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Development of hydroxylated naphthylchalcones as polyphenol oxidase inhibitors: Synthesis, biochemistry and molecular docking studies

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ABSTRACT

Polyphenol oxidase (Tyrosinase) has received great attention, since it is the key enzyme in melanin biosynthesis. In this study, novel hydroxy naphthylchalcone compounds were synthesized, and their inhibitory effects on mushroom tyrosinase activity were evaluated. The structures of the compounds synthesized were confirmed by ^{1}H NMR, ^{13}C NMR, FTIR and HRMS. Two of the compounds synthesized inhibited the diphenolase activity of tyrosinase in a dose dependent manner and exhibited much higher tyrosinase inhibitory activities (IC_{50} values of 10.4 μM and 14.4 μM, respectively) than the positive control, kojic acid (IC_{50}: 27.5 μM). Kinetic analysis showed that their inhibition was reversible. Both the novel compounds displayed competitive inhibition with their K_i values of 3.8 μM and 4.5 μM, respectively. Docking results confirmed that the active inhibitors strongly interacted with the mushroom tyrosinase residues. This study suggests hydroxy naphthylchalcone compounds to serve as promising candidates for use as depigmentation agents.

1. Introduction

Polyphenol oxidase (PPO), also known as tyrosinase [EC 1.14.18.1] is a multifunctional copper-containing enzyme, widely distributed in micro-organisms, animals, and plants [3,15]. The major rate limiting step in melanin biosynthesis involves the enzyme polyphenol oxidase that catalyzes two different reactions of melanin biosynthesis, the hydroxylation of L-tyrosine to L-DOPA (L-3,4-dihydroxy phenylalanine) and oxidation of L-DOPA to DOPA quinone [16]. 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 side-on coordination mode between two copper ions. Each of the copper ions is coordinated by three histidines in the protein matrix [19]. 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 [4,10]. Therefore, chelation of tyrosinase Cu^{2+} by synthetic compounds or agents from natural sources has been targeted as a way to inhibit or block tyrosinase catalysis [21]. An alternative solution to inhibit tyrosinase catalytic activity would be by effectively blocking access to the active site of enzyme.

Alterations in tyrosinase dysfunction could culminate with serious pigmentation disorders like melasma, chloasma, lentigo, age spots, inflammatory hypermelanosis and trauma-induced hyperpigmentation [17,1,26]. Thus use of tyrosinase inhibitors is becoming increasingly important in the cosmetic and medicinal industries due to their preventive effect on pigmentation disorders. In addition, tyrosinase is responsible for undesired enzymatic browning of fruits and vegetables that take place during senescence or damage in post-harvest handling, which makes the identification of novel tyrosinase inhibitors extremely important. Enzymatic browning could culminate with discoloration and a decline in the nutritional value of foods [18]. Recently, 4-hexyl resorcinol has found to be quite effective for browning control in fresh and dried fruit slices [20]. Despite reports of a large number of tyrosinase inhibitors, only a few are used today because of their limitations with regard to cytotoxicity, selectivity, and stability [12,6,25,5].

Studies have shown hydroxy-substituted chalcones to have good tyrosinase inhibition [14,11]. Previously, we have reported the synthesis of hydroxy substituted azachalcone compounds with potential inhibitory effects on mushroom tyrosinase activity [22]. Furthermore, several hydroxy-substituted 2-phenylnaphthalene derivatives were reported as potent tyrosinase inhibitors. Among them, 4-(6-hydroxy-2-naphthyl)-1,3-benzendiol (HNB) and 5-(6-hydroxy-2-naphthyl)-1,2,3-benzentriol (5HNB) were found to inhibit mushroom tyrosinase activity [24,9,2]. Hence, in our

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continuous effort to search for potential tyrosinase inhibitors and to develop a new template, we have attempted to design and synthesize a series of novel hydroxy substituted naphthylchalcone compounds for application as effective polyphenol oxidase inhibitors. We hypothesized that the ligand's hydroxyl group may block polyphenol oxidase activity by binding to the copper atoms in the active site of mushroom tyrosinase based on the fact that previous findings had shown the role of hydroxyl groups in tyrosinase inhibition [7,13]. Assays were performed with L-DOPA as the substrate, using kojic acid, a well-known strong tyrosinase inhibitor as the positive control. We have further investigated the kinetic parameters and inhibition mechanisms of active tyrosinase inhibitor compounds. In addition, we determined the IC_{50}, that is, the concentration of compound that causes 50% inhibition for kojic acid and the active inhibitors. Dose-dependent inhibition experiments were performed in triplicate to determine the IC_{50} of test compounds. To confirm our hypothesis, we simulated the docking between the ligands and tyrosinase using Discovery Studio 4.5. From the docking results, we checked for possible hydrogen-bonding and non-bonding interactions with the amino acid residues. For the control simulation, the docking simulation of kojic acid, a well-known tyrosinase inhibitor, with tyrosinase was also performed.

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) and kojic acid were purchased from Sigma–Aldrich Chemical Co.

2.2. General method for the synthesis of naphthylchalcones (**1a–5a**)

To a stirred solution of 2′-hydroxy-1′-acetonaphthone (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–5a**).

(**1a**). (2*E*)-3-(2,5-dimethoxyphenyl)-1-(3-hydroxynaphthalen-2-yl) prop-2-en-1-one. M.p 114–116 °C. ^{1}H NMR (500 MHz, $CDCl_3$): ∂ 12.54 (s, 1H, OH), 8.12 (dd, 1H, *J* = 12.5, H-10′), 7.96 (d, 1H, *J* = 13.5, H_α), 7.85 (s, 1H, H-6), 7.80 (dd, 1H, *J* = 10.0, H-5′), 7.67 (dd, 1H, *J* = 8.5, H-7′), 7.59 (dd, 1H, *J* = 9.0, H-8′), 7.52 (dd, 1H, *J* = 9.5, H-6′), 7.49 (d, 1H, *J* = 9.5, H-3′), 6.92 (d, 1H, *J* = 13.5, H_β), 6.57 (dd, 1H, *J* = 10.0, H-4), 6.49 (dd, 1H, *J* = 9.5, H-3), 3.82 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) ∂ 195.2 (C═O), 116.5 (C1′), 166.2 (C2′), 123.2 (C3′), 136.1 (C4′), 130.4 (C5′), 134.0 (C6′), 128.4 (C7′), 130.0 (C8′), 122.4 (C9′), 131.2 (C10′), 142.4 (vinylic), 131.7 (vinylic), 129.2 (C1), 156.6 (C2), 112.3 (C3), 126.4 (C4), 153.2 (C5), 112.2 (C6), 56.2 (Me); IR (KBr) *ν* (cm^{-1}): 3022, 2992, 2773, 1682, 1550, 1425, 1046, 814, 707; MS (ESI): 335.1 ([M+H])$^+$.

(**2a**). (2*E*)-3-(2,4-dimethoxyphenyl)-1-(3-hydroxynaphthalen-2-yl) prop-2-en-1-one. M.p 87–89 °C. ^{1}H NMR (500 MHz, $CDCl_3$): ∂ 12.72 (s, 1H, OH), 8.19 (dd, 1H, *J* = 15.0, H-10′), 7.98 (d, 1H, *J* = 15.0, H_α), 7.82 (dd, 1H, *J* = 12.0, H-5′), 7.78 (dd, 1H, *J* = 10.0, H-6), 7.63 (t, 1H, *J* = 8.5, H-7′), 7.57 (dd, 1H, *J* = 8.0, H-8′), 7.49 (dd, 1H, *J* = 9.0, H-6′), 7.47 (d, 1H, *J* = 9.0, H-3′), 7.09 (d, 1H, *J* = 15.0, H_β), 6.53 (t, 1H, *J* = 16.0, H-5), 6.44 (m, 1H, *J* = 9.0, H-3), 3.86 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) ∂ 194.6 (C═O), 114.1 (C1′), 165.0 (C2′), 122.2 (C3′), 137.1 (C4′), 130.4 (C5′), 132.6 (C6′), 126.1 (C7′), 128.4 (C8′), 124.4 (C9′), 133.2 (C10′), 140.8 (vinylic), 130.5 (vinylic), 119.2 (C1), 162.6 (C2), 99.3 (C3), 166.2 (C4), 107.2 (C5), 130.2 (C6), 55.6 (Me); IR (KBr) *ν* (cm^{-1}): 3018, 2957, 2781, 2614, 1679, 1562, 1427, 1375, 1218, 1102, 817, 717, 605; MS (ESI): 335.1 ([M+H])$^+$.

(**3a**). (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, H-6′), 7.69 (d, 1H, *J* = 8.5, H-5), 7.59 (dd, 1H, *J* = 8.5, H-4′), 7.02 (dd, 1H, *J* = 9.0, H-3′), 6.97 (m, 1H, *J* = 10.0, H-5′), 3.82 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) ∂ 194.2 (C═O), 130.2 (C6′), 119.3 (C1′), 118.2 (C5′), 137.0 (C4′), 118.4 (C3′), 162.9 (C2′), 128.2 (C1), 115.2 (C2), 112.2 (C3), 137.5 (C4), 122.4 (C5), 116.5 (C6), 125.5 (vinylic), 140.8 (vinylic); IR (KBr) *ν* (cm^{-1}): 3250, 3345, 3015, 2910, 2865, 2700, 1702, 1682, 1552, 1465, 1305, 979, 735, 680, 575; MS (ESI): 270.1 ([M+H])$^+$.

4a. (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])$^+$.

(**5a**). (2*E*)-3-(3,5-dimethoxyphenyl)-1-(3-hydroxynaphthalen-2-yl) prop-2-en-1-one. M.p 118–120 °C. ^{1}H NMR (500 MHz, $CDCl_3$): ∂ 12.62 (s, 1H, OH), 7.92 (dd, 1H, *J* = 10.0, H-10′), 7.85 (d, 1H, *J* = 10.5, H_α), 7.82 (dd, 1H, *J* = 10.5, H-5′), 7.69 (t, 1H, *J* = 9.5, H-7′), 7.58 (dd, 1H, *J* = 9.5, H-8′), 7.55 (m, 1H, *J* = 8.5, H-6′), 7.50 (d, 1H, *J* = 9.0, H-3′), 7.09 (dd, 2H, H-2 & H-6), 7.07 (d, 1H, *J* = 10.5, H_β), 6.75 (s, 1H, H-4), 3.90 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) ∂ 198.5 (C═O), 118.1 (C1′), 167.2 (C2′), 124.2 (C3′), 140.4 (C4′), 135.3 (C5′), 135.2 (C6′), 124.9 (C7′), 130.2 (C8′), 124.1 (C9′), 134.5 (C10′), 144.0 (vinylic), 133.2 (vinylic), 138.2 (C1), 106.6 (C2 & C6), 163.3 (C3 & C5), 106.2 (C4), 55.2 (Me); IR (KBr) *ν* (cm^{-1}): 3062, 2947, 2729, 2514, 1692, 1597, 1512, 1436,1208, 1054, 871, 732; MS (ESI): 335.1 ([M+H])$^+$.

2.3. Method for the synthesis of hydroxy derivatives of naphthylchalcones (**1b–5b**)

A solution of BBr_3 (2.5 mL for each methoxy group) was added to a cooled solution of the corresponding methoxy naphthylchalcone (1 mmol) in CH_2Cl_2 under argon. The cooling bath was removed and the dark solution was warmed to RT and stirred for 1–5 h. The dark solution was then poured into ice water and filtered. The aqueous layer was extracted with chloroform twice.