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Synthesis and Anticancer Activity Evaluation of New 1,2,4-Triazolyl-Quinazoline Hybrid Compounds and Their Pyrazolopyridine Analogs

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract
1,2,4-Triazolopyrimidinen and their isostericanalogs such as triazolopyridine have possessed their interest in, the literature, due to the reported broad spectrum of bioactivities, one of which is the anticancer activity. In the current study, new functionalized 1,2,4-triazoles-based substituted quinazolinesystems were prepared via multistep reactions starting from simple starting compounds. The reactions lead to the formation of the bi- and tricyclic 1,2,4-triazolopyridine and 1,2,4-triazolopyrimidine compounds from the S-alkyl hydrazide, the ester and the hydrazine derivatives of the quinazoline system, respectively. The structures were characterized and confirmed by NMR, mass, and IR spectra. The anticancer activity revealed that several of the triazolopyrimidines-basedquinazoline structures especially those with theN-alkyl substitution in the quinazoline system were the most potent derivatives with results comparable to doxorubicin reference drug, the reference potent compound in the current investigation.
Keywords: quinazoline, triazolopyrimidine, triazolopyridine, hydrazide, anticancer.

Introduction

Cancer is still one of the most important risks threatening large numbers of people who are infected with it and find treatment difficult. Concerted efforts to intensify and increase research aimed at discovering and finding a basis for chemical compounds to combat cancer have become an urgent matter to defeat this disease that threatens human health. The latter hypothesis is related to a strategy representing one of the most important approaches followed widely in medicinal chemistry research concerning anticancer candidates [1]. Quinazoline-containing compounds have been shown in the literature to possess anti-cancer [2, 3], anti-bacterial [4, 5], anti-psychotic [6], anti-fungal [7, 8], anti-inflammatory [9, 10], anti-diabetic [11], anti-malarial, anti-tuberculosis, antiviral, anti-obesity, anticonvulsant, antitumor activities and other applied fields.

The 1,2,4-triazole core is a vital component found in many different molecules with various applications including organic catalysis, pharmaceutical chemistry, and science of materials [12, 13].The importance of this heterocycle core is presented in the improvement of different functional ways to develop the 1,2,4-triazole derivatives. The main classification of 1,2,4-triazolopyridines contains 5-heterocyclic groups, three layouts of them are 1,2,3-triazoles and two of them are 1,2,4-triazoles [14]. Three have bridgehead nitrogen and two don't have. The triazole and its derived bicyclic, either condensed or isolated, acquired great interest as a biologically active motif[15-19].The triazolopyrimidine nucleus results from fusing the triazole moiety, a five-membered aromatic ring system containing three nitrogen atoms, with the pyrimidine moiety [20, 21]. The 1,2,4-triazolopyridine and 1,2,4-triazolopyrimidine quinazoline derivatives have highly biological activities [22, 23].

The molecular hybridization approach acquired considerable interest in the design of new candidate bioactive leads with possible anticancer activity[24-27]. Our hypothesis is the design of new hybrid products with more than one heterocyclic core in one hybrid structure. According to the above significances, we report here the synthesis of several novel quinazolinetriazolopyridine-based structures with investigation of their anti-cancer activity.

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2. Material and Methods

2.1. Chemistry

The utilized chemicals, solvents, and reagents were supplied from commercial suppliers and were of analytical grade (Sigma Aldrich, Fluka, Acros, BDH, or Merk), and employed without any additional purification. Infrared spectra were performed on a Nicolet FT-IR spectrophotometer in the range 4000-400 cm^{-1}. The ^1H NMR spectra were recorded in DMSO-d_6 at 300 MHz using a Varian Gemini 200 NMR spectrometer. Fast Atom Bombardment (FAB) mass spectra for the ligands were carried out on a Shimadzu Qp-2010 Plus spectrometer. Melting points have been measured by utilizing the Stuart melting point apparatus.Analysis of the elements (C, H, and N) was performed on a Perkin Elmer-2400 elemental analyzer at micro analytical center of Cairo University. The starting was prepared as reported by the isothiocyanate derivatives namely, ethyl isothiocyanate and 2-aminobenzoic acid [28].

Synthesis of 3-Substituted-2-hydrazinylquinazolin-4(3H)-one (2a,b)

Hydrazine hydrate (80%) (5 mmol) was added to a solution of quinazoline (1a or 1b) (1 mmol) and refluxed for 5 hours. Compounds 2a,b were obtained by filtering and washing the precipitated products with 100% ethanol after they had cooled [29].

2-Hydrazinyl-3-phenylquinazolin-4(3*H*)-one (2a)

White powder;mp: 204–205°C (lit. [29] 203-204 °C); Yield: 78%;IR (υ cm^{-1}): 3330 (NH_2), 3205 (NH), 1690 (C=O), 1616 (C=N); ^1H NMR (DMSO-d_6) δ (ppm): 8.22 (br s, 1H, NH), 7.90 (d, 1H, *J* = 7.8 Hz, H-5), 7.57 (t, 1H, *J* = 7.4 Hz, H-7), 7.55 (t, 2H, *J* = 7.2 Hz, H-3',5'), 7.54 (d, 2H, *J* = 7.6 Hz, H-2',6'), 7.28 (d, 1H, *J* = 7.8 Hz, H-8), 7.11 (t, 1H, *J* = 7.0 Hz, H-6), 7.08 (t, 1H, *J* = 7.2 Hz, H-4"), 4.37 (br s, 2H, NH_2); Anal. Calcd. C, 66.65; H, 4.79; N, 22.21 for $C_{14}H_{12}N_4O$ (252.27). Found: H, 4.65; N, 22.18; C, 66.63.

3-Quinazolin-4(3H)-one ethyl-2-hydrazinyl(2b)

White powder;mp: 202–203°C (lit. [29] 201-202 °C); yield: 76%, Internal reflection (υ cm^{-1}): 3335 (NH_2), 3190 (NH), 1690 (C=O), 1612 (C=N); ^1H NMR (DMSO-d_6) δ (ppm): 8.18 (br s, 1H, -NH), 7.56 (t, 1H, *J* = 7.0 Hz, H-7), 7.30 (d, 1H, *J* = 7.8 Hz, H-8), 7.10 (t, 1H, *J* = 7.6 Hz, H-5), 7.30 (d, 1H, *J* = 7.8 Hz, H-8),2.45 (q, 2H, *J* = 5.8 Hz, CH_2), 1.15 (t, 3H, *J* = 5.8 Hz, CH_3), 4.39 (br s, 2H, NH_2), and Anal. Calcd.;H, 5.92; N, 27.43; and C, 58.81 for $C_{10}H_{12}N_4O$ (204.23). Found: H, 5.75; N, 27.40; C, 58.80.

4-Substituted-1-mercapto-[1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one (3,4)

An ice-cooled solution of the hydrazide 2a,b (5 mmol) in absolute ethanol (20 mL) was progressively swirled in with a well-stirred solution of potassium hydroxide (10 mmol, 0.56 g) in absolute ethanol (10 mL). The later solution was then supplemented with CS_2 (20 mmol, 1.8 mL) dropwise, and the combination was then agitated at room temperature for an entire night. To obtain triazolopyrimidines 3 and 4, the precipitated solid that had formed was filtered out, rinsed with cold, dry diethyl ether, and then dried.

1-Quinazolin-5(4*H*)-one, metracho-4-phenyl-[1,2,4]triazolo[4,3-a] (3)

Chalky powder, yielding 73%; melting point: 315–317°C.IR (υ cm^{-1}): 3195 (NH), 1498 (C=S), 1672 (C=O); ^1H NMR (DMSO-d_6) δ (ppm): 10.44 (br s, 1H, -NH), 7.99 (d, 1H, *J* = 7.4 Hz, H-5), 7.88 (t, 1H, *J* = 7.0 Hz, H-7), 7.79 (t, 1H, *J* = 7.2 Hz, H-6), 7.50 (d, 2H, *J* = 7.6 Hz, H-2',6'), 7.43 (t, 2H, *J* = 7.0 Hz, H-3',5'), 7.28 (d, 1H, *J* = 7.4 Hz, H-8), 7.15 (d, 1H, *J* = 7.6 Hz, H-4'); ^{13}C NMR (DMSO-d_6) δ (ppm): 188.0 (C=S), 160.00 (C=O), 158.0 (C-2), 136.7 (C-1), 135.0 (C-9), 134.0 (C-6), 133.0 (C-7), 132.5 (C-10), 131.2 (C-5), 128.2 (C-2',6'), 125.9 (C-3',5'), 120.03 (C-4'); Anal. Calcd. C, 61.21; H, 3.42; N, 19.04 for $C_{15}H_{10}N_4OS$ (294.33). Found: H, 3.32; N, 19.04; C, 61.17.

4-Ethyl-1-mercapto-[1,2,4]triazolo[4,3-a]quinazolin-5(4*H*)-one (4)

Yellowish powder. Yield: 70%; m.p.173–175°C; IR (υ cm^{-1}): 3202 (NH), 1674 (C=O), 1610 (C=N); ^1H NMR (DMSO-d_6) δ (ppm): 11.2 (s, 1H, NH), 7.89 (d, 1H, *J* = 7.6 Hz, H-5), 7.51 (t, 1H, *J* = 7.0 Hz, H-7),7.22 (t, 1H, *J* = 7.2 Hz, H-6), 7.06 (d, 1H, *J* = 7.8 Hz, H-8), 3.32 (q, 2H, *J* = 6.2 Hz, CH_2), 1.13 (t, 3H, *J* = 6.2 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ (ppm): 171.0 (C=S), 164.0 (C=O), 151.0 (C-2), 134.4 (C-9), 133.2 (C-6), 128.0 (C-7), 133.0 (C-7), 126.5 (C-10), 125.6 (C-5), 122.0 (C-8), 39.9 (CH_2), 15.2 (CH_3); Anal. Calcd. C, 53.64; H, 4.09; N, 22.75; for $C_{11}H_{10}N_4OS$ (246.29). Found: H, 4.00; N, 22.45; C, 53.54.

Ethyl 2-((3-substituted-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetate (5a,b)

After 30 minutes of stirring, an equimolar combination of quinazoline 1a,b (20 mmol), and anhydrous potassium carbonate (20 mmol, 2.76 g) in dry DMF (20 mL) was added. Next, ethyl chloroacetate (20 mmol, 2.45 g) was added. Following a 24-hour stirring period at 70 °C, the reaction mixture was allowed to cool to room temperature before being added to ice-cold water. To obtain compounds 5a and 5b, respectively, the white precipitate that resulted was filtered out and refined by crystallization from ethanol.

Ethyl 2-((4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)thio)acetate(5a)

White powder, 92% yield; mp: 112-113 °C (lit. [30] 108-110 °C); IR (υ cm^{-1}), 1739 (C=O), 1687 (C=O), 1603 (C=N), 1554 (C-S); ^{1}H NMR ($CDCl_3$-d_1) δ (ppm): 8.22 (d, 1H, *J* = 7.8 Hz, H-5), 7.73 (t, 1H, *J* = 7.0 Hz, H-7), 7.55 (d, 1H, *J* = 7.6 Hz, H-8), 7.54 (t, 2H, *J* = 6.8 Hz, H-3',5'), 7.40 (d, 2H, *J* = 7.4 Hz, H-2',6'), 7.37 (t, 1H, *J* = 7.0 Hz, H-4'), 7.38 (t, 1H, *J* = 7.2 Hz, H-6), 4.22 (q, 2H, *J* = 5.8 Hz, CH_2), 3.89 (s, 2H, CH_2), 1.31 (t, 3H, *J* = 5.8 Hz, CH_3); Anal. Calcd. N, 8.23; H, 4.74; C, 63.51; for $C_{18}H_{16}N_2O_3S$ (340.40). Found: H, 4.55; N, 8.20; C, 63.48.

Ethyl 2-((3-ethyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetate (5b)

White powder, Yield: 90%; mp: 95-96 °C(lit. [30] 98-99 °C); ^{1}H NMR ($CDCI_3$-d_1) δ (ppm): 8.18 (d, 1H, *J* = 7.8 Hz, H-5), 7.64 (t, 1H, *J* = 7.0 Hz, H-7), 7.35 (d, 1H, *J* = 7.6 Hz, H-8), 7.25 (t, 1H, *J* = 6.8 Hz, H-6), 4.22 (q, 2H, *J* = 5.2 Hz, CH_2), 4.20 (s, 2H, CH_2), 4.01 (q, 2H, *J* = 5.8 Hz, CH_2), 1.39 (t, 3H, *J* = 5.2 Hz, CH_3), 1.29 (t, 3H, *J* = 5.8 Hz, CH_3), Anal. Calcd. H, 5.52; N, 9.58; and C, 57.52 for $C_{14}H_{16}N_2O_3S$ (292.35). C, 57.51; H, 5.50; N, 9.51 were found.

Synthesis of 4-Substituted-1-hydra4zinyl-[1,2,4] triazole [4,3-a]quinazolin-5(4H)-one (6,7)

A solution of ester (5a or 5b) (10 mmol) in 100% methanol (10 ml) was mixed with 20 ml of sodium methoxide and 10 mmol of thiocarbohydrazide. The reaction mixture was put onto crushed ice, refluxed for 20 hours, and then acidified with HCl. After filtering out the precipitate, washing it with water, drying it, and recrystallizing it from the ethanol, compounds 6 or 7 dark yellow powder was obtained.

1-Hydrazinyl-4-phenyl-[1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one (6)

Powder that is dark yellow, yielding 77% at 253-255°C. ^{1}H NMR (DMSO-d_6) δ (ppm): 10,04 (br s, 1H, -NH), 8.13 (d, 1H, *J* = 7.6 Hz, H-5), 7.53 (t, 1H, *J* = 7.2 Hz, H-7), 7.50 (t, 1H, *J* = 7.4 Hz, H-6), 7.42 (d, 2H, *J* = 7.6 Hz, H-2',6'), 7.31 (t, 2H, *J* = 7.0 Hz, H-3',5'), 7.25 (d, 1H, *J* = 7.5 Hz, H-8), 7.21 (t, 1H, *J* = 7.0 Hz, H-4'), 4.98 (br s, 2 H, NH_2); IR (υ cm-1), 3418.17 (NH_2), 3369.31 (NH), 1650.0 (C=O), 1601.55 (C=N); Anal. Calcd. C, 61.64; H, 4.14; N, 28.75; Found: C, 61.62; H, 4.10; N, 28.55 for $C_{15}H_{12}N_6O$ (292.30).

4-Ethyl-1-hydrazinyl-[1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one (7)

Dark yellow powder; melting point: 174–175°C; yield: 74%. ^{1}H NMR (DMSO-d_6) δ (ppm): 10,48 (br s, 1H, NH) , 8.12 (d, 1H, *J* = 7.8 Hz, H-5), 7.60 (t, 1H, *J* = 7.0 Hz, H-7), 7.25 (d, 1H, J = 7.6 Hz, H-8), 7.22 (t, 1H, *J* = 7.2 Hz, H-6), 4.18 (br s, 2 H, NH_2), 4.16 (q, 2H, *J* = 5.8 Hz, CH_2), 1.30 (t, 3H, *J* = 6.2 Hz, CH_3); Anal. Calcd. H, 4.95; N, 34.41; C, 54.09; for $C_{11}H_{12}N_6O$ (244.25). Found: C,54.00;H 4.85;N, 34.41.

Synthesis of 2-((3-Substituted-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetohydrazide (8a,b)

Compound (5a or 5b) (0.01mol) was dissolved in 20 milliliters of absolute ethanol, and hydrazine hydrate (80%) (0.05mol) was then added gradually. After agitating the mixture for a full day, the end product was properly cleaned with water, allowed to dry, and then recrystallized with ethanol to yield compounds 8a and 8b, respectively.

2-((4-Oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)thio) acetohydrazide (8a)

White powder; stated yield: 86%; melting point: 170–172°C(lit. [30] 173-175 °C);^{1}H NMR ($CDCL_3$-d_1) δ (ppm): 9.36 (s, 1H, -NH), 8.25 (d, 1H, *J* = 7.8 Hz, H-5), 7.77 (t, 1H, *J* = 7.2 Hz, H-7), 7.60 (d, 1H, *J* = 7.5 Hz, H-8), 7.55 (d, 2H, *J* = 7.0 Hz, H-3',5'), 7.46 (d, 2H, *J* = 7.5 Hz, H-2',6'), 7.32(t, 1H, *J* = 7.2 Hz, H-6), 7,25 (t, 1H, *J* = 7.0 Hz, H-4'), 4.30 (br s, 2H, NH_2), 3.70 (s, 2H, CH_2); Anal. Calcd. for $C_{16}H_{14}N_4O_2S$ (326.37): C, 58.88; H, 4.32; N, 17.17. Found: H, 4.30; N, 17.10; C, 58.82.

2-((3-Ethyl-4-oxo-3,4dihydroquinazolin-2-yl)thio)acetohydrazide (8b)

White powder; stated yield: 83%; melting point: 155–157°C (lit.[30] 152-154 °C); ^{1}H NMR ($CDCL_3$-d_1) δ (ppm): 8.38 (s, 1H, -NH), 8.21 (d, 1H, *J* = 7.6 Hz, H-5), 7.69 (t, 1H, *J* = 7.0Hz, H-7), 7.52 (d, 1H, *J* = 7.4 Hz, H-8), 7.25 (t, 1H, *J* = 6,2 Hz, H-6), 4.43 (br s, 2H, NH_2), 4.18 (s, 2H, CH_2), 3.90 (q, 2H, *J* = 6,5 Hz, CH_2), 1.36 (t, 3H, *J* = 6.8 Hz, CH_3); Anal. Calcd. N, 20.13; H, 5.07; C, 51.78; for $C_{12}H_{14}N_4O_2S$ (278.33). Found: H, 5.00; N, 20.02; C, 51.71.

Synthesis of Ethyl 2,7-bis(4-chlorophenyl)-6-((3-substituted-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-5-oxo-1,5,6,8a-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-8-carboxylate (9,10)

Five drops of tri ethyl amine were added to a solution of hydrazide (8a or 8b) (10 mmol), ethyl cyanoacetate (1.1 ml, 10 mmol), and p-chlorobenzaldehyde (10 mmol) in pure ethanol (20 ml). After 30 minutes of stirring the reaction mixture, the precipitate was removed by filtering, drying, and recrystallizing it from the ethanol to get triazolopyridine compounds 9 or 10, respectively.

Ethyl 2,7-bis(4-chlorophenyl)-5-oxo-6-((4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)thio)-1,5,6,8a-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-8-carboxylate (9)

Amorphous white powder with a yield of 86%, mp of 205-206°C, and ^{1}H NMR ($CDCL_3$-d_1) δ (ppm):9.68 (br s, 1H, -NH), 8.29 (d, 1H, *J* = 7.8 Hz,

H-5), 8.22 (t, 1H, *J* = 7.0 Hz, H-7), 8.20 (d, 1H, *J* = 7.6 Hz, H-8), 7.87 (t, 1H, *J* = 7.5 Hz, H-6), 7.74 (d, 2H, *J* = 7.6 Hz, H-3"',5'"), 7.63 (d, 2H, *J* = 7.5 Hz, H-2',6'), 7.61 (d, 2H, *J* = 7.6 Hz, H-3",5"), 7.58 (d, 2H, *J* = 7.2 Hz, H-2",6"), 7.43 (d, 2H, *J* = 7.6 Hz, H-2',6'), 7.33 (t, 2H, *J* = 7.0 Hz, H-3',5'), 4.51 (s, 1H, CH), 4,31 (s, 1H, CH), 4.16 (q, 2H, *J* = 6.2 Hz, CH2), 1.29 (t, 3H, *J* = 6.8 Hz, CH3); Anal. CoCalcd. C, 61.59; H, 3.69; N, 10.26 for $C_{35}H_{25}C_{l2}N_5O_4S$ (682.58). Found: H, 3.63; N, 10.20; C, 61.55.

Ethyl 2,7-bis(4-chlorophenyl)-6-((3-ethyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-5-oxo-1,5,6,8a-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-8-carboxylate (10)

White amorphous powder; Yield: 84%; mp: 187-189°C; ^{1}H NMR ($CDCL_3$-d_1) δ (ppm): 9.97 (br s, 1H, -NH), 7.80 (t, 1H, *J* = 7.6 Hz, H-7), 7.60 (d, 1H, *J* = 7.0 Hz, H-8), 7.58 (d, 2H, *J* = 7.5 Hz, d, 2H, *J* = 7.2 Hz, H-2",6"), 7.40 (d, 2H, *J* = 7.8 Hz, H-2',6'), 7.34 (t, 1H, *J* = 7.2 Hz, H-6), 7.29 (d, 2H, *J* = 7.0 Hz, H-3',5'), 4.61 (s, 1H, -NCH), 4,45 (s, 1H, -SCH), 4.24 (q, 2H, *J* = 5.8 Hz, CH_2 ester), 4.22 (q, 2H, *J* = 6.5 Hz, CH_2), 1.39 (t, 3H, *J* = 5.2 Hz, CH_3), ^{13}C NMR ($CDCL_3$-d_1) δ (ppm): 180.67 (C=O ester), 170.36 (C=O-N), 161.41 (C-4), 154.20 (N=C-S), 149.6 (N=C-NH), 146.50 (C-10), 143.70 (C=C-COOEt), 135.7 (C-4"), 135.6 (C-4'), 135.26 (C-1'), 133.50 (C-3",5"), 130.50 (C-1"), 129.23 (C-2",6"), 129.04 (C-4'), 128.52 (C-3',5'), 127.80 (C-2',6'), 127.06 (C-6), 125.98 (C-5,8), 121.3 (C-COO), 119.52 (C-9), 56.0 (CH), 40.91 (CH), 40.22 (CH_2), 40.12 (CH_2), 13.43 (CH_3), 13.33 (CH_3); Anal. Calcd. C, 58.68; H, 3.97; N, 11.04 for $C_{31}H_{25}C_{l2}N_5O_4S$ (634.53). Found: H, 3.85; N, 11.04; C, 58.64.

Synthesis of 2,7-Bis(4-chlorophenyl)-6-((3-substituted-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-5-oxo-1,5,6,8a-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-8-carbonitrile (11,12)

Five drops of tri ethyl amine were added to a mixture of hydrazide (8a or 8b) (10mmol), malononitrile (10mmol, 0.66g), and p-chlorobenzaldehyde (10mmol, 1.40g) in pure ethanol (20ml). For thirty minutes, the reaction mixture was agitated. Triazolo pyridine products 11 or 12, respectively, were obtained by filtering out, drying, and recrystallizing the precipitate that resulted from the reaction with ethanol.

Bis(4-chlorophenyl)-5-oxo-6-((4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)thio)-1,5,6,8a-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-8-carbonitrile (11*)*

White amorphous powder; Yield: 90%; mp:207-209°C; ^{1}H NMR ($CDCL_3$-d_1) δ (ppm): 9.64 (br s, 1H, -NH), 8.25 (d, 1H, *J* = 7.6 Hz, H-5), 7.85 (t, 1H, *J* = 7.2 Hz, H-7), 7.79 (d, 1H, *J* = 7.8 Hz, H-8), 7.73 (d, 2H, *J* = 7.5 Hz, H-3"',5'"), 7.70 (d, 2H, *J* = 7.6 Hz, H-2"',6'"), 7.65 (d, 2H, *J* = 7.2 Hz, H-3",5"), 7.56 (d, 2H, *J* = 7.8 Hz, H-2",6"), 7.50 (d, 2H, *J* = 7.5 Hz, H-3',5'), 7.45 (d, 2H, *J* = 7.2 Hz, H-2',6'), 7.33 (t, 1H, *J* = 7.0 Hz, H-6), 7.25 (t, 1H, *J* = 7.2 Hz, H-4'), 4.47 (s, 1H, CH), 4,30 (s, 1H, CH); Anal. Calcd. C, 62.37; H, 3.17; N, 13.22 for $C_{33}H_{20}C_{l2}N_6O_2S$ (635.52). Found: H, 3.12; N, 13.10; C, 62.31.

2,7-Bis(4-chlorophenyl)-6-((3-ethyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-5-oxo-1,5,6,8a-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-8-carbonitrile (12)

Amorphous white powder with a yield of 88%, mp of 185-187°C, and ^{1}H NMR($CDCL_3$-d_1) δ (ppm) of 8.27 (br s, 1H, -NH) ,7.81 (t, 1H, *J* = 7.4 Hz, H-7), 7.60 (d, 1H, *J* = 7.5 Hz, H-5), 8.15 (d, 1H, *J* = 7.8 Hz, H-5), 7.88 (m, 1H, H-8), 7.46 (d, 2H, *J* = 7.8 Hz, H-3",5"), 7.43 (d, 2H, *J* = 7.3 Hz, H-3',5'), 7.26 (d, 2H, *J* = 7.5 Hz, H-2',6'), 4.67 (s, 1H, CH), 4.45 (s, 1H, CH)), 4.28 (q, 2H, *J* = 6.8Hz, CH_2), 1.41 (t, 3H, *J* = 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$-d_1) δ (ppm): 180.18 (-NCO), 147.8 (C-2), 161.84 (C-4), 158.50 (-NCS), 151.5 (-NCNH), 146.90 (C-10), 143.70 (C=C-CN), 135.5 (C-4"), 134.89 (C-4'), 134,45 (C-1'), 133.99 (C-7), 130.90 (C-1"), 129.30 (C-2",6"), 129.11 (C-3",5"), 128.7 (C-3',5'). 121.20 (C-9), 127.59 (CN), 99.98 (-CCN), 52.54 (HC-NH), 40.47 (-SCH), 33.31 (-NCH2), 13.46 (CH3), 127.08 (C-6), 126.80 (C-5,8), 121.20 (C-9), 119.60 (CN), and Anal. Calcd. C, 59.29; H, 3.43; N, 14.31 for $C_{29}H_{20}C_{l2}N_6O_2S$ (587.48). Found: H: 3.24; N: 14.20; C: 59.20.

Synthesis of 2-((3-Substituted-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetic acid (13a,b)

A mixture of quinazoline (1a or 1b) (20mmol) and anhydrous potassium carbonate (2.76g,20mmol) in dry DMF (30ml) was allowed to stir for 30 minutes, and then, chloroacetic acid (20mmol) was added. Then stirring was continued for 36 h at 100 ˚C then the mix was cooled to the standard temperature and submerged in ice-cold water. An off-white residue was collected by filtration and purified by ethanol to get compounds 13a or 13b respectively.

2-((4-Oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)thio)acetic acid (13a)

An off-white powder was reported; yield: 78%; melting point: 180–182°C (lit.[31] 183-185 °C); IR (υ cm^{-1}); 1644 (C=O), 1609 (C=N), and 1553 (C-S). AnalCalcd. C, 61.53; H, 3.87; N, 8.97 for $C_{16}H_{12}N_2O_3S$ (312.34). Found: H, 3.65; N, 8.78; C, 61.46.

2-((3-Ethyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetic acid (13b)

a powder that is off white; stated Yield: 75%; melting point: 113–115°C (lit. [31] 110-112 °C);Anal.Calcd. C, 54.53; H, 4.58; N, 10.60 for

$C_{12}H_{12}N_2O_3S$ (264.30). Found: H, 4.47; N, 10.40; C, 54.51.

Synthesis of Prop-2-yn-1-yl 2-((3-substituted-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetate (14,15)

By adding propargyl alcohol (2.5 mmol) dropwise to a chloroacetic solution in a flask of acid derivatives (2 mmol) in 30 ml of 100% ethanol and one milliliter of sulfuric acid were heated to 80°C for 36 hourswhile swirling constantly. Compounds 14 and 15, respectively, were produced by washing the reaction mixture with 100% ethanol after it had reached room temperature and the residue had been filtered off.

Prop-2-yn-1-yl 2- (4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)thio)acetate(14)

Yellow powder; yield: 90%; mp: 253-255°C, ^{1}H NMR (DMSO-d_6) δ (ppm): 11.52 (s, 1H, OH), 7.92 (d, 1H, *J* = 7.6 Hz, H-5), 7.68 (t, 1H, *J* = 7.2 Hz, H-7), 7.47 (t, 1H, *J* = 7.4 Hz, H-6), 7.40 (d, 2H, *J* = 7.8 Hz, H-8), 7.30 (t, 2H, *J* = 7.5 Hz, C-2',6'), 7.28 (t, 2H, *J* = 7.0 Hz, H-3',5'), 7.19 (t, 1H, *J* = 7.2 Hz, H-4'), 3.31 (s, 2H, CH_2); Anal.Calcd. C, 65.13; H, 4.03; N, 7.99 for $C_{19}H_{14}N_2O_3S$ (350.39). Found: H = 4.00; N = 7.85; C = 65.05.

Prop-2-yn-1-yl2-((3-ethyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetate (15)

Gray powder; Yeild: 85%; mp: 170-172°C, ^{1}H NMR (DMSO-d_6) δ (ppm): 11.36 (s, 1H, OH),7.89 (d, 1H, *J* = 7.8 Hz, H-5), 7.62 (t, 1H, *J* = 7.0 Hz, H-7), 7.59 (d, 1H, *J* = 7.5 Hz, H-8), 7.17 (t, 1H, *J* = 7.3 Hz, H-6), 3.91 (q, 2H, *J* = 6.8 Hz, CH_2), 3.34 (s, 2H, CH_2), 1.12 (t, 3H, *J* = 6.8 Hz, CH_3); Anal. Calcd. C, 59.59; H, 4.67; N, 9.27 for $C_{15}H_{14}N_2O_3S$ (302.35). Found: H, 4.56; N, 9.10; C, 59.48.

2.2. Cytotoxic activity

Roswell Park Memorial Institute (RPMI) 1640 medium was purchased from Sigma Chem. Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and fetal calf serum (FCS) were purchased from Gibco, UK.Dimethyl sulfoxide (DMSO) and methanol were of HPLC grade, and all other reagents and chemicals were of analytical reagent grade.

2.2.1. In vitro anticancer activity:
Cell culture

HepG-2(Human liver carcinoma), HCT116 (human colorectal carcinoma), MCF-7 (human breast adenocarcinoma), and the normal human skin fibroblast(BJ-1) cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained in RPMI-1640 medium which was supplemented with 10% heat-inactivated FBS, 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2. All experiments were conducted thrice in triplicate (n = 3). All the values were represented as means ± SD.

Lactate dehydrogenase (LDH) assay

To determine the effect of each synthesized compound on membrane permeability in HepG2, MCF-7, and HCT-116 cancer cell lines as well as BJ-1 normal cell line, a lactate dehydrogenase (LDH) release assay was used[32-36]. The cells were seeded in 24-well culture plates at a density of 1×10^4 cells/well in 500 µL volume and allowed to grow for 18h before treatment. After treatment with a series of different concentrations of each compound or Doxorubicin® (positive control), the plates were incubated for 48h. Then, the supernatant (40 µL) was transferred to a new 96 well to determine LDH release and 6% triton X-100 (40 µL) was added to the original plate for determination of total LDH. An aliquot of 0.1 M potassium phosphate buffer (100 µL, pH 7.5) containing 4.6 mM pyruvic acid was mixed with the supernatant using repeated pipetting. Then, 0.1 M potassium phosphate buffer (100 µL, pH 7.5) containing 0.4 mg/mL reduced β-NADH was added to the wells. The kinetic changes were read for 1 min using an ELISA microplate reader in absorbance at wavelength 340 nm. This procedure was repeated with 40 µL of the total cell lysate to determine total LDH. The percentage of LDH release was determined by dividing the LDH released into the media by the total LDH following cell lysis in the same well.

2.2.2. Statistical analysis

All experiments were conducted in triplicate (n = 3). All the values were represented as mean ± SD. Significant differences between the means of parameters as well as IC_{50}s were determined by probit analysis using the SPSS software program (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Chemistry

The design and synthesis of new fused bicyclic and tricyclic systems incorporating the quinazoline and 1,2,4-triazole cores were based on starting with simply available compounds which were functionalized with active side chain centers for the formation of the suitable precursors. The latter can easily undergo heterocyclization to the desired products. Thus, the reaction of the starting *N*-substituted quinazoline-thione compound **1** with hydrazine hydrate afforded the corresponding derived 2-hydrazinyl products **2a,b**[37].The formedquinazolinehydrazinyl derivatives were then allowed to react with carbon disulfide in a basic medium and the4-substituted-1-mercapto-[1,2,4]triazolo[4,3-a] quinazoline-5 (4*H*)-products**3** and **4** resulted in good yields. The ^{1}H NMR spectrum

showed the NH signal at 10.50 ppm in addition to the signals attributed to phenyl and ethyl protons in addition to the disappearance of the signals related to the NH_2 group in the starting hydrazinyl compound.
The sulfanyl-substituted ester derivatives **5a,b** formed by esterification of the starting quinazoline thoil**1**were converted to the corresponding 4-substituted-1-hydrazinyl-[1,2,4] triazolo[4,3-a]quinazolin-5(4*H*)-one **6** and **7** via reaction with thiosemicarbzide[38]. The IR spectra of the later tricyclic products showed the characteristic absorptions of the NH and NH_2 groups at their normal regions (3300- 3450cm^{-1}) which were also confirmed by the corresponding ^1H NMR spectra presenting their signal at 4.8 and 10 ppm.

The sulfanyl-acetyl hydrazide**8a,b** was prepared from the starting **1a,b** and is found a useful key structure for the synthesis of functionalized bicyclic compounds[39]. Thus, reacting ethyl cyanoacetate and malononitrile with the acetyl hydrazides **8a,b** in the presence of para-chlorobenzaldehyde afforded the bicyclic structural products for which the spectral and analytical data revealed the formation of the quinazolyl-triazolopyridine hybrid products incorporating aryl substituents[40]. The observed absorption bands characterizing the -COOEt and -CN groups of the products and the disappearance of the amide carbonyl and NH_2 groups confirmed the structures. In addition, the NMR spectra also confirmed the product structure sincecompound 9's ethyl side chain exhibits distinctive triplet and quartet signals in addition to the NH, aryl, and other remaining signals of the assigned products.

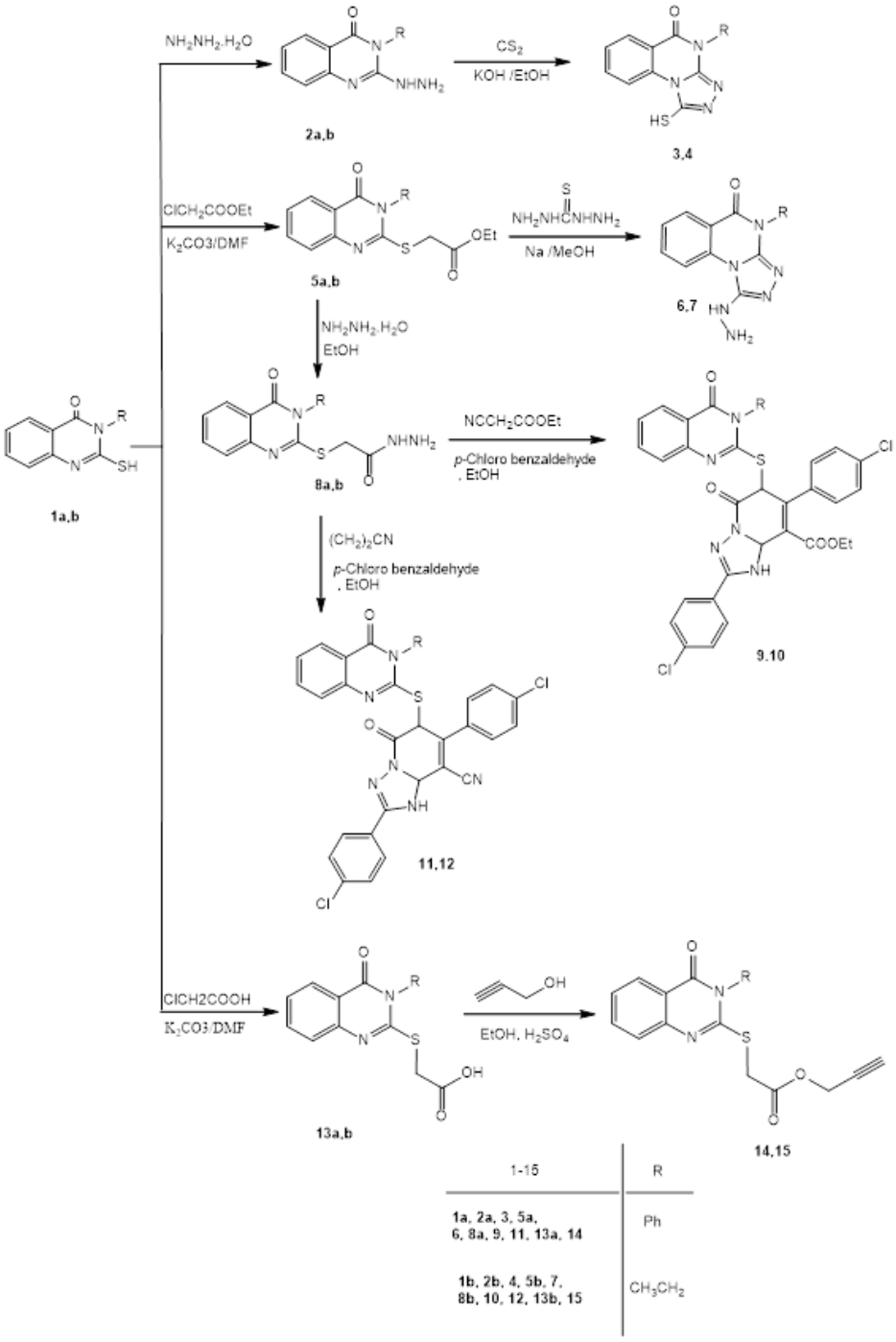
The terminal acetylenic derivative of the acetylthioquinazoline derivative was synthesized via thioalkylation of the starting quinazoline-thiol by reaction with chloroacetic acid followed by an esterification reaction with propargyl alcohol. The product may be a useful interesting structure for possible future synthetic processes owing to the expected chemical reactivities of the terminal alkyne group. The IR spectra showed the characteristic band of the acetylenic absorption in addition to the ester carbonyl confirming the formation of the acetylenic product. The structure was also confirmed by its ^1H NMR spectrum showing the signals of protons in the two methylene groups in the attached side chain, in addition to the signals of the quinazoline protons.

3.2. Cytotoxic activity

3.2.1. In Vitro Ant proliferative activity

Twenty compounds were examined in vitro for their activity against HCT-116, HepG2, and MCF-7 human cancer cells and one human healthy cell line (BJ-1) using the LDH assay. The percentages of dead cells were calculated and compared to those of the control. Activities of these compounds against the three carcinoma cell lines were compared to the activity of doxorubicin. All compounds suppressed three cancer cells (HCT-116, HepG2, and MCF-7) in a dose-dependent manner **(Figure 1 - 3)**. In the case of HepG2 human liver cancer cells: all the twenty compounds have less cytotoxic activities; three compounds (**9, 11,** and **6,** respectively) showed slightly less activities; four compounds (**12, 10, 2a,** and **4,** respectively) have moderate cytotoxic activities; the rest of the compounds have weak cytotoxic activities on HepG2 relative to that of doxorubicin **(Figure 1 &Table 1)**. In the case of MCF-7 human breast cancer cells: two compounds (**10** and **3,** respectively) have superior cytotoxic activities; one compound (**4**) has comparable cytotoxic activity; two compounds (**10** and **8a** respectively) have slightly less cytotoxic activities; nine compounds (**9, 14, 13a, 5a, 5b, 12, 13b, 7** and **8b,** respectively) have moderate cytotoxic activities; the rest of the compounds have weak cytotoxic activities on MCF-7 relative to the reference drug **(Figure 2 &Table 1)**. In the case of HCT 116 human colorectal carcinoma cells: both **Figure 3** and **Table 1** show that eight compounds (**3, 4, 13a, 13b, 8b, 10, 5b,** and **12**) respectively have superior cytotoxic activities; four compounds (**5a, 8a, 7, and 11**) respectively have moderate cytotoxic activities; the rest of the compounds have weak cytotoxic activities on HCT-116 relative to that of doxorubicin. In the case of the non-tumor fibroblast-derived cell line (BJ): both **Figure 4** and **Table 1** show that: twelve compounds (**13b, 5b, 10, 12, 3, 4, 8b, 13a, 5a, 11, 7,** and **8a**) respectively have more toxic activities; one compound (**9**) have a comparable toxic activity; the rest of the compounds have significantly less toxic activities on the healthy cells relative to that of doxorubicin.

By comparing the cytotoxicity results on all cancer types relative to normal cell lines, one can conclude that: one compound (**9**) is selectively active only on human liver cancer type; two compounds (**8a** and **11**) are selectively active only on human breast cancer type; three compounds (**10, 3** and **4**) are selectively active on both human colon and breast cancer types; five compounds (**5b, 8b, 12, 13a** and **13b**) are selectively active on only human colon cancer type and can be considered as good human colon anticancer candidate drugs rather than on liver or breast cancer types as their cytotoxic activities on this two cancer cells type are much greater than their cytotoxic activities on the normal cells compared to the drug reference.



In general, the formed products incorporated the triazolopyrimidine structural system-based quinazoline core revealed higher activity action than the substituted triazolopyridineanalogs. The latter products incorporating a bicyclic system in their afforded structures were also higher in activity than their precursors. There is an observed degree of selectivity for compound **4** toward MCF-7 when compared to its phenyl-substituted structure. Furthermore, most of the tested compounds with N-alkyl substituent showed improved activities more than their phenyl-substituted analogs.

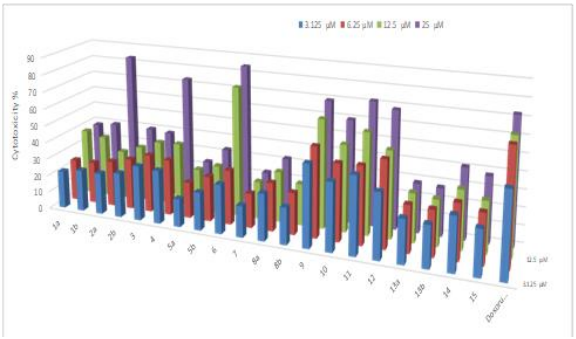


Figure 1 Dose-dependentant proliferative data of the twenty compounds onHepG2 cancer cells according to the LDH assay after 48 h of exposure.

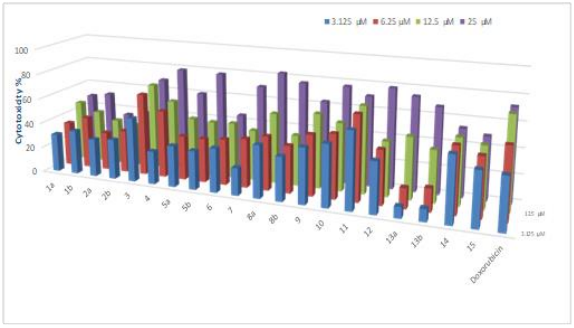


Figure2 Dose-dependent ant proliferative data of the twenty compounds onMCF-7 cancer cells according to the LDH assay after 48 h of exposure.

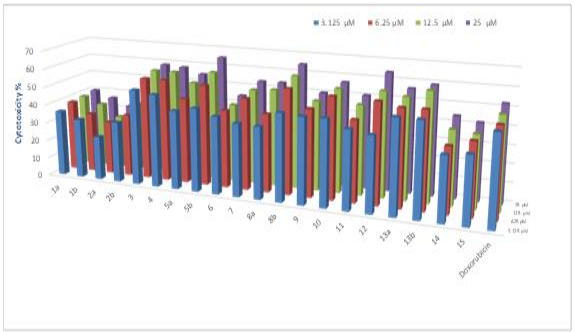


Figure 3 Dose-dependentantiproliferativedata of the twenty compounds onHCT-116 cancer cells according to the LDH assay after 48 h of exposure.

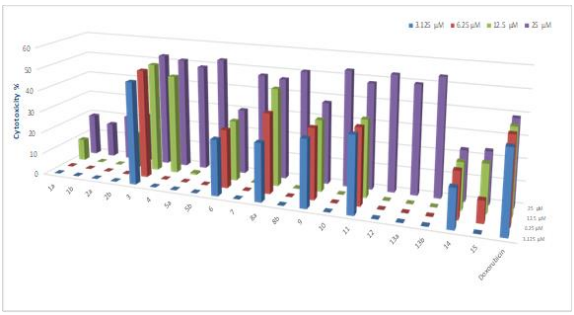


Figure 4 Dose-dependentant proliferative data of the twenty compounds onBJ-1normal cells according to the LDH assay after 48 h of exposure.

Table 1:The antiproliferative IC_{50} of the twenty compounds on the human cell lines according to the LDH assay.

Compound Code	IC_{50} (µM) ± SD			
	HepG-2	MCF-7	HCT-116	BJ-1
1a	32.6 ± 2.8	25.3 ± 1.9	31.3 ± 2.1	64.6 ± 4.1
1b	31.3 ± 3.1	23.9 ± 2.1	34.4 ± 2.4	78.0 ± 5.1
2a	15.0 ± 1.5	34.9 ± 2.5	38.4 ± 3.1	60.1 ± 4.5
2b	31.4 ± 2.9	31.8 ± 3.1	32.1 ± 2.3	55.7 ± 4.1
3	32.3 ± 2.5	4.8 ± 0.3	3.0 ± 0.2	23.7 ± 2.5
4	16.6 ± 1.1	5.8 ± 0.2	3.0 ± 0.2	24.3 ± 2.1
5a	52.2 ± 2.6	14.1 ± 0.8	11.9 ± 0.9	25.5 ± 2.3
5b	37.8 ± 3.2	14.5 ± 1.1	5.7 ± 0.2	23.5 ± 2.4
6	8.3 ± 0.6	27.5 ± 2.3	28.0 ± 1.3	41.4 ± 3.5
7	55.8 ± 2.9	15.7 ± 0.7	12.1 ± 0.7	26.3 ± 2.4
8a	38.6 ± 3.1	7.3 ± 0.6	11.9 ± 0.8	26.8 ± 1.1
8b	49.6 ± 3.3	15.7 ± 1.2	5.5 ± 0.3	24.5 ± 1.7
9	6.0 ± 0.5	10.7 ± 1.0	24.9 ± 1.5	33.3 ± 3.5
10	12.5 ± 2.1	6.2 ± 0.3	5.7 ± 0.1	23.5 ± 2.1
11	7.0 ± 0.5	4.7 ± 0.5	12.9 ± 0.9	25.9 ± 2.2
12	12.5 ± 2.0	14.6 ± 1.1	5.8 ± 0.2	23.6 ± 1.7
13a	43.9 ± 3.9	12.9 ± 0.9	3.1 ± 0.1	25.2 ± 1.9
13b	45.4 ± 2.9	15.4 ± 1.1	3.1 ± 0.2	23.3 ± 2.3
14	30.5 ± 2.7	12.1 ± 2.7	28.4 ± 1.9	54.5 ± 4.7
15	33.0 ± 2.9	25.4 ± 1.9	30.0 ± 3.1	52.9 ± 4.9
Doxorubicin	4.8 ± 0.5	5.6 ± 0.3	6.5 ± 0.5	32.1± 3.1

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