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CODEN: IAASCA **ORIGINAL ARTICLE**

Synthesis and Antifungal Evaluation Oxadiazole Derivatives

Kiran Mishra
Department of Applied Sciences
Universal Instt.of Engg.&Tech.,Lalau,Mohali,Pb.
Email: drmishrakiran@gmail.com m

ABSTRACT
The objective of present research work is to synthesis of a number of 1, 3, 4-oxadiazole derivatives for antifungal activity. Some of the synthesized compounds showed significant antifungal activity. KM-8 compound exhibited potent activity (MIC=12.5 µg/ml). The synthesized derivatives were characterized by means of TLC, IR, and 1HNMR spectral analysis for their structural confirmation. The derivatives which showed better antifungal activity may serve as leads for further optimization.
Keywords: Antifungal properties, TLC, IR, Oxadiazole

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INTRODUCTION

Heterocyclic is the largest classical division of medicinal chemistry and is Importance industrially and biologically. Development of novel chemotherapeutic agents an important and challenging task for the medicinal chemists and many research programs are directed towards the design and synthesis of new drugs for their chemotherapeutic usages. The majority of pharmaceutical and biologically active agrochemicals are heterocyclic while countless additives and modifiers used in industrial application ranging from reprography, Information storage, cosmetics and plastics are heterocyclic in nature. Oxadiazoles constitute an important class for new drug development in order to discover an effective compound against multi drug resistant microbial infection. A large number of heterocyclic compounds containing the 1, 3, 4-Oxadiazoles ring are associated with diverse pharmacological properties such as anti-inflammatory, antimicrobial, fungicidal and anti viral activity1-4. Substituted 1,3,4-oxadiazole have revealed antibacterial5, antimycobacterail6, anti fungal activity7, anti-inflammatory activity8, analgesic 9, anticonvulsant 10 and anticancer 11 properties. Prompted by the observed biological activities of the above mentioned derivatives and in continuation of our ongoing studies on novel biologically active molecules, we have designed and synthesized new 1,3,4-Oxadiazoles As a potential antimicrobial and Anti-Inflammatory agent. The results of this study are discussed in this paper.

MATERIALS AND METHODS

All melting points determined in open glass capillaries and are uncorrected. All the solvents and reagents used were of laboratory grade. All the reactions were monitored by TLC using benzene: methanol (9: 2), Ethyl acetate: n-Hexane (5: 5) and Methylene dichloride: Ethyl acetate (8: 2) as solvent System. TLC Plates were prepared by spreading method. These were dried in the air and then activated by heating in hot air oven at 1100C for 30-45 minutes. Iodine vapours were used for visualization of TLC plates. IR spectra in KBr were recorded on Perkin-Elmer infrared spectrophotometer (Vmax in cm-1) and 1H NMR spectra in DMSO-d6 on an EM-360 L (60 MHz) NMR spectrometer using TMS as internal references (chemical shifts in δ ppm). All the compounds have given satisfactory elemental analytical (C, N, H and S) and I R and 1H NMR spectra.

2, 4-Dichloro-5-fluorobenzoic acid(DM): 2, 4-Dichloro-5-fluorobenzoic acid was prepared from 2, 4-dichloro-5-fluoracetophenone by haloform reaction. To 2, 4-dichloro-5-fluoroacetophenone (0.1 M) in ethanol was added sodium hydroxide solution until pH was 10.0 to 10.20. Then chlorine gas was passed for several hours (care must be taken to maintain pH at 10.0 to 10.20), until solution smell strongly of antifungal activity data of 2-Aryl-5-(2, 4-dichloro-5-fluorophenyl) -1, 3, 4-oxadiazoles (2 a-i), 3-(Substituted methylamino)-5-(2, 4-dichloro-5-fluorophenyl)-1, 3, 4oxadiazol-2-thiones (4 a-i) and 2-Thioaryl-5-(2, 4-dichloro-5-fluorophenyl)-1, 3, 4-oxadiazoles (5 a-i). Then any resinous solid, if any was

removed. The clear sodium so obtained was neutralized with hydrochloric acid to get purr 2, 4-dichloro-5 fluorobenzoic acid, yield 89-90%, m. p. 144-1460C, 4-Fluoro-3-phenoxybenzoic acid.

Ethyl-2, 4-dichloro-5-fluorobenzoate: It has been prepared according to the general method of esterification. The compound after usual work up was obtained as pale yellow oil. It has been judged to be pure by thin layer chromatography (TLC).

2, 4-Dichloro-5-fluorobenzoyl hydrazine (DM-1): Ethyl-2, 4-dichloro-5-fluorobenzoate (0.1 M), hydrazine hydrate (0.15 M) and 20.0 ml of ethanol were refluxed with water bath for 4.0 hrs the excess of solvent was distilled off. The compound was recrystallized from ethanol, yield 98% m. p. 158-1620C,

2-Aryl-5-(2, 4-dichloro-5-fluorophenyl)-1, 3, 4-oxadiazoles (DM-2): A mixture of aroyl hydrazine (1.0 M), substituted benzoic / aryl Fluorobenzoic acid (1.0 M) and phosphorus oxychloride (2.5 M) were refluxed at 100-1100C for 6.0-7.0 hrs. Acetonitrile / Toluene were used as solvent. The excess of solvent was distilled off and recrystallized from suitable solvent to give oxadiazoles in 65-75% yield.

5-(2, 4)-Dichloro-5-fluorophenyl)-1, 3, 4-oxadiazol-2-thione (DM-3): 2, 4-Dichloro-5-fluorobenzoyl hydrazine (0.1 M), carbon disulphide (0.2 M) and potassium hydroxide (30%, 5 ml) were refluxed on water both for 2.0 hrs. The reaction mixture was cooled, acidified and the separated product was purified by recrystallization from ethanol, yield 85%, m. p. 156-1580C.

3-(Substituted methylamino)-5-(2, 4-dichloro-5-fluorophenyl)-1, 3, 4-oxadiazol-2-thiones (DM-4): 5-(2, 4- Dichloro-5-fluorophenyl)-1, 3, 4-oxadiazol-2-thione (1.0 M), formaldehyde (2.0 M) and a secondary amine (1.5 M) was taken in ethanol-1, 4-dioxane mixture and stirred at room temperature for 6.0-7.0 hrs (refluxed if necessary). The excess of solvent was removed under reduce pressure. The residue was poured into ice cold water the separated products were crystallized from 1, 4-Dioxane.

Chemistry

All the synthesized compounds with their characterization data m. p., molecular formula, elemental analysis and IR and 1H NMR spectra are recorded.

2, 4-Dichloro-5-fluorobenzoic acid: Yield: 78% ; Melting Point: 215 °C; IR(KBr, cm^{-1}): 3100 (Ar-C-H), 1609 (C=N), 1275 (C-O), 1189 (C-F), 1023 / 908 / 839 (C-Cl) cm-1. 1H NMR (DMSO-d6) δ: 7.5 (d, C-H, Ar-H), 7.7 (d, 1H, H-F meta), 7.9 (d, 1H, H-F ortho) 8.4 (d, 2H, Ar-H); Anal. Calcd. for C14H6N2OFCl3: C, 69.28; H, 3.96; N, 18.36; O, 8.39% **found;** C, 68.75; H, 3.45; N, 18.8; O, 9% Mol. Wt.: 381.

Ethyl-2, 4-dichloro-5-fluorobenzoate: Yield: 74% ; Melting Point: 283 °C; IR(KBr, cm^{-1}): 3075 (Ar-C-H), 2842 (CH3), 1690, 1616 (C=N), 1316 / 1216 (C=O), 1118 (C-F), 894 / 923 (C-Cl) cm-1. 1H NMR (DMSO-d6) δ: 4.2 (s, 3H, OCH3),7.21-7.98 (m, 6H, Ar-H); Anal. Calcd. for C14H5N2OFCl4: C, 53.11; H, 1.42; N, 8.25; O, 8.39% **found;** C, 53.09; H, 1.45; N, 8.27; O, 9% Mol. Wt.: 251.

2, 4-Dichloro-5-fluorobenzoyl hydrazine (KM-1): Yield: 70% ; Melting Point: 207 °C; IR(KBr, cm^{-1}) 3089 (Ar-C-H), 2850 (CH3), 1620 (C=N), 1316 / 1210 (C=O), 1122 (C-F) 885 / 997 (C-Cl) cm-1. 1H NMR (DMSO-d6) δ: 4.2 (s, 6H, 2-OCH3), 7.16-7.88 (m, 6H, Ar-H); Anal. Calcd. for C16H12N2O3FCl2: C, 51.86; H, 3.21; N, 7.59; O, 8.39% **found;** C, 51.89; H, 3.24; N, 7.56; O, 9% Mol. Wt.: 209.

2-Aryl-5-(2, 4-dichloro-5-fluorophenyl)-1, 3, 4-oxadiazoles (KM-2): Yield: 66% ; Melting Point: 238 °C; IR(KBr, cm^{-1}): 3072 (Ar-CH), 2840 (CH3), 1680, 1610 (C=N), 1312 / 1202 (C=O), 1128 (C-F), 892 / 925 (C-Cl) cm-1. 1H NMR (DMSO-d6) δ: 3.38 (s, 3H, -CH3), 7.19-7.84 (m, 6H, Ar-H); Anal. Calcd. for C15H9N2OFCl2: C, 55.76; H, 2.75; N, 8.69; O, 8.39% **found;** C, 55.72; H, 7.78; N, 8.66; O, 9% Mol. Wt.: 198.

IR and 1H NMR spectra of compound (KM-3): Yield: 57% ; Melting Point: 199 °C; IR(KBr, cm^{-1}): 3078 (Ar-C-H), 1595 (C=N), 1280 (C-O), 1185 (C-F), 1028 / 901 / 830 (C-Cl) cm-1. 1H NMR (DMSO-d6) δ: 7.53 (d, 2H, Ar-H), 7.79 (d, 1H, H-F meta), 7.82 (d, 1H, H-F ortho), 8.48 (d, 2H, Ar-H); Anal. Calcd. for C16H12N2OFCl2: C, 56.83; H, 3.42; N, 13.52; O, 8.39% **found;** C, 56.83; H, 3.45; N, 13.55; O, 9% Mol. Wt.: 223.

R and 1H NMR spectra of compound (KM-4): Yield: 66% ; Melting Point: 141 °C; IR(KBr, cm^{-1}): 3050 (Ar-C-H), 2956 (-N-CH2), 2837 (CH2-O-), 1685 (C-F), 1571 (C-S) cm-1. 1H NMR (DMSO-d6) δ: 2.65-2.85 (t, 4H, CH2-N-CH2), (t, 4H, CH2-OCH2), 4.42 (s, 2H, N-CH2-N), 7.9 (d, 1H, Ar-H H-F ortho), 7.6 (d, 1H, Ar-H, H-F meta). Anal. Calcd. for C18H22N5O2SFCl2 : C, 46.83; H, 4.73 N, 15.18; S, 6.95% **found;** C, 46.75; H, 4.76; N, 15.15; S, 6.92% Mol. Wt.: 207.

IR and 1H NMR spectra of compound (KM-5): Yield: 64% ; Melting Point: 139 °C; IR(KBr, cm^{-1}): 3035 (Ar-C-H), 2940 (-N-CH2), 2830 (CH2-O-), 1620 (C-N), 1550 (C-S) cm-1. 1H NMR (DMSO-d6) δ: 2.54-2.79 (t, 12H, CH2-C-CH2), 3.13-3.26 (t, 4H, CH2-O-CH2), 3.42 (s, 3H, N-CH3), 4.43 (s, 2H, N-CH2), 7.66 (d, 1H, Ar-H H-F meta), 7.93 (d, 1H, Ar-H H-F ortho), Anal. Calcd. for C18H23N6O2SFCl2 : C, 45.32; H, 4.85; N, 17.64; S, 6.73% **found;** C, 45.28; H, 4.82; N, 17.61; S, 6.70% Mol. Wt.: 321.

IR and 1H NMR spectra of compound (KM-6): Yield: 60% ; Melting Point: 135 °C; IR(KBr, cm^{-1}): 3065 (Ar-C-H), 2963 (-N-CH2), 2839 (CH2-O-), 1629 (C=N), 1530 (C=S) cm-1. 1H NMR (DMSO-d6) δ: 2.59-2.83

(t, 8H, CH2), 4.47 (S, 2H, N-CH2-N), 7.03-7.99 (m, 12H, Ar-H), 8.03 (d, 1H, Ar-H H-F meta), 8.30 (d, 1H, Ar-H H-F ortho); Anal. Calcd. for C19H24N5OSFCl2 : C, 49.52; H, 5.21; N, 15.19; S, 6.97% **found;** C, 49.56; H, 5.26; N, 15.21; S, 6.95% Mol. Wt.: 189.

IR and 1H NMR spectra of compound (KM-7): Yield: 63% ; Melting Point: 129 °C; IR(KBr, cm^{-1}): 3082 (Ar-C-H), 2969 (-N-CH2), 2845 (CH2-O-), 1639(C=N), 1582 (C=S) cm-1. 1H NMR (DMSO-d6) δ: 2.68-2.93 (t, 12H, CH2-N-CH2), 3.46 (S, 3H, NCH3), 4.49 (S, 2H, N-CH2-N), 7.01-7.68 (m, 6H, Ar-H), 7.90 (d, 1H, Ar-H H-F meta), 8.10 (d, 1H, Ar-HH-F ortho); Anal. Calcd. for C19H25N6OSFCl2 : C, 48.03; H, 5.29; N, 17.66; S, 6.70% **found;** C, 48.00; H, 5.26; N, 17.68; S, 6.73% Mol. Wt.: 235.

IR and 1H NMR spectra of compound(KM-8): Yield: 64% ; Melting Point: 138 °C; IR(KBr, cm^{-1}): 3072 (Ar-C-H), 2987 (-N-CH2), 2855 (CH2-O-), 1645 (C=F), 1535 (C=S) cm-1. 1H NMR (DMSO-d6) δ: 2.57-2.90 (t, 16H, CH2-N-CH2), 4.53 (s, 2H, NCH2- N), 7.69 (d, 1H, Ar-H H-F meta), 7.94 (d, 1H, Ar-H H-F ortho); Anal. Calcd. for C19H26N7OSFCl2 : C, 46.50; H, 5.36; N, 19.52; S, 6.53% **found;** C, 46.53; H, 5.30; N, 19.55; S, 6.53% Mol. Wt.: 271.

ANTIFUNGAL SCREENING [12, 13, 14, 15]

Synthesized compounds were screened for their antifungal efficacy.

In vitro Antifungal screening strategies

Comprehensive antifungal screening strategies include the following primary and secondary evaluation methods:

Materials and Method for antifungal screening a) Primary screening

Table 1: Detection of antifungal potential:

Method	**Agar diffusion quantitative bioassay (well plate method)**
Medium	Sabouraud'sDextrose agar (Hi Media).
Organismemployed	*Candida albicans* strain ATCC-3705 (NCIM 3471)
Inoculum	Growth from 3-4 old Sabouraud's Dextrose agar slope is uniformly suspended in 1 ml of sterile normal saline. Optical density of cell suspension is adjusted using Spectrophotometer at 640 nm to get absorbance 1.
Inoculum size	107 CFU/ml.
Stock solution	Prepared in dimetylsulfoxide for both synthesized compounds and standard drug.
Drug concentration	Ketoconazole at 6&12.5 µg/ml. Compounds at 6,12.5,25,50,100 µg/ml.
Incubation time	48-72hrs.
Incubation temp.	35-37o C.
Interpretation	Any compound showing inhibition zone exceeding 9mm diameter and quality of zone better than or comparable to Ketoconazole is considered to be endowed with antifungal potential.

b) Secondary Screening

Table 2: Determination of Minimum Inhibitory Concentration (MIC µg/ml)

Method	**MIC by Agar diffusion technique (well plate method).**
Medium	Sabouraud'sDextrose agar (Hi Media).
Compounds conc.	12.5, 25, 50, 100 µg/ml.
Organismemployed	*Candida albicans* strain ATCC-3705 (NCIM 3471).
Inoculum preparation	Growth from 3-4 old Sabouraud's Dextrose agar slopes is uniformily suspended in 1 ml of sterile normal saline. Optical density of cell suspension is adjusted using Spectrophotometer at 640 nm to get absorbance 1.[4]
Incubation time & temperature	48-72hrs at 30o C.
Endpoint definition	Concentration of compounds at which there is either complete disappearance or significant reduction in growth resulting in 5-10 colonies per spot is considered to be the MIC.
Interpretation	Synthesized compounds showing lower MIC than that shown by Ketoconazole would consider superior.

The synthesized compounds were evaluated for *in vitro* antifungal activity against *Candida albicans*(ATCC-3705). Ketoconazole was used as standard.

RESULT AND DISCUSSION

The new fluorophenyl oxadiazoles compounds (KM-1) to (KM-9) were prepared from fluorobenzoyl hydrazine and oxadiazole-2-thiones. Nine fluorophenyl oxadiazoles compounds were synthesized using

different aromatic amines and heterocycles as substituents. Synthesized compounds were characterized by chromatographic methods, Infrared spectroscopy and Nuclear Magnetic Resonance spectroscopy for their structural confirmation. These nine compounds were tested for antifungal activity by *in vitro* well plate method. Activity was presented in the form of zone of inhibition (mm) in agar culture plates and MIC. The zone of inhibition and MIC of the synthesized compounds are presented in table 4.

Table 3: Activity of Ketoconazole at different concentrations

Compound	Conc. in µg/ml	Zone of inhibition (mm)
Ketoconazole	12.5	22
	6	18

Table 4: In vitro antifungal activity of 2-Chloromethyl-1H-Benzimidazole) derivatives

S.No	Compound code	Conc. in µg/ml	Zone of inhibition (mm)
1	KM-1	100	20
2	KM-2	50	24
3	KM-3	100	22
4	KM-4	100	23
5	KM-5	100	25
6	KM-6	25	22
7	KM-7	25	21
8	KM-8	12.5	20

CONCLUSION

All the nine synthesized compounds screened for in vitro antifungal activity showed antifungal activity. KM-8 exhibited potent activity (MIC=12.5 µg/ml).In conclusion, these compounds provide preliminary insights into newer antifungal agents, which can help further modification to improve upon the activity profile.

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