Selvakumar S. *et al.* / *International Journal of Phytopharmacology.* 3(2), 2012, 163-172.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

PHARMACOLOGICAL EVALUATION OF SOME POTENT 2-SUBSTITUTED BENZIMIDAZOLYL CHALCONES FOR ANALGESIC, ANTI-INFLAMMATORY, ANTHELMINTIC AND CENTRAL NERVOUS SYSTEM ACTIVITIES

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ABSTRACT

The Chalcone nucleus based plant natural products are widely explored due to their array of biological activities. If some these medicinal plants not traceable we must go through new synthetic pharmaceuticals as a source of chalcone analogues. So we prepared a series of chalcone derived benzimidazoles by condensation reaction of 2-acetyl benzimidazole with various substituted aromatic aldehydes in presence of alkali. The yields of the synthesized chalcones were ranged 62-91%. The synthesized chalcones were screened for their In-vivo antinociceptive and central nervous system locomotor activities. All the synthesized chalcones exhibited moderate to good significant analgesic activity and showed significant appreciable anti-inflammatory activity (except 6e & 6h). The tested chalcones 6d, 6f, 6g and 6h has depicted good central nervous system depressant properties. The In-vitro anthelmintic screening of all benzimidazolyl chalcones indicates that, have pronounced potency when compared to albendazole.

Keywords: Chalcones, Benzimidazoles, Benzimidazolyl chalcones, α, β unsaturated carbonyl compounds, Analgesic, Anti-inflammatory, Anthelmintic, Central nervous system depressant activity.

INTRODUCTION

The Chalcone analogues are pharmacophores of various natural plant products such as flavonoids, isoflavonoids, aurones, tetralones, aziridines and clavicin etc. The chemical structure of chalcones consists of two aromatic rings joined by the reactive a three carbon keto ethylene group called α, β unsaturated carbonyl system. This α, β unsaturated ketone chain exerts capto-dative effect by electronic delocalization of a free-radical position via extensive mesomeric effects and free-radical stabilization which makes chalcones highly biologically active. This nucleus found in various natural plant products such as terpeneoids, dicoumarol, digitalis glycosides (John H. Block *et al*., 2004) and vitamin K etc. However in recent years the introduction of new synthetic chalcone derived products has out placed that of natural products such as warfarin for dicoumarol.

The Chalcone derivatives are important intermediate and also act as precursor for the synthesis of novel cyanopyridines, pyrazolines, isoxazoles,pyrimidines and tetrazole (Bhaskar HV *et al*., 2011) having various heterocyclic compounds. The Chalcone analogues are a chemical class that has depicted antioxidant (Hocine Aichaoui *et al*., 2009), antimicrobial (Balkrishna Tiwari *et al*., 2010), antiviral, antibacterial (Yeshwant B. Vibhute *et al*., 2011), antifungal (Gurubasavaraja

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Swamy PM *et al*., 2008), insecticidal, antitrichomonal, antitubercular (Louise DC *et al*., 2008), antileishmanial, antimalarial, inhibit eosinophilia (asthma), antiseptical (José Quincoces *et al*., 1998), analgesic/anti-inflammatory (H-L Dimitra *et al*., 2008; Gummudavelly S *et al*., 2009), cyclooxygenase inhibitors (Syed Nasir Abbas Bukhari *et al*., 2012), anti ulcerogenic, prostaglandin binding, anxiolytic (Jamal H *et al*., 2008), antiallergic, anaesthetic, antiplasmodial (Xiang Wu *et al*., 2006), hypotensive, antifibrogenic, immunosuppression, antineoplastic, cytotoxicity, anticancer (Geetha Achanta *et al*., 2006), antiproliferative (Piyush Trivedi *et al*., 2011), antileukemic, antitumor (Shoji Shibata, 1993), trypsin inhibitor and anthelmintic (Biswajit Chandra Das, 2010) activities.

Benzimidazole is a bicyclic heterocyclic compound and chemically known as 1,3 dideazapurine. This nucleus is structurally related to purine nucleoside bases so it interact with all types of the biological macromolecules and it is found in some natural products , such as vitamin B_{12}, marine natural product namely makaluvamins etc. The literature survey reports have been revealed that the 2- substituted 1H-benzimidazole compounds has reported to possess antibacterial (Chhonker YS *et al*., 2009: Yogendra KS *et al*., 2011), antifungal (Kabilan S *et al*., 2006; Michael D *et al*., 2004), antitubercular (Shahar Yar M *et al*., 2009), antiviral (Vinodkumar P *et al*., 2005), central nervous system depressant (Gummudavelly *et al*., 2011), analgesic (K.Anandarajagopal *et al*., 2010; Abidin Ayalp., 1989), anti-inflammatory (Shanmugapandiyan P *et al*., 2010), anticonvulsant, antidiabetic, anti-allergic (Srikanth L *et al*., 2011), anti asthmatic, spasmolytic, anticancer (R.Kalirajan *et al*., 2010), antioxidant, antiulcer (Thomas CK *et al*., 1998) and anthelminitic activities (Sreena K *et al*., 2009).

The various derivatives of 2 and 5 or 6 substituted 1H-benzimidazole compounds are reported to possess ths DNA topoisomerase inhibitor (A.S. Alpan *et al*., 2007), antiulcer, antibacterial, antifungal, HIV-RT inhibitor (Aysegul A Y *et al*., 2007), antifeedant, acaricidal (Manish C *et al*., 2011), antihistaminic, antiprotozoal, protein kinase Ck2 inhibitor, antioxidant, antimyobacterial, histamine H_4-receptor antagonist (Nadeem Siddiqui *et al*., 2010), cysticidal and anthelminitic activities (Townsend L.B *et al*., 1990). So our research work was planned to incorporate the chalcone nucleus with benzimidazole at 2^{nd} position to exhibit some better pharmacological actions. This benzimidazolyl chalcones has also displayed antimicrobial (B.A. Baviskar *et al*., 2009) and benzimidazole derivative of chalcones are useful for the development of new antimicrobial heterocyclic agents (Janardan SY *et al*., 2011). These observations prompted us to synthesize some 2- substituted benzimidazolyl chalcones and to screen for their analgesic, anti-inflammatory, anthelminitic and central nervous system locomotor activities.

EXPERIMENTAL

Synthesis of 2-hydroxy ethyl benzimidazole (3)

The ortho-phenylene diamine (1) and lactic acid (2) were weighed equimolecular quantity in a round bottom flask and refluxed for one hour. The mixture was cooled to room temperature and 10% potassium hydroxide solution was added slowly until the mixture is just alkaline to litmus to yield the product. The 2-hydroxy ethyl benzimidazole (3) was filtered with ice cold water and recrystalized from ethanol.

Synthesis of 2-acetyl benzimidazole (4)

The 0.01 molar 2-hydroxy ethyl benzimidazole (3) was taken in a round bottom flask, to this 10ml 25% sulphuric acid and 0.005 molar potassium dichromate were added, then the mixture was refluxed for one hour. The mixture was cooled to room temperature and poured in to a beaker containing ice cold water, with stirring to get the oxidized product. The product 2-acetyl benzimidazole (4) was filtered with ice cold water and recrystalized from ethanol.

Synthesis of benzimidazolyl chalcones (6a-j)

Weighed 0.01 molar 2-acetyl benzimidazole (4) and 10ml 30% potassium hydroxide solution was taken in a round bottom flask to this add 0.012 molar of various aromatic aldehydes (5a-j) namely, anisaldehyde (5a,(R_1&R_2=H,R_3=OCH_3,n=1)), benzaldehyde (5b, (R_1,R_2&R_3=H, n=1)), Cinnamaldehyde (5c, (R_1,R_2&R_3=H, n=2)), Orthochloro benzaldehyde (5d, (R_1=Cl,R_2&R_3=H, n=1)), Para dimethyl aminobenzaldehyde (5e,(R_1&R_2=H,R_3=$N(CH_3)_2$, n=1)), Para-fluorobenzaldehyde (5f,(R_1&R_2=H,R_3=F,n=1)), Orthonitro benzaldehyde (5g, (R_1=NO_2,R_2&R_3=H, n=1)), Paranitro benzaldehyde (5h,(R_1&R_2=H,R_3= NO_2, n=1)), salicylaldehyde (5i, (R_1=OH, R_2&R_3=H, n=1)) and vanillin (5j, (R_1 =H, R_2=OCH_3& R_3= OH, n=1)) separately, with stirring then reflux the mixture for 2 hours, cool to room temperature and pour in to a beaker containing ice cold water, with stirring to get a product. The precipitated chalcones was filtered with ice cold water, washed, dried and recrystalized from ethanol. The chemical names of above synthesized benzimidazolyl chalcones as follows: 1-(1H-benzoimidazol-2-yl)-3-(4-methoxy phenyl) prop-2-en-1-one (6a), 1-(1H-benzoimidazol-2-yl)-3-Phenyl Prop-2-en-1-one (6b), 1-(1H-benzoimidazol-2-yl)-5-Phenyl Penta-2,4-dien-1-one (6c), 1-(1H-benzoimidazol-2-yl)-3-(2-Chloro Phenyl) Prop-2-en-1-one (6d), 1-(1H-benzoimidazol-2-yl)-3-(4-(dimethyl amino) Phenyl) Prop-2-en-1-one (6e), 1-(1H-

benzoimidazol-2-yl)-3-(4-Fluoro Phenyl) Prop-2-en-1-one (6f), 1-(1H-benzoimidazol-2-yl)-3-(2-nitrophenyl) Prop-2-en-1-one (6g), 1-(1H-benzoimidazol-2-yl)-3-(4-nitrophenyl) Prop-2-en-1-one (6h), 1-(1H-benzoimidazol-2-yl)-3-(2-hydroxyphenyl) prop-2-en-1-one (6i), 1-(1H-benzo imidazol-2-yl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (6j).

The purity of the synthesized benzimidazolyl chalcones were checked by Thin Layer Chromatography using silica gel-60 F_{254} aluminium sheets using chloroform: ethanol (8:2) as eluent and identified in a ultra violet chamber. The chalcones were characterized by melting point and Rf values. The chemical structures of all synthesized benzimidazolyl chalcones had been established on the basis of their Infra-Red, ^{1}HNMR, ^{13}CNMR and mass spectral data (Sudeerbabu.I *et al*., 2012).

MATERIALS AND METHODS

Animals

The selected Albino mice and Albino rats of wistar strain either sex were procured from the animal house. The animals were maintained in polypropylene cages in standard environmental conditions at 25^0c-30^0c in a 12 hours light/dark cycle. The animals were fed with standard rodent laboratory pellet diet and water libitum. The experiments carried out according the protocols with guidelines duly approved by the Institutional Ethical Committee. Ethical Committee clearance approval was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for Purpose of Control and Supervision of Experiments on Animals).

Pharmacological Study

Testing for analgesic activity

The in-vivo analgesic activity was evaluated by writhing reflex syndrome test (H.Gerhard Vogel, 2002) using mice of either sex with a weight between 20 and 25 grams are used. The groups of five animals were used for controls and treated mice. The control group received only 1% CMC in water for injection. The tested concentrations of chalcones and standard drug diclofenac prepared at a doses level of 10, 20 & 30 mg/ kg were suspended in 1% carboxy methyl cellulose. The chalcones and standard drug administered orally by intragastric tube 60 min prior to intraperitoneal injection of 0.1ml of 3% v/v aqueous acetic acid in water for injection. The mice were then observed for a period of 15 min and the number of writhing reflex is recorded for each animal. For scoring purposes, a writhing reflex is characterized by stretching of the abdomen with simultaneous stretching of one hind limb. The total number of writhing reflex induced in these mices were counted visually for 15 minutes and the number of writhing reflex induced in tested groups were compared with those in control group. The formula for calculating percentage inhibition is: average writhing reflex in the control group minus writhing reflex in the drug group divided by writhing reflex in the control group times 100%. The time period with the maximum percentage of inhibition is considered the peak time. The analgesic activities were expressed as percentage protection and were analyzed statistically.

Estimation of inflammation

The in-vivo anti-inflammatory activity of synthesized benzimidazolyl chalcones was evaluated using carrageenan induced rat hind paw edema method (Atta-ur-Rahman *et al*., 2005). The Wister albino rats of either sex weighing 180-220 grams were used for experiment. The rats were divided into control, standard and tested synthesized chalcones groups, each group consisting of six animals. The standard diclofenac and test chalcones compounds were prepared as a suspension about using 1% sodium carboxy methyl cellose in water for injection. The first group was administered with 1% CMC suspension which served as control, second groups were treated with a doses of 10 mg/kg, 20 mg/kg & 30 mg/kg 1% CMC suspensions of diclofenac separately which served as standard groups and other groups were treated with a doses of 10 mg/kg, 20 mg/kg & 30 mg/kg in about 0.3ml suspension of 1% CMC suspensions of test chalcones compounds. The tested chalcones and standard drug administered orally by intragastric (stomach) tube 60 min before the induction of inflammation. An acute inflammation was induced by a subcutaneous injection of irritant 0.1ml of 1% carrageenen in to the plantar surface region of right hind paw before that rats were lightly anaesthetized with diethyl ether with chloroform. The rats paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The rats paw volume was measured using plethysmographically by using the mercury displacement technique, after carrageenan injection immediately and then after at hourly intervals for up to about 5 hours. Oedema is expressed as a mean increase in paw volume with respect to 1% CMC control. The variation of rats paw oedema volume up to 5 hours was calculated as percentage inhibition of oedema, compared with the volume measured immediately after injection of the irritant for each animal. Any significant increase or decrease in the volume of the paw compared to the control group was considered as anti-inflammatory response. The difference of average values between treated groups and control group is calculated for each one or two intervals and were statistically evaluated. The difference values at the tested time intervals provide some hints for the duration of the anti-inflammatory effect. The formula for calculating percentage inhibition of inflammation is: average mean edema of control group

minus average mean edema of treated group divided by average means edema of control group times 100%. The anti-inflammatory activity was measured in terms of percentage inhibition of edema at various time intervals and was analyzed statistically.

Evaluation for CNS-Locomotor activity

The central nervous system stimulant or depressant property of the benzimidazolyl chalcones for its locomotor activity was evaluated by using actophotometer (Ganesh A *et al*., 2011). The albino mice of either sex weighing 20-28 g were separated into control, standards and test groups of five mice in to each group. The 0.3ml of 1% CMC suspension vehicle was given for five days once daily before starting the experiment. The mices were fasted for six hours before experiment and they were allowed to adapt to the actophotometer cage environment for at least five minutes. The actophotometer was calibrated prior to experimentation. The mouse was placed individually in the digital actophotometer and the basal activity counts of each animal were noted for15 minutes 2 days before to start experiment. A count is recorded when the beam of light falling on the photoelectric cell of activity cage which connected a internal circuit with a counter, is cut off by mouse. This evaluation involves placing a mouse separately in an actophotometer which enables movement of the mice across a light beam to be measured as a locomotion count. The tested chalcones were administered orally by intragastric (stomach) tube at a doses of 10 mg/kg, 20 mg/kg & 30 mg/kg body weight of mouse in the form of suspension in 0.3ml of 1% CMC while two other groups administered with diazepam at a dose of 5mg/kg and caffeine at a dose of 10mg/kg body weight as a standard drugs, these also administered in the form of suspension in 0.3ml of 1% CMC. The control group mice received with 0.3ml suspension of 1% CMC in drinking water. The locomotor behaviour was observed after 60 minutes of administration of drugs, the activity cage counts were measured for a period of 15 minutes. The increase or decrease in the number of counts for each groups were recorded. The mean average scores for standard and chalcone groups were compared the results with control group. The percentage central nervous system stimulant or depressant activity was then calculated.

Anthelmintic Investigation

The synthesized benzimidazolyl chalcone were tested for anthelmintic activity by in-vitro bioassay method (B Lakshmanan *et al*., 2011). The south Indian adult earth worms *Pheretima posthuma of* 7-9cm in length and 0.2-0.3 cm in width were selected for the invitro anthelminitic activity due to its anatomical and physiological resemblance with the gastro intestinal worm parasites of human beings. The earth worms of nearly equal size (8 ± 1cm) were taken and than washed thoroughly with normal saline solution to remove all fecal and adhering soil materials before they were released in to petridishes which containing of 15 ml of normal saline solution with various drug concentration. The earth worms were separated into the control, standard and tested chalcones groups of five earthworms in each group. All the tested benzimidazolyl chalcones and the standard drug solutions were freshly prepared before commencement of the experiments. The control group petridish contains only 0.5ml of dimethylsulphoxide in 14.5ml of normal saline solution.

The standard drug albendazole and tested chalcones were prepared at a doses level of 10, 30,50 mg by dissolving in minimum quantity, about 0.5ml of dimethylsulphoxide and the volume was made up to 15 ml with normal saline, then poured into petridishes. The five earth worms were taken in each petridishes at room temperature. Then observe, the time taken for the induction of complete paralysis and time taken for death of individual earthworms were noted. The control group observed that the worms were still alive up to 48 hours.

The time taken for individual worm to become motionless and donot revive reflex even in normal saline was noted as paralysis time. The death time was ascertained by inducing external stimuli if not placing the individual worms in warm water at 50^{o}C which stimulate and induce movement of worms, if alive. The mean average paralysis time and mean average death time were calculated for each tested concentrations of the chalcones.

Statistical analysis

The recorded values of each groups were expressed as mean ± standard error mean (SEM). The values obtained from the pharmacological parameters in synthesized chalcones were compared with that control group if not standard group using One-way analysis of variance (ANOVA) was followed by Tukey multiple comparison tests. The criterion for statistical significance is P values must be less than 0.05. The value of $P < 0.001$ indicates excellent significance, $P< 0.01$ indicates good significance and $P < 0.05$ indicates satisfactory significant levels between the groups.

RESULTS AND DISCUSSION

Chalcone nucleus based natural products are used in Ayurveda, Sidddha system of medicines and pharmaceuticals due to their chemical structure. Many successful efforts have been made to improve their therapeutic properties by structural modifications and various synthetic routes. In this way our study based on the synthesis of various substituted benzimidazolyl

chalcones and evaluated for their antinociceptive, anthelmintic and central nervous system locomotor activity. The results of the In-vivo analgesic activity were displayed in Table-1. It revealed that all the synthesized benzimidazolyl chalcones at different tested concentrations 10mg/kg, 20mg/kg and 30mg/kg were showed significant analgesic activity against control, and also activity steeply increases when increasing concentration of chalcones. It has been observed that benzimidazolyl chalcones 6d & 6i were exhibited excellent and 6a, 6f & 6j were showed good analgesic activity when compared with that of the standard drug diclofenac. The benzimidazolyl chalcones 6g, 6e exerted moderate and 6b, 6c & 6h were possess weak analgesic activities. The anti-inflammatory screening data noticed in Table-2, it shows that some benzimidazolyl chalcones were given significant activity at 20mg/kg and 30mg/kg concentrations. In comparison with diclofenac, chalcones 6d, 6f, 6i, 6j were exhibited good and chalcone 6a displayed considerable anti-inflammatory activities. On the other hand, chalcones 6b, 6c have lowest and chalcones 6e, 6g, 6h were possessing bad anti-inflammatory activity. It was noticed that, anti-inflammatory activities increases when increasing concentration of chalcones (except 6e & 6h).

When structure activity relationship studies are concerned, benzimidazolyl chalcones bearing hydroxy, methoxy or dimethylamino electron donating substituted analogues and electron withdrawing substituents such as nitro, fluoro or chloro has shows more analgesic activity than unsubstituted chalcones. In the series, polar electron donating substituent like hydroxy and methoxy group is found to exhibit the more analgesic activity than nonpolar electron donating substituent like dimethylamino group. Meanwhile, the electronegative fluoro, chloro analogues also displayed good analgesic properties than bulky nitro substituted chalcones. All the tested ortho substituted chalcones were exert more potent analgesic activity than para substituted chalcones. The results shows that fluoro, chloro, hydroxy and methoxy substituted chalcones were showed appreciable anti-inflammatory properties but the bulky dimethylamino and nitro substituted chalcones exert bad anti-inflammatory activity than the unsubstituted chalcones. Moreover, the ortho hydroxyl, ortho chloro substituted chalcones (6d, 6i) produced excellent antinociceptive activity than that of para hydroxyl and para or meta methoxy substituted chalcones (6a, 6j). Among this para fluoro substituted chalcone (6f) marked equal antinociceptive potency to the para hydroxyl substituted chalcones (6j). Moreover extended conjugated analogue (6c) slightly reduces antinociceptive activity than parent benzimidazolyl chalcone (6b).

The central nervous system locomotor activity of the benzimidazolyl chalcones was evaluated by using actophotometer and the results are shown in Table-3. The data noticed that most of the chalcones were shows varying degrees of reducing locomotor activities against control group. All the synthesized chalcones were fails to show central nervous system stimulant activity and also most of the chalcones does not produce significant depressant activity at the tested 10mg/kg & 20mg/kg low concentrations but a all the chalcones produced significant decrease of locomotor activity were observed at 30mg/kg high concentration except 6i and 6j. Among this, the chalcones 6d, 6f, 6g, 6h were only expressed higher depressant activity when compared to diazepam and chalcones 6b, 6c revealed mild depressant properties. So the structure activity relationship proves that electron withdrawing substituents such as nitro, fluoro or chloro groups must be necessary to improve central nervous system depressant properties. Furthermore the chalcone 6d & 6g possess less number of cut-offs than 6f & 6h. The results also revealed that bulky electron withdrawing nitro group in a ortho position substituted chalcones (6g) produced better depressant activity than ortho chloro substituted chalcone (6d) but the para fluoro substituted chalcone (6f) elicits more central nervous depressant system activity than bulky para nitro substituted analogue. Meanwhile the non polar electron donating dimethylamino or methoxy substituted chalcones analogues (6e & 6a) shows more number of cut-offs and polar electron donating hydroxy substituted chalcones (6i & 6j) high number cut-offs values when compared to the unsubstituted chalcones analogues (6b & 6C). The extended conjugated analogue (6c) also slightly elevates the locomotor activity than parent benzimidazolyl chalcone (6b).

In-vitro anthelmintic screening results of benzimidazolyl chalcone were depicted in Table-4. The chalcones acquired the anthelmintic activity at minimal dose of 10 mg/dish. The investigation of all tested groups of chalcones at tested concentrations 10mg, 30mg and 50mg had shown significant anthelmintic activity compared to the standard drug albendazole. It was observed that while increasing the concentrations of chalcones and albendazole significantly reduced the time taken for paralysis and death as well. In which chalcones 6a, 6c, 6d, 6g, 6h and 6i showed appreciable action for time taken to paralysis and death when compared to the standard drug albendazole. A closer inspection of the data indicates that chalcones 6f and 6j exhibited higher potency than other tested chalcones while the chalcones 6b and 6e were registered comparable less activity with other tested chalcones for all the tested concentrations. The structure activity relationship studies revealed that polar electron donating substituent's like hydroxy and

methoxy group is found to increase the anthelmintic properties, where as non polar electron donating substituent dimethylamino group found to reduce activity. It has been observed that unsubstituted chalcones (6b, 6c) marked more effective anthelmintic activity than dimethylamino substituted chalcone (6e). The chalcones with methoxy substituent at the para position (6a) led to a enhance the anthelmintic activity than meta position substituted chalcone (6j). Moreover, the para hydroxy substituted chalcones (6j) have more potent anthelmintic activity than ortho hydroxyl substituted chalcone (6i). The para fluoro substituted chalcone (6f) have excellent potency than ortho chloro substituted chalcone (6d). The bulky electron withdrawing nitro group shows less potency than fluoro and chloro groups. Among this, para nitro substituted chalcone (6h) produced more activity than that of ortho nitro substituted chalcones (6g) and the extended conjugated analogue (6c) displayed better activity than parent benzimidazolyl chalcone (6b). Indeed, these all substituted and unsubstituted benzimidazolyl chalcones endowed with high anthelmintic potency.

Table 1. In vivo analgesic activity of synthesized benzimidazolyl chalcones (6a-j) by writhing reflex syndrome method

S.No	Groups	Number of writhing reflex up to 15 minutes			Percentage protection		
		10 mg/kg	20 mg/kg	30 mg/kg	10 mg/kg	20 mg/kg	30 mg/kg
1	6a	24.8±0.66***	19.6±0.50***	19.0±0.83***	24.39	40.24	42.07
2	6b	28.2±0.58***	24.4±0.40***	23.6±0.40***	14.02	25.60	28.04
3	6c	28.6±0.60***	24.8±0.58***	24.6±0.74***	12.80	24.39	25.00
4	6d	22.8±0.48***	17.0±0.77***	15.8±0.66***	30.48	48.17	51.82
5	6e	25.2±0.37***	20.6±0.50***	19.6±0.40***	23.17	37.19	40.24
6	6f	24.2±0.73***	17.6±0.67***	16.8±0.48***	26.21	46.34	48.78
7	6g	26.4±0.40***	21.8±0.58***	21.2±0.37***	19.51	33.53	35.36
8	6h	27.2±0.48***	23.6±0.67***	22.6±0.40***	17.07	28.04	31.09
9	6i	22.2±0.58***	16.0±0.54***	15.4±0.40***	32.31	51.21	53.04
10	6j	23.8±0.66***	18.2±0.58***	17.0±0.63***	27.43	44.51	48.17
11	STD	21.4±0.60***	12.8±0.58***	09.0± 0.31***	34.75	60.97	72.56
12	CL	32.8±.58			-		

*Each average value represents as the mean ± SEM (n=5). Significance level ***P<0.001 as compared with the respective control. STD-Diclofenac, CL-Control.*

Table 2. In vivo anti-inflammatory activity of synthesized benzimidazolyl Chalcones (6a-j) by carrageenan induced rat hind paw oedema method

Groups	Dose (mg/kg)	Rat paw edema volume				% protection		
		After 0 hr	After 1 hr	After 3 hr	After 5 hr	After 1 hr	After 3 hr	After 5 hr
6a	10	0.80±0.05	1.40±0.05	1.76±0.03	2.06±0.04	4.1	7.3	7.6
	20	0.80±0.07	1.26±0.04*	1.66±0.02**	1.86±0.04***	13.6	12.6	16.5
	30	0.83±0.06	1.23±0.03**	1.50±0.06***	1.63±0.08***	15.7	21.0	26.9
6b	10	0.80±0.07	1.43±0.06	1.86±0.04	2.20±0.05	2.0	2.1	1.3
	20	0.83±0.03	1.33±0.04	1.73±0.04	2.03±0.06	8.9	8.9	8.9
	30	0.80±0.05	1.36±0.06	1.60±0.05**	1.80±0.05***	6.8	15.7	19.2
6c	10	0.83±0.03	1.46±0.04	1.86±0.04	2.23±0.03	-	2.1	-
	20	0.80±0.05	1.40±0.05	1.73±0.04	2.06±0.06	4.1	8.9	7.6
	30	0.83±0.03	1.36±0.03	1.66±0.06*	1.83±0.06**	6.8	12.6	17.9
6d	10	0.83±0.03	1.33±0.06	1.63±0.03***	2.00±0.05**	8.9	14.2	10.3
	20	0.83±0.03	1.20±0.05**	1.43±0.03***	1.60±0.07***	17.8	24.7	28.2
	30	0.83±0.03	1.06±0.04***	1.26±0.04***	1.43±0.06***	27.3	33.6	35.8
6e	10	0.80±0.05	1.43±0.03	1.83±0.03	2.13±0.04	2.0	3.6	4.4
	20	0.83±0.06	1.40±0.05	1.80±0.05	2.10±0.04	4.1	5.2	5.8
	30	0.80±0.07	1.30±0.04	1.80±0.05	2.06±0.04	10.9	5.2	7.6

6f	10	0.83±0.03	1.36±0.06	1.70±0.04*	2.00±0.05**	6.8	10.5	10.3
	20	0.83±0.06	1.23±0.03**	1.56±0.03***	1.63±0.06***	15.7	17.8	26.9
	30	0.80±0.07	1.16±0.03***	1.36±0.06***	1.50±0.08***	20.5	28.4	32.7
6g	10	0.83±0.03	1.46±0.06	1.90±0.04	2.23±0.03	-	-	-
	20	0.80±0.05	1.43±0.03	1.83±0.03	2.13±0.04	2.0	3.6	4.4
	30	0.80±0.07	1.40±0.05	1.76±0.03	2.00±0.07	4.1	7.3	10.3
6h	10	0.80±0.05	1.46±0.04	1.90±0.04	2.23±0.03	-	-	-
	20	0.83±0.03	1.46±0.04	1.86±0.04	2.13±0.04	-	2.1	4.4
	30	0.83±0.06	1.43±0.03	1.73±0.04	2.06±0.06	2.0	8.9	7.6
6i	10	0.83±0.03	1.33±0.06	1.63±0.03***	1.66±0.02***	8.9	14.2	25.5
	20	0.80±0.07	1.16±0.03***	1.36±0.03***	1.50±0.06***	20.5	28.4	32.7
	30	0.80±0.05	1.03±0.03***	1.20±0.05***	1.40±0.07***	29.4	36.8	37.2
6j	10	0.80±0.05	1.36±0.06	1.70±0.04*	2.03±0.03*	6.8	10.5	8.9
	20	0.83±0.06	1.23±0.03**	1.53±0.04***	1.66±0.04***	15.7	19.4	25.5
	30	0.83±0.03	1.16±0.06***	1.33±0.06***	1.53±0.08***	20.5	30.0	31.3
STD	10	0.80±0.07	1.26±0.04*	1.43±0.06***	1.50±0.06***	13.6	24.7	32.7
	20	0.80±0.05	1.10±0.04***	1.16±0.06***	1.23±0.06***	24.6	38.9	44.8
	30	0.83±0.06	0.96±0.03***	1.03±0.03***	1.06±0.04***	34.2	45.7	52.4
CL		0.80±0.05	1.46±0.04	1.90±0.04	2.23±0.03	-	-	-

*Each average value represents the mean ± SEM (n=6). Significance levels *P<0.5, **P<0.01 and ***P<0.001 as compared with the respective control. STD-Diclofenac, CL-Control.*

Table 3. In vivo Central nervous system-locomotor activity of synthesized benzimidazolyl Chalcones (6a-j) by using actophotometer

Groups	Average number of movements in 15 minutes					
	10 mg/kg	Percentage	20 mg/kg	Percentage	30 mg/kg	Percentage
6a	152.6±1.03	99.6	149.8±1.82	97.7	132.6±2.11***	86.5
6b	150.2±0.96	98.0	148.8±1.68	97.1	128.2±2.26***	83.6
6c	150.6±0.81	98.3	148.0±2.07	96.6	121.8±2.15***	79.5
6d	146.2±1.06**	95.4	126.0±1.92***	82.2	85.8±2.35***	56.0
6e	152.2±1.11	99.3	150.0±1.58	97.9	135.2±1.98***	88.2
6f	147.2±1.02*	96.0	126.8±1.20***	82.7	90.0±2.42***	58.7
6g	144.2±0.96***	94.1	118.6±1.80***	77.4	78.6±3.07***	51.3
6h	148.2±1.06	96.7	131.2±1.59***	85.6	93.0±2.64***	60.7
6i	152.0±1.04	99.2	149.2±2.03	97.3	143.4±3.29	93.6
6j	153.0±1.09	99.8	152.4±1.56	99.4	149.2±2.47	97.3
Caffeine	208.4±1.63***	136.0	Not tested	--	Not tested	--
Diazepam	073.2±1.39*** (5 mg/kg)	47.7	Not tested	--	Not tested	--
control	153.2±1.02			Nil		

*Each average value represents the mean ± SEM (n=5). Significance levels *P<0.5, **P<0.01 and ***P<0.001 as compared with the respective control.*

Table 4. *In vitro* anthelminitic activity of the synthesized benzimidazolyl chalcones (6a-j)

Groups	Time taken for paralysis (P)			Time taken for death (D)		
	10 mg/group	30 mg/group	50 mg/group	10 mg/group	30 mg/group	50 mg/group
6a	29.30±1.24*	18.05±0.53**	16.15±0.46***	45.37±0.50***	33.05±0.61***	24.45±0.18***
6b	30.22±1.01**	20.05±0.58***	18.56±0.21***	51.25±0.65***	38.25±0.58***	29.07±0.16***
6c	32.10±1.17***	19.45±0.57***	18.09±0.47***	51.18±0.62***	37.44±0.64***	27.48±0.31***
6d	30.02±0.97**	18.38±0.51***	17.50±0.26***	48.02±0.78***	35.06±0.52***	25.12±0.26***
6e	29.56±0.80**	20.40±0.38***	20.25±0.27***	53.02±0.72***	39.47±0.63***	30.22±0.33***

6f	28.58±0.99*	17.22±0.59*	14.29±0.35**	40.26±0.66*	29.03±0.57*	21.05±0.15***
6g	30.43±1.33**	19.12±0.53***	19.05±0.29***	51.40±0.52***	37.20±0.59***	27.25±0.19***
6h	30.06±1.11**	18.55±0.59***	18.22±0.27***	49.44±0.70***	35.43±0.57***	25.38±0.20***
6i	29.57±1.18*	18.21±0.57**	17.32±0.34***	46.19±0.51***	33.40±0.40***	24.10±0.21***
6j	29.07±0.90*	17.25±0.53*	15.19±0.36***	42.27±0.64***	30.25±0.60***	22.18±0.24***
ALZ	23.51±0.91	14.55±0.62	12.33±0.21	37.24±0.33	26.28±0.26	19.12±0.07

*Each average value represents the mean ± SEM (n=5). Significance levels *P<0.5, **P<0.01 and ***P<0.001 as compared with the respective standard drug (ALZ- Albendazole). The control group worms were alive more than 48 hours.*

CONCLUSION

In summary, we have described the synthesis, a series of 2-substituted benzimidazolyl chalcones. The In-vivo analgesic activity data indicates that chalcones 6d, 6f, 6i and 6j have higher activity than other groups. The chalcones 6a, 6d, 6f, 6i and 6j analogues exhibited good anti-inflammatory activity. Among this chalcones 6d and 6i only exert high antinociceptive potency, so it proves that ortho electron donating hydroxy group substituted analogue and ortho electron withdrawing chloro substituted chalcone has given potent antinociceptive properties. On the other hand bulky electron donating dimethylamino and electro negative nitro substituted chalcones drastically reduce the anti-inflammatory activity than unsubstituted chalcones. The central nervous system activity data exhibiting that, the electron withdrawing group in ortho position substituted chalcones (6d,6g) produce slightly more central nervous system depressant activity than that of para substituted chalcones (6f,6h). The polar hydroxy analogue fails to produce considerable depressant activity. All the tested chalcones with endowed potent anthelmintic activity at 30mg and 50mg concentrations. Among this, para substituted polar electron donating substituted and para substituted electron withdrawing substituted analogues exposed excellent comparable activities against albendazole. Finally SAR studies concluded that ortho substituted chalcone analogues exhibit an elevated analgesic and anti-inflammatory activity than the corresponding para substituted and unsubstitued chalcones. In fact, the electron withdrawing substituted chalcones only exert central nervous system depressant activity. Indeed, these the para polar electron donating or para electron withdrawing substituted chalcones marked better anthelmintic activity than the corresponding ortho substituted and unsubstituted chalcones. Moreover the extended conjugated analogue (6c) depicted less antinociceptive activity but displayed comparablely more depressant and anthelmintic activity than parent benzimidazolyl chalcone (6b).

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