Synthesis of some quinazolinones inspired from the natural alkaloid L-norephedrine as EGFR inhibitors and radiosensitizers

Mostafa M. Ghorab, Maged S. Abdel-Kader, Ali S. Alqahtani, and Aiten M. Soliman

Department of Drug Radiation Research, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Nasr City P.O. Box 29, Cairo 11765, Egypt

ABSTRACT
A set of quinazolinones synthesized by the aid of L-norephedrine was assembled to generate novel analogues as potential anticancer and radiosensitizing agents. The new compounds were evaluated for their cytotoxicity against MDA-MB-231, MCF-7, HepG-2, HCT-116 cancer cell lines and EGFR inhibitory activity. The most active compounds 5 and 6 were screened against MCF-10A normal cell line and displayed lower toxic effects. They proved their relative safety with high selectivity towards MDA-MB-231 breast cancer cell line. Measurement of the radiosensitizing activity for 5 and 6 revealed that they could sensitize the tumour cells after being exposed to a single dose of 8 Gy gamma radiation. Compound 5 was able to induce apoptosis and arrest the cell cycle at the G2-M phase. Molecular docking of 5 and 6 in the active site of EGFR was performed to gain insight into the binding interactions with the key amino acids.

1. Introduction
Cancer is characterized by the disturbance of normal cellular processes required for cell growth, division and differentiation. Surgery, radiotherapy and chemotherapy, including immunotherapy, targeted and combined therapy, are different strategies advocated for cancer treatment. Protein kinases (PKs) play a pivotal role in cell proliferation by controlling signal transduction through the phosphorylation of different amino acid residues, namely tyrosine, threonine and serine. Tyrosine kinases (TKs) are divided into receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs) in human genome. RTKs are vital components of cellular signaling pathways that are active during embryonic development and adult homeostasis. Due to their role as growth factor receptors, many RTKs have been involved in the onset or progression of various cancers, either by mutations or receptor/ligand overexpression; thus, they are considered attractive candidates for therapeutic intervention. An example of RTK family members is epidermal growth factor receptor (EGFR). EGFR is a member of the ErB receptor family and plays an essential role in cell signaling. Signaling is initiated by binding ligands to the extracellular domain of the EGFR, activating kinases and promoting cancer cell survival, invasiveness and drug resistance. EGFR has a critical role in regulating several cellular functions such as cell growth, proliferation, differentiation and apoptosis, leading to the development of several types of solid tumors. EGFR (HER-1) and ERB-B2 (HER-2) are characterized in solid tumors as breast, ovary, lung and others. The inhibition of EGFR is classified as targeted therapy as it aims at the differences between cancer and normal cells and is characterized by its high selectivity and lowered side effects.

Quinazolines are fused heterocyclic ring systems known for their variable biological activity. They are well known for their inhibitory activity towards various protein kinase enzymes and their anticancer activity. For example, lapatinib, a dual reversible EGFR and HER2 inhibitor. Also, gefitinib and erlotinib are reversible EGFR inhibitors; they are examples of FDA approved small molecules TK inhibitors. Methaqualone, a potent hypnotic, was considered as an important landmark in synthetic anticonvulsants. The 3-[β-keto-gamma-(3-hydroxy-2-piperidyl)-propyl]-4-quinazolone (A) was the first isolated natural quinazolinone alkaloid known by its antimalarial activity. The quinazoline derivatives (B) and benzo[g]quinazoline (C) were reported to possess potent EGFR and HER2 inhibitory activity Figure 1. On the other hand, the Ephedra alkaloid, Norephedrine (NE) is a stereoisomer of phenylpropanolamine that is naturally occurring sympathomimetic. Investigation revealed that long-term use of NE caused severe side effects, including fatality. In addition to medicinal use, the properties of this alkaloid have attracted considerable attention in natural product chemistry field that leads to its use as a starting material in the preparation of chiral ligands for asymmetric catalytic synthesis.

In continuation of our studies aiming to find new leads with potential anticancer activities, various substituted quinazolinones have been designed to accommodate different electronic natures...
as heterocycles representing the primary scaffold in many cytotoxic agents hoping to develop potent and safe anticancer agents and EGFR inhibitors. All the synthesized compounds were screened against MDA-MB-231, MCF-7, HepG-2, HCT-116 cancer cell lines and the most potent compounds were evaluated against MCF-10A normal cells to determine the selectivity of the compounds on the different cell lines. Also, the in vitro EGFR inhibitory activity of the compounds was measured. The effect of the most potent compounds on cell cycle progression and the radiosensitizing activity were evaluated. Docking studies were carried out to allow us to design our target compounds.

2. Materials and methods

2.1. Chemistry

Melting points were determined uncorrected by a Gallen Kamp melting point apparatus (Sanyo Gallen Kamp, UK). Precoted silica gel plates (Kieselgel 0.25 mm, 60 F254, Merck, Germany) were used for TLC with solvent system of chloroform/methanol (8:2), spots were detected by UV light. IR spectra (KBr discs) were recorded for TLC with solvent system of chloroform/methanol (8:2), spots were detected by UV light. IR spectra (KBr discs) were recorded for TLC with solvent system of chloroform/methanol (8:2), spots were detected by UV light.

2.1.1. Methyl 2-(3-(1-hydroxy-1-phenylpropan-2-yl)thioureido)benzoate (3) & 3-(1-hydroxy-1-phenylpropan-2-yl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (4)

Methyl 2-isothiocyanatobenzoate 1 (0.193 g, 0.001 mol) was allowed to react with L-norephedrine (2-amino-1-phenylpropan-1-ol) (0.151 g, 0.001 mol) 2 in NMR tube in CDCl3 and measured immediately for 1H and 13C NMR to give 3. When the reaction was carried out in the presence of chloroform containing a catalytic amount of triethylamine the reaction gave 4 instead of 3 at room temperature. The product 4 was crystallized from ethanol. Derivative 3 was rapidly converted to 4 at room temperature.

3: 1H NMR (500 MHz, CDCl3): 0.99 (d, J = 6.0 Hz, 3H, CH3 of L-norephedrine), 2.87 (bs, 1H, NH), 3.83 (s, 3H, O–CH3), 4.81 (bs, 1H, N–CH), 5.13 (bs, 1H, O–CH), 6.64 (d, J = 6.8 Hz, 1H, OH), 7.05–7.93 (m, 9 aromatic), 10.31 (bs, 1H, NH). 13C NMR (126 MHz, CDCl3): 13.30 (CH3), 55.76 (O–CH3), 75.20 (N–CH), 118.80, 122.74, 123.69, 125.06, 126.92 (2), 128.35 (2), 131.44, 133.31, 142.37, 146.04, 157.15, 161.09. MS m/z (%): 296 (M+) (1.96), 179 (100).

4: Yield, 83%; m.p. 80.3°C. IR (KBr, cm⁻¹): 3445 (OH), 3244 (NH), 3088 (arom.), 2970, 2865 (aliph.), 1691 (CO), 1277 (CS). 1H NMR (500 MHz, CDCl3): 0.99 (d, J = 6.0 Hz, 3H, CH3 of L-norephedrine), 3.83 (s, 3H, O–CH3), 4.81 (bs, 1H, N–CH), 5.13 (bs, 1H, O–CH), 6.64 (d, J = 6.8 Hz, 1H, OH), 7.05–7.93 (m, 9 aromatic), 10.31 (bs, 1H, NH). 13C NMR (126 MHz, CDCl3): 13.30 (CH3), 55.76 (O–CH3), 75.20 (N–CH), 118.80, 122.74, 123.69, 125.06, 126.92 (2), 128.35 (2), 131.44, 133.31, 142.37, 146.04, 157.15, 161.09. MS m/z (%): 344 (M+) (1.96), 179 (100).

2.1.2. 3-(1-Hydroxy-1-phenylpropan-2-yl)-2-(methylthio)quinazolin-4(3H)-one (5)

A mixture of 4 (0.312 g, 0.001 mol) and methyl iodide (0.151 g, 0.001 mol) in dry acetonitrile (30 mL) containing K2CO3 was refluxed at room temperature. The product 5 was crystallized from ethanol.

5: Yield, 78%; m.p. 121.5°C. IR (KBr, cm⁻¹): 3450 (OH), 3244 (NH), 3088 (arom.), 2970, 2865 (aliph.), 1691 (CO), 1277 (CS). 1H NMR (500 MHz, CDCl3): 0.99 (d, J = 6.0 Hz, 3H, CH3 of L-norephedrine), 3.83 (s, 3H, O–CH3), 4.81 (bs, 1H, N–CH), 5.13 (bs, 1H, O–CH), 6.64 (d, J = 6.8 Hz, 1H, OH), 7.05–7.93 (m, 9 aromatic), 10.31 (bs, 1H, NH). 13C NMR (126 MHz, CDCl3): δ 13.30 (CH3), 55.76 (O–CH3), 75.20 (N–CH), 118.80, 122.74, 123.69, 125.06, 126.92 (2), 128.35 (2), 131.44, 133.31, 142.37, 146.04, 157.15, 161.09. MS m/z (%): 312 (M+) (10), 78 (100).

2.1.3. 3-(1-Hydroxy-1-phenylpropan-2-yl)-2-(methylthio)quinazolin-4(3H)-one (5)

A mixture of 4 (0.312 g, 0.001 mol) and methyl iodide (0.141 g, 0.001 mol) in dry acetonitrile (30 mL) containing K2CO3 was refluxed for 12 h. The obtained solid was crystallized from ethanol to give 5.

5: Yield, 78%; m.p. 121.5°C. IR (KBr, cm⁻¹): 3450 (OH), 3244 (NH), 3088 (arom.), 2970, 2871 (aliph.), 1691 (CO), 1277 (CS). 1H NMR (500 MHz, CDCl3): δ 1.71 (d, J = 6.5 Hz, 3H, CH3 of L-norephedrine), 2.47 (s, 3H, S–CH3), 4.45 (bt, 1H, N–CH), 5.57 (bs, 1H, O–CH), 5.87 (bs, 1H, O–CH), 6.78 (bs, 1H, OH), 7.10–7.38 (m, 7 aromatic), 7.69 (t, J = 6.5 Hz, 1H), 8.09 (d, J = 7.0 Hz, 1H). 13C NMR (126 MHz, CDCl3): δ 14.62 (CH3), 15.37 (S–CH3), 61.98 (N–CH), 72.80 (O–CH), 119.34, 125.51, 125.76, 126.07, 126.36 (2), 127.42, 127.58 (2), 134.45, 142.16, 146.04, 157.15, 161.09. MS m/z (%): 296 (M+−2CH3) (12), 180 (100).
7.37 from methanol. Fractions 10
from 5% methanol in chloroform afforded
134.72, 148.76, 154.84, 159.86. MS
124.48, 125.84, 126.14, 126.32 (2), 128.52 (2), 128.69, 133.66,
134.72, 148.76, 154.84, 159.86. MS m/z (%): 193 (M+) (95),
162 (100). Anal. Calc. For C_{17}H_{15}NO_5S (309): C, 66.00; H, 4.89; N, 13.58.
Found: C, 66.31; H, 5.16; N, 13.89.

11: Yield, 23%; m.p. 79.5°C (IR (KBr, cm\(^{-1}\)): 3460 (OH), 3391,
3132, 3215 (NH_2, NH), 3072 (arom.), 2948, 2815 (aliph.), 1678 (CO),
1680 (CN). MS 134.72, 148.76, 154.84, 159.86. MS m/z (%): 193 (M+) (95),
162 (100). Anal. Calc. For C_{17}H_{15}NO_5S (309): C, 66.00; H, 4.89; N, 13.58.
Found: C, 66.31; H, 5.16; N, 13.89.

11: Yield, 23%; m.p. 79.5°C (IR (KBr, cm\(^{-1}\)): 3460 (OH), 3391,
3132, 3215 (NH_2, NH), 3072 (arom.), 2948, 2815 (aliph.), 1678 (CO),
1680 (CN). MS 134.72, 148.76, 154.84, 159.86. MS m/z (%): 193 (M+) (95),
162 (100). Anal. Calc. For C_{17}H_{15}NO_5S (309): C, 66.00; H, 4.89; N, 13.58.
Found: C, 66.31; H, 5.16; N, 13.89.

11: Yield, 23%; m.p. 79.5°C (IR (KBr, cm\(^{-1}\)): 3460 (OH), 3391,
3132, 3215 (NH_2, NH), 3072 (arom.), 2948, 2815 (aliph.), 1678 (CO),
1680 (CN). MS 134.72, 148.76, 154.84, 159.86. MS m/z (%): 193 (M+) (95),
162 (100). Anal. Calc. For C_{17}H_{15}NO_5S (309): C, 66.00; H, 4.89; N, 13.58.
Found: C, 66.31; H, 5.16; N, 13.89.

11: Yield, 23%; m.p. 79.5°C (IR (KBr, cm\(^{-1}\)): 3460 (OH), 3391,
3132, 3215 (NH_2, NH), 3072 (arom.), 2948, 2815 (aliph.), 1678 (CO),
1680 (CN). MS 134.72, 148.76, 154.84, 159.86. MS m/z (%): 193 (M+) (95),
162 (100). Anal. Calc. For C_{17}H_{15}NO_5S (309): C, 66.00; H, 4.89; N, 13.58.
Found: C, 66.31; H, 5.16; N, 13.89.

11: Yield, 23%; m.p. 79.5°C (IR (KBr, cm\(^{-1}\)): 3460 (OH), 3391,
3132, 3215 (NH_2, NH), 3072 (arom.), 2948, 2815 (aliph.), 1678 (CO),
1680 (CN). MS 134.72, 148.76, 154.84, 159.86. MS m/z (%): 193 (M+) (95),
162 (100). Anal. Calc. For C_{17}H_{15}NO_5S (309): C, 66.00; H, 4.89; N, 13.58.
Found: C, 66.31; H, 5.16; N, 13.89.
2.1.8. 3-Methyl-2-phenyl-2H-oxazolo[2,3-b]quinazolin-5(3H)-one (15), 2,4-dimethyl-1,3-diphenyl-3,4-dihydro-2a,4a,9b-triazapentaleno[1,6-ab]napthalen-5(2aH)-one (16), 8,18-dimethyl-7,17-diphenyl-7,18-tetrahydro-1,6,3,8-dioxadiazecino[2,3-b:7,8-b’]quinazoline-10,20-dione (17) and 3-(1-hydroxy-1-phenylpropan-2-yl)-2-(1-hydroxy-1-phenylpropan-2-ylamino)quinazolin-4(3H)-one (18)

To a solution of 5 (0.326 g, 0.001 mol) in DMF (20 mL) containing K₂CO₃ (0.138 g, 0.001 mol), L-norephedrine 2 (0.151 g, 0.001 mol) was added and refluxed for 10 h. The reaction mixture progress was monitored by TLC. It showed the presence of four products 15, 16, 17 and 18 that were separated by silica gel column chromatography (45: C₂₂ i.d. cm, 40 gm) eluting with chloroform, followed by chloroform/methanol mixtures in a gradient system. Fractions 3–8 eluted with chloroform afforded 15 (111 mg) after crystallization from methanol. Fractions 11–12 eluted with chloroform afforded 17 (56 mg) after crystallization from methanol. Fractions 16–18 eluted with 2% methanol in chloroform afforded 16 (47 mg) after crystallization from methanol. Fractions 27–29 eluted with 5% methanol in chloroform afforded 18 (34 mg) after crystallization from methanol.

15: Yield, 40%; m.p. 136.6°C. IR (KBr, cm⁻¹): 3048 (arom.), 2970, 2816 (aliph.), 1690 (CO), 1621 (CN). ¹H NMR (500 MHz, DMSO-d₆): δ 0.95 (d, J = 6.7 Hz, 3H, CH₃ of L-norephedrine), 7.35–8.08 (m, 9H). ¹³C NMR (126 MHz, DMSO-d₆): δ 14.45 (CH₃), 77 (100). Anal. Calcd. For C₁₇H₁₄N₂O₂ (278): C, 73.37; H, 5.07; N, 10.07. Found: C, 73.65; H, 5.32; N, 10.32.

16: Yield, 12%; m.p. >350°C. IR (KBr, cm⁻¹): 3077 (arom.), 2927, 2846 (aliph.), 1693 (CO). ¹H NMR (500 MHz, DMSO-d₆): δ 1.23 (d, J = 11.5 Hz, 3H, CH₃ of L-norephedrine), 12.39 (d, J = 12.0 Hz, 3H, CH₃ of L-norephedrine), 4.73 (d, J = 11.0 Hz, 1H), 4.79 (d, J = 12.0 Hz, 1H), 113.76, 114.90, 115.49, 123.07, 127.69, 127.90, 128.41 (2), 129.17, 135.55, 135.63, 139.89, 140.12, 140.84, 150.24, 151.33, 162.77, 163.12. MS m/z (%): 278 (M⁺) (44), 235 (100). Anal. Calcd. For C₂₆H₂₄N₃O (394): C, 79.16; H, 6.13; N, 10.65. Found: C, 79.51; H, 6.35; N, 10.89.
Yield, 10%; semisolid. IR (KBr, cm\(^{-1}\)): 3101 (arom.), 2933, 2818 (aliph.), 1690 (2CO), 1622 (2CN). \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 0.60 (d, \(J = 6.2\) Hz, 6H, 2CH\(_3\) of L-norephedrine), 4.16 (P, \(J = 1.0\) Hz, 2H, 2 (N–CH)), 5.68 (d, \(J = 8.5\) Hz, 2H, 2 (O–CH)), 7.12–7.87 (m, 18H). \(^13\)C NMR (126 MHz, DMSO-\(d_6\)): \(\delta\) 13.74 (2), 53.02 (2) (N–CH), 79.94 (2) (O–CH), 113.57 (2), 123.47 (2), 126.53 (2), 127.99 (2), 128.58 (4), 128.81 (4), 132.72 (2), 136.34 (2), 139.62 (2), 149.71 (2), 158.88 (2), 161.80 (2). MS m/z (%): 556 (M\(^+\)) (13), 278 (100). Anal. Calcd. For C\(_{34}\)H\(_{28}\)N\(_4\)O\(_4\) (556): C, 73.37; H, 5.07; N, 10.07. Found: C, 73.08; H, 4.89; N, 9.11.

18: Yield, 8%; m.p. 88.8°C. IR (KBr, cm\(^{-1}\)): 3450 (2OH), 3321(NH), 2976, 2823 (aliph.), 1680 (CO), 1618 (CN). \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 1.58 (d, \(J = 6.6\) Hz, 6H, 2 (CH\(_3\)) of L-norephedrine), 4.15, 4.40 (bs, 1NH), 5.10 (bs, \(J = 11.5\) Hz, 2H, 2 (N–CH)), 5.24 (m, 2H, 2 (O–CH)), 5.62 (d, \(J = 5.3\) Hz, 2H, 2 (OH)), 7.01–7.55 (m, 14H, aromatic). \(^13\)C NMR (126 MHz, DMSO-\(d_6\)): \(\delta\) 15.31 (CH\(_3\)) (2), 56.53 (N–CH) (2), 74.05 (2), 115.14, 122.80 (2), 126.96, 127.63, 128.03, 128.30 (4), 128.65 (4), 135.28, 139.58 (2), 143.50, 150.47, 162.45. MS m/z: 430 (M\(^+\) + 1) (100). Anal. Calcd. For C\(_{26}\)H\(_{27}\)N\(_3\)O\(_3\) (429): C, 72.71; H, 6.34; N, 9.78. Found: C, 72.46; H, 6.08; N, 9.40.

### 2.2. Biological evaluation

#### 2.2.1. MTT assay

MDA-MB-231, MCF-7, HepG-2, HCT-116 cancer cell lines and MCF-10A normal cells were obtained from American Type Culture Collection. The 96-well plate was incubated for 24 h before the MTT assay. The cell layer was washed with 0.25% (w/v) Trypsin solution. Cells were cultured using DMEM supplemented with 10% foetal bovine serum, 10\(\mu\)g/mL insulin and 1% penicillin–streptomycin. Reconstituted MTT (10%) was added and incubated for 2h. Formazan crystals were dissolved by the MTT solubilizing solution after incubation. Absorbance was measured at a wavelength of 570 nm. \(\text{IC}_{50}\) was estimated according to the equation of Boltzmann sigmoidal concentration–response curve and compared to erlotinib and staurosporine.
2.2.2. EGFR assay
EGFR kinase kit (0.192 mg/mL) was obtained from Invitrogen. An ATP solution and a kinase/peptide mixture were developed just before use. The solution on the plate was mixed carefully and incubated for 1 h at 25°C. Then, 5 mL of the prepared solution was added to each well. The plate was incubated for 1 h and determined by an ELISA Reader (PerkinElmer, USA). Curve fitting using Graph Pad Prism 5 was constructed. Each experiment was repeated three times. IC₅₀ was represented as means ± SE.

2.2.3. Radiosensitizing evaluation
Irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, using Gamma cell-40 (¹³⁷Cs) source. The promising compounds 5 and 6 were selected to be re-evaluated for in vitro cytotoxic activity after the cells containing the compounds were gamma-irradiated at a dose level of 8 Gy with a dose rate of 0.758 rad/s for 17.59 min. Cytotoxicity was measured two days after irradiation. The IC₅₀ of the tested compounds is calculated using GraphPad Prism 5.

2.2.4. Cell cycle analysis
The MDA-MB-231 cells (10⁵/well) were incubated with compound 5 at its IC₅₀. After 24 h, the cells were washed twice with PBS, then collected and fixed with ice-cold ethanol 70% (v/v). The cells were re-suspended with 0.1 mg/mL RNase, stained with 40 mg/mL RNase, and analyzed by flow cytometry.

Scheme 3. Synthesis of compounds 9–11.
PI and examined using flow cytometry (FACScalibur-Becton Dickinson).

2.2.5. Apoptotic assay
Cells were prepared as previously mentioned. Treatment of cells \(10^5\) with Annexin V-FITC and propidium iodide (PI) was by apoptosis detection kit [BD Biosciences, San Jose, CA]. The binding of Annexin V-FITC and PI was examined using flow cytometry FACScalibur (BD Biosciences, San Jose, CA). CellQuest software was used for performing quadrant analysis of co-ordinate dot plots.

2.3. Molecular docking
Docking studies were performed using Molecular Operating Environment software (MOE, 2015.10) provided by chemical computing group, Canada. The software was used to carry out the docking of the promising compounds in the receptor’s active site. The protein crystal structure was obtained from the Protein Databank, PDB: 1M17 containing the EGFR enzyme co-crystallized with erlotinib. All the water molecules were removed, 3D protonation was performed. The pocket was determined by the alpha triangle matcher technique. The Energy Minimization was performed using MMFF94X force field with RMSD gradient of 0.001 kcal mol\(^{-1}\)Å\(^{-1}\) and the partial charges were calculated. The co-crystalized ligand was self-docked inside the active site. The compounds to be docked were drawn on ChemBioOffice 12 and copied as smiles to MOE followed by docking of 5 and 6.

3. Results and discussion
3.1. Chemistry
The behaviour of the methyl 2-isothiocyanatobenzoate 1 towards the natural alkaloid L-norephedrine 2 was studied. When the isothiocyanate 1 reacted with 2 in chloroform containing a catalytic amount of triethylamine (TEA) at room temperature the unexpected quinazolinone 4 was formed despite the prospective thiourea derivative 3. In a trial to obtain the open-chain thiourea derivative 3, NMR tube reaction was carried out between 1 and 2. The structure of 3 was confirmed by the presence of O–CH\(_3\) singlet signal at \(\delta_H\) 3.83; \(\delta_C\) 55.76 ppm in the \(^1H\) and \(^13C\) NMR, ester carbonyl at \(\delta_C\) 167.96 ppm, C=S at \(\delta_C\) 179.68 and the two NH singlets at \(\delta_H\) 2.87 and 10.31 ppm. The M\(^+\) at 344 \(m/z\) confirmed the structure of 3. While in NMR data of 4 the O–CH\(_3\), as well as one NH signal disappeared. The ester carbonyl signal in the \(^13C\) NMR of 3 was replaced by amide carbonyl at \(\delta_C\) 159.57 ppm in 4 while C=S appeared at \(\delta_C\) 176.01 ppm. Methylation of 4 by methyl iodide (MeI) in dry acetone in the presence of anhydrous K\(_2\)CO\(_3\) gave the corresponding 2-(methyl thio)quinazolin-4(3H)-one derivative 5. Both \(^1H\) and \(^13C\) NMR supported the structure of 5 by the appearance of S–CH\(_3\) signals at \(\delta_H\) 2.47; \(\delta_C\) 15.37 ppm. Moreover, the C=S signal at \(\delta_C\) 161.09 ppm in 4 was replaced by C=N signal at \(\delta_C\) 161.09 ppm in 5. On the other hand, the reaction of 1 with hydrazine hydrate afforded the reported quinazolinone derivative 6\(^26\), which was further reacted with MeI to give 7. The structure of 6 showed NH proton singlet at \(\delta_H\) 7.99 and carbon C=S signal at \(\delta_C\) 169.27 ppm. However, in 7 the C=S signal was replaced by C=N signal at \(\delta_C\) 160.94 ppm. The NMR data of 7 also showed the S–CH\(_3\) signals at \(\delta_H\) 2.44; \(\delta_C\) 14.09 ppm. Both \(^1H\) NMR of 6 and 7 showed signals for NH\(_2\) at \(\delta_H\) 6.39 and 5.77 ppm, respectively. The
MS spectra of 6 and 7 at 193 and 207 m/z, respectively were in full support of the proposed structures (Scheme 1).

When 1 reacted with 2 in DMF in the presence of a few drops of TEA yielded the thiazoloquinazolinone 8 rather than the expected 4 (Scheme 2, see Supplementary data 1). The structure of 8 was confirmed by different spectroscopic data and X-ray crystallographic analysis is displayed in Figure 2.

In Scheme 3, the interaction of 3 with hydrazine hydrate in absolute EtOH gives the expected product 11 in addition to two other derivatives 9 and 10 that were apparent in TLC. The structure of 8 was confirmed by different spectroscopic data and X-ray crystallographic analysis is displayed in Figure 2.

In Scheme 4, the reaction of 5 with 2 in DMF containing K2CO3 afforded the expected product 18 in addition to three other products 15, 16 and 17. The mechanism of formation of 15, 16 and 17 was explained in Figure 3. The 1H and 13C NMR data of 9 and 10 have common features like NH doublet at δH 8.01 and 8.12, O=C=N signal at δC 159.86 and 161.10 ppm, respectively. Both 9 and 10 showed signal at δC 154.84 and 152.85 ppm assigned for C=N carbons attached to another hetero atom, respectively. Compound 9 keeps the CH–O signals at δH 4.91 (bs); δC 73.43 ppm indicated a non-substituted OH group. All these data proved that 11 lack the ring structure present in 9 and 10. The M⁺ at 310 m/z further confirmed the formation of 11.

Treatment of 7 with 2 in EtOH 95%, in the presence of K2CO3 afforded a mixture of 12 and 13. While when the same reaction was repeated in DMF instead of EtOH the unexpected dimer 14 was formed. However, both reactions were expected to give 11 (Scheme 4). The formation of 12 and 13 was assumed to proceed via addition–elimination mechanisms, as depicted in Figure 4. The NMR data structure of 12 and 13 indicated the disappearance of the S–CH3 signals present in 7. 1H NMR spectrum of 12 showed NH2 signal at δH 5.50 and OH signal at δH 11.62 ppm. The mass spectrum of 12 showed an M⁺ at 177 m/z provided further evidence for replacing the S–CH3 with OH group. In 13 signals for an ethoxy group at δH 1.39 (t, J = 7.0, CH3), δC 14.61 ppm and δH 4.49 (q, J = 7.0, CH2), δC 64.79 ppm along with M⁺ at 205 m/z, besides the disappearance of the S–CH3 signals present in 7. The data of 14 indicated the replacement of NH2 signal by NH at δH 3.42 ppm. However, the MS data showed an M⁺ at 318 m/z, noting that 14 is formed via dimerization of 7, as shown in Scheme 4.

In Scheme 5, the reaction of 5 with 2 in DMF containing K2CO3 afforded the expected product 18 in addition to three other products 15, 16 and 17.
17 is present in Figures 5–7. The NMR data of 15 indicated the disappearance of the S–CH₃ signals present in 5 and the downfield shift of the CH–O signals from δₜ 5.57, δₜ 72.80 ppm in 5 to δₜ 6.18, δₜ 82.37 ppm in 15. These data were diagnostic for self-cyclization of 5–15 and were further supported by the mass data that showed M⁺ at 278 m/z. The reaction between one molecule of 5 and 2 according to Figure 6 resulted in the formation of the unique structure of 16. The ¹H and ¹³C NMR data of 16 indicated the presence of four CH–X groups at δₜ 4.73 (d, J = 11.0), δₜ 53.43; δₜ 4.79 (d, J = 12.0), δₜ 52.99, δₜ 5.33 (p, J = 7.0), δₜ 52.64 and δₜ 5.46 (p, J = 7.2), δₜ 51.18 ppm. The chemical shift indicated that none of the heteroatoms is oxygen, the formation of the complex ring structure involved water elimination and the presence of two methyl groups at δₜ 1.23 (d, J = 11.5), δₜ 16.49 and δₜ 1.24 (d, J = 12.0), δₜ 16.74 ppm. The M⁺ at m/z 394 was in complete agreement with the proposed structure of 16. The NMR data of 17 indicated the disappearance of the S–CH₃ signals and the OH signal at δₜ 5.87 ppm present in 5. The CH–O signals in 17 showed a downfield shift to δₜ 5.68 (d, J = 8.5), δₜ 79.94 ppm diagnostic for derivatized oxygen atom. Mass spectrum showed M⁺ at 556 m/z consistent with the molecular formula C₃₄H₂₈N₄O₄ surely prove the dimeric nature of 17. The addition of 2 via the elimination of S–CH₃ resulted in 18. The signals of two moieties of L-norephedrine were overlapped in both ¹H and ¹³C NMR spectra. HR ESI showed a quasi-molecular ion at 430.2127 m/z (calc. 430.2131) for M⁺ + 1 ion certainly supporting the structure of 18. The NH proton appeared as two broad singlets at 4.15, 4.40 each integrated for half proton diagnostic for the suggested tautomerisation in the structure. All the assignments of ¹H and ¹³C NMR signals were performed based on DEPT 135 as well as 2D NMR experiments including COSY, HSQC and HMBC (see Supplementary data 2).

### 3.2. Biological evaluation

#### 3.2.1. In vitro cytotoxic activity evaluation

The in vitro cell viability activity of the targeted compounds 4–18 was measured through MTT assay against a panel of cell lines MDA-MB-231, MCF-7, HepG-2 and HCT-116 human cancer cell lines derived from breast, liver and colon tumors. A closer look at Table 1 indicates that compounds 4–18 showed variable IC₅₀ values against the tested cell lines and was compared to erlotinib and staurosporine, as standards. Compound 5 was the most potent against all the cell lines with IC₅₀ ranging from 1.53 to

---

Figure 4. The mechanism of formation of compounds 12 and 13.
5.76 \mu M. Compound 6 takes second place after 5 as a promising homologue. Compounds 5, 6 and 15 displayed more potent activity against MDA-MB-231 cell line in comparison to erlotinib with IC\textsubscript{50} values = 1.53, 1.60 and 2.41 versus 3.73 \mu M. While the remaining compounds showed good to moderate activities towards the tested cell lines. The most potent compounds 5 and 6 were screened against MCF-10A normal breast cell line to determine their selectivity and relative safety towards normal cells. The compounds showed low cytotoxic effect with IC\textsubscript{50}=61.85 and 49.21 \mu M against MCF-10A cell line. Measuring the selectivity index\textsuperscript{10} indicates that compounds 5 and 6 showed the highest selectivity towards MDA-MB-231 followed by HepG-2 cell lines (Table 2).

### 3.2.2. EGFR kinase assay

All the newly synthesized compounds, 4–18, were subjected to EGFR-TK inhibitory assay. Furthermore, a representative compound eliciting superior EGFR inhibition was subjected to cell cycle analysis and apoptotic assay to investigate its effect on cell cycle progression and apoptosis. Table 1 shows the inhibition data of EGFR (IC\textsubscript{50} values) for the examined compounds, erlotinib and

![Scheme 5. Synthesis of compounds 15–18.](image-url)
Figure 5. Formation of compound 15.

Figure 6. Formation of compound 16.

Figure 7. Formation of compound 17.
Table 1. Antiproliferative and EGFR inhibitory activity of the target compounds 4–18.

| Cpd no. | Structure | IC_{50} (μM)^	ext{a} |
|---------|-----------|-----------------------|
|         |           | MDA-MB-231  | MCF-7  | HCT-116 | HepG-2 | EGFR  |
| 4       | ![Structure 4](image) | 35.24 ± 0.24 | 25.55 ± 0.02 | 23.49 ± 0.05 | 22.68 ± 0.61 | 1.39 ± 0.14 |
| 5       | ![Structure 5](image) | 1.53 ± 0.01  | 5.43 ± 0.14  | 5.76 ± 0.11  | 4.14 ± 0.03  | 0.76 ± 0.10  |
| 6       | ![Structure 6](image) | 1.60 ± 0.01  | 7.23 ± 0.07  | 10.6 ± 0.19  | 17.6 ± 0.15  | 2.13 ± 0.21  |
| 7       | ![Structure 7](image) | 23.48 ± 0.06 | 68.13 ± 1.21 | 59.25 ± 0.78 | 48.29 ± 0.93 | 3.44 ± 0.15  |
| 8       | ![Structure 8](image) | 38.19 ± 0.38 | 70.13 ± 0.87 | 49.22 ± 0.49 | 47.32 ± 0.47 | 3.74 ± 0.17  |
| 9       | ![Structure 9](image) | 84.15 ± 0.62 | >100          | 90.54 ± 0.73 | >100          | 9.78 ± 0.31  |
| 10      | ![Structure 10](image) | 21.86 ± 0.04 | 37.31 ± 0.29 | 46.27 ± 0.63 | 35.58 ± 0.36 | 3.09 ± 0.20  |

Copyright © 2023, Wiley Periodicals, Inc. All rights reserved.

(continued)
Table 1. Continued.

| Cpd no. | Structure | IC50 (μM)a |
|---------|-----------|------------|
|         |           | MDA-MB-231 | MCF-7 | HCT-116 | HepG-2 | EGFR     |
| 11      | ![Structure](image1) | 16.39 ± 0.06 | 39.42 ± 0.30 | 44.15 ± 0.22 | 33.65 ± 0.26 | 3.87 ± 0.11 |
| 12      | ![Structure](image2) | 57.78 ± 0.43 | 66.31 ± 0.72 | 50.75 ± 0.29 | 20.54 ± 0.31 | 7.39 ± 0.28 |
| 13      | ![Structure](image3) | 81.43 ± 0.27 | >100 | >100 | >100 | 8.35 ± 0.32 |
| 14      | ![Structure](image4) | 26.55 ± 0.04 | 30.12 ± 0.19 | 29.32 ± 0.16 | 41.25 ± 0.23 | 1.39 ± 0.27 |
| 15      | ![Structure](image5) | 2.41 ± 0.03 | 19.67 ± 0.13 | 26.51 ± 0.17 | 28.46 ± 0.40 | 1.09 ± 0.12 |
| 16      | ![Structure](image6) | 69.12 ± 0.21 | >100 | >100 | 76.65 ± 0.81 | 7.83 ± 0.19 |

(continued)
Table 1. Continued.

| Cpd no. | Structure | IC₅₀ (μM)a  | MDA-MB-231 | MCF-7 | HCT-116 | HepG-2 | EGFR |
|---------|-----------|-------------|------------|-------|---------|--------|------|
| 17      | ![Structure](image1) | 5.45 ± 0.06 | 20.14 ± 0.27 | 18.43 ± 0.20 | 24.30 ± 0.18 | 1.02 ± 0.05 |
| 18      | ![Structure](image2) | 62.94 ± 0.10 | 62.59 ± 0.18 | 44.90 ± 0.52 | 50.0 ± 0.13 | 0.99 ± 0.04 |
| Erlotinib | ![Structure](image3) | 3.73 ± 0.01 | 4.48 ± 0.02 | 2.78 ± 0.04 | 3.04 ± 0.20 | 0.31 ± 0.01 |
| Staurosporine | ![Structure](image4) | 25.26 ± 0.36 | 20.32 ± 0.31 | 10.31 ± 0.28 | 11.45 ± 0.19 | 0.82 ± 0.08 |

aThe results represent the mean of three different experiments ± SE.

Table 2. The selectivity index of compounds 5 and 6 towards the tested cell lines.

| Cpd no. | IC₅₀ (μM) | Selectivity index (SI) |
|---------|-----------|------------------------|
|         | MCF-10A   | MDA-MB-231 | MCF-7 | HCT-116 | HepG-2 | |
| 5       | 61.85 ± 2.14 | 40.42 | 11.39 | 10.74 | 14.93 | |
| 6       | 49.21 ± 1.52 | 30.75 | 6.81 | 4.64 | 23.10 | |

Table 3. IC₅₀ of compounds 5 and 6 on cancer cell lines after being subjected to irradiation.

| Cpd no. | IC₅₀ (μM)a after irradiation |
|---------|-----------------------------|
|         | MDA-MB-231 | MCF-7 | HCT-116 | HepG-2 |
| 5       | 0.78 ± 0.15 | 2.35 ± 0.18 | 2.28 ± 0.41 | 2.34 ± 0.23 |
| 6       | 1.04 ± 0.04 | 4.26 ± 0.01 | 6.98 ± 0.25 | 14.26 ± 0.11 |

aThe values represent the mean of three different experiments ± SE.
staurosporine, as reference standards. All analogues showed excellent EGFR inhibition potential ranging from 0.76 to 9.78 μM. The 3-(1-hydroxy-1-phenylpropan-2-yl)-2-(methylthio)quinazolin-4(3H)-one 5 demonstrated superior enzyme inhibition better than that expressed by erlotinib (IC\textsubscript{50} = 0.76 versus 0.92 μM). Compound 5 is the most active compound towards all the tested cell lines and EGFR inhibitory activity with relative safety towards normal cells and high selectivity towards MDA-MB-231 cell line. The activity of 5 displayed a remarkable decrease by the replacement of the methyl mercaptan with the thione group as in 4, while replacement with the 1-hydroxy-1-phenylpropan-2-ylamino group as in 18 demonstrates a narrow range change in activity from 0.76 to 0.99 μM. Furthermore, replacement of the 1-hydroxy-1-phenylpropan-2-yl in 4 with the amino group as in 6 leads to lowering the EGFR inhibitory activity (IC\textsubscript{50} 1.39 versus 2.13 μM). Also,
replacement of the thione group in 6 with methyl mercaptan 7, 1-hydroxy-1-phenylpropan-2-ylamino 11, hydroxy 12 or ethoxy group 13 reduces the activity. Regarding the two homologues 8 and 15, the replacement of sulphur with oxygen greatly enhances the EGFR activity, while the opposite occurs in 9 and 10. The two dimers 14 and 17 show very potent activity that demonstrates that a bulky rigid structure is favourable for binding with the receptor.

3.2.3. Radiosensitizing activity
Radiotherapy is second to surgery in cancer treatment. The major drawback of radiotherapy is its inability to differentiate between cancerous and normal tissues. Radiation causes ionization and excitation of atoms that result in the generation of short-lived free radicals. These free radicals can damage proteins and membranes, leading to single or double DNA strand breaks31,32. A radiosensitizing agent can induce tumor sensitization to ionizing radiation, thus lowering the required dose for treatment. This enhancement of radiation effects not only control the local tumors but also limit the metastatic spread. EGFR inhibitors can adopt another mechanism of action by inhibiting accelerated repopulation of tumor cells during fractionated radiotherapy as they block the membrane receptors of growth factors or interfere with the signaling pathways involved in cell proliferation33,34.

The ability of the most active compounds 5 and 6 to enhance gamma radiation-induced tumor cell death was examined. The results proved the ability of the two compounds to sensitize the cancerous cells to the lethal effects of ionizing radiation (Table 3). Compounds 5 and 6 showed enhanced cytotoxicity on all cell lines after irradiation with a single dose of 8 Gy gamma radiation. Compound 5 was more potent on all the tested cell lines with IC50 < 5 μM.

3.2.4. Effect on cell cycle progression
The therapeutic effect of the anticancer agent depends upon its ability to stop cell cycle progression by arresting cell division at certain checkpoints promoting apoptosis. These checkpoints exist at G1-S, S and G2-M phases35,36. The most potent and selective compound 5 was chosen to determine its ability to induce apoptosis using MDA-MB-231 cells according to the reported method37. The cells were treated with compound 5 at a concentration equals to its IC50 value on EGFR (0.76 μM) for 24 h. It is clear from Figures 8 and 9 that compound 5 interfered with the cell cycle in the G2-
Figure 13. 2D & 3D docking poses of compound 5 inside the active site of 1M17.

Figure 14. Superimposition of erlotinib (red) and compound 5 (magenta) showed that they adopt the same orientation inside the active site.
M phase. At that phase, accumulating cells reached 40.39% after treatment of control MDA-MB-231 cells (6.82%) with compound 5. Furthermore, compound 5 raised the percentage of cells at pri-G1 phase by 10 folds to reach 19.23% after being 1.91% in control cells. On the contrary, the cell population in G1 and S phases decrease after treatment with compound 5. So, compound 5 induces apoptosis through cell cycle arrest in the G2-M phase.

3.2.5. Apoptotic assay
Phosphatidylserine (PS) exposure on the outer plasma membrane was detected during apoptosis and forms the basis for Annexin V/PI (propidium iodide) double staining assay to detect apoptotic cell death. At early apoptosis, the cell membrane excludes viability dyes such as PI and permits the determination of apoptotic cell kinetics according to the cell cycle. To investigate the mode

Figure 15. 2D and 3D visuals of compound 6 inside 1M17 active site.
of induced cell death, MDA-MB-231 cells were incubated with compound 5 at 0.76 \( \mu \)M for 24 h. Compound 5 induced apoptosis (19.1\%) by more than 12 folds over the control (1.54\%). Compound 5 induced early apoptosis by 7.36\% and enhanced late apoptosis by 11.74\% compared with the untreated control cells (Figures 10 and 11).

### 3.3. Molecular docking

Molecular docking of compounds 5 and 6 was performed on the active site of EGFR co-crystallized with erlotinib (PDB: 1M17) (Figure 12)[40]. The active site of 1M17 consists mainly of these key amino acids; Met 769, Leu 694, Thr 766, Ala 719, Leu 764, Gln 767, Leu 768, Pro 770, Phe 771, Gly 772, Leu 820, Thr 830 and Asp 831. The ligand compounds 5 and 6 were docked into the active site of the target protein 1M17 and the binding affinities, energy scores and RMSD values for compounds were recorded. Validation of molecular docking showed that the RMSD values are within acceptable limits (less than 2 Å)[41]. The best binding affinity with the lowest energy score for the compounds was computed as \(-10.14 \text{ kcal mol}^{-1}\) (compound 5) and \(-10.03 \text{ kcal mol}^{-1}\) (compound 6). According to these findings, together with the above-mentioned biological evaluation, compounds 5 and 6 may act as effective docking material for EGFR tyrosine kinase. The 2D and 3D visuals of the interaction map for compound 5 can be seen in Figure 13. The hydrogen bond formation connected the CO of quinazolinone with Met 769 of the target protein with a length of 2.39 Å and Thr 766 by OH with 2.63 Å. Superimposing compound 5 with erlotinib showed that they adopt the same orientation inside the active site with RMSD = 1.243 Å (Figure 14). On the other hand, four conventional hydrogen bond interactions were observed between compound 6 and the macromolecule 1M17 as follows; the CO of the quinazolinone with Met 769 and Leu 768 with a recorded distance of 2.54 and 2.79 Å, respectively. In addition to Thr 766 that forms two hydrogen bonds with NH and CS at a distance of 2.88 and 3.01 Å (Figure 15). Overlaying of erlotinib and compound 6 can be observed in Figure 16 with RMSD = 1.236 Å.

### 4. Conclusion

In this study, a novel series of quinazolinone and fused quinazolinone derivatives synthesized by the aid of L-norephedrine were obtained. All these compounds showed variable anticancer activity against MDA-MB-231, MCF-7, HepG-2 and HCT-116 cancer cell lines and EGFR inhibitory activity comparable to erlotinib. The 3-(1-hydroxy-1-phenylpropan-2-yl)-2-(methylthio)quinazolin-4(3H)-one 5 and 3-amino-2-thioxo-2,3-dihydroquinazolin-4(1H)-one 6 were the most promising in this series towards the cancer cell lines and EGFR. Compounds 5 and 6 were further selected to measure their relative safety and selectivity towards normal cells. They showed mild cytotoxic activity towards MCF-10A normal cell line and high selectivity towards MDA-MB-231 cell line. Besides, they displayed radiosensitizing activity through their ability to sensitize the cancer cells to the lethal effect of gamma irradiation. The most potent compound in this series, 5, undergoes cell cycle analysis and annexin V/PI assay to detect apoptotic cell death. Compound 5 proved to arrest the cell cycle progression at the G2-M phase, induce early apoptosis and enhance late apoptosis. Moreover, molecular docking of compounds 5 and 6 showed the key interactions required for EGFR inhibition. Finally, compounds 5 and 6 could be considered as promising leads for the development of new anticancer and radiosensitizing agents.

### Acknowledgement

M. M. Ghorab and A. M. Soliman appreciate the staff members of gamma irradiation unit at the National Center for Radiation Research and Technology (NCRRT) for carrying out the irradiation process. A. S. Alqahtani is thankful to the Deanship of the Scientific Research and Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

### Disclosure statement

No potential conflict of interest was reported by the author(s).

### ORCID

Mostafa M. Ghorab [http://orcid.org/0000-0003-4250-0452](http://orcid.org/0000-0003-4250-0452)
References

1. Peitzsch C, Perrin R, Hill RP, et al. Hypoxia as a biomarker for radioresistant cancer stem cells. Int J Radiat Biol 2014;90: 636–52.

2. Sugrue T, Lowndes NF, Ceredig R. Hypoxia enhances the radioresistance of mouse mesenchymal stromal cells. Stem Cells 2014;32:2188–200.

3. Wang X-M, Xu J, Li Y-P, et al. Synthesis and anticancer activity evaluation of a series of [1,2,4]triazolo[1,5-α]-pyrimidines and related analogues in vitro and in vivo. Eur J Med Chem 2013;67:243–51.

4. Holohan C, Van Schaeybroeck S, Longley DB, Johnston P. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer 2013;13:714–26.

5. Xie L, Bourne PE. Developing multi-target therapeutics to fine-tune the evolutionary dynamics of the cancer ecosystem. Front Pharmacol 2015;6:209–14.

6. El Ella DAA, Ghorab MM, Heiba HI, Soliman AM. Synthesis of some new thiazolopyrane and thiazolopyranopyrimidine derivatives bearing a sulfonamide moiety for evaluation as anticancer and radiosensitizing agents. Med Chem Res 2012; 21:2395–407.

7. Hubbard SR, Miller W. Receptor tyrosine kinases: mechanisms of activation and signaling. Curr Opin Cell Biol 2007; 19:117–23.

8. Regad T. Targeting RTK signaling pathways in cancer. Cancers 2015;7:1758–84.

9. Baker SJ, Reddy EP, Medicine P. Targeted inhibition of kinases in cancer therapy. MT Sinai J Med 2010;77:573–86.

10. Flynn JF, Wong C, Wu JM. Anti-EGFR therapy: mechanisms and advances in clinical efficacy in breast cancer. J Oncol 2009;2009:526963.

11. Yewale C, Baradia D, Vhora I, et al. Epidermal growth factor receptor targeting in cancer: a review of trends and strategies. Biomaterials 2013;34:8690–707.

12. Ghorab MM, Alqahtani AS, Soliman AM, Askar AA. Novel N-(substituted) thioacetamide quinazolinone benzensulfonamides as antimicrobial agents. Int J Nanomedicine 2020;15:3161–80.

13. Soliman AM, Karam HM, Mekkawy MH, Ghorab MM. Antioxidant activity of novel quinazolinones bearing sulfonamide: potential radiomodulatory effects on liver tissues via NF-αB/PON1 pathway. Eur J Med Chem 2020;197:112333.

14. Soliman AM, Ghorab MM, Bua S, Supforat CT. Iodoquinazolinones bearing benzensulfonamide as human carbonic anhydrase I, II, IX and XII inhibitors: synthesis, biological evaluation and radiosensitizing activity. Eur J Med Chem 2020;200:112449.

15. Soliman AM, Karam HM, Mekkawy MH, Higgins M, et al. Radiomodulatory effect of a non-electrophilic NQO1 inducer identified in a screen of new 6-8-dilodoquinazolin-4(3H)-ones carrying a sulfonamide moiety. Eur J Med Chem 2020;200:112467.

16. Marzano G, Guiotto A, Chilin A. Quinazoline derivatives as potential anticancer agents: a patent review (2007–2010). Expert Opin Ther Pat 2012;22:223–52.

17. Wu P, Nielsen TE, Clausen MH. Tips. FDA-approved small-molecule kinase inhibitors. Trends Pharmacol Sci 2015;36: 422–39.

18. Ugale VG, Bari SB. Quinazolines: new horizons in anticonvulsant therapy. Eur J Med Chem 2014;80:447–501.

19. Karamouzis MV, Grandis JR, Argiris A. Therapies directed against epidermal growth factor receptor in aerodigestive carcinomas. JAMA 2007;298:70–82.

20. Ghorab MM, Alsaid MS, Soliman AM. Dual EGFR/HER2 inhibitors and apoptosis inducers: New benzo[gl]quinazoline derivatives bearing benzenesulfonamide as anticancer and radiosensitizers. Bioorg Chem 2018;80:611–20.

21. Soliman AM, Ghorab MM. Exploration of N-alkyl-2-[(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio]acetamide derivatives as anticancer and radiosensitizing agents. Bioorg Chem 2019;88:102956.

22. Lee YC, Chen YY, Lin JS, Chen YW, et al. Stereoselective synthesis of (1R, 25)-norephedrine by recombinant whole-cell biocatalysts coupling acetohydroxycid synthase I and α-transaminase. Process Biochem 2018;73:74–81.

23. Meadows M. FDA issues public health advisory on phenylpropanolamine in drug products. FDA Consum 2001;35:9.

24. Groeper JA, Hitchcock SR, Ferrence GM. A scalable and expedient method of preparing diastereomERICally and enantiomerically enriched pseudonorephedrine from norephedrine. Tetrahedron Asymmetry 2006;17:2884–9.

25. Alsaid MS, Ghorab MM, Alqasoumi SI, Abdel-Kader MS. Semisynthesis of some novel thiourea and carbamimidothioic acid derivatives using natural alkaloid L-norephedrine and their anticancer activity. Russ J Bioorganic Chem 2016; 42:567–73.

26. El-Hiti GA, Hussain A, Hegazy AS, Aloataib MH. Thioxoquinazolines: synthesis, reactions and biological activities. J Sulfur Chem 2011;32:361–95.

27. Ghorab MM, Alsaid MS, Shahat AA. Synthesis of some sulfonamide incorporating enaminone, quinolone mieties and thiazoloquinazoline derivative induce the cytoprotective enzyme NAD (P) H: quinone oxidoreductase 1. Biomed Res Int 2016;27:1.

28. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.

29. Ghorab MM, Al-Said MS, Abdel-Kader MS, et al. Absolute configuration of (15,25)-3-methyl-2-phenyl-2,3-dihydro-thiazolo[2,3-b]quinazolin-5-one. Acta Cryst E 2012;68:o927–8.

30. Hassan RM, Abd-Allah WH, Salman AM, et al. Design, synthesis and anticancer evaluation of novel 1,3-benzodioxoles and 1,4-benzodioxines. Eur J Pharm Sci 2019;139:105045.

31. Vonk J, Shackelford TK, eds. The Oxford handbook of comparative evolutionary psychology. P. E. Nathan (Editor-in-chief). Oxford Library of Psychology. New York: Oxford University Press; 2012:574.

32. Ghorab MM, Ragab FA, Heiba HI, Soliman AM. Anticancer and radio-sensitizing evaluation of some new sulfonamide derivatives bearing pyridine, thiophene, and hydrazono mieties. Res Chem Intermed 2017;43:4657–81.

33. Milas L, Fan Z, Andratschke NH, Ang KK. Epidermal growth factor receptor and tumor response to radiation: in vivo preclinical studies. Int J Radiat Oncol Biol Phys 2004;58:966–71.

34. Soliman AM, Alqahtani AS, Ghorab MM. Novel sulfonamide benzouazoquinazolines as dual EGFR/HER2 inhibitors, apoptosis inducers and radiosensitizers. J Enzyme Inhib Med Chem 2019;34:1030–40.

35. MacLachlan TK, Sang N, Giordano A. Cyclins, cyclin-dependent kinases and cdk inhibitors: implications in cell cycle
control and cancer. Crit Rev Eukaryot Gene Expr 1995;5:127–56.

36. Ghorab MM, Alsaid MS, Samir N, et al. Aromatase inhibitors and apoptotic inducers: design, synthesis, anticancer activity and molecular modeling studies of novel phenothiazine derivatives carrying sulfonamide moiety as hybrid molecules. Eur J Med Chem 2017;134:304–15.

37. Wang T-H, Wang H-S, Soong Y-K. Paclitaxel-induced cell death: where the cell cycle and apoptosis come together. Cancer 2000;88:2619–28.

38. Gorczyca W. Cytometric analyses to distinguish death processes. Endocr Relat Cancer 1999;6:17–9.

39. Andree H, Reutelingsperger C, Hauptmann R, et al. Binding of vascular anticoagulant alpha (VAC alpha) to planar phospholipid bilayers. J Biol Chem 1990;265:4923–8.

40. Stamos J, Sliwkowski MX, Eigenbrot C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. J Biol Chem 2002;277:46265–72.

41. Kramer B, Rarey M, Lengauer T. Evaluation of the FLEXX incremental construction algorithm for protein–ligand docking. Proteins 1999;37:228–41.