

Synthesis and Evaluation of Antiinflammatory, Analgesic and Ulcerogenic Potential of NSAIDs Bearing 1,3,4-Oxadiazole Scaffold

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Somani and Bhanushali: Nonulcerogenic 1,3,4-oxadiazole Derivatives of Diclofenac and Mefenamic Acid

Synthesis of 1,3,4-oxadiazole derivatives of diclofenac and mefenamic acid are described. The target compounds 5-[2-(2,6-dichloroanilino)benzyl]-2-aryl-1,3,4-oxadiazole (3a-3e) and 5-[2-(2,3-dimethylanilino)phenyl]-2-(aryl)-1,3,4-oxadiazole (6a-6e) were obtained by treating 2 and 5 with various aromatic acids using POCl₃ as dehydrating agent. They were purified and characterized by IR, ¹H-NMR and elemental analysis. These compounds were further subjected to antiinflammatory, analgesic and acute ulcerogenic activity. Compound 3c and 6d exhibited good antiinflammatory activity and compounds 3c, 3e, 6c, 6d, 6e were found to be non ulcerogenic.

Key words: Analgesic, antiinflammatory, diclofenac, mefenamic acid, ulcerogenic activity, 1,3,4-oxadiazole

Non steroidal antiinflammatory drugs (NSAIDs) are the first line drugs for the treatment of arthritis, pain and inflammatory disorder^[1]. The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme prostaglandin endoperoxidase, popularly known as cyclooxygenase (COX)^[2,3]. It was reported that COX exists in two isoforms, COX-1 and COX-2, which are regulated and expressed differently^[4-6]. COX-1 provides cytoprotection in the gastrointestinal tract (GIT), whereas inducible COX-2 selectively mediates inflammatory signals^[7-9]. Since most of the currently available NSAIDs in the market show greater selectivity for COX-1 than COX-2^[10], chronic use of NSAIDs, including diclofenac and mefenamic acid, may elicit appreciable GI irritation, bleeding and ulceration^[11]. GI damage from NSAID is generally attributed to two factors, local irritation by the direct contact of carboxylic acid (-COOH) moiety of NSAIDs with GI mucosal cells (topical effect) and decreased tissue prostaglandin production in tissues which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis are the effects shown by

the nonselective COX inhibitors^[12]. The ulcerogenesis due to carboxylate group can be minimized by modifying the acid functionality to ester^[13], amide or NO releasing group^[14,15]. Also derivatisation of the carboxylic acid group of NSAIDs to various five member heterocycles has been reported to retained antiinflammatory activity with reduced ulcerogenic potential^[16-19].

Among the various heterocycles, oxadiazole, especially 2,5-disubstituted-1,3,4-oxadiazole has unique features associated with it. It consists of toxophoric moiety (N-C-O)^[20] which enhances its potential as an effective biodynamic molecule, additionally the oxadiazole ring has been reported to be weak acidic in nature and it is an isoster for the ester functionality^[21]. The 2,5-disubstituted-1,3,4-oxadiazole is associated with diverse biological activities like anticonvulsant^[22], antidepressant^[23], non-ulcerogenic antiinflammatory^[24], anticancer^[25], antimicrobial-antiTB^[26] and antiangiogenic^[27] etc, which makes it biologically important scaffold.

Thus in our attempt to find new, safer and potent antiinflammatory agent and our continued interest in oxadiazole chemistry^[28-33], we built oxadiazole nucleus on the two most popular NSAIDs diclofenac

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and mefenamic acid and evaluated them for their antiinflammatory, analgesic and ulcerogenic activities.

MATERIALS AND METHODS

All the chemicals were obtained from commercial suppliers and used without further purification. All the melting points were determined on 'Veego' VMP-D apparatus and are uncorrected. Silica gel G plates of 3x8 cm (Sigma-Aldrich) were used for TLC and spots were located by UV or in iodine chamber. The IR spectra were recorded in the 4000-400 cm^{-1} range using KBr discs on FT-IR 8400 Shimadzu spectrometer. ^1H NMR spectra were recorded on Varian Mercury (300 MHz) spectrometer in CDCl_3 with TMS as an internal standard and values are expressed in ppm. Elemental analyses were performed for C, H, N and were found within $\pm 0.4\%$ of theoretical values.

Synthesis of methyl {2-[(2,6-dichlorophenyl)amino]phenyl}acetate (1) and methyl 2-[(2,3-dimethylphenyl)amino]benzoate (4):

In a solution of acids (diclofenac and mefenamic acid) (0.033 moles, 10 g) with absolute methanol (50 ml), was added 5 ml of conc. H_2SO_4 under stirring. It was then refluxed for 6-7 h on water bath, cooled and concentrated under reduced pressure. The residue was poured onto crushed ice and neutralized with 10% NaHCO_3 solution to yield solid precipitate. The solid was collected by filtration, washed twice with ice cold water and dried^[34].

Synthesis of 2-{2-[(2,6-dichlorophenyl)amino]phenyl}acetohydrazide (2) and 2-[(2,3-dimethylphenyl)amino]benzohydrazide (5):

To a solution of 1 (0.05 moles, 15.5 g), in absolute methanol (40 ml), 3.75 g of hydrazine hydrate (0.075 moles) was added and the reaction mixture was reflux for 15 h. It was then concentrated, cooled and poured onto ice cold water. The white solid thus separated out was filtered, dried and recrystallised from absolute ethanol^[35].

Synthesis of 5-[2-(2,6-dichloroanilino)benzyl]-2-aryl-1,3,4-oxadiazole (3a-3e) and 5-[2-(2,3-dimethylanilino)phenyl]-2-(aryl)-1,3,4-oxadiazole (6a-6e):

The mixture of 2 or 5 (1 g, 0.01 mole) and various aromatic acid (0.01 mole) was dissolved in 3-5 ml of phosphorus oxychloride (POCl_3). The reaction mixture was heated under reflux for 4 h. After

completion of the reaction, (monitored by TLC, ethyl acetate:hexane:: 0.5:1.5) mixture was neutralized with ice cold solution of 10% NaHCO_3 . The precipitate thus obtained was filtered, washed with ice cold water and purified by column chromatography^[36] (ethyl acetate:hexane:: 0.5:1.5).

5-[2-(2,6-Dichloroanilino) benzyl]-2-(phenyl)-1,3,4-oxadiazole (3a): Yield 71%, white crystalline solid, m.p. 80-82°, ^1H NMR (CDCl_3): δ 7.60-7.50 (m, 5H, Ar-H), 7.42 (d, 2H, Ar-H), 7.20-7.10 (m, 4H, Ar-H), 6.80-6.70 (t, 1H, Ar-H), 6.60 (s, 1H, NH), 1.55 (s, 2H, CH_2). IR (KBr, cm^{-1}): 3254, (NH), 3072 (Ar CH), 1606(C=N), 1101, 1000, 902(C-O-C). Rf; 0.84.

5-{[2-(2,6-Dichloroanilino)benzyl]-2-(3-chlorophenyl)}-1,3,4-oxadiazole (3b): Yield 72%, whitish yellow solid, m.p. 83-84°, ^1H NMR (CDCl_3): δ 7.60-7.50 (m, 4H, Ar-H), 7.48-7.40 (m, 3H, Ar-H), 7.30-7.10 (m, 3H, Ar-H), 6.80-6.70 (t, 1H, Ar-H), 6.68 (s, 1H, NH), 1.54 (s, 2H, CH_2). IR (KBr, cm^{-1}): 3252, (NH), 2953 (Ar CH), 1604(C=N), 1101, 1096 (C-O-C). Rf; 0.78.

5-{[2-(2,6-Dichloroanilino)benzyl]-2-(4-methylphenyl)}-1,3,4-oxadiazole (3c): Yield 79%, pinkish white solid, m.p. 79-80°, ^1H NMR (CDCl_3): δ 7.60-7.50 (m, 4H, Ar-H), 7.40 (d, 2H, Ar-H), 7.20-7.10 (d, 2H, Ar-H), 7.00 (d, 2H, Ar-H), 6.80-6.70 (t, 1H, Ar-H), 6.68 (s, 1H, NH), 1.50 (s, 2H, CH_2), 1.26 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3126, (NH), 2923 (Ar CH), 1589(C=N), 1101, 1090 (C-O-C). Rf; 0.81.

5-{[2-(2,6-Dichloroanilino)benzyl]-2-(pyridine-4-yl)}-1,3,4-oxadiazole (3d): Yield 74 %, white solid, m.p. 82-84°, ^1H NMR (CDCl_3): δ 7.60-7.50 (d, 2H, Ar-H), 7.52-7.40 (m, 4H, Ar-H), 7.30-7.20 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 6.81-6.70 (d, 2H, Ar-H), 6.68 (s, 1H, NH), 1.50 (s, 2H, CH_2). IR (KBr, cm^{-1}): 3326 (NH), 2929 (Ar CH), 1614 (C=N), 1172, 1092, 1022 (C-O-C). Rf; 0.79.

5-{[2-(2,6-Dichloroanilino)benzyl]-2-(1-naphthyl)}-1,3,4-oxadiazole (3e): Yield 80%, white crystalline solid, m.p. 88-90°, IR (KBr, cm^{-1}): 3128 (NH), 3028 (Ar CH), 1588 (C=N), 1103, 1010, 998 (C-O-C). Anal. Calcd for $\text{C}_{25}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}$: C, 68.18; H, 3.86; N, 9.54. Found C,68.11; H, 3.82; N, 9.55%. Rf; 0.80.

5-[2-(2,3-Dimethylanilino)phenyl]-2-(phenyl)-1,3,4-oxadiazole (6a): Yield 63%, yellow amorphous solid, m.p. 117-119°, IR (KBr, cm^{-1}): 3276 (NH), 2921 (Ar

CH), 1622 (C=N), 1103, 1165, 1010 (C-O-C). Calcd for $C_{22}H_{19}N_3O$: % C, 77.41; H, 5.57; N, 12.31. Found % C, 77.32; H, 5.50; N, 12.01. Rf; 0.80.

5-[2-(2,3-Dimethylanilino)phenyl]-2-(3-chlorophenyl)-1,3,4-oxadiazole (6b): Yield 70%, yellow amorphous solid, m.p. 117-118°, IR (KBr, cm^{-1}): 3211 (NH), 2921 (Ar CH), 1596 (C=N), 1157, 1089, 977 (C-O-C). Calcd for $C_{22}H_{18}ClN_3O$: % C, 67.64; H, 4.79; N, 11.18. Found %C, 67.43; H, 4.83; N, 11.00. Rf; 0.86.

5-[2-(2,3-Dimethylanilino) phenyl]-2-(4-methylphenyl)-1,3,4-oxadiazole (6c): Yield 65%, yellow amorphous solid, m.p. 121-123°, 1H NMR ($CDCl_3$): δ 8.30-8.20 (m, 4H, Ar- H), 7.95 (d, 2H, Ar- H), 7.90-7.80 (m, 3H, Ar- H), 7.42 (d, 2H, Ar- H), 7.23 (s, 1H, NH), 2.95 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 1.56 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3326 (NH), 2927 (Ar CH), 1627 (C=N), 1184, 1087, 1049 (C-O-C). Rf; 0.78.

5-[2-(2,3-Dimethylanilino)phenyl]-2-(pyridine-4-yl)-1,3,4-oxadiazole (6d): Yield 62%, yellow amorphous solid, m.p. 115-116°, 1H NMR ($CDCl_3$): δ 8.42 (d, 2H, Ar- H), 8.30-8.20 (d, 2H, Ar- H), 7.86-7.76 (m, 3H, Ar- H), 7.42 (d, 2H, Ar- H), 7.22 (s, 1H, NH), 2.90 (s, 3H, CH_3), 2.60 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3147 (NH), 2950 (Ar CH), 1608 (C=N), 1186, 1060, 986 (C-O-C). Rf; 0.81.

5-[2-(2,3-Dimethylanilino)phenyl]-2-(1-naphthyl)-1,3,4-oxadiazole (6e): Yield 74%, yellow solid, m.p. 113-115°, 1H NMR ($CDCl_3$): δ 8.20-8.10 (m 4H, Ar- H), 8.00-7.90 (m, 4H, Ar- H), 7.86-7.76 (m, 3H, Ar- H), 7.60-7.50 (m, 3H, Ar- H), 7.20 (s, 1H, NH), 2.90 (s, 3H, CH_3), 2.60 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3332, (NH), 2929 (Ar CH), 1627 (C=N), 1159, 1089 (C-O-C). Rf; 0.77.

Acute toxicity study:

The acute toxicity study was performed as per literature method^[37] on female swiss albino mice, weighing 20-25 g. Animals were kept overnight fasting (about 18 h) before administering the doses. Graded doses of compounds (175 mg/kg to 1750 mg/kg) were administered orally. Animals were observed for mortality at least once during the first 30 min, periodically during the first 24 h (with special attention given during the first 4 h) and daily thereafter, for a total of 14 days, except where they needed to be removed from the study and humanely killed for animal welfare reasons or were found dead.

Antiinflammatory activity:

This activity was performed as per the literature method^[38] on groups of six sprague dawley rats weighing 150-180 g each. A freshly prepared suspension of carrageenan (1.0% w/v, 0.1 ml) was injected in the planter region of right hind paw of each rat one hour after treating them with 10 mg/kg of test compounds. One group was treated as control and the animals of the other groups were pretreated with the test compounds suspended in 1.0% CMC with few drops of tween 80 given orally 1 h before the carrageenan treatment. The paw volume was measured after 1, 3 and 24 h of carrageenan treatment with the help of plethysmometer. The percent antiinflammatory activity was calculated according to the formula: % Antiinflammatory activity = $(V_c - V_t / V_c) \times 100$, where, V_t represents the mean increase in paw volume in rats treated with test compounds, V_c represents the mean increase in paw volume in control group of rats.

Analgesic activity:

The acetic acid-induced writhing test^[39] was performed for measure analgesic activity by an i.p. injection of 1% aqueous acetic acid solution in a volume of 0.1 ml. Each group of six albino mice was kept. Mice were kept individually in the test cage, before acetic acid injection and habituated for 30 min. Analgesic activity was tested after p.o. administration of test drugs at the dose of 10 mg/kg. All compounds were suspended in 1% CMC solution with few drops of tween 80. One group was kept as control and received p.o. administration of 1% CMC. After 1 h of administration of test compounds, 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 % pain 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.

Acute ulcerogenesis:

Acute ulcerogenesis test was done according to literature method^[40]. Albino rats have been divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after p.o. administration of test compounds or standard at the dose of 30 mg/kg. Control rats received

TABLE 2: MEAN % PAIN INHIBITION AFTER TREATMENT WITH COMPOUNDS 3A-3E, 6A-6E AND STANDARDS

Group	Writhes mean±SEM	%pain inhibition	Group	Writhes mean±SEM	%pain inhibition
Control	28±0.666 ^c	-	Control	28±0.666 ^c	-
3a	15±0.763 ^c	46.42	6a	17±1.201 ^c	39.28
3b	17±1.077 ^c	39.28	6b	16±0.666 ^c	42.85
3c	14±0.763 ^c	50.00	6c	13±0.966 ^c	53.57
3d	12±0.557 ^c	57.14	6d	14±0.654 ^c	50.00
3e	12±1.194 ^c	57.14	6e	13±1.536 ^c	53.57
Diclofenac	09±0.703 ^c	67.85	Mefenamic Acid	11±0.365 ^c	61.17

Data are expressed as mean±SEM, n = 6 animals per group. Data analysed by one way ANOVA followed by Dunnett's test, significant when compared with control ^ap<0.05, ^bp<0.01, ^cp<0.001.

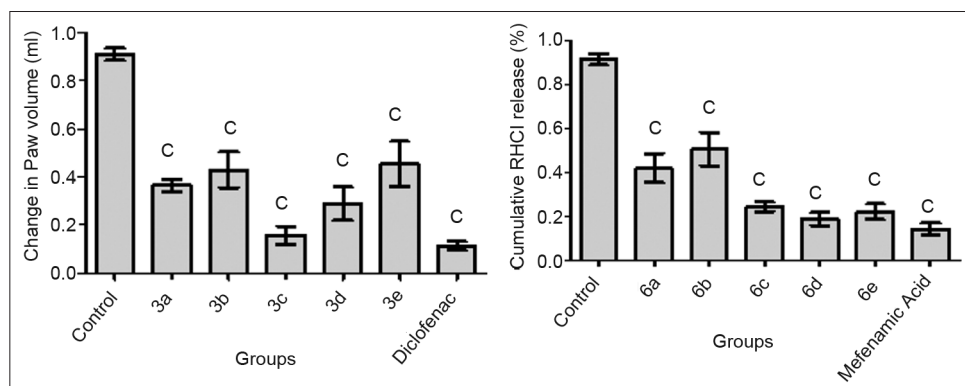


Fig. 2: Plot of mean paw volume (ml) after administration of compounds 3a-3e and 6a-6e.

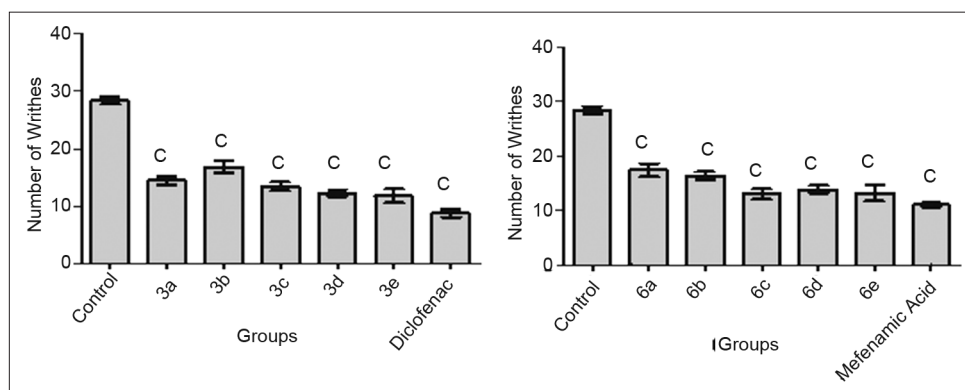


Fig. 3: Plot of number of writhes after i. p. administration of 1% acetic acid solution to animal treated with compounds 3a-3e, 6a-6e and standards.

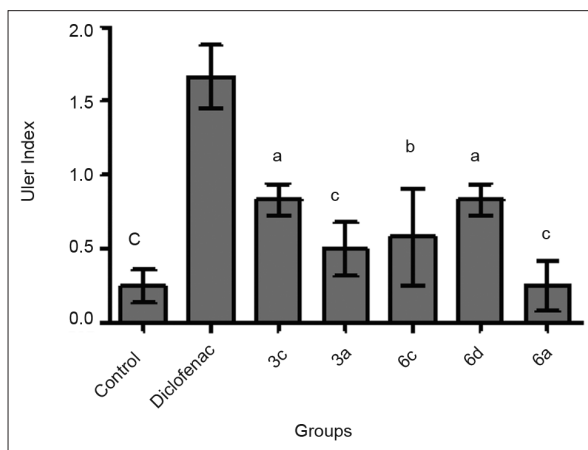


Fig. 4: Plot of mean ulcer sore of animal after the administration of test compounds.

widespread lined injury or widened injury. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Data are expressed as mean±S.E.M; Data analyzed by one way ANOVA followed by Dunnett's test, significance of the difference between the control group and rats treated with the test compounds. The difference in results was considered significant when $P<0.01$.

RESULTS AND DISCUSSION

The present study was aimed to replace the carboxylate functionality of diclofenac and

TABLE 3: MEAN ULCER SORE AND ULCER INDEX AFTER ADMINISTRATION OF COMPOUNDS

Group	Ulcer sore, mean±SEm	Ulcer index
Control	0.25±0.111 ^c	-
3c	0.83±0.105 ^c	0.58
3e	0.50±0.182 ^c	0.25
6c	0.58±0.327 ^c	0.33
6d	0.83±0.105 ^c	0.58
6e	0.25±0.170 ^c	0.00
Diclofenac	1.66±0.210 ^c	1.41
Mefenamic acid	2.00±0.258 ^{ns}	1.75 ^{ns}

Data expressed as mean±SEm, n = 6 animal per group. Data analyzed by one way ANOVA followed by Dunnett's test, significant when compare with standard (Diclofenac) ns = non significant ^ap<0.05, ^bp<0.01, ^cp<0.001.

mefenamic acid with less acidic 1,3,4-oxadiazole nucleus to protect the gastric mucosa from free carboxylate moiety. Five oxadiazole derivatives of diclofenac and mefenamic acid were synthesized as out line in scheme (fig. 1). The titled compounds 3a-3j and 6a-6j were obtained by reacting 2 and 5 respectively with various aromatic acids in the presence of POCl₃ as cyclodehydrating agent. Subsequent purification by column chromatography using ethyl acetate: hexane as a mobile phase and silica as a stationary phase yielded final compounds in moderate to higher yields. They were characterized by the IR (KBr) as sharp bands were observed around 3254 cm⁻¹ (NH stretch), 3056 cm⁻¹ (aromatic C-H stretch), 1608 cm⁻¹ (C=N), 1068-1020 cm⁻¹ (C-O-C stretch of oxadiazole ring) and their structures were further confirmed by ¹H NMR and elemental analysis.

The titled compounds were subjected to pharmacological evaluation which includes acute oral toxicity study, antiinflammatory, analgesic and acute ulcerogenic activities. The toxicity studies revealed that all compounds exhibited LD₅₀ values of 1750 mg/kg which is greater than the LD₅₀ of the diclofenac (95 to 1300 mg/kg)^[41] and mefenamic acid (525 mg/kg)^[42]. The antiinflammatory activity of the synthesized compounds (3a-3e and 6a-6e) showed that all of these compounds were sufficiently active in inhibiting the inflammation. However compound 3c and 6d exhibited percent inflammation inhibitions of 82.61% and 79.50% (Table 1 and fig. 2). The analgesic activity of the compounds (3a-3e and 6a-6e) was studied by using acetic acid induced writhing test in mice. The results of acetic acid induced writhing test showed that all compounds possess significant analgesic activity as compared with the control

group (Table 2 and fig. 3). Ulcerogenic effect of most active oxadiazole derivatives of diclofenac (3c, 3e) and mefenamic acid (6c, 6d, 6e) was evaluated for gastric ulcerogenic potential in rat stress model at 3 times the therapeutic doses. When compared with standard, these compounds showed less ulceration than the standard drugs. The results are shown in (Table 3 and fig. 4).

In summary, we disclosed that the effects of replacement of carboxylate functionality of the two most popular NSAIDs, diclofenac and mefenamic acid with 1,3,4-oxadiazole scaffold, with the objective of developing better antiinflammatory profile with minimum ulcerogenic activity and safety profile. The oxadiazole ring being weak acidic in nature and isoster for ester functionality, reduces the ulcerogenicity of the diclofenac and mefenamic acid and retain its antiinflammatory potential. However replacement with oxadiazole scaffold did not improve the analgesic activity of these molecules.

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Accepted 03 December 2011

Revised 30 November 2011

Received 04 April 2011

Indian J. Pharm. Sci., 2011, 73 (6): 634-640