 2H, CH_2), 3.88 (s, 3H, CH_3), 3.35–3.31 (m, 4H, OCH_2), 3.11–3.07 (m, 4H, NCH_2).

5.2.14. (*E*)-1-(2-Hydroxy-4-methoxy-5-(thiomorpholinomethyl)-phenyl)-3-phenylprop-2-en-1-one (6e)

From (*E*)-1-(5-((dimethylamino)methyl)-2-hydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (**6a**), yellow solid (38.2%) was obtained. Mp 154–156 °C; MS (API-ES positive) *m*/*z*: 370.1 $[M+H]^+$; IR (KBr, cm^{-1}): 3350, 3047, 2933, 1633, 1565,1496; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.35 (s, 1H, OH), 8.59 (s, 1H, Ar-2′-H), 8.07–8.03 (d, 1H, *J* = 16 Hz, CH=CH), 7.90–7.89 (m, 2H, Ar-2,6-H), 7.86–7.82 (d, 1H, *J* = 16 Hz, CH=CH), 7.43 (s, 3H, Ar-3,4,5-H), 6.64 (s, 1H, Ar-5′-H), 3.87 (s, 2H, CH_2), 3.49 (s, 3H, OCH_3), 2.67 (s, 4H, SCH_2), 2.60 (s, 2H, NCH_2).

5.2.15. (*E*)-1-(2-Hydroxy-4-methoxy-5-(4-methylpiperazin-1-ylmethyl)phenyl)-3-phenylprop-2-en-1-one (6f)

From (*E*)-1-(5-((dimethylamino)methyl)-2-hydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (**6a**), yellow solid (44.4%) was obtained. Mp 148–150 °C; MS (API-ES positive) *m*/*z*: 367.2 $[M+H]^+$; IR (KBr, cm^{-1}): 3361, 3038, 2783, 1632, 1570, 1498; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.38 (s, 1H, OH), 8.51 (s, 1H, Ar-2′-H), 8.20–8.16 (d, 1H, *J* = 16;Hz, CH=CH), 7.98–7.97 (m, 2H, Ar-2,6-H), 7.87–7.83 (d, 1H, *J* = 16 Hz, CH=CH), 7.48–7.47 (s, 3H, Ar-3,4,5-H), 6.57 (s, 1H, Ar-5′-H), 3.86 (s, 2H, CH_2), 3.44 (s, 3H, OCH_3), 2.41–2.38 (m, 8H, CH_2), 2.13 (s, 3H, NCH_3).

5.2.16. 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-8-(dimethylaminomethyl)-4*H*-chromen-4-one (8a)

To the solution of quercetin (**7**) (from *Sophora japonica* L. Mp 314 °C) (0.5 g, 1.66 mmol) in DMSO was added 37% formaldehyde solution (0.161 g, 1.99 mmol)) and 33% dimethylamine solution (0.27 g, 2.00 mmol). The solution was stirred at room temperature for 2 h, and the mixture was evaporated to dryness. The resulting residue was purified by TLC, silica-gel GF254, 1-butanol/water/acetic acid = 4:1:1, yellow solid. MS (API-ES positive) *m*/*z*: 360.1 $[M+H]^+$; ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.74 (m, 1H, Ar-6′-H), 7.60 (m, 1H, Ar-2′-H), 6.92 (m, 1H, Ar-5′-H), 6.19 (s, 1H, Ar-6-H), 3.86 (s, 2H, CH_2), 2.16 (s, 6H, NCH_3).

5.2.17. 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-8-(piperidin-1-ylmethyl)-4*H*-chromen-4-one (8c)

From 2-(3,4-dihydroxyphenyl)-8-((dimethylamino)methyl)-3,5,7-trihydroxy-4*H*-chromen-4-one (**8a**), yellow solid was obtained. MS (API-ES positive) *m*/*z*: 400.2 $[M+H]^+$; ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.72 (d, 1H, Ar-6′-H), 7.61 (d, 1H, Ar-2′-H), 6.92 (d, 1H, Ar-5′-H), 6.19 (s, 1H, Ar-6-H), 3.90 (s, 2H, CH_2), 2.84 (s, 4H, NCH_2), 1.62 (s, 4H, CH_2),1.48 (s, 2H, CH_2).

5.2.18. 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-8-(morpholinomethyl)-4*H*-chromen-4-one (8d)

From 2-(3,4-dihydroxyphenyl)-8-((dimethylamino)methyl)-3,5,7-trihydroxy-4*H*-chromen-4-one **(8a**), yellow solidwas obtained. MS (API-ES positive) *m*/*z*: 402.1 $[M+H]^+$; ^1H NMR (DMSO-d_6, 400 MHz)δ: 7.74 (d, 1H, Ar-6′-H), 7.60 (d, 1H, Ar-2′-H), 6.92 (d, 1H, Ar-5′-H), 6.19 (s, 1H, Ar-6-H), 3.86 (s, 2H, CH_2), 3.17 (s, 4H, OCH_2), 2.51 (s, 4H, NCH_2).

5.2.19. 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-((4-methylpiperazin-1-yl)methyl)-4*H*-chromen-4-one (8f)

From 2-(3,4-dihydroxyphenyl)-8-((dimethylamino)methyl)-3,5,7-trihydroxy-4*H*-chromen-4-one (**8a**), yellow solid was obtained. MS (API-ES positive) *m*/*z*: 415.2 $[M+H]^+$; ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.70 (d, 1H, Ar-6′-H), 7.57 (d, 1H, Ar-2′-H), 6.88 (d, 1H, Ar-5′-H), 6.17 (s, 1H, Ar-6-H), 3.96 (s, 2H, CH_2), 2.64 (t, 8H, NCH_2), 2.14 (s, 3H, NCH_3).

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