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Halogen Substituted Murrayanine Based Chalcone as Emerging Non-Steroidal Anti-Inflammatory Drug

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Abstract: *In the continuous effort to develop more potent and safe non-steroidal anti-inflammatory drug (NSAID), semi-synthetic halogen-containing murrayanine based chalcone was developed from murrayanine (1) and 1-(2,4-dichloro-5-fluorophenyl)ethanone (2) and screened for in vivo anti-inflammatory screening was performed in Swiss albino rats by employing the carrageenan-induced paw edema method. The in vivo anti-inflammatory activity chalcone compound (3) was found to be moderate as compared with the standard drug indomethacin in the carrageenan-induced paw edema method. The prop-2-ene-1-one compound demonstrated % edema reduction of 21.28%, 32.43%, and 45.77%, respectively over the 3 hrs duration. The compound may be believed to inhibit the inflammatory mediators like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX) through the electron-withdrawing substituents present. Although, no structure-activity-relationships (SARs) cannot be predicted from this study and need further studies. The current study will draw the attention of global (medicinal)-chemists towards the rational development of semi-synthetic NSAID compounds with pronounced edema reducing activity.*

Keywords: *Anti-inflammatory; Characterization; Edema; Murrayanine; Chalcone; Halogen.*

1. INTRODUCTION

Murrayanine, an active carbazole alkaloid present in *Murraya koenigii* L. (family: Rutaceae) have been known to traditionally used for centuries as astringent, febrifuge, analgesic, stimulant, etc [1]. Nascent report on anti-inflammatory perspective of this phytoconstituent has been reported, however, the intensity is quite low and is quite a misfit for any practical use. While knowing the fact, the semi-synthetic approach was implemented as a strategy to rationally develop hybrids which may demonstrate better pharmacological activity than its parent compound [2]. Moving in this path, our research group had fabricated few murrayanine based semi-synthetic hybrids which have displayed noteworthy edema reducing attribute owing to the presence of active functional group along with rational designing [3-10].

Chalcones (1, 3-diphenyl-2*E*-propene-1-one) is a natural product widely distributed in nature. These are open chain intermediate in aurone synthesis of the flavones pathway and are considered as the initiator of flavonoids and isoflavonoids compounds. The compounds have been known to have a very high degree of safety limit along with multiple pharmacological activities such as anti-cancer, anti-oxidant, anti-diabetic, anti-infective, anti-hypertensive, anti-hyperlipidemic, anti-arrhythmic, anti-obesity, etc [11-14]. This benzylideneacetophenone scaffold (both natural molecule and synthetically derived compounds) has been profoundly known to reduce the edema in experimental models, which inspired us in the development of chalcone based analogs having synergistic activity [15].

Similarly, in the continuous effort to develop more potent and safe non-steroidal anti-inflammatory drug (NSAID), semi-synthetic halogen-containing murrayanine based chalcone was developed and screened for *in vivo* anti-inflammatory screening was performed in Swiss albino rats by employing the carrageenan-induced paw edema method.

2. MATERIALS AND METHODS

2.1. Chemicals and Instrumentation

Murrayanine was obtained from powdered *M. koenigii* stem bark by soxhlation method by our own established method. The reactant was purchased from Sigma Aldrich, Germany through a local chemical vendor. The progress of the chemical reaction was determined by using the Merck® silica gel-G pre-coated TLC plates. Fourier Transformed Infrared (FT-IR) Spectroscopy (Shimadzu® IRAffinity-1), ^{1}H (proton)-NMR (Bruker Avance-II), Mass Spectroscopy (MICROMASS Q-TOF), and CHN analyses (PerkinElmer Elemental Analyzer 2400) were employed for the elucidation of the chemical structures.

2.2. Animals

After obtaining approval from CPCSEA (1389/a/10/CPCSEA) and Department Ethical Committee, the anti-inflammatory study was carried out on Swiss albino rats of age 5 to 6 weeks having 160-250 g body weight. The animals were kept under a controlled environment (25–26ºC temperature, humidity 50–65%, and 12 hr light and 12 hr dark) in the animal house. The rodents were kept two animals per cage, fed with standard pellets, and provided free access to water.

2.3. Synthesis of Target Compounds

By utilizing the Claisen-Schmidt reaction, the benzylideneacetophenone component was fabricated in the presence of ethanolic NaOH solution by using the starting material murrayanine **(1)**, having aldehydes portion which was reacted with the halogen-containing acetophenone reactant **(2)**. The β-hydroxyketone function was formed through an aldol condensation mechanism (**Scheme 1**) [16].

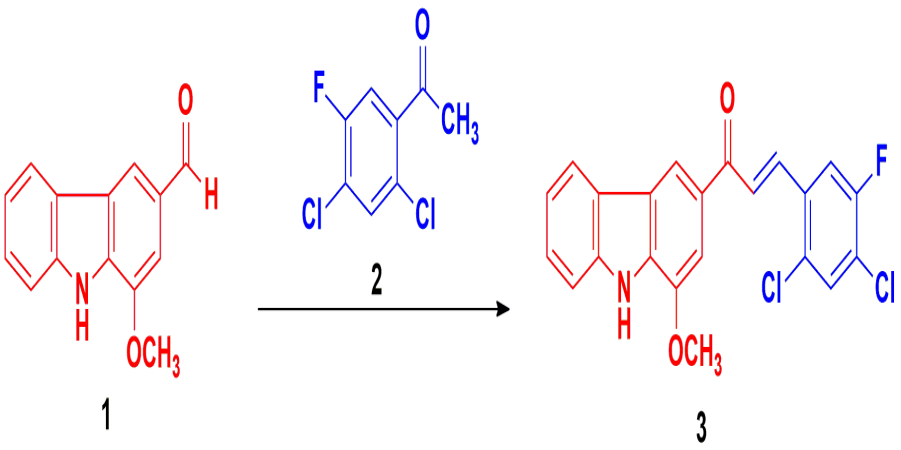

Fig1. *Development of Murrayanine based Halogen Substituted Chalcone.*

Synthetic protocol for (E)-3-(2,4-dichloro-5-fluorophenyl)-1-(1-methoxy-9H-carbazol-3-yl)prop-2-en-1-one

An equal amount of murrayanine **(1)** and 1-(2,4-dichloro-5-fluorophenyl)ethanone **(2)** were refluxed in presence of an aqueous solution of sodium hydroxide (20 mL) and ethanol (90%) solution (25 mL). The reaction was performed for the duration of 3 hrs and the refluxed content allowed standing overnight. Over crushed ice (containing a few drops of dilute HCl), the content was poured thoroughly and stirred vigorously with a rod. The separated product **(3)** was completely filtered under vacuum, washed thoroughly, dried, and later recrystallized.

80% yield; FTIR (KBr) υ (cm^{-1}): 3242 (-NH, stretching), 3111 (C-H, aromatic), 1729 (C=O), 1682 (C=C, alkene), 1641 (-NH, bending), 1605 (C=C, aromatic), 1354 (C-N), 1208 (C-O), 1088 (C-F), 768 (C-Cl); ^{1}H NMR (δ, ppm, $CDCl_3$): 10.11 (9, 1H), 8.38 (12, 1H), 7.33 (11, 1H), 6.8-8.5 (Aromatic, 8H), 3.92 (1, 3H). MS: M^+ 413, M+2 415. Anal. Calcd. for $C_{22}H_{14}Cl_2FNO_2$: C, 63.79; H, 3.41; N, 3.38. Found: C, 63.13; H, 3.02; N, 2.99.

2.4. Acute Toxicity Studies

The *in vivo* safety limit of the compound was estimated with accordance to the OECD protocol in the range of 25 mg/kg to 500 mg/kg. The safe dose was determined using the LD_{50} values [17].

2.5. Anti-Inflammatory Screening

The *in vivo* anti-inflammatory activity of the chalcone was screened by carrageenan-induced paw edema method. To minimize the difference during the screening, the rats were fasted overnight and fed orally with distilled water (5 mL). The control group was fed with 0.9% saline solution, the experimental group received 1% carrageenan solution at right hind paw in the subplanter region through the injection route. Before the commencement of the study, the rats received 150 mg/kg b.w. of chalcone (suspended in the vehicle) orally an hour before. The mercury digital micrometer was employed to determine the edema reducing potential of the murrayanine based chalcone for the duration of 3 hrs. The thickness of the rat paws was estimated by calculating the width of the injected paws and the non-injected paws [18].

2.6. Statistical Treatment

The data were analyzed statistically by one way ANOVA method followed by the Dunnett's multiple comparisons test. $P < 0.01$ was regarded statistically significant.

3. Results and Discussion

3.1. Chemistry

The structure of the murrayanine based compound was elucidated by spectroscopic techniques. The formation of the chalcone was ascertained from the appearance of ketonic carbonyl moiety at 1729 cm^{-1} from the earlier appeared aldehyde carbonyl moiety at 1753 cm^{-1}. Moreover, the formation of the alkene component at 1682 cm^{-1} confirmed the C=C bridge component of the chalcone scaffold. The alkene portion was corroborated through proton-NMR at 8.38 ppm and 7.33 ppm, respectively. The murrayanine portion was authenticated from the -NH part which appeared at 3242 cm^{-1} (-NH stretching), 1354 (C-N stretching), and 1641 cm^{-1} (-NH bending) in FT-IR. The ^{1}H-NMR peak at 10.11 ppm and 3.92 ppm additionally substantiated the -NH and methoxy part of the carbazole. Furthermore, the prominent vibrational peak at 1208 cm^{-1} represented the $-OCH_3$ part. The aromatic rings present in the molecule were verified from the rotational spectroscopy in the range of 6.8-8.5 ppm. Moreover, the FT-IR peaks at 3111 cm^{-1} and 1605 cm^{-1} presented the C=C stretching and C-H stretching. The presence of halogens; chlorine and fluorine in the compound were supported from the FT-IR peaks at 1088 cm^{-1} (C-F) and 768 cm^{-1} (C-Cl), respectively. Besides, the peak of M+2 in the mass spectra confirmed the halogens in the product. The mass spectra demonstrated the base peak corresponding to the molecular mass of the compound (M^+ 413). Few fragmented peaks were located significantly in the spectra. The estimated ratio of carbon, hydrogen, and nitrogen of the compound and its close semblance with the theoretical values certainly confirmed the formation of the halogen-containing murrayanine chalcone.

3.2. Acute Toxicity Study

The chalcone displayed no toxic symptoms over the increased dose of 25 mg/kg to 500 mg/kg. The safest dose for in vivo anti-inflammatory screening was screened at 150 mg/kg b.w.

3.3. Anti-inflammatory Activity

The *in vivo* anti-inflammatory activity chalcone compound **(3)** was found to be moderate as compared with the standard drug indomethacin in the carrageenan-induced paw edema method. The prop-2-ene-1-one compound demonstrated % edema reduction of 21.28%, 32.43%, and 45.77%, respectively over the 3 hrs duration **(Table 1)**. The compound may be believed to inhibit the inflammatory mediators like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX) through the electron-withdrawing substituents present. However, from the study, it can be concluded that the murrayanine hybrid did not perform very well in the anti-inflammatory screening which may be due to the very high lipophilic characteristic of the molecule. It may be predicted that the compound distributed over all the body tissues and the active fraction reached in an inadequate amount in the inflamed area, thereby inhibiting the restricted amount of inflammatory mediators.

Table1. *Exploring in vivo anti-inflammatory activity of the murrayanine Schiff's base derivatives.*

Group	Percentage (%) inhibition of edema		
	1 hr	2 hr	3 hr
3	21.28 ± 2.24	32.43 ± 1.89	45.77 ± 2.11
Indomethacin	40.99 ± 0.93	57.61 ± 1.17	75.12 ± 1.05

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

4. CONCLUSION

The investigation of the chalcone compound demonstrated moderate *in vivo* anti-inflammatory activity in the carrageenan-induced paw edema method as compared with the standard drug indomethacin. The murrayanine hybrid did not perform very well in the anti-inflammatory screening which may be due to the very high lipophilic characteristic of the molecule. Although, no structure-activity-relationships (SARs) cannot be predicted from this study and need further studies. The current study will draw the attention of global (medicinal)-chemists towards the rational development of semi-synthetic NSAID compounds with pronounced edema reducing activity.

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