Synthesis, in-vitro Anticancer Screening and Radiosensitizing Evaluation of some New N-(quinoxalin-2-yl)benzenesulfonamide Derivatives

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Key words
- sulfonamide
- quinoxaline
- anticancer
- radiosensitizing

Abstract

The objective of this work is to synthesize and investigate the anticancer activity of a new series of sulfaquinoxaline derivatives by incorporating biologically active moieties (thiourethane, thiazole, imidazole, imidazopyrimidine, imidazopyrimido-pyrimidine, thienopyrimidine, benzopyrimidinone, benzothiazole, thiazole and pyridine moieties). All the newly synthesized compounds were evaluated for their in-vitro anticancer activity against human liver cell line (HEPG2). All the tested compounds showed comparable activity to that of the reference drug 5-fluorouracil (IC_{50} = 40 µM), and the most potent compounds were found to be compounds **4** and **17** (IC_{50} = 4.29 and 11.27 µM, respectively). On the other hand, the most potent compounds **4** and **17** were evaluated as radiosensitizing agents.

Introduction

A series of structurally novel sulfonamide derivatives containing a N_1-substituted sulfonamide moiety were reported to show substantial antitumor activity in-vitro and/or in-vivo [1–5]. In order to explain this antitumor activity, several mechanisms were adopted, including carbonic anhydrase inhibition, cell cycle arrest at G1 phase, disruption of microtubules, and angiogenesis inhibition. The most prominent among these mechanisms was carbonic anhydrase inhibition [6–10]. Quinoxaline derivatives possess a wide range of biological activities [11–15]. Recently, several quinoxaline derivatives exhibited antitumor activity as potent and highly selective tyrosine kinase inhibitors [16–19]. Furthermore, chloroquinoxaline sulfonamide (CQS; **Fig. 1**), was reported to be an efficient antitumor agent against breast, lung, melanoma and ovarian carcinomas by causing cell cycle arrest at the G1 phase [20]. Based on the above informations, and due to the above mentioned anticancer activity of quinoxaline-containing compounds and due to our interest in synthesizing novel sulfonamide derivatives, the present investigation deals with the design and synthesis of some novel sulfaquinoxaline derivatives by substituting the amino group of sulfonamide by different biologically active moieties (thiourethane, thiazole, imidazole, imidazopyrimidine, imidazopyrimido-pyrimidine, thienopyrimidine, benzopyrimidinone, benzothiazole, thiazole and pyridine moieties) to be evaluated as antitumor agents against liver human tumor cell lines (HEPG2) to study their SAR. Moreover, we also aimed to evaluate these new compounds for their in vitro anticancer activity in combination with γ-irradiation.

received 14.09.2011
accepted 17.10.2011

Bibliography
DOI http://dx.doi.org/10.1055/s-0031-1295496
Arzneimittelforschung 2012; 62: 46–52

ISSN 0004-4172

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Materials and Methods

Chemistry

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. All compounds were within ± 0.4 % of the theoretical values. The IR spectra (KBr) were measured on Shimadzu IR 110 spectrophotometer (Shimadzu, Koyoto, Japan), ^{1}H-NMR spectra were obtained on a Bruker proton NMR-Avance 300 (300 MHz) (Bruker, Munuch, Germany), in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as internal standard. Mass spectra were run on HP Model MS-5988 (Hewlett Packard, Palo, Alto, California, USA). All reactions were monitored by thin layer

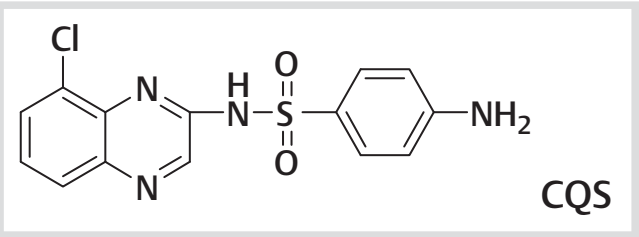

Fig. 1 Chloroquinoxaline.

chromatograph (TLC) using precoated Aluminium sheets Silica gel Merck 60 F254 and were visualized by UV lamp (Merck, Damstadt, Germany).

4-Isothiocyanato-N-quinoxaline-2-yl-benzene sulfonamide (1)

To a suspension of sulfaquinoxaline (3 g, 0.01 mol) in water (30 mL), thiophosgene (1 ml, 0.01 mol) was added and the reaction mixture was stirred for 1 h, where the red colour of the thiophosgene was disappeared and a white precipitate was formed. The precipitate was filtered off, washed with water and crystallized from ethanol to give 1. Yield %: 86, m.p.: 210–212 °C. IR (KBr, cm^{-1}): 3290 (NH), 3070 (CH arom.), 2106 (NCS), 1330, 1156 (SO_2). MS (m/z): 342 (M^+, 10.2%), 261 (27.94%), 236 (100%), 92 (71.14%).^{1}H-NMR DMSO-d_6 (ppm): 4.0 [s, 1H, NH, exchangeable with D_2O], 7.01, 7.5 [2d, 4H, Ar-H AB system], 7.68–8.05 [m, 4H, Ar-H], 8.07[s, 1H, CH-quinoxaline]. ^{13}C-NMR (DMSO-d_6): 124.1, 125.2, 126.9, 128.9, 134.8, 135.1, 135.6, 136.8 (NCS), 138.3, 161.9. Analysis Calc. for $C_{15}H_{10}N_4O_2S_2$: C, 52.61; H, 2.92; N, 16.37. Found: C, 52.40; H, 2.83; N, 16.11.

O-2-Hydroxyethyl4-(N-quinoxalin-2-yl-sulfamoyl)benzene-sulfonamide (2)

A mixture of **1** (0.4 g, 0.01 mol) and ethylene glycol (0.3 mL, 0.01 mol), was refluxed for 8 h, the solid obtained was precipitated on hot, filtered and crystallized from dioxane to give **2.** Yield %: 93, m.p.: 160–162 °C. IR (KBr, cm^{-1}): 3396 (0H), 3525, 3290 (2NH), 3080 (CH arom), 2944 (CHaliph), 1210 (C=S), 1396, 1142 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 3.8, 4.5 (2t, 4H, $2CH_2$), 4.2 [s, 2H, 2NH, exchangeable with D_2O], 6.7, 7.1 [2d, 4H, Ar-H AB system], 7.7–8.1 (m, 4H, Ar-H), 8.7(s,1H, CH quinoxaline), 11.5 (s,1H, OH). Analysis Calc. for $C_{17}H_{16}N_4O_4S_2$: C, 50.48; H, 3.99; N, 13.85. Found: C, 50.67; H, 3.75; N, 13.63.

O-Ethyl 4-(N-quinoxalin-2-ylsulfamoyl) benzenesulfonamide (3)

A mixture of **1** (0.4 g, 0.01 mol) and ethanol (3 mL, 0.01 mol), was refluxed for 8 h, the reaction mixture was poured into ice water, the solid obtained was filtered and crystallized from dioxane to give **3.** Yield %: 87, m.p.: 135–136 °C. IR (KBr, cm^{-1}): 3270, 3154 (2NH), 3080 (CH arom), 2986 (CHaliph), 1194 (C=S), 1378, 1114 (SO_2).^{1}H-NMR DMSO-d_6 (ppm): 1.3 (t, 3H, CH_3), 4.0 [s, 1H, NH, exchangeable with D_2O], 4.5(q, 2H, CH_2), 6.6, 7.4 [2d, 4H, Ar-H ABsystem], 7.7–8.1 (m, 4H, Ar-H), 8.3[s, 1H, SO_2NH, exchangeable with D_2O], 8.7(s,1H, CH quinoxaline). Analysis Calc. for $C_{17}H_{16}N_4O_3S_2$: C, 52.56; H, 4.15; N, 14.42. Found: C, 52.35; H, 3.98; N, 14.13.

O-Propyl 4-(N-quinoxalin-2-ylsulfamoyl) benzenesulfonamide (4)

A mixture of **1** (0.4 g, 0.01 mol) and propanol (3 mL, 0.01 mol), was refluxed for 3 h. The reaction mixture was poured into ice water and the solid obtained was filtered and crystallized from dioxane to give **4.** Yield %: 92, m.p.: 206–208 °C. IR (KBr, cm^{-1}): 3208, 3156 (2NH), 3070 (CH arom), 2966 (CHaliph), 1200 (C=S), 1348, 1140 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 0.9 (t, 3H, CH_3), 1.8 (m, 2H, CH_2), 4.4 (t, 3H, CH_2), 4.6 (s,1H, NH), 6.7, 7.5 [2d, 4H, Ar-H AB system], 7.7–8.1 (m, 4H, Ar-H), 8.4 [s, 1H, SO_2NH, exchangeable with D_2O], 8.7(s,1H, CH quinoxaline). Analysis Calc. for $C_{18}H_{18}N_4O_3S_2$: C, 53.71; H, 4.51; N, 13.92. Found: C, 53.52; H, 4.67; N, 13.65.

O-Butyl 4-(N-quinoxalin-2-ylsulfamoyl) benzenesulfonamide (5)

A mixture of **1** (0.4 g, 0.01 mol) and butanol (3 mL, 0.01 mol), was refluxed for 8 h, the reaction mixture was poured into ice water, filtered and crystallized from dioxane to give **5.** Yield %: 95, m.p.: 208–210 °C. IR (KBr, cm^{-1}): 3290, 3154 (2NH), 3060 (CH arom), 2956 (CHaliph), 1198 (C=S), 1342, 1140 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 0.95 (t, 3H, CH_3), 1.4, 1.7 (2m, 4H, $2CH_2$), 4.5(t, 3H, CH_2), 4.6 (s,1H,NH), 6.8, 7.5 [2d, 4H, Ar-H AB system], 7.8–8.2 (m, 4H,Ar-H), 8.5 (s,1H,SO_2NH), 8.7(s,1H, CH quinoxaline). Analysis Calc. for $C_{19}H_{20}N_4O_3S_2$: C, 54.79; H, 4.74; N, 13.45. Found: C, 54.45; H, 4.57; N, 13.85.

4-(4-Amino-5-cyano-2-thioxothiazol-3(2H)-yl)-N-(quinoxalin-2-yl)benzene-sulfonamide (6)

A mixture of **1** (0.4 g, 0.01 mol) and malononitrile (0.1 g, 0.001 mol) in ethanol (20 mL) and a catalytic amount of TEA in presence of sulphur (0.03 g, 0.001 mol), was refluxed for 5 h, the reaction mixture was poured into ice water, filtered and crystallized from dioxane to give **6.** Yield %: 92, m.p.: 158–160 °C. IR (KBr, cm^{-1}): 3312, 3220, 3160 (NH, NH_2), 3080 (CH arom), 2214 (CN), 1194 (C=S), 1342, 1138 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 2.2 [S, 2H, NH_2, exchangeable with D_2O], 4.9 (s,1H, NH), 6.7, 7.7 [2d, 4H, Ar-H AB system], 7.7–8.1 (m, 4H, Ar-H), 8.7 [s, 1H, SO_2NH, exchangeable with D_2O], 8.2 (s,1H, CH quinoxaline). ^{13}C-NMR (DMSO-d_6): 58.5 (CN), 112.2 (C-CN), 124.1, 125.2, 126.9, 128.9, 134.8, 135.1, 135.6, 137.1, 154.1 (C-NH_2), 161.9, 187.5 (C=S). MS (m/z): 440 (M^+, 0.1%), 323 (59.31%), 277 (100%), 235 (37.25%), 90 (26,75%). Analysis Calc. for $C_{18}H_{12}N_6O_2S_3$: C, 49.08; H, 2.75; N, 19.08. Found: C, 49.44; H, 2.83; N, 19.35.

4-(3-Cyano-6-imino-8-thioxo-5a,6,9,9a-tetrahydropyrazolo[1,5-a]pyrimido[3,4-e]pyrimidin-7(8H)-yl)-N-(quinoxalin-2-yl)benzenesulfonamide (7)

A mixture of **1** (0.4 g, 0.01 mol) and pyrazolo pyrimidine o-amino carbonitrile (0.3 g, 0.01 mol) in DMF (20 mL) and a catalytic amount of TEA, was refluxed for 5 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **7.** Yield %: 90, m.p.: 236–238 °C. IR (KBr, cm^{-1}): 3332, 3360, 3240 (3NH), 3075(CH arom), 2222 (CN), 1210 (C=S), 1328, 1148 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 4.4 (s, 2H, 2NH), 6.7, 7.5 [2d, 4H, Ar-H AB system], 7.6 [s, 1H, CH pyrazole], 7.7–8.1 (m, 4H, Ar-H), 8.2 [s, 1H, CH pyrimidine], 8.4 [s, 1H, SO_2NH, exchangeable with D_2O], 8.7(s,1H, CH quinoxaline). ^{13}C-NMR (DMSO-d_6): 66.9 (C-CN), 111.6, 114.1 (CN), 124.1, 125.2, 126.9, 128.9, 134.8, 135.1, 135.6, 138.9, 147, 147.1, 156.1, 156.2, 161.9, 166.9, 175.2 (C=S). MS (m/z): 526 (M^+,0.1%), 236 (12.56%), 184 (100%), 119

(12.91 %), 92 (4.79 %). Analysis Calc. for $C_{23}H_{16}N_{10}O_2S_2$: C, 52.26; H, 3.05; N, 26.50. Found: C, 52.55; H, 3.33; N, 26.61.

4-(5-Amino-3-(4-methoxyphenyl)-2,4-dithioxoimidazolidin-1-yl)-N-(quinoxalin-2-yl) benzenesulfonamide (8)

A mixture of **1** (0.4 g, 0.1 mol) and p-Methoxy cyano thio formanilide (0.2 g, 0.001 mol) in tetrahydrofuran (20 mL) and a catalytic amount of TEA, was refluxed for 6 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **8.** Yield %: 89, m.p.: 97–98 °C. IR (KBr, cm^{-1}): 3330, 3212, 3250 (NH, NH_2), 3065(CH arom), 2995 (CH aliph), 1250 (C=S), 1292, 1155 (SO_2). MS (m/z): 536 (M^+, 1.76 %), 256 (3.91 %), 165 (100 %), 150 (5.62 %), 64 (58.82 %). ^{1}H-NMR DMSO-d_6 (ppm): 1.7 [s, 3H, CH_3], 2.2 [s, 2H, NH_2, exchangeable with D_2O], 6.3, 6.5 [2d, 4H, Ar-H, AB system], 6.7, 7.1 [2d, 4H, Ar-H, AB system], 7.6–8.07 [m, 4H, Ar-H], 8.1 [s, 1H, CH quinoxaline], 8.3 [s, 1H, SO_2NH]. ^{13}C-NMR (DMSO-d_6): 102.1 (C-NH_2), 114.1, 124.1, 125.2, 126.9, 127.2, 128.9, 133.1, 133.9, 134.8, 135.1, 135.6, 143.3, 159.9 (OCH_3), 161.9. 176.2 (C=S), 196.1 (C=S). Analysis Calc. for $C_{24}H_{20}N_6O_3S_3$: C, 53.71; H, 3.76; N, 15.66. Found: C, 53.58; H, 3.65; N, 15.49.

6-Mercapto-1-phenyl-5-(4-(N-quinoxalin-2-yl-sulfamoyl) phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo [3,4-d] pyrimidine-4-carboxylic acid (9)

A mixture of **1** (0.4 g, 0.01 mol) and 4-(2-phenyl3-amino pyrazole)carboxylic acid (0.3 g ,0.001 mol) in dioxane (20 mL) and a catalytic amount of TEA, was refluxed for 6 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **9.** Yield %: 87, m.p.: 180–182 °C. IR (KBr, cm^{-1}): 3426 (OH), 3250, 3190 (2NH), 3080 (CH arom), 2929 (CH aliph), 1690 (C=O), 1280 (C=S), 1340, 1150 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 4.4 (s, 2H, 2NH), 6.7, 7.5 [2d, 4H, Ar-H AB system], 7.6 [s, 1H, CH pyrazole], 7.7–8.3 (m, 9H, Ar-H), 8.4 [s, 1H, SO_2NH, exchangeable with D_2O], 8.9 (s,1H, CH quinoxaline), 11.0 [s, 1H, COOH, exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 71.3 (C-COOH), 88.1 (C-SH), 99.5, 123.1, 124.1, 125.2, 126.2, 126.9, 128.9, 129.2, 134.8, 135.1, 135.6, 136.1, 138.9, 146.7, 152.1, 161.9, 169.1 (COOH). MS (m/z): 559 (M^+, 0.16 %), 264 (43.26 %), 111 (100 %), 95 (29.79 %), 75 (24.19 %). Analysis Calc. for $C_{26}H_{21}N_7O_4S_2$: C, 55.80; H, 3.78; N, 17.52. Found: C, 55.67; H, 3.65; N, 17.33.

4-Methyl-4-oxo-6-propionyl-2-thioxo-1,2-dihydrothieno[2,3-d]pyrimidin-3(4 H)-yl)-N-(quinoxalin-2-yl)benzenesulfonamide (10)

A mixture of **1** (0.4 g, 0.01 mol) and 3,5-(2amino-4-methyl thiophen)carboxylic acid ethyl ester (0.3 g ,0.001 mol) in dioxane (20 mL) and a catalytic amount of TEA, was refluxed for 6 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **10.** Yield %: 86, m.p.: 104–106 °C. IR (KBr, cm^{-1}): 3306, 3296 (2NH), 3095 (CH arom), 2984 (CH aliph),1674 (C=O), 1228 (C=S), 1320, 1104 (SO_2). MS (m/z): 537 (M^+, 14.38 %), 479 (11.81 %), 368 (100 %), 221 (97.12 %), 147 (50.16 %). ^{1}H-NMR DMSO-d_6 (ppm): 1.17 [s, 3 H, CH_3], 2.2 [s, 3 H, CH_3], 4.0 [s, 1H, NH, exchangeable with D_2O], 6.9, 7.5 [2d, 4H, Ar-H, AB system], 7.7–8.07 [m, 4H, Ar-H], 8.1 [s, 1H, CH quinoxaline], 8.3[s, 1H, SO_2NH]. Analysis Calc. for $C_{24}H_{19}N_5O_4S_3$: C, 53.62; H, 3.56; N, 13.03. Found: C, 53.77; H, 3.69; N, 13.25.

4-(6,8-Dichloro-4-oxo-2-thioxo-1,2-dihydroquinazolin-3(4H)-yl)-N-(quinoxalin-2-yl)benzenesulfonamide (11)

A mixture of **1** (0.4 g, 0.01 mol) and 3,5-dichloroanthranilic acid (0.3 g, 0.01 mol) in dioxane (20 mL) and a catalytic amount of TEA, was refluxed for 5 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **11.** Yield %: 92, m.p.: 250–252 °C. IR (KBr, cm^{-1}): 3350, 3240 (NH), 3072 (CH arom), 1690 (C=O), 1220 (C=S), 1310, 1160 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 4.0 [s, 2H, 2NH, exchangeable with D_2O], 6.9, 7.5[2d, 4H, Ar-H, AB system], 6.8, 7.2[2s, 2H, Ar-H], 7.7–8.07 [m, 4H, Ar-H], 8.2 [s, 1H, CH quinoxaline], 8.3 [s, 1H, SO_2NH]. MS (m/z): 529 (M^+, 10.81 %), 313 (46.82 %), 236 (100 %), 116 (28.60 %), 92 (69.07 %). Analysis Calc. for $C_{22}H_{13}Cl_2N_5O_3S_2$: C, 49.82; H, 2.47; N, 13.20. Found: C, 49.75; H, 2.33; N, 12.95.

4-(6-Methyl-4-oxo-2-thioxo-1,2-dihydroquinazolin-3(4H)-yl)-N-(quinoxalin-2-yl)benzenesulfonamide (12)

A mixture of **1** (0.4 g, 0.01 mol) and 2-amino 5-methyl benzoic acid (0.3 g ,0.01 mol) in dioxane (20 mL) and a catalytic amount of TEA, was refluxed for 6 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **12.** Yield %: 91, m.p.: 238–239 °C. IR (KBr, cm^{-1}): 3242, 3120 (2NH), 3036 (CH arom), 2860 (CH aliph.), 1620 (C=O), 1206 (C=S), 1206, 1186 (SO_2). MS (m/z): 475 (M^+,7 %), 410 (100 %), 266 (27.2 %), 160 (42.79 %), 90 (41.61 %). ^{1}H-NMR DMSO-d_6 (ppm): 2.7 [s, 3H, CH_3], 4.1 [s, 1H, NH, exchangeable with D_2O], 6.5, 7.3[2d, 4H, Ar-H, AB system], 7.5-8.07 [m, 7H, Ar-H], 8.1[s, 1H, CH quinoxaline], 8.3[s, 1H, SO_2NH]. Analysis Calc. for $C_{23}H_{17}N_5O_3S_2$: C, 58.09; H, 3.60; N, 14.73. Found: C, 58.45; H, 3.55; N, 14.85.

4-(Benzo[d]thiazol-2-yl-amino)-N-(quinoxalin-2-yl) benzenesulfonamide (13)

A mixture of **1** (0.4 g, 0.01 mol) and thiophenol (0.1 g ,0.01 mol) in DMF (20 mL) and a catalytic amount of TEA, was refluxed for 24 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **13.** Yield %: 94, m.p.: 190–192 °C. IR (KBr, cm^{-1}): 3374, 3288 (2NH), 3062 (CH arom), 1230 (C=S), 1300, 1150 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 4.0 [s, 2H, 2NH, exchangeable with D_2O], 6.9, 7.5 [2d, 4H, Ar-H, AB system], 7.2–8.1 [m, 9H, Ar-H], 8.2 [s, 1H, CH quinoxaline], 8.3 [s, 1H, SO_2NH]. MS (m/z): 433 (M^+, 16.89 %), 369 (100 %), 225 (73.21 %), 108 (13.02 %), 90 (27.65 %). Analysis Calc. for $C_{21}H_{15}N_5O_2S_2$: C, 58.18; H, 3.49; N, 16.16. Found: C, 58.55; H, 3.67; N, 16.43.

4-(9 H-Purin-8-ylamino)-N-(quinoxalin-2-yl) benzenesulfonamide (14)

A mixture of **1** (0.4 g, 0.01 mol) and 4, 5-diaminopyrimidine (0.2 g, 0.01 mol) in DMF (20 mL) and a catalytic amount of TEA, was refluxed for 8 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **14.** Yield %: 86, m.p.: 236–238 °C. IR (KBr, cm^{-1}): 3358, 3244 (2NH), 3070 (CH arom), 1236 (C=S), 1316, 1148 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 4.0 [s, 2 H, 2NH, exchangeable with D_2O], 6.9, 7.5 [2d, 4 H, Ar-H, AB system], 7.5–8.1 [m, 4 H, Ar-H], 8.2 [s, 1H, CH quinoxaline], 8.3 [s, 1H, SO_2NH], 8.9, 9.1 [2s, 2H, 2CH pyrimidine]. MS (m/z): 418 (M^+, 0.54 %), 388 (1.31 %), 236 (69.51 %), 145 (100 %), 92 (47.33 %). Analysis Calc. for $C_{19}H_{14}N_8O_2S$: C, 54.54; H, 3.37; N, 26.78. Found: C, 54.15; H, 3.23; N, 26.65.

N-(Quinoxalin-2-yl)-4-(5-thioxo-4,5-dihydro-1 H-1,2,4-triazol-3-ylamino)benzene-sulfonamide (15)

A mixture of **1** (0.4 g, 0.01 mol) and thiosemicarbazide (0.1 g, 0.01 mol) in DMF (20 mL) and a catalytic amount of TEA, was refluxed for 8 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **15.** Yield %: 90, m.p.: 198–200 °C. IR (KBr, cm^{-1}): 3 364, 3 256, 3 210, 3 160 (4NH), 3 066 (CH arom), 1 316, 1 146 (SO_2), 1 213 (C=S). ^{1}H-NMR DMSO-d_6 (ppm): 4.0 [s, 2 H, 2NH, exchangeable with D_2O], 6.9, 7.5 [2d, 4H, Ar-H, AB system], 7.2 [s, 2H, 2NH triazole, exchangeable with D_2O], 7.6–8.1 [m, 4H, Ar-H], 8.2 [s, 1H, CH quinoxaline], 8.3 [s, 1H, SO_2NH]. MS (m/z): 399 (M^+, 2.90%), 236 (100%), 145 (83.28%), 110 (3.70%), 92 (67.22%). Analysis Calc. for $C_{16}H_{13}N_7O_2S_2$: C, 48.11; H, 3.28; N, 24.55. Found: C, 47.95; H, 3.20; N, 24.49.

4-(O-pyridin-3-yl-carbamothioate)-N-(quinoxalin-2-yl) benzenesulfonamide (16)

A mixture of **1** (0.4 g, 0.01 mol) and 3-pyridinol (0.1 g, 0.01 mol) in DMF (20 mL) and a catalytic amount of TEA, was refluxed for 8 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **16.** Yield %: 95, m.p.: 258–260 °C. IR (KBr, cm^{-1}): 3 360, 3 250 (2NH), 3 070 (CH arom), 1 232 (C=S), 1 318, 1 148 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 4.3 [s, 2 H, 2NH, exchangeable with D_2O], 6.7, 7.3 [2d, 4H, Ar-H, AB system], 7.6–8.1 [m, 4H, Ar-H], 8.2[s, 1H, CH quinoxaline], 8.3 [s, 1H, SO_2NH], 8.4–8.7 [m, 4H, 4CH pyridine]. MS (m/z): 437 (M^+, 0.45%), 236 (100%), 156 (18.64%), 145 (48.85%), 92 (59.01%). Analysis Calc. for $C_{20}H_{15}N_5O_3S_2$: C, 54.91; H, 3.46; N, 16.01. Found: C, 54.95; H, 3.55; N, 16.35.

2-(4-Amino-5-mercapto-4 H-1,2,4-triazol-3-yl)-N-(4-(N-quinoxalin-ylsulfamoyl)phenyl)benzenesulfonamide (17)

A mixture of compound 1 (0.4 g, 0.001 mol) and 4-N-amino-3-hyrazine-5-thioxo-1,2,4-triazole (0.2 g, 0.001 mol) in DMF (20 mL) and a catalytic amount of TEA, was refluxed for 2 h, the reaction mixture was poured into ice water, filtered and crystallized from ethanol to give **17.** Yield %: 92, m.p.: 192–194 °C. IR (KBr, cm^{-1}): 3 336, 3 323, 3 272, 3 240, 3 212, 3 190 (4NH, NH_2), 3070 (CH arom),1232 (C=S), 1 324, 1 114 (SO_2). ^{1}H-NMR DMSO-d_6 (ppm): 2.2 [2, 2H, NH_2, exchangeable with D_2O], 3.1[s, 1H, SH], 4.0 [s, 3H, 3NH, exchangeable with D_2O], 6.9, 7.5 [2d, 4H, Ar-H, AB system], 7.6-8.1[m, 4H, Ar-H], 8.2[s, 1H, CH quinoxaline], 8.3 [s, 1H, SO_2NH]. MS (m/z): 488 (M^+, 0.1%), 313 (1.66%), 236 (16.71%), 108 (5.54%), 73 (100%). Analysis Calc. for $C_{217}H_{16}N_{10}O_2S_3$: C, 41.79; H, 3.30; N, 28.67. Found: C, 41.66; H, 3.25; N, 28,54.

In-vitro anticancer activity

The human tumor cell line (HEPG2) was available at the National Cancer Institute, Cairo, Egypt. Irradiation was performed in the National Cancer Institute, Cairo, Egypt using γ-cell-40 (^{60}CO) source. The anticancer activity of the newly synthesized compounds was mesured using the Sulfo-Rhodamine-B stain (SRB) assay by the method of Skehan et al. [21] (1990). Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compounds under test (5, 12.5, 25 and 40 µM) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Unbounded dye was removed by 4 washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared with the reference drug 5-flurouracil and the results are given in ◉ **Table 1**.

Radiosensitizing activity

The most potent compounds resulted from the in vitro anticancer screening; compounds **4** and **17**, 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 γ-cell-40 (^{60}CO) source. The surviving fractions were expressed as means ± standard error. The results were analyzed using 1-way ANOVA test and are given in ◉ **Table 2**.

Results and Discussion

▼

Chemistry

The isothiocyanate derivative **1** was synthesized by the reported method [22], due to the high reactivity if isothiocyanate group, compound **1** was employed as starting material for the synthesis of different sulfaquinoxaline derivatives through incorporation of different biologically active moieties, reaction of compound **1** with ethylene glycol yielded the corresponding thiourethane derivative **2**, while condensation of compound **1** with different aliphatic alcohols such as ethanol, propanol or butanol in DMF containing 3 drops of TEA gave compounds **3**, **4** and **5**, respectively, cyclization occurred when **1** was reacted with malononitrile in the presence of sulfur to the thiazole derivative **6**, the structure of the synthesized compounds were confirmed by microanalytical and spectral data, reaction of **1** with pyrazolopyrimidine o-amino carbonitrile yielded the pyrazolopyrimidopyrimidine derivative **7**, interaction of **1** with p-methoxy cyanothioformanilde, intramolecular cyclization occurred to give the imidazole derivative **8**. Refluxing **1** with 4-(2-phenyl 3-amino pyrazole)carboxylic acid in DMF yielded the purine analogue **9**. Refluxing **1** with 3,5-(2-amino-4-methylthiophen) carboxylic acid ester, 3,5-dichloroanthranilic acid or 2-amino-5-methyl benzoic acid resulted in the formation of pyrimido derivatives **10**, **11** and **12**, respectively. While, reaction of **1** with thiophenol or 4,5-diaminopyrimidine yielded compounds **13** and **14**, respectively. Double cyclization occurred when compound **1** was refluxed with thiosemicarbazide in DMF through elimination of H_2S which was detected by lead acetate paper to give the triazolo derivative **15**. Finally, interaction of **1** with 3-pyridinol and 4-N-amino-3-hydrazino-5-thioxo-1,2,4-triazole gave compounds **16** and **17**, respectively (◉ **Fig. 2**).

In-vitro anticancer evaluation

The newly synthesized compounds were evaluated for their in-vitro anticancer activity against human liver cancer cell line

Table 1 In-vitro anticancer screening of the synthesized compounds against human liver cell line (HEPG2).

Cpd. No.	Compound concentration (μM)				IC_{50} (μM)
	5 (μM)	12.5 (μM)	25 (μM)	40 (μM)	
	Surviving fraction (mean ± SE)[a]				
5-FU	0.921 ± 0.020	0.846 ± 0.020	0.761 ± 0.010	0.494 ± 0.030	40
1	0.934 ± 0.002	0.896 ± 0.009	0.848 ± 0.028	0.422 ± 0.047	38.12
2	0.855 ± 0.018	0.653 ± 0.031	0.493 ± 0.022	0.392 ± 0.012	23.35
3	0.882 ± 0.012	0.693 ± 0.041	0.625 ± 0.047	0.419 ± 0.022	32.21
4	0.395 ± 0.064	0.259 ± 0.014	0.346 ± 0.016	0.412 ± 0.012	4.29
5	0.921 ± 0.031	0.731 ± 0.043	0.610 ± 0.081	0.386 ± 0.015	30.87
6	0.925 ± 0.014	0.839 ± 0.007	0.642 ± 0.045	0.428 ± 0.317	34.63
7	0.892 ± 0.009	0.681 ± 0.016	0.553 ± 0.007	0.366 ± 0.015	26.84
8	0.912 ± 0.014	0.739 ± 0.019	0.654 ± 0.027	0.388 ± 0.044	32.48
9	0.896 ± 0.007	0.703 ± 0.017	0.611 ± 0.034	0.454 ± 0.008	34.36
10	0.894 ± 0.016	0.761 ± 0.031	0.538 ± 0.041	0.445 ± 0.044	29.26
11	0.927 ± 0.021	0.721 ± 0.021	0.583 ± 0.051	0.496 ± 0.062	40
12	0.758 ± 0.032	0.563 ± 0.026	0.437 ± 0.015	0.396 ± 0.004	16.64
13	0.91 ± 0.006	0.705 ± 0.039	0.481 ± 0.038	0.331 ± 0.012	23.35
14	0.93 ± 0.014	0.813 ± 0.015	0.725 ± 0.041	0.462 ± 0.049	38.12
15	0.923 ± 0.017	0.823 ± 0.027	0.569 ± 0.017	0.408 ± 0.006	29.79
16	0.886 ± 0.006	0.753 ± 0.021	0.603 ± 0.041	0.348 ± 0.009	30.06
17	0.888 ± 0.011	0.445 ± 0.025	0.316 ± 0.044	0.368 ± 0.028	11.27

[a] Each value is the mean of 3 experiments ± standard error

Table 2 In-vitro anticancer screening of compounds **4** and **17** against human liver cell line (HEPG2) in combination with radiation.

Cpd. No.	Control	Irradiated (8 Gy)	Compound concentration (μM) + irradiation (8 Gy)						IC_{50}(μM)
			Surviving fraction (mean ± SE) [a]						
			1	2.5	5	12.5	25	40	
4	1.000	0.927 ± 0.02*	0.93 ± 0.02*	0.47 ± 0.03*	0.29 ± 0.01*	0.16 ± 0.02*	0.24 ± 0.05*	0.21 ± 0.06*	2.41
17	1.000	0.927 ± 0.02*	0.98 ± 0.01*	0.81 ± 0.03*	0.67 ± 0.07*	0.23 ± 0.01*	0.11 ± 0.01*	0.16 ± 0.08*	6.97

[a] Each value is the mean of three values ± Standard Error

*Significant difference from control group at $p < 0.001$

(HEPG2). 5-Flurouracil, which is one of the most effective anticancer agents was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of liver cancer cell line (HEPG2). The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. ◉ **Table 1** shows the in-vitro anticancer activity of the synthesized compounds which exhibited significant activity compared to the reference drug. From the results in ◉ **Table 1**, it was found that some of the tested compounds were found to be equipotent while others showed lower IC_{50} than 5-fluorouracil (IC_{50} = 40 μM), where, the starting material **1** showed high IC_{50} compared to most of the tested compounds (IC_{50} = 38.12 μM), while, substitution on the isothiocyanato group resulted in an increase in the activity in most of the compounds and the most potent was the propyl thioureido compound **4** (IC_{50} = 4.29 μM), the thioureido **2** (IC_{50} = 23.35 μM), while, the thiouredo compounds **3** and **5** containing an aliphatic chain showed a slightly higher activity (IC_{50} = 32.21 and 30.87 μM, respectively). The pyrazolopyrimidopyrimidine **7** (IC_{50} = 26.84 μM), the benzopyrimidine **12** (IC_{50} = 16.64 μM), the benzothiophene **13** (IC_{50} = 23.3 μM) and the triazolo derivative **17** (IC_{50} = 11.27 μM) were the most active derivatives among those containing cyclic heterocyclic moieties as substituent on amino group of sulfonamide and they found to be more active than the reference drug. While compounds **3, 5, 6**, **8–11, 14–16** showed IC_{50} values ranging from 30.06–40 μM.

Radiosensitizing evaluation

The rationale for combining chemotherapy and radiotherapy is based mainly on 2 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. Cytotoxic agents can enhance 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 [23]. Consequently, the ability of the 2 most active compounds, compounds **4** and **17**, to enhance the cell killing effect of γ-irradiation was studied. From the results obtained in ◉ **Table 1**, compound **4** showed an in vitro cytotoxic activity with IC_{50} value of 4.29 μM, when the cells were subjected to different concentrations of the compound alone. While, when the cells were subjected to different concentrations of compound **4** (1, 2.5, 5, 12.5, 25, 40 μM), and irradiated with a single dose of γ-radiation at a dose level of 8 Gy, as shown in ◉ **Table 2**, the IC_{50} value was synergistically

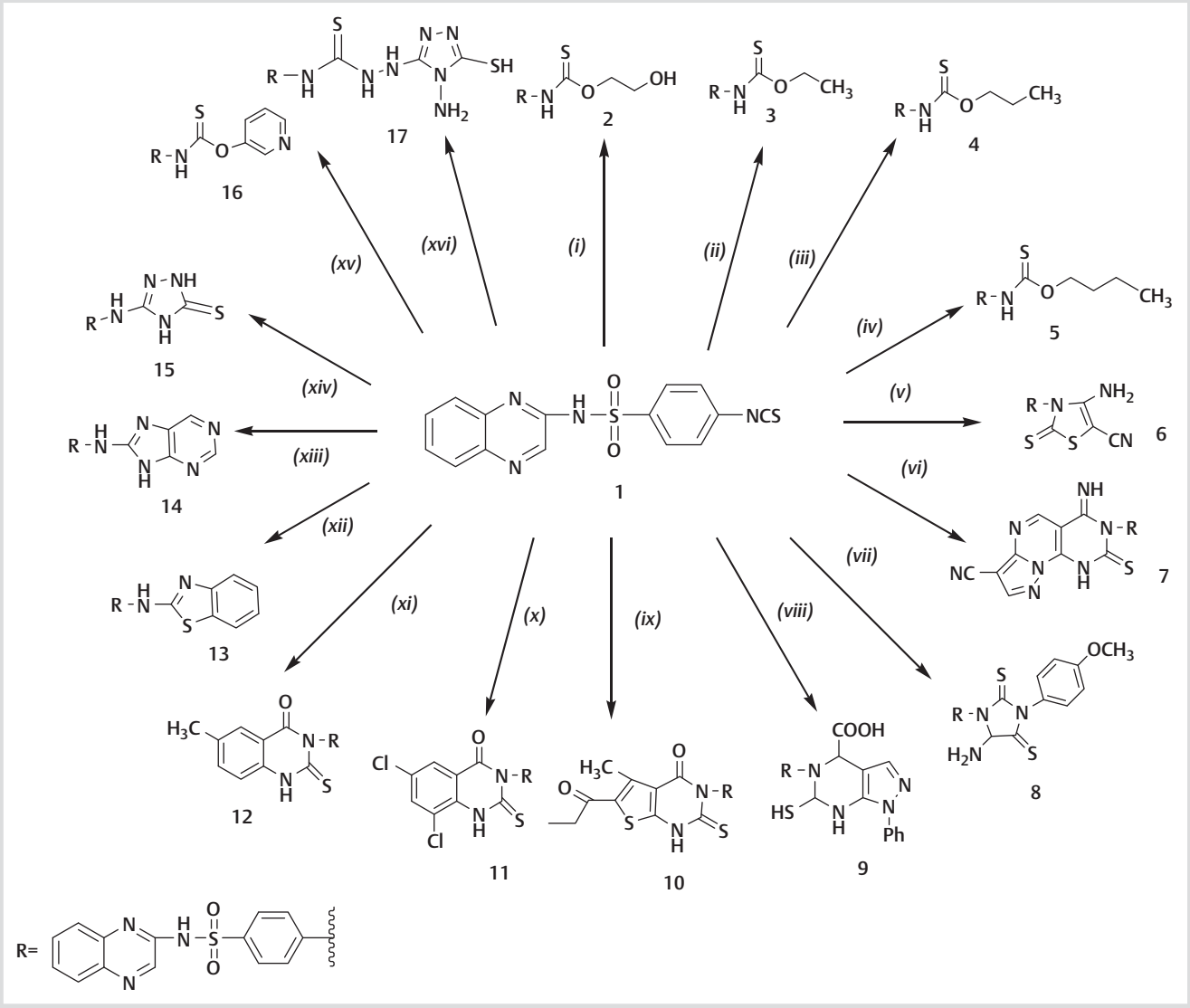

Fig. 2 Synthetic pathways for compounds 1–17. Conditions and reagents: (i) ethylen glycol, DMF, TEA, reflux 8 h; (ii) ethanol, DMF, TEA, reflux 8 h; (iii) propanol, DMF, TEA, reflux 3 h; (iv) butanol, DMF, TEA, 8 h; (v) malononitrile, S, DMF, TEA, reflux 5 h; (vi) pyrazolo pyrimidine o-amino carbonitrile, DMF, TEA, reflux 6 h; (vii) p-methoxy cyanothio formanilide, DMF, TEA, reflux 6 h; (viii) 4-(2 phenyl 3-amino pyrazole)carboxylic acid, DMF, TEA, reflux 1 h; (ix) 3,5-(2amino-4-methyl thiophen)carboxylic acid ethyl ester, DMF, TEA, reflux 5 h; (x) 3,5,dichloroanthranilic acid, DMF, TEA, reflux 5 h; (xi) 2-amino 5-methyl benzoic acid, DMF, TEA, reflux 6 h; (xii) thiophenol, DMF, TEA, reflux 24 h; (xiii) 4,5,diamino pyrimidine, DMF, TEA, reflux 8 h; (xiv) thiosemicarbazide, DMF, TEA, reflux 8 h; (xv) 3-pyridinol, DMF, TEA, reflux 5 h; (xvi) 4-N-amino-3-hydrazine-5-thioxo1,2,4,triazole, DMF, TEA, reflux 2 h.

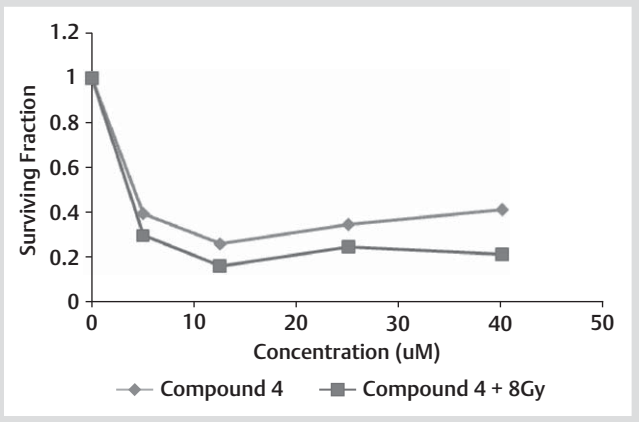

Fig. 3 Survival curve for HEPG2 cell line for compound 4 alone and in combination with γ-irradiation (8 Gy).

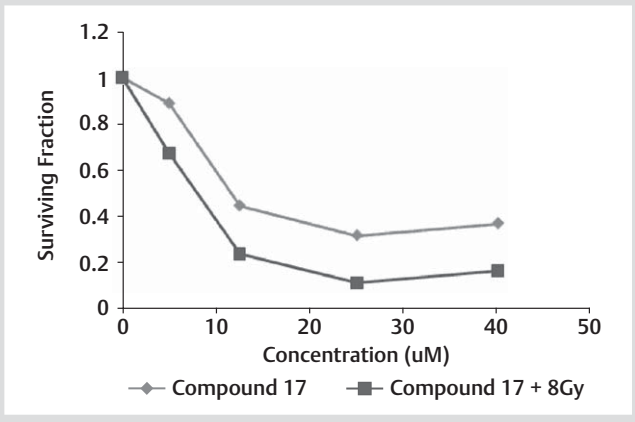

Fig. 4 Survival curve for HEPG2 cell line for compound 17 alone and in combination with γ-irradiation (8 Gy).

decreased to 2.41 µM (◉ **Fig. 3**). Similarly, compounds **17** showed IC_{50} value of 11.27 µM when used alone, as shown in ◉ **Table 1**. The IC_{50} value was decreased to 6.97 µM when the cells were treated with compounds **17** in combination with γ-radiation (◉ **Fig. 4**). From these results, we can conclude that using the combination of compounds **4** or **17** and ionizing radiation synergistically enhanced growth inhibition on liver cancer cells, compared with each agent alone.

Conclusion

We report here the synthesis of new sulfaquinoxaline derivatives. It was clearly observed from the results of in-vitro anticancer screening that the synthesized compounds exhibited significant anticancer activity on liver human tumor cell line (HEPG2). Combining these compounds with radiation enhances their activity, which demonstrates the importance of combination therapy for cancer patients as it allows a reduction of the individual doses, which in turn decreases the side effects of both drugs and radiation.

Conflict of Interest

The authors declare that they have no conflict of interest with respect to this paper.

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