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Novel 4-(4-substituted-thiazol-2-ylamino)-N-(pyridin-2-yl)-benzene-sulfonamides as Cytotoxic and Radiosensitizing Agents

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A series of novel 4-(4-substituted-thiazol-2-ylamino)-N-(pyridin-2-yl) benzene-sulfonamides were synthesized and screened for their cytotoxic activity against human breast cancer cell line (MCF-7). Compounds **6**, **7**, **9**, **10**, **11**, and **14** displayed significant activity against MCF-7 when compared to doxorubicin, which was used as a reference drug. The synergistic effect of Gamma radiation for the most active derivatives **7**, **9**, and **11** was also studied and their IC_{50} values markedly decreased to 11.9 μM, 11.7 μM, and 11.6 μM, respectively.

Key words: Sulfonamides, Thiazoles, Cytotoxic, Radiosensetizing activities

INTRODUCTION

Sulfonamide derivatives have been shown to display extensive *in vitro* and/or *in vivo* antitumor activity (Supuran and Scozzafava, 2000; Casini et al., 2002a; Abbate et al., 2004; Ghorab et al., 2006, 2009, 2010a, 2010b, 2010c, 2011; Ismail et al., 2006; Al-Said et al., 2010). Sulfonamide antitumor activity arises through a wide range of different mechanisms, such as cell cycle arrest in the G1 phase (Fukuoka et al., 2001) and inhibition of carbonic anhydrase (Supuran et al., 2001), histone deacetylases (HDACs) (Payne et al., 2008), methionine aminopeptidases (MetAPs) (Kawai et al., 2006), matrix metalloproteinase (MMPs) (Casini et al., 2002b), nicotinamide adenine dinucleotide (NADH) oxidase (Villar et al., 2004), cyclin-dependent kinase (CDK) (Huang et al., 2006), binding to β-Tubulin, and disruption of microtubule assembly (Kenneth et al., 2006).

Indisulam (E7070) is an example of an anticancer agent that contains sulfonamide moiety (Kesteren et al., 2002). A series of sulfonamide derivatives I bearing a thiazole moiety showed a significant tumor growth delay in mouse tumor xenograft models and some examples were reported to have > 100-fold selectivity for CDK4, as well as other examples that were equipotent against CDK1, CDK2, and CDK4 (Fischer and Lane, 2000). Recently, N^4 sulfapyridine derivatives II bearing a 2-substituted thiazolidine-4-one moiety were synthesized and showed more cytotoxic activity against human breast cancer cell line (MCF-7) and HeLa cell lines than 5-flurouracil and doxorubicin (Kamel et al., 2010) (Fig. 1).

Thus, with the goal of identifying new cytotoxic compounds, we designed and synthesized novel 4-(4-substituted-thiazol-2-ylamino)-N-(pyridin-2-yl) benzene-sulfonamide derivatives for use as potential cytotoxic agents and explored the effect of different substitutions at position 4 of the thiazole ring on their cytotoxic activity. The synergistic effect of γ-radiation with the most potent synthesized derivatives was also evaluated.

MATERIALS AND METHODS

Chemistry

Melting points were uncorrected and were determined using a Stuart melting point apparatus (Stuart

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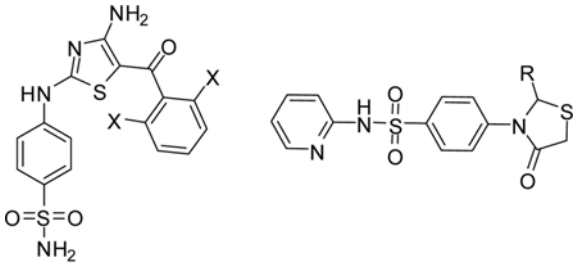


I II

Fig. 1. Sulfonamide derivatives bearing the thiazole moiety as cytotoxic agents

Scientific). Elemental analyses (C, H, N) were performed on Perkin-Elmer 2400 analyser (Perkin-Elmer) at the Microanalytical Laboratories of the Faculty of Science, Cairo University. All compounds were within ± 0.4% of the theoretical values. Infrared spectra (KBr) were determined using a Shimadzu IR-110 spectrophotometer (Shimadzu). ^{1}H-NMR spectra were carried out using BRUCKER proton NMR-Avance 300 (300 MHz, Bruker) in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a JEOL JMS AX-500 spectrometer (JEOL JMS), in electronic impact (EI). All reactions were monitored by thin layer chromatograph (TLC) using precoated Aluminium sheets Silica gel Merck 60 F254. Ethyl acetate-cyclohexane (2.5:7.5 mL) mixture was used as the eluting solvent and TLC sheets were visualized using a UV lamp (Merck).

4-(4-Hydroxythiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (3)

A solution of compound **2** (3.25 g, 0.01 mol) and Ammonium thiocyanate (0.76 g, 0.01 mol) in ethanol (20 mL) was refluxed for 1 h. The reaction mixture was filtered while hot and the obtained solid was crystallized from dioxane to give **3**: Yield 85%, m.p. 240-242ºC, IR (KBr, cm^{-1}): 3448 (OH), 3366, 3191 (NH), 3047 (CH arom.), 1602 (C=N), 1397, 1137 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.84 (s, 1H, CH, thiazole), 6.86-8.0 (m, 9H, Ar-H + NH, D_2O exchangeable), 10.9 (s, 1H, SO_2NH, D_2O exchangeable), 11.7 (s, 1H, OH, D_2O exchangeable). MS (*m/z*): 348 (M^+, 1.7%), 184 (100%). Anal. Calcd. for $C_{14}H_{12}N_4O_3S_2$ (348): C, 48.27; H, 3.44; N, 16.09. Found: C, 48.59; H, 3.26; N, 16.32.

4-(4-Chlorothiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (4)

A solution of compound **3** (3.48 g, 0.01 mol) in thionyl chloride (10 mL) was refluxed for 2 h, thionyl chloride was then removed by distillation under reduced pressure and the residual solid was washed twice with benzene and crystallized from dioxane to give **4**: Yield 95%, m.p. 198-200ºC, IR (KBr, cm^{-1}): 3192 (NH), 3040 (CH arom.), 1616 (C=N), 1390, 1165 (SO_2), 684 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.6 (s, 1H, CH, thiazole), 6.8-8.0 (m, 9H, Ar-H + NH, D_2O exchangeable), 10.9 (s, 1H, SO_2NH, D_2O exchangeable). MS (*m/z*): 366 (M^+, 3.46%), 78 (100%). Anal. Calcd. for $C_{14}H_{11}ClN_4O_2S_2$ (366): C, 45.90; H, 3.00; N, 15.30. Found: C, 45.63; H, 3.36; N, 15.5.

4-(4-Mercaptothiazol-2-ylamino)-N-(pyridin-2-yl)) benzenesulfonamide (5)

A mixture of compound **3** (3.48 g, 0.01 mol) and phosphorus pentasulfide (2.2 g, 0.01 mol) in pyridine (20 mL) was refluxed for 8 h, the reaction mixture was cooled and then poured onto ice-cold water and acidified with dilute hydrochloric acid. The solid obtained was crystallized from ethanol to give **5**: Yield 47%, m.p. 250-251ºC, IR (KBr, cm^{-1}): 3445, 3233 (NH), 3056 (CH arom.), 2553 (SH), 1590 (C=N), 1388, 1138 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.86 (s, 1H, CH, thiazole), 6.88-8.0 (m, 9H, Ar-H + NH, D_2O exchangeable), 10.9 (s, 1H, SO_2NH, D_2O exchangeable), 13.8 (s, 1H, SH, D_2O exchangeable). MS (*m/z*): 364 (M^+, 1.60%), 63 (100%). Anal. Calcd. for $C_{14}H_{12}N_4O_2S_3$ (364): C, 46.15; H, 3.29; N, 15.38. Found: C, 46.48; H, 2.99; N, 15.64.

4-(4-(Methylthio) thiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (6)

A mixture of compound **5** (3.64 g, 0.01 mol) and methyl iodide (1.52 mL, 1.42 g, 0.01 mol) in methanol (20 mL) was left at ambient temperature for 48 h. The sample was trhen heated under reflux for 6 h. The solvent was removed by evaporation and the oily residue was triturated with diethyl ether. The crude solid was collected, washed with cold ethanol and finally crystallized from propanol to give **6**: Yield 55%, m.p. 194-196ºC, IR (KBr, cm^{-1}): 3440, 3239 (NH), 3057 (CH arom.), 2928, 2860 (CH aliph), 1540 (C=N), 1390, 1140 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.2 (s, 3H, CH_3), 6.8 (s, 1H, CH, thiazole), 6.9-8.2 (m, 9H, Ar-H + NH, D_2O exchangeable), 10.8 (s, 1H, SO_2NH, D_2O exchangeable). Anal. Calcd. for $C_{15}H_{14}N_4O_2S_3$ (378): C, 47.61; H, 3.70; N, 14.81. Found: C, 47.34; H, 4.06; N, 14.64.

4-(4-Hydrazinylthiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (7)

Method A: A mixture of compound **6** (3.78 g, 0.01 mol) and hydrazine hydrate (0.01 mol) was refluxed in ethanol (20 mL) for 24 h. and H_2S was detected using lead acetate paper. The reaction mixture was cooled and poured onto ice-cold water. The solid obtained was crystallized from dioxane to give **7** with a 20%

yield.

Method B: A mixture of compound **4** (3.66 g, 0.01 mol) and hydrazine hydrate (0.01 mol) was refluxed in ethanol (20 mL) for 5 h. The reaction mixture was cooled and poured onto ice-cold water and acidified with dilute hydrochloric acid. The solid obtained was collected by filtration and crystallized from dioxane to give **7** at a yield higher than 58%, m.p. 210-212°C, IR (KBr, cm^{-1}): 3280, 3215, 3113 (3NH, NH_2), 3098 (CH arom.), 1600 (C=N), 1389, 1138 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.4 (s, 2H, NH_2, D_2O exchangeable), 4.7 (s, 1H, $NHNH_2$, D_2O exchangeable), 6.7 (s, 1H, CH, thiazole), 6.9-8.0 (m, 9H, Ar-H + NH, D_2O exchangeable), 10.2 (s, 1H, SO_2NH, D_2O exchangeable). Anal. Calcd. for $C_{14}H_{14}N_6O_2S_2$ (362): C, 46.40; H, 3.86; N, 23.20. Found: C, 46.67; H, 4.10; N, 23.38.

4-(5-Amino-7-(4-chlorophenyl)-6-cyano-7H-pyrano [2,3-d]thiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (8)

A mixture of compound **3** (3.48g, 0.01 mol) and 2-(4-chlorobenzylidene) malononitrile (1.88 g, 0.01 mol) in ethanol (20 mL) containing a catalytic amount of triethylamine (TEA), was refluxed for 5 h. The reaction mixture was cooled, poured onto ice-cold water and acidified with dilute hydrochloric acid. The obtained solid was crystallized from dioxane to give compound **8**: Yield 86%, m.p. 270-272°C, IR (KBr, cm^{-1}): 3250, 3191, 3113 (NH, NH_2), 3046 (CH arom.), 2939, 2825 (CH aliph.), 2219 (CN), 1603 (C=N), 1391, 1138 (SO_2), 780 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ ppm): 3.8 (s, 2H, NH_2, D_2O exchangeable), 4.1 (s, 1H, CH, pyran), 6.6-8.0 (m, 13H, Ar-H + NH, D_2O exchangeable), 8.9 (s, 1H, SO_2NH, D_2O exchangeable). MS (*m/z*): 536 (M^+, 1.00%), 168 (100%). Anal. Calcd. for $C_{24}H_{17}ClN_6O_3S_2$ (536): C, 53.73; H, 3.17; N, 15.67. Found: C, 53.39; H, 3.51; N, 15.45.

4-[3H-5-(4-Chlorophenyl)-thiazolo[4,5-b]-pyrano [2,3-d]pyrimidin-4-one]-N-(pyridin-2-yl) benzenesulfonamide (9)

A solution of compound **8** (5.36 g, 0.01 mol) in formic acid (20 mL) was refluxed for 5 h, the reaction mixture was cooled and then poured onto ice-cold water. The obtained solid was crystallized from ethanol to give **9**: Yield 67%, m.p. > 290°C, IR (KBr, cm^{-1}): 3273, 3120 (NH), 3046 (CH arom.), 2936, 2822 (CH aliph.), 1713 (C=O), 1589 (C=N), 1371, 1138 (SO_2), 778 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ ppm): 4.1 (s, 1H, CH, pyran), 6.8-8.0 (m, 15H, Ar-H + CH, pyrimidine + 2NH, D_2O exchangeable), 10.9 (s, 1H, SO_2NH, D_2O exchangeable). MS (*m/z*): 564 (M^+, 6.19%), 157 (100%). Anal. Calcd. for $C_{25}H_{17}ClN_6O_4S_2$ (564): C, 53.19; H, 3.01; N, 14.89. Found: C, 52.91; H, 2.78; N, 14.69.

4-(4-(2-Phenylhydrazinyl)thiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfon-amide (10), 4-(4-(2-(2,4-Dinitrophenyl)hydrazinyl)thiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (11)

A mixture of compound **4** (3.66 g, 0.01 mol) and phenylhydrazine (0.9 mL, 1.08 g, 0.01 mol) or 2,4-dinitrophenylhydrazine (1.98 g, 0.01 mol) in ethanol (20 mL) was refluxed for 5 h. The reaction mixture was cooled and poured onto cold water. The solid obtained was crystallized from dioxane to give **10**, **11** respectively.

Microanalytical and spectral data of **10**: Yield 72%, m.p.130-132°C, IR (KBr, cm^{-1}): 3364, 3313 (NH), 3057 (CH arom.), 1582 (C=N), 1389, 1143 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 3.5 (s, 2H, NHNH, D_2O exchangeable), 6.8 (s, 1H, CH, thiazole), 6.9-8.0 (m, 14H, Ar-H + NH, D_2O exchangeable), 10.1 (s, 1H, SO_2NH, D_2O exchangeable). MS (*m/z*): 438 (M^+, 0.74%), 61 (100%). Anal. Calcd. for $C_{20}H_{18}N_6O_2S_2$ (438): C, 54.79; H, 4.10; N 19.17,. Found: C, 54.55; H, 4.39; N, 18.98.

Microanalytical and spectral data of **11**: Yield 73%, m.p. 168-170°C, IR (KBr, cm^{-1}): 3421, 3347 (NH), 3068 (CH arom.), 1593 (C=N), 1520, 1387 (C-NO_2), 1335, 1135 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 3.7 (s, 2H, NHNH, D_2O exchangeable), 6.8 (s, 1H, CH, thiazole), 6.9-8.2 (m, 12H, Ar-H + NH, D_2O exchangeable), 9.7 (s, 1H, SO_2NH, D_2O exchangeable). MS (*m/z*): 528 (M^+, 1.71%), 61 (100%). Anal. Calcd. for $C_{20}H_{16}N_8O_6S_2$ (528): C, 45.45; H, 3.03; N, 21.21. Found: C, 45.80; H, 3.33; N, 21.53.

4-[4-(Isothiocyanato) thiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (12)

A mixture of compound **4** (3.66 g, 0.01 mol) and ammonium thiocyanate (0.76 g, 0.01 mol) in dry acetone (20 mL) was refluxed for 1 h. The reaction mixture was cooled and poured onto ice-cold water. The solid obtained was crystallized from ethanol to give **12**: Yield 69%, m.p. 215-217°C, IR (KBr, cm^{-1}): 3392, 3128 (NH), 3049 (CH arom.), 2051 (N=C=S), 1587 (C=N), 1390, 1129 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.8 (s, 1H, CH, thiazole), 6.9-8.0 (m, 9H, Ar-H + NH, D_2O exchangeable), 10.8 (s, 1H, SO_2NH, D_2O exchangeable). MS (*m/z*): 389 (M^+, 0.9%), 64 (100%). Anal. Calcd. for $C_{15}H_{11}N_5O_2S_3$ (389): C, 46.27; H, 2.82; N, 17.99. Found: C, 45.98; H, 3.04; N, 17.62.

4-(4-(4-Chlorophenylamino)thiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfon-amide (13)

A mixture of compound **4** (3.66 g, 0.01 mol) and 4-chloroaniline (1.27 g, 0.01 mol) in dimethylformamide (20 mL) was refluxed for 4 h. The reaction mixture

was cooled and poured onto cold water. The solid obtained was crystallized from ethanol to give **13**: Yield 64%, m.p. > 290°C, IR (KBr, cm^{-1}): 3386, 3345 (NH), 3076 (CH arom.), 1602 (C=N), 1397, 1130 (SO_2), 620 (C-Cl). MS (*m/z*): 457 (M^+, 0.27%), 54 (100%). Anal. Calcd. for $C_{20}H_{16}ClN_5O_2S_2$ (457): C, 52.51; H, 3.50; N, 15.31. Found: C, 52.19; H, 3.68; N, 15.54.

4-[4-(2-Aminobenzoic acid)thiazol-2-ylamino]-N-(pyridin-2-yl)benzenesulfon-amide (14)

A mixture of compound **4** (3.66 g, 0.01 mol) and anthranilic acid (1.37 g, 0.01 mol) in n-butanol (20 mL) was refluxed for 5 h. The reaction mixture was cooled and poured onto cold water. The solid obtained was crystallized from ethanol to give **14**: Yield 69%, m.p. > 290°C, IR (KBr, cm^{-1}): 3424 (OH), 3347, 3286 (NH), 3069 (CH arom.), 1681 (C=O), 1574 (C=N), 1390, 1142 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 6.5 (s, 1H, CH, thiazole), 6.8-7.9 (m, 14 H, Ar-H + 2NH, D_2O exchangeable), 10.9 (s, 1H, SO_2NH, D_2O exchangeable), 11.8 (s, 1H, OH, D_2O exchangeable). MS (*m/z*): 467 (M^+, 2.7%), 184 (100%). Anal. Calcd. for $C_{21}H_{17}N_5O_4S_2$ (467): C, 53.96; H, 3.64; N, 14.98. Found: C, 54.27; H, 3.45; N, 14.61.

In vitro cytotoxic screening

The cytotoxic activity of the newly synthesized compounds was evaluated against a human breast cancer cell line (MCF-7) *in vitro* using the Sulfo-Rhodamine-Bstain (SRB) assay as described previously (Skehan et al., 1990). The human tumor cell line (MCF-7) was provided by the National Cancer Institute, Cairo University, Egypt. The relationship between surviving fraction and drug concentration (μM) was plotted using the Microsoft Office Excel 2003 program to obtain the best fit survival curve for the breast tumor cell line after a specified time. The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated by solving the third order polynomial equation using software that was written on the 'Matlab R2008a' program.

Radiosensitizing evaluation

The most promising compounds based on the *in vitro* cytotoxic screening (**7**, **9**, and **11**) were used in subsequent experiments to assess the synergistic effects of combined treatment with γ-radiation on *in vitro* cytotoxicity against a human breast cancer cell line (MCF-7). Irradiation was performed at the National Center for Radiation Research and Technology, Atomic Energy Authority, using a Gamma cell-40 (^{137}Cs) source.

Cells were incubated with the selected compounds; **7**, **9**, and **11**, in molar concentrations of 10, 25, 50, 100 μM. After 2 h, Cells were subjected to a single dose of ã-radiation at a dose level of 8 Gy with a dose rate of 2 Gy/min. After 48 h, the surviving fractions were measured using an ELISA reader.

The cytotoxicity of the control group, the radiated group, the compound treated groups (10, 25, 50, and 100 μM) and the irradiated compound groups (10, 25, 50, and 100 μM) was measured using the SRB assay on the human breast cancer cell line (MCF-7).

Molecular docking study

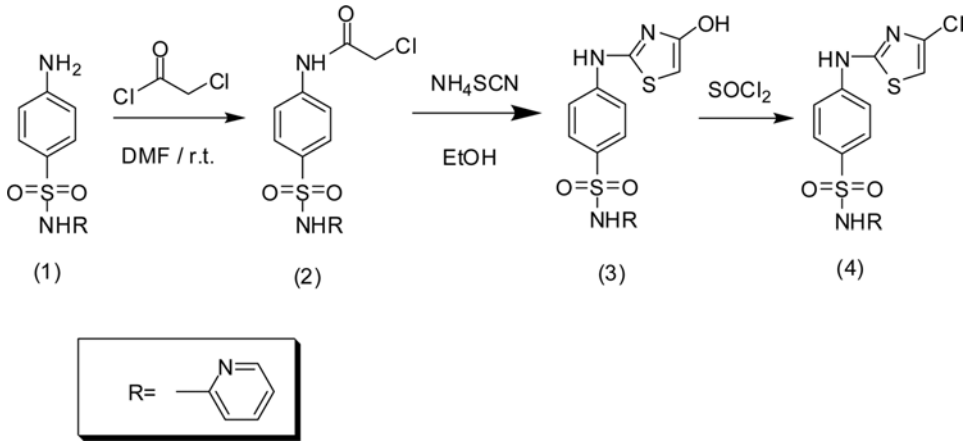
All molecular modeling studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE, 10.2008) software. All the minimizations were performed with MOE until a RMSD gradient of 0.05 kcal $mol^{-1}A^{o-1}$ with MMFF94X forcefield and the partial charges were automatically calculated.

The X-ray crystallographic structure of human CDK2 complexes with the sulfone inhibitor (PDB ID: 1FVV) was obtained from the protein data bank. The enzyme was prepared for docking studies where: (i) Ligand molecule was removed from the enzyme active site. (ii) Hydrogen atoms were added to the structure with their standard geometry. (iii) MOE Alpha Site Finder was used to search for the active sites in the enzyme structure and dummy atoms were created from the obtained alpha spheres. (iv) The obtained model was then used to predict the interaction between he ligand and enzymes at the active site

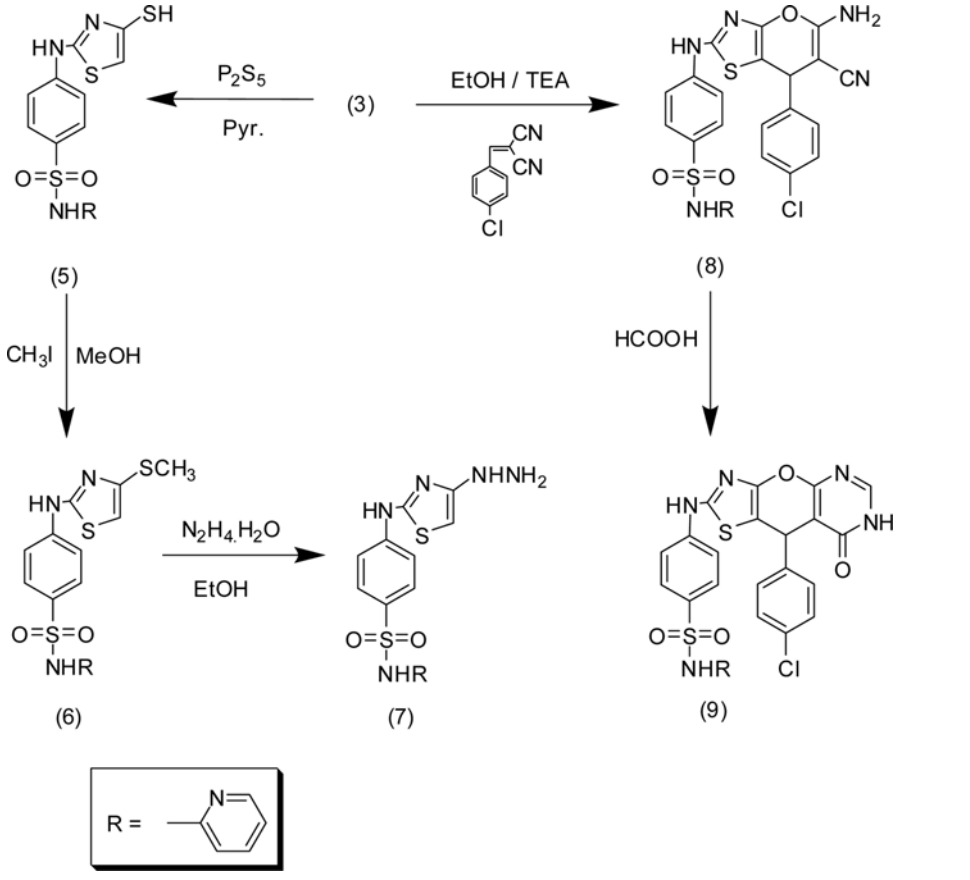
RESULTS AND DISCUSSION

Chemistry

2-Chloro-N-(4-(N-pyridin-2-ylsulfamoyl) phenyl) acetamide **2** was synthesized via reaction of sulfapyridine **1** with chloroacetyl chloride (Finkelstein, 1944). Refluxing compound **2** with ammonium thiocyanate in ethanol efficiently yielded 4-hydroxythiazole derivative **3** via intramolecular cyclization. Compound **4** was obtained by refluxing **3** with $SOCl_2$ (Scheme 1). When **3** was refluxed with phosphorus pentasulfide in pyridine, the 4-mercaptothiazole derivative **5** was produced. The thio methyl derivative **6** was obtained by refluxing compound **5** with methyl iodide in methanol. Treatment of **6** with hydrazine hydrate in ethanol for 24 h afforded the 4-hydrazino derivative **7** with a 20% yield. A higher yield of 45% was obtained by refluxing the 4-chloro derivative **4** with hydrazine hydrate in ethanol for 5 h. Reaction of **3** with 2-(4-chlorobenzylidene) malononitrile in ethanol containing a catalytic



Scheme 1. Synthetic pathway used to prepare the starting materials **3**, **4**

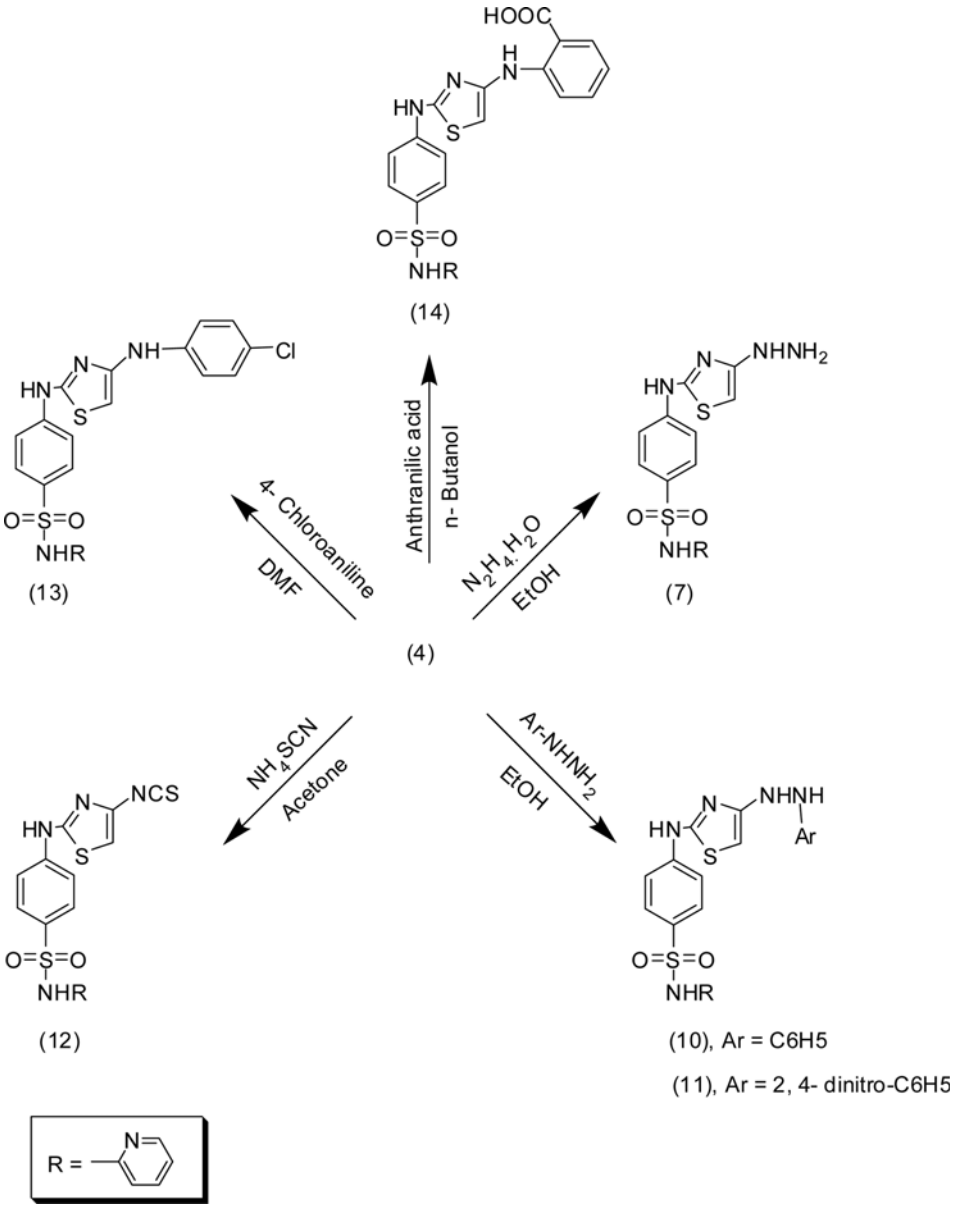


Scheme 2. Synthetic pathway used to prepare compounds **5**-**9**

amount of triethylamine (TEA), yielded the corresponding pyrano [2,3-d] thiazole derivative **8**, via the formation of an intermediate followed by intramolecular cyclization. Thiazolo [4,5-b]-pyrano[2,3-d] pyrimidine derivative **9** was obtained by refluxing **8** in formic acid. This reaction proceeded via condensation followed by cyclization (Scheme 2). Compounds **10**, **11**, **12**, **13**, and **14** were obtained by reacting 4-chlorothiazole derivative **4** with phenylhydrazine, 2,4 dinitrophenyl hydrazine, ammonium thiocynate, 4-chloroaniline and anthranilic acid, respectively (Scheme 3).

In vitro cytotoxic screening

In the *in vitro* cytotoxic screening of the synthesized compounds against human breast cancer cell line (MCF-7), the thiazolo [4,5-b]-pyrano [2,3-d] pyrimidine



Scheme 3. Synthetic pathway used to prepare compounds **7**, **10-14**

derivative **9** was shown to be highly cytotoxic against MCF-7 cells with an IC_{50} value of 12.0 μM, which was significantly better than doxorubicin, the reference drug (IC_{50} = 26.1 μM). 4-hydrazinyl thiazole derivatives **7**, **10**, and **11** also showed high activity with IC_{50} values of 21.7, 22.8, 20.0 μM, respectively, while the methylthio derivative **6**, and the anthranilic acid derivative **14** showed less activity with IC_{50} values of 23.2 and 25.8 μM, respectively (Table I).

Radiosensitizing evaluation

It has been reported that combining chemotherapy (CT) with radiotherapy (RT) is effective for treatment of cancer. The rationale for combining CT and RT is mainly based on two concepts; spatial cooperation, and enhancement of radiation effects. Spatial cooperation is effective if CT is sufficiently active to eradicate subclinical metastases and if the primary local tumor is effectively treated by RT. In this regard, no interaction between RT and CT is required. In regards to the former concept, radiation the initial radiation damage can be directly enhanced by incorporating drugs into DNA, inhibiting cellular repair, accumulating cells in a radiosensitive phase or eliminating radioresistant phase cells, and eliminating hypoxic cells (Nishimura, 2004).

Thus, the effect of combined treatment with the most active compounds (**7**, **9**, and **11**) and γ-radiation

Table I. *In vitro* anticancer screening of the synthesized compounds against the human breast cancer cell line (MCF-7)

Compd. No.	Compound concentration (μM)				IC_{50} (μM) 3rd order
	10 (μM)	25 (μM)	50 (μM)	100 (μM)	
	Surviving fraction (mean ± S.E.M.)[a]				
Doxorubicin	0.721 ± 0.02	0.546 ± 0.02	0.461 ± 0.01	0.494 ± 0.03	26.1
3	0.9294 ± 0.11	0.6825 ± 0.02	0.2583 ± 0.04	0.3387 ± 0.01	34.5
4	0.9032 ± 0.08	0.5566 ± 0.03	0.2540 ± 0.07	0.3272 ± 0.03	31.6
5	0.8624 ± 0.09	0.5843 ± 0.08	0.3262 ± 0.07	0.1474 ± 0.02	32.9
6	0.7797 ± 0.04	0.4775 ± 0.08	0.1452 ± 0.01	0.1448 ± 0.01	23.2
7	0.8047 ± 0.02	0.4019 ± 0.04	0.1122 ± 0.02	0.1521 ± 0.02	21.7
8	0.8502 ± 0.10	0.6243 ± 0.03	0.3514 ± 0.03	0.2735 ± 0.01	35.5
9	0.4921 ± 0.03	0.2722 ± 0.06	0.1659 ± 0.03	0.2160 ± 0.05	12.0
10	0.7347 ± 0.05	0.4925 ± 0.06	0.2606 ± 0.04	0.1716 ± 0.02	22.8
11	0.6666 ± 0.10	0.4294 ± 0.03	0.2076 ± 0.04	0.1975 ± 0.03	20.0
12	0.8940 ± 0.06	0.6655 ± 0.07	0.3155 ± 0.09	0.1533 ± 0.03	45.9
13	0.7831 ± 0.24	0.6426 ± 0.08	0.4252 ± 0.03	0.1397 ± 0.01	35.0
14	0.7510 ± 0.07	0.5662 ± 0.07	0.2576 ± 0.02	0.1972 ± 0.06	25.8

[a]Each value is the mean of three experiments ± S.E.M.

Table II. *In vitro* anticancer screening of compounds **7**, **9**, and **11** in combination with γ-radiation against the human breast cancer cell line (MCF-7)

Compd. No.	Control (cells only)	Irradiated control (8 Gy)	Compound concentration (μM) + Irradiation (8 Gy)				IC_{50} (μM)
			10	25	50	100	
			Surviving fraction (Means ± S.E.M.)[a]				
7	1.000	0.927 ± 0.02[b]	0.5256 ± 0.16[b]	0.1543 ± 0.04[b]	0.1020 ± 0.02[b]	0.2270 ± 0.07[b]	11.9
9	1.000	0.927 ± 0.02[b]	0.4902 ± 0.18[b]	0.2846 ± 0.04[b]	0.2468 ± 0.03[b]	0.2742 ± 0.04[b]	11.7
11	1.000	0.927 ± 0.02[b]	0.4286 ± 0.06[b]	0.3089 ± 0.05[b]	0.1081 ± 0.03[b]	0.1503 ± 0.03[b]	11.6

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

on cytotoxicity was evaluated. The IC_{50} values of the tested compounds were synergistically decreased to 11.9, 11.7, and 11.6 μM, respectively (Table II). Based on these results, we can conclude that the combination (treatment) of compounds **7**, **9** or **11** with the ionizing radiation synergistically enhanced inhibition of breast cancer.

Molecular docking study

Cyclin-dependant kinase 2 enzyme (CDK2) is one of the most important enzymes in the protein kinases family. It is responsible for G1/S phase in the cell cycle (Lucking et al., 2007), where it acts by transferring a phosphoryl group from a donor to an acceptor thus activating a cyclin protein and regulating cell division (Lucking et al., 2007). CDK2 interacts with Cyclin A and E to drive the cell from the G1 phase to S phase (Jeffrey et al., 1995). CDK2 is a monomer composed of 298 amino acids and consists of α-helix elements and a β-sheet (Morgan).

Understanding the binding mode of cyclin A and E to CDK2 was of great help in designing CDK2 inhibitors as antitumor agents. The CDK2/cyclin interface area is 3252 Å2 in Cyclin E and 2839 Å2 in Cyclin A (Jeffrey et al., 1995; Honda et al., 2005).

Two classes of synthetic inhibitors of CDK2 were examined to understand the binding mode to the active site of CDK2 and to identify the key amino acids in this site. Thiazolidinone inhibitors bind to CDK2 through two hydrogen bonds with Glu 81 and Leu 83 and the sulfonate group of these inhibitors interacts with Asp 86 and leu 10 of the backbone (Richardson et al., 2007). In contrast, aminopyrimidine inhibitors were shown to interact with Asp 86 and Ile 10 (Lucking et al., 2007).

Since our compounds were designed to contain aminopyridines and sulfone groups, we evaluated their docking on the active site of CDK2 and determined their interactions with amino acids on the active site of this enzyme in order to better understand their biological activity as cytotoxic agents.

A protein data bank file with the code 1FVV was selected for this purpose. The file contains the CDK2 enzyme co-crystallized with a sulfone ligand (Davis et

al., 2001). All docking procedures were achieved using MOE (Molecular Operating Enviroment) software 10.2008 provided by chemical computing group, Canada. Docking on the active site of CDK2 was performed for compounds **6**, **7**, **9**, **10**, **11**, and **14**, which showed good cytotoxic activity.

The docking protocol was verified by redocking of the co-crystallized ligand in the vicinity of the active site of the enzyme with energy score (S) = 18.1741 Kcal/mol and root mean standard deviation (RMSD) = 0.7723 (Fig. 2 and 3). The sulfone ligand interacts with the active site of CDK2 through four interactions: the sulfonate group interacts with Lys 89 through a hydrogen bond of 3.13 Å and Asp 86 through a hydrogen bond of 3.22 Å, the carbonyl group of pyrrolone interacts with Leu 83 through a hydrogen bond of 2.96 Å and the NH group of pyrrolone interacts with Glu 81 through a hydrogen bond of 1.98 Å. All the docked compounds bind to either Lys 89 or Asp 86 through SO_2 by one hydrogen bond. In addition, compound **11** binds to Asp 86 through NH (thiazole) and compound **14** binds to Leu 83 through NH as illustrated in Table III.

Compound **10** showed the best energy score (S) = 21.8927 Kcal/mol with two hydrogen bond interactions with Asp 86 through the NH group of its thiazole ring and with Lys 89 through its SO_2 group. This result suggests that compound **10** acts as a cytotoxic agent by inhibiting CDK2. This finding is supported by its IC_{50} value = 22.8 µM, which was better than Doxorubicin as illustrated in Fig. 4 and 5. Compounds **6**,**7**,**11** and **14** also act as cytotoxic agents through the same mechanism as compound **10**. However, compound **9** showed a poor energy score (S) = 10.7351 despite its IC_{50} value = 12.0 µM, suggesting that this compound functions as a cytotoxic agent through another mechanism.

Based on the above results, compounds **6**, **7**, **9**, **10**,

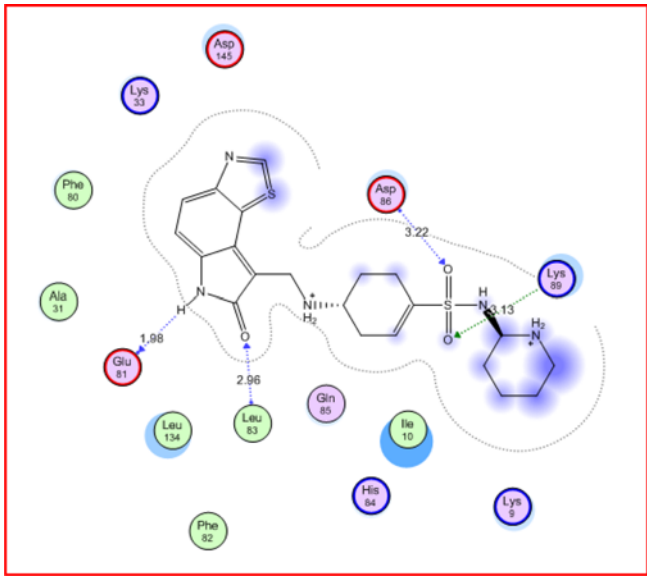


Fig. 2. Interactions between the ligand and the active site of CDK2

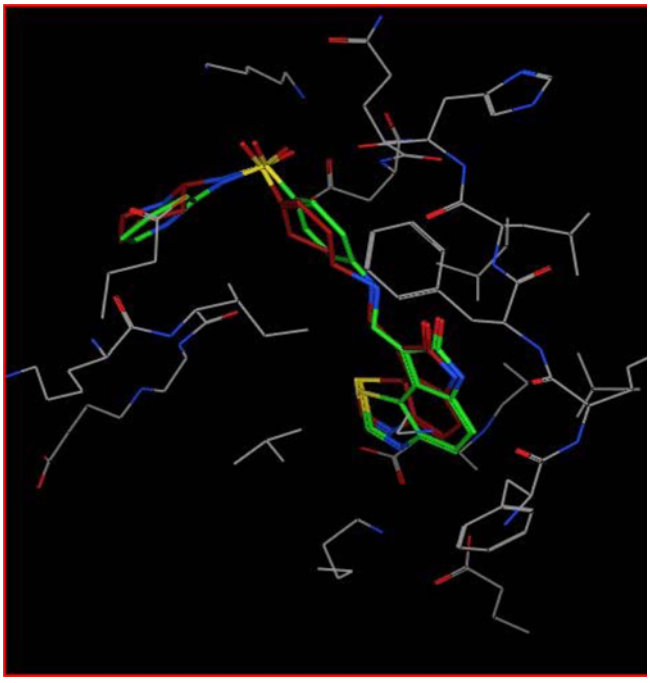


Fig. 3. Validation of the docking protocol on the active site of CDK2

Table III. Binding scores and interaction of the amino acids on the active site of CDK2 with the docked compounds

Compound No.	S Kcal/mol	Amino acids	Groups interacted	Length of H bonds (Å)
Co-crystallized Ligand	18.1741	Asp 86 Lys 89 Leu 83 Glu 81	O=S=O O=S=O C=O pyrrole NH pyrrole	3.72 3.13 2.96 1.98
6	15.9589	Lys 89	O=S=O	3.16
7	14.7205	Asp 86 Glu 51	O=S=O NH-NH_2	2.81 1.58
9	10.7351	Lys 89 Gln 131	O=S=O N (pyridine)	2.59 2.85
10	21.8927	Lys 89 Asp 86	O=S=O NH (thiazole)	2.77 1.72
11	17.5159	Lys 89 Asp 86	O=S=O NH (thiazole)	2.65 2.29
14	16.3041	Lys 89 Leu 83 Lys 33	O=S=O NH COO	2.74 1.68 2.79

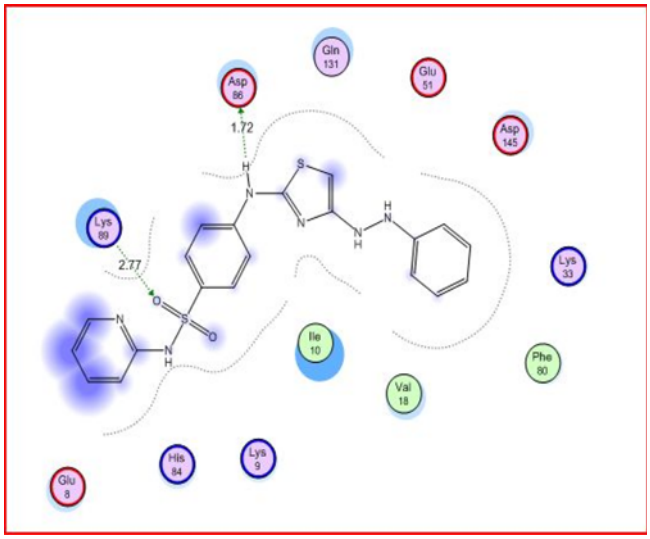


Fig. 4. Compound **10** on the active site of CDK2 2D

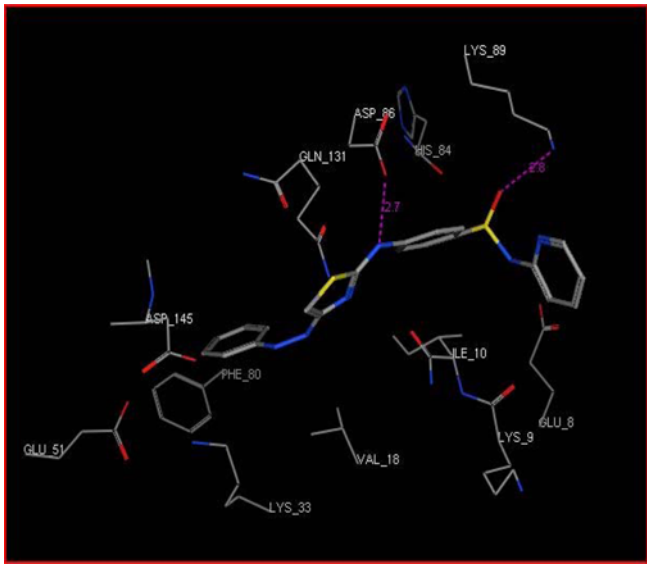


Fig. 5. Compound **10** on the active site of CDK2 3D

11, and **14** showed promising cytotoxic activity against human breast cancer cell line (MCF-7) with IC_{50} values of 23.2 μM, 21.7 μM, 12.0 μM, 22.8 μM, 20.0 μM, and 25.8 μM, respectively. Combination treatment using the three most active compounds **7**, **9**, and **11** and γ-radiation at the same concentrations enhanced the cytotoxic activity and the IC_{50} values were decreased to 11.9 μM, 11.7 μM, and 11.6 μM, respectively. All synthesized compounds, except compound **9**, appear to function as cytotoxic agents by inhibiting CDK2. Further studies should be conducted to determine the mechanism of action of this series of the synthesized compounds.

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