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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL BENZO[4,5]THIAZOLO[2,3-*C*][1,2,4]TRIAZOLE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS

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Abstract: In the present study, we have designed and synthesized a novel series of benzothiazolotriazole derivatives as potential anticancer agents. The anticancer activity of the newly synthesized compounds was evaluated against three cancerous cell lines; A549 (human lung adenocarcinoma), MCF-7 (human breast carcinoma) and Hep3B (human hepatocellular carcinoma) using MTT assay. Among this series, compounds **15** and **16** showed the most promising anticancer activity with IC_{50} values between 11.1 to 21.5 µM. Kinase profiling was performed for the most potent compounds **15** and **16** and it revealed weak inhibitory activity against 10 various kinases, where the highest inhibition was against CDK2/Cyclin A1 by compound **15**. Furthermore, caspase-3/7 assay also indicated that the same compound **15** has the ability to induce apoptosis through the activation of effector caspase-3/7 family. Therefore, it could be deduced that the *S*-benzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole is a promising novel anticancer scaffold with antiproliferative and apoptosis-inducing activities deserves to be taken up as a lead for further structural optimization.

Keywords: *S*-benzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole, anticancer, kinase, caspase3/7, apoptosis

Cancer remains one of the most life-threatening diseases, representing the second leading cause of death worldwide after cardiovascular diseases (1). It is estimated that 12 million people will die from cancer in 2030 which made the development of novel anticancer drugs one of the most intensely persuaded goals of modern drug design and discovery (2). Despite successes achieved, chemotherapy can offer only a modest increase in survival time in the majority of advanced disease cases (3). However, there is another optimistic view that in the coming years advances in prevention, detection and treatment will see cancer becoming considered not as a fatal but as a chronic disease (4). In the last few years, many efforts have been made to develop new strategies for finding safe and effective ways of treating this disease which include an increased in the understanding of the biological process involved in cancer cell survival (1). In this context, the major challenge is the development of more effective and safer drugs for the treatment of cancer. On the other hand, triazoles and their fused heterocyclic derivatives are well-known synthetic chemical entities that have received considerable attention owing to their synthetic and effective biological importance. Literature survey reveals that 1,2,4-triazole has been incorporated into several therapeutically interesting drug candidates with antimicrobial (5), anti-inflammatory (6), analgesic (7) and anticancer activities (8, 9). Vorozole **1** and Letrozole **2** incorporating substituted 1,2,4-triazole ring, are currently being used for the treatment of breast cancer (10), Figure 1. Meanwhile, the multi-functionalized benzothiazole scaffold represents a fruitful source of inspiration for drug designers for many years that has attracted special attention due to synthetic accessibility as well as their diverse biological activities, including antitumor (11), antimicrobial (12), antidiabetic (13),

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anticonvulsant (14) and anti-inflammatory (15). Ian Hutchinson et al. reported a novel set of metabolically stable fluorinated analogs of 2-(4-aminophenyl)benzothiazoles **3** that exhibited selective, potent, and unique *in vitro* antitumor activity (16). Moreover, a series of benzothiazole hydrazones has been synthesized and shown good antiproliferative activity against three cancer cell lines. Compound **4** was pointed as promising lead molecule for anticancer drug design, Figure 1 (17).

Based on the aforementioned data, it was of interest to hybridize the 1,2,3-triazole and benzothiazole in one scaffold (A) and explore the impact of this hybridization on the anticancer activity. Interestingly, scaffold A can be obtained also through restriction of the free rotation of the side chain in compound **4** which displayed promising activity as potential anticancer agent, Figure 2.

To study the structure activity relationship (SAR) of scaffold A, two different structural modifications were done. The first was variation of the linker between the fused system and small heterocycles. Therefore, linkers of different types and length were used to investigate their effect on anticancer activity of the new compounds. The second structural modification was achieved through variation of the small heterocyclic rings. Different heterocycles including oxadiazole, oxadiazol-2-ol, thiadiazol-2-amine, pyrazole, thiadiazole, triazol-5-thione and isatin were used, Figure 2.

EXPERIMENTAL PROTOCOLS

Chemistry

Chemical reagents and solvents were obtained from commercial sources (Sigma-Aldrich and Acros

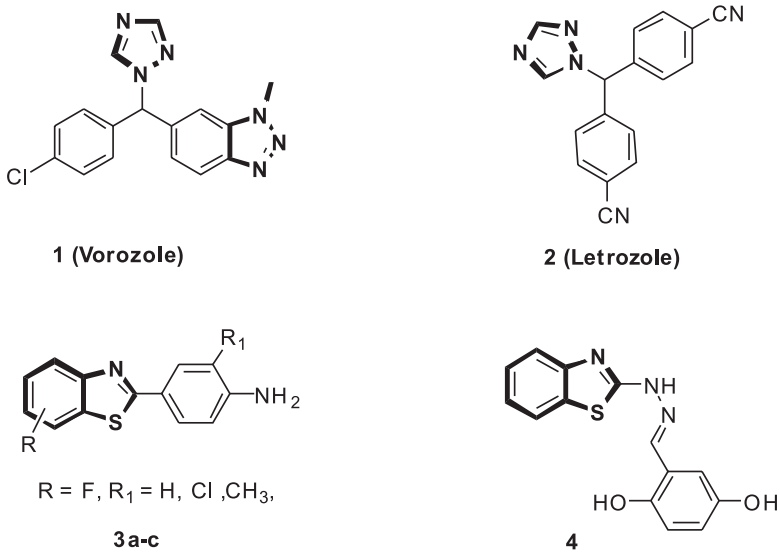


Figure 1. 1,2,4-Triazole/benzothiazole-based anticancer agents

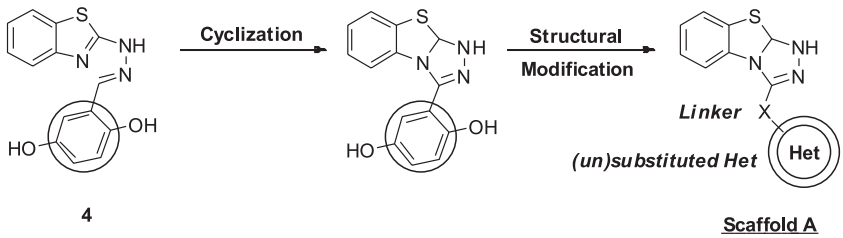


Figure 2. Rational design and structural modifications of scaffold A

companies). Solvents were dried by standard methods when necessary. Melting points (m.p.) were uncorrected and were carried out by open capillary tube method using IA 9100MK-Digital Melting Point Apparatus. Microanalyses were carried out at the microanalytical center, Faculty of Science, Cairo University. The proton magnetic resonance ^{1}H-NMR spectra were recorded on BRUKER APX400 spectrometer at 400 MHz in the specified solvent, chemical shifts were reported on the δ scale and were related to that of the solvent and *J* values are given in Hz. ^{13}C NMR spectra were obtained on a Bruker APX400 at 100 MHz at Faculty of Pharmacy, Beni-Suef University. Mass spectra were recorded on Finnigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI) at the microanalytical center, Faculty of Science, Cairo University. Thin layer chromatography, was done using Macherey-Nagel Alugram Sil G/UV254 silica gel plates and methylene choride-methanol (9.5 : 0.5) or hexane-ethyl acetate (8 : 2) as the eluting systems. The starting materials **5-9** were synthesized according to the previously reported methods (18-20).

4-amino-5-((benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-ylthio)methyl)-4H-1,2,4-triazole-3-thiol (10)

Carbon disulfide (124 µL, 1.59 mM) was added slowly to a solution of acid hydrazide **9** (837 mg, 3 mM) in ethanol (20 mL) containing potassium hydroxide (280 mg, 5 mM). The reaction mixture was allowed to stir at room temperature for 3 h where the potassium dithioate was formed. The precipitated was filtered off, dried and collected. A mixture of the formed potassium dithioate (1 mM) and hydrazine hydrate (5 mM, 250 mg) was refluxed in ethanol for 6 h. The solvent was evaporated and the residue was dissolved in water, neutralized with 10% HCl. The precipitate obtained was filtered, washed with water and dried to afford a white solid (70%) of compound **10**, m.p. 189-190°C. IRυ_{max}/cm^{-1} 3275 (NHs), 3065 (Ar C-H), 2923 (Aliphatic C-H), 2583 (S-H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.48 (s, 1H, SH), 8.09 (d, *J* = 8.3 Hz, 1H), 8.04 (d, *J* = 8.2 Hz, 1H), 7.61 (t, *J* = 7.8 Hz, 1H), 7.50 (t, *J* = 7.2 Hz, 8H), 5.62 (s, 2H, NH_2), 4.38 (s, 2H, SCH_2).^{13}C NMR (101 MHz, DMSO-d_6): δ 166.89, 161.79, 152.20, 141.50, 131.57, 130.58, 127.65, 127.00, 125.33, 114.77, 28.57 (SCH_2). MS (EI) m/z 335.00 (M^+). Analysis: calcd. for $C_{11}H_9N_7S_3$: C, 39.39; H, 2.70; N, 29.23%; found: C, 39.48; H, 2.49; N, 29.66%.

5-((benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-ylthio)methyl)-1,3,4-thiadiazole-2-thiol (11).

A mixture of the previously prepared potassium dithioate (2 mM) and conc. H_2SO_4 (5 mL) was stirred at rt overnight. The reaction mixture was poured onto water and neutralized with $NaHCO_3$ solution. The precipitate obtained was filtered, washed with water and dried to provide a white solid (52%) of compound **11**, m.p. 122-123°C. IRυ_{max}/cm^{-1} 3042 (Ar C-H), 2871 (Aliphatic C-H), 2561 (S-H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.90 (s, 1H, SH), 8.14 (d, *J* = 8.3 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.53 (t, *J* = 7.3 Hz, 1H), 4.11 (s, 2H, SCH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 169.77, 156.56, 143.02, 131.90, 129.87, 127.56, 126.92, 126.03, 125.37, 114.81, 36.31 (SCH_2). MS (EI) m/z 337.00 (M^+). Analysis: calcd. for $C_{11}H_7N_5S_4$: C, 39.15; H, 2.09; N, 20.75%; found: C, 39.52; H, 2.14; N, 20.65%.

4-amino-5-((benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-ylthio)methyl)-4H-1,2,4-triazole-3-thiol (12)

A mixture of the previously prepared potassium dithioate (1 mM) in ethanol (10 mL) was refluxed for 8 h. The reaction mixture was cooled and the solvent was evaporated in vacuo. Then, water was added to the residue and the solution was neutralized using 10% HCl to give a yellow solid (78%) of compound **12**, m.p. 164-165°C. IRυ_{max}/cm^{-1} 3027 (Ar C-H), 2941 (Aliphatic C-H), 2576 (S-H), 1267, 1143 (C-O, C-N). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.22 (s, 1H, SH), 8.14 (d, *J* = 8.3 Hz, 1H), 8.05 (d, *J* = 7.7 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 7.52 (t, *J* = 7.3 Hz, 1H), 4.11 (s, 2H, SCH_2).^{13}C NMR (101 MHz, DMSO-d_6): δ 178.27, 169.78, 152.21, 140.98, 131.93, 130.59, 127.01, 126.08, 125.34, 114.78, 36.24 (SCH_2). MS (EI) m/z 321.00 (M^+). Analysis: calcd. fo: $C_{11}H_7N_5OS_3$: C, 41.11; H, 2.20; N, 21.79%; found: C, 41.24; H, 2.37; N, 21.35%.

5-((benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-ylthio)methyl)-1,3,4-oxadiazol-2-ol (13)

To a solution of acid hydrazide **9** (0.796 mM) and triethylamine (332 µL, 2.38 mM) in 10 mL of THF, 1,1'-carbonyldiimidazole (95 mg, 1.59 mM) was added and the reaction mixture was stirred at 0°C for 2 h. The mixture was subsequently allowed to warm to room temperature and was left at stirring for overnight. The solvent was evaporated in vacuo and the obtained residue was dissolved in 1 N HCl and extracted with ethyl acetate. The organic extract was washed consecutively with $NaHCO_3$ saturated solution and brine. The organic layer was then separated, dried over sodium sulfate and concentrated in vacuo to furnish a white solid (62%) of compound **13**, m.p. 189-190°C. IRυ_{max}/cm^{-1} 3289 (OH), 3011

(Ar C-H), 2853 (Aliphatic C-H), 1261, 1157 (C-O, C-N). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.20 (s, 1H, OH), 8.20 (d, *J* = 8.1 Hz, 1H), 8.11 (d, *J* = 8.1 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.54 (t, *J* = 7.7 Hz, 1H), 4.33 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 157.46, 154.86, 153.60, 141.14, 131.92, 129.94, 127.59, 127.10, 126.13, 114.60, 29.94 (SCH_2). MS (EI) m/z 305.00 (M^+). Analysis: calcd. for $C_{11}H_7N_5O_2S_2$: C, 43.27; H, 2.31; N, 22.94%; found: C, 43.54; H, 2.43; N, 23.01%.

5-((benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-ylthio)methyl)-1,3,4-thiadiazol-2-amine (14)

A mixture of KSCN (1.5 g, 15 mM) and conc. HCl (2 mL) was added to a solution of acid hydrazide **9** (2.79 g, 10 mM) in CH_3OH (40 mL) with stirring at room temperature for 30 min then it was refluxed for 1 h. The resulting solid was treated with water and ethanol to afford thiosemicarbazide derivative **23** as off-white solid (75%). A mixture of **23** (0.3 mM) and conc. H_2SO_4 (10 mL) was stirred for 4 h at room temperature. The reaction mixture was poured onto cold water and the precipitate was filtered, washed with water, and dried providing a white solid (61%) of compound **14**, m.p. 132-133°C. IRυ$_{max}$/cm^{-1} 3297 (NHs), 3034 (Ar C-H), 2872 (Aliphatic C-H), 1258 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.13 (d, *J* = 8.0 Hz, 1H), 8.09 (d, *J* = 7.9 Hz, 1H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.53 (t, *J* = 7.4 Hz, 1H), 6.51 (s, 2H, NH_2) 4.12 (s, 2H, SCH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 169.80, 168.11, 156.56, 143.00, 131.90, 129.85, 127.56, 126.92, 126.04, 114.81, 36.34 (SCH_2). MS (EI) m/z 321.00 (M^++1). Analysis: calcd. for $C_{11}H_8N_6S_3$: C, 41.23; H, 2.52; N, 26.23%; found: C, 41.09; H, 2.67; N, 26.08%.

5-((benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-ylthio)methyl)-4H-1,2,4-triazole-3-thiol (15)

A mixture of the previously prepared thiosemicarbazide **23** (0.34 g, 1 mM) in KOH solution (10 mL, 7%) was refluxed for 3 h with continuous stirring. The reaction mixture was then allowed to cool and pH was adjusted to 6 using a solution of 10% HCl. The resulting precipitate was filtered off, washed with water and dried affording a yellow solid (53%) of compound **15**, m.p. 244-245°C. IRυ$_{max}$/cm^{-1} 3062 (Ar C-H), 2868 (Aliphatic C-H), 2573 (S-H), 1303 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.27 (s, 1H, SH), 8.13 (d, *J* = 8.1 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.63 (t, *J* = 7.7 Hz, 1H), 7.53 (t, *J* = 7.6 Hz, 1H), 4.12 (s, 2H, SCH_2), 3.66 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6): δ 169.77, 156.58, 143.00, 131.90, 129.86, 127.57, 126.93, 126.06, 125.40, 114.82, 36.36 (SCH_2). MS (EI) m/z 321.00 (M^++1). Analysis: calcd. for $C_{11}H_8N_6S_3$: C, 41.23; H, 2.52; N, 26.23%; found: C, 41.62; H, 2.43; N, 26.51%.

2-(benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-ylthio)-1-(3,5-dimethyl-1H-pyrazol-1-yl)ethanone (16)

Acetyl acetone (0.18 g, 1.8 mM) was added to a solution of acid hydrazide **9** (0.5 g, 1.8 mM) in gl. acetic acid (15 mL) and the reaction mixture was heated under reflux for 12 h. Then, the mixture was allowed to cool to room temperature. The precipitated solid was filtered off, washed with water, dried to give a yellow solid (63%) of compound **17**, m.p. 264-265°C. IRυ$_{max}$/cm^{-1} 3078 (Ar C-H), 2865 (Aliphatic C-H), 1657 (C=O), 1321 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.12 (d, *J* = 8.1 Hz, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H), 6.75 (s, 1H), 4.11 (s, 2H, SCH_2), 3.18 (s, 3H), 2.51 (s, 3H).^{13}C NMR (101 MHz, DMSO-d_6): δ 169.78 (C=O), 156.56, 142.99, 131.89, 129.84, 127.54, 126.90, 126.01, 114.79, 110.35, 36.34 (SCH_2), 21.39, 19.22. MS (EI) m/z 343.00 (M^+). Analysis: calcd. for $C_{15}H_{13}N_5OS_2$: C, 52.46; H, 3.82; N, 20.39%; found: C, 52.31; H, 3.57; N, 19.98%.

1-(2-(benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-ylthio)acetyl)-3-methyl-1H-pyrazol-5(4H)-one (17)

Ethyl acetoacetate (0.26 g, 2 mM) was added to a mixture of acid hydrazide **9** (0.5 g, 1.8 mM) and catalytic amount of piperidine (1 mL) in ethanol (15 mL). The reaction mixture was heated under reflux overnight. The volatiles were removed in vacuo and the solid formed was filtered off, washed with water, dried and recrystallized from ethanol to provide a white solid (64%) of compound **18**, m.p. 175-176°C. IRυ$_{max}$/cm^{-1} 3069 (Ar C-H), 2887 (Aliphatic C-H), 1713, 1658 (C=O), 1295 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.15 (d, *J* = 8.1 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 1H), 4.34 (s, 2H), 1.47 (s, 3H), 1.41 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 165.18 (C=O), 156.57 (C=O), 143.20, 131.89, 130.01, 127.56, 126.88, 126.00, 116.24, 114.84, 110.72, 42.91, 38.39 (SCH_2), 24.24 (CH_3). MS (EI) m/z 345.00 (M^+). Analysis: calcd. for $C_{14}H_{11}N_5O_2S_2$: C, 48.68; H, 3.21; N, 20.28%; found: C, 48.84; H, 3.43; N, 19.97%.

5-amino-1-(2-(benzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-ylthio)acetyl)-1H-pyrazole-4-carbonitrile (18)

A mixture of ethoxymethylenemalononitrile (0.24 g, 2 mM), acid hydrazide **9** (0.5 g, 1.8 mM)

and K_2CO_3 (0.6 g, 4 mM) in ethanol (20 mL) was heated under reflux for 12 h. The reaction mixture was allowed cool to room temperature, the formed precipitate was filtered off, washed with water and dried to afford a yellowish brown solid (70%) of compound **19**, m.p. 139-140°C. IRυ_{max}/cm^{-1} 3283 (NHs), 3071 (Ar C-H), 2926 (Aliphatic C-H), 2214 (CN), 1661 (C=O), 1284 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.77 (s, 1H, N=CH), 8.14 (d, *J* = 8.1 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.22 (s, 2H, NH_2), 4.11 (s, 2H).^{13}C NMR (101 MHz, DMSO-d_6): δ 169.76 (C=O), 156.58, 155.05, 143.00, 131.91, 129.87, 127.57, 126.93, 126.05, 125.33, 119.59, 116.05, 114.82 (CN), 36.41 (SCH_2). MS (EI) m/z 355.00 (M^+). Analysis: calcd. for $C_{14}H_9N_7OS_2$: C, 47.31; H, 2.55; N, 27.59%; found: C, 46.99; H, 2.74; N, 27.35%.

Pharmacological screening

Cell culture

The human cancer cell lines MCF-7, A549 and Hep3B were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in Dulbecco's modified Eagle's medium/F12 medium (DMEM/F-12), DMEM or RPMI-1640 media (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco) according to ATCC instructions. All the cell lines were maninated and treated at 37°C in a humidified incubator containing 5% CO_2.

Cell viability assay

All the synthesized S-Benzo[4,5]thiazolo[2,3-c][1,2,4]triazole derivatives were evaluated *in vitro* for their ability to inhibit cell viability against three different cancer cell lines, MCF-7, A549 and Hep3B, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as mentioned before (21, 22). Briefly, cells were seeded in 96-well plates for 24h then treated with test compounds in 5% FBS-supplemented media for 48 h. After treatment, cells were incubated in media containing 0.5 mg/mL MTT at 37°C for 2 h. Reduced MTT was solubilized in DMSO (200 µL/well) for determination of absorbance at 570 nm using a microplate reader, Table 1. For the NCI screening, Sulforhodamine B (SRB) method was used as mentioned before (23, 24), Table 2.

Kinase profiling assay protocol

Kinase profiling test was done at Reaction Biology Corp., PA, USA, following the previous report (25). The required cofactors were added individually to each kinase reaction. Testing compounds were dissolved in 100% DMSO to specific concentration. The substrate solutions were prepared in freshly prepared reaction buffer. The cofactors were added to the substrate solution. The kinase was added to the resulting solution. Subsequently, the solution was gently mixed, then the test compounds dissolved in DMSO were added into the kinase reaction mixture by Acoustic technology (Echo550; nanoliter range), incubate for 20 min at room temp. ^{33}P-ATP (specific activity 10 µCi/µL) was added into the reaction mixture to initiate the reaction. After incubation for 2 h at room temperature the radioactivity was detected by filter-binding method. Kinase activity data were expressed as the percent remaining kinase activity in test samples compared to vehicle (DMSO) reactions, Table 3.

Caspase activation assay

Caspase-Glo 3/7 luminescence assay kit (Promega, Madison, WI) was used to determine caspase 3/7 and caspase-8 activities in MCF-7 cells treated with the tested compounds. The assay kit was used according to the manufacturer's instructions and as mentioned before (26). Cells were plated at 1 x 10^4 (100 mL/well) into clear bottom, opaque wall 96-well tissue culture plates. Cells were incubated for 24 h. cells were treated with compounds **15**, and **16** for 24 h after removal of the medium. The activity of caspase 3/7 were assessed according to manufacturer's instructions. Luminometer was used to determine the luminescence of the plates and results were represented in Figure 4.

Statistical analysis

Results were analyzed for statistical significance using one-way analysis of variance followed by the Neuman-Keuls test for multiple comparisons using SPSS for Windows (SPSS, Chicago, IL, USA). Differences were considered significant at $p < 0.05$.

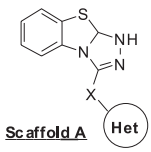
RESULTS AND DISCUSSION

Chemistry

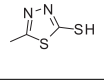
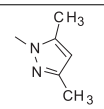
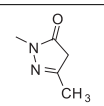
The synthetic pathways utilized to prepare different derivatives were illustrated in Schemes 1 and 2. The starting material benzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole-3-thiol **7** was prepared by the reaction of 2-hydrazinobenzothiazole **6** with carbon disulfide in alcoholic potassium hydroxide according to the reported methods (18, 27). The *S*-alkyla-

tion of **7** was carried out using ethyl bromoacetate in the presence of anhydrous potassium carbonate to afford ethyl *S*-triazolo[3,4-*b*]benzothiazol-3-ylthioacetate **8** in good yield which in turn was substituted with hydrazine monohydrate in absolute ethanol to give the acid hydrazide intermediate **9**. The acid hydrazide **9** was cyclized upon treatment with carbon disulfide flowed by hydrazine hydrate to yield compound **10**, conc. sulfuric acid to give compound **11** or by refluxing with ethanol to afford compound **12**. Furthermore, the reaction of compound **9** with 1,1'-carbonyldiimidazole (CDI) and KSCN furnishing the final targeted compounds **13-15**, Scheme 1. The characterization of compounds **10-12** was performed using spectral and elemental analyses. The IR spectrum of compound **10** revealed a stretching band at 3275 cm^{-1} indicated the amino group in compound **10**. Meanwhile, the ^{1}H-NMR of

Table 1. The anticancer screening results (in terms of IC_{50} values) of the newly synthesized S-benzothiazolotriazole derivatives (**10-18**) against three cancer cell lines; MCF-7, Hep3B and A549.



Scaffold A

Comp. No	Scaffold	X	Het	IC_{50} (μM)		
				MCF-7	Hep3B	A549
10	A	-S-CH_2-		29.6 ± 1.01	25.9 ± 2.20	35.5 ± 2.04
11	A	-S-CH_2-		20.1 ± 1.51	23.3 ± 1.32	36.2 ± 3.06
12	A	-S-CH_2-		23.5 ± 1.03	26.8 ± 1.73	34.2 ± 2.02
13	A	-S-CH_2-		35.9 ± 2.71	24.4 ± 1.42	40.2 ± 3.34
14	A	-S-CH_2-		> 60	> 60	> 60
15	A	-S-CH_2-		12.2 ± 1.11	14.3 ± 1.36	18.9 ± 1.57
16	B	-S-CH_2CO-		11.1 ± 1.21	13.2 ± 1.13	21.5 ± 1.42
17	B	-S-CH_2CO-		27.3 ± 2.42	25.2 ± 1.15	42.8 ± 2.65
18	B	-S-CH_2CO-		> 60	> 60	> 60
Doxorubicin	-	-	-	2.3 ± 0.15	2.4 ± 0.17	1.7 ± 0.31

Cells were treated with the new compound or vehicle for 48 h. and the results were presented as mean ± S.D. (n = 6)

Table 2. NCI anticancer screening data at single dose assay (10^{-5} M conc.) as percentage cell growth against sixty human cancer cell lines.

Subpanel tumor cell lines	Percentage cell growth			Subpanel tumor cell lines	Percentage cell growth		
	14	**17**	**18**		**14**	**17**	**18**
Leukemia				MDA-MB-435	110.40	108.36	106.86
CCRF-CEM	105.37	103.27	105.63	SK-MEL-2	106.46	101.61	115.27
HL-60(TB)	100.67	92.55	111.73	SK-MEL-28	116.46	121.16	99.89
K-562	99.68	96.87	111.41	SK-MEL-5	103.89	98.34	102.59
MOLT-4	100.54	91.66	107.97	UACC-257	104.80	98.34	111.71
RPMI-8226	106.64	105.93	105.82	UACC-62	104.05	104.18	100.68
SR	100.06	98.09	101.79	Ovarian Cancer			
Non-Small Cell Lung Cancer				IGROV1	103.12	113.68	104.82
A549/ATCC	108.84	101.91	106.84	OVCAR-3	106.73	102.41	111.94
EKVX	110.13	114.44	98.14	OVCAR-4	119.11	109.86	102.49
HOP-62	89.56	92.55	106.18	OVCAR-5	109.12	122.19	91.43
HOP-92	96.16	108.79	72.56	OVCAR-8	100.44	100.22	110.06
NCI-H226	100.71	105.11	95.98	NCI/ADR-RES	106.69	98.89	104.16
NCI-H23	100.39	102.36	103.62	SK-OV-3	99.42	94.58	108.91
NCI-H322M	103.72	105.86	104.16	Renal Cancer			
NCI-H460	103.54	105.52	101.29	A498	110.35	105.1198	101.48
NCI-H522	105.39	102.27	106.43	ACHN	100.71	97.81	100.50
Colon Cancer				CAKI-1	110.52	119.47	85.76
COLO 205	101.55	100.64	106.29	RXF 393	95.10	96.22	121.52
HCC-2998	116.22	109.23	112.34	SN12C	120.62	117.10	96.17
HCT-116	108.59	114.64	96.45	TK-10	98.12	102.28	111.74
HCT-15	110.08	107.17	109.64	UO-31	109.50	109.81	84.69
HT29	104.66	104.94	110.08	Prostate Cancer			
KM12	103.82	106.08	109.16	PC-3	93.19	95.31	93.84
SW-620	106.97	100.96	96.75	DU-145	111.54	99.05	113.87
CNS Cancer				Breast Cancer			
SF-268	102.75	98.14	102.07	MCF-7	98.08	97.09	101.63
SF-295	107.31	106.48	104.75	MDA-MB-231/ATCC	99.69	114.05	102.02
SF-539	102.18	105.21	93.70	HS 578T	107.84	103.53	95.65
SNB-75	92.66	99.03	82.02	BT-549	117.32	107.82	94.23
U251	106.64	100.04	107.76	T-47D	99.48	93.91	102.90
Melanoma				MDA-MB-468	118.24	113.03	107.99
LOX IMVI	96.98	104.71	95.53	CCRF-CEM	NT	NT	NT
MALME-3M	97.45	107.18	104.31	HL-60(TB)	NT	NT	NT
M14	104.38	103.75	102.79	K-562	NT	NT	NT

[a]Underlined values represent those < 90.00%. NT: Not Tested

compounds **10-12** showed a singlet signal at the range of 13.48-14.22 ppm assigned to the 3-thiol proton. A singlet peak at 5.62 ppm indicated the amino group in compound **10**. The mass spectra revealed the molecular ion peak at 335, 337 and 321. Moreover, the IR spectrum of compound **13** and **14** revealed a stretching band at 3289 and 3297 cm^{-1} indicated the hydroxy and amino groups, respectively. The ^{1}H-NMR of compounds **13-15** showed a singlet peak at 12.20 ppm assigned to the hydroxyl group, 6.51 ppm indicated the amino group and at 3.17 ppm for the thiol group.

On the other hand, the intermediate **9** was cyclized in a different way keeping the carbonyl group in the linker using a different set of cyclization reagents such as acetyl acetone, ethyl acetoacetate and ethoxymethylenemalononitrile providing the final compounds **16-18** as shown in Scheme 2. The IR spectrum of compounds **16-18** revealed absorption band at the range of 1657-1713 cm^{-1} assigned to the carbonyl groups. Another absorption band was appeared at 2214 cm^{-1} indicated the cyano group in compound **18**. Moreover, the ^{1}H-NMR revealed the methyl group in compounds **16** and **17** at the range of 1.47-3.18 ppm.

Pharmacological screening

Anticancer activity

To evaluate the anti-proliferative potential of the newly synthesized *S*-benzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole derivatives (**10-18**), all target compounds were subjected to *in vitro* anticancer screening against three cancerous cell lines; A549 (human lung adenocarcinoma), MCF-7 (human breast carcinoma) and Hep3B (human hepatocellular carcinoma) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay in six replicates according to the previous reported methodology (21, 22). The results were presented as IC_{50} values (the concentration caused an inhibition of 50%) where doxorubicin was used as positive control, Table 1. The results revealed that compound **14** and **18** were nearly inactive, with IC_{50} values above 60 µM, where the other derivatives revealed IC_{50} values in the range of 11.0-42.8 µM. In general, scaffold B showed a promising anticancer activity more than (scaffold A). Compounds **10-13** and **15** (scaffold A) displayed anticancer activity with IC_{50} values in the range of 12.2-40.2 µM, while those have scaffold B (**16** and **17**) showed IC_{50} in the range of 11.1-42.8 µM. Compound **16** with the 4,5-dimethyl-pyrazole heterocycle was the most potent among all the new compounds.

Structure activity relationship (SAR)

It was clear from the MTT results that compounds **11** and **12** bearing the 1,3,4-thiadiazolyl and 1,3,4-oxadiazolyl ring, respectively displayed different activity profile against A549 (human lung adenocarcinoma), MCF-7 (human breast carcinoma) and Hep3B (human hepatocellular carcinoma). Compound **14** with the 5-amino-1,3,4-thiadiazolyl ring lacks antiproliferative activity against the three cell lines at concentration up to 60 µM. Replacement of the amino group in the 1,3,4-thiadiazolyl ring with mercapto group in compound **11** resulted in enhanced anticancer activity with IC_{50} values in the range of 20.1-36.2 µM against the three cell lines. Moreover, replacement of the mercapto-thiadiazole heterocycle in compound **11** with substituted oxadiazole (**12** and **13**) resulted in slight decrease in activity, while upon replacement with mercapto-triazole (**15**) increased anticancer activity. Replacement of the substituted thiadiazole heterocycle in compound **11** dimethyl-pyrazole (**16**) gave the most active derivatives, but these activities were decreased or even eliminated on changing the substitution pattern on the pyrazole ring as in compound **17** and **18** (Fig. 3).

Table 3. Kinase activity% of compounds **15** and **16** against 10 kinases at 10 µM.

Kinases	Enzyme activity%		IC_{50} (M)		
	15	**16**	Staurosporine	GW5074	SB202190
ABL1	95.67	89.03	1.71E-08	-	-
AKT1	100.05	92.06	2.58E-09	-	-
BRAF	87.60	86.82	-	2.19E-08	-
c-Src	90.61	96.32	1.51E-09	-	-
CDK2/Cyclin A1	87.74	83.72	1.31E-09	-	-
EGFR	91.51	91.72	3.04E-08	-	-
FGFR1	98.32	91.31	1.76E-09	-	-
KDR/VEGFR2	93.59	85.27	2.28E-09	-	-
LCK	88.28	88.11	1.51E-09	-	-
P38a/MAPK14	95.44	110.53	-	-	1.86E-08

Compounds were tested in single dose duplicate mode at a concentration of 10 µM. Control compound, Staurosporine, was tested in 10-dose IC_{50} mode with 4-fold serial dilution starting at 20 µM. Alternate Control Compounds were tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 20 µM. Reactions were carried out at 1 µM ATP

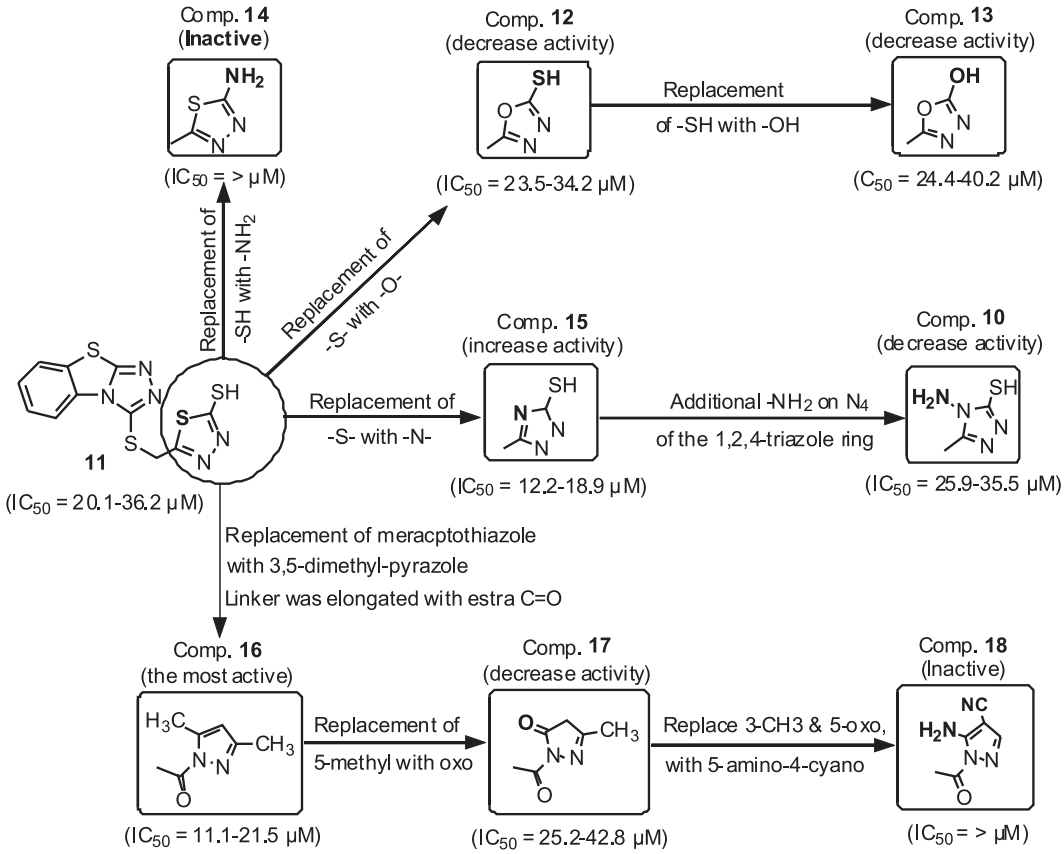


Figure 3. Structure-activity relationship (SAR) of compounds **10-18**

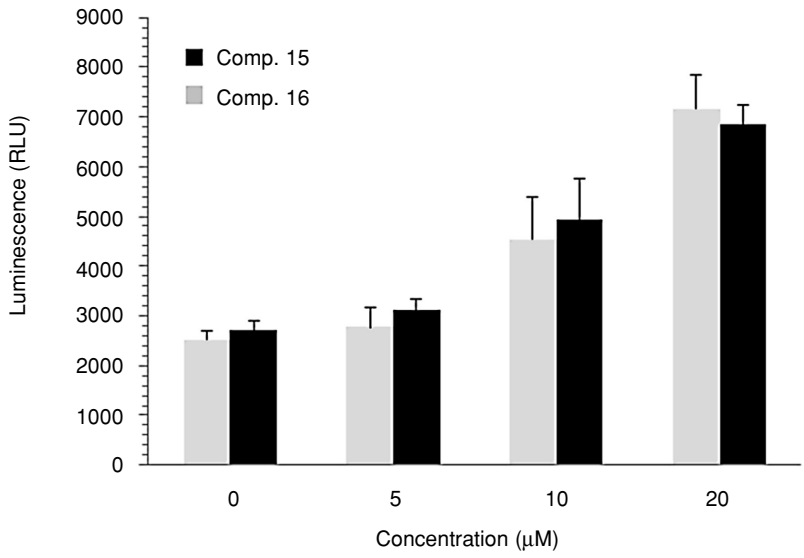


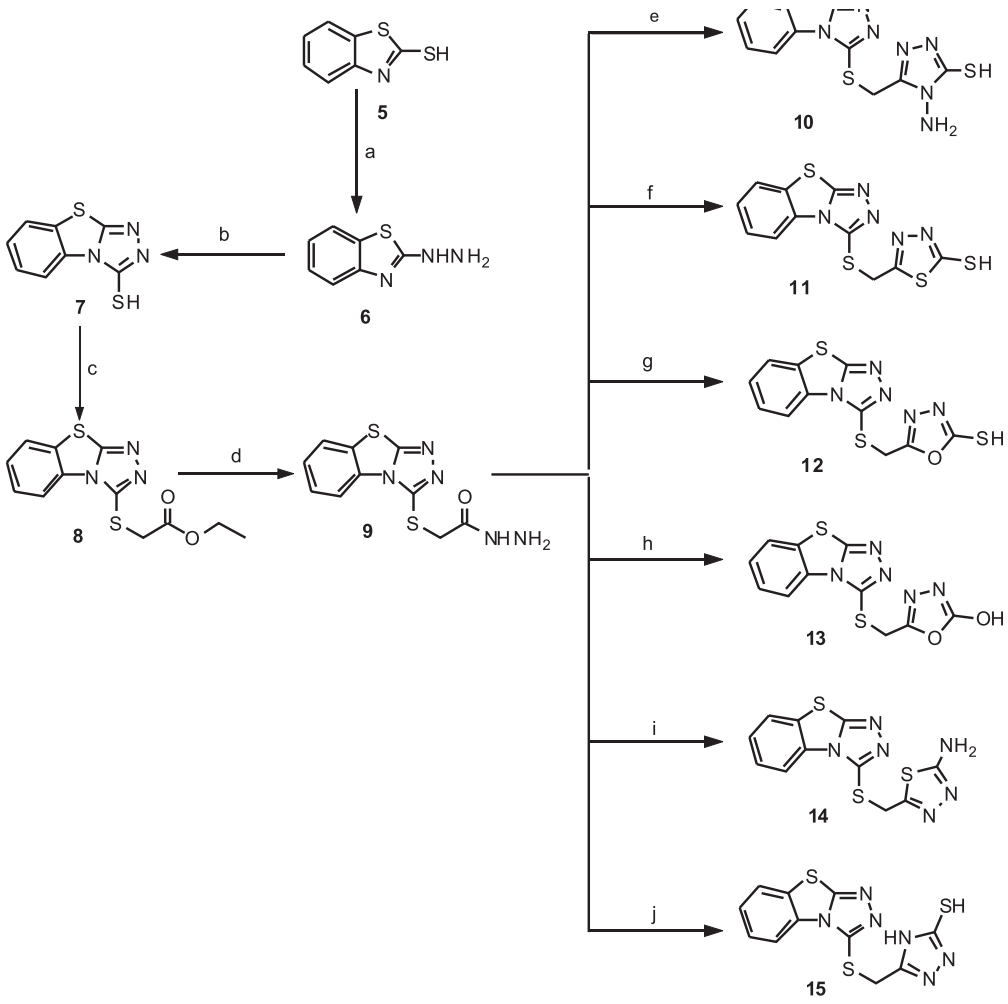
Figure 4. The effect of test compounds on caspases 3/7 activity in A549 cells. Caspase-3/7activities were measured using Caspase-Glo Assay Kit after the indicated treatment for 48 h. All data are depicted as mean ± S.D (n = 4). Significant difference from untreated control condition at $^{*}p < 0.05$, $^{\#}p < 0.01$

NCI anticancer screening

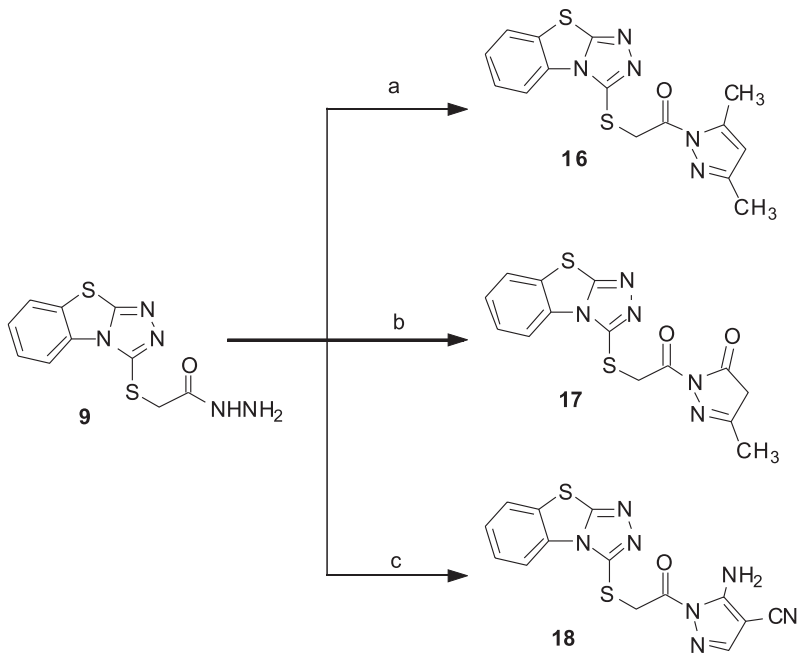
Three compounds (**14**, **17** and **18**) were further selected by National Cancer Institute (NCI), Bethesda, Maryland, USA to be tested using NCI-60 cell line antiproliferative screening program at a single dose (10^{-5} M). This screening assay is carried out against sixty different human cancer cell lines, representing melanoma, leukemia, lung, brain, colon, prostate, breast, kidney and ovary (23, 24). The results of the selected compounds against 60 cancer cell lines expressed in terms of growth percentage are summarized in Table 2. Based on the overall NCI results, it seems that the three compounds showed almost no activity which was in accordance with what we had in the MTT assay against three cancer cell lines. However, only compound **18** exhibited moderate activity against HOP-92 (non-small cell lung cancer cell line) and weak activity against SNB-75 (CNS cancer cell line) and UO-31 (renal cancer cell line).

Kinase profiling assay

In an attempt to explore the molecular targets that may contribute to the mechanism of action of the new compounds as anticancer agents, the most active compounds **15** and **16** were selected to be examined in a kinase profiling assay against 10 various kinases. The kinase profiling was performed at



Scheme 1. Reagents and reaction conditions: a) $NH_2NH_2.H_2O$, reflux, EtOH, 20 h; b) i. CS_2, KOH, EtOH, reflux; ii. Conc. HCl; c) Ethyl bromoacetate, K_2CO_3, reflux, acetone; d) $N_2H_4.H_2O$, reflux, EtOH, 1 h; e) i. CS_2, KOH, rt, 3 hr; ii. NH_2NH_2, reflux, 6 hr; f) i. CS_2, KOH, rt, 3 hr; ii. H_2SO_4, rt, 12 hr; g) i. CS_2, KOH, rt, 3 h; ii. EtOH, reflux, 8 h; h) CDI, Et_3N, THF, rt, overnight; i) i. KSCN, MeOH, HCl; ii. H_2SO_4, rt, 4 h; j) i. KSCN, MeOH, HCl; ii. KOH, reflux, 3 h



Scheme 2. Reagents and reaction conditions: a) Acetyl acetone, AcOH, reflux, 12 h; b) Ethyl acetoacetate, piperidine, EtOH, reflux, 8 h; c) Ethoxymethylenemalononitrile, K_2CO_3, reflux, EtOH, 6 h

Reaction Biology Corp., PA, USA using the radiolabeled ATP determination method as reported (25). The 10 kinases were selected from different groups, families and types including protein-tyrosine kinase and protein-serine/threonine kinase. The results revealed that the two compounds **15** and **16** exhibited weak inhibition against 10 kinases with inhibition% up to 16.28%, Table 3. The highest inhibition caused by compound **15** was against CDK2/Cyclin A1 (16.28%).

Caspase 3/7 assay

The activity of caspase-3/7 was assessed for the most two potent compounds (**15** and **16**) in MTT assay to investigate the apoptotic inducing mechanism. The activation of caspase-3/7 in A549 cells treated with test agents was measured using Caspase-Glo 3/7 luminescence assay kit (Promega, Madison, WI) according to the manufacturer's instructions and as reported (26), Figure 4. The results indicated that the two compounds showed an induction in caspase-3/7 activity at concentrations of 5 μM, 10 μM and 20 μM with nearly 3- to 4-fold, respectively compared to the control. Interestingly, compound **15** was more active than compound **16** at 5 μM and 10 μM which confirms the consistency of these results with that of the MTT assay. These results indicated that the tested compounds have the ability to induce apoptosis through the activation of effector caspase-3/7 family.

CONCLUSIONS

In summary, a set of *S*-benzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole-based derivatives has been synthesized and evaluated for their anticancer potential against three cancer cell lines (A-549, MCF-7, and Hep3B). Among this series, compounds **15** and **16** showed the most promising anticancer activity with IC_{50} values between 11.1 to 21.5 μM. Kinase profiling assay for compounds **15** and **16** revealed weak inhibitor activity against 10 various kinases, where the highest inhibition was for CDK2/Cyclin A1 (16.28%) by compound **15**. Furthermore, caspase-3/7 assay also indicated that the same compound **15** has the ability to induce apoptosis through the activation of effector caspase-3/7 family. Together, *S*-benzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole is a novel promising anticancer scaffold that could be used as a lead for further structural optimization.

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