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SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF NOVEL 1,3,4-OXADIAZOLE DERIVATIVES

Dhanya K, B.C.Revanasiddappa*

**Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences (NGSMIPS), Nitte (Deemed to be University), Mangalore-575018, (Karnataka) India*
Email: revan@nitte.edu.in

Abstract
A new series of novel 1,3,4-oxadiazoles**(3a-j)** were synthesized by reacting 4-nitro benzhydrazide**(1)** and substituted aromatic acids**(2)** in presence of phosphorus oxychloride. The title compounds were characterized by spectral data (IR, NMR, Mass). In-vitro Anti-inflammatory activity of all the newly synthesized compounds were evaluated by denaturation assay, anti-proteinase method, HRBC assay and Diclofenac sodium was used as standard drug. Some of the tested compounds showed good ant-inflammatory activity by denaturation assay.

INTRODUCTION
Heterocyclic chemistry, an ever-expanding field in which scientists constantly discover new compounds and innovative heterocyclic compound applications. Since the heterocyclic compounds are widely distributed, their study is of great importance. The heterocyclic nucleus, 1,3,4-oxadiazole has got wide attention in search of new therapeutic molecule. 1,3,4-oxadiazole is widely investigated for various applications and presently four isomers are available.
1,3,4-oxadiazoles are heterocyclic compounds with single oxygen and two nitrogen atom in a five-membered ring. These compounds as such is not commonly used in organic chemistry,but many of its derivatives have significant role in medicinal chemistry. Some of the medicinally important compounds are Raltegravir (HIV-integrase inhibitor drug), Fenadiazole (Hypnotic), Zibotentan (anticancer), Tiodazosin (alpha1-adrenergic antagonist), Furamizole (nitrofuran antibacterial).
The various derivatives of 1,3,4-oxadiazoles possess properties like anti-tumour[I], anti-oxidant[II] , STAT3 inhibitor[II] , anti-tubercular[III], anti-bacterial[IV] , antifunga[V] , anti-inflammatory[V] , ulcerogenic[V] , COX inhibitor[V], anti-thrombotic[VI], anti-convulsant[VII] , glycogen-phosphorylase inhibitor[VII] etc. They have wide number of uses in different areas of great practical significance. These include the drug synthesis, preparation of dyes, synthesis of polymers, use in photography as tone improvers[IX]
Inflammation is a network of response of body's immune system to a noxious stimuli like pathogen, damaged cells, or irritants. The main aim of inflammation is to remove the cause of

inflammation and the damage cell and to repair the damaged tissue. Inflammation involves a cascade of cellular and microvascular reactions which helps to fulfill its aim. The inflammation has been characterized by the major signs like redness, swelling, warmth, pain loss of function[X] Inflammation is categorized as acute inflammation and chronic inflammation. Acute inflammation is the first response of the body to stimuli, characterized by enhanced plasma and leukocyte movement to the injured portion of the tissue. It usually involve shorter duration. Chronic inflammation is slow and lasts for prolonged duration of time[XI]. Most of the features remain same when acute inflammation changes to chronic inflammation. But the composition of the white cells changes where short lived neutrophils are replaced by macrophages and lymphocytes[XII].

Keeping in view of the importance of biological and pharmacological activities associated with 1,3,4-oxadiazoles, it was considered of interest to design and synthesize some new derivatives of 1,3,4-oxadazoles followed by their anti-inflammatory activity.

MATERIALS AND METHODS

The starting material4-nitrobenzhydrazide was procured from Alfa aesar, Mumbai, India.All the reagents and solvents used for the synthesis are of laboratory grade chemicals. They were used without further purification. In open capillary tubes, melting points were determined and were uncorrected. IR spectra ($cm^{-1)}$ was recorded using Alpha Bruker FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded using Bruker spectrometer at 400 MHz with DMSO as a solvent. TMS was served as an internal standard. Mass spectra was carried out by Perkin Elmer Clarus 680 GC-MS. The purity of the compounds was determined using TLC plates.

General procedure for the synthesis of 1,3,4-oxadiazole derivatives (3a-j)

4-nitrobenzhydrazide**(1)** (0.01M) and substituted aromatic acids **(2)**(0.01M) were dissolved in 8ml of phosphorous oxychloride ($POCl_3$) and refluxed for 10-14 hrs in an oil bath. The contents were cooled to room temperature and ice chips were added with stirring. Then 10%$NaHCO_3$ solution was added little by little till the effervescence ceases. The precipitated compound was filtered, washed, dried and recrystallized from ethanol. The physical data of the compounds **(3a-j)** is given in table-1.

Scheme-01

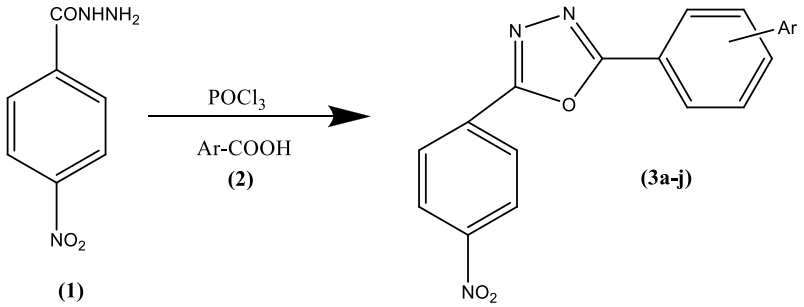

Table-1: Physical data of 1,3,4-oxdiazole derivatives(3a-j)

Comp	Ar-COOH	Molecular Formula	Molecular weight	Melting point (ºC)	Yield (%)
3a	C_6H_5	$C_{14}H_9N_3O_3$	267.23	111-13	69

3b	4-NH_2	$C_{14}H_{10}N_4O_3$	282.25	96-98	68
3c	4-NO_2	$C_{14}H_8N_4O_5$	312.23	125-27	69
3d	2,4-$(Cl)_2$	$C_{14}H_7Cl_2N_3O_3$	336.12	139-41	66
3e	4-OH	$C_{14}H_9N_3O_4$	283.23	156-58	68
3f	2-Br	$C_{14}H_8BrN_3O_3$	346.13	105-07	65
3g	3,5-$(NO_2)_2$	$C_{14}H_7N_5O_8$	373.23	165-67	64
3h	2-OH	$C_{14}H_9N_3O_4$	283.23	148-50	63
3i	4-OCH_3	$C_{15}H_{11}N_3O_4$	297.26	119-21	62
3j	2-Cl- 4-NO_2	$C_{14}H_7ClN_4O_4$	330.68	177-79	60

2-(4-nitrophenyl)-5-phenyl-1,3,4-oxadiazole(3a): IR(KBr)*ν*(cm^{-1}): 2949(C-H), 1606(C=N), 1550(C=C), 1077(C-O-C).^{1}H-NMR (400 MHz, DMSO): δ 8.47- 7.64(m,Ar-H, 9H). MS: 267.23 (M+)

4-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]aniline(3b):IR(KBr)*ν*(cm^{-1}): 3377(NH), 3074 (C-H), 1604(C=N), 1518(C=C),1072(C-O-C).^{1}H-NMR ^{1}H-NMR (400 MHz, DMSO):δ 6.31-8.28 (m, Ar-H, 8H), δ6.17(s, NH2, 2H). MS: 282.25(M+).

2,5-bis(4-nitrophenyl)-1,3,4-oxadiazole(3):IR(KBr)*ν*(cm^{-1}): 3004 (C-H), 1606 (C=N), 1554(C=C), 1071(C-O-C).

2-(2,4-dichlorophenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole(3d):IR(KBr)*ν*(cm^{-1}): 3098(C-H), 15919(C=N), 1551(C=C) 1043(C-O-C), 734(C-Cl)); ^{1}H-NMR (400 MHz, DMSO): δ 7.44-8.23 (m, Ar-H,7H).

4-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]phenol(3e):IR(KBr)*ν*(cm^{-1}): 3207(OH), 3070(CH), 1602(C=N), 1552(C=C), 1061(C-O-C). ^{1}H-NMR (400 MHz, DMSO): δ 6.51-8.15(m, Ar-H, 8H),10.23 (s, OH, IH).

2-(2-bromophenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole(3f):IR(KBr)*ν*(cm^{-1}): 3101 (CH), 1548(C=N), 1515(C=C), 1077(C-O-C), 768(C-Br)); ^{1}H-NMR (400 MHz, DMSO): δ 7.60-8.47 (m, Ar-H, 8H).

2,4-dinitro-6-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]phenol(3g):IR(KBr)*ν*(cm^{-1}): 2999(C-H), 1629(C=N), 1518(C=C), 1084(C-O-C).^{1}H-NMR (400 MHz, DMSO): δ 7.94-8.94 (m, Ar-H, 7H). MS: 373.23 (M+).

2-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]phenol(3h):IR(KBr)*ν*(cm^{-1}): 3104(OH), 2939(CH), 1621(C=N), 1517(C=C), 1059(C-O-C).^{1}H-NMR (400 MHz, DMSO): δ7.04-8.47 (m, Ar-H, 8H), 10.90(s, OH,1H). MS: 283.23 (M+).

2-(2-methoxyphenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole(3i):IR(KBr)*ν*(cm^{-1}): 2948(C-H), 1602(C=N), 1524(C=C), 1014(C-O-C).^{1}H-NMR (400 MHz, DMSO): δ 7.14-8.14 (m, Ar-H, 8H), δ 3.96 (s, OCH_3,3H), MS: 297.26 (M+).

2-(2-chloro-4-nitrophenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole(3j):IR(KBr)*ν*(cm^{-1}): 3323(OH), 3101(C-H), 1630(C=N), 1514(C=C), 1110(C-O-C),717(C-Cl).^{1}H-NMR (400 MHz, DMSO): δ 8.05-8.33(m, Ar-H, 7H).

Anti-inflammatory activity

Bovine serum albumin method[XIII]

Different concentration (10,20,30,40,50µg/ml) of test samples were prepared. To 0.05ml of test and standard drug, 0.45ml of bovine serum albumin solution was added and mixed. The pH was maintained to 6.3 with 1N HCl. The samples were incubated for 20 min at 37°C and heated at 57°C for 3 minutes. Cool the above solution and add 2.5ml of phosphate buffer. Triplicate experiment was carried out and the average was taken. The absorbance was measured at 416nm using UV-Visible spectrophotometer. The standard drug used was Diclofenac sodium.The IC_{50} values are tabulated in Table-2.The percentage inhibition was calculated with the below formula:

Percentage inhibition = (A_0-A_1/A_0) x100

Where A_0 = Control absorbance, A_1 = Test absorbance

Egg albumin denaturation method [XIV]

Different concentration (10,20,30,40,50µg/ml) of test and standard were prepared. To 2ml of sample, add 0.2ml egg albumin prepared from fresh hen's egg. The solution is then incubated at 37°C in BOD incubator for 15 minutes. Cool the solution and the pH was adjusted to 6.4 by adding 2.8ml of phosphate buffer. The standard drug used was Diclofenac sodium. The absorbance was measured at 660 nm. Percentage inhibition of denaturation was calculated using the above mentioned formula.The IC_{50} values are tabulated in Table-2.

Anti-Proteinase method [XV]

Different concentrations of samples wereprepared (10-50µg/ml). To 1ml of the sample solution, 0.06mg trypsin, 1ml of 20mM Tris HCl buffer of pH 7.4 was added. The reaction was kept under incubation for 5min at 37°C. 1ml of 0.8%casein was added. Again the reaction mixture was incubated for 20min. Finally 2ml 70% perchloric acid was added, to stop the reaction. The reaction mixture was centrifuged and the absorbance was measured at 210nm. Triplicate experiments were performed and the percentage inhibition was calculated as above.The IC_{50} values are tabulated in Table-2.

HRBC assay [XVI]

The red blood suspension was prepared by collecting the blood from a healthy human individual. Then the blood was subjected to centrifugation at 3000 RPM for 10 minutes. Using equal volume of normal saline, wash the centrifuged blood sample until the supernatant becomes clear. The volume of blood was measured and further reconstituted as 10%v/v HRBC suspension.

Different concentration(10-50µg/ml) of test and standard were prepared. To 1ml of test solution, phosphate buffer(1ml), hypo saline (2ml) and HRBC solution (0.5ml) was added. The reaction mixtures were incubated for 30min at37°C. The reaction mixture was then centrifuged at 3000RPM. The supernatant liquid of the reaction mixture was decanted. Using UV- spectrophotometer at 560 nm the hemoglobin content was estimated. The percentage inhibition was calculated using the formula mentioned earlier. Diclofenac was used as the standard drug.The IC_{50} values are tabulated in Table-2.

Table -2: Data of anti-inflammatory activity of compounds (3a-j)

Comp	IC_{50} Values			
	Bovine serum albumin denaturation assay	**Egg serum albumin denaturation assay**	**Anti-proteinase assay**	**HRBC Assay**
STD	22.57	21.84	28.81	23.09
3a	27.92	22.35	41.50	31.01
3b	34.44	29.15	41.12	45.36
3c	54.53	22.22	53.56	68.84
3d	77.98	36.87	46.78	78.64
3e	43.55	38.64	49.79	72.59
3f	21.77	26.67	40.62	26.69
3g	52.74	35.66	47.16	83.6
3h	21.74	28.88	41.45	50.65
3i	29.01	35.46	47.64	48.06
3j	21.31	37.09	36.32	69.98

RESULTS AND DISCUSSION

The main aim of the work was to synthesize and to evaluate anti-inflammatory activity of 1,3,4-oxadiazole derivatives. The reaction sequence is outlined in **Scheme 01.** The title compounds were synthesized by refluxing substituted aromatic acids and 4-nitrobenzhydrazide in presence of $POCl_3$ as cyclizing agent. All the newly synthesized compounds were characterized by the spectral data. The new compounds were purified by recrystallization technique and purity was evaluated by TLC using silica gel plates The physical data of the compounds is given in table-1.

All the newly synthesized compounds were evaluated for their In-vitro anti-inflammatory activity by Protein denaturation methods, anti-proteinase method and HRBC method. Diclofenac sodium was used as standard drug for comparison purpose. All the compounds were tested at a concentration of 10-50 µgm/ml. The IC_{5O} values are tabulated in table-2. In the anti-proteinase and HRBC methods all the tested compounds showed very weak to moderate activity when compared to the standard drug. In the bovine serum denaturation assay, the compounds **3f, 3h, 3j** showed potent activity and compound 3a also displayed good activity. The presence of electron withdrawing groups may be responsible for the potent activity. In the egg albumin denaturation assay, the compounds **3a, 3c** displayed good activity and compounds 3f, 3h also showed moderate activity.

CONCLUSION

A new series of 1,3,4-oxadiazoles were synthesized by reacting 4- nitro benzhydrazide and aromatic acids in $POCl_3$ medium. From the anti-inflammatory results it is concluded that the presence of electron withdrawing groups may be responsible for the potent activity. However the new compounds can be used as good anti-inflammatory agents, by the structural modifications.

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