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DESIGN, SYNTHESIS, MOLECULAR DOCKING AND ANTI-BREAST CANCER ACTIVITY OF NOVEL QUINAZOLINONES TARGETING ESTROGEN RECEPTOR α

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Abstract: A new series of 6,8-dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazoline derivatives **II**-**VI** were synthesized, their chemical structures were confirmed by spectroscopic means and elemental analyses. All these compounds were 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 IC_{50} 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

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 (6), 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 MCF-7 tumor cell line (34). 6,8-Dibromo substituted quinazolin-4-one **2** (Fig. 1) showed a greater activity than doxorubicin against MCF-7, their IC_{50} values were 1.7 and 29.6 µg/mL, respectively (11). In addition, 6-chloro-3-methyl-2-p-tolylquinazolin-4(3H)-one derivative **3** showed to be more active than doxorubicin against MCF-7 cell line (8). Moreover, 3H-quinazolin-4-one derivative **4** that bear aryl moiety at position 2 and 3 was found to be more active also than the reference drug doxorubicin against MCF-7 cell line (35).

In view of the aforementioned facts, and in continuation of our drug research program concerning synthesis of new safer and more biologically active quinazolinone derivatives, it was of interest to synthesize a new series of anilinoquinazoline compounds having the general formula **A** and **B**, in addi-

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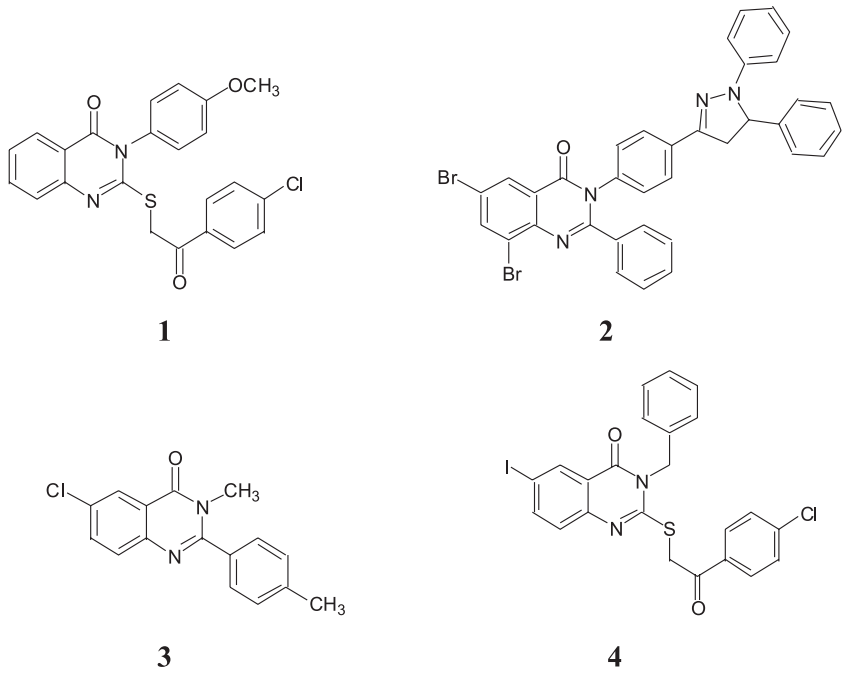


Figure 1. Substituted-3H-quinazolin-4-one derivatives active against the human breast cancer cell line (MCF-7)

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 substitution at both positions 2 and 3. Further substitution 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 performed against estrogen receptor α (ERα) subtype to predict the ability of these derivatives to act as modulators of the human estrogen receptor, in addition, 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. ^{1}H-NMR spectra were determined on a Varian-Gemini-300 MHz. and Jeol EX270 MHz NMR spectrometers. ^{13}C NMR (DMSO-d_6) 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 EI 70 eV (Thermo Inst. Sys. Inc. USA). Thin layer chromatography was carried out on silica gel 60 F_{254} (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 compounds (Scheme 1) was achieved by reacting compound **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 140^{o}C to afford compound **II**. Moreover, chemical structures of all the newly synthesized compounds (Scheme 1 and 2) were confirmed by IR, ^{1}H-NMR, ^{13}C 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^{o}C on sand bath for 1 h. After cooling, the crude mass was crystallized from ethanol twice to give white crystals of **II**. M.p. 230^{o}C, 70% yield. IR (KBr, cm^{-1}):

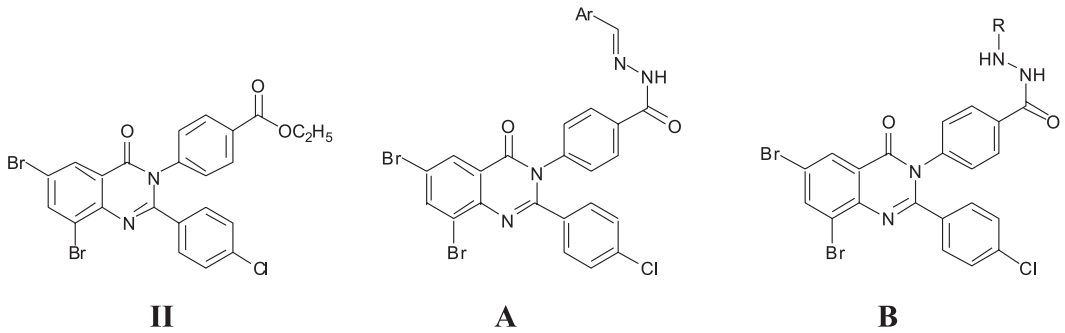
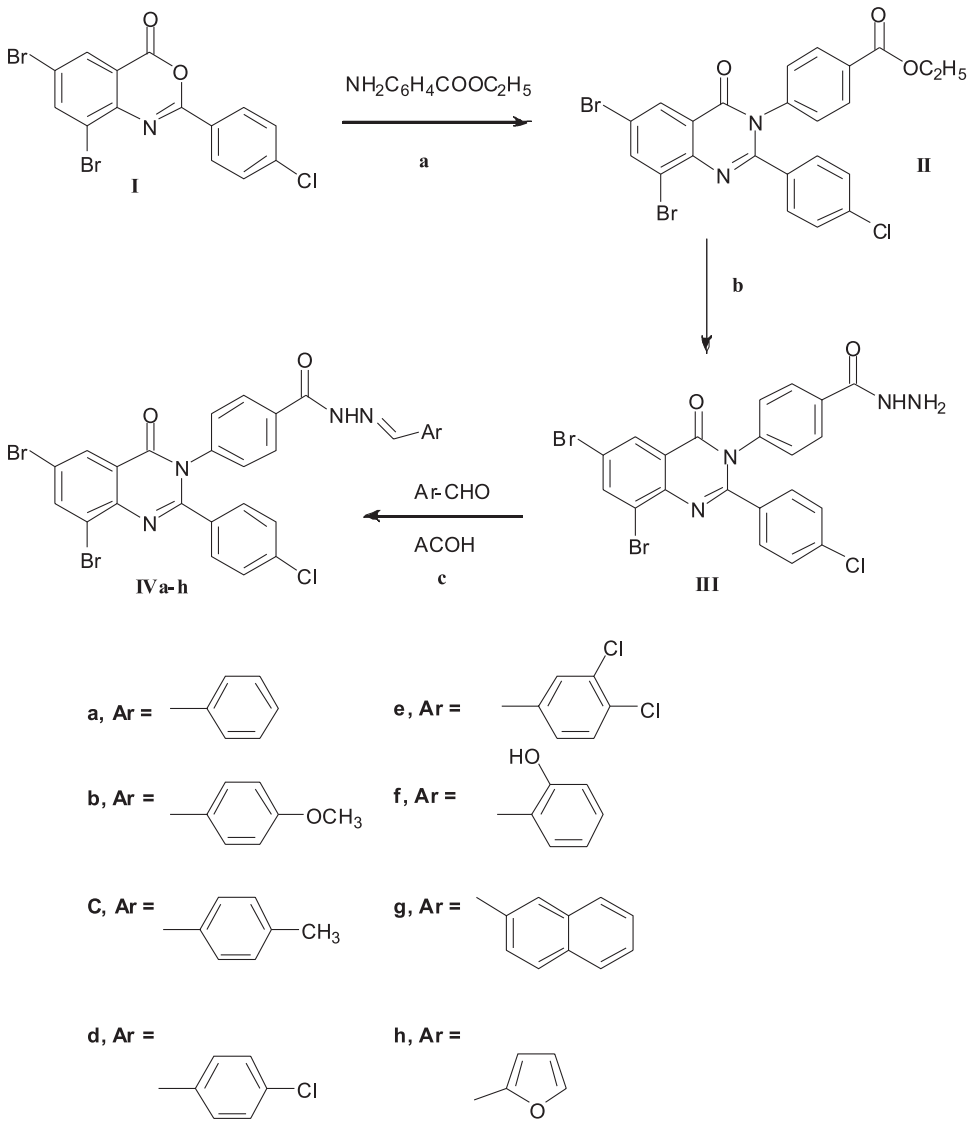
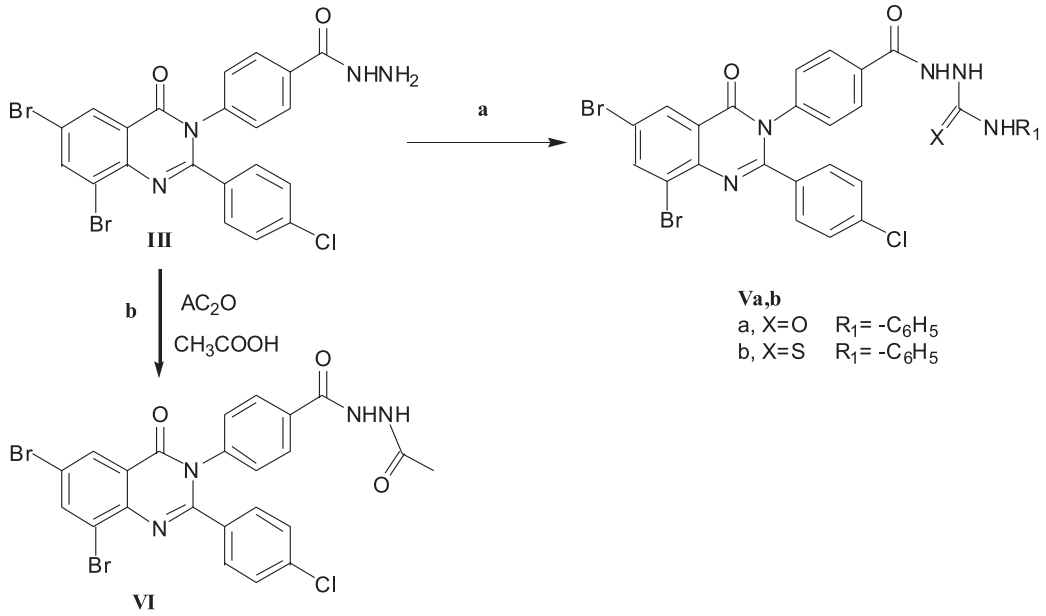


Figure 2. The formula of the novel synthesized 3H-quinazolin-4-one derivatives



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



Scheme 2. Reagents and conditions: **a**, phenyl iso-/isothio-cyanate, pyridine, reflux 8 h. **b**, reflux 8 h

3060 (CH, arom.), 1710, 1700 (2CO). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.3 (3H, t, CH_3, ethyl group), 4.3 (q, 2H, CH_2, ethyl group), 7.25-8.25 (m, 10H, arom. -H). ^{13}C NMR (DMSO-d_6, 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 $C_{23}H_{15}Br_2ClN_2O_3$, 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, NH_2), 3060 (CH, arom.), 1715 (CO, quinazoline ring), 1645 (CO, amide). ^{1}H-NMR (DMSO-d_6, δ ppm): 4.5 (s, 2H, NH2, exchangeable with D_2O), 7.53-8.25 (m, 10H, arom. -H), 9.8 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ, ppm): 167.2, 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^+. 547.91 (100.0%), 549.91 (44.9%), 545.91 (44.0%), 549.90 (27.4%). Analysis: for $C_{21}H_{13}Br_2ClN_4O_2$, 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 (substituted) benzylidene hydrazide (IV a-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). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.35-8.25 (m, 15H, arom.-H), 8.36 (s, 1H, CH=N), 11.800 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ,

ppm): 164.4, 163.3, 160.5, 154.3, 146.8, 139.2, 136.0, 135.4, 133.6, 131.2, 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 (100.0%), 637.94 (70.4%), 633.94 (44.0%), 636.94 (31.7%). Analysis: for $C_{28}H_{17}Br_2ClN_4O_2$, m.w. 636.72, calcd.: 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-methoxybenzylidene) hydrazide (IVb)

Crystallized from acetic acid to give yellow crystals, m.p. 190°C, 80% yield. IR (KBr, cm^{-1}): 3355 (NH), 1715, 1690 (2CO), 1598 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.83 (s, 3H, OCH_3), 7.00-8.25 (m, 14H, arom. -H), 8.36 (s, 1H, CH=N), 11.87 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 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, 114.4, 113.2, 55.7. MS (m/z, R.I.): M^+ 665.95 (100.0%), 667.95 (70.2%), 663.95 (44.1%), 666.95 (33.0%). Analysis: for $C_{29}H_{19}Br_2ClN_4O_3$, m.w. 666.75, calcd.: 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-methylbenzylidene) hydrazide (IVc)

Crystallized from ethanol to give white crystals, m.p. 200°C, 80% yield. IR (KBr, cm^{-1}): 3315 (NH), 1720, 1690 (2CO), 1600 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.35 (s, 3H, CH_3), 7.06-8.28 (m, 14H, arom. -H), 8.36 (s, 1H, CH=N), 11.8 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 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 $C_{29}H_{19}Br_2ClN_4O_2$, m.w. 650.75, calcd.: 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-chlorobenzylidene) hydrazide (IVd)

Crystallized from acetic acid to give brown crystals, m.p. 166°C, 75% yield. IR (KBr, cm^{-1}): 3420 (NH), 1720, 1690 (2CO), 1600 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.06-8.4 (m, 14H, arom. -H), 8.4 (s, 1H, CH=N), 11.7 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ, ppm): 164.2, 163.3, 160.5, 154.3, 146.7, 139.4, 136.9, 136.1, 135.7, 131.5, 131.3, 130.6, 129.7, 129.1, 128.9, 128.4, 126.7, 125.2, 124.3, 122.1, 112.7. MS (m/z, R.I.): M^+ 669.90 (100.0%), 671.90 (89.4%), 667.90 (38.6%), 670.90 (31.8%). Analysis: for $C_{28}H_{16}Br_2Cl_2N_4O_2$, m.w. 671.17, calcd.: C, 50.11; H, 2.40; N, 8.35%; found: C, 50.21; H, 2.50; N, 8.40%.

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

Crystallized from ethanol to give brown crystals, m.p.145°C, 82% yield. IR (KBr, cm^{-1}): 3325 (2NH), 1715, 1700 (2CO), 1605 (C=N). ^{1}H-NMR (DMSO-d_6, δ ppm): 7.3-8.2 (m, 13H, arom. -H), 8.54 (s, 1H, CH=N), 10.2 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ, ppm): 164.0, 163.2, 160.6, 154.5, 146.4, 139.4, 136.1, 135.9, 133.5, 133.2, 131.3, 130.6, 130.3, 129.6, 129.1, 128.9, 128.7, 128.4, 126.7, 124.2, 121.7, 112.5. MS (m/z, R.I.): M^+. 705.86 (100.0%), 703.86 (92.5%), 707.86 (32.8%), 701.86 (31.8%). Analysis: for $C_{28}H_{15}Br_2Cl_3N_4O_2$, m.w. 705.61, calcd.: C, 47.66; H, 2.14; N, 7.94%; found: C, 47.70; H, 2.30; N, 7.80%.

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

Crystallized from methanol to give yellowish white crystals, m.p.154°C, in 80% yield. IR (KBr, cm^{-1}): 3315 (NH), 1710, 1690 (2CO), 1600 (C=N). ^{1}H-NMR (DMSO-d_6, δ ppm): 7.2-8.3 (m, 14H, arom. -H), 8.54 (s, 1H, CH=N), 10.1 (s, 1H, NH, exchangeable with D_2O), 11.2 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δ, ppm): 163.5, 163.2, 160.1, 157.2, 154.3, 146.0, 139.2, 136.1, 135.7, 132.4, 131.3, 129.6, 129.1, 128.9, 128.4, 127.4, 126.7, 125.2, 124.5, 122.6, 121.4, 118.5, 113.2. MS (m/z, R.I.): M^+ 651.93 (100.0%), 653.93 (69.7%), 649.94 (44.1%), 652.94 (30.7%). Analysis: for $C_{28}H_{17}Br_2ClN_4O_3$, m.w. 652.72, calcd.: C, 51.52; H, 2.63; N, 8.58%; found: C, 51.39; H, 2.60; N, 8.65%.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazolin-3-yl] benzoic acid naphthalen-1-ylmethylene hydrazide (IVg)

Crystallized from ethanol to give yellwish brown crystals, m.p.195°C, 80% yield. IR (KBr, cm^{-1}): 3410 (NH), 1715, 1690 (2CO), 1595 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.35-8.25 (m, 17H, arom. -H), 8.36 (s, 1H, CH=N), 11.8 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ, ppm): 164.0, 163.2, 160.6, 154.3, 146.8, 139.4, 136.1, 135.7, 133.9, 131.3, 129.6, 129.1, 128.9,

128.6, 128.4, 128.2, 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 $C_{32}H_{19}Br_2ClN_4O_2$, 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). ^{1}H-NMR (DMSO-d_6, 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 D_2O). ^{13}C NMR (DMSO-d_6, 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, 125.2, 125.0, 122.5, 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 $C_{26}H_{15}Br_2ClN_4O_3$, 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 carbo(oxa/thioamide) (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). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.5-8.5 (m, 15H, arom. -H), 6.01, 9.02, 10.75 (3s, 3H, 3NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 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, 127.6, 126.7, 125.2, 124.5, 122.0, 121.4, 113.0. MS (m/z, R.I.): M^+ 666.94 (100.0%), 668.94 (69.8%), 664.95 (44.1%), 667.95 (30.7%). Analysis: for $C_{28}H_{18}Br_2ClN_5O_3$, m.w. 667.74, calcd.: C, 50.36; H, 2.72; N, 10.49%; found: C, 50.32; H, 2.59; N, 10.56%.

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

Crystallized from methanol to give brown crystals, m.p. 200°C, 65% yield. IR (KBr, cm^{-1}): 3285, 3265, 3200 (3NH), 3060 (CH arom.), 1685, 1660 (2CO), 1190 (C=S). ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.3-8.5 (m, 15H, arom. -H), 4, 10.04 and 12.2 (3s, 3H, 3NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ, ppm): 181.1, 164.8, 164.0, 160.6, 154.6, 139.4, 138.5, 136.1, 135.7, 131.3, 129.6, 129.1, 129.0, 128.9, 128.4, 128.0, 126.7, 126.5, 125.2, 124.5, 122.0, 113.9. MS (m/z, R.I.): M^+: 682.92 (100.0%), 684.92 (73.0%), 680.92 (43.2%), 683.92 (32.9%). Analysis: for $C_{28}H_{18}Br_2ClN_5O_2S$, m.w. 683.80, calcd.: C, 49.18; H, 2.65; N, 10.24%; found: C, 49.20; H, 2.70; N, 10.20%.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazolin-3-yl] benzoic acid N'-acetylhydrazide (VI)

A mixture of hydrazide **III** (5.48 g, 10 mmol), and a mixture of acetic anhydride (10 mL) and acetic acid (10 mL) was refluxed for 8 h. The precipitated solid formed upon cooling was filtered and recrystallized from ethanol to give orange crystals of **VI**. M.p. 240°C, 60% yield. IR (KBr, cm^{-1}): 3288, 3285 (2NH), 3065 (CH arom.), 1715, 1700, 1690 (3CO). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.1 (s, 3H, methyl), 7.5-8.5 (m, 10H, arom. -H) 10.00 and 10.2 (2s, 2H, 2NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ, ppm): 168.3, 164.8, 164.3, 160.6, 155.0, 139.9, 136.1, 135.7, 131.3, 129.5, 129.1, 128.9, 127.6, 126.7, 125.2, 124.9, 122.0, 112.9, 20.4. MS (m/z, RI): M^+ 589.92 (100.0%), 591.92 (45.5%), 587.92 (44.0%), 591.91 (27.3%). Analysis: for $C_{23}H_{15}Br_2ClN_4O_3$, m.w. 590.65, calcd.: C, 49.67; H, 2.90; N, 10.07%; found: C, 46.77; H, 2.56; N, 9.49%.

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 48°C, while trypsin-EDTA and FBS were stored frozen at -208°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 cm^2 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% CO_2 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 1×10^5 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 (5×10^3 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 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 a comparison with untreated cells. IC_{50} (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 moe 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 IC_{50} values (the dose that reduces survival to 50%) in µg/mL. It is evident that all of the tested compounds showed antitumor activities with IC_{50} 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 IC_{50} value less than that of DOX, Their IC_{50} 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_{50} values were 13.21, 13.31, 13.81 and 14.29 µg/mL, respectively). Compound **IVg** was the least active one, its IC_{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 **II** lead to better activity than doxorubicin against MCF-7 cell line.
2 Compounds with general formula **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 **IVe** which showed a closely similar activity to doxorubicin, IC_{50} 13.31 and 12.2 µg/mL, respectively, and compound **IVg** which was the least active.
3 Compounds with general formula **B** (Fig. 2), **III**, **Vb** and **VI** showed to be near the activity of doxorubicin against breast cancer cells, whoever, **Va** showed to be better active than doxorubicin.
4 The best active compound was **IVh** and the least active was **IVg** and both are quinazolin-4-one bearing Schiff base at the para position of 3-phenyl moiety.

ERα binding affinity assay

Docking studies indicated the ability of the best active compounds **IVh**, **IVf**, **IVb**, **IVc** and **Va** to quick fit the active site of ERα so the binding affinities of these compounds with ERα 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 ERα and this is considered an evidence that these compounds could act through ERα inhibition. The inhibitory range was 41-85%, compound **IVh** with a furan substituent at the para position of the 3-phenyl moiety showed to be the best active, its ERα inhibitory activity was 85% followed by compound **IVf** (71%) that has a substitution in the para position of the 3-phenyl with a phenyl moiety bearing an ortho hydroxyl group. Also compound **IVb** with a 4-methoxy group instead of 2-OH of **IVf** showed a percentage of inhibition at 66.7%.

Molecular docking

The estrogen receptor is a nuclear receptor which plays a critical role as a mediator for the actions of the estrogen hormones, ERα is predominant and highly expressed ER in breast cancer

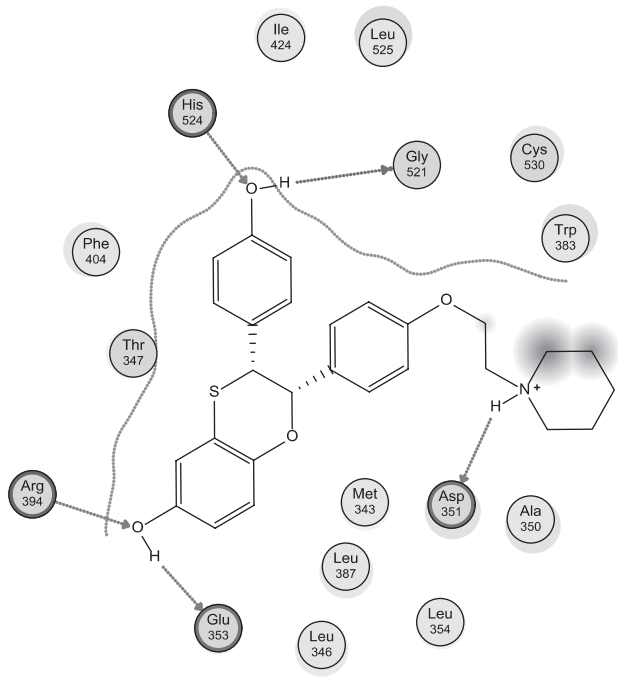


Figure 3. 2D interactions of dihydrobenzoxathin as a cocrystallized selective ligand in the active site of ERá subtype

Table 1. IC_{50} values (dose that inhibits 50% of cells) of the tested compounds and doxorubicin expressed in µg/mL ± S.E.

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

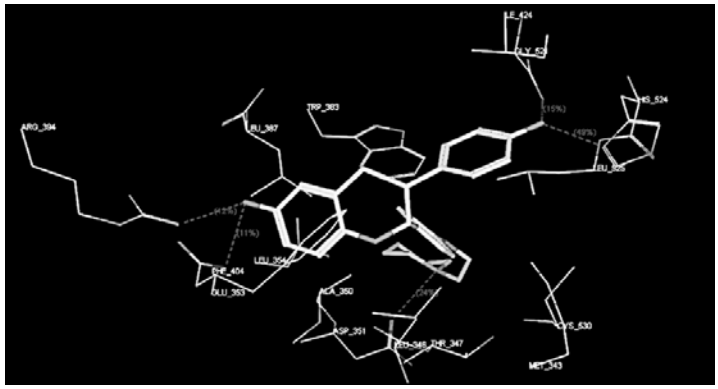


Figure 4. Redocked dihydrobenzoxathin ligand in the active site of ERα subtype, rmds = 0.921 and S = -26.18 kcal/mol

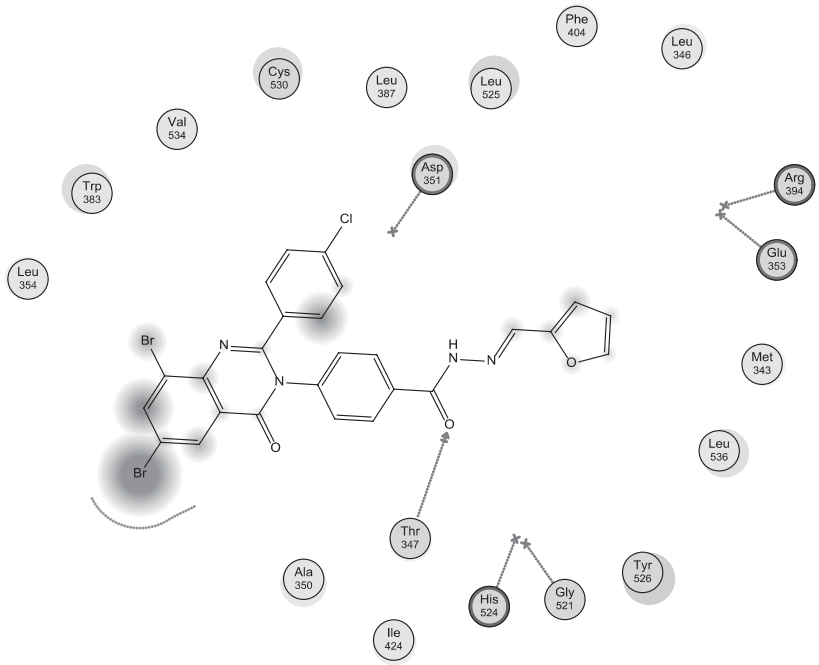


Figure 5. Compound **IVh** in the active site of ERα subtype

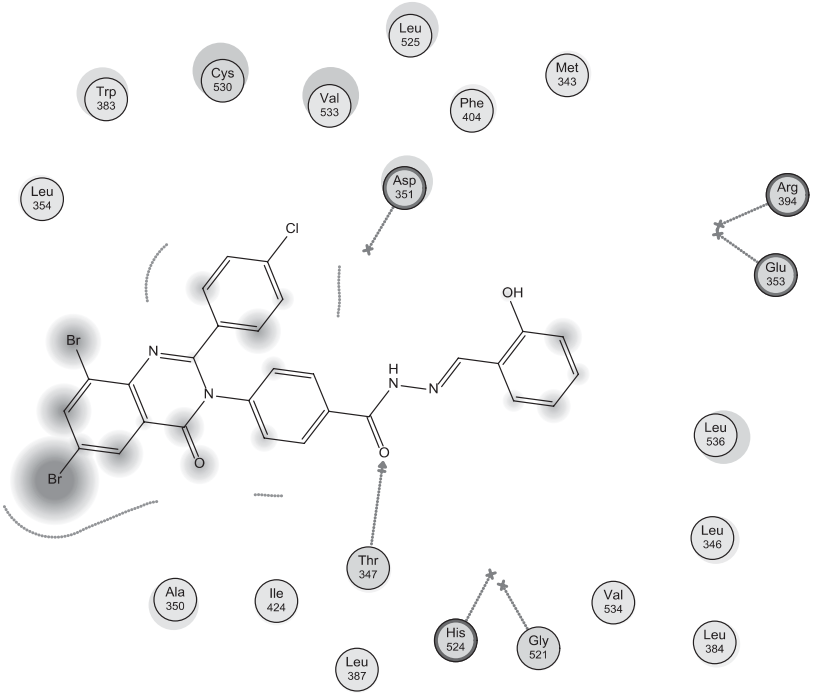


Figure 6. Compound **IVf** in the active site of ERα subtype

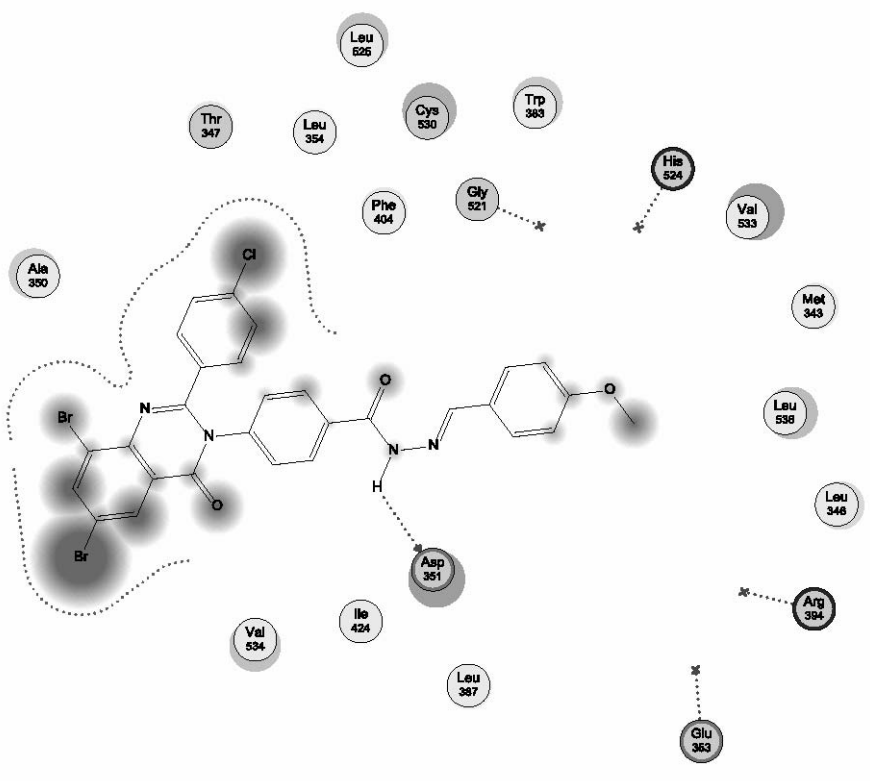


Figure 7. Compound **IVb** in the active site of ERα subtype

(45). 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 cocrystallized 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**.

Table 2. Estrogen receptor a inhibition %.

Comp. No.	Inhibition %
IVb	66.7
IVc	47.3
IVf	71.3
IVh	85
Va	41
Tamoxifen	100

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 carbothioamide moiety. On the other hand, Schiff bases substitution with bulky group (compound **IVg)** decreased anticancer activity compared to all other

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

Compound No.	S (kcal/mol)	Amino acid interaction
IVb	-14.23	Asp 351
IVc	-14.57	
IVf	-16.83	Thr 347
IVh	-25.29	Thr 347
Va	-13.52	
Benzoxathin	-26.18	Asp 351, His 524 Glu 353, Arg 394

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.

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