ISSN 1984-6428
ONLINE
www.orbital.ufms.br

| Vol 2 | | No. 2 | | April-June 2010 |

Full Paper

Synthesis and anticancer activity of some novel 3-(1,3,4-thiadiazol-2-yl)-quinazolin-4-(*3H*)-ones

Alex Joseph[*a], *Aravinda Pai*[a], *K. K. Srinivasan*[a], *Tukaram Kedar*[b], *Angel Treasa Thomas*[b], *Jessy E. M.*[a], *Rajeev K. Singla*[a]

[a]Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Science, Manipal University, Manipal-576104, Karnataka, India
[b]Department of Pharmaceutical Biotechnology, Manipal of College of Pharmaceutical Sciences, Manipal University, Manipal-576104, Karnataka, India

Received: 20 February 2010; revised: 21 May 2010; accepted: 03 June 2010. Available online: 27 December 2010.

ABSTRACT: *Quinazolinone is a versatile lead molecule for designing potential bioactive agents. The compounds that have quinazolin-4-ones moiety are associated with interesting biological activities such as antifungal, antibacterial, antiviral, antitubercular and anticancer. In view of this we have undertaken synthesis of various thiadiazol substituted quinazolin-4-(*3H*)-ones. Novel 3-(1,3,4-thiadiazol-2-yl)-quinazolin-4-(*3H*)-ones (5a-5h) were synthesized by reaction of 5-alkyl/aryl substituted 1,3,4-thiadiazoles with 2-methyl/phenyl-4H-1,3-benzooxazin-4-ones in the presence of pyridine. All the synthesized compounds were characterized by spectral and physical data.* In *vitro anticancer activity of all the synthesized compounds was determined by MTT assay on HeLa (Human cervical cancer cell) cells. Most active compounds found* in vitro *studies were further evaluated for their* in vivo *activity on Liquid tumor (Ehrlich's Ascites Carcinoma; EAC) induced mice. The anticancer activity of compound 5f was found to be comparable to that of cisplatin against HeLa cells and was also effective in preventing the growth of tumor in mice as indicated by decrease in progressive gain in body weight as well as increase in life span when compared to animals of control group.*

Keywords*: quinazolin-4-one; MTT; hela; liquid tumor; Ehrlich's ascites carcinoma*

Introduction

Cancer is a disease of striking significance in the world today. It represents the second leading cause of human mortality after cardiovascular diseases. In order to develop more effective and reliable anticancer agents, a large number of compounds possessing fused nitrogen-containing heterocyclic skeletons, such as 4-anilinoquinazol-

* Corresponding author. E-mail: alex.j.manu@gmail.com

-ines, quinazolinones, pyrazolopyrimidines, triazolopyrimidines, pyrrolopyrimidines, pyrazolopyridazines and imidazopyrazines, have been synthesized and many of them exhibited excellent anticancer activity [1, 2].

Recently, quinazolin-4(*3H*)-ones have aroused increasing attentions since they were proved to be the promising anticancer agents with an interactive mechanism with tubulin [3]. It is also reported that some 2-substituted quinazolin-4(*3H*)-ones analogs bearing halogen groups at 6 and 8 positions were reported for significant anticancer activity [4]. Owing to our interest in the anticancer profile of quinazolin-4(*3H*)-ones, we report herein the synthesis and *in vitro* and *in vivo* anticancer studies of novel 3-(1,3,4-thiadiazol-2-yl)-quinazolin-4-(*3H*)-ones with the hope to develop some promising anticancer agents.

Material and Methods

Chemistry

All melting points were determined in open capillary tubes in Toshniwal Melting point apparatus and are presented without any corrections. Using TLC, we assessed the reactions and the purity of the products using Merck Precoated silica gel GF aluminium plates and Toluene: Methanol (9:1) as solvent system. The infrared (IR) spectra were recorded on a FTIR-8310 Shimadzu spectrometer using potassium bromide pellets. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Bruker AMX 400 NMR spectrometer, using tetramethylsilane (TMS) as the internal standard and DMSO-d as solvent. The chemical shifts are expressed in part per million (ppm) downfield from the internal standard; the coupling constants are in Hz, and signals are quoted as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), or *m* (multiplet). The Mass spectral was recorded as EI spectra on Shimadzu GCMS QP 5050.

Synthesis of 2-methyl/phenyl-4H-1,3-benzooxazin-4-ones (2a-2c)

2-phenyl-4H-1,3-benzoxazin-4-ones (2a): To a stirred solution of anthranillic acid (0.01 M) (1) in pyridine (60 mL), benzoyl chloride (0.01 M) was added dropwise, maintaining the temperature near 8 ^{o}C for 1 h. Reaction mixture was stirred for another 1 h at room temperature and neutralized with 10 % w/v sodium bicarbonate solution . A pale yellow solid as crude product was precipitated out, which was filtered, washed with distilled water, and recrystallised from ethanol to give pure product 2a in a yield of 94% [5].

2-methyl-4H-1,3-benzoxazin-4-ones (2b/2c): A mixture of anthranilic acid or 3, 5 dibromo anthranilic acid (0.01 M) and acetic anhydride (0.02 M) were added to acetic acid (0.01 M). The reaction mixture was refluxed for 4 h. Acetic acid together with acetic anhydride was distilled off under reduced pressure and the solid separated was filtered and recrystallized from ethanol to offer pure product 2b in a yield of 89% [6].

Synthesis of 2-amino-5-aryl substituted-1,3,4-thiadiazoles (4a-4f)

Step 1. Aromatic aldehyde (0.2 M) in warm alcohol (300 mL) was added to a solution of thiosemicarbazide (0.2 M) in hot water (300 mL). The reaction mixture was stirred for a period of 1 h. After cooling down, the crude product was precipitated out, which was filtered and recrystallised from 50% aqueous ethanol.

Step 2. Thiosemicarbazone (0.005 M) obtained above was suspended in 300 mL distilled water in a 100 mL beaker. Ferric chloride (0.15 M) in 300 mL distilled water was added to it. After the reaction mixture was stirring for one hour at temperature of 80-90 °C, it was filtered while hot. A mixture of citric acid (0.11 M) and sodium citrate (0.05 M) was added to the filtrate and continue stirring for 15 minutes. After cooling the whole solution, it was taken in a bigger vessel (to account for the increase in volume) and neutralized with 10% aqueous ammonia. The precipitate which separated out was filtered and recrystallised from 25% aqueous ethanol to give pure product in the yield ranging from 72-84% [7].

Synthesis of 2-amino-5-ethyl-1,3,4-thiadiazoles (4g)

Propionic acid (0.15 M), concentrated sulfuric acid (25 mL) and thiosemicarbazide (0.125 M) were slowly heated to 80-90 °C on a thermostatically controlled water bath for 7 h. After cooling down, the reaction mixture was poured on to crushed ice and neutralized with 10% ammonia solution. The precipitate as crude product was filtered, washed several times with distilled water, and dried. It was recrystallised from hot water to offer pure product 4g in a yield of 76 %.

Synthesis of title compounds: 3-(1,3,4-Thiadiazol-2-yl)-quinazolin-4(3H) ones (5a-5h)

To a solution of 2-methyl/phenylsubstituted-4*H*-1,3-benzoxazin-4-ones (0.005 M) in 15 mL of dry pyridine, added 2-amino-5-alkyl/aryl substituted-1,3,4-thiadiazoles (0.005 M) in portions with constant stirring for 10 minutes. The reaction mixture was refluxed for 9 h. The hot solution was added to a beaker containing 100 g of crushed ice and 5 mL of concentrated HCl. The solid separated was filtered, dried and recrystallised from methanol to give pure product [8].

6,8-dibromo-3-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-methylquinazolin-4(3H)-one (5a): Prepared from 6, 8-dibromo-2-methyl-4*H*-1,3-benzooxazin-4-one (2c) and 5-ethyl-1,3,4-thiadiazol-2-amine (4g)**.** Yield 70%, M.P. 154-156 °C, IR (KBr) (cm^{-1}): 1444 (C-H def.), 1606 (C=N str.), 1750 (C=O str.), 1321 (C-N str.), 686 (C-S str.). ^{1}H-NMR (DMSO) δ ppm: 2.3 (s, 3H, 2nd position C$\underline{H}_3$), 3.2 (q, 2H, thiadiazole -C$\underline{H}_2$-CH$_3$), 1.4 (t, 3H, thiadiazole - CH$_2$-C$\underline{H}_3$), 7.8 (d, 1H, J = 2.12, Ar-H$_5$), 8.1 (d, 1H, J = 2.09, Ar-H$_7$). EIMS m/z: $[M+2]^+$ 430 ($C_{13}H_{10}Br_2N_4OS$).

3-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-phenylquinazolin-4(3H)-one (5b): Prepared from 2-phenyl-4*H*-1,3-benzooxazin-4-one (2a) and 5-ethyl-1,3,4-thiadiazol-2-amine (4g)**.** Yield 72%, M.P. 165-170 °C, IR (KBr) (cm^{-1}): 1410 (C-H def.), 1604(C=N str.), 1690(C=O str.), 1328 (C-N str.), 681 (C-S str.). ^{1}H-NMR (DMSO) δ ppm: 2.3 (s, 3H, 2nd position C$\underline{H}_3$), 3.31 (q, 2H, thiadiazole -C$\underline{H}_2$-CH$_3$), 1.5 (t, 3H, thiadiazole - CH$_2$-C$\underline{H}_3$), 7.6-8.4 (m, 9H, Ar-H). EIMS m/z: $[M]^+$ 334 ($C_{18}H_{14}N_4OS$).

2-methyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one (5c): Prepared from 2-methyl-4*H*-1,3-benzooxazin-4-one (**2b**) and 5-phenyl-1,3,4-thiadiazol-2-amine**.** Yield 69%, M.P. 147-150 °C, IR (KBr) (cm^{-1}): 1420 (C-H def.), 1610 (C=N str.), 1734 (C=O str.), 685(C-S str.). ^{1}H-NMR (DMSO) δ ppm: 2.4 (s, 3H, 2nd position C$\underline{H}_3$), 7.2-8.7 (m 9H, Ar-H). EIMS m/z: $[M]^+$ 320 ($C_{17}H_{12}N_4OS$).

6, 8-dibromo-2-methyl-3-(5-phenyl-1, 3, 4-thiadiazol-2-yl) quinazolin-4(3H)-one (5d): Prepared from 6,8-dibromo-2-methyl-4*H*-1,3- benzooxazin-4-one (2c) and 5-phenyl-1,3,4-thiadiazol-2-amine**.** Yield 74%, M.P. 170-174 °C, IR (KBr) (cm^{-1}): 1607 (C=N str.), 1732 (C=O str.), 675 (C-S str.). ^{1}H-NMR (DMSO) δ ppm: 2.7 (s, 3H, 2nd position C$\underline{H}_3$), 7.6-8.7 (m 7H , Ar-H). EIMS m/z: $[M+2]^+$ 480 ($C_{17}H_{10}Br_2N_4OS$),

6,8-dibromo-3-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)-2-methylquinazolin-4(3H)-one 5e): Prepared from 6,8-dibromo-2-methyl-4*H*-1,3-benzooxazin-4-one (2b) and 5-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine. Yield 70%, M.P. 160-162 °C, IR (KBr) (cm^{-1}): 1604 (C=N str.), 1728 (C=O str.), 683 (C-S str.). ^{1}H-NMR (DMSO) δ ppm: 2.65 (s, 3H, 2nd position C$\underline{H}_3$), 7.5-8.5 (m 6H , Ar-H). EIMS m/z: $[M+2]^+$ 514 ($C_{17}H_9Br_2ClN_4OS$).

3-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)-2-phenylquinazolin-4(3H)-one (5f): Prepared from 2-phenyl-4*H*-1,3-benzooxazin-4-one (2a) and 5-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine. Yield 75%, M.P. 154-158 °C, IR (KBr) (cm^{-1}): 1615 (C=N str.), 1735 (C=O str.), 685 (C-S str.). ^{1}H-NMR (DMSO) δ ppm: 7.2-8.5 (m 13H, Ar-H). EIMS m/z: $[M+2]^+$ 416 ($C_{22}H_{13}ClN_4OS$).

3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-2-methylquinazolin-4(3H)-one (5g): Prepared from 2-methyl-4*H*-1,3-benzooxazin-4-one (2b) and 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine. Yield 69%, M.P. 190-195 °C, IR (KBr) (cm^{-1}): 1610 (C=N str.), 1740 (C=O str.), 692 (C-S str.). ^{1}H-NMR (DMSO) δ ppm: 2.60 (s, 3H, 2nd position C$\underline{H}_3$), 7.4-8.5 (m 8H , Ar-H). EIMS m/z: $[M+1]^+$ 355 ($C_{17}H_{11}ClN_4OS$).

3-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)-2-phenylquinazolin-4(3H)-one (5h): Prepared from 2-phenyl-4*H*-1,3-benzoxazin-4-one (2a) and 5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine. Yield 71%, M. P. 180-184 °C, IR (KBr) (cm^{-1}): 1608 (C=N str.), 1725 (C=O str.), 1100 (C-O str.), 685 (C-S str.). ^{1}H-NMR (DMSO) δ ppm: 3.89 (s, 3H, -OC$\underline{H}_3$), 7.3-8.4 (m, 13H, Ar-H). EIMS m/z: $[M+2]^+$ 412 ($C_{23}H_{16}N_4O_2S$).

Biological Activity

Chemicals and cell culture

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was obtained from Sigma, USA, DMEM (Dulbecco's modified Eagles medium), fetal bovine serum (FBS) and antibiotic solution (containing penicillin and streptomycin) were obtained from Himedia, India, and dimethyl sulfoxide (DMSO) was obtained from Merck, India. Human cancer cell line HeLa (Human Cervical Cancer) cell line was provided by NCCS, Pune, India. Cells were grown in DMEM, containing L-glutamine and 25mM HEPES and supplemented with 10% fetal bovine serum, penicillin (100 units/mL), streptomycin (100 mcg/mL), and amphotericin B (0.25 mg/mL) at 37 ^{0}C, 5% CO_2, 100% humidity.

In-vitro cytotoxicity assay

Cells (1.5 x 10^4) were incubated with the compounds (5a–5h) dissolved in DMSO (final DMSO conc <0.1%) in triplicate wells to obtain drug concentration of 1–100 µg/mL. Cytotoxicity was measured after 72 h using MTT assay as described by Mosmann [9]. Each experiment was repeated thrice and mean IC_{50} values (half inhibitory concentration) have been reported.

In-vivo anticancer activity against EAC Cells by Liquid Tumor Model

The most active two compounds found in *in vitro* studies were selected for *in vivo* anticancer studies. Ehrlich ascites carcinoma (EAC) was used to develop the liquid tumor model. EAC was obtained from Cancer Research Institute, Mumbai and was propagated by serial transplantation in swiss albino mice in Central Animal Research Facility, Manipal University, India.

Acute Toxicity Studies

Acute toxicity studies were conducted to determine the safe dose as per OECD guidelines. Drugs were administered intraperitonially. After administration, the animals were observed continuously for 1 h, frequently for the next 4 h and then after 24 h [10].

The ascitic carcinoma bearing mice (donor) was taken 15 days after tumor transplantation. The ascitic fluid is drawn using a 22 gauge needle into sterile syringe. A small amount tested for microbial contamination. Tumor viability was determined by Trypan blue exclusion test and cells were counted using Haemocytometer. The Ascitic fluid was suitably diluted in phosphate buffer saline to get a concentration of 10^6 cells/mL of tumor cell suspension. This was injected intraperitonially to obtain ascitic tumor. The mice were weighed on the day of tumor inoculation and then for three subsequent days. Treatment was started 24 h after tumor inoculation. Cisplatin was used as positive

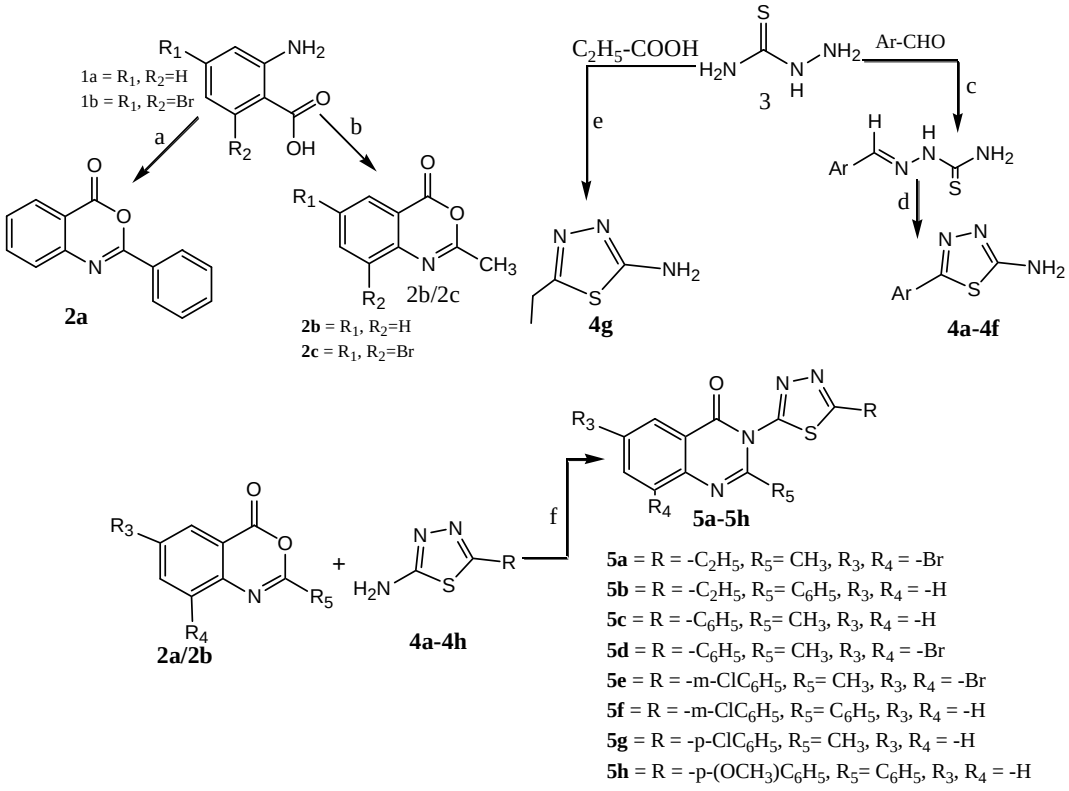
control and injected on two alternate days (3.5 mg/kg/bw) i.e. the 1st and 3rd day. Test compounds were administered at the selected two doses till 9th day intraperitonially. Tumor response was assessed on the basis of mean survival time (MST) and % increase in life span (% ILS) [11]. One way ANOVA followed by post Hoc Tukey test in SPSS 11.5 computer package was used for statistical analysis.

Results and Discussion

Chemistry

Literature review revealed that some 3-(alkyl/aryl substituted 1,3,4-thiadiazol-2-yl)-quinazolin-4(*3H*) ones have been earlier reported for their CNS depressant, anticonvulsant [6], MAO inhibitory , anticholinergics [12], antibacterial and antifungal activity [13]. Although some 2-substituted quinazolin-4(*3H*)-ones analogs has been reported for their anticancer activity, no reports are there on the anticancer activity of 3-(alkyl/aryl substituted 1,3,4-thiadiazol-2-yl)-quinazolin-4(*3H*) ones. Thus it was thought worthwhile to synthesize various 3-(alkyl/aryl substituted 1,3,4-thiadiazol-2-yl)-quinazolin-4(*3H*) ones and to evaluate them for anticancer activity.

Scheme 1



Reagents and conditions : a) C_6H_5-COCl/ Pyridine, Strried at room temperature for 1h b) $(CH_3CO)_2O$/ Re for 4 h c) Ar-CHO/C_2H_5-OH, Strried at room temperature for 1h d) $FeCl_3$, Strried at room temperature for 1h e) Propionic acid/Con H_2SO_4 / Strried at 80-90 ^{0}C for 7 h f) Pyridine reflux for 9 h

The title compounds 3-(alkyl/aryl substituted 1,3,4-thiadiazol-2-yl)-quinazolin-

4(*3H*) ones (5a-5h) were synthesized by the reaction of alkyl/aryl substituted 1,3,4-thiadiazoles (4a-4g) with 2-methyl/phenyl-4*H*-1,3-benzooxazin-4-ones (2a-2c) in the presence of pyridine. 2-methyl/phenyl-4*H*-1,3-benzooxazin-4-ones (2a-2c) were obtained by cyclocondensation of antranilic acid with acetic anhydride/benzoyl chloride. While 2-Amino-5-aryl/alkyl substituted-1,3,4-thiadiazole (4a-4g) were synthesized from aromatic aldehyde or aliphatic acid via thiosemicarbazone intermediate (Scheme 1). The structure of the all the synthesized compounds (5a-5h) were confirmed by ^{1}H-NMR, EIMS, IR spectroscopic and physicochemical data.

Biological Activity

In-vitro cytotoxicity assay

The effect of all the synthesized compounds on the viability of cancer cells was determined by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay on HeLa cells. The results are expressed in terms of IC_{50}, as mean±SEM (Table 1).

Table 1. Cytotoxicity of 3-(1, 3, 4-thiadiazol-2-yl)-quinazolin-4-(*3H*)-ones by MTT method on HeLa cells.

Compound code	***IC_{50}±SEM[a] µg/mL***
5a	84.35±3.26
5b	24.19±1.57
5c	> 100[b]
5d	7.32±2.24
5e	26.39±1.75
5f	9.43±1.08
5g	18.28±2.16
5h	16.74±1.84
Cisplatin	6.58±0.81

[a]Average of three determinations
[b]Concentration above 100 µg/mL not done

Most of the synthesized 3-(alkyl/aryl substituted 1, 3, 4-thiadiazol-2-yl)-quinazolin-4(*3H*) ones showed a cytotoxicity with IC_{50} less than 30 µg/mL. Among the synthesized title compounds 6,8-dibromo-2-methyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl) quinazolin-4(*3H*)-one (5d) and 3-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)-2-phenylquinazolin-4(*3H*)-one (5f) registered maximum cytotoxicity with IC_{50} of 7.32 and 9.43 µg/mL, respectively on HeLa cells. The standard drug cisplatin showed an IC_{50} of 6.58 µg/mL on HeLa cells. The cytotoxicity of 5d and 5f were comparable with that of cisplatin. Based on these results compounds 5d and 5f were selected for *in vivo* anticancer studies.

In-vivo anticancer activity against EAC Cells by liquid tumor model

In vivo anticancer activities of selected compounds were studied on swiss albino mice by liquid tumour model. Acute toxicity studies revealed that maximum tolerated safe dose for all the selected compounds were at 1000 mg/kg. Therefore the compounds were

studied at two dose levels, 50 and 100 mg/kg bw and were administered by i.p. route. Parameters such as percentage increase in body weight, mean survival time and percentage increase in life span were studied and the results are summarized in tables 2 and 3.

In the *in vivo* cancer model of Ehrlich Ascites Carcinoma (EAC), the compounds selected for the study (5d and 5f) significantly reversed the tumor induced changes in the parameters monitored such as percentage increase in body weight and percentage increase in life span.

Weight change: Control animals showed a progressive gain in body weight after the tumor inoculation. By day 13, they gained a maximum average weight of 43.9%. Among the compounds selected for the study 6, 8-dibromo-2-methyl-3-(5-phenyl-1, 3, 4-thiadiazol-2-yl) quinazolin-4(*3H*)-one (5d) and 3-(5-(3-chlorophenyl)-1, 3, 4-thiadiazol-2-yl)-2-phenylquinazolin-4(*3H*)-one (5f) significantly ($P<0.001$) reduced the tumor induced gain in weight as compared to the control at a both dose level studied. The percentage average weights gained by animals treated with these compounds were 13.1 and 8.8%, respectively for 5d and 5f at dose of 100 mg/kg. Cisplatin significantly ($P<0.001$) reduced the tumor induced gain in weight as compared to the respective control. The percentage average weight gained by animals treated with cisplatin at a dose of 3.5 mg/kg was 2.1%.

Mean survival time: MST for control group of animals was 17.0 days. MST of cisplatin treated group (32.0 days) was significantly ($p<0.01$) prolonged compared to that of control. The percentage increase in life span (%ILS) was 88.23. Among the compounds selected for the study, 3-(5-(3-chlorophenyl)-1, 3, 4-thiadiazol-2-yl)-2-phenylquinazolin-4(*3H*)-one (5f) was the most active compound showing an increase in life span of 51.76% at the dose level of 100 mg/kg as compared to the control group.

Table 2. Effect of compounds on the survival time in tumor induced mice.

Group	**Dose *(mg/kg)***	***Mean Survival Time (Days) (Mean ± SEM)***	***% ILS***
Control	-	17±0.707	-
Cisplatin	3.5	32±3.06[b]	88.23
5d	50	18.2±1.59	7.05
5d	100	19.2±1.01[a]	12.9
5f	50	22±0.4[b]	29.41
5f	100	25.8±1.52[b]	51.76

One way ANOVA followed by Post hoc Dunnett's test
a = $p<0.05$, b = $p<0.01$ Vs Control group

Table 3. Effect of selected compounds on percentage increase in body weight in tumor induced mice.

Compound code	***Percentage change in body weight as compared to day- 0 weight*** *(MEAN±S.E.M)*					
	3rd day	**5th day**	**7th day**	**9th day**	**11th day**	**13th day**
Control	11.2±1.02	18.2±2.56	24.5±1.45	28.5±2.27	36.7±5.3[b]	43.9±4.8[b]
Cisplatin	0.001±001[a]	0.53±0.017[b]	0.55±0.0[b]	0.56±0.2[b]	1.66±.01[a]	2.1±.01[a]
5d (50mg/kg)	1.4±1.5[b]	3.9±2.59[b]	10.5±2.0[b]	11.5±2.1[b]	14.4±2.9[a]	17.6±3.05[a]
5d (100mg/kg)	7.48±2.4[b]	2.1±1.55[b]	7.04±1.3[b]	3.41±2.7[b]	8±5[b]	13.1±5.8[b]
5f (50mg/kg)	3.14±2.17	5.28±2.1[a]	5.35±2.2[b]	6.99±2.1[b]	10.1±1.1[b]	8.8±3.8[a]
5f (100mg/kg)	0.88±3.59[a]	11.2±3.14	4.11±3.0[b]	3.9±4.6[b]	1.9±8.47	9.9±11.1

One way ANOVA followed by Post hoc Tukey test
a = $p<0.05$, b = $p<0.001$ Vs Control group

Conclusion

A series of 3-(1, 3, 4-thiadiazol-2-yl)-quinazolin-4-(*3H*)-ones (5a-5h) were synthesized and evaluated for *in vitro* and *in vivo* anticancer studies. The *in vitro* cytotoxicity of compounds 5d and 5f were found to be comparable with that of cisplatin. The *in vivo* anticancer activity of compound 5f was notable on liquid tumor (Ehrlich's Ascites Carcinoma; EAC) induced mice as indicated by decrease in progressive gain in body weight as well as increase in life span when compared to control group animals.

Acknowledgements

The authors express their gratitude to Manipal University for providing the necessary research facilities.

References and Notes

[1] Abdel-Rahman, T. M. *Boll. Chim Farm*. **1998**, *137*, 47.

[2] El-Bayouki, K, A. M.; Aly, M. M.; Mohamed, Y. A.; Basyouni, W. M.; Abbas, S. Y. *World J. Chemistry* **2009**, *4*, 170.

[3] Jack, B.; Jiang, D. P.; Hesson, B. A.; Dusak, D. L.; Dexter, G. J.; Kang, E. H. *J. Med. Chem*. **1990**, *33*, 1728.

[4] Murugan, V.; Caroline, C. T.; Ramasarma, G. V. S.; Kumar, E. P.; Suresh, B. *Indian J. Pharm. Sciences* **2003**, *65*, 386.

[5] Jatav, V.; Kashaw, S.; Mishra, P. *Med. Chem. Res*. **2008**, *17*, 169.

[6] Selvam, P. *Indian J. Pharm. Sciences* **2004**, **66**, 82.

[7] Jatav, V.; Mishra, P.; Kashaw, S.; Stables, J. P. *Eur. J. Med. Chem*. **2008**, *43*, 141.

[8] Satsangi, R. K. *J. Indian Chem. Soc*. **1978**, *55*, 478.

[9] Mosmann, T. *J. Immunol Methods* **1983**, *65*, 55.

[10] OECD Guidelines for acute toxicity studies. http://www.epa.gov/scipoly/sap/meeting/2001/december/12/up and down.pdf Access October, 2008.

[11] Echardt, A. E.; Malone, B. N.; Goldstein, I. *Cancer Res*. **1982**, *42*, 2977.

[12] Jatav, V.; Mishra, P.; Kashaw, S.; Stables, J. P. *Eur. J. Med. Chem*. **2008**, *43*, 1954.

[13] Satsangi, R. K. *Indian Drugs* **1979**, *17*, 81.