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Original article

Synthesis and anticancer activity of *N*-substituted 2-arylquinazolinones bearing *trans*-stilbene scaffold

Mohammad Mahdavi [a], Keyvan Pedrood [b], Maliheh Safavi [c], Mina Saeedi [d, e], Mahboobeh Pordeli [f], Sussan Kabudanian Ardestani [f], Saeed Emami [g], Mehdi Adib [b], Alireza Foroumadi [a], Abbas Shafiee [a, *]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran
[b] School of Chemistry, College of Science, University of Tehran, PO Box 14155-6455, Tehran, Iran
[c] Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Iran
[d] Medicinal Plants Research Center, Tehran University of Medical Sciences, Tehran, Iran
[e] Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran
[f] Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[g] Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

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ABSTRACT

A novel series of 2-arylquinazolinones **7a-o** bearing *trans*-stilbene moiety were designed, synthesized, and evaluated against human breast cancer cell lines including human breast adenocarcinoma (MCF-7 and MDA-MB-231) and human ductal breast epithelial tumor (T-47D). Among the tested compounds, the *sec*-butyl derivative **7h** showed the best profile of activity (IC_{50} < 5 μM) against all cell lines, being 2-fold more potent than standard drug, etoposide. Our investigation revealed that the cytotoxic activity was significantly affected by N3-alkyl substituents. Furthermore, the morphological analysis by acridine orange/ethidium bromide double staining test and flow cytometry analysis indicated that the prototype compound **7h** can induce apoptosis in MCF-7 and MDA-MB-231 cells.

1. Introduction

Cancer has been emerged as a major health problem and an important cause of mortality and morbidity worldwide [1]. Several main factors such as incorrect diet, genetic predisposition, and environmental contaminants contribute to the outbreak of cancers [2]. Although major advances in cellular and molecular biology have led to the improvement of chemotherapeutic management of cancer, the continued efforts to discover new anticancer agents in order to overcome resistance and toxicity concerns, is necessary [3].

To develop novel lead compounds as efficient anticancer agents, quinazoline derivatives attracted our attention. Quinazolines are considered as important bioactive scaffold due to their diverse biological activities including anti-inflammatory [4,5], antimicrobial [6–8], anti-virus [9], antimalarial [10], anti-tuberculosis [11], antioxidant [12], anti-hypertensive [13,14], anti-convulsant [15,16], anti-psychotic [17], anti-diabetes [18], and antihyperlipidemic [19] activities. In particular, many quinazoline derivatives have been reported as anti-cancer agents [20–26]. Furthermore, some quinazolinone analogs demonstrated potent anticancer activity *via* inhibition of dihydrofolate reductase enzyme [27]. Xia et al. reported a series of 2-aryl quinazolinones (Fig. 1) as antitumor agents and inhibitor of tubulin polymerization [28]. Accordingly the quinazoline scaffold is considered as an attractive heterocycle in the field of anti-cancer drug design and development.

In continuation of our efforts to develop anti-cancer agents [29–31], herein, we decided to investigate novel series of 3*H*-quinazolin-4-one derivatives bearing *trans*-stilbene moiety at 2-position. For this purpose, various 2-aryl quinazolinones **7a-o** were designed, synthesized and evaluated for their cytotoxic activity. Also, the structure-activity relationship of the corresponding

* Corresponding author.
E-mail address: ashafiee@ams.ac.ir (A. Shafiee).

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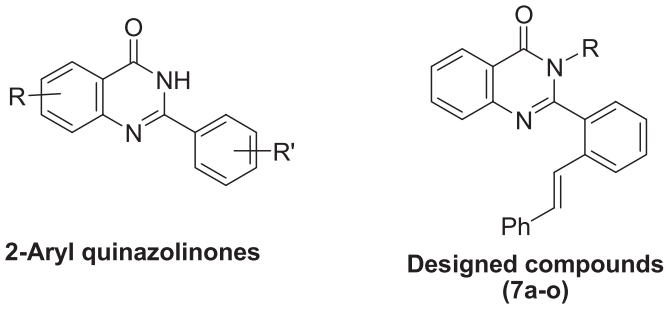


Fig. 1. Structures of 2-aryl quinazolines and designed copounds **7a-o** as cytotoxic agents.

compounds was studied (Fig. 1).

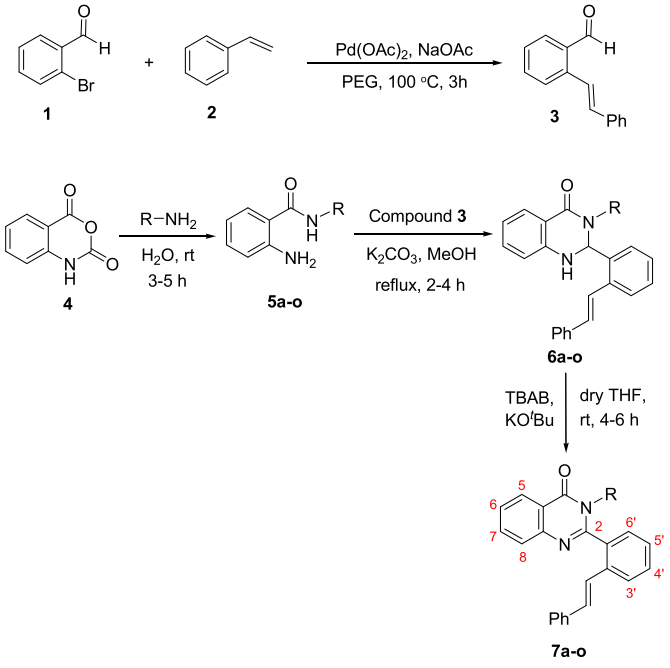
2. Results and discussion

2.1. Chemistry

The synthetic route to target compounds **7** was illustrated in Scheme 1. First, 2-bromobenzaldehyde (**1**) reacted with styrene (**2**) in the presence of palladium (II) acetate, and sodium acetate to give *trans*-stilbene **3** [32]. Also, the anthranilamide derivatives **5** were prepared from the reaction of isatoic anhydride (**4**) and appropriate primary amine in water at room temperature [33]. The reaction of compound **5** with aldehyde **3** in the presence of potassium carbonate in refluxing methanol afforded the corresponding 2,3-dihydroquinazolinone **6**. Finally, compounds **6a-o** were converted to quinazolinone derivatives **7a-o** using tetrabutylammonium bromide (TBAB) and potassium *tert*-butoxide in dry THF.

2.2. Cytotoxic activity

The cytotoxic activity of final compounds **7a-o** was evaluated against three human cancer cell lines including MCF-7, MDA-MB-231, and T-47D using MTT assay. The obtained IC_{50} values for the



Scheme 1. Synthesis of compounds **7**.

tested compounds were listed in Table 1, compared with the standard drug etoposide.

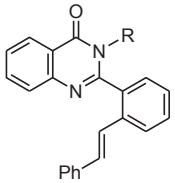
Structurally, tested compounds can be classified as *N*-alkyl, *N*-aryl, and *N*-benzyl derivatives (**7a-i**, **7j-l**, and **7m-o** respectively). In general, *N*-alkyl derivatives **7a** and **7c-h** showed significant cytotoxic activity against all cell lines. Among the *N*-aryl (**7j-o**) and *N*-benzyl derivatives (**7m-o**), only compound **7n** (R = 4-methylbenzyl) was active against all cell lines and also, *N*-phenyl analog **7j** showed inhibitory activity against MCF-7 cells.

The obtained IC_{50} values related to MCF-7 and T-47D cells revealed that compound **7h** had the highest activity against these cell lines. In the case of MDA-MB-231 cells, compounds **7e** and **7f** were the most potent compounds (IC_{50}s = 4.1 and 4.0 μM, respectively). The activities of compound **7c**, **7d**, and **7h** against MCF-7 were superior to that of standard drug, etoposide. Compounds **7a** and **7c-h** with IC_{50} values of 4.0–8.4 μM were more potent than etoposide against MDA-MB-231 cells. Against T-47D, compounds **7c-f** and **7h** showed higher activities when compared to etoposide. In particular, compounds **7h** displayed IC_{50} values of 3.8, 4.9 and 3.6 μM against MCF-7, MDA-MB-231, and T-47D cell lines, being 2-fold less than those of etoposide (IC_{50}s = 7.6, 10.3 and 8.9 μM, respectively).

As mentioned above, *N*-alkyl substituent was more biologically favored than *N*-aryl and *N*-benzyl groups. However, *N*-cyclopentyl analog **7i** showed no activity towards all cell lines and *N*-propyl derivative **7b** was active only against MCF-7. Interestingly, the susceptibility of all tested cell lines to the most of *N*-alkyl quinazolinones was similar and the observed IC_{50}s showed no significant differences. However, in the case of compound **7g**, the susceptibility of MCF-7 was significantly less than MDA-MB-231 and T-47D. In contrast, MCF-7 cells were susceptible to compounds **7b** at concentrations less than 30 μM but MDA-MB-231 and T-47D were resistant to this compound (IC_{50} > 100 μM).

The IC_{50} values of compounds **7j-o** demonstrated that the aryl or benzyl substituents were not favorable from anti-cancer activity point of view; however, 4-methyl substitution on benzyl moiety in compound **7n**, showed mild activity against all cells (IC_{50} values = 23.8–28.2 μM). It is speculated that the substituent at the

Table 1
Cytotoxic activities (IC_{50}s, μM) of compounds **7a-o** against human cancer cell lines.



Compound	R	MCF-7	MDA-MB-231	T-47D
7a	Ethyl-	8.7 ± 1.4	8.4 ± 2.1	10.2 ± 2.3
7b	Propyl-	29.4 ± 2.8	>100	>100
7c	Isopropyl-	6.7 ± 2.8	8.4 ± 0.1	6.2 ± 2.1
7d	Cyclopropyl-	5.3 ± 1.3	5.5 ± 0.8	6.8 ± 2.4
7e	Allyl-	10.3 ± 2.8	4.1 ± 2.2	5.8 ± 1.1
7f	Butyl-	9.8 ± 1.8	4.0 ± 0.9	7.9 ± 1.8
7g	Isobutyl-	23.3 ± 1.3	6.9 ± 0.4	9.3 ± 2.6
7h	*sec*-Butyl-	3.8 ± 2.1	4.9 ± 1.6	3.6 ± 1.2
7i	Cyclopentyl-	>100	>100	>100
7j	Phenyl-	44.9 ± 0.9	>100	>100
7k	*p*-Tolyl-	>100	>100	>100
7l	4-Methoxyphenyl-	>100	>100	>100
7m	Benzyl-	>100	>100	>100
7n	4-Methylbenzyl-	24.7 ± 1.1	23.8 ± 0.2	28.2 ± 1.5
7o	4-Methoxybenzyl-	>100	>100	>100
Etoposide		7.6 ± 2.1	10.3 ± 1.0	8.9 ± 2.1

N-3 position of quinazolinone ring interacts with a hydrophobic pocket in the target binding site by means of van der Waals interactions. Varying the length and bulk of the substituent (e.g. alkyl, aryl or benzyl groups) allows us to probe the depth and width of the pocket. It seems the depth and width of the pocket is favorable for *N*-alkyl groups such as ethyl, isopropyl, cyclopropyl, butyl, isobutyl, *sec*-butyl or allyl, but more bulky substituents (e.g., cyclopentyl, aryl or benzyl groups) may prevent the molecule from binding to the target. Furthermore, a great variation between isobutyl and *sec*-butyl derivatives (compounds **7g** and **7h**, respectively) was observed in biological activity against MCF-7. This could be due to the different bond connectivity of isobutyl and *sec*-butyl groups to the N-3 position which resulted in different steric effect on 2-aryl moiety.

2.3. Morphological analysis

Based on the molecular classification of breast carcinoma, MCF-7 and T-47D are luminal A cell lines and MDA-MB-231 is a triple negative breast cancer cell line. In this study, MCF-7 and MDA-MB-231 cells were selected as a representative for each class. Also, two potent compounds **7d** and **7h** which showed very good activity against all three cancer cell lines were nominated for more detailed investigations. Accordingly, compounds **7d** and **7h** were investigated in comparison with etoposide for identification of apoptosis induced by MCF-7 and MDA-MB-231 cells.

Morphological analysis of **7d** and **7h**-treated cells was accomplished using fluorescence microscopy after double staining with acridine orange/ethidium bromide. As shown in Figs. 2 and 3, morphological findings indicated that the compounds **7d** and **7h** reduced cell viability and induced apoptosis in MCF-7 and MDA-MB-231 cells.

2.4. Flow cytometry analysis

Flow cytometry analyses were performed to confirm apoptosis induced by representative compounds **7d** and **7h** in comparison to standard drug etoposide. Annexin V-FITC/PI double staining followed by flow cytometric analysis detects the externalization of phosphatidylserine in apoptotic cells using recombinant annexin V conjugated to green-fluorescent FITC dye and dead cells using propidium iodide (PI). Flow cytometric analysis revealed that cells undergo apoptosis after treatment with the test compounds. As shown in Figs. 4 and 5, the apoptotic index of the compounds **7d** and **7h** were compared with negative control and etoposide in MDA-MB-231 and MCF-7 cell lines. Although the tested compounds have caused necrosis in treated cells to some extent the results showed that the exposure of MDA-MB-231 cells to IC_{50} concentrations of **7h** and **7d** induced apoptosis in 16.55% and 22.41% of the cells after 12 h, respectively. The apoptosis inducing effect of compound **7d** in MDA-MB-231 cells is comparable with etoposide apoptosis inducing ability. The results also revealed that the percentage of MCF-7 cells undergoing apoptosis after 12 h of exposure to compounds **7h** and **7d** was higher than MDA-MB-231 (26.46% and 25.88% apoptosis in the **7h** and **7d**-treated cells, respectively). According to these data the cytotoxic activity of compounds **7h** and **7d** in MDA-MB-231 and MCF-7 cell lines occurs through apoptosis.

3. Conclusion

We described the synthesis of N3-substituted quinazolinones **7a-o** bearing *trans*-stilbene moiety as anti-proliferative agents against cancer cell lines. The results of cytotoxic assay against MCF-7, MDA-MB-231 and T-47D cells revealed that N3-alkyl derivatives had significant inhibitory activity against all tested cell lines. In contrast, the most of N3-aryl and N3-benzyl analogs were inactive. Among the tested compounds, the *sec*-butyl derivative **7h** showed

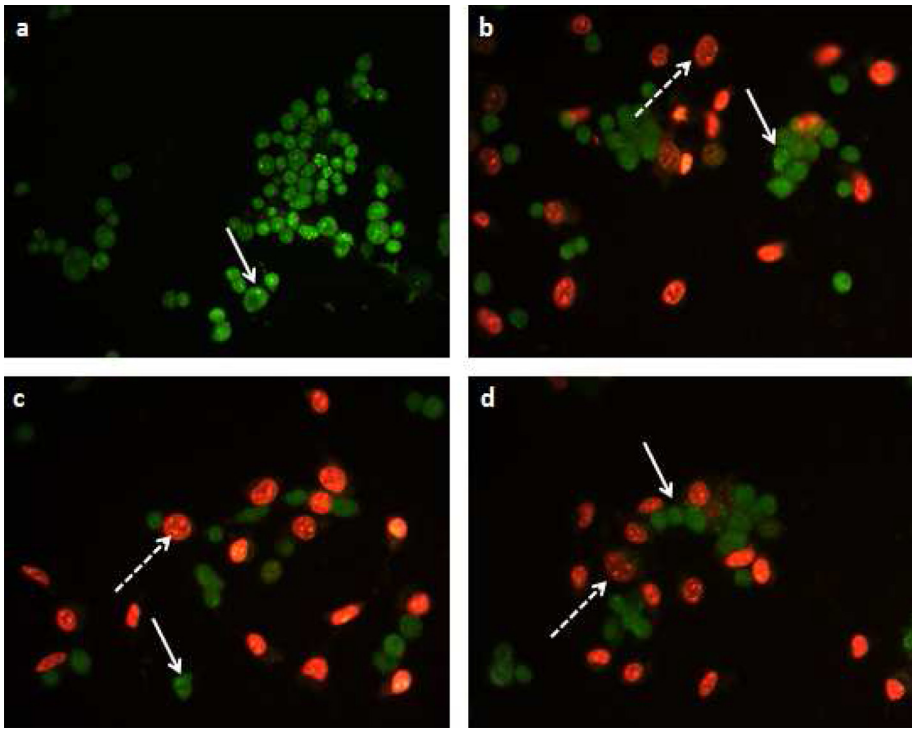


Fig. 2. Morphological analysis of MCF-7 cells by double staining method. a) Control condition, b) cells treated with IC_{50} value of etoposid for 24 h, c) cells treated with IC_{50} value of **7h** for 24 h. d) cells treated with IC_{50} value of **7d** for 24 h. White arrow indicates live cells, dashed arrow shows apoptosis.

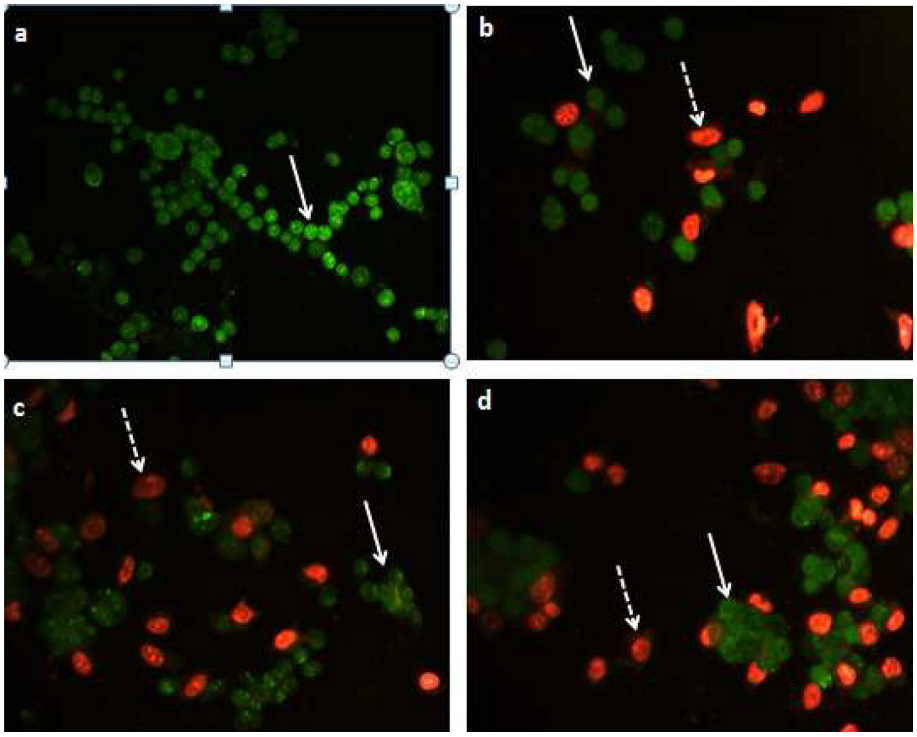


Fig. 3. Morphological analysis of MDA-MB-231 cells by double staining method. a) Control condition, b) cells treated with IC_{50} value of etoposid for 24 h, c) cells treated with IC_{50} value of **7h** for 24 h. d) cells treated with IC_{50} value of **7d** for 24 h. White arrow indicates live cells, dashed arrow shows apoptosis.

the best profile of activity as its IC_{50} was less than 5 μM for all tested cell lines. It was two times more potent than standard drug, etoposide. In addition to compound **7h**, the activities of isopropyl and cyclopropyl derivatives (compounds **7c** and **7d**, respectively) were higher than that of etoposide against all cell lines. The results of our study revealed that the N3-substituted quinazolinones having *trans*-stilbene moeity could be considered as a new bioactive structure and the optimum cytotoxic potency of the molecule would be achieved by introduction of an appropriate alkyl group such as cyclopropyl, isopropyl or *sec*-butyl on the 3-position of quinazoline ring. It seems that the substituent at the N-3 position of quinazolinone ring interacts with a hydrophobic pocket in the target binding site by means of van der Waals interactions. Thus, the length and bulk of the optimum substituents such as cyclopropyl, isopropyl or *sec*-butyl allows to favorable fitting to the pocket. However, more bulky substituents such as cyclopentyl, aryl or benzyl groups may prevent the prototype molecule from binding to the target and results in diminishing the activity.

4. Experimental

4.1. Chemistry

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on Bruker FT-400 and 500, using TMS as an internal standard. The IR spectra were obtained on a Nicolet Magna FT-IR 550 spectrophotometer (potassium bromide disks). Mass spectra were recorded on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. The elemental analysis was performed with an Elemetar Analysen system GmbH VarioEL CHNS mode. Purification of all product were conducted by column chromatography on silica gel using petroleum ether and ethyl acetate as eluent.

4.1.1. General procedure for the preparation of (E)-2-styrylbenzaldehyde (**3**)

A mixture of 2-bromobenzaldehyde (**1**, 1 mmol), styrene (**2**, 2 mmol), palladium(II) acetate (0.01 mmol), and sodium acetate (4 mmol) in polyethylene glycol (3 mL) was heated at 100 °C. After 3 h, the reaction mixture was poured into water (20 mL), extracted with diethyl ether (3 × 20 mL) and purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 15:1) (yield 90%).

4.1.2. General procedure for the preparation of 2-aminobenzamides ***5a-o***

A mixture of isatoic anhydride (**4**, 10 mmol) and appropriate amine (10 mmol) in water (20 mL) was stirred for 3–5 h at room temperature. After completion of the reaction (checked by TLC), the resulting off-white benzamides **5a-o** were filtered off and used for the next reaction without further purifications (yields 85–95%).

4.1.3. General procedure for the preparation of 2,3-dihydroquinazolin-4(1H)-one derivatives ***6a-o***

A mixture of 2-aminobenzamide **5a-o** (1 mmol), (*E*)-2-styrylbenzaldehyde (**3**, 1 mmol), and potassium carbonate (1.2 mmol) in MeOH (5 mL) was heated under reflux for 2–4 h. After completion of the reaction (checked by TLC), potassium carbonate was filtered off from the hot solution and pure products **6a-o** were obtained as white crystals after the solution was cooled down to room temperature (yields 90–95%).

4.1.4. General procedure for the preparation of quinazolin-4(3H)-one derivatives ***7a-o***

A mixture of 2,3-dihydroquinazolin-4(1*H*)-one **6a-o** (1 mmol), tetrabutylammonium bromide (TBAB, 1 mmol), potassium *tert*-butoxide (1 mmol, 0.11 g) in dry THF (3 mL) was stirred at room temperature for 4–6 h. Upon completion of reaction, the solvent was evaporated under vacuum. After addition of water to the

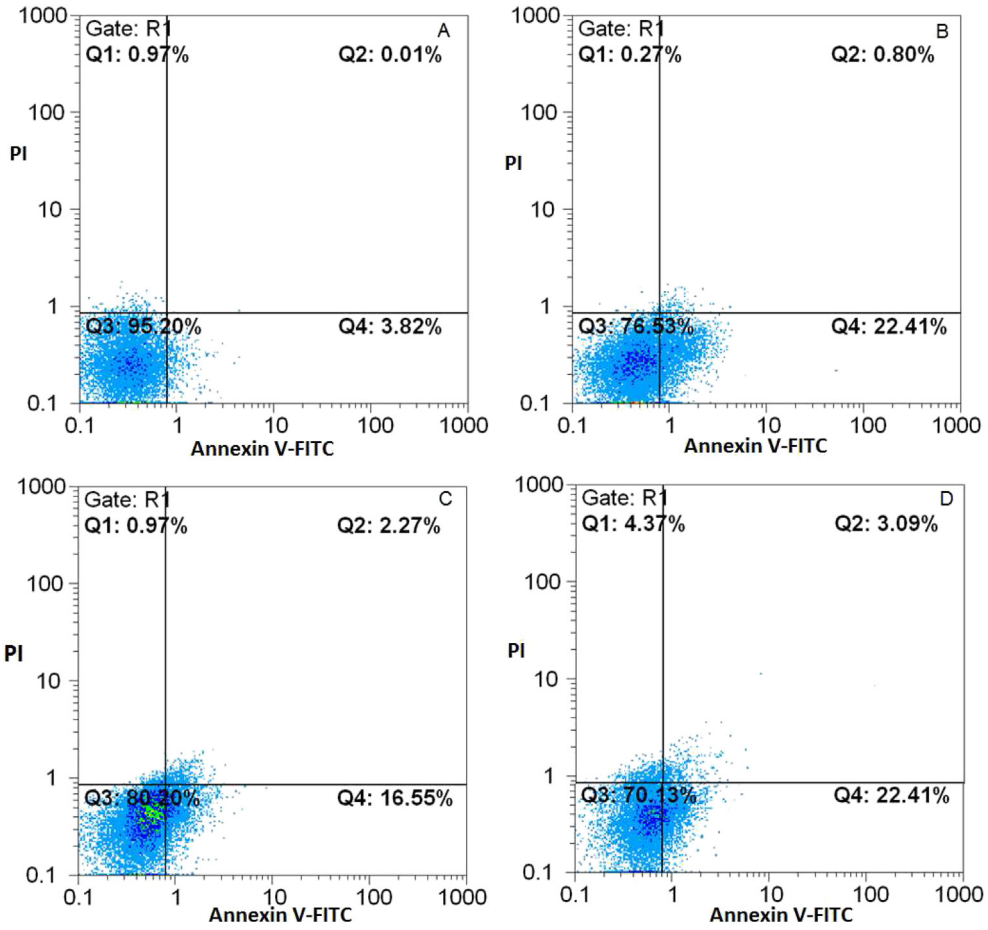


Fig. 4. Flow cytometric analysis of MDA-MB231 cells treated with synthetic compounds **7h** and **7d**. Cells were stained with Annexin V-FITC/PI and quantitated by flow cytometry. The cells treated with A) DMSO 1% (negative control); B) IC_{50} values of etoposide as positive control; C) IC_{50} values of compound **7h**; D) IC_{50} values of compound **7d** for 12 h.

residue, the mixture was extracted with diethyl ether (3 × 20 mL) and purified by column chromatography eluting with petroleum ether/ethyl acetate (10:1).

*4.1.4.1. (E)-3-Ethyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7a**).* Yield: 78%; white crystals; mp 154–156 °C. IR (KBr): 3055, 2974, 2867, 1671, 1597 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 1.17 (t, J = 6.8 Hz, 3H, CH_3), 3.72 (dq, J = 20.8, 6.8 Hz, 1H, CH_2), 4.20 (dq, J = 20.8, 6.8 Hz, 1H, CH_2), 6.90 (d, J = 16.4 Hz, 1H, CH), 7.16 (d, J = 16.4 Hz, 1H, CH), 7.22–7.29 (m, 3H, Ph), 7.32–7.35 (m, 2H, H6, H5′), 7.44–7.47 (m, 2H, H8, H3′), 7.54–7.60 (m, 2H, H7, H4′), 7.79–7.82 (m, 2H, Ph), 7.85 (d, J = 8.0 Hz, 1H, H6′), 8.41 (dd, J = 7.6, 0.8 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 13.8, 40.9, 121.1, 124.3, 125.7, 126.7, 126.8, 127.1, 127.6, 127.8, 128.2, 128.5, 128.7, 130.1, 132.2, 133.9, 134.4, 135.4, 136.6, 147.3, 155.4, 161.8. MS: m/z (%) = 352 (100) $[M]^+$, 323 (22), 275 (31), 247 (63). Anal. Calcd for $C_{24}H_{20}N_2O$: C, 81.79; H, 5.72; N, 7.95. Found: C, 81.57; H, 5.58; N, 8.18.

*4.1.4.2. (E)-3-Propyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7b**).* Yield: 80%; white crystals; mp 158–160 °C. IR (KBr): 3059, 3030, 2960, 2874, 1671, 1601, 1583 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 0.75 (t, J = 7.2 Hz, 3H, CH_3), 1.49–1.59 (m, 1H, CH_2), 1.60–1.72 (m, 1H, CH_2), 3.58 (ddd, J = 14.4, 9.2, 5.6 Hz, 1H, NCH_2), 4.10 (ddd, J = 14.4, 9.2, 5.6 Hz, 1H, NCH_2), 6.90 (d, J = 16.0 Hz, 1H, CH), 7.16 (d, J = 16.0 Hz, 1H, CH), 7.25–7.35 (m, 5H, Ph, H6, H5′), 7.43–7.45 (m, 2H, H8, H3′), 7.53–7.59 (m, 2H, H7, H4′), 7.80–7.82 (m, 2H, Ph), 7.85 (d, J = 8.0 Hz, 1H, H6′), 8.40 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 11.2, 21.9, 47.2, 121.0, 124.3, 125.7, 126.7, 126.8, 127.1, 127.6, 127.7, 128.2, 128.6, 128.7, 130.0, 132.1, 133.9, 134.4, 135.4, 136.6, 147.3, 155.5, 162.0. Anal. Calcd for $C_{25}H_{22}N_2O$: C, 81.94; H, 6.05; N, 7.64. Found: C, 82.14; H, 5.86; N, 7.80.

*4.1.4.3. (E)-3-Isopropyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7c**).* Yield: 75%; white crystals; mp 147–149 °C. IR (KBr): 3056, 2926, 2868, 1676, 1590 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 1.51 (d, J = 6.8 Hz, 3H, CH_3), 1.56 (d, J = 6.8 Hz, 3H, CH_3), 4.17 (septet, J = 6.8 Hz, 1H, CH), 6.95 (d, J = 16.0 Hz, 1H, CH), 7.15 (d, J = 16.0 Hz, 1H, CH), 7.22–7.32 (m, 3H, Ph), 7.33–7.36 (m, 2H, H8, H3′), 7.40 (td, J = 8.0 Hz, 1H, H5′), 7.43 (td, J = 8.0 Hz, 1H, H6), 7.52–7.58 (m, 2H, H7, H4′), 7.75–7.82 (m, 2H, Ph), 7.85 (d, J = 8.0 Hz, 1H, H6′), 8.40 (dd, J = 8.0, 0.8 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 19.3, 19.4, 54.1, 122.3, 124.6, 125.7, 126.5, 126.7, 127.0, 127.4, 127.8, 128.0, 128.2, 128.7, 129.8, 132.0, 134.2, 134.9, 135.3, 136.6, 146.9, 155.8, 162.4. Anal. Calcd for $C_{25}H_{22}N_2O$: C, 81.94; H, 6.05; N, 7.64. Found: C, 81.84; H, 6.22; N, 7.51.

*4.1.4.4. (E)-3-Cyclopropyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7d**).* Yield: 75%; white crystals; mp 177–179 °C. IR (KBr): 3062, 3029, 2923, 2852, 1685, 1587 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 0.56–0.62 (m, 2H, CH_2), 0.80–0.90 (m, 2H, CH_2), 2.92–2.96 (m, 1H, NCH), 7.15–7.32 (m, 7H, Ph, CH, H3′, H5′), 7.43 (dd, J = 8.0, 1.2 Hz, 1H, H8), 7.49 (m, 2H, H6, H4′), 7.56 (td, J = 8.0, 0.8 Hz, 1H, H7), 7.79–7.80 (m, 2H, Ph), 7.83 (d, J = 8.0 Hz, 1H, H6′), 8.37 (d, J = 8.0 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 9.9, 10.7, 29.2, 121.2, 125.0,

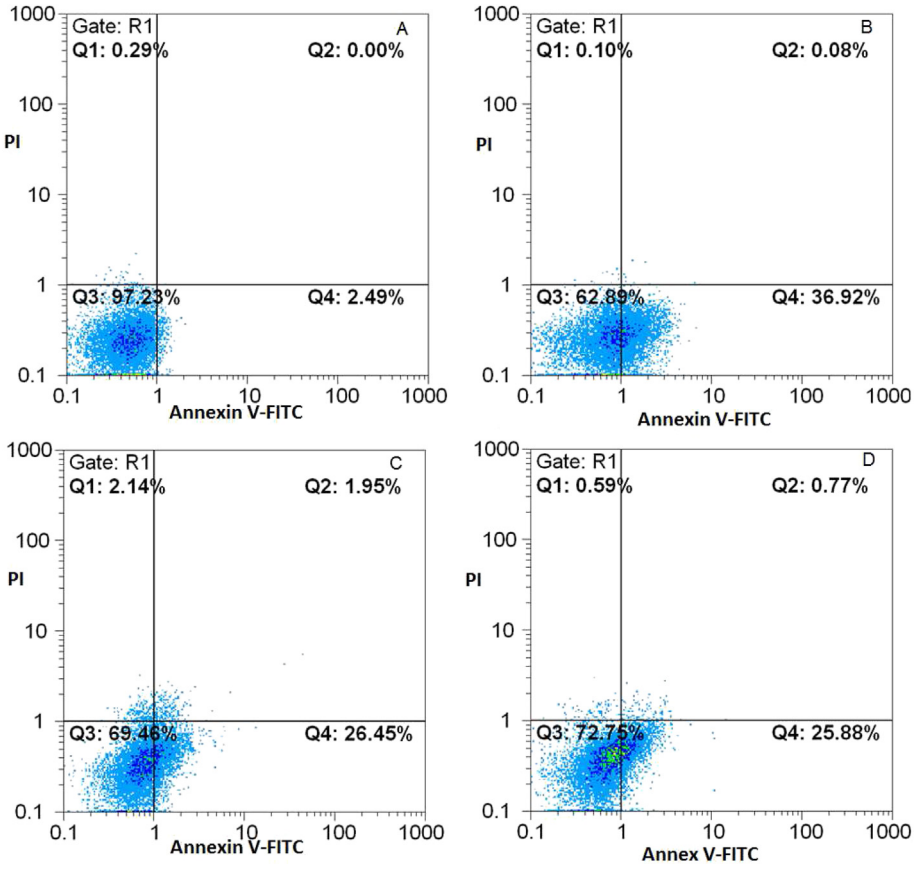


Fig. 5. Flow cytometric analysis of MCF-7 cells treated with synthetic compounds **7h** and **7d**. Cells were stained with Annexin V-FITC/PI and quantitated by flow cytometry. The cells treated with A) DMSO 1% (negative control); B) IC_{50} values of etoposide as positive control; C) IC_{50} values of compound **7h**; D) IC_{50} values of compound **7d** for 12 h.

125.8, 126.7, 126.8, 127.1, 127.5, 127.6, 128.2, 128.7, 129.0, 129.8, 131.8, 134.4, 134.9, 135.6, 136.7, 147.0, 156.9, 163.4. Anal. Calcd for $C_{25}H_{20}N_2O$: C, 82.39; H, 5.53; N, 7.69. Found: C, 82.50; H, 5.68; N, 7.80.

4.1.4.5. (E)-3-Allyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (***7e***). Yield: 78%; white crystals; mp 140–142 °C. IR (KBr): 3058, 3028, 2926, 2850, 1675, 1606 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 4.22 (dd, J = 15.3, 6.0 Hz, 1H, NCH_2), 4.80 (dd, J = 15.3, 5.3 Hz, 1H, NCH_2), 4.85 (d, J = 17.0 Hz, 1H, $=CH_2$), 5.07 (d, J = 10.1 Hz, 1H, $=CH_2$), 5.77 (dddd, J = 17.0, 10.2, 6.0, 5.3 Hz, 1H, CH), 6.89 (d, J = 16.1 Hz, 1H, CH), 7.13 (d, J = 16.1 Hz, 1H, CH), 7.23–7.33 (m, 5H, Ph, H6, H5′), 7.40–7.41 (m, 2H, H8, H3′), 7.54–7.58 (m, 2H, H7, H4′), 7.82–7.83 (m, 3H, Ph, H6′), 8.40 (d, J = 8.1 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 125 MHz): 47.7, 118.2, 120.9, 124.5, 125.7, 126.7, 126.9, 127.2, 127.5, 128.2, 128.3, 128.7, 128.9, 130.1, 131.4, 132.2, 133.5, 134.5, 135.4, 136.6, 147.2, 155.4, 161.7. Anal. Calcd for $C_{25}H_{20}N_2O$: C, 82.39; H, 5.53; N, 7.69. Found: C, 82.54; H, 5.31; N, 7.82.

4.1.4.6. (E)-3-Butyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (***7f***). Yield: 80%; white crystals; mp 132–134 °C. IR (KBr): 3069, 3015, 2957, 2925, 2863, 1679, 1590 cm^{-1}.^1H NMR ($CDCl_3$, 400 MHz): 0.72 (t, J = 7.5 Hz, 3H, CH_3), 1.10–1.21 (m, 2H, CH_2), 1.38–1.53 (m, 1H, CH_2), 1.59–1.70 (m, 1H, CH_2), 3.64 (ddd, J = 14.5, 9.2, 5.2 Hz, 1H, NCH_2), 4.13 (ddd, J = 14.5, 9.2, 5.2 Hz, 1H, NCH_2), 6.91 (d, J = 16.2 Hz, 1H, CH), 7.16 (d, J = 16.2 Hz, 1H, CH), 7.22–7.35 (m, 5H, Ph, H6, H5′), 7.44–7.47 (m, 2H, H8, H3′), 7.53–7.59 (m, 2H, H7, H4′), 7.79–7.86 (m, 2H, Ph), 7.85 (d, J = 8.0 Hz, 1H, H6′), 8.40 (d, J = 8.0 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 13.3, 19.9, 30.4, 45.4, 121.0, 124.4, 125.7, 126.7, 126.8, 127.1, 127.6, 127.7, 128.2, 128.7, 128.8, 130.1, 132.2, 133.9, 134.4, 135.4, 136.6, 147.3, 155.5, 162.0. Anal. Calcd for $C_{26}H_{24}N_2O$: C, 82.07; H, 6.36; N, 7.36. Found: C, 82.28; H, 6.51; N, 7.19.

4.1.4.7. (E)-3-Isobutyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (***7g***). Yield: 75%; white crystals; mp 131–133 °C. IR (KBr): 3058, 3028, 2961, 2869, 1675, 1595, 1588 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 0.70 (d, J = 7.0 Hz, 3H, CH_3), 0.79 (d, J = 7.0 Hz, 3H, CH_3), 1.98 (m, 1H, CH), 3.54 (dd, J = 13.5, 7.5 Hz, 1H, NCH_2), 4.11 (dd, J = 13.5, 7.5 Hz, 1H, NCH_2), 6.90 (d, J = 16.0 Hz, 1H, CH), 7.15 (d, J = 16.0 Hz, 1H, CH), 7.22–7.35 (m, 5H, Ph, H6, H5′), 7.41–7.48 (m, 2H, H8, H3′), 7.52–7.59 (m, 2H, H7, H4′), 7.81–7.84 (m, 3H, Ph, H6′), 8.40 (d, J = 8.0 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 20.0, 20.1, 27.7, 51.9, 120.9, 124.3, 125.6, 126.7, 127.0, 127.1, 127.6, 127.7, 128.2, 128.7, 129.4, 130.0, 132.2, 133.9, 134.4, 135.5, 136.6, 147.2, 155.8, 162.4. Anal. Calcd for $C_{26}H_{24}N_2O$: C, 82.07; H, 6.36; N, 7.36. Found: C, 82.31; H, 6.19; N, 7.51.

4.1.4.8. (E)-3-(sec-Butyl)-2-(2-styrylphenyl)quinazolin-4(3H)-one (***7h***). Yield: 75%; white crystals; mp 146–148 °C. IR (KBr): 3059, 2925, 2927, 1673, 1603, 1563 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 0.69 (t, J = 7.6 Hz, 3H, CH_3), 1.52 (d, J = 1.2 Hz, 3H, CH_3), 1.87–1.84 (m, 1H, CH_2), 2.16–2.28 (m, 1H, CH_2), 3.83 (sextet, J = 6.8 Hz, 1H, NCH), 6.92 (d, J = 16.4 Hz, 1H, CH), 7.03 (d, J = 16.4 Hz, 1H, CH), 7.23–7.33 (m, 5H, Ph, H6, H5), 7.35–7.45 (m, 2H, H8, H3′), 7.51–7.57 (m, 2H, H7, H4′), 7.76–7.81 (m, 2H, Ph), 7.84 (d, J = 8.0 Hz, 1H, H6′), 8.36–8.39 (m, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 11.5, 17.5, 26.3, 59.9, 122.1, 124.7, 125.6, 126.5, 126.7, 127.0, 127.3, 127.7, 127.9, 128.2, 128.7, 129.7, 131.6, 134.2, 134.8, 135.2, 136.5, 146.8, 156.1, 162.3. MS: *m/z* (%) = 380 (28) $[M]^+$, 323 (12), 247 (100), 233 (81). Anal. Calcd for $C_{26}H_{24}N_2O$: C, 82.07; H, 6.36; N, 7.36. Found: C, 81.89; H, 6.44; N, 7.50.

*4.1.4.9. (E)-3-Cyclopentyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7i**).* Yield: 78%; white crystals; mp 210–212 °C. IR (KBr): 3032, 2964, 2866, 1674, 1585 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 1.39–1.51 (m, 2H, CH_2), 1.66–1.78 (m, 2H, CH_2), 1.99–2.09 (m, 2H, CH_2), 2.32–2.43 (m, 2H, CH_2), 4.32 (pentet, J = 8.0 Hz, 1H, NCH), 6.96 (d, J = 16.2 Hz, 1H, CH), 7.15 (d, J = 16.2 Hz, 1H, CH), 7.22–7.35 (m, 5H, Ph, H6, H5′), 7.40–7.46 (m, 2H, H8, H3′), 7.52–7.58 (m, 2H, H7, H4′), 7.63–7.82 (m, 2H, Ph), 7.86 (d, J = 8.0 Hz, 1H, H6′), 8.37 (d, J = 8.0 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 26.1, 26.2, 29.0, 29.1, 61.8, 122.1, 124.6, 125.6, 126.4, 126.7, 127.1, 127.4, 128.0, 128.1, 128.2, 128.7, 129.8, 131.8, 134.2, 135.0, 135.2, 136.6, 146.9, 156.3, 161.9. Anal. Calcd for $C_{27}H_{24}N_2O$: C, 82.62; H, 6.16; N, 7.14. Found: C, 82.74; H, 6.31; N, 7.31.

*4.1.4.10. (E)-3-Phenyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7j**).* Yield: 80%; off-white crystals; mp 153–155 °C. IR (KBr): 3059, 3025, 2925, 1688, 1601, 1582 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 6.95 (d, J = 16.2 Hz, 1H, CH), 7.06–7.09 (m, 3H, Ph, CH), (t, J = 7.7 Hz, 1H, H5′), 7.20–7.21 (m, 3H, Ph), 7.25–7.28 (m, 3H, Ph), 7.31–7.34 (m, 2H, H6, H4′), 7.37–7.39 (m, 2H, H8, H3′), 7.52 (d, J = 7.7 Hz, 1H, H6′), 7.60 (td, J = 7.5, 2.0 Hz, 1H, H7), 7.85–7.88 (m, 2H, Ph), 8.42 (d, J = 7.5 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 125 MHz): 121.2, 125.3, 125.5, 125.6, 126.6, 126.9, 127.2, 127.4, 127.8, 128.1, 128.5, 128.6, 128.7, 129.4, 129.5, 129.6, 131.7, 134.0, 134.8, 135.3, 136.8, 147.2, 154.9, 162.1. MS: m/z (%) = 400 (97) $[M]^+$, 323 (100), 278 (13), 178 (17), 77 (50). Anal. Calcd for $C_{28}H_{20}N_2O$: C, 83.98; H, 5.03; N, 7.00. Found: C, 84.17; H, 5.24; N, 6.87.

*4.1.4.11. (E)-2-(2-Styrylphenyl)-3-(p-tolyl)quinazolin-4(3H)-one (**7k**).* Yield: 80%; white crystals; mp 186–188 °C. IR (KBr): 3061, 2922, 2855, 1683, 1582 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 2.26 (s, 3H, CH_3), 6.96 (m, 5H, CH, H2″, H3″, H5″, H6″), 7.10 (d, J = 16.4 Hz, 1H, CH), 7.19 (t, J = 7.6 Hz, 1H, H5′), 7.26–7.41 (m, 7H, Ph, H6, H8, H3′, H4′), 7.55 (d, J = 7.6 Hz, 1H, H6′), 7.60 (t, J = 7.6 Hz, 1H, H7), 7.87–7.89 (m, 2H, Ph), 8.43 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 21.5, 121.2, 125.4, 125.5, 126.7, 127.0, 127.3, 127.4, 127.8, 128.1, 128.2, 128.4, 128.5, 128.8, 129.4, 129.5, 131.6, 134.2, 134.8, 135.3, 136.9, 138.4, 147.3, 155.2, 162.3. MS: m/z (%) = 414 (100) $[M]^+$, 337 (77), 308 (19), 278 (10). Anal. Calcd for $C_{29}H_{22}N_2O$: C, 84.03; H, 5.35; N, 6.76. Found: C, 83.87; H, 5.19; N, 6.85.

*4.1.4.12. (E)-3-(4-Methoxyphenyl)-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7l**).* Yield: 80%; off-white crystals; mp 153–155 °C. IR (KBr): 3046, 2922, 2839, 1680, 1634, 1609 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 3.74 (s, 3H, OCH_3), 6.72 (d, J = 6.8 Hz, 2H, H3″, H5″), 6.98–7.02 (m, 3H, CH, H2″, H6″), 7.06 (d, J = 16.4 Hz, 1H, CH), 7.18 (t, J = 7.5 Hz, 1H, H5′), 7.28–7.40 (m, 7H, Ph, H6, H8, H3′, H4′), 7.55 (dd, J = 7.5, 1.6 Hz, 1H, H6′), 7.60 (td, J = 8.0, 2.5 Hz, 1H, H7), 7.86–7.88 (m, 2H, Ph), 8.43 (dd, J = 8.0, 2.5 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 55.3, 113.9, 121.2, 125.4, 125.6, 126.6, 127.1, 127.3, 127.4, 127.8, 128.1, 128.8, 129.3, 129.4, 129.5, 129.7, 131.6, 134.3, 134.8, 135.3, 136.9, 147.3, 155.4, 159.2, 162.4. Anal. Calcd for $C_{29}H_{22}N_2O_2$: C, 80.91; H, 5.15; N, 6.51. Found: C, 81.11; H, 5.28; N, 6.40.

*4.1.4.13. (E)-3-Benzyl-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7m**).* Yield: 90%; white crystals; mp 135–137 °C. IR (KBr): 3029, 2953, 2923, 2855, 1679, 1601, 1582 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 5.01 (d, J = 15.0 Hz, 1H, NCH_2), 5.42 (d, J = 15.0 Hz, 1H, NCH_2), 6.77 (d, J = 16.0 Hz, 1H, CH), 6.88–6.90 (m, 2H, Ph), 7.04 (d, J = 16.0 Hz, 1H, CH), 7.12–7.14 (m, 3H, Ph), 7.17 (dd, J = 7.6, 0.8 Hz, 1H, H3′), 7.23–7.32 (m, 6H, Ph, H8, H6, H5′), 7.51 (td, J = 7.6, 0.8 Hz, 1H, H4′), 7.61 (td, J = 7.6, 2.0 Hz, 1H, H7), 7.86 (d, J = 7.6 Hz, 1H, H6′), 7.82–7.85 (m, 2H, Ph), 8.47 (d, J = 7.6 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 48.2, 121.1, 124.3, 125.7, 126.8, 127.2, 127.3, 127.5, 127.6, 127.8, 127.7, 128.1, 128.3, 128.6, 129.0, 130.1, 132.0, 133.7, 134.6, 135.7, 136.3, 136.6, 147.3, 155.6, 162.4. Anal. Calcd for $C_{29}H_{22}N_2O$: C, 84.03; H, 5.35; N, 6.76. Found: C, 83.90; H, 5.48; N, 6.87.

*4.1.4.14. (E)-3-(4-Methylbenzyl)-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7n**).* Yield: 85%; white crystals; mp 130–132 °C. IR (KBr): 3054, 3025, 2957, 2921, 1662, 1595 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 2.19 (s, 3H, CH_3), 5.03 (d, J = 14.8 Hz, 1H, NCH_2), 5.29 (d, J = 14.8 Hz, 1H, NCH_2), 6.71 (d, J = 16.0 Hz, 1H, CH), 6.62 (d, J = 7.6 Hz, 2H, H3″, H5″), 6.79 (d, J = 7.6 Hz, 2H, H2″, H6″), 7.00 (d, J = 16.0 Hz, 1H, CH), 7.21–7.30 (m, 7H, Ph, H8, H6, H5′, H3′), 7.51 (t, J = 8.0 Hz, 1H, H4′), 7.59 (td, J = 8.0, 2.4 Hz, 1H, H7), 7.77 (d, J = 8.0 Hz, 1H, H6′), 7.80–7.85 (m, 2H, Ph), 8.46 (d, J = 8.0 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 21.0, 48.1, 121.1, 124.2, 125.6, 126.8, 127.2, 127.3, 127.5, 127.6, 127.7, 127.8, 128.1, 128.6, 129.0, 130.0, 131.8, 133.3, 133.8, 134.6, 135.7, 136.6, 137.3, 147.3, 155.7, 162.4. MS: m/z (%) = 428 (76) $[M]^+$, 323 (100), 247 (37), 105 (55). Anal. Calcd for $C_{30}H_{24}N_2O$: C, 84.08; H, 5.65; N, 6.54. Found: C, 84.21; H, 5.80; N, 6.37.

*4.1.4.15. (E)-3-(4-Methoxybenzyl)-2-(2-styrylphenyl)quinazolin-4(3H)-one (**7o**).* Yield: 85%; white crystals; mp 145–147 °C. IR (KBr): 3055, 2924, 2833, 1674, 1605, 1585 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 3.62 (s, 3H, OCH_3), 5.04 (d, J = 14.8 Hz, 1H, NCH_2), 5.23 (d, J = 14.8 Hz, 1H, NCH_2), 6.62 (d, J = 8.5 Hz, 2H, H3″, H5″), 6.71 (d, J = 16.0 Hz, 1H, CH), 6.79 (d, J = 8.5 Hz, 2H, H2″, H6″), 6.98 (d, J = 16.0 Hz, 1H, CH), 7.21–7.30 (m, 6H, Ph, H8, H5′, H3′), 7.33 (t, J = 7.5 Hz, 1H, H6), 7.52 (t, J = 7.5 Hz, 1H, H4′), 7.61 (td, J = 7.5, 2.4 Hz, 1H, H7), 7.80 (d, J = 7.5 Hz, 1H, H6′), 7.80–7.83 (m, 2H, Ph), 8.47 (d, J = 7.5 Hz, 1H, H5). ^{13}C NMR ($CDCl_3$, 100 MHz): 47.7, 55.1, 113.6, 121.1, 124.1, 125.7, 126.8, 127.2, 127.3, 127.5, 127.7, 128.1, 128.4, 128.6, 129.0, 129.2, 130.1, 131.7, 133.8, 134.6, 135.7, 136.6, 147.3, 155.6, 159.0, 162.5. Anal. Calcd for $C_{30}H_{24}N_2O_2$: C, 81.06; H, 5.44; N, 6.30. Found: C, 80.91; H, 5.23; N, 6.54.

4.2. Cell lines and cell culture

The human cancer cells MCF-7, MDA-MB-231 and T-47D were purchased from Pastor Institute of Iran. The cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and streptomycin (100 μg/mL) and penicillin (100 U/ml) at 37 °C in a humidified atmosphere with 5% CO_2 in air.

4.3. MTT assay

The cytotoxic activities of compounds **7a-o** were evaluated by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay as reported method [34,35]. The absorbance was read at 492 nm with an ELISA plate reader (Biotek Instruments, Winooski, Vt.). The inhibition percentage of compounds was calculated as: $OD_{\text{wells treated with DMSO1\%}} - OD_{\text{wells treated with compounds}}/OD_{\text{wells treated with DMSO1\%}}*100$ (OD = absorbance). Then, IC_{50} values were calculated by nonlinear regression analysis and expressed in mean ± SD.

4.4. Acridine orange/ethidium bromide double staining test

The acridine orange/ethidium bromide test was used to detect apoptosis induced by selected compounds **7d** and **7h** according to the previously described method [35,36].

4.5. Annexin V-FITC/PI double staining and flow cytometric analysis

The double staining test was performed using Annexin V-FITC Apoptosis Detection Kit (Bio Vision) as described in protocol. Using this technique, living cells (annexin V-$FITC^-$/PI^-), early apoptotic

cells (Annexin V-FITC$^+$/PI$^-$), late apoptotic or secondary apoptotic cells (Annexin V-FITC$^+$/PI$^+$), and necrotic cells (Annexin V-FITC$^-$/PI$^+$) were distinguished. In brief, the cancer cell lines were treated with IC_{50} of compounds **7d** and **7h**, and etoposide as reference drug. After 12 h incubation, cells were collected by centrifugation and resuspended in 500 μl of 1× Binding Buffer. Finally, the cells were double stained with Annexin V-FITC and 5 μl of PI and the samples were gently mixed and incubated for 5 min at room temperature in the dark before flow cytometry. Annexin V-FITC binding was analyzed by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (FL1) and PI staining by the phycoerythrin emission signal detector (FL2).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.03.057.

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