Indian Journal of Pharmaceutical Education and Research | Association of Pharmaceutical Teachers of India

Synthesis and Anticancer Activity of 3, 5-Diaryl 1, 2, 4-Oxadia ole Derivatives

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ABSTRACT

Submitted: 23/7/2010 *Revised: 13/11/2010* *Accepted: 20/3/2011*

3, 5- diaryl- 1, 2, 4-oxadiazole derivatives are synthesized by condensation reaction of amidoximes with aromatic acid chloride. Amidoximes are obtained from aromatic nitriles and acid chlorides from corresponding aromatic monocarboxylic acid derivatives. There are broad possibilities of preparing several such compounds in this series by using different aromatic nitriles as well as different aromatic monocarboxylic acid derivatives.

The synthesized compounds (**a-e**) were characterized by IR, ^{1}H NMR studies and evaluated for their anticancer activity using mice specific Ehrlich Ascites Carcinoma cell (EAC cells) in Swiss albino mice model. Synthesized compounds (**a-e**) (dose 25 mg/ kg body weight) show significant reduction of tumor cell count as well as tumor weight, where as life span of the treated mice also increased. The compound (**c**) showed significant inhibition of cancer cell growth as compared to others. The standard drug used for the study is 5-Fluorouracil (20mg/kg body weight).

Keywords- Amidoxime, 1, 2, 4-Oxadiazole, Acid chloride, EAC cells

INTRODUCTION

Drug discovery and development is a multidisciplinary, creative, innovative and highly regulated process. Finding a successful lead has been a great challenge in pharmaceutical research and also it is utmost important to take into account that is the formulation development. Lead candidates are those which show promising characteristics so that lead has to be developed into new drugs. Finding of 'lead' is not only the goal today but also leads has to be optimized. Optimization of 'lead' refers to the process used to manipulate the compound to improve its chemical stability, potency, biological or therapeutic effectiveness.

Nitrogen containing heterocyclic ring systems have a huge potential to become successful drug candidate that has been already prove in recent past. Here 1, 2, 4- oxadiazole is a five membered heterocyclic moiety having three hetero atoms out of these two nitrogen atoms and one ox ygen atom.1, 2, 4-oxadiazole is asymmetric system of oxadiazole series that is why position isomer is possible in case of non identical 3,5 disubstituted 1, 2, 4–oxadiazole. Number of reports have highlighted their synthetic chemistry and use, and most of the investigated report gives promising as well as satisfactory result which is statistically significant. A series of activities already shown by this candidate those are- novel ligands for the imaging of β-amyloid plaques in AD (Alzeimer's disease)[1], antiparasitic[2], anthelmintic[3], diuretic[4,5], anti-inflammatory[6,7], a novel apoptosis inducer with tumor-selective properties[8,9], antimicrobial[10], hypoglycemic[11], skeletal muscle relaxant[12], hypertensive activity[13], Anti-HIV[14].

There are broad possibilities for the synthesis of new compounds offering highly effective agents bio-isosteric with ester and amide groups, which is related to the high stability of the 1,2,4-oxadiazole ring with respect to metabolism in the entire physiological pH and temperature range. Being bioisosteric with ester and amide groups and producing a significant increase in the biological activity of 1, 2, 4-oxadiazoles have been extensively studied with a view to their use in the pharmaceutical chemistry[15-18]. Synthesis of some 3, 5-disubstituted 1, 2, 4-oxadiazole and their derivatives using the synthetic procedure based on the ring closure reactions of appropriate amidoxime with substituted acid chloride, Unless the substituent are very bulky the oxadiazole are volatile in nature rather unstable, its 3, 5-diaryl-substituted derivatives are more stable. The 3, 5-disubstituted 1, 2, 4-oxadiazoles are white crystalline compound which are insoluble in water but soluble in chloroform and in other organic solvent.

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MATERIALS AND METHOD

All the reagents and chemicals were procured from SD Fine Chemical New Delhi. They were of an analytical grade. Melting points were determined in open glass capillary tube using Veego melting point apparatus and were uncorrected.

3, 5-disubstituted-1, 2, 4-oxadiazole derivatives were prepared by using three step synthetic procedure.

STEP I- Synthesis of Chlorobenzamidoxime:

In a clean, dried 250 ml round bottomed flask a stirred solution of commercial 2-chlorobenzonitrile (0.048 mol, 6.56 gm) or 4-chlorobenzonitrile (0.048 mol, 6.56 gm) in ethanol (40ml), Hydroxylamine hydrochloride (0.12 mole, 8.36 gm) and Triethanolamine (32 ml) was added. The resultant solution was stirred and then refluxed on a heating mantle for 12 hours. The flask was then cooled to room temperature. The ethanol was evaporated under vacuum by rotary vacuum evaporator and then the mixture was poured in cool water (500 ml) and left to crystallize for overnight in refrigerator, the solid crystal obtained was separated by vacuum filtration and dried at 55^0C.

2-chlorobenzamidoxime Colorless crystals, M.P:- 116-118ºC lit[2].M.P: 119-121ºC, IR (KBr, ν_{max} cm^{-1}): 3488 (OH str), 3401(NH_2 str), 3152 (Ar C-H bend), 1646 (C=N bend) , 1379 ,1099 (C-N bend), 1560 (Ar C=C bend) , 759 (C-CI str).

4-chlorobenzamidoxime Colorless crystals. M. P: - 130-132 ^{0}C; lit[19]128-130^{o}C

STEP II- Synthesis of aromatic acid chloride:

In a clean and dry 100 ml round bottom flask chlorosubstituted-benzoic acid derivative (0.025 mole) was taken then thionyl chloride (0.05 mole) was added then the mixture was heated to reflux in a water bath for 2 hours. After that the extra thionyl chloride was removed by heating in water bath such a way that water vapor should not come in direct contact with acid chloride as it prone to hydrolysis. Then the acid chloride so formed is free from thionyl chloride and keep it in air tight condition.

STEP III- Synthesis of 3, 5-diaryl -1, 2, 4-oxadiazole:

In a clean, dried 100 ml round bottom flask, pyridine (10 ml) and 2-chlorobenzamidoxime/4-chlorobenzamidoxime (6 mmol) was taken, to this solution prepared chloro substituted benzoyl chloride was added (0.02 mol). Then the mixture was stirred in a magnetic stirrer at room temperature for 4 hours. Pyridine was removed under vacuum. Solid obtained was purified by column chromatography on silica gel with mixture of hexane / ethyl acetate as solvent.

3-(4-Chlorophenyl)-5-(phenyl)-1, 2, 4-oxadiazole (a)

Yield: 45%, Colorless crystals. M.P: 110-112^{o}C; literature.108-110 ^{0}C (*Silvio Luiz Machado et al*., 2005). Characterized by comparison of its ^{1}H NMR data with those of literature (*Silvio Luiz Machado et al*., 2005), IR (KBr, ν_{max} cm^{-1}): 3097 (Ar-CH bend), 747 (Ar-CI str) 1704 (C=N bend) 1241 (C-O str), 1610 (C=C bend). ^{1}H NMR ($CDCl_3$, 300MHz, δ ppm): 8.10 (d, 2H, J =8.7 Hz, H-2' and H-6'), 7.46 (d, 2H, J = 8.7 Hz, H-3' and H-5'), 8.20 (dd, 1H, J = 8.2 and 1.8 Hz, H-2" and H-6"), 7.56 –7.66 (m, 3H, H-3", H-4" and H-5").

3, 5 bis (4-Chlorophenyl)-1, 2, 4-oxadiazole (b)

Yield: 50%, Colorless crystals M.P: - 140-142^{0}C Solubility: - Soluble in chloroform, R_f Value - 0.64 (Solvent-Pet ether: Ethyl acetate 2:1) IR (KBr, ν_{max} cm^{-1}): 3097 (Ar-CH), 747 (Ar-CI str) 1696 (C=N bend) 1241 (C-O bend), 1610 (C=C bend). ^{1}H NMR ($CDCl_3$, 300MHz, δ ppm) 8.12 (d, 2H, J =8.7 Hz, H-2' and H-6'), 7.48 (d, 2H, J = 8.7 Hz, H-3' and H-5'), 8.15 (d, 2H, J = 8.7 Hz, H-2" and H- 6"), 7.53 (d, 2H, J = 8.7 Hz, H-3" and H- 5").

3- (2-Chlorophenyl)-5-phenyl-1, 2, 4-oxadiazole (c)

Yield: 40%, Colorless crystals M.P: - 163-166 ºC, Solubility- Soluble in chloroform, R_f Value- 0.67 (Solvent-Pet ether: Ethyl acetate 2:1). IR (KBr, ν_{max} cm^{-1}): 3064 (Ar-CH bend), 749 (Ar-CI str), 1685 (C=N bend), 1234 (C-O bend), 1610 (C=C bend). ^{1}H NMR ($CDCl_3$, 300MHz, δ ppm) 7.62 (t, 1H, H-5') 7.99 (d, 1H, J = 8.7 Hz, H-6'), 7.39-7.48 (m, 1H, H-4') 7.31 (d, 1H, J = 8.7 Hz, H- 3'), 8.20 (dd, 2H, J = 8.2 and 1.8 Hz, H-2" and H-6"), 7.56-7.66 (m, 3H, H-3", H-4" and H-5").

3-(2-chlorophenyl) 5-(4-chlorophenyl)-1, 2, 4-oxadiazole (d)

Yield: 40%, Colorless crystals M.P: - 148-150 ºC, Solubility- Soluble in chloroform, R_f Value- 0.74 (Solvent-Pet ether: Ethyl acetate= 2:1) IR (KBr, ν_{max} cm^{-1}): 3097 (Ar-CH), 747 (Ar-CI str) 1704 (C=N bend) 1241 (C-O bend), 1610 (C=C bend), ^{1}H NMR ($CDCl_3$, 300MHz,δ ppm) 7.62 (s 1H, H-5'), 7.99 (d, 1H, J =8.7 Hz, H- 6'), 7.39 (t, 1H, H-4') 7.31 (d, 1H, H-3'), 8.15 (d, 2H, J = 8.7 Hz, H- 2" and H- 6"), 7.53 (d, 2H, J=8.7 Hz, H-3" and H-5").

3-(2-Chlorophenyl) 5-(2, 4-dichlorophenyl) -1, 2, 4-oxadiazole (e)

Yield: 50%, Colorless crystals, M.P:-128-130^{0}C Solubility: - Soluble in chloroform, R_f Value- 0.80 (Solvent-Pet ether: Ethyl acetate 2:1) IR (KBr, ν_{max} cm^{-1}): 3064 (Ar-CH bend), 747 (Ar-CI str), 1700 (C=N bend), 1238 (C-O bend), 1610 (C=C bend). ^{1}H NMR ($CDCl_3$, 300MHz,δ ppm) 7.99 (d, 1H J=8.7 Hz, H-6'), 7.96 (d, 1H, J= 8.2 Hz, H-6"), 7.61 (d, 1H, J= 8.7

Hz, H-5"), 7.40 (t, 1H, H-4'), 7.30 (d, 1H, J= 8.7Hz, H -3'), 7.51(s, 1H, H-3"), 7.56 (d, 1H, J= 8.7 Hz, H- 5')

RESULT AND DISCUSSION

The synthesized compounds are characterized by their Physical parameter like solubility, R_f value, melting point determination and by their spectral analysis through ^{1}HNMR, IR spectroscopy. Anticancer activity of the synthesized compound has been done by measuring percentage tumor weight inhibition, percentage inhibition of tumor cell count and percentage increase of life span (in days) of the experimental animal. Tumor cells used for anticancer activity are EAC (Ehrlich Ascites Carcinoma) cells originated from human breast carcinoma by spontaneous passaging. It is an undifferentiated tumor, which has lost its epithelial character. Result for anticancer activity as shown in Table 1, were reported as the percentage of tumor weight inhibition (%TWI) and percentage of tumor cell count inhibition (%TCI) of the treated ascitic cells in comparison to untreated control ascitic cells. Compound d and e having anticancer potential shown in Table 1, where the growth percent inhibition of EAC cells is 30.20 to 34.80 % and result for survival time determination was shown in Table 2, the percentage increase of life span of test animals of treated group in comparison to EAC control group is from 33.33 to 40.00 %. The result of the present study in respect of synthesis, %TCI and percentage increase of life span are quite promising as well as significant. The observed values of the current research work shows an effective and interesting data regarding chemistry and anticancer potentiality of 3, 5-diaryl -1, 2, 4-oxadiazole derivatives.

EVALUATION OF ANTICANCER ACTIVITY

The synthesized compounds were subjected for assessment of anticancer activity. The test compounds (c and d) 25 mg/kg body weight and standard drug 5-Flurouracil 20mg/kg body weight were used.

Male and female mixed Swiss albino mice of about 8 weeks of age with an average body weight of 18-20 gm were used for the experiment. The animals were acclimatized to laboratory condition with 12-h/12-h cycles of light and dark at 25 ^{0}C for 10 days before commencement of the experiment.

Table 2: Survival Time Determination of the test animal

Group	Survival time (days) %	Increase of life span
II	18	-
III	30	40.00
IV	27	33.33

They were fed standard pellet diet and were given fresh water *ad libitum*.

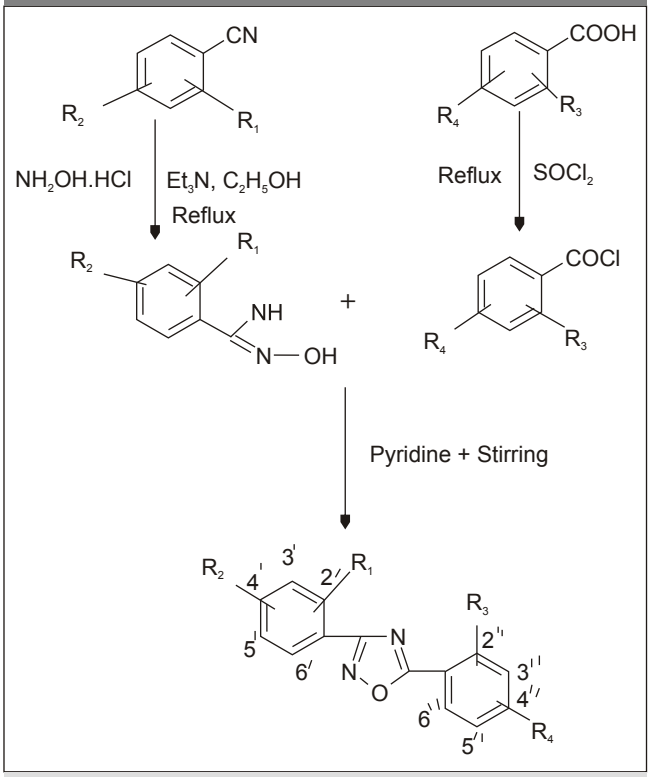
EAC cells were maintained *in vivo* in Swiss Albino mice by passaging every 10 days. EAC cells of 9 days old was used for screening of the synthesized compounds.

The test mice were divided into 5 groups (n = 12). EAC cells are collected from the donor mice and are suspended in sterile isotonic solution (0.9% w/v NaCl) and diluted accordingly by adding sterile normal saline so that the numbers of tumor cells per ml of this suspension are (2×10^6) those are counted under microscope with the help of haemocytometer. All the groups (except group-I) were treated with EAC cells (0.2 mL cell suspension means 2×10^6 cells/mice) intraperitoneally. 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). Phosphate buffer (*p*H-7.2) 5ml/kg, body weight per day was administered in group II (EAC control). The synthesized compounds (c, d -25 mg/kg, body weight/day) and the standard drug 5-Flurouracil (20mg/kg, body weight/day) were administered in groups (III-IV) and (V) respectively for 7 days intraperitonialy 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 weights of all the animals are recorded before they are sacrificed. The peritoneal cavity was dissected 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 3 mice in each group was calculated. The rest two was kept for determination of survival time. The fluid is sucked by adsorbent cotton. The

Table 1: Anticancer activity of synthesi ed compounds on Swiss albino mice

Group	Dose of drug mg/kg	Average tumor weight (g)	% TWI	Average cell Count(Number	Average cell Count/ml fluid	% TCI
I	-	-	-	-	-	-
II	-	3.50	0.00	43	2.68×10^8	0.00
III	25	1.90	47.10	28	1.75×10^8	34.80
IV	25	2.20	37.10	30	1.87×10^8	30.20
V	5	0.00	100.00	0	-	100.00

Scheme I



Where,
Compound a, $R^1 = R^3 = R^4 = H$, $R^2 = Cl$
Compound b, $R^1 = R^3 = H$, $R^2 = R^4 = Cl$
Compound c, $R^1 = Cl$, $R^2 = R^3 = R^4 = H$
Compound d, $R^1 = R^4 = Cl$, $R^2 = R^3 = H$
Compound e, $R^1 = R^3 = R^4 = Cl$, $R^2 = H$

weight of 6 mice after sacrifice was recorded and remaining animals kept for the observation of life span of the hosts.

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:

Percentage inhibition of Ascitic cells (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.

Survival time in days and percentage increase of life span of test animals in comparison to control animal is obtained by the following expression

% Increase of life span = (1-C/T) ×100

Where C is the Survival time in days of control animals, T is the Survival time in days of test animal.

The groups and the design of the experiment were as follows[20]:

Group-I: Normal saline (0.9 % NaCl, w/v; 5 ml / kg, of body weight).

Group-II: EAC (2×10^6 cells /mice) + Phosphate buffer (*p*H-7.2) (5 ml/kg, of body weight).

Group-III: EAC (2×10^6 cells /mice) + compound c (25 mg/kg, of body weight).

Group-IV: EAC (2×10^6 cells /mice) + compound d (25 mg/kg, of body weight).

Group-V: EAC (2×10^6cells/mice) + Standard drug 5-Flurouracil (20mg/kg,of body weight).

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

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

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.

CONCLUSION

3, 5-disubstituted-1, 2, 4-oxadiazole derivatives were synthesized and evaluated for their anticancer activity. The anticancer potential of the synthesized compounds were evaluated by measuring their ability to inhibit cancer cell growth in ascetic fluid of Swiss albino mice. The synthesized compounds significantly reduced the tumor weight and tumor cell count as compared to that of the EAC control group. The evidence presented herein indicates the anticancer activity of the 1, 2, 4-oxadiazole derivatives and therefore offers a unique opportunity for utilizing this nucleus as a leads in the research for novel therapeutic agents for anticancer drugs. The probable mechanism of reduction of tumor weight and tumor cell count is by inducing the apoptosis using a chemical genetics approach.

Thus, from the present study, it can be concluded that the synthesized Oxadiazole derivatives are biologically active and they can be a successful candidate having anticancer activity that will create an interest among future researchers to choose this nucleus for further development.

ACKNOWLEDGEMENTS

The authors are grateful to Jadavpur University for rendering the valuable support and providing necessary facilities to

carry out this research work, and also grateful to the University Grant Commission for providing the fellowship.

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