Molecular Docking Studies of (1E,3E,5E)-1,6-Bis(substituted phenyl)-hexa-1,3,5-triene and 1,4-Bis(substituted trans-styryl)benzene Analogs as Novel Tyrosinase Inhibitors

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We simulated the docking of the tertiary structure of mushroom tyrosinase with our compounds. From the structure-tyrosinase inhibitory activity relationship, it is notable that compounds 4, 8 and 11 showed similar or better activity rates than kojic acid which was used as a positive control. Compounds 17, 21, and 23 among benzene analogs that possess the same substituent showed significantly lower tyrosinase inhibitory effects. Therefore, we have confirmed that among the compounds showing better tyrosinase inhibitory effects than kojic acid, the compounds with triene analogs have better tyrosinase inhibitory effect than the compounds with benzene analogs. Docking simulation suggested the mechanism of compounds by several key residues which had possible hydrogen bonding interactions. The pharmacophore model underlined the features of active compounds, 4, 4′-(1E,3E,5E)-hexa-1,3,5-triene-1,6-diyl)diphenol, 5,5′-(1E,3E,5E)-hexa-1,3,5-triene-1,6-diyl)bis(2-methoxy-phenol), and 5,5′-(1E,3E,5E)-hexa-1,3,5-triene-1,6-diyl)dibenzene-1,3-diol among triene derivatives which had several hydrogen bond groups on both terminal rings. The soundness of the docking results and the agreement with the pharmacophores suggest that it can be conveniently exploited to design inhibitors with an improved affinity for tyrosinase.

Key words docking simulation; tyrosinase inhibitory activity; shared pharmacophore

Tyrosinase is involved not only in melanin synthesis in peripheral tissues but also in the substantia nigra of mice and humans.1,2) Recently, it has also been reported that secretion of neuronal tyrosinase plays an important role in antiapoptosis.3) Hence, over-expression of tyrosinase in brain might either be toxic, because of increased melanin precursors such as 3,4-dihydroxyphenylalanine (DOPA) and dopamine,4) or neuroprotective, because of the formation of neuromelanin.5) Tyrosinase has been also linked to Parkinson’s and other neurodegenerative diseases.6–8) Therefore, identification of tyrosinase inhibitors has promising potential in the treatment of such diseases.9)

In an effort to identify and explore new, potent, and safer tyrosinase inhibitors, we reported hydroxyl-substituted phenyl naphthalenes analogs of resveratrol,10) hydroxy-substituted phenyl-benzo[d]thiazole analogs,11) and 5-(substituted benzylidene)hydantoin analogs in our previous studies.12) We also found several potent tyrosinase inhibitors in natural materials10,13,14) that have an even greater potency than kojic acid, a well-known tyrosinase inhibitor.10,15) Accumulation of β-amyloid plaques in brain parenchyma and neurofibrillary tangles in the neuron16,17) has been linked to Alzheimer’s disease,18) which is a common neurodegenerative disorder.19) Many studies have reported that diphenyl triene,20) phe nolic bis-trans-styrylbenzenes21) and polyfluorinated bis-styrylbenzenes22) displayed high binding affinities to β-amyloid plaques. However, the association of these compounds with tyrosinase activity has not yet been reported.

We designed novel (1E,3E,5E)-1,6-bis(substituted phenyl)-hexa-1,3,5-triene and 1,4-bis(substituted trans-styryl)benzene analogs as potential tyrosinase inhibitors. We found that some of the compounds are more potent inhibitors than kojic acid, the standard tyrosinase inhibitor.23) Our kinetic and structure–activity relationship studies revealed novel clues to the identification, active site-binding and mechanism of action of the pharmacophores present in these inhibitors. Molecular docking was performed using AutoDock4.2 to examine the binding of the active compounds to the active site of the tyrosinase enzyme.

MATERIALS AND METHODS

Synthesis of Compound 1 A solution of trans-1,4-dibromobutene (2.0 g, 9.35 mmol) in triethylphosphite (4 mL) was refluxed for 5 h. After cooling, volatiles were evaporated. The residue was purified by silica gel column chromatography using hexane and ethyl acetate (EtOAc) (1 : 3) to give dichlorophenone 1 (3.12 g, 99%).

Synthesis of Compounds 3a–g tert-Butyldimethylsilyl chloride (1.25 eq×the number of hydroxyl groups) was added to a stirred solution of hydroxyl-substituted benzaldehydes (1.0 eq) and imidazole (2.5 eq×the number of hydroxyl groups) in methylene chloride and/or dimethylformamide (DMF) and the reaction mixture was stirred at room temperature and partitioned between methylene chloride and water. The organic layers were dried, filtered, and evaporated. The residue was purified by silica gel column chromatography using hexane and EtOAc (50:1) to give compounds 3a–g.

Synthesis of Compounds 4–8, 11, and 14 A solution of compound 1 (0.6 eq) in tetrahydrofuran (THF) and a solution of tert-butylimidethylsilyl (TBS)-protected benzaldehydes (1.0 eq, compounds 3a–g) in THF were added to a stirred suspension of NaH (1.5 eq) in THF and the reaction mixture was stirred at room temperature for 2–5 h. The reaction mixture

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was partitioned between methylene chloride and aqueous 
NH₄Cl solution and the organic layers were dried, filtered, 
and evaporated. The residue was purified by silica gel column 
chromatography using hexane and EtOAc (2:1) to give crude 
triene. To a solution of crude triene in THF and methanol 
(4:1) was added 12H Cl and the reaction mixture was stirred 
at room temperature overnight. The reaction mixture was par-
tioned between methylene chloride or EtOAc and water and 
the organic layers were washed with aqueous NaHCO₃ solu-
tion. The organic layers were dried, filtered, and evaporated 
and the residue was recrystallized or purified by silica gel 
column chromatography to give compounds 4–8, 11, and 14.

Synthesis of Compounds 9, 10, 12, and 13  A solution of 
compound 1 (0.6 eq) in THF and a solution of methoxy-substi-
tuted benzaldehydes (1 eq) in THF were added to a stirred 
suspension of NaH (1.5 eq) in THF and the reaction mixture 
was stirred at room temperature. The reaction mixture was 
partitioned between methylene chloride and aqueous NH₄Cl 
solution and the organic layers were dried, filtered, and evap-
orated. The residue was purified by silica gel column chroma-
tography using hexane and EtOAc (8:1) to give compounds 9, 
10, 12, and 13.

(E)-Tetraethyl but-2-ene-1,4-diyldiphenylophosphonate (I): Oil; 
reaction time, 5 h; yield, 99%; ¹H-NMR (400 MHz, CDCl₃) 
δ: 5.52 (m, 2H, 2×PhH), 4.01 (m, 8H, 4×OCH₂), 2.52 
(dd, 4H, J=4.0, 18.0 Hz, 1-CH₂, 4-CH₂), 1.23 (td, 12H, J=1.2, 6.8Hz, 4×CH₃); 
¹³C-NMR (100 MHz, CDCl₃) δ: 124.5 (d, J=1.5Hz, 2×Ph), 62.1 (d, J=6.1Hz, 4×OCH₂), 30.7 (d, J= 
4.6, 141.1 Hz, C₁, C₄), 16.6 (d, J=5.3Hz, 3×CH₃).

4-(tert-Butyldimethylsilyloxy)benzaldehyde (3a): Oil; reaction 
time, overnight; yield, 75%; ¹H-NMR (400 MHz, CDCl₃) 
δ: 9.84 (s, 1H, CHO), 7.75 (d, 2H, J=8.4Hz, 2-H, 6-H), 6.90 
(d, 2H, J=8.4Hz, 3-H, 5-H), 0.95 (s, 9H, t-Bu), 0.21 (s, 6H, 
2×SiCH₃); ¹³C-NMR (100 MHz, CDCl₃) δ: 191.0 (CHO), 161.7 
(C₄), 132.1 (C₂, C₆), 130.6 (Cl), 120.7 (C₃, C₅), 25.7 (3×CH₃), 18.4 (quaternary C), −4.2 (2×SiCH₃).

3,4-Bis(tert-butyldimethylsilyloxy)benzaldehyde (3b): Solid; reaction 
time, 6 h; yield, 74%; ¹H-NMR (500 MHz, CDCl₃) δ: 9.80 (s, 1H, CHO), 7.36 (d, 1H, J=7.5Hz, 6-H), 7.35 (s, 1H, 2-H), 6.94 (d, 1H, J=7.5Hz, 5-H), 0.99 (s, 18H, 2×t-Bu), 0.25 
(s, 6H, 2×SiCH₃); ¹³C-NMR (100 MHz, CDCl₃) δ: 157.8 (Cl₁, Cl₁'), 133.2 (Cl₄, Cl₄'), 132.4 (C₃, C₄'), 129.0 (C₄, C₄'), 128.3 (C₅, C₅'), 127.0 (C₇, C₇'), 116.3 (C₆, C₆'), 112.5 (C₃, C₃'); Anal. Calcd for C₈H₁₄O₃Si: C, 72.96; H, 5.54. Found: C, 73.06; H, 5.41.

4,4′-(1,1′,3,3′-di-tert-butylbiphenyl-2,2′-diyl)di(benzaldehyde) (3c): Solid; reaction time, 2h/overnight; yield, 2% 
(two-step yield); melting point, 255.9–258.6°C; ¹H-NMR (400 MHz, DMSO-d₆) δ: 9.55 (s, 2H, 2×OH), 7.23 (d, 4H, J=8.8Hz, 3-H, 5-H, 5′-H, 5″-H), 6.73 (ddd, 2H, J=3.2, 7.2, 15.6Hz, 2″-H, 5″-H), 6.69 (d, 4H, J=8.4Hz, 2-H, 6-H, 2-H, 6-H), 6.46 (d, 2H, J=15.6Hz, 1″-H, 6″-H), 6.41 (2H, J=3.2, 7.2Hz, 2″-H, 3″-H, 4″-H); ¹³C-NMR (100 MHz, DMSO-d₆) δ: 157.8 (Cl₁, Cl₁'), 133.2 (Cl₄, Cl₄'), 132.4 (C₃, C₄'), 129.0 (C₄, C₄'), 128.3 (C₅, C₅'), 127.0 (C₇, C₇'), 116.3 (C₆, C₆'), 112.5 (C₃, C₃'); Anal. Calcd for C₈H₁₄O₃Si: C, 73.06; H, 5.48. Found: C, 73.06; H, 5.41.

4,4′-(1,1′,3,3′-di-tert-butylbiphenyl-2,2′-diyl)bis(2-methoxy-
phenol) (6): Orange-colored solid; reaction time, 3.5h/ 
overnight; yield, 10% (two-step yield); melting point, 
214.3–215.7°C; ¹H-NMR (400 MHz, DMSO-d₆) δ: 9.11 (s, 2H, 2×OH), 7.04 (d, 2H, J=1.6Hz, 3-H, 3′-H), 6.83 (dd, 2H, J= 
1.6, 8.0Hz, 5-H, 5′-H), 6.81 (dd, 2H, J=3.2, 7.2, 16.0Hz, 2″-H, 5″-H), 6.69 (d, 2H, J=8.0Hz, 6-H, 6″-H), 6.47 (2H, J=16.0Hz, 1″-H, 6″-H), 6.42 (2H, J=3.2, 7.2Hz, 3″-H, 4″-H), 3.77 (s, 6H, 2×OCH₃); ¹³C-NMR (100 MHz, DMSO-d₆) δ: 145.8 (C₂, C₂'), 147.2 (Cl₁, Cl₁'), 133.2 (C₄, C₄'), 132.7 (C₃, C₃'), 129.6 (C₄, C₄'), 127.3 (C₇, C₇'), 120.6 (C₅, C₅'), 116.3 (C₆, C₆'), 110.2 (C₃, C₃'), 56.2 (2×OCH₃); Anal. Calcd for C₁₈H₁₄O₆: C, 74.06; H, 6.21. Found: C, 73.88; H, 6.04.

4,4′-(1,1′,3,3′-di-tert-butylbiphenyl-2,2′-diyl)bis(2-ethoxy-
phenol) (7): Beige solid; reaction time, 3.5h/overnight; yield, 
8% (two-step yield); melting point, 223.1–225.5°C; ¹H-NMR 
(400 MHz, DMSO-d₆) δ: 9.02 (s, 2H, 2×OH), 7.01 (s, 2H, 3-H, 3′-H), 6.83 (dd, 2H, J=2.0Hz, 5-H, 5′-H), 6.78 (dd, 2H, J=2.4, 6.5Hz).
(1E,3E,5E)-1,6-Bis(3,4,5-trimethoxyphenyl)hexa-1,3,5-triene (13): Light yellow solid; reaction time, 9.5h; yield, 27%; melting point, 203.5–207.8°C; 1H-NMR (500 MHz, CDCl3) δ: 6.81 (2dd, 2H, = J = 7.0, 15.1Hz, 2-H, 5-H), 6.65 (s, 4H, 2′-H, 2″-H, 6″-H), 6.53 (d, 2H, J = 15.1Hz, 1-H, 6-H), 6.52 (dd, 2H, J = 2.5, 7.0Hz, 3-H, 4-H), 3.91 (s, 12H, 3″-OCH3, 5″-OCH3, 3″-OCH3, 5″-OCH3), 3.87 (s, 6H, 4″-OCH3, 4″-OCH3); 13C-NMR (100 MHz, CDCl3) δ: 153.6 (C3′, C5′, C3″, C5″), 138.2 (C4′, C4″), 133.5 (C1′, C6′), 132.7 (C3′, C4′), 128.9 (C2′, C5′), 103.7 (C2′, C6′, C5′), 61.2 (4″-OCH3, 4″-OCH3), 56.3 (3″-OCH3, 3″-OCH3, 5″-OCH3, 5″-OCH3), Anal. Calcd for C23H22O6: C, 69.88; H, 6.84. Found: C, 69.94; H, 7.01.

4,4′-((1E,3E,5E)-Hexa-1,3,5-triene-1,6-diyl)bis(2,6-dimethoxyphenyl) (14): Dark brown solid; reaction time, 5h/overnight; yield, 7% (two-step yield); melting point, 217.5–220.6°C; 1H-NMR (500 MHz, CDCl3) δ: 6.75 (dd, 2H, J = 2.5, 7.0Hz, 2′″-H, 5″-H), 6.66 (s, 4H, 3-H, 5-H, 3″-H, 5″-H), 6.49 (d, 2H, J = 15.0Hz, 1″-H, 6″-H) 6.48 (dd, 2H, J = 2.5, 7.0Hz, 3″-H, 4″-H), 5.58 (s, 2H, 2×OCH3), 3.92 (s, 12H, 4″-OCH3, 4″-OCH3); 13C-NMR (100 MHz, CDCl3) δ: 147.4 (C2′, C6′, C5′, C3′), 130.5 (C1′, C1″), 133.0 (C1′, C1″), 128.7 (C2′, C6′, C5′, C3′, C6″), 127.5 (C2′, C5′), 114.3 (C3′, C5′, C3″, C5″), 55.5 (2×OCH3); Anal. Calcd for C23H22O6: C, 68.74; H, 6.29. Found: C, 68.71; H, 6.17.

Synthesis of Compound 15 A solution of α,α′-dichloro-p-xylene (40 g, 22.85 mmol) in triethylphosphite (29 mL) was refluxed for 24h. After cooling, volatiles were evaporated. The residue was purified by silica gel column chromatography using hexane and EtOAc (1:4) to give diphosphonate 15 (8.56 g, 99%).

Synthesis of Compounds 17–21, 23, and 26 A solution of 15 (0.6 eq) in THF and a solution of TBS-protected benzaldehydes (1.0 eq) in THF and a solution of methoxy-substituted benzaldehydes (0.6 eq) in THF were added to a stirred suspension of NaH (1.5 eq) in THF and the reaction mixture was stirred at room temperature for 4–8.5h. The reaction mixture was partitioned between methylene chloride and aqueous NH4Cl solution and the organic layers were dried, filtered and evaporated to give crude 1,4-bis-trans-styrylbenzenes. To a solution of crude 1,4-bis-trans-styrylbenzenes in THF and methanol (4:1) was added 12 n HCl and the reaction mixture was stirred at room temperature overnight. After evaporation of volatiles, the precipitates generated were filtered, washed with water and/or methylene chloride and/or hexane to give compounds 17–21, 23, and 26.
Synthesis of Compound 27 A solution of MEMCI (0.06 mL, 0.53 mmol) in methylene chloride (1 mL) was added to a stirred solution of 3-bromo-4-hydroxybenzaldehyde (80 mg, 0.40 mmol) and N,N'-disopropylethylamine (0.11 mL, 0.65 mmol) in methylene chloride (2 mL) and the reaction mixture was stirred at room temperature for 12 h and partitioned between methylene chloride and water. The organic layers were dried, filtered, and evaporated. The residue was purified by silica gel column chromatography using hexane and EtOAc (3:1) as the eluent to give MEM-protected benzaldehyde 16 (114.1 mg, 99%). A solution of compound 15 (89.6 mg, 0.24 mmol) in THF (2 mL) and a solution of compound 16 (114.1 mg, 0.39 mmol) in THF (2 mL) were added to a stirred suspension of NaH (23.7 mg, 0.59 mmol) in THF (2 mL) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was partitioned between methylene chloride and aqueous NH4Cl solution and the organic layers were dried, filtered and evaporated to give crude 1,4-bis-trans-styrylbenzenes. Hydrogen chloride (12%) was added to a solution of crude 1,4-bis-trans-styrylbenzenes in THF (2 mL) and methanol (1 mL) and the reaction mixture was stirred at room temperature for 2 d. The precipitates generated were filtered and washed with water and methylene chloride and hexane (2:1) to give compound 27.

Tetraethyl 1,4-Phenylenebis(methylene)phosphonate (15): White solid; reaction time, 24 h; yield, 99%; melting point, 72.5–72.8°C; 1H-NMR (400 MHz, CDCl3) δ: 7.19 (s, 4H, Ar-H), 3.95 (m, 8H, 4×OCH2), 3.07 (d, 4H, =J=20.4 Hz, 2×PXCH2), 1.18 (t, 12H, =J=7.2 Hz, 4×OCH2CH3); 13C-NMR (100 MHz, CDCl3) δ: 131.1 (d, 6H, J =5.3 Hz, C1, C4), 130.1 (d, J =1.5 Hz, C2, C3, C5, C6), 62.2 (d, J =6.8 Hz, OCH2), 33.6 (dd, =J=1.5 Hz, C18, OCH2), 16.5 (d, J =6.1 Hz, OCH2CH3).

3-Bromo-4-((2-methoxyethoxy)methylene)benzaldehyde (16): Oil; reaction time, 12 h; yield, 99%; 1H-NMR (500 MHz, CDCl3) δ: 9.86 (s, 1H, CHO), 8.09 (d, 1H, =J=2.0 Hz, 2-H), 7.79 (dd, 1H, =J=2.0, 8.5 Hz, 6-H), 7.32 (d, 1H, =J=8.55 Hz, 5-H), 5.44 (s, 2H, OCH3O), 3.88 (m, 2H, OCH2), 3.56 (m, 2H, OCH2), 3.36 (3H, OCH3).

4,4′-(1E,1′E)-2,2′-(1,4-Phenylene)bis(ethene-1,2-diyldi)phenol (17): Pale greenish solid; reaction time, 4.5 h/overnight; yield, 38.8% (two-step yield); melting point, 273.1–273.4°C; 1H-NMR (500 MHz, CDCl3): δ: 9.55 (s, 2H, 1-OH, 1′-OH), 7.50 (s, 4H, Ar-H), 7.41 (d, 4H, =J=8.55 Hz, 3-H, 3′-H, 5-H, 5′-H), 7.14 (d, 2H, =J=16.5 Hz, 2×vinylic H), 6.99 (d, 2H, =J=16.5 Hz, 2×vinylic H), 6.76 (d, 4H, =J=8.55 Hz, 2-H, 6-H, 2″-H, 6″-H). 13C-NMR (100 MHz, DMSO-d6) δ: 157.9 (Cl1, C1′), 136.9 (C′1″, C′4″), 128.9 (C′4, C′7), 128.7 (2×vinylic C), 128.5 (C3, C5, C3′, C5′), 127.0 (C2″, C3″, C5″, C6″), 125.5 (2×vinylic C), 116.2 (C2, C6, C2′, C6′); Anal. Calcled for C22H17O3C: C 84.05; H, 5.77. Found: C 83.99, H, 5.94.

4,4′-(1E,1′E)-2,2′-(1,4-Phenylene)bis(ethene-1,2-diyldi)benzene-1,3-diol (18): Greenish brown solid; reaction time, 5.5 h/overnight; yield, 14% (two-step yield); melting point, 235.1–235.9°C; 1H-NMR (400 MHz, CDCl3): δ: 8.98 (brs, 4H, 1-H, 1′-H, 2-OH, 2′-OH), 7.47 (s, 4H, Ar-H), 7.04 (d, 2H, =J=16.0 Hz, 2×vinylic H), 6.97 (s, 2H, 3-H, 3′-H), 6.91 (d, 2H, =J=16.0 Hz, 2×vinylic H), 6.84 (d, 2H, =J=8.4 Hz, 5-H, 5′-H), 6.70 (d, 2H, =J=8.4 Hz, 6-H, 6′-H); 13C-NMR (100 MHz, DMSO-d6) δ: 146.3 (C1, C1′), 146.1 (C2, C2′), 136.9 (C′1″, C′4″), 129.4 (C′4, C′7), 129.1 (2×vinylic C), 127.0 (C2″, C3″, C5″, C6″), 125.4 (2×vinylic C), 119.3 (C5, C5′), 116.4 (C6, C6′), 114.0 (C3, C3′); Anal. Calcled for C22H18O4C: C 76.29; H, 5.24. Found: C 76.04, H, 5.33.

5,5′-(1E,1′E)-2,2′-(1,4-Phenylene)bis(ethene-1,2-diyl)dibenzen-1,3-diol (23): Brown solid; reaction time, 4 h/overnight; yield, 26% (two-step yield); 1H-NMR (400 MHz, CDOD) δ: 7.48 (s, 4H, Ar-H), 7.02 (d, 2H, =J=16.4 Hz, 2×vinylic H), 6.98 (d, 2H, =J=16.4 Hz, 2×vinylic H), 6.47 (d, 4H,
1,4-Bis(2,4-dimethoxy styryl)benzene (24): Green yellow solid; reaction time, 2 d; yield, 35%; melting point, 181.3–182.6°C; 1H-nMR (400 MHz, DMSO-d6); 8.5 h/overnight; yield, 23% (two-step yield); melting point, 84.8°C.

Mushroom tyrosinase, L-tyrosinase [3-(4-hydroxy phenyl)-S-alanine, (2S)-2-amino-3-(4-hydroxy phenyl)-propiionic acid], and kojic acid [5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one] were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Assay to Measure Inhibitory Effects on Mushroom Tyrosinase Mushroom tyrosinase was used to measure tyrosinase activity for the entire study. Tyrosinase activity was determined as described previously with minor modification.  Briefly, 20 µL of aqueous mushroom tyrosinase solution (1000 units) was added to a 96-well microplate (Nunc, Denmark) in a 200 µL assay mixture containing 1 mM L-tyrosine solution and 50 mM phosphate buffer (pH 6.5). The assay mixture was incubated at 25°C for 30 min. Following incubation, the amount of dopachrome produced was determined spectrophotometrically at 492 nm (OD992) using a microplate reader (Hewlett Packard, Palo Alto, CA, U.S.A.). IC50 or inhibitory concentration 50, is the concentration of a compound that inhibits the maximal enzyme velocity by half. IC50 is derived from the X-axis on an inhibitor concentration versus product formation curve and is determined from the intersection of a linear regression line in a Lineweaver–Burk plot at varying L-tyrosine concentration. Reaction kinetics required modification of the Michaelis–Menten equation due to competitive inhibition by the compounds together with substrate inhibition by L-tyrosine. The results from 3 experiments are shown.

Kinetic Analysis of Tyrosinase Inhibition Varying L-tyrosine concentrations (0.25 to 2 mM) as a substrate, 20 µL of aqueous mushroom tyrosinase solution (1000 units), and 50 mM potassium phosphate buffer (pH 6.5) with or without test samples at various concentrations, were added to a 96-well plate to a total volume of 200 µL. Using a microplate reader, the initial rate of dopachrome formation was determined by the increase in absorbance in a 96-well plate at a wavelength of 492 nm per minute (ΔOD992/min). The Michaelis constant (Km) and maximal velocity (Vmax) of tyrosinase activity were determined using a Lineweaver–Burk plot at varying L-tyrosine concentration. Reaction kinetics required modification of the Michaelis–Menten equation due to competitive inhibition by the compounds together with substrate inhibition by L-tyrosine. The results of the 3 experiments are shown.

In Silico Docking between Tyrosinase and Target Compounds For docking simulations, we used the Dock6.3 and AutoDock4.2 program. Among the many tools available for in silico protein-ligand docking, AutoDock4.2 is the most commonly used because of its automated docking capability.  The 3D structure of tyrosinase was used the crystal structure of Agaricus bisporus (PDB ID: 2Y9X) without homology modeling. To define the docking pocket, we used a predefined active site of 2Y9X in complex with kojic acid which is used as the inhibitor of tyrosinase. We conducted simulations of the docking of tyrosinase to novel compounds and kojic acid. To prepare compounds for the docking simulation, we performed the following steps: (1) conversion of 2D structures into 3D structures, (2) calculation of charges, and (3) addition of hydrogen atoms using the ChemOffice program (http://www.cambridgeoft.com).

Pharmacophore Prediction of Inhibitor Compounds Pharmacophore is an ensemble of ligand features that is necessary for interaction with a specific receptor for biological response.  Because the three active inhibitors were similar with respect to their chemical structures and kinetic responses for tyrosinase inhibition, we searched the pharmacophores of inhibitor compounds. The pharmacophore model was generated by the LigandScout 3.0 program.  On the basis of the atom types, the chemical features of inhibitor compounds were defined as various pharmacophore elements, such as a hydrogen bond acceptor, a hydrogen bond donor, a positive
ionizable area, a negative ionizable area, hydrophobic interactions, and an aromatic ring. Specifically, we searched shared pharmacophore by aligning predicted pharmacophore of inhibitor compounds.

**Statistical Analysis** Inhibition of tyrosinase activity is expressed as a percentage of inhibition based on $100 - \frac{(A \times 100)}{B}$, where $A=\text{OD}_{492}$ with a test sample and $B=\text{OD}_{492}$ without a test sample. Data collected have a mean, standard error ($n=3$). Statistical significance of the differences among groups was determined by one-factor analysis of variance (ANOVA) followed by the Fisher’s protected least-significant difference post hoc test. Values of $p<0.05$ were considered statistically significant.
RESULTS AND DISCUSSION

The general strategy for the syntheses of the target compounds, (1\(E\),3\(E\),5\(E\))-1,6-bis(substituted phenyl)hexa-1,3,5-triene derivatives 4–14, and 1,4-bis(substituted trans-styryl)benzene derivatives 17–27 was based on the Horner–Emmons olefination reaction between diphosphonate 1 or 15 and an appropriate benzaldehyde, as outlined in Charts 1 and 2. Diphosphonates 1 and 15 were prepared by refluxing trans-1,4-dibromobutene or \(\alpha\),\(\alpha\)'-dichloro-\(p\)-xylene in triethylphosphite. Benzaldehydes bearing a phenolic hydroxyl group were protected with tert-butyldimethylsilyl chloride (TBSCl) or 2-methoxyethoxymethyl chloride (MEMCl) for the Horner–Emmons olefination reaction. Olefination reaction of diphosphonates 1 and 15 with benzaldehydes afforded only (\(E\))-olefin products, which was confirmed by \(J\) values (ca. 16 Hz) of vinylic proton peaks of \(^{1}H\)-NMR. Generally, \(J\) value of the vinylic proton peak of (\(E\))-olefin shows about 16 Hz, whereas that of (\(Z\))-olefin exhibits about 10 Hz. Compounds protected with TBS or MEM ether were kept under acidic conditions to produce the final compounds with a phenolic hydroxyl substituent.

Among the compounds (A and B), the compounds 4, 8, and 11 showed similar or better activity rates than kojic acid (56.16% at 50 \(\mu\)m) which was used as a positive control. The chemical structures of these compounds are shown in Table 1, and the benzene analogs (17, 21, 23) that possess the same substituent showed significantly lower tyrosinase inhibitory effects (Table 2). 4-Hydroxyphenyl triene 4 showed the most potent inhibitory activity among the synthesized compounds and its IC\(_{50}\) value was about 30-fold more potent than that of kojic acid.

### Table 1. Substitution Pattern and Tyrosinase Inhibition Effects of (1\(E\),3\(E\),5\(E\))-1,6-Bis(substituted phenyl)hexa-1,3,5-triene Analogs 4–14

<table>
<thead>
<tr>
<th>Compound</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>R(^3)</th>
<th>R(^4)</th>
<th>Tyrosinase inhibition (%)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>72.96±0.48</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>13.17±4.78</td>
</tr>
<tr>
<td>6</td>
<td>H</td>
<td>OMe</td>
<td>OH</td>
<td>H</td>
<td>8.67±3.39</td>
</tr>
<tr>
<td>7</td>
<td>H</td>
<td>OEt</td>
<td>OH</td>
<td>H</td>
<td>17.45±6.42</td>
</tr>
<tr>
<td>8</td>
<td>H</td>
<td>OH</td>
<td>OMe</td>
<td>H</td>
<td>66.45±1.35</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>10.84±6.88</td>
</tr>
<tr>
<td>10</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>H</td>
<td>9.61±4.59</td>
</tr>
<tr>
<td>11</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>51.32±1.38</td>
</tr>
<tr>
<td>12</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>4.00±4.72</td>
</tr>
<tr>
<td>13</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>OMe</td>
<td>3.97±4.13</td>
</tr>
<tr>
<td>14</td>
<td>H</td>
<td>OMe</td>
<td>OH</td>
<td>OMe</td>
<td>NI</td>
</tr>
</tbody>
</table>

\(^a\) Tyrosinase inhibition was measured using \(\alpha\)-tyrosine as the substrate at 50 \(\mu\)m. Values represent means±S.E. of three experiments, NI (no inhibition).

### Table 2. Substitution Pattern and Tyrosinase Inhibition Effects of 1,4-Bis(substituted trans-styryl)benzene Analogs 17–27

<table>
<thead>
<tr>
<th>Compound</th>
<th>R(^1)</th>
<th>R(^2)</th>
<th>R(^3)</th>
<th>R(^4)</th>
<th>Tyrosinase inhibition (%)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>7.02±4.29</td>
</tr>
<tr>
<td>18</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>NI</td>
</tr>
<tr>
<td>19</td>
<td>H</td>
<td>OMe</td>
<td>OH</td>
<td>H</td>
<td>NI</td>
</tr>
<tr>
<td>20</td>
<td>H</td>
<td>OEt</td>
<td>OH</td>
<td>H</td>
<td>22.12±3.12</td>
</tr>
<tr>
<td>21</td>
<td>H</td>
<td>OH</td>
<td>OMe</td>
<td>H</td>
<td>NI</td>
</tr>
<tr>
<td>22</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>H</td>
<td>NI</td>
</tr>
<tr>
<td>23</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>12.13±13.91</td>
</tr>
<tr>
<td>24</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>9.04±6.35</td>
</tr>
<tr>
<td>25</td>
<td>H</td>
<td>OMe</td>
<td>OMe</td>
<td>OMe</td>
<td>27.00±2.34</td>
</tr>
<tr>
<td>26</td>
<td>H</td>
<td>OMe</td>
<td>OH</td>
<td>OMe</td>
<td>1.46±2.61</td>
</tr>
<tr>
<td>27</td>
<td>H</td>
<td>Br</td>
<td>OH</td>
<td>H</td>
<td>10.33±4.05</td>
</tr>
</tbody>
</table>

\(^a\) Tyrosinase inhibition was measured using \(\alpha\)-tyrosine as the substrate at 50 \(\mu\)m. Values represent means±S.E. of three experiments, NI (no inhibition).
acid, which was used as a positive control (Table 3). As shown in Table 1, introduction of an additional hydroxyl, methoxy or ethoxy substituent (e.g., 5, 6, 7) at the R² position on the phenyl ring of compound 4 decreased the inhibitory activity, and replacement of the 4-hydroxy group of compound 4 by a methoxy group also reduced the inhibitory activity (e.g., 9). However, in case of replacement of the hydroxyl group of compound 4 by a methoxy group, introduction of an additional hydroxyl group at the R² position (e.g., 8) recovered the tyrosinase inhibitory effect. Triene derivatives bearing 3,4-dimethoxy (e.g., 10), 2,4-dimethoxy (e.g., 12), 3,4,5-trimethoxy (e.g., 13) or 3,5-dimethoxy-4-hydroxy (e.g., 14) substituent showed low inhibitory activity or no activity. A compound with a hydroxyl group at both the R² and R⁴ position (e.g., 11) also exhibited potent inhibitory activity.

We characterized the inhibition kinetics of compounds 4, 8, and 11, which showed a significant tyrosinase inhibitory effect. To explore the mechanism of active inhibitors, we conducted a study of the kinetic behavior of tyrosinase activity in the presence of inhibitors. We measured the reaction rates in the presence of active inhibitors at various concentrations of l-tyrosine as a substrate. As the concentrations of active inhibitors 4, 8, and 11 increased, Kₘ values gradually increased, but Vₘₐₓ values did not change, thereby indicating the inhibitors all act as competitive inhibitors of mushroom tyrosinase (Fig. 1). The general order of inhibition potency was found to be: compound 4 > compound 8 > compound 11 > kojic acid (Fig. 1, Table 3). A comparison of the Kₘ and Kᵢ values of the compounds with that of kojic acid revealed that they possess much higher affinity to tyrosinase than kojic acid. It is likely that this high potency renders them more selective toward tyrosinase than kojic acid.

Through simulating of docking, we were able to detail the binding configuration of tertiary structure of mushroom tyrosinase with active compounds, supporting the hypothesis that active compounds interact with residues in the active site of tyrosinase (Fig. 2). We found that kojic acid and the novel inhibitor compounds bound the inner region of tyrosine active site.
Copper ions are shown as gold balls. Kojic acid, which was used as a control compound, is shown in magenta. Compound 4 is shown in red, compound 8 is shown in cyan, and compound 11 is shown in blue. The binding energies of compounds were $-6.59 \text{ kcal/mol}$ (compound 4), $-6.05 \text{ kcal/mol}$ (compound 8), $-5.56 \text{ kcal/mol}$ (compound 11), and $-4.20 \text{ kcal/mol}$ (kojic acid). (Color images were converted into gray scale.)

![Docking Simulation](image1)

**Fig. 2. The Docking Simulation between Tyrosinase and Kojic Acid or Inhibitor Compounds**

Copper ions are shown as gold balls. Kojic acid, which was used as a control compound, is shown in magenta. Compound 4 is shown in red, compound 8 is shown in cyan, and compound 11 is shown in blue. The binding energies of compounds were $-6.59 \text{ kcal/mol}$ (compound 4), $-6.05 \text{ kcal/mol}$ (compound 8), $-5.56 \text{ kcal/mol}$ (compound 11), and $-4.20 \text{ kcal/mol}$ (kojic acid). (Color images were converted into gray scale.)

![Docking Simulation](image2)

**Fig. 3. Possible Hydrogen Bonding Interactions between Tyrosinase Residues and Inhibitor Compounds or Kojic Acid**

We searched for hydrogen binding interactions between tyrosinase and inhibitor compounds or kojic acid in the simulated docked structures. The pharmacophore model was generated using the LigandScout 3.0 program. The red arrow is a hydrogen bond acceptor, the green arrow is a hydrogen bond donor, and the yellow rings are hydrophobic regions. Only Met280 residue of the tyrosinase was responsible for the hydrogen bonding interactions with kojic acid. However, His296 (compounds 4, 11) residue of the tyrosinase was predicted to exert hydrogen bonding interactions with target compounds. (Color images were converted into gray scale.)

![Docking Simulation](image3)
had similar a tendency with the inhibition potency by kinetics results. Further, we found differences in the docking position of kojic acid compared to the novel compounds.

Additionally, we searched for hydrogen bonding interactions between tyrosinase and inhibitor compounds or kojic acid. Only the Met280 residue of the tyrosinase was responsible for the hydrogen bonding interactions with kojic acid, as shown in Fig. 3. However, His296 (compounds 4, 11) residue of tyrosinase was predicted to form hydrogen bonding interactions with the compounds (Fig. 3). Although compound 4 had one-hydrogen bonding interaction in the docking simulation with X-ray crystallography structure of tyrosinase, compound 4 was higher binding affinity than compounds 8 and 11 because the binding affinity of docking simulation is considered using various energy terms such as electrostatic energy, van der Waals energy and the solvation energy as well as hydrogen bonding interaction. These residues might function as key determinants of inhibitor activity and have an important effect on binding affinity. Shared pharmacophore results supported the hydrogen bonding interaction predicted by the docking simulation. The inhibitor compounds shared two features, hydrogen bonding acceptors on both terminal rings of the compounds (Fig. 4).

The docking simulation suggested the mechanism for enzyme binding by the compounds, and allowed us to identify the key residues that had possible hydrogen bonding interactions. The pharmacophore model underlined the features of inhibitor compounds required for optimal tyrosinase inhibition. The validity of the docking results and their agreement with the shared pharmacophores suggest that they can be exploited conveniently to design inhibitors with an improved affinity for tyrosinase.

CONCLUSION

(1(E,3E,5E))-1,6-Bis(substituted phenyl)hexa-1,3,5-triene and 1,4-bis(substituted trans-styryl)benzene analogs were synthesized and their tyrosinase inhibitory activity was evaluated. Among triene derivatives, compounds 4, 8, and 11 were found to be more potent than, or similar to, kojic acid, which was used as a positive control. In the kinetic analyses, we found that the compounds acted as competitive inhibitors of mushroom tyrosinase, with the same $V_{max}$ value regardless of the concentration of compounds. Therefore, the inhibitory activity of the compounds might result from binding at the same site as the copper-containing active site of mushroom tyrosinase. We also simulated the docking of mushroom tyrosinase of the tertiary structure with inhibitor compounds. The results suggest that the high affinity of the scaffold of (1E,3E,5E)-1,6-bis(substituted phenyl)hexa-1,3,5-triene analogs is most likely due to hydrogen-bonding interactions with one active site residues, His296 (compounds 4, 11). The pharmacophore model underlined the features of compounds with tyrosinase inhibitory activity, 4, 8, and 11, which had several hydrogen bond groups on both terminal rings.

The docking results supported a pharmacophore model which clarified the key features required for optimal tyrosinase inhibition. This study supports the possibility of compounds 4, 8, and 11 among of (1(E,3E,5E))-1,6-bis(substituted phenyl)hexa-1,3,5-triene analogs acting as pharmacophores in tyrosinase inhibition.

Acknowledgments

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REFERENCES


