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Development of a Chalcone-Based Therapeutic Candidate for Neuropathic Pain: Synthesis and Screening in AITC-Induced Paw Flinching Model

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Abstract

*A neotric chalcone derivative, (E)-3-(4-(dimethylamino) phenyl)-1-(2,4-dimethylphenyl) prop-2-en-1-one (**4**), synthesized and evaluated for its potential to alleviate neuropathic pain. The synthesis was achieved through a grinding method involving the condensation of 2,4-dimethyl acetophenone and 4-dimethylamino benzaldehyde in the presence of 10% sodium hydroxide. The compound was characterized using spectroscopic techniques, and its structure was confirmed as an E isomer. The biological activity of the compound was tested using an allyl isothiocyanate (AITC)-induced paw flinching model in rodents (rats), that exhibited a dose-dependent reduction in pain-related behavior. The chalcone with dose 100 mg/kg, test compound **4** reduced the number of flinches to 20, outperforming curcumin at the same dose but demonstrating less efficacy than the standard TRPA1 antagonist, HC-030031. These findings suggest that the synthesized chalcone has potential as a therapeutic candidate for neuropathic pain, though further structural optimization is needed to enhance its activity.*

Keywords: *Chalcone derivative, Neuropathic pain, Allyl isothiocyanate (AITC), Paw flinching model, TRPA1 antagonist, Structural optimization.*

1. Introduction

Neuropathic pain, long lasting pain condition ensuing nerve damage or dysfunction, poses suggestive challenges in clinical management (Cohen et al. 2021, Finnerup et al. 2020). Neuropathic pain is characterized by symptoms such as burning sensations, tingling, and spontaneous pain, which are often resistant to conventional analgesics (Shinu et al. 2022). The global burden of neuropathic pain is substantial, affecting millions of people and leading to decreased quality of life, increased healthcare costs, and a pressing need for effective therapeutic interventions (Andrew et al. 2014) The complexity of neuropathic pain mechanisms, involving multiple pathways such as ion channel dysregulation, inflammatory mediators, and neuroimmune interactions, limits the efficacy of current treatments (Fernandes et al. 2018, Viswanath et al. 2020). The available drugs, including gabapentinoids, tricyclic antidepressants, and opioids, often provide inadequate pain relief with related side effects including sedation, cognitive impairment and risk of addiction (Kim et al. 2020, Marciano et al. 2023). This highlights the urgent need for novel therapeutic agents that can effectively target the underlying mechanisms of neuropathic pain with better safety profiles.

Chalcones, a class of naturally occurring and synthetically accessible open-chain flavonoids, have garnered attention for their diverse pharmacological properties (Elkanzi et al. 2022, Afzal et al. 2019, Constantinescu et al. 2021, Shaik et al. 2017, Lokesh et al. 2017), including anti-inflammatory (Dhaliwal et al. 2022, Vasudha et al. 2024), antioxidant (Mittal et al. 2022, Konidala et al. 2021) and neuroprotective (Pérez-González et al. 2022, Adelusi et al. 2021, Martins et al. 2021) effects. These properties make chalcones promising candidates for the management of nociceptive pain. The structure-activity relationship (SAR) studies on chalcones have demonstrated that substitution at various positions on the aromatic rings can significantly modulate their biological activities. Specifically, chalcones with electron-donating groups, such as hydroxyl and methoxy, have shown enhanced anti-inflammatory and neuroprotective effects, which are critical in the context of neuropathic pain. Several chalcones have been reported in the literature for their potential in alleviating neuropathic pain (**Figure 1**). For instance, isoliquiritigenin (**1**) elicit its analgesic effect mainly by inhibiting Na_v channels (Miyamura et al. 2021) whereas compound **2** reduced mechanical allodynia and thermal hyperalgesia while inhibiting macrophage pro-inflammatory polarization in vincristine-induced peripheral neuropathy (VIPN) mice (Marchon et al. 2024). On the other hand, Hesperidin methyl chalcone (**3**) elucidated its anti-TRPV1 agonist induced inflammation and pain by inhibiting cytokine production, NF-κB activation and oxidative stress (Pinho-Ribeiro et al. 2024).

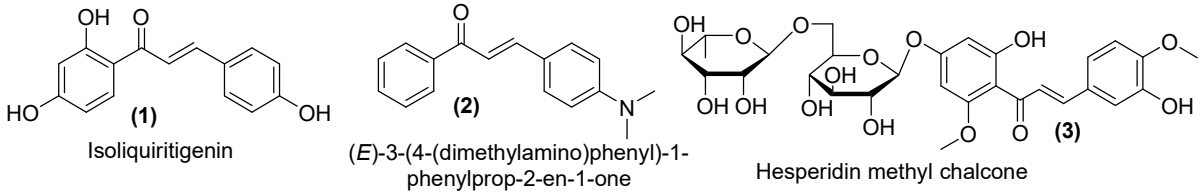


Figure 1. Substituted chalcones with potential action in neuropathic pain.

Despite the promising results of chalcones, there remains a critical need for the development of new lead compounds with improved potency, selectivity, and safety profiles. The heterogeneity of neuropathic pain pathophysiology necessitates the exploration of novel chalcone derivatives with useful actions. Additionally, the high attrition rate of drug candidates in clinical trials underscores the importance of identifying and optimizing new scaffolds that can address the unmet medical needs in neuropathic pain management. In this

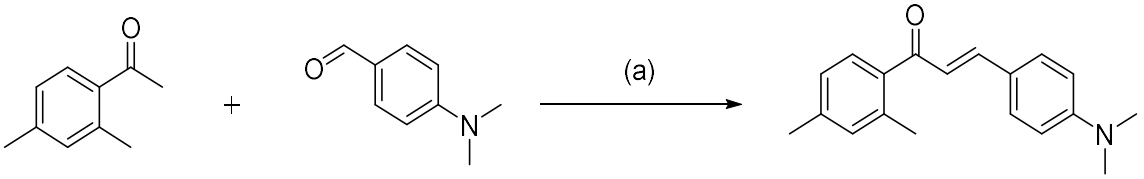
context, we have synthesized a innovative chalcone derivative -(*E*)-3-(4-(dimethylamino) phenyl)-1-(2,4-dimethylphenyl) prop-2-en-1-one (**4**) and evaluated its efficacy in a well-established animal model of neuropathic pain. Our study aims to expand the therapeutic arsenal against neuropathic pain by introducing a new chalcone with potential clinical applications. The present work focuses light to the understand chalcone pharmacology for evaluating ns new avenues for the development of effective neuropathic pain treatments.

2. Material and Methods

2.1. Chemicals and Instruments

The diluters like ethyl acetate, hexane and methanol of spectral grade were used directly deprived of additional purification were procured from S.D. Fine Chem. Ltd, Mumbai, India. The starting materials used for the synthesis 2',4'-Dimethyl acetophenone and 4-(dimethylamino)benzaldehyde were purchased from BLD Pharmatech Pvt Ltd, Hyderabad, Telangana, India and Sisco Research Laboratories Pvt Ltd, Maharashtra, India respectively. Allyl isothiocyanate (AITC) and the standard TRPA1 antagonist (HC-030031) were purchased form Sigma Aldrich-Merck, Karnataka, India. Thin-layer chromatography (TLC) was conducted on Merck precoated silica gel 60 F254 plates, with spot detection under UV light. Melting points were measured using a Boetius melting point apparatus in open capillaries and are uncorrected. FT-IR spectra were recorded on a Bruker alpha-T spectrometer, whereas 1H and 13C NMR spectra were measured on a Bruker 400 Avance NMR spectrometer with TMS as the internal standard, and mass spectra were recorded using an Agilent LC-MS spectrometer. Animals and the standard pellet diet for the animals were acquainted from Mahaveer Enterprise, Hyderabad, India.

2.2. Synthesis of (*E*)-3-(4-(dimethylamino)phenyl)-1-(2,4-dimethylphenyl)prop-2-en-1-one (4): We synthesized the target compound **4** by grinding 2,4-dimethyl acetophenone (0.001 mol) with 4-dimethylamino benzaldehyde (0.001 mol) and 10% sodium hydroxide, and the mixture was tirturated thoroughly using a mortar and pestle for 10 minutes. The resulting mixture was then allowed to solidify at room temperature for 30 minutes. The solid formed was dissolved in cold distilled water and then acidified with dilute hydrochloric acid, which caused a to form a dark orange chalcone precipitate. This precipitate was filtered, dried, and recrystallized from ethanol, producing chalcone with a 90% yield.



2',4'-Dimethyl acetophenone 4-(dimethylamino)benzaldehyde (*E*)-3-(4-(dimethylamino)phenyl)-1-(2,4-dimethylphenyl)prop-2-en-1-one (**4**)

Scheme 1. Synthesis of (*E*)-3-(4-(dimethylamino) phenyl)-1-(2,4-dimethylphenyl)prop-2-en-1-one (**4**); a. Grinding at room temperature, 10% NaOH.

2.3. Biological activity

2.3.1. AITC induced paw flinching behaviour

2.3.1.1. Experimental Animals

Albino Wistar rats of either sex weighing between 150-250 grams were selected for screening neuropathic pain. The animals survived in controlled environment with a 12-hour light-dark cycle, a relative humidity of 50±5% and a temperature of 21°C ±2°C. They were fed with a standard pellet diet ad libitum. All experiments adhered to the National Institutes of Health guidelines (Guide for the Care and Use of Laboratory Animals) and the standards set by the International Association for the Study of Pain to minimize the animals used and reduce discomfort (Zimmermann et al., 1983, National Institutes of Health 1985). Animals were ascribed randomly to different groups to eliminate bias and the researcher conducting the behavioral observations is blinded to the treatment groups to ensure objective assessment of the results. The study received approval from the Animal Ethics Committee, Narasaraopeta Institute of Pharmaceutical Sciences, Narasaraopeta, Andhra Pradesh, India.

2.3.1.2. Screening model

The neuropathic pain activity was evaluated using the paw flinching behavior induced by Allyl thiocyanate (AITC) in rats. The animals were divided into ten groups, with each group containing six animals. AITC was used as the neuropathic pain inducer for all the groups. Healthy, overnight-fasted animals were selected for the screening process. The vehicle (80% DMSO + 20% Tween), standard drug [TRPA1 antagonist (HC-030031)], and different doses of chalcone (compound **4**) were administered through the intraperitoneal route. The animals were administered with the standard and graded doses of chalcones, followed by intraplantar injection of 30 µl of AITC dissolved in vehicle. Among the six groups, one group (Group-1) served as the negative control group treated with only vehicle, and a second group (Group-2) served as a standard that was administered with vehicle and TRPA1 antagonist at a dose of 10 mg/kg/2ml. Rest of the groups received compound **4** at doses of 3, 10, 30, and 100 mg/kg/2 ml respectively. Each animal was placed separately to prevent interaction, and the number of flinches was recorded for one hour. The flinching behaviour was recorded at 5-minutes interval at the late phase (from 20-45 minutes after drug administration).

2.3.1.3. Statistical Analysis

All data were presented as the mean ± standard error of the mean (SEM). Group averages were analyzed using one-way ANOVA, and Tukey's post hoc test was applied when necessary. Statistical analysis was conducted on the raw data with GraphPad Prism 5 (GraphPad Software, Inc.), and a P-value below 0.0001 was deemed statistically significant.

3. Results and Discussion

3.1. Chemistry

The target compound (4) was prepared grinding supported condensation between 2,4-dimethyl acetophenone and 4-dimethylamino benzaldehyde, with 10% sodium hydroxide serving as catalysts, as depicted in Scheme 1. The orange color product's (**Figure 2**) purity was verified through thin-layer chromatography, followed by the determination of its melting point. The compound's structure was characterized using spectroscopic techniques, and the corresponding data are provided below. Based on the observed coupling constant (J = 17 Hz), the compound is inferred to adopt an *E* isomeric configuration.

Figure 2. The orange-colored target compound **4** is visually depicted above.

(E)-3-(4-(dimethylamino)phenyl)-1-(2,4-dimethylphenyl)prop-2-en-1-one (**4**): Dark orange color powder (0.212 g, Yield: 90%), m.p. 106 °C, R_f = 0.52 (20% ethyl acetate and *n*-hexane), MS (*m*/*z*, %) 280.1 (M + 1, 99.56); FT-IR (KBr, cm^{-1}): 1533.22 (CH=CH band of -COCH=CH), 1599.32 (Aromatic -C=C- stretching), 1663.58 (strong C=O band of -COCH=CH); ^{1}H-NMR (400 MHz, DMSO) δ (ppm): 2.32 (s, 6H- $N(CH_3)_2$), 2.98 (s, 3H, $-CH_3$), 3.03 (s, 3H $-CH_3$), 7.33 (d, 1H, J = 17Hz), 7.69 (d, 1H, J = 17 Hz), 6.70-7.57 (m, 7H, Ar- H); ^{13}C-NMR spectrum (100 MHz, DMSO) δ (ppm): 19.77 (Ar-CH_3), 20.73 (Ar-CH_3), 194.39 (C-1), 131.59 (C-2), 145.56 (C-3), 110.93 (-$N(CH_3)_2$), 111.64, 120.73, 121.48, 125.99, 128.05, 130.34, 136.01, 136.75, 139.68, 145.56, 151.83, 154.08 (Ar-C).

3.2. Biological activity

The animals in each group were administered with standard drug and test compounds (curcumin and chalcone), followed by the inducer allyl isothiocyanate. The number of flinches exhibited by the animals was recorded at 5-minute intervals. The mean and standard error of the mean (SEM) values for the total number of flinches observed in the animals for each 5-minute interval, following treatment with the standard drug TRPA1 antagonist (10 mg/kg/2ml), curcumin and chalcone (compound **4**) at doses of 3, 10, 30, and 100 mg/kg/2ml, along with a negative control group. The total number of flinches for all the ten groups is represented in **Tables-1**.

Table 1. AITC induced paw flinching behavior

		Mean flinches (Mean ± S.E.M)					
Srl. No.	**Treatment groups**	**20 min**	**25 min**	**30 min**	**35 min**	**40 min**	**45 min**

1	Vehicle (80% DMSO + 20% Tween)	16.00 ±5.68	21.00 ±6.07	20.50 ±5.08	25.00 ±6.09	32.83 ±3.44	34.00 ± 9.23
2	TRPA1 antagonist (10 mg/kg/2ml) + Vehicle	2.33 ±0.84	1.83 ±0.70	1.67 ±0.49	1.33 ±0.49	0.83 ±0.48	0.67 ± 0.33
3	Chalcone (3 mg/kg/2ml) + Vehicle	17.50 ± 2.36	19.33± 1.96	18.08± 0.74	16.75± 1.65	26.42± 7.74	21.25± 3.35
4	Chalcone (10 mg/kg/2ml) + Vehicle	15.33 ± 2.55	17.33± 1.84	15.75± 0.79	14.58± 2.01	23.75± 7.61	18.75± 3.42
5	Chalcone (30 mg/kg/2ml) + Vehicle	5.33± 1.20	3.00 ±0.58	5.00 ±0.97	3.33 ±0.61	2.00 ±0.45	3.00 ± 0.52
6	Chalcone (100 mg/kg/2ml) + Vehicle	5.17± 0.83	4.83 ±1.40	2.83 ±1.11	2.83 ±0.75	3.83 ±0.60	0.83± 0.31

The vehicle-treated group exhibited a total of 149 flinches during the late phase. In contrast, the group treated with the TRPA1 antagonist showed only 8 flinches throughout the observation period. The animals treated with the test compound **4** at doses of 3, 10, 30, and 100 mg/kg exhibited a dose-dependent reduction in the number of flinches, with flinch counts of 119, 105, 22, and 20, respectively. Figure 3 compares the total number of flinches in the test drug-treated groups.

Figure 3 compares the total number of flinches in groups treated with the standard drugs. Statistical analysis, performed using one-way ANOVA with GraphPad Prism 5, yielded a p-value of less than 0.0001, indicating statistical significance. From the data, it was observed that compound **4** at a dose of 100mg/kg produced a total of 20 flinches over the entire observation period. The reduction in flinches by chalcone derivative **4** suggests that it is effective in treating neuropathic pain. However, its activity was less than the standard TRPA1 antagonist- HC-030031. These results indicated that the synthesized chalcone is effective to treat neuropathic pain. However, less activity of chalcone than the standard drug warrants structural modification to enhance the activity.

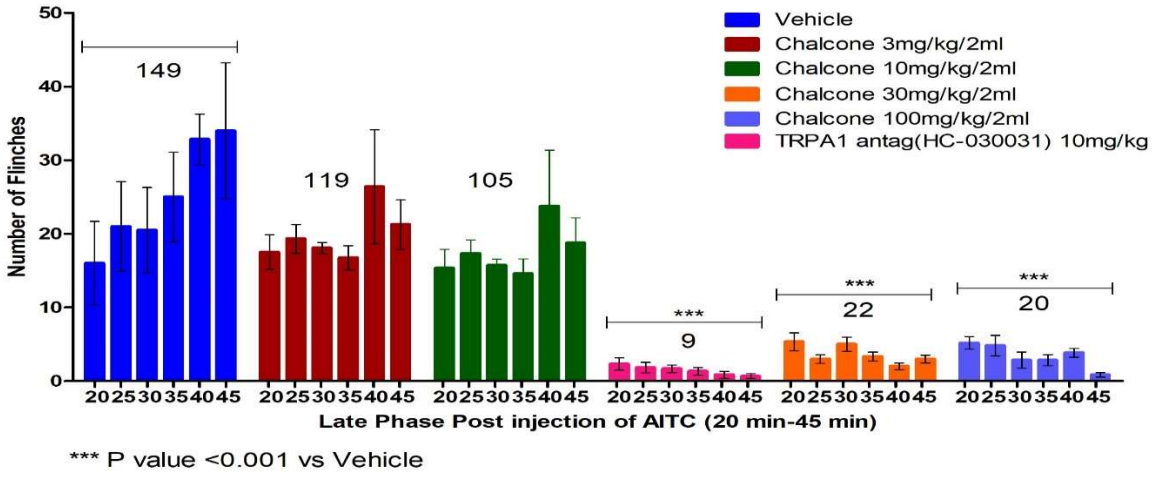


Figure 3. Comparative analysis of flinches elicited in Chalcone treated groups

4. Conclusion

The synthesis and characterization of the chalcone derivative (E)-3-(4-(dimethylamino) phenyl)-1-(2,4-dimethylphenyl) prop-2-en-1-one were successfully achieved, and its efficacy in reducing neuropathic pain was demonstrated in an AITC-induced paw flinching model. The compound showed a dose-dependent reduction in pain-related flinching, with significant efficacy at higher doses. It exhibited less activity compared to the standard TRPA1 antagonist. These results highlight the potential of chalcone derivatives as candidates for neuropathic pain treatment, while also indicating that structural modifications may further enhance their therapeutic efficacy.

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Conflict of interest

None

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