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Antiproliferative BT008B inhibits neovascularization in inflammatory ascites tumour model

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ABSTRACT

Inflammation is one of the hall mark of cancer, inflammatory angiogenesis plays vital role in inducing cancer, were tumour microenvironment orchestring different inflammatory cytokines. Benzophenone compounds have proven their ability as potent anticancer moiety among them BT008B, a benzophenone tagged pyridine molecule was screened against different cancer cell lines and showed its effect as potent cytotoxic molecule against EAC and DLA with 3.8µM and 8.5µM respectively. Further, BT008B showed its antiproliferative effect in *in-vivo* ascites tumour model system with the decrease in the ascites volume, cell count, tumour growth in BT008B treated mice compared to the untreated and the survivality of the treated mice was increased. Peritoneal angiogenesis of the BT008B was reduced compared to the untreated mice.

KEY WORDS: Inflammation, angiogenesis, Benzophenone

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INTRODUCTION

Inflammation is amultifaceted biological reaction of vascular tissues to harmful spurs, such as pathogens, damaged cells or irritants[1]. In recent years, inflammation has taken its stage to be listed as the seventh hallmark of cancer[2]. About 25% of all cancers are linked to chronic inflammation and inflammation induced through external sources like exposure to environmental or to the infectious pathogens these studies was confirmed through epidemiological and clinical studies[3]. Inflammation and cancer are linked to each other, chronic inflammation may lead to cancer were inflammatory cells infiltrate tumours leading to cancer[4, 5] .Tumour microenvironment reminds you of an inflammatory site were recruitment of numerous pro inflammatory cytokines is seen.Although it is now clear that proliferation of cells alone does not cause cancer, sustained cell proliferation in an environment rich in inflammatory cells, growth factors, activated stroma, and DNA-damage-promoting agents, certainly potentiates and/or promotes neoplastic risk[6].Angiogenesis is the process which involves growth and remodelling of new vascular system from the initial blood vesselleading to formation of neo branching of complex network system of blood vessels[7]**.** Chronic inflammation and angiogenesis are the two process that are coordinated with each other in the development of cancer.Inhibiting inflammatory angiogenesis is one of the key strategies in blocking the cancer. In the quest of searching anticancer drugs Benzophenones was our answer.Benzophenones, The pharmacologically active molecules which are available by both natural and synthetic means[8]these derivatives are used in medicine research for their familiar effectiveness against various pathological conditions including cancer[9,10].Benzophenone conjugated analogues are known as potent inhibitors targeting angiogenesis[11,12]. Benzophenone tagged pyridine While, pyridine or nicotinic acid is well known for its biological activity against carcinogenesis[13,14]our group has identified one of the compound Benzophenone tagged pyridine BT008B (6-Hydroxy-nicotinic acid N0-{2-[2-chloro-6-fluoro-4-(4-fluoro-benzoyl)-phenoxy]-acetyl}-hydrazide) which is known for its potentiality to induce apoptosis by degrading ICAD from CAD and inducing nuclear translocation of CAD[15]by taking the following consideration compound BT008B was chosen to screen against multiple cancer cell lines both *in vitro* and *in vivo* system.

MATERIALS AND METHOD

Cell culture and In-vitro treatment

Human cancer cell lines lung adenocarcinoma (A549), breast adenocarcinoma(MCF-7), melanoma (A375),Hepatocellular carcinoma (HUH-7, HepG2) and murine origin Dalton's Ascites Lymphoma (DLA),Ehrlich-Lettre Ascites-E (EAC),melanoma (B16F10), cells were cultured in

DMEM and RPMI media (Gibco-Invitrogen, USA), respectively with 10% FBS(In vitrogen, USA), with necessary antibiotic and antimitotic (Sigma Aldrich, USA) supplied with 5% co_2 at 37 ^{0}c. The cytotoxic effect of BT008B (0, 10, 20, 50, and 100 µM for 48 hrs)was evaluated by cytotoxic assays trypan blue dye exclusion assay as reported earlier with an suitable vehicle and positive control.

Trypan blue dye exclusion assay

Cytotoxic effect of BT008B was checked through trypan blue dye exclusion assay against A549, MCF-7, and Huh-7, Hepg2, B16F10, A375, DLA and EAC cell lines. Cells were cultured and treated with or without compound BT008B and incubated for 48hr with the supply of 5% co2 and after 48hrs cells were collected and determination of Ic50 value of BT008B was done by resuspending the cells in 0.4% trypan blue[16, 17].

Animal models and ethics

Healthy Swiss albino mice aged 5–6 weeks, weighing 25–30 g were used throughout the study. The mice were grouped in polyacrylic cages with not more than ten animals per cage with adequate food and water supply. The present study involving all the animal experimentations was approved by the Institutional Animal Ethics Committee (IAEC), National College of Pharmacy, Shivamogga, India, in accordance with the CPCSEA guidelines for laboratory animal facility (NCP/IAEC/CL/101/05/2012-13).

Determining the LD50 value of compound BT008B

Normal Swiss albino mice dived into 5 groups (n = 6) were subjected to the acute toxicity studies. The LD50 of compound BT008Bwas determined by intraperitoneal administration of BT008Bas per the standard CPCSEA guidelines. To check the adverse effect of the compound BT008B (75 mg/kg body weight) in Swiss albino mice haematological and serum profiles was done as per the methods described previously[18]

Tumour models and treatment

In-vivo EAC Ascites tumour model system was developed in Swiss albino mice, EAC cells (1×10^6 cells) were injected into the peritoneal region of normal Swiss albino mice and weight of transplanted mice was monitored daily. On the onset of tumour growth i.e. on the fourth day after transplantation compound BT008B was treated with the concentration of 50 and 75mg/kg body weight of the mice and appropriate Vehicle control was kept. On the 10th day mice were sacrificed

and tumour parameters like ascites volume, packed cell volume, tumour growth and survivality of the control and treated mice was noted [19].

Peritoneal angiogenesis

Ascites tumour was induced by injecting EAC tumour cells into peritoneal region of Swiss albino mice after the onset of tumour i.e. on the 4th day compound BT008B was treated (50, 75 mg/Kg body weight) for three doses to the intraperitoneal region on every alternative days on the 10th day mice was sacrificed .The peritoneal region of BT008B treated and untreated mice was observed and the micro vessel density in the peritoneal region was observed.

RESULTS

BT008B screened against multiple cell line to check its cytotoxic effect

BT008B(Figure1) was screened against human origin A549, MCF-7, Huh-7, Hepg2, B16F10, A375 and murine origin DLA and EAC cell lines.BT008B showed more cytotoxic effect in EAC with IC_{50} value of 3.8 µM and in DLA IC_{50} value was found to be 8.5 µM,effect of BT008B on A549, MCF-7, Huh-7, HepG2, B16F10, A375 was not found promising (Figure2).

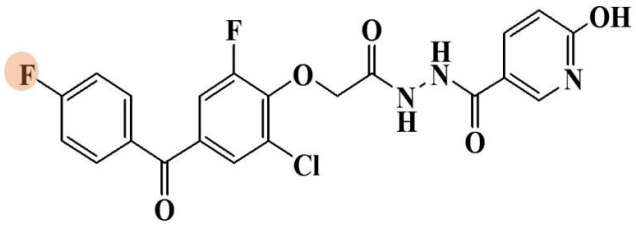

Figure 1. Structure of compound BT008B (6-Hydroxy-nicotinic acid N0-{2-[2-chloro-6-fluoro-4-(4-fluoro-benzoyl)-phenoxy]-acetyl}-hydrazide).

BT008B induces antineoplastic effect in Ascites tumour model system

To evaluate the *In vivo*antineoplasticeffect of BT008B, EAC ascites model system was developed by injecting 1×10^6 EAC cells into the peritoneum region of Swiss albino mice after the onset tumour BT008B was treated in dose dependent manner i.e. 50mg and 75 mg/Kg body weight, for three doses in alternative days.

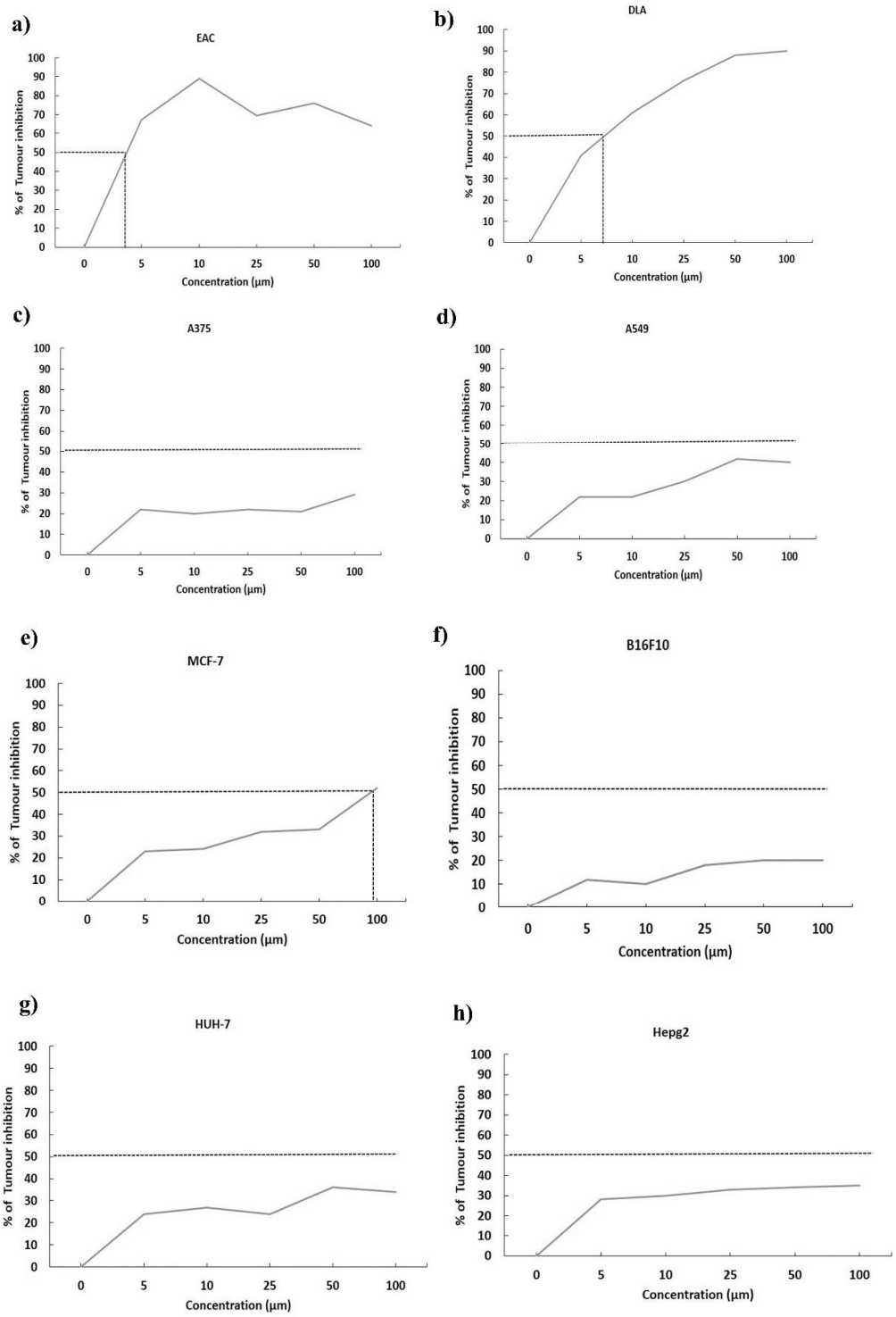

Figure2. Graphs showing IC_{50} value of BT008B In-vitro cytotoxic effect of Compound BT008B against multiple cancer cell lines.

It was found that there was decrease in the tumour in dose dependent manner, ascites volume, cell count and tumour volume was found to be low in BT008B treated mice compared to the untreated mice(Figure3). There was decrease in the micro vessel density of the BT008B treated mice compared to untreated and there was increase in the survivality of the BT008B treated mice compared to the untreated mice (Figure 4).

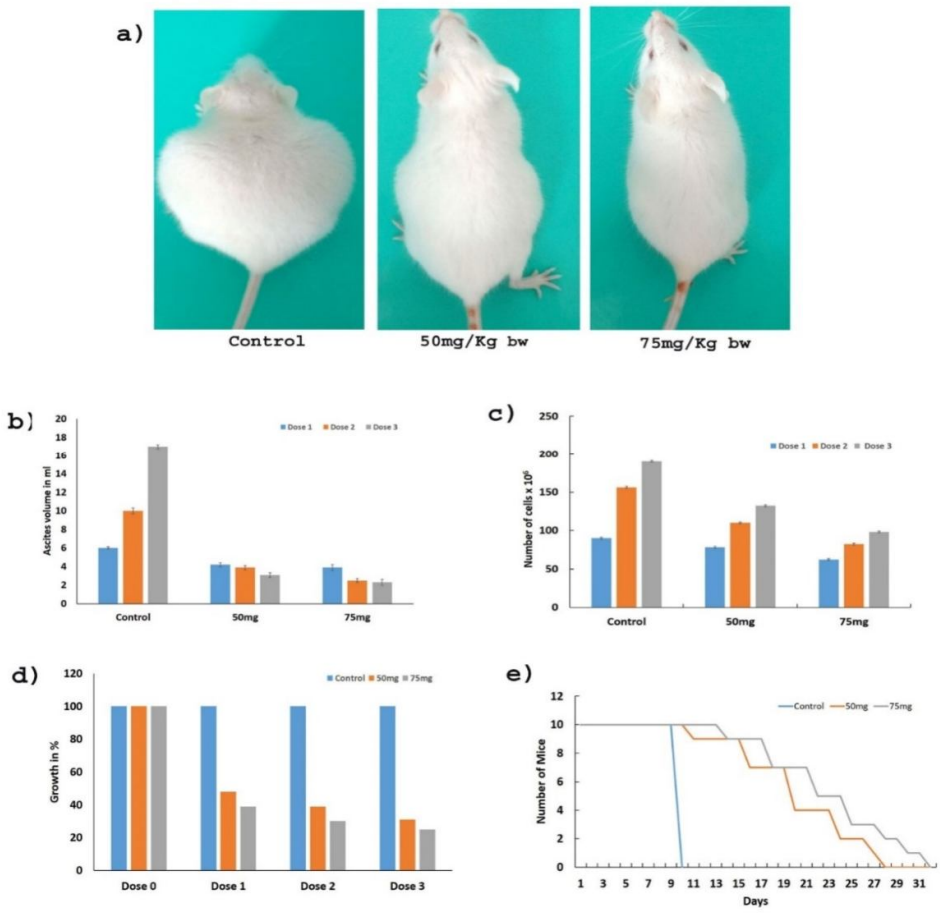

Figure 3. ***In vivo*** **screening of BT008B against EAC cells compound BT008B was given in two different concentration 50mg and 75mg a) Physical morphology of tumour bearing mice and treated mice with two different concentration, b) Dose dependent decrease in ascites volume compared to the control, c) Decreased cell count in BT008B treated mice In dose dependent manner compared to the untreated mice, d) Decrease in the tumor growth in BT008B treated mice, e) Prolonged survivality of BT008B treated mice can be seen compared to the control mice**

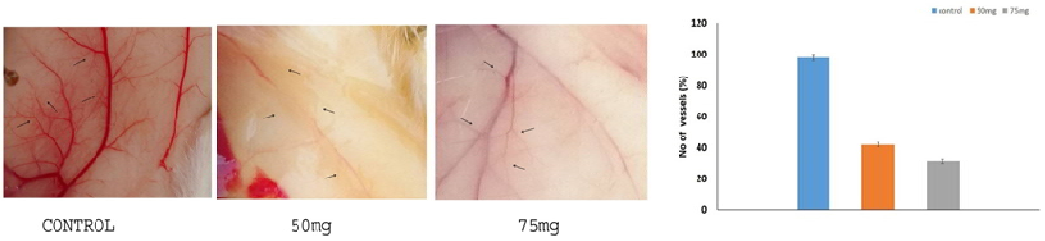

Figure 4. Reduced peritoneal angiogenesis in dose dependent manner in BT008B treated mice

DISCUSSION

Inflammation is one of the emerging hallmark for cancer[2], inflammatory environment mimics the tumour microenvironment [3]. Angiogenesis is development of new blood vesselsin controlled manner[20]angiogenesis plays major role in tumourgensis during tumour condition there is increase in the angiogenesis, which help tumour cells in providing essential nutrients and growth factors. Targeting inflammatory angiogenesis plays vital step in reducing tumour [4, 5]. Benzophenones are known for their potent anticancer activity[9, 10]. Previously our group as reported different benzophenone tagged molecules acting as potent anticancer drugs against various cancer cell lines[11, 12&18] as in the process of developing the drug benzophenone was tagged with pyridine molecule, pyridine molecule is known for its cytotoxic effect[13,14] benzophenone tagged pyridine molecule BT008B (6-Hydroxy-nicotinic acid N0-{2-[2-chloro-6-fluoro-4-(4-fluoro-benzoyl)-phenoxy]-acetyl}-hydrazide) is one such kind of molecule which showed its potency against tumour cells, the flour group present in the molecule (Fig.1) plays vital role in making compound more cytotoxic molecule this was checked through structural activity relationship[15] further BT008B was screened against EAC,DLA,A375,A459,B16F10,HuH-7 and HepG2 cell lines through cytotoxic assay tryphan blue dye exclusion, cells were treated with or without BT008B in concentration dependent manner(0-100µm) and incubated for 48 hrs with the supply of 5% Co_2 in humidified condition , BT008B found to be more cytotoxic in EAC and DLA cell lines with the Ic_{50} value of 3.9µm and 8.5µm and its effect on other cell lines was not much promising (Fig.2). Further the antiproliferative effect of BT008B was checked through *In-vivo* EAC ascites tumour model system which is one of the foremost inflammatory angiogenesis model system were tumour cells are cultured in peritoneum region of mice, BT008B was treated with two different dose 50mg and 75mg after the onset of tumour in mice and body weight of treated mice and untreated mice was observed daily on the 10th day mice was scarified and different tumour parameters like cell count ,ascites volume, tumour growth, survivality was checked there was drastic decrease in the ascites volume of treated mice compared to the untreated, cell count was low in BT008B treated compared to control mice and

tumour growth was reduced in compound treated mice compared to the control mice and survivality of the BT008B was increased two folds more compared to the untreated mice. Peritoneal angiogenesis induced by ascites tumour was measured, increased micro vessel in the peritoneum region of mice indicates the angiogenesis, and peritoneal angiogenesis was reduced in BT008B treated mice compared to the control this was inferred through reduced count of microvessel density in BT008B treated mice compared to the control. By considering the above results BT008B acts as potent angiopreventive drug.

CONFLICT OF INTEREST: The authors declare that there are no conflicts of interest.

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