Bioorganic & Medicinal Chemistry Letters 19 (2009) 1532–1533

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Studies on depigmenting activities of dihydroxyl benzamide derivatives containing adamantane moiety

Ho Sik Rho *, Heung Soo Baek, Soo Mi Ahn, Jae Won Yoo, Duck Hee Kim, Han Gon Kim

R & D Center, AmorePacific Corporation, 314-1, Bora-daong, Giheung-gu, Yongin-si, Kyounggi-do 449-729, Republic of Korea

ARTICLE INFO

Article history:
Received 16 August 2008
Revised 20 December 2008
Accepted 25 December 2008
Available online 1 January 2009

Keywords:
Depigmenting activity
Tyrosinase
Dihydroxyl benzamide
Adamantane moiety

ABSTRACT

Six diphenolic compounds containing adamantane moiety were synthesized and evaluated as potent inhibitors on tyrosinase activity and melanin formation in melan-a cells. The inhibitory activity of 4-adamantyl resorcinol **1** was similar to that of 4-*n*-butyl resorcinol in both assays. However, dihydroxyl benzamide derivatives **6a–e** showed different inhibitory patterns. All derivatives significantly suppressed the cellular melanin formation without tyrosinase inhibitory activities. These behaviors indicated that the introduction of amide bond changes the binding mode of dihydroxyl groups to tyrosinase. Among derivatives, **6d** (3,4-dihydroxyl compound) and **6e** (2,3-dihydroxyl compound) showed stronger inhibitory activities (IC_{50} = 1.25 μM and 0.73 μM, respectively) as compared to 4-*n*-butyl resorcinol (IC_{50} = 21.64 μM) and hydroquinone (IC_{50} = 3.97 μM). This study showed that the position of dihydroxyl substituent at aromatic ring is important for the intercellular inhibition of melanin formation, and also amide linkage and adamantane moiety enhance the inhibition.

Melanogenesis is the process by which the synthesis and distribution of melanin within skin and hair follicles.[1] Melanin has important role in protecting the skin against the harmful effect of UV-irradiation. However, increase in the levels of epidermal melanin synthesis and uneven distribution can cause esthetic problems such as freckles, melasma and aged spot. In melanocyte, melanin are synthesized by the action of tyrosinase which catalyzes the hydroxylation of tyrosine to DOPA and the oxidation of DOPA to dopaquinone.[2] Therefore, the inhibition of tyrosinase to treat the pigmentation disorders has been a recent subject of many studies. Certain polyphenolic compounds, containing resorcinol moiety, showed potent depigmenting activities and their inhibitory mechanisms were well investigated.[3] Recently, the effects and mechanism of 4-*n*-butyl resorcinol has been clearly elucidated.[4] The hypopigmentary effect of 4-*n*-butyl resorcinol results from its direct inhibition of tyrosinase. The structure of 4-*n*-butyl resorcinol consists of main two parts such as resorcinol moiety and *n*-butyl group. *n*-Butyl group was introduced as a hydrophobic moiety to increase the depigmenting activity of resorcinol. The present work was designed to develop more potent depigmenting agent containing diphenolic moiety such as catechol and resorcinol (Fig. 1).

Considering cell permeability, we introduced adamantane moiety[5] expecting an increase in tyrosinase inhibitory activity and enhancing cell penetration. 4-Adamantyl resorcinol **1**[6] was synthesized in one step through the reaction of adamantylation of resorcinol **2** using 1-adamantanol in CF_3COOH at reflux condition (Scheme 1).[7]

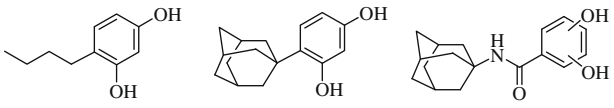
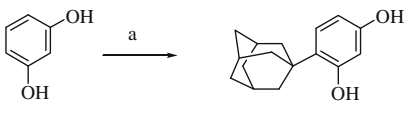


Figure 1. Structure of 4-*n*-butyl resorcinol and diphenolic compounds containing adamantane moiety **1** and **6a–e**.

The synthetic pathways of dihydroxyl benzamide derivatives containing adamantane moiety are shown in Scheme 2.

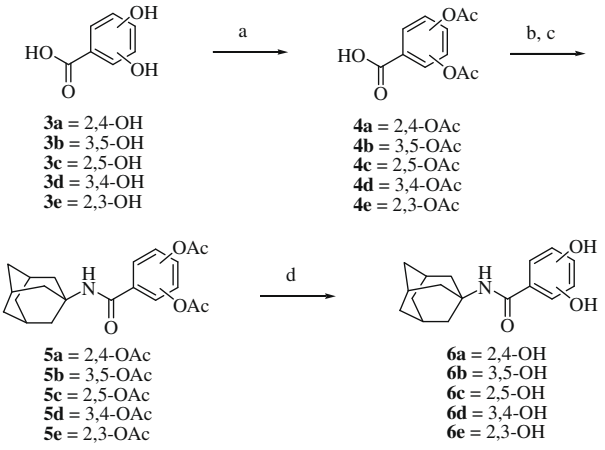
The starting materials, dihydroxy benzoic acids (**3a–e**) were reacted with acetic anhydride in the presence of Et_3N and catalytic amount of dimethylaminopyridine (DMAP) in THF to afford diacetoxy benzoic acids. Diacetoxy benzoic acids (**4a–e**) were treated with ethylchloroformate and *N*-methyl morpholine in THF to convert the carboxylic acids to an anhydrides. The anhydrides were re-



Scheme 1. Reaction conditions; (a) 1-adamantanol, CF_3COOH, reflux.

* Corresponding author. Tel.: +82 31 280 5805; fax: +82 31 281 8397.
E-mail address: thiocarbon@freechal.com (H.S. Rho).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.12.106



Scheme 2. Reaction conditions: (a) acetic anhydride, Et_3N, DMAP (cat), THF; (b) ethylchloroformate, *N*-methylmorpholine, THF; (c) adamantamine.HCl, Et_3N, DMF (d) (d) KOH, H_2O.

acted immediately adamantamine.HCl to produce the corresponding amide derivatives (**5a**–**e**). The hydrolysis of compounds (**5a**–**e**) afforded desired final products, dihydroxyl benzamides (**6a**–**e**).[8]

Above six synthesized compounds were evaluated for mushroom tyrosinase activity[9] and their ability to inhibit melanin formation by a murine melanocytes cell line (Melan-a).[10] Their activities were compared with 4-*n*-butyl resorcinol and hydroquinone (Table 1).

Consistent with previous reports, 4-*n*-butyl resorcinol induced a strong inhibition of the tyrosianse activity (IC_{50} = 0.15 μM). Its strong activity was also detected in melan-a cells (IC_{50} = 21.64 μM). When the *n*-butyl group was replaced by adamantane moiety, the resulting compound **1** showed slightly lower tyrosinase inhibitory activity (IC_{50} = 0.90 μM) but more potent depigmenting activity (IC_{50} = 8.82 μM) in cell based assay. These results indicate that adamantane group enhances the tyrosinase inhibitory activity and cell penetration of resorcinol like *n*-butyl group. Encouraged by this result, we synthesized five dihydroxyl benzamide derivatives containing adamantane moiety and evaluated their activities. Dihydroxy benzoic acids were conjugated with adamantamine. Surprisingly, in tyrosinase assay, *N*-adamantyl-2,4-dihydroxy benzamide **6a** showed no inhibitory activity (IC_{50} > 200 μM). All other derivatives (**6b**, **6c**, **6d** and **6e**), having different dihydroxyl positions, also showed no inhibitory activities. Introduction of amide linkage caused a negative influence on the binding to tyrosinase. However, unexpected results were obtained in melan-a cells. Compound **6a** exhibited potent depigmenting activity (IC_{50} = 34.15 μM). When 3,5-dihydroxyl, and 2,5-dihydroxyl derivatives (**6b** and **6c**) were tested, increased activities were detected (respectively, IC_{50} = 12.47 μM and IC_{50} = 9.82 μM). *N*-Adamantyl-3,4-dihydroxy benzamide **6d** showed a better inhibitory activity (IC_{50} = 1.25 μM). Among all the derivatives tested, compound **6e** containing 2,3-dihydroxyl groups exhibited stronger inhibitory activity (IC_{50} = 0.73 μM). Its activity was about five times more potent than that of hydroquinone (IC_{50} = 3.97 μM). Although five dihydroxyl benzamide derivatives (**6a–e**) had no tyrosinase inhibitory activities, there were potent depigmenting activities in cell based assay. Inhibition pathways of **6a–e** may be different from those of compound **1** and 4-*n*-butyl resorcinol. From these result, compound **6d** and **6e** can be considered as a good candidate for depigmenting agent because their activities were more potent than that of hydroquinone known to be the most powerful depigmenting agent. Further studies on their depigmenting mechanism are underway in our laboratory.

Table 1
Depigmenting activities of 4-*n*-butyl resorcinol and diphenolic compounds containing adamantane moiety **1** and **6a**–**6e**

Compound	Tyrosinase IC_{50}[a] (μM)	Pigmentation IC_{50}[a] (μM)	% Survival of melan-a cell
4-*n*-Butyl resorcinol	0.15	21.64	98.21 (80 μM)
Compound **1**	0.90	8.82	91.73 (20 μM)
6a (2,4-OH)	>200	34.15	99.24 (50 μM)
6b (3,5-OH)	>200	12.47	91.33 (30 μM)
6c (2,5-OH)	>200	9.82	99.10 (20 μM)
6d (3,4-OH)	>200	1.25	95.23 (30 μM)
6e (2,3-OH)	>200	0.73	96.17 (20 μM)
Hydroquinone	9.81	3.97	87.27 (10 μM)

[a] Values were determined from logarithmic concentration-inhibition curves and are given as means of three experiments.

References and notes

1. Spritz, R. A.; Hearing, V. J. *Adv. Hum. Genet.* **1994**, *22*, 1.
2. Marmol, V. D.; Beermann, F. *FEBS Lett.* **1996**, *381*, 165.
3. (a) Khatib, S.; Nerya, O.; Musa, R.; Shumuel, M.; Tamir, S.; Vaya, J. *Bioorg. Med. Chem.* **2005**, *13*, 433; (b) Shimizu, K.; Kondo, R.; Sakai, K.; Takeda, N.; Nagahata, T.; Oniki, T. *Lipids* **2001**, *36*, 1321; (c) Shimizu, K.; Kondo, R.; Sakai, K. *Planta Med.* **2000**, *66*, 11; (d) Nerya, O.; Vaya, J.; Musa, R.; Izrael, S.; Ben-Arie, R.; Tamir, S. *J. Agric. Food Chem.* **2003**, *51*, 1201.
4. Kim, D. S.; Kim, S. Y.; Park, S. H.; Choi, Y. G.; Kwon, S. B.; Kim, M. K.; Na, J. I.; Youn, S. W.; Park, K. C. *Biol. Pharm. Bull.* **2005**, *12*, 2216.
5. (a) Rho, H. S.; Baek, H. S.; Lee, B. S.; Kim, J. H.; Kim, D. H.; Chang, I. S. *Bull. Korean Chem. Soc.* **2006**, *27*, 115; (b) Baek, H. S.; Rho, H. S.; Yoo, J. W.; Ahn, S. M.; Lee, J. Y.; Lee, J.; Kim, M. K.; Chang, I. S. *Bull. Korean Chem. Soc.* **2008**, *29*, 43.
6. Compound **1**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.98 (s, 1H), 8.89 (s, 1H), 6.80 (d, 1H, J = 8.4 Hz), 6.22 (s, 1H), 6.10 (d, 1H, J = 8.4 Hz), 1.99 (s, 9H), 1.69 (s, 6H). IR ν_{max} (KBr) 3486, 3390, 2903, 1614 cm^{-1}. FABMS: (*m*/*e*) 259 [M+H]$^+$.
7. Shokova, E.; Tafeenko, V.; Kovalev, V. *Tetrahedron Lett.* **2002**, *43*, 5153.
8. We previous reported dihydroxyl benzamide derivatives **6a–6e** on Korean Patent 0,643,514, 2005 and 0,740,575, 2007. Compound **6a**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.62 (s, 1H), 10.07 (s, 1H), 7.73 (d, 1H, J = 8.7 Hz), 7.65 (s, 1H), 6.21 (m, 2H), 2.05 (s, 9H), 1.65 (s, 6H). IR ν_{max} (KBr) 3429, 3221, 2904, 1650, 1583 cm^{-1}. FABMS: (*m*/*e*) 302 [M+H]$^+$. Compound **6b**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.36 (s, 2H), 7.34 (s, 1H), 6.57 (s, 2H), 6.29 (s, 1H), 2.02 (s, 9H), 1.63 (s, 6H). IR ν_{max} (KBr) 3430, 3221, 2907, 1652, 1584 cm^{-1}. FABMS: (*m*/*e*) 302 [M+H]$^+$. Compound **6c**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.14 (s, 1H), 8.93 (s, 1H), 8.04 (s, 1H), 7.26 (s, 1H), 6.71 (m, 2H), 2.05 (s, 9H), 1.65 (s, 6H). IR ν_{max} (KBr) 3429, 3222, 2905, 1651, 1580 cm^{-1}. FABMS: (*m*/*e*) 302 [M+H]$^+$. Compound **6d**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.18 (bs, 1H), 9.02 (bs, 1H), 7.19 (s, 1H), 7.17 (s, 1H), 7.10 (d, 1H, J = 8.1 Hz), 6.71 (d, 1H, J = 8.1 Hz). 2.03 (s, 9H), 1.63 (s, 6H). IR ν_{max} (KBr) 3428, 3220, 2903, 1650, 1582 cm^{-1}. FABMS: (*m*/*e*) 302 [M+H]$^+$. Compound **6e**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.20 (s, 1H), 9.21 (s, 1H), 7.92 (s, 1H), 7.33 (d, 1H, J = 1.5 Hz), 6.89 (d, 1H, J = 1.5 Hz), 6.64 (m, 1H), 2.07 (s, 9H), 1.65 (s, 6H). IR ν_{max} (KBr) 3427, 3223, 2901, 1650, 1580 cm^{-1}. FABMS: (*m*/*e*) 302 [M+H]$^+$.
9. Measurements of mushroom tyrosinase activity: Mushroom tyrosinase, L-tyrosine were purchased from Sigma Chemical. The reaction mixture for mushroom tyrosinase activity consisted of 150 μl of 0.1 M phosphate buffer (pH 6.5), 3 μl of sample solution, 8 μl of mushroom tyrosinase (2100 U/ml, 0.05 M phosphate buffer at pH 6.5), and 36 μl of 1.5 mM L-tyrosine. Tyrosinase activity was determined by reading the optical density at 490 nm on a microplate reader (Bio-Rad 3550, Richnmond, CA, U.S.A.) after incubation for 20 min at 37 °C. The inhibitory activity of the sample was expressed as the concentration that inhibits 50% of the enzyme activity (IC_{50}).
10. Measurements of melanin content and cell viability: Melanin content and cell number were measured in melan-a melanocytes. Murine melan-a melanocytes were originally derived from C57BL/6 J (black, a/a) mice, a kind gift from Prof. Dorothy C. Bennett (St. George's Hospital, London, U.K.). The melanin content was measured using the method reported by Hosoi et al. with a slight modification.[11] The cells (2×10^5 cells/ml) were seeded into 24-well plates and the test compounds were added in triplicate. The medium was changed daily and after 4 d of culture, the cells were lysed with 1 ml of 1 N NaOH. Then 200 μl of each crude cell extract was transferred into 96-well plates. The relative melanin content was measured at 400 nm with a microplate reader (Molecular Devices). Cell viability was determined using a modified crystal violet assay.[12] After removing the medium from each well, the cells were washed with PBS and stained with 0.1% crystal violet in 10% ethanol for 5 min at room temperature. The cells were then rinsed four times with distilled water, and crystal violet retained by adherent cells was extracted with 95% ethanol at room temperature for 10 min. Crystal violet absorption was measured at 590 nm (Molecular Devices Co., Sunnyvale, CA, U.S.A.).
11. (a) Hosoi, J.; Abe, E.; Suda, T.; Kuroki, T. *Cancer Res.* **1985**, *45*, 1474; (b) Cho, Y.; Kim, K.-H.; Shim, J.-S.; Hwang, J.-K. *Biol. Pharm. Bull.* **2008**, *31*, 986.
12. Dooley, T. P.; Gadwood, R. C.; Kilgore, K.; Thomasco, L. M. *Skin Pharmacol.* **1994**, *7*, 188.