Synthesis and Biological Evaluation of Caffeic Acid 3,4-Dihydroxyphenethyl Ester

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A high-yield synthesis of caffeic acid 3,4-dihydroxyphenethyl ester (**1**) has been achieved through Knoevenagel condensation of 3,4-dihydroxybenzaldehyde and 3,4-dihydroxyphenethyl monomalonate as the key step. Compound **1** was tested against a 56-cell-line cytotoxicity panel and for its free-radical-scavenging activity in the DPPH test.

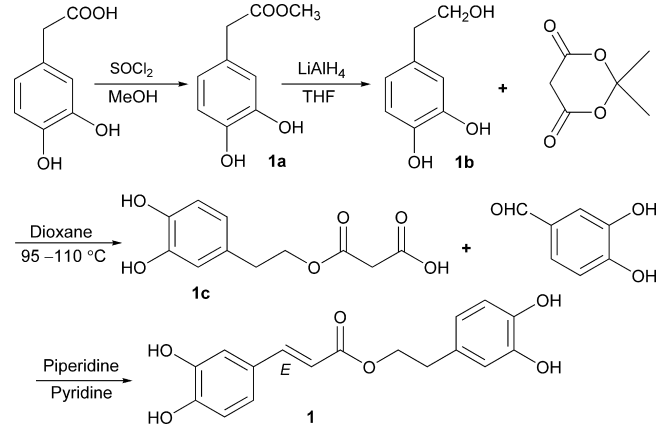
Phenolic compounds are prominent plant-derived components of the human diet and many folk medicines, and include (−)-epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, caffeic acid phenethyl ester (CAPE), a phenolic acid ester from propolis, and curcumin, a phenolic diarylheptanoid of curry powder and the rhizomes of *Curcuma longa*, a traditional Chinese medicine. These three compounds have received extensive attention because of their broad spectrum of biological properties including anti-inflammatory,[1] antioxidative,[2] neuroprotective,[3] cardioprotective,[4] and antitumor activities.[5] Recent research has clarified that these phenolic compounds exhibit antitumor effects by modulating multiple cellular targets and signal pathways,[5] in a fashion that interrupts the carcinogenic process.[5c,d,6a] It has been proposed that modulating multiple targets could be beneficial for the prevention and treatment of complex human diseases such as cancer.[5d,6b−d]

Caffeic acid 3,4-dihydroxyphenethyl ester (**1**, CADPE) was originally isolated from *Teucrium pilosum* as a substance named teucrol in 2000.[7] However, no activity was reported therein. In the course of the investigation on the active ingredients of *Sarcandra glabra*, a traditional Chinese medicine with reported antitumor activity,[8] our group found that **1** isolated from an active fraction of this plant inhibited the growth of solid tumor S180 in mice after administration of **1** by oral (35 mg/kg), intraperitoneal (5 mg/kg), intramuscular (10 mg/kg), and tail intravenous (5 mg/kg) means for 10 days.[9] Jung et al. reported that **1** suppressed tumor growth and angiogenesis by inhibiting the activity of signal transducers and activated transcription 3 (STAT 3), the expression of hypoxia inducible factor-1α (HIF-1α), and vascular endothelial growth factor (VEGF), using a mouse xenograft model implanted with Caki-I human renal carcinoma cells.[10]

Caffeic acid 3,4-dihydroxyphenethyl ester (**1**) is a phenolic alcohol with a phenolic acid ester motif. While this class of compounds is structurally uncomplicated, their reported syntheses have had poor yields[11a,c,d] and are not easily amenable to the preparation of derivatives,[11a,b] because of the use of harsh reaction conditions, the heavy burden of protecting groups needed, and the large excess of one of the reactants required.[11b] Previously, we synthesized **1** from 3,4-dihydroxyphenethyl alcohol and caffeic acid with excess phenolic alcohol using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as a coupling reagent.[9] However, this method had a very low yield, and the target product was not easily purified by chromatography.

In the present study, we present an efficient method through Knoevenagel condensation of 3,4-dihydroxybenzaldehyde and 3,4-dihydroxyphenethyl monomalonate as the key step to generate **1** in high yield, and we report the cytotoxic activity of this compound on 56 cell lines from nine different human cancers and its free-radical-scavenging activity.

Scheme 1. Synthetic Route Leading to Caffeic Acid 3,4-Dihydroxylphenethyl Ester (**1**)



The methodology for preparation of **1** is outlined in Scheme 1. The preparation started from 3,4-dihydroxybenzoic acid (67.26 g), which was treated with $SOCl_2$ in MeOH to furnish 3,4-dihydroxyphenylacetic acid methyl ester **1a** (66.46 g, 91.2% yield). The phenolic alcohol **1b** (49.91 g, 92.5% yield) was obtained by reduction of **1a** (63.76 g) with $LiAlH_4$ in anhydrous THF at room temperature, and then **1b** (46.25 g) was refluxed with Meldrum's acid in dioxane at 95−110 °C to form malonic acid phenolic alcohol monoester **1c** (62.48 g, 86.7% yield). Finally, 3,4-dihydroxybenzaldehyde (41.44 g) was reacted with **1c** (48.04 g) in pyridine catalyzed by piperidine (Knoevenagel condensation)[11d,12] to afford **1** (54.72 g, 86.5% yield) at a purity of 97.6%, as determined by HPLC. The identity of **1** was confirmed on the basis of comparison of its NMR spectroscopic and HRESIMS data with literature values.[7]

Caffeic acid phenethyl ester, an analogue of **1**, has been synthesized by different methods such as acid-catalyzed esterification, transesterification, DCC coupling reactions, esterification via acyl chlorides, and Witting reactions.[11] However, these reported methods have some disadvantages.[11] Previously, we prepared **1** in a very low yield from 3,4-dihydroxyphenethyl alcohol and caffeic acid through DCC coupling reactions.[9] Unfortunately, attempted alternative methods such as esterification via acyl chlorides and acid-catalyzed esterification did not afford the expected product, **1**. The Knoevenagel condensation of malonic acid 3,4-dihydroxyphenethyl monoester and 3,4-dihydroxybenzaldehyde presented in this study is an efficient method to prepare sufficient quantities of **1** to facilitate an ongoing preclinical investigation on its potential for the treatment of cancer.

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Compound **1** was tested for its activity against 56 cell lines from nine different human cancers including leukemia, breast, CNS, colon, ovarian, melanoma, lung, renal, and prostate, by the National Cancer Institute (Bethesda, MD).[13] The results (Figures S1 and S2, and Tables S1 and S2, Supporting Information) indicated that **1** exhibited cytotoxic activities for the tested cell lines with most GI_{50}'s at 10^{-7} to 10^{-6} M.

Compound **1** was also evaluated for its free-radical-scavenging activity using the DPPH scavenging assay.[14] The results showed that **1** has potent free-radical-scavenging activity with an EC_{50} of 4.7 ± 0.2 μg/mL. It is well known that oxidative stress produced by free radicals plays an important role in the carcinogenesis process.[15] Compound **1** has a strong free-radical-scavenging activity and may be effective against oxidative stress and therefore has the potential for the prevention and treatment of cancer.

Experimental Section

General Experimental Procedures. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected. UV spectra were recorded in MeOH with a Hewlett-Packard 8435 spectrometer. NMR experiments were performed on a Bruker 600 MHz NMR instrument, and NMR data are reported as δ (ppm) values referenced to the solvent used. HRESIMS were acquired on an electrospray instrument (MDS Sciex Pulsar Qstar). Silica gel (60−200 mesh) and Diaion HP-20 for open column chromatography were obtained from Sigma-Aldrich. TLC was conducted on precoated silica gel F254 plates (Merck) with detection under UV light at 254 nm. HPLC analysis was performed on an Agilent 1100 HPLC system with an Agilent 1100 diode array detector using a Hypersil ODS column (column A, 250 × 4.6 mm, 5 μm, Supelco). 3,4-Dihydroxyphenylacetic acid, 3,4-dihydroxybenzaldehyde, Meldrum's acid, thionyl chloride ($SOCl_2$), lithium aluminum hydride ($LiAlH_4$), pyridine, piperidine, anhydrous tetrahydrofuran (THF), anhydrous dioxane, anhydrous methanol (MeOH), and α,α-diphenyl-β-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich.

3,4-Dihydroxyphenylacetic Acid Methyl Ester (1a). A quantity of $SOCl_2$ (34.8 mL, 0.48 mol) was added dropwise to a stirred solution of 3,4-dihydroxyphenylacetic acid (67.26 g, 0.4 mol) in anhydrous methanol (800 mL) at 0 °C. The mixture was stirred at room temperature for 7−8 h. After removal of the solvent under reduced pressure, the residue was subjected to column chromatography on silica gel eluting with hexane−EtOAc (4:1) to afford the desired compound **1a** (66.46 g, 91.2% yield) as a colorless oil: ^{1}H NMR (600 MHz, MeOH-d_4) δ 3.43 (2H, s, H-7), 3.61 (3H, s, OCH_3), 6.55 (1H, dd, J = 8.0, 2.0 Hz, H-6), 6.71 (1H, d, J = 8.0 Hz, H-5), 6.72 (1H, d, J = 2.0 Hz, H-2); ^{13}C NMR (150 MHz, MeOH-d_4) δ 41.6 (CH_2, C-7), 52.9 (CH_3, OCH_3), 116.8 (CH, C-5), 117.8 (CH, C-2), 122.1 (CH, C-6), 127.4 (C, C-1), 145.9 (C, C-4), 146.7 (C, C-3), 175.1 (C, C-8).

3,4-Dihydroxybenzyl Alcohol (1b). To a stirred solution of **1a** (63.76 g, 0.35 mol) in THF (4000 mL) was added $LiAlH_4$ (26.56 g, 0.7 mol). The mixture was stirred at room temperature for 9−10 h, and then a 5% HCl water solution (1500 mL) was added to the mixture. The acidic solution was extracted with EtOAc (1000 mL × 3). The organic layers were combined and then evaporated to dryness under vacuum. The residue was applied to column chromatography using silica gel eluting with hexane−EtOAc (2:1) to furnish **1b** (49.91 g, 92.5% yield) as a light yellow oil: ^{1}H NMR (MeOH-d_4, 600 MHz) δ 2.66 (2H, t, J = 7.2 Hz, H-7), 3.69 (2H, t, J = 7.2 Hz, H-8), 6.53 (1H, dd, J = 8.0, 2.0 Hz, H-6), 6.71 (1H, J = 2.0 Hz, H-2), 6.73 (1H, d, J = 8.0 Hz, H-5); ^{13}C NMR(MeOH-d_4, 150 MHz) δ 39.6 (CH_2, C-7), 64.8 (CH_2, C-8), 116.8 (CH, C-5), 117.5 (CH, C-2), 121.8 (CH, C-6), 132.2 (C, C-1), 144.5 (C, C-4), 146.1 (C, C-3).

Malonic Acid 3,4-Dihydroxyphenethyl Monoester (1c). A mixture of Meldrum's acid (57.65 g, 0.4 mol) and 3,4-dihydroxyphenethyl alcohol **1b** (46.25 g, 0.3 mol) in dioxane (1000 mL) was refluxed at 95−110 °C for 5−6 h. After removal of the dioxane, the residue was separated using a column of Diaion HP-20, eluting with 30% methanol in water, to give **1c** (62.48 g, 86.7% yield) as a light yellow oil: ^{1}H NMR (MeOH-d_4, 600 MHz) δ 2.79 (2H, t, J = 6.7 Hz, H-7), 3.23 (2H, s, H-10), 4.24 (2H, t, J = 6.7 Hz, H-8), 6.66 (1H, dd, J = 8.1, 1.7 Hz, H-6), 6.78 (1H, d, J = 1.7 Hz, H-2), 6.80 (1H, d, J = 8.1 Hz, H-5); ^{13}C NMR (MeOH-d_4, 150 MHz) δ 34.9 (CH_2, C-7), 45.3 (CH_2, C-10), 67.7 (CH_2, C-8), 117.5 (CH, C-5), 118.0 (CH, C-2), 122.5 (CH, C-6), 132.2 (C, C-1), 144.1 (C, C-4), 145.5 (C, C-3), 172.6 (C, C-9), 175.0 (C, C-11); HRESIMS m/z 263.05293 $[M + Na]^+$ (calcd for $C_{11}H_{12}NaO_6$, 263.05316).

Caffeic Acid 3,4-Dihydroxyphenethyl Ester (1). Piperidine (20 mL) was added to a mixture of **1c** (48.04 g, 0.2 mol) and 3,4-dihydroxybenzaldehyde (41.44 g, 0.3 mol) in pyridine (200 mL). The mixture was stirred at room temperature until the reaction completely finished using TLC to monitor the reaction. The reaction mixture was concentrated under vacuum to produce a residue, which was dissolved in EtOAc (200 mL) and then washed with 5% HCl (50 mL × 2) and distilled water (50 mL × 2). The EtOAc extract was applied to a column of Diaion HP-20, eluting with 65% methanol, to furnish **1** (54.72 g, 86.5% yield) as a white solid: mp 110−111 °C; UV(MeOH) λ_{max} (log ε) 223 (4.45), 246 (sh), 278 (4.40), 326 (4.46) nm; HRESIMS m/z 339.08408 $[M + Na]^+$ (calcd for $C_{17}H_{16}NaO_6$, 339.08446); ^{13}C and ^{1}H NMR data see Supporting Information (Table S3). The purity of **1** was determined by HPLC as 97.6%.

Cytotoxicity Assay. The cytotoxicity of **1** was assayed by the National Cancer Institute (Bethesda, MD) using the methodology of the 60-cell-line cancer screen.[13]

Free-Radical-Scavenging Activity. The α,α-diphenyl-β-picrylhydrazyl (DPPH) scavenging assay was carried out according to a procedure described previously.[14] Briefly, different concentrations of **1** (100 μL) were mixed with 900 μL of a 0.04 mg/mL methanolic solution of DPPH. The mixture was kept at room temperature for 20 min, and then the UV absorbance at 517 nm was measured. The inhibition percentage was calculated using the following equation: $I = [(A_c - A_s)/A_c] \times 100$, where I is the inhibition percentage, A_c is the absorbance of the negative control (containing 100 μL of methanol instead of the test samples), and A_s is the absorbance of the samples. The inhibition percentage was plotted against the concentration of **1**, and the EC_{50} value (mean ± SD) was determined by linear regression analysis of three determinations.

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Supporting Information Available: The cytotoxic activity of **1**; NMR data and NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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