Synthesis and Evaluation of 1-Benzhydryl-sulfonyl-piperazine Derivatives as Inhibitors of Tumor Growth and Tumor Angiogenesis of Mouse Ehrlich Ascites Tumor *In Vivo*

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Abstract: A series of novel 1-benzhydryl-sulfonyl-piperazine derivatives **3(a-e)** were synthesized by nucleophilic substitution reaction of 1-benzhydryl-piperazine with different sulfonyl chlorides and were characterized by ^{1}H NMR, LC/MS, FTIR and elemental analysis. In the present study, the compounds **3(a-e)** exhibited *in vivo* inhibition of Ehrlich ascites tumor (EAT) cell growth and increased the Median Survival Time (MST) and %ILS of EAT bearing mice. Further treatment of derivatives *in vivo* resulted in reduction of EAT cell number and ascites formation. The efficacy of the derivatives to inhibit the angiogenesis *in vivo* was evaluated in tumor bearing mice peritoneum and chorio allantoic membrane (CAM) model. The compounds suppressed the blood vessel formation *in vivo* in mice peritoneum and in CAM. Among the compounds studied, **3e** demonstrated highest tumor inhibitory and anti-angiogenic effects against mouse tumor. However, this phenomenon needs detailed investigation.

Key Words: 1-benzhydryl-piperazine, Sulfonyl chlorides, EAT cells, MST, Anti-angiogenesis, Chorio allantoic membrane.

INTRODUCTION

Angiogenesis is the formation of new capillary blood vessels from preexisting vessels and is considered as a tightly regulated phenomenon that plays essential roles in reproductive functions, wound healing, and embryonic development [1]. However, angiogenesis may become pathological when capillary growth gets uncontrolled and the resulting excessive neovascularization may then sustain the development of numerous pathologies including retinopathies, hemangiomas, rheumatoid arthritis, psoriasis and tumor growth [1]. It is clearly established that the aggressive growth of tumors and their metastasis is strictly dependent on angiogenesis [2], thus suggesting that the inhibition of angiogenesis may prove as an effective approach for blocking tumor progression. This has led to the development of a new class of novel products having antiangiogenic activity. One of the main strategies of cancer treatment is developing molecules of anti-angiogenic activity [3-6]. The novel synthetic compounds like TNP-470 and thalidomide have shown anti-tumor effect in many of the cell lines both *in vivo* and *in vitro* [7, 8].

Piperazines are currently the most important building blocks in drug discovery, with a great number of positive hits encountered in biological screens of this heterocycle and its congeners. The piperazine template forms the molecular backbone, possesses versatile binding properties with a frequently occurring binding motif, and provides potent and selective ligands for a wide range of different biological targets in Medicinal Chemistry. The piperazine scaffold and its analogues are important pharmacophores that can be found in biologically active compounds across a number of different therapeutic areas [9].

The piperazine containing epoxides are protease inhibitors, which are used in cardiovascular diseases [10-12]. Protease inhibitors such as NCO-700, have been examined in cancer therapy mainly as anti-metastatic agents [13-15]. These agents have been shown to inhibit tumor cell derived proteases that are important for the cancer cell to escape the vasculature and invade the sub-endothelial space and outlying tissues. Unfused aromatic systems containing terminal piperazino substituent have shown excellent anti-cancer activity and DNA interaction [16], piperazine scaffold was also found in anti-tumor drugs against colon, prostate, breast, lung and leukemia cancers [17]. MST-16[4,4-1,2-(ethanediyl)bis(1-isobutoxycarbonyloxy-methyl-2,6-piperazinedione)] was recently approved as an oral anticancer drug for clinical use in Japan [18]. Chloroalkyl piperazines have shown potent bioactivity and increased anti-cancer activity [19]. The primary anti-cancer activities of chloroalkyl piperazine porphyrins toward bel-7404 liver cancer cell were investigated *in vitro* and they exhibited potential anti-cancer activities [20].

In continuation of our research on anticancer activity of heterocycles [21-23], we have further investigated the tumor growth inhibitory and anti-angiogenic effects of the novel piperazine analogs on Ehrlich's ascites tumor cells grown in the peritoneal cavity of Swiss albino mice. The results suggest that synthetic piperazine analogs probably target multiple steps in tumor growth and neovascularization.

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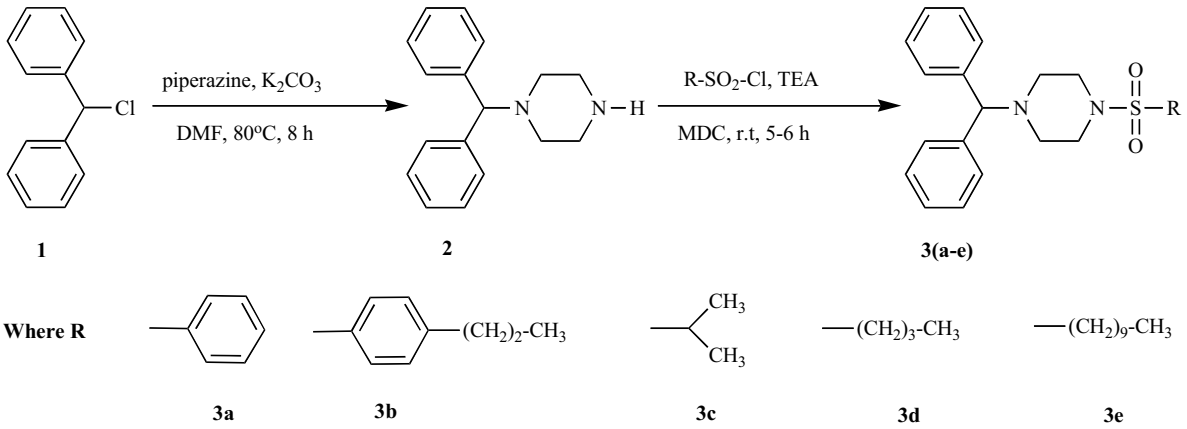

Scheme 1.

MATERIALS AND METHODS

General

Melting points determined using SELACO-650 hot stage melting point apparatus were uncorrected. Infrared (IR) spectra were recorded using a Jasco FTIR-4100 series. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Shimadzu AMX 400-Bruker, 400MHz spectrometer using DMSO as a solvent and TMS as internal standard (chemical shift in δ ppm). Spin multiplets are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Mass and purity were recorded on a LC-MSD-Trap-XCT. Elemental (CHNS) analyses were obtained on Vario EL III Elementar. Silica gel column chromatography was performed using Merck 7734 silica gel (60-120 mesh) and Merck made TLC plates.

CHEMISTRY

1-Benzhydryl-piperazine derivatives **3**(**a-e**) were prepared as per the method summarized in Scheme **1**. The nucleophilic substitution reactions of 1-benzhydryl-piperazine (**2**) with different aliphatic and aromatic sulfonyl chlorides (**R-SO$_2$-Cl**) were carried out in the presence of triethylamine and dichloromethane as solvent with a good yield ranging from 78-86% having high purity. Synthesized molecules **3**(**a-e**) were structurally characterized by ^{1}H NMR, LC/MS, IR and elemental analysis. The *N*-substitution of 1-benzhydryl-piperazine with different sulfonyl chlorides was confirmed by the disappearance of N-H group in IR and ^{1}H NMR data. Compounds **3**(**a-e**) were also confirmed by IR data, which showed asymmetric stretching frequency of O=S=O at 1350 cm^{-1} and symmetric stretching frequency at 1280 cm^{-1}. Several new derivatives of 1-benzhydryl-piperazine **3**(**a-e**) were synthesized in order to screen their anti-angiogenic efficacy. The products obtained were purified by column chromatography using hexane: ethyl acetate (8:2) as an eluent. The physical data and purity of all the synthesized compounds are given in Table **2**.

General Procedure for Synthesis of 1-benzhydryl-piperazine (2)

A solution of piperazine dihydrochloride (10.0 g, 62.86 mmol) in dimethyl formamide was taken, anhydrous potassium carbonate (43.44 g, 314.3 mmol) was added and stirred for 10 min, and then benzhydryl chloride (11.46 g, 56.58

Table 1. Effect of Novel 1-benzhydryl-sulfonyl-piperazines 3(a-e) on Survival of Mice Bearing Ehrlich Ascites Tumor

Treatment Groups	No of Animals	Schedule Days	ΔMST (Days)	ILS (%)	Av. Wt• Changes
Control (0.1% DMSO)	10	7, 9,11	16	-----	8.71 ± 0.33
3a-100	10	7, 9,11	21 *	31.25	-3.48 ± 0.4*
3b-100	10	7, 9,11	19*	18.75	-2.1 ± 0.2*
3c-100	10	7, 9,11	26*	62.5	-2.88 ± 0.6*
3d-100	10	7, 9,11	24*	50.0	-2.6 ± 0.6*
3e-100	10	7, 9,11	31*	93.75	-4.56 ± 0.1*

3(a-e) 100 indicate dose in mg/kg body wt.

Δ Median survival time (MST) and ILS % was calculated from the mortality data within the observation period.

•Determined on 12th day of treatment.

*Significant difference from control ($p<0.05$).

mmol) was added. The reaction mixture was heated at 80°C for 8 h, and monitored by TLC. After completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. Finally, water wash was given to the organic layer and dried with anhydrous sodium sulphate. The solvent was evaporated and the crude product obtained was purified by column chromatography over silica gel (60-120 mesh) using chloroform: methanol (9:1) as an eluent.

General Procedure for Synthesis of 1-benzhydryl-sulfonyl-piperazine Derivatives 3(a-e)

A solution of 1-benzhydryl-piperazine **2** (1.0 eq) in dry dichloromethane was taken and cooled to 0-5°C in an ice bath. Triethylamine (3.0 eq) was added to the cold reaction mixture and stirred for 10 min, and then appropriate sulfonyl chlorides (1.0 eq) were added. The reaction mixture was stirred for 5-6 h at room temperature, and monitored by TLC. After completion, the solvent was removed under reduced pressure and the residue was taken in water and extracted with ethyl acetate. The organic layer was washed with 10% ammonium chloride solution and finally water wash was given to the organic layer and dried with anhydrous sodium sulphate. The solvent was evaporated and the crude product obtained was purified by column chromatography over silica gel (60-120 mesh) using hexane:ethyl acetate (8:2) as an eluent.

Synthesis of 1-benzenesulfonyl-4-benzhydryl-piperazine (3a)

This was obtained from 1-benzhydryl-piperazine (**2**) (0.5 g, 1.98 mmol), benzenesulfonyl chloride (0.349 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product was a white crystalline solid (0.622 g).

^{1}H NMR (DMSO-d_6, 400MHz) δ: 7.48 (d, 2H, Ar-H), 7.40 (m, 6H, Ar-H), 7.27 (t, 5H, Ar-H), 7.15 (t, 2H, Ar-H), 4.26 (s, 1H, -CH-), 3.2 (br s, 4H, -CH_2-), 2.50 (br s, 4H, -CH_2-).

MS(ESI+ion): m/z = 393.61.

IR (KBr, cm^{-1}): 3026, 2925, 2960, 1319, 1150.

Anal. calcd. for $C_{23}H_{24}N_2O_2S$(in %): C-70.38, H-6.16, N-7.14, S-8.17. Found C-70.34, H-6.11, N-7.10, S-8.15.

Synthesis of 1-benzhydryl-4-(4-propyl-benzenesulfonyl)-piperazine (3b)

This was obtained from 1-benzhydryl-piperazine (**2**) (0.5 g, 1.98 mmol), 4-propyl-benzenesulfonyl chloride (0.377 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product was a white crystalline solid (0.671 g).

^{1}H NMR (DMSO-d_6, 400MHz) δ: 7.62 (d, 2H, Ar-H), 7.49 (d, 2H, Ar-H), 7.34 (d, 4H, Ar-H), 7.26(t, 4H, Ar-H), 7.16 (t, 2H, Ar-H), 4.28 (s, 1H, -CH), 2.9 (br s, 4H, -CH_2-), 2.41 (br s, 4H, -CH_2-), 2.7 (t, 2H, -CH_2-), 1.62-1.68 (m, 2H, -CH_2-), 0.93 (t, 3H, -CH_3).

MS(ESI+ion): m/z = 435.56

IR (KBr, cm^{-1}): 3045, 2962, 2819, 1346, 1281.

Anal. calcd. for $C_{26}H_{30}N_2O_2S$ (in %): C-71.86, H-6.96, N-6.45, S-7.38. Found C-71.84, H-6.92, N-6.43, S-7.40.

Synthesis of 1-benzhydryl-4-(propane-2-sulfonyl)-piperazine (3c)

This was obtained from 1-benzhydryl-piperazine (**2**) (0.5 g, 1.98 mmol), propane-2-sulfonyl chloride (0.282 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product was a light brown crystalline solid (0.589 g).

^{1}H NMR (DMSO-d_6, 400MHz) δ: 7.43 (d, 4H, Ar-H), 7.27 (t, 4H, Ar-H), 7.17 (t, 2H, Ar-H), 4.33 (s, 1H, -CH-), 3.23 (br s, 4H, -CH_2-) 3.1 (m, 1H, -CH-), 2.35 (br s, 4H, -CH_2-), 1.1-1.2 (m, 6H, -$(CH_3)_2$-).

MS(ESI+ion): m/z = 359.47.

IR (KBr, cm^{-1}): 3029, 2959, 2850, 1346, 1285.

Anal. calcd. for $C_{20}H_{26}N_2O_2S$ (in %): C-67.01, H-7.31, N-7.81, S-8.94. Found C-67.04, H-7.27, N-7.77, S-8.90.

Synthesis of 1-benzhydryl-4-(butane-1-sulfonyl)-piperazine (3d)

This was obtained from 1-benzhydryl-piperazine (**2**) (0.5 g, 1.98 mmol), butane-1-sulfonyl chloride (0.310 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product was a off-white amorphous solid (0.634 g).

^{1}H NMR (DMSO-d_6, 400MHz) δ: 7.43 (d, 4H, Ar-H), 7.28 (t, 4H, Ar-H), 7.18 (t, 2H, Ar-H), 4.35 (s, 1H, -CH-), 3.28 (br s, 4H, -CH_2-), 3.1 (t, 2H, -CH_2-), 2.35 (br s, 4H, -CH_2-), 1.62-1.68 (m, 2H, -CH_2-), 1.37-1.43 (m, 2H, -CH_2-), 0.89 (t, 3H, -CH_3-).

MS(ESI+ion): m/z = 373.26.

IR (KBr, cm^{-1}): 3033, 2950, 2852, 1356, 1275.

Anal. calcd. for $C_{21}H_{28}N_2O_2S$ (in %): C-67.71, H-7.58, N-7.52, S-8.61. Found C-67.69, H-7.55, N-7.50, S-8.63.

Synthesis of 1-benzhydryl-4-(decane-1-sulfonyl)-piperazine (3e)

This was obtained from 1-benzhydryl-piperazine (**2**) (0.5 g, 1.98 mmol), decanesulfonyl chloride (0.476 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product was a white glassy solid (0.732 g).

^{1}H NMR (DMSO-d_6, 400MHz) δ: 7.43 (d, 4H, Ar-H), 7.27 (t, 4H, Ar-H), 7.18 (t, 2H, Ar-H), 4.32 (s, 1H, -CH-), 3.26 (br s, 4H, -CH_2-), 2.35 (br s, 4H, -CH_2-), 3.08 (t, 2H, -CH_2-), 1.6-1.68 (m, 2H, -CH_2-), 1.35-1.42 (m, 14H, -(CH_2)-), 0.88 (t, 3H, -CH_3).

MS(ESI+ion): m/z = 457.72.

IR (KBr, cm^{-1}): 3029, 2918, 2850, 1346, 1285.

Anal. calcd. for $C_{27}H_{40}N_2O_2S$ (in %): C-71.01, H-8.83, N-6.13, S-7.02. Found C-71.03, H-8.86, N-6.15, S-7.05.

Biological Assay

In Vivo Anti-Cancer and Anti-Angiogenic Effects of Novel 1-benzhydryl-sulfonyl-piperazine Derivatives

Animals and Tumor

Inbred Swiss albino mice of 6-8 weeks old and weighing 25±5g of either sex were used in the experiments. Ehrlich ascites tumor was grown in adult Swiss albino mice intrape-

ritoneally. Experimental animals were prepared by injecting $5x10^6$ viable tumor cells into intraperitoneal cavity. Tumor growth was followed by recording the animal weights. EAT cells started exponential growth phase from the 7^{th} day after tumor cell injection and the animal succumbed to ascites tumor burden 14 to 18 days after injection.

Animal Survival

Seven days after tumor cell injection the animals were divided into groups of ten each and were treated as follows: Control: 0.2 ml of 0.1% DMSO was given on day 7, 9 and 11 of tumor transplantation. Compound treated groups; the compounds **3**(**a-e**) were given to five different groups of tumor bearing mice. Three doses of compounds **3**(**a-e**) (100 mg /kg body wt) were injected intraperitoneally into the mice on day 7, 9 and 11 of tumor transplantation. All the mice were weighed initially on the day of tumor inoculation followed by weekly intervals. Animal survival was recorded up to 40 days. The tumor response was assessed on the basis of median survival time (MST) and percent increase in life span (% ILS) [24]. MST and % ILS were calculated from the mortality data within the observation period. Enhancement of life span by 25% are more over that of the control was considered as effective anti-tumor response [24].

In Vivo tumor Growth Inhibition

After 7 days of tumor cell injection the animals were divided into 5 groups of ten each; the control group received 0.2 ml of 0.1% DMSO on day 7, 9 and 11 after tumor transplantation. The compounds **3**(**a-e**) were given to five different groups of tumor bearing mice as scheduled above. The tumor inhibitory effect of the compounds on EAT cell growth was assessed by counting cell number and ascites volume. On day 12 the control and compounds **3**(**a-e**) treated tumor bearing mice were sacrificed, and an incision was made in the abdominal region. EAT cells along with the ascites fluid were harvested in a beaker containing 2 ml saline and centrifuged at 3000 rpm for 10 min at 4^o C. The volume of ascites fluid was obtained by subtracting the volume of saline added from the volume of the supernatant. The harvested EAT cells were resuspended in 0.9% saline and counted using a haemocytometer.

Anti-Angiogenic Effects of the Compounds

Peritoneal Angiogenesis

The peritoneum of the mice were cut open and the inner lining of the peritoneal cavities were examined for angiogenesis in both control and compound **3**(**a-e**) treated tumor bearing mice and these were photographed.

Angioinhibitory Effects of the Compounds on In Vivo Chorio Allantoic Membrane

Chorio allantoic membrane assay was performed according to the method of Chandhu and Sharada [25]. The fertilized eggs were divided into different treatment groups, which included control, the vehicle treated group and compounds **3**(**a-e**) treated groups with minimum of six eggs in each group. They were maintained separately and observations were made. The fertilized eggs were incubated for 5 days at 37°C in a humidified and sterile atmosphere. Window was made on the egg shell to assess the developmental stage of the embryo and were resealed and incubation was continued. On day 11 the windows were opened and the compounds **3**(**a-e**) **(**0.1mM) or vehicle were loaded on the cover slips separately, air dried and inverted over the CAM and the windows were closed. The window was resealed and the embryo was allowed to develop further. The windows were opened and observed on day 13 and inspected for changes in the microvessel density in the area under the cover slip and examined under a microscope and photographed.

Statistical Analysis

All data were analyzed by one way ANOVA and observed for significance at $P< 0.05$ level.

RESULTS

Growth Inhibition of Ehrlich Ascites Tumor *In Vivo*

Animal Survival

The vehicle treated control animals developed tumor and died in 14-18 days; and the MST was 16 days. Three doses of 100 mg/ kg body weight of 1-benzhydryl-sulfonyl-piperazine derivatives treatment **3**(**a-e**) on 7, 9 and 11 days after tumor transplantation increased the MST and %ILS but could not completely eliminate the tumor and protect against mortality. The compound **3a** showed 21 days of MST with 31.25 %ILS, but the compound **3b** was ineffective and produced 18.75 %ILS, which is less than the effective anti-tumor response (25 %ILS). The compound **3c** increased the %ILS which was double that produced by the compound **3a**. The %ILS reached 50% in **3d** treated group. The highest tumor response of 93.75 % ILS was observed in **3e** treated group. The decrease in animal body weights in compound treated groups as compared to control is an indication of inhibition of tumor cell growth (Table **1**).

Table 2. Physical Data and Purity of Synthesized Compounds 3(a-e)

Compound	Yield (%)	M.P (^{0}C)	Purity
3a	80	157-159	99.1
3b	78	169-171	98.5
3c	83	88-90	96.8
3d	86	103-105	98.8
3e	81	66-68	97.2

Ascites Volume and Cell Number

The inhibitory effect of 1-benzhydryl-sulfonyl-piperazine derivatives **3**(**a-e**) on EAT cells *in vivo* was evaluated in terms of total number of cells survived and volume of ascites in treated and control mice. The mean cell numbers and ascites volume in control animals was found to be 1760.40 ± 1.12 $X10^6$ cells /mouse (Fig. **1**) and 7.09 ± 1.2 ml respectively (Fig. **2**). Among the treated compounds **3**(**a-e**), the best response was obtained with the compound **3e**. This compound decreased the mean ascites volume to 2.0±1.33 ml with corresponding reduction in mean cell number to 830±0.95$X10^6$ cells/mouse. The compound **3d** showed mean ascites volume of 3.5±1.05 ml, which is 50% less than the control. The compounds **3a**, **3b** and **3c** were also equally efficient in delaying tumor growth as they showed significant decrease ($P< 0.05$) in mean value of cell number and ascites volume (Figs. **1** and **2**).

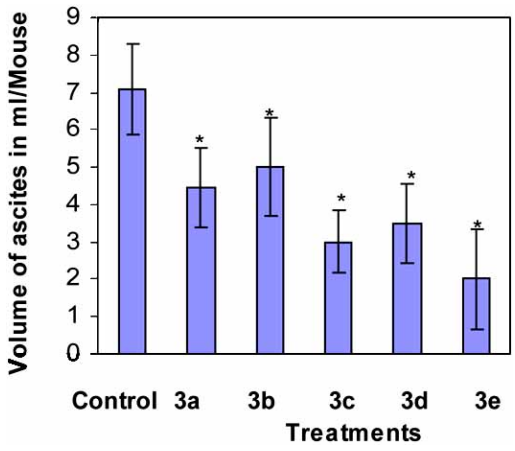

Fig. (1). Effects of 1-benzhydryl-sulfonyl-piperazine derivatives **3(a-e)** on ascites volume of EAT bearing mice. The bar graph represents the effect of the compounds on ascites volume. All the treatments showed a significant decrease in ascites volume from that of control ($p< 0.05$). The error bars represent standard deviation of the mean.

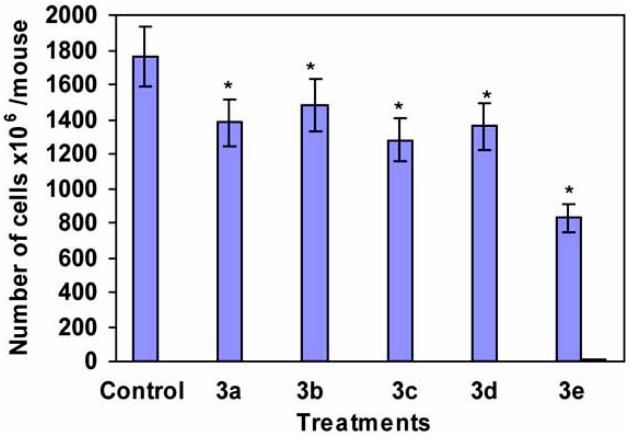

Fig. (2). Effects of 1-benzhydryl-sulfonyl-piperazine derivatives **3(a-e)** on cell number of EAT bearing mice. The bar graph represents the effect of compounds on cell number. All the treatments showed a significant decrease in cell number compared to control ($p< 0.05$). The error bars represent standard deviation of the mean.

Inhibition of Tumor Induced Neovascularization by 1-benzhydryl-sulfonyl-piperazine Derivatives

Examination of peritoneal vasculature of EAT bearing mice is a reliable method of *in vivo* angiogenesis assay. It is evident from (Fig. **3**) that more number of blood vessels were present in the peritoneum of control EAT bearing mice compared to those observed in compounds treated mice.

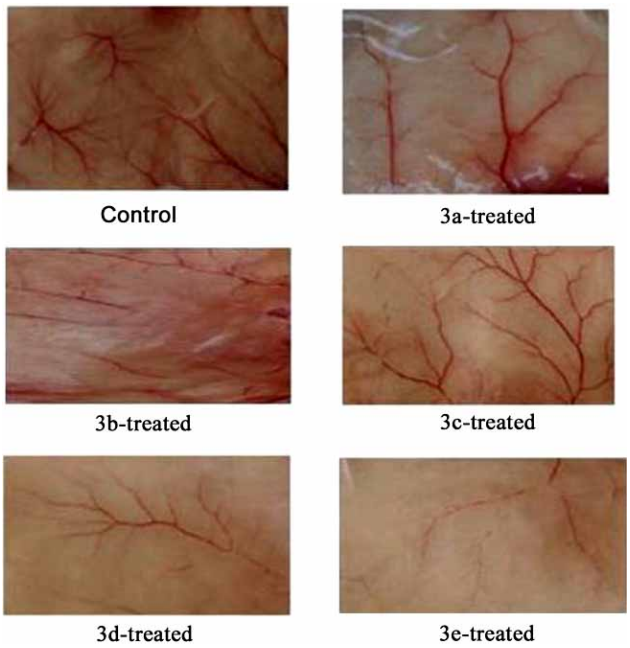

Fig. (3). Suppression of *in vivo* angiogenesis by 1-benzhydryl-sulfonyl-piperazine derivatives **3(a-e)**. Peritoneal lining of tumor bearing mice treated with vehicle (0.1% DMSO) and piperazine derivatives were inspected for anti-angiogenesis effects**.** Inhibition of angiogenesis were prominent in compound treated mice compared to control.

Angioinhibitory Effects of the 1-benzhydryl-sulfonyl-piperazine Derivatives on CAM

The anti-angiogenic activity was evaluated by observing for neovascularization under the cover slip (Fig **4**). The growth of blood vessels were markedly enhanced in control CAM, which was treated with 0.1% DMSO. The compounds **3**(**a-e**) treated CAM exhibited decreased growth of blood vessels and this is evident from the avascular zone, which is indicated as dotted circles in the photographs.

DISCUSSION

These results demonstrate the tumoricidal effects of compounds **3**(**a-e**) on growth of tumor. The compounds **3**(**a-e**) increased the MST and %ILS of tumor bearing mice. The delay in tumor growth by the compounds was supported by the decrease in cell number and ascites volume. These findings suggest that the effect of these compounds is on the actively dividing tumor cells. Our findings agree with the earlier studies on *in vitro* effects of piperizine derivatives of butyric acid on growth inhibition of human erythroleukemia K5-62 cells and myeloid leukemia HL-60 cells [26]. Piperazines were shown to inhibit topoisomarase II activity [27]. David *et al.* reported the interaction of DNA with un-

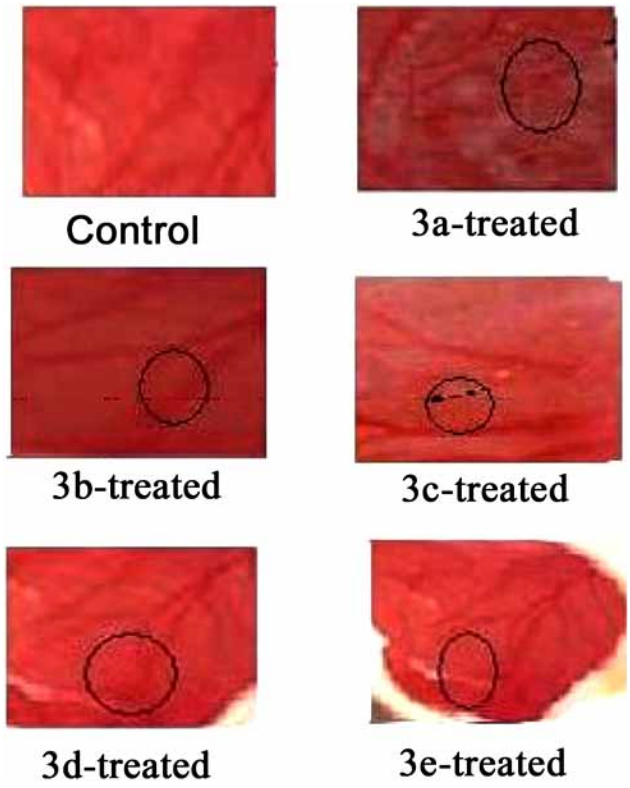

Fig. (4). CAM assay model 1-benzhydryl-sulfonyl-piperazine derivatives **3(a-e)** or the vehicle was applied on the CAM of 11 days old chick embryo. Decreased vasculature was observed in treated groups compared to control. Dotted circles indicate the area covered by the cover slip.

fused aromatic system containing terminal piperizino substituents [16]. Bisdioxopiperazines have been reported for their anti-tumor effects against two experimental lung cancer models *in vivo* [28].

The results of studies on **3**(**a-e**) compounds demonstrated that in addition to being good anti-tumor agents, the compounds can also act as effective angiogenic inhibitors. These compounds prevented the process of angiogenesis both in mouse peritoneum and chick embryo model. Vascularization is a complex phenomenon involving proliferation of endothelial cells and their migration towards the cancer mass that secretes a diverse growth factors [29]. Piperazine derivatives are protease inhibitors [12] and the angioinhibitory effect observed may be due to inhibition of growth factors. Piperazine analogs have shown typical characteristics of anti-metastatic agent, which targets small nodule of tumors. This special targeting on metastases ought to be more useful in clinical cancer treatment [30].

The synthesis of 1-benzhydryl-sulfonyl-piperazines with different sulfonyl chlorides containing aromatic, substituted aromatic and also aliphatic groups from lower alkyl chain to higher alkyl chains produced novel piperazine derivatives **3**(**a-e**). Among these compounds **3**(**a-e**), compound **3a** exhibited higher inhibition of tumor growth compared to **3b**. The structure has activity relationship, compound **3a** has a phenyl ring whereas **3b** substituted with a propyl alkyl chain in the phenyl ring, and hence exhibited lower tumor growth inhibition. Similarly, among alkyl series, the alkyl chain length has shown to modulate the activity of the compounds **3**(**c-e**). The compound **3e** showed good tumor growth inhibition compared to **3c** and **3d**. The anti-tumor and anti-angiogenic effects of the compound **3e** was found to be maximum, which may be related to the presence of a higher alkyl decane chain. The compound **3c** with a lower isopropyl alkyl chain showed better tumor growth inhibition compared to compound **3d**, which has butyl chain. The additional modification and diversification of functional groups in order to improve the anti-cancer activity is currently in progress.

CONCLUSION

Synthesis of 1-benzhydryl-sulfonyl-piperazine derivatives gave good yield with purity. The results of the present findings are encouraging and all the synthesised compounds **3**(**a-e**) when tested have shown good anti-tumor and anti-angiogenic effects against mouse Ehrlich ascites tumor. Further studies on piperazine derivatives will be of great importance since these compounds may prove as potential therapeutic agents for treatment of cancer.

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