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Design, synthesis and inhibitory activities of naringenin derivatives on human colon cancer cells

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ABSTRACT

Based on the previous result, several naringenin derivatives modified at position 7 with bulky substituents were designed and synthesized, and their inhibitory effects on HCT116 human colon cancer cells were tested using a clonogenic assay. The half maximal inhibitory concentrations (IC_{50}) of five naringenin derivatives ranged between 1.20 μM and 20.01 μM which are much better than naringenin used as a control. In addition, new structural modification at C-4 of flavanone results in improving both the anti-cancer effect and anti-oxidative effect. In vitro cyclin dependent kinase 2 (CDK2) binding assay was carried out based on the previous results. To elucidate the possible interaction between naringenin derivatives and CDK2, in silico docking study was performed. This result demonstrates the rationale for the different inhibitory activities of the naringenin derivatives. These findings could be used for designing cancer therapeutic or preventive flavanone-derived agents.

Colon cancer is the fourth most commonly diagnosed malignant disease and is prevalent where the people have adopted western diets and also among the elderly. Its symptom is worsening constipation or bloody stool. When such symptom arises, complete treatment is late and 5 years survival is <60%, and thus, a periodic colonoscopy is desired.[1] Main treatment for colon cancer is surgery, radiation, or chemotherapy which is applied as adjuvant therapy in many cases. Chemotherapeutic agents such as oxaliplatin, leucovorin, and irinotecan are known, however, they are associated with severe adverse effects. Therefore, there is a need for more potent and less toxic drugs.[2]

Flavanones, 2-phenylchroman-4-one, belong to the family of flavonoids, many of which are produced as secondary metabolites in the plant kingdom. They have three-ring skeletons, C6-C3-C6, and the rings are referred to as A-, C-, and B-rings, respectively. Their functional groups are attached to the main skeleton through oxygen or carbon linkages.[3] The biological activities of flavonoids depend on the degree of condensation in their structures and the position and number of substitutions, such as hydroxy groups, glucosides, isoprenyl units, homodimers, and heterodimers.[4] Flavanones compared to flavones, 2-phenyl-4*H*-chromen-4-one, have more molecular flexibility due to the absence of a carbon–carbon double bond in the C-ring. In our previous studies, we found that a methoxy or hydroxy substituent at C-7 position of flavanone resulted in better inhibitory effects on HCT116 human colon cancer cell lines.[5] Based on this result, we tried to design flavanone derivatives with bulkier substituents at C-7 position and elucidate their inhibitory effects.

Since the anti-cancer activity of naringenin, 4′,5,7-trihydroxyflavanone against colon cancer cells has been reported, several flavanone derivatives were designed from naringenin.[6] Besides, naringenin plays a key role as an estrogenic substance in humans and as an endogenous regulator in plants.[7] Various flavonoid derivatives modified at C-7 position have been reported, but in flavanone, especially naringenin, derivatives modified at position 7 have rarely been studied.[8,9] Naringenin derivatives designed in this study have a common moiety, so that they may result in small changes in their biological activities. Therefore, one of long-term survival assays, clonogenic assay, was applied here.[10] As a control, naringenin was used.

Naringenin contains three hydroxy groups (Fig. 1). *O*-substitutions can be easily at carried out at the 4′- and 7-hydroxy groups, however, the 5-hydroxy group forms a hydrogen bond (H-bond) with the ketone at C-4, making it less accessible. The treatment of cancer cell lines such as breast cancer cell line Michigan Cancer Foundation 7 (MCF7) with flavanone derivatives shows different effects according to the variation in functional groups on C-4′

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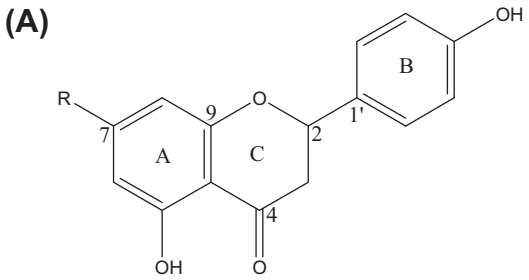
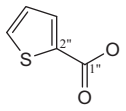
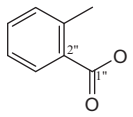
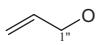
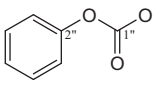


derivative	R
naringenin	OH
N1	
N2	
N3	
N4	
N5	

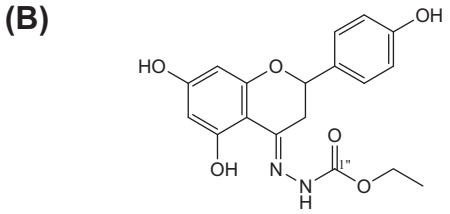


Figure 1. (A) Structures of synthetic naringenin derivatives **N1**–**N5** and (B) the structure of derivative **N6**.

and C-7.[11] In this study, five naringenin derivatives modified at position 7 with thiophenecarboxylate (**N1**), methylbenzoate (**N2**), isobutyrate (**N3**), allyloxy (**N4**), and phenyl carbonate (**N5**) groups were synthesized. All derivatives except 7-allyl substituted naringenin are novel.[12] The aim of this research is to discover potent anti-cancer agents showing better inhibitory effects on human colon cancer cell lines than naringenin whose anti-cancer activity on colon cancer has already been reported.

(±)-Naringenin was purchased from INDOFINE chemical company (Hillsborough, NJ, USA). All the *O*-alkylated naringenin derivatives **N1**–**N5** were efficiently synthesized from the coupling reaction of naringenin and the corresponding acid halides (derivatives **N1**, **N2**, **N3**, and **N5**) or alkyl halide (derivative **N4**) as shown in Scheme 1. Because of the hydrogen bond between hydroxy group at 5 position and ketone group at 4 position, alkylation at 7-position was very selective and gave the 7-*O*-alkylated naringenin products in good yield. Derivative **N6** was obtained from the reaction of naringenin and ethyl carbazate at 110–120 °C in a reasonable yield. The compounds used for synthesis were supplied by a local company in Korea.

All nuclear magnetic resonance (NMR) measurements were performed according to the methods published previously (Supplementary data). [13,14]

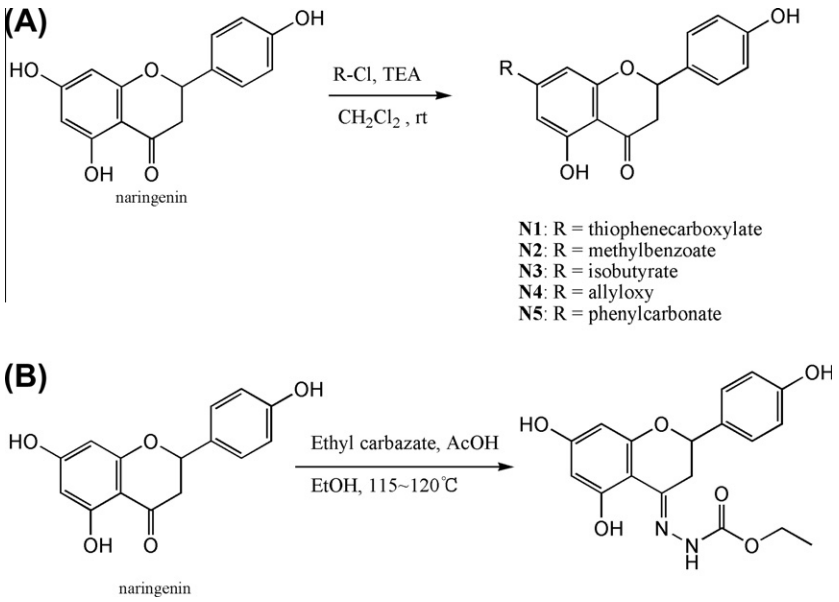
HCT116 human colon cancer cells (Health Protection Culture Collections, Salisbury, UK) were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; HyClone, Logan, UT). HCT116 cells (5×10^3 cells/well) were seeded onto 24-well tissue culture plates (BD Falcon, Franklin Lakes, NJ) in the absence or presence of different concentrations of six naringenin derivatives and naringenin, and incubated for 7 days.[10] HCT116 cells were tested on four plates treated with 0, 10, 20, and 40 μM of samples (Fig. 2). The colonies that formed were fixed with 6% glutaraldehyde and stained with 0.1% crystal violet, as described previously.[15] The clonogenic survival densities were measured using the densitometry (Multi-Guage, Fujifilm, Japan) and their half maximal inhibitory concentrations (IC_{50}) were calculated using SigmaPlot software (SYSTAT, Chicago, IL).

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay was used to screen for anti-oxidative effects. DPPH radical-scavenging effects were tested according to the method reported previously.[16]

In order to confirm whether naringenin derivatives including naringenin inhibit cyclin dependent kinase 2 (CDK2), in vitro CDK2 binding assay was performed using EMD Millipore's KinaseProfiler service assay protocol (EMD Millipore Corporation, Billerica, MA).

All docking experiments were carried out on an Intel Core 2 Quad Q6600 (2.4 GHz) Linux PC with Sybyl 7.3 (Tripos, St. Louis, MO).[17] Of many crystallographic structures of CDK2 deposited in protein data bank, 2r3j.pdb was selected because its crystal structure was determined with an inhibitor with bicyclic cores and the structure of the ligand, 3-bromo-5-phenyl-*N*-(pyridin-3-ylmethyl)pyrazolo[1,5-a]pyrimidin-7-amine, was similar to naringenin derivatives modified at position 4 or 7 (Supplementary data Fig. 1).[18] It was originated from *Homo sapiens* and its resolution was 1.65 Å. Its apo-protein without its ligand was obtained from energy minimization using the Sybyl/FlexX Single Receptor Module. Since the Sybyl program provides flexible docking procedure, the binding pocket was defined first. The docking radius was set to 6.5 Å and the residues for docking were selected; Ile10, Ala31, Glu81, Phe82, Leu83, His84, Gln131, and Leu134. Naringenin derivatives including naringenin were used as ligands. Their three dimensional (3D) structures were constructed based on the X-ray crystallographic structure of naringenin contained in chalcone isomerase as a ligand, 1eyq.pdb deposited in the protein data bank and subjected to energy minimization using the molecular mechanics algorithms provided by Sybyl 7.3.[19] Minimization was stopped upon convergence of the total energy (0.05 kcal/mol Å). Systematic conformational searches were performed because rotational bonds existed in the compounds.[15] The conformers with the lowest energy were selected for the docking. They were docked into apo-protein CDK2. The docking process was iterated 30 times and 30 docking poses were obtained. Among them, the results showing the similar docking pose comparing to the ligand contained in 2r3j.pdb were selected. The residues surrounding the derivatives were analyzed using LigPlot provided by the European Bioinformatics Institute.[20] All 3D images were constructed using PyMOL program (The PyMOL Molecular Graphics System, Version 1.0r1, Schrödinger, LLC.).

The naringenin derivatives synthesized here were characterized using NMR spectroscopy and mass spectrometry (MS). The NMR data of naringenin, which have been reported previously, could be applied to the derivative **N1**, 5-hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl thiophene-2-carboxylate, since the structure is similar with the exception of the thiophene-2-carboxylate moiety.[21] The ^{13}C peaks in the B- and C-rings were easily assigned (Supplementary data Fig. 2). Previous reports did not show the



Scheme 1. (A) Synthesis of naringenin derivatives **N1**–**N5** modified at C-7 and (B) of ethyl carbamate derivative **N6** modified at C-4.

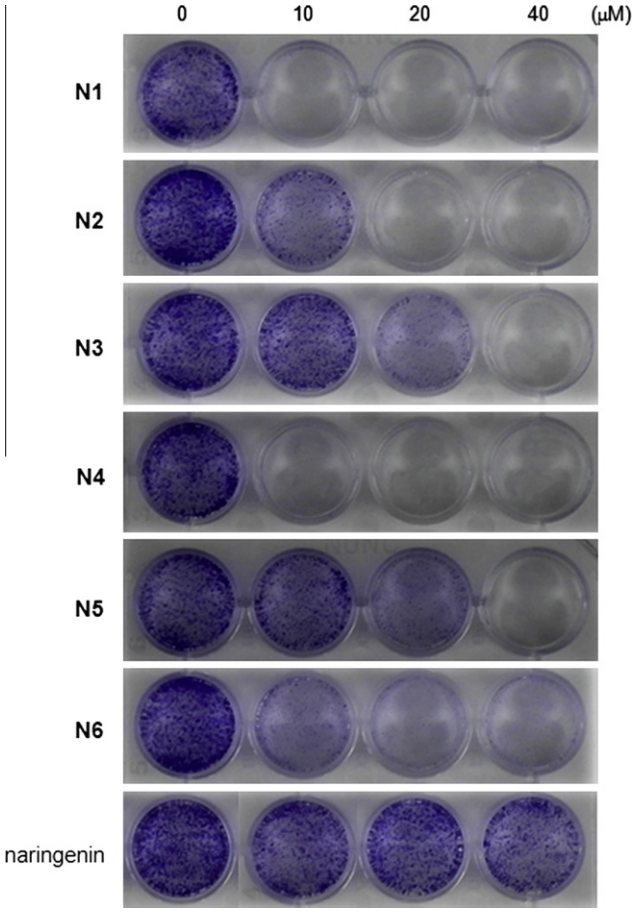


Figure 2. The effects of naringenin derivatives, **N1**–**N6** including naringenin on the clonogenicity of HCT116 human colon cancer cells. HCT116 cells (5×10^3 cells/well) were plated and cultured for seven days in the absence or presence of different concentrations of each derivative. Similar results were obtained from two other independent experiments.

^{1}H chemical shifts of 4′-OH and 5-OH, but we were able to assign those (Supplementary data Fig. 3). Two hydroxy protons were observed at 9.65 and 12.01 ppm. The former was long-range coupled to two ^{13}C peaks at 115.4 and 158.0 ppm in the HMBC spectrum, which were assigned as C-3′/C-5′ and C-4′, respectively (Supplementary data Fig. 4). Thus, the former proton was assigned as 4′-OH and the later as 5-OH. The protons and carbons in the A-ring showed different chemical shifts compared to naringenin because of the 7-thiophene-2-carboxylate group. The proton peak of 5-OH was long-range coupled to the doublet carbon peak at 102.8 ppm and the singlet peak at 106.1 ppm. They were assigned to C-6 and C-10, respectively. The ^{1}H peak at 6.50 ppm, which was directly attached to C-6 in the HMQC spectrum (Supplementary data Fig. 5), showed a cross peak with the ^{1}H peak at 6.51 ppm in the COSY spectrum (Supplementary data Fig. 6). Thus, it was assigned as H-8. Since the ^{13}C peak at 162.5 ppm in HMBC was long-range coupled to 5-OH, it was assigned as C-7. Three protons at 7.29, 8.00, and 8.07 ppm were correlated with each other in COSY and were assigned as the protons of the 7-thiophene-2-carboxylate group. Here, the proton at 7.29 ppm showed a doublet of doublet coupling; thus, it was assigned as H-4″. Because H-6 at 6.50 ppm was long-range coupled to the ^{13}C peak at 159.2 ppm in HMBC, it was assigned as C-1″ of the 7-thiophene-2-carboxylate group. The ^{13}C peak at 128.9 ppm showed long-range couplings with H-6 and H-8 in HMBC; thus, it was assigned as C-2″. Likewise, the ^{13}C peak at 157.6 ppm showed long-range coupling with H-6 and was assigned as C-9. A singlet carbon at 162.4 ppm was assigned as C-5. The chemical shift of C-3″ in 7-thiophene-2-carboxylate should be shifted farther downfield than that of C-5″. Therefore, the first of two undetermined ^{13}C peaks at 135.7 and 135.9 ppm was assigned as C-5″. To confirm the result, high-resolution mass spectrometry (HRMS) was carried out on a Hybrid LC-Quadrupole-TOF Tandem Mass Spectrometer (Applied Biosystems, Carlsbad, CA). The calculated mass and the experimental mass agree with each other (Supplementary data Fig. 7). The spectral data and chemical yield of derivative **N1** are listed in the references and notes section.[22]

Derivatives **N2**, 5-hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl 2-methylbenzoate, **N3**, 5-hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl isobutyrate, **N4**, 7-(allyloxy)-5-hydroxy-2-(4-hydroxyphenyl)chroman-4-one, and **N5**, 5-hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl phenyl carbonate, were determined based on the same procedure as derivative **N1**, and their NMR assignments and their HRMS data are listed in the references and notes section.[23–27]

The IC_{50} values of five derivatives (**N1**–**N5**) on HCT116 human colon cancer cells were 1.20, 6.03, 15.87, 1.91, and 20.01 μM, respectively. Molecular volumes increased from the substituents at position 7 compared to naringenin were calculated using the Sybyl 7.3 program (Tripos, St. Louis, MO). The volume differences between naringenin and derivatives **N1**–**N5** were 69.2, 96.7, 61.2, 41.1, and 86.7 $Å^3$, respectively. This result does not show a correlation with the biological activities mentioned above. However, the expectation based on our previous results may be proved from the current results,[5] because the IC_{50} value obtained from the treatment of naringenin was 36.75 μM. As a result, all derivatives showed much better activities than naringenin, and the bulky substituent at C-7 position of flavanone can increase the inhibitory effect on HCT116 colon cancer cells.

Like most flavonoids, the common biological activity of flavanone is an anti-oxidative effect.[28,29] In this experiment, radical-scavenging effects were tested for the five naringenin derivatives. The values for derivatives **N1**–**N5** were 11.2%, 14.2%, 15.4%, 20.7%, and 15.9%, respectively, compared to 89.1% for vitamin C. Under the same conditions, naringenin showed 59.3% anti-oxidative effect. Thus, modification at position 7 with thiophenecarboxylate, methylbenzoate, isobutyrate, allyloxy, and phenyl carbonate groups decreased the radical-scavenging effects drastically. As mentioned above, because the 5-hydroxy group of naringenin forms a H-bond with the ketone at C-4, *O*-substitution at C-5 is not easy. We tried to prepare an alternative substitution and designed another naringenin derivative **N6** modified at position 4 in which the ketone group at C-4 was substituted with *N*-methylene ethyl carbamate (Fig. 1B). Its structure was determined based on the same procedure as other derivatives. Its scavenging effect was 70.6% which shows that a modification of the ketone group at C-4 increased the radical-scavenging effect. The same clonogenic assay for the naringenin derivative **N6** was performed and its IC_{50} value was 4.35 μM as shown in Figure 2. This result is significant for suggesting new structural modification at C-4 and C-5 to increase both the anti-cancer effect and anti-oxidative effect.

From the cell based clonogenic assay, it was concluded that naringenin derivatives inhibit effectively HCT116 cancer cells, but their target protein in the cell was not revealed. Cell proliferation is tightly regulated by cell cycle progression, which is divided into four distinct phases, G1 (Gap1), S (DNA synthesis), G2 (Gap2) and M (mitosis) phases. Cell cycle progression is controlled through the actions of various classes of cyclin and cyclin-dependent protein kinase (CDK) complexes. During early G1 progression, CDK4 and CDK6 are associated with D-type cyclins (D1, D2, and D3). In late G1, CDK2 and E-type cyclin (E1 and E2) complexes are required for entry into S phase. As cells enter into S phase, CDK2 and A-type cyclin (A1 and A2) complexes play important roles in S phase progression. As the cell moves into G2 phase, CDK1 and B-type cyclin (B1 and B2) complexes regulate G2/M transition and M phase progression. Previously, we and others have demonstrated that naringenin derivatives block G1 cell cycle progression in HCT116 colon cancer cells[5,30] and in vascular smooth muscle cells.[31] Since G1 cell cycle progression is controlled by CDK2 and the inhibition of CDK2 alone may be sufficient to induce G1 cell cycle arrest,[32] we tried to perform in vitro CDK2 binding assay. CDK2/cyclinE was prepared in 8 mM 3-(*N*-morpholino)propanesulfonic acid buffer (MOPS, pH7.0, 0.2 mM EDTA, 0.1 mg/ml histone H1, 10 mM MgAcetate, γ-^{33}P-ATP) and its specific activity was 500 cpm/pmol. Its enzymatic activity was detected using scintillation counting. When any inhibitor was not added into the enzyme, scintillation count was 12,637 whose activity was set to 100%. Ten micromolar of ATP was added into the enzyme. Naringenin derivatives were provided as substrates. All experiments were iterated 3 times and standard deviation was within 5%. When naringenin was added into CDK2, the enzyme activity was 85.1% (scintillation count, 10,749). That is, naringenin showed the 14.9% inhibitory effect on the enzymatic activity of CDK2 at 10 μM. The inhibitory activities for derivatives **N1**–**N6** were 83.6%, 48.9%, 42.1%, 84.0%, 42.0%, and 62.3%, respectively. This result does show a correlation with the IC_{50} values of derivatives **N1**–**N6** on HCT116 human colon cancer cells. Likewise, six derivatives showed better inhibitory activities than naringenin in in vitro CDK2 binding assay as well as cell based assay.

To elucidate the possible interaction between naringenin derivatives including naringenin itself and CDK2, molecular modeling

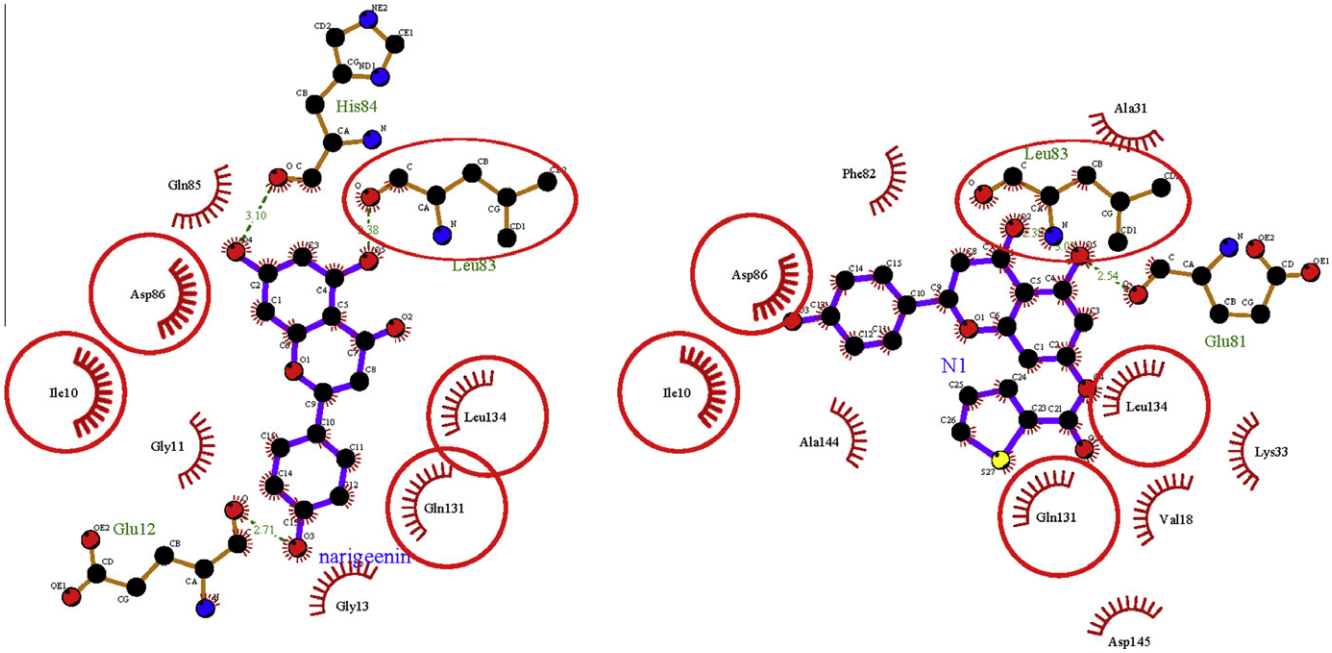


Figure 3. The LigPlot analyses of (left) naringenin docked into CDK2 (2r3j.pdb) and (right) the derivative **N1** modified at position 7 with thiophenecarboxylate where red half circles and green dashed lines denote hydrophobic interactions and H-bonds, respectively.

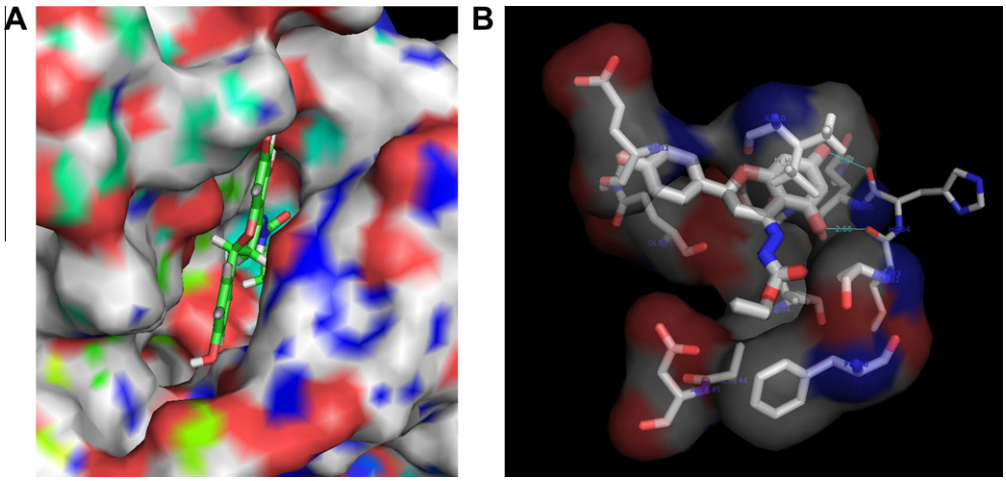


Figure 4. The 3D images of (A) naringenin derivative **N6** modified at position 4 in which the ketone group at C-4 was substituted with N-methylene ethyl carbamate docked into CDK2 (2r3j.pdb), and (B) H-bonds between the CDK2 protein and derivative **N6** and residues participating in hydrophobic interactions. The images were constructed using PyMOL.

was carried out. Naringenin docked well into the CDK2 apo-protein and its docking score was −11.97 kcal/mol (Supplementary data Fig. 8A). Seven residues residing in the binding site of the CDK2 ligand (protein data bank accession id: 2r3j.pdb) had hydrophobic interactions with naringenin: Ile10, Gly11, Gly13, Gln85, Asp86, Gln131, and Leu134. Three residues, Glu12, Leu83, and His84, interacted with naringenin via H-bonds (Supplementary data Fig. 8B). Naringenin derivatives modified at position 7, **N1**–**N5**, docked well into the CDK2 apo-protein too and their docking scores were −11.50, −12.80, −9.99, −12.87, and −13.85 kcal/mol, respectively (Supplementary data Figs. 9A–E). The LigPlot analysis of derivative **N1** modified at position 7 with thiophenecarboxylate provided information about the residues forming H-bonds and hydrophobic interactions with the ligand as shown in Figure 3; five residues, Ile10, Leu83, Asp86, Gln131, and Leu134 were found in both naringenin and **N1**. The docking poses of two ligands docked into CDK2 were different with each other as shown in Figure 3.

While naringenin formed three H-bonds at 4′-O, 5-O, and 7-O with three residues such as Glu12, Leu83, and His84, respectively, derivative **N1** formed three H-bonds at 4′-O, 5-O, and 7-O with two residues such as Leu83 (two H-bonds) and Glu81. The thiophenecarboxylate group of derivative **N1** was placed inside the hole formed by Ile10, Gly11, and Glu12. Docking poses of derivatives **N2**–**N5** docked into CDK2 showed similar results as that of derivative **N1**. As shown in Supplementary data Figs. 9C and E, isobutyrate group of derivative **N3** and phenyl carbonate group of **N5**

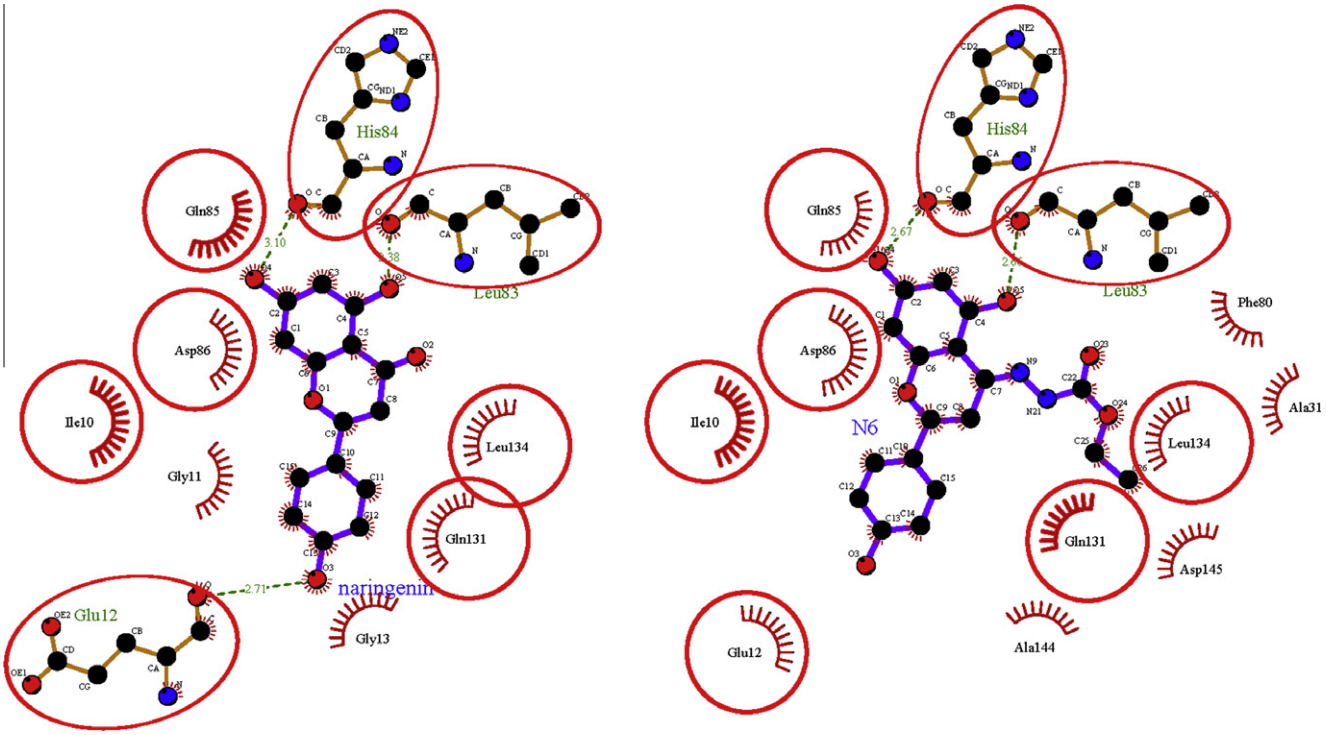


Figure 5. The LigPlot analyses of (left) naringenin docked into CDK2 (2r3j.pdb) and (right) the derivative **N6** modified at position 4 where red half circles and green dashed lines denote hydrophobic interactions and H-bonds, respectively.

were not positioned inside the hole. The docking poses obtained here may be related with their biological activities. While the IC_{50} values of three derivatives **N1**, **N2**, and **N4** on HCT116 cancer cells were lower than 7 μM, those of **N3** and **N5** were upper than 15 μM. Naringenin derivative **N6** modified at position 4 in which the ketone group at C-4 was substituted with *N*-methylene ethyl carbamate docked CDK2 well too as shown in Figure 4A. Its docking score was −14.82 kcal/mol. There were two H-bonds between the protein and derivative **N6**, and ten residues participate in hydrophobic interactions (Fig. 4B).

Eight residues, Ile10, Glu12, Leu83, His84, Gln85, Asp86, Gln131, and Leu134 were observed near both naringenin and derivative **N6**. While naringenin formed three H-bonds at 4′-O, 5-O, and 7-O with Glu12, Leu83, and His84, respectively as mentioned above, derivative **N6** formed two H-bonds at 5-O and 7-O with Leu83 and His84, respectively (Fig. 5). However, while seven residues participated in the hydrophobic interactions with naringenin, ten residues did with derivative **N6**. Although less H-bonds were observed for derivative **N6**, more hydrophobic interactions existed with CDK2. In addition, as shown in Figure 4A, the *N*-methylene ethyl carbamate group substituted at position 4 in derivative **N6** docked inside the binding pocket. These results may explain why derivative **N6** (4.35 μM) showed stronger inhibitory effect than naringenin (36.75 μM).

In silico docking study performed here demonstrates the rationale for the different inhibitory activities of six naringenin derivatives including five novel compounds. Their inhibitory effects on HCT116 human colon cancer cells ranged between 1.20 μM and 20.01 μM which are much better than naringenin used as a control. Besides, in CDK2 binding assay, six derivatives showed better inhibitory activities than naringenin too. The inhibitory effects of flavanones on colon cancer cells have been reported,[5] but their structures are quite different with the naringenin derivatives synthesized here. The study on in vitro CDK2 binding assay of flavanones has never been reported yet except Western blot analysis on two flavanone derivatives, silibinin and tomentodiplacone B.[33,34] In conclusion, the current results of cell-based clonogenic assay on HCT116 colon cancer cells, in vitro CDK2 binding assay, and in silico CDK2 docking study for naringenin derivatives are the first time. As expected based on the previous results,[5] the bulky substituent at C-7 position of flavanone can increase the inhibitory effect on human colon cancer cells as well as on CDK2 activity. In addition, new structural modification at C-4 results in improving both the anti-cancer effect and anti-oxidative effect. These findings could be used for designing cancer therapeutic or preventive flavanone-derived agents.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.130.

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22. *Spectral data of 5-hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl thiophene-2-carboxylate (**N1**).* Color: yellow sticky liquid; Yield: 63.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H, 5-OH), 9.65 (s, 1H, 4′-OH), 8.07 (d, 1H, H-3″, *J* = 4.0 Hz), 8.00 (d, 1H, H-5″, *J* = 3.8 Hz), 7.35 (d, 2H, H-2′/H-6′, *J* = 8.4 Hz), 7.29 (dd, 1H, H-4″, *J* = 3.8, 4.0 Hz), 6.82 (d, 2H, H-3′/H-5′, *J* = 8.4 Hz), 6.51 (d, 1H, H-8, *J* = 2.0 Hz), 6.50 (d, 1H, H-6, *J* = 2.0 Hz), 5.58 (dd, 1H, H-2, *J* = 2.6, 12.9 Hz), 3.42 (dd, 1H, H-3, *J* = 12.9, 17.2 Hz), 2.80 (dd, 1H, H-3, *J* = 2.6, 17.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 198.4 (C-4), 162.5 (C-7), 162.4 (C-5), 159.2 (C-1″), 158.0 (C-4′), 157.6 (C-9), 135.9 (C-3″), 135.7 (C-5″), 131.4 (C-1′), 128.9 (C-2″), 128.5 (C-4″), 128.5 (C-2′/C-6′), 115.4 (C-3′/C-5′), 106.1 (C-10), 102.8 (C-6), 101.9 (C-8), 72.0 (C-2), 42.4 (C-3); HRMS (*m*/*z*): Calcd for $C_{17}H_{16}O_4$ $(M)^+$: 405.0709; Found: 405.0407 (Supplementary data Fig. 7).
23. *Spectral data of 5-hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl 2-methylbenzoate (**N2**).* Color: yellow sticky liquid; Yield: 66.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (s, 1H, 5-OH), 9.64 (s, 1H, 4′-OH), 8.03 (d, 1H, H-4″, *J* = 7.4 Hz), 7.56 (dd, 1H, H-5″, *J* = 7.4, 7.6 Hz), 7.38 (m, 1H, H-6″), 7.38 (m, 1H, H-7″), 7.37 (d, 2H, H-2′/H-6′, *J* = 8.6 Hz), 6.82 (d, 2H, H-3′/H-5′, *J* = 8.6 Hz), 6.53 (d, 1H, H-6, *J* = 2.1 Hz), 6.52 (d, 1H, H-8, *J* = 2.1 Hz), 5.60 (dd, 1H, H-2, *J* = 2.9, 13.1 Hz), 3.42 (dd, 1H, H-3, *J* = 13.1, 17.2 Hz), 2.81 (dd, 1H, H-3, *J* = 2.9, 17.2 Hz), 2.56 (s, 3H, 2″-CH3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 198.4 (C-4), 164.3 (C-1″), 162.4 (C-5), 162.4 (C-7), 158.2 (C-9), 158.0 (C-4′), 140.5 (C-3″), 133.3 (C-5″), 132.0 (C-7″), 131.0 (C-4″), 128.6 (C-2′/C-6′), 128.5 (C-1′), 127.9 (C-2″), 126.3 (C-6″), 115.3 (C-3′/C-5′), 106.0 (C-10), 103.0 (C-6), 102.1 (C-8), 79.0 (C-2), 42.4 (C-3), 21.3 (3″–CH_3); HRMS (*m*/*z*): Calcd. for $C_{17}H_{16}O_4$ $(M)^+$: 413.1001; Found: 413.1005 (Supplementary data Fig. 10).
24. *Spectral data of 5-hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl isobutyrate (**N3**).* Color: light yellow sticky liquid; Yield: 68.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H, 5-OH), 10.87 (bs, 1H, 4′-OH), 7.57 (d, 2H, H-2′/H-6′, *J* = 8.5 Hz), 7.17 (d, 2H, H-3′/H-5′, *J* = 8.5 Hz), 5.94 (d, 1H, H-6, *J* = 2.0 Hz), 5.91 (d, 1H, H-8, *J* = 2.0 Hz), 5.59 (dd, 1H, H-2, *J* = 2.1, 13.0 Hz), 3.26 (dd, 1H, H-3, *J* = 13.0, 17.1 Hz), 2.84 (m, 1H, H-2″), 2.81 (dd, 1H, H-3, *J* = 2.1, 17.1 Hz), 1.23 (d, 6H, 2″-CH3, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.0 (C-4), 175.1 (C-1″), 166.9 (C-7), 163.6 (C-5), 162.8 (C-9), 150.8 (C-4′), 136.3 (C-1′), 128.0 (C-2′/C-6′), 122.0 (C-3′/C-5′), 101.9 (C-10), 96.1 (C-6), 95.2 (C-8), 78.2 (C-2), 42.2(C-3), 33.4 (C-2″), 18.8 (2″–CH_3); HRMS (*m*/*z*): Calcd for $C_{17}H_{16}O_4$ $(M)^+$: 365.1001; Found: 365.1005 (Supplementary data Fig. 10).
25. *Spectral data of 7-(allyloxy)-5-hydroxy-2-(4-hydroxyphenyl)chroman-4-one (**N4**).* Color: yellow sticky liquid; Yield: 59.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (s, 1H, 5-OH), 9.60 (s, 1H, 4′-OH), 7.32 (d, 2H, H-2′/H-6′, *J* = 8.5 Hz), 6.81 (d, 2H, H-3′/H-5′, *J* = 8.5 Hz), 6.15 (d, 1H, H-8, *J* = 2.2 Hz), 6.09 (d, 1H, H-6, *J* = 2.2 Hz), 5.98 (m, 1H, H-2″), 5.47 (dd, 1H, H-2, *J* = 2.9, 12.9 Hz), 5.38 (dd, 1H, H-3″, *J* = 1.3, 17.3 Hz), 5.26 (dd, 1H, H-3″, *J* = 1.3, 10.5 Hz), 4.61 (m, 1H, H-1″), 3.28 (dd, 1H, H-3, *J* = 12.9, 17.1 Hz), 2.72 (dd, 1H, H-3, *J* = 2.9, 17.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.9 (C-4), 166.2 (C-7), 163.1 (C-5), 162.8 (C-9), 157.7 (C-4′), 132.8 (C-2″), 128.6 (C-1′), 128.4 (C-2′/C-6′), 118.0 (C-3″), 115.1 (C-3′/C-5′), 102.6 (C-10), 95.2 (C-6), 94.3 (C-8), 78.6 (C-2), 68.7 (C-1″), 42.0 (C-3); HRMS (*m*/*z*): Calcd for $C_{17}H_{16}O_4$ $(M)^+$: 335.0895; Found: 335.0892 (Supplementary data Fig. 10).

26. *Spectral data of 5-hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl phenyl carbonate (**N5**).* Color: yellow sticky liquid; Yield: 48.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H, 5-OH), 10.88 (s, 1H, 4′-OH), 7.63 (m, 2H, H-4″/H-6″), 7.50 (m, 2H, H-2′/H-6′), 7.46 (m, 2H, H-3″/H-7″), 7.45 (m, 2H, H-3′/H-5′), 7.33 (dd, 1H, H-5″, J = 7.3, 7.7 Hz), 5.98 (d, 1H, H-8, J = 2.1 Hz), 5.95 (d, 1H, H-6, J = 2.1 Hz), 5.63 (dd, 1H, H-2, J = 2.9, 12.8 Hz), 3.28 (dd, 1H, H-3, J = 12.8, 17.1 Hz), 2.83 (dd, 1H, H-3, J = 2.9, 17.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.8 (C-4), 166.7 (C-7), 163.5 (C-5), 162.6 (C-9), 151.5 (C-2″), 150.6 (C-4′), 150.6 (C-1″), 136.9 (C-1′), 129.7 (C-2′/C-6′), 128.1 (C-4″/C-6″), 126.5 (C-5″), 121.4 (C-3″/C-7″), 121.2 (C-3′/C-5′), 101.7 (C-10), 96.0 (C-6), 95.1 (C-8), 77.8 (C-2), 42.0 (C-3); HRMS (m/z): Calcd for $C_{17}H_{16}O_4$ (M)$^+$: 415.0794; Found: 415.0791 (Supplementary data Fig. 10).
27. *Spectral data of (E)-ethyl 2-(5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-ylidene)hydrazinecarboxylate (**N6**).* Color: light yellow sticky liquid; Yield: 83.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.63 (s, 1H, 5-OH), 10.50 (s, 1H, 7-OH), 9.74 (bs, 1H, NH), 9.74 (bs, 1H, 4′-OH), 7.29 (d, 2H, H-2′/H-6′, J = 8.5 Hz), 6.81 (d, 2H, H-3′/H-5′, J = 8.5 Hz), 5.91 (d, 1H, H-6, J = 2.2 Hz), 5.87 (d, 1H, H-8, J = 2.2 Hz), 5.06 (dd, 1H, H-2, J = 11.7, 2.9 Hz), 4.16 (m, 2H, H-2″), 3.23 (dd, 1H, H-3, J = 2.9, 17.1 Hz), 2.78 (dd, 1H, H-3, J = 11.7, 17.1 Hz), 1.24 (m, 3H, H-3″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.9 (C-5), 160.3 (C-7), 158.6 (C-4′), 157.6 (C-9), 154.1 (C-1″), 150.3 (C-4), 129.9 (C-1′), 128.0 (C-2′/C-6′), 115.2 (C-3′/C-5′), 98.3 (C-10), 96.8 (C-6), 95.0 (C-8), 75.8(C-2), 61.0 (C-2″), 31.7 (C-3), 14.6 (C-3″); HRMS (m/z): Calcd for $C_{17}H_{16}O_4$ (M)$^+$: 381.1062; Found: 381.1064 (Supplementary data Fig. 10).

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