

Original Article

EDEMA REDUCING PROSPECTIVE OF MURRAYANINE BASED CHALCONE COMPOUND AS EMERGING NSAID AGENT

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ABSTRACT

Objective: In this study substituted murrayanine based chalcone compound (E)-3-(5-chloro-2-hydroxy-4-methylphenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one has been synthesized and screened for anti-inflammatory activity. **Methods:** The compound was synthesized by conventional method of chalcone synthesis and the *in vivo* anti-inflammatory activity was screened in Swiss albino rats employing the carrageenan-induced paw edema method. **Results:** The synthesized compound was characterized by spectroscopic techniques (FT-IR; ¹H-NMR; and Mass) and elemental analyses. The compound displayed % edema reduction of 24.91%, 34.49%, and 51.31%, respectively in the carrageenan-induced paw edema method over the 3 hrs duration. The molecule presented a fairly significant activity owing to inhibition of the inflammatory mediators like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX) through the various electron-donating and electron-withdrawing substituents present in the B-ring of the chalcone scaffold. The activity was not analogous with that of the standard drug indomethacin which exhibited 74.59% in the third hr which indicated that still much improvement is needed for the development of better anti-inflammatory candidates. **Conclusion:** The study will undoubtedly motivate the researchers working on pharmacologically active low-molecular-weight ligands in the better and rational development of the molecules.

Keywords: Anti-inflammatory; Characterization; Edema; Murrayanine; Chalcone; Synthesis.

INTRODUCTION

Inflammation is a coordinated biological process persuaded by any tissue injury or due to the aggression of any infection. Inflammatory diseases involve a series of disorders like ankylosing spondylitis, osteoarthritis, systemic lupus erythematosus, rheumatic fever, polyarthritis nodosa, rheumatoid arthritis, etc., which leads to granuloma formation, edema, leukocyte infiltration, etc [1]. The process of inflammation initiates by a series of events where arachidonic acid metabolism occurs. Therefore, the process is a defense reaction of the body to eradicate or restrict the broadening of the agents causing inflammation [2]. Two isoforms of cyclooxygenases (constitutive COX-1 and inducible COX-2) and several forms of lipoxygenase (most prominent 5-LOX) enzymes, which transform arachidonic acid into the effective biologically active lipid mediators that involved in the inflammatory processes [3]. Lipoxygenases (LOX) enzymes; particularly the isoform 5-LOX is predominantly involved in the inflammatory and allergic reactions as it facilitates the synthesis of the more potent inflammatory mediator; leukotrienes (LTs). In several human ailments like psoriasis, asthma, rheumatoid arthritis, colitis ulcerosa, and allergic rhinitis, a very high level of LTs have been observed [4]. The best way of reducing the inflammation is the complete termination of COX-1, COX-2, 5-LOX, and LT production, which can be achieved directly or indirectly by the inhibition of the pathway.

Murrayanine is a carbazole alkaloid present in *Murraya koenigii* L. (family: Rutaceae) with ethnopharmacological importance like stimulant, analgesic, astringent, febrifuge, etc [5]. Murrayanine as a single component is not much active for exhibiting any pharmacological properties. Murrayanine demonstrated mild edema reducing potential and is not suitable for pharmacotherapy. For enhancing the biological effect, semi-synthesis remained an emerging approach for fabricating hybrids [6]. In order to enhance the pharmacotherapeutic potential of the natural product and to develop safe drug candidates, murrayanine hybrids have been synthesized by our group [7-14]. Chalcones

(benzylideneacetophenone or 1,3-diphenyl-2E-propene-1-one) are safe natural product with multifarious pharmacological perspectives such as anti-diabetic, anti-cancer, anti-infective, anti-hypertensive, etc [15-18]. These are open chain intermediate in aurone synthesis of the flavones pathway and are considered as the initiator of flavonoids and isoflavonoids compounds. The scaffold is distributed all over nature and its synthetically derived components have been well-known to express anti-inflammatory, anti-gout, anti-ulcer, etc [19].

Similarly, in this study, substituted murrayanine based chalcone compound has been synthesized by using the conventional method and screened for *in vivo* anti-inflammatory activity in Swiss albino rats employing the carrageenan-induced paw edema method.

MATERIALS AND METHODS

Chemicals and Instrumentation

From the powdered *M. koenigii* stem bark, the starting material murrayanine was obtained by the soxhlation process, based on our established method. The reactant, 1-(5-chloro-2-hydroxy-4-methylphenyl)ethanone was procured from Sigma Aldrich, Germany. The synthesized compound was characterized by spectroscopic techniques [FT-IR (Shimadzu® IRAffinity-1); ¹H-NMR (Bruker Avance-II); and Mass (MICROMASS Q-TOF)] and elemental analyses (PerkinElmer Elemental Analyzer 2400). The reaction progress was judged by the Merck® pre-coated silica gel-G TLC plates.

Animals

The *in vivo* anti-inflammatory study was performed on Swiss albino rats (5-6 weeks age, 140-240g body weight) after getting approval from CPCSEA (1389/a/10/CPCSEA) and Department Ethical Committee. In the animal house, two rodents were kept each cage under a controlled environment (25-26°C temperature, humidity

50–65%, and 12 hr light and 12 hr dark). The animals were provided free access to water and fed with standard rodent pellet.

Synthesis of target compounds

The synthesis involved Claisen-Schmidt reaction where murrayanine (**1**), the starting material having an aldehyde function reacts with the reactant (**2**) containing an acetyl function (acetophenone) in the presence of ethanolic NaOH solution to form β -hydroxyketone function (benzylideneacetophenone) via aldol condensation mechanism (Scheme 1) [20].



Scheme 1: Fabrication of a diversely substituted murrayanine chalcone.

Synthetic protocol for (E)-3-(5-chloro-2-hydroxy-4-methylphenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one

An equal molar concentration of murrayanine (**1**) and 1-(5-chloro-2-hydroxy-4-methylphenyl)ethanone (**2**) were taken in a round bottom flask and refluxed for the duration of 3 hrs in the presence of ethanol (90%) solution (25 mL) and added with an aqueous solution of sodium hydroxide (20 mL) dropwise intermittently. The reaction mixture was made to stand overnight and poured over crushed ice containing few drops of HCl (dilute) with vigorous stirring. The product (**3**) was separated, filtered with Buchner funnel under vacuum system, thoroughly washed with cold water, dried completely, and suitably recrystallized.

76% yield; FTIR (KBr) ν (cm^{-1}): 3417 (-OH), 3280 (-NH, stretching), 3045 (C-H, aromatic), 1724 (C=O), 1679 (C=C, alkene), 1635 (-NH, bending), 1612 (C=C, aromatic), 1349 (C-N), 1233 (C-O), 796 (C-Cl); ^1H NMR (δ , ppm, CDCl_3): 10.18 (9, 1H), 8.42 (12, 1H), 7.21 (11, 1H), 6.9–8.6 (Aromatic, 8H), 5.56 (18, 1H), 3.95 (1, 3H), 2.47 (16, 3H). MS: M^+ 391, $M+2$ 393. Anal. Calcd. for $\text{C}_{23}\text{H}_{18}\text{ClNO}_3$: C, 70.50; H, 4.63; N, 3.57. Found: C, 70.17; H, 4.14; N, 3.01.

Acute toxicity studies

Based on the OCED guidelines, the safe dose of the chalcone compound was determined in the escalating range of 25–500 mg/kg in Swiss albino rats and the LD_{50} value was suitably established [21].

Anti-inflammatory screening

The chalcone was screened for its potential in reducing the carrageenan-induced edema in Swiss albino rats. Before the commencement of screening, the rats were fasted overnight and fed with distilled water through oral route to reduce any interference in the results. The experimental group was given 1% carrageenan solution injected in the subplanter region of the right hind paw. An hour before, the rats were administered with 150 mg/kg b.w. of the molecule (suspended in the vehicle) through the oral route. The control group received 0.9% saline solution (vehicle). The edema reducing potential of the compound was measured by mercury digital micrometer for the duration of 3 hrs. The thickness of the rat paws was determined by the disparity in the width of the injected paws and the non-injected paws [22].

Statistical treatment

The obtained edema reducing data was statistically analyzed by one-way ANOVA method followed by the Dunnett's multiple comparisons test. The value $P < 0.01$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Chemistry

The formation of the chalcone scaffold was ascertained from the formation of the alkene component (bridge function) which was perceived from the peak in the FT-IR spectra at 1679 cm^{-1} .

Additionally, the alkene component was confirmed by the proton-NMR spectroscopy which represented the protons at 8.42 ppm and 7.21 ppm, respectively. The presence of the murrayanine component was corroborated from both FT-IR and ^1H -NMR spectra. The carbazole moiety was validated from the N-H stretching, N-H bending, C-N stretching, C-O stretching ($-\text{OCH}_3$) in FT-IR spectra at 3280 cm^{-1} , 1635 cm^{-1} , 1349 cm^{-1} , and 1233 cm^{-1} . Analogously, the ^1H -NMR spectra presented peaks at 10.18 ppm (carbazole) and 3.95 ppm (methoxy protons). The aromatic components were bear out from the FT-IR spectra which represented C-H stretching and C=C stretching at 3045 cm^{-1} and 1612 cm^{-1} , respectively, as well as from proton-NMR peak in the range of 6.9–8.6 ppm. The presence of the B-ring of the chalcone was proved from the appearance of 796 cm^{-1} (signifying the carbon-chlorine bond) and 3417 cm^{-1} (symbolizing the $-\text{OH}$ moiety) peaks. The ^1H -NMR spectra characterized the hydroxyl proton and methyl proton at 5.56 ppm and 2.47 ppm, respectively, which supported the presence of ring-B of the chalcone compound. The halogen ($-\text{Cl}$) was also substantiated from the mass spectra where $M+2$ peaks were clearly seen along with the beak peak which corresponds with the molecular mass of the compound. The comparable practically obtained ratio of CHN with that of theoretical value authenticated the formation of the murrayanine based chalcone compound.

Acute toxicity study

The safety limits in the escalated dose of 25–500 mg/kg showed no toxic symptoms and the safest dose for *in vivo* anti-inflammatory screening was performed at 150 mg/kg b.w.

Anti-inflammatory activity

The screened *in vivo* anti-inflammatory activity of the murrayanine based chalcone presented a fairly significant activity owing to inhibition of the inflammatory mediators like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX) through the various electron-donating and electron-withdrawing substituents present in the B-ring of the chalcone scaffold. The compound displayed % edema reduction of 24.91%, 34.49%, and 51.31%, respectively in the carrageenan-induced paw edema method over the 3 hrs duration (Table 1). The activity was not analogous with that of the standard drug indomethacin which exhibited 74.59% in the third hr which indicated that still much improvement is needed for the development of better anti-inflammatory candidates. More study is required to establish a crystal clear structure-activity-relationship (SAR) of the scaffold. However, it may be predicted that the combination of both electron-donating and electron-withdrawing substituents in the B-ring of the chalcone component express better edema reducing attributes as compared to substituting a large number of electron-withdrawing substituents. As the electron-withdrawing substituents render the molecule more lipophilic which rapidly distributes in the whole body and reaches the inflamed area in a very limited amount, thereby reducing the pharmacological effect drastically. The present molecule may be regarded as optimized for the activity and can be further developed in a rational approach for better responses.

Table 1: *In vivo* anti-inflammatory activity of the diversely substituted murrayanine chalcone derivative.

Group	Percentage (%) inhibition of edema		
	1 hr	2 hr	3 hr
3	24.91 \pm 1.72	34.49 \pm 1.33	51.31 \pm 1.84
Indomethacin	38.63 \pm 1.99	53.22 \pm 1.21	74.59 \pm 1.57

$n = 6$; ED_{50} of 150 mg/kg b.w. in male adult albino mice; $P < 0.01$

CONCLUSION

This study highlighted the *in vivo* anti-inflammatory potential of the diversely substituted murrayanine chalcone derivative in Swiss albino rats. The molecule presented a fairly significant activity owing to inhibition of the inflammatory mediators like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX) through the various electron-donating and electron-withdrawing substituents present in the B-ring of the chalcone scaffold. A hopeful SAR has been predicted for the study, however, more study is required for

the complete establishment of the SAR. Although, the combination of electron-donating and electron-withdrawing substituents presented an optimum activity and it requires further development in a rational approach for the better responses. The study will undoubtedly motivate the researchers working on pharmacologically active low-molecular-weight ligands in the better and rational development of the molecules.

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CONFLICT OF INTEREST

None declared.

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