Chem Biol Drug Des 2013; **82:** 39–47

Research Article

Microwave-Assisted Synthesis and Tyrosinase Inhibitory Activity of Chalcone Derivatives

Jinbing Liu*, Changhong Chen, Fengyan Wu and Liangzhong Zhao

Department of Biology and Chemical Engineering, Shaoyang University, Shao Shui Xi Road, Shaoyang, 422100, China
**Corresponding author: Jinbing Liu, xtliujb@yahoo.com.cn, jliu1238@umd.edu*

A series of chalcones and their derivatives were synthesized, and their inhibitory effects on the diphenolase activity of mushroom tyrosinase were evaluated. The results showed that some of the synthesized compounds exhibited significant inhibitory activity, and four compounds exhibited more potent tyrosinase inhibitory activity than the reference standard inhibitor kojic acid (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one). Specifically, 1-(-1-(4-methoxyphen- yl)-3-phenylallylidene) thiosemicarbazide (18) exhibited the most potent tyrosinase inhibitory activity with IC_{50} value of 0.274 μM. The inhibition mechanism analysis of 1-(-1-(2,4-dihydroxyphenyl)-3-phenylallylidene) thiosemicarbazide (16) and 1-(-1-(4-methoxyphenyl)-3-phenylallylidene) thiosemicarbazide (18) demonstrated that the inhibitory effects of the two compounds on the tyrosinase were irreversible. Preliminary structure activity relationships' analysis suggested that further development of such compounds might be of interest.

Key words: chalcone derivatives, inhibition mechanism, microwave-assisted synthesis, tyrosinase inhibitors

Received 2 December 2012, revised 26 January 2013 and accepted for publication 14 February 2013

Tyrosinase(monophenol or o-diphenol, oxygen oxidoreductase, EC 1.14.18.1, syn. polyphenol oxidase), also known as polyphenol oxidase (PPO), is a copper-containing monooxygenase that is widely distributed in microorganisms, animals, and plants (1). Tyrosinase could catalyze two distinct reactions involving molecular oxygen in the hydroxylation of monophenols to o-diphenols (monophenolase) and in the oxidation of o-diphenols to o-quinones (diphenolase) (2). Due to the high reactivity, quinones could polymerize spontaneously to form high-molecular weight brown pigments (melanins) or react with amino acids and proteins to enhance brown color of the pigment produced (3,4). Hyperpigmentations, such as senile lentigo, melasma, freckles, and pigmented acne scars, are of particular concern to women. Therefore, the regulation of melanin synthesis by inhibiting the tyrosinase enzyme is a current research topic in the context of preventing hyperpigmentation. The treatment usually involves the use of medicines or medicinal cosmetics containing depigmenting agents or skin-whitening agents (5,6). In clinical usage, tyrosinase inhibitors are used for treatments of dermatological disorders related to melanin hyperaccumulation and are essential in cosmetics for depigmentation (7–11), such as age spots and freckle, caused by the accumulation of an excessive level of epidermal pigmentation (12,13).

Previous reports confirmed that tyrosinase not only was involved in melanism animals, but also was one of the main causes of most fruits and vegetables quality loss during postharvest handling and processing, leading to faster degradation and shorter shelf life (14). Tyrosinase has also been linked to Parkinson's and other neurodegenerative diseases (15). In insects, tyrosinase is uniquely associated with three different biochemical processes, including sclerotization of cuticle, defensive encapsulation and melanization of foreign organism, and wound healing (16). These processes provide potential targets for developing safer and effective tyrosinase inhibitors as insecticides and ultimately for insect control. Thus, the development of safe and effective tyrosinase inhibitors is of great concern in the medical, agricultural, and cosmetic industries. However, only a few such as kojic acid (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one), arbutin, tropolone, ascorbic acid derivatives, hydroxystilbene derivatives, and 1-phenyl-2-thiourea are used as therapeutic agents and cosmetic products (12,17).

Chalcones (1,3-diaryl-2-propen-1-ones) constitute an important class of natural products belonging to the flavonoids family of widespread occurrence in plants. They have been reported to exhibit a wide range of pharmaceutical effects including antioncogenetic, anti-inflammatory, anti-ulcerative, antimalarial, antiviral, antifungal, and antibacterial activities (18–20). Recently, chalcones were highlighted as a new class of tyrosinase inhibitor in several publications (21,22). The literature survey revealed that compounds with thiourea moieties have been reported to demonstrate a wide range of pharmacological activities, which include antibacterial, antifungal, anticonvulsant, and tyrosinase inhibitory activity, such as phenylthioureas,

 doi: 10.1111/cbdd.12126

alkylthioureas, and 1,3-bis-(5-methanesulfonylbutyl)thiourea, displayed weak or moderate tyrosinase inhibitory activity (23,24). More recently, our investigations also demonstrated that thiosemicarbazide derivatives exhibited potent inhibitory activities against mushroom tyrosinase (25,26). Stimulated by these results, in continuation of our research work on safe and effective tyrosinase inhibitors, in the present investigation, we synthesized a series of chalcones and their Schiff bases, and their inhibitory activities against mushroom tyrosinase were evaluated using kojic acid (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one) as comparing substance. Meanwhile, the structure–activity relationships of these compounds were also primarily discussed. Microwave-assisted synthesis is an efficient and eco-friendly synthetic strategy and has now become a powerful tool for green chemistry. Microwave irradiation has been applied to organic reactions in the absence of solvent or in the presence of a solid support, such as clays, alumina, and silica, resulting in shorter reaction times and better product yields than those obtained using conventional heating (27–30). In this article, some compounds were also synthesized by microwave irradiation.

Methods and Materials

Chemical reagents and instruments

Melting points (m.p.) were determined with WRS-1B melting point apparatus, and the thermometer was uncorrected. NMR spectra were recorded on Bruker 400 spectrometersat 25 °C in $CDCl_3$ or DMSO-d_6. All chemical shifts (δ) are quoted in parts per million downfield from TMS, and coupling constants (J) are given in hertz. Abbreviations used in the splitting pattern were as follows: s = singlet, d = doublet, t = triplet, q = quintet, m = multiplet; LC-MS spectra were recorded using the LCMS-2010A. All reactions were monitored by TLC (Merck Kieselgel 60 F254, Shanghai, China), and the spots were visualized under UV light. Infrared (IR) spectra were recorded as potassium bromide pellets on VECTOR 22 spectrometer.

Tyrosinase, L-3, 4-dihydroxyphenylalanine (L-DOPA), and kojic acid were purchased from Sigma–Aldrich Chemical Co (Shanghai, China). Other chemicals were purchased from commercial suppliers and were dried and purified when necessary.

The general procedures for the synthesis of substituted chalcone compounds (1–12)

Method 1

To the mixture of substituted acetophenone (5.0 mmol) and benzaldehyde (5.0 mmol), ethanol (20 mL) and 30% NaOH in H_2O (1 mL) were added. The reaction mixture was taken in round-bottomed flask placed in a microwave reactor and irradiated at 800W for 3 min. Then, this reaction mixture was poured over crushed ice and acidified with 10% HCl solution (10 mL) to form precipitate. The precipitate was filtered and washed with appropriate amount of water. The crude product was recrystallized from 95% ethanol to afford substituted chalcone **1**–**12**.

Method 2

Substituted acetophenone (5.0 mmol) and benzaldehyde (5.0 mmol) were dissolved in ethanol (20 mL), and 30% NaOH (1 mL) was added to the mixture. The reaction mixture was stirred at room temperature. The completion of the reaction was checked by TLC. The mixture was neutralized with 10% HCl solution (10 mL) to form precipitate. The precipitate was filtered and washed with appropriate amount of water. The crude product was recrystallized from 95% ethanol to afford substituted chalcone **1**–**12**.

1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one (1)

Yield 63.2%. Yellow solid, mp 78.3–80.3 °C; IR(KBr): 1736, 1653, 1549, 1333, 1217, 1151, 1084, 1030, 977, 827, 761/cm. ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.98(d, 2H, J = 8.4 Hz, ph-H), 7.84(d, 1H, J = 15.6 Hz, =CH), 7.66–7.63 (m, 2H, ph-H), 7.50(d, 1H, J = 15.6 Hz, =CH), 7.49(d, 2H, J = 8.4 Hz, ph-H), 7.43–7.42(m, 3H, ph-H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 189.2, 145.3, 139.2, 136.5, 134.7, 130.7, 129.9, 129.1, 128.9, 128.5, 121.5.

1-(3-hydroxyphenyl)-3-phenylprop-2-en-1-one (2)

Yield 81.2%. Yellow solid, mp 128.6–130.3 °C; IR(KBr): 3208, 1648, 1570, 1445, 1346, 1259, 1188, 1030, 972, 856, 757/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 7.90–7.84 (m, 3H, ph-H), 7.75 (d, 1H, J = 15.6 Hz, =CH), 7.88 (d, 1H, J = 8.1 Hz, ph-H), 7.48–7.46 (m, 4H, ph-H), 7.41 (d, 1H, J = 15.6 Hz, =CH), 7.09 (d, 1H, J = 8.1 Hz, ph-H).

1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one (3)

Yield 67.2%. Yellow solid, mp 179.0–181.2 °C; IR(KBr): 1736, 1653, 1549, 1333, 1217, 1151, 1084, 1030, 977, 827, 761/cm. ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.98 (d, 2H, J = 8.4 Hz, ph-H), 7.84 (d, 1H, J = 15.6 Hz, =CH), 7.66–7.63 (m, 2H, ph-H), 7.50 (d, 1H, J = 15.6 Hz, =CH), 7.49 (d, 2H, J = 8.4 Hz, ph-H), 7.43–7.42 (m, 3H, ph-H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 189.2, 145.3, 139.2, 136.5, 134.7, 130.7, 129.9, 129.1, 128.9, 128.5, 121.5.

1-(2,4-dihydroxyphenyl)-3-phenylprop-2-en-1-one (4)

Yield 51.3%. Yellow solid, mp 139.0–141.4 °C; IR(KBr): 3237, 1752, 1628, 1549, 1491, 1441, 1358, 1225, 1142, 968, 860, 769/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 8.25 (d, 1H, J = 9.0 Hz, ph-H), 8.03 (d, 1H, J = 15.3 Hz, =CH), 7.93–7.90 (m, 2H, ph-H), 7.85 (d, 1H, J = 15.3 Hz, =CH), 7.49–7.47 (m, 3H, ph-H), 6.48 (d, 1H, J = 9.0 Hz, ph-H), 6.34 (s, 1H, ph-H).

1-(2-hydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (5)

Yield 40.4%. Yellow solid, mp 87.7–99.0 °C; IR(KBr): 3370, 1595, 1483, 1242, 1163, 1105, 1076, 1022, 964, 860, 786/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 8.32 (d, 1H, J = 9.0 Hz, ph-H), 8.06 (d, 1H, J = 15.3 Hz, =CH), 7.94–7.91 (m, 2H, ph-H), 7.86 (d, 1H, J = 15.3 Hz, =CH), 7.49–7.48 (m, 3H, ph-H), 6.60(d, 1H, J = 9.0 Hz, ph-H), 6.54 (s, 1H, ph-H), 3.86 (s, 3H, OCH3).

1-(4-methoxyphenyl)-3-phenylprop-2-en-1-one (6)

Yield 77.4%. White solid, mp 104.6–108.6 °C; IR(KBr): 2894, 1736, 1653, 1503, 1342, 1259, 1225, 1184, 1101, 1035, 968, 827, 761/cm. ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.06 (d, 2H, J = 8.8 Hz, ph-H),7.83 (d, 1H, J = 15.6 Hz, =CH), 7.66–7.63 (m, 2H, ph-H), 7.56 (d, 1H, J = 15.6 Hz, =CH), 7.42–7.41 (m, 3H, ph-H), 7.00 (d, 2H, J = 8.8 Hz, ph-H), 3.89 (s, 3H, OCH3); ^{13}CNMR (100 MHz, $CDCl_3$) δ 188.7, 163.4, 144.0, 135.1, 131.1, 130.8, 130.3, 128.9, 128.3, 121.9, 113.9, 55.5.

1-(4-(methoxymethoxy)phenyl)-3-phenylprop-2-en-1-one (7)

Yield 57.2%. Yellow solid; IR(KBr): 2897, 2843, 1731,1636, 1512, 1371, 1342, 1221, 1134, 1068, 1010, 956, 840, 790/cm. ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.04 (d, 2H, J = 8.8 Hz, ph-H),7.83 (d, 1H, J = 15.6 Hz, =CH), 7.66–7.63 (m, 2H, ph-H), 7.56 (d, 1H, J = 15.6 Hz, =CH), 7.42–7.41 (m, 3H, ph-H), 7.12 (d, 2H, J = 8.8 Hz, ph-H), 5.23 (s, 2H, OCH2), 3.50 (s, 3H, OCH3); ^{13}CNMR (100 MHz, $CDCl_3$) δ 188.9, 161.0, 144.2, 135.1, 132.0, 130.7, 130.4, 128.9, 128.4, 121.9, 115.9, 94.1, 56.3.

1-(4-fluorophenyl)-3-phenylprop-2-en-1-one (8)

Yield 56.5%. Yellow solid, mp 76.2–78.3 °C; IR(KBr): 1742, 1644, 1536, 1333, 1217, 1151, 1035, 981, 836, 761/cm. ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.10 (d, 2H, J = 7.6 Hz, ph-H), 7.85 (d, 1H, J = 15.6 Hz, =CH),7.65–7.62 (m, 2H, ph-H), 7.66–7.64 (m, 2H, ph-H), 7.54 (d, 1H, J = 15.6 Hz, =CH), 7.44(d, 2H, J = 7.6 Hz, ph-H),7.21–7.17(m, 4H, ph-H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 188.9, 145.1, 134.8, 134.6, 131.2, 131.0, 130.6, 129.0, 128.5, 121.6, 115.8, 115.6.

1-(4-chlorophenyl)-3-phenylprop-2-en-1-one (9)

Yield 50.9%. Yellow solid, mp 97.1–98.3 °C; IR(KBr): 1736, 1653, 1549, 1333, 1217, 1151, 1084, 1030, 977, 827, 761/cm. ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.98 (d, 2H, J = 8.4 Hz, ph-H), 7.84 (d, 1H, J = 15.6 Hz, =CH), 7.66–7.63 (m, 2H, ph-H), 7.50 (d, 1H, J = 15.6 Hz, =CH), 7.49 (d, 2H, J = 8.4 Hz, ph-H), 7.43–7.42 (m, 3H, ph-H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 189.2, 145.3, 139.2, 136.5, 134.7, 130.7, 129.9, 129.1, 128.9, 128.5, 121.5.

1-(4-bromophenyl)-3-phenylprop-2-en-1-one (10)

Yield 75.4%. Yellow solid, mp 100.5–102.7 °C; IR(KBr): 1736, 1648, 1557, 1391, 1337, 1213, 1155, 1072, 1030, 977, 827, 761/cm. ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.89 (d, 2H, J = 8.8 Hz, ph-H),7.83 (d, 1H, J = 15.6 Hz, =CH), 7.65–7.63 (m, 4H, ph-H), 7.49 (d, 1H, J = 15.6 Hz, =CH), 7.43–7.41 (m, 3H, ph-H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 189.3, 145.4, 136.9, 134.6, 131.9, 130.7, 130.0, 129.0, 128.5, 127.9, 121.4.

1-(3-chlorophenyl)-3-phenylprop-2-en-1-one (11)

Yield 56.6%. Yellow solid, mp 95.9–97.0 °C; IR(KBr): 1771, 1648, 1599, 1325, 1283, 1205, 1047, 972, 856, 757/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 8.22 (s, 1H, ph-H), 8.15 (d, 1H, J = 8.7 Hz, ph-H), 8.01 (d, 1H, J = 15.6 Hz, =CH), 7.95–7.93 (m, 2H, ph-H), 7.82 (d, 1H, J = 15.6 Hz, =CH), 7.75–7.73 (m, 2H, ph-H), 7.49–7.47 (m, 3H, ph-H).

1-(3-bromophenyl)-3-phenylprop-2-en-1-one (12)

Yield 67.2%. Yellow solid, mp 87.0–91.3 °C; IR(KBr): 1736, 1653, 1541, 1337, 1205, 1151, 1068, 981, 856, 757/cm. ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H, ph-H), 7.95 (d, 1H, J = 8.8 Hz, ph-H),7.84 (d, 1H, J = 15.6 Hz, =CH), 7.72 (d, 1H, J = 8.8 Hz, ph-H), 7.66–7.64 (m, 2H, ph-H), 7.48 (d, 1H, J = 15.6 Hz, =CH), 7.43–7.36 (m, 4H, ph-H); ^{13}CNMR (100 MHz, $CDCl_3$) δ 189.0, 145.7, 140.0, 135.6, 134.6, 131.5, 130.8, 130.2, 129.0, 128.6, 127.0, 123.0, 121.4.

The general procedures for the synthesis of substituted chalcone thiosemicarbazide Schiff base compounds (13–21)

Method 1

To the mixture of substituted chalcone (5.0 mmol) and thiosemicarbazide (5.0 mmol), ethanol (20 mL) and catalytic amount of acetic acid were added. The reaction mixture was taken in round-bottomed flask placed in a microwave reactor and irradiated at 800W for 8 min. The reaction mixture was allowed to cool to room temperature, and precipitate was formed. The precipitate was filtered and washed with appropriate amount of ethanol. The crude product was recrystallized from 95% ethanol to afford substituted chalcone Schiff base **13**–**21**.

Method 2

To the mixture of substituted chalcone (5.0 mmol) and thiosemicarbazide (5.0 mmol), ethanol (20 mL) and catalytic amount of acetic acid were added. The reaction mixture was refluxed at 80 °C over a silicone oil bath for 6–24 h. The completion of the reaction was checked by TLC. The reaction mixture was allowed to cool to room temperature, and precipitate was formed. The precipitate was filtered

and washed with appropriate amount of ethanol. The crude product was recrystallized from 95% ethanol to afford substituted chalcone Schiff base **13**–**21**.

1-(-1-(2-hydroxyphenyl)-3-phenylallylidene) thiosemicarbazide (13)

Yield 42.9%. Yellow solid, mp 201.3–207.6 °C; IR(KBr): 3395, 1648, 1557, 1499, 1283, 1217, 1113, 1076, 985, 881, 761/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.44 (s, 1H, NH), 8.64 (s, 1H, NH), 8.16 (s, 1H, NH), 8.13 (d, 1H, J = 9.0 Hz, ph-H), 7.96 (d, 1H, J = 15.6 Hz, =CH), 7.70 (d, 2H, J = 8.7 Hz, ph-H), 7.43–7.41 (m, 4H, ph-H), 7.28 (d, 1H, J = 15.6 Hz, =CH), 6.46 (d, 1H, J = 9.0 Hz, ph-H), 6.34 (s, 1H, ph-H).

1-(-1-(3-hydroxyphenyl)-3-phenylallylidene) thiosemicarbazide (14)

Yield 63.1%. Yellow solid, mp 178.7–180.1 °C; IR(KBr): 3378, 1615, 1570, 1487, 1433, 1234, 1159, 1076, 968, 827, 757/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 11.07 (s, 1H, NH), 9.54 (s, 1H, NH), 8.46 (s, 1H, NH), 7.79 (d, 1H, J = 15.6 Hz, =CH), 7.75–7.72 (m, 3H, ph-H), 7.47 (d, 1H, J = 8.1 Hz, ph-H), 7.41–7.37 (m, 4H, ph-H), 7.24 (d, 1H, J = 15.6 Hz, =CH), 7.01 (d, 1H, J = 8.1 Hz, ph-H).

1-(-1-(4-hydroxyphenyl)-3-phenylallylidene) thiosemicarbazide (15)

Yield 37.9%. Yellow solid, mp 200.1–204.5 °C; IR(KBr): 3341, 1603, 1487, 1333, 1279, 1221, 1159, 1122, 964, 873, 836, 761/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.90 (s, 1H, NH), 9.76 (s, 1H, NH), 8.58 (s, 1H, NH), 8.02 (d, 2H, J = 8.4 Hz, ph-H),7.75 (d, 1H, J = 15.0 Hz, =CH), 7.74–7.20 (m, 2H, ph-H), 7.49 (d, 1H, J = 15.0 Hz, =CH), 7.49–7.47 (m, 3H, ph-H), 7.00 (d, 2H, J = 8.4 Hz, ph-H).

1-(-1-(2,4-dihydroxyphenyl)-3-phenylallylidene) thiosemicarbazide (16)

Yield 32.1%. Yellow solid, mp 185.1–188.9 °C; IR(KBr): 3361, 1599, 1516, 1495, 1445, 1288, 1230, 1163, 1101, 997, 831, 699/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.28 (s, 1H, NH), 8.34(s, 1H, NH), 8.33 (d, 1H, J = 8.4 Hz, ph-H), 8.11(s, 1H, NH), 7.57 (d, 2H, J = 8.4 Hz, ph-H), 7.47 (d, 1H, J = 15.6 Hz, =CH), 7.42–7.40 (m, 2H, ph-H), 7.34–7.30 (m, 2H, ph-H), 7.01 (d, 1H, J = 15.6 Hz, =CH), 7.01–6.96 (m, 2H, ph-H). MS (ESI): m/z(100%) 312 (M-1).

1-(-1-(2-hydroxy-4-methoxyphenyl)-3-phenylallylidene)thiosemicarbazide (17)

Yield 17.5%. Yellow solid, mp 116.5–122.6 °C; IR(KBr): 3407, 1599, 1487, 1433, 1259, 1196, 1155, 1097, 831, 695/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.34 (s, 1H, NH), 8.24 (s, 1H, NH), 8.21 (d, 1H, J = 8.7 Hz, ph-H), 8.03 (s, 1H, NH), 7.56 (d, 2H, J = 6.9 Hz, ph-H), 7.47 (d, 1H, J = 15.6 Hz, =CH), 7.46–7.37 (m, 3H, ph-H), 6.60 (d, 1H, J = 15.6 Hz, =CH), 6.59–6.54 (m, 2H, ph-H), 3.77 (s, 3H, OCH3). MS (ESI): m/z (100%) 326 (M-1).

1-(-1-(4-methoxyphenyl)-3-phenylallylidene) thiosemicarbazide (18)

Yield 28.7%. Yellow solid, mp 158.6–165.7 °C; IR(KBr): 1595, 1483, 1242, 1163, 1105, 1076, 1022, 964, 860, 786/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.96 (s, 1H, NH), 9.92 (s, 1H, NH), 8.53 (s, 1H, NH), 7.77 (d, 1H, J = 15.6 Hz, =CH), 7.74–7.72 (m, 2H, ph-H), 7.61 (d, 2H, J = 8.7 Hz, ph-H),7.41 (d, 1H, J = 15.6 Hz, =CH), 7.39–7.36 (m, 3H, ph-H), 7.01 (d, 2H, J = 8.7 Hz, ph-H), 3.81 (s, 3H, OCH3). MS (ESI): m/z(100%) 312 (M + 1).

1-(-1-(4-fluorophenyl)-3-phenylallylidene) thiosemicarbazide (19)

Yield 32.9%. Yellow solid, mp 168.9–171.0 °C; IR(KBr): 1603, 1487, 1333, 1279, 1221, 1159, 1122, 1075, 964, 873, 761/cm. ^{1}HNMR (400 MHz, DMSO-d_6) δ11.09 (s, 1H, NH), 8.86 (s, 1H, NH), 8.32 (s, 1H, NH), 7.82 (d, 1H, J = 15.6 Hz, =CH), 7.75 (d, 2H, J = 7.5 Hz, ph-H), 7.71–7.68 (m, 2H, ph-H), 7.49–7.47 (m, 2H, ph-H), 7.47 (d, 1H, J = 15.6 Hz, =CH), 7.39–7.25 (m, 3H, ph-H); ^{13}CNMR (100 MHz, DMSO-d_6) δ 196.4, 179.4, 147.6, 140.1, 136.3, 131.9, 129.8, 129.1, 128.5, 121.6, 119.2, 115.7. MS (ESI): m/z (100%) 300 (M + 1).

1-(-1-(3-chlorophenyl)-3-phenylallylidene) thiosemicarbazide (20)

Yield 45.8%. Yellow solid, mp 175.2–178.3 °C; IR(KBr): 1595, 1565, 1466, 1321, 1279, 1134, 1072, 968, 865, 836, 753/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 11.13 (s, 1H, NH), 9.12 (s, 1H, NH), 8.57 (s, 1H, NH), 8.37 (s, 1H, ph-H), 8.12 (d, 1H, J = 8.7 Hz, ph-H), 8.09 (d, 1H, J = 15.6 Hz, =CH), 7.78–7.74 (m, 2H, ph-H), 7.56 (d, 1H, J = 16.0 Hz, =CH), 7.51–7.49 (m, 2H, ph-H), 7.46–7.42 (m, 3H, ph-H).

1-(-1-(3-bromophenyl)-3-phenylallylidene) thiosemicarbazide (21)

Yield 39.4%. Yellow solid, mp 159.7–162.2 °C; IR(KBr): 1595, 1491, 1445, 1317, 1292, 1205, 1122, 1064, 968, 873, 757/cm. ^{1}HNMR (300 MHz, DMSO-d_6) δ 11.16 (s, 1H, NH), 8.40 (s, 1H, NH), 8.14 (s, 1H, NH), 7.99 (s, 1H, ph-H), 7.88 (d, 1H, J = 8.7 Hz, ph-H),7.80 (d, 1H, J = 15.9 Hz, =CH), 7.60–7.30 (m, 2H, ph-H), 7.64 (d, 1H, J = 8.7 Hz, ph-H), 7.44–7.40 (m, 4H, ph-H), 6.81 (d, 1H, J = 15.9 Hz, =CH).

Assay of the diphenolase activity

The spectrophotometric assay for tyrosinase was performed according to the method reported by our groups

with some slight modifications (14,25). Briefly, all the synthesized compounds were screened for the diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. All the compounds were dissolved in DMSO. The final concentration of DMSO in the test solution was 2.0%. Phosphate buffer, pH 6.8, was used to dilute the DMSO stock solution of test compounds. Thirty units of mushroom tyrosinase (0.5 mg/mL) were first pre-incubated with the compounds, in 50 mM phosphate buffer (pH 6.8), for 10 min at 25 °C. Then, the L-DOPA (0.5 mM) was added to the reaction mixture, and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm of formation of the DOPA chrome for 1 min. The measurement was performed in triplicate for each concentration and averaged before further calculation. IC_{50} value, a concentration giving 50% inhibition of tyrosinase activity, was determined by interpolation of the dose–response curves. The percent of inhibition of tyrosinase reaction was calculated as following:

$$\text{Inhibition rate}(\%) = [(B - S)/B] \times 100$$

Here, the *B* and *S* are the absorbances for the blank and samples. Kojic acid (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one) was used as reference standard inhibitor for comparison.

Results and Discussion

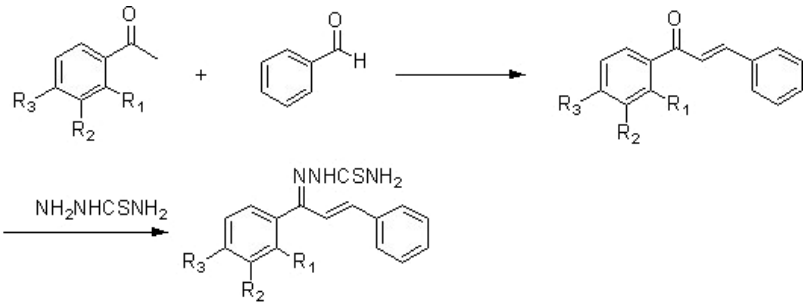
Synthesis

In the present investigation, substituted chalcones have been prepared by the Claisen–Schmidt condensation of substituted acetophenone and benzaldehyde (Scheme 1). The crude product was recrystallized from 95% ethanol to afford substituted chalcone. Chalcone Schiff bases were synthesized from corresponding chalcone and thiosemicarbazide in present of acetic acid as catalyst. The rapid, highly yielding, and eco-friendly microwave-assisted organic syntheses were also used to synthesize substituted chalcones and their Schiff bases. The majority of microwave-assisted reactions were conducted in polar, highly microwave absorbing solvent. The yields of these compounds are from moderate to good, and all the target compounds were characterized by IR, NMR. Some target compounds were also characterized by MS.

Tyrosinase inhibitory activity

For evaluating the tyrosinase inhibitory activity, all the synthesized compounds were subjected to tyrosinase inhibition assay with L-DOPA as substrate, according to the method reported by our groups with some slight modifications. The tyrosinase inhibitory activity of kojic acid (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one) was ever reported; therefore, it was selected as comparing substance. Figure 1 showed that the remaining enzyme activity rapidly decreased with the increasing concentrations of compound **18**. The IC_{50} values of substituted chalcones and their Schiff bases against tyrosinase were summarized in Table 1, and IC_{50} values of all these compounds were determined by interpolation of the dose–response curves and given as means of three experiments.

Our results showed that most of compounds exhibited potent inhibition on mushroom tyrosinase with IC_{50} values ranged from 0.274 to 148.41 μM. Specifically, compounds **14**–**21** bearing thiosemicarbazide showed more potent inhibitory activities than the other compounds. In addition, compounds **16**–**19** demonstrated more potent inhibitory



1. R^1 = OH, R^2 = H, R^3 = H;
2. R^1 = H, R^2 = OH, R^3 = H;
3. R^1 = H, R^2 = H, R^3 = OH;
4. R^1 = OH, R^2 = H, R^3 = OH ;
5. R^1 = OH, R^2 = H, R^3 = OMe;
6. R^1 = H, R^2 = H, R^3 = OMe;
7. R^1 = H, R^2 = H, R^3 = OCH2OCH3;
8. R^1 = H, R^2 = H, R^3 = F;
9. R^1 = H, R^2 = H, R^3 = Cl;
10. R^1 = H, R^2 = H, R^3 = Br;
11. R^1 = H, R^2 = Cl, R^3 = H;
12. R^1 = H, R^2 = Br, R^3 = H;
13. R^1 = OH, R^2 = H, R^3 = H;
14. R^1 = H, R^2 = OH, R^3 = H;
15. R^1 = H, R^2 = H, R^3 = OH;
16. R^1 = OH, R^2 = H, R^3 = OH;
17. R^1 = OH, R^2 = H, R^3 = OMe ;
18. R^1 = H, R^1 = H, R^3 = OMe;
19. R^1 = H, R^2 = H, R^3 = F;
20. R^2 = H, R^2 = Cl, R^3 = H ;
21. R^1 = H , R^2 = Br, R^3 = H

Scheme 1: Synthesis of the substituted chalcones and their thiosemicarbazide Schiff base.

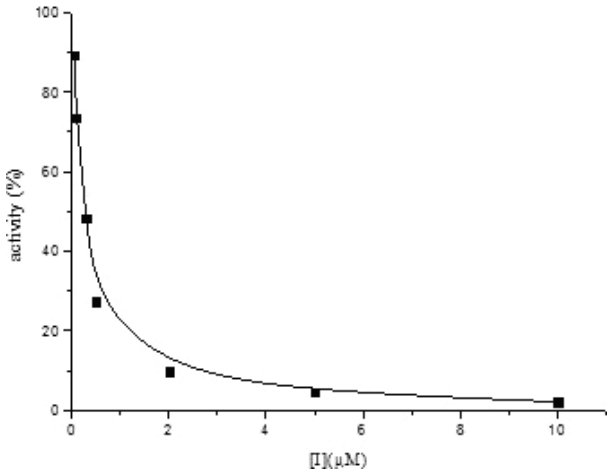


Figure 1: Effect of compound **18** on the diphenolase activity of mushroom tyrosinase for the catalysis of L-DOPA at 25 °C.

Table 1: Tyrosinase inhibitory activities of the synthesized compounds

Compounds	CLogP[a]	IC_{50} (μM)[b]	Percent of inhibition[c]
1	3.96		11.2
2	3.50		18.35
3	3.50		29.06
4	3.45	110.22	75.2
5	4.03		31.5
6	3.84	141.80	54.11
7	3.35		13.26
8	3.84		40.2
9	4.41		40.25
10	4.56		37.22
11	4.41	117.18	65.91
12	4.56		NA[d]
13	4.92		47.97
14	4.92	148.41	59.14
15	4.92	77.87	71.61
16	4.42	19.79	95.49
17	5.00	18.81	95.58
18	5.28	0.274	98.64
19	5.29	7.56	96.59
20	5.86	23.91	85.68
21	6.01	52.68	76.78
Kojic acid		23	11.2

[a]Value of CLogP was obtained by ChemBioDraw Ultra 12.0.
[b]Values were determined from logarithmic concentration–inhibition curves (at least seven points) and are given as means of three experiments.
[c]Percent of inhibition of tyrosinase reaction at the 200 μM.
[d]Not active at 200 μM concentration.

activity than the reference standard inhibitor kojic acid, and 1-(-1-(4-methoxyphenyl)-3-phenylallylidene)thiosemicarbazide (**18**) exhibited the most potent tyrosinase inhibitory activity with IC_{50} value of 0.274 μM. 1-(-1-(2-hydroxyphenyl)-3-phenylallylidene)thiosemicarbazide (**13**) bearing thiosemicarbazide exhibited low tyrosinase inhibitory activity, and the percent of inhibitory is 47.97% at the 200 μM, but it still showed higher activity against tyrosinase than 1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one (**1**). The results showed substituted chalcone Schiff bases exhibited more potent inhibitory activities than corresponding substituted chalcones. Most of the chalcone analogs showed low activities against tyrosinase. The results showed that the group of thiosemicarbazide played important role in increasing activities against tyrosinase. These may be related to the structure of tyrosinase contained a type-3 copper center with a coupled dinuclear copper active site in the catalytic core. Tyrosinase inhibition of compounds **14**–**21** depended on the competency of the sulfur atom to chelate with the dicopper nucleus in the active site, and tyrosinase would lose its catalyzing ability after forming complex (31).

From the tyrosinase inhibitory activities of compounds **1**–**3**, although these compounds show low activities, we can find 1-(2-hydroxyphenyl)-3- phenylprop-2-en-1-one (**3**) exhibited more potent inhibitory activity than the other compounds. This suggested that hydroxyl group at the position of benzene ring might play an important role in determining this kind of compounds inhibitory activities. This may be related to the steric hindrance. Comparing to the tyrosinase inhibitory activities of compounds **1**–**4**, compound **4** exhibited the most potent tyrosinase inhibitory activity with IC_{50} value of 110.22 μM. This result showed that the number of hydroxyl group may be an important effecting factor of inhibitory activities. However, from the Table 1, we can find that compound **6** (CLogP = 3.84) exhibited higher activity than the compound **3** (CLogP = 3.50). This suggested that methoxyl group at the 4-position of benzene ring might be beneficial to improve this kind of compounds inhibitory activities. This may be related to the lipophilicity. 1-(3-bromophenyl)-3-phenylprop-2-en-1-one (**12**) showed the most potent inhibitory activity in all the halogen substituted chalcone. Comparing the inhibitory activities of compounds **8**–**10** with halogen atom at the 4th position of benzene ring and compounds **11**–**12** with halogen atom at the 3th position of benzene ring, with the increase in the radius of halogen atom, the inhibitory activities decreased gradually. Comparing to the inhibitory activities of all the thiosemicarbazide Schiff bases, 1-(-1-(4-methoxyphenyl)-3-phenylallylidene)thiosemicarbazide (**18**) exhibited the most potent tyrosinase inhibitory activity with IC_{50} value of 0.274 μM. From the tyrosinase inhibitory activities of compounds **13**–**18**, the same result can be also obtained, hydroxyl group at the position of benzene ring and the number hydroxyl group might play an important role in determining this kind of Schiff base compounds inhibitory activities, and methoxyl group at the 4th position of benzene ring might be beneficial to improve this kind of Schiff base compounds inhibitory activities. Comparing to the tyrosinase inhibitory activities of compounds **19**–**21**, the result showed that 1-(-1-(4-fluorophenyl)-3-phenylallylidene) thiosemicarbazide (**19**) have better activity than the other two compounds; with the increase in the radius of halogen

atom, the inhibitory activities also decreased gradually. According to the ClogP values of compounds **19**–**21**, the inhibitory activities of these compounds may be also related to the lipophilicity.

Inhibitory mechanism

The inhibitory mechanisms of the selected 1-(-1-(2,4-dihydroxyphenyl)- 3-phenylallylidene)thiosemicarbazide (**16**) and 1-(-1- (4-methoxyphenyl)-3-phenylallylidene)thiosemicarbazide (**18**) on mushroom tyrosinase for the oxidation of L-DOPA were determined (32). Figures 2 and 3 showed the relationship between enzyme activity and concentration in the presence of different concentrations of the above-mentioned compounds. The results displayed that the plots of *V* versus *[E]* gave a family of parallel straight lines with the same slopes. These results demonstrated that the inhibitory effects of compounds **16**, **18** on the tyrosinase were irreversible, suggesting that thiosemicarbazide Schiff bases of substituted chalcone compounds effectively inhibited the enzyme by binding to its binuclear active site irreversibly. The result may be related to the structure of tyrosinase; within the structure, there are two copper ions in the active center of tyrosinase and a lipophilic long-narrow gorge near to the active center. Compounds **16** or **18** could exhibit strong affinity for copper ions in the active center and form a reversible non-covalent complex with the tyrosinase, and this then reacts to produce the covalently modified 'dead-end complex' (13,33–35).

Conclusions

In conclusion, we have design, synthesized, and tested a series of chalcones and their thiosemicarbazones as inhibitors of tyrosinase for defining their structure–activity relationship (SAR) studies. As a result, 1-(-1-(4-methoxyphenyl)-3-phenylallylidene)thiosemicarbazide (**18**) proved the best tyrosinase inhibitor among synthesized compounds. Preliminary structure activity relationships (SARs) analysis indicated that (i) thiosemicarbazide moiety might play an important role in determining their inhibitory activities, because of the sulfur atom could chelate with the dicopper nucleus in the active site, and tyrosinase would lose its catalyzing ability after forming complex; (ii) methoxyl group at the 4th position of benzene ring might be beneficial to improve the inhibitory activities of tyrosinase; (iii) hydroxyl group at the position of benzene ring and the number hydroxyl group might affect inhibitory activity. The inhibition mechanism analysis of 1-(-1-(2,4-dihydroxyphenyl) -3-phenylallylidene) thiosemicarbazide (**16**) and 1-(-1- (4-methoxyphenyl)-3-phenylallylidene)thiosemicarbazide (**18**) demonstrated that the inhibitory effects of the two compounds on the tyrosinase were irreversible.

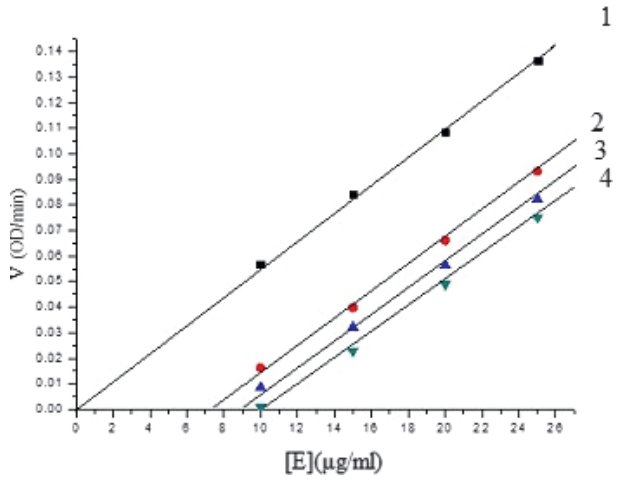


Figure 2: The effect of concentrations of tyrosinase on its activity for the catalysis of L-DOPA at different concentration of compound **16**. The concentrations of compound **16** for curves 1–4 are 0, 5, 10 and 50μM, respectively.

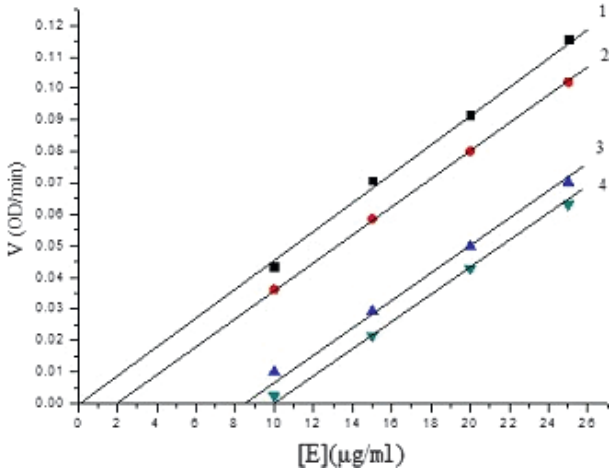


Figure 3: The effect of concentrations of tyrosinase on its activity for the catalysis of L-DOPA at different concentration of compound **18**. The concentrations of compound **18** for curves 1–4 are 0, 0.2, 0.3 and 0.5 μM, respectively.

Acknowledgments

This work was financially supported by the Foundation of Hunan Provincial Science & Technology Department, China (2012FJ4350), the Key Subject Foundation of Shaoyang University (2008XK201) and the Hunan Province University Students Research Fund (Xiang Jiao Tong [2010]244-320).

References

1. Song K.K., Huang H., Han P., Zhang C.L., Shi Y., Chen Q.X. (2006) Inhibitory effects of *cis*- and *trans*-isomers of 3,5-dihydroxystilbene on the activity of

mushroom tyrosinase. Biochem Biophys Res Commun;342:1147–1151.
2. Chen Q.X., Liu X.D., Huang H. (2003) Inactivation kinetics of mushroom tyrosinase in the dimethyl sulfoxide solution. Biochemistry (Mosc);68:644–649.
3. Kubo I., Nihei K.I., Shimizu K. (2004) Oxidation products of quercetin by mushroom tyrosinase. Bioorg Med Chem Lett;12:5343–5347.
4. Matsuura R., Ukeda H., Sawamura M. (2006) Tyrosinase inhibitory activity of citrus essential oils. J Agric Food Chem;54:2309–2313.
5. Wang H.-M., Chen C.-Y., Chen C.-Y. (2010) (–)-*N*-Formylanonaine from *Michelia alba* as human tyrosinase inhibitor and antioxidant. Bioorg Med Chem;18:5241–5247.
6. Tripathi R.K., Hearing V.J., Urabe K., Aroca P., Spritz R.A. (1992) Mutational mapping of the catalytic activities of human tyrosinase. J Biol Chem;267:23707–23712.
7. Shiino M., Watanabe Y., Umezawa K. (2001) Synthesis of N-substituted N-nitrosohydroxylamines as inhibitors of mushroom tyrosinase. Bioorg Med Chem;9:1233–1240.
8. Gillbro J.M., Marles L.K., Hibberts N.A., Schallreuter K.U. (2004) Autocrine catecholamine biosynthesis and the beta-adrenoceptor signal promote pigmentation in human epidermal melanocytes. J Invest Dermatol;123:346–353.
9. Spencer J.D., Chavan B., Marles L.K., Kauser S., Rokos H., Schallreuter K.U. (2005) A novel mechanism in control of human pigmentation by {beta}-melanocyte-stimulating hormone and 7-tetrahydrobiopterin. J Endocrinol;187:293–302.
10. Schallreuter K.U., Hasse S., Rokos H., Chavan B., Shalbaf M., Spencer J.D., Wood J.M. (2009) Cholesterol regulates melanogenesis in human epidermal melanocytes and melanoma cells. Exp Dermatol;18:680–688.
11. Wood J.M., Decker H., Hartmann H., Chavan B., Rokos H. (2009) Senile hair graying: H_2O_2-mediated oxidative stress affects human hair color by blunting methionine sulfoxide repair. FASEB J;23:2065–2075.
12. Gupta A.K., Gover M.D., Nouri K.N., Taylor S. (2006) The treatment of melasma: a review of clinical trials. J Am Acad Dermatol;55:1048–1065.
13. Thanigaimalai P., Hoang T.A.L. (2010) Structural requirement(s) of *N*-phenylthioureas and benzaldehyde thiosemicarbazones as inhibitors of melanogenesis in melanoma B 16 cells. Bioorg Med Chem Lett;20:2991–2993.
14. Yi W., Cao R. (2010) Synthesis and biological evaluation of novel 4-hydroxybenzaldehyde derivatives as tyrosinase inhibitors. Eur J Med Chem;45:639–646.
15. Zhu Y.J., Zhou H.T., Hu Y.H. (2011) Antityrosinase and antimicrobial activities of 2-phenylethanol, 2-phenylacetaldehyde and 2-phenylacetic acid. Food Chem;124:298–302.
16. Ashida M., Brey P. (1995) Role of the integument in insect defense: pro-phenol oxidase cascade in the cuticular matrix. Proc Natl Acad Sci USA;92:10698–10702.
17. Battaini G., Monzani E., Casella L., Santagostini L., Pagliarin R. (2000) Inhibition of the catecholase activity of biomimetic dinuclear copper complexes by kojic acid. J Biol Inorg Chem;5:262–268.
18. Kim B.T., O.K.-J., Chun J.C., Hwang K.J. (2008) Synthesis of dihydroxylated chalcone derivatives with diverse substitution patterns and their radical scavenging ability toward DPPH free radicals. Bull Korean Chem Soc;29:1125–1130.
19. Rao Y.K., Fang S.H., Tzeng Y.M. (2004) Differential effects of synthesized 2′-oxygenated chalcone derivatives: modulation of human cell cycle phase distribution. Bioorg Med Chem;12:2679–2686.
20. Bandgaretal B.P., Gawande S.S., Bodade R.G., Totre J.V. (2010) Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory and antioxidant agents. Bioorg Med Chem;18:1364–1370.
21. Nerya O., Musa R., Khatib S., Tamir S., Vaya J. (2004) Chalcones as potent tyrosinase inhibitors: the effect of hydroxyl positions and numbers. Phytochemistry;65:1389–1395.
22. Seo W.D., Ryu Y.B., Crutis-Long M.J., Lee C.W., Park K.H. (2010) Evaluation of anti-pigmentary effect of synthetic sulfonylamino chalcone. Eur J Med Chem;45:2010–2017.
23. Klabunde T., Eicken C. (1998) Crystal structure of a plant catechol oxidase containing a dicopper center. Nat Struct Biol;5:1084–1090.
24. Ley J.P., Bertram H. (2001) Hydroxy- or methoxy-substituted benzaldoximes and benzaldehyde-*O*-alkyloximes as tyrosinase inhibitors. Bioorg Med Chem;9:1879–1885.
25. Liu J.B., Yi W., Wan Y.Q., Ma L., Song H.C. (2008) 1-(1-Arylethylidene)thiosemicarbazide derivatives: a new class of tyrosinase inhibitors. Bioorg Med Chem;14:1096–1102.
26. Liu J.B., Cao R.H., Yi W., Ma C.M., Wan Y.Q., Zhou B.H., Ma L., Song H.C. (2009) A class of potent tyrosinase inhibitors: alkylidenethiosemicarbazide compounds. Eur J Med Chem;44:1773–1778.
27. Paweczyk A., Zaprutko L. (2009) Microwave assisted synthesis of unsaturated jasmone heterocyclic analogues as new fragrant substances. Eur J Med Chem;44:3032–3039.
28. Kappe C.O. (2008) Microwave dielectric heating in synthetic organic chemistry. Chem Soc Rev;37:1127–1139.
29. Kappe C.O. (2004) Controlled microwave heating in modern organic synthesis. Angew Chem Int Ed;43:6250–6284.
30. Shi F., Dai A.X., Zhang S., Zhang X.H., Tu S.J. (2011) Efficient microwave-assisted synthesis of novel

3-aminohexahydrocoumarin derivatives and evaluation on their cytotoxicity. Eur J Med Chem;46:953–960.
31. Gerdemann C., Eicken C., Krebs B. (2002) The crystal structure of catechol oxidase: new insight into the function of type-3 copper proteins. Acc Chem Res;35:183–191.
32. Yin S.J., Si Y.X., Chen Y.F., Qian G.Y. (2011) Mixed-type inhibition of tyrosinase from *Agaricus bisporus* by terephthalic acid: computational simulations and kinetics. Protein J;30:273–280.
33. Tsou C.L. (1988) Kinetics of substrate reaction during irreversible modification of enzyme activity. Adv Enzymol Relat Areas Mol Biol;61:381–436.
34. Garcia-Canovas F., Tudela J., Varon R., Vazquez A.M. (1989) Experimental methods for kinetic study of suicide substrates. J Enzyme Inhib;3:81–90.
35. Espín J.C., Wichers H.J. (2001) Effect of captopril on mushroom tyrosinase activity in vitro. Biochim Biophys Acta;1544:289–300.