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Design, synthesis and biological evaluation of hydroxy substituted amino chalcone compounds for antityrosinase activity in B16 cells

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ABSTRACT

A series of hydroxy substituted amino chalcone compounds have been synthesized. These compounds were then evaluated for their inhibitory activities on tyrosinase and melanogenesis in murine B16F10 melanoma cell lines. The structures of the compounds synthesized were confirmed by ^{1}H NMR, ^{13}C NMR, FTIR and HRMS. Two novel amino chalcone compounds exhibited higher tyrosinase inhibitory activities (IC_{50} values of 9.75 μM and 7.82 μM respectively) than the control kojic acid (IC_{50}: 22.83 μM). Kinetic studies revealed them to act as competitive tyrosinase inhibitors with their K_i values of 4.82 μM and 1.89 μM respectively. Both the compounds inhibited melanin production and tyrosinase activity in B16 cells. Docking results confirm that the active inhibitors strongly interact with mushroom tyrosinase residues. This study suggests that the depigmenting effect of novel amino chalcone compounds might be attributable to inhibition of tyrosinase activity, suggesting amino chalcones to be a promising candidate for use as depigmentation agents or as anti-browning food additives.

1. Introduction

Melanogenesis is considered as a major defense mechanism of human skin against UV light. However, an excessive accumulation of the pigment, melanin could lead to serious aesthetic issues. Tyrosinase [EC 1.14.18.1], also known as polyphenol oxidase is responsible for melanization in animals, and also plays an important role in cuticle formation in insects. Alterations in tyrosinase dysfunction could culminate with serious maladies including Café au lait macules, ephelides (freckles), solar lentigo (age spots), and melasma [1–5]. Tyrosinase is a key enzyme in the melanogenic pathway responsible for the hydroxylation of L-tyrosine to 3,4-dihydroxy phenylalanine (L-DOPA) and oxidation of L-DOPA to dopaquinone [6]. Therefore, tyrosinase inhibitors should be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation and also important in cosmetics for whitening and depigmentation after sunburn. In addition, tyrosinase is responsible for undesired enzymatic browning of fruits and vegetables that take place during senescence or damage in post-harvest handling. This could culminate with a decline in the functional and organoleptic qualities, such as darkening, softening and off-favour development including a loss in the nutritional value of foods which makes the identification of novel tyrosinase inhibitors extremely important [7,8].

From a structural perspective, tyrosinase has two copper ions in its active site which play a vital role in its catalytic activity. At the active site of tyrosinase, a dioxygen molecule binds in a side-on coordination between two copper ions. Each of the copper ions is coordinated by three histidines in the protein matrix [9]. The copper atoms participate directly in hydroxylation of monophenols to diphenols (cresolase activity) and in the oxidation of *o*-diphenols to *o*-quinones (catechol oxidase activity) that enhance the production of the brown color [10].

Previously, we have reported the synthesis of hydroxy substituted azachalcone compounds with potential inhibitory effects on mushroom tyrosinase activity [11]. Presence of the 2′-hydroxyl group on ring A of the chalcone was considered important as it was involved in binding to the Cu atoms in the tyrosinase active site. Studies have shown the presence of an electron-donating group at *para* position to increase the mushroom tyrosinase inhibitory activity [12]. It was interesting to note that the scaffold of the amino chalcone was structurally quite similar to the substrate L-DOPA. In addition, amino chalcones have been reported to have promising biological activities [13,14]. Also, naphthalene derivatives of oxyresveratrol have served to be a favourable scaffold with potent tyrosinase inhibition [15]. On the basis of these findings, we considered that it might be interesting to synthesize a series of hydroxyphenyl and hydroxynaphthyl

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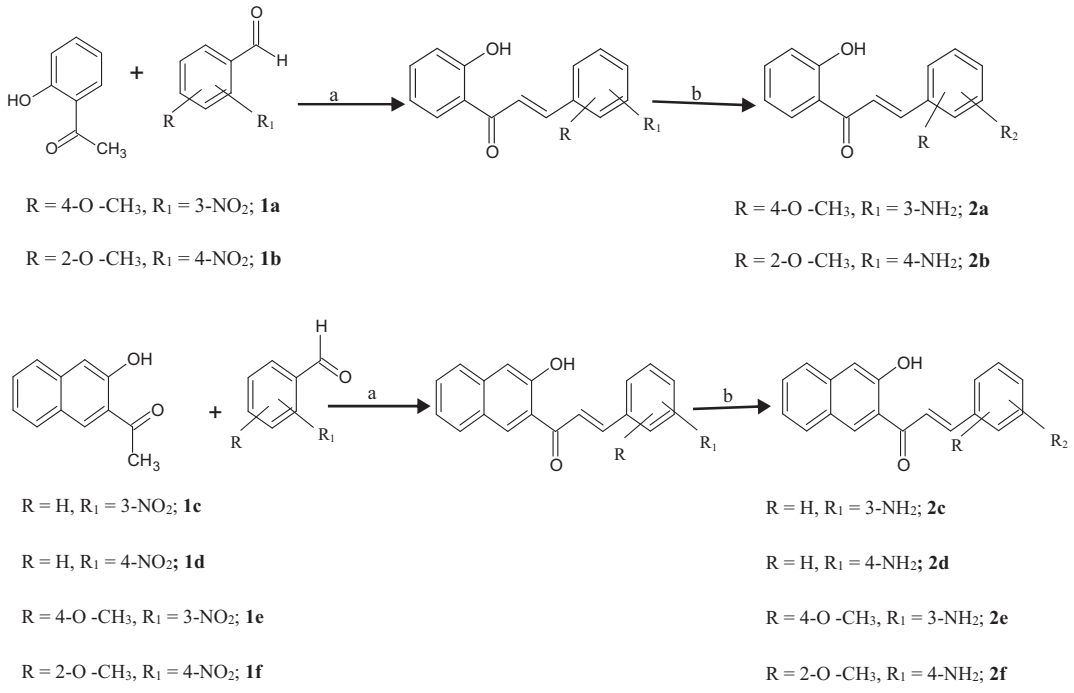

Scheme 1. (General method for the synthesis of nitro chalcones (**1a–1f**) and amino chalcones (**2a–2f**). Reagents and conditions: (a) MeOH, NaOH, 0 °C, 24 hrs; (b) ammonium formate, palladium on carbon, MeOH, RT.

substituted amino chalcone compounds for use as depigmentation agents and as anti-browning food additives.

In the first step, nitro chalcones were synthesized by the base-catalyzed Claisen–Schmidt condensation of an aldehyde and an appropriate ketone in a polar solvent like methanol. These nitro chalcones were then successfully reduced to their corresponding primary amines in the presence of palladium/carbon, using ammonium formate as the hydrogen source (Scheme 1). The reaction was based on a facile mechanism of catalytic hydrogen transfer hydrogenation, an extension of the 'Leuckart reaction' [16]. The structures of the compounds synthesized were confirmed by ^{1}H NMR, ^{13}C NMR, FTIR and HRMS. Assays were performed with L-DOPA as the substrate, using kojic acid, a well-known strong tyrosinase inhibitor as the positive control. For the most promising inhibitors, Lineweaver–Burk kinetic analysis was performed to determine the mechanism of inhibition. Further, we have integrated our experimental results with computational simulation methods to obtain a relative insight into the molecular mechanisms governing the mode of inhibition. Previous studies have indicated the use of chalcones as anticancer agents [17]. This prompted us to further investigate the effect of aminochalcone compounds on melanin formation in murine B16F10 cells.

2. Materials and methods

2.1. Chemical reagents and instruments

Melting points (Mp) were determined with WRS-1B melting point apparatus and the thermometer was uncorrected. NMR spectra were recorded on Agilent 500 spectrometer at 25 °C in $CDCl_3$ or DMSO-d_6. All chemical shifts (∂) are quoted in parts per million downfield from TMS and coupling constants (*J*) are given in Hz. Abbreviations used in the splitting pattern were as follows: *s* = singlet, *d* = doublet, *t* = triplet, *m* = multiplet. HRMS spectra were recorded using the Agilent Technologies 6520 LC/MS-QTOF. All reactions were monitored by TLC (Merck Kieselgel 60 F254) and the spots were visualized under UV light. Infrared (IR) spectra were recorded on Thermo Scientific NICOLET 6700 FT-IR spectrometer. Tyrosinase, L-3,4-dihydroxyphenylalanine (L-DOPA), kojic acid and α-MSH (alpha-melanocyte stimulating hormone) were purchased from Sigma–Aldrich Chemical Co.

2.2. General method for the synthesis of nitro chalcone derivatives (*1a–1f*)

To a stirred solution of the appropriate ketone (1 mM) and a substituted aldehyde (1 mM) in 25 ml methanol, was added pulverized NaOH (2 mM) and the mixture was stirred at room temperature for 24–36 h. The reaction was monitored by TLC using *n*-hexane: ethyl acetate (7:3) as mobile phase. The reaction mixture was cooled to 0 °C (ice-water bath) and acidified with HCl (10% v/v aqueous solution) to afford total precipitation of the compounds. In most cases, a yellow precipitate was formed, which was filtered and washed with 10% aqueous HCl solution. In the cases where an orange oil was formed, the mixture was extracted with CH_2Cl_2, the extracts were dried (Na_2SO_4) and the solvent was evaporated to give the respective chalcone (**1a–1f**).

(**1a**). (2*E*)-1-(2-hydroxyphenyl)-3-(4-methoxy-3-nitrophenyl) prop-2-en-1-one Mp: 110–112 °C; ^{1}H NMR (500 MHz, $CDCl_3$): ∂ 12.47 (s, 1H, OH), 8.10 (s, 1H), 7.86 (d, 1H, *J* = 12.5, H_β), 7.74 (d, 1H, *J* = 10.0, H-6′), 7.72 (d, IH, *J* = 12.5, H_α), 7.65 (d, 1H, *J* = 9.5, H-5), 7.54 (d, 1H, *J* = 8.0, H-6), 7.49 (t, 1H, *J* = 8.5, H-4′), 6.92 (dd, 1H, *J* = 9.5, H-3′), 6.90 (dd, 1H, *J* = 9.0, H-5′), 3.86 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) ∂ 192.22 (C=O), 130.52 (C6′), 119.25 (C1′), 118.27 (C5′), 136.77 (C4′), 118.28 (C3′), 161.92 (C2′), 126.90 (C1), 120.54 (C2), 137.22 (C3), 147.50 (C4), 112.42 (C5), 114.23 (C6), 56.67 (Me), 122.40 (vinylic), 141.83 (vinylic); IR (KBr) ν (cm^{-1}): 3200, 3070, 2914, 2864, 2720, 1686, 1578, 1550, 1468, 1349, 970, 720, 580; MS (ESI): 270.1 $([M + H])^{+}$.

(**1b**). (2*E*)-1-(2-hydroxyphenyl)-3-(2-methoxy-4-nitrophenyl) prop-2-en-1-one Mp: 123–125 °C; ^{1}H NMR (500 MHz, $CDCl_3$): ∂ 12.22 (s, 1H, OH), 8.22 (s, 1H), 7.95 (d, 1H, *J* = 14.0, H_β), 7.86 (m, 1H, *J* = 9.5, H-6), 7.80 (d, IH, *J* = 14.0, H_α), 7.75 (d, 1H, *J* = 10.5,