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Evaluation of antitumor activity of novel triazole derivatives

Bhavana V.[1], Praveen Singh[1], Krishna K. L.[1*] and Purohit M. N.[2]

[1]*Department of Pharmacology, JSS College of Pharmacy, JSS University, Mysore-570015, Karnataka, India*
[2]*Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS University, Mysore-570015, Karnataka, India*

ABSTRACT

In the present study, the three novel triazole derivatives (13c, 11b & 16c) were evaluated for their anti-tumor activity against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice. The test compounds were subjected for in-vitro preliminary cytotoxicity screening by trypan blue exclusion and MTT assay on cancer cell lines. In EAC ascites tumor model, the anti tumor activity of 13c, 11b and 16c was evaluated at the doses of 50mg/kg b.w by measuring the mean survival time, body weight changes, haematological parameters (WBC & RBC count and haemoglobin content) and cyclophosphamide was used as a standard. In addition, the effect of test compound on DNA was evaluated by DNA fragmentation assay. Among all the test compounds, 13c showed promising cytotoxic activity as compared to 11b and 16c when tested against cancer cell lines. In EAC model, the novel triazole compounds (13c, 11b and 16c) at a dose of 50mg/kg markedly enhanced the mean survival time of tumor bearing mice and activity was found more with compound 13c. The haematological parameters were also revealed anticancer activity of triazole derivatives whereas compound 13c has shown better activity when compared with other two compounds. In the mechanistic studies, the induction of apoptosis was confirmed by agarose gel electrophoresis and DNA binding studies revealed the binding efficacy of the compounds. From the present study it can be concluded that compound 13c has shown better anticancer activity when tested in-vitro and in-vivo than the 11b and 16c. However this study gives a lead for further research to establish the anticancer activity of these novel triazole derivatives.

Key words: anti-tumor, triazole derivatives, trypan blue exclusion assay, MTT assay.

INTRODUCTION

Cancer is a disease in which a group of cells display uncontrolled growth, metastasis and invasion. Carcinogenesis, the process by which cancers is generated, is a multistep mechanism resulting from the accumulation of errors in vital regulatory pathways. It is initiated in a single cell, which then multiplies and acquires additional changes that give it a survival advantage over its neighbours. The altered cells must be amplified to generate billions of cells that constitute a cancer. On the global scale, the disease is been ranked as second leading cause of morbidity next to cardiovascular diseases. It has been proposed that by 2050, the global burden is expected to be 27 million new cases and 17.5 million cancer deaths. The disease accounted for 14.1 million cases in 2012 and is projected for a continuous rise, with an estimated 8.2 million deaths in 2012. In India the prevalence of cancer is estimated to be around 2.5 million, with about 8, 00,000 new cases and 5,50,000 deaths per annum [1] . In India cancer is the 9th common cause for the deaths among children between 5 to 14 years of age [2] . Treatment of cancer depends upon the type, location, and stage of the cancer as well as the person's health and wishes. The most commonly employed treatments are [3] surgery, radiation therapy, chemotherapy, immunotherapy, hormone therapy. Many drugs are available to treat cancer, but still more efficacious drugs are needs to be discovered. In this regard, this study was designed to evaluate novel 1, 2 4 triazole derivatives for possible anticancer activity.

MATERIALS AND METHODS:

2.1 Animals:
The experiments were carried out on Swiss albino mice of either sex weighing 25-35gm. Animals used in the study were procured from JSS Medical College, Central animal house facility, Mysore. Animals were acclimatized to the experimental condition for one week prior to the experiment. Animals were maintained under controlled conditions of temperature (27 ± 2º C) and were caged in sterile polypropylene cages containing sterile paddy husk as bedding material with maximum of six animals in each cage. The mice were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Ethical Committee, JSS College of Pharmacy, Mysore, Karnataka. (Approval number:- 156/2014)

2.2 Materials:
A) Cell lines: Ehrlich Ascitic Carcinoma (EAC) cell line was obtained from Amala Cancer Research Centre, Trissur, Kerala, India. The cells were maintained as ascites tumor in Swiss albino mice by intraperitoneal inoculation.
B) Triazole derivatives: Three Triazole derivatives (13c, 11b and 16c) were obtained as samples from Dr. Madhusudan. N. Purohit, Professor, Dept. of Pharmaceutical Chemistry, JSS College of Pharmacy, Mysore.

2.3 Trypan blue assay [4, 5]: Trypan blue exclusion assay method was employed to assess the cytotoxic potential of novel triazole derivatives on EAC & HCT cell lines. The ascetic fluid was withdrawn from the peritoneum of EAC cell line bearing animal. It was washed with phosphate buffer saline (PBS). Fixed numbers of EAC cell lines were treated with various concentrations of test compounds and cell viability was checked after 3 hr of incubation at room temperature. Cyclophosphamide was used as a standard to compare the cytotoxic potentials of test compounds. The percentage cytotoxicity was reported.

2.4 MTT Assay [6]: The MTT assay, based on the conversion of the yellow tetrazolium salt-MTT, to purple-formazan crystals by metabolically active cells, provides a quantitative determination of viable cells. The cytotoxicity activity of novel triazole derivatives was determined against EAC cell lines. Cells were plated on to 96 well plates and allowed to grow in CO_2 incubator for 24 h (37˚C, 5 % CO_2). The medium is then removed and replaced by fresh medium containing different concentrations of sample for 48 h. The cells are incubated for 24-48 h (37 ˚C, 5 % CO_2). Then, 20 µL MTT ([3- (4, 5 dimethylthiazol-yl)-2, 5-diphenyltetrazolium bromide]) stock solution (5 mg/mL in PBS) is added to each well and incubated for 5 h. The medium was removed and 200 µL DMSO was added to each well to dissolve the MTT metabolic product. Then the plate was shaken at 150 rpm for 5 min and the optical density was measured at 560nm. The results are expressed as % viability.

2.5 DNA Fragmentation assay [7] :
Ascetic fluid (2 ml) was aspirated from an EAC bearing mice and the cells were washed with 1ml of PBS. To the cell pellet, 10 ml of 70% chilled ethanol was added, mixed well and vortexed. The entire mixture was centrifuged at 1000rpm which leads to the partial lysis of the cells. Cells were fixed for 2hrs on ice. Cells were stored in the fixative at -20ºC for several weeks. After fixation, the cells were centrifuged for 5min at 3000 rpm. The ethanol was thoroughly decanted. 50µl of DNA extraction buffer was added to cell pellet. Tubes were transferred to a 37ºC water bath, capped and incubated for 30min on the shaker incubator. Cell suspension was centrifuged for 10min at 3650 rpm. The supernatant was reserved for the analysis of low molecular weight DNA by gel-electrophoresis. 5µl of 2 mg/ml DNAse –free RNAse solution was added to the supernatant. The tubes were incubated for 30min at 37ºC. After incubation of 30min, 5µl of 1mg/ml of Proteinase K was added and incubated for 30min. 1.5g of agarose was added into 100ml of TAE buffer. The solution was heated for 10min for complete dissolution of the agarose. 5µl of ethidium bromide was added into the gel solution. Gel was poured into the gel-tray while warm and allowed to set. Gel electrophoresis assembly was set up using electrophoresis buffer to fill the reservoir. Sample was prepared by adding 5µl of gel loading dye to entire sample content. Samples were loaded into the wells and electrophoresis was run at 60V, for 90 min. The power was turned off when the dye from the loading buffer migrates from the sufficient distance for separation of DNA fragments. For the visualization purpose, the gel was stained with ethidium bromide for 20-30 min. The gel was transferred onto a UV transilluminator and the illumination was observed. The bands were visualized using gel-documentation system.

2.6 Acute toxicity [8] :
Oral acute toxicity test was done by OECD 423 guideline for novel triazole compounds 13c, 11b and 16 c.

***2.7 In-vivo* anticancer activity:**
2.7.1 Induction of Ascites tumor (EAC) [9, 10]:
EAC cells were obtained from the peritoneal cavity of an EAC bearing mouse, after 15 days of tumor transplantation. The ascitic fluid was drawn intraperitonially using a sterile syringe and a small amount was tested

for microbial contamination. The ascitic fluid was suitably diluted in phosphate buffer saline to obtain a stock cell concentration of 10^7 cells per ml. For ascitic tumor 2.5×10^6 EAC cells (0.25 ml of stock suspension) was injected intraperitoneally to each mouse. Treatment was started after 24 h tumor inoculation and continued for 15 days daily dosing regimen.

2.7.2 Parameters monitored:

a. Percentage increase in body weight as compared to day "0"weight

The percentage increase in the body weight was calculated from the day of inoculation at 3 days interval.

b. Mean survival time (MST) and Increase in life span [%ILS]

Total number of days an animal survived from the day of tumor inoculation was counted. Subsequently the mean survival time was calculated.

c. Haematological parameters:-

In order to assess the influence of treatment on the haematological status of animals, blood was collected from retro orbital plexus of the animals in to sodium citrate treated micro centrifuge tubes and following parameters were determined- total RBC count, total WBC count, and haemoglobin content.

2.8 Carbon clearance test [11, 12]:

Swiss albino mice of 25-30g of body weight were used as a experimental animal. Animals were randomized into five groups (Control, Standard, 13c, 11b and 16c) comprising of 6 animals each. Animals were given the test sample for five days. After 48hr of i.p injection the mice were administered with carbon ink suspension at a dose of 0.1ml/10g through the tail-vein. Blood samples were taken from the tail vein at 5min, 15min. Blood samples were mixed with 0.1% sodium carbonate solution (4ml) for the lysis of erythrocytes. Absorbance was measured at 675 nm using the spectrophotometer. The phagocytic activity is expressed by the phagocytic index which measures all the reticuloendothelial system function in contact with the circulating blood. The clearance rate is expressed as the half life period of carbon ink in the blood

2.9 Statistical analysis

The values were expressed as Mean± Standard Error of Mean (S.E.M.) of the indicated number of experiments animals. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Values were significant if p value <0.05.

RESULTS AND OBSERVATION

3.1 Trypan Blue Exclusion Assay on EAC cells:

In vitro cytotoxicity of triazole derivatives on EAC cells was determined by trypan blue exclusion assay. The compounds showed concentration dependant cytotoxicity on EAC cells at 3h of incubation. In this short term cytotoxicity assay 13c showed more cytotoxicity with an IC_{50} of 0.611±0.02μg/ml, when compared to the IC_{50} of 11b 0.701±0.05μg/ml and 16c 0.791±0.06 μg/ml. In control group the EAC cells were treated with 0.5% sodium CMC at different concentrations and the 100% cytotoxicity was observed (Table 1).

Table 1. Cytotoxic potential of 13c, 11b and 16c on EAC cells by trypan blue exclusion assay (% cytotoxicity)

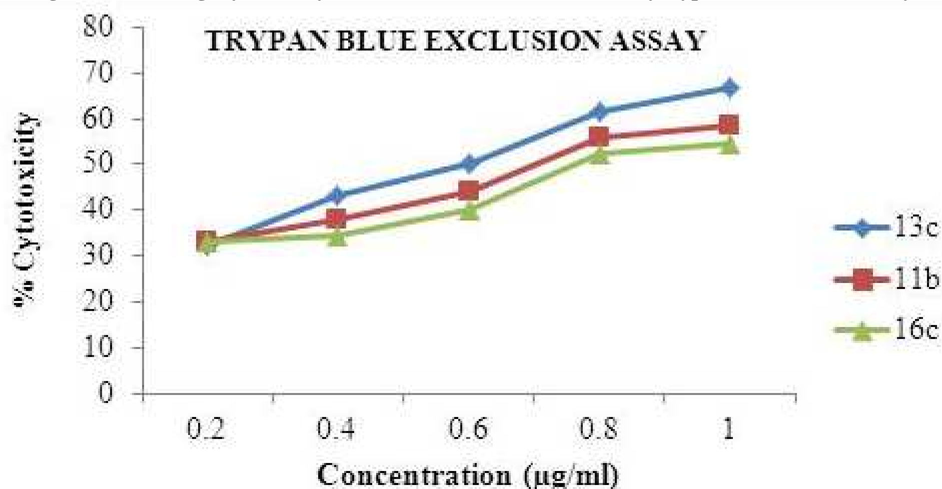
Concentration (μg/ml)	% Cytotoxicity		
	13c	11b	16c
0.2	29.34±0.02	29.28±0.01	29.23±0.09
0.4	42.67±0.03	38.75±0.09	35.59±0.08
0.6	49.11±0.01	45.21±0.07	41.20±0.06
0.8	60.41±0.04	56.92±0.08	53.37±0.04
1.0	65.88±0.01	59.41±0.02	55.71±0.05
IC_{50}	0.611±0.02	0.701±0.05	0.791±0.06

Values are Mean±SEM, n=3

3.2 Trypan Blue Exclusion Assay on HCT cells:

Figure 1 shows the cytotoxic potentials of novel triazole compounds on HCT cell line. All compound showed concentration dependant cytotoxicity on HCT cells at 3h of incubation. Compound 13c showed more cytotoxicity with an IC_{50} of 0.583±0.01μg/ml, when compared to 11b (0.715±0.02μg/ml) and 16c (0.812±0.03μg/ml).

Figure 1. Percentage cytotoxicity of 13c, 11b and 16c on HCT cells by trypan blue exclusion assay



3.3 MTT assay on EAC cell lines

All three compounds showed significant cytotoxicity in EAC cells at 72 h of drug incubation. The IC_{50} values were found to be 0.58±0.03µg/ml, 0.68±0.02µg/ml and 0.78±0.01µg/ml respectively for 13c, 11b and 16c.

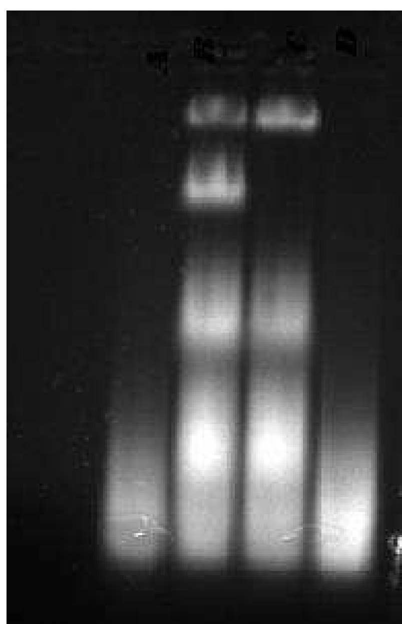
Table 2. Percentage cytotoxicity of 13c, 11b and 16c on EAC cells by MTT assay

Concentration (µg/ml)	% Cytotoxicity		
	13c	**11b**	**16c**
0.2	31.05±0.01	30.15±0.07	29.83±0.03
0.4	43.27±0.06	39.27±0.01	35.78±0.02
0.6	51.07±0.03	45.51±0.03	41.63±0.01
0.8	60.71±0.01	57.86±0.01	53.48±0.01
1.0	66.79±0.03	60.05±0.02	55.82±0.02
IC_{50}	0.58±0.03	0.68±0.02	0.78±0.01

Values are Mean±SEM, n=3

3.4 DNA Fragmentation Assay

Figure 2. DNA fragmentation of EAC cell lines



A B C D

A-Control, B-DNA with cyclophosphamide, C- DNA with 13c compound, D- DNA with 11b compound.

In the DNA fragmentation assay, compound 13 c induced good fragmentation of EAC cell lines and the activity was similar to the standard drug cyclophosphamide. The activity of 13c is more than the other compounds tested (Figure 2).

3.5 Acute toxicity tests

The compounds 13c, 11b, 16c were found to be safe upto the dose of 500 mg/kg body weight. Based on this, the dose for *in-vivo* activity was chosen.

3.6 *In-vivo* antitumor activity against EAC model

3.6.1 Body weight changes

Substantial increase in body weight was observed in EAC inoculated control mice with a maximum gain of (60.59±1.30%) on day 15 compared to day 0. The Standard Cyclophosphamide treatment significantly reduced bodyweight (7.25±0.94%) compared to control. 13c treatment at a dose of 50 mg/kg significantly reduced the tumor induced % increase in the bodyweight (12.55±1.08%) when compared to control and the efficacy was comparable to standard. The activity was found more with compound 13c than 11b and 16c (Table 3).

Table 3. Antitumor activity of 13c, 11b and 16c on EAC inoculated mice (% body weight changes)

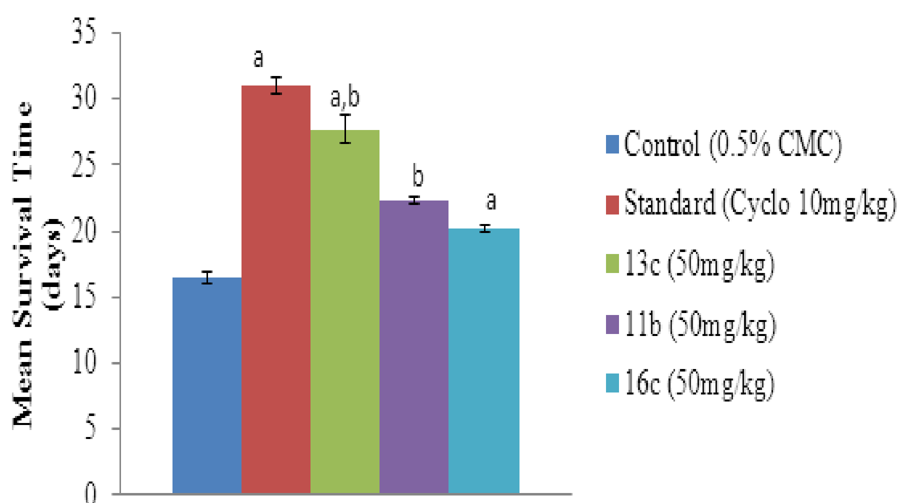
	% increase in body weight				
	Day-3	**Day-6**	**Day-9**	**Day-12**	**Day-15**
Control	17.32±0.98	25.11±1.14	29.46±0.98	49.93±1.25	60.59±1.30
Cyclophosphamide	7.97±0.94^{a}	11.66±0.57^{a}	16.36±1.06^{a}	11.72±1.45^{a}	7.25±0.94^{a,b}
13c (50mg/kg)	9.77±0.7^{a}	15.86±0.99	21.14±1.13^{a}	18.56±1.30^{a}	12.55±1.08
11b (50mg/kg)	11.99±1.38^{a}	18.99±1.20^{a,b}	25.77±1.89	22.44±1.97^{a,b}	15.44±0.67^{a}
16c (50mg/kg)	15.66±0.79^{b}	20.22±2.08^{a}	26.99±1.87^{b}	23.66±2.48	16.66±1.21^{b}

Values are Mean ± SEM, n=6, Statistical analysis- One way ANOVA a – Significant when compared with control animals (P<0.05)
b – Significant when compared with cyclophosphamide treated animals (P<0.05)

3.6.2. Mean survival time and % increase in life span

Mean survival time of EAC inoculated mice was 16.50±0.42 days. Standard Cyclophosphamide treatment at 10 mg/kg significantly enhanced the mean survival time to 31.00±0.63 days when compared to control. The compound 13c at a dose of 50 mg/kg significantly increased the mean survival time (MST) to 27.66±1.05 when compared to control. The percentage increase in life span (% ILS) of animals treated with the compounds at 50 mg/kg was 63.63%, 35.33 and 23.18 respectively for 13c, 11b and 16c whereas cyclophosphamide had shown 87.87%. (Figure 3).

Figure 3. Antitumor effect of 13c, 11b and 16c on EAC inoculated mice (Mean survival time in days)



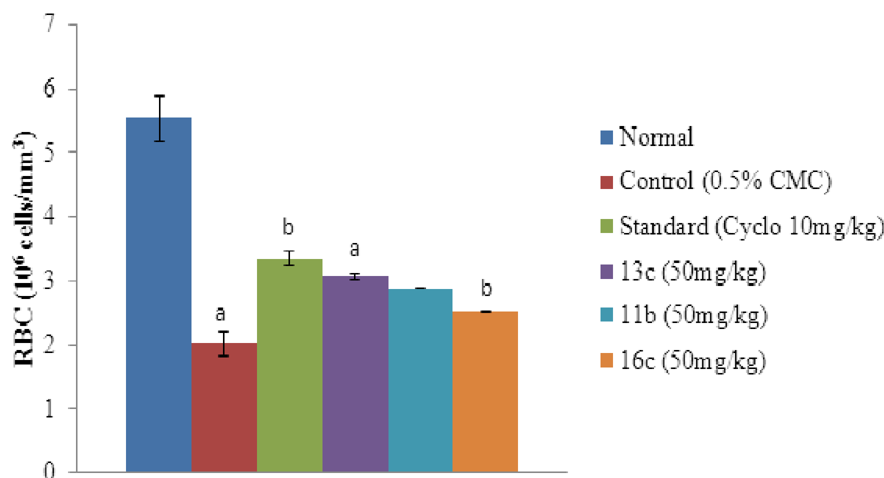
Values are Mean ± SEM, n=6, Statistical analysis- One way ANOVA
a – Significant when compared with control animals (P<0.05)
b – Significant when compared with cyclophosphamide treated animals (P<0.05)

3.6.3 Haematological parameters

To assess the influence of 13c, 11b and 16c treatment on haematological parameters, the total RBCs, WBCs and haemoglobin content of all the treatment groups were checked on 15th day of tumor inoculation.

3.6.3.1 Effect on total RBC:
A significant reduction in total RBCs count was observed in EAC inoculated control mice (2.01±0.20 million cells/mm^3) when compared with the normal mice (5.53±0.34 million cells/mm^3). Treatment with Cyclophosphamide 10mg/kg significantly reversed this reduction to (3.34±0.11 million cells/mm^3) as compared to control. The text compound 13c, 11b and 16c at 50 mg/kg doses increased the total RBC count to 3.05±0.04, 2.88±0.00 and 2.52±0.01 million cells/mm^3 when compared to control (Figure 4).

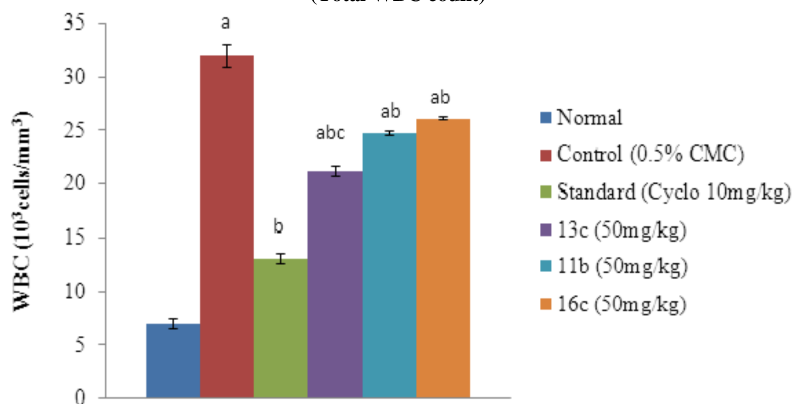
Figure 4. Effect of 11b, 13c and 16c on EAC inoculated mice (Total RBC count)



Values are Mean ± SEM, n=6, Statistical analysis- One way ANOVA
a – Significant when compared with normal animals (P<0.05)
b – Significant when compared with control animals (P<0.05)

3.6.3.2 Effect on total WBC:
A significant increase in total WBCs count was observed in EAC inoculated control mice (31.87±1.02 thousand cells/mm^3) when compared to normal animal (7.01± 0.53 thousand cells/mm^3). Standard Cyclophosphamide treatment at a dose of 10 mg/kg resulted in the reduction in WBC count to (13.00±0.42 thousand cells/mm^3) when compared with control. The text compound 13c,11b and 16c at 50 mg/kg significantly reversed the elevated WBC, to 21.17±0.51 thousand cells/mm^3, 24.75±0.19 thousand cells/mm^3 and 26.08±0.13 thousand cells/mm^3 when to compared to control (Figure 5).

Figure 5. Effect of 11b, 13c and 16c on EAC inoculated mice (Total WBC count)

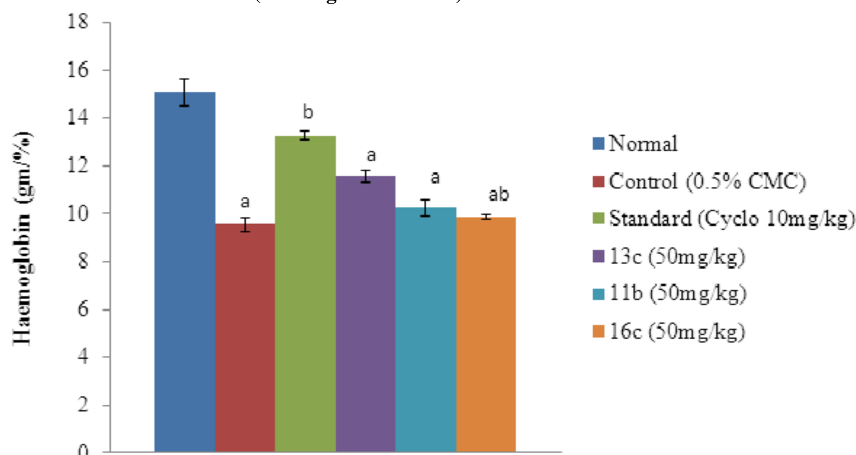


Values are Mean ± SEM, n=6, Statistical analysis- One way ANOVA
a – Significant when compared with normal animals (P<0.05)
b – Significant when compared with control animals (P<0.05)
c – Significant when compared with cyclophosphamide treated animals (P<0.05)

3.6.3.3 Effect on haemoglobin content:
A significant reduction in haemoglobin level was observed in EAC inoculated control (9.55±0.32 gm%) mice as compared to normal (15.06± 0.55 gm%). Standard Cyclophosphamide treatment at a dose of 10 mg/kg significantly reversed the tumor induced reduction in haemoglobin level to (13.27±0.18 gm%). The text compound 13c, 11b and 16c at 50 mg/kg significantly reversed the decrease in haemoglobin level to 11.56±0.21 gm%, 10.24±0.34 gm% and

9.88±0.10 gm% when compared to control (Figure 6).

Figure 6. Effect of 11b, 13c and 16c on EAC inoculated mice (Haemoglobin content)



Values are Mean ± SEM, n=6, Statistical analysis- One way ANOVA
a – Significant when compared with normal animals (P<0.05)
b – Significant when compared with control animals (P<0.05)

3.6.3.4 Carbon clearance test:-

At the dose of 50mg/kg the compound 13c showed highly significant increase in phagocytic index 0.456±0.002 respectively in treated animals when compared with control group (Table 4).

Table 4. Immonomodulatory effect of 13c, 11b and 16c (Phagocytic index)

Group	Phagocytic index
Control (0.5% CMC)	0.286±0.003
Levimasole 50mg/kg	0.476±0.002
13c (50mg/kg)	0.456±0.002[a]
11b(50mg/kg)	0.441±0.003[a]
16c(50mg/kg)	0.419±0.003

Values are Mean ± SEM, n=6, Statistical analysis- One way ANOVA
a – Significant when compared with control animals (P<0.05)
b – Significant when compared with cyclophosphamide treated animals (P<0.05

DISCUSSION

Cancer is one of the major causes of death and the number of new cases and the number of individuals living with cancer is expanding continuously. Cancer still remains as major threat against human race**.** Extensive progress has been made to fight against cancer. Conventional cancer chemotherapy is highly inadequate as a result of the lack of selectivity between cancer cells and normal cells. This calls for novel cancer therapies for selectively targeting cancers without toxicity to normal tissues. The discovery of novel anticancer agents that will hopefully provide the desired degree of selectivity for Cancer cells versus normal tissues has been fuelled by the unveiling of a host of novel potential molecular targets through the application of molecular biology methods to cancer biology.

Based on the reports available on the molecules of our interest, we have taken up this study, to investigate the *in vivo* antitumor activity of the novel triazole derivatives which showed effective *in-vitro* cytotoxic activity. The ‘‘appropriate’’ transplantable mouse tumor model, was used in the drug development programs and to investigate the antineoplastic effects of several chemical compounds. Hence in the present study *in vivo* antitumor efficacy was assessed using transplantable tumor bearing (Ehrlich’s) models in mice respectively.

The trypan blue exclusion assay is a simple and reliable method for preliminary screening of cytotoxicity potential of test compounds. In the present study two cell lines i.e. EAC and HCT were employed to check the cytotoxicity potential of novel triazole derivatives. The compounds 13c, 11b and 16c exhibited good cytotoxicity on EAC cell lines whereas 13c was found to be most promising molecules. The same results were observed by the test compounds on HCT cell lines.

MTT assay is based on the reduction of MTT (3-(4-5- dimethyl thiazolyl)-2-5- diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product and it showed 66.79±0.03, 60.05±0.02 and 55.82±0.02 %

of cytotoxicity respectively by 13c, 11b and 16c at concentration 1.0µg/ml. Thus IC_{50} values of all compounds showed significant anticancer activity by MTT assay but compound 13c exhibited more cytotoxic potential among the compound tested.

DNA fragmentation is a key feature in apoptosis (programed cell death). It is characterized by the activation of endogenous endonucleases, with subsequent cleavage of chromatin DNA into inter-nucleosomal fragments composed of 180 base pairs and their multiples. Among the most prolific areas of research in this arena has been the quest for novel anti-cancer agents. Therapeutic to manipulate apoptosis have immense potential. EAC cells treated with test compound 13c and 11b demonstrated DNA fragmentation as a result of DNA damage when compared to control. However 13c compound was effective in inducing apoptosis as indicated by DNA fragmentation.

Based on encouraging results of the compound 13c, 11b and 16c in *in vitro* study, we further proceeded with the *in vivo* anti-tumor study of all three compounds in transplantable tumor bearing mice to find out the most promising compound in *in-vivo* model. Prior to *in vivo studies,* acute toxicity of 3 compounds was determined in Swiss albino mice as per OECD- 423 guidelines. No mortality was observed when 500 mg/kg was administered. So $1/10^{th}$ of acute toxicity dose was chosen for *in-vivo* studies.

The Ehrlich ascites tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behaviour which grows in almost all mice strains [13]. It has been reported that Ehrlich ascitic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense oedema formation, cellular migration, and a progressive ascitic fluid formation [14]. The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells [15]. In this ascites tumor model, on 15th day of inoculation, a substantial increase in body weight was seen in tumor control animals, owing to the rapid and progressive accumulation of ascites tumor cells. Maximum increment in body weight was 60.59±1.30 % in tumor bearing control mice. Treatment with 13c, 11b and 16c caused marked reduction in the body weight of the animals as compared to control indicating the inhibition of tumor cell progression. 16c treatment did not show enhanced MST and compound 13c and 11b treatment enhanced the MST of tumor bearing mice. The survival rate observed was 63.63 % by compound 13c at dose of 50mg/kg. Since the prolongation of life span is a reliable criterion for judging the anticancer efficacy of any compound. 13c at 50 mg/kg was found to be most promising and the efficacy was comparable to standard cyclophosphamide treatment (87.87 %).

The influence of tumor growth on blood cells is well established, myelosuppression and anaemia have been frequently observed in ascites carcinoma. Hence to assess the influence of 13c, 11b and 16c on the haematological parameters (total RBC & WBC count, haemoglobin content), all the treated groups were checked on 15th day of tumor inoculation. In tumor control animals there was a significant increase in the WBC count. Treatment of 13c, 11b and 16c at the dose of 50mg/kg reversed the enhanced WBC count in treated group which was more significant to that of control. Anaemia (reduced haemoglobin) encountered in ascites carcinoma mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number. The major problems of cancer chemotherapy with the conventional drugs are myelosuppression and anaemia. RBC count was much decreased in the control group which was restored by the treatment of 13c, 11b and 16c at the dose of 50mg/kg. Ascites tumor bearing mice reduced haemoglobin content which is a hallmark in iron deficiency. The treatment with 13c, 11b and 16c at 50mg/kg reversed the tumor induced alternation in haematological parameters such as elevation in the haemoglobin content and total RBC count.

Since most of the anticancer agents exhibits myelosuppression, hence apart from evaluation of anticancer property of all test compounds subjected for investigation of immunomodulatory activity by Carbon clearance test. Phagocytic activity of reticuloendothelial systems (RES) was assayed by carbon clearance test. Phagocytic index was calculated as a rate of carbon elimination of reticuloendothelial systems by carbon clearance test. The reticuloendothelial system consist of the spleen, thymus and other lymphoid tissues, together with cells lining the sinuses of the spleen, bone marrow, and lymph nodes and capillary endothelium of the liver (Kupffers cells), and of the adrenal and pituitary glands. These comprise the sessile or fixed macrophages. In addition, free macrophages, such as the blood monocytes, other leucocytes and the tissue macrophages, are transported by the body fluids or wander through the tissues [16]. The RES is best defined functionally by its ability to scavenge debris or other foreign matter and forms first line of defence. The rate of removal of carbon particles, by the sessile intravascular phagocytes in the liver and spleen, from the bloodstream is a measure of reticuloendothelial phagocytic activity [17]**.** Phagocytosis represents an important innate defence mechanism against ingested particulates including whole pathogenic microorganisms. The specialized cells that are capable of phagocytosis include blood monocytes, neutrophils and tissue macrophages [18]**.**

When the carbon suspension is injected intravenously clearance of carbon from blood by macrophages is governed

by an exponential equation. The increase in clearance response decrease in phagocytic index reveals that treated animals decrease the macrophages activity. The test compound 13c at dose of 50mg/kg increased the clearance of carbon particles from the blood as indicated by the significant increase in the phagocytic index treated animals when compared to control.

CONCLUSION

From the above observations it can be concluded that compound-13c showing promising activity than the compound 11b and 16c. Further detailed investigations are needed to explore the mechanism of action of this novel molecule which may bring promising results in cancer chemotherapy.

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