Quinazoline is one of the most widespread scaffolds in medicinal chemistry and its derivatives were reported to possess diverse biological applications like antiviral (1, 2), antibacterial (3, 4), antifungal (3, 5), antimalarial (7-11), anticancer (7-11), antihypertensive (12), anti-inflammatory (13, 14), analgesic and COX-II inhibitors (15-18).

In spite of development of novel antitumor drugs in recent years, cancer is considered the major leading cause of death. The literature survey revealed that anilinoquinazoline containing compounds were recently approved for the treatment of HER2-positive metastatic breast cancer (19-27). The presence of substituted aromatic ring at position 3 and at position 2 is necessary requirement for its medicinal properties (9, 10). On the other hand, diverse chemotherapeutic agents contain pharmacophores like Br (28), Schiff bases (29-31), carboxamide (32) and carbothioamide (33) are known to contribute to the enhancement of the antitumor activity.

Abdel Gawad et al. reported that 10 µM of compound 1 (Fig. 1) revealed 34.3 percentage growth inhibition when tested in vitro against human breast cancer cell line (MCF-7) using resazurin reduction assay method and doxorubicin as a reference drug. Most of the tested compounds showed better activity than doxorubicin. Compound IVh was the best active one, its IC50 is 8.52 µg/mL. Molecular docking studies for the best active compounds IVb, IVc, IVf, IVh and Va were performed on the active site of estrogen receptor α (ERα) subtype to explore the estrogen receptor binding ability of these compounds. All the docked compounds showed good fitting score energy with the active site of ERα subtype and compound IVh showed the best docking score energy (-25.3 kcal/mol). Estrogen binding evaluation assay was performed for the docked compounds to ensure that their activity against MCF7 go through inhibition of ERα, they showed ERα inhibition at 41–85% and compound IVh was the most active one (85%).

Keywords: synthesis, quinazolines, 6,8-dibromo-4(3H)-quinazolinone, breast cancer
tion to ethyl benzoate ester as a substituent at position 3 (compound II) (Fig. 2); all these compounds bear Br atom at position 6 and 8, and have aryl substi-
tution at both positions 2 and 3. Further substitu-
tion of the 3-phenyl moiety at para position with
ester, hydrazide, carboxamide, thiocarboxamide and Schiff bases was also performed aiming to obtain
new quinazolin-4-one derivatives with high activity
against breast cancer cells.

The estrogen receptors are considered as an
important pharmaceutical targets for the treatment
of variety of diseases such as osteoporosis and
breast cancer (36), as a result molecular docking
studies for the best active compounds were per-
formed against estrogen receptor α (ERα) subtype
to predict the ability of these derivatives to act as
modulators of the human estrogen receptor, in addi-
tion, the binding affinities of these compounds with
ERα were assessed to ensure their mode of action.

EXPERIMENTAL

Chemistry

All melting points are uncorrected, elemental
analyses were carried out in the micro analytical
units of National Research Centre and Cairo
University, Egypt. IR spectra were recorded on
FTIR spectrophotometer-Nexus 670-Nicolet, USA
and Perkin Elmer-9712 spectrophotometer. 1H-
NMR spectra were determined on a Varian-Gemini-
300 MHz. and Jeol EX270 MHz NMR spectrometers.
13C NMR (DMSO-d6) spectra were recorded at 100.62 MHz at the aforementioned research center
in Cairo University. Mass spectra were recorded on
Finnigan Mat SSQ 7000 mode El 70 eV (Thermo
Inst. Sys. Inc. USA). Thin layer chromatography
was carried out on silica gel 60 F254 (Merck) thin
layer chromatography plates using a chloroform,
petroleum ether/methanol/mixture (7 : 4 : 1 v/v/v) as
the mobile phase. Synthesis of the desired com-
pounds (Scheme 1) was achieved by reacting com-
pound I, which was synthesized following the same
method used for the preparation of 6,8-dibromo-2-
phenyl-4H-benzo-[1,3]-oxazin-4-one (37) with
ethyl-p-aminobenzoate at 140OC to afford com-
pound II. Moreover, chemical structures of all the
newly synthesized compounds (Scheme 1 and 2)
were confirmed by IR, 1H-NMR, 13C NMR and mass
spectra.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-
quinazolin-3-yl] benzoic acid ethyl ester (II)

A mixture of benzoxazine derivative I (4.1 g.
10 mmol) and ethyl p-aminobenzoate (1.65 g. 10
mmol) was heated together upon fusion at 140°C on
sand bath for 1 h. After cooling, the crude mass was
crystallized from ethanol twice to give white crys-
tals of II. M.p. 230°C, 70% yield. IR (KBr, cm⁻¹):
Figure 2. The formula of the novel synthesized 3H-quinazoline-4-one derivatives

Scheme 1. Reagents and conditions: a, fusion 140°C on sand bath, 1 h. b, hydrazine hydrate 98%, abs. ethanol, reflux 8 h. c, reflux 5 h in glacial acetic acid
3060 (CH, arom.), 1710, 1700 (2CO), 1.3 (3H, t, CH₃, ethyl group), 4.3 (q, 2H, CH₂, ethyl group), 7.25-8.25 (m, 10H, arom. -H). 13C NMR (DMSO-d₆, δ ppm): 165.9, 164, 160.6, 154.3, 139.4, 137, 135.7, 131.3, 130.1, 129.1, 128.9, 126.7, 125.2, 124.3, 123.7, 122, 113, 60.9, 14.1. MS (m/z, R.I.): M⁺ 561.91 (100.0%), 563.91 (69.4%), 559.91 (44.1%). Analysis: for C₂₃H₁₅Br₂ClN₂O₃, m.w. 562.64, calcd.: C, 49.10; H, 2.69; N, 4.98%; found: C, 49.50; H, 2.65; N, 5.10%.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazolin-3-yl] benzoic acid hydrazide (III)

A solution of the ester derivative II (5.62 g, 10 mmol) and hydrazine hydrate 98% (1.6 g, 50 mmol) in absolute ethanol (20 mL) was refluxed for 8 h. Upon cooling, the formed precipitate was filtered off and recrystalized from ethanol to give the hydrazide derivative III. M. p. 170°C, 80% yield. IR (KBr, cm⁻¹): 3315, 3140 (NH, NH₂), 3060 (CH, arom.), 1715 (CO, quinazoline ring), 1645 (CO, amide), 1672, 164.1, 160.4, 154.1, 139.2, 136.3, 131.2, 131.1, 130.1, 129.4, 128.8, 128.6, 128.2, 127.5, 125.2, 124.5, 122.2, 113.5. MS (m/z, R.I.): M⁺ 561.91 (100.0%), 563.91 (69.4%), 559.91 (44.1%). Analysis: for C₂₃H₁₅Br₂ClN₂O₃, m.w. 562.64, calcd.: C, 49.10; H, 2.69; N, 4.98%; found: C, 49.50; H, 2.65; N, 5.10%.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazolin-3-yl] benzoic acid (substituted) benzylidene hydrazide (IVa-h)

General method: A mixture of compound III (5.48 g, 10 mmol) and the appropriate aldehyde, namely: benzaldehyde, p-anisaldehyde, p-tolualdehyde, p-chlorobenzaldehyde, 3,4-dichlorobenzaldehyde, p-hydroxybenzaldehyde, naphthalene-2-carboxaldehyde or furan-2-carboxaldehyde (20 mmol) in glacial acetic acid (30 mL), was refluxed for 5 h. The reaction mixture was cooled and ice water was added, the formed precipitate was filtered off, washed with water and crystallized from the proper solvent to obtain the desired Schiff bases (IVA-h), respectively.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazolin-3-yl] benzoic acid benzylidene hydrazide (IVA)

Crystallized from methanol to give yellow crystals, m.p. 150°C, 70% yield. IR (KBr, cm⁻¹): 3370 (NH), 1710, 1685 (2CO), 1596 (C=N). 13C NMR (DMSO-d₆, δ ppm): 7.53-8.25 (m, 10H, arom.-H). MS (m/z, R.I.): M⁺ 547.91 (100.0%), 549.91 (44.9%), 545.91 (44.0%), 549.90 (27.4%). Analysis: for C₂₁H₁₃Br₂ClN₄O₂, m.w. 548.61, calcd.: C, 49.97; H, 2.39; N, 10.21%; found: C, 49.70; H, 2.59; N, 10.40%.
4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-
quinazolin-3-yl] benzoic acid (4-methoxybenzyl-
dene) hydrazide (IVb)

Crystallized from acetic acid to give yellow
-crystals, m.p. 166 °C, 75% yield. IR (KBr, cm⁻¹):
Crystallized from acetic acid to give brown
-crystals, m.p. 200 °C, 80% yield. IR (KBr, cm⁻¹):
Crystallized from methanol to give yellowish
-white crystals, m.p. 154°C; in 80% yield. IR (KBr, cm⁻¹):
Crystallized from ethanol to give yellow
-crystals, m.p. 190°C, 80% yield. IR (KBr, cm⁻¹):
Crystallized from ethanol to give brown crys-
tals, m.p. 145°C, 82% yield. IR (KBr, cm⁻¹): 3325
(2NH), 1715, 1700 (2CO), 1605 (C=O). ¹H-NMR
(DMSO-d₆, δ ppm): 8.34 (s, 1H, CH=O), 5.19 (s, 2H,
CH₂), 4.96-5.06 (2H, arom. -H), 4.85 (s, 1H, CH=CH),
11.87 (s, 1H, NH, exchangeable with D₂O). ¹C NMR
(DMSO-d₆, δ ppm): 164.0, 163.2, 162.9, 160.6, 160.6,
164.3, 164.8, 139.4, 136.1, 135.7, 131.3, 130.2,
129.6, 129.1, 128.9, 128.4, 126.7, 126.0, 125.2,
124.5, 122.0, 144.4, 113.2, 55.7. MS (m/z, R.I.):
M⁺ 665.95 (100%), 667.95 (70.2%), 663.95 (44.1%),
666.95 (33.0%). Analysis: for C₂₉H₁₅Br₂Cl₂N₄O₂,
m.w. 666.75, calcld.: C, 52.24; H, 2.52; N, 8.56%.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-
quinazolin-3-yl] benzoic acid (4-methoxybenzyl-
dene) hydrazide (IVc)

Design, synthesis, molecular docking and anti-breast cancer activity of... 119

ppm): 164.4, 163.3, 160.5, 154.3, 146.8, 139.2,
136.0, 135.4, 133.6, 131.0, 129.6, 129.2,
129.1, 128.9, 128.8, 128.4, 126.7, 125.2, 124.7,
122.0, 112.9. MS (m/z, R.I.): M⁺ 635.94 (100%),
637.94 (70.4%), 633.94 (44.0%), 636.94 (31.7%).
Analysis: for C₁₉H₁₂Br₂ClN₂O₂, m.w. 637.72, calcld.: C,
52.82; H, 2.69; N, 8.80%; found: 55.70; H, 2.65;
N, 8.60%.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-
quinazolin-3-yl] benzoic acid (4-methoxybenzyl-
dene) hydrazide (IVd)

Crystallized from acetic acid to give brown
-crystals, m.p. 190°C, 80% yield. IR (KBr, cm⁻¹):

3325 (NH), 1715, 1690 (2CO), 1598 (C=N). ¹H-
NMR (DMSO-d₆, δ ppm): 7.83 (s, 3H, OCH₃),
7.00-8.25 (m, 14H, arom. -H), 8.36 (s, 1H,
CH=CH), 11.87 (s, 1H, NH, exchangeable with D₂O). ¹C NMR
(DMSO-d₆, δ ppm): 164.0, 163.2, 162.9, 160.6,
154.3, 146.8, 139.4, 136.1, 135.7, 131.3, 130.2,
129.6, 129.1, 128.9, 128.4, 126.7, 126.0, 125.2,
124.5, 122.0, 144.4, 113.2, 55.7. MS (m/z, R.I.):
M⁺ 665.95 (100%), 667.95 (70.2%), 663.95 (44.1%),
666.95 (33.0%). Analysis: for C₂₉H₁₅Br₂Cl₂N₄O₂,
m.w. 666.75, calcld.: C, 52.24; H, 2.87; N, 8.40%;
found: C, 52.20; H, 2.90; N, 8.56%.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-
quinazolin-3-yl] benzoic acid (4-methylbenzyl-
dene) hydrazide (IVe)

Crystallized from methanol to give lemonish
-white crystals, m.p. 154°C; in 80% yield. IR (KBr, cm⁻¹):
Crystallized from methanol to give white crys-
tals, m.p. 200°C, 80% yield. IR (KBr, cm⁻¹):
Crystallized from acetic acid to give brown
-crystals, m.p. 145°C, 82% yield. IR (KBr, cm⁻¹): 3325
(2NH), 1715, 1700 (2CO), 1605 (C=O). ¹H-NMR
(DMSO-d₆, δ ppm): 8.34 (s, 1H, CH=O), 5.19 (s, 2H,
CH₂), 4.96-5.06 (2H, arom. -H), 4.85 (s, 1H, CH=CH),
11.87 (s, 1H, NH, exchangeable with D₂O). ¹C NMR
(DMSO-d₆, δ ppm): 163.9, 163.0, 160.5, 154.7,
146.4, 140.7, 139.9, 136.0, 135.7, 131.1, 130.5,
129.6, 129.1, 128.9, 128.4, 126.7, 126.1, 125.2,
124.4, 122.1, 113.5, 21.3. MS (m/z, R.I.): M⁺ 649.95
(100.0%), 651.95 (69.7%), 647.96 (44.1%), 650.96
(31.8%). Analysis: for C₁₉H₁₂Br₂Cl₂N₄O₂, m.w.
650.75, calcld.: C, 53.52; H, 2.94; N, 8.61%;
found: C, 53.49; H, 3.00; N, 8.67%.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-
quinazolin-3-yl] benzoic acid (4-chlorobenzyl-
dene) hydrazide (IVf)

Crystallized from acetic acid to give brown
-crystals, m.p. 166°C, 75% yield. IR (KBr, cm⁻¹):
Crystallized from methanol to give yellowish
-white crystals, m.p. 154°C; in 80% yield. IR (KBr, cm⁻¹):

128.6, 128.4, 128.1, 127.2, 126.9, 126.7, 126.2, 125.2, 124.5, 122.0, 113.5. MS (m/z, R.I.): M+ 685.95 (100.0%), 687.95 (69.7%), 683.96 (44.1%), 686.96 (35.0%). Analysis: for C32H19Br2ClN4O2, m.w. 686.78, calcd.: C, 55.96; H, 2.79; N, 8.16%; found: C, 55.81; H, 2.60; N, 8.20%.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazolin-3-yl] benzoic acid furan-2-ylmethylene hydrazide (IVh)

Crystallized from methanol to give white crystals, m.p. 240°C, 75% yield. IR (KBr, cm⁻¹): 3415 (NH), 1720, 1690 (2CO), 1605 (C=N). ¹H-NMR (DMSO-d₆, δ, ppm): 6.5-8.25 (m, 13H, arom, -H and furan -H ), 8.3 (s, 1H, CH=N), 11.1 (s, 1H, NH, exchangeable with D₂O). ¹³C NMR (DMSO-d₆, δ, ppm): 163.6, 163.2, 160.0, 154.1, 144.2, 139.4, 136.1, 135.7, 131.0, 129.6, 129.1, 128.9, 128.4, 126.7, 126.2, 125.2, 124.5, 122.0, 118.9, 113.2, 112.6. MS (m/z, R.I.): M+ 625.92 (100.0%), 627.92 (46.4%), 623.92 (43.9%), 626.92 (28.3%). Analysis: for C₂₆H₁₅Br₂ClN₄O₃, m.w. 626.68, calcd.: C, 49.83; H, 2.41; N, 8.94%; found: C, 49.79; H, 2.30; N, 8.97%.

2-(4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-quinazolin-3(4H)-yl)benzoyl)-N-phenylhydrazine carboxamide (Va, b)

General method: a mixture of compound III (5.48 g, 10 mmol), the appropriate iso/isothiocyanate, namely: phenyl isocyanate or phenyl isothiocyanate (10 mmol) in pyridine (20 mL) was refluxed for 8 h. The solvent was poured on crushed ice containing few drops of HCl. The solid product was filtered off and washed with water to obtain the desired products Va, b, respectively.

2-{4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-quinazolin-3(4H)-yl]benzoyl}-N-phenylhydrazine carboxamide (Va)

Crystallized from acetic acid to give white crystals, m.p. 194°C, 60% yield. IR (KBr, cm⁻¹): 3313, 3270, 3200 (3NH), 3064 (CH arom.), 1709, 1669, 1659 (3CO). ¹H-NMR (DMSO-d₆, δ, ppm): 7.5-8.5 (m, 15H, arom. -H), 6.01, 9.02, 10.75 (3s, 3H, 3NH, exchangeable with D₂O). ¹³C NMR (DMSO-d₆, δ, ppm): 164.8, 164.2, 160.6, 154.0, 153.6, 139.4, 136.1, 135.7, 131.0, 129.6, 129.1, 128.9, 128.6, 126.7, 125.2, 124.5, 122.0, 121.4, 113.0. MS (m/z, R.I.): M⁺ 666.92 (100.0%), 668.94 (69.8%), 664.95 (44.1%), 667.95 (30.7%). Analysis: for C₂₈H₁₈Br₂ClN₅O₃, m.w. 667.74, calcd.: C, 50.32; H, 2.59; N, 10.49%; found: C, 49.79; H, 2.30; N, 10.20%.

Cell culture and treatment

All reagents were handled in a sterile fume hood. DMEM medium and fetal bovine serum (FBS) were purchased from Gibco; phosphate-buffered saline pH 7.4 (PBS) and trypsin-EDTA were obtained from Sigma-Aldrich. Alamar blue or resazurin (Promega, Mannheim, Germany) reduction assay was used to assess the cytotoxicity of the studied samples. The growth medium (DMEM medium with 10% FBS, 100 U/mL penicillin, and 100 mg/L streptomycin), and alamar blue were stored at 4°C, while trypsin-EDTA and FBS were stored frozen at -20°C and thawed before use; PBS was stored at room temperature. The MCF-7 cells
were obtained from the German Cancer Research Center (DKFZ). Cells were cultured in 50 cm² culture flasks (Corning) using DMEM medium supplemented with 10% FBS, penicillin (100 IU/mL), and streptomycin (100 mg/mL). The culture was maintained at 37°C in an atmosphere of 5% CO₂ and 95% relative humidity. The cells were transferred to a new flask every 2 days and treated with trypsin-EDTA to detach them from the flask. Cells were counted under a microscope using a hemacytometer (Hausser Scientific). Cell solutions were diluted with growth medium to a concentration of 1 × 10⁵ cells/mL and transferred to a 96-well plate, and treated with gradient concentrations of test compounds.

**Resazurin cell growth inhibition assay**

The assay tests cellular viability and mitochondrial function. Briefly, adherent cells were grown in tissue culture flasks as previously described (38-42) and then harvested by treating the flasks with 0.025% trypsin and 0.25 mM EDTA for 5 min. Once detached, cells were washed, counted and an aliquot (5 × 10⁴ cells) was placed in each well of a 96-well cell culture plate in a total volume of 100 µL. Cells were allowed to attach overnight and then were treated with samples. The final concentration of samples ranged from 0 to 100 µM. After 48 h, 20 µL resazurin 0.01% w/v solution was added to each well and the plates were incubated at 37°C for 1–2 h. Fluorescence was measured on an automated 96-well Infinite M2000 Pro plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. After 48 h incubation, plates were treated with resazurin solution as described above and then were treated with resazurin 0.01% w/v solution was added to each well and the plates were incubated at 37°C for 1–2 h. Fluorescence was measured on an automated 96-well Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. After 48 h incubation, plates were treated with resazurin solution as above mentioned. Doxorubicin was used as positive control. Each assay was done at least three times, with two replicates each. The viability was compared based on contrast with untreated control. IC₁₀ (on cancer cells) were the concentration of sample required to inhibit 50% of the cell proliferation and were calculated from a calibration curve by a linear regression using Microsoft Excel.

**ERα binding assay**

The fluorescent estrogen ligand (self-made) was added to the recombinant ERα and screening buffer (ES2 Screening Buffer, Invitrogen, USA) to make the final concentration 9 nM for fluorescent estrogen and 30 nM for ERα. Test compounds were dissolved in DMSO (1 µL) and screening buffer (49 µL) to obtain the required concentration. The fluorescent estrogen/ER complex was added to this 50 µL to make the final volume of 100 µL. Fifty µL estradiol buffer (1 nM) and 50 µL fluorescent estrogen/ER complex was used as a positive control. A negative control containing 50 µL screening buffer and 50 µL fluorescent estrogen/ER complex was used to determine the theoretical maximum polarization. The microplate was incubated in the dark at room temperature for 1.5 h and was shaken on a plate shaker. Finally, the polarization values were read on a Safire microplate reader.

**Molecular docking**

Molecular modeling studies were performed using Molecular Operating Environment (MOE, 10.2008) software on an Intel Core i5, 2.53 GHz processor, 4 GB memory with Windows XP 32-bit operating system. Preparation of the synthesized molecules was carried out by Chem Draw ultra to generate their 2D structures that were saved as mol, then their 3D, protonation and energy minimization were done by MOE software, with RMSD gradient of 0.05 kcal/mol, MMFF94X force field and automatic calculation of the partial charges. ERα subtype cocrystallized with benzoxathin ligand was obtained from protein data bank (43), the pdb file is 1SJ0, all bound water molecules were removed, hydrogens were added, the active site was detected and saved as mole file for docking. This model was then used to predict the interactions and binding score energy between the new compounds and the active site.

**RESULTS AND DISCUSSION**

**In vitro cytotoxic activity against MCF-7**

In this present work novel series of quinazolin-4(3H)-ones were synthesized. Synthetic Schemes 1, 2 illustrate the way used for the synthesis of target compounds. All synthesized compounds were screened for their in vitro cytotoxic and growth inhibitory activities against MCF-7 cell line, in comparison with the activity of the known anticancer reference drug doxorubicin (DOX) as a reference standard. The cytotoxic activities of our tested compounds were expressed as IC₅₀ values (the dose that reduces survival to 50%) in µg/mL. It is evident that all of the tested compounds showed antitumor activities with IC₅₀ values ranging from 8.52 to 25.96 µg/mL (Table 1).

Compounds IVh, IVf, IVc, Va, IVb, IVa, IVd and II exerted powerful cytotoxic effects against MCF-7 with very low IC₅₀ value less than that of DOX. Their IC₅₀ range was 8.52-11.44 µg/mL. Compounds VI, IVe, III and Vb exerted cytotoxic
effect against MCF-7 cells nearly as DOX (IC\textsubscript{50} values were 13.21, 13.31, 13.81 and 14.29 µg/mL, respectively). Compound \textit{IVg} was the least active one, its IC\textsubscript{50} value is 25.9 µg/mL.

From these observed results we can conclude that:

1. Substituting the para position of the 3-phenyl moiety with ethyl ester \textit{II} lead to better activity than doxorubicin against MCF-7 cell line.

2. Compounds with general formula \textit{A} (Fig. 2), that have a Schiff base in addition to the quinazolin-4-one scaffold showed better activity than doxorubicin against the same cell line except compound \textit{IVe} which showed a closely similar activity to doxorubicin, IC\textsubscript{50} 13.31 and 12.2 µg/mL, respectively, and compound \textit{IVg} which was the least active.

3. Compounds with general formula \textit{B} (Fig. 2), \textit{III}, \textit{Vb} and \textit{VI} showed to be near the activity of doxorubicin against breast cancer cells, whoev- er, \textit{Va} showed to be better active than doxorubicin.

4. The best active compound was \textit{IVh} and the least active was \textit{IVg} and both are quinazolin-4-one bearing Schiff base at the para position of 3-phenyl moiety.

\textbf{ER\textalpha binding affinity assay}

Docking studies indicated the ability of the best active compounds \textit{IVh}, \textit{IVf}, \textit{IVb}, \textit{IVe} and \textit{Va} to quick fit the active site of ER\textalpha so the binding affinities of these compounds with ER\textalpha were assessed using fluorescence polarization procedure (44) and tamoxifen was used as a positive control. The obtained results (Table 2) exhibited a good binding affinities with ER\textalpha and this is considered an evidence that these compounds could act through ER\textalpha inhibition. The inhibitory range was 41-85%, compound \textit{IVh} with a furan substituent at the para position of the 3-phenyl moiety showed to be the best active, its ER\textalpha inhibitory activity was 85% followed by compound \textit{IVf} (71%) that has a substitu- tion in the para position of the 3-phenyl with a phenyl moiety bearing an ortho hydroxyl group. Also compound \textit{IVb} with a 4-methoxy group instead of 2-OH of \textit{IVf} showed a percentage of inhibi- tion at 66.7%.

\textbf{Molecular docking}

The estrogen receptor is a nuclear receptor which plays a critical role as a mediator for the actions of the estrogen hormones, ER\textalpha is predomi- nant and highly expressed ER in breast cancer

Figure 3. 2D interactions of dihydrobenzoxathin as a cocrystallized selective ligand in the active site of ER\textalpha subtype
Table 1. IC_{50} values (dose that inhibits 50% of cells) of the tested compounds and doxorubicin expressed in µg/mL ± S.E.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} ± S.E.</th>
<th>Compound</th>
<th>IC_{50} ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>11.44 ± 1.56</td>
<td>IVf</td>
<td>9.1 ± 0.22</td>
</tr>
<tr>
<td>III</td>
<td>13.81 ± 2.73</td>
<td>IVg</td>
<td>25.96 ± 1.39</td>
</tr>
<tr>
<td>IVa</td>
<td>10.12 ± 3.23</td>
<td>IVh</td>
<td>8.52 ± 0.20</td>
</tr>
<tr>
<td>IVb</td>
<td>9.26 ± 0.14</td>
<td>Va</td>
<td>9.23 ± 1.66</td>
</tr>
<tr>
<td>IVc</td>
<td>9.23 ± 1.34</td>
<td>Vb</td>
<td>14.29 ± 1.71</td>
</tr>
<tr>
<td>IVd</td>
<td>11.12 ± 0.25</td>
<td>VI</td>
<td>13.21 ± 1.21</td>
</tr>
<tr>
<td>IVe</td>
<td>13.31 ± 0.23</td>
<td>Doxorubicin</td>
<td>12.2 ± 2.12</td>
</tr>
</tbody>
</table>

Figure 4. Redocked dihydrobenzoxathin ligand in the active site of ERα subtype, rmds = 0.921 and S = -26.18 kcal/mol

Figure 5. Compound IVh in the active site of ERα subtype
Figure 6. Compound IVf in the active site of ERα subtype

Figure 7. Compound IVb in the active site of ERα subtype
Docking the best active compounds into the active site of ERα subtype to explore their ability of binding to this receptor was performed using Molecular Operating Environment (MOE, 10.2008) software and the PDB file that was obtained from protein data bank is 1SJ0 (43), refined, protonated and saved as moe file for docking. This file represents the human ERα subtype co-crystallized with benzoxathin ligand (Fig. 3), to perform accurate docking protocol. Validation process was performed by redocking this ligand to the active site of the ERα, the docking score energy was -26.18 kcal/mol, rmds value was 0.921 and amino acid interactions was Gly 521, Glu 353, Arg 394 and His 524 (Fig. 4). All the docked compounds showed good score energy range from -13.5 to -25.3 kcal/mol (Table 3), that indicates their ability to fit into the active site of ERα subtype. Moreover, compound IVh (Fig. 5) showed amino acid interaction with Thr 347, compound IVf (Fig. 6) showed amino acid interactions with the same amino acid and compound IVb (Fig. 7) with Asp 351 amino acid. The best fitting score energy was shown by compound IVh.

CONCLUSION

In this study novel series II-VI of compounds having quinazolin-4(3H)-one scaffold was synthesized. All synthesized compounds were screened against MCF-7 cell line in order to determine their possible anti-breast cancer activity. Most of the tested compounds have shown promising activities against the human breast cancer cell line at very low concentrations and some of them showed better activity than doxorubicin. Substituting the para position of 3-phenyl moiety with ethyl ester II gave better activity against MCF-7 than doxorubicin, the best active of compounds with the general formula A (Fig. 2) was IVh with the least bulky substitution on Schiff base (furan moiety), whereas substitution with naphthalene moiety IVg dramatically decreased the activity. Substituting the aryl moiety in Schiff base with 3,4-dichloro IVe decreased the activity compared to mono chloro substituent IVd. All compounds having the general formula A (Schiff base derivatives) showed better activity than doxorubicin except IVe and IVg as they contain 3,4-dichlorophenyl and naphthalene moiety, respectively. None of compounds with the general formula B (Fig. 2) showed to be more active than doxorubicin except Va which is substituted with carboxamide at the para position of 3-phenyl moiety, the other derivatives III, Vb, VI showed activity nearly similar to doxorubicin. It is interesting to note that a minor alteration in the molecular configuration of investigated compounds may have a pronounced effect on anticancer activity, e.g., compound Va having phenylhydrazine carboxamide moiety showed higher anticancer activity than compound Vb that have phenylhydrazine carbodiimide moiety. On the other hand, Schiff bases substitution with bulky group (compound IVg) decreased anticancer activity compared to all other

Table 2. Estrogen receptor α inhibition %.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVb</td>
<td>66.7</td>
</tr>
<tr>
<td>IVc</td>
<td>47.3</td>
</tr>
<tr>
<td>IVf</td>
<td>71.3</td>
</tr>
<tr>
<td>IVh</td>
<td>85</td>
</tr>
<tr>
<td>Va</td>
<td>41</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. Binding score energy of the best active compounds and benzoxathin ligand with ERα subtype.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>S (kcal/mol)</th>
<th>Amino acid interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVb</td>
<td>-14.23</td>
<td>Asp 351</td>
</tr>
<tr>
<td>IVc</td>
<td>-14.57</td>
<td></td>
</tr>
<tr>
<td>IVf</td>
<td>-16.83</td>
<td>Thr 347</td>
</tr>
<tr>
<td>IVh</td>
<td>-25.29</td>
<td>Thr 347</td>
</tr>
<tr>
<td>Va</td>
<td>-13.52</td>
<td></td>
</tr>
<tr>
<td>Benzoxathin</td>
<td>-26.18</td>
<td>Asp 351, His 524</td>
</tr>
</tbody>
</table>
Schiff bases, especially compound IVh, which is the best active one. Docking IVb, IVc, IVf, IVh and V compounds against the active site of ERα subtype was performed to investigate their binding mode with this receptor which is a key target in treatment of breast cancer. All compounds showed a good fitting score energy, evaluating ERα inhibitory activity revealed good inhibitory properties of these compounds. In light of obtained results we can conclude that 6,8-dibromo-2-(4-chlorophenyl)-4H-quinazolin-4-one with para substituted phenyl moiety at position 3 represent a useful scaffold for designing new promising active drugs in breast cancer treatment with the ability of binding to human ERα subtype which is the key target in breast cancer treatment.

REFERENCES


Received: 7. 12. 2014