Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2682–2684

N-Benzylbenzamides: A new class of potent tyrosinase inhibitors

Sung Jin Cho,[a] Jung Seop Roh,[b] Won Suck Sun,[c] Sung Han Kim[d] and Ki Duk Park[e,*]

[a]*Marine Biotechnology Laboratory, School of Earth and Environmental Sciences, Seoul National University, Seoul 151-747, Republic of Korea*
[b]*HAITAI Confectionary R&D Center, Seoul, Republic of Korea*
[c]*Life Science Division, Life Science R&D Center, SK Chemicals, Suwon, Republic of Korea*
[d]*Nutrex Technology, 1446-11 Seocho-dong, Seoul 137-864, Republic of Korea*
[e]*Laboratory of Cellular Function Modulator, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Republic of Korea*

Received 25 November 2005; revised 27 January 2006; accepted 8 February 2006
Available online 2 March 2006

Abstract—A series of potent inhibitors of tyrosinase and their structure–activity relationships are described. *N*-Benzylbenzamide derivatives (**1**–**21**) with hydroxyl(s) were synthesized and tested for their tyrosinase inhibitory activity. With this series, compound **15** provided a potent tyrosinase inhibition: it effectively inhibited the oxidation of L-DOPA catalyzed by mushroom tyrosinase with an IC_{50} of 2.2 μM.

Tyrosinase (E.C. 1.14.18.1), also known as polyphenol oxidase, is a multifunctional copper-containing enzyme widely distributed in nature. It is the key enzyme in the undesirable browning of fruits and vegetables, and coloring of skin, hair, and eyes in animals.[1,2] It catalyzes two distinct reactions of melanin biosynthesis: the hydroxylation of monophenols to *o*-phenols, and conversion of the *o*-diphenols to the corresponding *o*-quinones, which are the initial steps in the pathway. These quinones are highly reactive and tend to polymerize spontaneously to form brown pigments of high molecular weight, namely melanin, which determine the color of mammalian skin and hair.[3] This enzymatic oxidation of L-tyrosine to melanin is of considerable importance because melanin has many functions, and alterations in melanin synthesis occur in many disease states. Therefore, tyrosinase inhibitors have become increasingly important in the food industry as well as in medicinal and cosmetic products.[4,5]

Many tyrosinase inhibitors are polyphenol derivatives of *trans*-stilbene or chalcone which have been investigated intensively.[3,6] They are constructed with one of two distinct substructures: a 4-substituted resorcinol or catechol moiety. It was suggested that 4-substituted resorcinol-type inhibitors bind to the enzyme binuclear active site.[7] The catechol structure may behave as a chelator to the copper ions in the tyrosinase. These polyphenols generally compete in inhibition with tyrosinase.[6] Recently, a naturally occurring tyrosinase inhibitor, *N*-(*p*-coumaroyl)serotonin, has been described, which contains two aromatic rings connected by an amide bond (Fig. 1).[8]

In our continuing search for tyrosinase inhibitor, a new group of compounds, with a similar structure to the referenced inhibitors, was designed, synthesized, and tested. *N*-Benzylbenzamides also consist of two aromatic rings like stilbene separated by three atoms like chalcone

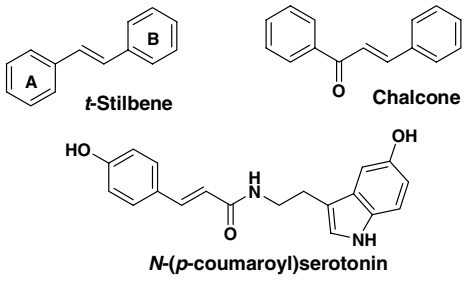


Figure 1. Structure of tyrosinase inhibitors.

Keywords: Tyrosinase inhibitor; *N*-Benzylbenzamides; 4-Substituted resorcinol; SAR.
* Corresponding author. Tel.: +82 42 860 4564; fax: +82 42 860 2675; e-mail: kdpark@kribb.re.kr

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.02.018

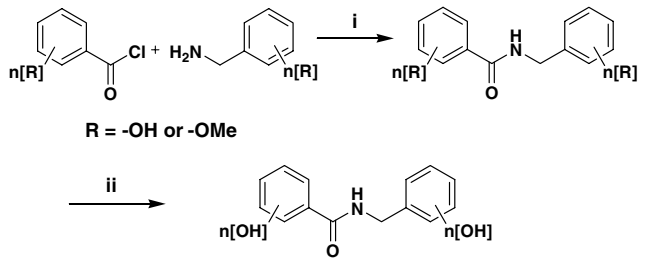
and connected by an amide bond like *N*-(*p*-coumaroyl)serotonin.

To evaluate this class of compounds further, a number of analogues were constructed with various hydroxyl substituents at both aromatic rings of the molecule. Our hypothesis was that the position of the hydroxyl(s) attached to the rings of *N*-benzylbenzamide must be of major importance in that activity in the same manner of stilbene and chalcone. Therefore, changing the position of hydroxyl(s) would be very useful to find a potent tyrosinase inhibitor.

N-Benzylbenzamides were synthesized according to the details in Scheme 1. Thus, commercially available benzyl amine, with hydroxyl(s) or methoxy group(s), was first reacted with an acyl chloride, with methoxy group(s), to give the hydroxy or methoxy substituted *N*-benzylbenzamide. The methoxy group(s) was then demethylated with boron tribromide to give the desired final compound in good yields.[9]

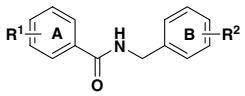
The two aromatic rings in *N*-benzylbenzamides are not symmetric, thus the inhibitory effects on tyrosinase were tested and compared according to the position of hydroxy group(s) on both rings A and B.[10] The in vitro results for compounds **1**–**21** are given in Table 1. Compounds **5**, **10**, **15**, and **20** with resorcinol on ring B (2′,4′-OH) demonstrated high tyrosinase inhibitory activity. However, compounds **17**–**19** and **21** with resorcinol on ring A (2,4-OH) remarkably decreased inhibitory activity. Especially, compound **5** exhibited 30 times the activity than compound **19** which has opposite positions of hydroxyls on its rings to compound **5**. These observations suggests that 2′,4′-substituted resorcinol substructure on ring B plays an important role in determining their activity. Among compounds **5**, **10**, **15**, and **20**, compound **15** with resorcinols on both rings A and B (3,5,2′,4′,-OH) exerted the most potent inhibitory activity with an IC_{50} of 2.2 μM.

In compounds **6**, **11**, **16**, and **21**, the catechol subunit on ring B (3′,4′-OH) was oxidized by tyrosinase to its *o*-quinone derivatives in addition to the oxidization of original substrate, L-DOPA. Tyrosinase inhibitory activity of those compounds thus could not be determined being tested because the absorbance at 475 nm in the test was abnormally increased by those *o*-quinone derivatives. Interestingly, tyrosinase inhibitory activity was not so much affected by the catechol subunit on ring A in compounds **9** and **10**. It is likely that 3,4-OH of ring A is not oxidized by tyrosinase as cofactor like another diphenol.



Scheme 1. Reagents and conditions: (i) TEA, THF, rt, 80–93%; (ii) BBr_3, CH_2Cl_2, −20 °C, 65–78%.

Table 1. The inhibitory effect of *N*-benzylbenzamide (**1**) and its derivatives (**2**–**21**) on mushroom tyrosinase activities



Compound	R^1	R^2	IC_{50}[a] (μM)
1	H	H	1990
2	3,4,5-OH	H	780
3	3,4,5-OH	4′-OH	1180
4	3,4,5-OH	3′,4′,5′-OH	555
5	3,4,5-OH	2′,4′-OH	17
6	3,4,5-OH	3′,4′-OH	nd[b]
7	3,4-OH	H	>2000
8	3,4-OH	4′-OH	>2000
9	3,4-OH	3′,4′,5′-OH	280
10	3,4-OH	2′,4′-OH	11
11	3,4-OH	3′,4′-OH	nd
12	3,5-OH	H	700
13	3,5-OH	4′-OH	710
14	3,5-OH	3′,4′,5′-OH	705
15	3,5-OH	2′,4′-OH	2.2
16	3,5-OH	3′,4′-OH	nd
17	2,4-OH	H	1660
18	2,4-OH	4′-OH	1820
19	2,4-OH	3′,4′,5′-OH	550
20	2,4-OH	2′,4′-OH	29
21	2,4-OH	3′,4′-OH	nd
Kojic acid			16.3

[a] Values were determined from logarithmic concentration–inhibition curves (at least eight points) and are given as means of three experiments.

[b] Means not determined because of promoting effect which could act as cofactor like diphenol.

The kinetic behavior of the oxidation of L-DOPA catalyzed by tyrosinase at different concentrations of compound **15** was studied. The inhibition data were further analyzed by a Dixon plot, which showed that, the plot of $1/V$ versus [I] was characterized by straight lines at different fixed substrate concentrations intersecting in the second quadrant (Fig. 2A). The replot of slopes represented a straight line not going through the origin reflecting the linear mixed-type inhibition (Fig. 2B). In the Lineweaver–Burk plot, straight lines at different fixed inhibitor concentrations intersected with a common intersection point in the second quadrant (data not shown). The K_I value estimated from this Dixon plot was 1.3 μM as shown in Figure 2A.

In similar experiment testing, the tyrosinase effect of chalcone derivatives substituted with hydroxyl(s), the inhibitory potency was determined by the position rather than the number of hydroxyl(s).[11] In this study as well, the position of hydroxyl(s) was the major factor affecting potency. Besides, removal of the hydroxyl or its substitution with another small group (OMe, NO_2, Cl, and F) significantly reduced the activity toward mushroom tyrosinase (data not shown). Therefore, the

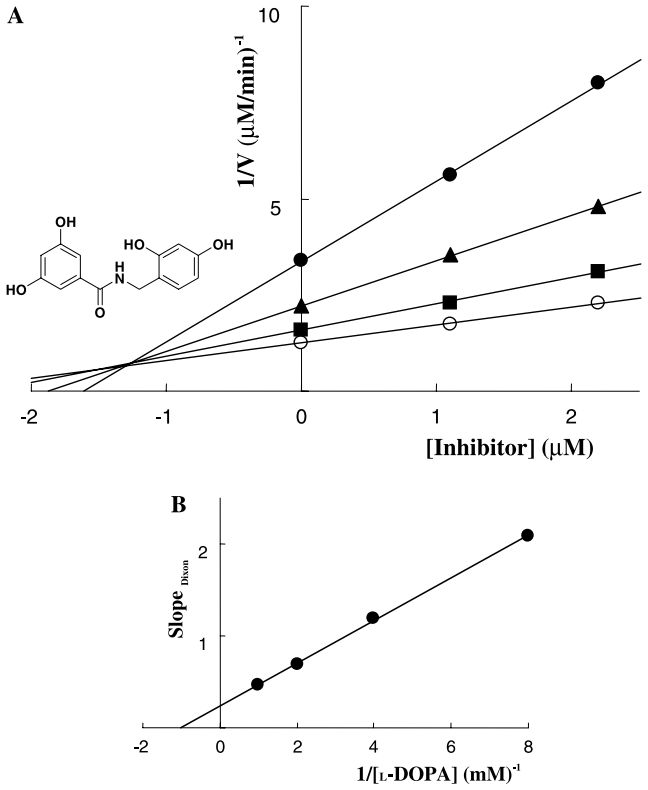


Figure 2. (A) Dixon plot for the inhibitory effect of compound **15** on L-DOPA oxidation catalyzed by mushroom tyrosinase. The inhibitor concentrations used were 0, 1.1, and 2.2 μM. The L-DOPA concentrations used were 1 mM (○), 0.5 mM (■), 0.25 mM (▲), and 0.125 mM (●). (B) Secondary replot of slopes from Dixon plot versus l/[L-DOPA]. Values are means of three separate experiments.

presence and the position of hydroxyl(s) proved to be important for their activity in this class of compounds.

In summary, a series of *N*-benzylbenzamides has been established as a new class of tyrosinase inhibitors. Structural feature of resorcinol on aromatic ring B (2′,4′-OH) was most important for potent inhibitory activity. Compound **15**, *N*-(2,4-dihydroxybenzyl)-3,5-dihydroxybenzamide,[12] was found to be eight times more potent than the positive control, kojic acid.

Acknowledgment

We thank Nutrex Technology for the financial support.

References and notes

1. Kubo, I.; Kinst-Hori, I.; Chaudhuri, S. K.; Kubo, Y.; Scanchez, Y.; Ogura, T. *Bioorg. Med. Chem.* **2000**, *8*, 1749.
2. Perez-Gilabert, M.; Garcia-Carmona, F. *Biochem. Biophys. Res. Commun.* **2001**, *285*, 257.
3. Seo, S. Y.; Sharma, V. K.; Sharma, N. *J. Agric. Food Chem.* **2003**, *51*, 2837.
4. Mosher, D. B.; Pathak, M. A.; Zpatrick, T. B.. In *Update: Dermatology in General Medicine*; Fitzpatrick, T. B., Eisen, A. Z., Wolff, K., Freedberg, I. M., Austen, K. F., Eds.; McGraw-Hill: New York, 1983; Vol. 1, p 205.
5. Maeda, K.; Fukuda, M. *J. Soc. Cosmet. Chem.* **1991**, *42*, 361.
6. Briganti, S.; Camera, E.; Picardo, M. *Pigment Cell Res.* **2003**, *16*, 101.
7. Shimizu, K.; Kondo, R.; Sakai, K. *Planta Med.* **2000**, *66*, 11.
8. Roh, J. S.; Han, J. Y.; Kim, J. H.; Hwang, J. K. *Biol. Pharm. Bull.* **2004**, *27*, 1976.
9. All compounds provided satisfactory spectral data (^{1}H NMR and LC–MS) and were homogeneous by TLC.
10. The tyrosinase assay was performed by the method of Masamoto et al. with slight modifications.[13] First, 65 μl of 2.5 mM L-DOPA solution, 10 μl of DMSO with or without a sample, and 105 μl of 0.1 M phosphate buffer (pH 6.8) were mixed. The mixture was preincubated at 25 °C for 10 min before 20 μl of 1380 U/ml tyrosinase in aqueous solution was added, and the reaction was monitored at 475 nm. A control reaction was conducted with DMSO. The OD values were measured by a UV spectrophotometer at 475 nm using an ELISA Microplate Reader (PowerWaveX, Bio-Tek, USA). Kojic acid was used as a reference.
11. Khatib, S.; Nerya, O.; Musa, R.; Shmuel, M.; Tamir, S.; Vaya, J. *Bioorg. Med. Chem.* **2005**, *13*, 433.
12. Spectral data for compound **15**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.85 (s, 1H), 9.79 (s, 1H), 9.26 (s, 2H), 8.00 (br s, 1H), 7.02 (s, 2H), 6.72 (d, 2H, J = 7.6 Hz), 6.47 (s, 1H), 6.17 (d, 2H, J = 7.6 Hz), 6.11 (s, 1H), 4.02 (d, 2H). MS (EI) *m*/*z*: 275 (M^+).
13. Masamoto, Y.; Ando, H.; Murata, Y.; Shimoishi, Y.; Tada, M.; Takahata, K. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 631.