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Original article

Anticancer and radio-sensitizing evaluation of some new thiazolopyrane and thiazolopyranopyrimidine derivatives bearing a sulfonamide moiety

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ABSTRACT

Recently, it has been reported that compounds bearing a sulfonamide moiety posses many types of biological activities, including anticancer activity. There are a variety of mechanisms for their anticancer activity, and the most prominent mechanism is the inhibition of carbonic anhydrase (CA) isozymes. The present work reports the synthesis of some new thiazolo[4,5-*b*]pyrane, thiazolo[4,5-*b*]pyrano[2,3-*d*] pyrimidine derivatives bearing a sulfonamide moiety. The design of the structures of these compounds complies with the general pharmacophoric requirements for CA inhibiting anticancer drugs. The newly synthesized compounds were evaluated for their *in vitro* anticancer activity against human breast cancer cell line (MCF7). Most of the screened compounds showed interesting cytotoxic activities compared to doxorubicin as a reference drug. Compounds **5**, **6**, **10** and **12** (IC_{50} values 39.4 μM, 41.6 μM, 35.72 μM and 34.64 μM, respectively) exhibited higher cytotoxic activities than the reference drug doxorubicin (IC_{50} = 71.8 μM). Additionally, the previously mentioned compounds were evaluated again for their ability to enhance the cell killing effect of γ–radiation.

1. Introduction

Sulfonamides constitute an important class of drugs, with several types of pharmacological activities such as antibacterial [1], hypoglycemic [2], diuretic [3,4], anti-carbonic anhydrase [3,5], antithyroid agents [6]. Recently, a host of structurally novel sulfonamide derivatives have been reported to show substantial anticancer activities *in vitro* and/or *in vivo* [7–11]. It has been known that aryl/heteroaryl sulfonamides may act as antitumor agents through a variety of mechanisms such as cell cycle perturbation in the G1 phase, disruption of microtubule, angiogenesis inhibition and functional suppression of the transcriptional activator NF-Y. The most prominent mechanism was the inhibition of carbonic anhydrase isozymes [12–16].

Also, it was found that thiazole and fused thiazole derivatives are known to possess several biological activities including anticancer activity [17–23]. In the light of these facts, the present work reports the synthesis of some new thiazolo[4,5-*b*]pyrane and thiazolo[4,5-*b*]pyrano[2,3-*d*]pyrimidine derivatives bearing a free sulfonamide moiety, in order to study their structure activity relationship, hoping that the new synthesized compounds might show significant anticancer activity. This anticancer activity is tested against a human breast cancer cell line (MCF7).

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.

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 radio resistant 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 [24]. We also aimed to evaluate the most potent compounds for their *in vitro* anticancer activity in combination with γ -radiation, to evaluate their ability to enhance the cytotoxic activity of γ-radiation.

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2. Discussion

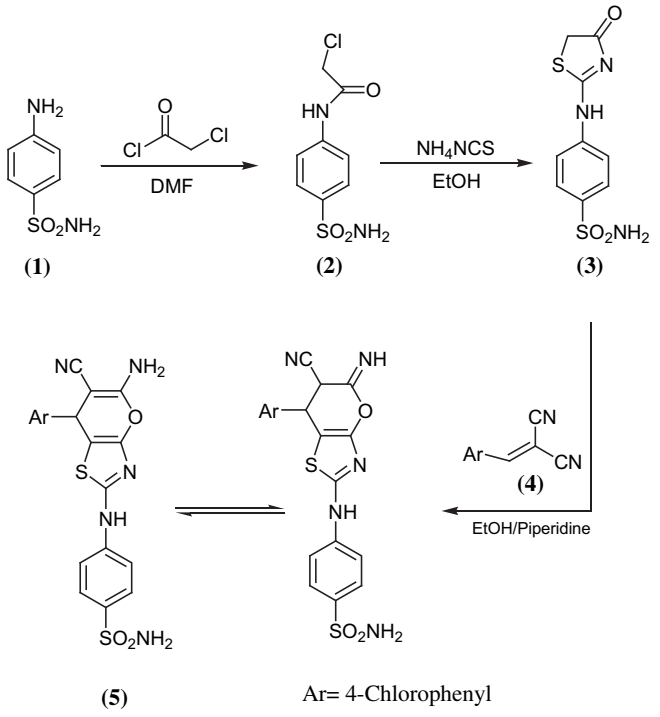
2.1. Chemistry

The newly synthesized compounds were obtained starting with 4-(4-oxo-4,5-dihydrothiazol-2ylamino)benzenesulfonamide **3**, which was prepared according to the reported procedure [25], by refluxing the *N*-chloroacetyl derivative **2** with ammonium thiocyanate in ethanol, through intramolecular cyclization and elimination of 1 mol of ammonium chloride. Treatment of compound **3** with 2-(4-chlorobenzylidene) malononitrile **4** in ethanol containing a catalytic amount of piperidine, as a base catalyst, resulted in intramolecular cyclization affording the 5-amino-6-cyano-thiazolo [4,5-*b*]pyrane derivative **5**. Structure of compound **5** was confirmed from its microanalytical and spectral data. IR spectrum of **5** exhibited bands at 3328, 3227, 3200 cm^{-1} for NH and NH_2, and a band at 2193 $cm^{-1}$for (C≡N). ^{1}H-NMR spectra in DMSO-d_6 revealed a singlet at 4.8 ppm for CH and also a singlet at 7.1 ppm for NH_2 (Scheme 1).

The thiazolopyranopyrimidine derivative **6** was obtained by refluxing compound **5** in formic acid. This reaction proceeded via condensation followed by elimination of 2 mol of water. IR spectrum of compound **6** showed the absence of (C≡N) band and the presence of band at 1709 cm^{-1} corresponding to the (C═O).When compound **5** was refluxed in acetic anhydride for 20 min and 10 h, the monoacetyl derivative **7** and the diacetyl derivative **8** were obtained, respectively. Structures of compounds **7** and **8** were confirmed from their microanalytical and spectral data. ^{1}H-NMR spectrum of **7** in DMSO-d_6 was characterized by a singlet at 1.08 ppm (CH_3).While, the ^{1}H-NMR spectrum of compound **8** revealed two singlets at 2.4 and 2.7 ppm ($2CH_3$). Stirring of compound **5** in conc. H_2SO_4 at room temperature for 6 h caused partial hydrolysis of the cyano group, yielding the carboxamide derivative **9.** IR spectrum of carboxamide derivative **9** showed the absence of the (C≡N) group and the presence of a band at 1680 cm^{-1} corresponding to (C═O) group. Mass spectrum of **9** showed a molecular ion peak *m/z* at 478 [M^+] (2.65), with a base peak at 333 (100). Additionally, the pyrimido derivative **10** was obtained by the reaction of compound **5** with formamide, where intramolecular cyclization occurred through elimination of one molecule of water. IR spectrum of **10** revealed the absence of (C≡N) band, which confirms the cyclization and the formation of the thiazolo[4,5-*b*]pyrano[2,3-*d*]pyrimidine system (Scheme 2).

Treatment of compound **5** with aromatic aldehydes yielded the corresponding Schiff's bases **11** and **12**. The structures of synthesized compounds **11** and **12** were supported from their microanalytical and spectral data. ^{1}H-NMR spectrum in DMSO-d_6 of **11** and **12** revealed singlets at 9.6 and 9.9 ppm for (N═CH–), respectively. Mass spectrum of **11** showed a molecular ion peak *m/z* at 548 [M^+] (0.76), with a base peak at 77 (100). The reactivity of compound **5** toward sulphonyl chlorides in benzene containing a catalyatic amount of pyridine was also studied. Reaction of compound **5** with benzene sulphonylchloride and toluene sulphonylchloride afforded the sulfonamide derivatives **13** and **14**, respectively. Mass spectrum of **13** showed a molecular ion peak *m/z* at 600 [M^+] (5.91), with a base peak at 56 (100). ^{1}H-NMR spectrum in DMSO-d_6 of **14** showed singlet at 2.3 ppm for (CH_3) group (Scheme 3).

Fusion of compound **5** with urea or thiourea, yielded the 6-oxo- and 6-thioxo-thiazolo[4,5-*b*]pyrano[2,3-*d*]pyrimidine derivatives **15** and **16,** respectively. IR spectra of compounds **15** and **16** revealed the absence of (C≡N) band, and the presence of a band at 1650 cm^{-1} for (C═O) for the 6-oxo- derivative **15**, and a band at 1250 cm^{-1} corresponding to (C═S) for 6-thioxo- derivative **16**. Mass spectrum of **16** showed a molecular ion peak *m/z* at 519 [M^+] (10.00), with a base peak at 77 (100). While refluxing compound **5** with urea in the presence of sodium ethoxide as a catalyst yielded the ureido derivative **17**. IR spectrum of compound **17** showed the presence of bands at 2201 cm^{-1} for (C≡N) and at 1728 cm^{-1} for (C═O). Also, stirring of compound **5** with chloroacetyl chloride in dimethylformamide at room temperature for 6 h, yielded the 2-chloroacetyl derivative **18**. IR spectrum of compound **18** showed the presence of (C≡N) band at 2204 cm^{-1} and a band at 1653 cm^{-1} for (C═O). ^{1}H-NMR spectrum in DMSO-d_6 revealed singlet at 4.2 ppm corresponding to (CH_2Cl) group and a singlet at 8.2 ppm for the (NH) group (Scheme 4).



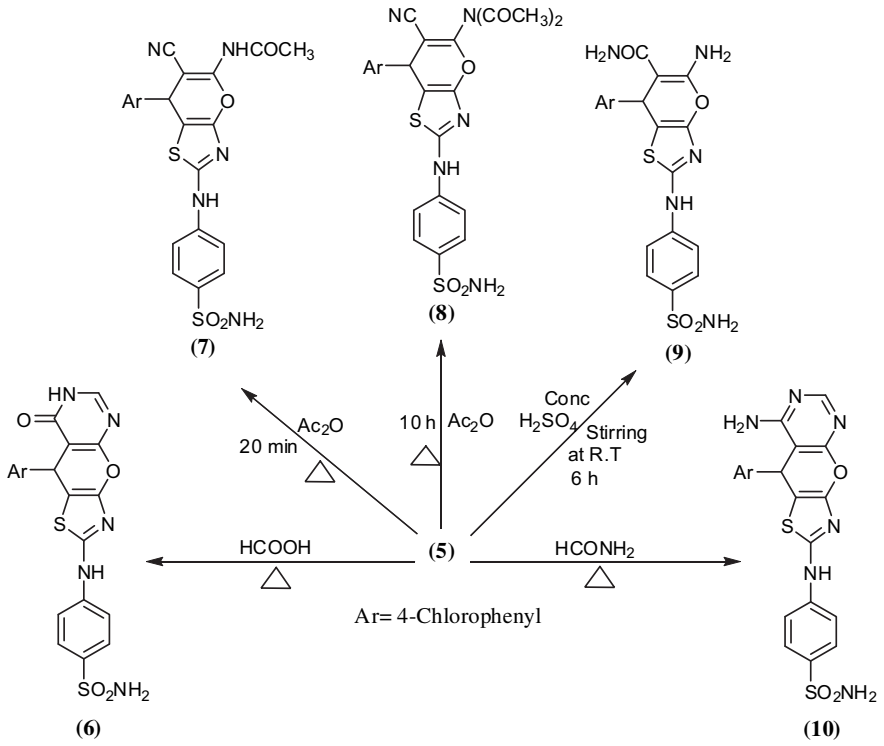
Scheme 1.

2.2. In vitro anticancer screening

The newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line, MCF7.

Doxorubicin, 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 breast cancer cell line (MCF7). 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* cytotoxic activity of the synthesized compounds. Most of the synthesized compounds exhibited significant activity compared to the reference drug.

From the results in Table 1, it was found that compound **12** with IC_{50} value 34.64 μM was the most potent compound in this screening, and exhibited a higher cytotoxic activity when compared with the reference drug doxorubicin (IC_{50} = 71.8 μM), followed by compounds **5**, **6**, **7**, **9**, **10**, **11**, **13**, **16**, **17** with IC_{50} values ranging from 35.79 μM to 52.77 μM which showed also better activity than doxorubicin. Compounds **8, 14** of IC_{50} value (74.3 μM, 71.74 μM, respectively) are nearly as active as doxorubicin. While compound **15** of IC_{50} value 84.13 μM showed lower activity than that of the reference drug.



Scheme 2.

2.3. Radio-sensitizing evaluation

The ability of the most four active compounds, **5, 6, 10** and **12**, to enhance the cell killing effect of γ-irradiation was studied. From the obtained results in Table 1, compound **12** showed cytotoxic activity with IC_{50} value of 34.64 μM, when the cells were subjected to different concentrations of the compound alone. While when the cells were subjected to the same concentrations of compound **12**, 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 decreased to 17.03 μM (Fig. 1).

Similarly, compounds **5**, **6** and **10** showed IC_{50} values of 39.4, 41.6 and 35.72 μM, respectively, when used alone (Table 1). The IC_{50} values were decreased to 26.25, 28.41 and 23.68 μM when the cells were treated with compound **5, 6** and **10** in combination with a single dose of γ-radiation at a dose level of 8 Gy (Fig. 2, 3 and 4).

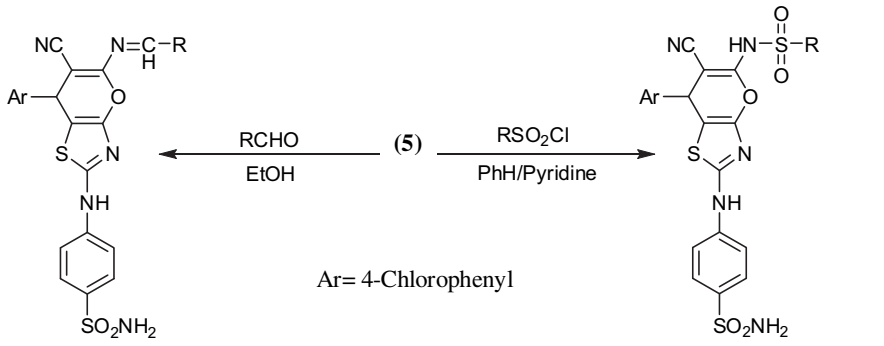
3. Conclusion

We report here the synthesis of some new thiazolo[4,5-*b*]pyrane and thiazolo[4,5-*b*]pyrano[2,3-*d*]pyrimidine derivatives bearing a sulfonamide moiety. Most of these new compounds exhibited significant anticancer activity, when compared to doxorubicin as a reference drug. Moreover, the most four active compounds showed the ability to sensitize cancer cells to the lethal effects of ionizing radiation.

4. Experimental

4.1. Chemistry

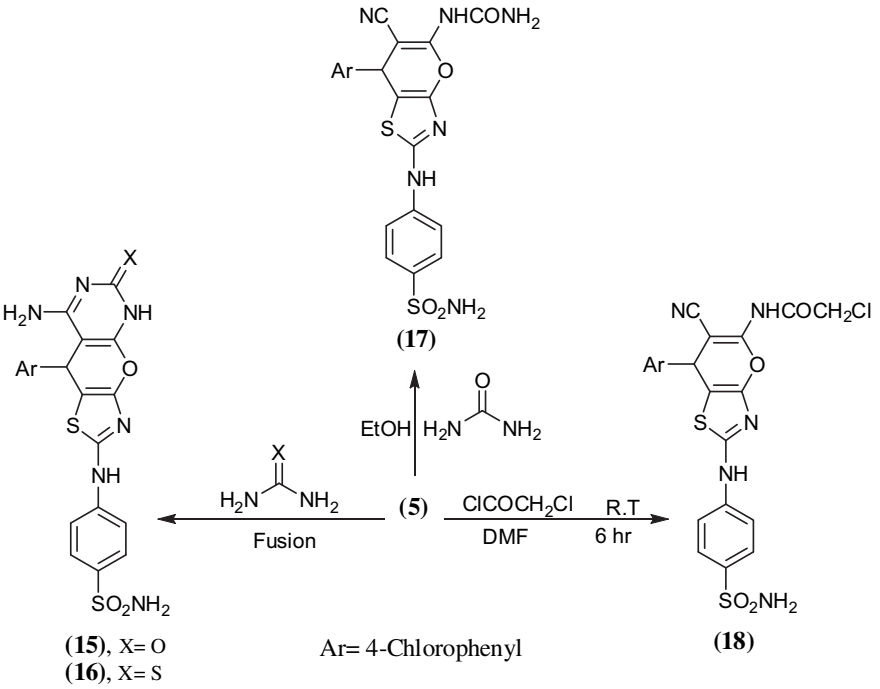
Melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). Elemental analysis (C, H, N) were performed on Perkin–Elmer 2400 analyzer



(11), R= Phenyl
(12), R= 4-Chlorophenyl

(13), R= Phenyl
(14), R= 4-Methylphenyl

Scheme 3.



Scheme 4.

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

4.1.1. 4-(4-Oxo-4,5-dihydrothiazol-2-ylamino)benzenesulfonamide (3)

Prepared according to the reported procedure [25].

4.1.2. 4-(5-Amino-7-(4-chlorophenyl)-6-cyano-7H- thiazolo[4,5-b] pyrane-2-yl amino)benzenesulfonamide (5)

A mixture of 4-(4-oxo-4, 5-dihydrothiazol-2-ylamino) benzenesulfonamide **3** (0.27 g, 0.001 mol) and 2-(4-chlorobenzylidene) malononitrile **4** (0.18 g, 0.001 mol) in ethanol (20 mL) containing 5 drops of piperidine was refluxed for 5 h. The reaction mixture was cooled then poured onto cold water, the obtained solid was crystallized from dioxane to give compound **5**: yield, 77%; m.p. 200–202 °C; IR, (KBr, cm^{-1}): 3328, 3227, 3200 (NH, NH_2), 3058 (CH arom.), 2939, 2870 (CH aliph.), 2193 (C≡N), 1626 (C=N), 1330, 1156 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 4.6 [s, 1H, NH, exchangeable with D_2O], 4.8 [s, 1H, CH pyrane], 7.1 [s, 2H, NH_2, exchangeable with D_2O], 7.3–8.0 [m, 10H, ArH + SO_2NH_2, exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6) δ: 25.6, 56.8, 115.5, 117.5, 128.7, 129.1, 130.4, 131.3, 132.8, 143.7, 157.2, 170.2. MS *m/z* (%): 460 [M$^+$] (11.95), 56 (100).Anal. Calcd. For $C_{19}H_{14}ClN_5O_3S_2$

Table 1
In vitro anticancer screening of the synthesized compounds against human breast cell line (MCF7).

Compound	Compound Concentration (μM)				IC_{50} (μM)
	10	**25**	**50**	**100**	
	Surviving Fraction (Means ± SE)[a]				
Doxorubicin	0.721 ± 0.02	0.546 ± 0.02	0.461 ± 0.01	0.494 ± 0.03	71.8
5	0.848 ± 0.03	0.360 ± 0.01	0.168 ± 0.01	0.214 ± 0.01	39.4
6	0.794 ± 0.01	0.515 ± 0.03	0.204 ± 0.01	0.175 ± 0.01	41.6
7	0.801 ± 0.01	0.447 ± 0.01	0.183 ± 0.01	0.252 ± 0.01	41.91
8	0.913 ± 0.01	0.511 ± 0.02	0.234 ± 0.01	0.617 ± 0.03	74.3
9	0.815 ± 0.02	0.499 ± 0.02	0.169 ± 0.01	0.210 ± 0.01	41.8
10	0.62 ± 0.03	0.454 ± 0.02	0.186 ± 0.01	0.207 ± 0.01	35.79
11	0.93 ± 0.04	0.206 ± 0.01	0.295 ± 0.01	0.324 ± 0.01	45.22
12	0.634 ± 0.02	0.240 ± 0.01	0.257 ± 0.01	0.304 ± 0.01	34.64
13	0.697 ± 0.02	0.492 ± 0.02	0.239 ± 0.01	0.361 ± 0.01	47.46
14	0.92 ± 0.05	0.52 ± 0.02	0.2 ± 0.01	0.6 ± 0.03	71.74
15	0.9 ± 0.02	0.56 ± 0.01	0.265 ± 0.01	0.654 ± 0.02	84.13
16	0.814 ± 0.04	0.639 ± 0.04	0.241 ± 0.01	0.325 ± 0.02	52.77
17	0.701 ± 0.04	0.717 ± 0.02	0.309 ± 0.01	0.228 ± 0.01	49.80
18	0.702 ± 0.03	0.531 ± 0.01	0.25 ± 0.01	0.252 ± 0.01	43.75

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

Table 2
In vitro anticancer screening of compounds **5, 6, 10** and **12** against human breast cell line (MCF7) in combination with γ-radiation.

Compd. no.	Control	Irradiated (8 Gy)	Compound Concentration (μM) + Irradiation (8 Gy)				IC_{50} (μM)
			10	**25**	**50**	**100**	
	Surviving Fraction (Means ± SE)[a]						
5	1.000	0.927 ± 0.02[b]	0.706 ± 0.02[b]	0.203 ± 0.01[b]	0.062 ± 0.01[b]	0.097 ± 0.01[b]	26.25
6	1.000	0.927 ± 0.02[b]	0.650 ± 0.02[b]	0.371 ± 0.01[b]	0.060 ± 0.01[b]	0.040 ± 0.01[b]	28.41
10	1.000	0.927 ± 0.02[b]	0.504 ± 0.02[b]	0.341 ± 0.02[b]	0.064 ± 0.01[b]	0.086 ± 0.01[b]	23.68
12	1.000	0.927 ± 0.02[b]	0.462 ± 0.02[b]	0.124 ± 0.01[b]	0.143 ± 0.01[b]	0.182 ± 0.01[b]	17.03

[a] Each value is the mean of three values ± Standard Error.
[b] Significant difference from control group at $p < 0.001$.

(459.93): C, 49.62; H, 3.07; N, 15.23. Found: C, 49.70; H, 3.10; N, 15.34.

4.1.3. 4-(9-(4-Chlorophenyl)-8-oxo-7, 9-dihydrothiazolo[4,5-b] pyrano[2,3-d]pyrimidin-2-ylamino) benzenesulfonamide (6)

A solution of compound **5** (0.91 g, 0.002 mol) in formic acid (30 mL) was refluxed for 5 h, the reaction mixture was cooled then poured onto cold water, the obtained solid was recrystallized from dioxane to give compound **6**: Yield, 72%; m.p. 172–174 °C; IR, (KBr, cm^{-1}): 3310, 3241, 3186 (NH, NH_2), 3053 (CH arom.), 2942, 2850 (CH aliph.), 1709 (C=O), 1599 (C=N), 1370, 1155 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 4.6 [s, 1H, NH, exchangeable with D_2O], 4.8 [s, 1H, CH pyrano], 7.1 [s, 1H, CH pyrimidine], 7.3–8.0 [m, 10H, Ar–H + SO_2NH_2, exchangeable with D_2O], 8.1 [s, 1H, NH pyrimidine, exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6) δ: 28.0, 103.3, 113.1, 125.9, 128.1, 129.3, 130.5, 131.0, 131.9, 134.9, 143.7, 155.89, 162.2. MS *m/z* (%): 488 [M^+] (57.77), 449 (100). Anal. Calcd. For $C_{20}H_{14}ClN_5O_4S_2$ (487.94): C, 49.23; H, 2.89; N, 14.35. Found: C, 49.34; H, 3.10; N, 14.41.

4.1.4. N-(7-(4-chlorophenyl)-6-cyano-2-(4-sulfamoylphenyl-amino)-7H- thiazolo[4,5-b]pyrane-5-yl)acetamide (7)

A solution of compound **5** (0.91 g, 0.002 mol) in acetic anhydride (20 mL) was refluxed for 20 min, the reaction mixture was then concentrated, the solid separated was recrystallized from ethanol to give compound **7**: Yield, 75%; m.p. 162–164 °C; IR, (KBr, cm^{-1}): 3341, 3224 (NH, NH_2), 3090 (CH arom.), 2970, 2860 (CH aliph.), 2205 (C≡N), 1719 (C=O), 1590 (C=N), 1364, 1163 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 1.08 [s, 3H, $COCH_3$], 4.6 [s, 1H, NH, exchangeable with D_2O], 4.8 [s, 1H, CH pyrane], 7.3–8.0 [m, 10H, Ar–H + SO_2NH_2, exchangeable with D_2O], 8.2 [s, 1H, NHCO, exchangeable with D_2O]. Anal.Calcd.For $C_{21}H_{16}ClN_5O_4S_2$ (501.97): C, 50.25; H, 3.21; N, 13.95. Found: C, 50.36; H, 3.33; N, 14.05.

4.1.5. N-acetyl-N-(7-(4-chlorophenyl)-6-cyano-2-(4-sulfamoylp-henylamino)-7H- thiazolo[4,5-b]pyrane-5-yl)acetamide (8)

A solution of compound **5** (0.91 g, 0.002 mol) in acetic anhydride (20 mL) was refluxed for 10 h, the reaction mixture was then concentrated, the solid separated was crystallized from ethanol to give compound **8**: Yield, 70%; m.p. 234–236 °C; IR, (KBr, cm^{-1}): 3253, 3209 (NH, NH_2), 3097 (CH arom.), 2972, 2855 (CH aliph.), 2205 (C≡N), 1721,1669 (2 C=O), 1590 (C=N), 1364, 1162 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 2.4, 2.7 [2s, 6H, $2COCH_3$] 4.6 [s, 1H, NH, exchangeable with D_2O], 4.8 [s, 1H, CH pyrane], 7.3–8.0 [m, 10H, Ar–H + SO_2NH_2, exchangeable with D_2O]. MS *m/z* (%): 543 [M-1] (3.19), 55 (100). Anal. Calcd. For $C_{23}H_{18}ClN_5O_5S_2$ (544.00): C, 50.78; H, 3.34; N, 12.87. Found: C, 50.65; H, 3.20; N, 12.70.

4.1.6. 5-Amino-7-(4-chlorophenyl)-2-(4-sulfamoylphenylamino)-7H- thiazolo[4,5-b]pyrane -6-carboxamide (9)

A solution of compound **5** (0.9 g, 0.002 mol) in conc. H_2SO_4 (10 mL) was stirred for 6 h at room temperature, then the reaction mixture was poured onto cold water. The obtained solid was recrystallized from ethanol to give compound **9**: Yield, 77%; m.p. 290–292 °C; IR, (KBr, cm^{-1}): 3342, 3232 (NH, NH_2), 3090 (CH arom.), 2981, 2866 (CH aliph.), 1680 (C=O), 1590 (C=N), 1334, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 3.9 [s, 1H, NH, exchangeable with D_2O], 4.5 [s, 1H, CH pyrane], 6.7 [s, 2H, NH_2, exchangeable with D_2O], 7.3–8.0 [m, 10H, Ar-H + SO_2NH_2, exchangeable with D_2O], 8.2 [s, 2H, $CONH_2$, exchangeable with D_2O] .MS *m/z* (%): 478 [M^+] (2.65), 333 (100). Anal. Calcd. For $C_{19}H_{16}ClN_5O_4S_2$ (477.94): C, 47.75; H, 3.37; N, 14.65. Found: C, 47.86; H, 3.43; N, 14.75.

4.1.7. 4-(8-Amino-9(4-chlorophenyl)-9H- thiazolo[4,5-b]pyrano [2,3-d]pyrimidine-2-ylamino) benzenesulfonamide (10)

A solution of compound **5** (0.91 g, 0.002 mol) in formamide (30 mL) was refluxed for 5 h, the reaction mixture was cooled and

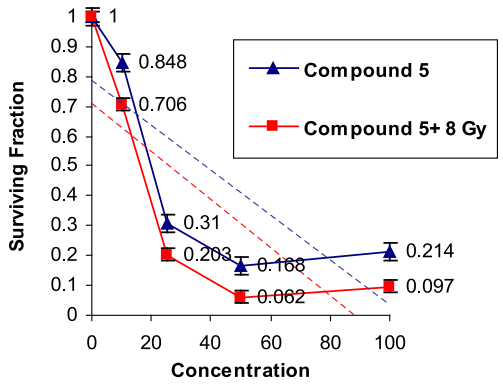


Fig. 1. Survival curve for MCF7 cell line for compound **5** alone or in combination with γ- radiation (8 Gy).

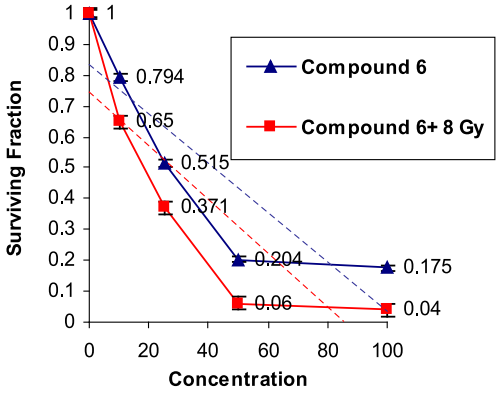


Fig. 2. Survival curve for MCF7 cell line for compound **6** alone or in combination with γ- radiation (8 Gy).

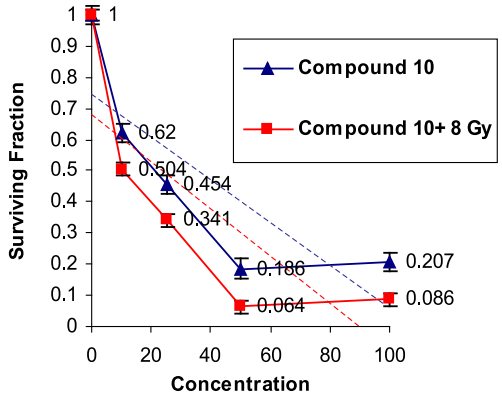


Fig. 3. Survival curve for MCF7 cell line for compound **10** alone or in combination with γ- radiation (8 Gy).

then poured onto cold water, the obtained solid was crystallized from dioxane to give compound **10**: Yield, 75%; m.p. 250–252 °C; IR, (KBr, cm^{-1}): 3330, 3270, 3209 (NH, NH_2), 3080 (CH arom.), 2989, 2870 (CH aliph.), 1619, 1595 (2 C=N), 1398, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 3.9 [s, 1H, NH, exchangeable with D_2O], 4.8 [s, 1H, CH pyrano], 7.3–8.0 [m, 10H, Ar-H + SO_2NH_2, exchangeable with D_2O], 8.2 [s, 1H, CH pyrimidine], 10.2 [s, 2H, NH_2, exchangeable with D_2O]. MS *m/z* (%): 487 [M^+] (3.50), 125 (100). Anal. Calcd. For $C_{20}H_{15}ClN_6O_3S_2$ (486.95): C, 49.33; H, 3.10; N, 17.26. Found: C, 49.40; H, 3.24; N, 17.35.

4.1.8. 4-(5-(Arylideneamino)-7-(4-chlorophenyl)-6-cyano-7H-thiazolo[4,5-b]pyrane -2-ylamino) benzenesulfonamide (11 and 12)

A mixture of compound **5** (0.91 g, 0.002 mol) and benzaldehyde or *p*-chlorobenzaldehyde (0.002 mol) in 20 mL ethanol was refluxed for 5 h. The reaction mixture was then poured onto cold water, the obtained solid was recrystallized from dioxane to give compounds **11**and **12** respectively.

4.1.8.1. 4-(5-(Benzylideneamino)-7-(4-chlorophenyl)-6-cyano-7H-thiazolo[4,5-b]pyrane -2-ylamino) benzenesulfonamide (11). Yield, 80%; m.p. 260–262 °C; IR, (KBr, cm^{-1}): 3335, 3295, 3231 (NH, NH_2), 3080 (CH arom.), 2947, 2850 (CH aliph.), 2194 (C≡N), 1594 (C=N), 1327, 1153 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 3.8 [s, 1H, NH, exchangeable with D_2O], 4.5 [s, 1H, CH pyrane], 7.3–8.0 [m, 15H, Ar–H + SO_2NH_2, exchangeable with D_2O], 9.6 [s, 1H, N=CH]. MS *m/z* (%): 548 [M^+] (0.76), 77 (100). Anal. Calcd. For $C_{26}H_{18}ClN_5O_3S_2$ (548.04): C, 56.98; H, 3.31; N, 12.78. Found: C, 56.81; H, 3.20; N, 12.58.

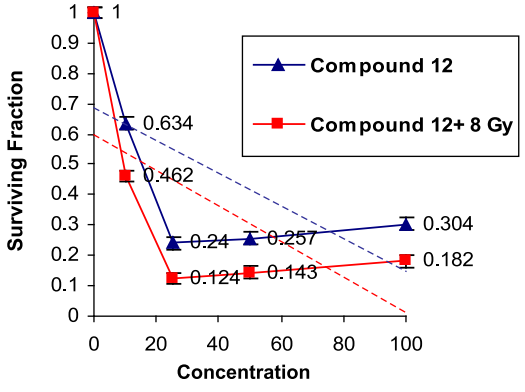


Fig. 4. Survival curve for MCF7 cell line for compound **12** alone or in combination with γ- radiation (8 Gy).

4.1.8.2. 4-(5-(4-Chlorobenzylideneamino)-7-(4-chlorophenyl)-6-cyano-7H- thiazolo[4,5-b]pyrane -2-ylamino)benzenesulfonamide (12). Yield, 78%; m.p. 223–225 °C; IR, (KBr, cm^{-1}): 3319, 3246, 3191 (NH, NH_2), 3070 (CH arom.), 2940, 2830 (CH aliph.), 2202 (C≡N), 1581 (C=N), 1331, 1153 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 3.8 [s, 1H, NH, exchangeable with D_2O], 4.5 [s, 1H, CH pyrane], 7.3–8.0 [m, 14H, Ar–H + SO_2NH_2, exchangeable with D_2O], 9.9 [s, 1H, N=CH]. ^{13}C-NMR (DMSO-d_6) δ: 31.5, 113.8, 117.3, 127.2, 129.1, 131.1, 132.5, 133.1, 134.4, 143.7, 157.7, 163.7.MS *m/z* (%): 582 [M^+] (1.82), 101 (100). Anal. Calcd. For $C_{26}H_{17}Cl_2N_5O_3S_2$ (582.48): C, 53.61; H, 2.94; N, 12.02. Found: C, 53.74; H, 3.15; N, 12.16.

4.1.9. 4-(5-(Benzylideneamino)-7-(4-chlorophenyl)-6-cyano-7H-thiazolo[4,5-b]pyrane -2-ylamino)benzenesulfonamide(13) and 4-(7-(4-chlorophenyl)-6-cyano-5-(4-methylbenzylideneamino)-7H-thiazolo[4,5-b]pyrane -2-ylamino)benzenesulfonamide (14)

A mixture of compound **5** (0.91 g, 0.002 mol) and the appropriate sulphonyl chloride derivative (0.002 mol) in benzene containing 3 drops of pyridine was refluxed for 8 h. The obtained solid was recrystallized from ethanol to give compounds **13** and **14** respectively.

4.1.9.1. 4-(5-(Benzylideneamino)-7-(4-chlorophenyl)-6-cyano-7H-thiazolo[4,5-b]pyrane -2-ylamino)benzenesulfonamide (13). Yield, 80%; m.p. 278–280 °C; IR, (KBr, cm^{-1}): 3325, 3248, 3220 (NH, NH_2), 3046 (CH arom.), 2930, 2870 (CH aliph.), 2194 (C≡N), 1634 (C=N), 1325, 1156 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 3.8 [s, 1H, NH, exchangeable with D_2O], 4.5 [s, 1H, CH pyrane], 7.3–8.0 [m, 15H, Ar–H + SO_2NH_2, exchangeable with D_2O], 12.0 [s, 1H, $NHSO_2$, exchangeable with D_2O]. MS *m/z* (%): 600 [M^+] (5.91), 56 (100). Anal. Calcd. For $C_{25}H_{18}ClN_5O_5S_3$ (600.09): C, 50.04; H, 3.02; N, 11.67. Found: C, 50.18; H, 3.17; N, 11.76.

4.1.9.2. 4-(7-(4-Chlorophenyl)-6-cyano-5-(4-methylbenzylidene-amino)-7H- thiazolo[4,5-b]pyrane -2-ylamino)benzenesulfonamide (14). Yield, 75%; m.p. 280–282 °C; IR, (KBr, cm^{-1}): 3325, 3250, 3215 (NH, NH_2), 3090 (CH arom.), 2950, 2860 (CH aliph), 2203 (C≡N), 1629 (C=N), 1327, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 2.3 [s, 3H, CH_3], 3.6 [s, 1H, NH, exchangeable with D_2O], 4.7 [s, 1H, CH pyrane], 7.3–8.0 [m, 14H, Ar–H + SO_2NH_2, exchangeable with D_2O], 11.8 [s, 1H, $NHSO_2$, exchangeable with D_2O]. MS *m/z* (%): 613 [M-1] (11.34), 63 (100).Anal. Calcd. For $C_{26}H_{20}ClN_5O_5S_3$ (614.12): C, 50.85; H, 3.28; N, 11.40. Found: C, 50.63; H, 3.17; N, 11.30.

4.1.10. 4-(8-Amino-9(4-chlorophenyl)-6-oxo-5,9-dihydrothiazolo [4,5-b]pyrano[2,3-d]pyrimidine-2-yl amino) benzenesulfonamide (15) and 4-(8-amino-9(4-chlorophenyl)-6-thioxo-5,9-dihydrothiazolo[4,5-b]pyrano[2,3-d]pyrimidine-2-yl amino) benzenesulfonamide (16)

A mixture of compound **5** (0.91 g, 0.002 mol) and urea or thiourea (0.002 mol) was fused together in an oil bath at 250 °C for 15 min, the fused mass was dissolved in dimethylformamide and poured onto cold water, the solid obtained was recrystallized from ethanol to give compounds **15** and **16** respectively:

4.1.10.1. 4-(8-Amino-9(4-chlorophenyl)-6-oxo-5,9-dihydrothiazolo [4,5-b]pyrano[2,3-d]pyrimidine-2-yl amino) benzenesulfonamide (15). Yield, 70%; m.p. 310–312 °C; IR, (KBr, cm^{-1}): 3327, 3260, 3184 (NH, NH_2), 3080 (CH arom.), 2930, 2860 (CH aliph), 1690 (C=O), 1620 (C=N), 1327, 1153 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 3.6 [s, 1H, NH, exchangeable with D_2O], 4.8 [s, 1H, CH pyrane], 6.9 [s, 1H, NH pyrimidine, exchangeable with D_2O], 7.3–8.0[m, 10H, Ar–H + SO_2NH_2], 10.6 [s, 2H, NH_2, exchangeable with D_2O]. MS *m/z* (%): 503 [M^+] (4.92), 51 (100). Anal. Calcd. For $C_{20}H_{15}ClN_6O_4S_2$ (502.95): C, 47.76; H, 3.01; N, 16.71. Found: C, 47.61; H, 2.88; N, 16.65.

4.1.10.2. 4-(8-Amino-9(4-chlorophenyl)-6-thioxo-5,9-dihydrothiazolo[4,5-b]pyrano[2,3-d]pyrimidine-2-yl amino) benzenesulfonamide (16). Yield, 76%; m.p. 300–302 °C; IR, (KBr, cm^{-1}): 3315, 3270, 3174 (NH, NH_2), 3080 (CH arom.), 2950, 2870 (CH aliph.), 1596 (C=N), 1250 (C=S), 1330, 1152 (SO_2). MS *m/z* (%): 519 [M^+] (10.00), 77 (100). Anal. Calcd. For $C_{20}H_{15}ClN_6O_3S_3$ (519.02): C, 46.28; H, 2.91; N, 16.19. Found: C, 46.38; H, 3.18; N, 15.96.

4.1.11. 4-(7-(4-Chlorophenyl)-6-cyano-5-ureido-7H- thiazolo[4,5-b]pyrane -2-yl amino)benzenesulfonamide (17)

A mixture of compound **5** (0.91 g, 0.002 mol) and urea (0.12 g, 0.002 mol) and sodium ethoxide (0.03 g, 0.002 mol) in ethanol (20 mL) was refluxed for 5 h. The reaction mixture was then poured onto cold water, the obtained solid was crystallized from dioxane to give compound **17:** Yield, 80%; m.p. 240–242 °C; IR, (KBr, cm^{-1}): 3338, 3294, 3250 (NH, NH_2), 3095 (CH arom.), 2970, 2860 (CH aliph.), 2201 (C≡N), 1728 (C=O), 1560 (C=N), 1331, 1156 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 3.8 [s, 1H, NH, exchangeable with D_2O], 4.5 [s, 1H, CH pyrane], 6.5 [s, 1H, NHCO, exchangeable with D_2O], 7.3–8.0 [m, 10H, Ar-H + SO_2NH_2], 8.3 [s, 2H, $CONH_2$, exchangeable with D_2O]. MS *m/z* (%): 502 [M^+] (7.63), 55 (100). Anal. Calcd. For $C_{20}H_{15}ClN_6O_4S_2$ (502.95): C, 47.76; H, 3.01; N, 16.71. Found: C, 47.88; H, 3.13; N, 16.85.

4.1.12. 2-Chloro-N-(7-(4-chlorophenyl)-6-cyano-2-(4-sulfamoylphenylamino)-7H- thiazolo[4,5-b]pyrane -5-yl)acetamide (18)

A mixture of compound **5** (0.91 g, 0.002 mol) and chloroacetylchloride (0.22 g, 0.002 mol) was stirred in dimethylformamide for 6 h at room temperature, then the reaction mixture was poured onto cold water. The obtained solid was crystallized from ethanol to give compound **18**: Yield, 68%; m.p. 164–166 °C; IR, (KBr, cm^{-1}): 3339, 3244, 3194 (NH, NH_2), 3089 (CH arom.), 2970, 2860 (CH aliph.), 2204 (C≡N), 1690 (C=O), 1615 (C=N), 1331, 1155 (SO_2), 750 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ: 3.8 [s, 1H, NH, exchangeable with D_2O], 4.2 [s, 2H, CH_2Cl], 4.7 [s, 1H, CH pyrane], 7.3–8.0 [m, 10H, Ar-H + SO_2NH_2], 8.2 [s, 1H, NHCO, exchangeable with D_2O]. MS *m/z* (%): 537 [M^+] (0.53), 90 (100). Anal. Calcd. For $C_{21}H_{15}Cl_2N_5o_4S_2$ (536.41): C, 47.02; H, 2.82; N, 13.06. Found: C, 47.26; H, 3.04; N, 13.18.

4.2. In vitro anticancer screening

Human tumor breast cell line (MCF7) was used in this study. The cytotoxic activity was measured *in vitro* for the newly synthesized compounds using the Sulfo-Rhodamine B stain (SRB) assay using the method of Skehan et al. [26]. The *in vitro* anticancer screening was done by the pharmacology unit at the National Cancer Institute, Cairo University.

Cells were plated in 96-multiwell plate (10^4cells/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 dimethylsulfoxide. Different concentrations of the compound under test (10, 25, 50, and 100 μM) 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% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (wt/vol) SRB 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 ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve for breast tumor cell line after the specified time. The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared to the reference drug doxorubicin (CAS, 25316-40-9). The surviving fractions were expressed as means ± standard error and the results are given in Table 1.

4.3. Radio-sensitizing evaluation

The most potent compounds resulted from the *in vitro* anticancer screening, the thiazolo[4,5-*b*]pyrane derivatives **5** and **12** and the thiazolo[4,5-*b*]pyrano[2,3-*d*]pyrimidine derivatives **6** and **10**, were selected to be reevaluated again for 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.

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.

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