

RESEARCH ARTICLE

Synthesis, antitumor activity and molecular docking study of some novel 3-benzyl-4(3H)quinazolinone analogues

Ibrahim A. Al-Suwaidan¹, Alaa A.-M. Abdel-Aziz^{1,2}, Taghreed Z. Shaver³, Rezk R. Ayyad³, Amer M. Alanazi¹, Ahmad M. El-Morsy⁴, Menshawy A. Mohamed^{4,5}, Naglaa I. Abdel-Aziz², Magda A.-A. El-Sayed⁶, and Adel S. El-Azab^{1,4}

¹Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, ²Department of Medicinal Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura, Egypt, ³Department of Pharmaceutical Chemistry, and ⁴Department of Organic Chemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, ⁵Department of Pharmaceutical Chemistry, College of Pharmacy, Salman Bin Abdulaziz University, AlKharj, Saudi Arabia, and ⁶Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura, Egypt

Abstract

A novel series of 3-benzyl-substituted-4(3H)-quinazolinones were designed, synthesized and evaluated for their *in vitro* antitumor activity. The results of this study demonstrated that 2-(3-benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N-(3,4,5-trimethoxyphenyl)acetamide, 2-(3-benzyl-6,7-dimethoxy-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N-(3,4,5-trimethoxyphenyl)acetamide and 3-(3-benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N-(3,4,5-trimethoxyphenyl)-propanamide have shown amazing broad spectrum antitumor activity with mean GI₅₀ (10.47, 7.24 and 14.12 μM, respectively), and are nearly 1.5–3.0-fold more potent compared with the positive control 5-FU with mean GI₅₀, 22.60 μM. On the other hand, compounds **6** and **10** yielded selective activities toward CNS, renal and breast cancer cell lines, whereas compound **9** showed selective activities towards leukemia cell lines. Molecular docking methodology was performed for compounds **7** and **8** into ATP binding site of EGFR-TK which showed similar binding mode to erlotinib, while compound **11** into ATP binding site of B-RAF kinase inhibited the growth of melanoma cell lines through inhibition of B-RAF kinase, similar to PLX4032.

Introduction

Cancer is a disease characterized by a shift in the controlled mechanisms that govern cell proliferation and differentiation¹. Malignancy is caused by abnormalities in cells, which might be due to inherited genes or caused by outside exposure of the body to chemicals, radiation, or even infectious agents^{2,3}. Several techniques have been adopted for the treatment and eradication of cancerous cells. These techniques involved surgery, radiation, immunotherapy, chemotherapy and chemoprevention. Ideal anticancer drugs would eradicate cancer cells without harming normal tissues. Unfortunately, no currently available agents meet this criterion, and clinical use of drugs involves a weighing of benefits against toxicity in a search of favorable therapeutic index⁴. Many of chemotherapeutic agents currently used in cancer therapy are agents which inhibit tumor growth by inhibiting the replication and transcription of DNA. The wide occurrence of the heterocycles in bioactive natural products made them important synthetic targets. Quinazolines represent a class of heterocyclic compounds of great importance in biological chemistry^{5–11}.

Keywords

5-FU, erlotinib, *in vitro* antitumor evaluation, molecular docking, NCI, quinazoline

History

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Quinazolines are frequently used in medicine because of their wide spectrum of biological activities^{5–11}. In the course of identifying various chemical substances which may serve as leads for designing novel antitumor agents, we are particularly interested in the present work with quinazoline derivatives which have been identified as a new class of cancer chemotherapeutic agents with significant therapeutic efficacy against solid tumors^{10,11}. It is well known that quinazoline derivatives are potent inhibitors of epidermal growth factor receptor (EGFR)^{12–17}. The EGFR is cellular trans-membrane tyrosine kinases that are over-expressed in a significant number of human tumors (e.g., breast, ovarian, colon, renal, and prostate)^{18,19}. Overexpression of EGFR family receptors have been observed in these tumors, approximately in 60% of all tumors. A number of small molecule EGFR kinase inhibitors have been evaluated in cancer clinical trials^{12–19}. For example (Figure 1), anilinoquinazoline-containing compounds gefitinib (IressaTM),^{13c–19} erlotinib (TarcevaTM),^{15a} lapatinib (TykerbTM, also known as GW-572016) and Vandetanib (ZactimaTM) were recently approved for the treatment of breast cancer and non-small-cell lung cancer^{15b–17}. On the other hand, melanoma is the most aggressive type of skin cancer in which V600E-B-RAF kinase is over-expressed in 63% of all malignant melanomas^{20a}. Vemurafenib (PLX4032) is a drug which targets inhibition of V600E-B-RAF kinase^{20a}. In 2011, it was approved by the U.S. Food and Drug Administration (FDA) for the treatment of late-stage melanoma, so inhibition of V600E-B-RAF

Address for correspondence: Adel S. El-Azab, Pharmaceutical Chemistry Department, College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh-11451, Saudi Arabia. Tel: +966 14679096. Fax: +966 1 467-6383. E-mail: adelazaba@yahoo.com

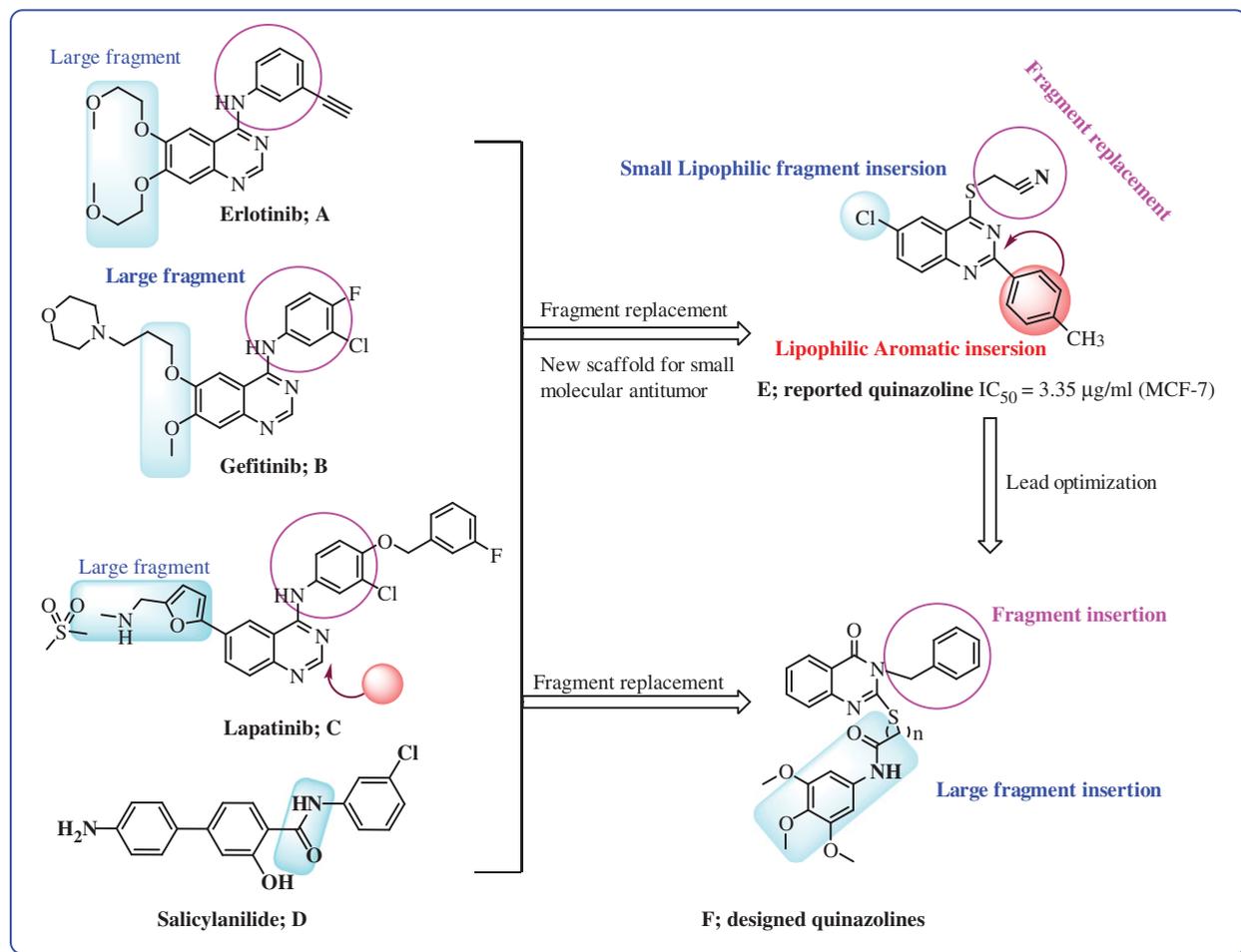


Figure 1. Reported and designed quinazoline derivatives as antitumor.

kinase is a very potential avenue for the treatment of melanoma^{20a}. Moreover, a series of salicylanilides were synthesized and their inhibitory activity against tyrosine kinases was determined. Some of them indeed have proven to be potent and selective EGFR tyrosine kinase inhibitors^{20b} (Figure 1). Many more compounds are still under evaluation in clinical trials for the treatment of cancer^{18b,19}.

Continuing our studies on quinazoline derivatives as attractive candidates as antitumor agents^{10b,11a,20b}, we have designed a number of new quinazoline derivatives containing benzyl group with different fragments and biologically evaluated the *in vitro* antitumor activities (Figure 1). In the present study, the substitution pattern at the 6 and 7 positions of quinazoline pharmacophore was selected so as to confer different electronic environment that would affect the lipophilicity, and hence the activity of the target molecules. The objectives of forming these hybrids are an attempt to reach an active antitumor agent with potentiated activity and selectivity toward cancerous cells. Molecular docking methodology was used to identify the structural features required for the antitumor properties of these new series.

Materials and methods

Chemistry

Melting points were recorded on Barnstead 9100 Electrothermal melting apparatus at the Pharmaceutical Chemistry Department, King Saud University, Riyadh, Saudi Arabia. IR spectra (KBr) were recorded on a FT-IR Perkin-Elmer spectrometer (νcm^{-1}) at Research Center, King Saud University, Riyadh, Saudi Arabia. Nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were

recorded on Bruker 700 MHz or 500 MHz spectrometer using DMSO- d_6 or CDCl_3 as solvents at Research Center, King Saud University, Riyadh, Saudi Arabia. The chemical shifts are expressed in δ ppm using TMS as internal standard. Mass spectra were recorded on a Perkin-Elmer, Clarus 600T GC/MS, or Varian TQ 320 GC/MS/MS mass spectrometers at Research Center, King Saud University, Riyadh, Saudi Arabia. Single crystal X-ray crystallography was recorded on Bruker APEXII CCD diffractometer at Chemistry Department, University of Malaya, Kuala Lumpur, Malaysia and structure refinement and resolve was carried out using SHELXS97^{21a}. Solvent evaporation was performed under reduced pressure using Buchan Rotatory Evaporator at the Pharmaceutical Chemistry Department, King Saud University, Riyadh, Saudi Arabia. Thin-layer chromatography was performed on precoated (0.25 mm) silica gel GF254 plates (E. Merck, Germany); compounds were detected with 254 nm UV lamp. Silica gel (60–230 mesh) was employed for routine column chromatography separations. Compounds **1–3** and **14–15** were prepared according to reported procedure^{11b}, while compounds **13** and **21** were prepared according to the reported method^{7a,21b} respectively.

General procedure for the synthesis of 3-benzyl-2-mercapto-substitutedquinazolin-4(3H)-one (**1–4**)

A mixture of substituted anthranilic acid (10 mmol) and benzylisothiocyanate (10 mmol, 1.49 g) in 30 ml absolute ethanol containing triethylamine (11 mmol, 1.1 g) was heated under reflux for 2 h. The reaction mixture was filtered while hot, the solvent was removed under reduced pressure and the solid obtained was dried and recrystallized from ethanol.

3-Benzyl-2-mercaptoquinazolin-4(3H)-one (1)

Yield 95%, m.p. 240–242 °C; ¹H NMR (700 MHz, DMSO-d₆): δ 13.06 (s, 1H), 7.96 (d, 1H, *J* = 15.34 Hz), 7.75 (t, 1H, *J* = 14.0 Hz), 7.43 (d, 1H, *J* = 15.34 Hz), 7.34–7.29 (m, 5H), 7.24 (d, 1H, *J* = 12.5 Hz), 5.67 (s, 2H). ¹³C NMR (175 MHz, DMSO-d₆): δ 49.2, 115.9, 116.2, 125.0, 127.4, 127.6, 127.8, 128.7, 136.1, 137.0, 139.6, 159.8, 176.0.

3-Benzyl-6-chloro-2-mercaptoquinazolin-4(3H)-one (2)

Yield 95%, m.p. 248–251 °C; ¹H NMR (700 MHz, DMSO-d₆): δ 13.17 (s, 1H), 7.88 (s, 1H), 7.80 (d, 1H, *J* = 8.36 Hz), 7.34 (d, 1H, *J* = 16.73 Hz), 7.33–7.24 (m, 4H), 7.23 (s, 1H), 5.65 (s, 2H). ¹³C NMR (175 MHz, DMSO-d₆): δ 49.3, 117.3, 118.4, 126.7, 127.4, 127.7, 128.6, 128.9, 136.0, 136.8, 138.4, 159.0, 175.9.

3-Benzyl-2-mercapto-6-methylquinazolin-4(3H)-one (3)

Yield 95%, m.p. 259–261 °C; ¹H NMR (700 MHz, DMSO-d₆): δ 13.01 (s, 1H), 7.75 (s, 1H), 7.58 (d, 1H, *J* = 15.34 Hz), 7.34–7.29 (m, 5H), 7.23 (s, 1H), 5.67 (s, 2H), 2.35 (s, 3H). ¹³C NMR (175 MHz, DMSO-d₆): δ 20.9, 49.1, 115.7, 116.1, 127.1, 127.3, 127.5, 128.6, 134.7, 137.1, 137.2, 137.6, 159.8, 175.5.

3-Benzyl-2-mercapto-6,7-dimethoxyquinazolin-4(3H)-one (4)

Yield 82%, m.p. 268–270 °C; IR (KBr, cm⁻¹) ν: 3180 (NH), 1684 (CO), 1251 (CS); ¹H NMR (500 MHz, DMSO-d₆): δ 12.86 (s, 1H), 7.30–6.94 (m, 7H), 5.66 (s, 2H), 3.85 (s, 6H), 8.04 (d, 1H, *J* = 1.5 Hz), 7.82 (d, 2H, *J* = 8.0 Hz), 7.68 (d, 1H, *J* = 8.5 Hz), 7.37–7.22 (m, 2H), 2.33 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-d₆): δ 48.6, 55.7, 56.0, 97.7, 106.7, 107.9, 126.8, 127.1, 128.1, 135.0, 136.8, 146.7, 155.3, 158.8, 174.3. MS: (M, 328).

General procedure for the synthesis of compounds 5–12

A mixture of appropriate 2-mercapto-3-benzylquinazolin-4(3H)-one (**1**) (2 mmol) and/or the appropriate 2-chloro-*N*-phenylacetamide and/or phenyl 2-chloroacetate (2 mmol) in 15 ml acetone containing anhydrous potassium carbonate (3 mmol, 415 mg) was stirred at room temperature for 10–12 h. The reaction mixture was filtered, the solvent was removed under reduced pressure and the solid obtained was dried and recrystallized from ethanol.

2-(3-Benzyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)acetamide (5)

Yield 92%, m.p.; 201–203 °C; IR (KBr, cm⁻¹) ν: 3287 (NH), 1674, 1655 (CO); ¹H NMR (500 MHz, DMSO-d₆): δ 9.88 (s, 1H), 8.34 (d, 1H, *J* = 7.5 Hz), 7.80 (t, 1H, *J* = 7.0 Hz), 7.70 (d, 1H, *J* = 8.0 Hz), 7.51 (t, 1H, *J* = 7.0 Hz), 7.40–7.31 (m, 5H), 6.73 (s, 2H), 5.42 (s, 2H), 3.99 (s, 2H), 3.78 (s, 3H), 3.74 (s, 6H). ¹³C NMR (125 MHz, DMSO-d₆): δ 36.3, 47.8, 56.0, 61.0, 96.8, 119.6, 124.8, 126.7, 127.8, 128.0, 128.1, 128.8, 134.1, 134.5, 134.7, 135.0, 146.4, 153.3, 158.0, 161.3, 166.3. MS: (M, 491).

2-(3-Benzyl-6-chloro-4-oxo-3,4-dihydroquinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)acetamide (6)

Yield 88%, m.p. 190–192 °C; IR (KBr, cm⁻¹) ν: 3243 (NH), 1663, 1650 (CO); ¹H NMR (500 MHz, DMSO-d₆): δ 9.48 (s, 1H), 8.28 (s, 1H), 7.71 (dd, 1H, *J* = 7.0, 1.5 Hz), 7.62 (d, 1H, *J* = 8.5 Hz), 7.37–7.31 (m, 5H), 6.71 (s, 2H), 5.41 (s, 2H), 3.99 (s, 2H), 3.79 (s, 3H), 3.76 (s, 6H). ¹³C NMR (125 MHz, DMSO-d₆): δ 36.3, 48.0, 56.0, 61.0, 97.0, 120.6, 126.6, 127.2, 127.8, 128.2, 128.8, 132.4, 133.9, 134.5, 134.7, 135.3, 145.0, 153.3, 158.1, 160.4, 166.0. MS: (M, 526 and M + 2, 528).

2-(3-Benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)acetamide (7)

Yield 87%, m.p.; 199–200 °C; IR (KBr, cm⁻¹) ν: 3310 (NH), 1685, 1654 (CO); ¹H NMR (500 MHz, DMSO-d₆): δ 9.94 (s, 1H), 8.13 (s, 1H), 7.62 (s, 2H), 7.38–7.30 (m, 5H), 6.73 (s, 2H), 5.42 (s, 2H), 3.97 (s, 2H), 3.79 (s, 3H), 3.75 (s, 6H), 2.63 (s, 3H). ¹³C NMR (125 MHz, DMSO-d₆): δ 21.3, 36.3, 47.8, 56.0, 61.0, 96.9, 119.3, 124.7, 127.4, 127.8, 128.1, 128.8, 134.1, 134.5, 134.9, 136.3, 137.1, 144.5, 153.3, 157.0, 161.4, 166.4. MS: (M, 505).

2-(3-Benzyl-6,7-dimethoxy-4-oxo-3,4-dihydroquinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)acetamide (8)

Yield 86%, m.p. 205–206 °C; IR (KBr, cm⁻¹) ν: 3304 (NH), 1677, 1656 (CO); ¹H NMR (500 MHz, DMSO-d₆): δ 9.71 (s, 1H), 7.63 (s, 1H), 7.37–7.34 (m, 5H), 7.04 (s, 1H), 6.73 (s, 2H), 5.42 (s, 2H), 4.00 (s, 2H), 3.98 (s, 6H), 3.78 (s, 3H), 3.74 (s, 6H). ¹³C NMR (125 MHz, DMSO-d₆): δ 36.2, 47.8, 56.0, 56.4, 56.5, 61.0, 97.0, 105.6, 106.6, 112.7, 127.7, 128.0, 128.8, 134.1, 134.7, 135.0, 142.8, 148.9, 153.4, 155.6, 155.7, 160.9, 166.4. MS: (M, 551).

3-(3-Benzyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)propanamide (9)

Yield 90%, m.p. 195–196 °C; IR (KBr, cm⁻¹) ν: 3292 (NH), 1685, 1662 (CO); ¹H NMR (500 MHz, DMSO-d₆): δ 10.40 (s, 1H), 8.12 (d, 1H, *J* = 7.5 Hz), 7.83 (t, 1H, *J* = 7.0, 7.5 Hz), 7.61 (d, 1H, *J* = 8.0 Hz), 7.48 (t, 1H, *J* = 7.5 Hz), 7.36–7.27 (m, 5H), 7.01 (s, 2H), 5.33 (d, 2H, *J* = 7.0 Hz), 4.74 (d, 1H, *J* = 6.5 Hz), 3.73 (s, 6H), 3.61 (s, 3H), 1.60 (s, 3H). ¹³C NMR (125 MHz, DMSO-d₆): δ 17.7, 46.7, 47.0, 55.6, 60.0, 97.0, 118.8, 125.8, 126.2, 126.6, 126.7, 127.4, 128.6, 133.6, 134.9, 135.5, 146.7, 152.7, 156.2, 160.8, 169.0. MS: (M, 505).

3-(3-Benzyl-6-chloro-4-oxo-3,4-dihydroquinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)propanamide (10)

Yield 89%, m.p. 196–198 °C; IR (KBr, cm⁻¹) ν: 3307 (NH), 1683, 1663 (CO); ¹H NMR (500 MHz, DMSO-d₆): δ 10.41 (s, 1H), 8.04 (s, 1H), 7.88 (d, 1H, *J* = 7.0 Hz), 7.69 (d, 1H, *J* = 7.0 Hz), 7.43–7.28 (m, 5H), 7.0 (s, 2H), 5.31 (s, 2H), 4.71 (d, 1H, *J* = 6.5 Hz), 3.71 (s, 6H), 3.60 (s, 3H), 1.58 (d, 3H, *J* = 6.5 Hz). ¹³C NMR (125 MHz, DMSO-d₆): δ 17.6, 48.8, 47.2, 55.6, 60.1, 96.8, 120.0, 125.6, 126.8, 127.5, 128.0, 128.6, 130.2, 133.5, 134.9, 135.0, 135.2, 145.4, 152.7, 157.0, 159.9, 169.0. MS: (M, 539, M + 2, 541).

3-(3-Benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)propanamide (11)

Yield 85%, m.p. 217–219 °C; IR (KBr, cm⁻¹) ν: 3293 (NH), 1686, 1662 (CO); ¹H NMR (500 MHz, DMSO-d₆): δ 10.42 (s, 1H), 7.91 (s, 1H), 7.62 (d, 1H, *J* = 7.0 Hz), 7.51 (d, 1H, *J* = 7.0 Hz), 7.43–7.25 (m, 5H), 7.01 (s, 2H), 5.30 (s, 2H), 4.72 (d, 1H, *J* = 6.5 Hz), 3.72 (s, 6H), 3.60 (s, 3H), 2.42 (s, 3H), 1.58 (d, 3H, *J* = 6.5 Hz). ¹³C NMR (125 MHz, DMSO-d₆): δ 17.6, 20.7, 46.6, 46.8, 55.6, 60.0, 96.8, 118.5, 125.7, 125.9, 126.7, 127.4, 128.6, 133.5, 135.0, 135.6, 136.0, 136.2, 144.9, 152.9, 155.1, 160.7, 169.1. MS: (M, 519).

3-(3-Benzyl-6,7-dimethoxy-4-oxo-3,4-dihydroquinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)propanamide (12)

Yield 84%, m.p. 150–152 °C; IR (KBr, cm⁻¹) ν: 3275 (NH), 1684, 1670 (CO); ¹H NMR (500 MHz, DMSO-d₆): δ 10.34 (s, 1H), 7.43–7.27 (m, 7H), 7.05 (s, 2H), 5.31 (s, 2H), 4.69 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 3.72 (s, 3H), 3.61 (s, 1H), 3.51 (s, 3H),

1.59 (s, 3H). ^{13}C NMR (125 MHz, DMSO- d_6): δ 17.6, 46.5, 46.8, 55.6, 55.7, 60.1, 96.8, 105.6, 106.7, 11.6, 126.7, 127.4, 128.5, 133.6, 135.1, 135.7, 143.1, 148.2, 152.7, 154.1, 155.0, 160.1, 169.2. MS: (M, 565).

Ethyl 2-(3-benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)acetate (14)

A mixture of 3-benzyl-2-mercapto-6-methylquinazolin-4(3H)-one (**3**) (10 mmol, 2.82 g) ethyl 2-bromoacetate (11 mmol, 1.84 g), and anhydrous potassium carbonate (12 mmol, 1.66 g) in (100 ml) dry acetone was stirred at room temperature for 6 h. The reaction mixture was filtered, the solvent was removed under reduced pressure, and the solid obtained was dried and recrystallized from ethanol. Yield 95%; m.p.; 130–133 °C; ^1H NMR (700 MHz, DMSO- d_6): δ 7.90 (s, 1H), 7.64 (d, 1H, $J = 12.55$ Hz), 7.38 (d, 1H, $J = 12.55$ Hz), 7.34 (d, 2H, $J = 9.76$ Hz), 7.428 (s, 3H), 5.33 (s, 2H), 4.13 (q, 3H, $J = 9.76$ Hz), 4.08 (s, 2H), 2.43 (s, 3H), 1.19 (s, 3H). ^{13}C NMR (175 MHz, DMSO- d_6): δ 14.6, 21.2, 34.6, 47.4, 61.5, 118.8, 126.2, 126.4, 127.2, 127.9, 129.0, 136.0, 136.4, 136.7, 145.2, 155.5, 161.2, 168.7. MS: (M, 368).

2-(3-Benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)acetohydrazide (15)

A solution of the ethyl 2-(3-benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)acetate (**14**) (3.68 g, 0.01 mol) and hydrazine hydrate (85 %, 3 ml) in ethanol (40 ml) was heated under reflux for 2 h. The obtained solid was filtered, dried, and recrystallized from ethanol. Yield 95%, m.p. 207–210 °C; ^1H NMR (700 MHz, DMSO- d_6): δ 9.36 (s, 1H), 7.91 (s, 1H), 7.66 (d, 1H, $J = 13.94$ Hz), 7.50 (d, 1H, $J = 15.34$ Hz), 7.38–7.27 (m, 5H), 5.34 (s, 2H), 4.35 (s, 2H), 3.93 (s, 2H), 2.44 (s, 3H). ^{13}C NMR (175 MHz, DMSO- d_6): δ 21.2, 35.0, 47.3, 118.9, 126.3, 126.4, 127.2, 127.9, 129.0, 136.1, 136.3, 136.6, 145.2, 155.5, 161.3, 166.6. MS: (M, 354).

2-(3-Benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N'-substituted benzylideneacetohydrazide (16–18)

A solution of acid hydrazide **15** (1 mmol, 354 mg) in methanol (10 mL) and the appropriate aldehyde (1 mmol) was stirred at room temperature for 12–15 h. The reaction mixture was washed with hexane, filtered and dried.

2-(3-benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N'-benzylideneacetohydrazide (16)

Yield 92%; m.p. 225–227 °C; ^1H NMR (700 MHz, DMSO- d_6): δ 11.81 (s, 0.37 H), 11.65 (s, 0.63 H), 8.27 (s, 0.37 H), 8.07 (s, 0.63 H), 7.90 (s, 1H), 7.70 (s, 2H), 7.63–7.59 (m, 1H), 7.45–7.41 (m, 3H), 7.35–7.32 (m, 6H), 5.36 (s, 2H), 4.56 (s, 1.2 H), 4.09 (s, 0.8 H), 2.41 (s, 3H). ^{13}C NMR (175 MHz, DMSO- d_6): δ 21.2, 34.3, 47.3, 118.9, 126.3, 126.4, 127.2, 127.3, 127.5, 127.9, 129.0, 129.3, 130.5, 134.6, 136.1, 136.3, 136.6, 143.8, 145.3, 147.1, 155.7, 161.1, 164.0, 169.0. MS: (M, 422).

2-(3-benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N'-(2-fluorobenzylidene)acetohydrazide (17)

Yield 92%; m.p. 219–221 °C; ^1H NMR (700 MHz, DMSO- d_6): δ 11.95 (s, 0.33 H), 11.76 (s, 0.67 H), 8.50 (s, 0.33 H), 8.28 (s, 0.67 H), 7.90 (s, 2H), 7.63–7.59 (m, 1H), 7.47–7.45 (m, 1H), 7.35–7.20 (m, 8H), 5.36 (s, 2H), 4.56 (s, 1.2 H), 4.08 (s, 0.8 H), 2.41 (s, 3H). ^{13}C NMR (175 MHz, DMSO- d_6): δ 21.2, 34.2, 37.3, 116.5, 118.9, 122.1, 125.3, 125.4, 126.4, 126.7, 126.8, 127.2, 127.8, 127.9, 129.1, 132.2, 136.1, 136.2, 136.6, 155.8, 160.5, 161.9, 164.1, 169.3. MS: (M, 460).

2-(3-Benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N'-(2-methoxybenzylidene)acetohydrazide (18)

Yield 90%; m.p. 203–205 °C; ^1H NMR (700 MHz, DMSO- d_6): 11.79 (s, 0.36 H), 11.60 (s, 0.64 H), 8.61 (s, 0.36 H), 8.40 (s, 0.64 H), 7.90 (s, 1H), 7.82 (d, 0.64 H, $J = 11.15$ Hz), 7.77 (d, 0.36, $J = 12.55$ Hz), 7.63 (d, 0.36 H, $J = 13.94$ Hz), 7.60 (d, 0.64 H, $J = 13.94$ Hz), 7.45 (d, 0.36, $J = 13.94$ Hz), 7.40 (d, 0.64 H, $J = 13.94$ Hz), 7.35–7.34 (m, 6H), 7.31 (d, 1H, $J = 11.15$ Hz), 7.30 (d, 0.36 H, $J = 5.76$ Hz), 7.29 (d, 0.64, $J = 25.1$ Hz), 5.36 (s, 2H), 4.55 (s, 1.2 H), 4.06 (s, 0.8 H), 3.86 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (175 MHz, DMSO- d_6): δ 21.2, 34.3, 47.3, 56.2, 112.3, 118.9, 121.1, 122.5, 125.9, 126.3, 127.2, 127.8, 129.0, 131.8, 132.0, 136.1, 136.6, 139.5, 142.6, 145.3, 155.7, 155.9, 158.1, 161.2, 163.8, 169.1.

2-(3-Benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N'-(4-oxo-2-phenylthiazolidin-3-yl)acetamide (19)

A solution of 2-(3-benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N'-benzylideneacetohydrazide (**16**) (0.5 mmol, 221 mg) in acetic acid (10 ml) was refluxed for 12 h with thiglycolic acid (3 mmol, 271 mg). The reaction mixture was cooled, the solvent was removed under reduced pressure; the residue was neutralized with 10% sodium carbonate solution. The solid obtained was filtered, dried and recrystallized from acetic acid. Yield 61%; m.p. 207–209 °C; ^1H NMR (700 MHz, DMSO- d_6): 11.44 (s, 1H), 7.95 (d, 1H, $J = 8.36$ Hz), 7.64 (d, 1H, $J = 13.95$ Hz), 7.52–7.11 (m, 11H), 5.34 (s, 2H), 5.09 (s, 2 H), 4.38–4.30 (m, 1H), 3.94–3.88 (m, 1H), 3.76–3.59 (m, 1H), 2.46 (s, 3H). ^{13}C NMR (175 MHz, DMSO- d_6): 20.7, 21.2, 25.3, 43.6, 46.7, 114.0, 115.6, 120.0, 126.1, 126.7, 126.9, 127.1, 127.3, 127.4, 127.5, 127.7, 127.9, 128.7, 128.8, 129.4, 136.2, 136.6, 137.0, 137.7, 137.9, 145.6, 150.6, 154.6, 161.9, 162.4. MS: (M, 516).

2-Mercapto-5H-[1,3,4]thiadiazolo[2,3-b]quinazolin-5-one (20)

A solution of 3-benzyl-2-mercaptoquinazolin-4(3H)-one (**13**) (354 mg, 1 mmol), carbon disulphide (760 mg, 10 mmol) and potassium hydroxide (170 mg, 3 mol) in ethanol (10 ml) was stirred at room temperature for 4 h the reaction mixture was heated under reflux for 6 h. The reaction mixture was cooled and the solvent was evaporated under reduced pressure. The solid obtained was dissolved in water, acidified with concentrated hydrochloric acid; the solid obtained was filtered, washed with water, dry and crystallized from ethanol. Yield 90%; m.p. 198–200 °C; ^1H NMR (500 MHz, DMSO- d_6): δ 14.72 (s, 1H), 7.55 9 (d, 1H, $J = 7.5$ Hz), 7.28 (t, 1H, $J = 7.0$ Hz), 6.90 (d, 1H, $J = 8.0$ Hz), 6.68 (t, 1H, $J = 7.5$ Hz), ^{13}C NMR (125 MHz, DMSO- d_6): δ 101.1, 113.7, 114.0, 125.0, 130.7, 145.1, 158.6, 173.8. MS: (M, 235).

2-(Methylthio)-5H-[1,3,4]thiadiazolo[2,3-b]quinazolin-5-one (21)

A solution of 2-mercapto-5H-[1,3,4]thiadiazolo[2,3-b]quinazolin-5-one (**20**) (470 mg, 2 mmol), in 10 ml acetone containing anhydrous potassium carbonate (3 mmol, 415 mg) was stirred with iodomethane (426 mg, 3 mmol) at room temperature for 6 h. The reaction mixture was filtered, the solvent was removed under reduced pressure, and the solid obtained was washed with water, dried and recrystallized from ethanol. Yield 76%; m.p. 96–98 °C; ^1H NMR (500 MHz, CDCl₃): δ 8.42 (d, 1H, $J = 7.5$ Hz), 7.79 (t, 1H, $J = 7.0$ Hz), 7.63 (d, 1H, $J = 8.0$ Hz), 7.49 (t, 1H, $J = 7.0$ Hz), 2.84 (s, 3H), ^{13}C NMR (125 MHz, CDCl₃): δ = 15.3, 118.9, 126.2, 127.6, 134.8, 147.2, 156.2, 157.1, 158.5. MS: (M, 249).

Antitumor screening

A primary anticancer assay was performed for an approximately 60 human tumor cell line panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, MD^{22–26}.

Docking methodology

Docking studies have been performed using MOE 2008.10 (2008). Docking procedure was followed using the standard protocol implemented in MOE 2008.10 and the geometry of resulting complexes was studied using the MOE's Pose Viewer utility²⁷.

Results and discussion

Chemistry

Synthesis of 2-mercapto-3-benzylquinazolin-4(3H)-one derivatives (**1–4**) as key intermediates was achieved by the reaction of various anthranilic acid with benzylisothiocyanate in absolute ethanol in 82–88% yield. Compounds **1–4** were treated with 2-chloro-*N*-(3,4,5-trimethoxyphenyl)acetamide or 3-chloro-*N*-(3,4,5-trimethoxyphenyl)propanamide in anhydrous acetone in the presence of potassium carbonate to give the corresponding 2-(quinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)acetamide or 2-(quinazolin-2-ylthio)-*N*-(3,4,5-trimethoxyphenyl)propanamide **5–12** in 84–92% yield (Scheme 1).

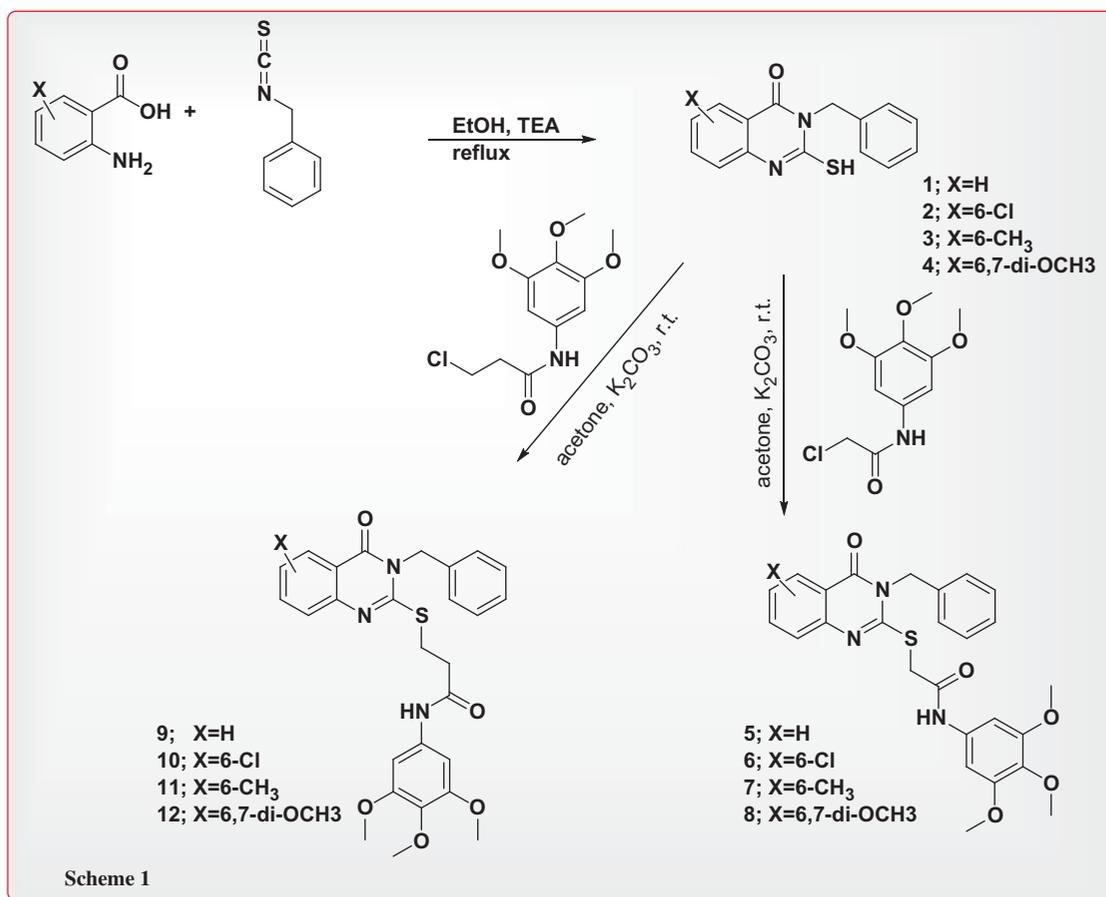
On the other hand, 3-benzyl-2-mercapto-6-methylquinazolin-4(3H)-one (**3**) was reacted with ethyl 2-bromoacetate in dry acetone in the presence of potassium carbonate at room temperature to afford ethyl 2-(3-benzyl-6-methyl-4-oxo-3,4-

dihydroquinazolin-2-ylthio)acetate (**14**), which was treated with hydrazine hydrate in ethanol at room temperature to furnish 2-(3-benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)acetohydrazide (**15**) as key intermediate in 90% yield. Schiff's bases **16–18** were obtained in 90–92% yields, by reaction of the acid hydrazide **15** with various aldehydes in methanol at room temperature, compound **16** was subjected cyclization via boiling with acetic acid containing thioglycolic acid to give 2-phenylthiazolidin-4-one **19** in 61% yield. Reaction of 3-amino-2-mercaptoquinazolin-4(3H)-one (**13**) with carbon disulfide in ethanol containing potassium hydroxide gave 2-mercapto-5H-[1,3,4]thiadiazolo[2,3-b]quinazolin-5-one (**20**) in 90% yield, the last compound was alkylated by stirring with iodomethane in acetone containing potassium carbonate to yield 2-(methylthio)-5H-[1,3,4]thiadiazolo[2,3-b]quinazolin-5-one (**21**) in 76% yield. The structure of compound **21** was also confirmed by X-ray crystallography analysis^{21b} (Scheme 2) and the molecular solid state structure and numbering system are indicated in Figure 2.

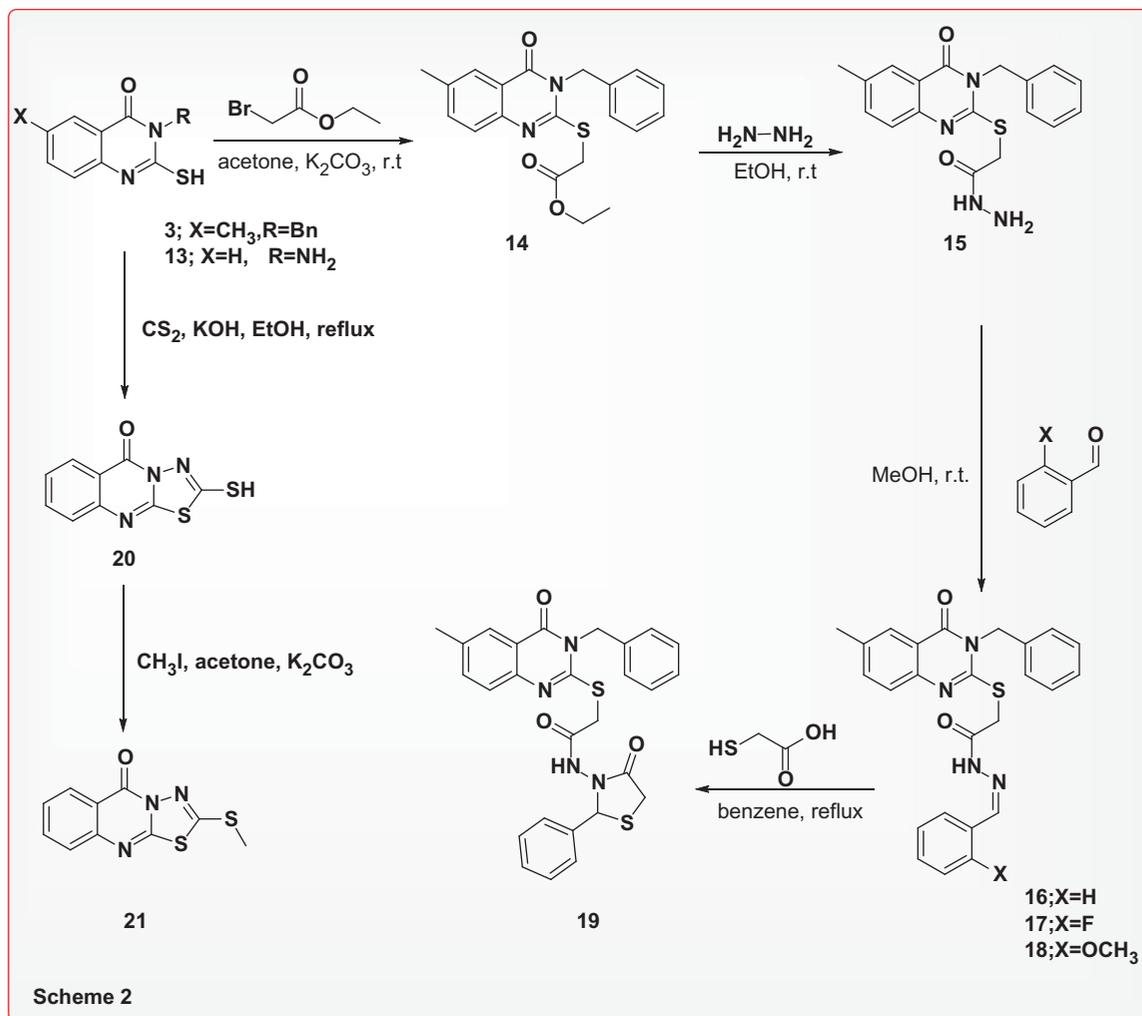
Antitumor activity

In vitro antitumor evaluation of eight compounds indicated in Table 1 was selected by National Cancer Institute, Bethesda, Maryland, USA, on the basis of degree of the structure variation and computer modeling techniques for evaluation of their anticancer activity.

A single dose (10 μ M) of the test compounds **5–11** and **21** was used in the full NCI 60 cell lines panel assay^{22–26}. The data reported as mean-graph of the percent growth of the treated cells, and presented as percentage growth inhibition (GI %) caused by the test compounds (Table 1).



Scheme 1. Reactions of 2-mercapto-3-benzylquinazolin-4(3H)-one (**1**) with 2-chloro-*N*-(3,4,5-trimethoxyphenyl)acetamide and 3-chloro-*N*-(3,4,5-trimethoxyphenyl)propanamide.



Scheme 2. Synthesis, reactions of 3-benzyl-2-mercaptoquinazolin-4(3H)-one and 2-(3-benzyl-6-methyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)acetohydrazide.

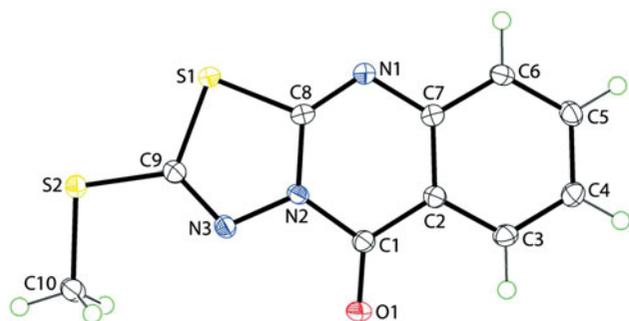


Figure 2. X-ray crystal structure and numbering system of compound **21**.

The compounds **5–11** displayed significant activities in the *in vitro* screening on the tested cell lines in 10 μ M concentration with positive cytotoxic effect (PCE) of 23/53, 52/53, 52/53, 49/49, 41/57, 46/57 and 46/58, respectively, whereas 7/58 for compound **21** (Table 2). Compounds **5–11** showed cytotoxic effects on the most of the cancer cell lines with mean growth inhibition percentages (MGI %) of 10%, 35%, 44%, 50%, 19%, 29% and 34%, respectively (Table 2). Compound **21** showed slight selective activity against HOP-62, NCI-H226, NCI-H522, SNB-75, OVCAR-4, PC-3 and MCF7 cancer cell lines with GI values of 13%, 20%, 11%, 28%, 11%, 11% and 21%, respectively (Table 1).

With regard to broad spectrum antitumor activity, close examination of the data presented in Table 1, revealed that compounds **7**, **8** and **11** are the most active members of this study, showing effectiveness toward numerous cell lines belong to different tumor subpanels. Consequently, compounds **7**, **8** and **11** were selected and tested against a panel of 60 different tumor cell lines at a 5-log dose range^{22–26}. Three response parameters, GI₅₀, TGI, and LC₅₀ were calculated for each cell line, using the known drug 5-Fluorouracil (5-FU) as a positive control. Compounds **7**, **8** and **11** exhibited remarkable growth inhibitory activity pattern against renal cancer (GI₅₀ = 4.26, 3.47 and 12.30, μ M), non-small cell lung cancer (GI₅₀ = 5.65, 3.92 and 7.83 μ M), breast cancer (GI₅₀ = 4.16, 4.16 and 6.16 μ M), ovarian cancer (GI₅₀ = 9.54, 6.30 and 10.23 μ M) and melanoma cancer (GI₅₀ = 6.58, 4.24 and 3.70 μ M), CNS (GI₅₀ = 5.43, 2.50 and 3.38 μ M), prostate cancer (GI₅₀ = 27.22, 4.89 and 18.8 μ M), respectively. Compounds **7**, **8** and **11** are almost 1.5–3.0 fold more active than the positive control 5-FU, with GI₅₀, (10.47, 7.24 and 14.12 μ M), TGI (58.8, 36.30 and 60.25 μ M) and LC₅₀ (>100, 87.09 and 95.49 μ M) values, respectively (Table 3). Comparison of the antitumor activities of compounds **7**, **8** and **11** with the activities of Gefitinib and Erlotinib showed that compounds **7**, **8** and **11** (Table 4) possess activities almost equal to or higher than those of gefitinib and erlotinib against most cell lines except non-small lung (EKVX and NCI-H522), melanoma (SK-MEL-28), ovarian cancer (IGROV1 and SK-OV-3) renal cancer (ACHN and TK-10) and breast cancer (MDA-MB-468). On the other hand,

Table 1. Percentage growth inhibition (GI %) of *in vitro* subpanel tumor cell lines at 10 μ M concentration.

Subpanel tumor cell lines	% Growth inhibition (GI %) ^a							
	5	6	7	8	9	10	11	21
Leukemia								
CCRF-CEM	nt	nt	nt	nt	26	22	–	–
HL-60(TB)	nt	nt	nt	nt	–	–	–	–
K-562	nt	nt	nt	nt	19	33	23	–
MOLT-4	nt	nt	nt	nt	31	40	–	–
PRMI-8226	nt	nt	nt	nt	44	58	17	–
SR	nt	nt	nt	nt	nt	34	–	nt
Non-small cell lung cancer								
A549/ATCC	13	33	34	72	28	41	58	–
EKVX	11	17	16	25	nt	nt	nt	nt
HOP-62	35	–	55	nt	–	43	91	13
NCI-H226	45	54	L	55	nt	69	73	20
HOP-92	10	nt	76	nt	46	nt	nt	–
NCI-H23	–	24	26	31	11	–	–	–
NCI-H322M	11	18	37	35	25	17	29	–
NCI-H460	–	36	43	32	–	19	52	–
NCI-H522	30	43	69	nt	96	L	35	11
Colon cancer								
COLO 205	–	17	12	–	–	–	–	–
HCC-2998	–	10	25	23	–	–	–	–
HCT-116	14	49	57	48	17	48	66	–
HCT-15	–	12	16	10	–	28	24	–
HT29	–	44	30	39	–	24	22	–
KM12	–	49	43	23	13	nt	nt	–
SW-620	–	19	19	–	–	14	16	–
CNS cancer								
SF-268	14	41	31	61	19	–	60	–
SF-295	–	16	21	55	25	34	41	–
SF-539	46	L	89	94	28	74	67	–
SNB-19	–	12	11	37	16	–	27	–
SNB-75	23	47	L	L	45	74	L	28
U251	25	45	43	94	20	30	56	–
Melanoma								
LOX IMVI	–	26	39	32	18	–	29	–
MALME-3 M	17	39	51	55	19	16	67	–
M14	22	27	35	61	–	25	13	–
MDA-MB-435	–	22	33	24	11	12	–	–
SK-MEL-2	–	23	43	55	16	27	48	–
SK-MEL-28	–	22	25	40	–	30	27	–
SK-MEL-5	–	55	61	33	13	26	10	–
UACC-257	–	31	39	80	11	39	43	–
UACC-62	–	57	47	24	34	47	15	–
Ovarian cancer								
IGROV1	–	16	26	25	–	–	–	–
OVCAR-3	–	23	57	31	12	–	33	–
OVCAR-4	–	19	77	52	37	39	L	11
OVCAR-5	–	15	–	–	–	12	–	–
OVCAR-8	55	32	59	L	21	27	71	–
NCI/ADR-RES	–	33	34	nt	18	16	17	–
SK-OV-3	28	28	61	53	–	19	70	–
Renal cancer								
786-0	–	42	57	L	17	–	28	–
A498	17	89	86	L	36	65	33	–
ACHN	–	43	63	34	22	55	47	–
CAKI-1	12	24	18	16	28	37	35	–
RXF 393	29	70	76	64	–	43	64	–
SN12C	–	12	17	31	18	21	11	–
TK-10	–	28	45	L	–	–	–	–
UO-31	23	38	29	52	55	26	29	–
Prostate cancer								
PC-3	–	29	26	65	34	57	17	10
DU-145	–	28	20	46	–	–	17	–
Breast cancer								
MCF7	–	30	30	18	16	30	23	21
MDA-MB-231/ATCC	15	56	65	59	36	49	48	–
HS 578T	45	53	56	76	23	60	94	–
BT-549	28	69	47	87	12	27	–	–
T-47D	–	45	88	27	22	36	65	–
MDA-MB-468	nt	nt	nt	nt	–	–	32	–

nt = not tested; –, GI < 10%; L, compound proved lethal to the cancer cell line.

Table 2. Mean growth inhibition percentage and positive cytotoxic effect of quinazolinone derivatives.

Compd No	60 cell lines assay in one dose 10.0 μ M concentration	
	MGI%	PCE
5	10	23/53
6	35	52/53
7	44	52/53
8	50	49/49
9	19	41/57
10	29	46/57
11	34	46/58
20	0	7/58

PCE: Positive cytotoxic effect; the ratio between number of cell lines with percentage growth inhibition >10% and total number of cell lines.

MGI%: Mean growth inhibition percentage.

compounds **6** and **10** yielded selective activities toward CNS, renal and breast cancer cell lines, compound **9** showed selective activities toward leukemia cell lines, whereas compound **5** possessed moderate antitumor activity.

Regarding the activity toward individual cell lines, compounds **9** and **10** showed selective activity against leukemia cell lines CCRF-CEM, K-562, MOLT-4 and PRMI-8226 with GI values of 26%, 22%, 19%, 23%, 31%, 40% and 44%, 58% respectively. Compound **10** revealed selective activities towards SR leukemia cell lines with GI values of 34%, whereas compound **11** disclosed weak activity against K-562 and PRMI-8226 cell lines with GI values of 23% and 17%.

In non-small cell lung, A549/ATCC cell line proved to be selectively sensitive to **6**, **7**, **8**, **9**, **10** and **11** with GI values of 33%, 34%, 72%, 28%, 41% and 58%, respectively. In addition, compounds **5**, **10** and **11** proved to be susceptible to the HOP-62, NCI-H226 and NCI-H522 cell lines with GI values of 35%, 43%, 91%, 45%, 69%, 73%, 30%, lethal and 35%, respectively. Compounds **6**, **7** and **8** have dedicated activity against NCI-H226, NCI-H23, NCI-H322M and NCI-H460 cell lines with GI values of 54%, lethal, 55%, 24%, 26%, 31%, 18%, 37%, 35%, 36%, 43% and 32% respectively. Compounds **7** and **9** showed strong activity against HOP-92 and NCI-H522 cell lines in 76%, 46%, 69% and 96% respectively, Meanwhile, compounds **10** and **11** showed certain activity against NCI-H322M and NCI-H460 cell lines in 17%, 29%, 19% and 52% respectively, whereas compounds **7**, **8** and **9** showed activity in 55%, 25% and 25% against HOP-62, EKVX and NCI-H322M cancer cells respectively.

In activity against colon cancer, compounds **6**, **7**, **8**, **10** and **11** showed GI values of 49%, 57%, 48%, 48%, 66%, 44%, 30%, 39%, 24% and 22% with colon HCT-116 and HT29 cell lines, respectively, while compounds **7** and **8** demonstrated moderate activities against HCC-2998 cancer cell line with GI value 25% and 23%, respectively. On the other hand, compounds **10** and **11** verified sensitivity in 28% and 24% to colon HCT-15 cancer cells, compounds **6**, **7** and **8** displayed modest activities against KM12 cancer cell with GI value 49%, 43% and 23% respectively.

In investigation of activity toward CNS cancer, compounds **5**, **6**, **7**, **8**, **9**, **10** and **11** showed strong potency against CNS cancer SF-539, SNB-75 and U251 cell lines with GI values of 46%, lethal, 89%, 94%, 28%, 74%, 67%, 23%, 47%, lethal, lethal, 45%, 74%, lethal, 25%, 45%, 43%, 94%, 20%, 30 and 56%, respectively. Compounds **7**, **8**, **9** and **11** showed GI values of 31%, 61%, 19%, 60%, 21%, 55%, 25% and 41% to CNS cancer SF-268 and SF-295 cell lines, respectively. CNS cancer SNB-19 proved to be sensitive to compounds **8** and **11** with GI values of 37% and 27%, while compounds **6** and **10** showed certain potency to SF-268, and SF-295 cancer cells with GI values of 41% and 34% respectively.

Table 3. Compounds **7**, **8** and **11** median growth inhibitory (GI₅₀, μM), total growth inhibitory (TGI, μM) and median lethal (LC₅₀, μM) concentration of *in vitro* subpanel tumor cell lines.

Compd.	Activity	Subpanel tumor cell lines									
		Leukemia	NSC lung cancer	Colon cancer	CNS cancer	Melanoma	Ovarian cancer	Renal cancer	Prostate cancer	Breast cancer	MG-MID ^a
7	GI ₅₀	84.5	5.65	51.9	5.43	6.56	9.54	4.26	27.22	4.16	10.47
	TGI	b	42.65	b	33.65	19.85	60.25	39.81	b	67.09	58.8
	LC ₅₀	b	b	b	97.72	b	b	b	b	b	b
8	GI ₅₀	86.09	3.92	36.43	2.50	4.24	6.30	3.47	4.89	4.16	7.24
	TGI	b	35.89	72.21	84.7	45.47	26.0	26.76	b	27.28	36.30
	LC ₅₀	b	71.03	93.32	56.75	b	93.11	98.0	b	70.79	87.09
11	GI ₅₀	b	7.83	63.09	3.38	3.70	10.23	12.30	18.80	6.16	14.12
	TGI	b	40.36	b	18.28	79.03	87.09	45.49	b	57.32	60.25
	LC ₅₀	b	94.40	b	69.50	b	97.72	b	b	b	95.49
5-FU	GI ₅₀	15.1	b	8.4	72.1	70.6	61.4	45.6	22.7	76.4	22.60
	TGI	b	b	b	b	b	b	b	b	b	b
	LC ₅₀	b	b	b	b	b	b	b	b	b	b

^aFull panel mean-graph midpoint (μM).^bCompounds showed values >100 μM.Table 4. GI₅₀ values (μM) of compounds **7**, **8**, **11**, Gefitinib and Erlotinib over the most cell lines of non-small lung cancer, colon cancer, CNS cancer, melanoma, Ovarian Cancer, Renal Cancer and breast cancer subpanels.

Subpanel tumor cell lines	GI ₅₀ (μM)				
	7	8	11	Erlotinib	Gefitinib
Non-small cell lung cancer					
A549/ATCC	10.5	5.13	8.08	7.94	7.94
EKVX	8.59	5.12	nt	0.05	0.05
HOP-62	2.30	2.03	2.94	12.58	10.00
NCI-H226	3.20	2.66	2.66	6.30	15.84
NCI-H23	8.30	4.17	26.6	19.95	15.84
NCI-H460	7.24	6.72	nt	5.01	6.30
NCI-H522	4.63	3.65	11.3	1.00	6.30
HOP-92	nt	nt	1.90	6.30	7.94
NCI-H322M	nt	nt	70.4	0.05	0.08
NCI-H460	nt	nt	5.31	5.01	6.30
Colon cancer					
COLO 205	>100	15.3	>100	31.62	6.30
HCC-2998	>100	50.6	>100	79.34	10.00
HCT-116	3.38	6.74	4.35	5.01	7.94
HT29	>100	16.6	>100	50.11	3.98
KM12	29.9	>100	>100	63.09	7.94
CNS cancer					
SF-268	6.91	3.57	4.21	19.95	7.94
SF-295	7.01	1.94	nt	15.84	1.99
SF-539	3.46	2.37	2.50	12.58	10.00
SNB-19	21.4	2.93	3.17	3.98	12.58
SNB-75	1.82	1.57	1.90	12.58	6.30
U251	3.93	3.18	7.06	19.95	10.00
Melanoma					
LOX IMVI	55.0	4.71	6.40	5.01	7.94
MALME-3M	4.07	3.30	4.73	5.01	3.16
M14	8.38	16.1	24.4	6.30	5.01
MDA-MB-435	9.55	4.91	>100	15.84	3.16
SK-MEL-2	3.38	3.41	4.40	12.58	12.58
SK-MEL-28	7.04	3.02	6.30	31.62	0.31
SK-MEL-5	3.67	-	32.4	15.84	3.98
UACC-257	4.86	3.55	4.96	100	6.30
UACC-62	2.93	2.35	15.7	1.25	5.01
Ovarian cancer					
IGROV1	32.5	7.34	27.7	0.25	0.20
OVCAR-3	9.36	7.68	5.05	3.16	5.01
OVCAR-4	3.41	2.11	3.95	19.95	7.94
OVCAR-5	58.0	>100	>100	19.95	10.00
OVCAR-8	4.42	3.46	5.01	7.94	10.00

(continued)

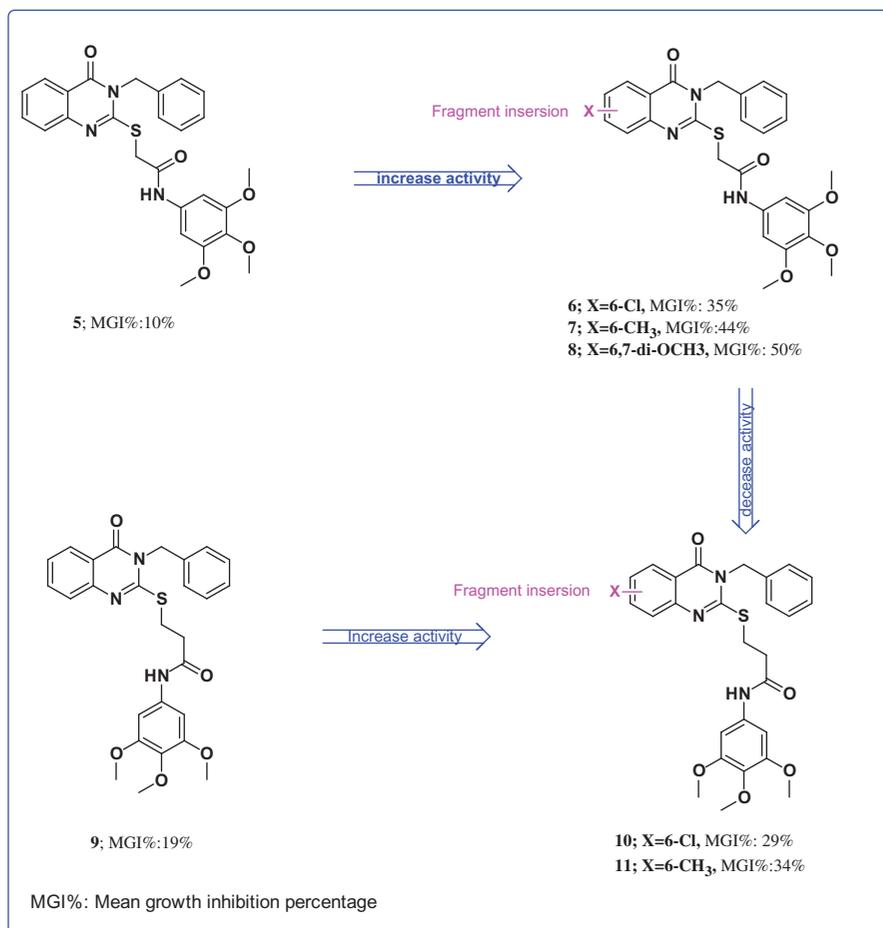
Table 4. Continued

Subpanel tumor cell lines	GI ₅₀ (μM)				
	7	8	11	Erlotinib	Gefitinib
NCI/ADR-RES	8.97	3.30	9.25	6.30	12.58
SK-OV-3	3.06	2.91	4.75	0.39	0.63
Renal cancer					
786-0	3.20	3.51	4.17	5.01	7.94
A498	1.01	1.75	15.8	1.58	0.40
ACHN	3.49	4.63	3.72	0.15	0.20
CAKI-1	5.08	3.05	>100	0.10	0.16
RXF 393	2.24	2.55	3.48	6.30	5.01
SN12C	25.4	4.70	78.0	6.3	6.30
TK-10	5.58	4.06	7.43	0.10	0.10
UO-31	5.97	1.95	11.6	1.99	1.25
Breast cancer					
MCF7	9.98	7.93	32.0	100	10.00
MDA-MB-231/ATCC	3.42	3.73	4.20	1.99	12.58
HS 578T	3.23	2.46	2.18	6.30	10.00
BT-549	2.98	2.02	5.02	39.81	7.94
T-47D	4.01	6.57	7.04	3.16	6.30
MDA-MB-468	4.11	5.72	5.39	0.20	0.01

In activity against melanoma, compounds **6**, **7** and **8** are active against LOX IMVI, MALME-3M, M14, MDA-MB-435, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257 and UACC-62 cell lines with GI values from 22% to 80%. Compound **5** showed moderate activities against M14 cell line in 22% and 25%, whilst compounds **9** and **10** possessed activity against UACC-62 cell line with GI values of 34% and 47%. Melanoma SK-MEL-2, SK-MEL-28 and UACC-257 cell lines were sensitive to compounds **10** and **11** in 27%, 48%, 30%, 27%, 39% and 43%, respectively, at the same time as compound **11** was active to LOX IMVI and MALME-3M cell lines in 29% and 67%, while compound **10** had slight activity against SK-MEL-5 cell line with GI value of 26%.

In investigation of activity toward ovarian cancer, compounds **5**, **6** and **7** showed moderate activities against ovarian OVCAR-8 and SK-OV-3 cell lines with GI values of 55%, 32%, 59%, 28%, 28% and 61% respectively. Ovarian OVCAR-3 and OVCAR-4 cell lines were responsive to compounds **6**, **7** and **8** with GI values of 23%, 57%, 31%, 19%, 77% and 52% respectively, while compounds **9** and **10** were active against OVCAR-8 and OVCAR-4 cell lines with GI values of 21%, 27%, 37% and 39%.

Figure 3. The antitumor activity correlation of the newly synthesized compounds.



respectively. Ovarian NCI/ADR-RES and IGROV1 cell lines were receptive to compounds **6**, **7** and **7**, **8** with GI values of 33%, 34% and 26%, 25%, respectively, additionally compounds **8** and **11** demonstrated lethal activities to OVCAR-8 and OVCAR-4 cell lines, respectively. OVCAR-3, OVCAR-8, SK-OV-3 cell lines were sensitive to compound **11** with GI values of 33%, 71% and 70%, respectively, just as SK-OV-3 cell line was perceptive to compound **8** with GI values of 53%.

In renal cancer, compounds **6**, **7**, **8** and **11** were active against 786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10 and UO-31 cell lines with GI values from 24%-lethal. Renal A498, ACHN, CAKI-1, SN12C and UO-31 cell lines were sensitive to compounds **9** and **10** with GI values of 36%, 65%, 22%, 55%, 28%, 37%, 18%, 21%, 55% and 26%, respectively, whereas RXF 393 cell line was responsive to compounds **5** and **10** with GI values of 29% and 43%, respectively.

Prostate PC-3 and DU-145 cell lines proved to be selectively sensitive to compounds **6**, **7** and **8** with GI value of 29%, 26%, 65%, 28%, 20% and 46%, respectively, while compounds **9** and **10** possessed selective activity towards PC-3 cell line with GI value of 34% and 57%, respectively.

Activity against breast cancer; breast MCF7, MDA-MB-231/ATCC, HS 578T, BT-549, T-47D cell lines possessed convinced response to compounds **6**, **7**, **8**, **9**, **10** and **11** with GI values from 23% to 94%. Compound **5** showed GI effectiveness against breast HS 578T and BT-549 cell lines with values of 45% and 28%, additionally compound **11** had selective activity against breast MDA-MB-468 cell line with GI value of 32%.

Structure activity relationship

The antitumor activity correlation of the newly synthesized compounds (Figure 3) revealed that compound **5** showed

moderate antitumor potency. The introduction of 6-Cl, 6-CH₃ or 6,7-dimethoxy groups at quinazolinone moiety of compound **5** produced compounds **6–8** with dramatically increased the antitumor activity, also the induction of chloro or methyl function at position 6 of quinazolinone nucleus of compound **9** produced compounds **10**, **11** with remarkable improved antitumor potency. Additionally, replacement of the 6-Cl function at quinazolinone ring of compounds **6** and **10** by 6-CH₃ group produced compounds **7** and **11** with significant enhanced antitumor activity, as well substitution of the 6-Cl-function at quinazolinone nucleus of compound **6** with 6,7-dimethoxy functions gave compound **8** with advanced antitumor achievement, which implied that electron donating functions favor the activity rather than electron withdrawing moieties. Moreover, changeover of the 6-CH₃ group of quinazolinone ring of compound **7** with 6,7-dimethoxy group enhanced the antitumor activity of compound **8**. On the other hand, replacement of acetamide function of compounds **6–8** by propanamide function produced compounds **9–11** with reduction of the antitumor activity, which indicated that the length of the carbon chain linking is a vital factor for the antitumor activity. Accordingly, the *N*-substituted phenylacetamides with one carbon length favor the activity (compounds **6–8**) rather than the propanamides with two carbon lengths (compounds **9–11**).

Molecular docking studies

The level of antitumor activities of the compounds **7** and **8** over breast cancer, colon, renal and small lung cancer cells, in which EGFR kinase is highly expressed^{18,19,27}, prompted us to perform molecular docking into the ATP binding site of EGFR kinases to predict if these compounds had analogous binding mode to the EGFR kinase inhibitor. We assumed that the active target

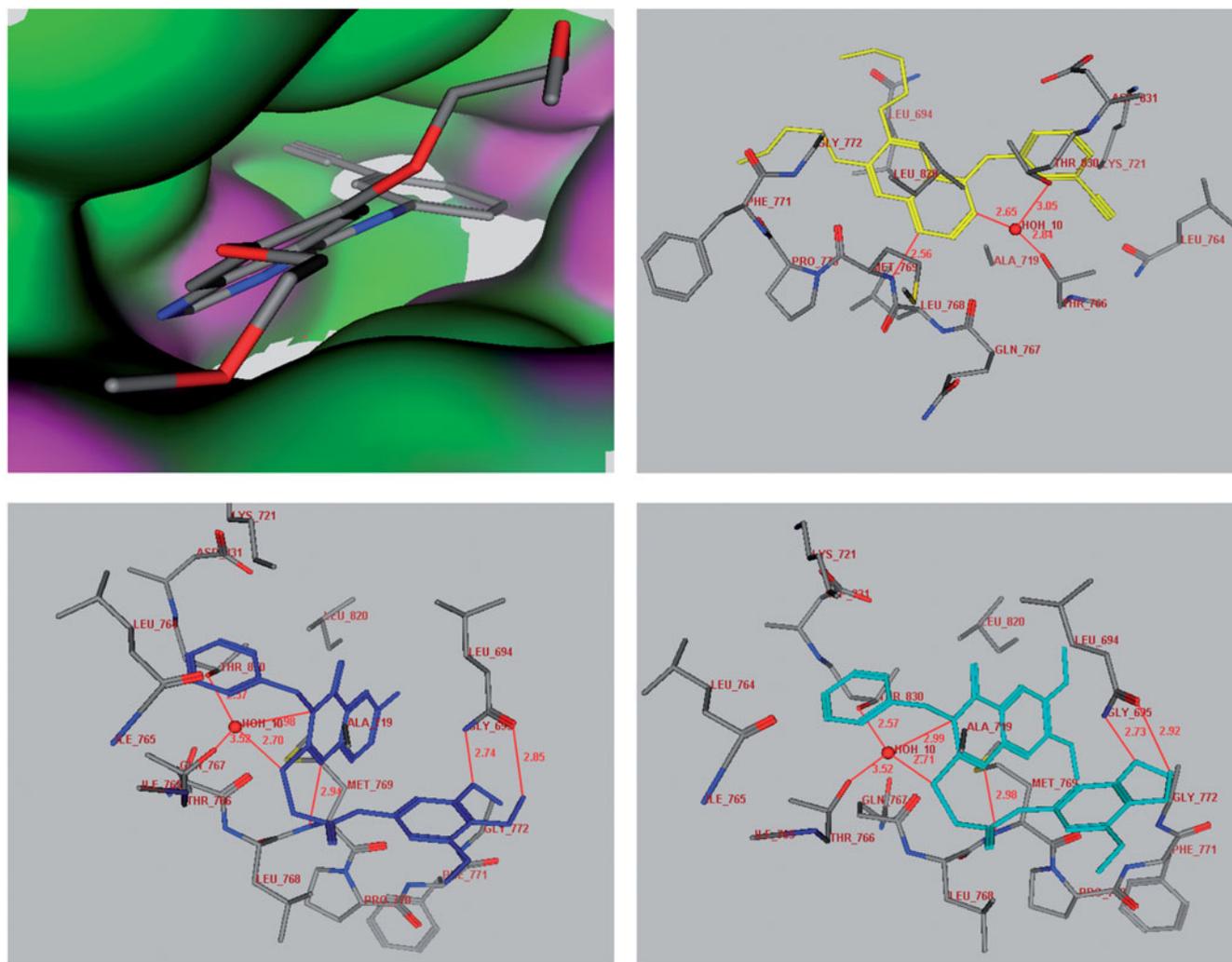


Figure 4. Docking of the erlotinib inhibitor (Upper panel in yellow), compounds **7** (Lower left panel in blue) and **8** (Lower right panel in cyan) into the active site of epidermal growth factor receptor. Hydrogen bonds are shown in red.

compounds **7** and **8** might demonstrate antiproliferative activity against breast cancer, colon, renal and small lung cell lines through inhibition of EGFR. Compounds **7** and **8** were docked into receptor active site of EGFR along with their inhibitor. All calculations were performed using MOE 2008.10 software²⁸ installed on 2.0G Core 2 Duo. The crystal structure of EGFR with erlotinib (*Tarceva*TM) (PDB code: 1M17)^{18,19,29–31} was obtained from protein data bank (PDB). The automated docking program of MOE 2008.10 was used to dock compounds **7** and **8** along with the inhibitor erlotinib into ATP binding site of EGFR. The complexes were energy-minimized with a MMFF94 force field³¹ till the gradient convergence 0.01 kcal/mol was reached. The binding energies of compounds **7**, **8** and erlotinib docked into the active site of EGFR were -21.04 , -22.56 and -24.00 kcal/mol, respectively (Figure 4). These docking studies have revealed that the quinazoline ring binds to a narrow hydrophobic pocket in the N-terminal domain of EGFR-TK where N-1 of the quinazoline ring interacts with the backbone NH of Met-769 via a hydrogen bond, and similarly, a water (HOH-10) molecule-mediated hydrogen bonding interaction is observed among the N-3 and S-atom of the quinazoline ring and the Thr-830 and Thr-766 side chain. These interactions revealed the importance of quinazoline ring for binding and the subsequent inhibitory capacity. Compounds **7**, **8** complexed with EGFR-TK in a fashion similar to erlotinib and showed the occurrence of three hydrogen

bonds with Met-769- (2.94–2.98Å) and HOH-10 (2.70–2.71Å and 2.98–2.99Å)-mediated hydrogen bonding interaction with Thr-830 and Thr-766 side chain. Moreover, the anilide trimethoxyphenyl fragment showed two additional hydrogen bonds with Gly-695 (2.74–2.73Å and 2.85–2.92Å).

On the other hand, the level of antitumor activities of the compound **11** over melanoma cancer cells, in which V600E-B-RAF kinase is highly expressed^{18,19,28}, prompted us to perform molecular docking into the ATP binding site of V600E-B-RAF kinase to predict if this compound **11** has analogous binding mode to the V600E-B-RAF kinase inhibitors. The crystal structure of V600E-BRAF kinase in complex with PLX4032 (PDB code: 3OG7)^{28,32,33} was obtained from protein data bank (PDB). The binding energies of compound **11** and PLX4032 docked into the active site of V600E-BRAF kinase were -30.65 and -36.11 kcal/mol, respectively (Figure 5). The docking study has revealed that the ligand **11** has bound in the active site of one of the protomers in the protein dimer through the formation of four hydrogen bonds with Gln-530 (2.85Å), Cys-532 (2.92Å), Leu-505 (2.13Å) and Thr-529 (2.20Å). Moreover, there was one arene π - π interaction between the binding site and the ligand which occurred between quinazoline fragment and Trp-531. The results of this molecular docking study can support the postulation that our active compound may inhibit the growth of melanoma cell lines through inhibition of B-RAF kinase, similar to PLX4032³³.

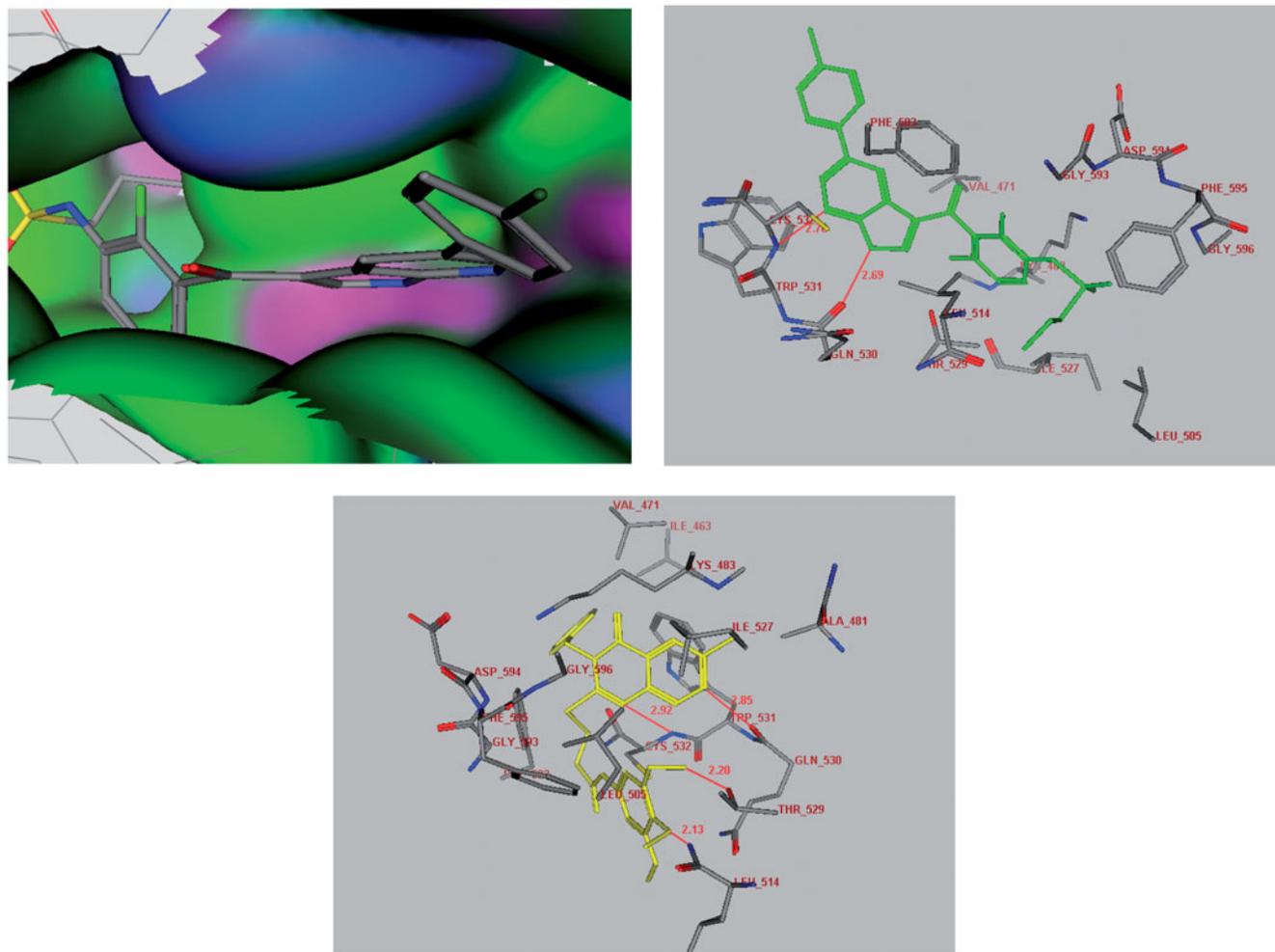


Figure 5. Docking of the PLX4032 inhibitor (Upper panel in green) and compound **11** (Lower panel in yellow) into the V600E-B-RAF kinase domain. Hydrogen bonds are shown in red.

Conclusion

A new derivatives of 3-benzyl-4(3H)-quinazolinones were synthesized and evaluated for their *in vitro* antitumor activity. The results of this study indicated that compounds **7**, **8** and **11** possess amazing broad spectrum antitumor activity with mean GI_{50} (10.47, 7.24 and 14.12 μ M respectively) and are nearly 1.5–3.0 fold more potent compared with the positive control 5-FU with mean GI_{50} 22.60 μ M.

Declaration of interest

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Supplementary material available online
NMR Chart for the synthesized compounds