Design, synthesis, molecular modeling and anti-breast cancer activity of novel quinazolin-4-one derivatives linked to thiazolidinone, oxadiazole or pyrazole moieties

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, molecular modeling and anti-breast cancer activity of novel quinazolin-4-one derivatives linked to thiazolidinone, oxadiazole or pyrazole moieties

Marwa F. Ahmed · Amany Belal · Mahmoud Youns

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Abstract 6,8-Dibromo-2-(4-chlorophenyl)-quinazolin-4-one linked directly to oxadiazole **5**, pyrazole **6** or through amide linkage to thiazolidinone **2a–d** and **3a–h** were synthesized; their chemical structures were confirmed by spectral and elemental analyses. Their anti-breast cancer activity was evaluated against human breast cancer cell line (MCF-7) using resazurin reduction method and doxorubicin as a reference drug. Linking quinazolin-4-one scaffold to oxadiazole or pyrazole gave compounds **5** and **6** with a closely similar activity as doxorubicin; their IC_{50} was 23, 22 and 22 nmol/ml, respectively; however, the hybridization of quinazolin-4-one with thiazolidinone gave much better activity than doxorubicin. The most active compounds of the hybrid molecules between quinazolin-4-one and thiazolidinone are **2c**,**d** and **3a**,**f**. Their IC_{50} range was (3–9 nmol/ml). In an attempt to explore the mode of action of the best active compounds, docking on the ATP binding site of EGFR was performed. In vitro screening of these four compounds against EGFR tyrosine kinases showed inhibitory activity range 54–77.2 %.

Keywords Quinazolin-4-one · Thiazolidinone · Oxadiazole · Pyrazole · MCF-7

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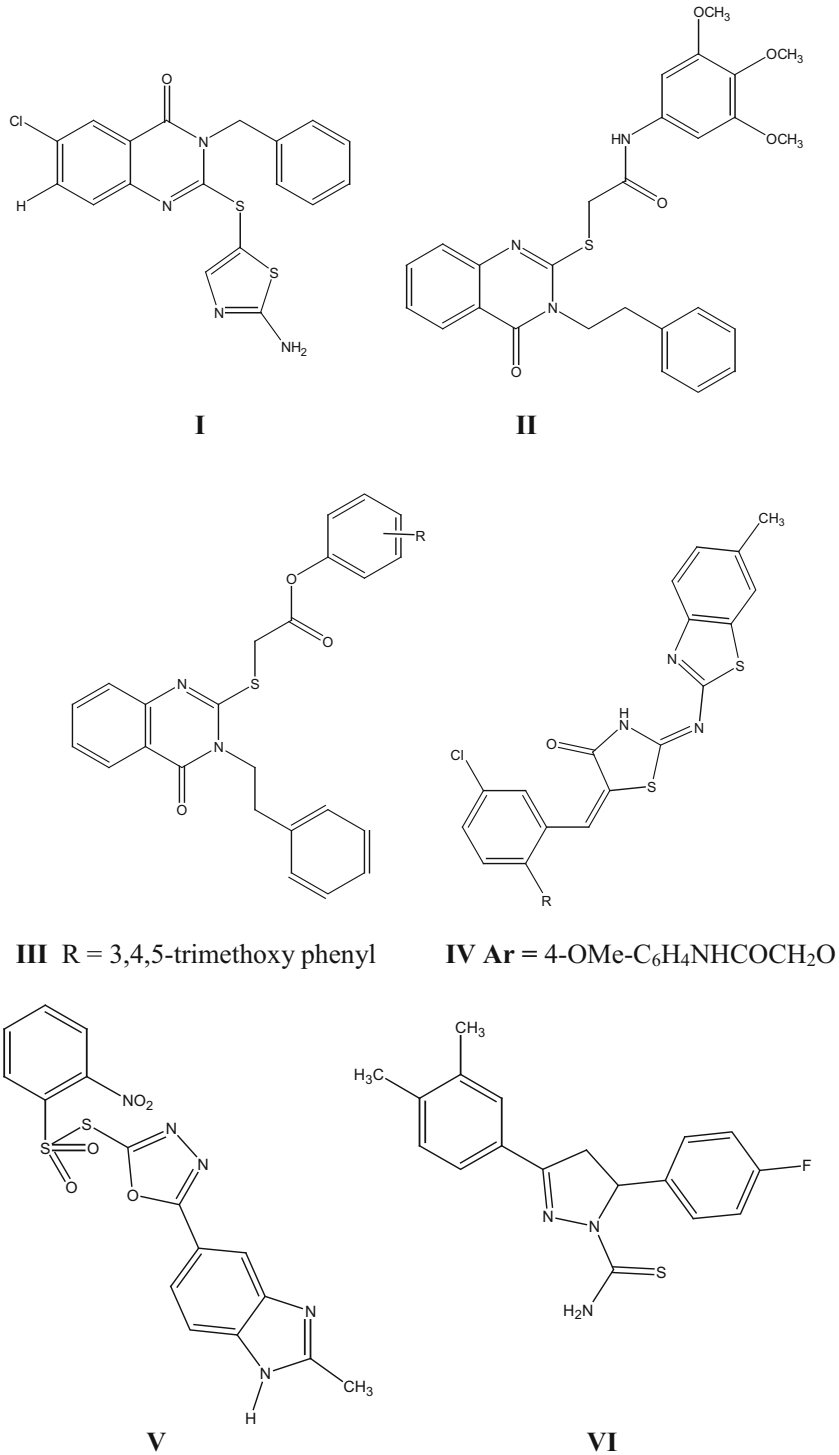
Introduction

Breast cancer occurs in humans and other mammals and originates from the tissues of the breast, either from milk ducts known as ductal carcinomas or from the lobules that supply milk for the ducts called lobular carcinomas (Sariego, 2010). The majority of human breast cancer cases are in women; however, it can also occur in men (Weiss *et al.*, 2005). Breast cancer is the most common tumor among women worldwide. Its incidence is increasing around the world, and it is believed to be the leading cause of cancer mortality, according to American Cancer Society. The estimated new breast cancer cases in USA (2014) are 235,030 cases, 232,670 of them estimated in women and the other 2360 in men. In addition, the estimated breast cancer deaths are 40,430 for the same year (Siegel *et al.*, 2014).

Quinazolines play a major role in the field of medicinal chemistry; they are of particular interest and considered as promising scaffolds in the search for new bioactive agents due to their diverse pharmacological activities such as anti-inflammatory (Alagarsamy *et al.*, 2009), antimicrobial (Mohamed *et al.*, 2010), antihypertensive (Ismail *et al.*, 2006), anticonvulsant (Georgey *et al.*, 2008), cholinesterase inhibitors (Decker, 2005), anticancer (Chandrika *et al.*, 2008) and anti-diabetic (Dongamanti *et al.*, 2012). Moreover, quinazolin-4-one derivative **I** (Fig. 1) bearing phenyl moiety at position 2 and 3 was synthesized and showed to inhibit about twenty percent of MCF-7 cells at 10 μM

Fig. 1 Active compounds against MCF-7 cell line



concentration (Al-Omary *et al.*, 2013). Another quinazolin-4-one having a phenyl moiety at the same positions 2 and 3 compound **II** (Fig. 1) showed a 49 % growth of MCF-7 inhibition at the same concentration 10 μM; replacing the NH **II** with O **III** (Fig. 1) affected the activity and caused a 34 % growth inhibition, so it was observed that a minor change in the side chain has a great effect on the activity against MCF-7 cell line (Al-Suwaidan *et al.*, 2013). In addition, thiazolidinone **IV** (Havrylyuk *et al.*, 2010), oxadiazole **V** (Galal *et al.*, 2010) and pyrazole (Lv *et al.*, 2010) **VI** (Fig. 1) containing compounds proved to have a great activity against breast cancer cells. Based on these findings, quinazolin-4-one scaffold substituted with phenyl at position 2,3 linked directly to oxadiazole **5**, pyrazole **6** or

through amide linkage to thiazolidinone **2a** was synthesized, screened against MCF-7 cell line using doxorubicin as a reference drug; **2a** was the best active one and showed better activity than both compounds **5**,**6** and doxorubicin itself. Furthermore, synthesis for a series **2a–d**, **3a–h** of quinazolin-4-one hybrid molecules with thiazolidinone was performed aiming to obtain potent and active compounds helpful in breast cancer treatment.

Epidermal growth factor receptors are family of receptor tyrosine kinases that play a crucial rule in cell growth regulation and survival (Olayioye *et al.*, 2000); they are highly expressed in a number of human tumors as breast cancer, colon, prostate and ovarian cancer, thus considered as attractive targets for the design and development of new anticancer active agents. It was also reported that quinazoline derivatives have a potent inhibitory activity against EGFR (Barlesi *et al.*, 2005; Burris *et al.*, 2005; Hennequin *et al.*, 2002; Kopper, 2008; Wood *et al.*, 2004); hence, docking the best active compounds **2c**,**d** and **3a**,**f** into ATP binding site of EGFR was performed to explore the possible interactions and amino acid binding for these compounds with EGFR.

Result and discussion

Chemistry

Compounds **1a–d** and **4** were synthesized according to the reported methods (Ahmed *et al.*, 2016); cyclocondensation of the Schiff bases **1a–d** with mercapto acetic acid in glacial acetic acid afforded the corresponding quinazolin-4-one linked to thiazolidinone nucleus through amide linkage **2a–d**, respectively. Their IR spectrum revealed the appearance of an additional absorption band corresponding to the carbonyl of thiazolidinone nucleus, and ^{1}H-NMR spectra showed the appearance of two signals at 3.8–3.9 and 5.9 which are characteristic for CH_2 and CH–Ar in thiazolidinone moiety. Moreover, compounds **2b** and **2c** showed signals at 3.8 and 2.3 ppm corresponding to OCH_3 and CH_3, respectively. To make substitution at position 5 of the thiazolidinone nucleus aiming to obtain another new derivatives of quinazolinone–thiazolidinone hybrid molecules, reacting **2a–d** with paraformaldehyde and 2-ry amines as piperidine or morpholine to give the corresponding Mannich bases **3a–h** (Scheme 1), ^{1}H-NMR showed the appearance of morpholine and piperidine protons and a signal for CH_2–N protons at 2.9 ppm in addition to the other signals characterizing the structures.

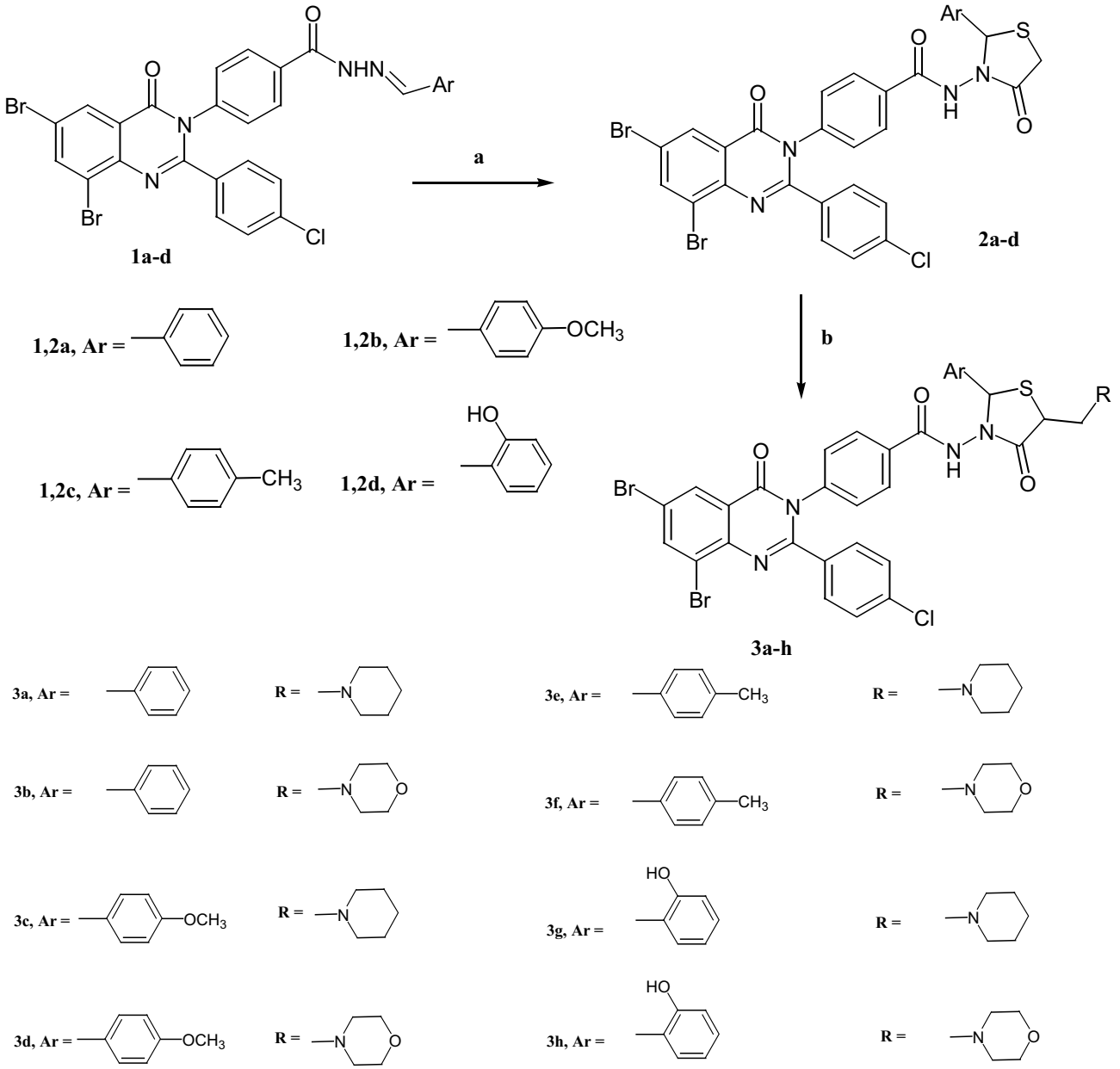
To prepare a hybrid molecule of quinazolin-4-one with oxadiazole, heating the acid hydrazide **4** with acetic anhydride was done and the corresponding methyl oxadiazole derivative **5** was obtained. Furthermore, reaction of **4** with diketonic esters such as ethyl acetoacetate afforded the corresponding pyrazolyl-quinazolinone molecule **6** (Scheme 2), microanalysis and mass spectrum confirmed both structures **5**,**6**, and in addition the ^{1}H-NMR of compound **5** showed a characteristic signal for the CH_3 group at 2.6 ppm.

Biological screening

All the synthesized compounds were screened for their in vitro cytotoxic and growth inhibitory activities against MCF-7 cell line using resazurin cell growth inhibition assay method in comparison with the activity of the known anticancer drug doxorubicin. The cytotoxic activities of our tested compounds were expressed as IC_{50} values (the dose that reduces survival to 50 %). The tested compounds showed anti-breast cancer activity with IC_{50} values ranging from 3 to 23 nmol/ml (Table 1). The two series **2a–d** and **3a–h** showed better activity than doxorubicin except **3d** that showed to be closely similar to the reference drug. Compounds **5** and **6** showed less to similar activity as doxorubicin. The best active compounds are **2c**,**d** and **3a**,**f**; their IC_{50} values range from 3 to 9 nmol/ml. In vitro screening of these four compounds against EGFR-TKs was also performed, they showed inhibitory activity range from 54 to 77.2 % (Table 3), and the best activity was assigned for compound **3f** that showed inhibitory activity at 77.2 %.

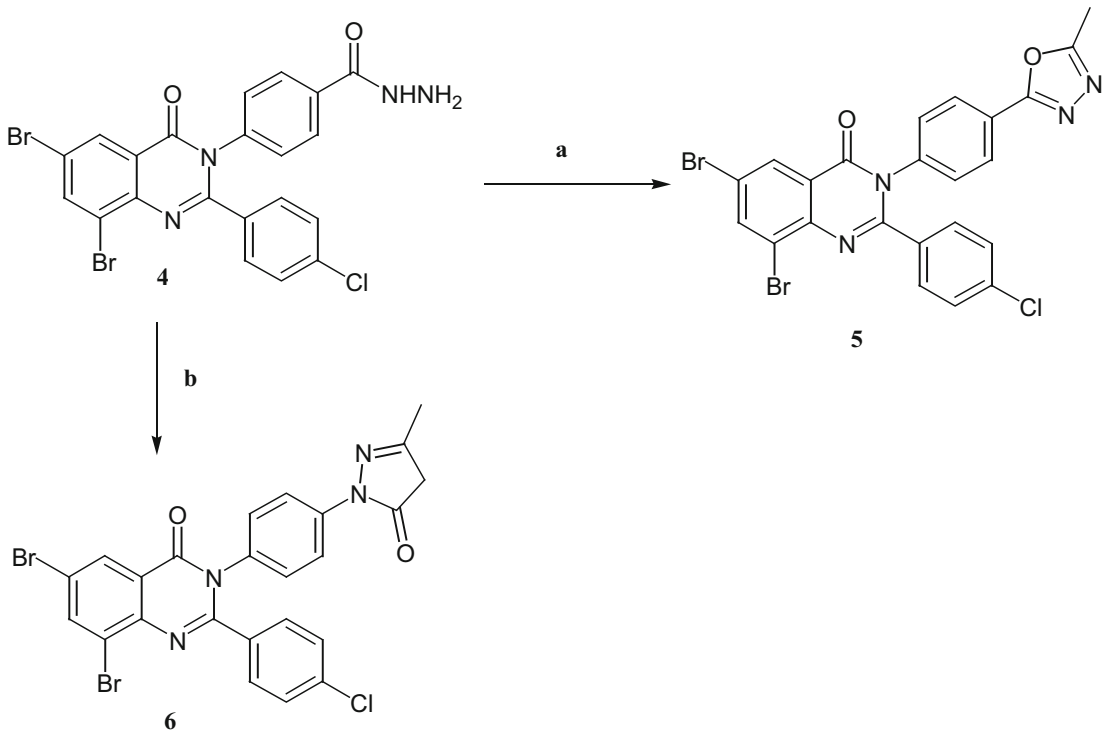
Molecular docking studies

The epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase that transmits signals for the cell causing proliferation, differentiation or motility. Overexpression of the EGFR is noticed in numerous human malignancies; hence, targeting the ATP binding site of the EGFR by small molecules that act as tyrosine kinase inhibitors is considered a strategy for cancer treatment (Yun *et al.*, 2007). Moreover, several 4-anilinoquinazolines act as tyrosine kinase inhibitors (TKIs) which are currently in clinical investigation as gefitinib (Iressa) (Wakeling *et al.*, 2002), erlotinib (Tarceva) (Pollack *et al.*, 1999) and lapatinib (Rusnak *et al.*, 2001). Molecular docking studies for the most active compounds **2c**,**d** and **3a**,**f** that showed to be more potent than doxorubicin against MCF-7 were performed on the ATP binding site of EGFR to explore possible interactions and amino acid binding with these receptors. To perform accurate validation of the docking protocol, docking of IRE should be carried out to study the root mean standard deviation (RMSD), scoring energy (S) and amino acid interactions. Docking was performed using London dG force, and the results were refined using force field energy. IRE was fitted in the pocket of EGFR



Scheme 1 Synthesis of compounds **2a–d** and **3a–h**. Reagents and conditions: *a* thioglycolic acid, glacial acetic acid, reflux 6 h. *b* Paraformaldehyde, secondary amine, DMF, reflux 8 h

with $S = -22.28$ kcal/mol, RSMD = 1.52 and showed interactions with two amino acids Asp 855 and Lys 745 by two hydrogen bonds (Fig. 2). The aforementioned docking protocol was used for all the docked compounds **2c**,**d** and **3a**,**f** on the active site of EGFR. Docking scores, amino acids interactions and interacting groups are shown in Table 2. All the docked compounds exhibited proper fitting on the active site with docking score energy range from −13.15 to −19.16 kcal/mol (Figs. 3, 4, 5, 6). Compound **3f** fit with the active site with the best fitting score energy (−19.16 kcal/mol) and amino acid interaction with Cys 797, Lys 728 and Lys 716 (Fig. 6). Compound **2c** showed amino acid interactions with Asp 855 and Lys 754 with score energy −13.37 kcal/mol (Fig. 3). Docking score energy of compounds **2d** and **3a** are −13.15 and −13.49 kcal/mol, respectively (Figs. 4, 5). These results support the postulation of the ability of these compounds to act as EGFR-TKIs and give a promising venue for further investigation of the quinazolin-4-one hybrid molecules with thiazolidinone (Table 3).



Scheme 2 Synthesis of compounds **5** and **6**. Reagents and conditions: *a* acetic anhydride, reflux 6 h, *b* ethyl acetoacetate, absolute ethanol, reflux 6 h

Table 1 IC_{50} values expressed in μmol/ml (nmol/ml) of the new compounds and doxorubicin against MCF-7 cell line

Compound number	IC_{50}	Compound number	IC_{50}
2a	0.011 (11)	**3d**	0.023 (23)
2b	0.016 (16)	**3e**	0.014 (14)
2c	0.006 (6)	**3f**	0.009 (9)
2d	0.009 (9)	**3g**	0.011 (11)
3a	0.003 (3)	**3h**	0.018 (18)
3b	0.012 (12)	**5**	0.023 (23)
3c	0.012 (12)	**6**	0.022 (22)
Doxorubicin			0.022 (22)

Conclusion

We have synthesized three hybridized molecules of quinazolin-4-one with thiazolidinone **2a**, oxadiazole **5** or pyrazole **6**, and we have evaluated their anti-breast cancer activity against MCF-7 cell line. The best activity was assigned for compound **2a**, so more derivatives of this hybrid molecule were synthesized **2a–d**, **3a–h** and screened also against the same cell line. From the observed results, we can conclude that:

1. Linking quinazolin-4-one with thiazolidinone in one molecule **2a** gave better activity against MCF-7 cell line than linking it with oxadiazole **5** or pyrazole **6**; the obtained hybrid molecule **2a** showed better activity than doxorubicin itself.
2. All hybrid molecules that have no substitution at position 5 of the thiazolidinone nucleus **2a–d** showed better activity than doxorubicin.
3. Substituting position 5 of thiazolidinone moiety **3a–h** gave better active compounds than doxorubicin except compound **3d**; moreover, substituting this position with morpholine decreased the activity than substituting it with piperidine except compound **3f**.
4. The best active compounds **2c**,**d** and **3a**,**f** that showed IC_{50} range 3–9 nmol/ml are quinazolin-4-one and thiazolidinone hybrid molecules. In vitro screening of these compound**s** against EGFR tyrosine kinases revealed inhibitory activity range 54–77.2 %, and the best inhibitory activity 77.2 % was shown by compound **3f** followed by **2c** (70 %).
5. Molecular docking studies for the best active compounds **2c**,**d** and **3a**,**f** against ATP binding site of EGFR proved their ability to quick fit this active site; docking results supported the in vitro EGFR tyrosine kinase inhibitory activities.

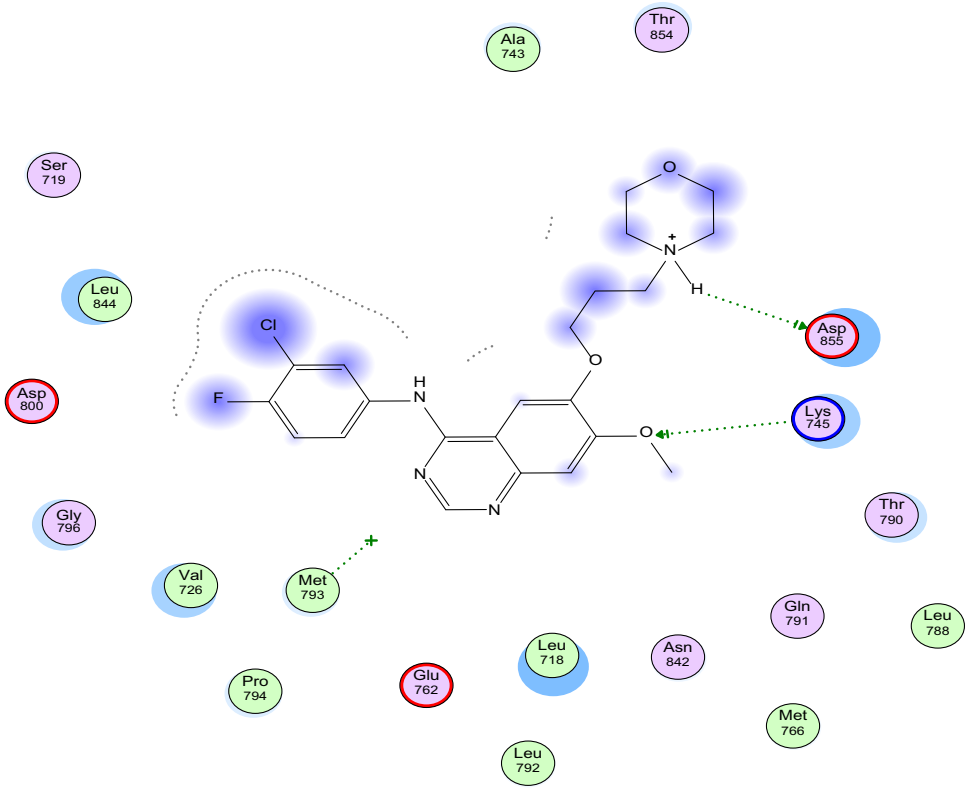


Fig. 2 IRE in the ATP binding site of EGFR (RMDS = 1.52, $S = -22.28$ kcal/mol)

Table 2 Amino acid interactions and the binding scores of the docked compounds on the active site EGFR

Compound number	S (kcal/mol)	Amino acid interactions	Interacting groups
2c	−13.37	Asp 855	NH of the amide spacer
		Lys 745	CO of thiazolidinone
2d	−13.15		
3a	−13.49		
3f	−19.16	Lys 728	O of morpholine
		Lys 716	O of morpholine
		Cys 797	CO of the amide spacer
IRE	−22.28	Asp 855	NH of morpholine
		Lys 745	O of OCH_3

Experimental

Chemistry

Melting points were detected using Electrothermal Stuart SMP3 digital melting point apparatus and were uncorrected. Elemental analyses were carried out in the microanalytical units of National Research Centre and Cairo University, Egypt. IR spectra were recorded on FT-IR spectrophotometer (Nexus 670-Nicolet, USA) and PerkinElmer-9712 spectrophotometer. ^{1}H-NMR spectra were determined on a Varian-Gemmi-300 MHz and Joel-Ex270 MHz NMR spectrometer using TMS as an internal standard. ^{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 F254 (Merck) thin-layer chromatography plates using a chloroform, petroleum ether, methanol mixture (7:4:1 v/v) as the mobile phase. Compounds **1a–d** and **4** were synthesized with the same previously reported methods (Ahmed *et al.*, 2016).

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazolin-3-yl]-benzoic acid benzylidene-hydrazide (**1a**) Yellow crystals (methanol); yield 70 %; mp 150–152 °C; IR (KBr) νmax 3370 (NH), 1710, 1685 (2CO) and 1596 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.35–7.39 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.5–7.64 (3H, m, H-3‴, H-4‴, H-5‴), 7.65–7.79 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.1 (1H, s, H-5), 8.2 (1H, s, H-7), 8.36 (1H, s, CH=N), and at 11.80 (1H, s, NH, exchangeable with D2O); ^{13}C NMR (100 MHz, DMSO-d_6): 164.4 (C=N), 163.3 (C=O), 160.5 (C=O), 154.3(Ar/olefinic

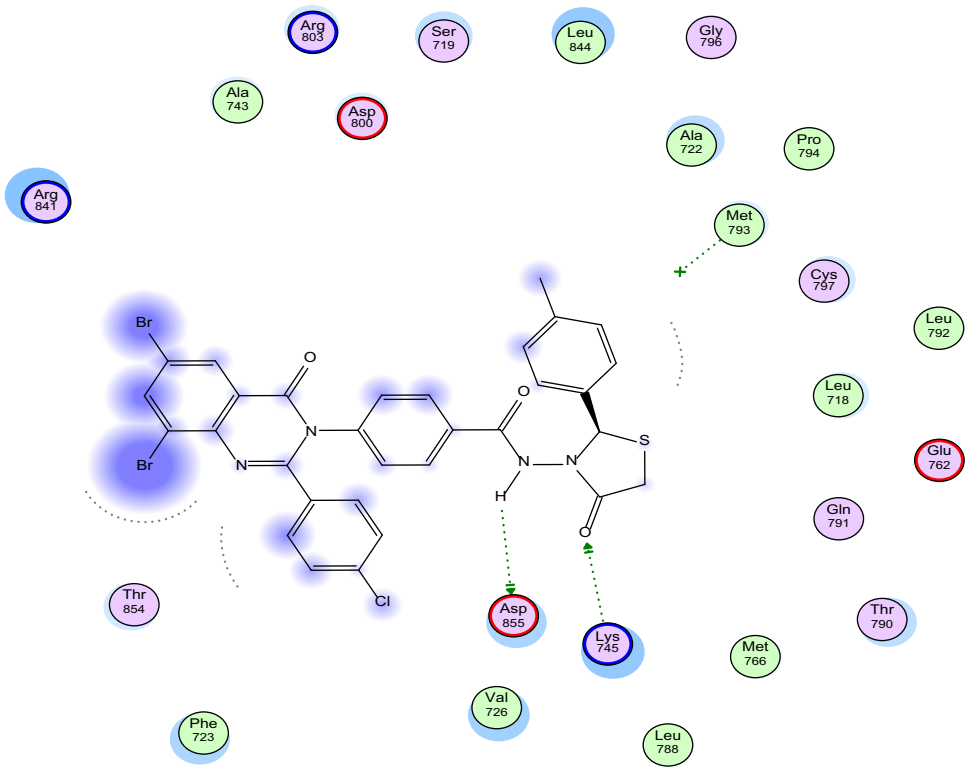


Fig. 3 Compound **2c** in the ATP binding site of EGFR ($S = -13.37$ kcal/mol)

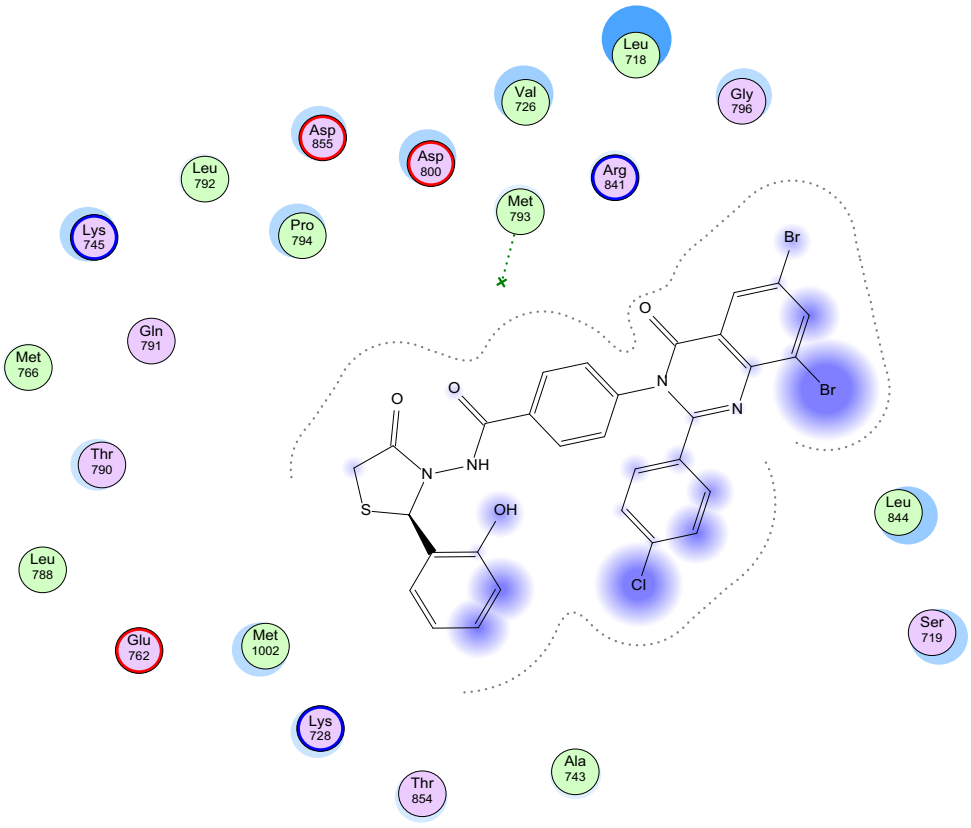


Fig. 4 Compound **2d** in the ATP binding site of EGFR ($S = -13.15$ kcal/mol)

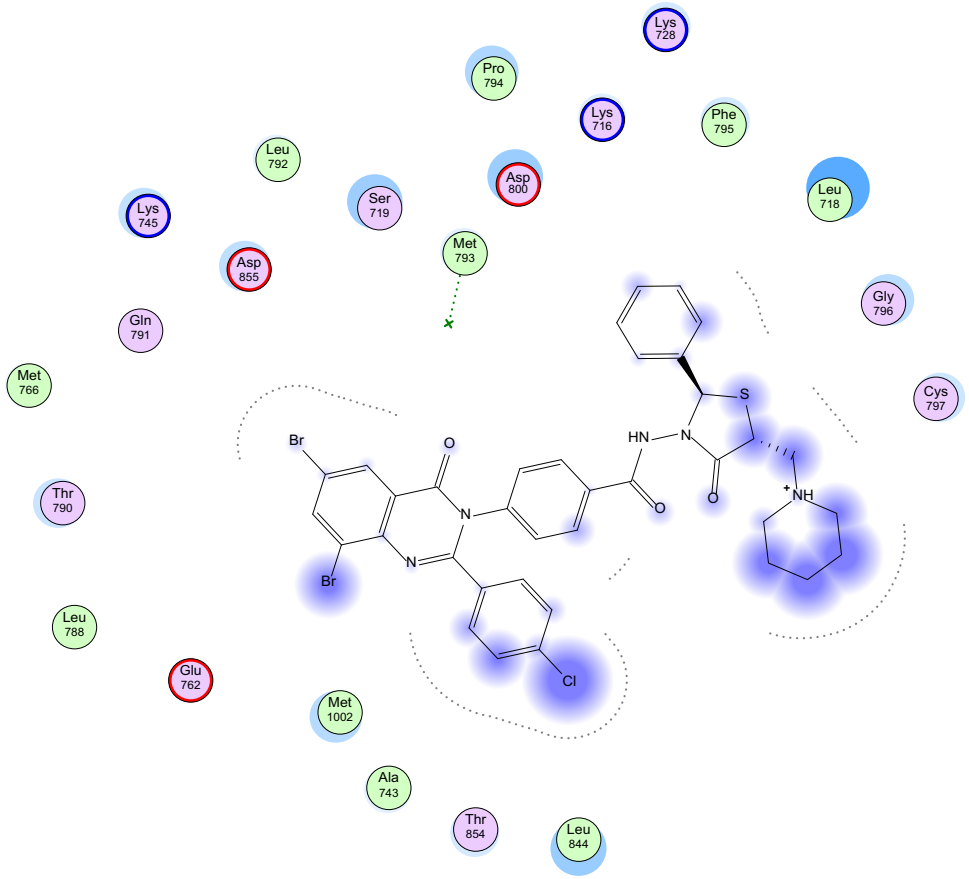


Fig. 5 Compound **3a** in the ATP binding site of EGFR ($S = -13.49$ kcal/mol)

carbon), 146.8 (CH), 139.2 (Ar/olefinic carbon), 136 (Ar/olefinic carbon), 135.4 (Ar/olefinic carbon), 133.6 (Ar/olefinic carbon), 131.2 (Ar/olefinic carbon), 131 (Ar/olefinic carbon), 129.6 (Ar/olefinic carbon), 129.2 (Ar/olefinic carbon), 129.1(Ar/olefinic carbon), 128.9 (Ar/olefinic carbon), 128.8 (Ar/olefinic carbon), 128.4 (Ar/olefinic carbon), 126.7 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.7 (Ar/olefinic carbon), 122 (Ar/olefinic carbon), 112.9 (Ar/olefinic carbon); MS: $m/z = 635.94$; Anal. Calcd for: C28H17Br2ClN4O2: C, 52.82; H, 2.69; N, 8.80 %. Found C, 55.70; H, 2.65; N, 8.6 %.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazolin-3-yl]-benzoic acid (4-methoxy-benzylidene)-hydrazide (**1b**) Yellow crystals (acetic acid); yield 80 %; mp 190–192 °C; IR (KBr) νmax 3355 (NH), 1715, 1690 (2CO) and 1598 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.83$(3H, s, OCH_3), 7.0–7.3 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.4–7.6 (2H, m, H-3‴, H-5‴), 7.7–7.79 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.1 (1H, s, H-5), 8.25 (1H, s, H-7), 8.36 (1H, s, CH=N), and at 11.87 (1H, s, NH, exchangeable with D2O); ^{13}C NMR (100 MHz, DMSO-d_6): 164 (C=N), 163.2 (C=O), 162.9 (Ar/olefinic carbon), 160.6 (C=O), 154.3 (Ar/olefinic carbon), 146.8 (CH), 139.4 (Ar/olefinic carbon), 136.1 (Ar/olefinic carbon), 135.7 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 130.2 (Ar/olefinic carbon), 129.6 (Ar/olefinic carbon), 129.1 (Ar/olefinic carbon), 128.9 (Ar/olefinic carbon), 128.4 (Ar/olefinic carbon), 126.7 (Ar/olefinic carbon), 126 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.5 (Ar/olefinic carbon), 122 (Ar/olefinic carbon), 114.4 (Ar/olefinic carbon), 113.2 (Ar/olefinic carbon), 55.7 (CH_3); MS: $m/z = 665.95$; Anal. Calcd for: $C_{29}H_{19}Br_2ClN_4O_3$: 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-methyl-benzylidene)-hydrazide (**1c**) White crystals (ethanol); yield 80 %; mp 200–202 °C; IR (KBr) νmax 3315 (NH), 1720, 1690 (2CO) and 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.35$(3H, s, CH_3), 7.06–7.25 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.35–7.59 (2H, m, H-3‴, H-5‴), 7.7–7.79 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.2 (1H, s, H-5), 8.28 (1H, s, H-7), 8.36 (1H, s, CH=N), and at 11.8 (1H, s, NH, exchangeable with D2O); ^{13}C NMR (100 MHz, DMSO-d_6): 163.9 (C=N), 163(C=O), 160.5 (C=O), 154.7 (Ar/olefinic carbon), 146.4 (CH), 140.7 (Ar/olefinic carbon), 139.9 (Ar/olefinic carbon), 136 (Ar/olefinic carbon), 135.7 (Ar/olefinic carbon), 131.1 (Ar/olefinic carbon), 130.5 (Ar/olefinic carbon), 129.6 (Ar/olefinic carbon), 129.1 (Ar/

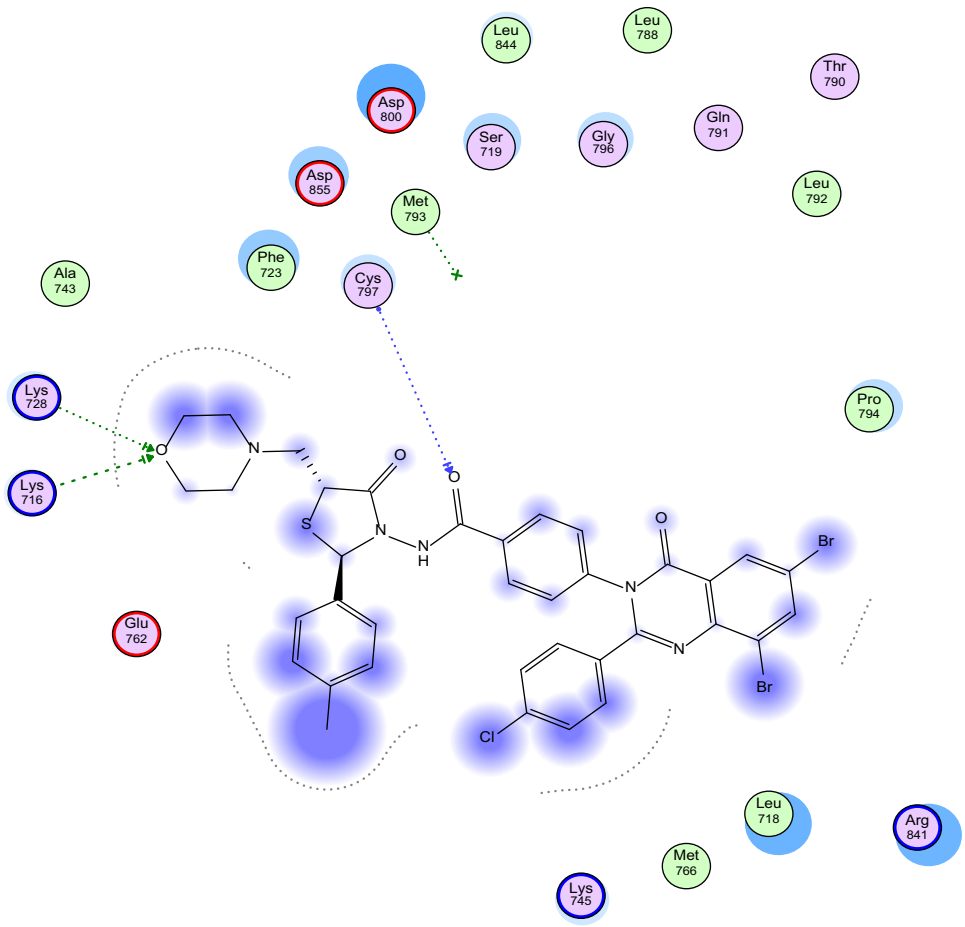


Fig. 6 Compound **3f** in the ATP binding site of EGFR ($S = -19.16$ kcal/mol)

Table 3 EGFR tyrosine kinase assay of compounds **2c**,**d** and **3a**,**f** at single dose (10 μM)

Compound number	% Inhibition of EGFR tyrosine kinase
2c	70
2d	54
3a	54.3
3f	77.2
Gefitinib	100

olefinic carbon), 128.9 (Ar/olefinic carbon), 128.4 (Ar/olefinic carbon), 126.7 (Ar/olefinic carbon), 126.1 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.4 (Ar/olefinic carbon), 122.1 (Ar/olefinic carbon), 113.5 (Ar/olefinic carbon), 21.3 (CH_3); MS: $m/z = 649.95$; Anal. Calcd for: $C_{29}H_{19}Br_2ClN_4O_2$: 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 (2-hydroxy-benzylidene)-hydrazide (**1d**) Yellowish white crystals (methanol); yield 80 %; mp 154–156 °C; IR (KBr) νmax 3315 (NH), 1710, 1690 (2CO) and 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 7.2$–7.45 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.5–7.7 (2H, m, H-3‴, H-5‴), 7.73–8.00 (6H, m, H-3′, H-5′, H-3″, H-5″, H-4‴, H-6‴), 8.24 (1H, s, H-5), 8.3 (1H, s, H-7), 8.54(1H, s, CH=N), 10.1 (1H, s, NH, exchangeable with D2O) and at 11.2 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6): 163.5 (C=N), 163.2 (C=O), 160.1 (C=O), 157.2 (Ar/olefinic carbon), 154.3 (Ar/olefinic carbon), 146 (CH), 139.2 (Ar/olefinic carbon), 136.1 (Ar/olefinic carbon), 135.7 (Ar/olefinic carbon), 132.4 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 129.6 (Ar/olefinic carbon), 129.1 (Ar/olefinic carbon), 128.9 (Ar/olefinic carbon), 128.4 (Ar/olefinic carbon), 127.4 (Ar/olefinic carbon), 126.7 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.5 (Ar/olefinic carbon), 122.6 (Ar/olefinic carbon), 121.4 (Ar/olefinic carbon), 118.5 (Ar/olefinic carbon), 113.2 (Ar/olefinic carbon); MS: $m/z = 651.93$; Anal. Calcd for: C28H17Br2ClN4O3: 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-oxoquinazolin-3(4H)-yl)-N-(4-oxo-2-(aryl) thiazolidin-3-yl)benzamide (**2a–d**) *General method*: A mixture of compounds

1a–d (10 mmol) and thioglycolic acid (0.35 ml; 10 mmol) in glacial acetic acid (20 ml) was refluxed for 6 h. The excess solvent was evaporated under reduced pressure, and the obtained residue was poured on crushed ice. The solid product was filtered off and washed with water to obtain the desired products **2a–d**, respectively.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(4-oxo-2-phenyl thiazolidin-3-yl)benzamide (**2a**) Orange crystals (methanol); yield 75 %; mp 210 −212 °C; IR (KBr) νmax 3270 (NH), 3067 (CH, aromatic), 2940 (CH, thiazolidinone), 1720 (CO, cyclic amide thiazolidinone), 1690 (CO, cyclic amide quinazoline), 1660 (CONH) and 1595 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.90$ (2H, s, CH_2, thiazolidinone ring), 5.92 (1H, s, CH of thiazolidinone ring), 7.35–7.45 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.5–7.59 (3H, m, H-3‴, H-4‴, H-5‴), 7.65–7.79 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.1 (1H, s, H-5), 8.3 (1H, s, H-7), 10.2 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 168.8 (C=O), 164 (C=N), 163.7 (C=O), 160.6 (C=O), 154.3 (Ar/olefinic carbon), 139.2 (Ar/olefinic carbon), 136.1 (Ar/olefinic carbon), 135.7 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 130.1 (Ar/olefinic carbon), 129.6 (Ar/olefinic carbon), 129.2 (Ar/olefinic carbon), 128. 8 (Ar/olefinic carbon), 128.6 (Ar/olefinic carbon), 128.2 (Ar/olefinic carbon), 127.6 (Ar/olefinic carbon), 127.1 (Ar/olefinic carbon), 126.9 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.5 (Ar/olefinic carbon), 122 (Ar/olefinic carbon), 113.2 (Ar/olefinic carbon), 57.3 (CH), 35.6 (CH_2); MS: $m/z = 675.96$; Anal. Calcd for: $C_{30}H_{19}Br_2ClN_4O_3S$: C, 50.69; H, 2.69; N, 7.88 %. Found C, 50.60; H, 2.72; N, 7.76 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(2-(4-methoxy phenyl)-4-oxothiazolidin-3-yl)benzamide (**2b**) White crystals (acetic acid); yield 75 %; mp 155–157 °C; IR (KBr) νmax 3365 (NH), 3070 (CH, aromatic), 2950 (CH, thiazolidinone), 1715 (CO, cyclic amide thiazolidinone), 1690 (CO, cyclic amide quinazoline), 1665 (CONH) and 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.9$ (2H, s, CH_2, thiazolidinone ring), 3.8 (3H, s, OCH_3), 5.90 (1H, s, CH of thiazolidinone ring), 7.3–7.5 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.55–7.64 (2H, m, H-3‴, H-5‴), 7.7–8.1 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.2 (1H, s, H-5), 8.45 (1H, s, H-7), 9.27 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 170 (C=O), 165.2 (C=N), 164.5(C=O), 161.6 (C=O), 159 (Ar/olefinic carbon), 155.2 (Ar/olefinic carbon), 140.2 (Ar/olefinic carbon), 136.4 (Ar/olefinic carbon), 135.9 (Ar/olefinic carbon), 131.5 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 130.1 (Ar/olefinic carbon), 129.6 (Ar/olefinic carbon), 129 (Ar/olefinic carbon), 128.6 (Ar/olefinic carbon), 128.2 (Ar/olefinic carbon), 127.9 (Ar/olefinic carbon), 125.4 (Ar/olefinic carbon), 124.6 (Ar/olefinic carbon), 122 (Ar/olefinic carbon), 114.2 (Ar/olefinic carbon), 113.4 (Ar/olefinic carbon), 58.4 (CH), 55. 8 (CH_3), 36.4 (CH_2); MS: $m/z = 739.93$; Anal. Calcd for: $C_{31}H_{21}Br_2ClN_4O_4S$: C, 50.26; H, 2.86; N, 7.56 %. Found C, 52.30; H, 2.90; N, 7.69 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(4-oxo-2-p-tolyl thiazolidin-3-yl)benzamide (**2c**) Yellowish white crystals (ethanol); yield 70 %; mp 179–181 °C; IR (KBr) νmax 3365 (NH), 3070 (CH, aromatic), 2930 (CH, thiazolidinone), 1700 (CO, cyclic amide thiazolidinone), 1685 (CO, cyclic amide quinazoline), 1665 (CONH) and 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.34$ (3H, s, CH_3), 3.9 (2H, s, CH_2, thiazolidinone ring), 5.90 (1H, s, CH of thiazolidinone ring), 7.25–7.45 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.5–7.65 (2H, m, H-3‴, H-5‴), 7.7–8.1 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.2 (1H, s, H-5), 8.3 (1H, s, H-7), 9.25 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 166.9 (C=O), 164.2 (C=N), 163.5(C=O), 161.2 (C=O), 154.3 (Ar/olefinic carbon), 139.2 (Ar/olefinic carbon), 136. 8 (Ar/olefinic carbon), 136.2 (Ar/olefinic carbon), 136.1 (Ar/olefinic carbon), 135.7 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 129.6 (Ar/olefinic carbon), 129.1 (Ar/olefinic carbon), 128.9 (Ar/olefinic carbon), 128.6 (Ar/olefinic carbon), 127.6 (Ar/olefinic carbon), 126.7 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.5 (Ar/olefinic carbon), 122.1 (Ar/olefinic carbon), 113.2 (Ar/olefinic carbon), 57.4 (CH), 35.4 (CH_2), 21.3 (CH_3); MS: $m/z = 739.93$; Anal. Calcd for: $C_{31}H_{21}Br_2ClN_4O_3S$: C, 51.73; H, 2.92; N, 7.73 %. Found C, 51.88; H, 2.70; N, 7.67 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(2-(2-hydroxy phenyl)-4-oxothiazolidin-3-yl)benzamide (**2d**) White crystals (methanol); yield 65 %; mp 140–142 °C; IR (KBr) νmax 3360 (NH), 3060 (CH, aromatic), 2930 (CH, thiazolidinone), 1720 (CO, cyclic amide thiazolidinone), 1685 (CO, cyclic amide quinazoline), 1665 (CONH) and 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 4.1$ (2H, s, CH_2, thiazolidinone ring), 6.00 (1H, s, CH of thiazolidinone ring), 7.5–7.59 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.6–7.7 (2H, m, H-3‴, H-5‴), 7.73–8.00 (6H, m, H-3′, H-5′, H-3″, H-5″, H-4‴, H-6‴), 8.25 (1H, s, H-5), 8.4 (1H, s, H-7), 9.50 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 170.2 (C=O), 164.6 (C=N), 163.9 (C=O), 161.5 (C=O), 155.5 (Ar/olefinic carbon), 140.2 (Ar/olefinic carbon), 137.3 (Ar/olefinic carbon), 136.1 (Ar/olefinic carbon), 135.2 (Ar/olefinic carbon), 132.7 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 130.2 (Ar/olefinic carbon), 129.6 (Ar/olefinic carbon), 129.1 (Ar/olefinic carbon), 128.7 (Ar/olefinic carbon), 128.5 (Ar/olefinic carbon), 127.6 (Ar/olefinic

carbon), 126.5 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.7 (Ar/olefinic carbon), 123.2 (Ar/olefinic carbon), 115.4 (Ar/olefinic carbon), 57.9 (CH), 36.4 (CH_2); MS: $m/z =$ 743.88; Anal. Calcd for: $C_{30}H_{18}Br_2Cl_2N_4O_3S$: C, 48.35; H, 2.43; N, 7.52 %. Found C, 48.15; H, 2.32; N, 7.49 %.

4-(2-Phenyl-6,8-dibromo-2-(4-chlorophenyl)-4-oxo-(4H) quinazolin-3-yl)-N-[5-piperidino methyl and/or morpholinomethyl)-4-oxo-2-arylthiazolidin-3-yl]benzamides (**3a**–**h**): *(Mannich bases) General method*: A mixture of compounds (**2a**–**d**), paraformaldehyde (0.045 g; 5 mol) and the appropriate secondary amine (5 mmol), namely piperidine and/or morpholine in DMF (20 ml), was refluxed for 8 h. The excess solvent was evaporated under vacuum, and the obtained residue was poured on crushed ice. The solid product was filtered off and washed with water to obtain the desired products **3a**–**h**, respectively.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(4-oxo-2-phenyl-5-(piperidin-1-ylmethyl)thiazolidin-3-yl)benzamide (**3a**) Yellow crystals (methanol); yield 60 %; mp 130–132 °C; IR (KBr) νmax 3365 (NH), 3090 (CH, aromatic), 1710 (CO, cyclic amide thiazolidinone), 1685 (CO, cyclic amide quinazoline), 1665 (CONH) and 1610 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.53$ (6H, m, $3CH_2$ of piperdine), 2.45 (4H, m, CH_2NCH_2 of piperdine), 2.9 (2H, s, $–CH_2–$N), 3.73 (1H, s, CH, thiazolidinone ring), 5.90 (1H, s, CH of thiazolidinone ring), 7.5–7.62 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.65–7.79 (3H, m, H-3‴, H-4‴, H-5‴), 7.8–8.0 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.15 (1H, s, H-5), 8.4 (1H, s, H-7), 10.3 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 173.4 (C=O), 165.4 (C=N), 164.7 (C=O), 160.5 (C=O), 154.9 (Ar/olefinic carbon), 139.4 (Ar/olefinic carbon), 139.2 (Ar/olefinic carbon), 136.3 (Ar/olefinic carbon), 135.7 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 129.9 (Ar/olefinic carbon), 129.4 (Ar/olefinic carbon), 128.9 (Ar/olefinic carbon), 128.6 (Ar/olefinic carbon), 127.4 (Ar/olefinic carbon), 127.1 (Ar/olefinic carbon), 126.9 (Ar/olefinic carbon), 126.7 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.5 (Ar/olefinic carbon), 124 (Ar/olefinic carbon), 114.5 (Ar/olefinic carbon), 56.9 (CH_2), 54.9 (CH), 53.9 (CH_2), 52.3 (CH), 25.9 (CH_2), 24.5 (CH_2); MS: $m/z = 807.01$; Anal. Calcd for: $C_{36}H_{30}Br_2ClN_5O_3S$: C, 53.51; H, 3.74 N, 8.67 %. Found C, 53.48; H, 3.70; N, 8.53 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(5-(morpholinomethyl)-4-oxo-2-phenylthiazolidin-3-yl)benzamide (**3b**) Brown crystals (ethanol); yield 70 %; mp 222–224 °C; IR (KBr) νmax 3360 (NH), 3086 (CH, aromatic), 1695 (CO, cyclic amide thiazolidinone), 1685 (CO, cyclic amide quinazoline), 1660 (CONH) and 1601 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.6$ (4H, t, $2CH_2$ of morpholine), 2.8 (2H, s, $–CH_2–$N), 3.4 (4H, t, $2CH_2$ of morpholine) 3.65 (1H, s, CH, thiazolidinone ring), 5.90 (1H, s, CH of thiazolidinone ring), 7.4–7.50 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.6–7.69 (3H, m, H-3‴, H-4‴, H-5‴), 7.71–7.79 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.00 (1H, s, H-5), 8.1 (1H, s, H-7), 10.1 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 174.5 (C=O), 165.7 (C=N), 165.2(C=O), 161.3 (C=O), 155.9 (Ar/olefinic carbon), 139.7 (Ar/olefinic carbon), 139.5 (Ar/olefinic carbon), 137.3 (Ar/olefinic carbon), 136.7 (Ar/olefinic carbon), 131.5 (Ar/olefinic carbon), 129.7 (Ar/olefinic carbon), 129.2 (Ar/olefinic carbon), 128.7 (Ar/olefinic carbon), 128.4 (Ar/olefinic carbon), 127.9 (Ar/olefinic carbon), 127.4 (Ar/olefinic carbon), 126.4 (Ar/olefinic carbon), 126.1 (Ar/olefinic carbon), 125.6 (Ar/olefinic carbon), 124.7 (Ar/olefinic carbon), 124 (Ar/olefinic carbon), 117.5 (Ar/olefinic carbon), 66.7 (CH_2), 56.6 (CH_2), 54.9 (CH), 52.9 (CH_2), 52.4 (CH); MS: $m/z = 808.99$; Anal. Calcd for: $C_{35}H_{28}Br_2ClN_5O_4S$: C, 51.90; H, 3.48; N, 8.65 %. Found C, 51.80; H, 3.50; N, 8.77 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(2-(4-methoxy phenyl)-4-oxo-5-(piperidin-1-ylmethyl)thiazolidin-3-yl)benzamide (**3c**) White crystals (methanol); yield 60 %; mp 196–197 °C; IR (KBr) νmax 3360(NH), 3070(CH, aromatic), 1695(CO, cyclic amide thiazolidinone), 1685 (CO, cyclic amide quinazoline), 1665 (CONH) and 1601 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.5$ (6H, m, $3CH_2$ of piperdine), 2.6 (4H, m, CH_2NCH_2 of piperdine), 2.9 (2H, s, $–CH_2–$N), 3.71 (1H, s, CH, thiazolidinone ring), 3.83(3H, s, OCH_3), 5.72 (1H, s, CH of thiazolidinone ring), 7.5–7.7 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.74–7.82 (2H, m, H-3‴, H-5‴), 7.85–8.2 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.3 (1H, s, H-5), 8.5 (1H, s, H-7), 11.3 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 175.2 (C=O), 166.5 (C=N), 165.9(C=O), 163.5 (C=O), 157.4 (Ar/olefinic carbon), 140.4 (Ar/olefinic carbon), 140.2 (Ar/olefinic carbon), 137.3 (Ar/olefinic carbon), 136.7 (Ar/olefinic carbon), 133.3 (Ar/olefinic carbon), 130.9 (Ar/olefinic carbon), 129.4 (Ar/olefinic carbon), 129.2 (Ar/olefinic carbon), 128.6 (Ar/olefinic carbon), 127.7 (Ar/olefinic carbon), 127.4 (Ar/olefinic carbon), 127.1 (Ar/olefinic carbon), 126.9 (Ar/olefinic carbon), 125.7 (Ar/olefinic carbon), 125.5 (Ar/olefinic carbon), 125 (Ar/olefinic carbon), 116.2 (Ar/olefinic carbon), 57.9 (CH_2), 56.3 (CH_3), 55.4 (CH), 54.2 (CH_2), 53.4 (CH), 26.7 (CH_2), 25.5 (CH_2); MS: $m/z = 837.02$; Anal. Calcd for: $C_{37}H_{32}Br_2ClN_5O_4S$: C, 53.03; H, 3.85; N, 8.36 %. Found C, 53.07; H, 3.90; N, 8.43 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(2-(4-methoxy phenyl)-5-(morpholinomethyl)-4-oxothiazolidin-3-yl)benzamide (**3d**) Yellowish white crystals (acetic acid); yield 65 %; mp 175–177 °C; IR (KBr) νmax 3350 (NH), 3065(CH, aromatic), 1695 (CO, cyclic amide thiazolidinone), 1685 (CO, cyclic amide quinazoline), 1660 (CONH) and 1601 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.8$ (4H, t, $2CH_2$ of morpholine), 2.9 (2H, s, $–CH_2–N$), 3.4 (3H, s, OCH_3), 3.7 (4H, t, $2CH_2$ of morpholine) 3.8 (1H, s, CH, thiazolidinone ring), 5.95 (1H, s, CH of thiazolidinone ring), 7.6–7.8 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.82–7.95 (2H, m, H-3‴, H-5‴), 8.0–8.3 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.4 (1H, s, H-5), 8.6 (1H, s, H-7), 10.5 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 173.5 (C=O), 166.2 (C=O), 164.6 (C=N), 163.4 (C=O), 155.9 (Ar/olefinic carbon), 139.9 (Ar/olefinic carbon), 139.6 (Ar/olefinic carbon), 138.2 (Ar/olefinic carbon), 137.2 (Ar/olefinic carbon), 132.5 (Ar/olefinic carbon), 130.6 (Ar/olefinic carbon), 130.2 (Ar/olefinic carbon), 128.9 (Ar/olefinic carbon), 128.3 (Ar/olefinic carbon), 128.0 (Ar/olefinic carbon), 127.6 (Ar/olefinic carbon), 126.7 (Ar/olefinic carbon), 126.4 (Ar/olefinic carbon), 125.4 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 125 (Ar/olefinic carbon), 118.5 (Ar/olefinic carbon), 65.6 (CH_2), 57.6 (CH_2), 55.8 (CH_3), 55.1 (CH), 53.2 (CH_2), 52.6 (CH); MS: $m/z = 839.00$; Anal. Calcd for: $C_{36}H_{30}Br_2ClN_5O_5S$: C, 51.48; H, 3.60; N, 8.34 %. Found C, 51.50; H, 3.70; N, 8.40 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(4-oxo-5-(piperidin-1-ylmethyl)-2-p-tolylthiazolidin-3-yl)benzamide (**3e**) Yellow crystals (ethanol); yield 65 %; mp 212–214 °C; IR (KBr) νmax 3360 (NH), 3090 (CH, aromatic), 1720(CO, cyclic amide thiazolidinone), 1690 (CO, cyclic amide quinazoline), 1665 (CONH) and 1610 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.53$ (6H, m, $3CH_2$ of piperdine), 2.34 (3H, s, CH_3), 2.5 (4H, m, CH_2NCH_2 of piperdine), 2.9 (2H, s, $–CH_2–N$), 3.75 (1H, s, CH, thiazolidinone ring), 5.90 (1H, s, CH of thiazolidinone ring), 7.5–7.65 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.69–7.74 (2H, m, H-3‴, H-5‴), 7.79–8.1 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.2 (1H, s, H-5), 8.3 (1H, s, H-7), 9.3 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 172.3 (C=O), 163.2 (C=N), 162.4 (C=O), 160.5 (C=O), 154.2 (Ar/olefinic carbon), 139.2 (Ar/olefinic carbon), 139 (Ar/olefinic carbon), 136.3 (Ar/olefinic carbon), 136.5 (Ar/olefinic carbon), 132.1 (Ar/olefinic carbon), 129.7 (Ar/olefinic carbon), 129.2 (Ar/olefinic carbon), 129 (Ar/olefinic carbon), 127.6 (Ar/olefinic carbon), 126.7 (Ar/olefinic carbon), 126.3 (Ar/olefinic carbon), 126.1 (Ar/olefinic carbon), 124.9 (Ar/olefinic carbon), 123.3 (Ar/olefinic carbon), 122.5 (Ar/olefinic carbon), 120 (Ar/olefinic carbon), 114.1 (Ar/olefinic carbon), 56.7 (CH_2), 53.4 (CH), 52.2 (CH_2), 50.4 (CH), 24.7 (CH_2), 23.5 (CH_2), 22.4 (CH_3); MS: $m/z = 821.03$; Anal. Calcd for: $C_{37}H_{32}Br_2ClN_5O_3S$: C, 54.06; H, 3.92 N, 8.52 %. Found C, 54.12; H, 3.79; N, 8.53 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(5-(morpholinomethyl)-4-oxo-2-p-tolylthiazolidin-3-yl)benzamide (**3f**) White crystals (ethanol); yield 60 %; mp 165–167 °C; IR (KBr) νmax 3355(NH), 3060 (CH, aromatic), 1695 (CO, cyclic amide thiazolidinone), 1680 (CO, cyclic amide quinazoline), 1660 (CONH) and 1600 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.34$ (3H, s, CH_3), 2.7 (4H, t, $2CH_2$ of morpholine), 2.9 (2H, s, $–CH_2–N$), 3.6 (4H, t, $2CH_2$ of morpholine) 3.75 (1H, s, CH, thiazolidinone ring), 5.90 (1H, s, CH of thiazolidinone ring), 7.53–7.69 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.71–7.77 (2H, m, H-3‴, H-5‴), 7.79–8.1 (6H, m, H-3′, H-5′, H-3″, H-5″, H-2‴, H-6‴), 8.15 (1H, s, H-5), 8.25 (1H, s, H-7), 10.3 (1H, s, NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): 171.2 (C=O), 162.5 (C=N), 161.2 (C=O), 160.5 (C=O), 153.2 (Ar/olefinic carbon), 138.7 (Ar/olefinic carbon), 138.4 (Ar/olefinic carbon), 137.2 (Ar/olefinic carbon), 137.0 (Ar/olefinic carbon), 130.9 (Ar/olefinic carbon), 130.6 (Ar/olefinic carbon), 130.0 (Ar/olefinic carbon), 127.9 (Ar/olefinic carbon), 127.3 (Ar/olefinic carbon), 126.2 (Ar/olefinic carbon), 125.5 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.3 (Ar/olefinic carbon), 124.1 (Ar/olefinic carbon), 122.2 (Ar/olefinic carbon), 120.4 (Ar/olefinic carbon), 114.9 (Ar/olefinic carbon), 63.7 (CH_2), 55.4 (CH_2), 53.1 (CH), 50.6 (CH_2), 49.9 (CH), 23.8 (CH_3); MS: $m/z = 823.01$; Anal. Calcd for: $C_{36}H_{30}Br_2ClN_5O_4S$: C, 52.48; H, 3.67; N, 8.50 %. Found C, 52.38; H, 3.70; N, 8.48 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(2-(2-hydroxy phenyl)-4-oxo-5-(piperidin-1-ylmethyl)thiazolidin-3-yl)benzamide (**3g**) Brown crystals (methanol); yield 60 %; mp 145–147 °C; IR (KBr) νmax 3365 (NH), 3100 (CH, aromatic), 1725 (CO, cyclic amide thiazolidinone), 1710 (CO, cyclic amide quinazoline), 1670 (CONH) and 1615 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.53$ (6H, m, $3CH_2$ of piperdine), 2.5 (4H, m, CH_2NCH_2 of piperdine), 2.9 (2H, s, $–CH_2–N$), 3.75 (1H, s, CH, thiazolidinone ring), 5.90 (1H, s, CH of thiazolidinone ring), 7.45–7.55 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.64–7.76 (2H, m, H-3‴, H-5‴), 7.79–8.00 (6H, m, H-3′, H-5′, H-3″, H-5″, H-4‴, H-6‴), 8.3 (1H, s, H-5), 8.4 (1H, s, H-7), 9.3 (1H, s, NH, exchangeable with D_2O), 10.2 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6): 173.4 (C=O), 164 (C=N), 163.7 (C=O), 160.6 (C=O), 154.3 (Ar/olefinic carbon), 153.7 (Ar/olefinic carbon), 139.4 (Ar/olefinic carbon), 136.1 (Ar/olefinic carbon), 135.7 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 129.6 (Ar/olefinic carbon),

129.1 (Ar/olefinic carbon), 128.9 (Ar/olefinic carbon), 128.5 (Ar/olefinic carbon), 128 (Ar/olefinic carbon), 127.6 (Ar/olefinic carbon), 126.7 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.5 (Ar/olefinic carbon), 122 (Ar/olefinic carbon), 121.2 (Ar/olefinic carbon), 115.1 (Ar/olefinic carbon), 113.2 (Ar/olefinic carbon), 56.6 (CH_2), 53.9 (CH_2), 52.4 (CH), 48.6 (CH), 25.9 (CH_2), 24.5 (CH_2); MS: $m/z = 823.01$; Anal. Calcd for: $C_{36}H_{30}Br_2ClN_5O_4S$: C, 52.48; H, 3.6 N, 8.50 %. Found C, 52.43; H, 3.55; N, 8.53 %.

4-(6,8-Dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-N-(2-(2-hydroxy phenyl)-5-(morpholinomethyl)-4-oxothiazolidin-3-yl)benzamide (**3h**) White crystals (ethanol); yield 60 %; mp 160–162 °C; IR (KBr) νmax 3360(NH), 3065 (CH, aromatic), 1670 (CO, cyclic amide thiazolidinone), 1685 (CO, cyclic amide quinazoline), 1660 (CONH) and 1610 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.7$ (4H, t, $2CH_2$ of morpholine), 2.9 (2H, s, $–CH_2–N$), 3.6 (4H, t, $2CH_2$ of morpholine) 3.75 (1H, s, CH, thiazolidinone ring), 5.90 (1H, s, CH of thiazolidinone ring), 7.5–7.65 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.67–7.79 (2H, m, H-3‴, H-5‴), 8.0–8.2 (6H, m, H-3′, H-5′, H-3″, H-5″, H-4‴, H-6‴), 8.3 (1H, s, H-5), 8.4 (1H, s, H-7), 9.3 (1H, s, NH, exchangeable with D_2O), 10.34 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6): 172.4 (C=O), 163.6 (C=N), 162.4 (C=O), 161.5 (C=O), 155.9 (Ar/olefinic carbon), 140.7 (Ar/olefinic carbon), 140.4 (Ar/olefinic carbon), 139.2 (Ar/olefinic carbon), 139.0 (Ar/olefinic carbon), 132.5 (Ar/olefinic carbon), 130.6 (Ar/olefinic carbon), 130.0 (Ar/olefinic carbon), 129.5 (Ar/olefinic carbon), 129.3 (Ar/olefinic carbon), 127.2 (Ar/olefinic carbon), 126.5 (Ar/olefinic carbon), 126.2 (Ar/olefinic carbon), 125.5 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.4 (Ar/olefinic carbon), 122.6 (Ar/olefinic carbon), 116.7 (Ar/olefinic carbon), 66.7 (CH_2), 56.4 (CH_2), 52.9 (CH_2), 52.4 (CH), 48.6 (CH); MS: $m/z = 824.98$; Anal. Calcd for: $C_{35}H_{28}Br_2ClN_5O_5S$: C, 50.90; H, 3.42; N, 8.48 %. Found C, 50.89; H, 3.42; N, 8.48 %.

4-[6,8-Dibromo-2-(4-chlorophenyl)-4-oxo-4H-quinazolin-3-yl]-benzoic acid hydrazide (**4**) Orange crystals (methanol); yield 80 %; mp 170/172 °C; IR (KBr) νmax 3315, 3140 (NH, NH2), 3060 (CH, aromatic), 1715 (CO, quinazoline ring) and 1645 (CO, amide) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 4.5$ (2H, s, NH2, exchangeable with D2O), 7.53–7.6 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.72–7. 8 (4H, m, H-3′, H-5′, H-3″, H-5″), 8.15 (1H, s, H-5), 8.25 (1H, s, H-7), and at 9.8 (1H, s, NH, exchangeable with D2O); ^{13}C NMR (100 MHz, DMSO-d_6): 167.2 (C=O), 164.1 (C=N), 160.4 (C=O), 154.1(Ar/olefinic carbon), 139.2 (Ar/olefinic carbon), 136.3 (Ar/olefinic carbon), 131.2 (Ar/olefinic carbon), 131.1 (Ar/olefinic carbon), 130.1 (Ar/olefinic carbon), 129.4 (Ar/olefinic carbon), 128.8 (Ar/olefinic carbon), 128.6 (Ar/olefinic carbon), 128.2 (Ar/olefinic carbon), 127.5 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 124.5 (Ar/olefinic carbon), 122.2 (Ar/olefinic carbon), 113.5(Ar/olefinic carbon); MS: $m/z = 547.91$; Anal. Calcd for: $C_{21}H_{13}Br_2ClN_4O_2$: C, 49.97; H, 2.39; N, 10.21 %. Found C, 49.70; H, 2.59; N, 10.40 %.

6,8-Dibromo-2-(4-chlorophenyl)-3-(4-(5-methyl-1,3,4-oxadiazol-2-yl) phenyl) quinazolin-4(3H)-one (**5**) A mixture of the hydrazide **4** (5.48 g; 10 mmol) and acetic anhydride (20 ml) was refluxed for 6 h. The precipitated solid formed upon cooling, was filtered and recrystallized as yellowish white crystals (ethanol); yield 70 %; mp 174–176 °C; IR (KBr) νmax 3075 (CH, aromatic), 1703 (CO) and 1610 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.6$ (3H, s, methyl), 7.5–7.7 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.8–8.0 (4H, m, H-3′, H-5′, H-3″, H-5″), 8.15 (1H, s, H-5), 8.25 (1H, s, H-7); ^{13}C NMR (100 MHz, DMSO-d_6): 164.9 (C=N), 164.5 (C=N), 164 (C=N), 160.5 (C=O), 154.9 (Ar/olefinic carbon), 139.4 (Ar/olefinic carbon), 135.4 (Ar/olefinic carbon), 132.2 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 129.5 (Ar/olefinic carbon), 129.1 (Ar/olefinic carbon), 129 (Ar/olefinic carbon), 127.5 (Ar/olefinic carbon), 126.3 (Ar/olefinic carbon), 125.2 (Ar/olefinic carbon), 121.5 (Ar/olefinic carbon), 122 (Ar/olefinic carbon), 113.5 (Ar/olefinic carbon), 20.5 (CH_3); MS: $m/z = 571.91$; Anal. Calcd for: $C_{23}H_{13}Br_2ClN_4O_2$: C, 48.24; H, 2.29; N, 9.78 %. Found C, 48.34; H, 2.33; N, 9.68 %.

6,8-Dibromo-2-(4-chlorophenyl)-3-(4-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)phenyl)quinazolin-4(3H)-one (**6**) A mixture of the hydrazide **4** (5.48 g; 10 mmol), and ethyl acetoacetate (1.3 g; 10 mmol) in absolute ethanol (20 ml) was refluxed for 6 h. The precipitated solid formed upon cooling, was filtered and recrystallized as white crystals (methanol); yield 70 %; mp 290–292 °C; IR (KBr) νmax 3065 (CH, aromatic), 1720 (CO, cyclic amide pyrazol), 1685 (CO, cyclic amide quinazoline) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.9$ (3H, s, CH_3), 2.8 (2H, s, CH_2 of pyrazol), 7.55–7.76 (4H, m, H-2′, H-6′, H-2″, H-6″), 7. 8–8.0 (4H, m, H-3′, H-5′, H-3″, H-5″), 8.15 (1H, s, H-5), 8.25 (1H, s, H-7); ^{13}C NMR (100 MHz, DMSO-d_6): 175.5 (C=O), 164 (C=N), 162.9 (C=N), 160.6 (C=O), 154.3 (Ar/olefinic carbon), 139.9 (Ar/olefinic carbon), 135.6 (Ar/olefinic carbon), 134.2 (Ar/olefinic carbon), 131.3 (Ar/olefinic carbon), 129.2 (Ar/olefinic carbon), 128 (Ar/olefinic carbon), 126.5 (Ar/olefinic carbon), 125.3 (Ar/olefinic carbon), 122 (Ar/olefinic carbon), 121.7 (Ar/olefinic carbon), 113.5 (Ar/olefinic carbon), 42.5 (CH_2), 16.5 (CH_3); MS: $m/z = 585.92$; Anal. Calcd for: $C_{24}H_{15}Br_2ClN_4O_2$: C, 52.20; H, 2.92; N, 10.15 %. Found C, 50.14; H, 2.58; N, 9.55 %.

Pharmacological screening

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 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

Alamar blue or resazurin (Promega, Mannheim, Germany) reduction assay was used to assess the cytotoxicity of the studied samples. The assay tests cellular viability and mitochondrial function. Briefly, adherent cells were grown in tissue culture flasks as previously described (Youns *et al.*, 2009, 2010, 2011; Youns and Fathy, 2013) 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 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} values (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.

In vitro inhibition studies of EGFR tyrosine kinase

Compounds **2c**, **2d**, **3a**, **3f** were tested in vitro for inhibition of EGFR tyrosine kinase, using Kinase-Glo Plus luminescence kinase assay kit. In this method, tested compounds were dissolved in DMSO and tested at a single concentration of 10 µM. They were then added to reaction plates containing the EGFR tyrosine kinase in assay buffer [20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 10 mM $MgCl_2$, 1 mM ethylene glycol tetraacetic acid (EGTA), 0.02 % Brij35, 0.02 mg/ml bovine serum albumin (BSA), 0.1 mM Na_3VO_4, 2 mM dithiothreitol (DTT), 1 % DMSO]. Reactions were initiated by addition of a mixture of ATP (Sigma, St. Louis MO) and 33P ATP (PerkinElmer, Waltham MA) to a final concentration of 10 µM. Reactions were carried out at room temperature for 120 min, followed by spotting of the reactions onto P81 ion exchange filter paper (Whatman Inc., Piscataway, NJ). Unbound phosphate was removed by extensive washing of filters in 0.75 % phosphoric acid (Ma *et al.*, 2008). Results are presented as percentage of enzyme inhibition and compared with those of gefitinib as a reference EGFR-TK inhibitor.

Molecular modeling

Molecular modeling studies were carried out on an Intel Core i5, 2.53 GHz processor, 4 GB memory with Windows XP 32-bit operating system using Molecular Operating Environment (MOE, 10.2008) software. Energy minimization was performed with MOE with RMSD gradient of 0.05 kcal/mol, MMFF94X force field and automatic calculation of the partial charges. The X-ray crystallographic structure of IRE (3-chloro-4-fluoro-*N*-[(4*Z*)-7-methoxy-6-(3-morpholin-4-ylpropoxy)quinazolin-4(1*H*)-ylidene]aniline) co-crystallized with EGFR was obtained from the protein data bank (PDB ID: 2ITO) (http://www.rcsb.org/pdb/explore/explore.do?structureId=2ITO). The ATP binding site of EGFR was prepared for docking studies where: (1) IRE was removed from the active site. (2) Hydrogen atoms were added to the structure with their standard geometry. (3) MOE Alpha Site Finder was used for the active sites detection. (4) The obtained model was then used in predicting interactions at the active site between the new compounds and EGFR.

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