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Original article

Synthesis of 6-aminomethyl derivatives of benzopyran-4-one with dual biological properties: Anti-inflammatory-analgesic and antimicrobial

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ABSTRACT

A series of 6-aminomethyl-2-aryl-1-benzopyran-4-one derivatives (**10–24**) were synthesized. The compounds were tested for anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation actions. Among the tested compounds, six compounds **11**, **13**, **16**, **18**, **21** and **23** showed higher degree of anti-inflammatory activity (>75% activity of standard drug ibuprofen). In addition to remarkable anti-inflammatory activity, analgesic activity was found to be comparable with that of the standard drug ibuprofen. Compounds **16** and **21** showed a significant GI protection (with respect to ulcerogenesis) and a marked decrease in lipid peroxidation values whereas compounds **11** and **16** were found to possess antimicrobial activity against *Staphylococcus aureus*, *Escherichiacoli*, *Rhizopus oryza* and *Penicillum citrum* with an MIC of 10 μg/mL.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been recognized as vital therapeutic agents for the alleviation of pain and inflammation associated with a numerous pathologic conditions *viz.* arthritis, bursitis, tendinitis. However, chronic administration of NSAIDs has been associated with clinically significant complications such as gastrointestinal (GI) symptoms including mucosal damage, bleeding, nausea, heartburn, dyspepsia, and abdominal pain; renal toxicity, etc. [1,2]. Polytherapy for inflammatory conditions associated with microbial infections increases the risk for developing NSAID-related complications especially in elderly, patients with prior history of peptic ulcer disease, patients with impaired liver or kidney functions and patients taking anticoagulants, corticosteroids, etc. concurrently. Hence, there is a pressing need for the drugs having both anti-inflammatory and antimicrobial activities, both from the pharmacoeconomic as well as the patient compliance point of view [3].

Substituted flavonoids are an important bioactive molecules possessing antiplatelet aggregation [4], antihypertensive [5,6], antifungal [7,8], anti-inflammatory [9,10], antitumor [11–13], antimalarial [14,15], anti-HIV [16], hypoglycemic [17,18], antibacterial [19], analgesic [20], antioxidant [21,22] properties. Although not fully understood, several cellular mechanisms have been proposed to explain the *in-vitro* and *in-vivo* anti-inflammatory activity of flavonoids [23]. One of the important mechanism is modulation of the expression of pro-inflammatory genes such as cyclooxygenase (COX), lipoxygenase (LOX), nitric oxide synthases and several pivotal cytokines, *via* transcription factors (nuclear factor-kappa B and mitogen-activated protein kinase signaling); and thus decreasing the levels of prostanoids and leukotrienes.

The activated inflammatory cells could be involved in the pathogenesis of mucosal damage. Gastric mucosal cells metabolise arachidonic acid *via* both the LOX and COX pathways [23] 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

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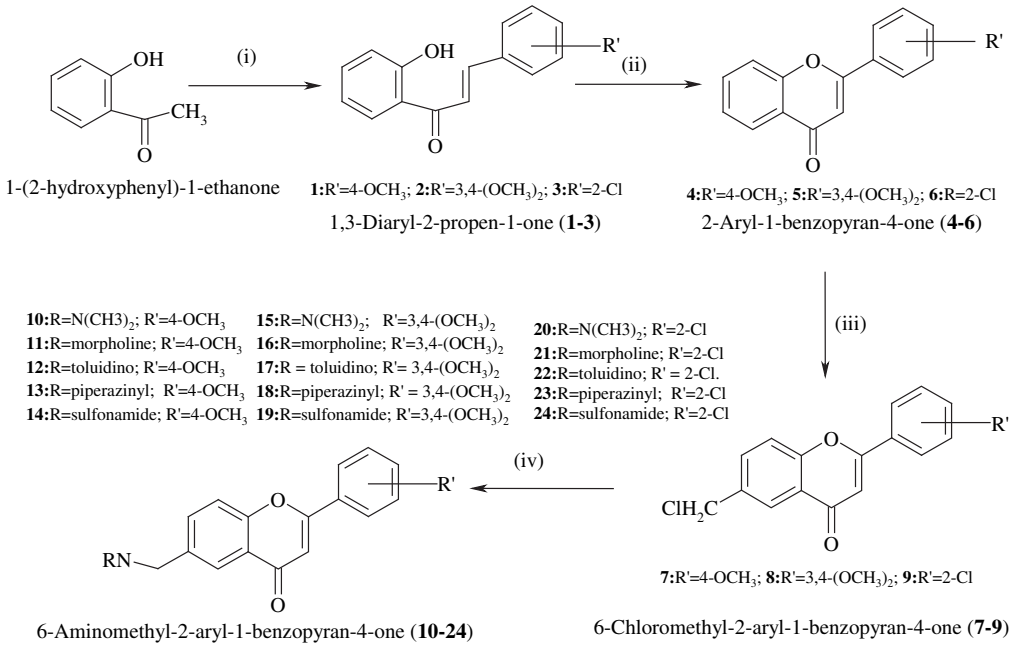


Reagents and reaction conditions: (i) ArCHO, KOH, room temperature; (ii) DMSO, I_2, reflux; (iii) HCl, CH_3COOH, HCHO, 0^0C; (iv) RNH_2, steam bath

Scheme 1.

mucosal injury. Flavonoid's antioxidant and free radical scavenging activity largely depends upon their molecular structure.

Compounds containing an amino group in the structure occupy a prominent position in medicinal chemistry due to a number of physiological actions; and its removal will lead to a virtual loss of their activity [24]. We therefore, envisaged incorporating an amino link attached to the flavone moiety thereby improving the known bioactions of flavonoids. Thus, the chloromethylation (at position-6) followed by substitution of the chlorine of the chloromethyl group by several amines was carried out. The synthesized derivatives were then tested for antimicrobial, anti-inflammatory and analgesic actions; for the GI protection from ulcerogenesis and for lipid peroxidation (LPO) effect. The effect of molecular variations on the anti-inflammatory and antimicrobial activities of flavonoid nucleus has also been reported.

2. Chemistry

The method utilized for the synthesis of 6-aminomethyl-2-aryl-1-benzopyran-4-one derivatives **10**–**24** has been outlined in Scheme 1. The arylketones and arylaldehydes were commercially available and the 1,3-diaryl-2-propen-1-one **1**–**3** and 2-aryl-1-benzopyran-4-one **4**–**6** were easily prepared by following the previously reported methods in high yield [25]. The obtained 2-aryl-1-benzopyran-4-one derivatives **4**–**6** were chloromethylated (replacement of a hydrogen atom by a chloromethyl group) by reaction with paraformaldehyde and hydrogen chloride in presence of acetic acid at position-6 to give 6-chloromethyl-2-aryl-1-benzopyran-4-one derivatives **7**–**9**. Chloromethylation is similar in some respect to that of Friedel Craft's reaction. The mechanism of chloromethylation is based upon an electrophilic attack on an aromatic ring to give the benzylic alcohol, which is then converted into the chloromethyl derivative by hydrogen chloride. The chloromethylated benzopyran-4-ones derivatives were further condensed with different amines to give 6-aminomethyl-2-aryl-1-benzopyran-4-one derivatives **10**–**24**.

The IR spectral data of all the compounds showed two peaks each around 3060 cm^{-1} and 1690 cm^{-1} indicating the presence of olefinic linkage and conjugated carbonyl group of chromone ring respectively. ^1H NMR and ^{13}C NMR spectral data of all the compounds showed characteristic peak at appropriate δ-values. Mass spectral data of 6-aminomethyl-2-aryl-1-benzopyran-4-one gave M^+ peak in reasonable intensities. The molecular ion or other related ions produced the appropriate isotopic abundances due to presence of chlorine atom(s). The analytical data has been mentioned in Table 1.

In order to support the assignments made through one-dimensional NMR, HMBC was recorded for compound-8 as representative and the noticed correlations are given in Table 2 which supports that the chloromethylation takes place at position-6 of the flavonoid ring.

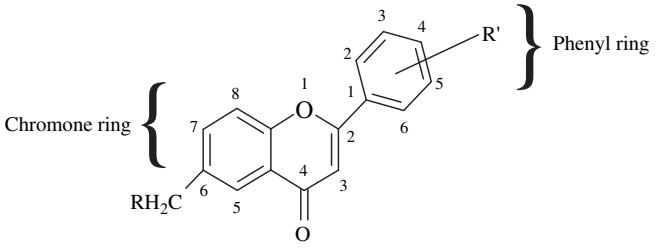
3. Pharmacological results and discussion

3.1. Anti-inflammatory activity

Compounds **7**–**24** were tested *in-vivo* for their anti-inflammatory properties by carrageenan-induced rat paw edema method [26]. All the compounds were administered orally (*p.o.*) and assayed at the dose level of 20 mg/kg of body weight. Ibuprofen was used as a standard drug for comparison. The results of the pharmacological evaluation are enlisted in Table 3. Six of the eighteen tested compounds exhibited statistically significant anti-inflammatory activity with respect to standard drug. The maximum activity was shown by the compounds bearing morpholine moiety as an amino linkage i.e. compounds **16** and **21** with 81.48% and 82.51% inhibition respectively. In addition, four compounds, **11**, **13**, **18** and **23** showed 75.30%, 69.13%, 72.63% and 73.86% inhibition respectively. From the above pharmacological results following remarks can be made.

- With compounds bearing morpholine linkage at 6-position of benzopyran-4-one the best result was shown by compound **21** with a chloro substitution at *ortho position* on the phenyl ring.

Table 1
Physical constants of 6-substitutedmethyl-2-aryl-1-benzopyran-4-one derivatives.



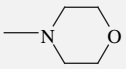
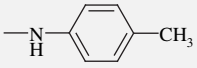
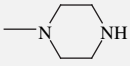
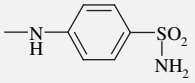
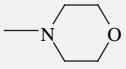
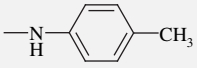
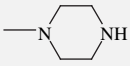
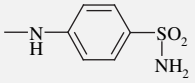
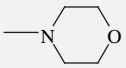
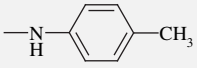
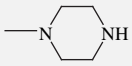
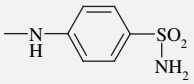
Comp. no.	R	R'	M.pt. (°C)	Yield (%)	R_f	Molecular formula
7	Cl	4-OCH_3	170	46	0.91	$C_{17}H_{13}O_3Cl$
8	Cl	$(OCH_3)_{3,4}$	171	52	0.70	$C_{18}H_{15}O_4Cl$
9	Cl	2-Cl	178	38	0.82	$C_{16}H_{10}O_2Cl_2$
10		4-OCH_3	156	44	0.68	$C_{19}H_{19}O_3N$
11		4-OCH_3	146	48	0.72	$C_{21}H_{21}O_4N$
12		4-OCH_3	136	46	0.68	$C_{24}H_{21}O_3N$
13		4-OCH_3	140	50	0.84	$C_{21}H_{22}O_3N_2$
14		4-OCH_3	160	52	0.92	$C_{23}H_{20}O_5N_2S$
15		$(OCH_3)_{3,4}$	166	42	0.88	$C_{20}H_{20}O_4N$
16		$(OCH_3)_{3,4}$	156	54	0.80	$C_{22}H_{23}O_5N$
17		$(OCH_3)_{3,4}$	150	38	0.86	$C_{25}H_{23}O_4N$
18		$(OCH_3)_{3,4}$	150	42	0.84	$C_{22}H_{24}O_4N_2$
19		$(OCH_3)_{3,4}$	178	46	0.87	$C_{24}H_{22}O_6N_2S$
20		2-Cl	156	48	0.75	$C_{18}H_{15}O_2NCl$
21		2-Cl	148	52	0.73	$C_{20}H_{18}O_3NCl$
22		2-Cl	162	50	0.87	$C_{23}H_{18}O_2NCl$

Table 1 (*continued*)

Comp. no.	R	R'	M.pt. (°C)	Yield (%)	R_f	Molecular formula
23		2-Cl	142	54	0.79	$C_{20}H_{19}O_2N_2Cl$
24		2-Cl	172	48	0.81	$C_{22}H_{17}O_4N_2SCl$

* R_f values for compounds **2**–**13** in solvent system (toluene:ethylacetate:formic acid, 5:4:1) and for compound 14–18 in solvent system (petrol:toluene:ethylacetate, 10:5:3); ** The micro analysis values for C, H and N were within ±4% of the theoretical values.

A *para* substitution on the phenyl ring with methoxy group (compound **11**) decreases activity whereas inhibition increases with an additional methoxy group on phenyl ring (3,4 dimethoxy) (compound **16**).

- Presence of piperazinyl moiety at 6-position of benzopyran-4-one showed good activity. Compound **23** with a chloro substitution at *ortho position* on phenyl ring showed 73.86% inhibition. Presence of methoxy group on the phenyl ring (compound **13**) decreases the activity to 68.93% which increases to 72.22% with an additional methoxy group on phenyl ring (compound **16**).
- The presence of sulfanilamide as amino linkage showed decrease in anti-inflammatory activity (58–60% inhibition) and the activity decreases further when the group is replaced by *p*-toluidine or dimethylamine.

Test compounds exhibiting significant anti-inflammatory activity **11**, **13**, **16**, **18**, **21** and **23** were evaluated for their analgesic, ulcerogenic and LPO actions.

3.2. Analgesic activity

The compounds that exhibited above 75% of anti-inflammatory activity of ibuprofen were evaluated for analgesic effects using acetic acid-induced writhing method [27].

The result of analgesic activity (Table 4) indicates that the compounds **16** and **21** showed 53.61% and 54.81% protection against acetic acid-induced writhing and were comparable to standard ibuprofen (65.06%). Compounds **11** and **23** also showed good analgesic activity (50.80% and 48.39%).

Structure–activity relationship showed that the morpholine and piperazinyl derivatives of benzopyran-4-one were good anti-inflammatory agents with analgesic activity equal to that of ibuprofen.

3.3. Acute ulcerogenesis

The compounds which were screened for analgesic activity were further tested for their acute ulcerogenic activity. Compounds **11**, **13**, **16**, **18**, **21** and **23** were tested according to the method reported by Cioli et al. [28]. The tested compounds showed low ulcerogenic activity ranging from 0.1 ± 0.1 to 0.4 ± 0.4 whereas the standard drug ibuprofen showed high severity index of 0.9 ± 0.36. The maximum reduction in ulcerogenic activity (0.1 ± 0.1) was found in the morpholine derivatives of benzopyran-4-one. The other tested compounds also exhibited better GI safety profile as compared to the standard drug ibuprofen. Results are tabulated in Table 4.

3.4. Lipid peroxidation (LPO)

LPO 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 often affects polyunsaturated fatty acids forming malondialdehyde (MDA).

The colorimetric reaction of thiobarbituric acid (TBA) with MDA, a secondary product of LPO has been widely adopted as a sensitive assay method for measuring LPO in animal tissues. It is used as an index for 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 Thiobarbituric acid reactive substance (TBARS) test [29].

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 LPO. The LPO was measured as nmoles of MDA/mg of protein using Ohkawa et al. method [30]. Ibuprofen exhibited high lipid peroxidation 0.608 ± 0.001 nmol MDA/mg of protein whereas control group showed 0.238 ± 0.002. It was found that all the aminomethyl derivatives of benzopyran-4-one showing less ulcerogenic activity also showed reduction in lipid peroxidation (Table 4). 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 gastric mucosa.

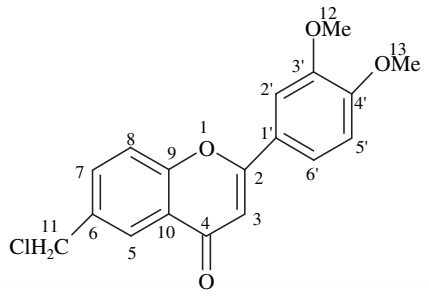
3.5. Antimicrobial activity

The compounds were evaluated for their antimicrobial activity using turbidity method [31] against *Staphylococcus aureus* (ATCC-29737) representing Gram-positive bacteria, *Escherichia coli* (ATCC-8739) representing Gram negative bacteria and *Rhizopus oryza* (NRRL-21498) and *Penicillum citrum* (NCIM-768) representing fungi. The results of antimicrobial effect of all the tested compounds were reported as minimal inhibitory concentrations (MICs, μg/mL), and are shown in Table 5. The results revealed that most of the newly synthesized compounds exhibited promising antifungal activities. Compounds **11** and **16** were most active against both bacterial and fungal strain with MIC of 10 μg/mL. However, compound **19** was active against fungal strain with MIC of 10 μg/mL. Compound **7** was active against *S. aureus* with MIC of 10 μg/mL. Compounds **14** and **15** were active against *R. oryza* with MIC of 10 μg/mL. Compound **18** was most active against *E. coli* with MIC of 10 μg/mL.

4. Conclusion

Eighteen new benzopyran-4-one derivatives were synthesized and screened for anti-inflammatory, analgesic, ulcerogenic, lipid peroxidation and antimicrobial activities. It is interesting to note that six aminomethyl derivatives of benzopyran-4-one were found to have anti-inflammatory properties comparable to standard drug ibuprofen. When these compounds were subjected to analgesic

Table 2
The data of ^{13}C and ^{1}H and HMBC correlations for compound-**8**.



Carbon number	^{13}C (δ)	^{1}H(δ)	HMBC correlation (from C to H)
2	162.4	–	C-2/H-2'; C-2/H-6'; C-2/H-3
3	105.6	7.04 (s, 1H)	–
4	177.2	–	C-4/H-3; C-4/H-5
5	107.5	8.28 (d, 1H)	C-5/H-7; C-5/H-11
6	141.4	–	C-6/H-7; C-6/H-5; C-6/H-11
7	123.7	7.70 (d, 1H)	C-7/H-5; C-7/H-8; C-7/H-11
8	119.6	7.49 (m, 1H)	C-8/H-7
9	155.5	–	C-9/H-5; C-9/H-7; C-9/H-8
10	124.2	–	C-10/H-3; C-10/H-5; C-10/H-8
11	65.5	4.66 (s, 2H)	C-11/H-5; C-11/H-7
12	55.3	3.99 (s, 3H)	C-12/H-2'
13	56.8	3.99 (s, 3H)	C-13/H-5'
1'	121.5	–	C-1'/H-3; C-1'/H-2'; C-1'/H-6'
2'	115.3	7.24 (d, 1H)	C-2'/H-6'
3'	148.5	–	C-3'/H-2'; C-3'/H-5'; C-3'/H-12
4'	149.3	–	C-4'/H-2'; C-4'/H-6'; C-4'/H-13
5'	110.9	7.49 (m, 1H)	C-5'/H-6'
6'	128.3	7.49 (m, 1H)	C-6'/H-2'; C-6'/H-5'

activity by acetic acid-induced writhing method in mice, all compounds exhibited moderate to good activity. These compounds were also showed superior GI safety profile along with reduction in lipid peroxidation as compared with ibuprofen.

Compounds **11** and **16** were most active against both bacterial and fungal strains with MIC of 10 μg/mL. However, compound **19** was active only against fungal strain with MIC of 10 μg/mL.

Among the newer derivatives, two compounds **11** and **16** emerged as lead compounds. It is conceivable that these derivatives could be further modified to develop potent and safer anti-inflammatory and analgesic agents with an additional antimicrobial activity. 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; bs, broad 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. Compounds **1**–**3** and **4**–**6** were synthesized according to reported literature procedures [25].

Table 3
Anti-inflammatory activity of the synthesized compounds **7**–**24**.

Compound	Paw volume ± SEM[a]		% Inhibition ± SEM[b]	
	After 2 h	After 3 h	After 2 h	After 3 h
Control	0.75 ± 0.01	0.81 ± 0.02	–	–
Ibuprofen	0.14 ± 0.01***	0.085 ± 0.02***	80.88 ± 2.02	89.50 ± 2.56
7	0.50 ± 0.03***	0.49 ± 0.04***	32.22 ± 5.04***	38.68 ± 5.85***
8	0.5 ± 0.02***	0.41 ± 0.02***	33.33 ± 3.15***	49.38 ± 2.68***
10	0.48 ± 0.02***	0.38 ± 0.03***	36 ± 3.31***	53.08 ± 3.96***
11	0.28 ± 0.02***	0.2 ± 0.01***	62.66 ± 3.24***	75.30 ± 2.01**
12	0.61 ± 0.02**	0.39 ± 0.02***	18.66 ± 3.83***	51.02 ± 2.75***
13	0.45 ± 0.02***	0.25 ± 0.01***	39.55 ± 3.27***	68.93 ± 1.75***
14	0.56 ± 0.03***	0.32 ± 0.02***	25.33 ± 4.35***	60.49 ± 2.29***
15	0.52 ± 0.02***	0.39 ± 0.03***	29.77 ± 3.34***	51.02 ± 4.10***
16	0.28 ± 0.00***	0.15 ± 0.00***	61.77 ± 1.17***	80.86 ± 1.04*
17	0.61 ± 0.03**	0.39 ± 0.01***	18.66 ± 4.10***	50.82 ± 1.36***
18	0.34 ± 0.01***	0.22 ± 0.01***	54 ± 2.37***	72.22 ± 1.82***
19	0.54 ± 0.02***	0.32 ± 0.02***	27.11 ± 3.99***	60.49 ± 2.99***
20	0.47 ± 0.01***	0.37 ± 0.03***	36.22 ± 2.58***	54.32 ± 4.01***
21	0.26 ± 0.01***	0.14 ± 0.00***	64.44 ± 1.36***	82.51 ± 1.07*
22	0.60 ± 0.02**	0.38 ± 0.01***	19.77 ± 3.38***	52.67 ± 1.30***
23	0.33 ± 0.02***	0.21 ± 0.01***	56 ± 2.92***	73.86 ± 1.87***
24	0.50 ± 0.02***	0.33 ± 0.02***	32.22 ± 2.78***	58.23 ± 2.84***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
[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 $n = 6$.

5.1.1. General procedure for the synthesis of 6-chloromethyl-2-aryl-1-benzopyran-4-one derivatives 7–9

2-Aryl-1-benzopyran-4-one derivatives (**4**–**6**) (0.01 mol) were dissolved in acetic acid (80%; 75 mL) and paraformaldehyde (0.04 mol; 1.2 g) was added. The solution was maintained at 90–95 °C and hydrogen chloride gas passed until a shining crystalline product separated (1.5 h). The reaction mixture was cooled when more products separated which was recrystallized from dioxane to give TLC pure 6-chloromethyl-2-aryl-1-benzopyran-4-one derivatives.

Table 4
Analgesic effects along with ulcerogenic and lipid peroxidation effect of some 6-substitutedmethyl-2-aryl-1-benzopyran-4-one derivatives.

Compound	Severity index[a]	Lipid peroxidation[c]	Peripheral analgesic activity (Writhing test) % protection
Control	0.00 ± 0.00	0.241 ± 0.007[b],***	
Ibuprofen	0.9 ± 0.36*	0.607 ± 0.008[a],***	65.06 ± 1.39
11	0.2 ± 0.12	0.526 ± 0.023[a,b],***	50.80 ± 2.14***
13	0.4 ± 0.4	0.599 ± 0.008[a,b],**	40.36 ± 1.19***
16	0.1 ± 0.1	0.331 ± 0.006[a,b],***	53.61 ± 0.92***
18	0.4 ± 0.29	0.525 ± 0.017[a,b],***	45.38 ± 1.01***
21	0.1 ± 0.1	0.352 ± 0.014[a,b],***	54.81 ± 0.92***
23	0.3 ± 0.12*	0.403 ± 0.005[a,b],***	48.39 ± 1.05***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
[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 5
Antibacterial and antifungal study; MIC results of 7–24.

Compounds	*Staphylococcus aureus* (ATCC-29737)	*Escherichia coli* (ATCC-8739)	*Rhizopus oryza* (NRRL-21498)	*Penicillium citrum* (NCIM-768)
Norfloxacin	6.25	6.25	–	–
Fluconazole	–	–	6.25	6.25
7	10	50	10	>100
8	10	50	10	>100
10	50	50	10	50
11	10	10	10	10
12	50	50	50	>100
13	50	50	50	>100
14	25	50	10	25
15	25	25	10	50
16	10	10	10	10
17	25	50	25	25
18	25	10	25	50
19	25	10	10	10
20	>100	50	>100	>100
21	50	>100	25	25
22	>100	>100	50	>100
23	>100	>100	50	50
24	50	50	>100	25

* MIC (μg/mL) = minimum inhibitory concentration, i.e., the lowest concentration to completely inhibit microbial growth.

*5.1.1.1. 6-Chloromethyl-2-(4-methoxyphenyl)-1-benzopyran-4-one (**7**).*

Yield: 46%, m.p. 170 °C. ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3H, OCH_3), 4.63 (s, 2H, CH_2), 7.06 and 7.84 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.42 (m, 2H, H-7,8, chromone ring), 7.69 (s, 1H, H-3, chromone ring), 8.28 (d, 1H, H-5, chromone ring). ^{13}C NMR ($CDCl_3$) δ 154.31 (C-2), 133.21 (C-3), 174.21 (C-4), 106.5 (C-5), 146.06 (C-6), 128.44 (C-7), 131.24 (C-8), 146.15 (C-9), 117.19 (C-10), 131.29 (C-1'), 127.4 (C-2',6'), 128.1 (C-3',5'), 131.27 (C-4'), 66.21 (CH_2). MS: *m*/*z* 300(M^+), 265. IR (cm^{-1}, KBr): 3060, 1690. Anal. Calcd. for $C_{17}H_{13}ClO_3$: C, 67.89; H, 4.36. Found: C, 67.68; H, 4.35.

*5.1.1.2. 6-Chloromethyl-2-(3,4-dimethoxyphenyl)-1-benzopyran-4-one (**8**).* Yield: 52%, m.p. 171 °C. ^{1}H NMR ($CDCl_3$) δ 3.99 (s, 6H, 2 × OCH_3), 4.66 (s, 2H, CH_2), 7.04 (s, 1H, H-3, chromone ring) 7.24 (d, 1H, H-2, phenyl) 7.49 (m, 3H, H-5,6, phenyl & H-8, chromone ring), 7.70 (d, 1H, H-7, chromone ring), 8.28 (d, 1H, H-5, chromone ring). MS: *m*/*z* 330(M^+), 295. IR (cm^{-1}, KBr): 3058, 1692. Anal. Calcd. for $C_{18}H_{15}ClO_4$: C, 65.36; H, 4.57. Found: C, 65.52; H, 4.59.

*5.1.1.3. 6-Chloromethyl-2-(2-chlorophenyl)-1-benzopyran-4-one (**9**).*

Yield: 38%, m.p. 178 °C. ^{1}H NMR ($CDCl_3$) δ. 4.20 (s, 2H, CH_2), 7.32 (t, 1H, H-4, phenyl), 7.52 (dd, 1H, H-6, phenyl), 7.72 (m, 3H, H-3,5, phenyl & H-7, chromone ring), 7.84 (m, 1H, H-8, chromone ring), 8.10 (s, 1H, H-3, chromone ring), 8.18 (d, 1H, H-5, chromone ring), MS: *m*/*z* 305(M^+), 270. IR (cm^{-1}, KBr): 3072, 1698. Anal. Calcd. for $C_{16}H_{10}Cl_2O_2$: C, 62.97; H, 3.30. Found: C, 62.78; H, 3.31.

*5.1.2. General procedure for the synthesis of 6-aminomethyl-2-aryl-1-benzopyran-4-one derivatives **10**–**24***

The appropriate amines (0.04 mol) were added to solution of compounds **7**–**9** (0.01 mol) in alcohol (75 mL) and the reaction mixture was heated on a steam bath for 6 h. The residue obtained on removal of alcohol was treated with dilute hydrochloric acid and filtered. The filtrate was neutralized with sodium-bi-carbonate solution and the product obtained was crystallized from alcohol.

*5.1.2.1. 6-Dimethylaminomethyl-2-(4-methoxyphenyl)-1-benzopyran-4-one (**10**).* Yield: 44%, m.p. 156 °C. ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 6H, 2 × NCH_3), 3.87 (s, 3H, OCH_3), 5.32 (s, 2H, CH_2), 7.23 and 7.62 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.51 (m, 2H, H-3,7, chromone ring), 7.67 (m, 1H, H-8, chromone ring), 8.23 (d, 1H, H-5, chromone ring). ^{13}C NMR ($CDCl_3$) δ 153.98 (C-2), 133.32 (C-3), 173.91 (C-4), 106.7 (C-5), 146.09 (C-6), 128.48 (C-7), 131.18 (C-8), 145.95 (C-9), 117.21 (C-10), 131.4 (C-1'), 128.1 (C-2',6'), 128.6 (C-3',5'), 131.57 (C-4'), 66.42 (CH_2), 29.2 (CH_3), 28.8 (CH_3). MS: *m*/*z* 308(M^+), 265. IR (cm^{-1}, KBr): 3062, 1692. Anal. Calcd. for $C_{19}H_{19}NO_3$: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.72; H, 6.17; N, 4.54.

*5.1.2.2. 6-Morphilinomethyl-2-(4-methoxyphenyl)-1-benzopyran-4-one (**11**).* Yield: 48%, m.p. 146 °C. ^{1}H NMR ($CDCl_3$) δ 2.58 (t, 4H, H-3,5, morpholine ring), 3.45 (s, 2H, CH_2), 3.72 (t, 4H, H-2,6, morpholine ring), 3.92 (s, 3H, OCH_3) 7.02 and 8.12 (d each, 2 × A_2B_2, *p*-substituted phenyl), 7.3 (m, 2H, H-3,8, chromone ring), 7.66 (d, 1H, H-7, chromone ring), 8.27 (d, 1H, H-5, chromone ring). MS: *m*/*z* 350(M^+), 265. IR (cm^{-1}, KBr): 3060, 1688. Anal. Calcd. for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.64; H, 6.04; N, 3.97.

*5.1.2.3. 2-(4-Methoxyphenyl)-6-(4-toluidinomethyl)-1-benzopyran-4-one (**12**).* Yield: 46%, m.p. 136 °C. ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3) 4.03 (s, 2H, CH_2), 6.42 and 6.87 (d, each, 2 × A_2B_2, *p*-disubstituted phenyl), 6.96 and 7.23 (d, each, 2 × A_2B_2, phenyl), 7.32 (m, 2H, H-3,7, chromone ring), 7.64 (m, 1H, H-8, chromone ring), 8.12 (d, 1H, H-5, chromone ring). MS: *m*/*z* 370 (M^+), 265. IR (cm^{-1}, KBr): 3192, 3050, 1672. Anal. Calcd. for $C_{24}H_{21}NO_3$: C, 77.61; H, 5.70; N, 3.77. Found: C, 77.82; H, 5.67; N, 3.78.

*5.1.2.4. 2-(4-Methoxyphenyl)-6-(4-piperazinomethyl)-1-benzopyran-4-one (**13**).* Yield: 50%, m.p. 140 °C. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 4H, H-3,5, piperazine ring), 2.65 (s, 4H, H-2,6, piperazine ring), 3.49 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 6.92 and 7.39 (d, each, 2 × A_2B_2, phenyl), 7.62 (m, 1H, H-7, chromone ring), 7.72 (m, 1H, H-8, chromone ring), 7.78 (s, 1H, H-3, chromone ring), 8.14 (d, 1H, H-5, chromone ring), 9.28 (bs, 1H, NH). **MS: *m*/*z* 349 (M^+), 265.** IR (cm^{-1}, KBr): 3360, 3058, 1676. Anal. Calcd. for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99. Found: C, 71.86; H, 6.34; N, 7.97.

*5.1.2.5. 4-{4-Oxo-2-(4-methoxyphenyl)-6-benzopyranylmethylamino}-1-benzene-sulfonamide (**14**).* Yield: 52%, m.p. 160 °C. ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3H, OCH_3), 4.21 (s, 2H, CH_2), 4.6 (s, 2H, NH_2), 6.69 and 7.78 (d, each, 2 × A_2B_2, *p*-disubstituted phenyl), 6.716 (m, 2H, H-3,8, chromone ring), 7.06 and 7.42 (d, each, 2 × A_2B_2, phenyl), 7.62 (d, 1H, H-7, chromone ring), 8.19 (d, 1H, H-5, chromone ring), 9.62 (bs, 1H, NH). **MS: *m*/*z* 436 (M^+), 265.** IR (cm^{-1}, KBr): 3220, 3190, 3100, 3068, 1680. Anal. Calcd. for $C_{23}H_{20}N_2O_5S$: C, 63.29; H, 4.62; N, 6.42. Found: C, 63.38; H, 4.64; N, 6.41.

*5.1.2.6. 6-Dimethylaminomethyl-2-(3,4-dimethoxyphenyl)-1-benzopyran-4-one (**15**).* Yield: 42%, m.p. 166 °C. ^{1}H NMR ($CDCl_3$) δ 2.61 (s, 6H, 2 × NCH_3) 3.98 (s, 6H, 2 × OCH_3), 5.34 (s, 2H, CH_2), 7.03 (d, 1H, H-5, phenyl), 7.44 (m, 4H, H-2,6, phenyl & H-3,7, chromone ring), 7.71 (m, 1H, H-8, chromone ring), 8.22 (d, 1H, H-5, chromone ring). **MS: *m*/*z* 338 (M^+), 295.** IR (cm^{-1}, KBr): 3064, 1696. Anal. Calcd. for $C_{20}H_{20}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.82; H, 6.25; N, 4.14.

*5.1.2.7. 2-(3,4-Dimethoxyphenyl)-6-morphilinomethyl-1-benzopyran-4-one (**16**).* Yield: 54%, m.p. 156 °C. ^{1}H NMR ($CDCl_3$) δ 2.61 (s, 4H, H-2,6, morpholine ring), 3.47 (s, 2H, CH_2), 3.70 (s, 4H, H-3,5, morpholine ring), 3.97 (s, 6H, 2 × OCH_3), 6.99 (d, 1H, H-5, phenyl), 7.41 (m, 2H, H-2, phenyl & H-7, chromone ring), 7.65 (m, 2H, H-6, phenyl & H-8, chromone ring), 7.92 (s, 1H, H-3, chromone ring), 8.23 (d, 1H, H-5, chromone ring). **MS: *m*/*z* 381 (M^+), 295.** IR (cm^{-1}, KBr): 3064, 1692. Anal. Calcd. for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 69.34; H, 6.06; N, 3.65.

*5.1.2.8. 2-(3,4-Dimethoxyphenyl)-6-(4-toluidinomethyl)-1-benzopyran-4-one (**17**).* Yield: 38%, m.p. 150 °C. ^{1}H NMR ($CDCl_3$) δ 2.21 (s,

3H, CH_3), 3.75 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.23 (s, 2H, CH_2), 6.45 and 6.91 (d, each, $2 \times A_2B_2$, *p*-disubstituted phenyl), 6.98 (d, 1H, H-5, phenyl), 7.38 (m, 4H, H-2,6, phenyl & H-3,7, chromone ring), 7.64 (m, 1H, H-8, chromone ring), 8.22 (d, 1H, H-5, chromone ring), 9.48 (bs, 1H, NH). **MS: *m/z* 401(M^+), 372, 295.** IR (cm^{-1}, KBr): 3196, 3056, 1680. Anal. Calcd. for $C_{25}H_{23}NO_4$: C, 74.79; H, 5.77; N, 3.49. Found: C, 74.84; H, 5.74; N, 3.48.

*5.1.2.9. 2-(3,4-Dimethoxyphenyl)-6-(4-piperazinomethyl)-1-benzopyran-4-one (**18**).* Yield: 42%, m.p. 150 °C. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 4H, H-3,5, piperazine ring), 2.65 (s, 4H, H-2,6, piperazine ring), 3.29 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 7.11 (d, 1H, H-5, phenyl), 7.46 (m, 1H, H-2, phenyl), 7.65 (m, 2H, H-6, phenyl & H-7, chromone ring), 7.78 (m, 1H, H-8, chromone ring), 8.02 (s, 1H, H-3, chromone ring), 8.05 (d, 1H, H-5, chromone ring), 9.42 (bs, 1H, NH). **MS: *m/z* 380(M^+), 295.** IR (cm^{-1}, KBr): 3364, 3062, 1678. Anal. Calcd. for $C_{22}H_{24}N_2O_4$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.58; H, 6.34; N, 7.37.

*5.1.2.10. 4-{4-Oxo-2-(3,4-dimethoxyphenyl)-6-benzopyranylmethylamino}-1-benzene-sulfonamide (**19**).* Yield: 46%, m.p. 178 °C. ^{1}H NMR ($CDCl_3$) δ 3.58 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2), 6.611 (d, 2H, H-2,6, sulphanilamide ring), 6.716 (s, 1H, H-3, chromone ring), 6.960 (s, 2H, NH_2), 7.13 (d, 1H, H-5, phenyl), 7.422 (m,5H, H-2, phenyl, H-3,5, sulphanilamide ring, H-5, chromone ring & NH), 7.707 (d, 1H, H-6, phenyl), 7.841 (m, 1H, H-7, chromone ring), 8.09 (d, 1H, H-8, chromone ring). **MS: *m/z* 466(M^+), 295.** IR (cm^{-1}, KBr): 3218, 3192, 3110, 3072, 1682. Anal. Calcd. for $C_{24}H_{22}N_2O_6S$: C, 61.79; H, 4.75; N, 6.00. Found: C, 61.88; H, 4.74; N, 6.01.

*5.1.2.11. 2-(2-Chlorophenyl)-6-dimethylaminomethyl-1-benzopyran-4-one (**20**).* Yield: 48%, m.p. 156 °C. ^{1}H NMR ($CDCl_3$) δ 2.62 (s, 6H, $2 \times NCH_3$), 5.42 (s, 2H, CH_2), 7.31 (t, 1H, H-4, phenyl), 7.46 (dd, 1H, H-6, phenyl), 7.59 (d, 2H, H-3,5, phenyl), 7.67 (m, 2H, H-3,7, chromone ring), 7.82 (m, 1H, H-8, chromone ring), 8.28 (d, 1H, H-5, chromone ring). **MS: *m/z* 313(M^+), 314(M + 1), 270.** IR (cm^{-1}, KBr): 3062, 1694. Anal. Calcd. for $C_{18}H_{15}NO_2Cl$: C, 68.90; H, 5.14; N, 4.46. Found: C, 68.72; H, 5.16; N, 4.44.

*5.1.2.12. 2-(2-Chlorophenyl)-6-morphilinomethyl-1-benzopyran-4-one (**21**).* Yield: 52%, m.p. 148 °C. ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 4H, H-2,6, morpholine ring), 3.72 (s, 4H, H-3,5, morpholine ring), 3.82 (s, 2H, CH_2), 7.41 (m, 3H, H-3,4, phenyl & H-7, chromone ring), 7.65 (m, 4H, H-5,6, phenyl & H-3,8, chromone ring), 8.08 (d, 1H, H-**5**, chromone ring). **MS: *m/z* 355(M^+), 356(M + 1), 270.** IR (cm^{-1}, KBr): 3058, 1690. Anal. Calcd. for $C_{20}H_{18}NO_3Cl$: C, 67.51; H, 5.10; N, 3.94. Found: C, 67.58; H, 5.13; N, 3.96.

*5.1.2.13. 2-(2-Chlorophenyl)-6-(4-toluidinomethyl)-1-benzopyran-4-one (**22**).* Yield: 50%, m.p. 162 °C. ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H, CH_3), 4.38 (s, 2H, CH_2), 6.74 and 6.86 (d, each, $2 \times A_2B_2$, *p*-disubstituted phenyl), 7.33 (t, 1H, H-4, phenyl), 7.46 (dd, 1H, H-6, phenyl), 7.62 (d, 2H, H-3,5, phenyl), 7.64 (m, 3H, H-3,7,8, chromone ring), 8.14 (d, 1H, H-5, chromone ring), 9.64 (bs, 1H, NH). **MS: *m/z* 375(M^+), 376(M + 1), 270.** IR (cm^{-1}, KBr): 3190, 3048, 1676. Anal. Calcd. for $C_{23}H_{18}NO_2Cl$: C, 73.50; H, 4.83; N, 3.73. Found: C, 73.62; H, 4.85; N, 3.71.

*5.1.2.14. 2-(2-Chlorophenyl)-6-(4-piperazinomethyl)-1-benzopyran-4-one (**23**).* Yield: 54%, m.p. 142 °C. ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 4H, H-3,5, piperazine ring), 2.75 (s, 4H, H-2,6, piperazine ring), 4.22 (s, 2H, CH_2), 7.34 (t, 1H, H-4, phenyl), 7.49 (dd, 1H, H-6, phenyl), 7.68 (m, 3H, H-3,5, phenyl & H-7, chromone ring), 7.82 (m, 1H, H-8, chromone ring), 8.02 (s, 1H, H-3, chromone ring), 8.14 (d, 1H, H-5, chromone ring), 9.82 (bs, 1H, NH). **MS: *m/z* 354(M^+), 355(M + 1), 270.** IR (cm^{-1}, KBr): 3358, 3060, 1680. Anal. Calcd. for $C_{20}H_{19}N_2O_2Cl$: C, 67.70; H, 5.40; N, 7.89. Found: C, 67.86; H, 5.41; N, 7.87.

*5.1.2.15. 4-{4-oxo-2-(2-chlorophenyl)-6-benzopyranylmethylamino}-1-benzene-sulfonamide (**24**).* Yield: 48%, m.p. 172 °C. ^{1}H NMR ($CDCl_3$) δ 4.12 (s, 2H, CH_2), 6.72 (d, 2H, H-2,6, sulphanilamide ring), 6.84 (s, 1H, H-3, chromone ring), 6.98 (s, 2H, NH_2), 7.65 (m,5H, H-4, phenyl, H-3,5, sulphanilamide ring & NH), 7.82 (m, 3H, H-3,5,6, phenyl), 7.96 (m, 1H, H-7, chromone ring), 8.18 (d, 1H, H-8, chromone ring). **MS: *m/z* 440(M^+), 441(M + 1), 270.** IR (cm^{-1}, KBr): 3222, 3190, 3110, 3070, 1684. Anal. Calcd. for $C_{22}H_{17}N_2O_4SCl$: C, 59.93; H, 3.89; N, 6.35. Found: C, 59.82; H, 3.90; N, 6.34.

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. [26]. 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) were measured and the percentage inhibition of edema was calculated using the formula:

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

5.3. Analgesic activity

Compounds which showed anti-inflammatory activity above 75% of ibuprofen were screened for analgesic activity. Analgesic activity was done by acetic acid induce writhing method [27].

Swiss albino mice (25–30 g) of either sex were divided into group of six in each. A 1% aqueous acetic acid solution (*i.p.* injection in a volume of 0.1 mL) was used as writhing induced agent. 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. Group I was taken as control and received CMC suspension only, group II received reference drug ibuprofen and rest of the groups were treated with test drugs (20 mg/kg) suspended in 1.0% CMC orally. 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.

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

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

5.4. Acute ulcerogenesis

Acute ulcerogenesis test was done according to Cioli et al. [28]. 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 (LPO)

LPO in the gastric mucosa was determined according to the method of Ohkawa et al. [30]. 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 aluminium 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 supernatant was read at 540 nm at room temperature against the blank on UV spectrophotometer.

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

5.6. Antibacterial activity

The newly prepared compounds were screened for their antibacterial activity against *E. coli* (ATCC-8739) and *S. aureus* (ATCC-29737) bacterial strains at a concentration of 100 μg/mL by turbidity method [31] using norfloxacin as standard. Antifungal activity of the compounds was determined against *R. oryza* (NRRL-21498) and *P. citrum* (NCIM-768) using fluconazole as standard. Compounds inhibiting growth of one or more of the above microorganisms were tested for minimum inhibitory concentration (MIC).

6. Statistical analysis

Data were expressed as the mean ± standard error (S.E.) of the means. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with post hoc analysis. The Turkey test was applied to identify significance among groups.

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