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Synthesis and anti-melanogenic activity of hydroxyphenyl benzyl ether analogues

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ABSTRACT

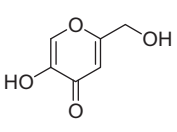
In order to develop potent skin whitening agents, we have synthesized 17 hydroxyphenyl benzyl ether compounds and tested their melanin synthesis inhibitory activity, DPPH free radical scavenging activity and tyrosinase inhibitory activity. Compounds **32**, **35** and **36** possessing 4-hydroxyphenyl benzyl ether structure showed excellent inhibitory capacity with almost 50-fold than arbutin used as a reference in the inhibition test of α-MSH stimulated melanin synthesis in B-16 cells. 4-Hydroxyphenyl benzyl ether compounds also showed good antioxidant activity in the DPPH free radical scavenging test. The tyrosinase function was effectively inhibited by 3,5-dihydroxyphenyl benzyl ether analogues, especially compounds **18**, **22**, and **24**.

1. Introduction

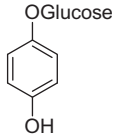
Melanin is one of the most widely distributed pigments and found in bacteria, fungi, plants and animals.[1] Melanin pigments are known to be biosynthesized in a lysosome-like organells, termed melanosomes, of a specialized pigment producing cells called melanocytes, through the process of melanogenesis. Natural melanin is a mixed-type copolymer of black–brown eumelanin and red–yellow sulfur-containing pheomelanin. The type and amount of melanin synthesized by melanocytes and its distribution in the surrounding keratinocytes determines the actual color of the skin.[2] Various dermatological disorders result in the accumulation of excessive levels of epidermal pigmentation. These hyperpigmented lentigines include melasma, age spots or liver spots, and sites of actinic damage.[3,4]

Tyrosinase is a multifunctional copper containing glycoprotein and induces the browning of fruits, vegetables and mammalian melanogenesis. This is also known as polyphenol oxidase. A number of studies have been conducted on targeting tyrosinase to relieve melanogenesis since its discovery.[5,6] Tyrosinase, known to be the most critical and rate limiting enzyme during melanin biosynthesis, exists ubiquitously from microorganisms to plants and animals.[7] It catalyzes first two steps in the melanin biosynthesis, converting L-tyrosine to dopa and then dopaquinone (*o*-quinone type). Due to the essential role of tyrosinase, the approach to inhibit tyrosinase function is most common to achieve skin depigmentation.[8]

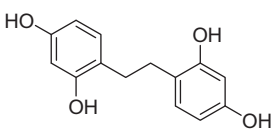
Kojic acid (**1**)[9] and arbutin (**2**)[10] are well-known hypopigmenting agents. Recently, bibenzyl analogues (**3**) are reported to have potent anti-tyrosinase activity with almost 20-fold stronger than kojic acid.[11]



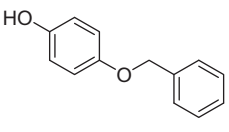
Kojic acid (1)



Arbutin (2)



Bibenzyl analogue (3)



Monobenzone (4)

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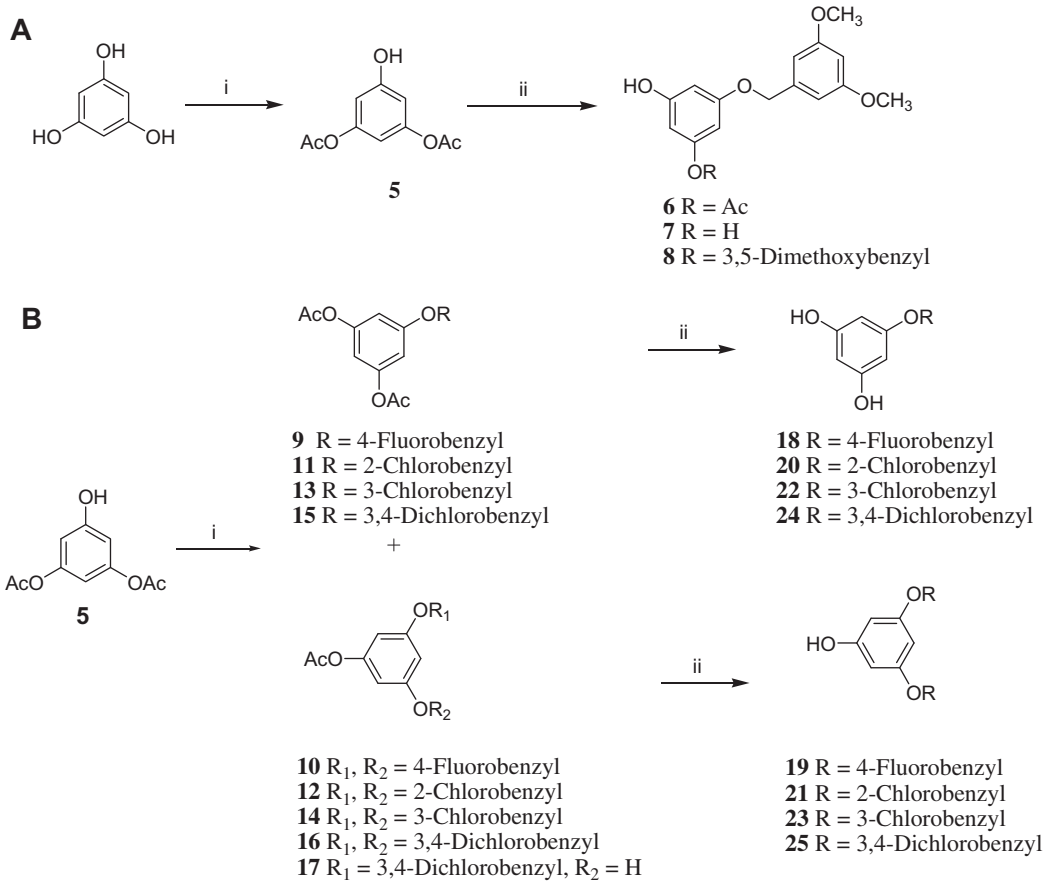


Scheme 1. Synthesis of hydroxyphenyl benzyl ether analogues. Reagents and conditions: (A) (i) Ac_2O, pyridine, reflux; (ii) 3,5-dimethoxybenzyl bromide, K_2CO_3, acetone, reflux. (B) (i) Benzyl halide, K_2CO_3, acetone, reflux; (ii) 1.0 M KOH, ethanol, reflux.

Hydroquinone is known to inhibit tyrosinase enzyme and thus stop conversion of DOPA to melanin.[12] Monobenzone, a monobenzyl ether of hydroquinone (MBEH), has similar biological property to hydroquinone, which is subjected to selective uptake by melanocytes and is metabolized into reactive free radicals, resulting in permanently destroying melanocytes.[13]

As an effort to develop potential skin whitening agents we designed and synthesized 3,5-dihydroxyphenyl or 4-hydroxyphenyl benzyl ether analogues possessing halogens or methoxy groups on the aromatic ring in benzyl part. We chose halogens and methoxy groups as mild electron withdrawing and donating groups to evaluate the effect of electron disposition in the benzyl part. Compounds prepared were tested for the tyrosinase inhibitory and anti-melanogenesis activities. Since anti-oxidation can be a method to reduce the free radical formation and auto-oxidation, we also conducted anti-oxidation activity of compounds using DPPH free radical scavenging test.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of 3,5-dihydroxyphenyl benzyl ether compounds 6–8, and 18–25

Synthetic methods are straightforward. Compound **5** was selected as starting compound for preparation of 3,5-dihydroxyphenyl benzyl ether compounds **6–25**. It was prepared by di-acetylation of phloroglucinol with acetic anhydride in pyridine. Williamson ether synthesis of compound **5** with 3,5-dimethoxybenzyl bromide with K_2CO_3 in acetone provided compound **6** (14%), **7** (33%) and **8** (5%) (Scheme 1A). In the ^{1}H NMR spectrum, compound **6** showed a singlet peak at δ 2.24 corresponding to acetyl protons. Further coupling reaction of compound **5** with corresponding benzyl halide under the same condition in Scheme 1A provided monobenzyl (**9**, **11**, **13**, **15**) and dibenzyl (**10**, **12**, **14**, **16**) ether compounds at the same time. In the reaction of 3,4-dichlorobenzyl chloride reaction, compound **17** was also isolated from product mixture, which lost one acetyl group in the course of reaction. Subsequent alkaline hydrolysis of acetyl protecting group gave desired benzyl ether compounds **18–25** in 62–99% reaction yields (Scheme 1B). All the spectral data were consistent with proposed structures.

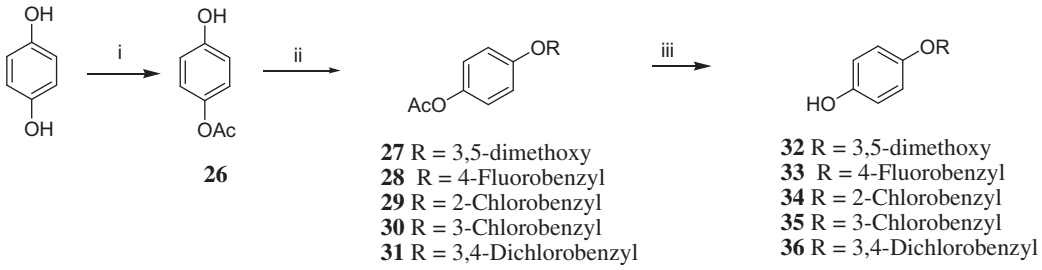
2.1.2. Synthesis of 4-hydroxyphenyl benzyl compounds 32–36

Compound **26** was selected as protected starting compound for preparation 4-hydroxyphenyl benzyl ether compounds **32–36** It was prepared by acetylation of hydroquinone with acetic anhydride in pyridine. Williamson ether reaction of compound **26** with corresponding benzyl halide in the presence of K_2CO_3 in acetone afforded acetyl protected benzyl ether compounds **27–31** in 61–71% reaction yields. Subsequent alkaline hydrolysis of compounds gave desired benzyl ether compounds **32–36** in 86–96% reaction yields (Scheme 2). All the spectral data were consistent with proposed structures.

2.2. Pharmacological evaluation

2.2.1. α-MSH-induced melanin synthesis inhibitory activity in vitro

Melanin synthesis inhibition was studied in mouse melanoma B-16 cells. Most 3,5-dihydroxyphenyl benzyl ether compounds



26

27 R = 3,5-dimethoxy
28 R = 4-Fluorobenzyl
29 R = 2-Chlorobenzyl
30 R = 3-Chlorobenzyl
31 R = 3,4-Dichlorobenzyl

32 R = 3,5-dimethoxy
33 R = 4-Fluorobenzyl
34 R = 2-Chlorobenzyl
35 R = 3-Chlorobenzyl
36 R = 3,4-Dichlorobenzyl

Scheme 2. Synthesis of compounds **32**–**36** from hydroquinone. Reagents and conditions: (i) Ac_2O, pyridine, reflux; (ii) benzyl halide, K_2CO_3, acetone, reflux; (iii) 1.0 M KOH, ethanol, reflux.

were not active to α-melanocyte-stimulating hormone (α-MSH) induced B-16 cells at 10 μM concentration. But 4-hydroxyphenyl benzyl compounds showed better melanin synthesis inhibitory activities than 3,5-dihydroxyphenyl benzyl ether compounds without cell toxicity within tested concentration range. The results are listed in Table 1. Of the compounds, **32**, **35** and **36** were potent for inhibiting melanin synthesis without cell toxicity up to 10 μM. Compounds **33** and **34** showed good melanin synthesis inhibitory activity at 10 μM concentration, but they were toxic at 30 μM concentration. From a view of structural point, the results showed that the substitution of chlorine group (compounds **34**, **35**, and **36**) might enhance the melanin synthesis inhibitory activity than that of fluorine (compound **33**). Location of chlorine also affected the activity of these series of compounds (compound **35** vs. **34**). These observations imply that 4-hydroxyphenyl benzyl ether moiety possessing proper types and location of substituents is very important to inhibit melanin synthetic pathways.

2.2.2. DPPH Free radical scavenging activity

Polyphenol compounds are well known with their antioxidant property. Since our compounds also have phenolic hydroxyl group, we intended to test anti-oxidative activity of compounds whether antimelanogenic activity of the compounds is induced by antioxidant function. The anti-oxidative activity of compounds was tested by measuring their ability to convert DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical to DPPH-H (1,1-diphenyl-2-picryl hydrazine). It was monitored by measuring the change in light absorption at 517 nm using microplate reader. The results are indicated in Table 2. 3,5-Dihydroxyphenyl benzyl ether compounds were weak anti-oxidants. But most 4-hydroxyphenyl benzyl compounds showed moderate antioxidant activity except **36**. These results are parallel with melanin synthesis inhibitory activity stream. Both compounds **32** and **35** were most active among the series of compounds. From the results, it seems that 4-hydroxyphenyl moiety is important for the anti-oxidation activity in these compounds.

Table 1
Inhibitory activity of compounds on α-MSH-induced melanogenesis

Compound	% Inhibition at 10 μM	IC_{50}[a] (μM)
Arbutin	ND[b]	99.0
6	ND	ND
7	19	ND
8	6	ND
17	ND	ND
18	14	ND
19	12	ND
20	11	ND
21	33	ND
22	18	ND
23	21	ND
24	15	ND
25	4	ND
32	>100	1.93
33	42	ND
34	55	ND
35	78	1.83
36	87	1.87

[a] The IC_{50} value was expressed as the mean of 50% inhibitory concentrations of triplicate determinations.
[b] Not determined.

Table 2
DPPH free radical scavenging activity of compounds

Compound	% Inhbition at 100 μM	EC_{50}[a] (μM)
Vitamin C	93.9	26.2 ± 1.5
6	1.7	ND[b]
7	46.8	128.7 ± 25.7
8	27.4	ND
18	46.6	140.0 ± 85.9
19	21.2	ND
20	42.8	165.4 ± 7.7
21	15.6	ND
22	49.0	119.4 ± 14.7
23	22.1	ND
17	8.00	ND
24	37.3	ND
25	17.0	ND
32	54.1	76.3 ± 3.9
33	48.2	101.7 ± 7.2
34	46.5	113.1 ± 3.3
35	54.7	76.8 ± 3.20
36	33.2	ND

[a] Concentration for scavenging of 50% of DPPH free radical was expressed in mean value ± standard deviation obtained in triplicate.
[b] Not determined.

2.2.3. Tyrosinase inhibitory activity

Compounds were evaluated for tyrosinase inhibitory activity using L-tyrosine as a substrate. The percentage inhibition is presented in Table 3. The test results of compounds were contradictory to that of melanin synthesis inhibition. 4-Hydroxyphenyl benzyl compounds were all inactive to tyrosinase function (data not shown). However some of 3,5-dihydroxyphenoxy benzyl compounds **18**, **22** and **24** showed superior tyrosinase inhibitory activities to that of arbutin. The results suggest that 3,5-dihydroxyphenyl moiety with proper species and location of substituents in this series is necessary for inhibiting tyrosinase function. These results were inconsistent with previous observation[14] which implied the importance of *p*-hydroxyphenyl group for the tyrosinase inhibitory process due to its structural resemblance to L-tyrosine, a substrate for tyrosinase during early stage of melanogenesis.

3. Conclusion

We have synthesized 17 hydroxyphenylbenzyl ether compounds and tested their melanin synthesis inhibitory activity, DPPH free radical scavenging activity and tyrosinase inhibitory activity. In the

Table 3
Tyrosinase inhibitory activity of compounds

Compound	% Inhbition at 100 μM	IC_{50} (μM)
Arbutin	ND[a]	1550
6	ND	ND
7	27.5	ND
8	0	ND
17	ND	ND
18	55.5	90.0
19	5.5	ND
20	45.5	ND
21	3.5	ND
22	70.5	54.7
23	1.5	ND
24	66.0	66.4
25	0	ND
32	0	ND
33	1.5	ND
34	0	ND
35	1.0	ND
36	9.5	ND

[a] Not determined.

inhibition test of α-MSH-induced melanin synthesis using B-16 cells, compounds **32**, **35** and **36** possessing 4-hydroxyphenyl benzyl ether structure moiety showed excellent inhibitory capacity with almost 50-fold than arbutin used as a reference. The structure-activity relationship analysis revealed that species and location of substituents are also important factor for the inhibition of melanogenesis. 4-Hydroxyphenyl benzyl ether compounds also showed moderate antioxidant activity in DPPH free radical scavenging test. But 3,5-dihydroxyphenyl benzyl ether analogues were mostly weaker than 4-hydroxyphenyl benzyl ether compounds. The tyrosinase function was effectively inhibited by 3,5-dihydroxyphenyl benzyl ether analogues, especially compounds **18**, **22**, and **24**. These three compounds possess 3,5-dihydroxyphenyl moiety, which may reflect the importance of this core for tyrosinase inhibitory activity in the hydroxyphenyl benzyl ether analogues. The results, however, was discrepant with those of the previous DPPH free radical scavenging test and tyrosinase inhibitory activity of 4-hydroxy-2′-nitrodiphenyl ether analogues.[14] At this point, although we don't have a clear clue for the discrepancy, we suspect that the electron disposition property between benzyl and nitrophenyl groups affect the biological functions of the 4-hydroxyphenyl benzyl ether and 4-hydroxy-2'-nitrodiphenyl ether analogues. Because there was no clear correlation between inhibition of melanongenesis and tyrosinase function of the compounds **32**, **35**, and **36**, anti-melanogenic activity of the compounds might be derived from affecting on the other pathways including antioxidation during the melanin biosynthesis process. Further mechanism study will enhance the possibility of the compounds to be developed as potential candidate for skin whitening agent.

4. Experimental

4.1. General

Most of the chemicals and reagents used were purchased from Aldrich Chemical Co. and some were from other companies like Junsei, Acros Organics, Tokyo Chemicals. Melting points were measured in open capillaries with Barnstead Electrothermal melting point apparatus, Manual MEL-TEMP (Model No: 1202D) without correction. Chromatographic separations were monitored by thin-layer chromatography (TLC) using commercially available pre-coated Merck Kieselgel 60 F_{254} plate (0.25 mm) and detected by visualizing under UV at 254 and 365 nm. Silica gel column chromatography (SGC) was carried out with Merck Kieselgel 60 (0.040–0.063 mm). All solvents used for chromatography were directly used without distillation. NMR spectra were recorded on Varian AS 400 (^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz) with tetramethylsilane (TMS) as an internal standard. Chemical shift (δ) values are expressed in ppm and coupling constant (J) values in hertz (Hz). GC mass data were recorded using Agilent-5975C MSD (USA) and Shimadzu GCMS-QP2010 (Japan).

4.2. General procedure 1

To the solution of acetoxyphenol in acetone were added sequentially potassium carbonate and benzyl halide under nitrogen at rt. The mixture was allowed to reflux overnight. After cooling to rt, the mixture was filtered and washed with acetone. The filtrate was concentrated under reduced pressure and purified of by SGC to afford acetoxyphenylbenzyl ether compound.

4.3. General procedure 2

To the acetylated compound were added 1.0 M KOH (aq.) and ethanol in 1:1 (v/v) ratio. The reaction mixture was refluxed (1 h). After cooling to rt, the mixture was neutralized with 1.0 M HCl solution. Solid formed was collected by filtration, washed with water and dried to give a desired product. In some cases, where there was no precipitation, reaction mixture was extracted with EtOAc and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure. Wherever necessary, it was further purified by SGC to yield the hydroxyphenyl benzyl ether compound.

4.4. 3,5-Diacetoxy phenol (5)

To a solution of phloroglucinol (4.00 g, 31.72 mmol) in pyridine (50 mL) was added acetic anhydride (8.09 g, 79.30 mmol) under stirring. The reaction mixture was refluxed overnight (120–130 °C) and then pyridine was removed under reduced pressure. The residue was purified by SGC (EtOAc/*n*-hexane = 1:5) to afford compound **5** (4.95 g, 74.5%) as a white solid. R_f = 0.56 (EtOAc/*n*-hexane = 1:1); mp 110–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 6H), 6.44 (d, 2H, J = 2.0 Hz), 6.45 (t, 1H, J = 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.4, 107.2, 107.4, 151.7, 157.4, 169.8 ppm.

4.5. 5-(3,5-Dimethoxybenzyloxy)benzene-1,3-diol (6), 3-(3,5-dimethoxybenzyloxy)-5-hydroxyphenyl acetate (7) and 3,5-bis(3,5-dimethoxybenzyloxy)phenol (8)

Using the general procedure 1, a reaction mixture of compound **5** (1.07 g, 5.06 mmol), 3,5-dimethoxy benzyl bromide (1.17 g, 5.06 mmol) and K_2CO_3 (1.40 g, 10.14 mmol) in acetone (25 mL) was refluxed (15 h). Purification by SGC (EtOAc/*n*-hexane = 1:4) provided compound **6** (240 mg, 14.2%) as a light brown viscous liquid, compound **7** (462 mg, 33.1%) and compound **8** (98 mg, 4.5%) as light brown viscous liquid. Compound **6**: R_f = 0.56 (EtOAc/*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.24 (s, 3H), 3.75 (s, 6H), 4.86 (s, 2H), 6.16 (t, 1H, J = 2.0 Hz), 6.25 (t, 1H, J = 2.0 Hz), 6.27 (t, 1H, J = 2.0 Hz), 6.39 (t, 1H, J = 2.0), 6.52 (d, 2H, J = 2.0, Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.3, 55.5, 70.2, 100.3, 100.9, 102.4, 105.5, 139.1, 152.1, 157.7, 160.4, 161.1, 170.2 ppm; EI-MS (*m/z*) 318.1 $[M]^+$. Compound **7**: R_f = 0.39 (EtOAc/*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (s, 6H), 4.86 (s, 2H), 5.94 (t, 1H, J = 2.0 Hz), 6.04 (d, 2H, J = 2.4 Hz), 6.40 (t, 1H, J = 2.0 Hz), 6.54 (d, 2H, J = 2.0, Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 55.6, 70.2, 95.6, 96.3, 100.1, 105.6, 139.4, 157.6, 160.9, 161.1 ppm; EI-MS (*m/z*) 276.0 $[M]^+$. Compound **8**: R_f = 0.22 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (s, 12H), 4.90 (s, 4H), 5.86 (brs, 1H), 6.10 (s, 2H), 6.22 (s, 1H), 6.42 (s, 2H), 6.57 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 55.5,

70.1, 95.0, 95.6, 100.1, 105.5, 139.3, 157.6, 160.8, 161.1 ppm; EI-MS (*m/z*) 426.1 $[M]^+$.

4.6. 5-(4-Fluorobenzyloxy)-1,3-phenylene diacetate (9) and 3,5-bis(4-fluorobenzyloxy)phenyl acetate (10)

Using the general procedure 1, a reaction mixture of compound **5** (0.40 g, 1.90 mmol), 4-fluorobenzyl bromide (0.36 g, 1.90 mmol) and K_2CO_3 (5.30 g, 3.80 mmol) in acetone (15 mL) was refluxed (15 h). Purification by SGC (EtOAc/*n*-hexane = 1:7) yielded compound **9** (369 mg, 26.2%) as a cream colored solid and compound **10** (191 mg, 2.6%) as a white solid. Compound **9**: R_f = 0.45 (EtOAc/*n*-hexane = 1:3); mp 112–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 6H), 4.94 (s, 2H), 6.55 (t, 1H, *J* = 1.6 Hz), 6.61 (d, 2H, *J* = 1.6 Hz), 7.05 (dd, 2H, *J* = 7.2, 8.4 Hz), 7.35 (dd, 2H, *J* = 5.6, 8.0, Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.2, 69.9, 106.3, 108.3, 115.6, 115.8, 129.6, 132.1, 151.8, 159.8, 161.5, 164.0, 169.1 ppm; EI-MS (*m/z*) 318.1 $[M]^+$. Compound **10**: R_f = 0.50 (EtOAc/*n*-hexane = 1:3); mp 132–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H), 4.97 (s, 4H), 6.37 (d, 2H, *J* = 2.0 Hz), 6.47 (t, 1H, *J* = 1.6 Hz), 7.08 (dd, 4H, *J* = 8.0, 8.0 Hz), 7.38 (dd, 4H, *J* = 6.0, 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.4, 69.8, 100.0, 101.6, 115.7, 115.9, 129.7, 132.4, 152.4, 160.3, 161.6, 164.0, 169.5 ppm; EI-MS (*m/z*) 384.1 $[M]^+$.

4.7. 5-(2-Chlorobenzyloxy)-1,3-phenylene diacetate (11) and 3,5-bis(2-chlorobenzyloxy)phenyl acetate (12)

Using the general procedure 1, a reaction mixture of compound **5** (0.30 g, 1.43 mmol), 4-fluorobenzyl bromide (0.29 g, 1.43 mmol) and K_2CO_3 (0.39 g, 2.85 mmol) in acetone (15 mL) was reluxed (15 h). Purification by SGC (EtOAc/*n*-hexane = 1:7) yielded compound **11** (250 mg, 52.3%) as an off-white solid and compound **12** (277 mg, 67.6%) as light brown liquid. Compound **11**: R_f = 0.47 (EtOAc/*n*-hexane = 1:3); mp 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.27 (s, 6H), 5.12 (s, 2H), 6.57 (t, 1H, *J* = 2.0 Hz), 6.64 (d, 2H, *J* = 1.6 Hz), 7.26–7.32 (m, 2H), 7.40 (dd, 1H, *J* = 2.0, 7.2 Hz), 7.52 (dd, 1H, *J* = 2.8, 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.3, 67.8, 106.4, 108.7, 127.3, 129.1, 129.4, 129.6, 132.8, 134.2, 151.9, 159.8, 169.1 ppm; EI-MS (*m/z*) 334.0 $[M]^+$. Compound **12**: R_f = 0.60 (EtOAc/*n*-hexane = 1:3); mp 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 3H), 5.12 (s, 4H), 6.40 (d, 2H, *J* = 2.0 Hz), 6.52 (t, 1H, *J* = 2.4 Hz), 7.24–7.31 (m, 4H), 7.39 (dd, 2H, *J* = 1.6, 7.2 Hz), 7.52 (dd, 2H, *J* = 2.4, 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.4, 67.6, 100.0, 101.8, 127.2, 129.0, 129.3, 129.6, 132.8, 134.4, 152.4, 160.2, 169.4 ppm; EI-MS (*m/z*) 416.0 $[M]^+$.

4.8. 5-(3-Chlorobenzyloxy)-1,3-phenylene diacetate (13) and 3,5-bis(3-chlorobenzyloxy)phenyl acetate (14)

Using the general procedure 1, a reaction mixture of compound **5** (0.40 g, 1.90 mmol), 3-chlorobenzyl bromide (0.39 g, 1.90 mmol) and K_2CO_3 (0.53 g, 3.80 mmol) in acetone (15 mL) was refluxed (12 h). Purification by SGC (EtOAc/*n*-hexane = 1:6) afforded compound **13** (352 mg, 55.3%) and compound **14** (206 mg, 26.0%) as cream colored solids. Compound **13**: R_f = 0.41 (EtOAc/*n*-hexane = 1:3); mp 66–68 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 6H), 4.96 (s, 2H), 6.56 (t, 1H, *J* = 2.0 Hz), 6.61 (d, 2H, *J* = 2.0 Hz), 7.24–7.30 (m, 3H), 7.39 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3, 69.7, 106.3, 108.5, 125.6, 127.6, 128.5, 130.1, 134.7, 138.4, 151.8, 159.7, 169.0 ppm; EI-MS (*m/z*) 334.0 $[M]^+$. Compound **14**: R_f = 0.48 (EtOAc/*n*-hexane = 1:3); mp 76–78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.27 (s, 3H), 4.95 (s, 4H), 6.36 (d, 2H, *J* = 1.6 Hz), 6.45 (t, 1H, *J* = 2.0 Hz), 7.24–7.30 (m, 6H), 7.39 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 21.3, 69.6, 100.0, 101.8, 125.6, 127.6, 128.4, 130.1, 134.7, 138.6, 152.4, 160.1, 169.4 ppm; EI-MS (*m/z*) 416.0 $[M]^+$.

4.9. 5-(3,4-Dichlorobenzyloxy)-1,3-phenylene diacetate (15), 3,5-bis(3,4-dichlorobenzyloxy)phenyl acetate (16) and 3-(3,4-dichlorobenzyloxy)-5-hydroxyphenyl acetate (17)

Using the general procedure 1, a reaction mixture of compound **5** (0.23 g, 1.10 mmol), 3,4-dichlorobenzyl chloride (0.22 g, 1.10 mmol) and K_2CO_3 (0.30 g, 2.20 mmol) in acetone (15 mL) was refluxed (12 h). Purification by SGC (EtOAc/*n*-hexane = 1:6) afforded compound **15** (70 mg, 17.3%) as a light gray liquid, compound **16** (60 mg, 11.2%) and compound **17** (60 mg, 16.7%) as white solids. Compound **15**: R_f = 0.39 (EtOAc/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.26 (s, 6H), 4.94 (s, 2H), 6.57 (t, 1H, *J* = 2.0 Hz), 6.60 (d, 2H, *J* = 2.0 Hz), 7.21 (d, 1H, *J* = 8.0 Hz), 7.43 (d, 1H, *J* = 8.0 Hz), 7.49 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 21.3, 69.1, 106.3, 108.7, 126.8, 129.4, 130.8, 132.3, 132.9, 136.6, 151.9, 159.5, 169.0 ppm; EI-MS (*m/z*) 368.0 $[M]^+$. Compound **16**: R_f = 0.60 (EtOAc/*n*-hexane = 1:3); mp 166–168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 3H), 4.95 (s, 4H), 6.35 (d, 2H, *J* = 2.0 Hz), 6.42 (t, 1H, *J* = 2.0 Hz), 7.22 (dd, 2H, *J* = 2.0, 8.0 Hz), 7.45 (d, 2H, *J* = 8.0 Hz), 7.50 (d, 2H, *J* = 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.4, 69.0, 100.0, 101.9, 126.7, 129.4, 130.8, 132.4, 133.0, 136.9, 152.5, 160.0, 169.4 ppm; EI-MS (*m/z*) 484.0 $[M]^+$. Compound **17**: R_f = 0.23 (EtOAc/*n*-hexane = 1:3); mp 124–126 °C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD) δ 2.23 (s, 3H), 4.88 (s, 2H), 6.19 (d, 2H, *J* = 1.2 Hz), 6.28 (t, 1H, *J* = 1.6 Hz), 7.18 (dd, 1H, *J* = 1.6, 8.0 Hz), 7.40 (d, 1H, *J* = 8.4, Hz), 7.46 (d, 1H, *J* = 1.6, Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.5, 68.7, 100.3, 102.6, 113.0, 126.7, 129.3, 130.7, 132.1, 132.8, 137.1, 152.2, 158.4, 160.0, 170.0 ppm; EI-MS (*m/z*) 326.0 $[M]^+$.

4.10. 5-(4-Fluorobenzyloxy)benzene-1,3-diol (18)

Using the general procedure 2, compound **9** (140 mg, 0.44 mmol) was hydrolyzed in1.0 M KOH (7 mL) and ethanol (7 mL). After extraction with EtOAc (3 × 30 mL), compound **18** (103 mg, 99.7%) was obtained as a brick red solid. R_f = 0.10 (EtOAc/*n*-hexane = 1:3); mp 80–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD) δ 4.87 (s, 2H), 5.91 (t, 1H, *J* = 1.6 Hz), 5.96 (d, 2H, *J* = 1.2 Hz), 6.99 (dd, 2H, *J* = 8.8, 8.8 Hz), 7.31 (dd, 2H, *J* = 5.6, 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) 69.4, 94.5, 96.0, 115.4, 115.6, 129.6, 132.9, 158.6, 160.7, 161.4, 163.8 ppm; EI-MS (*m/z*) 234.0 $[M]^+$.

4.11. 3,5-Bis(4-fluorobenzyloxy)phenol (19)

Using the general procedure 2, compound **10** (99 mg, 0.25 mmol) was hydrolyzed in1.0 M KOH (3 mL) and ethanol (3 mL). After extraction with EtOAc (2 × 30 mL), compound **19** (87 mg, 98.8%) was obtained as an off-white solid. R_f = 0.33 (EtOAc/*n*-hexane = 1:3); mp 82–84 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (s, 4H), 5.34 (s, 1H), 6.10 (d, 2H, *J* = 1.6 Hz), 6.20 (t, 1H, *J* = 1.6 Hz), 7.07 (dd, 4H, *J* = 7.6, 8.8 Hz), 7.37 (dd, 4H, *J* = 5.6, 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 69.6, 95.0, 95.7, 115.6, 115.8, 129.6, 132.7, 157.7, 160.8, 161.5, 163.9 ppm; EI-MS (*m/z*) 342.1 $[M]^+$.

4.12. 5-(2-Chlorobenzyloxy)benzene-1,3-diol (20)

Using the general procedure 2, compound **11** (146 mg, 0.43 mmol) was hydrolyzed in 1.0 M KOH (6 mL) and ethanol (6 mL). After extraction with EtOAc (3x30 mL), compound **20** (103 mg, 94.3%) was obtained as a brick red solid. R_f = 0.10 (EtOAc/*n*-hexane = 1:3); mp 130–132 °C; ^{1}H NMR (400 MHz,

$CDCl_3/CD_3OD$) δ 5.08 (s, 2H), 5.98 (t, 1H, J = 0.8 Hz), 6.05 (d, 2H, J = 1.2 Hz), 7.22–7.29 (m, 2H), 7.37 (dd, 1H, J = 1.2, 7.2 Hz), 7.51 (dd, 1H, J = 1.2, 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) 67.2, 94.4, 96.0, 127.0, 128.9, 129.0, 129.3, 132.6, 134.8, 158.5, 160.6 ppm; EI-MS (*m/z*) 250.0 $[M]^+$.

4.13. 3,5-Bis(2-chlorobenzyloxy)phenol (21)

Using the general procedure 2, compound **12** (85 mg, 0.20 mmol) was hydrolyzed in 1.0 M KOH (3 mL) and ethanol (3 mL). Solid formed after neutralization with 1.0 M HCl was collected by filtration and dried to obtain compound **21** (67 mg, 88.0%) as an off-white solid. R_f = 0.43 (EtOAc/*n*-hexane = 1:3); mp 92–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.92 (s, 1H), 5.11 (s, 4H), 6.13 (s, 2H), 6.26 (s, 1H), 7.25–7.29 (m, 4H), 7.38 (d, 2H, J = 7.6 Hz), 7.52 (d, 2H, J = 6.8 Hz, H-3′, 3″); ^{13}C NMR (100 MHz, $CDCl_3$) 67.4, 95.2, 95.7, 127.0, 129.0, 129.2, 129.6, 132.8, 134.7, 157.6, 160.8 ppm; EI-MS (*m/z*) 374.0 $[M]^+$.

4.14. 5-(3-Chlorobenzyloxy)benzene-1,3-diol (22)

Using the general procedure 2, compound **13** (143 mg, 0.43 mmol) was hydrolyzed in 1.0 M KOH (7 mL) and ethanol (7 mL). Solid formed after neutralization with 1.0 M HCl was collected by filtration and dried to obtain compound **22** (71 mg, 66.3%) as a cream colored solid. R_f = 0.14 (EtOAc/*n*-hexane = 1:3); mp 130–132 °C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 4.93 (s, 2H), 5.97 (s, 1H), 6.03 (s, 2H), 7.27–7.32 (m, 3H), 7.39 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) 69.3, 94.8, 96.3, 125.6, 127.6, 128.2, 130.0, 134.6, 139.2, 158.3, 160.7 ppm; EI-MS (*m/z*) 250.0 $[M]^+$.

4.15. 3,5-Bis-(3-chlorobenzyloxy)phenol (23)

Using the general procedure 2, compound **14** (95 mg, 0.23 mmol) was hydrolyzed in 1.0 M KOH (2 mL) and ethanol (2 mL). Solid formed after neutralization with 1.0 M HCl was collected by filtration and dried to yield compound **23** (72 mg, 84.6%) as an off-white solid. R_f = 0.34 (EtOAc/*n*-hexane = 1:3); mp 80–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.96 (s, 4H), 4.99 (s, 1H), 6.09 (s, 2H), 6.18 (s, 1H), 7.25–7.29 (m, 4H), 7.40 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 69.4, 95.1, 95.7, 125.6, 127.6, 128.4, 130.1, 134.7, 139.0, 157.6, 160.7 ppm; EI-MS (*m/z*) 374.0 $[M]^+$.

4.16. 5-(3,4-Dichlorobenzyloxy)benzene-1,3-diol (24)

Using the general procedure 2, compound **15** (55 mg, 0.15 mmol) was hydrolyzed in 1.0 M KOH (4 mL) and ethanol (4 mL). After extraction with EtOAc (2x20 mL), compound **24** (39 mg, 91.8%) was obtained as a cream colored solid. R_f = 0.10 (EtOAc/*n*-hexane = 1:3); mp 146–148 °C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 4.89 (s, 2H), 5.93 (t, 1H, J = 2.0 Hz), 5.96 (d, 2H, J = 2.0 Hz), 7.19 (d, 1H, J = 8.4 Hz) 7.39 (d, 1H, J = 8.0 Hz), 7.47 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) 68.6, 94.5, 96.2, 126.7, 129.3, 130.6, 131.9, 132.7, 137.6, 158.6, 160.5 ppm; EI-MS (*m/z*) 284.0 $[M]^+$.

4.17. 3,5-Bis-(3,4-dichlorobenzyloxy)phenol (25)

Using the general procedure 2, compound **16** (40 mg, 0.08 mmol) was hydrolyzed in 1.0 M KOH (2 mL) and ethanol (2 mL). Solid formed after neutralization with 1.0 M HCl was collected by filtration and dried to obtain compound **25** (24 mg, 66.0%) as an off-white solid. R_f = 0.35 (EtOAc/*n*-hexane = 1:3); mp 140–142 °C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 4.92 (s, 4H), 6.07 (s, 2H), 6.08 (s,1H), 7.21 (dd, 2H, J = 1.6, 8.0 Hz), 7.42 (d, 2H, J = 8.0 Hz), 7.49 (d, 2H, J = 1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) 68.7, 94.2, 95.8, 126.7, 129.4, 130.7, 132.1, 132.9, 137.3, 158.8, 160.4 ppm; EI-MS (*m/z*) 443.9 $[M]^+$.

4.18. 4-Acetoxy phenol (26)

After dissolving hydroquinone (4.00 g, 36.33 mmol) in pyridine (50 mL), was added drop wise acetic anhydride (5.56 g, 54.5 mmol) under stirring. Reaction mixture was allowed to reflux (120–130 °C) overnight. Pyridine was removed under reduced pressure before purification by SGC (EtOAc/*n*-hexane = 1:4) to yield compound **26** (3.45 g, 62.4%) as a white solid. R_f = 0.53 (EtOAc/*n*-hexane = 1:3); mp 146–148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.27 (s, 3H), 6.74 (dd, 2H, J = 2.8, 8.8 Hz), 6.89 (dd, 2H, J = 2.8, 8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.3, 116.3, 122.5, 144.0, 154.0, 170.9 ppm.

4.19. 4-(3,5-Dimethoxybenzyloxy)phenyl acetate (27)

Using the general procedure 1, a reaction mixture of compound **26** (0.41 g, 2.7 mmol), 3,5-dimethoxy benzyl bromide (0.63 g, 2.7 mmol) and K_2CO_3 (0.75 g, 5.40 mmol) in acetone (15 mL) was refluxed (20 h). Purification by SGC (EtOAc/*n*-hexane = 1:4) was done to afford compound **27** (494 mg, 60.5%) as a cream colored semi-solid. R_f = 0.42 (EtOAc/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H), 3.77 (s, 6H), 4.96 (s, 2H), 6.41 (t, 1H, J = 2.4 Hz), 6.57 (d, 2H, J = 2.4 Hz), 6.94 (d, 2H, J = 8.0 Hz), 6.54 (d, 2H, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.1, 55.4, 70.4, 100.0, 105.3, 115.5, 122.4, 139.3, 144.5, 156.5, 161.1, 169.9 ppm; EI-MS (*m/z*) 302.1 $[M]^+$.

4.20. 4-(4-Fluorobenzyloxy)phenyl acetate (28)

Using the general procedure 1, a reaction mixture of compound **26** (0.29 g, 1.94 mmol), 4-fluorobenzyl bromide (0.36 g, 1.94 mmol) and K_2CO_3 (0.54 g, 3.88 mmol) in acetone (15 mL) was refluxed (15 h). Purification by SGC (EtOAc/*n*-hexane = 1:5) afforded compound **28** (357 mg, 70.7%) as an off-white solid. R_f = 0.52 (EtOAc/*n*-hexane = 1:3); mp 102–104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H), 5.02 (s, 2H), 6.95 (dd, 2H, J = 1.6, 8.8 Hz), 7.01 (dd, 2H, J = 1.2, 9.2 Hz), 7.08 (dd, 2H, J = 7.2, 8.0 Hz), 7.40 (dd, 2H, J = 5.6, 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.3, 70.0, 115.6, 115.8, 122.6, 129.6, 132.7, 144.7, 156.5, 161.5, 170.1 ppm; EI-MS (*m/z*) 260.1 $[M]^+$.

4.21. 4-(2-Chlorobenzyloxy)phenyl acetate (29)

Using the general procedure 1, a reaction mixture of compound **26** (0.23 g, 1.48 mmol), 2-chlorobenzyl bromide (0.30 g, 1.48 mmol) and K_2CO_3 (0.40 g, 2.96 mmol) in acetone (15 mL) refluxed (15 h). Purification by SGC (EtOAc/*n*-hexane = 1:6) afforded compound **29** (277 mg, 67.6%) as a light brown liquid. R_f = 0.58 (EtOAc/*n*-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.26 (s, 3H), 5.14 (s, 2H), 6.96 (dd, 2H, J = 2.4, 9.2 Hz), 7.01 (dd, 2H, J = 2.4, 9.2 Hz), 7.24–7.28 (m, 2H), 7.38 (dd, 1H, J = 2.0, 7.2 Hz), 7.53 (dd, 1H, J = 2.0, 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 21.3, 67.7, 115.7, 122.6, 127.2, 129.0, 129.2, 129.6, 132.8, 134.8, 144.8, 156.4, 170.0 ppm; EI-MS (*m/z*) 276.0 $[M]^+$.

4.22. 4-(3-Chlorobenzyloxy)phenyl acetate (30)

Using the general procedure 1, a reaction mixture of compound **26** (0.34 g, 2.23 mmol), 3-chlorobenzyl bromide (0.46 g, 2.23 mmol) and K_2CO_3 (0.62 g, 4.46 mmol) in acetone (15 mL) was refluxed (15 h). Purification by SGC (EtOAc/*n*-hexane = 1:5) afforded compound **30** (470 mg, 76.0%) as a white crystalline solid. R_f = 0.51 (EtOAc/*n*-hexane = 1:3); mp 94–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H,), 5.02 (s, 2H), 6.96 (dd, 2H, J = 2.0, 8.8 Hz), 7.02 (dd, 2H,

J = 2.0, 8.8 Hz), 7.27–7.31 (m, 3H, H-4'), 7.43 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 21.3, 69.8, 115.7, 122.7, 125.6, 127.6, 128.4, 130.1, 134.7, 139.1, 144.8, 156.4, 170.1 ppm; EI-MS (m/z) 276.0 $[M]^+$.

4.23. 4-(3,4-Dichlorobenzyloxy)phenyl acetate (31)

Using the general procedure 1, a reaction mixture of compound **26** (0.39 g, 2.56 mmol), 3,4-dichlorobenzyl chloride (0.50 g, 2.56 mmol) and K_2CO_3 (0.70 g, 5.12 mmol) in acetone (15 mL) was refluxed (15 h). Purification by SGC (EtOAc/n-hexane = 1:6) afforded compound **31** (515 mg, 64.7%) as a white crystalline solid. R_f = 0.57 (EtOAc/n-hexane = 1:3); mp 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 3H), 4.98 (s, 2H), 6.92 (dd, 2H, J = 1.2, 9.2 Hz), 7.01 (dd, 2H, J = 1.2, 8.8 Hz), 7.24 (dd, 1H, J = 1.2, 8.4 Hz), 7.45 (d, 1H, J = 8.0 Hz), 7.52 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 21.3, 69.2, 115.7, 122.7, 12637, 129.4, 130.8, 132.2, 133.0, 137.3, 144.9, 156.2, 170.0 ppm; EI-MS (m/z) 310.0 $[M]^+$.

4.24. 4-(3,4-Dimethoxybenzyloxy)phenol (32)[15]

Using the general procedure 2, compound **27** (190 mg, 0.63 mmol) was hydrolyzed with 1.0 M KOH (3 mL) and ethanol (3 mL). After extraction with EtOAc (3x30 mL), compound **32** (141 mg, 86.3%) was obtained as a dark brown liquid. R_f = 0.23 (EtOAc/n-hexane = 1:3); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD) δ 3.75 (s, 6H), 4.89 (s, 2H), 6.38 (t, 1H, J = 2.4 Hz), 6.55 (d, 2H, J = 2.4 Hz), 6.72 (dd, 2H, J = 2.4, 9.2 Hz), 6.80 (dd, 2H, J = 2.4, 9.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) 55.3, 70.8, 99.86, 105.3, 116.0, 116.1, 139.9, 150.7, 152.3, 160.9 ppm; EI-MS (m/z) 260.1 $[M]^+$.

4.25. 4-(4-Fluorobenzyloxy)phenol (33)[16]

Using the general procedure 2, compound **28** (205 mg, 0.78 mmol) was hydrolyzed with 1.0 M KOH (3 mL) and ethanol (3 mL). Solid formed after neutralization with 1.0 M HCl was collected by filtration to obtain compound **33** (155 mg, 90.3%) as an off-white solid. R_f = 0.34 (EtOAc/n-hexane = 1:3); mp 124–126 °C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD) δ 4.9 (s, 2H), 6.71 (dd, 2H, J = 2.0, 8.8 Hz), 6.78 (dd, 2H, J = 1.2, 8.8 Hz), 7.01 (dd, 2H, J = 7.6, 7.6 Hz), 7.40 (dd, 2H, J = 5.6, 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) 70.4, 115.4, 155.6, 116.0, 116.2, 129.5, 133.2, 151.0, 152.2, 161.3, 163.8 ppm; EI-MS (m/z) 218.1 $[M]^+$.

4.26. 4-(2-Chlorobenzyloxy)phenol (34)

Using the general procedure 2, compound **29** (184 mg, 0.66 mmol) was hydrolyzed with 1.0 M KOH (3 mL) and ethanol (3 mL). After extraction with EtOAc (3 × 30 mL), compound **34**(143 mg, 91.6%) was obtained as a gray crystalline solid. R_f = 0.33 (EtOAc/n-hexane = 1:3); mp 64–66 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.79 (brs, 1H), 5.10(s, 2H), 6.77 (dd, 2H, J = 2.4, 9.2 Hz), 6.88 (dd, 2H, J = 2.4, 9.2 Hz), 7.22–7.30 (m, 2H), 7.39 (dd, 1H, J = 2.0, 7.6 Hz), 7.54 (dd, 1H, J = 2.0, 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 68.2, 116.2, 116.3, 127.1, 129.0, 129.1, 129.6, 132.8, 135.2, 150.1, 153.0 ppm; EI-MS (m/z) 234.0 $[M]^+$.

4.27. 4-(3-Chlorobenzyloxy)phenol (35)[17]

Using the general procedure 2, compound **30** (310 mg, 1.12 mmol) was hydrolyzed in 1.0 M KOH (4 mL) and ethanol (4 mL). Solid formed after neutralization with 1.0 M HCl was collected by filtration and dried to obtain the compound **35** (242 mg, 92.1%) as an off-white solid. R_f = 0.41 (EtOAc/n-hexane = 1:3); mp 112–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (br s, 1H), 4.99 (s, 2H), 6.77 (dd, 2H, J = 2.4, 9.2 Hz), 7.02 (dd, 2H, J = 2.4, 9.2 Hz), 7.27–7.30 (m, 3H), 7.43 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 70.2, 116.2, 116.3, 125.6, 127.6, 128.2, 130.0, 134.7, 139.6, 150.1, 152.9 ppm; EI-MS (m/z) 234.0 $[M]^+$.

4.28. 4-(3,4-Dichlorobenzyloxy)phenol (36)

Using the general procedure 2, compound **31** (150 mg, 0.48 mmol) was hydrolyzed in 1.0 M KOH (3 mL) and ethanol (3 mL). Solid formed after neutralization with 1.0 M HCl was collected by filtration and dried to yield compound **36** (124 mg, 95.6%) as a gray solid. R_f = 0.33 (EtOAc/n-hexane = 1:3); mp 100–102 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.70 (brs, 1H), 4.94 (s, 2H), 6.76 (d, 2H, J = 8.8 Hz), 6.82 (d, 2H, J = 8.4 Hz), 7.23 (d, 1H, J = 8.4 Hz), 7.43 (d, 1H, J = 8.0 Hz), 7.51 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 69.6, 116.3, 116.4, 126.7, 129.5, 130.7, 132.1, 132.9, 137.8, 150.2, 152.7 ppm; EI-MS (m/z) 268.0 $[M]^+$.

4.29. DPPH free radical scavenging assay

DPPH free radical scavenging assay was carried out as described by Bondet et al.[18] with slight modification. All compounds used were dissolved in ethanol (Sigma, USA). In order to determine the capacity to scavenge the stable free radical, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma, USA), 50 μL of each compound solution was added to each well in 96-well plate containing 50 μL of 200 μM DPPH stock solution. After incubation for 30 min. with shaking at room temperature, the absorbance was recorded at 517 nm using a microplate reader (VERSAmax, Molecular Devices). The percent DPPH scavenging activity was expressed as follow:

$$\text{DPPH scavenging activity } (\%) = [(A_c - A_b) - (A_s - A_b)]/(A_c - A_b) \times 100$$

where A_c was the absorbance of the DPPH solution without compound, A_s was that of the DPPH solution with compound, and A_b was that of the blank. Experiments for each compound were performed in triplicate and vitamin C was used as a positive control. For compounds showing more than 40% of DPPH scavenging activity from screening test with 100 μM concentration in final, The EC_{50} values for DPPH radical scavenging activities were calculated using the data graphing software, TableCurve 2D (Systat Software Inc.) with the concentration range from 20 to 200 μM. The EC_{50} value was defined as a concentration that could scavenge 50% DPPH free radical.

4.30. Tyrosinase inhibition assay in vitro

B16 cells were stimulated with 10 nM α-MSH alone for 48 h and disrupted by sonication on ice. After centrifugation, supernatants were dialyzed against 50 mM sodium phosphate buffer (pH 6.8) and then used as the tyrosinase sources. Enzyme activity was determined by L-tyrosine oxidation velocity as described previously.[19] Briefly, sample was incubated with cell-free tyrosinase sources and then reacted with 5 mM L-tyrosine as a substrate. The initial velocity of dopachrome formation was determined by the increasing rate of absorbance values at 475 nm. One unit of the enzyme activity was defined as the conversion of 1 nmol L-tyrosine to dopachrome per min.

4.32. α-MSH-induced melaninsynthesis inhibition assay in vitro

Melanin production inhibition assay was carried out by the method of Hosoi et al.[20] with slight modification. The mouse melanoma B-16 cells were seeded at a density 2.5 × 10^3 cells per well in Dulbecco's modified eagle medium (DMEM) supplemented with 10% Fetal bovine serum (FBS) using a 96-well culture plates. The

cells were grown in a 5% CO_2–95% air atmosphere at 37 °C for 1 day. Then, α-MSH, together with various sample concentrations, was added to the wells. The cells were incubated for 3 days at 37 °C and then equilibrated to ambient temperature and atmosphere. The absorbance of each well was measured in a reader at 405 nm. The absorbance values were compared with a standard curve obtained with synthetic melanin (Sigma–Aldrich) which was dissolved in 0.85 N KOH and diluted in DMEM-10% FBS culture medium, and distributed in 96-well plates to measure the optical density (OD) at 405 nm. The inhibitory activity was calculated from the change from the initial rate of OD.

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