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Original article

Aroylpropionic acid based 2,5-disubstituted-1,3,4-oxadiazoles: Synthesis and their anti-inflammatory and analgesic activities

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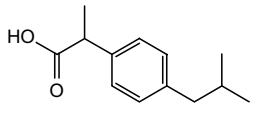
ABSTRACT

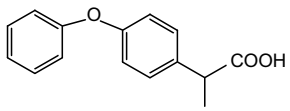
Synthesis and biological evaluation of various aroylpropionic acid derivatives containing 1,3,4-Oxadiazole nucleus is reported here. The compounds (**3a**–**w**) were synthesized by cyclization of 3-aroylpropionic acids into 1,3,4-oxadiazole nucleus by treating with various aryl acid hydrazides in the presence of $POCl_3$. The structures of new compounds are supported by IR, ^1H NMR and MS data. These compounds were tested in vivo for their anti-inflammatory activity. All the compounds tested showed anti-inflammatory activity. The compounds which showed activity comparable to the standard drug ibuprofen were screened for their analgesic, ulcerogenic and lipid peroxidation activities. Seven (**3c**, **g**, **i**, **j**, **m**, **o**, **p**) out of 23 new compounds showed very good anti-inflammatory activity in the carrageenan-induced rat paw edema test with very less ulcerogenic action. The compounds, which showed less ulcerogenic action, also showed reduced malondialdehyde production (MDA), which is one of the byproducts of lipid peroxidation. Compound **3i** and **o** showed 89.50 and 88.88% of inhibition in paw edema, 69.80 and 66.25% protection against acetic acid induced writhings and 0.7 and 0.65 of severity index respectively, compared to 90.12, 72.50 and 1.95 values of ibuprofen. The study showed that the cyclization of carboxylic group of aroylpropionic acids into an oxadiazole nucleus resulted in compounds having good anti-inflammatory and analgesic effects with reduced gastric irritation.

1. Introduction

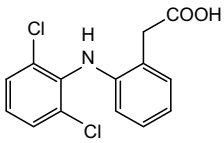
Aryl/aroyl acetic/propionic acid derivatives are widely used as anti-inflammatory and analgesic agents. But almost all the derivatives of this category like ibuprofen (**1**), fenbuprofen (**2**), diclofenac (**3**), fenbufen (**4**) under current clinical usage suffer from a common drawback of gastrointestinal toxicity due to direct contact of free carboxylic group with gastrointestinal mucosa and inhibition of cyclooxygenase enzyme non-selectively [1–3].



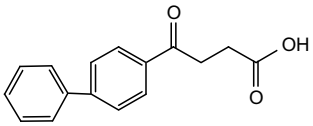
Ibuprofen (1)



Fenbuprofen (2)



Diclofenac (3)



Fenbufen (4)

Studies have shown that derivatization of the carboxylate function of these agents resulted in reduced ulcerogenic effect while sparing the anti-inflammatory and analgesic activities [4–8]. Furthermore substituted 1,3,4-oxadiazole derivatives also have been reported to show broad spectrum of biological activities including anticancer [9], antibacterial [10], anti-inflammatory and analgesic activities [11–17]. It was of interest to convert the free carboxylic function of these aroylpropionic acid derivatives into 1,3,4-oxadiazole nucleus and screen the synthesized compounds for their biological activity. In this paper we report 1,3,4-oxadiazole derivatives of three different 3-aroylpropionic acids as safer agents for treatment of inflammatory conditions (Table 1). The synthesized compounds were found to possess an interesting profile of anti-inflammatory and analgesic activities with significant reduction in the ulcerogenic effect (Table 2).

2. Chemistry

The synthesis of the title compounds (**3a**–**w**) is outlined in Scheme 1. The required 3-aroylpropionic acids (**1a**–**c**) were prepared by condensing appropriate aromatic compound with succinic anhydride in presence of anhydrous aluminium chloride following Friedel-Craft's acylation reaction conditions [18]. The acid hydrazides (**2a**–**j**) were prepared by esterification of different aryl acids followed by treatment with hydrazine hydrate in absolute ethanol [19]. Treatment of 3-aroylpropionic acids (**1a**–**c**) with aryl

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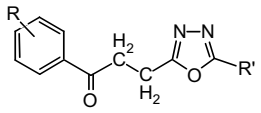
Table 1
Physical constants of the newly synthesized 2,5-substituted-1,3,4-oxadiazole derivatives (**3a**–**w**)



3a-w

Compound	R	R′	MP (°C)	Yield (%)	Compound	R	R′	MP (°C)	Yield (%)
3a	H	C_6H_5	167–169	48	**3m**	2,4-$(CH_3)_2$	4-Cl C_6H_5	118–120	56
3b	H	2-Cl C_6H_5	158–160	65	**3n**	2,4-$(CH_3)_2$	4-NO_2 C_6H_5	108–110	38
3c	H	4-Cl C_6H_5	197–199	56	**3o**	2,4-$(CH_3)_2$	4-OCH_3 C_6H_5	134–136	52
3d	H	4-NO_2 C_6H_5	164–166	41	**3p**	2,4-$(CH_3)_2$	3,4-$(OCH_3)_2$ C_6H_5	122–124	56
3e	4-CH_3	C_6H_5	176–177	62	**3q**	4-CH_3	$CH_2C_6H_5$	166–168	54
3f	4-CH_3	2-Cl C_6H_5	164–166	58	**3r**	4-CH_3	CH_2O C_6H_5	152–154	62
3g	4-CH_3	4-Cl C_6H_5	152–154	60	**3s**	2,4-$(CH_3)_2$	$CH_2C_6H_5$	126–128	61
3h	4-CH_3	4-NO_2 C_6H_5	148–150	46	**3t**	2,4-$(CH_3)_2$	CH_2O C_6H_5	130–132	39
3i	4-CH_3	4-OCH_3 C_6H_5	140–142	56	**3u**	H	CH_2 O 2-$C_{10}H_7$	145–148	41
3j	4-CH_3	3,4-$(OCH_3)_2$ C_6H_5	136–138	66	**3v**	4-CH_3	CH_2O 2-$C_{10}H_7$	160–162	46
3k	2,4-$(CH_3)_2$	C_6H_5	132–134	61	**3w**	4-CH_3	CH_2O 1-$C_{10}H_7$	144–146	56
3l	2,4-$(CH_3)_2$	2-Cl C_6H_5	126–128	52					

acid hydrazides (**2a**–**j**) in phosphorus oxychloride resulted in cyclization of the carboxylic function of 3-aroylpropionic acid into title compounds (**3a**–**w**) in 38–66% yield after crystallization with methanol. The purity of compounds was checked by TLC in benzene:acetone (8:2). The structure of all the synthesized compounds was established by ^{1}H NMR, IR, Mass spectral analysis. The result of elemental analysis of the synthesized compounds were in all cases within ±0.4% of the theoretical values.

3. Results and discussion

Protocol of animal experiments has been approved by the Institutional Animal Ethics Committee (IAEC). The anti-inflammatory activity of newly synthesized compounds (**3a**–**w**) was carried out on Wistar rats by Winter et al. [20] method using ibuprofen as reference compound. Analgesic activity was carried out on albino mice by Seigmund et al. [21] method and ulcerogenic activity was carried out by Cioli et al. [22] method. Lipid peroxidation studies were carried by Ohkawa et al. [23] method.

3.1. Anti-inflammatory activity

The anti-inflammatory activity of compounds was carried out at an equimolar oral dose relative to 20 mg kg^{-1} of ibuprofen. The percent edema inhibition relative to control was measured after 2 and 3 h of the treatment. The inhibition of swelling in carrageenan-induced edema in rat paw brought about by oral administration of the drugs is shown in Table 2. The percentages of swelling of the drugs were calculated using Eq. (1).

$$\text{Inhibition } (\%) = \{[(V_t - V_O)\ \text{control} - (V_t - V_O)\ \text{treated}]/ \times (V_t - V_O)\ \text{control}\} \times 100 \quad (1)$$

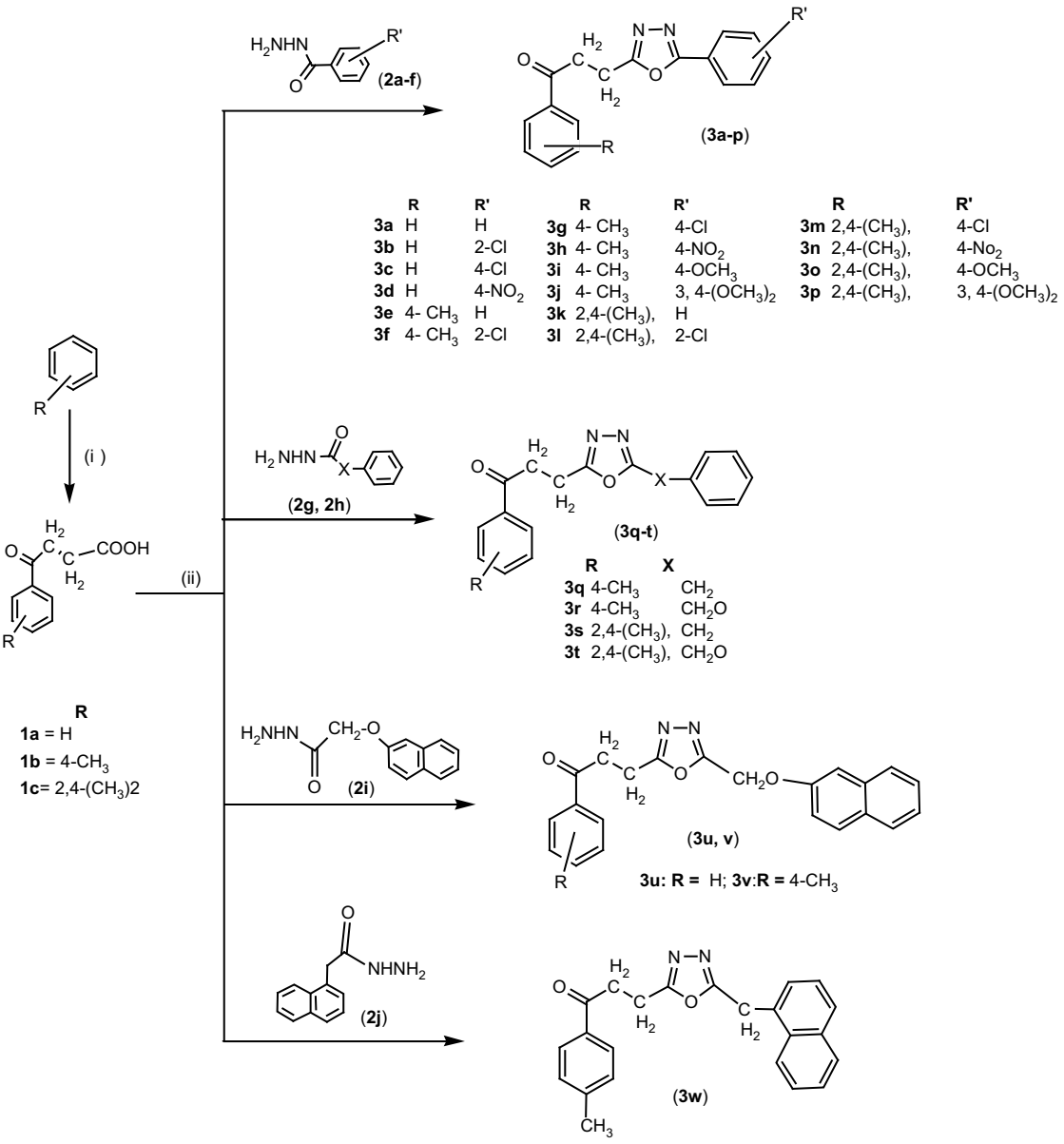
(V_t and V_O relates to the average volume in the hind paw of the rats ($n = 6$) before any treatment and after anti-inflammatory agent treatment, respectively).

All the synthesized compounds tested for anti-inflammatory activity showed inhibition of edema ranging from 24 to 90%. Compounds (**3c**, **g**, **i**, **j**, **m**, **o**, **p**) showed very good anti-inflammatory activity. 2-[3-(4-Methylphenyl)-propane-3-one]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles (**3i**) (89.50%) and 2-[3-(2,4-dimethylphenyl)-propane-3-one]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles (**3o**) (88.88%) were equipotent to ibuprofen (90.12%) in inhibiting the paw edema in rats. Compound 2-(3-phenyl propane-3-one)-5-(4-chlorophenyl)-1,3,4-oxadiazole (**3c**), 2-[3-(4-methyl phenyl)-propane-3-one]-5-(4-chlorophenyl)-1,3,4-oxadiazole (**3g**) showed anti-inflammatory activity 81.46 and 81.48%, respectively, compared to the standard drug. Compounds **3j**, **m**, **n** and **p** showed inhibition in edema equivalent to 80% of inhibition by ibuprofen. Oxadiazoles having substituted phenyl ring at 5th position were in general more active than unsubstituted ones, indicating that the presence of functional groups may be helpful in properly orienting the molecule in active site. The presence of 4-methoxyphenyl or 3,4-dimethoxyphenyl substitution at 5th position of oxadiazole

Table 2
Percentage of inhibition caused by synthesized compounds in carrageenan-induced edema in rats after 2 and 3 h of test drug administration

Compound	Anti-inflammatory activity (% inhibition ± S.E.M.)		Compound	Anti-inflammatory activity (% inhibition ± S.E.M.)	
	2h	**3h**		**2h**	**3h**
3a	18.66 ± 4.34	29.42 ± 3.22	**3n**	40.0 ± 3.18	69.13 ± 1.68*
3b	26.89 ± 3.20	35.80 ± 2.67	**3o**	62.66 ± 3.29	88.88 ± 2.09*
3c	41.97 ± 3.76	81.46 ± 1.52*	**3p**	58.66 ± 3.49	75.31 ± 2.04*
3d	40.88 ± 3.29	62.66 ± 3.29*	**3q**	24.66 ± 1.94	51.85 ± 2.40
3e	33.33 ± 3.20	49.3 ± 2.72	**3r**	25.33 ± 2.70	41.97 ± 1.71
3f	36.21 ± 2.30	57.33 ± 2.39	**3s**	25.33 ± 4041	49.98 ± 3.4
3g	62.667 ± 1.52	81.48 ± 0.64*	**3t**	27.55 ± 3.11	45.67 ± 2.76
3h	52.0 ± 2.98	60.49 ± 2.14	**3u**	29.78 ± 3.39	51.03 ± 4.16
3i	69.33 ± 1.94	89.50 ± 2.60*	**3v**	24.66 ± 2.48	49.38 ± 3.40
3j	53.31 ± 2.39	72.63 ± 2.25*	**3w**	24.00 ± 3.87	37.03 ± 2.24
3k	36.0 ± 3.89	58.02 ± 2.16	Ibuprofen	80.89 ± 3.11	90.12 ± 1.79*
3l	33.54 ± 2.93	60.49 ± 2.35	Control	–	–
3m	53.33 ± 2.39	72.63 ± 2.25*			

$n = 6$, *$p < 0.01$ compared to control.



Scheme 1. Reagent and conditions: (i) succinic anhydride, anhydrous $AlCl_3$, reflux; (ii) POCl3, reflux.

ring seems important for good anti-inflammatory activity. Presence of other electronegative group at *para* position of phenyl ring at 5th position of oxadiazole nucleus also resulted in increased activity.

However, replacement of substituted phenyl with benzoyl group resulted in decrease in activity. Loss of activity was also observed when phenyl group at 5th position oxadiazole nucleus was replaced by bulky group like naphthoyl. Statistical significance testing using one-way analysis of variance showed that the anti-inflammatory activity of ibuprofen and all the newly synthesized compounds were effective in comparison with the control group ($p < 0.01$). Since compounds (**3i** and **o**) showed anti-inflammatory activity at par to ibuprofen; therefore, it was of interest to study the anti-inflammatory of these compounds for longer duration (12 h). It was observed that the compounds were practically effective in inhibiting the edema for longer duration (8 h) compared to ibuprofen (Table 3). Compounds, which showed more than 60% inhibition in edema, were further screened for their analgesic effect and ulcerogenic profile.

3.2. Analgesic activity

Compounds (**3c**, **d**, **g**–**j**, **l**–**p**) showing more than 60% of inhibition in swelling induced by carrageenan were further tested for analgesic activity at the same dose as used for anti-inflammatory activity. The percent protection in mice brought about by administration of the drugs is shown in Table 4. The compounds tested showed analgesic activity in the range of 47–70%. The percent protection was calculated using Eq. (2).

$$\text{Protection } (\%) = 100 - [\text{number of writhings in test/number of writhing in control} \times 100] \quad (2)$$

Table 3
Percentage of inhibition caused by ibuprofen, **3j** and **t** in carrageenan-induced edema in rats

	Time (h)				
	2	3	4	8	12
Ibuprofen	80.89 ± 2.05*	90.12 ± 1.79*	65.45 ± 1.68*	46.72 ± 1.12*	39.06 ± 1.22*
3i	69.33 ± 1.94*	89.50 ± 2.60*	77.14 ± 2.23*	63.39 ± 1.68*	42.71 ± 1.54*
3o	62.66 ± 3.29*	88.88 ± 2.09*	75.70 ± 1.98*	57.14 ± 1.59*	44.82 ± 1.86*

Data are represented as mean ± SEM, $n = 6$, $^{*}p < 0.01$ compared to control.

Compound **3i** and **o** showed 69.80 and 66.25% of protection respectively, against acetic acid induced writhings compared to 72.50% protection with ibuprofen. Similar pattern in analgesic effect of compounds was observed as was seen in anti-inflammatory activity. Compound bearing electronegative group at 4th position of phenyl ring were good in analgesic effect compared to other substitutions.

3.3. Acute ulcerogenesis

The compounds which were screened for analgesic activity were studied for their gastric irritation activity. The ulcerogenic effect of ibuprofen and newly synthesized compounds were studied at 60 mg kg^{-1} in rats. It was observed that the ulcerogenic effect of test compounds (**3c**, **d**, **g**–**j**, **l**–**p**) was appreciably less than ibuprofen. Less number of ulcers were seen in animals treated with test compounds compared with the animals treated with ibuprofen. The tested compounds showed severity index ranging from 0.65 to 1.15, whereas the standard drug ibuprofen showed high severity index of 1.95 comparatively (Table 4). Compounds **3i**, **m** and **o** showed severity index of 0.7, 0.71 and 0.65 respectively, which is less than half the value of ibuprofen (1.95). These findings support the statement that the derivatization of carboxylic function of aroylpropionic acids results in decreased gastric irritation and that these newly synthesized compounds may be considered as safer drugs for treating inflammation conditions.

3.4. Lipid peroxidation

Compounds, which are less irritant to gastric mucosa, are also reported to show reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation [23]. Therefore by determining the MDA levels it can be ascertained that the compounds are less irritant to gastric mucosa. To correlate the ulcerogenic profile of compounds the lipid peroxidation values were also determined. Compounds which were screened for ulcerogenic activity were therefore analyzed for lipid peroxidation. The lipid peroxidation was measured as nmoles of MDA per 100 mg of tissue. Animals treated with ibuprofen exhibited 7.51 whereas control group showed 2.59 and the groups treated with synthesized compounds showed lipid peroxidation in the range of (3–5.5) (Table 4). These findings further confirm that the synthesized compounds are less irritant to gastric mucosa.

Table 4
Percentage protection in acetic acid induced writhings by test compounds and corrosive effects observed on gastric mucosa of rats treated with single oral dose of test compounds

Compound number	Analgesic activity		Ulcerogenic activity (S.I.)	nmols of MDA content ± S.E.M. per 100 mg tissue
	No. of writhings ± SEM	% Protection		
3c	8.99 ± 1.12	63.00*	1.12 ± 0.12**	5.32 ± 0.65$^{\Upsilon}$
3d	10.17 ± 0.79	58.20*	0.98 ± 0.18**	4.95 ± 0.56$^{\Upsilon}$
3g	10.00 ± 0.82	58.89*	0.98 ± 0.25**	4.25 ± 0.35$^{\Upsilon}$
3h	11.33 ± 0.89	53.43*	0.95 ± 0.32**	3.95 ± 0.85$^{\Upsilon}$
3i	7.33 ± 0.66	69.80*, $^{\Upsilon}$	0.70 ± 0.21**	3.15 ± 0.46$^{\Upsilon}$
3j	9.15 ± 0.76	62.39*	0.87 ± 0.14**	3.85 ± 0.35$^{\Upsilon}$
3l	10.49 ± 0.95	56.80*	0.89 ± 0.36**	4.15 ± 0.74$^{\Upsilon}$
3m	9.63 ± 0.86	60.42*	0.71 ± 0.51**	3.26 ± 0.23$^{\Upsilon}$
3n	12.83 ± 0.95	47.20*	0.87 ± 0.18**	4.42 ± 0.52$^{\Upsilon}$
3o	8.21 ± 1.32	66.25*, $^{\Upsilon}$	0.65 ± 0.21**	3.79 ± 0.52$^{\Upsilon}$
3p	10.66 ± 1.21	56.10*	1.12 ± 0.13**	5.12 ± 0.36$^{\Upsilon}$
Ibuprofen	6.67 ± 0.68	72.50*, $^{\Upsilon}$	1.95 ± 0.10**	7.51 ± 0.65
Control	24.33 ± 0.24	–	–	2.59 ± 0.20

Severity index (S.I.): mean score of each treated group minus the mean score of the control group.
$^{*}p < 0.01$ compared to control, $^{**}p < 0.001$ compared to control, $^{*\Upsilon}p < 0.001$ compared to control.
$^{\Upsilon}p < 0.001$ compared to control.

All the above observations suggest that cyclization of carboxylic group of aroylpropionic acids into oxadiazole nucleus results in safe anti-inflammatory agents which is supported by the fact that the synthesized compounds were less corrosive to GIT as indicated by severity index and lipid peroxidation values. Further studies to acquire more information about structure–activity relationship are in progress in our laboratory.

4. Conclusions

Various derivatives of aroylpropanoic acid containing oxadiazole nucleus were successfully synthesized and screened for anti-inflammatory, analgesic, ulcerogenic activities and lipid peroxidation studies. Some of the synthesized compounds (**3c**, **g**, **i**, **j**, **m**, **o**, **p**) were very safe with anti-inflammatory and analgesic activities comparable to ibuprofen. All the synthesized compounds showed anti-inflammatory activity but only compounds **3c**, **d**, **g**, **h**–**j**, **l**–**p** were tested for analgesic and ulcerogenic activities and lipid peroxidation studies. The tested compounds were found good in analgesic effects and less irritant to gastric mucosa as indicated by severity index. The lipid peroxidation values of the compounds tested were also less than the standard drug. The results obtained support the statement that the synthesized compounds may be used as safer anti-inflammatory agents.

5. Experimental

All the reagents and solvents used were procured from E Merck (India) Ltd., ibuprofen was obtained as gift sample from Sun Pharmaceuticals Pvt. Ltd. All the solvents used were dried and distilled before use. Animals were procured from Institutional animal house. Melting points were determined in open-end capillary tubes using Kjeldhal flask containing liquid paraffin and are uncorrected. IR spectra were recorded in KBr on Bio-Rad FTIR spectrometer FTS 135 and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Brucker Avance (300 MHz) in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. Mass spectra were recorded on JEOL 5x102/DA-6000 Mass Spectrometer/Data system using Argon/Xenon (6 kV, 10 mA) as the FAB gas. The purity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel G (Merck), visualized by iodine vapour or UV. Developing solvents were benzene:acetone (8:2).

5.1. Synthesis of 3-aroylpropionic acid derivatives (*1a–c*)

Synthesis of 3-aroylpropionic acid derivatives (**1a**–**c**) was carried out by reported procedure given in Organic Synthesis Collective Vol III [18].

5.2. General procedure for 2-[3-(substituted phenyl)propan-3-one]-5-(aryl)-1,3,4-oxadiazole (*3a–w*)

Appropriate aryl acid hydrazide (**2**) (1 mmol) was dissolved in phosphorus oxychloride (5 mL) and to it was added appropriate propanoic acid (**1**) (1 mmol). The reaction mixture, after refluxing for

5 h, was cooled to room temperature and poured onto crushed ice. The contents were neutralized with sodium bicarbonate solution (20%), a solid mass separated out which was filtered, washed with water and dried. It was crystallized from methanol/ethanol.

*5.2.1. 2-(3-Phenyl propane-3-one)-5-phenyl-1,3,4-oxadiazole (**3a**)*

IR (cm^{-1}, KBr): 2963, 1670, 1445, 1056, 783. ^1H NMR (DMSO-d_6) δ 2.62 and 3.28 (t, each, 2 × CH_2), 7.49–7.52 (m, 3H, H-3,4,5,phenyl), 7.73–7.77 (m, 2H, H-2,6,phenyl), 7.87–7.92 (m, 5H, phenyl); Mass (*m*/*z*) 278 (M^+).

*5.2.2. 2-(3-Phenyl propane-3-one)-5-(2-chlorophenyl)-1,3,4-oxadiazole (**3b**)*

IR (cm^{-1}, KBr): 2982, 1665, 1475, 1070, 812. ^1H NMR (DMSO-d_6) δ 2.64 and 3.32 (t, each, 2 × CH_2), 7.52–7.56 (m, 4H, H-3,4,5,6,*o*-chloro phenyl), 7.76–7.91 (m, 5H, phenyl); Mass (*m*/*z*) 312 (M^+).

*5.2.3. 2-(3-Phenyl propane-3-one)-5-(4-chlorophenyl)-1,3,4-oxadiazole (**3c**)*

IR (cm^{-1}, KBr): 3005, 1650, 1456, 1071, 805; ^1H NMR (DMSO-d_6) δ 2.65 and 3.36 (t, each, 2 × CH_2), 7.31–7.33 and 7.47–7.51 (m, 4H, *p*-chlorophenyl), 7.66–7.69 (m, 5H, phenyl); Mass (*m*/*z*) 312 (M^+).

*5.2.4. 2-(3-Phenyl propane-3-one)-5-(4-nitrophenyl)-1,3,4-oxadiazole (**3d**)*

IR (cm^{-1}, KBr): 2991, 1671, 1485, 1078, 812; ^1H NMR (DMSO-d_6) δ 2.61 and 3.31 (t, each, 2 × CH_2), 7.26–7.98 (complex m, 8H, 2 × phenyl); Mass (*m*/*z*) 323 (M^+).

*5.2.5. 2-[3-(4-Methylphenyl)-propane-3-one]-5-phenyl-1,3,4-oxadiazole (**3e**)*

IR (cm^{-1}, KBr): 2970, 1650, 1455, 1058, 793; ^1H NMR (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 2.68 and 3.32 (t, each, 2 × CH_2), 7.29–7.32 (m, 3H, H-3,4,5,phenyl), 7.73–7.75 (m, 2H, H-2,6,phenyl), 7.28 and 7.87 (d, each, A_2B_2, *p*-methyl phenyl); Mass (*m*/*z*) 292 (M^+).

*5.2.6. 2-[3-(4-Methylphenyl)-propane-3-one]-5-(2-chlorophenyl)-1,3,4-oxadiazole (**3f**)*

IR (cm^{-1}, KBr): 2990, 1656, 1470, 1069, 810; ^1H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 2.68 and 3.33 (t, each, 2 × CH_2), 7.32–7.36 (m, 4H, H-3,4,5,6,*o*-chloro phenyl), 7.34 and 7.86 (d, each, A_2B_2, *p*-methyl phenyl); Mass (*m*/*z*) 326 (M^+).

*5.2.7. 2-[3-(4-Methylphenyl)-propane-3-one]-5-(4-chlorophenyl)-1,3,4-oxadiazole (**3g**)*

IR (cm^{-1}, KBr): 3005, 1660, 1462, 1071, 809; ^1H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 2.67 and 3.35 (t, each, 2 × CH_2), 7.31, 7.47, 7.66, 7.89 (d, each, 2 × A_2B_2, 2 × phenyl); Mass (*m*/*z*) 326 (M^+).

*5.2.8. 2-[3-(4-Methylphenyl)-propane-3-one]-5-(4-nitrophenyl)-1,3,4-oxadiazole (**3h**)*

IR (cm^{-1}, KBr): 2996, 1650, 1490, 1075, 818; ^1H NMR (DMSO-d_6) δ 2.37 (s, 3H, CH_3), 2.62 and 3.23 (t, each, 2 × CH_2), 7.26–7.98 (complex m, 8H, 2 × phenyl); Mass (*m*/*z*) 337 (M^+).

*5.2.9. 2-[3-(4-Methylphenyl)-propane-3-one]-5-(4-methoxyphenyl)-1,3,4-oxadiazoles (**3i**)*

IR (cm^{-1}, KBr): 3010, 1665, 1490, 1062, 795; ^1H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 2.64 and 3.23 (t, each, 2 × CH_2), 7.09 and 7.78 (d, each, A_2B_2, *p*-methyl phenyl), 7.46 and 7.96 (d, each, A_2B_2, *p*-methoxyl phenyl); Mass (*m*/*z*) 322 (M^+).

*5.2.10. 2-[3-(4-Methylphenyl)-propane-3-one]-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole (**3j**)*

IR (cm^{-1}, KBr): 3010, 1655, 1484, 1060, 822; ^1H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 3.97 (two closely spaced singlets, 6H, 2 × OCH_3), 2.68 and 3.31 (t, each, 2 × CH_2), 6.99 (d, 1H, H-5, dimethoxyphenyl), 7.15 (d, 1H, H-2, dimethoxyphenyl), 7.39 (dd, 1H, H-6, dimethoxyphenyl), 7.24 and 7.86 (d, each, A_2B_2, *p*-methyl phenyl); Mass (*m*/*z*) 352 (M^+).

*5.2.11. 2-[3-(2,4-Dimethylphenyl)-propane-3-one]-5-phenyl-1,3,4-oxadiazole (**3k**)*

IR (cm^{-1}, KBr): 2940, 1655, 1458, 1059, 755; ^1H NMR (DMSO-d_6) δ 2.34 and 2.51 (s, each, 2 × CH_3), 2.76 and 3.27 (t, each, 2 × CH_2), 7.07–7.21, 7.38–7.40, 7.62–7.66, 7.75–7.78 (m, each, 8H, 2 × phenyl); Mass (*m*/*z*) 306 (M^+).

*5.2.12. 2-[3-(2,4-Dimethylphenyl)-propane-3-one]-5-(2-chlorophenyl)-1,3,4-oxadiazole (**3l**)*

IR (cm^{-1}, KBr): 2955, 1650, 1465, 1070, 807; ^1H NMR (DMSO-d_6) δ 2.35 and 2.49 (s, each, 2 × CH_3), 2.78 and 3.27 (t, each, 2 × CH_2), 7.11–7.16 (m, 2H, H-3,5,xylene), 7.32–7.37 (m, 4H, H-3,4,5,6,*o*-chloro phenyl), 7.65 (d, 1H, H-6, xylene); Mass (*m*/*z*) 341 (M^+).

*5.2.13. 2-[3-(2,4-Dimethylphenyl)-propane-3-one]-5-(4-chlorophenyl)-1,3,4-oxadiazole (**3m**)*

IR (cm^{-1}, KBr): 2955, 1666, 1510, 1072, 821; ^1H NMR (DMSO-d_6) δ 2.39 and 2.52 (s, each, 2 × CH_3), 2.81 and 3.29 (t, each, 2 × CH_2), 7.15–7.20 (m, 2H, H-3,5,phenyl), 7.66 (d, 1H, H-6, phenyl), 7.71 and 7.78 (d, each, A_2B_2, *p*-chlorophenyl); Mass (*m*/*z*) 341 (M^+).

*5.2.14. 2-[3-(2,4-Dimethylphenyl)-propane-3-one]-5-(4-nitrophenyl)-1,3,4-oxadiazole (**3n**)*

IR (cm^{-1}, KBr): 2956, 1665, 1504, 1073, 825; ^1H NMR (DMSO-d_6) δ 2.38 and 2.53 (s, each, 2 × CH_3), 2.79 and 3.28 (t, each, 2 × CH_2), 7.13–7.17 (m, 2H, H-3,5,phenyl), 7.67 (d, 1H, H-6, phenyl), 7.75 and 7.83 (d, each, A_2B_2, *p*-nitrophenyl); Mass (*m*/*z*) 365 (M^+).

*5.2.15. 2-[3-(2,4-Dimethylphenyl)-propane-3-one]-5-(4-methoxyphenyl)-1,3,4-oxadiazole (**3o**)*

IR (cm^{-1}, KBr): 2945, 1655, 1461, 1057, 783; ^1H NMR (DMSO-d_6) δ 2.33 and 2.48 (s, each, 2 × CH_3), 2.77 and 3.27 (t, each, 2 × CH_2), 7.05–7.13 (m, 2H, H-3,5,phenyl), 7.63 (d, 1H, H-6, phenyl), 6.97 and 7.68 (d, each, A_2B_2, *p*-methoxyphenyl); Mass (*m*/*z*) 336 (M^+).

*5.2.16. 2-[3-(2,4-Dimethylphenyl)-propane-3-one]-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole (**3p**)*

IR (cm^{-1}, KBr): 2962, 1655, 1471, 1071, 806; ^1H NMR (DMSO-d_6) δ 2.35 and 2.49 (s, each, 2 × CH_3), 2.78 and 3.27 (t, each, 2 × CH_2), 3.93 and 3.98 (s, each, 6H, 2 × OCH_3), 7.03–7.56 (m, 6H, 2 × phenyl); Mass (*m*/*z*) 366 (M^+).

*5.2.17. 2-[3-(4-Methylphenyl)-propane-3-one]-5-benzyl-1,3,4-oxadiazole (**3q**)*

IR (cm^{-1}, KBr): 2969, 1650, 1480, 1056, 805; ^1H NMR (DMSO-d_6) δ 2.37 (s, 3H, CH_3), 2.66 and 3.31 (t, each, 2 × CH_2), 4.12 (s, 2H, CH_2), 7.42–7.46 (m, 3H, H-3,4,5,phenyl), 7.61–7.65 (m, 2H, H-2,6,phenyl), 7.33 and 7.81 (d, each, A_2B_2, *p*-methyl phenyl); Mass (*m*/*z*) 306 (M^+).

*5.2.18. 2-[3-(4-Methylphenyl)-propane-3-one]-5-phenoxymethyl-1,3,4-oxadiazole (**3r**)*

IR (cm^{-1}, KBr): 2990, 1655, 1485, 1055, 790; ^1H NMR (DMSO-d_6) δ 2.38 (s, 3H, CH_3), 2.68 and 3.29 (t, each, 2 × CH_2), 4.56 (s, 2H, OCH_2), 7.48–7.51 (m, 3H, H-3,4,5,phenyl), 7.64–7.67 (m, 2H, H-2,6,phenyl), 7.28 and 7.79 (d, each, A_2B_2, *p*-methyl phenyl); Mass (*m*/*z*) 322 (M^+).

*5.2.19. 2-[3-(2,4-Dimethylphenyl)-propane-3-one]-5-benzyl-1,3,4-oxadiazole (**3s**)*

IR (cm^{-1}, KBr): 2908, 1654, 1451, 1061, 780; ^1H NMR (DMSO-d_6) δ 2.33 and 2.50 (s, each, 2 × CH_3), 2.75 and 3.28 (t, each, 2 × CH_2),

4.11 (s, 2H, CH_2), 7.05–7.75 (complex m, 8H, 2 × phenyl); Mass (*m*/*z*) 320 (M^+).

*5.2.20. 2-[3-(2,4-Dimethylphenyl)-propane-3-one]-5-phenoxymethyl-1,3,4-oxadiazole (**3t**)*

IR (cm^{-1}, KBr): 2916, 1650, 1463, 1060, 762; ^1H NMR (DMSO-d_6) δ 2.36 and 2.53 (s, each, 2 × CH_3), 2.77 and 3.31 (t, each, 2 × CH_2), 4.59 (s, 2H, OCH_2), 7.09–7.93 (complex m, 8H, 2 × phenyl); Mass (*m*/*z*) 336 (M^+).

*5.2.21. 2-(3-Phenyl propane-3-one)-5-(1-naphthoxymethyl)-1,3,4-oxadiazole (**3u**)*

IR (cm^{-1}, KBr): 3015, 1655, 1470, 1056, 790; ^1H NMR (DMSO-d_6) δ 2.63 and 3.29 (t, each, 2 × CH_2), 2.75 (s, 2H, CH_2), 7.36–7.39 and 7.79–7.81 (m, each, 7H, naphthyl), 7.29–7.33 (m, 5H phenyl); Mass (*m*/*z*) 358 (M^+).

*5.2.22. 2-[3-(4-Methylphenyl)-propane-3-one]-5-(2-naphthoxymethyl)-1,3,4-oxadiazole (**3v**)*

IR (cm^{-1}, KBr): 2985, 1655, 1460, 1062, 823; ^1H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 2.69 and 3.31 (t, each, 2 × CH_2), 2.92 (s, 2H, CH_2), 7.28–7.31 and 7.83–7.88 (m, each, 7H, naphthyl), 7.34 and 7.86 (d, each, A_2B_2, *p*-methyl phenyl); Mass (*m*/*z*) 372 (M^+).

*5.2.23. 2-[3-(4-Methylphenyl)-propane-3-one]-5-(1-naphthoxymethyl)-1,3,4-oxadiazole (**3w**)*

IR (cm^{-1}, KBr): 3005, 1650, 1460, 1060, 810; ^1H NMR (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 2.65 and 3.25 (t, each, 2 × CH_2), 2.85 (s, 2H, CH_2), 7.36–7.39 and 7.79–7.82 (m, each, 7H, naphthyl), 7.29 and 7.88 (d, each, A_2B_2, *p*-methyl phenyl); Mass (*m*/*z*) 372 (M^+).

5.3. Anti-inflammatory activity

The anti-inflammatory activity was evaluated using carrageenan-induced paw odema on rat [20] method. Wistar rats (150–200 g) were divided into groups of six animals each. Group I served as the control group without using the drug, group II received ibuprofen 20 mg kg^{-1}, other groups received test drugs in dose molecularly equivalent to the ibuprofen. Drug solutions were prepared as a homogeneous suspension in aqueous solution of sodium CMC (0.5% w/v) and were administered orally to the animals. Thirty minutes after administration of drugs, each rat received a sub-planter injection of 0.1 mL of 1% carrageenan solution in its left hind paw. The measurement of the hind paw volume was carried out using a Ugo Basile Plethysmometer before any treatment (V_o) and in any interval (V_t) after the administration of the drugs. All the results are expressed as mean ± S.E.M. Statistical evaluation was performed using analysis of variance followed by *t*-test for sub-group comparison.

5.4. Analgesic activity

Compounds **3c**, **d**, **g**–**j**, **l**–**p** were tested for analgesic activity. Analgesic activity was carried out by using acetic acid induced writhing method [21] in Swiss albino mice (25–30 g) of either sex. A 1% v/v solution of acetic acid was used as writhing inducing agent. Since the maximum anti-inflammatory effect was observed at 3 h post-treatment the analgesic effect was also studied after 3 h of drug administration. Test compounds were administered orally 3 h prior to acetic acid injection. Mice were divided into groups of six animals each. Group I served as a control group without using the drug, while group II received ibuprofen 20 mg kg^{-1}, other groups received test drugs in dose molecularly equivalent to 20 mg kg^{-1} of ibuprofen. All drugs were prepared as a homogeneous suspension in aqueous solution of sodium CMC (0.5% w/v) and administered orally to animals. Acetic acid was administered intraperitoneally 1 mL per 100 g body weight of the animal. Number of writhings were counted for 10 min in control, standard and test compounds and compared. Analgesic activity was measured as percent decrease in writhings in comparison to control. All the results are expressed as mean ± S.E.M. Statistical evaluation was performed using analysis of variance followed by *t*-test for sub-group comparison.

5.5. Acute ulcerogenesis

The studies were carried out on healthy Wistar rats (150–200 g) at a dose three times the anti-inflammatory dose viz. 60 mg kg^{-1}. The animals were divided into different groups of six each, group I served as control and received vehicle only and groups II received pure ibuprofen 60 mg kg^{-1}. Other groups were administered test compounds in dose molecularly equivalent to 60 mg kg^{-1} of ibuprofen. The animals were fasted 8 h prior to a single dose of each of the vehicle, standard and test compounds, respectively, and sacrificed 17 h later during which period food and water were available. The gastric mucosa of the rats was examined by means of a 4× binocular magnifier. For each stomach the severity of mucosal damage was assessed according to the following scoring system: 0 – no lesions of upto five punctiform lesions; 1 – more than five punctiform lesions; 2 – one to five small ulcers; 3 – more than five small ulcers of one large ulcer; 4 – more than one large ulcer [22]. The mean score of each treated group minus the mean score of the control group was considered as the 'severity index' of gastric damage (level of significance $p < 0.001$).

5.6. Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al. [23]. After screening the animals for ulcerogenic effect of synthesized drugs the gastric mucosa of animals was scraped with two glass slides, weighed (100 mg) and homogenized in 1.8 mL of 1.15% ice-cold KCl solution. The homogenate was supplemented with 0.2 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of acetate buffer (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid (TBA). The mixture was heated at 95 °C for 60 min. The cooled reactants were shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm after supplementing with 5 mL of a mixture of *n*-butanol and pyridine (15:1 v/v). The supernatant organic layer was collected and absorbance was measured at 532 nm on UV spectrophotometer. The results are expressed as nmols of MDA per 100 mg tissue, using extinction coefficient 1.56 × 105 cm^{-1} M^{-1}.

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