Research in Pharmaceutical Sciences, August 2017; 12(4): 330-336
Received: December 2016
Accepted: March 2017

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Synthesis and antimicrobial evaluation of some 2,5 disubstituted 1,3,4-oxadiazole derivatives

Elham Jafari[1], Tahereh Mohammadi[1], Ali Jahanian-Najafabadi[2], and Farshid Hassanzadeh[1,*]

[1]*Department of Medicinal Chemistry and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.*
[2]*Department of Pharmaceutical Biotechnology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.*

Abstract

1,3,4-oxadiazoles are interesting compounds because of their valuable biological effects such as cytotoxic, antibacterial, antifungal, and anti-tubercular activities. Ethyl mandelate was treated with hydrazine hydrate to yield the corresponding acylhydrazide. Some of the 2,5 disubstituted 1,3,4-oxadiazole derivatives were prepared from acylhydrazide using three different procedures. In the first procedure, acylhydrazide was reacted with nitro or chloro aroyle chloride to afford a diacylhydrazide which was cyclized to 2,5-disubstituted 1,3,4-oxadiazole in the presence of phosphoryl chloride as dehydrating agent. In the second procedure, furan-oxadiazole derivative was directly prepared from carboxylic acid and acylhydrazide in one step. In the third procedure, acyl hydrazide was condensed with 5-nitrofuraldehyde to yield 5-nitrofuran-2-yl) methylene)-2-phenyl acetohydrazide intermediate which was cyclized to form the nitrofuran-oxadiazole derivative by acetic anhydride as dehydrating agent. The structures of these compounds have been elucidated by spectral IR and ^{1}H-NMR analysis. All the newly synthesized compounds were screened for their antibacterial and antifungal activities. Compounds **F_3** and **F_4** showed remarkable antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* bacteria.

Keywords: Oxadiazole; Mandelate; Antimicrobial

INTRODUCTION

Infectious diseases are responsible for nearly one-half of all deaths in tropical countries. Although deaths from bacterial and fungal infections have declined in the developed countries, these infections are still major problem in undeveloped territories. One of the attractive backbones for scientists in production of new therapeutic agents is oxadiazole structure (1-4). There are four known isomers of this five-membered heterocycle including: 1,2,4-, 1,2,3-, 1,2,5-, and 1,3,4-oxadiazole (5,6). However, 1,3,4-oxadiazole is more important because of its remarkable biological activities. Compounds containing 1,3,4-oxadiazole structure possess various pharmacological effects including antibacterial, antifungal (1,4-10), anti-tubercular (11), anticonvulsant, anti-allergic, anti-inflammatory, cytotoxic, and insecticidal activities (1,6,12). This structure can be used as a bioisoester for carboxylic acids, esters, and carboxamides (6). Tiodazosin, nosapidil, and furamizole are derivatives of oxadiazole which have been introduced as antihypertensive and antibacterial agents, respectively (12). Various 2,5 substituted 1,3,4-oxadiazoles have been shown to be active against a wide range of gram-positive and gram-negative bacteria (6,13,14).

It is generally accepted that lipophilic substitutions may facilitate transport of drug molecules through biological membrane of microorganisms thereby improving their antimicrobial activities (10,15). The presence of electronegative groups such as Cl or NO_2 on phenyl ring enhances the antimicrobial activities of drug moieties (10,14,15).

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.212051

*Corresponding author: F. Hassanzadeh
Tel: +98-3137927096, Fax: +98-3136680011
Email: Hassanzadeh@pharm.mui.ac.ir

Substitution of oxadiazoles with nitro furan ring has shown significant antibacterial activities against staphylococcal strains at 4 to 32 μg/mL (6).

Many methods have been reported for the synthesis of both symmetrical and asymmetrical 2,5-diaryl(alkyl)-1,3,4-oxadiazoles. One of the methods involves hydrazinolysis of the ethyl ester with hydrazine hydrate to yield the corresponding acylhydrazide. The resultants can be reacted with different aroyle chloride to afford intermediates which can be subsequently cyclized to 2,5-disubstituted 1,3,4-oxadiazole in the presence of a suitable dehydrating agent like phosphoryl chloride. Other dehydrating agents commonly used are CF_3COOH, H_2SO_4, P_2O_5, H_3PO_4, PCl_5 (10,13). Literature survey revealed that some oxadiazoles could be directly prepared from carboxylic acids and acylhydrazides in one step which involves longer reaction time and production of by-products (13,14,16). Another method is reaction of tetrazoles with acyl chlorides in the presence of pyridine (12). Therefore, the procedure started with N,N-diacyl hydrazines intermediate which is easier and more convenient to get target compounds.

Mandelic acid (MA) is an alpha-hydroxy acid used as an antibacterial in the treatment of urinary tract and skin infections such as acne. Some of the MA derivatives have shown antibacterial activities against *Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa),* and *Candida albicans (C. albicans)* (17,18).

Due to the remarkable antimicrobial effects of many 1,3,4-oxadiazole and MA derivatives, some novel structural hybrids of 1,3,4-oxadiazole derivatives and benzyl group derived from ethylmandelate were synthesized and evaluated for their antibacterial and antifungal activities.

MATERIALS AND METHODS

Chemistry

Melting points were determined in open capillaries using electrothermal 9200 melting point apparatus (England) and are uncorrected. IR (KBr discs) was recorded with a WQF-510 FT-IR spectrophotometer (China). ^{1}H-NMR spectra were recorded on Bruker 400 MHz spectrometers (Germany) using tetramethyl-silane (TMS) as internal standard and either DMSO-d6 or $CDCl_3$ as solvents. Mass spectra were recorded on Finnigan TSQ-70 Mass spectrometer (United States). All chemicals were purchased from Merck Company (Germany).

Synthesis of compounds

In this study, we have synthesized some new 1,3,4-oxadiazole derivatives substituted at 2,5 positions. Ethyl mandelate was reacted with hydrazine hydrate to obtain acylhydrazide as crystalline product. Acylhydrazide was reacted with 4-chlorobenzoyl chloride or 4-nitro benzoyl chloride to afford intermediate which subsequently was cyclized to 2,5-disubstituted 1,3,4-oxadiazole in the presence of a suitable dehydrating agent like phosphoryl chloride. These compounds were re-crystallized by ethanol to obtain target compounds (12,13). In another procedure, acylhydrazide and furan-2-carboxylic acid refluxed in $POCl_3$ for 1 h on water bath. The reaction mixture was poured into crushed ice with stirring. The resultant solid was collected, and purified by column chromatography (19-22) (Fig. 1).

Reaction between acylhydrazide and 5-nitro-2-furaldehyde in ethanol (95%) with glacial acetic acid as the catalyst produced N-acylhydrazone in high yield and purity. Treatment of N-acylhydrazone with acetic anhydride afforded oxadiazole derivative substituted with nitrofuran (23,24) (Fig. 1). The structures of synthesized compounds were confirmed by IR and ^{1}H-NMR.

Details of preparation procedures of synthesized compounds

*2-hydroxy-2-phenyl-acetohydrazide (**I$_1$**)*

Ethyl mandelate (100 mmol, 13.8 mL) was added dropwise to a solution of hydrazine hydrate (100 mmol, 3.2 mL) in ethanol (8 mL) and was refluxed for 4 h. The ethanol was partially removed by vacuum distillation. On cooling the resultant mixture, white needle crystals of 2-hydroxy-2-phenyl-cetohydrazide began to separate. It was collected, filtered, and recrystallized from ethanol.

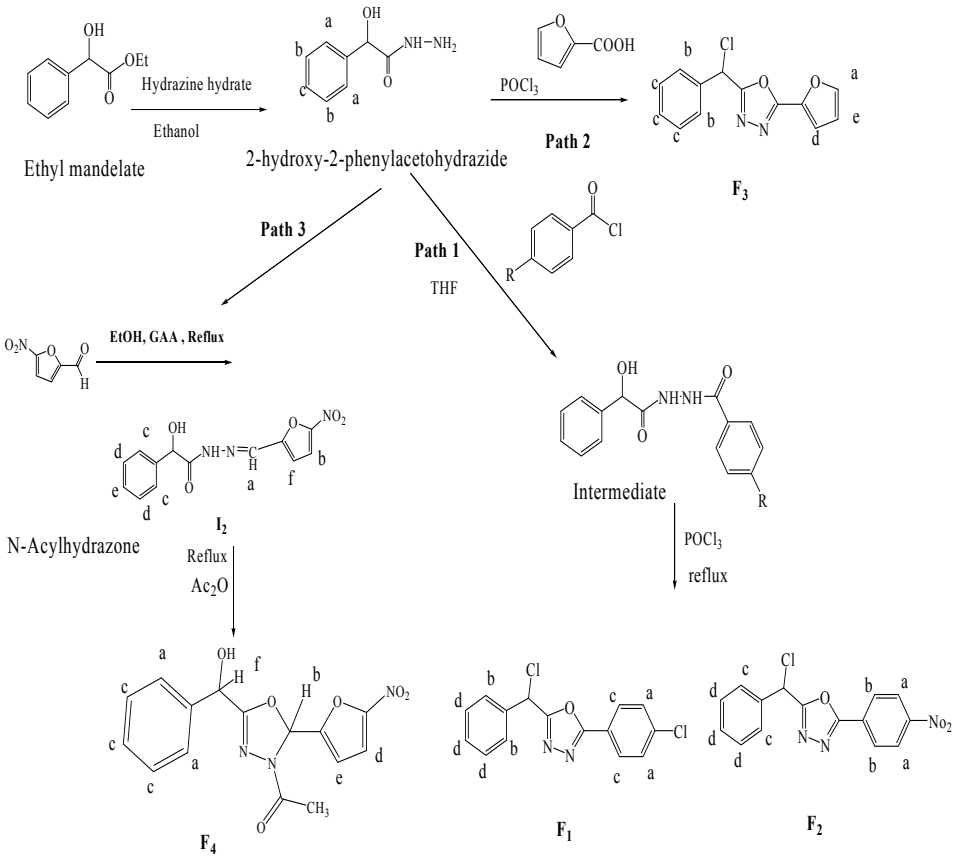

Fig. 1. General reaction scheme for preparation of the final compounds; GAA stands for glacial acetic acid.

Diacyl hydrazide intermediates

A solution of the 4-chloro benzoyl chloride (2 mmol, 350 mg) or 4-nitro-benzoyl chloride (2 mmol, 370 mg) in tetrahydrofuran (THF) (3 mL) was added dropwise to the mixture of 2-hydroxy-2-phenyl-acetohydrazide (2 mmol, 330 mg) and THF (5 mL) over 1 h, while stirring at 0 °C. The reaction mixture was stirred for 3 h at room temperature. The solvent was removed in vacuum to furnish a semisolid material which was washed with saturated aqueous $NaHCO_3$ solution then extracted with ethyl acetate. The organic layer was evaporated to afford the intermediate.

*2,5-disubstituted 1,3,4-oxadiazoles (**F_1** and **F_2**)*

A mixture of obtained diacyl hydrazide of 4-chloro benzoyl chloride (0.66 mmol, 200 mg) or 4-nitro-benzoyl chloride (0.67 mmol, 200 mg) and $POCl_3$ (4 mL) was refluxed for 2-3 h. After cooling to room temperature, the reaction mixture was gradually poured into crushed ice with stirring. The aqueous layer was extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate, and finally distilled under reduced pressure to remove the solvent. The resulting solid was recrystallized using ethanol to give the target compounds.

*2-(Chloro(phenyl)methyl)-5-(furan-2-yl)-1,3,4-oxadiazole (**F_3**)*

A mixture of 2-hydroxy-2-phenyl-acetohydrazide (6 mmol, 990 mg) and the furan-2-carboxylic acid (5 mmol, 560 mg) in $POCl_3$ (4 mL) was heated to 70 °C for about 3 h on water bath. The progress of the reaction was monitored on TLC. The reaction mixture was poured into crushed ice with stirring. The resultant solid was collected, washed with water, and purified by column chromatography chloroform:methanol 19:1 to afford an orange powder (40% yield).

*2-Hydroxy-N'-((5-nitrofuran-2-yl) methylene)-2-phenylacetohydrazide (**I_2**)*

To a stirred solution of 2-hydroxy-2-phenyl-acetohydrazide (1 mmol, 160 mg) and 5-nitrofuraldehyde (1 mmol, 140 mg) in

ethanol, glacial acetic acid (0.2 mL) was added dropwise.

The resulting mixture was refluxed for 5 h, after which the solution was poured into ice water. The precipitate was collected by filtration and dried under reduced pressure to give hydrazone intermediate as brown solid (89% yield).

*1-(5-(Hydroxy (phenyl) methyl)-2-(5-nitrofuran-2-yl)-1,3,4-oxadiazole-3(2H)-yl)ethanone (**F₄**)*

5-Nitrofuran derivative of oxadiazole was synthesized by addition of acetic anhydride (2 mL) to hydrazone (0.34 mmol, 100 mg), and the resulting solution was refluxed for 1 h. The reaction mixture was poured into ice water, and the resulting solid was filtered, washed with water, and purified by PTLC to obtain a yellow powder**,** (54% yield).

Antimicrobial activity

The employed microorganisms for antimicrobial studies were *S. aureus,* PTCC (Persian Type Culture Collection) 1337 as gram-positive*; Escherichia coli (E. coli),* PTCC 1338 and *P. aeruginosa*, PTCC 1074 as gram-negative bacteria; and *C. albicans,* PTCC 5027 as fungus. Mueller Hinton agar, Mueller Hinton broth and Sabouraud dextrose agar were purchased from Merck (Germany). RPMI1640 culture medium was purchased from Gibco (USA).

Microplate alamar blue assay for antimicrobial evaluation

The inocula of bacterial and fungal strains (1.5×10^8 CFU/mL) were prepared from Mueller Hinton agar and Sabouraud dextrose agar cultures, respectively. Prepared suspensions of bacteria were adjusted to 0.5 McFarland standard turbidity and the fungal suspensions turbidity was measured spectrometrically at 580 nm.

Finally, prepared inoculum densities for bacterial and fungal strains were equal to 1.0×10^5 CFU/mL and 1.0×10^6 CFU/mL, respectively.

The Synthesized compounds were dissolved in DMSO (0.5 mL) and diluted with water up to 1 mL to obtain concentration of 5120 µg/mL as stock solutions. The stock solution was serially diluted to give concentrations of 2560 to 40 µg/mL.

Bacterial suspension (20 µL) was distributed in all 96 wells of microplate. Then 20 µL of each concentration of the compounds were added to wells with the exception of those wells acting as positive control (containing standard antibiotic) and growth control (containing culture media without testing materials). After adding alamar blue (20 µL) to all 96 wells the total volume in each well reached to 200 µL using culture medium. The final concentrations of the compounds in the wells were 512, 256, 32, 16, 8, and 4 µg/mL. After incubation, the lowest concentration which required inhibiting the growth of microorganism was regarded as minimum inhibitory concentration (MIC). The test was carried out in triplicates.

Following a broth micro dilution MIC test, from each well that showed no growth, contents were removed and spread onto Mueller Hinton agar plates for bacteria and Saboraud dextrose agar for fungi to determine minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC).

RESULTS

Chemistry of synthesized compounds

*2-Hydroxy-2-phenyl-acetohydrazide (**I₁**)*

White crystal, (89% yield), m.p.: 125-128 °C (lit.132-133 °C), ($C_8H_{10}N_2O_2$); IR (KBr, cm^{-1}), 3338 (NH_2), 3208 (N-H), 3062 (C-H Ar), 1695 (C=O), ^{1}HNMR, δH (400 MHz; DMSO), 9.24 (1H, s, NH), 7.50 (2H, d, J = 7.2 Hz, H^a, Ar), 7.40 (2H, t, J = 7.2 Hz, H^b, Ar), 7.34 (1H, t, J = 7.2 Hz, H^c, Ar), 6.05 (1H, d, J = 5.2 Hz, CH), 5.01 (1H, d, J = 5.2 Hz, OH), 4.3 (2H, s, NH_2).

*2-(Chloro (phenyl) methyl)-5-(4-chlorophenyl)-1, 3, 4-oxadiazole (**F₁**)*

Light yellow, (50% yield), m.p.: > 265 °C, MS (m/z,): found: 305 $C_{15}H_{10}Cl_2N_2O$; requires: 305, IR (KBr, cm^{-1}), 3010 (CH, Ar), 1012 (C-O-C, ether), 1599 (C=C), 850 (C-Cl). ^{1}HNMR, δH (400 MHz; $CDCl_3$), 8.1 (2H, d, J = 8 Hz, H^a, Ar), 7.74 (2H, dd, J = 2 Hz, J = 6.4 Hz, H^b, Ar), 7.6 (2H, d, J = 8Hz, H^c, Ar), 7.54 (3H, m, H^d, Ar), 6.3 (1H, s, oxadiazole ring -C<u>H</u>-Cl).

*2-(Chloro (phenyl) methyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (**F_2**)*

Pale yellow (45% yield), m.p.:154-155 °C, MS (m/z): found 315 $C_{15}H_{10}ClN_3O_3$; requires: 315, IR (KBr, cm^{-1}), 3103 (CH, Ar), 1338, 1516 (NO_2), 1012 (COC, ether), 700 (C-Cl), ^{1}H-NMR, δH (400 MHz; $CDCl_3$),8.38 (2H, d, *J* = 7.2Hz, H^a, Ar), 8.26 (2H, d, *J* = 7.2 Hz, H^b, Ar), 7.64 (2H, dd, *J* = 2Hz, *J* = 8 Hz, H^c, Ar), 7.45 (3H, m, H^d, Ar), 6.30 (1H, s, oxadiazole ring -CH-Cl).

*2-(Chloro(phenyl)methyl)-5-(furan-2-yl)-1,3,4-oxadiazole (**F_3**)*

Orange powder (40% yield), m.p.: 227.5-228.3 °C, $C_{13}H_9ClN_2O_2$, IR (KBr, cm^{-1}), 1103, 1024 (C-O-C, ether), 806 (C-Cl). ^{1}HNMR, δH (400 MHz; $CDCl_3$),7.58 (1H, dd, *J* = 4 Hz, *J* = 0.4 Hz, H^a, furan), 7.55 (2H, dd, *J* = 8 Hz, *J* = 4 Hz, H^b, Ar), 7.35 (3H, m, H^c, Ar), 7.1 4 (1H, dd, *J* = 4 Hz, *J* = 0.4 Hz, H^d, furan), 6.53 (1H, m, H^e, furan), 6.19 (1H, s, oxadiazole ring-CH-Cl).

*2-Hydroxy-N'-((5-nitrofuran-2-yl) methylene)-2-phenylacetohydrazide (**I_2**)*

Brown solid (89% yield), m.p.: 178-180.5 °C, ($C_{13}H_{11}N_3O_5$); IR (KBr, cm^{-1}), (3400, OH), 3216 (NH), 1674 (C=N), 1300, 1550 (NO_2), ^{1}HNMR, δH (400 MHz; DMSO), 11.89 (1H, s, NH,),8.44 (1H, s, H^a),7.77 (1H, d, *J* = 4Hz, H^b, nitro furan ring), 7.48 (2H, d, *J* = 7.2 Hz, H^c, Ar), 7.36 (2H, t, *J* = 7.2 Hz, H^d, Ar), 7.3 (1H, t, *J* = 7.2 Hz, H^e, Ar), 7.19(1H, d, *J* = 4 Hz, H^f, nitrofuran ring), 6.52 (1H, d, *J* = 4.4 Hz, C<u>H</u>), 5.13 (1H, d, *J* = 4.4 Hz, O<u>H</u>).

*1-(5-(Hydroxy (phenyl) methyl)-2-(5-nitrofuran-2-yl)-1,3,4-oxadiazole-3(2H)-yl)ethanone (**F_4**)*

Yellow powder**,** (54% yield), m.p.:182-183 °C, ($C_{15}H_{13}N_3O_6$); IR (KBr, cm^{-1}), 3427 (OH), 1685 (C=N), 1236 (COC, ether), 1352, 1560, (NO_2), ^{1}HNMR, δH (400 MHz; $CDCl_3$), 9.07 (1H, s, OH),7.63 (2H, d, *J* = 8Hz, H^a, Ar),7.54 (1H, s, H^b),7.36-7.43 (4H, m, H^c and H^d),6.85 (1H, d, *J* = 4Hz, H^e, nitrofuran ring), 6.69 (1H, s, H^f), 2.22 (3H, s, CH_3).

Antimicrobial results

The MIC of all the tested compounds was evaluated at concentrations of 4 to 512 µg/mL. Compounds **F_3**, **F_4**, and **I_2** showed significant inhibition at 8, 4, and 4 µg/mL concentrations, respectively against *S. aureus* as gram-positive bacteria. **F_3**, **F_4**, and **I_2** exhibited maximum activity at 16, 16, and 8 µg/mL, respectively, against *E. coli* as gram-negative bacteria. Compounds **F_1** and **F_2** displayed high fungi static and fungicidal activities against *C. albicans* at 32 µg/mL concentration. Results of MIC, MBC, and MFC are depicted in Tables 1 and 2.

Table 1. MIC results of synthesized compounds against bacteria and fungi.

No	Gram-positive bacteria MIC (µg/mL)	Gram-negative bacteria MIC (µg/mL)		Fungus MIC (µg/mL)
	S. aureus	***E. coli***	***P. aeruginosa***	***C. albicans***
I_1	-	-	-	512
F_1	-	-	512	32
F_2	-	-	512	32
F_3	8	16	-	-
F_4	4	16	-	-
I_2	4	8	512	-

Ciprofloxacin 50 µg/mL (standard antibacterial agent) and ketoconazol 50 µg/mL (standard antifungal agent).

Table 2. MFC and MBC results of synthesized compounds against bacteria and fungi**.**

No	Gram-positive bacteria MBC	Gram-negative bacteria MBC		Fungus MFC
	S. areus	***E. coli***	***P. aeruginosa***	***C. albicans***
I_1	NA	NA	NA	512
F_1	NA	NA	-	32
F_2	NA	NA	-	32
F_3	16	32	NA	NA
F_4	8	16	NA	NA
I_2	4	8	NA	NA

NA: Not applicable.

DISCUSSION

Oxadiazoles constitute an important class for new drug development with various biological properties (1,4-12). Substituted 1,3,4-oxadiazoles at 2,5 positions are particularly important because of their antimicrobial activities against a wide range of gram-positive and gram-negative bacteria (6,13,14,25,26).

Structure-activity relationships have shown that presence of electronegative groups such as Cl or NO_2 on phenyl ring can enhance their antimicrobial effects (10,14,15). Substituted oxadiazole with nitro furan or furan ring showed significant antimicrobial activities (6). Due to valuable antimicrobial effects of oxadiazole scaffold, in this study, a new series of 2,5 disubstituted 1,3,4-oxadiazoles derivatives were synthesized and their antimicrobial activity assessed using microplate alamar blue assay.

Substitution of chlorine or nitro group on the phenyl ring (**F_1** and **F_2**) rendered reasonable antifungal activity compared to other tested compounds. While the furan-derivatives (**F_3** and **F_4**) showed remarkable antibacterial activities.

The results of antibacterial screening showed that compounds **F_3**, **F_4**, and **I_2** (furan and nitro furan derivatives) had the highest activities against *S. aureus* at 8, 4, and 4 µg/mL concentrations. It seems that presence of hydroxyl group of mandelate residue in compound **F_4** and **I_2** might improve activities in gram-positive bacteria. These compounds **F_3**, **F_4**, and **I_2** showed the best activities against *E. coli* at 16, 16, and 8 (µg/mL) concentrations, respectively. Presence of a para-substituted-phenyl group at position 5 of oxadiazole in compounds **F_1** and **F_2** may be responsible for fungistatic and fungicidal activities against *C. albicans* in comparison with furan derivative of oxadiazole.

CONCLUSION

Oxadiazole derivatives preparation from N, N-diacylhydrazine intermediate in two steps is easier and more convenient than direct preparation from carboxylic acids and acylhydrazides in one step. Direct one-step method with mild condition was used for the synthesis of furan-oxadiazole derivative. Condensation reaction between the 2-hydroxy-2-phenyl-acetohydrazide and 5-nitro-2-furaldehyde in ethanol (95%) with glacial acetic acid as the catalyst produced N-acylhydrazone in high yields. Nitro-furan-oxadiazole compound easily obtained by reacting N-acylhydrazone with acetic anhydride in moderate yield. According to the antimicrobial results, furan-derivatives of oxadiazoles showed potential antibacterial activities and a para-substituted phenyl derivatives of oxadiazole exhibited significant antifungal activities.

ACKNOWLEDGEMENTS

The content of this paper is extracted from the Pharm. D thesis (No. 293189) which was financially supported by the Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, I.R. Iran

REFERENCES

1. Sahu VKR, Singh AK, Yadav D. Review article on 1,3,4-oxadiazole derivatives and its pharmacological activities. Int J ChemTech Res.2011;3(3):1362-1372.
2. Husain A, Ajmal M. Synthesis of novel 1, 3, 4-oxadiazole derivatives and their biological properties. Acta Pharm. 2009;59(2):223-233.
3. Singh AK, Lohani M, Parthsarthy R. Synthesis, characterization and anti-inflammatory activity of some 1,3,4-oxadiazole derivatives. Iran J Pharm Res. 2013;12(2):319-323.
4. Joshi SD, Vagdevi HM, Vaidya VP, Gadaginamath GS. Synthesis of new 4-pyrrol-1-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: A novel class of potential antibacterial and antitubercular agents. Eur J Med Chem. 2008;43(9):1989-1996.
5. Redhu S, Kharb R. Recent updates on chemistry and pharmacological aspects of 1, 3, 4-oxadiazole scaffold. Int J Pharm Innov. 2013;3(1):93-110.
6. de Oliveira CS, Lira B, Barbosa-Filho JM, Lorenzo JG, de Athayde-Filho PF. Synthetic approaches and pharmacological activity of 1,3,4-Oxadiazoles: a review of the literature from 2000-2012. Molecules. 2012;17(2):10192-10231.
7. Bhat K, Sufeera K, Chaitanya SK. Synthesis, characterization and biological activity studies of 1, 3, 4-Oxadiazole analogs**.** J Young Pharm. 2011;3(4):310-314.

8. Azzawi AMA, Al-Obiadi KKH. Synthesis and antimicrobial screening new bisshiff bases and their acetyl oxadiazole azetidinone derivatives from pyromelliticdiimide. Int J Res Pharm Chem. 2016;6(1),1-8.
9. Malhotra M, Sanduja M, Samad A, Deep A. New oxadiazole derivatives of isonicotinohydrazide in the search for antimicrobial agents: synthesis and *in vitro* evaluation. J Serb Chem Soc. 2012;77(1):9-16.
10. Singh I, Kumar A. Synthesis and antibacterial activity of 2-(substituted phenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazoles. Chem Sci Trans. 2015;4(1): 133-136.
11. Yar MS, Siddiqui AA, Ali MA. Synthesis and anti tuberculostatic activity of novel 1,3,4-Oxadiazole derivatives. J Chin Chem Soc. 2007;54(1):5-8.
12. Singh R, Chouhan A. Various approaches for synthesis of 1, 3, 4-Oxadiazole derivatives and their pharmacological activity. World J Pharm Pharm Sci. 2014;3(10):1474-1505.
13. Zheng X, Li Z, Wang Y, Chen W, Huang Q, Liu C, Song G. Syntheses and insecticidal activities of novel 2,5-disubstituted 1,3,4-oxadiazoles. J Fluorine Chem. 2003;123(2):163-169.
14. Kumar R, Yar MS, Rai AK, Chaturvedi S. Synthesis and biological evaluation of some novel 1, 3, 4-oxadiazoles derived from bi phenyl 4- carboxylic acid. Der Pharmacia Lettre. 2013;5(1):366-370.
15. Salih N, Salimon J, Hameed A, Yousif E. Synthesis, characterization and antimicrobial evaluation of some 1,3,4-oxadiazole derivatives. Int J PharmTech Res. 2011;3(2):1097-1102.
16. Khan K, Rani M, Ambreen N,Muhammad A, Hussain S, Perveen S, *et al*. 2, 5-disubstituted-1,3,4-oxadiazoles: thymidine phosphorylase inhibitors. Med Chem Res. 2013;22:6022-6028.
17. Motamedifar M, Bazargani A, Namazi MR, Sarai E, Sedigh H. Antimicrobial activity of mandelic acid against methicillin-resistant *Staphylococcus aureus*: a novel finding with important practical implications. World Appl Sci J. 2014;31(5):925-929.
18. Stan CD, Ştefanache A, Tataringa G, Dragan M, Tuchilus CG. Microbiological evaluation and preservative efficiency of new mandelic acid derivatives in ointments. FARMACIA. 2015;63(4):577-580.
19. Isobe T, Tsutomu I. 2-chloro-1,3-dimethyl-imidazolinium chloride. 2. Its application to the construction of heterocycles through dehydration reactions. J Org Chem. 1999;64(19):6989-6992.
20. Shridhar A. Synthesis of some novel bis 1,3,4-oxadiazole fused azo dye derivatives as potent antimicrobial agents. Int Res J Pure Appl Chem. 2011;1(3):119-129.
21. Shi W, Qian X, Song G, Zhang R, Li R. Shynthesis and insecticidal activities of novel 2-fluorophenyl-5-aryl/cyclopropyl-1,3,4-oxadiazoles. J fluor chem. 2000;106(2):173-179.
22. Shet Prakash M, Vaidya VP. Synthesis, characterization and antimicrobial studies of novel N-substituted napthofuran carboxamides. J Pharm SciTec. 2011;3(10):996- 1002.
23. de Oliveira CS, Lira BF, dos Santos Falcão-Silva V, Siqueira-JP JR, Barbosa-Filho JM, de athayde-Filho PF. Synthesis, molecular properties prediction, and anti-staphylococcal activity of N-Acylhydrazones and new 1,3,4-oxadiazole derivatives. Molecules. 2012;17(5):5095-5107.
24. Lee L, Robb LM, Lee M, Davis R, Mackay H, Chavda S, *et al*. Design, synthesis, and biological evaluations of 2,5-diaryl-2,3-dihydro-1,3,4-oxadiazoline analogs of combretastatin-A4. J Med Chem. 2010;53(1):325-334.
25. Vittal S, Poojary B, Bansal P, Nandagokula C, Tangavelu AM, Shenoy S. Synthesis, characterization and antioxidant activity of some 1,3,4-oxadiazoles carrying 4-(methylsulfonyl)benzyl moiety. Der Pharma Chemic. 2011;3(6):138-146.
26. Prasanna Kumar BN, Mohana KN, Mallesha L, Harish KP. Synthesis and *in vitro* antimicrobial evaluation of new 1,3,4-Oxadiazoles bearing 5-chloro-2-methoxyphenyl moiety. Int J Med Chem. 2013;2013:725673.