Journal of Industrial and Engineering Chemistry 91 (2020) 285–295

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Highly Regioselective Preparation and Characterization of New 6-*O*-Substituted Dieckol Derivatives

Yongkyun Kim[a], Jooseok Shin[a], Su Min Kang[a], Jinyoung Song[b], Hyeon-Cheol Shin[c], Young-Sam Keum[b], Hye Jeong Hwang[c,*], Kwangyong Park[a,*]

[a] School of Chemical Engineering and Material Science, Chung-Ang University, 84 Heukseok-ro, Dongjak-gu, Seoul 03722, Republic of Korea
[b] College of Pharmacy and Integrated Research Institute for Drug Development, Dongguk University, 32 Dongguk-ro, Goyang, Gyeonggi-do 10326, Republic of Korea
[c] Center for Molecular Intelligence, The State University of New York, Korea, 119 Songdomunhwa-ro, Yeonsu-gu, Incheon 21985, Republic of Korea

ARTICLE INFO

Article history:
Received 24 June 2020
Received in revised form 31 July 2020
Accepted 9 August 2020
Available online 16 August 2020

Keywords:
Substituted dieckol
Regioselective substitution
Free-radical scavenging activity
Reducing power
Anticancer activity

ABSTRACT

Dieckol is a brown algae-derived polyphenol that has broad bioactivity and low toxicity. Therefore, it is a promising lead compound for the development of therapeutic agents to treat various pathological conditions, including viral infections, allergies, diabetes, skin ageing, cancers, and neurodegenerative conditions. To exploit the pharmacological potential of dieckol, its stability, solubility, pharmacokinetics, and drug delivery must be improved. This can be achieved by the controlled modification of the hydroxyl groups, but the presence of eleven nearly equivalent hydroxyl groups makes the task highly challenging. In this study, the regioselectivities in five substitution reactions of the hydroxyl groups of dieckol under various S_N2 reaction conditions were investigated. After reaction optimization, five substituents (methyl, benzyl, methoxymethyl, 3-hydroxypropyl, and 3-(ethoxycarbonyl)propyl) could be introduced at the 6-*O*position of dieckol with surprisingly high regioselectivity, as confirmed by 2D-NMR spectroscopic analyses. The prepared dieckol derivatives showed antioxidant and anticancer activities analogous to those of unmodified dieckol, indicating that the mono-*O*-substitutions did not affect the biochemical and biological characteristics of dieckol. Therefore, the proposed methodology for the mono-*O*-substitution of a specific oxygen of dieckol is a powerful tool to add various pharmaceutical attributes to dieckol, thus contributing to the development of various dieckol-based drug candidates.

1 Introduction

Phlorotannins are phloroglucinol-based polyphenols predominantly extracted from marine brown algae and are well-known for their therapeutic properties [1–6]. Among the many phlorotannin compounds, those having a dibenzo-*p*-dioxin skeleton are termed eckols. Since eckol was first isolated from *Ecklonia kurome* Okamura, various eckol derivatives including 7-phloroeckol, 2-phloroeckol, dieckol, 6,6-bieckol, 8,8'-bieckol, and phlorofucofuroeckol-A have been isolated from several other species of brown algae and reported to exhibit interesting bioactivities [7–10].

In particular, dieckol, a dimerized analog of eckol bearing an ether linkage between the dibenzo-*p*-dioxin skeleton and phloroglucinol, has attracted significant attention and has been thoroughly investigated because of its potent antioxidant [11,12], anti-inflammatory [13,14], antiviral [15,16], anti-allergy [17], antidiabetic [18,19], skin anti-aging [20], and anticancer properties [21–27]. Additionally, it is a potential drug candidate for the treatment of osteoclastogenesis as well as Alzheimer's and Parkinson's diseases [28–32]. Moreover, dieckol has been shown to have excellent safety for oral administration, as determined by in-depth good laboratory practice (GLP) studies and several human

Abbreviations: DMF, *N*,*N*-Dimethylformamide; GLP, good laboratory practice; DPPH, 2,2-diphenyl-1-picryhydrazyl; TPTZ, 2,4,6-tripyridyl-s-triazine; ABTS, [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TLC, thin-layer chromatography; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; ESI, electrospray ionization; TOF, time of flight; UHR-MS, ultrahigh-resolution mass spectrometry; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple-bond correlation; RPMI, Roswell Park Memorial Institute; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.

* Corresponding author.
E-mail addresses: hyejeong.hwang@sunykorea.ac.kr (H.J. Hwang), kypark@cau.ac.kr (K. Park).

https://doi.org/10.1016/j.jiec.2020.08.012
1226-086X/

trials evaluating botanical extracts containing dieckol as the major component [33–36]. In summary, dieckol is a highly promising lead compound for the development of a spectrum of drugs for the treatment of various diseases.

In the development of drug candidates for a specific disease via the optimization of a lead compound, precise chemical control over the introduction of new attributes is required, and the original biochemical or biological properties must be maintained or improved. However, synthetic methodologies for the modification of dieckol or other eckol analogs are scarce. In a study, the methylation and acetylation of all eleven hydroxyls of dieckol has been reported [37]. Subsequently, a study on the mono-*O*-propargylation of dieckol was reported [38,39], and this is, to date, the only example of mono-*O*-substitution reaction. However, the exact position of the mono-*O*-propargylation on dieckol was unclear. This lack of precise synthetic approaches limits the exploitation of the pharmaceutical potential of dieckol.

Our research regarding the elucidation and application of the bioactivities of dieckol necessitates the regioselective introduction of various chemical moieties on specific hydroxyl groups in dieckol; hence, precise control of the reaction conditions is required to achieve regioselective modification of a single hydroxyl group of the eleven nearly equivalent ones. Exact structural identification of the product structure necessitated careful 2D-nuclear magnetic resonance (NMR) analyses of nuclear Overhauser effect spectroscopy (NOESY), ^{1}H–^{13}C heteronuclear multiple-bond correlation (HMBC) spectra, and heteronuclear single quantum coherence (HSQC) spectra. Herein, we report the development of a regioselective methodology for the mono-*O*-substitution of dieckol (Scheme 1) and the structural identification of the products. Furthermore, we report the biochemical and biological characteristics of the derivatives based on antioxidative and anticancer bioassays, and compare them to those of unmodified dieckol.

2 Experimental

2.1 Materials

Dieckol was obtained from Botamedi Inc. (Jeju, Korea). *N,N*-Dimethylformamide (DMF), dimethyl sulfate, benzyl bromide, 2,2-diphenyl-1-picryhydrazyl (DPPH), 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tripyridyl-*s*-triazine (TPTZ), and penicillin were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Ethyl-4-bromo butyrate, 3-bromo-1-propanol, and 3,4-dihydro-2*H*-pyran were purchased from Alfa Aesar (Ward Hill, MA, USA). Chloromethyl methyl ether was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Acetone was dehydrated with 4-Å molecular sieves before use. Analytical thin-layer chromatography (TLC) was performed using Merck Kieselgel 60 F_{254} pre-coated plates (0.25 mm) with a fluorescent indicator and visualized under UV light (254 and 365 nm). Column chromatography was performed on silica gel 60, 70–230 mesh. Roswell Park Memorial Institute (RPMI) 1640 medium, Dulbecco's Modified Eagle Medium (DMEM), and fetal bovine serum (FBS) were obtained from Welgene Inc. (Kyungsan, Korea). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was purchased from Molecular Probes Inc. (Eugene, OR, USA).

2.2 Analytical Methods

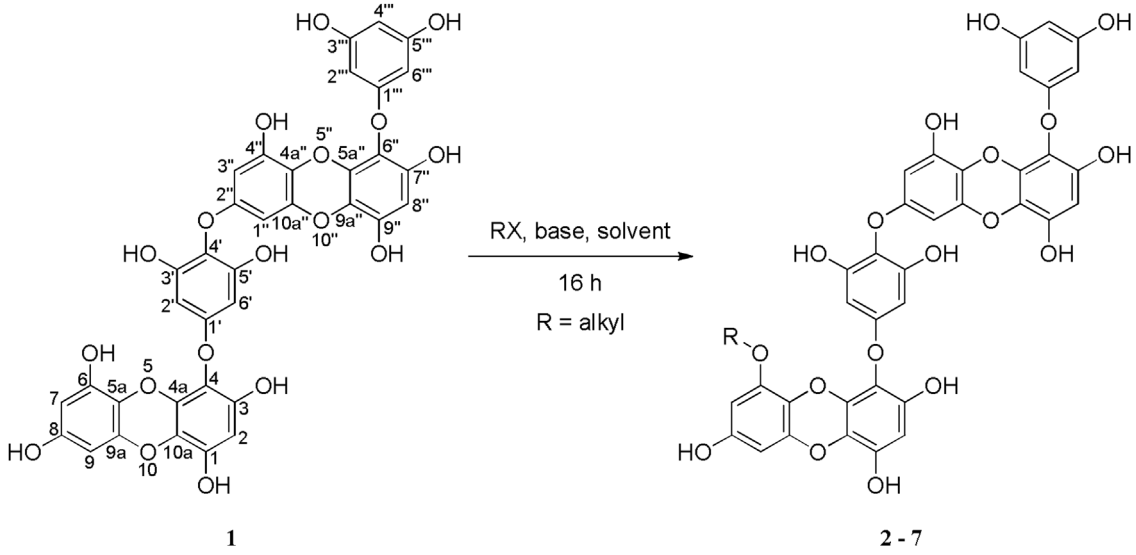
^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) were acquired on Varian NMR system 600-MHz spectrometers (VNS, Varian, Palo Alto, CA, USA) using DMSO-d_6 as a solvent. Chemical shifts were referenced to the residual solvent peaks (δ_H 2.50 and δ_C 39.5 for DMSO-d_6 in ^{1}H NMR and ^{13}C NMR, respectively). All coupling constants, *J*, are reported in hertz (Hz). Ultrahigh-resolution mass spectrometry (UHR-MS) analysis was conducted on a Bruker HPLC system (Compact, Bruker, Billerica, MA, USA) with electrospray ionization (ESI) in positive ion mode. UHR-MS was measured employing Quadrupole and time of flight (TOF) parallel conjugation methods. Spectrophotometric measurements of all antioxidant activities were performed in 96-well microplates on a UV–VIS spectrophotometer (SPECTROstar^{Nano} BMG Labtech, Ortenberg, Germany).

2.3 Synthesis and Analysis

All reactions were performed under an inert atmosphere of N_2.

2.3.1 Analysis of 4-[4-[[6-(3,5-dihydroxyphenoxy)-4,7,9-trihydroxydibenzo[b,e][1,4]dioxin-2-yl]oxy]-3,5-dihydroxyphenoxy] dibenzo[b,e][1,4]dioxin-1,3,6,8-tetrol (1, dieckol).

TLC R_f = 0.18 (Chloroform: Methanol: Water = 60:30:4, v/v/v); ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.67 (s, 1H, $C_{4''}$-OH), 9.57 (s, 1H, C_6-



1 **2 - 7**

Scheme 1. Regioselective mono-*O*-substitution of dieckol.

OH), 9.47 (s, 1H, C_1-OH), 9.42 (s, 1H, $C_{9''}$-OH), 9.32 (s, 2H, $C_{3',5'}$-OH), 9.25 (s, 1H, C_3-OH), 9.20 (s, 1H, $C_{7''}$-OH), 9.19 (s, 1H, C_8-OH), 9.13 (s, 2H, $C_{3''',5'''}$-OH), 6.16 (s, 1H, C_2-H), 6.14 (s, 1H, $C_{8''}$-H), 6.02 (d, J = 2.84 Hz, 1H, $C_{3''}$-H), 5.99 (d, J = 2.73 Hz, 1H, C_7-H), 5.95 (s, 2H, $C_{2',6'}$-H), 5.82 (d, J = 2.84 Hz, 1H, $C_{1''}$-H), 5.81 (d, J = 2.73 Hz, 1H, C_9-H), 5.80 (t, J = 2.09 Hz, 1H, $C_{4'''}$-H), 5.72 (d, J = 2.09 Hz, 2H, $C_{2''',6'''}$-H); ^{13}C-NMR (150 MHz, DMSO-d_6) δ 160.69 (s, 1 C, 1'''-C), 159.20 (s, 1 C, 3'''-C), 159.20 (s, 1 C, 5'''-C), 156.30 (s, 1 C, 1'-C), 154.63 (s, 1 C, 2''-C), 153.47 (s, 1 C, 8-C), 151.55 (s, 1 C, 5'-C), 151.55 (s, 1 C, 5'-C), 146.49 (s, 1 C, 7''-C), 146.45 (s, 1 C, 6-C), 146.36 (s, 1 C, 4''-C), 146.31 (s, 1 C, 3-C), 142.99 (s, 1 C, 9a-C), 142.80 (s, 1 C, 10a''-C), 142.37 (s, 1 C, 1-C), 142.27 (s, 1 C, 9''-C), 137.63 (s, 1 C, 4a-C), 137.46 (s, 1 C, 5a''-C), 124.62 (s, 1 C, 4'-C), 124.42 (s, 1 C, 4a''-C), 123.63 (s, 1 C, 10a-C), 123.55 (s, 1 C, 9a''-C), 122.99 (s, 1 C, 5a-C), 122.68 (s, 1 C, 4-C), 122.61 (s, 1 C, 6''-C), 98.90 (s, 1 C, 7-C), 98.75 (s, 1 C, 8''-C), 98.63 (s, 1 C, 2-C), 98.46 (s, 1 C, 3''-C), 96.63 (s, 1 C, 4'''-C), 94.90 (s, 1 C, 2'-C), 94.90 (s, 1 C, 6'-C), 94.27 (s, 1 C, 9-C), 94.05 (s, 1 C, 2'''-C), 94.05 (s, 1 C, 6'''-C), 93.94 (s, 1 C, 1''-C)

2.3.2 Preparation of 4-[4-[[6-(3,5-dihydroxyphenoxy)-4,7,9-trihydroxydibenzo[b,e][1,4]dioxin-2-yl]oxy]-3,5-dihydroxyphenoxy]-6-methoxydibenzo[b,e][1,4]dioxin-1,3,8-triol (2).

Dry acetone (100 mL) was added to a mixture of dieckol (300 mg, 0.404 mmol) and anhydrous potassium carbonate (55.8 mg, 0.404 mmol) in a round-bottomed flask under N_2 atmosphere. After stirring for 10 min, dimethyl sulfate (38.3 μL, 0.404 mmol) was added in small portions. The reaction mixture was stirred at 25 °C for 16 h, diluted with EtOAc (200 mL) and washed successively with 1% aqueous HCl, water, and saturated aqueous NaCl. Thereafter, the organic fraction was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (MeOH: $CHCl_3$ = 1:10) to afford 6-*O*-methyl dieckol (196 mg, 64%) as a pale yellow powder.

TLC R_f = 0.35 ($CHCl_3$: MeOH: H_2O = 60:30:4, v/v/v); ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.66 (s, 1H, $C_{4''}$-OH), 9.53 (s, 1H, C_1-OH), 9.42 (s, 2H, $C_{8,9''}$-OH), 9.33 (s, 2H, $C_{3',5'}$-OH), 9.30 (s, 1H, C_3-OH), 9.20 (s, 1H, $C_{7''}$-OH), 9.13 (s, 2H, $C_{3''',5'''}$-OH), 6.19 (s, 1H, C_2-H), 6.15 (s, 1H, $C_{8''}$-H), 6.09 (d, J = 2.68 Hz, 1H, C_7-H), 6.04 (d, J = 2.86 Hz, 1H, $C_{3''}$-H), 5.98 (d, J = 2.64 Hz, 1H, C_9-H), 5.96 (s, 2H, $C_{2',6'}$-H), 5.81 (t, J = 2.09 Hz, 1H, $C_{4'''}$-H), 5.79 (d, J = 2.86 Hz, 1H, $C_{1''}$-H), 5.73 (d, J = 2.09 Hz, 2H, $C_{2''',6'''}$-H), 3.66 (s, 3H, C_6-OCH_3); ^{13}C-NMR (150 MHz, DMSO-d_6) δ 160.70 (s, 1 C, 1'''-C), 159.21 (s, 1 C, 3'''-C), 159.21 (s, 1 C, 5'''-C), 156.28 (s, 1 C, 1'-C), 154.63 (s, 1 C, 2''-C), 153.85 (s, 1 C, 8-C), 151.58 (s, 1 C, 3'-C), 151.58 (s, 1 C, 5'-C), 148.66 (s, 1 C, 6-C), 146.49 (s, 1 C, 7''-C), 146.46 (s, 1 C, 3-C), 146.36 (s, 1 C, 4''-C), 142.81 (s, 1 C, 10a''-C), 142.75 (s, 1 C, 9a-C), 142.42 (s, 1 C, 1-C), 142.29 (s, 1 C, 9''-C), 137.47 (s, 1 C, 5a''-C), 137.43 (s, 1 C, 4a-C), 124.68 (s, 1 C, 4'-C), 124.47 (s, 1 C, 4a''-C), 123.85 (s, 1 C, 5a-C), 123.56 (s, 1 C, 10a-C), 123.56 (s, 1 C, 9a''-C), 122.72 (s, 1 C, 4-C), 122.62 (s, 1 C, 6''-C), 98.85 (s, 1 C, 2-C), 98.75 (s, 1 C, 8''-C), 98.61 (s, 1 C, 3''-C), 96.63 (s, 1 C, 4'''-C), 96.39 (s, 1 C, 7-C), 95.64 (s, 1 C, 9-C), 94.91 (s, 1 C, 2'-C), 94.91 (s, 1 C, 6'-C), 94.07 (s, 1 C, 2'''-C), 94.07 (s, 1 C, 6'''-C), 93.81 (s, 1 C, 1''-C), 56.59 (s, 1 C, 6-CO*CH*$_3$); HRMS (ESI) $[M + Na]^+$ calculated for $C_{37}H_{24}O_{18}Na^+$ m/z = 779.0860, found m/z = 779.0858.

2.3.3 Preparation of 4-[4-[[6-(3,5-dihydroxyphenoxy)-4,7,9-trihydroxydibenzo[b,e][1,4]dioxin-2-yl]oxy]-3,5-dihydroxyphenoxy]-6-benzyloxydibenzo[b,e][1,4]dioxin-1,3,8-triol (3).

Dry acetone (100 mL) was added to a mixture of dieckol (300 mg, 0.404 mmol) and anhydrous potassium carbonate (55.8 mg, 0.404 mmol) in a round-bottomed flask under N_2 atmosphere. After stirring for 10 min, benzyl bromide (48.1 μL, 0.404 mmol) was added in small portions. The reaction mixture was stirred at 25 °C for 16 h, diluted with EtOAc (200 mL) and washed successively with 1% aqueous HCl, water, and saturated aqueous NaCl. The organic fraction was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (MeOH: $CHCl_3$ = 1:10) to afford 6-*O*-benzyl dieckol (222 mg, 66%) as a pale yellow powder.

TLC R_f = 0.42 ($CHCl_3$: MeOH: H_2O = 60:30:4, v/v/v); ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.63 (s, 1H, $C_{4''}$-OH), 9.56 (s, 1H, C_1-OH), 9.42 (s, 1H, C_8-OH), 9.41 (s, 1H, $C_{9''}$-OH), 9.35 (s, 2H, $C_{3',5'}$-OH), 9.33 (s, 1H, C_3-OH), 9.18 (s, 1H, $C_{7''}$-OH), 9.12 (s, 2H, $C_{3''',5'''}$-OH), 7.33 (t, J = 7.34 Hz, 2H, C_6-$OCH_2C(CH)_2$*(CH)*$_2$CH), 7.27 (t, J = 7.34 Hz, 1H, C_6-$OCH_2C(CH)_2(CH)_2$*CH*), 7.24 (d, J = 7.34 Hz, 2H, C_6-OCH_2C*(CH)*$_2$$(CH)_2$CH), 6.21 (s, 1H, C_2-H), 6.15 (d, J = 2.64 Hz, 1H, C_7-H), 6.14 (s, 1H, $C_{8''}$-H), 6.04 (d, J = 2.84 Hz, 1H, $C_{3''}$-H), 6.01 (s, 1H, C_9-H), 6.00 (s, 2H, $C_{2',6'}$-H), 5.80 (t, J = 2.08 Hz, 1H, $C_{4'''}$-H), 5.78 (d, J = 2.84 Hz, 1H, $C_{1''}$-H), 5.72 (d, J = 2.08 Hz, 2H, $C_{2''',6'''}$-H), 4.97 (s, 2H, C_6-O*CH*$_2$$C(CH)_2(CH)_2$CH); ^{13}C-NMR (150 MHz, DMSO-d_6) δ 160.69 (s, 1 C, 1'''-C), 159.20 (s, 1 C, 3'''-C), 159.20 (s, 1 C, 5'''-C), 156.24 (s, 1 C, 1'-C), 154.62 (s, 1 C, 2''-C), 153.73 (s, 1 C, 8-C), 151.65 (s, 1 C, 3'-C), 151.65 (s, 1 C, 5'-C), 147.43 (s, 1 C, 6-C), 146.60 (s, 1 C, 3-C), 146.46 (s, 1 C, 7''-C), 146.32 (s, 1 C, 4''-C), 142.85 (s, 1 C, 9a-C), 142.76 (s, 1 C, 10a''-C), 142.54 (s, 1 C, 1-C), 142.28 (s, 1 C, 9''-C), 137.45 (s, 1 C, 5a''-C), 137.42 (s, 1 C, 6-$COCH_2$*C*$(CH)_2(CH)_2$CH), 137.23 (s, 1 C, 4a-C), 128.74 (s, 2 C, 6-$COCH_2C(CH)_2$*(CH)*$_2$CH), 127.94 (s, 1 C, 6-$COCH_2C(CH)_2(CH)_2$*CH*), 127.28 (s, 2 C, 6- $COCH_2$*C(CH)*$_2$$(CH)_2$CH), 124.69 (s, 1 C, 4'-C), 124.62 (s, 1 C, 5a-C), 124.45 (s, 1 C, 4a''-C), 123.63 (s, 1 C, 10a-C), 123.55 (s, 1 C, 9a''-C), 122.60 (s, 1 C, 6''-C), 122.39 (s, 1 C, 4-C), 98.83 (s, 1 C, 2-C), 98.74 (s, 1 C, 8''-C), 98.71 (s, 1 C, 3''-C), 98.43 (s, 1 C, 7-C), 96.63 (s, 1 C, 4'''-C), 96.25 (s, 1 C, 9-C), 94.52 (s, 1 C, 2'-C), 94.52 (s, 1 C, 6'-C), 94.06 (s, 1 C, 2'''-C), 94.06 (s, 1 C, 6'''-C), 93.85 (s, 1 C, 1''-C), 70.59 (s, 1 C, 6-CO*CH*$_2$$C(CH)_2(CH)_2$CH); HRMS (ESI) $[M + Na]^+$ calculated for $C_{43}H_{28}O_{18}Na^+$ m/z = 855.1173, found m/z = 855.1171.

2.3.4 Preparation of 4-[4-[[6-(3,5-dihydroxyphenoxy)-4,7,9-trihydroxydibenzo[b,e][1,4]dioxin-2-yl]oxy]-3,5-dihydroxyphenoxy]-6-(methoxymethoxy)dibenzo[b,e][1,4]dioxin-1,3,8-triol (4).

Dry acetone (70 mL) was added to a mixture of dieckol (200 mg, 0.269 mmol) and anhydrous potassium carbonate (37.2 mg, 0.269 mmol) in a round-bottomed flask under N_2 atmosphere. After stirring for 10 minutes, chloromethyl methyl ether (20.5 μL, 0.269 mmol) was added in small portions. The reaction mixture was stirred at 25 °C for 16 h, diluted with EtOAc (200 mL) and washed successively with 1% aqueous HCl, water, and saturated aqueous NaCl. Following this, the organic fraction was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (MeOH: $CHCl_3$ = 1:10) to afford 6-*O*-methoxymethyl dieckol (120.6 mg, 57%) as a pale yellow powder.

TLC R_f = 0.34 ($CHCl_3$: MeOH: H_2O = 60:30:4, v/v/v); ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.68 (s, 1H, $C_{4''}$-OH), 9.59 (s, 1H, C_1-OH), 9.46 (s, 1H, C_8-OH), 9.45 (s, 1H, $C_{9''}$-OH), 9.35 (s, 1H, C_3-OH), 9.34 (s, 2H, $C_{3',5'}$-OH), 9.22 (s, 1H, $C_{7''}$-OH), 9.14 (s, 2H, $C_{3''',5'''}$-OH), 6.20 (s, 1H, C_2-H), 6.18 (d, J = 2.68 Hz, 1H, C_7-H), 6.14 (s, 1H, $C_{8''}$-H), 6.07 (d, J = 2.68 Hz, 1H, C_9-H), 6.03 (d, J = 2.85 Hz, 1H, $C_{3''}$-H), 5.95 (s, 2H, $C_{2',6'}$-H), 5.80 (t, J = 2.04 Hz, 1H, $C_{4'''}$-H), 5.76 (d, J = 2.85 Hz, 1H, $C_{1''}$-H), 5.72 (d, J = 2.04 Hz, 2H, $C_{2''',6'''}$-H), 4.94 (s, 2H, C_6-O*CH*$_2$$OCH_3$), 3.25 (s, 3H, C_6-OCH_2O*CH*$_3$); ^{13}C-NMR (150 MHz, DMSO-d_6) δ 160.69 (s, 1 C, 1'''-C), 159.20 (s, 1 C, 3'''-C), 159.20 (s, 1 C, 5'''-C), 156.23 (s, 1 C, 1'-C), 154.60 (s, 1 C, 2''-C), 153.63 (s, 1 C, 8-C), 151.60 (s, 1 C, 3'-C), 151.60 (s, 1 C, 5'-C), 146.58 (s, 1 C, 3-C), 146.47 (s, 1 C, 7''-C), 146.34 (s, 1 C, 4''-C), 145.60 (s, 1 C, 6-C), 142.98 (s, 1 C, 9a-C), 142.79 (s, 1 C, 10a''-C), 142.49 (s, 1 C, 1-C), 142.29 (s, 1 C, 9''-C), 137.47 (s, 1 C, 5a''-C), 137.24 (s, 1 C, 4a-C), 125.55 (s, 1 C, 5a-C), 124.66 (s, 1 C, 4'-C), 124.48 (s, 1 C, 4a''-C), 123.57 (s, 1 C, 10a-C), 123.55 (s, 1 C, 9a''-C), 122.63 (s, 1 C, 4-C), 122.61 (s, 1 C, 6''-C), 101.08 (s, 1 C, 7-C), 98.89 (s, 1 C, 2-C), 98.73 (s, 1 C, 8''-C), 98.71 (s, 1 C, 3''-C), 97.65 (s, 1 C, 9-C), 96.62 (s,

1 C, 4"'-C), 95.93 (s, 1 C, 6-COCH_2OCH$_3$), 94.74 (s, 1 C, 2'-C), 94.74 (s, 1 C, 6'-C), 94.06 (s, 1 C, 2"'-C), 94.06 (s, 1 C, 6"'-C), 93.73 (s, 1 C, 1"-C), 56.06 (s, 1 C, 6-COCH$_2$OCH_3); HRMS (ESI) $[M+Na]^+$ calculated for $C_{38}H_{26}O_{19}Na^+$ m/z = 809.0966, found m/z = 809.0962.

2.3.5 Preparation of 4-[4-[[6-(3,5-dihydroxyphenoxy)-4,7,9-trihydroxydibenzo[b,e][1,4]dioxin-2-yl]oxy]-3,5-dihydroxyphenoxy]-6-[3-(ethoxycarbonyl)propoxy]dibenzo[b,e][1,4]dioxin-1,3,8-triol (5)

Dry acetone (120 mL) was added to a mixture of dieckol (300 mg, 0.404 mmol) and anhydrous potassium carbonate (279 mg, 2.02 mmol) in a round-bottomed flask under N_2 atmosphere. After stirring for 10 min, ethyl 4-bromobutyrate (578 μL, 4.04 mmol) was added in small portions. The reaction mixture was stirred at reflux for 16 h, and then diluted with EtOAc (200 mL). It was washed successively with 1% aqueous HCl, water, and saturated aqueous NaCl. The organic fraction was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude compound was purified by column chromatography (MeOH: $CHCl_3$ = 1:10) to afford 6-*O*-3-(ethoxycarbonyl)propyl dieckol (183 mg, 53%) as a pale yellow powder.

TLC R_f = 0.43 ($CHCl_3$: MeOH: H_2O = 60:30:4, v/v/v); ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.62 (s, 1H, C$_{4''}$-OH), 9.52 (s, 1H, C$_1$-OH), 9.39 (s, 1H, C$_{9''}$-OH), 9.38 (s, 1H, C$_8$-OH), 9.29 (s, 1H, C$_3$-OH), 9.23 (s, 2H, C$_{3',5'}$-OH), 9.16 (s, 1H, C$_{7''}$-OH), 9.10 (s, 2H, C$_{3''',5'''}$-OH), 6.17 (s, 1H, C$_2$-H), 6.12 (s, 1H, C$_{8''}$-H), 6.03 (d, J = 2.59 Hz, 1H, C$_7$-H), 6.02 (d, J = 2.80 Hz, 1H, C$_{3''}$-H), 5.97 (d, J = 2.59 Hz, 1H, C$_9$-H), 5.92 (s, 2H, C$_{2',6'}$-H), 5.80 (t, J = 1.99 Hz, 1H, C$_{4'''}$-H), 5.73 (d, J = 2.80 Hz, 1H, C$_{1''}$-H), 5.70 (d, J = 1.99 Hz, 2H, C$_{2''',6'''}$-H), 4.01 (q, J = 7.12 Hz, 2H, C$_6$-OCH$_2$CH$_2$CH$_2$COOCH_2CH$_3$), 3.81 (t, J = 6.35 Hz, 2H, C$_6$-OCH_2CH$_2$CH$_2$COOCH$_2$CH$_3$), 2.31 (t, J = 7.30 Hz, 2H, C$_6$-OCH$_2$CH$_2$$CH_2$COOCH$_2CH_3$), 1.77 (m, J = 6.35 Hz, 7.30 Hz, 2H, C$_6$-OCH$_2$$CH_2CH_2$COOCH$_2CH_3$), 1.14 (t, J = 7.12 Hz, 3H, C$_6$-OCH$_2$CH$_2$CH$_2$COOCH$_2$$CH_3$); ^{13}C-NMR (150 MHz, DMSO-d_6) δ 173.02 (s, 1 C, 6-OCH$_2$CH$_2$CH$_2$$C$OOCH$_2CH_3$), 160.70 (s, 1 C, 1'"-C), 159.19 (s, 1 C, 3"'-C), 159.19 (s, 1 C, 5"'-C), 156.30 (s, 1 C, 1'-C), 154.63 (s, 1 C, 2"-C), 153.74 (s, 1 C, 8-C), 151.57 (s, 1 C, 3'-C), 151.57 (s, 1 C, 5'-C), 147.72 (s, 1 C, 6-C), 146.60 (s, 1 C, 3-C), 146.45 (s, 1 C, 7"-C), 146.32 (s, 1 C, 4"-C), 142.78 (s, 1 C, 9a-C), 142.78 (s, 1 C, 10a"-C), 142.45 (s, 1 C, 1-C), 142.28 (s, 1 C, 9"-C), 137.47 (s, 1 C, 5a"-C), 137.29 (s, 1 C, 4a-C), 124.67 (s, 1 C, 5a-C), 124.67 (s, 1 C, 4'-C), 124.47 (s, 1 C, 4a"-C), 123.67 (s, 1 C, 10a-C), 123.58 (s, 1 C, 9a"-C), 122.69 (s, 1 C, 4-C), 122.62 (s, 1 C, 6"-C), 98.75 (s, 1 C, 2-C), 98.75 (s, 1 C, 3"-C), 98.75 (s, 1 C, 8"-C), 98.51 (s, 1 C, 7-C), 96.63 (s, 1 C, 4"'-C), 96.28 (s, 1 C, 9-C), 94.70 (s, 1 C, 2'-C), 94.70 (s, 1 C, 6'-C), 94.08 (s, 1 C, 2"'-C), 94.08 (s, 1 C, 6"'-C), 93.73 (s, 1 C, 1"-C), 68.74 (s, 1 C, 6-OCH_2CH$_2$CH$_2$COOCH$_2$CH$_3$), 60.23 (s, 1 C, 6-OCH$_2$CH$_2$CH$_2$COOCH_2CH$_3$), 30.06 (s, 1 C, 6-OCH$_2$CH$_2$$CH_2$COOCH$_2CH_3$), 24.74 (s, 1 C, 6-OCH$_2$$CH_2CH_2$COOCH$_2CH_3$), 14.47 (s, 1 C, 6-OCH$_2CH_2CH_2$COOCH$_2$$CH_3$); HRMS (ESI) $[M+Na]^+$ calculated for $C_{42}H_{33}O_{20}Na^+$ m/z = 879.1384, found m/z = 879.1382.

2.3.6 Preparation of 4-[4-[[6-(3,5-dihydroxyphenoxy)-4,7,9-trihydroxydibenzo[b,e][1,4]dioxin-2-yl]oxy]-3,5-dihydroxyphenoxy]-6-(3-hydroxypropoxy)dibenzo[b,e][1,4]dioxin-1,3,8-triol (7).

Dry acetone (120 mL) was added to a mixture of dieckol (300 mg, 0.404 mmol) and anhydrous potassium carbonate (391 mg, 2.83 mmol) in a round-bottomed flask under N_2 atmosphere. After stirring for 10 min, 2-(3-bromopropoxy)tetrahydro-2*H*-pyran (683 μL, 4.04 mmol) was added in small portions. The reaction mixture was stirred at reflux for 16 h and then diluted with EtOAc (200 mL). Following this, it was washed successively with 1% aqueous HCl, water, and saturated aqueous NaCl. The organic fraction was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (MeOH: $CHCl_3$ = 1:10) to afford 6-*O*-3-(tetrahydro-2*H*-pyran-2-yl) oxypropyl dieckol (**6**)(189 mg, 53%) as a pale yellow powder. Subsequently, anhydrous ethanol (0.6 mL) was added to a mixture of **6** (200 mg, 0.226 mmol) and pyridinium *p*-toluenesulfonate (5.68 mg, 0.023 mmol) in a round-bottomed flask under N_2 atmosphere. The reaction mixture was stirred at 55 °C for 24 h, diluted with EtOAc (200 mL), and washed successively with aqueous $NaHCO_3$, water, and saturated aqueous NaCl. The organic fraction was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (MeOH: $CHCl_3$ = 1:10) to afford 6-*O*-3-hydroxypropyl dieckol (149 mg, 82%)

TLC R_f = 0.17 ($CHCl_3$: MeOH: H_2O = 60:30:4, v/v/v); ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.63 (s, 1H, C$_{4''}$-OH), 9.51 (s, 1H, C$_1$-OH), 9.41 (s, 1H, C$_{9''}$-OH), 9.36 (s, 1H, C$_8$-OH), 9.28 (s, 1H, C$_3$-OH), 9.26 (s, 2H, C$_{3',5'}$-OH), 9.17 (s, 1H, C$_{7''}$-OH), 9.11 (s, 2H, C$_{3''',5'''}$-OH), 6.16 (s, 1H, C$_2$-H), 6.13 (s, 1H, C$_{8''}$-H), 6.05 (d, J = 2.65 Hz, 1H, C$_7$-H), 6.01 (d, J = 2.85 Hz, 1H, C$_{3''}$-H), 5.95 (d, J = 2.65 Hz, 1H, C$_9$-H), 5.93 (s, 2H, C$_{2',6'}$-H), 5.78 (t, J = 2.08 Hz, 1H, C$_{4'''}$-H), 5.77 (d, J = 2.85 Hz, 1H, C$_{1''}$-H), 5.71 (d, J = 2.08 Hz, 2H, C$_{2''',6'''}$-H), 4.48 (t, J = 5.27 Hz, 1H, C$_6$-OCH$_2$CH$_2$CH$_2$$OH$), 3.87 (t, J = 6.25 Hz, 2H, C$_6$-OCH_2CH$_2$CH$_2$OH), 3.45 (q, J = 5.76 Hz, 2H, C$_6$-OCH$_2$CH$_2$$CH_2$OH), 1.69 (m, J = 6.25 Hz, 2H, C$_6$-OCH$_2$$CH_2CH_2$OH); ^{13}C-NMR (150 MHz, DMSO-d_6) δ 160.69 (s, 1 C, 1'"-C), 159.20 (s, 1 C, 3"'-C), 159.20 (s, 1 C, 5"'-C), 156.35 (s, 1 C, 1'-C), 154.64 (s, 1 C, 2"-C), 153.73 (s, 1 C, 8-C), 151.53 (s, 1 C, 3'-C), 151.53 (s, 1 C, 5'-C), 148.03 (s, 1 C, 6-C), 146.60 (s, 1 C, 3-C), 146.47 (s, 1 C, 7"-C), 146.32 (s, 1 C, 4"-C), 142.82 (s, 1 C, 9a-C), 142.82 (s, 1 C, 10a"-C), 142.43 (s, 1 C, 1-C), 142.27 (s, 1 C, 9"-C), 137.47 (s, 1 C, 5a"-C), 137.32 (s, 1 C, 4a-C), 124.66 (s, 1 C, 4'-C), 124.46 (s, 1 C, 4a"-C), 124.38 (s, 1 C, 5a-C), 123.69 (s, 1 C, 10a-C), 123.58 (s, 1 C, 9a"-C), 122.77 (s, 1 C, 4-C), 122.64 (s, 1 C, 6"-C), 98.76 (s, 1 C, 8"-C), 98.70 (s, 1 C, 2-C), 98.62 (s, 1 C, 3"-C), 97.92 (s, 1 C, 7-C), 96.64 (s, 1 C, 4"'-C), 95.85 (s, 1 C, 9-C), 94.84 (s, 1 C, 2'-C), 94.84 (s, 1 C, 6'-C), 94.08 (s, 1 C, 2"'-C), 94.08 (s, 1 C, 6"'-C), 93.83 (s, 1 C, 1"-C), 66.48 (s, 1 C, 6-OCH_2CH$_2$CH$_2$OH), 57.34 (s, 1 C, 6-OCH$_2$CH$_2$$CH_2$OH), 32.54 (s, 1 C, 6-OCH$_2$$CH_2CH_2$OH); HRMS (ESI) $[M+Na]^+$ calculated for $C_{39}H_{28}O_{19}Na^+$ m/z = 823.1122, found m/z = 823.1121.

2.4 Antioxidant Activity Assay

2.4.1 DPPH radical scavenging ability assay

The radical scavenging activities of the dieckol derivatives were determined using stable DPPH radical according to the procedure described by Blois [40]. For the working solution, DPPH was dissolved in ethanol at a mass concentration of 0.025 g/L. The dieckol derivatives were dissolved and diluted with ethanol to various concentrations, thus obtaining the sample solutions. The sample solutions (5.0 μL) were placed in a 96-well plate, followed by the addition of 195 μL of the DPPH working solution to each well. After 20 min of reaction at 25 °C, the absorbance of the solution was measured at 515 nm on a spectrophotometer. The measurements were made in triplicate. The DPPH radical scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution (no sample). DPPH scavenging ability is expressed as SE_{50} (μM) and the inhibition percentage was calculated as DPPH scavenging effect (SE, %) = $[A_0 - A_1]/A_0 \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.4.2 ABTS radical scavenging ability assay

The ABTS method described by Re et al. [41] was implemented with some modifications. Equal volumes of stock solutions of ABTS (7.00 mM) and potassium persulfate (2.45 mM) were mixed and allowed to react for 12–16 h in the dark at room temperature to generate the free radical cations. The thus-formed $ABTS^+$ solution was diluted with ethanol, and the absorbance of the working solution was adjusted to 0.70 ± 0.02 at 734 nm. The dieckol derivatives were dissolved and diluted with ethanol to various concentrations to obtain the sample solutions. The sample

solutions (5 μL) were placed in a 96-well plate, and 195 μL of ABTS working solution was added to each well. After 15 min of incubation at 25 °C, the absorbance of the solution was measured in triplicate at 734 nm on a spectrophotometer. The ABTS radical scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution (no sample). ABTS scavenging ability is expressed as SE_{50} (μM) and the inhibition percentage calculated using the following formula: ABTS scavenging effect (SE, %) = $[A_0–A_1]/A_0 \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.4.3 Ferric reducing antioxidant power (FRAP) assay

The ferric ion reducing ability of the derivatives was measured according to the method described by Benzie and Strain [42] with some modifications. Stock solutions of 10 mM of TPTZ in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$, and 0.30 M sodium acetate buffer (pH 3.6) were prepared. The freshly prepared FRAP reagent contained 2.5 mL TPTZ solution, 2.5 mL ferric chloride solution, and 50 mL acetate buffer. The dieckol derivatives were dissolved and diluted with ethanol to obtain sample solutions of varying concentrations. The sample solutions (5.0 μL) were placed into a 96-well plate, and 195 μL FRAP reagent was added to each well. After the reaction mixture had been incubated at 37 °C for 30 min, the absorbance of the solution at 593 nm was measured using a spectrophotometer. The measurements were made in triplicate. The results represent the relative reducing power of each compound in comparison with that of unmodified dieckol.

2.5 In vitro anti-cancer assay

Breast cancer cell lines BT-20 and MDA-MB-231 and ovarian cancer cell line SNU-840 were purchased from Korea Cell Line Research Foundation (Seoul, Korea). The BT-20 and SNU-840 cell lines were cultured in RPMI 1640 supplemented with 10% FBS and penicillin (100 μg/mL). The MDA-MB-231 cell line was cultured in DMEM supplemented with 10% FBS and penicillin (100 μg/mL). The cytotoxicity was assessed using the MTT assay. Briefly, the cells (2×10^5) were seeded in a 96-well plate containing culture medium. Following 24 h of incubation at 37 °C, various concentrations of dieckol and its derivatives were added. After 48 h, 100 μL of the MTT reagent (final concentration = 0.50 mg/mL) was added, and the plates were incubated for an additional 4 h. The medium was discarded, and the formazan blue that had formed in the cells was dissolved in 100 μL of DMSO. The optical density was measured at 570 nm using a microplate spectrophotometer (BIO-RAD xMark™; Bio-Rad Laboratories, Inc. CA, USA). The median inhibitory concentration (IC_{50}) was defined as a 50% decrease in the number of cells compared to the untreated control. Results are represented as the mean of three independent experiments.

3 Results and Discussion

3.1 Synthesis and Analysis

Our studies of the regioselective mono-*O*-substitution of dieckol commenced with the optimization of the reaction conditions for the methylation of dieckol; parameters such as the choice and amount of methylating agent and base were varied, as well as solvents, reaction temperature, and time (Table 1). Dimethyl sulfate, methyl triflate, and methyl iodide were tested as methylating agents, and K_2CO_3, NaH, and KO^{t}Bu were evaluated as bases. Each reaction was performed in acetone or DMF at various temperatures from −78 to 35 °C. Although the product of each reaction was not isolated, TLC and HPLC analyses provided sufficiently reliable information regarding the relative reaction efficiencies to allow us to determine the optimal reaction conditions. As a result of the optimization studies, the reaction of dieckol with one equivalent of dimethyl sulfate in the presence of one equivalent of K_2CO_3 in dry acetone for 16 h at room temperature delivered the highest yield of a single regioisomer. The optimized reaction produced a 64% isolated yield of 6-*O*-methyl dieckol as a pale-yellow powder.

UHR-MS (quadrupole-TOF) revealed a peak at m/z = 756.576, indicative of the molecular formula $C_{37}H_{24}O_{18}$, corresponding to 26 degrees of unsaturation. The ^{1}H-NMR spectrum indicated the presence of 10 hydroxyl groups, represented by signals at δ = 9.66 (s, 1H), 9.53 (s, 1H), 9.42 (s, 2H), 9.33 (s, 2H), 9.30 (s, 1H), 9.20 (s, 1H), and 9.13 (s, 2H), and one new methoxy group represented by a signal at δ = 3.66 (s, 3H). The ^{13}C-NMR spectrum also confirmed the successful introduction of a single methyl group, indicated by a singlet at δ = 56.59 (Table 2). Even though ^{1}H and ^{13}C NMR spectroscopy and UHR-MS spectrometry clearly indicated the formation of a single mono-*O*-methyl dieckol regioisomer as a major product, careful two-dimensional NMR studies were necessary to elucidate the exact structures of the products.

All aromatic protons and carbons could be assigned through the interpretation of HSQC spectroscopic data: δ_H 6.19–δ_C 98.85 (2-C), δ_H 6.15–δ_C 98.75 (8”-C), δ_H 6.09–δ_C 96.39 (7-C), δ_H 6.04–δ_C 98.61 (3”-C), δ_H 5.98–δ_C 95.64 (9-C), δ_H 5.96–δ_C 94.91 (2’,6’-C), δ_H 5.81–δ_C 96.63 (4”’-C), δ_H 5.79–δ_C 93.81 (1”-C), and δ_H 5.73–δ_C 94.07 (2”’,6”’-C).

The connectivity of all protons and carbon atoms was elucidated via HMBC spectroscopy. In the HMBC spectrum, the methyl protons at δ 3.66 show a correlation with the aromatic carbon at δ 148.66. This correlation clearly indicates the attachment of an *O*-methyl moiety at the 6-C position of dieckol (Figs. 1a and 2).

The relative configuration of **2** was confirmed by NOESY experiments. In the NOESY spectrum, the 6-*O*-methyl group was identified by key NOEs of C_6-OCH_3 (δ_H 3.66)/C_7-H (δ_H 6.09), C_8-OH (δ_H 9.42)/C_9-H (δ_H 5.98), and C_8-OH (δ_H 9.42)/C_7-H (δ_H 6.09) (Figs. Fig. 1b and 1c, and 2). All HMBC and NOESY correlations are listed in Table 2.

Table 1
Reaction conditions and yields for the mono-*O*-substitution of dieckol (**1**).

Entry	RX (eq.)	Product	R	Base (eq.)	Solvent	Temperature	Yield (%)
1	Dimethyl sulfate (1.0)	**2**	Methyl	K_2CO_3 (1.0)	Acetone	25 °C	64
2	Benzyl bromide (1.0)	**3**	Benzyl	K_2CO_3 (1.0)	Acetone	25 °C	66
3	Methoxymethyl chloride (1.0)	**4**	Methoxymethyl	K_2CO_3 (1.0)	DMF	25 °C	57
4	Ethyl 4-bromobutyrate (10.0)	**5**	3-(Ethoxycarbonyl)propyl	K_2CO_3 (5.0)	Acetone	56 °C	53
5	2-(3-Bromopropoxy)tetrahydro-*2H*-pyran (10.0)	**6**	3-(Tetrahydro-2*H*-pyran-2-yl)oxypropyl	K_2CO_3 (7.0)	Acetone	56 °C	53

Table 2
^{1}H, ^{13}C, HMBC, and NOESY data of 2 (δ in ppm, data obtained in DMSO-d_6).

No.	δ_C	δ_H	(J in Hz)	HMBC (H → C)	NOESY
1	142.42	9.53		1-C, 2-C, 10a-C	C_2-H
2	98.85	6.19		1-C, 3-C, 4-C	C_1-H, C_3-H
3	146.46	9.30		2-C, 3-C, 4-C	C_2-H
4	122.72	-			
4a	137.43	-			
5a	123.85	-			
6	148.66	-			
7	96.39	6.09	d (2.66)	5a-C, 6-C, 8-C, 9-C	C_8-H, C_6-OCH_3
8	153.85	9.42		7-C, 8-C	C_7-H, C_9-H
9	95.64	5.98	d (2.66)	5a-C, 7-C, 8-C, 9a-C	C_8-H
9a	142.75	-			
10a	123.56	-			
1'	156.28	-			
2'	94.91	5.96		1'-C, 2'-C, 3'-C, 4'-C, 5'-C, 6'-C	$C_{3'}$-H, $C_{5'}$-H
3'	151.58	9.33		2'-C, 3'-C, 4'-C, 5'-C, 6'-C	$C_{2'}$-H, $C_{6'}$-H
4'	124.68	-			
5'	151.58	9.33		2'-C, 3'-C, 4'-C, 5'-C, 6'-C	$C_{2'}$-H, $C_{6'}$-H
6'	94.91	5.96		1'-C, 2'-C, 3'-C, 4'-C, 5'-C, 6'-C	$C_{3'}$-H, $C_{5'}$-H
1"	93.81	5.79	d (2.86)	2"-C, 3"-C, 4a"-C, 10a"-C	
2"	154.63	-			
3"	98.61	6.04	d (2.86)	1"-C, 2"-C, 4"-C, 4a"-C	$C_{4"}$-H
4"	146.36	9.66		4"-C, 4a"-C	$C_{3"}$-H
4a"	124.47	-			
5a"	137.47	-			
6"	122.62	-			
7"	146.49	9.20		6"-C, 7"-C, 8"-C	$C_{8"}$-H
8"	98.75	6.15		6"-C, 7"-C, 9"-C	$C_{7"}$-H, $C_{9"}$-H
9"	142.29	9.42		8"-C, 9"-C, 9a"-C	$C_{8"}$-H
9a"	123.56	-			
10a"	142.81	-			
1"'	160.70	-			
2"'	94.07	5.73	d (2.09)	1"'-C, 2"'-C, 3"'-C, 4"'-C, 5"'-C, 6"'-C	$C_{3"'}$-H, $C_{5"'}$-H
3"'	159.21	9.13		2"'-C, 3"'-C, 4"'-C, 5"'-C, 6"'-C	$C_{2"'}$-H, $C_{3"'}$-H, $C_{6"'}$-H
4"'	96.63	5.81	t (2.09)	2"'-C, 3"'-C, 5"'-C, 6"'-C	$C_{3"'}$-H
5"'	159.21	9.13		2"'-C, 3"'-C, 4"'-C, 5"'-C, 6"'-C	$C_{2"'}$-H, $C_{3"'}$-H, $C_{6"'}$-H
6"'	94.07	5.73	d (2.09)	1"'-C, 2"'-C, 3"'-C, 4"'-C, 5"'-C, 6"'-C	$C_{3"'}$-H, $C_{5"'}$-H
6-OCH_3	56.59	3.66		6-C	C_7-H

The other four mono-*O*-substituted dieckols were similarly prepared by employing the optimized reaction conditions. One deviation was that DMF was used as a solvent for the reaction with methoxymethyl chloride because the reagent did not dissolve well in acetone (Table 1). All reactions proceeded smoothly to deliver the corresponding substituted products as pale-yellow powders in excellent yields. This is a significant accomplishment considering the presence of 11 similarly reactive hydroxyl groups in dieckol.

The general features of the ^{1}H and ^{13}C NMR spectra of **3**–**7** resemble those of the spectra of **2**, except for the peaks attributed to 6-*O*-substituted moieties. All aromatic protons and carbons of **3**–**7** were assigned with the aid of HSQC data. Comprehensive ^{1}H and ^{13}C NMR data are listed in Table 3. The exact positions of *O*-substitutions were identified by HMBC and NOESY spectroscopic analyses (Table S1–S4).

The presence of an *O*-benzyl moiety at the 6-C position of dieckol was verified by the correlation of the benzylic protons at δ 4.97 with the aromatic carbon at δ 147.43 (6-C) in the HMBC spectrum, and the key NOEs of C_6-OCH_2C(CH)$_2$(CH)$_2$CH (δ_H 4.97)/C_7-H (δ_H 6.15), C_8-OH (δ_H 9.42)/C_9-H (δ_H 6.01) and C_8-OH (δ_H 9.42)/C_7-H (δ_H 6.15) in the NOESY spectrum of 6-*O*-benzyl dieckol (**3**) (Table S1).

The presence of an *O*-methoxymethyl moiety at the 6-C position of dieckol was verified by the correlation of the methylene protons of the methoxymethyl group at δ 4.94 with 6-C at δ 145.60 in the HMBC spectrum, and the key NOEs of C_6-OCH_2CH_3 (δ_H 4.94)/C_7-H (δ_H 6.18), C_8-OH (δ_H 9.46)/C_9-H (δ_H 6.07) and C_8-OH (δ_H 9.46)/C_7-H (δ_H 6.18) in the NOESY spectrum of 6-*O*-methoxymethyl dieckol (**4**) (Table S2).

The presence of an *O*-(ethoxycarbonyl)propyl moiety at the 6-C position of dieckol was verified by the correlation of the methylene protons of ethoxycarbonylpropyl group at δ 3.81 with 6-C at δ 147.72 in the HMBC spectrum, and the key NOEs of C_6-O$CH_2CH_2CH_2COOCH_2CH_3$ (δ_H 3.81)/C_7-H (δ_H 6.03), C_8-OH (δ_H 9.38)/C_9-H (δ_H 5.97), and C_8-OH (δ_H 9.38)/C_7-H (δ_H 6.03) in the NOESY spectrum of 6-*O*-(ethoxycarbonyl)propyl dieckol (**5**) (Table S3).

The presence of an *O*-hydroxypropyl moiety at the 6-C position of dieckol was confirmed by the correlation of the methylene protons of the hydroxypropyl group at δ 3.87 with 6-C at δ 148.03 in the HMBC spectrum, and the key NOEs of C_6-O$CH_2CH_2CH_2OH$ (δ_H 3.87)/C_7-H (δ_H 6.05), C_8-OH (δ_H 9.36)/C_9-H (δ_H 5.95), and C_8-OH (δ_H 9.36)/C_7-H (δ_H 6.05) in the NOESY spectrum of 6-*O*-hydroxypropyl dieckol (**7**) (Table S4).

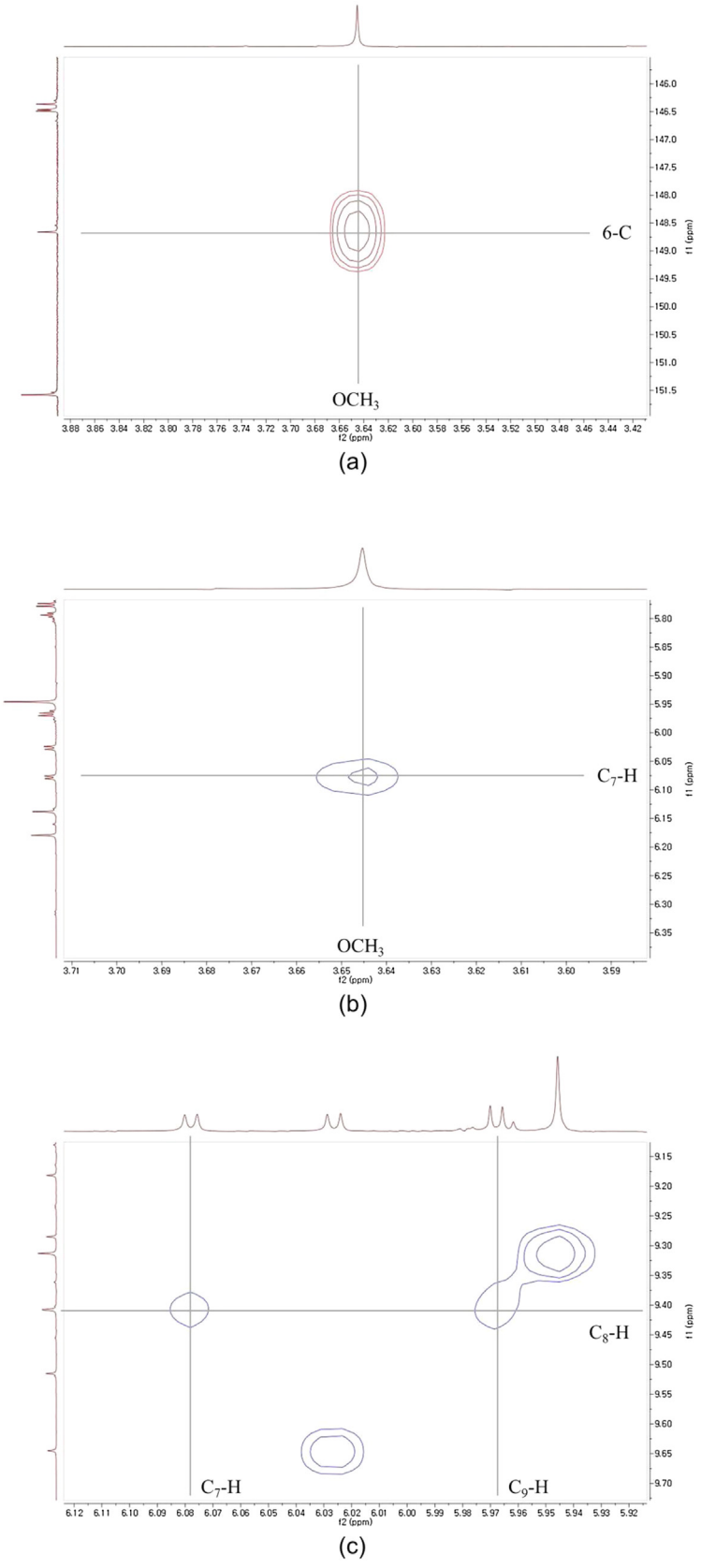


Fig. 1. Key HMBC (a) and NOESY (b) and (c) spectra of 2.

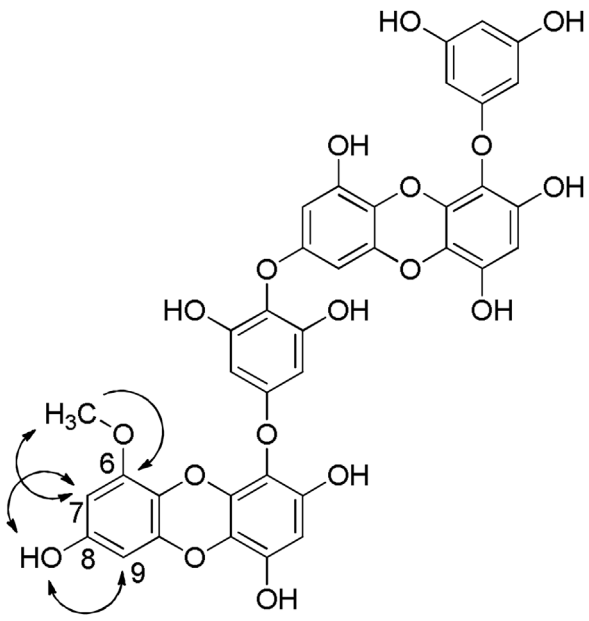


Fig. 2. Key HMBC (H to C, →) and NOESY (↔) correlations of 2.

After optimization of the reaction conditions, all five substituents (methyl, benzyl, methoxymethyl, 3-(ethoxycarbonyl) propyl, and 3-hydroxypropyl) could be efficiently introduced at the 6-*O* position of dieckol with surprisingly high regioselectivity, as confirmed by the detailed 2D-NMR spectroscopic analyses. This result is significantly varied from previous reports that claimed that the propargyl group is mainly bound to the 8-*O* position under similar reaction conditions [38,39]. It was initially anticipated that the reactions would preferentially occur at the 1, 8, 3‴, or 5‴ positions of dieckol, where the steric environment is more favorable for the access of the electrophiles, assuming that the 11 hydroxyl groups of dieckol are chemically equivalent. However, the reactions occurred predominantly at the 6-*OH* position of dieckol, regardless of the bulkiness of the electrophile. Even more surprisingly, no reaction occurred at the 4″-*OH* position, which has a similar molecular environment to 6-*OH*. Possibly, the proton of 6-*OH* is more acidic than the other 10 hydroxyl groups, including 4″-*OH*. Hence, it is necessary to comparatively evaluate the acidity of each hydroxyl group through additional theoretical and experimental studies.

3.2 Antioxidant activity

The importance of antioxidant activity in pharmaceuticals is increasing because age-related and oxidative-stress-associated chronic degenerative diseases such as cancers, cardiovascular

Table 3
^{1}H-NMR and ^{13}C-NMR data of 1–7 (δ in ppm, data obtained in DMSO-d_6).

No.	**1**		**2**		**3**		**4**		**5**		**7**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	142.37	9.47	142.42	9.53	142.54	9.56	142.49	9.59	142.45	9.52	142.43	9.51
2	98.63	6.16	98.85	6.19	98.83	6.21	98.89	6.20	98.75	6.17	98.70	6.16
3	146.31	9.25	146.46	9.30	146.60	9.33	146.58	9.35	146.60	9.29	146.60	9.28
4	122.68	-	122.72	-	122.39	-	122.63	-	122.69	-	122.77	-
4a	137.63	-	137.43	-	137.23	-	137.24	-	137.29	-	137.32	-
5a	122.99	-	123.85	-	124.62	-	125.55	-	124.67	-	124.38	-
6	146.45	9.57	148.66	-	147.43	-	145.60	-	147.72	-	148.03	-
7	98.90	5.99	96.39	6.09	98.43	6.15	101.08	6.18	98.51	6.03	97.92	6.05
8	153.47	9.19	153.85	9.42	153.73	9.42	153.63	9.46	153.74	9.38	153.73	9.36
9	94.27	5.81	95.64	5.98	96.25	6.01	97.65	6.07	96.28	5.97	95.85	5.95
9a	142.99	-	142.75	-	142.85	-	142.98	-	142.78	-	142.82	-
10a	123.63	-	123.56	-	123.63	-	123.57	-	123.67	-	123.69	-
1'	156.30	-	156.28	-	156.24	-	156.23	-	156.30	-	156.35	-
2'	94.90	5.95	94.91	5.96	94.52	6.00	94.74	5.95	94.70	5.92	94.84	5.93
3'	151.55	9.32	151.58	9.33	151.65	9.35	151.60	9.34	151.57	9.23	151.53	9.26
4'	124.62	-	124.68	-	124.69	-	124.66	-	124.67	-	124.66	-
5'	151.55	9.32	151.58	9.33	151.65	9.35	151.60	9.34	151.57	9.23	151.53	9.26
6'	94.90	5.95	94.91	5.96	94.52	6.00	94.74	5.95	94.70	5.92	94.84	5.93
1″	93.94	5.82	93.81	5.79	93.85	5.78	93.73	5.76	93.73	5.73	93.83	5.77
2″	154.63	-	154.63	-	154.62	-	154.60	-	154.63	-	154.64	-
3″	98.46	6.02	98.61	6.04	98.71	6.04	98.71	6.03	98.75	6.02	98.62	6.01
4″	146.36	9.67	146.36	9.66	146.32	9.63	146.34	9.68	146.32	9.62	146.32	9.63
4a″	124.42	-	124.47	-	124.45	-	124.48	-	124.47	-	124.46	-
5a″	137.46	-	137.47	-	137.45	-	137.47	-	137.47	-	137.47	-
6″	122.61	-	122.62	-	122.60	-	122.61	-	122.62	-	122.64	-
7″	146.49	9.20	146.49	9.20	146.46	9.18	146.47	9.22	146.45	9.16	146.47	9.17
8″	98.75	6.14	98.75	6.15	98.74	6.14	98.73	6.14	98.75	6.12	98.76	6.13
9″	142.27	9.42	142.29	9.42	142.28	9.41	142.29	9.45	142.28	9.39	142.27	9.41
9a″	123.55	-	123.56	-	123.55	-	123.55	-	123.58	-	123.58	-
10a″	142.80	-	142.81	-	142.76	-	142.79	-	142.78	-	142.82	-
1‴	160.69	-	160.70	-	160.69	-	160.69	-	160.70	-	160.69	-
2‴	94.05	5.72	94.07	5.73	94.06	5.72	94.06	5.72	94.08	5.70	94.08	5.71
3‴	159.20	9.13	159.21	9.13	159.20	9.12	159.20	9.14	159.19	9.10	159.20	9.11
4‴	96.63	5.80	96.63	5.81	96.63	5.80	96.62	5.80	96.63	5.78	96.64	5.78
5‴	159.20	9.13	159.21	9.13	159.20	9.12	159.20	9.14	159.19	9.10	159.20	9.11
6‴	94.05	5.72	94.07	5.73	94.06	5.72	94.06	5.72	94.08	5.70	94.08	5.71

Table 4
Antioxidant activities and cytotoxicities of dieckol derivatives.

Compound	R	Antioxidant activity[d]			Cytotoxicity[d] (IC_{50}[c])		
		DPPH SE_{50}[a]	ABTS SE_{50}[a]	FRAP[b]	BT-20	MDA-MB-231	SNU-840
1	H	9.17 ± 0.67	3.90 ± 0.37	1	12.05 ± 2.64	5.42 ± 1.73	6.08 ± 2.81
3	Benzyl	16.85 ± 1.33	5.53 ± 0.72	0.438 ± 0.029	28.40 ± 3.02	8.54 ± 3.74	16.96 ± 8.28
4	Methoxymethyl	6.61 ± 0.84	3.50 ± 0.18	0.793 ± 0.023	4.63 ± 1.32	6.69 ± 3.49	9.71 ± 3.05
5	3-(Ethoxycarbonyl)propyl	6.33 ± 0.98	3.09 ± 0.09	0.892 ± 0.046	8.34 ± 5.00	7.29 ± 2.92	20.27 ± 5.01
7	Hydroxypropyl	11.06 ± 0.67	4.18 ± 0.24	0.793 ± 0.230	6.24 ± 4.32	9.15 ± 3.78	11.94 ± 4.49

[a] SE_{50} is defined as the concentration of the sample required to scavenge 50% free radical.
[b] Relative Reducing Power in comparison with dieckol.
[c] IC_{50} is defined as the concentration that results in a 50% decrease in the number of cells compared to that of the control cultures.
[d] The values are expressed as mean ± SD

diseases, and neurodegenerative diseases are becoming more prevalent. The antioxidant activity of dieckol has been studied by many research groups and found to be high in comparison with well-known synthetic and natural antioxidants [43–48]. For example, in comparison with the antioxidant activities of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ascorbic acid and alpha-tocopherol, that of dieckol has been shown to be 3–19 times greater [43–47]. In addition, in comparison with natural polyphenols such as epigallocatechin gallate (EGCG), catechin, and resveratrol, dieckol shows 1–25 times the antioxidant activity [44,45,47]. Furthermore, in comparison with eckol, its monomeric homolog, the antioxidant activity of dieckol is 2.6 times greater [43–45,48]. On the other hand, a bioactivity of permethylated dieckol has been reported to be significantly lower than that of unmodified dieckol, indicating that the presence of hydroxyl groups is important for dieckol's pharmacological activity [37]. Therefore, the antioxidant activities of the dieckol derivatives prepared in this study were investigated and compared with those of dieckol to assess the sensitivity of the antioxidant activity to structural modification. To investigate the antioxidant activity, which is strongly related to the presence of phenolic hydroxyl groups, the radical scavenging activities and reducing power were studied. The radical scavenging activity was evaluated for a neutral radical, DPPH, and a cationic radical, $ABTS^+$, and the reducing ability was evaluated using the FRAP assay. As evident from the data in Table 4, all the investigated derivatives exhibited radical scavenging activities (DPPH SE_{50} = 6.6–16.9 μM; $ABTS^+$ SE_{50} = 3.1–5.53 μM) comparable to that of dieckol **1** (DPPH SE_{50} = 9.2 μM; ABTS SE_{50} = 3.9 μM), indicating that mono-*O*-substitution preserves the electronic structure of dieckol. Moreover, the nature of the group introduced at the 6-*O* position determined whether the derivatives exhibited stronger or weaker antioxidant activity compared with that of dieckol, and similar trends were observed in both the DPPH and $ABTS^+$ radical scavenging assays. Specifically, 6-*O*-(ethoxycarbonyl)propyl dieckol (**5**) showed notably enhanced activity (SE_{50} lowered by 31% and 21% for DPPH and ABTS, respectively), whereas 6-*O*-benzyl dieckol (**3**) showed reduced activity (SE_{50} increased by 84% and 42% for DPPH and $ABTS^+$, respectively). In the FRAP assay, all the derivatives exhibited somewhat diminished reducing ability (44–89%) compared to that of dieckol, indicating that the capping of the 6-*O* position contributes to a partial loss of the electron-transfer potential of unmodified dieckol. Once more, **5** exhibited the highest activity, and **3** showed the lowest activity among the derivatives. Overall, the results suggest that the antioxidant activity of dieckol can be finely tuned by the introduction of specific moieties, which has significant implications for the development of novel redox-based therapeutic agents based on dieckol.

3.3 Cytotoxicity against cancer cells

Chemotherapy drugs for cancer treatment are limited because of their serious side effects. Because the principle of chemotherapy is based on toxicity toward rapidly replicating cells, these chemotherapeutic drugs not only kill cancer cells but also rapidly replicating normal cells, such as those in the gut, hair, blood, and liver [49]. Recently, natural antioxidant polyphenols have shown great promise in solving such problems either as a stand-alone therapy or as sensitizers for traditional chemotherapeutic drugs [23–26,50–54]. Notably, dieckol has been shown to have potent tumor-selective cytotoxicity in several cancer cell lines, including breast, ovary, liver, and lung [23–26], and powerful sensitizing and normal-cell protective effect in combination with the well-known chemotherapeutic agent cisplatin [23,27]. To utilize the promising anticancer properties of dieckol in a pharmaceutically competitive manner, it is crucial to optimize its polarity, pK_a, solubility, and metabolic stability via controlled structural modification without altering its bioactivity. In addition, to enable the study of the absorption, distribution, metabolism, and excretion characteristics of dieckol in animal models, regioselective monosubstitution with radio-labelled reagents is necessary. Therefore, the cytotoxicities of dieckol and its various monosubstituted derivatives against cancer cells should be assessed and compared.

The cytotoxicity was assessed using the MTT assay against two breast cancer cell lines (BT-20 and MDA-MB-231) and one ovarian cancer cell line (SNU-840). The investigated dieckol derivatives exhibited comparable values of cytotoxicity (IC_{50}) against all three cell lines, although there was some variation depending on the type of substituent (Table 4). Notably, **4**, **5**, and **7** displayed substantially higher activities than dieckol against the BT-20 cell line. Overall, these observations confirm that the anticancer properties of dieckol are well-maintained after mono-*O*-substitution, which indicates that mono-*O*-substitution could be a promising route for the preparation of novel dieckol-based anticancer agents.

4 Conclusion

Despite their medical potential, synthetic dieckol derivatives have not been developed as pharmaceutical agents because of the structural complexity of dieckol; in particular, regioselective derivatization is highly challenging. In this study, the selective substitution of a single hydroxyl group of the 11 seemingly equivalent hydroxyl groups in dieckol was achieved via optimized S_N2 reaction conditions using various reactants. The exact position of the substitution was confirmed to be the 6-*O* position of dieckol based on multiple 2D-NMR spectroscopic analyses. Furthermore, the prepared dieckol derivatives exhibit antioxidant and anticancer activities comparable to those of intact dieckol, indicating that precise mono-*O*-substitution does not affect the original biochemical and biological characteristics of dieckol. Therefore, the methodology for the substitution of a specific hydroxyl group in dieckol proposed in this study could contribute to the development of various novel drug candidates based on the biological activity of dieckol. The mechanism for the regioselective substitution at the 6-*O* position of dieckol will be investigated in the future.

Acknowledgments

This research was supported by the Chung-Ang University Graduate Research Scholarship in 2018 and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology [2017R1D1A1B03031524]

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.jiec.2020.08.012.

References

[1] J.H.I. Martinez, H.G.T. Vastaneda, J. Chromatogr. Sci 51 (2013) 825–838 https://doi.org/10.1093/chromsci/bmt045.
[2] Y. Li, I. Wijesekara, Y. Li, S. Kim, Process Biochemistry 46 (2011) 2219–2224 https://doi.org/10.1016/j.procbio.2011.09.015.
[3] G. Lopes, P.B. Andrade, P. Valentao, Molecules 22 (2017) 56 https://doi.org/10.3390/molecules22010056.
[4] K.K.A. Sanjeewa, E. Kim, K. Son, Y. Jeon, J. Photochem. Photobiol. B 162 (2016) 100–105 https://doi.org/10.1016/j.jphotobiol.2016.06.027.
[5] S. Eom, Y. Kim, S. Kim, Food Chem. Toxicol 50 (2012) 3251–3255 https://doi.org/10.1016/j.fct.2012.06.028.
[6] S. Lee, Y. Jeon, Fitoterapia 86 (2013) 129–136 https://doi.org/10.1016/j.fitote.2013.02.013.
[7] Y. Fukuyama, I. Miura, Z. Kinzyo, H. Mori, M. Kido, Y. Nakayama, M. Takahashi, M. Ochi, Chem. Lett (1985) 739–742 https://doi.org/10.1246/cl.1985.739.
[8] G.P. Rosa, W.R. Tavares, P.M.C. Sousa, A.K. Pages, A.M.L. Seca, D.C.G.A. Pinto, Mar. Drugs 18 (2020) 8 https://doi.org/10.3390/md18010008.
[9] N.C. Afonso, M.D. Catarino, A.M.S. Silva, S.M. Cardoso, Antioxidants 8 (2019) 365 https://doi.org/10.3390/antiox8090365.
[10] P. Koirala, H.A. Jung, J.S. Choi, Arch. Pharm. Res 40 (2017) 981–1005 https://doi.org/10.1007/s12272-017-0948-4.
[11] G.N. Ahn, K.N. Kim, S.H. Cha, C.B. Song, J. Lee, M.S. Heo, I.K. Yeo, N.H. Lee, Y.H. Jee, J.S. Kim, M.S. Heu, Y.J. Jeon, Eur. Food Res. Technol 226 (2007) 71–79.
[12] M.S. Lee, B. Lee, K.E. Park, T. Utsuki, T. Shin, C.W. Oh, H.R. Kim, Food Chem 174 (2015) 538–546 https://doi.org/10.1016/j.foodchem.2014.11.090.
[13] N.J. Kang, D.H. Koo, G.J. Kang, S.C. Han, B.W. Lee, Y.S. Koh, J.W. Hyun, N.H. Lee, M.H. Ko, H.K. Kang, E.S. Yoo, Biomol. Ther 23 (2015) 238–244 https://doi.org/10.4062/biomolther.2014.141.
[14] H.J. Choi, J.H. Park, B.H. Lee, H.Y. Chee, K.B. Lee, S.M. Oh, Appl. Biochem. Biotechnol 173 (2014) 957–967 https://doi.org/10.1007/s12010-014-0910-6.
[15] J.Y. Park, J.H. Kim, J.M. Kwon, H.J. Kwon, H.J. Jeong, Y.M. Kim, D. Kim, W.S. Lee, Y.B. Ryu, Bioorg. Med. Chem 21 (2013) 3730–3737 https://doi.org/10.1016/j.bmc.2013.04.026.
[16] Y.B. Ryu, H.J. Jeong, S.Y. Yoon, J.Y. Park, Y. Kim, S.J. Park, M.C. Rho, S.J. Kim, W.S. Lee, J Agric. Food Chem 59 (2011) 6467–6473 https://doi.org/10.1021/jf2007248.
[17] G. Yang, J.W. Oh, H.E. Lee, B.H. Lee, K.M. Lim, J.Y. Lee, Invest. Dermatol 136 (2016) 1062–1066 https://doi.org/10.1016/j.jid.2015.12.046.
[18] E.A. Kim, S.H. Lee, J.H. Lee, N. Kang, J.Y. Oh, S. Heui, G. Ahn, S.C. Ko, S.P. Fernando, S.Y. Kim, S.J. Park, Y.T. Kim, Y.J. Jeon, RSC Advances 6 (2016) 78570–78575 https://doi.org/10.1039/C6RA12724J.
[19] H.J. Jeon, K.Y. Yoon, E.J. Koh, J. Choi, K.J. Choi, K.J. Kim, H.S. Choi, B.Y. Lee, J. Food Nutr. Res 3 (2015) 648–652 https://doi.org/10.12691/jfnr-3-10-5.
[20] M.J. Joe, S.N. Kim, H.Y. Choi, W.S. Shin, G.M. Park, D.W. Kang, Y.K. Kim, Biol. Pharm. Bull 29 (2006) 1735–1739 https://doi.org/10.1248/bpb.29.1735.
[21] V. Sadeeshkumar, A. Duraikannu, S. Ravichandran, W.S. Fredrick, R. Sivaperumal, P. Kodisundaram, Biomed. Pharmacother 84 (2016) 1810–1819 https://doi.org/10.1016/j.biopha.2016.10.091.
[22] Y.X. Li, Y. Li, S.K. Kim, Environ. Toxicol. Pharmacol 39 (2015) 259–270 https://doi.org/10.1016/j.etap.2014.11.027.
[23] J.H. Ahn, Y.I. Yang, K.T. Lee, J.H. Choi, J. Cancer Res. Clin. Oncol 141 (2015) 255–268 https://doi.org/10.1007/s00432-014-1819-8.
[24] C.H. Wang, X.F. Li, L.F. Jin, W. Zhao, G.J. Zhu, J. Biochem. Mol. Toxicol 33 (2019) e22346 https://doi.org/10.1002/jbt.22346.
[25] E.K. Kim, Y. Tang, Y.S. Kim, J.W. Hwang, E.J. Choi, J.H. Lee, S.H. Lee, Y.J. Jeon, P.J. Park, Mar Drugs 13 (2015) 1785–1797 https://doi.org/10.3390/md13041785.
[26] J.S. Yoon, A.K. Yadunandam, S.J. Kim, H.C. Woo, H.R. Kim, G.D. Kim, Journal of Natural Medicines 67 (2013) 519–527 https://doi.org/10.1007/s11418-012-0709-0.
[27] Y.I. Yang, J.H. Ahn, Y.S. Choi, J.H. Choi, Gynecol Oncol 136 (2015) 355–364 https://doi.org/10.1016/j.ygyno.2014.11.015.
[28] B.W. Choi, H.S. Lee, H. Shin, B.H. Lee, Phytother. Res 29 (2015) 549–553 https://doi.org/10.1002/ptr.5282.
[29] H.A. Jung, A. Roy, J.H. Jung, J.S. Choi, Arch. Pharm. Res 40 (2017) 480–491 https://doi.org/10.1007/s12272-017-0904-3.
[30] J.A. Nho, Y.S. Shin, H.R. Jeong, S. Cho, H.J. Heo, G.H. Kim, D.O. Kim, J. Microbiol. Biotechnol 30 (2020) 359–367 https://doi.org/10.4014/jmb.1910.10068.
[31] J. Lee, M. Jun, Mar Drugs 17 (2019) 91 https://doi.org/10.3390/md17020091.
[32] S. Kim, S.S. Kang, S.I. Choi, G.H. Kim, J.Y. Imm, J. Microbiol. Biotechnol 29 (2019) 11–20 https://doi.org/10.4014/jmb.1810.10005.
[33] D. Turck, J.L. Bresson, B. Bulingame, T. Dean, S. Fairweather-Tait, M. Heinonen, K.I. Hirsch-Ernst, T. Mangelsdorf, H.J. McArdle, A. Naska, M. Neuhauser-Berthold, G. Nowicka, K. Pentieva, Y. Sanz, A. Siani, A. Sjodin, M. Stern, D. Tome, M. Vinceti, P. Willatts, K.H. Engel, R. Marchelli, A. Poting, M. Poulsen, J.R. Schlatter, R. Ackerl, H.V. Loveren, EFSA Journal 15 (2017) 5003 https://doi.org/10.2903/j.efsa.2017.5003.
[34] FDA-regulated Phase I Clinical trial, Phase I Study of PH100 (Ecklonia Cava Phlorotannins) NCT04335045. https://clinicaltrials.gov/ct2/show/NCT04335045?term=NCT04335045&draw=2&rank=1, 2020 (Last Update Posted 6 April 2020).
[35] H.J. Lee, O. Kwon, J.Y. Kim, J. Functional Foods 46 (2018) 356–364 https://doi.org/10.1016/j.jff.2018.04.062.
[36] E.K. Choi, S.H. Park, K.C. Ha, S.O. Noh, S.J. Jung, H.J. Chae, S.W. Chae, T.S. Park, Int. J. Pharmacol 11 (2015) 798–805 https://doi.org/10.3923/ijp.2015.798.805.
[37] T.H. Kim, T. Lee, S. Ku, J. Bae, Bioorg. Med. Chem. Lett 22 (2012) 3710–3712 https://doi.org/10.1016/j.bmcl.2012.04.026.
[38] J.H. Kwak, Y. He, B. Yoon, S. Koo, Z. Yang, E.J. Kang, B.H. Lee, S. Han, Y.C. Yoo, K.B. Lee, J.S. Kim, Chem. Commun 50 (2014) 13045–13048 https://doi.org/10.1039/c4cc04270k.
[39] J.H. Kwak, Z. Yang, B. Yoon, Y. He, S. Uhm, H. Shin, B.H. Lee, Y.C. Yoo, K.B. Lee, S. Han, J.S. Kim, Biomaterials 61 (2015) 52–60 https://doi.org/10.1016/j.biomaterials.2015.04.045.
[40] M.S. Blois, Nature 181 (1958) 1199–1200 https://doi.org/10.1038/1811199a0.
[41] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Free Radical Biol, Med. 26 (1999) 1231–1237 https://doi.org/10.1016/S0891-5849(98)00315-3.
[42] I.F. Benzie, J.J. Strain, Anal. Biochem 239 (1996) 70–76 https://doi.org/10.1006/abio.1996.0292.
[43] M. Yotsu-Yamashita, S. Kondo, S. Segawa, Y.C. Lin, H. Toyohara, H. Ito, K. Konoki, Y. Cho, T. Uchida, Mar Drugs 11 (2013) 165–183 https://doi.org/10.3390/md11010165.
[44] J. A. Kim, J.M. Lee, D.B. Shin, N.H. Lee, Food Sci. Biotechnol. 13 (2004) 476-480.
[45] T. Shibata, K. Ishimaru, S. Kawaguchi, H. Yoshikawa, Y. Hama, J Appl Phycol. 20 (2007) 705–711 https://doi.org/10.1007/s10811-007-9254-8.
[46] A.R. Kim, T.S. Shin, M.S. Lee, J.Y. Park, K.E. Park, N.Y. Yoon, J.S. Kim, J.S. Choi, B.C. Jang, D.S. Byun, N.K. Park, H.R. Kim, J Agric Food Chem. 57 (2009) 3483–3489 https://doi.org/10.1021/jf900820x.
[47] K. Kang, Y. Park, H.J. Hwang, S.H. Kim, J.H. Lee, H.C. Shin, Arch Pharm Res. 26 (2003) 286–293 https://doi.org/10.1007/BF02976957.
[48] Y. Li, Z.J. Qian, B.M. Ryu, S.H. Lee, M.M. Kim, S.K. Kim, Bioorg Med Chem. 17 (2009) 1963–1973 https://doi.org/10.1016/j.bmc.2009.01.031.
[49] The American Cancer Society medical and editorial content team, Chemotherapy Side Effects. https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/chemotherapy/chemotherapy-side-effects.html, 2020 (Last Revised 1 May 2020).
[50] O. Bender, A. Atalay, Marmara Pharm J. 22 (2018) 173–179 https://doi.org/10.12991/mpj.2018.54.
[51] M. Hashemzaei, A.D. Far, A. Yari, R.E. Heravi, K. Tabrizian, S.M. Taghdisi, S.E. Sadegh, K. Tsarouhas, D. Kouretas, G. Tzanakakis, D. Nikitovic, N.Y. Anisimov, D. A. Spandidos, A.M. Tsatsakis, R. Rezaee, Oncol Rep 38 (2017) 819–828 https://doi.org/10.3892/or.2017.5766.

[52] A. Pouyafar, M.Z. Heydarabad, S.B. Aghdam, M. Khaksar, A. Azimi, R. Rahbarghazi, M. Talebi, J Cell Biochem (2019) Online ahead of print. https://doi.org/10.1002/jcb.28129.
[53] D. Catanzaro, E. Ragazzi, C. Vianello, L. Caparrotta, M. Montopoli, Nat Prod Commun. 10 (2015) 1365–1368 https://doi.org/10.1177/1934578×1501000813.
[54] C.C. Chou, J.S. Yang, H.F. Lu, S.W. Ip, C. Lo, C.C. Wu, J.P. Lin, N.Y. Tang, J.G. Chung, M.J. Chou, Y.H. Teng, D.R. Chen, Arch Pharm Res. 33 (2010) 1181–1191 https://doi.org/10.1007/s12272-010-0808-y.