Development of Whitening Agents by Synthesis of Polyhydroxy Aromatic Compounds

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Key words

skin lightener, polyhydroxy aromatic, melanin, anti-oxidant, tyrosinase, melanogenesis, natural product , synthesis

Abstract

Some natural polyhydroxy aromatic compounds have inhibitory activity against tyrosinase, key enzyme for formation of melanin pigment. We examined the structure-activity relationship of the natural polyhydroxy aromatic compounds and synthesized a number of new derivatives through various methods. Skin lightening effects of these compounds were examined through inhibition of mushroom tyrosinase and inhibition of melanogenesis on B-16 melanoma cells. These new compounds showed strong inhibitory activity against tyrosinase (IC_{50}: 1.0 mg/ml~130mg/ml). Good lightening effects due to inhibition of melanogenesis were observed from several resorcinol and pyrogallol derivatives. In toxicological tests such as skin primary irritation and sensitization, the above compounds were sufficiently safe for cosmetic use.

1. Introduction

Many Asian women have wanted to keep their skin as white as possible. Skin lightners not only make the user's skin whiter and brighter but also prevent freckles and brown spots, a serious aesthetic problem in today's society. The development of potent active agents has positively exploded for cosmetic use in last five years and is taking markets all over Asia Pacific.

Many plant extracts have been examined to develop such active agents by several research groups. Some of the plant extracts, especially polyhydroxy aromatic compounds, have shown excellent inhibitory effects against melanin biosynthesis, which is the most determinant factor for skin color [1].

We have been interested in the extracts from Licorice, Mori Cortex Radicis [2] and Paper-mulberry [3] that should inhibit the action of mushroom tyrosinase. The active components of those extracts have common structure that is composed of some anti-oxidant and polyhydroxy aromatic moiety such as 2,4-resorcinol group. (Figure 1)

We assumed that both parts are essential for inhibition of tyrosinase, key enzyme for melanin biosynthesis. The 2,4-resorcinol part should block tyrosinase active site to exert inhibitory effect and the anti-oxidant parts should deactivate copper-containing tyrosinase by the reduction of oxytyrosinase ($Cu^{2+}Cu^{2+}O_2$) to deoxytyrosinase ($Cu^{+}Cu^{+}$) [4].

Based on the above assumption, we designed new tyrosinase inhibitors that mimicked the structure of natural products to minimize unexpected side effects. This new agents had an overall flavonoid structure composed of well-known vitamin E moiety and 2,4-resorcinol part (Figure 2). We also synthesized several derivatives of compound 1 to improve their activity and stability.

2. Material and Methods

1) Synthesis

Syntheses of the flavonoids 1, 5 and 6 were performed by well known methods as shown in Figure 3 [5].

We prepared 1,3-diphenyl derivatives 9, 10, 13 and 14 by the cleavage of C_1 - O_{10} bond of flavonoid derivatives. Compounds 13 and 14 were mono-methoxylated to improve their stability (Figure 4).

We also prepared 1,3-diphenyl derivative 15 containing chroman moiety (vitamin E Type). Its structure is similar to that of glabridin, the most active component extracted from licorice [2] (Figure 5).

Other derivatives 16, 17, 18 and 19 were synthesized by same synthetic methodology.

2) Assay

1) Inhibition of tyrosinase [6]: The reaction mixture consisted of 0.05 M phosphate buffer(pH 6.8, 2.3 ml), 1.5 mM L-tyrosine solution (0.4 ml) and 2,000 U/ml mushroom tyrosinase (Sigma), in 0.05 M phosphate buffer (pH 6.8, 0.1 ml). A sample solution (0.2 ml) was added to reaction mixture and incubated at 37°C for 10 min. The optical density at 475 nm was measured by a spectrophotometer. The inhibitory activity of the sample was expressed as the concentration which inhibits 50% of the enzyme activity (IC_{50}).

The percent inhibition of tyrosinase reaction was calculated as follows

$$\% \text{ inhibition} = \{(A-B)/A\} \times 100$$

A = Absorbance at 475 nm without test samples after incubation
B = Absorbance at 475 nm with test samples after incubation

2) Inhibition of melanin synthesis in B-16 melanoma cell [7]: Cells were seeded into 60 mm petridishes at a density of 5×10^5 cells per dish. After cells were attached, medium was replaced with fresh medium containing various concentrations of chemicals. Then cells were cultured for 2 days and the medium was replaced with fresh medium, further incubated for a day. Then cells were harvested with a cell scraper, counted with a haemacytometer and collected by centrifugation.

Melanin was extracted and measured according to the method of Maeda with some modifications. Briefly, cell pellets were resuspended in 1 ml of distilled water, frozen at -20°C and thawed at 37°C. This freezing-thawing process was performed 3 times. Perchloric acid was added to the cell suspensions at a final concentration of 0.5 N. The tubes were set on ice for 10 min and centrifuged at 15,000 gravities for 5 min. The pellets were extracted with 0.5 N perchloric acid for 2 times, with cold ethanol/ether (3:1) for 2 times, and with ether. The resulting pellets were dried in air and 1ml of 1N NaOH was added to each tube. The tubes were incubated in a boiling water bath for 10 min to dissolve the pellets. Melanin contents were measured by reading the absorbance at 400 nm and expressed as $A_{400}/10^6$ cells.

3. Results and Discussion

We examined the structures of tyrosinase inhibitors from several plant extracts and assumed that both antioxidant and binder of tyrosinase should be essential for inhibition of melanin biosynthesis. To confirm the feasibility of our assumption, we designed and synthesized new tyrosinase inhibitors.

Our initial target molecule was compound 1 that has modified vitamin E structure and has flavonoid skeleton, similar to that of isocathechin [8] that has the inhibitory activity against melanin synthesis. The modification of natural product skeleton was expected to minimize unexpected side effects and to improve some drawback of natural products such as stability and/or bioavailability.

Compound 1 was synthesized by known method. Compound 6 and 7, derivatives of 1, were also prepared by the minor modification of synthetic pathway as shown in Figure 3.

The reaction of tyrosinase was mainly oxidative hydroxylation from tyrosine to DOPA quinone. We speculated that a reduced form of tyrosinase inhibitor should be more effective for inhibition of melanin synthesis via interruption of the oxidative hydroxylation. Accordingly, flavonoid compounds 7 and 8 were reduced to 1,3-diphenyl propane derivatives by the cleavage of C_1-O_{10} bonds under dissolving metal condition using Na/liquid ammonia [9] (Figure 4).

We also prepared another 1,3-diphenyl propane derivative 16 to contain vitamin E moiety and to have the similar backbone to that of glabridin, the most active component extracted from licorice. This compound was expected to have enhanced antioxidant effect (Figure 5).

For the evaluation of efficacy of our agents, we prepared more derivatives 17, 18 and 19 by same synthetic methology and 1-alkyl-2,4-resorcinol 16 derivative. Binding parts of these compounds 17, 18 and 19 were changed without any transformation of antioxidant parts. Compound 16, no antioxidant property, was easily prepared from protection, reduction, akylation and deprotection of 2,4-dihydroxy-benzaldehyde (Figure 6).

We examined these synthethic derivatives on inhibition of tyrosinase activity. These results were listed in Table 1.

The activity against mushroom tyrosinase mainly depended on the structure of binding part without any participation of antioxidant. The analysis of the inhibitory activity was diagramed in Figure 7.

Regardless of the properties of anti-oxidants, 2,4-resorcinol derivatives 16 and 20 were the most effective on tyrosinase inhibition. Even compound 16, no anti-oxidant, showed strong activity on tyrosinase inhibition. *p*-Alkyl phenol derivative 21 showed week activity that were similar to that of arbutin which had *para*-sugar-

substituted phenol skeletone. 3,5-Resorcinol derivative 22 was less effective than 2,4-resorcinol derivative but was comparable with that of 3,4,5-trihydroxybenzyl derivative 23.

It was reported that the two active copper (II) ions should bind to the hydoxy group of tyrosine and should convert tyrosine to dopa-quinone by oxidative hydroxylation in the active site of tyrosinase. The 2,4-resorcinol group containing two hydroxy groups should bind to tyrosinase more efficiently than tyrosine containing only one hydroxy group and should inhibit the binding of tyrosin by the blocking of tyrosinase active site.

We assumed that the two Cu(II) ions should be placed symmetrically in the active site of tyrosinase and expected that 3,5-resorcinol derivative should be more effective than 2,4-resorcinol derivative due to their symmetry. However, 3,5-resorcinol derivative was less effective than 2,4-resorcinol derivative. It can be speculated that tyrosinase active site is distorted and more efficiently bound to 2,4-resorcinol derivative than 3,5-resorcinol derivative.

We also examined melanogenesis on B-16 melanoma cells. The results with 30 mg/ml concentration of the compounds were summarized on Table 2. Melanin contents were expressed as $A_{490}/10^6$ cells.

Although the antioxidant parts of these polyhydroxy-aromatic compounds did not show any crucial role for good inhibitory activity on mushroom tyrosinase, they were essential for inhibition of melanogenesis on B-16 melanoma cells. The analysis of the inhibitory activity was diagramed in Figure 8.

Compound 16, containing no antioxidant, showed poor activity on B-16 melanoma cells although it showed high efficacy on mushroom tyrosynase. Compounds 1, 5 and 6, having flavonoid type antioxidant 25, did not significiently inhibit melanogenesis on B-16 melanoma cells. Compounds 17, 18 and 19, having less efficent tyrosinase inhibitor 27, had no significant inhibitory activity for melanogenesis. Compound 9, 10, 13, 14 and 15, having anti-oxidant *via* free carbon chain 26, were highly effective for inhibition of melanogenesis. Compound 14, expected the highest radical scavenger effect, showed the highest activity. This type of molecule 26 did not show any significant difference of activity for melanogenesis by the change of functionality of R_1 (methyl or H) although that should influence the activity of radical scavenger effect. The change of functionality of R_2 (H or OH) in 26 also did not show any significant effect for melanogenesis. Because compounds 13 and 14 (R_1 = Me in 26) were much more stable to air-oxidation than compounds 9 and 10 (R_1 = H in 26) and compounds 10 and 14 (R_2 = OH in 26) were synthesized more easily than compounds 9 and 13 (R_2 = H), compound 14 (R_1 = Me and R_2 = OH in 26) was expected to be the most promising agent for further development.

By this time, the research of tyrosinase inhibitor was mainly focused on chelator of copper ion in tyrosinase active site and reducing agent that converted quinone derivatives to phenol derivatives respectively. However, it was reported that reducing agent or anti-oxidant should convert activated oxytyrosinase ($Cu^{2+}Cu^{2+}O_2$) to deactivated deoxytyrosinase ($Cu^{1+}Cu^{1+}$) simultaneously [4]. We have shown that both binding agent and anti-oxidant should be required for efficient inhibition of melanogenesis. Moreover, intramolecular system should be much more effective than intermolecular system. The plausible mechanism of our system is that the binding part should be attached and fixed to the active site of tyrosinase by the chelation on copper (II) ion and then anti-oxidant(or reducing agent) should deactivate the tyrosinase by the conversion of copper (II) ion to copper (I) ion. The high activity of 1,3-diphenyl propane derivative 26 compared to that of flavonoid 25 can be explained by that the type of molecule 26 is the reduced form of the type of molecule 25 [10].

References

1. M. Duggan, J. Wilmott and A. Znaiden, *Cosmetic & Toiletries* **102**(3), 97 (1987)
2. T. Ikeda and T. Tsutsumi, *Fragrance J.*, **6**, 59 (1990)
3. D.I. Jang, B.G. Lee, C.O. Jeon and N.S. Jo *Proceedings, 2nd Scientific Conference of ASCS* 295 (1995)
4. J. F. Maddaluno and K. F. Faull, *Experimentia*, **44**, 885 (1988)
5. K. B. Main, *J. Chem. Soc. Perkin rans* **2** 1309 (1982)
6. A. Vanni, D. Gastaldi and G. Giunta, *Annali di Chimica* **80**, 36 (1990)
7. K. Maeda and M. Fukuda, *J. Soc. Cosmet. Chem.*, **42**, 361 (1991)
8. K. Obayashi, A. Iwamoto, S. Sakaki, Y.Okano, H. Masaki and M. Ito, Proceedings 19th IFSCC Congress, Vol.1, 17 (1996).
9. H. G. Krishnamurty and S. Sathyanarayana, *Synthetic Communication*, **19** (1&2), 119 (1989)
10. J. Ikuta, Y. Hano, T. Nomura, Y. Kawakami and T. Sato, *Chem. Pham. Bull*, **34**(5), 1968 (1986)

Table 1. Inhibitory activity against mushroom tyrosinase of test materials

Material	*IC_{50} (mg/ml)*
1	0.5
5	5.0
6	5.0
9	0.9
10	0.5
13	0.1
14	0.1
15	0.1
16	0.5
17	130.0
18	28.0
19	25.0
Arbutin	131.0
Kojic acid	4.1
Glabridin[a]	0.4

a) Cited from T. Ikeda and T. Tsutsumi, *Fragrance J.* 6, 59 (1990)

Table 2. Inhibitory effect of materials on B-16 mouse melanoma cells

Compound	Control test compound	With 30 mg/ml
1	0.199 ± 0.006	0.154 ± 0.002
5	0.199 ± 0.006	0.170 ± 0.006
6	0.199 ± 0.006	0.229 ± 0.009
9	0.191 ± 0.009	0.120 ± 0.001
10	0.191 ± 0.009	0.132 ± 0.008
13	0.191 ± 0.009	0.120 ± 0.001
14	0.191 ± 0.009	0.102 ± 0.001
15	0.210 ± 0.009	0.053 ± 0.009
16	0.188 ± 0.009	0.175 ± 0.009
17	0.191 ± 0.009	0.198 ± 0.009
18	0.191 ± 0.009	0.165 ± 0.009
19	0.191 ± 0.009	0.145 ± 0.009

Figure 1. Typical structure of active ingredient for inhibition of melanogenesis

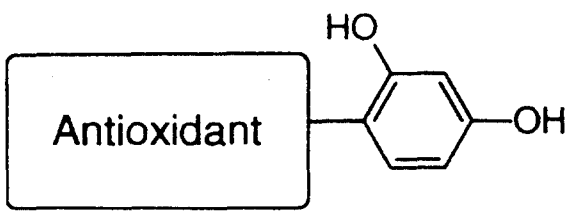
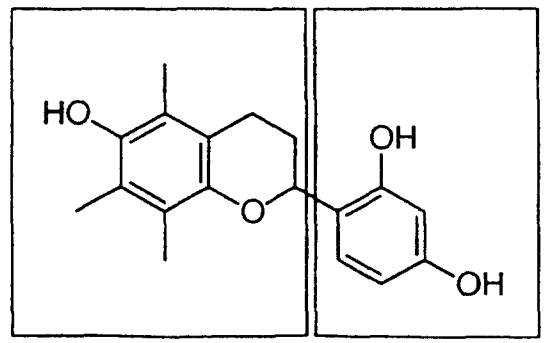


Figure 2. Initial target molecule of flavonoid type

Antioxidant part
- vitamin E type structure
- deactivation of reaction pathway

2,4-Resorcinol part
- binding to tyrosinase active site
- blocking of active site without any reaction



1

Figure 3. Synthesis of flavonoid derivatives

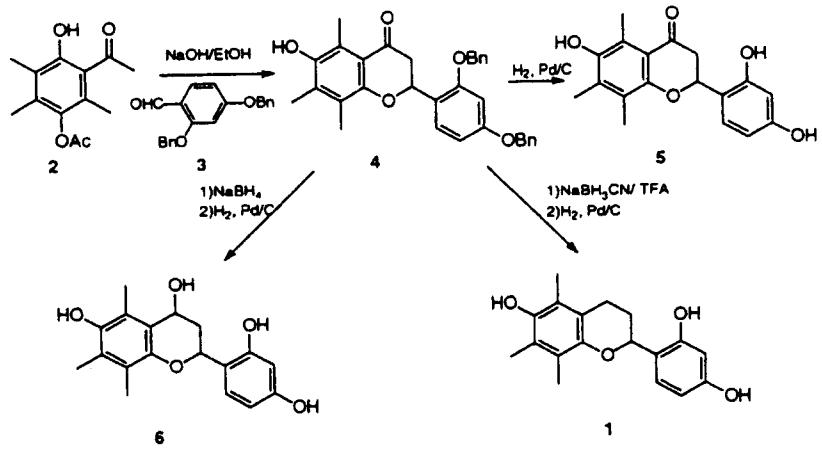
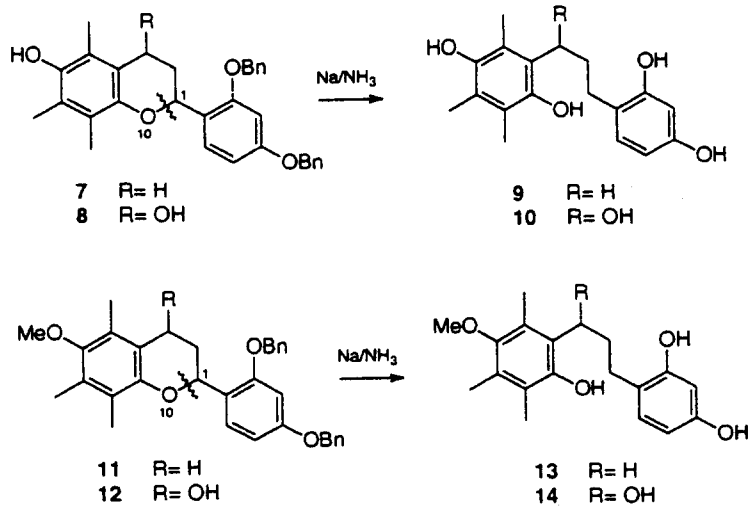


Figure 4. Synthesis of 1,3-diphenyl propane derivatives



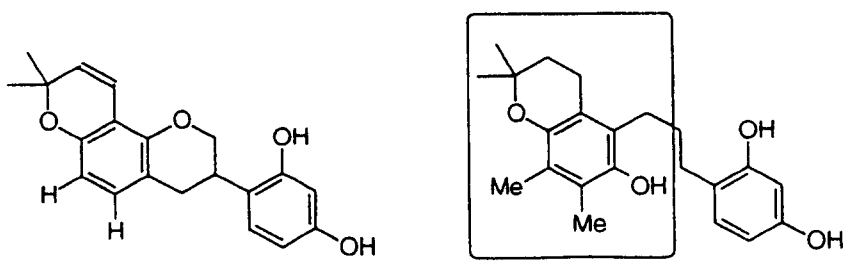
7 R= H
8 R= OH

9 R= H
10 R= OH

11 R= H
12 R= OH

13 R= H
14 R= OH

Figure 5. 1,3-Diphenyl propane derivatives containing chroman moiety



Glabridin
(Extract from licorice)

Vitamin E type

15

Figure 6. Synthetic derivatives

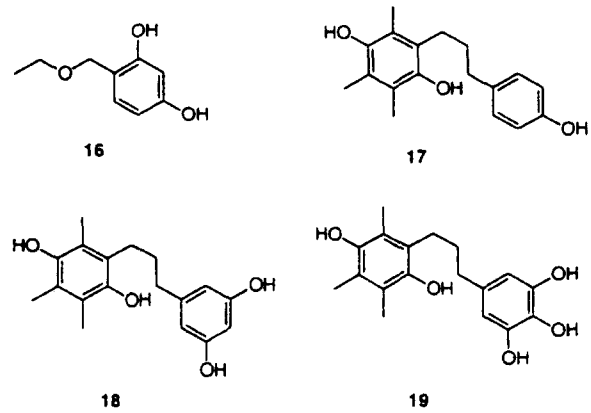


Figure 7. Relation of structure and inhibition on mushroom tyrosinase

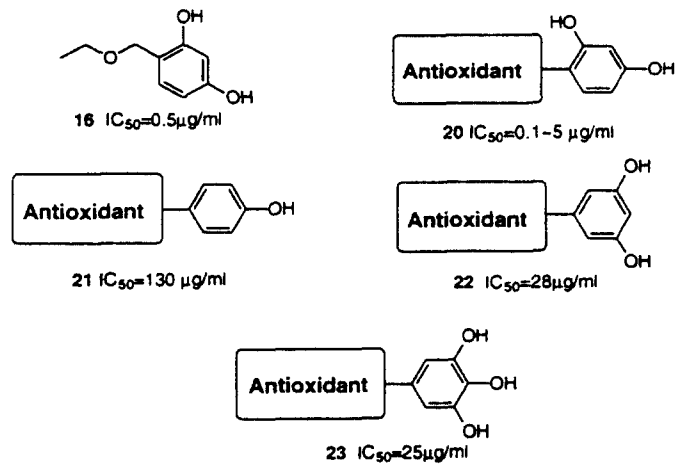


Figure 8. Relation of structure and inhibition on B-16 mouse melanoma cells