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Synthesis of 4′-Thiosemicarbazonegriseofulvin and Its Effects on the Control of Enzymatic Browning and Postharvest Disease of Fruits

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Supporting Information

ABSTRACT: 4′-Thiosemicarbazonegriseofulvin, a new thiosemicarbazide derivative of griseofulvin, was synthesized and evaluated for its potential in the control of enzymatic browning and postharvest disease of fruits. Browning on fruits is mainly due to the enzymatic oxidation of phenolic compounds catalyzed by tyrosinase. 4′-Thiosemicarbazonegriseofulvin could effectively inhibit the activity of tyrosinase, and its 50% inhibitory concentration (IC_{50}) against tyrosinase was determined to be 37.8 μM. It was a reversible and noncompetitive inhibitor of tyrosinase, and its inhibition constant (K_I) was determined to be 38.42 μM. The antifungal activity of 4′-thiosemicarbazonegriseofulvin was studied against four fungi (*Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium solani*, and *Colletotrichum truncatum*) that often cause postharvest diseases of fruits. The results showed that 4′-thiosemicarbazonegriseofulvin could also strongly inhibit the mycelial growth of the four target fungi; the 50% lethal concentration (LC_{50}) values were 5.4, 7.0, 15.3, and 1.5 mM, respectively.

KEYWORDS: *4′-thiosemicarbazonegriseofulvin, synthesis, antibrowning activity, antifungal activity, postharvest disease*

INTRODUCTION

Browning and postharvest disease of fruits cause considerable losses to the food industry.[1,2] The mechanical damage and collision that may likely occur to fruits during postharvest handling and processing will accelerate the browning.[3] These not only reduce the visual quality but also result in undesirable changes in flavor and nutrient loss. The browning is mainly due to the enzymatic oxidation of phenolic compounds catalyzed by tyrosinase (EC 1.14.18.1).[4−7] Tyrosinase can catalyze monophenols into *o*-diphenols and *o*-diphenols into quinones, respectively.[8,9] Quinones will be converted into brown pigments and undergo further nonenzymatic polymerization.[10] Thus, tyrosinase inhibitors will be effective antibrowning agents; Enokitake mushroom extracts,[11] *Artocarpus heterophyllus* extracts,[12] oxyresveratrol and *Morus alba* L. extract[13] and so on are all antibrowning agents.

Fruit is vulnerable to attack by several fungal pathogens because of its high amount of nutrients, water content, and low pH. Moreover, fruit loses most of its intrinsic resistance to fungal pathogens after harvest. Therefore, postharvest disease of fruits could cause severe losses to the food industry.[14,15] Seen in this light, adequate control of postharvest diseases is a prerequisite for the production of a stable and profitable food supply. The pathogens that cause the most important postharvest diseases are such species belonging to *Alternaria*, *Aspergillus*, *Botrytis*, *Fusarium*, *Geotrichum*, *Gloeosporium*, *Mucor*, *Monillinia*, *Penicillium*, *Colletotrichum*, and other genera.[16−22] Griseofulvin is a representative antifungal antibiotic and has been widely used as an antifungal drug. Its antifungal activity has been demonstrated on many filamentous fungi.[23−25] However, it has little effect on antibrowning of fruit. The present study aimed to prepare a new thiosemicarbazide derivative of griseofulvin (4′-thiosemicarbazonegriseofulvin) and investigated its inhibitory effects on both tyrosinase activity and antifungal properties.

MATERIALS AND METHODS

Materials. Griseofulvin (99.9% powder) was purchased from Shanghai Pharmaceuticals Holding Co., Ltd. (Shanghai, China). Mushroom tyrosinase (EC 1.14.18.1) with 6680 U/mg was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and L-3,4-dihydroxyphenylalanine (L-DOPA) were obtained from Aldrich (St. Louis, MO, USA). *Fusarium oxysporum* (FJAT-3701), *Fusarium moniliforme* (FJAT-165), *Fusarium solani* (FJAT-176), and *Colletotrichum truncatum* (FJAT-9253) were from Fujian Academy of Agricultural Sciences. All other reagents were local products of analytical grade.

Synthesis of 4′-Thiosemicarbazonegriseofulvin. 4′-Thiosemicarbazonegriseofulvin was prepared by the reaction of griseofulvin and thiosemicarbazide in an acidic solution of ethanol, as previously described.[26] Griseofulvin (10 mmol) and acetic acid (10 mL) were added to a solution of thiosemicarbazide (10 mmol) in methanol (60 mL). The mixture was stirred and refluxed for 72 h and cooled to room temperature. Then the reaction mixture was kept under 4 °C overnight and filtered. The product was purified by recrystallization in ethanol. The structure of the product was established by spectroscopic methods (HRMS and NMR; see the Supporting Information).

4′-Thiosemicarbazonegriseofulvin (the structure is shown in Figure 1a) was obtained as a white powder: yield, 85.4%; ^{1}H NMR ($CDCl_3$,

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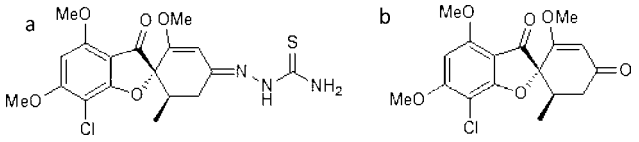

Figure 1. Chemical structures of (a) 4′-thiosemicarbazonegriseofulvin and (b) griseofulvin.

600 MHz), δ 7.25 (HN, 1H, s), 5.61 (CH, 2/3H, s), 5.72 (CH, 1/3H, s), 6.12 (bezene, H, s), 6.35, 8.68 (NH_2, 4/3 H, s), 6.32, 8.97 (NH_2, 2/3 H, s), 3.98, 4.06 (CH_3O–benzene, 6H, s), 3.60 (3′-CH_3O, 2H, s), 3.67 (3′-CH_3O, H, s), 2.72 (CH, 2/3H, m), 3.10 (CH, 1/3H, m), 1.26 (CH_2, 2H, m), 0.96 (CH_3, 3H, m); HRMS calcd for $[M]^+$ 426.08904, found 426.08917.

Enzymatic Activity Assay. The enzyme activity assay was performed as reported previously.[27] In this investigation, L-DOPA was used as a substrate for the activity assay of tyrosinase. The reaction media (3 mL) for activity assay contained 0.5 mM L-DOPA in 50 mM sodium phosphate buffer (pH 6.8). 4′-Thiosemicarbazonegriseofulvin and griseofulvin were dissolved in DMSO and diluted to appropriate concentrations. The final concentration of DMSO in the test solution was 3.3%. Controls, without inhibitors but containing 3.3% DMSO in the reaction media, were routinely carried out. The reaction was carried out at a constant temperature of 30 °C. The inhibition type was assayed by the Lineweaver–Burk plot, and the inhibition constant was determined by the second plots of the apparent K_m/V_m or $1/V_m$ versus the concentration of the inhibitor. A Beckman UV-650 spectrophotometer was used for absorbance and kinetic parameters.

Antifungal Activity Assay. The antifungal activity of 4′-thiosemicarbazonegriseofulvin and griseofulvin was tested against four target fungi: *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum*. Proper concentrations of 4′-thiosemicarbazonegriseofulvin and griseofulvin in DMSO were evenly spread on the culture medium PDA, in sterilized Petri dishes, and dried for 2 h under a laminar flow, and then *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum* were inoculated in the middle with 100 μL of fungal conidial suspension ($\sim 1 \times 10^5$ CFU/mL). In parallel, control experiments with only DMSO were also performed. The Petri dishes were incubated at 28 ± 1 °C, and the fungal colony diameter was measured daily. The inhibition rates of 4′-thiosemicarbazonegriseofulvin and griseofulvin were calculated after 7 days of incubation when mycelium in control completely covered the dishes. It was expressed as an average diameter and calculated using the equation

$$\text{inhibition rate}\ (\%) = \frac{D_c - D_t}{D_c} \times 100$$

where D_c and D_t represent mycelium growth diameter in control and treated Petri plates, respectively. The antifungal effects were measured in triplicate. Lethal concentration 50 (LC_{50}) values on the target fungi were analyzed using probit analysis and considered to be significantly different if 95% confidence intervals did not overlap. The statistical analyses were done using the China-DPS program.

Effect of 4′-Thiosemicarbazonegriseofulvin on Hyphal Morphology. The effects of 4′-thiosemicarbazonegriseofulvin on hyphal growth of *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum* were viewed under a microscope. Ten milliliters of agar medium inoculated with fresh fungal culture ($\sim 1 \times 10^5$ CFU/mL) was poured into a Petri dish as the upper layer. After solidification, two wells of 8 mm diameter were created. 4′-Thiosemicarbazonegriseofulvin solution (16 mM) and control DMSO were deposited in wells. Petri dishes were then incubated at 28 ± 1 °C for 72 h. The hyphae closely around the inhibition zones were collected and observed by Leica DMI 3000 M inverted microscope (Leica Corp.).

RESULTS AND DISCUSSION

Effects of 4′-Thiosemicarbazonegriseofulvin and Griseofulvin on the Activity of Mushroom Tyrosinase. The effects of 4′-thiosemicarbazonegriseofulvin and griseofulvin (the chemical structure is shown in Figure 1b) on the activity of mushroom tyrosinase were studied by using L-DOPA as substrate. The enzyme activity was monitored by following the increasing absorbance at 475 nm accompanying the oxidation of L-DOPA. Griseofulvin had little effect on the activity of tyrosinase (data not shown). 4′-Thiosemicarbazonegriseofulvin could effectively inhibit the activity of tyrosinase with dose dependence (Figure 2). When the concentration of

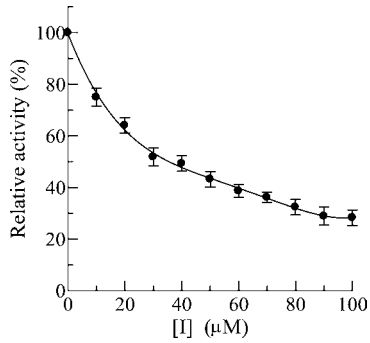

Figure 2. Effect of 4′-thiosemicarbazonegriseofulvin on the activity of mushroom tyrosinase for the oxidation of L-DOPA.

4′-thiosemicarbazonegriseofulvin was enhanced to 20 μM, the relative activity of tyrosinase was only 64%. The 50% inhibitory concentration (IC_{50}) of 4′-thiosemicarbazonegriseofulvin against tyrosinase was estimated to be 37.8 μM. It was much better than that of arbutin (IC_{50} = 5.30 mM) as previously reported,[28] which is well-known as a tyrosinase inhibitor.

Inhibition Mechanism of 4′-Thiosemicarbazonegriseofulvin on the Activity of Mushroom Tyrosinase. The inhibition mechanism of 4′-thiosemicarbazonegriseofulvin on tyrosinase was investigated, and the result is shown in Figure 3.

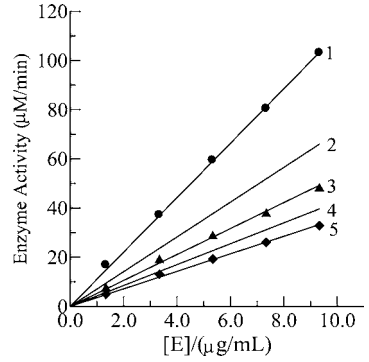

Figure 3. Determination of the inhibitory mechanism of 4′-thiosemicarbazonegriseofulvin on mushroom tyrosinase. The concentrations of 4′-thiosemicarbazonegriseofulvin for curves 1–5 were 0, 20, 40, 60, and 80 μM, respectively.

The plots of enzyme activity versus the concentration of enzyme in the presence of different concentrations of 4′-thiosemicarbazonegriseofulvin gave a set of straight lines, which all passed through the origin, indicating that the inhibition mechanism of 4′-thiosemicarbazonegriseofulvin on tyrosinase was a reversible reaction course. The presence of 4′-thiosemicarbazonegriseofulvin did not bring down the amount of the efficient enzyme, but resulted only in the descending activity of the enzyme.

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Inhibition Type of 4′-Thiosemicarbazonegriseofulvin on the Activity of Mushroom Tyrosinase. The inhibitory type of 4′-thiosemicarbazonegriseofulvin on tyrosinase was determined by Michaelis–Menten kinetics. Plots of $1/v$ versus $1/[S]$ observed a set of lines with different slopes and intersecting one another in the *X*-axis (Figure 4a). This

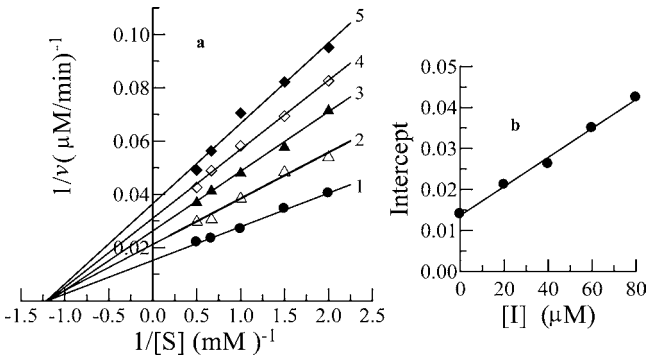

Figure 4. Determination of the inhibitory type and inhibition constants of 4′-thiosemicarbazonegriseofulvin on mushroom tyrosinase: (a) Lineweaver–Burk plots for inhibiton of 4′-thiosemicarbazonegriseofulvin on mushroom tyrosinase (the concentrations of 4′-thiosemicarbazonegriseofulvin for curves 1–5 were 0, 20, 40, 60, and 80 μM, respectively); (b) secondary plot of K_m versus concentration of 4′-thiosemicarbazonegriseofulvin to determine the inhibition constant (K_I).

meant 4′-thiosemicarbazonegriseofulvin could decrease the value of V_m but had no effect on the K_m of tyrosinase. Thus, 4′-thiosemicarbazonegriseofulvin was a noncompetitive inhibitor of tyrosinase, it could bind with both free tyrosinase and the enzyme–substrate complex, and the equilibrium constants are the same. The equilibrium constant (K_I) was obtained to be 38.42 μM from Figure 4b.

Antifungal Activity Assay. The antifungal activity of 4′-thiosemicarbazonegriseofulvin and griseofulvin against *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum* was evaluated from inhibition rates on the mycelium growth. After 7 days of incubation, the inhibition rates were calculated. 4′-Thiosemicarbazonegriseofulvin had potential antifungal activity on all target fungi (Figure 5). It was most efficient on *F. moniliforme* and *C. truncatum* at the concentration of 0.25 mM with inhibition rates of 23.0 and 22.4%, respectively. However, at the concentration of 1 mM, 4′-thiosemicarbazonegriseofulvin was most efficient on *C. truncatum* with an inhibition rate of 61.9%, which was similar to the effects at the concentrations of 4 and 16 mM. We estimated LC_{50} values of 4′-thiosemicarbazonegriseofulvin against *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum* were 5.4, 7.0, 15.3, and 1.5 mM by probit analysis. Griseofulvin was employed as the positive control; its LC_{50} values against *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum* were 3.7, 7.43, 52.86, and 0.39 mM, respectively. 4′-Thiosemicarbazonegriseofulvin was shown to have better antifungal activity than griseofulvin. Our findings also demonstrated that the conjugation of griseofulvin with thiosemicarbazide did not reduce the antifungal activity of griseofulvin.

Effect of 4′-Thiosemicarbazonegriseofulvin on Hyphal Morphology. The alteration of *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum* hyphae grown on PDA amended with 4′-thiosemicarbazonegriseofulvin was viewed under an inverted microscope. The hyphae of the four target fungi treated with DMSO as control treatment were thick, elongated, even, and smooth surfaced, whereas degenerative changes were observed in the hyphal morphology in all 4′-thiosemicarbazonegriseofulvin treatments (Figure 6). The growth of four target fungi hyphae was strongly inhibited after treatment with 16 mM 4′-thiosemicarbazonegriseofulvin, as indicated by mycelia sparsity, asymmetry, swelling, curling, and twisting.

Browning and postharvest disease are two main issues of the fruit industry. Griseofulvin is a broad-spectrum antifungal drug; it inhibits fungal mitosis by disrupting the mitotic spindle

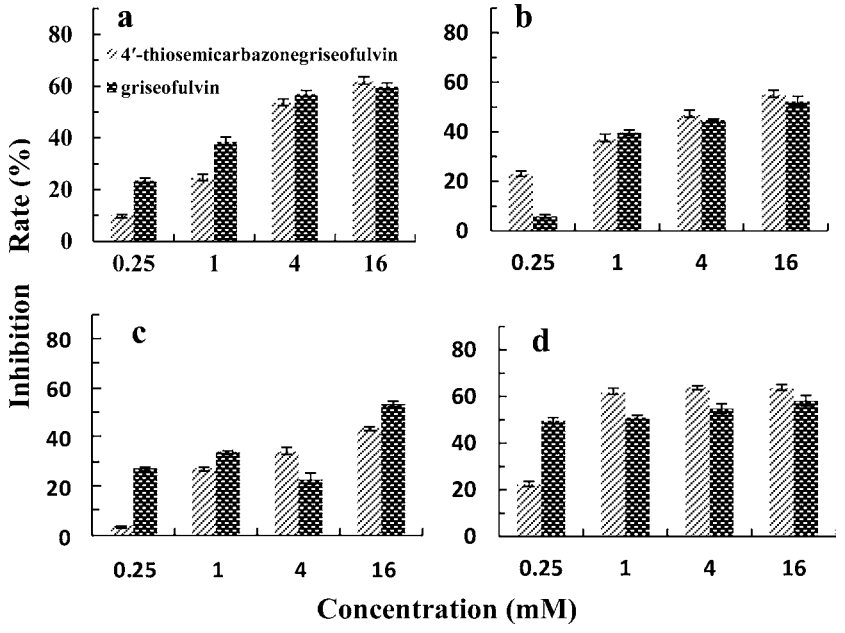

Figure 5. Effects of 4′-thiosemicarbazonegriseofulvin and griseofulvin on mycelium growth of (a) *F. oxysporum*, (b) *F. moniliforme*, (c) *F. solani*, and (d) *C. truncatum*. The concentrations of these compounds were 0.25, 1, 4, and 16 mM, respectively. The bars on each column show standard error.

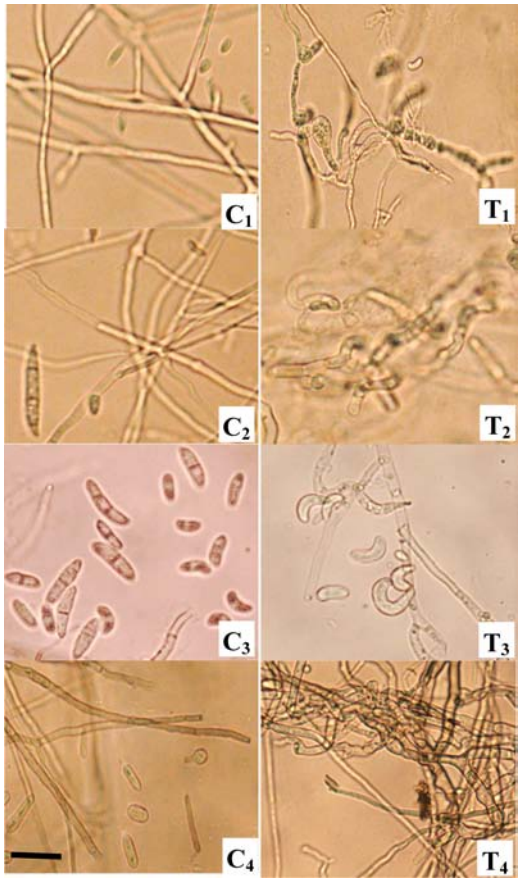

Figure 6. Micrographs of *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum* treated with 4′-thiosemicarbazonegriseofulvin or control. C_1, C_2, C_3, and C_4 display micrographs of *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum* mycelium incubation in controlled DMSO. T_1, T_2, T_3, and T_4 display micrographs of *F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum* mycelium incubation in 16 mM 4′-thiosemicarbazonegriseofulvin. (Bar = 20 μm.)

through interaction with polymerized microtubules.[29] However, it has little effect on antibrowning of fruit. In this study we conjugated a thiosemicarbazide group, which may be the functional group of the inhibitors of tyrosinase, with griseofulvin. It was interesting to find that the new compound (4′-thiosemicarbazonegriseofulvin) could effectively inhibit the activity of tyrosinase with an IC_{50} value of 37.8 μM, which is much better than that of arbutin, a well-known tyrosinase inhibitor. It also strongly inhibited the mycelium growth of four target fungi (*F. oxysporum*, *F. moniliforme*, *F. solani*, and *C. truncatum*), which can often cause postharvest disease of fruits. Thus, the conjugation of thiosemicarbazide with griseofulvin could improve the inhibitory activity against tyrosinase but would not reduce the antifungal activity of griseofulvin.

■ ASSOCIATED CONTENT

Supporting Information

HRMS and ^{1}H NMR spectra of 4′-thiosemicarbazonegriseofulvin. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‖The authors contributed equally in this work.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Segovia-Bravo, K. A.; Jaren-Galan, M.; Garcia-Garcia, P.; Garrido-Fernandez, A. Characterization of polyphenol oxidase from the Manzanilla cultivar (*Olea europaea pomiformis*) and prevention of browning reactions in bruised olive fruits. *J. Agric. Food Chem.* **2007**, *55*, 6515−6520.

(2) Wan, Y. K.; Tian, S. P.; Qin, G. Z. Enhancement of biocontrol activity of yeasts by adding sodium bicarbonate or ammonium molybdate to control postharvest disease of jujube fruits. *Lett. Appl. Microbiol.* **2003**, *37*, 249−253.

(3) Arias, E.; Gonzalez, J.; Oria, R.; Lopez-Buesa, P. Ascorbic acid and 4-hexylresorcinol effects on pear PPO and PPO catalyzed browning reaction. *J. Food Sci.* **2007**, *72*, 422−429.

(4) Yan, Q.; Cao, R.; Yi, W.; Chen, Z.; Wen, H.; Ma, L.; Song, H. Inhibitory effects of 5-benzylidene barbiturate derivatives on mushroom tyrosinase and their antibacterial activities. *Eur. J. Med. Chem.* **2009**, *44*, 4235−4243.

(5) Qiu, L.; Chen, Q. H.; Zhuang, J. X.; Zhong, X.; Zhou, J. J.; Guo, Y. J.; Chen, Q. X. Inhibitory effects of α-cyano-4-hydroxycinnamic acid on the activity of mushroom tyrosinase. *Food Chem.* **2009**, *112*, 609−613.

(6) Wang, B. S.; Chang, L. W.; Wu, H. C.; Huang, S. L.; Chu, H. L.; Huang, M. H. Antioxidant and antityrosinase activity of aqueous extracts of green asparagus. *Food Chem.* **2011**, *127*, 141−146.

(7) Zheng, Z. P.; Cheng, K. W.; Chao, J.; Wu, J.; Wang, M. Tyrosinase inhibitors from paper mulberry (*Broussonetia papyrifera*). *Food Chem.* **2008**, *106*, 529−535.

(8) Guo, Y. J.; Pan, Z. Z.; Chen, C. Q.; Hu, Y. H.; Liu, F. J.; Shi, Y.; Yan, J. H.; Chen, Q. X. Inhibitory effects of fatty acids on the activity of mushroom tyrosinase. *Appl. Biochem. Biotechnol.* **2010**, *162*, 1564−1573.

(9) Li, Z. C.; Chen, L. H.; Yu, X. J.; Hu, Y. H.; Song, K. K.; Zhou, X. W.; Chen, Q. X. Inhibition kinetics of chlorobenzaldehyde thiosemicarbazones on mushroom tyrosinase. *J. Agric. Food Chem.* **2010**, *53*, 12537−12540.

(10) Huang, Q. S.; Zhu, Y. J.; Li, H. L.; Zhuang, J. X.; Zhang, C. L.; Zhou, J. J.; Li, W. G.; Chen, Q. X. Inhibitory effects of methyl *trans*-cinnamate on mushroom tyrosinase and its antimicrobial activities. *J. Agric. Food Chem.* **2009**, *57*, 2565−2569.

(11) Jang, M. S.; Sanada, A.; Ushio, H.; Tanaka, M.; Ohshima, T. Inhibitory effects of 'Enokitake' mushroom extracts on polyphenol oxidase and prevention of apple browning. *Lebensm.−Wiss. Technol.* **2002**, *35*, 697−702.

(12) Zheng, Z. P.; Cheng, K. W.; To, J. T. K.; Li, H.; Wang, M. Isolation of tyrosinase inhibitors from *Artocarpus heterophyllus* and use of its extract as antibrowning agent. *Mol. Nutr. Food Res.* **2008**, *52*, 1530−1538.

(13) Li, H.; Cheng, K. W.; Cho, C. H.; He, Z.; Wang, M. Oxyresveratrol as an antibrowning agent for cloudy apple juices and fresh-cut apples. *J. Agric. Food Chem.* **2007**, *55*, 2604−2610.

(14) Janisiewicz, W. J.; Korsten, L. Biological control of postharvest diseases of fruits. *Annu. Rev. Phytopathol.* **2002**, *40*, 411−441.

(15) Nunes, C. A. Biological control of postharvest diseases of fruit. *Eur. J. Plant Pathol.* **2011**, 1−16.

(16) Schirra, M.; D'Aquino, S.; Cabras, P.; Angioni, A. Control of postharvest diseases of fruit by heat and fungicides: efficacy, residue

levels, and residue persistence. A review. *J. Agric. Food Chem.* **2011**, *59*, 8531−8542.
(17) Kobiler, I.; Akerman, M.; Huberman, L.; Prusky, D. Integration of pre-and postharvest treatments for the control of black spot caused by *Alternaria alternata* in stored persimmon fruit. *Postharvest Biol. Technol.* **2011**, *59*, 166−171.
(18) Kong, Q.; Shan, S.; Liu, Q.; Wang, X.; Yu, F. Biocontrol of *Aspergillus flavus* on peanut kernels by use of a strain of marine *Bacillus megaterium*. *Int. J. Food Microbiol.* **2010**, *139*, 31−35.
(19) Sharma, R.; Singh, D.; Singh, R. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: a review. *Biol. Control.* **2009**, *50*, 205−221.
(20) Brenes, M.; García, A.; De Los Santos, B.; Medina, E.; Romero, C.; De Castro, A.; Romero, F. Olive glutaraldehyde-like compounds against plant pathogenic bacteria and fungi. *Food Chem.* **2011**, *125*, 1262−1266.
(21) Zong, Y.; Liu, J.; Li, B.; Qin, G.; Tian, S. Effects of yeast antagonists in combination with hot water treatment on postharvest diseases of tomato fruit. *Biol. Control* **2010**, *54*, 316−321.
(22) Dang, K. T. H.; Singh, Z.; Swinny, E. E. Impact of postharvest disease control methods and cold storage on volatiles, color development and fruit quality in ripe 'Kensington Pride' mangoes. *J. Agric. Food Chem.* **2008**, *56*, 10667−10674.
(23) Cole, G. W.; Stricklin, G. A comparison of a new oral antifungal, terbinafine, with griseofulvin as therapy for tinea corporis. *Arch. Dermatol.* **1989**, *125*, 1537−1539.
(24) Robinson, H. M., Jr.; Robinson, R. C. Griseofulvin, an antifungal antibiotic. *Int. Rec. Med.* **1959**, *172*, 737−742.
(25) Gupta, A. K.; Williams, J. V.; Zaman, M.; Singh, J. In vitro pharmacodynamic characteristics of griseofulvin against dermatophyte isolates of *Trichophyton tonsurans* from tinea capitis patients. *Med. Mycol.* **2009**, *47*, 796−801.
(26) Zhu, Y. J.; Song, K. K.; Li, Z. C.; Pan, Z. Z.; Guo, Y. J.; Zhou, J. J.; Wang, Q.; Liu, B.; Chen, Q. X. Antityrosinase and antimicrobial activities of *trans*-cinnamaldehyde thiosemicarbazone. *J. Agric. Food Chem.* **2009**, *57*, 5518−5523.
(27) Pan, Z. Z.; Li, H. L.; Yu, X. J.; Zuo, Q. X.; Zheng, G. X.; Shi, Y.; Liu, X.; Lin, Y. M.; Liang, G.; Wang, Q. Synthesis and antityrosinase activities of alkyl 3,4-dihydroxybenzoates. *J. Agric. Food Chem.* **2011**, *59*, 6645−6649.
(28) Song, K. K.; Qiu, L.; Huang, H.; Chen, Q. X. The inhibitory effect of tyrosinase by arbutin as cosmetic additive. *J. Xiamen Univ. (Nat. Sci.)* **2003**, *42*, 791−794.
(29) Crackower, S. The effects of griseofulvin on mitosis in *Aspergillus nidulans*. *Can. J. Microbiol.* **1972**, *18*, 683−687.