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Short communication

Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2,6-dichloroanilino) phenyl]acetic acid derivatives

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Abstract

The synthesis of a group of 1,3,4-oxadiazoles, 1,2,4-triazoles, 1,3,4-thiadiazoles and 1,2,4-triazine derived from 2-[(2,6-dichloroanilino) phenyl] acetic acid is described. The structures of new compounds are supported by IR, ^{1}H-NMR and Mass spectral data. These compounds were tested in vivo for their anti-inflammatory activity. The compounds, which showed activity comparable to the standard drug diclofenac, were screened for their analgesic, ulcerogenic and lipid peroxidation activities. Ten new compounds, out of 28 showed very good anti-inflammatory activity in the carrageenin induced rat paw edema test, with significant analgesic activity in the acetic acid induced writhing test together with negligible ulcerogenic action. The compounds, which showed less ulcerogenic action, also showed reduced malondialdehyde content (MDA), which is one of the byproduct of lipid peroxidation. The study showed that the compounds inhibited the induction of gastric mucosal lesions and it can be suggested from our results that their protective effects may be related to inhibition of lipid peroxidation in the gastric mucosa.

Keywords: Diclofenac; 1,3,4-Oxadiazole; 1,3,4-Thiadiazole; 1,2,4-Triazole; 1,2,4-Triazine; Anti-inflammatory activity; Analgesic activity; Ulcerogenic activity; Lipid peroxidation

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever and inflammation, particularly arthritis [1–3]. Among the most popular NSAIDs, diclofenac has been approved in 120 countries since its introduction 25 years ago and ranked 30th among the top 200 drugs with respect to new prescriptions [4].

The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme cyclooxygenases (COXs) [5,6]. Recently, it was discovered that COX exists in two isoforms, COX-1 and COX-2, which are regulated differently [7–9]. COX-1 provides cytoprotection in the gastrointestinal (GI) tract whereas inducible COX-2 mediates inflammation [10–12]. Since most of the NSAIDs in the market show greater selectivity for COX-1 than COX-2 [13], chronic use of NSAIDs, including diclofenac may elicit appreciable GI irritation, bleeding and ulceration [14]. The incidence of clinically significant GI side effects due to NSAIDs is high (30%) and causes some patients to abandon NSAID therapy [4]. GI damage from NSAID is generally attributed to two factors. Local irritation by the carboxylic acid moiety, common to most NSAIDs (topical effect) and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis [5,15].

Synthetic approaches based upon NSAIDs chemical modification have been taken with the aim of improving NSAID safety profile. Studies by others described the derivatization of the carboxylate function [16–18] of representative NSAID resulted in an increased anti-inflammatory activity with reduced ulcerogenic effect. Furthermore, it has been reported in literature that certain compounds bearing 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole nucleus possess significant anti-inflammatory activity [19–23]. In our attempt to discover new and useful agents for treatment of inflammatory diseases, we have replaced the carboxylic acid group of diclofenac with additional heterocycles, which have been

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found to possess an interesting profile of anti-inflammatory activity with significant reduction in their ulcerogenic effect. The heterocycles reported here are 1,3,4-oxadiazoles, 1,2,4-triazoles, 1,3,4-thiadiazoles and 1,2,4-triazine.

2. Chemistry

The acid hydrazide **2** was prepared by esterification of 2-[(2,6-dichloroanilino) phenyl]acetic acid followed by treatment with hydrazine hydrate in absolute ethanol. The reaction of hydrazide **2** with carbon disulphide in alkaline medium afforded, after acidic treatment, 5-[2-(2,6-dichloroanilino)benzyl]2-mercapto-1,3,4-oxadiazole **3**. Various 2-aryl substituted-1,3,4-oxadiazole **5a–d** were prepared by treatment of hydrazide with appropriate aromatic acids in presence of phosphorus oxychloride. 3-[2-(2,6-Dichloroanilino)benzyl]5-oxo-1,2,5,6-tetrahydro-1,2,4-triazine **6** was prepared by condensation of hydrazide with chloroacetamide (Fig. 1).

Furthermore, hydrazide **2** on treatment with various aryl isothiocyanate gave N^1-[2-(2,6-dichloroanilino) phenylacetyl]N^4-alkyl/arylthiosemicarbazides **7a–g**. The thiosemicarbazides were oxidatively cyclised to 2-arylamino-5-substituted-1,3,4-oxadiazoles **8a–g** by elimination of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide. The thiosemicarbazides **7a–g** on heating with 4 N-NaOH in ethanol underwent smooth cyclisation through dehydration to afford 5-substituted-4-aryl-3-mercapto-4H-1,2,4-triazoles **9a–g**. 2-Arylamino-5-substituted–1,3,4-thiadiazoles **10a–g** were obtained by cyclisation of **7a–g** by treating with cold concentrated sulphuric acid.

The structures of various compounds synthesized were assigned on the basis of elemental analysis and spectral data. The IR, ^{1}H-NMR and Mass spectral data of compounds are given in experimental protocols. Physical data for the compounds are given in Table 1.

3. Pharmacological results and discussion

3.1. Anti-inflammatory activity

The anti-inflammatory activity of the synthesized compounds **3, 4, 5b, 5d, 6, 8a, 8b, 8d, 8e, 8f, 8g, 9a, 9b, 9d, 9e, 9g, 10a, 10c, 10d, 10e, 10g** was evaluated by carrageenan induced paw edema method of Winter et al. [27]. The compounds were tested at 10 mg/kg oral dose and were compared with the standard drug diclofenac at the same oral dose. The tested compounds showed anti-inflammatory activity ranging from 44.05% to 84.61% (Table 2), whereas the standard drug diclofenac showed 80.76% inhibition after 4 h. The anti-inflammatory activity of 1,3,4-oxadiazole derivatives is in the range from 44.05% to 84.61%. When the 2-amino group of the oxadiazole nucleus **4** was replaced by a 2-mercapto group **3**, the activity was found to be decreased, whereas when the 2-amino group was replaced by 2-alkyl/aryl amino group there was sharp increase in the activity. The compound **8a** having *n*-butyl amino group showed the greatest activity (84.61%). It was observed that oxadiazole derivatives having *p*-methyl, *o*-methoxy and *p*-fluoro phenyl amino group at second position showed better activity (83.65%, 82.69% and 80.76%) than the standard drug. Rest of the oxadiazole derivatives showed moderate activity.

The anti-inflammatory activity of 1,2,4-triazole derivatives was found between 63.46% and 82.69%. The highest activity (82.69%) was found in the triazole derivative **9e** having *p*-methyl phenyl group at fourth position. When this group was replaced by *n*-butyl group **9a**, the activity was found to be decreased (79.80%), whereas in case of oxadiazole derivative **8a**, the compound having *n*-butyl group showed greatest activity. It was observed that triazole derivative having *o*-methoxy phenyl **9g** and *p*-fluoro phenyl group **9d**, also showed good activity viz. 81.90% and 79.80%, respectively. Other derivatives showed moderate activity.

The 1,3,4-thiadiazole derivatives of diclofenac showed anti-inflammatory activity from 79.04% to 82.85%. The maximum activity (82.85%) was shown by thiadiazole derivative **10d** having *p*-fluoro phenyl amino group at second position. When this group was replaced by *n*-butyl amino group the activity was found to be decreased but equivalent to the standard drug (80.76%). Furthermore, thiadiazole derivatives having *p*-methyl phenyl amino **10e**, *o*-methoxy phenyl amino **10g** and *p*-chloro phenyl amino groups **10c** at second position showed good activity, viz. 80.00%, 80.76% and 79.04%, respectively.

When the hydrazide of diclofenac was treated with 2-chloroacetamide, 1,2,4-triazine derivative was obtained, which also showed anti-inflammatory activity equivalent to diclofenac (80.76%).

3.2. Analgesic activity

1,3,4-Oxadiazole derivatives **8a, 8d, 8e** and **8g** showed analgesic activity ranging from 78.57% to 81.86%, better than the standard drug diclofenac (70.32%). *p*-Fluoro phenyl amino group, present at the second position on oxadiazole ring **8d**, showed the maximum activity (81.86%). When this group was replaced by *n*-butyl **8a** and *o*-methoxy phenyl **8g**, the activity was found to be slightly decreased (81.31%). When these groups were replaced by *p*-methyl phenyl amino group **8e**, the activity was further decreased (78.57%). The result shows that an electron-withdrawing group increases the analgesic activity of the compound.

When 1,3,4-oxadiazole nucleus was replaced by 1,2,4-triazole nucleus, the analgesic activity was found to be decreased. Two triazole derivatives **9e** and **9g** screened, showed 72.52% and 73.62% analgesic activity, respectively. 1,3,4-Thiadiazole derivatives **10a, 10d** and **10g** were also screened and showed 77.47%, 76.92% and 72.52% analgesic activity, respectively. The compound having *p*-fluoro phenyl amino

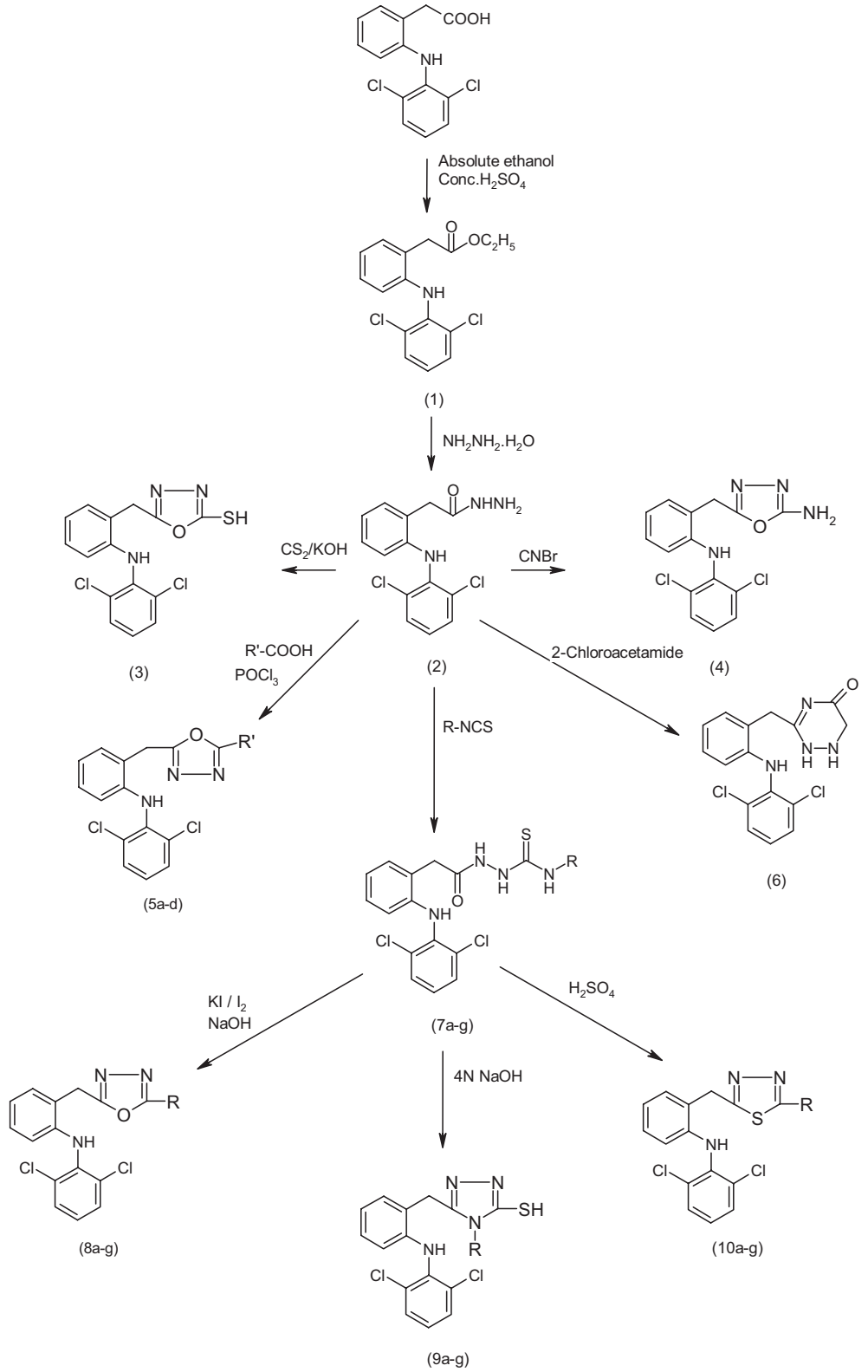
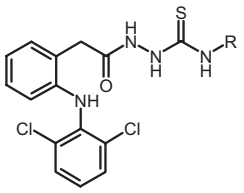


Fig. 1. Synthetic pathways to oxadiazole (**3, 4, 5a-d, 8a-g**), triazine (**6**), triazole (**9a-g**) and thiadiazole (**10a-g**) derivatives of diclofenac.

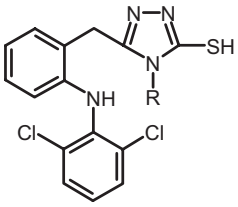
10d and *n*-butyl amino group **10a** at second position of thiadiazole derivative showed slight difference in their activity. When these groups were replaced by *o*-methoxy phenyl amino group **10g** there was decrease in the activity.

Cyclisation of carboxyl group of diclofenac into a 1,2,4-triazine nucleus showed 79.12% analgesic activity, which was better than 1,2,4-triazole and 1,3,4-thiadiazole derivatives.

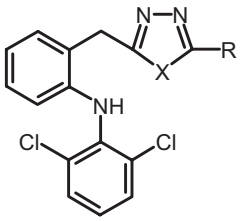
Table 1
Physical data of 2-[(2,6-dichloroanilino)phenyl]acetic acid derivatives



7a-g



9a-g



X= O (3,4,5,8); S (10)

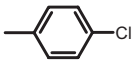
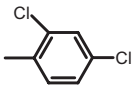
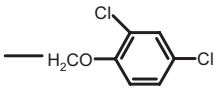
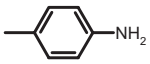
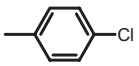
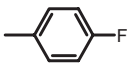
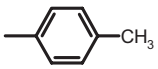
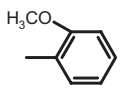
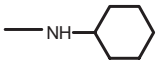
Compound	R	Yield (%)	MP (°C)	Molecular formula	Molecular weight
3	–SH	79	240	$C_{15}H_{11}ON_3SCl_2$	352.24
4	$–NH_2$	74	>360	$C_{15}H_{12}ON_4Cl_2$	335.20
5a		68	190	$C_{21}H_{14}ON_3Cl_3$	430.72
5b		70	120	$C_{21}H_{13}ON_3Cl_4$	465.17
5c		76	70	$C_{22}H_{15}O_2N_3Cl_4$	495.20
5d		71	295	$C_{21}H_{16}ON_4Cl_2$	411.29
7a	$–CH_2–CH_2–CH_2–CH_3$	74	190	$C_{19}H_{22}ON_4SCl_2$	425.30
7b		82	178	$C_{21}H_{24}ON_4SCl_2$	451.42
7c		80	174	$C_{21}H_{17}ON_4SCl_3$	479.82
7d		78	140	$C_{21}H_{17}ON_4SCl_2F$	463.36
7e		68	176	$C_{22}H_{20}ON_4SCl_2$	459.40
7f		65	146	$C_{22}H_{20}ON_4SCl_2$	459.40
7g		72	150	$C_{22}H_{20}O_2N_4SCl_2$	475.40
8a	$–NH–CH_2–CH_2–CH_2–CH_3$	52	280	$C_{19}H_{20}ON_4Cl_2$	391.30
8b		40	220	$C_{21}H_{22}ON_4Cl_2$	417.34

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Table 1
(continued)

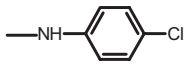
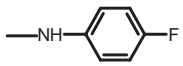
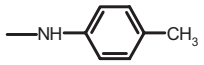
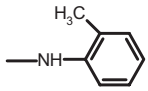
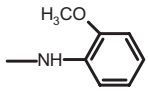
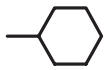
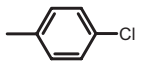
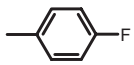
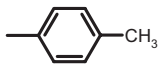
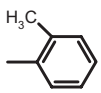
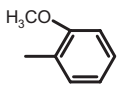
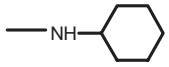
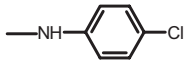
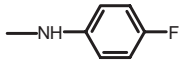
Compound	R	Yield (%)	MP (°C)	Molecular formula	Molecular weight
8c		65	>360	$C_{21}H_{15}ON_4Cl_3$	445.74
8d		47	80	$C_{21}H_{15}ON_4Cl_2F$	429.28
8e		33	300	$C_{22}H_{18}ON_4Cl_2$	425.32
8f		55	320	$C_{22}H_{18}ON_4Cl_2$	425.32
8g		46	260	$C_{22}H_{18}O_2N_4Cl_2$	441.32
9a	–CH_2–CH_2–CH_2–CH_3	84	168	$C_{19}H_{20}N_4SCl_2$	407.37
9b		78	220	$C_{21}H_{22}N_4SCl_2$	433.41
9c		80	290	$C_{21}H_{15}N_4SCl_3$	461.80
9d		86	188	$C_{21}H_{15}N_4SCl_2F$	445.35
9e		79	222	$C_{22}H_{18}N_4SCl_2$	441.39
9f		80	240	$C_{22}H_{18}N_4SCl_2$	441.39
9g		75	246	$C_{22}H_{18}ON_4SCl_2$	457.38
10a	–NH–CH_2–CH_2–CH_2–CH_3	28	Semisolid	$C_{19}H_{20}N_4SCl_2$	407.37
10b		32	Semisolid	$C_{21}H_{22}N_4SCl_2$	433.41
10c		44	120	$C_{21}H_{15}N_4SCl_3$	461.80
10d		56	124	$C_{21}H_{15}N_4SCl_2F$	445.35

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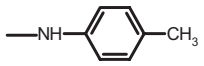
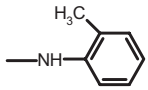
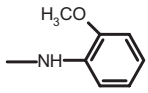
Table 1
(continued)

Compound	R	Yield (%)	MP (°C)	Molecular formula	Molecular weight
10e		48	Semisolid	$C_{22}H_{18}N_4SCl_2$	441.39
10f		33	Semisolid	$C_{22}H_{18}N_4SCl_2$	441.39
10g		37	60	$C_{22}H_{18}ON_4SCl_2$	457.38

Satisfactory analysis for C,H,N was obtained for all the compounds within ± 0.4% of the theoretical values.

3.3. Acute ulcerogenesis

The compounds, which showed anti-inflammatory activity comparable to that of the standard drug diclofenac and also showed high analgesic activity, were screened for their ulcerogenic activity.

The tested compounds showed significant reduction in ulcerogenic activity ranging from 0.417 ± 0.08 to 1.50 ± 0.00, whereas the standard drug diclofenac showed high severity index of 2.416 ± 0.20. The ulcerogenic activity of 1,3,4-oxadiazole derivatives **8a, 8d, 8e** and **8g** ranges from 0.50 ± 0.00 to 0.666 ± 0.10. Compound **8e** and **8g** having *p*-methyl phenyl and *o*-methoxy phenyl amino group showed minimum ulcerogenic activity (0.500 ± 0.00 and 0.517 ± 0.11, respectively). Moreover their anti-inflammatory activity was found to be high viz. 83.65% and 82.69%, respectively. The other two oxadiazole derivatives **8a** and **8d** also showed reduction in the ulcerogenic activity (0.666 ± 0.10 and 0.583 ± 0.08, respectively) in comparison to the standard drug.

The triazole derivatives **9e** and **9g** also showed reduction in ulcerogenic activity (0.583 ± 0.08 and 0.517 ± 0.11). It was interesting to note that the compound **8g** and **9g** having oxadiazole and triazole nucleus showed the same severity index (0.517 ± 0.11). 1,3,4-Thiadiazole derivative showed minimum ulcerogenic activity, when compared to oxadiazole

Table 2
Biological data of diclofenac derivatives

Compound	Anti-inflammatory activity (% inhibition ± S.E.M.)	Analgesic activity (% inhibition ± S.E.M.)	Ulcerogenic activity (severity index ± S.E.M.)	nmol MDA content ± S.E.M.)/ 100 mg tissue
Control	–	–	0.000 ± 0.00	3.371 ± 0.01
Diclofenac	80.76 ± 3.71 [a]	70.32 ± 1.31 [b]	2.416 ± 0.20	9.155 ± 0.14
3	44.05 ± 3.76 [c]	–	–	–
4	69.23 ± 3.71 [a]	–	–	–
5b	77.14 ± 2.20 [b]	–	–	–
5d	61.76 ± 5.61 [c]	–	–	–
6	80.76 ± 2.43 [a]	79.12 ± 0.58 [b]	1.500 ± 0.00 [a]	5.928 ± 0.06 [b]
8a	84.61 ± 1.00 [a]	81.31 ± 0.85 [b]	0.666 ± 0.10 [b]	6.110 ± 0.06 [b]
8b	68.26 ± 2.31 [a]	–	–	–
8d	80.76 ± 1.96 [a]	81.86 ± 0.92 [b]	0.583 ± 0.08 [b]	5.715 ± 0.14 [b]
8e	83.65 ± 1.84 [a]	78.57 ± 0.85 [b]	0.500 ± 0.00 [b]	5.833 ± 0.19 [b]
8f	54.28 ± 5.76 [a]	–	–	–
8g	82.69 ± 1.65 [a]	81.31 ± 0.85 [b]	0.516 ± 0.11 [b]	5.742 ± 0.17 [b]
9a	79.80 ± 1.84 [a]	–	–	–
9b	63.46 ± 2.38 [a]	–	–	–
9d	79.80 ± 1.28 [a]	–	–	–
9e	82.69 ± 1.65 [a]	72.52 ± 1.00 [b]	0.583 ± 0.08 [b]	5.876 ± 0.13 [b]
9g	81.90 ± 1.27 [b]	73.62 ± 1.16 [b]	0.517 ± 0.11 [b]	5.854 ± 0.14 [b]
10a	80.76 ± 1.98 [a]	77.47 ± 0.85 [b]	0.417 ± 0.08 [b]	5.788 ± 0.10 [b]
10c	79.04 ± 1.64 [b]	–	–	–
10d	82.85 ± 1.65 [b]	76.92 ± 0.58 [b]	0.500 ± 0.00 [b]	5.808 ± 0.02 [b]
10e	80.00 ± 2.22 [a]	–	–	–
10g	80.76 ± 1.18 [b]	72.52 ± 1.00 [b]	0.500 ± 0.00 [b]	5.904 ± 0.14 [b]

Anti-inflammatory and analgesic activities of the test compounds were compared w.r.t. control. Ulcerogenic and lipid peroxidation were compared w.r.t. standard drug i.e. diclofenac. Data were analyzed by Student's *t*-test for $n = 6$.
[a] $P < 0.001$; [b] $P < 0.0001$; [c] $P < 0.01$.

and triazole derivatives. The compound **10a** having *n*-butyl amino group showed the minimum severity index (0.417 ± 0.83). The other two compounds **10d** and **10g** having *p*-fluoro phenyl amino and *o*-methoxy phenyl amino group showed the same severity index (0.500 ± 0.00).

Cyclisation of carboxyl group of diclofenac into 1,2,4-triazine nucleus also showed reduction in ulcerogenic activity (1.500 ± 0.00). Thus it was concluded that cyclisation of carboxylic group of diclofenac into oxadiazole, triazole, thiadiazole and triazine nucleus resulted in the significant decrease of ulcerogenic activity while retaining their high anti-inflammatory properties.

3.4. Lipid peroxidation

It has been reported in literature that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation [24,25]. 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 is measured as nmol of MDA/100 mg of tissue. The diclofenac (standard drug) showed the maximum lipid peroxidation (9.155 ± 0.14), whereas the control group showed 3.370 ± 0.01. It was found that all the cyclised derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation (Table 2). Thus these studies showed that synthesized compounds 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.

4. Conclusion

Various oxadiazoles, triazoles, thiadiazoles and triazine derivatives of 2-[(2,6-dichloroanilino)phenyl]acetic acid were prepared with the objective of developing better anti-inflammatory molecules with minimum ulcerogenic activity. It was interesting to note that six cyclised compounds **8a, 8e, 8g, 9e, 9g** and **10d** were found to have anti-inflammatory activity greater than the standard drug (diclofenac, 80.76%) at 10 mg/kg p.o. Furthermore, four compounds **6, 8d, 10a** and **10g** exhibited anti-inflammatory activity equivalent to the standard drug against carrageenin induced paw edema test in rats. When these compounds were subjected to the analgesic activity, against acetic acid induced writhing test in mice, showed increased activity than their reference drug.

The presence of *o*-methoxy phenyl amino, *p*-methyl phenyl amino, *p*-fluoro phenyl amino and *n*-butyl amino group at second position in the oxadiazole and thiadiazole nucleus increases the anti-inflammatory activity whereas presence of cyclohexyl amino and *o*-methyl phenyl amino group decreases the anti-inflammatory activity. It was further noted that the presence of *o*-methoxy and *p*-methyl phenyl group at fourth position of triazole nucleus increases the anti-inflammatory activity than the standard drug, whereas presence of *n*-butyl group and *p*-fluoro phenyl group showed anti-inflammatory activity slightly less than the standard drug.

These compounds tested for ulcerogenic activity showed significant decrease in the activity than the standard drug. It was noted that thiadiazole derivatives showed maximum reduction in the ulcerogenic activity followed by triazoles and oxadiazoles. It was further concluded that presence of *o*-methoxy phenyl amino and *p*-methyl phenyl amino at the second position of oxadiazole and thiadiazole nucleus showed maximum anti-inflammatory activity, minimum ulcerogenic activity along with minimum lipid peroxidation.

5. Experimental protocols

5.1. Chemistry

Melting points were measured in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Perkin Elmer 157 infracord spectrometer (ν_{max} in cm^{-1}) and ^{1}H-NMR spectra on a Bruker DRX-300 (300 MHz FT NMR) spectrophotometer using TMS as internal reference (Chemical shift δ in ppm). Mass spectra were recorded at Jeol SX-102 (FAB) spectrometer. Purity of the compounds was checked on silica gel G plates using iodine vapours as visualising agent. 2-[(2,6-Dichloroanilino)phenyl]acetic acid (Diclofenac) was procured from Pharmax Corporation Pvt., New Delhi, India.

*5.1.1. Ethyl-[2-(2,6-dichloroanilino)phenyl]acetate (**1**)*

It was prepared by the procedure given in literature [26].

*5.1.2. [2-(2,6-Dichloroanilino)phenyl]acetic acid hydrazide (**2**)*

Compound **1** (0.01 mol) and hydrazine hydrate (0.02 mol) were refluxed in absolute ethanol (50 ml) for 20 h. The mixture was concentrated, cooled and poured in ice cold water. The solid thus separated out was filtered, dried and recrystallized from ethanol, yield: 72%; m.p. 134–136 °C. IR spectra of the compound showed bands at 3325 (N–H); 2970 (C–H); 1638 (C=O). ^{1}H-NMR ($CDCl_3$, δ ppm): 3.59 (s, 2H, CH_2CO); 4.14 (s, 2H, NH_2); 6.82–6.98 (m, 4H, 3,4,5,6 ArH); 7.21–7.27 (m, 3H, dichloro ArH); 7.54 (s, 1H, NH); 8.27 (s, 1H, CONH). Mass spectra of compound exhibited molecular ion peak at *m/z* 309 (M^+), other important fragments were observed at 310 ($M^+ + 1$), 311 ($M^+ + 2$), 313 ($M^+ + 4$), 278, 250, 214.

*5.1.3. 5-[2-(2,6-Dichloroanilino)benzyl]2-mercapto-1,3,4-oxadiazole (**3**)*

A mixture of **2** (0.005 mol), KOH (0.005 mol) and carbon-disulphide (5 ml) in ethanol (50 ml) was refluxed on a steam

bath for 12 h. The solution was then concentrated, cooled and acidified with dilute HCl. The solid mass that separated out was filtered, washed with ethanol, dried and recrystallized from ethanol (Table 1). IR spectra of the compound showed bands at 3354 (N–H); 1525 (C–N); 1182 (C=S). ^{1}H-NMR ($CDCl_3$, δ ppm): 4.13 (s, 2H, CH_2); 7.25–7.30 (m, 4H, 3,4,5,6 ArH); 7.51–7.53 (m, 3H, dichloro ArH); 7.90 (s, 1H, NH); 10.51 (s, 1H, SH).

*5.1.4. 5-[2-(2,6-Dichloroanilino)benzyl]2-amino-1,3,4-oxadiazole (**4**)*

To an ethanolic solution of **2** (0.001 mol), cyanogen bromide (0.001 mol) was added. The reaction mixture was warmed at 55–60 °C for 90 min. The resulting solution was cooled and neutralized with sodium bicarbonate solution. The solid thus separated out was filtered, washed with water, dried and recrystallized from methanol (Table 1). IR spectra of the compound showed bands at 3447 (NH_2); 2975 (C–H); 1431 (C–N). ^{1}H-NMR (DMSO-d_6, δ ppm): 4.15 (s, 2H, CH_2); 6.20 (s, 2H, NH_2); 6.73–7.20 (m, 4H, 3,4,5,6 ArH); 7.55–7.67 (m, 3H, dichloro ArH); 7.82 (s, 1H, NH). Mass spectra of compound exhibited molecular ion peak at *m/z* 335 (M^+), other important fragments were observed at 337 (M^+ + 2), 339 (M^+ + 4), 289, 237.

*5.1.5. General procedure for the preparation of 5-[2-(2,6-dichloroanilino)benzyl]2-aryl-1,3,4-oxadiazoles (**5a–d**)*

Compound **2** (0.001 mol) and appropriate aromatic acid (0.001 mol) was dissolved in phosphorus oxychloride and refluxed for 20 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus separated out was filtered, washed with water, dried and recrystallized from ethanol (Table 1). IR spectra of the compound **5a–d** showed bands at 3450–3430 (N–H); 2970–2950 (CH_2); 1490–1450 (C–N). ^{1}H-NMR of **5b** ($CDCl_3$, δ ppm): 3.71 (s, 2H, CH_2); 6.65 (s, 1H, NH); 7.10–7.23 (m, 4H, 3,4,5,6 ArH); 7.28–7.43 (m, 6H, dichloro ArH). ^{1}H-NMR of **5c** ($CDCl_3$, δ ppm): 3.80 (s, 2H, CH_2); 4.54 (s, 2H, OCH_2); 6.67 (s, 1H, NH); 7.06–7.20 (m, 4H, 3,4,5,6 Ar–H); 7.32–7.52 (m, 6H, dichloro ArH). Mass spectra of compound **5c** exhibited molecular ion peak at *m/z* 495 (M^+), other important fragments were observed at 497 (M^+ + 2), 499 (M^+ + 4), 295 and 246. ^{1}H-NMR of **5d** ($CDCl_3$, δ ppm): 2.88 (s, 2H, CH_2); 6.67–6.94 (m, 4H, 3,4,5,6 Ar–H); 7.03 (s, 2H, NH_2); 7.16–7.39 (m, 4H, 2,3,5,6 Ar–H); 7.44–7.78 (m, 4H, dichloro ArH and 1NH).

*5.1.6. 3-[2-(2,6-Dichloroanilino)benzyl]5-oxo-1, 2,5,6-tetra-hydro-1, 2,4-triazine (**6**)*

To compound **2** (0.01 mol) was added chloroacetamide (0.01 mol) and dimethyl formamide (80 ml) and the reaction mixture was refluxed for 25 h. It was then concentrated and cooled, whereupon the solid separated out, which was filtered, washed with ethanol and recrystallized from DMF/water, yield: 54%; m.p. 176 °C. IR spectra of the compound showed bands at 3385 (N–H); 1640 (C=O); 1474 (C–N). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.81 (s, 2H, CH_2 of triazinone); 3.96 (s, 2H, CH_2); 6.39–6.46 (bs, 2H, 2NH); 6.98–7.50 (m, 7H, ArH); 8.02 (s, 1H, NH).

*5.1.7. General procedure for the preparation of N^1-[2-(2,6-dichloroanilino)phenyl acetyl]N^4-alkyl/arylthiosemicarbazides (**7a–g**)*

A mixture of **2** (0.10 mol), alkyl/aryl isothiocyanate (0.10 mol) and ethanol (50 ml) was refluxed on steam bath for 8 h. It was then concentrated, cooled and kept overnight in refrigerator. The solid thus separated out, was filtered, washed with ethanol, dried and recrystallized from ethanol (Table 1). IR spectra of the compounds **7a–g** showed bands at 3300–3280 (N–H); 2960–3000 (CH); 1680–1670 (C=O); 1217–1190 (C=S). Mass spectra of compound **7a** exhibited molecular ion peak at *m/z* 424 (M^+), other important fragments were 425 (M^+ + 1), 426 (M^+ + 2), 428 (M^+ + 4), 278, 214 and 148. ^{1}H-NMR of **7b** ($CDCl_3$, δ ppm): 0.99–1.88 (m, 11H, C_6H_{11}); 3.72 (s, 2H, CH_2–CO); 6.40–6.45 (d, 1H, cyclohexyl-NH); 6.82–7.04 (m, 4H, 3,4,5,6-Ar–H); 7.25–7.28 (m, 3H, dichloro Ar–H); 7.61 (s, 1H, NH); 8.57 (s, 1H, CSNH); 9.64 (s, 1H, CONH).

*5.1.8. General procedure for the preparation of 5-[2-(2,6-dichloroanilino)benzyl]2-alkyl/arylamino-1,3,4-oxadiazoles (**8a–g**)*

A suspension of **7a–g** (0.002 mol) in ethanol (50 ml) was dissolved in aqueous sodium hydroxide (5 N, 1 ml) with cooling and stirring, resulting in a clear solution. To this, iodine in potassium iodide solution (5%) was added gradually with stirring till the colour of iodine persisted at room temperature. The reaction mixture was then refluxed for 1 h on steam bath. It was then cooled and poured over crushed ice. The solid mass that separated out was filtered, dried and recrystallized from ethanol (Table 1). IR spectra of the compound **8a–g** showed bands at 3440–3420 (N–H); 2985–2965 (CH_2); 1441–1425 (C–N). ^{1}H-NMR of **8a** (DMSO-d_6, δ ppm): 0.82–0.84 (t, 3H, CH_3), 1.14–1.17 (m, 2H, CH_3CH_2); 1.52–1.58 (m, 2H, $CH_3CH_2CH_2$); 3.94–3.99 (m, 2H, $NHCH_2$); 4.21 (s, 2H, CH_2); 6.20–6.25 (t, 1H, $NHCH_2$); 6.58–7.24 (m, 4H, 3,4,5,6 ArH); 7.42–7.55 (m, 3H, dichloro ArH); 8.51 (s, 1H, NH). Mass spectra of compound **8a** exhibited molecular ion peak at *m/z* 391 (M^+), other important fragments were at 393 (M^+ + 2), 395 (M^+ + 4), 376, 289 and 176. ^{1}H-NMR of **8b** (DMSO-d_6, δ ppm): 1.13–1.69 (complex m, 11H, cyclohexyl H); 3.94 (s, 2H, CH_2); 6.86–6.90 (m, 4H, 3,4,5,6-ArH); 7.10–7.29 (m, 3H, dichloro ArH); 7.39 (s, 1H, cyclohexyl NH); 7.41 (s, 1H, NH). ^{1}H-NMR of **8d** ($CDCl_3$, δ ppm): 4.40 (s, 2H, CH_2); 7.40–7.56 (complex m, 12H, 11ArH and 1NH); 8.61 (s, 1H, NH). ^{1}H-NMR of **8e** (DMSO-d_6, δ ppm): 2.39 (s, 3H, CH_3); 3.39 (s, 2H, CH_2); 6.59–7.51 (complex m, 12H, 11ArH and 1NH); 8.47 (s, 1H, NH). ^{1}H-NMR of **8f** (DMSO-d_6, δ ppm): 2.27 (s, 3H, CH_3); 3.36 (s, 2H, CH_2); 6.59–7.54 (complex m, 12H, 11ArH and 1NH); 8.50 (s, 1H, NH). ^{1}H-NMR of **8g** (DMSO-d_6, δ ppm): 3.69 (s, 3H, OCH_3); 4.01 (s, 2H, CH_2); 6.58–7.45 (complex m, 12H, 11ArH and 1NH); 8.90 (s, 1H, NH).

*5.1.9. General procedure for the preparation of 5-[2-(2,6-dichloro-anilino)benzyl]4-alkyl/aryl-3-mercapto-4H-1,2,4-triazoles (**9a–g**)*

A suspension of **7a–g** (0.002 mol) in ethanol (25 ml) was dissolved in aqueous sodium hydroxide (4 N, 2 ml) and gently refluxed for 2 h. The resulting solution was concentrated, cooled and filtered. The filtrate was adjusted to pH 5–6 with dilute acetic acid and was kept aside for 1 h. The crystals produced were filtered, washed with water, dried and recrystallized from ethanol (Table 1). IR spectra of the compounds **9a–g** showed bands at 3375–3360 (N–H); 2960–2926 (CH_2); 1450–1435 (C–N); 1220–1199 (C=S). ^{1}H-NMR of **9a** ($CDCl_3$, δ ppm): 0.90–0.97 (t, 3H, CH_3); 1.34–1.46 (m, 2H, CH_3CH_2); 1.61–1.72 (m, 2H, $CH_3CH_2CH_2$); 3.98–4.03 (t, 2H, NCH_2); 4.14 (s, 2H, C_6H_4–CH_2); 6.93–7.03 (m, 4H, 3,4,5,6 ArH); 7.13–7.19 (m, 3H, dichloro ArH); 7.26 (s, 1H, NH); 11.46 (s, 1H, SH). ^{1}H-NMR of **9b** ($CDCl_3$, δ ppm): 0.85–1.85 (m, 11H, C_6H_{11}); 4.21 (s, 2H, CH_2); 6.96–7.04 (m, 4H, 3,4,5,6 Ar–H); 7.13–7.35 (m, 4H, dichloro ArH and 1NH); 10.53 (s, 1H, SH). ^{1}H-NMR of **9c** ($CDCl_3$, δ ppm): 3.97 (s, 2H, CH_2); 6.44–6.82 (m, 4H, 3,4,5,6 ArH); 6.98–7.11 (m, 4H, *p*-Cl phenyl ArH); 7.20–7.34 (m, 3H, dichloro ArH); 7.55 (s, 1H, NH); 11.51 (s, 1H, SH). Mass spectra of compound **9c** exhibited molecular ion peak at *m/z* 460 (M^+), other important fragments were found at 462 ($M^+ + 2$), 464 ($M^+ + 4$), 364, 251 and 129. ^{1}H-NMR of **9d** ($CDCl_3$, δ ppm): 3.95 (s, 2H, CH_2); 6.46–6.85 (m, 4H, 3,4,5,6 ArH); 6.90–7.12 (m, 4H, *p*-F phenyl ArH); 7.22–7.38 (m, 3H, dichloro ArH); 7.56 (s, 1H, NH); 11.51 (s, 1H, SH). ^{1}H-NMR of **9e** ($CDCl_3$, δ ppm): 1.95 (s, 3H, CH_3); 3.82 (s, 2H, CH_2); 6.27–6.83 (m, 4H, ArH); 6.91–7.13 (m, 4H, 3,4,5,6 ArH); 7.30–7.43 (m, 3H, dichloro ArH); 8.21 (s, 1H, NH); 10.81 (s, 1H, SH). ^{1}H-NMR of **9f** ($CDCl_3$, δ ppm): 1.81 (s, 3H, CH_3); 3.82 (s, 2H, CH_2); 6.24–6.81 (m, 4H, ArH); 6.93–7.18 (m, 4H, 3,4,5,6 ArH); 7.31–7.45 (m, 3H, dichloro ArH); 8.08 (s, 1H, NH); 10.82 (s, 1H, SH). ^{1}H-NMR of **9g** (DMSO-d_6, δ ppm): 3.69 (s, 2H, CH_2); 3.84 (s, 3H, OCH_3); 6.54–6.71 (m, 4H, ArH); 6.95–7.20 (m, 4H, 3,4,5,6 Ar-H); 7.43–7.51 (m, 4H, dichloro ArH and 1NH); 10.83 (s, 1H, SH).

*5.1.10. General procedure for the preparation of 5-[2-(2,6-dichloroanilino)benzyl]2-alkyl/arylamino-1,3,4-thiadiazoles (**10a–g**)*

The thiosemicarbazide **7a–g** (0.002 mol) was added gradually with stirring to ice cold concentrated sulphuric acid (10 ml) and the reaction mixture was further stirred for 4 h in an ice bath. It was then poured over crushed ice and solid thus separated was filtered, washed with water and recrystallized from methanol (Table 1). ^{1}H-NMR of **10a** (DMSO-d_6, δ ppm): 1.16–1.21 (t, 3H, CH_3), 1.23–1.28 (m, 2H, CH_3CH_2); 1.39–1.48 (m, 2H, $CH_3CH_2CH_2$); 3.26–3.35 (m, 2H, $NHCH_2$); 4.15 (s, 2H, CH_2); 6.86–6.90 (t, 1H, NH–CH_2); 7.10–7.25 (m, 4H, 3,4,5,6 ArH); 7.39–7.41 (m, 3H, dichloro ArH); 8.23 (s, 1H, NH). ^{1}H-NMR of **10c** ($CDCl_3$, δ ppm); 3.79 (s, 2H, CH_2), 6.95–7.57 (complex m, 12H, 11ArH and 1NH), 8.30 (s, 1H, NH). ^{1}H-NMR of **10d** ($CDCl_3$, δ ppm); 3.80 (s, 2H, CH_2), 6.98–7.61 (complex m, 12H, 11ArH and 1NH), 8.32 (s, 1H, NH). ^{1}H-NMR of **10e** (DMSO d_6, δ ppm); 2.21 (s, 3H, CH_3); 4.16 (s, 2H, CH_2), 6.86–7.41 (complex m, 12H, 11ArH and 1NH), 7.55 (s, 1H, NH). ^{1}H-NMR of **10g** (DMSO d_6, δ ppm); 3.82 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 6.35–7.81 (complex m, 12H, 11Ar–H and 1NH), 8.28 (s, 1H, NH). Mass spectra of compound **10g** exhibited molecular ion peak at *m/z* 456 (M^+), other important fragments were found at 458 ($M^+ + 2$), 460 ($M^+ + 4$), 424, 350 and 107.

5.2. Pharmacology

The compounds **3, 4, 5b, 5d, 6, 8a, 8b, 8d, 8e, 8g, 9a, 9d, 9e, 9f, 9g, 10a, 10c, 10d, 10e** and **10g** were evaluated for their anti-inflammatory activity. Diclofenac, the parent compound was used as a reference drug. The experiments were performed on albino rats of Wistar strain of either sex, weighing 180–200 g. The animals were maintained at 25 ± 2 °C, 50 ± 5% relative humidity, 12 h light/dark cycle. Food and water were freely available upto the time of experiments. The test compounds were dissolved in 1% carboxy methyl cellulose (CMC) solution.

5.2.1. Anti-inflammatory activity

This activity was performed by the following procedure of Winter et al. [27] on groups of six animals each. A freshly prepared suspension of carrageenin (1.0% w/v, 0.1 ml) was injected in the planter region of right hind paw of each rat. One group was kept as control and the animals of the other group were pretreated with the test drugs suspended in 1.0% CMC given orally 1 h before the carrageenin treatment. The volume was measured before and after 4 h of carrageenin treatment with the help of pleythysmometer. The percent anti-inflammatory activity was calculated according to the formula given below:

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

where V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents the mean increase in paw volume in control group of rats.

Data are expressed as mean ± S.E.M., 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 difference in results was considered significant when $P < 0.01$.

5.2.2. Analgesic activity

The acetic acid induced writhing test [28] was performed by an i.p. injection of 1% aqueous acetic acid solution in a volume of 0.1 ml. In each group six albino mice were kept. 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. The compounds, which exhibited good anti-inflammatory activity comparable to that of diclofenac, were screened for analgesic activity. All compounds were dissolved in 1% CMC solution. One group was kept as control and received p.o. administration of 1% CMC. Diclofenac was used as reference drug. 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.

Data are expressed as mean ± S.E.M., 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 difference in results was considered significant when $P < 0.01$.

5.2.3. Acute ulcerogenesis

Acute ulcerogenesis test was done according to Cioli et al. [29]. 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 diclofenac at the dose of 30 mg/kg. Control rats received p.o. administration of vehicle (suspension of 1% methyl cellulose). 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 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.

Data are expressed as mean ± S.E.M., 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 difference in results was considered significant when $P < 0.01$.

5.2.4. Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al. [30]. After screening for ulcerogenic activity, the gastric mucosa was scrapped 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 sulfate (SDS), 1.5 ml of acetate buffer (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid (TBA). The mixture was heated at 95 °C for 60 min. 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^{-1} M^{-1}.

Data are expressed as mean ± S.E.M., 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 difference in results was considered significant when $P < 0.01$.

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