Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 1, 2012

Research Article

SYNTHESES, ANTIBACTERIAL AND ANTIFUNGAL SCREENING OF SOME 1,3,4-OXADIAZOLE ANALOGUES

AJAY KUMAR[1], VARSHA KASHAW[2], GUNJAN SHUKLA[3], VIKAS MISHRA[3], SUSHIL K. KASHAW [3]*

[1]University Institute of Pharmacy, C.S.J.M. University, Kanpur (U.P.), [2]Sagar Institute of Pharmaceutical Sciences, Sagar, (M.P.) , [3]Division of Medicinal and Computational Chemistry, Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar-(M.P.)
Email: sushilkashaw@gmail.com

Received: 28 Oct 2011, Revised and Accepted: 29 Nov 2011

ABSTRACT

Eight novel 2-amino-5-aryl-1,3,4-oxadiazole analogues were synthesized and screened for antibacterial and antifungal activity by liquid dilution method used for the determination of MIC (minimum inhibitory concentration). Structure of the synthesized compounds was confirmed by means of their IR, ^{1}H-NMR, Mass spectroscopy and elemental analysis. Bacterial strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus Subtilis* and fungal strains of *Aspergillus niger* and *Candida albicans* were used in the study. Norfloxacin and clotrimazole was used as the standard positive control for antibacterial and antifungal activities respectively. MIC of the synthesized compounds ranged between 20-56 µg/mL and 42-78 µg/mL for antibacterial and antifungal activities respectively.

Keywords: 1,3,4-Oxadiazole analogues, Antibacterial, Antifungal and MIC method.

INTRODUCTION

Biological activity of synthetic compounds for potential antimicrobial activity continues to be an important strategy for the initial identification of new drugs with possible clinical values. 1,3,4-Oxadiazoles were associated with broad spectrum of biological activities, including antituberculosis[1], anticonvulsant[2], antibacterial[3], antifungal[4], anti-inflammatory[5], antitumors[6], CNS stimulant[7], antimalarial[8] and anticancer[9] .

In continuation of our earlier research here in this paper we report the syntheses, antibacterial and antifungal activities of some novel 1,3,4-oxadiazole analogues.

MATERIAL AND METHODS

The synthesis of the title compounds is given in scheme-1. Melting point was determined by open capillaries in a liquid paraffin bath and are uncorrected. The purity of the synthesized compounds were ascertained by TLC on silica gel-G plate. The structure of the synthesized compounds was confirmed on the basis of elemental analysis and spectral studies. IR spectra were recorded on Perkin Elmer spectrum RX1 in KBr plates, ^{1}H-NMR spectra were recorded on Bruker DRX-300 spectrometer, using DMSO as the solvent, Mass spectra were recorded on Micromass Quanttro II by chemical ionization (CI) method, using DMSO as the solvent and elemental analysis (Nitrogen & Oxygen) were undertook with elementar vario EL III carlo Erba 1108 elementar analyzer.

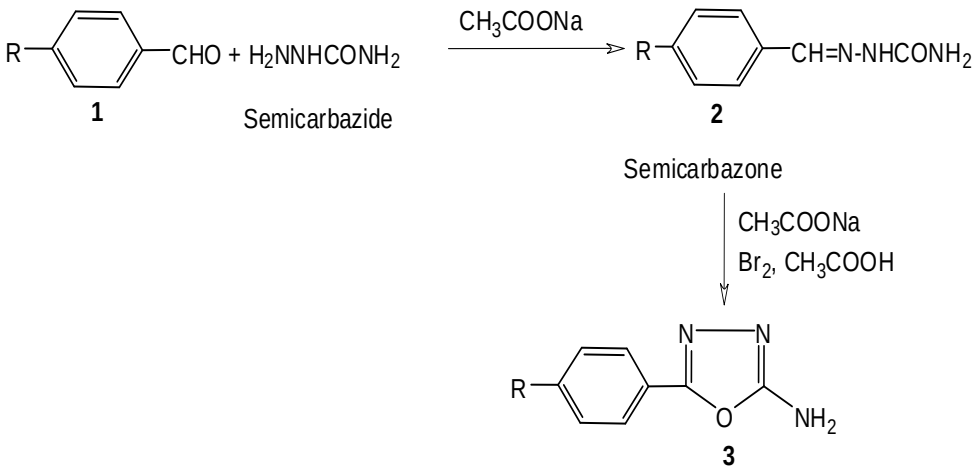
Synthesis of 2-amino-5-aryl-1,3,4-oxadiazoles

Benzaldehyde Semicarbazone

Semicarbazide hydrochloride (0.1M) and sodium acetate (0.2M) was added and dissolved in 15-20mL of distilled water placed in flat-bottomed flask. In a separate beaker containing required aromatic aldehyde (1) (0.1M) was dissolved in aldehyde free alcohol. This ethanolic aromatic aldehyde solution was added slowly to the solution of semicarbazide hydrochloride. The obtained precipitate was filtered, dried and recrystallized from 95% ethanol. TLC of the compounds were performed on silica gel-G using $CHCl_3$: CH_3OH (8:2) as solvent system. IR (KBr) cm^{-1}: 3283, 3168 (-CONH), 1703 (Amido carbonyl), 3061.8 (aromatic C-H), 1588, 1448 (-C=C-ring) , 759 (out of plane aromatic C-H bending).

2-Amino-5-aryl-1,3,4-oxadiazole

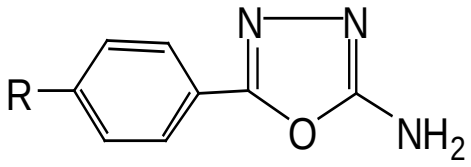
Semicarbazone (2) (0.1M) and sodium acetate (0.2M) was dissolved in 300-400 mL of glacial acetic acid with continuous stirring. Bromine (7 mL in 50 mL of GAA) was added slowly to it. Solution was stirred for an hour and then poured on crushed ice to separate the solid. The product was dried and recrystallized from ethanol (95%). TLC of the compounds were performed on silicagel-G using $CHCl_3$: CH_3OH (8:2) as solvent system. IR (KBr) cm^{-1}: 3412, 3290 (-NH str.), 1040 (C-O-C), 1661 (-C=N), 1594, 1481. ^{1}H-NMR (in □ ppm): 7.55-8.44 (m, 4-H, Ar-H), 7.44 (2H, Ar-NH_2).Physical and spectroscopic data is given in Table 1 and 2 respectively.



Scheme 1: Scheme for the synthesis of title compounds

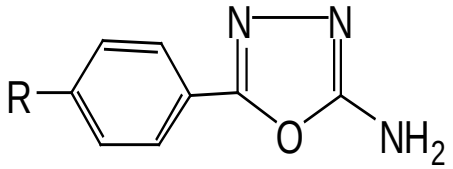
Compounds	R
A	*m*- chloro
B	*p*-chloro
C	*m*-bromo
D	*p*-bromo
E	*m*-methoxy
F	*p*-methoxy
G	*m*-nitro
H	*p*-nitro

Table 1: Physical data of synthesized compounds



R	Mol. Formula	M.P.	% Yield	Percentage Nitrogen and carbon Found/ (calculated)
m-chloro	$C_8H_6N_3OCl$	237-240^{0}C	73.06%	N=21.20 (21.40) C=48.90 (49.10)
p-chloro	$C_8H_6N_3OCl$	288-290^{0}C	75.04%	N=21.02 (21.40) C=49.07 (49.10)
m-bromo	$C_8H_6N_3OBr$	238-240^{0}C	83.06%	N=16.50 (17.50) C=39.80 (40.00)
p-bromo	$C_8H_6N_3OBr$	259-260^{0}C	81.4%	N=17.03 (17.50) C=39.89 (40.00)
m-methoxy	$C_9H_9N_3O_2$	256-258^{0}C	62.4%	N=20.80 (21.90) C=56.02 (56.54)
p-methoxy	$C_9H_9N_3O_2$	232^{0}C	86.03%	N=21.70 (21.90) C=56.25 (56.54)
m-nitro	$C_8H_6N_4O_3$	245-247^{0}C	83.0%	N=27.06 (27.18) C=46.42 (46.60)
p-nitro	$C_8H_6N_4O_3$	260-262^{0}C	78.78%	N=27.04 (27.18) C=46.56 (46.60)

Table 2: Spectral data of the synthesized compounds



R	νmax cm $^{-1}$	δ	m/z (Molecular ion)
m-chloro	3296.1 (-N-H), 3094.8, 3021.9 (aromatic -C-H), 1691.6 (-C=N), 1565.2, 1404.4 (-C=C ring), 1106.8 (aromatic-C-Cl str), 1215.1 (-C-O-C-)	7.38 - 7.76 (m,4H, Ar-H)	196(M+1)
p-chloro	3293.9 (-N-H), 3108.3 (aromatic-C-H), 1660.9(-C=N), 1596.3,1484.9 (-C=C-ring), 1039.8 (-C-O-C-), 1090.2 (aromatic -C-Cl)	7.57 - 7.80 (m,4H, Ar-H), 7.29 (2H, Ar- NH_2)	196 (M+1)
m-bromo	3290.4 (-N-H), 3020.4 (aromatic -C-H), 1652.3 (-C=N), 1595.3, 1565.6, 1474.4, (-C=C ring), 1214.5 (-C-O-C ring), 755.7 (aromatic -C-Br)	7.70-7.89 (4H, Ar-H), 7.37-7.52 (2H, Ar-NH_2)	241 (M+1)
p-bromo	3412 .6 (asymmetric -N-H), 3290.7 (symmetric -N-H), 3112.9 (aromatic -C-H), 1661.0 (-C=N), 1594.3, 1481.1,1396.4 (C=C ring), 583.2 (aromatic -C-Br)	7.72 (m,4H, Ar-H), 7.30 (2H, Ar-NH_2,)	241 (M+1)
m-methoxy	3469.2 (asymmetric -N-H), 3281.8 (symmetric -N-H), 3064.0 (aromatic -C-H), 1271.9 (asymmetric -C-O-C of aryl alkyl ether), 1018.1 (symmetric -C-O-C str of aryl alkyl ether), 1181.5 (-C-O-C)	6.86-8.14 (m,4H, Ar-H) 6.61 (2H, Ar-NH_2), 3.80-3.90 (3H, Ar-OCH_3)	192 (M+1)
p-methoxy	3315.5 (asymmetric -N-H), 3021.1 (aromatic -C-H), 1602.9 (-C=N), 1502.5, 1426.1 (-C=C ring), 1215.4 (-C-O-C),	7.06-7.11 (m,4H, Ar-H), 7.71-7.74(2H, Ar-NH_2), 3.81 (3H, Ar-OCH_3)	192 (M+1)
m-nitro	3415.3 (asymmetric -N-H), 3304.02 (symmetric -N-H), 1676.0 (-C=N), 1522.5 (asymmetric -N-O of Ar-NO_2), 1353.8 (symmetric –N-O- of Ar-NO_2), 1043.4 (-C-O-C)	7.55-847 (m,4H, Ar-H), 7.44 (2H, Ar-NH_2)	207 (M+1)

p-nitro	3272.1 (-N-H), 1657.1 (-C=N), 1530.0 (asymmetric -N-O of Ar-NO_2), 1338.1 (symmetric -N-O of Ar-NO_2), 1039.4 (-C-O-C), 856.7 (-C-N- of Ar-NO_2)	8.01-8.38 (m,4H, Ar-H), 7.52 (2H, Ar-NH_2)	207 (M+1)

Screening for Antimicrobial Activity

In the present study, liquid dilution method was used for the determination of minimum inhibitory concentration of the synthesized compounds. The MIC was taken as the lowest concentration (highest dilution) without visible growth. Bacterial strains of *Bacillus Subtilis* (MTCC-619), *Staphylococcus aureus* (MTCC-96), *Psuedomonas aeruginosa* (MTCC-424) and *Escherichia coli* (MTCC-40) and fungal strains of *Aspergillus niger* (MTCC-282) and *Candida albicans* (MTCC-227) were obtained from Institute of Microbial Technology, Chandigarh, INDIA. Nutrient broath was used as the growth medium for the bacteria and Sabourauds medium was used for *Aspergillus niger* and Malt yeast medium was used for *Candida albincans.* The incubation was done in electrically heated incubator at 37^{0}C for 48 h for bacteria and for 27^{0}C (for three days) for fungal culture.

Preparation of Solution of Synthesized Compounds

A stock solution of standard drug (100 µg/ml) was prepared in N,N-dimethyl formamide (DMF). The required concentration of the standard drug was prepared by diluting the stock solution by same solvent. A stock solution of each synthesized compound (1000 µg/ml) were prepared in N,N-dimethyl formamide (DMF) The required concentration of the synthesized compound for antibacterial studies were prepared by appropriate dilution of the stock solution by same solvent. A loopful of the original lyophilized microbial strain was transferred into the required medium aseptically and incubated at 37^{0}C for 48 h for bacteria and at 27^{0}C for three days for fungi (Table 3). The sterilization of the culture medium, culture tubes and other materials were done by autoclaving at 15 lb/sq. inch pressure for 20 min.

Determination of Minimum Inhibitory concentration (MIC)

A set of '6' sterilized test tubes were taken and different solutions were transferred aseptically to each test tube as per the quantities given in following Table 3. Test Tube No. 05, 06 was control. Test tube 05 contained bacteria but no inhibitor, to confirm the viability of the culture in the presence of DMF. Test tube 06 contained neither inhibitor nor organism, which confirmed the sterility of the culture. All the test tubes were incubated for the period as mentioned above and examined for growth of the test organism. The MIC of the test compound was between the lowest concentration inhibiting growth and the highest concentration allowing growth. These two concentrations for each compound were noted. The exact MIC of the each compound was determined by repeating the experiment, using range of concentration between these two concentrations.

Table 3: Concentration of the compounds used to determine MIC

Test Tube No.	Bacterial Strain (ml)	Test Compound (ml)	Nutrient Broth (ml)	Final conc. of test comp (µg/ml)	Solvent blank (DMF)
01	0.1	0.2	9.7	20	—
02	0.1	0.4	9.5	40	—
03	0.1	0.6	9.3	60	—
04	0.1	0.8	9.1	80	—
05	0.1	—	9.4	—	0.5 ml.
06	0.1	—	9.9	—	—

Table 4: Antibacterial and antifungal activities of the synthesized compounds (mic in µg/ml)

Compounds	Antibacterial activity in (µg/ml)				Antifungal activity in (µg/ml)	
R	*S. aureus*	*B. Subtilis*	*P.aeruginosa*	*E.coli*	*A.niger*	*C. Albicans*
3[a]	26	24	28	30	48	46
3b	20	22	24	28	42	44
3c	30	30	34	36	64	68
3d	28	28	30	36	62	62
3e	42	46	48	50	78	74
3f	38	42	46	44	74	72
3g	36	42	54	56	66	70
3h	34	40	52	52	62	54
Norfloxacin	6	8	8	4	-	-
Clotrimazole	-	-	-	-	6	8

RESULTS AND DISCUSSION

The percentage yield of the compounds, together with melting point and elemental (nitrogen and oxygen) analysis are reported in Table 1. The structure of the compounds was confirmed on the basis of IR. Compound (2) showed absorption bands at 3283, 3168 (-CONH), 1703 (Amido carbonyl), 3061 (aromatic C-H), 1588, 1448 (C=C ring), 785 (out of plane aromatic C-H bending). The structure of compounds were confirmed on the basis of m.p., IR, Mass and ^{1}H-NMR spectra. The entire spectral data specific for the group substituted or added to the nucleus of synthesized compounds are given in Table 2. A perusal of Table-4 showed that the MIC of the synthesized compound ranged between 20-56 µg/mL and 42-78 µg/mL for bactericidal and fungicidal activity respectively. A closer look at the table-4 showed that the introduction of a substituted benzaldehyde at position 5th of the 1,3,4-Oxadiazole nucleus improved the bactericidal activity of final compound (3) as against compound (2). As in the case of bactericidal activity their has been improvement in the fungicidal activity of the compounds with substituted benzeldehyde at position 5th of the nucleus. The most active compound was 3b. Presence of *p*-Cl in the phenyl ring at position 5th made compounds favorable for the cidal activity.

ACKNOWLEDGEMENT

The authors are thankful to RSIC, CDRI Lucknow for IR, ^{1}H-NMR and Nitrogen analysis and sophisticated Instruments division of NIPER, Chandigarh for Mass spectra.

REFERENCES

1. Zhang ZY, Chen LM Microwave-prompted Rapid and Efficient Synthesis of Diacyl Thiosemicarbazides and Semicarbazides in Solvent and Catalyst Free Condition. Chem J Chin Univ 1981; 50:1027-1030.
2. Chopleo CB, Myers PL, Smith ACB, Stillings MR, TullochI F, Walter DS Substituted 1,3,4-thiadiazoles with anticonvulsant activity. J Med Chem 1988; 31: 7-11.
3. Hill J., "*Comprehensive Heterocyclic Chemistry",* edited by A.R. Katritzky and C.W. Rees (Pergmon Press) 1986, 6, 267.
4. Adams SS, Cliffe EE, Lessel B, Nicholson JS Some biological properties of 2-(4-isoburylphenyl)-propionic acid. J Pharm Sci 1967; 56: 1986-1690.
5. Chimirri A, Grasso S, Monforte P, Fenech, G, Zappalà M, Monforte AM Compounds with potential anti-tumor activity VII. Synthesis and anti-tumor activity of 1-aryl-*N,N´*-di(1,3,4-thiadiazol-2-yl)methylenediamines. Eur J Med Chem 1989; 24: 131-135.
6. Miyahara M, Nakadate M, Tanno M, Kamiya S 1-(2,6-Dichlorophenyl)-N,N′-di(1,3,4-thiadiazol-2-yl) methylenedi amine 1, whose significant activity against P388. Chem Pharm Bull 1982; 30: 4402-4409.
7. Mehta DK, Das R, Dua K Synthesis, antimicrobial and anti-inflammatory activity of some new 1,3,4-oxadiazoles and 1,3,4-oxadiazole-2-thione derivatives as mannich bases containing furan moiety. Int J Chem Sci 2009; 7: 225-234.
8. Mishra AR, Singh DV, Mishra RM Synthesis and antifungal activity of new 1,3,4-oxadiazolo[3,2-b]-s-triazine-5-ones and their thiones analogues. Ind J Heterocycl Chem 2005; 14: 289-92.
9. Nagalakshmi G Synthesis, antimicrobial and anti-inflammatory activity of 2,5-disubstituted-1,3,4-oxadiazoles. Ind J Pharm Sci 2008; 70: 49-55
10. Katritzky A.R. and Rees C.W.*, "Comprehensive Heterocyclic Chemistry"*, I[st] ed., Pergamon Press, Oxford, 1984, 6, 445.