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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Investigation on the substitution effects of the flavonoids as potent anticancer agents: a structure–activity relationships study

Xiao-Bing Wang · Wei Liu · Lei Yang · Qing-Long Guo · Ling-Yi Kong

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Abstract Three series of flavonoid analogues substituted with different aminomethyl substitutions at C-6, C-7, and C-8 were designed and synthesized for the structure–activity relationship studies as potent anticancer agents. The prepared analogues were evaluated for their in vitro inhibitory activity against the growth of the hepatic cancer cell lines HepG2 and SMMC-7721. Structure–activity relationships indicated that not only the compounds with amino methyl groups were more active than those without the groups in the same series but also the compounds substituted by aminomethyl groups at position C-8 were more active than those at positions C-6 and C-7.

Keywords Flavonoid · Drug design · Cytotoxicity · Structure–activity relationship

Introduction

Flavonoids are a large class of natural compounds with a C6–C3–C6 skeleton substituted by various groups. They have been receiving great interests because of a wide spectrum of biological activities exhibited, such as antiallergic, antiinflammatory, antimicrobial, anticancer effects (Ververidis *et al.*, 2007; Spencer, 2008; Cushnie and Lamb, 2005; Lotito and Frei, 2006). Noteworthily, flavonoids bearing alkyl substituents at C-8 were frequently found with interesting pharmacological properties (Adinarayana and Ramachandraiah, 1987; Barron and Ibrahim, 1996). Some good examples (Scheme 1) are kushenol C (**1**) with potent antitumor activity both in vitro and in vivo (Sun *et al.*, 2007), phellamurin (**2**) with DNA cleavage activity (Ma *et al.*, 2005), icariin (**3**), the main active component of *Epimedium koreanum*, with myriads of activities (Makarova *et al.*, 2007; He *et al.*, 1995; Liu *et al.*, 2005), puerarin (**4**) with preventive and treating effects on arrhythmia (Chai *et al.*, 1985), and the synthetic flavonoids, flavone-8-acetate acid (**5**) having strong antitumor activity (Atassi *et al.*, 1985; Naik *et al.*, 1988). Of these compounds, flavopiridol (**6**), a semisynthetic flavonoid that inhibits tumor growth both in vitro and in vivo, is the first potent cyclin-dependent kinase (CDK) inhibitor entering clinical trials. It inhibits the activity of several CDKs, specifically CDK1, CDK2, and CDK4, inducing a G_1- or G_2-phase cell cycle arrest (De Azevedo *et al.*, 1996; Losiewicz *et al.*, 1994; Carlson *et al.*, 1996; Kaur *et al.*, 1992; Czech *et al.*, 1995; Parker *et al.*, 1998). Its success stimulates ensuing researches on the synthesis of related flavonoid analogues. Its SAR studies have shown that *N*-methyl-piperidine attached to the skeleton with carbon–carbon bond formation was the key pharmacophore. Some ideas that the substitution at position C-8 may be the key pharmacophore to their bioactivity and the bioactivity may increase progressively with the substitution changed from position C-6 to C-7 and finally to C-8 were achieved based on the above investigations. Bearing this in mind, we focused our attention on the substitution effects of the flavonoids as potent anticancer agents and designed three

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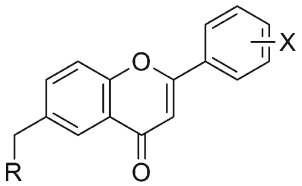
series of flavonoids analogues. Compounds substituted by various groups containing nitrogen at position C-6 (series A, Table 1), C-7 (series B, Table 2), and C-8 (series C, Table 3) were designed. We reported herein the synthesis, biological evaluation, and structure–activity relationship of these flavonoid analogues as anticancer agents.

Results and discussion

Chemistry

Mannich reaction, a directive tactics to make an amino alkylation of an acidic proton placed next to a carbonyl

Table 1 The target compounds in series A



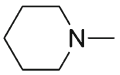
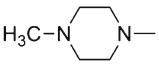
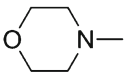
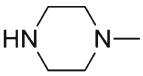
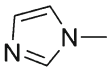
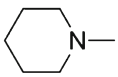
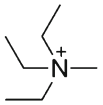
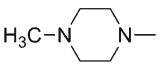
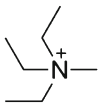
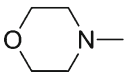
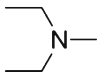
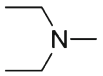
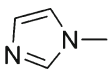
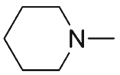
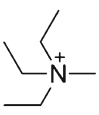
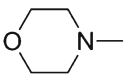
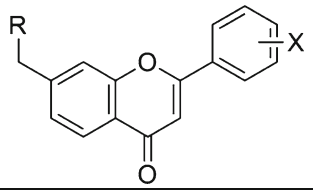
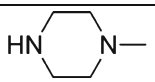
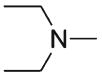
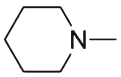
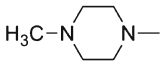
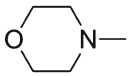
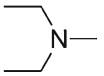
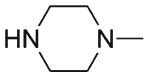
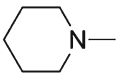
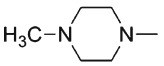
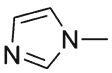
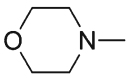
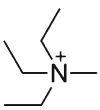
Compound	R	X	Compound	R	X
17a	H	4 -NO_2	**17n**		4 -Cl
17b	H	4 -Cl	**17o**		4 -Cl
17c	H	2 -Cl	**17p**		4 -Cl
17d	H	H	**17q**		4 -Cl
17e	H	4 -NH_2	**17r**		4 -Cl
17f		4 -NO_2	**17s**		4 -Cl
17g		4 -NO_2	**17t**		4 -Cl
17h		4 -NO_2	**17u**		2 -Cl
17i		4 -NO_2	**17v**		H
17j		4 -NO_2	**17w**		4 -NH_2
17k		4 -NO_2	**17x**		1-3B
17l		4 -NO_2	**17y**		1-6B
17m		4 -Cl			

Table 2 The target compounds in series B

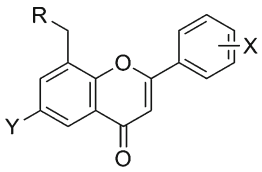


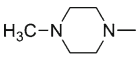
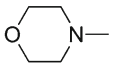
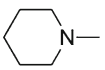
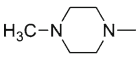
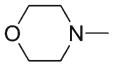
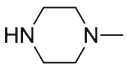
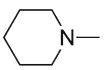
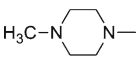
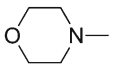
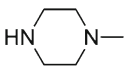
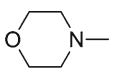
Compound	R	X	Compound	R	X
18a	H	4 -NO_2	**18j**		4 -NO_2
18b	H	4 -Cl	**18k**		2 -Cl
18c	H	2 -Cl	**18l**		2 -Cl
18d	H	H	**18m**		2 -Cl
18e	H	4 -NH_2	**18n**		2 -Cl
18f		4 -NO_2	**18o**		2 -Cl
18g		4 -NO_2	**18p**		2 -Cl
18h		4 -NO_2	**18q**		4 -Cl
18i		4 -NO_2	**18r**		4 -Cl

functional group, was used as the normal preparation method of these kinds of compounds. For example, series of C-8 nitrogen-containing flavonoid analogues had been designed and synthesized through Mannich reaction (Zhang *et al.*, 2008; Liu *et al.*, 2007). In this study, three series of compounds with the substitution positions varied among C-6, C-7, and C-8 were designed to investigate the substitution effects of these flavonoids on their activities. However, Mannich reaction is not suitable for the direct synthesis of these compounds. So the oriented synthesis routes were outlined as shown in Schemes 1 and 2. The synthetic procedures of the target compounds can be divided into two parts: (i) the synthesis of the methylflavones, **17a–17d**, **18a–18d**, and **19a–19d**, and (ii) the amino methylflavones, **17e–17y**, **18e–18r**, and **19e–19w** (Schemes 2, 3, and 4).

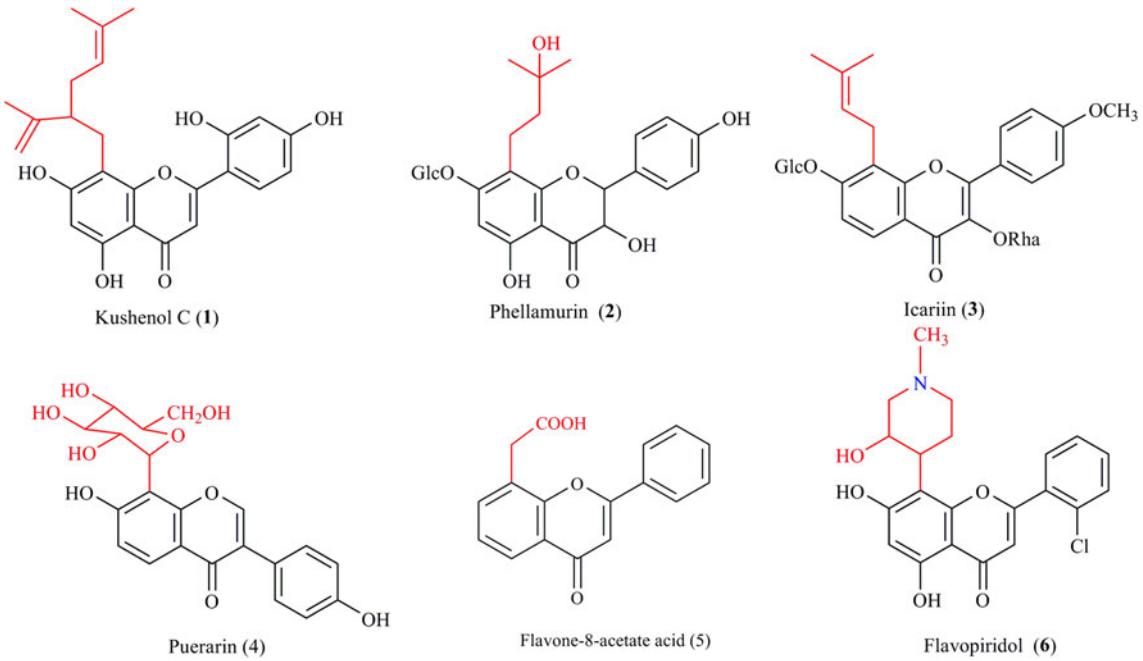
Compounds **17a–17y** in series A were prepared using *p*-cresol as the starting material. Compound **20a** was first esterified with acetic anhydride to yield *p*-tolyl acetate **21a**. The Fries rearrangement of **21a** led to a high yield of 2-hydroxy-5-methylacetophenone **22a**, which was condensed with different substituted benzoyl chlorides through the Baker–Venkataraman method to produce *β*-diketone **23a–23d**. The classic Baker–Venkataraman transformation involves two steps: 2-Hydroxy-5-methyl- acetophenone and benzoyl chloride are first converted into a benzoyl ester in pyridine, and then treated with a base, such as KOH, affording *β*-diketone through Claisen rearrangement. In this study, the two Baker–Venkataraman transformation steps were reduced to one by using K_2CO_3 in acetone, and then the obtained *β*-diketone **23a–23d** was treated with NaOAc/HOAc to give desired methylflavones **17a–17d** as the important intermediates. The bromomethylflavones **24a–24c** were prepared through free-radical reaction of methylflavones **17a–17c** with *N*-bromosuccinimide (NBS) in CCl_4 using benzoyl peroxide (BPO) as an inducer. The

Table 3 The target compounds in series C and their CDK/cyclin B inhibitory activity



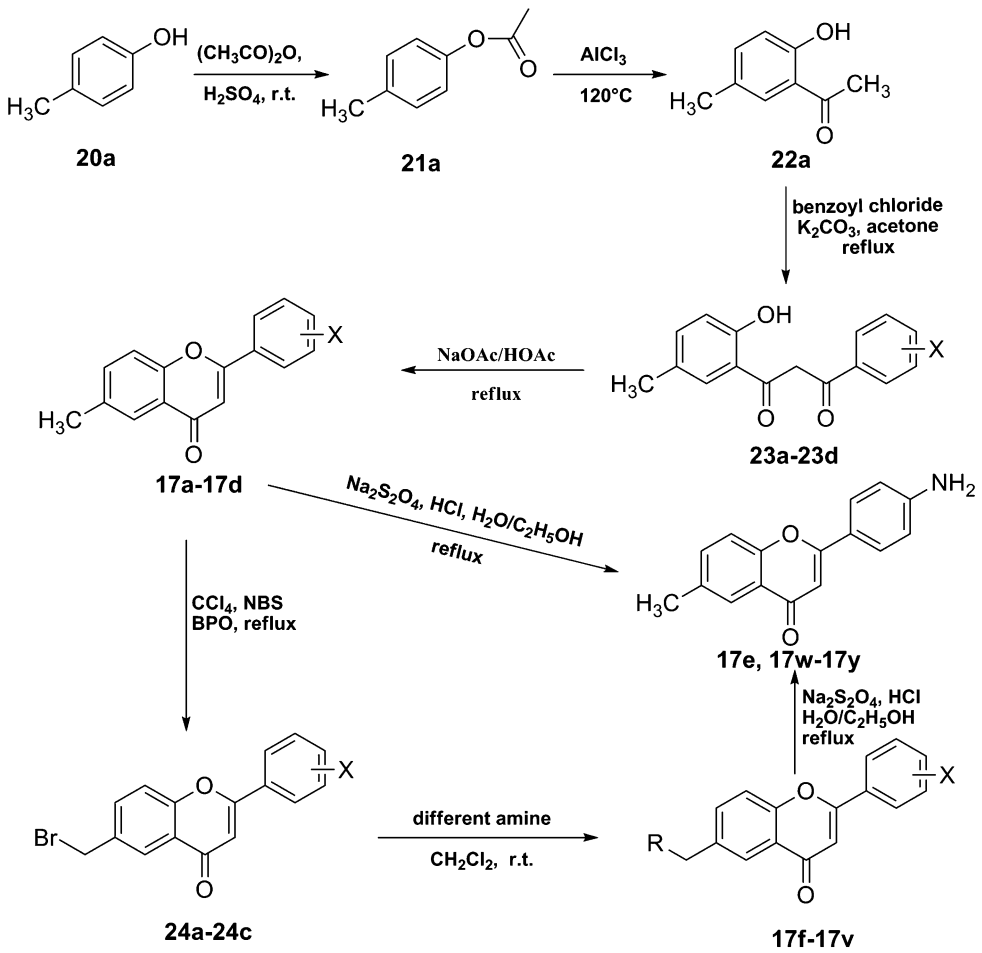
Compound	R	X	Y	CDK/ Cyclin B (IC_{50}, μM)
19a	H	4′-NO_2	H	ND*
19b	H	4′-NO_2	Cl	ND
19c	H	4′-Cl	Cl	ND
19d	H	2′-Cl	Cl	ND
19e	H	4′-NH_2	Cl	ND
19f		4′-NO_2	H	2.21
19g		4′-NO_2	H	8.13
19h		4′-NO_2	H	ND
19i		4′-NO_2	Cl	ND
19j		4′-NO_2	Cl	ND
19k		4′-NO_2	Cl	6.22
19l		4′-NO_2	Cl	ND
19m		4′-NO_2	Cl	4.84
19n		4′-NO_2	Cl	ND
19o		4′-NO_2	Cl	2.14
19p		2′-Cl	Cl	2.06
19q		2′-Cl	Cl	ND
19r		2′-Cl	Cl	ND
19s		2′-Cl	Cl	ND
19t		2′-Cl	Cl	ND
19u		2′-Cl	Cl	ND
19v		2′-Cl	Cl	ND
19w		4′-Cl	Cl	2.17

ND the CDK/cyclin B inhibitory activity was not detected in the assay

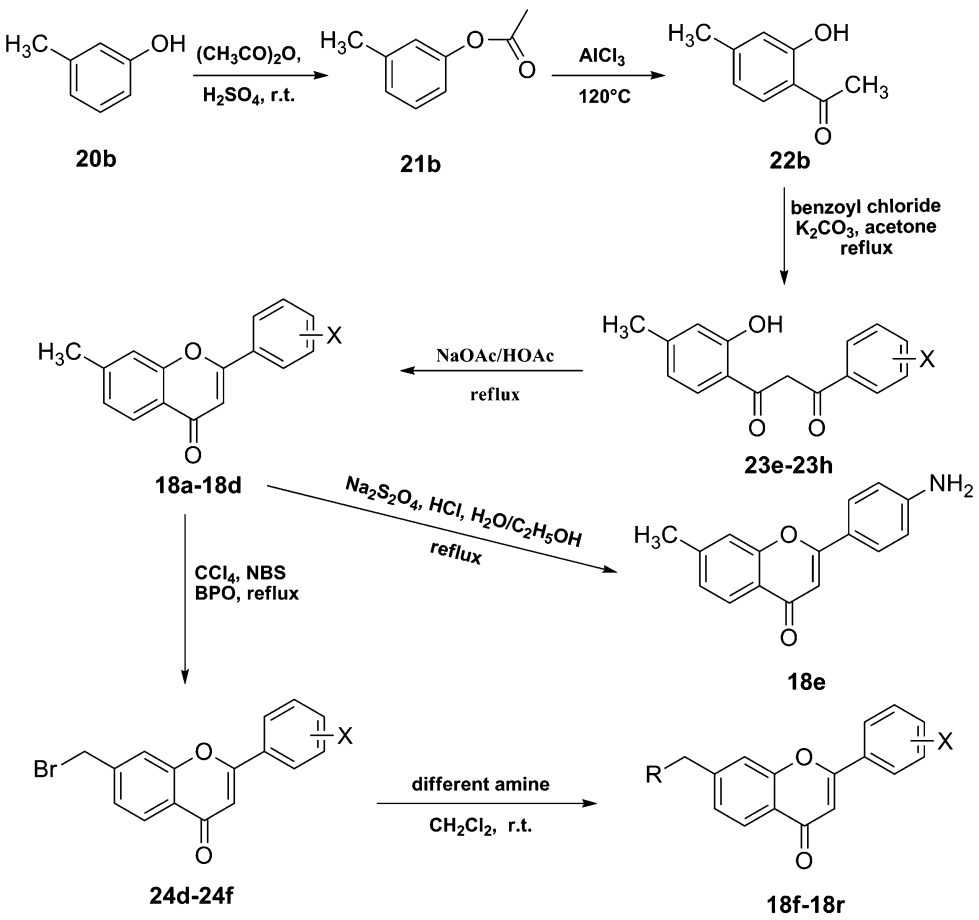


Scheme 1 Some bioactivity flavonoids

Scheme 2 Synthesis of the target compounds in A



Scheme 3 Synthesis of the target compounds in B



target molecules **17f–17y** were prepared by reaction of **24a–24c** with optional amines, diethylamine, piperidine, piperazine, *N*-methylpiperazine, morphine, dimethylamine, imidazole, triethylamine, and so on. Compounds **17a** and **17f–17i** were reduced by sodium dithionite to give the corresponding aminoflavones **17e** and **17w–17y**, respectively.

Similar to the protocols for the synthesis of the compounds in series A, compounds **18a–18r** in series B were synthesized using *m*-cresol as the starting material. In the preparation of the compounds in series C, 8-aminomethylflavones, an initial attempt to use *o*-cresol as the starting material was made (Scheme 5). The result was disappointing as the Fries rearrangement of **21c** majorly produced the *para* product **22c′**. Although the *ortho* product **22c** was the key intermediate for the synthesis of the 8-alkylated flavones, as a result, only four compounds **19f–19h** were obtained using this way. To enhance the yield of *ortho* products, 4-chloro-2-methylphenol (**20d**) was used as the starting material in terms of the characteristic of the Fries rearrangement, which was exclusively converted to its *ortho* products. In this manner, 8-substituted flavone derivatives **19i–19w** were also successfully synthesized.

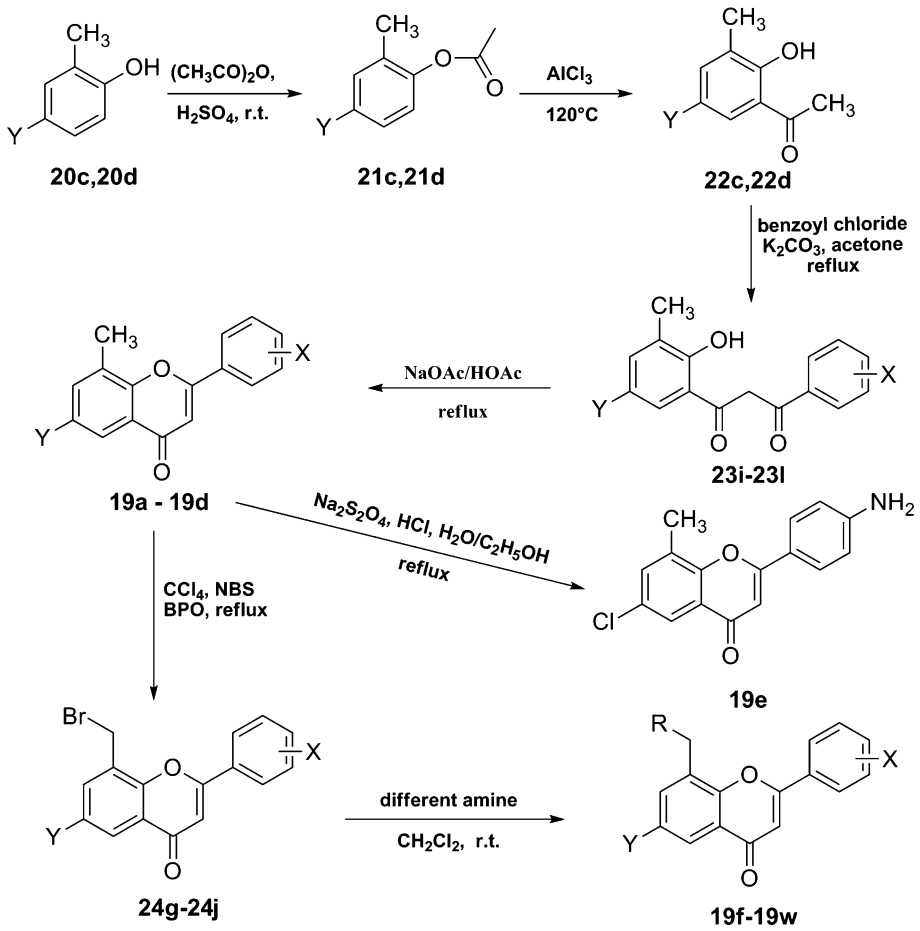
CDK1/cyclin B inhibitory activity

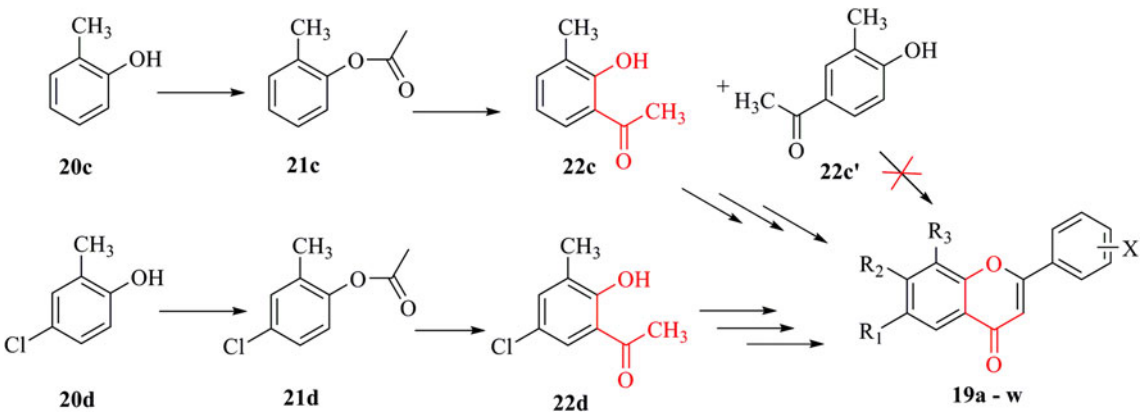
Compounds **19a–19w** in series C were assayed for their CDK1/cyclin B inhibitory activity with Z′-LYTE™ kinase assay kits (Table 3). Some compounds, especially those containing a piperazine moiety, e.g., **19g** (IC_{50} = 8.13 μM), **19k** (IC_{50} = 6.22 μM), **19m** (IC_{50} = 4.84 μM), exhibited potent CDK1/cyclin B inhibitory activity. However, their activities were still many times weaker than that of flavopiridol (Kim *et al.*, 2000), which might be because of the lack of the hydrogen-bonding donors, e.g., hydroxyl groups.

Cytotoxicity

The anticancer activities of all the synthesized compounds were evaluated by MTT method using hepatic cancer cell lines HepG2 and SMMC-7721 (Table 4). Almost all the flavones with amino methyl groups, such as **17e–17y**, **18e–18r**, and **19e–19w**, were more active than those without amino methyl groups, such as **17a–17d**, **18a–18d**, and **19a–19d**, which suggested the key role of the N-containing substituents for activities. Potential bioactivities were found for 11 out of 50 tests assays in series A (ca. 22%), 13 of 36

Scheme 4 Synthesis of the target compounds in C





Scheme 5 Synthetic routes of the key methylflavones in series C

(ca. 36%) in series B, and 31 of 46 (ca. 67%) in series C in two cell lines. Compounds with substitution at position C-8 were more active than those at positions C-7 and C-6. Compounds **17h**, **18h**, **19g**, and **19k**, for example, have the same substituent group, 4-methylpiperazinylmethyl, at ring A of the flavone nucleus but with different substitution positions. The 8-substituted derivatives **19g** and **19k** exhibited potent cytotoxic activity against hepatic cancer cell line SMMC-7721 with IC_{50} at 1.19 and 5.21 μM, respectively, as compared with those for **18h** (7-substituted) and **17h** (6-substituted) at 10.7 and 19.0 μM, respectively. The bioactivity of all the target compounds increased when the substituent positions varied from C-6 to C-7 and C-8, indicating the essential role of the substituent position for the biological activity of these flavonoids as anticancer agents. Furthermore, almost all the target compounds containing piperidine, piperazine, and *N*-methylpiperazine groups exhibited cytotoxic activity to HepG2 and SMMC-7721

Table 4 Cytotoxicity of the present compounds against two tumor cells

Compound	MTT/IC_{50} (μM)		Compound	MTT/IC_{50} (μM)		Compound	MTT/IC_{50} (μM)	
	HepG2	SMMC-7721		HepG2	SMMC-7721		HepG2	SMMC-7721
17a	ND	ND	**17w**	ND	ND	**19b**	ND	ND
17b	ND	ND	**17x**	2.64	3.68	**19c**	ND	ND
17c	6.23	15.1	**17y**	ND	ND	**19d**	ND	ND
17d	ND	ND	**18a**	ND	ND	**19e**	11.9	ND
17e	21.3	ND	**18b**	6.83	ND	**19f**	28.8	ND
17f	ND	ND	**18c**	28.3	ND	**19g**	2.94	1.19
17g	ND	ND	**18d**	ND	ND	**19h**	ND	ND
17h	ND	19	**18e**	40.5	ND	**19i**	1.19	2.99
17i	ND	ND	**18f**	ND	ND	**19j**	8.91	1.09
17j	ND	ND	**18g**	ND	ND	**19k**	1.76	5.21
17k	ND	ND	**18h**	ND	10.7	**19l**	ND	ND
17l	ND	ND	**18i**	ND	ND	**19m**	0.243	1.1
17m	ND	ND	**18j**	4.53	2.03	**19n**	6.19	56.53
17n	14	16.2	**18k**	10.7	ND	**19o**	2.94	1.2
17o	ND	ND	**18l**	3.64	ND	**19p**	7.94	0.81
17p	ND	ND	**18m**	14.2	12.9	**19q**	10.7	3.4
17q	2.69	1.51	**18n**	ND	ND	**19r**	ND	3.1
17r	ND	ND	**18o**	2.58	1.91	**19s**	6.81	13.2
17s	ND	ND	**18p**	ND	28.2	**19t**	18.7	2.69
17t	ND	ND	**18q**	ND	ND	**19u**	1.87	1.14
17u	61	ND	**18r**	ND	ND	**19v**	3.27	1.07
17v	ND	ND	**19a**	ND	ND	**19w**	6.33	15.3
Control[a]	6.11	3.20						

ND the bioactivity of the compound was not detected

[a] Gambogic acid was chosen as the control in the MTT assay

cells. For example, the following IC_{50} values have been obtained for HepG2 and SMMC-7721 cells, respectively using the following compounds: **17q** with a piperazine 2.69 and 1.51 μM, **18j** with a piperazine 4.53 and 2.03 μM, **18o** with a piperazine 2.58 and 1.91 μM, **19g** with an *N*-methylpiperazine as 2.94 and 1.19 μM, **19j** with a piperidine 8.91 and 1.09 μM, **19k** with an *N*-methylpiperazine 1.76 and 5.21 μM, and **19m** with a piperazine as 0.243 and 1.1 μM. The results combined suggested that 8-aminomethyl flavonoids with free hydroxyl groups have the potential to be developed as anticancer agents in the future.

Conclusions

In conclusion, three series of flavonoid analogues were designed and synthesized. The CDK1/cyclin B inhibitory activity of the compounds in series C was measured using Z′-LYTE™ kinase assay kits, and some compounds were found to show inhibitory activity. The cytotoxic activity of all the synthesized compounds was assayed using MTT method. Structure–activity relationships studies indicated that compounds with substitutions at position C-8 were more active than those at positions C-7 and C-6, and in the same series, compounds with the amino methyl groups were more active than those without such functionalities.

Experimental

General

NMR spectra were recorded on Bruker AC 300 and 500 spectrometer using TMS as internal standard. ESI–MS was detected with HP-1100 Series LC/MSD ion trap mass spectrometer. The IR (KBr) spectra were obtained on a Nicolet Impact 410 spectrometer. Flash chromatography was performed with 230–400 mesh silica gel. TLC was carried out using commercially available precoated silica gel 60 F_{254} (Qingdao Marine Chemical Co. Ltd).

Unless otherwise stated, chemicals and solvents were of reagent grade and used as obtained from commercial sources without further purification. All the reactions were monitored by TLC and MS.

General procedure for the synthesis of the methylflavones

One drop of concentrated sulfuric acid was added to a mixture of optionally substituted phenol (0.10 mol) and acetic anhydride (10 g, 0.1 mol). After the mixture was stirred for ½ h, water was added to the solution. The solution was extracted with EtOAc, washed with water and brine, dried over Na_2SO_4, then the solvent was evaporated under reduce pressure to obtain phenyl acetates **21a–21d** as an oil. Optional phenyl acetates **21a**–**21d** (1 mol) were mixed with anhydrous $AlCl_3$ (2 mol) at 120 ~ 180°C for 2 h, respectively, then cooled down to room temperature. The solution of ice water and HCl with 1:1 (V:V) was added to the mixture slowly, *o*-hydroxyacetophenone derivatives **22a–22d**, the most important intermediate for preparing the designed flavone, were synthesized. A solution of the *o*-hydroxyacetophenone derivatives **22a–22d** (20 mmol) in acetone (60 ml) was added with appropriately substituted benzoyl chloride (20 mmol) using dry K_2CO_3 (12 g) as the base under refluxing for 12 h. The mixture was filtered and the precipitate was decomposed with 10% glacial acetic acid to give yellow powder as *β*-diketone **23a–23l**. The resulted *β*-diketone **23a–23l** was treated with NaOAc/HOAc (8 g/40 ml) under reflux for 12 h and cooled to room temperature. Water was added to the mixture, then filtered, and washed with hot water, saturated $NaHCO_3$ solution, and cold water successively to give the methylflavones **17a–17d, 18a–18d,** and **19a–19d.**

*6-Methyl-2-(4-nitrophenyl)-4H-chromen-4-one (**17a**)*

Yield 74.9%; IR (KBr) υ_{max} 3,442, 1,640, 1,618, 1,573, 1,522, 1,483, 1,347, 1,042, 851, 824, 691 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.38 (d, 2H, $J = 8.1$ Hz, H-3′, H-5′), 8.11 (d, 2H, $J = 8.1$ Hz, H-2′, H-6′), 8.03 (d, 1H, $J = 2.1$ Hz, H-5), 7.55 (dd, 1H, J = 8.6, 2.1 Hz, H-7), 7.50 (d, 1H, $J = 8.6$ Hz, H-8), 6.89 (s, 1H, H-3), and 2.49 (s, 3H, CH_3); ESI–MS *m/z* 282 $[M + H]^+$.

*2-(4-Chlorophenyl)-6-methyl-4H-chromen-4-one (**17b**)*

Yield 82% IR (KBr) υ_{max} 3,444, 3,075, 1,641, 1,620, 1,574, 1,486, 1,432, 1,364, 1,090, 1,039, 1,009, 902, 820 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.09 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.82 (s, 1H, H-5), 7.65 (m, 2H, H-7, H-8), 7.63 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.01 (s, 1H, H-3), and 2.43 (s, 3H, CH_3); ESI–MS *m/z* 271 $[M + H]^+$.

*2-(2-Chlorophenyl)-6-methyl-4H-chromen-4-one (**17c**)*

Yield 80%; IR (KBr) υ_{max} 3,444, 3,070, 1,658, 1,620, 1,485, 1,440, 1,340, 1,224, 1,072, 1,025, 813, 761 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 7.89 (s, 1H, H-5), 7.82 (d, 1H, $J = 8.6$ Hz, H-7), 7.69 (d, 1H, $J = 8.6$ Hz, H-8), 7.60 (m, 3H, H-3′, H-4′, H-5′, H-6′), 6.61 (s, 1H, H-3), and 2.46 (s, 3H, CH_3); ESI–MS *m/z* 271 $[M + H]^+$.

*6-Methyl-2-phenyl-4H-chromen-4-one (**17d**)*

Yield 78%; IR (KBr) υ_{max} 3,451, 1,644, 1,615, 1,568, 1,483, 1,451, 1,360, 1,223, 1,139, 1,041, 902, 822, 771 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.91 (s, 1H, H-5), 7.89 (m, 2H, H-7, H-8), 7.49 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 6.79 (s, 1H, H-3), and 2.44 (s, 3H, CH_3); ESI–MS *m/z* 237 $[M + H]^+$.

*7-Methyl-2-(4-nitrophenyl)-4H-chromen-4-one (**18a**)*

Yield 72%; IR (KBr) υ_{max} 3,450, 3,073, 1,632, 1,568, 1,518, 1,424, 1,376, 1,346, 1,046, 852, 834, 692 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.38 (d, 2H, $J = 8.1$ Hz, H-2′, H-6′), 8.11 (d, 2H, $J = 8.1$ Hz, H-3′, H-5′), 7.42 (s, 1H, H-5), 7.28 (m, 2H, H-6, H-8), 6.88 (s, 1H, H-3), and 2.55 (s, 3H, CH_3); ESI–MS *m/z* 282 $[M + H]^+$.

*2-(4-Chlorophenyl)-7-methyl-4H-chromen-4-one (**18b**)*

Yield 76%; IR (KBr) υ_{max} 3,449, 3,069, 1,639, 1,571, 1,491, 1,411, 1,370, 1,277, 1,233, 1,161, 1,093, 1,046, 1,012, 907, 826 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.07 (d, 2H, $J = 8.4$ Hz, H-2′, H-6′), 7.91 (d, 1H, $J = 8.5$ Hz, H-5), 7.62 (d, 2H, $J = 8.4$ Hz, H-3′, H-5′), 7.55 (s, 1H, H-8), 7.30 (d, 1H, $J = 8.5$ Hz, H-6), 7.00 (s, 1H, H-3), and 2.47 (s, 3H, CH_3); ESI–MS *m/z* 271 $[M + H]^+$.

*2-(2-Chlorophenyl)-7-methyl-4H-chromen-4-one (**18c**)*

Yield 69%; IR (KBr) υ_{max} 3,442, 1,643, 1,612, 1,418, 1,365, 1,230, 1,140, 1,039, 911, 868, 823, 752 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.98 (d, 1H, $J = 8.1$ Hz, H-5), 7.80 (m, 1H, H-6′), 7.61 (m, 3H, H-3′, H-4′, H-5′), 7.50 (s, 1H, H-8), 7.37 (d, 1H, $J = 8.1$ Hz, H-6), 6.59 (s, 1H, H-3), and 2.54 (s, 3H, CH_3); ESI–MS *m/z* 271 $[M + H]^+$.

*7-Methyl-2-phenyl-4H-chromen-4-one (**18d**)*

Yield 73%; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.12 (1H, s, H-8), 7.95(d, 1H, $J = 7.8$ Hz, H-5), 7.61(br, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 7.34(d, 1H, $J = 7.8$ Hz, H-6), 7.02 (s, 1H, H-3), and 2.46 (s, 3H, CH_3); ESI–MS *m/z* 237 $[M + H]^+$.

*8-Methyl-2-(4-nitrophenyl)-4H-chromen-4-one (**19a**)*

Yield 78%; IR (KBr) υ_{max} 3,442, 3,076, 1,662, 1,523, 1,484, 1,416, 1,347, 1,328, 1,037, 850, 758 cm^{-1}; ^1H NMR

(DMSO-d_6, 300 MHz) δ 8.40 (br, 4H, H-2′, H-3′, H-5′, H-6′), 7.90 (d, 1H, $J = 2.4$ Hz, H-5), 7.74 (d, 1H, $J = 2.4$ Hz, H-7), 7.43(m, 1H, H-6), 7.26 (s, 1H, H-3), and 2.62 (s, 3H, –CH_3); ESI–MS *m/z* 282 $[M + H]^+$.

*6-Chloro-8-methyl-2-(4-nitrophenyl)-4H-chromen-4-one (**19b**)*

Yield 78%; IR (KBr) υ_{max} 3,443, 3,071, 1,667, 1,582, 1,523, 1,465, 1,347, 1,300, 1,041, 882, 864, 852 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.41 (d, 2H, $J = 9.0$ Hz, H-3′, H-5′), 8.02 (d, 2H, $J = 9.0$ Hz, H-2′, H-6′), 7.98 (d, 1H, $J = 2.1$ Hz, H-5), 7.48 (d, 1H, $J = 2.1$ Hz, H-7), 6.83 (s, 1H, H-3), and 2.15 (s, 3H, CH_3); ESI–MS *m/z* 316 $[M + H]^+$.

*6-Chloro-2-(4-chlorophenyl)-8-methyl-4H-chromen-4-one (**19c**)*

Yield 76%; IR (KBr) υ_{max} 3,444, 3,069, 1,636, 1,594, 1,492, 1,465, 1,415, 1,361, 1,291, 1,095, 1,042, 880, 836 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.03 (s, 1H, H-5), 7.85 (d, 2H, $J = 8.4$ Hz, H-3′, H-5′), 7.52(d, 2H, $J = 8.4$ Hz, H-2′, H-6′), 7.27 (s, 1H, H-7), 6.81 (s, 1H, H-3), and 2.59 (s, 3H, CH_3); ESI–MS *m/z* 305 $[M + H]^+$.

*6-Chloro-2-(2-chlorophenyl)-8-methyl-4H-chromen-4-one (**19d**)*

Yield 76%; IR (KBr) υ_{max} 3,442, 1,637, 1,595, 1,581, 1,493, 1,465, 1,405, 1,361, 1,291, 1,163, 1,095, 1,041, 1,011, 880, 837 cm^{-1}; ^1H NMR (CD_3COCD_3, 300 MHz) δ 7.89 (m, 3H, H-3′, H-5, H-6′), 7.65 (m, 3H, H-4′, H-5′, H-7), 6.64 (s, 1H, H-3), and 2.54 (s, 3H, CH_3); ESI–MS *m/z* 305 $[M + H]^+$.

General procedure for the synthesis of the aminomethylflavones

To a solution of methylflavones **17a–17c**, **18a**, **18b**, and **19a–19d** (5 mmol) in CCl_4 (150 ml) were added *N*-bromosuccinimide (5 mmol) and a catalytic amount of benzyl peroxide, and the mixture was refluxed for 12 h. The mixture was hot-filtered and evaporated to dryness to give **24a–24i**, respectively, and they were used without further purification. Bromomethylflavones **24a–24j** (1 mmol) were individually mixed with different amine (10 mmol) in CH_2Cl_2 at room temperature with stirring for ½ h, and the solvent was evaporated to dryness. HCl solution was added to adjust the pH 3 ~ 4 and filtered. Then, the pH of the solution was adjusted to 10 with ammonia water, and precipitation appeared. The precipitation was filtered and recrystallized to give the target molecules **17f–17v**, **18f–18r,** and **19f–19w**, respectively.

*6-((Diethylamino)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**17f**)*

Yield 42.3%; IR (KBr) υ_{max} 3,450, 2,968, 2,796, 1,639, 1,573, 1,522, 1,481, 1,347, 1,042, 851, 822, 690 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.39 (d, 2H, $J = 8.2$ Hz, H-3′, H-5′), 8.12 (d, 2H, $J = 8.2$ Hz, H-2′, H-6′), 7.83 (d, 1H, $J = 8.1$ Hz, H-8), 7.57 (d, 1H, $J = 8.1$ Hz, H-7), 7.27 (s, 1H, H-5), 6.91 (s, 1H, H-3), 3.67 (s, 2H, Ar–CH_2–), 2.54 (q, 4H, $J = 6.9$ Hz, N(CH_2CH_3)$_2$), and 1.06 (t, 6H, $J = 6.9$ Hz, $NCH_2(CH_3)_2$); ESI–MS *m/z* 353 $[M + H]^+$.

*2-(4-Nitrophenyl)-6-(piperidin-1-ylmethyl)-4H-chromen-4-one (**17g**)*

Yield 37.6%; IR (KBr) υ_{max} 3,443, 1,640, 1,569, 1,518, 1,483, 1,414, 1,346, 1,038, 851, 757, 693 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.39 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.96 (s, 1H, H-8), 7.79 (s, 1H, H-5), 7.23 (s, 1H, H-3), 3.56 (s, 2H, Ar–CH_2–), 2.36 (m, 4H, N(CH_2CH_2)$_2$$CH_2$), 1.69 (m, 2H, N($CH_2CH_2$)$_2CH_2$), and 1.50 (m, 4H, N($CH_2CH_2$)$_2$$CH_2$); ESI–MS *m/z* 365 $[M + H]^+$.

*6-((4-Methylpiperazin-1-yl)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**17h**)*

Yield 34.6%; IR (KBr) υ_{max} 3,453, 2,929, 1,639, 1,521, 1,482, 1,452, 1,349, 1,133, 1,041, 904, 853 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.39 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.96 (s, 1H, H-8), 7.79 (s, 2H, H-5, H-7), 7.23 (s, 1H, H-3), 3.59 (s, 2H, Ar–CH_2–), 2.38 (m, 8H, N(CH_2CH_2)$_2$$NCH_3$), and 2.16 (s, 3H, NC$H_3$); ESI–MS *m/z* 380 $[M + H]^+$.

*6-(Morpholinomethyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**17i**)*

Yield 45.0%; IR (KBr) υ_{max} 3,423, 1,639, 1,618, 1,520, 1,452, 1,346, 1,120, 1,084, 904, 866 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.39 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.30 (s, 1H, H-5), 8.12 (d, 1H, $J = 8.5$ Hz, H-8), 7.94 (d, 1H, $J = 8.5$ Hz, H-7), 7.30 (s, 1H, H-3), 4.52 (s, 2H, Ar–CH_2–), 3.95 (m, 2H, N(CH_2CH_2)$_2$O), 3.75 (m, 2H, N(CH_2CH_2)$_2$O), 3.26 (m, 2H, N(CH_2CH_2)$_2$O), and 3.13 (m, 2H, N(CH_2CH_2)$_2$O); ESI–MS *m/z* 367 $[M + H]^+$.

*2-(4-Aminophenyl)-6-((dimethylamino)methyl)-4H-chromen-4-one (**17j**)*

Yield 27.1%; IR (KBr) υ_{max} 3,451, 1,641, 1,520, 1,482, 1,448, 1,349, 1,124, 1,037, 901, 852, 694 cm^{-1}; ^1H NMR

(DMSO-d_6, 500 MHz) δ 8.40 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.11 (s, 1H, H-5), 7.93 (d, 1H, $J = 8.6$ Hz, H-8), 7.85 (d, 1H, $J = 8.6$ Hz, H-7), 7.26 (s, 1H, H-3), 3.95 (s, 2H, Ar–CH_2–), and 2.44 (s, 6H, N(CH_3)$_2$); ESI–MS *m/z* 325 $[M + H]^+$.

*6-((1H-imidazol-1-yl)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**17k**)*

Yield 30.5%; IR (KBr) υ_{max} 3,443, 1,639, 1,572, 1,516, 1,483, 1,444, 1,347, 1,184, 1,040, 912, 852, 823, 693 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.39 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.94 (s, 1H, H-2″), 7.86 (s, 1H, H-5), 7.78 (m, 2H, H-7, H-8), 7.59 (s, 1H, H-5″), 7.24 (s, 1H, H-4″), 6.69 (s, 1H, H-3), and 5.38 (s, 2H, Ar–CH_2–); ESI–MS *m/z* 348 $[M + H]^+$.

*N,N-Diethyl-N-((2-(4-nitrophenyl)-4-oxo-4H-chromen-6-yl)methyl)ethanaminium (**17l**)*

Yield 51.2%; IR (KBr) υ_{max} 3,442, 1,639, 1,572, 1,521, 1,488, 1,448, 1,411, 1,347, 1,198, 1,037, 910, 852, 694 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.41 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.26 (s, 1H, H-5), 7.98 (m, 2H, H-7, H-8), 7.32 (s, 1H, H-3), 4.70 (s, 2H, Ar–CH_2–), 3.23 (q, 6H, $J = 7.0$ Hz, N(CH_2CH$_3$)$_3$), and 1.35 (t, 9H, $J = 7.0$ Hz, N(CH$_2$CH_3)$_3$); ESI–MS *m/z* 381 $[M + H]^+$.

*2-(4-Chlorophenyl)-6-((diethylamino)methyl)-4H-chromen-4-one (**17m**)*

Yield 46%; IR (KBr) υ_{max} 3,444, 2,967, 1,642, 1,621, 1,576, 1,495, 1,448, 1,408, 1,360, 1,095, 1,037, 910, 827 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.12 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.97 (s, 1H, H-5), 7.78 (d, 1H, $J = 9.0$ Hz, H-7), 7.73 (d, 1H, $J = 9.0$ Hz, H-8), 7.65 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.06 (s, 1H, H-3), 3.65 (s, 2H, Ar–CH_2–), 2.50 (q, 4H, $J = 7.0$ Hz, N(CH_2CH$_3$)$_2$), and 0.99 (t, 6H, $J = 7.0$ Hz, N(CH$_2$CH_3)$_2$); ESI–MS *m/z* 342 $[M + H]^+$.

*2-(4-Chlorophenyl)-6-(piperidin-1-ylmethyl)-4H-chromen-4-one (**17n**)*

Yield 48%; IR (KBr) υ_{max} 3,431, 2,935, 1,643, 1,620, 1,594, 1,494, 1,453, 1,408, 1,366, 1,094, 1,038, 1,011, 906, 829 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.15 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 8.05 (s, 1H, H-5), 7.87 (m, 2H, H-7, H-8), 7.68 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.12 (s, 1H, H-3), 3.32 (s, 2H, Ar–CH_2–), 2.50 (m, 4H, N(CH_2CH$_2$)$_2$CH$_2$), and 1.73 (br, 6H, N(CH$_2$CH_2)$_2$CH_2); ESI–MS *m/z* 354 $[M + H]^+$.

*2-(4-Chlorophenyl)-6-((4-methylpiperazin-1-yl)methyl)-4H-chromen-4-one (**17o**)*

Yield 52%; IR (KBr) υ_{max} 3,443, 2,930, 2,797, 1,638, 1,619, 1,575, 1,492, 1,452, 1,407, 1,361, 1,278, 1,091, 1,040, 1,011, 903, 832 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.13 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.94 (s, 1H, H-5), 7.66 (s, 2H, H-7, H-8), 7.61 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.07 (s, 1H, H-3), 3.59 (s, 2H, Ar–CH_2–), 2.42 (br, 8H, N(CH_2CH_2)$_2$NCH$_3$), and 2.18 (s, 3H, NCH_3); ESI–MS *m/z* 369 $[M + H]^+$.

*2-(4-Chlorophenyl)-6-(morpholinomethyl)-4H-chromen-4-one (**17p**)*

Yield 56%; IR (KBr) υ_{max} 3,443, 2,852, 2,803, 1,638, 1,575, 1,494, 1,454, 1,409, 1,131, 1,112, 1,091, 1,010, 856, 833 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.97 (s, 1H, H-5), 7.77 (m, 2H, H-7, H-8), 7.66 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.08 (s, 1H, H-3), 3.59 (s, 6H, Ar–CH_2–, N(CH$_2$CH_2)$_2$O), and 2.39 (br, 4H, N(CH_2CH$_2$)$_2$O); ESI–MS *m/z* 356 $[M + H]^+$.

*2-(4-Chlorophenyl)-6-(piperazin-1-ylmethyl)-4H-chromen-4-one (**17q**)*

Yield 51%; IR (KBr) υ_{max} 3,439, 2,923, 1,645, 1,550, 1,494, 1,454, 1,408, 1,362, 1,113, 1,091, 1,038, 1,010, 756 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.13 (d, 2H, $J = 8.1$ Hz, H-2′, H-6′), 7.93 (s, 1H, H-5), 7.75 (m, 2H, H-7, H-8), 7.65 (d, 2H, $J = 8.1$ Hz, H-3′, H-5′), 7.08 (s, 1H, H-3), 3.55 (s, 2H, Ar–CH_2–), 2.70 (br, 4H, N(CH$_2$CH_2)$_2$NH), 2.48(s, 1H, N*H*), and 2.32(br, 4H, N(CH_2CH$_2$)$_2$NH); ESI–MS *m/z* 355 $[M + H]^+$.

*2-(4-Chlorophenyl)-6-((dimethylamino)methyl)-4H-chromen-4-one (**17r**)*

Yield 39%; IR (KBr) υ_{max} 3,441, 2,969, 2,770, 1,642, 1,622, 1,577, 1,495, 1,455, 1,408, 1,364, 1,134, 1,095, 1,029, 1,012, 903, 840, 820 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.13 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.94 (s, 1H, H-5), 7.74 (m, 2H, H-7, H-8), 7.65 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.06 (s, 1H, H-3), 3.52 (s, 2H, Ar–CH_2–), and 2.18 (s, 6H, N(CH_3)$_2$); ESI–MS *m/z* 314 $[M + H]^+$.

*6-((1H-imidazol-1-yl)methyl)-2-(4-chlorophenyl)-4H-chromen-4-one (**17s**)*

Yield 47%; IR (KBr) υ_{max} 3,433, 1,640, 1,620, 1,578, 1,493, 1,452, 1,408, 1,369, 1,285, 1,232, 1,093, 1,038, 911, 827 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.12 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.91 (s, 1H, H-2″), 7.84 (s, 1H,

H-5), 7.79 (d, 1H, $J = 8.5$ Hz, H-5″), 7.73 (d, 1H, $J = 8.5$ Hz, H-4″), 7.64 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.26 (s, 1H, H-8), 7.07 (s, 1H, H-7), 6.95(s, 1H, H-3), and 5.36 (s, 2H, Ar–CH_2–); ESI–MS *m/z* 337 $[M + H]^+$.

*N-((2-(4-chlorophenyl)-4-oxo-4H-chromen-6-yl)methyl)-N,N-diethylethanaminium (**17t**)*

Yield 52%; IR (KBr) υ_{max} 3,439, 2,997, 1,642, 1,618, 1,577, 1,495, 1,449, 1,408, 1,360, 1,095, 1,036, 908, 829 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.20 (br, 1H, H-5), 8.15 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.92 (m, 2H, H-7, H-8), 7.65 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.15 (s, 1H, H-3), 4.65 (s, 2H, Ar–CH_2–), 3.19 (q, 6H, $J = 7.0$ Hz, N(CH_2CH$_3$)$_3$), and 1.31 (t, 9H, $J = 7.0$ Hz, N(CH$_2$CH_3)$_3$); ESI–MS *m/z* 370 $[M]^+$.

*N-((2-(2-chlorophenyl)-4-oxo-4H-chromen-6-yl)methyl)-N,N-diethylethanaminium (**17u**)*

Yield 51.2%; IR (KBr) υ_{max} 3,442, 1,639, 1,572, 1,521, 1,488, 1,448, 1,411, 1,347, 1,198, 1,037, 910, 852, 694 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.41 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.26 (s, 1H, H-5), 7.98 (m, 2H, H-7, H-8), 7.32 (s, 1H, H-3), 4.70 (s, 2H, Ar–CH_2–), 3.23 (q, 6H, $J = 7.0$ Hz, N(CH_2CH$_3$)$_3$), and 1.35 (t, 9H, $J = 7.0$ Hz, N(CH$_2$CH_3)$_3$); ESI–MS *m/z* 381 $[M + H]^+$.

*6-((Diethylamino)methyl)-2-phenyl-4H-chromen-4-one (**17v**)*

Yield 51.2%; IR (KBr) υ_{max} 3,442, 2,974, 2,793, 1,647, 1,621, 1,483, 1,454, 1,364, 1,225, 1,198, 1,043, 901, 831, 771, 687 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.11 (m, 2H, H-2′, H-6′), 7.99 (s, 1H, H-5), 7.76 (m, 2H, H-7, H-8), 7.60 (br, 3H, H-3′, H-4′, H-5′), 7.03 (s, 1H, H-3), 3.66 (s, 2H, Ar–CH_2–), 2.49 (q, 4H, $J = 6.5$ Hz, N(CH_2CH$_3$)$_2$, and 1.00 (t, 6H, $J = 6.5$ Hz, N(CH$_2$CH_3)$_2$); ESI–MS *m/z* 308 $[M + H]^+$.

*7-((Diethylamino)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**18f**)*

Yield 43.2%; IR (KBr) υ_{max} 3,443, 2,971, 2,797, 1,636, 1,523, 1,437, 1,346, 1,115, 1,042, 910, 851, 757, 692 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.37 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.00 (d, 1H, $J = 8.0$ Hz, H-5), 7.74 (s, 1H, H-8), 7.47 (d, 1H, $J = 8.0$ Hz, H-6), 7.20 (s, 1H, H-3), 3.70 (s, 2H, Ar–CH_2–), 2.56 (q, 4H, $J = 6.9$ Hz, N(CH_2CH$_3$)$_2$), and 1.01 (t, 6H, $J = 6.9$ Hz, N(CH$_2$CH_3)$_2$); ESI–MS *m/z* 353 $[M + H]^+$.

*2-(4-Nitrophenyl)-7-(piperidin-1-ylmethyl)-4H-chromen-4-one (**18g**)*

Yield 34.8%; IR (KBr) υ_{max} 3,425, 2,937, 1,635, 1,521, 1,438, 1,346, 1,106, 1,043, 910, 851, 756, 692 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.39 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.01 (d, 1H, $J = 9.0$ Hz, H-5), 7.77 (s, 1H, H-8), 7.48 (d, 1H, $J = 9.0$ Hz, H-6), 7.22 (s, 1H, H-3), 3.68 (s, 2H, Ar–CH_2–), 2.46 (m, 4H, N(CH_2CH$_2$)$_2$CH$_2$), 1.56 (m, 4H, N(CH$_2$CH_2)$_2$CH$_2$), and 1.43 (m, 2H, N(CH$_2$CH$_2$)$_2$CH_2); ESI–MS *m/z* 365 $[M + H]^+$.

*7-((4-Methylpiperazin-1-yl)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**18h**)*

Yield 36.3%; IR (KBr) υ_{max} 3,443, 2,938, 2,803, 1,635, 1,519, 1,437, 1,347, 1,160, 1,135, 1,045, 909, 850, 812, 755, 691 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.39 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.02 (d, 1H, $J = 9.2$ Hz, H-5), 7.75 (s, 1H, H-8), 7.47 (d, 1H, $J = 9.2$ Hz, H-6), 7.23 (s, 1H, H-3), 3.67 (s, 2H, Ar–CH_2–), 2.50 (brs, 8H, N(CH_2CH_2)$_2$NCH$_3$), and 2.30 (s, 3H, NCH_3); ESI–MS *m/z* 380 $[M + H]^+$.

*7-(Morpholinomethyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**18i**)*

Yield 43.1%; IR (KBr) υ_{max} 3,451, 2,964, 1,634, 1,521, 1,436, 1,346, 1,113, 1,008, 910, 850 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.40 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.03 (d, 1H, $J = 9.1$ Hz, H-5), 7.77 (s, 1H, H-8), 7.49 (d, 1H, $J = 9.1$ Hz, H-6), 7.23 (s, 1H, H-3), 3.66 (s, 2H, –CH_2–), 3.61 (t, 4H, $J = 4.5$ Hz, N(CH_2CH$_2$)$_2$O), and 2.43 (t, 4H, $J = 4.5$ Hz, N(CH$_2$CH_2)$_2$O); ESI–MS *m/z* 367 $[M + H]^+$.

*2-(4-Nitrophenyl)-7-(piperazin-1-ylmethyl)-4H-chromen-4-one (**18j**)*

Yield 30.7%; IR (KBr) υ_{max} 3,443, 2,807, 1,634, 1,521, 1,436, 1,346, 1,139, 1,008, 910, 850 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.40 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.03 (d, 1H, $J = 8.1$ Hz, H-5), 7.77 (d, 1H, $J = 8.1$ Hz, H-6), 7.48 (s, 1H, H-8), 7.23 (s, 1H, H-3), 3.65 (s, 2H, Ar–CH_2–), 2.83 (m, 4H, N(CH$_2$CH_2)$_2$NH)), and 2.43 (m, 4H, N(CH_2CH$_2$)$_2$NH)); ESI–MS *m/z* 366 $[M + H]^+$.

*2-(4-Chlorophenyl)-7-((diethylamino)methyl)-4H-chromen-4-one (**18k**)*

Yield 41.2%; IR (KBr) υ_{max} 3,444, 2,970, 2,803, 1,638, 1,572, 1,492, 1,439, 1,371, 1,094, 1,042, 909, 826 cm^{-1};

^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.16 (d, 2H, J = 7.8 Hz, H-2′, H-6′), 8.00 (d, 1H, J = 9.0 Hz, H-5), 7.73 (s, 1H, H-8), 7.66 (d, 2H, J = 7.8 Hz, H-3′, H-5′), 7.47 (d, 1H, J = 9.0 Hz, H-6), 7.06 (s, 1H, H-3), 3.71 (s, 2H, Ar–CH_2–), 2.54 (q, 4H, J = 6.9 Hz, N(CH_2CH$_3$)$_2$), and 1.02 (t, 6H, J = 6.9 Hz, N(CH$_2$CH_3)$_2$); ESI–MS *m/z* 342 $[M + H]^+$.

*2-(4-chlorophenyl)-7-(piperidin-1-ylmethyl)-4H-chromen-4-one (**18l**)*

Yield 43%; IR (KBr) υ_{max} 3,442, 2,933, 2,794, 1,642, 1,570, 1,492, 1,437, 1,345, 1,106, 1,042, 909, 831 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.15 (d, 2H, J = 7.8 Hz, H-2′, H-6′), 7.99 (d, 1H, J = 9.0 Hz, H-5), 7.70 (s, 1H, H-8), 7.65(d, 2H, J = 7.8 Hz, H-3′, H-5′), 7.44 (d, 1H, J = 9.0 Hz, H-6), 7.06 (s, 1H, H-3), 3.60 (s, 2H, Ar–CH_2–), 2.38 (br, 4H, N(CH_2CH$_2$)$_2$CH$_2$), 1.52(br, 4H, N(CH$_2$CH_2)$_2$CH$_2$), and 1.41 (br, 2H, N(CH_2CH$_2$)$_2$CH_2); ESI–MS *m/z* 354 $[M + H]^+$.

*2-(4-Chlorophenyl)-7-((4-methylpiperazin-1-yl)methyl)-4H-chromen-4-one (**18m**)*

Yield 46%; IR (KBr) υ_{max} 3,428, 2,790, 1,641, 1,569, 1,491, 1,436, 1,409, 1,370, 1,283, 1,093, 1,042, 1,011, 907, 830 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.14 (d, 2H, J = 6.9 Hz, H-2′, H-6′), 8.00 (d, 1H, J = 7.8 Hz, H-5), 7.69 (s, 1H, H-8), 7.65 (d, 2H, J = 6.9 Hz, H-3′, H-5′), 7.43 (d, 1H, J = 7.8 Hz, H-6), 7.06 (s, 1H, H-3), 3.63 (s, 2H, Ar–CH_2–), 2.65 (br, 4H, N(CH$_2$CH_2)$_2$NCH$_3$), 2.44 (br, 4H, N(CH_2CH$_2$)$_2$NCH$_3$), and 2.21 (s, 3H, NCH_3); ESI–MS *m/z* 369 $[M + H]^+$.

*2-(4-Chlorophenyl)-7-(morpholinomethyl)-4H-chromen-4-one (**18n**)*

Yield 52%; IR (KBr) υ_{max} 3,444, 2,805, 1,641, 1,570, 1,492, 1,437, 1,409, 1,371, 1,351, 1,112, 1,005, 907, 865, 810 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.13 (d, 2H, J = 9.1 Hz, H-2′, H-6′), 7.99 (d, 1H, J = 9.0 Hz, H-5), 7.71 (s, 1H, H-8), 7.64 (d, 2H, J = 9.1 Hz, H-3′, H-5′), 7.45 (d, 1H, J = 9.0 Hz, H-6), 7.04 (s, 1H, H-3), 3.64 (s, 2H, Ar–CH_2–), 3.62 (t, 4H, N(CH$_2$CH_2)O), and 2.51(t, 4H, J = 4.4 Hz, N(CH_2CH$_2$)$_2$O); ESI–MS *m/z* 356 $[M + H]^+$.

*2-(4-Chlorophenyl)-7-(piperazin-1-ylmethyl)-4H-chromen-4-one (**18o**)*

Yield 36.7%; IR (KBr) υ_{max} 3,443, 2,927, 1,640, 1,569, 1,436, 1,410, 1,371, 1,112, 1,094, 908, 829 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.15 (d, 2H, J = 8.4 Hz, H-2′, H-6′), 8.00 (d, 1H, J = 8.1 Hz, H-5), 7.72 (s, 1H, H-8), 7.66 (d, 1H, J = 8.4, H-3′, H-5′), 7.45 (d, 1H, J = 8.1 Hz, H-6), 7.07 (s, 1H, H-3), 3.61 (s, 2H, Ar–CH_2–), 2.74 (m, 4H, CH$_2$N(CH$_2$CH_2)$_2$NH)), and 2.36 (m, 4H, CH$_2$N(CH_2CH$_2$)$_2$NH)); ESI–MS *m/z* 355 $[M + H]^+$.

*2-(4-Chlorophenyl)-7-((dimethylamino)methyl)-4H-chromen-4-one (**18p**)*

Yield 38%; IR (KBr) υ_{max} 3,443, 2,976, 1,637, 1,572, 1,492, 1,442, 1,373, 1,096, 1,032, 908, 823 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.15 (d, 2H, J = 9.2 Hz, H-2′, H-6′), 8.00 (d, 1H, J = 9.0 Hz, H-5), 7.73 (s, 1H, H-8), 7.65 (d, 2H, J = 9.2 Hz, H-3′, H-5′), 7.46 (d, 1H, J = 9.0 Hz, H-6), 7.07 (s, 1H, H-3), 3.63 (s, 2H, Ar–CH_2), and 2.25 (s, 6H, N(CH_3)$_2$); ESI–MS *m/z* 314 $[M + H]^+$.

*7-((1H-imidazol-1-yl)methyl)-2-(4-chlorophenyl)-4H-chromen-4-one (**18q**)*

Yield 39%; IR (KBr) υ_{max} 3,441, 1,629, 1,571, 1,490, 1,437, 1,410, 1,372, 1,096, 1,011, 909, 831 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.09 (d, 2H, J ,= 8.6 Hz, H-2′, H-6′), 8.00 (d, 1H, J = 9.0 Hz, H-5), 7.87 (s, 1H, H-8), 7.64 (d, 2H, J = 8.6 Hz, H-3′, H-5′), 7.62 (s, 1H, H-2″), 7.57 (d, 1H, J = 9.0 Hz, H-6), 7.36 (d, 1H, J = 5.5 Hz, H-4″), 7.06 (s, 1H, H-3), 7.00 (d, 1H, J = 5.5 Hz, H-5″), and 5.42 (s, 2H, Ar–CH_2–); ESI–MS *m/z* 337 $[M + H]^+$.

*N-((2-(4-chlorophenyl)-4-oxo-4H-chromen-7-yl)methyl)-N,N-diethylethan-amiium (**18r**)*

Yield 46%; IR (KBr) υ_{max} 3,444, 2,985, 1,639, 1,562, 1,492, 1,440, 1,411, 1,369, 1,311, 1,095, 1,012, 909, 834 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.15 (d, 2H, J = 8.5 Hz, H-2′, H-6′), 8.10 (d, 1H, J = 9.2 Hz, H-5), 8.03 (d, 1H, J = 1.8 Hz, H-8), 7.66 (d, 2H, J = 8.5 Hz, H-3′, H-5′), 7.62 (dd, 1H, J = 9.2, 1.8 Hz, H-6), 7.13 (s, 1H, H-3), 4.67 (s, 2H, Ar–CH_2–), 3.26 (q, 6H, J = 7.2 Hz, N(CH_2CH$_3$)$_3$, and 1.33 (t, 9H, J = 7.2 Hz, N(CH$_2$CH_3)$_3$; ESI–MS *m/z* 370 $[M]^+$.

*8-((Diethylamino)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**19f**)*

Yield 43.2%; IR (KBr) υ_{max} 3,445, 2,970, 1,655, 1,522, 1,417, 1,347, 1,117, 1,066, 851, 758, 696 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.41 (br, 4H, H-2′, H-3′, H-5′, H-6′), 7.98 (d, 1H, J = 8.1 Hz, H-5), 7.91 (d, 1H, J = 8.1 Hz, H-7), 7.51(d, 1H, J = 8.1 Hz, H-6), 7.25 (s, 1H, H-3), 3.98 (s, 2H, Ar–CH_2–), 2.60 (q, 4H, J = 6.8 Hz, N(CH_2CH$_3$)$_2$), and 1.03 (t, 6H, J = 6.8 Hz, N(CH$_2$CH_3)$_2$); ESI–MS *m/z* 353 $[M + H]^+$.

*8-((4-Methylpiperazin-1-yl)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**19g**)*

Yield 45%; IR (KBr) υ_{max} 3,439, 2,923, 1,645, 1,481, 1,460, 1,416, 1,376, 1,281, 1,145, 1,036, 1,009, 847, 756 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.20 (br, 2H, H-3′, H-5′), 7.95 (br, 1H, H-5), 7.88 (m, 2H, H-2′, H-6′), 7.55 (m, 1H, H-6), 6.73 (s, 1H, H-3), 3.69 (s, 2H, Ar–CH_2–), 2.34 (br, 4H, N($CH_2CH_2)_2$NCH_3), 2.05 (br, 4H, N($CH_2CH_2)_2$NCH_3), and 1.01 (s, 3H, NCH_3); ESI–MS *m/z* 380 $[M + H]^+$.

*8-(Morpholinomethyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**19h**)*

Yield 56%; IR (KBr) υ_{max} 3,444, 2,855, 1,640, 1,582, 1,520, 1,377, 1,346, 1,116, 1,009, 853 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.42 (m, 4H, H-2′, H-3′, H-5′, H-6′), 8.01 (d, 1H, $J = 7.8$ Hz, H-5), 7.85 (d, 1H, $J = 7.8$ Hz, H-7), 7.50 (t, 1H, $J = 7.8$ Hz, H-6), 3.90 (s, 2H, Ar–CH_2), 3.60 (t, 4H, $J = 4.2$ Hz, N($CH_2CH_2)_2$O), and 2.50 (t, 4H, $J = 4.2$ Hz, N($CH_2CH_2)_2$O); ESI–MS *m/z* 367 $[M + H]^+$.

*6-Chloro-8-((diethylamino)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**19i**)*

Yield 43.2%; IR (KBr) υ_{max} 3,444, 2,970, 1,651, 1,523, 1,450, 1,346, 1,117, 849 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.41 (br, 4H, H-2′, H-3′, H-5′, H-6′), 7.90 (br, 2H, H-5, H-7), 7.29 (s, 1H, H-3), 3.97 (s, 2H, Ar–CH_2–), 2.60 (q, 4H, $J = 6.9$ Hz, N($CH_2CH_3)_2$), and 1.03 (t, 6H, $J = 6.9$ Hz, N($CH_2CH_3)_2$); ESI–MS *m/z* 387 $[M + H]^+$.

*6-Chloro-2-(4-nitrophenyl)-8-(piperidin-1-ylmethyl)-4H-chromen-4-one (**19j**)*

Yield 38%; IR (KBr) υ_{max} 3,442, 2,936, 1,649, 1,524, 1,450, 1,346, 1,299, 11,107, 849 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.41 (br, 4H, H-2′, H-3′, H-5′, H-6′), 7.92 (s, 1H, H-5), 7.87 (s, 1H, H-7), 7.32 (s, 1H, H-3), 3.87 (s, 2H, Ar–CH_2), 2.51 (br, 4H, N($CH_2CH_2)_2CH_2$), 1.55 (br, 4H, N($CH_2CH_2)_2CH_2$), and 1.43 (br, 2H, N($CH_2CH_2)_2$ CH_2); ESI–MS *m/z* 399 $[M + H]^+$.

*6-Chloro-8-((4-methylpiperazin-1-yl)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**19k**)*

Yield 47%; IR (KBr) υ_{max} 3,424, 2,939, 2,803, 1,645, 1,579, 1,524, 1,454, 1,347, 1,298, 1,142, 849 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.40 (br, 4H, H-2′, H-3′, H-5′, H-6′), 7.91 (s, 1H, H-5), 7.86 (s, 1H, H-7), 7.31 (s, 1H, H-3), 3.88 (s, 2H, Ar–CH_2–), 2.51 (br, 4H, N($CH_2CH_2)_2$NCH_3), 2.39 (br, 4H, N($CH_2CH_2)_2$NCH_3), and 2.15 (br, 3H, N($CH_2CH_2)_2$NCH_3); ESI–MS *m/z* 414 $[M + H]^+$.

*6-Chloro-8-(morpholinomethyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**19l**)*

Yield 62%; IR (KBr) υ_{max} 3,442, 2,854, 1,649, 1,581, 1,525, 1,451, 1,347, 1,115, 1,010, 850 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.42 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.94 (s, 1H, H-5), 7.91 (d, 1H, H-7), 7.34 (s, 1H, H-3), 3.91(s, 2H, Ar–CH_2–), 3.61(t, 4H, $J = 4.2$ Hz, N($CH_2CH_2)_2$O), and 2.51 (t, 4H, $J = 4.2$ Hz, N($CH_2CH_2)_2$O); ESI–MS *m/z* 401 $[M + H]^+$.

*6-Chloro-2-(4-nitrophenyl)-8-(piperazin-1-ylmethyl)-4H-chromen-4-one (**19m**)*

Yield 46%;IR (KBr) υ_{max} 3,435, 1,651, 1,523, 1,447, 1,405, 1,347, 1,163, 1,116, 1,089, 851 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.41 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.92 (br, 2H, H-5, H-7), 7.32 (s, 1H, H-3), 3.93(s, 2H, Ar–CH_2), 2.63(s, 4H, N($CH_2CH_2)_2$NH), 2.50 (t, 4H, $J = 4.2$ Hz, N($CH_2CH_2)_2$NH), and 2.27(s, 1H, N*H*); ESI–MS *m/z* 400 $[M + H]^+$.

*6-Chloro-8-((dimethylamino)methyl)-2-(4-nitrophenyl)-4H-chromen-4-one (**19n**)*

Yield 40%; IR (KBr) υ_{max} 3,442, 2,943, 2,821, 1,649, 1,580, 1,523, 1,451, 1,346, 1,037, 849 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.39 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.89 (s, 1H, H-5), 7.85 (s, 1H, H-7), 7.29 (s, 1H, H-3), 3.82 (s, 2H, Ar–CH_2), and 2.29 (s, 6H, N($CH_3)_2$); ESI–MS *m/z* 359 $[M + H]^+$.

*8-((1H-imidazol-1-yl)methyl)-6-chloro-2-(4-nitrophenyl)-4H-chromen-4-one (**19o**)*

Yield 49%; IR (KBr) υ_{max} 3,431, 1,649, 1,580, 1,523, 1,460, 1,347, 1,081, 851 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.38 (m, 4H, H,-2′, H-3′, H-5′, H-6′), 8.08 (m, 1H, H-2″), 7.96 (s, 1H, H-5), 7.56 (s, 1H, H-5″), 7.33 (br, 2H, H-4″, H-7), 7.02 (s, 1H, H-3), and 5.72 (s, 2H, Ar–CH_2–); ESI–MS *m/z* 382 $[M + H]^+$.

*6-Chloro-2-(2-chlorophenyl)-8-((diethylamino)methyl)-4H-chromen-4-one (**19p**)*

Yield 49%; IR (KBr) υ_{max} 3,444, 2,972, 1,667, 1,649, 1,595, 1,455, 1,363, 1,194, 1,120, 1,071, 1,033, 857, 778 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.88 (m, 3H, H-3′, H-5, H-6′), 7.64 (m, 3H, H-4′, H-5′, H-7), 6.74 (s, 1H,

H-3), 3.82 (s, 2H, Ar–CH_2–), 2.54 (q, 4H, $J = 6.9$ Hz, N(CH_2CH$_3$)$_2$), and 0.95 (t, 6H, $J = 6.9$ Hz, N(CH$_2$CH_3)$_2$); ESI–MS *m/z* 376 $[M + H]^+$.

*6-Chloro-2-(2-chlorophenyl)-8-(piperidin-1-ylmethyl)-4H-chromen-4-one (**19q**)*

Yield 35%; IR (KBr) υ_{max} 3,444, 2,937, 1,644, 1,593, 1,449, 1,430, 1,352, 1,271, 1,030, 855, 796 cm^{-1}; ^1H NMR (DMSO-d$_6$, 300 MHz) δ 7.94 (s, 1H, H-5), 7.90 (m, 2H, H-3′, H-6′), 7.62 (m, 3H, H-4′, H-5′, H-7), 6.76 (s, 1H, H-3), 3.74 (s, 2H, Ar–CH_2–), 2.42 (br, 4H, N(CH_2CH$_2$)$_2$CH$_2$), 1.50 (br, 4H, N(CH$_2$CH_2)$_2$CH$_2$), and 1.39 (br, 2H, N(CH$_2$CH$_2$)$_2$CH_2); ESI–MS *m/z* 388 $[M + H]^+$.

*6-Chloro-2-(2-chlorophenyl)-8-((4-methylpiperazin-1-yl)methyl)-4H-chromen-4-one (**19r**)*

Yield 48%; IR (KBr) υ_{max} 3,448, 2,939, 2,802, 1,710, 1,637, 1,581, 1,462, 1,355, 1,280, 1,161, 1,142, 1,012, 853, 762 cm^{-1}; ^1H NMR (DMSO-d$_6$, 300 MHz) δ 7.93 (s, 1H, H-5), 7.86 (m, 2H, H-3′, H-6′), 7.64 (m, 3H, H-4′, H-5′, H-7), 6.76 (s, 1H, H-3), 3.76 (s, 2H, Ar–CH_2–), 2.46 (br, 4H, N(CH_2CH$_2$)$_2$NCH$_3$), 2.36 (br, 4H, N(CH$_2$CH_2)$_2$NCH$_3$), and 2.17 (s, 3H, NCH_3); ESI–MS *m/z* 403 $[M + H]^+$.

*6-Chloro-2-(2-chlorophenyl)-8-(morpholinomethyl)-4H-chromen-4-one (**19s**)*

Yield 61%; IR (KBr) υ_{max} 3,444, 2,968, 2,817, 1,646, 1,592, 1,450, 1,354, 1,271, 1,138, 1,116, 1,014, 854, 767 cm^{-1}; ^1H NMR (DMSO-d$_6$, 300 MHz) δ 7.94 (s, 1H, H-5), 7.88 (m, 2H, H-3′, H-6′), 7.64 (m, 3H, H-4′, H-5′, H-7), 6.76 (s, 1H, H-3), 3.77 (s, 2H, Ar–CH_2), 3.59 (t, 4H, $J = 4.5$ Hz, N(CH$_2$CH_2)$_2$O), and 2.44 (t, 4H, $J = 4.5$ Hz, N(CH_2CH$_2$)$_2$O); ESI–MS *m/z* 390 $[M + H]^+$.

*6-Chloro-2-(2-chlorophenyl)-8-(piperazin-1-ylmethyl)-4H-chromen-4-one (**19t**)*

Yield 52%; IR (KBr) υ_{max} 3,442, 2,937, 2,808, 1,648, 1,593, 1,450, 1,354, 1,294, 1,197, 1,131, 1,072, 854, 762 cm^{-1}; ^1H NMR (DMSO-d$_6$, 300 MHz) δ 7.94 (s, 1H, H-5), 7.87 (m, 2H, H-3′, H-6′), 7.65 (m, 3H, H-4′, H-5′, H-7), 6.76 (s, 1H, H-3), 3.75 (s, 2H, Ar–CH_2–), 2.78 (br, 4H, N(CH$_2$CH_2)$_2$NH), 2.43 (br, 4H, N(CH_2CH$_2$)$_2$NH), and 2.10 (s, 1H, NH); ESI–MS *m/z* 389 $[M + H]^+$.

*6-Chloro-2-(2-chlorophenyl)-8-((dimethylamino)methyl)-4H-chromen-4-one (**19u**)*

Yield 51%; IR (KBr) υ_{max} 3,448, 3,065, 2,930, 2,826, 1,640, 1,508, 1,452, 1,396, 1,353, 1,266, 1,199, 1,086, 1,073, 1,028, 857, 770, 715 cm^{-1}; ^1H NMR (DMSO-d$_6$, 300 MHz) δ 7.93 (s, 1H, H-5), 7.86 (m, 2H, H-3′, H-6′), 7.65 (m, 3H, H-4′, H-5′, H-7), 6.76 (s, 1H, H-3), 3.72 (s, 2H, Ar–CH_2), and 2.23 (s, 6H, N(CH_3)$_2$); ESI–MS *m/z* 348 $[M + H]^+$.

*8-((1H-imidazol-1-yl)methyl)-6-chloro-2-(2-chlorophenyl)-4H-chromen-4-one (**19v**)*

Yield 41%; IR (KBr) υ_{max} 3,419, 1,649, 1,593, 1,462, 1,439, 1,361, 1,345, 1,286, 1,232, 1,158, 1,111, 1,088, 1,026, 898, 856, 784, 760 cm^{-1}; ^1H NMR (DMSO-d$_6$, 300 MHz) δ 7.99 (s, 1H, H-5″), 7.83 (s, 1H, H-5), 7.68 (m, 4H, H-2′, H-3′, H-4′, H-5′), 7.49 (s, 1H, H-5″), 7.20 (s, 1H, H-7), 6.94 (s, 1H, H-4″), 6.80 (s, 1H, H-3), and 5.54 (s, 2H, Ar–CH_2–); ESI–MS *m/z* 348 $[M + H]^+$.

*6-Chloro-2-(4-chlorophenyl)-8-(morpholinomethyl)-4H-chromen-4-one (**19w**)*

Yield 62%; IR (KBr) υ_{max} 3,445, 1,650, 1,595, 1,493, 1,451, 1,357, 1,117, 1,093, 1,012, 827 cm^{-1}; ^1H NMR (DMSO-d$_6$, 300 MHz) δ 8.16 (d, 2H, $J = 8.7$ Hz, H-3′, H-5′), 7.90 (d, 1H, $J = 2.4$ Hz, H-5), 7.85 (d, 1H, $J = 2.4$ Hz, H-7), 7.68 (d, 2H, $J = 8.7$ Hz, H-2′, H-6′), 7.15 (s, 1H, H-3), 3.88 (s, 2H, Ar–CH_2–), 3.61 (t, 4H, $J = 4.5$ Hz, N(CH$_2$CH_2)$_2$O), and 2.51 (t, 4H, $J = 4.5$ Hz, N(CH_2CH$_2$)$_2$O); ESI–MS *m/z* 390 $[M + H]^+$.

General procedure for the reduction of the nitro group

Compounds **17a**, **17f**, **17g**, **17i**, **18a**, and **19b** (5 mmo1) were individually suspended in 100 ml of a solution of ethanol in water [V(EtOH):V(H_2O) = 2:1] and heated under reflux. Sodium dithionite (6 mmoI) was gradually added to the flask until the compound was dissolved completely to give a yellow solution. An excess of approximately 0.5 g of sodium dithionite was then added to the solution, and the solution was refluxed for another 1 h. Ethanol was distilled at a reduced pressure, and the solution was poured to 80 ml of concentrated HCl and heated further until the smell of SO_2 disappeared. The solution was filtered hot and cooled, then neutralized with ammonia to give **17e**, **17w**, **17x**, **17y**, **18e**, and **19e**, respectively.

*2-(4-Aminophenyl)-6-methyl-4H-chromen-4-one (**17e**)*

Yield 89%; IR (KBr) υ_{max} 3,435, 3,355, 3,235, 1,633, 1,604, 1,558, 1,516, 1,484, 1,448, 1,367, 1,338, 1,255, 1,187, 1,035, 902, 826, 812 cm^{-1}; ^1H NMR (DMSO-d$_6$, 300 MHz) δ 6.93 (br, 3H, H-5, H-2′, H-6′), 6.74 (br, 2H, H-7, H-8), 5.83 (br, 3H, H-3, H-3′, H-5′), 5.15 (s, 2H, NH_2), and 2.50 (s, 3H, CH_3); ESI–MS *m/z* 252 $[M + H]^+$.

*2-(4-Aminophenyl)-6-((diethylamino)methyl)-4H-chromen-4-one (**17w**)*

Yield 81%; IR (KBr) υ_{max} 3,443, 2,968, 1,630, 1,617, 1,563, 1,515, 1,483, 1,452, 1,365, 1,186, 833 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.92 (s, 1H, H-5), 7.78 (d, 2H, $J = 7.7$ Hz, H-2′, H-6′), 7.70 (d, 1H, $J = 7.4$ Hz, H-7), 7.64 (d, 1H, $J = 7.4$ Hz, H-8), 6.68 (d, 2H, $J = 7.4$ Hz, H-3′, H-5′), 5.99 (s, 2H, NH_2), 2.50 (q, 4H, $J = 6.9$ Hz, $N(CH_2CH_3)_2$), and 1.06 (t, 6H, $J = 6.9$ Hz, $N(CH_2CH_3)_2$); ESI–MS *m/z* 323 $[M + H]^+$.

*2-(4-Aminophenyl)-6-(piperidin-1-ylmethyl)-4H-chromen-4-one (**17x**)*

Yield 80%; IR (KBr) υ_{max} 3,455, 2,934, 1,633, 1,565, 1,514, 1,453, 1,365, 1,187, 1,038, 834 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.41 (s, 1H, H-5), 8.18 (m, 2H, H-7, H-8), 7.80 (d, 2H, $J = 8.54$ Hz, H-2′, H-6′), 6.75 (s, 1H, H-3), 6.70 (d, 2H, $J = 8.54$ Hz, H-3′, H-5′), 6.10 (s, 2H, NH_2), 2.50 (br, 4H, $N(CH_2CH_2)_2CH_2$), and 1.64 (br, 6H, $N(CH_2CH_2)_2CH_2$); ESI–MS *m/z* 335 $[M + H]^+$.

*2-(4-Aminophenyl)-6-(morpholinomethyl)-4H-chromen-4-one (**17y**)*

Yield 45.0%; IR (KBr) υ_{max} 3,455, 1,633, 1,571, 1,452, 1,366, 1,188, 1,111, 862 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 7.91 (s, 1H, H-5), 7.78 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.70 (d, 1H, $J = 8.5$ Hz, H-7), 7.66 (d, 1H, $J = 8.5$ Hz, H-8), 6.71 (s, 1H, H-3), 6.68 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 3.58 (m, 4H, NCH_2CH_2O), 3.35 (m, 2H, Ar–CH_2–), and 2.38 (m, 4H, $N(CH_2CH_2)_2O$); ESI–MS *m/z* 337 $[M + H]^+$.

*2-(4-Aminophenyl)-7-methyl-4H-chromen-4-one (**18e**)*

Yield 86%; IR (KBr) υ_{max} 3,443, 1,631, 1,518, 1,496, 1,425, 1,375, 1,346, 1,251, 1,187, 1,161, 852, 820 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.87 (d, 1H, $J = 7.8$ Hz, H-5), 7.76 (d, 2H, $J = 11.4$ Hz, H-2′, H-6′), 7.51 (s, 1H, H-8), 7.25 (d, 1H, $J = 7.8$ Hz, H-6), 6.68 (d, 2H, $J = 11.4$ Hz, H-3′, H-5′), 6.66 (s, 1H, H-3), 6.00 (s, 2H, NH_2), and 2.45 (s, 3H, CH_3); ESI–MS *m/z* 525 $[2\ M + Na]^+$.

*2-(4-Aminophenyl)-6-chloro-8-methyl-4H-chromen-4-one (**19e**)*

Yield 89%; IR (KBr) υ_{max} 3,443, 3,071, 1,667, 1,582, 1,523, 1,465, 1,347, 1,325, 1,300, 1,041, 882, 852 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.77 (d, 2H, $J = 8.1$ Hz, H-2′, H-6′), 7.74 (s, 1H, H-5), 7.71 (s, 1H, H-7), 6.67 (d, 2H, $J = 8.1$ Hz, H-3′, H-5′), 6.75 (s, 1H, H-3), 6.07 (s, 2H, NH_2), and 2.49 (s, 3H, CH_3); ESI–MS *m/z* 286 $[M + H]^+$.

CDK1/cyclin B activity assays

The CDK1/cyclin B (Invitrogen Corporation, CA, USA) enzyme activity was measured by a fluorescence kinetic assay using the Z′-LYTE™ kinase assay kit (Invitrogen Corporation, CA, USA) according to the protocol. The assay was carried out at room temperature (25°C) in a Corning® 384-well assay plate. The final assay volume was 20 µl, including 2.5 µl 4× test compound, 5 µl 2× peptide substrate/CDK1/cyclin B mixture, 2.5 µl 4× ATP solution, 5 µl development reagent solution, and 5 µl stop reagent. The final concentration of the assay constituents was 250 ng/ml CDK1/cyclin B, 2-µM peptide substrate, and 25-µM ATP. Continuous kinetic monitoring of enzyme activity was performed on Thermo Scientific Varioskan Flash with $\lambda_{ex} = 400$, $\lambda_{em} = 445$, and $\lambda_{em} = 520$ nm.

Cytotoxicity

The cytotoxic activities of the compounds were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay. Cells HepG2 and SMMC-7721, growing at the logistic stage, were seeded in 100 µl Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37°C in a CO_2 incubator. Compounds **17a–17y**, **18a–18r**, and **19a–19w** and gambogic acid as control were diluted to the desired concentrations in the culture medium. After 48 h of incubation, media were removed, 10-µl MTT (5 mg/ml) was added to each well, and the plates were further incubated for 4 h. The supernatant from each well was carefully removed, formazon crystals were dissolved in 100 µl of DMSO, and absorption at 570-nm wavelength were recorded, respectively.

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