Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: informahealthcare.com/journals/ienz20

Synthesis and biological evaluation of polyhydroxy benzophenone as mushroom tyrosinase inhibitors

Jianlong Wu, Xuesen Hu & Lin Ma

To cite this article: Jianlong Wu, Xuesen Hu & Lin Ma (2011) Synthesis and biological evaluation of polyhydroxy benzophenone as mushroom tyrosinase inhibitors, Journal of Enzyme Inhibition and Medicinal Chemistry, 26:3, 449-452, DOI: 10.3109/14756366.2010.521745

To link to this article: https://doi.org/10.3109/14756366.2010.521745

Journal of Enzyme Inhibition and Medicinal Chemistry, 2011; 26(3): 449–452

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.3109/14756366.2010.521745

informa
healthcare

SHORT COMMUNICATION

Synthesis and biological evaluation of polyhydroxy benzophenone as mushroom tyrosinase inhibitors

Jianlong Wu, Xuesen Hu, and Lin Ma

School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, P. R. China

Abstract

A series of polyhydroxy benzophenone were synthesized and evaluated as mushroom tyrosinase inhibitors. The results demonstrated that most of the target compounds had remarkable inhibitory activities on mushroom tyrosinase. Among all these compounds, 2,3,4,3′,4′,5′-hexahydroxy-diphenylketone **10** was found to be the most potent tyrosinase inhibitor with IC_{50} value of 1.4 μM. In addition, the inhibition kinetics analyzed by Lineweaver–Burk plots revealed that such compounds were competitive inhibitors. These results suggested that such compounds might be utilized for the development of new candidate for treatment of dermatological disorders.

Keywords: Polyhydroxy benzophenone, tyrosinase, inhibitors

Tyrosinase (EC 1.14.18.1; polyphenol oxidase, PPO), a multifunctional copper-containing enzyme, catalyzed two distinct reaction of melanin synthesis: the hydroxylation of monophenols and the oxidation of the *o*-phenols.[1,2] Thereby, the enzyme converts tyrosine to 3,4-dihydroxyphenylalanine (L-dopa) and oxidizes L-dopa to form dopaquinone, which plays important roles in the process of melanin biosynthesis.[3] Tyrosinase is widespread in plants and animals. Therefore, tyrosinase inhibitors have become increasingly important in agriculture,[2,4] cosmetic industry,[5] and medication[6] due to decreasing the excessive accumulation of pigmentation resulting from the enzyme action.[7–12]

Presently, tyrosinase inhibitors have been established as important constituents of cosmetic materials, food preservative, and depigmenting agents for hyperpigmentation. Many tyrosinase inhibitors have been reported, for example, hydroquinone,[13–16] kojic acid,[17] azelaic acid,[18,19] electron-rich phenols,[20] corticosteroids,[21,22] resinoids,[23,24] resveratrol,[25] oxyresveratrol,[26] and arbutin have been utilized as cosmetic agents. Most of these compounds have the structures of benzene rings and hydroxyl radicals.

In the last decades, polyhydroxy benzophenones have been widely utilized for the production and development of plastic, resin, coating materials, synthetic rubber, light-sensitive materials, and cosmetic materials. However, its other properties such as antioxidant activity and tyrosinase inhibitory activity have not been reported yet.

Since polyhydroxy benzophenones have the similar structures to the tyrosinase inhibitors mentioned earlier, we speculated that polyhydroxy benzophenones might exhibit potent tyrosinase inhibitory activity. Therefore, our team synthesized a series of polyhydroxy benzophenones and evaluated their inhibitory effects on the diphenolase activity of mushroom tyrosinase. To the best of our knowledge, this is the first time to report the inhibitory effects on the diphenolase activity of mushroom tyrosinase of polyhydroxy benzophenones.

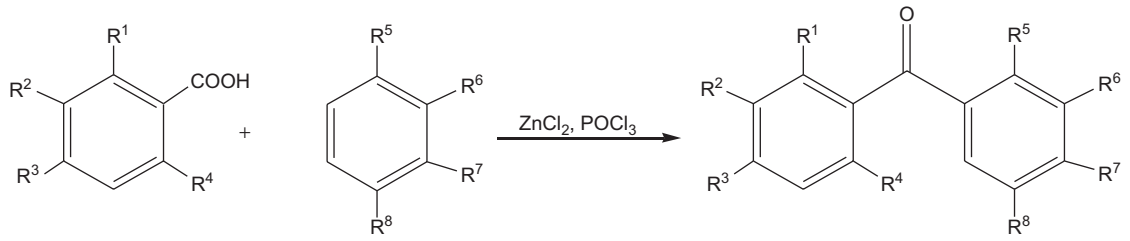
The synthetic routes of compounds **1–18**[1] were outlined in Scheme 1, and the chemical structures of polyhydroxy benzophenones **1–18** were given in Table 1.

Tyrosinase inhibition assays were performed according to the developed method described earlier by

[1]*The synthesis of compounds **1–18***: To the mixture of appropriate phenol (50 mmol) and polyhydroxybenzoic acid (55.6 mmol), anhydrous zinc chloride (27.2 g) and phosphorus oxychloride (25 mL) were added. The reaction mixture was refluxed for 2.5 h in the water bath of 70°C with stirring. Then add the mixture to appropriate ice and cool to 4°C for 24 h. The precipitate solid was filtered, washed with 3% sodium bicarbonate twice, and purified by recrystallization from boiling water to afford compounds **1–18** (Figure 1). The synthetic route of compounds **1–18** is shown in Scheme 1. Compound **10**: 2,3,4,3′,4′,5′-hexahydroxydiphenylketone (10). Yield: 90%; yellow powders; mp 272–273°C; ^{1}H NMR (dimethyl sulfoxide-d6, 300 MHz) δ (ppm): 6.4 (1H, d, J = 6.9 Hz, ArH), 6.6 (2H, s, ArH), 7.0 (1H, d, J = 4.2 Hz, ArH), 8.6 (1H, s, OH-R_3), 8.9 (1H, s, OH-R_2), 9.3 (2H, s, OH-R_6, OH-R_8), 10.0 (1H, s, OH-R_7), 12.1 (1H, s, OH-R_1). ^{13}C NMR(dimethyl sulfoxide-d6, 300 MHz) δ: ^{13}C NMR(dimethyl sulfoxide-d6, 300 MHz) δ: 198.60($C_{carbonyl}$), 175.94 (C_{phenyl}-4), 152.43 (C_{phenyl}-3′), 145.96 (C_{phenyl}-3,5), 138.27 (C_{phenyl}-4′), 133.45 (C_{phenyl}-2′), 128.61 (C_{phenyl}-5′), 125.31 (C_{phenyl}-6′), 113.76 (C_{phenyl}-1′), 109.64 (C_{phenyl}-2,6), 107.83 (C_{phenyl}-1).

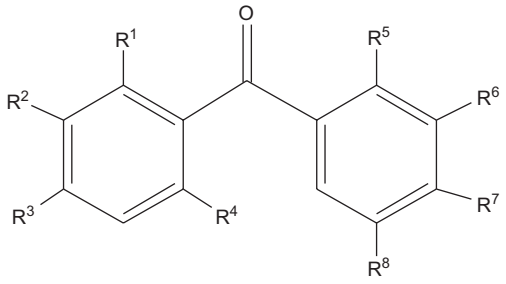
Address for Correspondence: Lin Ma, School of Chemistry and Chemical Engineering, Sun Yat-sen University, 135 Xin Gang Xi Road, Guangzhou 510275, P. R. China. E-mail: cesmal@mail.sysu.edu.cn

(Received 07 July 2010; revised 01 September 2010; accepted 02 September 2010)



Scheme 1. Synthetic route of polyhydroxy benzophenones **1–18**.

Table 1. Chemical structure of polyhydroxy benzophenones **1–18** and inhibitory effects on mushroom tyrosinase of polyhydroxy benzophenones and kojic acid as reference inhibitor.



Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	IC_{50} (μmol/L)
1	H	H	OH	H	H	H	OH	H	NT[a]
2	OH	H	OH	H	H	H	OH	H	NT
3	H	H	OH	H	H	OCH_3	OH	H	NT
4	OH	H	OH	H	OH	H	OH	H	≥200
5	OH	H	OH	H	H	OCH_3	OH	H	≥200
6	OH	H	OH	H	H	OH	OH	OH	17.4
7	OH	OH	OH	H	H	H	OH	H	≥100
8	OH	OH	OH	H	OH	H	OH	H	10.1
9	OH	OH	OH	H	H	OCH_3	OH	H	6.4
10	OH	OH	OH	H	H	OH	OH	OH	1.4
11	OH	H	OH	OH	OH	H	OH	H	≥200
12	OH	H	OH	H	H	OH	H	H	≥200
13	OH	H	OH	H	OH	H	H	H	7.3
14	H	H	OH	H	H	OH	OH	OH	98.3
15	OH	OH	OH	H	OH	H	H	H	8.8
16	OH	H	OH	OH	H	H	H	H	≥200
17	OH	OH	OH	H	H	H	H	H	10.5
18	OH	OH	H	H	H	H	H	H	≥200
Kojic acid[30]	—	—	—	—	—	—	—	—	19.0

[a]NT: not tested.

Hearing[27] with some modification.[2] The IC_{50} values of all compounds investigated were summarized in Table 1 and Figure 1. As the results reported in Table 1, compounds **6**, **8**, **9**, **10**, **13**, **15**, and **17** exhibited more potent tyrosinase inhibitory activities than kojic acid, especially compound **10** (IC_{50} = 1.4 μmol/L), which demonstrated excellent *in vivo* activity.

Of all polyhydroxy benzophenones **1–18**, compounds **1**, **18**, and **3**, which are dihydroxy-substituted diphenylketones, display none or very weak inhibitory effect on tyrosinase, whereas trihydroxy-, tetrahydroxy-, pentahydroxy-, and hexahydroxy-substituted diphenylketones exhibited a significant increase inhibitory activity on tyrosinase along the number of hydroxyl group grows. It seems to be a tendency that the inhibitory activity of polyhydroxy benzophenones on tyrosinase increase along the number of hydroxyl group grows.

Interestingly, most of the compounds that display potent tyrosinase inhibitory activities are substituted by hydroxyl group on positions R^1, R^2, and R^3. The result

[2]*Tyrosinase Inhibition Assay*: In brief, a 10 μL sample was added to an assay mixture containing with 10 μL tyrosinase solution (0.5 mg/mL) and 900 μL phosphate buffer (pH 6.8) to a total volume assay mixture of 920 μL, for 20 min at 25°C. Then 80 μL of L-dopa (1.50 mg/mL) was added to the reaction mixture and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm of the L-dopa for 1 min. IC_{50} value, a concentration giving 50% inhibition of tyrosinase activity, was determined by interpolation of the dose–response curves. Here, kojic acid (IC_{50} = 19.0 μmol/L) was used as the reference inhibitors.

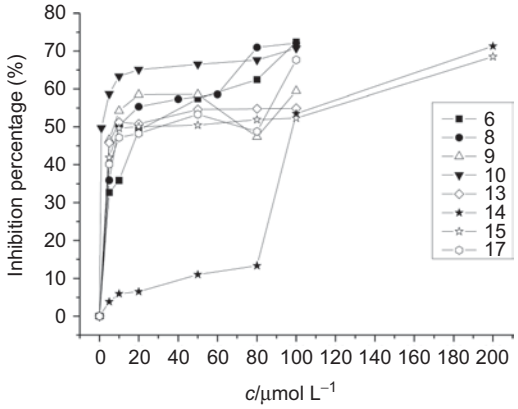


Figure 1. Dose-dependent inhibition of mushroom tyrosinase by polyhydroxy benzophenones.

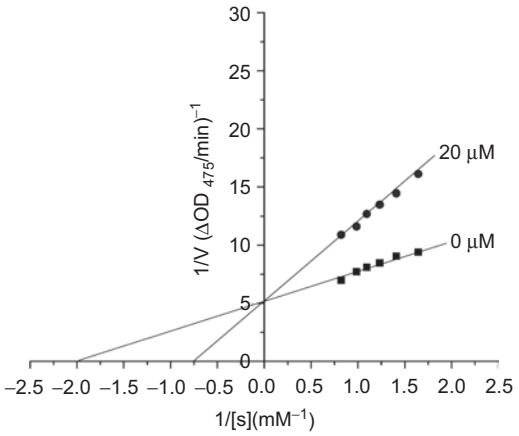


Figure 2. Lineweaver–Burk plots for inhibition of 2,3′,4,4′,5′-pentahydroxydiphenylketone on mushroom tyrosinase for the catalysis of dopa at 25°C, pH 6.8. Concentrations of 2,3′,4,4′,5′-pentahydroxydiphenylketone (compound **6**) for curves were 0 and 20 μmol/L, respectively.

suggests that the introduction of hydroxyl group on positions R^1, R^2, and R^3 strengthens the tyrosinase inhibitory activity. Compared with compounds **2** and **12**, compound **13** (IC_{50} = 7.3 μmol/L) bearing a hydroxyl substituent at position R^5 displays potent inhibitory activity. When position R^7 is replaced by hydroxyl group, such as compounds **4** ($IC_{50} \geq 200$ μmol/L) and **7** ($IC_{50} \geq 100$ μmol/L), a significant decrease in inhibition potency was observed, whereas the IC_{50} values of compounds **13** and **17** are 7.3 μmol/L and 10.5 μmol/L, respectively.

In addition, compounds substituted by methoxyl group exhibited more potent tyrosinase inhibition potencies than unsubstituted compounds with similar structure, such as compounds **9** and **7** or compounds **2** and **5**.

As the compounds that showed potent inhibitory activities on tyrosinase have similar structures to each other, we can get the conclusion that they have the same inhibition type on mushroom tyrosinase. Therefore, 2,3′,4,4′,5′-pentahydroxy-diphenylketone (compound **6**) had been chosen to investigate the kinetic behavior of mushroom tyrosinase during the oxidation of L-dopa (Figure 2)[3]. [29] The result shows that 2,3′,4,4′,5′-pentahydroxy-diphenylketone is a competitive inhibitor because increasing its concentration resulted in a family of lines with a common intercept on the $1/V$ axis but with different slopes.

In conclusion, in the present investigation, a series of polyhydroxy benzophenone were synthesized and evaluated as mushroom tyrosinase inhibitors. The results demonstrated that most of target compounds had remarkable inhibitory activities on mushroom tyrosinase. Particularly, compounds **6**, **8**, **9**, **10**, **13**, **15**, and **17** exhibited more potent tyrosinase inhibitory activities than kojic acid. This research indicates that both the number and position of hydroxyl groups in diphenylketone seem to play a critical role in exerting the inhibitory effect on dopa oxidase activity of tyrosinase. In addition, the inhibition kinetics analyzed by Lineweaver–Burk plots revealed that such compounds were competitive inhibitors. These results suggested that such compounds might be utilized for the development of new candidate for treatment of dermatological disorders.

[3]*Kinetic Assay of Tyrosinase Inhibition*: Various concentrations of L-dopa (0.1–1.2 mM) as the substrate, 40 μL of mushroom tyrosinase (1 units/mL), and 50 mM potassium phosphate buffer (pH 6.8) were added to test tube in a total volume assay mixture of 1000 μL. The initial rate of dopa chrome formation in the reaction mixture was determined by the increase of absorbance at 475 nm/min (ΔOD_{475}/min). Michaelis constant (K_m) and maximal velocity (V_{max}) of the tyrosinase activity were determined by the Lineweaver–Burk plot.

Declaration of interest

This work was supported by the Undergraduate Research Programmes of Sun Yat-sen University, China (2009-73).

References

1. Song K. K., Huang H., Han P., Zhang C. L., Shi Y., Chen Q. X., Inhibitory effects of *cis*- and *trans*-isomers of 3,5-dihydroxystilbene on the activity of mushroom tyrosinase. Biochem. Biophys. Res. Commun. 2006, 342, 1147–1151.
2. Yokochi N., Morita T., Yagi T., Inhibition of diphenolase activity of tyrosinase by vitamin B6 compounds. J. Agric. Food Chem. 2003, 51, 2733–2736.
3. Hearing V. J., Tsukamoto K. Enzymic control of pigmentation in mammals. FASEB J. 1991, 5, 2902–2909.
4. Nerya O., Musa R., Khatib S., Tamir S., Vaya J., Chalcones as potent tyrosinase inhibitors: the effect of hydroxyl positions and numbers. Phytochemistry 2004, 65, 1389–1395.
5. Maeda K., Fukuda M., *In vitro* effectiveness of several whitening cosmetic components in human melanocytes. J. Soc. Cosmet. Chem. 1991, 42, 361–363.
6. Maeda K., Fukuda M., Arbutin: mechanism of its depigmenting action in human melanocyte culture. J. Pharm. Exp. Ther. 1996, 276, 765–769.
7. Chen Q. X., Kubo I., Kinetics of mushroom tyrosinase inhibition by quercetin. J. Agric. Food Chem. 2002, 50, 4108–4112.
8. Shiino M., Watanabe Y., Umezawa K., Synthesis of N-substituted *N*-nitrosohydroxylamines as inhibitors of mushroom tyrosinase. Bioorg. Med. Chem. 2001, 9, 1233–1240.
9. Seo S. Y., Sharma V. K., Sharma N., Mushroom tyrosinase: recent prospects. J. Agric. Food Chem. 2003, 51, 2837–2853.

10. Kubo I., Kinst-Hori I., 2-Hydroxy-4-methoxybenzaldehyde. A potent tyrosinase inhibitor from African medicinal plants. Planta Med. 1999, 65, 19–22.
11. Okombi S., Rival D., Bonnet S., Mariotte A. M., Perrier E., Boumendjel A., Discovery of benzylidenebenzofuran-3(2*H*)-one (aurones) as inhibitors of tyrosinase derived from human melanocytes. J. Med. Chem. 2006, 49, 329–333.
12. Khatib S., Nerya O., Musa R., Tamir S., Pete T., Vaya J., Enhanced substituted resorcinol hydrophobicity augments tyrosinase inhibition potency. J. Med. Chem. 2007, 50, 2676–2681.
13. Arandt K. A., Fitzpatrick T. B., Topical use of hydroquinine as a depigmenting agent. J. Am. Med. Assoc. 1965, 194, 117–119.
14. Fitztrick T. B., Arandt K. A., El-Mofty A. M. M. A., Hydroquinone and psoralens in the therapy of hypermelanosis and vitiligo. Arch. Dermatol. 1966, 93, 589–600.
15. Kligman A. M., Willis I., A new formula for depigmenting human skin. Arch. Dermatol. 1975, 111, 40–48.
16. Bleehen S. S., Skin bleaching preparations. J. Soc. Cosmet. Chem. 1977, 28, 407–412.
17. Mishima Y., Hatta S., Ohyama Y., Inazu M., Induction of melanogenesis suppression: cellular pharmacology and mode of differential action. Pigment Cell Res. 1998, 1, 367–374.
18. Breathnach A. C., Nazzaro-Porro M., Passi S., Azelaic acid therapy in disorders of pigmentation. Clin. Dermatol. 1989, 7, 106–119.
19. Verallo-Rowell V. M., Verallo V., Graupe K., Lopez-Villafuerte L., Garcia-Lopez M., Double-blind comparison of azelaic acid and hydroquinone in the treatment of melasma. Acta Dermatol. Venereol. 1989, 143, 58–61.
20. Jimbow K., *N*-Acetyl-4-*S*-cysteaminylphenol as a new type of depigmenting agent for the melanoderma of melasma patients. Arch. Dermatol. 1991, 127, 1528–1534.
21. Neering H., Treatment of melasma (chloasma) by local application of steroid cream. Dermatologica 1975, 151, 349–353.
22. Kanwar A. J., Dhar S., Kaur S., Treatment of melasma with potent topical corticosteroids. Dermatology 1994, 188, 170.
23. Griffiths C. E. M., Finkel L. J., Ditre C. M., Hamilton T. A., Ellis C. N., Voorhees J. J., Topical tretinonin (retinoic acid) improves melasma: a vehicle-controlled clinical trial. Br. J. Dermatol. 1993, 129, 415–421.
24. Kimbrough-Green C. K., Griffiths C. E. M., Finkel L. J., Hamilton T. A., Burengo-Ransby S. M., Ellis C. N., Voorhees J. J., Topical retinoic acid (tretinoin) for melasma in black patients: a vehicle-controlled clinical trial. Arch. Dermatol. 1994, 130, 727–733.
25. Kenji O., Toshiyuki T., Tet suro I., Inhibitory effects of resveratrol derivatives from Dipterocarpaceae plants on tyrosinase activity. J. Biosci. Biotechnol. Biochem. 2003, 67, 1587–1589.
26. Shin N H., Ryu S. Y., Choi E J., Oxyresveratrol as the potent inhibitor on DOPA oxidase activity of mushroom tyrosinase. J. Biochem. Biophys. Res. Commun. 1998, 243, 801–803.
27. Hearing V. J., Jr., Methods in enzymology, Academic, 1987, 142, 155.
28. Chen Q. X., Song K. K., Qiu L., Liu X. D., Huang H., Guo H. Y., Inhibitory effects on mushroom tyrosinase by *p*-alkoxybenzoic acids. Food Chem. 2005, 91, 269–274.