Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 2 pp. 307–318, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

ANTICANCER ACTIVITY OF NEWLY SYNTHESIZED TRIAZOLOPYRIMIDINE DERIVATIVES AND THEIR NUCLEOSIDE ANALOGS

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Abstract: New substituted triazolopyrimidne derivatives were synthesized starting from 1,2,3-triazolo-4-carboxamide derivative. The *N*- and *S*-glycoside derivatives of the synthesized triazolopyrimidine ring system as well as their acyclic sugar analogs were also synthesized. The cytotoxicity and *in vitro* anticancer evaluation of the prepared compounds have been assessed against three different human tumor cell lines including human breast MCF-7, lung A549 and colon HCT116 cancer cell lines. The results revealed that the prepared compounds exert their actions in MCF-7 and A549. MCF-7 cells are more sensitive to the tested compounds than the other cell lines. Compounds **2**, **3**, **9** and **10** revealed promising anticancer activities compared to the activity of the commonly used anticancer drug, doxorubicin in both MCF-7 and A549 cell lines.

Keywords: triazolopyrimidine, glycosides, anticancer, MCF-7, A549, HCT116

Although there have been great advances in the detection and treatment of cancer, it remains one of the greatest medical challenges, with the incidence of some malignancies continuing to increase (1). For many tumor types, established treatments such as cytotoxic chemotherapy and radiotherapy provide only transient therapeutic benefits despite severe side effects (2). Therefore, the need for better treatments has stimulated research to develop new efficient chemotherapeutic agents for management of cancer.

The search for new heterocyclic compounds and novel methods for their synthesis is a major topic in contemporary medicinal chemistry (3-6) representing one of the ways to face cancer diseases. Pyrimidines has gained considerable attention because of their role in biological systems, particularly in nucleic acids, which contain pyrimidines and purines as the main nucleobases. It has been noticed that introduction of an additional ring to the pyrimidines core tends to exert profound influence in conferring novel biological activities in these molecules (7-9). Consequently, the aza analogs of purines, mainly the triazolo[x,y-z]pyrimidines, also are important (8). The study of compounds incorporating the triazolopyrimidine has been developed due to their varied effects in diverse domains. Triazolopyrimidines (TPs), a subtype of purine analogs, have been the subject of chemical and biological studies due to their interesting pharmacology including antihypertensive, cardiac stimulant, antimalarial, antifungal, anti-HBV, antimicrobial, anticancer, antipyretic, analgesic, antiinflammatory, potential herbicidal, and leishmanicidal activities (10–25). In addition, triazolopyrimidines are versatile ligands and their derived coordination compounds can be considered as model systems for metal–ligand interactions observed in biological systems (17, 26). The simple molecule of Trapidil, the most widely known triazolopyrimidine derivative acts as a platelet-derived growth factor antagonist and as a phosphodiesterase inhibitor (11).

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Cevipabulin and its analogs represent a class of triazolo[1,5-a]pyrimidines and were proved to be potent anticancer agents with an unique mechanism of action in promoting tubulin polymerization (17).

On the other hand, the acyclic nucleoside analogs with modification of both the glycon part and the heterocyclic core have stimulated extensive research as biological inhibitors (27-29). Nucleosides and their analogs have been known to possess antibiotic, antiviral, and antitumor activity (22–33). We have been interested in our previous work in developing new bioactive modified heterocyclic sugar derivatives by attachment of sugar moieties to newly synthesized heterocycles (34-39) in an ongoing search for new compounds with potential biological activity.

In the same direction and in continuing effort to find more potent and selective anticancer compounds, herein, we synthesized triazolopyrimidine compounds, their glycosides and acyclic sugar derivatives. Their biological activities against three different human breast MCF-7, lung A549 and colon HCT116 cancer cell lines were evaluated.

EXPERIMENTAL

Chemistry

All melting points were measured on Electro thermal IA 9000 series digital melting point apparatus. The IR spectra were recorded in potassium bromide discs on a PyeUnicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded at 270 MHz on a Varian Mercury VX-300 NMR spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) were run in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses were carried out at the Micro analytical Centre of Cairo University, Giza, Egypt. All reactions were followed by TLC using silica gel, aluminum sheets 60 F_{254}, (Merck). The anticancer screening was run in the United States National Institute of Health (NIH)/National Cancer Institute (NCI). Compounds **1** and **2** were prepared according to a previously reported method (40-43).

3-(4-Methoxybenzyl)-5-thioxo-5,6-dihydro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(4H)-one (3)

To a solution of the carboxamide derivative **2** (10 mmol) in dimethylformamide (30 mL), sodium hydroxide (12 mL, 10%) was added dropwise at 0°C, the reaction mixture was stirred at 0°C for one hour, then carbon disulfide (14 mmol) was added and the temperature of the reaction was raised up to room temperature, then refluxed in water bath for 8 h. The solvent was distilled off under vacuum and the residue was dissolved in ice-water and then acidified with diluted hydrochloric acid to afford compound **3**.

3-(4-Methoxybenzyl)-3H-[1,2,3]triazolo[4,5-d] pyrimidin-7(6H)-one (4)

A mixture of the triazole derivative **2** (10 mmol) and triethyl orthoformate (20 mmol) in ethanol (25 mL) was heated at 100°C for 8 h. The excess of triethyl orthoformate was removed under vacuum and the residue was dissolved in ethanol, then allowed to stand at room temperature overnight to afford compound **4**.

5-(4-Chlorophenyl)-3-(4-methoxybenzyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (5)

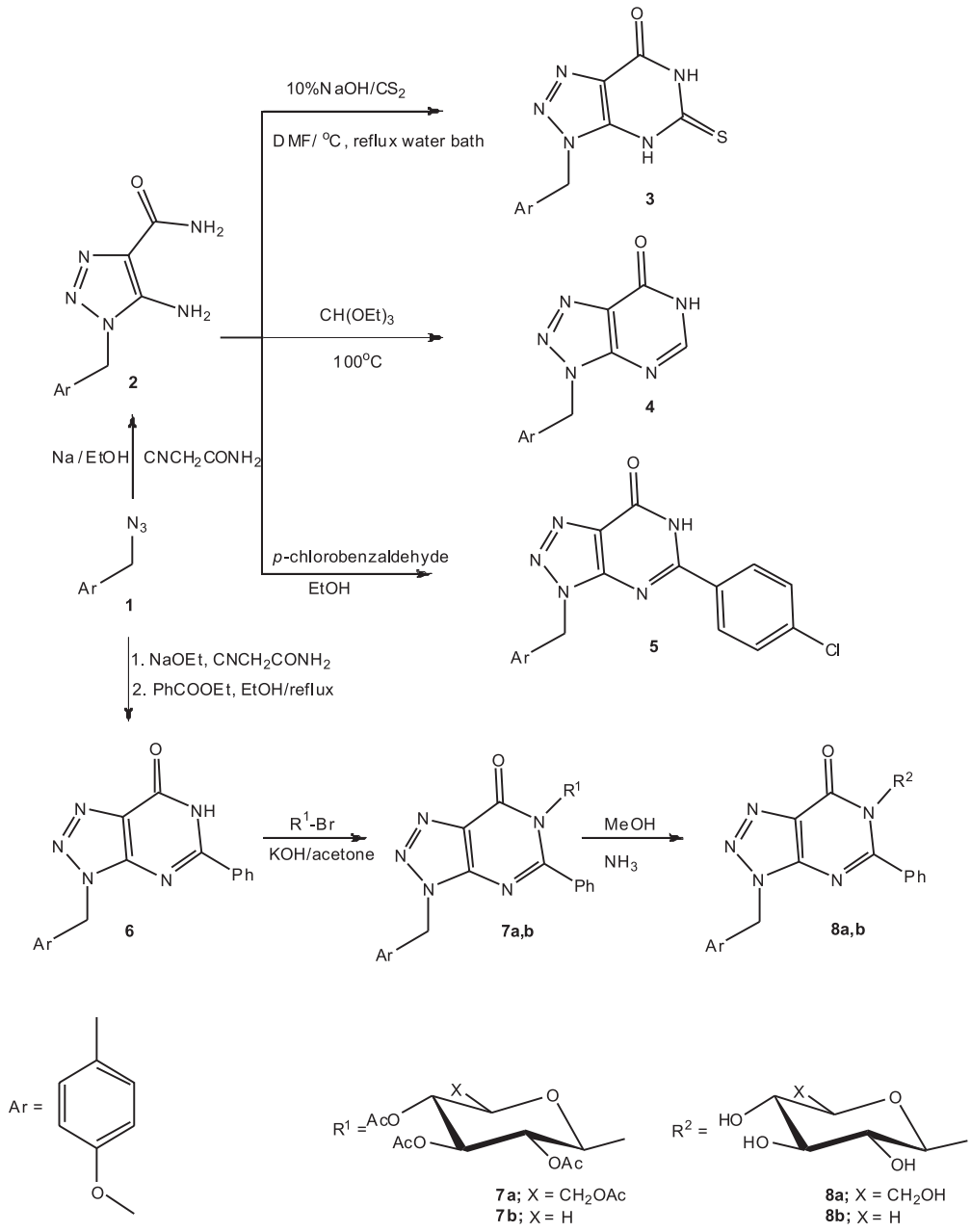
A solution of the carboxamide derivative **2** (10 mmol) and *p*-chlorobenzaldehyde (10 mmol) in ethanol (30 mL) was heated under reflux for 8 h. The solvent was distilled off *in vacuo* and the residue was recrystallized from ethanol-water mixture (1 : 1) to afford compound **5**.

3-(4-Methoxybenzyl)-5-phenyl-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (6)

To a stirred solution of EtONa (2.76 g, 0.12 g atom of Na) in 30 mL of absolute EtOH, cyanacetamide (2.51 g, 3 mmol) was added. The mixture was refluxed for 0.5 h, then a solution of the azide **1** (3 mmol) and ethyl benzoate (3 mmol) in absolute EtOH (10 mL) was added drop by drop and the mixture was refluxed for 6 h, then cooled and concentrated under reduced pressure. To the residue, water (20 mL) was added and the solution was acidified with 4 M acetic acid to pH = 5. The precipitated solid was filtered and crystallized from ethanol.

6-(Glucosyl)-3-(4-methoxybenzyl)-5-phenyl-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (7a,b)

To a solution of compound **6** (5 mmol) in aqueous potassium hydroxide [(10 mmol in distilled water (16 mL)] was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galacto, glucopyranosyl bromide, or 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (5 mmol) in acetone (20 mL). The reaction mixture was stirred at room temperature for 10-12 h (TLC). The solvent was evaporated under reduced pressure at 40°C and the residue was washed with distilled water to remove potassium bromide formed. The product was dried, and crystallized from ethanol to give compounds **7a**,**b**, respectively.



Scheme 1. Synthesis of triazolopyrimidine derivatives.

3-(4-Methoxybenzyl)-6-(D-glucopyranosyl)-5-phenyl-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (8a,b)

Dry gaseous ammonia was passed through a solution of a protected nucleoside **7a**,**b** (0.3 mmol) in dry methanol (12 mL) at 0°C for 1 h, and then the mixture was stirred at 0°C for ca. 5 h. The solvent was evaporated under reduced pressure at 40°C to give a solid residue, which was crystallized from ethanol to give compounds **8a** and **8b**, respectively.

5-(2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosylthio)-3-(4-methoxybenzyl)-3*H*-[1,2,3]triazolo[4,5-d]pyrimidin-7(6*H*)-one (9)

To a solution of compound **3** (5 mmol) in aqueous potassium hydroxide [(10 mmol in distilled water (16 mL)] was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-glucopyranosyl bromide (5 mmol) in acetone (20 mL). The reaction mixture was stirred at room temperature for 8 h (TLC). The

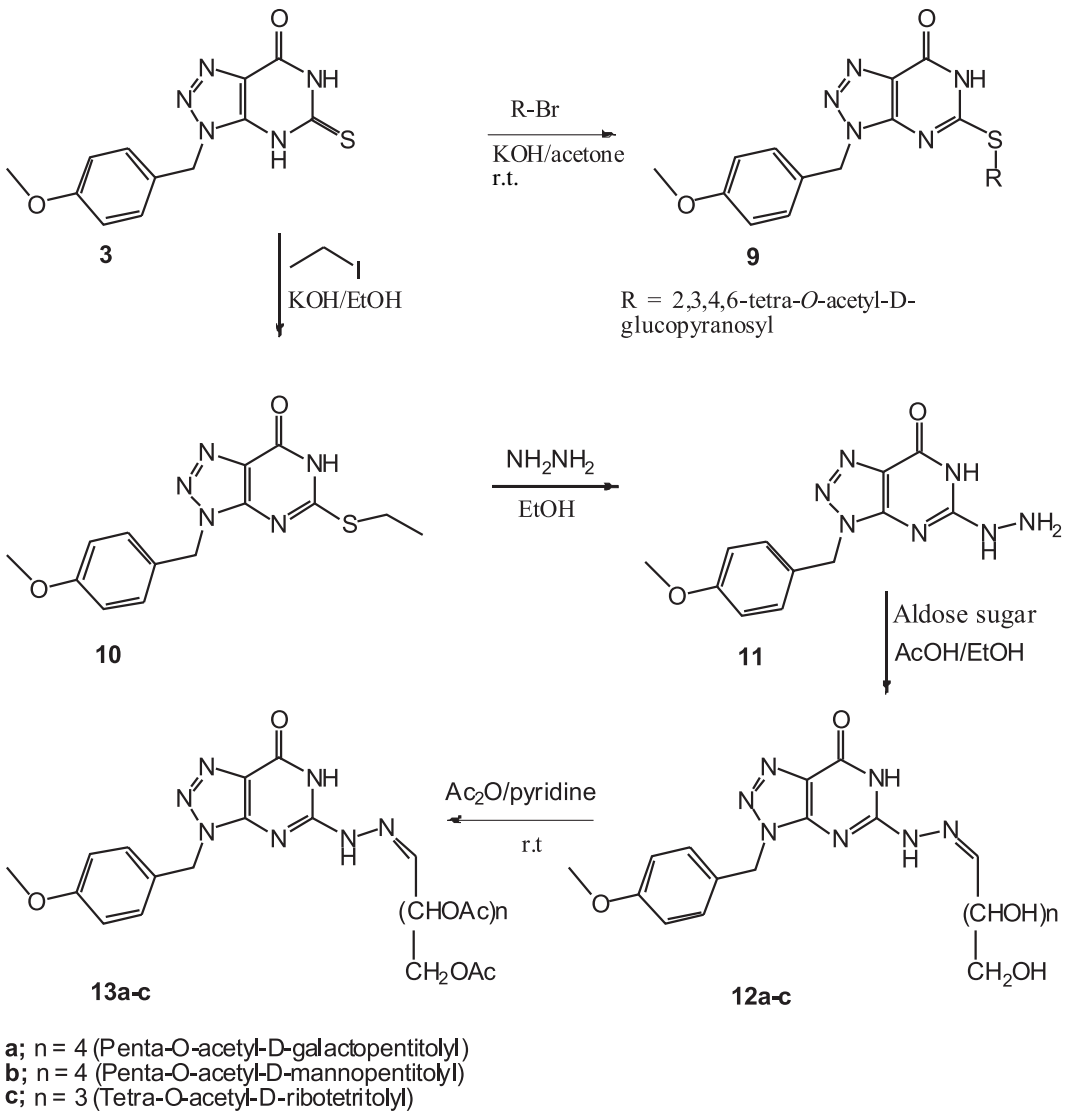
solvent was evaporated under reduced pressure at 40°C and the residue was washed with distilled water to remove potassium bromide formed. The product was dried, and crystallized from ethanol to give compound **9**.

5-(Ethylthio)-3-(4-methoxybenzyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (10)

To a solution of the pyrimidine thione **3** (10 mmol) in 50 mL ethanol, potassium hydroxide (10 mmol) in water (5 mL) was added and a greenish precipitate was formed. Ethyl iodide (10 mmol) was added and a white precipitate was formed. The reaction mixture was stirred at room temperature for 4 h and refluxed for another 5 h. The resulting precipitate was filtered off and crystallized from ethanol.

5-Hydrazinyl-3-(4-methoxybenzyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (11)

A solution of compound **10** (10 mmol) and hydrazine hydrate (15 mmol) in ethanol was heated under reflux for 6 h. The solution was cooled and the resulting precipitate was filtered and crystallized from ethanol.



3 9 10 11 13a-c 12a-c

a; n = 4 (Penta-O-acetyl-D-galactopentitolyl)
b; n = 4 (Penta-O-acetyl-D-mannopentitolyl)
c; n = 3 (Tetra-O-acetyl-D-ribotetritolyl)

Scheme 2. Synthesis of triazolopyrimidine sugar derivatives

Table 1. *In vitro* cytotoxicity activity of the synthesized compounds in three human cancer cell lines as measured with SRB assay.

Compound	IC_{50} (μg/mL)		
	MCF-7	A549	HCT116
Doxorubicin	2.90 ± 0.27	4.30 ± 0.40	4.80 ± 0.50
DMSO	N.A.	N.A.	N.A.
2	4.80 ± 0.55	8.20 ± 0.78	75.40 ± 8.80
3	2.80 ± 0.33	4.20 ± 0.50	75.00 ± 7.30
4	N.A.	N.A.	N.A.
6	14.70 ± 1.75	33.20 ± 5.00	N.A.
7a	N.A.	N.A.	N.A.
9	3.90 ± 0.42	8.00 ± 0.83	80.70 ± 9.30
10	3.60 ± 0.39	6.50 ± 0.67	66.80 ± 7.50
11	22.70 ± 2.50	28.00 ± 3.29	88.50 ± 9.40
12a	N.A.	N.A.	N.A.

Data were expressed as the mean ± standard error (SE) of six independent experiments. N.A. is no activity.

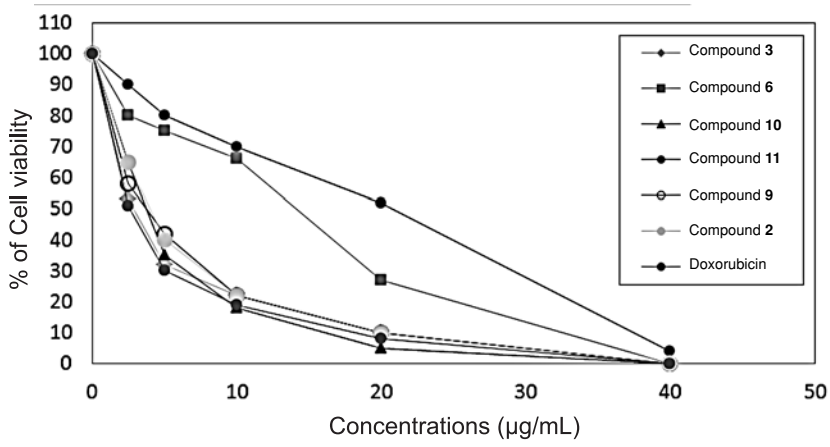


Figure 1. Effect of treatment with various concentrations of prepared compounds on breast MCF-7 cell line cytotoxicity (IC_{50}) as measured with SRB method

(Z)-3-(4-Methoxybenzyl)-5-(hydrazinylsugar)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (12a-c)

General procedure

To a well-stirred solution of the respective monosaccharide (0.01 mol) in water (2 mL), and glacial acetic acid (0.2 mL) was added the hydrazine derivative **11** (10 mmol) in ethanol (15 mL). The mixture was heated under reflux for 4-6 h (TLC) and the resulting solution was concentrated and left to cool. The precipitate formed was filtered off, washed with water, then dried and crystallized from ethanol-DMF (2 : 1, v/v).

(Z)-3-(4-Methoxybenzyl)-5-(per-*O*-acetylhydrazinylsugar)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-one (13a-c)

General procedure

To a solution of the hydrazinyl sugar derivative **12a-c** (5 mmol) in pyridine (10 mL) was added acetic anhydride (6 mmol) and the mixture was stirred at room temperature for 7 h. The resulting solution was poured onto crushed ice, and the product that separated out was filtered off, washed with sodium hydrogen carbonate and water, then dried to afford compounds **13a-c**.

Table 2. Physical and analytical data of the synthesized compounds.

Compd. no.	M.p. (°C)	Yield (%)	Mol. formula	Analysis (%) calc. / found		
				C	H	N
3	36-238	77	$C_{12}H_{11}N_5O_2S$	49.82 49.59	3.83 3.69	24.21 24.14
4	155-156	76	$C_{12}H_{11}N_5O_2$	56.03 55.89	4.31 4.26	27.22 27.17
5	190-192	79	$C_{18}H_{14}ClN_5O_2$	58.78 58.68	3.84 3.80	19.04 19.18
6	261-263	78	$C_{18}H_{15}N_5O_2$	64.86 64.70	4.54 4.42	21.01 20.90
7a	148-150	58	$C_{32}H_{33}N_5O_{11}$	57.92 57.59	5.01 4.88	10.55 10.39
7b	151-153	72	$C_{29}H_{29}N_5O_9$	58.88 58.70	4.94 4.85	11.84 11.75
8a	199-200	66	$C_{24}H_{25}N_5O_7$	58.18 58.05	5.09 4.90	14.13 13.98
8b	200-202	62	$C_{23}H_{23}N_5O_6$	59.35 59.18	4.98 5.05	15.05 14.82
9	> 300	73	$C_{26}H_{29}N_5O_{11}S$	50.40 50.62	4.63 4.63	11.30 11.19
10	223-225	74	$C_{14}H_{15}N_5O_2S$	52.98 52.81	4.76 4.72	22.07 21.98
11	296-298	79	$C_{12}H_{13}N_7O_2$	50.17 50.05	4.56 4.45	34.13 34.02
12a	153-156	79	$C_{18}H_{23}N_7O_7$	48.11 47.92	5.16 5.10	21.82 21.69
12b	158-160	77	$C_{18}H_{23}N_7O_7$	48.11 48.02	5.16 5.12	21.82 21.71
12c	156-159	75	$C_{17}H_{21}N_7O_6$	48.69 48.55	5.05 5.02	23.38 23.25
13a	188-191	79	$C_{28}H_{33}N_7O_{12}$	50.99 50.80	5.04 4.91	14.86 14.71
13b	193-195	80	$C_{28}H_{33}N_7O_{12}$	50.99 50.75	5.04 4.92	14.86 14.69
13c	193-195	80	$C_{25}H_{29}N_7O_{10}$	51.11 50.95	4.98 4.92	16.69 16.51

Biological evaluation

Chemicals

Fetal bovine serum (FBS) and L-glutamine were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, and streptomycin were obtained from Sigma Chemical Company (Saint Louis, MO, USA).

Cell lines and culturing

Anticancer activity screening for the tested compounds utilizing three different human tumor cell lines including human breast MCF-7, lung A549 and colon HCT116 cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/mL) and streptomycin (100 µg/mL) at 37°C in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50×10^6 were grown in a 25 cm^2 flask in 5 mL of complete culture medium.

In vitro cytotoxicity assay

The cytotoxicity activity was measured *in vitro* using the sulforhodamine-B stain (SRB) assay

according to the previously reported standard procedure (44). Cells were inoculated in 96-well microtiter plate (10^4 cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO at 1 mg/mL immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentrations of tested compounds and doxorubicin were added to the cells. Six wells were prepared for each individual dose. Cells were incubated with the compounds for 48 h, at 37°C and in atmosphere of 5% CO_2. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. 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 is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1. The results were compared to the antiproliferative effects of the reference control - doxorubicin (45).

Statistical analysis

The results are reported as the mean ± standard error (SE) for at least six times experiments.

RESULTS AND DISCUSSION

Chemistry

In this investigation, the starting carboxamide key derivative **2** was synthesized according to a pre-

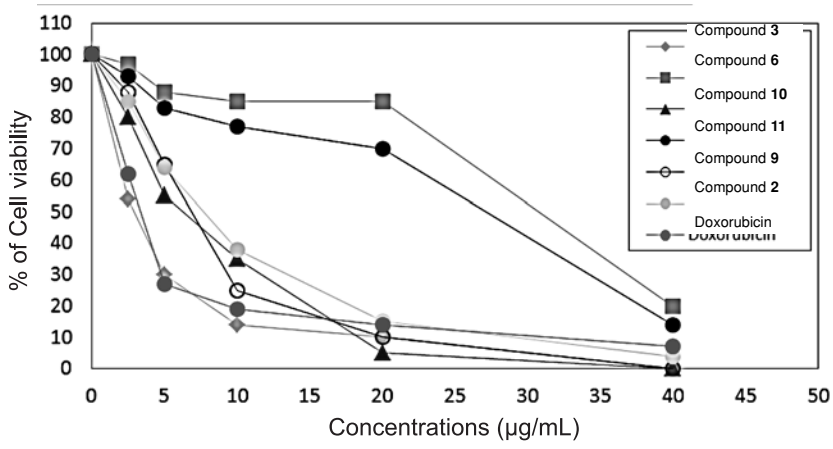


Figure 2. Effect of treatment with various concentrations of prepared compounds on lung A549 cell line cytotoxicity (IC_{50}) as measured with SRB method

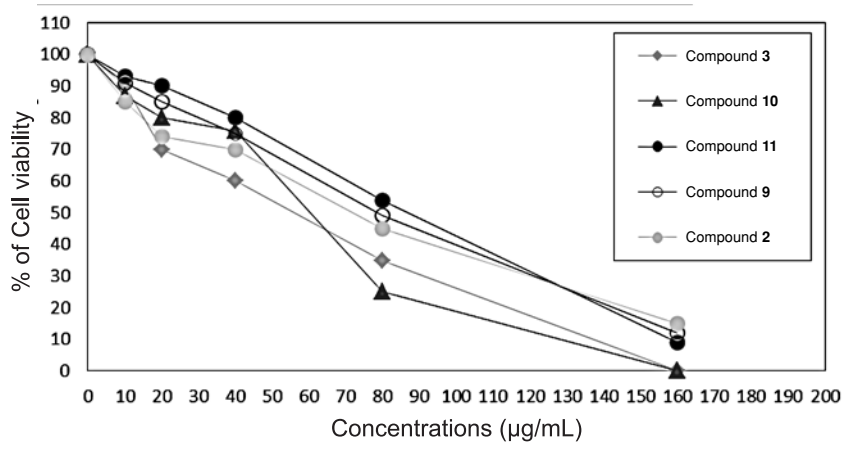


Figure 3. Effect of treatment with various concentrations of prepared compounds on colon HCT116 cell line cytotoxicity (IC_{50}) as measured with SRB method

Table 3. Spectral data for the synthesized compounds.

Comp. no.	IR [KBr; ν cm^{-1}], ^{1}H NMR [δ, ppm], ^{13}C NMR [δ, ppm], MS [m/z]
3	IR: 3285 (NH), 1662 (C=O). ^{1}H NMR (DMSO-d_6): 3.71 (s, 3H, OCH_3), 5.21 (s, 2H, CH_2), 6.90 (m, 2H, Ar-H), 7.26 (d, 2H, *J* = 7.6 Hz, Ar-H), 12.57 (s, 1H, NH), 13.95 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6): 50.0 (CH_2), 56.5 (CH_3), 115.9-154.7 (Ar-C and pyrimidyl C), 159.98 (C=O), 175.9 (C=S). MS: *m*/*z*: 289 [M^+].
4	IR: 3250 (NH), 1658 (C=O). ^{1}H NMR (DMSO-d_6): 3.81 (s, 3H, OCH_3), 5.30 (s, 2H, CH_2), 6.95 (d, 2H, Ar-H), 7.22 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.92 (s, 1H, pyrimidine H-2), 9.28 (s, 1H, NH). MS: *m*/*z*: 257 [M^+].
5	IR: 3290 (NH), 1661 (C=O). ^{1}H NMR (DMSO-d_6): 3.80 (s, 3H, OCH_3), 5.31 (s, 2H, CH_2), 6.95 (m, 2H, Ar-H), 7.27 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.48 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.66 (d, 2H, *J* = 7.6 Hz, Ar-H), 9.28 (s, 1H, NH). MS: *m*/*z*: 368 [M^+].
6	IR: 3277 (NH), 1664 (C=O). ^{1}H NMR (DMSO-d_6): 3.72 (s, 3H, OCH_3), 5.32 (s, 2H, CH_2), 6.93 (m, 2H, Ar-H), 7.39 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.59-7.64 (m, 3H, Ar-H), 7.98-8.19 (m, 2H, Ar-H), 12.83 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 49.9 (CH_2), 57.5 (CH_3), 115.8-151.9 (Ar-C and pyrimidyl C), 158.8 (C=O), 159.9 (pyrimidyl C-2). MS: *m*/*z*: 333 [M^+].
7a	IR: 1735 (C=O), 1668 (C=O). ^{1}H NMR ($CDCl_3$): 1.77, 1.95, 2.02, 2.06 (4s, 12H, 4 CH_3CO), 3.71 (s, 3H, OCH_3), 4.03 (m, 1H, H-5), 4.09 (dd, 1H, $J_{6,6'}$ = 11.4 Hz, $J_{5,6}$ = 2.8 Hz, H-6), 4.16 (m, 1H, H-6'), 4.56 (t, 1H, $J_{3,4}$ = 9.3 Hz, H-4), 5.12 (dd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 5.36 (t, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 5.41 (s, 2H, CH_2), 5.87 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 6.92 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.38 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.63 (m, 3H, Ar-H), 8.19 (m, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): 20.67, 20.75, 20.79, 20.89 (4CH_3CO), 50.2 (CH_2), 56.3 (OCH_3), 62.2 (C-6), 67.7 (C-4), 68.6 (C-3), 70.7 (C-2), 72.5 (C-5), 93.8 (C-1), 114.6-152.7 (Ar-C and pyrimidyl 2C), 159.7 (C=N), 162.8 (C=O), 169.6, 169.9, 170.1, 140.4 (4CH_3C=O).
7b	IR: 1738 (C=O), 1660 (C=O). ^{1}H NMR ($CDCl_3$): 1.80, 1.92, 2.04 (3s, 9H, 3 CH_3CO), 3.71 (s, 3H, OCH_3), 4.09 (dd, 1H, *J* = 11.4 Hz, *J* = 2.8 Hz, H-5), 4.18 (m, 1H, H-5'), 4.78 (t, 1H, $J_{3,4}$ = 9.3 Hz, H-4), 4.95 (dd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 5.11 (t, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 5.35 (s, 2H, CH_2), 5.88 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 6.93 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.39 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.63 (m, 3H, Ar-H), 8.18 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$): 20.6, 20.7, 20.8 (3CH_3CO), 50.2 CH_2), 56.3 (OCH_3),62.2 (C-5), 67.8 (C-4), 69.5 (C-3), 70.9 (C-2), 94.1 (C-1), 114.5-152.8 (Ar-C and pyrimidyl 2C), 159.5 (C=N), 162.8 (C=O), 169.5, 169.9, 140.5 (3CH_3C=O).
8a	IR: 3475-3440 (OH), 1662 (C=O). ^{1}H NMR (DMSO-d_6): 3.72 (s, 3H, OCH_3), 3.88-4.05 (m, 2H, H-6,6'), 4.27 (m, 1H, H-5), 4.77-5.10 (m, 3H, H-4,3 and OH), 5.31 (m, 3H, CH_2 and OH), 5.38 (m, 1H, H-2), 5.60 (m, 1H, OH), 5.64 (s, 1H, OH), 5.86 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 6.93 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.37 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.68 (m, 3H, Ar-H), 8.17 (m, 2H, Ar-H).
8b	IR: 3480-3445 (OH), 1662 (C=O). ^{1}H NMR (DMSO-d_6): 3.74 (s, 3H, OCH_3), 3.85-4.04 (m, 2H, H-5,5'), 4.77-5.03 (m, 2H, H-4,3), 5.15-5.28 (m, 3H, CH_2 and OH), 5.35 (m, 1H, H-2), 5.62 (m, 1H, OH), 5.66 (s, 1H, OH), 5.88 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 6.94 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.38 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.69 (m, 3H, Ar-H), 8.17 (m, 2H, Ar-H).
9	IR: 1695 (C=O), 1734 (C=O). ^{1}H NMR ($CDCl_3$): 1.89, 1.93, 1.98, 2.03 (4s, 12 H, CH_3CO), 3.77 (s, 3H, OCH_3), 3.99 (m, 1H, H-5), 4.05 (dd, 1H, *J* = 2.8, *J* = 11.0, H-6), 4.15 (dd, *J* = 2.8 Hz, *J* = 11.4 Hz, 1H, H-6'), 4.94 (t, $J_{3,4}$ = 9.3 Hz, 1H, H-4), 5.20-5.27 (m, 3H, CH_2 and H-3), 5.33 (t, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 5.77 (d, $J_{1,2}$ = 10.2 Hz, 1H, H-1), 6.94 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.40 (d, 2H, *J* = 8.4 Hz, Ar-H), 12.02 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): 20.05, 20.18, 20.66, 20.82 (4CH_3CO), 50.5 CH_2), 56.8 (OCH_3), 62.1 (C-6), 7.5 (C-4), 68.9 (C-3), 70.7 (C-2), 72.5 (C-5), 90.2 (C-1), 116.6-154.4 (Ar-C and pyrimidyl 2C), 158.9 (C=N), 162.6 (C=O), 169.1, 169.8, 170.2, 170.5 (4CH_3C=O).

Table 3. cont.

Comp. no.	IR [KBr; ν cm^{-1}], ^{1}H NMR [δ, ppm], ^{13}C NMR [δ, ppm], MS [m/z]
10	IR: 3275 (NH), 1664 (C=O). ^{1}H NMR (DMSO-d_6): 1.33 (t, 3H, *J* = 5.2 Hz, CH_3CH$_2$), 3.72 (s, 3H, OCH$_3$), 4.42 (q, 2H, *J* = 5.2 Hz, CH$_3$CH_2), 5.61 (s, 2H, CH$_2$),6.92 (d, 2H,*J* = 7.6 Hz,Ar-H), 7.34 (d, 2H, *J* = 7.6 Hz, Ar-H), 12.87 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): 15.6 (CH$_3$), 25.4 (CH$_2$), 49.9 (CH$_2$), 55.6 (OCH$_3$), 114.6-156.3 (Ar-C and pyrimidyl 2C), 159.6 (C=N), 162.7 (C=O). MS: *m*/*z*: 317 [M$^+$].
11	IR: 3340 and 3305 (NH$_2$), 3255 (NH), 1668 (C=O). ^{1}H NMR (DMSO-d_6): 3.73 (s, 3H, OCH$_3$), 5.32 (s, 2H, CH$_2$),5.68 (bs, 2H, NH$_2$), 6.91 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.43 (d, 2H, *J* = 7.6 Hz, Ar-H), 10.02 (bs, 1H, NH), 12.63 (bs, 1H, NH). MS: *m*/*z*: 287 [M$^+$].
12a	IR: 3495 (OH), 3269 (NH), 1664 (C=O). ^{1}H NMR (DMSO-d_6): 3.31-3.43 (m, 2H, H-6, H-6'), 3.61-3.65 (m, 1H, H-5), 3.69-3.79 (m, 2H, H-3,4), 3.82 (s, 3H, OCH$_3$), 4.31 (t, 1H, *J* = 5.8 Hz, H-2), 4.51 (m, 1H, OH), 4.92 (d, 1H, *J* = 6.3 Hz, OH), 5.21 (m, 1H, OH), 5.40 (s, 2H, CH$_2$), 5.73 (t, 1H, *J* = 4.5 Hz, OH), 5.80 (t, 1H, *J* = 4.5 Hz, OH), 6.98 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.27 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.49 (d, 1H, *J* = 7.5 Hz, H-1), 9.36 (s, 1H, NH), 10.08 (bs, 1H, NH).
12b	IR: 3482 (OH), 3238 (NH), 1669 (C=O). ^{1}H NMR (DMSO-d_6): 3.30-3.43 (m, 2H, H-6, H-6'), 3.59-3.64 (m, 1H, H-5), 3.70-3.78 (m, 2H, H-3,4), 3.84 (s, 3H, OCH$_3$), 4.30 (t, 1H, *J* = 5.8 Hz, H-2), 4.51 (m, 1H, OH), 4.94 (d, 1H, *J* = 6.3 Hz, OH), 5.22 (m, 1H, OH), 5.40 (s, 2H, CH$_2$), 5.72 (t, 1H, *J* = 4.5 Hz, OH), 5.82 (t, 1H, *J* = 4.5 Hz, OH), 6.97 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.27 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.49 (d, 1H, *J* = 7.5 Hz, H-1), 9.41 (s, 1H, NH), 10.12 (bs, 1H, NH).
12c	IR: 3472 (OH), 3218 (NH), 1666 (C=O). ^{1}H NMR (DMSO-d_6): 3.30-3.43 (m, 2H, H-5, H-5'), 3.59-3.64 (m, 1H, H-4), 3.70-3.78 (m, 2H, H-3,2), 3.81 (s, 3H, OCH$_3$), 4.50 (m, 1H, OH), 4.94 (d, 1H, *J* = 6.3 Hz, OH), 5.19 (m, 1H, OH), 5.38 (s, 2H, CH$_2$), 5.74 (t, 1H, *J* = 4.4 Hz, OH), 6.97 (d, 2H, *J* = 7.6 Hz,Ar-H), 7.30 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.51 (d, 1H, *J* = 7.5 Hz, H-1), 9.39 (s, 1H, NH), 10.08 (bs, 1H, NH).
13a	IR: 3248 (NH), 1736 (C=O), 1662 (C=O). ^{1}H NMR (CDCl$_3$): 1.83, 1.98, 2.05, 2.10, 2.14 (5s, 15H, 5CH$_3$), 3.84 (s, 3H, OCH$_3$), 4.10 (dd, 1H, *J* = 11.2 Hz, *J* = 2.4 Hz, H-6), 4.18 (dd, 1H, *J* = 10.6 Hz, *J* = 2.4 Hz, H-6'), 4.58 (m, 1H, H-5), 5.28 (dd, 1H, *J* = 3.2 Hz, *J* = 6.5 Hz, H-4), 5.38 (t, 1H, *J* = 6.5 Hz, H-3), 5.42 (s, 2H, CH$_2$), 5.68 (dd, 1H, *J* = 3.2 Hz, *J* = 6.2 Hz, H-2), 7.02 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.25 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.51 (d, 1H, *J* = 6.8 Hz, H-1), 9.36 (s, 1H, NH), 10.88 (bs, 1H, NH).
13b	IR: 3242 (NH), 1736 (C=O), 1660 (C=O). ^{1}H NMR(CDCl$_3$): 1.84, 1.98, 2.05, 2.11, 2.15 (5s, 15H, 5CH$_3$), 3.82 (s, 3H, OCH$_3$), 4.11 (dd, 1H, *J* = 11.2 Hz, *J* = 2.4 Hz, H-6), 4.18 (dd, 1H, *J* = 10.6 Hz, *J* = 2.4 Hz, H-6'), 4.55 (m, 1H, H-5), 5.20 (dd, 1H, *J* = 3.2 Hz, *J* = 6.5 Hz, H-4), 5.38 (t, 1H, *J* = 6.5 Hz, H-3), 5.43 (s, 2H, CH$_2$), 5.69 (dd, 1H, *J* = 3.2 Hz, *J* = 6.2 Hz, H-2), 7.04 (d, 2H,*J* = 7.6 Hz, Ar-H), 7.25 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.52 (d, 1H, *J* = 6.2 Hz, H-1), 9.39 (s, 1H, NH), 10.74 (bs, 1H, NH).
13c	IR: 3240 (NH), 1735 (C=O), 1664 (C=O). ^{1}H NMR(CDCl$_3$): 1.86, 2.02, 2.11, 2.14 (4s, 12H, 4CH$_3$), 3.83 (s, 3H, OCH$_3$), 4.09 (dd, 1H, *J* = 11.2 Hz, *J* = 2.4 Hz, H-5), 4.16 (dd, 1H, *J* = 10.6 Hz, *J* = 2.4 Hz, H-5'), 4.82 (m, 1H, H-4), 5.38 (dd, 1H, *J* = 3.8 Hz, *J* = 6.4 Hz, *J* = 6.5 Hz, H-3), 5.40 (s, 2H, CH$_2$), 5.69 (dd, 1H, *J* = 3.2 Hz, *J* = 6.4 Hz, H-2), 7.10 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.27 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.50 (d, 1H, *J* = 6.2 Hz, H-1), 10.02 (s, 1H, NH), 11.02 (bs, 1H, NH).

viously reported procedure (40-43). Compound **2** was used for the preparation of various functionalized triazolopyrimidine derivatives. Reaction of the carboxamide **2** with carbon disulfide in the presence of 10% sodium hydroxide afforded the triazolopyrimidine thione derivative **3** in 77% yield. When compound **2** was allowed to react with triethyl orthoformate, the N-1 substituted triazolopyrimidine derivative **4** was obtained. Reaction of the key compound **2** with 4-chlorobenzaldehyde afforded the 2-chlorophenyl derivative **5**. On the other hand, preparation of the 2-phenyl derivative **6** in 78% yield was started with azide **1** by its reaction with cyanoacetamide and ethyl benzoate. The structures of compounds **3**-**6** were confirmed by their IR, NMR and elemental analyses. Their IR spectra revealed the absence of the charac-

teristic NH_2 absorption bands. The ^{1}H NMR spectra showed, in addition to the aryl and methylene protons, two signals for the NH groups for the thione derivative **3** and one NH signal for compounds **4** and **6**. Furthermore, the ^{1}H NMR spectra of compounds **5** and **6** showed the signals corresponding to protons of the added aryl groups (Scheme 1).

Glycosylation of the pyrimidine derivative **6** was carried out by reaction with the acetylated gluco- and xylopyranosyl bromide derivatives in the presence of potassium hydroxide and produced the corresponding glycosides **7a**,**b**. The ^{1}H-NMR of **7a** showed the anomeric proton of the sugar moiety at δ 5.87 ppm as a doublet, with coupling constants equal to 10.2 Hz indicating the β-orientation of the glycosidic bond. The ^{13}C NMR spectra of **7a** showed a signal at δ 93.8 ppm corresponding to the anomeric C-1, which also confirmed the β-configuration.

Treatment of the acetylated glycosides **7a**,**b** with methanolic ammonia afforded the free hydroxyl glycosides **8a**,**b**, respectively, whose spectral data agree with the assigned structures. Their IR spectra showed characteristic absorption bands for the free hydroxyl groups and their ^{1}H NMR spectra showed the hydroxyl protons signals and revealed the absence of the acetyl-methyl signals which agreed with the assigned structures.

The attachment of thioglycosyl moiety to the triazolopyrimidine ring system was carried out by reaction of the triazolopyrimidyl thione derivative **3** with glucosyl bromide in the presence of potassium hydroxide and produced the corresponding thioglycoside **9**. The formation of glycosylthione derivative rather than the *N*-substituted analog was confirmed on the basis of its spectral data. The ^{1}H NMR spectrum of the produced glycoside showed the anomeric proton of the sugar moiety at δ 5.77 ppm as a doublet, with coupling constants equal to 10.2 Hz indicating the β-orientation of the thioglycosidic bond. The anomeric proton of β-*N*-glycosides having an adjacent C=S was reported to appear at higher chemical shift (δ 6.9–7.2 ppm) due to the anisotropic deshielding effect of the C=S (46-48). The ^{13}C NMR spectra of **9** showed a signal at δ 90.2 ppm corresponding to the anomeric C-1, which also confirmed the β-configuration. The absence of a peak corresponding to the C=S group indicates that the attachment of the sugar has taken place at the sulfur atom and not on the nitrogen atom.

Alkylation of the triazolopyrimidine thione **3** with ethyl iodide in alkaline medium afforded the 2-ethylmercapto derivative **10** in 74% yield. Hydrazinolysis of the produced *S*-ethyl compound gave the required 2-hydrazino derivative **11** in good yield. The ^{1}H-NMR spectrum of compound **10** showed the signals of the ethyl group as triplet and quartet which disappeared in the spectra of the corresponding hydrazine derivative **11**, whereas the NH_2 and NH signals appeared at δ 5.68 and 10.02 ppm, respectively, in addition to signals of the aromatic protons.

When the hydrazine derivative **11** was allowed to react with a number of monosaccharides, namely D-galactose, D-mannose and D-ribose in an aqueous ethanolic solution and with catalytic amount of acetic acid, the corresponding hydrazinyl sugar derivatives were obtained in 75-79% yields. The structures of the produced compounds were confirmed by the analytical and spectral data. The IR spectra of **12a-c** showed the presence of characteristic absorption bands corresponding to the hydroxyl groups in the region 3472–3495 cm^{-1}. The ^{1}H NMR spectra showed the signals of the sugar chain protons at δ 3.30–5.82 ppm, the C-1 methine proton as doublet in the range δ 7.49–7.51 ppm in addition to signals of the methylene and aromatic protons. This high chemical shift value of H-1 indicates the acyclic form of the sugar moiety. The H-1 of sugar moieties in the cyclic form is reported to appear at lower chemical shift values (41). Acetylation of compounds **12a-c** with acetic anhydride in pyridine at room temperature lead to the formation of per-*O*-acetylated derivatives **13a-c**, respectively, in 79-80% yield. The IR spectra showed the presence of absorption bands in the carbonyl frequency region in the range 1731-1736 cm^{-1}. Their ^{1}H NMR spectra revealed the absence of the hydroxyl signals and showed the presence of the acetyl methyl protons at δ 1.83-2.15 ppm in addition to the signals of the sugar chain and aromatic protons.

***In vitro* cytotoxicity activity**

As shown in Table 1, the cytotoxicity of the synthesized compounds was tested using SRB assay in MCF7 and A549 and HCT116 cancer cell lines. For comparison, doxorubicin was used as standard drug, while treatment with DMSO was used as control cancer cells.

Studying the anticancer activity of the new compounds against MCF-7 cell line, revealed that compounds **4**, **7a** and **12a** showed no anticancer activity. Compound **3** exhibited higher potency against MCF-7 cell line with IC_{50} = 2.80 ± 0.33 µg/mL, which is lower than that of doxorubicin (IC_{50} 2.90 ± 0.27 µg/mL). Moreover, the results showed that compounds **2**, **9**, and **10** were found to be potent and selective similar to doxorubicin against MCF-7

cell line with IC_{50} 3.60 ± 0.39, 3.90 ± 4.20 and 4.80 ± 0.55 µg/mL, respectively (Table 1 and Fig. 1).

The cytotoxicity of compounds **2**, **3**, **4**, **6**, **7a**, **9**, **10**, **11** and **12a** was tested against lung cancer cell line A549. For comparison, doxorubicin was also tested. All of the tested compounds except **4**, **7a** and **12a** exhibited anticancer activity and compound **3** (IC_{50} 4.20 ± 0.50 µg/mL) was more potent than doxorubicin (IC_{50} 4.30 ± 0.40 µg/mL), while compound **9**, **10** and **2** were found to be potent near to doxorubicin with IC_{50} 6.50 ± 0.67, 8.00 ± 0.83 and 8.20 ± 0.78 µg/mL respectively. The order of activity was **3**, **10**, **9**, **2**, **11**, **6** in a descending order (Table 1 and Fig. 2).

The results of colon cancer HCT116 cell line revealed that although compounds **4**, **6**, **7a** and **12a** did not exert any activity against the cell, the rest of compounds **2**, **3**, **9**, **10** and **11** had very little anticancer activity compared to doxorubicin (IC_{50} = 4.80 ± 0.50 µg/mL) (Table 1 and Fig. 3).

In conclusion, the tested compounds exert anticarcinogenic activity in breast MCF-7 and lung A549 cancer cell lines through reducing the cell proliferation and resulted in significant growth inhibitory, especially, compounds **2**, **3**, **9** and **10** which revealed promising activity compared to the activity of the commonly used anticancer drug, doxorubicin. The present study reveals that MCF-7 cells are more sensitive to the tested compounds than the other cell lines.

From the above obtained results (Table 1), we can conclude that the attachment of thioxo group at position 2 in the triazolopyrimidine moieties resulted in an enhanced activity. It is obvious that the activity was reduced in other derivatives which do not incorporate such functionality in their structures. Moreover, the attachment of glucosyl moiety to the triazolopyrimidine nucleus through a thioglucosidic linkage increased the activity. This was not the case in nearly similar structures in which the glycosyl moiety is attached to the triazolopyrimidine ring system through C-N linkage. In the present work, the most active compounds were the triazolopyrimidine derivatives **2**, **3**, **9**, and **10** when compared to the reference drug. The difference in activity between the compounds may be attributed to the indicated attachments to the pyrimidine ring of the molecule.

Acknowledgment

The researchers extend their appreciation to the Deanship of Scientific Research at Aljouf University for funding the work through the research group project No. 33/70.

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Received: 21. 10. 2014