

DESIGN, SYNTHESIS AND ANTICANCER ACTIVITY EVALUATION OF NEW QUINAZOLINE DERIVATIVES LINKED TO THIAZOLIDINONE, AZETIDINONE OR OXADIAZOL MOIETIES

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Abstract: Novel series of 4-substituted 6,8-dibromo-2-(4-chlorophenyl)-quinazoline have been designed and synthesized. All new derivatives were tested *in vitro* against MCF-7. Compounds **VIc** and **VIIb** exerted powerful cytotoxic activity with low IC₅₀ (6.3 and 6.9 μM) compared to doxorubicin 7.72 μM. Compounds **VIa**, **Vb** and **Vc** showed moderate cytotoxic effects with IC₅₀ range (10.0 – 16.7) μM, respectively. Compounds **Va**, **VIIc**, **VIIa**, **IVb**, **IVc**, **VIb** and **IVa** showed promising cytotoxic effects with IC₅₀ range (20.3 – 40 μM, respectively.). Exploring their apoptotic effect; all compounds activated apoptotic cascade in MCF-7. Compounds **VIc** and **VIIb** increased CASP3 activity more than doxorubicin.

Keywords: quinazoline, cytotoxicity, MCF-7, CASP3

Breast cancer is the second leading cause of cancer death in women. The American Cancer Society's estimates about 252710 new cases of breast cancer in 2017. Breast cancer starts when cells in the breast begin to grow out of control. The tumor is malignant if the cells can grow into surrounding tissues or metastasize to distant areas of the body (1).

A failure of apoptosis is considered to be a key event in cancer formation and progression (2, 3). Apoptosis or programmed cell death is important in cancer because the suppression of apoptosis is an important event in both cancer initiation and progression. Most cytotoxic anticancer agents cause tumor regression, by inducing apoptosis.

The key mediators of the process of apoptosis are the caspases (4, 5). Currently, there are at least 14 different caspases. The caspases involved in apoptosis are divided into two main groups, the initiator caspases and the downstream effector caspases.

Caspase 3 plays a key role in both the death receptor pathway and the mitochondrial pathway. In addition, caspase3 activation is required for apopto-

sis induction in response to chemotherapeutic drugs as 5-fluorouracil, and doxorubicin (6-9). Breast carcinomas exhibited higher rates of apoptosis than either fibroadenomas or normal breast tissue (10).

Quinazoline scaffolds have attracted a great attention because of their ready accessibility and diverse chemical reactivity. Quinazolines have a wide range of biological activities such as anticancer (11-16), antibacterial (17-19), anti-inflammatory (20), antifungal (21, 22) and antimalarial activity (23). Development of new quinazoline derivatives as anticancer drugs is a promising field. 6,8 -disubstituted quinazoline derivatives have good anticancer activity (24, 13). Moreover, quinazoline derivatives bearing phenyl moiety at position 2 show potent cytotoxic activity (25, 15).

Substitution of quinazoline at position 4 showed promising cytotoxic activity (26-31). Our aim in this study is continuation of our drug research program concerning the synthesis of new safer and more biologically active quinazoline derivatives (12-16), our interest was to design and synthesize new 6,8 dibromo quinazoline derivatives having 4-chlorophenyl moiety at position 2 and different sub-

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stitution at position 4 aiming to obtain new quinazoline derivatives with high activity against breast cancer.

EXPERIMENTAL

Chemistry

Elemental analyses were made at Cairo University, Egypt. IR spectra were measured on Perkin Elmer-9712 spectrophotometer. Varian-Gemini-300 MHz. and Joel-Ex270 MHz NMR spectrometer was used to measure ^1H -NMR and ^{13}C NMR (DMSO-d_6) spectra. Finnigan Mat SSQ 7000 mode EI 70 eV was used to measure Mass spectra. Compounds **I** was synthesized with the same previously reported methods (32).

Ethyl 2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetate (**II**)

A mixture of **I** (0.01 mol), ethyl chloroacetate (0.015 mol) and anhydrous potassium carbonate (2.0 g) in dry acetone (50 mL) was refluxed for 12 h. The reaction mixture filtered while hot. The filtrate was cooled, poured into crushed ice, filtered and crystallized from ethanol (33).

2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetohydrazide (**III**)

A mixture of **II** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol (30 mL) were refluxed for 5 h. The reaction mixture was concentrated and left to cool, filtered and crystallized from ethanol (33).

(E)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N'-(substituted)benzylidene acetohydrazide (**IVa-c**)

General method: A mixture of compound **III** (0.01 mol) and the appropriate aldehyde, namely: benzaldehyde, 3,4,5 trimethoxybenzaldehyde and/or p-chloro benzaldehyde (0.01 mol) was refluxed for 8 h in glacial acetic acid. Then the reaction was cooled and poured into crushed ice, the formed precipitate was filtered and crystallized from ethanol to obtain the desired Schiff bases (**IVa-c**) respectively.

(E)-N'-benzylidene-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetohydrazide (**IVa**)

Crystallized from ethanol, m.p. 170°C , 75% yield. Analysis: calcd. for $\text{C}_{23}\text{H}_{15}\text{Br}_2\text{ClN}_4\text{O}_2$: C, 48.07; H, 2.63; N, 9.75%; found: C, 47.90; H, 2.54; N, 9.66%. IR: $\nu_{\text{max}}/\text{cm}^{-1}$ 3240 (NH), 3010 (C-H aromatic), 1700 (C=O), 1620 (C=N) and at 1580 (C=C). ^1H -NMR (DMSO-d_6 , ppm): δ 4.7 (s, 2H,

OCH_2), 7.5-8.1 (m, 9H, Ar-H), 8.2 (s, 1H, Quin-H), 8.3 (s, 1H, Quin-H), 8.9 (s, 1H, CH=N) and 11.5 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 182, 173, 160, 150, 144, 141, 135, 133, 132, 131, 130, 129, 128, 127, 124, 123, 121, 116, 70. MS: m/z = 578

(E)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N'-(3,4,5-trimethoxybenzylidene)acetohydrazide (**IVb**)

Crystallized from ethanol, m.p. 120°C , 80% yield. Analysis: calcd. for $\text{C}_{26}\text{H}_{21}\text{Br}_2\text{ClN}_4\text{O}_5$: C, 46.98; H, 3.18; N, 8.43%; found: C, 46.80; H, 3.10; N, 8.47%. IR: $\nu_{\text{max}}/\text{cm}^{-1}$ 3240 (NH), 3020 (C-H aromatic), 1700 (C=O), 1620 (C=N) and at 1590 (C=C). ^1H -NMR (DMSO-d_6 , ppm): δ 3.8 (s, 9H, OCH_3), 4.6 (s, 2H, OCH_2), 7.6-8.2 (m, 6H, Ar-H), 8.3 (s, 1H, Quin-H), 8.4 (s, 1H, Quin-H), 8.8 (s, 1H, CH=N) and 11.0 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 184, 162, 153, 150, 143, 141, 134, 133, 132, 131, 130, 129, 128, 127, 124, 123, 121, 116, 104, 69, 60, 56. MS: m/z = 668.

(E)-N'-(4-chlorobenzylidene)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetohydrazide (**IVc**)

Crystallized from methanol, m.p. 210°C , 75% yield. Analysis: calcd. for $\text{C}_{23}\text{H}_{14}\text{Br}_2\text{Cl}_2\text{N}_4\text{O}_2$: C, 45.35; H, 2.32; N, 9.20%; found: C, 45.30; H, 2.29; N, 9.36%. IR: $\nu_{\text{max}}/\text{cm}^{-1}$ 3200 (NH), 3010 (C-H aromatic), 1710 (C=O), 1620 (C=N) and at 1580 (C=C). ^1H -NMR (DMSO-d_6 , ppm): δ 4.8 (s, 2H, OCH_2), 7.2 (d, 2H, J = 8.4 Hz, Ar-H), 7.4 (d, 2H, J = 8.4 Hz, Ar-H), 7.7 (d, 2H, J = 8.5 Hz, Ar-H), 7.9 (d, 2H, J = 8.5 Hz, Ar-H), 8.2 (s, 1H, Quin-H), 8.3 (s, 1H, Quin-H), 8.5 (s, 1H, CH=N) and 11.3 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 185, 175, 162, 150, 143, 141, 135, 133, 132, 131, 130, 129, 128, 127, 124, 123, 121, 116, 69. MS: m/z = 613

2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N-(4-oxo-2-(substituted phenyl)thiazolidin-3-yl)acetamide (**Va-c**)

General method: A mixture of compounds **IV a-c** (0.01 mol) in glacial acetic acid (25 mL) and thioglycolic acid (0.01 mol) was refluxed for 10 h. Then the reaction mixture was poured on crushed ice. The solid product was filtered off to give the desired products **V a-c** respectively.

2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N-(4-oxo-2-phenylthiazolidin-3-yl)acetamide (**V a**)

Crystallized from glacial acetic acid m.p. 150°C, 70% yield. Analysis: calcd. for $C_{25}H_{17}Br_2ClN_4O_3S$: C, 46.28; H, 2.64; N, 8.64%; found: C, 46.3; H, 2.70; N, 8.59%. IR: $\nu_{\max}/\text{cm}^{-1}$ 3350 (NH), 3050 (C-H aromatic), 1730 (C=O), 1710 (C=C=O), 1650 (C=N) and at 1600 (C=C). ^1H NMR (DMSO- d_6 , ppm): δ 3.7-3.8 (m, 2H, CH_2 , thiazolidinone ring), 4.8 (s, 2H, OCH_2), 5.9 (s, 1H, CH, thiazolidinone ring), 7.2-7.8 (m, 9H, Ar-H), 8.3 (s, 1H, Quin-H), 8.4 (s, 1H, Quin-H), and at 11.1 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO- d_6): 180, 168, 166, 162, 149, 141, 139, 134, 132, 129, 128, 127, 126, 124, 121, 115, 70, 57, 36. MS: m/z = 652

2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N-(4-oxo-2-(3,4,5-trimethoxyphenyl)thiazolidin-3-yl)acetamide (V b)

Crystallized from ethanol m.p. 270°C, 65% yield. Analysis: calcd. for $C_{28}H_{23}Br_2ClN_4O_6S$: C, 45.52; H, 3.14; N, 7.58%; found: C, 45.40; H, 3.10; N, 7.60%. IR: $\nu_{\max}/\text{cm}^{-1}$ 3300 (NH), 3100 (C-H aromatic), 1720 (C=O), 1700 (C=C=O), 1660 (C=N) and at 1600 (C=C). ^1H NMR (DMSO- d_6 , ppm): δ 3.7 (s, 9H, OCH_3), 3.9-4.0 (m, 2H, CH_2 , thiazolidinone ring), 4.9 (s, 2H, OCH_2), 6.0 (s, 1H, CH, thiazolidinone ring), 7.3-8.3 (m, 6H, Ar-H), 8.4 (s, 1H, Quin-H), 8.6 (s, 1H, Quin-H) and at 10.8 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO- d_6): 186, 170, 166, 162, 152, 150, 141, 137, 134, 133, 132, 129, 128, 124, 123, 121, 116, 106, 70, 60, 57, 56, 35. MS: m/z = 742

N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetamide (V c)

Crystallized from ethanol m.p. 115°C, 70% yield. Analysis: calcd. for $C_{25}H_{16}Br_2Cl_2N_4O_3S$: C, 43.95; H, 2.36; N, 8.20%; found: C, 43.90; H, 2.29; N, 8.25%. IR: $\nu_{\max}/\text{cm}^{-1}$ 3200 (NH), 3150 (C-H aromatic), 1720 (C=O), 1700 (C=C=O), 1650 (C=N) and at 1600 (C=C). ^1H NMR (DMSO- d_6 , ppm): δ 3.8-3.7 (m, 2H, CH_2 , thiazolidinone ring), 4.8 (s, 2H, OCH_2), 5.8 (s, 1H, CH, thiazolidinone ring), 7.2 (d, 2H, J = 8.3 Hz, Ar-H), 7.4 (d, 2H, J = 8.3 Hz, Ar-H), 7.7 (d, 2H, J = 8.5 Hz, Ar-H), 7.9 (d, 2H, J = 8.5 Hz, Ar-H), 8.2 (s, 1H, Quin-H), 8.4 (s, 1H, Quin-H), and at 10.8 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO- d_6): 184, 168, 165, 162, 150, 141, 137, 134, 132, 130, 129, 128, 124, 121, 116, 69, 57, 35. MS: m/z = 688

N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(6,8-dibromo-2-(substituted phenyl)quinazolin-4-yloxy)acetamide (VI a-c)

To a solution of compounds **IV a-c** (0.01 mol) in dry benzene (30 mL) containing triethylamine (1 mL) as a base, chloroacetyl chloride (0.015 mol) was added. Then the reaction was stirred for 30 min and refluxed for 30h. The reaction was filtered while hot and the filtrate was poured onto crushed ice with continuous stirring. The solid obtained was cooled in the refrigerator for 5 days.

N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetamide (VI a)

Crystallized from glacial acetic acid m.p. 210°C yield (65%). Analysis: calcd. for $C_{25}H_{16}Br_2Cl_2N_4O_3$: C, 46.11; H, 2.48; N, 8.60%; found: C, 46.24; H, 2.45; N, 8.58%. IR: $\nu_{\max}/\text{cm}^{-1}$ 3250 (NH), 3050 (C-H aromatic), 1720 (C=O), 1700 (C=O), 1620 (C=N) and at 1600 (C=C). ^1H NMR (DMSO- d_6 , ppm): δ 4.8 (s, 2H, OCH_2), 5.0 (d, 1H, J = 6 Hz, CH, azetidinone), 5.4 (d, 1H, J = 6.5 Hz, CH, azetidinone), 7.4-7.9 (m, 9H, Ar-H), 8.0 (s, 1H, Quin-H), 8.2 (s, 1H, Quin-H), and at 10.8 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO- d_6): 180, 166, 163, 162, 150, 143, 141, 134, 132, 129, 128, 126, 124, 123, 121, 116, 69, 64, 62. MS: m/z = 656

N-(3-chloro-2-oxo-4-(3,4,5-trimethoxyphenyl)azetidin-1-yl)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetamide (VIb)

Crystallized from glacial acetic acid m.p. 140°C yield (70%). Analysis: calcd. for $C_{28}H_{22}Br_2Cl_2N_4O_6$: C, 45.37; H, 2.99; N, 7.56%; found: C, 45.32; H, 2.97; N, 7.58%. IR: $\nu_{\max}/\text{cm}^{-1}$ 3300 (NH), 3050 (C-H aromatic), 1730 (C=O), 1720 (C=O), 1620 (C=N) and at 1600 (C=C). ^1H NMR (DMSO- d_6 , ppm): δ 3.9 (s, 9H, OCH_3), 4.8 (s, 2H, OCH_2), 5.1 (d, 1H, J = 6 Hz, CH, azetidinone), 5.3 (d, 1H, J = 6.5 Hz, CH, azetidinone), 6.8-7.8 (m, 6H, Ar-H), 8.0 (s, 1H, Quin-H), 8.1 (s, 1H, Quin-H) and at 11.2 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO- d_6): 185, 167, 164, 162, 152, 150, 141, 137, 134, 132, 129, 128, 124, 123, 121, 116, 102, 69, 64, 63, 60, 56. MS: m/z = 746

N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetamide (VI c)

Crystallized from ethanol m.p. 260°C yield (65%). Analysis: calcd. for $C_{25}H_{15}Br_2Cl_3N_4O_3$: C, 43.80; H, 2.21; N, 8.17%; found: C, 43.79; H, 2.19; N, 8.19%. IR: $\nu_{\max}/\text{cm}^{-1}$ 3300 (NH), 3050 (C-H aromatic), 1740 (C=O), 1710 (C=O), 1630 (C=N) and at 1600 (C=C). ^1H NMR (DMSO- d_6 , ppm): δ 4.6 (s,

2H, OCH₂), 5.0 (d, 1H, $J = 6$ Hz, CH, azetidinone), 5.4 (d, 1H, $J = 6.5$ Hz, CH, azetidinone), 7.3 (d, 2H, $J = 8.4$ Hz, Ar-H), 7.4 (d, 2H, $J = 8.4$ Hz, Ar-H), 7.7 (d, 2H, $J = 8.5$ Hz, Ar-H), 7.9 (d, 2H, $J = 8.5$ Hz, Ar-H), 8.1 (s, 1H, Quin-H), 8.3 (s, 1H, Quin-H) and at 10.8 (s, 1H, NH, exchangeable with D₂O). ¹³C NMR (DMSO-d₆): 183, 166, 164, 162, 151, 141, 134, 131, 129, 128, 127, 124, 123, 122, 114, 70, 63, 62 MS: $m/z = 690$

1-(5-(((6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)methyl)-2-(substituted phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (VII a-c)

General method: A mixture of **IV a-c** (0.005 mol) and acetic anhydride (25 mL) was refluxed for 10 h. The reaction mixture was cooled, filtered and recrystallized from appropriate solvent systems.

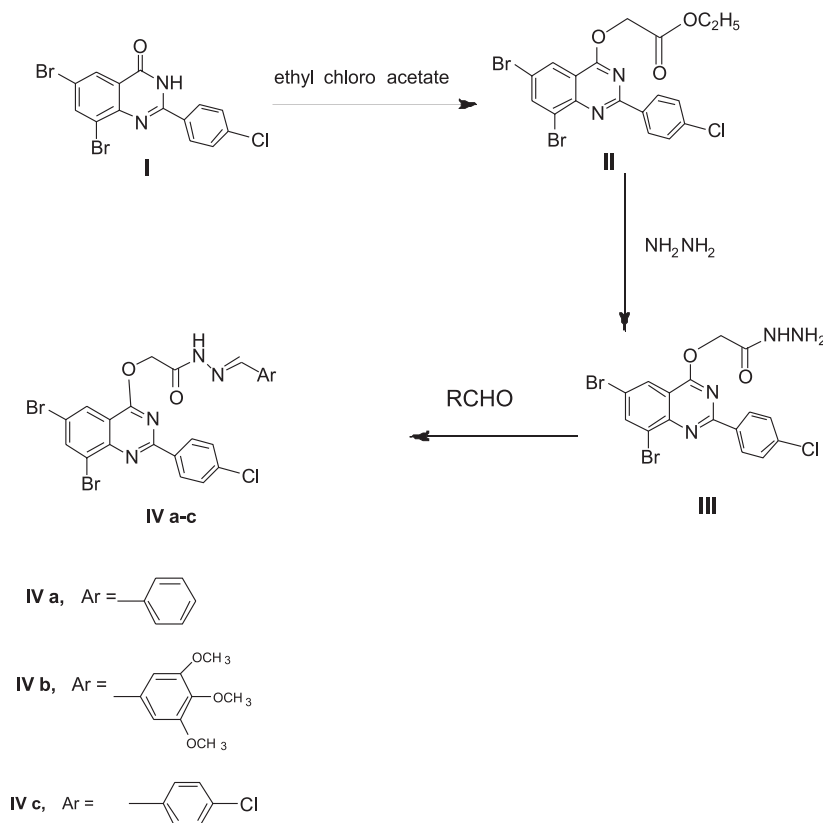
1-(5-(((6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)methyl)-2-phenyl-1,3,4-oxadiazol-3(2H)-yl)ethanone (VII a)

Crystallized from ethanol, m.p. 165°C, 60% yield. Analysis: calcd. for C₂₅H₁₇Br₂ClN₄O₃: C, 48.69; H, 2.78; N, 9.09%; found: % C 48.72; H,

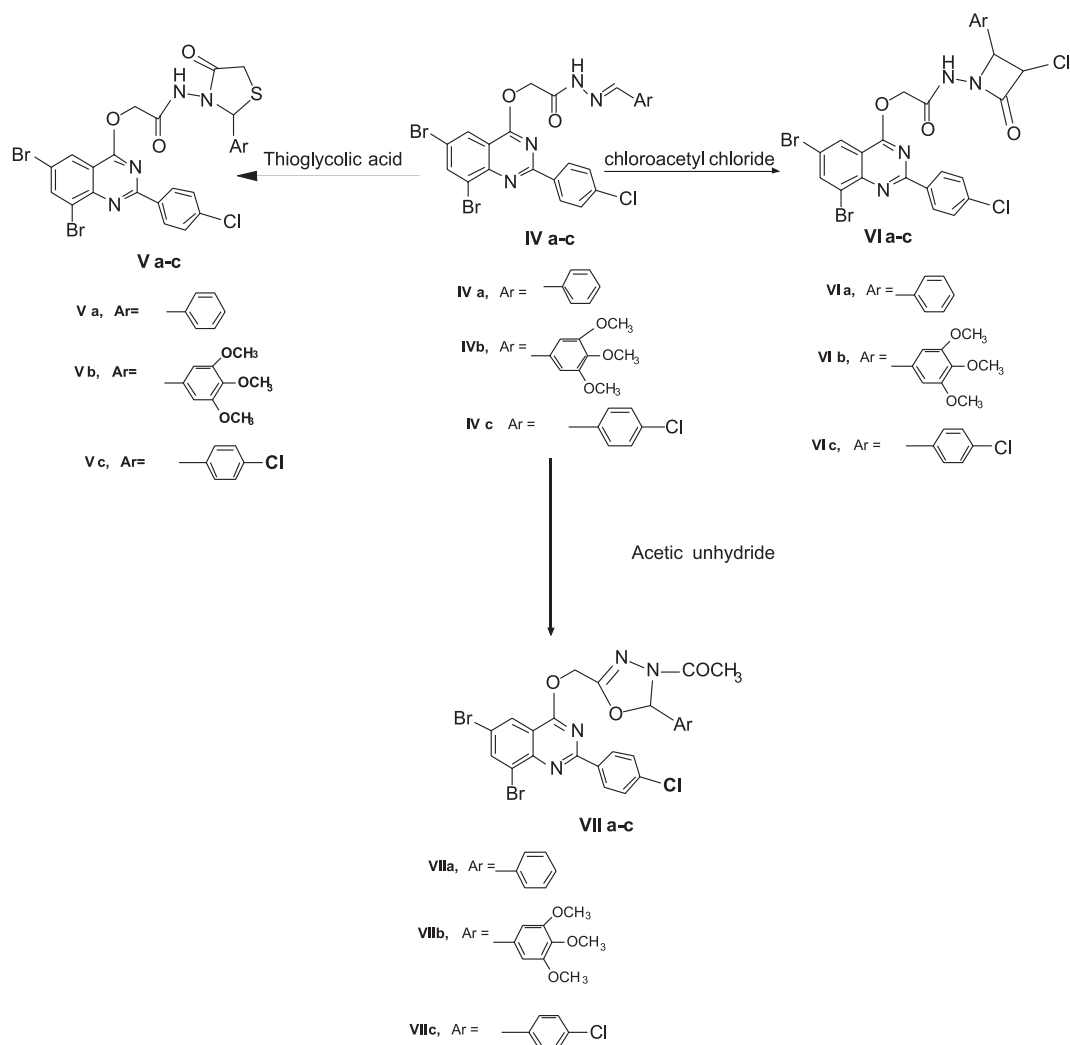
2.74; N, 9.00%. IR: $\nu_{\max}/\text{cm}^{-1}$ 3100 (C-H aromatic), 1720 (C=O), 1610 (C=N) and at 1595 (C=C). ¹H-NMR (DMSO-d₆, ppm): δ 1.9 (s, 3H, COCH₃), 4.7 (s, 2H, OCH₂), 6.2 (s, 1H, CH) and at 7.4-7.9 (m, 9H, Ar-H), 8.1 (s, 1H, Quin-H), 8.3 (s, 1H, Quin-H). ¹³C NMR (DMSO-d₆): 180, 168, 162, 158, 149, 141, 140, 134, 132, 129, 128, 126, 125, 122, 121, 116, 83, 73, 23. MS: $m/z = 620$

1-(5-(((6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)methyl)-2-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (VIIb)

Crystallized from methanol, m.p. 140°C, 60% yield. Analysis: calcd. for C₂₈H₂₃Br₂ClN₄O₆: C, 47.58; H, 3.28; N, 7.93%; found: C 47.54; H, 3.24; N, 7.89%. IR: $\nu_{\max}/\text{cm}^{-1}$ 3100 (C-H aromatic), 1730 (C=O), 1620 (C=N) and at 1600 (C=C). ¹H-NMR (DMSO-d₆, ppm): δ 1.8 (s, 3H, COCH₃), 3.7 (s, 9H, OCH₃), 4.7 (s, 2H, OCH₂), 6.6 (s, 1H, CH), 7.2-8.2 (m, 6H, Ar-H), 8.3 (s, 1H, Quin-H) and at 8.5 (s, 1H, Quin-H). ¹³C NMR (DMSO-d₆): 185, 170, 162, 159, 152, 149, 141, 137, 134, 132, 129, 127, 125, 122, 121, 115, 104, 84, 73, 60, 56, 24. MS: $m/z = 710$



Scheme 1. Synthesis of compounds **II-IVa-c**

Scheme 2. Synthesis of compounds **Va-c**, **VI a-c** and **VII a-c**

1-(2-(4-chlorophenyl)-5-((6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)methyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (VII c)

Crystallized from methanol, m.p. 205^o C, 60% yield. Analysis: calcd. for C₂₅H₁₆Br₂Cl₂N₄O₃: C, 46.12; H, 2.48; N, 8.60%; found: C 46.20; H, 2.45; N, 8.54%. IR: $\nu_{\text{max}}/\text{cm}^{-1}$ 3000 (C-H aromatic), 1720 (C=O), 1620 (C=N) and at 1600 (C=C). ¹H-NMR (DMSO-d₆, ppm): δ 1.6 (s, 3H, COCH₃), 4.5 (s, 2H, OCH₂), 6.4 (s, 1H, CH), 7.5 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.6 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.7 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.9 (d, 2H, *J* = 8.5 Hz, Ar-H), 8.3 (s, 1H, Quin-H) and at 8.4 (s, 1H, Quin-H). ¹³C NMR (DMSO-d₆): 183, 169, 162, 158, 149, 141, 138, 134, 132, 130, 127, 124, 122, 121, 116, 83, 72, 23. MS: *m/z* = 656

Biological studies:

Cell viability assay (SulfoRhodamine B assay)

The Sulphorhodamine-B (SRB) assay method was used in the evaluation of the cytotoxic activity of the newly synthesized compounds (34). The concentration of the seeded cells was 1000-2000 cells/well, 100 μL /well in a 96 well microtiter plates for 24 h, then the incubation of the tested compounds of cells took place using the following concentrations (0, 6.25, 12.5, 25, 50, 100 $\mu\text{g/mL}$) and in doxorubicin for 48 h. For each concentration, three wells were used, after that fixation of the cells took place for 1 h at 4^oC using 10% trichloroacetic acid (150 μL /well), then washed 3 times by distilled water. The SRB 0.4% dissolved in 1% acetic acid 70 μL /well was used for staining of wells at room temperature, then washed with 1% acetic acid.

Then air drying of the plates for 24 h without exposure to UV. The solubilization of the dye was done for 5 min using 150 µL/well of 10 mM Tris-EDTA (pH 7.4) for 5 min, on a shaker at 1600 rpm. For each well, the optical density (OD) was measured with the spectrophotometer at 545 nm with the ELISA microplate reader. The % of surviving cells was calculated and then plotted against the different concentrations of the tested compounds in order to obtain the survival curve. The sigmoidal concentration – response curve fitting models (SigmaPlot software) was used to calculate the IC₅₀ values.

Apoptotic effect (Caspase-3 activity)

The Human CASP3 assay is an enzyme-linked immunosorbent assay for Caspase-3 activities quantitative measurement used for the supernatant cell cultures in vitro (32). After six hours treatment with the tested compounds or doxorubicin of the cell lines (MCF-7), the activity of CASP3 was determined according to the protocol of the manufacturer (Cat. KA1868, Ver01, Abnova). The précised microplate reader was used in the measurement of the absorbance at 450 nm (StatFax 2100, USA).

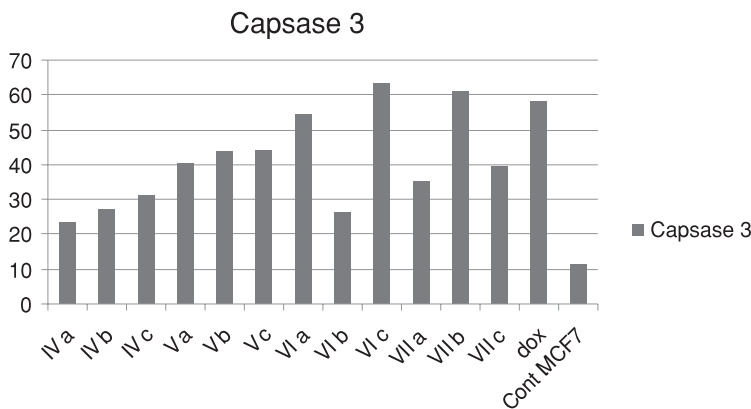


Figure 1. Enzymatic activity of caspase-3 relative to untreated cells

Table 1. Cytotoxicity of all synthesized compounds against human breast cancer cell line (MCF-7).

Compound No.	Sample conc. (µg/mL)						IC ₅₀ µM ± SD
	Growth inhibition%						
	0	6.25	12.5	25	50	100	
IV a	0	19.00	40.29	52.22491	68.03	85.75	40.01 ± 0.059
IV b	0	20.40	41.33	59.67231	72.94	88.08	26.37 ± 0.094
IV c	0	24.20	45.59	61.08732	73.74	89.08	26.95 ± 0.075
V a	0	27.20	48.85	68.53472	79.57	90.03	20.33 ± 0.091
V b	0	28.13	52.85	74.28784	81.64	88.82	13.53 ± 0.055
V c	0	26.36	50.15	69.67045	81.21	89.49	16.77 ± 0.064
VI a	0	49.73	63.54	79.81754	89.29	92.72	10.04 ± 0.075
VI b	0	19.00	34.09	55.42729	70.32	86.12	29.41 ± 0.086
VI c	0	59.57922	70.47	83.91361	92.10	95.08	6.34 ± 0.095
VII a	0	22.71	39.11	63.69391	77.28	87.43	24.84 ± 0.093
VII b	0	56.89	67.138	82.2938	90.82	94.80	6.93 ± 0.071
VII c	0	23.83	42.56	66.65425	80.26	87.43	20.58 ± 0.068
Doxorubicin	0	64.69	82.07	88.69857	94.73	95.66	7.72 ± 0.075

Table 2. Apoptotic effect "in terms of caspase-3 concentration ($\mu\text{g/mL}$) of tested compounds after 6 h treatment of human breast cancer cell line (MCF-7).

Compound No.	Caspase 3 conc. $\mu\text{g/mL}$
IVa	23.27
IVb	27.24
IVc	30.78
Va	40.19
Vb	43.85
Vc	44.23
VIa	54.33
VIb	26.51
VIc	63.42
VIIa	35.27
VIIb	61.14
VIIc	39.45
dox	58.48
Cont.MCF7	11.32

RESULTS AND DISCUSSION

Chemistry

Compounds I-III were prepared according to reported methods (32, 33) (Scheme 1). Schiff bases IVa-c were prepared by reaction of III with appropriate aldehyde namely benzaldehyde, 3,4,5 trimethoxy-benzaldehyde and/or p-chloro benzaldehyde respectively (Scheme 1). The reaction of hydrazide IVa-c with thioglycolic acid in glacial acetic acid affords compounds Va-c respectively (Scheme 2). 2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N-(4-oxo-2-thiazolidin-3-yl)acetamide derivatives VIa-c were prepared by refluxing compound IVa-c with chloroacetyl chloride (Scheme 2). Compounds VII a-c were achieved by reaction of IV a-c with acetic anhydride (Scheme 2).

Biological activity

In vitro cytotoxic activity

In this present work new series of 4-substituted 6,8-dibromo-2-(4-chlorophenyl)-quinazoline derivatives were synthesized (Schemes 1 and 2). Cytotoxic activity of new compounds was evaluated on breast (MCF7) cancer cell line in vitro, using sulphorhodamine-B assay method (34) and doxorubicin as a reference drug.

The response parameter calculated was the IC_{50} value (the dose that reduces survival to 50%) in μM . By investigating the cytotoxic activity of the 4-sub-

stituted 6,8-dibromo-2-(4-chlorophenyl)-quinazoline derivatives (Table 1), it was observed that all tested compounds showed remarkable cytotoxic activity against MCF-7 with IC_{50} values range of 6.3-40.0 μM . Compounds **VIc** and **VIIb** exerted powerful cytotoxic activity with low IC_{50} (6.3 and 6.9 μM), compared to doxorubicin 7.72 μM .

Compounds **VIa**, **Vb** and **Vc** showed moderate cytotoxic effects with IC_{50} range (10.0 – 16.7) μM , respectively. Compounds **Va**, **VIIc**, **VIIa**, **IVb**, **IVc**, **VIIb** and **IVa** showed promising cytotoxic effects with IC_{50} range (20.3 – 40 μM , respectively).

In vitro apoptotic effect

The human CASP3 assay was used in the determination of CASP3 activity in vitro. (Table 2) represented enzyme activity relative to the untreated cells. All our tested compounds caused a significant increase in CASP3 activity against MCF-7 compared to doxorubicin. Compounds **VIc** and **VIIb** increased CASP3 activity even more than doxorubicin, while compounds **VIa**, **Vb**, **Vc**, **Va**, **VIIc**, **VIIa**, **IVb**, **IVc**, **VIIb** and **IVa** showed a moderate increase in CASP3 activity relative to untreated cells more or less similar to doxorubicin (Fig. 1). The reconstituting of CASP3 expression could augment the sensitivity of CASP3-deficient breast cancer (MCF-7) cells going in apoptosis in the response to doxorubicin and other apoptotic stimuli. These results gave the hypothesis that the CASP3 expression's loss may represent an important mechanism of cell survival in patients having breast cancer.

Structure activity relationship

Substitution of 6,8-dibromo-2-(4-chlorophenyl)-quinazoline moiety with Schiff bases at position 4 gave compounds IVa-c with moderate anticancer activity against MCF-7 and moderate CASP3 activity. Cyclization of compounds IVa-c with thioglycolic acid gives thiazolidine moiety (compounds Va-c) with higher cytotoxic and CASP3 activity. On the other hand, cyclization of Schiff bases IVa-c with chloroacetyl chloride gives azetidine moiety which gives powerful cytotoxic and CASP3 activity of compounds VIa and VIc. Cyclization of compounds IVa-c with acetic anhydride gives oxadiazole moiety which shows marked increase of cytotoxic and CASP3 activity only in compound VIIb

CONCLUSION

In summary, novel series of 4-substituted 6,8-dibromo-2-(4-chlorophenyl)-quinazoline deriva-

tives that may increase CASP3 activity have been designed and synthesized. All new derivatives were tested *in vitro* against MCF-7. Compounds **VIc** and **VIIb** exerted powerful cytotoxic activity with low IC₅₀ (6.3 and 6.9 μ M). All compounds activated apoptotic cascade in MCF-7. As expected, compounds **VIc** and **VIIb** exhibited remarked increase in CASP3 activity.

Acknowledgment

This work was financially supported by The Deanship of Scientific Research, Taif University, Kingdom of Saudi Arabia, grant no. 1-438-5497.

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Received: 08. 01. 2018