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Original research paper

Synthesis, *in vitro* anticancer screening and radiosensitizing evaluation of some new 4-[3-(substituted)thioureido]-*N*-(quinoxalin-2-yl)-benzenesulfonamide derivatives

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Sulfonamides and quinoxaline derivatives possess many types of biological activities and have been recently reported to show substantial antitumor activity. This paper reports the synthesis of novel thioureido sulfaquinoxaline derivatives. All the newly synthesized compounds were evaluated for their *in vitro* anticancer activity against a human liver cell line (HEPG2) and showed higher activity than the reference drug doxorubicin. 4-(3-(4-Ethylbenzoate)thioureido)-*N*-(quinoxalin-2-yl)benzenesulfonamide (**9**) (IC_{50} = 15.6 μmol L^{-1}), *N*-(pyridin-2-yl)-4-(3-(4-(*N*-quinoxalin-2-yl-sulfamoyl)phenyl)thioureido)benzenesulfonamide (**10**) (IC_{50} = 26.8 μmol L^{-1}) and *N*-(quinoxalin-2-yl)-4-(3-(4-(*N*-thiazol-2-ylsulfamoyl)phenyl)thioureido)benzenesulfonamide (**11**) (IC_{50} = 24.4 μmol L^{-1}) were the most potent compared to doxorubicin (IC_{50} = 71.8 μmol L^{-1}). The most potent compounds **9**, **10** and **11** were evaluated as radiosensitizing agents by subjecting the compounds to γ-irradiation (8 kGy).

Keywords: quinoxaline, sulfonamides, anticancer activity, radiosensitizing effect

Quinoxaline derivatives show very interesting biological properties such as antibacterial (1), antiviral (2), antifungal (3), anthelmintic (4) and insecticidal (5). They were proven to be efficient anticancer agents when tested against several cell lines. Several quinoxaline derivatives were reported to be potent and highly selective epidermal growth factor receptor tyrosine kinase inhibitors (7). On the other hand, thioureido sulfonamide derivatives were reported to show significant anticancer activity by acting as carbonic anhydrase inhibitors (8). In the light of these facts and as a continuation of our previous work (9–12), we planned to synthesize novel thioureido sulfaquinoxaline

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derivatives hoping that the new compounds might show significant anticancer activity and to study their structure-activity relationships. Moreover, this research was also aimed to evaluate the new compounds for their *in vitro* anticancer activity in combination with γ-irradiation.

EXPERIMENTAL

Melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, UK). Elemental analyses (C, H, N) were performed on a Perkin-Elmer 2400 analyzer (Perkin-Elmer, USA) at the Microanalytical Laboratories of the Faculty of Science, Cairo University. All compounds were within ±0.4 % of theoretical values. The IR spectra (KBr) were measured on a Shimadzu IR 110 Spectrophotometer (Shimadzu, Japan), ^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker proton NMR-Avance 300 instrument (300 MHz) (Bruker, Germany), in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as internal standard. Mass spectra were run using an HP Model MS-5988 (Hewlett Packard, USA). All reactions were monitored with a thin layer chromatography using precoated aluminum sheets (Silica gel Merck 60 F_{254}) and were visualized with a UV lamp (Merck, Germany). All chemicals (sulfaquinoxaline and doxorubicin) were commercially supplied from Sigma-Aldrich (USA).

Syntheses

4-Isothiocyanato-N-*quinoxaline-2-yl-benzenesulfonamide (**2**).* – To a suspension of sulfaquinoxaline **1** (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 until red color of the thiophosgene disappeared and a white precipitate was formed. The precipitate was filtered off and washed with water to give compound **2**.

4-(3-Pyridin-2-yl-thioureido)-N-*(quinoxalin-2-yl)benzenesulfonamide (**3**).* – A mixture of **2** (0.342 g, 0.01 mol) and 2-aminopyridine (0.094 g, 0.01 mol) in dimethylformamide (DMF) (20 mL) containing 3 drops of triethylamine (TEA), was refluxed for 5 h. The solid obtained was precipitated while hot and filtered to give compound **3**.

4-(3-(5-Chloropyridin-2-yl)thioureido)-N-*(quinoxalin-2-yl)benzenesulfonamide (**4**).* – A mixture of **2** (0.342 g, 0.01 mol) and 2-amino-5-chloropyridine (0.128 g, 0.001 mol) in DMF (20 mL), containing 3 drops of TEA, was refluxed for 5 h. The solid obtained was precipitated while hot and filtered to give compound **4**.

4-(3-(5-Bromopyridin-2-yl)thioureido)-N-*(quinoxalin-2-yl)benzenesulfonamide (**5**).* – A mixture of **2** (0.342 g, 0.001 mol) and 2-amino-5-bromopyridine (0.173 g, 0.001 mol) in DMF (20 mL), containing 3 drops of TEA, was refluxed for 5 h. The solid obtained was precipitated while hot and filtered to give compound **5**.

4-(3-(4-Bromophenyl)thioureido)-N-*(quinoxalin-2-yl)benzenesulfonamide (**6**).* – The mixture of **2** (0.342 g, 0.01 mol) and *p*-bromoaniline (0.171 g, 0.001 mol) in dioxane (20 mL), with 3 drops of TEA, was refluxed for 1 h, the solid obtained was precipitated when cold and filtered to give compound **6**.

4-(3-(4-Fluorophenyl)thioureido)-N-*(quinoxalin-2-yl)benzenesulfonamide* (**7**). – A mixture of **2** (0.342 g, 0.01 mol) and *p*-fluoroaniline (0.111 g, 0.001 mol) in dioxane (20 mL), with 3 drops of TEA, was refluxed for 1 h. The solid obtained was precipitated when cold and filtered to give compound **7**.

4-(3-(4-Chlorophenyl)thioureido)-N-*(quinoxalin-2-yl)benzenesulfonamide (8)*. – A mixture of **2** (0.342 g, 0.001 mol) and *p*-chloroaniline (0.127 g, 0.001 mol) in dioxane (20 mL), with 3 drops of TEA, was refluxed for 1 h, the solid obtained was precipitated while hot and filtered to give compound **8**.

4-(3-(4-Carbethoxy)thioureido)-N-*(quinoxalin-2-yl)benzenesulfonamide (9)*. – A mixture of **2** (0.342 g, 0.001 mol) and benzocaine (0.165 g, 0.001 mol) in dioxane (20 mL), with 3 drops of TEA, was refluxed for 5 h. The solid obtained was precipitated when hot and filtered to give compound **9**.

Table I. Physical and analytical data of newly synthesized compounds

Compd.[a]	M.p. (°C)[b]	Yield (%)	Mol. formula (M_r)	Analysis calc./found (%)		
				C	H	N
2	210–212	86	$C_{15}H_{10}N_4O_2S_2$ (342.40)	52.62 52.40	2.94 2.83	16.36 16.11
3	244–246	83	$C_{20}H_{16}N_6O_2S_2$ (436.51)	55.03 55.43	3.69 3.90	19.25 18.98
4	240–242	91	$C_{20}H_{15}ClN_6O_2S_2$ (470.96)	51.01 50.90	3.21 2.85	17.84 17.50
5	246–248	93	$C_{20}H_{15}BrN_6O_2S_2$ (514.41)	46.61 46.25	2.93 2.90	16.31 15.90
6	220–222	85	$C_{21}H_{16}BrN_5O_2S_2$ (513.42)	49.03 49.35	3.14 3.50	13.61 13.85
7	177–180	92	$C_{21}H_{16}FN_5O_2S_2$ (453.51)	55.62 55.15	3.56 3.48	15.44 15.39
8	128–130	90	$C_{21}H_{16}ClN_5O_2S_2$ (469.97)	53.67 53.33	3.43 3.25	14.90 14.67
9	135–136	89	$C_{24}H_{21}N_5O_4S_2$ (507.58)	56.79 57.10	4.17 4.46	13.80 13.95
10	248–249	87	$C_{26}H_{21}N_7O_4S_3$ (591.68)	52.78 52.45	3.58 3.18	16.57 16.23
11	251–252	94	$C_{24}H_{19}N_7O_4S_4$ (597.71)	48.23 48.44	3.20 3.38	16.40 16.60

[a] Starting material **1** is not included in the table since it is commercially available.
[b] Crystallization solvent was ethanol.

Table II. Spectral data of newly synthesized compounds

Compd.[a]	IR (ν, cm^{-1})	^1H NMR (DMSO-d_6) (δ, ppm)	^{13}C NMR (DMSO-d_6) (δ, ppm)	MS
2	3290 (NH), 3070 (CH arom.), 2106 (NCS), 1330, 1156 (SO_2)	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)	124.1, 125.2, 126.9, 128.9, 134.8, 135.1, 135.6, 136.8 (NCS), 138.3, 161.9	342 (M^+, 10.2 %), 236 (100 %) ($C_{10}H_{11}N_3O_2S$, *N*-(5,6-dihydro--pyrazin-2-yl)--benzenesulfonamide)
3	3436, 3360, 3252 (3NH), 3072 (CH arom.) 1318, 1150 (SO_2), 1234 (C=S)	4.0 (s, 3H, NH, exchangeable with D_2O), 6.5–6.7 (m, 2H, 2CH pyridine), 6.74, 7.68 (2d, 4H, Ar-H AB system), 7.88–8.05 (m, 4H, Ar-H), 8.07 (s, 1H, CH--quinoxaline), 8.11, 8.3 (2t, 2H, 2CH pyridine)	–	436 (M^+, 0.8 %), 236 (100 %) ($C_{10}H_{11}N_3O_2S$, *N*-(5,6-dihydro--pyrazin-2-yl)--benzenesulfonamide)
4	3436, 3358, 3246 (3NH), 3070 (CH arom), 1316, 1148 (SO_2), 1232 (C=S)	4.0 (s, 3H, NH, exchangeable with D_2O), 6.74, 7.68 (2d, 4H, Ar-H AB system), 7.8–8.05 (m, 4H, Ar-H), 8.1 (s, 1H, CH-quinoxaline), 8.3 (t, 3H, 3CH pyridine)	–	470 (M^+, 6.05 %), 84 (100 %) ($C_4H_8N_2$, 1,2,3,6--tetrahydropyrazine)
5	3338, 3258, 3224 (3NH), 3072 (CH arom.), 1232 (C=S), 1318, 1148 (SO_2)	4.0 (s, 3H, NH, exchangeble with D_2O), 6.4, 7.6 (2d, 4H, Ar-H AB system), 7.88–8.0 (m, 4H, Ar-H), 8.1 (s, 1H, CH-quinoxaline), 8.23 (t, 3H, 3CH pyridine)	–	515 (M^+, 0.5 %), 236 (100 %) ($C_{10}H_{11}N_3O_2S$, *N*-(5,6-dihydropy razin-2-yl)-benzenesulfonamide)
6	3442, 3360, 3198 (3NH), 3072 (CH arom), 1236 (C=S), 1314, 1148 (SO_2)	4.0 (s, 3H, NH, exchangeable with D_2O), 6.35, 7.18 (2d, 4H, Ar-H AB system), 6.74, 7.68 (2d, 4H, Ar-H AB system), 7.7–8.07 (m, 4H, Ar-H), 8.05 (s, 1H, CH-quinoxaline)	–	513 (M^+, 0.8 %), 149 (100 %) ($C_5H_{15}N_3S$, *N*--(methylthio) piperazine-2--amine)
7	3355, 3314, 3212 (3NH), 3016(CH arom), 1228 (C=S), 1338, 1152 (SO_2)	4.0 (s, 3H, NH, exchangeable with D_2O), 6.44, 6.72 (2d, 4H, Ar-H AB system), 6.74, 7.68 (2d, 4H, Ar-H AB system), 7.72–8.1 (m, 4H, Ar-H), 8.2 (s, 1H, CH-quinoxaline)	–	453 (M^+, 1.5 %), 111 (100 %) ($C_5H_9N_3$, 2,3--dihydro-2,6-dimethylpyrazine)

8	3320, 3236, 3163 (3NH), 3072 (CH arom), 1299 (C=S), 1362, 1143 (SO_2)	4.0 (s, 3H, 3NH, exchangeable with D_2O), 6.4, 7.02 (2d, 4H, Ar-H AB system), 6.74, 7.68 (2d, 4H, Ar-H AB system), 7.78–8.1 (m, 4H, Ar-H), 8.3 (s, 1H, CH-quinoxaline)	–	469 (M^+, 5 %), 126 (100 %) ($C_7H_{14}N_2$, N^1--methylcyclohex-5-ene-1,2-diamine)
9	3510, 3450, 3208 (3NH), 3072 (CH arom), 2992 (CH aliph), 1710 (C=0), 1282 (C=S), 1310, 1114 (SO_2).	1.2 (t, 3H, CH_3), 4.0 (s, 3H, 3NH, exchangeable with D_2O), 4.3 (q, 2H, CH_2), 6.57, 7.52 (2d, 4H, Ar-H AB system), 6.74, 7.6 (2d, 4vH, Ar-H, AB system), 7.7–8.1 (m, 4H, Ar-H), 8.6 (s,1H, CH quinoxaline)	–	–
10	3440, 3360, 3250 (3NH), 3070 (CH arom), 1314, 1148 ($2SO_2$)	4.0 (s, 4H, 4NH, exchangeable with D_2O), 6.4, 7.02 (2d, 8H, Ar-H AB system), 7.2–7.5 (m, 4H, 4CH-pyridine), 7.8–8.1 (m, 4H, Ar-H), 8.2 (s, 1H, CH-quinoxaline)	–	591 (M^+, 0.8 %), 264 (29.93 %), 111 (100 %) ($C_5H_9N_3$, 2,3-dihydro-2,6-dimethylpyrazine)
11	3436, 3360, 3252 (3NH), 3072 (CH arom), 1264 (C=S), 1310, 1148 (SO_2)	4.0 (s, 4H, 4NH, exchangeable with D_2O), 6.74, 7.68 (2d, 8H, Ar-H AB system), 7.7–8.02 (m, 4H, Ar-H), 8.2, 8.32 (2d, 2H, 2CH-thiazole), 8.4 (s, 1H, CH-quinoxaline)	–	597 (M^+, 3 %), 236 (100 %) ($C_{10}H_{11}N_3O_2S$, *N*-(5,6-dihydropyrazin-2-yl)--benzenesulfonamide)

[a] Starting material **1** is not included in the table since it is commercially available.

N-*(Pyridin-2-yl)-4-(3-(4-*(N-*quinoxalin-2-yl-sulfamoyl)phenyl)thioureido)benzenesulfonamide (**10**)*. – A mixture of **2** (0.342 g, 0.01 mol) and sulfapyridine (0.233 g, 0.01 mol) in DMF (20 mL), with 3 drops of TEA, was refluxed for 5 h. The solid was precipitated when hot and filtered to give compound **10**.

N-*(Quinoxalin-2-yl)-4-(3-(4-*(N-*thiazol-2-ylsulfamoyl)phenyl)thioureido)benzenesulfonamide (**11**)*. – A mixture of **2** (0.342 g, 0.001 mol) and sulfathiazole (0.239 g, 0.001 mol) in DMF (20 mL), with 3 drops of TEA, was refluxed for 5 h. The solid was precipitated when hot and filtered to give compound **11**.

In vitro *anticancer screening*

The human tumor cell line (HEPG2) was obtainedfrom the National Cancer Institute, Cairo, Egypt. Irradiation was performed using a Gamma cell-40 (^{60}Co) source

[radioactivity of one gram of ^{60}Co is 44 TBq (about 1100 Curies)]. The antitumor activity of the newly synthesized compounds was measured by the sulfo-rhodamine-B stain (SRB) assay as reported by Skehan *et al.* (13). Cells were plated in 96-multiwell plates (10^4 cells per well) for 24 h before treatment with the compounds to allow attachment of cells to the plate wall. Tested compounds were dissolved and diluted with DMSO. Different concentrations of the compounds under test (5, 12.5, 25 and 40 μmol L^{-1}) 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 an atmosphere of 5 % CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4 % (*m/V*) SRB dissolved in 1 % acetic acid. Unbounded dye was removed through four washes with 1 % acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader (SPR-960B Sunostick Medical Technology, UK). Negative control was added using cell lines with the solvent without the drug. The relation between the surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after a specified time. The concentration required for 50 % inhibition of cell viability (IC_{50}) was calculated and compared with the reference drug doxorubicin and the results are given in Table III.

Radiosensitizing activity

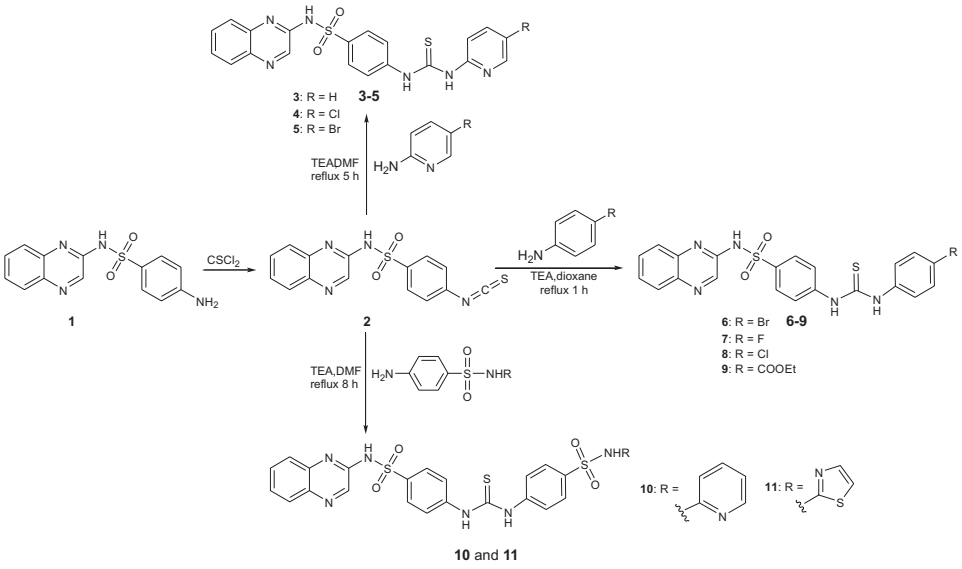
The most potent compounds resulting from the *in vitro* anticancer screening (compounds **9**, **10** and **11**) were selected in order to evaluate their *in vitro* anticancer activity in combination with γ-radiation. This study was conducted to evaluate the ability of these compounds to enhance the cell killing effect of γ-radiation. Negative control was added using cell lines with the solvent without the drug and radiation alone. Cells were subjected to a single dose of γ-irradiation at a dose level of 8 kGy with a dose rate of 2 kGy min^{-1}. The surviving fractions were expressed as the mean ± standard error. The results were analyzed using the 1-way ANOVA test and are given in Table IV.

RESULTS AND DISCUSSION

Chemistry

4-Isothiocyanato-*N*-quinoxaline-2-yl-benzene sulfonamide **2** was prepared by the reaction of *N*-quinoxaline-2-yl-benzenesulfonamide with thiophosgene as reported earlier (14). Compound **2** was used to prepare different sulfonamide derivatives by incorporating different biologically active moieties. Compound **2** reacted with 2-aminopyridine to obtain the corresponding thioureido derivative **3**, while its interaction with 2--aminopyridine, 2-amino-5-chloropyridine and 2-amino-5-bromopyridine yielded compounds **4** and **5**, respectively (Scheme 1).

The isothiocyanato derivative **2** was further reacted with different aniline derivatives to obtain the corresponding thioureido derivatives. Thus, its interaction with 4-bromoaniline, 4-fluoroaniline, 4-chloroaniline and benzocaine yielded compounds **6**, **7**, **8**, and **9**, respectively.



Scheme 1

Another sulfonamide molecule was incorporated into isothiocyanate **2** through its reaction with sulphapyridine and sulphathiazole and compounds **10** and **11** were obtained, respectively (Scheme 1).

In vitro *anticancer activity*

Newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against a human liver cancer cell line (HEPG2). Doxorubicin, one of the most effective anticancer agents, was used as the reference drug. The relationship between the surviving fraction and drug concentration was plotted to obtain the survival curve of the liver cancer cell line. The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50 % inhibition of cell viability. Table III shows the *in vitro* anticancer activity of the synthesized compounds, which exhibited significant activity compared to the reference drug. It was found that in the negative control, the solvent had no effect on the cells as the surviving fraction was 1.00, all the tested compounds showed lower IC_{50} than doxorubicin (IC_{50} = 71.8 μmol L^{-1}), with compound **9** (IC_{50} = 15.6 μmol L^{-1}) being the most potent in this screening. This might be due to incorporation of ethylbenzoate group since the corresponding halogenated compounds **6**, **7** and **8** exhibited lower activity (IC_{50} = 31.2–39.6 μmol L^{-1}). Incorporation of pyridine moiety yielding moderate activity in compound **3** (IC_{50} = 30.0 μmol L^{-1}) and this activity slightly increased to 27.6 and 28 μmol L^{-1}, respectively, in compounds **4** and **5** containing halogenated pyridine moieties. Replacement of pyridine with sulfapyridine moiety in compound **10** yielded an increased activity (IC_{50} = 26.8 μmol L^{-1}) which was also in-

Table III. In vitro *anticancer screening of synthesized compounds against the human liver cell line (HEPG 2)*

Compd.	Control	Compound concentration (μmol L^{-1})				IC_{50} (μmol L^{-1})
		5	12.5	25	40	
		Surviving fraction[a]				
Dox	1.00	0.721 ± 0.020	0.546 ± 0.020	0.461 ± 0.010	0.494 ± 0.030	71.8
2	1.00	0.934 ± 0.002	0.896 ± 0.009	0.848 ± 0.028	0.422 ± 0.047	45.0
3	1.00	0.822 ± 0.058	0.646 ± 0.026	0.530 ± 0.030	0.447 ± 0.022	30.0
4	1.00	0.876 ± 0.032	0.692 ± 0.024	0.456 ± 0.023	0.377 ± 0.015	27.6
5	1.00	0.811 ± 0.085	0.657 ± 0.016	0.503 ± 0.022	0.375 ± 0.043	28.0
6	1.00	0.921 ± 0.008	0.694 ± 0.033	0.621 ± 0.032	0.478 ± 0.081	35.2
7	1.00	0.861 ± 0.017	0.745 ± 0.015	0.557 ± 0.003	0.407 ± 0.027	31.2
8	1.00	0.818 ± 0.044	0.723 ± 0.012	0.622 ± 0.014	0.553 ±0.065	39.6
9	1.00	0.371 ± 0.074	0.298 ± 0.033	0.416 ± 0.004	0.394 ± 0.021	15.6
10	1.00	0.748 ± 0.025	0.540 ± 0.003	0.433 ± 0.076	0.437 ± 0.003	26.8
11	1.00	0.588 ± 0.021	0.507 ± 0.058	0.451 ± 0.004	0.406 ± 0.016	24.4

[a] Mean ± SEM, $n = 3$.
Dox – doxorubicin

creased upon adding the sulfathiazole moiety in compound **11** (IC_{50} = 24.4 μmol L^{-1}). Finally, it was observed that the isothiocyanate derivative **2** was the least potent in this study (IC_{50} = 45 μmol L^{-1}).

Radiosensitizing evaluation

The rationale for combining chemotherapy and radiotherapy is mainly based 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. 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. Consequently, the ability of the most active compounds (**9**, **10** and **11**) to enhance the cell killing effect of γ-irradiation was studied. From the results given in Tables III and IV, it was concluded that in the negative control, radiation alone had a low effect on the cells, compound **9** showed *in vitro* cytotoxic activity with the IC_{50} value of 15.6 μmol L^{-1} when the cells were subjected to different concentrations of the compound alone. However, when the cells were subjected to the same concentrations of compound **9** and irradiated with a single dose of γ-radiation of 8 kGy,

Table IV. In vitro *anticancer screening of compounds **9**, **10** and **11** against the human liver cell line (HEPG2) in combination with γ-irradiation*

Compd.	Control	Compound concentration (μmol L^{-1}) + γ-irradiation (8 kGy) Surviving fraction[b]					IC_{50} (μmol L^{-1})
		0[a]	5	12.5	25	40	
9	1.00	0.927 ± 0.02[c]	0.27 ± 0.08[c]	0.20 ± 0.01[c]	0.31 ± 0.01[c]	0.29 ± 0.01[c]	4.8
10	1.00	0.927 ± 0.02[c]	0.55 ± 0.01[c]	0.34 ± 0.02[c]	0.23 ± 0.01[c]	0.13 ± 0.01[c]	12.3
11	1.00	0.927 ± 0.02[c]	0.39 ± 0.02[c]	0.31 ± 0.01[c]	0.25 ± 0.01[c]	0.21 ± 0.01[c]	14.7

[a] Irradiation (8 Gy) only.
[b] Mean ± SEM, $n = 3$.
[c] Significant difference from control group: $p < 0.001$.

as shown in Table IV, the IC_{50} value was synergistically decreased to 4.8 μmol L^{-1}. Similarly, compounds **10** and **11** showed IC_{50} values of 26.8 and 24.4 μmol L^{-1}, respectively, when used alone, but their IC_{50} values decreased to 12.3 and 14.7 μmol L^{-1}, respectively, when the cells were treated with compounds **10** or **11** in combination with γ-irradiation. These results point to the conclusion that using a combination of compounds **9**, **10** or **11** and ionizing radiation synergistically enhances growth inhibition of liver cancer cells compared to the use of each agent alone.

CONCLUSIONS

The above results allow the conclusion that administration of the tested compounds to the human liver (HEPG2) cell lines showed promising anticancer activity. The most potent compounds being4-(3-(4-ethylbenzoate)thioureido)-*N*-(quinoxalin-2-yl)benzenesulfonamide (**9**) (IC_{50} = 15.6 μmol L^{-1}), *N*-(pyridin-2-yl)-4-(3-(4-(*N*-quinoxalin-2-yl-sulfamoyl)phenyl)thioureido)benzenesulfonamide (**10**) (IC_{50} = 26.8 μmol L^{-1}) and *N*-(quinoxalin-2-yl)-4-(3-(4-(*N*-thiazol-2-ylsulfamoyl)phenyl)thioureido)benzenesulfonamide (**11**) (IC_{50} = 24.4 μmol L^{-1}). Combining these compounds, at the same concentrations, with radiation enhances their activity. This indicates the importance of a combination therapy for cancer patients to reduce the side effects of both drugs and radiation.

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S A Ž E T A K

Sinteza, *in vitro* antitumorsko ispitivanje i radiosenzitirajuće vrednovanje novih derivata 4-[3-(supstituiranih)tioureido]-*N*-(kinoksalin-2-il)benzensulfonamida

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Derivati sulfonamida i kinoksalina imaju raznoliko biološko djelovanje, između ostalog i antitumorsko djelovanje. U radu je opisana sinteza novih derivata tioureido sulfakinoksalina. Svim novim spojevima ispitano je antitumorsko djelovanje *in vitro* na humanoj staničnoj liniji jetre (HEPG 2). Svi ispitani spojevi pokazuju jači učinak nego referentni lijek doksorubicin. Najjači učinak imali su 4-(3-(4-etilbenzoat)tioureido)-*N*-(kinoksalin-2-il)benzensulfonamid (**9**) (IC_{50} = 15,6 μmol L^{-1}), *N*-(piridin-2-il)-4-(3-(4-(*N*-kinoksalin-2-il-sulfamoil)fenil)tioureido)-benzensulfonamid (**10**) (IC_{50} = 26,8 μmol L^{-1}) i *N*-(kinoksalin-2-il)-4-(3-(4-(*N*-tiazol-2-ilsulfamoil)fenil)tioureido)benzensulfonamid (**11**) (IC_{50} = 24,4 μmol L^{-1}), dok je IC_{50} vrijednost bila 71,8 μmol L^{-1}. Najaktivniji spojevi **9**, **10** i **11** evaluirani su kao radiosenzitirajuća sredstva nakon izlaganja spojeva γ-zračenju (8 kGy).

Ključne riječi: kinoksalin, sulfonamid, antitumorsko djelovanje, radiosenzitirajući učinak

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