Original Article

Synthesis and Evaluation of Anticancer Activity of 1, 3, 4-Oxadiazole Derivatives against Ehrlich Ascites Carcinoma Bearing Mice and Their Correlation with Histopathology of Liver

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ABSTRACT

A series of 2, 5-*disubstituted* 1, 3, 4-Oxadiazole derivatives (4**A**-4**G**) have been synthesized with the help of different aromatic benzaldehyde and final compounds were characterized by FT-IR, ^{1}H NMR and Mass spectroscopy. The anticancer study was investigated against Ehrlich Ascites Carcinoma (EAC) bearing albino mice. The synthesized (4**A**-4**G**) compounds were administered intraperitoneally at dose of 20-25 mg/kg; body weight per day for 7 days after 24 hour of tumor inoculation in mice. The standard compound used was 5-FU (20 mg/kg; body, weight). Synthesized compounds (4**A**-4**G**) remarkably decreased the body weight, tumor volume, packed cell volume, viable cell count and increased in tumor weight (%) inhibition, tumor cells (%) inhibition, the life span, nonviable cell count of EAC tumor bearing mice when compared with the control group. The liver section of EAC treated control group (**II**) was compared with the drug treated groups (**III-X**). The histopathological observations of test groups suggested that normal architecture of liver nucleus, parenchyma, and hepatic cells were regenerated, which was damaged in EAC control group. All the synthesized compounds (4**A**-4**G**) showed significant anticancer activity in EAC bearing mice which encourages us to develop/improve similar other compounds and test them for their anticancer activity.

Key word: Synthesis, 1, 3, 4 oxadiazole, EAC cell, Anticancer activity, FT-IR, NMR.

INTRODUCTION

Cancer ranked second to cardiovascular disease as a cause of mortality it is likely to become the most common disease in the near future.[1] In modern medicine, chemotherapy, radiotherapy and surgery are the chief modes of cancer treatment. Chemopreventive agents have a narrow margin of safety and the therapy may fail due to drug resistance and dose limiting toxicities, which may severely affect the host normal cells.[2] Therefore, identification of novel potent, less toxic and selective mechanism of action anticancer agents remains one of the most imperative health problems.[3]

1,3,4-Oxadiazoles are an important class of heterocyclic compounds[4] with a broad range of biological activities such as anti-inflammatory, analgesic and ulcerogenicity,[5] apoptosis-inducer,[6] antimycobacterial,[7] antifungal,[8] antitumor,[3,6,9] P-Glycoprotein Inhibitors,[10] pesticides and insecticides,[11] 4-Hydroxylase Inhibitors,[12] anticonvulsant activity[13] etc.

Moreover, it is considered that the presence of toxophoric –N=C–O– linkage[14] in 1, 3, 4-oxadiazole ring might be responsible for their potent pharmacological activities. Further1, 3, 4-oxadiazole heterocyclic are very good bioisosters of amide and ester functionalities with substantial improvement in biological activity by participating in hydrogen bonding interactions with different receptor.[15,16]

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Schiff bases containing the >C= N group are known to have antitumor activity, more of this compounds have been synthesized in order to find greater antitumor activity.[17] Thus the present investigation was designed to report some new derivatives of oxadiazole containing >C= N group at its 2 position. Considering the potential of this class of compounds some new *2, 5, disubstituted* 1, 3, 4-oxadiazole derivatives were synthesized (4**A**-4**G**) as showed in Scheme -1, studied for their anticancer activity and histopathological study of liver was performed.

Chemistry

Semicarbazone (2**A**-2**G**) were synthesized by using different aromatic aldehyde (1**A**-1**G**), semicarbazide hydrochloride and sodium acetate. (Vogel's, 1996) .Then 2-Amino-5-aryl-1, 3, 4-oxadiazole (3**A**-3**G**) was prepared by using Semicarbazone (2A-2G), sodium acetate and bromine in glacial acetic acid with the help of Magnetic stirring. 2-Amino-5-aryl-1, 3, 4-oxadiazoles were refluxed with required aromatic aldehyde (5-6 hours) in ethanol to form final Schiff bases of 2-amino-5-aryl-1, 3, 4-oxadiazoles (4**A**-4**G**) derivatives then the final compound was dried and recrystallized from alcohol. The structures of these compounds were characterized by FT-IR, ^{1}H NMR and LC MS/MS Mass spectroscopy.

MATERIALS AND METHODS

General

All the chemical (synthetic grade) were procured from Merck, SRL and SD Fine Chemicals. Melting points were determined in open glass capillaries and are uncorrected (VEEGO, VMP-DS). The purity of compounds was checked by TLC on micro plates using Silica-gel-G, solvent system chloroform: methanol (6: 1) with detecting agent. Infrared spectra in KBr were recorded on Shimazdzu 470 Infrared Spectrophotometer. ^{1}H NMR spectra were recorded on a Brucker DPX (300 MHz) NMR spectrometer in DMSO-d_6 using TMS as an internal reference, chemical shifts are expressed in (ppm) and mass were recorded on (API-2000) LCMS/MS. All the compounds have given satisfactory ^{1}H NMR, Mass and FT- IR spectra.

Synthesis

Semicarbazone [2A-2G]

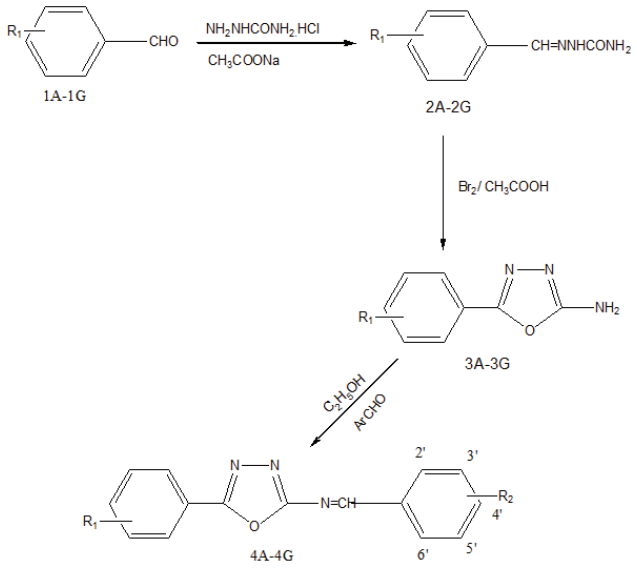
Semicarbazones were synthesized by using different aromatic aldehyde (1**A**-1**G**) (0.5g), semicarbazide hydrochloride (1.0 g) and sodium acetate (1.50 g) were taken in 100 ml conical flask and dissolved in 30-40ml of distilled water. After half an hour stirring, the precipitate was filtered and recrystallized from alcohol. (Vogel's, 1996).

2-Amino-5-aryl-1, 3, 4-oxadiazoles [3A-3G]

Semicarbazone (2**A**-2**G**) (0.01 M) and sodium acetate (0.02 M) were dissolved in 30–40 ml of glacial acetic acid taken in a (100 ml) round-bottomed flask equipped with a separating funnel for the addition of bromine. Bromine (0.7 ml in 5 ml glacial acetic acid) was added drop by drop, while stirring magnetically. After half an hour stirring, the solution was poured on crushed ice. The resulting solid was separated, dried and recrystallized from aldehyde free ethanol.[18]

Schiff bases of 5-aryl-2-substituted-1, 3, 4-oxadiazoles [4A-4G]

A solution of [**3A-3G**] (0.01 M) was taken in 20 ml alcohol a round-bottomed flask. Required aldehyde (0.01M) dissolved in 15 ml alcohol was then added to it. The mixture was refluxed for 5–6 h. The volume of alcohol was reduced to half by distillation under reduced pressure. The resulting solution was poured on crushed ice. The precipitate was separated, dried and recrystallized from alcohol.[9]



Scheme 1 Synthetic pathway for compounds 4A-4G

Compound	R_1	R_2
4A.	4 -OH,	4' -N $(CH_3)_2$
4B	2 -Cl	2' -NO_2
4C	3 -Br	4' -Cl
4D	4 -N $(CH_3)_2$,	4' -N $(CH_3)_2$
4E	2-Cl,	4' -N $(CH_3)_2$
4F	3 -Br	4' -OH
4G	4 -OCH_3,	4' -N $(CH_3)_2$

Compound:4A.[5-{-4-Hydroxy-Phenyl)-2-imino--(4-dimethyl-amino-phenyl) 1, 3, 4- Oxadiazole] (C): M.P: 220-221-^{O} C. Yield: 70%. Chemical formula: $C_{17}H_{16}O_2N_4$. FT-IR (cm $^{-1}$): 3452, 3283 (ArC-OH); 2913, 3068, 3154 (ArC-H); 1657 (C=N), 1250 (C-O-C), 1601 (C=C), 1438, 1509 (N=CH). ^{1}H NMR (300 MH$_Z$, DMSO d_6 p p m): δ 10.08 (S, 1H, N=CH): δ 7.74 (d, 2H, J= 9Hz, ArH 2, 3); δ 7.66 (d, 2H, J= 9Hz, ArH 4, 5,); δ 2.93-2.85 (d, 6H, N(CH_2)$_2$); δ 7.13-7.06 (m, 4H, ArH); δ 9.91 (s, 1H, OH). m/z: 310.50/308.50 (M^+ /M^-).

Compound: 4B. [5-{(-2-Chloro-Phenyl)-2-imino--(2-nitro-phenyl)}-1, 3, 4- Oxadiazole] : M. p: **214-216 ^{O} C.** Yield: 80%. Chemical formula: $C_{15}H_9O_3N_4Cl$. FT-IR (cm $^{-1}$): 2991 (ArCH), 1515 (ArC-NO_2) 742(ArC-Cl), 1658 (C=N), 1280 (C-O-C), 1600(C=C), 1426, 1515 (N=CH). ^{1}H NMR (300 MHZ, DMSO d_6 p p m): δ 10.48.(S, 1H, N=CH); δ 6.57-7.46 (m, 4H, ArH 2'3' 5' 6'); δ 8.23-8.17 (m, 4H, ArH 2, 3, 4, 5,). m/z: 329.50/327.50(M^+ /M^-).

Compound: 4C. [5-{(3-Bromo-Phenyl)-2-imino-(4-chloro-phenyl)}-1, 3, 4-Oxadiazole]: M. p: 222-224^{O} C. Yield: 75%. Chemical formula: $C_{15}H_9ON_3BrCl$. FT-IR (cm $^{-1}$): 2991, 3068 (ArC-H); 1682 (C=N), 1220 (C-O-C), 1600 (C=C) 1426, 1515 (C=NH), 851,742 (ArC-Cl), 562, 627 (ArC-Br),. ^{1}H NMR (300 MHZ, DMSO d_6 p p m): δ 10.35 (S, 1H, N=CH); δ 7.52 (d, 2H, J= 6Hz, ArH 2' 3'); δ 7.35 (d, 2H, J= 6Hz, ArH 5', 6',); δ 8.06 (S 1H, ArH , 2); δ 7.78 (s, 1H, ArH, 4); δ 7.30-7.35 (t, 1H, ArH 5); 6.60(s 1H, ArH, 6).

Compound: 4D. [5-{-4-dimethyl amino-Phenyl)-2-imino-(4-dimethyl amino-phenyl) 1, 3, 4- Oxadiazole]: M.P: 185-187^{O} C. yield-70%. Chemical formula: $C_{19}H_{21}ON_5$. FT-IR (cm $^{-1}$): 2938, 3153 (ArC-H); 1682 (C=N), 1221 (C-O-C), 1558 (C=C), 1435, 1493 (N=CH). ^{1}H NMR (300 MHZ, DMSO d_6 p p m): δ 10.20 (S, 1H, N=CH); δ 2.96-2.93 (d, 6H, N(CH_2)$_2$); δ 3.34 (s, 6H, N(CH_2)$_2$); δ 6.79 (d, 2H, J= 9Hz, ArH 2 , 3); δ 6.70 (d, 2H, J= 9Hz, ArH 5 ,6); δ 7.56-748.(m, 4H, ArH 2', 3', 4', 5',).

Compound: 4E. [5-{ 2 Chloro-Phenyl)-2-imino-(4-dimethylamino-phenyl) 1, 3, 4- xadiazole] : M.P: 173-175^{-O} C. Yield: 61%. Chemical formula: $C_{17}H_{15}ON_4Cl$. FT-IR (cm- ^{1}): 3134 (ArC-H), 1651 (C=N), 1253 (C-O-C), 1507 (C=C), 1439, 1357 (N=CH), 765(ArC-Cl). ^{1}H NMR (300 MHZ, DMSO d_6 p p m): δ 10.50 (S, 1H, N=CH); δ 8.23-8.18 (m, 4H, ArH 3, 4, 5, 6); δ 7.69 (d, 2H, J= 6Hz, ArH 2', 3'); δ 7.35 (d, 2H, J= 6Hz, ArH 5', 6'); δ 3.04 (s, 6H, N(CH_3)$_2$). m/z: 327.50/325.50(M^+ /M^-).

Compound: 4F. [5-{(3-Bromo-Phenyl)-2-imino-(4-hydroxy-phenyl)}-1, 3, 4-Oxadiazole]: M. p: 222-224^{O} C. Yield: 55%. Chemical formula: $C_{15}H_{10}O_2N_3Br$. FT-IR (cm $^{-1}$): 3463 (Ar-OH) 2984, 3062 (ArC-H); 1699 (C=N), 1227 (C-O-C), 1584 (C=C) 1471, 1443 (C=NH), 559, 680 (ArC-Br),.^{1}H NMR (300 MHZ, DMSO d_6 p p m): δ 10.36 (S, 1H, N=CH); δ 7.78-7.76 (d, 2H, J= 6Hz, ArH 2' 3'); δ 7.48-7.46 (d, 2H, ArH 5', 6',); δ 7.89 (S 1H, ArH , 2); δ 7.71-7.69 (d, 1H, J= 6Hz, ArH, 4); δ 7.64-7.62 (d, 1H, J= 6Hz, ArH, 6); 7.36-7.31 (t 1H, ArH, 5); δ 8.06 (s, IH, OH). m/z: 344.90/342.90 (M^+ /M^-).

Compound: 4G.[5-{(4 Methoxy-Phenyl)-2-imino--(4amino-dimethyl-phenyl)}-1, 3, 4- Oxadiazole]: M. p: 218-221 ^{O} C. yield-71%. Chemical formula: $C_{18}H_{18}O_2N_4$.. FT-IR (cm $^{-1}$): 3111 (ArCH), 1657 (C=N), 1077 (C-O-C), 1503 (C=O), 1563 (C=C), 1474, 1515 (C=NH). ^{1}H NMR (300 MHZ, DMSO d_6 p p m): δ 8.57. (S, 1H, N=CH): δ 1.58 (s, 3H, CH_3O); δ 3.84-3.93 (d, 6H, N (CH_3)$_2$); δ 6.98 (d, 2H, J= 9Hz, ArH 2' 3'); δ 6.92 (d, 2H, J= 9Hz ArH 5' 6'); δ 7.57 (d, 2H, J=9Hz, ArH 2, 3,); δ 7.50 (d, 2H, J=Hz, ArH 5, 6,). m/z: 323/321(M^+ /M^-).

Biological Experimental Section

Animals

Studies were carried out using male Swiss albino mice of about 8 weeks of age with an average body weight of 18-20 g. The animals were obtained from animal supplier Rita Ghosh, Kolkata, India. They were grouped and housed in polyacrylic cages and maintained under standard laboratory conditions (temperature 30^0 C) with dark and light cycle (12/12h) and fed standard pellet diet, fresh water *ad libitum*. The mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee. The recommendations of Jadavpur University Institutional Animal Ethics Committee [Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA registration. no. 0367/01/C/CPCSEA) India for the care and use of laboratory animals were strictly followed throughout the study and these were in accordance with the NIH (USA) guidelines.

Tumor cell

A tumor cell used for anticancer activity is EAC (Ehrlich Ascites Carcinoma) cells originated from human breast carcinoma. It is an undifferentiated tumor, which has lost its epithelial character. Ehrlich Ascites Carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute, Kolkata, India. The (EAC) cells were maintained *in vivo* in Swiss albino mice by intra

peritoneal inoculation of 2 $\times 10^6$ cells/ mouse after 10 days. EAC cells of 9 days old were used for screening of the compounds.

Experimental procedure

Male Swiss albino mice of 8 weeks old with an average body weight of 18 to 20 g were used. All mice were kept on basal metabolic diet with water *ad libitum*. Male Swiss albino mice were divided into 10 groups (n = 12). EAC cells are collected from the donor mice and are suspended in sterile isotonic solution (0.9% w/v NaCl). The numbers of tumor cells per ml of this suspension are counted under microscope with the help of hemocytometer. All the groups were treated with EAC cells (0.2 ml of 2 $\times 10^6$ cells/mouse) intraperitoneally except the normal group. This was taken as day zero. In this instance, the tumor cells multiply relatively freely within the peritoneal cavity and ascites develops. A day of incubation allows for establishing the disease in the body before starting the drug administration. On the first day, 5 ml/kg body weight of normal saline (0.9% NaCl W/v) was administered in group I (Normal). Normal saline 5ml/kg; body weight per day was administered in-group II (EAC control). The synthesized compounds (4A-4G were administered at dose of 25, 20, 20, 25, 20, 20, 25mg mg/kg; body weight/day) and the standard drug 5-Fluorouracil (20 mg/kg; body weight/day) was administered in groups (III-IX) and (X) respectively for 7 days intraperitoneally at 24 hr interval. Thus 7 doses of the drug are administered to each mouse in the test group. On the 9th day food and water were with hold 18 hr before the starting the testing operation. The weight of all the animals was recorded before they are sacrificed. The peritoneal cavity was dissected and by a syringe the ascitic 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 6 mice in a group was calculated. The fluid is sucked by adsorbent cotton. The weight of 6 mice after sacrifice was recorded and remaining animals kept for observation of life span of the hosts.

The evaluation of the test drug is made by comparing the cell count of the test with that of the control. The percentage inhibition of cell count is obtained by following expression: (TCI) = (1-T/C) **×100, where T is the average number of** Ascitic cells /ml in test animals, C is the average number of the Ascitic cells /ml in control animals.

The anti-tumor activity of the compounds was measured in EAC animals with respect to the following parameters such as: Body weight, Tumor weight, Tumor cell count, Tumor growth response, Tumor volume, Viable and non viable tumor cell count, Mean survival time and percentage increase in life span, Hematological studies.[19-23]

Acute Toxicity study: Male Albino mice of 10 animals per group and weighting between 18-20g were administered with graded doses of (50 -300 mg/kg, Body weight, i. p) of the synthesized compounds (4A-4G). Death of the animals was observed 48h treatment of the administered synthesized (4A-4G) compounds. The toxicological effects were observed in terms of mortality and expressed as LD_{50} (Ghosh *et al.,* 1984). During the experiment on above said dose regimen 50% death was observed of synthesized compounds(4A-4G) respectively (250, 200, 200, 250, 2000, 200, 250mg/kg, Body weight, i. p). From present study it could be concluded that synthesizes compounds (4A-4G) were safe unto 25, 20, 20, 25, 20, 20, 25mg/kg body weight i. p.

RESULT AND DISCUSSION

Cancer is a pathological state where uncontrolled proliferation of the cancer cells is found. The anticancer properties of the synthesized compounds were evaluated by measuring their ability to inhibit cancer cell growth in ascitic fluid of Swiss albino mice. Percentage tumor weight inhibition (% TWI) and percentage inhibition of ascitic cells or percentage of tumor cell count inhibition (%TCI) were measured on treated EAC cells when compared to untreated control cells. Compounds (4A-4G) having anticancer potential are shown in the **Table 1.** Among all the test compounds, compound 4E exhibited highest tumor weight inhibition (73.15%) and tumor cell count inhibition (65.07%) at the dose of **20** mg/kg; body weight, (i.p.) as compared to control. Standard drug showed about 95.78% tumor weight inhibition and 96.09 % tumor cell count inhibition. The rest of the compounds showed 57.63%, 60.52%, 60.52%, 62.63%, 62.63% and 63.68% of % TWI and 39.35%, 50.41%, 52.00%, 57.01%, 58.87% and 60.17% of % TCI respectively. According to the standard of National Cancer Institute, a substance is considered active if it exhibits the tumor growth inhibition of 50 %. All the tested compounds were found to show inhibition of tumor growth above 50 % (except compound 4D) which supports the efficacy of the oxadiazole derivatives to serve as potent anti cancer agents against EAC cells.

In EAC bearing mice a regular rapid increase in ascites tumor volume was observed. Ascites fluid is considered to be direct nutritional source for tumor cells and a rapid increase in ascites fluid with tumor growth would be a means to meet the nutritional requirement of tumor

Table 1: Results of anticancer activity of the tested (A-G) compounds on % TWI and %TCI.

Group	Compounds	Dose of drug (mg /kg)	Avg tumor weight (g)	% TWI	Avg cell count (Number)	%TCI
I	Normal Control	-------	------	--------	------	--------
II	Induced control	--------	1.90	0.00	79.96 ± 0.28	0.00
III	A	25	0.75 ± 0.00*	60.52	38.38 ± 0.51*	52.00
IV	B	25	0.71 ± 0.00*	62.63	34.35 ± 0.53*	57.01
V	C	25	0.75 ± 0.00*	60.52	31.86 ± 0.43*	60.15
VI	D	25	0.81 ± 0.00*	57.63	52.49 ± 0.62*	39.35
VII	E	25	0.51 ± 0.00*	73.15	27.96 ± 0.51*	65.07
VIII	F	25	0.69 ± 0.00*	63.68	32.88 ± 0.48*	58.87
IX	G	25	0.71 ± 0.00*	62.63	39.65 ± 0.25*	50.41
X	5-Fluorouracil	20	---------	95.78	--------	96.09

Value are Mean ± SEM. n=6 animal in each group. *P< 0.05 is considered significant when III, IV, V, VI, VII, VIII, IX, groups were compared with group II. (When considered both Avg Tumor weight and Avg Cell count).

cells.[23,24] This might be the possible reason that the EAC cell-bearing mice (group II) showed a significant increase in body weight as compared with the normal mice in group-I (negative control).

5-Flurouracil treated group and test compounds (4A-4G) treated groups showed a significant ($p<0.001$) reduction in the body weight of EAC cell-bearing mice when compared with EAC control. The drugs decreased the tumor volume, packed cell volume, viable cell count and increased the life span, percentage of trypan blue positive stained dead cells in tumor bearing mice (**Table 2)**.

Results for the test compound indicate that there is decrease in the nutritional fluid volume, arresting the tumor growth and the prolongation of the life span of animals and might be act as antineoplastic agents.

A complete blood count provides detailed information about three types of blood cells: red blood cells (RBC), white blood cells (WBC) and platelets. These blood cells are made in the bone marrow. Furthermore, hematological characteristics have been widely used in the diagnosis of variety of diseases (like cancer) and pathologies induced by industrial compounds, drugs, dyes, heavy metals, pesticides and several others.[25,26,28,29]

RBC (known as erythrocytes) is very important for the transport of oxygen from the lungs to the tissues and hemoglobin concentration is directly correlated with the RBC count. This close correlation between erythrocyte count and haemoglobin concentration was also reported for other vertebrates including man.[30]

WBC formed in the bone marrow either enters the blood or migrates to key organs such as the spleen, lymph nodes, or gut. The increased number of leukocytes can occur abnormally as a result of an infection, cancer**,** or toxic chemical. Such increase of WBC may be due to the activation of the defense mechanism of animals and their immune system.[27]

Myelosuppression and anemia are the two major problems in cancer chemotherapy.[24] (Price *et al.,* 1958; Hogland HC *et al.*, 1982) The anemia in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and increase in WBC and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions or due to cancer. Treatment with (4A-4G) brought back significantly ($p<0.05$) the hemoglobin (Hb) content, RBC and WBC count more or less to normal levels. He differential count and the percentage of neutrophil was increased, while the lymphocytes count was decreased in synthesized compounds treated groups bearing EAC cell lines when compared with EAC control mice. Other parameters like HCT (%), MCV (fl), MCH (Pg), MCHC (g/ dL), PLT (10^9 / L) were showen in **Table 3**. This clearly indicates that synthesized (4A-4G) compounds possess protective action on the hemopoietic system.

Carcinogenesis is associated with cirrhosis and cirrhosis correlates with primary liver disease. Liver is easily affected by various types of diseases, cancer being one of them. EAC easily affects the liver of mice. From the experiment the liver section of EAC treated control group was compared with normal, 5-Flurouracil and drug treated groups (4A-4G). The histopathological observations of test groups suggested normal architecture of liver tissue, less thick wall central vein (**CV**), deformed necrosis of tissue (**N**) and nucleus, parenchyma, hepatic cells were regenerated, which was damaged in EAC treated control group. (Figure 1A-10)

Table 2: Results of anticancer activity of the tested (A-G) compounds on mean survival time (MST), increase in life (%ILS), tumor volume, packed cell volume, cell count.

Parameter	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII	Group-VIII	Group-IX	Group-XI
	EAC+(2×10^6cell/ml per mice)	EAC+A (25mg/kg)	EAC+B (25mg/kg)	EAC+C (25mg/kg)	EAC+D (25mg/kg)	EAC+E (25mg/kg))	EAC+F (25mg/kg)	EAC+G (25mg/kg)	EAC+5-FU (20mg/kg)
Body weight(g)	22.54±0.012	19.31±0.004*	19.16±0.006*	19.96±0.012*	20.27±0.010*	18.90±0.004*	19.18±0.005*	19.56± 0.004*	18.19± 0.004*
Mean survival time (days)	16.50±84	31.78±0.44*	35.33±0.50*	36.00±73*	26.16±87*	36.33±80*	35.66±84*	28.00±0.89*	41.50±0.71*
Increase in life span(%ILS)	---------	92.60	114.12	118.18	58.54	120.18	116	69.69	151.51
Tumor volume(ml)	1.19±0.06	0.77±0.02*	0.73±0.04*	0.60±0.02*	0.90±0.01*	0.40±0.01*	0.63±0.27*	0.83±0.02*	--------
Pack cell volume(ml)	0.85±0.01	0.36±0.01*	0.30±0.00*	0.13±0.00*	0.56±0.02*	0.10±00*	0.13±0.00*	0.46±0.02*	----------
Viable cell count($\times10^6$ cells/ml)	74.27±0.51	28.00±0.57*	24.32±0.53*	21.34±0.43*	42.06±0.62*	15.37±0.51*	20.50±0.56*	28.13±0.94*	--------
Non-viable cell count($\times10^6$ cells/ml)	5.69±0.28	10.38±0.63*	10.03±0.37*	10.52±0.34*	10.43±0.20	12.56±0.48*	12.38±0.25*	11.52±0.43	---------

Value are Mean ± SEM. n=6 animal in each group. * P<0.01 is considered significant when (III), (IV), (V), (VI), (VII), (VII), (IX), (X) groups were compared with EAC control group (II).

Table 3: Results of anticancer activity of the tested (A-G) compounds on hematological parameters.

Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII	Group-VIII	Group-IX
	Normal control (0.9%NaCl ml/mice)	EAC control (2×10^6cell/ml per mice)	EAC + Compound A (25mg/kg)	EAC + Compound B (25mg/kg)	EAC + Compound C (25mg/kg)	EAC + Compound D (25mg/kg)	EAC + Compound E (25mg/kg)	EAC + Compound F (25mg/kg)	EAC + Compound G (25mg/kg)
Hemoglobin (g %)	13.30±0.06*	9.04±0.49	10.57±0.50*	10.28±0.23*	10.96±0.59*	9.74±0.30*	12.32±0.30	11.25±0.39*	10.60±0.32*
RBC($\times10^{12}$/L)	9.53±0.01*	3.84±0.05	6.76 ± 0.07*	5.78±0.07*	6.25±0.10*	4.56±0.04	7.56±0.05*	7.13±0.03*	6.74±0.16*
WBC($\times10^9$/L)	5.36±0.07*	19.10±0.04	10.06±0.04*	11.35±0.12*	10.13±0.05*	14.13±0.06*	8.41±0.31*	9.06±0.02*	9.90±0.18
Monocyte (%)	1.93±0.00*	1.06±0.02	1.52±0.01*	1.63±0.06*	1.50±0.05*	1.25±0.00*	1.71±0.01*	1.34±0.00*	1.41±0.00*
Neutrophil(%)	17.97±0.00*	80.79±0.34	51.42±0.33*	55.96±0.17*	58.12±0.25*	71.70±0.35*	33.25±0.08*	61.91±0.0.47*	64.60±0.23*
Lymphocyte (%)	81.23±0.05*	24.11±0.09	48.53±0.30*	56.31±0.00*	67.98±0.17*	38.84±0.41*	73.31±0.07*	70.02±0.29*	45.28±0.08*
HCT (%)	39.29±0.26*	28.48±0.12	35.19±0.43*	31.39±0.41*	35.52±0.47*	31.45±0.30*	37.21±0.63	35.74±0.41	34.01±0.22*
MCV(fL)	78.52±0.19*	39.22±0.37	46.22±0.25*	44.65±0.14*	44.91±0.29*	40.11±0.25*	64.79±0.51*	59.23±0.11*	51.34±0.09*
MCH(Pg)	29.19±0.15*	11.24±0.05	15.29±0.03*	14.73±0.08*	14.64±0.08*	13.02±0.21*	25.12±0.06*	22.25±0.07*	16.08±0.04*
MCHC(g/dL)	35.79±*	30.28±0.28	32.87±0.05*	33.34±0.09*	33.16±0.23*	30.97±0.17*	34.56±0.18*	33.28±0.32*	32.81±0.17*
PLT(10^9 / L)	410.50±0.42*	297.50±0.42	335.50±0.61*	350.16±0.47*	340.50±0.42*	301.16±0.30*	387.83±0.47*	351.66±0.42*	340.50±0.42*

Value are Mean ± SEM. n=6 animal in each group. *P< 0.05 is considered significant when (I), (III), (IV), (V), (VI), (VII), (VIII), (IX) groups were compared with EAC control group (II). Hb: Hemoglobin, RBC: Red Blood Cells, WBC: White Blood Cells, M: Monocyte, N: Neutrophils, L: Lymphocytes, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, PLC: Platelet Count.

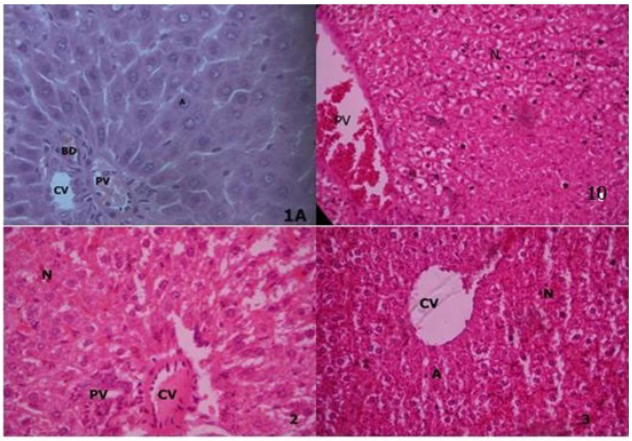


Fig. 1A, 2, 3, 10

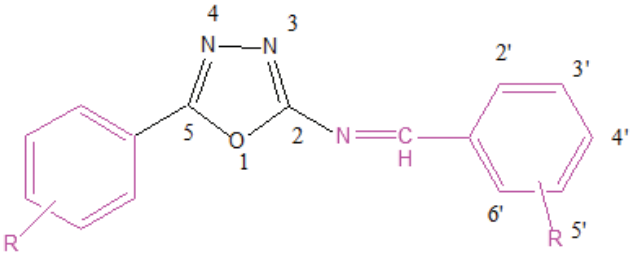
Figure 1A: The liver section of the normal mice was composed of a number of lobules and hepatic tissue showing a thin walled central vein (CV) from which cords of hepatic cells radiate. Branches of the hepatic portal vein (PV), hepatic artery (A) and bile ductules (BD) forming the portal triads are also seen (×100).

Figure 2: Photomicrograph of mice liver in EAC treated with 5-Fluorouracil showing more or less normal structure of the liver tissue, showing thin wall central vein(CV), hepatic portal vein (PV) and large neoplastic hepatocytes (N) are regenerated look like to normal liver section (×100).

Figure 10: Photomicrograph of mice liver in control group showing thick walled central vein (CV) plates of highly differentiated, large neoplastic hepatocytes (N) (prominent nucleoli and finely granular cytoplasm) without discernible hepatic architecture (× 100).

Structural features of the synthesized compounds for their antitumor activity

In general, the anti cancer activity seemed to be dependent on the nature of the substituents rather the basic skeleton of the molecule.[33] Within the Oxadiazole series it was noticed that the substituent at the position 5 had great influence on the anticancer activity. Substitution with various pharmacophore at this position may give rise to the novel molecules with enhanced anticancer properties.



The Study indicates that groups like 4-hydroxy-phenyl (4A) 3-bromo phenyl(4C, 4F), 2-chloro phenyl(4B, 4E) *substituent* at 5-position and 2-nitrophenyl(4B), 4-chlorophenyl (4C), 4-hydroxy phenyl (4F) *substituent* at position 2 linking by im ino bridge to 1, 3, 4-oxadiazole showed significant anti-tumor activity.

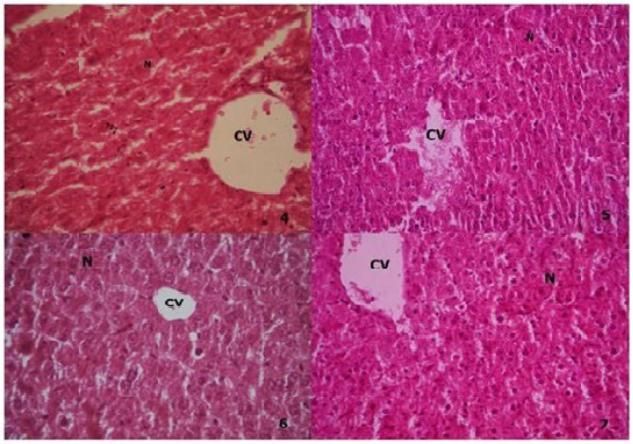


Fig. 4, 5, 6, 7

Figure: 3, 4, 5, 6, 7, 8 & 9 Photomicrograph of mice liver in EAC treated with drugs (4A-4G) showing more or less structural damages of the liver tissue, showing damage central vein(CV), degenerated hepatocytes and deformed necrosis of tissue (N)(× 100).

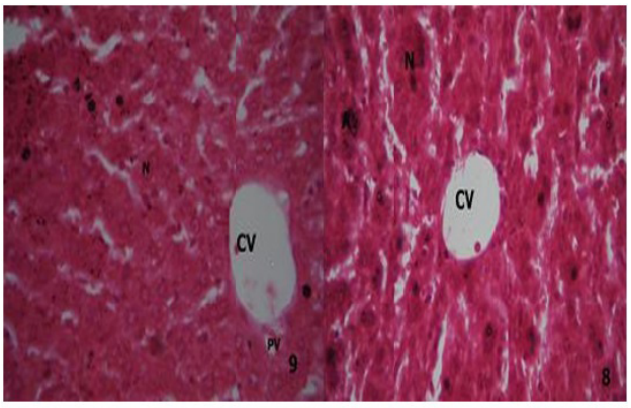


Fig: 8, 9

The activity of the compounds get increased due to the attachment of the phenyl ring with the substitution of the electron withdrawing/donating group at 5th position of the Oxadiazole ring and also due to the presence of the N=CH linked Schiff base side chains at 2 position. The exact mechanism of action of 1, 3, 4-Oxadiazole derivatives are still unknown. The probable mechanism may be due to multiple events, can increase percentage inhibition of ascitic cells or percentage of tumor cell count inhibition or act as decreasing the nutritional fluid volume or arresting the tumor growth or act as apoptosis inducer. A novel analog, 5-(3-chlorothiophen-2- yl)-3-(5-chloro-pyridin-2-yl)-1, 2, 4-oxadiazole was identified as a lead compound to induce apoptosis *in vivo* anticancer activity.[31] Another novel series of 3, 5-[1, 2, 4]-diaryl-oxadiazoles act as apoptosis-inducer in cancer treatment with no measurable effects on normal cells.[32] Therefore the synthesized 1, 3, 4 oxadiazole com-

pounds with the above mentioned substituents could be a potential anticancer agent.

CONCLUSION

The synthesized compounds (4G-AG) showed significant result in different parameters of EAC bearing mice and markedly increase the average life span of experimental animals. From the present study, result indicate that the oxadiazole compounds can potentially be developed into useful anticancer agents or further work to develop or improve similar and related compounds and test them for a wide range of biological activity.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

ABBREVIATIONS USED

EAC: Ehrlich Ascites Carcinoma; **5FU:** 5- Fluorouracil DMSO: Dimethyl sulfoxide; **FTIR:** Fourier Transform Infrared; **HNMR:** Proton Nuclear Magnetic Resonance; **LCMS:** Liquid Chromatography Mass Spectrometry; **CPCSEA:** The Committee for the Purpose of Control and Supervision of Experiments on Animals; **LD50:** Lethal Dose; **TWI:** Tumor Wight Inhibition; **TCI:** Tumor Cells Inhibition; **HCT:** Hematocrit; **MCV:** Mean Corpuscular Volume; **MCH:** Mean Corpuscular Hemoglobin; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **PLC:** Platelet Count.

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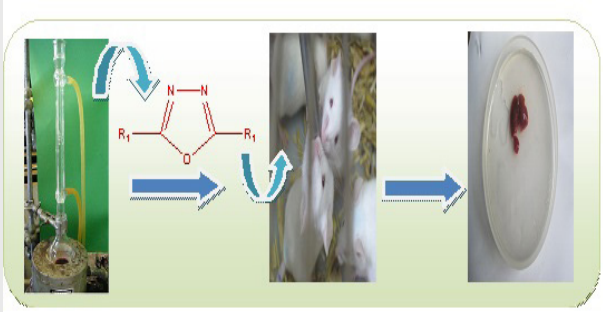
PICTORIAL ABSTRACT



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SUMMARY

- A series of 2, 5-*disubstituted* 1, 3, 4-Oxadiazole derivatives (4A-4G) have been synthesized with the help of different aromatic Benzaldehyde and final compounds were characterized by FT-IR, ^{1}H NMR and Mass spectroscopy.
- The anticancer study was investigated against Ehrlich Ascites Carcinoma (EAC) bearing albino mice. The synthesized (4A-4G) compounds were administered intraperitoneally at dose of 20-25 mg/kg; body weight per day for 7 days after 24 hour of tumor inoculation in mice.
- The standard compound used was 5-FU (20 mg/kg; body, weight). Synthesized compounds (4A-4G) remarkably decreased the body weight, tumor volume, packed cell volume, viable cell count and increased in tumor weight (%) inhibition, tumor cells (%) inhibition, the life span, nonviable cell count of EAC tumor bearing mice when compared with the control group. The liver section of EAC treated control group (II) was compared with the drug treated groups (III-X).
- The histopathological observations of test groups suggested that normal architecture of liver nucleus, parenchyma, and hepatic cells were regenerated, which was damaged in EAC control group.
- All the synthesized compounds (4A-4G) showed significant anticancer activity in EAC bearing mice which encourages us to develop/improve similar other compounds and test them for their anticancer activity.

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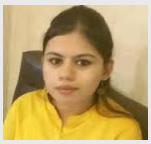

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