

Synthesis and Assessment of Anti-inflammatory Activity of a Chlorosubstituted Chalcone Derivatives and using the Semi-empirical Methods to Measure the Linked Physicochemical Parameters

ABSTRACT

The present research was planned to synthesize chlorinated chalcone derivatives by condensing acetophenone with chlorinated benzaldehyde in presence of sodium hydroxide. The anti-inflammatory activity was evaluated practically by measuring cotton bellets induced granuloma. Theoretical evaluation was made by using HyperChem software. Chlorinated chalcone derivatives (CHD) showed comparable anti-inflammatory effect to diclofenac sodium, and less than dexamethasone.

KEYWORDS chalcone, granuloma, anti-inflammatory, computational study, benzaldehyde, aldol condensation

INTRODUCTION

Chalcones are naturally occurring compound. It was first extracted from Chinese liquorice (*Glycyrrhizae inflata*)¹. The stable chalcones in plants can't be isolated due to the presence of the enzyme chalcone synthase which converts chalcone into flavanone².

These compounds go through many chemical reactions and it is helpful in the synthesis of heterocyclic compounds such as isoxazole, quinolinone, benzofuranone, indols and flavones, etc. Additionally, these compounds are vital intermediates in many addition reactions of nucleophiles owing to the inductive polarization of the carbonyl-functional group at the β -position³⁻⁵. Many methods are reported for preparation of chalcones, one method involve conjugation or binding of acetophenone derivatives with benzaldehyde, or substituted benzaldehyde in alkaline condition, followed by spontaneous dehydration^{6,7}. They have a wide range of pharmacological activities depending on the substituted group of the two benzene rings of the chalcone⁸. Chalcone derivatives exhibit many medicinal activities like anti-inflammatory analgesic^{9,10}, anticancer¹¹, antiviral¹², antifungal^{13,14}, antimicrobial^{15,16}, antioxidant¹⁷, anti-histamine¹⁸ and anti-hyperglycemic activities¹⁹. The aim of this study is to synthesis of a chlorinated chalcone derivative and to study their anti-inflammatory potency.

EXPERIMENTAL

Instrumentation

All compounds used were of analytical grade, supplied by Fluka. IR spectra were done using FT-IR spectrophotometer, Shimadzo, Japan. Elemental analysis was done at Al Albayt University, Jordan. The animals used were supplied from the College of veterinary Medicine, Basrah University. Statistical study was done by using Microsoft Office 2007, and the computational study performed using HyperChem Software, Department of Chemistry College of Science, Basrah University.

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Synthesis of chlorinated chalcone derivatives

Chlorinated chalcone derivative (CHD) was synthesized by binding of acetophenone with chloro-substituted aromatic aldehyde (chloro-substituted benzaldehyde) in the presence of sodium hydroxide NaOH (Aldol condensation). To the mixture of phenyl methyl ketone (acetophenone, 25 mmol) and chloro-substituted-benzaldehyde (30 mmol) in 30 mL ethanol; 5 mL of 20% ethanolic sodium hydroxide solution was added gradually, drop by drop at ambient temperature with slight stirring for 2 hr. The reaction mixture was set overnight, then it was filtered; the collected solid product was washed with water (2×10 mL). The product was dried to obtain the chlorinated product²⁰. The yellow product then re-crystallized using absolute ethanol. The melting point (m.p.) were checked and recorded, Infrared spectra and C,H,N analysis were recorded as shown in Figure 1.

Evaluation of the anti-inflammatory activity of chlorinated chalcone derivatives

Thirty rats weighing 180–220 g of both sexes were separated into, five groups, each of six rats chosen randomly. Cotton pellets or balls weighing 20 mg were sterilized

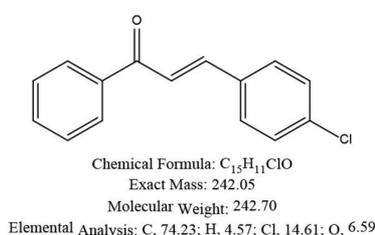
using an autoclave for 30 min. Four pellets were set in subcutaneously (s.c.) into the both sides of the abdominal region under light ether anesthesia. The first group act as a control and given the vehicle only (0.5% carboxymethyl cellulose). Second and third group of animals were provided with standard drug, diclofenac sodium (25 mg/kg) and dexamethasone (2 mg/kg)²¹. The other group was treated with chlorinated chalcone derivatives (100 mg/kg). All the doses were given orally. By the 8th day, the animals were scarified and the pellets with the granuloma tissues were taken, dried in an oven at 60 °C, weighed and compared with control²² (Table 1).

Computational studies

The most common, and popular computational, (Semi-empirical) method used, today in the field of Cheminformatics, is PM3, was used to estimate, total energy, heat of formation (ΔH), dipole moment, hydrophobicity (log-p), the highest energy of the occupied molecular orbital EHOMO, the lowest energy of the occupied molecular orbital ELUMO, EHOMO-ELUMO gap as, following in Table 2.

Even with using structure activity-relationship-techniques (SAR) to study a particular, pharmacological

[E]-3-[4 chlorophenyl]-1 phenylprop-2-en-1-one (compound I)



Color: yellow

Yield: 85%.

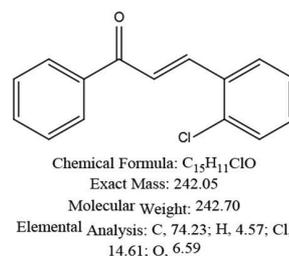
M.p.: 113, reported 113-117[31]

FT-IR [KBr, n, cm⁻¹]: 1656 [C=O], 1611, 1571 [C=C], 1220, 1035

[C-Cl]

Anal.calcd. for C, 74.32, H, 4.57. Found: C, 74.09; H, 4.61.

(E)-3-(2-chlorophenyl)-1-phenylprop-2-en-1-one (compound II)



Color: yellow

Yield: 81%.

M.p.: 48, reported 49-50[31]

FT-IR [KBr, n, cm⁻¹]: 1655 cm⁻¹ (C=O), 1620 cm⁻¹

1597 cm⁻¹(C=C), 1217 cm⁻¹, 1012 cm⁻¹(C-Cl)

Anal.calcd. for C, 74.32, H, 4.57. Found: C, 74.19; H, 4.59.

Fig. 1 Chemical structure, melting point, infrared spectra and C,H,N analysis of the titled compounds.

Table 1 The anti-inflammatory activity of the synthesized compound [CHD] compared to dexamethasone and diclofenac.

Group	Total wt [mg]	% Total inhibition	Exudate wt [mg]	% Edema inhibition	Granuloma wt [mg]	% Inhibition granuloma
Control	59.28 ± 6.3	0.00	21.76 ± 3.7	0.00	37.52 ± 3.6	0.00
Compound 1	46.06 ± 6.4	0.22	12.54 ± 6.7	0.42	33.52 ± 5.9	0.11
Compound 2	42.81 ± 7.2	0.28	11.78 ± 4.3	0.46	31.04 ± 6.1	0.17
Dexamethasone	38.50 ± 4.5	0.35	16.64 ± 7.4	0.24	21.86 ± 6.5	0.42
Diclofenac	53.50 ± 5.7	0.10	17.10 ± 5.1	0.21	36.40 ± 7.4	0.03

Table 2 Physico-chemical properties of chalcone derivatives (computational data).

Properties	2-Chlorochalcone	4-Chlorochalcone
EHOMO [ev]	-9.372677	-9.069714
ELUMO [ev]	-0.9412275	-0.9830234
GAP	8.4314495	8.0866906
Surface area [approx]	399.98	401.9
Surface area [grid]	440.74	445.65
Volume	718.17	718.17
Log P	4.19	2.86
Refractivity	71.68	79.78
Polarizability	27.42	27.42
Dipole moment [Debye]	2.28865	3.101
Mass	242.70	242.70
Total energy [a. u.]	-93.912682329	-93.909368829
Binding energy [kcal/mol]	-3197.5109828	-3195.4317326
Electronic energy [kcal/mol]	-349250.0581166	-346265.37669665
Heat of formation [kcal/mol]	27.100172	29.5892674

activity, we need certain linked physico-chemical, parameters in order to expect the more powerful new medicinal molecule²³. Observation of 2-chlorochalcone and 4-chlorochalcone products on their hydrophobic (log p) and electronic effect (HOMO-LUMO) parameter can be seen on Table 2. 2-chlorochalcone has the highest log p value at 4.19, and 2.86 for the 4-chlorochalcone. It means that 2-chlorochalcone tends to be at the highest non-polar phase²⁴. The difference between HOMO and LUMO- energy (HOMO-LUMO gap) is very important at measuring the molecules stability. Molecules with large HOMO-LUMO gap have a high stability so it has a low reactivity for chemical reaction²⁵. Experimental compound with largest HOMO-LUMO gap is 2-chlorochalcone at 8.431 eV. Since decrease of the energy gap usually leads to easier polarization of the molecule.

RESULTS AND DISCUSSION

Chemical synthesis of CHD gives a good yield (80–85%). The chemical structure of the titled products confirmed through FT-IR, spectroscopy and elemental analysis(C,H,N). While the overall weight of the inflammatory lesion was decreased in all groups as compared to control, the tested compound leads to anti-inflammatory effects less or fewer than that of dexamethasone and higher than diclofenac, as compared to control using unpaired t-test as shown in Table 1.

Relating to the effects of CHD and the standard on the exudate formation, all of them formed a considerable decline in exudate production in comparison to control; nevertheless, when analyzed with ANOVA, the inhibition in exudates revealed to be more than dexamethasone and

diclofenac. For granuloma formation, just dexamethasone results in considerable diminish in the weight of granuloma as compared with control (using an unpaired t-test). CHD produced moderate inhibition percent while diclofenac leads to a little percent of inhibition.(Fig. 2 and 3)

The Aldol condensation reaction is a significant C-C bond formation, for the production of chalcones²⁶. It is commonly carried out by using of strong base, or acid as catalyst²⁷. It involve the reaction of two aldehyde or ketone molecules, one of them, at least, has α hydrogen atom. If there's no α hydrogen, the reaction will change to Cannizzaro reaction and gives the corresponding alcohol and the carboxylate anion. In our experiment, we use NaOH as a catalyst. This method gave a good yield of the products in a short period of time without the formation of side products. Aldol condensation could be prepared by under acidic conditions, using thionyl chloride as a source for the hydrochloric acid to act as a catalyst²⁸.

CHD has been reported to have anti-inflammatory activity due to their effect on nitric-oxide formation²⁹. This changes in the action results from the introduction of the halogen molecule on the parent unsubstituted chalcone, which led to change the physico-chemical properties of these derivatives³⁰.

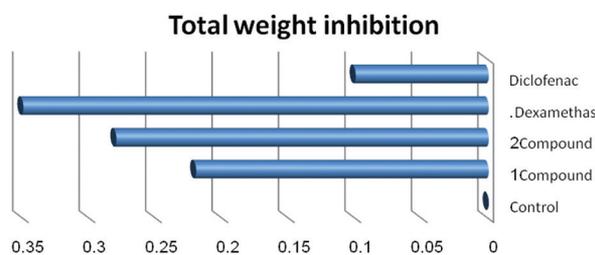


Fig. 2 Total percent of inhibition in the total granuloma weight.

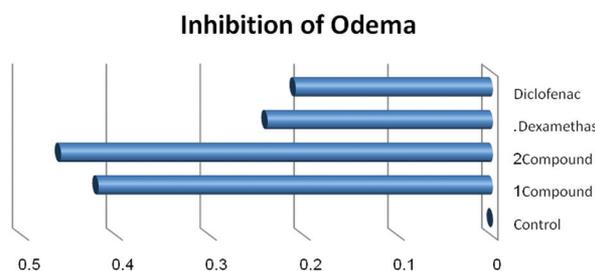


Fig. 3 Total percent of inhibition of the odema.

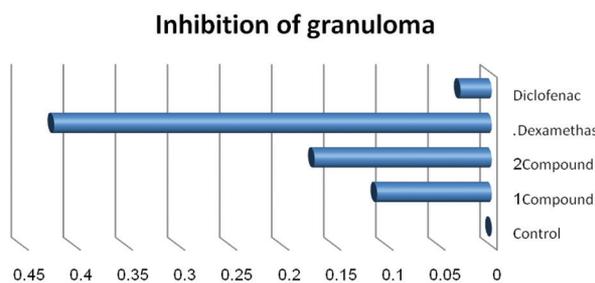


Fig. 4 Total percent of inhibition of the granuloma tissue.

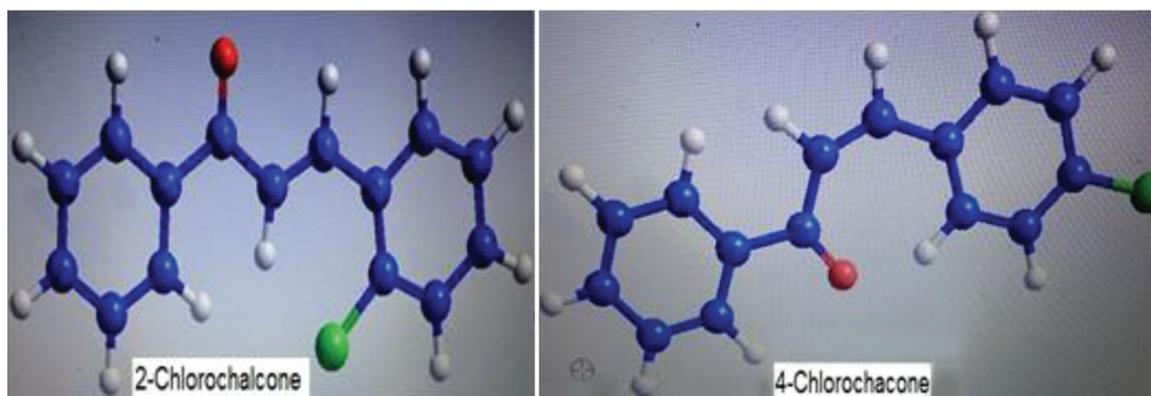


Fig. 5 Energy minimized 3D structure (molecular modeling).

CHD reduces the activities of many enzymes, particularly mammalian- α -amylase, cyclooxygenase enzyme (COX), nitric oxide synthase, monoamine-oxidase enzyme (MAO), tyrosinase, aldolreductase, α -glucosidase, and ant mitotic activity³¹. It seems that the anti-inflammatory effects of chalcone derivatives may be associated with inhibition or suppression of the inflammatory mediators like TNF- α , NO, COX-2, and interleukins³².

Some chalcones confirmed to have the ability to block voltage-dependent potassium-channels³³. The anti-inflammatory activity of CHD was made *in vivo* to establish the influence on both edema and granulation tissue formation. CHD showed effective-anti-inflammatory activity, which is relatively comparable to that expressed by dexamethasone. Similar results were obtained by Yadav-*et al*¹⁰. who revealed that the anti-inflammatory effect of chalcones was elevated when electron-withdrawing group [EWG] incorporated in the chalcone nucleus. Electron withdrawal group like halogen or nitro group at the position number 2 of the phenyl moiety of the substituted benzaldehyde will increase the activity to a greater extent than position number 4 by inhibiting COX-2 enzyme and reduce production of NO³⁴.

The biological activity of chalcone derivatives were correlated to different molecular properties. The reactivity of the chemical compounds, vary as its chemical structure altered. The chemical structure alteration will bring out a modification in the biological properties.

In conclusion, the chlorinated chalcone derivative was synthesized using NaOH catalytic system with excellent yield; CHD shows anti-inflammatory activity comparable to dexamethasone and better than diclofenac in cotton pellet-induced granuloma model.

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