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SYNTHESIS OF SOME NOVEL 2-[5-MERCAPTO-3-(SUBSTITUED) [1,2,4] TRIAZOL-4-YL]-ISOINDOLE-1,3-DIONE DERIVATIVES AND EVALUATION OF THEIR ANTICANCER ACTIVITY ON EAC BEARING MICE MODEL

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ABSTRACT

Objective(s): *A series of titled compounds were synthesized using conventional and microwave assisted fusion of phthalic anhydride moiety with mercaptotriazole via N-N bond. All the steps for synthesis have been done by conventional methods except the final step, which have been done also by microwave irradiation method for comparative study and in vivo anticancer activity was evaluated.* ***Materials and Methods****: In the present study six new isoindole-1,3-dione derivatives were synthesized. The structures of final synthesized compounds were assigned by using FT-IR, ^{1}H NMR, LC-MS and their anticancer activity was evaluated on the Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice model. Compounds were administered orally at dose of 100 mg/kg b.w. for 9 days. The effect of all the compounds on tumor growth inhibition was evaluated by studying the parameters such as tumor volume, percentage of the total cell count (viable and nonviable), the mean survival time, increasing life span and hematological parameters of the treated animal on nine groups of albino mice.* ***Results:*** *The result obtained confirms superiority of microwave irradiation method over conventional heating by increasing the yield value within a short period of reaction time. All compounds showed significant (P<0.01) anticancer activity compared to EAC control group and the compound 5e and 6e were found to be more potent.* ***Conclusion:*** *it can be concluded that isoindole-1,3-dione derivatives might have potent anticancer activity and microwave irradiation method was much better than traditional reflux method.*

KEY WORDS

Anticancer, EAC cell, Fluorouracil, Isoindoline-13-dione, Mercaptotriazole, µw

INTRODUCTION

Cancer is a frightful disease & major global challenge[1] because it is the second most common cause of death worldwide after cardiovascular diseases. Cancer is a non-communicable disease, but it spread like a communicable disease. So, it is more dangerous than any other disease except AIDS. Cancer is not a modern disease, but as cancer risk increases steeply with age, it is more common nowadays due to increasing life expectancy. Extensive research has been carried out to combat this silent killer. Various synthetic drugs have been developed from different chemical entities. Various techniques like surgery, immunotherapy, radiotherapy and chemotherapy are used to treat cancer. But the above-mentioned treatment procedures are expensive and beyond the reach of common people. Our primary focus is to develop an anticancer drug which will be cheap, easily available and more effective than the existing drugs.

It is also reported in the history of medicinal chemistry that nitrogen containing heterocyclics compound have wide range of application. Maximum numbers of disease treatment are only possible with nitrogen containing chemical entity.

Nitrogen containing heterocyclics are present in the structure of a large number and variety of synthetic compounds, and as well as in natural products with a wide range of application in the field of medicine. Phthalimide (IUPAC Name- Isoindole-1, 3-Dione) is bicyclic non-aromatic nitrogen heterocyclic compound. It is an imide, which is a chemical compound with two carbonyl groups bound to a primary amine or ammonia. It is a white solid at room temperature.

Isoindole-1, 3-Dione derivatives exhibit anxiolytic[2], anti-cyclooxygenases (COX-1 and COX-2)[3], antidiabetic[4], antimycobacterial[5], antiangigenic[6], anti-inflammatory[7,8], anticonvulsant[9], antimicrobial[7] anticancer[2,10,11], activities due to presence of –CO-N(R)-CO-. The chemical core of phthalimides (-CO-N(R)-CO-) shows they are hydrophobic and this increases their potential to cross biological membranes in vivo.To increase the biological activity of phthalimide derivatives, a molecular hybridization approach was used to introduce 1, 2, 4 triazole pharmacophore by thermal and microwave assisted power.

Now-a-days, so many non-conventional methods has been applied in the field of medicinal chemistry, among them microwave technology is the most attractive to researcher, chemist, biotechnologist throughout the globe as it has several benefits over conventional heating like uniform heating through the material, high processing speed, decreases the unwanted side of reaction, purity in the final product, improve reproducibility, loss of environment heat can be avoided with low operating cost. This potent versatile technique is a vital part of green chemistry as it is pollution free and eco-friendly methods[12,13].

Here in we have tried to explore some novel 2-[5-Mercapto-3-(substituted)-[1,2,4]triazol-4-yl]phthalamic acid and 2-[5-Mercapto-3-(substitued)-[1,2,4]triazol-4-yl]-isoindole-1,3-dione derivatives by thermal and microwave assisted power for comparative study of the two methods and evaluate their possible anticancer activity against EAC cell line bearing mice model. EAC (Ehrlich Ascites Carcinoma) cell line is the tumor cells used for this *in vivo* anticancer study. The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma[14]**.**

MATERIALS AND METHODS

Materials

O-Anisicacid, *p*-anisic acid, *o*-chlorobenzoic acid, *p*-chlorobenzoic acid, *o*-toluic acid and *p*-toluic acid were commercially available and obtained from LobaChemie Pvt. Ltd. (India). Ethanol, methanol, hydrazine hydrate, carbon disulfide, potassium hydroxide, glycerol, acetonitrile, hydrochloric acid, sulfuric acid, ethyl acetate and petroleum benzene were purchased from Merck (Mumbai, India) and Spectrochem (Mumbai, India) and Phthalic anhydride was purchased from Sigma Aldrich Chemical Co. All solvents and reagents were used without further purification. Tumor cells (EAC) used for this *in vivo* anticancer study was obtained from Chittaranjan National Cancer Institute, Kolkata, India. The EAC 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 haemocytometer and were adjusted at 2 $\times 10^7$ cells/mL. EAC cells suspension (0.1 ml) was injected (i.p.) in each mouse.

Animals and its maintenance

Swiss male albino mice of about 8 weeks old with an average body weight of 18-20 g were obtained from 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 of title compounds (1e – 6e):

Aromatic acids (**1a–6a**) were esterified with dry methanol in the presence of concentrated sulfuric acid and the resulting methyl benzoates (**1b–6b**) were refluxed with hydrazine hydrate in ethanol to give aroylhydrazines (**1c–6c**). Aroylhydrazines were refluxed with $NH_2NH_2.H_2O$, CS_2 and KOH in ethanol to yield 1, 2, 4-triazole derivatives (**1d–6d**) in good to excellent yield. The prepared (**1d–6d**) derivatives were heated with phthalic anhydride in the presence of glycerol at 200°C for 4 hours to give **(1e-6e)** in good yield (15, 16). Remarkably, microwave (Microwave condition was

done by using CEM Discover System, Model no. 908010 and Serial no. DU9636) assisted formation of the same compounds from (**1d–6d**) was comparatively efficiently giving inspiring yield in shorter reaction times. These derivatives were confirmed by FT-IR, ^{1}H NMR & Mass spectroscopy. Physical analysis of synthesized compounds **(1e-6e)** was tabulated in Table 1.Comparative statement of synthesis under conventional and microwave technique were given in Table 2

Table 1: Physical analysis of synthesized compounds (1e-6e)

Compound	Mol. formula	Mol.Wt	Solvent used for crystallization	Mp (°C)
1e	$C_{17}H_{12}N_4O_3S$	352.37	Acetonitrile – water (5:3)	211 - 213
2e	$C_{17}H_{12}N_4O_3S$	352.37	Acetonitrile – water (5:3)	211 - 213
3e	$C_{17}H_{12}N_4O_2S$	336.37	Acetonitrile – water (1:5)	203 - 206
4e	$C_{17}H_{12}N_4O_2S$	336.37	Acetonitrile – water (1:5)	207-209
5e	$C_{16}H_9ClN_4O_2S$	356.79	Acetonitrile – water (1:5)	229 - 231
6e	$C_{16}H_9ClN_4O_2S$	356.79	Acetonitrile – water (1:5)	217-220

Table 2: Comparative statement of synthesis under conventional and microwave technique

Compound code and Compound IUPAC Name	Conventional		Microwave	
	Yield (%)	Time (h)	Yield (%)	Time (m)
1e:2-[5-Mercapto-3-(4-methoxyphenyl)-[1,2,4] triazol-4-yl]-isoindole-1,3-dione	49	4	70	20
2e:2-[5-Mercapto-3-(2-methoxyphenyl)- [1,2,4] triazol-4-yl]-isoindole-1,3-dione	55	4	65	25
3e: 2-[5-Mercapto-3-(4-methylphenyl)- [1,2,4] triazol-4-yl]-isoindole-1,3-dione	57	4	69	30
4e:2-[5-Mercapto-3-(2-methylphenyl)- [1,2,4] triazol-4-yl]-isoindole-1,3-dione	58	4	65	30
5e:2-[5-Mercapto-3-(4-chlorophenyl)-[1,2,4] triazol-4-yl]-isoindole-1,3-dione	62	4	74	25
6e:2-[5-Mercapto-3-(2-chlorophenyl)- [1,2,4] triazol-4-yl]-isoindole-1,3-dione	60	4	77	20

Preparation of methyl benzoates (1b-6b):

In a clean dried round bottomed flask, dried methanol (60 ml, 1.50 mol) and concentrated sulfuric acid (2-3 ml) were taken. Disubstituted benzoic acid (0.06 mol) (**1a - 6a**) was added and refluxed for 6hrs. The reaction mixture was cooled to room temperature and the solvent was removed by rotary evaporator to get crude products **(1b-6b).** Crystallization from an appropriate solvent to get pure disubstituted methyl benzoates (**1b-6b).**

Preparation of substituted benzoic acidhydrazides (1c-6c):

Di substituted methyl benzoates **(1b-6b)** (0.04 mol) were refluxed with an excess of hydrazine hydrate (6.00 gm, 0.12 mol) for 10 minutes. Then the reaction mixture was further refluxed for 4 h after adding of absolute alcohol (10 ml) to get a clear solution. It was concentrated and cooled to room temperature. The crystals of acid hydrazide were dried and crystallized from 75% ethanol or ethyl acetate or pet ether to get pure substituted benzoic acid hydrazide(**1c-6c).**

Preparation of 4-Amino -5-mercapto-3-(substituted phenyl)-1, 2, 4-triazole (1d-6d):

The acid hydrazide (0.01 mol) was added to absolute alcohol containing potassium hydroxide (KOH) (1.6 gm) at room temperature. Carbon disulfide (CS_2) (1.8 ml) was added and the mixture stirred at room temperature for 10 h. The mixture was diluted with ether and stirred for a further 1 hr. The potassium salts was used for the next step without further purification. Hydrazine hydrate (99%) (0.02 mol 1.00 gm) was gradually added to the above potassium salt, then dissolved in water (20 ml) with stirring and the mixture was refluxed gently for 5 h. The color of the reaction mixture changed to a dark green color with evolution of hydrogen sulfide. It was then cooled to 5°C and acidified. The separated solid was filtered and recrystallized with an appropriate solvent to get pure 1, 2, 4triazole (**1d-6d).**

Preparation of 2-[5-Mercapto-3-(substituted)-[1,2,4] triazol-4-yl]-isoindole-1,3-dione (1e-6e):

Triazole derivatives **(1d-6d)** (0.01mole) in glycerol (20ml) were heated with phthalic anhydride (0.01mole) for 4 h. The crude product separated from reaction mixture on cooling. It was collected by filtration, washed

with water repeatedly and dried. Then it was recrystallized from acetonitrile- water mixture to yield pure (**1e-6e).**

Microwave assisted synthesis of 2-[5-Mercapto-3-(substituted)-[1,2,4] triazol-4-yl]-isoindole-1,3-dione (1e-6e):

Triazole derivatives **(1d-6d)** (0.01mole) were subjected to **µw** irradiation (100W) with phthalic anhydride (0.01mole) in presence of glycerol (20ml) at 185°C for 20 min. The reaction mixture gave on cooling a colourless solid. The resulting solid was filtered, washed with water repeatedly, and dried. Then this crude product was recrystallized from acetonitrile- water mixture to yield pure (**1e-6e).**

2.4.1 Acute toxicity study:

The acute toxicity of the synthesized compounds (**1e-6e)** were determined according to the OECD guideline no. 420. Male albino mice weighing 18-20 g were used for this study. The synthesized compounds were given at 50,100, 500, 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 death was observed in any group after 24h. 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 9 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 $\times 10^6$ cells/mouse) intraperitoneally except the negative control group (Group I). This was taken as day zero. In this state, the tumor cells multiply relatively freely within the peritoneal cavity and ascites develops. Animals were allowed for 24 hrs incubation to set the disease condition in their body before starting the administration of synthesized compounds (1e-6e, for 100mg/kg b.w orally /day) and standard drug (5 Fluorouracil, 20 mg/kg b.w. Intraperitoneally). On the first day, 5 ml/kg b.wt of normal saline (0.9 % NaCl w/v) was administered in group I (Normal). Normal saline (0.9 % w/v, NaCl), 5ml/kg, b. wt per day was administered in-group II (EAC control). The synthesized compounds (1e-6e, 100mg/kg body weight /day, orally) and the standard drug 5-Fluorouracil (20 mg/kg, body weight/day, intraperitoneally) were administered in groups (III-VIII) and (IX) 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 haematological and biochemical parameters[14,15,17,18]. 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 percentage increase in life span 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*[14].

Tumor Volume- The ascitic fluid was collected from the peritoneal cavity, and volume was measured by taking it in a graduated centrifuge tube.

Tumor cell count- The ascitic fluid 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 the numbers of cells in the 64 small squares were counted with the help of microscope under 40X magnification.

$$\text{Percentage inhibition of ascitic cells (\%TCI)} = \left(1 - \frac{T}{C}\right) \times 100$$

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

Viable/nonviable tumor cell count-The viability and nonviability 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 nonviable. These viable and nonviable cells were counted.

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

Effect on Body Weight- The effect of the synthesized test compounds and standard drug on body weight of the animals was checked by measuring body weight of the mice at 3 days interval and percent change of body weight for each group was calculated.

Percentage Increase in Life Span (%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.

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

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

Here, time is denoted by days.

Hematological Parameters- Blood collected from the animals were used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) and white blood cell (WBC).

Serum Biochemical Parameters-Serum biochemical parameters like serum glutamate oxaloacetate (SGOT) and serum glutamate pyruvate transaminase (SGPT) were also estimated from EDTA free blood sample.

Statistical analysis- All data are expressed as mean ± SEM. (n = 6 mice per group). The data were analysed by one-way ANOVA between the treated groups and the EAC control followed by Dunnett's Multiple Comparison test.

Count of viable cells, non-viable cells and total cells- The ascitic fluid ofdissected 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 the numbers of total EAC cells, viable and nonviable cells in the 64 small squares were counted with help of microscope.

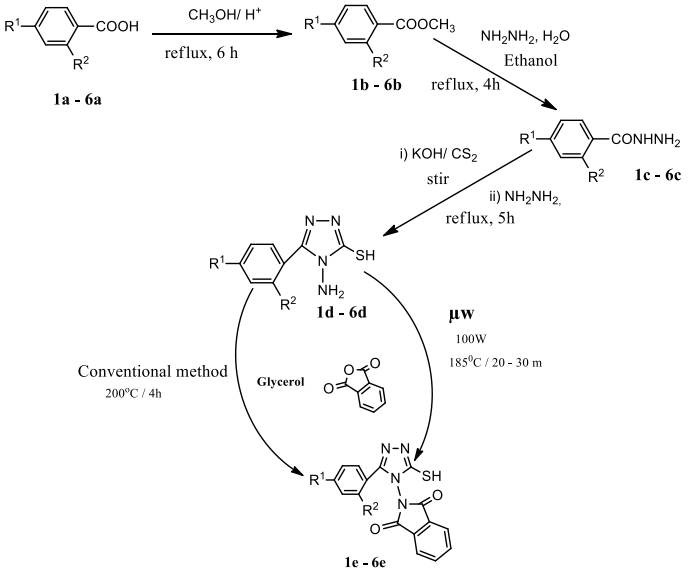
***Haematological parameters*-**Before dissection of the animals, blood sample were collected through orbital plexus through heparinized tubes with the help of thin capillary. Blood sample was taken up to 0.5 marks of the WBC pipette and RBC pipette and it was diluted up to 11 marks (it becomes 20 times dilution) and 101 marks (it becomes 200 times dilution) by using WBC and RBC diluting fluid respectively.

Then a drop of this diluted sample was placed in the Haemocytometer chamber, and the numbers of cells were counted. Sahli's Hemaoglobinometer is used for determination of hemoglobin content.

RESULTS

Synthesis:

In this present study, titled compounds were synthesized by fusion of two bio-active component i.e. phthalic anhydride and triazole. In figure 1, it revealed that final step done in two condition i.e. conventional heating and microwave irradiation because microwave-assisted method proved to be an eco-friendly, quick, efficient, safe and energetically profitable method and also gives better yield when compare with the conventional method (Table 2). Various triazole derivatives (1d-6d) were synthesized from different aromatic acid. Physical analysis of synthesized compounds was given Table 1. Melting points were determined with Veego melting point apparatus. Synthesized compounds were assigned by spectral data (FT-IR, ^{1}HNMR, LC-MS.) as given in Table 3. The structures of all the compounds were confirmed by ^{1}H NMR spectra with Brucker DPX 300 MHz Instrument (DMSO-d_6 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. Infrared (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.



1e: R^1=OMe, R^2 = H; **2e:** R^1=H, R^2 = OMe; **3e:** R^1=Me, R^2=H;
4e: R^1= H, R^2=Me; **5e:** R^1= Cl, R^2=H; **6e:** R^1= H; R^2=Cl.

Figure 1: Scheme of synthesis of 2-[5-Mercapto-3-(substituted)- [1,2,4] triazol-4-yl]-isoindole-1,3-dione

***In vivo* anticancer activity:**

The anticancer property of the synthesized compounds was assessed by their ability to inhibit cancer cell growth in ascitic fluid of swiss albino mice. Various parameters like tumor volume (%TVI) (Table 5), total cell count (Table 4 & 5), viable and nonviable cell count (Table 4), median/ mean survival time (Table 5), percentage increase in life span (%ILS) (Table 5), haematological parameters (Table 6) and serum biochemical parameters (Table 6) have been taken to be considered to establish the potency of the anticancer activity of the synthesized compounds.

The tumor volume and viable cell count were found to be significantly increased and non-viable cell count was significantly decreased in EAC control animals when compared with negative control animals (Table 4 & 5).

Administration of synthesized compounds (1e – 6e) at the dose of 100mg/kg body weight significantly ($P < 0.01$) decreased tumor volume, total cell count, viable cell count. Non-viable cell count was significantly ($P < 0.01$) higher in synthesized compounds treated animals, when explored with respect to EAC control animals.

There was increased level of WBC and decreased level of haemoglobin (Hb) and RBC in EAC control group as compared to normal control group (Table 6). But treatment with synthesized compound at the doses of 100 mg/kg body weight in EAC bearing animals significantly increased both the RBC count, Hb content and significantly reduced the WBC count as compared with the EAC control animals (Table 6).

The biochemical parameters like SGPT and SGOT in the EAC control group were significantly increased as compared to the negative control group. Treatment with synthesized compounds (1e-6e) in EAC bearing mice were significantly decreased the SGPT and SGOT as compared to EAC control mice (Table 6).

Table 3: Spectral data of synthesized compounds (1e-6e)

Compound code	FT-IR (KBr, V_{max}cm-1):	1H NMR (300 MHz, DMSO-d6 ppm):	(LC/MS): m/z (M+/M-)
1e	3346(N-H stretch), 2976(Ar–C-H), 2502(SH), 1794(C=O), 1666(C=N), 1599(N-H bending), 1395(C-N), 1206(C=S).	δ 3.75(3H, s, CH_3); 7.43 – 8.17(8H, m, Ar-Hs).	353.5
2e	3346(N-H stretch), 3098(Ar–C-H), 2535(SH), 1726(C=O), 1666(C=N), 1599(N-H bending), 1288(C-N), 1174 (C=S).	3.94(3H, s, CH_3); 7.08 – 7.83(8H, m, Ar-Hs).	353.2
3e	3087(N-H stretch), 3018 (Ar–C-H), 2574(SH), 1730(C=O), 1667(C=N), 1617(N-H bending), 1312(C-N), 1181 (C=S).	2.35(3H, s, CH_3); 6.82-7.97(8H, m, Ar-Hs); 9.91(1H, s, SH).	337.5
4e	3206(N-H stretch), 2991(Ar–C-H), 2535(SH), 1738(C=O), 1645(C=N), 1606(N-H bending), 1279(C-N), 1201(C=S).	2.20(3H, s, CH_3); 7.43-7.97(8H, m, Ar-Hs); 8.986(1H, s, SH).	337.1
5e	3138(N-H stretch), 3076 (Ar–C-H), 2533(SH), 1734(C=O), 1670(C=N), 1588(N-H bending), 1296(C-N), 1201 (C=S).	2.05(s, SH, 1H) 7.50-7.61(4H, m, Ar-Hs); 7.97-8.01 (4H, m, Ar-Hs,).	358
6e	3214(N-H stretch), 3076 (Ar–C-H), 2520(SH), 1734(C=O), 1670(C=N), 1588(N-H bending), 1257(C-N), 1113 (C=S).	2.07(s, SH, 1H) 7.50-7.63(4H, m, Ar-Hs); 7.94-8.02 (4H,m, Ar-Hs,).	358.4

Table 4: Effect of synthesized compounds on Tumor volume (ml), Total cell count(x10^7), viable cell, Non-viable cell in EAC bearing mice.

Group	Compound	Dose of drug (mg /kg)	Tumor volume (mL)	Total cell count (x10^7)	Viable cell count (x10^7)	Non-viable cell count (x10^7)
I	Negative control	----	---	----	----	----
II	EAC +Control	----	6.12±0.15	7.58±0.09	7.44±0.09	0.14±0.01
III	EAC + 1e	100	3.61±0.21*	3.46±0.43*	2.96 ±0.41*	0.50±0.14*
IV	EAC + 2e	100	3.34±0.29*	4.41±0.59*	4.04± 0.61*	0.36±0.17*
V	EAC + 3e	100	4.07±0.30*	4.82±0.04*	3.51±0.12*	1.30±0.09*
VI	EAC + 4e	100	2.36±0.20*	3.76±0.36*	3.34±0.29*	0.55±0.06*
VII	EAC + 5e	100	2.05±0.22*	2.75±0.11*	1.40±0.17*	0.13±0.16*
VIII	EAC + 6e	100	2.47±0.35*	2.00±0.09*	1.18±0.12*	0.81±0.13*
IX	EAC + 5-FU	20	0.28±0.07*	0.43±0.01*	0.06±0.007*	0.36±0.01*

Each value represents the mean ± SEM, Where n = 6. *Experimental groups were compared with EAC control group (P < 0.01).

Table 5: Percentage inhibition of total cell count (TCI), Percentage inhibition of tumor volume (TVI), mean survival time (MST), percentage increase in life span (%ILS) of synthesized compounds in EAC bearing mice.

Group	Compound	Dose of drug (mg /kg)	% TCI	% TVI	MST (in days)	% ILS
I	Negative control	----	----		----	----
II	EAC +Control	----	0.00	0.00	17.35	0.00
III	EAC + 1e	100	54.35	41.01	20.22	16.54
IV	EAC + 2e	100	41.82	45.42	22.89	31.93
V	EAC + 3e	100	36.41	33.49	18.02	3.86
VI	EAC + 4e	100	50.39	61.43	33.77	94.63
VII	EAC + 5e	100	63.72	66.50	37.41	115.62
VIII	EAC + 6e	100	73.61	59.64	28.02	61.49
IX	EAC + 5-FU	20	94.32	95.42	48.08	177.11

Table 6: Effect of synthesized compounds on haematological parameters like RBC count (10^{12}/L), WBC count (x10^9/L), Haemoglobin (g/dL) and Serum biochemical parameters like SGPT, SGOT in EAC bearing mice

Compound	Dose of drug (mg /kg)	WBC count	RBC count	Haemoglobin	SGPT	SGOT
Negative control	----	5.26±0.06*	9.84 ± 0.09*	14.29±0.15*	37.93± 2.605*	133.7±8.265*
EAC +Control	----	20.35 ± 0.43	3.46 ± 0.13	5.38 ±0.15	92.46 ±4.892	251.7±9.168
EAC + 1e	100	14.17±0.06*	4.15± 0.04**	7.54± 0.12*	61.59 ± 5.237*	85.80±1.946*
EAC + 2e	100	13.24±0.06*	5.28±0.11*	8.59 ±0.06*	64.34 ±1.908*	85.78±5.438*
EAC + 3e	100	17.37±0.06*	4.45 ±0.04*	8.20 ±0.04*	47.32 ± 6.614*	63.93±2.722*
EAC + 4e	100	6.00±0.03*	7.82±0.05*	13.08±0.05*	61.59±5.328*	102.9±2.442*
EAC + 5e	100	8.19±0.05*	7.23 ±0.05*	12.45±0.01*	61.23±5.345*	121.6±3.427*
EAC + 6e	100	7.62±0.13*	6.87±0.38*	12.06±0.02*	66.64±6.169*	149.8±3.390*
EAC + 5-FU	20	6.21±0.14*	8.55±0.07*	13.46±0.06*	53.85±5.849*	50.80±5.340*

Each value represents the mean ± 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).

DISCUSSION

In this present study, titled compounds were synthesized by fusion of two bio-active component i.e. phthalic anhydride and triazole. Phthalimide derivatives have antidiabetic property[19]as they have α-glucosidase inhibitory activity and have potent anti-cancer activity[20] as they may be as apoptosis inducer. 1,2,4-Triazole derivatives are biologically active and also have potent antiproliferative activity (15) due to N-C-S linkage. According to Lamie*et al* 2015[21], the biological activity of phthalimide derivatives can be increased by introducing other pharmacophore subunits i.e. pyrazoles, diazoles, benzo-oxazoles, benzo-imidazoles and benzo-thiazoles. Then we have tried to synthesize a big pharmacophore. In figure 1, it revealed that final step done in two condition i.e. conventional heating and microwave irradiation[22] because microwave-assisted method proved to be an eco-friendly, quick, efficient, safe and energetically profitable method and also gives better yield when compare with the conventional method (Table 2).

Synthesized compounds are hydrophobic due to presence of –CO-N(R)-CO- in their chemical core. So, they easily cross biological membranes in case in vivo biological evaluation.

The present study was undertaken to evaluate the anticancer activity of synthesized compounds at the dose of 100 mg/kg b.w. in EAC bearing mice. It observed that EAC cell were increased regularly so ascitic fluid volume were also increased within a very short period of time in EAC tumor bearing mice. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells[14]. Treatment with synthesized compounds (1e-6e) decreased the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug is the prolongation of the life span of animals. It may be concluded that compounds treated group (Gr III-VIII) enhanced survival time when compare with EAC control group (Gr II). So, Table 5 indicates the anticancer efficacy of synthesized compounds. The compound 4e and 5e were found to increase life span (%ILS) more significantly. It may be by decreasing the nutritional fluid volume or decreasing the ascites fluid volume and arresting the tumor growth.

Usually myelosuppression and anemia (reduced hemoglobin) have been frequently observed in cancer chemotherapy[15]. The anemiaen countered in ascites carcinoma is mainly due to iron deficiency, either by hemolytic or myelopathic conditions which finally lead to reduced RBC number[15]. In this study, we observed that elevated WBC count, reduced hemoglobin and RBC count in EAC control mice. All the phthalimide derivatives specially 4e, 5e and 6e are tried to maintain normal hemoglobin level, normal values of RBC and WBC, thus prove that hematopoietic protecting activity of phthalimide derivatives.

Enzyme in serum has been considered as possible indicators of neoplasia and as aids following the progression and regression of disease[14]. Generally, most of the synthetic anticancer drugs are cytotoxic in nature, so it may be detrimental to liver by producing toxic metabolites. In the present study, EAC control group exhibited increased levels of tissue enzymes such as SGPT and SGOT. Treatment with synthesized compounds restored the elevated biochemical parameters more or less to normal range, indicating the protection of tumor cell induced hepatotoxicity by synthesized compounds.

CONCLUSION

A novel series of 2-[5-Mercapto-3-(substituted)-[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 anticancer activity has been evaluated against EAC bearing mice model. Microwave irradiation method only applied in the final step to assess its productivity by comparing with the yield value and reaction time of conventional heating. If microwave assisted synthesis will be carried out in every reaction step, then it should be a novel promising approach to us. All the final compounds exhibited significant *in-vivo* anticancer activity. Compound 6e has highest percentage (73.61%) of tumor cell count inhibition and compound 5e has highest percentage of (66.50%) of tumor volume inhibition among the tested compounds.

The result of the present study encourages us to develop similar other related compounds by using microwave irradiation and test them for a wide range of anticancer activity. The 2-[5-Mercapto-3-(substitued)-[1,2,4] triazol-4-yl]-isoindole-1,3-dione is a bio active template i.e, it should be considered as valuable leads

which will help the researcher to obtain more potent, selective and less toxic anticancer agent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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