Novel Quinazoline Derivatives Bearing a Sulfapyridine Moiety as Anticancer and Radiosensitizing Agents

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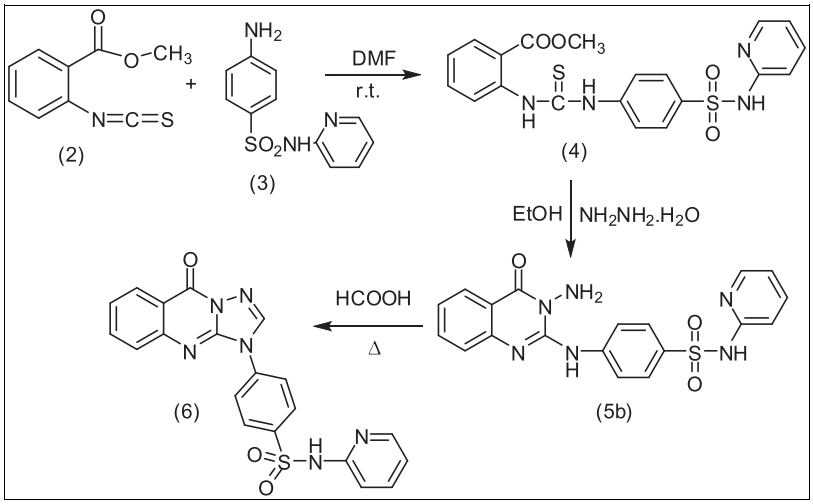

Quinazoline derivatives posses many types of biological activities and have recently been reported to show substantial antitumor activity *in vitro* and/or *in vivo*. There is a variety of mechanisms for their anticancer activity. The present work reports the possible utility of methyl anthranilate in the synthesis of some new quinazoline derivatives, bearing a substituted sulfonamide moiety. All the newly synthesized compounds were evaluated for their *in vitro* anticancer activity against human liver cancer cell line, using doxorubicin as a reference drug. In addition, the most active compounds **14** and **15** were selected and evaluated for their ability to enhance the cell killing effect of γ-radiation.

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INTRODUCTION

Quinazolines constitute an important class of heterocyclic compounds, which represent building block for approximately 150 naturally occurring alkaloids [1,2] and have relieved an increasing interest from medicinal chemists because of their wide range of biological and pharmaceutical activities including antidiabetic [3] antihyperglycemic [4], antihypertensive [5], antihistaminic [6], antioxidant [7] analgesic, anti-inflammatory [8], antipsychotic, muscle relaxant [9], anticonvulsant [10],antiparkinsonian [11], antitoxoplasmic [12], antitubercular [13], antibacterial [14], antifungal [15], and antiviral [16] activities. Quinazoline derivatives have been reported to show substantial antitumor activity *in vitro* and/or *in vivo* [17–21].

From the literature survey, it was found that quinazoline derivatives act as antitumor agents through a variety of mechanisms such as dihydrofolate reductase inhibition [22], microtubule polymerization inhibition [23], epidermal growth factor receptor inhibition [24], cyclin-dependent kinase inhibition [25], tymidylate synthase inhibition [26], selective erB2 receptor tyrosine kinase inhibition [27], selective c-Src kinase inhibition [28], and tumor suppressor protein (p-53) reactivator [29].

According to the data, quinazoline derivatives show inhibition of the growth of human hepatocelluar cancer cells [30,31] and have synergistic effect when combined with γ-radiation through potentiatiation of the antitumor effect of single and multiple fractions of γ-radiation [32,33].

Sulfonamides constitute an important class of drugs, with several types of pharmacological activities including antibacterial [34], carbonic anhydrase inhibitors [35], diuretic [36], hypoglycemic [37], and antithyroid activity [38]. Also, some structurally novel sulfonamide derivatives have recently been reported to show substantial antitumor activity *in vitro* and/or *in vivo* [39–42].

In the light of these facts, we planned to synthesize novel quinazoline derivatives, in order to study their structure activity relationship, and hoped that the new compounds might show significant anticancer activity.

Moreover, we also aimed to evaluate most active compounds for their *in vitro* anticancer activity in combination with γ-radiation, to evaluate their ability to enhance the cytotoxic activity of γ-radiation.

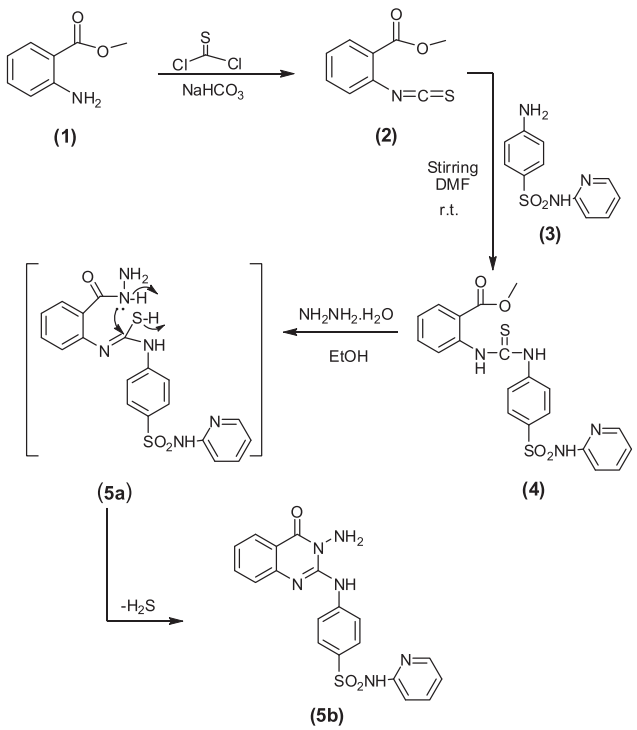
RESULTS AND DISCUSSION

The starting material methyl-2-isothiocyanatobenzoic acid **2** was synthesized from the reaction of methyl anthranilate with thiophosgene [43]. The reactivity of isothiocyanato derivative **2** toward nitrogen nucleophile was investigated. When compound **2** was reacted with sulfapyridine **3** in dimethylformamide at room temperature, the thioureido derivative **4** was obtained in good yield (Scheme 1). The structure of compound **4** was supported by elemental analysis, IR, ^{1}H NMR, and mass spectral data. IR spectrum of compound **4** showed the absence of NCS band and the presence of bands at 3300 and 3238 cm^{-1} (3NH), 1651 cm^{-1} (C═O), 1262 cm^{-1} (C═S), 1389 and 1133 cm^{-1} (SO_2), and 1262 cm^{-1} (C═S). The ^{1}H NMR spectrum of compound **4** in (DMSO-d_6) revealed signal at 3.6 ppm because of (OCH_3) group. The mass spectrum of compound **4** revealed a molecular ion peak *m/z* at 429 [$M^+ - CH_3$] (0.4%), with a base peak *m/z* at 226 (100%).

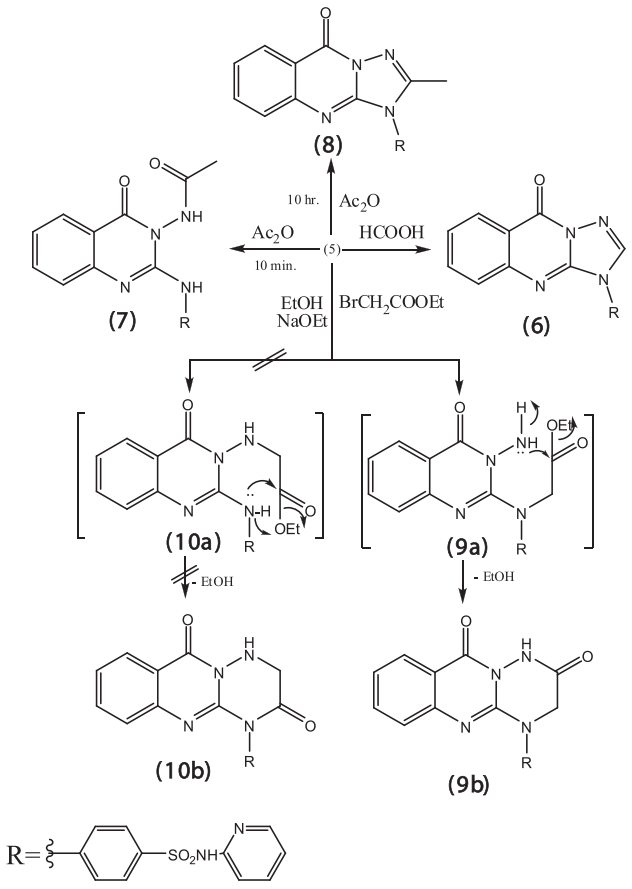
Treatment of compound **4** with hydrazine hydrate in ethanol afforded the corresponding N-amino derivative **5b**. The formation of compound **5b** was assumed to occur via elimination of 1 mol H_2S of intermediate **5a** followed by intramolecular cyclization [44] to give the N-amino derivative **5b** (lead acetate paper). The IR spectrum of compound **5** showed bands at 3410, 3308, and 3183 cm^{-1} (NH, NH_2), 1660 cm^{-1} (C═O), and 1383 and 1133 cm^{-1} (SO_2). The ^{1}H NMR spectrum of compound **5** in (DMSO-d_6) revealed signals at 6.8 ppm assigned to (NH_2) group and 13.1 ppm corresponding to (SO_2NH) group. The mass spectrum of compound **5b** revealed a molecular ion peak *m/z* at 408 [M^+] (2.1%), with a base peak *m/z* at 343 (100%).

The reactivity of N-amino derivative **5b** was studied. Thus, interaction of compound **5b** with formic acid yielded the 1,2,4-triazolo[5,1-*b*]quinazoline derivative **6** (Scheme 2). The structure of compound **6** was established by elemental analysis, IR, ^{1}H NMR, and mass spectroscopy. The IR spectrum showed bands at 3227 cm^{-1} (NH), 1663 cm^{-1} (C═O), and 1395 and 1139 cm^{-1} (SO_2). The ^{1}H NMR spectrum of compound **6** in (DMSO-d_6) revealed signals at 8.0 ppm assigned to CH of triazole ring and a multiplet at 6.9–7.8 ppm for aromatic protons. The mass spectrum of compound **6** revealed a molecular ion peak *m/z* at 418 [M^+] (1.65%), with a base peak *m/z* at 88 (100%). When compound **5b** was refluxed with acetic anhydride for short time (10 min), the corresponding mono acetyl derivative **7** was obtained whereas for long time (10 h), gave the cyclic compound triazoloquinazoline **8** (Scheme 2). The structure of compounds **7** and **8** was established by elemental analysis, IR, and ^{1}H NMR spectral data. The IR spectrum of compound **7** showed bands at 3190 cm^{-1} (NH), 1721 and 1662 cm^{-1} (2 C═O), and 1393and 1142 cm^{-1} (SO_2).

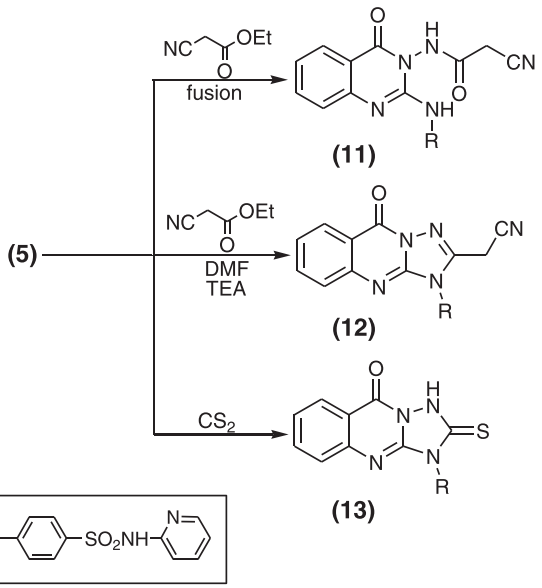
Scheme 1



Scheme 2



Scheme 3



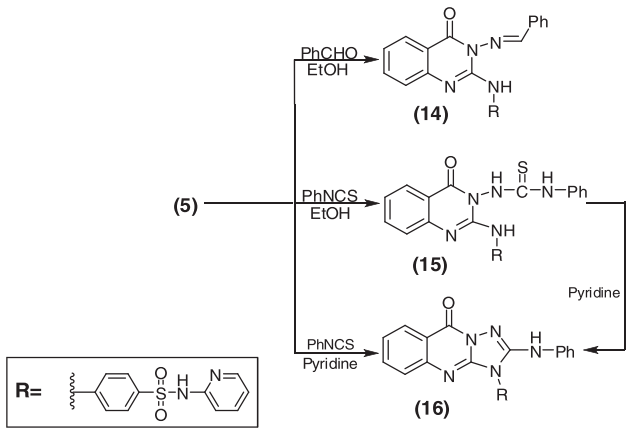
The ^{1}H NMR spectrum of compound **7** in (DMSO-d_6) revealed signals at 2.6 ppm for ($COCH_3$) group and 11.7 ppm because of (NH) group. The IR spectrum of compound **8** showed bands at 3272 cm^{-1} (NH), 1686 cm^{-1} (C═O), and 1359 and 1169 cm^{-1} (SO_2). Its ^{1}H NMR spectrum in (DMSO-d_6) revealed signals at 1.2 ppm assigned to (CH_3) group.

Reaction of **5b** with ethyl bromoacetate in refluxing sodium ethoxide yielded the triazinoquinazoline **9b** via the formation of the intermediate **9a** rather than its isomeric structure **10b** (Scheme 2). Structure **9b** was suggested rather than structure **10b**, on the basis of the assumption that the reaction basic condition allowed it to proceed through formation of sodium salt on the less basic NH, and elimination of sodium bromide followed by cyclization [45]. The IR spectrum of the isolated compound **9b** showed bands at 3484 cm^{-1} (NH), and 1743 and 1675 cm^{-1} (2 C═O), which was at less frequency than that expected for structure **10b**. Further evidence was the ^{1}H NMR spectrum, which showed a singlet at 3.9 ppm for the methylene protons.

When compound **5b** was reacted with ethyl cyanoacetate under condition of fusion, the 3-cyanoacetamidoquinazoline derivative **11** was obtained in good yield, whereas in the presence of triethylamine as catalyst, the cyclic triazoloquinazoline **12** was obtained (Scheme 3). The structure of compounds **11** and **12** was established by elemental analysis, IR, ^{1}H NMR, and mass spectral data. IR spectrum of compound **11** showed bands at 3223 cm^{-1} (NH), 2208 cm^{-1} (C ≡ N), 1652 cm^{-1} (C═O), and 1398 and 1139 cm^{-1} (SO_2). The ^{1}H NMR spectrum of compound **11** in (DMSO-d_6) exhibited signals at 4.1 ppm for (CH_2) group and 13.1 ppm because of (SO_2NH) group. The mass spectrum of compound **11** revealed a molecular ion peak *m/z* at 475 [M^+] (0.15%), with a base peak *m/z* at 373 (100%). The IR spectrum of compound **12** showed bands at 3356 cm^{-1} (NH), 2206 cm^{-1} (C ≡ N), 1645 cm^{-1} (C═O), and 1393 and 1139 cm^{-1} (SO_2). The ^{1}H NMR spectrum of compound **12** in (DMSO-d_6) revealed signal at 4.2 ppm corresponding to (CH_2) group. The mass spectrum of compound **12** revealed a molecular ion peak *m/z* at 457 [M^+] (18.8%), with a base peak *m/z* at 92 (100%). On the other hand, refluxing compound **5** with carbon disulfide in pyridine yielded the 2-thioxo-1,2,4-triazolo[5,1-*b*]quinazoline derivative **13**. Its IR spectrum showed bands at 3223 cm^{-1} (NH), 1663 cm^{-1} (C═O), 1391 and 1130 cm^{-1} (SO_2), and 1250 cm^{-1} (C═S). The ^{1}H NMR spectrum of compound **13** in (DMSO-d_6) revealed signal at 13.1 because of (SO_2NH) group. The mass spectrum of compound **13** revealed a molecular ion peak *m/z* at 450 [M^+] (7.0%), with a base peak *m/z* at 344 (100%).

The Schiff's base derivative **14** was obtained via reaction of compound **5b** with benzaldehyde in refluxing ethanol (Scheme 4). The structure of compound **14** was confirmed by elemental analysis, IR, ^{1} NMR, and mass spectral data. The IR spectrum of compound **14** showed bands at 3319 and 3203 cm^{-1} (NH), 1665 cm^{-1} (C═O),

Scheme 4



and 1394 and 1141 cm^{-1} (SO_2). Its ^1H NMR spectrum in (DMSO-d_6) revealed signal at 8.2 ppm because of (N=CH). The mass spectrum of compound **14** revealed a molecular ion peak *m/z* at 496 [M^+] (0.5%), with a base peak *m/z* at 463 (100%). Our work was extended to study the reactivity of compound **5b** toward phenyl isothiocyanate. Thus, reaction of **5b** with phenyl isothiocyanate in refluxing ethanol furnished the corresponding thioureido derivative **15**, whereas in refluxing pyridine, the triazoloquinazoline **16** was obtained. The structure of compounds **15** and **16** was established by elemental analyses, IR, ^1H NMR, and mass spectral data. The IR spectrum of compound **15** showed bands at 3240 and 3203 cm^{-1} (NH). The ^1H NMR spectrum of compound **15** in (DMSO-d_6) revealed signals at 4.5 ppm assigned to (2NH) of thioureido group. The IR spectrum of compound **16** showed bands at 3463 and 3234 cm^{-1} (NH), 1663 cm^{-1} (C=O), and 1356 and 1139 cm^{-1} (SO_2). The mass spectrum of compound **16** revealed a molecular ion peak *m/z* at 509 [M^+] (15.6%), with a base peak *m/z* at 57 (100%). Also, compound **16** was obtained by refluxing compound **15** in pyridine (mp and mixed mp).

***In vitro* anticancer screening.** The results of anticancer activity (Table 1) indicated that compounds **14** containing benzylidenamino at position-3 with the biologically active sulfonamide moiety at position-2 (IC_{50} value = 22.11 μM) and **15** having phenylthioureido at position-3 with sulfonamide moiety at position-2 (IC_{50} = 19.70 μM) were found to be potent than all the synthesized compounds and less active than doxorubicin as reference drug.

Radiosensitizing evaluation. The most potent compounds resulted from the *in vitro* anticancer screening; the quinazolone derivatives **14** and **15** were selected to be evaluated again for their *in vitro* anticancer activity alone and in combination with γ-radiation. This study was conducted to evaluate the ability of these compounds to enhance the cell killing effect of γ-radiation. Cells were subjected to a single dose of γ-radiation at a dose level of 8 Gy with a dose rate of 2 Gy/min. Irradiation was performed in the National Cancer Institute, Cairo University, using Gamma cell-40 (^{60}Co) source. The surviving fractions were expressed as means ± standard error. The results were analyzed using 1-way ANOVA test and given in Table 2.

EXPERIMENTAL

Melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). Elemental analysis (C, H, N) were performed on Perkin-Elmer 2400 analyser (Perkin-Elmer, Norwalk, CT, USA) at the microanalytical laboratories of the Faculty of Science, Cairo University.

Table 1

In vitro cytotoxic activity of the synthesized compounds against human liver cell line (HEPG2).

	Surviving fraction (means ± SE)[#]				
	Compound concentration (μ*M*)				
Comp. No.	50	25	12.5	5	IC_{50}
Dox.	0.1011 ± 0.0473	0.0633 ± 0.0101	0.0615 ± 0.0116	0.0967 ± 0.0247	**4.15**
4	0.3497 ± 0.0064	0.3114 ± 0.0164	0.4781 ± 0.0007	0.9047 ± 0.0337	**26.90**
5b	0.3536 ± 0.0181	0.3901 ± 0.0166	0.6979 ± 0.0395	0.7773 ± 0.0680	**30.32**
6	0.3822 ± 0.0174	0.4047 ± 0.0318	0.5438 ± 0.0580	0.9456 ± 0.0176	**30.84**
7	0.4997 ± 0.0110	0.4843 ± 0.0340	0.5541 ± 0.0354	0.8744 ± 0.0087	**38.42**
8	0.6051 ± 0.0245	0.5998 ± 0.0327	0.8783 ± 0.0345	0.8567 ± 0.0630	**55.32**
9	0.7637 ± 0.0666	0.9102 ± 0.0698	0.9473 ± 0.0346	1.0110 ± 0.0162	**105.63**
11	0.3255 ± 0.0407	0.3544 ± 0.0123	0.7150 ± 0.0449	0.8220 ± 0.0558	**29.16**
12	0.4675 ± 0.0285	0.4477 ± 0.0246	0.6388 ± 0.0655	0.7573 ± 0.0208	**35.67**
13	0.4596 ± 0.0207	0.4440 ± 0.0387	0.7931 ± 0.0812	0.8379 ± 0.0120	**37.63**
14	0.4404 ± 0.1207	0.4886 ± 0.0965	0.4242 ± 0.0930	0.2313 ± 0.0133	**22.11**
15	0.3182 ± 0.0156	0.3588 ± 0.0246	0.3878 ± 0.0149	0.4880 ± 0.0178	**19.70**
16	0.3634 ± 0.0255	0.2911 ± 0.0051	0.6162 ± 0.0437	0.8643 ± 0.0150	**28.30**

[#]Each value is the mean of three values ± standard error.

Table 2

In vitro anticancer screening of compounds **14** and **15** against human liver cell line (HEPG2) in combination with γ-radiation.

			Surviving fraction (means ± SE)[#]				
	Control	Irradiated (8 Gy)	Compound concentration (μ*M*) + irradiation (8 Gy)				
Comp. No.			5	12.5	25	50	IC_{50} (μ*M*)
Dox.	1.000	0.927 ± 0.02*	0.113 ± 0.0012*	0.4266 ± 0.04702*	0.53 ± 0.08505*	0.96 ± 0.111*	**3.67**
14	1.000	0.927 ± 0.02*	0.498 ± 0.0712*	0.3111 ± 0.01396*	0.3221 ± 0.01176*	0.3442 ± 0.02365*	**17.92**
15	1.000	0.927 ± 0.02*	0.4766 ± 0.03177*	0.2911 ± 0.03588*	0.3001 ± 0.00329*	0.2992 ± 0.04411*	**15.71**

[#]Each value is the mean of three values ± standard error.
*Significant difference from control group at $p < 0.001$.

All compounds were within ±0.4% of the theoretical values. The IR spectra (KBr) were measured on Shimadzu IR 110 spectrophotometer (Shimadzu, Koyoto, Japan), and 1H NMR spectra were obtained on a Bruker proton NMR-Avance 300 (300 MHz) (Bruker, Munich, Germany), in DMSO-d_6 as a solvent, usingTMS as internal standard. Mass spectra were run on HP Model MS-5988 (Hewlett Packard, Palo, Alto, California, USA). All reactions were monitored by TLC using precoated Aluminum sheets Silica gel Merck 60 F254 and were visualized by UV lamp (Merck, Darmstadt, Germany).

Methyl 2-isothiocyanatobenzoate (2). This was prepared according to the previously reported procedure [43].

Methyl 2-(3-(4-(N-pyridin-2-ylsulfamoyl)phenyl)thioureido) benzoate (4). A mixture of methyl-2-isothiocyanatobenzoate **2** (1.9 g, 0.01*M*) and pyridin-2-yl benzenesulfonamide **3** (2.49 g, 0.01 mol) in dimethylformamide (20 mL) was stirred at room temperature for 5 h. The reaction mixture was poured onto cold water, and the obtained solid was crystallized from dioxane to give compound **4**: yield, 94%; mp 235–237°C; IR (KBr, cm^{-1}): 3300, 3238 (NH), 3041 (CH arom.), 1651 (C═O), 1262 (C═S), 1389, 1133 (SO_2). ^{1}H NMR (DMSO-d_6) δ: 3.6 [s,3H,OCH_3], 7.0–8.0 [m,14H,Ar–H + 2NH], 10.26 [s,1H, SO_2NH, D2O-exchangeable]. MS, *m/z* (%): 429 [$M^+ - CH_3$] (0.4), 226 (100). *Anal*. Calcd for $C_{20}H_{18}N_4O_4S_2$: C, 54.28; H, 4.10; N, 12.66. Found: C, 54.44; H, 3.99; N, 12.87.

4-(3-Amino-4-oxo-3,4-dihydroquinazolin-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (5b). A mixture of compound **4** (4.4 g, 0.01 mol) and hydrazine hydrate (1 g, 0.02 mol) in ethanol (30 mL) was refluxed till evolution of hydrogen sulfide gas was ceased (lead acetate paper) (14 h.). The reaction mixture was filtered while hot, and the solid obtained was crystallized from dioxane to give compound **5**: yield, 80%; mp 255–257°C; IR (KBr, cm^{-1}): 3410, 3308, 3183 (NH, NH_2), 3045 (CH arom.), 1660 (C═O), 1383 1133 (SO_2). ^{1}H NMR (DMSO-d_6)δ: 6.8 [s,2H,NH_2, D_2O-exchangeable], 7.3–8.0 [m,13H,Ar–H + NH], 13.2 [s,1H, SO_2NH, D_2O-exchangeable]. MS, *m/z* (%): 408 [M^+] (2.1), 343 (100). *Anal*. Calcd for $C_{19}H_{16}N_6O_3S$: C, 55.87; H, 3.95; N, 20.58. Found: C, 55.97; H, 3.75; N, 20.88.

4-(9-Oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(pyridin-2-yl) benzenesulfonamide (6). A solution of compound **5b** (4.0 g, 0.01 mol) in formic acid (20 mL) was refluxed for 5 h. The reaction mixture was cooled and then poured onto cold water, and the obtained solid was crystallized from dioxane to give compound **6**: yield, 71%; mp 281–283°C; IR (KBr, cm^{-1}): 3227 (NH), 3045 (CH arom.), 1663 (C═O), 1395, 1139 (SO_2). ^{1}H NMR (DMSO-d_6)δ: 6.9–7.8 [m,12H,Ar–H], 13.1 [s,1H, SO_2NH, D_2O-exchangeable], 8.0 [s,1H,CH triazole]. MS, *m/z* (%): 418 [M^+] (1.65), 88(100). *Anal*. Calcd for $C_{20}H_{14}N_6O_3S$: C, 57.41; H, 3.37; N, 20.08. Found: C, 57.17; H 3.15; N, 19.8.

N-(4-oxo-2-(4-(N-pyridin-2-ylsulfamoyl)phenylamino)quinazolin-3(4H)-yl)acetamide (7). A solution of compound **5b** (4.0 g, 0.01 mol) in acetic anhydride (20 mL) was refluxed For 10 min. The formed solid mass was collected and crystallized from ethanol to give compound **7**: yield, 68%; mp 149–151°C; IR (KBr, cm^{-1}): 3190 (NH), 3065 (CH arom.), 1721, 1662 (2 C═O), 1393, 1142 (SO_2). ^{1}H NMR (DMSO-d_6)δ: 2.6 [s,3H, $COCH_3$], 7.3–8.2 [m,13H,Ar–H + NH], 11.7 [s,1H, SO_2NH, D_2O-exchangeable,]. *Anal*. Calcd for $C_{21}H_{18}N_6O_4S$. C, 55.99; H, 4.03; N, 18.66. Found: C, 55.97; H, 3.88; N, 18.88.

2-(4-Methyl-9-oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(pyridin-2-yl)benzenesulfonamide (8). A solution of compound **5b** (4.0 g, 0.01 mol) in acetic anhydride (20 mL) was refluxed for 10 h. The formed solid mass was collected and crystallized from ethanol to give compound **8**: yield, 66%; mp 296–298°C; IR (KBr, cm^{-1}): 3272 (NH), 1686 (C═O), 1359, 1169 (SO_2). ^{1}H NMR (DMSO-d_6)δ: 1.2 [s,3H,CH_3], 7.2–8.2 [m,12H,Ar–H], 13.4 [s,1H, SO_2NH, D_2O-exchangeable]. *Anal*. Calcd for $C_{21}H_{16}N_6O_3S$: C, 58.32; H, 3.73; N, 19.43. Found: C, 58.44; H 3.78; N, 19.22.

4-(2,10-Dioxo-2,3-dihydro-1H-[1,2,4]triazino[3,2-b]quinazolin-4(10H)-yl)-N-(pyridin-2-yl)benzenesulfonamide (9). A mixture of compound **5b** (4.0 g, 0.01 mol) and ethyl bromoacetate (1.69 g, 0.01 mol) and sodium ethoxide (0.68 g, 0.01 mol) in ethanol (30 mL) was refluxed for 8 h. The reaction mixture was filtered while hot, and the solid obtained was crystallized from dioxane to give compound **9**: yield, 78%; mp 182–184°C; IR (KBr, cm^{-1}): 3484 (NH) 3065 (CH arom.), 2922, 2846 (CH aliph.), 1743, 1675 (2C═O), 1366, 1136 (SO_2). ^{1}H NMR (DMSO-d_6)δ: 3.9 [s,2H, CH_2CO], 7.2–7.9 [m,12H,Ar–H], 8.2 [s,1H, NH, D_2O-exchangeable], 13.2 [s,1H, SO_2NH, D_2O-exchangeable]. *Anal*. Calcd for $C_{21}H_{16}N_6O_4S$: C, 56.24; H, 3.60; N, 18.74. Found: C, 55.97; H 3.75; N, 18.55.

2-Cyano-N-(4-oxo-2-(4-(N-pyridin-2-ylsulfamoyl)phenylamino) quinazolin-3(4H)-yl)acetamide (11). A solution of compound **5b** (4.0 g, 0.01 mol) and ethyl cyanoacetate (10 mL) was refluxed for 8 h. The formed solid mass was collected and crystallized from methanol to give compound **11**: yield, 84%; mp >300°C; IR (KBr, cm^{-1}): 3223 (NH), 2934, 2867 (CH aliph.), 2208 (CN), 1652 (C═O), 1398, 1139 (SO_2).

^{1}H NMR (DMSO-d_6)δ: 4.1 [s,2H, CH_2CN], 7.4–8.0 [m,14H, Ar–H + 2NH], 13.1 [s,1H, SO_2NH, D_2O-exchangeable]. MS, *m/z* (%): 475 [M^+] (0.15), 373 (100). *Anal*. Calcd for $C_{22}H_{17}N_7O_4S$: C, 55.57; H, 3.60; N, 20.62. Found: C, 55.44; H 3.88; N, 20.42.

4-(2-(Cyanomethyl)-9-oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(pyridin-2-yl)benzenesulfonamide (12). A mixture of compound **5b** (4.0 g, 0.01 mol) and ethyl cyanoacetate (10 mL) in dimethylformamide (10 mL) containing three drops of triethylamine was refluxed for 8 h. The formed solid mass was collected and crystallized from dioxane to give compound **12**: yield, 84%; mp >300°C; IR (KBr, cm^{-1}): 3356 (NH), 2928, 2870 (CH aliph.), 2206 (CN), 1645 (C═O), 1393, 1139 (SO_2). ^{1}H NMR (DMSO-d_6)δ: 4.2 [s,2H,CH_2CN], 7.3–8.0 [m,12H,Ar–H], 13.4 [s,1H, SO_2NH, D_2O-exchangeable].MS, *m/z* (%): 457 [M^+] (18.8), 92 (100). *Anal*. Calcd for $C_{22}H_{15}N_7O_3S$: C, 57.76; H, 3.30; N, 21.43. Found: C, 57.97; H, 3.65; N, 21.22.

4-(2-Sulfanylidine-9-oxo-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(pyridin-2-yl)benzenesulfonamide (13). A solution of compound **5b** (4.0 g, 0.01 mol) in carbondisulfide (1.52 g, 0.02 mol) in pyridine (20 mL) was refluxed for 10 h. The reaction mixture was cooled and then poured onto cold water, and the obtained solid was crystallized from acetic acid to give compound **13**: yield, 68%; mp >300°C; IR (KBr, cm^{-1}): 3223 (NH), 3038 (CH arom.), 1663 (C═O), 1203 (C═S), 1391, 1130 (SO_2),. ^{1}H NMR (DMSO-d_6)δ: 7.2–8.0 [m,12H,Ar–H + NH], 13.1 [s,1H, SO_2NH, D_2O-exchangeable]. MS, *m/z* (%): 450 [M^+] (7.0), 344 (100). *Anal*. Calcd for $C_{20}H_{14}N_6O_3S_2$: C, 53.32; H, 3.13; N, 18.66 . Found: C, 53.11;H 2.93;N, 18.88.

N-benzyl-4-(3-(benzylideneamino)-4-oxo-3,4-dihydroquinazolin-2-ylamino)benzenesulfonamide (14). A mixture of compound **5b** (4.0 g, 0.01 mol) and benzaldehyde (1 g, 0.01 mol) in ethanol (30 mL) was refluxed for 12 h. The reaction mixture was filtered while hot, and the solid obtained was crystallized from dioxane to give compound **14**: yield, 72%; mp 223–225°C; IR (KBr, cm^{-1}): 3319, 3203 (NH), 2923, 2845 (CH aliph.), 1665 (C═O), 1394, 1141 (SO_2). ^{1}H NMR (DMSO-d_6)δ: 6.9–8.1 [m, 18H,Ar–H + NH], 8.2 (s, 1H,N═CH), 9.8 [s,1H, SO_2NH, D_2O-exchangeable]. ^{13}C-NMR (DMSO-d_6): 112.6, 113.0, 115.7 (2), 119.4, 121.6, 126.7, 127.8 (2), 127.9, 129.3 (2), 130.6 (2), 130.9, 132.8, 134.5, 135.1, 139.0, 141.9, 145.3, 148.2, 149.7, 154.6, 161.3, 165.1. MS, *m/z* (%): 496[M^+] (0.5), 463(100). *Anal*. Calcd for $C_{26}H_{20}N_6O_3S$: C, 62.89; H, 4.06; N, 16.39. Found: C, 62.91; H, 4.1; N, 16.42.

4-(4-oxo-3-(3-phenylthioureido)-3,4-dihydroquinazolin-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (15). A mixture of **5b** (4.0 g, 0.01*M*) and phenyl isothiocyanate (1.35 g, 0.01 mol) in ethanol (30 mL) was refluxed for 5 h. The reaction mixture was filtered while hot, and the solid obtained was crystallized from ethanol to give compound **15**: yield, 51%; mp:76–78°C. IR (KBr, cm^{-1}): 3240, 3203 (NH), 3036 (CH arom.), 1663 (C═O), 1236 (C═S), 1320, 1100 (SO_2). ^{1}H NMR (DMSO-d_6)δ: 4.5 (s,2H,2NH of thioureido, D_2O-exchangeable), 7.1–7.7 [m, 20H,Ar–H + 3NH], 11.2 (s,1H,SO_2NH, D_2O-exchangeable). ^{13}C-NMR (DMSO-d_6): 110.5, 114.6, 115.2 (2), 119.7, 121.3, 123.8, 125.6 (2), 128.0, 128.4 (2), 129.6, 130.5 (2), 131.1, 132.7, 138.6, 139.9, 149.0, 149.7, 151.3, 154.2, 163.9, 168.0, 179.3. MS, *m/z* (%): 543[M^+] (5.01), 135 (100). *Anal*. Calcd for $C_{26}H_{21}N_7O_3S_2$: C, 57.44; H, 3.89; N, 18.04. Found: C, 57.65; H, 3.73; N, 17.88.

4-(9-oxo-2-(phenylamino)-[1,2,4]triazolo[5,1-b]quinazolin-3(9H)-yl)-N-(pyridin-2-yl)benzenesulfonamide (16). A mixture of compound **5b** (4.0 g, 0.01 mol) and phenyl isothiocyanate (1.35 g, 0.01 mol) in pyridine (30 mL) was refluxed for 12 h. The reaction mixture was poured onto ice/water acidified by dil HCl, and the solid obtained was crystallized from dioxane to give compound **16**: yield, 73%; mp: 298–300°C; IR (KBr, cm^{-1}): 3463, 3234 (NH), 3045 (CH arom.), 1663 (C═O), 1385, 1139 (SO_2). ^{1}H NMR (DMSO-d_6)δ: 7.2–8.1[m,18H,Ar–H + NH], 13.1 [s,1H,SO_2NH, D_2O-exchangeable].MS, *m/z* (%): 509 [M^+] (15.6), 57 (100). *Anal*. Calcd for $C_{26}H_{19}N_7O_3S$: C, 61.29; H, 3.76; N, 19.24. Found: C, 61.44; H, 3.92; N, 19.50.

***In vitro* anticancer screening.** Human tumor liver cell line (HEPG2) was used in this study. The cytotoxic activity was measured *in vitro* for the newly synthesized compounds using the Sulfo-Rhodamine-B stain assay using the method of Skehan et al. [46]. The *in vitro* anticancer screening was performed by the pharmacology unit at the National Cancer Institute, Cairo University.

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in dimethyl sulfoxide. Different concentrations of the compound under test (5, 12.5, 25, and 50 μ*M*) were added to the cell monolayer. Triplicate wells were prepared for each individual concentration. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in atmosphere of 5% CO2. After 48 h, cells were fixed, washed, and stained for 30 min with 0.4% (wt/vol) Sulfo-Rhodamine-B stain dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an enzyme-linked immunosorbent assay reader. The relation between surviving fraction and drug concentration is plotted to obtain the survival curve for liver tumor (HEPG2) cell line after the specified time. The molar concentration required for 50% inhibition of cell viability (IC50) was calculated and compared with the reference drug doxorubicin (CAS, 25316-40-9). The surviving fractions were expressed as means ± standard error.

Radiosensitizing evaluation. The rationale for combining chemotherapy and radiotherapy is based mainly on two ideas, one being spatial cooperation, which is effective if chemotherapy is sufficiently active to eradicate subclinical metastases and if the primary local tumor is effectively treated by radiotherapy. In this regard, no interaction between radiotherapy and chemotherapy is required. The other idea is the enhancement of radiation effects by direct enhancement of the initial radiation damage by incorporating drugs into DNA, inhibiting cellular repair, accumulating cells in a radiosensitive phase or eliminating radioresistant phase cells, eliminating hypoxic cells, or inhibiting the accelerated repopulation of tumor cells. Virtually, all chemotherapeutic agents have the ability to sensitize cancer cells to the lethal effects of ionizing radiation [47].

Calculation of IC_{50}. The surviving fractions of cells for each tested compound and the reference drug in concentrations (5, 12.5, 25, and 50 μ*M*) were the average of three tests. Surviving fraction of cells in control test (0 μ*M*) was considered as 100% viable cells. Concentrations of the tested compounds and the reference drug were plotted against surviving fractions of the cells using Microsoft Excel (2007). A trend line was drawn for each curve, and a corresponding equation was

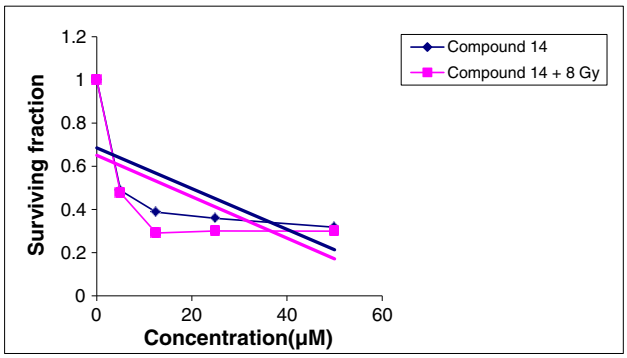


Figure 1. Survival curve for HEPG2 cell line for compound **14** alone or in combination with γ-irradiation (8 Gy).

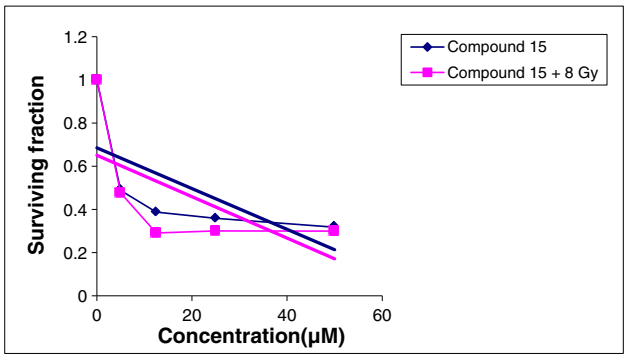


Figure 2. Survival curve for HEPG2 cell line for compound **15** alone or in combination with γ-irradiation (8 Gy).

obtained. Each equation was solved considering the surviving fraction of cells as 50% and the concentration obtained in μ*M* representing IC_{50}.

CONCLUSION

From the previous results, we can conclude that administration of the tested compounds **14** and **15** on human liver (HEPG2) cell line showed promising cytotoxic activity with IC_{50} value of 22.11 and 19.70 μ*M*, respectively. Combining these compounds with radiation at the same concentrations resulted in a remarkable improvement of their activity with IC_{50} value of 17.92 and 15.71 μ*M*, respectively. This demonstrates the importance of the combination therapy (CT and RT) for the patients with cancer to decrease the side effects of both drugs and radiation (Figures 1 and 2).

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