Asian Journal of Research in Biochemistry

5(4): 1-15, 2019; Article no.AJRB.53368
ISSN: 2582-0516

In vivo Biochemical Evaluation of Some Synthesize Thiazole Derivatives Containing Coumarin Moiety as Antioxidant and Antitumor Agents

F. Z. Mohammed[1], Lamiaa A. A. Barakat[2], Emad M. Gad[3] and N. M. Shetya[4*]

[1]Department of Biochemistry, Faculty of Science, Zagazig University, Egypt.
[2]Department of Biochemistry, Faculty of Science, Port-Said University, Egypt.
[3]Department of Chemistry, Faculty of Science, Suez Canal University, Egypt.
[4]Department of Biochemistry, Faculty of Science, Suez Canal University, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2019/v5i430104
Editor(s):
(1) Dr. V. Spirina Liudmila, Professor, Department of Biochemistry and Molecular Biology, Laboratory of Tumor Biochemistry, Siberian State Medical University, Russia.
Reviewers:
(1) Kuntal Manna, Tripura University, India.
(2) Mustafa Ceylan, Gaziosmanpasa Univesity, Türkiye.
(3) Byron Baron, Universty of Malta, Malta.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53368

Original Research Article

Received 10 October 2019
Accepted 19 December 2019
Published 26 December 2019

ABSTRACT

Background: Coumarin and thiazole derivatives have been used traditionally for many centuries. Because they have anti-tumor, antioxidant activities, and induced apoptosis.
Aim: The present study aims to investigate the *in vivo* antitumor, and antioxidant activities of potassium salt of 5-(4 -chlorophenyl)-2-[(7-hydroxy coumarin-3-ylethylidene) acetyl hydrazine]-1,3 thiazole (5a) and 5-(4 -chlorophenyl)-2-[(8-hydroxy coumarin-3-ylethylidene) acetyl hydrazine]-1,3 thiazole (5b).
Materials and Methods: Toxicity has been determined for the synthesized compound. Anti-cancer and anti-oxidant activities were studied through the evaluation of tumor cell viability, lifespan prolongation and antioxidant estimation.
Results: Doses of up to 500 mg/kg showed good protection in 5a and 5b compounds. Results of in vivo anti-tumor activity against Ehrlich ascites carcinoma (EAC) cells for the compounds studied showed a significant reduction in the volume and number of ascites. And increased in life span. In therapeutic and preventive groups, compounds 5a and 5b have anti-oxidant properties by a

**Corresponding author: E-mail: nada.shetya11@gmail.com;*

significant decrease in malondialdehyde and significant increased catalase and GSH. Also, compounds 5a and 5b induced apoptosis by increase Caspase-3 and BCL-2 associated protein (BAX), Compared to the group of positive controls.
Conclusion: It was concluded that compounds 5a and 5b have a potent antitumor and antioxidant activity against Ehrlich ascites carcinoma in mice.

Keywords: Thiazoles; cytotoxicity; antioxidant; antitumor; Ehrlich ascites carcinoma; in vivo.

1. INTRODUCTION

Cancer is a wide range of diseases that are associated with abnormal growth of cells. According to the World Health Organization, approximately one-third of deaths from cancer are related to poor nutrition and daily habits such as high-fat diets, lack of exercise, low fiber intake, tobacco, and alcohol consumption. On the other hand, one-fifth of cancer deaths are associated with viral infections such as hepatitis B and C viruses and human papillomavirus in economically less developed countries [1].

In 2012, GLOBOCAN reported around the world 14.1 million new cases and 8.2 million cancer-related deaths [2]. The design and development of new particles for cancer treatment have spurred tremendous interest in many industrial and academic research laboratories around the world. The biological activities of different compounds, either artificial or natural, have been investigated extensively around the world. The applications of these compounds in medical research have increased widely to develop the treatment methods of various diseases. Unfortunately, several of these compounds are not suitable for therapeutic use because of their toxicity level, carcinogenic effect, and mutagenic property. As technology and knowledge advance, it is possible to synthesize an active compound of the molecular structure with improved therapeutic activity and reduced toxicity [3,4]. Coumarins are bioactive compounds of both nature and synthetic origin, and due to their useful and diverse pharmaceutical and biological activities, there has been a growing interest in their synthesis [5].

Coumarin compounds possessing anti-bacterial [6,7], Antifungal [8-10], anticoagulant [11], antituberculosis [12], anti-inflammatory [11], antitumor [13,14], anti-human immunodeficiency virus (HIV) activities [15]. Its substitution effectively influences the biological activities of the synthesized coumarin derivatives. These substitutions in the coumarin nucleus are very important in the design and development of new coumarin derivatives with remarkable biological properties for the prediction of structure-activity relationship (SAR) analysis. Thiazole, you know. Compounds play an important role in nature and have a number of biological effects, including antitumors. [16-18], antibacterial [19], antimicrobial [20], anti-viability [21], anti-inflammatory [22], Syk inhibitor [23], antiviral [24], antiproliferative [25] and anticandidal activity [26].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals

2,4-dihydroxybenzaldehyde, 2,3 - dihydroxybenzaldehyde, ethyl acetoacetate, piperidine, thiosemicarbazide, ethanol, acetic acid, p-chlorophenacyl bromide, sodium acetate, potassium hydroxide were obtained from El-Gomhoria Chemical Co. Port-said. All chemicals were used as received without extra purification.

2.1.2 Animals

Female Swiss albino mice aged 8 weeks, weighing 22 to 25 g of body weights, were raised at the Faculty of Science, Port-said University's experimental animal house. The animals were kept in temperature, humidity, and light-controlled environments. They were fed on a standard commercial diet and adlibitum tap water.

2.1.3 Tumors

Ehrlich ascites carcinoma (EAC) was initially delivered by the National Cancer Institute in Cairo, Egypt, and maintained ascites at 8 or 10-day intervals in our laboratory in female Swiss albino mice through serial intraperitoneal (I.P) inoculation.

2.2 Methods

2.2.1 Chemistry

The 7-hydroxy and 8-hydroxy-3-acetylcoumarins (2a, b) were prepared using 2,4-

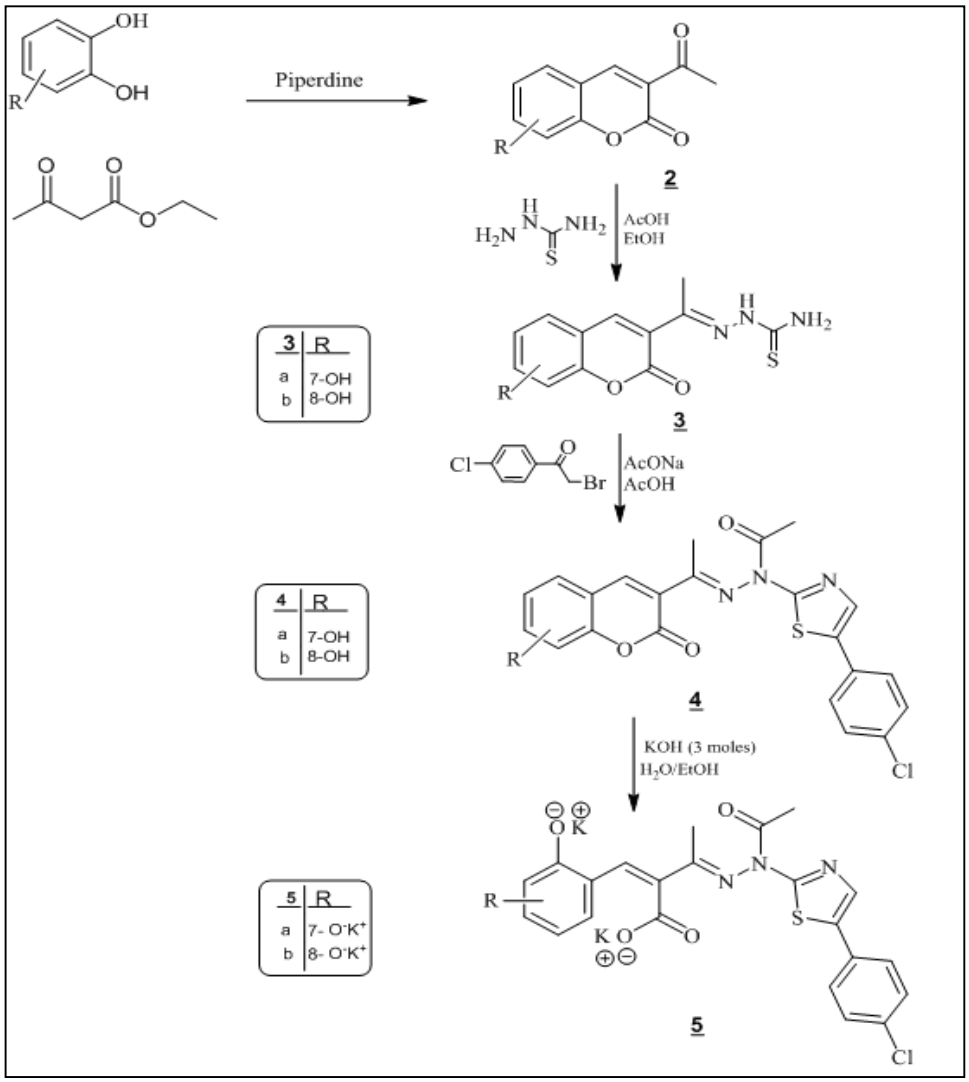
dihydroxybenzaldehyde cyclo condensation and 2,3-dihydroxybenzaldehyde in the presence of piperidine as a catalyst using the literature method [1,2]. As a key starting material. Treatment of 3-acetylcoumarin derivatives (2a,b) with thiosemicarbazide in ethanol in the presence of acetic acid as catalyst under reflux led to the formation of 1-[(7-hydroxy and 8-hydroxycoumarin-3-ylethylidene) amino]-thioureas (3a,b).

5-(4-chlorophenyl)-2-[(7-hydroxy and/or 8-hydroxy coumarin-3-ylethylidene) acetyl hydrazino]-1,3-thiazoles (4a,b) were prepared via the cyclization of thiourea derivatives (3a,b) with 4-chlorophenacyl bromide in acetic acid in the presence of fused sodium acetate under reflux. Preparation of the compounds 2-5 was accomplished by means of a common synthetic procedure shown in Scheme 1.

The potassium salt of compounds (5a,b) was formed via the dissolving thiazole derivatives (4a,b) in equal two equivalent moles of potassium hydroxide solution (equal volumes from water and ethanol) and the removal of the solvent at room temperature.

2.2.2 Determination of median lethal dose (LD 50) of the synthesized compounds

Approximate LD50 of newly synthesized compounds in mice has been determined using the Meier and Theakston methods [27].



Scheme 1. Synthesis of 1,3 thiazole derivatives (4 and 5)

2.2.3 Experimental design

60 Swiss albino mice were divided into 6 groups of 10 mice each: group I "acted as a negative control group" injected with sterile saline for 10 days (day after day); group 2 "positive control;" intraperitoneal (i.p.) injected with 2.5x106 of Ehrlich ascites carcinoma "EAC" cells. Group 3"5a therapeutic group, injected intraperitoneal (I.P.) with 2.5 mg/kg one day after EAC injection and repeated doses five times during the experiment; Group 4" 5a Preventive Group: injected I.P. with compound 5a (2.5 mg/Kg) before injection with EAC cells (2.5×106 cells/mouse) and repeated dose five times during the experiment. Group 5 "5b therapeutic group", injected i.p. with 5 mg/kg one day after EAC injection and repeated dose five times during the experiment. Group 6 " injected I.P. with compound 5b (5 mg/Kg) before injection with EAC cells (2.5×106 cells/mouse) and repeated dose five times during the experiment. At the end of the experiment, EAC cells were harvest from each mouse in a centrifuge tube containing heparinized saline. Note the volume of ascetic fluid in each mouse in each group. Each sample of cells was undergoing viability tests of EAC cells, and antioxidants assays were performed Zahran, F et al. [28].

2.2.4 Life span prolongation

Life span calculation was done using the method described by Mazumdar et al. [29].

2.2.5 Evaluation of Tumor volume

The volume of the EAC was detected by measuring tube in milliliters (ml) [30].

2.2.6 Determination of cell viability

100 µl of Ehrlich ascites cells were stained with trypan blue for 15 minutes, and the number of viable cells was counted using homocytometers as follows;

Number of cells / ml = average × 25 ×104× dilution factor

2.2.7 Evaluation of antioxidant parameters

Malondialdehyde level (MDA), catalase level (CAT), and reduced glutathione level (GSH) are calculated by Satoh [31]; Aebi [32] methods.

2.2.8 Evaluation of apoptosis assays

The caspase-3 activity was calculated by the caspase-3 colorimetric kit according to the method of Casciola [33].

2.3 Statistical Analysis

Statistical analysis was conducted using version 14 of SPSS Software II [34]. Data were expressed as mean ± SE. Student (t) test and One-way ANOVA test were performed to detect the significance variation between test groups and control.

3. RESULTS

3.1 Determination of Median Lethal Dose LD50 of Synthetic Compounds

The acute toxicity was estimated by intraperitoneal administration of compounds to determine the median lethal dose (LD50) of compounds 5a and 5b. Our results showed that in compounds 5a and 5b, doses up to 500 mg/kg are considered safe, where no mortality was observed.

3.2 The Effect of Test Compounds 5a and 5b on Tumor Volume, Viability of EAC Cells

Table 1 summarized the effect of compounds 5a and 5b on EAC cell volume and count. The mean count of EAC cells in the positive control group was found to be 233 ×106 cells, which significantly decreased to 165, 137, 144, 171(106) by 29.2% and 41.6%; by 38.2 % and 26.6% (P <0. 01), respectively in therapeutic and preventive groups of compounds 5a and 5b; respectively.

Also, the mean volume of EAC in the positive control group was found to be 3.65 (mL). While This volume was significantly decreased in compounds 5a and 5b to 1.92, 1.4, 2.25, 1.25 mL by 47.4%, and 61.6%; by 38.4%, and by 65.8%, (P <0. 01); respectively in therapeutic and preventive groups compared to the positive control group.

3.3 Effect of Tested Compounds on Life Span in All Studied Groups

Table 2 illustrated the life span prolongation of the studied tested compounds.

The mean life span prolongation in the positive control group was found to be 14 days compounds 5a and 5b therapeutic and preventive groups showed a significantly increased in the life span prolongation to 20 days by 42.9% (T/c ratio = 142.9%), 19 days by 35.7% (T/c ratio = 135.7%), 15 days by 7.14% (T/c ratio = 107.1%), 23 days by 64.3% (T/c ratio = 164.3%), respectively; compared to the positive control group.

3.4 Antioxidants Assays

3.4.1 The Effect of compounds 5a and 5b on oxidant and Antioxidants in EAC of all studied groups

Table 3 illustrated the EAC antioxidant activity of the investigated compounds.

The mean values of EAC MDA levels in the positive control group were found to be 14.22±0.323 (nmol/g.tissue). Treatments by compounds 5a and 5b showed significant decrease to 7.59±0.37, 4.03±0.11(nmol/g.tissue); 7.75±0.323, 5.29±0.39 (nmol/g.tissue) by 46.62% and 71.66%; 45.49% and 62.79 ($p<0.001$) for both therapeutic and preventive groups, respectively; compared to the positive control group as showed in Fig. 1.

Also, EAC catalase activity in the positive control group was found to be 292.4±23.75 (U/g tissue). Treatments with compounds (5a, 5b) showed, significant increase in EAC catalase To 853.3±43.3 and 504.6±22.48(U/g); 664.2±24.5, 576.1±74 (U/g) by 191.8%, 72.57%; 127.15%, 97.02% in both therapeutic and preventive groups, respectively; compared to the positive control group ($p<0.001$) as showed in Fig. 3.

Meanwhile, the mean values of EAC Glutathione reduced levels in the positive control group were found to be 516.2±33.27 (nmol/g.tissue). Treatments with compounds (5a, 5b) showed, significant increase to 1372.97±59.37 and 1274±71.09 (nmol/g.tissue); 1066±52.12 and 867.56±94.11 (nmol/g.tissue) by 165.97% and 146.85%; 106.57% and 68.1% ($p<0.001$) respectively; compared to the positive control group; as showed in Fig. 5.

3.4.2 The Effect of compound 5a and 5b on oxidant and Antioxidants in the liver of all studied groups

Table 4 illustrated the liver antioxidant activity of the investigated compounds.

The mean values of liver MDA were found to be 4.73 ±0.097 (nmol/g.tissue) in the negative control group. These values were increased in the positive control group to 6.13± 0.22 (nmol/g.tissue) by 29.6%, ($p>0.05$). Treatments by compounds 5a and 5b show reduction of liver MDA levels to 5.99±0.25, 2.78±0.15 (nmol/g.tissue); 5.6± 0.17 and 5.59±0.21 (nmol/g.tissue) by 2.3% and 54.6% ; 8.6% and 8.8% for both therapeutic and preventive group, respectively; compared to the positive control group ($p>0.05$) showed in Fig. 2.

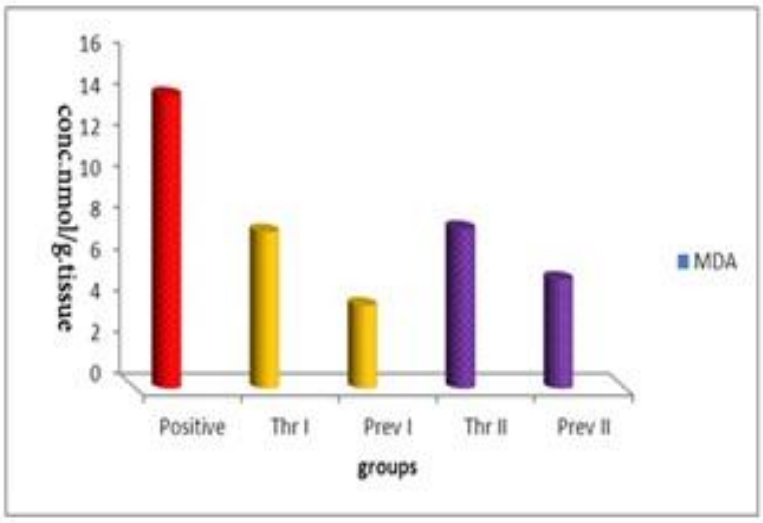


Fig. 1. EAC MDA in all studied groups

**There is a significant decrease in EAC MDA levels in compound 5a and 5b therapeutic and preventive groups*

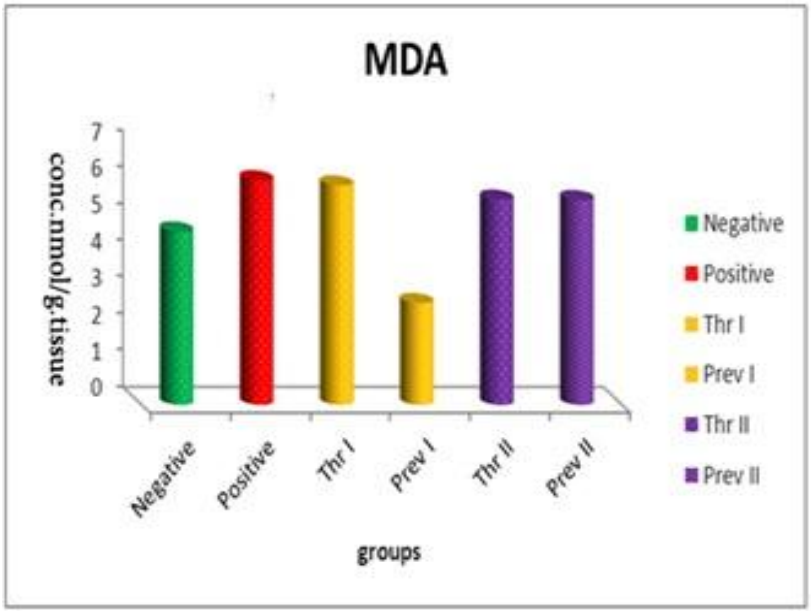


Fig. 2. Liver MDA in all studied groups

**There is a significant decrease in liver MDA levels in compound 5a and 5b therapeutic and preventive groups*

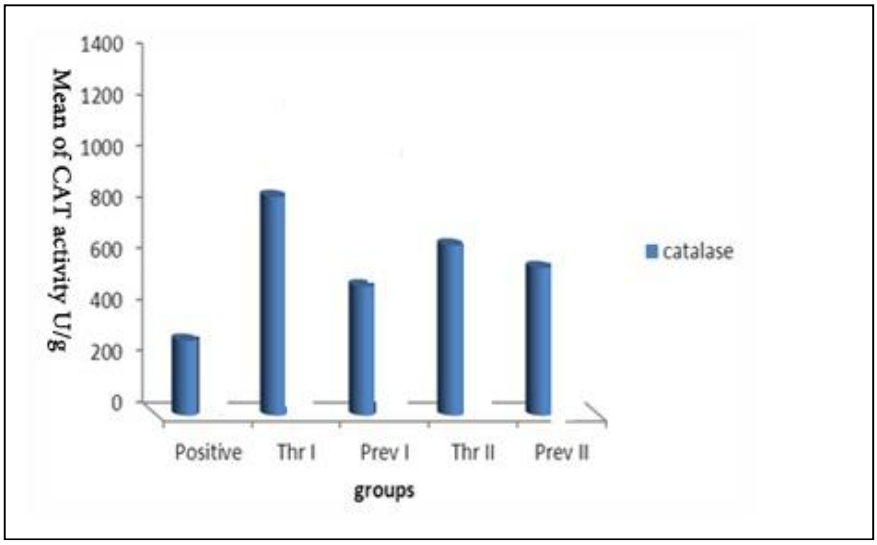


Fig. 3. EAC Catalase in all studied groups

**There is a very highly significant increase in EAC catalase in compounds 5a&5b therapeutic and preventive groups*

Also, the mean values of liver Catalase were very highly significant decreased to 333.4± 7.35(u/g) in the positive control group compared to the negative control group 529± 19.05(u/g) by 36.98%, ($p<0.001$). Treatments with compounds (5a, 5b) show very highly significant increase in liver catalase activities to 463.4±4.9 and 703.1±21.7(u/g); 812.6± 22.06 and 940.2±17.7 (u/g) by 38.9% and 110.9%;143.7% and 182%, ($p<0.001$) in both therapeutic and preventive group, respectively; compared to the positive control group showed in Fig. 4.

On the other hand, the mean values of glutathione reduced in liver samples were very highly significant decreased to 142.4± 1.51 (nmol/g.tissue) in the positive control group compared to the negative control group 468±

7.71 (nmol/g.tissue) by 69.6%, ($p<0.001$). While, the mean Glutathione reduced activities very highly significant increased to 254.84±24.8, 265.32±28.6 (nmol/g.tissue); 175.59± 8.83 and 253.27±18.41 (nmol/g.tissue) by 78.96% and 86.32%; 23.3% and 77.85% in both therapeutic and preventive groups; ($p<0.001$) respectively, compared to the positive control group as showed in Fig. 6.

3.5 Apoptosis Assays

3.5.1 Effect of tested compounds on caspase-3 and Bax (BCL-2 associated protein) in EAC of all studied groups

Table 6 illustrated the EAC apoptosis assays of the investigated compounds.

The mean values of EAC Caspase 3 activity in positive control were found to be 0.270±0.004 (ng/mL). Treatments by Compounds 5a and5b showed highly significant increase to 0.398±0.042 and 0.76±0.1371(ng/mL); 1.18±0.0725 and 0.7±0.044 (ng/mL) by 47.4%, 181.5%; 337%, 159.25% in both therapeutic and preventive groups, respectively. Compared to the positive control group ($p<0.01$), as shown in Fig. 7.

It also showed very highly significant increase in EAC BAX to be 3.44±0.126 and 3.98±0.24 (ng/mL); 6.34±0.78 and 3.85 ±0.23 (ng/mL) by 60.74% and 86.3%; 196.3% and 80.3% in both therapeutic and preventive groups, respectively. Compared to the positive control group 2.14±0.125 (ng/mL). ($p<0.001$) showed in Fig. 9.

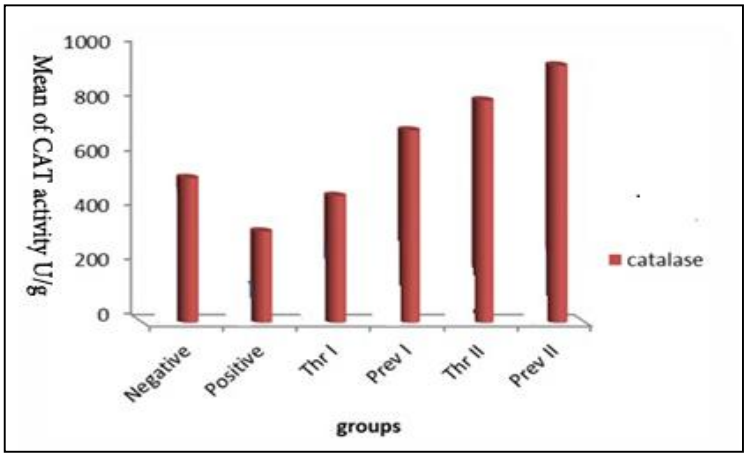


Fig. 4. liver Catalase in all studied groups

**There is a very highly significant increase in liver catalase in compounds 5a&5b therapeutic and preventive groups*

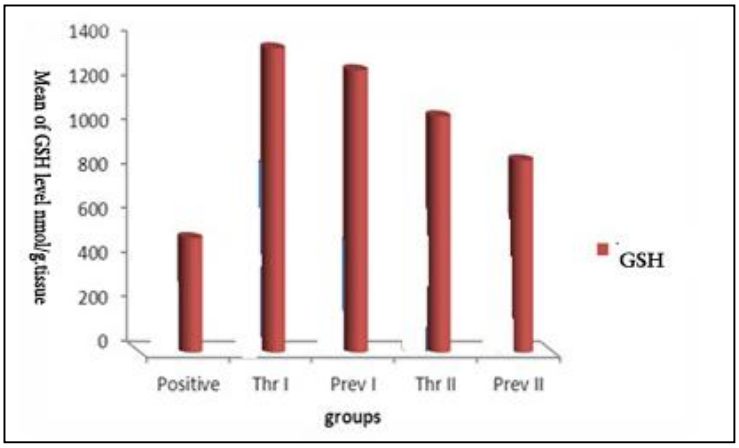


Fig. 5. EAC Glutathione reduced in all studied groups

**There is a very highly significant increase in EAC Glutathione reduced levels in compounds 5a&5b therapeutic and preventive groups*

Table 1. Effect of compounds 5a and 5b on volume and count of EAC in treated groups

Group	Positive control		Therapeutic I		Preventive I		Therapeutic II		Preventive II	
	Mean	%Change	Mean	%Change	Mean	%Change	Mean	%Change	Mean	%Change
Volume of ascites fluid (ml)	3.65	…..	1.92	47.40%	1.4	61.60%	2.25	38.40%	1.25	65.80%
Count of EAC cells (×106)	233	…..	165	29.20%	137	41.60%	144	38.20%	171	26.60%

**There is a decrease in count and volume of EAC in treated*

Table 2. Effect of compounds 5a and 5b on life span prolongation

Live span prolongation	Positive control	Therapeutic I	Preventive I	Therapeutic II	Preventive II
Days	14	20	19	15	23
% Change	……..	42.90%	35.70%	7.14%	64.30%
T/C ratio (%)	…….	142.90%	135.70%	107.10%	164.30%

**There is an increase in life span in both therapeutic and preventive groups*

Table 3. Anti-oxidants effect of compounds 5a&5b in EAC cells

Variable	Positive control		therapeutic I		Preventive 1		Therapeutic II		Preventive II	
	Mean ± SE.	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang
MDA(nmol/g.tissue)	14.22***±0.323	…..	7.59***±0.37	-46.62%	4.03***±0.113	-71.66%	7.75***±0.323	-45.49%	5.29***±0.39	-62.79%
Catalase(U/gT)	292.4*** ±23.75	…..	853.3***±43.31	191.82%	504.6*** ±22.48	72.57%	664.2 ***±24.5	127.15%	576.1*** ±41.74	97.02%
Gluathione reduced(nmol/g.tissue)	516.2*** ±33.27	…..	1372.97***±59.37	165.97%	1274.28 ***±71.09	146.85%	1066.29*** ±52.12	106.57%	867.56 ***±94.11	68.10%

****P value <0.001 was considered very highly significant, *There is a very highly significant reduction in EAC MDA levels in compounds 5a&5b therapeutic and preventive groups, **There is a very highly significant increase in both EAC catalase and G.reduced activity in compounds 5a &5b therapeutic and preventive groups*

Table 4. Anti-oxidants effect of compounds 5a&5b in liver cells

Variable	Negative control		Positive control		therapeuticl		Preventive I		Therapeutic II		Preventive II	
	Mean ± SE.	% Change	Mean ± SE.	% Change	Mean ± SE.	% Change	Mean ± SE.	% Change	Mean ± SE.	% Change	Mean ± SE.	% Change
MDA(nmol/g.tissue)	4.73*** ±0.097	……	6.13***±0.225	29.60%	5.99***±0.255	-2.30%	2.78***±0.151	-54.60%	5.6***±0.1788	-8.60%	5.59*** ±0.216	-8.80%
Catalase(U/gT)	529***±19.05	…….	333.4*** ±7.35	-36.98%	463.4 ***±4.92	38.90%	703.1*** ±21.72	110.90%	812.6***±22.06	143.70%	940.2*** ±17.74	182%
Glutathione reduced (nmol/g.tissue)	468 *** ±7.71	……	142.4*** ±1.51	-69.60%	254.84***±24.82	78.96%	265.32 ***±28.64	86.32%	175.59 ***±8.833	23.30%	253.27*** ±18.41	77.85%

****P value <0.001 was considered very highly significant, *There is a very highly significant reduction in liver MDA levels in compounds 5a&5b therapeutic and preventive groups, **There is a very highly significant increase in both liver catalase and G.reduced activity in compounds 5a &5b therapeutic and preventive groups*

Table 5. Effect of compounds 5a & ab on caspase-3 activity and bax levels in EAC cells

Variable	Positive control		therapeutic I		Preventive 1		Therapeutic II		Preventive II	
	Mean ± SE.	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang
caspase-3	0.27***±0.004		0.398*** ±0.042	47.40%	0.76***±0.137	181.50%	1.18***±0.072	337.00%	0.7***±0.044	159.25%
bax	2.14***±0.125		3.44***±0.126	60.74%	3.986***±0.247	86.30%	6.34***±0.783	196.30%	3.858***±0.2313	80.30%

*P value <0.001 was considered very highly significant, **There is a very highly significant increase in EAC caspase-3 and Bax levels in compounds 5a&5b therapeutic and preventive groups*

Table 6. Effect of compounds 5a & 5b on caspase-3 activity and bax levels in liver of all studied groups

Variable	Negative control		Positive control		therapeuticl		Preventive I		Therapeutic II		Preventive II	
	Mean ± SE .	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang	Mean ± SE.	% Chang
caspase-3	3.076***±0.1199		0.9*** ±0.245	-70.57%	1.93***±0.137	113.25%	2.98***±0.04	229.30%	2.41***±0.03	166.29%	2.17***±0.18	139.77%
bax	17.84±0.204		9.92***±0.357	-44.39%	10.16***±0.336	2.42%	12.2***±0.593	22.98%	9.93***±0.08	0.10%	10.29***±0.238	3.72%

*P value <0.001 was considered very highly significant, **There is a very highly significant increase in liver caspase-3 and Bax levels in compounds 5a&5b therapeutic and preventive groups*

3.5.2 Effect of tested compounds on caspase-3 and Bax (BCL-2 associated protein) in the liver of all studied groups

Table 7 illustrated the liver apoptosis assays of the investigated compounds.

The mean values of Caspase-3 in the liver tissue were found to be 3.07 ± 0.119 (ng/ml) in negative control group .These values were decreased in the positive control group to 0.905± 0.245 (ng/mL) by 70.57%, ($p<0.01$). While, the mean values of Caspase-3 activities for both therapeutic and preventive groups treatments with Compound (5a, 5b) showed highly significant increase to be 1.93±0.13, 2.98± 0.04 (ng/ml); 2.41±0.038 and 2.17±0.18 (ng/ml) by 113.25% and 229.4%; 116.9% and 139.77% ($p<0.01$), respectively; compared to the positive control group ($p<0.01$) showed in Fig. 8.

Also, the mean values of BAX levels in the liver tissue were found to be 17.84±0.204 (ng/ml) in the negative control group. These values were very highly significant, decreased to 9.92± 0.35(ng/mL) in the positive control group by ($p<0.001$). the mean values of BAX levels were very highly significant increased for both therapeutic and preventive groups treatments with Compound (5a, 5b) to be 10.16±0.33 and 12.2± 0.59(ng/ml); 9.93 ±0.08 and 10.29±0.23 (ng/ml) by 2.42%, 22.98%, 0.1% and 3.72% ($p<0.01$), respectively; compared to the positive control group showed in Fig. 10.

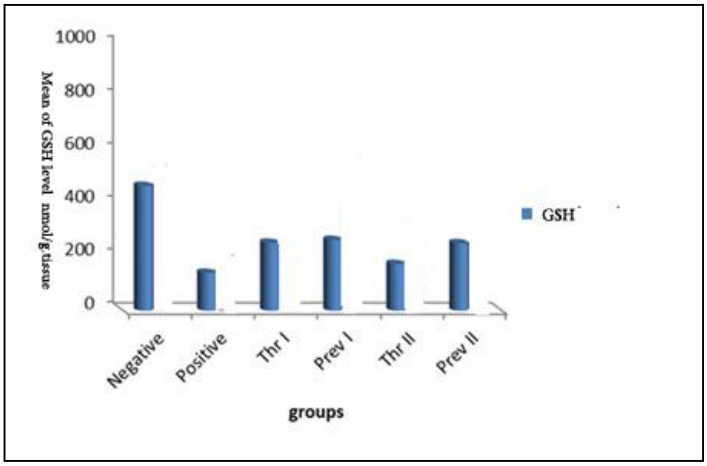


Fig. 6. Liver Glutathione reduced in all studied groups

**There is a very highly significant increase in liver Glutathione reduced levels in compounds 5a&5b therapeutic and preventive groups*

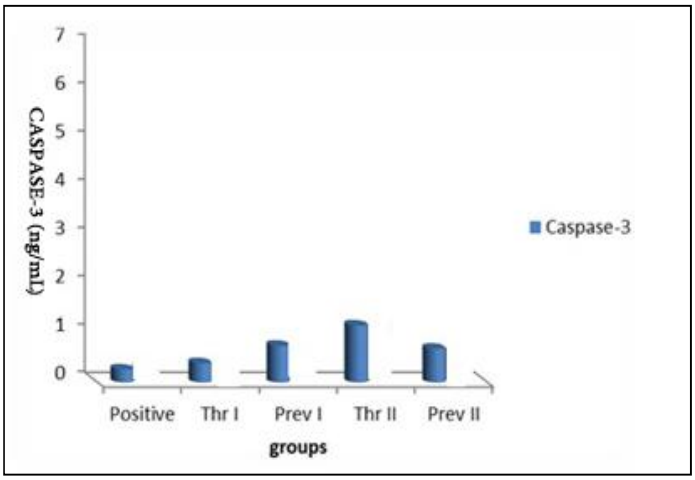


Fig. 7. EAC Caspase-3 in all studied groups

**There is a very highly significant increase in EAC caspase-3 in compounds 5a and 5b therapeutic and preventive groups*

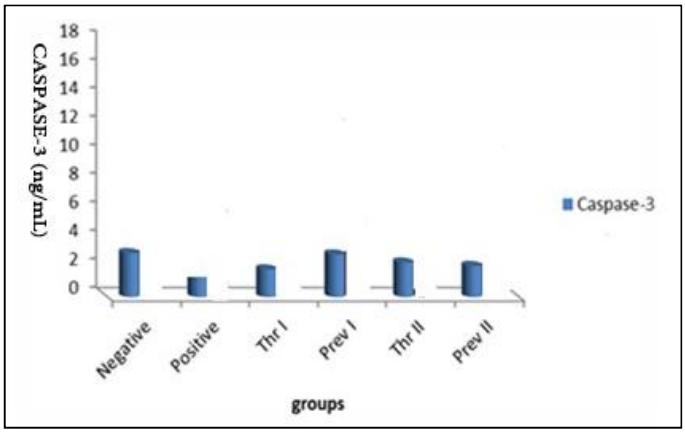


Fig. 8. Liver Caspase-3 in all studied groups

**There is a very highly significant increase in liver caspase-3 in compounds 5a and 5b therapeutic and preventive groups*

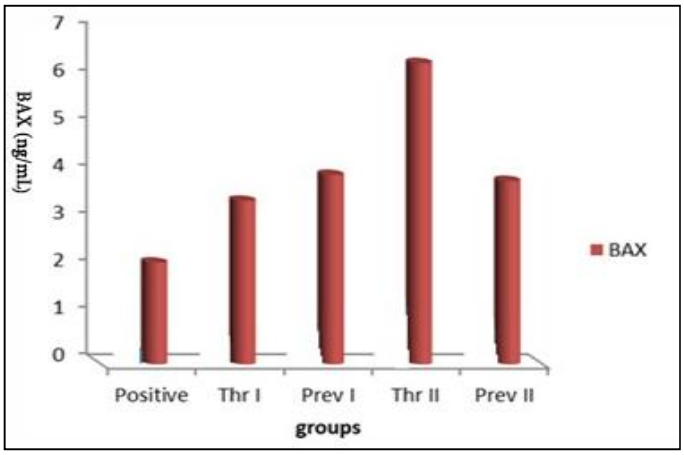


Fig. 9. EAC BAX level in all studied groups

**There is a very highly significant increase in EAC Bax in compounds 5a and 5b therapeutic and preventive groups*

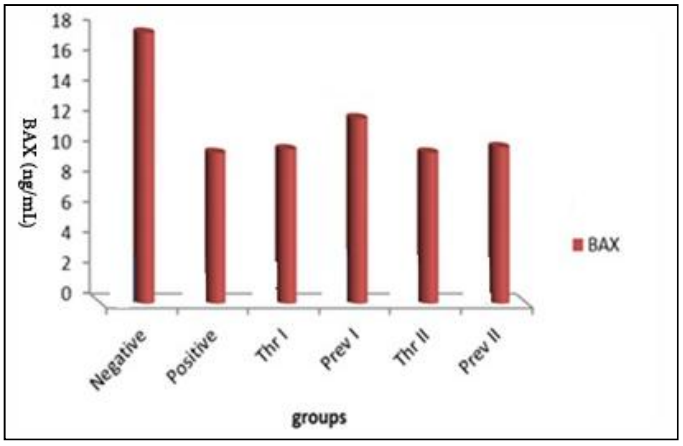


Fig. 10. Liver BAX level in all studied groups

**There is a very highly significant increase in liver Bax levels in compound 5a and 5b therapeutic and preventive groups*

4. DISCUSSION

Cancer is a disease caused by dysregulated cellular signaling process in cell homeostasis and proliferation and caused by a combination of genetic mutation and internal or external oncogenic stimuli [35]. Chemotherapy in cancer therapy [36] is proven to be an effective and safe treatment option that uses multiple drugs and hormonal agents alone or in combination [37]. The synthetic drugs used for chemotherapy, in addition to being toxic to cancer cells, also have a lethal effect on normal cells due to their non-specificity, which only works on cell division [38].

This paper describes the evaluation of the anti-tumor, anti-oxidant effects of synthetic potassium salt of 5-(4 -chlorophenyl)-2-[(7-hydroxy coumarin-3-ylethylidene) acetyl hydrazine]-1,3 thiazole (5a) and potassium salt of 5-(4 -chlorophenyl)-2-[(8-hydroxy coumarin-3-ylethylidene) acetyl hydrazine]-1,3 thiazole (5b) . Our results found that these doses were significantly prolonged the life span to 20 days by 42.9%, 19 days by 35.7%, 15 days by 7.14%, 23 days by 64.3%, in therapeutic and preventive groups; respectively compared to the positive control group as shown in Table 1.

A rapid increase in the volume of ascetic tumors was observed in EAC tumor-bearing mice, and treatment with compounds 5a and 5b increased the life span of EAC-bearing mice by reducing the viability of EAC cells and decreasing the tumor volume due to present of coumarin and thiazole ring in compounds 5a and 5b, which have been shown to possess anticancer properties. Such results are consistent with the observation of Gabr et al. [39]. The thiazolylcoumarin derivatives have the highest in vitro antitumor activity in mice against EAC cells. Increasing the life span of EAC inoculated mice (%ILS), decreasing the number of viable tumor cells.

Our results found that compounds 5a & 5b treatment also decreased the levels of MDA (malondialdehyde) and raised the values of GSH (reduced glutathione), Catalase. These results prove the cytotoxic efficacy of compounds 5a & 5b on tumor cells. Also, in this research, a decrease in CAT and GSH and an increase in MDA levels were noticed in EAC bearing mice. Compound 5a & 5b treatment prevents the reduction in antioxidant levels and rises in MDA levels in EAC bearing mice, which prove the potency of these compounds in protecting against oxidative stress by normalization of antioxidants values in EAC bearing mice. Lowering in antioxidants and raising in MDA is assign to cancer. Flow cytometric analysis shows that the arrest of a cell cycle mostly occurs in the S phase then leads to apoptosis because the aneuploid ratio is permanently and strongly relate to the S phase, which produces proliferation. This may indicate that compounds 5a & 5b interfere with DNA replication. Apoptosis is a programmed cell death process in which biochemical events lead to cell changes and death that are characteristic. As a biological phenomenon and defective apoptotic processes involved in many diseases, apoptosis is essential. Excessive apoptosis triggers atrophy, whereas inadequate apoptosis contributes to the uncontrolled proliferation of cells, which leads to cancer. Some factors, such as fas receptors and caspases, promote apoptosis, while individual members of the protein family Bcl-2 inhibit apoptosis. Caspase-3 is also required for some typical hallmarks of apoptosis [40]. The two major pro-apoptotic Bcl-2 proteins, Bax and Bak, induce apoptosis through the development of mitochondrial outer membrane permeabilization (MOMP) complex [41]. MOMP formation results in the release from the mitochondrial intermembrane space of cytochrome c and other factors, which in turn activates the critical caspase cascade. The interactions between the Bcl-2 protein family's anti-apoptotic and pro-apoptotic proteins either inhibit or activate MOMP and determine the cell's fate [42].

The present study showed treatment with compounds 5a and 5b cause an increase in liver and EAC caspase-3 activity and increase Bax level compared with positive control group. Therefore, the results of the present study suggested that treatment with compounds 5a and 5b may have induced apoptosis. Apoptosis induction is considered to be an active cancer therapy strategy [43].

5. CONCLUSION

It was concluded that compounds 5a and 5b have a potent antitumor and antioxidant activity against Ehrlich ascites carcinoma in mice. The compound 5b is more active than compound 5a.

ETHICAL APPROVAL

As per international standard or university standard was written, ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. De Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M. Global burden of cancers attributable to infections in 2008: A review and synthetic analysis. Lancet Oncol. 2012;13:607–615.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A, Global cancer statistics, 2012. CA-Cancer J Clin. 2015; 65:87–108.
3. Kostova I. Synthetic and natural coumarins as cytotoxic agents. Curr. Med. Chem. Anti-Cancer Agents. 2005;5:29–46.
4. Abdul Rahman FS, Yusufzai SK, Osman H, Mohamad D. Synthesis, characterisation and cytotoxicity activity of thiazole substitution of coumarin derivatives (characterisation of coumarin derivatives). Physical Science. 2016;27:77–87.
5. Salem MA, Marzouk MI, El-Kazak AM. Synthesis and characterization of some new coumarins with in vitro antitumor and antioxidant activity and high protective effects against DNA damage. Molecules. 2016;21:249–269.
6. Vazquez-Rodriguez S, Lopez RL, Matos MJ, Armesto-Quintas G, Serra S, Uriarte E, Santana L, Borges F, Crego AM, Santos Y. Design, synthesis and antibacterial study of new potent and selective coumarin–chalcone derivatives for the treatment of tenacibaculosis. Bioorg Med Chem. 2015;23:7045–7052.
7. Azelmat J, Fiorito S, Taddeo VA, Genovese S, Epifano F, Grenier D. Synthesis and evaluation of antibacterial and anti-inflammatory properties of naturally occurring coumarins. Phytochem Lett. 2015;13:399–405 .
8. Karatas MO, Olgundeniz B, Gunal S, Ozdemir I, Alici B, Cetinkaya E. Synthesis, characterization and antimicrobial activities of novel silver (I) complexes with coumarin substituted N-heterocyclic carbene ligands. Bioorg Med Chem. 2015;24:643–650.
9. Patil SA, Prabhakara CT, Halasangi BM, Toragalmath SS, Badami PS. DNA cleavage, antibacterial, antifungal and anthelmintic studies of Co (II), Ni (II) and Cu (II) complexes of coumarin Schiff bases: Synthesis and spectral approach. Spectrochim Acta A Mol Biomol Spectrosc. 2015;137:641–651.
10. Shaikh MH, Subhedar DD, Khan FA., Sangshetti JN, Shingate BB. 1, 2, 3-Triazole incorporated coumarin derivatives as potential antifungal and antioxidant agents. Chin Chem Lett. 2016;2:295–301.
11. Abdelgadir HA, Van Staden J. Ethnobotany, ethnopharmacology and toxicity of *Jatropha curcas* L. (Euphorbiaceae): A review. S Afr J Bot. 2013;88:204–218.
12. Arshad A, Osman H, Bagley MC, Lam CK, Mohamad S, Mohdzahariluddin AS. Synthesis and antimicrobial properties of some new thiazolyl coumarin derivatives. Eur J Med Chem. 2011;46:3788–3794.
13. Nocentini A, Carta F, Ceruso M, Bartolucci G, Supuran CT. Click-tailed coumarins with potent and selective inhibitory action against the tumor-associated carbonic anhydrases IX and XII. Bioorg Med Chem. 2015;23:6955–6966.
14. Elhalim SA, Ibrahim IT. Radioiodination of 2, 3-dimethyl-4H-furo [3, 2-c] coumarin and biological evaluation in solid tumor bearing mice. Appl Radiat Isot. 2015;95:153–158.
15. Matsuda K, Hattori S, Kariya R, Komizu Y, Kudo E, Goto H, Taura M, Ueoka R, Kimura S, Okada S. Inhibition of HIV-1 entry by the tricyclic coumarin GUT-70 through the modification of membrane fluidity. Biochem Biophys Res Commun. 2015;457:288–294.
16. Leoni A, Locatelli A, Morigi R, Rambaldi M. Novel thiazole derivatives: A patent review (2008–2012. Part 2). Exp. Opin. Ther. Pat. 2014;24:759–777.
17. Tung TT, Oanh DTK, Dung PTP, Hue VTM, Park SH, Han BW, Kim Y, Hong JT, Han SB, Nam NH. New benzothiazole/ thiazole-containing hydroxamic acids as potent histone deacetylase inhibitors and antitumor agents. Med. Chem. 2013;9: 1051–1057.
18. Ghorab MM, Al-Said MS. Antitumor activity of novel pyridine, thiophene and thiazole derivatives. Arc. Pharm. Res. 2012;35: 965–973.
19. Li JR, Li DD, Wang RR, Sun J, Dong JJ, Du QR, Fang F, Zhang WM, Zhu HL. Design and synthesis of thiazole

derivatives as potent FabH inhibitors with antibacterial activity. Eur. J. Med. Chem. 2014;75:438–447.
20. Bondock S, Fadaly W, Metwally MA. Synthesis and antimicrobial activity of some new thiazole, thiophene and pyrazole derivatives containing benzothiazole moiety. Eur. J. Med. Chem. 2010; 45:3692–3701.
21. Lin XD, Yang HW, Ma S, Li WW, Zhang CH, Wang WJ, Xiang R, Li LL, Yang SY. Discovery of 6-phenylimidazo [2,1-b]thiazole derivatives as a new type of FLT3 inhibitors. Bioorg. Med. Chem. Lett. 2015;25:4534-4538.
22. Geronikaki A, Hadjipavlou-Litina D, Zablotskaya A, Segal I. Organosilicon-containing thiazole derivatives as potential lipoxygenase inhibitors and anti-inflammatory agents. Bioinorg. Chem. 2007;92145.
23. Thoma G, Veenstra S, Strang R, Blanz J, Vangrevelinghe E, Berghausen J, Lee CC, Zerwes HG. Orally bioavailable Syk inhibitors with activity in a rat PK/PD model. Bioorg. Med. Chem. Lett. 2015;25: 4642–4647.
24. Barradas JS, Errea MI, D'Accorso NB, Sepulveda CS, Damonte EB. Imidazo [2,1-b]thiazole carbohydrate derivatives: Synthesis and antiviral activity against Junin virus, agent of Argentine hemorrhagic fever. Eur. J. Med. Chem. 2011;46:259–264.
25. Grozav A, G˘ain˘a LI, Pileczki V, Crisan O, Silaghi-Dumitrescu L, Therrien B, Zaharia V, Berindan-Neagoe I. The Synthesis and Antiproliferative Activities of New Arylidene-Hydrazinyl-Thiazole Derivatives. Int. J. Mol. Sci. 2014;15:22059–22072
26. Altıntop MD, Özdemir A, Turan-Zitouni G, Ilgın S, Atlı Ö, Demirci F, Kaplancıklı ZA. Synthesis and in vitro evaluation of new nitro-substituted thiazolyl hydrazone derivatives as anticandidal and anticancer agents. Molecules. 2014;19:14809–14820.
27. Meier J, Theakston RDG. Approximate LD50 determination of snake venuoms using eight to ten experimental animals. Toxicon. 1985;24:395-401.
28. Zahran F, Keshta AT, EL-Deen IM, Elbehary MM. Mode of action of potassium salt of 2-thioxo-4-hydroxycoumarin [3, 4-b] pyrimidine and 9-bromo-2-thioxohydroxycoumarin [3,4-b] pyrimidine against Ehrlich Ascites carcinoma cells. Biol. Chem. Res. 2014;76-89.
29. Mazumdar UK, Gupta M, Maiti S, Mukherjee D. Antitumor activity of *Hygrophila spinosa* on *Ehrlich ascites* carcinoma and sarcoma-180 induced mice. Indian J Exp Biol. 1997;35:473-477.
30. Sneha Mishra, Ankit Kumar Tamta, Mohsen Sarikhani, Perumal Arumugam Desingu, Shruti M. Kizkekra, Anwit Shriniwas Pandit, Shweta Kumar, Danish Khan, Sathees C. Raghavan, Nagalingam R. Sundaresan, subcutaneous ehrlich ascites carcinoma mice model for studying cancer-induced cardiomyopathy. Scientific Reports. 2018;8(Article number: 5599).
31. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin. Chim. Acta. 1978;90(1):37-43.
32. Aebi H. Methods enzymol. 1984;105:121-126.
33. Casciola RL, Nicholson DW, Chong T, Roan KR, Thornberry N A, Miller D K, Rosen A. Apopain/CPP32 cleaves proteins that are essential for cellular repair: A fundamental principle of apoptotic death. J. Exp. Med. 1996;183:1957-1964.
34. Levesque R, SPSS Inc. SPSS Programming and Data Management, 4th Edition: A guide for SPSS and SAS Users. Chicago, II; 2007.
35. Cantor JR, Sabatini DM. cancer cell metabolism: One hallmark, many faces. Cancer Discov. 2012;2:881-898.
36. Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, et al. Resistance to cancer chemotherapy: Failure in drug response from ADME to P-gp. Cancer Cell Int. 2015;15:71.
37. Raguz S, Yague E. Resistance to chemotherapy: New treatments and novel insights into an old problem. Br J Cancer. 2008;99:387-391.
38. Alison MR. Cancer. Encyclopedia of life sciences. Nature Publishing Group; 2001.
39. Gabr MT, El-Gohary NS, El-Bendary ER, El-Kerdawy MM, Ni N. Microwave assisted synthesis and antitumor evaluation of a new series of thiazolyl coumarine derivatives. EXCLI Journal. 2017;6:1114-1131.
40. Kalimuthu S, Se-Kwon K. Cell survival and Apiptosis signaling as therapeutic target for cancer: Marine bioactive compounds. Int J Mol Sci. 2013;14:2334-2354.
41. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. Science. 2004;305:626–629.

42. Certo M, Moore VDG, Nishino M, et al. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell. 2006; 9:351–365.

43. Yusof YAM, Md-Saad S, Makpol S, Shamaan NA, Ngah WZW. Hot water extract of Chlorella vulgaris induced DNA damage and apoptosis. Clinics. 2010;65: 1371–1377.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53368