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Research Article

SYNTHESIS OF NEW FLUORINATED CHALCONE DERIVATIVE WITH ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

In an effort to synthesize a new fluorinated chalcone derivative with potent anti-inflammatory activity, Claisen-Schmidt condensation method was followed using $SOCl_2$/ETOH as a catalyst to synthesize (E)-3-(4-fluorophenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one with excellent yield. The structure of the synthesized compound has been characterized by TLC, melting point, UV, and IR Spectroscopy, and elemental microanalysis (CHNO). This compound has been evaluated for anti-inflammatory activity using cotton pellet-induced granuloma in rats as a model, and found comparable to dexamethasone in this regard.

Keywords: Fluorinated chalcones, (E)-3-(4-fluorophenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one, Anti-inflammatory activity, Granuloma, rats

INTRODUCTION

Chalcone [1,3-diphenyl-2-propene-1-one] and related compounds "chalconoids" are those in which two aromatic rings are linked by a reactive keto ethylenic group (–CO–CH=CH–) that forms the central core for a variety of important biological compounds, and known collectively as chalcones. Chalcones has a conjugated double bonds and an entirely delocalized π-electron system on both benzene rings; such system has relatively low redox potentials and have a greater probability of undergoing electron transfer reactions[1]. They are precursors in flavonoid biosynthesis[2], and their colors attributed to the presence of the chromophore (–CO–CH=CH–) and other auxochromes (Scheme 1)[3]. Many methods are available for synthesis of chalcones, but they mostly prepared by condensing an aromatic ketones with an aromatic aldehydes in presence of suitable condensing agents. The simplest method involves Claisen-Schmidt condensation of equimolar quantities of acetophenone or a substituted acetophenone with benzaldehyde, or substituted benzaldehydes under an acidic condition (HCl), i.e., formed in situ by the reaction of $SOCl_2$ with absolute ethanol followed by dehydration to yield the anticipated chalcone derivative[4]. These compounds undergo a variety of chemical reactions and found useful in the synthesis of variety of heterocyclic compounds like isoxazoles, quinolinones, benzofuranones, indols, flavones, etc. Furthermore, these compounds are important intermediates in many addition reactions of nucleophiles due to the inductive polarization of the carbonyl group at the β-position (Scheme 2)[5,6]. Chalcones have a unique chemical structure and display wide range of pharmacological activities depending on the nature, number, and position of the substituent(s) on both benzene rings of the chalcone. Depending on these factors, some of chalcone derivatives exhibit numerous biological activities such as anti-inflammatory, analgesic[7,8], anticancer[9], antiviral and antimicrobial[10], antioxidant[11], anti-histamine[12] and anti-hyperglycemic activities[13]. They also inhibit the activities of many enzymes, especially the mammalian alpha-amylase, cyclooxygenase (COX), monoamine oxidase (MAO), tyrosinase, aldose reductase, α-glucosidase, and antimitotic activity[14,15]. Some chalcones demonstrated the ability to block voltage-dependent potassium channels[16]. The present study was designed to synthesize a novel fluorinated chalcone derivative and evaluate its anti-inflammatory activity in animal model of chronic inflammation.

MATERIALS AND METHODS

Synthesis of (E)-3-(4-fluorophenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one (FCD)

Fluorinated Chalcone derivative (FCD) was synthesized by Claisen-Schmidt condensation using $SOCl_2$/EtOH. This general procedure was utilized starting with aromatic ketones and aromatic aldehydes to prepare the corresponding chalcone derivative (Scheme 3). To a stirred mixture of the p-hydroxyacetophenone (10 mmol/1.36 gm) and p-flourobenzaldehyde (Himedia, India) (10 mmol/1.07 ml) in 5 ml absolute ethanol; 0.5 ml of thionyl chloride was added in drop wise manner over a 5 min with vigorous stirring continued for 3-4 hr at room temperature. The solution turned deep red immediately; when stirred for 20-30 min, the mixture became coagulated. After completion of the reaction, the reaction mixture was allowed to stand overnight. The reaction mixture was precipitated by the addition of 5 ml distilled water and the mixture was filtered; the obtained solid was washed successively with water (2×30ml), absolute ethanol (2×10ml) and cold diethyl ether (2×10ml), and allowed to dry to get (E)-3-(4-fluorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (FCD) (Scheme 3). This product was recrystallized from absolute ethanol. The progress of the reaction was monitored by ascending thin layer chromatography which was run on Kieslgel GF_{254} (60) aluminum plates (E. Merck, Germany) in order to check the purity of the product. FCD was revealed either by derivatization or reactivity toward iodine vapor or by irradiation with UV_{254} light. The melting point was determined by open capillary method (Thomas Hoover, England); Infra-red spectra were recorded in KBr disc (Shimadzu FTIR 8400 spectrophotometer, Japan) and elemental microanalysis was performed at the College of pharmacy, University of Baghdad using C,H,N,O analyzer (Euro-vector EA3000A, Italy).

Evaluation of the anti-inflammatory activity of FCD

The chalcone derivative (FCD) was suspended in 0.5% carboxymethyl cellulose solution to produce a stock suapension of 100 mg/ml, from which different doses were prepared according to the body weight of the animals. Sprague–Dawley rats weighing 180–220 g of both sexes were kept in the animal house of the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, at 25 ± 2 °C and light:dark cycle of 12:12 h for 1 week before starting experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 12 hr before the experiment, though water was allowed *ad libitum*. All experiments were performed according to the guidelines of laboratory animals' care and the ethical guidelines for the investigations on experimental animals. Twenty four rats allocated into four groups for the study of the anti-inflammatory activity of FCD in rat model of cotton pellet-induced granuloma. The anti-inflammatory activity of FCD was evaluated using a standard method of cotton pellets-induced granuloma[17]. Cotton pellets weighing 10 ± 1 mg were sterilized in an autoclave for 30 min at 120 °C under 15 lb pressure. Four pellets were implanted subcutaneously (s.c.) into the ventral region, two on either side, in each rat under light ether anesthesia.

The FCD (200 mg/kg), diclofenac sodium (25 mg/kg) and dexamethasone (2 mg/kg) doses and vehicle were administered orally using oral gavage needle for seven consecutive days from the day of cotton pellet implantation. On 8th day the animals were anaesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed for the determination of wet weight, and then dried in an incubator at 60°C for 18 hr until a constant weight obtained (all the exudates dried); after that the dried pellets were weighed again. The exudate amount (mg) was calculated by subtracting the constant dry weight of pellet from the immediate wet weight of pellet. The granulation tissue formation (dry weight of granuloma) was calculated after deducting the weight of cotton pellet (10 mg) from the constant dry weight of pellet and taken as a measure of granuloma tissue formation. The percent inhibitions of exudate and granuloma tissue formation were determined.

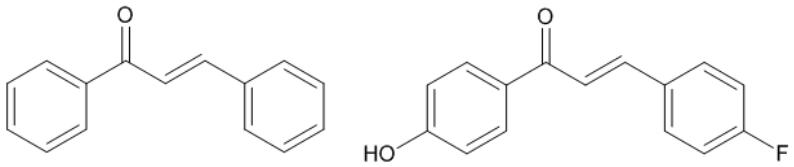
RESULTS

Synthesis of FCD

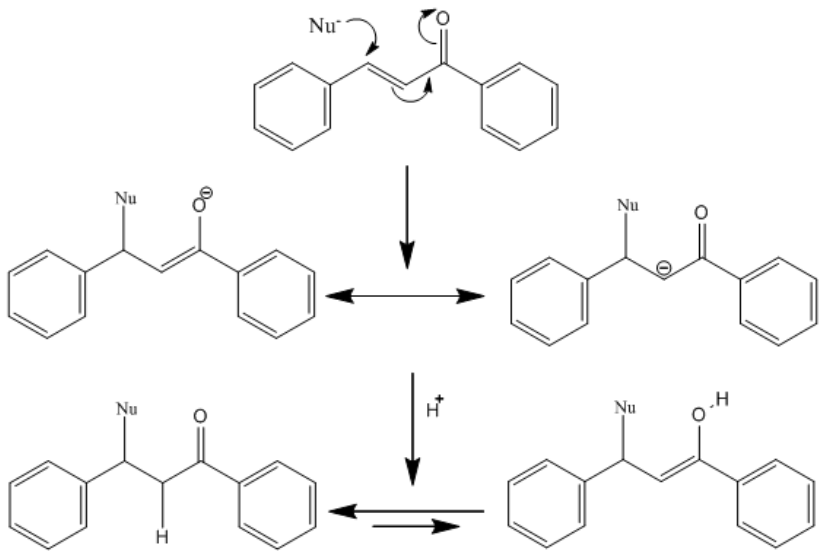
In table 1, FCD showed a good yield (92%) with a melting point around 192-194°C, while showing a single spot with R_f value of 0.3 when eluted on a TLC plate using petroleum spirit (40-60): ethyl acetate (70:30) solvent system. The spectral analysis in the UV region exhibited a λ_{max} between 300-350 nm. The IR spectrum in KBr discs showed many characteristic absorption bands at *v* values 1651, 1612, 1571, 1217, and 1031 cm^{-1}. The elemental microanalysis of the FCD revealed that the C and H% were 74.968% and 4.407% respectively (Table 2).

Anti-inflammatory activity of the FCD

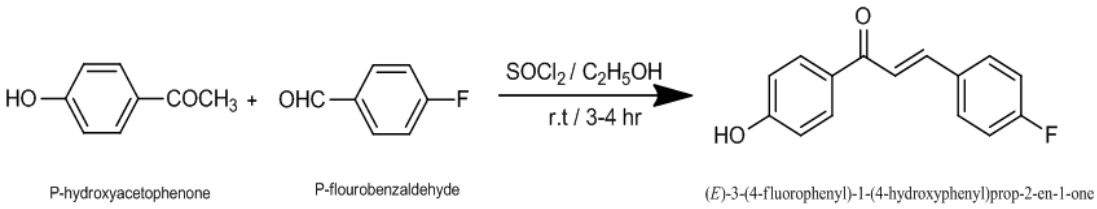
In table 3, although the total wt of inflammatory lesion was reduced by both diclofenac and FCD, they are non-significantly different compared with control when evaluated by unpaired *t*-test; only dexamethasone produces significantly different decrease in this respect. However, when the percent change in total wt compared using ANOVA, FCD shows comparative effect to dexamethasone and higher reduction compared with diclofenac (Figure 1). Regarding the effects of FCD and the standard drugs on exudates formation, all produced significant decrease in exudates formation compared to control (Table 3); however, when analyzed with ANOVA, the percent decrease in exudates shown to be comparable (P>0.05; figure 2).Concerning the effect on granuloma production, only dexamethasone produced significant decrease in the weight of granulation tissue compared with control (using unpaired t-test), while FCD fails to achieve significant difference although > 12% reduction was reported. However, when the percent inhibition produced by the three compounds compared using ANOVA, FCD produced comparable effect to that reported for dexamethasone (Figure 3).



Scheme 1: chemical structures of chalcone and its fluorinated derivative



Scheme 2: Nucleophilic conjugate additions of the chalcone



Scheme 3: Synthetic pathway of (E)-3-(4-fluorophenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one (FCD)

Table 1: Physicochemical data for the synthesized compound

Chemical name	Molecular formula	Molecular weight	Appearance	Yield (%)	M.P (°C)	R_f
(E)-3-(4-fluoro phenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	$C_{15}H_{11}FO_2$	242	Pink powder	92	192-194	0.3

Table 2: IR spectral data and elemental analysis of synthesized compound

IR Spectral data	Elemental analysis (calculated) %		
	C	H	N
IR (KBr) *v* cm^{-1} 3115 cm^{-1}(-OH), 1651 cm^{-1} (C=O), 1612 cm^{-1} 1571 cm^{-1}(C=C), 1217 cm^{-1},1031 cm^{-1}(C-F)	74.968 (74.37)	4.407 (4.58)	-

Table 3: The anti-inflammatory activity of Chalcone derivative (FCD) on the cotton pellet-induced granuloma in rats compared to Dexamethasone and Diclofenac

Animal groups	Total Wt post-challenge (mg)	% inhibition Total	Exudate Wt (mg)	% inhibition Edema	Granuloma Wt (mg)	% Inhibition Granuloma
Control	47.42±5.2	0	17.0±1.8	0	30.4±3.4	0
Test compound	36.85±4.0	23.17±8.5 [a]	9.8±1.2*	42.3±6.8 [a]	26.7±5.0	21.3±12.8 [a]
Dexamethasone	30.8±2.5*	35.0±5.2 [a]	13.0±0.6*	23.6±5.0 [a]	17.8±2.1*	41.1±6.8 [a]
Diclofenac	42.8±1.8	10.3±3.4 [a,b]	11.8±0.7*	30.8±4.1 [a]	31.0±2.3	6.8±4.5 [a,b]

Values are presented as mean±SEM; number of rats=6 in each group; * significantly different compared to control (P<0.05) using unpaired t-test; values with non-identical superscripts (a,b) within the same parameter are considered significantly different (P<0.05) using ANOVA and *post hoc* analysis.

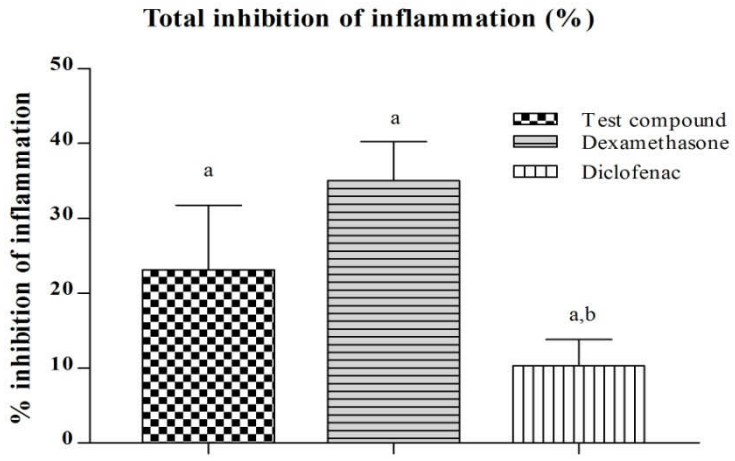


Fig. 1: % inhibition of the total chronic inflammatory response by the chalcone derivative (FCD) compared to Dexamethasone and Diclofenac according to cotton pellet-induced granuloma in rats; values are presented as mean±SEM; values with non-identical letters (a,b) are considered significantly different (P<0.05) using ANOVA and *post hoc* analysis.

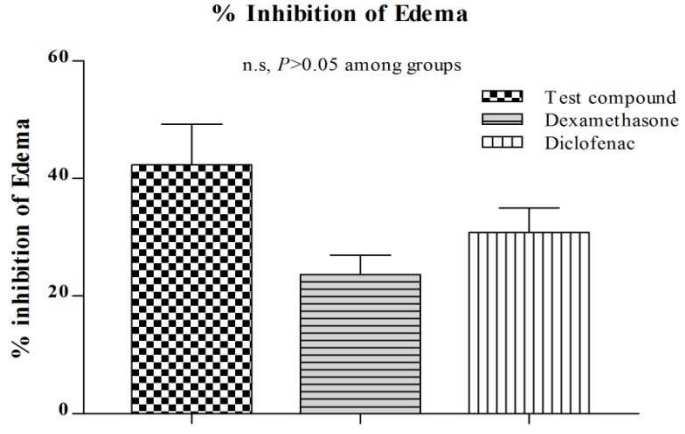


Fig. 2: % inhibition of the Edema by the chalcone derivative (FCD) compared to Dexamethasone and Diclofenac according to cotton pellet-induced granuloma in rats; values are presented as mean±SEM; n.s= non-significant differences (P>0.05).

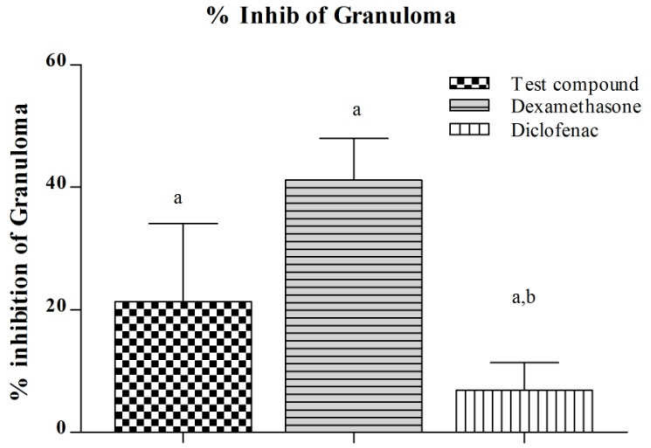


Fig. 3: % inhibition of granuloma formation by the chalcone derivative (Code ---) compared to Dexamethasone and Diclofenac according to cotton pellet-induced granuloma in rats; values are presented as mean±SEM; values with non-identical letters (a,b) are considered significantly different (*P*<0.05) using ANOVA and *post hoc* analysis.

may be attributed to alterations in the physic-chemical properties of these derivatives[25]. The anti-inflammatory activity of FCD was studied *in vivo* to determine the influence on both edema and granulation tissue formation, where FCD demonstrates effective anti-inflammatory activity in term of attenuation of granulation tissue formation, which is comparable to that produced by dexamethasone, greater than the influence on edema formation. Similar results were reported by Yadav *et al* (2011), who indicated that the anti-inflammatory activity of chalcone derivatives was increased when electron withdrawing groups (Halogen or hydroxyl moiety) included in the chalcone nucleus[8]. Similarly, the compound '4-fluoro/4-chloro chalcone showed more activity comparable to indomethacin due to -F/-Cl groups present in the compound[26]. In conclusion, the fluorinated chalcone derivative (FCD) was synthesized using $SOCl_2$/EtOH catalytic system with excellent yield; FCD shows anti-inflammatory activity comparable to dexamethasone in cotton pellet-induced granuloma model.

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DISCUSSION

The Claisen-Schmidt condensation is an important C-C bond formation for the synthesis of 1,3-diaryl-2-propen-1-ones (chalcones)[18]. It is generally carried out by the use of strong bases such as NaOH or KOH in polar solvents (MeOH or DMF) or the use of an acid as HCl, BF_3, B_2O_3, p-toluenesulfonic acid, etc[19]. Many modified methods for the synthesis of chalcones have been reported, such as using natural phosphate, lithium nitrate, amino grafted zeolites, zinc oxide, water, Na_2CO_3, PEG_{400}, silicasulfuric acid, $ZrCl_4$ and ionic liquids[20]. Chalcones can be synthesized using basic alumina under microwave irradiation; however, many of these methods had limited applications due to harsh reaction condition, poisonous reagents, strong acidic or basic conditions, prolonged reaction-times, poor yields and low selectivity, and several modifications had been made to overcome such problems[21]. Accordingly, the present study was designed to use an efficient protocol for synthesizing chalcone derivative using $SOCl_2$/EtOH catalytic system, and to explore the potential of the FCD as anti-inflammatory agent. Such protocol gave an excellent yield of the intended product in a short period of time without formation of any side product. As previously reported, the use of 0.05 ml of $SOCl_2$ in the catalytic system was accompanied with a good product yield[22]. Chalcones exist as either E or Z isomers. E isomer is the most stable form[23], and accordingly we follow this approach and synthesized (E)-3-(4-fluorophenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one (FCD) in the present study. In the present study, the use of such volume of $SOCl_2$ was found to be inadequate to produce a good yield of FCD, and many attempts tried to improve the yield by manipulating the volume of $SOCl_2$; then 0.5 ml was shown to be the optimal volume required for this catalyst mixed with 5 ml of absolute ethanol during the reaction, which results in the excellent yield obtained. The structure of FCD was confirmed by chromatographic and spectral data. The melting point of this compound was observed to be different from the melting points of the starting ingredients, which confirm the successful synthesis of the product. The purity of FCD was checked by monitoring a single spot on the TLC plate which in turn proves its purity, and the structure was determined by spectral analysis. The λ_{max} of the FCD was observed at 320 nm and this indicates the presence of an α,β-unsaturated carbonyl moiety. The IR absorption bands at ν values of 1651, 1612, and 1571 cm^{-1} confirmed the presence of a conjugated carbonyl group (C=O) and (C=C), respectively. The experimentally determined elemental microanalysis results were compatible with the calculated data.

Fluorinated chalcone derivatives have been reported to poses anti-inflammatory activity due to their influence on nitric oxide production[24]. The beneficial changes in such type of biological activity often results from introduction of fluorine in the molecule

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