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Synthesis and Analgesic Activity of Some Chalcones

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A series of novel 1-(4'-aminophenyl)-3-(substituted aryl/heteroaryl)-2-propen-1-ones (**1-16**) have been synthesized by treating 4-amino acetophenone with various substituted aromatic and unsubstituted heterocyclic aldehydes in presence of methanol and aqueous alkaline solution at room temperature. Their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, EI-MS spectra and elemental analyses data. The synthesized compounds were investigated for their analgesic activity. Compounds **6** and **15** exhibited maximum analgesic activity. Chalcones with electron releasing substituent like amino, hydroxyl, methyl, halogens etc exhibited good analgesic activity.

Key Words: Chalcone, Synthesis, Analgesic activity.

INTRODUCTION

Chalcones or 1,3-diaryl-2-propen-1-ones (Fig. 1), belong to the flavonoid family. Chemically they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system. A vast number of naturally occurring chalcones are polyhydroxylated in the aryl rings and are used as drugs or food preservatives either as a compound or chalcone rich plant extract because of their radical quenching properties[1]. Chalcones abundantly present in nature from fern to higher plants display a number of interesting biological activities such as antimalarial[2], antimicrobial[3], antiangiogenic[4], antiviral[5], anti-HIV[6], anticancer[7], antioxidant[8], antiinflammatory[9], analgesic[10], antihypergly-cemic[11], antitubercular[12] and antileishmanial[13] activities.

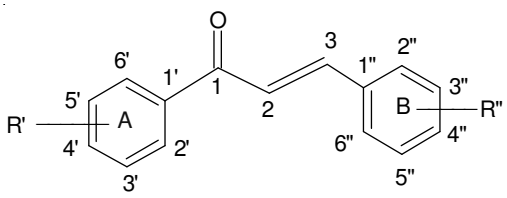


Fig. 1. General structure and numbering of chalcone

Inflammation continues to be an area of great interest for research, probably due to the unavailability of a safer and more effective antiinflammatory agent. Chalcones like flavonoids are known for their ability to strengthen capillary walls to inhibit cyclooxygenase and lipooxygenase enzymes and also found to be useful in the treatment of heavy menstrual bleeding with no apparent cause. They also possessed antiinflammatory and analgesic activities[10,14]. Free radical reactions have been implicated in the pathology of many human diseases/disease conditions like atherosclerosis, ischemic heart disease, aging process, inflammation, diabetes, immunosupression, neurodegenerative diseases, *etc*.[15]. Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing tissue injury like inflammation[16]. No drugs having antiinflammatory activity solely based on antioxidant effects are currently available and perhaps chalcones because of their potential antioxidant effect may provide leads to such type of antiinflammatory and analgesic agents[17]. Hence they were screened for analgesic activity. In general practice chemotherapeutic, analgesic and antiinflammatory drugs are prescribed simultaneously.

Keeping the above discussion in view, we screened the analgesic activity for already synthesized chalcones[18,19] which have been proved as antimicrobials and antiinflammatory agents.

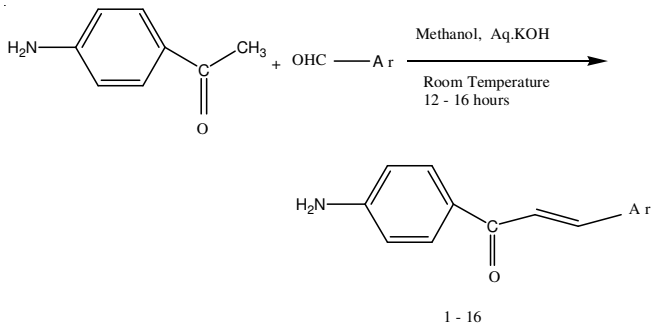
EXPERIMENTAL

Melting points were determined in open capillaries and were uncorrected by melting point determining apparatus (SISCO). Purity of the compounds were checked by TLC. FT-IR spectra (KBr, cm^{-1}) were recorded on a Perkin-Elmer BXF1 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR ($CDCl_3$) spectra on a Bruker AMX 400 and Bruker AMX 100 spectrophotometer respectively using tetramethylsilane (TMS) as an internal standard and the values are expressed in δ ppm. EI-MS recorded on a Agilent 1100 EI-Mass spectrophotometer.

The elemental analyses of the synthesized compounds were recorded on Carlo Erba 1108 elemental analyzer and were within ± 0.4 % of the theoretical values, unless otherwise noted. When possible, the data obtained were compared with the existing structure identification in the literature.

Dr. Reddy's Laboratories Ltd., Hyderabad, India, supplied the ibuprofen as a gift sample. Silica gel (100-200 mesh) for column chromatography and thin layer chromatography (TLC) plastic sheets silica gel 60 F_{254} were purchased from Merck (Darmstadt, Germany). 4-Aminoacetophenone, all derivatives of aldehydes, potassium hydroxide (all from Merck, Germany). All other materials and solvents used were of analytical reagent quality.

General procedure for the synthesis of chalcones[18,19] (1-12): Potassium hydroxide (10 % aqueous solution, 1 mL) was added to a stirred solution of 4-aminoacetophenone (1 mmol) and substituted aromatic aldehyde (1 mmol) in methanol (10 mL) (**Scheme-I** and Table-1). Stirring was carried out for at least 12-16 h at room temperature and each hour the reaction mixture was analyzed by TLC. Then the reaction mixture was poured into ice water, acidified with dil. HCl and extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with water, dried and concentrated *in vacuuo*. The residue was purified on a column of silica gel using hexane-ethyl acetate (4:1) as eluant.



Scheme-I: Synthesis of 4'-aminochalcones

TABLE-1
SUBSTITUTED 4'-AMINOCHALCONES

Compd.	Ar	Compd.	Ar
1	3-Bromophenyl	9	3,4,5-Trimethoxyphenyl
2	4-Methylphenyl	10	4-Dimethylaminophenyl
3	2-Chlorophenyl	11	9-Anthracenyl
4	4-Chlorophenyl	12	4-Nitrophenyl
5	2,4-Dichlorophenyl	13	3-Pyridinyl
6	4-Fluorophenyl	14	2-Pyridinyl
7	4-Methoxyphenyl	15	4-Pyridinyl
8	3,4-Dimethoxyphenyl	16	2-Quinolinyl

4'-aminochalcones were synthesized by the reaction between 4-aminoacetophenone and various substituted aromatic and heteroaromatic aldehydes by Claisen-Schmidt condensation at room temperature in the presence of methanol.

(E)-1-(4'-Aminophenyl)-3-(3''-bromophenyl)-2-propen-1-one, (1): Orange yellow crystals, yield 81 %, m.p. 168 °C, IR (KBr, ν_{max}, cm^{-1}): 3414, 3326 (NH_2), 1652 (C=O), 1626 (HC=CH), 1304 (C-N), 1177 (C-Br). ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.20 (2H, br s, NH_2, exchangeable with D_2O), 6.72 (1H, d, *J* = 16.0 Hz, CO-CH=), 7.27 (2H, d, *J* = 10.2 Hz, C-3' and 5'-H), 7.57-7.49 (3H, m, C-4'', 5'' and 6''-H), 7.70 (1H, d, *J* = 16.0 Hz, Ar-CH=), 7.80 (1H, s, C-2''-H), 7.95 (2H, d, *J* = 10.0 Hz, C-2' and 6'-H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 187.59 (C-1), 151.31 (C-4'), 141.26 (C-3), 137.63 (C-1''), 132.79 (C-1'), 131.19 (C-2' and 6'), 130.65 (C-4''), 130.39 (C-5''), 128.43 (C-2''), 127.11 (C-6''), 123.50 (C-3''), 123.06 (C-2) and 114.01 (C-3' and 5'). EI-MS, m/z (% relative intensity): 301 (M^+, 87), 302 [{M+H}$^+$, 100], 209 (M^+-C_6H_6N, 38), 181 (M^+-C_7H_6NO, 18), 146 (M^+-C_6H_4Br, 22), 155 (M^+- C_9H_8NO, 12), 120 (M^+-C_8H_6Br, 17), 92 (M^+-C_9H_6OBr) mass units. Exact mass of molecular ion: m/z = 301.0548, calculated for $C_{15}H_{12}NOBr$: 301.0551. Analysis ($C_{15}H_{12}NOBr$) calcd. (found) %: C, 59.66 (59.62); H, 4.00 (3.98). Chalcones **2-12** were prepared similarly by using different arylaldehydes and characterized as chalcone **1**.

General procedure for the synthesis of chalcones[18,19] (13-16): Potassium hydroxide (5 % aqueous solution, 1 mL) was added to a stirred solution of 4-aminoacetophenone (1 mmol) and a heteroaromatic aldehyde derivative (1 mmol) in methanol (10 mL) (**Scheme-I** and Table-1). Stirring was carried out for at least 12-16 h at room temperature and each hour the reaction mixture was analyzed by TLC. Then the reaction mixture was poured into ice water and mix it well. Extracted the chalcone with ethyl acetate (3 × 50 mL). The organic layer was separated, washed with water, dried and concentrated *in vacuuo*. The residue was purified on a column of silica gel using hexane-ethyl acetate (2:3) as eluant.

(E)-1-(4'-Aminophenyl)-3-(3''-pyridinyl)-2-propen-1-one (13): Orange red crystals, yield 62.06 %, m.p. 160-163 °C, IR (KBr, ν_{max}, cm^{-1}): 3437, 3354 (NH_2), 1636 (C=O), 1595 (HC=CH), 1422 (C=N), 1342 (C-N). ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.13 (2H, br s, NH_2, exchangeable with D_2O), 6.63 (2H, d, *J* = 8.8 Hz, C-3' and 5'-H), 7.26 (1H, d, *J* = 8.0 Hz, C-5''-H), 7.52 (1H, d, *J* = 15.6 Hz, CO-CH=), 7.68 (1H, d, *J* = 16.0 Hz, Ar-CH=), 7.86 (2H, d, *J* = 8.4 Hz, C-2' and 6'-H), 8.54-8.52 (1H, m, C-4''-H), 8.78 (2H, d, *J* = 9.0 Hz, C-2'' and 6''-H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 185.58 (C-1), 154.01 (C-4'), 150.39 (C-2''), 149.93 (C- 6''), 137.83 (C-3), 134.72 (C-4''), 131.21 (C-2' and C-6'), 130.98 (C-3''), 125.15 (C-1'), 124.48 (C-2), 123.80 (C-5'') and 112.76 (C-3' and 5'). EI-MS, m/z (% relative intensity): 224 (M^+, 84), 225 [{M+H}$^+$, 100], 196 (M^+-CO, 24), 132 (M^+-C_6H_6N, 42), 120 (M^+-C_7H_6N, 29), 92 (M^+-C_8H_6NO, 37) mass units. Exact mass of molecular ion: m/z = 224.1256, calculated for $C_{14}H_{12}N_2O$: 224.1279. Analysis ($C_{14}H_{12}N_2O$) calcd. (found) %: C, 75.06 (75.00); H, 5.39 (5.38). Chalcones **14-16** were prepared similarly by using different heteroaromatic aldehydes and characterized as chalcone **13**.

Wistar albino mice (M/S Ghosh Enterprises, Calcutta, West Bengal, India) of female sex, weighing between 20-25 g were used in the experiment. The mice were maintained under standard laboratory conditions at 25 ± 2 °C, relative humidity 50 ± 15 % and normal photo period (12 h dark/12 h light). Commercial pellet diet (Ratan Brothers, India) and water were provided *ad libitum*. The principles of Laboratory Animal Care (NIH, 1985) were followed and instructions given by our institutional animal ethical committee were maintained throughout the experiment.

Analgesic activity: The analgesic activity was determined by tail flick method[20,21]. Wistar albino mice of either sex (20-25 g) in the groups of six animals each were selected by random sampling technique. All groups were fasted for 24 h before administering the drug with water *ad libitum*. Stock suspension of 1 % sodium CMC (sodium carboxymethylcellulose) was prepared by triturating 1 g of sodium CMC in 100 mL of distilled water and used for suspending the test compounds and reference drug.

Ibuprofen at a dose level of 25 mg/kg was administered as a reference drug for comparison. The test compounds at dose level of 25 mg/kg were administered orally by intragastric tube. The animals were held in position by a suitable restrained with the tail extending out and the tail (up to 5 cm) was then dipped in a beaker of water maintained at 55 ± 5 °C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. The reading was recorded at 0, 1, 2, 4, 6 and 8 h, respectively. A cut off point of 10 s was observed to prevent the tail damage. The percentage of protection in the control, standard and drug treated animals were calculated by using the formula.

$$\% \text{ Analgesic activity (PAA)} = [(Rt/Rc)-1] \times 100$$

where Rt and Rc are the reaction time in test and control, respectively.

Statistical analysis: Biological data were expressed as mean ± SEM and were analyzed by the One-way ANOVA followed by the Dunnett's t-test using computerized Graph Pad Instat version 3.05 (Graph Pad software, U.S.A). The results are shown in Figs. 2 and 3.

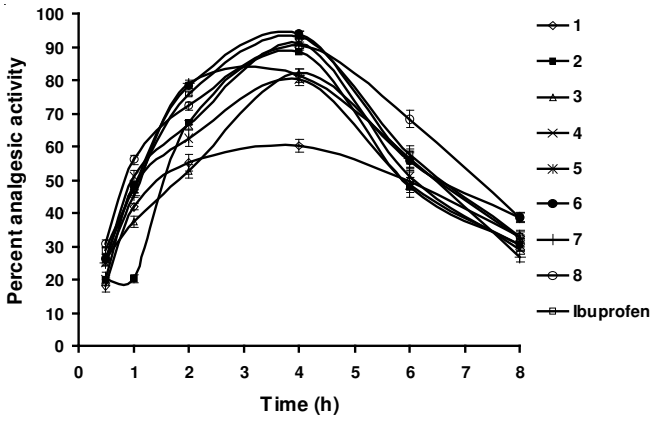


Fig. 2. Analgesic activity of chalcones **1** to **8**; Values are means ± SEM of the number of animals (in parentheses). Latencies to thermal stimuli were measured at several times (0, 0.5, 1, 2, 4, 6 and 8 h) after drug administration. Dosage: Ibuprofen-25 mg/kg and test compounds-25 mg/kg body wt. *P < 0.01 *vs.* control group at the same period of time. One-way ANOVA followed by Dunnet's t-test

RESULTS AND DISCUSSION

The result of analgesic activity (Figs. 2 and 3) revealed that the chalcones exhibited moderate to considerable activity when compared with control and reference standard ibuprofen and in particular compound **6** having 4"-fluorophenyl ring and **15** having 4"-pyridinyl ring showed maximum activity. Particularly both the compound **6** and **15** showed maximum activity at third hour interval and also maximum number of chalcones started their analgesic activity first hour of interval.

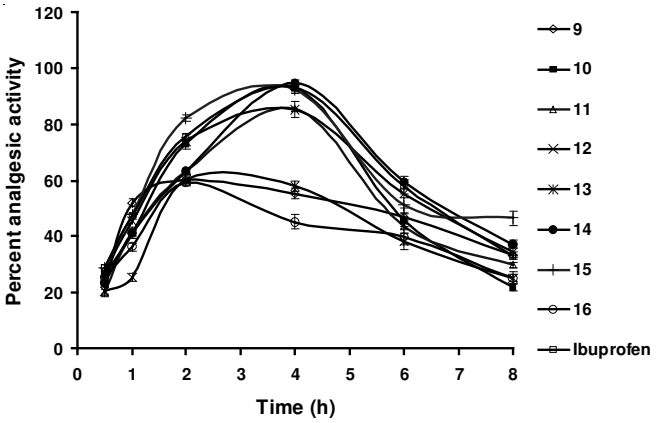


Fig. 3. Analgesic activity of chalcones **9** to **16**; Values are means ± SEM of the number of animals (in parentheses). Latencies to thermal stimuli were measured at several times (0, 0.5, 1, 2, 4, 6 and 8 h) after drug administration. Dosage: Ibuprofen-25 mg/kg and test compounds-25 mg/kg body wt. *P < 0.01 vs control group at the same period of time. One-way ANOVA followed by Dunnet's t-test

Flavonoids constitute a large group of naturally occurring compounds found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. A variety of *in vivo* and *in vitro* experiments have shown that selected flavonoids possess anti-allergic, antiinflammatory, antiviral and antioxidant activities. Certain flavonoids possess potent inhibitory activity against a wide array of enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A2 and other[10]. Flavonoids are known to inhibit prostaglandins, a group of powerful pro-inflammatory signaling molecules, chalcones are reported to inhibit chemiluminescence *in vitro*, cell migration and eicosanoid and TNF-α in mouse air pouch injected with zymosan known to act as promising antiinflammatory agents because of suppression of chemical mediators released from mast cells and neutrophils[22,23] and also shown to be 5-lipo-oxygenase inhibitors as well as an inhibitors of peroxidation in rat liver microsomes[24], apart from their analgesic activity[10]. Because of chalcones proved to have antioxidant activity and expected that these analgesic activity caused by their free radical scavenging activity.

Conclusion

In conclusion, all these observations suggested that a combination of these might have contributed to the observed analgesic activity in this study but requires further studies to establish a specific mode of action, if any. Hence this present study may provide a useful direction to research on chalcones and related compounds, with a potential to get promising therapeutic agents from this structural class of compounds.

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