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## Novel Chalcones as Potent Anti-Inflammatory Drugs

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### Abstract

Chalcones are important key intermediates for synthesizing heterocyclic compounds like pyrazolines, isoxazolines, pyridine, pyrimidines etc. Chalcones exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and anti-ulcer actions. They also inhibit enzymes such as aldose reductase and xanthine oxidase. They are potent antioxidants and have free radical scavenging abilities. In the present paper different chalcones were synthesized by condensing appropriate acetophenone with sodium hydroxide by Claisen Schimdt reaction. All the synthesized chalcones were then tested for the anti-inflammatory activity by using carrageenan induced hind paw edema model in Wistar strain albino rats. The activity of all the synthesized chalcones showed moderate to strong and equal activity to that exhibited by standard drug Celecoxib at 3h and 5h.

**Keywords:** Chalcones, Antiinflammatory Activity, Carrageenan.

### INTRODUCTION

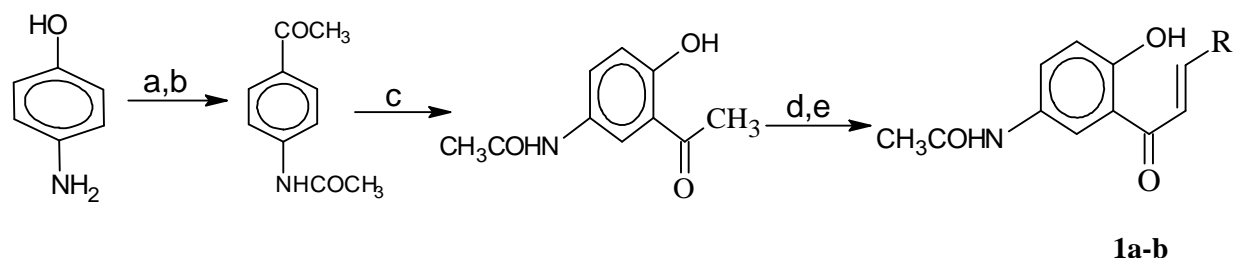
The term “chalcone” is a generic term used to describe compounds with the 1,3-diphenylprop-2-en-1-one framework. Chalcones are also intermediates in the Auwers synthesis of flavones. Chalcone (and related compounds “chalconoids”) is an aromatic ketone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones. Chalcones are naturally occurring compounds found in various plant species like Angelica, Glycyrrhiza, Humulus and Scutellaria, which are widely used as traditional folk remedies. Chalcones are intermediates in the biosynthesis of flavonoids, which are substances widespread in plants and with an array of biological activities. These are abundant in edible plants and are considered to be precursors of flavonoids and isoflavonoids. Methyl hydroxychalcone found in cinnamon, was thought to be insulin mimetic, improving insulin response of diabetics. It has since been determined that a flavonoid is responsible for the insulin-like biological activity<sup>1,2</sup>. Chalcones show antibacterial, antimicrobial, antifungal, antitumor and anti-inflammatory properties. Chalcones are important key intermediates in the synthesis of flavanones, flavones, flavonols and heterocyclic compounds like pyrazolines, isoxazolines etc. Chalcones and flavones are reported to possess many useful properties including anti-inflammatory<sup>3,4,5</sup>, antioxidant<sup>6,7</sup>, antitumour and anticancer<sup>8</sup> activities. Chalcone (C6–C3–C6 compound) is one of the subgroups of the flavonoid family. These molecules possess interesting biological activities including cyto-toxic<sup>9,10</sup>, antimalarial<sup>11</sup>, antileishmanial<sup>12</sup>, anti-HIV<sup>13</sup>, antifungal, antimicrobial<sup>14</sup> and as tyrosine kinase inhibitors<sup>15</sup> [26]. As part of our continuous search for potential anti-inflammatory drug candidates in the present study we have synthesized some different chalcones and examined their in vivo anti-inflammatory activity.

### RESULT AND DISCUSSION

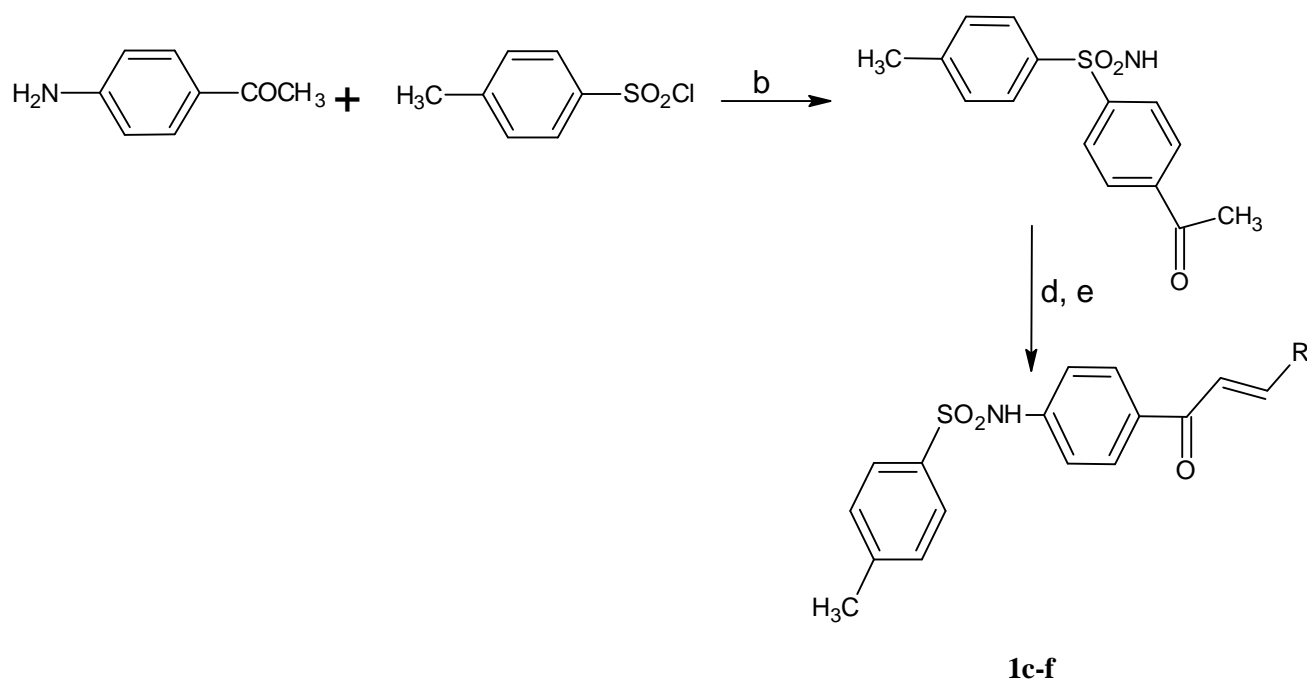
#### Chemistry

The chalcones (1a-f) were synthesized according to the steps outlined in Scheme 1 and 2. The chalcones (1a-f) were prepared by condensing aromatic aldehydes and acetophenones in presence of sodium hydroxide in

ethanol at 0-5 °C for 12h. All the synthesized compounds were confirmed by the various analytical techniques (UV, IR, NMR, MS and elemental analysis).



**SCHEME 1**



**SCHEME-2**

a =  $(\text{CH}_3\text{CO})_2\text{O}$ , b = pyridine, c = anhydrous  $\text{AlCl}_3$ , d= Aromatic aldehydes, e=NaOH

**Scheme 1&2. General procedure for the synthesis of chalcones (1a-f).**

**1a:** R = 2-Chlorophenyl

**1b:** R = 3,4,5-trimethoxyphenyl

**1c:** R = Phenyl

**1d:** R = 4-Chlorophenyl

**1e:** R = 2-Chlorophenyl

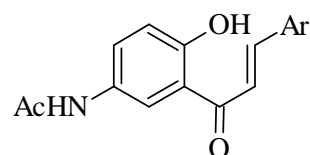
**1f:** R = 3,4-dimethoxyphenyl

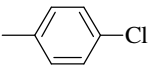
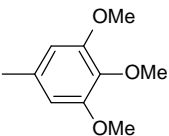
## PHARMACOLOGY

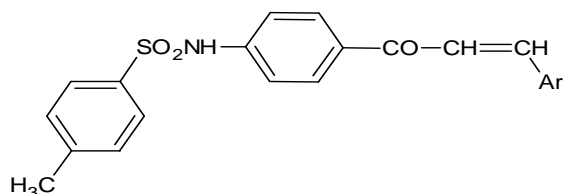
### Antinflammatory Activity

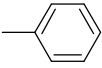
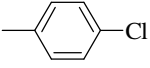
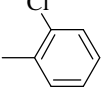
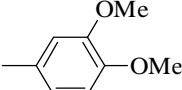
The in vivo anti-inflammatory activity for all synthesized compounds (at 0.05 mmol/kg dose) was evaluated using carrageenan-induced rat paw edema model by adopting the earlier reported method of Winter et al.<sup>16</sup> (Table 1 and 2). Two paracetamol based chalcones (**1a** and **1b**) were evaluated for their anti-inflammatory potentials. Both compounds showed the activity level similar to that of reference drug at both time points (3h & 5h). Anti-inflammatory potential of chalcone bearing toluene sulfonamide moiety (**1c** to **1f**) has also been evaluated. Compound **1c** showed percentage protection of 60.5% at 3h and 56.3% at 5h. Introduction of electron donating group in B-ring enhances the activity (**1c** vs **1d**, **1c** vs **1f**) at the 3h time point and (**1c** vs **1e**) at the 5h time point.

**Table-1: Effect of chalcones of 4-amino phenol on carageenan induced hind paw edema in rats**



Group no.	Treatment	Ar	Dose Per kg b.w.	Increase in paw volume ml ± SEM after carrageenan administration <sup>a</sup>	
				3 hours	5 hours
I	Vehicle	—	10 ml/kg	0.38 ± 0.0307	0.42 ± 0.0401
II	Celecoxib	—	20 mg	0.066 ± 0.020 (82.6%) <sup>b</sup>	0.06 ± 0.02 (85.7 %)
III	1a		20 mg	0.05 ± 0.04 (86.8 %)	0.05 ± 0.0244 (88.1%)
IV	1b		20 mg	0.05 ± 0.0244 (86.8 %)	0.03 ± 0.04 (92.8 %)

**Table-2: Effect of chalcones of p-toluene sulfonamides on carrageenan induced hind paw edema in rats**


Group no.	Treatment	Ar	Dose Per kg b.w.	Increase in paw volume ml $\pm$ SEM after carrageenan administration	
				3 hours	5 hours
I	Vehicle	—	10 ml/kg	0.38 $\pm$ 0.0307	0.42 $\pm$ 0.0401
II	Celecoxib	—	20 mg	0.066 $\pm$ 0.0210 (82.6%)	0.06 $\pm$ 0.02 (85.7 %)
III	1c		20 mg	0.15 $\pm$ 0.034 (60.5 %)	0.183 $\pm$ 0.031 (56.3 %)
IV	1d		20 mg	0.066 $\pm$ 0.154 (82.4 %)	0.166 $\pm$ 0.021 (60.3 %)
V	1e		20 mg	0.0166 $\pm$ 0.021 (56.1 %)	0.083 $\pm$ 0.017 (80.1 %)
VI	1f		20 mg	0.083 $\pm$ 0.017 (78.1 %)	0.133 $\pm$ 0.084 (68.2 %)

All data are significantly different compared to control values,  $P < 0.05$

<sup>a</sup> Values are expressed as mean + SEM and analysed by ANOVA followed by Dunnett's test.

<sup>b</sup>Values in parenthesis (percent inhibitions).

## CONCLUSION

The present study describes the synthesis of different chalcones by Claisen Schimdt reaction. All the synthesized compounds exhibited moderate to strong anti-inflammatory activity. The chalcones **1a** and **1b** have higher anti-inflammatory activity.

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**EXPERIMENTAL SECTION****Chemistry**

Melting points were determined by open capillary tubes and are uncorrected. All the Fourier Transform Infra Red (FTIR) spectra were recorded on a Brukers Vector 22 spectrophotometer in film;  $\lambda_{\max}$  values are given in  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR spectra were recorded on a Bruker Spectrospin DPX 300-MHz spectrometer using deuterated  $\text{CDCl}_3$  as a solvent and tetramethyl silane (TMS) as an internal standard. Chemical shifts are given in (ppm) scale and coupling constants (J values) are expressed in Hz. Mass spectra (MS) were scanned by affecting FAB ionization JEOL-JMS-DX 303 system, equipped with direct inlet probe system and Maldi by using 4800 MALDI-TOF/TOF MS. The  $m/z$  values of the more intense peaks are mentioned. Purity of the compounds was checked on TLC plates (silica gel G) which were visualized by exposing to iodine vapors. Elemental analysis was carried out on CHNS Elementar (Vario EL III).

**Synthesis of Chalcones (1a-f)**

To a hot solution of acetophenone (2g, 1 mole) and desired aromatic aldehyde (1g, 1 mole) in ethanol (25 ml), a sodium hydroxide solution (25 ml of 30% solution) was added gradually with shaking. The reaction mixture was shaken for 10-15 minutes and then on dilution with ice-cold acidulated water gave solid precipitate. It was filtered and washed with sodium bicarbonate solution and then with water. It was crystallized from chloroform: methanol.

**5'-acetamido-2'-hydroxy-4-Chloro chalcone (1a)**

Orange crystalline compound, yield 70%, m.p.  $169-170^\circ\text{C}$  (reported  $198-199^\circ\text{C}$ ),  $R_f = 0.24$  [toluene:ethyl formate:formic acid; 7.5:2:0.5]. **UV  $\lambda_{\max}$  (MeOH):** 219, 244, 323 nm. **IR  $\nu_{\max}$  (Solvent, in  $\text{cm}^{-1}$ ):** 3281 (OH), 1658 (CO), 1584 (olefinic C=C bond), 1486, 1365, 1181  $\text{cm}^{-1}$ .  **$^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$   $\delta$ ):** 2.14 (3H, s,  $\text{COCH}_3$ ), 6.91 (1H, d,  $J = 8.83$  Hz, H-3'), 7.15 (1H, s, NH), 7.25 (1H, d,  $J = 8.78$  Hz, H-2'), 7.33 (2H, d,  $J = 7.76$  Hz, H-3, H-5), 7.52 (3H, m, H- $\alpha$ , H-2, H-6), 7.79 (1H, d,  $J = 15.40$  Hz, H- $\beta$ ), 8.37 (1H, s, H-6'), 12.55 (1H, s, Chelated OH). **ESI-MS ( $m/z$ ):** 314 [M-1]. Anal. Calcd for  $\text{C}_{17}\text{H}_{14}\text{ClNO}_3$ . Calculated; C = 64.67, H = 4.47, N = 4.44. Found; C = 64.60, H = 4.43, N = 4.4.

**5'-acetamido-2'-hydroxy-3, 4, 5-trimethoxychalcone (1b)**

Orange crystalline compound, yield=57%, m.p.  $186^\circ\text{C}$  (reported  $186-188^\circ\text{C}$ ),  $R_f = 0.28$  [toluene:ethyl formate:formic acid; 7.5:2:0.5]. **UV  $\lambda_{\max}$  (MeOH):** 219, 248, 318 nm. **IR  $\nu_{\max}$  (Solvent, in  $\text{cm}^{-1}$ ):** 3327 (OH), 1642 (CO), 1580 (olefinic C=C bond), 1501, 1296, 1125, 1023  $\text{cm}^{-1}$ .  **$^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$   $\delta$ ):** 2.14 (3H, s,  $\text{COCH}_3$ ), 3.85 (3H, s,  $\text{OCH}_3$ , C-4), 3.87 (6H, s, 2\*  $\text{OCH}_3$ , C-3,5), 6.83 (2H, s, H-2, H-6), 6.93 (1H, d,  $J = 8.83$  Hz, H-3'), 7.08 (1H, s, NH), 7.29 (1H, d,  $J = 8.85$  Hz, H-4'), 7.42 (1H, d,  $J = 15.33$  Hz, H- $\alpha$ ), 7.78 (1H, d,  $J = 15.3$  Hz, H- $\beta$ ), 8.26 (1H, s, H-6'), 12.55 (1H, s, Chelated OH). **ESI-MS ( $m/z$ ):** 370 [M-1], 394 [M+Na]. Anal. Calcd for  $\text{C}_{20}\text{H}_{21}\text{NO}_6$ : Calculated; C = 64.68, H = 5.70, N = 3.77. Found; C = 64.62, H = 5.65, N = 3.76.

**p-Toluene sulphonamide chalcone (1c)**

Off white crystalline compound, yield = 66 %, m.p.  $180-182^\circ\text{C}$  [reported m.p.  $165^\circ\text{C}$ ],  $R_f = 0.50$ , [Chloroform: Methanol; 96:4], Anal. Calcd for  $\text{C}_{22}\text{H}_{19}\text{NO}_3\text{S}$ : Calculated; C = 70.00, H = 5.07, N = 3.71, S = 8.50. Found; C = 69.94, H = 5.03, N = 3.70, S = 8.48

**p-Toluene sulphonamide-4-chlorochalcone (1d)**

Light yellow crystalline compound, yield = 72 %, m.p.  $198-200^\circ\text{C}$ ,  $R_f = 0.54$ , [toluene: ethyl formate: formic acid 7.5: 2: 0.5], **IR  $\nu_{\max}$  (Solvent, in  $\text{cm}^{-1}$ ):** 3125  $\text{cm}^{-1}$  (secondary amine), 1650  $\text{cm}^{-1}$  (CO), 1596  $\text{cm}^{-1}$  (olefinic C=C bond), 1326 and 1156  $\text{cm}^{-1}$  ( $\text{SO}_2\text{NH}$ ).  **$^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$   $\delta$ ):** 2.31 (3H, s,  $\text{CH}_3$ ), 6.99 (1H, s, NH), 7.12 (2H, d,  $J = 8.4$  Hz, H-3'', H-5''), 7.20 (2H, d, merged with solvent residual peak, H-3, H-5), 7.34 (3H, m, H- $\alpha$ , H-3', H-5'), 7.48 (2H, d,  $J = 8.4$  Hz, H-2'', H-6''), 7.66 (3H, m, H-2, H-6, H- $\beta$ ), 7.86 (2H, d,  $J = 8.4$  Hz, H-2', H-6'). **ESI-MS ( $m/z$ ):** 410 [M-1], 434 [M+Na]. Anal. Calcd for  $\text{C}_{22}\text{H}_{18}\text{ClNO}_3\text{S}$ : Calculated; C = 64.15, H = 4.40, N = 3.40, S = 7.78. Found; C = 64.09, H = 4.36, N = 3.39, S = 7.76

***p*-Toluene sulphonamide-2- chloroalcone (1e)**

Off white crystalline compound, yield = 75 %, m.p. 144-146<sup>0</sup>C, R<sub>f</sub> = 0.63, [toluene: ethyl formate: formic acid 7.5: 2: 0.5], **IR**  $\nu_{\max}$  (Solvent, in  $\text{cm}^{-1}$ ): 3437  $\text{cm}^{-1}$ , 3205  $\text{cm}^{-1}$  (secondary amine), 1671  $\text{cm}^{-1}$  (CO), 1607  $\text{cm}^{-1}$  (olefinic C=C bond), 1329 & 1156  $\text{cm}^{-1}$ (SO<sub>2</sub>NH). **<sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>,  $\delta$ ):** 2.26 (3H, s, CH<sub>3</sub>), 7.17 – 8.15 (14H, m, olefinic protons, & aromatic protons), 10.88 (1H, s, NH). **ESI-MS (m/z):** 410 [M-1], 434 [M+Na]. Anal. Calcd for C<sub>22</sub>H<sub>18</sub>ClNO<sub>3</sub>S: Calculated; C = 64.15, H = 4.40, N= 3.40, S = 7.78, Found; C = 64.09, H = 4.36, N= 3.39, S = 7.76

***p*-Toluene sulphonamide- 3,4-dimethoxyalcone (1f)**

Light yellow crystalline compound, yield = 68 %, m.p. 170-172<sup>0</sup>C, R<sub>f</sub> = 0.52, [toluene: ethyl formate: formic acid 7.5: 2: 0.5], **IR**  $\nu_{\max}$  (Solvent, in  $\text{cm}^{-1}$ ): 3240  $\text{cm}^{-1}$ , 3021  $\text{cm}^{-1}$  (secondary amine), 1601  $\text{cm}^{-1}$  (CO), 1510  $\text{cm}^{-1}$  (olefinic C=C bond), 1341 & 1160  $\text{cm}^{-1}$ (SO<sub>2</sub>NH), 1263  $\text{cm}^{-1}$ . **<sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>,  $\delta$ ):** 2.39 (3H, s, CH<sub>3</sub>), 3.94 (3H, s, OCH<sub>3</sub>), 3.95 (3H, s, OCH<sub>3</sub>), 6.90 (1H, d, J= 8.1 Hz, H-5), 7.14 – 7.36 (7H, m, H-3', H-5', H-3'', H-5'', H- $\alpha$ , H-2, H-6), 7.75 (1H, d, J= 15 Hz, H- $\beta$ ), 7.75 (2H, d, J= 8.4 Hz, H-2'', H-6''), 7.93 (2H, d, J= 8.4 Hz, H-2', H-6'). **ES<sup>+</sup>-MS (m/z):** 438 [M+1], 439 [M+2], 355, 290, 289. Anal. Calcd for C<sub>24</sub>H<sub>23</sub>NO<sub>5</sub>S: Calculated; C = 65.89, H = 5.30, N= 3.20, S = 7.33, Found; C = 65.82, H = 5.25, N= 3.19, S = 7.31.

**PHARMACOLOGICAL STUDY****Anti-inflammatory Activity**

Carrageenan induced hind paw edema method was used for evaluating anti-inflammatory activity<sup>16</sup>. Wistar rats (either sex) weighing 150-175g were procured from Central Animal House facility of Jamia Hamdard, New Delhi (Registration no. 173/CPCSEA). The experiments were performed in accordance with the guidelines for the care and use of laboratory animals, laid down by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Social Justice and Empowerment, Govt. of India, Jan. 2000. Overnight fasted rats (16 h) were divided into 24 groups of 6 animals each. One group of rats, which served as control was given vehicle (1% CMC in water in a volume of 10 ml/kg) only. Test compounds (0.05 mmol/kg b.w), suspended in vehicle (10 ml/kg) were administered orally to respective groups. After 30 minutes, all animals were injected with 0.1 ml of 1 % carageenan solution (prepared in normal saline) in the subplantar aponeurosis of left hind paw to induce inflammation and the volume of injected paw was measured by using plethysmometer immediately (at 0 h). The paw volume was again measured after 3 h and 5 h. The average paw volume in a group of treated rats was compared with vehicle (control group) and the percentage inhibition of edema was calculated by using the formula:

The percentage inhibition of edema was calculated by applying the following formula.

$$\% \text{ Inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

V<sub>c</sub> = Volume of edema in control group.

V<sub>t</sub> = volume of edema in test group.

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## CONFLICT OF INTEREST

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