Breast cancer is the second leading cause of cancer death in women. The American Cancer Society’s estimates about 252,710 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 apoptosis 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 antimarial 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).

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-

**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 Vla, Vb and Vc showed moderate cytotoxic effects with IC50 range (10.0 – 16.7) µM, respectively. Compounds Va, Vlle, Vlla, IVb, IVc, VIIb 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

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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 measured 1H-NMR and 13C NMR (DMSO-d₆) 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).

**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.

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

Crystallized from methanol, m.p. 210°C, 75% yield. Analysis: calcd. for C₂₃H₁₄Br₂Cl₂N₄O₂: C, 45.35; H, 2.29; N, 9.20%; found: C, 45.30; H, 2.29; N, 9.20%. IR: \( \nu_{\text{max}}/\text{cm}^{-1} \) 3200 (NH), 3010 (C-H aromatic), 1700 (C=O), 1620 (C=N) and at 1580 (C=C). 1H-NMR (DMSO-d₆, ppm): 4.8 (s, 2H, OCH₂), 7.2 (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, CH=N) and 11.3 (s, 1H, NH, exchangeable with D₂O). 13C NMR (DMSO-d₆): 185, 175, 162, 150, 143, 141, 134, 133, 132, 131, 129, 128, 127, 124, 123, 112, 69, 68. MS: m/z = 613
Crystallized from glacial acetic acid m.p. 150°C, 70% yield. Analysis: calcd. for C_{25}H_{17}Br_{2}ClN_{4}O_{3}S: C, 46.28; H, 2.64; N, 8.64%; found: C, 46.3; H, 2.70; N, 8.59%. IR: \nu_{max}/cm^{-1}: 3350 (NH), 3050 (C=H aromatic), 1730 (C=C=O), 1710 (C=C=O), 1650 (C=N) and at 1600 (C=C). \textit{H} NMR (DMSO-d_{6}, ppm): 6 3.7-3.8 (m, 2H, CH, 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), and at 11.1 (s, 1H, NH, exchangeable with D_{2}O). 13C NMR (DMSO-d_{6}): 6 180, 168, 166, 162, 149, 141, 139, 134, 132, 129, 128, 127, 126, 124, 121, 115, 70, 57, 36. MS: m/z = 652

To a solution of compounds \textit{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 30 h. 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-yl)oxo)acetamide (\textit{VI} a)

Crystallized from glacial acetic acid m.p. 210°C yield (65%). Analysis: calcd. for C_{25}H_{15}Br_{2}Cl_{3}N_{4}O_{3}: C, 45.32; H, 2.97; N, 7.58%. IR: \nu_{max}/cm^{-1}: 3300 (NH), 3050 (C=H aromatic), 1730 (C=C=O), 1720 (C=C=O), 1630 (C=N) and at 1600 (C=C). \textit{H} NMR (DMSO-d_{6}, ppm): 6 3.9-4.0 (m, 2H, CH, 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, NH, exchangeable with D_{2}O). 13C NMR (DMSO-d_{6}): 6 186, 167, 165, 162, 150, 141, 137, 134, 132, 129, 128, 127, 126, 124, 111, 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-yl)oxo)acetamide (\textit{VI} b)

Crystallized from ethanol m.p. 115°C yield (70%). Analysis: calcd. for C_{28}H_{23}Br_{2}Cl_{2}N_{4}O_{6}: C, 46.37; H, 2.21; N, 8.17%. IR: \nu_{max}/cm^{-1}: 3300 (NH), 3050 (C=H aromatic), 1730 (C=C=O), 1720 (C=C=O), 1620 (C=N) and at 1600 (C=C). \textit{H} NMR (DMSO-d_{6}, ppm): 6 3.9-4.0 (m, 2H, CH, thiazolidinone ring), 6.8-7.8 (m, 6H, Ar-H), 8.0 (s, 1H, Quin-H), and at 11.2 (s, 1H, NH, exchangeable with D_{2}O). 13C NMR (DMSO-d_{6}): 6 180, 166, 163, 162, 150, 143, 134, 132, 129, 128, 126, 124, 123, 121, 116, 69, 64, 62 MS: m/z = 746

N-(3-chloro-2-oxo-4-(3,4,5-trimethoxyphenyl)azetidin-1-yl)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yl)oxo)acetamide (\textit{VI} c)

Crystallized from ethanol m.p. 140°C yield (70%). Analysis: calcd. for C_{25}H_{16}Br_{2}Cl_{2}N_{4}O_{3}: C, 45.90; H, 3.05; N, 8.50%. IR: \nu_{max}/cm^{-1}: 3300 (NH), 3050 (C=H aromatic), 1730 (C=C=O), 1720 (C=C=O), 1620 (C=N) and at 1600 (C=C). \textit{H} NMR (DMSO-d_{6}, ppm): 6 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 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_{2}O). 13C NMR (DMSO-d_{6}): 6 186, 167, 164, 162, 152, 150, 141, 137, 134, 132, 129, 128, 124, 123, 121, 69, 64, 60, 56 MS: m/z = 746

N-(3-chloro-2-oxo-4-(phenylazetidin-1-yl)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yl)oxo)acetamide (\textit{VII} a-c)
2H, OCH$_2$), 5.0 (d, 1H, $J = 6$ Hz, CH, azetidinone), 5.4 (d, 1H, $J = 8.4$ 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 D$_2$O). $^{13}$C NMR (DMSO-d$_6$): 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 C$_{25}$H$_{17}$Br$_2$ClN$_4$O$_3$: C, 48.69; H, 2.78; N, 9.09%; found: C 48.72; H, 2.74; N, 9.00%. IR: $\nu_{max}$/cm$^{-1}$ 3100 (C-H aromatic), 1720 (C=O), 1610 (C=N) and at 1595 (C=C). $^1$H-NMR (DMSO-d$_6$, ppm): $\delta$ 1.9 (s, 3H, COCH$_3$), 4.7 (s, 2H, OCH$_2$), 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) and at 10.8 (s, 1H, NH, exchangeable with D$_2$O). $^{13}$C NMR (DMSO-d$_6$): 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 C$_{28}$H$_{23}$Br$_2$ClN$_4$O$_6$: C, 47.58; H, 3.28; N, 7.93%; found: C 47.54; H, 3.24; N, 7.89%. IR: $\nu_{max}$/cm$^{-1}$ 3100 (C-H aromatic), 1730 (C=O), 1620 (C=N) and at 1600 (C=C). $^1$H-NMR (DMSO-d$_6$, ppm): $\delta$ 1.8 (s, 3H, COCH$_3$), 3.7 (s, 9H, OCH$_3$), 4.7 (s, 2H, OCH$_2$), 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). $^{13}$C NMR (DMSO-d$_6$): 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
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°C, 60% yield. Analysis: calcd. for C_{25}H_{16}Br_{2}Cl_{2}N_{4}O_{3}: 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-d\textsubscript{6}, ppm): \( \delta \) 1.6 (s, 3H, COCH\textsubscript{3}), 4.5 (s, 2H, OCH\textsubscript{2}), 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-d\textsubscript{6}): 183, 169, 162, 158, 149, 141, 138, 134, 132, 130, 127, 124, 122, 121, 116, 83, 72, 23. MS: m/z = 656

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

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 µg/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°C 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](image)

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

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Sample conc. (µg/mL)</th>
<th>IC₅₀ µM ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6.25</td>
</tr>
<tr>
<td>IV a</td>
<td>0</td>
<td>19.00</td>
</tr>
<tr>
<td>IV b</td>
<td>0</td>
<td>20.40</td>
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<td>IV c</td>
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<td>V a</td>
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<td>V b</td>
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</tr>
<tr>
<td>V c</td>
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</tr>
<tr>
<td>VI a</td>
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<tr>
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<td>23.83</td>
</tr>
<tr>
<td>Doxorubicin</td>
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</tr>
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</table>
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 (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, VIb 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, Ve, Va, VIIc, VIIa, IVb, IVc, VIb 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 thio glycolic 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 µM). All compounds activated apoptotic cascade in MCF-7. As expected, compounds VIc and VIIb exhibited remarked increase in CASP3 activity.

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