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Synthesis and Characterization of 2, 5-Disubstituted-1, 3, 4-oxadiazoles as Potential Anti-inflammatory Agents

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ABSTRACT

The aim of this study was to design, synthesize, and investigate the *in vivo* anti-inflammatory activity of some novel 2, 5-disubstituted - 1, 3, 4-oxadiazole derivatives. Ethyl-4-acetamido phenoxy acetate (I) was prepared by the condensation of ethyl chloroacetate with starting material p-acetamidophenol in the presence of dry acetone and anhydrous potassium carbonate. Hydrazinolysis of (I) with hydrazine hydrate results in the formation of 4-acetamidophenoxy acetyl hydrazide (II), which on cyclisation with various substituted aromatic carboxylic acids in the presence of phosphorous oxychloride affords various 2, 5-disubstituted - 1, 3, 4-oxadiazole derivatives (IIIa-IIIi). The newly synthesized compounds were characterized by IR, ^{1}HNMR, and MS spectral data. The titled compounds were screened for *in vivo* anti- inflammatory activity using the carrageenan-induced paw edema method. A few of them manifested promising activity when compared with the standard drug Diclofenac sodium.

Key words: Anti-inflammatory activity, 1, 3, 4 - oxadiazoles, Ethyl - 4- acetamido phenoxy acetate, 4 - acetamido phenoxy acetyl hydrazide

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INTRODUCTION

In the family of heterocyclic compounds, nitrogen containing heterocycles with an oxygen atom are considered to be an important class of compounds in medicinal chemistry because of their interesting diversified biological application.[1] During the past few years, considerable evidence has been accumulated that demonstrates the efficacy of 1,3,4-oxadiazoles including antibacterial,[2] anti-inflammatory,[3] anti-malarial,[4] anti-tubercular,[5] anti-hypoglycemic,[6] anti-cancer,[7] anti-leishmanial,[8] anti-viral,[9] anti-convulsant,[10] and insecticidal properties.[11] A survey of literature revealed that a slight modification in the structure can result in qualitative as well as quantitative changes in the activity, which prompted us to undertake the synthesis of various new 2-aryl-5-substituted -1,3,4-oxadiazole derivatives with the aim of having improved activity and lesser toxicity. The synthesized compounds were evaluated for *in vivo* anti-inflammatory activity using a carrageenan-induced rat paw edema method and the results are compared with the standard drug Diclofenac sodium.

EXPERIMENTAL

Melting points of the synthesized compounds were determined using a Thomas Hoover melting point apparatus and were uncorrected. The infrared radiation (IR) spectra of the synthesized compounds were recorded using KBr pellet on a Perkin Elmer fourier transform infrared radiation (FTIR) spectrophotometer and the frequencies are recorded in wave numbers. Proton nuclear magnetic resonance (^{1}HNMR) spectra were recorded on

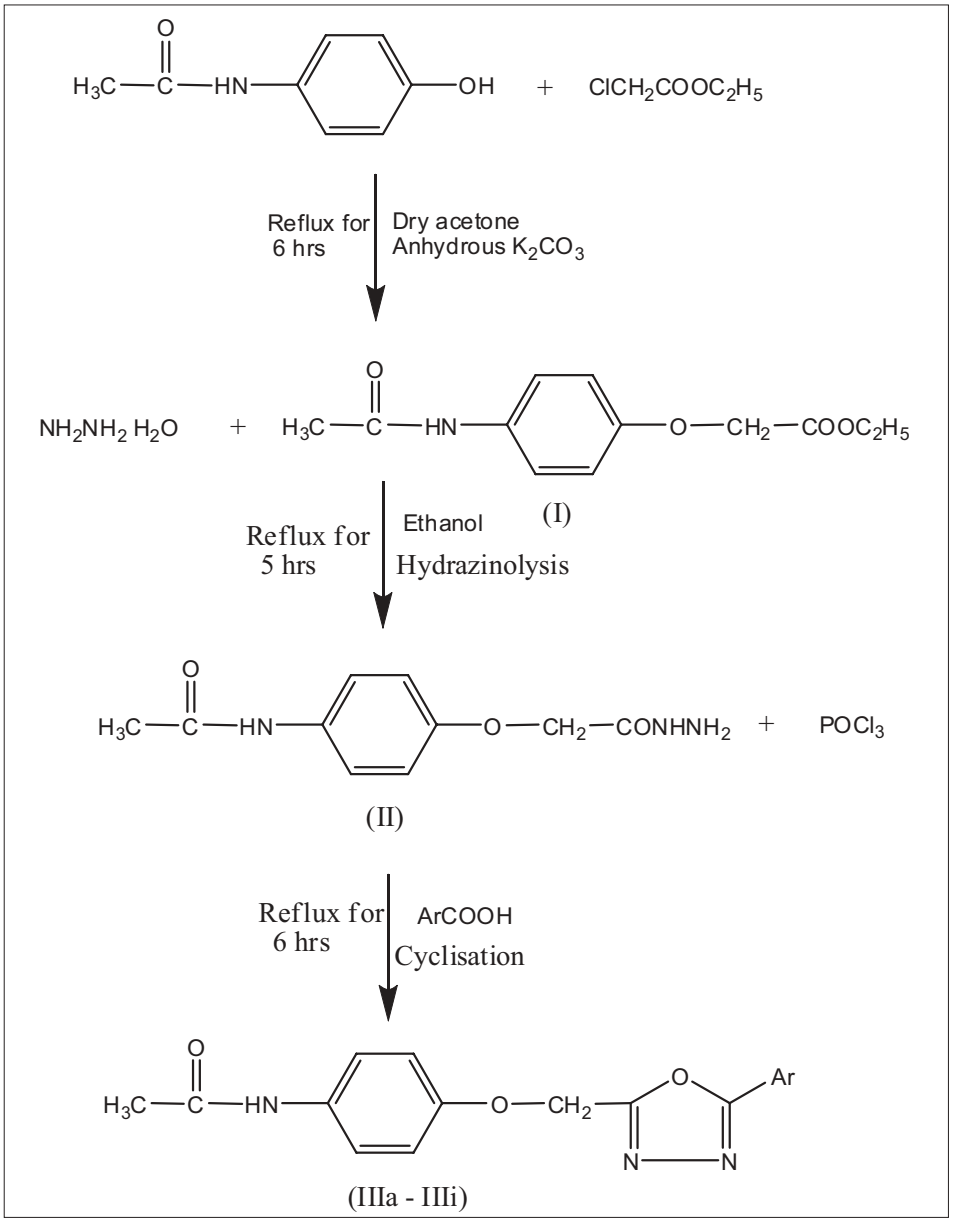
a Bruker Avance II (400 MHz) spectrometer and their chemical shifts are recorded in δ (parts per million) units with respect to tetramethyl silane (TMS) as an internal standard. The mass spectra were recorded on a Jeol-JMS-D 300 mass spectrometer operating at 70 eV. The purity of the compounds were checked by a thin layer chromatography carried out on precoated silica gel plates using several solvent mixtures of chloroform, acetone, and toluene as a mobile phase and UV light as a detector. The physical constants of the titled compounds are reported in Table 1.

Procedure for the preparation of Ethyl-4-acetamido phenoxy acetate (I)

A mixture of p-acetamido phenol (0.01 mol) and ethyl chloroacetate (0.01 mol) was refluxed using dry acetone in the presence of anhydrous potassium carbonate for 6h. The reaction mixture was cooled and poured into crushed ice. The solid product obtained was filtered, dried, and recrystallized using ethanol.

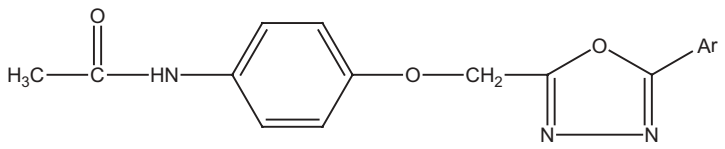
Procedure for the synthesis of 4-Acetamido phenoxy acetyl hydrazide (II)

To the product (I) (0.01 mol), hydrazine hydrate (0.04 mol) was added and refluxed in ethanol for 5 h. The reaction mixture was cooled and poured into crushed ice. The separated solid was filtered, dried, and recrystallized using



Scheme 1: Synthetic route of the compounds

Table 1: Physical constants of 2-(4-Acetamidophenoxymethyl)-5-aryl-l,3,4-oxadiazole



Compd. Code	Ar	M.F.	M. Wt.	Yield(%)	m.p (oC)	R_f Values
III a	3-Cl-C_6H_4	$C_{17}H_{14}N_3O_3Cl$	343	60.20	112 - 115	0.72
III b	4-Cl-C_6H_4	$C_{17}H_{14}N_3O_3Cl$	343	56.32	120 - 122	0.76
III c	2,4-(Cl)2-C_6H_3	$C_{17}H_{13}N_3O_3Cl_2$	378	40.28	175 - 178	0.78
III d	4-OH-C_6H_4	$C_{17}H_{14}N_3O_4$	324	67.34	115 - 118	0.71
III e	2,4-$(OH)_2$-C_6H_3	$C_{17}H_{15}N_3O_3$	309	56.12	280 - 282	0.75
III f	4-NO_2-C_6H_4	$C_{17}H_{14}N_4O_5$	354	48.62	180 - 182	0.73
III g	3,5-$(NO_2)_2$-C_6H_3	$C_{17}H_{13}N_5O_7$	399	70.12	120 - 122	0.79
III h	4-NH_2-C_6H_4	$C_{17}H_{16}N_4O_3$	324	54.82	98-100	0.77
III i	2-OCH_3-C_6H_4	$C_{18}H_{17}N_3O_4$	339	49.24	210 - 212	0.74

ethanol.

Preparation of 2- (4-Acetamido phenoxy methyl) - 5-aryl substituted - 1, 3, 4-oxadiazole derivatives (IIIa - IIIi)

To the product (II) (0.01 mol), various aromatic acids (0.01 mol) were added and refluxed in the presence of phosphorous oxychloride for 6 h. The reaction mixture was cooled and poured into crushed ice. The resulting content was neutralized with a sodium bicarbonate solution, filtered, dried, and recrystallized using ethanol. The formation of 1, 3, 4-oxadiazole was confirmed by the difference in mp and R_f values. The physical data are recorded in Table 1.

4 - ((5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-methoxy)-phenyl)-acetamide (IIIa)

IR (KBr) (cm^{-1}): 3352 (Ar-NH), 1687 (C=N), 1644 (C=C), 1137 (-C-O-C-), 799 (C-Cl), 3122 (Ar-CH).^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 2.02 (s, 3H, CH_3), 8.20 (s, 1H, NH), 5.28 (s, 2H, CH_2), 7.53 (m, 8H, Ar-H). LC-MS (m/z): 344 (M+1).

4- ((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-methoxy)-phenyl)-acetamide (III b)

IR (KBr) (cm^{-1}): 3412 (Ar-NH), 1680 (C=N), 1643 (C=C), 1128 (-C-O-C), 795 (C-Cl), 3146 (Ar-CH).^{1}HNMR (DMSO-d_6, 400 MHz), δ (ppm): 2.08 (s, 3H, CH_3), 8.32 (s, 1H, NH), 5.14 (s, 2H, CH_2), 7.58 (m, 8H, Ar-H). EI - MS (m/z): 344 (M+1).

4- ((5-(2,4-Dichlorophenyl)-1,3,4-oxadiazol-2-yl)-methoxy)-phenyl)-acetamide (IIIc)

IR (KBr) (cm^{-1}): 3496 (Ar-NH), 1616 (C=N), 1584 (C=C), 1102 (-C-O-C-), 774 (C-Cl), 3128 (Ar-CH).^{1}HNMR (DMSO-d_6, 400 MHz), δ (ppm): 2.18 (s, 3H, CH_3), 8.24 (s, 1H, NH), 5.16 (s, 2H, CH_2), 7.51 (m, 7H, Ar-H). EI - MS (m/z) : 378 (M^+).

4- ((5-(4-Hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-methoxy)-phenyl)-acetamide (IIId)

IR (KBr) (cm^{-1}): 3339 (Ar-NH), 1730 (C=N), 1598 (C=C), 1130 (-C-O-C), 3121 (Ar-CH).^{1}HNMR (DMSO-d_6, 400 MHz), δ (ppm): 2.42 (s, 3H, CH_3), 8.36 (s, 1H, NH), 5.34 (s, 2H, CH_2), 5.02 (s, 1H, OH), 7.64 (m, 8H, Ar-H). EI - MS (m/z) : 324 (M^+).

4- ((5-(2,4-Dihydroxyphenyl)-1,3,4-oxadiazol-2-yl)-methoxy)-phenyl)-acetamide (IIIe)

IR (KBr) (cm^{-1}): 3397 (Ar-NH), 1679 (C=N), 1611 (C=C), 1136 (-C-O-C), 3136 (Ar-CH).^{1}HNMR (DMSO-d_6, 400 MHz), δ (ppm): 2.42 (s, 3H, CH_3), 8.40 (s, 1H, NH), 5.23 (s, 2H, CH_2), 7.64 (m, 7H, Ar-H). EI - MS (m/z) : 310 (M +1).

4- ((5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl)-methoxy)-phenyl)-acetamide (IIIf)

IR (KBr) (cm^{-1}): 3393 (Ar-NH), 1651 (C=N), 1604 (C=C), 1022 (-C-O-C-), 1384 (N=0), 3142 (Ar-CH).^{1}HNMR (DMSO-d_6, 400 MHz), δ (ppm): 2.22 (s, 3H, CH_3), 8.14 (s, 1H, NH), 5.34 (s, 2H, CH_2), 7.60 (m, 8H, Ar-H). EI - MS (m/z) : 355 (M+1).

4- ((5-(3,5-Dinitrophenyl)-1,3,4-oxadiazol-2-yl) methoxy)phenyl)acetamide (IIIg)

IR (KBr) (cm^{-1}): 3447 (Ar-NH), 1630 (C=N), 1598 (C=C), 1154 (-C-O-C-), 1383 (N=O), 3124 (Ar-CH).^{1}HNMR (DMSO-d_6, 400 MHz), δ (ppm): 2.30 (s, 3H, CH_3), 8.16

Table 2: Anti-inflammatory activity of synthesized compounds against carrageenan induced paw edema in rats

Compound Code	Dose mg / kg	Mean paw edema volume ± SEM	% Inhibition ± SEM
III a	50 mg / kg	0.26 ± 0.02	41.90 ± 1.16 **
III b	50 mg / kg	0.21 ± 0.01	53.40 ± 1.63*
III c	50 mg / kg	0.32 ± 0.02	22.99 ± 1.31***
III d	50 mg / kg	0.31 ± 0.01	27.26 ± 1.18***
III e	50 mg / kg	0.29 ± 0.03	28.28 ± 1.32***
III f	50 mg / kg	0.22 ± 0.02	50.56 ± 1.31*
III g	50 mg / kg	0.33 ± 0.02	26.13 ± 1.32***
III h	50 mg / kg	0.27 ± 0.01	39.76 ± 1.32**
III i	50 mg / kg	0.18 ± 0.03	56.65 ± 1.64*
Control	5 ml / kg	0.43 ± 0.03	-
Diclofenac Sodium	20 mg / kg	0.12 ± 0.02	78.20 ± 1.24**

*P < 0.0001, **P<0.001, ***P<0.01 calculated by student's t test in comparison with control (n=6).

(s, 1H, NH), 5.34 (s, 2H, CH_2), 7.42 (m, 7H, Ar-H). EI - MS (m/z) : 400 (M+1).

4- ((5-(4-Aminophenyl)-1,3,4-oxadiazol-2-yl)methoxy) phenyl)acetamide (IIIh)

IR (KBr) (cm^{-1}): 3430 (Ar-NH), 1601 (C=N), 1513 (C=C), 1181 (-C-O-C-) 3142 (Ar-CH).^{1}HNMR (DMSO-d_6, 400 MHz), δ (ppm): 2.34 (s, 3H, CH_3), 8.22 (s, 1H, NH), 5.30 (s, 2H, CH_2), 4.20 (s, 2H, NH_2), 7.63 (m, 8H, Ar-H). EI - MS (m/z) : 325 (M+1).

4- ((5-(2-Methoxyphenyl)-1,3,4-oxadiazol-2-yl) methoxy)phenyl)acetamide (IIIi)

IR (KBr) (cm^{-1}): 3480 (Ar-NH), 1664 (C=N), 1584 (C=C), 1144 (-C-O-C-), 3128 (Ar-CH).^{1}HNMR (DMSO-d_6, 400 MHz), δ (ppm): 2.52 (s, 3H, CH_3), 8.34 (s, 1H, NH), 5.48 (s, 2H, CH_2), 7.54 (m, 8H, Ar - H), 3.74 (s, 3H, OCH_3). EI - MS (m/z) : 340 (M+1).

Evaluation of Biological Activity

Anti-inflammatory activity: The synthesized compounds were screened for *in vivo* anti-inflammatory activity using the carrageenan-induced rat paw edema method.[12] The percentage inhibition of paw edema was calculated for the synthesized compounds using the formula[13] and compared with control and the standard drug Diclofenac sodium.

$$\% \text{ Inhibition} = \frac{1 - V_t}{V_c} \times 100$$

Where V_t and V_c represent edema volume in test compounds and control, respectively. The results are summarized in Table 2.

RESULTS AND DISCUSSION

The structures of synthesized compounds were confirmed by thin layer chromatography (TLC), mp, IR, ^{1}HNMR, and mass spectrometry (MS) spectral analysis. The compounds (IIIa- IIIi) were obtained by the treatment of p-acetamidophenol with ethylchloroacetate in the presence of acetone and anhydrous potassium carbonate yields ester ethyl-4- acetamido phenoxy acetate (I). The compound I, on further treatment with hydrazine hydrate in ethanol, affords 4-acetamido phenoxy acetyl hydrazide (II) and finally hydrazide reacted with different aromatic acids in the presence of phosphorous oxychloride yields different oxadiazole derivatives (Scheme 1). The yield was found to be in the range of 50-70%. The titled compounds were confirmed by IR spectral data showing characteristic bands at 3200-3500 cm^{-1} indicating the presence of -OH and -NH stretching, sharp bands in the range between 1650-1730 cm^{-1} indicated the presence of C=N group. Compounds (IIIf-IIIg) were confirmed by stretching at 1384 cm^{-1} due to the presence of the -NO_2 group. Similarly, compounds IIIa, IIIb, and IIIc were further confirmed by a sharp absorption peak at 799 cm^{-1} denoting the presence of the C-Cl group. Compounds IIIh and IIIi were confirmed by ^{1}HNMR spectral analysis. The NMR proton singlet peak at δ 8.24 ppm and 3.73 ppm revealed the presence of -NH_2 and -OCH_3 groups. Further appearance of molecular ion peak at 342 (M+1) and 355 (M+1) confirmed the structures of compounds IIIe and IIIf. The synthesized compounds with functional groups such as -3Cl, -4NH_2, -4NO_2, and -4Cl were found to have moderate anti-inflammatory activity. Compounds IIIc, IIId, IIIe, and IIIg exhibited less activity, whereas compound IIIi i.e., 4-((5-(2-Methoxyphenyl)-1, 3, 4-oxadiazol-2-yl)methoxy)phenyl) acetamide was found to possess better activity with 56% inhibition.

CONCLUSIONS

The yield of all 2, 5-Disubstituted-1,3,4-oxadiazole derivatives were found to be in the range of 50-70%.The purity of compounds were ascertained by melting point and TLC. The assigned structure was further established by IR, ^{1}HNMR and MS spectral studies.

The acute anti-inflammatory activity of the synthesized compounds was screened using the carrageenan-induced paw edema method in rats. Diclofenac sodium was used as a reference drug. In the prepared oxadiazole series, it seemed that compound IIIa [4-((5-(3-Chlorophenyl) -1,3,4-oxadiazol-2-yl) methoxy) phenyl)acetamide], IIIf [4-((5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl)methoxy) phenyl)acetamide] and IIIh [4-((5-(4-Aminophenyl)-1,3,4-oxadiazol -2-yl)methoxy)phenyl)acetamide] showed moderate activity, when compared with the standard drug Diclofenac sodium. But the compound IIIi exhibited the highest anti-inflammatory activity with a percentage inhibition of 56.65.

From the present study, it may be concluded that the oxadiazole compounds can potentially be developed into useful anti-inflammatory agents that can prompt future researchers to synthesize a series of oxadiazole derivatives containing a wide variety of substituents with the aim of obtaining novel heterocyclic systems with enhanced activity. Further work to develop and improve similar and related compounds and test them for manifold biological activity is in progress in our laboratory.

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