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In vivo anti-tumour activity of novel Quinazoline derivatives

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Abstract. – OBJECTIVE: The two scaffold Quinazoline analogues (Compound 21, NSC: 95112/753439 and Compound 12, NSC: D-104834/758270) in three different concentrations were evaluated for their anti-tumor activity against Ehrlich ascites carcinoma (EAC) and two different concentration were evaluated for their anti-tumor activity against Dalton's ascites lymphoma (DLA) bearing Swiss albino mice.

MATERIALS AND METHODS: The *in vivo* anti-tumor potency of Quinazoline bases was assessed in EAC model by measuring the increase in mean survival time of the drug treated over untreated control mice and treated standard (Gefitinib) mice. Their toxicity was assessed *in vivo* in normal, standard, and EAC-bearing mice by measuring the drug-induced changes in haematological parameters. The *in vivo* anti-tumor potency of Quinazoline bases was assessed in DLA model by measuring solid tumor volume, solid tumor weight and % inhibition of the tumor weight of the drug treated over untreated control mice and treated standard (Gefitinib) mice.

RESULTS AND CONCLUSIONS: Among the two quinazoline bases studied, 3-(2-chloro benzylideneamine)-2-(furan-2-yl) quinazoline-4(3h)-one (Compound 21) at an optimal dose of 20 mg/kg body weight was found to enhance the mean survival time of infected mice. Haematological parameters and mean survival time in tumor bearing mice were found to be significantly restored towards normal after treatment with Compound 21 at 20 mg/kg body weight of mice in EAC model. Tumor volume and tumor weight in tumor bearing mice were found to be significantly restored towards normal after treatment with Compound 12 at 20 mg/kg body weight of mice in DLA model. Compound 21 at a prime dose of 20 mg has shown promising anticancer activity *in vivo* against EAC and DLA models when compared to standard drug with minimum toxic effects.

Key Words:
Quinazolines, Ehrlich's ascites carcinoma, Dalton's lymphoma ascites, Epidermal growth factor receptor.

Introduction

Cancer is a disease of striking significance in the world today. It is the second leading cause of death in the world after cardiovascular diseases and it is projected to beginning the primary cause of death there within the coming years[1,2]. The identification of novel structures that can be potentially useful in designing new, potent selective and less toxic anticancer agents is still a major challenge to medicinal chemistry researchers[3]. Despite of the important advances achieved over recent decades in the research and development of various cancerostatic drugs, current antitumor chemotherapy still suffers from two major limitations–the first is the lack of selectivity of conventional chemotherapeutic agents for cancer tissues, bringing about unwanted side effects. The second is the acquisition by cancer cells of multiple-drug resistance. Unwanted side effects of antitumor drugs could be overcome with agents capable of discriminating tumor cells from normal proliferative cells and the resistance is minimized using combined modality approach with different complementary mechanism of action[4].

The current scenario highlights the need for the discovery and development of new lead compounds of simple structure, exhibiting optimal *in vivo* antitumor potency and new mechanisms of action. Recent advances in clinical techniques, including large co-operative studies are allowing more rapid and reliable evaluation of new drugs.

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The combination of these advantages with improved preliminary screening systems is enhancing the emergence of newer and more potent compounds. In this regard, it should be emphasized that National Cancer Institute (NCI) *in vitro* primary anticancer drug screen represents a valuable research tool to facilitate the drug discovery of new structural/ mechanistic types of antitumor agents[5].

Rapid scientific advances in recent years have enhanced understanding of the biology of cancer. Consequently, several novel targets have been identified. The various targets of cancer upon which vast research is being done includes Growth factor receptor tyrosine kinases (RTKs) and serine/threonine kinase signal transduction pathway targets; Cell cycle targets; apoptosis-related targets; Extracellular matrix targets, Tumour angiogenesis and metastasis targets; and Cell life-span targets[6].

The extensive research on above targets leads to the discovery of several drugs which were selective and effective in treatment of some cancers, eg. epidermal growth factor receptor (EGFR) targeting small molecule inhibitors, like: Imatinib, Gefitinib, Erlotinib and mono clonal antibodies, like: Cetuximab and Panitumumab. The other molecules which are in clinical developmental stages include small molecule inhibitors like: afatinib, vandetanib and mono clonal antibodies like: zalutumumab, nimotuzumab, and matuzumab[7-10].

Tyrosine kinases have emerged as a new promising target for cancer therapy[11]. Receptor tyrosine kinases (RTKs) play important roles in activating numerous signal transduction pathways within cells, leading to cellular proliferation, differentiation, and various regulatory mechanisms[12,13]. Mutated or over expressed tyrosine kinases frequently associated with tumour growth[14,15]. The epidermal growth factor receptor (EGFR) is a cell surface receptor belonging to the family of receptor protein tyrosine kinases which is activated by the binding of epidermal growth factor (EGF)[16]. EGFR family of tyrosine kinases is the most widely investigated for cancer. EGFR over expression is associated with several cancers like: ovarian, head and neck, oesophageal, cervical, bladder, breast, colorectal, gastric, and endometrial cancer[17]. Inhibitors of the EGFR RTKs are, therefore, expected to have great therapeutic potential in the treatment of malignant and non-malignant epithelial diseases. A great number of different structural classes of tyrosine kinase inhibitors have been reported and reviewed[18,19].

The most promising small molecule selective EGFR-TK inhibitors include quinazolines. Quinazoline containing conventional drugs are enjoying a greater share in the oncology market which includes drugs[20-22]. Recently, quinazolines have emerged as novel molecules for inhibition of a diverse range of receptor tyrosine kinases. The research aimed at further exploration of the SAR of this novel quinazoline core has led to discovery of highly selective compounds that target EGFR. The novel compounds synthesized in the present study were meant to act via competing with ATP for binding at the catalytic domain of tyrosine kinase.

Taking inspiration from above mentioned facts and in continuation of our research for newer anticancer agents 23-27 in the present study, we are reporting *in vivo* study of two promising compounds (Compound 21, NSC: 95112/753439 and Compound 12, NSC: D-104834/758270) using two different anti-tumour models namely, Ehrlich ascites carcinoma (EAC) induced ascites tumour model and Dalton's ascites lymphoma (DLA) induced solid tumour models, which were found to be most potent compounds from five dose *in vitro* screening assay against 60 NCI cancer cell lines panel at National Cancer Institute (NCI), Bethesda, MD, USA as reported in our previously published paper[28,29].

Materials and Methods

Rational and Design

In recent years, quinazolines have emerged as a versatile template for inhibition of a diverse range of receptor tyrosine kinases. The most widely studied of these is the epidermal growth factor receptor (EGFR), with the small-molecule inhibitor gefitinib being the first agent from this class to be approved for the treatment of non-small cell lung cancer refractory to prior chemotherapeutic intervention[30,31]. Subsequent research aimed at further exploration of the structure activity relationship (SAR) of this novel template has led to discovery of highly selective compounds that target EGFR. These compounds act via competing with ATP for binding at the catalytic domain of tyrosine kinase. Later on, a great structural variety of compounds of structurally diverse classes have proved to be highly potent and selective ATP-competitive inhibitors. The ATP binding site has the following features:

Adenine region- Contains two key Hydrogen bonds formed by the interaction of N-1 and N-6 amino group of the adenine ring. Many potent inhibitors use one of these Hydrogen bonds. Sugar region – A hydrophilic region, except a few e.g. EGFR. Hydrophobic pocket – Though not used by ATP, but plays an important role in inhibitor selectivity. Hydrophobic channels – It is not used by ATP and may be exploited for inhibitor specificity. Phosphate binding region – This is used for improving inhibitor selectivity[11].

In this study, we present a new sub-family of compounds containing 2, 3, 7-trisubstituted quinazolinones core as EGFR inhibitors. Our strategy is directed toward designing a variety of ligands with diverse chemical properties hypothesizing that the potency of these molecules might be enhanced by adding alternative binding group such as furan and phenyl ring at position 2-, and imines at position 3-of the quinazoline ring. In this way, such substitution pattern could target different regions of the ATP-binding site of the protein kinase domain to create differentially selective molecules. The design of our ligands was done based on previous Quantitative Structure-Activity Relationship (QSAR) of 4-anilinoquinazolines as EGFR inhibitors[23-25]. We introduced larger moiety at 3 position of the quinazoline such as substituted arylidene moiety in a fashion similar to lapatinib which binds in the ATP-binding cleft, so that the bulky group could be oriented deep in the back of the ATP binding site and makes predominantly hydrophobic interactions with the protein mimicking the 3-chloro-4-[(3-fluorobenzyl)oxy]aniline group of lapatinib (Figure 1).

Chemistry of Compounds

The scaffold Quinazoline analogues, which has come out with promising result in five dose screening assay against 60 NCI cancer cell line panel at National Cancer Institute (NCI), Bethesda, Maryland, USA, Compound 21, (NSC: 95112/753439) and Compound 12 (NSC: 104834/758270) were synthesized and characterized as per protocol mentioned in our previously published paper[28,29]. The structures of the Quinazoline analogues along with their NSC code are given in Chart 1.

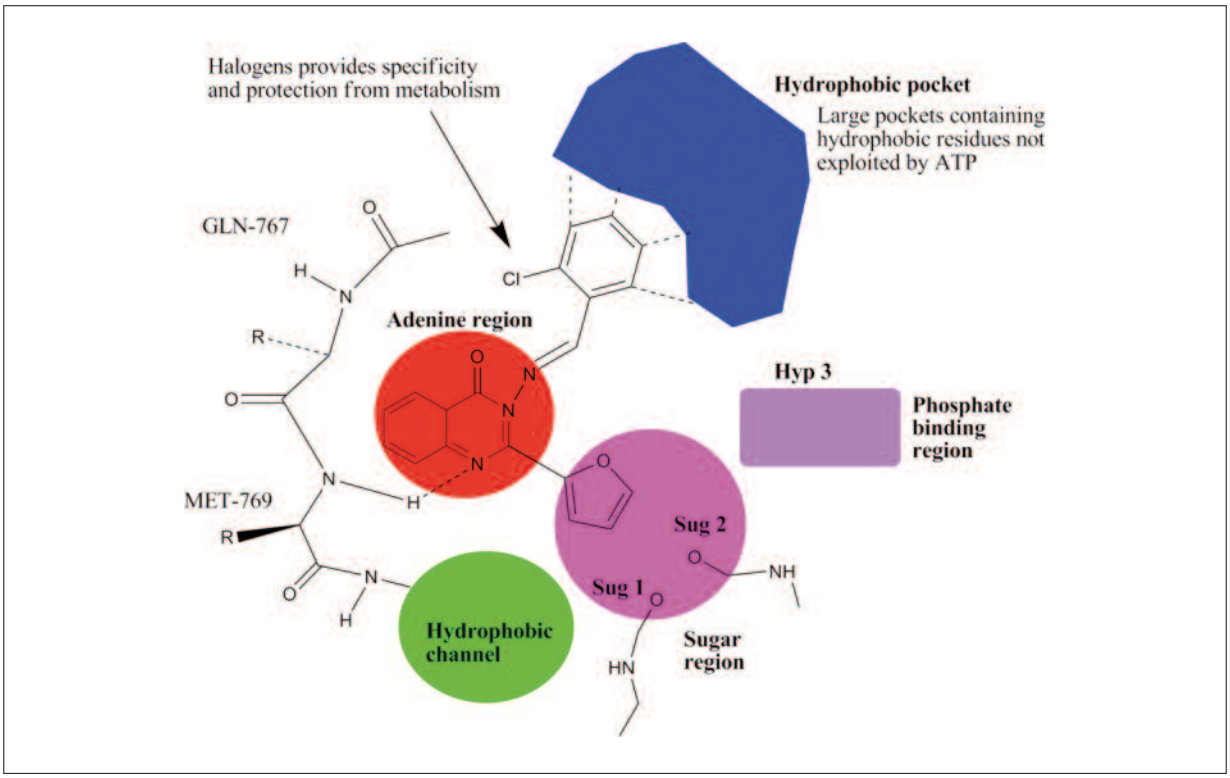

Figure 1. Proposed hypothetical model of the highly active 2, 3-disubstituted quinazoline (Compound 21) bound to ATP binding site of EGFR protein tyrosine kinase.

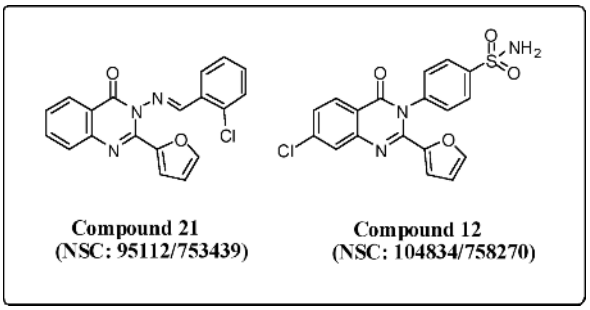

Chart 1. Quinazoline with NCI NSC code.

Drugs and Chemicals

The investigated compounds were suspended in a 0.25% carboxy methylcellulose (CMC) solution just prior to administration and administered intraperitoneally (*ip*). Gefitinib (Natco Pharma, Hyderabad, India) was prepared as suspension in 0.25% CMC. All the other laboratory chemicals used in the present study were from Sigma-Aldrich (St Louis, MO, USA).

Animals

Adult female swiss albino mice of 6-8 weeks old weighing 25-30 g, inbred at Central Animal Facility, JSS Medical College, Karnataka, India was used throughout the study. Animals were housed in polypropylene cages containing sterile paddy husk as bedding material under hygienic conditions with a maximum of four animals in a cage. They were maintained under controlled conditions (10:14 h light:-dark), temperature (23±3°C). Animals were fed on autoclaved standard mice food pellets (Hindustan Lever Ltd., Mumbai, India) and water ad libitum. The animal experiments were performed according to the rules and regulations of the Institutional Animal Ethics Committee (IAEC).

Tumour Cell Lines

Dalton's lymphoma ascites (DLA) and Ehrlich's ascites carcinoma (EAC) cells originally obtained from Amala Cancer Research Centre, Thrissur, Kerala, India, were maintained and propagated as ascites tumor in Swiss albino mice by serial intraperitoneal transplantation[32,33].

Statistical Analysis

The data are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed by one-way ANOVA, followed by Tukey's post hoc test using Graph Pad Prism 5.02. Differences were considered significant $p<0.05$.

Determination of Maximum Tolerable Dose

Maximum tolerable dose of the Compound-21 and Compound-12 were determined by following the OECD (Organization for Economic Cooperation and Development) guidelines – 2001. In brief, Swiss albino mice deprived of food for 18h, were administered various doses of Compound-21 and Compound-12. Animals were observed for any symptoms of toxicity continuously for 4 h, then after 24 h and finally the number of survivors was noted after a period of 72 h. Depending on the results obtained, the therapeutic doses for further studies were selected.

In vivo Anti-tumour Activity Against Ehrlich's Ascites Carcinoma (EAC) Model in Mice

EAC cells were aspirated from EAC tumor bearing mice using a 23 gauge needle into a sterile syringe. Cells viability was determined by trypan blue exclusion test and cells were counted using hemocytometer. The ascitic fluid was suitably diluted in normal saline or sterile phosphate buffered saline (PBS) to get a concentration of 10×10^6 cells/ml of cell suspension. From this stock suspension, 0.25 ml (2.5 million cells) was injected intraperitoneally to each mouse. After 24 hrs of tumour inoculation the tumor-bearing animals were randomly divided into 9 groups of six animals each and treated with test compounds or vehicle as follows:

Group 1: The normal mice.
Group 2: The EAC-bearing mice (Control).
Group 3: The EAC-bearing mice treated with Gefitinib 25 mg/kg, i.p.
Groups 4, 5 and 6: The EAC bearing mice treated with Compound-21 at 5, 10, 20 mg/kg, i.p.
Groups 7, 8 and 9: The EAC bearing mice treated with Compound-12 at 5, 10, 20 mg/kg, i.p.

Standard (**Gefitinib**) and the test compounds were administered intraperitoneally on days1, 3, 5, 7, 10, 12 and 14 (6doses). The control group was treated with same volume of the vehicle [0.25% CMC (Carboxy Methyl Cellulose)]. Every third day animals were weighed to assess the tumor growth. The percentage increase in weight was calculated using the following formula[34];

$$\% \text{ Increase in Weight} = \frac{(\text{Animal weight on respective day} - \text{Animal weight on day 0})}{\text{Animal weight on day 0}} \times 100$$

Mortality of the animals was recorded to calculate Mean survival time (MST). The percentage increase in lifespan (ILS) was calculated by the formula as follows[32];

$$\text{Increase of life Span} = \frac{[\text{MST of treated group-MST of control group}]}{\text{MST of control group}} \times 100$$

$$\text{where MST} = \frac{\Sigma \text{ survival time (days) of each mice in a group}}{\text{Total number of mice}}$$

Haematological Parameters

To study the influence of treatment on haematological parameters, blood was collected from similar set of animals, on 15th day from retro-orbital plexus. The blood was collected into a microcentrifuge tubes containing EDTA and to determine (1) haemoglobin count, (2) erythrocyte count and (3) leukocyte count were determined in peripheral blood of mice[35].

In vivo Anti-tumour Activity Against Dalton's Lymphoma Ascites (dla) Induced Solid Tumour Model in Mice

DLA cells were aspirated from peritoneal cavity of DLA tumor bearing mice using a sterile syringe with 23 gauge needle. Cells viability was determined by trypan blue exclusion test and cells were counted using hemocytometer. The ascitic fluid was suitably diluted in normal saline or sterile PBS to get a concentration of 10×10^6 cells/ml of cell suspension. From this stock suspension, 0.1 ml (1 million cells) was injected subcutaneously into right limb of each mouse. Twenty four hours after tumor inoculation the tumor-bearing animals were randomly divided into 8 groups of six animals each and treated with test compounds or vehicle as follows:

Group 1: The normal mice.
Group 2: The DLA-bearing mice (Control).
Group 3: The DLA-bearing mice treated with Gefitinib 25 mg/kg, i.p
Groups 4 and 5: The DLA bearing mice treated with Compound-21 at 10, 20 mg/kg, i.p.
Groups 7, 8 and 9: The DLA bearing mice treated with Compound-12 at 10, 20 mg/kg, i.p.

The development of tumour in animals was determined by measuring the diameter of tumor growth in two perpendicular planes using Vernier callipers.

The tumor volume was calculated using the formula:

$$V = 4/3\pi a^2 b/2$$

where: a is minor diameter and b is major diameter [36].

At the end of the fifth week, animals were sacrificed under anaesthesia using diethyl ether, tumor extirpated and weighed. The percent inhibition was calculated by the formula:

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

where: A is average tumour weight of the control group and B that of the treated group[37].

Results

Maximum Tolerable Dose

The maximum tolerable dose (MTD) of Compound-21 and Compound-12 in mice was found to be 200 mg/kg body weight. Based on the MTD their 1/30th, 1/20th, 1/10th doses 5 mg/kg, 10 mg/kg, and 20 mg/kg i.p. was chosen for the study in, *in vivo* models.

Effect on Mean Survival Time

Mean survival time (MST) of EAC inoculated control mice was 17.12 ± 0.23 days. Gefitinib at 25 mg/kg significantly enhanced the mean survival time of tumor bearing mice to 21.75 ± 0.67 days when compared to control. Both compounds displayed a dose dependent increase in % life span when compared to control (Table I).

Compound-21 and Compound-12 at 20 mg/kg enhanced the life span to 45.9% and 35% respectively at dose of 20 mg/kg. The efficacy of Compound-21 and Compound-12 (20 mg/kg) in enhancing life span of tumor bearing animal was comparable to that of Gefitinib 25 mg/kg which was 27% as shown in (Table I and Figure 2).

Effect on Body Weight Changes

Substantial increase in body weight was observed in EAC inoculated control mice with a maximum gain (31.98 ± 3.80%) compared to day 0. Gefitinib significantly reduced bodyweight (16.15%) compared to control. Compound-21 and Compound-12 treatment at a dose of 20

Table I. Effect of Compound-21 and Compound-12 on Mean survival time of EAC inoculated mice.

Group	Mean ± SEM	% Increase in life span (% ILS)
Control	17.125 ± 0.23	0
Gefitinib (25 mg/kg)	21.75 ± 0.67*	27
Compound 21 (5 mg/kg)	18.75 ± 0.59	9.4
Compound 21(10 mg/kg)	20.125 ± 0.63*	17.5
Compound 21 (20 mg/kg)	25 ± 0.62*	45.9
Compound 12 (5 mg/kg)	18.62 ± 0.82	8.7
Compound 12 (10 mg/kg)	20 ± 0.73*	16.7
Compound 12 (20 mg/kg)	23.12 ± 0.58*	35

*All the values are mean ± SEM of six mice, where *$p < 0.05$ compared to control compared to standard. All data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison test.

mg/kg caused significant reduction in the body weight (15.7 and 16.9%) when compared to control. The reduction in the body weight at lower doses of Compound-21 and Compound-12 (5 and 10 mg/kg), were not found significant. As shown in (Table II and Figure 3).

Effect on Haematological Parameters

The red blood cells (RBC) counts were restored back by both compounds Compound-21 and Compound-12 in a dose dependent manner. However, it was not efficiently as Gefitinib. Though Compound-12 could also improve RBC count it was not effective as Compound-21. The haemoglobin levels were in the normal range in all the treated groups. The white blood cells (WBC) levels were brought down by both the groups in a dose dependent manner. However, it was not effective as gefitinib. The detailed data obtained from each haematological parameter is given in Figures 4, 5 and 6.

Effect on DLA Induced Solid Tumour

The DLA inoculation significantly increased the tumor volume to 20.93 cm^3 in control mice on 30th day. Gefitinib treatment at 25 mg/kg significantly decreased the tumor volume (9.35 cm^3) when compared to control. Compound-21 and Compound-12 treatment at both doses caused significant reduction in the tumor volume when compared to control. Maximum reduction in the tumor volume (6.9 cm^3) was exhibited by Compound 21 at 20 mg/kg as shown in Table III and Figure 7. At the end of fourth week, the weight of solid tumor in control mice was 8.73 ± 0.16 g.

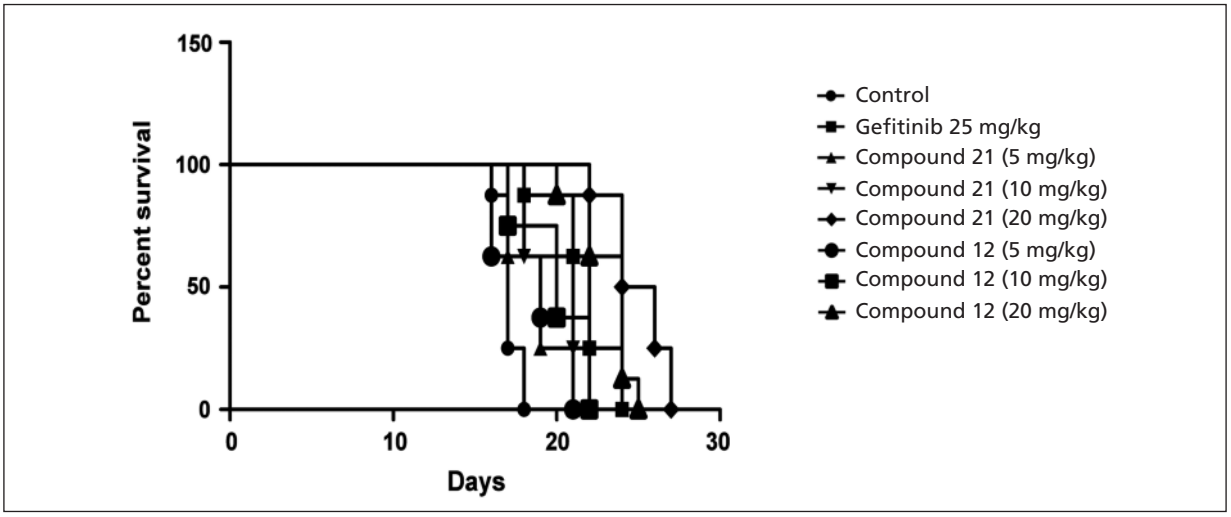

Figure 2. Kaplan Meier estimate of survival of EAC inoculated mice following treatment with Compound 21 and Compound 12 (5 mg/kg, 10 mg/kg, 20 mg/kg) and Gefitinib 25 mg/kg. *All the values are mean ± SEM of six mice, where *$p < 0.05$ compared to control compared to standard. All data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

Table II. Effect Compound 21 and Compound 12 on body weight changes in EAC inoculated mice.

S.No	Group	Percentage increase in body weight on respective days					
		Day 3	Day 5	Day 7	Day 9	Day 12	Day 15
01	Control	10.13 ± 1.40	14.76 ± 1.62	17.54 ± 2.18	21.77 ± 3.18	26.85 ± 3.62	31.98 ± 3.80
02	Gefitinib (25 mg/kg)	1.64 ± 2.32*	3.52 ± 2*	6.49 ± 3.66*	9.89 ± 4.23*	14.77 ± 4.62*	16.13 ± 5.05*
03	Compound 21 (5 mg/kg)	3.78 ± 4.26	6.24 ± 2.97	9.48 ± 5.03	14.94 ± 4.69	16.05 ± 6.03	19.27 ± 6.24
04	Compound 21 (10 mg/kg)	2.95 ± 2.15	4.93 ± 2.32	8.60 ± 3.97	11.21 ± 5.03	14.23 ± 5.63	17.72 ± 6.16
05	Compound 21 (20 mg/kg)	1.79 ± 2.31*	3.83 ± 3.78*	6.67 ± 3.90*	9.66 ± 4.98*	13.82 ± 6.17*	15.76 ± 6.24*
06	Compound 12 (5 mg/kg)	4.64 ± 3.61	7.15 ± 2.45	10.34 ± 4.36	15.67 ± 5.91	18.94 ± 5.39	20.49 ± 6.84
07	Compound 12 (10 mg/kg)	3.17 ± 3.45	5.23 ± 2.72	9.12 ± 3.17	11.98 ± 4.73	14.79 ± 4.93	18.45 ± 6.76
08	Compound 12 (20 mg/kg)	1.97 ± 2.91*	4.08 ± 3.98*	6.97 ± 4.90*	10.14 ± 3.98*	14.32 ± 4.17*	16.69 ± 6.74*

*All values represent mean ± SEM of six animals. Data was analyzed by one way ANOVA followed post hoc Tukey's multiple comparison test. Where $^{a}p < 0.05$ when compared to control.

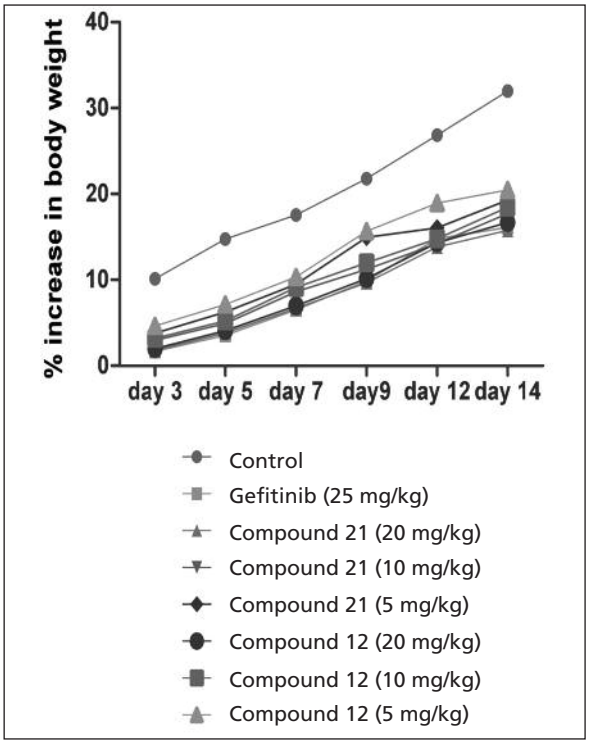

Figure 3. Effect Compound 21 and Compound 12 on body weight changes in EAC inoculated mice. *All values represent mean ± SEM of six animals. Data was analyzed by one way ANOVA followed post hoc Tukey's multiple comparison test. Where $*p < 0.05$ when compared to control.

Gefitinib at a dose of 25 mg/kg significantly reduced the solid tumor weight by 4.08 ± 0.19 when compared with control. Treatment with Compound-21 and Compound-12 at both doses (10 and 20 mg/kg) caused significant reduction in the solid tumor weight when compared with control. Compound-21 and Compound-12 at a dose of 20 mg/kg were found most effective in reducing the tumour weight by 68 and 66.42% respectively when compared to standard as shown in Table IV and Figure 8.

Discussion

The search for selective and less toxic molecules for cancer treatment is an ongoing process. Rapid scientific advances in recent years have enhanced our understanding of the biology of cancer. Consequently, several novel targets have been identified. Tyrosine kinases have emerged as a new promising target for cancer therapy. Generally, anticancer screening involves use of expensive and sophisticated

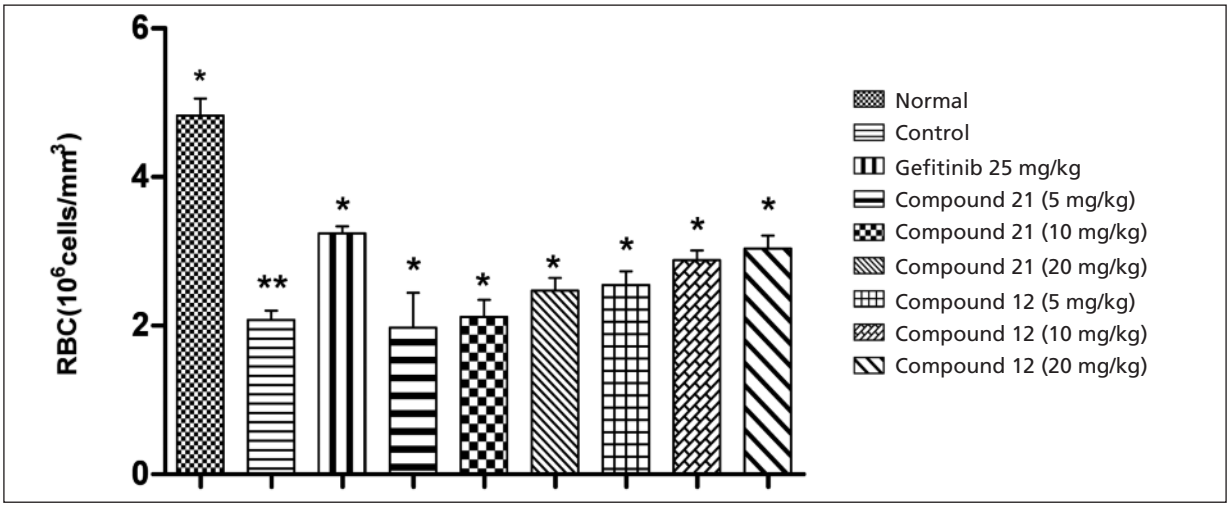

Figure 4. Effect of Compound 21 and Compound 12 on total RBC in EAC inoculated mice. *All the values are mean ± SEM of six mice, *$p < 0.05$ compared to normal, **$p < 0.05$ compared to control. All data was analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

techniques viz. using human cell lines and transplantable or implanted tumor mice models. The appropriate transplantable mouse tumors models still have their place in the drug development programs and are used to investigate the antineoplastic effects of several chemical compounds[38].

The results in the present investigation demonstrate the significant anti-tumor activity of investigational Compound 21 against both ascites and solid tumors. Although, the other investigational Compound 12 also displayed anti-tumor potential against both tumor models, it was less effective when compared with Compound 21. The maximum tolerable dose of both investigational compounds in Swiss albino mice was found to be 200 mg/kg. The three doses 5 mg/kg, 10 mg/kg, 20 mg/kg were chosen based on MTD for *in vivo* studies in ascites tumour bearing model.

In the ascites tumour model, a substantial increase in body weight of the animals was observed in EAC-bearing control mice owing to the rapid and progressive accumulation of ascites tumor cells. Treatment with Compound-21 and Compound-12 at 20 mg/kg significantly caused marked reduction in the body weight of the animals as compared to control indicating the inhibition of tumor cell progression. More-

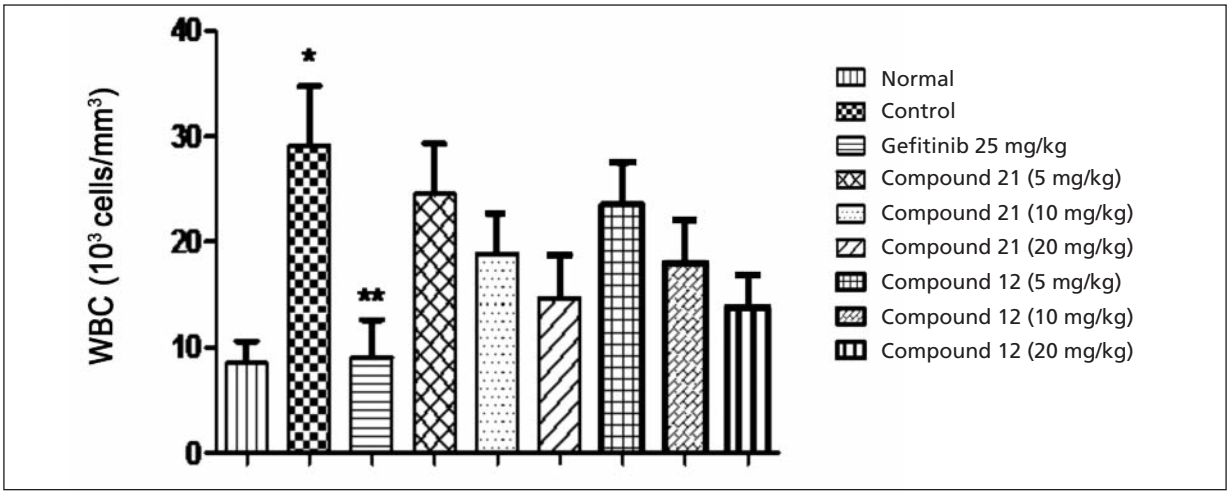

Figure 5. Effect of Compound 21 and Compound 12 on total WBC in EAC inoculated mice. *All values represent mean ± SEM of six animals. Data was analyzed by one way ANOVA followed post hoc Tukey's multiple comparison test. Where *$p < 0.05$ when compared to normal, **$p < 0.05$ when compared to control.

Table III. Effect of Compound 21 and Compound 12 against DLA induced solid tumour volume in mice.

S.No		Percentage increase in solid tumour volume				
	Days	Control	Standard	Compound 21 (10 mg/kg)	Compound 21 (20 mg/kg)	Compound 12 (10 mg/kg)	Compound 12 (20 mg/kg)
01	Day 5	0.12 ± 0.67	0.02 ± 0.39*	0.03 ± 0.53*	0.02 ± 0.46*	0.08 ± 0.73	0.04 ± 0.59*
02	Day 10	0.91 ± 0.79	0.31 ± 0.57*	0.34 ± 0.76*	0.12 ± 0.61*	0.45 ± 0.46	0.16 ± 0.34*
03	Day 15	2.39 ± 0.93	0.96 ± 0.71*	0.81 ± 0.83*	0.38 ± 0.71*	1.19 ± 0.63	0.43 ± 0.67*
04	Day 20	10.81 ± 0.57	4.76 ± 0.83*	5.37 ± 0.94*	2.69 ± 0.25*	6.23 ± 0.94	2.87 ± 0.55*
05	Day 25	16.38 ± 0.31	6.21 ± 0.96*	7.63 ± 0.43*	4.14 ± 0.62*	8.65 ± 0.49	4.45 ± 0.52*
06	Day 30	20.93 ± 0.57	9.35 ± 0.69*	10.42 ± 0.61*	6.90 ± 0.81*	11.34 ± 0.68	7.13 ± 0.19*

*All values represent mean ± SEM of six animals. Data were analyzed by one way ANOVA followed post hoc Tukey's multiple comparison tests. Where $*p < 0.05$ when compared to control.

over Compound-21 and Compound-12 treatment enhanced the MST of tumor bearing mice significantly in a dose dependent manner and maximum enhancement in the survival rate was observed at a dose of 20 mg/kg which was 45.9% and 35% respectively. Since the prolongation of life span is a reliable criterion for judging the anticancer efficacy of any compound[39], an enhancement of life span by 25% or more over that of control is considered as effective anti-tumour response[40].

The enhancement in MST of Compound-21 and Compound-12 (20 mg/kg) treatment in animals was comparable to gefitinib treatment 27%. Progression of tumor was accompanied by following haematological changes when compared to normal mice, gradual decrease in haemoglobin content, erythrocyte count and gradual increase in leukocytes which was observed in control mice. Myelosuppression and anaemia have been frequently observed in ascites carcinoma. In EAC control mice elevated WBC count, and reduced haemoglobin and RBC count was observed. The major problems of cancer chemotherapy with the conventional drugs are myelosuppression and anaemia. The Compound-21 and Compound-12 reversed the EAC induced alteration in haematological parameters such as elevation of haemoglobin content and total RBC count and reduction of elevated total WBC count. These findings substantiate that Compound-21 and Compound-12 treatment is devoid of one of the most common side effects of cancer chemotherapy with anticancer activity. From the ascites tumor model two best doses were selected to determine its efficacy against DLA induced solid tumour model. In DLA induced solid tumour model, Compound-21 and Compound-12 at both doses (10 and 20 mg/kg) was effective in decreasing the solid tumor growth and solid tumor volume when compared to control.

Conclusions

From the above observations it can be concluded that Compound-21 and Compound-12, at a dose of 20 mg/kg, optimally inhibited the growth of EAC and DLA cells *in vivo*. This is evident from reduced tumor weight and enhanced life span in EAC challenged mice, and reduction in tumor weight and volume of the

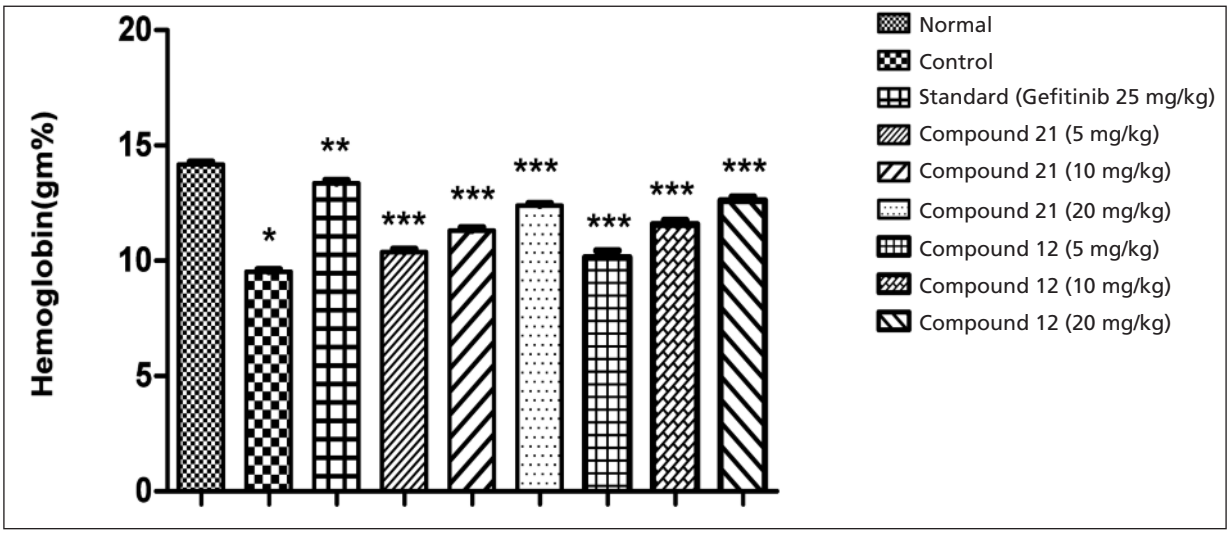

Figure 6. Effect of Compound 21 and Compound 12 on haemoglobin count in EAC inoculated mice. *All values represent mean ± SEM of six animals. The data was analyzed by one way ANOVA followed post hoc Tukey's multiple comparison test. Where *$p < 0.05$ when compared to normal, **$p < 0.05$ when compared to control, ***$p < 0.05$ when compared to standard.

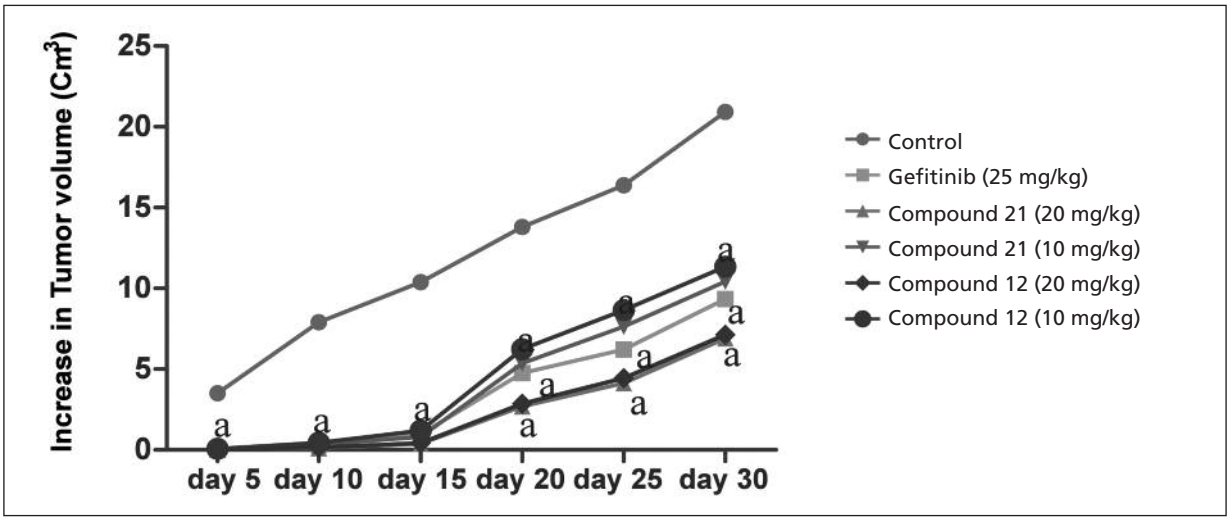

Figure 7. Effect of Compound 21 and Compound 12 against DLA induced solid tumour volume in mice. *All values represent mean ± SEM of six animals. Data were analyzed by one way ANOVA followed post hoc Tukey's multiple comparison tests. Where *$p < 0.05$ when compared to control.

Table IV. Effect of Compound 21 and Compound 12 against DLA induced solid tumour weight in mice.

S. No	Group	Tumour weight on 30th day	% Inhibition
1	Control	8.73 ± 0.16	0
2	Gefitinib (25 mg/kg)	4.08 ± 0.19*	54 ± 4.46*
3	Compound 21 (10 mg/kg)	4.54 ± 1.71*	48 ± 2.14*
4	Compound 21 (20 mg/kg)	3.01 ± 0.21*	68 ± 3.15**
5	Compound 12 (10 mg/kg)	4.95 ± 1.9*	46.56 ± 1.64*
6	Compound 12 (20 mg/kg)	3.51 ± 0.21*	66.42 ± 2.89**

*All values represent mean ± SEM of six animals. Data were analyzed by one way ANOVA followed post hoc Tukey's multiple comparison test. Where *$p < 0.05$ when compared to control, **$p < 0.05$ when compared to standard.

solid tumor in DLA induced solid tumor mice. Moreover, the treatment with Compound-21 and Compound-12 (20 mg/kg) significantly restored the deviated haematological parameters in EAC challenged mice. But results substantiate that Compound 21 was most effective of the both compounds when evaluated *in vivo*. It is an effective antineoplastic agent with less toxic effects. 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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