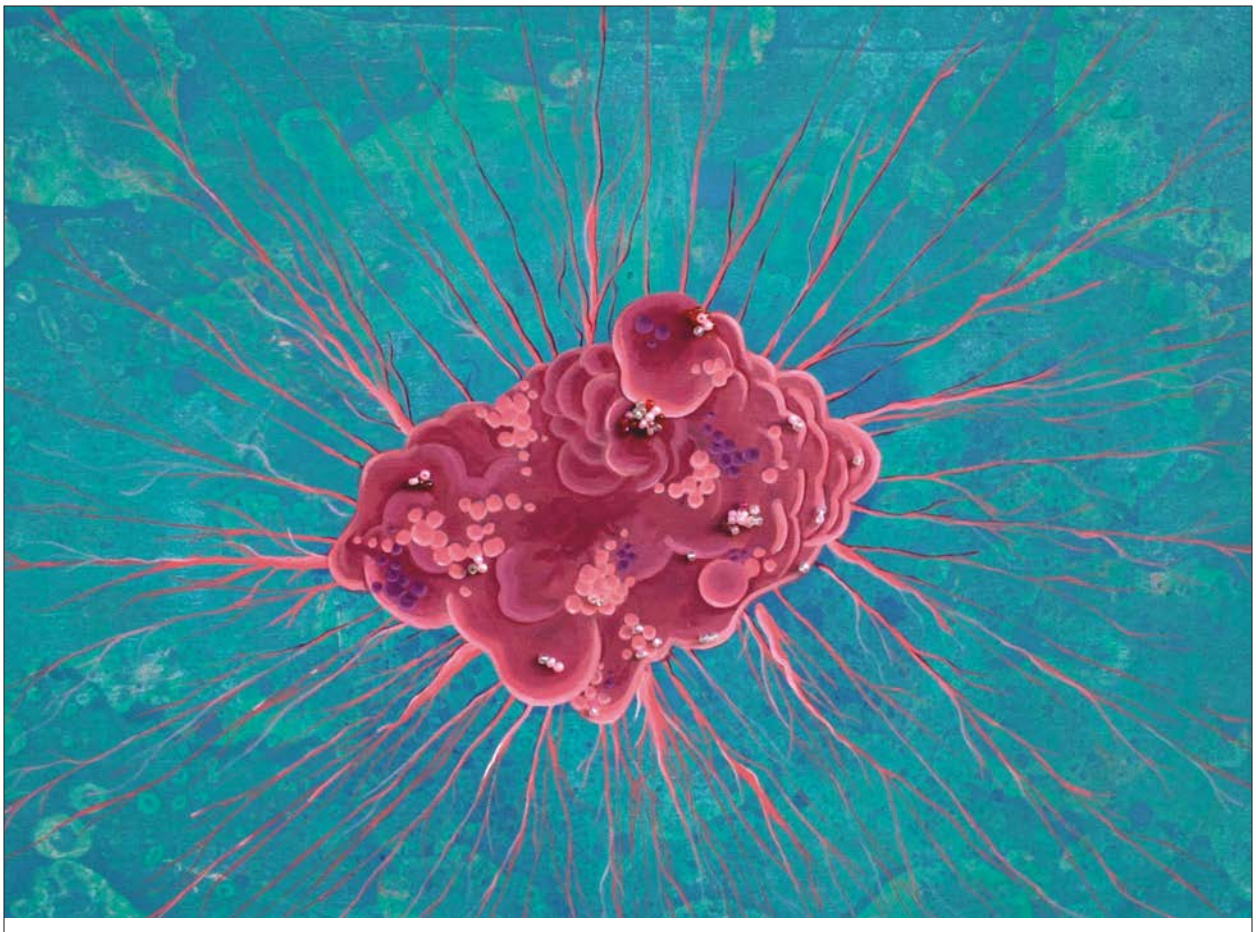

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Anti-tumor Activity of Some 1,3,4-thiadiazoles and 1,2,4-triazine Derivatives against Ehrlichs Ascites Carcinoma

[1,3]Sabry A. EL-Naggar, [2]Ahmed A. El-Barbary, [1]Merveet A. Mansour, [1]Fawzya Abdel-Shafy and [1]Shaimaa Talat

[1]Department of Zoology, Faculty of Science, Tanta University, Egypt
[2]Department of Chemistry, Faculty of Science, Tanta University, Tanta, Egypt
[3]Department of Biology, Faculty of Science, Al-Jouf University, KSA

Corresponding Author: Dr. Sabry A. El-Naggar, Department of Zoology, Faculty of Science, Tanta University, Egypt Tel: (002) 040-3412712

ABSTRACT

Recently, identifying new chemo-preventive agents to replace the current chemotherapies consider one of the most important approaches which could be crucial for cancer treatment. The present study was conducted to evaluate the anti-tumor activity of some newly synthesized heterocyclic 1,3,4-thiadiazole and 1,2,4-triazine derivatives. Different groups of mice were inoculated with Ehrlichs Ascites Carcinoma cells (EAC) intra-peritoneal (i.p.) (2×10^6 cells mouse^{-1}). After one day of inculcation, mice were treated either with cisplatin (reference drug) or with twenty five different new derivatives of 1,3,4-thiadiazoles or 1,2,4-triazines. The anti-tumor activity of these derivatives against EAC-bearing mice were monitored through the changes in the total body weight, total ascetic volume, the number of live and dead tumor cells, median survival time (MST) and some biochemical parameters. The results showed that only five compounds of 1,3,4-thiadiazoles significantly inhibited the tumor progression after 14 days of the treatment. Interestingly, two of these compounds increased the life span of the tumored mice by 34 and 40% when compared with the untreated group. In contrast, all 1,2,4-triazine derivatives didn't show any potential anti-tumor activity against EAC-model. In conclusion, screening of 1,3,4-thiadiazole derivatives showed a potential activity against EAC while, 1,2,4-triazine derivatives didn't show any marked anti-tumor activity.

Key words: Synthetic, organic compounds, 1,3,4-thiadiazoles, 1,2,4-triazines, anti-tumor, efficacy, EAC-model

INTRODUCTION

Cancer represents the largest cause of death all over the world (Abdullaev *et al*., 2000). It claims over 6 million lives annually and nearly ten million new cases are diagnosed globally every year (Abdullaev, 2001). Despite the progress in the efficacy of surgical operations to remove tumor, treatment with chemotherapy and/or radiotherapy, the outcomes are still limited. Even though, the efficacy of the treatment with chemotherapies, the existing drugs have showed severe side effects upon the treatment such as pachtaxel (Vassilakopoulou *et al*., 2010), doxorubicin (Susa *et al*., 2009; Mohan *et al*., 2010) and cyclophosphamide (Dantas *et al*., 2010). To overcome the sever toxicity; several approaches were applied. Our recent studies showed that adoptive immunotherapy with

T-cells, dendritic cells-based vaccination were considered as a potential approach for cancer treatment (Díaz-Montero *et al.*, 2007; Salem *et al.,* 2007, 2009). Recently, the natural medicinal plants are also used for cancer treatment (El-Gawish and El-Sayed Aly, 2001; Khorshid and Moshref, 2006; Khorshid, 2009; Ali *et al.*, 2010; Bisht *et al.*, 2011). One of the essential approaches is to find new synthetic drugs to cure the tumor (Nulgumnalli *et al.*, 2009). In this line, several studies were conducted to identify a novel chemical entity having a broad range of therapeutic activity with fewer side effects (Dantas *et al.*, 2010; Ghorab *et al.*, 2011). It has been shown that new derivatives of quinoline and cis-bis{imidazo(1, 2-a) pyridine} dichloroplatinum (II) had potential anti-tumor effect against ovarian and breast cancer cell lines *in vitro*, respectively (Huq *et al.*, 2006; Rasoul-Amini *et al.*, 2006). It has been reported that different derivatives of 1,3,4-thiadiazoles and 1,2,4-triazines have been evaluated as anti-tumor agents against EACs in mice (Grasso *et al.*, 1984). In addition, some triazoles such as 8,9-dimethoxy-1,2,4-triazolo[4,3-c]quinazolines and 7,8,9,10-tetrahdro-1,2,4-triazolo [4,3-a]phthalazines were evaluated as anti-tumor agents (Hardtmann and Kathawala, 1977; Kadota and Honda, 1978). Furthermore, it has been reported that 3-amino-1,2,4-triazole showed some degree of protection against experimentally induced tumors, although it is also classified as a carcinogen (Feinstein *et al.*, 1978). A number of various thio-ethers derived from 3-hydrazono-5,6-diphenyl-1,2,4-triazines displayed a significant activity against leukemia/lymphoma, CNS, ovarian and small cell lung cancer (Abdel-Rahman *et al.*, 1993). Also, several new 3-thiono-1,2,4-triazine derivatives inhibited the development of chicken fibroblast (Mordarski *et al.*, 1980). In a different study, it has been reported that series of 8-aryl-2,6,7,8-tetrahydroimidazo[2,1-c][1,2,4]triazine-3,4-diones and 8-aryl-4-imino-2,3,7,8-tetrahydroimidazo[2,1-c][1,2,4]triazin-3(6H)-ones were tested in vitro against human LS180, HeLa, T47D, A549 and RPMI 8226 carcinoma (Sztanke *et al.*, 2007). Recently, Mohareb and Mohamed (2011) reported that 1,3,4-triazines showed high inhibitory effects against breast adeno carcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268). The aim of the present study was to investigate the anti-tumor activity of some newly synthesized heterocyclic compounds containing 1,3,4-thiadiazoles and 1,2,4-triazine moities.

MATERIALS AND METHODS

Chemical compounds

Synthesis of 1,3,4-thiadiazoles and 1,2,4-triazine derivatives: These new synthetic derivatives, 1,3,4-thiadiazoles and 1,2,4-triazine were prepared approximately 18 months ago. The twenty five new compounds were prepared at Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt. All melting points were uncorrected and performed by the open capillary melting point apparatus. Microanalyses were performed by Microanalysis Unit, Central Laboratory, Tanta University, Tanta, Egypt.

Reference drug: Cisplatin was commercially available obtained from (Sigma Co.) and injected under the sterile conditions and kept at 4°C until used. Mice were treated with 2 mg kg^{-1} of cisplatin or with 10 mg kg^{-1} of the twenty-five different derivatives of 1,3,4-thiadiazoles or 1,2,4-triazines (Table 1) daily for six days.

Tumor cell line and animals: Ehrlich ascites carcinoma (EAC) cells were originally obtained from the National Cancer Institute (Cairo University, Egypt). The tumor line was maintained in female mice and has been propagated in our laboratory by weekly intra-peritoneal (i.p.) inoculation of

Table 1: Nomenclature of the screened compounds against EAC-model *in vivo*

Abb.	Chemical nomenclature
Start N.	5-Amino-1,3,4-thiadiazol-2-thiol
Cpd.1	3,4,5-Triacetoxy-6-(5-amino-[1,3,4]thiadiazol-2-ylsulfanyl)-tetrahydro-pyran-2-ylmethyl ester
Cpd.2	(6-(5-Mercapto-[1,3,4]thiadiazol-2-ylimino)- 1,2,3,4,5-pentabenzoyl-oxy)-hexane
Cpd.3	6-Bromo-3-(5-mercapto-[1,3,4]thiadiazol-2-yl)-2-methyl-3H-quinazolin-4-one
Cpd.4	6,8-Dibromo-3-(5-mercapto-[1,3,4]thiadiazol-2-yl)-2-methyl-3H-quinazolin-4-one
Cpd.5	3-(5-Mercapto-[1,3,4] thiadiazol-2-phenyl-3Hquinazolin-4-one
Cpd.6	2-[1-(5-Amino-[1,3,4]thiadiazol-2-ylsulfanyl)-ethyl]-benzo[d][1,3] oxazin-4-one
Cpd.7	N-(5-mercapto-1,3,4-thiadiazol-2-yl)-2-(phenylsulfonamido)acetamide
Cpd.8	N-(5-mercapto-1,3,4-thiadiazol-2-yl)-2-(methyl phenylsulfonamido) acetamide
Cpd.9	N-(5-mercapto-[1,3,4]thiadiazol-2-yl)-2-(toluene-4-sulfonylamino)-propionamide
Cpd.10	[(5-Mercapto-[1,3,4]thiadiazol-2-ylamino)-(4-methoxyphenyl)-methyl]-phosphouic acid diethyl ester
Cpd.11	2-Thiono-1,3,4-thiadiazolyl-propane-1,3sultam
Cpd.12	2-Thiono-1,3,4-thiadiazolyl-butane-1,4-sultam
Cpd.13	5-(3-(5-Amino-1,3,4-thiadiazol-2-ylthio)propylthio)-1,3,4-thiadiazol-2-amine
Cpd.14	5-(3-Bromopropylthio)-1,3,4-thiadiazol-2-amine
Cpd.15	2-(5-(5-Oxo-2-phenyloxazolidin-3-yl)-1,3,4-thiadiazol-2-ylthio)acetic acid
Cpd.16	2-(5-(5-Oxo-2-(4-chlorophenyl)oxazolidin-3-yl)-1,3,4-thiadiazol-2-ylthio)acetic acid
Cpd.17	2-Benzylthio-1,3,4-thiadiazolyl-propane-1,3-sultam
Cpd.18	(E)-6-(5-(benzylthio)-1,3,4-thiadiazol-2-ylimino)hexane-1,2,3,4,5-pentanol
Cpd.19	2-(5-Mercepto-1, 3, 4-thiadiazol-2-ylsulfonyl) acetaldehyde
Cpd.20	3-(5-Amino-2-thioxo-[1,3,4]thiadiazol-3-ylmethyl)-3-oxo-butyric acid ethyl ester
Cpd.1T	(E)-N-((4-oxo-3-styryl-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazine-7-yl)methyl)benzenesulfonamide
Cpd.2T	N-(1-(3-benzyl-4-oxo-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazine -7-yl)ethyl)-4-methylbenzenesulfonamide
Cpd.3T	3-Mercapto-6-methyl-1-4-(thiophen-2-ylmethyleneamino)-1,2,4-triazin-5(4H)-one
Cpd.4T	Mercapto-6-methyl-5-thioxo-1,2,4-triazin-4(5H)yl)acetamide
Cpd.5T	4-Amino-6-methyl-1,2,4-triazine-3,5(2H,4H)-dithione

about 2 million cells per mouse. The tumor cells multiplied relatively freely within the peritoneal cavity. The EAC cells were obtained from donor mice on the eight day of tumor growth. The cells were withdrawn by sterile disposable syringe and diluted with physiological saline. The viability of the cells was 97% as judged by trypan blue exclusion assay and counted with haemocytometer.

Adult swiss female albino mice weighting 20±2 g were used and obtained from Teodor Bilharis Institute (TBI), Imbaba, Giza, Cairo, Egypt). Animals were kept in clean and dry plastic cages, as 6 animals per cage in 12 h/12 h dark/light cycle under normal laboratory condition of temperature and humidity, fed with rodent pellets and tap water *ad libitum*.

Experimental protocol: Female swiss albino mice were divided into 28 groups of 7 animals each, as follows: group-1: naive mice only; group-2: EAC-bearing mice (positive controls); group-3: EAC-bearing mice treated with cisplatin (2 mg kg^{-1}) daily for 6 days i.p. groups 4:28: EAC bearing mice treated with twenty five different derivatives of 1,3,4-thiadiazoles or 1,2,4-triazines (10 mg/kg/day). Briefly, after 24 h of inoculation, mice were delivered the doses for six consecutive days. After fourteen days mice were sacrificed, blood samples were collected from all groups and the plasma quickly separated and frozen at -20°C until used.

The anti-tumor activity measurements: The anti-tumor activity was measured in EAC bearing mice with respect to the following parameters; the changes in the total body weight; tumor volume;

total tumor cell counts. In brief, mice were dissected and the ascitic fluid was collected from the peritoneal cavity and the volume of the fluid was measured by taking it in a graduated centrifuge tube. To count the tumor cells, the ascitic fluid was diluted 1:10-1:15 and the cells were counted by staining with trypan blue.

Estimation of percentage increase life span (% ILS): The effect of new organic synthetic compounds on tumor growth was monitored by recording the mortality daily for a period of experiment and percentage increase in life span (% ILS) was calculated.

$$\% \text{ ILS} = \frac{\text{Mean survival of treated group-Mean survival of control group}}{\text{Mean survival of control group}} \times 100$$

$$\text{Mean survival time (MST)} = \frac{\text{Day of 1st death} + \text{Day of last death}}{2}$$

Estimation of the biochemical parameters: After the collection of blood samples, mice were sacrificed and their liver were excised, rinsed in ice-cold normal saline, blotted dry and weighed. A 10% w/v homogenate was prepared in normal saline. The homogenate was centrifuged at 4000 rpm for 10 min at 4°C. The supernatant thus obtained was used for the estimation of transaminases (AST and ALT), alkaline phosphatase (ALP). In addition, some of oxidative stress parameters such as catalase, superoxide dismutase (SOD), glutathione reduced (GSH) and lipid peroxidation (MDA) were assessed only in the groups of mice which showed potential antitumor activity.

Statistical analysis: All values were expressed as Mean±SD. Statistical analysis was performed by student's t-test $p<0.05$ were considered as significant when compared to control.

RESULTS

In this study twenty-five new synthesized organic 1,3,4-thiadiazolyl and 1,2,4-triazine derivatives were evaluated as anti-tumor agents using EAC model *in vivo* (Table 1). The results showed that only five compounds of 1,3,4-thiadiazoles namely 3,4,5-triacetoxy-6-(5-amino-[1,3,4] thiadiazol-2-ylsulfanyl)-tetrahydro-pyran-2-ylmethyl ester (Cpd.1), N-(5-mercapto-1,3,4-thiadiazol-2-yl)-2-(phenylsulfonamido) acetamide (Cpd.7), 2-thiono-1,3,4-thiadiazolyl-butane-1,4-sultam (Cpd.12), 2-(5-(5-oxo-2-phenyloxazolidin-3-yl)-1,3,4-thiadiazol-2-ylthio) acetic acid (Cpd.15) and 2-(5- (5-oxo-2- (4-chlorophenyl)oxazolidin-3-yl)-1,3,4-thiadiazol-2-ylthio) acetic acid (Cpd.16) significantly decreased the total ascetic volume as compared to the untreated tumor bearing mice. As shown in Table 2, the highest reduction in the ascetic volume was 87.5% (Cpd.12) and the lowest was 58% (Cpd.7). In contrast, all of 1,2,4-triazine compounds and the rest of 1,3,4-thiadiazolyl under the evaluation did not show any activity against EAC model *in vivo* (Table 3).

The results showed that all the screened compounds which caused decrease in the total ascetic volume after treatment, significantly showed decrease in the total number of tumor cell counts. As shown in Table 2, among all the tested new compounds of 1,3,4-thiadiazoles, Cpd.1 and Cpd.15 showed highest decrease in the total cell count of tumor cells representing -66 and -70.5%, respectively. As shown in Table 3, treatment with compounds 1T, 2T, 3T and 5T increased both

Table 2: Screening of the anti-tumor activities of some 1,3,4-thiadiazoles derivatives by using of EAC model *in vivo*

	Ascitic volume (mL)		Tumor cell count $\times 10^6$			
Abbrev.	Day 14	% Chnge	Live	Dead	Total	% Change
Untreated	8±1.2	--	85±5.5	17±8.51	02±5.1	--
Cisplatin	ND	ND	ND	ND	ND	ND
Cpd1	3±5.0*	-62.5	28±3.1*	7±11.5*	35±2.1*	-66
Cpd.3	6±1.8	-25.0	83±11	7±1.2*	90±.2	-12
Cpd.12	1±1.0	*-87.5	22±2.1*	26±16.5	48±2.7*	-53
Cpd18	9±1.0	12.5	116±23	19±17	131±6	.028
Cpd19	7±3.0	-12.5	78±2.3	13±6	100±3.1	-2
Control	7.0±1.2	---	74±3.0	4±0.9	78±2.6	---
Cisplatin	ND	ND	ND	ND	ND	ND
Start.	N 5.0±1.3	-28.5	58.0±5.6	3±0.5	61±4.5	-21.7
Cpd.4	9.0±1.4	28.5	116.5±7.	76.0±0.5	122.5±2.0	57
Cpd.15	1.8±3.0*	-74.0	21.0±3.1*	1±0.1*	23±0.9*	-70.5
Cpd.16	1.8±0.7*	-74.0	45.0±3.4*	4±0.2	49±2.5*	-37
Cpd.17	7.0±2.6	0	80.5±6.1	55±4.0	86±1.7	10
Control	12.0±3.5	--	112±22.8	3.8±1.6	116±12.3	--
Cisplatin	ND	ND	ND	ND	ND	ND
Cpd.2	4.7±1.4*	-61.0	93±4.1	3±0.6	96±3.2	-17
Cpd.7	5.0±1.2*	-58.0	79±3.5	4±1.7	83±3.6	-28
Cpd.10	7.0±1.0	-42.0	106±10.1	4±0.5	110±13.0	-5
Cpd.11	6.6±1.2*	-45.0	93±3.5	2.2±1.6	95±2.8	-18
Cpd.13	8.0±1.8	-33.0	85±2.9	3±0.7	88±1.6	-24
Cpd.14	6.0±2.0*	-50.0	83±5.2	2.8±0.2	86±3.7	-26
Control	4.0±1.3	--	85±1	8±1.2	93±0.6	--
Cisplatin	ND	ND	ND	ND	ND	ND
Cpd.5	5.6±1.2	4010	4±9.1	2±0.2	106±8.0	14
Cpd.6	4.5±0.9	12.5	86±5.1	5±0.2	91±4.0	-2
Cpd.8	5.5±0.7	37.5	71±3.7	6.5±1.0	77±4.1	-17
Cpd.9	4.6±1.6	15.0	131±9.9*	27±1.1*	157±13.1*	69

Each reading represent Mean±SD of n = 7. ND: Not determine. *Significance of difference from $p<0.05$ was analyzed by one-way ANOVA test

of the total ascetic volume and the total cell count as compared to their counterparts. Interestingly, among these compounds, Cpd 1T and Cpd5T showed the highest values in the total ascetic volume and the total cell count, respectively.

The results also showed that treatment with Cpds. 1, 7, 12, 15 and Cpd. 16 significantly decreased the total body weight as compared to the untreated bearing mice, where the Cpd16 showed the maximum decrease (5.3%) (Table 4). After the treatment with some of 1,3,4-thiadiazoles and 1,2,4-triazine compounds, the body weight of mice was increased and with no any anti-tumor activity as shown in Table 2 and 3. The only five 1,3,4-thiadiazoles derivatives which showed potential anti-tumor activity were used to determine the mean survival time (MST) as shown in Table 5. The results showed that all the selected compounds increased the MST as compared to their counterparts. Interestingly, as compared to the untreated mice, the treatment with Cpd.15 or 16 increased the MST by 44 and 34%, respectively as shown in Table 5.

As shown in Table 6, AST enzyme increased in cisplatin-treated mice. In contrast, groups of mice which treated with Cpds. 1, 12 or 15 showed insignificant changes in AST as compared with naive mice. Additionally, a significant decrease in the AST activity as compared to their

Table 3: Screening of the anti-tumor activities of some 1,2,4-triazine derivatives by using EAC model *in vivo*

	Ascitic volume (mL)		Tumor cell count $\times 10^6$			
Abbrev	Day 14	% Change	Live	Dead	Total	% Change
Control	4.0±1.3	--	85±1.0	8±1.2	93±0.6	--
Cisplatin	ND	ND	ND	ND	ND	ND
Cpd.1T	6.5±2.4	*62.5	117±5.8*	15±1.1*	132±15.5*	42
Cpd.2T	5.9±1.5	47.51	58±1.6*	12±2.5*	170±17.3*	83
Cpd.3T	6.1±1.6*	52.5	85±2.0	5±1.1	90±3	-3
Cpd.4T	4.6±0.7	15	137±7.5*	13±1.8*	150±125*	61
Cpd.5T	5.8±0.8*	45	160±9.6*	15±2.8*	175±10.6*	88

Each reading represent Mean±SD of n = 7. ND: Not determine. *Significance of difference from $p<0.05$ was analyzed by one-way ANOVA test

Table 4: Assessment the changes in the total body weight after inoculation with EAC cells and treatment with 1,3,4-thiadiazoles derivatives for six consecutive days

	Total body weight				
Abbrev	D0	$D4^{th}$	$D8^{th}$	$D14^{th}$	% Change
Untreated	24.4±0.7	24.9±0.5	28.6±2.1	31.4	28.7
Cisplatin	24.5±1.0	22.6±1.9	18.9±2.1	16.9±2.1	-31.0
Cpd.1	24.0±0.8	23.9±1.7	26.7±2.9	26.8±2.1	11.6
Cpd.7	23.0±0.9	22.8±1.5	25.6±2.9	26.0±0.5	13.0
Cpd.12	24.7±2.9	23.6±3.7	29.4±3.4	27.1±3.1	9.7
Cpd.15	21.6±2.9	21.6±3.8	21.2±3.5	21.8±1.9*	0.9
Cpd.16	22.5±1.5	21.8±1.6	23.0±0.8	21.3±2.2*	-5.3

Each reading represent Mean±SD of n = 7. *Significance of difference from $p<0.05$ was analyzed by one-way ANOVA test

Table 5: Shows the mean survival time (MST) of mice inoculated with EAC cells on day zero and treated with some derivatives of 1,3,4-thiadiazoles for six consecutive days

	Mean survival time (days)			
Groups	Dose (mg kg^{-1})	MST[a]	% T/C	% ILSb
Untreated	--	15.8±1.1	--	--
Cisplatin	2	31.0±1.4	196.2	96.2
Cpd.1	10	20.4±2.1	129.0	29.0
Cpd.7	10	17.8±2.5	112.7	12.7
Cpd.12	10	20.4±3.1	129.7	29.0
Cpd.15	10	22.8±2.5*	144.0	44.0
Cpd.16	10	21.2±2.8*	134.0	34.0

[a]Mean survival time. [b]Percentage increase in life span. *Significance of difference from $p<0.05$ was analyzed by one-way ANOVA test

counterparts was noticed when the tumor-bearing mice treated with Cpd7 or Cpd16. The values of AST enzyme in these groups were found to be 28.0±2.9 and 38.8±2.1 UL^{-1}, respectively. Interestingly, ALT activity decreased in EAC-bearing mice and all groups treated with the tested compounds as compared with naive mice (Table 6). Of note, treatment with cisplatin, return the level of ALT enzyme into the normal range. Furthermore, as compared to the naive mice, the level of creatinine and urea increased in all the treated groups with new synthetic derivatives as well as in the untreated EAC-bearing mice (Table 6). Interestingly, as compared to naive mice, the treatment with cisplatin or Cpd. 7 recorded the highest values of creatinine among all the

Table 6: Effect of the treatment with 1, 3, 4-thiadiazolyl Cp derivatives on the kidney function parameters (Creatinine and Urea) on tumor bearing mice

	Liver functions (homogenate 1%)		Kidney functions (homogenate 1%)	
Groups	AST[a] (U L^{-1})	ALT[b] (U L^{-1})	Creatinine (mg dL^{-1})	Urea (mg dL^{-1})
Naive	45.0±4.7	48.0±1.5	1.2±0.08	38±3.7
Untreated	51.0±4.3	44.0±2.3	1.3±0.3	51±9.7
Cisplatin	56.0±1.3*	49.0±0.6	1.7±0.1*	55±4.7*
Cpd.1	50.5±3.2	35.5±1.2	1.6±0.5	62±4.3*
Cpd.7	28.0±2.9*	12.0±0.6	1.7±0.1*	63±5.7*
Cpd.12	42.0±2.9	41.0±1.5	1.4±0.4	51±6.5*
Cpd.15	42.0±3.4	46.0±1.4	1.7±0.2*	59±3.1*
Cpd.16	38.8±2.1*	44.0±0.7	1.5±0.2	41±3.8

Each reading represent Mean±SD of n = 7. [a]Aspartate aminotransferasc, [b]Alamine aminotransferasc. *Significance of difference from $p<0.05$ was analyzed by one-way ANOVA test

Table 7: Effect of the treatment with 1, 3, 4-thiadiazolyl Cp derivatives on the lipid peroxidation glutathione content and antioxidant enzymes (SOD and Catalase) in the liver of EAC-bearing mice

	Liver (homogenate 10%)			
Groups	MDA[a] (umole g^{-1})	GSH[b] (mg g^{-1}. tissue)	SOD[c] (U g^{-1}. tissue)	Catalase (mol min g^{-1}. tissue)
Naive	162.0±9.10	2.5±0.15	4.7±0.20	235.3±19.2
Untreated	227.5±16.2*	1.6±0.20	2.1±0.15	154.0±15.9
Cisplatin	213.0±10.6*	1.7±0.20	1.5±0.10	113.6±12.2
Cpd.1	160.0±14.0	1.7±0.20	2.1±0.15	112.3±8.3
Cpd.7	116.6±11.5*	1.4±0.11	1.1±0.12*	91.5±7.5*
Cpd.12	310.0±20.9*	1.9±0.15	2.0±0.15	131.6±9.2
Cpd.15	203.0±11.5*	1.9±0.10	2.6±0.10	105.0±5.0
Cpd.16	176.7±12.5	1.7±0.15	1.6±0.13	103.0±4.5

Each reading represent Mean±SD of n = 7. [a]Malonaldehyde, [b]Glutathione, [c]Super oxided dismutase. *Significance of difference from $p<0.05$ was analyzed by one-way ANOVA test

other groups. As compared to naive mice, except Cpd. 16, all compounds increased the level of urea in serum samples.

Determination of antioxidant enzymes and lipid peroxidation: In the present study, the levels of MDA were significantly increased in EAC bearing mice, treated with cisplatin, Cpds. 12, 15 or Cpd. 16-treated mice. In contrast, the treatment with Cpd. 7 significantly reduced the MDA levels when compared with naive mice (Table 7). The results showed that among the tested compounds, the treatment with Cpd. 12 showed the highest value of MDA which was found to be 310.0±20.9 nmole g^{-1}, while the treatment with Cpd. 1 recorded the lowest value which was found to be 160.0±14.0 nmole g^{-1}. The levels of reduced GSH were found to be significantly reduced in cisplatin or treated with Cpds. 1, 7, 12, 15 or Cpd. 16 when compared with naive mice (Table 7). Also, the level of antioxidant enzyme significantly reduced in EAC bearing mice alone or all other treated groups. Interestingly, similar to MDA, Cpd. 7 showed the lowest values of SOD and catalase (Table 7).

DISCUSSION

Several studies were conducted to minimize the side effects after treatment with the chemotherapeutic agents using different modalities (Dantas *et al.*, 2010; Mohan *et al.*, 2010;

Ghorab *et al.*, 2011; Sodde *et al.*, 2011). One of these modalities is to seek for new drugs with fewer side effects. In the present study, twenty new synthetic organic derivatives of 1,3,4-thiadiazoles and five 1,2,4-triazines were evaluated as anti-tumor agents. The evaluated compounds had varying degrees of anti-tumor activities using the EAC-bearing mice as compared with the reference drug; cisplatin. The results showed among the twenty new derivatives of 1,3,4-thiadiazoles, as shown in Table 2, only five compounds were significantly decrease the total ascetic volume by 87.5, 58, 62.5, 74 and 74%, compared to the untreated tumor bearing mice. The present work was in agreement with the previous study by Grasso *et al.* (1984) who reported that thiadiazole derivatives had anti-tumor activity. Furthermore, Cpds.1 and 15, showed the highest decrease in the total cell count of tumor cells representing -66 and -70.5%, respectively. The results showed that among the five selected compounds, only two of them 15 and 16 significantly increased the MST by 44 and 34%, respectively. This increase in the MST should explain the presence of anti-tumor activity of these compounds based on the prolongation of the life span of animals (Clarkson and Burchenal, 1965).

Abdel-Rahman (2001) showed that a number of various thioethers derived from 3-hydrazono-5,6-diphenyl-1,2,4-triazines displayed a significant activity against Leukemia/Lymphoma and small cell lung cancer. Furthermore, several new 3-thiono-1,2,4-triazine derivatives showed anti-tumor activity *in vitro* and *in vivo* systems (Mordarski *et al.*, 1980). In contrast, the present work showed that all the tested 1,2,4-triazines did not show any promising activity against EAC model *in vivo*.

This study showed that AST enzyme increased in both of the untreated group (tumor alone) and cisplatin-treated mice. In contrast, groups of mice which treated with Cpd.12 or Cpd.15, showed non-significant changes in AST as compared with naive mice. These results could explain that these compounds have no remarkable liver toxicity. Interestingly, ALT activity decreased in EAC-bearing mice and in all groups treated with new synthetic compounds as compared with naive mice. This study was in agreement with the study which showed that the treatment with cisplatin return the level of ALT enzyme into the normal range (Fahim *et al.*, 1993). Malondialdehyde (MDA) is formed during oxidative degeneration as a product of free oxygen radicals (Valenzuela, 1991; Hanafi and Mansour, 2010) which is accepted as an indicator of lipid peroxidation (Neilsen *et al.*, 1997). The present work indicates that thiobarbituric acid reactive substances levels in the tested cancerous tissues are higher than those in normal tissues. These results are in agreement with the Louw *et al.* (1997) and De Cavanagh *et al.* (2002). This emphasizes the reduction in free radical yield and the subsequent decrease in harm and damage to the cell membrane and decrease in MDA production. In the present study, the levels of MDA were significantly increased in EAC bearing mice, treated with cisplatin, Cpds. 12, 15 or Cpd. 16-treated mice. In contrast, the treatment with Cpds. 1 or Cpd. 7 significantly reduced the MDA levels when compared with naive mice.

Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system (Sinclair *et al.*, 1990). In this study, GSH levels were found to be significantly lower than that in the EAC control mice. The levels of reduced GSH were significantly decreased in EAC-bearing mice. Also, the levels of reduced GSH were found to be significantly reduced in cisplatin or treated with Cpds. 1,7,15 or Cpd. 16, when compared with naive mice. In this study, as compared to the naive mice, the levels of SOD and catalase were decreased in all other groups. These results were in agreement with the study which showed tumor growth led to the inhibitions of SOD and CAT activities (Marklund *et al.*, 1982).

CONCLUSION

In present study, it was noted that among the twenty five compounds of 1,3,4-thiadiazoles and 1,2,4-triazines which screened as anti-cancer agents, only five 1,3,4-thiadiazoles derivatives were significantly reduced tumor growth, viability of tumor cells and raising life span as compared with those of EAC control mice. Therefore, it can be concluded that these compounds demonstrated remarkable anti-tumor effect in EAC-bearing mice.

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