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Short communication

Fenbufen based 3-[5-(substituted aryl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-ones as safer antiinflammatory and analgesic agents

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ABSTRACT

The synthesis of a series of 3-[5-(substituted aryl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-ones derived from 4-oxo-4-(biphenyl-4-yl)butanoic acid (fenbufen) is described. The structures of these compounds were supported by IR, ^{1}H NMR, mass spectrometric data and elemental analysis. These compounds were tested for their antiinflammatory, analgesic, ulcerogenic and lipid peroxidation actions. A few compounds were found to have very good antiinflammatory activity in carrageenan induced rat paw edema test, while a fair number of compounds showed significant analgesic activity in acetic acid induced writhing test. The newly synthesized compounds showed very low ulcerogenic action with reduced malondialdehyde (MDA) content, which is one of the byproducts of lipid peroxidation. In vitro COX-1 and COX-2 isozyme inhibition studies were also performed on some of the selected compounds. Compound **4i** and **4h** were found to be more selective towards COX-2 as indicated by COX-2 selectivity index of 36.06 and 29.05 (COX-2 $IC_{50} = 1.5$ μM and 1.8 μM) respectively.

1. Introduction

Non-steroidal antiinflammatory drugs (NSAIDs) are the most commonly prescribed medications in the world. They are used for the treatment of pain, fever and inflammation, particularly arthritis [1,2]. The most prevalent side effects of the use of non-steroidal antiinflammatory drugs are the occurrence of gastrointestinal damage with gastric upset and irritation being the major problems [3,23]. The search for safer NSAIDs continues with the failure of anticipated 'Ideal' antiinflammatory agents, the coxibs, on long-term usage [4,5].

Fenbufen **3** is an example of a well-known aroylpropanoic acid class of antiinflammatory drugs and available in the market under the name of Cinopal [6]. Aroylpropanoic acids including fenbufen are good antiinflammatory agents but produce gastrointestinal side effects [6,7]. Synthetic approaches based upon chemical modification have been taken with the aim of improving NSAIDs' safety profile [7–12]. Studies by us [7,8] and others [9–12] have shown that derivatization of the carboxylate function of some NSAIDs resulted in an increased antiinflammatory activity with reduced ulcerogenic effect.

During recent years there has been a large investigation on different classes of oxadiazole compounds many of which were found to possess an extensive spectrum of pharmacological activities. In particular, compounds bearing 1,3,4-oxadiazole nucleus are known to exhibit unique antiedema and antiinflammatory activities [13–15]. Differently substituted oxadiazole moiety has also been found to have other interesting activities such as analgesic, antibacterial, antifungal, anticonvulsant, anticancer, etc. [16–20].

In our attempt to discover safer agents for treatment of inflammatory conditions, we have replaced the carboxylic acid group of fenbufen with an additional heterocycle i.e. 1,3,4-oxadiazole. These new compounds have been found to possess potential antiinflammatory and analgesic activities with significant reduction in their ulcerogenic effect.

2. Chemistry

3-[5-(Substituted aryl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-ones **4a–l** described in this study are shown in Table 1,

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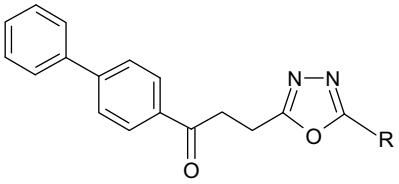
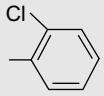
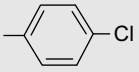
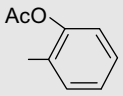
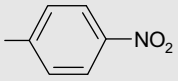
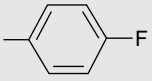
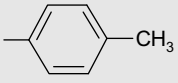
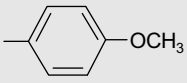
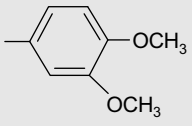
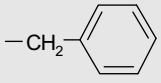
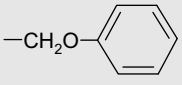
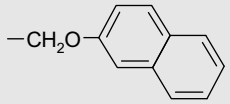
Table 1
Biological activities of the compounds **4a**–**l**.



Compound No.	R	Antiinflammatory activity (% inhibition)	Analgesic activity (% protection)	Ulcerogenic activity (severity index)	Lipid peroxidation[a]
4a		43.7 ± 1.6**	51.3 ± 0.4**	0.4 ± 0.1**	4.3 ± 0.2**
4b		46.8 ± 1.0**	66.1 ± 0.6**	0.5 ± 0.1**	5.7 ± 0.1**
4c		53.1 ± 1.3	61.9 ± 0.2**	0.6 ± 0.1**	5.8 ± 0.1**
4d		43.7 ± 1.1**	56.3 ± 0.4**	0.7 ± 0.1**	5.8 ± 0.1**
4e		56.2 ± 1.1	45.8 ± 0.3**	0.5 ± 0.2**	4.3 ± 0.2**
4f		53.1 ± 1.3	72.5 ± 0.2**	0.6 ± 0.2**	4.6 ± 0.1**
4g		50.0 ± 1.1*	45.1 ± 0.1**	0.5 ± 0.2**	5.8 ± 0.2**
4h		59.4 ± 2.3	51.3 ± 0.0**	0.5 ± 0.1**	6.1 ± 0.1**
4i		62.5 ± 1.1*	57.3 ± 0.0**	0.7 ± 0.1**	4.9 ± 0.1**
4j		50.0 ± 1.1*	54.1 ± 0.6	0.5 ± 0.2**	5.8 ± 0.1**
4k		53.1 ± 1.2	55.8 ± 0.0**	0.6 ± 0.1**	5.8 ± 0.1**

(*continued on next page*)

Table 1 (*continued*)

Compound No.	R	Antiinflammatory activity (% inhibition)	Analgesic activity (% protection)	Ulcerogenic activity (severity index)	Lipid peroxidation[a]
4l		59.4 ± 1.4	68.6 ± 0.0**	0.6 ± 0.2**	5.9 ± 0.1**
Diclofenac		65.6 ± 1.3	70.3 ± 0.1	2.3 ± 0.2	8.9 ± 0.1
Fenbufen		56.3 ± 1.3	54.1 ± 0.0	1.5 ± 0.2	6.8 ± 0.1
Control			–	0.00	3.8 ± 0.1

*$P < 0.05$ compared to the parent drug (fenbufen), and data were given in mean ± SEM and analyzed by ANOVA.
**$P < 0.01$ compared to the parent drug (fenbufen), and data were given in mean ± SEM and analyzed by ANOVA.
[a] Expressed as nmol MDA content/100 mg tissue.

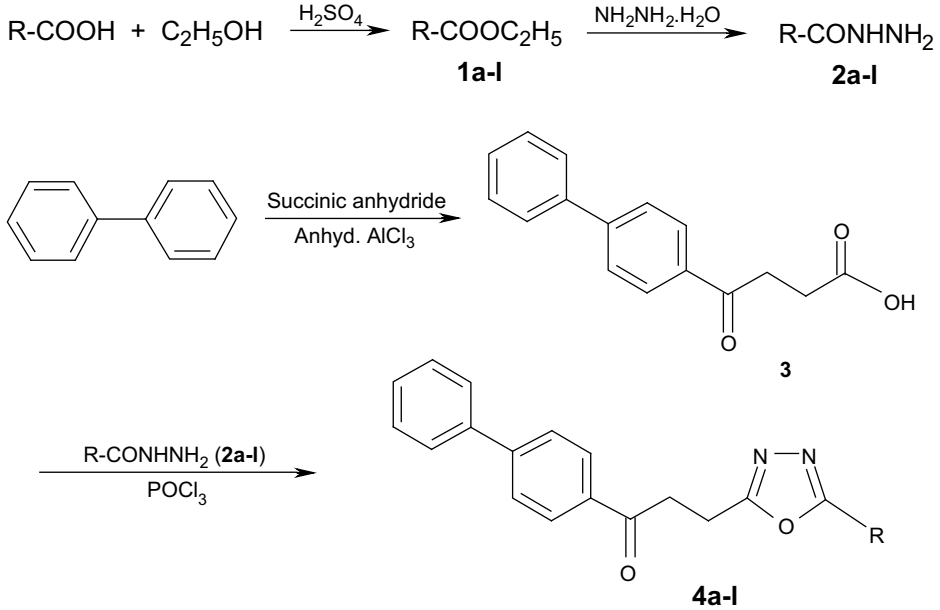
and the reaction sequence for the preparation is outlined in Scheme 1. The required 4-oxo-4-(biphenyl-4-yl)butanoic acid (fenbufen) **3** was prepared by condensing biphenyl with succinic anhydride in the presence of anhydrous aluminium chloride following Friedel–Craft's acylation reaction conditions [7]. Reaction between fenbufen and aryl acid hydrazides **2a–l** in phosphorous oxychloride (reaction time varies from 2 to 5 h) afforded title compounds **4a–l** in 56–71% yield. The purity of compounds was checked by TLC and elemental analysis.

Analytical and spectral data (^{1}H NMR, IR, mass spectra) of the synthesized compounds were in full agreement with the proposed structures. In general, The ^{1}H NMR spectral data of the title compounds showed two triplets of two protons each approximately at around δ 2.5 and δ 3.5 ppm, respectively which are assigned to two methylene protons (–CH_2–CH_2–). Other signals were observed at appropriate places. The mass spectra showed acylium fragments containing biphenyl and aryl moieties as major peaks followed by peaks with loss of CO besides the molecular ion peaks in reasonable intensities supporting the structure. In the case of aryl group having chlorine atom as a substituent, the molecular ion or other related ions produced the appropriate isotopic abundances. The infrared spectral data (cm^{-1}; KBr) revealed bands at 2975–2945 (CH_2), 1650–1665 (C=O) and 1440–1420 (C–N).

3. Pharmacological results and discussion

3.1. Antiinflammatory activity

Compounds **4a–l** were evaluated for their in-vivo antiinflammatory activity by carrageenan induced paw edema method [21]. The protocol of animal experiments has been approved by the Institutional Animal Ethics Committee (IAEC). The compounds were tested at 10 mg/kg oral dose and were compared with the standard drug diclofenac and the parent drug fenbufen at the same oral dose. The tested compounds showed antiinflammatory activity ranging from 43.75% to 62.50% (Table 1). Compounds **4c**, **4e**, **4f**, **4h**, **4k** and **4l** were equipotent to fenbufen while **4i** proved to be more potent than fenbufen, its antiinflammatory activity being comparable with that of diclofenac. Data show that the presence of 2-naphtyloxymethyl, 4-methoxyphenyl or 3,4-dimethoxy phenyl substitution at the 5 position of the oxadiazole ring can either maintain or improve the antiinflammatory activity in this series of fenbufen derivatives.



R=C_6H_5-, 2-Cl-C_6H_4-, 4-Cl-C_6H_4-, 2-OAc-C_6H_4-, 4-NO_2-C_6H_4-,4-F-C_6H_4-, 4-CH_3-C_6H_4-
4-OCH_3-C_6H_4-,3,4-$(OCH_3)_2$-C_6H_3-, $C_6H_5CH_2$-, C_6H_5-OCH_2-, 2-$C_{10}H_7$-OCH_2-

Scheme 1.

3.2. Analgesic activity

The analgesic activity of the synthesized compounds was evaluated by acetic acid induced writhing test [22]. The newly synthesized compounds showed activity ranging from 45.12% to 72.52%. The activity showed that compound **4f** exhibited maximum analgesic activity (72.52%) and its activity was better than that of the standard drug diclofenac (70.32%). Compound **4l** showed good activity (68.57%). Seven compounds (**4b**, **4c**, **4d**, **4f**, **4i**, **4k** and **4l**) out of twelve new compounds have been found to possess better analgesic activity (55.80–72.52%) than that of fenbufen (54.12%). The results are presented in Table 1.

It is concluded that the presence of 4-fluorophenyl or 2-naphthyloxymethyl or chlorophenyl substitution at position 5 in the oxadiazole ring causes significant increase in analgesic activity.

3.3. Acute ulcerogenesis

All compounds were screened for their ulcerogenic activity according to Cioli et al. [23]. The tested compounds showed significant reduction in ulcerogenic activity ranging from 0.417 to 0.666, whereas the standard drug diclofenac and the parent drug fenbufen showed high severity index, 2.332 and 1.500 (Table 1). The results indicate that compounds showed very low ulcerogenic potential. Thus it is concluded that transformation of the carboxylic group of fenbufen into oxadiazole nucleus resulted in marked decrease in ulcerogenic activity while retaining their high antiinflammatory and analgesic properties.

3.4. Lipid peroxidation study

Lipid peroxidation refers to the oxidative degradation of lipids. This process proceeds by free radical chain reaction in which free radicals abstract electrons from the lipids in cell membrane and consequently damages the cell. It most often affects polyunsaturated fatty acids forming malondialdehyde (MDA). It is evident that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation [24]. Therefore, an attempt was made to correlate the low ulcerogenesis of the compounds with that of lipid peroxidation. The lipid peroxidation was measured as nmol of MDA/100 mg of tissue [25]. Diclofenac and fenbufen (standard and parent drugs) produced high lipid peroxidation, 8.911 and 6.842 respectively, whereas the control group showed 3.788 nmol/100 mg of tissue (Table 1). It was found that all compounds showing low ulcerogenic activity also reduced lipid peroxidation. Therefore, the study indicated that these oxadiazole derivatives have inhibited the induction of gastric mucosal lesions and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa.

3.5. In vitro cyclooxygenase (COX) inhibition assays

The compounds (**4e**, **4h**, **4i** and **4l**), which showed significant antiinflammatory activity (>55% of inhibition in edema), were further evaluated for their ability to inhibit cyclooxygenase (COX) according to the method described by Jashim Uddin et al. [26]. In vitro COX-1 and COX-2 enzyme inhibition data showed that the compounds were more selective towards COX-2 than COX-1. Compound **4i** and **4h** with 4-methoxy and 3,4-dimethoxy substitution showed a selectivity index of 36.1 and 29 for COX-2 vs COX-1. These compounds (**4i** and **4h**) have IC_{50} value (COX-2) of 1.5 μM and 1.8 μM and IC_{50} value (COX-1) of 54.1 μM and 52.3 μM, respectively, suggesting that methoxy group helps the compound in orientation more favorable for COX-2 blocking. The parent drug fenbufen showed selectivity index of 0.48.

4. Experimental protocols

4.1. Chemistry

Melting points were determined in open capillary tubes and are uncorrected. Microanalysis of the compounds was done on Perkin–Elmer model 240 analyzer and the values were found within ±0.4% of the theoretical values. The IR spectra were measured as potassium bromide pellets using a Perkin–Elmer 1725X spectrophotometer. ^{1}H NMR spectra were recorded on Bruker spectropsin DPX-300 MHz with tetramethylsilane (TMS) as an internal standard. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Spectral data are consistent with assigned structures. The progress of each reaction was monitored on silica gel G plates using iodine vapors as visualizing agent.

4.1.1. Aryl acid ethyl esters (**1a–l**) and their hydrazides (**2a–l**)

These compounds were obtained by the method reported in literature [27].

*4.1.1.1. Benzoic acid hydrazide (**2a**).* Yield: 68%, m.p. 94–96 °C. ^{1}H NMR ($CDCl_3$) δ 4.29 (s, 2H, NH_2), 7.47 (m, 3H, H-3,4,5), 7.63 (m, 2H, H-2,6), 8.10 (s, 1H, CONH). MS: *m*/*z* 136 (M^+), 105, 78, 77.

*4.1.1.2. 2-Chlorobenzoic acid hydrazide (**2b**).* Yield: 72%, m.p. 88 °C. ^{1}H NMR ($CDCl_3$) δ 4.50 (s, 2H, NH_2), 7.19–7.25 (m, 4H, phenyl), 8.21 (s, 1H, CONH). MS: *m*/*z* 170 (M^+), 105, 77.

*4.1.1.3. 4-Chlorobenzoic acid hydrazide (**2c**).* Yield: 71%, m.p. 98–100 °C. ^{1}H NMR ($CDCl_3$) δ 4.57 (s, 2H, NH), 7.41 and 7.52 (d, each, 2× A_2B_2, *p*-disubstituted phenyl), 8.27 (s, 1H, CONH). MS: *m*/*z* 170 (M^+), 139, 105, 77.

*4.1.1.4. 2-Acetoxybenzoic acid hydrazide (**2d**).* Yield: 85%, m.p. 82 °C. ^{1}H NMR ($CDCl_3$) δ 4.03 (s, 3H, OCH_3), 4.88 (s, 2H, NH), 7.17–7.21 (m, 4H, phenyl), 8.42 (s, 1H, CONH). MS: *m*/*z* 194 (M^+), 105, 77.

*4.1.1.5. 4-Nitrobenzoic acid hydrazide (**2e**).* Yield: 75%, m.p. 70 °C. ^{1}H NMR ($CDCl_3$) δ 4.79 (s, 2H, NH), 7.73 and 7.91 (d, each, 2× A_2B_2, *p*-disubstituted phenyl), 8.60 (s, 1H, CONH). MS: *m*/*z* 181 (M^+), 135, 105, 77.

*4.1.1.6. 4-Fluorobenzoic acid hydrazide (**2f**).* Yield: 78%, m.p. 96–98 °C. ^{1}H NMR ($CDCl_3$) δ 4.60 (s, 2H, NH), 7.48 and 7.79 (d, each, 2× A_2B_2, *p*-disubstituted phenyl), 9.01 (s, 1H, CONH). MS: *m*/*z* 154 (M^+), 105, 77.

*4.1.1.7. 4-Methylbenzoic acid hydrazide (**2g**).* Yield: 70%, m.p. 74 °C. ^{1}H NMR ($CDCl_3$) δ 1.77 (s, 3H, CH_3), 4.08 (s, 2H, NH), 6.88 and 7.53 (d, each, 2× A_2B_2, *p*-disubstituted phenyl), 8.19 (s, 1H, CONH). MS: *m*/*z* 150 (M^+), 119, 91, 77.

*4.1.1.8. 4-Methoxybenzoic acid hydrazide (**2h**).* Yield: 68%, m.p. 78 °C. ^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3H, OCH_3), 4.14 (s, 2H, NH), 6.96 and 7.60 (d, each, 2× A_2B_2, *p*-disubstituted phenyl), 8.21 (s, 1H, CONH). MS: *m*/*z* 166 (M^+), 135, 107, 77.

*4.1.1.9. 3,4-Dimethoxybenzoic acid hydrazide (**2i**).* Yield: 74%, m.p. 71 °C. ^{1}H NMR ($CDCl_3$) δ 3.96 (two closely spaced singlets, 2×

OCH_3), 4.53 (s, 2H, NH), 6.98 (d, 1H, H-5), 7.13 (d, 1H, H-2), 7.31 (dd, 1H, H-6), 8.19 (s, 1H, CONH). MS: *m*/*z* 196 (M^+), 166.

*4.1.1.10. Phenylacetic acid hydrazide (**2j**).* Yield: 68%, m.p. 82 °C. ^{1}H NMR ($CDCl_3$) δ 3.58 (s, 2H, CH_2), 4.15 (s, 2H, NH), 6.98–7.18 (m, 5H, phenyl), 8.25 (s, 1H, CONH). MS: *m*/*z* 150 (M^+), 119, 78, 77.

*4.1.1.11. Phenoxyacetic acid hydrazide (**2k**).* Yield: 72%, m.p. 78 °C. ^{1}H NMR ($CDCl_3$) δ 4.56 (s, 2H, OCH_2), 5.08 (s, 2H, NH), 7.21–7.28 (m, 5H, phenyl), 8.63 (s, 1H, CONH). MS: *m*/*z* 166 (M^+), 135, 77.

*4.1.1.12. 2-Naphthoxy acetic acid hydrazide (**2l**).* Yield: 76%, m.p. 105–107 °C. ^{1}H NMR ($CDCl_3$) δ 4.78 (s, 2H, OCH_2), 4.97 (s, 2H, NH), 7.19 (m, 2H, H-1,3), 7.94 (m, 5H, H-4,5,6,7,8), 8.56 (s, 1H, CONH). MS: *m*/*z* 216 (M^+), 128, 127.

*4.1.2. 4-Oxo-4-(biphenyl-4-yl)butanoic acid (fenbufen) (**3**)*

Fenbufen was prepared by the method reported in literature [7]. Yield: 70%, m.p. 180 °C, ^{1}H NMR ($CDCl_3$) δ 2.82 and 3.37 (t, each, 2× CH), 7.45 (m, 3H, H-3,4,5, phenyl), 7.64 (m, 2H, H-2,6, phenyl), 7.70 and 8.07 (d, each, 2× A_2B_2, *p*-substituted phenyl). MS: *m*/*z* 254 (M^+), 181, 153, 77.

*4.1.3. General procedure for the synthesis of 3-[5-(substituted aryl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-ones (**4a**–**l**)*

Compounds **2a**–**l** (0.001 mol) were dissolved in phosphorous oxychloride (5 mL) and to it was added **3** (equimolar, 0.001 mol). The reaction mixture, after refluxing for 2–5 h, was cooled to room temp. and poured onto crushed ice. On neutralization of the contents with sodium bicarbonate solution (20% w/v) a solid mass separated out which was filtered, washed with water, dried and recrystallized from methanol to give **4a**–**l**.

*4.1.3.1. 3-(5-Phenyl-1,3,4-oxadiazol-2-yl)-1-(biphenyl-4-yl)propan-1-one (**4a**).* Yield: 62%, m.p. 148 °C. IR (cm^{-1}, KBr): 2975, 1650, 1420. ^{1}H NMR ($CDCl_3$) δ 2.51 and 3.47 (t, each, 2× CH_2), 7.48 (m, 6H, H-3,4,5, 2× phenyl), 7.62 (m, 4H, H-2,6, 2× phenyl), 7.65 and 8.14 (d, each, 2× A_2B_2, *p*-disubstituted phenyl). MS: *m*/*z* 354 (M^+), 181, 105, 78, 77. Anal. $C_{23}H_{18}N_2O_2$ (C, H, N).

*4.1.3.2. 3-[5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-one (**4b**).* Yield: 58%, m.p. 158–160 °C. IR (cm^{-1}, KBr): 2967, 1650, 1435. ^{1}H NMR ($CDCl_3$) δ 2.55 and 3.43 (t, each, 2× CH_2), 7.29 (m, 4H, H-3,4,5,6, *o*-chloro phenyl), 7.57 (m, 5H, phenyl), 7.73 and 7.84 (d, each, 2× A_2B_2, *p*-disubstituted phenyl). MS: *m*/*z* 388 (M^+), 181, 153, 78, 77. Anal. $C_{23}H_{17}N_2O_2Cl$ (C, H, N).

*4.1.3.3. 3-[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-one (**4c**).* Yield: 61%, m.p. 146 °C. IR (cm^{-1}, KBr): 2970, 1655, 1430. ^{1}H NMR ($CDCl_3$) δ 2.51 and 3.57 (t, each, 2× CH_2), 7.32 (m, 3H, H-3,4,5, phenyl), 7.38 (m, 2H, H-2,6, phenyl), 7.45 and 7.64 (d, each, 2× A_2B_2, *p*-disubstituted phenyl; biphenyl), 7.11 and 7.83 (d, each, 2× A_2B_2, *p*-chloro phenyl). MS: *m*/*z* 388 (M^+), 181, 153, 77. Anal. $C_{23}H_{17}N_2O_2Cl$ (C, H, N).

*4.1.3.4. 3-[5-(2-Acetoxyphenyl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-one (**4d**).* Yield: 66%, m.p. 138–140 °C. IR (cm^{-1}, KBr): 2955, 1660, 1425. ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H, $OCOCH_3$), 2.61 and 3.52 (t, each, 2× CH_2), 7.48 (m, 3H, H-3,4,5, phenyl), 7.65 (m, 2H, H-2,6, phenyl), 7.25 (m, 4H, H-3,4,5,6, *o*-disubstituted phenyl), 7.77 and 7.84 (d, each, 2× A_2B_2, *p*-disubstituted phenyl). MS: *m*/*z* 412 (M^+), 181, 153, 93, 92, 78, 77. Anal. $C_{25}H_{20}N_2O_4$ (C, H, N).

*4.1.3.5. 3-[5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-one (**4e**).* Yield: 56%, m.p. 122 °C. IR (cm^{-1}, KBr): 2965, 1663, 1433. ^{1}H NMR ($CDCl_3$) δ 2.61 and 3.57 (t, each, 2× CH_2), 7.44 (m, 3H, H-3,4,5, phenyl), 7.62 (m, 2H, H-2,6, phenyl), 7.71 and 7.85 (d, each, 2× A_2B_2, *p*-nitro phenyl), 7.74 and 7.98 (d, each, 2× A_2B_2, *p*-disubstituted phenyl). MS: *m*/*z* 399 (M^+), 181, 153, 77. Anal. $C_{23}H_{17}N_3O_4$ (C, H, N).

*4.1.3.6. 3-[5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-one (**4f**).* Yield: 71%, m.p. 136–138 °C. IR (cm^{-1}, KBr): 2970, 1653, 1420. ^{1}H NMR ($CDCl_3$) δ 2.55 and 3.52 (t, each, 2× CH_2), 7.48 (m, 3H, H-3,4,5, phenyl), 7.66 (m, 2H, H-2,6, phenyl), 7.43 and 7.54 (d, each, 2× A_2B_2, *p*-fluoro phenyl), 7.05 and 7.84 (d, each, 2× A_2B_2, *p*-disubstituted phenyl). MS: *m*/*z* 372 (M^+), 181, 153, 77. Anal. $C_{23}H_{17}FN_2O_2$ (C, H, N).

*4.1.3.7. 3-[5-(4-Methylphenyl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-one (**4g**).* Yield: 58%, m.p. 144 °C. IR (cm^{-1}, KBr): 2975, 1655, 1428. ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H, CH_3), 2.53 and 3.37 (t, each, 2× CH_2), 6.64 and 7.78 (d, each, 2× A_2B_2, *p*-disubstituted phenyl), 7.42 (m, 5H, phenyl), 6.91 and 7.63 (d, each, 2× A_2B_2, *p*-methyl phenyl). MS: *m*/*z* 368 (M^+), 181, 153, 91, 77. Anal. $C_{24}H_{20}N_2O_2$ (C, H, N).

*4.1.3.8. 3-[5-(4-Methoxyophenyl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-one (**4h**).* Yield: 65%, m.p. 154–156 °C. IR (cm^{-1}, KBr): 2971, 1650, 1433. ^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3H, OCH_3), 2.61 and 3.19 (t, each, 2× CH_2), 6.76 and 7.87 (d, each, 2× A_2B_2, *p*-disubstituted phenyl), 7.53 (m, 5H, phenyl), 7.13 and 7.71 (d, each, 2× A_2B_2, *p*-methoxy phenyl). MS: *m*/*z* 384 (M^+), 153, 77. Anal. $C_{24}H_{20}N_2O_3$ (C, H, N).

*4.1.3.9. 3-[5-(3,4-Dimethoxy phenyl)-1,3,4-oxadiazol-2-yl]-1-(biphenyl-4-yl)propan-1-one (**4i**).* Yield: 62%, m.p. 150 °C. IR (cm^{-1}, KBr): 2970, 1655, 1425. ^{1}H NMR ($CDCl_3$) δ 3.95 (two closely spaced singlets, 6H, 2× OCH_3), 2.57 and 3.45 (t, each, 2× CH_2), 6.96 (d, 1H, H-5, dimethoxy phenyl), 7.13 (d, 1H, H-2, dimethoxy phenyl), 7.34 (dd, 1H, H-6, dimethoxy phenyl), 7.46 (m, 3H, H-3,4,5, phenyl), 7.66 (m, 2H, H-2,6, phenyl), 7.71 and 8.17 (d, each, 2× A_2B_2, *p*-disubstituted phenyl). MS: *m*/*z* 414 (M^+), 181, 153, 77. Anal. $C_{25}H_{22}N_2O_4$ (C, H, N).

*4.1.3.10. 3-(5-Benzyl-1,3,4-oxadiazol-2-yl)-1-(biphenyl-4-yl)propan-1-one (**4j**).* Yield: 56%, m.p. 158 °C. IR (cm^{-1}, KBr): 2965, 1651 1430. ^{1}H NMR ($CDCl_3$) δ 2.39 and 3.58 (t, each, 2× CH_2), 4.11 (s, 3H, CH_2), 7.39 (m, 6H, H-3,4,5, 2× phenyl), 7.60 (m, 4H, H-2,6, 2× phenyl), 7.65 and 7.81 (d, each, 2× A_2B_2, *p*-disubstituted phenyl). MS: *m*/*z* 368 (M^+), 181, 153, 91, 77. Anal. $C_{24}H_{20}N_2O_2$ (C, H, N).

*4.1.3.11. 3-(5-Phenoxymethyl-1,3,4-oxadiazol-2-yl)-1-(biphenyl-4-yl)propan-1-one (**4k**).* Yield: 68%, m.p. 164 °C. IR (cm^{-1}, KBr): 2970, 1653, 1422. ^{1}H NMR ($CDCl_3$) δ 2.51 and 3.47 (t, each, 2× CH_2), 4.55 (s, 3H, OCH_2), 7.46 (m, 6H, H-3,4,5, 2× phenyl), 7.66 (m, 4H, H-2,6, 2× phenyl), 7.71 and 7.88 (d, each, 2× A_2B_2, *p*-disubstituted phenyl). MS: *m*/*z* 384 (M^+), 181, 135, 77. Anal. $C_{24}H_{20}N_2O_3$ (C, H, N).

*4.1.3.12. 3-[(5-(2-Naphthoxymethyl)-1,3,4-oxadiazol-2-yl)-1-(biphenyl-4-yl)propan-1-one (**4l**).* Yield: 61%, m.p. 166 °C. IR (cm^{-1}, KBr): 2965, 1655, 1440. ^{1}H NMR ($CDCl_3$) δ 2.51 and 3.47 (t, each, 2× CH_2), 4.89 (s, 3H, OCH_2), 7.19 (2H, H-1,3, naphthoxy), 7.48 (m, 3H, H-3,4,5, phenyl), 7.67 (m, 2H, H-2,6, phenyl), 7.75 and 8.11 (d, each, 2× A_2B_2, *p*-disubstituted phenyl), 7.94 (m, 5H, H-4,5,6,7,8, naphthoxy). MS: *m*/*z* 434 (M^+), 181, 153, 128, 77. Anal. $C_{23}H_{18}N_2O_2$ (C, H, N).

4.2. Pharmacology

4.2.1. Antiinflammatory activity

Antiinflammatory activity test was performed following the method of Winter et al. [21]. Freshly prepared suspension of carrageenan (0.05 mL, 1% w/v solution in 0.9% saline) was injected under the plantar aponeurosis of the right hind paw of each rat. Animals were divided in groups of six in each group. One group was kept as control and the animals of other groups were pre-treated with the test drugs suspended in 1% carboxymethylcellulose (CMC) given orally 30 min before carrageenan injection. The paw volume was measured before and after 4 h of carrageenan injection by plethysmograph. The percentage inhibition of inflammation was calculated by applying the following formula:

$$\text{Antiinflammatoryactivity (\% inhibition)} = (V_c - V_t)/V_c \times 100$$

where V_c = edema volume in control group, V_t = edema volume in groups treated with test compounds.

4.2.2. Analgesic activity

Analgesic activity was carried out by acetic acid induced writhing method [22] using albino mice (25–30 g) of either sex on groups of six animals each. A 1% aqueous acetic acid solution (i.p. injection; 0.1 mL) was used as writhing inducing agent. Mice were kept individually in the test cage, before acetic acid injection and habituated for 30 min. Screening of analgesic activity was performed after p.o. administration of test drugs at the dose of 10 mg/kg. All compounds were dissolved in 1% carboxymethylcellulose (CMC) solution. One group was kept for the control experiment and received p.o. administration of 1% CMC. Diclofenac and fenbufen were used as reference drugs. After 1 h of drug administration 0.10 mL of 1% acetic acid solution was given to mice intraperitoneally. Stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted for 5–15 min of acetic acid injection. The analgesic activity was expressed in terms of % inhibition. % Analgesic activity = $\{(n-n')/n\} \times 100$ where n = mean number of writhes of control group and n' = mean number of writhes of test group. The percent protection in mice brought about by administration of the drugs is shown in Table 1.

4.2.3. Acute ulcerogenesis

Acute ulcerogenesis test was done according to Cioli et al. [23]. Albino rats (150–200 g) were divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after p.o. administration of test compounds or diclofenac or fenbufen at the dose of 30 mg/kg. Control rats received p.o. administration of vehicle (suspension of 1% methylcellulose). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The gastric mucosa of the rats was examined by means of a magnifying glass. For each stomach, the severity of mucosal damage was assessed by measuring severity index i.e. severity of drug to cause mucosal damage. It is measured according to the following scoring system: 0.5 – redness; 1.0 – spot ulcers; 1.5 – hemorrhagic streaks; 2.0 – ulcers >3, but ≤5; 3.0 – ulcers > 5.

The mean score of each treated group minus the mean score of the control group was considered as severity index of gastric mucosal damage.

4.2.4. Lipid peroxidation study

Lipid peroxidation of the synthesized compounds was determined according to the method of Ohkawa et al. [25]. After evaluating ulcerogenic activity, the gastric mucosa was scrapped with two glass slides, weighed (100 mg) and homogenized in 1.8 mL of 1.15% cold potassium chloride solution. The homogenate was supplemented with 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of acetate buffer (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid. The mixture was heated at 95 °C for 1 h. After cooling, the reactants were supplemented with 5 mL of the mixture of *n*-butanol:pyridine (15:1 v/v), shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm. The supernatant organic layer was taken out and absorbance was measured at 532 nm on UV spectrophotometer. The results were expressed as nmol MDA/100 mg tissue, using extinction coefficient 1.56×10^5/cm/M.

4.2.5. In vitro cyclooxygenase (COX) inhibition assays

The ability of the test compounds listed in Table 2 to inhibit ovine COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immunoassay (EIA) kit (catalog no. 560101, Cayman Chemical, Ann Arbor, MI, USA) according to the methodology described by Jashim Uddin et al. [26]. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid (AA) to PGH_2. $PGF_{2\alpha}$ produced from PGH_2 by reduction with stannous chloride is measured by enzyme immunoassay (ACE™ competitive EIA). Stock solution of test compounds was dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (960 μL, 0.1 M Tris–HCl pH 8.0 containing 5 mM EDTA and 2 mM phenol) with either COX-1 or COX-2 (10 μL) enzyme in the presence of heme (10 μL) were added 10 μL of various concentrations of test drug solutions (0.01, 0.1, 1, 10, 50 and 100 μL in a final volume of 1 mL). These solutions were incubated for a period of 5 min at 37 °C after which 10 μL of AA (100 μM) solution were added and the COX reaction was stopped by the addition of 50 μL of 1 M HCl after 2 min. $PGF_{2\alpha}$ produced from PGH_2 by reduction with stannous chloride was measured by enzyme immunoassay. This assay is based on the competition between PGs and PG–acetylcholine esterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells since the concentration of PG tracer is held constant while the concentration of PGs varies. This antibody–PG complex binds to a mouse anti-rabbit monoclonal antibody that had been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagent, which contains the substrate to acetylcholine esterase, is added to the well. The product of this enzymatic reaction produces a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation: Absorbance α [Bound PG tracer] α1/PGs. Percent inhibition was calculated by the comparison of compound treated to various control incubations. The concentration of test compound causing 50% inhibition (IC_{50}, μM) was

Table 2
In vitro COX-inhibition data for **4e**, **4h**, **4i** and **4l**.

Compound	COX-1[a] (IC_{50}, μM)	COX-2[a] (IC_{50}, μM)	COX-2 SI[b]
4e	42.6	8.2	5.19
4h	52.3	1.8	29.05
4i	54.1	1.5	36.06
4l	48.4	2.5	19.36
Celecoxib	33.1	0.07	472
Fenbufen	3.9	8.1	0.48

[a] Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit (catalog no. 560101, Cayman Chemicals, MI, USA) and the deviation from the mean is <10% of the mean value.

[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

calculated from the concentration–inhibition response curve (duplicate determinations).

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