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Synthesis of (2*R*,3*R*)-*epi*gallocatechin-3-*O*-(4-hydroxybenzoate), a novel catechin from *Cistus salvifolius*, and evaluation of its proteasome inhibitory activities

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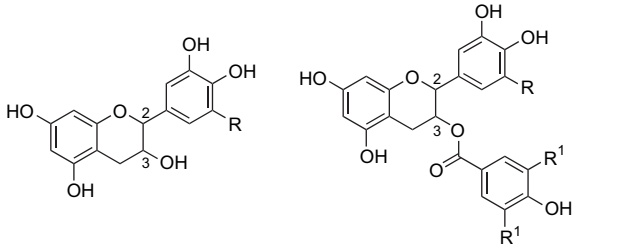
Abstract—The total and semi syntheses of (2*R*,3*R*)-*epi*gallocatechin-3-*O*-(4-hydroxybenzoate), a novel catechin from *Cistus salvifolius*, were accomplished. The proteasome inhibition and cytotoxic activities of the synthetic compound and its acetyl derivative were studied and compared with (2*R*,3*R*)-*epi*gallocatechin-3-gallate (EGCG), the active component from green tea.

1. Introduction

The *Cistus* species are indigenous in the Mediterranean area and traditionally used in folk medicine for the treatment of fever, diarrhea, skin diseases, rheumatism, and other inflammatory diseases.[1] In Turkey, tea prepared from the leaves of *Cistus salvifolius* has been used for the treatment of cancer.[2] While a number of polyphenols have been found within the genus, the phenolic pattern of *Cistus salvifolius* L. appeared to differ markedly from other *Cistus* species.[3] The ethyl acetate-soluble fraction from the aqueous acetone extract of *C. salvifolius* was found to contain the following catechins: catechin (**1**, C), *epi*catechin (**2**, EC), gallocatechin (**3**, GC), *epi*gallocatechin (**4**, EGC), *epi*catechin-3-gallate (**5**, ECG), gallocatechin-3-gallate (**6**, GCG), *epi*gallocatechin-3-gallate (**7**, EGCG), and a novel compound, *epi*gallocatechin-3-*O*-(4-hydroxybenzoate) (**8**) (Fig. 1). The structure of **8** was deduced mainly through the ^{1}H NMR of its acetate (**9**).[3] The 2*R*,3*R* absolute configuration in **8** was assigned solely on the basis of the optical rotation of **9** {$[\alpha]_D^{20}$ −60.1 (*c* 0.06, acetone)}.

Tea, produced from the plant *Camellia sinensis*, has been consumed by human beings for thousands of years. Tea catechins constitute about 30–40% of the water-soluble compounds in brewed green tea. The major tea catechins are compounds **1**–**7**.[4] Of these, EGCG (**7**) is by far the most abundant and has been reported to have various biological activities, which may account for the beneficial effects attributed to green tea.[5,6] Compound **8**, on the other hand, had not been reported to exist in green tea. Regular drinking of green tea has been associated with reduced risk of several forms of cancer.[7] It is known that a number of cancer-related proteins are affected by tea polyphenols, in particular by EGCG. However, the exact mechanism of tea-mediated cancer prevention is still unknown.[7] Recently, we proposed that inhibition of proteasome may be a key mechanism in the cancer prevention activity of green tea.[8] Furthermore, it is the gallate ester bond-containing tea polyphenols (e.g., **5**–**7**) but not the flavan-3-ols (e.g., **1**–**4**), which inhibit the proteasomal chymotrypsin-like (β5) activities of the proteasome.[9]



1: 2,3-*trans*; R=H; C
2: 2,3-*cis*, R=H; EC
3: 2,3-*trans*; R=OH; GC
4: 2,3-*cis*, R=OH; EGC

5: 2,3-*cis*, R=H, R^1=OH; ECG
6: 2,3-*trans*; R=OH, R^1=OH; GCG
7: 2,3-*cis*, R=OH, R^1=OH; EGCG
8: 2,3-*cis*, R=OH, R^1=H

Figure 1. Green tea catechins.

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Because of our interest in the synthesis of EGCG,[10] its analogs,[11] and their role as proteasome inhibitors in cancer protection,[12] we became interested in the synthesis of compound **8** and its activity in proteasome inhibition.

2. Results and discussion

2.1. De novo synthesis of compound 8

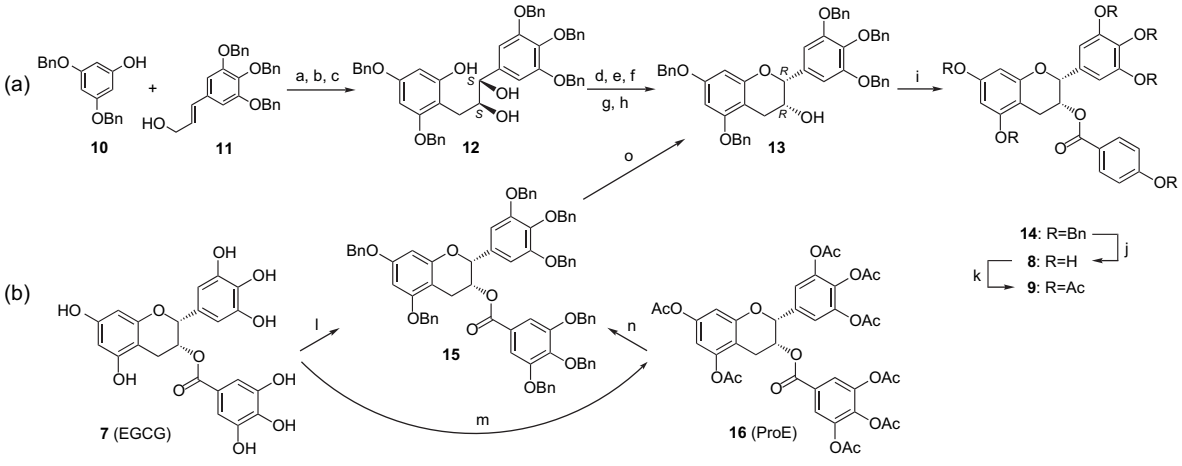
Previously, we had reported on the enantioselective synthesis of the benzylated *epi*gallocatechin **13** [(−)-(2*R*,3*R*)-5,7-bis(benzyloxy)-2-[3,4,5-tris(benzyloxy)phenyl]chroman-3-ol] starting from 3,5-bis(benzyloxy)phenol (**10**) and 3,4,5-tris-(benzyloxy)cinnamyl alcohol (**11**) (Scheme 1a).[10] Acylation of **13** with 4-(benzyloxy)benzoyl chloride, prepared from 4-benzyloxybenzoic acid and oxalyl chloride, gave the protected ester **14**. Alternately, compound **13** can be acylated with 4-benzyloxybenzoic acid directly using DCC/DMAP as the coupling conditions. Hydrogenolysis of **14** afforded compound **8** in good yield. Acetylation of **8** with acetic anhydride gave the acetate **9** (Scheme 1), which showed the same ^{1}H NMR as those reported for **9** isolated from *C. salvifolius*. This confirms the structure of the natural compound **8**. Since the total synthesis gave enantioselectively the 2*R*,3*R* absolute configuration and since the synthetic **9** has the same spectroscopic data and identical optical rotation {[α]$_D^{20}$ −60 (*c* 0.2, acetone)} as the isolated compound **9**, this confirms the stereochemistry of the natural product.

2.2. Semi syntheses of compound 8 from natural EGCG

Even though the above total synthesis was able to give compounds **8** and **9**, it involved a number of steps including the preparation of the starting materials **10** and **11**, and the overall yield was low (<19% based on **11**). This prompted us to examine the synthesis of **8** from the natural (−)-EGCG (**7**), which is readily available commercially. Although direct benzylation of EGCG with different benzylating agents (BnCl, BnBr), different bases (NaH, K_2CO_3, K_2CO_3/KI), and solvents (dimethylformamide (DMF), acetone) at different temperatures was tried, the conditions of BnCl/NaH/DMF/−60 °C/N_2 were found to be the best giving a complex mixture from which the benzylated derivative **15** could be obtained in pure form in slightly over 20% after purification (Scheme 1b). The complex mixture was presumably due to the formation of both *O*- and *C*-benzylated products with electron-rich phenolic compounds under basic conditions.[13] We also tried acid-catalyzed benzylation by using benzyl trichloroacetimidate,[14] which was also unsuitable. However, by converting EGCG to the peracetate **16** in quantitative yield first,[15] we were able to hydrolyze the acetyl function of **16** and use an in situ benzylation method to form the benzylated compound **15** in a cleaner manner and improved isolated yield (31%). In any case, hydrolysis of compound **15** with sodium hydroxide in methanol gave **13** in 90% yield (Scheme 1b). Compound **13**, obtained in this manner, can be similarly converted to **8** and **9** as shown in Scheme 1.

2.3. Proteasome inhibition of compounds 8 and 9

The eukaryotic proteasome is a large multi-catalytic, multi-subunit protease complex possessing three distinct activities, which are associated with three different β subunits, chymotrypsin-like (with β5 subunit), trypsin-like (with β2 subunit), and peptidyl-glutamyl peptide-hydrolyzing-like (PGPH- or caspase-like; with β1 subunit).[16] Inhibition of the chymotrypsin-like, but not the trypsin-like, activity of the proteasome has been found to be associated with induction of tumor cell apoptosis.[17] The discovery of new proteasome inhibitors with little or no toxicity is highly desirable in anticancer therapy.[18] We have previously reported that (−)-EGCG inhibits the chymotrypsin-like activity of the proteasome in vitro (IC_{50} 86–194 nM) and in intact tumor cells (1–10 μM)[9] leading to accumulation of proteasome target proteins (such as IκB-α, p27, and Bax) and apoptosis in human cancer cell lines, as measured by activation of caspases and cleavage of poly(ADP-ribose) polymerase (PARP).[19] We have now compared the IC_{50} of EGCG (0.2 μM) with compound **8** (40.4 μM) for their inhibitory activity against the chymotrypsin-like activity of a purified proteasome in vitro (Table 1). It is clear that the loss of the two hydroxyl groups on the gallate ring has led to much reduced inhibitory activity (about 200-fold).



Scheme 1. (a) 25% H_2SO_4/SiO_2; (b) TBSCl, imidazole; (c) AD-mix-α, $MeSO_2NH_2$; (d) TBAF; (e) $CH(OEt)_3$, PPTS; (f) K_2CO_3; (g) Dess–Martin periodinane; (h) L-Selectride; (i) 4-benzyloxybenzoic acid, DCC, DMAP; (j) $Pd(OH)_2$, H_2; (k) Ac_2O, DMAP; (l) NaH, BnCl; (m) Ac_2O, pyridine; (n) NaH, BnCl, H_2O; (o) NaOH, MeOH.

Table 1. Inhibition of proteasome activity by EGCG and analogs

Compound	IC_{50} (μM)
(−)-EGCG	0.2±0.02
8	40.4±0.17
16 (ProE)	*n.a.*[a]
9	*n.a.*[a]

[a] Indicates that the inhibitory activity of the purified proteasome at 25 μM was <25%.

A critical issue concerning the potential application of EGCG as an anticancer agent is its known poor bioavailability.[20] The low bioavailability was thought to be partly due to the poor stability of EGCG in alkaline or in neutral solutions.[21] Another factor may be due to in vivo metabolic transformation of EGCG into various metabolites.[22] To improve the stability and the potency of (−)-EGCG, we recently proposed acetylated EGCG (**16**, ProE) as a potential prodrug.[15] As prodrugs rationally have low cytotoxicity prior to the metabolic activation, we found that **16** did not inhibit a purified 20S proteasome activity in vitro (Table 1) but inhibited the proteasome activity in intact tumor cells in vivo inducing cell death.[15] It was reported that **16** was rapidly converted to EGCG (**7**) by HCT116 human colon cancer cells resulting in a 2.8–30-fold greater intracellular concentration of EGCG as compared to treatment with EGCG itself.[23] These authors further showed that intragastric administration of **16** to CF-1 mice resulted in higher bioavailability compared to that of equimolar doses of EGCG.[23] We have therefore examined the biological activity of compound **9**, the acetylated derivative of **8**. Interestingly, even though **9** is not active as an inhibitor of purified proteasome (Table 1), it showed intracellular proteasome inhibition of chymotrypsin-like activity (Fig. 2A) in cultured Leukemia Raji B cells, similar to that of ProE. Proteasome inhibition was confirmed by the levels of accumulation of ubiquitinated proteins and Bax (Fig. 2B).

The acetylated compound **9** induced time-dependent cell death observed by trypan blue dye exclusion more than the

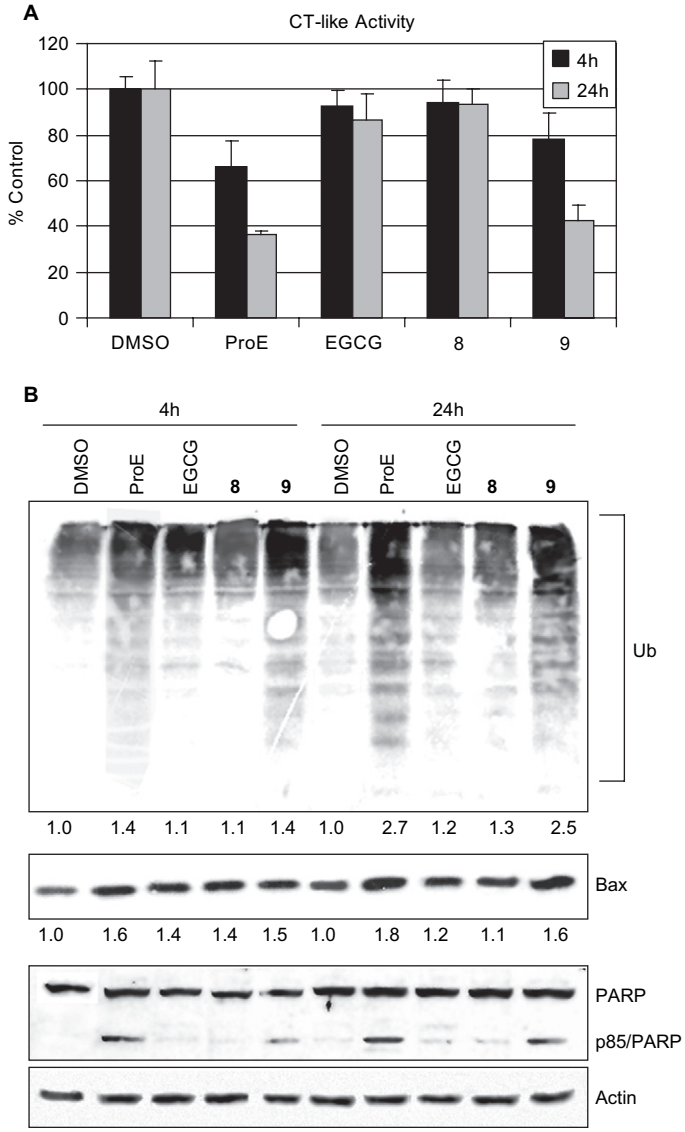


Figure 2. ProE and **9** are proteasome inhibitors in cultured tumor cells. Leukemia Raji B cells were treated, harvested, and analyzed for the chymotrypsin-like activity, as described in Section 4 (A). Western blot analysis using specific antibodies to ubiquitin, the proteasome target protein Bax, PARP, and actin (B).

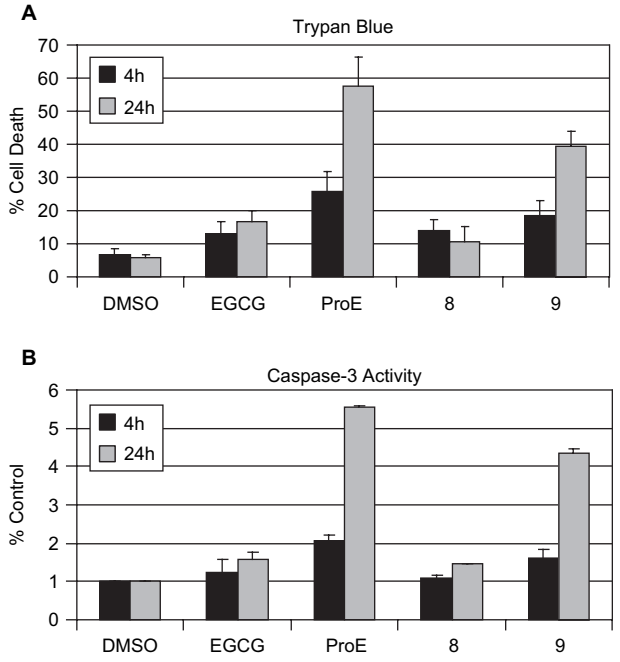


Figure 3. Acetylated compound **9** induces time-dependent cell death in cultured tumor cells. Leukemia Raji B cells were treated with the solvent (DMSO) or 25 μM of the indicated analog for 4 or 24 h, followed by trypan blue dye exclusion assay. The data represented are the mean number of dead cells over total cell population±SD after treatment (A). A cell-free caspase-3/-7 activity assay was performed as described in Section 4. Caspase-3/-7 activity is represented as a percentage of the solvent (DMSO) control (B).

parent compound **8** and EGCG, though not quite to the same extent as ProE (Fig. 3A). To determine whether the observed cell death (Fig. 3A) was due to apoptosis, the cell extracts from the same experiments were used for measurement of caspase-3 activation (Fig. 3B) and PARP cleavage (Fig. 2B). Indeed, after 24 h treatment, compound **9** induced nearly 4.3-fold increase in caspase-3 activity in Raji B cells compared with the control experiment. These results indicate that the acetylated compound **9** is more potent than either its parent compound **8** or EGCG itself in inhibiting proteasome activity and inducing apoptotic cell death in a time-dependent manner.

3. Conclusion

In conclusion, we have confirmed by synthetic studies the structure and the absolute configurations of a novel catechin (2*R*,3*R*)-*epi*gallocatechin-3-*O*-(4-hydroxybenzoate) (**8**) isolated from *C. salvifolius*. Compound **8** is less potent than EGCG in proteasome inhibition in vitro. However, its acetyl derivative **9** was found to be nearly comparable to ProE (**16**) in inducing tumor cell death by apoptosis.

4. Experimental

4.1. General

The starting materials and reagents, purchased from commercial suppliers, were used without further purification. Literature procedures were used for the preparation of (−)-(2*R*,3*R*)-5,7-bis(benzyloxy)-2-[3,4,5-tris(benzyloxy)-phenyl]chroman-3-ol[12] (**13**) and (−)-(2*R*,3*R*)-5,7-diacetoxy-2-(3,4,5-triacetoxyphenyl)chroman-3-yl-(3,4,5-triacetoxy)benzoate (**16**).[15]

Anhydrous tetrahydrofuran (THF) was distilled under nitrogen from sodium benzophenone ketyl. Anhydrous dichloromethane (CH_2Cl_2) was distilled under nitrogen from CaH_2. Anhydrous DMF was distilled under nitrogen from CaH_2. Reaction flasks were flame-dried under a stream of N_2. All moisture-sensitive reactions were conducted under a nitrogen atmosphere. Flash chromatography was carried out using silica gel 60 (70–230 mesh). The melting points were uncorrected. ^1H and ^{13}C NMR (500 MHz) spectra were measured with TMS as internal standard when $CDCl_3$ and CD_3OD were used as solvent. High-resolution electrospray ionization (HRESI) mass spectra were recorded using a QTOF-2 Micromass spectrometer.

4.1.1. (−)-(2*R*,3*R*)-5,7-Bis(benzyloxy)-2-[3,4,5-tris(benzyloxy)-phenyl]chroman-3-yl-(4-benzyloxy)benzoate (14) with $(COCl)_2$, DMAP. To a solution of 4-benzyloxybenzoic acid (85.1 mg, 373 μmol) in dry CH_2Cl_2 (7.00 mL) was added oxalyl chloride (3.00 mL, 34.4 mmol). The mixture was refluxed at 65 °C for 2 h. After the solution of the reaction mixture was evaporated, the resulting oil was dried completely under a reduced pressure for 2 h. The obtained white solid was dissolved in CH_2Cl_2 (1.00 mL) and cooled to 0 °C. 4-Dimethylaminopyridine (DMAP, 55.6 mg, 455 μmol) was added to the solution and the mixture was stirred for 5 min. A solution of (−)-(2*R*,3*R*)-5,7-bis(benzyloxy)-2-[3,4,5-tris(benzyloxy)phenyl]chroman-3-ol (**13**, 138 mg, 182 μmol) in dry CH_2Cl_2 (1.00 mL) was added dropwise at 0 °C and the mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the resulting oil was purified by flash SiO_2 column chromatography (hexane/EtOAc, 5:1) to give 161 mg (91%) of the title compound as a pale yellow amorphous solid: $[\alpha]_D^{20}$ −42 (*c* 0.70, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.87 (d, J=7.5 Hz, 2H), 7.40–7.10 (m, 30H), 6.84 (d, J=7.5 Hz, 2H), 6.71 (s, 2H), 6.27 (br s, 1H), 6.23 (br s, H), 5.59 (br s, 1H), 5.01–4.85 (m, 10H), 4.72–4.67 (m, 2H), 3.10–2.97 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 165.3, 162.9, 159.0, 158.2, 155.8, 153.1, 138.5, 138.1, 137.3, 137.1, 137.1, 136.3, 133.6, 132.1, 128.9, 128.9, 128.8, 128.6, 128.4, 128.3, 128.3, 128.2, 128.0, 128.0, 127.8, 127.8, 127.7, 127.7, 127.4, 122.9, 114.7, 106.9, 101.2, 95.0, 94.2, 78.1, 75.3, 71.4, 70.4, 70.3, 70.2, 68.2, 26.4; LRMS (ESI) *m/z* 989 (M+Na); HRMS *m/z* calculated for $C_{64}H_{54}O_9Na$ (M+Na) 989.3666, found 989.3616.

4.1.2. Benzylated (−)-EGCG, [(−)-(2*R*,3*R*)-5,7-bis(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl-(3,4,5-trisbenzyloxy)benzoate (15)]. *Method 1*: to a solution of EGCG (**7**, 458 mg, 1.00 mmol) in dry DMF (10.0 mL) under nitrogen at −60 °C, NaH (60% dispersion in mineral oil, 200 mg, 5.00 mmol) was added. After 2 h, benzyl chloride (920 μL, 8.00 mmol) was added via a syringe. The mixture was stirred at −60 °C for an additional 30 min and then at room temperature for 24 h. The reaction was quenched by adding hydrochloric acid (1 N, 2.00 mL) and water (15.0 mL). The aqueous layer was extracted with EtOAc×3. The combined organic layers were washed with water and brine, then dried over sodium sulfate

(Na_2SO_4), and evaporated in vacuo. The residue was purified by flash SiO_2 column chromatography (hexane/EtOAc, 5:1) to afford the crude benzylated EGCG as pale yellow oil. After precipitation of the crude mixture by mixed-solvent (hexane/EtOAc/dichloromethane), 248 mg (21%) of the title compound was obtained as a white solid.

Method 2: to a mixture of (−)-(2*R*,3*R*)-5,7-diacetoxy-2-(3,4,5-triacetoxyphenyl)chroman-3-yl-(3,4,5-triacetoxy)-benzoate (**16**, 794 mg, 1.00 mmol), benzyl chloride (920 μL, 8.00 mmol), NaH (60% dispersion in mineral oil, 200 mg, 5.00 mmol), and DMF (20.0 mL), water (144 μL, 8.00 mmol) was added at 0 °C dropwise over a period of 10 min. After stirring overnight at room temperature, the reaction mixture was diluted with EtOAc and washed with water and brine, then dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by flash SiO_2 column chromatography (hexane/EtOAc, 5:1) and subsequently precipitation by hexane/EtOAc/dichloromethane to afford 366 mg (31%) of the title compound as a white solid: $[\alpha]_D^{20}$ −44 (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.42–7.18 (m, 42H), 6.73 (s, 2H), 6.39 (d, *J*=2.0 Hz, 1H), 6.34 (d, *J*=2.0 Hz, 1H), 5.66 (s, 1H), 5.05–4.90 (m, 13), 4.79 (d, *J*=11.0 Hz, 1H), 4.67 (d, *J*=11.0 Hz, 1H), 3.12 (dd, *J*=17.5, 4.5 Hz, 1H), 3.06 (dd, *J*=17.5, 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.0, 159.1, 158.3, 155.9, 153.1, 152.6, 143.0, 138.7, 138.0, 137.7, 137.1, 137.0, 137.0, 136.7, 133.5, 128.8, 128.8, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.7, 127.7, 127.5, 125.2, 109.4, 107.0, 101.2, 94.9, 94.2, 78.2, 75.4, 75.3, 71.4, 71.3, 70.4, 70.3, 68.5, 26.5; LRMS (ESI) *m/z* 1201 (M+Na); HRMS *m/z* calculated for $C_{78}H_{66}O_{11}Na$ 1201.4503, found 1201.4519.

4.1.3. Benzylated (−)-EGC, [(−)-(2*R*,3*R*)-5,7-bis(benzyloxy)-2-[3,4,5-tris(benzyloxy)phenyl]chroman-3-ol (13)]. To a solution of (−)-(2*R*,3*R*)-5,7-bis(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl-(3,4,5-tris)benzyloxy)-benzoate (**15**, 236 mg, 200 μmol) in MeOH (15.0 mL), sodium hydroxide (8.00 mg, 200 μmol) was added. The resulting mixture was stirred at room temperature for 4 h, concentrated and water was added, then the mixture was extracted with EtOAc×3. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by Flash SiO_2 column chromatography (hexane/EtOAc, 4:1) to afford 136 mg (90%) of the title compound as a white solid. The spectroscopic data of the compound were identical to that of compound **13** reported in the literature.

4.1.4. (2*R*,3*R*)-5,7-Bis(benzyloxy)-2-[(3,4,5-tris(benzyloxy)-phenyl]chroman-3-yl-(4-benzyloxy)benzoate (14) with DCC, DMAP. To a solution of 4-benzyloxylbenzoic acid (34.2 mg, 150 μmol) in dry CH_2Cl_2 (10.0 mL), dicyclohexylcarbodiimide (DCC, 31.0 mg, 150 μmol) was added. The resulting mixture was stirred at room temperature for 4 h and cooled to 0 °C. DMAP (1.20 mg, 100 μmol.) was added and then a solution of (2*R*,3*R*)-5,7-bis(benzyloxy)-2-[3,4,5-tris(benzyloxy)phenyl]chroman-3-ol (**13**, 75.7 mg, 100 μmol) in dry CH_2Cl_2 (2.00 mL) was added dropwise. The mixture was refluxed for 24 h and concentrated. After EtOAc (1.00 mL) was added and the mixture was cooled in fridge, the urea byproduct was filtered, and the filtrate was evaporated in vacuo. The resulting residue was purified by flash SiO_2 column chromatography (hexane/EtOAc, 5:1) to afford 72.5 mg (75%) of the desired compound as a pale yellow amorphous solid. The spectroscopic data of the compound were identical to that of compound **14** obtained above.

4.1.5. (2*R*,3*R*)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-4′-hydroxybenzoate (8). Under a hydrogen atmosphere, palladium hydroxide on carbon powder, 20% Pd (5.00 mg) was added to a solution of (2*R*,3*R*)-5,7-bis(benzyloxy)-2-[(3,4,5-tris (benzyloxy)phenyl]chroman-3-yl-(4′-benzyloxy)benzoate (**14**, 48.4 mg, 50.0 μmol) in EtOAc (5.00 mL). The resulting mixture was stirred at room temperature until TLC showed that the reaction was completed. Then the mixture was filtered to remove the catalyst. The filtrate was evaporated in vacuo to afford 20.4 mg (96%) of the title compound as a white foam solid: $[\alpha]_D^{20}$ −57 (*c* 0.23, MeOH); ^{1}H NMR (CD_3OD) δ 7.78 (d, *J*=8.0 Hz, 2H), 6.83 (d, *J*=8.0 Hz, 2H), 6.63 (s, 2H), 6.05 (d, *J*=2.0 Hz, 1H), 6.03 (d, *J*=1.5 Hz, 1H), 5.54 (m, 1H), 5.09 (s, 1H), 3.05 (dd, *J*=17.0, 4.5 Hz, 1H), 2.95 (dd, *J*=17.0, 2.5 Hz, 1H); ^{13}C NMR (CD_3OD) δ 165.3, 161.9, 157.1, 156.8, 156.4, 145.6, 131.9, 130.1, 121.9, 115.3, 105.9, 98.2, 95.7, 95.0, 77.4, 68.9, 25.8; LRMS (ESI) *m/z* 449 (M+Na); HRMS *m/z* calculated for $C_{22}H_{18}O_9H$ (M+H) 427.1029, found 427.1028.

4.1.6. (2*R*,3*R*)-5,7-Diacetoxy-2-(3,4,5-triacetoxyphenyl)-chroman-3-yl-4′-acetoxybenzoate (9). To a solution of (2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-4′-hydroxybenzoate (**8** (17.1 mg, 40.0 μmol) and Ac_2O (28.4 μL, 300 μmol) in dry MeCN (5.00 mL) at −40 °C, DMAP (5.00 mg, 40.0 μmol) was added. The resulting mixture was stirred for 1 h. Saturated $NaHCO_3$ aqueous solution was added and the mixture was allowed to warm to room temperature. The mixture was extracted with EtOAc×3 and the combined organic phases were washed with water and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by flash SiO_2 column chromatography (hexane/EtOAc, 1:1) to afford 24.4 mg (90%) of the title product as a white solid: Mp 109–111 °C; $[\alpha]_D^{20}$ −60 (*c* 0.2, acetone); ^{1}H NMR ($CDCl_3$) δ 7.87 (d, *J*=8.0 Hz, 2H), 7.27 (s, 2H), 7.09 (d, *J*=8.0 Hz, 2H), 6.74 (d, *J*=2.0 Hz, 1H), 6.59 (d, *J*=2.0 Hz, 1H), 5.62 (s, 1H), 5.20 (s, 1H), 3.06 (m, 2H), 2.28 (s, 6H), 2.27 (s, 3H), 2.24 (s, 3H), 2.23 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 168.9, 168.6, 168.4, 167.5, 166.7, 165.0, 154.7, 154.4, 149.7, 149.7, 143.4, 135.4, 134.2, 131.4, 126.9, 121.5, 118.6, 109.5, 108.9, 108.0, 76.4, 67.5, 25.9, 21.1, 20.7, 20.6, 20.1; LRMS (ESI) *m/z* 701 (M+Na); HRMS *m/z* calculated for $C_{34}H_{30}O_{15}Na$ 701.1482, found 701.1472.

4.2. Cell culture

Human Raji B cells were cultured in RPMI supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cell cultures were maintained in a 5% CO_2 atmosphere at 37 °C.

4.3. Inhibition of purified 20S proteasome activity

Measurement of the chymotrypsin-like activity of the 20S proteasome was performed by incubating 35 μg of purified rabbit 20S proteasome with 40 μM of fluorogenic peptide

substrate, Suc-Leu-Leu-Val-Tyr-AMC, with or without a tea polyphenol at various concentrations, as described previously.[9]

4.4. Cell extract preparation and western blotting

Whole cell extracts were prepared as described previously.[9] Analysis of Bax, PARP, and ubiquitinated proteins was performed using monoclonal or polyclonal antibodies, according to previously reported protocols.[9] Densitometry was quantified using AlphaEase FC software (Alpha Innotech Corporation, San Leandro, CA, USA).

4.5. Inhibition of proteasome activity in intact tumor cells

Cells were treated with each compound at 25 μM for 4 or 24 h, harvested, and lysed as described previously. Whole cell extracts (10 μg) were incubated with Suc-Leu-Leu-Val-Tyr-AMC (40 μM) fluorogenic substrate at 37 °C in 100 μL of assay buffer (50 mM Tris–HCl, pH 8) for 2.5 h. After incubation, production of hydrolyzed 7-amino-4-methylcoumarin (AMC) groups was measured using a Victor 3 Multilabel Counter with an excitation filter of 380 nm and an emission filter of 460 nm (Perkin–Elmer, Boston, MA, USA).

4.6. Trypan blue dye exclusion assay

The trypan blue dye exclusion assay was used to ascertain cell death in Jurkat T cells treated with compounds at 25 μM for 4 or 24 h.[12]

4.7. Induction of caspase-3 activity

Cells were treated with each compound at 25 μM for 4 or 24 h, harvested, and lysed as described previously.[9] Ac-DEVD-AMC (40 μM) was then incubated with the prepared cell lysates for 2.5 h and the caspase-3 activity was measured as described previously.[21,22]

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References and notes

1. Attaguile, G.; Russo, A.; Campisi, A.; Savoca, F.; Acquaviva, R.; Ragusa, N.; Vanella, A. *Cell Biol. Toxicol.* **2000**, *16*, 83.
2. Guvenc, A.; Yildiz, S.; Ozkan, A. M.; Erdurak, C. S.; Coskun, M.; Yilmaz, G.; Okuyama, T.; Okada, Y. *Pharm. Biol.* **2005**, *43*, 178.
3. Danne, A.; Petereit, F.; Nahrstedt, A. *Phytochemistry* **1994**, *37*, 533.
4. Haslam, E. *Plant Polyphenols: Vegetable Tannins Revisited*; Cambridge University Press: Cambridge, 1989.
5. Fujiki, H.; Suganuma, M,; Okabe, S.; Sueoka, N.; Komori, A.; Sueoka, E.; Kozu, T.; Tada, Y.; Suga, K.; Imai, K.; Nakachi, K. *Mutat. Res.* **1998**, *402*, 307.
6. Dreosti, I. E. *Nutr. Rev.* **1996**, *54*, S51.
7. Chen, D.; Daniel, K. G.; Kuhn, D. J.; Kazi, A.; Bhuiyan, M.; Li, L.; Wang, Z.; Wan, S. B.; Lam, W. H.; Chan, T. H.; Dou, Q. P. *Front. Biosci.* **2004**, *9*, 2618.
8. Landis-Piwowar, K. R.; Milacic, V.; Chen, D.; Yang, H.; Zhao, Y.; Chan, T. H.; Yan, B.; Dou, P. Q. *Drug Resist. Updat.* **2006**, *9*, 263.
9. Nam, S.; Smith, D. M.; Dou, Q. P. *J. Biol. Chem.* **2001**, *276*, 13322.
10. Li, L.; Chan, T. H. *Org. Lett.* **2001**, *5*, 739.
11. Wan, S. B.; Chen, D.; Dou, Q. P.; Chan, T. H. *Bioorg. Med. Chem.* **2004**, *12*, 3521.
12. (a) Smith, D. M.; Daniel, K. G.; Wang, Z.; Guida, W. C.; Chan, T. H.; Dou, Q. P. *Proteins* **2004**, *54*, 58; (b) Kuhn, D. J.; Lam, W. H.; Kazi, A.; Daniel, K. G.; Song, S.; Chow, L. M. C.; Chan, T. H.; Dou, Q. P. *Front. Biosci.* **2005**, *10*, 1010.
13. Tueckmantel, W.; Kozikowski, A. P.; Romanczyk, L. J. *J. Am. Chem. Soc.* **1999**, *121*, 12073.
14. Wessel, H. P.; Iversen, T.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2247.
15. Lam, W. H.; Kazi, A.; Kuhn, D. J.; Chow, L. M. C.; Chan, A. S. C.; Dou, Q. P.; Chan, T. H. *Bioorg. Med. Chem.* **2004**, *12*, 5587.
16. Schwartz, A. L.; Ciechanover, A. *Ann. Rev. Med.* **1999**, *50*, 57.
17. Groll, M.; Ditzel, L.; Löwe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. *Nature* **1997**, *386*, 463.
18. Adam, J. *Cancer Cell* **2004**, *5*, 417.
19. Li, B.; Dou, Q. P. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3850.
20. Sang, S.; Lambert, J. D.; Yang, C. S. *J. Sci. Food Agric.* **2006**, *86*, 2256.
21. Chen, Z.; Zhu, Q. Y.; Tsang, D.; Huang, Y. *J. Agric. Food Chem.* **2001**, *49*, 477.
22. Lu, H.; Meng, X.; Yang, C. S. *Drug Metab. Dispos.* **2003**, *31*, 572.
23. Lambert, J. D.; Sang, S.; Hong, J.; Kwon, S. J.; Lee, M. J.; Ho, C. T.; Yang, C. S. *Drug Metab. Dispos.* **2006**, *34*, 2111.