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Synthesis and anticancer evaluation of 5-benzyl-1,3,4-thiadiazol-2-amine derivatives on Ehrlich ascites carcinoma bearing mice

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ABSTRACT

*5-benzyl-1,3,4-thiadiazol-2-amine derivatives (**2-12**) were synthesized by reaction of 5-benzyl-1,3,4-thiadiazol-2-amine with different substituted phenyl isocyanates or phenyl isothiocyanates. Structures of all compounds were established on the basis of FT-IR, ^{1}HNMR, and LC-MS spectral data. The effects of all compounds on in-vivo tumor growth inhibition were evaluated by studying the parameters such as tumor volume, tumor cell count (viable and non-viable), haematological values, and mean survival time of carcinoma cells bearing mice. Among the tested compounds, compound **2** (54.63%), **3** (60.8%), and **11** (51.54%) showed significant tumor growth inhibition and compared favourably with the standard anticancer drug 5-fluorouracil.*

Key words: 1,3,4-Thiadiazoles, Synthesis, Ehrlich ascites carcinoma cells, Anticancer activity

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INTRODUCTION

1,3,4-thiadiazole is a planar aromatic [1] five member ring system displays exceptional chemical behaviour and a broad spectrum of versatile biological activities probably by virtue of −N=C−S− group. 1,3,4-thiadiazole is also a mesoionic compound associated with conjugated p and π electrons and distinct regions of positive and negative charges. 1,3,4-thiadiazole scaffolds are incorporated in many compounds, exhibited the diverse biological activities such as antimicrobial [2-4], antifungal [5-7], anticonvulsant [8,9], analgesic and anti-inflammatory [10-12], carbonic anhydrase inhibitors [13-15], antidiabetic [16], antiviral [17], antituberculosis [18,19], antiparasitic [20,21], antidepressant and anxiolytic [22,23] and antioxidant [24,25].

Literature survey of 1,3,4-thiadiazole derivatives revealed that these compounds are well known for their *in vivo* and *in vitro* anticancer activities. 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole [26], a promising anticancer compound which was found to inhibit the proliferation of tumour cells of nervous system and peripheral cancers including colon adrenocarcinoma and lung carcinoma, by decreasing cell division and inhibiting metastasis. 5-aryl-(2-thienylamino)-1,3,4-thiadiazoles [27] were studied for their anti proliferative activity against six cancer cell lines. Among the tested compounds, 5-(4-methoxyphenyl)-N-(5-phenylthiophen-3-yl)-1,3,4-thiadiazol-2-amine and 4-(5-((5-(tolyl)thio phen-3-yl)amino)-1,3,4-thiadiazol-2-yl)phenol exhibited potent tumor growth inhibition with IC_{50} < 10 μM. 2-arylamino-5-aryl-1,3,4-thiadiazole [28] derivatives with 3,4,5-trimethoxy phenyl substituent at C-5 position of 1,3,4-thiadiazole ring showed potent anticancer activity against human prostate, breast and pancreatic cancer cell lines with twofold selectivity. 1,3,4-thiadiazole derivatives [29] were screened for FAK inhibitory activity and the compound, N-(5-(2,3-dihyrobenzo[b][1,4]dioxin-6-yl)-1,3,4-thiadiazol-2-yl)cinnamamide showed potent activity against HEPG2 cancer cell line. 5-substituted-2-(2,4-dihydroxy phenyl)-1,3,4-thia diazoles [30] were synthesized and evaluated for antiproliferative activity. Among the tested compounds, 4-(5-(4-(tert-butyl)phenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol and 4-(5-((4methoxybenzyl)oxy)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol showed higher inhibitory activity against T47D cells than standard drug cisplatin.

MATERIALS AND METHODS

General

All chemicals obtained from Sigma, Alfa-Aesar, and Merck were used without further purification. TLC was carried out using precoated silica gel 60 F254 sheet (Merck) and visualised at 366, 254 nm UV light. Melting points were determined in open capillary tubes using digital electrothermal melting point apparatus (VEEGO, VMP-DS) and were uncorrected. IR spectra were recorded on Bruker Alpha spectrophotometer and expressed in wave number ν_{max} cm^{-1} using ZnSe ATR. The ^1H NMR Chemical Shifts δ ppm were determined in DMSO-d_6 as solvent and tetramethylsilane as internal standard on Bruker Avance 600 MHz spectrophotometer. Triple quadrupole mass spectra were performed as Q1 scan on Applied Biosystem-Sciex API 2000.

Chemistry

Synthesis of 5-benzyl-1, 3, 4-thiadiazol-2-amine (1)

It was synthesised from the reported method [31], a mixture of 0.5 mole of phenylacetyl chloride and 0.25 mole of thiosemicarbazide was heated with stirring until the evolution of hydrogen chloride had ceased. Then the reaction mixture was cooled, dissolved in 100 ml of water and filtered. The cooled filtrate was rendered alkaline by slowly adding 50% sodium hydroxide solution. The solid material which precipitated was filtered off, washed with water and dried. White solid, yield 55%, m.p. 202-203 °C, IR (ZnSe ATR): ν_{max} cm^{-1}) 3732.92 (NH_2), 1628.54 (NH_2), 1143.72 (C-N), 1490.86 (CH_2), 1517.66 (C=N), 3080.73 (Ar-H), ^1H NMR (600 MHz, DMSO-d_6): δ ppm 7.325 (t, 2H, Ar-H), 7.272-7.236 (m, 3H, Ar-H), 7.026 (s, 2H, NH_2), 4.136 (s, 2H, CH_2), LC/MS (m/z): 192.0 ($M+H^+$), Anal. Calc. For $C_9H_9N_3S$ (191.25)

General method for synthesis of compounds (2-12)

A mixture of 20 mmoles of 5-benzyl-1,3,4-thiadiazol-2-amine **(1)** and 20 mmoles of substituted phenylisocyanates / phenylisothiocyanates in 100 ml acetontrile was refluxed at 80 ^0C for 24 hours on hot water bath and the hot reaction mixture was filtered, the obtained residue was washed with hot methanol, and dried. The product was recrystallised from methanol-dimethyl formamide (4:1) solvent system.

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(4-chlorophenyl) urea (2)

White solid, yield 72%, m.p. 282-284 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3364.59 (NH), 1557.80 (NH), 1635.96 (C=O), 1539.86 (C=N), 1487.75 (CH_2), 3025.36 (Ar-H), ^1H NMR (600 MHz, DMSO-d_6): δ ppm 10.900 (s, 1H, NH), 9.180 (s, 1H, NH), 7.494 (d, 2H, J = 7.847Hz, Ar-H), 7.365-7.323 (m, 6H, Ar-H), 7.275 (t, 1H, Ar-H), 4.291 (s, 2H, CH_2), LC/MS (m/z): 345.1 ($M+H^+$), Anal. Calc. For $C_{16}H_{13}ClN_4OS$ (344.82)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(4-bromophenyl) urea (3)

White solid, yield 67%, m.p. 289-291 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3363.59 (NH), 1578.67 (NH), 1699.77 (C=O), 1532.93 (C=N), 1483.83 (CH_2), 3025.12 (Ar-H), ^1H NMR (600 MHz, DMSO-d_6): δ ppm 10.931 (s, 1H, NH), 9.196 (s, 1H, NH), 7.474 – 7.451 (m, 4H, Ar-H), 7.365 – 7.323 (m, 4H, Ar-H), 7.275 (t, 1H, Ar-H), 4.291 (s, 2H, CH_2), LC/MS (m/z): 388.8 ($M-H^+$), Anal. Calc. For $C_{16}H_{13}BrN_4OS$ (389.27)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(p-tolyl) urea (4)

White solid, yield 73%, m.p. 274-276 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3370.27 (NH), 1592.70 (NH), 1712.14 (C=O), 1538.73 (C=N), 1450.19 (CH_2), 3029.53 (Ar-H), ^1H NMR (600 MHz, DMSO-d_6): δ ppm 10.773 (s, 1H, NH), 8.894 (s, 1H, NH), 7.364 – 7.259 (m, 7H, Ar-H), 7.099 (d, 2H, Ar-H), 4.294 (s, 2H, CH_2), 2.238 (s, 3H, CH_3), LC/MS (m/z): 325.0 ($M+H^+$), Anal. Calc. For $C_{17}H_{16}N_4OS$ (324.40)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(2,6-dimethylphenyl) urea (5)

White solid, yield 60%, m.p. 295-297 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3381.56 (NH), 1553.03 (NH), 1662.65 (C=O), 1516.46 (C=N), 1493.09 (CH_2), 3024.82 (Ar-H), ^1H NMR (600 MHz, DMSO-d_6): δ ppm 10.978 (s, 1H, NH), 8.077 (s, 1H, NH), 7.348 – 7.304 (m, 4H, Ar-H), 7.256 (t, 1H, Ar-H), 7.100 – 7.059 (m, 3H, Ar-H), 4.278 (s, 2H, CH_2), 2.037 (s, 6H, CH_3), LC/MS (m/z): 339.3 ($M+H^+$), Anal. Calc. For $C_{18}H_{18}N_4OS$ (338.43)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(4-isopropylphenyl) urea (6)

White solid, yield 63%, m.p. 252-254 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3370.92 (NH), 1647.64 (NH), 1652.82 (C=O), 1517.32 (C=N), 3030.30 (Ar-H), 2862.10 (CH, aliphatic), 1473.30 (CH_2), 1373.53 (CH_3), ^1H NMR (600 MHz, DMSO-d_6): δ ppm 10.750 (s, 1H, NH), 8.874 (s, 1H, NH), 7.364 – 7.322 (m, 6H, Ar-H), 7.272 (t, 1H, Ar-H), 7.161 (d, 2H, J = 8.4Hz, Ar-H), 4.279 (s, 2H, CH_2), 2.837 (t, 1H, CH), 1.169 (s, 6H, CH_3), LC/MS (m/z): 351.0 ($M-H^+$), Anal. Calc. For $C_{19}H_{20}N_4OS$ (352.46)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(4-nitrophenyl) urea (7)
White solid, yield 70%, m.p. 299-301 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3363.36 (NH), 1595.28 (NH), 1715.71 (C=O), 1556.08 (C=N), 3010.12 (Ar-H), 1493.46 (CH_2), 1507.70 (NO_2), ^{1}H NMR (600 MHz, DMSO-d_6): δ ppm 11.183 (s, 1H, NH), 9.849 (s, 1H, NH), 8.1855 (d, 2H, Ar-H), 7.7475 (d, 2H, Ar-H), 7.373 – 7.332 (m, 4H, Ar-H), 7.285 (t, 1H, Ar-H), 4.290 (s, 2H, CH_2), LC/MS (m/z): 354.1 ($M-H^+$), Anal. Calc. For $C_{16}H_{13}N_5O_3S$ (355.37)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(4-methoxyphenyl) urea (8)
White solid, yield 68%, m.p. 253-255 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3371.92 (NH), 1598.80 (NH), 1691.16 (C=O), 1548.27 (C=N), 1490.76 (CH_2), 2992.43 (Ar-H), 1043.03 (C-O-C), ^{1}H NMR (600 MHz, DMSO-d_6): δ ppm 10.750 (s, 1H, NH), 8.817 (s, 1H, NH), 7.363 – 7.319 (m, 6H, Ar-H), 7.271 (t, 1H, Ar-H), 6.875 (d, 2H, J = 9Hz, Ar-H), 4.289 (s, 2H, CH_2), 3.709 (s, 3H, CH_3), LC/MS (m/z): 339.1 (($M-H^+$), Anal. Calc. For $C_{17}H_{16}N_4O_2S$ (340.40)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(4-ethoxyphenyl) urea (9)
White solid, yield 55%, m.p. 249-251 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3381.37 (NH), 1592.77 (NH), 1721.95 (C=O), 1546.69 (C=N), 2972.90 (Ar-H), 1495.67(CH_2), 1044.17 (C-O-C), ^{1}H NMR (600 MHz, DMSO-d_6): δ ppm 10.742 (s, 1H, NH), 8.737 (s, 1H, NH), 7.363 – 7.320 (m, 6H, Ar-H), 7.272 (t, 1H, Ar-H), 6.856 (d, 2H, J = 9Hz, Ar-H), 4.261 (s, 2H, CH_2), 3.967 (q, 2H, CH_2), 1.295 (t, 3H, CH_3), LC/MS (m/z): 353.0 ($M-H^+$)1, Anal. Calc. For $C_{18}H_{18}N_4O_2S$ (354.43)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-phenylthiourea (10)
White solid, yield 75%, m.p. 260-262 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3627.05 (NH), 1539.43 (NH), 1336.69 (C=N), 1453.60 (CH_2), ^{1}H NMR (600 MHz, DMSO-d_6): δ ppm 14.241 (s, 1H, NH), 10.438 (s, 1H, NH), 7.662 (d, 2H, J = 9Hz, Ar-H), 7.376 – 7.258 (m, 8H, Ar-H), 4.138 (s, 2H, CH_2), LC/MS (m/z): 325.1 ($M-H^+$), Anal. Calc. For $C_{16}H_{14}N_4S_2$ (326.44)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(4-chlorophenyl) thiourea (11)
White solid, yield 66%, m.p. 260-262 °C, IR (ZnSe ATR) ν_{max} cm^{-1} 3627.01 (NH), 1541.40 (NH), 1430.64 (C=N), 1489.08 (CH_2), ^{1}H NMR (600 MHz, DMSO-d_6): δ ppm 14.340 (s, 1H, NH), 10.472 (s, 1H, NH), 7.714 (d, 2H, J = 8.4Hz, Ar-H), 7.376 – 7.329 (m, 6H, Ar-H), 7.286 (t, 1H, Ar-H), 4.231(s, 2H, CH_2), LC/MS (m/z): 361.3 ($M+H^+$), Anal. Calc. For $C_{16}H_{13}ClN_4S_2$ (360.88)

1-(5-benzyl-1, 3, 4-thiadiazol-2-yl)-3-(4-methoxyphenyl) thiourea (12)
White solid, yield 62%, m.p. 277-279 °C, IR (ZnSe ATR): ν_{max} cm^{-1} 3636.83 (NH), 1534.16 (NH), 1450.09 (C=N), 1333.68 (CH_2), 1281.34 (C-O-C), ^{1}H NMR (600 MHz, DMSO-d_6): δ ppm 14.136 (s, 1H, NH), 10.274 (s, 1H, NH), 7.480 (d, 2H, J = 7.8, Ar-H), 7.371 – 7.199 (m, 7H, Ar-H), 4.180 (s, 2H, CH_2), 3.610 (s, 3H, CH_3), LC/MS (m/z): 357.0 ($M+H^+$), Anal. Calc. For $C_{17}H_{16}N_4OS_2$ (356.46)

BIOLOGICAL ACTIVITY

Antitumor activity

Swiss albino mice with an average body weight (20-22 g) were used for the study. All the animals were housed in polypropylene cages and the standard pellet diets were given after dividing into fourteen groups of eight animals each. The groups except group I (normal control) were injected with 0.2ml of 2 x 10^6 viable EAC cells in ice cold normal saline (0.9%) intraperitonially from the donor mouse [32]. Animals were allowed for 18 h incubation to establish the disease in body. On first day, 5ml/kg body weight of normal saline (0.9%) was administered to normal control group (I) and EAC control group (II). The synthesized compounds [**2-12]** (30 mg/kg body weight/day) and the standard drug 5-Fluorouracil (20 mg/kg body weight/day) was injected intraperitonially to groups (III-XIII) and XIV, respectively for 9 days with 24 h interval. After 24 h of last treatment, half of animals in each group were sacrificed and the remaining animals in each group were used for body weight and survival studies. The intraperitonial tumor cells were harvested using normal saline. The cells were then stained with trypan blue dye (0.4% in normal saline) and cell count was determined in a Neubauer′s counting chamber. The total number of cells in every animal of the treated groups was compared with those of the control group. The percentage tumor cell growth inhibition was calculated by using the formula, % tumor cell growth inhibition = (1– T/C) ×100 where, T = Mean of number of tumor cells in the treated group of mice and C = Mean of number of tumor cells in the control group of mice [33-36]. The effect of compounds was expressed in terms of percentage inhibition of tumor growth and other related parameters.

Tumor volume and tumor weight

The mice were dissected, the ascetic fluid from peritoneal cavity was collected the in graduated centrifuge tubes with the help of sterile syringe, and the tumor cell volume was measured. The tumor weight was measured from the difference in weight of mice before dissection and after collection of ascetic fluid.

Viable and non-viable cell count

The ascetic fluid was diluted, and the cell suspension was stained with the trypan blue (0.4% in normal saline) and allowed to stand for few minutes at room temperature. The cell count was measured in a Neubeauer counting chamber, and the unstained (viable) and stained (non-viable) cells were counted.

Haematological parameters

The blood was drawn from each mouse by retro orbital puncture method under anaesthesia and performed the estimation of haematological parameters such as white blood cells count, red blood cells count and haemoglobin content.

Survival time

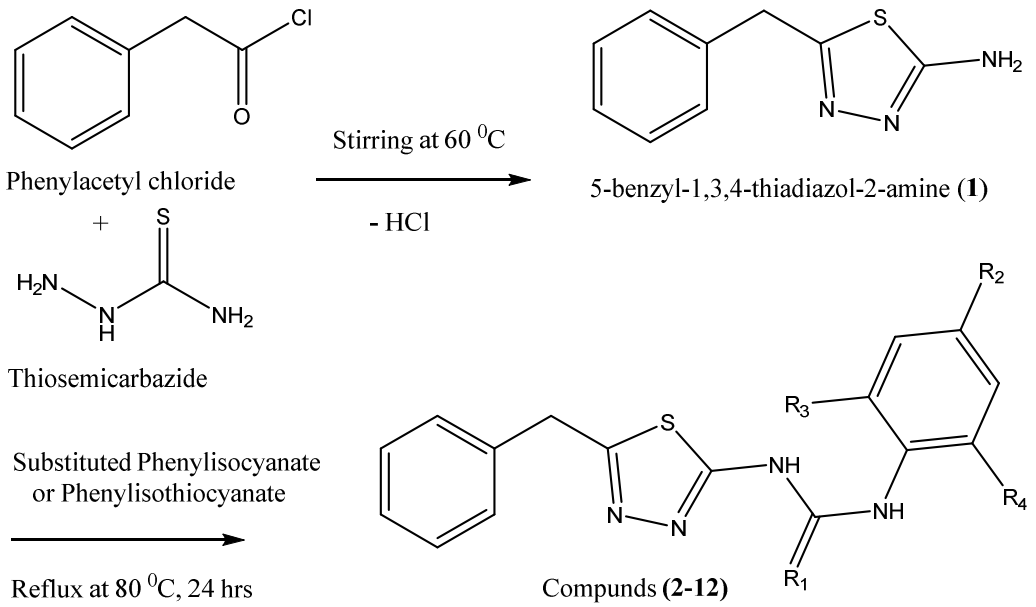
The sets of animal groups for the study on body weight changes and survival time, were separately maintained [37,38]. The effect of the tested compounds on increase in life span was measured by calculating the average survival time. Mean survival time = (Day of first death + Day of last death)/2. The percentage increase in life span was measured using the following formula. Percentage increase in life span (%ILS) = [(Mean survival time of the treated group/Mean survival time of the control group)−1] x 100.

RESULTS AND DISCUSSION

Chemistry

The ring closure reaction of phenylacetyl chloride with thiosemicarbazide, afforded the 5-benzyl-1,3,4-thiadiazol-2-amine (**1**) and structure was established by IR spectra absorption peaks in the regions 3732.92 (NH_2), 1628.54 (NH_2) ν_{max} cm^{-1}, and ^1H NMR spectra chemical shifts δ ppm at 7.026 (NH_2). 5-benzyl-1,3,4-thiadiazol-2-amine (**1**), the amino group compound **1** was then converted into urea derivatives (**2-12**) by heating under reflux with different substituted phenylisocyanates or phenylisothiocyanates in acetonitrile. The disappearance of absorption peak ν_{max} cm^{-1} at 3732.92 (NH_2), 1628.54 (NH_2), chemical shift δ ppm at 7.026 (NH_2) and the appearance of amide functional group (−HN−CO−NH−), proved the formation of compounds (**2-12**)**.**

Scheme



Compound	R_1	R_2	R_3	R_4
2.	O	Cl	H	H
3.	O	Br	H	H
4.	O	CH_3	H	H
5.	O	H	CH_3	CH_3
6.	O	$CH(CH_3)_3$	H	H
7.	O	NO_2	H	H
8.	O	OCH_3	H	H
9.	O	OCH_2CH_3	H	H
10.	S	H	H	H
11.	S	Cl	H	H
12.	S	OCH_3	H	H

Antitumor activity

Antitumor activities were evaluated against Ehrlich ascites carcinoma cells in Swiss albino mice by observing the parameters such as survival time, tumor volume, tumor cell growth inhibition and haematological parameters. The result of present study indicated that among the tested compounds, compound **3** showed the highest mean survival time 30.48±0.10 days (58.2%) whereas it was 19.27±0.06 days, and 37.42±0.09 days (94.0%) for control group and 5-Fluorouracil treated mice, respectively. The tested compounds significantly increased the mean survival time of EAC treated mice [Table no.1].

Table 1: Effects of compounds (2-12) on survival time of EAC cell bearing mice

Experimental groups	Compounds	Mean survival time (days)	Percentage increase in life span (%ILS)
I	Normal control	-	-
II	EAC control	19.27±0.06	-
III	**2**	30.33±0.52	57.4
IV	**3**	30.48±0.10	58.2
V	**4**	24.83±0.41	28.9
VI	**5**	24.14±0.21	25.3
VII	**6**	24.11±0.21	25.1
VIII	**7**	29.29±0.41	52.0
IX	**8**	28.03±0.39	45.5
X	**9**	26.15±0.15	35.7
XI	**10**	24.23±0.49	25.7
XII	**11**	25.24±0.39	30.9
XIII	**12**	27.19±0.13	41.1
XIV	**5 - FU**	37.42±0.09	94.0

All values were expressed in mean ± SD; n = 8, Days of treatment = 9, 5-FU = 5-Fluorouracil

In the treated group with compound **3**, the tumor volume 3.08±0.25 ml, packed cell volume 3.08±0.25 ml, viable tumor cell count 4.45±0.14 x 10^7 ml^{-1} were significantly reduced, while non-viable tumor cell count was increased to 3.62±0.07 x 10^7 ml^{-1} [Table no.2].

Table 2: Effects of compounds (2-12) on ascitic tumor volume, packed cell volume, viable, and non-viable cells of EAC cell bearing mice

Experimental groups	Compounds	Tumor Volume (ml)	Packed Cell Volume (ml)	Viable Cell (x 10^7 ml^{-1})	Non-viable Cell (x 10^7 ml^{-1})
I	Normal control	-	-	-	-
II	EAC control	5.30±0.18	2.78±0.25	11.35±0.15	0.51±0.09
III	**2**	3.13±0.28	1.65±0.24	5.15±0.24	3.18±0.04
IV	**3**	3.08±0.25	1.48±0.17	4.45±0.14	3.62±0.07
V	**4**	3.88±0.33	1.78±0.28	7.56±0.21	1.34±0.07
VI	**5**	4.10±0.14	2.18±0.17	8.18±0.25	1.09±0.11
VII	**6**	3.93±0.22	1.95±0.13	7.93±0.20	1.22±0.13
VIII	**7**	3.35±0.24	1.55±0.24	6.83±0.13	1.79±0.06
IX	**8**	3.48±0.22	1.85±0.13	7.06±0.26	1.63±0.04
X	**9**	3.70±0.18	1.58±0.22	7.32±0.15	1.51±0.05
XI	**10**	4.18±0.22	1.86±0.17	8.17±0.19	1.15±0.15
XII	**11**	3.80±0.18	1.70±0.18	5.50±0.29	2.90±0.06
XIII	**12**	3.25±0.24	1.63±0.21	6.93±0.18	1.71±0.10
XIV	**5 - FU**	2.20±0.22	0.83±0.26	1.10±0.20	4.55±0.57

All values were expressed in mean ± SD; n = 8, Days of treatment = 9, 5-FU = 5-Fluorouracil

The WBC count was decreased in the treated group with compound **3** (12.23±0.07 x 10^3 cells/mm^3) with respect to the control group (18.56±0.28 x 10^3 cells/mm^3) and decreased RBC count and haemoglobin content of control group (2.57±0.27 x10^6 cells/mm^3, 9.26±0.31 gm/dl, respectively) were restored to near normal values in the treated group with compound **3** (3.23±0.05x10^6 cells/mm^3, 6.83±0.05 gm/dl, respectively [Table no.3].

Table 3: Effects of compounds (2-12) on haematological parameters of haemoglobin, red blood cells and white blood cells of EAC cell bearing mice

Experimental Groups	Compounds	Hb (gm/dL)	RBC ($x10^6$ cells/mm^3)	WBC ($x10^3$ cells/mm^3)
I	Normal control	13.43±0.37	5.64±0.10	9.31±0.10
II	EAC control	9.26±0.31	2.57±0.27	18.56±0.28
III	**2**	6.41±0.29	3.18±0.14	12.42±0.22
IV	**3**	6.83±0.05	3.23±0.05	12.23±0.07
V	**4**	6.03±0.21	2.62±0.09	12.80±0.10
VI	**5**	5.73±0.20	2.16±0.10	13.15±0.15
VII	**6**	5.95±0.10	2.28±0.01	12.96±0.12
VIII	**7**	6.49±0.13	3.11±0.12	12.49±0.16
IX	**8**	6.21±0.10	2.88±0.06	12.65±0.22
X	**9**	6.14±0.15	2.73±0.05	12.78±0.07
XI	**10**	5.75±0.21	2.59±0.04	13.19±0.13
XII	**11**	6.10±0.17	2.64±0.04	12.89±0.04
XIII	**12**	6.29±0.09	2.99±0.08	12.63±0.21
XIV	**5 - FU**	11.58±0.07	5.14±0.17	11.55±0.09

All values were expressed in mean ±SD; n = 8, Days of treatment = 9, 5-FU = 5-Fluorouracil

CONCLUSION

According to the obtained results, the synthesized compounds showed significant increase in mean survival time of treated groups and they were able to restore the changes in the haematological parameters. The treated groups exhibited significant decrease in tumor weight, tumor volume and packed cell volume and viable cell count while it was found the increase in non-viable cell count. Among the screened compounds, compound **3** showed highest tumor cell growth inhibition (60.8%), **2** (54.63%) and **11** (51.54%) also showed significant tumor cell growth inhibition and compared with the anticancer drug 5-fluorouracil (90.31%).

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