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Pharmaceutical Sciences **RESEARCH ARTICLE……!!!**

MICROWAVE ASSISTED SYNTHESIS, CHARACTERIZATION AND EVALUATION OF THE ANTI-MICROBIAL ACTIVITY OF 2,5-DISUBSTITUTED-1,3,4-OXADIAZOLE

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KEYWORDS:

2,5-disubstituted-1,3,4-oxadiazoles, Anti-microbial activity.

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ABSTRACT

The synthesis of 2-furyl-5-(substituted)-1,3,4-oxadiazoles was carried out by microwave irradiation of 2-furoic acid and ethanol followed by subsequent hydrazinolysis with hydrazine hydrate. Finally furan-2-acid hydrazide was treated with appropriate carboxylic acid in the presence of phosphorous oxychloride to produce title compounds. The structures of the newly synthesized compounds were established on the basis of physicochemical analysis and spectral analysis such as IR, H^1NMR and Mass spectral data.

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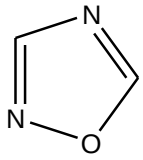
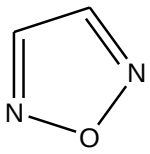
INTRODUCTION:

The use of microwave irradiation in organic chemistry has exploded over the last few years. Two of the main advantages of this technology are the potential for dramatically shortened reaction times and access to reaction conditions that are not attainable under conventional thermal heating. Combining the speed of microwave assisted synthesis with the statistical design of experiments affords a powerful tool for the rapid and comprehensive development of optimized reaction conditions. Herein, we report the application of this approach to the development and synthesis of 1,3,4-oxadiazoles.

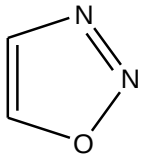
Oxadiazoles are five membered heterocyclic compounds with two nitrogen atoms and one oxygen atom. Depending on the positions of hetero atoms, they are named as 1,2,3; 1,2,4; 1,2,5; 1,3,4-oxadiazoles .1,2,4-Oxadiazole, 1,2,5-Oxadiazole, and 1,3,4-Oxadiazole are known, but the 1,2,3-isomer is unstable and reverts to the diazoketone tautomer. The stable oxadiazoles appear in a variety of pharmaceutical drugs including raltegravir, butalamine, fasiplon, oxolamine, and pleconaril.



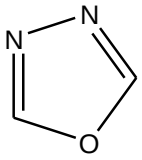
1,2,4-Oxadiazole



1,2,5-Oxadiazole



1,2,3-Oxadiazole



1,3,4-Oxadiazole

1,3,4-oxadiazoles are known to possess anti-bacterial, anti-fungal, anti-inflammatory, anti-tubercular, anticancer, anti-convulsant and analgesic activities. Similarly , the furan moiety are known to display antibacterial, antifungal, antihypertensive, diuretic activities and are also useful in stomach, renal, biliary and colic disorders. In view of this, an attempt was made to incorporate the 1,3,4-oxdiazoles with the furan moiety to probe how this combination will influence the biological activity.

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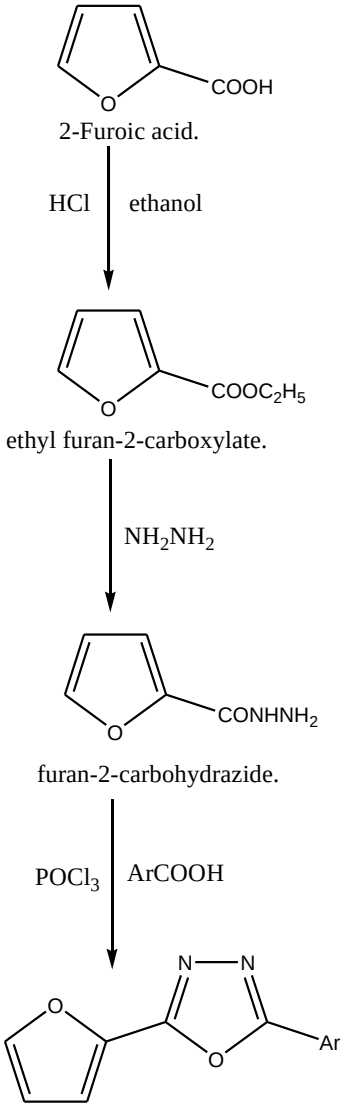
MATERIALS AND METHODS

SYNTHETIC SCHEME:[13-17]



2,5-disubstituted-1,3,4-oxadiazole.

4(a-g)

EXPERIMENTAL WORK.[18-22]

I. PREPARATION OF FURAN-2-CARBOXYLIC ACID ETHYL ESTER.

In a double necked flask, a mixture of 11.2g furoic acid and 60ml ethanol were refluxed by microwave irradiation at 360W for 12mins. The reaction is catalyzed using 1ml HCl.

II. PREPARATION OF 2-FUROIC ACID HYDRAZIDE.

A mixture of ethyl-2-furoate (2g) and hydrazine hydrate (6.9ml) were directly irradiated under microwave without any solvent for 60-100 sec. at 360W. The yield of the hydrazide is 79-90%.

III. PREPARATION OF 2-FURYL-5(SUBSTITUTED)-1,3,4-OXADIAZOLE DERIVATIVES.

A mixture of furoic acid hydrazide (0.01mole), aromatic acid (0.01 mole) and phosphorous oxychloride were taken in a double necked round bottom flask. The reaction mixture is irradiated for 6 min. at 210 watts. The reaction mixture is cooled and poured into crushed ice. It was then neutralized with sodium bicarbonate and the resulting solid was filtered, dried and recrystallized with methanol.

Melting point of the synthesized compounds was taken with the help of Thiele tube apparatus. Purity of the compounds was checked by TLC using Silica gel G as the stationary phase and ethyl acetate: acetone (9:1) as the mobile phase. The spot is visualized by using iodine vapors or U.V. light. The physical property data of the synthesized compounds has been given.

The IR spectra of all the compounds were recorded in FT-IR (Model: Shimadzu IR Affinity-1) using KBr pellets in the region of 4000-500 cm^{-1}. The H^1NMR spectra were recorded in Bruker Avaze III at a frequency of 400 MHz and the Mass spectra were recorded on Varian 1200 L Single Quadrupole. The characterization data of the oxadiazole derivatives has been given.

RESULTS AND DISCUSSIONS

PHYSICAL PROPERTY DATA OF OXADIAZOLE DERIVATIVES.

S.NO.	Compound.	Derivatives	Mol. Formula	M.P. (˚C)	Yield.	Rf values.	Physical state
1.	4.a)	p-chlorobenzoic acid.	$C_{12}H_7N_2O_2Cl$	111-102	82%	0.56	Yellow crystals.
2.	4.b)	p-nitrobenzoic acid.	$C_{12}H_7N_3O_4$	230-231	72%	0.62	Yellow crystals.
3.	4.c)	3,5-dinitro-benzoic acid.	$C_{12}H_6N_4O_6$	147-148	74%	0.69	Yellow crystals.
4.	4.d)	Benzoic acid	$C_{12}H_8N_2O_2$	95-96	68%	0.52	White crystals.
5.	4.e)	o-aminobenzoic acid	$C_{12}H_9N_3O_2$	107-108	62%	0.49	Orange crystals.
6.	4.f)	p-hydroxy-benzoic acid.	$C_{12}H_8N_2O_3$	121-122	60%	0.55	Yellow crystals.
7.	4.g)	Salicylic acid.	$C_{12}H_8N_2O_3$	120-121	72%	0.53	Yellow crystals.

CHARACTERIZATION:

The structure of the oxadiazoles (4.a) to 4.b)) has been confirmed by the IR, H^1NMR and Mass spectral data. The Characterization data of the synthesized compounds has been reported as below:-

4. a) 2-furyl-5-(p-chlorophenyl)-1,3,4-oxadiazole

IR (KBr) (cm^{-1}): 3001.54 (C-H), 1620.27 (C=N), 1481.33 (C=C), 1095 (C-O), 840.96 (disubstitution at para position), 740.67 (C-Cl). H^1NMR (DMSO-d_6, 400 MHz), δ (ppm): 8.098-7.694 (m, 4H, CH), 7.692-7.690 (d, 1H, CH), 7.469-7.452 (t, 1H, CH), 6.838-6.834 (d, 1H, CH). GC-MS (m/z): 246 (M^+).

4. b) 2-furyl-5-(p-nitrophenyl)-1,3,4-oxadiazole.

IR (KBr) (cm^{-1}): 3070.68 (C-H), 1635.64 (C=N), 1527.62 (C-C), 1450.47 (C=C), 1527 & 1350 (N=0), 1103.28 (C-O), 1018.41(C-O-C), 840.96 (disubstitution at para position). H^1NMR (DMSO-d_6, 400 MHz), δ (ppm): 8.094-7.695 (m, 4H, CH), 7.691-7.689 (d, 1H, CH), 7.461-7.458 (t, 1H, CH), 6.833-6.830 (d, 1H, CH). GC-MS (m/z): 302 (M^+).

4. c) 2-furyl-5-(3,5-dinitrophenyl)-1,3,4-oxadiazole.

IR (KBr) (cm^{-1}): 3109.25 (C-H), 1604.77 (C=N), 1519.91 (C-C), 1442.75 (C=C), 1519 & 1350 (N=0), 1172.28 (C-O), 1026.13(C-O-C), 864.11 (1,3,5-trisubstitution). H^1NMR (DMSO-d_6, 400 MHz), δ (ppm): 8.097-7.694 (m, 4H, CH), 7.692-7.690 (d, 1H, CH_2), 7.462-7.457 (t, 1H, CH), 6.834-6.830 (d, 1H, CH). GC-MS (m/z): 257 (M^+).

4. d) 2-furyl-5-phenyl-1,3,4-oxadiazole.

IR (KBr) (cm^{-1}): 3062.96 (C-H), 1635.66 (C=N), 1506.32 (C-C), 1480.23 (C=C), 1081.14 (C-O), 1018.41(C-O-C), 840.26 (disubstitution at para position). H^1NMR (DMSO-d_6, 400 MHz), δ (ppm): 8.092-7.694 (m, 4H, CH), 7.692-7.690 (d, 1H, CH), 7.463-7.458 (t, 1H, CH), 6.831-6.830 (d, 1H, CH). GC-MS (m/z): 212 (M^+).

4. e) 2-furyl-5-(o-aminophenyl)-1,3,4-oxadiazole.

IR (KBr) (cm^{-1}): 3335.55 & 3400 (N-H), 3100.24 (C-H), 1630.27 (C=N), 1530.77 (C-C), 1485.33 (C=C), 1281.73 (C-N), 1085.57 (C-O), 1027.13(C-O-C). H^1NMR (DMSO-d_6, 400 MHz), δ (ppm):

8.090-7.693 (m, 4H, CH), 7.691-7.690 (d, 1H, CH), 7.463-7.457 (t, 1H, CH), 6.831-6.830 (d, 1H, CH). GC-MS (m/z): 227 (M^+).

4. f) 2-furyl-5-(p-hydroxyphenyl)-1,3,4-oxadiazole.

IR (KBr) (cm^{-1}): 3500-3200 (O-H), 3035.25 (C-H), 1620.32 (C=N), 1520.27 (C-C), 1452.55 (C=C), 1281.73, 1107.72 (C-O), 1044.62(C-O-C), 840.77 (disubstitution at para position). H^1NMR (DMSO-d_6, 400 MHz), δ (ppm): 8.094-7.693 (m, 4H, CH), 7.692-7.691 (d, 1H, CH), 7.462-7.456 (t, 1H, CH), 6.831-6.830 (d, 1H, CH). GC-MS (m/z): 228 (M^+).

4. g) 2-furyl-5-(o-hydroxyphenyl)-1,3,4-oxadiazole.

IR (KBr) (cm^{-1}): 3500-3200 (O-H), 3110.33 (C-H), 1640.22 (C=N), 1505.89 (C-C), 1452.42 (C=C), 1281.73, 1070.88 (C-O), 1022.62(C-O-C), 740.67 (disubstitution at para position). H^1NMR (DMSO-d_6, 400 MHz), δ (ppm): 8.097-7.694 (m, 4H, CH), 7.692-7.690 (d, 1H, CH), 7.462-7.457 (t, 1H, CH), 6.834-6.830 (d, 1H, CH). GC-MS (m/z): 228 (M^+).

ANTI-BACTERIAL ACTIVITY SCREENING: [23-25]

The cup plate method has been employed for the anti-bacterial screening of the synthesized compounds. The values of the zone of inhibition for each of the compounds have been tabulated.

DATA OF THE ANTI-BACTERIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS.

S.NO.	Compoud	Average zone of inhibition(mm)					
		E. coli			*P. aeruginosa*		
		150µg/ ml	300µg/ ml	500µg/ ml	150µg/ m l	300µg/m l	500µg/m l
1.	**4.a)**	12	15	18	5	9	12
2.	**4.b)**	11	14	19	5	7	11
3.	**4.c)**	12	15	20	5	8	12
4.	**4.d)**	10	12	15	4	6	10
5.	**4.e)**	9	11	14	3	5	8
6.	**4.f)**	8	11	14	4	6	9
7.	**4.g)**	11	14	16	2	4	8
8.	**Netilmicin**	13	16	20	14	18	22
9.	**Control**	-	-	-	-	-	-

ANTI-FUNGAL ACTIVITY SCREENING: [26-28]

The standard cup plate method has been employed for the anti-fungal screening of the synthesized compounds. The standard drug taken is Griseofulvin. The values of inhibition for the synthesized compounds have been tabulated.

DATA OF THE ANTI-FUNGAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS.

S.NO.	Compound. (1000µg/ml)	Average diameter of the zone of inhibition(mm).	
		Candida albicans.	***Aspergillus niger.***
1.	4.a)	17	14
2.	4.b)	18	14
3.	4.c)	18	15
4.	4.d)	14	13
5.	4.e)	14	14
6.	4.f)	12	11
7.	4.g)	14	13
8.	Griseofulvin (1000µg/ml)	20	18
9.	Control	-	-

CONCLUSION:

- In the current study Oxadiazole derivatives were obtained by an efficient synthetic route. The yield of all the synthesized compounds was found to be in the range of 60-82%. The titled compounds were characterized by physiochemical properties like melting point and R_f value. The structures of the synthesized compounds were confirmed by IR, H^1NMR and Mass spectra. The spectral data also supported the assigned structure by showing the characteristic absorption peaks.
- The synthesized compounds were screened for their anti-bacterial tests against *E. coli* and *S.aureus*. Compounds 4.a), 4.b) and 4.c) showed good activity against *E.coli* and compounds 4.d) and 4.g) showed moderate activity. Compounds ARD-5 and ARD-6 showed weak activity against *E.coli*.

  Compounds 4.a), 4.b) and 4.c) showed good activity, while compounds 4.d), 4.e), 4.f) and 4.g) showed weak activity against S. aureus.
- All the compounds were screened for their anti-fungal activity against Candida albicans and *Aspergillus niger* using Griseofulvin as the standard.

  Zone of inhibition was measured. Griseofulvin exhibited a zone of inhibition of 20mm for *C.albicans* and 18mm for *A.niger*. The screening results revealed that the compounds ARD-2 and ARD-3 exhibited good activity towards C. albicans and 4.a), 4.b), 4.c) and 4.e)

showed good activity towards *A. niger* compared to that of the standard drug. The compounds 4.d), 4.e) and 4.g) are moderately active towards *C.albicans*. Compounds 4.f) showed weak activity against both the fungal organisms.

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