

**RESEARCH ARTICLE****Synthesis, Characterization, and Biological Evaluation of Some Novel Phthalimide Derivatives**

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**ABSTRACT**

**Objective:** Different Phthalimide derivatives (4,5,6,7-tetrachloro-2-[1,2,4]triazol-4-yl]-isoindole-1,3-dione derivatives) were synthesized and biological activities of them were evaluated. **Materials and Methods:** In the present study, four new phthalimide derivatives were synthesized. The structures of final compounds were characterized on the basis of spectral data. Then, biological evaluation of all the synthesized compounds means *in vivo* anticancer activity was evaluated on the Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice model, and *in vitro* antioxidant activity was assessed using 1,1-diphenyl-2-picryl hydrazine (DPPH) radical scavenging assay. **Results:** The titled compounds (2A-2D) were found to reduce tumor volume, viable cell count and increase non-viable cell count, and percentage increase in life span. All compounds showed significant activity in quenching DPPH free radical. **Conclusion:** All compounds showed significant ( $P<0.01$ ) anticancer activity compared to EAC control group. All synthesized compound exhibited considerable antioxidant activity against free radicals, and they showed significant  $IC_{50}$  values and can, thus, ensure protection against oxidative stress.

**Keywords:** 1,1-diphenyl-2-picryl hydrazine, anticancer, antioxidant, ehrlich ascites carcinoma cell, phthalimide

**INTRODUCTION**

Cancer is a frightful disease and major global challenge<sup>[1]</sup> because it is the second most common cause of death worldwide after cardiovascular diseases. Cancer is a non-communicable disease, but it spreads like a communicable disease in spite of its non-contagious character. Extensive research has been carried out to combat this silent killer. Various synthetic drugs have been developed from different chemical entities. Various techniques such as surgery, immunotherapy, radiotherapy, and chemotherapy are used to treat cancer. Maximum number of disease treatment is only possible with nitrogen containing chemical entity. Among them, phthalimide (IUPAC name - Isoindole-1, 3-Dione) is bicyclic non-aromatic nitrogen containing heterocyclic compound which possesses a structural feature  $-CO-N(R)-CO-$  and an imide ring.<sup>[2]</sup>

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Phthalimide has received attention due to their diverse biological activities such as androgen receptor antagonists,<sup>[3]</sup> anticonvulsant,<sup>[4]</sup> antimicrobial,<sup>[5]</sup> hypoglycemic,<sup>[6]</sup> anti-inflammatory,<sup>[7]</sup> antitumor,<sup>[8,9]</sup> anxiolytics,<sup>[10]</sup> anti HIV-1 activities,<sup>[11]</sup> antitubercular,<sup>[12]</sup> antivirus,<sup>[13]</sup> histone deacetylase inhibitory,<sup>[14]</sup> liver X receptor antagonist,<sup>[15]</sup> leukotriene D4 receptor antagonist,<sup>[16]</sup> and antioxidant.<sup>[17]</sup>

Antioxidants are the reducing agents which used to stabilize some free radicals. Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules in the mitochondrial respiratory chain reaction, atmospheric pollutants, and from drugs. These free radicals play a vital role in damaging the various cellular macromolecules and cause various pathological conditions such as diabetes mellitus, atherosclerosis, myocardial infarction, arthritis, anemia, asthma, inflammation, neurodegenerative diseases, and carcinogenesis.<sup>[18,19]</sup> In human body, this oxidative damage can be prevented by enzymatic and non-enzymatic antioxidant. However, this

protective mechanisms may be disrupted in various pathological processes and thereby cause damage. This situation can be overcome by providing natural (vegetables and fruits) antioxidants into the human body as promising prophylactic agents. Butylated hydroxy anisole and butylated hydroxy tolune are commercially available synthetic antioxidant, but they are reported to be toxic animals including human beings.<sup>[17-20]</sup>

Therefore, the development of some novel tetrachlorophthalimide derivatives by fusion of tetra chlorophthalic anhydride moiety with mercaptotriazole through N-N bond to achieve the generation of the molecules with potential anticancer and antioxidant activities are of great interest in the field of pharmaceutical industries.

## MATERIALS AND METHODS

## Materials

Anhydrous glycerol was purchased from M/S Merck (Mumbai, India) and tetrachlorophthalic anhydride was purchased from Loba Chemie Pvt. Ltd. (India). 1,1-diphenyl-2-picryl hydrazine (DPPH) was purchased from Sigma-Aldrich Chemical Co. Various substituted triazole derivatives were synthesized according to our previous publication. Tumor cells (Ehrlich ascites carcinoma [EAC]) used for this *in vivo* anticancer study were obtained from Chittaranjan National Cancer Institute, Kolkata, India. The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma.<sup>[21]</sup> These tumor cells were maintained *in vivo* in Swiss albino mice by intraperitoneal inoculation of  $2 \times 10^6$  cells/mouse after every 10 days. The viable EAC cells were counted (Trypan blue indicator) under the microscope with the help of hemocytometer and were adjusted at  $2 \times 10^7$  cells/mL. EAC cells suspension (0.1 ml) was injected (i.p.) in each mouse.

## Instruments

Melting points were determined with Veego melting point apparatus. Synthesized compounds were assigned by spectral data (Fourier-transform infrared [FT-IR], nuclear magnetic resonance [ $^1\text{H}$ NMR], and liquid chromatography-mass spectrometry [LC-MS]). The structures of all the compounds were confirmed by  $^1\text{H}$  NMR spectra with Brucker DPX 300 MHz

Instrument (dimethyl sulfoxide [DMSO]-d<sub>6</sub> and TMS) and mass spectrometric detection was performed by API 2000 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, ON, Canada) equipped with an Turbo electrospray ionization (ESI) interface. IR spectra were recorded on a Bruker FTIR spectrophotometer (Model: Alpha, PN: 1005151/06, SN: 200667, Germany) with the help of OPUS 7.2 spectroscopy software. UV-visible spectrophotometer (SpectraMAX M5) was used for *in vitro* antioxidant study.

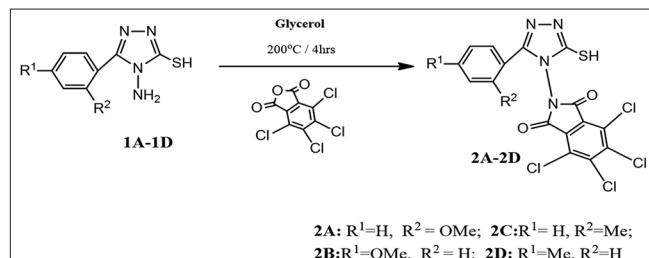
## **Animals and its maintenance**

Swiss male albino mice of about 8 weeks old with an average body weight (b.w.) of 18–20 g were obtained from the Indian Institute of Chemical Biology (IICB), Kolkata, India. The mice were grouped and housed in suitable polyacrylic cages for acclimatized to the laboratory environment (temperature 30°C with dark/light cycle 12/12 h). All mice are kept on basal metabolic diet with water *ad libitum* for 10 days before commencement of the experiment. All methodology related to animal experiment were assessed and permitted by the University Animals Ethical Committee (Registration No: 1805/GO/Re/S/15/CPCSEA), Jadavpur University, India.

## Experimental work

Synthesis

Various 4-amino-5-mercaptopro-3-(substituted phenyl)-1, 2, 4-triazole derivatives were synthesized from various aromatic acids according to literature method.<sup>[22,23]</sup> Then, synthesized triazole derivatives (1A–1D) (0.01mole) were refluxed with tetrachlorophthalic anhydride (0.01 mole) in the presence of glycerol [Figure 1]. The crude products were separated from reaction mixture on cooling. These were collected by filtration, washed with water repeatedly, and dried. Then, final compounds were recrystallized from acetonitrile-water mixture



**Figure 1:** Synthesis scheme

to yield pure titled compounds. These derivatives were confirmed by FT-IR, <sup>1</sup>H NMR, and mass spectroscopy.

### **Physical and spectral data of some representative titled compounds (2A–2D)**

#### **4,5,6,7-tetrachloro-2-[5-mercaptop-3-(2-methoxyphenyl)-[1,2,4]triazol-4-yl]-isoindole-1,3-dione (2A)**

Molecular formula: C<sub>17</sub>H<sub>8</sub>Cl<sub>4</sub>N<sub>4</sub>O<sub>3</sub>S, molecular weight: 490.15, recrystallized with acetonitrile–water (4:3), melting point - 199–203 °C, %yield: 58.76, (LC/MS): m/z 491.1 (M<sup>+</sup>/M<sup>-</sup>), FT-IR (KBr, V<sub>max</sub> cm<sup>-1</sup>): 3390 (N-H stretch), 3014 (Ar-C-H), 2558 (SH), 1743 (C=O), 1680 (C=N), 1631 (N-H bending), 1314 (C-N), 1180 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub> ppm): δ 2.20 (3H, s, CH<sub>3</sub>); 7.43–7.60 (8H, m, Ar-Hs); 8.001(1H, s, SH).

#### **4,5,6,7-tetrachloro-2-[5-mercaptop-3-(4-methoxyphenyl)-[1,2,4]triazol-4-yl]-isoindole-1,3-dione (2B)**

Molecular formula: C<sub>17</sub>H<sub>8</sub>Cl<sub>4</sub>N<sub>4</sub>O<sub>3</sub>S, molecular weight: 490.15, recrystallized with acetonitrile–water (4:3), melting point - 195–200 °C, %yield: 63.53, (LC/MS): m/z 490.9 (M<sup>+</sup>/M<sup>-</sup>), FT-IR (KBr, V<sub>max</sub> cm<sup>-1</sup>): 3583 (N-H stretch), 2986 (Ar-C-H), 2540 (SH), 1729 (C=O), 1682 (C=N), 1598 (N-H bending), 1363 (C-N), 1201 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub> ppm): δ 3.99 (3H, s, CH<sub>3</sub>); 7.08 – 7.25 (2H, m, Ar-Hs); 7.58–7.83 (2H, m, Ar-Hs).

#### **4,5,6,7-tetrachloro-2-[5-mercaptop-3-(2-methyl-phenyl)-[1,2,4]triazol-4-yl]-isoindole-1,3-dione (2C)**

Molecular formula: C<sub>17</sub>H<sub>8</sub>Cl<sub>4</sub>N<sub>4</sub>O<sub>2</sub>S, molecular weight: 474.15, recrystallized with acetonitrile–water (3:5), melting point - 214–217 °C, %yield: 70.01, (LC/MS): m/z 475.2 (M<sup>+</sup>/M<sup>-</sup>), FT-IR (KBr, V<sub>max</sub> cm<sup>-1</sup>): 3335 (N-H stretch), 3135 (Ar-C-H), 2552 (SH), 1739 (C=O), 1683 (C=N), 1559 (N-H bending), 1304 (C-N), 1177 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub> ppm): δ 2.20 (3H, s, CH<sub>3</sub>); 7.43–7.85 (8H, m, Ar-Hs); 8.98 (1H, s, SH).

#### **4,5,6,7-tetrachloro-2-[5-mercaptop-3-(4-methyl-phenyl)-[1,2,4]triazol-4-yl]-isoindole-1,3-dione (2D)**

Molecular formula: C<sub>17</sub>H<sub>8</sub>Cl<sub>4</sub>N<sub>4</sub>O<sub>2</sub>S, molecular

weight: 474.15, recrystallized with acetonitrile–water (3:5), Melting point - 221–224 °C, %Yield: 70.68, (LC/MS): m/z 475.3 (M<sup>+</sup>/M<sup>-</sup>), FT-IR (KBr, V<sub>max</sub> cm<sup>-1</sup>): 3343 (N-H stretch), 3185 Ar-C-H), 2552 (SH), 1740 (C=O), 1661 (C=N), 1588 (N-H bending), 1314 (C-N), 1180 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub> ppm): δ 2.20 (3H, s, CH<sub>3</sub>); 7.43–7.60 (8H, m, Ar-Hs); 8.001(1H, s, SH).

### **Acute toxicity study**

Male albino mice weighing 18–20 g were used for acute toxicity study. The synthesized compounds (2A–2D) were given at 50,100, 500, and 2000 mg/kg b.w. p.o. None of the synthesized compounds showed any significant changes in the skin, fur, eyes, and other behavioral patterns in mice at any of the tested dose levels. No mortality was observed in any groups after 24 h (OECD guideline no. 420). The test samples were found to be safe up to the dose of 2000 mg/kg.

### **In vivo anticancer study**

Male Swiss albino mice were divided into seven groups of 12 animals in each. All mice are kept on basal metabolic diet with water *ad libitum* during the experiment. All the groups were treated with EAC cells (0.1 ml of 2 × 10<sup>6</sup> cells/mouse) intraperitoneally except the negative control group (Group I). This was taken as day 0. In this state, the tumor cells multiply relatively freely within the peritoneal cavity and ascites develops. Animals were allowed for 24 h incubation to set the disease condition in their body before starting the administration of synthesized compounds (2A–2D, for 100mg/kg b.w orally/day) and standard drug (5 fluorouracil, 20 mg/kg b.w. intraperitoneally). On the 1<sup>st</sup> day, 5 ml/kg b.w of normal saline (0.9 % NaCl w/v) was administered in Group I (normal). Normal saline (0.9 % w/v, NaCl), 5ml/kg, b.w/day was administered in Group II (EAC control). The synthesized compounds (2A–2D, 100 mg/kg b.w./day, orally) and the standard drug 5-fluorouracil (20 mg/kg, b.w./day, intraperitoneally) were administered in Groups III–VI and VII, respectively, for 9 days at 24 h interval. Thus, 9 doses of the synthesized compounds and standard drug were administered to each mouse in the test group. After administration of last dose, 6 mice from each group were kept fasting for 18 h and

blood was collected by cardiac puncture for the estimation of hematological and biochemical parameters.<sup>[21,22,24,25]</sup> The animals then sacrificed for the study of antitumor activity. Rest of the animals in each groups were kept alive with food and water *ad libitum* to check Increase in life span (ILS) of the tumor host to determine the mean survival time (MST).

The anticancer activity of the test compounds was measured against EAC animals with respect to the following parameters according to Dolai *et al.*<sup>[21]</sup>

#### Tumor volume

The ascitic fluid was collected from the peritoneal cavity, and the volume was measured by taking it in a graduated centrifuge tube.<sup>[21]</sup>

#### Tumor cell count

The ascitic fluid was taken in a white blood cell (WBC) pipette and diluted 100 times. Then, a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the numbers of cells in the 64 small squares were counted with the help of microscope under  $\times 40$  magnification.<sup>[21]</sup>

#### Percentage inhibition of ascitic cells

$$(\%TCI) = \left( 1 - \frac{T}{C} \right) \times 100$$

Where T is the total number of ascitic cells/ml in test animals and C is the total number of the ascitic cells/mL in EAC control animals.

#### Viable/non-viable tumor cell count

The viability and non-viability of the cell were checked by trypan blue assay. The cells were stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable, and those that took the dye were non-viable. These viable and non-viable cells were counted.<sup>[21]</sup>

$$\text{Cell count} = \frac{\text{number of cells} \times \text{dilution factor}}{\text{area} \times \text{thickness of liquid film}}$$

#### %ILS

The effects of synthesized test compounds are seen to increase the life span which was calculated on the basis of mortality of the experimental mice.<sup>[21]</sup>

$$\%ILS = \left\{ \frac{\text{mean survival time of treated group}}{\text{mean survival time of control group}} - 1 \right\} \times 100$$

$$Q \text{ Mean survival time (MST)} = \frac{\text{first death} + \text{last death}}{2}$$

Here, time is denoted by days.

#### Hematological parameters

Blood collected from the animals was used for the estimation of hemoglobin (Hb) content, red blood cell (RBC), and WBC.<sup>[21]</sup>

#### Statistical analysis

All data are expressed as mean $\pm$ SEM ( $n = 6$  mice per group). The data were analyzed by one-way ANOVA between the treated groups and the EAC control followed by Dunnett's multiple comparison test.<sup>[21]</sup>

#### Count of viable, nonviable, and total cells

The ascitic fluid of dissected mice of each group was taken in a WBC pipette and diluted 100 times. Then, a drop of the diluted cell suspension was placed on the Neubauer counting chamber and a number of total EAC, viable, and nonviable cells in the 64 small squares were counted with help of microscope.<sup>[21]</sup>

#### Hematological parameters

Before dissection of the animals, blood sample was collected through orbital plexus through heparinized tubes with the help of thin capillary. Blood sample was taken up to 0.5 marks of the WBC pipette and RBC pipette, and it was diluted up to 11 marks (it becomes 20 times dilution) and 101 marks (it becomes 200 times dilution) using WBC and RBC diluting fluid, respectively.<sup>[21]</sup>

Then, a drop of this diluted sample was placed in the hemocytometer chamber, and a number of cells were counted. Sahli's Hemoglobinometer is used for the determination of Hb content.

## In vitro antioxidant study

### DPPH radical scavenging activity

The antioxidant study of synthesized compounds was determined using DPPH. This *in vitro* assay was monitored according to the Blois method. Here, ascorbic acid was used as standard antioxidant. The stock solutions of test compounds (1 mg/mL) were prepared in DMSO, and DPPH (0.004%) solution was prepared in 95% methanol. Freshly prepared DPPH solution was taken in test tubes, and solution of synthesized compounds (100 µg) was then added to every test tube except control and standard group.<sup>[26]</sup>

The mixture was shaken vigorously and allowed to react at room temperature and kept in darkness for 10 min/20 min/5 h.

A freshly prepared DPPH solution shows a deep color with an absorption maximum at 517 nm. When the purple color changes to yellow, it leads to decrease absorbance. Hence, the absorbance of the resulting solution was measured at 517 nm using a UV-Visible spectrophotometer (Spectra MAX M5), and scavenging of DPPH free radicals was calculated as follows:<sup>[26]</sup>

$$\% \text{ radical scavenging activity} = \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}} \times 100$$

Where control absorbance is the measurement of DPPH solution without compound and sample

**Table 1:** DPPH scavenging activity of the compounds

Compounds code	DPPH assay in %
2A	41.3
2B	38.6
2C	72.4
2D	75.5
Ascorbic acid	90.2

DPPH: 1,1-diphenyl-2-picryl hydrazine

absorbance is the measurement of DPPH solution with compound. Tests were carried out in triplicate and tabulated in Table 1.

## RESULTS AND DISCUSSION

### Synthesis

In this present study, some novel tetrachlorophthalimide derivatives were synthesized from various substituted 1,2,4-triazole derivatives by fusion of tetrachlorophthalic anhydride in the presence of glycerol. 1, 2, 4-triazole derivatives were (used as starting material) prepared from different aromatic acids. Final compounds were characterized by their physicochemical properties.

### In vivo anticancer activity

Anticancer activities were evaluated against EAC cells in Swiss albino mice by observing the parameters such as percentage inhibition of tumor volume (%TVI), percentage inhibition of total cell count (%TCI), percentage of viable and non-viable cell count, %ILS, and MST. Compounds (2A-2D) having anticancer potential are shown in Tables 2 and 3. Hematological parameters such as WBC count ( $\times 10^9/\text{L}$ ), RBC count ( $10^{12}/\text{L}$ ), and Hb (g/dL) content have been taken [Table 4] to be considered to establish the significance of the synthesized compounds as an anticancer agent. All the compounds have significantly reduced the TCI and tumor volume when compared to EAC control group. Among them, compound 2D exhibited tumor volume inhibition (71.07%) and highest TCI inhibition (76.72%) at the dose of 100 mg/kg, b.w., orally as compared to control. Standard drug (5-FU) showed about 95.97% of tumor volume inhibition and 95.23 % of tumor

**Table 2:** Effect of synthesized compounds on tumor volume (ml), MST, and %ILS in EAC bearing mice

Group	Compound	Dose	Tumor volume (mL)	% TVI	MST (in days)	% ILS
I	Negative control	-	-	-	-	-
II	EAC+control	-	6.95±0.45	0.00	17.35	0.00
III	EAC+2A	(100 mg/kg)	3.33±0.43*	52.08	28.22	62.65
IV	EAC+2B	(100 mg/kg)	2.45±0.30*	64.74	33.89	95.33
V	EAC+2C	(100 mg/kg)	2.89±0.16*	58.41	38.02	119.13
VI	EAC+2D	(100 mg/kg)	2.01±0.26*	71.07	44.07	154
VII	EAC+5-FU	(20 mg/kg)	0.28±0.07*	95.97	48.08	177.11

Each value represents the mean±SEM, Where n=6.\*Experimental groups were compared with EAC control group ( $P<0.01$ ), MST: Mean survival time, EAC: Ehrlich ascites carcinoma, ILS: Increase in life span

**Table 3:** Effect of synthesized compounds on TCI ( $\times 10^7$ ), viable cell, and non-viable cell count in EAC bearing mice

Group	Compound	TCI ( $\times 10^7$ )	%TCI	Viable cell count ( $\times 10^7$ )	Non-viable cell count ( $\times 10^7$ )	% of viable cells	% of non-viable cells
I	Negative control	-	-	-	-	-	-
II	EAC+control	9.11 $\pm$ 0.16	0.00	7.96 $\pm$ 0.30	1.15 $\pm$ 0.24	87.38	12.62
III	EAC+2A	4.39 $\pm$ 0.47*	51.81	2.56 $\pm$ 0.50*	1.83 $\pm$ 0.41*	76.50	23.46
IV	EAC+2B	4.05 $\pm$ 0.56*	55.54	2.35 $\pm$ 0.45*	1.70 $\pm$ 0.28*	58.00	42.00
V	EAC+2C	3.53 $\pm$ 0.30*	61.25	1.85 $\pm$ 0.32*	1.68 $\pm$ 0.06*	52.00	48.00
VI	EAC+2D	2.12 $\pm$ 0.24*	76.72	0.54 $\pm$ 0.22*	1.58 $\pm$ 0.06*	25.47	74.53
VII	EAC+5-FU	0.43 $\pm$ 0.01*	95.23	0.06 $\pm$ 0.007*	0.37 $\pm$ 0.01*	13.97	86.03

Each value represents the mean $\pm$ SEM, Where n=6. \*Experimental groups were compared with EAC control group ( $P<0.01$ ), TCI: Total cell count Inhibition, EAC: Ehrlich ascites carcinoma

**Table 4:** Effect of synthesized compounds on hematological parameters

Group	Compound	WBC count	RBC count	Hemoglobin (g/dl)
I	Negative control	5.58 $\pm$ 0.06*	9.79 $\pm$ 0.09*	14.25 $\pm$ 0.15*
II	EAC+control	19.80 $\pm$ 0.43	2.23 $\pm$ 0.13	6.44 $\pm$ 0.12
III	EAC+2A	15.05 $\pm$ 0.06*	4.09 $\pm$ 0.04**	9.53 $\pm$ 0.06*
IV	EAC+2B	11.22 $\pm$ 0.06**	6.13 $\pm$ 0.48**	7.04 $\pm$ 0.47*
V	EAC+2C	8.09 $\pm$ 0.05*	8.09 $\pm$ 0.05*	11.08 $\pm$ 0.05**
VI	EAC+2D	7.32 $\pm$ 0.13*	6.97 $\pm$ 0.11*	10.76 $\pm$ 0.02*
VII	EAC+5-FU	6.40 $\pm$ 0.14*	8.41 $\pm$ 0.09*	13.18 $\pm$ 0.06*

Each value represents the mean $\pm$ SEM, Where n=6. \*Experimental groups were compared with EAC control group ( $P<0.01$ ), \*\*Experimental groups were compared with EAC control group ( $P<0.05$ ), EAC: Ehrlich ascites carcinoma, WBC: White blood cell, RBC: Red blood cell

cell count inhibition. The rest of the compounds showed 52.08%, 64.74%, and 58.41% of %TVI and 51.81%, 55.54%, and 61.25% of %TCI, respectively.

All of these compounds significantly reduced the number of viable cells and increased the number of non-viable cell comparing to the EAC control group. Compound 2D showed the maximum percentage (74.53%) of non-viable cells causing destruction of the EAC cells [Table 3].

One of the most reliable criteria for judging the efficiency of any anticancer drug is the prolongation of the life span of animals [Table 2]. All the compounds (2A-2D) significantly increase %ILS (62.65%, 95.33%, 119.13%, and 154%, respectively) compared to the induced control group. Compound 2D (154%) showed maximum %ILS among the test compounds, whereas standard drug shows 177.11% compared to the EAC control group [Table 2].

Hematological characteristics have been widely used in diagnosis of variety of diseases (like cancer) and other pathological condition. A complete blood count provides detailed information about three types of blood cells: RBC, WBC, and platelets. RBC is important to

transfer of oxygen from the lungs to the tissues, and Hb concentration is directly correlated with the RBC count. This close correlation between erythrocyte count and Hb concentration was also reported for other vertebrates including man.<sup>[27]</sup>

WBC formed in the bone marrow either enters the blood or migrates to key organs such as the spleen, lymph nodes, or gut. The increased number of leukocytes can occur abnormally as a result of an infection, cancer, or toxic chemical. Such increase of WBC may be due to the activation of the defense mechanism of animals and their immune system.<sup>[28]</sup>

Myelosuppression and anemia (reduced Hb) have been frequently observed in cancer chemotherapy. Due to iron deficiency or due to hemolytic or myelopathic conditions, anemia is occurred in tumor bearing mice.<sup>[29,30]</sup> There was increased level of WBC and decreased level of hemoglobin (Hb) and RBC in EAC control group as compared to normal control group (Table 4). After treatment with synthesized compounds (2A-2D) at the dose of 100 mg/kg body weight in EAC bearing mice significantly increased RBC count, Hb content and significantly decreased the WBC count as compared with the EAC control group [Table 4].

## In vitro antioxidant study

Antioxidant properties of phthalimide derivatives were evaluated by the DPPH free radical scavenging assay to find a new source of antioxidant. This *in vitro* assay provides a method to evaluate the antioxidant activity in a relatively short period, and the scavenging activity may be attributed to the hydrogen donating ability of the compounds. The results of the screening are shown in Table 1 as comparable with ascorbic acid, known antioxidant.

Phthalimide derivatives have shown to scavenge DPPH free radicals. All compounds showed considerable activity in quenching DPPH free radical more effectively with significant IC<sub>50</sub> values and can thus ensure protection against oxidative stress caused by free radical.

## CONCLUSION

Novel derivatives of 4,5,6,7-tetrachloro-2-[5-mercaptop-3-(substitued)-[1,2,4]triazol-4-yl]-isoindole-1,3-dione possessing -CO-N(R)-CO- in their chemical core have been synthesized using simple synthetic procedures, and their *in vivo* anticancer activity and *in vitro* antioxidant study have been done. All the final compounds (2A-2D) exhibited significant result in different parameters of EAC bearing mice and markedly increase the average life span of experimental animals. Compound 2D [4,5,6,7-tetrachloro-2-(3-mercaptop-5-(4-methylphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione] has highest percentage (76.72%) of tumor cell count inhibition and highest percentage of (71.07%) of tumor volume inhibition among the tested compounds. It can be concluded that all the synthesized 4,5,6,7-tetrachloro-2-[5-mercaptop-3-(substitued)-[1,2,4]triazol-4-yl]-isoindole-1,3-dione (2A-2D) derivatives exhibited significant antioxidant activity. However, among them, compound 2C and 2D antioxidant activity was more promising than others.

The result of the present study indicates that bioactive compounds of 4,5,6,7-tetrachloro-2-[5-mercaptop-3-(substituted)-[1,2,4]triazol-4-yl]-isoindole-1,3-dione can potentially be developed and evaluated them for a wide range of biological activities.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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