

Relationship between Structure and Antiproliferative Activity of 1-Azaflavanones

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Abstract. The synthesis of 19 derivatives of 2-phenyl-3,4-dihydroquinolin-4(1H)-one, as aza analogs of flavanones, was carried out and these compounds were further screened for their antiproliferative activity toward HL60 promyelocytic leukemia cells. In comparison with flavanone the replacement of C-ring ether oxygen atom with a nitrogen atom potentiated activity by more than 100-fold. It was suggested that the aromaticity of the B-ring contributes greatly to the activity of 1-azaflavanones.

Flavonoids, one of the prevalent components of the human diet (1), are present in significant amounts in many commonly consumed vegetables and fruits, especially in citrus plants (2-4). Many clinical trials and meta-analyses have suggested positive associations between flavonoid intake and human health (5, 6). Differences in the chemical structures of flavonoid subclasses, such as flavones, flavanones, flavan-3-ols, and isoflavones, alter both their biological efficacy and bioavailability (7, 8). Numerous biological activities have been attributed to flavonoids, including antiproliferative (9, 10), antioxidative (11), anti-inflammatory (12, 13) and differentiation-inducing activities (14, 15). Although considerable progress in the research for new biologically active compounds has been achieved, the study of bioactivity remains significant due to the continuous demand for novel compounds.

Recently several aza-analogs have been synthesized and screened for their biological properties. 2-Phenyl-2,3-dihydroquinolin-4(1H)-ones (PDQOs), 1-aza analogs of flavanones, attract considerable attention, not only as antimetastatic antitumor agents (16, 17), but also as building blocks for creating further diversity in the synthesis of other

active compounds (18-20). Although many efforts have been made to obtain new PDQOs/1-azaflavanones (21), little is known about their biological activity.

In the course of our investigation on the relationship between flavanone structures and their antiproliferative activity, the role of the C-ring ether moiety drew our attention. In order to determine how the ring ether moiety contributes to the antiproliferative activity of flavanone, we synthesized 19 PDQOs/1-azaflavanones and determined their activities.

Materials and Methods

General procedures. Chemicals and solvents from commercial sources were used without further purification, unless specified. Reactions were carried out under argon and monitored by thin-layer chromatography on silica gel (mesh size 60, F₂₅₄) with visualization under UV light. Standard and flash column chromatography employed silica gels (Merck 60, 230-400 mesh). Experimental procedures were not optimized. Nuclear magnetic resonance (NMR) spectra were recorded on a 400-MHz JEOL ECP-400 spectrometer, and chemical shifts values are expressed in ppm (δ) in relation to the residual 1H signal of the solvents. Unless otherwise specified, compounds were dissolved in ²HCCl₃. Electrospray and atmospheric pressure chemical ionization mass spectrometry were performed on a Thermo Exactive instrument.

Synthesis of 1-azaflavanone.

General procedure for synthesis of PDQOs (Figure 1, 1-8, 11-19). To a suspension of sodium amide (3.29 g, 84.3 mmol) in dry tetrahydrofuran (THF) (120 ml) 2-aminoacetophenone (2.00 g, 14.8 mmol) and then benzaldehyde (1.88 g, 17.8 mmol) were added. The reaction mixture was stirred at room temperature for 30 min and then was poured into iced water. The mixture was neutralized with dilute sulfuric acid and was then extracted with dichloromethane and washed with aqueous sodium hydrogen carbonate and water. The solvent was removed under reduced pressure, and the residue was chromatographed over a silica gel [hexane/ethyl acetate (EA); 8:2] to obtain 2'-aminochalcone as a thick yellow oil. To a solution of 2'-aminochalcone (2.6 g, 11.7 mmol) in 150 ml of ethanol, 10 ml of 50% sodium acetate in water were added, and the mixture was heated to reflux under argon for 72 h. After being cooled to room temperature, the mixture was diluted with water, and extracted with dichloromethane (DCM). The organic phase was washed with

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saturated brine and dried over anhydrous MgSO_4 . The solvent was removed under reduced pressure, and the residue was chromatographed over a silica gel (hexane/EA; 9:1) to obtain the desired products as a yellow solid.

Preparation of (3-methylbut-2-en-1-yl)oxy derivatives of 2-phenyl-2,3-dihydroquinolin-4(1H)-one (9 and 10). To a solution of **5** (0.08 g, 0.344 mmol) in 20 ml acetone, K_2CO_3 (0.183 g, 1.00 mmol) and 1-bromo-3-methylbut-2-ene (0.075 g, 0.501 mmol), were added and the reaction mixture was refluxed for 18 h. The organic solvent was removed under reduced pressure and the residue was partitioned between DCM and water. The organic phase was dried over anhydrous MgSO_4 and the solvent was removed under reduced pressure. The residue was chromatographed over a silica gel (hexane/EA; 5:5) to obtain the desired product as a yellow solid.

Cell proliferation assay. HL60 cells were obtained from the RIKEN Bioresource Center (Tsukuba, Japan), and were maintained in RPMI 1640 medium, supplemented with 10% fetal bovine serum. The level of cellular proliferation for HL60 cells grown in 96-well microplates, was measured by using alamar blue (Life Technologies Ltd., Japan, Tokyo). To each well 100 μl of HL60 cell suspension (1.0×10^4 cells/100 μl) were inoculated and then 100 μl of medium containing serial dilution of the samples were assayed. After 3 days of incubation, 20 μl of alamar blue were aseptically added to each well, and cells were incubated for approximately 20 h. Inhibition of cellular proliferation (as a percentage that of untreated control) was calculated with the following equation;

Inhibition of cellular proliferation (%)

$$= 100 - \frac{[(A_{570} - A_{595}) \text{ of test agent dilution}] - [(A_{570} - A_{595}) \text{ of blank}]}{[(A_{570} - A_{595}) \text{ of positive growth control}] - [(A_{570} - A_{595}) \text{ of blank}]}$$

where A_{570} and A_{595} are the absorbance at 570 nm and 595 nm, respectively.

Cell cycle analysis. The cell cycle distribution was monitored by flow cytometry. Cells were treated with samples at a concentration of 0.40 μM for 24 h, and were then fixed with 70% ethanol, for at least 2 h at -20°C . Fixed cells were stained with a solution containing 20 $\mu\text{g/ml}$ propidium iodide, 200 $\mu\text{g/ml}$ RNase A (Sigma-Aldrich Japan, Tokyo), and 0.1% Triton X-100 for 20 min in the dark. Cell cycle distribution was analyzed using BD FACSCalibur flow cytometry (BD Japan, Tokyo).

Determination of the apoptotic DNA ladder. Fixed cells were centrifuged, resuspended in 100 μl of DNA extraction buffer (0.2 M Na_2HPO_4 , 0.1 M citric acid and 0.5% Triton X-100, pH 7.8), and were then incubated for 1 h at 37°C . After centrifugation, the supernatant was collected and incubated with 5 μl RNase A solution (100 mg/ml) for 1 hour at 37°C , followed by digestion with 5 ml proteinase K solution (20 mg/ml) for 1 h at 37°C . After electrophoresis of the extracted DNA, the gels were stained and photographed.

Results and Discussion

In this article, 19 PDQOs as 1-azaflavanone analogs which had a modified B-ring moiety, were synthesized from the cyclization of substituted 2'-aminochalcones, that were prepared from Claisen-Schmidt condensations between 2-aminoacetophenone and the substituted benzaldehydes (13). A base-catalyzed cyclization of the 2'-aminochalcones *via* conjugate addition of nitrogen nucleophiles to α,β -unsaturated carbonyl compounds was noteworthy due to its simplicity, low cost and efficiency. The antiproliferative activity of PDQOs/1-azaflavanones was determined using promyelocytic leukemic HL60 cells, based on alamar blue assay (14). The results are summarized in Table II.

Among the compounds tested, **1** showed the most potent activity [half maximal inhibitory concentration (IC_{50})=0.40 μM], followed by the 2'-methyl derivative (IC_{50} =0.50 μM for **2**) of PDQO/1-azaflavanone. In comparison with flavanone, the replacement of the C-ring ether oxygen atom of flavanone with a nitrogen atom increased its activity by more than 100-fold.

The importance of the C-ring NH group existing as a free form was supported by the findings that more potent activity was found in the 2'-methyl derivative (**2**), rather than the 2'-hydroxyl derivative (IC_{50} =4.8 μM , **5**), whose hydroxyl group can act both as an acceptor and as a donor of hydrogen bonding with the C-ring NH group.

The antiproliferative activity was significantly reduced when an alkyl group was attached at the 4' position (IC_{50} =5.4 μM for **3** and >400 μM for **4**) of PDQOs/1-azaflavanones. In addition, alkylation of 4'-hydroxyl derivative (IC_{50} =1.8 μM for **6**) remarkably lowered the activity (IC_{50} >400 μM for **8** and 63 μM for **10**). Thus we speculated that the size of the B-ring moiety plays an important role in antiproliferative activity and that the binding pocket for this portion of the PDQO/1-azaflavanone molecule is quite small. However, replacement of the 4' hydrogen atom of **1** with a fluorine atom (**13**) significantly reduced its activity, although these derivatives have a similar size B-ring moiety. Similarly, the activity of the 4'-methyl derivative (IC_{50} =5.4 μM for **3**) was diminished by replacement with a trifluoromethyl group (IC_{50} =>400 μM for **14**). Furthermore, the introduction of any functionality on the B-ring of the 1-azaflavanone analog, such as methyl, hydroxyl, alkoxyl and halogen, resulted in reduction of activity, demonstrating the importance of the aromaticity of the B-ring moiety. The decrease in antiproliferative activity seems to be correlated with reduction of B-ring aromaticity of PDQOs/1-azaflavanones caused by attachment of electron-donating and/or -withdrawing groups.

Bioisosteric replacement of the phenyl B-ring in PDQOs/1-azaflavanones with various heteroaromatic rings also led to decreased activity. Specifically, activity of **15** and **17**, both of which have a pyridyl group instead of phenyl group as the B-ring moiety, was completely diminished.

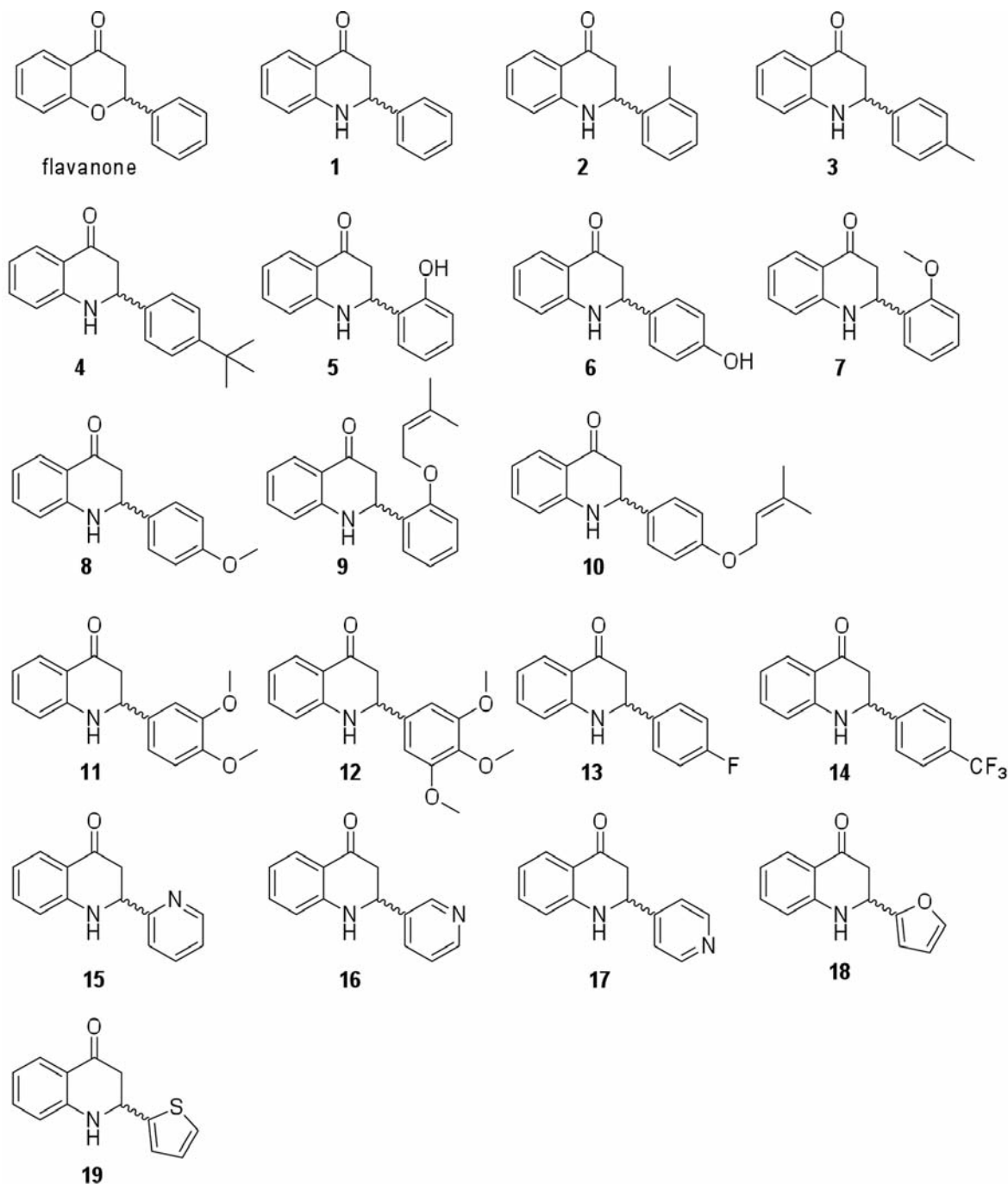


Figure 1. Structure of 1-azaflavanones (see Table I for names of compounds).

Compound **19** ($IC_{50}=5.1 \mu M$), possessing a thiophenyl group as the B-ring moiety, exhibited more potent activity than did **18**, with a furanyl group ($IC_{50}=280 \mu M$). It should be noted that the aromaticity of thiophene ring is higher than that of the furan ring due to the lower electronegativity of the sulfur atom than that of oxygen atom. While these results suggested

that the aromaticity of the B-ring may be involved in interactions with biomolecules, further studies are necessary before robust conclusions can be drawn.

In order to determine whether the antiproliferative activity of **1** was associated with apoptosis and/or cell cycle arrest, we conducted flow cytometric analysis of propidium iodide-stained

Table I. Analytical data of synthesized compounds.

Compound (Nos.)	2-Phenyl-2,3-dihydroquinolin-4(1H)-one (1)
Weight (yield)	1.00 g (38.5%)
HRMS (M + H) ⁺	calcd for C ₁₅ H ₁₃ NO, 223.0994; found 223.0995
¹ H-NMR	7.92 (dd, J=8.2, 1.8 Hz, 1H), 7.85 (m, 2H), 7.68 (m, 4H), 6.81 (dt, J=8.8, 1.4 Hz, 1H), 6.74 (dd, J=8.8, 1.4 Hz, 1H), 4.60 (dd, J=14.22, 4.26 Hz, 1H), 2.89 (dd, J=16.89, 14.22 Hz, 1H), 2.77 (dd, J=16.89, 4.26 Hz, 1H)
Compound (Nos.)	2-(2-Methylphenyl)-2,3-dihydroquinolin-4(1H)-one (2)
Weight (yield)	0.239 g (13.0%)
HRMS (M + H) ⁺	calcd for C ₁₆ H ₁₆ NO, 238.1232; found 238.1235
¹ H-NMR	7.89 (dd, J=7.68, 1.48 Hz, 1H), 7.68 (dd, J=8.8, 1.48 Hz, 1H), 7.35 (m, 5H), 6.8 (dt, J=7.7, 0.72 Hz, 1H), 6.72 (d, J=8.4 Hz, 1H), 5.05 (dd, J=13.2, 4.4 Hz, 1H), 2.85 (dd, J=16.48, 13.2 Hz, 1H), 2.77 (dd, J=16.48, 4.4, 1H), 2.36 (s, 3H)
Compound (Nos.)	2-(4-Methylphenyl)-2,3-dihydroquinolin-4(1H)-one (3)
Weight (yield)	0.339 g (23.6%)
HRMS (M + H) ⁺	calcd for C ₁₆ H ₁₆ NO, 238.1232; found 238.1233
¹ H-NMR	7.88 (dd, J=7.68, 1.1 Hz, 1H), 7.3 (m, 5H), 6.8 (dt, J=7.7, 0.72 Hz, 1H), 6.7 (d, J=8.4 Hz, 1H), 4.7 (dd, J=13.9, 3.7 Hz, 1H), 2.90 (dd, J=16.12, 13.92 Hz, 1H), 2.77 (dd, J=16.12, 3.64 Hz, 1H), 2.36(s, 3H)
Compound (Nos.)	2-(4-tert-Butylphenyl)-2,3-dihydroquinolin-4(1H)-one (4)
Weight (yield)	0.350 g (20.3%)
HRMS (M + H) ⁺	calcd for C ₁₉ H ₂₂ NO, 280.1701; found 280.1704
¹ H-NMR	8.04 (dd, J=7.68, 1.48 Hz, 1H), 7.34 (dd, J=7.72, 1.48 Hz, 1H), 7.31 (dt, J=6.96, 1.84 Hz, 1H), 7.14 (m, 5H), 6.63 (dt, J=6.69, 1.08 Hz, 1H), 4.99 (dd, J=15.68, 4.25 Hz, 1H), 2.73 (dd, J=15.68, 12.97 Hz, 1H), 2.67 (dd, J=15.68, 4.25 Hz, 1H), 1.28 (s, 9H)
Compound (Nos.)	2-(2-Hydroxyphenyl)-2,3-dihydroquinolin-4(1H)-one (5)
Weight (yield)	0.240 g (32.0%)
HRMS (M + H) ⁺	calcd for C ₁₅ H ₁₄ NO ₂ , 240.1025; found 240.1025
¹ H-NMR	8.04 (dd, J=7.68, 1.48 Hz, 1H), 7.34 (dd, J=7.72, 1.48 Hz, 1H), 7.31 (dt, J=6.96, 1.84 Hz, 1H), 7.14 (m, 5H), 6.63 (dt, J=6.69, 1.08 Hz, 1H), 4.99 (m, 1H), 2.73 (m, 2H)
Compound (Nos.)	2-(4-Hydroxyphenyl)-2,3-dihydroquinolin-4(1H)-one (6)
Weight (yield)	0.214 g (65.3%)
HRMS (M + H) ⁺	nd
¹ H-NMR	7.71 (dd, J=8.04, 1.08 Hz, 1H), 7.34 (m, 3H), 6.7 (d, J=8.4 Hz, 2H), 6.7 (d, J=8.76 Hz, 2H), 6.70 (dt, J=8.08, 1.1 Hz, 1H), 4.68 (dd, J=13.2, 4.0 Hz, 1H), 2.82 (dd, J=16.12, 13.20 Hz, 1H), 2.62 (dd, J=16.12, 4.0 Hz, 1H)
Compound (Nos.)	2-(2-Methoxyphenyl)-2,3-dihydroquinolin-4(1H)-one (7)
Weight (yield)	0.470 g (58.0%)
HRMS (M + H) ⁺	nd
¹ H-NMR	7.88 (dd, J=8.0, 1.5 Hz, 1H), 7.49 (dd, J=7.7, 1.5 Hz, 1H), 7.30 (m, 2H), 7.00 (dt, J=8.0, 1.1 Hz, 1H), 6.92 (d, J=8.4 Hz, 1H), 6.76 (t, J=7.7 Hz, 1H), 6.72 (d, J=8.4 Hz, 1H), 5.18 (dd, J=11.7, 4.4 Hz, 1H), 3.85 (s, 3H), 2.90 (dd, J=16.1, 4.4 Hz, 1H), 2.75 (dd, J=16.1, 11.4, 1H)
Compound (Nos.)	2-(4-Methoxyphenyl)-2,3-dihydroquinolin-4(1H)-one (8)
Weight (yield)	0.08 g (19.9%)
HRMS (M + H) ⁺	calcd for C ₁₆ H ₁₆ NO, 254.1181; found 254.1185
¹ H-NMR	7.88 (dd, J=8.0, 1.4 Hz, 1H), 7.38 (d, J=4.7 Hz, 2H), 7.33 (dt, J=1.48, 7.4 Hz, 1H), 6.93 (d, J=4.7 Hz, 2H), 6.78 (t, J=7.4 Hz, 1H), 5.70 (d, J=8.8 Hz, 1H), 4.70 (dd, J=13.9, 3.7 Hz, 1H), 3.82 (s, 3H), 2.90 (dd, J=16.1, 13.9 Hz, 1H), 2.75 (dd, J=16.1, 3.7 Hz, 1H)
Compound (Nos.)	2-(2-(3-methylbut-2-enyloxy)phenyl)-2,3-dihydroquinolin-4(1H)-one (9)
Weight (yield)	0.0697 g (67.9%)
HRMS (M + H) ⁺	calcd for C ₂₀ H ₂₁ NO ₂ , 307.1571; found 307.1572
¹ H-NMR	7.86 (dd, J=8.08, 1.84 Hz, 1H), 7.47 (dd, J=1.48, 7.72 Hz, 1H), 7.25-7.29 (m, 3H), 6.97 (dt, J=0.7, 7.32 Hz, 1H), 6.90 (d, J=8.44 Hz, 1H), 6.76 (dt, J=0.7, 7.32 Hz, 1H), 6.68 (d, J=8.44 Hz, 1H), 5.45 (m, 1H), 5.19 (dd, J=4.4, 11.7 Hz, 1H), 4.55 (d, J=6.6 Hz, 2H), 2.81-2.95 (m, 2H), 1.78 (s, 3H), 1.73 (s, 3H)
Compound (Nos.)	2-(4-(3-methylbut-2-enyloxy)phenyl)-2,3-dihydroquinolin-4(1H)-one (10)
Weight (yield)	0.0688 g (67.0%)
HRMS (M + H) ⁺	nd

Table I. continued

Table I. *continued*

¹ H-NMR	7.86 (dd, <i>J</i> =8.08, 1.84 Hz, 1H), 7.36 (d, <i>J</i> =8.8 Hz, 2H), 7.33 (dt, <i>J</i> =1.1, 8.04 Hz, 1H), 6.92 (d, <i>J</i> =8.8 Hz, 2H), 6.78 (dt, <i>J</i> =1.1, 7.5 Hz, 1H), 6.68 (d, <i>J</i> =8.44 Hz, 1H), 5.49 (m, 1H), 4.69 (dd, <i>J</i> =3.64, 13.92 Hz, 1H), 4.51 (d, <i>J</i> =6.6 Hz, 2H), 2.87 (dd, <i>J</i> =13.92, 16.48 Hz, 1H), 2.73 (dd, <i>J</i> =3.67, 16.08 Hz, 1H), 1.80 (s, 3H), 1.75 (s, 3H)
Compound (Nos.)	2-(3,4-Dimethoxyphenyl)-2,3-dihydroquinolin-4(1 <i>H</i>)-one (11)
Weight (yield)	0.301 g (58.0%)
HRMS (M + H) ⁺	nd
¹ H-NMR	7.88 (dd, <i>J</i> =8.0, 1.5 Hz, 1H), 7.33 (dt, <i>J</i> =7.0, 1.5 Hz, 1H), 7.0 (dd, <i>J</i> =8.0, 2.2 Hz, 2H), 6.87 (d, <i>J</i> =8.0 Hz, 1H), 6.8 (t, <i>J</i> =7.3 Hz, 1H), 4.71 (dd, <i>J</i> =13.9, 3.7 Hz, 1H), 3.89 (s, 6H), 2.88 (m, 1H), 2.77 (dd, <i>J</i> =16.5, 3.7 Hz, 1H)
Compound (Nos.)	2-(3,4,5-Trimethoxyphenyl)-2,3-dihydroquinolin-4(1 <i>H</i>)-one (12)
Weight (yield)	0.591 g (43.0%)
HRMS (M + H) ⁺	calcd for C ₁₈ H ₂₀ NO ₄ , 314.1392; found 314.1396
¹ H-NMR	7.88 (dd, <i>J</i> =8.0, 1.5 Hz, 1H), 7.37 (dt, <i>J</i> =7.0, 1.5 Hz, 1H), 6.8 (t, <i>J</i> =7.7, 1H), 6.7 (d, <i>J</i> =8.4 Hz, 1H), 6.6 (s, 2H), 4.7 (dd, <i>J</i> =13.5, 3.7 Hz, 1H), 3.85 (s, 9H), 2.90 (t, <i>J</i> =16.5, 1H), 2.77 (dd, <i>J</i> =16.5, 3.6, 1H)
Compound (Nos.)	2-(4-Fluorophenyl)-2,3-dihydroquinolin-4(1 <i>H</i>)-one (13)
Weight (yield)	0.312 g, yield; 24.5%
HRMS (M + H) ⁺	calcd for C ₁₅ H ₁₃ FN, 242.0981; found 242.0982
¹ H-NMR	7.88 (dd, <i>J</i> =8.08, 1.48 Hz, 1H), 7.45 (m, 2H), 7.36 (dt, <i>J</i> =7.32, 1.80 Hz, 1H), 7.10 (m, 2H), 6.82 (dt, <i>J</i> =7.32, 1.08 Hz, 1H), 6.72 (d, <i>J</i> =7.72 Hz, 1H), 4.76 (dd, <i>J</i> =13.6, 4.04 Hz, 1H), 2.88 (dd, <i>J</i> =16.48, 13.6 Hz, 1H), 4.47 (br. s, 1H), 2.78 (dd, <i>J</i> =16.48, 4.04 Hz, 1H)
Compound (Nos.)	2-(4-Trifluoromethylphenyl)-2,3-dihydroquinolin-4(1 <i>H</i>)-one (14)
Weight (yield)	0.610 g (10.6%)
HRMS (M + H) ⁺	calcd for C ₁₆ H ₁₃ F ₃ NO, 292.0949; found 292.0951
¹ H-NMR	7.88 (dd, <i>J</i> =8.08, 1.48 Hz, 1H), 7.67 (d, <i>J</i> =8.44 Hz, 2H), 7.60 (d, <i>J</i> =8.40 Hz, 2H), 7.38 (dt, <i>J</i> =7.32, 1.84 Hz, 1H), 6.84 (dt, <i>J</i> =7.32, 1.08 Hz, 1H), 6.74 (dd, <i>J</i> =8.76 Hz, 1H), 4.86 (dd, <i>J</i> =12.8, 4.76 Hz, 1H), 4.50 (br. s, 1H), 2.90 (dd, <i>J</i> =16.1, 12.8 Hz, 1H), 2.82 (dd, <i>J</i> =16.1, 4.76 Hz, 1H)
Compound (Nos.)	2-(Pyridin-2-yl)-2,3-dihydroquinolin-4(1 <i>H</i>)-one (15)
Weight (yield)	0.122 g (27.4%)
HRMS (M + H) ⁺	calcd for C ₁₄ H ₁₃ N ₂ O, 225.1028; found 225.1031
¹ H-NMR	8.63 (d, <i>J</i> =4.04 Hz, 1H), 7.84 (dd, <i>J</i> =8.08, 1.48 Hz, 1H), 7.75 (dt, <i>J</i> =7.68, 1.48 Hz, 1H), 7.38 (m, 3H), 6.79 (m, 2H), 5.80 (br. s, 1H), 4.92 (dd, <i>J</i> =12.44, 4.40 Hz, 1H), 3.06 (dd, <i>J</i> =16.12, 4.4 Hz, 1H), 2.98 (dd, <i>J</i> =16.12, 12.48 Hz, 1H)
Compound (Nos.)	2-(Pyridin-3-yl)-2,3-dihydroquinolin-4(1 <i>H</i>)-one (16)
Weight (yield)	0.137 g (14.7%)
HRMS (M + H) ⁺	nd
¹ H-NMR	8.73 (d, <i>J</i> =2.2 Hz, 1H), 8.62 (dd, <i>J</i> =5.08, 1.84 Hz, 1H), 7.88 (m, 2H), 7.39 (m, 2H), 6.84 (dd, <i>J</i> =6.0, 2.0 Hz, 1H), 6.74 (dd, <i>J</i> =8.1 Hz, 1H), 4.81 (dd, <i>J</i> =13.2, 4.0 Hz, 1H), 4.61 (br. s, 1H), 2.87 (dd, <i>J</i> =16.12, 12.84 Hz, 1H), 2.83 (dd, <i>J</i> =16.12, 4.04 Hz, 1H)
Compound (Nos.)	2-(Pyridin-4-yl)-2,3-dihydroquinolin-4(1 <i>H</i>)-one (17)
Weight (yield)	0.0802 g (10.2%)
HRMS (M + H) ⁺	nd
¹ H-NMR	8.63 (d, <i>J</i> =6.24 Hz, 2H), 7.85 (dd, <i>J</i> =6.24, 1.44 Hz, 1H), 7.39 (m, 3H), 6.82 (dt, <i>J</i> =8.08, 1.1 Hz, 1H), 6.76 (d, <i>J</i> =8.08 Hz, 1H), 4.79 (m, 1H), 4.56 (br. s, 1H), 2.83 (m, 2H)
Compound (Nos.)	2-(Furan-2-yl)-2,3-dihydroquinolin-4(1 <i>H</i>)-one (18)
Weight (yield)	0.189 g (16.0%)
HRMS (M + H) ⁺	calcd for C ₁₃ H ₁₂ NO ₂ , 214.0869; found 214.0870
¹ H-NMR	7.86 (dd, <i>J</i> =8.08, 1.48 Hz, 1H), 7.38 (dd, <i>J</i> =1.8, 0.72 Hz, 1H), 7.34 (dt, <i>J</i> =6.96, 1.44 Hz, 1H), 6.79 (dt, <i>J</i> =7.72, 0.76 Hz, 1H), 6.70 (d, <i>J</i> =8.44 Hz, 1H), 6.33 (dd, <i>J</i> =3.28, 1.84 Hz, 1H), 6.26 (d, <i>J</i> =3.28 Hz, 1H), 4.84 (dd, <i>J</i> =9.52, 5.16 Hz, 1H), 4.81 (br. s, 1H), 3.04 (dd, <i>J</i> =16.48, 9.88 Hz, 1H), 2.97 (dd, <i>J</i> =16.12, 5.12 Hz, 1H)
Compound (Nos.)	2-(Thiophen-2-yl)-2,3-dihydroquinolin-4(1 <i>H</i>)-one (19)
Weight (yield)	0.345 g (23.0%)
HRMS (M + H) ⁺	calcd for C ₁₃ H ₁₂ NOS, 230.0640; found 230.0640
¹ H-NMR	7.87 (dd, <i>J</i> =7.68, 1.48 Hz, 1H), 7.36 (dt, <i>J</i> =8.76, 1.80 Hz, 1H), 7.28 (dd, <i>J</i> =5.12, 1.08 Hz, 1H), 7.07 (d, <i>J</i> =2.96 Hz, 1H), 6.99 (dd, <i>J</i> =4.76, 3.32 Hz, 1H), 6.81 (m, 1H), 6.72 (d, <i>J</i> =8.40 Hz, 1H), 5.06 (dd, <i>J</i> =11.0, 5.48 Hz, 1H), 2.99 (dd, <i>J</i> =16.48, 13.2 Hz, 1H), 2.98 (dd, <i>J</i> =16.12, 3.28 Hz, 1H)

nd; Not determined.

Table II. Antiproliferative activity of 1-azaflavanones.

Compound	IC ₅₀ (μM)
Flavanone	45
1	0.40
2	0.50
3	5.4
4	>400
5	4.8
6	1.8
7	10
8	>400
9	13
10	63
11	>400
12	215
13	84
14	>400
15	>400
16	77
17	>400
18	280
19	5.1

IC₅₀: Half maximal inhibitory concentration.

nuclei. As shown in Figure 2, the cell cycle histogram demonstrates that **1** at 0.40 μM (*i.e.* its IC₅₀) increased the S, as well as sub-G₁ fraction, showing that the antiproliferative activity of **1** resulted, in part, from cell cycle arrest at the S phase and in part from apoptosis induction. To further elucidate the induction of apoptosis, a DNA ladder analysis was carried out (Figure 3). After 24 h, **1** at 0.40 μM caused apoptosis DNA ladders in HL60 cells. Interestingly, flavanone had almost no effect on the cell cycle distribution and no effect on DNA ladder analysis, at the same concentration as its IC₅₀ (45 μM).

In summary, the data presented here demonstrate that the enhancement of activity, by replacing the C-ring ether oxygen atom of flavanone with a nitrogen atom, resulted partly from cell cycle arrest at the S phase and from apoptosis induction. A close correlation between the antiproliferative activity and B-ring aromaticity of 1-azaflavanones is also suggested.

References

- 1 Erdman JW Jr., Balentine D, Arab L, Beecher G, Dwyer JT, Folts J, Harnly J, Hollman P, Keen CL, Mazza G, Messina M, Scalbert A, Vita J, Williamson G and Burrowes J: Flavonoids and heart health: proceedings of the ILSI North America Flavonoids Workshop, May 31-June 1, 2005, Washington, DC. J Nutr 137: 718S-737S, 2007.
- 2 Kawaii S, Tomono Y, Katase E, Ogawa K, Nonomura-Nakano M, Nesumi H, Yoshida T, Sugiura M and Yano M: Quantitative study of fruit flavonoids in citrus hybrids of King (*C. nobilis*) and Mukaku Kishu (*C. kinokuni*). J Agric Food Chem 49: 3982-3986, 2001.

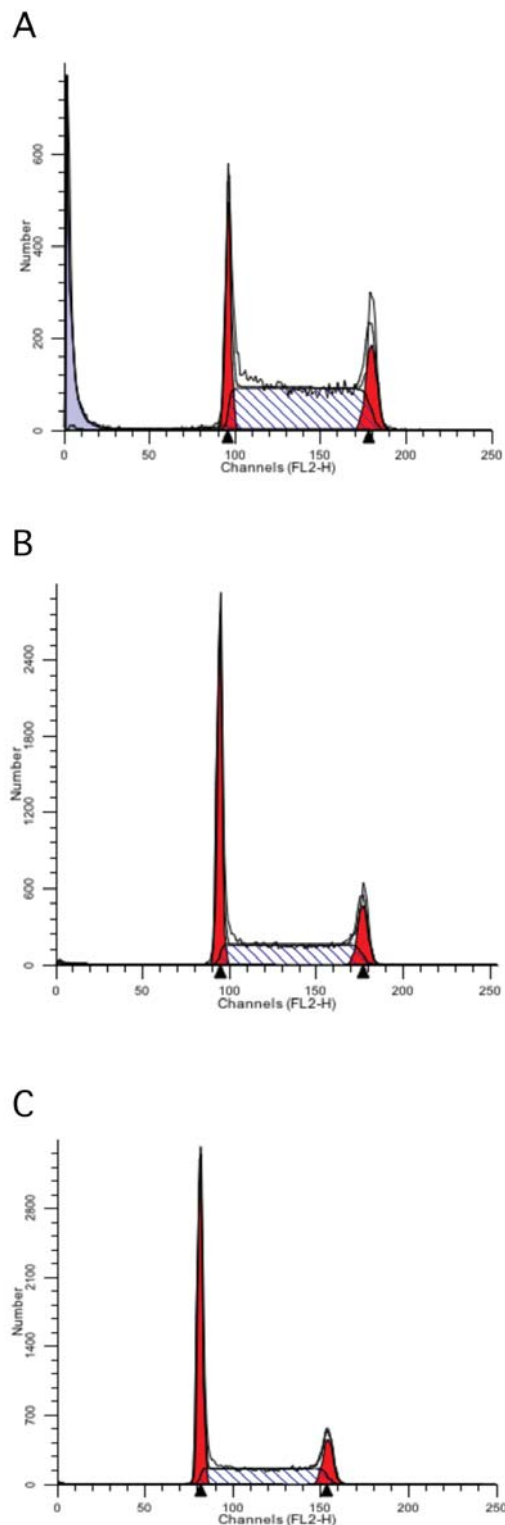


Figure 2. Cell cycle distribution of HL60 cells treated with **1** (A), flavanone (B) and blank control (0.4% DMSO) (C). A: G₁: 19.02%, G₂: 13.40%, S: 67.58%, G₂/G₁: 0.70, subG₁: 21.03%. B: G₁: 38.06%, G₂: 12.92 %, S: 49.02%, G₂/G₁: 0.34, subG₁: 1.50%. C: G₁: 44.82%, G₂: 12.08%, S: 43.09%, G₂/G₁: 0.27, subG₁: 0.91%.

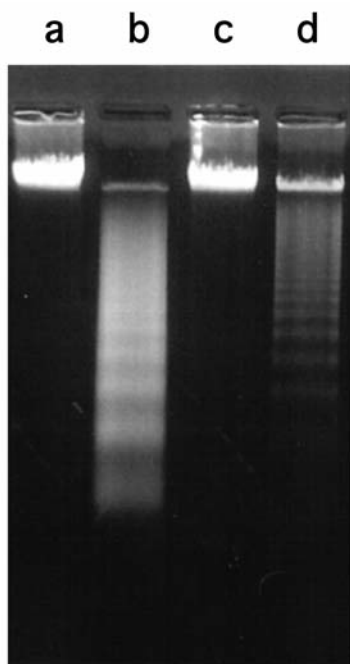


Figure 3. A degradation of chromosomal DNA into small fragments was detected by DNA ladders on agarose gels (lane d). Lane a: blank control (0.4% DMSO); b: positive control (1 µg/ml of actinomycin D); c: flavanone at 45 µM; d: 1 at 0.40 µM.

- 3 Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M, Koizumi M, Ito C and Furukawa H: Quantitative study of flavonoids in leaves of citrus plants. *J Agric Food Chem* 48: 3865-3871, 2000.
- 4 Kawaii S, Tomono Y, Katase E, Ogawa K and Yano M: Quantitation of flavonoid constituents in citrus fruits. *J Agric Food Chem* 47: 3565-3571, 1999.
- 5 Prasain JK, Carlson SH and Wyss JM: Flavonoids and age-related disease: risk, benefits and critical windows. *Maturitas* 66: 163-171, 2010.
- 6 Ekström AM, Serafini M, Nyrén O, Wolk A, Bosetti C and Bellocchio R: Dietary quercetin intake and risk of gastric cancer: results from a population-based study in Sweden. *Ann Oncol* 22: 438-443, 2011.
- 7 Williamson G and Manach C: Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am J Clin Nutr* 81: 243S-255S, 2005.
- 8 Manach C, Williamson G, Morand C, Scalbert A and Rémésy C: Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* 81: 230S-242S, 2005.
- 9 Kawaii S, Tomono Y, Katase E, Ogawa K and Yano M: Antiproliferative activity of flavonoids on several cancer cell lines. *Biosci Biotechnol Biochem* 63: 896-899, 1999.
- 10 Tsimplouli C, Demetrios C, Hadzopoulou-Cladaras M, Pantazis P and Dimas K: *In vitro* activity of dietary flavonol congeners against human cancer cell lines. *Eur J Nutr* 51: 181-190, 2012.
- 11 Terao J: Dietary flavonoids as antioxidants. *Forum Nutr* 61: 87-94, 2009.
- 12 González R, Ballester I, López-Posadas R, Suárez MD, Zarzuelo A, Martínez-Augustín O and Sánchez de Medina F: Effects of flavonoids and other polyphenols on inflammation. *Crit Rev Food Sci Nutr* 51: 331-362, 2011.
- 13 Korkina L, Kostyuk V, De Luca C and Pastore S: Plant phenylpropanoids as emerging anti-inflammatory agents. *Mini Rev Med Chem* 11: 823-835, 2011.
- 14 Kawaii S, Tomono Y, Katase E, Ogawa K and Yano M: HL-60 differentiating activity and flavonoid content of the readily extractable fraction prepared from citrus juices. *J Agric Food Chem* 47: 128-135, 1999.
- 15 Kawaii S, Tomono Y, Katase E, Ogawa K and Yano M: Effect of citrus flavonoids on HL-60 cell differentiation. *Anticancer Res* 19: 1261-1269, 1999.
- 16 Xia Y, Yang ZY, Xia P, Bastow KF, Tachibana Y, Kuo SC, Hamel E, Hackl T and Lee KH: Antitumor agents. 181. Synthesis and biological evaluation of 6,7,2',3',4'-substituted-1,2,3,4-tetrahydro-2-phenyl-4-quinolones as a new class of antimitotic antitumor agents. *J Med Chem* 41: 1155-1162, 1998.
- 17 Zhang SX, Feng J, Kuo SC, Brossi A, Hamel E, Tropsha A and Lee KH: Antitumor agents. 199. Three-dimensional quantitative structure-activity relationship study of the colchicine binding site ligands using comparative molecular field analysis. *J Med Chem* 43: 167-176, 2000.
- 18 Pejović A, Damjanović I, Stevanović D, Vukićević M, Novaković SB, Bogdanović GA, Radulović N and Vukićević RD: Antimicrobial ferrocene containing quinolones: Synthesis, spectral, electrochemical and structural characterization of 2-ferrocenyl-2,3-dihydroquinolin-4(1H)-one and its 6-chloro and 6-bromo derivatives. *Polyhedron* 31: 789-795, 2012.
- 19 Jiang C, Yang L, Wu WT, Guo QL and You QD: De novo design, synthesis and biological evaluation of 1,4-dihydroquinolin-4-ones and 1,2,3,4-tetrahydroquinazolin-4-ones as potent kinesin spindle protein (KSP) inhibitors. *Bioorg Med Chem* 19: 5612-5627, 2011.
- 20 Mphahlele MJ and Oyeyiola FA: Suzuki-Miyaura cross-coupling of 2-aryl-6,8-dibromo-1,2,3,4-tetrahydroquinolin-4-ones and subsequent dehydrogenation and oxidative aromatization of the resulting 2,6,8-triaryl-1,2,3,4-tetrahydroquinolin-4-ones. *Tetrahedron* 67: 6819-6825, 2011.
- 21 Chandrasekhar S, Vijeender K and Sridhar C: L-Proline-catalyzed one-pot synthesis of 2-aryl-2,3-dihydroquinolin-4(1H)-ones. *Tetrahedron Lett* 48: 4935-4937, 2007.
- 22 Donnelly JA and Farrell DF: The chemistry of 2'-amino analogs of 2'-hydroxychalcone and its derivatives. *J Org Chem* 55: 1757-1761, 1990.
- 23 Uzunoglu S, Karaca B, Atmaca H, Kisim A, Sezgin C, Karabulut B and Uslu R: Comparison of XTT and alamar blue assays in the assessment of the viability of various human cancer cell lines by AT-101 (–/– gossypol). *Toxicol Mech Methods* 20: 482-486, 2010.

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