Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1284–1287
Bioorganic & Medicinal Chemistry Letters

3-Hydroxychromones as cyclin-dependent kinase inhibitors: Synthesis and biological evaluation

Jinho Lee,[a,*] Taesik Park,[b] Shinwu Jeong,[b,†] Kyoung-Hee Kim[b] and Changyong Hong[b]

[a]*Department of Chemistry, Keimyung University, 1000 Sindang-Dong, Dalseo-Gu, Daegu 704-701, South Korea*
[b]*LG Life Sciences, Science Town, Taejon 305-380, South Korea*

Received 1 September 2006; revised 20 November 2006; accepted 4 December 2006
Available online 15 December 2006

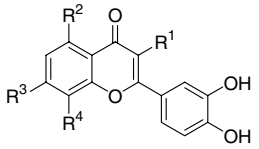
Abstract—A novel series of 3-hydroxychromones were prepared and found to be CDK inhibitors. Isothiazolidine 1,1-dioxide analogues showed potent CDK1 and CDK2 inhibitory activities and inhibited proliferation of EJ, HCT116, SW620, and MDAMB468 cancer cells.

Cyclin-dependent kinases (CDKs) play significant roles in cell cycle progression, and their activities are regulated precisely by multiple mechanisms.[1] The activities of CDKs are regulated positively by the various cyclin proteins and negatively by the CDK inhibitors such as the Cip/Kip and INK protein families. In various human tumors, the abnormal high expression of the cyclins and/or loss of the CDK inhibitors are known to cause the dysregulation of the activities of CDKs.[2] Such altered activations of CDKs in tumorigenesis have instigated intensive searches for CDK inhibitors as a potential anti-cancer drug.[3,4]

Flavonoids are phenylchromone derivatives that are ubiquitous in all plants. It has been shown that they have a variety of biological activities including anticarcinogenic activity.[5,6] Flavonoids displayed either inhibition or induction of the enzymes involved in the regulation of the cell proliferation;[7] for example, flavopiridol showed inhibitory activity against multiple kinases and was the first small molecule that went to the clinical trial as a CDK inhibitor.[8] In an effort to search for CDK inhibitors, flavonol had been found as a hit. Preliminary screening of the commercially available flavonoids showed that at least one hydroxyl group at either R^1 or R^2 of chromone was needed to have the inhibitory activities against CDK2 (Table 1). As far as the hydroxyl group was present at R^1, any additional hydroxyl group at R^2 or R^3 did not affect the inhibitory activities (**1**, **2**, **6**). Since flavopiridol has the hydroxyl group at R^2 which is involved in H-bond interactions with the enzyme,[9] compounds having hydroxyl group at R^1 instead of R^2 were expected to have different binding orientations, compared to flavopiridol, and to show different structure–activity relationships.

Efforts to improve the potency of the 3-hydroxychromone **6** initially focused on the modification of R^4 with amine derivatives. 3-Hydroxychromones were prepared in two steps.[11] However, two different synthetic routes were used depending on the type of R^4 as shown in

Table 1. Structure and CDK2 inhibitory activity of flavones[a]

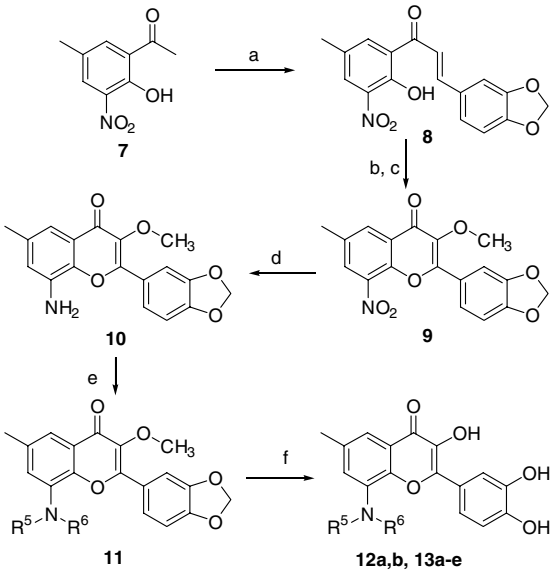


Compound	R^1	R^2	R^3	R^4	CDK2 IC_{50}[b] (μM)
1	OH	OH	OH	H	40
2	OH	H	OH	H	5
3	H	OH	OH	H	20
4	H	H	OH	OH	>500
5	H	H	H	H	>500
6	OH	H	H	H	4

[a] The CDK2 inhibitory assays were performed as described in Ref. 10.
[b] Values are means of three experiments.

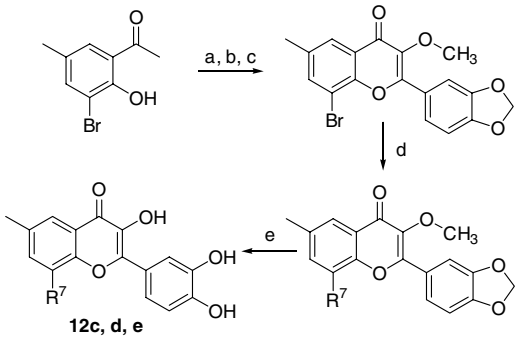
Keywords: 3-Hydroxychromone; Isothiazolidine 1,1-dioxide; CDK2.
* Corresponding author. Tel.: +82 53 580 5183; fax: +82 53 580 5164; e-mail: jinho@kmu.ac.kr
† Present address: USC/Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, 1441 East Lake Avenue, Los Angeles, CA 90089, USA.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.12.011



Scheme 1. General synthetic route for 3-hydroxychromones. Reagents and conditions: (a) piperonal, NaOH, 80% aq EtOH (95%); (b) H_2O_2, 10% aq NaOH, MeOH (62%); (c) CH_3I, K_2CO_3, acetone (94%); (d) Pd/C, H_2, MeOH/DCM (1:1) (quantitative); (e) **12b**: CH_3CHO, $NaBH(AcO)_3$, 1,2-DCE (42%), **13a**: (1) $Cl(CH_2)_3COCl$, Et_3N, DCM; (2) NaH, DMF (26%, two steps yield), **13b**: succinic anhydride, Et_3N, DMF (55%), **13c**: $ClCO(CH_2)_3COCl$, K_2CO_3, DMF (37%), **13d**: (1) $CbzNHCH_2CO_2H$, *i*-PrOCOCl, *N*-methylmorpholine, THF (75%); (2) $(n\text{-}Bu)_4NF$, THF (59%), **13e**: Cl_3COCCl_3, $CH_3NH(CH_2)_2OCH_3$, Et_3N, DMF (61%); (f) BBr_3, DCM (30–84%).

Schemes 1 and 2. 8-Amino-3-hydroxychromone **10** was prepared according to the procedure outlined in Scheme 1. The coupling reaction between 1-(2-hydroxy-5-methyl-3-nitro-phenyl)-ethanone and benzo[1,3]dioxole-5-carbaldehyde provided compound **8** which was converted to 3-hydroxychromone by NaOH in the presence of hydrogen peroxide. Protection of the hydroxyl group followed by reduction of nitro group produced the compound **10** which was converted to various functionalities either by alkylation or amide coupling reaction. Compound **13b** was obtained by treatment of **11**, product of the reaction between **10** and succinic anhydride, with BBr_3.



Scheme 2. General synthetic route for 3-hydroxychromones. Reagents and conditions: (a) piperonal, NaOH, 80% aq EtOH (65%); (b) H_2O_2, 10% aq NaOH, MeOH (78%); (c) CH_3I, K_2CO_3, acetone (79%); (d) $Pd_2(DBA)_3$, DPPF toluene; **12c**: pyrrolidine (82%), **12d**: piperidine (68%), **12e**: *N*-methylpiperazine (63%); (e) BBr_3, DCM (75–81%).

Alternatively, cyclic amines were introduced at R^4 by the palladium-catalyzed coupling reactions[12] of 8-bromochromone with the corresponding amines as shown in Scheme 2.

Alkylation and amidation of amine either open-chains or cyclic forms did not improve the potency against CDK2, **12a–e**, **13a–d** (Table 2). However, the presence of methyl group at the nitrogen of hydantoin improved the activity by 2 order of magnitude compared to hydantoin itself (**13d** vs **13e**). $1\lambda^6$-Isothiazolidine-1,1-dione **15a** provided a similar enzymatic potency with *N*-methylhydantoin, but showed improved cellular activity.

An X-ray crystal structure analysis showed that the binding mode of 3-hydroxychromone was different from that of flavopiridol, as expected (Fig. 1).

The binding mode was analogous to that of fisetin bound to CDK6.[14] 3-Hydroxyl and 4-keto groups made two hydrogen bonds with carbonyl oxygen of Glu 81 and the amide nitrogen of Leu 83, respectively. In contrast, flavopiridol used its 5-hydroxyl group for the hydrogen bond with Glu 81. The chromone ring made many van der Waals contacts with the hinge region of CDK2. The dihydroxyphenyl ring rotated about 30 de-

Table 2. CDK2 inhibitory activities of 3-hydroxychromones

Compound	R^5, R^6, or R^7	CDK2 IC_{50}[a] (μM)	EJ cytotoxicity[b] GI_{50} (μM)
12a	H, H	0.63	
12b	CH_3CH_2, CH_3CH_2	1.6	
12c	$CH_2(CH_2)_2CH_2$	3.0	
12d	$CH_2(CH_2)_3CH_2$	2.6	
12e	$CH_2CH_2N(CH_3)CH_2CH_2$	0.64	
13a	$CH_2(CH_2)_2CO$	1.6	
13b	$CO(CH_2)_2CO$	1.5	1.2
13c	$CO(CH_2)_3CO$	4.4	
13d	$CONHCH_2CO$	4.0	
13e	$CON(CH_3)CH_2CO$	0.056	1.1
15a	$SO_2(CH_2)_2CH_2$	0.087	0.6

[a] Values are means of three experiments.

[b] Exponentially growing cells were treated with test compounds at various concentrations for 48 h, and then the cell numbers were measured. The compound concentration with 50% growth inhibition activity was determined.[13] Values are means of three experiments.

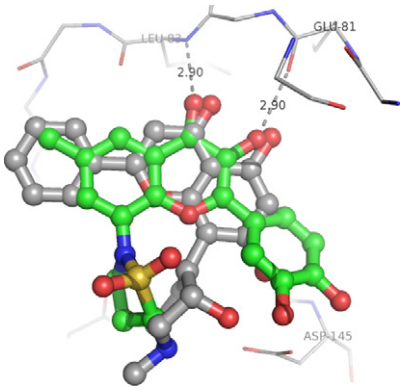


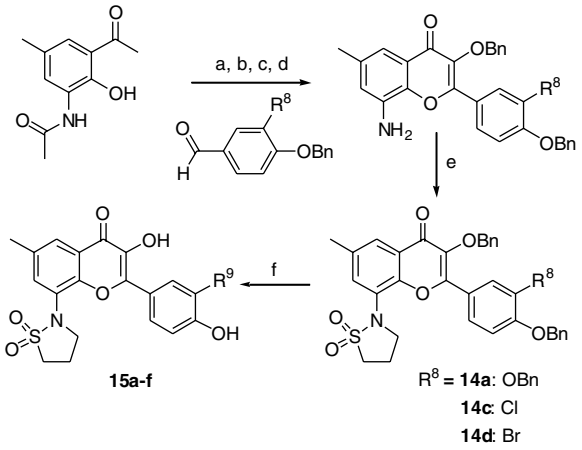
Figure 1. Comparison of the binding mode of 3-hydroxychromone **15a** (in green) and deschloro-flavopiridol (in gray) in the CDK2 ATP-binding site.

grees from the plane of chromone and bound complementarily to the pocket formed by Lys 33, Val 64, Phe 80, and Ala 144. Its 2-hydroxyl group formed third hydrogen bond with the side chain of Asp 145. $1\lambda^6$-Isothiazolidine-1,1-dione rotated about 80 deg from the plane of chromone making close contacts with side chains of Gly 11, Val 18, and Gln 131.[15]

Since the introduction of $1\lambda^6$-isothiazolidine-1,1-dione at R^4 improved the potency in both CDK2 enzyme and EJ cell line, further modifications of catechol had been performed while keeping $1\lambda^6$-isothiazolidine-1,1-dione.

The synthetic methods for the modification of catechol are outlined in Scheme 3. The synthesis of $1\lambda^6$-isothiazolidine-1,1-dione was performed in two consecutive steps without purification of intermediate.

Compound **15e** was obtained by palladium-catalyzed cyanation of **14d** according to the procedure described by Maligres et al.[16] Introduction of trifluoromethyl moiety was accomplished by treatment of aryl bromide **14d** with methyl 2,2-difluoro-2-(fluorosulfonyl)-acetate in

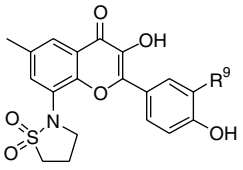


Scheme 3. General synthetic route for 3-hydroxychromones bearing isothiazolidine-1,1-dione. Reagents and conditions: (a) NaOH, 80% aq EtOH (59–78%); (b) H_2O_2, 10% aq. NaOH, MeOH (59–93%); (c) $C_6H_5CH_2Br$, K_2CO_3, acetone (47–56%), (d) 1—$(Boc)_2O$, DMAP, DCM; 2—NaOH, MeOH; (3) AcCl, MeOH (52–94%, three steps yield); (e) 1—$Cl(CH_2)_3SO_2Cl$, Et_3N, DMAP, DCM; 2—1N aq NaOH, DMF (**14a**: 74%, **14c**: 82%, **14d**: 67%, two steps yield); (f) **15a**: Pd/C, H_2, MeOH/DCM (1:1) (54%), **15b**: Pd/C, H_2 (82% from **14d**), **15c**: Pd/C, H_2 (58%), **15d**: BBr_3, DCM (69%), **15e**: (1) $Pd_2(dba)_3$, DPPF, $Zn(CN)_2$, DMF (33% from **14d**); (2) Pd/C, H_2 (86%), **15f**: (1) $FSO_2CF_2CO_2CH_3$, CuI, DMF (76% from **14d**); (2) Pd/C, H_2 (91%).

the presence of CuI according to the procedure described by Njoroge et al.[17]

The substitution of the hydroxyl group with Cl or Br (**15c**, **15d**, respectively) provided highly potent compounds against both CDK1 and CDK2 and the proliferation of various human cancer cell lines, such as EJ (bladder carcinoma), HCT116, SW620 (colon carcinoma), and MDAMB468 (breast carcinoma) (Table 3). The introduction of CF_3 **15f** increased the selectivity on CDK1 over CDK2 but caused poor cellular activity. The results described in Table 3 indicate that inhibition of both CDK1 and CDK2 may be required to achieve potent anti-proliferative activity in cancer cells.

Table 3. CDK2 inhibitory activities of 3-hydroxychromones bearing $1\lambda^6$-isothiazolidine-1,1-dione



Compound	R^9	IC_{50}[a] (μM)		Cytotoxicity[b] GI_{50} (μM)			
		CDK1	CDK2	EJ	HCT116	SW620	MDAMB468
15a	OH	0.13	0.087	0.6	0.28	0.9	1.0
15b	H	0.45	0.25	0.27	0.34	0.57	0.62
15c	Cl	0.033	0.19	0.065	0.037	0.064	0.18
15d	Br	0.019	0.075	0.064	0.017	0.052	0.23
15e	CN	0.16	1.4	[c]	[c]	[c]	[c]
15f	CF_3	0.063	1.7	1.3	0.91	[c]	[c]

[a] Values are means of three experiments.
[b] Tests were performed as shown in Table 2. Values are means of three experiments.
[c] Not determined.

In summary, we have discovered a novel series of 3-hydroxychromone derivatives that were effective cyclin-dependent kinase inhibitors and potent growth inhibitors of human cancer cell lines.

References and notes

1. (a) Morgan, D. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 261; (b) Meijer, L., Guidet, S., Philippe, M., Eds.; *Progress in Cell Cycle Research*; 1997, Vol. 3, Plenum Press: New York.
2. Sellers, W. R.; Kaelin, W. G., Jr. *J. Clin. Oncol.* **1997**, *15*, 3301.
3. (a) Sausville, E. A.; Zaharevits, D.; Gussio, R.; Meijer, L.; Mouarn-Leost, M.; Kunick, C.; Schultz, R.; Lahusen, T.; Headlee, D.; Stinson, S.; Arbuck, S. G.; Senderowicz, A. *Pharmacol. Ther.* **1999**, *82*, 285; (b) Mani, S.; Wang, C.; Wu, K.; Francis, R.; Pestell, R. *Exp. Opin. Investig. Drugs* **2000**, *9*, 1849; (c) Knockaert, M.; Greengard, P.; Meijer, L. *Trends Pharm. Sci.* **2002**, *23*, 417; (d) Senderowicz, A. M. *Cancer Biol. Ther.* **2003**, *2*, s084; (e) Blagden, S.; de Bono, J. *Curr. Drug Targets* **2005**, *6*, 325; (f) Shapiro, G. I. *J. Clin. Oncol.* **2006**, *24*, 1770.
4. (a) Kim, K. S.; Kimball, S. D.; Misra, R. N.; Rawlins, D. B.; Hunt, J. T.; Xiao, H.-Y.; Lu, S.; Qian, L.; Han, W.-C.; Shan, W.; Mitt, T.; Cai, Z.-W.; Poss, M. A.; Zhu, H.; Sack, J. S.; Tokarski, J. S.; Chang, C.-Y.; Pavletich, N.; Kamath, A.; Humphreys, W. G.; Marathe, P.; Bursuker, I.; Kellar, K. A.; Roongta, U.; Batorsky, R.; Mulheron, J. G.; Bol, D.; Fairchild, C. R.; Lee, F. Y.; Webster, K. R. *J. Med. Chem.* **2002**, *45*, 3905; (b) McClue, S. J.; Blake, D.; Clarke, R.; Cowan, A.; Cummings, L.; Fischer, P. M.; MacKenzie, M.; Melville, J.; Stewart, K.; Wang, S.; Zhelev, N.; Zheleva, D.; Lane, D. P. *Int. J. Cancer* **2002**, *102*, 463; (c) Lin, R.; Lu, Y.; Wetter, S. K.; Connolly, P.; Turchi, I. J.; Murray, W. V.; Emanuel, S. L.; Gruninger, R. H.; Fuentes-Pesquera, A. R.; Adams, M.; Pandey, N.; Moreno-Mazza, S.; Middleton, S. A.; Jolliffe, L. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2221; (d) Bathini, Y.; Singh, I.; Harvey, P. J.; Keller, P. R.; Singh, R.; Micetich, R. G.; Fry, D. W.; Dobrusin, E. M.; Toogood, P. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3881; (e) Byth, K. F.; Culshaw, J. D.; Green, S.; Oakes, S. E.; Thomas, A. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2245; (f) Schoepfer, J.; Fretz, H.; Chaudhuri, B.; Muller, L.; Seeber, E.; Meijer, L.; Lozach, O.; Vangrevelinghe, E.; Furet, P. *J. Med. Chem.* **2002**, *45*, 1741.
5. Middleton, E., Jr.; Kandaswami, C.; Theoharides, T. C. *Pharmacol. Rev.* **2000**, *52*, 673.
6. Ren, W.; Qiao, Z.; Wang, H.; Zhang, L. *Med. Res. Rev.* **2003**, *29*, 519.
7. (a) López-Lázaro, M. *Curr. Med. Chem.-Anti-Canc. Agents* **2002**, *2*, 691; (b) Casagrande, F.; Darbon, J.-M. *Biochem. Pharmacol.* **2001**, *61*, 1205.
8. Sedlacek, H. H.; Czech, J.; Naik, R.; Kaur, G.; Worland, P.; Losiewicz, M.; Parker, B.; Carlson, B.; Smith, A.; Senderowicz, A.; Sausville, E. *Int. J. Oncol.* **1996**, *9*, 1143.
9. Azevedo, W. F.; Mueller-Dieckmann, H. J.; Schulfe-Gahmen, U.; Worland, P. J.; Sausville, E.; Kim, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2735.
10. The inhibitory activity against CDK2 was measured by referring to Kitagawa, M.; Higashi, H.; Takahashi, I. S.; Okabe, T.; Ogino, H.; Taya, Y.; Hishimura, S.; Okuyama, A. *Oncogene* **1994**, *9*, 2549.
11. Kauffman, J. M.; Aziz, M. A. *J. Heterocycl. Chem.* **1993**, *30*, 1549.
12. (a) Wolfe, J. P.; Wagaw, S.; Buchwald, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 7215; (b) Driver, M. S.; Hartwig, J. F. *J. Am. Chem. Soc.* **1996**, *118*, 7217; (c) Wolfe, J. P.; Wagaw, S.; Marcoux, J-F.; Buchwald, S. L. *Acc. Chem. Res.* **1998**, *31*, 805.
13. Shoemaker, R. H. *Nat. Rev. Cancer* **2006**, *6*, 813.
14. Lu, H.; Debbie, J.; Chang, D. J.; Baratte, B.; Meijer, L.; Schulze-Gahmen, U. *J. Med. Chem.* **2005**, *48*, 737.
15. The coordinate has been deposited in the Protein Data Bank: PDB ID 2DUV.
16. Maligres, P. E.; Waters, M. S.; Fleitz, F.; Askin, D. *Tetrahedron Lett.* **1999**, *40*, 8193.
17. Njoroge, F. G.; Vibulbhan, B.; Rane, D. F.; Bishop, W. R.; Petrin, J.; Patton, R.; Bryant, M. S.; Chen, K.-J.; Nomeir, A. A.; Lin, C.-C.; Liu, M.; King, I.; Chen, J.; Lee, S.; Yaremko, B.; Dell, J.; Lipari, P.; Malkowski, M.; Li, Z.; Catino, J.; Doll, R. J.; Girijavallabhan, V.; Ganguly, A. K. *J. Med. Chem.* **1997**, *40*, 4290.