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Evaluation of Anticancer Activity of Some 1,3,4-Oxadiazole Derivatives Against *Ehrlich ascites carcinoma* Bearing Mice

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Synthesis of carboxymethyl derivatives of 1,3,4-oxadiazole-2-thione (**a-e**) were carried out by ring closure reaction with appropriate acid hydrazide, carbon disulfide and potassium hydroxide solution by conventional refluxing method. The attachment of an acetic acid moiety at the thiol equivalent site was tried for new biological properties. Synthesized compounds were characterized by IR, ^{1}H NMR studies and their anticancer activity were evaluated. Tumour cells used for anticancer activity were EAC (Ehrlich ascites carcinoma) cells originated from human breast carcinoma. Male Swiss albino mice were used as test animals. The standard drug used was cisplatin (1 mg/kg, body weight). The compound (**a**) showed significant inhibition of cancer cell growth as compared to others. The result of the present investigation encourage us to develop and improve similar other related compounds and test them for a wide range of biological activity.

Key Words: 1,3,4-Oxadiazole, Anticancer activity, Cell count, Tumour weight inhibition.

INTRODUCTION

Although oxadiazoles have been known for last 90 years, it is only in the last three to four decades that investigations in this field have been intensified. Today oxadiazoles are used in the most diverse areas, for example in drug synthesis, scintillation materials and in the dye industry. 1,3,4-Oxadiazoles are well known to have a wide range of biological activities such as antiinflammatory[1], antiparasitic[2], antihyperglycemic[3], apoptosis-inducing[4], antiproliferation[5], antitumour[6], antitrypanosomal[7] and antimicrobial activities[8].

Carboxymethyl derivatives of *p*-substituted/*o*-substituted and 2,4-disubstituted oxadiazole-2-thione were synthesized by ring closure reaction with appropriate acid hydrazide following conventional refluxing method[9-13]. The synthesized compounds were characterized by IR and ^{1}H NMR studies. The compounds synthesized were 2-carboxymethylthio-5-(2-iodo phenyl)-1,3,4-oxadiazole (**a**), 2-carboxymethylthio-5-(2,4-dichloro phenyl)-1,3,4-oxadiazole (**b**), 2-carboxymethylthio-5-(2-methyl phenyl)-1,3,4-oxadiazole (**c**), 2-carboxymethylthio-5-(2-chloro phenyl)-1,3,4-oxadiazole (**d**), 2-carboxymethylthio-5-(4-methoxy phenyl)-1,3,4-oxadiazole (**e**).

The structures of the synthesized compounds **a-e** are shown in Fig. 1. In the present study, the anticancer activity of some carboxymethyl derivatives of substituted 1,3,4-oxadiazole-2-thiones is reported.

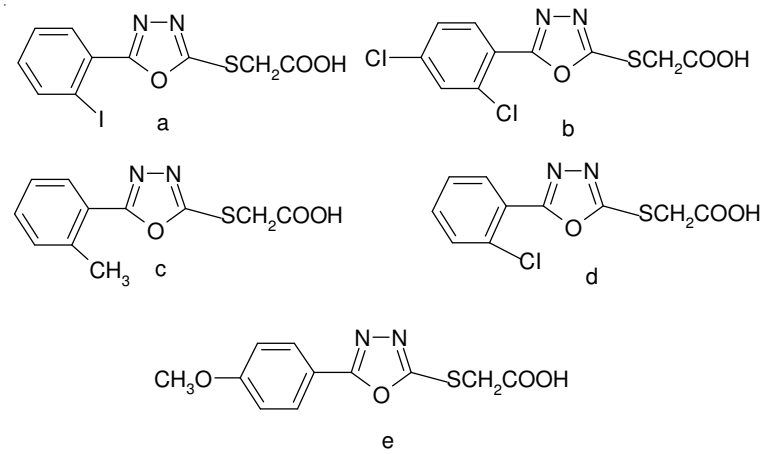

Fig. 1. Structure of compounds **a-e** synthesized and evaluated for anticancer activity

EXPERIMENTAL

Evaluation of anticancer potential

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 obtained from the animal supplier Rita Ghosh, Kolkata, India. The animals were grouped and housed in polyacrylic cages and maintained under standard laboratory conditions (temperature 30 ºC) with dark and light cycle (12/12 h). They were fed standard pellet diet and were given 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.

Tumour cells: Tumour cell used for anticancer activity is *Ehrlich ascites carcinoma* cells originated from human breast carcinoma. It is an undifferentiated tumour, which has lost its epithelial character. *Ehrlich ascites carcinoma* (EAC) cells were obtained from Chittaranjan National Cancer Institute, Kolkata, India. The *Ehrlich ascites carcinoma* cells were maintained *in vivo* in Swiss albino mice by intraperitoneal inoculation of 2×10^6 cells/mouse after 10 days. *Ehrlich ascites carcinoma* cells of 9 days old were used for the screening of the compounds.

Experimental procedure[12,14]: All the mice were kept on basal metabolic diet with water *ad libitum*. Male Swiss albino mice were divided into 8 groups (n = 6). *Ehrlich ascites carcinoma* cells were 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 haemocytometer.

All the groups were treated with EAC cells (0.2 mL of 2×10^6 cells/mouse) intraperitoneally except the normal group. This was taken as day zero. In this instance, the tumour cells multiply relatively freely within the peritoneal cavity and ascites develops. A day of incubation was allowed 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 (0.9 % NaCl), 5 mL/kg, body weight per day was administered in-group II (EAC control). The synthesized compounds (**a, b, c, d, e**, 25 mg/kg, body weight/day) and the standard drug cisplatin (1 mg/kg, body weight/day) were administered in groups (III-VII) and (VIII) respectively for 7 days orally at 24 h interval. Thus 7 doses of the drug were administered to each mouse in the test group. On the 9th day food and water were withdrawn 18 h before the starting of the testing operation. The weight of all the animals were recorded before they were 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 the 6 mice in a group was calculated. The fluid is sucked by adsorbent cotton. The weight of the 6 mice after sacrifice was recorded.

The evaluation of the test drug was 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:

$$\text{Percentage inhibition of ascitic cells (TCI)} = (1\text{-}T/C) \times 100$$

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 groups and the design of the experiment were as follows:

Group-I: Normal saline (0.9 % NaCl, w/v, 5 mL/kg; body weight).
Group-II: EAC (2×10^6 cells/mice) + normal saline (vehicle, 5 mL/kg; body weight).
Group-III: EAC (2×10^6 cells/mice) + compound **a** (25 mg/kg; body weight).
Group-IV: EAC (2×10^6 cells/mice) + compound **b** (25 mg/kg; body weight).
Group-V: EAC (2×10^6 cells/mice) + compound **c** (25 mg/kg; body weight).
Group-VI: EAC (2×10^6 cells/mice) + compound **d** (25 mg/kg; body weight).
Group- VII: EAC (2×10^6 cells/mice) + compound **e** (25 mg/kg; body weight).
Group-VIII: EAC (2×10^6 cells/mice) + standard drug cisplatin (1 mg/kg; body weight).

The antitumour activity of the compounds were measured in EAC animals with respect to the following parameters such as: (i) **Body weight:** Body weight the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and on the final day before sacrifice in order to evaluate the relative change. (ii) **Tumour weight:** The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The tumour weight is calculated from the difference in weight of mice before dissection and after collection of ascitic fluid after dissection. (iii) **Tumour 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.

Statistical analysis: Data were expressed as the mean SEM. The data were analyzed statistically using S.P.S.S. Version 10. Software using ANOVA, Followed by Dunnet's multiple comparison test (DMRT). The minimum level of significance was fixed at $p < 0.05$.

RESULTS AND DISCUSSION

Carboxymethyl derivatives of various *p*-substituted/*o*-substituted oxadiazole-2-thione were synthesized and evaluated for their anticancer activity.

In EAC-bearing mice a regular rapid increase in ascites tumour volume was observed. Ascitic fluid is considered to be direct nutritional source for tumour cells and a rapid increase in ascitic fluid with tumour growth would be a means to meet the nutritional requirement of tumor cells[15].

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. Tumour weight inhibition (% TWI) and percentage inhibition of ascitic cells or percentage of tumour cell count inhibition (% TCI) of the treated EAC cells were compared to untreated control cells. Compounds **a-e** having anticancer potential are shown in the Table-1, where the growth per cent inhibition of the EAC cells is from 22.14 to 46.36 % as compared to the standard drug cisplatin.

TABLE-1
RESULTS OF ANTICANCER ACTIVITY OF THE TESTED COMPOUNDS **a-e**

Group	Compd.	Dose of drug (mg/kg)	Avg. tumour weight (g)	TWI (%)	Avg. cell count (Number)	TCI (%)
I	Control	–	–	–	–	–
II	Induced control	-	3.40±0.07	0	179.16±0.77	0
III	**a**	25	1.25±0.06*	63.23	96.00±0.63*	46.36
IV	**b**	25	2.16±0.05*	34.47	123.33±1.09*	22.14
V	**c**	25	1.90±0.07*	44.11	105.83±0.68*	41.34
VI	**d**	25	2.2±0.09*	35.29	110.66±1.54*	38.23
VII	**e**	25	1.58±0.11*	53.52	112.16±0.61*	37.39
VIII	Standard	1	0	100	0	100

Values are Mean ± SEM, n = 6 animal in each group. *$p < 0.05$ is considered significant when III, IV, V, VI, VII, group compared with group II.

Thus compounds **a-e** has antitumour activity against EAC bearing mice. The exact mechanism of action of 1,3,4-oxadiazole derivatives are not known. It may be due to multiple events or act as apoptosis inducer and thereby reducing tumour weight as well as cells inhibition.

The compound **a** showed significant inhibition of cancer cell growth as compared to others.

Conclusion

From the present study, it can be concluded that the oxadiazole compounds can potentially be developed into useful anticancer agents. These anticancer findings are very much encouraging and extend present research in two directions. The first one is to synthesize other similar derivatives in order to increase their anticancer activity. The second is to carry out more investigation of the synthesized compound in term of any possible cytotoxicity, antiinflammatory, antidiabetic, antitussive, MAO inhibitor and apoptosis inducer activity. Further work to develop and/or improve similar and related compounds and test them for a wide range of biological activity is in progress.

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