Acta Poloniae Pharmaceutica – Drug Research, Vol. 75 No. 3 pp. 669–677, 2018 ISSN 0001-6837
Polish Pharmaceutical Society

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL SUBSTITUTED QUINAZOLINE DERIVATIVES AS ANTITUMOR AGENTS

MARWA FARAG AHMED[1, 2]* and NAJA MAGDY[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Taif University, Taif 21974, Kingdom of Saudi Arabia
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Helwan University, Cairo, Egypt
[3]Department of Pharmacology and toxicology, Faculty of Pharmacy, Taif University, Kingdom of Saudi Arabia

Abstract: New series of 6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy derivatives were synthesized and their cytotoxic activity on MCF7, HEPG2 and HCT116 cell lines were evaluated. Compounds **XI and XIIIb** were two times more active than doxorubicin on MCF7 cancer cell line. Compound **VIIIa** was 3 times more active than doxorubicin on HEPG2 cancer cell line. While compounds **XII, XIIIa** and **XIIIb** were more potent than doxorubicin on HCT116 cancer cell line. IC_{50} of all newly synthesized compounds were evaluated in vitro for their inhibition of EGFR tyrosine kinase. All compounds show good inhibitory activity on EGFR tyrosine kinase with IC_{50} range (6.19-19.87) µM.

Keywords: quinazoline cytotoxicity Tyrosine kinases

Cancer is considered one of the most health problems in the world due to many factors (1-3). In 2017 it is estimated that 1 688 780 new cancer cases will be diagnosed. There are about 600 920 Americans are expected to die of cancer in 2017 (4). Many cytotoxic drugs are used to treat cancer but they are associated with several drawbacks and are not able to differentiate between normal and cancerous cell types, therefor discovery of new anticancer agents continues to be a great challenge (5). Receptor tyrosine kinase plays an important role in signal transduction pathway which regulates cell differentiation and division (6, 7). Compounds that inhibit tyrosine kinase activity are of potential interest as antitumor agents (8, 9). Receptor tyrosine kinase is a very promising molecular target for cancer therapy but most of the patients developed resistance to the EGFR inhibitors (10, 11). Therefore, many efforts are being undertaken to develop new and more potent EGFR inhibitors with improved anti-tumor activities. Quinazoline derivatives are of particular interest as they are considered as a new class of chemotherapeutic agents (12-20). The Food and Drug Administration (FDA) has approved many quinazoline derivatives such as gefitinib, erlotinib, lapatinib and vandetanib for clinical use as anticancer drugs. Moreover, many quinazoline derivatives are potent inhibitors of epidermal growth factor receptor (EGFR) (21-25). This research reports synthesis of new quinazoline derivatives, evaluation of their antitumor activity and their inhibition of tyrosine kinase EGFR.

EXPERIMENTAL

Chemistry

Elemental analyses were carried out in Cairo University, Egypt. IR spectra were recorded on Perkin Elmer-9712 spectrophotometer. ^{1}H-NMR spectra were determined on a Varian-Gemini-300 MHz. and Joel-Ex270 MHz NMR spectrometer. ^{13}C NMR (DMSO-d_6) spectra were recorded at 100.62 MHz at the aforementioned research center. Mass spectra were recorded on Finnigan Mat SSQ 7000 mode EI 70 ev. Compounds **I-V** were synthesized with the same previously reported methods (26).

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

A mixture of **V** (0.01 mol), ethylchloroacetate (0.015 mol) and anhydrous potassium carbonate (2.0 g) in dry acetone (50 mL) were refluxed for 12 h.

* Corresponding author: e-mail: marwafarag80@yahoo.com

The reaction mixture filtered while hot. The filtrate was cooled, poured into crushed ice, filtered and crystallized from ethanol. m.p. 220°C, 70% yield. Analysis calculated for $C_{18}H_{13}Br_2ClN_2O_3$ calcd.: C, 43.19; H, 2.62; N, 5.60%; found: C, 43.13; H, 2.60; N, 5.51. IR: $\nu_{max.}$/cm$^-$ 3010 (C-H aromatic), 1720 (C=O), 1630 (C=N) and at 1600 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ 1.2 (t, 3H, CH_3), 4 (q, 2H, CH_2), 5.1 (s, 2H, OCH_2) and 7.5-8.2 (m, 6H, Ar-H). ^{13}C NMR (DMSO-d_6): 180, 170, 162, 150, 140, 134, 132, 129, 128, 125, 121, 115, 64, 60, 15. MS: m/z = 500, M + 2 = 502, M + 4 = 504.

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

A mixture of **VI** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol (30 mL) were refluxed for 5 h. The reaction mixture was concentrated and left to cool, filtered and crystallized from ethanol. m.p. 140°C, 80% yield. Analysis calculated for $C_{16}H_{11}Br_2ClN_4O_2$ calcd.: C, 39.50; H, 2.28; N, 11.5%; found: C, 39.6; H, 2.30; N, 11.6%. IR: $\nu_{max.}$/cm$^-$ 3300 (NH_2), 3200 (NH),3000 (C-H aromatic), 1700 (C=O), 1620 (C=N) and at 1610 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ 4.2 (2H, s, NH_2, exchangeable with D_2O), 5.1 (s, 2H, OCH_2), 7.5-8.2 (m, 6H, Ar-H) and 9.1 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 182, 166, 161, 150, 142, 134, 130, 129, 128, 124, 123,121, 70. MS: m/z = 486, M + 2 = 488, M + 4 = 490.

(E)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N'-(substituted)benzylidene acetohydrazide (VIII a-e)

General method: A mixture of compound **VII** (0.01 mol) and the appropriate aldehyde, namely: 2-flurobenzaldehyde, 3- flurobenzaldehyde, 4- fluro benzaldehyde, 3-methylbenzaldehyde and/or furan-2-carboxaldehyde (0.01 mol) in glacial acetic acid (30 mL), was refluxed for 6 h. The reaction mixture was cooled and poured into crushed ice, the formed precipitate was filtered off and crystalized from ethanol to obtain the desired Schiff bases (**VIII a-e**) respectively.

(E)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N'-(2-fluorobenzylidene)acetohydrazide (VIII a)

Crystallized from methanol, m.p. 110°C, 75% yield. Analysis calculated for $C_{23}H_{14}Br_2ClFN_4O_2$ calcd.: C, 46.61; H, 2.38; N, 9.45%; found: C, 46.70; H, 2.4; N, 9.56%. IR: $\nu_{max.}$/cm^{-1} 3220 (NH), 3010 (C-H aromatic), 1710 (C=O), 1620 (C=N) and at 1580 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ4.6 (s, 2H, OCH_2), 7.45-8.3 (m, 10H, aromatic-H), 8.9 (1H, s, CH=N) and 11 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 184, 173, 162, 154, 144, 141, 134, 133, 132, 131, 130, 129, 128, 127, 124, 123, 121, 116, 69. MS: m/z = 592, M + 2 = 594, M + 4 = 596.

(E)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N'-(3-fluorobenzylidene)acetohydrazide (VIII b)

Crystallized from methanol, m.p. 190°C, 70% yield. Analysis calculated for $C_{23}H_{14}Br_2ClFN_4O_2$ calcd.: C, 46.61; H, 2.38; N, 9.45%; found: C, 46.59; H, 2.30; N, 9.39%. IR: $\nu_{max.}$/cm^{-1} 3200 (NH), 3010 (C-H aromatic), 1710 (C=O), 1620 (C=N) and at 1580 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ4.7 (s, 2H, OCH_2), 7.5-8.4 (m, 10H, aromatic-H), 9 (1H, s, CH=N) and 11.2 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 183, 174, 162, 154, 143, 141, 134, 133, 132, 131, 130, 129, 128, 127, 124, 123, 121, 116, 69.5. MS: m/z = 592, M + 2 = 594, M + 4 = 596.

(E)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N'-(4-fluorobenzylidene)acetohydrazide (VIII c)

Crystallized from ethanol, m.p. 205°C, 70% yield. Analysis calculated for $C_{23}H_{14}Br_2ClFN_4O_2$ calcd.: C, 46.61; H, 2.38; N, 9.45%, found: C, 46.52; H, 2.35; N, 9.30%. IR: $\nu_{max.}$/cm^{-1} 3200 (NH), 3000 (C-H aromatic), 1710 (C=O), 1610 (C=N) and at 1580 (C=C) . ^{1}H-NMR (DMSO-d_6, ppm): δ4.8 (s, 2H, OCH_2), 7.4-8.5 (m, 10H, aromatic-H), 8.9 (1H, s, CH=N) and 11 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 182, 174, 165, 155, 143, 141, 135, 133, 132, 131, 130, 129, 128, 127, 124, 123, 121, 118, 70. MS: m/z = 592, M + 2 = 594, M + 4 = 596.

(E)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N'-(3-methylbenzylidene)acetohydrazide (VIII d)

Crystallized from ethanol, m.p. 115°C, 75% yield. Analysis calculated for $C_{24}H_{17}Br_2ClN_4O_2$ calcd.: C, 48.97; H, 2.91; N, 9.52%; found: C, 48.90; H, 2.80; N, 9.50%. IR: $\nu_{max.}$/cm^{-1} 3150 (NH), 3000 (C-H aromatic), 1700 (C=O), 1610 (C=N) and at 1600 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ 2.5 (s, 3H, CH_3), 4.9 (s, 2H, OCH_2), 7.2-8.0 (m, 10H, aromatic-H), 8.5 (1H, s, CH=N) and 10.9 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 180, 173, 160, 150, 146, 141, 138, 134, 133, 132, 131, 129.4, 129, 128, 127, 126, 124, 123, 120, 115, 69, 21. MS: m/z = 588, M + 2 = 590, M + 4 = 592.

(E)-2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)-N'-(furan-2-ylmethylene)acetohydrazide (VIII e)

Crystallized from ethanol, m.p. 170°C, 70% yield. Analysis calculated for $C_{21}H_{13}Br_2ClN_4O_3$ calcd.: C, 44.67; H, 2.32; N, 9.92%; found: C, 44.64; H, 2.29; N, 9.90%. IR: $\nu_{max.}$/cm^{-1} 3200 (NH), 3000 (C-H aromatic), 1710 (C=O), 1610 (C=N) and at 1600 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ 4.6 (s, 2H, OCH_2), 6.9-7.9 (m, 9H, aromatic-H), 8.2 (1H, s, CH=N) and 10.5 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 181, 172, 162, 150, 149, 144, 141, 137, 134, 132, 130, 129, 124, 123, 121, 119, 116, 112, 68. MS: m/z = 564, M + 2 = 566, M + 4 = 568.

2-((6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)methyl)-5-methyl-1,3,4-oxadiazole (IX)

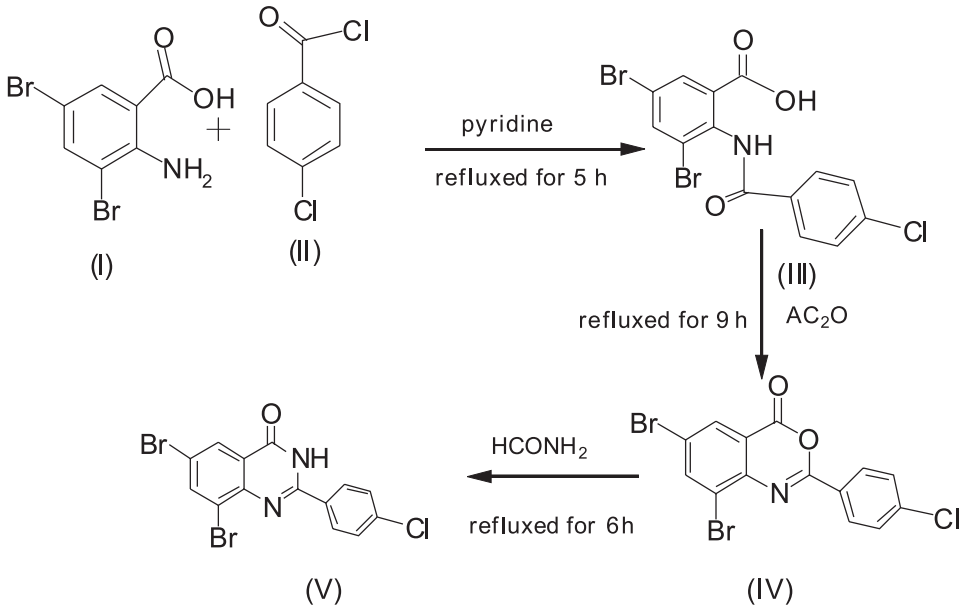
A mixture of the hydrazide **VII** (0.01 mol) and acetic anhydride (30 mL) was refluxed for 6 h. The precipitated solid formed upon cooling, was filtered and recrystallized from ethanol, m.p. 155°C, 80% yield. Analysis calculated for $C_{18}H_{11}Br_2ClN_4O_2$ calcd.: C, 42.34; H, 2.17; N, 10.97%; found: C, 42.30; H, 2.14; N, 10.90%. IR: $\nu_{max.}$/cm^{-1} 3000 (C-H aromatic), 1610 (C=N) and at 1600 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ 2.6 (s, 3H, CH_3), 5.4 (s, 2H, OCH_2) and 7.5-8.2 (m, 6H, aromatic-H). ^{13}C NMR (DMSO-d_6): 185, 164, 162, 150, 141, 135, 133, 129, 128, 124, 123, 121, 117, 72, 21. MS: m/z = 510, M + 2 = 512, M + 4 = 514.

N'-(2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetyl)isonicotinohydrazide (X)

A mixture of hydrazide **VII** (0.01 mol) and isonicotinoyl chloride (0.015 mol) in dimethyl formamide (25 mL) was heated under reflux for 6 h. The reaction mixture was cooled, poured onto crushed (100 mL) ice. The solid was filtered, washed with water, dried and crystallized from methanol, m.p. 245°C, 80% yield. Analysis calculated for $C_{22}H_{14}Br_2ClN_5O_3$ Calcd.: C, 44.66; H, 2.39; N, 11.84%; Found: C, 44.69; H, 2.40; N, 11.90%. IR: $\nu_{max.}$/cm^{-1} 3200 (NH), 3010 (C-H aromatic), 1750 (C=O), 1700 (C=O), 1620 (C=N) and at 1600 (C=C) . ^{1}H-NMR (DMSO-d_6, ppm): 4.5 (s, 2H, OCH_2) and 7.9-8.9 (m, 10H, aromatic-H), 10.0 (1H, s, NH, exchangeable with D_2O) and 10.5 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 180,166, 165, 164, 162, 152, 149, 142, 141, 134, 132, 129, 128, 124, 123, 121, 116, 69.5. MS: m/z = 591, M + 2 = 593, M + 4 = 595.

N'-(2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetyl)nicotinohydrazide (XI)

A mixture of hydrazide **VII** (0.01 mol) and nicotinoyl chloride (0.015 mol) in dimethyl formamide (25 mL) was heated under reflux for 6 h. The reaction mixture was cooled, poured onto crushed (100 mL) ice. The solid was filtered, washed with water, dried and crystallized from methanol, m.p. 245°C, 80% yield. Analysis calcu-



Scheme 1. Synthesis of compounds **III -V**

lated for $C_{22}H_{14}Br_2ClN_5O_3$ calcd.: C, 44.66; H, 2.39; N,11.84%; found: C, 44.62; H, 2.35; N, 11.80%. IR: $\nu_{max.}$/cm^{-1} 3200 (NH), 3000 (C-H aromatic), 1750 (C=O), 1700 (C=O), 1620 (C=N) and at 1600 (C=C) . ^{1}H-NMR (DMSO-d_6, ppm): 4.6 (s, 2H, OCH_2) and 7.7-9.2 (m, 10H, aromatic-H), 10.2 (1H, s, NH, exchangeable with D_2O) and 10.6 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 184,166 164, 162, 150, 149, 148, 141, 135, 134, 132, 130, 129, 128, 125, 124, 123, 121, 116, 70. MS: m/z = 591, M + 2 = 593, M + 4 = 595.

4-chloro-N'-(2-(6,8-dibromo-2-(4-chlorophenyl) quinazolin-4-yloxy)acetyl)benzohydrazide (XII)

A mixture of hydrazide **VII** (0.01 mol) and 4-chlorobenzoyl chloride (0.015 mol) in dimethyl formamide (25 mL) was heated under reflux for 6 h. The reaction mixture was cooled, poured onto crushed (100 mL) ice. The solid was filtered, washed with water, dried and crystallized from methanol, m.p. 180^{o}C, 80% yield. Analysis calculated for $C_{23}H_{14}Br_2Cl_2N_4O_3$ calcd.: C, 44.19; H, 2.26; N, 8.96%; found: C, 44.15; H, 2.24; N, 8.64%. IR: $\nu_{max.}$/cm^{-1} 3300 (NH), 3020 (C-H aromatic), 1760 (C=O), 1720 (C=O), 1650 (C=N) and at 1610 (C=C) . ^{1}H-NMR (DMSO-d_6, ppm): 4.5 (s, 2H, OCH_2) and 7.5-8.0 (m, 10H, aromatic-H), 10.0 (1H, s, NH, exchangeable with D_2O) and 10.2 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 182, 165 164, 162, 152, 141, 138, 134, 132, 131, 129, 128, 124, 123, 121, 119, 68. MS: m/z = 625, M + 2 = 627, M + 4 = 629.

2-(2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetyl)-N-(methyl or substituted phenyl) hydrazinecarbothioamide (XIII a-c)

General method: a mixture of hydrazide **VII** (0.01 mol), the appropriate isothiocyanate, namely: methyl isocyanate, 4-methoxy phenyl isothiocyanate and/or 4-trifluro methyl phenyl isothiocyanate (10 mmol) in pyridine (25 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 **XIII a-c,** respectively.

2-(2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetyl)-N-methylhydrazinecarbothioamide (XIIIa)

Crystallized from ethanol, m.p. 125^{o}C, 80% yield. Analysis calculated for $C_{18}H_{14}Br_2ClN_5O_2S$ calcd.: C, 38.63; H, 2.52; N, 12.51%, found: C, 38.59; H, 2.49; N, 12.50%. IR: $\nu_{max.}$/cm^{-1} 3300 (NH), 3200 (NH), 3000 (C-H aromatic), 1720 (C=O), 1620 (C=N) and at 1600 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ 2.8 (s, 3H, CH_3), 4.5 (s, 2H, OCH_2), 7.6-8.5 (m, 6H, aromatic-H), 9.5 (1H, s, NH, exchangeable with D_2O), 10.2 (1H, s, NH, exchangeable with D_2O) and 10.4 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 186, 182, 166, 162, 150, 141, 135, 133, 130, 129, 124, 123, 121, 116, 75, 31. MS: m/z = 559, M + 2 = 561, M + 4 = 563.

2-(2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetyl)-N-(4-methoxyphenyl)hydrazinecarbothioamide (XIIIb)

Crystallized from ethanol, m.p. 205^{o}C, 70% yield. Analysis calculated for $C_{24}H_{18}Br_2ClN_5O_3S$ calcd.: C, 44.23; H, 2.78; N, 10.75%; found: C, 44.46; H, 2.75; N, 10.60%. IR: $\nu_{max.}$/cm^{-1} 3250 (NH), 3200 (NH), 3010 (C-H aromatic), 1700 (C=O), 1610 (C=N) and at 1600 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ 3.8 (s, 3H, OCH_3), 4.8 (s, 2H, OCH_2), 6.3-8.1 (m, 10H, aromatic-H), 10.1 (1H, s, NH, exchangeable with D_2O), 10.5 (1H, s, NH, exchangeable with D_2O) and 11.2 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 188, 184, 166, 160,159, 150, 142, 134, 132, 130, 129, 128, 127, 124, 123, 121, 116, 114, 70, 55. MS: m/z = 651, M + 2 = 653, M + 4 = 655.

2-(2-(6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)acetyl)-N-(4-(trifluoromethyl)phenyl) hydrazinecarbothioamide (XIIIc)

Crystallized from ethanol, m.p. 185^{o}C, 70% yield. Analysis calculated for $C_{24}H_{15}Br_2ClF_3N_5O_2S$ calcd.: C, 41.79; H, 2.19; N, 10.15%; found: C, 41.75; H, 2.24; N, 10.20%. IR: $\nu_{max.}$/cm^{-1} 3300 (NH), 3200 (NH), 3000 (C-H aromatic), 1700 (C=O), 1620 (C=N) and at 1600 (C=C). ^{1}H-NMR (DMSO-d_6, ppm): δ 4.6 (s, 2H, OCH_2), 6.0-8.3 (m, 10H, aromatic-H), 9.1 (1H, s, NH, exchangeable with D_2O), 11.5 (1H, s, NH, exchangeable with D_2O) and 12.2 (1H, s, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): 184, 181, 167, 162, 150, 141, 139, 134, 132, 130, 129, 128, 127, 126, 124, 123, 121, 118, 115, 69. MS: m/z = 689, M + 2 = 691, M + 4 = 693.

Pharmacological screening

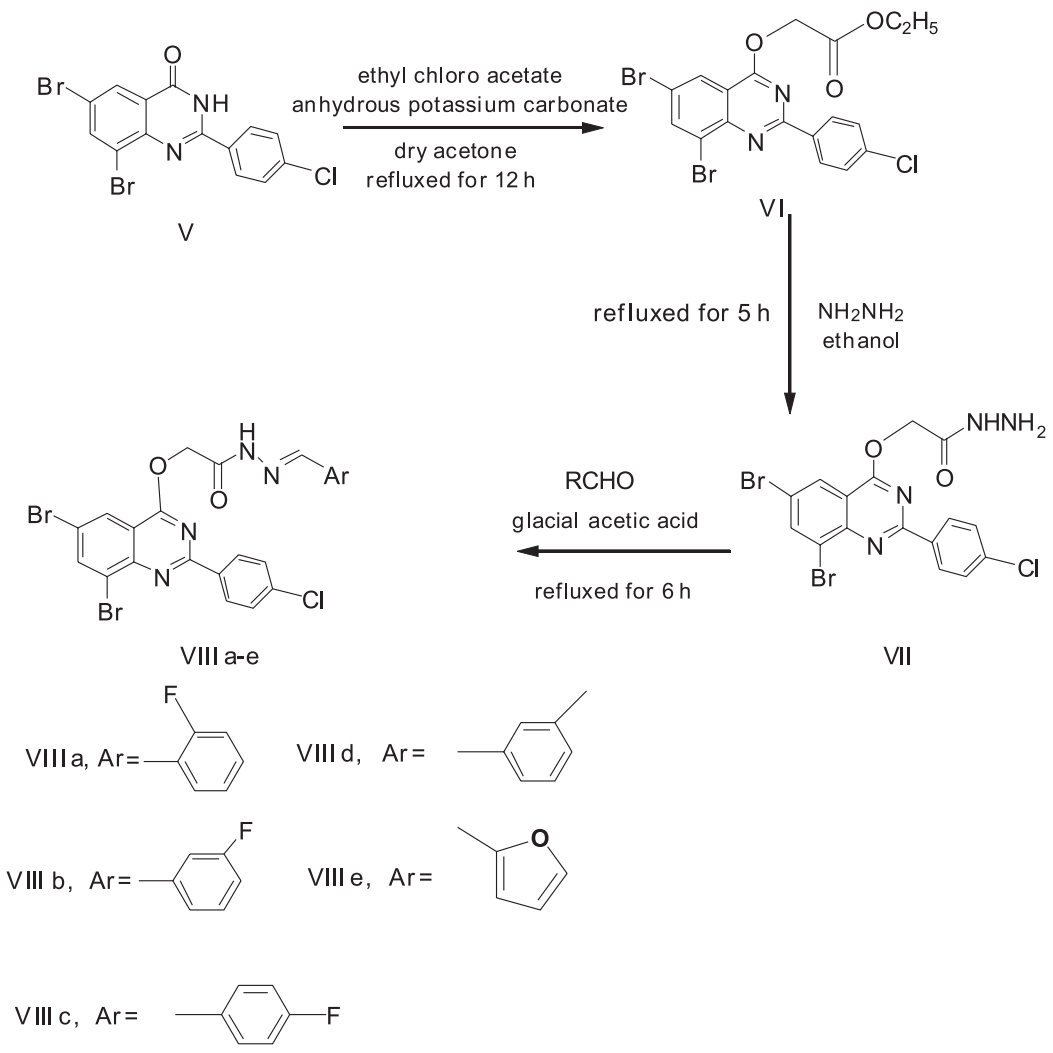
***In vitro* cytotoxicity**

Newly synthesized quinazoline derivatives were tested on MCF7, HCT116 and HEPG2 cancer cell lines for their cytotoxic activity using sulphorhodamine-B (SRB) assay method (27). Three cancer cell lines were obtained from the American Type Culture Collection (ATCC, Minnesota, U.S.A.). All Chemicals were purchased from Sigma Aldrich

Chemical Company. Seeding cells were counted and placed in each well of a 96-well cell culture plate at a concentration of 1000-2000 cells/well in a total volume of 100 µL. They were incubated with various concentrations (0, 6.25, 12.5, 25, 50, 100 µg/mL) of the tested compounds and doxorubicin in DMSO for 48 h (each repeated three times). 10% trichloro acetic acid was added to cells for 1 h at 4°C, washed with distilled water and stained for 10-30 min. at room temperature with 0.4% SRB, dissolved in 1% acetic acid. Acetic acid 1% was added to remove unbound dye till colorless drainage obtained. The plates were subjected to air drying, 24 h not exposed to UV. The dye was solubilized with 100 mm^3 of 10 mM Tris-base solution (PH 7.4) for 5 min on a shaker at 1600 rpm. Absorbance was measured at 545 nm with an ELISA microplate reader. IC_{50} values were calculated using sigmoidal concentration– response curve fitting models (Sigmaplot software).

EGFR TK inhibition assay

IC_{50} of all newly synthesized compounds were evaluated in vitro using Kinase-Glo Plus luminescence kinase assay kit for inhibition of EGFR tyrosine kinase as reported method (28). New synthesized compounds were dissolved in DMSO, added to reaction plates containing the EGFR tyrosine kinase in assay buffer [10 mM $MgCl_2$, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 1 mM ethylene glycol tetraacetic acid (EGTA), 0.02 mg/mL bovine serum albumin (BSA), 0.02% Brij35, 0.1 mM Na_3VO_4, 2 mM dithiothreitol (DTT), 1% DMSO]. A mixture of



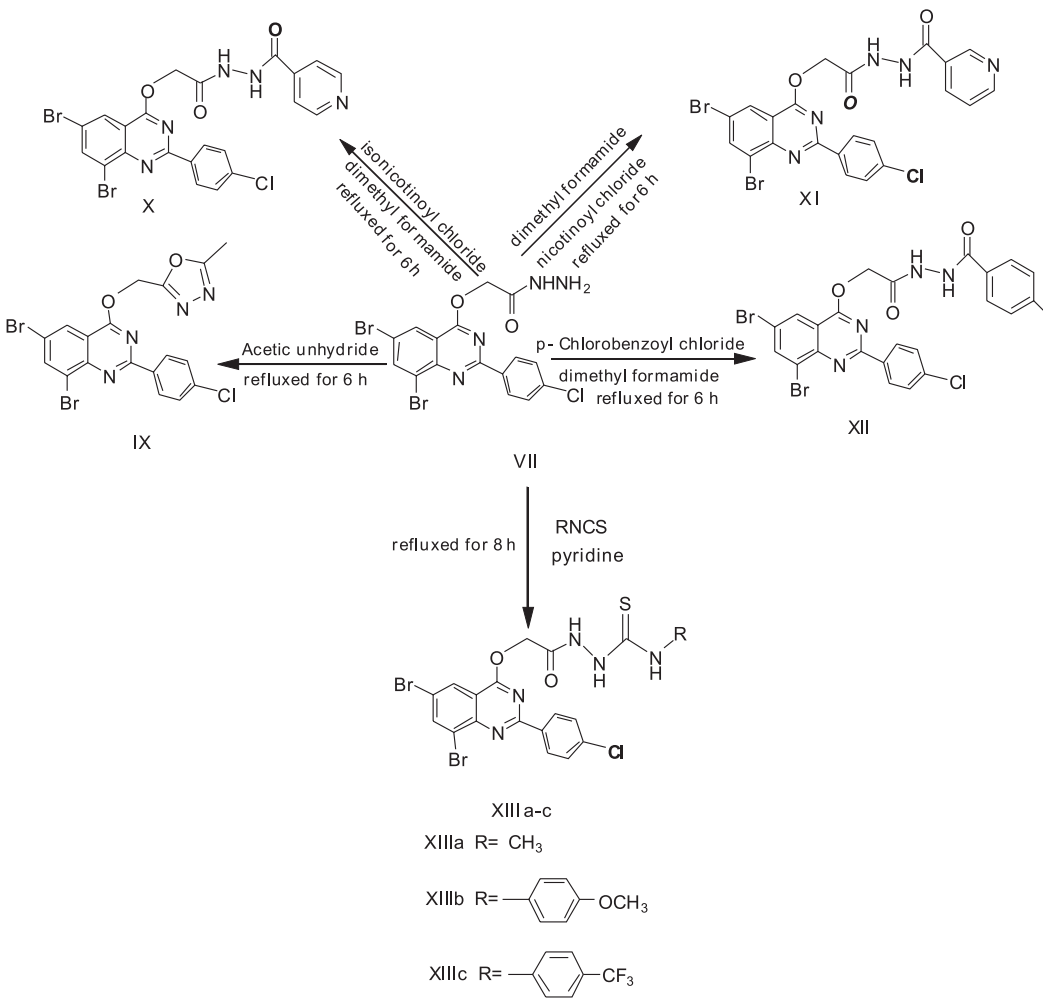
Scheme 2. Synthesis of compounds **VI –VIIIa-e**

ATP (Sigma, St. Louis MO) and 33P ATP (Perkin Elmer, Waltham MA) was added to a final concentration of 10 μM of the tested compounds. Reactions were carried out at room temperature for 2 h, followed by spotting of the reactions onto P81 ion exchange filter paper (Whatman Inc., Piscataway, NJ). Unbound phosphate was removed by washing of filters in 0.75% phosphoric acid. Results are presented in comparison to Erlotinib as a reference EGFR-TK inhibitor.

RESULTS AND DISCUSSION

Chemistry

Compounds I-V were prepared according to reported methods (26) (Scheme 1). The reaction of V with ethyl chloroacetate in the presence of anhydrous potassium carbonate gave compound VI. Hydrazide VII was obtained by refluxing compound VI with hydrazine hydrate (Scheme 2). Schiff bases VIIIa-e were prepared by reaction of VII with appropriate aldehyde namely 2-flurobenzaldehyde, 3- flurobenzaldehyde, 4- fluro benzaldehyde, 3-methylbenzaldehyde and/or furan-2-carboxaldehyde respectively (Scheme 2). 2-((6,8-dibromo-2-(4-chlorophenyl)quinazolin-4-yloxy)methyl)-5-methyl-1,3,4-oxadiazole IX were prepared by refluxing compound VII with acetic anhydride (Scheme 3). Compounds X, XI and XII were achieved by reaction of VII with isonicotinoyl chloride, nicotinoyl chloride or 4-chlorobenzoyl chloride in dimethyl formamide. The reaction of hydrazide VII with methyl isocyanate, 4-methoxy phenyl isothiocyanate and/or 4-trifluro methyl phenyl isothiocyanate in pyridine affords compounds XIIIa-c respectively (Scheme 3).



Scheme 3. Synthesis of compounds **IX-XIIIa-c**

Table 1. In vitro anticancer activity of all new compounds on breast (MCF-7), liver (HEPG-2) and colon (HCT116) cancer cell lines.

Comp. no.	MCF-7	HEPG-2	HCT116
	IC_{50} (µM ± S.D.)		
VI	28.27 ± 0.081	21.45 ± 0.072	34.74 ± 0.011
VII	17.80 ± 0.024	18.82 ± 0.011	15.88 ± 0.031
VIIIa	21.34 ± 0.027	3.32 ± 0.015	14.20 ± 0.035
VIIIb	12.63 ± 0.019	15.81 ± 0.012	23.52 ± 0.032
VIIIc	13.46 ± 0.018	9.12 ± 0.011	10.52 ± 0.012
VIIId	34.40 ± 0.045	6.82 ± 0.026	35.35 ± 0.032
VIIIe	51.18 ± 0.052	31.26 ± 0.141	38.77 ± 0.24
IX	35.76 ± 0.001	24.13 ± 0.214	21.91 ± 0.436
X	39.01 ± 0.021	14.96 ± 0.075	17.26 ± 0.011
XI	3.49 ± 0.023	18.49 ± 0.033	10.48 ± 0.033
XII	5.00 ± 0.042	14.32 ± 0.08	7.79 ± 0.010 1
XIIIa	5.11 ± 0.023	22.49 ± 0.011	6.02 ± 0.012
XIIIb	4.86 ± 0.012	12.24 ±0.089	7.35 ± 0.0116
XIIIc	9.06 ± 0.112	6.85 ± 0.210	8.88 ± 0.102
Dox.	8.46 ± 0.052	10.85 ± 0.035	8.83 ± 0.013

Table 2. IC_{50} EGFR inhibition of all new derivatives.

Comp. no.	EGFR IC_{50} µM
VI	18.44
VII	16.46
VIIIa	6.19
VIIIb	14.19
VIIIc	11.37
VIIId	7.90
VIIIe	19.87
IX	19.07
X	12.07
XI	16.43
XII	11.58
XIIIa	18.65
XIIIb	10.64
XIIIc	8.65
Erlotinib	2

Biological activity

In vitro cytotoxic activity

In this work, we synthesized a new series of quinazoline derivatives and they are tested for their cytotoxic activity on the breast (MCF7), liver (HEPG2) and colon (HCT116) cell lines, using sulphorhodamine-B assay method (24). IC_{50} values (the dose that causes 50% inhibition of viable cells) of the tested compounds were evaluated (Table 1). All compounds showed promising cytotoxic activity on the three cancer cell lines. Cytotoxic activity of the tested compounds against (MCF7) cancer cells showed that compounds XI, XII, XIIIa, XIIIb were more potent than doxorubicin, their IC_{50} were 3.4, 5.0, 5.1 and 4.8 µM respectively, compared with doxorubicin (8.46 µM). Compounds XI and XIIIb were two times more active than doxorubicin. Compounds VII, VIIIb, VIIIc and XIIIc showed potent cytotoxic activity with IC_{50} range 9.06 –17.8 µM. Compounds VI, VIIIa, VIIId, VIIIe, IX and X showed good cytotoxic activity with IC_{50} range 21.3–51.1 µM.

Cytotoxic activity of the tested compounds against (HEPG2) cancer cells showed that compounds VIIIa, VIIIc, VIIId and XIIIc exerted remarkable cytotoxic activity with IC_{50} 3.32, 9.12, 6.82 and 6.85 compared with doxorubicin (10.85 µM). Compound VIIIa was 3 times more active than doxorubicin. Compounds VII, VIIIb, X, XI, XII and XIIIb show potent cytotoxic activity with IC_{50} range 12.24 –18.82 µM. Compounds VI, VIIIe, IX and XIIIa showed good cytotoxic activity with IC_{50} range 21.4–31.2 µM.

On the other hand compounds XII, XIIIa, XIIIb and XIIIc were the best active on (HCT116)

cancer cells with IC_{50} 7.79, 6.02, 7.35 and 8.8 µM compared with doxorubicin (8.83µM).While compounds VII, VIIIa, VIIIc, X and XI show potent cytotoxic activity with IC_{50} range 10.48 –17.26 µM. Compounds VI, VIIIb, VIIId, VIIIe and IX showed good cytotoxic activity with IC_{50} range 21.9–38.7 µM.

Structure activity relationship

Substitution with N-(4-(trifluoromethyl)phenyl) hydrazinecarbothioamide acetyl oxy moiety at the same position afforded compound XIIIc with broad spectrum activity on MCF7, HCT116 and HEPG2 cell lines.

While substitution of quinazoline at position 4 with 4- chlorobenzohydrazide acetyl oxy, N-methylhydrazinecarbothioamide acetyl oxy or N-(4-methoxyphenyl)hydrazinecarbothioamide acetyl oxy moiety gave compounds XII, XIIIa and XIIIb with broad spectrum activity on MCF7 and HCT116 cell lines.

On the other hand substitution with nicotinohydrazide acetyl oxy moiety at position 4 afforded compound XI with high selectivity towards MCF7 cell line, Moreover substitution at the same position with N'-(2-fluorobenzylidene)acetohydrazide oxy, N'-(4-fluorobenzylidene)acetohydrazide oxy or N'-(3-methylbenzylidene)acetohydrazide oxy moiety afforded compounds VIIIa, VIIIc and VIIId respectively which had high selectivity on HEPG2 cell lines.

EGFR inhibition

In this work, all synthesized compounds were screened for their IC_{50} inhibitory activity against EGFR-TK. IC50 range of the tested compounds is 6.19-19.87 µM. Interestingly, the cytotoxic properties of these newly synthesized compounds showed significant correlation with their EGFR inhibitory activity (Table 2).

CONCLUSION

New series of 4-substituted quinazoline derivatives were synthesized and tested for their cytotoxic activity *in vitro* against MCF7, HEPG2 and HCT116 cell lines.

Most of the tested compounds showed potent cytotoxic activity. Compound XI and XIIIb were two more than potent doxorubicin on MCF7 cancer cell line. Compound VIIIa was more active than doxorubicin on HEPG2 cancer cell line. Compounds XII, XIIIa and XIIIb were more potent than doxorubicin on HCT116 cancer cell line. All compounds were tested for their inhibitory activity against EGFR-TK and show good results. The present work led to the discovery of new cytotoxic compounds having quinazoline pharmacophore.

Acknowledgement

This work was financially supported by The Deanship of Scientific Research, Taif University, Kingdom of Saudi Arabia, grant no. 1-437-4782.

REFERENCES

1. Scheurle D., Jahanzeb M., Aronsohn R.S., Watzek L., Narayanan R.H.: Anticancer Res. 20, 2091 (2000).
2. Cox G., Vyberg M., Melgaard B., Askaa J., Oster A., O'Byrne K. J.: Int. J. Cancer. 92, 480 (2001).
3. Gullick W.: J. Med. Bull. 47, 87 (1991).
4. https://www.cancer.org/ research/cancer-facts-statistics/all-cancer-facts-figures/ cancer-facts-figures-2017.html (accessed on 10. 02. 2017).
5. Moscatello D.K., Holgado-Mudruga M., Godwin A.K., Ramirez G., Gunn G. et al.: Cancer Res. 55, 5536 (1995).
6. Gilman A.: J. Surg. 105, 574 (1963).
7. Kohn K.W.: Cancer Res. 56, 5533 (1996).
8. Bridges A.J., Zhou H., Cody D.R., Rewcastle G.W., McMichael A. et al: J. Med. Chem. 39, 267 (1996).
9. Lüth A., Löwe W.: Eur. J. Med. Chem. 43, 1478 (2008).
10. Kris M.G., Natale R.B., Herbst R.S., Lynch T.J., Prager D. et al.: J. Am. Med. Assoc. 290, 2149 (2003).
11. Kobayashi S., Boggon T.J., Dayaram T., Janne P.A., Kocher O. et al.: Engl. J. Med. 352, 786 (2005)
11. Khodarahmi G., Asadi P., Hassanzadeh F., Khodarahmi E.: J. Res. Med. Sci. 20, 1094 (2015).
12. Asadi P., Khodarahmi G., Farrokhpour H., Hassanzadeh F., Saghaei L.: Res. Pharm. Sci. 12, 233 (2017).
13. Asadi P., Khodarahmi G., Jahanian-Najafabadi A., Saghaie L., Hassanzadeh F.: Chem. Biodiversity 14, e1600411 (2017).
14. Fry D.W., Kraker A.J., McMichael A., Ambroso L.A., Nelson J.M. et al.: J. Science. 265, 1093 (1994).
15. Ward W.H.J., Cook P.N., Slater A.M., Davies D.H., Holdgate G.A., Green L.R.: Biochem. Pharmacol. 48, 659 (1994).

16. Discafani C.M., Carroll M.L., Floyd M.B.Jr., Hollander I.J., Husain Z. et al.: Biochem. Pharmacol. 57, 917 (1999).
17. Ahmed M.F., Youns, M.: Arch. Pharm. Chem. Life Sci. 346, 610 (2013).
18. Ahmed M.F., Belal A.: Res. Chem. Intermed., 42, 659 (2016).
19. Ahmed M.F., Hashim A.A.: Res. Chem. Intermed. 42, 1777 (2016).
20. Fry D.W., Bridges A.J., Denny W.A., Doherty A., Gries K.D. et al.: Proc. Natl. Acad. Sci. U.S.A. 95, 12022 (1998).
21. Lv P.C., Li H.Q., Sun J., Zhou Y., Zhu H.L.: Bioorg. Med. Chem. 18, 4606 (2010).
22. Li H.Q., Yan T., Yang Y., Shi L., Zhou C.F., Zhu H. L.: Bioorg. Med. Chem. 18, 305 (2010).
23. Ahmed M.F., Belal A., Youns M.: Med. Chem. Res. 24, 2993 (2015).
24. Ahmed M.F., Belal A.: Arch. Pharm. Chem. Life Sci. 348, 487 (2015).
25. Mphahlele M.J., Paumo H.K., El-Nahas A.M, El-Hendawy M.M.: Molecules 19, 795 (2014).
26. Skehan P., Storeng R., Scudiero D., Monks A., McMahon I. et al.: J. Nat. Cancer Inst. 82, 1107 (1990).
27. Ma H., Deacon S., Horiuchi K.: Expert Opin Drug Discov. 3, 607 (2008).

Received: 20. 06. 2017