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Graphical Abstract

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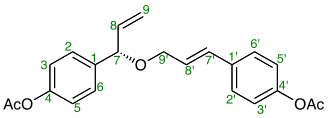
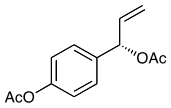
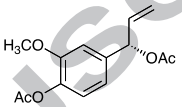


galanganol D diacetate (**1**)



1'*S*-1'-acetoxychavicol acetate (**2**)



1'*S*-1'-acetoxyeugenol acetate (**3**)

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ABSTRACT

An aqueous acetone extract from the fruit of *Alpinia galanga* (Zingiberaceae) demonstrated inhibitory effects on melanogenesis in theophylline-stimulated murine B16 melanoma 4A5 cells (IC_{50} = 7.3 μg/mL). Through bioassay-guided separation of the extract, a new 7-*O*-9'-linked neolignan, named galanganol D diacetate (**1**), was isolated along with 16 known compounds including 14 phenylpropanoids (**2**–**15**). The structure of **1**, including its absolute stereochemistry in the C-7 position, was elucidated by means of extensive NMR analysis and total synthesis. Among the isolates, **1** (IC_{50} = 2.5 μM), 1'*S*-1'-acetoxychavicol acetate (**2**, 5.0 μM), and 1'*S*-1'-acetoxyeugenol acetate (**3**, 5.6 μM) exhibited a relatively potent inhibitory effect without notable cytotoxicity at effective concentrations. The following structural requirements were suggested to enhance the inhibitory activity of phenylpropanoids on melanogenesis: (i) compounds with 4-acetoxy group exhibit higher activity than those with 4-hydroxy group; (ii) 3-methoxy group dose not affect the activity; (iii) acetylation of the 1'-hydroxy moiety enhances the activity; and (iv) phenylpropanoid dimers with the 7-*O*-9'-linked neolignan skeleton exhibited higher activity than those with the corresponding monomer. Their respective enantiomers [**1'** (IC_{50} = 1.9 μM) and **2'** (4.5 μM)] and racemic mixtures [(±)-**1** (2.2 μM) and (±)-**2** (4.4 μM)] were found to exhibit melanogenesis inhibitory activities equivalent to those of the naturally occurring optical active compounds (**1** and **2**). Furthermore, the active compounds **1**–**3** inhibited tyrosinase, tyrosine-related protein (TRP)-1, and TRP-2 mRNA expressions, which could be the mechanism of melanogenesis inhibitory activity.

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1. Introduction

The Zingiberaceae plant, *Alpinia galanga* Swartz, is widely cultivated in China, India, and Southeast Asian countries such as Thailand, Indonesia, and the Philippines. The fruit and rhizomes of this plant have been extensively used as a spice or ginger substitutes to flavor food. In the traditional medicine, the fruit has also been used for the treatment of stomach ache, dyspepsia, emesis, diarrhea, asthma, osteoarthritis, and rheumatoid arthritis.[1,2] Previous chemical studies on the fruit or seeds of *A. galanga,* revealed the presence of several phenylpropanoids,[2–4] sesquiterpenes,[2,5] and diterpenes.[2,6,7] In addition, the biological activities such as antitumor[2,4,7] and antifungal[7] activities of the extract and/or constituents have been reported. During the course of our characterization studies on this plant material, we have reported gastroprotective,[8] anti-allergic,[9] and anti-inflammatory constituents[10–12] from the rhizomes of *A. galanga*. Continuing studies on the bioactive properties of this plant material revealed that the aqueous acetone extract from the fruit of *A. galanga* had an inhibitory effect against melanogenesis in theophylline-stimulated murine B16 melanoma 4A5 cells. Through bioassay-guided separation, a new 7-*O*-9'-linked neolignan, named galanganol D diacetate (**1**), was isolated along with 16 known compounds including 14 phenylpropanoids (**2**–**15**). This study details the isolation, structure determination, and total synthesis of the new neolignan (**1**), as well as the melanogenesis inhibitory activities of the isolates.

2. Results and discussion

2.1. Effects of 80% aqueous acetone extract from *A. galanga* fruit and its fractions on theophylline-stimulated melanogenesis in murine B16 melanoma 4A5 cells

Melanin is a broad term for a group of natural pigments found in bacteria, fungi, plants, and animals. It is a heterogeneous, polyphenol-like biopolymer with a complex structure, and its color varies from yellow to black through its development. The color of the mammalian skin and hair is determined by several factors, the most important one being the degree and distribution of melanin pigmentation. The role of melanin is to protect the skin from UV damage by absorbing UV light and removing reactive oxygen species.[13,14] However, excess production of melanin due to a prolonged exposure to sunlight causes dermatologic disorders such as melisma, freckles, post-inflammatory melanoderma, and solar lentigines.[13,15–17] Melanin is secreted from melanocytes distributed in the basal layer of the dermis. Melanocytes are known to be stimulated by various factors including UV radiation,[18] α-melanocyte-stimulating hormone (α-MSH),[19] or a phosphodiesterase inhibitor, such as theophylline.[20]

Dried fruit of *A. galanga* was used to obtain an 80% aqueous acetone extract (8.62% from the dried material). The aqueous acetone extract was partitioned using EtOAc–H_2O (1:1, v/v) to yield an EtOAc-soluble fraction (5.09%) and an aqueous phase. The latter was subjected to Diaion HP-20 column chromatography ($H_2O \rightarrow$ MeOH) to yield H_2O- and MeOH-eluted fractions (0.75% and 0.96%, respectively). As shown in Table 1, the aqueous acetone extract was found to inhibit melanogenesis (IC_{50} = 7.3 μg/mL) in theophylline-stimulated murine B16 melanoma 4A5 cells without inducing cytotoxicity at the effective concentration [cell viability in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay: 107.8 ± 4.0% for cell viability at 30 μg/mL]. Through bioassay-guided separation, the EtOAc-soluble and the MeOH-eluted fractions were found to be active for melanogenesis inhibition (IC_{50} = 3.9 and 30.7 μg/mL, respectively), without cytotoxicities [116.3 ± 9.7% and 117.3 ± 0.7%, respectively, for cell viability at 30 μg/mL], whereas the H_2O-eluted fraction showed no notable activity.

Table 1. Inhibitory effects of the 80% aqueous acetone extract and its fractions from the fruit of *Alpinia galanga* on melanogenesis and viability in B16 4A5 cells

	Inhibition (%)					
	0 μg/mL	3 μg/mL	10 μg/mL	30 μg/mL	100 μg/mL	IC_{50} (μg/mL)
80% Aqueous acetone extract	0.0 ± 4.1	31.8 ± 7.0[b]	57.1 ± 5.9[b]	78.0 ± 3.0[b]		7.3
	(100.0 ± 1.6)	(103.6 ± 1.1)	(109.1 ± 3.1)	(107.8 ± 4.0)		
EtOAc-soluble fraction	0.0 ± 8.8	38.0 ± 8.2[b]	59.2 ± 4.2[b]	88.2 ± 5.5[b]		3.9
	(100.0 ± 8.1)	(104.8 ± 11.0)	(102.1 ± 8.4)	(116.3 ± 9.7)		
MeOH-eluted fraction	0.0 ± 4.0	4.8 ± 3.8	19.2 ± 2.2	43.2 ± 3.8[b]	82.4 ± 2.0[b]	30.7
	(100.0 ± 5.6)	(110.2 ± 6.5)	(104.6 ± 4.2)	(117.3 ± 0.7)	(136.8 ± 6.8)	
H_2O-eluted fraction	0.0 ± 9.6	12.5 ± 4.1	0.3 ± 7.0	8.1 ± 11.1	14.3 ± 7.2	>100
	(100.0 ± 5.4)	(105.4 ± 5.4)	(115.1 ± 8.0)	(125.4 ± 8.1)	(141.7 ± 8.2)	

Each value represents mean ± S.E.M. (N = 4).
Significantly different from control, [a]$p < 0.05$, [b]$p < 0.01$.
Values in parenthesis indicate cell viability (%) in MTT assay.

2.2. Chemical constituents from *A. galanga* fruit

The EtOAc-soluble fraction was subjected to normal-phase silica gel and reversed-phase ODS column chromatographies and finally HPLC to obtain galanganol D diacetate (**1**, 0.00292% from the dried fruit) together with 14 phenylpropanoids, 1'*S*-1'-acetoxychavicol acetate[8,12] (**2**, 0.0977%), 1'*S*-1'-acetoxteugenol acetate[8,12] (**3**, 0.119%), 1'*S*-1'-hydroxychavicol acetate[8,12] (**4**, 0.00430%), 1'*S*-1'-hydroxyeugenol acetate[3] (**5**, 0.0675%), 1'*S*-1'-acetoxydihydrochavicol acetate[3] (**6**, 0.00028%), 1-(4-hydroxyphenyl)-1-propanone[21] (**7**, 0.00024%), *trans*-*p*-coumaryl acetate[12] (**8**, 0.00140%), *trans*-*p*-acetoxycinnamoyl alcohol[12] (**9**, 0.00162%), *trans*-*p*-coumaryl alcohol[8,12] (**10**, 0.00168%), *trans*-*p*-coumaryl aldehyde[22,24] (**11**, 0.00026%), *trans*-*p*-coumaryl alcohol γ-*O*-methyl ether[23] (**12**, 0.00131%), *trans*-coniferyl alcohol 4-*O*-acetate[23,25] (**13**, 0.00041%), *trans*-coniferyl alcohol[21] (**14**, 0.00869%), and *trans*-coniferyl aldehyde[21] (**15**, 0.00036%) (Figure 1), as well as 4-hydroxybenzaldehyde[21] (0.00678%) and 4-hydroxy-3-methoxybenzaldehyde[21] (0.00043%).

2.3. Structure of galanganol D diacetate (1)

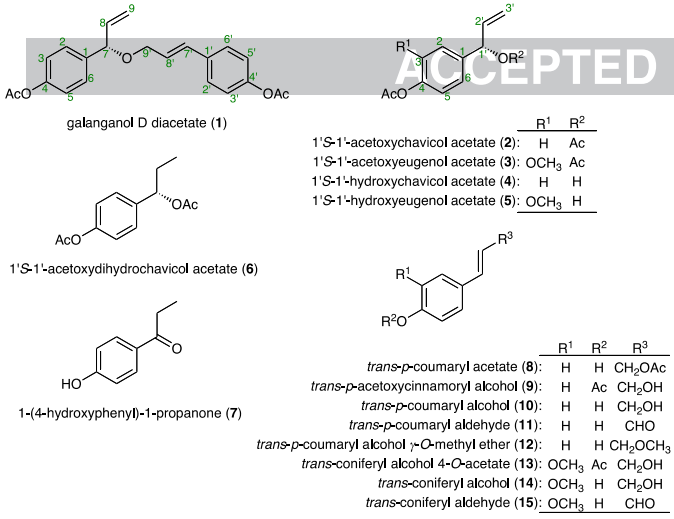


Figure 1. Neolignan and phenylpropanoid constituents (**1**–**15**) from the fruit of *Alpinia galanga*

Galanganol D diacetate (**1**) was obtained as a pale yellow oil with negative optical rotation ($[\alpha]_D^{24}$ –6.2 in $CHCl_3$). The positive-ion ESIMS spectrum of **1** showed a quasimolecular ion peak at *m/z* 389 $[M + Na]^+$, and the molecular formula was determined as $C_{22}H_{22}O_5$ by high-resolution MS measurement. The UV spectrum exhibited absorption maxima at 225 and 271 nm, while the IR spectrum showed absorption bands at 1767, 1651, 1508, and 1460 cm^{-1} ascribable to conjugated olefin function and aromatic ring. The ^1H and ^{13}C NMR spectra of **1** (pyridine-d_5, Table 2), which were assigned with the aid of DEPT, ^1H–^1H COSY, HMQC, and HMBC experiments (Figure 2), showed signals assignable to a methylene bearing an oxygen function [δ 4.14, 4.20 (1H each, both dd, J = 1.6, 5.6 Hz, H_2-9')], mono- and a *trans*-di-substituted olefins {δ [5.22 (1H, dd, J = 1.6, 10.4 Hz), 5.39 (1H, dd, J = 1.6, 16.8 Hz), H_2-9], 6.03 (1H, ddd, J = 6.4, 10.4, 16.8 Hz, H-8), 6.38 (1H, dd, J = 5.6, 16.0 Hz, H-8'), 6.70 (1H, d, J = 16.0 Hz, H-7')}, and two *para*-substituted aromatic rings [δ 7.21, 7.29, 7.47, 7.53 (2H each, all d, J = 8.0 Hz, H_2-2',6', 2,6, 3',5', 3,5)], together with two acetyl groups [δ 2.20, 2.21 (3H each, both s, H_3-OAc)]. As shown in Figure 2, the,

Table 2. ^1H (800 MHz) and ^{13}C NMR (200 MHz) data of galanganol D diacetate (**1**)

	1 (in pyridine-d_5)		**1** (in $CDCl_3$)	
position	***δ_H (J Hz)***	***δ_C***	***δ_H (J Hz)***	***δ_C***
1		139.5		138.5
2,6	7.29 (2H, d, 8.0)	128.4	7.08 (2H, d, 8.0)	127.4
3,5	7.53 (2H, d, 8.0)	122.2	7.38 (2H, d, 8.0)	121.5
4		151.1		150.12[d]
7	4.98 (1H, d, 6.4)	81.6	4.85 (1H, d, 7.2)	81.6
8	6.03 (1H, ddd, 6.4, 10.4, 16.8)	139.5	5.95 (1H, ddd, 7.2, 10.4, 16.8)	138.6
9	5.22 (1H, dd, 1.6, 10.4)	116.2	5.23 (1H, dd, 1.6, 10.4)	116.6
	5.39 (1H, dd, 1.6, 16.8)		5.30 (1H, dd, 1.6, 16.8)	
4-OCOCH$_3$		169.3[a]		169.41[e]
4-OCOC*H*$_3$	2.21 (3H, s)	20.9[b]	2.30 (3H, s)[c]	21.13[f]
1'		135.4		134.6
2',6'	7.21 (2H, d, 8.0)	127.8	7.04 (1H, d, 8.0)	128.0
3',5'	7.47 (2H, d, 8.0)	122.4	7.37 (2H, d, 8.0)	121.6
4'		151.1		150.09[d]
7'	6.70 (1H, d, 16.0)	131.1	6.58 (1H, dd, 1.6, 16.0)	131.3
8'	6.38 (1H, dd, 5.6, 16.0)	127.4	6.25 (1H, dd, 5.6, 16.0)	126.4
9'	4.14 (1H, dd, 1.6, 5.6)	69.1	4.13 (1H, dd, 1.6, 5.6)	68.8
	4.20 (1H, dd, 1.6, 5.6)		4.16 (1H, dd, 1.6, 5.6)	
4'-OCOCH$_3$		169.4[a]		169.40[e]
4'-OCOC*H*$_3$	2.20 (3H, s)	20.9[b]	2.29 (3H, s)[c]	21.11[f]

[a–f]May be interchangeable.

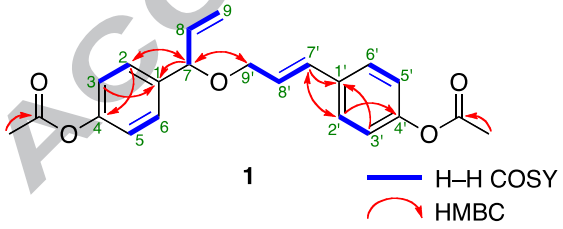
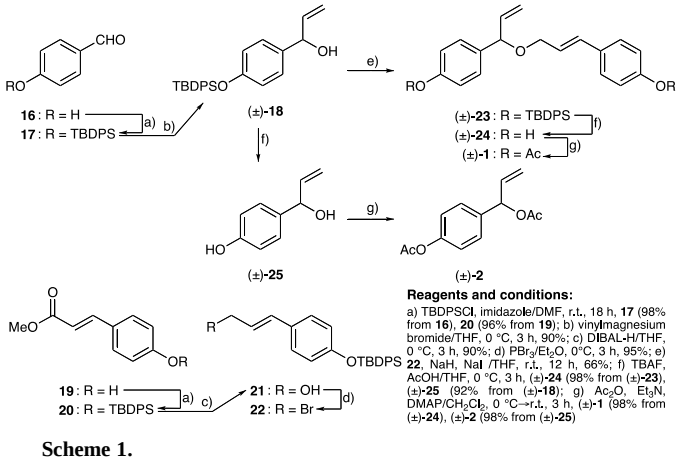


Figure 2. ^1H–^1H COSY and HMBC correlations of **1**

^1H–^1H COSY experiment on **1** indicated the presence of partial structures shown by bold lines. In the HMBC experiment of **1**, long-range correlations were observed between the following proton and carbon pairs: H_2-2,6 and C-4, 7; H_2-3,5 and C-1; H-7 and C-1, 2,6, 9'; H_2-2',6' and C-4', 7'; H_2-3',5' and C-1; H-7' and C-1', 2',6'.

The 7-*O*-9'-linked neolignan skeleton in **1** was also confirmed by a synthetic study executed as depicted in Scheme 1. Protection of a hydroxy group in 4-hydroxybenzaldehyde (**16**) as a *tert*-butyldiphenylsilyl (TBDPS) ether, **17**, followed by the Grignard



Scheme 1.

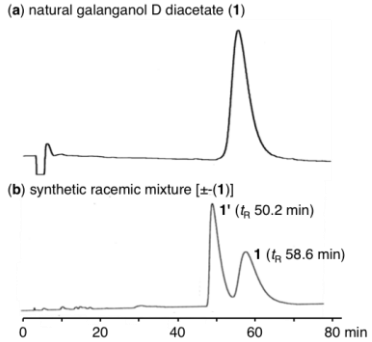
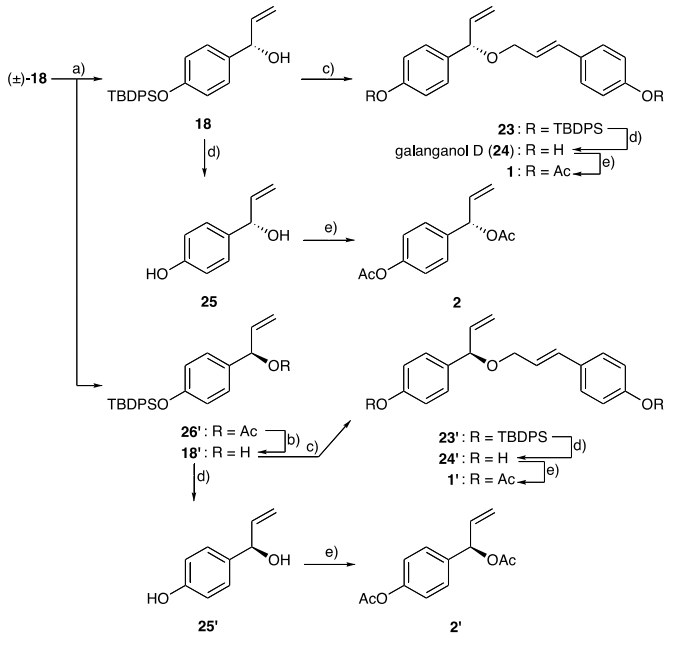


Figure 3. Chiral HPLC chromatograms of natural galanganol D diacetate (**1**) and synthetic racemic mixture [(±)-**1**]

HPLC conditions: column, Ceramospher Chiral RU-1 (4.6 × 250 mm, i.d.); detection, UV (230 nm); mobile phase, MeOH; column temperature, 40 °C; flow rate, 0.9 mL/min.

reaction with vinylmagnesium bromide gave a racemic mixture of a secondary alcohol of (±)-**18** with an 88% yield. However, preparation of the counterpart bromoalkene **22** commenced with *p*-hydroxycoumaric acid methyl ester (**19**), which was readily



Reagents and conditions:

a) lipase PS, BHT/vinyl acetate–THF (1:1, v/v), 65 °C, 24 h, **26'** (46%), **18** (45%); b) lipase PS/0.1 M phosphate buffer (ph 7.0), r.t., 24 h, 78%; c) **22**, NaH, NaI /THF, r.t., 12 h, **23** (78% from **18**), **23'** (75% from **18'**); d) TBAF, AcOH/THF, 0 °C, 3 h,**24** (99% from **23**), **24'** (99% from **23'**), **25** (90% from **18**), **25'** (94% from **28'**); e) Ac_2O, Et_3N, DMAP/CH_2Cl_2, 0 °C→r.t., 3 h, **1** (99% from **24**), **1'** (99% from **24'**), **2** (99% from **25**), **2'** (98% from **25'**)

Scheme 2.

prepared from commercially available *p*-hydroxycoumaric acid. In brief, protection of a hydroxy group in **19** by TBDPS chloride gave the corresponding silyl ether, **20**. Reduction of **20** with diisobutylaluminium hydride (DIBAL-H) in tetrahydrofuran (THF), followed by bromination with phosphorus tribromide (PBr_3) in diethylether (Et_2O), provided bromoalkene **22** with 82% yield, from **19**. Coupling of (±)-**18** and **22** proceeded with sodium iodide (NaI) in the presence of sodium hydride (NaH) in THF to afford a 7-*O*-9'-linked neolignan (±)-**23** with 66% yield. Removal of the TBDPS group with *tert*-butylammonium fluorolide (TBAF) and acetic acid (AcOH) in THF and successive acetylation of a resulting diol (±)-**24** with acetic anhydride (Ac_2O) in the presence of triethylamine (Et_3N) and 4-dimethylaminopyridine (DMAP) in dichloromethane (CH_2Cl_2) furnished a racemic mixture (±)-**1** with 96% yield from (±)-**23**. The synthetic product (±)-**1** was unambiguously identified by comparison of their physical and spectral data with those of the natural product **1** without the inactive optical rotation. As shown in Figure 3, compound (±)-**1** was able to separate by chiral HPLC,[26–28] and each peak was observed at the following retention time (t_R 50.2 and 58.6 min). Of them, the latter peak was unambiguously assigned by comparison of the retention time with that of the natural product **1**. Using this HPLC analytical method, the optical purity of natural product **1** was identified as over 99% ee.

Next, we attempted to synthesize optically active galanganol D diacetate (**1**) and its enantiomer **1'** by enzymatic optical resolution. As outlined in Scheme 2, using the racemic alcohol (±)-**18** as the substrate, a lipase-catalyzed, enantioselective esterification was carried out. Ogino *et al.* reported that highly optically pure 1'*S*-1'-acetoxychavicol acetate (**2**) and its enantiomer **2'** (99.7% ee and 99.1% ee, respectively) were obtained from the corresponding racemic alcohol using lipase PS (Amano Enzyme Inc., Aichi, Japan).[29] As reported, the esterification of (±)-**18** with lipase PS in vinyl acetate–THF (1:1, v/v) under reflux (65 °C) after 24 h gave a mixture of unreacted alcohol **18** (45%) and the 1'-*O*-acetate **26'** (46%). Since a natural product 1'*S*-1'-acetoxychavicol acetate (**2**), $[\alpha]_D^{25}$ –60.0 {lit. $[\alpha]_D^{25}$ –60.1}[29] both in EtOH, was obtained by means of deprotection of the TBDPS group and then by the acetylation from the unreacted alcohol **18**, the absolute stereochemistry of **18** was determined to be the *S* orientation. On the other hand, deacetylation of the 1'-*O*-acetate **26'** with lipase PS in 0.1 M phosphate buffer (pH 7.0) after 24 h gave an enantiomer of the alcohol **18'** with 78% yield. Enantiomer of the natural product, 1'*R*-1'-acetoxychavicol acetate (**2'**), $[\alpha]_D^{25}$ +59.9 {lit. $[\alpha]_D^{25}$ +60.0}[29] both in EtOH, was obtained from **18'**. Therefore, the 1'*R* orientation in **18'** was elucidated. Through a similar procedure mentioned above, each alcohol (**18** and **18'**) was coupled with **22** to give **23** and **23'** (78 and 75%, respectively). For **23** and **23'**, through deprotection of the TBDPS group and then by the acetylation, a desired neolignan **1** (98% yield with 99% ee) and its enantiomer **1'** (98% yield with over 99% ee) was afforded. Thus, the absolute stereochemistry at C-7 in **1** was determined to be *S* orientation. To our knowledge, this is the first article to report on the isolation of 7-*O*-9'-linked neolignan from natural resources.

2.4. Effects of the constituents and related compounds on melanogenesis in theophylline-stimulated B16 melanoma 4A5 cells

In our previous investigation of compounds from natural medicines possessing melanogenesis inhibitory activity, it was reported that dimeric pyrrolidinoindoline-,[30] aporphine-,[31,32] benzylisoquinoline-,[32] and phenanthridine-type[33] alkaloids, phenylethanoid glycosides,[33] and methoxyflavones[34] exhibited significant positive effects against theophylline-stimulated melanogenesis in B16 melanoma 4A5 cells. As a continuing study, the effects of the isolates from the fruit of *A. galanga* and their analogs on melanogenesis inhibitory activity were examined. Among the isolates, galanganol D diacetate (**1**, IC_{50} = 2.5 μM), 1'*S*-1'-acetoxychavicol acetate (**2**, 5.0 μM), 1'*S*-1'-acetoxyeugenol acetate (**3**, 5.6 μM), 1'*S*-1'-acetoxydihydrochavicol acetate (**6**, 10.5 μM), *trans*-*p*-coumaryl aldehyde (**11**, 18.3 μM), *trans*-*p*-coumaryl alcohol γ-*O*-methyl ether (**12**, 19.5 μM), *trans*-coniferyl alcohol 4-*O*-acetate (**13**, 16.9 μM), and *trans*-coniferyl aldehyde (**15**, 15.3 μM), significantly inhibited melanogenesis at a lower concentration than the IC_{50} value of 20 μM (Table 3). In addition, 1'*S*-1'-hydroxychavicol acetate (**4**, 88.5 μM), *trans*-*p*-acetoxycinnamoyl alcohol (**9**, 29.4 μM), *trans*-coniferyl alcohol (**14**, 16.1 μM), and 4-hydroxy-3-methoxybenzaldehyde (49.2 μM), exhibited moderate inhibitory activities. These active constituents were more potent than the positive control, arbutin (IC_{50} = 174 μM), a commercially used tyrosinase inhibitor without notable cytotoxic effects at the effective concentrations.[30–34] Especially, the melanogenesis inhibitory activity of **1**–**3** were found to be more than 25–70-fold that of arbutin (Figure 4). The following structural requirements were suggested to enhance the inhibitory activity of phenylpropanoids on melanogenesis: (i) compounds with 4-acetoxy group exhibit higher activity those with 4-hydroxy group [**9** > *trans*-*p*-coumaryl alcohol (**10**, IC_{50} > 100 μM), **13** > *trans*-coniferyl alcohol (**14**, > 100 μM)]; (ii) 3-methoxy group does not affect the activity (**2** ≒ **3**, **4** ≒ **5**, **9** ≒ **13**, **11** ≒ **15**); (iii) acetylation of the 1'-hydroxy moiety enhances the activity [**2** > **4**, **3** > 1'*S*-1'-hydroxyeugenol acetate (**5**, IC_{50} > 100 μM)], and (iv) phenylpropanoid dimers having the 7-*O*-9'-linked neolignan skeleton exhibited higher activity than those having the corresponding monomer (**1** > **2**). To clarify the relationships on the absolute stereochemistry of the 7-position in the 7-*O*-9'-linked neolignans in **1**, galanganol D (**24**), and the 1'-acethoxy

moiety in **2**, the melanogenesis inhibitory activities of the corresponding enantiomers (**1'**, **24'**, and **2'**) and the racemic mixtures [(±)-**1**, (±)-**24**, and (±)-**2**] were also compared. The inhibitory activities were found to show equivalent IC_{50} values regardless of the stereochemistry [1.9–2.5 μM for **1**, **1'**, and (±)-**1**; 3.0–3.3 μM for **24**, **24'**, and (±)-**24**; and 4.4–5.0 μM for **2**, **2'**, (±)-**2**, respectively].

2.5. Effects on mushroom tyrosinase

Tyrosinase, a copper-containing enzyme widely distributed in microorganisms, animals, and plants, is a key enzyme in melanin biosynthesis, and determines the color of skin and hair.[35] It catalyzes oxidation of both L-tyrosine and L-DOPA, following another oxidation of L-DOPA to dopaquinone, and finally oxidative polymerization via several dopaquinone derivatives to yield melanin. Tyrosinase inhibitors are clinically used for the treatment of several dermatologic disorders associated with melanin hyperpigmentation.[36,37] In addition, the inhibitors are also commonly used as additives in cosmetics for skin whitening and/or depigmentation.[14,38] As shown in Table S1, all of the tested compounds showed no inhibitory activities when both L-tyrosine and L-DOPA were used as substrates. This suggests that tyrosinase inhibition is barely involved in the mechanisms of action of these melanogenesis inhibitors.

Table 3. Inhibitory effects of constituents from the fruit of *Alpinia galanga* and related compounds on melanogenesis and viability in B16 4A5 cells

	Inhibition (%)					IC_{50}
	0 μM	0.3 μM	1 μM	3 μM	10 μM	(μM)
Galanganol D diacetate (**1**)	0.0 ± 9.7	-5.9 ± 6.4	10.0 ± 4.6	58.5 ± 9.1[b]	117.1 ± 4.3[b]	2.5
	(100.0 ± 1.2)	(98.1 ± 1.2)	(82.9 ± 9.2)	(68.5 ± 12.8[c])	(26.5 ± 8.4[c])	
Enantiomer of **1** (**1'**)	0.0 ± 4.6	10.8 ± 4.4	31.8 ± 5.6[b]	71.4 ± 1.4[b]	137.3 ± 3.0[b]	1.9
	(100.0 ± 8.6)	(106.8 ± 1.8)	(101.8 ± 10.0)	(81.5 ± 14.5)	(29.7 ± 7.3[c])	
Racemic mixture of **1** ((±)-**1**)	0.0 ± 1.2	10.3 ± 2.6	32.4 ± 4.3[b]	57.2 ± 4.2[b]	-5.5 ± 6.2	2.2
	(100.0 ± 1.5)	(105.3 ± 1.3)	(99.3 ± 0.7)	(85.2 ± 1.4)	(12.9 ± 0.1[c])	
1'*S*-1'-Acetoxychavicol acetate (**2**)	0.0 ± 9.8	9.3 ± 5.0	6.2 ± 1.0	29.8 ± 2.1[b]	73.1 ± 8.7[b]	5.0
	(100.0 ± 5.6)	(102.8 ± 3.0)	(101.8 ± 1.2)	(99.9 ± 2.9)	(99.9 ± 2.9)	
1'*R*-1'-Acetoxychavicol acetate (**2'**)	0.0 ± 5.6	8.4 ± 3.7	21.2 ± 8.2	36.8 ± 7.9[b]	69.2 ± 3.1[b]	4.5
	(100.0 ± 1.7)	(96.6 ± 3.9)	(98.8 ± 4.2)	(95.2 ± 1.6)	(88.7 ± 1.6)	
Racemic mixture of **2** ((±)-**2**)	0.0 ± 9.1	5.0 ± 3.8	4.4 ± 7.5	35.1 ± 6.8[b]	102.7 ± 2.2[b]	4.4
	(100.0 ± 3.5)	(101.5 ± 3.1)	(103.8 ± 3.6)	(107.5 ± 4.2)	(31.8 ± 3.1[c])	
1'*S*-1'-Acetoxyeugenol acetate (**3**)	0.0 ± 3.6	-4.9 ± 8.8	15.3 ± 3.8	16.8 ± 3.3	84.4 ± 7.3[b]	5.6
	(100.0 ± 4.9)	(92.0 ± 2.4)	(104.8 ± 2.7)	(101.1 ± 2.9)	(42.3 ±7.2[c])	
Galanganol D **(24**)	0.0 ± 3.5	40.4 ± 2.9[b]	46.4 ± 4.1[b]	46.4 ± 5.1[b]	70.5 ± 2.8[b]	3.3
	(100.0 ± 2.7)	(110.7 ± 4.1)	(119.1 ± 6.9)	(120.5 ± 5.2)	(114.4 ± 4.1)	
24'	0.0 ± 7.4	32.9 ± 5.4[b]	47.1 ± 6.1[b]	47.7 ± 8.6[b]	80.2 ± 7.1[b]	3.2
	(100.0 ± 3.1)	(106.5 ± 2.9)	(106.9 ± 4.0)	(102.6 ± 5.4)	(93.9 ± 3.1)	
Racemic mixture of **24** ((±)-**24**)	0.0 ± 6.5	26.7 ± 1.3	43.6 ± 2.7[b]	50.9 ± 5.0[b]	77.4 ± 2.3[b]	3.0
	(100.0 ± 2.0)	(102.0 ± 4.6)	(95.1 ± 3.9)	(104.3 ± 1.3)	(104.6 ± 1.9)	

	Inhibition (%)					IC_{50}
	0 μM	3 μM	10 μM	30 μM	100 μM	(μM)
1'*S*-1'-Hydroxychavicol acetate (**4**)	0.0 ± 5.7	-6.7 ± 4.4	-0.4 ± 4.4	14.9 ± 4.3	56.7 ± 3.7[b]	88.5
	(100.0 ± 3.6)	(87.9 ± 2.0)	(97.2 ± 2.3)	(101.1 ± 5.3)	(113.9 ± 4.0)	
1'*S*-1'-Hydroxyeugenol acetate (**5**)	0.0 ± 9.8	5.0 ± 10.3	10.9 ± 9.3	11.7 ± 9.4	22.9 ± 2.5	
	(100.0 ± 5.9)	(104.5 ± 4.4)	(114.1 ± 7.9)	(113.1 ± 7.7)	(123.9 ± 9.1)	
1'*S*-1'-Acetoxydihydrochavicol acetate (**6**)	0.0 ± 4.9	41.6 ± 2.0[b]	44.5 ± 2.8[b]	74.1 ± 1.1[b]	105.2 ± 1.2[b]	10.5
	(100.0 ± 4.3)	(105.9 ± 4.8)	(105.0 ± 3.3)	(96.5 ± 3.4)	(94.5 ± 1.4)	
1-(4-Hydroxyphenyl)-1-propanone (**7**)	0.0 ± 5.3	4.1 ± 9.4	-3.6 ± 4.8	-1.9 ± 6.6	-3.6 ± 10.6	
	(100.0 ± 3.8)	(110.8 ± 7.4)	(115.9 ± 5.0)	(117.4 ± 6.2)	(125.4 ± 6.1)	
trans-*p*-Coumaryl acetate (**8**)	0.0 ± 4.8	4.1 ± 6.7	0.0 ± 8.2	-3.0 ± 6.3	-13.4 ± 10.5	
	(100.0 ± 3.1)	(99.2 ± 4.0)	(100.0 ± 3.6)	(96.1 ± 5.4)	(90.1 ± 5.7)	
trans-*p*-Acetoxycinnamoyl alcohol (**9**)	0.0 ± 8.5	10.7 ± 5.5	18.0 ± 4.4[b]	96.9 ± 1.4[b]	94.8 ± 1.4[b]	29.4
	(100.0 ± 5.1)	(92.9 ± 1.4)	(85.8 ± 3.8)	(65.5 ± 1.6[c])	(44.9 ± 1.3[c])	
trans-*p*-Coumaryl alcohol (**10**)	0.0 ± 5.4	-3.4 ± 9.0	-2.8 ± 5.0	35.8 ± 7.2[b]	46.9 ± 5.4[b]	
	(100.0 ± 11.5)	(98.9 ± 6.0)	(98.4 ± 9.8)	(116.2 ± 8.4)	(110.2 ± 9.8)	
trans-*p*-Coumaryl aldehyde (**11**)	0.0 ± 5.2	8.9 ± 3.6	12.8 ± 4.9	67.4 ± 9.4[b]	104.5 ± 2.5[b]	18.3
	(100.0 ± 9.1)	(102.7 ± 9.1)	(108.3 ± 9.5)	(94.3 ± 9.3)	(66.8 ± 5.2[c])	
trans-*p*-Coumaryl alcohol γ-*O*-methyl ether (**12**)	0.0 ± 6.6	18.2 ± 3.9	25.1 ± 5.2	56.9 ± 7.3[b]	81.7 ± 3.7[b]	19.5
	(100.0 ± 5.7)	(108.9 ± 7.0)	(103.6 ± 6.7)	(93.6 ± 6.2)	(107.3 ± 8.6)	
trans-Coniferyl alcohol 4-*O*-acetate (**13**)	0.0 ± 8.4	5.5 ± 3.7	12.9 ± 8.9	102.3 ± 1.8[b]	63.3 ± 6.5[b]	16.9
	(100.0 ± 2.2)	(90.6 ± 3.0)	(85.9 ± 5.8)	(60.1 ± 3.3[c])	(41.7 ± 3.3[c])	
trans-Coniferyl alcohol (**14**)	0.0 ± 8.9	35.9 ± 7.9[b]	44.5 ± 3.4[b]	64.2 ± 3.1[b]	-3.0 ± 4.6	16.1
	(100.0 ± 2.3)	(98.9 ± 6.0)	(98.2 ± 4.3)	(88.0 ± 0.9)	(33.1 ± 0.7[c])	
trans-Coniferyl aldehyde (**15**)	0.0 ± 8.1	17.0 ± 9.1	23.7 ± 9.3	100.0 ± 3.3[b]	87.7 ± 1.1[b]	15.3
	(100.0 ± 5.9)	(100.9 ± 5.3)	(85.8 ± 7.0)	(74.2 ± 3.2[c])	(44.7 ± 3.8[c])	
4-Hydroxybenzaldehyde	0.0 ± 4.6	11.3 ± 3.7	4.2 ± 1.7	7.9 ± 5.4	3.5 ± 3.3	

	(100.0 ± 5.0)	(107.4 ± 1.7)	(103.2 ± 1.2)	(107.4 ± 4.8)	(103.0 ± 2.9)	
4-Hydroxy-3-methoxybenzaldehyde	0.0 ± 3.3	-8.4 ± 10.4	8.4 ± 9.9	49.7 ± 2.7[b]	96.1 ± 2.2[b]	49.2
	(100.0 ± 1.4)	(108.1 ± 5.7)	(104.4 ± 2.3)	(104.1 ± 3.4)	(105.9 ± 5.5)	

	Inhibition (%)					IC_{50}
	0 μM	30 μM	100 μM	300 μM	1000 μM	(μM)
Arbutin[30–34]	0.0 ± 1.4	20.4 ± 0.5	38.1 ± 0.9[b]	61.5 ± 0.6[b]	83.7 ± 0.5[b]	174
	(100.0 ± 2.1)	(82.4 ± 3.0)	(78.1 ± 1.9)	(79.8 ± 2.2)	(53.1 ± 1.8)	

Each value represents mean ± S.E.M. (N = 4).

Significantly different from control, [a]$p < 0.05$, [b]$p < 0.05$.

[c]Cytotoxic effects were observed, and values in parenthesis indicate cell viability (%) in MTT assay.

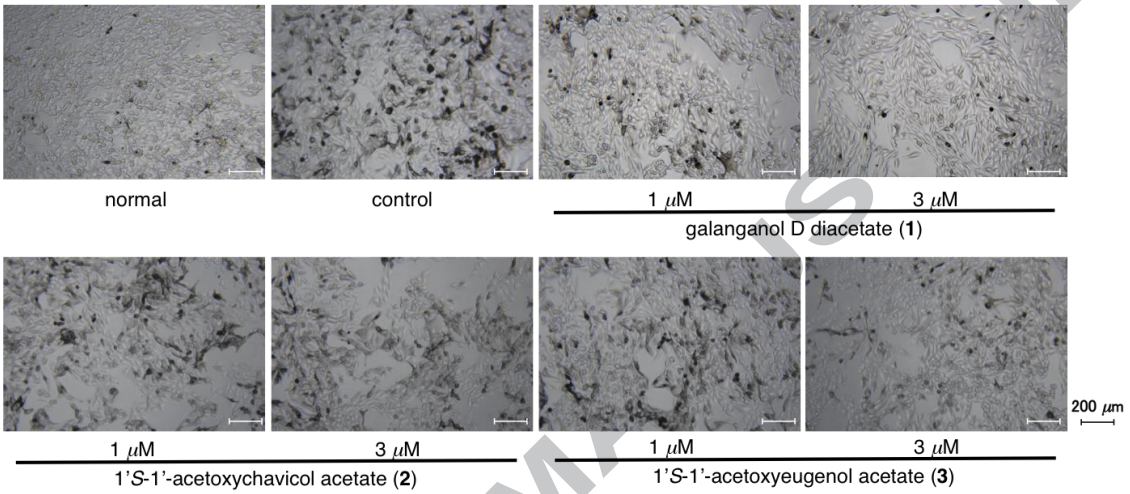


Figure 4. Theophylline-stimulated B16 melanoma 4A5 cells 72 h after treatment with **1**–**3**

The images are representative of several experiments.

2.6. Effects on expression of tyrosinase and TRP-1 and 2

The TRP enzyme family (tyrosinase, TRP-1, and TRP-2) catalyzes the major steps in melanin synthesis.[39] To clarify the mechanisms of action of these active constituents, we examined the effects of galanganol D diacetate (**1**) and its enantiomer (**1'**) and racemic mixture (±)-**1**, and the principal active phenylpropanoids (**2** and **3**) on the expression of mRNAs for tyrosinase, TRP-1, and TRP-2 in B16 melanoma 4A5 cells. As presented in Table 4, compounds **1**, **1'**, and (±)-**1** inhibited mRNA expressions for tyrosinase at 10 μM. In addition, compound **2** inhibited tyrosinase, TRP-1, and TRP-2 mRNA expression at 10 μM, whereas compound **3** inhibited TRP-1 and TRP-2 mRNA expression at 3–10 μM.

In conclusion, we isolated a new rare 7-*O*-9'-linked neolignan, galanganol D diacetate (**1**), from the 80% aqueous acetone extract of the fruit of *A. galanga*, along with 16 known compounds including 14 phenylpropanoids (**2**–**15**). The stereostructure of **1** was determined by means of extensive NMR analysis and total synthesis. Several compounds in the extract, including a new neolignan (**1**), were identified as melanogenesis inhibitors, and some of them were found to be more potent than arbutin, a clinically used depigmentation agent. In particular, **1** (IC_{50} = 2.5 μM), 1'*S*-1'-acetoxychavicol acetate (**2**, 5.0 μM), and 1'*S*-1'-acetoxyeugenol acetate (**3**, 5.6 μM) showed relatively potent inhibitory effects without notable cytotoxicity at the effective concentrations. Several structural requirements of phenylpropanoids for the melanogenesis inhibitory activity were suggested. Compounds **1**, **1'**, (±)-**1**, **2**, and **3** inhibited tyrosinase, TRP-1, and TRP-2 mRNA expressions, which could be the mechanism of melanogenesis inhibitory activity. Further studies on the mechanism of action of these highly active constituents are in progress.

Table 5. Effects of **1**–**3**, and related compounds on expression of tyrosinase, TRP-1, and TRP-2 mRNA in B16 4A5 cells

	Tyrosinase mRNA / β-actin mRNA		
	0 μM	3 μM	10 μM
Galanganol D diacetate (**1**)	1.00 ± 0.07	0.87 ± 0.11	0.53 ± 0.13[b]
Enantiomer of galanganol D (**1'**)	1.00 ± 0.07	0.84 ± 0.18	0.56 ± 0.07[b]
Racemic mixture of **1** ((±)-**1**)	1.00 ± 0.07	0.77 ± 0.14	0.60 ± 0.10[b]
1'S-1'-Acetoxychavicol acetate (**2**)	1.00 ± 0.09	1.00 ± 0.12	0.49 ± 0.06[b]
1'S-1'-Acetoxyeugenol acetate (**3**)	1.00 ± 0.09	1.10 ± 0.17	0.80 ± 0.08
	TRP-1 mRNA / β-actin mRNA		
	0 μM	3 μM	10 μM
Galanganol D diacetate (**1**)	1.00 ± 0.12	0.76 ± 0.25	0.99 ± 0.29

Enantiomer of galanganol D (**1'**)	1.00 ± 0.12	0.92 ± 0.06	0.94 ± 0.31
Racemic mixture of **1** ((±)-**1**)	1.00 ± 0.12	0.79 ± 0.23	0.81 ± 0.14
1'*S*-1'-Acetoxychavicol acetate (**2**)	1.00 ± 0.03	0.86 ± 0.24	0.50 ± 0.23[b]
1'*S*-1'-Acetoxyeugenol acetate (**3**)	1.00 ± 0.03	0.52 ± 0.10[b]	0.29 ± 0.03[b]

	TRP-2 mRNA / β-actin mRNA		
	0 μM	3 μM	10 μM
Galanganol D diacetate (**1**)	1.00 ± 0.21	0.72 ± 0.24	1.43 ± 0.27
Enantiomer of galanganol D (**1'**)	1.00 ± 0.21	1.67 ± 0.51	0.88 ± 0.33
Racemic mixture of **1** ((±)-**1**)	1.00 ± 0.21	0.86 ± 0.25	0.81 ± 0.16
1'*S*-1'-Acetoxychavicol acetate (**2**)	1.00 ± 0.07	1.08 ± 0.18	0.34 ± 0.05[b]
1'*S*-1'-Acetoxyeugenol acetate (**3**)	1.00 ± 0.07	0.89 ± 0.20	0.88 ± 0.16

Each value represents mean ± S.E.M. (N = 3).
Significantly different from control, [a]$p < 0.05$, [b]$p < 0.01$.

3. Experimental

3.1. General

The following instruments were used to obtain physical data: specific rotations, SEPA-300 digital polarimeter (Horiba Ltd., Kyoto, Japan, l = 10 cm); UV spectra, UV-1600 spectrometer (Shimadzu Co., Kyoto, Japan); IR spectra, FTIR-8100 spectrometer (Shimadzu Co.); ^{1}H NMR spectra, JNM-ECA800 (800 MHz), JNM-ECA600 (600 MHz), and JNM-ECS400 (400 MHz) spectrometers (JEOL Ltd., Tokyo, Japan); ^{13}C NMR spectra, JNM-ECA800 (200 MHz), JNM-ECA600 (150 MHz), and JNM-ECS400 (100 MHz) spectrometers (JEOL Ltd.) with tetramethylsilane as an internal standard; ESIMS and HRESIMS, Exactive Plus mass spectrometer (Thermo Fisher Scientific Inc., MA, USA); HPLC detector, SPD-10A*vp* UV-VIS detector (detection: 230 nm, Shimadzu Co.); HPLC column, Cosmosil 5C_{18}-MS-II (Nacalai Tesque Inc., Kyoto, Japan), 4.6 mm i.d. × 250 mm and 20 mm i.d. × 250 mm for analytical and preparative studies, respectively; Chiral HPLC column, Ceramospher Chiral RU-1 (Shiseido Co., Ltd., Tokyo, Japan), 4.6 mm i.d. × 250 mm.

The following experimental conditions were used for column chromatography (CC): highly porous synthetic resin, Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan); normal-phase silica gel CC, silica gel 60N (Kanto Chemical Co., Ltd., Tokyo, Japan; 63–210 mesh, spherical, neutral); reversed-phase ODS CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., Aichi, Japan; 100–200 mesh); TLC, pre-coated TLC plates with silica gel 60F_{254} (Merck, Darmstadt, Germany, 0.25 mm, normal-phase) and silica gel RP-18 WF_{254S} (Merck, Darmstadt, Germany, 0.25 mm, reversed-phase); reversed-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was carried out by spraying 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Dehydrated DMF, THF, and CH_2Cl_2 were purchased from Kanto Chemical. Lipase PS from *Burkholderia cepacia* was from Amano Enzyme. Other chemicals were from Wako Chemicals, Nacalai Tesque, or Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

3.2. Plant Material

The fruit of *Alpinia galanga* was collected from Nakhonsithammarat Province, Thailand, in September 2011. The plant material was identified by one of the authors (S. C.). A voucher specimen (2011.09. Raj-01) of this plant is on file in our laboratory.

3.3. Extraction and Isolation

Dried fruit of *A. galanga* (3.0 kg) were extracted four times with 80% aqueous acetone at room temperature for over night. Evaporation of the combined extracts under reduced pressure provided an aqueous acetone extract (258.7 g, 8.62%). An aliquot (230.0 g) was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (131.7 g, 5.09%) and an aqueous phase. The aqueous phase was subjected to Diaion HP-20 CC (3.0 kg, H_2O → MeOH) to give H_2O-eluted (19.4 g, 0.75%) and MeOH-eluted (24.8 g, 0.96%) fractions, respectively. An aliquot (110.0 g) of the EtOAc-soluble fraction was subjected to normal-phase silica gel CC [3.0 kg, *n*-hexane–EtOAc (10:1 → 5:1 → 3:1→ 1:1, v/v) → EtOAc → MeOH] to give 11 fractions [Fr. 1 (1.16 g), Fr. 2 (2.91 g), Fr. 3 (2.95 g), Fr. 4 (6.45 g), Fr. 5 (9.80 g), Fr. 6 (11.47 g), Fr. 7 (1.25 g), Fr. 8 (10.03 g), Fr. 9 (2.80 g), Fr. 10 (16.28 g), and Fr. 11 (37.70 g)]. Fraction 4 (6.45 g) was subjected to reversed-phase silica gel CC [200 g, MeOH–H_2O (60:40 → 80:20, v/v) → MeOH] to afford eight fractions [Fr. 4-1 (62.4 mg), Fr. 4-2 [= 1'*S*-1'-acetoxychavicol acetate (**2**, 2.06 g, 0.951%)], Fr. 4-3 (239.1 mg), Fr. 4-4 (53.2 mg), Fr. 4-5 (802.6 mg), Fr. 4-6 (252.8 mg), Fr. 4-7 (187.6 mg), and Fr. 4-8 (2.80 g)]. Fraction 4-3 (239.1 mg) was purified by HPLC [MeOH–H_2O (70:30, v/v)] to give **2** (56.1 mg, 0.00258%) and 1'*S*-1'-acetoxydihydrochavicol acetate (**6**, 5.2 mg, 0.00028%). Fraction 5 (9.80 g) was subjected to reversed-phase silica gel CC [300 g, MeOH–H_2O (60:40 → 80:20, v/v) → MeOH] to afford nine fractions [Fr. 5-1 (63.8 mg), Fr. 5-2 (323.8 mg), Fr. 5-3 [= 1'*S*-1'-acetoxyeugenol acetate (**3**, 2.57 g, 0.119%)], Fr. 5-4 (483.9 mg), Fr. 5-5 (223.9 mg), Fr. 5-6 (2.08 g), Fr. 5-7 (827.2 mg), Fr. 5-8 (140.8 mg), and Fr. 5-9 (2.78 g)]. Fraction 5-2 (323.8 mg) was purified by HPLC [MeOH–H_2O (40:60, v/v)] to give 1-(4-hydroxyphenyl)-1-propanone (**7**, 5.2 mg, 0.00024%), *trans*-*p*-coumaryl alcohol (**10**, 8.3 mg, 0.00038%), *trans*-*p*-coumaryl aldehyde (**11**, 5.7 mg, 0.00026%), *trans*-*p*-coumaryl alcohol γ-*O*-methyl ether (**12**, 28.3 mg, 0.00131%), and 4-hydroxy-3-methoxybenzaldehyde (9.3 mg, 0.00043%). Fraction 5-6 (502.0 mg) was purified by HPLC [MeOH–H_2O (80:20, v/v)] to give galanganol D diacetate (**1**, 15.2 mg, 0.00292%) and *trans*-*p*-coumaryl acetate (**8**, 0.00140%). Fraction 6 (11.47 g) was subjected to reversed-phase silica gel CC [450 g, MeOH–H_2O (50:50 →80:20 → 90:10, v/v) → MeOH] to afford 10 fractions [Fr. 6-1 (51.9 mg), Fr. 6-2 (324.1 mg), Fr. 6-3 (193.4 mg), Fr. 6-4 (509.4 mg), Fr. 6-5 (1.72 g), Fr. 6-6 (1.96 g), Fr. 6-7 (1.84 g), Fr. 6-8 (516.9 mg), Fr. 6-9 (1.72 g), and Fr. 6-10 (3.14 g)]. Fraction 6-2 (324.1 mg) was purified by HPLC [CH_3CN–H_2O (15:85, v/v)] to give 4-hydroxybenzaldehyde (146.4 mg, 0.00547%). Fraction 6-4 (509.4 mg) was purified by HPLC [CH_3CN–H_2O (25:75, v/v)] to give 1'*S*-1'-hydroxchavicol acetate (**4**, 92.9 mg, 0.00430%), 1'*S*-1'-hydroxyeugenol acetate (**5**, 145.9 mg, 0.00675%), *trans*-*p*-acetoxycinnamoyl alcohol (**9**, 34.9 mg, 0.00162%), *trans*-coniferyl alcohol 4-*O*-acetate (**13**, 8.9 mg, 0.00041%), and *trans*-coniferyl aldehyde (**15**, 7.7 mg, 0.00036%). Fraction 8 (10.03 g) was subjected to reversed-phase silica gel CC [300 g, MeOH–H_2O (30:70 → 60:40 → 80:20, v/v) → MeOH] to afford seven fractions [Fr. 8-1 (175.0 mg), Fr. 8-2 (296.2 mg), Fr. 8-3 (92.9 mg), Fr. 8-4 (286.0 mg), Fr. 8-5 (298.4 mg), Fr. 8-6 (2.12 g), and Fr. 8-7 (2.84 g)]. Fraction 8-2 (296.2

mg) was purified by HPLC [MeOH–H_2O (30:70, v/v)] to give **10** (28.1 mg, 0.00130%). Fraction 8-5 (298.4 mg) was purified by HPLC [MeOH–H_2O (50:50, v/v)] to give *trans*-coniferyl alcohol (**14**, 187.9 mg, 0.00869%).

3.3.1. Galanganol D diacetate (1). Colorless oil, $[\alpha]_D^{24}$ –6.2 (*c* 0.55, $CHCl_3$). Positive-ion ESIMS: *m/z* 389 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{22}H_{22}O_5Na$ $[M + Na]^+$: 389.1359. Found: 389.1346. UV [MeOH, nm (log ε)]: 225 (3.62), 271 (3.19). IR (film): 1767, 1651, 1508, 1460, 1370, 1194, 1165 cm^{-1}. ^1H NMR (800 MHz, pyridine-d_5 and $CDCl_3$) δ: given in Table 2. ^{13}C NMR (200 MHz, pyridine-d_5 and $CDCl_3$) δ_C: given in Table 2.

3.4. Synthesis

3.4.1. 4-*O*-*tert*-Butyldiphenylsiloxybenzaldehyde (17)

A solution of 4-hydroxybenzaldehyde (**16**, 2.44 g, 20.0 mmol) in dry DMF (200 mL) was treated with imidazole (2.04 g, 1.50 equiv.) and TBDPSCl (6.60 g, 4.2 mL, 1.20 equiv.) at 0 °C for 18 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The EtOAc extract was washed with brine then dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC [300 g, *n*-hexane–EtOAc (40:1, v/v)] to give **17** (7.10 g, 98%); as a colorless oil. Positive-ion ESIMS: *m/z* 361 $[M + H]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{23}H_{25}O_2Si$ $[M + H]^+$: 361.1618. Found: 361.1618. IR (film): 1697, 1597, 1508, 1273, 1157, 1115 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.09 (9H, s, *tert*-Bu), 6.84 (2H, d, *J* = 8.7 Hz, H-3,5), 7.34–7.45 (6H, m, arom.), 7.62 (2H, d, *J* = 8.7 Hz, H-2,6), 7.66–7.69 (4H, m, arom.), 9.79 (1H, s, -C*H*O). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 19.4 (s, *tert*-Bu), 26.3 (3C, q, *tert*-Bu), 120.3 (2C, d, C-3,5), 128.0 (4C, d, arom.), 130.2 (s, C-1), 130.2 (2C, d, C-2,6), 131.7 (2C, d, arom.), 131.9 (2C, s, arom.), 135.4 (4C, d, arom.), 161.2 (s, C-4), 190.9 (d, -*C*HO).

3.4.2. Racemic 4-*O*-*tert*-butyldiphenylsilyl-1'-hydroxychavicol ((±)-18)

To a solution of **17** (5.40 g, 15.0 mmol) in dry THF (150 mL), vinylmagnesium bromide (1 M in THF, 18.0 mL) was added dropwise at 0 °C for 3 h. The reaction mixture was poured into aqueous saturated ammonium chloride (NH_4Cl) and extracted with EtOAc. The EtOAc extract was washed with brine then dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC [180 g, *n*-hexane–EtOAc (20:1, v/v)] to give (±)-**18** (5.20 g, 90%); as a colorless oil. Positive-ion ESIMS: *m/z* 411 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{25}H_{28}O_2SiNa$ $[M + Na]^+$: 411.1751. Found: 411.1755. IR (film): 1605, 1508, 1258, 1169, 1111 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.07 (9H, s, *tert*-Bu), 5.03 (1H, d, *J* = 5.5 Hz, H-7), [5.12 (1H, dd, *J* = 1.4, 10.1 Hz), 5.26 (1H, dd, *J* = 1.4, 18.3 Hz), H_2-9], 5.98 (1H, ddd, *J* = 5.5, 10.1, 18.3 Hz, H-8), 6.72 (2H, d, *J* = 8.7 Hz, H-3,5), 7.07 (2H, d, *J* = 8.7 Hz, H-2,6), 7.30–7.42 (6H, m, arom.), 7.67–7.69 (4H, m, arom.). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 19.4 (s, *tert*-Bu), 26.5 (3C, q, *tert*-Bu), 76.7 (d, C-7), 114.7 (t, C-9), 119.7 (2C, d, C-3,5), 127.4 (2C, d, C-2,6), 127.8 (4C, d, arom.), 129.9 (2C, d, arom.), 132.8 (2C, s, arom.), 135.0 (s, C-1), 135.5 (4C, d, arom.), 140.3 (d, C-8), 155.2 (s, C-4).

3.4.3. Lipase-catalyzed esterification of (±)-18

General procedure was carried out according to the previously reported method.[29] In a solution of (±)-**18** (2.00 g, 5.00 mmol) and 2,6-di-*tert*-butyl-*p*-cresol (a polymerization inhibitor for vinyl acetate; 50.0 mg, 0.22 mmol) in a mixture of freshly distilled vinyl acetate (30 mL) and dry THF (30 mL), lipase PS (3.60 g) was suspended and stirred for 24 h at 65 °C in the dark. After cooling, lipase was removed by filtration and the filtrate was evaporated to dryness. The obtained residue was purified by normal-phase silica gel CC [100 g, *n*-hexane–EtOAc (20:1 → 15:1, v/v)] to give **26'** (970 mg, 45%) and unreacted **18** (880 mg, 45%).

3.4.3.1. (1'*R*)-4-*O*-*tert*-Butyldiphenylsilyl-1'acetoxychavicol (26'). Colorless oil, $[\alpha]_D^{25}$ +27.0 (*c* 0.52, $CHCl_3$). Positive-ion ESIMS: *m/z* 453 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{27}H_{30}O_3SiNa$ $[M + Na]^+$: 453.1856. Found: 453.1855. UV [MeOH, nm (log ε)]: 225 (3.62), 271 (3.19). IR (film): 1740, 1609, 1508, 1427, 1369, 1173, 1111 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.08 (9H, s, *tert*-Bu), 2.05 (3H, s, -O*Ac*), [5.16 (1H, dd, *J* = 6.4, 10.1 Hz), 5.20 (1H, dd, *J* = 6.4, 16.9 Hz), H_2-9], 5.92 (1H, ddd, *J* = 6.0, 10.1, 16.9 Hz, H-8), 6.14 (1H, d, *J* = 6.0 Hz, H-7), 6.72 (2H, d, *J* = 8.2 Hz, H-3,5), 7.07 (2H, d, *J* = 8.2 Hz, H-2,6), 7.33–7.45 (6H, m, arom.), 7.68–7.70 (4H, m, arom.). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 19.4 (s, *tert*-Bu), 21.3 (q, -O*Ac*), 26.5 (3C, q, *tert*-Bu), 75.7 (d, C-7), 116.4 (t, C-9), 119.6 (2C, d, C-3,5), 127.8 (4C, d, arom.), 128.4 (2C, d, C-2,6), 129.9 (2C, d, arom.), 131.2 (s, C-1), 132.7 (2C, s, arom.), 135.5 (4C, s, arom.), 136.3 (d, C-8), 155.5 (s, C-4), 170.0 (s, -O*Ac*).

3.4.3.2. (1'*S*)-4-*O*-*tert*-Butyldiphenylsilyl-1'hydroxychavicol (18). Colorless oil, $[\alpha]_D^{25}$ –1.6 (*c* 0.43, $CHCl_3$). Positive-ion ESIMS: *m/z* 411 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{25}H_{28}O_2SiNa$ $[M + Na]^+$: 411.1751. Found: 411.1753.

3.4.4. Lipase-catalyzed hydrolysis of 26'

Lipase PS (250 mg) and **26'** (860 mg, 2.00 mmol) were suspended in 0.1 M phosphate buffer (8.0 mL, pH 7.0) and stirred for 24 h at room temperature. The reaction mixture was diluted with brine and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC [2.5 g, *n*-hexane–EtOAc (15:1, v/v)] to give **18'** (610 mg, 78%); as a colorless oil, $[\alpha]_D^{25}$ +1.6 (*c* 0.53, $CHCl_3$). Positive-ion ESIMS: *m/z* 411 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{25}H_{28}O_2SiNa$ $[M + Na]^+$: 411.1751. Found: 411.1749.

3.4.5. 4-*O*-*tert*-Butyldiphenylsilyl-(*E*)-*p*-coumaric acid methyl ester (20)

A solution of (*E*)-*p*-coumaric acid methyl ester (**19**, 2.70 g, 15.0 mmol) in dry DMF (150 mL) was treated with imidazole (1.20 g, 1.20 equiv.) and TBDPSCl (14.1 mL, 1.05 equiv.) at 0 °C for 18 h. The reaction mixture was poured into H_2O and extracted with EtOAc. Work-up of the EtOAc extract as described in Section **3.4.1.** gave a residue, which was purified by normal-phase silica gel CC [200 g, *n*-hexane–EtOAc (40:1, v/v)] to give **20** (6.00 g, 96%); as a colorless oil. Positive-ion ESIMS: *m/z* 417 $[M + H]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{26}H_{29}O_3Si$ $[M + H]^+$: 417.1880. Found: 417.1862. IR (film): 1709, 1601, 1508, 1427, 1261, 1169, 1111 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.09 (9H, s, *tert*-Bu), 3.75 (3H, s, -OC*H*$_3$), 6.22 (1H, d, *J* = 16.0 Hz, H-8), 6.74 (2H, d, *J* = 8.7 Hz, H-3,5), 7.26 (2H, d, *J* = 8.7 Hz, H-2,6), 7.34–7.45 (6H, m, arom.), 7.56 (1H, d, *J* = 16.0 Hz, H-7), 7.68–7.71 (4H, m, arom.). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 19.4 (s, *tert*-Bu), 26.4 (3C, q, *tert*-Bu), 51.5 (q, -O*C*H$_3$), 115.3 (d, C-8), 120.2 (2C, d, C-3,5), 127.4 (s, C-1), 127.9 (4C, d, arom.), 129.5 (2C, d, C-2,6), 130.1 (2C, d, arom.), 132.4 (2C, s, arom.), 135.4 (4C, d, arom.), 144.6 (d, C-7), 157.7 (s, C-4), 167.7 (s, C-9).

3.4.6. 4-*O*-*tert*-Butyldiphenylsilyl-(*E*)-*p*-coumaryl alcohol (21)

To a solution of **20** (5.00 g, 12.0 mmol) in dry THF (120 mL), a solution of diisobutylaluminium hydride (DIBAL-H ,1.0 M in THF, 26.4 mL, 2.20 equiv.) was added drop wise and the mixture was stirred at 0 °C for 3 h. The resulting mixture was poured into saturated aqueous NH_4Cl and extracted with EtOAc. The EtOAc extract was washed with brine then dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC [180 g, *n*-hexane–EtOAc (15:1, v/v)] to give **21** (4.20 g, 90%); as a colorless oil. Positive-ion ESIMS: *m/z* 411 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{25}H_{28}O_2SiNa$ $[M + Na]^+$: 411.1750. Found: 411.1750. IR (film): 3476, 1643, 1257, 1173, 1111 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.08 (9H, s, *tert*-Bu), 4.23 (1H, d, J = 5.0 Hz, H-9), 6.15 (1H, dd, J = 5.0, 16.0 Hz, H-8), 6.46 (1H, d, J = 16.0 Hz, H-7), 6.70 (2H, d, J = 8.7 Hz, H-3,5), 7.11 (2H, d, J = 8.7 Hz, H-2,6), 7.33–7.44 (6H, m, arom.), 7.68–7.70 (4H, m, arom.). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 19.4 (s, *tert*-Bu), 26.4 (3C, q, *tert*-Bu), 63.9 (t, C-9), 119.8 (2C, d, C-3,5), 126.2 (d, C-8), 127.4 (2C, d, C-2,6), 127.8 (4C, d, arom.), 129.6 (s, C-1), 129.9 (2C, d, arom.), 131.1 (d, C-7), 132.7 (2C, s, arom.), 135.5 (4C, d, arom.), 155.4 (s, C-4).

3.4.7. 4-*O*-*tert*-Butyldiphenylsilyl-(*E*)-*p*-coumaryl bromide (22)

To a solution of the alcohol **21** (3.90 g, 10.0 mmol) in dry diethyl ether (Et_2O, 100 mL), phosphorus tribromide (PBr_3, 1.05 mL, 1.10 mmol) was added drop wise at 0 °C. After stirring for 3 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$, and extracted with *n*-hexane. The *n*-hexane extract was washed with brine then dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent under reduced pressure gave **22** (4.30 g, 95%); as a colorless oil. Positive-ion ESIMS: *m/z* 371 $[M – Br]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{25}H_{27}OSi$ $[M – Br]^+$: 371.1826. Found: 371.1825. IR (film): 1639, 1605, 1508, 1258, 1111 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.07 (9H, s, *tert*-Bu), 4.10 (2H, d, J = 7.8 Hz, H_2-9), 6.17 (1H, dt, J = 15.6, 7.8 Hz, H-8), 6.50 (1H, d, J = 15.6 Hz, H-7), 6.69 (2H, d, J = 8.7 Hz, H-3,5), 7.11 (2H, d, J = 8.7 Hz, H-2,6), 7.33–7.43 (6H, m, arom.), 7.67–7.70 (4H, m, arom.). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 19.5 (s, *tert*-Bu), 26.5 (3C, q, *tert*-Bu), 34.3 (t, C-9), 120.0 (2C, d, C-3,5), 123.1 (d, C-8), 127.9 (6C, d, C-2,6 and arom.), 128.9 (s, C-1), 130.0 (2C, d, arom.), 132.7 (2C, s, arom.), 134.4 (d, C-7), 135.5 (4C, d, arom.), 156.0 (s, C-4).

3.4.8. Coupling reaction of secondary alcohol ((±)-18, 18, or 18') and bromide 22

A solution of (±)-**18** (780 mg, 2.00 mmol) in dry THF (10 mL) was treated with sodium hydride (NaH, 50.4 mg, 1.05 equiv.) at room temperature for 30 min. Then the reaction mixture was treated with **22** (900 mg, 2.0 mmol, 1.00 equiv.) and sodium iodide (NaI, 30.0 mg, 0.20 mmol, 0.1 equiv.) at room temperature for 12 h. The resulting mixture was poured into saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine then dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC [50 g, *n*-hexane–EtOAc (40:1, v/v)] to give (±)-**23** (1.00 g, 66%). According to the similar procedure, optically active **23** (590 mg, 78%) or **23'** (570 mg, 75%) was obtained from **18** or **18'** (390 mg, 1.00 mmol).

3.4.8.1. Racemic compound (±)-23. Colorless oil. Positive-ion ESIMS: *m/z* 781 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{50}H_{54}O_3Si_2Na$ $[M + Na]^+$: 781.3504. Found: 781.3501. UV [MeOH, nm (log ε)]: 225 (3.62), 271 (3.19). IR (film): 1647, 1508, 1458, 1396, 1172, 1111 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.11 (18H, s, *tert*-Bu), 3.97–4.06 (2H, m, H_2-9'), 4.71 (1H, d, J = 6.9 Hz, H-7), [5.15 (1H, dd, J = 1.4, 11.9 Hz), 5.20 (1H, dd, J = 1.4, 18.3 Hz),H_2-9], 5.90 (1H, ddd, J = 6.9, 11.9, 18.3 Hz, H-8), 6.07 (1H, dt, J = 16.0, 6.4 Hz, H-8'), 6.39 (1H, d, J = 16.0 Hz, H-8'), [6.71 (2H, d, J = 8.7 Hz), 6.75 (2H, d, J = 8.7 Hz), H-3,5 and H-3',5'], [7.07 (2H, d, J = 8.7 Hz), 7.11 (2H, d, J = 8.7 Hz), H-2,6 and H-2',6'], 7.34–7.50 (12H, m, arom.), 7.71–7.73 (8H, m, arom.). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 19.4, 19.8 (2C each, both s, *tert*-Bu), 26.46, 26.49 (6C each, both q, *tert*-Bu), 68.8 (t, C-9'), 81.4 (d, C-7), 116.0 (t, C-9), 119.6, 119.7 (2C each, both d, C-3,5 and C-3',5'), 124.0 (d, C-8'), 127.4, 127.9 (2C each, both C-2,6 and C-2',6'), 127.71, 127.74 (8C each, both d, arom.), 129.79 (s, C-1'), 129.83, 129.9 (4C each, both d, arom.), 132.0 (d, C-7'), 132.8, 132.9 (2C each, both s, arom.), 133.3 (s, C-1), 135.4, 135.5 (8C each, both d, arom.), 138.9 (d, C-7), 155.0, 155.2 (2C each, both s, C-4 and C-4').

3.4.8.2. Compound 23. Colorless oil, $[\alpha]_D^{25}$ –5.1 (*c* 3.75, $CHCl_3$). Positive-ion ESIMS: *m/z* 781 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{50}H_{54}O_3Si_2Na$ $[M + Na]^+$: 781.3504. Found: 781.3504.

3.4.8.3. Compound 23'. Colorless oil, $[\alpha]_D^{25}$ +5.1 (*c* 5.75, $CHCl_3$). Positive-ion ESIMS: *m/z* 781 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{30}H_{54}O_3Si_2Na$ $[M + Na]^+$: 781.3504. Found: 781.3508.

3.4.9. Deprotection of phenylpropanoid ((±)-18, 18, or 18')

To a solution of (±)-**18** (194.5 mg, 0.50 mmol) in dry THF (2.0 mL) in the presence of acetic acid (AcOH, 36.0 mg, 34.3 μL), tetra-*n*-butylammonium fluoride (1 M in THF, 0.6 mL) was added dropwise at 0 °C. After stirring for 3 h, the reaction mixture was poured into brine, then extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, then dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC [10 g, *n*-hexane–EtOAc (2:1, v/v)] to give (±)-**25** (69.0 mg, 92%). According to a similar procedure, optically active **25** (68.0 mg, 90%) or **25'** (71.0 mg, 94%) was obtained from **18** or **18'** (194.5 mg, 0.50 mmol).

3.4.9.1. Racemic 1'-hydroxychavicol ((±)-25). Colorless oil. Negative-ion ESIMS: *m/z* 149 $[M – H]^-$. High-resolution negative-ion ESIMS: Calcd for $C_9H_9O_2$ $[M – H]^-$: 149.0597. Found: 149.0595. UV [MeOH, nm (log ε)]: 229 (4.94), 273 (4.38). IR (film): 3422, 1639, 1512, 1447, 1377, 1238, 1173 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ: 4.93 (1H, d, J = 6.0 Hz, H-7), [4.99 (1H, dd, J = 1.4, 10.5 Hz), 5.14 (1H, dd, J = 1.4, 17.4 Hz), H_2-9], 5.90 (1H, ddd, J = 6.0, 10.5, 17.4 Hz, H-8), 6.64 (2H, d, J = 8.2 Hz, H-3,5), 7.06 (2H, d, J = 8.2 Hz, H-2,6). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 75.8 (d, C-7), 114.3 (t, C-9), 116.0 (2C, d, C-3,5), 128.9 (2C, d, C-2,6), 135.4 (s, C-1), 142.4 (d, C-8), 157.9 (s, C-4).

3.4.9.2. Compound 25. Colorless oil, $[\alpha]_D^{24}$ –49.5 (*c* 1.00, EtOH). Negative-ion ESIMS: *m/z* 149 $[M – H]^-$. High-resolution negative-ion ESIMS: Calcd for $C_9H_9O_2$ $[M – H]^-$: 149.0597. Found: 149.0595.

3.4.9.3. Compound 25'. Colorless oil, $[\alpha]_D^{24}$ +49.5 (*c* 1.00, EtOH). Negative-ion ESIMS: *m/z* 149 $[M – H]^-$. High-resolution negative-ion ESIMS: Calcd for $C_9H_9O_2$ $[M – H]^-$: 149.0597. Found: 149.0595.

3.4.10. Deprotection of 7-*O*-9' linked neolignan ((±)-23, 23, or 23')

According to a similar procedure as described in Section **3.4.9.**, racemic (±)-**24** (138.0 mg, 98%), optically active galanganol D (**24**, 140.0 mg, 99%) or **24'** (140.0 mg, 99%) was obtained from (±)-**23**, **23** or **23'** (380.0 mg, 0.50 mmol).

3.4.10.1. Racemic compound (±)-24. Colorless oil. Negative-ion ESIMS: *m/z* 281 $[M - H]^-$. High-resolution negative-ion ESIMS: Calcd for $C_{18}H_{17}O_3$ $[M - H]^-$: 281.1172. Found: 281.1182.

3.4.10.2. Galanganol D (24). Colorless oil, $[\alpha]_D^{25}$ –17.4 (*c* 1.50, $CHCl_3$). Negative-ion ESIMS: *m/z* 281 $[M - H]^-$. High-resolution negative-ion ESIMS: Calcd for $C_{18}H_{17}O_3$ $[M - H]^-$: 281.1172. Found: 281.1183. UV [MeOH, nm (log ε)]: 231 (4.99), 261 (4.26). IR (film): 3421, 1639, 1512, 1446, 1377, 1173, 1111 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ: 3.99–4.09 (2H, m, H_2-9'), 4.80 (1H, d, *J* = 6.4 Hz, H-7), [5.12 (1H, dd, *J* = 1.4, 10.6 Hz), 5.25 (1H, dd, *J* = 1.4, 16.0 Hz), H_2-9], 5.92 (1H, ddd, *J* = 6.6, 10.6, 16.0 Hz, H-8), 6.15 (1H, dt, *J* = 15.6, 6.4 Hz, H-8'), 6.51 (1H, d, *J* = 16.0 Hz, H-7'), [6.78 (2H, d, *J* = 8.2 Hz), 6.81 (2H, d, *J* = 8.7 Hz), H-3,5 and H-3',5'], [7.18 (2H, d, *J* = 8.2 Hz), 7.28 (2H, d, *J* = 8.7 Hz), H-2,6 and H-2',6'], 8.31, 8.39 (1H each, both br s, -OH). ^{13}C NMR (100 MHz, acetone-d_6) δ_C: 69.3 (t, C-9'), 82.2 (d, C-7), 115.3 (t, C-9), 115.9, 116.2 (2C each, both d, C-3,5 and C-3',5'), 124.3 (d, C-8'), 128.5, 129.1 (2C each, both d, C-2,6 and C-2',6'), 129.5 (s, C-1'), 132.0 (d, C-7'), 133.1 (s, C-1), 140.7 (d, C-8), 157.7, 158.0 (2C each, both s, C-4 and C-4').

3.4.10.3. Compound 24'. Colorless oil, $[\alpha]_D^{24}$ +17.4 (*c* 1.58, $CHCl_3$). Negative-ion ESIMS: *m/z* 281 $[M - H]^-$. High-resolution negative-ion ESIMS: Calcd for $C_{18}H_{17}O_3$ $[M - H]^-$: 281.1172. Found: 281.1184.

3.4.11. Acetylation of phenylpropanoids ((±)-25, 25, or 25')

To a solution of (±)-**25** (15.0mg, 0.10 mmol) in dry CH_2Cl_2 (1.0 mL) in the presence of triethylamine (Et_3N, 25.3 mg, 35 μL, 2.5 equiv.) and 4-dimethylaminopyradine (DMAP, 1.2 mg, 0.01 mmol, 0.1 equiv.), acetic anhydride (Ac_2O, 20 μL, 2.1 equiv.) was added drop wise at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was poured into ice-cold water, and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$, and brine, then dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC [12 g, *n*-hexane–EtOAc (6:1, v/v)] to give (±)-**2** (22.9 mg, 98%). According to a similar procedure, optically active **2** (23.2 mg, 99%) or **2'** (22.9 mg, 98%) was obtained from **25** or **25'** (15.0 mg, 0.10 mmol).

3.4.11.1. Racemic compound (±)-2. Colorless oil. Positive-ion ESIMS: *m/z* 257 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{13}H_{14}O_4Na$ $[M + Na]^+$: 257.0784. Found: 257.0788.

3.4.11.2. Compound 2. Colorless oil, $[\alpha]_D^{24}$ –61.2 (*c* 0.80, $CHCl_3$), $[\alpha]_D^{25}$ –60.0 (*c* 0.75, EtOH). Positive-ion ESIMS: *m/z* 257 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{13}H_{14}O_4Na$ $[M + Na]^+$: 257.0784. Found: 257.0786.

3.4.11.3. Compound 2'. Colorless oil, $[\alpha]_D^{24}$ +61.2 (*c* 0.84, $CHCl_3$), $[\alpha]_D^{25}$ +59.9 (*c* 0.79, EtOH). Positive-ion ESIMS: *m/z* 257 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{13}H_{14}O_4Na$ $[M + Na]^+$: 257.0784. Found: 257.0787.

3.4.12. Acetylation of 7-*O*-9'-linked neolignans ((±)-24, 24, or 24')

According to a similar procedure as described in Section **3.4.11.**, racemic (±)-**1** (53.8 mg, 98%), optically active **1** (54.9 mg, 99%) or **1'** (54.4 mg, 99%) was obtained from (±)-**24**, **24**, or **24'** (42.3 mg, 0.15 mmol).

3.4.12.1. Racemic compound (±)-1. Colorless oil. Positive-ion ESIMS: *m/z* 389 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{22}H_{22}O_5Na$ $[M + Na]^+$: 389.1359. Found: 389.1353.

3.4.12.2. Compound 1. Colorless oil, $[\alpha]_D^{25}$ –6.1 (*c* 2.04, $CHCl_3$). Positive-ion ESIMS: *m/z* 389 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{22}H_{22}O_5Na$ $[M + Na]^+$: 389.1359. Found: 389.1355.

3.4.12.3. Compound 1'. Colorless oil, $[\alpha]_D^{24}$ +6.1 (*c* 1.85, $CHCl_3$). Positive-ion ESIMS: *m/z* 389 $[M + Na]^+$. High-resolution positive-ion ESIMS: Calcd for $C_{22}H_{22}O_5Na$ $[M + Na]^+$: 389.1359. Found: 389.1359.

3.5. Bioassay

3.5.1. Reagents for bioassays

Dulbecco's modified Eagle's medium (DMEM, 4.5 g/L glucose) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco (Invitrogen, Carlsbad, CA, USA). Other chemicals were purchased from Wako Pure Chemical Co., Ltd (Osaka, Japan). The 24-well multiplate and 96-well microplates (Sumilon) were purchased from Sumitomo Bakelite Co., Ltd (Tokyo, Japan).

3.5.2. Cell culture

Murine B16 melanoma 4A5 cells (RCB0557)[40] were obtained from Riken Cell Bank (Tsukuba, Japan), and the cells were grown in DMEM supplemented with 10% FBS, penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C in 5% CO_2/air. The cells were harvested by incubation in phosphate-buffered saline (PBS) containing 1 mM EDTA and 0.25% trypsin for ca. 5 min at 37 °C and were used for the subsequent bioassays.

3.5.3. Melanogenesis and cell viability

Effects on theophylline-stimulated melanogenesis and viability in B16 melanoma 4A5 cells were examined according to the protocol described previously.[31–34]

3.5.4. Mushroom tyrosinase

Tyrosinase activities using L-tyrosine or 3,4-dihydroxyphenyl-L-alanine (L-DOPA) as a substrate were determined according to the protocol described previously.[30,32–34]

3.5.5. Expressions of tyrosinase, TRP-1, and TRP-2 mRNA

The expressions of tyrosinase, TRP-1, and TRP-2 mRNA were assessed according to the previously reported method.[30,33,34]

3.6. Statistical analysis

Values are expressed as mean ± S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analyses. Probability (*p*) values less than 0.05 were considered significant.

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