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Design, synthesis, and antiproliferative and CDK2-cyclin A inhibitory activity of novel flavopiridol analogues

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Abstract—The design and synthesis of a small library of 8-amidoflavone, 8-sulfonamidoflavone, 8-amido-7-hydroxyflavone, and heterocyclic analogues of flavopiridol is reported. The potential activity of these compounds as kinase inhibitors was evaluated by cytotoxicity studies in MCF-7 and ID-8 cancer cell lines and inhibition of CDK2-Cyclin A enzyme activity in vitro. The antiproliferative and CDK2-Cyclin A inhibitory activity of these analogues was significantly lower than the activity of flavopiridol. Molecular docking simulations were carried out and these studies suggested a different binding orientation inside the CDK2 binding pocket for these analogues compared to flavopiridol.

1. Introduction

The cyclin-dependent kinases (CDK) represent a class of enzymes that play a central role in cell-cycle progression and cellular proliferation.[1] CDKs are multi-subunit enzymes composed of at least a catalytic subunit (CDK) and a regulatory subunit (cyclin). They exert their effect via activation of host proteins through phosphorylation of key serine or threonine residues by ATP. The phosphorylated proteins modulate the activity of a variety of cellular proteins. It is widely accepted that inhibition of CDKs could provide control of the inappropriate cellular proliferation characteristic of certain cancers. A number of reviews have recently appeared which detail the possible use of CDKs as novel therapeutic targets for cancer chemotherapy.[2] Inhibitors developed to date can be divided into three general classes: (i) ATP-competitive inhibitors that contain key structural hydrogen-bonding motifs to bind to the ATP pocket; (ii) noncompetitive inhibitors that bind to the region of natural peptide inhibitors, typically these are small synthetic peptides (~20 residues); and (iii) dual inhibitors, representing molecules incorporating both of these attributes.[3]

Three common classes have emerged among the kinase inhibitors found to date: bis-indoles, purine-containing analogues, and flavones (Fig. 1). Staurosporine, an ATP-competitive PKC inhibitor,[4] and UCN-1 are representative compounds in the bis-indole class. A number of purine-containing compounds that bind to the ATP pocket have also shown promise, including purvalanols A and B,[5] and olomucine.[4c,5] Representative com-

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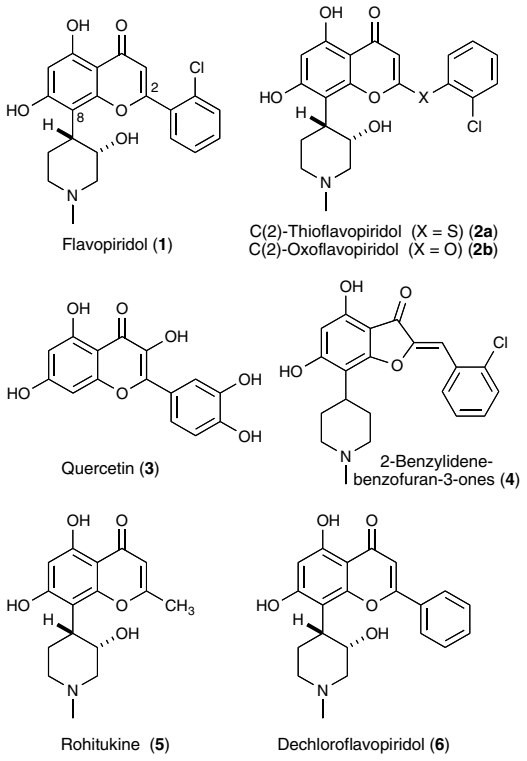


Figure 1. Structures of flavopiridol and representative flavopiridol analogues.

pounds in the flavone class of CDK inhibitors include flavopiridol (**1**),[6] 2-thioflavopiridol (**2a**),[7] 2-oxoflavopiridol (**2b**),[7] and quercetin (**3**),[8] and related analogues such as **4**.[7] Flavopiridol is a novel semi-synthetic derivative of the plant alkaloid rohitukine (**5**), itself isolated from an Indian plant, *Dysoxylum* sp.[9]

Flavopiridol is the first CDK inhibitor to undergo clinical trials against a variety of cancers.[10] Flavopiridol was shown to inhibit the proliferation of mammalian cell lines at nanomolar concentrations. Flavopiridol is non-selective, showing in vitro activity against CDK1, CDK2, CDK4 and protein-tyrosine kinase, with some activity for the EGF-receptor tyrosine kinase.[6e]

Due to the overall success of flavopiridol, and because of the availability of the X-ray structure of dechloroflavopiridol (**6**), co-crystallized with CDK2, revealing key hydrogen bonds (Fig. 2A), [6g–i] we decided to pursue the development of prototypical libraries based on the flavone scaffold. In spite of flavopiridol's potent activity, two major challenges remain; the development of analogues with improved kinase inhibitory selectivity and higher binding affinity.[3,11]

Recent work by Aronov and Murcko on kinase inhibitors suggests a distinct structural pattern for ''frequent-hitters'' emphasizing a five-point-of-attachment pharmacophore for the ATP binding site of kinases.[12] Flavopiridol and the analogues to be described herein depart from this structural pattern, and therefore can be reasonably supposed to show selectivity. SAR studies demonstrated that the flavone class of CDK inhibitory compounds is amenable to structural modifications at the C2 and C8 positions of the flavone core.[3a]

Another important consideration is that high activity and selectivity require the formation of at least two key hydrogen bonds between the substrate and the ATP binding pocket.[3] To this date, none of the flavone inhibitors have shown picomolar potencies and therefore it has been hypothesized that an additional binding interaction will be required to achieve both better potency and selectivity.[3a]

Accordingly, we initially designed a key 8-aminoflavone intermediate (Fig. 2B), which was designed to retain the hydrogen-bonding interactions with Glu81, Leu83, possibly also Wat327 (Fig. 2A), and the interactions of the 2-(2-chlorophenyl) group of **1** with the protein, avoiding the 'frequent-hitters' pharmacophore.[12] The 8-amino group provides a new site for introduction of various hydrogen bond donor/acceptor motifs aimed at providing additional interactions with the ATP binding pocket and surrounding areas so as to potentially impart potency and selectivity. Guided by the aforementioned SAR studies,[3] we initiated the synthesis of four classes of 8-amino-modified flavones related to flavopiridol (Fig. 3). The syntheses of the key 8-aminoflavone intermediates **10** and **16** are outlined in Schemes 1 and 2, respectively.

2. Chemistry

The 8-aminoflavone **10** was synthesized from 2′,6′-dihydroxyacetophenone (**7**) in four steps (Scheme 1). Reaction of **7** with two equivalents of 2-chlorobenzoyl chloride and a catalytic amount of dimethylaminopyridine (DMAP) in pyridine provided 2′,6′-di(2-chlorobenzoyl)acetophenone. Subsequent Baker–Venkataraman rearrangement[13] using DBU produced flavone **8** in excellent yield over two steps. Nitration with nitric acid and glacial acetic acid at 55 °C generated a 1:1 mixture of 8-nitro- and 6-nitroflavones **9a** and **9b**, along with traces of the 6,8-dinitroflavone. The nitroflavones **9a** and **9b** were separated using silica gel column chromatography. The corresponding 8-aminoflavone **10** was obtained by reduction of **9a** in the presence of tin chloride dihydrate. The structures of the two nitroflavones **9a** and **9b** (**9a** is less polar than **9b**) were assigned after X-ray crystallography of **10**.

The 8-amino-7-hydroxyflavone **16** was synthesized from 2′,4′,6′-trihydroxyacetophenone (**11**) in five steps (Scheme 2). Acetophenone **11** was treated with three equivalents of 2-chlorobenzoyl chloride and a catalytic amount of DMAP in pyridine to form 2′,4′,6′-tris(2-chlorobenzoyl)acetophenone, which was treated with KOH (5.0 equiv.) at room temperature for 1.5 h to furnish intermediates **12** and **13** (3:1 ratio for**12**:**13**).[14] The mixture of **12** and **13** was subjected to H_2SO_4 and glacial acetic acid conditions at 100 °C to provide **14** in 31% yield. Nitration of **14** gave a mixture of regioisomers **15a** and **15b** (1:1 ratio) and a trace amount of **15c**. The nitro compound **15a** was obtained after column

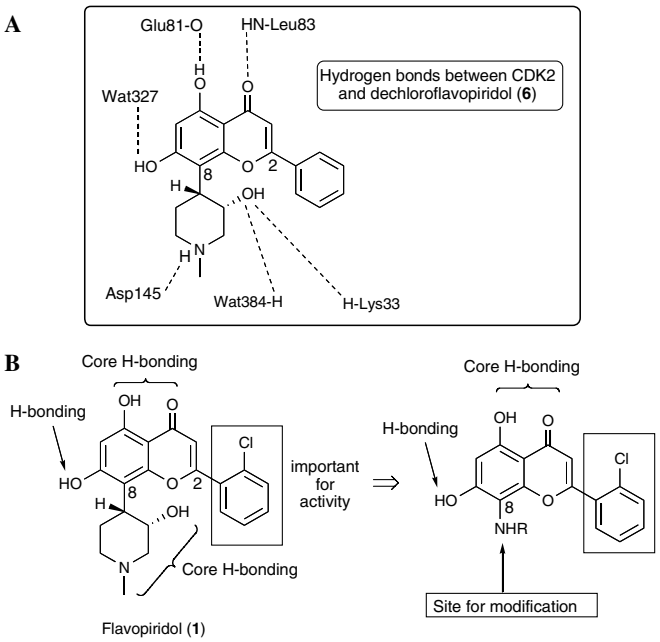


Figure 2. Key H-bonding interactions between CDK2 and dechloroflavopiridol (A). SAR relationship for flavopiridol and targeted compounds (B).

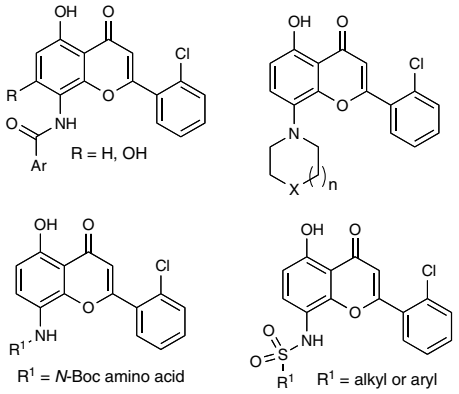
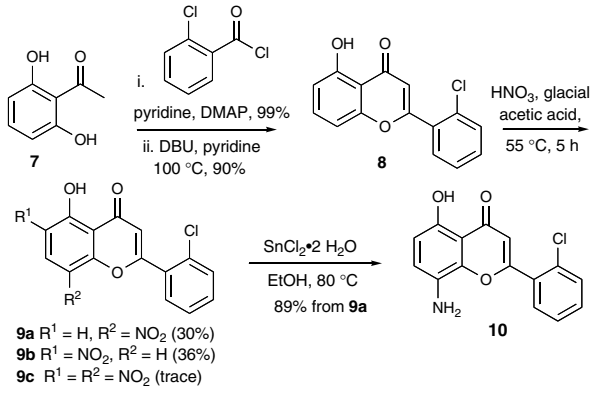
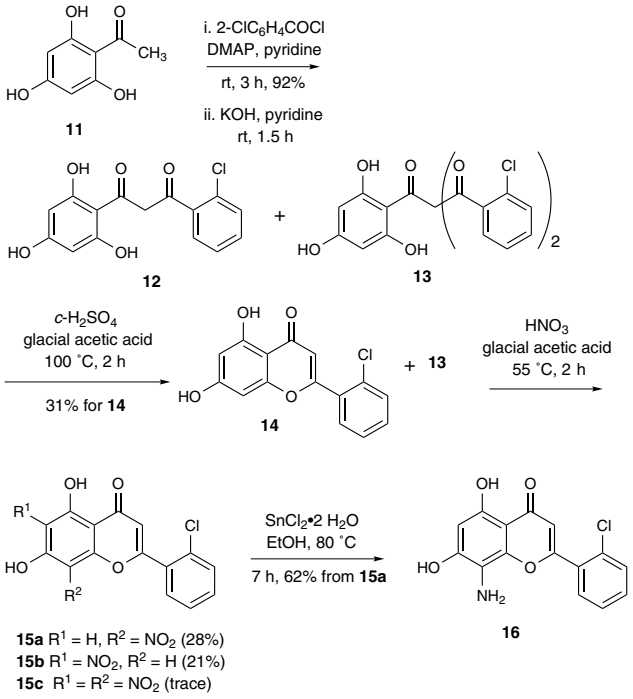


Figure 3. Targeted classes of flavopiridol analogues.



Scheme 1.

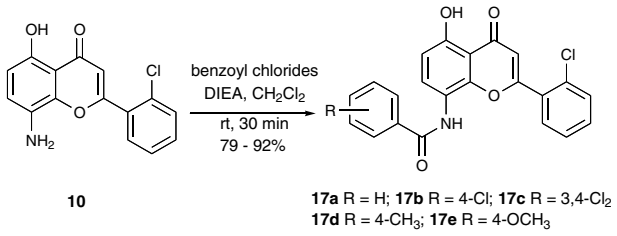


Scheme 2.

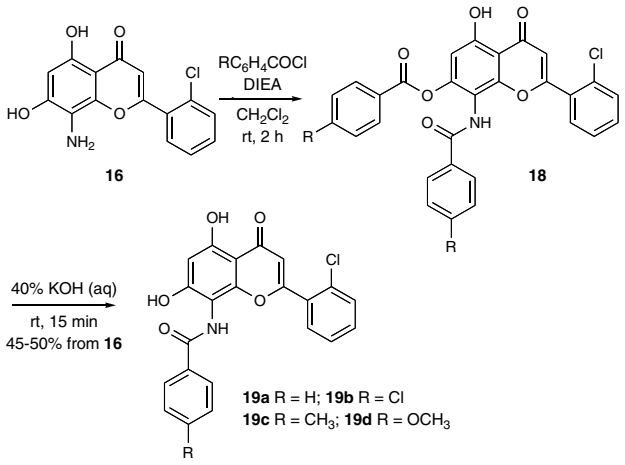
chromatography and **15b** was separated from **15c** by reverse-phase HPLC on a C-18 column. Subsequent reduction of **15a** using tin chloride dihydrate afforded the targeted 8-amino-2-(2-chlorophenyl)-5,7-dihydroxychromen-4-one (**16**) in good yield.

Parallel synthesis was employed to react various benzoyl chlorides with 8-aminoflavone **10** using Hünig's base to generate 8-amidoflavones **17a**–**e** (Scheme 3).

Acylation of **16** furnished *N*,*O*-bis-acylated products **18**, which were hydrolyzed with KOH to obtain N-acylated products **19** (Scheme 4). The structure of **18b** (R = Cl),



Scheme 3.

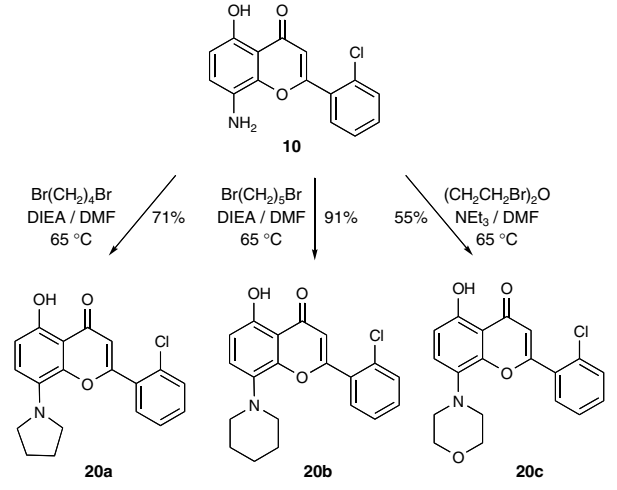


Scheme 4.

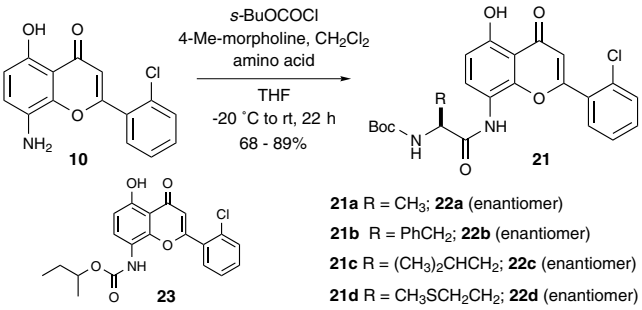
as well as the assignment of regioisomers **15a** and **15b**, was confirmed by X-ray crystallography of **18b** (R = Cl).

Heterocyclic analogues at C8 were prepared using various alkyl dibromides, which were reacted with 8-aminoflavone **10** under basic conditions to afford the corresponding C8-pyrrolidinyl flavopiridol analogue **20a**, C8-piperidinylflavopiridol analogue **20b**, and C8-morpholinylflavopiridol analogue **20c** (Scheme 5).

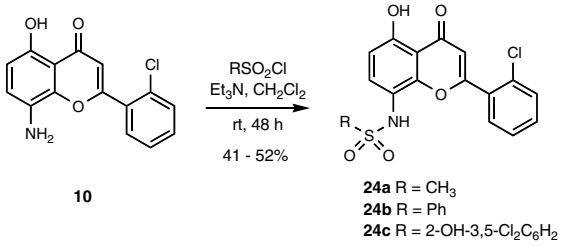
Reactions between *N*-Boc-protected amino acids and 8-aminoflavone **10** were carried out using 4-methyl morpholine (NMM) as base in THF with *sec*-butyl chloroformate activation (formation of the mixed anhydride) to yield amides **21a–d** and **22a–d** (Scheme 6).



Scheme 5.



Scheme 6.



Scheme 7.

8-Aminoflavone **10** also reacted with *sec*-butyl chloroformate itself to give by-product **23** in less than 10% yield.

The sulfonamido flavones **24a–c** were prepared by treating 8-aminoflavone **10** with a variety of sulfonyl chlorides under basic conditions to yield products **24** in moderate yields (Scheme 7).

3. Biological evaluation and computational study

Flavopiridol (**1**) and compounds **17–24** were tested for their anti-proliferative properties against ovarian cancer ID-8 and breast cancer MCF-7 cell lines and their activities are shown in Table 1. The data reveal that all compounds in Table 1 were less active than flavopiridol. The effect on growth of MCF-7 and ID-8 cells measures the biological efficacy of the flavone derivatives and is a cumulative effect of the inhibition of the target enzyme, the ability of the compounds to reach their intracellular target, and stability and solubility of the compounds. A measure of the inhibitory activity of compounds to its intended enzyme target is an important parameter for effective drug design. Therefore, we determined the effect of the flavone derivatives on CDK2-Cyclin A enzymatic activity in vitro. The enzyme activity was measured using a peptide with a recognition sequence specific for CDK2-Cyclin A which contains a chelation enhanced fluorophore, sox (8-hydroxy-5-(*N*,*N*-dimethylsulfonamido)-2-methylquinoline).

Table 1. Antiproliferative and CDK2-Cyclin A inhibitory activities of 8-aminoflavopiridol analogues

Compound	ID-8[a] IC_{50} (μM)	MCF-7[a] IC_{50} (μM)	CDK2-Cyclin A (μM)
Flavopiridol (**1**)	0.0070	0.026	1.5
17a	13	7.1	417
17b	5.5	4.6	417
17c	13	15	217
17d	16	10	N.D.
17e	5.0	3.5	383
19a	N.D.	8.5	91
19b	N.D.	13	339
19c	N.D.	9.7	90
19d	N.D.	13	54
20a	9.3	20	417
20b	5.7	17	417
20c	7.9	20	417
21a	104	5.5	N.D.
21b	12	4.5	417
21c	17	2.5	417
21d	13	2.8	N.D.
22a	9.8	5.5	417
22b	5.3	7.1	417
22c	5.1	4.0	417
22d	6.2	20	N.D.
23	18	30	417
24a	16	25	219
24b	9.5	16	178
24c	24	17	94

[a] Proliferation experiments were performed as previously described.[16]
[b]CDK2-Cyclin A enzymatic assay is described in Section 4 procedures section. N.D., not determined.

Phosphorylation of the peptide by the enzyme leads to chelation of Mg^{2+} and formation of a bridge between the sox moiety and the phosphate resulting in an increase in fluorescence signal.[15] Table 1 shows the IC_{50} for the compounds. Flavopiridol inhibits CDK2-Cyclin A activity with an IC_{50} of 1.5 μM. None of the derivatives are good inhibitors with **19d** being the best with an IC_{50} of 54 μM. The results are very similar to the cytotoxicity of these compounds to MCF-7 and ID-8 cells.

In an attempt to understand the decrease of potency of the new analogues compared to flavopiridol, molecular docking simulations of flavone derivatives binding to the CDK2 ATP receptor were carried out using the FlexX program.[17] The results of these studies, revealed a different binding orientation inside the CDK2 binding pocket for these analogues compared to flavopiridol (Fig. 4). The receptor model was constructed from the bare uninhibited CDK2 crystal structure including all residues within 7.0 Å of the documented binding pocket.[18] Thirty docking poses were requested for each ligand, and structural predictions were based on the conformation of that pose achieving the top FlexX score. The difference in orientation likely arises from the presence of a second carbonyl in molecule **19b** (Fig. 4b). This second carbonyl, located on the semi-flexible C8-amide chain, can sample a range of torsional orientations and thus is able to adapt to a more optimal electrostatic interaction with Lys33 than is possible for the rigid carbonyl on the chromenone ring.

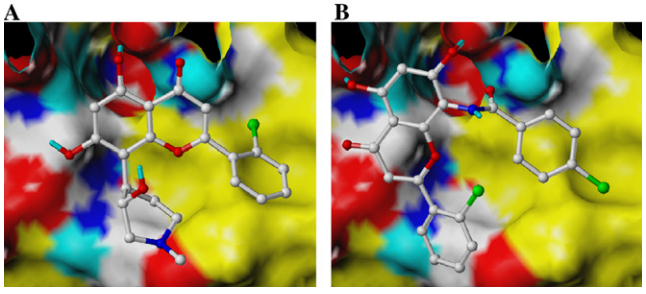


Figure 4. Docked conformations in the CDK2 receptor for flavopiridol (**1**, A) and flavopiridol analogue **19b** (B).

These computational studies have led us to pursue rational design-guided efforts in the preparation of analogues where the amino group has been altered to a bioisosteric equivalent. In this series, a nanomolar inhibitor was discovered. This work will be detailed in a future publication.

In conclusion, we have designed and synthesized a small chemical library of 8-amidoflavone, 8-sulfonylamidoflavone, 8-amido-7-hydroxyflavone, and heterocyclic analogues of flavopiridol. The cytotoxicity of these compounds was evaluated in MCF-7 and ID-8 cell lines, and several were shown to inhibit ID-8 and MCF-7 cancer cell proliferation in the single digit micromolar range. None of the compounds were effective inhibitors of CDK2-Cyclin A. Computational studies have directed us toward novel C8 bioisosteric analogues with promising bioactivity.

4. Experimental

Column chromatography was carried out employing silica gel (230–400 mesh). Analytical thin-layer chromatography (TLC) was performed on a silica gel $60F_{254}$ plate. THF and CH_2Cl_2 were freshly distilled or purified over an aluminum column before use. Other anhydrous solvents were purchased.

^1H NMR and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer (300 and 75.6 MHz, respectively), a 400 MHz spectrometer (400 and 100 MHz, respectively), or a 500 MHz spectrometer (500 and 125.5 MHz, respectively). NMR spectra were recorded in $CDCl_3$ unless otherwise indicated. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; br s, broad singlet, br d, broad doublet. High-resolution mass spectrometry (HRMS) spectra were obtained on a double-focusing mass spectrometer. X-ray crystallography data were collected using an area detector mounted on a D8 platform goniometer using graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). The X-ray crystallographic data for compounds **10** and **18b** have been deposited with the Cambridge Crystallographic Data Center and have been allocated the deposition numbers CCDC 293761 (**10**) and CCDC 293762 (**18b**). Optical

rotations were obtained at room temperature. Melting points are uncorrected.

4.1. 2-(2-Chlorophenyl)-5-hydroxychromen-4-one (8)

To a solution of 2′,6′-dihydroxyacetophenone (2.5 g, 16 mmol) in pyridine (20 mL) were added 2-chlorobenzoyl chloride (5 mL, 38 mmol) and a catalytic amount of dimethylaminopyridine (DMAP). The reaction mixture was stirred at room temperature for 2 h and then poured into ice water (150 mL). The solution was extracted with ethyl acetate (EtOAc). The organic layer was washed with 3*N*-HCl, $NaHCO_3$ (satd aq),and brine. The organic layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue was purified by flash silica gel column chromatography (1:9 EtOAc/hexane) to afford 7.0 g (99%) of 2′,6′-di(2-chlorobenzoyl)acetophenone as a white solid: mp 67–68 °C; R_f 0.43 (2:1 hexane/EtOAc); ^{1}H NMR (300 MHz): δ 8.02 (d, J = 8.4 Hz, 2H), 7.3–7.6 (m, 7H), 7.27 (d, J = 8.4 Hz, 2H), 2.51 (s, 3H); ^{13}C NMR (75.6 MHz): δ 198.3, 168.3, 147.8, 134.7, 133.7, 132.1, 131.5, 131.0, 128.5, 128.4, 127.0, 120.8, 31.5; HRMS (FAB) m/z calcd for $C_{22}H_{15}O_5Cl_2$ $[M+H]^+$ 429.0297; found 429.0278. To a solution of 2′,6′-di(2-chlorobenzoyl)acetophenone (7.0 g, 16 mmol) in pyridine (40 mL) was added DBU (5.8 mL, 38 mmol). The reaction mixture was heated at 100 °C for 11 h and then poured into ice water (150 mL). The solution was extracted with EtOAc. The organic layer was washed with 3*N*-HCl, $NaHCO_3$ (satd aq), and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash silica gel column chromatography (1:9 EtOAc/hexane) to afford 3.9 g (90%) of **8** as a white solid: mp 168–168.5 °C; R_f 0.59 (2:1 hexane/EtOAc); ^{1}H NMR (400 MHz): δ 12.5 (s, 1H), 7.66 (dd, J = 1.8, 7.5 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.49 (dt, J = 1.6, 7.8 Hz, 1H), 7.44 (dt, J = 1.2, 7.5 Hz, 1H), 6.97 (d, J = 8.5 Hz, 1H), 6.85 (d, J = 8.3 Hz, 1H), 6.63 (s, 1H); ^{13}C NMR (100 MHz): δ 183.8, 164.1, 161.2, 157.2, 136.0, 133.3, 132.5, 131.8, 131.3, 131.0, 127.6, 112.0, 111.9, 111.2, 107.6; HRMS (FAB) m/z calcd for $C_{15}H_{10}O_3Cl$ $[M+H]^+$ 273.0318; found 273.0323.

4.2. 2-(2-Chlorophenyl)-5-hydroxy-8-nitrochromen-4-one (9a)

To a solution of **8** (360 mg, 1.32 mmol) in glacial acetic acid (10 mL) was added nitric acid (1.3 mL). The reaction mixture was heated at 55 °C (external temperature) for 5 h and then poured into ice water (100 mL). The solution was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (1:9 EtOAc/hexane) to afford 150 mg (30%) of **9a** as a white solid, 180 mg (36%) of **9b** as a pale yellow solid and a trace of **9c**. Compound **9a**: mp 194–195 °C; R_f 0.57 (1:1 hexane/EtOAc); ^{1}H NMR (300 MHz): δ 13.7 (s, 1H), 8.45 (d, J = 9.3 Hz, 1H), 7.98 (m, 1H), 7.65–7.45 (m, 3H), 7.01 (s, 1H), 6.90 (d, J = 9.3 Hz, 1H); ^{13}C NMR (75.6 MHz): δ 182.4, 166.7, 163.4, 133.4, 133.1, 132.9, 131.4, 131.2, 129.7, 127.7, 112.6, 111.5, 110.3; HRMS (FAB) m/z calcd for $C_{15}H_9O_5NCl$ $[M+H]^+$ 318.0169; found 318.0166.

Compound **9b**: mp 200–200.5 °C; R_f 0.45 (1:1 hexane/EtOAc); ^{1}H NMR (300 MHz): δ 14.5 (s, 1H), 8.41 (d, J = 9.3 Hz, 1H), 7.66 (dd, J = 1.5, 7.5 Hz, 1H), 7.40–7.62 (m, 3H), 7.04 (d, J = 9.3 Hz, 1H), 6.77 (s, 1H); ^{13}C NMR (75.6 MHz): δ 183.3, 164.6, 159.4, 157.7, 133.1, 132.9, 132.4, 131.2, 130.7, 130.3, 127.5, 112.2, 111.7, 107.5; HRMS (FAB) m/z calcd for $C_{15}H_9O_5NCl$ $[M+H]^+$ 318.0169; found 318.0169.

4.3. 8-Amino-2-(2-chlorophenyl)-5-hydroxychromen-4-one (10)

To a solution of **9a** (541 mg, 1.7 mmol) in ethanol (200 mL) was added tin chloride hydrate (2.3 g, 10.2 mmol). The reaction mixture was heated at 80 °C for 5 h and then the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (500 mL) and the solution was washed with water. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to obtain 435 mg (89%) of **10** as a reddish powder: mp 197–198 °C; R_f 0.52 (1:1 hexane/EtOAc); ^{1}H NMR (400 MHz): δ 11.65 (s, 1H), 7.64 (dd, J = 1.7, 7.5 Hz, 1H), 7.57 (dd, J = 1.3, 8.0 Hz, 1H), 7.51 (dt, J = 1.7, 7.9 Hz, 1H), 7.45 (dt, J = 1.4, 7.4 Hz 1H), 7.06 (d, J = 8.7 Hz, 1H), 6.73 (d, J = 8.7 Hz, 1H), 6.57 (s, 1H), 3.77 (br s, 2H); ^{13}C NMR (100 MHz): δ 183.4, 163.2, 152.1, 144.2, 132.8, 132.1, 131.4, 130.9, 130.7, 127.2, 126.8, 111.3, 111.0, 110.9; HRMS (FAB) m/z calcd for $C_{15}H_{11}O_3NCl$ $[M+H]^+$ 288.0427; found 288.0415.

4.4. 2-(2-Chlorophenyl)-5,7-dihydroxychromen-4-one (14)

To a solution of **11** (2.0 g, 0.011 mol) in pyridine (20 mL) were added 2-chlorobenzoyl chloride (5 mL, 0.022 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred at room temperature for 3 h and then was poured into ice water (150 mL). The solution was extracted with EtOAc. The organic layer was washed with 3 N HCl, $NaHCO_3$ (satd aq), and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was recrystallized with EtOAc/hexane to afford 5.8 g (92%) of 2′,4′,6′-tri(2-chlorobenzoyloxy)acetophenone as a white solid: mp 141–142 °C; R_f 0.78 (1:1 hexane/EtOAc); ^{1}H NMR (300 MHz)): δ 8.05 (m, 3H), 7.3–7.6 (m, 9H), 2.52 (s, 3H); ^{13}C NMR (75.6 MHz): δ 197.5, 162.8, 162.7, 151.9, 148.4, 134.9, 133.8, 133.7, 133.5, 132.5, 132.2, 131.6(2), 131.5, 128.5, 128.1, 127.0, 126.9, 126.7, 114.6, 31.5; HRMS (FAB) m/z calcd for $C_{29}H_{18}O_7Cl_3$ $[M+H]^+$ 583.0118; found 583.0120. To a solution of 2′,4′,6′-tri(2-chlorobenzoyloxy)acetophenone (5.0 g, 8.6 mmol) in pyridine (40 mL) was added KOH (2.0 g, 5 equiv). The reaction mixture was stirred at room temperature for 1.5 h and then poured into ice-cold 10% AcOH (150 mL). The solution was extracted with EtOAc. The organic layer was washed with 3*N*-HCl,

$NaHCO_3$ (satd aq), and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to obtain a mixture of **12** and **13** (3:1 ratio by ^1H NMR). The mixture was then treated with concd H_2SO_4 (3 mL) in glacial acetic acid (50 mL). The solution was heated at 100 °C for 2 h and then poured into ice water (100 mL). The solution was extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to obtain a mixture of unreacted **13** and **14**. The mixture was purified by silica gel column chromatography (1:9 EtOAc/CH_2Cl_2) to obtain a mixture of **13** (0.36 g, 10%) as a pale yellow solid, and **14** (0.76 g, 31%) as a white solid. **13**: mp 253–254 °C; R_f 0.84 (1:1 acetone/CH_2Cl_2); ^1H NMR (acetone-d_6, 300 MHz): δ 12.3 (s, 1H), 10.0 (br s, 1H) 7.66 (dd, J = 1.7, 7.7 Hz, 1H), 7.60 (m, 1H), 7.35–7.55 (m, 5H), 7.34 (dt, J = 1.2, 7.4 Hz, 1H), 6.50 (s, 1H), 6.36 (s, 1H); ^{13}C NMR (acetone-d_6, 75.6 MHz): δ 189.8, 180.5, 165.9, 163.7, 158.7, 136.4, 133.8, 133.7, 133.2, 132.8, 132.0, 131.8, 131.4, 130.7, 128.0, 127.8, 124.5, 105.2, 100.6, 95.2; HRMS (FAB) m/z calcd for $C_{22}H_{13}O_5Cl_2$ $[M+H]^+$ 427.0140; found 427.0140. Compound **14**: mp 258 °C dec; R_f 0.82 (1:1 acetone/CH_2Cl_2); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.7 (br s, 1H), 11.0 (br s, 1H) 7.78 (d, J = 7.8 Hz, 1H), 7.67 (d, J = 7.9 Hz, 1H), 7.61 (t, J = 7.4 Hz, 1H), 7.54 (t, J = 7.4 Hz, 1H), 6.57 (s, 1H), 6.41 (s,1H), 6.25 (s,1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 181.5, 164.7, 162.8, 161.5, 157.8, 131.7, 131.6, 131.4, 130.9, 130.5, 127.8, 110.6, 103.9, 99.2, 94.1; HRMS (FAB) m/z calcd for $C_{15}H_{10}O_4Cl$ $[M+H]^+$ 289.0268; found 289.0263.

4.5. 2-(2-Chlorophenyl)-5,7-dihydroxy-8-nitrochromen-4-one (15a)

To a solution of **14** (0.5 g, 1.73 mmol) in glacial acetic acid (10 mL) was added nitric acid (1.5 mL). The reaction mixture was heated at 55 °C for 2 h and then poured into ice water (100 mL). The solution was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to give a mixture of products **15**. The residue was purified by silica gel column chromatography (1:4 EtOAc/CH_2Cl_2) to afford 160 mg (28%) of **15a** was pale yellow solid. Elution with methanol provided a mixture of **15b** and **15c**. This mixture was purified by prep-HPLC (C-18 column: 4.6 × 150 mm, water/acetonitrile) to yield 121 mg (21%) of **15b** as a yellow solid. Compound **15a**: mp 223–225 °C; R_f 0.52 (2:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 14.0 (s, 1H), 12.0 (d, J = 9.3 Hz, 1H), 7.97 (m, 1H), 7.40–7.70 (m, 3H), 7.06 (s, 1H), 6.51 (s, 1H); ^{13}C NMR (100 MHz): δ 181.6, 167.5, 163.1, 163.0, 153.8, 133.2, 133.0, 131.6, 131.2, 129.7, 127.8, 118.3, 113.3, 105.8, 101.0; HRMS (FAB) m/z calcd for $C_{15}H_9O_6NCl$ $[M+H]^+$ 334.0118; found 334.0115. Compound **15b**: ^1H NMR (DMSO-d_6, 500 MHz): δ 13.0 (s, 1H), 7.77 (d, J = 7.5 Hz, 1H), 7.69 (d, J = 7.9 Hz, 1H), 7.64 (t, J = 7.7 Hz, 1H), 7.57 (t, J = 7.5 Hz, 1H), 6.84 (s, 1H), 6.42 (s, 1H); ^{13}C NMR (75.6 MHz): δ 180.8, 162.4, 162.2, 157.7, 149.8, 133.1, 132.7, 131.3, 130.7, 130.2, 129.9, 121.8, 111,6, 102.8, 99.1; HRMS (FAB) m/z calcd for $C_{15}H_9O_6NCl$ $[M+H]^+$ 334.0118; found 334.0129.

4.6. 8-Amino-2-(2-chlorophenyl)-5,7-dihydroxychromen-4-one (16)

To a solution of **15a** (130 mg, 0.37 mmol) in ethanol (70 mL) was added tin chloride dihydrate (530 mg, 2.35 mmol). The reaction mixture was heated at 80 °C for 7 h and then the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (300 mL) and the solution was washed with water. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to afford 73 mg (62%) of **16** as a reddish solid: mp 227 °C (dec); R_f 0.31 (1:1 hexane/EtOAc); ^1H NMR (DMSO-d_6, 400 MHz): δ 11.9 (s, 1H), 7.88 (dd, J = 1.5, 7.6 Hz, 1H), 7.68 (dd, J = 1.0, 7.1 Hz, 1H), 7.61 (dt, J = 1.5, 7.5 Hz, 1H), 7.54 (dt, J = 1.2, 7.5 Hz, 1H), 6.52 (s, 1H), 6.32 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 182.3, 172.1, 162.7, 151.9, 144.1, 133.0, 132.1, 132.0, 131.5, 131.0, 128.2, 116.8, 110.4, 104.0, 99.0; HRMS (FAB) m/z calcd for $C_{15}H_{11}O_4NCl$ $[M+H]^+$ 304.0377; found 304.0370.

4.7. General procedure for reactions of 10 with benzoyl chlorides

The procedure for the synthesis of **17a** was also used for the preparation of **17b**–**17e**.

4.7.1. *N*-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-yl]benzamide (17a). To a solution of **10** (14.0 mg, 0.0435 mmol) in CH_2Cl_2 (2 mL) were added DIEA (17 μL, 0.087 mmol) and benzoyl chloride (11 μL, 0.087 mmol). The reaction mixture was stirred at room temperature for 30 min and then quenched with ice water (20 mL). The solution was extracted with EtOAc and then the organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (1:4 EtOAc/hexane) to provide 16 mg (85%) of **17a** as a yellow solid: mp 224–225 °C; R_f 0.76 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.1 (s, 1H), 8.60 (d, J = 9.0 Hz, 1H), 8.27 (br s, 1H), 7.91 (d, J = 7.3 Hz, 2H), 7.65 (dd, J = 1.3, 7.3 Hz, 1H), 7.6–7.4 (m, 4H), 7.50 (d, J = 7.2 Hz, 2H), 6.92 (d, J = 9.0 Hz, 1H), 6.62 (s, 1H); ^{13}C NMR (100 MHz): δ 182.8, 163.2, 156.5, 146.3, 134.2, 132.4 (2), 131.9, 131.1, 130.9, 130.7, 128.8, 128.7, 128.5, 127.4, 126.9, 117.9, 111.4, 111.1, 110.2; HRMS (FAB) m/z calcd for $C_{22}H_{15}O_4NCl$ $[M+H]^+$ 392.0690; found 392.0714.

4.7.2. 4-Chloro-*N*-[2-(2-chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-yl]benzamide (17b). 15.1 mg (82%); mp 246–247.5 °C; R_f 0.73 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.1 (s, 1H), 8.56 (d, J = 9.0 Hz, 1H), 8.22 (br s, 1H), 7.85 (d, J = 8.5 Hz, 2H), 7.64 (dd, J = 1.7, 7.7 Hz, 1H), 7.61 (dd, J = 1.2, 7.0 Hz 1H), 7.56 (dt, J = 1.6, 7.4 Hz, 1H), 7.49 (d, J = 8.5 Hz, 2H), 7.46 (dt, J = 1.2, 7.4 Hz, 1H), 6.92 (d, J = 9.0 Hz, 1H), 6.62 (s, 1H); ^{13}C NMR (100 MHz): δ 183.0, 164.4, 163.4, 156.9, 146.8, 138.5, 132.7, 132.5, 132.0, 131.4,

131.1, 130.8, 129.2, 128.7, 128.5, 127.7, 117.9, 111.6, 111.3, 110.4; HRMS (FAB) *m/z* calcd for $C_{22}H_{14}O_4NCl_2$ $[M+H]^+$ 426.0300; found 426.0277.

4.7.3. 3,4-Dichloro-*N*-[2-(2-chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-yl]benzamide (17c). 15.8 mg (79%); mp 222–223 °C; R_f 0.91 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.0 (s, 1H), 8.54 (d, J = 9.0 Hz, 1H), 8.21 (br s, 1H), 8.02 (d, J = 2.0 Hz, 1H), 7.74 (dd, J = 2.1, 8.4 Hz, 1H), 7.64 (m, 3H), 7.56 (dt, J = 1.6, 7.5 Hz, 1H), 7.48 (dt, J = 1.2, 7.6 Hz, 1H), 6.92 (d, J = 9.0 Hz, 1H), 6.63 (s, 1H); ^{13}C NMR (100 MHz): δ 182.7, 163.2, 162.9, 156.8, 148.3, 136.4, 133.9, 133.3, 132.6, 132.2, 131.2, 130.9, 130.8, 130.5, 129.1, 128.1, 127.6, 126.0, 117.4, 111.3, 111.1, 110.2; HRMS (FAB) *m/z* calcd for $C_{22}H_{13}O_4NCl_3$ $[M+H]^+$ 459.9910; found 459.9903.

4.7.4. *N*-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-yl]-4-methylbenzamide (17d). 16.9 mg (92%); mp 252.5–254 °C; R_f 0.72 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.1 (s, 1H), 8.61 (d, J = 8.9 Hz, 1H), 8.26 (br s, 1H), 7.81 (d, J = 7.9 Hz, 2H), 7.64 (d, J = 7.5 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.53 (t, J = 7.3 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.31 (d, J = 7.9 Hz, 2H), 6.91 (d, J = 9.0 Hz, 1H), 6.62 (s, 1H), 2.44 (s, 3H); ^{13}C NMR (100 MHz): δ 183.1, 165.5, 163.4, 156.7, 146.5, 142.8, 132.7, 131.6, 131.4, 131.2, 131.0, 129.6, 128.7, 127.7, 127.2, 118.4, 111.6, 111.4, 110.5, 21.6; HRMS (FAB) *m/z* calcd for $C_{23}H_{17}O_4NCl$ $[M+H]^+$ 426.0846; found 426.0842.

4.7.5. *N*-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-yl]-4-methoxybenzamide (17e). 15.8 mg (86%); mp 210–210.5 °C; R_f 0.76 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.0 (s, 1H), 8.56 (d, J = 9.0 Hz, 1H), 8.20 (br s, 1H), 7.87 (d, J = 8.7 Hz, 2H), 7.64 (dd, J = 1.3, 7.7 Hz, 2H), 7.59 (d, J = 7.8 Hz, 1H), 7.52 (dt, J = 1.3, 7.9 Hz, 1H), 7.45 (t, J = 7.6 Hz, 3H), 6.98 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 9.0 Hz, 1H), 6.60 (s, 1H); ^{13}C NMR (100 MHz): δ 183.0, 165.0, 163.3, 162.6, 156.5, 146.4, 132.6, 131.2, 131.1, 130.9, 128.9, 128.7, 127.6, 126.5, 118.4, 114.1, 111.5, 111.2, 110.4, 55.5; HRMS (FAB) *m/z* calcd for $C_{23}H_{17}O_5NCl$ $[M+H]^+$ 422.0795; found 422.0781.

4.8. 2-(2-Chlorophenyl)-8-(4-chlorophenylamido)-5-hydroxy-4-oxo-4*H*-chromen-7-yl 4-chlorobenzoate (18b)

To a solution of **16** (30.0 mg, 0.099 mmol) in CH_2Cl_2 (3 mL) were added DIEA (34.5 μL, 0.20 mmol) and benzoyl chloride (25.2 μL, 0.20 mmol). The reaction mixture was stirred at room temperature for 40 min and then quenched with ice water (20 mL). The solution was extracted with EtOAc and the organic layer was dried over anhydrous Na_2SO_4 and the removed solvent under reduced pressure. The residue was purified by silica gel column chromatography (1:5 EtOAc/hexane) to yield 20 mg (50%) of **18b** as a yellow solid: mp 241 °C; R_f 0.57 (1:1 hexane/EtOAc); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.8 (s, 1H), 10.3 (s, 1H), 7.83 (d, J = 1.2 Hz, 1H), 7.66 (m, 5H), 7.55 (d, J = 1.3, 2H), 7.40 (m, 4H), 7.09 (s, 1H), 6.87 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 183.3, 171.4, 163.5, 161.5, 157.2, 156.3, 147.2, 133.1, 132.8, 131.6, 131.5, 131.3, 131.2, 129.5, 128.5, 127.4, 111.0, 104.9, 104.5, 99.8; MS (FAB) *m/z* calcd for $C_{29}H_{17}Cl_3NO_6$ $[M+H]^+$ 581.8; found 582.1.

4.9. General procedure for reactions with benzoyl chlorides

The procedure for the synthesis of **19a** was also used for the preparation of **19b**–**19d**.

4.9.1. *N*-[2-(2-Chlorophenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-8-yl]benzamide (19a). To a solution of **16** (30.0 mg, 0.099 mmol) in CH_2Cl_2 (2 mL) were added DIEA (19 μL, 0.11 mmol) and benzoyl chloride (13 μL, 0.11 mmol). The reaction mixture was stirred at room temperature for 2 h and then quenched with ice water (20 mL). The solution was extracted with EtOAc and then the solvent was removed until the volume of the solution was around 6 mL. To the solution was added 40% KOH (4 mL). The solution was stirred at room temperature for 15 min. and then the organic layer was washed with water, 3*N*-HCl, and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (1:5 EtOAc/hexane) to yield 20 mg (50%) of **19a** as a yellow solid: mp 205–206 °C; R_f 0.44 (1:1 hexane/EtOAc); ^1H NMR (300 MHz): δ 12.2 (s, 1H), 11.2 (br s, 1H), 8.50 (br s, 1H), 7.93 (dd, J = 1.3, 7.2 Hz, 2H), 7.62 (dt, J = 1.6, 7.6 Hz, 2H), 7.4–7.6 (m, 5H), 6.54 (s, 1H), 6.53 (s, 1H); ^{13}C NMR (100 MHz): δ 181.7, 174.3, 164.8, 167.4, 162.7, 159.7, 157.1, 148.8, 133.0, 132.7, 131.2 (2), 131.1, 129.1, 127.8, 127.6, 111.6, 106.8, 104.8, 102.7; HRMS (FAB) *m/z* calcd for $C_{22}H_{15}O_5NCl$ $[M+H]^+$ 408.0639; found 408.0638.

4.9.2. 4-Chloro-*N*-[2-(2-chlorophenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-8-yl]benzamide (19b). 20 mg (45%); mp 234 °C; R_f 0.38 (1:1 hexane/EtOAc); ^1H NMR (acetone-d_6, 500 MHz): δ 12.6 (s, 1H), 9.21 (br s, 1H), 8.04 (d, J = 8.4 Hz, 2H), 7.84 (dd, J = 1.6, 7.7 Hz, 1H), 7.54 (dt, J = 1.3, 8.0 Hz, 1H), 7.51 (dd, J = 1.6, 7.9 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.44 (dt, J = 1.3, 8.0 Hz, 1H), 6.61 (s, 1H), 6.39 (s, 1H); ^{13}C NMR (acetone-d_6, 125.5 MHz): δ 182.6, 166.8, 163.0, 161.3, 153.6, 138.3, 133.4, 133.1, 132.9, 131.9, 131.8, 131.6, 130.3, 129.3, 128.3, 111.8, 105.8, 105.3, 100.6; HRMS (FAB) *m/z* calcd for $C_{22}H_{14}O_5NCl_2$ $[M+H]^+$ 442.0249; found 442.0250.

4.9.3. *N*-[2-(2-Chlorophenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-8-yl]-4-methylbenzamide (19c). 21 mg (49%); mp 210–212 °C; R_f 0.50 (1:1 hexane/EtOAc); ^1H NMR (DMSO-d_6, 300 MHz): δ 12.7 (s, 1H), 11.1 (br s, 1H), 9.52 (s, 1H), 7.86 (d, J = 7.8 Hz, 2H), 7.76 (d, J = 7.5 Hz, 1H), 7.4–7.7 (m, 3H), 7.29 (d, J = 7.8 Hz, 2H), 6.69 (s, 1H), 6.42 (s, 1H), 2.36 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 181.5, 166.0, 162.1, 161.2, 159.3, 153.4, 141.3, 132.7, 131.3, 131.1, 130.7, 130.4, 128.8, 127.7, 127.6, 110.5, 105.0, 103.7, 98.9, 20.9;

HRMS (FAB) *m/z* calcd for $C_{23}H_{17}O_5NCl$ $[M+H]^+$ 422.0795; found 422.0786.

4.9.4. *N*-[2-(2-Chlorophenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-8-yl]-4-methoxybenzamide (19d). 24 mg (55%); mp 208 °C; R_f 0.53 (1:1 hexane/EtOAc); ^1H NMR (300 MHz):δ 12.2 (s, 1H), 11.2 (br s, 1H), 8.38 (br s, 1H), 7.88 (d, J = 8.8 Hz, 2H), 7.62 (m, 2H), 7.54 (dt, J = 1.6, 7.5 Hz, 1H), 7.46 (dt, J = 1.0, 7.5 Hz, 1H), 6.97 (d, J = 8.8 Hz, 2H), 6.52 (s, 1H), 6.51 (s, 1H), 3.88 (s, 3H); ^{13}C NMR (100 MHz): δ 181.7, 166.9, 163.4, 162.6, 159.4, 157.1, 148.6, 132.6, 132.4, 131.2, 131.1 129.5, 127.7, 124.3, 114.3, 111.6, 106.9, 104.7, 102.7, 55.6, 29.7; HRMS (FAB) *m/z* calcd for $C_{23}H_{17}O_6NCl$ $[M+H]^+$ 438.0744; found 438.0729.

4.10. General procedure for the formation of heterocyclic analogues

The procedure for the synthesis of **20a** was also used for the preparation of **20b** and **20c**.

4.10.1. 2-(2-Chlorophenyl)-5-hydroxy-8-pyrrolidin-1-yl-chromen-4-one (20a). To a solution of **10** (20 mg, 0.070 mmol) in DMF (1 mL) were added DIEA (100 μL) and 1,4-dibromobutane (40 μL, 0.35 mmol). The reaction mixture was heated at 65 °C for 18 h and then the solution was diluted with EtOAc (50 mL). The solution was washed with Na_2CO_3 (50 mL) and then the organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (1:5 EtOAc/hexane) to afford 17 mg (71%) of **20a** as a yellow solid; mp 144–145 °C; R_f 0.68 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.0 (s, 1H), 7.64 (dd, J = 1.8, 7.4 Hz, 1H), 7.55 (dd, J = 1.2, 7.9 Hz, 1H), 7.45 (m, 2H), 7.08 (d, J = 8.9 Hz, 1H), 6.78 (d, J = 8.9 Hz, 1H), 6.59 (s, 1H), 3.33 (t, J = 6.4 Hz, 4H), 1.94 (apparent quintet, J = 3.4, 6.5 Hz, 4H); ^{13}C NMR (100 MHz): δ 183.8, 163.0, 152.5, 147.2, 132.8, 132.0, 131.8, 131.3, 130.8, 130.7, 127.2, 121.5, 111.7, 111.4, 110.9, 51.0, 24.9; HRMS (FAB) *m/z* calcd for $C_{19}H_{17}O_3NCl$ $[M+H]^+$ 342.0897; found 342.0882.

4.10.2. 2-(2-Chlorophenyl)-5-hydroxy-8-piperidin-1-yl-chromen-4-one (20b). 23 mg (91%); mp 120–121 °C; R_f 0.87 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.2 (s, 1H), 7.75 (dd, J = 2.0, 7.3 Hz, 1H), 7.56 (dd, J = 1.7, 7.6 Hz, 1H), 7.45 (m, 2H), 7.27 (d, J = 8.8 Hz, 1H), 6.78 (d, J = 8.8 Hz, 1H), 6.75 (s, 1H), 3.00 (t, J = 5.1 Hz, 4H), 1.73 (quintet, J = 5.9, 11.4 Hz, 4H), 1.58 (quintet, J = 6.1, 11.6 Hz, 2H); ^{13}C NMR (100 MHz): δ 183.8, 162.8, 155.2, 149.9, 134.1, 132.8, 132.0, 131.4, 131.1, 130.8, 127.2, 125.7, 111.8, 111.4, 110.7, 53.4, 26.4, 24.2; HRMS (FAB) *m/z* calcd for $C_{20}H_{19}O_3NCl$ $[M+H]^+$ 356.1053; found 356.1030.

4.10.3. 2-(2-Chlorophenyl)-5-hydroxy-8-morpholin-4-yl-chromen-4-one (20c). 14 mg (55%); mp 137–139 °C; R_f 0.81 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.2 (s, 1H), 7.69 (dd, J = 1.7, 7.7 Hz, 1H), 7.57 (dd, J = 1.2, 7.9 Hz, 1H), 7.47 (m, 2H), 7.28 (d, J = 8.8 Hz, 1H), 6.80 (d, J = 8.8 Hz, 1H), 6.71 (s, 1H), 3.85 (t, J = 4.5 Hz, 4H), 3.08 (t, J = 4.6 Hz, 4H); ^{13}C NMR (100 MHz): δ 183.6, 163.1, 155.9, 150.0, 132.7, 132.5, 132.2, 131.4, 131.1, 130.8 127.3, 125.8, 111.9, 111.4, 110.9, 67.2, 52.1; HRMS (FAB) *m/z* calcd for $C_{19}H_{17}O_4NCl$ $[M+H]^+$ 358.0846; found 358.0828.

4.11. General procedure for the reactions with amino acids

The procedure for the synthesis of **21a** was also used for the preparation of **21b**–**21d**.

4.11.1. (*R*)-{1-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-ylcarbamoyl]ethyl}carbamic acid *tert*-butyl ester (21a). To a solution of D-*N*-Boc-alanine (19 mg, 0.1 mmol) in THF (2 mL) were added 4-methylmorpholine (10 μL, 0.1 mmol) and *iso*-butylchloroformate (10 μL, 0.08 mmol) at −20 °C. 8-Amino-2-(2-chlorophenyl)-5-hydroxychromen-4-one (**10**, 24 mg, 0.084 mmol) was dissolved in THF (1 mL) and then the solution was added into the reaction mixture at −20 °C and stirred at the same temperature for 30 min. The reaction mixture was stirred at room temperature for 22 h and then the solution was diluted with EtOAc (30 mL). The organic layer was washed with brine and then dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (1:5 EtOAc/hexane) to afford a mixture of 32 mg (84%) of **21a** as a yellow solid and 3 mg (9%) of **23** as a yellow solid. **21a**: mp 174–175 °C; R_f 0.65 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.1 (s, 1H), 8.60 (br s, 1H), 8.38 (d, J = 9.0 Hz, 1H), 7.71 (d, J = 6.8 Hz, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.51 (dt, J = 1.4, 7.3 Hz, 1H), 7.45 (t, J = 7.3 Hz, 1H), 6.81 (d, J = 9.0 Hz, 1H), 6.63 (s, 1H), 5.05 (br s, 1H), 4.35 (br s, 1H), 1.44 (d, J = 9.6 Hz, 3H), 1.30 (s, 9H); ^{13}C NMR (100 MHz): δ 182.9, 171.0, 163.1, 156.7, 155.8, 146.6, 132.7, 132.4, 131.1, 130.8, 128.5, 127.5, 117.8, 111.8, 111.0, 110.3, 80.4, 50.8, 28.1, 17.7; HRMS (FAB) *m/z* calcd for $C_{23}H_{24}O_6N_2Cl$ $[M+H]^+$ 459.1323; found 459.1315; $[\alpha]_D^{20}$ +6.1 (*c* 0.85, $CHCl_3$). Compound **23**: mp 136.5–137 °C; R_f 0.87 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.0 (s, 1H), 8.21 (br s, 1H), 7.64 (dd, J = 1.7, 7.6 Hz, 1H), 7.59 (dd, J = 1.1, 7.9 Hz, 1H), 7.53 (dt, J = 1.8, 7.5 Hz, 1H), 7.46 (dt, J = 1.3, 7.6 Hz, 1H), 6.87 (br s, 1H), 6.86 (d, J = 9.0 Hz, 1H), 6.59 (s, 1H), 3.97 (d, J = 6.6 Hz, 2H), 1.99 (m, 1H), 0.96 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz): δ 183.0, 163.3, 132.8, 132.4, 131.2, 130.9, 130.8, 127.4, 111.5, 111.2, 110.4, 71.7, 27.9, 19.0; HRMS (FAB) *m/z* calcd for $C_{20}H_{19}O_5NCl$ $[M+H]^+$ 388.0952; found 388.0944.

4.11.2. (*R*)-{1-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-ylcarbamoyl]-2-phenylethyl}carbamic acid *tert*-butyl ester (21b). 31 mg (68%); mp 217–218 °C; R_f 0.75 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.0 (s, 1H), 8.43 (d, J = 9.0 Hz, 1H), 8.05 (br s, 1H), 7.61 (dd, J = 1.3, 7.7 Hz, 1H), 7.56 (dd, J = 1.3, 7.9 Hz, 1H), 7.52 (dt, J = 1.7, 7.9 Hz, 1H), 7.48 (dt, J = 1.6, 7.5 Hz, 1H), 7.1–7.25 (m, 5H), 6.83 (d, J = 9.0 Hz, 1H), 6.58 (s, 1H), 5.08 (br s, 1H), 4.49 (br s, 1H), 3.16 (d, J = 6.3 Hz, 2H), 1.32 (s, 9H); ^{13}C NMR (100 MHz): δ 182.9, 169.6, 163.1, 156.7, 155.5, 146.2,

136.2, 132.7, 132.4, 131.2, 131.0, 130.8, 129.2, 128.7, 128.1, 127.5, 127.0, 117.5, 111.6, 111.1, 110.3, 80.5, 56.7, 38.3, 28.1; HRMS (FAB) *m/z* calcd for $C_{29}H_{28}O_6N_2Cl$ $[M+H]^+$ 535.1636; found 535.1641; $[\alpha]_D^{20}$ +38 (*c* 0.22, $CHCl_3$).

4.11.3. (*R*)-{1-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-ylcarbamoyl]-3-methylbutyl}carbamic acid *tert*-butyl ester (21c). 35 mg (84%) was prepared in a similar procedure as **21a**; mp 169 °C; R_f 0.80 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.1 (s, 1H), 8.43 (br s, 1H), 8.35 (d, J = 9.0 Hz, 1H), 7.71 (d, J = 6.7 Hz, 1H), 7.58 (dd, J = 1.2, 7.8 Hz, 1H), 7.51 (dt, J = 1.7, 7.4 Hz, 1H), 7.46 (dt, J = 1.4, 7.5 Hz, 1H), 6.82 (d, J = 9.0 Hz, 1H), 6.63 (s, 1H), 4.96 (br s, 1H), 4.27 (br s, 1H), 1.77 (m, 2H), 1.55 (m, 1H), 1.33 (s, 9H), 0.95 (d, J = 3.0 Hz, 3H), 0.94 (d, J = 2.9 Hz, 3H); ^{13}C NMR (100 MHz): δ 182.9, 171.0, 163.1, 156.8, 155.7, 146.8, 132.7, 132.4, 131.1, 130.8, 128.8, 127.5, 117.6, 111.8, 111.1, 110.4, 80.3, 53.8, 40.9, 28.2, 24.8, 22.9, 22.0; HRMS (FAB) *m/z* calcd for $C_{26}H_{30}O_6N_2Cl$ $[M+H]^+$ 501.1792; found 501.1787; $[\alpha]_D^{20}$ +19 (*c* 0.58, $CHCl_3$).

4.11.4. (*R*)-{1-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-ylcarbamoyl]-3-methylsulfanylpropyl}carbamic acid *tert*-butyl ester (21d). 36 mg (82%); mp 162–163 °C; R_f 0.65 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.1 (s, 1H), 8.51 (br s, 1H), 8.39 (d, J = 9.0 Hz, 1H), 7.70 (d, J = 7.0 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.52 (dt, J = 1.7, 7.5 Hz, 1H), 7.47 (dt, J = 1.2, 7.5 Hz, 1H), 6.84 (d, J = 9.0 Hz, 1H), 6.61 (s, 1H), 5.24 (d, J = 5.4 Hz, 1H), 4.45 (m, 1H), 2.59 (m, 2H), 2.21 (m, 1H), 2.06 (s, 3H), 2.00 (m, 1H), 1.35 (s, 9H); ^{13}C NMR (100 MHz): δ 183.1, 170.1, 163.3, 157.1, 146.8, 133.0, 132.6, 131.3, 131.2, 131.0, 128.7, 127.6, 117.7, 112.0, 111.3, 110.5, 80.7, 54.3, 31.2, 30.4, 28.3, 15.4; HRMS (FAB) *m/z* calcd for $C_{25}H_{28}O_6N_2ClS$ $[M+H]^+$ 519.1357; found 519.1350; $[\alpha]_D^{20}$ +11 (*c* 0.15, $CHCl_3$).

4.12. (*S*)-(1-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-ylcarbamoyl]ethyl)carbamic acid *tert*-butyl ester (22a)

34 mg (89%); mp 176 °C; HRMS (FAB) *m/z* calcd for $C_{23}H_{24}O_6N_2Cl$ $[M+H]^+$ 459.1323; found 459.1311; $[\alpha]_D^{20}$ −5.9 (*c* 0.80, $CHCl_3$).

4.13. (*S*)-{1-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-ylcarbamoyl]-2-phenylethyl}carbamic acid *tert*-butyl ester (22b)

32 mg (72%); mp 217–218 °C; HRMS (FAB) *m/z* calcd for $C_{29}H_{27}O_6N_2Cl$ M^+ 534.1558; found 534.1549; $[\alpha]_D^{20}$ −35 (*c* 0.46, $CHCl_3$).

4.14. (*S*)-{1-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-ylcarbamoyl]-3-methylbutyl}carbamic acid *tert*-butyl ester (22c)

31 mg (74%) was prepared in a similar procedure as **21a**; mp 170–171 °C; HRMS (FAB) *m/z* calcd for $C_{26}H_{30}O_6N_2Cl$ $[M+H]^+$ 501.1792; found 501.1788; $[\alpha]_D^{20}$ −18 (*c* 0.24, $CHCl_3$).

4.15. (*S*)-{1-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-ylcarbamoyl]-3-methylsulfanyl-propyl}carbamic acid *tert*-butyl ester (22d)

31 mg (71%); mp 159–160 °C; HRMS (FAB) *m/z* calcd for $C_{25}H_{28}O_6N_2ClS$ $[M+H]^+$ 519.1357; found 519.1335; $[\alpha]_D^{20}$ −12 (*c* 0.49, $CHCl_3$).

4.16. General procedure for reactions with sulfonyl chlorides

The procedure for the synthesis of **24a** was also used for the preparation of **24b** and **24c**.

4.17. *N*-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-yl]methanesulfonamide (24a)

To a solution of **10** (30 mg, 0.104 mmol) in CH_2Cl_2 (3 mL) were added Et_3N (22 μL, 0.16 mmol) and methylsulfonyl chloride (10 μL, 0.13 mmol). The reaction mixture was stirred at room temperature for 48 h and then the solution was diluted with EtOAc (30 mL). The organic layer was washed with brine and then dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (1:5 EtOAc/hexane) to afford 15 mg (52%) of **24a** as a yellow solid: mp 162–164 °C; R_f 0.59 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.3 (s, 1H), 7.79 (d, J = 8.9 Hz, 1H), 7.63 (dd, J = 1.6, 7.6 Hz, 1H), 7.59 (dd, J = 1.4, 8.0 Hz, 1H), 7.54 (dt, J = 1.4, 8.0 Hz, 1H), 6.47 (dt, J = 1.7, 7.5 Hz, 1H), 6.88 (d, J = 8.9 Hz, 1H), 6.61 (s, 1H), 6.54 (br s, 1H), 2.99 (s, 3H); ^{13}C NMR (100 MHz): δ 183.0, 163.8, 159.2, 149.2, 132.9, 132.8, 132.1, 131.3, 131.0, 130.9, 127.8, 115.7, 112.2, 112.1, 110.9, 40.2; HRMS (FAB) *m/z* calcd for $C_{16}H_{13}O_5NSCl$ $[M+H]^+$ 366.0203; found 366.0193.

4.18. *N*-[2-(2-Chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-yl]benzenesulfonamide (24b)

18 mg (41%); mp 176–177 °C; R_f 0.64 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.3 (s, 1H), 7.78 (d, J = 8.9 Hz, 1H), 7.59 (dd, J = 1.3, 8.3 Hz, 2H), 7.55 (m, 2H), 7.40 (m, 2H), 7.32 (dd, J = 1.2, 7.7 Hz, 1H), 7.18 (t, J = 7.6 Hz, 2H), 6.85 (d, J = 8.9 Hz, 1H), 6.68 (br s, 1H), 6.48 (s, 1H); ^{13}C NMR (100 MHz): δ 182.8, 163.0, 159.5, 149.7, 139.0, 133.9, 133.3, 132.8, 132.7, 131.3, 130.9, 130.5, 128.9, 127.5, 127.2, 115.2, 112.0, 111.8, 110.5; HRMS (FAB) *m/z* calcd for $C_{21}H_{15}O_5NSCl$ $[M+H]^+$ 428.0359; found 428.0352.

4.19. 3,5-Dichloro-*N*-[2-(2-chlorophenyl)-5-hydroxy-4-oxo-4*H*-chromen-8-yl]-2-hydroxybenzenesulfonamide (24c)

23 mg (44%); mp 192–194 °C; R_f 0.32 (1:1 hexane/EtOAc); ^1H NMR (400 MHz): δ 12.4 (br s, 1H), 8.17 (br s, 1H), 7.72 (d, J = 8.9 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.54 (dt, J = 2.4, 8.0 Hz, 1H), 7.46 (m, 2H), 7.36 (d, J = 2.4 Hz, 1H), 7.28 (d, J = 2.4 Hz, 1H), 6.87 (d, J = 8.9 Hz, 1H), 6.86 (br s, 1H), 6.55 (s, 1H); ^{13}C NMR (100 MHz): δ 182.7, 163.3, 160.7, 135.0,

134.9, 133.0, 132.7, 131.6, 130.8, 130.3, 127.9, 126.9, 125.5, 124.2, 113.4, 112.3, 112.1, 110.7; HRMS (FAB) *m/z* calcd for $C_{21}H_{13}O_6NSCl_3$ $[M+H]^+$ 511.9529; found 511.9534.

4.20. CDK2-CyclinA activity assays

The CDK2-Cyclin A (Invitrogen Corporation, CA, USA) enzyme activity was measured by a fluorescence kinetic assay using the Biosource Omnia Ser/Thr recombinant kit 7 (Invitrogen Corporation, CA,USA). The assay was carried out in a volume of 30 μl at room temperature in 384-well black polystyrene plates. The final concentrations of the assay constituents were 1 μM CDK2-Cylin A, 10 μM peptide substrate, and 1 mM ATP. The compounds were solubilized in DMSO and added to the reaction mixture at six concentrations with the highest concentration of 100 μM for flavopiridol and 417 μM for the rest of the flavone derivatives. The final concentration of DMSO in the mixture was 1% for flavopiridol and 17% for the derivatives. The presence of 17% DMSO did not significantly affect the activity of the enzyme. Continuous kinetic monitoring of enzyme activity was performed on Spectramax Gemini reader (λ_{ex} 355 nm and λ_{em} 460 nm) and controlled by the Softmax software. The experiment was carried out in triplicate and the percent inhibition of enzyme activity was calculated for all the compounds at each concentration. The IC_{50} values were obtained from the non-linear curve fitting of the plot of percent inhibition versus inhibitor concentration [I] using the equation, % inhibition = $100/\{1+ (IC_{50}/[I])^k\}$, where k is the Hill coefficient. For compounds where no inhibition was observed, the IC_{50} value is reported at the highest concentration tried, that is, 417 μM.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.10.063.

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