Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN 2250 – 2688

Received: 08/06/2012
Revised: 15/06/2012
Accepted: 22/06/2012

Harish Rajak,Vijay Patel, Deepak K Jain, Pramod K Dewangan, Jawahar S Dangi
Medicinal Chemistry Research Laboratory, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur-495 009, (CG) India

Ravichandran Veerasamy
Faculty of Pharmacy, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, Malaysia

Prabodh C Sharma
Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136 119, (Haryana) India

Arun K Gupta
B.P.S. Mahila Vishwavidyalaya, Khapur-Kalan,Sonepat-131001, (Haryana) India

Correspondence
Harish Rajak
Medicinal Chemistry Research Laboratory, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur-495 009, (CG) India
E mail: dsresearch@rediffmail.com

Structure-Activity Relationships Among Novel 1,3,4-oxadiazole Analogues for Their Anti-inflammatory Activity

Harish Rajak,Vijay Patel, Deepak K Jain, Pramod K Dewangan, Prabodh C Sharma, Ravichandran Veerasamy, Arun K Gupta and Jawahar S Dangi

ABSTRACT

In pursuit of novel Non-Steroidal Anti-inflammatory Drugs (NSAID's) and the significance of oxadiazoles as anti-inflammatory agent encouraged us to perform the synthesis of 1,3,4-oxadiazole analogues hitherto unreported for their anti-inflammatory activities. The structures of the compounds were confirmed by elemental analysis, IR, ^{13}C-NMR and mass spectral data. The anti-inflammatory potential of the compounds were investigated using carrageenan-induced rat paw oedema method and cotton pellet-induced granuloma method. Some of the compounds displayed appreciable anti-inflammatory activity at doses of 100 mg/kg against Carrageenan-induced rat paw oedema assay, moist cotton pellet-induced and dry cotton pellet-induced granuloma method, respectively. Structure-activity relationships among synthesized compounds were established.

Keywords: 1,3,4-oxadiazole, carrageenan-induced rat paw oedema method, cotton pellet-induced granuloma method

1. INTRODUCTION

The inflammatory mechanisms in the body are very complicated and they cannot be attributed to a single mediator or factor. Inflammation mediators, such as histamine, serotonin and arachidonic acid metabolites are well-known for their role in generation of the inflammatory reactions[1,2]. Inflammatory process comprises of two phases: acute and chronic. Acute inflammation is characterized by fever, pain, and oedema, while chronic inflammation is characterized by cellular proliferation.

Non-steroidal anti-inflammatory drugs (NSAID's) are widely used for the treatment of pain, fever and inflammation, particulary arthritis [3,4]. The pharmacological activity of NSAID's is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme cyclooxygenases (COXs). On chronic use of NSAID's, one of prominent side effect is formation of gastric ulcers. This adverse effect may be attenuated in the presence of an inhibitor of 5-lipoxygenase (5-LO). 1,3,4-oxadiazoles found to possess anti-inflammatory properties by virtue of dual mechanism i.e. inhibit both COXs and LO reducing the gastric ulcer formation [5,6].

Numerous studies have been performed with the aim of exploring anti-inflammatory properties of 1, 3, 4 oxadiazole analogues [7-13]. These studies had shown that 1,3,4-oxadiazole analogues are equipotent with Phenylbutazone, Naproxen and other NSAID's. Keeping these above facts in view we considered, it of interest to synthesize novel 1,3,4-oxadiazole analogues for their anti-inflammatory activities.

2. MATERIALS AND METHODS

2.1 Chemistry

The chemicals used for the experimental work were commercially procured from various chemicals units like Sigma Aldrich Germany, Qualigens Mumbai, S.D.fine chemical Mumbai, E.Merck Bombay, Loba chemicals Bombay and Samar chemical India. The solvents and reagents were of AR grade and some were LR grade purified before the use. The silica G (60-120 mesh) used for analytical chromatography (TLC) was obtained from Merck India ltd. Mumbai. Melting points were determined by open capillary method and are uncorrected. Elemental estimation was done using elemental analyzer Heraeus Carlo Erba 1108, IR spectra were recorded on Perkin Elmer IR spectrophotometer (KBr disc), ^{1}H-NMR spectra on Bruker DRX300 NMR spectrometer (DMSO-d_6, TMS) and the electrospray mass spectra on MICROMASS QUATTRO II triple quadrupole mass spectrometer (Methanol). The title compounds were prepared using the Scheme described in Figure-1.

Preparation of Semicarbazone (II)

Semicarbazide HCl (0.01mol) and crystallized sodium acetate (0.02 mol) was dissolved in 8-10 ml of water, aldehyde (0.01 mol) was added and shaken well. If the mixture was turbid, alcohol (acetone free) was added until a clear solution was obtained; the mixture was shaken for few minutes and allowed to stand. Crystals were filter off, washed with a little cold water and recrystallized from water or from methanol or ethanol either alone or dilute with water.

Preparation of 2-amino-5-aryl-1,3,4-oxadiazole (III)

Semicarbazone (0.01 mol) and sodium acetate (0.02 mol) were dissolved in 30-40 ml of glacial acetic acid taken in a round bottomed flask equipped with a separating funnel for the addition of bromine. Bromine (0.7 ml in 5 ml of glacial acetic acid) was added slowly to it, while stirring magnetically. After 30 min of stirring, the solution was poured on crushed ice. The resulting solid was separated, dried, and recrystallized from ethanol.

Preparation of ethyl-2-aryl-6-methylimidazo-[2,1-b]-1,3,4-oxadiazole-5-carboxylate (IV)

0.01 mol of 2-amino-5-methyl-1,3,4-oxadiazole (III) was refluxed with 0.01 mol of ethyl-2-chloroacetoacetate in 50 ml of ethanol (70%) in the presence of 0.2 g pyridine for 5h. The solid that separate was recrystallized with water.

Preparation of 2-aryl-6-methylimidazo-[2,1-b]-1,3,4-oxadiazole-5-Carbohydrazide (V)

0.01 mol of IV was refluxed with 0.04 mol 0f the hydrazine hydrate (80%) in 20 ml ethanol for 2 h. The solid that separate was recrystallized with ethanol (60%).

Preparation of 2-aryl-6-methyl-N'-substituted phenylmethylidene-Imidazo-[2,1-b]-1,3,4-oxadiazole -5- carbohydrazide (01-18)

0.005 mol of V was refluxed with 0.005 mol of the appropriate aldehyde in 50 ml ethanol (60%) for 5 h. The solid that separated was washed & recrystallized with ethanol (80%).

2-(4-Chloro-phenyl)-6-methyl-imidazo[2,1-b][1,3,4]oxadiazole-5-carboxylic acid (4-methoxy-benzylidene)-hydrazine (1)

Yield 66%, m.p. 258-260 °C; IR (cm^{-1}) (KBr): 1089.71 (C-O str of 1,3,4-oxadiazole nucleus), 1660.60 (C=N str of 1,3,4-oxadiazole nucleus), 1600.81 & 1487.01 (C=C str of aromatic ring), 1780.17 (C=O str of amide group), 3286.48 (N-H str of amide group), 833.19 (C-H def of Disubstituted (para) aromatic ring), 742.54 (Ar-Cl str of substituted aromatic ring), 2852.52 (methyl group); ^{1}H-NMR (DMSO-d_6, TMS, δ ppm): 7.3-7.6 (Ar-H), 2.5 (CH_3), 7.9 (CONHN=CH), 7.7 (CONHN=CH), 3.4 (OCH_3); ESI-MS (Methanol) *m/z* 409.6 ([M+H]$^+$).

2-(4-Chloro-phenyl)-6-methyl-imidazo[2,1-b][1,3,4] oxadiazole-5-carboxylic acid (4-chloro-benzylidene)-hydrazine (4)

Yield 68%, m.p. 252-253 °C; IR (cm^{-1}) (KBr): 1086.86 (C-O str of 1,3,4-oxadiazole nucleus), 1658.67 (C=N str of 1,3,4-oxadiazole nucleus), 1600.87 & 1485.21 (C=C str of aromatic ring), 1782.10 (C=O str of amide group), 3294.19 (N-H str of amide group), 831.26 (C-H def of disubstituted (para) aromatic ring), 742.42 (Ar-Cl str of substituted aromatic ring), 2852.52 (methyl group); ^{1}H-NMR (DMSO-d_6, TMS, δ ppm): 7.2-7.7 (Ar-H), 2.4 (CH_3), 7.8 (CONHN=CH), 7.6 (CONHN=CH); ESI-MS (Methanol) *m/z* 418.02 ([M+H]$^+$).

2-(4-Chloro-phenyl)-6-methyl-imidazo[2,1-b][1,3,4] oxadiazole-5-carboxylic acid (4-methoxy-benzylidene)-hydrazine (7)

Yield 61, m.p. 268-270 °C; IR (cm^{-1}) (KBr): 1080.06 (C-O str of 1,3,4-oxadiazole nucleus), 1662.52 (C=N str of 1,3,4-oxadiazole nucleus), 1606.59 & 1502.44 (C=C str of aromatic ring), 1762.82 (C=O str of amide group), 3323.12 (N-H str of amide group), 831.28 (C-H def of disubstituted (para) aromatic ring), 746.40 (Ar-Cl str of substituted aromatic ring), 1024.13 (C-O str of 4-Methoxybenzene), 1259.43 (C-H str of methoxy group), 2852.52

(methyl group); ^{1}H-NMR (DMSO-d_6, TMS, δ ppm): 7.3-7.7 (Ar-H), 2.4 (CH_3), 7.9 (CONHN=CH), 7.7 (CONHN=CH), 3.8 ($ArOCH_3$); ESI-MS (Methanol) *m/z* 405.06 ($[M+H]^+$).

2.2 Pharmacology

Models of acute inflammation, which is induced by formalin, dextran, histamine, serotonin, bradykinin, prostaglandin and carrageenan are used to investigate anti-inflammatory effects of drugs [1]. Carrageenan-induced inflammation model is a COX-dependent reaction and is used to determine COX inhibition[14]. Models of chronic inflammation, which is provoked by subcutaneous (*sc*) implantation of foreign bodies are used to investigate the effects of drugs on a chronic phase of inflammation. The cotton pellet Granuloma method is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the cotton pellets correlates with the amount the amount of the granulomatous tissue [15].

For anti-inflammatory activity, adult albino rats of either sex weighing 150-175 g were divided in groups of six (n=6). The rats were acclimatized to laboratory conditions for one week before commencement of experiment. They were allowed free access to standard dry pellet diet and water ad libitum. All the test compounds and the reference drug were administered orally, suspended in 1% Carboxymethylcellulose (CMC). Acute oral toxicity test was performed for all the synthesized compounds according to organization of economic co-operation and development (OECD) guidelines. Statistical analyses were carried out with the single tailed t- test. A level of $P<0.001$ was adopted as the test of significance. Procedure employed for anti-inflammatory evaluation was reviewed and approved by the University animal ethical committee.

Acute oral toxicity [16]

It was performed as per OECD-423 guidelines (acute toxic class method).

Carrageenan-induced rat paw oedema test

The anti-inflammatory activity of the synthesized compounds was assessed by rat paw oedema assay [17] utilizing 0.1 mL, 1% carrageenan as phlogistic agent. The anti-inflammatory activity of test compounds and standard reference drug was determined by the formula % anti-inflammatory activity= (1-Vt/Vc) ×100, Where Vt represents the mean increase in paw volume in rat treated with test compounds and Vc represents the mean increase in paw volume in control group of rats.

Cotton pellets-induced granuloma method

Anti-inflammatory activity was determined as per the reported protocol [15].

3. RESULTS AND DISCUSSION

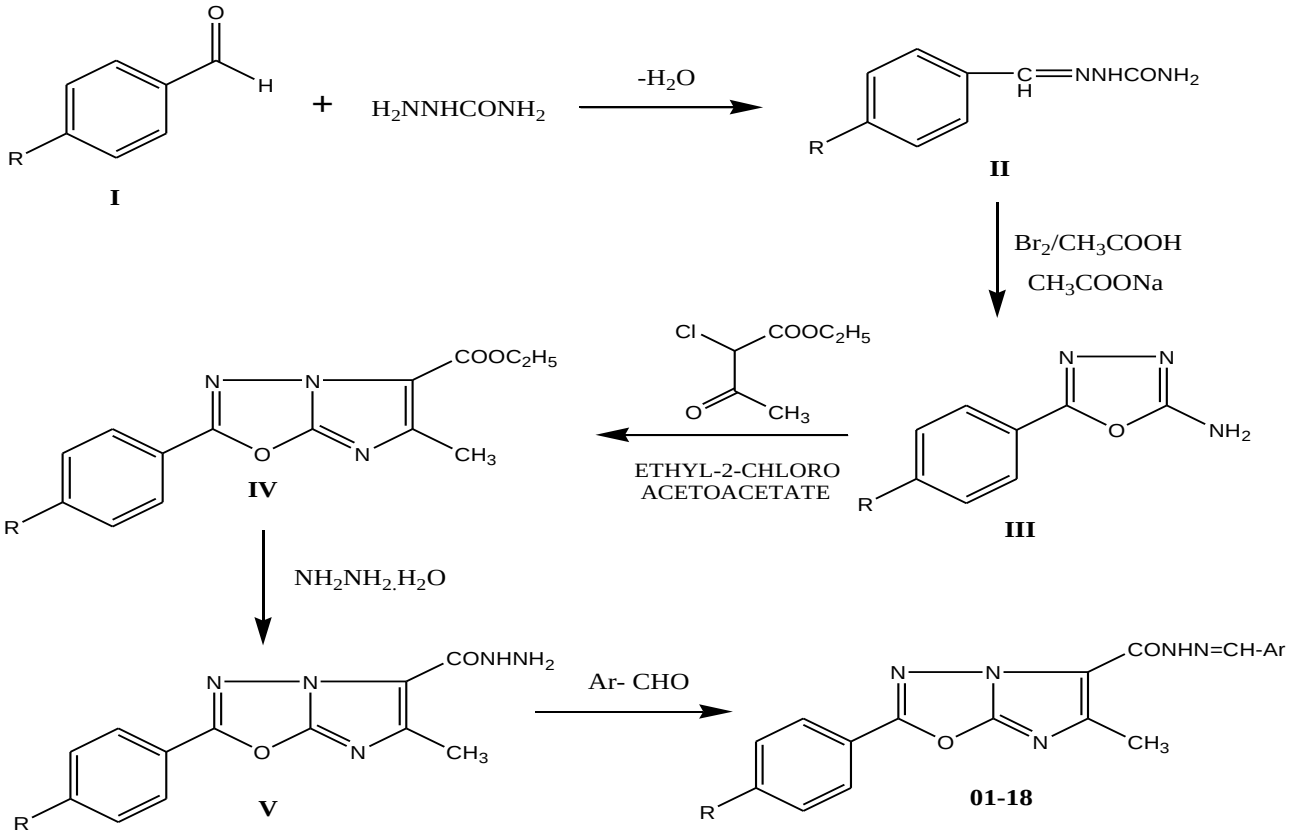
The structures of the title compounds were elucidated on the basis of elemental analysis, IR, ^{1}H-NMR and mass spectral data. The spectroscopic data of title compounds were in conformity with the assigned structure.

All the compounds were found to possess diverse anti-inflammatory properties in both the models selected in the present anti-inflammatory studies. The anti-inflammatory activity of oxadiazoles at doses of 100 mg/kg is indicated by their ability to provide 18-67%, 11-34% and 13-58% protection against Carrageenan-induced rat paw oedema assay, moist cotton pellet-induced and dry cotton pellet-induced granuloma method respectively (Table-1).

A result of carrageenan-induced rat paw oedema method indicates that, among the synthesized compounds maximum anti-inflammatory activity was exhibited by compound no. 4 with 67.79% protection and minimum activity was shown by compound no. 13 with 18.64% protection. Other compounds showing considerable activity were 1, 4, 7, 8, 9 and 12.

In order to evaluate the anti-inflammatory potential of synthesized oxadiazoles in chronic inflammatory conditions, test compounds were also subjected to cotton pellet-induced granuloma method. Almost similar results were observed in these studies except that compounds are less effective in chronic inflammatory conditions than in acute inflammatory conditions. Maximum activity was shown by compound no. 4 with 35.76% and 58.93% protection in moist and dry cotton pellet-induced granuloma method respectively. Minimum activity was exhibited by compound no. 13 with 11.26% and 13.37% protection in moist and dry cotton pellets-induced granuloma method, respectively. Other compounds with moderate anti-inflammatory activities were 1, 3, 7, 8 and 9.

The result of structure-activity studies among the synthesized oxadiazole analogues showed that anti-inflammatory activity is sensitive to structural changes. Among all the synthesized oxadiazole analogues, the most active compound 4 found to possess chloro group on benzene ring attached to C-5 of oxadiazole nucleus and para-chlorophenyl group attached to imine moiety.



Compd No	R	Ar	Compd No	R	Ar
1	Cl	-C_6H_4 (p-OCH_3)	**10**	OCH_3	-C_6H_4 (p-CH_3)
2	Cl	-C_6H_4 (p-CH_3)	**11**	OCH_3	-C_6H_4 (m-Cl)
3	Cl	-C_6H_4 (p-OH)	**12**	OCH_3	-C_6H_4 (o-Cl)
4	Cl	-C_6H_4 (p-Cl)	**13**	CH_3	-C_6H_4 (p-CH_3)
5	Cl	-C_6H_4 (m-Cl)	**14**	CH_3	-C_6H_4 (p-OCH_3)
6	Cl	-C_6H_4 (o-Cl)	**15**	CH_3	-C_6H_4 (p-OH)
7	OCH_3	-C_6H_4 (p-OCH_3)	**16**	CH_3	-C_6H_4 (p-Cl)
8	OCH_3	-C_6H_4 (p-OH)	**17**	CH_3	-C_6H_4 (m-Cl)
9	OCH_3	-C_6H_4 (p-CH_3)	**18**	CH_3	-C_6H_4 (o-Cl)

Figure 1: Scheme for Synthesis of Imidazo-[2,1-b]-1,3,4-oxadiazole Analogues

Table No 1: Anti-inflammatory Activity[i] of the Synthesized Oxadiazoles (100 mg/kg. p.o.) using Carrageenan-induced Rat Paw Oedema method and Cotton Pellets Induced Granuloma Method

Compd code	Carrageenan-induced Rat Paw oedema method		Cotton Pellets Induced Granuloma Method			
	Mean increase in Paw volume mL±SEM[ii]	Percentage Protection	Weight of moist cotton pellet±SEM[iii] (mg)	Percentage inhibition	Weight of dry cotton pellet±SEM[iv] (mg)	Percentage inhibition
1.	0.19±0.008	67.79	136.48±0.72	33.88	20.88±0.27	55.95
2.	0.32±0.007	47.76	156.72±0.60	24.07	25.92±0.42	45.32
3.	0.26±0.01	55.93	153.56±0.42	25.60	23.00±0.55	51.48
4.	0.20±0.03	66.10	134.60±0.51	34.77	19.47±0.47	58.93
5.	0.40±0.009	32.20	168.87±0.45	18.19	31.31±0.38	33.95
6.	0.35±0.007	40.67	166.06±0.06	19.55	28.52±0.48	39.84
7.	0.24±0.008	59.32	146.33±0.65	29.08	23.78±0.55	49.84
8.	0.21±0.005	64.40	148.63±0.72	27.99	21.52±0.31	54.60
9.	0.28±0.009	52.54	152.27±0.82	26.23	25.50±0.54	46.21
10.	0.32±0.007	45.76	156.96±0.44	23.96	26.38±0.42	44.35
11.	0.39±0.01	37.28	166.52±0.62	19.30	28.60±0.66	39.67
12.	0.89±0.009	50.84	165.14±0.67	19.99	28.81±0.48	39.23
13.	0.48±0.003	18.64	183.16±0.65	11.26	41.07±0.33	13.37
14.	0.32±0.006	45.76	161.43±0.76	21.79	26.59±0.44	43.91
15.	0.40±0.001	32.20	168.11±0.68	21.79	29.35±0.55	38.09
16.	0.38±0.01	35.59	166.52±0.62	19.30	28.58±0.66	39.71
17.	0.43±0.005	27.11	171.33±0.73	16.99	33.72±0.34	28.87
18.	0.46±0.005	22.03	172.88±0.60	16.24	33.17±0.51	30.03
Control	0.59±0.074	-	206.42±0.35	-	47.41±0.35	-

[1] The results were statistically significant ($P<0.001$) during all the observations.
[1] The mean increase in paw volume in rats treated with Indomethacin (10 mg/kg) observed in this experiment was 0.11±0.006 mL with percentage protection of 81.35 and $P<0.0001$.
[1] The mean increase in weight of moist cotton pellet in rats treated with Indomethacin (10 mg/kg) observed in this experiment was 102.38±0.46 mg with percentage protection of 50.40 and $P<0.0001$.
[1] The mean increase in weight of dry cotton pellet in rats treated with Indomethacin (10 mg/kg) observed in this experiment was 21.72±0.30 mg with percentage protection of 54.18 and $P<0.0001$.

Significant anti-inflammatory activity of compound 4 may be attributed to the electronegativity of chloro group which can withdraw electron more can strongly than methoxy and methyl group present in some of the compounds. Other point to be considered is that among all the compounds 13, 15, 16, 17 and 18 possess very weak anti-inflammatory activity. Compound with chloro group i.e. 1, 3 and 4 possess considerable activity in comparison to compounds with methyl group i.e. 13, 17 and 18. This may be due to the reason that chloro group is capable of withdrawing electrons more strongly than methyl group, due to its electronegative nature. Replacement of methyl group on aryl moiety with other group's i.e. chloro, hydroxy, or methoxy groups resulted in considerably increased anti-inflammatory activities in both the anti-inflammatory models.

4. CONCLUSION

In conclusion, 18 novel 1, 3, 4 oxadiazole analogues were synthesized for their potential anti-inflammatory activities, using carrageenan induced rat paw oedema method and cotton pellet-induced granuloma method. Among all the compounds, most potent anti-inflammatory compounds were 1, 7, 8, 9 and 12. In general, all the oxadiazoles possess greater anti-inflammatory activity in carrageenan-induced rat paw oedema method than in cotton pellet-induced granuloma method indicating that presently studied oxadiazoles are more effective in acute inflammatory conditions than in chronic inflammatory conditions. In our laboratory, research work is going on to explore the anti-inflammatory potential of oxadiazole analogues.

5. ACKNOWLEDGEMENT

The help rendered by RSIC, CDRI Lucknow for spectral and elemental analysis is gratefully acknowledged.

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