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Short communication

Synthesis and pharmacological evaluation of 2(3*H*)-furanones and 2(3*H*)-pyrrolones, combining analgesic and anti-inflammatory properties with reduced gastrointestinal toxicity and lipid peroxidation

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ABSTRACT

A series of 3-arylidene-5-(4-chloro-phenyl)-2(3*H*)-furanones (**2**–**13**) and their nitrogen analogues 1-benzylpyrrolones (**14**–**18**) were synthesized. The compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation actions. Some of the newly synthesized compounds showed good anti-inflammatory and analgesic activities with low GI toxicity and reduced lipid peroxidation. The biological activity was found to improve upon replacement of oxygen of furanone ring with benzylamine moiety i.e. 1-benzylpyrrolones. Similarly, compounds containing halogen group(s), compounds **15** and **17**, showed higher degree of anti-inflammatory activity and their activity was comparable to that of the standard. These compounds showed interesting profile of analgesic activity in acetic acid induced writhing test (peripheral effect) and in the hot-plate test (central effect). The compounds were also tested for their ulcerogenic and lipid peroxidation action and showed superior GI safety profile along with reduction in lipid peroxidation as compared to that of the standard.

1. Introduction

2-Furanone, also known as furan-2-one, is a heterocyclic chemical compound classified as a lactone. It is a common component of natural products synthesized by biochemical pathways in organisms, especially plants of the genus *Angelica*. 2-Furanone is also known as *γ-crotonolactone* or *β-angelica lactone*. It is the simplest butenolide compound and is colloquially called "butenolide" in the context of natural product synthesis. Compounds derived from furanones are generated by some plants exposed to high heat in brush fires and trigger seed germination in plants whose reproduction depends on exposure to fire [1].

In the last few decades, the chemistry of furanones has received considerable attention owing to their synthetic and effective biological importance. Furanone moieties have been incorporated into a wide variety of therapeutically interesting drug candidates having anti-inflammatory [2], cardiotonic activity [3], analgesic [4], anticancer [5], anti-convulsant [6], anti-microbial [7] and antiviral activities [8]. There are marketed drugs containing furanone ring, e.g., basidalin as anticancer, ascorbic acid as an antioxidant, narthogenin and butalactin as antibiotic, rofecoxib as a specific COX-2 inhibitor. Moreover, there are many furanones which are patented as an anti-inflammatory agent e.g., 3-alkyl-5-hydroxy-3,4-dihydrofuran-2-ones (United States Patent 5202350), 4-[3,6-dihydro-6-hydroxy-5-(3-phenylpropyl)-2*H*-pyran-2-yl]-5-hydroxy-2(5*H*)-furanone (European Patent EP042-9287).

We have previously described the synthesis of a series of new anti-inflammatory and analgesic agents with reduced gastric ulceration having furanone structure [9]. A recent literature survey revealed that many of the halogen containing heterocycles has attracted attention due to the ability of halogen to act as polar hydrogen or hydroxyl mimic. Substitution of hydrogen by halogen has been a strategy in designing molecules having potential biological activities [10,11]. Prompted by these investigations and in continuation of our research work [9,12] on studies of novel *O*- & *N*-heterocyclic compounds of pharmaceutical interest, it was thought to be interesting to study various compounds containing 2(3*H*)-furanone and 2(3*H*)-pyrrolone moieties.

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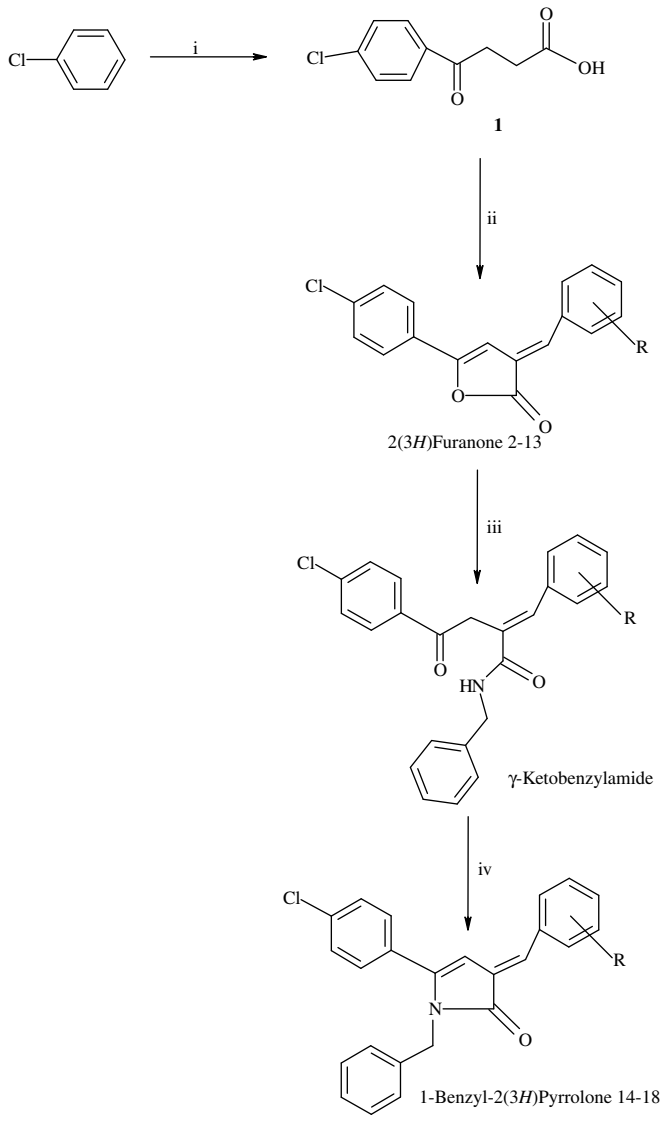
2. Chemistry

Overall eighteen compounds were synthesized. Synthesis of twelve 2(3*H*)-furanones were brought about by two-step reaction using Friedel–Craft's acylation reaction and modified Perkin reaction conditions (Scheme 1). The 3-arylidene-5-(4-chloro-phenyl)-2(3*H*)-furanones **2**–**13** were prepared by condensing different aromatic aldehydes with 3-(4-chloro-benzoyl)propionic acid **1** in presence of triethylamine and acetic anhydride under anhydrous conditions. The required 3-(4-chloro-benzoyl)-propionic acid **1** was prepared by reacting dry chlorobenzene with succinic anhydride in presence of anhydrous aluminum chloride following Friedel–Craft's acylation reaction conditions. In an attempt to prepare the 2(3*H*)-furanones in higher yields, two different synthetic methods were applied. Thus the condensation of above-mentioned 3-(4-chloro-benzoyl)propionic acid with aromatic aldehydes was carried out using: (a) 15 mL acetic anhydride (method i) [9] and (b) 5–8 drops acetic anhydride; one pot synthesis i.e. fusion reaction (method ii) by heating on a heating mantle. Substrates were mixed together in molar ratio (3 mmol; 1:1). It was found that both methods led directly to the formation of desired 2(3*H*)-furanones. Higher yields (on an average 75%) with reduced reaction time for 2(3*H*)-furanones were achieved when the reaction was performed by one pot synthesis (method ii). Mixed melting points of the 2-(3*H*)-furanones (**2**–**13**) obtained by two above-mentioned methods have not shown any depression. The IR, ^{1}H NMR and MS spectral data of these compounds were also identical.

Some of the 2(3*H*)-furanones (**2**–**13**) synthesized above were further converted to nitrogen derivatives i.e. 1-benzylpyrrolones (**14**–**18**). The 3-arylidene-5-(4-chloro-phenyl)-1-benzyl-2(3*H*)-pyrrolones (**14**–**18**) were synthesized by reacting appropriate 2(3*H*)-furanone with benzylamine in dry benzene to give γ-keto-benzylamide, which was then lactamized (cyclized) in 6 N HCl to give the corresponding 1-benzylpyrrolone. Calculations of δ-values using incremental parameters for the hydrogen (semicyclic double bond) suggest (*E*)-configuration.

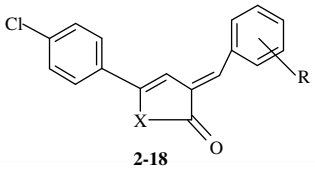
In the ^{1}H NMR spectral data all the compounds showed two singlets of one proton each around δ 6.5 and δ 7.4 which could be assigned to the ring βH and the olefinic hydrogen of the arylidene substituent. Other peaks were observed at appropriate δ-values. The fragmentation pattern observed on electron impact mass spectrum can be summarized as follows:

The 3-arylidene-5-(4-chloro-phenyl)-2(3*H*)-furanones gave M^+ peak in reasonable intensities. The major fragment appears to be $Cl–C_6H_4–C{\equiv}O^+$ arising from the heterocyclic oxygen and γ-carbon with its substituent. Subsequently it loses CO to give $Cl–C_6H_4^+$. There appeared a peak at *m*/*z* 77 that corresponds to $C_6H_5^+$. Occasionally the aryl ring of the arylidene moiety also appeared as Ar^+. In case of 1-benzylpyrrolones, loss of 91 mass units corresponding to benzyl moiety from the molecular ion is observed along with peaks at *m*/*z* 91, 77. Other pathway is via $Cl–C{\equiv}N^+H$ arising from C-2 and its substituent, which appears to be novel. This also loses HCN to give $Cl–C_6H_4^+$. The molecular ion or other related ions produced the appropriate isotopic abundances due to presence of chlorine atom(s). The analytical data are mentioned in Table 1.



Scheme 1. (i) Anhydrous $AlCl_3$, succinic anhydride; (ii) aryl aldehyde, Ac_2O, triethylamine, reflux; (iii) dry benzene, benzylamine; (iv) 6 N HCl.

Table 1
Physical constants of 2(3*H*)-furanones and 1-benzyl-2(3*H*)-pyrrolones.



2-18

Compounds	X	R	Rf value[a]	Molecular formula[b]
2	O	H	0.76	$C_{17}H_{11}O_2Cl$
3	O	2-Chloro	0.74	$C_{17}H_{10}O_2Cl_2$
4	O	3-Chloro	0.74	$C_{17}H_{10}O_2Cl_2$
5	O	4-Chloro	0.82	$C_{17}H_{10}O_2Cl_2$
6	O	2-Nitro	0.82	$C_{17}H_{10}O_4NCl$
7	O	3-Nitro	0.80	$C_{17}H_{10}O_4NCl$
8	O	4-Nitro	0.82	$C_{17}H_{10}O_4NCl$
9	O	4-Fluoro	0.78	$C_{17}H_{10}O_2ClF$
10	O	4-Methoxy	0.78	$C_{18}H_{13}O_3Cl$
11	O	3,4-Dimethoxy	0.76	$C_{19}H_{15}O_4Cl$
12	O	2,4-Dichloro	0.72	$C_{17}H_9O_2Cl_3$
13	O	4-Acetoxy-3-methoxy	0.72	$C_{20}H_{15}O_5Cl$
14	$–NCH_2C_6H_5–$	H	0.71	$C_{24}H_{18}ONCl$
15	$–NCH_2C_6H_5–$	4-Chloro	0.70	$C_{24}H_{17}ONCl_2$
16	$–NCH_2C_6H_5–$	4-Nitro	0.76	$C_{24}H_{17}O_3N_2Cl$
17	$–NCH_2C_6H_5–$	4-Fluoro	0.78	$C_{24}H_{17}ONClF$
18	$–NCH_2C_6H_5–$	4-Hydroxy-3-methoxy	0.78	$C_{25}H_{20}O_3NCl$

[a] Rf values for compounds **2**–**13** in solvent system (toluene:ethylacetate:formic acid, 5:4:1) and for compounds **14**–**18** in solvent system (petrol:toluene:ethylacetate, 10:5:3).

[b] The micro analysis values for C, H and N were within ± 4% of the theoretical values.

3. Pharmacological results and discussion

3.1. Anti-inflammatory activity

The *in-vivo* anti-inflammatory activity of the synthesized compounds **2**–**18** was evaluated by carrageenan-induced rat paw edema method [13]. Ibuprofen was used as a standard drug for comparison. All the compounds were administered orally and assayed at a dose level of 20 mg/kg of body weight. The obtained pharmacological results revealed that replacement of oxygen atom of furanone ring with benzylamine moiety resulted in markedly increase in anti-inflammatory activity (Table 2).

Following points could be made after analyzing the anti-inflammatory results:

- Among the compounds bearing benzylamine moiety i.e. 1-benzyl-2(3*H*)-pyrrolones, the maximum activity was shown by the 3-(4-chloro-benzylidene)-5-(4-chloro-phenyl)-1-benzyl-2(3*H*)-pyrrolone **15** and 3-(4-fluoro-benzylidene)-5-(4-chloro-phenyl)-1-benzyl-2(3*H*)-pyrrolone **17** with 88.88% and 89.5% inhibition respectively. The other three compounds, 3-(benzylidene)-5-(4-chloro-phenyl)-1-benzyl-2(3*H*)-pyrrolone **14**, 3-(4-nitro-benzylidene)-5-(4-chloro-phenyl)-1-benzyl-2(3*H*)-pyrrolone **16** and 3-(4-hydroxy-3-methoxy-benzylidene)-5-(4-chloro-phenyl)-1-benzyl-2(3*H*)-pyrrolone **18**, showed 37.03, 19.75 and 41.97% inhibition, respectively.
- Among the compounds of 2(3*H*)-furanone series, good results were obtained with 3-(2-chloro-benzylidene)-5-(4-chloro-phenyl)-2(3*H*)-furanone **3**, 3-(4-chloro-benzylidene)-5-(4-chloro-phenyl)-2(3*H*)-furanone **5**, 3-(4-fluoro-benzylidene)-5-(4-chloro-phenyl)-2(3*H*)-furanone **9** and 3-(2,4-dichloro-benzylidene)-5-(4-chloro-phenyl)-2(3*H*)-furanone **12** with inhibition range of 69.13–81.48%.
- Compound **17** having fluoro at 4-position showed higher anti-inflammatory activity than compound **15** having chloro group at 4-position which indicates that activity increases with an increase in electronegativity on phenyl moiety. That effect further increases on replacement of oxygen moiety of furanone ring with benzylamine moiety.
- DFP [3-(2-propyloxy)-(4-methyl-sulfonylphenyl)-(5,5-dimethyl)-furanone], a furanone derivative shows selective cyclooxygenase-2 inhibition [4]. The compounds under investigation also have furanone nucleus in their structure. It gives the impression that these compounds would also possess selective cyclooxygenase-2 inhibition. The studies are under progress in our laboratory to acquire more information regarding their mechanism of action or biological targets.

Test compounds that exhibited good anti-inflammatory activity, namely 2(3*H*)-furanones **3**, **5**, **9** and **12** and 1-benzylpyrrolones **15** and **17**, were further evaluated for their analgesic, ulcerogenic and lipid peroxidation actions.

3.2. Analgesic activity

The compounds **3**, **5**, **9**, **12**, **15** and **17** were evaluated for their peripherally and centrally mediated analgesic effects using acetic acid induced writhing method [14] and Turner hot-plate method [15], respectively.

The results of analgesic activity (Table 3) indicated that few tested compounds showed central analgesic activity (hot-plate test), compounds **15** and **17** showed activity comparable to that of standard ibuprofen. Compounds **3**, **5**, **9** and **12** showed less central analgesic activity. Also, Table 3 shows that, compounds **3**, **9**, **15** and **17** were found to have significant peripheral analgesic activity (acetic acid induced writhing method) in the range of 50–59.03%, which was comparable to that of standard drug ibuprofen (65.06%).

These findings present an important advantage of compound **15** having chloro group at 4-position and compound **17** having fluoro group at 4-position of phenyl moiety as anti-inflammatory with high analgesic activity equal to that of ibuprofen.

3.3. Acute ulcerogenesis

The compounds which were screened for analgesic activity were further tested for their ulcerogenic activity. Compounds **3**, **5**, **9**, **12**, **15** and **17** were tested according to the method reported by Cioli et al. [16]. The tested compounds showed low ulcerogenic activity ranging from 0.1 ± 0.1 to 0.4 ± 0.1 whereas the standard drug ibuprofen showed high severity index of 0.9 ± 0.1. The maximum reduction in ulcerogenic activity (0.1 ± 0.1) was found in the chloro derivatives of furanones and benzylpyrrolones. The benzylpyrrolone derivatives **17** and **18** showing high anti-inflammatory activity and analgesic activity also showed reduction in ulcerogenic activity.

Table 2
Anti-inflammatory activity along with ulcerogenic and lipid peroxidation effect of the synthesized compounds **2**–**18**.

Compounds	Paw volume ± SEM[a]		% Inhibition ± SEM[b]		Severity index[a]	Lipid peroxidation[c]
	After 2 h	After 3 h	After 2 h	After 3 h		
Control	0.75 ± 0.01	0.81 ± 0.02	–	–	0.00 ± 0.00	0.238 ± 0.002[b],***
Ibuprofen	0.14 ± 0.01***	0.085 ± 0.02***	80.88 ± 2.02	89.50 ± 2.56	0.9 ± 0.36*	0.608 ± 0.001[a],***
2	0.60 ± 0.02**	0.518 ± 0.02***	18.88 ± 3.94***	36.00 ± 3.13***	0.9 ± 0.36*	0.522 ± 0.002[a,b],***
3	0.28 ± 0.01***	0.15 ± 0.00***	62.66 ± 1.50***	81.48 ± 0.63*	0.2 ± 0.12	0.494 ± 0.001[a,b],***
5	0.45 ± 0.02***	0.25 ± 0.01***	40 ± 3.13***	69.13 ± 1.65***	0.2 ± 0.12	0.403 ± 0.002[a,b],***
6	0.72 ± 0.01	0.66 ± 0.01**	4 ± 1.37***	18.51 ± 2.29***	0.6 ± 0.36	0.429 ± 0.001[a,b],***
8	0.72 ± 0.00	0.518 ± 0.01***	4 ± 0.91***	36.00 ± 1.36***	0.4 ± 0.40	0.549 ± 0.001[a,b],***
9	0.44 ± 0.02***	0.24 ± 0.01***	41.33 ± 3.04***	70.37 ± 1.56***	0.4 ± 0.10	0.329 ± 0.001[a,b],***
10	0.72 ± 0.00	0.548 ± 0.02***	4 ± 0.76***	32.30 ± 2.78***	0.5 ± 0.27	0.545 ± 0.001[a,b],***
11	0.68 ± 0.01*	0.518 ± 0.01***	9.33 ± 2.57***	36.00 ± 1.69***	0.3 ± 0.12*	0.533 ± 0.001[a,b],***
12	0.35 ± 0.01***	0.221 ± 0.01***	53.33 ± 2.36***	72.63 ± 2.21***	0.1 ± 0.10	0.388 ± 0.002[a,b],***
13	0.57 ± 0.02***	0.51 ± 0.01***	24 ± 3.81***	37.03 ± 2.20***	0.3 ± 0.12*	0.597 ± 0.001[a,b],**
14	0.57 ± 0.02***	0.51 ± 0.01***	24 ± 3.81***	37.03 ± 2.20***	0.6 ± 0.36	0.605 ± 0.001[a],***
15	0.31 ± 0.02***	0.09 ± 0.01***	58.66 ± 3.44***	88.88 ± 2.06	0.1 ± 0.10	0.310 ± 0.001[a,b],***
16	0.66 ± 0.02*	0.65 ± 0.03**	12 ± 3.07***	19.75 ± 4.54***	0.4 ± 0.10*	0.403 ± 0.002[a,b],***
17	0.31 ± 0.02***	0.085 ± 0.01***	58.66 ± 3.13***	89.50 ± 1.79	0.2 ± 0.12	0.401 ± 0.002[a,b],***
18	0.48 ± 0.02***	0.47 ± 0.03***	36 ± 3.83***	41.97 ± 3.74***	0.5 ± 0.27	0.558 ± 0.001[a,b],***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
[a] Relative to their respective control and data were analyzed by one-way ANOVA followed by Turkey test for $n = 6$.
[b] Relative to the standard (Ibuprofen) and data were analyzed by one-way ANOVA followed by Turkey test for $n = 6$.
[c] Lipid peroxidation activity is expressed as nmoles of MDA/mg of protein.

Table 3
Analgesic effects the some 2(3*H*)-furanones and 1-benzyl-2(3*H*)-pyrrolones.

Compounds	Dose (mg/kg)	Central analgesic activity [Hot-plate test (Reaction time)]		Peripheral analgesic activity (Writhing test)	
		0 min	60 min	No. of writhes/ 30 min	% Protection
Control	1 mL	9.39 ± 0.029	10.57 ± 0.026		
Ibuprofen	20	10.18 ± 0.034	14.40 ± 0.024***	29 ± 1.154***	65.06
3	20	9.87 ± 0.028	12.69 ± 0.030***	39 ± 1.897***	53.01
5	20	10.1 ± 0.023	12.67 ± 0.029***	46 ± 1.612***	44.57
9	20	10.30 ± 0.016	12.9 ± 0.026***	41 ± 1.932***	50.60
12	20	9.14 ± 0.020	11.86 ± 0.023***	42 ± 1.264***	49.39
15	20	8.46 ± 0.029	11.55 ± 0.026***	35 ± 1.238***	57.83
17	20	9.76 ± 0.024	13.3 ± 0.019***	34 ± 1.290***	59.03

***$p < 0.001$, Relative to the control and data were analyzed by one-way ANOVA followed by Turkey test for $n = 6$.

The other tested compounds also exhibited better GI safety profile as compared to the standard drug ibuprofen. Results are presented in Table 2.

3.4. Lipid peroxidation

The activated inflammatory cells could be involved in the pathogenesis of mucosal damage. Gastric mucosal cells metabolise arachidonic acid via both the lipoxygenase and cyclooxygenase pathways [17] and the presence of inflammatory cells infiltrate in the gastric mucosa results in the production of large quantities of oxygen derived free radicals that could cause cell damage and leads ultimately to mucosal injury.

Lipid peroxidation refers to the oxidative degradation of lipids. This process proceeds by free radical chain reaction in which free radicals steal electrons from the lipids in cell membrane and consequently damages the cell. It most often affects polyunsaturated fatty acids forming malondialdehyde (MDA).

The colorimetric reaction of thiobarbituric acid (TBA) with MDA, a secondary product of lipid peroxidation (LPO) has been widely adopted as a sensitive assay method for measuring LPO in animal tissues. It is used as an index of the extent to which LPO has progressed. Since the assay procedure estimates the amount of TBA reactive substances e.g., MDA, it is also referred to as TBARS (Thiobarbituric Acid Reactive Substance) test.

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 using Ohkawa et al. method [18]. The lipid peroxidation was measured as nmoles of MDA/mg of protein. Ibuprofen exhibited high lipid peroxidation 0.608 ± 0.001 whereas control group showed 0.238 ± 0.002. It was found that all the furanone and its benzyl pyrrolones derivatives showed less ulcerogenic activity along with reduced lipid peroxidation (Table 2).

4. Conclusion

Seventeen new 2(3*H*)-furanones and their nitrogen analogues i.e. 1-benzyl-2(3*H*)-pyrrolones were successfully synthesized. Biological evaluation results showed that the compounds are promising anti-inflammatory and analgesic agents with lesser GI toxicity and reduced lipid peroxidation. 1-Benzyl-2(3*H*)-pyrrolones showed better anti-inflammatory activity than their parent compounds i.e. 2(3*H*)-furanones. Similarly, compounds containing halogen group(s) showed improved anti-inflammatory activity. These compounds showed superior GI safety profile along with reduction in lipid peroxidation as compared to that of ibuprofen. The furanone and 1-benzylpyrrolone derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of pain and inflammation.

Among the newer derivatives, two compounds **15** and **17** emerged as lead compounds. It is conceivable that these derivatives could be further modified to develop potent and safer anti-inflammatory and analgesic agents. Further studies to acquire more information about quantitative structure–activity relationship (QSAR) are in progress in our laboratory.

5. Experimental protocols

5.1. Chemistry

Chemicals were purchased from Merck and Sigma-Aldrich as 'synthesis grade' and used without further purification. Melting points (m.p.) were determined in open capillary tubes and are given uncorrected. Elemental analyses were performed on a Perkin–Elmer analyzer and were in range of ± 0.4% for each element analyzed (C, H, N). The IR spectra were measured as potassium bromide pellets using a Perkin–Elmer 1725X spectrophotometer. ^{1}H NMR spectra were recorded on Bruker spectropsin DPX-300 MHz in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard; chemical shifts (δ) are reported in parts per million (*ppm*) downfield from TMS. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet. Mass spectroscopic analyses for compounds were performed on a JEOL JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Spectral data are consistent with assigned structures. The molecular ion for compounds containing chloro group was calculated according to ^{35}Cl isotope. Thin-layer chromatography was carried out to monitor the reactions using silica gel (Merck No. 5554). Dry solvents were used throughout.

5.1.1. Preparation of 3-(4-chloro-benzoyl) propionic acid (*1*)

Succinic anhydride (10 g, 10 mmol) was reacted with dry chlorobenzene (50 mL) in presence of anhydrous aluminum chloride (15 g, 11.25 mmol). The reaction mixture was heated on a heating mantle for 2 h. It was purified by dissolving in sodium hydroxide solution, filtering, followed by addition of hydrochloric acid. The solid mass so obtained was filtered, washed with cold water, dried and crystallized from methanol to give 65 g (62%) of the desired compound as a off-white crystalline solid, m.p. 124 °C which gave effervescence with sodium bicarbonate solution, ^{1}H NMR ($CDCl_3$) δ 2.81 and 3.38 (t each, 2 × CH_2), 7.45, 7.92 (d each, 2 × A_2B_2, *p*-substituted phenyl).

5.1.2. General procedure for the synthesis of 3-arylidene-5-(4-chloro-phenyl)-2(3H)-furanones *2*–*13*

5.1.2.1. Method i. A solution of 3-(4-chloro-benzoyl)propionic acid (3 mmol) and aromatic aldehyde (equimolar, 3 mmol) in acetic anhydride (15 mL) with triethylamine (3–4 drops) was refluxed for 4 h under anhydrous conditions. After completion of reaction, the contents were poured into crushed ice in small portions while stirring. A colored solid mass separated out, which was filtered, washed with water and crystallized from a mixture of methanol/chloroform (1:1) to give **2**–**13**.

5.1.2.2. Method ii. 3-(4-Chloro-benzoyl)propionic acid (3 mmol) and aromatic aldehyde (equimolar, 3 mmol) in acetic anhydride (5–8 drops) were fused together for 5 min then triethylamine (2 drops) was added and the contents were refluxed for 15 min. A colored solid mass formed, which was crystallized from a mixture of methanol/chloroform (1:1) to give **2**–**13**.

5.1.2.3. 3-(Benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (2). Yield: 52%, m.p. 194 °C. ^{1}H NMR ($CDCl_3$) δ 6.93 (s, 1H, furanone ring), 7.41 (m,

1H, olefinic H), 7.62 and 7.68 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.41 (m, 5H, H-2,3,4,5,6, arylidene). ^{13}C NMR ($CDCl_3$) δ 170.6 (C-1), 127.2 (C-2), 118.3 (C-3), 129.3 (C-4), 98.4 (C-5), 130.0 (C-6), 132.8 (C-7,11), 120.6 (C-8,10), 124.3 (C-9), 153.7 (C-12), 119.8 (C-13,17), 131.0 (C-14,16), 156.6 (C-15). MS: *m*/*z* 282(M^+), 139, 111, 77. IR (cm^{-1}, KBr): 1763, 1602, 836. Anal. Calcd. for $C_{17}H_{10}ClO_2$: C, 72.22; H, 3.92. Found: C, 72.43; H, 3.90.

*5.1.2.4. 3-(2-Chloro-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**3**).* Yield: 64%, m.p. 220 °C. ^{1}H NMR ($CDCl_3$) δ 6.84 (s, 1H, furanone ring), 7.46 (s, 1H, olefinic H), 7.44 and 7.67 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.64 (m, 4H, H-3,4,5,6, arylidene). MS: *m*/*z* 317(M^+), 139, 111. IR (cm^{-1}, KBr): 1768, 1605, 831. Anal. Calcd. for $C_{17}H_{10}Cl_2O_2$: C, 64.38; H, 3.18. Found: C, 64.20; H, 3.19.

*5.1.2.5. 3-(3-Chloro-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**4**).* Yield: 60%, m.p. 240 °C. ^{1}H NMR ($CDCl_3$) δ 6.78 (s, 1H, furanone ring), 7.42 (s, 1H, olefinic H), 7.52 and 7.69 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.36 (m, 3*H*, H-4,5,6, arylidene), 7.62 (s, 1H, H-2). MS: *m*/*z* 317(M^+), 139, 111, 77. IR (cm^{-1}, KBr): 1773, 1605, 832. Anal. Calcd. for $C_{17}H_{10}Cl_2O_2$: C, 64.38; H, 3.18. Found: C, 64.19; H, 3.16.

*5.1.2.6. 3-(4-Chloro-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**5**).* Yield: 68%, m.p. 284 °C. ^{1}H NMR ($CDCl_3$) δ 6.87 (s, 1H, furanone ring), 7.45 (s, 1H, olefinic H), 7.55 and 7.69 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.41 and 7.53 (d each, 2 × A_2B_2, arylidene). MS: *m*/*z* 317(M^+), 139, 77. IR (cm^{-1}, KBr): 1763, 1603, 835. Anal. Calcd. for $C_{17}H_{10}Cl_2O_2$: C, 64.38; H, 3.18. Found: C, 64.55; H, 3.20.

*5.1.2.7. 3-(2-Nitro-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**6**).* Yield: 70%, m.p. 240 °C. ^{1}H NMR ($CDCl_3$) δ 6.60 (s, 1H, furanone ring), 7.66 (s, 1H, olefinic H), 7.54 and 7.68 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.84 (s, 1H, H-5, arylidene), 8.20 (d, 1H, H-3, arylidene), 8.43 (m, 2H, H-4,6, arylidene). MS: *m*/*z* 327(M^+), 122, 111, 77. IR (cm^{-1}, KBr): 1769, 1608, 828. Anal. Calcd. for $C_{17}H_{10}ClNO_4$: C, 62.30; H, 3.08; N, 4.27. Found: C, 62.48; H, 3.09; N, 4.26.

*5.1.2.8. 3-(3-Nitro-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**7**).* Yield: 72%, m.p. 226 °C. ^{1}H NMR ($CDCl_3$) δ 6.67 (s, 1H, furanone ring), 7.62 (s, 1H, olefinic H), 7.52 and 7.69 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.64 (dd, 1H, H-5, arylidene), 7.81 (d, 1H, H-6, arylidene), 8.16 (d, 1H, H-4, arylidene), 8.23 (s, 1H, H-2, arylidene). MS: *m*/*z* 327(M^+), 139, 122, 77. IR (cm^{-1}, KBr): 1771, 1606, 826. Anal. Calcd. for $C_{17}H_{10}ClNO_4$: C, 62.30; H, 3.08; N, 4.27. Found: C, 62.12; H, 3.05; N, 4.29.

*5.1.2.9. 3-(4-Nitro-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**8**).* Yield: 74%, m.p. 264 °C. ^{1}H NMR ($CDCl_3$) δ 6.82 (s, 1H, furanone ring), 7.56 (s, 1H, olefinic H), 7.50 and 7.63 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.59 and 8.12 (d each, 2 × A_2B_2, arylidene). MS: *m*/*z* 327(M^+), 139, 111. IR (cm^{-1}, KBr): 1765, 1608, 835. Anal. Calcd. for $C_{17}H_{10}ClNO_4$: C, 62.30; H, 3.08; N, 4.27. Found: C, 62.47; H, 3.10; N, 4.28.

*5.1.2.10. 3-(4-Fluoro-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**9**).* Yield: 56%, m.p. 220 °C. ^{1}H NMR ($CDCl_3$) δ 6.87 (s, 1H, furanone ring), 7.42 (s, 1H, olefinic H), 7.63 and 7.68 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.14 (m, 2H, H-3,5, arylidene), 7.42 (m, 3H, H-2,6, arylidene). MS: *m*/*z* 300(M^+), 139, 77. IR (cm^{-1}, KBr): 1761, 1601, 833. Anal. Calcd. for $C_{17}H_{10}ClFO_2$: C, 67.90; H, 3.35. Found: C, 67.68; H, 3.36.

*5.1.2.11. 3-(4-Methoxy-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**10**).* Yield: 64%, m.p. 214 °C. ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 3*H*, OCH_3), 6.90 (s, 1H, furanone ring), 6.93 and 7.45 (d each, 2 × A_2B_2, arylidene), 7.42 (s, 1H, olefinic H), 7.50 and 7.67 (d each, 2 × A_2B_2, *p*-substituted phenyl). MS: *m*/*z* 312(M^+), 111. IR (cm^{-1}, KBr): 1755, 1597, 844. Anal. Calcd. for $C_{18}H_{13}ClO_3$: C, 69.13; H, 4.19. Found: C, 68.93; H, 4.17.

*5.1.2.12. 3-(3,4-Dimethoxy-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**11**).* Yield: 64%, m.p. 218 °C. ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 6H, 2 × OCH_3), 6.85 (s, 1H, furanone ring), 6.98 (d, 1H, H-5, arylidene), 7.17 (s, 1H, H-2, arylidene), 7.39 (d, 1H, H-6, arylidene), 7.59 (s, 1H, olefinic H), 7.45 and 7.74 (d each, 2 × A_2B_2, *p*-substituted phenyl). MS: *m*/*z* 342(M^+) 139, 111. IR (cm^{-1}, KBr): 1771, 1598, 837. Anal. Calcd. for $C_{19}H_{15}ClO_4$: C, 66.58; H, 4.41. Found: C, 66.80; H, 4.43.

*5.1.2.13. 3-(2,4-Dichloro-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**12**).* Yield: 52%, m.p. 172 °C. ^{1}H NMR ($CDCl_3$) δ 6.41 (s, 1H, furanone ring), 7.42 (s, 1H, olefinic H), 7.3 and 7.48 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.54 (m, 2H, H-3,5, arylidene), 7.37 (m, 1H, H-6, arylidene). MS: *m*/*z* 351(M^+), 139, 111, 77. IR (cm^{-1}, KBr): 1776, 1611, 833. Anal. Calcd. for $C_{17}H_9Cl_3O_2$: C, 58.07; H, 2.58. Found: C, 57.26; H, 2.57.

*5.1.2.14. 3-(4-Acetoxy-3-methoxy-benzylidene)-5-(4-chloro-phenyl)-2(3H)-furanone (**13**).* Yield: 52%, m.p. 148 °C. ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H, $OCOCH_3$), 3.90 (s, 3H, OCH_3), 6.67 (s, 1H, furanone ring), 7.32 and 7.64 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.42 (s, 1H, olefinic H), 7.36 (d, 1H, H-5, arylidene), 7.54 (m, 2H, H-2,6, arylidene). MS: *m*/*z* 370(M^+), 139, 77. IR (cm^{-1}, KBr): 1767, 1597, 829. Anal. Calcd. for $C_{20}H_{15}ClO_5$: C, 64.79; H, 4.08. Found: C, 64.58; H, 4.09.

*5.1.3. General procedure for the synthesis of 3-arylidene-5-(4-chloro-phenyl)-1-benzyl-2(3H)-pyrrolones (**14**–**18**)*

Synthesis of these compounds involved the following two steps:

- Synthesis of γ-ketobenzylamide

Furanone (3 mmol) and benzylamine (4 mmol) were refluxed in dry benzene for 2 h. On completion of reaction, excess benzene was distilled off and a solid mass so obtained was washed with petroleum ether and dried. The compound obtained was used without crystallization.

- Cyclization of γ-ketobenzylamide

γ-Ketobenzylamide (3 mmol) was refluxed in 6 N hydrochloric acid (20 mL) for 1 h. The contents were then cooled and a solid mass so obtained was collected, washed with water and crystallized from methanol to give **14**–**18**.

*5.1.3.1. 3-Benzylidene-5-(4-chloro-phenyl)-1-benzyl-2(3H)-pyrrolone (**14**).* Yield: 44%, m.p. 124 °C. ^{1}H NMR ($CDCl_3$) δ 4.82 (s, 2H, CH_2), 6.25 (s, 1H, furanone ring), 7.55 (s, 1H, olefinic H), 7.05 and 7.64 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.46 (m, 3*H*, H-3,4,5, arylidene), 7.62 (m, 2H, H-2,6, arylidene), 7.37 (m, 5H, H-2,3,4,5,6, benzyl). ^{13}C NMR ($CDCl_3$) δ 173.2 (C-1), 126.9 (C-2), 118.3 (C-3), 127.1 (C-4), 100.0 (C-5), 129.5 (C-6), 132.1 (C-7,11), 119.4 (C-8,10), 124.0 (C-9), 152.5 (C-12), 124.0 (C-13,17), 131.2 (C-14,16), 155.3 (C-15), 44.6 (C-1′), 133.6 (C-2′), 128.5 (C-3′,7′), 129.1 (C-4′,6′), 119.8 (C-5′). MS: *m*/*z* 371(M^+), 282, 139, 91, 77. IR (cm^{-1}, KBr): 1752, 1613, 805. Anal. Calcd. for $C_{24}H_{18}ClNO$: C, 77.52; H, 4.88; N, 3.77. Found: C, 77.24; H, 4.87; N, 3.78.

*5.1.3.2. 3-(4-Chloro-benzylidene)-5-(4-chloro-phenyl)-1-benzyl-2(3H)-pyrrolone (**15**).* Yield: 48%, m.p. 136 °C. ^{1}H NMR ($CDCl_3$) δ 4.82 (s, 2H, CH_2), 6.18 (s, 1H, furanone ring), 7.37 and 7.55 (d each, 2 × A_2B_2, arylidene), 7.46 (s, 1H, olefinic H), 7.04 and 7.31 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.24 (m, 5H, H-2,3,4,5,6, benzyl). MS: *m*/*z* 406 (M^+), 315, 111, 91, 77. IR (cm^{-1}, KBr): 1749, 1593, 811. Anal. Calcd. for

$C_{24}H_{17}Cl_2NO$: C, 70.95; H, 4.22; N, 3.45. Found: C, 71.15; H, 4.20; N, 3.43.

*5.1.3.3. 3-(4-Nitro-benzylidene)-5-(4-chloro-phenyl)-1-benzyl-2(3H)-pyrrolone (**16**).* Yield: 46%, m.p. 200 °C. ^{1}H NMR ($CDCl_3$) δ 6.19 (s, 1H, furanone ring), 7.50 (s, 1H, olefinic H), 7.04 and 7.75 (d each, $2 \times A_2B_2$, *p*-substituted phenyl), 7.34 and 8.25 (d each, $2 \times A_2B_2$, arylidene), 7.24 (m, 5H, H-2,3,4,5,6, benzyl). MS: *m*/*z* 416 (M^+), 325, 139, 91, 77. IR (cm^{-1}, KBr): 1739, 1613, 795. Anal. Calcd. for $C_{24}H_{17}ClN_2O_3$: C, 69.15; H, 4.11; N, 6.72. Found: C, 68.97; H, 4.09; N, 6.70.

*5.1.3.4. 3-(4-Fluoro-benzylidene)-5-(4-chloro-phenyl)-1-benzyl-2(3H)-pyrrolone (**17**).* Yield: 48%, m.p. 146 °C. ^{1}H NMR ($CDCl_3$) δ 4.85 (s, 2H, CH_2), 6.19 (s, 1H, furanone ring), 7.49 (s, 1H, olefinic H), 7.18 and 7.31 (d each, $2 \times A_2B_2$, *p*-substituted phenyl), 7.11 and 7.61 (d each, $2 \times A_2B_2$, arylidene), 7.21 (m, 5H, H-2,3,4,5,6, benzyl). MS: *m*/*z* 389 (M^+), 298, 139, 91, 77. IR (cm^{-1}, KBr): 1736, 1606, 813. Anal. Calcd. for $C_{24}H_{17}ClFNO$: C, 73.94; H, 4.40; N, 3.59. Found: C, 73.68; H, 4.38; N, 3.40.

*5.1.3.5. 3-(4-Hydroxy-3-methoxy-benzylidene)-5-(4-chloro-phenyl)-1-benzyl-2(3H)-pyrrolone (**18**).* Yield: 48%, m.p. 150 °C. ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3*H*, OCH_3), 4.84 (s, 2H, CH_2), 5.8 (s, 1H, OH), 6.21 (s, 1H, furanone ring), 7.07 and 7.35 (d each, $2 \times A_2B_2$, *p*-substituted phenyl), 6.96 (d, 1H, H-6, arylidene), 7.11 (d, 1H, H-5, arylidene), 7.21 (s, 1H, H-2, arylidene), 7.49 (s, 1H, olefinic H), 7.24 (m, 5H, H-2,3,4,5,6, benzyl). MS: *m*/*z* 417 (M^+), 326, 91, 77. IR (cm^{-1}, KBr): 1748, 1606, 815. Anal. Calcd. for $C_{25}H_{20}ClNO_3$: C, 71.85; H, 4.82; N, 3.35. Found: C, 71.67; H, 4.80; N, 3.33.

5.2. Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema method of Winter et al. [13]. The experiment was performed on Albino rats of Wistar strain of either sex, weighing 180–200 g. The animals were randomly divided into groups of six. Group I was kept as control, and received only 0.5% carboxymethyl cellulose (CMC) solution. Groups II was kept as standard and received ibuprofen (20 mg/kg *p.o.*). Carrageenan 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, 30 min after the administration of the test compounds and standard drugs. The paw volume was measured by saline displacement shown on screen of digital Plethysmometer (Ugo Basile) at 2 and 3 h after carrageenan injection. Thus the edema volume in control group (V_c) and edema volume in groups treated with test compounds (V_t) was measured and the percentage inhibition of edema was calculated using the formula: anti-inflammatory activity (% inhibition) $= (V_c - V_t)/V_c \times 100$.

5.3. Analgesic activity

Experimental models used in this study were selected to investigate both centrally and peripherally mediated analgesic effects of the tested compounds. For this purpose; the acetic acid abdominal constriction method was used to elucidate the peripheral effect and the hot-plate test to reveal central activity of the tested compounds.

5.3.1. Writhing test

The abdominal constriction in mice (Writhing effect) was determined by acetic acid induced writhing method [14] in Swiss Albino mice (25–30 g) of either sex. A 1% aqueous acetic acid solution (i.p. injection in a volume of 0.1 mL) was used as writhing induced agent. In each group six 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 a dose of 20 mg/kg. The compounds, which exhibited good anti-inflammatory activity comparable to that of ibuprofen, 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. Ibuprofen 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 percentage inhibition.

$$\% \text{ Analgesic activity} = \{(n - n')/n\} \times 100$$

where, n = mean number of writhes of control group, n' = mean number of writhes of test group.

5.3.2. Hot-plate test

The experiment was carried out as described by Turner [15] using hot-plate apparatus, maintained at 53 ± 0.5 °C. Swiss Albino mice (25–30 g) of either sex in a group of six animals were used. The mice were divided and received the same doses of tested compounds and ibuprofen as mentioned before. The reaction time of the mice to the thermal stimulus was the time interval between placing the animal on the hot plate and when it licked its hind paw or jumped. Reaction time was measured prior to aqueous suspension of synthesized compounds and drug treatment (0 min) and 1 h after oral administration of the same.

5.4. Acute ulcerogenesis

Acute ulcerogenesis test was done according to Cioli et al. [16]. Albino rats (150–200 g) were divided into different groups consisting of six animals in each group. Ulcerogenic activity evaluated after *p.o.* administration of test compounds or ibuprofen at the dose of 60 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 with 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 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.

5.5. Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al. [18]. After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides and 10% of that tissue was homogenized at 10,000 rpm in 1.8 mL of 1.15% ice-cold KCl solution. 1 mL of suspension medium was taken from the supernatant, 0.5 mL of 30% trichloroacetic acid (TCA) followed by 0.5 mL of 0.8% thiobarbituric acid (TBA) reagent were added to it. The tubes were covered with aluminum foil and kept in a shaking water bath for 30 min at 80 °C. After 30 min tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 3000 rpm for 15 min. The absorbance of

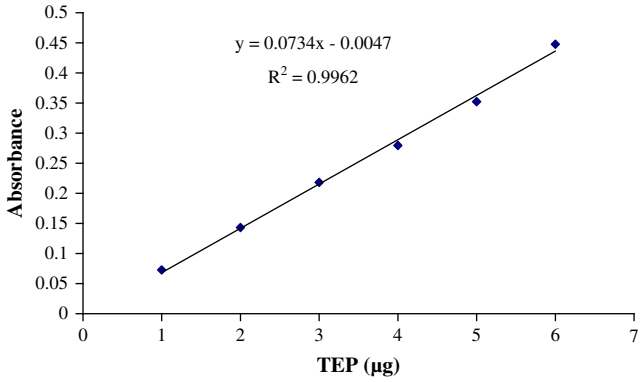


Fig. 1. Calibration standard curve for TBARS concentration.

supernatant was read at 540 nm at room temperature against the blank on UV spectrophotometer.

The standard curve (Fig. 1) was used for estimating the concentration of malondialdehyde (MDA) prepared by using 1,1,3,3, tetraethoxypropane. The results are presented as nM MDA/mg of protein.

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