Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 278–281
Bioorganic & Medicinal Chemistry Letters

Nitrogen-containing flavonoids as CDK1/Cyclin B inhibitors: Design, synthesis, and biological evaluation

Tao Liu,[a] Zhongmiao Xu,[a] Qiaojun He,[b] Yanhong Chen,[b] Bo Yang[b] and Yongzhou Hu[a,*]

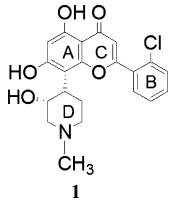
[a]*ZJU-ENS Joint Laboratory of Medicinal Chemistry, School of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310031, PR China*

[b]*Department of Pharmacology, School of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310031, PR China*

Received 24 May 2006; revised 27 July 2006; accepted 31 July 2006
Available online 7 November 2006

Abstract—A novel series of nitrogen-containing flavonoids **5a**–**l**, **6a**,**b**, and **7a**,**b** were designed and synthesized as cyclin-dependent kinases (CDKs) inhibitors. The representative compounds **5a**, **5b**, **5e**, and **5g** showed potent CDK1/Cyclin B inhibitory activities. All compounds displayed a significant growth inhibitory action in vitro against Bel-7402, PC-3, ECA-109, A-549, HL-60, and MCF-7 cancer cell lines. Flow cytometry analysis showed that **5b** induced apoptosis in PC-3 cells.

Cyclin-dependent kinases (CDKs) are a prominent family of protein kinases, which play a key role in regulation of the cell cycle.[1] Abnormal CDKs' control of the cell cycle has been strongly linked to the molecular pathology of cancer. CDKs have thus become attractive therapeutic targets for cancer therapy.[2] A number of CDKs inhibitors have been developed toward the clinical trials against various cancer diseases.[3] Among them, flavopiridol (**1**) is a pioneering benchmark CDKs inhibitor, which shows inhibitory activity against a broad range of kinases including CDK1, CDK2, and CDK4, and is currently in Phase II clinical trials as an antitumor agent.[4] The SAR studies of flavopiridol reveal that the presence of the nitrogen atom on the D ring is very important for CDKs inhibitory activity.[5] The *o*-chloro substituent on B ring leads to a tenfold increase in activity compared with that of none.[6]



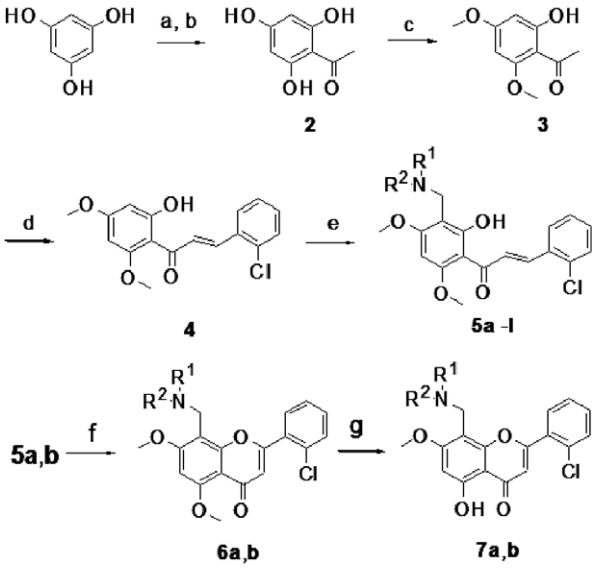
1

On the other hand, it is well known that 2′-hydroxychalcones and flavones are two forms of flavonoids. They have been shown to possess a variety of biological activities, one area of particular interest is their potential as anticancer agents. Pouget reported that 2′-hydroxychalcones were potent inhibitors of MCF-7 human breast cancer cell growth.[7] Rao also reported that hydroxyflavones and methoxyflavones displayed a significant growth inhibitory action against Jurkat, PC-3, and Colon 205 cancer cell lines.[8] These prompted us to design a new series of nitrogen-containing flavonoid analogues of **1** in an attempt to improve potency against the CDK1/Cyclin B and to carry out a structure–activity relationship study. In the course of an analogue generation program, the side chain containing nitrogen atom and 2-chlorobenzene were chosen as the two pharmacophores, and were integrated into the skeletons of 2′-hydroxychalcones and flavones. In this communication, we describe the synthesis of the nitrogen-containing chalcone and flavone derivatives, the evaluation of these compounds as CDK1/Cyclin B inhibitor, and their antiproliferative activity in vitro against various human cancer cell lines.

Nitrogen-containing chalcones **5a**–**l** and flavones **6a**,**b**, **7a**,**b** were prepared as shown in Scheme 1. Phloroacetophenone **2**, 2-hydroxy-4,6-dimethoxyacetophenone **3** and 2′-hydroxy-4′,6′-dimethoxychalcone **4** were synthesized following the method described in the literature with minor modifications.[9–11] Condensation of **4** with

Keywords: Cyclin-dependent kinases (CDKs) inhibitor; Antiproliferative activity; Flavonoids; Apoptosis.
* Corresponding author. Tel.: +86 13957120059; fax: +86 571 87217412; e-mail: huyz@zjuem.zju.edu.cn

0960-894X/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2006.07.088



Scheme 1. Preparation of chalcone and flavone derivatives. Reagents and conditions: (a) CH_3CN, $ZnCl_2$, ether, HCl(g), 0–5 °C, 90–95%; (b) H_2O, reflux, 90%; (c) Me_2SO_4, K_2CO_3, acetone, rt, 90%; (d) 2-chlorobenzaldehyde, KOH, EtOH, H_2O, rt, 90–93%; (e) secondary amine, 37% HCHO, isopropanol, HCl, reflux, 47–60%; (f) I_2, concd H_2SO_4, DMSO, 80–85 °C, 81–85%; (g) $AlCl_3$, CH_3CN, reflux, 44–54%.

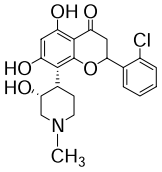
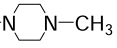
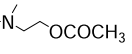
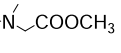
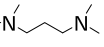
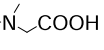
the various secondary amines and formaldehyde in the presence of hydrochloride in isopropanol yielded the desired derivatives **5a**–**l**.[12] Cyclization of chalcones **5a** or **5b** in the presence of catalytic amount of iodine and conc. sulfuric acid in DMSO afforded dimethoxyflavones **6a** or **6b**.[13] Treatment of **6a** or **6b** with aluminium trichloride gave 5-hydroxy-7-methoxy flavones **7a** or **7b**.[14] We tried many O-demethylation reagents, such as BBr_3, HBr/HOAc, pyridinium hydrochloride and Me_3SiI, but failed to remove the 7-*O*-methyl-protective group.

The single crystal X-ray analysis of compound **5a** we reported previously indicated that the morpholinomethyl group was dominant at position 3′ of chalcone core.[15] It was confirmed that the synthesized chalcone was of *trans*-configuration, which was also proved by the data of the ^1H NMR spectra (J = 15.2–16.0 Hz).[16]

The synthesized compounds **5a**–**l**, **6a**,**b**, **7a**,**b** were assayed for their CDK1/Cyclin B inhibitory activity according to the procedure instruments of CycLex cdc2 (CDK1) kinase assay kit (CycLex Co., Ltd, Japan).[17] The results obtained are summarized in Table 1. We also tested the synthesized compounds for their antiproliferative activity in vitro against several cancer cell lines by MTT assay.[18] These included human liver carcinoma (Bel-7402), human prostate carcinoma (PC-3), human esophageal carcinoma (ECA-109), human lung carcinoma (A-549), human leukemia (HL-60) and human breast carcinoma (MCF-7) .The data are listed in Table 2.

As shown in Table 1 most of tested compounds displayed potent CDK1/Cyclin B inhibitory activity. The largest fluctuation in CDK1/Cyclin B inhibitory activity

Table 1. CDK1/Cyclin B inhibitory activity of the compounds **5a**–**5l**, **6a**,**b**, and **7a**,**b**

Compound	NR^1R^2	IC_{50}[a] (μM)
Flavopiridol		0.4[b]
5a		0.0475
5b		0.0569
5c		1.246
5d		>3.0
5e		0.0497
5f		0.5034
5g		0.0758
5h		>3.0
5i		0.3235
5j		>3.0
5k		0.4684
5l		—
6a		>3.0
6b		0.1547
7a		>3.0
7b		0.1382

[a] The IC_{50} values were the mean values of three repeated experiments.
[b] From the literature.[19]

Table 2. Antiproliferative activity of the compounds **5a**–**l, 6a**,**b**, and **7a**,**b**

Compound	IC_{50}[a,b] (μM)					
	Bel-7402	PC-3	ECA-109	A-549	HL-60	MCF-7
5a	22.7	17.2	6.2	4.8	6.9	17.2
5b	3.1	1.7	2.5	2.8	3.6	2.6
5c	6.8	3.1	6.9	2.6	14.2	3.9
5d	7.6	4.7	7.1	4.1	10.9	5.2
5e	6.7	4.2	7.1	3.6	30.9	2.7
5f	21.8	0.4	2.6	2.1	19.2	0.09
5g	23.3	0.003	1.4	1.3	0.2	6.8
5h	64.4	6.2	0.8	6.9	19.7	10.0
5i	8.1	10.0	3.9	3.3	10.8	5.1
5j	8.5	7.2	13.9	9.1	0.4	10.6
5k	30.9	14.5	3.8	3.1	11.4	3.2
5l	35.2	12.5	44.4	79.4	80.3	73.2
6a	61.3	57.8	61.3	>150.0	67.3	Nd[c]
6b	44.2	15.5	23.7	>150.0	34.5	23.7
7a	>150.0	42.0	86.6	>150.0	26.9	124.4
7b	118.0	63.4	21.5	45.5	48.1	Nd[c]

[a] The IC_{50} values represent the concentration which results in a 50% decrease in cell growth after 72 h incubation.
[b] The IC_{50} values were the mean values of three repeated experiments.
[c] Nd means not detected.

was observed for heterocyclic substituted chalcones **5a–d**. Compounds with the morpholinomethyl (e.g., **5a**) or piperidinylmethyl substituent (e.g., **5b**) at position 3′ of chalcone moiety showed IC_{50} values of 0.0475 μM and 0.0569 μM in CDK1/Cyclin B inhibitory assay, respectively, which were about 7- or 8-fold more potent than flavopiridol. Whereas the pyrrolidinylmethyl analogue **5c** was significantly less active, and 4-methylpiperazinylmethyl analogue **5d** was proved to be the least active molecule in this series. These indicated that the position 3′ was not tolerant of the smaller heterocycle of the substituent and introduction of additional alkaline atom in heterocycle would be detrimental to CDK1/Cyclin B inhibitory potency. A similar trend was seen in the compounds **5e–g**. The bulkier diethylaminomethyl analogue **5e** increased the activity as compared to **5f** which had the dimethylaminomethyl group at position 3′ of chalcone. In addition, a comparison of the potencies of **5g** and **5h–k** suggested that substitution of the *N*-ethyl with an *N*-hydroxyethyl, *N*-acetyloxyethyl, *N*-methoxycarbonylmethyl or *N*-dimethylaminopropyl group reduces the activity (Table 1). In the flavone series, no significant difference between methoxyflavone and hydroxyflavone (e.g., **6a** vs **7a**, **6b** vs **7b**) was found, whereas the substituent at position 8 of flavone moiety was important for CDK1/Cyclin B inhibitory potency, as an example in Table 1, piperidinylmethyl analogues (e.g., **6b**, **7b**) were about 12- or 21-fold more potent than morpholinomethyl analogues (e.g., **6a**, **7a**).

Despite their potency effects against CDK1/Cyclin B, all tested chalcone compounds showed moderate to strong antiproliferative activity against Bel-7402, PC-3, ECA-109, A549, HL60, and MCF-7 cells (Table 2). Among them, compound **5b** exhibited significant antiproliferative activity, with IC_{50} values below 4 μM, against all of the cancer lines. On the other hand, the flavones (**6a**,**b**, **7a**,**b**) had negligible antiproliferative activity against all cancer lines tested, as their IC_{50} values were generally higher than 20 μM.

The antiproliferative mechanism of compound **5b**, a representative from this class, was further explored in PC-3 cells. PC-3 cells were incubated with 7.2 μM **5b** for 12 h and 24 h, and DNA analysis was conducted by flow cytometry to determine cell cycle progression and apoptosis.[20] We found that, compared to control cells, cells exposed to **5b** slightly arrested at the G_2/M phase of the cell cycle at 12 h (14.0%), and subsequently accumulated in the sub-G_0 phase at 12 h (6.1%) and 24 h (34.1%) (Table 3 and Fig. 1). Because the increase of cells in the sub-G_0 phase generally indicated the increase of apoptotic cell death, **5b** may induce apoptosis in PC-3 cells. These results suggest that **5b**-mediated G_2/M arrest and apoptosis might involve in antiproliferative function of **5b**.

To confirm that the induction of cell death by **5b** was related to apoptosis, we next examined cellular changes such as chromatin condensation by **5b** using staining method with both acridine orange (AO) and ethidium bromide (EB).[21] The result showed that the amount of alive cells exhibited a declining trend, while the cell population of which chromatin was condensed in the nucleus exhibited an increasing manner with the increasing concentrations of **5b** (Fig. 2), further suggesting that **5b** could induce apoptosis to exert antiproliferative activity in PC-3 cells.

Table 3. Effects of **5b** on cell cycle progression in PC-3 cells

Treated time (h)	G_0/G_1 (%)	S (%)	G_2/M (%)	Sub-G_0 (%)
0	60.4	29.2	10.1	1.1
12	60.2	25.8	14.0	6.1
24	60.7	29.9	9.8	34.1

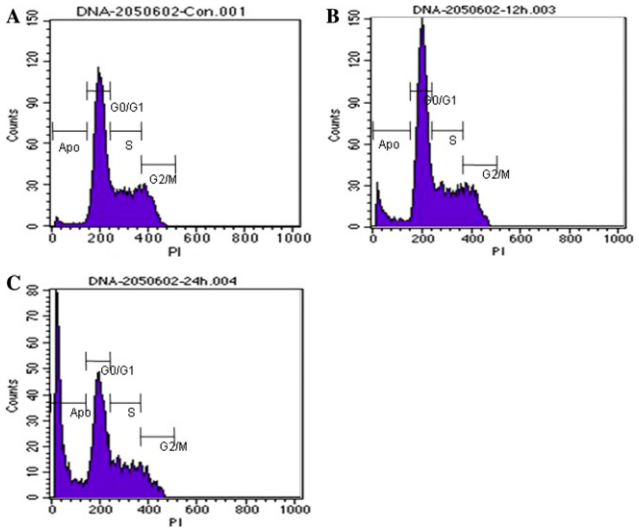


Figure 1. Compound **5b** induced apoptosis in PC-3 cell. Cells were treated without (Control, A) or with 7.2 μM **5b** for 12 h (B) and 24 h (C), respectively. Apo, apoptosis.

In summary, a novel series of nitrogen-containing chalcone and flavone derivatives were prepared. Among them, chalcone derivatives were found to be inhibitors of CDK1/Cyclin B in vitro. They also inhibited the growth of various human cancer cells including Bel-7402, PC-3, ECA-109, A549, HL60, and MCF-7. Representative compound **5b** was used to investigate the antiproliferative mechanism in PC-3 cells. Apoptosis pathway contributed to the antiproliferative activity. The results provide valuable information for the design of CDK1/Cyclin B inhibiting compounds with enhanced anticancer effect. Future progress on related series will be reported in due course.

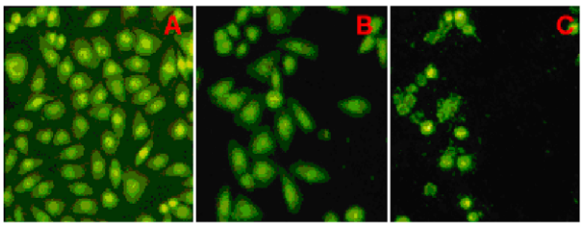


Figure 2. The effects of **5b** on the morphology of PC-3 cell. PC-3 cells were treated with DMSO (A, Control), 1.8 μM **5b** (B), and 7.2 μM **5b** (C) for 48 h. The increase of chromatin condensation by **5b** was found in PC-3 cells.

References and notes

1. Harper, J. W.; Adams, P. D. *Chem. Rev.* **2001**, *101*, 2511.
2. Sielecki, T. M.; Boylan, J. F.; Benfiled, P. A.; Trainor, G. T. *J. Med. Chem.* **2000**, *43*, 1.
3. Moon, M. J.; Lee, S. K.; Lee, J. W.; Song, W. K.; Kim, S. W.; Kim, J. I.; Cho, C.; Choi, S. J.; Kim, Y. C. *Bioorg. Med. Chem.* **2006**, *14*, 237.
4. Zhai, S.; Senderowicz, A. M.; Sausville, E. A.; Figg, W. D. *Ann. Pharmacother.* **2002**, *36*, 905.
5. Murthi, K. K.; Dubay, M.; McClure, C.; Brizuela, L.; Boisclair, M. D.; Worland, P. J.; Mansuri, M. M.; Pal, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1037.
6. Huwe, A.; Mazitschek, R.; Giannis, A. *Angew. Chem., Int. Ed.* **2003**, *42*, 2122.
7. Pouget, C.; Lauthier, F.; Simon, A.; Fagnere, C.; Basly, J. P.; Delage, C.; Chulia, A. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3095.
8. Rao, Y. K.; Fang, S. H.; Tzeng, Y. M. *Bioorg. Med. Chem.* **2005**, *13*, 6850.
9. Gulati, K. C.; Seth, S. R.; Venkataraman, K. *Org. Synth.* **1943**, *Coll. Vol. 2*, 522.
10. Juntend, K. Y. T.; Junte, T. S. T. E.P. Patent 292,576, 1988; *Chem. Abstr.* **1988**, *110,* 23539.
11. Bu, X. Y.; Zhao, L. Y.; Li, Y. L. *Synthesis* **1997**, *11*, 1246.
12. Wilds, A. L.; Nowak, R. M.; McCaleb, K. E. *Org. Synth.* **1963**, *Coll. Vol. 4*, 281.
13. Li, J. F.; Mao, G. J.; Miao, Y. Y. *Heilongjiang Med. Pharm.* **2002**, *25*, 15.
14. Solladie, G.; Gehrold, N.; Maignan, J. *Tetrahedron: Asymmetry* **1999**, *10*, 2739.
15. Liu, T.; Xu, Z. M.; Hu, Y. Z. *Acta Crystallogr., Sect. E* **2005**, 3389, CCDC deposition number 287623.
16. Compound **5a**: yellow powder, mp 162–164 °C; IR (KBr): 3446, 3023, 2954, 2935, 2840, 1627, 1272, 748 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 8.04 (d, 1H, J = 15.6 Hz, =*CH*–), 7.69–7.70 (m, 1H, Ar-6-H), 7.57 (d, 1H, J = 15.6 Hz, O=C–*HC*=), 7.41–7.43 (m, 1H, Ar-3-H), 7.28–7.30 (m, 2H, Ar-4-H and 5-H), 6.02 (s, 1H, Ar-5′-H), 3.91 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.71 (t, 4H, J = 4.4 Hz, –H_2C–O–CH_2–), 3.67 (s, 2H, –CH_2), 2.56 (t, 4H, J = 4.4 Hz, –H_2C–N–CH_2–); Anal. Calcd for $C_{22}H_{24}ClNO_5$: C, 63.02; H, 5.66; N, 3.16. Found: C, 63.23; H, 5.79; N, 3.35; MS (ESI): *m*/*z* 418 $[M+1]^+$. Compound **5b**: yellow powder, mp 159–162 °C; IR (KBr): 3506, 3031, 2928, 2847, 1627, 1273, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 7.94 (d, 1H, J = 16.0 Hz, =*CH*–), 7.66–7.69 (m, 1H, Ar-6-H), 7.36–7.38 (m, 1H, Ar-3-H), 7.23–7.26 (m, 2H, Ar-4-H and 5-H), 7.22 (d, 1H, J = 16.0 Hz, =CH–), 5.99 (s, 1H, Ar-5′-H), 3.82 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.66 (s, 2H, –CH_2), 2.43–2.47 (m, 4H, –H_2C–N–CH_2–), 1.55–1.59 (m, 4H, –CH_2–CH_2–), 1.41–1.43 (m, 2H, –CH_2–); Anal. Calcd for $C_{23}H_{26}ClNO_4$: C, 66.22; H, 6.15; N, 3.30. Found: C, 66.42; H, 6.30; N, 3.37; MS (ESI): *m*/*z* 416 $[M+1]^+$. Compound **6a**: white powder, mp 170–172 °C; IR (KBr): 3067, 2940, 2845, 1662, 1594, 1276, 1225, 764 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): δ 7.71 (dd, 1 H, J = 7.6, 2.0 Hz, Ar-6′-H), 7.47 (dd, 1H, J = 7.6, 2.0 Hz, Ar-3′-H), 7.36 (m, 2H, Ar-4′-H and Ar-5′-H), 6.57 (s, 1H, 3-H), 6.39 (s, 1H, 6-H), 3.97 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.67 (s, 2H, CH_2), 3.61 (t, 4H, J = 4.8 Hz, –CH_2–O–CH_2–), 2.43 (t, 4H, J = 4.8 Hz, –CH_2–N–CH_2–); Anal. Calcd for $C_{22}H_{22}ClNO_5$: C, 63.54; H, 5.33; N, 3.37. Found: C, 63.37; H, 5.40; N, 3.35; MS (ESI): *m*/*z* 416 $[M+1]^+$.
17. CDK1/Cyclin B kinase assay procedure: Add 3000 nM, 300 nM, and 30 nM of tested compounds to the wells, respectively, and incubate for 30 min at 30 °C. Wash the wells with wash buffer. Add 100 μL of Anti-phospho Cdc7 T376 Monoclonal Antibody TK-3H7, incubate for 30 min at room temperature. Wash the wells with wash buffer again. Add 100 μL of HRP-conjugated anti-mouse IgG, incubate for 30 min at room temperature. Wash the wells with wash buffer again. Add 100 μL of substrate reagent and incubate for 5–15 min at room temperature. Add 100 μL of stop solution. Measure absorbance at 450 nm.
18. Mosman, T. *J. Immunol. Methods* **1983**, *55*, 5563.
19. Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. *J. Med. Chem.* **2000**, *43*, 1.
20. Lee, S. K.; Heo, Y. H.; Steele, V. E.; Pezzuto, J. M. *Anticancer Res* **2002**, *22*, 97.
21. Ye, F.; Wu, J.; Dunn, T.; Yi, J.-Z.; Tong, X.-D.; Zhang, D. *Cancer Lett.* **2004**, *211*, 39.