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SYNTHESIS AND EVALUATION OF 1, 3, 4-OXADIAZOLE SUBSTITUTED DICLOFENAC ACID: ANTI-INFLAMMATORY ACTIVITY WITH REDUCED ULCEROGENICITY

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ABSTRACT

Background: A new series of 1,3,4-oxadiazole substituted diclofenac acid derivatives has been synthesized and evaluated *in vivo* for anti-inflammatory activity and ulcerogenic effects. It was observed that the nature of substituent at carboxyl group on the diclofenac moiety remarkably affects the anti-inflammatory activity and ulceration index. In the present paper, the results of synthesis and pharmacological findings of a series of Schiff bases of 1, 3, 4-oxadiazole substituted diclofenac acid are described. Both, the moieties are important due to their versatile biological actions. **Findings:** The present work encompasses the functionalization of oxadiazole system onto the diclofenac acid moiety for the synthesis of various compounds. The structures of the newly synthesized compounds were characterized by TLC, FT-IR, ^{1}H-NMR and elemental analysis. The compounds were also tested *in vivo* for their anti-inflammatory activity and ulcerogenicity index. Among the synthesized derivatives *6a-g*, **6c** was found to be most active in comparison to the standard. **Conclusion:** Synthesized and recorded anti-inflammatory and ulcerogenicity activity of some new 1,3,4-oxadiazole substituted diclofenac acid derivatives.

KEYWORDS: NSAIDs, Diclofenac Acid, Oxadiazole, Carrageenan.

BACKGROUND

Inflammation is a tissue reaction against infection, injury, irritation etc. and is a part of a host defense mechanism. There are number of tissue factors which are involved in the inflammatory reaction and can be suppressed by anti-inflammatory drugs.[1] Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, inflammation and arthritis which acts by blocking prostaglandins (PGs) generation.[2] Unfortunately, the same groups of chemicals i.e. prostaglandins, are also present in the gastric mucosa lining of the stomach and duodenum. So most of the NSAIDs tend to cause gastrointestinal hemorrhage, ulceration of the stomach or duodenum, dyspepsia, heartburn and other side effects.[3]

Diclofenac acid is an aryl-acetic acid derivative which inhibits the PG synthesis by COX inhibition and is one of the most frequently used NSAIDs worldwide. The drug was first approved in the United States in 1988 and currently over 5 million prescriptions are filled annually.[4] Some of the evidences also indicates that diclofenac acid also inhibits the lipoxygenase pathways, thus reducing the formation of leukotrienes which are also known as pro-inflammatory autacoids. It may also inhibit the phospholipase A_2 as a part of its mechanism of action. These additional actions may explain its high potency on broad basis.[5] But the presence of the free carboxylic acid group in the diclofenac moiety, is mainly responsible for the associated side effects.[6,7] A number of synthetic approaches have been developed which are associated with the chemical modification of –COOH moiety with the aim of improving safety profile and in turn therapeutic window of diclofenac acid. Several studies have been described for the derivatization of the carboxylate function of diclofenac acid, with less acidic azoles, *viz.* 1, 3, 4-oxadiazole, etc. and resulted in an increased anti-inflammatory activity with reduced ulcerogenicity index.[8-10]

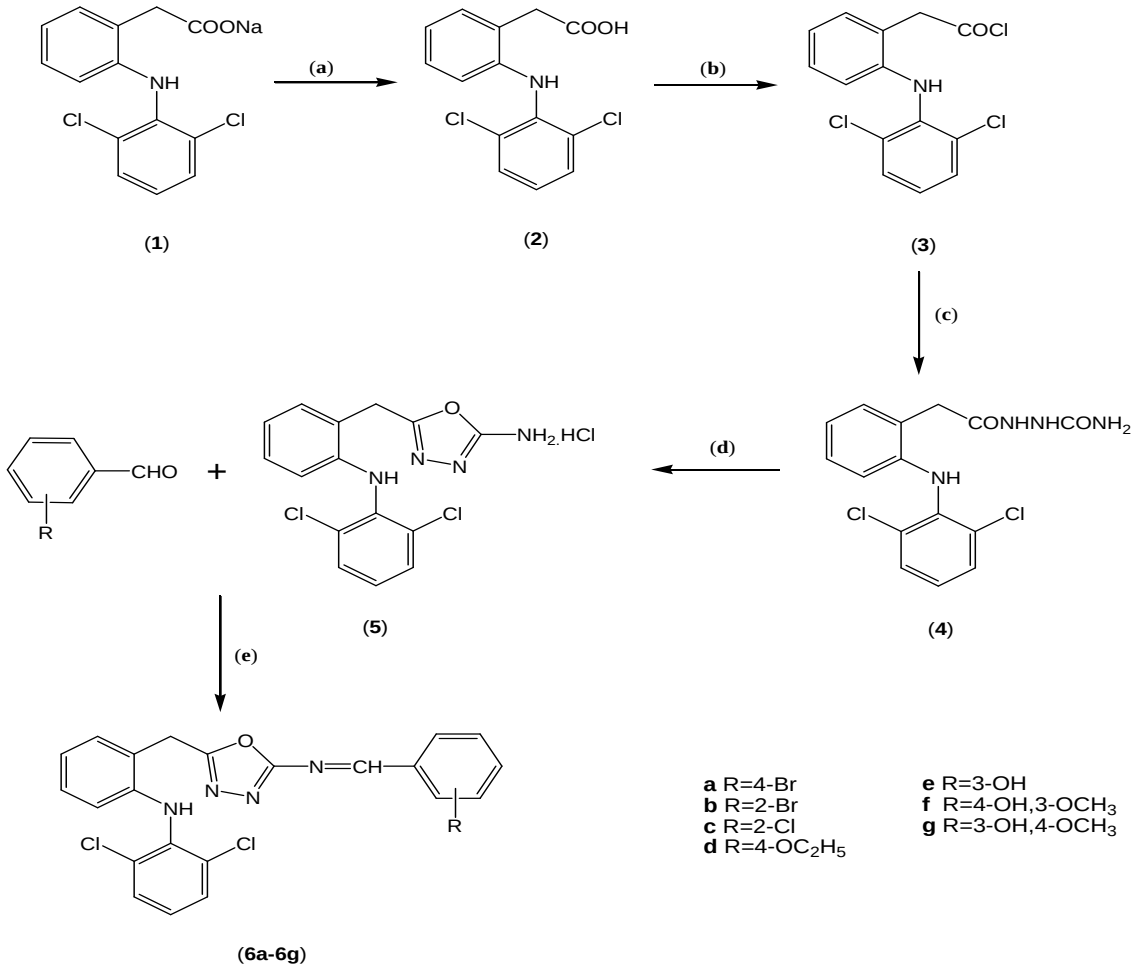
In view of these observations, we decided to study the impact of substitution at the carboxyl moiety of diclofenac acid with various benzylidene substituted 1,3,4-oxadiazole derivatives. The newly synthesized

derivatives have been evaluated for anti-inflammatory activity and ulcerogenicity index.[11]

Method

Chemistry

The synthetic route followed for the synthesis of various diclofenac acid containing 1, 3, 4-oxadiazole derivatives is depicted in *Scheme-1*. The key compound, 2-(2, 6-dichloroanilino)-phenyl acetic acid (**2**) was synthesized from diclofenac sodium in acidic medium according to the methods reported earlier.[12] The carboxylate function of 2-(2, 6-dichloroanilino)-phenyl acetic acid was converted to its chloride in the presence of thionyl chloride to gave the chloride salt of diclofenac (**3**). The compound **3** was further reacted with the semicarbazide in basic medium in the presence of methanol to give [2-(2, 6-dichloroanilino)-phenylacetyl]-semicarbazide (**4**). The compound **4** was cyclised with the help of iodine and sodium hydroxide in the presence of methanol to give 5-{2-[(2, 6-dichlorophenyl)-amino]-benzyl}-1, 3, 4-oxadiazol-2-amine hydrochloride (**5**). Treatment of **5** with substituted various aromatic aldehydes (**6a-g**) in the presence of methanol afforded the desired target compounds **7a-g.** The structures of the synthesized compounds were characterized by spectral and elemental analysis.



Scheme-1: Reagents and conditions : (a) Distt. H_2O, Conc. HCl, (b) $SOCl_2$, (c) Semicarbazide salt, NaOH, Methanol, (d) I_2/KI,NaOH, Methanol, (e) Methanol/ Acetic acid, NaOH

Pharmacological evaluation

The synthesized derivatives **7a-g** were evaluated *in vivo* for anti-inflammatory activity and ulcerogenicity index using carrageen induced rat paw edema and pyloric ligation method against the standard drug. The results so obtained are summarized in ***Table 1 and 2***.

RESULTS AND DISCUSSIONS

Chemistry

The synthesis of diclofenac acid derivatives **7a–g** described in this paper were prepared according to the synthetic Scheme 1. The sodium salt of diclofenac was acidified to produce the starting material, diclofenac acid (**1**). The acid was refluxed with thionyl chloride to produce **3**, which further on treatment with the semicarbazide hydrochloride in basic conditions formed the compound **4.** The semicarbazide derivatve of diclofenac was cyclized in the presence of I_2/KI and sodium hydroxide in methanol to from 1,3,4-oxadiazole

derivative **5**. Condensation of **5** with various aromatic aldehydes **6a–g** in methanol at reflux led to the generation of Schiff derivatives **7a–g** in 80–92% yield (Scheme 1). The structures of the synthesized compounds were confirmed by ^{13}C-NMR ^{1}H-NMR, elemental analysis and IR spectral data. As a representative example, the ^{1}H-NMR and ^{13}C-NMR spectra of (5-(2-(2,6-dichlorophenylamino)benzyl)-N-(2-chlorobenzylidene)-1,3,4-oxadiazole-2-amine (**7c**) are characterized as follows: the three singlet at 3.39, 7.73, and 10.26 ppm with two & one proton integration corresponds to Ar–C*H*$_2$–, –N=*H*C–, Ar-N*H*-Ar-, respectively. The doublet signal at 6.91 ppm, multiplet signal at 7.37 ppm and 7.61 ppm with two, four and three proton integration represents aromatic rings, respectively. The aliphatic carbon of methylene (-*C*H$_2$-) appears at 23.1 ppm, aromatic carbon (Ar-*C*) showed peaks between 119.2-140.6 ppm and carbon attached with nitrogen (*C*=N) in chain appears at 162.5 ppm whereas for the oxadiazole (*C*=N) at 167.4 ppm. The elemental analysis of the synthesized compounds showed in agreement with their molecular formula. The IR data provided functional group evidence for the formation of the expected structures.

Pharmacological evaluation

Table 1 summarizes the anti-inflammatory activity of the various newly synthesized 1, 3, 4-oxadiazole derivatives of diclofenac acid. In comparison to the standard drug the synthesized compounds exhibited remarked activity against the induced inflammation. Among the synthesized compounds, the *chloro* substituted aromatic ring at 2nd position of oxadiazole ring (**6c**) found to be the most active. While other derivatives showed comparably similar activity with the standard.

The newly synthesized compounds were also screened for acute ulcerogenicity by pyloric ligation model. The mucosal damage was examined by using 4x binocular magnifier and severity index was determined. Close inspection showed the ulcerogenicity index (tab. 2) of various synthesized compounds ranging from 0.23± 0.12 to 0.83± 0.16, in comparison to standard 1.76±0.15.

It can be concluded that the presence of electron withdrawing substitution at the aromatic ring on position 2 of oxadiazole ring greatly affects the anti-inflammatory activity and ulcerogenicity.

Experimental

Chemistry

All melting points were obtained using glass capillary tubes on Veego melting point apparatus and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using precoated plates with silica gel G (Merck 60 F_{254}) and the spots were visualized in iodine chamber. Infrared (IR) spectra were recorded on Agilent Technology cary 600 series Fourier Transform-Infrared spectrophotometer using potassium bromide pellets (υ_{max} in cm^{-1}). ^{13}C- and ^{1}H-NMR spectroscopy were performed using a Bruker model 400 MHz spectrometer in deuterated dimethylsulfoxide (DMSO-d_6). The results are reported in parts per million (ppm) downfield from tetramethylsilane (Me_4Si) as internal standard. The spin multiplicities are indicated as symbols, s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and the coupling constants (*J*) are given in Hertz (Hz). Anhydrous sodium sulfate was used as drying agent and all the solvents were freshly distilled off and dried prior to use according to the standard procedures.

General procedure for the synthesis of 2-(2, 6-dichloroanilino)-phenylacetic acid (2)

Diclofenac sodium (**1**) (0.5 g, 1.57 mmol) was dissolved in water (10 ml) followed by the addition of concentrated hydrochloric acid slowly to hydrolyze the sodium salt. The acid so obtained was filtered, washed with ice-cold water and dried to obtain **2** (0.42 g, 91 %), mp 154-156 ^{0}C (Lit[12,13] 155 ^{0}C).

General procedure for the synthesis of 2-[(2, 6-dichloroanilino) phenyl]acetyl chloride (3)

Diclofenac acid (**2**, 1g, 3.37 mmol) was refluxed in the presence of thionyl chloride (10 ml). The completion of reaction was monitored with help of thin layer chromatography (TLC). After completion of the reaction, excess thionyl chloride was distilled off under vacuum. Ice-cold water was added to the residue so obtained, pH was adjusted to neutral. The resulting precipitate was filtered off, washed with cold water and dried to obtain **3** (1.01 g, 98 %), mp 138-140 ^{0}C (Lit[13]). **FT-IRυ_{max} (KBr):** 3442 (N-H), 3076 (Ar-CH), 2921 (CH_2), 1759 (C=O), 1567 (C=C), 1173 (C-N) and 791 cm^{-1} (C-Cl); **Calcd. for $C_{14}H_{10}Cl_3NO$ (%):** C, 53.46; H, 3.20; N, 4.45. **Found (%):** C, 53.40; H, 3.11; N, 4.15.

General procedure for the synthesis of 2-[{(2, 6-dichloroanilino) phenyl}acetyl] semicarbazide (4)

The chloride derivative of diclofenac (**3**) (0.5 g, 1.58 mmol) and semicarbazide hydrochloride (1.5 g, 13.44 mmol) was dissolved in methanol and refluxed for 24 h in the presence of basic conditions. The completion of reaction was monitored by thin layer chromatography (TLC). The excess solvent was removed under pressure and the product so obtained was dissolved in ice cold water and neutralized. The resulting precipitate was filtered, washed with cold water and dried to obtain the desired product **4** (0.39 g, 70 %), mp 176-178 ^{0}C.**FT-IRυ_{max} (KBr):** 3392 (N-H), 3241 (Asym-NH_2), 3185 (Sym-NH_2), 3078 (Ar-CH), 2922 (CH_2), 1696 (C=O), 1566 (C=C), 1195 (C-N) and 791 cm^{-1} (C-Cl); **Calcd. for $C_{15}H_{14}Cl_2N_4O_2$ (%):** C, 51; H, 3.99; N, 15.86. **Found (%):** C, 49.05; H, 4.02; N, 14.96.

General procedure for the synthesis of 5-{2-[(2,6-dichlorophenyl)amino]benzyl}-1,3,4-oxadiazol-2-amine hydrochloride (5)

The mixture of semicarbazide derivative of diclofenac (**4**) (0.5 g, 1.41 mmol) and aqueous sodium hydroxide (5 N) was stirred at 4 ^{0}C in methanol. To the reaction mixture aqueous potassium iodide (5 %) solution was added gradually while stirring, till the color of iodine persists at room temperature. The reaction mixture was refluxed for 6-8 h and completion was monitored with the help of thin layer chromatography (TLC). The excess solvent was distilled off and acidified to get the solid product, which was filtered, washed with cold water and dried to obtain the desired product **5** (0.4 g, 83 %), mp 122-124 ^{0}C (Lit[14,15] 124 ^{0}C). **FT-IRυ_{max} (KBr):** 3274 (N-H), 3079 (Ar-CH), 2987 (CH_2), 1727 (C=O), 1610 (C=N), 1577 (C=C), 1320 (C-N), 1164 (C-O-C) and 779 cm^{-1} (C-Cl); **Calcd. for $C_{15}H_{14}Cl_2N_4O_2$ (%):** C, 48.47; H, 3.52; N, 15.07. **Found (%):** C, 48.17; H, 3.72; N, 15.17.

General procedure for the synthesis of various schiff bases i.e. 5-(2-(2,6-dichlorophenylamino)benzyl)-N-(substitutedbenzylidene)-1,3,4-oxadiazole-2-amine (7a-g)

The compound 5 (0.5 g, 1.34 mmol) was refluxed with the corresponding aromatic aldehyde (1.0 g, 5.4 mmol) in the presence of methanol (60 ml) for 48 h under basic conditions. The completion of the reaction was monitored with the help of thin layer chromatography (TLC). The residue obtained after the removal of solvent under reduced pressure was dissolved in diethyl ether, precipitates so obtained was filtered off, dried and crystallized from ethanol to obtain the desired Schiff bases **7a-g**.

5-(2-(2,6-dichlorophenylamino)benzyl)-N-(4-bromobenzylidene)-1,3,4-oxadiazole-2-amine (7a)

Yield 0.51 g, 76.11 %; Mp >300 ^{0}C; **FT-IRυ_{max} (KBr):** 3423 (N-H), 3174 (Ar-CH), 1639 (C=N), 1561 (C=C), 1221 (C-N), 1020 (C-O-C), 779 (C-Cl) and 529 (C-Br) cm^{-1}; **^{1}H-NMR (DMSO-d_6):** δ 3.21 (s, 2H, -CH_2), 6.16 (d, 2H, Ar-*H*, J_o = 8.1 Hz), 6.71-6.76 (d, 2H, Ar-*H*, J_o = 7.8 Hz), 7.24-7.37 (m, 4H, Ar-*H*, J_o = 7.5 Hz), 7.47-7.71 (m, 3H, Ar-*H*), 7.83 (s,1H, -C*H*=N) and 10.29 ppm (s, 1H, -N*H*); **^{13}C-NMR (DMSO-d_6):** δ 21.8 (-CH_2), 118.2-138.6 (Ar-*C*), 160.1 (*C*=N) and 166.4 ppm (*C*=N, oxadiazole); **Calcd. for $C_{22}H_{15}Cl_2N_4OBr$ (%):** C, 52.61; H, 3.01; N, 11.15. **Found (%):** C, 53.21; H, 3.89; N, 12.08.

5-(2-(2,6-dichlorophenylamino)benzyl)-N-(2-bromobenzylidene)-1,3,4-oxadiazole-2-amine (7b)

Yield 0.54 g, 80.59 %; Mp >300 ^{0}C; **FT-IRυ_{max} (KBr):** 3423 (N-H), 3174 (Ar-CH), 2924 (CH_2), 1699 (C=N), 1588 (C=C), 1190 (C-N), 1026 (C-O-C), 745 (C-Cl) and 681 (C-Br) cm^{-1}; **^{1}H-NMR (DMSO-d_6):** δ 3.47 (s, 2H, -CH_2), 6.46-6.98 (m, 4H, Ar-*H*), 7.03-7.33 (m, 4H, Ar-*H*), 7.35-7.88 (m, 3H, Ar-*H*), 8.68 (s, 1H, -C*H*=N) and 9.0 ppm (s, 1H, N*H*); **^{13}C-NMR (DMSO-d_6):** δ 22.2 (-CH_2), 119.5-140.6 (Ar-*C*), 161.1 (*C*=N) and 165.4 ppm (*C*=N, oxadiazole); **Calcd. for $C_{22}H_{15}Cl_2N_4OBr$ (%):** C, 52.61; H, 3.01; N, 11.15. **Found (%):** C, 53.86; H, 4.11; N, 12.05.

5-(2-(2,6-dichlorophenylamino)benzyl)-N-(2-chlorobenzylidene)-1,3,4-oxadiazole-2-amine (7c)

Yield 0.6 g, 98.6 %; Mp >300 ^{0}C; **FT-IRυ_{max} (KBr):** 3423 (N-H), 3174 (Ar-CH), 2925 (CH_2), 1700 (C=N), 1569 (C=C), 1116 (C-N), 1026 (C-O-C) and 783 (C-Cl) cm^{-1}; **^{1}H-NMR (DMSO-d_6):** δ 3.39 (s, 2H, -CH_2), 6.77 (t, 2H, Ar-*H*), 6.19-7.64 (d, 2H, Ar-*H*), 7.37 (m, 4H, Ar-*H*), 7.61 (m, 3H, Ar-*H*, J_o = 7.8 Hz), 7.73 (s, 1H, -C*H*=N) and 10.26 ppm (s, 1H, N*H*); **^{13}C-NMR (DMSO-d_6):** δ 23.1 (-CH_2), 119.2-140.6 (Ar-*C*), 162.5 (*C*=N) and 167.4 ppm (*C*=N, oxadiazole); **Calcd. for $C_{22}H_{15}Cl_3N_4O$ (%):** C, 57.72; H, 3.30; N, 12.24. **Found (%):** C, C, 56.72; H, 4.10; N, 14.04.

5-(2-(2,6-dichlorophenylamino)benzyl)-N-(4-ethoxybenzylidene)-1,3,4-oxadiazole-2-amine (7d)

Yield 0.56 g, 90 %; Mp >300 ^{0}C; **FT-IRυ_{max} (KBr):** 3441 (N-H), 3179 (Ar-CH), 2925 (CH_2), 1639 (C=N), 1242 (C-N), 1021 (C-O-C) and 802 (C-Cl) cm^{-1}; **^{1}H-NMR (DMSO-d_6):** δ 1.06-1.11 (t, 3H, -CH_3 J_o = 7.2 Hz), 2.50 (s, 2H, -CH_2), 3.37-3.39 (q, 2H, -OCH_2, J_o = 7.2 Hz), 6.19-6.22 (d, 2H, Ar-*H*, J_o = 8.1 Hz), 6.77 (d, 2H, Ar-*H*), 7.29-7.37 (m, 4H, Ar-*H*), 7.61-7.71 (m, 3H, Ar-*H*), 7.73 (s, 1H, -C*H*=N) and 10.26 ppm (s, 1H, N*H*); **^{13}C-NMR (DMSO-d_6):** δ 14.8 (-CH_3), 21.8 (-CH_2), 64.7 (-OCH_2), 118.2-138.6 (Ar-*C*), 160.1 (*C*=N) and 166.4 ppm (*C*=N, oxadiazole); **Calcd. for $C_{24}H_{20}Cl_2N_4O_2$ (%):** C, 61.67; H, 4.31; N, 11.99. **Found (%):** C, 62.08; H, 5.02; N, 12.09.

5-(2-(2,6-dichlorophenylamino)benzyl)-N-(4-hydroxybenzylidene)-1,3,4-oxadiazole-2-amine (7e)

Yield 0.54 g, 91.52 %; Mp >300 ^{0}C; **FT-IRυ_{max} (KBr):** 3625 (O-H), 3423 (N-H), 3174 (Ar-CH), 2925 (Ali-CH_2), 1700 (C=N), 1569 (C=C), 1220 (C-N), 1017 (C-O-C) and 782 (C-Cl) cm^{-1}; **^{1}H-NMR (DMSO-d_6):** δ 2.27 (s, 2H, -CH_2), 6.19-6.21 (d, 2H, Ar-*H*, J_o = 8.4 Hz), 6.68-6.76 (d, 2H, Ar-*H*, J_o = 8.7 Hz), 7.29-7.40 (m, 4H, Ar-*H*), 7.55-7.69 (m, 3H, Ar-*H*, J_o = 7.8 Hz), 7.72(s, 1H, -C*H*=N), 9.49 (s, 1H, N*H*) and 10.27 ppm (s, 1H, O*H*); **^{13}C-NMR (DMSO-d_6):** δ 22.1 (-CH_2), 117.1-139.6 (Ar-*C*), 161.5 (*C*=N) and 169.4 ppm (*C*=N, oxadiazole); **Calcd. for $C_{22}H_{16}Cl_2N_4O_2$ (%):** C, 60.14; H, 3.67; N, 12.57. **Found (%):** C, 61.07; H, 4.07; N, 13.07.

5-(2-(2,6-dichlorophenylamino)benzyl)-N-(4-hydroxy-3-methoxybenzylidene)-1,3,4-oxadiazole-2-amine (7f)

Yield 0.58 g, 92.06 %; Mp >300 ^{0}C; **FT-IRυ_{max} (KBr):** 3438 (O-H), 3288 (N-H), 3183 (Ar-CH), 2925 (CH_2), 1639 (C=N), 1563 (C=C), 1242 (C-N), 1021 (C-O-C) and 806 (C-Cl) cm^{-1}; **^{1}H-NMR (DMSO-d_6):** δ 2.27 (s, 2H, -CH_2), 3.31 (s, 3H, -OCH_3), 6.19-6.21 (d, 2H, Ar-*H*, J_o = 8.4 Hz), 6.68-6.76 (d, 2H, Ar-*H*, J_o = 8.7 Hz), 7.28-

7.40 (m, 4H, Ar-*H*), 7.56-7.70 (m, 3H, Ar-*H*, J_o = 7.8 Hz), 7.73 (s, 1H, -C*H*=N), 9.59 (s, 1H, N*H*) and 10.28 ppm (s, 1H, O*H*); **^{13}C-NMR (DMSO-d_6):** δ 23.1 (-*C*H_2), 56.2 (-*C*H_2), 119.2-140.6 (Ar-*C*), 162.5 (*C*=N) and 167.4 ppm (*C*=N, oxadiazole); **Calcd. for $C_{23}H_{18}Cl_2N_4O_3$ (%):** C, 58.85; H, 3.86; N, 11.94. **Found (%):** C, 59.05; H, 4.16; N, 10.94.

5-(2-(2,6-dichlorophenylamino)benzyl)-N-(3-hydroxy-4-methoxybenzylidene)-1,3,4-oxadiazole-2-amine (7g)

Yield 0.62 g, 98.41 %; Mp >300 ^{0}C; **FT-IRυ_{max} (KBr):** 3430 (O-H), 3266 (N-H), 3013 (Ar-CH), 2915 (CH_2), 1650 (C=N), 1590 (C=C), 1250 (C-N), 1030 (C-O-C) and 830 (C-Cl) cm^{-1}; **^{1}H-NMR (DMSO-d_6):** δ 2.25 (s, 2H, -CH_2), 3.39 (s, 3H, -OCH_3), 6.20 (d, 2H, Ar-*H*, J_o = 8.4 Hz), 6.71 (d, 2H, Ar-*H*, J_o = 8.7 Hz), 7.31-7.42 (m, 4H, Ar-*H*), 7.55-7.69 (m, 3H, Ar-*H*, J_o = 7.8 Hz), 7.75 (s, 1H, -C*H*=N), 9.68 (s, 1H, N*H*) and 10.30 ppm (s, 1H, O*H*); **^{13}C-NMR (DMSO-d_6):** δ 22.1 (-*C*H_2), 55.8 (-*C*H_2), 120.1-137.6 (Ar-*C*), 160.5 (*C*=N) and 165.4 ppm (*C*=N, oxadiazole); **Calcd. for $C_{23}H_{18}Cl_2N_4O_3$:** C, 58.85; H, 3.86; N, 11.94. **Found (%):** C, 57.95; H, 4.01; N, 12.04.

Pharmacological evaluation

The synthesized derivatives were evaluated for anti-inflammatory and reduced ulcerogenicity in comparison to the standard *diclofenac sodium*. The animals were housed at 25 ± 2 °C, 50 ± 5% relative humidity and 12 h light/dark cycle. The animals were fasted for 24 h prior to the experiment and provided water *ad libitum*. The experiments were performed according to the ethical guidelines for the investigation on experimental animals.

Anti-inflammatory activity

The synthesized derivatives **6a-g** were evaluated for their anti-inflammatory activity using carrageenan induced rat paw edema method.[16,17] The experiments were performed on male albino rats of Wistar strain, weighing (190-210 gm). The animals were marked on both the hind paws (right and left) just beyond tibia-tarsal junction, to ensure that every time the paw is dipped in the mercury column upto the mark. The animals were divided into 9 groups and each group contains 6 animals: The control group is named as *I*, standard group as *II* and test compounds as *III-IX* for the pharmacological evaluation. The group *I* was injected with saline only, group *II* was injected with the standard drug in saline (*diclofenac sodium 10 mg/kg*) and group *III-IX* were injected with the synthesized derivatives *7a-g* in saline (*10 mg/kg*) *via* subcutaneously route. Carrageenan (0.1 ml; 1% w/v) was injected to each animal in each group before measuring the paw volume. The paw volume was measured using the mercury displacement technique with the help of plethysmograph at 0, 15, 30, 60, 120, 180, 240, and 300 minutes. The percent difference between volume of right and left paw of each rat was calculated. The results are expressed as % difference in right and left paw volumes. (**Table 1**)

Table-1: Anti-inflammatory activity of the synthesized derivatives using carrageenan induced method

S. No.	Compound No. (Code)	Anti-inflammatory activity (% inhibition) (Time in min.)								
		Normal	0	15	30	60	120	180	240	300
1	6a (RS-5)	7.72	49.22	45.21	43.25	43.91	30.26	26.84	18.60	12.21
2	6b (RS-6)	6.65	39.10	31.98	25.98	28.86	21.65	20.98	15.43	11.86
3	6c (RS-7)	5.24	35.48	34.43	27.27	22.71	15.15	14.67	13.78	10.67
4	6d (RS-8)	9.37	46.82	41.56	30.31	28.41	25.18	19.56	18.46	14.64
5	6e (RS-9)	8.78	36.98	39.98	30.98	28.86	21.65	20.98	17.43	12.86
6	6f (RS-10)	8.31	49.22	45.21	43.25	43.91	30.26	26.84	18.60	14.21
7	6g (RS-11)	6.69	45.21	40.18	34.12	28.91	22.26	21.84	17.60	12.21
8	Control	8.21	38.50	39.51	41.15	45.50	57.76	62.56	66.76	70.61
9	Diclofenac sodium	11.14	39.64	36.20	26.29	23.59	22.76	19.08	17.4	13.89

Acute ulcerogenicity studies

The ulcerogenicity index was also calculated for the newly synthesized compounds to evaluate the ulcerogenicity. The ulcerogenesis test was performed according to the reported literature.[18] Albino rats have been divided into different groups consisting of six animals in each group as discussed earlier. Ulcerogenic activity was evaluated after p.o. administration of test compounds or standard at the dose of 30 mg/kg. Control rats received p.o. administration of vehicle (suspension of 1% carboxy methyl cellulose). After the drug treatment, the rats were fed normal diet for and then sacrificed after 24 h. The stomach was removed, opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The mucosal damage was examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following scoring system: 0.0 score was given to normal stomach (no injury, bleeding and latent injury). 0.5 score was to latent injury or widespread bleeding (>2 mm). 1.0 was to slight injury (2–3 dotted lines), 2.0 for severe injury (continuous lined injury or 5–6 dotted injuries). 3.0 to very severe injury (several continuous lined injuries) and 4.0 for wide spread 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 was obtained $P<0.001$.

Table 2: The ulcerogenicity index of the synthesized compounds using pyloric ligation model

S. No.	Compound No. (Code)	Ulcerogenicity index	
		Ulcer score, Mean±SEM	Ulcer index
1	6a (RS-5)	0.39±0.14	0.04
2	6b (RS-6)	0.43±0.12	0.08
3	6c (RS-7)	0.36±0.13	0.01
4	6d (RS-8)	0.23±0.12	0.12
5	6e (RS-9)	0.73±0.11	0.38
6	6f (RS-10)	0.83±0.16	0.48
7	6g (RS-11)	0.50±0.14	0.15
8	Control	0.35±0.11	-
9	Diclofenac sodium	1.76±0.15	1.41

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) p<0.001.

CONCLUSION

The diclofenac acid derivatives (**7a-g**) described in this paper were synthesized in five elaborate steps from commercially available diclofenac sodium as a starting material. All the synthesized compounds were investigated for their *in vivo* anti-inflammatory and ulcerogenicity index. Compound **7c** exhibited very good activity with maximum inhibition in the inflammation and reduced ulcer index score in comparison to the standard drug. The other synthesized derivatives also showed comparable or better activity when compared to the standard drug as diclofenac sodium.

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Authors' Contributions

KP, DG and AK carried out the biological studies of synthesized compounds and helped to draft the manuscript. DY participated in the design of the study. RS performed the synthesis, characterization of compounds and drafted the manuscript. RY conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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