Tert-butyl Group Substitution in the Ring-B of Murrayanine-Chalcone leads to Higher Expression of Edema Reduction

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Abstract

Inspiring from the fact that the free-radicals are the main culprit in the precipitation of inflammation, cancer, and several other diseases, a hybrid molecule comprising of murrayanine (carbazole moiety), chalcone, and tert-butyl group was fabricated by our group, and it showed enhanced anti-oxidant activity due to the synergistic effect of the three individual components. These three components, in individuality, have a strikingly high anti-oxidant activity. Similarly, motivating from the above reports and data obtained from the previously performed anti-oxidant studies, at present the developed chalcone molecule was screened for its anti-inflammatory potentials for treating chronic conditions such as rheumatoid arthritis which involves the participation of free-radicals and the management requires complete free-radical scavenging. The in vivo anti-inflammatory screening was performed by employing the carrageenan-induced paw edema method. The compound through the tert-butyl group exhibited potential anti-inflammatory activity with 64.69% inhibition of edema after 3 hrs, probably by the inhibition of the mediators like cyclooxygenase-1/2 and lipoxygenase. As compared to the previously synthesized murrayanine-chalcones, either unsubstituted or substituted by electron-withdrawing / electron-donating groups, the chalcone exhibited much better activity. The study opened new avenues of research by encouraging medicinal chemists in understanding the strategies and approach toward fabricating more potent analogs.

Keywords: *Murrayanine, Chalcone, tert-butyl, Anti-inflammatory, Anti-oxidant, Edema*

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory condition caused by the auto-immune response in the human body [1]. It is often characterized by inflamed joints and massive infiltration of macrophages and T-cells which produces reactive oxygen species (ROS) and reactive nitrogen species (RNS) and aggravates the pathological conditions. The enhanced levels of isoprostanes and prostaglandins in the serum are the chief markers of oxidative stress [2].

Millions of patients of age more than 50 of both the sexes are affected by this disease across the globe (in both developing and developed nations) and is expected to rise nearly twice by the end of the year 2050 [3].

In general, the human body produces more than 20,000 free-radicals every day which has a delirious effect on molecular constitution [4]. Additionally, the long duration exposure to the environmental contaminants such as industrial effluents, contaminated low-grade food additives, cigarette smoking and exceptional lifestyle practices, and excessive alcohol consumption doubles up these inflammatory conditions [5]. Thereby, it can be predicted that person with a chronic inflammatory state has two-fold oxidative stress than a normal disease-free individual, which sturdily supported the direct relationship between free-radical and chronic inflammatory state [6]. At present, there are a number of non-steroidal anti-inflammatory agents (NSAIDs) which are generally prescribed by medical practitioners for the management of these conditions [7].

Inspiring from the fact that the free-radicals are the main culprit in precipitating the inflammation, cancer, and several other diseases [8], a hybrid molecule comprising of murrayanine (carbazole moiety), chalcone, and tert-butyl group was fabricated by our group, and it showed enhanced anti-oxidant activity due to the synergistic effect of the three individual components. These three components, in individuality, have a strikingly high anti-oxidant activity [9].

Murrayanine is a carbazole-based alkaloid obtained from *Murraya koenigii* (Family: Rutaceae) having a noteworthy anti-oxidant effect [10]. The semi-synthetic derivatives have extraordinarily higher anti-oxidant and edema reducing perspectives [11-13]. Individually, the carbazole synthetic molecules have both inflammation controlling and free-radical scavenging potentials [14]. Chalcones are the low-molecular-weight natural ligands having tremendous anti-oxidant and anti-inflammatory effect [15-16]. The artificially developed commercial anti-oxidants such as tert-butylhydroquinone (TBHQ), 2-tert-butyl-4-methoxyphenol (BHA), 2,4,6-tri-tert-butylphenol (TBP), and 2,6-di-tert-butyl-4-methylphenol (BHT), have tert-butyl group which scavenges the free-radicals and have been recently screened for edema reducing potentials where an outstanding anti-inflammatory activity have been perceived [17].

Similarly, motivating from the above reports and data obtained from the previously performed anti-oxidant studies, at present the developed chalcone molecule (Figure 1) was screened for its anti-inflammatory potentials for treating chronic conditions such as rheumatoid arthritis which involves the participation of free-radicals and the management requires complete free-radical scavenging. The *in vivo* anti-inflammatory screening was performed in Swiss albino rats by employing the carrageenan-induced paw edema method**.**

MATERIALS AND METHODS

Chemicals

The analytical grade chemicals, solvents, and reagents for anti-inflammatory screening were procured from HiMedia Ltd., India. The (*E*)-3-(4-(tert-butyl)phenyl)-1-(1-methoxy-9*H*-carbazol-3-yl)prop-2-en-1-one was one of our previous reports and taken from our compound library [9].

Animals

The anti-inflammatory screening was performed on Swiss albino rats of age 5-6 weeks and weights in the range of 190-260 g were employed after obtaining ethical permission from DEC and CPCSEA. The experimental animals were kept in polypropylene cages under the hygienic conditions of 25–26ºC / 50–55% RH / 12 dark 12 light cycle in the registered departmental animal house.

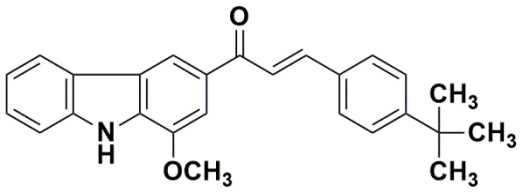

Figure 1. Tert-butyl group containing murrayanine-chalcone.

Table 1. *In vivo* anti-inflammatory potential of (*E*)-3-(4-(tert-butyl)phenyl)-1-(1-methoxy-9*H*-carbazol-3-yl)prop-2-en-1-one.

Compound	Percentage (%) inhibition of edema		
	1 hr	**2 hr**	**3 hr**
Chalcone	39.88** ± 1.97	50.72* ± 1.46	64.69* ± 1.55
Indomethacin	45.21** ± 1.33	58.33* ± 1.62	77.16** ± 1.41

n = 6; ED_{50} of 200 mg/kg b.w. in male adult albino mice; **P < 0.01; *P< 0.05

Acute toxicity studies

An acute toxicity study was performed to estimate the highest safe dose which will exert the maximum therapeutic effect without showing any distinct sign and symptoms of toxicity along with the mortality. The protocol involved injecting the chalcone compound in escalating dose range of 25 mg/kg to 500 mg/kg in adult male albino rats. The lethal dose (LD_{50}) was established based on calculating the dose at which 50% animal died [18].

Anti-inflammatory screening

The *in vivo* anti-inflammatory screening of chalcone was performed according to the standard carrageenan-induced paw edema method. The albino rats were fasted overnight to reduce the inconsistencies while recording the edema. 5 mL distilled water was administered orally before commencing the study. An hour before the induction of inflammation by injecting 1% carrageenan solution at the subplanter region of the right hind paw through subcutaneous route, the chalcone molecule (200 mg/kg b.w.) was suspended in the saline solution and administered orally. The control group was administered saline solution (0.9%). The thickness of each rat paw was measured using the mercury digital micrometer for the duration of 3 hrs at an interval of 1 hr. The disparity in the width of non-injected paws and injected paws were determined to calculate appropriately the edema reducing potential of the chalcone compound. The data were expressed as mean ± standard error [19].

Statistical treatment

The procured anti-inflammatory data were treated by one-way ANOVA method followed by treating with Dunnett's multiple comparison test. A P value of <0.01 was considered as statistically significant.

RESULTS AND DISCUSSION

Anti-inflammatory activity

A significant high inflammatory activity has been noticed for the chalcone compound and also demonstrated an analogous activity with

that of indomethacin. The compound through the tert-butyl group exhibited potential anti-inflammatory activity with 64.69% inhibition of edema after 3 hrs (Table-1), probably by the inhibition of the mediators like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX). As compared to the previously synthesized murrayanine-chalcones, either unsubstituted or substituted by electron-withdrawing / electron-donating groups, the tert-butyl group containing chalcone exhibited much better activity [12-13]. The reason may be better free-radical (hydroxyl, superoxide anion, reactive nitrogen species, etc.) scavenging by the synergistic activity of the three components (murrayanine, chalcone, and tert-butyl), which are generated by the inflammatory mediators.

CONCLUSION

This motivating research highlighted that hybridization of three components; murrayanine (carbazole), chalcone, and tert-butyl exhibited a strikingly high edema reducing activity (64.69% inhibition in 3 hrs) by scavenging the free-radical (hydroxyl, superoxide anion, reactive nitrogen species, etc.) produced by the mediators like COX-1/2 and lipoxygenase LOX, through synergistic activity. The study also revealed that this murrayanine-chalcone molecule expressed higher pharmacological activity than the previously developed electron-withdrawing / electron-donating groups containing murrayanine-chalcone compounds. The study opened new avenues of research by encouraging medicinal chemists in understanding the strategies and approach toward fabricating more potent analogs.

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CONFLICT OF INTEREST

No conflict of interest declared.

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