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Synthesis of some novel 3,5-*di*substituted 1,3,4-oxadiazole derivatives and anticancer activity on EAC animal model

Sasmita Dash · B. Ashok Kumar · Jagadish Singh · B. C. Maiti · T. K. Maity

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Abstract A series of novel 3,5-disubstituted 1,3,4-oxadiazole-2-thione derivatives (**1e**, **2e**, **3e**, **4e**, and **5e**) have been synthesized from different substituted aromatic acids. The structure determination of these compounds have been made on the basis of IR, ^{1}H NMR, and Elemental analysis. The effect of all the compounds on tumor growth inhibition was evaluated by studying the parameters—tumor volume, percentage of the tumor cell count (viable and nonviable), hematological values, and the mean survival status of the treated animals on eight groups of Swiss albino mice. Compounds were given at the dose of 50 mg/kg body weight intraperitoneally and all exhibited the significant ($P < 0.001$) anticancer activity compared to control. 5-Fluorouracil was used as a standard drug (20 mg/kg body weight i.p.) in the study. All the compounds demonstrated a prominent anticancer activity. The study supported the derivatives of oxadiazoles for the development as potent anticancer molecules.

Keywords 1,3,4-Oxadiazoles · Synthesis · EAC cell · Anti cancer activity

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Introduction

1,3,4-Oxadiazole ring is associated with number of biological properties like anti-inflammatory (Raman *et al*., 1993; Sahin *et al*., 2001), hypoglycemic (Husain *et al*., 1986), antifungal (Giri *et al*., 1976), antibacterial (Misra, 1983; Oludotun *et al*., 2009) and anticancer activities (Sengupta *et al*., 2008). It also has gained a significant interest in medicinal chemistry for a number of biological targets including benzodiazepine receptor agonists (Trully *et al*., 1991), 5-HT receptor agonists (Cehn *et al*., 1994), muscarinic agonists (Swan *et al*., 1991), 5-HT antagonists (Ladduwahetty *et al*., 1996), antirhinoviral compounds (Diana *et al*., 1994), and anti-inflammatory agents (Omar *et al*., 1996). Certain 1,3,4-oxadiazole derivatives and their Mannich bases are reported to possess anti-inflammatory, antitubercular (Mamolo *et al*., 2005), antifungal (Kucukguzel *et al*., 1999), and anticancer activities (Loetchutinat *et al*., 2003). It has also been reported that incorporation of various chemical moieties with Mannich base of 1,3,4-oxadiazoles give the compounds of compact structure and expected anticancer activities (Ladduwahetty *et al*., 1996). Therefore, the development of novel and simple synthetic routes to achieve the generation of the molecules with potential anticancer activity are of great interest in the field of pharmaceutical industries.

Thus the aim was to synthesize some 3,5-*di*substituted 1,3,4-oxadiazole-2-thiones derivatives using the synthetic procedure based on the ring closure reactions of appropriate acid hydrazides with carbon disulfide and potassium hydroxide solution to form the 1,3,4-oxadiazole derivatives. Among the compounds, thione is the preferred form because when it is transferred to its thiolic structure it possesses an aromatic units as well as an acidic function. The attachment of an acidic moiety at the thiol equivalent

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side results in necessary aromaticity in the ring. Keeping this fact in mind, the parent 1,3,4-oxadiazole thiones are converted to their acetic acid derivatives to achieve better results.

Chemistry

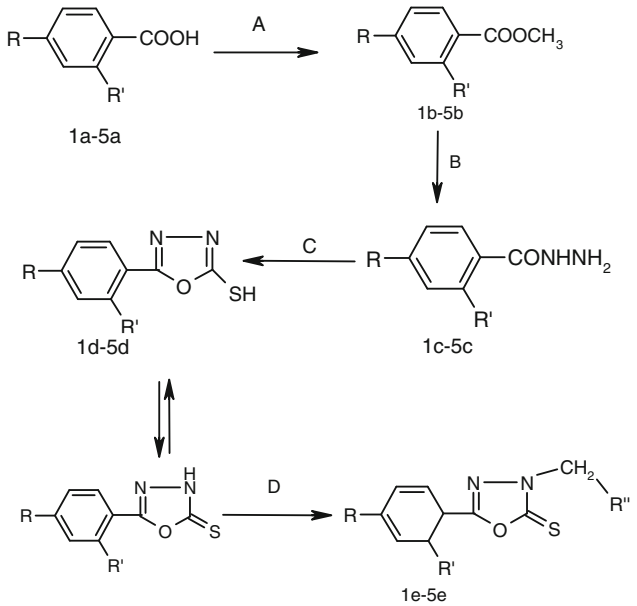
Aromatic acid **1a**–**5a** was esterified with methanol using sulfuric acid according to the procedure depicted in Scheme 1, and the resulting compounds **1b**–**5b** were refluxed with hydrazine hydrate in ethanol to give aroyl hydrazine **1c**–**5c**. Aroyl hydrazines were refluxed with CS_2 and KOH in ethanol to get cyclized to form aryl derivatives of 1,3,4-oxadiazole-2-thione (**1d**–**5d**) in good yield. Then the aryl derivatives of 1,3,4-oxadiazoles were reacted with formaldehyde (40%) and a primary amine (i.e., 2-amino-pyridine or aniline) to form 3,5-substituted aryl derivative of 1,3,4-oxadiazole-2-thione by continuous stirring at room temperature. Then it was kept overnight in refrigerator and filtered to give the desired product. The structure of these compounds have been elucidated by spectral and elemental (IR, ^{1}H NMR, and C, H, N analysis) analysis.

Experimental protocols

General

All the chemicals used in synthesis were supplied from Merck and SRL fine Chemicals, Mumbai, and all are of synthetic grade. Melting point of the synthesized compounds was determined using a Digital Electrothermal Melting point apparatus (VEEGO, VMP-DS) and are uncorrected. For monitoring the chemical reactions and purity of the intermediates and final compounds, thin layer chromatography (TLC) was carried out using Silica gel G coated plates with the appropriate solvent system of

Scheme 1 Synthetic pathway for compounds **1e**–**5e**



Where,

A=CH_3OH / H+ B = NH_2NH_2. H_2O
C=CS2 / KOH D = Formalin (HCHO) 40%, 2-Aminopyridine, Aniline

While, For 1e, R= NO_2 For 2e, R= NO_2
R'= H, R'= H
R''= 2- Amino pyridine R''= Aniline

For 3e, R= Cl For 4e, R= OH
R'= Cl R'= H
R''= 2- Amino pyridine R'= 2- Amino pyridine

For 5e, R=CH_3
R'=H
R= 2-Amino pyridine

different ratios. Then the plates were visualized under ultraviolet light at a wavelength (λ) of 254 nm to determine the R_f value. IR spectra of all the synthesized compounds were performed as KBr discs on a Shimazdu 470 IR spectrophotometer. The structure of all the compounds was confirmed by ^{1}H NMR spectra with Brucker DPX 300 MHz Instrument (DMSO-d_6, $CDCl_3$ and TMS) and Elemental analysis using CHN analyzer 2400 ser II (Perkin Elmer).

Synthesis

*Aryl substituted methyl benzoate **[1b–5b]***

In a clean, dried 250-ml round bottomed flask, methanol (36 g, 45 ml, and 1.13 mol) and concentrated H_2SO_4 (1 ml) were taken. To this, the aryl substituted aromatic acid (0.06 mol) was added and refluxed for 6 h. The reaction mixture was then cooled to room temperature. After cooling, crystals of the aryl substituted methyl benzoate were filtered and washed successively with saturated solution of sodium bicarbonate and water. It was then recrystallized from aqueous ethanol (Ladduwahetty *et al*., 1996).

*Aryl substituted benzoic acid hydrazide **[1c–5c]***

Hydrazine hydrate (6.18 g, 6 ml, and 0.12 mol) was placed in a small, round bottomed flask fitted with a reflux condenser. Aryl substituted methyl benzoate (0.04 mol) was added to this and gently heated under reflux for 10 min. Sufficient quantity of absolute alcohol was added through the condenser to get a clear solution (about 10 ml), then the reaction mixture was refluxed further for 3 h. The product obtained was concentrated and cooled to room temperature. The concentrated solution was filtered to get the crystals of acid hydrazide and recrystallized from aqueous ethanol.

*5-Aryl-2,3-dihydro-1,3,4-oxadiazole-2-thione **[1d–5d]***

In a clean, dried 250-ml round bottomed flask, ethanol (120 ml) and potassium hydroxide (1.68 g, 0.03 mol, and dissolved in 6 ml of water) were taken. To this, the aryl substituted benzoic acid hydrazide (0.03 mol) was added. Then to the above clear solution, carbon disulfide (22.8 g, 18 ml, and 0.6 mol) was added and refluxed for 4 h. A solid appeared initially dissolved on heating. Then 60 ml of ethanol was distilled off and cooled to room temperature. The content was poured into water ($\sim$25 ml) and acidified with ice cold concentrated HCl (3 ml, 0.03 mol). A solid separated was filtered and washed with ice cold water. The resulting compound 5-aryl-2,3-dihydro-1,3,4-oxadiazole-2-thione was recrystallized with aqueous ethanol.

*5-Aryl-3-substituted 2,3-dihydro-1,3,4-oxadiazole-2-thione **[1e–5e]***

A 40% formalin (1.5 ml, 0.02 mol) was added to a stirred solution of 5-aryl-2,3-dihydro-1,3,4-oxadiazole-2-thione (0.02 mol) in absolute ethanol (40 ml). An ethanolic solution (10 ml) of the appropriate amine (0.02 mol) was added to a little portion at a time to the above reaction mixture, stirred for 3 h at room temperature, and left overnight in a refrigerator. The precipitate formed was filtered, washed with cold ethanol, dried, and recrystallized from ethanol.

*Compound **1e**: 5-(4-nitrophenyl)-3-[(2-pyridylamino) methyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione* M.P.: 190°C (61%); $R_f = 0.43$; solvents used for TLC: Ethanol:Benzene (1:2); IR (KBr, V_{max} cm^{-1}) 3297, 3105 (N–H and C–H), 1622 (C=N), 1611 (C=C), 1549 (C–NO_2), 1511 (C=C aromatic stretch), 1381 (C=S), 1343 (C=N, aromatic 20 amine, stretch), 1144 (C–O–C). ^{1}H NMR (DMSO-d_6), δ ppm: 8.38–8.36 [d, 2H, $J = 3$ Hz, (5,6)nitrophenyl], 8.06–8. 04 [d, 2H, $J = 3$ Hz, (2,3)nitro-phenyl], 7.89, 7.91 [d, 1H (6) pyridine], 7.65, 7.68, 7.71 [t, NH, hydrazide], 6.60, 6.62 [d, 1H, (3) pyridine], 6.67, 6.67 [m, 2H, (4,5)pyridine]. 5. 68–5.70 [d, 2H, N–CH_2] Anal. Calcd. for $C_{14}H_{11}O_3N_5S$: C, 51.06; H, 3.37; N, 21.27; found: C, 50.79; H, 3.35; N, 21.80.

*Compound **2e**: 5-(4-nitrophenyl)-3-[anilinomethyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione* M.P.: 209°C (88%); $R_f = 0.62$; solvents used for TLC: Ethanol:Benzene (1:4); IR (KBr, V_{max} cm^{-1}) 3342–3061 (N–H and aromatic C–H stretching), 1523 (C=N), 1597 (C=C), 1346 (C–NO_2 aromatic), 1241 (C=S) and 1253 (C–O–C). ^{1}H NMR ($CDCl_3$), δ ppm: 8.08–8.05 [d, 2H, $J = 3$ Hz, (2,3)nitro-phenyl], 8.32, 8.34 [d, 2H, $J = 2$ Hz, (5,6)nitro-phenyl], 7.23,7.25 [d, 2H, (2,6) phenyl], 6.09, 6.92 [d, 2H, (3,5) phenyl], 6.81, 6.83 and 6.86 [t, 1H, (4) phenyl], 6.99, 7.02, 7.05, [t, NH, hydrazide], 5.58–5.56 [d, 2H, methylene (N-CH_2)]. Anal. Calcd. for $C_{15}H_{12}O_3N_5S$: C, 54.87; H, 3.68; N, 17.06; found: C, 54.84; H, 3.61; N, 16.61.

*Compounds **3e**: 5-(2,4-dichlorophenyl)-3-[(2-pyridylamino) methyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione* M.P.: 199°C (60%); $R_f = 0.56$; solvents used for TLC: Ethanol:Chloroform (1:2); IR (KBr, V_{max} cm^{-1}) 3450 (N–H and C–H). 3060 (C–H, methylene). 2920 (C–H). 1645 (C=N). 1590 (C–C=C, stretch, aromatic). 1481 (C=S). 1247 and 1150 (C–O–C). 839 and 769 (C–Cl, aromatic).^{1}H NMR (DMSO-d_6), δ ppm: 7.59 [S, aromatic peak of proton associated with C_3 proton due to Cl] 8.04 [S, 1H, (6) pyridine], 7.46, 7.49, 7.52 [t, 1H, hydrazide], 6.69, 6.72 [d, 1H, (3) pyridine], 6.59, 6.55, 6.59 [t, 2H, (4,5)pyridine].

Anal. Calcd. for $C_{15}H_{11}ON_3\ CI_2\ S$: C, 47.60; H, 2.85; N, 15.86; found: C, 6.71; H, 2.76; N, 15.08.

Compound ***4e****: 5-(4-hydroxyphenyl)-3-[(2-pyridylamino) methyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione* M.P.: 168°C (75%); $R_f = 0.69$; solvents used for TLC: Ethylacetate:Benzene (1:2); IR (KBr, V_{max} cm^{-1}): 3320–3236 (O–H and N–H). 3007 (C–H, alkyl, methylene, stretch). 1603 (C=N). 1508 (C–C=C, aromatic, stretch). 1443 (C=S). 1253 and 1163 (C–O–C, stretch). ^{1}H NMR (DMSO-d_6), δ ppm: 10.44 [s, 1H, OH], 8.04 [S, 1H, (6) pyridine], 7.66, 7.69 [d, 2H, (2,6)phenyl], 7.45, 7.48, 7.50 [t, 1H, hydrazide], 6.91, 6.93 [d, 2H, (3,5)phenyl], 6.71, 6.73 [d, 1H, (3) pyridine], 6.52, 6.55, 6.58 [t, 2H, (4,5)pyridine], 5.61,5.64 [d, 2H, methylene].Anal. calcd. For $C_{14}H_{13}O_2N_4S$: C, 55.99; H, 4.03; N, 18.65; found: C, 55.83; H, 3.78; N, 18.49.

Compound ***5e****: 5-(4-methylphenyl)-3-[(2-pyridylamino) methyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione* M.P.: 205°C (69%); $R_f = 0.81$; solvents used for TLC: Ethylacetate: Benzene (1:2); IR (KBr, V_{max}, cm^{-1}): 3339 (N–H and C–H, stretch). 1598, 1503 (C=C and C=N, stretch, pyridine). 1426 (C=S) and 1253 (C–O–C, asymmetric stretch). ^{1}H NMR ($CDCl_3$), δ ppm: 8.18 [s, 1H, (6) pyridine], 7.78, 7.80 [d, 2H, (2,6)phenyl], 7.26, 7.28 [d, 2H, (3,5)phenyl], 7.47, 7.50, 7.52 [t, 1H, NH, hydrazide], 5.83, 5.85 [d, 1H, (3) pyridine], 5.83–5.85 [d, 2H, methylene]. 2.59 (S, 3H, Ar–CH_3). Anal. Calcd. for $C_{15}H_{13}ON_4S$: C, 60.38; H, 4.73; N, 18.7: found: C, 59.66; H, 4.52; N, 18.78.

Biological testing

Anticancer activity

Determination of anticancer activity

The 10-week-old Swiss albino mice with an average body weight of 18–20 g were used in the study. All the animals were kept on basal metabolic diet after dividing into eight groups of six animals each. The groups except normal control (group I) were injected with 0.2 ml of 2×10^6 viable EAC cells in ice cold normal saline (0.9%) intraperitoneally (Aspirated from EAC bearing mice at the log phase 7–8th day). Animals were allowed for 18 h incubation to establish the disease in the body before starting the drug administration. On first day, 5 ml/kg body weight of normal saline (0.9% W/V NaCl) and phosphate buffer (pH 7.2) was administered to group I (Normal) and group II (EAC control), respectively. The synthesized compounds [**1e**–**5e**] (50 mg/kg body weight/day) and the standard drug (5-Fluorouracil) (20 mg/kg body weight/day) were administered intraperitoneally (i.p.) to groups (III–VII) and VIII, respectively, for 7 days at 24 h interval. On 8th day, Ehrlich cells were harvested, washed five times with 0.9% saline and resuspended in Dulbecco's phosphate-buffered saline (PBS). Ehrlich cells were counted microscopically at 100X magnification. Cell viability was confirmed by exclusion of 0.25% trypan blue dye.

The evaluation of the test drug was made by comparing the cell count of the test with that of control. The percentage inhibition of cell count was obtained by the formula $TCI = (1 - T/C) \times 100$, where T is the average number of Ascitic cells/ml in test animals and C is the average number of the ascitic cells/ml in control animals. The anticancer activity of the compounds was measured in EAC-treated animals with respect to the following parameters (Qureshi *et al*., 1993; Zarafoenetis, 1969; Qureshi *et al*., 2001; Al-Harbi *et al*., 1995) such as,

Tumor volume and tumor weight

The mice were dissected, the ascitic fluid was collected from the peritoneal cavity, and the tumor volume was noted. The tumor weight was calculated from the difference in weight of mice before dissection and after collection of ascitic fluid.

Cancer is a pathological state where uncontrolled proliferation of the cancer cells is found. The synthesized compounds **1e**–**5e** have reduced the growth of the Ehrlich ascites carcinoma cells in mouse model. The efficacy of the compounds was expressed in terms of percentage inhibition of tumor formation and other relevant parameters described based on anticancer activity. Among the test compounds, compound **4e** exhibited highest inhibition (73.9%) of tumor at the dose of 50 mg/kg body weight (i.p.) when compared with control. The standard drug showed 93% inhibition. The rest of the compounds **1e**, **2e**, **3e**, and **5e** inhibited the tumor formation 60.8, 56.5, 52.2, and 56.5%, respectively.

It was also observed that the volume of EAC was significantly ($P < 0.001$) reduced when the EAC implanted animals treated with compounds (**1e**–**5e**) and standard drug (5-Fluorouracil) for 7 days.

Viable and non-viable tumor cell count

The ascitic fluid was diluted 100 times, and the cell suspension was mixed with the trypan blue and allowed to stand for few minutes at room temperature. The cell suspension was then placed over the Neubeauer counting chamber, and the stained (Non viable) and unstained (Viable) cells were counted in the 64 small squares.

The number of viable EAC cells was significantly ($P < 0.001$) reduced, whereas the nonviable cells were

found to be increased in drug and standard treated animal groups on comparison with the EAC control animals.

Hematological parameters

The blood was collected by retro-orbital puncture under anesthesia and performed the estimation of hematological parameters like hemoglobin content (Hb), red blood cells (RBC) count, and white blood cells (WBC) count.

The count of red blood cells and the content of hemoglobin were observed to be restored significantly ($P < 0.001$) in all the drug-treated animals except group **1e** which was less significant ($P < 0.01$) than that of the EAC control. The reliable criteria for judging the value of any anticancer drug is prolongation of life span and decrease of WBC from blood (Latha and Panikkar, 1998). This fact was supported from the result that the count of WBC was also decreased significantly ($P < 0.001$) than that of EAC control.

Survival time

The sets of animal groups allotted for observations on body weight changes and survival were maintained separately. These animals were observed for their weight changes and mortality day-to-day until their death or up to a maximum of 62 days. The effect of the compounds on life span was measured by calculating median survival time (MST) and average survival time (AST). The MST and AST were calculated as follows: MST = (first death + last death of animal)/2; AST = sum of the animal death in different days/*N*, where *N* is number of animals. The mortality rate was calculated in all the groups of animals, and the percentage increase in life span was calculated using the following formula. Percentage Increase in Life Span (%ILS) = Mean survival of treated group/Mean survival of control group × 100 and Mean Survival = (Day of first death + Day of last death)/2.

Treatment of synthesized compounds for the period of 7 days increased the survival time of the EAC-treated animals significantly ($P < 0.01$) as shown in Table 2.

Statistical analysis

The values were recorded as means ± SD. The data were analyzed by using ANOVA; differences below the 0.001 level ($P < 0.05$) were considered as statistically significant.

Discussion

It was clearly understood from the result that all final [**1e–5e**] compounds and the standard drug exhibited a prominent anticancer activity against the EAC implanted mice models. According to the standard of National Cancer Institute, a substance is considered active if it exhibits the tumor growth inhibition by 50%. All the tested compounds were found to show inhibition of tumor growth above 50% which supports the efficacy of the oxadiazole derivatives to serve as potent anticancer agents against EAC cells.

The tumorigenesis and its progression have been reported to be accompanied by the following changes compared with normal cytogenesis: (1) gradual decrease in hemoglobin content and erythrocyte count, (2) gradual increase in leukocytes, thrombocytes, and splenic cellularity, and (3) reversal of the lymphoid–myeloid ratio in the differential WBC count. It was observed in the study that there was notable decrease in the hemoglobin level and erythrocyte count in animals of EAC control group; however, the level was effectively improved and restored toward the normal group by treatment with the synthesized compounds [**1e–5e**]. The leukocytes count was drastically increased in the tumor bearing mice which was significantly brought back toward normal level on the treatment (Tables 1, 2)

Table 1 Anticancer activity of oxadiazole derivatives against EAC bearing mice

Group	Compounds	RBC (10^6 cells)	WBC (10^3 cells)	Tumor volume (ml)	% of Viable cell	% of Non-viable cell
I	Normal Saline	11.04 ± 0.33	4.47 ± 0.15	–	–	–
II	Induce control	2.77 ± 0.13	10.31 ± 0.23	3.35 ± 0.07	85.17 ± 0.95	14.83 ± 0.95
III	**1e**	3.57 ± 0.20*	7.73 ± 0.21***	1.60 ± 0.06***	36.83 ± 1.49***	63.17 ± 1.49
IV	**2e**	5.68 ± 0.30***	5.57 ± 0.14***	1.57 ± 0.03***	33.50 ± 1.15***	66.50 ± 1.15
V	**3e**	7.23 ± 0.07***	4.47 ± 0.12***	0.92 ± 0.08***	27.33 ± 0.88***	72.67 ± 0.88
VI	**4e**	6.25 ± 0.16***	4.97 ± 0.10***	0.88 ± 0.09***	22.50 ± 0.99***	77.50 ± 0.99
VII	**5e**	8.27 ± 0.16***	4.62 ± 0.14***	0.60 ± 0.04***	24.83 ± 1.19***	75.17 ± 1.19
VIII	5-Fluorouracil	8.32 ± 0.15***	4.81 ± 0.14***	0.25 ± 0.04***	14.83 ± 1.19***	85.17 ± 1.19

The results given are mean ± SEM; number of animal used ($n = 6$)

Experimental groups were compared with Induce control; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.01$

Table 2 Anticancer activity of oxadiazole derivatives against EAC bearing mice

Group	Compounds	HB	%IALS	%IMLS
I	Normal Saline	13.92 ± 0.10	–	–
II	Induce control	4.97 ± 0.19	–	–
III	**1e**	7.717 ± 0.10	38.54	36.92
IV	**2e**	11.75 ± 0.08	46.35	44.61
V	**3e**	12.28 ± 0.15	53.64	47.69
VI	**4e**	12.08 ± 0.16	80.72	76.92
VII	**5e**	13.77 ± 0.17	73.95	73.84
VIII	5-Fluorouracil	13.35 ± 0.15	92.70	90.76

$P < 0.001$, when compare all treated groups with phosphate buffer control in HB parameter. Other parameters are no need analysis by ANOVA

Though all the compounds exhibited prominent anticancer activity, the efficacy of the compounds against different biochemical parameters studied varied from each other. For example, the count of RBC, WBC, and hemoglobin content levels in drug-treated animals have been restored significantly when compared to the EAC control values which is near to saline control value (Fig. 1). However, compound 5e having substituted with *o*-amino pyridine and *p*-methyl phenyl groups at 3rd and 5th positions of oxadiazole and 5-Flurouracil exhibited prominent recovery in the RBC level and hemoglobin content than those of other compounds, whereas the WBC level was found to be decreased significantly in compounds **3e**, **4e**, **5e**, and 5-Flurouracil-treated groups. One of the common side effects associated with most of the treatment of anticancer drugs is anemia and elevation of WBC level. The study indicates that compounds [**1e**–**5e**] did not adversely affect the process of haematopoiesis instead it was found to be restored. This exhibits their efficacy as a potent source for development of anticancer molecule with decreased or negligible adverse effects.

The present study clearly demonstrated the tumor inhibitory activity of the oxadiazole derivatives against transplantable tumor cell line (Table 1). In the EAC bearing mice, cells were present in the peritoneal cavity, and the compounds were administered directly into the peritoneum. Thus, tumor inhibition might be due to the direct effect of the compounds on the tumor cells. The standard drug 5-fluorouracil acts cytostatically by interfering with nucleotide metabolism in S phase of the cell cycle. The reduced tumor cell count and increased percentage of nonviable cell count in the study led us to the assumption that the test compounds might be acting by interfering with S phase of cell cycle through apoptosis. However, the exact molecular mechanism behind the action of these compounds is yet to be ascertained.

In general, the anticancer activity seemed to be dependent on the nature of the substituent rather the basic skeleton of the molecule (Adnan *et al.*, 2007). Within the oxadiazole series, it was noticed that the substituent at the position 5 has great influence on the anticancer activity. Substitution of various pharmacopores at this position may give rise to the novel molecules with enhanced anticancer properties. The activity of the compounds increased due to the attachment of the phenyl ring with the substitution of the electron withdrawing/donating group at 5th position of the oxadiazole group and also due to the presence of the Mannich side chain. Among the synthesized compounds, those substituted with hydroxyl, methyl, and 2,4-dichloro phenyl group showed significant anticancer activity compared to other substituents. Another reason for the increasing activity might be due to the chlorine atoms which are expected to have more receptor binding capacity.

Compound **4e** substituted with 2-amino pyridine through a methylene bridge at 3rd position and by *p*-hydroxy phenyl group at 5th position of the 1,3,4-oxadiazoles exhibited marked cytotoxicity to the EAC cells which was confirmed from the percentage of non viable

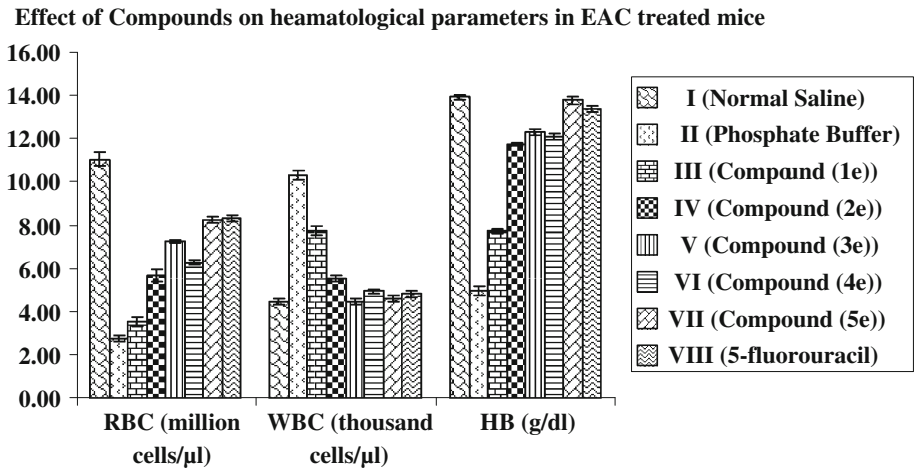


Fig. 1 Anticancer activity of oxadiazole derivatives against EAC bearing mice

(77.50 ± 0.99) and viable cell (22.50 ± 0.99) counts. Replacement of *p*-hydroxyl group of phenyl ring at 5th position with methyl group (**5e**) decreased the ratio of percentage of non viable (75.17 ± 1.19) and viable cell (24.83 ± 1.19) counts. It exhibited reduced cytotoxic efficacy of the electron donating methyl group in the molecule. Substitution of *p*-hydroxyl group of phenyl ring with the electron withdrawing nitro group showed 63.17 ± 1.49% and 36.83 ± 1.14% of nonviable and viable cells count. They were found to be less significant than those of other compounds in the study. Inclusion of aniline group at 3rd position of oxadiazole ring through methylene linkage and the *p*-nitro group of phenyl ring at 5th position produced **2e**, and it showed nonviable (66.50 ± 1.15%) and viable (33.50 ± 1.15%) cells count. Substitution of 2, 4-*di*chloro phenyl at 5th position of the compound **3e** showed 72.67 ± 0.88% of nonviable and 27.33 ± 0.88% of viable cells.

The anticancer efficacy of the compounds was also supported by the enhanced survival time of the EAC bearing mouse (Table 2). Compounds **4e** and **5e** were found to increase % IALS and % IMLS more significantly. Hence, it can be concluded that the Mannich base derivatives of oxadiazoles are effective source of novel drug development for cancer.

Conclusion

A series of 3,5-*di*substituted 1,3,4-oxadiazole-2-thione derivatives possessing Mannich base have been synthesized using simple synthetic procedures, and their anticancer activity against EAC cancer cell has been evaluated. All the final compounds exhibited good anticancer activity. Compound **4e** with the 73.9% of tumor inhibition was found to be the most active among all. This might be due to the hydrophilic *p*-hydroxyl group of phenyl ring substituted at position 5. Further exploration of derivatives of this class is on the progress. From the above experiments and observations, it can be concluded that the 1,3,4-oxadiazole derivatives are potential molecule to be developed as significant anticancer drug.

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