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Synthesis and antiproliferative evaluations of certain 2-phenylvinylquinoline (2-styrylquinoline) and 2-furanylvinylquinoline derivatives

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ABSTRACT

The present study describes the synthesis of 2-phenylvinylquinoline (styrylquinoline) and 2-furanylvinylquinoline derivatives and evaluation for their antiproliferative activities. (*E*)-2-Styrylquinolin-8-ol (**14a**) was inactive against a 3-cell line panel consisting of MCF-7 (Breast), NCI-H460 (Lung), and SF-268 (CNS). Replacement of the phenyl ring with 5-nitrofuran-2-yl group significantly enhanced antiproliferative activity in which (*E*)-2-(2-(5-nitrofuran-2-yl)vinyl)quinolin-8-ol (**14i**) and its 4-substituted derivatives **15–19** exhibited strong inhibitory effects against the growth of all three cancer cells. These compounds were further evaluated for their IC_{50} against the growth of MCF-7, LNCaP, and PC3. Results indicated that a hydrogen bond donating oxime derivative **19a** was more active than its hydrogen bond accepting methyloxime derivative **19b**. For the inhibition of LNCaP, the potency decreased in an order **14i** > **19a** > **19b** > **15** > **18** > **16**. Compound **14i** is the most active with an IC_{50} value of 0.35 and 0.14 μM, respectively, against the growth of LNCaP and PC3 cancer cells. Therefore, compound **14i** was evaluated by flow cytometric analysis for its effects on cell cycle distributions. Results indicated that **14i** effectively induced cell cycle arrest at S phase for both cell lines, which consequently trigger late apoptosis for both LNCaP and PC3 cells.

1. Introduction

We have synthesized certain furo[2,3-*b*]quinoline derivatives and evaluated for their antiproliferative activities on the ground that this tricyclic furo[2,3-*b*]quinoline moiety is an isosteric isomer of the versatile acridine ring.[1–6] Among them, 1-[4-(furo[2,3-*b*]quinolin-4-ylamino)phenyl]ethanone (CIL-102) (mean GI_{50} = 0.025 μM) was more active than both clinically used anticancer drugs, *m*-AMSA (mean GI_{50} = 0.44 μM) and Daunomycin (mean GI_{50} = 0.044 μM) against the full panel of 60 human tumor cell lines derived from nine cancer cell types.[1,2] CIL-102 had been selected as a lead compound and was evaluated by flow cytometric analysis for its effects on cell cycle distributions. Results indicated that CIL-102 inhibits cell proliferation by the alteration of cell division, accumulation of cells in the G2/M phase followed by the cell apoptosis which is similar to microtubule-targeting agents such as paclitaxel but distinct from that of amsacrine.[4] This is interesting because 4-anilinofuro[2,3-*b*]quinoline derivatives, if proved to be active, could be developed as a new structural type of potential anti-microtubule drug candidates.

Quinoline moiety is present in many classes of biologically active compounds.[7–11] The biological activities of these quinoline derivatives depends not only on the bicyclic heteroaromatic pharmacophore but also on the nature of the peripheral substituents and their spatial relationship. Recently, we have synthesized 2-phenylquinoline and 2-furanylquinoline derivatives which can be considered as the isomers of acridine and furo[2,3-*b*]quinoline, respectively, in which the phenyl and furanyl moiety, respectively, is appended on the C-2 position instead of fused with bicyclic quinoline.[3,12–14] The 2-phenylquinoline derivatives can also be considered as aza-analogues of 2-phenylnaphthalene skeleton which consists of a large number of antitumor compounds.[15–18] In continuation of our search for potential drug candidates, the present study describes the synthesis and antiproliferative evaluations of 2-phenylvinylquinoline (styrylquinoline) and 2-furanylvinylquinoline derivatives, the insertion of a vinyl bridge between the 2-aryl moiety and the bicyclic quinoline ring. Styrylquinoline derivatives have gained strong attention recently due to their extensive biological activities.[19–23]

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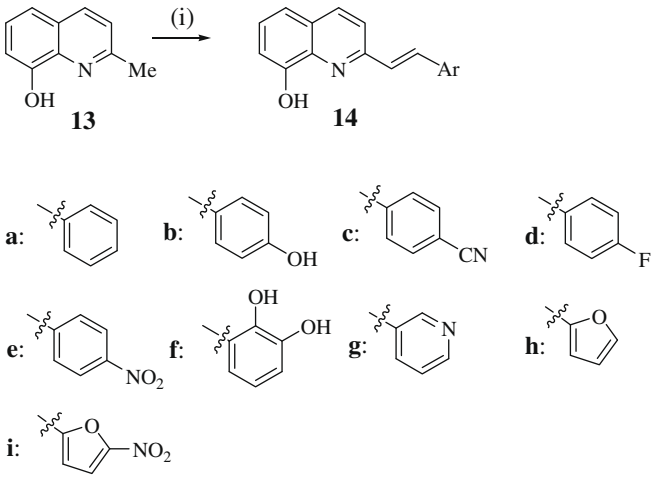
2. Chemistry

Reaction of 2-methoxyaniline (**2**) and ethyl acetoacetate gave 4-hydroxy-2-methyl-8-methoxyquinoline (**4**)[24] as described in Scheme 1. Condensation of **4** with benzaldehyde in acetic anhydride gave (*E*)-4-hydroxy-8-methoxy-2-styrylquinoline (**6**) in a good overall yield. The known (*E*)-4-hydroxy-2-styrylquinoline (**5**)[25] was prepared under the same reaction conditions from commercially available 4-hydroxy-2-methylquinoline (**3**) and benzaldehyde. Treatment of **6** with $POCl_3$ afforded (*E*)-4-chloro-8-methoxy-2-styrylquinoline (**8**) which was reacted with 4-aminoacetophenone to give (*E*)-1-(4-(8-methoxy-2-styrylquinolin-4-ylamino)phenyl)ethanone (**10**) in a good overall yield. Accordingly, compound **9** was obtained from (*E*)-4-chloro-2-styrylquinoline (**7**) and 4-aminoacetophenone. Treatment of **9** and **10** with NH_2OH in EtOH gave (*E*)-1-{4-[(*E*)-2-styrylquinolin-4-ylamino]phenyl}-ethanone oxime (**11a**) and its derivative **12a**, respectively. The configuration of the oxime moiety was confirmed by the chemical shift of ^{13}C NMR spectra in which the Me moiety (δ 11.53 ppm for **11a** and 11.45 ppm for **12a**) of the *E*-form isomer shifted more upfield (δ approximately at 11.50 ppm) than its *Z*-form counterpart (δ approximately at 18.80 ppm).[26] Accordingly, compounds **11b** and **12b** were synthesized from their respective ketone precursors **9** and **10** with NH_2OMe under the same reaction conditions.

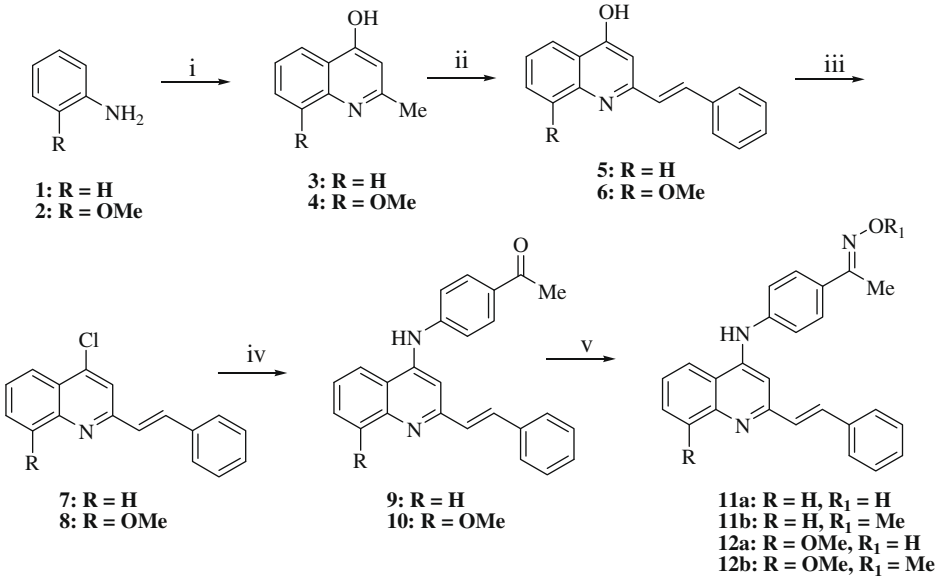
We have also prepared certain 8-hydroxy-2-styrylquinoline derivatives in which the styryl moiety was substituted or replaced with various heterocycles as described in Scheme 2. Reaction of 8-hydroxy-2-methylquinoline (**13**) with benzaldehyde in acetic anhydride afforded 2-styrylquinolin-8-ol (**14a**)[20] in 45% yield. Accordingly, compounds **14b–i** were obtained from **13** and various aldehydes in a fairly good yield. (*E*)-1-{4-{2-[(*E*)-2-(5-Nitrofuran-2-yl)vinyl]quinolin-4-ylamino}phenyl}ethanone oxime (**19a**) and its methyloxime derivative **19b** were prepared from **3** and 5-nitro-2-furaldehyde (Scheme 3) by the same reaction sequences for the preparation of **11a** and **11b**.

3. Results and discussion

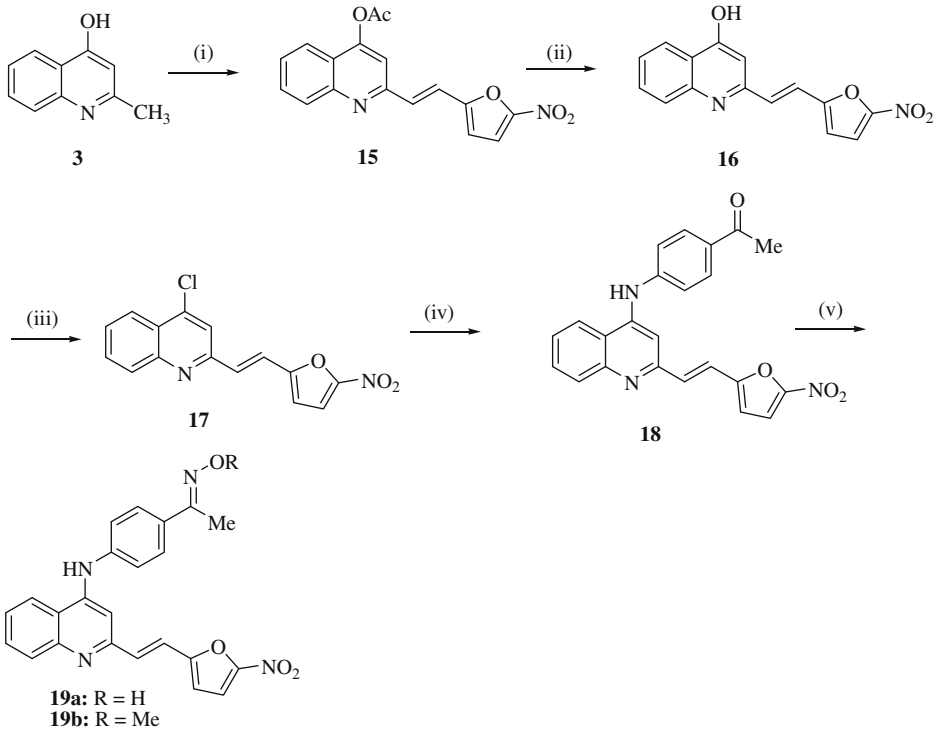
All compounds were evaluated in vitro against a 3-cell line panel consisting of MCF-7 (Breast), NCI-H460 (Lung), and SF-268 (CNS). In this protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at a concentration of 4.0 μg/mL and the culture incubated for 48 h. End-point determinations are made with sulforhodamine B, a protein-binding dye. Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells and results are summarized in Tables 1 and 2. With an exception of (*E*)-1-{4-[8-methoxy-(*E*)-2-styrylquinolin-4-ylamino]phenyl}ethanone oxime (**12a**) which exhibited a marginal antiproliferative activity, all the 2-styrylquinolines **5–10**, **11a**, **11b**, and **12b** are inactive at a concentration of 4.0 μM (Table 1). (*E*)-2-Styrylquinolin-8-ol (**14a**) was inactive although 8-hydroxyquinoline constitutes a number of biologically active compounds.[10,23] Introduction of an electron-donating group such as OH or an electron-withdrawing group such as CN, F, or NO_2 at the 2-styryl moiety of **14a** did not improve antiproliferative activity. Replacement of the phenyl ring with its heterocyclic isosteric isomers such as pyridine or furan ring proved to be no improvement in which compounds **14g** and **14h** were inactive. However, replacement of the phenyl ring with 5-nitrofuran-2-yl group significantly enhanced



Scheme 2. Reagents and conditions: (i) aldehyde and Ac_2O, 150 °C, 30 h.

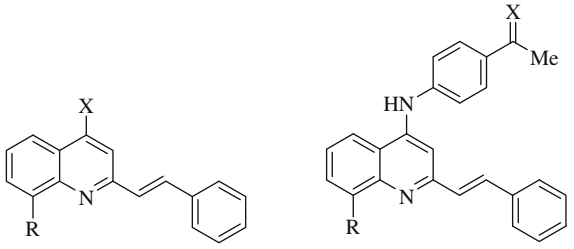


Scheme 1. Reagents and conditions: (i) (a) ethyl acetoacetate, AcOH, benzene, 80 °C, 24 h; (b) phenyl ether, 250 °C, 1 h; (ii) benzaldehyde, Ac_2O, 150 °C, 30 h; (iii) $POCl_3$, 80–90 °C, 1 h; (iv) 4-aminoacetophenone, EtOH, reflux, 2 h; (v) hydroxyamine or methylhydroxyamine, K_2CO_3, EtOH, rt, 1 h.



Scheme 3. Reagents and conditions: (i) (5-nitrofuran-2-yl)methylene diacetate, Ac_2O, 150 °C, 30 h; (ii) pyridine/H_2O (v/v 4:1), 100 °C, 1 h; (iii) $POCl_3$, 80–90 °C, 1 h; (iv) 4-aminoacetophenone, EtOH, reflux, 2 h; (v) hydroxyamine or methylhydroxyamine, K_2CO_3, EtOH, rt, 1 h.

Table 1
Preliminary antiproliferative activities of 2-phenylvinylquinoline derivatives in vitro[a]

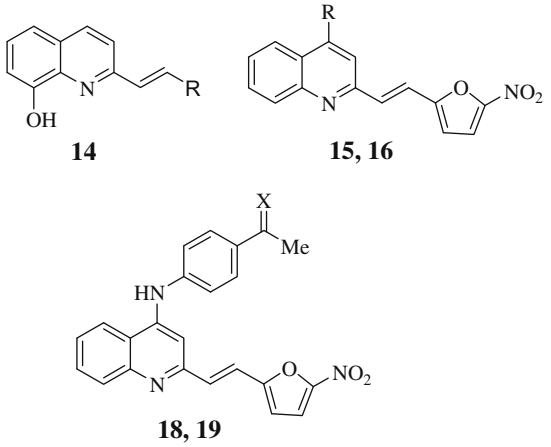


	R	X	Survival (% control)		
			MCF-7	NCI-H460	SF-268
5	H	OH	119	118	120
6	OMe	OH	104	111	119
7	H	Cl	120	109	118
8	OMe	Cl	99	109	81
9	H	O	83	52	107
10	OMe	O	95	75	114
11a	H	NOH	54	67	90
11b	H	NOMe	108	79	112
12a	OMe	NOH	40	<20	63
12b	OMe	NOMe	97	87	97

[a] Cells were cultured with agents at a concentration of 4.0 μg/mL for 72 h before growth and viability were assessed using the MTS assay.

antiproliferative activity in which (*E*)-2-(2-(5-nitrofuran-2-yl)vinyl)quinolin-8-ol (**14i**) exhibited a strong inhibitory effect against the growth of all three cancer cells (Table 2). These results indicated that the substituent of 2-(5-nitrofuran-2-yl)vinyl moiety is crucial for antiproliferative activity. Therefore, a number of (*E*)-2-(2-(5-nitrofuran-2-yl)vinyl)quinoline derivatives **15–19** had been synthesized and evaluated. Among them, (*E*)-2-(2-(5-nitrofu-

Table 2
Preliminary antiproliferative activities of 2-arylvinylquinoline derivatives in vitro[a]



	R	X	Survival (% control)		
			MCF-7	NCI-H460	SF-268
14a	Ph	—	110	55	72
14b	Ph-4-OH	—	108	52	78
14c	Ph-4-CN	—	107	66	90
14d	Ph-4-F	—	103	66	82
14e	Ph-4-NO_2	—	89	52	77
14f	Ph-2,3-diOH	—	95	53	52
14g	Pyridin-3-yl	—	115	63	89
14h	Furan-2-yl	—	110	70	76
14i	5-NO_2-furan-2-yl	—	21	<20	<20
15	OAc	—	<20	<20	<20
16	OH	—	59	<20	<20
18	—	O	<20	<20	<20
19a	—	NOH	<20	<20	<20
19b	—	NOMe	<20	<20	<20

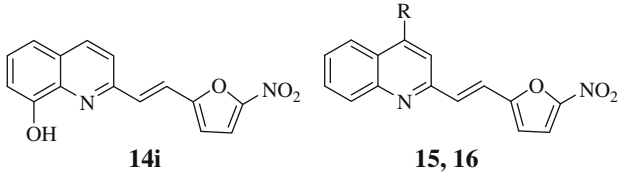
[a] Cells were cultured with agents at a concentration of 4.0 μg/mL for 72 h before growth and viability were assessed using the MTS assay.

ran-2-yl)vinyl)quinolin-4-ol (**16**) was less active than its 4-acetyl derivative **15** against the growth of MCF-7 which implied that a bulky group at C-4 position is favorable. This SAR can also be applied in which significant antiproliferative activities were observed for C-4 bulky derivatives **18**, **19a**, and **19b**.

(*E*)-2-(2-(5-Nitrofuran-2-yl)vinyl)quinolin-8-ol (**14i**) and its 4-substituted derivatives **15–19** which exhibited potent antiproliferative effects were further evaluated for their inhibitory activity against the growth of MCF-7, LNCaP, and PC3 and the IC_{50} values are given in Table 3. A hydrogen bond donating oxime derivative **19a** was found to be more active than its methyloxime derivative **19b**. For the inhibition of LNCaP, the potency decreased in an order **14i** > **19a** > **19b** > **15** > **18** > **16**. Compound **14i** is the most active with an IC_{50} value of 0.35 and 0.14 μM, respectively, against the growth of LNCaP and PC3 cancer cells. Therefore, compound **14i** was selected as a lead compound and was evaluated by flow cytometric analysis for its effects on cell cycle distributions. Results indicated that **14i** effectively induced cell cycle arrest of LNCaP at S phase, about 37% at a concentration of 0.5 μM for 48 h as shown in Figure 1. For the PC3 cells, compound **14i** induced cell cycle arrest at S phase, about 44% at a concentration of 0.2 μM for 24 h. Our results indicated that **14i** induce higher percentage of PC3 cells than that of LNCaP cells arrested at S phase by using the respective dose near IC_{50} and the respective time course related to doubling time of cell cycle.[27,28]

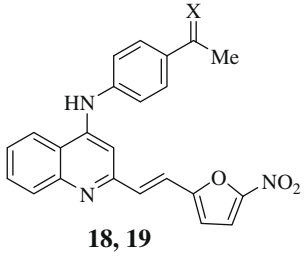
We further asked if **14i**-induced cell cycle arrest at S phase might trigger apoptosis for LNCaP and PC3 cells by Annexin-V and propidium iodide (PI) staining with flow cytometry. The FITC-conjugated Annexin-V staining can detect apoptotic cells by binding to their exposed phosphatidylserine, and PI is included with Annexin-V because dead cells or cells in the late stage of apoptosis will allow PI to diffuse into the cell and bind to DNA.[29,30] Apoptotic cells can be sorted into two quadrants, the upper right representing late apoptosis (both Annexin-V and PI positive) and the lower right representing early apoptosis (Annexin positive and PI negative), among four quadrants in this dual dot plot by flow cytometry. As shown in Figure 2A, treatment of LNCaP cells with 0.5 μM of **14i** for 24 h, we observed that only 1% increase of cells appears in the upper right quadrant and prolong treatment

Table 3
Antiproliferative activities (IC_{50}) of 5-nitrofuran-2-ylvinylquinoline derivatives[a]



14i

15, 16



18, 19

	R	X	MCF-7	LNCaP	PC3
14i	—	—	0.30 ± 0.04	0.35 ± 0.06	0.14 ± 0.02
15	OAc	—	0.71 ± 0.17	1.61 ± 0.33	0.86 ± 0.10
16	OH	—	2.21 ± 0.54	5.69 ± 2.75	1.37 ± 0.35
18	—	O	0.43 ± 0.10	4.12 ± 1.13	0.45 ± 0.10
19a	—	NOH	0.25 ± 0.03	0.95 ± 0.08	0.19 ± 0.03
19b	—	NOMe	0.40 ± 0.04	1.04 ± 0.08	0.52 ± 0.06

[a] Values are given in μM and are means of three experiments.

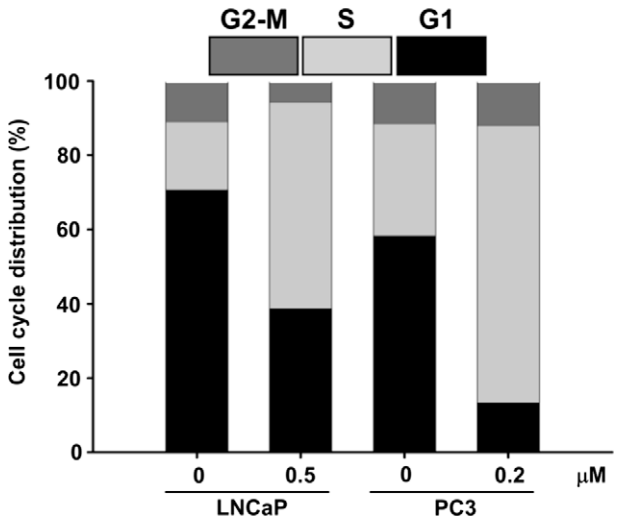


Figure 1. Compound **14i** induced cell cycle arrest at S phase for LNCaP and PC3 cells. LNCaP and PC3 cells were incubated in the presence or absence of **14i** for 48 and 24 h, respectively, and then the cells were harvested for flow cytometry analysis of cell cycle. The experimental details were described in Section 5.4. Representative cell cycle distribution of LNCaP and PC3 cells after treatment of **14i** was shown ($n = 3$).

of LNCaP cells with the compound for 48 h significantly increases the cells in this quadrant up to 24%. In contrast, treatment of PC3 cells with 0.2 μM of **14i** for either 24 h or 48 h induces the significant amounts of cells toward the upper right quadrant (Fig. 2B). This different outcome between LNCaP and PC3 cells is simply just due to the different doubling time of them. Since PC3 cells have a short doubling time of about 24 h, the treatment of **14i** for 24 h might induce the most cells arrested at S phase, resulting in entering into apoptosis. However, LNCaP cells have long doubling time of about 60 h, reflecting that late apoptosis appears after 48 h of treatment. Thus, we concluded that compound **14i** can induce cell cycle arrest at S phase for both cell lines, which consequently trigger late apoptosis for both LNCaP and PC3 cells. Moreover, we also examined the cleavage pattern of poly(ADP-ribose) polymerase (PARP) to confirm that **14i** induced an apoptotic response in PC3 cells by western blotting. Our results indicated that the native 116 kDa PARP protein is cleaved into its characteristic 85 kDa fragment upon treatment with 0.4 and 0.6 μM of **14i** in PC3 cells (Fig. 3).

4. Conclusion

We have synthesized and evaluated antiproliferative activities of certain 2-phenylvinylquinoline (styrylquinoline) and 2-furanylvinylquinoline derivatives. Among them, (*E*)-2-(2-(5-nitrofuran-2-yl)vinyl)quinolin-8-ol (**14i**) exhibited a strong inhibitory effect against the growth of MCF-7 (Breast), NCI-H460 (Lung), and SF-268 (CNS). Compound **14i** was evaluated by flow cytometric analysis for its effects on cell cycle distributions of LNCaP and PC3 cells. Results indicated that **14i** effectively induced cell cycle arrest at S phase for both cell lines, which consequently trigger late apoptosis. Structural optimization and mechanism studies of **14i** are currently under investigation.

5. Experimental

5.1. General

Melting points were determined on a Electrothermal IA9100 melting point apparatus and are uncorrected. IR Spectra: Nicolet Magana-IR 550 infrared spectrophotometer using KBr pellets

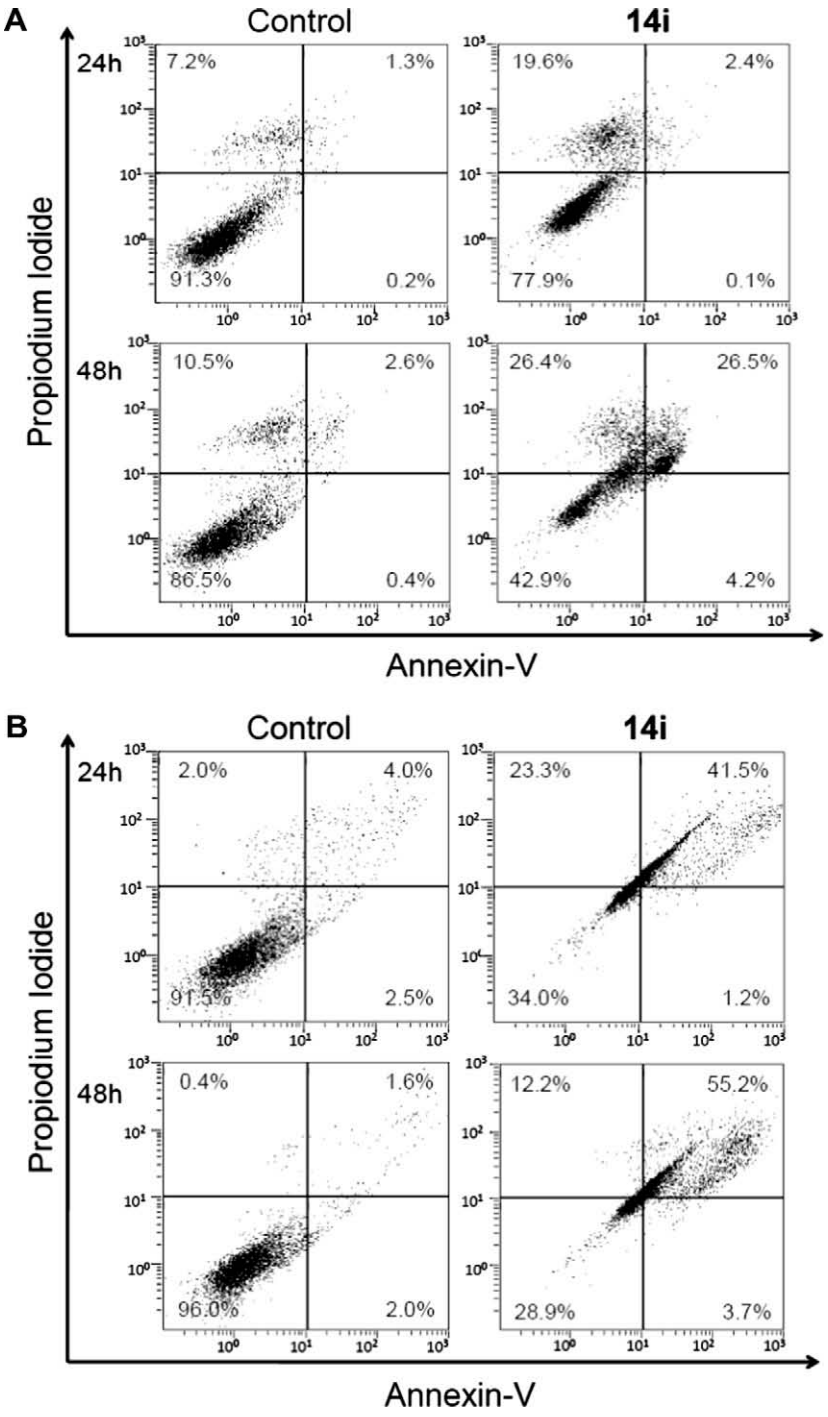


Figure 2. Compound **14i** possesses both necrosis- and apoptosis-inducing effects on (A) LNCaP and (B) PC3 cells. LNCaP or PC3 cells were incubated in the presence or absence of **14i** for 24 and 48 h and then the cells were harvested for flow cytometry analysis of Annexin-V staining. The experimental details were described in Section 5.5. Representative cell distribution by PI and Annexin-V staining were shown ($n = 3$).

(solid), and reported on wavenumber (cm^{-1}). Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a Varian Gemini 200 spectrometer or Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on Silica Gel 60 F-254 plates purchased from E. Merck and Co. The low resolution EIMS data was collected on a Bruker APEX II mass spectrometer. The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University and National Taiwan University using Heraeus CHN-O Rapid EA, and all values are within ±0.4% of the theoretical compositions.

5.1.1. 8-Methoxy-2-methylquinoline-4(1*H*)-one (4)

A mixture of *o*-anisidine (2.46 g, 20 mmol), ethyl acetoacetate (2.60 g, 20 mmol), acetic acid (0.1 mL) and benzene (150 mL) was heated at 80 °C for 24 h (TLC monitoring). After cooling, the solvent was removed in vacuo, and the residue was heated at 250 °C for 1 h (TLC monitoring) in diphenyl ether (80 mL). After cooling, the solvent was added hexane and the resulting precipitate was collected,

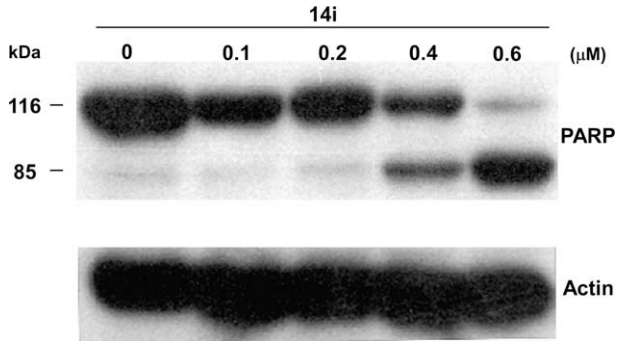


Figure 3. Compound **14i** induced apoptosis in PC3 cells as assessed by Western blot. PC3 cells were seeded onto 10-cm dish, cultured for 12 h and then treated with 0, 0.1, 0.2, 0.4, and 0.6 μM of **14i**, respectively, for 24 h. Then the cells were harvested for Western blot. The experimental details were described in Section 5.6. Actin was used as a control of constant protein loading of sampling. Representative immunoblots for PARP, its degradation product and actin loading controls were shown ($n = 3$).

washed with hexane, and crystallized from EtOH to give **4** (1.68 g, 45%). Mp 238–239 °C (lit.: 230–232 °C).[24] IR (KBr): 3366, 1659, 1635, 1594, 1545, 1518, 1424, 1265, 1198, 1065. ^{1}H NMR (400 MHz, DMSO-d_6): 2.37 (s, 3H, Me), 3.99 (s, 3H, OMe), 5.92 (d, 1H, J = 1.2 Hz, 3-H), 7.20–7.22 (m, 2H, 5- and 7-H), 7.61 (m, 1H, 6-H), 11.01 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 19.52, 56.10, 109.09, 110.98, 116.09, 122.43, 125.47, 130.86, 148.21, 149.58, 176.47. Anal. Calcd for $C_{11}H_{11}NO_2{\cdot}1.0H_2O$: C, 63.74; H, 6.33; N, 6.75. Found: C, 63.77; H, 6.39; N, 6.79.

5.1.2. (*E*)-2-Styryl-1-4-dihydroquinoline-4-ol (5)

A mixture of 4-hydroxy-2-methylquinoline (0.32 g, 2 mmol), benzaldehyde (0.85 g, 8 mmol) and acetic anhydride (100 mL) was heated at 150 °C for 30 h (TLC monitoring). After cooling, the solvent was removed in vacuo, and the residue was heated at 100 °C for 1 h (TLC monitoring) in pyridine/water (v/v = 4:1) (100 mL). After cooling, the solvent was removed in vacuo to provide the crude product, which was purified by FC (MeOH/CH_2Cl_2 = 1:30) to give **5** (0.23 g, 48%). Mp 220–223 °C (lit.: 279 °C).[25] IR (KBr): 3141, 1655, 1555, 1506, 1433, 1393, 1261, 1207, 1071, 1034. ^{1}H NMR (400 Hz, DMSO-d_6): 7.14 (s, 1H, 3-H), 7.43 (d, 1H, J = 16.4 Hz, =CH), 7.46–7.54 (m, 3H, Ar-H), 7.65 (m, 1H, Ar-H), 7.77 (m, 2H, Ar-H), 7.90–8.00 (m, 3H, Ar-H), 8.24 (m, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6): 104.02, 119.41, 120.42, 121.25, 123.83, 126.27, 128.00 (2C), 129.24 (2C), 129.29, 130.40, 132.92, 133.93, 134.84, 139.55, 171.25. Anal. Calcd for $C_{17}H_{13}NO{\cdot}0.2H_2O$: C, 81.37; H, 5.39; N, 5.58. Found: C, 81.54; H, 5.29; N 5.61.

5.1.3. (*E*)-8-Methoxy-2-styrylquinoline-4-ol (6)

This compound was obtained from **4** and benzaldehyde as described for **5** and was crystallized from EtOH in 55% yield. Mp 238–240 °C. IR (KBr): 3348, 1677, 1626, 1587, 1551, 1492, 1432, 1266, 1064. ^{1}H NMR (400 Hz, DMSO-d_6): 4.03 (s, 3H, OCH_3), 6.58 (d, 1H, J = 1.2 Hz, 3-H), 7.23–7.26 (m, 2H, 5- and 7-H), 7.36–7.51 (m, 4H, Ar-H), 7.61–7.66 (m, 4H, Ar-H), 10.97 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 56.17, 104.22, 111.26, 112.50, 112.76, 116.04, 119.33, 122.79, 122.87, 126.27 (2C), 130.75 (2C), 144.68, 147.02, 148.52, 151.66, 176.35. Anal. Calcd for $C_{18}H_{15}NO_2{\cdot}0.3H_2O$: C, 76.46; H, 5.57; N, 4.95. Found: C, 76.46; H, 5.44; N, 4.88.

5.1.4. (*E*)-4-Chloro-2-styrylquinoline hydrochloride (7)

A mixture of **5** (0.50 g, 2 mmol) and $POCl_3$ (20 mL) was heated at 80–90 °C for 1 h (TLC monitoring). After cooling, the mixture was slowly poured into ice water (150 mL). The precipitate was collected, washed with H_2O, and then crystallized from EtOH to give **7** (0.47 g, 90%). Mp 180–182 °C. IR (KBr): 1655, 1629, 1585, 1496, 1338, 1197. ^{1}H NMR (400 Hz, DMSO-d_6): 7.44–7.51 (m, 3H, Ar-H), 7.63 (d, 1H, J = 16.4 Hz, =CH), 7.74–7.84 (m, 3H, Ar-H), 8.02 (m, 1H, Ar-H), 8.16 (d, 1H, J = 16.4 Hz, =CH), 8.26 (m, 2H, Ar-H) 8.45 (s, 1H, 3-H). ^{13}C NMR (100 MHz, DMSO-d_6): 119.84, 123.64, 124.19, 124.75, 125.86, 127.87 (2C), 128.15, 128.75, 129.18 (2C), 129.25, 130.11, 132.79, 135.35, 139.47, 154.64. Anal. Calcd for $C_{17}H_{12}ClN{\cdot}1.7H_2O{\cdot}1.0HCl$: C, 61.35; H, 4.97; N, 4.21. Found: C, 61.21; H, 5.03; N, 4.23.

5.1.5. (*E*)-4-Chloro-8-methoxy-2-styrylquinoline-4-ol hydrochloride (8)

This compound was obtained from **6** and $POCl_3$ as described for **7** and was crystallized from EtOH in 90% yield. Mp 129–131 °C. IR (KBr): 1631, 1584, 1550, 1489, 1455, 1405, 1326, 1257, 1201, 1112, 1014. ^{1}H NMR (400 Hz, DMSO-d_6): 4.08 (s, 3H, OCH_3), 7.42–7.52 (m, 4H, Ar-H), 7.71–7.78 (m, 5H, Ar-H), 8.08 (d, 1H, J = 16.4 Hz, =CH), 8.49 (s, 1H, 3-H). ^{13}C NMR (100 MHz, DMSO-d_6): 56.46, 111.50, 115.25, 119.90, 124.64, 125.68, 127.78 (2C), 129.03, 129.16 (2C), 129.29, 129.88, 135.60, 138.79, 144.47, 153.31, 153.79. Anal. Calcd for $C_{18}H_{14}ClNO{\cdot}1.2H_2O{\cdot}1.0HCl$: C, 61.10; H, 4.96; N, 3.96. Found: C, 61.02; H, 4.91; N, 3.89.

5.1.6. (*E*)-1-[4-(2-Styrylquinolin-4-ylamino)phenyl]ethanone hydrochloride (9)

A mixture of **7** (0.54 g, 2 mmol), 4-aminoacetophenone (0.27 g, 2 mmol) and EtOH (30 mL) was refluxed for 2 h (TLC monitoring). The mixture was then cooled and evaporated in vacuo to yield a yellow residue, treated with H_2O (50 mL), and the resulting precipitate was filtered and washed with H_2O. The crude product was crystallized from EtOH to give **9** (0.67 g, 93%). Mp 298–300 °C. IR (KBr): 3311, 1674, 1637, 1585, 1547, 1493, 1442, 1357, 1268, 1183, 1183. ^{1}H NMR (400 Hz, DMSO-d_6): 2.64 (s, 3H, CH_3), 7.35 (s, 1H, 3-H), 7.44–7.49 (m, 3H, Ar-H), 7.54 (d, 1H, J = 16.4 Hz, =CH), 7.70–7.79 (m, 5H, Ar-H), 8.02 (m, 1H, 7-H), 8.13–8.18 (m, 3H, Ar-H), 8.36 (d, 1H, J = 8.8 Hz, 5-H), 8.79 (d, 1H, J = 8.4 Hz, 8-H), 10.98 (br s, 1H, HCl), 14.65 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.72, 99.64, 112.55, 117.23, 120.17, 123.61, 124.06 (2C), 126.83, 127.97 (2C), 129.11 (2C), 130.02 (2C), 130.42, 134.01, 134.47, 134.85, 138.87, 140.47, 142.15, 150.56, 153.52, 196.87. Anal. Calcd for $C_{25}H_{20}N_2O{\cdot}1.5H_2O{\cdot}1.0HCl$: C, 70.15; H, 5.66; N, 6.54. Found:C, 70.06; H, 5.73; N 6.58.

5.1.7. (*E*)-1-[4-(8-Methoxy-2-styrylquinolin-4-ylamino)phenyl]ethanone hydrochloride (10)

This compound was obtained from **8** and 4-aminoacetophenone as described for **9** and was crystallized from EtOH in 90% yield. Mp 179–181 °C. IR (KBr): 3343, 1677, 1625, 1586, 1550, 1492, 1432, 1266, 1160, 1067. ^{1}H NMR (400 Hz, DMSO-d_6): 2.63 (s, 3H, CH_3), 4.13 (s, 3H, OCH_3), 7.43–7.50 (m, 4H, Ar-H), 7.59 (d, 1H, J = 8.0 Hz, Ar-H), 7.67–7.75 (m, 5H, Ar-H), 7.89 (d, 1H, J = 16.4 Hz, =CH), 8.13 (m, 2H, Ar-H), 8.46 (d, 1H, J = 8.4 Hz, Ar-H), 11.00 (br s, 1H, HCl), 13.35 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.72, 56.87, 97.46, 113.18, 114.86, 118.46, 120.14, 123.74 (2C), 127.30, 127.95 (2C), 129.11 (2C), 129.64, 130.05 (2C), 130.36, 134.32, 135.02, 140.38, 142.26, 149.24, 150.72, 153.01, 196.90. Anal. Calcd for $C_{26}H_{22}N_2O_2{\cdot}1.2H_2O{\cdot}1.5HCl$: C, 66.31; H, 5.56; N, 5.95. Found: C, 66.48; H, 5.89; N, 5.98.

5.1.8. (*E*)-1-{4-[(*E*)-2-Styrylquinolin-4-ylamino]phenyl}ethanone oxime (11a)

A mixture of **9** (0.36 g, 1 mmol), $NH_2OH{\cdot}HCl$ (0.34 g, 5.0 mmol), and K_2CO_3 (0.21 g, 1.5 mmol) in EtOH (30 mL) was stirred at room temperature for 1 h (TLC monitoring). The mixture was evaporated under reduced pressure and then H_2O (80 mL) was added. The resulting precipitate was collected, washed with H_2O, and crystallized from EtOH to give **11a** (0.30 g, 85%). Mp 268–270 °C. IR (KBr):

3253, 1579, 1520, 1430, 1385, 1301, 1273, 1178, 1004. ^{1}H NMR (400 Hz, DMSO-d_6): 2.23 (s, 3H, CH_3), 7.17 (s, 1H, 3-H), 7.37–7.60 (m, 5H, Ar-H), 7.70–7.77 (m, 4H, Ar-H), 8.01 (m, 1H, 7-H), 8.22 (d, 1H, J = 16.8 Hz, =CH), 8.42 (d, 1H, J = 8.4 Hz, 5-H), 8.83 (d, 1H, J = 8.0 Hz, 8-H), 10.98 (s, 1H, NOH), 11.36 (br, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 11.53, 99.03, 116.81, 120.26, 122.81, 123.59, 124.91 (2C), 126.79, 126.94 (2C), 127.98 (2C), 129.09 (2C), 130.32, 133.88, 134.94, 135.42, 137.71, 138.88, 140.13, 150.22, 152.28, 154.09. Anal. Calcd for $C_{25}H_{21}N_3O{\cdot}0.4H_2O$: C, 77.66; H, 5.68; N, 10.87. Found: C, 77.56; H, 5.59; N, 10.63.

5.1.9. (*E*)-1-{4-[2-(*E*)-Styrylquinolin-4-ylamino]phenyl}ethanoneomethyl oxime (11b)

A mixture of **9** (0.36 g, 1 mmol), $NH_2OCH_3{\cdot}HCl$ (0.42 g, 5.0 mmol), and K_2CO_3 (0.21 g, 1.5 mmol) in EtOH (30 mL) was stirred at room temperature for 1 h (TLC monitoring). The mixture was evaporated under reduced pressure and then H_2O (80 mL) was added. The resulting precipitate was collected, washed with H_2O, and crystallized from EtOH to give **11b** (0.30 g, 80%). Mp 146–147 °C. IR (KBr): 3421, 1572, 1534, 1440, 1389, 1321, 1255, 1050. ^{1}H NMR (400 Hz, DMSO-d_6): 2.21 (s, 3H, CH_3), 3.93 (s, 3H, OMe), 7.26 (s, 1H, 3-H), 7.31–7.51 (m, 6H, Ar-H), 7.63 (m, 1H, Ar-H), 7.69–7.88 (m, 6H, Ar-H), 8.07 (d, 1H, J = 7.6 Hz, 5-H), 8.51 (d, 1H, J = 8.0 Hz, 8-H), 9.91 (br, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 12.22, 61.64, 100.75, 118.30, 121.72, 122.74 (2C), 125.00, 125.24, 125.52, 127.17 (2C), 127.59 (2C), 128.96 (2C), 129.35, 129.77, 131.74, 132.06, 135.69, 136.10, 140.30, 150.44, 153.06, 153.52. Anal. Calcd for $C_{26}H_{23}N_3O{\cdot}0.1H_2O$: C, 78.98; H, 5.92; N, 10.63. Found: C, 78.80; H, 5.92; N, 10.54.

5.1.10. (*E*)-1-{4-[8-Methoxy-(*E*)-2-styrylquinolin-4-ylamino]phenyl}ethanone oxime hydrochloride (12a)

This compound was obtained from **10** and $NH_2OH{\cdot}HCl$ as described for **11a** and was crystallized from EtOH in 90% yield. Mp 303–304 °C. IR (KBr): 3230, 1611, 1583, 1525, 1460, 1427, 1389, 1312, 1258, 1062. ^{1}H NMR (400 Hz, DMSO-d_6): 2.21 (s, 3H, CH_3), 4.10 (s, 3H, OCH_3), 7.33 (s, 1H, 3-H), 7.39–7.56 (m, 7H, Ar-H), 7.62–7.70 (m, 4H, Ar-H), 7.82 (m, 2H, Ar-H), 8.25 (d, 1H, J = 8.8 Hz, Ar-H), 10.44 (br s, 1H, NH), 11.30 (s, 1H, NOH). ^{13}C NMR (100 MHz, DMSO-d_6): 11.45, 56.65, 97.55, 112.33, 114.39, 118.45, 122.01, 123.91 (2C), 126.67, 126.91 (2C), 127.77 (2C), 129.04 (2C), 129.92, 130.9, 131.66, 134.61, 135.28, 138.42, 150.39, 151.04, 152.35. Anal. Calcd for $C_{25}H_{21}N_3O{\cdot}2.4H_2O{\cdot}1.0HCl$: C, 65.40; H, 5.88; N, 9.15. Found: C, 65.36; H, 5.69; N, 8.81.

5.1.11. (*E*)-1-{4-[8-Methoxy-(E)-2-styrylquinolin-4-ylamino]phenyl}ethanoneomethyl oxime hydrochloride (12b)

This compound was obtained from **10** and $NH_2OCH_3{\cdot}HCl$ as described for **11b** and was crystallized from EtOH in 80% yield. Mp 154–156 °C. IR (KBr): 3362, 1624, 1591, 1554, 1490, 1435, 1278, 1049. ^{1}H NMR (400 Hz, DMSO-d_6): 2.24 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 4.13 (s, 3H, OCH_3), 7.34 (s, 1H, 3-H), 7.42–7.46 (m, 3H, Ar-H), 7.55–7.48 (m, 3H, Ar-H), 7.66–7.71 (m, 4H Ar-H), 7.81 (d, 1H, J = 16.4 Hz, =CH), 7.85 (m, 2H, Ar-H), 8.38 (d, 1H, J = 8.8 Hz, Ar-H), 10.85 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 12.22, 56.81, 61.71, 96.72, 113.01, 114.67, 118.12, 120.48, 124.40 (2C), 127.07, 127.28 (2C), 127.90 (2C), 129.07 (2C), 129.79, 130.23, 133.90, 135.06, 138.43, 139.80, 149.34, 150.51, 153.23, 153.44. Anal. Calcd for $C_{27}H_{25}N_3O_2{\cdot}2.0H_2O{\cdot}1.0HCl$: C, 65.38; H, 6.10; N, 8.47. Found: C, 65.22; H, 6.11; N, 8.41.

5.1.12. 2-Styrylquinolin-8-ol (14a)

This compound was obtained from 8-hydroxy-2-methylquinoline and benzaldehyde as described for **5** and was crystallized from EtOH in 45% yield. Mp 104–105 °C (lit.: 96–100 °C).[26] IR (KBr): 3399, 2362, 1594, 1559, 1506. ^{1}H NMR (400 Hz, DMSO-d_6): 7.18 (dd, 1H, J = 7.6, 1.2 Hz, 7-H), 7.30 (dd, 1H, J = 8.4, 1.2 Hz, 5-H), 7.32–7.43 (m, 5H, Ar-H), 7.62–7.65 (m, 3H, Ar-H), 7.71 (d, 1H, J = 16.4 Hz, =CH), 8.11 (d, 1H, J = 8.8 Hz, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): 110.19, 117.64, 120.29, 127.23 (2C), 127.29, 127.44, 128.02, 128.75, 128.81 (2C), 134.39, 136.32, 136.43, 137.92, 152.00, 153.57. Anal. Calcd for $C_{17}H_{13}NO{\cdot}0.2H_2O$: C, 80.81; H, 5.42; N, 5.54. Found: C, 80.89; H, 5.36; N, 5.40.

5.1.13. 2-[2-(4-Hydroxyphenyl)vinyl]quinolin-8-ol (14b)

This compound was obtained from 8-hydroxy-2-methylquinoline and 4-hydroxybenzaldehyde as described for **5** and was crystallized from EtOH in 48% yield. Mp 125–126 °C (lit.: 235 °C).[31] IR (KBr): 3399, 1588, 1562, 1510. ^{1}H NMR (400 MHz, DMSO-d_6): 6.89 (m, 2H, Ar-H), 7.15–7.29 (m, 3H, Ar-H), 7.38 (m, 1H, 6-H), 7.53 (m, 2H, Ar-H), 7.59 (d, 1H, J = 8.4 Hz, 3-H), 7.65 (d, 1H, J = 16.4 Hz, =CH), 8.08 (d, 1H, J = 8.4 Hz, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): 110.10, 115.82 (2C), 117.70, 120.25, 125. 91, 127.01, 127.29, 128.85 (2C), 129.27, 133.94, 136.32, 137.95, 151.85, 153.94, 156.33. Anal. Calcd for $C_{17}H_{13}NO_2{\cdot}0.1H_2O$: C, 77.01; H, 5.02; N, 5.28. Found: C, 76.63; H, 5.14; N, 5.18.

5.1.14. 4-[2-(8-Hydroxyquinolin-2-yl)vinyl]benzonitrile (14c)

This compound was obtained from 8-hydroxy-2-methylquinoline and 4-cyanobenzaldehyde as described for **5** and was crystallized from EtOH in 62% yield. Mp 235–236 °C. IR (KBr): 3347, 2220, 1598, 1555, 1506. ^{1}H NMR (400 MHz, $CDCl_3$): 7.20 (dd, 1H, J = 7.6, 1.2 Hz, 7-H), 7.33 (dd, 1H, J = 8.4, 1.2 Hz, 5-H), 7.40–7.46 (m, 2H, Ar-H), 7.65 (d, 1H, J = 8.4 Hz, 3-H), 7.67–7.72 (m, 5H, Ar-H), 8.17 (d, 1H, J = 8.4 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.42, 111.68, 117.74, 118.79, 120.60, 127.55 (2C), 127.76, 127.92, 131.47, 132.05, 132.59 (2C), 136.74, 138. 00, 140.78, 152.09, 152.41. Anal. Calcd for $C_{18}H_{12}N_2O$: C, 79.39; H, 4.44; N, 10.29. Found: C, 79.50; H, 4.53; N, 10.22.

5.1.15. 2-[2-(4-Fluorophenyl)vinyl]quinolin-8-ol (14d)

This compound was obtained from 8-hydroxy-2-methylquinoline and 4-fluorobenzaldehyde as described for **5** and was crystallized from EtOH in 59% yield. Mp 107–108 °C. IR (KBr): 3398, 2364, 1630, 1594, 1561, 1510. ^{1}H NMR (400 MHz, $CDCl_3$): 7.07–7.13 (m, 2H, Ar-H), 7.18 (dd, 1H, J = 7.6, 1.2 Hz, 7-H), 7.27 (d, 1H, J = 16.0 Hz, =CH), 7.30 (dd, 1H, J = 8.4, 1.2 Hz, 5-H), 7.40 (dd, 1H, J = 8.4, 7.6 Hz, 6-H), 7.57–7.62 (m, 3H, Ar-H), 7.69 (d, 1H, J = 16.0 Hz, =CH), 8.11 (d, 1H, J = 8.8 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.16, 115.86 (2C, J = 21.3 Hz), 117.62, 120.33, 127.31, 127.44, 127.81 (J = 2.3 Hz), 128.85 (2C, J = 8.4 Hz), 130.54 (J = 3.0 Hz), 133.03, 136.44, 137.97, 151.99, 153.39, 162.98 (J = 247.0 Hz). Anal. Calcd for $C_{17}H_{12}FNO$: C, 76.97; H, 4.56; N, 5.28. Found: C, 76.99; H, 4.63; N, 5.26.

5.1.16. 2-[2-(4-Nitrophenyl)vinyl]quinolin-8-ol (14e)

This compound was obtained from 8-hydroxy-2-methylquinoline and 4-nitrobenzaldehyde as described for **5** and was crystallized from EtOH in 80% yield. Mp 200–201 °C. IR (KBr): 3342, 1591, 1508. ^{1}H NMR (400 MHz, $CDCl_3$): 7.21 (dd, 1H, J = 7.6, 1.2 Hz, 7-H), 7.34 (dd, 1H, J = 8.4, 1.2 Hz, 5-H), 7.43–7.50 (m, 2H, Ar-H), 7.67 (d, 1H, J = 8.4 Hz, 3-H), 7.75–7.78 (m, 3H, Ar-H), 8.19 (d, 1H, J = 8.4 Hz, 4-H), 8.28 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.52, 117.76, 120.65, 124.20 (2C), 127.65, 127.82 (2C), 128.05, 131.59, 132.20, 136.82, 138.00, 142.71, 147.43, 152.11, 152.27. Anal. Calcd for $C_{17}H_{12}N_2O_3$: C, 69.86; H, 4.14; N, 9.58. Found: C, 69.89; H, 4.17; N, 9.46.

5.1.17. 3-[2-(8-Hydroxyquinolin-2-yl)vinyl]benzene-1,2-diol (14f)

This compound was obtained from 8-hydroxy-2-methylquinoline and 2,3-duhydroxybenzaldehyde as described for **5** and was

crystallized from EtOH in 41% yield. Mp 173–174 °C. IR (KBr): 3342, 1600, 1505. ^{1}H NMR (400 Hz, DMSO-d_6): 6.68–6.80 (m, 2H, Ar-H), 7.06–7.12 (m, 2H, Ar-H), 7.32–7.38 (m, 2H, Ar-H), 7.47 (d, 1H, J = 16.4 Hz, =CH), 7.80 (d, 1H, J = 8.8 Hz, 3-H), 8.12 (d, 1H, J = 16.4 Hz, =CH), 8.26 (d, 1H, J = 8.8 Hz, 4-H), 8.92 (br s, 1H, OH), 9.53 (br s, 2H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): 111.27, 115.23, 117.58, 118.16, 119.12, 120.16, 123.67, 126.78, 127.55, 128.14, 130.36, 136.41, 138.20, 144.36, 145.60, 152.87, 154.27. Anal. Calcd for $C_{17}H_{13}NO_3{\cdot}0.5H_2O$: C, 70.81; H, 4.90; N, 4.85. Found: C, 70.46; H, 5.10; N, 4.82.

5.1.18. 2-(2-Pyridin-3-ylvinyl)quinolin-8-ol (14g)

This compound was obtained from 8-hydroxy-2-methylquinoline and pyridine-3-carboxaldehyde as described for **5** and was crystallized from EtOH in 51% yield. Mp 138–139 °C. IR (KBr): 3041, 2363, 1721, 1562, 1502. ^{1}H NMR (400 MHz, $CDCl_3$): 7.18 (dd, 1H, J = 7.2, 1.2 Hz, 7-H), 7.32 (dd, 1H, J = 8.4, 1.2 Hz, 5-H), 7.33–7.44 (m, 3H, Ar-H), 7.63 (d, 1H, J = 8.8 Hz, 3-H), 7.69 (d, 1H, J = 16.4 Hz, =CH), 7.95 (m, 1H, pyridinyl-H), 8.16 (d, 1H, J = 8.8 Hz, 4-H), 8.57 (dd, 1H, J = 4.8, 1.6 Hz, pyridinyl-H), 8.84 (d, 1H, J = 2.4 Hz, pyridinyl-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.33, 117.69, 120.39, 123.69, 127.66 (2C), 130.06, 130.40, 132.08, 133.34, 136.62, 137.98, 149.07, 149.48, 152.08, 152.76. Anal. Calcd for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.28. Found: C, 77.12; H, 4.90; N, 11.15.

5.1.19. 2-(2-Furan-2-ylvinyl)quinolin-8-ol (14h)

This compound was obtained from 8-hydroxy-2-methylquinoline and furfural as described for **5** and was crystallized from EtOH in 63% yield. Mp 173–174 °C. IR (KBr): 3336, 1736, 1641, 1591, 1552, 1504. ^{1}H NMR (400 MHz, $CDCl_3$): 6.48 (dd, 1H, J = 3.2, 1.6 Hz, furanyl-H), 6.55 (d, 1H, J = 3.6 Hz, furanyl-H), 7.15 (dd, 1H, J = 7.2, 1.6 Hz, 7-H), 7.22 (d, 1H, J = 16.0 Hz, =CH), 7.27 (dd, 1H, J = 8.0, 1.2 Hz, 5-H), 7.38 (dd, 1H, J = 8.0, 7.2 Hz, 6-H), 7.48 (d, 1H, J = 1.6 Hz, furanyl-H), 7.51 (d, 1H, J = 8.8 Hz, 3-H), 7.55 (d, 1H, J = 16.0 Hz, =CH), 8.07 (d, 1H, J = 8.8 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.06, 111.43, 112.06, 117.63, 120.85, 121.53, 125.88, 127.15, 127.38, 136.36, 138.00, 143.28, 151.92, 152.62, 153.21. ESIMS (m/z): 238 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.62; H, 4.70; N, 5.78.

5.1.20. 2-[2-(5-Nitrofuran-2-yl)vinyl]quinolin-8-ol (14i)

This compound was obtained from 8-hydroxy-2-methylquinoline and 5-nitro-2-furaldehyde diacetate as described for **5** and was crystallized from EtOH in 51% yield. Mp 179–180 °C (lit.: 192 °C).[32] IR (KBr): 3423, 2363, 1554, 1503. ^{1}H NMR (400 MHz, $CDCl_3$): 6.72 (d, 1H, J = 4.0 Hz, furanyl-H), 7.20 (dd, 1H, J = 8.0, 1.2 Hz, 7-H), 7.33 (dd, 1H, J = 8.0, 1.2 Hz, 5-H), 7.40 (d, 1H, J = 3.6 Hz, furanyl-H), 7.45 (dd, 1H, J = 8.0, 8.0 Hz, 6-H), 7.52–7.61 (m, 3H, Ar-H), 8.16 (d, 1H, J = 8.4 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.52, 112.68, 113.84, 117.80, 119.00, 121.56, 128.03, 128.26, 132.60, 136.92 (2C), 138.11, 151.25, 152.11, 154.91. ESIMS (m/z): 283 $[M+H]^+$. Anal. Calcd for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.92. Found: C, 63.58; H, 3.70; N, 9.83.

5.1.21. (*E*)-2-[2-(5-Nitrofuran-2-yl)vinyl]quinolin-4-yl acetate (15)

A mixture of 2-methylquinoline (0.72 g, 5 mmol), (5-nitrofuran-2-yl)methylene diacetate (4.87 g, 20 mmol) and acetic anhydride (150 mL) was heated at 150 °C for 30 h (TLC monitoring). After cooling, the solvent was removed in vacuo to provide the crude product, which was purified by flash column chromatography (FC, silica gel use CH_2Cl_2 as eluent) to give **15** (0.64 g, 45%). Mp >320 °C. IR (KBr): 1770, 1594, 1567, 1540, 1508, 1469, 1354, 1238, 1168, 1069. ^{1}H NMR (400 MHz, DMSO-d_6): 2.53 (s, 3H, CH_3), 7.23 (d, 1H, J = 4.0 Hz, furanyl-3-H), 7.57 (d, 1H, J = 16.4 Hz, =CH), 7.66 (m, 1H, Ar-H), 7.78–7.87 (m, 3H, Ar-H), 8.01 (d, 1H, J = 8.4 Hz, Ar-H), 8.07 (d, 1H, J = 8.8 Hz, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6): 20.78, 113.12, 114.50, 115.33, 120.43, 121.60, 127.45, 129.05, 130.91, 132.51, 149.15, 151.44, 154.53, 154.79, 154.86, 168.65. Anal. Calcd for $C_{17}H_{12}N_2O_5$: C, 62.97; H, 3.73; N, 8.64. Found: C, 62.98; H, 4.12; N, 8.46.

5.1.22. (*E*)-2-[2-(5-Nitrofuran-2-yl)vinyl]quinolin-4-ol (16)

A mixture of **15** (0.97 g, 3 mmol) was heated at 100 °C for 1 h (TLC monitoring) in pyridine/water (v/v = 4:1) (50 mL). After cooling, the solvent was removed in vacuo to provide the crude product, which was purified by FC ($MeOH/CH_2Cl_2$ = 1:20) to give **16** (0.44 g, 52%). Mp 305–307 °C. IR (KBr): 3452, 1626, 1594, 1538, 1508, 1470, 1389, 1352, 1242, 1017. ^{1}H NMR (400 MHz, DMSO-d_6): 6.47 (d, 1H, J = 1.6 Hz, 3-H), 7.15 (d, 1H, J = 3.6 Hz, furanyl-3-H), 7.25 (d, 1H, J = 16.4 Hz, =CH), 7.32 (m, 1H, Ar-H), 7.61–7.70 (m, 3H, Ar-H), 7.81 (d, 1H, J = 4.0 Hz, furanyl-4-H), 8.05 (dd, 1H, J = 8.0, 1.2 Hz, 5-H), 11.69 (br s, 1H, 4-OH). ^{13}C NMR (100 MHz, DMSO-d_6): 107.93, 114.75, 115.22, 118.34, 120.79, 123.37, 124.79, 125.31, 126.49, 132.22, 140.17, 145.25, 151.64, 153.88, 177.07. Anal. Calcd for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.92. Found: C, 63.47; H, 3.69; N, 9.73.

5.1.23. (*E*)-4-Chloro-2-[2-(5-nitrofuran-2-yl)vinyl]quinoline (17)

A mixture of **16** (1.41 g, 5 mmol), $POCl_3$ (100 mL) was heated at 80–90 °C for 1 h (TLC monitoring). After cooling, the mixture was slowly poured into ice water (200 mL). The precipitate was collected, washed with H_2O, and then crystallized from EtOH to give 17 (1.42 g, 95%). Mp 206–207 °C (lit.: 205 °C).[33] IR (KBr): 1637, 1581, 1515, 1486, 1450, 1391, 1350, 1332. 1249, 1041. ^{1}H NMR (400 MHz, DMSO-d_6): 7.24 (d, 1H, J = 4.0 Hz, furanyl-3-H), 7.57 (d, 1H, J = 16.4 Hz, =CH), 7.74–7.92 (m, 4H, Ar-H), 8.07 (d, 1H, J = 8.4 Hz, 8-H), 8.18 (d, 1H, J = 8.4 Hz, 5-H), 8.22 (s, 1H, 3-H). ^{13}C NMR (100 MHz, DMSO-d_6): 114.73, 115.31, 120.90, 120.93, 123.63, 124.96, 128.39, 129.50, 131.37, 131.77, 141.89, 148.29, 151.50, 154.16, 154.70. Anal. Calcd for $C_{15}H_9ClN_2O_3$: C, 59.92; H, 3.02; N, 9.32. Found: C, 59.58; H, 3.40; N, 9.23.

5.1.24. (*E*)-1-{4-{2-[2-(5-Nitrofuran-2-yl)vinyl]quinolin-4-ylamino}phenyl}ethanone hydrochloride (18)

A mixture of **17** (0.60 g, 2 mmol) and 4-aminoacetophenone (0.27 g, 2 mmol) in EtOH/HCl (30:1 mL) was refluxed for 2 h (TLC monitoring). The mixture was then cooled and evaporated in vacuo to yield a yellow residue, treated with H_2O (50 mL), and the resulting precipitate was filtered and washed with H_2O. The crude product was crystallized from EtOH to give 17a (0.64 g, 80%). Mp 275–276 °C. IR (KBr): 3432, 1675, 1587, 1548, 1518, 1464, 1357, 1274, 1244, 1197, 1026. ^{1}H NMR (400 MHz, DMSO-d_6): 2.60 (s, 3H, Me), 7.46 (s, 1H, 3-H), 7.51 (d, 1H, J = 16.0 Hz, =CH), 7.55 (d, 1H, J = 4.0 Hz, furanyl-3-H), 7.59 (m, 2H, Ar-H), 7.68 (m, 1H, Ar-H), 7.90 (m, 1H, Ar-H), 8.01 (m, 2H, Ar-H), 8.08 (m, 2H, Ar-H), 8.12 (d, 1H, J = 4.0 Hz, furanyl-4-H), 8.48 (m, 1H, Ar-H), 10.12 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.60, 102.69, 115.22, 115.56, 118.77, 121.57, 122.87 (2C), 124.89, 126.35, 127.90, 128.87, 129.18, 130.14 (2C), 130.96, 132.34, 132.74, 143.97, 148.21, 150.25, 151.45, 196.56. ESIMS (m/z): 400 $[M+H]^+$. Anal. Calcd for $C_{23}H_{17}N_3O_4{\cdot}1.0HCl$: C, 63.38; H, 4.16; N, 9.64. Found: C, 63.58; H, 4.25; N, 9.39.

5.1.25. (*E*)-1-{4-{2-[(*E*)-2-(5-Nitrofuran-2-yl)vinyl]quinolin-4-ylamino}phenyl}ethanone oxime hydrochloride (19a)

A mixture of **18** (0.40 g, 1 mmol), $NH_2OH{\cdot}HCl$ (0.34 g, 5.0 mmol), and K_2CO_3 (0.21 g, 1.5 mmol) in EtOH (30 mL) was stirred at room temperature for 1 h (TLC monitoring). The mixture was evaporated under reduced pressure and then H_2O (80 mL) was added. The resulting precipitate was collected, washed with H_2O,

and crystallized from EtOH to give **19a** (0.35 g, 85%). Mp 289–290 °C. IR (KBr): 3255, 1628, 1589, 1529, 1474, 1441, 1375, 1341, 1244, 1014. ^{1}H NMR (400 MHz, DMSO-d_6): 2.21 (s, 3H, Me), 7.22 (d, 1H, J = 3.2 Hz, furanyl-3-H), 7.29 (s, 1H, 3-H), 7.48 (d, 1H, J = 16.4 Hz, =CH), 7.50 (m, 2H, Ar-H), 7.72–8.00 (m, 6H, Ar-H), 8.11 (d, 1H, J = 8.4 Hz, 5-H), 8.67 (d, 1H, J = 8.4 Hz, 8-H), 10.60 (br s, 1H, NH), 11.32 (s, 1H, NOH). ^{13}C NMR (100 MHz, DMSO-d_6): 11.48, 99.63, 100.87, 115.09 (2C), 115.42, 116.23, 117.41, 121.64, 123.22, 124.32, 126.19, 126.86, 127.01 (3C), 133.69, 135.04, 138.06, 149.32, 151.92, 152.37, 153.42. ESIMS (m/z): 415 $[M+H]^+$. Anal. Calcd for $C_{23}H_{18}N_4O_4{\cdot}0.2H_2O{\cdot}1.0HCl$: C, 60.79; H, 4.30; N, 12.33. Found: C, 60.86; H, 4.59; N, 12.20.

5.1.26. (*E*)-1-{4-{2-[(*E*)-2-(5-Nitrofuran-2-yl)vinyl]quinolin-4-ylamino}phenyl}ethanone *O*-methyl oxime hydrochloride (19b)

A mixture of **18** (0.40 g, 1 mmol), $NH_2OCH_3{\cdot}HCl$ (0.41 g, 5.0 mmol), and K_2CO_3 (0.21 g, 1.5 mmol) in EtOH (30 mL) was stirred at room temperature for 1 h (TLC monitoring). The mixture was evaporated under reduced pressure and then H_2O (80 mL) was added. The resulting precipitate was collected, washed with H_2O, and crystallized from EtOH to give **19b** (0.34 g, 80%). Mp 230–231 °C. IR (KBr): 3384, 1633, 1592, 1547, 1521, 1473, 1448, 1355, 1246, 1055. ^{1}H NMR (400 MHz, DMSO-d_6): 2.20 (s, 3H, Me), 3.92 (s, 3H, OMe), 7.12 (d, 1H, J = 3.6 Hz, furanyl-3-H), 7.23 (s, 1H, 3-H), 7.34 (d, 1H, J = 16.4 Hz, =CH), 7.51 (d, 2H, J = 8.8 Hz, Ar-H), 7.65–7.72 (m, 3H, Ar-H), 7.79 (d, 2H, J = 8.8 Hz), 7.89–7.97 (m, 2H, Ar-H), 8.52 (d, 1H, J = 8.4 Hz, 8-H), 10.42 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 12.67, 62.11, 99.95, 115.45, 116.53, 117.86, 122.51, 123.27, 124.07, 124.35 (2C), 126.27, 127.29, 127.76 (2C), 133.90, 134.00, 139.10, 141.14, 149.98, 152.15, 153.10, 153.79, 154.06. ESIMS (m/z): 429 $[M+H]^+$. Anal. Calcd for $C_{24}H_{20}N_4O_4{\cdot}0.6H_2O{\cdot}1.0HCl$: C, 60.60; H, 4.70; N, 11.78. Found: C, 60.70; H, 5.04; N, 11.77.

5.2. Cell Growth Inhibitory Assay

Human breast carcinoma MCF-7 cells, nonsmall cell lung carcinoma H460 cells, and human glioma (SF-268) cells were maintained in RPMI-1640 medium supplied with 5% fetal bovine serum. Cell in logarithmic phase were cultured at a density of 5000 cells/mL/well in a 24-well plate. The cells were exposed in 4.0 μg/mL of the tested drugs for 72 h. The MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay was used to evaluate the effect of the tested compounds on cell growth as described previously.[34]

5.3. Measurements of IC_{50} in LNCAP and PC3 cells

LNCaP and PC3 cells were obtained from the American Type Culture Collection. These cells were maintained in RPMI-1640 supplemented with 10% nonheat inactivated fetal bovine serum (FBS) (Invitrogen). Exponentially growing cells (5×10^3 cells) were plated in 96-well plates and incubated with various concentrations of compounds through a series of 2× fold-dilution from 10 to 0.02 μM. Each concentration was tetra-plicate. Incubation was carried out at 37 °C for 48 h. ATPLite assay reagents (Perkin-Elmer) were added and luminescence measured by TopCounter (Perkin-Elmer). The concentration that killed 50% of cells (IC50) was determined from the curve by calculating the concentration of agent that reduced the readout of luciferase activity in treated cells, compared to control cells by SigmaPlot (SYSTAT).

5.4. Flow cytometric analysis of cell cycle

About 5×10^5 cells of LNCaP and PC3 were seeded onto 10-cm dish, cultured for 12 h and then treated with 0.5 μM and 0.2 μM of **14i** for 48 and 24 h, respectively. The cells were harvested by trypsinization, centrifuged, washed with PBS and collected by centrifugation. The cells were fixed with ice-cold 70% ethanol for 30 min, washed with PBS and centrifuged to remove supernatant. The cells were re-suspended by PBS containing contain 0.05% Triton X-100 and RNAase A (40 μg/mL) and incubated at 37 °C for 1 h and propidium iodide (PI) was added into the cell suspension to final concentration 50 μg/mL for another 1 h incubation. The cells were harvested by centrifugation, washed with PBS and centrifuged to remove supernatant. Finally, the cells were re-suspended by PBS and analyzed by Coulter EPICS XL Flow Cytometry (Beckman Coulter) with Win cycle software (Beckman Coulter).

5.5. Flow cytometric analysis of Annexin-V staining

About 5×10^5 cells of LNCaP and PC3 were seeded onto 10-cm dish, cultured for 12 h and then treated with 0.5 μM and 0.2 μM of **14i**, respectively, for 24 and 48 h. The cell media were removed into a centrifugation tube. The attached cells were washed by PBS and harvested by trypsinization. The washed PBS and harvested cells were collected into the above centrifugation tube, which contained the cell media. The tubes were centrifuged to remove supernatant and the cell pellets were washed by PBS twice. After centrifugation, the cell pellets were re-suspended with 200 μL Annexin-V binding buffer (Strong Biotech), which contain Annexin-V (1 μg/mL) and PI (2.5 μg/mL), and then incubated at room temperature for 15 min. Finally Annexin-V buffer were added into this cell suspension to final 1000 μL and analyzed by Coulter EPICS XL Flow Cytometry (Beckman Coulter) with XL SYSTEM II software (Beckman Coulter).

5.6. Analysis of the cleavage of poly (ADP-ribose) polymerase (PARP) in PC3 cells by Western blot

About 5×10^5 cells of PC3 were seeded onto 10-cm dish, cultured for 12 h and then treated with 0, 0.1, 0.2, 0.4 and 0.6 μM of **14i**, respectively, for 24 h. Then the cells were subjected to sonification and centrifuged to remove the cell debris, and the supernatants were collected. Protein concentration was determined by a BCA Protein Assay Kit (PIERCE). About 60 μg of protein/per well were resolved in NuPAGE Novex Tris–Acetate Mini Gel (Invitrogen). After electrophoresis, the proteins were transferred to a nitrocellulose membrane. The transferred membranes were blocked in 5% (w/v) nonfat dry milk in TBST (0.5 M NaCl, 20 mM Tris–HCl, 0.05% (v/v) Tween 20, pH 7.4) and probed for the respective anti-PARP antibody (Santa Crutz) and anti-actin antibody (Santa Crutz), followed by incubation with a secondary antibody containing horseradish peroxidase (anti-mouse, anti-goat; Jackson ImmunoResearch) and visualization with a SuperSignal West Pico Rabbit IgG Detection kit (PIERCE) by the ChemiDoc XRS system (Bio-Rad).

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