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IN VIVO EVALUATION OF NEWLY DESIGNED RHODAMINE -BASED DERIVATIVES AGAINST EHRLICH ASCITES CARCINOMA BEARING MICE MODEL

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ABSTRACT

In recent years, heterocyclic compounds are acquiring more importance because of their wide spectrum of biological and pharmacological activities. Also, exhibit exciting medicinal properties including anticancer. The rhodanine (2-thioxothiazolidin-4-one) derivatives are considered as the privileged heterocyclic system in modern medicinal chemistry especially in the discovery of new anticancer agents due to their great affinity to many anticancer biotargets. In the present study, newly designed rhodamine -based derivative 8-imino-9-(4-nitrophenyl)-7-phenyl-5,7,8,9-tetrahydro-2H thiazolo [5',4':5,6] pyrano [2,3-d] pyrimidine-2,6(3H)-dithione (N_2) was synthesized to evaluate its anticancer potential against Ehrlich Ascites tumor (EAC) bearing mice. The antitumor effect was assessed by evaluating tumor volume, tumor cell count, survival time and increase in the life span of EAC bearing mice also improvement of hematological status and food intake. The therapeutic impact of three consecutive doses of N2 was used comparing with the traditional drug 5-FU (5-flurouracil) and their mix. A total of 90 female adult Swiss albino mice were randomly divided into nine groups (n=10). Inoculation of $2.5x10^6$ EAC in all treated groups followed by different doss's injection of the (N2) compound to prove its antitumor effect, the ability to improve hematological parameters and food intake compared to positive EAC (Ehrlich ascites carcinoma) group. Normal mice were treated with compound of dose (100 mg/kg) to show its low toxicity effect compared to negative normal group and also treated with DMSO to the same reason. Our results demonstrated that the new compound N2 significantly reduced ascites tumor volume, cell number, and increased the life span of EAC-bearing mice. In addition, the new compound N2 can return the hematological parameters to their normal levels mice also managed to regain their appetite by increasing the dose in dose dependent manner. The highest dose of the compound and the 5-FU gave similar results and their mix group gave us the best result ever. From our findings, it is noted that the N2 derivative may be possible candidate for anticancer therapy with the ability to inhibit tumor cell proliferation.

KEYWORDS: Heterocycles; 2-Thioxothiazolidin-4-one, Rhodanine, Ehrlich ascites carcinoma; 5-flurouracil; food intake; Hematological parameters.; Lifespan.

INTRODUCTION

Cancer (otherwise termed as malignant, neoplasm), is a category of disorders in which abnormal cells develop and spread uncontrollably[1] and has the ability to spread.[2] throughout your body. The spread of cancer cells, known as metastasis, leads to death[3] worldwide based on the world health organization (WHO) population-based data.[4] Cancer is considered the second leading cause of death (14%), following cardiovascular disorders[5] (46%). No communicable diseases, including cancer, caused 86% of the deaths in Egypt.

Symptoms of cancer vary depending on the type and location of the disease[6], and its treatment is based on the different internal and external factors causing cancer. Different screening tests are used to detect cancer, and a variety of therapies are now available, including gene therapy, chemotherapy, surgery, radiation therapy, and immunotherapy.[7] In the future, up to 2030, Cancer is predicted to be diagnosed in 22.2 million people. Therefore, identification of novel potent, less toxic and selective mechanism of action anticancer agents remains one of the most imperative health problems.[7]

Ehrlich ascites carcinoma is a spontaneous influence can affect murine mammary adenocarcinoma carried in outbred mice by serial successive intraperitoneal (i/p) passages. lacking H-2 histocompatibility antigens are responsible for its rapid growth.[8] EAC cells are undifferentiated, hyper-diploid in nature[9], aggressive and completely tumorigenic. EAC 's metastatic behavior spreads to the lungs, liver, spleen, kidney, bone,

diaphragm, blood, and adrenal glands causing enlargement and dysfunction of these organs.[10] EAC generate free neoplastic cells in peritoneal ascites fluid and may be passaged and maintained in mice's peritoneal cavities with an increase in the number of passes enhances the aggressiveness of EAC cells. One significant advantage of utilizing ascites carcinoma cells the total sum of cells administered in mice can be controlled. Since neoplastic cells proliferate in free suspension, there is no need for Mechanical tension required for cell breakdown. So Ehrlich ascites tumor was chosen as a rapidly growing experimental tumor model where various experimental designs for anticancer agents can be applied.[11]

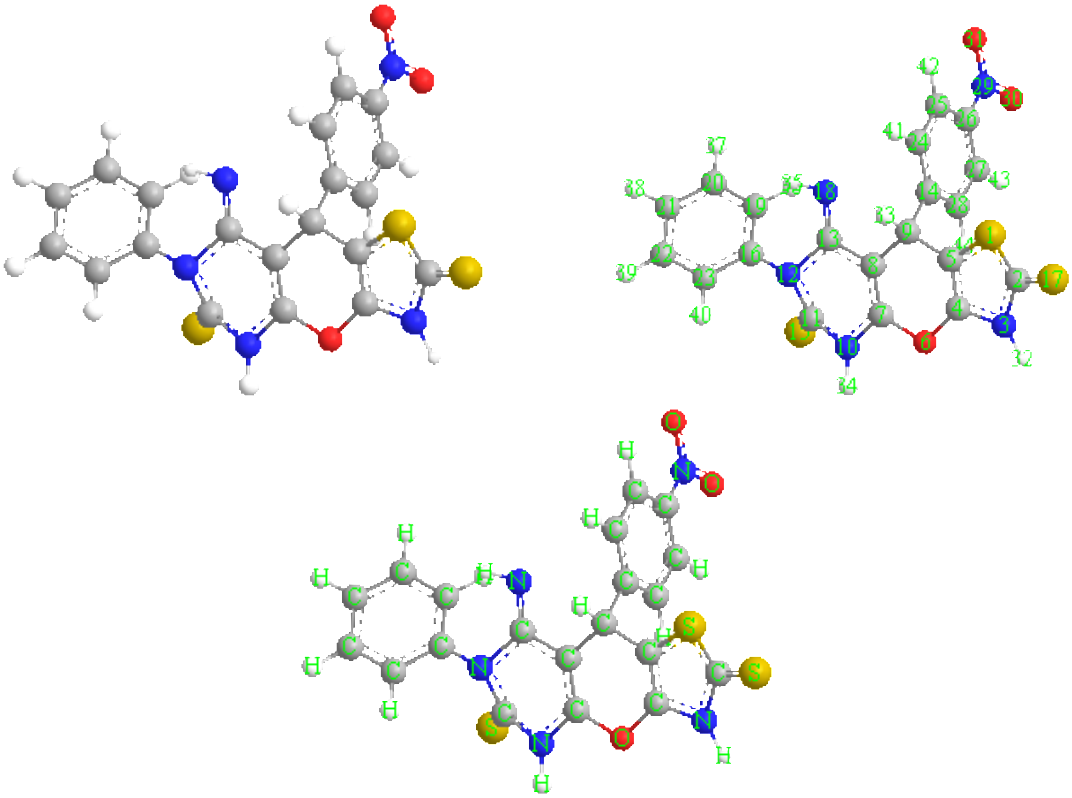
Heterocyclic compounds have remarkable and unique therapeutic effects, including anticancer capabilities.[12] The usage of heterocycles is a valuable tool for modifying the solubility, lipophilicity, polarity, and hydrogen bonding capacity of biologically active compounds, resulting in the optimization of the ADME/Tox characteristics of medications or drug candidates.[13] Otherwise rhodanine consider as important class of heterocyclic compounds[4] with a broad spectrum of biological activities; they are used as antimicrobial[14], antiviral[15], antitubercular[16], anti-inflammatory[17], antidiabetic[18], anticancer and antitumor agents.[19-22]

Rhodanines were found to induce apoptosis through the modulation of the Bcl-2 family proteins[23,24] or through the modulation of other key signaling proteins.[25,26] Moreover, rhodanines were also reported to reveal their anticancer activity through the inhibition of the phosphatase of regenerating liver (PRL-3).[27] In view of the abovementioned findings and in continuation to our effort to the synthesis a variety of heterocyclic ring systems for biological and pharmacological evaluation[28– 32] N1 andN2 were designed, synthesized to fulfill the objectives of the target anticancer activity.

MATERIAL AND METHODS

Chemicals

Rhodanine powder, Malononitrile, DMF, DMSO, dioxane and ethanol was purchased from Sigma (St.Louis,MO,USA) and Merck Chemical Co. (Germany).5-flurouracil (used as a standard drug) was purchased from local pharmacy. The rest of reagents and materials were available in chemistry department, Faculty of Science, Damietta university. Chemicals and reagents of the highest available pure grade were used. Solvents were redistilled by standard techniques before use. New synthetic compound (N1) 5-amino -7-(4-nitrophenyl)-2-thioxo-3,7-dihydro2H-pyrano[2,3-d]thiazole -6-carbonitrile and (N2)8-imino-9-(4-nitrophenyl)-7-phenyl-5,7,8,9-tetrahydro-2H-thiazolo [5',4':5,6] pyrano[2,3-d] pyrimidine-2,6(3H)-dithione derivativeس was synthesized in our laboratory, faculty of science Damietta university.



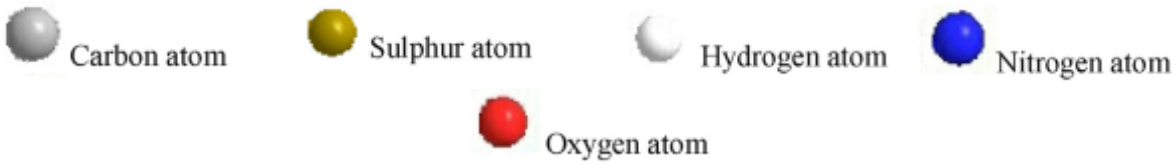


Fig. 1. The molecular structures forN2.

EXPERIMENTAL ANIMAL

Nanty Swiss albino adult female mice with an a mean average body weight of 20 to 25**.** They were housed in polyethylene cages in a controlled environment and kept in agreement with institutional and national official guidelines under standard conditions (temperature: 23+1 ^{o}C and 12/12 h dark/light cycle and controlled humidity conditions according to the agreed rules of the criteria outlined in the in the "Guide for the care and Use of Laboratory Animals" prepared by the National Institute of Health (NIH)[33] **Albus, U. (2012).**

Ehrlich Ascites Carcinoma model preparation

Ehrlich ascites carcinoma (EAC) cells were collected from the ascetic fluid of parent female Swiss albino mouse harboring 8–10 days old ascetic tumor. Viability is calculated by trypan blue assay and 2.5 x 1o^{6} EAC cells were injected intraperitoneally in each mouse selected for the experiment on day 0. The next day, animals were randomized and divided into nine groups. Five treatment groups contained 10 animals each and received different doses of the new synthesized compound, 5-FU and their mix. One positive EAC control untreated group contained 10 animals and didn't receive any treatment to show the efficacy of the synthesized compound in the treated groups.

Experimental design

Mice were selected and divided randomly into nine group (n=10).

Group 1: normal healthy negative control mice didn't receive any treatment.

Group II: EAC untreated positive control mice injected with (2.5 x 10^{6} EAC cells in 0.2 ml saline / mouse) for once at day zero.

Group III: Mice were injected with (2.5 x 10^{6} EAC cells in 0.2 ml saline / mouse) for once at day zero (the first day of the experiment) and the day after that they were treated with 5-flurouracil of dose (51 mg/kg) dissolved for one time (0.2 ml / mouse)

Group IV, group V and group VI: Mice were injected with (2.5 x 10^{6} EAC cells in 0.2 ml saline / mouse) for once at day zero and day after that they were treated with three different doses of the freshly prepared new synthesized rhodanine base heterocyclic compound (50 ,75, 100 mg/kg) respectively dissolved in (0.2 ml DMSO / mouse /day after day) for ten days.

Group VII: Mice were injected with (2.5 x 10^{6} EAC cells in 0.2 ml saline / mouse) for once at day zero and day after that they were treated with combination of the freshly prepared new synthesized rhodanine -base heterocyclic compound of dose (100mg/kg) and 5-flurouracil of dose (51 mg/kg) dissolved in (0.2 ml DMSO / mouse /day after day) for ten days.

Group VIII: normal healthy mice treated with the new freshly prepared rhodanine heterocyclic compound (N2) of dose (100 mg/kg) dissolved in (0.2 ml DMSO /mouse/day after day) for ten days.

Group IX: normal healthy mice treated with (0,2 ml DMSO / mouse / day after day) for ten days.

On day 10, mice of all different groups were fasted for 18 hours then sacrificed and for the detection of the effect of different doses of the (N2) compound on the hematological status of EAC bearing mice. Different samples of Blood were withdrawn from mice heart and collected tubes for hemoglobin, red blood cells (RBCs), white blood cells (WBCs) and platelets detection.

Evaluation of antitumor effect on tumor proliferation

1.Tumor growth response

Cancer anti-proliferation effects of **N2** compound was assessed by observation of the change with respect to tumor volume, body weight, viable /non-viable tumor cell count median survival time (MST) and percentage increase in life span (%ILS).

1.i.Tumor volume

The ascitic fluid was taken from the peritoneal cavity of the mice. Using graduated centrifuge tube, the packed cell volume was measured by centrifugation for 10 min at 1000 rpm.

1.ii. Tumor cell count

Anti-tumor potential was evaluated by measuring tumor cell count and inhibition of EAC growth. Ascetic fluid of EAC tumor bearing mice was collected in a Wintrobe's tube. The total number of tumor cells was counted by the Trypan blue exclusion assay.[34] The total number of EAC cells was calculated by using the formula:

Total EAC count = Mean number of unstained cells × volume of count samples × dilution × 10^{4} /m

1.iii.Viable/non-viable tumor cell count

The viability of EAC cells was determined by trypan blue exclusion method [34](Mcliman WFet-al.,1957), where the total and viable cells (non stained) were counted at magnification x40.

The ascitic fluid was collected with a help of WBC pipette and diluted 100 times. The trypan blue (0.4% in normal saline) dye was used to stain the cells.

Thus, the viable and non-viable cells were counted and determined in the studied groups.

$$\text{Cell count} = \frac{\text{No. of cells} \times \text{Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

1.iv.Median survival time (MST) and percentage increase in life span (%ILS).

MST was monitored by recording the mortality daily for 8 weeks and % ILS was calculated using the following equations

MST= (day of first death + day of last) /2

%ILS= [(MST of treated group /MST of control group) - 1] x100

1.iv.Body weight

Body weights of the experimental mice were recorded both in the treated and control groups at the beginning of the experiment (day 0) and sequentially on the 3rd, 6th and 9th day during the treatment period to evaluate the relative change. The percentage increase in weight was calculated as per the following equation:

$$\%\ \text{increase in weight} = \left(\frac{\text{Animal weight on respective day} - \text{Animal weight on day 0}}{\text{Animal weight on day 0}}\right) \times 100$$

1.v.Hematological parameters evaluation

The hematological status can be predicted by the evaluation of the hematological parameters that can reflect the general health of body.[35,36] Hematological parameters. Red blood cells (RBCs), white blood cells (WBCs), platelets (PLT) counts and haemoglobin (Hb) level were estimated using the methods of D'Armour et al.,[37] Dacie and Lewis,[38] Brecher and Cronkite,[39] and Drabkin and Austin,[40] respectively.

1.vi. Food intake

Each cage of mice received food on a daily basis. The remaining food was collected and weighed the next day. This value was subtracted from the quantity of diet delivered the previous day to determine the daily food intake. The mice's health was checked on a daily basis for gross toxicity assessment caused by the ehrlish ascites carcinoma injection and also for assessment of the antitumor effect of the new synthesized compound after treatment.

Statistical analysi

The SPSS software version 25 was used for statistical analysis. The findings were recorded as mean ± SE. To identify the significant variation between test groups and controls, the student (t) test and One-way ANOVA test were used.

RESULTS

Table (1) summarizes the effect of N2 on EAC cells volume count, viability% and packed EAC dead cell %. The results showed increased tumor volume in the EAC cell-bearing mice (group II) animal, whereas on treatment with the compound N2 decreased the tumor volume. The mean volume of EAC in positive control group was found to be 3.82±0.2(ml) this value was significantly decreased by 56,66 and 82% in50,75,100 mg/kg dose of N2treated groups respectively, also 5-FUtreated group reduced it by 80% while the mix of N2and 5-FU afforded the best result ever by 90%, compared to the positive control group, Fig (1).

The mean of EAC cells count in the positive control group was found to be (214±6.2) $x10^6$ cell/ml, which significantly decreased gradually by increasing the dose of the new compound by 102±5.4, 80±4.4and 74±3.8 and therefore the cell viability % compared to the EAC control group. The treatment with 5-flurouracil gave results similar to the highest dose of the newly compound (100mg/kg). it is found that dose dependent reduction in all previous ant proliferative parameters as shown in table (1) and Fig (2).

The mean body weight value in the positive control group was found to be increased 33.1±2.6 (gm) involving the tumor volume compared to negative control group 23.7±1.0 (gm) while rhodamine derivative N2 50, 75,100 mg/kg and 5-FU downregulated it near or as in the negative control group and DMSO control group.

A significant decrease in body weight changes % recorded gradually (30.41±2.6, 27.24±1.5, 18.95±1.2 %) by the treatment with the different doses of the new compound in a dose dependent manner (50,75,100 mg/kg) respectively. In the case of the mix treated mice, gain in body weight was only 16.025±1.8 % indicative of its efficiency in bringing down the progression of cancer.

It can be hypothesized that increased body weight in the positive control group was attributed to increased tumor growth volume but down regulated near or as in negative control group in treated groups due to tumor growth inhibiting property of N2 (Table 3, Fig1).

Survival parameters

The effects of the compound on survival of tumor-bearing mice are shown in Table 3. Results showed that the survival time of treated mice was markedly increased by the different doses of the compound in a dose-dependent manner. Both the compound at a high dose of 100 mg/kg and the mixed groups recorded the highest MST against untreated EAC-bearing mice (62.5 and 67). So, they had a high percentage of the life span (81% and 94%), respectively. The treatment with standard drug (5-FU) recorded a significant increase in MST and IL%, which were 61.5 and 78.3%, respectively, compared with the untreated EAC group. Treating with the new complex raised life span to (33.3%), (68%), and (81.1%) gradually by increasing the doses (50, 75, and 100mg/kg) in a dose-dependent manner compared to EAC bearing mice (untreated EAC group).The four remaining mice from each group (normal, complex at a dose of 100 mg/kg, and solvent (DMSO) treated) survived for an

extended period of time after the MST, ILS% recording. It was time for more than two months without any abnormalities in their activities and behavior. And this observation proved the non-toxicity of the compound.

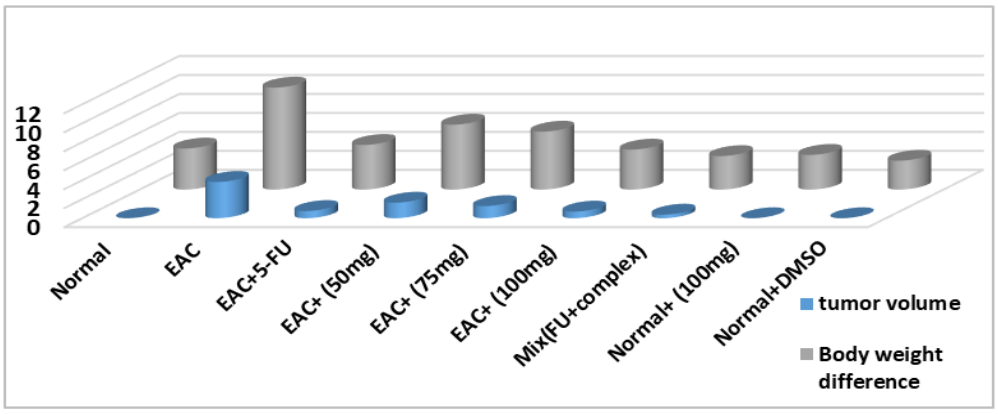


Fig. (1) Effect on tumor volume and body weight difference in all studied groups.

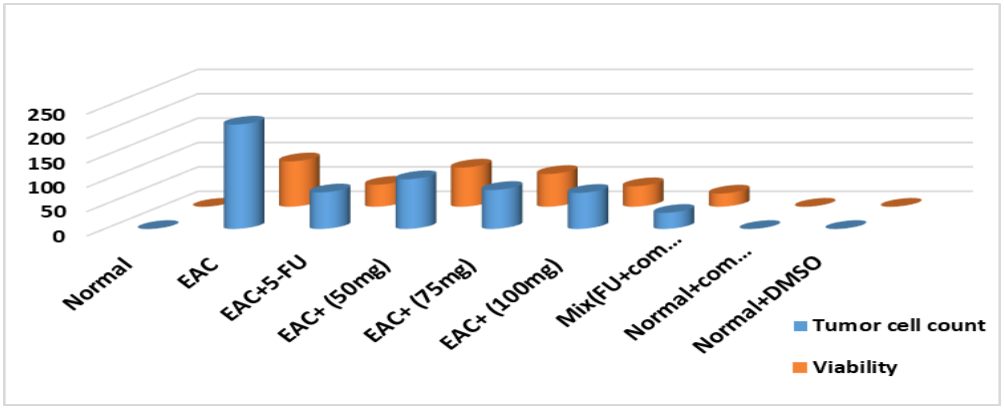


Fig (2) Effect on tumor cell count and viability% in all studied groups.

The effect on the hematological parameters in all studied groups

The treatment with the new heterocyclic compound can restore the normal hematological status in dose dependent manner compared to the Ehrlich bearing mice. By increasing the dose of the new compound, the hematological parameters improved that reflected on the general health body. It was found that all the hematological parameters of the untreated EAC group had extremely significant (P1<0.001) differences in their levels compared to the normal control group. The levels of RBC count, Hb and platelets decreased (5.27±0.21, 13.07±0.19, and 214.5±8.31) respectively. However, the WBC count increased by 13.07±0.19 by Ehrlich ascites carcinoma cells injection into the mice of the untreated group. It was observed with the treatment that the mice's health improved, which included an improvement in the hematological state, which was reflected in the levels of the hematological parameters. there was extremely significant (P1<0.001) changes in the hematological parameters of all the treated group compared to the Ehrlich bearing mice . Red blood cells (RBCs) levels recorded 6.23±0.18, 6.84±0.21 and 7.35±0.26 when Ehrlich bearing mice were treated with the three different doses (50, 75, and 100 mg/kg) respectively. Hemoglobin (Hb) restored its normal level gradually by increasing the dose of the treatment to reach their normal level of the different treated groups. Hemoglobin recorded 11.19±0.09, 11.51±0.29 and 12.24±0.04 by the treatment with the different doses (50, 75, 100) respectively. platelets levels were increased gradually 273.03±8.2, 299±6.1 and 359.5±10.2 by the treatment with 50, 75 and 100 mg/kg of the new heterocyclic compound. WBCs levels were decreased and improved gradually to reach 11.56±0.21, 10.37±0.34 and 8.16±0.14 by the treatment with 50, 75, 100 mg/kg respectively. The treatment with 5-flurouracil caused also extremely significant(P1<0.001) differences in all parameter levels (RBCs, WBCs, Hb, and PLT) compared to the untreated group to be 7.2±0.07, 8.27±0.18, 12.04±0.04 and 361.8±10.2 respectively. The treatment with mix restored the parameter levels to their normal level compared to the normal group. so, there was non-significant P1=0.692, P1=0.085, and P1=0.396 differences in (RBCs, WBCs, Hb and PLT) levels of the mix group to reach 7.91±0.29, 7.85±0.21, 12.33±0.08 and 379.8±4.4 respectively. These results were able to prove the antitumor effective therapeutic ability of the new heterocyclic compound. Besides, to prove the non-toxicity of the compound on healthy cells, the dose (100 mg/kg) of the new complex was injected into healthy mice and the hematological parameters were measured, and it was found that it gave the same levels of parameters as the normal group, and this was statistically

clarified by the presence of non-significant P1=0.204, P1=0.373 and P1=0.631differences in the measured parameters (WBCs, Hb, and PLT) compared to the normal group respectively. The RBCs, WBCs, Hb and Platelets levels were 7.54±0.11, 7.96±0.09, 12.44±0.09 and 382±2.6 respectively. DMSO was also able to maintain the normal levels of the parameter, which confirmed its safe use as a solvent on normal cells of mice as shown in table (2)&Fig(3).

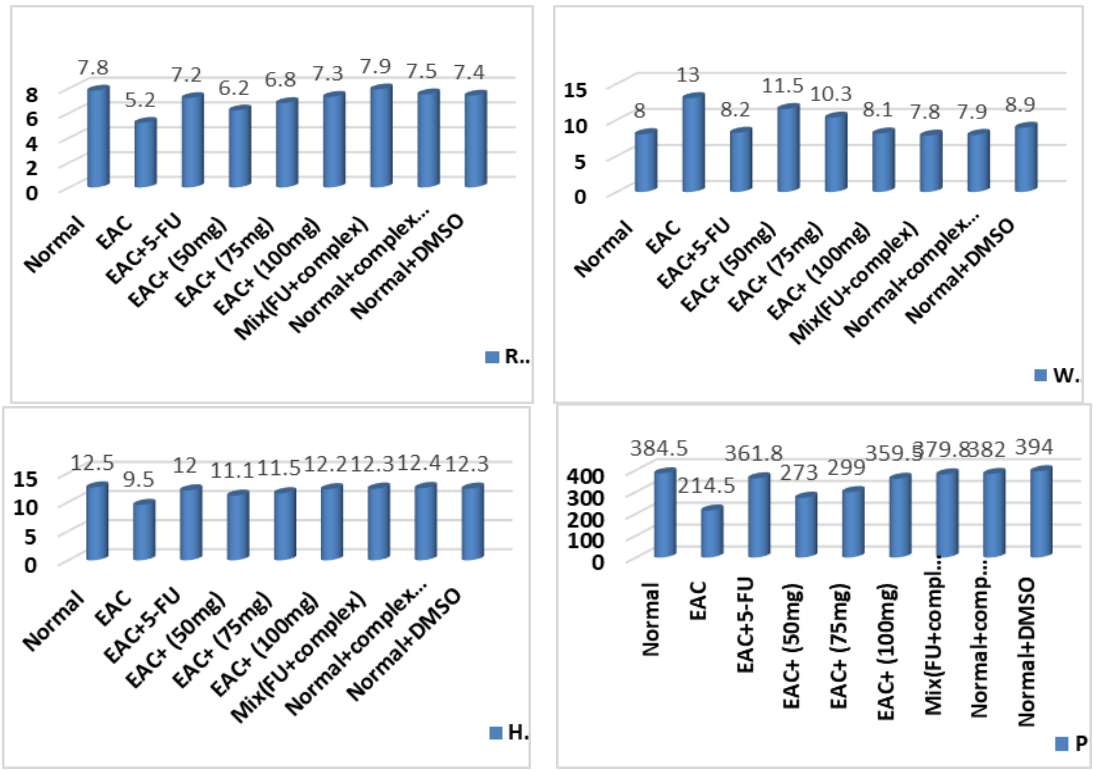


Fig. (3): Effect of the different doses of the newly synthesized compound, 5-FU, Mix, normal and EAC injection on RBCs count, WBCs count, Hgb and platelets.

Effect of the newly synthesized rhodamine derivative on the food intake of mice

It was found from the results that there was a direct relationship between the appetite of mice, which is reflected in the amount of food eaten and their physical health. Ehrlish bearing mice were recorded the lowest amount of the food intake (2.8±0.01 gram/day/mouse). The treatment with the new complex increased the amount of food intake (3±0.05, 3.2±0.04, 4±0.06 gram/day/mouse) gradually by increasing the dose 50, 75, 100mg/kg. Mice were treated with 5-flurouracil and regained their appetite well compared to ehrlish bearing mice and consumed (3.5±0.03 gram/day/mouse). The food intake of normal healthy mice group was (5.5±0.2 gram/day/mouse). Groups of healthy mice were treated with the new complex (100 mg/kg) and DMSO did not give significantly different results compared to normal group (5±0.07, 5.3±0.08) respectively. The mix group gave the best results (4.8±0.07 gram/day/mouse) compared with other treated groups.

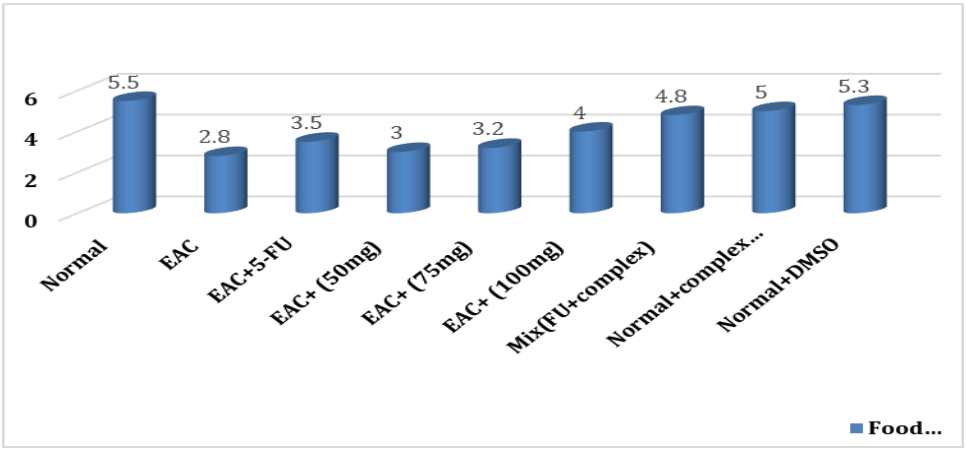


Figure (4): The effect on the food intake by mice of the different studied group (n=6).

Table (1). Effect of synthesized compound N2 on tumor cell count, tumor volume, viability% and packed EAC dead cell% in all the studied groups (n=6).

Variable	Normal	EAC	EAC+5-flurouracil	EAC+ compound (N2) (50mg/kg)	EAC+ compound (75mg/kg)	EAC+ compound (100mg/kg)	Mix(5FU+N2 compound (100mg/kg)	Normal+ compound (100mg/kg)	Normal+ DMSO
Tumor cell count ($x10^6$cells/mL)	0	214±6.2	75±3.3	102±5.4	80±4.4	4±3.8	33±1.9	0	0
tumor volume (mL)	0	3.82±0.2	0.76±0.01	1.64±0.02	1.28±0.03	0.7±0.55	0.38±0.01	0	0
Viability (EAC%)	0	93.04±3.1	45.1±2.3	80.3±4.7	67.23±3.0	42±3.6	27±1.4	0	0
packed EAC dead cells(Cytotoxicity %)	0	6.96±0.54	54.9±5.1	19.7±0.3	32.77±1.88	58±2.7	72.1±3.7	0	0

Table (2). Effect of N2 compound on hematological parameters in all studied groups (n=6).

parameters	Normal	EAC	EAC+5-flurouracil	EAC+complex (50mg/kg)	EAC+complex (75mg/kg)	EAC+complex (100mg/kg)	Mix(5-FU+complex 100mg)	Normal+ complex (100mg)	Normal+ DMSO
RBCs (10^6/mm³)	7.85±0.12	5.27±0.21 P1<0.001	7.2±0.07 P1<0.001 P2<0.001	6.23±0.18 P1<0.001 P2<0.001	6.84±0.21 P1<0.001 P2<0.001	7.35±0.26 P1=0.002 P2<0.001	7.91±0.29 P1=0.692 P2<0.001	7.54±0.11 P1=0.001 P2<0.001	7.4±0.14 P1<0.001 P2<0.001
WBCs (10^3/mm³)	8.04±0.11	13.07±0.19 P1<0.001	8.27±0.18 P1=0.031 P2<0.001	11.56±0.21 P1<0.001 P2<0.001	10.37±0.34 P1<0.001 P2<0.001	8.16±0.14 P1=0.156 P2<0.001	7.85±0.21 P1=0.085 P2<0.001	7.96±0.09 P1=0.204 2<0.001	8.97±0.31 P1<0.001 P2<0.001
Hb (g/dl)	12.5±0.14	9.54±0.29 P1<0.001	12.04±0.04 P1<0.001 2<0.001	11.19±0.09 P1<0.001 P2<0.001	11.51±0.29 P1<0.001 P2<0.001	12.24±0.04 P1=0.001 P2<0.001	12.33±0.08 P1=0.028 P2<0.001	12.44±0.09 P1=0.373 P2<0.001	12.35±0.13 P1=0.074 P2<0.001
PLT (10^3/mm³)	384.5±12.0	214.5±8.31 P1<0.001	361.8±10.2 P1=0.006 P2<0.001	273.03±8.2 P1<0.001 P2<0.001	299±6.1 P1<0.001 P2<0.001	359.5±10.2 P1=0.003 P2<0.001	379.8±4.4 P1=0.396 P2<0.001	382±2.6 P1=0.631 P2<0.001	394±8.17 P1=0.142 P2<0.001

Values are expressed as mean±SD

P1, compared to normal control whereas P2, compared to EAC control group; p>0.05 is considered non-significant; p <0.05 is considered significant; p <0.001 is considered extremely significant

Abbreviations: RBCs: Red blood cells; WBCs: White blood cells; Hb:Hemoglobin; PLT: platlet

Table (3). Effect of synthesized compound N2 on body weight and the body weight changes% in all the studied groups.

P1, compared to normal control whereas P2, compared to EAC control group; p>0.05 is considered non-significant; p <0.05 is considered significant; p <0.001 is considered extremely significant (Data represented as mean ± standard division)

Group	Normal	EAC	EAC+5-flurouracil	EAC+ compound (50mg/kg)	EAC+ compound (75mg/kg)	EAC+compound (100mg/kg)	Mix(5-FU+compound 100mg/kg)	Normal+ compound(100mg/kg)	Normal+ DMSO
Weight at Day 0 (gm/day)	19.43±2.6	22.43±2.1 P1=0.012	22.28±1 P1=0.005 P2=0.843	22.51±1.1 P1=0.003 P2=0.921	22.42±0.9 P1=0.003 P2=0.989	22.27±0.8 P1=0.004 P2=0.829	22.07±1 P1=0.008 P2=0.645	20.93±1.2 P1=0.123 P2=0.077	20.45±1.4 P1=0.299 P2=0.027
Weight at Day 3 (gm/day)	20.3±2.7	25.24±1.8 P1<0.001	23.99±1.1 P1=0.001 P2=0.088	23.9±1.3 P1=0.001 P2=0.095	24.48±0.9 P1<0.001 P2=0.264	23.86±1.2 P1=0.002 P2=0.066	23.28±0.8 P1=0.004 P2=0.007	21.76±1.2 P1=0.145 P2<0.001	21.16±1.3 P1=0.388 P2<0.001
Weight at Day 6 (gm/day)	21.32±2.2	30.01±3.2 P1<0.001	25.58±0.7 P1<0.001 P2=0.001	26.8±1 P1<0.001 P2=0.009	26.62±0.7 P1<0.001 P2=0.005	25.66±1.1 P1<0.001 P2=0.001	24.9±1.1 P1<0.001 P2<0.001	23.01±1.4 P1=0.061 P2<0.001	21.96±1.2 P1=0.44 P2<0.001
Weight at Day 9 (gm/day)	23.7±1.0	33.1±2.6 P1<0.001	26.99±0.7 P1<0.001 P2<0.001	29.35±1.1 P1<0.001 P2=0.001	28.53±0.9 P1<0.001 P2<0.001	26.47±0.8 P1<0.001 P2<0.001	25.61±0.9 P1=0.001 P2<0.001	24.59±1 P1=0.101 P2<0.001	23.5±0.7 P1=0.499 P2<0.001
Body weight difference(gm/day)	4.33±0.3	10.75±1.4	4.71±5.8	6.84±1.1	6.107±0.77	4.217±0.74	3.53±0.67	3.66±0.34	3.049±0.7
BW% change (%)	22.26±2.4	47.93±2.7	21.15±1.7	30.41±2.6	27.24±1.5	18.95±1.2	16.025±1.8	17.503±1.2	14.908±1.0

Data represented as mean ± standard division

Table (4). Effect of N2 compound on survival time against EAC induced animals.

Group	First death	Last death	MST	IL%
Normal negative control	_	_	90<	>160.8
Normal treated with DMSO	–	_	90<	>160.8
Normal treated with 100mg/kg 0f complex	_	_	90<	>160.8
EAC positive control	23	46	34.5	0
EAC treated with 5-FU	54	69	61.5	78.3
EAC treated with 50 mg/kg of complex	42	50	46	33.3
EAC treated with 75 mg/kg of complex	51	65	58	68
EAC treated with 100 mg/kg of complex	53	72	62.5	81
Mix	59	75	67	94

DISCUSSION

Heterocyclic compounds associated with a broad spectrum of biological and pharmacological activities. Thiazole derivatives, are a category of those compounds that have proven considerable antitumor compounds. Due to various possibilities of chemical derivatization of the rhodanine ring, rhodanine based compounds will probably remain a privileged scaffold in drug discovery.[41] The most effective anticancer were supposed to be DNA damaging agents (Gurova, K. (2009).[42] Based on their mode of action, these medications may be classified into many types. They include antitumor antibiotic, topoisomerase inhibitors, antimetabolite, hormones and hormones antagonists, Herbal remedies, alkylating agents and other medications.[43]

5-flurouracil is an antimetabolite medication that is commonly used in cancer therapy (Sethy, C., et al., 2021).[44] 5-FU inhibits thymidylate synthase (TS) and induces the incorporation of its metabolites into RNA and DNA, resulting in anticancer activity (Longley, D. B., et al., 2003).[45] Despite these developments, drug resistance continues to be a significant barrier to the therapeutic use of 5-FU (He, L., et al., 2018).[46]

The present study was performed to demonstrate the efficacy of thiazolo [5',4':5,6] pyrano[2,3-d] pyrimidine hybrid derivative N2 as anticancer agents against Ehrlich Ascites Carcinoma and gives information for further investigations into the molecule's antitumor potential. The anti-tumor activity of N2 compound was measured in EAC animals with respect to the following parameters: Body weight, Tumor weight, Tumor cell count, Tumor growth response, Tumor volume, Viable and non-viable tumor cell count, mean survival time and percentage increase in life span, hematological parameters was also studies.

New anticancer drug candidates are frequently designed using small compounds against specific targets or tumor types. Due of the sensitivity of different cancer types, such therapeutic candidates must be more effective and have fewer side effects than publicly known cytotoxic medicines[47]**(Kumar, D., et al., 2018)**.

The effect of N2 on tumor volume, viable and non-viable cell count was measured the result showed significant reduction in tumor volume, packed cell volume, viable tumor cell count and increase the tumor weight inhibition (%), ascites cells inhibition (%) and the non-viable cell count when compared to EAC tumor control, along with a life span increase. Therefore, it may be hypothesized that the increase in life span may be due to the decrease in nutritional ascitic volume and a delay in cell proliferation.[48] This confirms the anticancer activity of N2compound against EAC cells because prolongation of life span is a dependable principle for judging the anticancer efficacy of any compound.[49] also, a considerable increase in body weight of the animals was observed in EAC control mice due to the fast and progressive accumulation of ascites tumor cells. Treatment with the N2 compound caused a marked reduction in the body weight, indicating the feature of inhibition of tumor cells progression and the antitumor nature of N2.

The full CBC, also known as a hemogram, is a critical test for a variety of medical conditions, including anaemia, infection, and leukaemia. It includes the total number of red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs). Any abnormality (increases or decreases) in the quantity of blood cells or associated indices detected by the CBC might suggest a medical problem underlying the diagnosis[50] **(Yassin, 2013)**. The major problems encountered in cancer chemotherapy are myeloid suppression and anemia,[51,52] which are mainly because of iron deficiency, either due to haemolytic or myelopathic conditions finally leads to reduction in RBC count or hemoglobin content. The results showed the destructive effect caused by the injection of Ehrlich ascites carcinoma in mice on the blood parameters compared to the negative normal control. Hematological parameters, showed reduced decreased HB, RBCS and elevated he total WBC

count[52] in EAC bearing group because of iron deficiency, either due to hemolytic or myeloid pathic conditions. While the treatment with the different concentrations (50,75, 100 mg/kg) of the new synthesized compound N2 were able to increase the levels of Hb, RBCs, PLTs and decrease WBCs to reach to near its normal levels gradually in dose dependent manner compared to Ehrlish bearing mice. This indicated that the N2 compound exhibits haematopoietic protecting activity without myelotoxicity, the most common side effect of cancer chemotherapy. The treatment with 5-Flurouracil gave the same therapeutic effect of the highest dose of the new compound. Otherwise, the treatment with the combination of 5-FU and the new compound N2was able to return RBCs, hemoglobin, platelets, and WBCs to their normal levels and gave results better than the therapeutic effect of each one alone. On the other hand, there was no significant difference in the hematological parameters between the groups of healthy mice treated with the high dose (100 mg/kg) of the thiazolo [5',4':5,6] pyrano[2,3-d] pyrimidine N2 , DMSO groups and negative normal groups. This proved that the thiazolo [5',4':5,6] pyrano[2,3-d] pyrimidine N2 is not toxic and that it is safe for healthy cells. From this point, it was also found that the mice's appetite was not affected by the treatment with the different doses of the new compound, but there was a significant increase in food intake compared to the positive control group. Ehrlich ascites carcinoma causes oxidative stress due to the imbalance between the reactive oxygen species and the antioxidants inside the cell **Ellethy A. T. (2019).**[53] Oxidative stress can cause cell, protein, and DNA damage, which contributes to ageing. It may also have a role in the development of a variety of health problems **(Pizzino, G., et al., 2017).**[54] Injecting mice with Ehrlich ascites carcinoma induced a sudden drop in food consumption because it caused a deterioration in the general health of mice as a result of the failure of the vital functions of the liver, spleen, kidneys and other organs **(Kapoor, R., et al., 2014).**[55] The healthy mice were treated with a dose (100 mg/kg) of the new compound didn't affect their food consumption compared with the normal control group.

CONCLUSION

Finally, our study shows that the newly synthesized heterocyclic compounds N2 can be considered as a potent anticancer agent with minimal side effects as it exhibited a significant antitumor activity in EAC-bearing mice that is almost comparable to that of the reference standard, 5FU.

The treatment of mice bearing tumor's with N2 resulted in a restoration to normal hematological status by raising RBC count, hemoglobin, and platelets while reducing WBC count. By gradually increasing the injected dose, it was also able to improve the appetite of mice and hence increase the amount of food consumption.

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