Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5650–5653

Chemical transformations of oxyresveratrol (*trans*-2,4,3′,5′-tetrahydroxystilbene) into a potent tyrosinase inhibitor and a strong cytotoxic agent

Kittisak Likhitwitayawuid,[a,*] Acom Sornsute,[a] Boonchoo Sritularak[a] and Poonsakdi Ploypradith[b]

[a]*Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
[b]*Chulabhorn Research Institute, Vibhavadee-Rangsit Highway, Bangkok 10210, Thailand*

Received 4 April 2006; revised 29 July 2006; accepted 2 August 2006
Available online 17 August 2006

Abstract—From oxyresveratrol (*trans*-2,4,3′,5′-tetrahydroxystilbene **1**), seven derivatives were prepared, including *trans*-2-methoxy-4,3′,5′-trihydroxystilbene (**2**), *trans*-2,3′-dimethoxy-4,5′-dihydroxystilbene (**3**), *trans*-4,3′-dimethoxy-2,5′-dihydroxystilbene (**4**), *trans*-2,4,3′,5′-tetramethoxystilbene (**5**) and *cis*-2,4,3′,5′-tetramethoxystilbene (**6**), 2,4,3′,5′-tetrahydroxybibenzyl (**7**), and 2,4,3′,5′-tetramethoxybibenzyl (**8**). The tetrahydroxybibenzyl **7**, a hydrogenation product of **1**, exhibited more potent tyrosinase inhibitory activity than the parent compound, without cytotoxicity. A kinetic study revealed that **7** was a reversible and non-competitive inhibitor of mushroom tyrosinase with L-dopa as the substrate. Analysis of the K_i values indicated that **7** has a slightly higher affinity to the enzyme than **1**. Compound **6**, a tetra-*O*-methylated analogue of **1** with *cis*-configuration, was deprived of inhibitory effect on the enzyme tyrosinase, but showed very strong cytotoxicity against the human cancer cells KB, BC, and NCI-H187, with potency comparable to those of the anticancer agents ellipticine and doxorubicin. Data on the tyrosinase inhibitory activity and cytotoxicity of **1**–**8** indicated that *O* methylation on stilbene **1** destroyed anti-tyrosinase activity but generated cytotoxicity. Thus, facile preparations of a potent tyrosinase inhibitor (**7**) and a strong cytotoxic agent (**6**) from the natural product **1** were achieved through simple chemical reactions.

Oxyresveratrol (*trans*-2,4,3′,5′-tetrahydroxystilbene, **1**) is a major constituent of the heartwood of *Artocarpus lakoocha* Roxb.[1,2] The compound is the active principle of 'Puag-Haad,' a dried aqueous extract traditionally used in Thailand as an anthelmintic.[3] Recent studies have revealed that **1** also possesses potent inhibitory activity against tyrosinase,[4,5] a key enzyme in the biosynthetic pathway of melanin pigments in both plants and animals. Further investigations have shown that the compound has strong skin depigmenting effects in both animals and humans.[6] As a strong tyrosinase inhibitor, **1** has potential applications as a skin-whitening agent in cosmetic preparations or as an anti-browning agent for food products of plant origin. Other interesting biological activities reported for **1** include antiherpetic and anti-HIV,[2] antiinflammatory,[7] antioxidative,[8] and antiapoptotic and neuroprotective activities.[9] Since **1** can be obtained in large amounts from the widely available *A. lakoocha*,[2–4] the compound appears to be an attractive target for chemical studies in terms of structure–activity relationships. In the present communication, we describe our efforts to prepare, from **1**, analogues with higher potency and selectivity with regard to their cytotoxic and anti-tyrosinase activities.

The reaction of **1** with CH_3I (3 equiv, reflux, 24 h) gave three partially methylated products, namely *trans*-2-methoxy,4,3′,5′-trihydroxystilbene (**2**), *trans*-2,3′-dimethoxy-4,5′-dihydroxystilbene (**3**), and *trans*-4,3′-dimethoxy-2,5′-dihydroxystilbene (**4**). Full *O*-methylation of **1** was achieved when CH_3I (6 equiv) was employed, leading to the formation of *trans*-2,4,3′,5′-tetramethoxystilbene (**5**). Attempts to prepare other mono-, di-, and tri-methoxy derivatives of **1** using CH_3I or $(CH_3)_2SO_4$ in various conditions were not successful. When *trans*-tetramethoxystilbene **5** was subjected to a photochemical reaction, the *cis*-isomer **6** was formed. Catalytic Pd/C hydrogenation of **1** and **5** gave bibenzyl compounds **7** and **8**, respectively. The positions of the

Keywords: Stilbene; Tyrosinase inhibitor; Cytotoxicity; Oxyresveratrol.
* Corresponding author. Fax: +662 2188357; e-mail: Kittisak.L@chula.ac.th

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.08.018

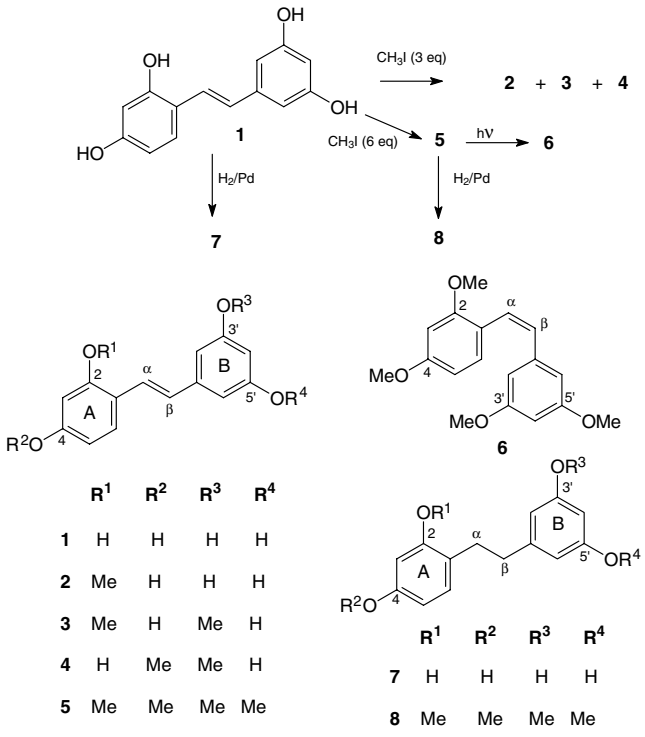

Figure 1. Chemical transformations of **1** into **2**–**8**.

methoxy groups in **2**–**4** were determined by NOESY experiments. The *cis*-configuration of **6** was established from the coupling constant between H-α and H-β (J = 12.1 Hz) ($J_{\alpha\beta}$ = 16.5 Hz in **5**), and from the NOESY effect observed between H-6 and H-2′(6′). These natural and synthetic stilbenoids (**1**–**8**) (Fig. 1) were then evaluated for tyrosinase inhibitory activity and cytotoxic potential.[10]

For the evaluation of tyrosinase inhibitory activity, assays were performed with L-dopa as the substrate as previously described, using kojic acid, a well-known strong tyrosinase inhibitor, as the positive control.[4,11–13] Table 1 summarizes the percentages of inhibition and the IC_{50} values of **1**–**8**, as compared with kojic acid. It should be mentioned that oxyresveratrol (**1**) was earlier reported to be a stronger tyrosinase inhibitor than kojic acid,[5] and this was confirmed in this study, as reflected in their respective IC_{50} values of 12.7 and 133.4 μM (Table 1). For the *O*-methylated products (**2**–**6** and**8**), none of them showed significant inhibitory activity (IC_{50} > 100 μM). The loss of activity in **2**–**6** and **8** was caused by the disappearance of the 4-alkyl resorcinol-like structure of ring A.[12–14] Compound **7**, a bibenzyl derivative prepared from **1**, showed more inhibitory activity than did the parent compound, being about 8-fold stronger in view of the IC_{50} value. Further analysis of the data obtained for **7** indicated that this compound, as well as **1** and kojic acid, inhibited the enzyme in a dose-dependent manner (Fig. 2). The higher tyrosinase inhibitory activity of **7**, as compared with **1**, was probably due to its bibenzyl structure which gave more flexibility and thus allowed the phenolic groups to interact with the enzyme more effectively. Kinetic studies were then conducted on **7**, in comparison with **1**, with regard to the ability to inhibit mushroom tyrosinase with L-dopa as the substrate. As summarized in Table 2, the V_{max} (ΔA_{490}/min) for the dopa oxidase activity of the enzyme was 2.0×10^{-1}, and the K_m value was 0.7 mM L-dopa. It can be seen from the Lineweaver–Burk plot of $1/V$

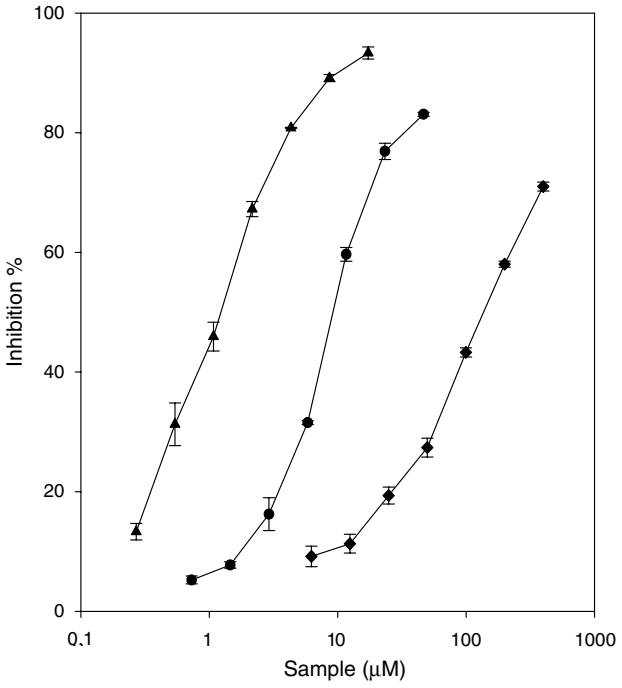

Figure 2. Dose-dependent inhibitory effects on mushroom tyrosinase by oxyresveratrol (**1**), 2,4,3′,5′-tetrahydroxybibenzyl (**7**), and kojic acid. Samples shown are oxyresveratrol (circle, ●), 2,4,3′,5′-tetrahydroxybibenzyl (triangle, ▲), and kojic acid (diamond, ♦).

Table 1. Tyrosinase inhibitory activity of **1**–**8**

Compound	% inhibition (at 100 μM)	IC_{50} (μM)
2,4,3′,5′-Tetrahydroxystilbene (**1**)	83.5 ± 0.9	12.7
trans-2-Methoxy-4,3′,5′-trihydroxystilbene (**2**)	4.8 ± 1.6	>100
trans-2,3′-Dimethoxy-4,5′-dihydroxystilbene (**3**)	<0 ± 1.4	>100
trans-4,3′-Dimethoxy-2,5′-dihydroxystilbene (**4**)	<0 ± 3.6	>100
trans-2,4,3′,5′-Tetramethoxystilbene (**5**)	<0 ± 2.6	>100
cis-2,4,3′,5′-Tetramethoxystilbene (**6**)	<0 ± 1.9	>100
2,4,3′,5′-Tetrahydroxybibenzyl (**7**)	96.3 ± 0.6	1.6
2,4,3′,5′-Tetramethoxybibenzyl (**8**)	2.3 ± 3.2	>100
Kojic acid	41.1 ± 1.4	133.4

Table 2. Kinetic parameters of mushroom tyrosinase in the presence of **1** or **7**

Inhibitor	Dose (μM)	K_m (M)	V_{max} (ΔA_{490}/min)	K_i (M)
None	—	0.7×10^{-3}	2.0×10^{-1}	—
7	1.2	0.7×10^{-3}	1.6×10^{-1}	4.8×10^{-6}
7	2.4	0.7×10^{-3}	1.4×10^{-1}	5.6×10^{-6}
1	9.2	0.7×10^{-3}	1.4×10^{-1}	2.2×10^{-5}
1	18.4	0.7×10^{-3}	1.2×10^{-1}	2.8×10^{-5}

values with different concentrations of L-dopa (Fig. 3) that the presence of **7** at different concentrations (1.2 and 2.4 μM) did not affect the K_m value (0.7 mM L-dopa) of the enzyme, but decreased the V_{max} values to 1.6×10^{-1} and 1.4×10^{-1}, respectively (Table 2). Therefore, **7** was a non-competitive inhibitor of tyrosinase on L-dopa with the K_i values of 4.8–5.6 μM (Table 2). A previous kinetic study on mushroom tyrosinase with L-dopa as the substrate indicated that **1** was a non-competitive inhibitor.[15] This is in agreement with the results of our investigation, as reflected in the unchanged K_m value (0.7 mM L-dopa) in the presence of **1** (Table 2). When the K_i values of **1** (2.2×10^{-5}–2.8×10^{-5} M) and **7** (4.8×10^{-6}–5.6×10^{-6} M) were compared (Table 2), it was found that **7** had about 5-fold higher affinity to the enzyme than **1**. The higher potency of the former is probably due to this kinetic property.

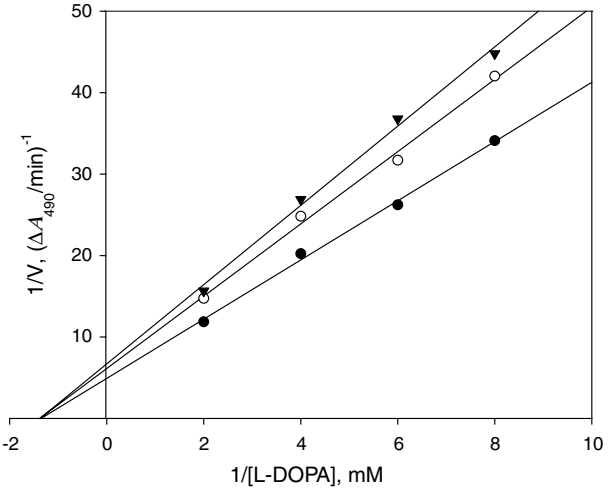

Figure 3. Lineweaver–Burk plot of mushroom tyrosinase in the presence of 2,4,3′,5′-tetrahydroxybibenzyl (**7**). Data were obtained as mean values of $1/V$, inverse of the increase of absorbance at the wavelength 490 nm per min (ΔA_{490}/min), with different concentrations of L-dopa as substrate. Inhibitors of the enzyme were 2,4,3′,5′-tetrahydroxybibenzyl (**7**) with 2.4 μM (triangle, ▼), 1.2 μM (circle, ○), and no 2,4,3′,5′-tetrahydroxybibenzyl (**7**) (circle, ●).

A study of the cytotoxicity of **1**–**8** was carried out using a battery of cancer cell lines, including KB, BC, and NCI-H187, with the anticancer agents ellipticine and doxorubicin as positive controls.[16,17] Table 3 illustrates the IC_{50} values for these compounds. It is obvious that while the parent compound **1** was not cytotoxic, all of its *O*-methylated derivatives (**2**–**6**) demonstrated cytotoxic potential, ranging from weak to very strong activity. Thus, it appears that the introduction of methoxy groups to the aromatic rings generated cytotoxicity. It should be noted that the *cis*-polymethoxystilbene **6** was more potent than the *trans*-isomer **5**. The bibenzyl structures **7** and **8**, however, were devoid of cytotoxicity. Therefore, it could be deduced from these data that the requirements for cytotoxicity of the stilbenoids were: (1) polymethoxy groups, (2) unsaturation at α- and β-carbons, and (3) *cis*-configuration. In view of the IC_{50} value, **6** showed more cytotoxicity than the positive control ellipticine in all cell lines. However, when compared with doxorubicin, **6** was equally active in KB but less active in BC and NCI-H187 cells. Structurally, stilbene **6** could be considered, as related to combretastatins, a group of natural products possessing strong anticancer potential, which were first isolated from *Combretum caffrum* (Combretaceae).[18] Studies have shown that *trans*-polymethoxystilbenoids exert their cytotoxicity by inhibition of cytochrome P_{450} 1B1,[19] whereas *cis*-analogues have inhibitory effects on tubulin polymerization of cancer cells.[18] More studies on **6**, such as in vitro assays in other cancer cell lines and in vivo experiments in animals, as well as examinations of the mechanism of action, are needed before the anticancer potential of this compound can be fully determined.

In summary, this study showed that a more potent tyrosinase inhibitor (**7**) can be obtained from oxyresveratrol (**1**) through a single-step reduction reaction. The bibenzyl structure **7** was a non-competitive inhibitor of mushroom tyrosinase with higher affinity to the enzyme

Table 3. Cytotoxicity of **1**–**8**

Compound	IC_{50} (μM)		
	KB	BC	NCI-H187
2,4,3′,5′-Tetrahydroxystilbene (**1**)	NA[a]	NA	NA
trans-2-Methoxy-4,3′,5′-trihydroxystilbene (**2**)	NA	66.6	NA
trans-2,3′-Dimethoxy-4,5′-dihydroxystilbene (**3**)	5.5	10.8	10.9
trans-4,3′-Dimethoxy-2,5′-dihydroxystilbene (**4**)	16.5	13.9	33.5
trans-2,4,3′,5′-Tetramethoxystilbene (**5**)	8.6	5.6	8.0
cis-2,4,3′,5′-Tetramethoxystilbene (**6**)	0.3	1.0	0.3
2,4,3′,5′-Tetrahydroxybibenzyl (**7**)	NA	NA	NA
2,4,3′,5′-Tetramethoxybibenzyl (**8**)	NA	NA	NA
Ellipticine	2.4	2.7	1.9
Doxorubicin	0.3	0.5	0.1

[a] NA, no activity, showing less than 50% inhibition at 20 μg/ml.

than **1**, and exhibited no toxicity in human cells such as KB, BC, and NCI-H187. The present study also presented a process for the conversion of the non-cytotoxic stilbene **1** into a strongly cytotoxic compound **6** through two simple chemical reactions. The results from this investigation reflect the importance of natural products chemistry as a tool for finding and developing useful bioactive compounds.

Acknowledgments

We are grateful to the Asahi Glass Foundation for partial financial support (for the study of tyrosinase inhibitory activity). Thanks are due to the National Center for Genetic Engineering and Biotechnology for the assays of cytotoxicity.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.08.018.

References and notes

1. Mongkolsuk, S.; Alexander, R.; Towers, R. *J. Chem. Soc.* **1957**, 2231.
2. Likhitwitayawuid, K.; Sritularak, B.; Benchanak, K.; Lipipun, V.; Mathew, J.; Schinazi, R. F. *Nat. Prod. Res.* **2005**, *19*, 177.
3. Poopyruchpong, N.; Rungruangsak, K.; Nimmanpisut, S.; Panijpan, B.; Ratanabanangkoon, K. *J. Sci. Soc. Thai.* **1978**, *4*, 163.
4. Sritularak, B.; De-Eknamkul, W.; Likhitwitayawuid, K. *Thai. J. Pharm. Sci.* **1998**, *22*, 149.
5. Kim, Y. M.; Yun, J.; Lee, C. K.; Lee, H.; Min, K. R.; Kim, Y. *J. Biol. Chem.* **2002**, *277*, 16340.
6. Tengamnuay, P.; Pengrungruangwong, K.; Likhitwitayawuid, K. In *Proceedings of the IFCC Conference Cosmetics: Where Science Meets Dream*, Seoul, Korea, September 22–24, 2003, pp 201–212.
7. Chung, K. O.; Kim, B. Y.; Lee, M. H.; Kim, Y. R.; Chung, H. Y.; Park, J. H.; Moon, J. O. *J. Pharm. Pharmacol.* **2003**, *55*, 1695.
8. Lorenz, P.; Roychowdhury, S.; Engelmann, M.; Wolf, G.; Horn, T. F. W. *Nitric Oxide* **2003**, *9*, 64.
9. Andrabi, S. A.; Spina, M. G.; Lorenz, P.; Ebmeyer, U.; Wolf, G.; Horn, T. F. W. *Brain Res.* **2004**, *1017*, 98.
10. Full experimental details about the preparations and biological evaluations of derivatives of **1** are available as Supporting Information which can be obtained upon request to the corresponding author.
11. Likhitwitayawuid, K.; Sritularak, B.; De-Eknamkul, W. *Planta Med.* **2000**, *66*, 275.
12. Sritularak, B. Chemical constituents of *Artocarpus lakoocha* and *A. gomezianus*. MS thesis, Chulalongkorn University, 1998; pp 101–102.
13. Likhitwitayawuid, K.; Sritularak, B. *J. Nat. Prod.* **2001**, *64*, 1457.
14. Shimizu, K.; Kondo, R.; Sakai, K. *Planta Med.* **2000**, *66*, 11.
15. Shin, N. H.; Ryu, S. Y.; Choi, E. J.; Kang, S. H.; Chang, I. M.; Min, K. R.; Kim, Y. *Biochem. Biophys. Res. Commun.* **1998**, *243*, 801.
16. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
17. Plumb, J. A.; Milroy, R.; Kaye, S. B. *Cancer Res.* **1989**, *49*, 4435.
18. Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, *50*, 119.
19. Guengerich, F. P.; Chun, Y. J.; Kim, D.; Gillam, E. M. J.; Shimada, T. *Mutation Res.* **2003**, *523–524*, 173.