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MICROWAVE ASSISTED SYNTHESIS, CHARACTERIZATION AND IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF 2,5-DISUBSTITUTED-1,3,4-OXADIAZOLE

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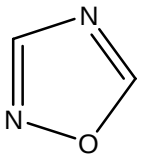
ABSTRACT

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. The synthesized derivatives of 1,3,4-oxadiazole are screened for their in-vitro anti-inflammatory activity.

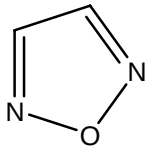
INTRODUCTION[1-12]

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.

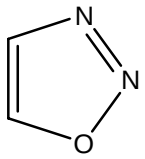
Oxadiazoles are five membered heterocyclic compounds with two nitrogen atoms and one oxygen atom. Depending on the positions of hetero atoms, they are named as 1,2,3; 1,2,4; 1,2,5; 1,3,4-oxadiazoles .1,2,4-Oxadiazole, 1,2,5-Oxadiazole, and 1,3,4-Oxadiazole are known, but the 1,2,3-isomer is unstable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



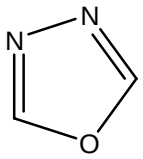
1,2,4-Oxadiazole



1,2,5-Oxadiazole



1,2,3-Oxadiazole

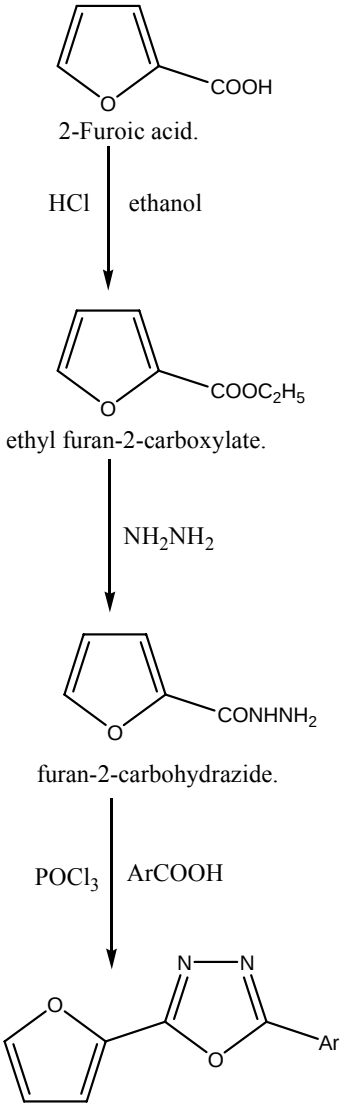


1,3,4-Oxadiazole

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.

MATERIALS AND METHODS

SYNTHETIC SCHEME[13-17]



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

4(a-g)

EXPERIMENTAL WORK[18-22]

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.

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%.

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.

SCREENING OF ANTI-INFLAMMATORY ACTIVITY [23-27]

Cyclooxygenase Assay Method: The assay mixture contains Tris HCl buffer, glutathione, hemoglobin and the enzyme mixture (containing COX).Reaction was started by the addition of arachidonic acid and terminated after 20 minutes by the addition of 0.2ml 10% TCA in 1N HCl and 0.2 ml of thiobarbituric acid followed by incubation at 37°C.The contents are heated in a boiling water bath for 20minutes, cooled and centrifuged at 100rpm for 3 minutes. The supernatant was measured at 632nm for COX activity. The percentage inhibition is given as:

$$\% \text{ inhibition} = (C-T)/C$$

Where, C = Optical density of the control.

T = Optical density of the test.

The results are tabulated in the table.

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-dinitrobenzoic 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-hydroxybenzoic 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-INFLAMMATORY ACTIVITY SCREENING

In-vitro anti-inflammatory screening was performed against COX enzyme for sample ARD-4 in concentrations of 100µg/ml, 500µg/ml and 1000µg/ml. The percentage inhibition of COX enzyme in three different concentrations is reported in the table as below:-.

DATA OF THE IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF THE SYNTHESIZED OXADIAZOLE DERIVATIVES

SAMPLE	CONCENTRATION	OD(632 nm)	% INHIBITION
4.a)	100 µg/ml	0.195	66.28 %
	500 µg/ml	0.174	69.68 %
	1000 µg/ml	0.105	81.78 %
4.b)	100 µg/ml	0.195	66.28 %
	500 µg/ml	0.175	69.42 %
	1000 µg/ml	0.108	81.52 %
4.c)	100 µg/ml	0.196	66.15 %
	500 µg/ml	0.176	69.20 %
	1000 µg/ml	0.107	81.67 %
4.d)	100µg/ml	0.194	66.20 %
	500µg/ml	0.174	69.68 %

	1000µg/ml	0.106	81.53 %
4.e)	100 µg/ml	0.198	66.10 %
	500 µg/ml	0.176	69.20 %
	1000 µg/ml	0.108	81.23 %
4.f)	100 µg/ml	0.196	66.15 %
	500 µg/ml	0.174	69.68 %
	1000 µg/ml	0.105	81.78 %
4.g)	100 µg/ml	0.192	66.52 %
	500 µg/ml	0.172	69.88 %
	1000 µg/ml	0.104	81.87 %
Standard (Diclofenac)	100µg/ml	0.012	95.34 %
CONTROL	-	0.574	100 %

CONCLUSION

In the current study 2,5-disubstituted-1,3,4- oxadiazoles 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.

Anti-inflammatory potential of the synthesized derivatives of 1,3,4-ozadiazole were screened against cyclooxygenase enzyme produced by human lymphocytes. It was reported that the oxadiazole derivatives exhibit inhibition of COX ranging from 66.52 % to 66.10 %. Therefore, they were reported to possess moderate anti-inflammatory potential as compared to that of the control, Diclofenac which showed inhibition of 95.34 % at the same concentration of 100 µg/ml.

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