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Synthesis of some 1,3,4-oxadiazole derivatives as potential anti-inflammatory agents

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The synthesis of some new 1,3,4-oxadiazole derivatives and 1,2,4-triazine-5-one has been described. IR, ^{1}H NMR and mass spectral data support the structures of newly synthesized compounds. All the compounds have been tested *in vivo* for their anti-inflammatory activity by carrageenin-induced rat paw edema method. The compounds, which show good anti-inflammatory activity, have been screened for their ulcerogenic and lipid peroxidation activities.

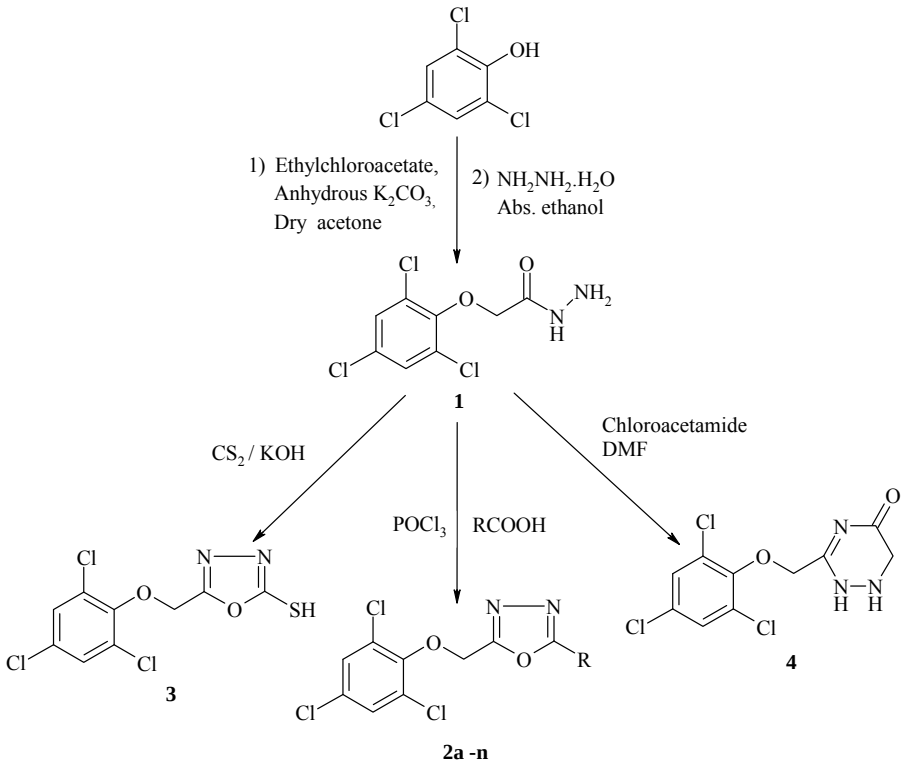
Keywords: 1,3,4-Oxadiazoles, 1,2,4-triazine-5-one, anti-inflammatory, ulcerogenicity, lipid peroxidation, ibuprofen

The non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of rheumatoid arthritis, and other inflammatory diseases. However, their long term use has been associated with gastro-intestinal ulceration, bleeding and naphrotoxicity[1]. The tendency of many acidic drugs to accumulate in the stomach walls soon after oral absorption, as evidenced by radio autography, has been considered as contributory factor to GI irritation[2]. In addition, cyclooxygenase (COX) inhibition resulting in decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining gastrointestinal health and homeostasis[3,4].

It was observed from the literature that certain five membered heterocyclic compounds possess interesting anti-inflammatory activity with lesser gastro-intestinal side effects[5,6]. Among them the compounds bearing 1,3,4-oxadiazole nucleus have been reported to have significant anti-inflammatory activity[7-9]. Moreover, the triazine nucleus and derivatives of 2-aryl acetic acids have been found to possess good anti-inflammatory activity[10,11]. 2,6-Dichloroanilino phenyl acetic acid (diclofenac) represents one of the most active acid derivative used as an anti-inflammatory drug[12]. In continuation of our research programme on the synthesis of five-membered heterocyclic compounds of acetic acid derivatives[13,14] we report, herein, the synthesis of 1,3,4-oxadiazole derivatives of acetic acid having a 2,4,6-trichloro-phenoxy group instead of 2,6-dichloroanilino phenyl group.

2,4,6-Trichlorophenoxyacetic acid hydrazide **1** was prepared by treating 2,4,6-trichlorophenol with ethyl-chloroacetate in the presence of anhydrous K_2CO_3, followed by reaction with hydrazine hydrate in absolute ethanol. 2-Substituted aryl-5-(2,4,6-trichloro-phenoxymethyl)-1,3,4-oxadiazoles **2a-n** were prepared by the treatment of the hydrazide **1** with various aromatic acids or arylalkanoic acids in the presence of phosphorous oxychloride. The reaction of hydrazide **1** with carbondisulphide in alkaline medium afforded, after acidic treatment, 5-(2,4,6-tri-chlorophenoxymethyl)-2-mercapto-1,3,4-oxadiazole **3**. Furthermore, 3-(2,4,6-trichlorophenoxymethyl)-1,2,5,6-tetrahydro-1,2,4-triazine-5-one **4** was synthe-sized by condensation of hydrazide with chloro-acetamide (**Scheme I**, **Table I**).

The IR spectrum of the compound **2e** showed absorption peaks at 3072 cm^{-1} due to the stretching of aromatic CH. The C=N stretching vibration appeared at 1575 cm^{-1}. The peak at 1052 cm^{-1} appeared for C-O-C stretching of oxadiazole nucleus. The absorption at 796 cm^{-1} was obtained due to C-Cl stretching vibrations. The ^{1}H NMR (DMSO-d_6) spectra of compound **2e** displayed two singlet at δ 5.21 and δ 5.35 showing the presence of OCH_2 proton attached to 2′,4′-dichlorophenyl ring and 3,4,5-trichlorophenyl ring respectively. The 5 aromatic protons of 3,4,5-trichlorophenyl and 2′, 4′-dichlorophenyl rings were



Scheme I

observed as a multiplet at δ 7.06-7.41. The mass spectra of **2e** showed molecular ion peak M^+ at *m/z* 452 corresponding to molecular formula $C_{16}H_9Cl_5N_2O_3$. Other fragments obtained at *m/z* 237, 209, 195.

The IR spectrum of the compound **3** showed characteristic absorption peaks at 3079 cm^{-1} (C-H aromatic), 1623 cm^{-1} (C=N), 1168 cm^{-1} (C=S), 1057 cm^{-1} (C-O-C) and 834 cm^{-1} (C-Cl). The ^1H NMR (DMSO-d_6) spectra of compound **3** showed a singlet at δ 5.17 confirming the presence of OCH_2 protons. The signal of SH proton was observed as broad singlet at δ 10.84. The aromatic protons were observed as a singlet at δ 7.50.

The IR spectrum of the compound **4** showed absorption peaks at 3380 cm^{-1} (NH), 3062 cm^{-1} (C-H aromatic), 1605 cm^{-1} (C=N), 854 cm^{-1} (C-Cl). The ^1H NMR (DMSO-d_6) spectra of compound **4** showed a singlet at δ 2.50 confirming the presence of triazinone ring. The singlet of OCH_2 ring was observed at δ 4.54. Two broad singlets of NH protons of triazinone ring were appeared at δ 4.56 and δ 5.02. The two protons of phenyl ring were observed as a singlet at δ 7.73.

Biological Studies

Adult male Wistar strain rats of either sex, weighing 180-200 gm were used. The animals were allowed food and water *ad libitum*. They were housed in room at 25 ± 2 °C, and 50 ± 5% relative humidity with 12 hr light/dark cycle. The animals were randomly allocated into groups at the beginning of all the experiments. All the test compounds and the reference drug were administered orally, suspended in 0.5% carboxymethyl cellulose (CMC) solution.

Anti-inflammatory activity. The test was performed by the method of Winter *et al.*[15] on the groups of six animals in each. Carrageenin solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 mL was injected subcutaneously into the sub plantar region of the right hind paw of each rat, 1 hr after the administration of the test compounds (70 mg/kg, p.o.). One group was kept as control, received only 0.5% carboxymethyl cellulose solution. The right hind paw volume was measured before and after 4 hr of carrageenin treatment by means of a pleythysmometer. The percent anti-inflammatory activity was calculated according to the following formula.

$$\text{Percent anti-inflammatory activity} = (V_c\text{-}V_t/V_c) \times 100$$

where, V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents

Table I — Physical data of compounds **2a-n**, **3** and **4**

Compd	R	Yeild (%)	m.p. (°C)	Mol. formula	Found (Calcd)% N
2a	Phenyl	67.0	145	$C_{15}H_9Cl_3N_2O_2$	7.68 (7.87)
2b	2-Chlorophenyl	76.5	178	$C_{15}H_8Cl_4N_2O_2$	7.37 (7.18)
2c	4-Chlorophenyl	80.1	122	$C_{15}H_8Cl_4N_2O_2$	6.92 (7.18)
2d	2,4-Dichlorophenyl	70.3	170	$C_{15}H_7Cl_5N_2O_2$	6.37 (6.59)
2e	2,4-Dichlorophenoxymethyl	74.2	128	$C_{16}H_9Cl_5N_2O_3$	5.83 (6.16)
2f	4-Aminophenyl	78.5	108	$C_{15}H_{10}Cl_3N_3O_2$	10.97 (11.33)
2g	2-Aminophenyl	59.6	146	$C_{15}H_{10}Cl_3N_3O_2$	11.12 (11.33)
2h	4-Nitrophenyl	64.5	188	$C_{15}H_8Cl_3N_3O_4$	10.54 (10.48)
2i	2-Acetoxyphenyl	61.0	167	$C_{17}H_{11}Cl_3N_2O_4$	6.55 (6.77)
2j	1-(4-isobutylphenyl)ethyl	81.3	108	$C_{21}H_{21}Cl_3N_2O_2$	6.17 (6.37)
2k	1-(2-Fluoro-4-biphenylyl)ethyl	67.7	160	$C_{23}H_{16}Cl_3FN_2O_2$	5.69 (5.86)
2l	1-(6-Methoxynaphth-2-yl)ethyl	60.2	206	$C_{22}H_{17}Cl_3N_2O_3$	6.27 (6.04)
2m	Naphth-2-ylmethyl	52.6	172	$C_{20}H_{13}Cl_3N_2O_2$	6.48 (6.67)
2n	2-(2,6-Dichloroanilino)benzyl	65.9	193	$C_{22}H_{14}Cl_5N_3O_2$	7.74 (7.93)
3	--	56.4	160	$C_9H_5Cl_3N_2O_2S$	9.13 (8.98)
4	--	63.5	154	$C_{10}H_8Cl_3N_3O_2$	13.51 (13.62)

the mean increase in paw volume in control group of rats.

Data are expressed as mean ± SEM, the student′s *t*-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds. The anti-inflammatory activity of the newly synthesized compounds **2a-n, 3** and **4** were compared with the standard drug ibuprofen at the same oral dose. Ibuprofen showed 86.36% inhibition of rat paw edema whereas the tested compounds showed inhibition ranging from 50.00% to 72.72% after 4 hr **(Table II)**. The oxadiazole derivatives **2d** (R = 2,4-dichlorophenyl) and **2j** [R = 1-(4-isobutylphenyl) ethyl] showed the maximum anti-inflammatory activity, and when these groups were replaced by 4-aminophenyl **2f** and 4-nitrophenyl **2h** the activity was found to be minimum. Replacement of aryl group at position 5 of the oxadiazole nucleus by mercapto (-SH) group showed significant activity (69.55%). Rest of the compounds showed moderate activity.

Acute Ulcerogenicity. Acute ulcerogenicity was determined according to Cioli *et al.*[16]. The animals were allocated into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after oral administration of the test compounds or ibuprofen at the dose of 210 mg/kg. Control group received only 0.5% carboxymethyl cellulose solution. Food but not water was removed 24 hr before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 hr and then sacrificed. The stomach was removed and open along the greater curvature, washed with distilled water and cleaned gently by dipping in normal 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: 0.5: 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 control group was regarded as severity index of gastric mucosal damage.

Table II — Biological data of compounds **2a-n**, **3** and **4**

Compd	Anti-inflammatory activity (% inhibition ± SEM)	Ulcerogenic activity (Severity index ± SEM)	nmol MDA content ± SEM / 100 mg tissue
Control	--	0.00	3.269 ± 0.05
Ibuprofen	86.36 ± 2.03*	2.00 ± 0.13*	6.154 ± 0.18*
2a	57.73 ± 1.91*	--	--
2b	59.10 ± 2.03*	--	--
2c	62.27 ± 2.79*	--	--
2d	72.72 ± 3.31*	0.500 ± 0.00*	4.263 ± 0.15*
2e	66.81 ± 3.03*	--	--
2f	50.00 ± 3.03*	--	--
2g	66.81 ± 1.91*	--	--
2h	50.00 ± 2.03*	--	--
2i	66.81 ± 3.03*	--	--
2j	72.72 ± 3.31*	0.667 ± 0.10*	4.551 ± 0.17*
2k	68.18 ± 2.03*	0.583 ± 0.08*	4.364 ± 0.16*
2l	70.00 ± 1.91*	0.417 ± 0.08*	4.087 ± 0.06*
2m	60.90 ± 1.91*	--	--
2n	63.66 ± 3.31*	--	--
3	69.55 ± 1.91*	0.500 ± 0.00*	4.327 ± 0.15*
4	66.66 ± 2.50*	--	--

Anti-inflammatory activity of the test compounds were compared w.r.t control. Ulcerogenic and lipid peroxidation activity were compared w.r.t standard drug i.e. ibuprofen. Data were analysed by student′s *t* test for n=6; *p> 0.0001.

Data are expressed as mean ± SEM, the student′s *t*-test was applied to determine the significance of the difference between the standard group and rats treated with the test compounds. The compounds which showed significant anti-inflammatory activity were further screened for their ulcerogenicity. A significant reduction in ulcerogenic activity was observed by the tested compounds with the severity index ranging from 0.417 ± 0.08 to 0.667 ± 0.10 (**Table II**), whereas the standard drug ibuprofen showed a high severity index of 2.00 ± 0.13. The compound **2l** [R = 1-(6-methoxynaphth-2-yl) ethyl] showed maximum reduction (severity index = 0.417 ± 0.08), whereas the compound **2j** [R = 1-(4-isobutylphenyl) ethyl] showed minimum reduction (severity index = 0.667 ± 0.10) in ulcerogenic activity.

Lipid Peroxidation. Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa *et al.*[17]. After screening for ulcerogenic activity, the gastric mucosa 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 (*p*H-3.5) and 1.5 mL of 0.8% thiobarbituric acid (TBA). The mixture was heated at 95°C for 60 min. After cooling the reactants were supplemented with 5 mL of the mixture of *n*-butanol and pyridine (15:1), shaked 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^{-1} M^{-1}.

Data are expressed as mean ± SEM, the student′s *t*-test was applied to determine the significance of the difference between the standard group and rats treated with the test compounds. It has been reported that the compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) tissue content, a byproduct of lipid peroxidation. Therefore an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of lipid peroxidation. All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation.

The lipid peroxidation was measured in nmole of MDA/100 mg of tissue. The ibuprofen (standard drug) showed the maximum lipid peroxidation (6.154±0.18), whereas the control group showed 3.269±0.05. It was

found that all the synthesized compounds showing less ulcerogenicity also showed reduced lipid peroxidation (**Table II**). Thus, this study showed that the tested compounds have inhibited the induction of gastric lesions and the result further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa.

Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Nicolet, 5PC FTIR spectrometer (ν_{max} in cm^{-1}) and ^1H NMR spectra were recorded in DMSO-d_6 on a Bruker DRX-300 (300 MHz FT NMR) spectrometer using TMS as internal reference (chemical shift in δ ppm). Mass spectra were recorded at Jeol SX-102 spectrometer. Chemical were purchaged from Merck Chemical Company, S. D. Fine (India) and Qualigens (India). Purity of the compounds was checked on silica gel G plates using iodine vapours as visualizing agent. Ethyl-2-(2,4,6-trichlorophenoxy) acetate was prepared by the procedure given in literature[18].

2,4,6-Trichlorophenoxyacetic acid hydrazide 1. In a 100 mL round bottom flask, a mixture of ethyl-2-(2,4,6-trichlorophenoxy) acetate (0.01 mole), hydrazine hydrate (0.02 mole) and absolute ethanol (50 mL) was added. A condenser with calcium chloride guard tube was attached to the flask and mixture was refluxed for 24 hr on a water bath. The mixture was concentrated, cooled and poured into crushed ice. It was kept for 4-5 hr at room temperature and solid mass separated out was filtered, dried and recrystallized from ethanol. IR (KBr): 3359 (NH), 3079 (C-H), 1623 (C=N), 1669 (C=O), 757 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 3.99 (s, 2H, OCH_2), 4.58 (s, 2H, NH_2), 7.35 (s, 2H, ArH), 8.06 (bs, 1H, CONH).

2-Substitutedaryl-5-(2,4,6-trichlorophenoxy methyl)-1,3,4-oxadiazoles 2a-n. A mixture of hydrazide **1** (0.001 mole), aromatic acid/aryl alkanoic acid (0.001 mole) and phosphorous oxychloride (15 mL) were refluxed at 110-120°C for 15 hr. After cooling to room temperature the reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus separated out was filtered, dried and recrystallized from methanol. **2d**: IR (KBr): 3080 (C-H), 1611 (C=N), 1048 (C-O-C), 807 cm^{-1} (C-Cl); **2d**: ^1H NMR (DMSO-d_6): δ 5.17 (s, 2H, OCH_2), 7.26-7.96 (m, 4H, ArH); **2e**: IR (KBr): 3072 (C-H), 1575 (C=N), 1052 (C-O-C), 796 cm^{-1} (C-Cl); **2e**: ^1H NMR (DMSO-d_6): δ 5.21 (s, 2H, dichloro OCH_2), 5.35 (s, 2H, trichloro OCH_2), 7.06-7.41 (m, 5H, ArH); **2e**: MS: m/z 452 (M^+), 453 (M^++1), 454 (M^++2), 237, 209, 195; **2f**: IR (KBr): 3379 (NH_2), 3072 (C-H), 1604 (C=N), 1062 (C-O-C), 816 cm^{-1} (C-Cl); **2f:** ^1H NMR (DMSO-d_6): δ 4.50 (s, 2H, OCH_2), 5.08 (s, 2H, NH_2), 7.67-7.87 (m, 6H, ArH); **2h**: IR (KBr): 3083 (C-H), 1553 (C=N), 1340 (NO_2), 1050 (C-O-C), 808 cm^{-1} (C-Cl); **2h**: ^1H NMR (DMSO-d_6): δ 5.36 (s, 2H, OCH_2), 7.36 (s, 2H, ArH), 8.31 (d, J = 9 Hz, 2H, $ArNO_2$), 8.40 (d, J = 9 Hz, 2H, $ArNO_2$); **2h**: MS: m/z 394 (M^+), 395 (M^++1), 396 (M^++2), 244, 150, 122; **2j**: IR (KBr): 2970 (C-H Ali), 1615 (C=N), 1071 (C-O-C), 797 cm^{-1} (C-Cl); **2j**: ^1H NMR (DMSO-d_6): δ 0.88 [dd, J = 6.5 Hz, 6H, $(CH_3)_2$], 1.56 (d, J = 7 Hz, 3H, CH_3), 1.84 (m, 1H, CH), 2.45 (d, J = 7 Hz, 2H, CH_2), 3.69 (q, J = 7 Hz, 1H, $CH\text{-}CH_3$), 4.64 (s, 2H, OCH_2), 7.11-7.61 (m, 6H, ArH); **2j**: MS: m/z 438 (M^+), 439 (M^++1), 440 (M^++2), 395, 189, 161; **2k**: IR (KBr): 3068 (C-H), 1613 (C=N), 1074 (C-F), 1063 (C-O-C), 765 cm^{-1} (C-Cl); **2k**: ^1H NMR (DMSO-d_6): δ 1.60 (d, J = 7 Hz, 3H, CH_3), 3.78 (q, J = 7 Hz, 1H, CH), 4.61 (s, 2H, OCH_2), 7.18-7.53 (m, 10H, ArH); **2k**: MS: m/z 476 (M^+), 477 (M^++1), 478 (M^++2), 249, 227, 199, 185; **2l**: IR (KBr): 3080 (C-H), 1610 (C=N), 1068 (C-O-C), 805 cm^{-1} (C-Cl); **2l**: ^1H NMR (DMSO-d_6): δ 1.58 (d, J = 7 Hz, 3H, CH_3), 3.80-3.84 (q, J = 7Hz, 1H, CH), 3.87 (s, 3H, CH_3), 4.62 (s, 2H, OCH_2), 7.10-7.43 (m, 6H, naphthyl), 7.68 (s, 2H, phenyl).

5-(2,4,6-Trichlorophenoxymethyl)-2-mercapto-1,3,4-oxadiazole 3. A mixture of hydrazide **1** (0.005 mole), potassium hydroxide (0.005 mole) and carbondisulphide (5 mL) in ethanol (50 mL) was refluxed on water bath for 18 hr. The solution was then concentrated, cooled and acidified with dilute hydrochloric acid. The solid that separated out was filtered and recrystallized from ethanol. IR (KBr): 3079 (C-H), 2736 (SH), 1623 (C=N), 1057 (C-O-C), 734 cm^{-1} (C-Cl); ^1H NMR (DMSO-d_6): δ 5.17 (s, 2H, OCH_2), 7.50 (s, 2H, ArH), 10.84 (bs, 1H, SH).

3-(2,4,6-Trichlorophenoxymethyl)-1,2,5,6-tetrahydro-1,2,4-triazin-5-one 4. A mixture of hydrazide **1** (0.01 mole), chloroacetamide (0.01 mole) and dimethylformamide (60 mL) was refluxed for 30 hr. The resulting solution was then concentrated, cooled and poured into ice cold water. The solid thus separated out was washed with water, dried and recrystallized from ethanol. IR (KBr): 3380 (NH), 3062 (C-H), 1640 (C=O), 1605 (C=N), 854 cm^{-1} (C-

Cl); ^{1}H NMR (DMSO-d_6): δ 2.50 (s, 2H, CH_2-triazinone), 4.54 (s, 2H, OCH_2), 4.56 (s, 1H, NH), 5.02 (s, 1H, NH), 7.73 (s, 2H, ArH).

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