Available online on 15.03.2024 at ijmspr.com

International Journal of Medical Sciences and Pharma Research

Open Access to Medical Science and Pharma Research

Open Access **Research Article**

Synthesis and Evaluation of Antifungal Activity of Novel 1,3,4-Oxadiazole Derivatives

Aswathy Ramesh [1]*, Dr Rakesh Kumar Jat [2], Dr R Arunkumar [3]

[1] *PhD scholar, JJT University, Rajasthan, Jhunjhunu, India*
[2] *HOD, Department of Pharmacy, JJT University, Rajasthan, Jhunjhunu, India*
[3] *Principal, Holygrace Academy of Pharmacy, Thrissur, Kerala, India*

Article Info:

Article History:

Received 23 December 2023
Reviewed 04 February 2024
Accepted 26 February 2024
Published 15 March 2024

Abstract

Here new eight derivatives of 1,3,4-oxadiazole were synthesised from salicylic acid and phenyl acetic acid by ring condensation mechanism. The synthesized derivatives were characterized and structures were confirmed by various spectral analysis. Antifungal activity studies revealed that Ox1, Ox2 and Ox4 exhibit promising activity.

Keywords: 1,3,4-oxadiazole, antifungal activity, Disc diffusion method, MIC,

Cite this article as:

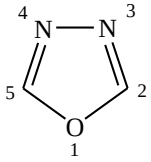
Ramesh A, Jat RK, Arunkumar R, Synthesis and Evaluation of Antifungal Activity of Novel 1,3,4-Oxadiazole Derivatives, International Journal of Medical Sciences & Pharma Research, 2024; 10(1):22-25 *DOI: http://dx.doi.org/10.22270/ijmspr.v10i1.85*

*Address for Correspondence: Ramesh Aswathy, PhD scholar, JJT University, Jhunjhunu, Rajasthan, India

1. INTRODUCTION

Oxadiazole is five membered heterocyclic compounds with two nitrogen atoms and one oxygen atom. They are synthesized by ring condensation and rearrangements. They are considered to be derived from furan by the replacement of two methine (-CH=) groups by two pyridine-type nitrogen (-N=). This reduces aromaticity of the resulting oxadiazole ring to such an extent that the ring exhibits character of the conjugated diene[2]. Depending on the positions of hetero atoms, there are four isomeric type and are named as 1,2,3; 1,2,4; 1,2,5 and 1,3,4 oxadiazoles.

1, 3, 4-oxadiazoles are thermally stable neutral aromatic heterocycle with molecular formula $C_2H_2N_2O$. It is symmetrical and planar molecule with resonance energy 167.4 kJ/mol. The bond length reflects π-electron delocalization. The C=N bond lengths are very close to that in acyclic compounds (1.27 A0) and hence show dienic character. The numbering is done as follows



1, 3, 4- oxadiazoles are well known compounds that are found to possess a broad spectrum of biological activities. They are associated with antibacterial, antifungal, tuberculostatic, anticonvulsant, analgesic, anti-inflammatory, diuretic, antiemetic and insecticidal properties. Recently they were found to possess anti-inflammatory, antitumor and antiviral activities[13]. A variety of pharmaceutical drugs containing stable oxadiazoles are available including butalamine (vasodialator), fasiplon (antiretroviral), oxolamine (cough suppressant) and pleconaril (antiviral)[5].

. In this present work, the derivatives of 1, 3, 4-oxadiazole were synthesized from salicylic acid and phenyl acetic acid, through a three-step process and *In-silico* studies were done by using different softwares like ChemDraw, Molinspiration, PASS[3]. Those compounds which satisfied the Lipinski rule of five were selected for wet lab synthesis[6]. Newly synthesized compounds having high Pa value were evaluated for its antifungal 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

2-,5- disubstituted 1,3,4-oxadiazole derivatives are synthesized by ring condensation reaction of different carboxylic acids with salicylic acid hydrazide and phenyl acetic acid hydrazide in the presence of phosphorus oxychloride.

2.2.1 Synthesis of methyl salicylate[9]

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 (80oC). 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[8]

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 Ox1-Ox4

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, mp: 170-1730C. 2.2.4 Synthesis of ethyl phenyl acetate (Ur-Rehman et al., 2012)

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.

2.2.4 Synthesis of ethyl phenyl acetate[15]

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 Ox5-Ox8

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 synthesised derivatives were 2-(5-(4-hydroxyphenyl)-1, 3, 4-oxaddiazol-2-yl) phenol (**Ox1**), 2-(5-styryl-1, 3, 4-oxadiazol-2-yl) phenol (**Ox2**), 2-(5-(4-chlorophenyl)-1, 3, 4-oxadiazol-2-yl) phenol (**Ox3**), 2-(5-(3, 5-dinitrophenyl)-1, 3, 4-oxadiazol-2-yl) phenol (**Ox4**), 2-benzyl-5-(3, 5-dinitrophenyl)-1, 3, 4-oxadiazol(**Ox5**), 2-benzyl-5-styryl-1, 3, 4-oxadiazol (**Ox6**), 1-(5-benzyl-1, 3, 4-oxadiazol-2-yl)-2-phenylethanamine (**Ox7**), 2-benzyl-5-(4-chlorophenyl)-1, 3, 4-oxadiazole (**Ox8**). The structures of the compounds were confirmed by spectral analysis and are given in Fig 1

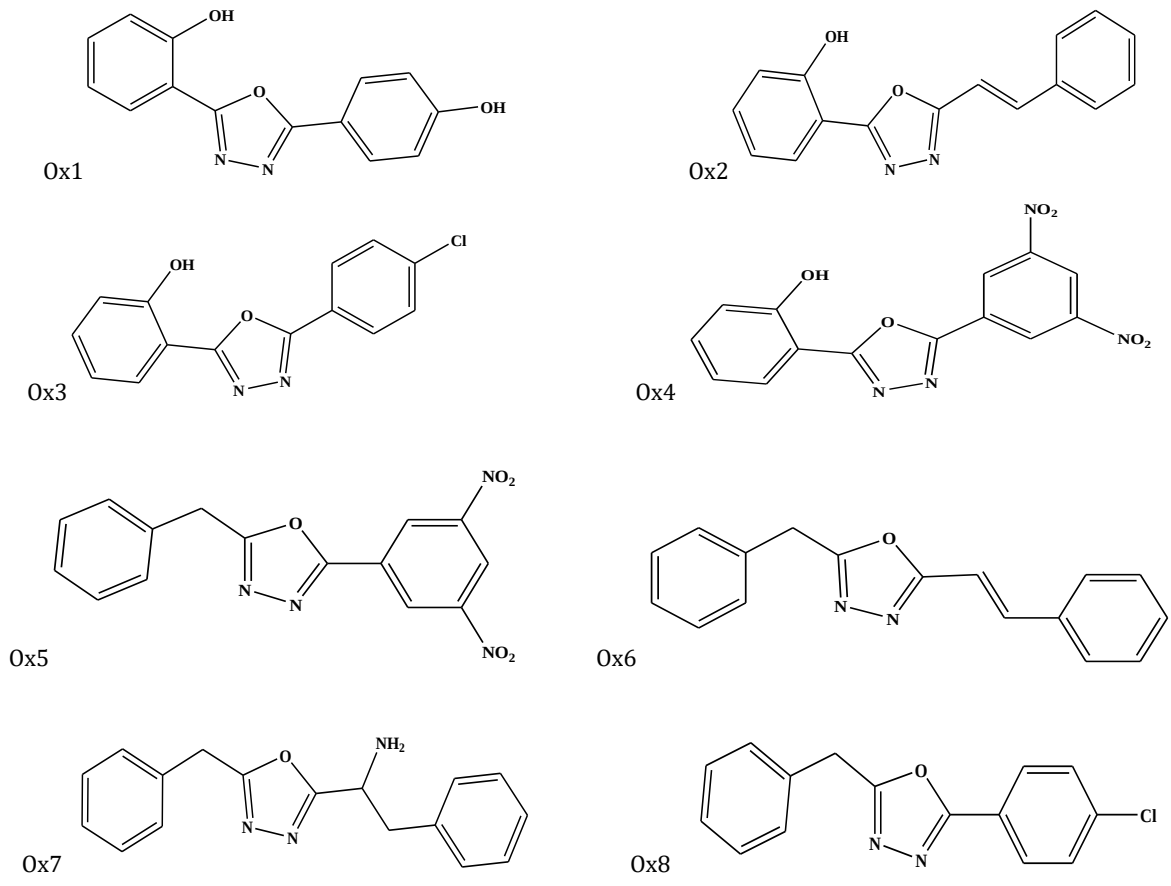


Figure 1: Structures of new derivatives of 1,3,4-oxadiazoles

2.3 Test microbes

Candida albicans (diploid fungus) was obtained from Maratha Mandal's NGH Institute of Dental Sciences and Research Centre.

2.4 Antifungal studies[4]

The antifungal activity of the derivatives having high value in activity prediction software, PASS, were done by Disc Diffusion Method in Brain Heart Infusion agar medium.

Standard solution: Fluconazole – 50 µg/ml

Agar plates were brought to room temperature before use. The inoculum was prepared by transferring the colonies to the plates using a loop or swab. The turbidity was visually adjusted with broth to equal that of a 0.5 McFarland turbidity standard that had been vortexed. Alternatively, the suspension can be standardized with a photometric device.

A sterile cotton swab dipped in the inoculum, within 15 min of adjusting the inoculum to McFarland 0.5 turbidity standard and was rotated against the wall of the tube above the liquid to remove excess inoculum, was swabbed three times on the entire surface of the agar plate to inoculate it. the plates were rotated approximately 600 times in between streaking to ensure even distribution. The formation of aerosols can be prevented by avoiding hitting the sides of petriplate. The inoculated plates were allowed to stand for at least 3 min but no longer than 15 min before making wells. 75µl, 50µl, 25µl, 10µl and 5µl of sample solutions was added into respective wells of 5mm diameter on each inoculated plate.

Plates were incubated within 15 min of sample application for 18-24 hrs at 37 ^{0}C in an incubator and were inverted and stacked no more than five high. The plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of inhibition zone was measured to the nearest whole millimetre by holding the measuring device.

Minimum inhibitory concentration[12]:

In the initial tube 20 µl of drug was added into the 380 µl of BHI broth. 9 dilutions of each drug were done with BHI for MIC. For dilutions, 200 µl of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200 µl was transferred to the first tube containing 200 µl of BHI broth. This was considered as 10-1 dilution. From 10-1 diluted tube 200µl was transferred to second tube to make 10-2 dilution. The serial dilution was repeated up to 10-9 dilution for each drug.

5 µl of required organisms was taken from the maintained stock cultures, and was added into 2ml of BHI (brain heart infusion) broth. 200 µl of this culture suspension was added to each serially diluted tube and were incubated for 24 hrs and observed for turbidity

3. RESULT AND DISCUSSION

The synthesized compounds Ox1, Ox2 and Ox4 having high Pa value in PASS result, were evaluated for its antifungal activity against *C.albicans* using Fluconazole (50µg/ml) as standard. The result is summarized in Table 1. Result of MIC was given in Table 2. The figures 1,2 and 3 shows zone of inhibition. Figure 4,5 and 6 shows MIC of test compounds.

Table 1: Antifungal activity of compounds at different concentration (disc diffusion method)

Compound Code	Candida albicans			
	50µg/ml	100µg/ml	250µg/ml	500µg/ml
Ox1	25mm	26mm	30mm	33mm
Ox2	22mm	25mm	26mm	28mm
Ox4	25mm	28mm	30mm	30mm
Control	00mm	00mm	00mm	00mm
Fluconazole (50µg/ml)	24mm	ND	ND	ND

ND: not done

Figure 1: Zone of inhibition of Ox1

Figure 2: Zone of inhibition of Ox2

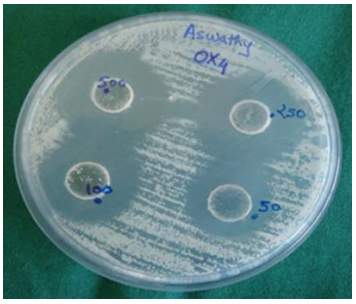


Figure 3: Zone of inhibition of Ox3

Table 2: MIC Results (by tube dilution method)

Fungal strain	Code	Concentration (µg/ml)									
		100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
C. albicans	Ox1	S	S	S	S	S	R	R	R	R	R
	Ox2	S	S	S	R	R	R	R	R	R	R
	Ox4	S	S	S	S	R	R	R	R	R	R

S-Sensitive, R- Resistant

Compound Ox1 and Ox4 showed significant Disc Diffusion of 25mm at a concentration of 50µg/ml when compared to other compounds. Compounds Ox2 displayed Disc Diffusion of 25mm at a concentration of 100µg/ml. The standard value of Disc Diffusion for Fluconazole at 50µg/ml was 24mm.

Figure 4: MIC of Ox1

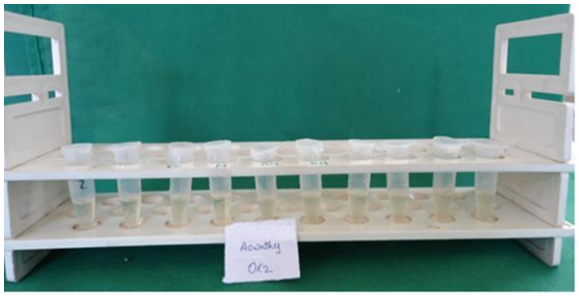


Figure 5: MIC of Ox2

Figure 6: MIC of Ox4

The compound Ox1 was found to be sensitive at concentration 6.25 µg/ml. Compound Ox4 showed sensitivity at 12.5µg/ ml. Standard value of MIC for fluconazole is 16µg/ml. Therefore compound Ox1and Ox4 were found to possess better antifungal activity of which Ox1 is highly sensitive towards *C.albicans*.

4. SUMMARY AND CONCLUSION

In this study, on the basis of literature survey[1,7,10,11]on 1, 3, 4-oxadiazole and its derivatives, less harmful, effective scheme of synthesis[14] was selected. In-silico modelling of proposed derivatives was done with the help of reliable software, and those obeying Lipinski rule of five was selected for wet lab synthesis. The characterization of the derivatives was done by spectral analysis. Pharmacological activity was predicted using PASS software. The derivatives having high Pa value were subjected to evaluation of antifungal activity. The result revealed that they possess promising antifungal activity.

The compounds Ox1 and Ox4 showed promising antifungal activity against C.albicans of which Ox1 showed better activity compared to standard drug fluconazole at a concentration 500 µg/ 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.

REFERENCES

1. Chandrakantha B, Shetty P, Nambiar V, Isloor N, Isloor AM, Synthesis, characterization and biological activity of some new 1, 3, 4-oxadiazole bearing 2-flouro-4-methoxyphenyl moiety, European Journal of Medicinal Chemistry, (2010); 45:1206 – 1210 https://doi.org/10.1016/j.ejmech.2009.11.046 PMid:20004043

2. De-Oliveira CS, Lira BF, Barbosa-Filho JM, Lorenzo JGF, de-Menezes CP, de-Lima O, dos-Santos JMCG, de-Athayde-Filho PF, Synthesis and testing of 3-acetyl-2, 5-disubstituted-2, 3-dihydro-1, 3, 4-oxadiazole derivatives for antifungal activity against selected Candida species, Journal of the Brazilian Chemical Society, 2013; 24:1-5 https://doi.org/10.1590/S0103-50532013000100016

3. Filimonov DA, Poroikov VV, Karaicheva EI, Computer-aided prediction of biological activity spectra of chemical substances on the basis of their structural formulae: computerized system PASS, Experimental and Clinical Pharmacology (Rus.), 1995; 4:56-62

4. Henry DI, Clinical Microbiology Procedure Handbook, American society for microbiology/ Washington, D.C., 1992 ;1

5. / Joule JA, Mills K, Smith G F. Heterocyclic Chemistry http://www.en.wikipedia.org/wiki/Oxadiazole , 3:452

6. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Advanced Drug Delivery Reviews, 2001; 2:3-25.

7. Parikh PK, Marvaniya HM, Prof.Dr.Sen DJ, Synthesis and biological evaluation of 1, 3, 4-oxadiazole derivatives as potential antibacterial and antifungal agents, International Journal of Drug Development and Research, 2011; 3(2):248-255

8. Pattan SR, Rabra PA, Pattan JS, Bukitager AA, Wakale VS, Musmade DS, Synthesis and evaluation of some novel substituted 1, 3, 4-oxadiazole and pyrazole derivatives for antitubercular activity, Indian Journal of Chemistry, 2009; 48B:1453-1456 https://doi.org/10.1002/chin.201006125

9. Pavia, Lampman, Kriz, Engel, Introduction to Organic Laboratory Techniques: A Microscale Approach, Saunders College Publishing, 1999

10. Prakash O, Kumar M, Kumar R, Sharma C, Aneja KR, Hypervalent iodine (III) mediated synthesis of novel unsymmetrical 2, 5-disubstituted 1, 3, 4-oxadiazoles as antibacterial anti-fungal agents, European Journal of Medicinal Chemistry, 2010; 45:4252-4257 https://doi.org/10.1016/j.ejmech.2010.06.023 PMid:20630627

11. Saini R, Rai AK, Kesari AN, Yar MS, Synthesis and biological evaluation of 2, 5-disubstituted-1, 3, 4-oxadiazoles, Asian Journal of Research and Chemistry, 2009; 2: 34-36.

12. Schwalve, Moore and Goodwin, Antimicrobial susceptibility testing protocols, CRC Press, 2007 https://doi.org/10.1201/9781420014495

13. Sharma S, Sharma PK, Kumar N, Dhude R, A review: oxadiazole: their Chemistry and Pharmacological potential, Der Pharma Chemica, 2010; 2: 253-263

14. Tomi IHR, Al-Qaisi AHJ, Al-Qaisi ZHJ, Synthesis, characterization and effect of bis-1, 3, 4-oxadiazole containing glycine moiety on the activity of some transferase enzymes. IBN Al-Haitham, Journal for Pure and Applied Sciences, 2010; 23:1-13. https://doi.org/10.1016/j.jksus.2010.06.002

15. Ur-Rehman A, Siddiqui S, Abbasi MA, Abbas N, Khan KM, Shahid M, Mahmood Y, Akthar MN, Lajis NH, Synthesis, antibacterial screening and haemolytic activity of S-substituted derivatives of 5-benzyl-1, 3, 4-oxadiazole-2-thiol, International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4:676-680