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Original Article

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTICANCER ACTIVITY OF SOME NEW SCHIFF BASES OF 1, 3, 4-THIADIAZOLE DERIVATIVES

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ABSTRACT

Objective: N-benzylidene-5-phenyl-1, 3, 4-thiadiazol-2-amine derivatives: Synthesis and anticancer activity.

Methods: In the present study five new derivatives of N-benzylidene-5-phenyl-1, 3, 4-thiadiazol-2-amine (Schiff bases containing 1, 3, 4-thiadiazole) were synthesized according to the literature methods and were characterized by FT-IR, ^{1}H NMR spectroscopy and C, H, N analysis. Anticancer activity was evaluated in Male Swiss albino mice using Ehrlich's Ascites carcinoma cells. Compounds were administered at a dose of 25 mg/kg, body weight intraperitoneally.

Results: The compounds were found to reduce tumor volume, viable cell count and increase the tumor weight (%) inhibition, ascites cells (%) inhibition, non-viable cell count and increase in life span (%ILS). All the compounds exhibited significant ($P < 0.01$) anticancer activity compared to control and the compound 2d & 4d was found to be most potent.

Conclusion: It is concluded that synthesized Schiff bases of 2-amino-5-aryl-1, 3, 4-thiadiazoles derivatives are biologically active and developed into useful anticancer agents.

Keywords: 1, 3, 4-Thiadiazole, Anticancer activity, Tumor cell count, Tumor weight inhibition.

INTRODUCTION

Cancer continues to be the leading cause of mortality and claims over 6 million lives each year all over the world [1]. Extensive research has been carried out since the past few decades to find relief and cure to this silent-killer. Heterocyclic chemistry plays an important role in this field of synthesis of anti neoplastic agents. 2, 5-disubstituted-1, 3, 4-thiadiazole derivatives are important classes of sulfur and nitrogen containing organic compounds. The area of the synthesis of 1, 3, 4-thiadiazole rings continues to grow, and organic chemistry will provide more and better methods for the synthesis of this interesting heterocyclic compound, allowing the discovery of new drug candidates that are more active, more specific and safer.

They present antimicrobial activity [2, 3], antidepressant [4], antituberculosis [5], anti-inflammatory [6], anticonvulsants [7], antihypertensive [8], antioxidant [9], antitumor [10] and anticancer [10-12] activities. 1, 3, 4-Thiadiazole derivatives have also been reported as potent Abl tyrosine kinase inhibitors and cyto-differentiating agents responsible for their antitumor activity [12]. 1, 3, 4- Thiadiazoles exhibit wide spectrum of biological activities, possibly due to presence of toxophoric >N-C-S- moiety [13].

A Schiff base (or azomethine) is a functional group that contains a carbon nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group but not hydrogen[14]. Schiff bases are usually synthesized from the condensation of primary amines and active carbonyl group [15]. Schiff bases are characterized by the −N=CH− (imines) group which is important for elucidating the mechanism of transamination and racemisation reactions in biological systems and are also known to have biological activities such as antimicrobial [16, 17], antifungal [17], antitubercular [18] and antitumor [19, 20] activity. Here we can assume that compounds containing Schiff bases of 1, 3, 4-thiadiazole derivatives may be a potential source for the development of anticancer drugs. Considering the potential of this class of compounds some new 2, 5, di-substituted 1, 3, 4-thiadiazole derivatives were synthesized (1d-5d) as shown in Scheme -1, and was studied for their anticancer activity.

MATERIALS AND METHODS

Chemistry

All the melting points were determined by the open capillary method and are uncorrected. The purity of compounds was checked by TLC on micro plates using Silica-gel-G with detecting agent. IR spectra were recorded on Perkin Elmer IR spectrophotometer (KBr disc) and ^{1}HNMR spectra on Bruker DRX300 NMR spectrometer (DMSO-d_6, $CDCl_3$, and TMS).

The title compounds were prepared by following steps

Step 1: Synthesis of thiosemicarbazones (III)

Aromatic aldehyde **I** (0.05 mol) dissolved in 60 mL warm alcohol and thiosemicarbazide **II** (0.05 mol) dissolved in 100 mL in warm water were mixed slowly with continuous stirring. The product was separated immediately on cooling, then filtered with suction, dried and recrystallized with 75% ethanol to yield **III** [21].

Step 2: Synthesis of 2-amino-5-aryl-1, 3, 4-thiadiazoles (IV)

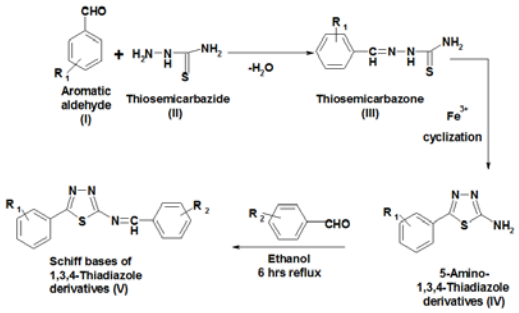
Thiosemicarbazone III (0.015 mol) was suspended in 300 mL warm water. To this ferric chloride (0.045 mol) in 100 mL water was added quantitatively, slowly with constant stirring. The contents were heated at 80–90^{0}C for 45 minute. The solution was filtered hot, and then 100 mL citrate buffer containing citric acid (0.033 mol) and sodium citrate (0.015 mol) were added. The resulting mixture was neutralized with aq. Ammonia (10%) at pH 7. The required amine was separated out, filtered with suction, dried and recrystallized with an appropriate solvent to get IV [21, 22].

Step 3: Synthesis of Schiff bases of 2-amino-5-aryl-1, 3, 4-thiadiazole derivatives (V)

A solution of **IV** (0.002 mol) was prepared in 20 mL alcohol in a round-bottomed flask. Required aldehyde (0.002 mol) dissolved in 15 mL ethanol was then added to it. The mixture was refluxed for 6 hours. The volume of alcohol was reduced to half by distillation

under reduced pressure in rotary vacuum evaporator. The resulting solution was poured on crushed ice. The precipitated compounds **V** was separated by filtration and dried. Finally compounds were recrystallized with appropriate solvent [23, 24].

General methodology



1d: R_1= p-OH, R_2= *p*-Cl, 2d: R_1= o-OH, R_2= *p*-N $(CH_3)_2$, 3d: R_1= p-Cl, R_2= o-OH, 4d: R_1= p-Cl, R_2= *p*-OH, 5d: R_1= o-Cl, R_2= *p*-OH

Compound-1d: N-(4-chlorobenzylidene)-5-(4-hydroxyphenyl)-1, 3, 4-thiadiazol-2-amine

Recrystallized with ethanol. Yield: - 76.82%, M. p.- 203-205°C, Molecular Weight- 315.78, Molecular Formula- $C_{15}H_{10}N_3OSCl$. FT-IR (KBr, V_{max} cm^{-1}): 3396, 3242 (O–H and N–H), 3156 (Ar–CH), 833, 725 (Ar-C-Cl), 1695 (C=N), 660 (C-S), 1599(C=C), 1035, 1166 (N=CH).^{1}H NMR (300 MHz, DMSO-d_6 ppm): δ 9.91 (s, 1H, –N=CH), δ 6.85 (d, 2H, J=9Hz, Ar–H), δ 6.95 (d, 2H, J=9Hz, Ar–H), δ 7.68 (d, 2H, J=3Hz, Ar–H), δ 7.65 (d, 2H, J=3Hz, Ar–H), δ 7.91 (s, 2H, –OH). Anal. Calcd. For$C_{15}H_{10}N_3OSCl$, C, 57.00, H, 3.16, N, 13.30and found: C, 57.83 H, 3.00, N, 13.10,

Compound-2d: N-(4-dimethylaminobenzylidene)-5-(2-chlorophenyl) - 1, 3, 4-thiadiazol-2-amine

Recrystallized with ethanol. Yield- 81.45%, M. p.- 175-176°C, Molecular Weight- 342.85, Molecular Formula- $C_{17}H_{15}N_4SCl$. FT-IR (KBr, V_{max} cm^{-1}): 3106 (Ar–CH), 721 (Ar-C-Cl), 1637 (C=N), 692 (C-S), 1582(C=C), 1064, 1132 (N=CH). ^{1}H NMR (300 MHz, DMSO-d_6 ppm): δ 8.81 (s, 1H, –N=CH), δ 3.06 (s, 6H, $-N(CH_3)_2$), δ 7.69 (d, 2H, J=3Hz, Ar–H), δ 7.59 (d, 2H, J=3Hz, Ar–H), δ 7.88 (s, 1H, Ar–H), δ 7.19 (s, 1H, Ar–H), δ 7.16 (s, 1H, Ar–H). Anal. Calcd. For$C_{17}H_{15}N_4SCl$. C, 59.50, H, 4.37 N, 16.33and found: C, 59.46, H, 4.57, N, 16.40

Compound-3d: N-(2-hydroxybenzylidene)-5-(4-chlorophenyl) - 1, 3, 4-thiadiazol-2-amine

Recrystallized with ethanol. Yield- 79.95%, M. p.- 218-220°C, Molecular Weight- 315.78, Molecular Formula- $C_{15}H_{10}N_3OSCl$. FT-IR (KBr, V_{max} cm^{-1}): 3273 (O–H and N–H), 3092, 2963 (Ar–CH), 828 (Ar-C-Cl), 1631 (C=N), 694 (C-S), 1512(C=C), 1090, 1135 (N=CH). ^{1}H NMR (300 MHz, DMSO-d_6 ppm): δ 9.92 (s, 1H, –N=CH), δ 4.36 (s, 2H, –OH), δ 7.76 (d, 2H, J=9Hz, Ar–H), δ 7.63 (d, 2H, J=9Hz, Ar–H), δ 6.79 (s, 1H, Ar–H), δ 7.23 (s, 1H, Ar–H), δ 7.04 (s, 1H, Ar–H). Anal. Calcd. For$C_{15}H_{10}N_3OSCl$. C, 57.00, H, 3.16, N, 13.30 and found: C, 57.40, H, 3.28, N, 13.42.

Compound-4d: N-(4-hydroxybenzylidene)-5-(4-chlorophenyl) - 1, 3, 4-thiadiazol-2-amine

Recrystallized with ethanol. Yield- 74.14%, m. p.- 210-212°C, Molecular Weight- 315.78, Molecular Formula- $C_{15}H_{10}N_3OSCl$. IR (KBr, V_{max} cm^{-1}): 3274 (OH, NH), 3091 (Ar–CH), 785 (Ar-C-Cl), 1633 (C=N), 696 (C-S), 1513 (C=C), 1090, 1136 (N=CH). ^{1}H NMR (300 MHz, DMSO-d_6 ppm): ^{1}H NMR (300 MHZ, DMSO d_6 p p m): δ 9.76 (s, 1H, –N=CH), δ 8.86 (s, 2H, –OH), δ 8.03 (d, 2H, Ar–H), δ 7.97 (d, 2H, J=9Hz, Ar–H), δ 7.76 (d, 2H, J=9Hz, Ar–H), δ 7.63 (s, 2H, Ar–H). Anal. Calcd. For $C_{15}H_{10}N_3OSCl$. C, 57.00, H, 3.16, N, 13.30 and found: C, 57.09, H, 3.41, N, 13.36.

Compound-5d: N-(4-hydroxybenzylidene)-5-(2-chlorophenyl) - 1, 3, 4-thiadiazol-2-amine

Recrystallized with ethanol. Yield- 71.52%, M. p. - 195-197°C, Molecular Weight- 315.78, Molecular Formula- $C_{15}H_{10}N_3OSCl$. IR (KBr, V_{max} cm^{-1}): 3287 (OH and NH), 755 (Ar-C-Cl), 1637 (C=N), 626 (C-S), 1509 (C=C), 1064, 1132 (N=CH. ^{1}H NMR (300 MHz, DMSO-d_6 ppm): ^{1}H NMR (300 MHZ, DMSO d_6 p p m): δ 8.01 (d, 2H, J = 3 Hz, Ar–H), δ 7.99 (d, 2H, J = 3 Hz, Ar–H), δ 7.60 (s, 1H, Ar–H), δ 7.43-7.43 (m, 1H, Ar-H). Anal. Calcd. For $C_{15}H_{10}N_3OSCl$. C, 57.00, H, 3.166, N, 13.30 and found: C, 57.12, H, 3.01, N, 13.50,

Anticancer evaluation

Experimental animals- Male Swiss albino mice of about 8 weeks old with an average body weight of 18-20 g were used for the experiment. The animals were acclimatized to the laboratory environment and facilitated with 12-hrs light & dark cycles at room temperature and they were fed with standard pellet diet and fresh water *ad libitum* for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Tumor cells- Tumor cells used for anticancer activity were EAC (Ehrlich Ascites Carcinoma) cells originated from human breast carcinoma by spontaneous passaging. It is an undifferentiated tumor, which has lost its epithelial character. On subcutaneous inoculation, it grows in the form of solid nodes and upon intraperitoneal inoculation ascites rich tumor cells will be produced. EAC were maintained *in vivo* in Swiss Albino mice by passaging 0.1 mL containing $2x10^6$ cells every 10 days. EAC cells of 9 days old are used for the screening of synthesized compounds.

Experimental procedure-Male Swiss Albino mice of 8 weeks old with an average body weight of 18-20 grams were used. All mice are kept on basal metabolic diet with water *ad libitum*. Male Swiss albino mice were divided into 8 groups (n = 12). EAC cells were collected from the donor mice and are suspended in sterile isotonic solution (0.9% w/v NaCl). The number of tumor cells per mL of this suspension was counted under the microscope with the help of haemocytometer. All the groups were treated with EAC cells (0.1 mL containing $2x10^6$ cells/mice) intraperitoneally except the normal group (I). This was taken as day '0'. Group-I served as normal saline control (5 ml/kg i. p.) and Group-II served as EAC control.

After 24 hrs of tumor inoculation Group III-VII received the synthesized compounds 1d-5d at a dose of 25mg/kg, body weight/day and Group VIII received the standard drug 5-Fluorouracil at a dose of 20mg/kg, body weight/day respectively for 9 consecutive days. Weights of the animals were recorded at 3 days interval. Twenty-four hours of last dose and 18 h of fasting, 6 animals of each group were sacrificed to measure antitumor and hematological parameters and the rest were kept with food and water *ad libitum* to check percentage increase in life span of the tumor host. The weights of all the animals were recorded before they were sacrificed.

The animals were anaesthetized and dissected to expose the peritoneal cavity and by a syringe, the ascetic fluid was withdrawn to a suitable volume, collected in sterile ice-cold saline and preserved in ice bath. The total number of living cells/mL in the peritoneal fluid of the 6 mice in a group is calculated. The fluid is sucked by adsorbent cotton. The weight of 6 mice after sacrifice was recorded. After sacrificing the animals, blood was collected to evaluate the hematological parameters [23, 25-28].

The anti-tumor activity of the compounds were measured in EAC animals with respect to the following parameters such as

i. Tumor weight- The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The tumor weights were calculated from the difference in weight of mice before dissection and after collection of ascitic fluid after dissection.

ii. Tumor Volume- The ascitic fluid was collected from the peritoneal cavity, and volume was measured by taking it in a graduated centrifuge tube.

iii. Tumor cell count- The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the numbers of cells in the 64 small squares were counted with the help of the microscope under 40X magnification.

$$\text{Percentage inhibition of ascitic cells (\%TCI)} = \left(1 - \frac{T}{C}\right) \text{x } 100$$

Where T is the total number of ascitic cells /ml in test animals, C is the total number of the ascitic cells /mL in control animals.

iv. Viable/nonviable tumor cell count-The viability and nonviability of the cell were checked by trypan blue assay. The cells were stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable, and those that took the dye were nonviable. These viable and nonviable cells were counted.

$$\text{Cell count} = \frac{\text{number of cells } \times \text{ dilution factor}}{\text{area x thickness of liquid film}}$$

v. *Effect on Body Weight*- The effect of the synthesized test compounds and standard drug on body weight of the animals were checked by measuring body weight of the mice at 3 days interval and percent change of body weight for each group was calculated.

vi. Percentage Increase in Life Span (%ILS)-The effects of synthesized test compounds on percentage increases in life span were calculated on the basis of mortality of the experimental mice.

$$\%\text{ILS} = \left\{\frac{\text{mean survival time of treated group}}{\text{mean survival time of control group}} - 1\right\} \text{x } 100$$

$$\because \text{ Mean survival time (MST)} = \frac{\text{first death } + \text{ last death}}{2}$$

Here, time is denoted by days.

vii. Hematological Parameters- Blood collected from the animals were used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) count, and white blood cell (WBC) count by standard procedures.

viii. Statistical analysis- All data are expressed as mean ± SEM. (n = 6 mice per group). Statistical significance (p) calculated by one-way ANOVA between the treated groups and the EAC control followed by Dunnett's Multiple Comparison Test of significance where $p < 0.05$, $p < 0.01$ and $p < 0.001$ considered to be significant, highly significant and most significant respectively.

Count of viable cells, non-viable cells and total cells-The ascitic fluid of dissected mice of each group was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the numbers of total EAC cells, viable and nonviable cells in the 64 small squares were counted with help of microscope.

Haematological parameters-Before dissection of the animals, blood sample were collected through orbital plexus through heparinized tubes with the help of thin capillary. Blood sample was taken up to 0.5 marks of the WBC pipette and RBC pipette and it was diluted up to 11 marks (it becomes 20 times dilution) and 101 marks (it becomes 200 times dilution) by using WBC and RBC diluting fluid respectively.

Then a drop of this diluted sample was placed in the Haemocytometer chamber, and the numbers of cells were counted. Sahli's Hemaoglobinometer is used for determination of hemoglobin content.

DISCUSSION

1, 3, 4-Thiadiazole and its derivatives are important class of organic compounds with diverse activities in various fields such as biological, agricultural and industrial.

The anticancer property of the synthesized compounds was evaluated by their ability to inhibit cancer cell growth in ascitic fluid of swiss albino mice. Various parameters like percentage inhibition of tumor weight (%TWI), percentage inhibition of tumor volume (%TVI), percentage inhibition of total cell count (%TCI), viable and nonviable cell count, effect on body weight, percentage increase in life span (%ILS) and haematological parameters have been taken to be considered to establish the potency of the anticancer activity of the synthesized compounds. All the compounds have significantly reduced the tumor weight and tumor volume when compared to EAC control group. Percentage inhibition of tumor weight (%TWI) and tumor volume (%TVI) have been observed for test compounds were 38.99% to 68.17% and 37.77% to 63.65% respectively. The compounds 2d (68.17% and 63.17%) and 4d (67.89% and 63.65%) showed the maximum activities for the %TWI and %TVI respectively but the values of these compounds are nearly same for both the cases (table 1, fig. 1).

Table 1: Tumor weight and tumor volume inhibition of the tested compounds

Group	Compound	Dose of drug(mg /kg)	Tumor weight (gram)	% TWI	Tumor volume (mL)	%TVI
I	Normal	-	-	-	-	-
II	EAC control	-	3.18 ± 0.21	0.00	1.26 ± 0.08	0.00
III	EAC + **1d**	25	1.13 ± 0.08*	64.49	0.54 ± 0.05*	56.51
IV	EAC + **2d**	25	1.01 ± 0.08*	68.17	0.46 ± 0.04*	63.17
V	EAC + **3d**	25	1.76 ± 0.19*	44.80	0.78 ± 0.04*	38.25
VI	EAC + **4d**	25	1.02 ± 0.09*	67.89	0.45 ± 0.05*	63.65
VII	EAC + **5d**	25	1.94 ± 0.25*	38.99	0.78 ± 0.03*	37.77
VIII	EAC + 5-FU	20	0.25± 0.14*	92.15	0.12 ± 0.011*	90.48

Each value represents the mean ± SEM (n = 6 mice per group), *Experimental groups were compared with EAC control group ($p < 0.001$).

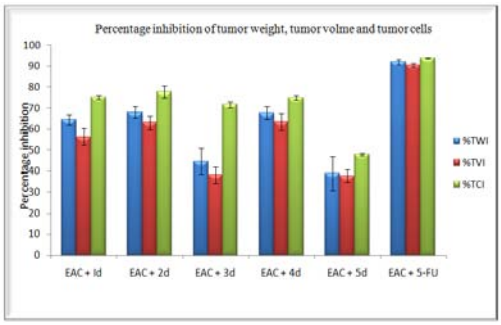


Fig. 1: Percentage inhibition of tumor weight (%TWI), tumor volume (%TVI) and total cells in ascitic fluid (%TCI) compared to the EAC control group

Percentage inhibitions of total cell count (%TCI) have been observed for these compounds are 48.22% to 77.85%, where compound 2d showed the maximum activity (77.85%). Compounds 1d, 3d and 4d (75.30%, 71.78% and 75.17% respectively) also reduce tumor cells significantly (table 2, fig. 1). All of these compounds significantly reduced the number of viable cells and increase the number of nonviable cell comparing to the EAC control group. Compound 1d showed the maximum percentage (80.12%) of nonviable cells causing destruction of the EAC cells (table 2).

Table 2: Count of viable, non-viable, Total EAC cells and % inhibition of total cell count

Group	Compound	Viable cell count ($x10^7$)	Non-viable cell count ($x10^7$)	% of viable cells	% of non-viable Cells	Total cell count ($x10^7$)	%TCI
I	Normal	--	--	--	--	--	--
II	EAC + control	7.37 ± 0.18	0.30 ± 0.02	96.05	3.95	7.67 ± 0.19	0.00
III	EAC + **1d** (25mg/kg)	0.69 ±0.15*	1.20 ± 0.19*	36.52	63.48	1.89 ± 0.07*	75.30
IV	EAC + **2d** (25mg/kg)	1.36 ±0.22*	0.34 ± 0.03**	80.12	19.88	1.70 ± 0.21*	77.84
V	EAC + **3d** (25mg/kg)	1.69 ±0.08*	0.47 ± 0.04	77.97	22.03	2.16 ± 0.11*	71.78
VI	EAC + **4d** (25mg/kg)	1.58 ±0.09*	0.32 ± 0.02**	83.31	16.69	1.90 ± 0.09*	75.17
VII	EAC + **5d** (25mg/kg)	3.71 ±0.04*	0.26 ± 0.01**	93.25	6.75	3.97 ± 0.22*	48.22
VIII	EAC + 5-Fluorouracil (20mg/kg)	0.06 ±0.01*	0.40 ± 0.023**	12.47	87.53	0.46 ± 0.02*	94.04

Each value represents the mean ± SEM (n = 6 mice per group). ∗Experimental groups were compared with EAC control group ($P < 0.001$). **Experimental groups were compared with EAC control group ($P < 0.05$).

Tumor-bearing mice (EAC control) showed a significant ($p < 0.05$) increase in body weight as compared with group I (normal control). Treatment with synthesized compounds (1d-5d, 25 mg/kg, body weight) significantly reduced the increase in body weight of EAC bearing mice. Whereas at the dose of 25 mg /kg of compounds 1d, 2d and 4d showed highest retardation of increase in body weight (6.87%, 5.93% and 7.09% respectively) nearly as same as normal group (5.88%) was statistically significant ($p < 0.05$) at 9 days after tumor implantation when compare with EAC control group (12.48%) (table 3, fig. 2).

Table 3: Percentage change of body weight of animals at different intervals was calculated by comparing with the day '0' of the experiment

Group	Compound	% change of body weight after day 3	% change of body weight after day 6	% change of body weight after day 9
I	normal	2.13 ± 0.64	3.62 ± 0.37**	5.88 ± 0.54***
II	EAC + control	4.91 ± 0.76	9.25 ± 0.88	12.48 ± 0.81
III	EAC + 1d (25mg/kg)	3.02 ± 0.51*	9.32 ± 0.89	6.87 ± 0.87**
IV	EAC + 2d (25mg/kg)	-6.65 ± 1.92***	11.13 ± 0.71*	5.93 ± 0.92***
V	EAC + 3d (25mg/kg)	1.96 ± 1.58	5.92 ± 0.97*	10.23 ± 0.98
VI	EAC + 4d (25mg/kg)	-0.30 ± 0.76**	7.49 ± 1.84	7.09 ± 0.78**
VII	EAC + 5d (25mg/kg)	3.51 ± 0.31*	8.53 ± 0.78*	11.65 ± 0.62*
VIII	EAC + 5-FU (20mg/kg)	1.18 ± 0.71	2.06 ± 0.48***	2.12 ± 1.51***

Each value represents the mean ± SEM (n = 6 mice per group).∗Experimental groups were compared with EAC control group ($P < 0.05$). **Experimental groups were compared with EAC control group ($P < 0.01$). ***Experimental groups were compared with EAC control group ($P < 0.001$).

One of the most reliable criteria for judging the efficiency of any anticancer drug is the prolongation of the life span of animals. It may be concluded that synthesized drugs act by decreasing the nutritional fluid volume and arresting tumor growth increases the life span of EAC-bearing mice [29]. All the compounds (1d-5d) significantly increase percentage increase in life span (%ILS) (94.59%, 110.81%, 48.64%, 137.83% and 70.27% respectively) compared to the induced control group. Thus, compounds (1d-5d) have notable anti-tumor activity against EAC bearing mice. Compound 4d (137.83%) showed maximum %ILS among the test compounds whereas standard drug shows 164.86% compared to the EAC control group (table 3).

Table 4: Percentage increase in life span (%ILS)

Group	Compound	MST (in days)	% ILS
I	normal	--	--
II	EAC + control	18.5	0.00
III	EAC + 1d (25mg/kg)	36	94.59
IV	EAC + 2d (25mg/kg)	39	110.81
V	EAC + 3d (25mg/kg)	27.5	48.64
VI	EAC + 4d (25mg/kg)	44	137.83
VII	EAC + 5d (25mg/kg)	31.5	70.27
VIII	EAC + 5-FU (20mg/kg)	49	164.86

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions[29, 30]. The haematological parameters in the EAC control mice were compared with drug treated groups, shown increased in hemoglobin content in the drugs treated groups as compared to EAC control mice and moderate changes in RBC count were also observed in the drug treated mice. The total WBC counts were significantly higher in the EAC treated mice when compared with normal mice. Whereas, the percentage of WBC count is significantly reduced in synthesized drug treated groups of EAC bearing mice as compared to EAC control mice (table 5, fig. 3).

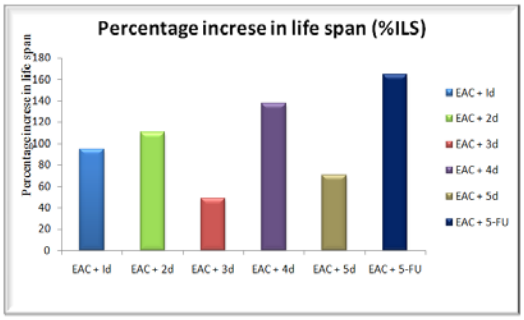


Fig. 2: Percentage increase in life span (%ILS) compared to EAC control group

Table 5: Haematological parameters- WBC, RBC and Haemoglobin content

Group	Compound	WBC count (x10⁹/L)	RBC count (10[12]/L)	Haemoglobin (g/dL)
I	normal	5.88 ± 0.35*	9.78 ± 0.35*	14.30 ± 0.52*
II	EAC + control	18.80 ± 0.53	2.79 ± 0.25	8.92 ± 0.28
III	EAC + 1d (25mg/kg)	7.46 ± 0.38	5.16 ± 0.29*	11.95 ± 0.39*
IV	EAC + 2d (25mg/kg)	9.22 ± 0.41*	6.51 ± 0.47*	13.33 ± 0.30*
V	EAC + 3d (25mg/kg)	10.12 ± 0.45*	3.59 ± 0.18	9.08 ± 0.36**
VI	EAC + 4d (25mg/kg)	9.64 ± 0.43*	6.24 ± 0.34*	13.13 ± 0.47*
VII	EAC + 5d (25mg/kg)	12.22 ± 0.41*	4.20 ± 0.24**	9.53 ± 0.38**
VIII	EAC + 5-Fluorouracil (20mg/kg)	6.24 ± 0.36**	7.83 ± 0.39*	13.28 ± 0.52*

Each value represents the mean ± SEM (n = 6 mice per group). *Experimental groups were compared with EAC control group (P < 0.001). **Experimental groups were compared with EAC control group (P < 0.05).

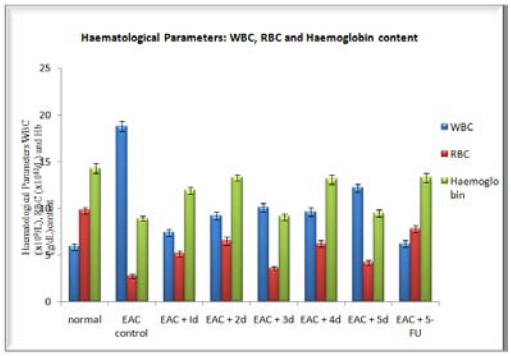


Fig. 3: Haematological parameters- WBC (x10⁹/L), RBC (10[12]/L) and Haemoglobin (g/dL) content of different groups

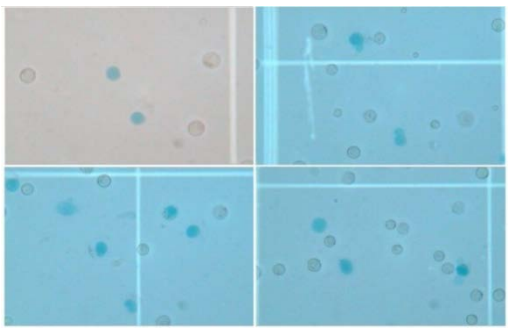


Fig. 4: Tumor cell count. The blue stained cells are non-viable whereas the unstained cells are viable EAC cell. Dye used was trypan blue

CONCLUSION

All the final synthesized compounds exhibited good anticancer activity. Compounds **1d, 2d, 3d** and **4d** were found to have greater anticancer activity. Percentage inhibitions of total cell count (%TCI) by these compounds are 75.30%, 77.84%, 71.78% and 75.17% respectively, where standard reference drug 5-fluorouracil showed 94.04%. Compound **5d** has moderate (48.22%) anticancer activity. The results of the present investigation encourage us to develop similar other related compounds and test them for a wide range of anticancer activity. Thus from the present study, it can be concluded that the synthesized Schiff bases of 2-amino-5-aryl-1, 3, 4-thiadiazoles derivatives are biologically active and they can potentially be developed into useful anticancer agents that can prompt future researcher to choose this nucleus to synthesize a series of other derivatives containing wide varieties of substituent with the aim of obtaining some novel heterocyclic systems with enhanced anticancer effectiveness.

CONFLICT OF INTERESTS

Declared None

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