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Novel Hybrid Molecules Based on (−)-Epigallocatechin Gallate as Potent Anti-adipogenic Agents

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A series of novel (−)-epigallocatechin gallate (EGCG)–phloroglucinol hybrid compounds 1–4 has been successfully synthesized by employing a simple and efficient methodology using a dielectric barrier discharge (DBD) plasma irradiation. The new hybrid structures were determined by interpretation of spectroscopic data, with the absolute configurations being established by analysis of the circular dichroism (CD) spectra. The novel hybrids 1 and 2 showed highly improved anti-adipogenic potencies toward both pancreatic lipase and preadipocytes differentiation in 3T3-L1 compared to the original EGCG and phloroglucinol. A novel hybrid 1 represent an interesting subclass of anti-adipogenic candidates that need further research.

Key words hybrid compound; (−)-epigallocatechin gallate; phloroglucinol; dielectric barrier discharge plasma; adipogenesis

Introduction

Obesity is a major risk factor for hypertension, diabetes mellitus, hyperlipidemia, osteoarthritis, coronary heart disease, and arteriosclerosis, collectively known as metabolic syndrome.1 Obesity mainly causes from extra energy eating than energy expenditure and is generally recognized as a serious health problem. Studies researching treatments for obesity in the last two decades have faced extensive challenges. Numerous studies have also clarified the distinct mechanisms of action such as pancreatic lipase suppression, suppression of adipocyte differentiation, and control of lipid metabolism. Current one of the most effective approaches for the therapeutic intervention of obesity contains inhibition of triglyceride hydrolysis into absorbable glycerol and fatty acids in the intestinal tract by inhibition of pancreatic lipase and lipid accumulation by disruption adipogenesis using effective anti-obesity chemical agents.3 Pancreatic lipase is known an pharmacological important enzyme in the hydrolysis and absorption of dietary lipids and suppression of pancreatic lipase is recognized to be a better therapeutic strategy for treating diet-induced obesity.5 Obesity is also understood to be associated with excess adipocyte tissue mass caused by overall increases of adipocytes differentiated from preadipocytes.5 Therefore, regulation of lipid absorption and accumulation via suppression of pancreatic lipase activity and adipogenesis is considered to be an major targets in the development of new effective anti-obesity agent.

Molecular hybridization is an emerging approach for the valuable designing of new prototypes and structural modifications based on the recognition of pharmacophoric comprising units. The synthesis of hybrid molecules and their evaluation as diverse range of pharmacological agents and as new potent drugs has been under constant escalation for the last two decades.5 Hybrid molecules, obtained by the combination of structural features of two differently active fragments, are the most popular chemical entities to work upon for developing modified scaffolds with much improved and remarkable capacities in the area of biology as well as medicinal chemistry. Naturally occurring bioactive natural products such as polyphenols have recently attracted a great deal of attention with regards to their advantageous effects on human health. A recent investigation showed that molecular hybridization of each naturally available scaffolds is a valuable chemical strategy for creation of structurally modified new entities with greatly improved pharmacological efficacies.7

(−)-Epigallocatechin gallate (EGCG) is only one of commonly consumed polyphenols found in the leaves of *Camellia sinensis* and has been extensively studied and is believed to have potent pharmacological capacities responsible for a number of health advantages, especially anti-obesity properties.5 However, the low bioavailability of EGCG along with potential confounders may have contributed to the inconsistent outcome of human studies. However, (−)-EGCG is quite unstable under oxidative conditions and the low bioavailability.

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Thus, there have been recent investigations of the effects of its structural modifications, which are closely connected to various biological actions. Both synthetic and naturally occurring phloroglucinol analogues have attracted attention and revealed a vast range of biological efficacies including anti-obesity properties. Although many phloroglucinol derivatives have displayed promising capacities as a new class of drug candidate in several bioassay systems, very few have extended clinical therapeutic purposes. These two pharmacologically substantial backbones were interestingly worked upon to synthesize their hybrid molecules.

Pharmacologically active natural products and their semi-synthetic derivatives are used extensively in therapy of several diseases. Discovery of novel chemical entities of natural origin with improvement of biological capacity without side effects is extremely required. However, the meager amounts of bioactive compounds of natural origin have quite restricted their application and most synthetic protocols of creating pharmacological agents need prolonged reaction times, harsh reactions, and tedious extraction processes. Dielectric barrier discharge (DBD) plasma is recognized to mediate specific chemical changes associated to enough production of reactive oxygen and nitrogen species. A recent exploration presented that non-thermal plasma irradiation is a characteristic green chemical technique for creation of structurally modified natural products with improved biological efficacy and yields.

Therefore, some novel hybridization approaches develop to clear these problems on the basis of increasing environmental and economic problem. However, the beneficial use of plasma irradiation technique for induction of specific chemical reactions during hybridization of pharmacological active molecules with each different frameworks is still very limited. We report herein the convenient structural hybridization of (−)-EGCG and phloroglucinol units assisted by non-thermal DBD plasma treatment. The present research resulted in the rare hybrid molecules 1–4 of (−)-EGCG and phloroglucinol with considerably enriched bioactivities based on and in vitro assay toward pancreatic lipase and 3T3-L1 preadipocytes differentiation compared to the original (−)-EGCG and phloroglucinol.

**Results and Discussion**

DBD plasma apparatus irradiation was accompanied as previously described manner. A prepared mixture containing (−)-epigallocatechin gallate (100 mg) and phloroglucinol (400 mg) in methanol (1.0 L) was straight subjected to DBD plasma for 2 h, during which time hybridization patterns were detected using reversed-phase HPLC. Cautious chromatographic purification lead to the isolation of four unusual methylene-linked (−)-EGCG-phloroglucinol hybrids 1–4 (Fig. 1). The novel hybrid structures were elucidated by interpretation of their spectroscopic data, with the absolute configurations being established by analysis of the circular dichroism (CD) spectra.

Compound 1 was purified as a yellow amorphous optically active powder, $[\alpha]_{D}^{25}$ $-52.5^\circ$ (c 0.1, MeOH). The negative ion mode high resolution (HR) FABMS measurement of compound 1 presented a pseudomolecular ion peak at $m/z$ 871.1715 [M−H]− (calcd for C₄₃H₃₆O₂₀), which was consistent with a molecular formula of C₄₃H₃₆O₂₀. The maximal absorption maxima at 210 nm and a broad band around 275 nm in the UV spectrum indicated the existence of a flavan-3-ol framework. The 1H-NMR spectrum of 1 (Table 1) displayed the resonance of three sets of three 1,3,4,5-tetrasubstituted aromatic singlets at δH 6.98 (2H, s, H-2/uni²033, 6/uni²033), 6.60 (2H, s, H-2/uni²032, 6/uni²032), and 6.00 (2H, s, H-17/uni²034, 19/uni²034), indicating the presence of three symmetrical aromatic moieties. The spectrum also included resonances attributable to three isolated aromatic singlets at δH 6.13 (1H, s, H-8), 5.99 (1H, s, H-5²⁰), and 5.98 (1H, s, H-12²⁰). Also observed were two oxygenated methine protons at δH 5.45 (1H, m, H-3) and 4.98 (1H, H, brs, H-2), which displayed the diagnostic broad singlets at H-2 and H-3 associated with the 2,3-cis configuration, and one methylene at δH 3.02 (1H, dd,
Apart from these resonances, the $^1$H-NMR spectrum of I in the aliphatic region also revealed the signals for three typical isolated methylene protons at $\delta_H$ 3.67 (2H, s, H-21$^c$), 3.69 (2H, s, H-21$^c$), and $\delta_H$ 3.65 (2H, s, H-21$^c$). Consistent with these $^1$H-NMR interpretations, the $^1$C-NMR and heteronuclear single quantum coherence (HSQC) analyses of I further inferred the presence of another 30 carbon resonances, including 36 aromatic carbons, three aliphatic signals, one carbonyl carbon, and three additional signals at $\delta_C$ 16.9 (C-14$^{14}$), 16.7 (C-7$^{14}$), and 16.6 (C-21$^{14}$), comprised of one EGCG, three phloroglucinols, and three methylene units. The linkage positions of the three methylene bridge functionalities in I were unambiguously assigned through key heteronuclear multiple bond correlation (HMBC) relationships as shown in Fig. 2. The small $J_{2,3}$ value indicated a cis configuration between H-2 and H-3 of the flavan unit.$^{18}$ The absolute stereochemistry at C-3 and C-2 chiral centers were established by the diagnostic CD spectral comparison with authentic analogous.$^{19}$ The CD measurement of I revealed a small distinct negative Cotton effect around 280 nm and a high-amplitude negative Cotton effect band at 220 nm, representing 2$R$,3$R$-configuration of the EGCG unit.

Table 1. $^1$H- and $^13$C-NMR Shifts of Compounds 1-4$^{a}$

| Position | $\delta_H$ (J in Hz) | $\delta_C$ (J in Hz) | $\delta_H$ (J in Hz) | $\delta_C$ (J in Hz) | $\delta_H$ (J in Hz) | $\delta_C$ (J in Hz) |
|----------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| 1        | 4.98 (br s)          | 77.1                 | 5.21 (br s)          | 78.9                 | 4.96 (br s)          | 77.1                 |
| 2        | 5.45 (m)             | 68.8                 | 5.48 (m)             | 68.5                 | 5.40 (m)             | 69.0                 |
| 3        | 3.02 (dd, 17.4, 4.8) | 26.1                 | 3.04 (dd, 17.4, 4.8) | 25.9                 | 3.00 (dd, 17.4, 4.8) | 26.1                 |
| 4        | 2.94 (dd, 17.4, 1.2) | 2.97 (dd, 17.4, 1.2) | 2.89 (dd, 17.4, 1.2) | 2.96 (dd, 17.4, 1.2) |         |                      |
| 4a       | —                    | 99.6                 | —                    | 98.8                 | —                    | 99.4                 |
| 5        | —                    | 151.9                | —                    | 151.6                | —                    | 152.9                |
| 6        | —                    | 107.0                | 6.08 (s)             | 96.5                 | —                    | 107.2                |
| 7        | —                    | 153.6                | —                    | 153.6                | —                    | 153.0                |
| 8        | 6.13 (s)             | 96.2                 | —                    | 106.8                | 6.12 (s)             | 95.3                 |
| 8a       | —                    | 155.3                | —                    | 155.2                | —                    | 156.9                |
| 1’       | —                    | 129.6                | —                    | 128.5                | —                    | 129.6                |
| 2’       | 6.60 (s)             | 105.7                | 6.80 (s)             | 106.0                | 6.60 (s)             | 105.7                |
| 3’       | —                    | 145.2                | —                    | 145.5                | —                    | 145.2                |
| 4’       | —                    | 132.1                | —                    | 132.8                | —                    | 132.1                |
| 5’       | —                    | 145.2                | —                    | 145.5                | —                    | 145.2                |
| 6’       | 6.60 (s)             | 105.7                | 6.80 (s)             | 106.0                | 6.60 (s)             | 105.7                |
| 1”       | —                    | 120.5                | —                    | 120.4                | —                    | 120.4                |
| 2”       | 6.98 (s)             | 109.0                | 7.11 (s)             | 109.2                | 6.96 (s)             | 109.0                |
| 3”       | —                    | 144.9                | —                    | 145.0                | —                    | 144.9                |
| 4”       | —                    | 138.0                | —                    | 138.2                | —                    | 138.1                |
| 5”       | —                    | 144.9                | —                    | 145.0                | —                    | 144.9                |
| 6”       | 6.98 (s)             | 109.0                | 7.11 (s)             | 109.2                | 6.96 (s)             | 109.0                |
| 7”       | —                    | 165.6                | —                    | 165.6                | —                    | 165.9                |
| 1”’      | —                    | 105.9                | —                    | 105.4                | —                    | 105.2                |
| 2”’      | —                    | 154.0                | —                    | 154.0                | —                    | 153.9                |
| 3”’      | —                    | 109.2                | —                    | 104.9                | 5.97 (s)             | 95.2                 |
| 4”’      | —                    | 153.3                | —                    | 153.7                | —                    | 155.0                |
| 5”’      | 5.99 (s)             | 95.3                 | 5.99 (s)             | 95.3                 | 5.97 (s)             | 95.2                 |
| 6”’      | —                    | 154.1                | —                    | 151.8                | —                    | 153.9                |
| 7”’      | 3.67 (s)             | 16.7                 | 3.71 (s)             | 16.6                 | 3.62 (s)             | 16.4                 |
| 8”’      | —                    | 106.5                | —                    | 106.2                | —                    | 106.2                |
| 9”’      | —                    | 153.4                | —                    | 154.9                | —                    | 154.9                |
| 10”’     | —                    | 106.8                | 5.97 (s)             | 95.5                 | —                    | 157.0                |
| 11”’     | —                    | 157.1                | —                    | 157.0                | —                    | 157.0                |
| 12”’     | 5.98 (s)             | 95.5                 | 5.97 (s)             | 95.5                 | —                    | 154.9                |
| 13”’     | —                    | 157.2                | —                    | 154.9                | —                    | 154.9                |
| 14”’     | 3.69 (s)             | 16.9                 | 3.64 (s)             | 16.2                 | —                    | 155.1                |
| 15”’     | —                    | 105.1                | —                    | —                    | —                    | —                    |
| 16”’     | —                    | 155.1                | —                    | —                    | —                    | —                    |
| 17”’     | 6.00 (s)             | 95.4                 | —                    | —                    | —                    | —                    |
| 18”’     | —                    | 152.9                | —                    | —                    | —                    | —                    |
| 19”’     | 6.00 (s)             | 95.4                 | —                    | —                    | —                    | —                    |
| 20”’     | —                    | 155.1                | —                    | —                    | —                    | —                    |
| 21”’     | 3.65 (s)             | 16.6                 | —                    | —                    | —                    | —                    |

$^a$ $^1$H NMR measured at 600 MHz, $^13$C-NMR measured at 150 MHz, obtained in acetone-$d_6$ + D$_2$O with TMS as internal standard. Assignments based on HSQC and HMBC NMR spectra. $^b$ $J$ values (Hz) are given in parentheses.
Therefore, the absolute structure of unusual hybrid molecule 1 was assigned the trivial name triphloroegcg.

The negative-ion mode HRFABMS of compound 2 presented a pseudomolecular ion peak at \( m/z \) 733.1410 [M−H]−, consistent with the molecular formula of \( C_{36}H_{30}O_{17} \). The 1D (\(^1\)H- and \(^13\)C-) NMR spectroscopic data of these two molecules 1 and 2 were exhibited to be quite comparable. The \(^1\)H-NMR spectrum of 2 (Table 1) exhibited three sets of meta-coupled aromatic resonances at \( \delta_H \) 7.11 (2H, s, H-2′, 6′), 6.80 (2H, s, H-2′, 6′), and 5.97 (2H, s, H-10′, 12′), and two isolated aromatic singlet protons at \( \delta_H \) 6.08 (1H, s, H-6) and 5.99 (1H, s, H-5′), and two diagnostic oxygenated methine protons of flavan-3-ol subunit at \( \delta_H \) 5.48 (1H, s, H-6) and 5.99 (1H, s, H-5′), and two diagnostic oxygenated methine protons of flavan-3-ol subunit at \( \delta_H \) 5.48 (1H, m, H-3) and 5.21 (1H, 1H, br's, H-2), and one methylene at \( \delta_H \) 3.04 (1H, dd, \( J = 17.4, 4.8 \) Hz, H-4) and 2.97 (1H, dd, \( J = 17.4, 2.4 \) Hz, H-4), indicating the presence of two phloroglucinol and one EGCG moieties. In addition to these distinctive magnetic resonances, \(^1\)H- and \(^13\)C-NMR spectra of 2 appeared two extra methylene functionalities at \( \delta_H \) 3.71 (\( \delta_C \) 16.6) and 3.64 (\( \delta_C \) 16.2). The connection points of two methylene bridges in the new hybrid molecule were unambiguously determined by the key HMBC cross peaks between the CH₂ groups and aromatic carbons as displayed in Fig. 2. The same 2\(R\),3\(R\)-configuration depicted in novel structure 2 was based on the diagnostic small \( J_{2,3} \) coupling constant\(^{18} \) and a negative Cotton effect at around 280 nm and a strong negative Cotton effect at around 220 nm of its CD spectrum\(^{19} \) (Fig. 2). Thus, the absolute structure of 2 was proposed as diphloroegcg as shown in Fig. 1.

The \(^1\)H- and \(^13\)C-NMR spectral data of new hybrid molecules 3 and 4 were nearly identical (Table 1), and the same molecular formula of \( C_{29}H_{24}O_{14} \) was assigned for 3 (\( m/z \) 559.1088) and 4 (\( m/z \) 559.1089) on the basis of their HRFABMS [M−H]−. These two compounds were suggested to be phloroglucinol-flavan-3-ol hybrids with a phloroglucinol unit substituted in A-ring by comparison of their 1D NMR data with those of compounds 3 and 4. Further investigation
of their two-dimensional NMR (2D-NMR) (HSQC, HMBC, and nuclear Overhauser effect spectroscopy (NOESY)) spectral data resulted in the structural isomer for both 3 and 4. The only difference between 3 and 4 was evident only in the linkage points of phloroglucinol units at C-8 or C-6 in A-ring. The connective position of phloroglucinol through a methylene bridge was clearly assigned at C-6 or C-8 from the occurrence of the key HMBC correlations (Fig. 2), respectively. This is also consistent with the only other evident distinct difference of the chemical shift of H-2 in the 1H-NMR of 3 and 4, because the difference of anisotropic effects. In their CD spectra, closely similar negative Cotton effects at 280 and 220 nm were observed for 3 and 4, respectively. This indicated the absolute stereochemistry of C-2 and C-3 to be 2R and 3R for both new hybrids. Thus, compounds 3 and 4 are isomers and were structurally assigned as the trivial names phloroegcg (3) and isophloroegcg (4), respectively.

Table 2. Inhibitory Effects of Isolated Compounds 1–4 on Pancreatic Lipase

| Compound                      | IC$_{50}$ value (µM)$^a$ | Compound                      | IC$_{50}$ value (µM)$^a$ |
|-------------------------------|--------------------------|-------------------------------|--------------------------|
| (−)-EGCG                      | 97.9 ± 6.3               | 1                             | 19.5 ± 1.0               |
| Phloroglucinol                | >500                     | 2                             | 29.2 ± 1.5               |
| Plasma treated (−)-EGCG (2h)  | 45.5 ± 2.9$^{(a)}$       | 3                             | 46.4 ± 3.1               |
|                               |                          | 4                             | 51.9 ± 3.6               |
|                               |                          |                               | 0.6 ± 0.2                |

$^a$ All compounds were examined in triplicate. $^b$ Results expressed as IC$_{50}$ value using µg/mL unit. $^c$ Used as positive control.

The pharmacological potential of (−)-EGCG-phloroglucinol...
hybrid on obesity have been extremely rare studied. Hybrid molecules 1–4 obtained in the present study were evaluated in terms of their inhibitory abilities toward pancreatic lipase25 (Table 2). Among these compounds, EGCG-triphloroglucinol (I) and EGCG-diphloroglucinol (2) were found to exhibit more highly enhanced inhibitory activities than those of the parent (−)-EGCG and phloroglucinol toward pancreatic lipase with IC50 values of 19.5 ± 1.0 and 29.2 ± 1.5 µM (Table 2), which inferred that degrees of phloroglucinol oligomerization on EGCG subunit through a methylene bridge functionality enriched inhibitory activities against pancreatic lipase is might chief for the observed capacity.

In the present investigation, all isolated novel hybrid molecules 1–4 were also assessed for their capacity to inhibit adipocyte differentiation of 3T3-L1 cells based enzyme-linked immunosorbert assay (ELISA) method with Oil Red O staining.26 The maximum non-toxic concentration of the tested hybrids for 3T3-L1 adipocytes was estimated on the basis of cell viability at concentrations up to 25 µM over the course of 48 h. No cytotoxicity was confirmed for any of the subjected compounds at concentrations up to 25 µM in the 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The all isolated hybrids were tested on the basis anti-adipogenic properties in the 3T3-L1 preadipocytes over a differentiation period of eight days. The results indicated that the unprecedented (−)-EGCG-phloroglucinol hybrid 1 possessing methylene linkages functionality induced potent inhibition of fat accumulation at concentrations of 3 and 1.5 µM, by approximately 31.4, and 49.9%, respectively, without any cytotoxicity. Interestingly, the degrees of condensation of phloroglucinol on EGCG subunit linked through the methylene bridges strongly affected the blocking of adipocyte differentiation of 3T3-L1 cells.

Hybrid molecules with methylene linkage originated natural products are extremely rare, and the application of chemical hybridization linked through methylene linkage with potent antiadipogenic capability induced by cold plasma irradiation is still not postulated. Recent advances have recommended that unstable reactive oxygen species and free radicals generated by nonthermal DBD plasma might be conveniently transformed to new molecules with significantly improved bioactivity.27 The present investigation deals with the hybridization and isolation of structurally novel EGCG-phloroglucinol through a methylene bridge that is distinctly associated with enhanced anti-adipogenic efficacies.

In the present study, we verified that (−)-EGCG and phloroglucinol is readily hybridized into four novel compounds 1–4. The new hybrid 1 showed more potent anti-adipogenic properties toward pancreatic lipase and 3T3-L1 adipocytes than the original compounds. These results will facilitated structure–activity relationship studies of the anti-adipogenic effects of (−)-EGCG-phloroglucinol connected through methylene bridge compare to other types of hybrid molecules. This investigation demonstrated a convenient hybridization of major natural product induced by nonthermal plasma and provide a unique approach to semisynthesis of (−)-EGCG-phloroglucinol hybridization with highly enriched potency for anti-adipogenic capacity. Further systematic study into the influences of DBD plasma treatment on structure hybridization and biological potencies of other natural products is now in progress.

**Experimental**

**General Experimental Procedures** (−)-EGCG and phloroglucinol were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). UV spectra were run on a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) and CD spectra were recorded on a JASCO J-720W spectrometer (JASCO Inc., Tokyo, Japan). NMR spectroscopic data were recorded at room temperature on a Varian VNS600 instrument (Varian, Palo Alto, CA, U.S.A.). Chemical shifts are provided in δ (ppm) values relative to those of the solvent acetone-d6 (δH 2.04; δC 29.8) on a tetramethylsilane scale. The standard pulse sequences programmed into the instruments were used for each 2D measurement. The JCH value was fixed at 7 Hz in the HMBC spectra. FABMS were performed on a Micro Mass Auto Spec OA-TOF mass spectrometer (Micromass, Manchester, U.K.) operated in the negative-ion mode. Column chromatography was conducted using YMC GEL ODS AQ 120–50S (YMC Co., Kyoto, Japan). Reversed-phase HPLC analysis was operated on a YMC-Pack ODS A-302 column (4.6 mm i.d. × 150 mm; YMC Co.).

**Sample Preparation and Isolation Procedure** The plasma irradiation apparatus consisted of a process chamber with a DBD apparatus and power supply. The material of the chamber is a Teflon with relatively low chemical reactivity, and the inside of the chamber is 150 × 150 × 275 (h) mm. The DBD device is consisted of four surface DBD sources. Each source is made of a fused silica plate with a thickness of 0.6 mm and a size of 100 × 100 mm2 and two electrodes of a metal sheet based on nickel-chromium alloy. One electrode consists of 6 × 6 open surface patterns, and each pattern is a rounded square and the size is 9 × 9 mm2. The other electrode is no open area. The power supply consists of an arbitrary waveform generator (Tektronix AFG3021C) and a high voltage power amplifier (Trek 5/80). A sinusoidal waveform with a frequency 2.5 kHz and a peak-to-peak voltage of 4 kV was applied between the two electrodes during the operation, and surface discharge was generated at the open boundary with 36 patterns in ambient air without any gas supply. The high voltage probe (Tektronix P6015A), 10:1 voltage probe (Tektronix P2100), and 100 nF capacitor were used to measure the dissipated power by plasma. The temperature inside the chamber was measured using a digital hydrometer before and after sample treatment. The dissipated power by plasma is 65 (±5%) W, and the temperature increased from 20°C up to 50°C during the operation. The sample solution (100 mg EGC and 400 mg phloroglucinol in 1.0L MeOH), which was located in a glass dish at the bottom of the container, was exposed to DBD plasma for 2h, respectively, while stirring with a magnetic bar during plasma exposure. The methanolic solution treated by DBD plasma was evaporated to remove the solvent immediately. The dried material was then dissolved in EtOAc (3 × 50 mL) to yield the dried EtOAc-soluble portion (401.7 mg). Among the dried products, the sample solution containing EGC and phloroglucinol treated for 2 h induced the improvement of inhibitory capacity in the in vitro pancreatic lipase assay with an IC50 value of 45.5 ± 2.9 µg/mL, relative to that of parent EGC and phloroglucinol. A part of the treated sample (400 mg) was passaged to column chromatography over a YMC GEL ODS AQ 120–50S column (1.0 cm i.d. × 41 cm) with distilled water containing

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growing amounts of MeOH in a stepwise gradient to isolate pure new hybrids 1 (3.9 mg, tR 16.4 min), 2 (18.0 mg, tR 15.0 min), 3 (62.9 mg, tR 14.8 min), and 4 (42.4 mg, tR 13.1 min) (Fig. 1). HPLC analysis was performed on a YMC-Pack ODS A-302 column (4.6 mm i.d. × 150 mm; YMC Co., Ltd.) and the solvent system comprised of a slight gradient that was started with 8% MeCN in 0.1% HCOOH/H2O (detection: UV 280 nm; flow rate: 1.0 mL/min; at 40 °C), increased to 80% MeCN over 25 min, and then increased to MeCN over 30 min.

Tryphloegog (1) Yellow amorphous powder. [α]D25 −52.5 (c 0.1, MeOH); UV (MeOH) λ max (log ε): 210 (4.00), 275 (2.13) nm. CD (MeOH) β max (Δε): 212 (−23.3), 278 (−4.1) nm. 1H- and 13C-NMR NMR, see Table 1. FABMS m/z 871 [M−H]−, HRFABMS m/z 871.1715 [M−H]− (Calcd for C43H35O20, 871.1722).

Diphloroegcg (2) Yellow amorphous powder. [α]D25 −62.4 (c 0.1, MeOH); UV (MeOH) λ max (log ε): 209 (3.95), 275 (2.10) nm. CD (MeOH) β max (Δε): 212 (−13.7), 279 (−3.3) nm. 1H- and 13C-NMR NMR, see Table 1. FABMS m/z 733 [M−H]−, HRFABMS m/z 733.1410 [M−H]− (Calcd for C36H29O17, 733.1405).

Phloroegcg (3) Yellow amorphous powder. [α]D25 −55.1 (c 0.1, MeOH). UV (MeOH) λ max (log ε): 209 (4.02), 280 (2.13) nm. CD (MeOH) β max (Δε): 213 (−15.1), 279 (−3.1) nm. 1H- and 13C-NMR NMR, see Table 1. FABMS m/z 595 [M−H]−, HRFABMS m/z 595.1088 [M−H]− (Calcd for C34H29O15, 595.1088).

Isophloroegcg (4) Yellow amorphous powder. [α]D25 −91.3 (c 0.1, MeOH). UV (MeOH) λ max (log ε): 209 (4.01), 274 (2.13) nm. CD (MeOH) β max (Δε): 214 (−29.0), 278 (−5.3) nm. 1H- and 13C-NMR NMR, see Table 1. FABMS m/z 595 [M−H]−, HRFABMS m/z 595.1089 [M−H]− (Calcd for C34H29O15, 595.1088).

Inhibitory Effects of Pancreatic Lipase

The capacity of the compounds to inhibit porcine pancreatic lipase was evaluated using the previously reported method with minor modification. Briefly, an enzyme buffer was prepared by mixing 5 mg of the substrate solution (10 mM of p-nitrophenylbutyrate in dimethyl formamide) added and the enzymatic reactions allowed to proceed for 15 min at 37 °C. p-Nitrophenol at 405 nm was measured

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Conflict of Interest The authors declare no conflict of interest.

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