Design, Synthesis and Evaluation of Chalcone Derivatives as Anti-Inflammatory, Antioxidant and Antiulcer Agents

Alka N. Choudhary*, Arun Kumar and Vijay Juyal

Medicinal Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Bhimtal campus, Kumaun University, Bhimtal-263136, Nainital, Uttarakhand, India

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Abstract: In the present study, a series of chalcone derivatives were designed based on QSAR analysis. The designed compounds were synthesized by Claisen Schmidt condensation and evaluated for anti-inflammatory, antioxidant and antiulcer activities. The results of the best 2D & 3D QSAR models suggested that by introducing electron releasing groups at R_2 and introducing heteroatom with increasing bulkiness at R_4 in the benzylideneacetophenone nucleus will increase the activity. The structures of the compounds were established by IR, ^{1}H NMR and mass spectral analysis. All the compounds were evaluated for their anti-inflammatory (carrageenan-induced rat paw edema assay), antioxidant (inhibition of lipid peroxidation) and antiulcer activity (indomethacin-induced gastric damage). Of 10 compounds screened, compounds 1e and 1d exhibited promising anti-inflammatory activity with 68-70% inhibition at 100mg/kg , inhibition of lipid peroxidation with IC_{50} 2.47 & 3.1 µg/ml respectively. The Compounds 1e, 1j and 1d exhibited good gastro protective action as indicated by their low ulcer score. Overall, 1e was obtained as lead compound with promising anti-inflammatory, antioxidant and antiulcer activities.

Keywords: Antioxidants, Antiulcer, Chalcones, Inflammation, Rheumatoid Arthritis, NSAIDs.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) still remain among the most extensively used drugs world wide and have been used in the treatment of inflammatory conditions like rheumatoid arthritis, osteoarthritis, orthopedic injuries, postoperative pain, etc [1-2]. However, the use of conventional NSAIDs has been restricted due to their side effects especially gastric erosion and ulcers [3-5]. Thus, there is an urgent need for new targets that are required for the design and development of novel anti-inflammatory agents as an alternative to NSAIDs. Reactive oxygen species (ROS) in the form of super oxide anion ($O_2^{-\cdot}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide attack various biological macromolecules (proteins, enzyme, DNA, etc) under 'oxidative stress' conditions, giving rise to a number of inflammatory, metabolic disorders, cellular aging, reperfusion damage and cancer [6-7]. Interestingly a number of therapeutically useful NSAIDS have been shown to act by virtue of their free radical scavenging activity [8-10]. Antioxidants are the compounds that prevent oxidative damage induced by free radicals and reactive oxygen species. Thus, antioxidant therapy has also gained immense importance in the treatment of above-mentioned diseases [11].

Chalcones, or 1,3 –diaryl-2-propen-1-ones, belong to the flavanoid family [12]. Chemically they consist of open-chain flavanoids in which the two aromatic rings are joined by a three-carbon α, β unsaturated carbonyl system [13]. A vast number of naturally occurring chalcones are polyhydroxylated in the aryl rings. The radical quenching properties of the phenolic groups present in many chalcones have raised interest in using the compounds [14-15]. The aim of the present study is to design, synthesize & evaluate chalcone derivatives based on best QSAR model. A Quantitative structure–activity relationship (QSAR) enables the investigators to establish a reliable quantitative structure–activity and structure–property relationships to derive QSAR model [16] to predict the activity of novel molecules prior to their synthesis.

2. EXPERIMENTAL

2.1. QSAR Analysis

Nineteen compounds belonging to chalcone derivatives were taken from literature [17] (Table **1**). All the biological activity data had been converted to negative logarithmic mole dose (pIC50) for QSAR analysis. The correlations were sought between inhibitory activity and various substituents constants at position R_1, R_2, R_3& R_4 of molecule and indicator variable for the presence of methoxy at R_5 (I_V1) and presence of hydroxy group in the ring system at R_6 position (I_V2).

The value of substituent constants like hydrophobic (π), steric (molar refractivity MR), hydrogen acceptor (HA), and hydrogen donor (HD) and electronic (field effect F, resonance effect or R) and Hammett's constant (σ) were taken from literature for position R_1, R_2, R_3 & R_4. The series was also subjected to Molecular Modeling studies and Quantum mechanical calculations were performed using CS Chem. Office version 10.0 (Cambridge soften ware) running on a P-IV processor [18]. All molecules were built using Chemdraw Ultra ver 10.0 and subjected to energy minimization using Allinger's MM2 force field. The minimization is continued until the root mean square (RMS) gradient value reaches a value smaller than 0.1 kcal/molA°.

*Address correspondence to this author at the Medicinal Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Bhimtal campus, Kumaun University, Bhimtal-263136, Nainital, Uttarakhand, India; Tel: +919411313837; Fax: 05942-248307;
E-mail: alka_pharma@rediffmail.com

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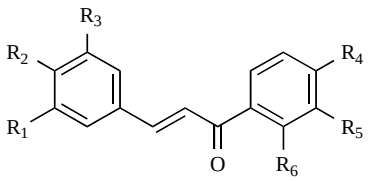
Table 1. Chalcone Derivatives and their Biological Activities



No	R_1	R_2	R_3	R_4	R_5	R_6	$pIC_{50}(BA_2)$
1	CH_3O	OH	H	H	H	H	-1.25
2	CH_3O	OH	H	CH_3	H	H	-1.09
3	CH_3O	OH	H	CH_3O	H	H	-1.26
4	CH_3O	OH	H	Cl	H	H	-0.85
5	CH_3O	OH	H	H	H	OH	-1.17
6	CH_3O	OH	CH_3O	CH_3	H	H	-0.107
7	CH_3O	OH	CH_3O	CH_3O	H	H	-0.704
8	CH_3O	OH	H	OH	CH_3O	H	-1.35
9	CH_3O	OH	H	F	H	H	-1.31
10	CH_3O	OH	CH_3O	F	H	H	-0.62
11	t-Bu	OH	t-Bu	F	H	H	-0.55
12	t-Bu	OH	t-Bu	OH	CH_3O	H	-0.29
13	CH_3O	OH	H	OH	H	H	-1.29
14	i-Pr	OH	i-Pr	F	H	H	-0.70
15	t-Bu	OH	t-Bu	CH_3	H	H	-0.55
16	t-Bu	OH	t-Bu	C_2H_5O	H	H	-0.26
17	t-Bu	OH	t-Bu	CH_3O	H	H	-0.29
18	t-Bu	OH	t-Bu	OH	H	H	-0.22
19	t-Bu	OH	t-Bu	Cl	H	H	-0.26

The Hamiltonian approximations Austin model -1(AM-1) method and RHF (restricted Hartee-Fork:closed shell) wave function was adopted for reoptimization until the root mean square (RMS) gradient attains a value smaller than 0.001 kcal/molA° by the use of GAMESS module. Different combinations of descriptors were subjected to sequential regression analysis employing VALSTAT software [19]. In stepwise multiple linear regression analysis [20] the independent variables are individually added or deleted from the model at each step of the regression depending on the Fischer ratio values selected to enter and to remove until the 'best' model is obtained.

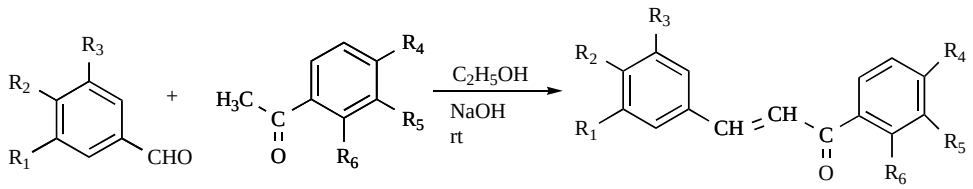
2.2. Synthesis & Characterization of Designed Compounds

The designed compounds, having higher predicted pIC_{50} value than observed pIC_{50} value, were synthesized.

a. General Method of Preparation of Chalcones

A mixture of benzaldehyde derivatives (0.01 mol) and acetophenone derivatives (0.01 mol) was dissolved in 10 ml rectified spirit in a 250 ml round-bottomed flask equipped with a magnetic stirrer. Then 10 ml sodium hydroxide solution (1g in 10ml H_2O) was added drop wise to the reaction mixture on vigorous stirring for 30 minutes when solution became turbid. The reaction temperature was maintained between 20-25˚ C using a cold water bath on the magnetic stirrer. After vigorous stirring for 4-5 hours the reaction mixture was neutralized by 0.1-0.2N HCl whereby the precipitation occurred. On filtering off, the crude chalcones were dried in air and recrystallized by rectified spirit [21]] (Scheme **1**).

The melting points were recorded in open capillaries with electrical melting point apparatus and were uncorrected. IR spectra (nujol) were recorded using Perkin-Elmer FTIR-RX_1 spectrophotometer. A ^{1}HNMR spectrum was recorded on Bruker Avance (400 MHz) spectrometer in DMSO solutions, with tetra methyl silane (TMS) as internal standard. Mass spectra were recorded on a Waters Q-T of micro MS. All the reagents and solvents used were of analytical grade and were used as supplied unless otherwise stated. Progress of the reactions was monitored using TLC, performed on aluminium plates precoated with silica gel-G, using chloroform: methanol (9:1) as the solvent systems and the spots were visualized by exposure to iodine vapors.



Scheme 1. Synthesis of Chalcone derivatives.

2.3. Pharmacological Evaluation

All the experiments were carried out using Wistar rats of either sex produced from IVRI, Bareilly, U.P. India. The animals were housed, 12 hr. light and 12 hr. dark cycle in the departmental animal house with free access to water and standard diet. All experiments were performed as per the norms of the ethical committee and the studies were approved and clearance obtained by the 'Institutional Review Board'.

a. Anti-Inflammatory Activity

Anti-inflammatory activity was measured using carrageenan-induced rat paw edema assay [22]. Groups of 6 rats of both sexes (pregnant females excluded) were given a three doses (25, 50,100 mg/kg, i.p.) of a test compound. After 1h, 0.1 mL of 1% carrageenan suspension in 0.9% NaCl solution was injected into sub planer tissue of the right hind paw. The linear paw circumference was measured at 3rd and 4th hours. The mean paw edema value for the test group was compared with mean value for the control group. Anti-inflammatory activity was measured as the percentage of reduction in edema level when drug was present, relative to control. Indomethacin (10mg/kg, i.p.) was administered as reference drug whereas 10% Tween 80 was used as negative control.

b. Determination of the Inhibition of Lipid Peroxidation

Rat liver was used as the source of polyunsaturated fatty acids for determining the extent of lipid per oxidation [23]. Liver was collected immediately after sacrificing animals by cervical dislocation under ether anesthesia. The liver was homogenized with 40mM Tris-HCl buffer (pH7) and centrifuged at 3000 rpm for 10 min to get a clear supernatant. Reaction mixture (4ml) containing 0.5 ml of supernatant (liver), 3.2 ml of synthesized chalcones (1a-1o) in different concentrations (0.1-25.6µg/ml and 100µL of each of 0.15 M KCl ,15mM $FeSO_4$ and 6mM ascorbic acid was incubated at 37˚C for 1hr.Trichloroacetic acid (TCA)(1ml,10%) was added to the reaction mixture and the sample was centrifuged at 3000 rpm for 20 min at 4˚C to remove insoluble proteins. Supernatant (1ml) was removed and 1ml Thiobarbituric acid (TBA) (0.8%) was added to this fraction followed by heating at 90˚C for 20 min in a water bath. After cooling the colored TBA-MDA complex extracted with organic solvent (2ml butanol), absorbance was measured at 532 nm. All the assays were carried out in duplicate. Percentage inhibition was calculated using the formula.

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/ A_{control}] \text{ X100.}$$

Where, $A_{control}$ is absorbance of the control reaction (containing all regents except test compounds) and A_{sample} is absorbance of test compound. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the samples and % inhibition of free radical formation

c. Antiulcer Activity

Anti-ulcer activity was measured using indomethacin induced gastric ulcer model [24]. The animals were starved for 24 h, groups of 6 rats of both sexes (pregnant females excluded) were given a dose of 100 mg/kg, i.p. of test compounds 30 min prior to indomethacin administration (48mg/kg). Four hours later, animals were sacrificed by cervical dislocation and stomach quickly removed, open out along the greater curvature, carefully cleaned out with a gentle stream of running distilled water and ulcers formed on the granular mucosa were counted. The percentage of inhibition of ulcers calculated using the formula.

$$\% \text{ inhibition of ulcers} = \text{C-T/C X 100}$$

2.4. Statistical Analysis

The results were subjected to statistical analysis by using Student's t-test comparing the control with treated group. Statistical significance was considered at $p<0.05$. The result values were expressed as mean ± S.E.M (standard error of mean).

3. RESULTS AND DISCUSSION

3.1. QSAR Analysis

The data set was subjected to stepwise multiple linear regression analysis, in order to develop 2D-QSAR between inhibitory activities as dependent variables and substituent constants as independent variable, The statistically significant equation [Eqn.1] with coefficient of correlation (r = 0.946) was obtained.

$$BA = 0.354(\pm 0.473)\ R_2 + 1.256(\pm 0.073)\ MR_4 - 1.023(\pm 0.543)\ \sigma p_2 - 3.865$$

$$n=19,\ r = 0.946,\ r^2 = 0.894,\ SE = 0.159,\ F = 40.365 \quad \text{(Eqn. 1)}$$

The model indicates that steric i.e., molar refractivity (MR) at R_4 contributed positively, while on R_2 position in parent moiety, electronic effect (R) contributed positively and Hammet constant (σp) contributed negatively to QSAR model. The study suggested that bulkier substitution at R_4 is favorable for the lipid peroxidation inhibitory action and introduction of electron releasing groups at R_2 position may prove to be helpful in development of more potent inhibitors.

The correlation between different physicochemical and topological descriptors as independent variable and

Table 2. Descriptors (3D) Contributing to the Lipid Peroxidation Inhibitory Activity

No	SD	MTI	CAA	CC	TOE	Ov
1	68	514.41	249.69	19	-16.6644	1.486
2	70	690.49	269.83	20	-12.7252	1.505
3	76	879.14	277.73	21	-11.8878	1.517
4	69	702.69	264.42	20	-16.6644	1.504
5	74	661.45	256.38	20	-10.4083	1.468
6	78	904.60	294.95	22	-19.3784	1.548
7	84	1107.5	303.77	23	-13.7152	1.554
8	82	971.00	281.91	22	-12.8624	1.493
9	76	711.00	255.14	20	-11.7541	1.482
10	84	907.99	279.47	22	-18.6602	1.53
11	84	1634.6	350.02	26	-11.55	1.529
12	90	1672.6	379.44	28	-13.7209	1.57
13	74	608.12	257.44	20	-16.6643	1.497
14	80	1288.2	327.4	24	-17.3392	1.549
15	78	1645.0	364.60	26	-12.86	1.551
16	86	1912.3	391.73	28	-11.6174	1.585
17	84	1783.0	372.63	27	-12.1718	1.562
18	82	1632.4	353.96	26	-11.9929	1.535
19	77	1728.2	360.61	26	-12.1419	1.542

inhibitory data as dependent variable was established *via* molecular modeling using 3D-QSAR. Statistical processing by stepwise regression method gave many QSAR equations. Only those parameters having intercorrelation below 0.6 and confidence interval limit >95% were considered to select the best model. The descriptors found in the best 3D QSAR equations of chalcone derivatives are summarized in Table **2**.

The best equations along with its statistical measures are given below.

BA_2 = 0.189(± 0.066) SD + 0.226(± 0.085) MTI + 0.001(± 0.0003) CAA -4.838

n=19, r=0.907, r^2=0.822, variance=0.034, SEE=0.191, F=23.202 (Eqn. 2)

BA_2= 1.567(± 0.538) CC + 0.220(± 0.088) TOE + 0.012(± 0.0006)Ov -5.265

n=19, r=0.905, r^2=0.819, variance=0.037, SEE=0.202, F=20.924 (Eqn. 3)

Eqns. 2 & 3 having nearly same variance i.e., 82% but eqn 2 having intercorrelation among the physicochemical descriptors is less than eqn 3 which is desired for robustness of the regression expression (Table **3** & **4**). The overall high

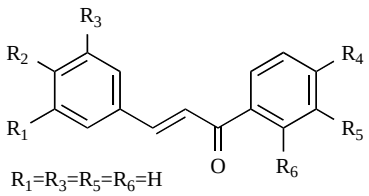
Table 3. Correlation Matrix for Equation-2

	SD	MTI	CAA
SD	1.000		
MTI	0.140	1.000	
CAA	0.237	0.067	1.0000

Table 4. Correlation Matrix for Equation-3

	CC	TOE	Ov
CC	1.000		
TOE	0.238	1.000	
Ov	0.669	0.486	1.000

Table 5. General Structure of Designed Compounds



S.No	R_2	R_4	Predicted pIC_{50} (μM)
1	$N(CH_3)_2$	Cl	1.680
2	$N(CH_3)_2$	Br	2.160
3	$N(CH_3)_2$	I	1.833
4	$N(CH_3)_2$	OCH_3	2.278
5	$N(CH_3)_2$	OC_2H_5	2.949
6	$N(CH_3)_2$	NH_2	0.686
7	OC_2H_5	Cl	1.506
8	OC_2H_5	Br	1.854
9	OC_2H_5	I	1.851
10	OC_2H_5	OCH_3	2.011
11	OC_2H_5	OC_2H_5	2.236
12	OC_2H_5	NH_2	0.663
13	OCH_3	NH_2	0.395
14	OH	Cl	0.463
15	OH	Br	0.569
16	OH	I	0.520
17	OH	OC_2H_5	0.718
18	OH	NH_2	0.567

value r-value (0.907) and low standard error of estimate prove (Eqn 2) to be the best model describing the activity.

The study revealed that topological descriptors i.e., molecular topological index (MTI), sum of valence degree (SD), steric descriptor i.e., connolly's solvent accessible area (CAA) parameters play a significant role in the model to explain the variance in activity. Molecular topological index contributed positively to the model, which suggests that increased activity can be achieved by introduction of the heteroatom and flexibility of substituent side chain, also supported by the positive contribution of sum of valence degree (SD) that illustrates that presence of heteroatom is favorable for the activity [25-26]. Connolly's accessible area, steric descriptors [27]. The descriptors bears positive coefficient in this equation, suggesting increase in the bulkiness of the substituents is favorable for the activity, also suggested by the 2D QSAR studies that steric effect is much important on R_4 position of the parent structure so increase of bulkiness at R_4 position may prove to be helpful in development of more potent inhibitors.

From the results of 2D and 3D QSAR analysis, it is concluded that by introducing electron releasing groups at R_2 and introducing heteroatom with increasing bulkiness at R_4 will increase the activity. Based on inference of 2D & 3D QSAR analyses, following compounds were designed and their activities were predicted with the help of best equations (Table **5**).

3.2. Synthesis & Characterization of Chalcone Derivatives

The designed compounds, having higher predicted pIC_{50} value than observed pIC_{50} value, were synthesized. The synthesis was based on Claisen Schmidt reaction, which is condensation reaction of substituted benzaldehyde with substituted acetophenones in the presence of sodium hydroxide and ethanol. The products were characterized by comparison of their spectral and physical data with those of authentic samples (Table **6** & **7**).

3.3. Biological Evaluation

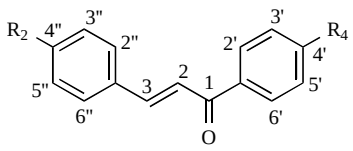
a. Anti-Inflammatory Activity

All the synthesized compounds were evaluated for anti-inflammatory activity by carrageenan-induced rat paw edema assay. The basis for the determination of anti-inflammatory activity at third and fourth hour is that with carrageen –induced rat paw model peak inflammatory

Table 6. The Chemical Profile of the Synthesized Compounds

Code	R_2	R_4	Molecular Formula	M.Wt	Yield %	R_f	mp(°C)
1a	$N(CH_3)_2$	Cl	$C_{17}H_{16}ClNO$	285.092	58	0.45	190-192
1b	$N(CH_3)_2$	Br	$C_{17}H_{16}BrNO$	329.219	55	0.41	220-222
1c	$N(CH_3)_2$	I	$C_{17}H_{16}INO$	377.211	53	0.39	221-223
1d	$N(CH_3)_2$	OCH_3	$C_{18}H_{19}NO_2$	281.141	18	0.52	195-197
1e	$N(CH_3)_2$	OC_2H_5	$C_{19}H_{21}NO_2$	295.370	16	0.51	207-209
1f	OC_2H_5	Cl	$C_{17}H_{15}ClO_2$	286.076	74	0.61	182-184
1g	OC_2H_5	Br	$C_{17}H_{15}BrO_2$	330.025	72	0.58	212-215
1h	OC_2H_5	I	$C_{17}H_{15}IO_2$	378.204	72	0.54	210-213
1i	OC_2H_5	OCH_3	$C_{18}H_{18}O_3$	282.125	68	0.74	186-188
1j	OC_2H_5	OC_2H_5	$C_{19}H_{20}O_3$	296.141	67	0.71	195-198

Table 7. IR, ^{1}HNMR and Mass Spectral Data of Synthesized Compounds

No.	IR(cm^{-1})	NMR (δ ppm) 	Mass (ESI)
1a	1647(>C=O in conjugation with C=C), 1590,1552 (>C=C< in conjugation with C=O), 1527,1460 (C=C aromatic stretching), 725, 1227 (C-Cl & C-N stretching)	7.86(dd, J=8Hz, J=16Hz 2H, Ar 2',H 6'H), 7.76(d, J=16Hz, $1H_3$, =CH), 7.51 (d, J=12Hz 2H, Ar 2",6"-H), 7.42(d, J=8Hz, 2H, Ar 3', 5'H), 7.25(d, J=16Hz, $1H_2$,=CH), 6.66 (dd, J=8Hz, J= 12HZ, 2H, Ar 3", 5"-H), 3.04(s,6H,-N($C\underline{H}_3$)$_2$)	*m/z*(relative intensity%): 286$[M+1]^+$(54)
1b	1647(>C=O in conjugation with C=C),1588,1551 (>C=C< in conjugation with C=O), 1528,1460 (C=C aromatic stretching), 665,1226 (C-Br & C-N stretching)	7.85 (dd, J=8Hz, J=4Hz, 2H, Ar 2',6'H), 7.77(d, J=12Hz, $1H_3$, =CH), 7.60(dd, J= 8Hz, J=4Hz, 2H, Ar 3', 5'H), 7.52 (d, J=8Hz, 2H, Ar 2",6"-H),7.24(d, J=16Hz, $1H_2$, =CH), 6.67 (d, J=8Hz, 2H, Ar 3", 5"- H), 3.04(s,6H,-N($C\underline{H}_3$)$_2$	*m/z*(relative intensity%): 352$[M+Na]^+$(74), 330$[M+1]^+$ (20)
1c	1648 (>C=O in conjugation with C=C), 1576,1534(>C=C< in conjugation with C=O), 1508,1449(C=C aromatic stretching), 565,1218(C-I & C-N stretching)	7.82 (d, J=12Hz, 2H, Ar 2',6'H), 7.71 (d, J=16Hz, $1H_3$, =CH), 7.66(d, J=8Hz, 2H, Ar 3', 5'H), 7.54 (d, J=12Hz, 2H, Ar 2",6"-H), 7.30(d,J=16Hz, $1H_2$,=CH), 6.69 (d,J=8Hz, 2H, Ar 3", 5"- H) 3.06(s,6H,-N($C\underline{H}_3$)$_2$)	*m/z*(relative intensity%): 378$[M+1]^+$ (86)
1d	1642 (>C=O in conjugation with C=C), 1567,1556 (>C=C< in conjugation with C=O), 1529,1457 (C=C aromatic stretching), 1257& 1064 (C-O asymmetric & symmetric stretching)	7.86 (dd, J=4Hz, J=4Hz, J=4Hz, 2H, Ar 2',6'H), 7.80(d, J=16Hz, $1H_3$, =CH), 7.61(dd, J=4Hz, J=8Hz, 2H, Ar 3', 5'H), 7.58 (d, J=4Hz,2H,Ar2",6"H),7.36(d, J=16Hz,$1H_2$,=CH), 6.93 (dd, J=4Hz, J=8Hz, 2H, Ar 3", 5"- H),3.84(s,3H,-O$C\underline{H}_3$), 3.04(s,6H,-N($C\underline{H}_3$)$_2$	*m/z*(relative intensity%): 304[281+Na]$^+$(100)
1e	1640 (>C=O in conjugation with C=C), 1584,1566 (>C=C< in conjugation with C=O) 1510,1461(C=C aromatic stretching) 1267 & 1070 (C-O asymmetric & symmetric stretching)	7.85 (dd, J=4Hz, J=8Hz,2H, Ar 2',6'H),7.76(d, J=16Hz, $1H_3$, =CH), 7.61(dd, J=4Hz, J=8Hz, 2H, Ar 3', 5'H), 7.57 (d, J=8Hz, 2H, Ar 2",6"-H), 7.36(d, J=16Hz, $1H_2$,=CH), 6.90 (d, J=8Hz, 2H, Ar 3", 5"- H), 1.41-1.45(t, 3H, -$C\underline{H}_3$), 4.05-4.10 (q, 2H, -$C\underline{H}_2$), 3.06(s,6H,-N($C\underline{H}_3$)$_2$)	*m/z*(relative intensity%): 296$[M+1]^+$ (100) 318 [295 +Na]$^+$ (82)
1f	1658 (>C=O in conjugation with C=C),1602(>C=C< in conjugation with C=O), 1510,1459(C=C aromatic stretching),721(C-Cl stretching), 1269 & 1036 (C-O asymmetric & symmetric stretching)	7.93 (dd, J=4Hz, J=8Hz, 2H, Ar 2',6'H), 7.75(d, J=16Hz, $1H_3$, =CH), 7.56 (d, J=4Hz, 2H, Ar 2",6"-H),7.43(dd, J=4Hz, J=8Hz 2H, Ar 3', 5'H), 7.36(d, J=16Hz,$1H_2$,=CH), 6.89 (dd, J=4Hz, J=8Hz 2H, Ar 3", 5"- H),1.40-1.44(t, 3H, -$C\underline{H}_3$), 4.03-4.08 (q, 2H, -$C\underline{H}_2$)	*m/z*(relative intensity%): 287$[M+1]^+$ (65)
1g	1657 (>C=O in conjugation with C=C), 1584,1566(>C=C< in conjugation with C=O), 665(C-Br stretching), 1267 & 1070 (C-O asymmetric & symmetric stretching) 1510,1461 (C=C aromatic stretching)	7.85 (dd, J=4Hz, J=8Hz,2H, Ar 2',6'H),7.76(d, J=16Hz, $1H_3$, =CH), 7.61(dd, J=4Hz, J=8Hz, 2H, Ar 3', 5'H), 7.57 (d, J=8Hz, 2H, Ar 2",6"-H), 7.36(d, J=16Hz, $1H_2$,=CH), 6.90 (d, J=8Hz, 2H, Ar 3", 5"- H), 1.41-1.45(t, 3H, -$C\underline{H}_3$), 4.05-4.10 (q, 2H, -$C\underline{H}_2$)	*m/z*(relative intensity%): 331$[M+1]^+$(24)

(Table 7). Contd…..

1h	1653 (>C=O in conjugation with C=C), 1591,1459 (>C=C< in conjugation with C=O, C=C aromatic stretching), 1254 & 1026 (C-O asymmetric & symmetric stretching), 461 (C-I stretching)	7.88 (d,J=8Hz, 2H, Ar 2',6'H), 7.77 (d, J=8Hz, 2H, Ar 2",6"-H), 7.71(d, J=12Hz, 1H_3, =CH),7.64(d, 2H, Ar 3', 5'H), 7.45(d, J=16Hz, 1H_2,=CH), 6.91 (d,J=12Hz, 2H, Ar 3", 5"- H), 1.42-1.46(t, 3H, -CH_3), 4.03-4.08 (q, 2H, CH_2)	*m/z*(relative intensity%): 379$[M+1]^+$(41)
1i	1654(>C=O in conjugation with C=C), 1596,1571(>C=C< in conjugation with C=O), 1507,1461(C=C aromatic stretching), 1256 & 1075 (C-O asymmetric & symmetric stretching)	8.00 (dd, J=4Hz, J=8Hz, 2H, Ar 2',6'H), 7.74 (d, J=16Hz, 1H_3, =CH), 7.56 (d, J=8Hz, 2H, Ar 2",6"-H), 7.39(d, J=16Hz,1H_2, =CH), 6.94 (dd,J=4Hz, J=8Hz, 2H, Ar 3', 5'H), 6.89 (d, J=4Hz, 2H, Ar3" 5"- H), 1.40-1.43(t, 3H, -CH_3), 4.02-4.07 (q, 2H, -CH_2); 3.85(s,3H,-OCH_3)	*m/z*(relative intensity%): 305$[M+Na]^+$(100), 283$[M+1]^+$(17)
1j	1640 (>C=O in conjugation with C=C), 1596,1570(>C=C< in conjugation with C=O), 1510,1458(C=C aromatic stretching) 1253 & 1045 (C-O asymmetric & symmetric stretching)	8.00 (dd, J=4Hz, J=8Hz, 2H, Ar 2',6'H), 7.75(d, J= 12Hz, 1H_3, =CH), 7.59 (d, 2H, Ar 2",6"-H), 7.40(d, J=16Hz, 1H_2,=CH), 6.93 (d, J= 4Hz, 2H, Ar 3', 5'H), 6.89 (d, J=4Hz, 2H, Ar 3", 5"- H); 1.40-1.45(m, 6H, -CH_3), 4.03-4.12 (m, 4H, -CH_2),	*m/z*(relative intensity%): 297$[M+1]^+$(15), 319$[M+Na]^+$(100), 615$[2M+Na]^+$ (22)

Table 8. Anti-Inflammatory Activity of the Synthesized Compounds

Compound	Dose mg/kg, i.p	Change in paw edema mean±SEM in mm		% Activity	
		3rd hour	4th hour	3rd hour	4th hour
1a	25	5.15±1.93	5.19±2.23	9.3	8.6
	50	4.62±1.72	4.70±1.89	18.6	17.2
	100	3.95±2.56[a]	2.85±1.89[a]	30.4	49.8
1b	25	4.68±1.32	3.51±1.43[a]	17.6	38.2
	50	4.39±2.15	2.92±1.67[a]	22.7	48.5
	100	3.78±2.13[a]	2.29±1.36[b]	33.4	59.8
1c	25	4.97±1.78	4.01±1.89[a]	12.5	29.4
	50	4.53±1.67	3.62±2.03[a]	20.2	36.2
	100	3.85±2.08[a]	2.85±1.48[b]	32.2	57.5
1d	25	4.29±1.45[a]	3.34±1.98[a]	24.4	41.1
	50	3.40±1.83[a]	2.67±2.34[b]	40.1	52.9
	100	2.76±2.34[a]	1.80±2.61[b]	51.4	68.3
1e	25	4.11±2.40[a]	3.37±1.56[a]	27.6	40.6
	50	3.12±1.37[a]	2.57±1.73[b]	45.0	54.7
	100	2.64±2.41	1.72±2.53	53.5	69.7
1f	25	5.23±1.32	5.27±1.34	7.8	7.2
	50	4.79±1.63	4.85±1.90	15.6	14.6
	100	3.98±2.04[a]	2.90±1.89[a]	29.9	48.9
1g	25	4.98±2.38	4.15±2.00[a]	12.2	26.9
	50	4.52±1.42[a]	3.66±2.13[a]	20.3	35.5
	100	3.86±2.44[a]	2.67±1.45[a]	32.0	52.9
1h	25	5.14±1.57	4.38±1.43[a]	9.5	22.8
	50	4.64±2.31[a]	4.64±1.85[a]	18.2	33.8
	100	3.90±2.34[a]	2.81±1.38[a]	31.3	50.5
1i	25	4.78±1.63	3.57±2.07[a]	15.8	37.1
	50	4.47±1.61[a]	2.99±1.32[a]	21.3	47.5
	100	3.79±2.03[a]	2.21±1.52[b]	33.2	61.8
1j	25	4.57±2.43	3.47±2.04[a]	19.5	38.9
	50	3.45±1.31[a]	2.81±2.34[a]	39.2	50.5
	100	2.89±2.49[a]	2.34±2.73[b]	49.1	58.8
Control		5.68±1.21			
Indomethacin	10	2.02±1.42[b]	2.13±2.11[b]	64.4	62.5

n = 6, Values are mean ± S.E.M., [a] =p<0.05, [b]= p<0.001, significantly different from control.

response at 3-5 h. From the results obtained (summarized in Table **8**), all the synthesized compounds show a dose dependent inhibition of edema, with an increase in activity from 25 mg/kg to 50 mg/kg and to 100mg/kg doses.

At 25 and 50 doses all the compounds except 1a and 1f, showed increase in activity from third hour to fourth hour. At 100 mg/kg, 1d and 1e at the fourth hour showed greater activity in comparison with the reference drug. However, the anti-inflammatory activity of the reference drug (indomethacin) was reduced during the same time. This may probably due to difference in the mechanism of action of these compounds. It can be deduced from the above that the synthesized compounds have antioxidant properties (like other flavanoids).

This is evident in the increase in activities at the fourth hour in comparison with indomethacin, which had reduced activity at this time; indomethacin is, however, a well-known non-specific COX enzyme inhibitor. This result points to the conclusion that the compounds synthesized may not act in the same manner as indomethacin. The anti-inflammatory response of synthesized chalcones may be by scavenging of OH or super oxide radicals, and interrupting ROS mediated signaling pathways such as inhibiting lipid per oxidation, inhibit the activation of NF-κB[28].

b. In Vivo Antioxidant Activity (Inhibition of Lipid Peroxidation)

All the synthesized compounds were evaluated for antioxidant activity by ferrous sulphate induced lipid peroxidation. It is based on the inhibition of lipid peroxidation, provides a measure of how efficiently antioxidants protect against lipid peroxidation.

From the results obtained, all compounds effectively inhibit lipid peroxidation in a concentration dependent manner measured in terms of pink colored TBA-MDA complex (Table **9**).

The experimental data concerning the effect on lipid peroxidation of synthesized compounds was related to their chemical structures designed by best QSAR model. Two dimethylamino chalcone (1e &1d), predicted as active by best QSAR model, together with 1f predicted to be low activity, was confirmed by *in vitro* inhibition of lipid peroxidation assay method. Compounds 1e & 1d exhibited the highest lipid peroxidation inhibitory activity (IC_{50} 2.47μg/ml,3.1 μg/ml), where as compound 1f having lowest activity (IC_{50} 17.6 μg/ml). For these chalcones (compounds 1d, 1e, 1i and 1j), presence of electron donating groups on p-position of both rings A and B seems to enhance activity, whereas the presence of electron donating groups on p-position of rings A and electron withdrawing groups on p-position of ring B leads to compounds 1a.1b, 1c,1f,1g, and1h with good to moderate activity.

The compound 1e and 1d exhibited the highest activity (IC_{50} 2.47μg/ml,3.1 μg/ml). The lipid per oxidation inhibitory activity of the compound is related with their electron donating ability to a lipid radical and converting it to a non-radical form. These reducing compounds can terminate radical chain reactions and reduce hydro peroxides and epoxides to less reactive derivatives. In lipid per oxidation assay, malondialdehyde (MDA) is formed during the oxidative degeneration as a product of free radicals, which is accepted as an indicator of lipid per oxidation [29]. The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. The synthesized chalcones caused a reduction in cell membrane damage by decreasing the MDA production. They showed a concentration dependent inhibition of the lipid per oxidation. The results obtained in the present study may be attributed to several reasons like inhibition of ferryl-perferryl complex formation, scavenging of OH or super oxide radicals or by the changing the ratio of Fe^{3+}/Fe^{2+}, reducing the rate of conversion of ferrous to ferric or by chelation of iron itself[30].

c. Antiulcer Activity

All compounds were evaluated for their gastro protective properties in the rats by indomethacin-induced gastric damage. Gastro protective activity was measured in terms of % anti-ulcer activity as represented in Table **10**, in which all compounds at the tested dose level exhibited varying degree of activity against ulceration induced by indomethacin.

Table 9. Effect of Synthesized Compounds on Lipid Peroxidation Inhibition

S N	Quantity in micrograms (μg/ml), Mean ± S.E.M.									IC_{50}(μg/ml)	
	0.1	0.2	0.4	0.8	1.6	3.2	6.4	12.8	25.6	*Ex	*Pr
1a	0.3±0.35	1.08±1.3	1.7±0.82	4.5±1.8	6.1±1.7	13.5±1.9	27.2±1.3	53±1.8	-	12.02	5.95
1b	0.7±0.14	1.5±0.78	2.2±1.3	6.7±1.3	16.2±2.4	34±2.9	51±2.1	-	-	5.70	2.27
1c	0.2±0.47	1.06±0.94	1.5±1.5	4.3±1.5	5.8±2.6	12.7±1.6	26.8±2.1	54.3±2.4	-	11.84	5.71
1d	1.2±0.14	2.7±1.76	4.1±2.3	18±3.2	39.2±3.7	73.0±3.2	85.2±2.9	-	-	3.1	1.46
1e	1.7±0.45	4.2±2.3	9.0±2.4	25.1±2.1	58.0±1.9	88.1±4.7	93.2±2.6	-	-	2.47	0.32
1f	0.1±0.31	0.9±1.7	1.2±1.3	4.2±1.6	5.6±2.4	12.1±1.7	26.5±3.2	48.3±2.6	64.1±3.5	17.6	9.86
1g	0.5±0.36	1.3±0.73	2.2±0.85	6.5±0.6	15.9±2.7	32±2.4	51.6±2.3	-	-	5.9	4.62
1h	0.47±1.3	1.25±1.22	2.23±2.1	6.2±1.4	11±1.8	21.1±1.2	45.3±1.5	64.0±2.7	-	9.14	5.29
1i	0.8±0.43	1.6±0.43	2.4±2.1	7.1±2.2	17.2±0.9	39.0±3.2	64.0±1.8	-	-	4.8	2.82
1j	0.8±0.58	2.4±1.1	3.7±1.32	9.4±1.3	22.5±2.3	48.1±2.1	79.0±1.9	-	-	3.87	1.78

*Ex = Experimental value, *Pr = Predicted value.

Table 10. The Gastroprotective Activity of the Test Compounds

Compounds	Doses (mg/kg, p.o.)	No. of ulcer spots counted	% Anti-ulcer activity
1a	100	8.20±0.36[a]	67.1
1b	100	11.30±0.47[b]	54.6
1c	100	16.40±0.39[b]	34.1
1d	100	6.10±0.87[a]	75.5
1e	100	5.10±0.74[a]	79.5
1f	100	10.30±0.38[a]	58.6
1g	100	12.30±0.45[b]	50.6
1h	100	17.00±0.31[b]	31.7
1i	100	9.50±0.63[a]	61.8
1j	100	5.70±0.92[a]	77.1
Indomethacin	50	24.90±0.63	

n = 6, Values are mean ± S.E.M., [a] =p<0.05, [b]= p<0.001, significantly different from control.

The Compounds 1e, 1j & 1d showed excellent activity (72-79%), whereas compounds1a &1i exhibited good to moderate (61-69%) activity. In addition, compounds 1h and 1c showed very less gastro protective action as indicated by their high ulcer score.

The synthesized chalcones caused a reduction in gastric damage. The result obtained in the present study may be attributed to their antioxidant activity through scavenging of ROS and protection of GPO [31].

CONCLUSION

In summary we have designed, synthesized and evaluated novel chalcones for their biological studies. 4-Dimethylamino-4'-ethoxychalcone (1e) and 4-Dimethylamino-4'-methoxychalcone (1d) have increasing anti-inflammatory activity than reference drug. Moreover, the same compounds also obtained promising antioxidant and antiulcer activities. The results of this study may find a lead (1e) towards the development of new therapeutic agent against inflammation.

CONFLICT OF INTEREST

Declared none.

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