Available online on 15.03.2024 at ijmspr.com

International Journal of Medical Sciences and Pharma Research

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Pharmacological Evaluation of Novel 1,3,4-Oxadiazole Derivatives

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Article Info:

Article History:

Received 24 December 2023
Reviewed 02 February 2024
Accepted 27 February 2024
Published 15 March 2024

Abstract

A series of 2, 5- disubstituted- 1, 3, 4-oxadiazole derivatives (Ox1-Ox10) are synthesized by the ring condensation reaction followed by rearrangement of salicylic acid and phenyl acetic acid with various aromatic acids in presence of phosphorous oxychloride as cyclizing agent. Structure of the new derivatives are confirmed by spectral analysis. Those derivatives having high Pa value in PASS software are subjected to antibacterial, anticancer and antidiabetic studies which yield promising reports. This study helps and stimulate the researcher to exploit the oxadiazole nuclei for the development of more active less harmful drugs.

Keywords: 1,3,4-oxadiazole, antibacterial activity, anticancer activity, antidiabetic activity

Cite this article as:

Ramesh A, Jat RK, Arunkumar R, Pharmacological Evaluation of Novel 1,3,4-Oxadiazole Derivatives, International Journal of Medical Sciences & Pharma Research, 2024; 10(1):26-34 *DOI: http://dx.doi.org/10.22270/ijmspr.v10i1.88*

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1. INTRODUCTION

Oxadiazoles ($C_2H_2N_2O$) are heterocyclic compounds with a five membered ring as the basic nucleus in which three carbon atoms are replaced by heteroatoms. These compounds come under azole family in which 2 nitrogen atoms and one oxygen atom are present in the compound instead of carbon. By their multiple action in both medicinal as well as material chemistry, Oxadiazoles attracted the researcher's attention[5].

1, 3, 4-oxadiazoles are thermally stable neutral aromatic nuclei that are found to possess a broad spectrum of biological activities. They are associated with wide variety of pharmacological activities like antibacterial, antifungal, tuberculostatic, anticonvulsant, analgesic, anti-inflammatory, diuretic, antiemetic and insecticidal properties. Recently they were found to possess anti-inflammatory, antitumor and antiviral activities[15]. And hence, an intensified investigations are going on in the field of oxadiazoles due to tremendous outbreak in lifestyle diseases.

In the present study novel derivatives of 1,3,4-oxadiazole are synthesized and are subjected to pharmacological evaluation. Most of the derivatives showed promising activity.

2. MATERIALS AND METHODS

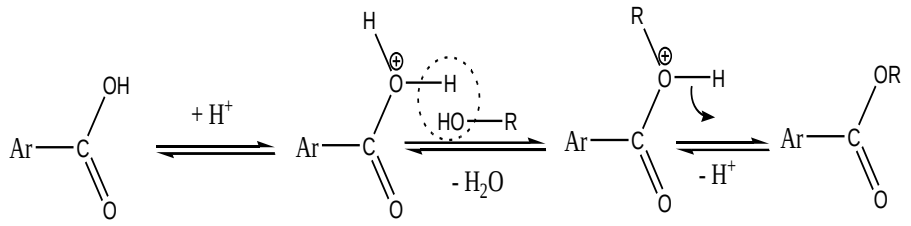
2.1 Chemicals

All the chemicals and reagents used in the present work were of analytical grade and obtained from Nice Chemicals, Mumbai and Chemco, Mumbai.

2.2 Synthesis of compounds

30 analogues, that are selected from series designed using ChemDraw Ultra and Chem 3D Ultra, are subjected to molecular properties prediction using Molinspiration software. Those derivatives satisfying the Lipinski rule of five[10] were then subjected to PASS software to predict the general biological potential of the novel proposed compounds. Derivatives with maximum PASS[4] value for the desired activities are then synthesized in the laboratory using the selected scheme (given Fig 1) based on literature survey[20].

Step 1:



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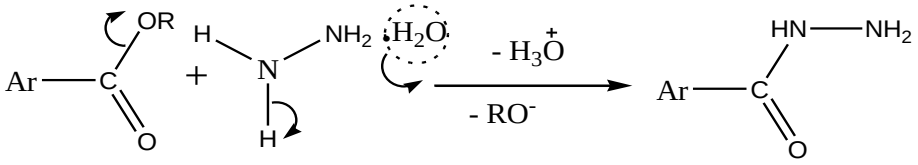

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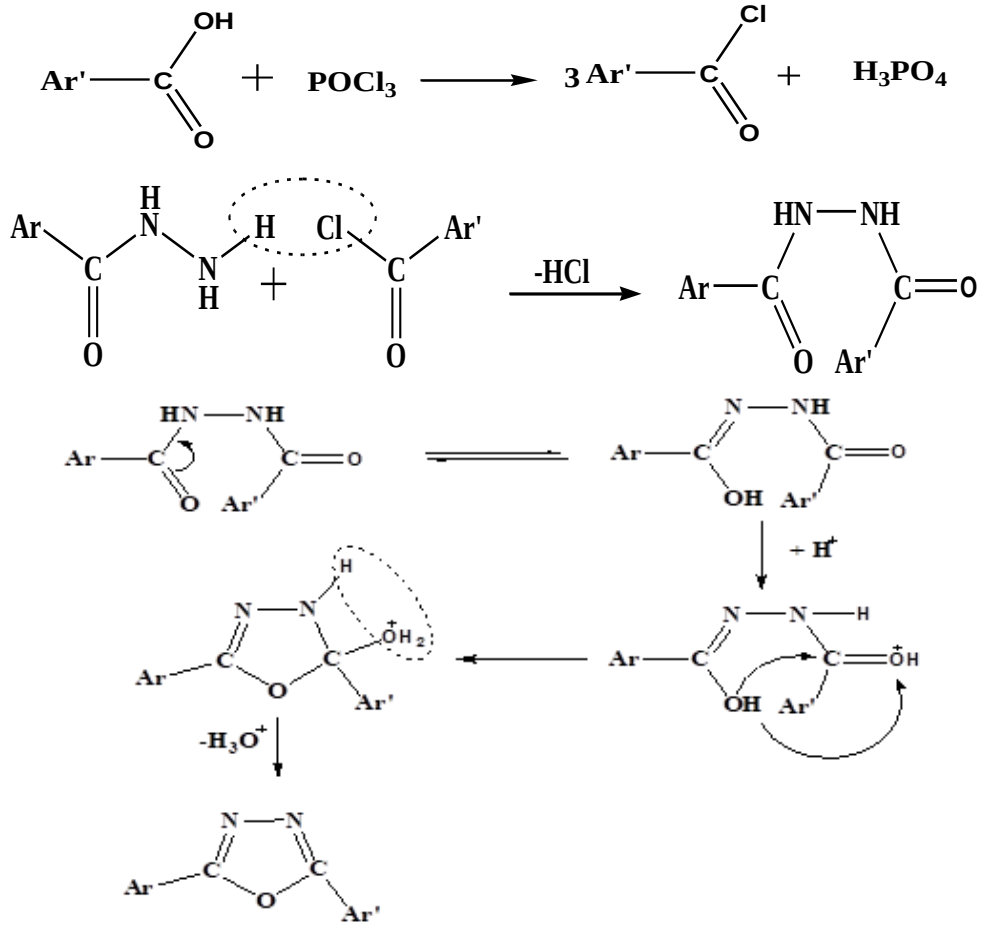

Figure 1: Mechanism of synthesis

2.2.1 Synthesis of methyl salicylate[13]

A mixture of salicylic acid (0.47mol; 0.65g) and methanol (2ml) was taken in a 5ml round bottom flask. The flask was stirred well until the solid dissolves. Drop by drop addition of 0.75 ml of concentrated H2SO4 was done with constant agitation. The flask was then connected to a water condenser capped with a drying tube that has been loosely packed with CaCl2. Reflux the content for 75 min (80°C). The solution was cooled to room temperature. Then extracted the solution with CHCl3 (1 ml x 3). The organic layer was collected. The combined solution was treated with 1 ml of aqueous 5% NaHCO3 solution. The solution was mixed well and was allowed to stand for separation the organic layer was collected and transferred to a dry vial. It was then allowed to evaporate over anhydrous Na2SO4. The product was purified.

2.2.2 Synthesis of salicyl hydrazide[12]

A mixture of methyl salicylate (0.1 mol; 15.2 ml) and hydrazine hydrate (0.2 mol; 10 ml) were boiled in 50 ml of 95% ethanol by connecting the flask to a condenser which promote flow back and reheating for 7-8 hrs. The water content in the succedent mixture was reduced, cooled and poured to crushed ice. The remaining mass thus separate out was filtered, dried and purified by recrystallization, mp.142-1440C.

2.2.3 Synthesis of 0x1-0x4, 0x9, 0x10

A mixture of salicyl hydrazide (0.1mol; 1.52g) and carboxylic acid (0.1mol) was liquified in phosphorus oxy chloride (5ml) and boiled under reflux for 35 min. The liquid mixture was gently poured over broken ice and then neutralised with 5% NaHCO3. The solution was kept overnight. The separated solid mass was filtered, dried and purified by recrystallization using ethanol.

2.2.4 Synthesis of ethyl phenyl acetate[21]

A mixture of phenyl acetic acid (0.1 mol; 13.6 g), ethanol (0.4 mol; 18.4 ml) and concentrated H2SO4 (0.05 mol; 4.9 ml) were taken in a 50 ml round bottom flask and was refluxed for 3 hrs. The solution was cooled to room temperature, neutralised with 10% Na2CO3. The solution was extracted with diethyl ether (10 ml x 4) in a separating funnel. The upper ethereal layer was collected, purified by distillation on rotary evaporator.

2.2.5 Synthesis of phenyl acetic acid hydrazide

A mixture of ethyl phenyl acetate (15 ml) and methanol (10 ml) was taken in a 50ml round bottom flask. The reaction vessel was cooled to 0-5 0C. To this solution, hydrazine hydrate (15 ml) was added drop wise with occasional stirring. Then the solution was stirred for 60 min. After the evaporation of methanol, crude precipitate was collected and washed with n-hexane, mp: 113-1160C.

2.2.6 Synthesis of 0x5-0x8

A mixture of phenyl acetic acid hydrazide (0.1 mol; 1.50 g) and different carboxylic acid (0.1 mol) was dissolved in phosphorus oxy chloride (5 ml). The mixture was refluxed for 35 min. The reaction mixture was slowly poured over crushed ice and then neutralized with 5% NaHCO3. The solution was kept overnight. The separated solid mass was filtered, dried and purified by recrystallization with ethanol.

The synthesized derivatives are 2-(5-(4-hydroxyphenyl)-1, 3, 4-oxaddiazol-2-yl) phenol (0x1), 2-(5-styryl-1, 3, 4-oxadiazol-2-yl) phenol (0x2), 2-(5-(4-chlorophenyl)-1, 3, 4-oxadiazol-2-yl) phenol (0x3), 2-(5-(3, 5-dinitrophenyl)-1, 3, 4-oxadiazol-2-yl) phenol (0x4), 2-benzyl-5-(3, 5-dinitrophenyl)-1, 3, 4-oxadiazol(0x5), 2-benzyl-5-styryl-1, 3, 4-oxadiazol (0x6), 1-(5-benzyl-1, 3, 4-oxadiazol-2-yl)-2-phenylethanamine (0x7), 2-benzyl-5-(4-chlorophenyl)-1, 3, 4-oxadiazole (0x8), [2-(5- ((2, 4- dichloro phenoxy)methyl)- 1, 3, 4- oxadiazol-2-yl) phenol (0x9), [2- (5- (2-hydroxy-(phenyl))- [1, 3, 4]- oxadiazol- 2-yl) phenyl acetate(0x10). Fig 2 shows the structures.

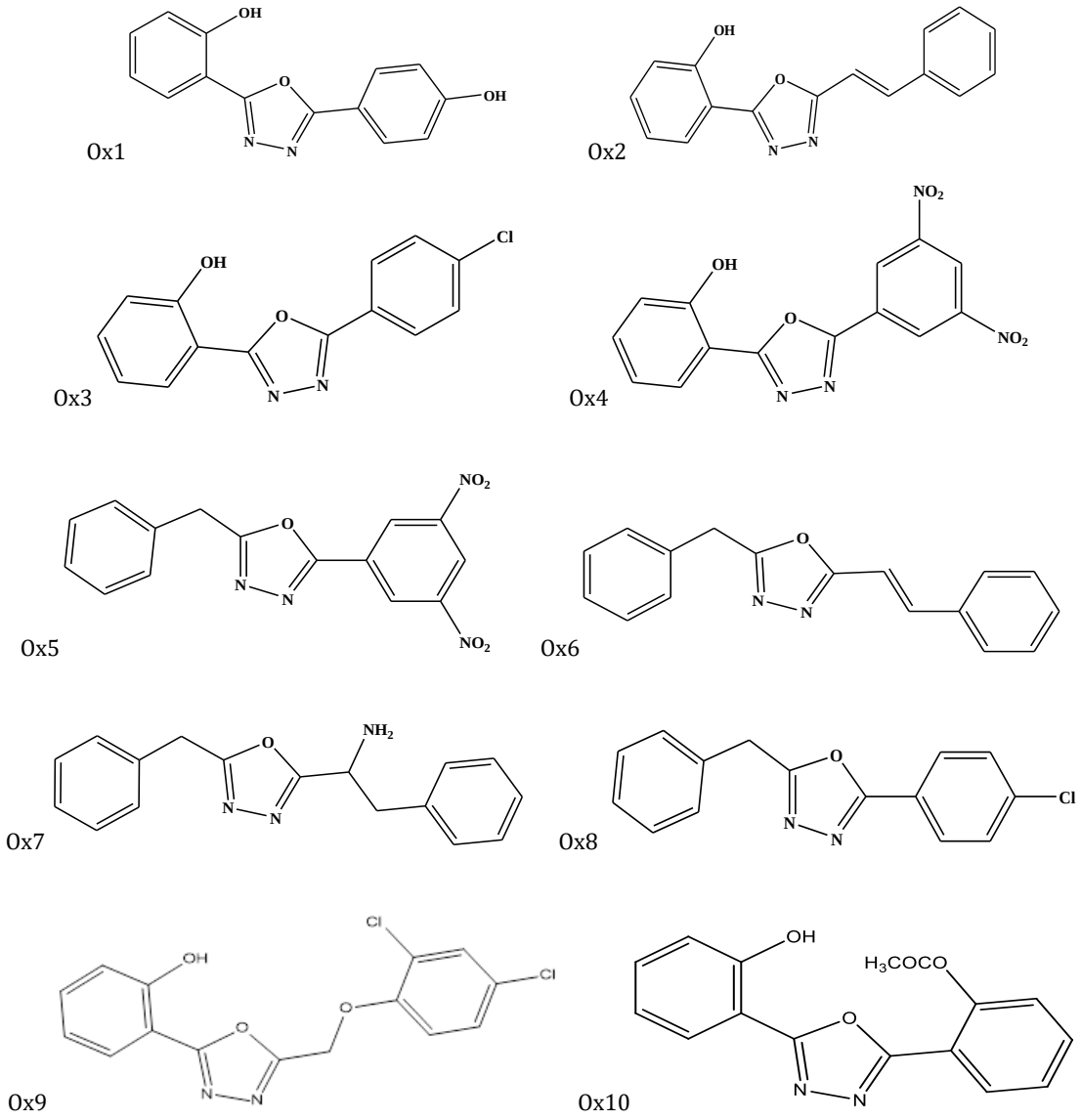

Figure 2: Structures of new derivatives of 1,3,4-oxadiazoles

The structure of synthesized derivatives are confirmed by their spectral analysis. Finally, the derivatives were subjected to Docking studies using ArgusLab[7] and Schrodinger software and those derivatives having high docking scores are evaluated for their desired biological activity in laboratory.

2.3 Pharmacological evaluation

2.3.1 Antibacterial Screening[6]

The following micro-organisms were used to study the anti-bacterial activity:

Staphylococcus aureus - Gram positive organism.

Escherichia coli - Gram negative organism.

Nutrient agar broth was melted in an autoclave. The broth was then poured to petri-dishes (30 ml each) and allowed it to set. The culture media thus prepared was then inoculated with 0.5 ml of 24 hrs old culture. In each petri-dish, four wells of 6mm diameter were made at equal distance by digging the surface with an aseptic cork-borer and the separated agar was discarded. The sample solutions of 0.1ml volume having specific concentration were added to the wells using sterile micropipette. Separate petri-dishes were prepared for both standard and the control (DMF) and were noted. Amoxicillin (50 µg/0.1 ml) were used as standard drug. The sample was allowed to spread in the agar medium by placing the petri dish in refrigerator for a time period of half an hour and then incubated at 37± 0.5^{0}C for one day. The appearance of clear zone where microbial growth is inhibited is a sign of antibacterial activity. The dimension of clear zone was individually measured and the average value was calculated for each test compound. The results obtained are collated with that of reference drug amoxicillin.

Tube dilution method[14] is used to find minimum inhibitory concentration.

Procedure for Standardization of inoculum:100ml of inoculum was prepared by incorporating 1ml stock culture of bacterium in nutrient broth. It was then maintained at 37^{o}C for one day and allowed to incubate. Further growth was suppressed by incubating at 4^{o}C. 1ml of this culture was introduced to 9ml sterile water taken in another test tube. This dilution was carried to 10-fold serially from 10^{-1} to 10^{-10} dilution. 10^{-7} to 10^{-9} dilutions were taken and 0.2 ml of the diluted culture were inoculated on solid nutrient agar plate. The colony forming unit (CFU) was counted after incubation at 37^{o}C for 24 hrs. From this suitable inoculum load of cells/ml of selected broth was prepared. This selected broth is called working stock culture.

Procedure for Antibacterial activity: The new compounds were subjected to antibacterial studies using Staphylococcus aureus and E.coli. The standard solution of synthesized compounds was prepared in 1000 µg/ml concentration. 8 assay tubes were taken for each bacterium and 1ml working stock was added in each tube except in first tube. To this first tube, 1.8ml stock culture was added. It was then mixed thoroughly with 0.2ml of test solution and transfer 1 ml of this solution to the next tube. Repeating the step to get concentration of test solutions in the following order.100 microgram/ml, 50µmicrogram/ml, 25microgram/ml, 12.5 microgram/ml, 6.25 microgram/ml, 3.125 microgram/ml and 1.5 microgram /ml. The standard drug Amoxicillin was prepared in the same manner to produce different dilutions ranging from 100µg -1.5µg/ml. The 1 ml working stock was taken as the positive control. 1 ml of solvent DMSO was taken as the negative control. The assay tubes were incubated at 37^{o}C for 24hrs and observation were done at end of 24hrs. With the aid of test sample containing lowest concentration that blocked the turbidity formation, inhibition can be determined after observing the amount of growth in the tube which cause cloudiness. Lowest concentration of antibiotic that caused complete inhibition of bacterial growth was taken as MIC of the compound against that microorganism. The cloudiness was examined after incubating the tubes for one day.

2.3.2 Anticancer activity[16]

In this method mice with tumour cells (Dalton's lymphoma ascites cells) were selected and these cells from the peritoneal cavity was collected. Then using phosphate-buffered-saline or normal saline solution, these cells were thoroughly washed. The ability of these cells to survive was done by method using trypan-blue method. A suspension of alive cells with 1X105 cells in 0,1ml proportion was introduced to different test tubes each of which contain test compound with various concentration. By adding enough quantity of PBS, 1ml volume was attained. A tube was kept as a control in which no other additives except cell suspension was present. These mixtures were then allowed to incubate at a temperature of 37^{o}C for a time period of three hours. To this suspension 0.1ml trypan blue having 1% concentration was mixed and it was then placed on a haemocytometer immediately after standing for two to three minutes. When observed blue coloured cells give the number of dead cells as live cells fails to take up the colour. The result was calculated by counting separately the total number of coloured and colourless cells.

2.3.3 Antidiabetic activity[22]

Test Procedure: The activity was determined using a modified assay of that described in the Worthington Enzyme Manual[23]. 500ml of α-amylase standard solution was prepared by dissolving 0.5mg/ml of the same in sodium phosphate buffer with molarity 0.02 and a pH of 6.9 was adjusted by the addition of 0.006 M NaCl and different concentrations (in µg) of extract as inhibitor were incubated initially around 10 minutes by maintaining a temperature of 37oC. Then each tube was added with another 500µL of solution in which 1% starch dissolved in 0.02M sodium phosphate buffer having 6.9 pH value. It was again incubated for 5 more minutes at room temperature then 1.0 ml of indicator DNSA reagent was added to stop the reaction. After the addition, the tubes were placed in boiling water bar and allow to incubate. When the time reached 5 minutes, it was cooled until room temperature is obtained. Sufficient amount of distilled water was added to make the final volume to 10ml. with the aid of spectrophotometer operating in the UV-Visible range, the optical density was recorded at 540 nanometre wavelengths. The absorbance readings were compared with the controls and blank that contained buffer instead of sample extract.

3. RESULT AND DISCUSSION

Among the designed derivatives subjected to various software for their properties and activity prediction, ten derivatives are chemically synthesized in the laboratory. Of which, six derivatives (Ox1, Ox2, Ox3, Ox4, Ox9, Ox10) were synthesized by the reaction between salicylic acid and various aromatic acids and other four derivatives (Ox5, Ox6, Ox7, Ox8) were synthesized by using phenyl acetic acid as starting material. The synthesized derivatives were confirmed by IR, NMR and MASS spectral analysis[17]. Table 1, 2 and 3 shows the readings

Table 1: Characterization by IR

Compounds	IR peaks (cm-1)
Ox1	3360 (O-H), 3275 (phenolic OH), 1361 (OH bend), 1242 (CCO), 1446 (C double bond N), 1269 (CN), 1570 (NN), 1215 (COC), 3055 (aromatic CH)
Ox2	3356 (OH), 3178 (phenolic OH), 1332 (OH bend), 1246 (CCO), 1203 (COC), 3059 (aromatic CH), 1448 (C double bond N), 1332 (CN), 1560 (NN), 1670 (C double bond C)
Ox3	3213 (phenolic OH), 1400 (OH bend), 1233 (CCO), 301(aromatic CH), 1255(COC), 1481 (C double bond N), 1255 (CN), 1591 (NN), 1091 (C6H5Cl), 637 (CCl)
Ox4	3431 (OH), 3527 (phenolic OH), 1340 (OH bend), 1226 (CCO), 3057 (aromatic CH), 1205 (COC), 1448 (C double bond N), 1286 (CN), 1570 (NN), 1313; 1589 (NO2)
Ox5	2869 (CH2), 3145 (aromatic CH), 1281 (COC), 1531 (C double bond N), 1338 (CN), 1562 (NN), 1632; 1429 (NO2)
Ox6	2521 (CH2), 3025 (aromatic CH), 1218 (COC), 1448 (C double bond N), 1282 (CN), 1579 (NN), 1587; 1494 (C double bond C)
Ox7	2975 (CH2), 3195 (aromatic CH), 1193 (COC), 1433 (C double bond N), 1245 (CN), 1594 (NN), 3030 (NH2)
Ox8	2924 (CH2), 3061 (aromatic CH), 1282 (COC), 1483 (C double bond N), 1323 (CN), 1575 (N-N), 1091 (chlorobenzene), 821 (CCl)
Ox9	3320 (phenolic OH), 1350 (OH bend), 1243 (CCO), 2945 (CH2), 3090 (aromatic CH), 1430 (aromatic C double bond C), 1290 (COC), 1490 (C double bond N), 1250 (CN), 1560 (NN), 1070 (dichlorobenzene), 752 (CCl)
Ox10	3229 (phenolic OH), 1323 (OH bend), 1207 (C-C-O), 2849 (aromatic CH), 1441 (aromatic C double bond C), 1290 (COC), 1480 (C double bond N), 1233 (CN), 1555 (NN), 1603 (O(C double bond O)CH3), 1651 (acetyl C double bond O)

Table 2: Characterization by ^{1}HNMR

COMPOUND	1HNMR (ppm)
Ox4	7.037-7.944- multiplet, aromatic proton (7H) 10.864-singlet, OH (1H) TOTAL 8 PROTONS
Ox8	7.323 - 7.954 – multiplet, aromatic proton (9H) 2.504 – singlet, CH2 (2H) TOTAL 11 PROTONS
Ox9	7.835 - 7.022 – multiplet, aromatic proton (7H) 6.902 – singlet, OH (1H) 4.781 – singlet, CH2 (2H) TOTAL 10 PROTONS
Ox10	6.959 - 7.939 – multiplet, aromatic proton (8H) 7.261 – singlet, CH3 (3H) 10.405 – singlet, OH (1H) TOTAL 12 PROTONS

Table 3: Mass spectral data

Compound	Peak value	Molecular formula
Ox4	328.4718	(C14H8N4O6)
Ox8	269.6911	(C15H11N2OCl)
Ox9	337.1573	(C15H10N2O3Cl2)
Ox10	296.2772	(C16H12N2O4)

3.1 Anti-bacterial effect of derivatives

Fig 3 and Fig 4,5 shows zone of inhibition and MIC respectively.

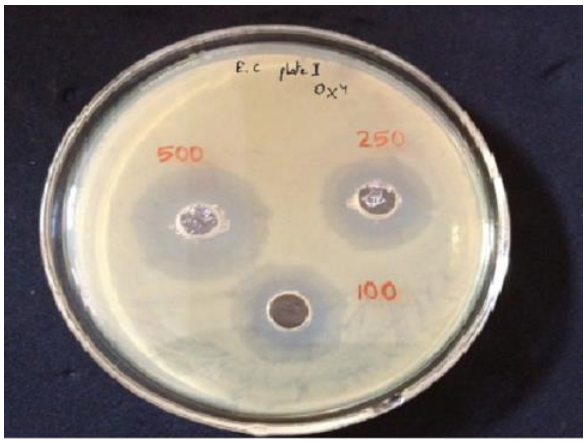

Figure 3: Photo of Antibacterial activity of Ox4 on E. coli

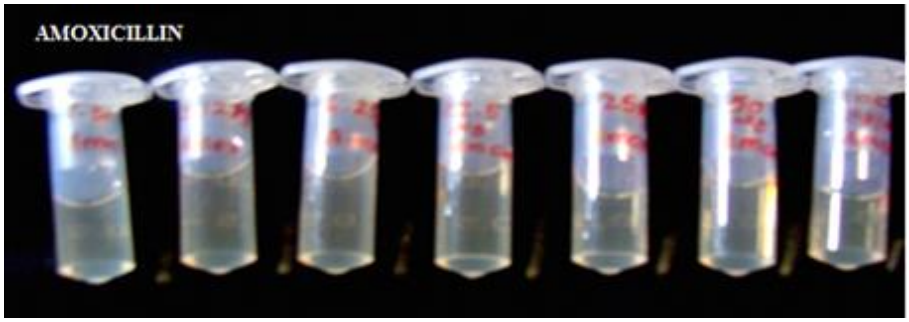

Figure 4: Photos of MIC values of Amoxicillin

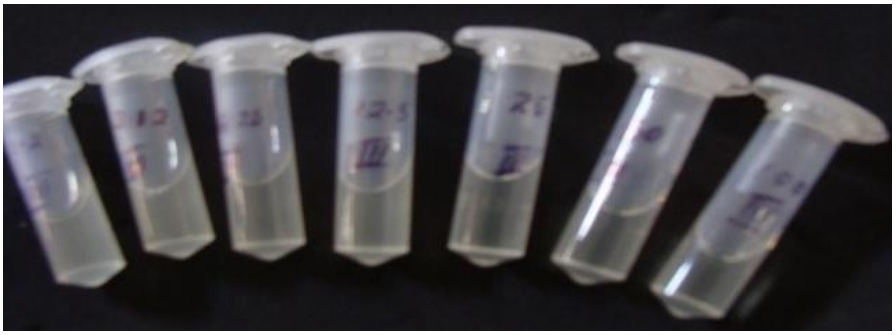

Figure 5: Photos of MIC values of Ox4

Table 4 and Table 5 shows the average restriction area in disc diffusion method and minimum inhibitory concentration in tube dilution method respectively.

Table 4: Antibacterial activities of synthesized compounds (disc diffusion method)

Sample	AVERAGE RESTRICTION SECTOR (mm)					
	Staphylo-coccus aureus			Escherichia-coli		
	100 µg/ 100µl	250µg/ 100µl	500µg/ 100µl	100µg/ 100µl	250µg/ 100µl	500µg/ 100µl
Control(DMSO)	00	00	00	00	00	00
Ox2	00	00	00	20	23	26
Ox4	00	09	11	22	25	28
Amoxicillin (50µg/ml)	13			19		

Readings are mean of three individual measurements

Table 5: Minimum Inhibitory Concentrations (MIC)

Sample	*Staphylococcus aureus* (Gram positive)	*Escherichia coli* (Gram negative)
Ox2	50	12.5
Ox4	25	12.5
Amoxicillin	3.12µg/ml	6.25µg/ml

The derivatives **Ox2** and **Ox4** showed significant anti- bacterial activity against *E.coli* and **Ox4** showed moderate inhibitory activity against Gram positive organism (*S.aureus*).

3.2 Anticancer activity of derivatives

Fig 6 shows graphical representation of cytotoxic activity of Ox8. Table 6 shows the reading of trypan blue exclusion method.

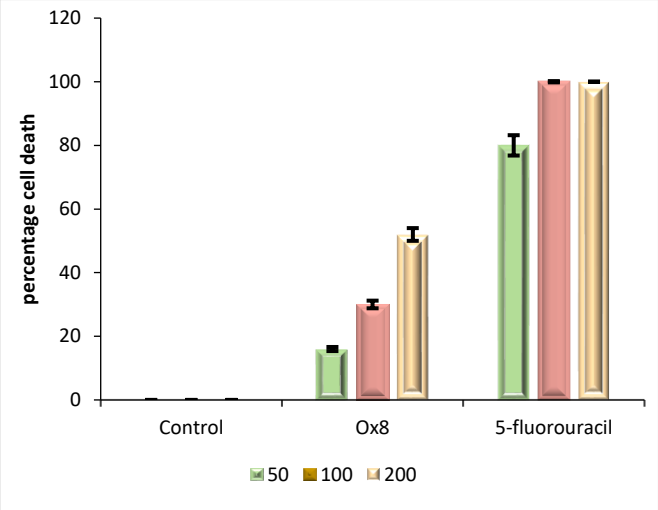

Figure 6: Cytotoxicity of Ox8 (trypan blue exclusion method)

Table 6: Cytotoxic effect of Ox8 (trypan blue exclusion method)

Compounds	Concentration (µg/ml)	Percentage cell death (%)
control (5% DMSO)	200	0
	100	0
	50	0
Ox8	200	52
	100	30
	50	16
+ve control (5-fluorouracil)	200	100
	100	100
	50	80

Compound **Ox8** having high docking score (-11.3906 Kcal/mol) when docked with 3VO3, was evaluated for its in-vitro cytotoxicity against DLA cells and was found to have 52% cytotoxic activity at 200 µg/ml

3.3 Anti- diabetic activity of derivatives

Fig 7 and Fig 8 shows the antidiabetic activity of Ox10 and standard drug Acarbose respectively. Table 7 and 8 shows the corresponding readings.

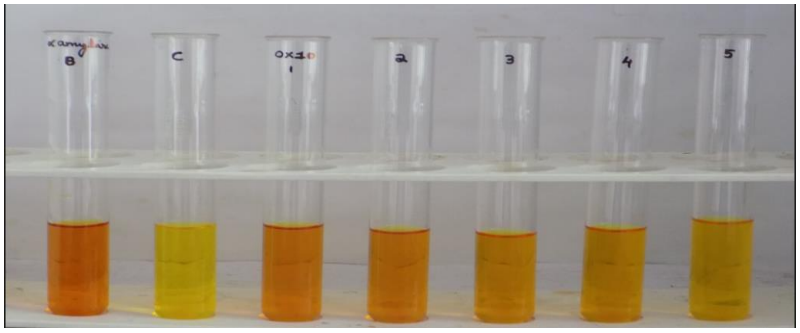

Figure 7: Alpha amylase inhibitory activity of Ox10

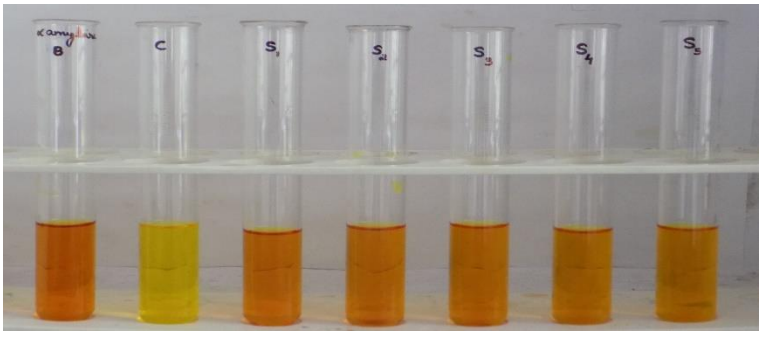

Figure 8: Alpha amylase inhibitory activity of standard drug Acarbose

Table 7: Antidiabetic activity of Ox10 (by DNSA Method)

Sample	Concentration (µg)	OD at 540nm	% of Inhibition
Blank	-	0.446	-
Control	-	0.029	-
OX 10	6.25	0.309	32.85
	12.5	0.263	43.88
	25	0.194	60.43
	50	0.145	72.18
	100	0.106	81.53

Table 8: Antidiabetic activity of standard drug Acarbose (by DNSA Method)

Sample	Concentration (µg)	OD at 540nm	% of Inhibition
Blank	-	0.446	-
Control	-	0.029	-
Acarbose (standard)	6.25	0.344	24.46
	12.5	0.307	33.33
	25	0.286	38.36
	50	0.214	55.63
	100	0.189	61.63

Among the 10 selected derivatives, **Ox10** having high docking score (-8.8683 Kcal/mol) when docked with 1B2Y, was evaluated for its alpha-amylase inhibitory activity using DNSA method. Ox10 was found to have 81.53% of inhibition at 100 g/ml when compared to standard Acarbose.

4. SUMMARY AND CONCLUSION

The detailed literature studies revealed that various oxadiazole derivatives have biological activities like anti-bacterial[8], anti-fungal[18], anti-inflammatory[1], anti-tubercular[2], anticancer[9], anticonvulsant[3], analgesic activities[11], antiviral activity[19] etc.

In this study, a series of 2, 5-disubstituted- 1, 3, 4- oxadiazole derivatives were prepared from salicylic acid and phenyl acetic acid. The compounds Ox2 and Ox4 showed promising antibacterial activity against *E.coli* while Ox4 showed better activity compared to the standard drug amoxicillin at a concentration 500 µg/ml.

Result of docking studies showed that compound Ox8 (-11.3906 Kcal/mol) and Ox10 (-8.8683 Kcal/mol) with high docking scores possess good interaction with the enzyme and hence possess better anticancer and antidiabetic activity respectively when compared to other derivatives.

The compound Ox8 shows 52% cytotoxic activity against DLA cells at a concentration 200 g/ml when compared to standard drug 5-fluorouracil.

The compound Ox10 shows 81.53 % of inhibition at a dose of 100g/ml when compared to standard drug Acarbose which shows 61.63% of inhibition at a dose of 100µg/ml.

Acknowledgement

Authors are grateful to Shri Jagdishprasad Jhabarmal Tibrewala University, and St. Josephs College of Pharmacy, Cherthala for providing the necessary facilities to carry out this research work.

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