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ORIGINAL ARTICLE

2nd Cancer Update

Preliminary investigation of cytotoxic potential of 2-quinolone derivatives using *in vitro* and *in vivo* (solid tumor and liquid tumor) models of cancer

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Abstract 2-Quinolone analogs are powerful inhibitors of farnesyl transferase, and are a novel class of anticancer drugs. The present study focused on continual efforts to elucidate the anticancer activity of synthesized 2-quinolone derivatives without *N*-methyl or 3-aryl substitution. Three derivatives namely JST, JST2 and JST13 were synthesized in our lab and screened for *in vitro* and *in vivo* anticancer activities. Significant cytotoxicity was observed in MCF-7 cells treated with JST2 and JST13. Both the derivatives' treatment showed damage to the DNA. *In vivo* studies for JST2 and JST13 were performed at two doses 100 and 200 mg/kg using Ehrlich ascites carcinoma (liquid) and Dalton lymphoma ascites (solid) models. Both derivatives showed a significant reduction in the tumor progression by increasing the mean life span and by improving the haematological profile and antioxidant status of the liver in a liquid tumor model. More prominent effect was observed in a solid tumor model by reduction in solid tumor weight and tumor volume.

1. Introduction

Cancer has the second highest mortality rate after cardiovascular diseases throughout the world. Even though remarkable progress has been made by medical science, the availability of safe and specific anticancer drugs has remained a major challenge in clinical practice. Development of tumor resistance against available treatment modes like radiotherapy, chemotherapy together with patient noncompliance raises the demands for the introduction of newer drugs in cancer management. Hence, finding a reliable molecule for cancer treatment remained the prime aim of research scientists. Considering all these points, a number of natural and synthetic molecules/compounds have been studied for cancer drug

Abbreviations: DLA, Dalton lymphoma ascites; EAC, Ehrlich ascites carcinoma; JST, 7-Amino-4-methylquinoline-2(1H)-one; JST2, N(1H)-7-(2′-chloro benzoylamino)-4-methyl quinoline-2-(1H)-one; JST13, N(1H)-7-(1′-napthoyl benzoylamino)-4-methyl quinoline-2-(1H)-one); IMLS, Increase in mean life span; MST, Mean survival time.

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discovery and some of them were found promising. Among synthetic molecules, 2-quinolone and its derivatives have emerged as one of the promising lead molecules in cancer treatment. Besides its anticancer activity, 2-quinolone also has been reported to possess antimalarial, antiprotozoal activity (Anquetin et al., 2006), antibacterial (Kulkarni et al., 1991), antiviral (Dodia and Shah, 2001), antihypertensive, 5-HT antagonistic, cardiotonic (Leclerc et al., 1986), diuretic, and anti-inflammatory activities (Kulkarni et al., 1990).

A few studies demonstrated the participation of 2-quinolones in the regulation of signal transduction, cell growth and apoptosis (Chen et al., 2007) as a novel class of anti-cancer agents (Brunner et al., 2003) which act by inhibiting the farnesyl transferase enzyme. These derivatives of 2-quinolone were found to be having either 3-aryl and *N*-methyl substitution (Joseph et al., 2002) or 3,4,6-substitution (Wall et al., 2008). Tipifarnib, a 3-aryl-2-quinolone derivative acts by inhibiting the farnesyl transferase enzyme and is currently under clinical trial. Assuming that 2-quinolone analogs are powerful inhibitors of farnesyl transferase (Li et al., 2005), a series of compounds without *N*-methyl or 3-aryl substitution have been synthesized in our lab and explored for anticancer activity (Kumar et al., 2012). Those compounds were found to induce apoptosis in MCF-7 cells through the Bax induced pathway. In the present study we are continuing to elucidate the anticancer activity of similar compounds synthesized in our lab for their cellular cytotoxicity and DNA level toxicity using human breast cancer cell line (MCF-7) and animal models of cancer (solid and liquid tumor models).

2. Materials and methods

2.1. Materials

Minimum essential media (MEM), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) were obtained from Sigma Chemicals Co., St. Lious, MO, USA and DMSO from SD-fine chemicals, Mumbai, India. Cisplatin was obtained from Dabur Pharma Ltd., New Delhi (Chemoplat®). Other chemicals were obtained from local reliable vendors.

2.2. Test drugs preparation

All *in vitro* cytotoxic studies were performed using DMSO (0.1%) as solvent. In control samples equimolar amount of DMSO was maintained to rule out its cytotoxic effect. For *in vivo* screening the test drugs were suspended in 2% gum acacia immediately before use and given orally. The standard anticancer drug (Cisplatin) was injected intraperitoneally.

2.3. Cell line and culture media

The Ehrlich Ascites Carcinoma (EAC), liquid tumor model cells and Dalton Lymphoma Ascites (DLA), solid tumor model cells were obtained from Dr. Ramdasan Kuttan (Director, the Amala Cancer Research Center, Amala Nagar, Thrissur, Kerala, India) and were maintained and propagated by serial intraperitoneal transplantation of the cells into an aseptic environment. The cells propagated for 12–14 days were used in the experiments. For *in vitro* cytotoxicity studies the human breast adenocarcinoma cell line (MCF7) was used, procured from National Centre for Cell Science, Pune, and maintained in our tissue culture lab. Cells were routinely grown in the 25 cm^2 culture flasks with loosened caps containing minimum essential medium (MEM) supplemented with 10 percent fetal calf serum and 50 μg/ml gentamicin sulfate at 37°C in an atmosphere of 5% CO_2 in humidified air in a CO_2 incubator.

2.4. Animals

Animal care and handling was done as per the guidelines issued by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Govt. of India. Prior permission was taken from the Institutional Animal Ethics Committee for conducting the study (clearance certificate No. IAEC-KMC/07/2007–2008). Eight to ten week old Swiss albino mice weighing 26–30 g were selected from an inbred colony maintained under controlled conditions of temperature (23 ± 2 °C), humidity (50 ± 5%) and light (14 and 10 h of light and dark, respectively) at the Central Animal Research Facility, Manipal University. Four animals were housed in each polypropylene cage containing paddy husk as bedding. The animals were provided with sterile food and water *ad libitum*.

2.5. Test compounds

The test compounds used for the study were JST (7-amino 4-methylquinolin-2(1H)-one), JST2 (N(1H)-7-(2′-chloro benzoylamino)-4-methyl quinoline-2-(1H)-one), and JST13 (N(1H)-7-(1′-napthoyl benzoylamino)-4-methyl quinoline-2-(1H)-one). These were synthesized and characterized in our synthetic laboratory (Jayashree et al., 2010). The structures are given in Fig. A.1.

3. Experimental pharmacology

3.1. ***In vitro*** *cytotoxicity in human MCF-7 breast cancer cell line*

A known number of cells (2×10^3 cells/well in 100 μl of medium) were seeded in 96 well plates and incubated for 24 h for the attachment of cells. Desired concentrations of test compounds were prepared in 0.1% DMSO prior to the experiment. The reactant mixtures were diluted with media and cells were treated with different concentration ranges of the drug (1–200 μM). After 72 h the media were removed and the cell cultures were incubated with 100 μl MTT reagent (1 mg/ml) for 4 h at 37°C, the formazan produced was then solubilized by the addition of 100 μl DMSO. The suspension was placed on a microvibrator for 5 min and then absorbance was recorded at 540 nm by ELISA reader and then percentage cytotoxicity was calculated (Francis and Rita, 1986).

3.2. DNA fragmentation studies

MCF-7, breast cancer cells (1×10^5 cells/ml) were seeded in 25 cm^2 bottles and incubated for 24 h for the attachment of cells. Desired concentrations of test compounds below the CTC_{50} were exposed to the MCF-7 cells and incubated overnight. The cells were lysed and DNA was extracted with digestion buffer (pH 7.5) containing 0.5% SDS, 25 mM Tris–HCl,

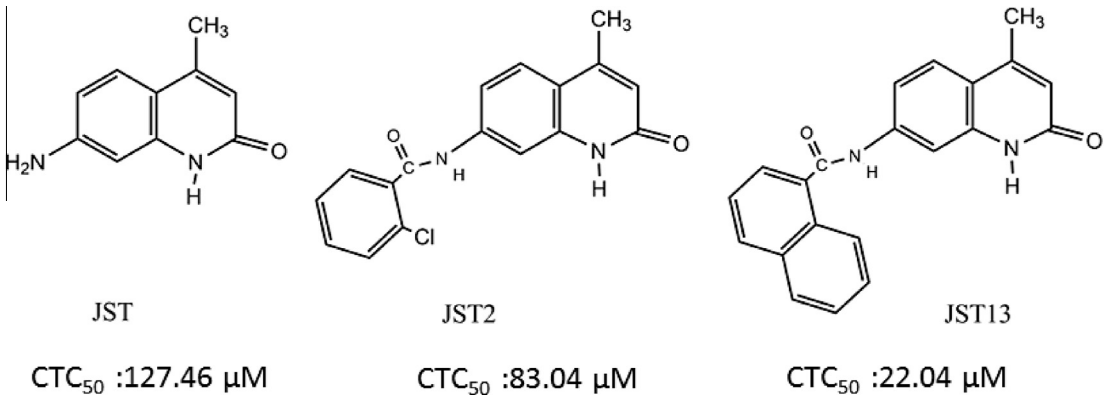

Figure A.1 Structure of compounds.

0.5% mg/ml proteinase K and 5 mM EDTA at 55 °C overnight. After extracting the cell lysates with phenol:choloroform:isoamyl alchol (25:24:1) and chloroform:isoamyl alcohol (24:1), 3 M sodium acetate (pH 5.2) and absolute ethanol was added for the precipitation of DNA, and was washed, dried and resuspended with Tris–EDTA buffer containing RNase A (100 µg/ml) at 37°C for 30 min. Approximately 10 µg of DNA was electrophoresed on a 1% agarose gel and was visualized with ethidium bromide staining under UV light (Raj et al., 2010).

3.3. Toxicological study [OECD-425]

Two compounds viz JST2 and JST13 that showed promising results in the *in vitro* studies were subjected to toxicological studies as per the OECD-425 guideline to obtain a safe dose in mice.

3.4. In vivo cytotoxic activity in EAC inoculated mice (liquid tumor model)

3.4.1. Effect on mean survival time (MST) and percentage increase in mean life span (% IMLS) in EAC inoculated mice

The number of cells required to develop tumor in our laboratory animals were standardized. The desired EAC cells (2.5×10^6 cells/mice) were injected intraperitoneally into each mouse under aseptic conditions (Kviecinski et al., 2008). The day of tumor inoculation was considered as zero day and animals were grouped randomly after 24 h of cell inoculation as mentioned below.

Group 1 (2% acacia) animals in this group were treated with 10 ml/kg 2% gum acacia by oral route.
Groups 2 and 3 (JST13) these groups of animals received JST13 in the dose of 100 and 200 mg/kg, respectively by oral route.

Groups 4 and 5(JST2) animals received JST2 100 and 200 mg/kg by the oral route.
Group 6 (cisplatin) animals were injected cisplatin (3.5 mg/kg) by the i.p. route.

The doses of the test compound chosen were less than 1/10th of the oral safe dose established in toxicity studies. The drug treatment was started on day 1 and the test compounds were administered p.o. on 1st, 3rd, 5th, 7th, 9th, 11th, and 13th days of tumor inoculation. The standard drug cisplatin 3.5 mg/kg, i.p. at a single dose was injected on day one. The animals were monitored for 45 days and MST, % IMLS was calculated. The MST was calculated by dividing the total number of days the animals survived to the total number of animals. The % IMLS time was calculated as follows,

[(MST in the treated group/MST in the control group) − 1] × 100. (Hazra et al., 2002).

3.4.2. In vivo cytotoxic activity on the hemotopoietic system in EAC inoculated mice (n = 4)

The experimental design was the same as described above in Section 3.4.1. A few drops of blood were withdrawn on day 14 from the retro orbital plexus of the animals. The blood samples were analyzed for total WBC and RBC count and hemoglobin estimation (Brandao et al., 2008). Serum total protein was determined by Cobas C-111 auto-analyzer using kits manufactured by Roche Diagnostic Division.

3.4.3. Effect of test compounds on liver antioxidant enzymes in EAC inoculated mice (n = 4)

Adopting the experimental design was the same as described above in Section 3.4.1, animals were sacrificed by cervical dislocation on day 14 and the livers were dissected out after transcardial perfusion with ice-cold saline. Liver was blot dried, weighed and a 10% homogenate was prepared with ice cold potassium chloride (150 mM) using a homogenizer (Yamato LSG LH-21, Japan). The homogenate was used for the estimation of catalase (Miao, 1994), glutathione *S*-transferase (Jakoby, 1985) and lipid peroxidation (Konings and Driver, 1979). Amount of antioxidant enzyme was expressed as units/mg of tissue.

3.5. In vivo cytotoxic activity in DLA inoculated mice (solid tumor model)

3.5.1. Maintenance of cell lines

Dalton's lymphoma ascites cell lines (DLA) were maintained and propagated intraperitoneally by serial transplantation in adult male Swiss albino mice. Fifteen days after tumor transplantation, the DLA bearing mouse (donor) was taped for the ascitic fluid collection. The ascitic fluid was drawn with an 18-gauge needle into a sterile syringe. The drawn fluid was checked for cell viability by trypan blue dye exclusion test. Cells were counted using Neubauer chamber. The ascitic fluid

was suitably diluted in saline. Around 1 million cells (0.1 ml of 10×10^6 cells suspension) were injected subcutaneously to the right hind limb of male mice to obtain a solid tumor. Treatment was started from 24 h after tumor inoculation. Cisplatin was injected on alternate days for 2 days. Compounds were administered daily for 7 days orally from day 1.

3.5.2. Parameters monitored

- Tumor volume.
- Tumor weight.

3.5.2.1. Tumor volume. The radii of the developing tumors were measured using Vernier calipers at 3 day intervals for 1 month and the tumor volume was calculated using the formula $V = 4/3\pi ab^2$, where a & b represent the major and minor diameters, respectively (Natesan et al., 2007).

3.5.2.2. Tumor weight. At the end of the fourth week, animals were sacrificed under anesthesia using diethyl ether; the tumor was extirpated and weighed (Rajkapoor et al., 2007).

The percentage inhibition was calculated by the formula,

$$\% \text{ Inhibition} = 1 - B/A \times 100$$

where A is the average tumor weight of the control group.

B is the average tumor weight of the treated group.

4. Statistical analysis

Data were represented as mean ± SEM. Statistical analysis of the data was carried out by one way ANOVA (GraphPAD Prism Vesrion 5.02, Instat Software La Jolla, CA, USA) followed by Tukey post hoc test. A value of $p < 0.05$ was considered to be significant.

5. Results

*5.1. **In vitro** cytotoxicity in MCF-7 cells by MTT assay*

To check the cytotoxicity in human cells, the breast adenocarcinoma (MCF-7) cells were treated with test compounds. All three compounds showed significant cytotoxicity in MCF7 cells at 72 h of drug incubation. The CTC_{50} of JST, JST2, JST13, were found to be 127.46 μM, 83.04 μM, 22.04 μM, respectively. (Fig. A.2)

5.2. DNA fragmentation

Concentration below the CTC_{50} was selected to study its effect on DNA damage to the cancer cells MCF-7. A single clear band of intact DNA was observed in untreated cells, whereas a smear of DNA, showing clear damage to the DNA was observed in JST2 and JST13 treated cells at concentrations of 80 and 20 μM respectively (Fig. A.3).

5.3. Change in mean survival time (MST) in EAC inoculated mice

A drastic fall in MST was observed in EAC inoculated control mice when compared with the treated EAC mice. In EAC inoculated control mice first mortality was observed on day 12 and all the animals died by day 18. The MST of EAC control mice (15.16 days) was significantly improved by the cisplatin treatment (34.16 days). The MSTs of JST2 treatment at 100 and 200 mg/kg were found to be 19.87 days and 27.62 days respectively. Similarly JST13 at 100 and 200 mg/kg increased the MST to 20.12 days and 23 days respectively. Among these only the JST2 treatment at 200 mg/kg dose significantly increased the MST compared to the control. The% ILS in JST2 at 100 and 200 mg/kg was found to be in a dose dependent manner −31.04 and 82.14. (Table B.1) (Fig. A.4).

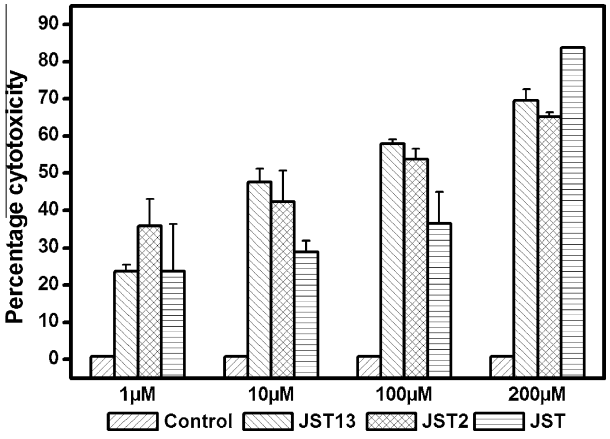

Figure A.2 *In vitro* cytotoxic effect of synthesized compounds in MCF-7 (human breast adenocarcinoma cell) by MTT assay after 72 h of exposure. All the values are expressed as mean ± SEM of three samples.

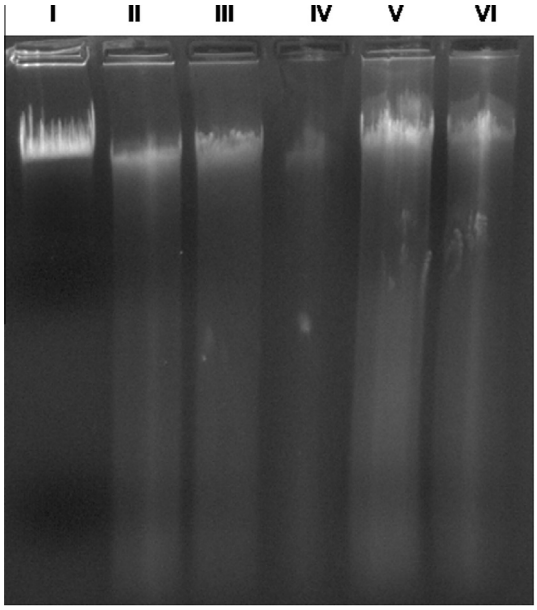

Figure A.3 DNA fragmentation studies by agarose gel electrophoresis (1%). Lane 1: MCF-7 cells without treatment, Lane II: MCF-7 cells + JST13 (10 μM), Lane III: MCF-7 cells + JST13 (20 μM), Lane IV: MCF-7 cells + JST (120 μM), Lane V: MCF-7 cells + JST2 (40 μM), Lane VI: MCF-7 cells + JST2 (80 μM).

Table B.1 Effect of synthesized compounds on mean survival time in EAC inoculated mice.

Treatment	Dose	MST (days)	% IMLS
Control (n = 6)	2% Acacia	15.16 ± 0.87	—
Cisplatin (n = 6)	3.5	34.16 ± 5[a]	125.27 ± 23
JST 13 (n = 8)	100	20.12 ± 0.89	32.69 ± 5.9
JST 13 (n = 8)	200	23 ± 0.8	51.64 ± 5.28
JST 2 (n = 8)	100	19.87 ± 0.23	31.04 ± 1.39
JST 2 (n = 8)	200	27.62 ± 3.94[a]	82.14 ± 25.99

All the values are mean ± SEM of indicated No. of mice.
[a] $p < 0.05$ compared to control.

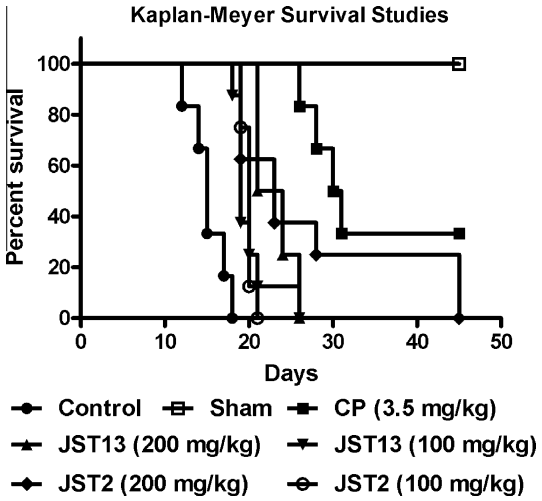

Figure A.4 Survival study of synthesized compounds. Where n = 6 for control and cisplatin; n = 8 for remaining treatment.

5.4. Change in body weight in EAC inoculated mice

EAC inoculated mice gained the body weight progressively. The maximum gain (47%) in tumor weight was observed on day 15 of tumor inoculation. Cisplatin (CP) administration on day 1 significantly ($p < 0.05$) reduced the elevated body weight on every monitored day. In drug treated groups only JST 13 at 200 mg/kg was found effective in reducing tumor weight on all monitored days. However, at lower dose (100 mg/kg) both the drugs marginally reduced the elevated body weight (Fig. A.5).

5.5. Effect of test compounds on haematological parameters and total protein in EAC inoculated mice

More than two fold increase in WBC count was observed in EAC inoculated mice when compared to the sham animal. The CP administration significantly ($p < 0.05$) reversed the EAC elevated WBC count. However, none of the tested compounds significantly ($p < 0.05$) reduced the WBC counts in EAC mice. Significant fall in the RBC count was also observed in EAC inoculated mice. Both cisplatin and test compounds marginally improved the RBC count in EAC inoculated mice. In drug treated mice a marginal improvement in hemoglobin levels was observed (Table B.2).

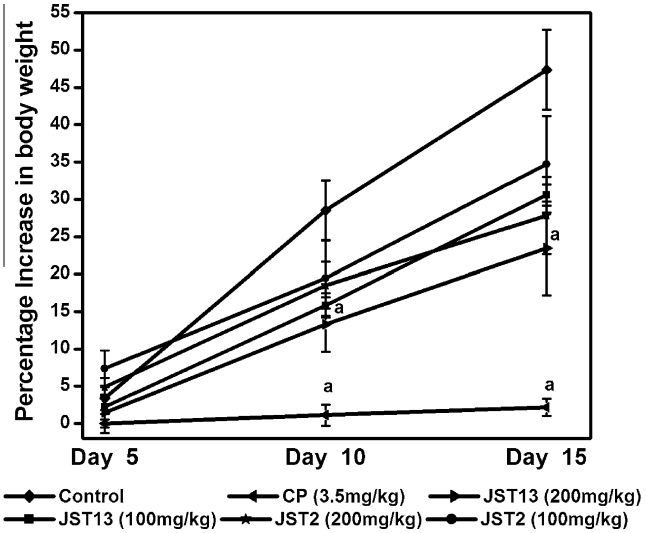

Figure A.5 Effect of synthesized compounds on body weight changes in EAC inoculated mice. All the values are mean ± SEM of indicated No. of mice, [a]$p < 0.05$ compared to control.

Total protein level was found to be increased in EAC control mice. After CP administration total protein level was significantly ($p < 0.05$) reversed. JST2 at 200 mg/kg was found to be most effective in total protein level among the test drugs (Table B.3).

5.6. Effect of test compounds on liver antioxidant enzymes in EAC inoculated mice

The level of lipid peroxidation was significantly increased ($p < 0.05$) in the EAC control group as compared to the normal group (Fig. A.6). After the administration of different doses of JST2 and JST13 (100 mg/kg and 200 mg/kg) in EAC treated mice, the lipid peroxidation decreased as compared to the control ($p < 0.05$) except for JST2 at 100 mg/kg. Tumor inoculation of EAC drastically increased GST level in the control group as compared to sham from 37.47 to 86.06 μmol of CDNB-GSH formed/min/mg of tissue (Fig. A.6). CAT level was reduced in the control but it was increased in the drug treated group in a dose dependent manner. As compared to the control, both the molecules viz JST2 and JST13 at 200 mg/kg increased the CAT level by 54.68% and 70.47%, respectively (Fig. A.6).

5.7. Effect of test compounds against DLA induced solid tumor volume in mice

At the end of the fourth week, significant development of solid tumors was observed in all the mice that were inoculated with DLA cells, which were quantified by increased tumor volume. Treatment with CP 3.5 mg/kg at a single dose on day 1 significantly reduced (95.96%) the tumor volume. JST13 at 200 mg/kg and 100 mg/kg and JST2 at 200 mg/kg reduced the solid tumor volume significantly ($p < 0.05$) at the end of fourth week. JST2 at 200 mg/kg inhibited the tumor growth by 81.31% whereas JST13 at 200 mg/kg reduced the tumor growth by 75.50% (Fig. A.7).

Table B.2 Effect of synthesized compounds on haematological parameters in EAC inoculated mice.

Group (n = 4)	Dose (mg/kg)	WBC (10^3 cells/mm^3)	RBC (millions/mm^3)	Hemoglobin (g%)
Sham	–	6.25 ± 0.46	3.38 ± 0.22	17.35 ± 1.02
Control	2% Acacia	18.11 ± 1.54[a]	2.45 ± 0.40	11 ± 1.68[a]
Cisplatin	3.5	5.57 ± 1.26[b]	3.40 ± 0.19	14.15 ± 0.99
JST13	100	16.86 ± 0.48	2.52 ± 0.48	13.7 ± 0.78
JST13	200	13.34 ± 1.58	2.89 ± 0.10	14.15 ± 0.39
JST2	100	15.4 ± 1.32	2.48 ± 0.13	11.7 ± 0.67
JST2	200	14.32 ± 1.25	2.92 ± 0.32	13.45 ± 0.52

All the values are mean ± SEM of four samples.
[a] $p < 0.05$ compared to sham.
[b] $p < 0.05$ compared to control.

Table B.3 Effect of synthesized compound on total protein level in serum of EAC inoculated mice.

Group (n = 4)	Dose (mg/kg)	Total protein (g/dl)	% Increase
Control	–	6.65 ± 0.30	–
Control	2% Acacia	12.85 ± 0.51[a]	–
Cisplatin	3.5	7.22 ± 0.78[b]	43.77 ± 6.05
JST13	100	6.52 ± 0.29[b]	49.22 ± 2.27
JST13	200	5.65 ± 0.60[b]	56.03 ± 4.7
JST2	100	7.22 ± 0.65[b]	43.13 ± 4.08
JST2	200	4.6 ± 0.61[b]	64.20 ± 4.76

All the values are mean ± SEM of four samples.
[a] $p < 0.05$ compared to sham.
[b] $p < 0.05$ compared to control.

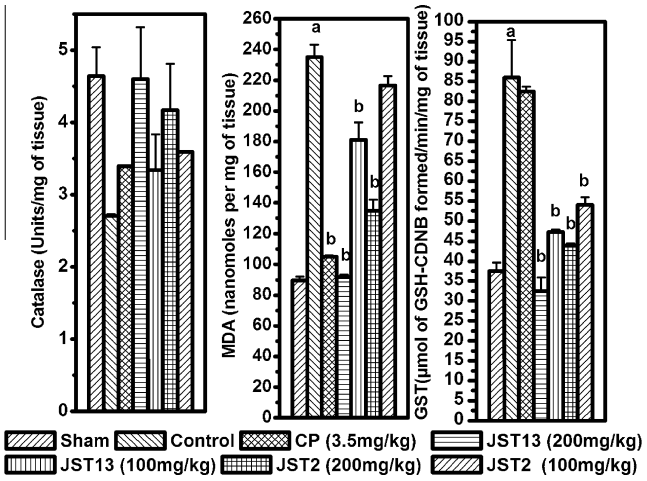

Figure A.6 Effect of test compounds on liver antioxidant enzymes. All the values are mean ± SEM of four samples, [a]$p < 0.05$ compared to sham; [b]$p < 0.05$ compared to control. All the values are mean ± SEM of three mice, [a]$p < 0.05$ compared to control.

5.8. Effect of test compounds against DLA induced solid tumor weight in mice

JST2 and JST13 were found significantly ($p < 0.05$) effective in reducing the tumor weight at end of fourth week. In all the groups of drug treated mice significant ($p < 0.05$) reduction in tumor weight was observed as compared to the vehicle treated group. Both compounds were found more effective at

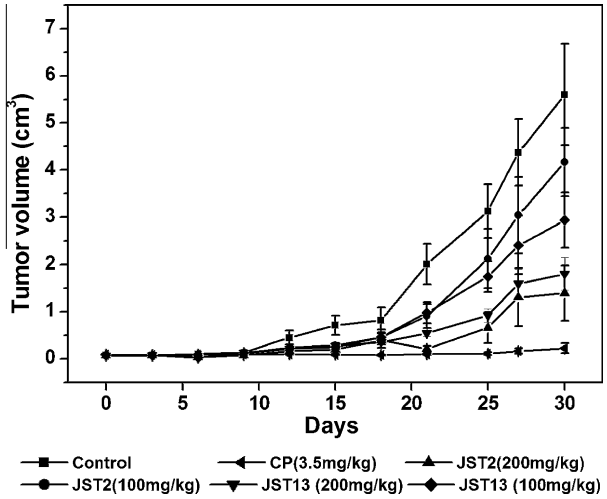

Figure A.7 Effect of synthesized compounds against DLA induced solid tumor volume. All the values are mean ± SEM of six mice.

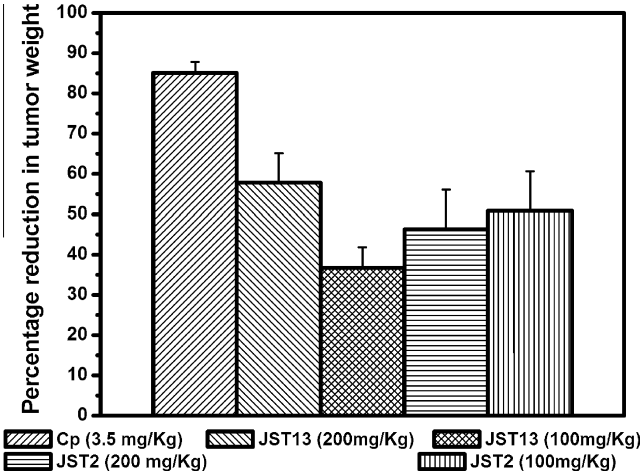

Figure A.8 Effect of synthesized compounds against DLA induced solid tumor weight. All the values are mean ± SEM of six mice.

higher dose (200 mg/kg) as compared to lower dose (100 mg/kg). The standard drug CP was found to be very effective in reducing the tumor weight by 85.11%. However, between the four treatments, the JST2 at 200 mg/kg was found to be the most effective treatment in reducing the tumor weight by 60%. The JST13 at 100 mg/kg was found to be the least effective agent in reducing the tumor weight by 36.69% (Fig. A.8).

6. Discussion

Farnesyl transferase inhibitors have emerged as a novel class of anti-cancer agents (Brunner et al., 2003). Tipifarnib, a 3-aryl-2-quinolone derivative acts as an anticancer agent by similar mechanism (inhibiting farnesyl transferase) and is currently under clinical trials. Assuming that 2-quinolone analogs are powerful inhibitors of farnesyl transferase (Li et al., 2005), and a novel class of anticancer drugs, a series of 2-quinolone analogs were synthesized in our lab. Some of them are already reported to have anticancer activity in our earlier study (Kumar et al., 2012). But the study was lacking the elucidation of *in vitro* and *in vivo* antitumor activities of three compounds (JST, JST2 and JST13). These three compounds were screened for *in vitro* cytotoxicity, and selected compounds were further explored for anticancer activity using tumor bearing mice. Significant cytotoxicity was observed in JST2 and JST13 treated samples and CTC_{50} values for these compounds were found at 83.04 μM and 22.04 μM, respectively. Concentrations below the CTC_{50} for both the compounds were used to check the effect on DNA. JST2 and JST13 showed clear damage to the DNA, which confirms their ability to kill cancer cells. Previous studies support our present finding and reveal that quinolone derivatives are effective against a wide range of cancer cells (Joseph et al., 2002; Kumar et al., 2012). In the present study cell death was assessed at 72 h, which also supports their anti-proliferative activity. Quinolone analog inhibits tubulin polymerization and induces apoptosis via Cdk1-involved signaling pathways (Chen et al., 2007). Further it has been reported that the 2-phenyl-4-quinolone analog causes cell cycle arrest at G2/M phase, and a subsequent apoptosis (Chen et al., 2007). Hence a similar mechanism could be possible here, because all of our test compounds possess quinolone as the basic moiety, though experimental confirmation is still required to correlate this point.

In vivo results obtained indicate the possible therapeutic potential of test compounds. Ehrlich tumor cells are hastily growing cells with the ability to grow in almost all strains of mice (Segura et al., 2000). Implantation of the tumor cells induces a local inflammatory reaction followed by a series of responses like increasing vascular permeability, an intense edema formation, cellular migration, and results in a progressive ascitic fluid formation (Fecchio et al., 1990). The ascitic fluid is vital for tumor growth and it is a nutritional source for tumor cells (Shimizu et al., 2004). Drug treatment on 1st, 3rd, 5th, 7th, 9th, 11th, and 13th day of inoculation marginally reduced the tumor weight in EAC inoculated mice. However, JST13 at 200 mg/kg was found most effective in reducing the tumor weight. Interestingly on day 5 none of the drugs were found effective in reducing the tumor weight/development.

The reliable criterion for judging the antitumor activity of any molecule is the prolongation of life span even for human and for tumor inoculated mice (Gupta et al., 2004). Usually in untreated mice, EAC inoculation causes 100% mortality within 18 days, and our present data support this fact. An enhancement of life span by 25% or more over that of the control was considered as effective antitumor response (Hazra et al., 2002). Treatment with JST2 and JST13 also delayed the onset of mortality and increased the life span of EAC inoculated mice by 82.14% and 51.64%, respectively. Reduced tumor progression and increased life span in tumor bearing mice support the antitumor activity of JST2 and JST13. However, JST13 at 200 mg/kg was found the most effective in reducing the tumor development and progression. Incidence of EAC induced anemia is usually observed in mice on the 14th day of tumor transplantation (Price and Greenfield, 1958). In the present study significant reduction in hemoglobin and RBC was observed in the EAC vehicle treated mice. However mice treated with test compounds showed only marginal improvement in hemoglobin levels. Similarly no significant improvement in the RBC and WBC count was observed in mice treated with test compounds. Total protein level was found to be increased in the EAC vehicle treated mice. The protein levels in serum were elevated in EAC control animals. Treatment with the test compounds significantly reversed the rise. A similar finding was reported on EAC cells using a plant extract (Rajkapoor et al., 2004).

Correlation between free radical generation and cancer development is well documented. Oxidative/electrophilic stress is generally perceived as one of the major causes of carcinogenicity, and antioxidants are considered as effective against cancer development. Excessive free radical generation in oxidative stress causes damages in macromolecules such as lipids, proteins and carbohydrates (Fenninger and Mider, 1954). The lipids undergo peroxidation at the primary site and the products of lipid peroxidation generate more free radicals, which migrate along with circulation to the other sites (Yagi, 1991). Endogenous antioxidant systems such as glutathione and enzymes like catalase and superoxide dismutase, play an important role in preventing and inactivating them (Sinclair et al., 1990). For examples, decreased catalase (Sun et al., 1989) and enhanced malondialdehyde (MDA) levels are documented in carcinogenesis. (Daniela et al., 2006). Many tumor cells are also noted to have pro-oxidant status and promote generalized oxidative stress (Sun, 1990). In the present study a drastic fall in liver catalase levels and elevation in MDA was observed in EAC inoculated mice which supports the role of oxidative stress in cancer progression. Antioxidant molecules such as sesamol (Kaur and Saini, 2000), dehydrozingerone (Ruby et al., 1995), curcumin (Mosley et al., 2007) etc. are known to have protective effects on oxidative stress and cancer (Jiau-Jian and Larry, 1997). Antioxidants protective role in cancer through the differential regulation of transcriptional activator activities and the redox modulation of gene expression is well documented (Storz et al., 1990). We have observed that both the compounds enhance catalase levels in EAC inoculated mice, which support the endogenous antioxidant defense mechanism. Significant reduction in lipid peroxidation was also observed in tumor bearing mice treated with the test compound. Hence the observed antitumor activity may be due to *in vivo* antioxidant properties of JST2 and JST13.

The Dalton's lymphoma transplanted mice were used to assess the effect of the test compound on solid tumors. In DLA-

tumor-bearing mice, a regular rapid increase in tumor volume was observed starting from day 6. Both the studied compounds significantly reduced the tumor size and tumor weight on the 4th week of tumor implantation. Treatment with standard drug (cisplatin 3.5 mg/kg) at two doses on day 1 and 2 significantly reduced the tumor volume and tumor size. Mice treated with JST13 at both the doses (200 mg/kg and 100 mg/kg) showed a significant reduction in development. However, JST2 was found significantly effective only at a higher dose (200 mg/kg), though there was a marginal reduction at a lower dose also.

7. Conclusion

Present study shows that JST2 and JST13 reduce Ehrlich tumor progression in mice. However efficacy was more prominent against Dalton's lymphoma. The exact mechanism responsible for their antitumor activity may be due to its ability to damage the DNA of cancer cells, which have to be studied further in detail. There is possibility of multiple mechanisms playing in the anticancer activities of these compounds. As discussed earlier 2-quinolone analogs inhibit farnesyl transferase, thus retards further growth by inhibiting the Ras mediated pathway and they also cause apoptosis by DNA damage. Hence these mechanisms cannot be ignored. Furthermore in EAC models elevated catalase levels and reduced MDA were observed in drug treated mice. Hence elevated catalase levels and reduced lipid peroxidation may have contributed to the antitumor activity of JST2 and JST13. By this study we can conclude that 2-quinolone derivatives JST2 and JST13 are active against both EAC and DLA models. More prominent activity was observed with the solid tumor model.

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