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Synthesis, SAR and Biological Evaluation of Chalcone Derivatives as Antiulcer Agents

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ABSTRACT

Non-steroidal Anti-Inflammatory Drugs (NSAIDs) are a group of often chemically unrelated compounds with some common therapeutic actions and side effects. However, the use of conventional NSAIDs has been restricted due to their side effects especially gastric erosion and ulcer so; the attention is focused on chalcones having gastric protectant and anti-inflammatory, activities by virtue of free radical scavengers. In the present study, alkoxy chalcones were synthesized by Claisen–Schmidt condensation reaction and evaluated for an antiulcer activity in the rats by indomethacin-induced gastric damage. The structures of the compounds were established by IR, ^{1}H NMR and Mass spectral analysis. Possible correlation between observed biological activities and substituents at different positions on rings was also studied. The spectral analysis reveals that all the compounds 1a-1j was in good agreement with the standard values reported in the literature for these types of structures. Most of the synthesized compounds have shown good to moderate activity. Compounds with electron releasing groups (1i, 1j & 1g) at para position of both rings (R_1 & R_2) showed excellent antiulcer activity whereas compounds having pharmacophore such as iodo & bromo electron withdrawing groups (1b & 1h) at R_2 position has mild action. The result obtained in the present study may be attributed to their antioxidant activity through scavenging of ROS, protection of glutathione peroxidase.

Keywords: Antiulcer, Chalcone, Substituted benzylidene acetophenone

INTRODUCTION

Non-steroidal Anti-inflammatory Drugs (NSAID's) are widely used in the treatment of pain and inflammation. NSAID's reduce the pain and swelling associated with arthritis by blocking the metabolism of Arachidonic Acid (AA) through the enzyme Cyclooxygenase (COX) and thereby the production of prostaglandins and leukotrienes [1-3]. However, the use of conventional NSAIDs has been restricted due to their side effects especially gastric erosion and ulcer [4] so; the attention is focused on chalcones having gastric protectant and antiinflammatory, activities by virtue of free radical scavengers [5,6]. Chalcones, or 1,3-diaryl-2-propen-1-ones, belong to the flavanoid family [7]. Chemically they consist of open-chain flavanoids in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system [8]. 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 [9,10]. Chalcones have been reported to possess many useful properties, including anti-inflammatory [11], antiulcer [12], antioxidant [13], antimicrobial [14], antitumor and anticancer [15] activities.

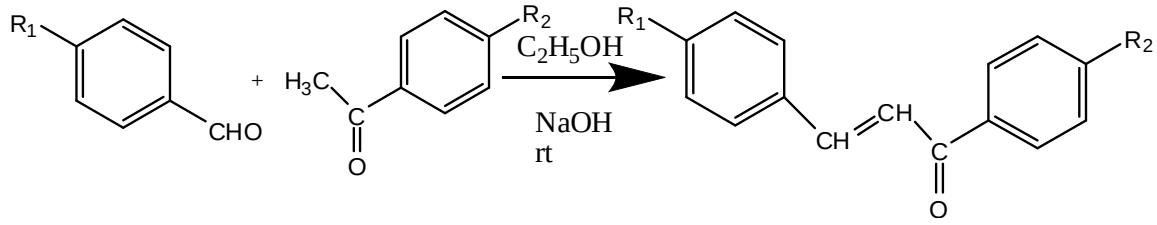
EXPERIMENTAL SECTION

General

The melting points were recorded in open capillaries with electrical melting point apparatus and were uncorrected. IR spectra (KBr disks) were recorded using Perkin-Elmer FTIR-RX_1 spectrophotometer. A ^{1}H-NMR spectrum was recorded on Bruker Avance (400 MHz) spectrometer in $CDCl_3$ solutions, with Tetramethylsilane (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 (92:8) as the solvent systems and the spots were visualized by exposure to iodine vapors.

Synthesis of chalcones

Chalcones were synthesized by base catalyzed Claisen-Schmidt condensation reaction of appropriately substituted acetophenones and aldehydes by known literature method [16]. 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 NaOH solution (1g in 10 ml H_2O) was added drop wise to the reaction mixture on vigorous stirring for 30 min 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 h the reaction mixture was neutralized by 0.1-0.2 N HCl whereby the precipitation occurred. On filtering off, the crude chalcones were dried in air and recrystallized by rectified spirit. The residue was purified on column chromatography (Silica gel with 10% ethyl acetate in hexane) to afford pure chalcones (Scheme 1).



Scheme 1: Synthesis of chalcone derivative

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 h. light and 12 h. 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'.

Antiulcer activity

Antiulcer activity was measured using indomethacin induced gastric ulcer model [17]. 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 (48 mg/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.

$$\% \text{ inhibition of ulcers} = \frac{C-T}{C} \times 100$$

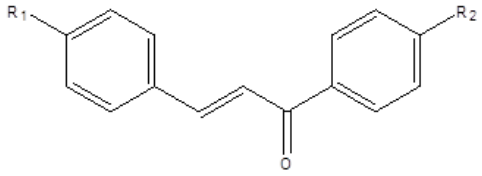
Statistical analysis

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).

RESULT AND DISCUSSION

The synthesis was based on Claisen Schmidt reaction, which is condensation reaction of substituted benzaldehyde with substituted acetophenones in the presence of sodium hydroxide (10%) and ethanol. The products were characterized by comparing physical and their spectral data (Table 1).

Table 1: Physical constants of the synthesized compounds



Comp code	R_1	R_2	Molecular formula	Molecular Wt	Reaction time (h)	Yield[a] %	M.P. (°C)
1a	OCH_3	Br	$C_{16}H_{13}BrO_2$	317.17	4	88	197-199
1b	-OCH_3	I	$C_{16}H_{13}IO_2$	364.17	5	80	199-201
1c	-OCH_3	-OCH_3	$C_{17}H_{18}O_3$	268.30	4	68	175-177
1d	-OCH_3	-OC_2H_5	$C_{18}H_{18}O_3$	282.33	4.5	65	186-188
1e	-OH	-OCH_3	$C_{16}H_{14}O_3$	254.28	3	88	238-240
1f	H	-OC_2H_5	$C_{17}H_{16}O_2$	252.11	4	84	140-143
1g	-OC_3H_7	Cl	$C_{18}H_{19}ClO_2$	302.11	5	66	132-135
1h	-OC_3H_7	Br	$C_{18}H_{19}BrO_2$	344.04	5	58	176-178
1i	-OC_3H_7	-OCH_3	$C_{19}H_{20}O_3$	296.36	5	76	196-198
1j	-OC_3H_7	-OC_2H_5	$C_{20}H_{22}O_3$	310.39	5	42	173-175

[A]isolated yield

The spectral detail of synthesized compounds is given below:

3-(4-methoxy phenyl)-1-(4-bromophenyl)-2-propen-1-one (1a): IR (nujol) cm^{-1}: 1658 (>C=O in conjugation with C=C), 1596, 1540 (>C=C< in conjugation with C=O), 722 (-Br); ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.85 (d, 2H, Ar 3', 5'H), 7.87 (d, 2H, Ar 2',6'H), 7.58 (d, $1H_a$, J=16 Hz, =CH),

7.61 (d, 1H$_b$, J =16Hz, =CH), 6.92 (d, 2H, Ar 2",6"-H), 6.94 (d, 2H, Ar 3", 5"- H), 3.84 (s, 3H, Ar 4"-OCH_3); Mass spectrum (EI, *m/z*): 318 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z*=317.175, calculated for $C_{16}H_{13}BrO_2$: 317.177.

3-(4-methoxy phenyl)-1-(4-iodophenyl)-2-propen-1-one (1b): IR (nujol) cm^{-1}: 1656 (>C=O in conjugation with C=C), 1597, 1541 (>C=C< in conjugation with C=O), 660 (-I); ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.72 (d, 2H, Ar 3', 5'H), 7.83 (d, 2H, Ar 2',6'H), 7.58 (d, 1H$_a$, J = 16 Hz, =CH), 7.60 (d, 1H$_b$, J =16 Hz, =CH), 6.92 (d, 2H, Ar 2",6"-H), 6.94 (d, 2H, Ar 3", 5"- H), 3.85 (s, 3H, Ar 4"-OCH_3);Mass spectrum (EI, *m/z*): 365 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z*=364.175, calculated for $C_{16}H_{13}IO_2$: 364.178.

3-(4-methoxy phenyl)-1-(4-methoxyphenyl)-2-propen-1-one (1c): M.p. 175-177°C, IR (nujol) cm^{-1}: 1655 (>C=O in conjugation with C=C), 1599, 1528 (>C=C< in conjugation with C=O), 1017 (-OCH_3); ^{1}H-NMR ($CDCl_3$), δ (ppm): 6.98 (d, 2H, Ar 3', 5'H), 8.02 (d, 2H, Ar 2',6'H), 7.41 (d, 1H$_a$, J=16 Hz, =CH), 7.76 (d, 1H$_b$, J=16 Hz, =CH), 7.61 (d, 2H, Ar 2",6"-H), 6.92 (d, 2H, Ar 3", 5"- H), 3.89 (s, 3H, Ar 4"-OCH_3); Mass spectrum (EI, *m/z*): 270 ($M^{+\cdot}$+2) Exact mass of molecular ion *m/z*=268.303, calculated for $C_{17}H_{18}O_3$: 268.304.

3-(4-methoxy phenyl)-1-(4-ethoxyphenyl)-2-propen-1-one (1d): IR (nujol) cm^{-1}: 1654 (>C=O in conjugation with C=C), 1599,1526 (>C=C< in conjugation with C=O), 1048(-OC_2H_5), 1026(-OCH_3); ^{1}H-NMR ($CDCl_3$), δ (ppm): 1.40-1.42 (t, 3H, -CH_3), 4.02-4.06 (q, 2H, -CH_2), 6.95 (d, 2H, Ar 3', 5'H), 8.02 (d, 2H, Ar 2',6'H), 7.50 (d, 1H$_a$, J=16 Hz, =CH), 7.76 (d, 1H$_b$, J=16 Hz, =CH), 7.61 (d, 2H, Ar 2",6"-H), 6.91 (d, 2H, Ar 3", 5"- H), 3.83 (s, 3H, Ar"-OCH_3). Mass spectrum (EI, *m/z*): 283 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z*=282.329, calculated for $C_{18}H_{18}O_3$: 282.330.

3-(4-hydroxy phenyl)-1-(4-methoxyphenyl)-2-propen-1-one (1e): IR (nujol) cm^{-1}: 1640 (>C=O in conjugation with C=C), 1598,1528 (>C=C< in conjugation with C=O), 1020(-OCH_3), 3659(-OH); ^{1}H-NMR ($CDCl_3$), δ (ppm): 3.87 (s, 3H, Ar-4'-OCH_3), 6.89 (d, 2H, Ar 3', 5'H), 7.94 (d, 2H, Ar 2',6'H), 7.51 (d, 1H$_a$, J=16 Hz, =CH), 7.71 (d, 1H$_b$, J =16 Hz, =CH), 7.45 (d, 2H, Ar 2",6"-H), 6.96 (d, 2H, Ar 3", 5"- H), 8.03(s, 1H, Ar 4"- OH); Mass spectrum (EI, *m/z*): 255 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z*=254.278, calculated for $C_{16}H_{14}O_3$: 254.280.

3-phenyl-1-(4-ethoxyphenyl)-2-propen-1-one (1f): IR (nujol) cm^{-1}: 1648 (>C=O in conjugation with C=C), 1576, 1534 (>C=C< in conjugation with C=O), 1048 (-OC_2H_5); ^{1}H-NMR ($CDCl_3$), δ (ppm): 1.39-1.43(t, 3H, -CH_3), 4.03-4.08 (q, 2H, -CH_2), 6.95 (d, 2H, Ar 3', 5'H), 8.02 (d, 2H, Ar 2',6'H), 7.51 (d, 1H$_a$, J=16 Hz, =CH), 7.76 (d, 1H$_b$, J=16Hz, =CH), 7.61 (d, 2H, Ar 2",6"-H), 7.36-7.39 (m, 3H, Ar 3",4", 5"- H); Mass spectrum (EI, *m/z*): 253($M^{+\cdot}$+1) Exact mass of molecular ion *m/z*=252.1150, calculated for $C_{17}H_{16}O_2$: 252.1151.

3-(4-propoxy phenyl)-1-(4-chlorophenyl)-2-propen-1-one (1g): IR (nujol) cm^{-1}: 1651 (>C=O in conjugation with C=C), 1596 (>C=C< in conjugation with C=O), 1520, 1458 (C=C aromatic stretching), 732 (C-Cl stretching), 1245 & 1012 (C-O asymmetric & symmetric stretching); ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.81 (dd, J=4 Hz, J=8 Hz, 2H, Ar 2',6'H),7.70 (d, J=16 Hz, 1H$_3$, =CH), 7.56 (dd, J=4 Hz, J=8Hz, 2H, Ar 3', 5'H), 7.47 (d, J=8 Hz, 2H, Ar 2",6"-H), 7.36 (d, J=16 Hz, 1H$_2$,=CH), 6.92 (d, J=8Hz, 2H, Ar 3", 5"- H), 1.71-1.75 (m, 2H, -C<u>H</u>$_2$), 4.05-4.10 (t, 2H, -C<u>H</u>$_2$), 0.92-0.96 (t, 3H, -C<u>H</u>$_3$); Mass spectrum (EI, *m/z*): 304 ($M^{+\cdot}$+2) Exact mass of molecular ion *m/z*=302.11 calculated for $C_{18}H_{19}ClO_2$: 302.11.

3-(4-propoxy phenyl)-1-(4-bromophenyl)-2-propen-1-one (1h): IR (nujol) cm^{-1}: 1654 (>C=O in conjugation with C=C), 1602 (>C=C< in conjugation with C=O), 1532, 1473 (C=C aromatic stretching), 668 (C-Br stretching), 1250 & 1022 (C-O asymmetric & symmetric stretching); ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.84 (dd, J=4 Hz, J=8 Hz, 2H, Ar 2',6'H), 7.74 (d, J=16 Hz, 1H$_3$, =CH), 7.54 (dd, J=4 Hz, J=8 Hz, 2H, Ar 3', 5'H), 7.34 (d, J=8 Hz, 2H, Ar 2",6"-H), 7.40 (d, J=16 Hz, 1H$_2$, =CH), 6.56 (d, J=8Hz, 2H, Ar 3", 5"- H), 1.71-1.75 (m, 2H, -C<u>H</u>$_2$), 4.05-4.10 (t, 2H, -C<u>H</u>$_2$), 0.92-0.96(t, 3H, -C<u>H</u>$_3$); Mass spectrum (EI, *m/z*): 345 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z*=344.23 calculated for $C_{18}H_{19}BrO_2$: 344.04.

3-(4-propoxy phenyl)-1-(4-methoxyphenyl)-2-propen-1-one (1i): IR (nujol) cm^{-1}: 1642 (>C=O in conjugation with C=C), 1588 (>C=C< in conjugation with C=O), 1520, 14532 (C=C aromatic stretching), 1245 & 1012 (C-O asymmetric & symmetric stretching); ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.88 (dd, J=4 Hz, J=8 Hz, 2H, Ar 2',6'H), 7.68 (d, J=16 Hz, 1H$_3$, =CH), 7.48 (dd, J=4 Hz, J=8 Hz, 2H, Ar 3', 5'H), 7.30 (d, J=8 Hz, 2H, Ar 2",6"-H), 7.42 (d, J=16 Hz, 1H$_2$,=CH), 6.56 (d, J=8 Hz, 2H, Ar 3", 5"- H), 1.71-1.75 (m, 2H, -C<u>H</u>$_2$), 4.05-4.10 (t, 2H, -C<u>H</u>$_2$), 0.92-0.96 (t, 3H, -C<u>H</u>$_3$), 3.80 (s, 3H, Ar-4'-OCH_3); Mass spectrum (EI, *m/z*): 297 ($M^{+\cdot}$+1). Exact mass of molecular ion *m/z*=296.14 calculated for $C_{19}H_{20}O_3$: 296.36

3-(4-propoxy phenyl)-1-(4-ethoxyphenyl)-2-propen-1-one (1j): IR (nujol) cm^{-1}: 1648 (>C=O in conjugation with C=C), 1596 (>C=C< in conjugation with C=O), 1542, 1528 (C=C aromatic stretching), 1258 & 1018 (C-O asymmetric & symmetric stretching); ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.88 (dd, J=4 Hz, J=8 Hz, 2H, Ar 2',6'H), 7.68 (d, J=16 Hz, 1H$_3$, =CH), 7.48 (dd, J=4 Hz, J=8 Hz, 2H, Ar 3', 5'H), 7.30 (d, J=8 Hz, 2H, Ar 2",6"-H), 7.42 (d, J=16 Hz, 1H$_2$, =CH), 6.56 (d, J=8 Hz, 2H, Ar 3", 5"- H), 1.71-1.75 (m, 2H, -C<u>H</u>$_2$), 4.05-4.07 (t, 2H, -C<u>H</u>$_2$), 0.92-0.96 (t, 3H, -C<u>H</u>$_3$), 3.80 (s, 3H, Ar-4'-OCH_3), 1.39-1.43 (t, 3H, -CH_3), 4.16-4.20 (q, 2H, -CH_2); Mass spectrum (EI, *m/z*): 311 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z*=310.16 calculated for $C_{20}H_{22}O_3$:310.39.

The titled compounds were confirmed by IR spectral data showing sharp bands in the range between 1630-1660 cm^{-1} indicated the presence of C=O group. The compounds (1a-1j) were characterized by ^{1}H-NMR spectra carried out in dimethylsulphoxide (DMSO), where vinylic protons, α proton attributed to proton at position 2 and β proton attributed to proton at position 3, showed two sets of doublets at δ=7.24-7.45 and 7.71-7.80 respectively, having the coupling constant J between 13-16 Hz and thus showing the trans (E) configuration of the carbon-carbon double bonds of α, β-unsaturated carbonyl system. The molecular ion peaks of all synthesized compounds were shown as $[M+1]^+$ or as sodium bound pseudo molecular ions $[M+Na]^+$. 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 % antiulcer activity as represented in Table 2, in which all compounds at the tested dose level exhibited varying degree of activity against ulceration induced by indomethacin.

Table 2: The antiulcer activity of the test compounds

Compounds	Doses (mg/kg, p.o.)	No. of ulcer spots counted	% Antiulcer activity
1a	100	12.49 ± 0.36[a]	49.8
1b	100	14.89 ± 0.47[b]	40.2
1c	100	9.63 ± 0.39[a]	61.3
1d	100	6.82 ± 0.87[a]	72.6
1e	100	7.59 ± 0.74[a]	69.5
1f	100	17.13 ± 0.38[b]	41.8

1g	100	11.91 ± 0.49[a]	52.2
1h	100	12.31 ± 0.54[a]	50.6
1i	100	6.63 ± 0.72[a]	73.4
1j	100	7.43 ± 0.59[a]	70.2
Indomethacin	100	24.90 ± 0.63	

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

The compounds 1i 1d and 1j exhibited excellent (70-73%) gastro protective action as indicated by their low ulcer score. The Compound 1b&1f showed mild antiulcer activity. Indomethacin causes gastric erosions with increased lipid peroxidation and decreased Glutathione Peroxidase (GPO) activity, (a highly active peroxidase in the gastric mucosa plays a vital role in scavenging H_2O_2 to prevent oxidative damage) [18,19]. The synthesized chalcones, being aromatic electron donor, is a suitable substrate for peroxidase, caused a reduction in gastric damage [20]. The result obtained in the present study may be attributed to their antioxidant activity through scavenging of lipid peroxides and other free radicals and protection of Glutathione Peroxidase (GPO).

CONCLUSION

A series of chalcones were synthesized and evaluated for their antiulcer activity properties in the rats by indomethacin induced gastric damage. All the synthesized chalcones caused a reduction in gastric damage. The result obtained in the present study may be due to their antioxidant activity through scavenging of reactive oxygen species, protection of glutathione peroxidase.

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