Syntheses and Anticancer Activities of Novel Glucosylated (-)-Epigallocatechin-3-Gallate Derivatives Linked via Triazole Rings

Cheng-Ting Zi
Yunnan Agricultural University https://orcid.org/0000-0002-2367-3617
Bo-Ya Shi (shiboya7@163.com)
Yunnan Agricultural University
Ze-Hao Wang
Yunnan Agricultural University
Ning Zhang
Yunnan Agricultural University
Yin-Rong Xie
Yunnan Agricultural University
Xiu-Li Sun
Yunnan Agricultural University
Hao-Nan Yang
Yunnan Agricultural University
Yi-Long Wu
Yunnan Agricultural University
Xuan-Jun Wang
Yunnan Agricultural University
Jun Sheng
Yunnan Agricultural University

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Abstract

Novel glucosylated (-)-epigallocatechin-3-gallate derivatives **10** – **13** having the EGCG analogues conjugated to the D-glucosyl azide were synthesized by carrying out the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, and were evaluated for their cytotoxicities against a panel of five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW480) using MTT assays. Compounds **10** and **11** showed the highest levels of cytotoxicity against the HL-60 cells with IC_{50} values of 4.57 μM and 3.78 μM, respectively, and showed moderate selectivity towards cancer cell lines. Compound **11** was also shown to induce apoptosis in HL-60 cells. Most notably, inclusion of the perbutyrylated glucose residue in an EGCG derivative was concluded to lead to increased anticancer activity.

Introduction

Tea (*Camellia sinensis* (Linnaeus) O. Kuntze) was first taxonomically described in 1753 by Carl Linnaeus in *SpeciesPlantarum*. Two species of tea have been identified, namely black tea (*Thea bohea*) and green tea (*Thea viridis*) [1]. Four varieties of *Camellia sinensis* have been identified, including *Camellia sinensis* var. *assamica*, *Camellia sinensis* var. *sinensis*, *Camellia sinensis* var. *dehungensis*, and *Camellia sinensis* var. *pubilimba*, which are mainly distributed in the understory of forests of broad-leaved evergreen trees at altitudes of between 100–2,200 m [2]. The Chinese have been using tea as a drink since 3000 BC, and the subspecies: var. *sinensis* (China tea) and var. *assamica* (Assam tea) are found in China.

Green tea is one of the drinks most widely consumed by people around the world, perhaps second most after water, due to its health, sensory, stimulant, relaxing and cultural properties [3]. Catechins are the primary compounds responsible for the claimed health benefits of green tea, including its antioxidant and anti-inflammatory properties. The major catechins in green tea including (-)-epicatechin (EC, **1**), (-)-epicatechin-3-gallate (ECG, **2**), (-)-epigallocatechin (EGC, **3**) and (-)-epigallocatechin-3-gallate (EGCG, **4**) (Figure 1) have been reported to display numerous biological activities [4–6]. EGCG is the most abundant catechin found in green tea and has been reported to display physiological activities stronger than those of the other catechins [7–9] and to display many types of biological activities including anti-oxidative, anti-inflammatory, anti-cancer, anti-infection and neuroprotective activities [10–12].

However, the use of EGCG is often hindered by problems such as being easily oxidized, readily degraded in aqueous solution and poorly intestinal absorbed in the intestines [13, 14]. To obtain more potent analogues and overcome this problem of poor intestinal absorption, many semisynthetic derivatives such as permethyl EGCG [15], peracetyl EGCG [16], EGCG monoester derivatives [17], and EGCG glycosides [13, 18–21] have been developed. In recent years, the use of glycoconjugates of small-molecule anticancer drugs has become an attractive strategy for improving drug efficacy [22, 23]. In our previous study, we reported the syntheses and cytotoxicities of glucosylated EGCG derivatives, we found that, in aqueous solution, EGCG glucosides displayed higher activities against cells of human breast cancer cell lines and higher levels of stability than did EGCG [21].

Due to the ability of terminal alkyne and an azides to undergo copper-catalyzed [3 + 2]-cycloadditions with azides to generate substituted triazole rings [24] and due to butyrate having been shown to be a histone deacetylase (HDAC) inhibitor and to display anticancer effects with promising therapeutic potential [25], we set out in the current work to chemically synthesized glucosylated (-)-epigallocatechin-3-gallate derivatives linked via triazole rings and to characterize their in vitro anticancer activities against five human cancer cell lines, including HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer) and SW480 (colon cancer). In addition, chemical informatics analyses of these compounds were carried out, and the chemical properties of the compounds were correlated with their anticancer activity.

Results And Discussion

Chemistry

The novel triazole-linked glucose-(-)-epigallocatechin-3-gallate derivatives **10** – **13** were synthesized according to the synthetic route shown in Scheme 1. Here, 2,3,4,6-tetra-O-butyryl-1-azido-α-D-glucopyranose **7** was readily prepared from D-glucose by using a method similar to that reported in the literature [26]. And O-alkylated EGCG analogues **8** and **9** were prepared in 30%–45% yields by reacting EGCG (**4**) with potassium carbonate (K_2CO_3) and propargyl bromide at room temperature for 12 h. Then, compound **7** was allowed to react with EGCG analogues **8** and **9** at their terminal alkynes in the presence of copper (II) acetate and sodium ascorbate in *t*-BuOH–H_2O (1:1) as the solvent for 2 h to yield 2,3,4,6-tetra-O-butyryl-*α*-D-glucopyranosyloxy-1,2,3-triazol-1-yl-(-)-epigallocatechin-3-gallates **10** and **11** in 77–80% yields [24, 27]. Finally, compounds **10** and **11** were treated with potassium hydroxide (KOH) solution (dissolved in CH_3OH) in CH_3OH at 0 ºC for 72 h to yield triazole-linked glucose-EGCG derivatives **12** and **13** with 52% to 55% yields [28].

The structures of all of the synthesized compounds were characterized using ^{1}H-NMR spectroscopy, ^{13}C-NMR spectroscopy, electrospray ionization mass spectrometry (ESI-MS), and high-resolution mass spectrometry (HRESI-MS). ESI-MS and HRESI-MS of all compounds showed the $[M + Na]^+$/$[M + H]^+$/$[M – H]^-$ species. In the ^{1}H-NMR spectra, resonance of the C^{14}-H/$C^{14'}$-H signal (δ 8.19–8.20 ppm) in the aromatic region confirmed the formation of the triazole ring. The structures were further confirmed upon analysis of their ^{13}C-NMR spectra, which showed the two characteristic carbon signals at 145.4–146.7 ppm ($\delta_{C\text{-}14}$/$\delta_{C\text{-}14'}$) and the others at 124.7–126.9 ppm ($\delta_{C\text{-}13}$/$\delta_{C\text{-}13'}$), corresponding to the triazole residue. The synthesized compounds **12** and **13** were further analyzed using 2D-NMR spectroscopy (Fig. 2); the heteronuclear multiple bond correlation (HMBC) of compound **12** showed a strong correlation between $C^{12'}$-H (5.11 ppm) and C-4" (139.4 ppm) of the D ring. This signal indicated an attachment of the triazole-linked ring to the C-4" of EGCG. Similarly, the HMBC of compound **13** indicated the occurrence of coupling between $C^{12'}$-H (5.11 ppm) and C-4" (139.4 ppm) of the D ring and also between C^{12}-H (5.21 ppm) and C-4' (136.3 ppm) of the B ring. These results indicated that the attachment of two triazole-linked ring one to the C-4' and C-4" position of EGCG. The chemical shift for the proton at C^4-H was observed to the coupled

with C-2 and C-3, those for $C^{2'}$-H and $C^{6'}$-H occurred with C-2 and $C^{2''}$-H, that for $C^{6''}$-H occurred with C-11, and that for C^{2}-H occurred with C-11 in both compounds **12** and **13**.

In vitro anti-proliferative activity

The triazole-linked glucose-(-)-epigallocatechin-3-gallate derivatives **10** – **13** were evaluated for their cytotoxicities against five human cancer cell lines, including HL-60, SMMC-7721, A-549, MCF-7 and SW480. The compounds EGCG and cisplatin were used as positive controls. The screening procedure was based on the standard MTT method [26]. Their activities were expressed as IC_{50} values (concentration of drug inhibiting 50% cell growth) and the data are presented in Table 1.

The compounds having a free glucose residue namely compounds **12** and **13** show weaked activity levels (IC_{50} > 40 μM) toward cells of the three cancer cell lines SMMC-7721, A-549 and SW480. In contrast, the derivatives containing each a each a perbutyrylated glucose residue, namely compounds **10** and **11** showed higher activity levels, and they showed the highest cytotoxicity levels agaisnt HL-60 cells, with IC_{50} values of 4.57 μM and 3.78 μM, respectively; they were also found to more potent than the control drug EGCG, which displayed IC_{50} > 40 μM against each of the five cancer cell lines. Interestingly, all of the EGCG derivatives showed good levels of cytotoxicity against MCF cells with IC_{50} values in the range 28.24–39.89 μM. Based on these results taken together, we concluded that perbutyrylation of the glucose residue of the EGCG scaffold lead to increased anticancer activity.

Selectivities of the compounds

To evaluate the degrees of selectivity of the most cytotoxic compounds, namedly **11** and **12**, their growth inhibitory effects on cells of a normal human bronchial epithelial cell line (BEAS-2B) were measured (Table 1). The selectivity index (SI) values of compounds **11**, **12** and cisplatin are presented in Table 2. Compounds **11** and **12** showed moderate selectivity toward cancer cell lines with SI values in the range of 1.0–8.4 for all cells tested.

Induction of cell apoptosis

Given that the EGCG derivative **11** exhibited significant inhibitory activity of cancerous cell growth in HL-60 cells, we studied further the ability of compound **11** to induce cell death through apoptosis. To carry out this study, the tested HL-60 cells were stained with annexin V, and compound **11** was administered at a concentration of 8 μM. Significantly higher amounts of compound **11** were detected in HL-60 cells undergoing apoptosis than in the untreated control (Fig. 3A-B). We also determined the expression levels of caspase-3 and PARP, which are the hallmarks of apoptosis and play crucial roles in the cellular process. For this purpose, samples of HL-60 cells were treated with compound **11**, respectively, concentrations of 2, 4 and 8 μM for 12 h and the expression levels of caspase-3, PARP, cleaved-caspase-3 and cleaved-PARP were monitored using western blot analysis. The treatment of HL-60 cells with compound **11** was found to be associated with increased levels of expression of cleaved-caspase-3 and cleaved-PARP in a dose-dependent manner (Fig. 3C). Compared to the untreated control, compound **11**

apparently induced a significant increase in the expressed levels cleaved-caspase-3 and cleaved-PARP and a decrease in those of caspase-3 and PARP.

Physicochemical property

As compounds **12** and **13** showed each weak activity in vitro, we calculated the Clogp values of **10** – **13** by MarvinSketch version 5.3.8 [29], and the data are shown in Table 3. ClogP values of only 0.50 and –2.08 were calculated for, respectively, compounds **12** and **13**. But higher ClogP values of 6.84 and 10.61 were calculated for, respectively, compounds **10** and **11**. Based on these results, compounds **12** and **13** were expected to display lower of cell permeability than were compounds **10** and **11**.

Solubility

We also determined the water solubilities of the EGCG and EGCG derivatives (**10**–**13**), and these results are shown in Table 4. The solubility of EGCG was measured to be 16.40 mM, whereas those of compounds **10**–**13** were measured to be 5.71 mM, 1.05 mM, 281.17 mM and 512.88 mM, respectively. That is compounds **12** and **13** (those each having a free glucose residue) showed, respectively, 17 and 31 times higher levels of solubility in water than did EGCG; while compounds **10** and **11** (the derivatives each containing a perbutyrylated glucose residue showed respectively, solubility levels only fractions (0.3 and 0.06) of that of EGCG. Taken together, these data indicated a trend of higher aqueous solubility levels for those compounds containing more glycol units.

Conclusion

In summary, a series of novel glucosylated (-)-epigallocatechin-3-gallate derivatives have been synthesized by carrying out the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. According to the results of in vitro proliferation inhibitory activity against a panel of five human cancer cells (HL-60, SMMC-7721, A-549, MCF-7 and SW480), Compounds **10** and **11** showed the highest levels of cytotoxicity against the HL-60 cells with IC_{50} values of 4.57 μM and 3.78 μM, respectively. Compounds with perbutyrylated glucose residue was concluded to lead to increased anticancer activity. Further research for modifying and enhancing their biological activity potential is undergoing based on the present data.

Experimental

Materials and measurements

D-glucose and *n*-butyric anhydride were purchased from Aladdin Chemical Co., Ltd (Guangzhou, China); (-)-epigallocatechin-3-gallate was obtained from Chengdu Proifa Technology Development Co., Ltd (Chengdu, China); and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents were commercially available and used without further purification unless indicated otherwise. The melting points were measured by using an X-4 melting point apparatus and were uncorrected. Optical rotations data were obtained using a Jasco P-

1020 Automatic Digital Polariscope. MS data were obtained in ESI mode using an API Qstar Pulsar instrument; HRMS data were obtained in ESI mode using LCMS-IT-TOF apparatus (Shimadzu, Kyoto, Japan); ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker DRX-500 instrument (Bruker BioSpin GmbH, Rheinstetten, Germany) with tetramethylsilane (TMS) as an internal standard. Column chromatography (CC) was performed with a silica gel (200 – 300 mesh; Qingdao Makall Group CO., LTD; Qingdao; China). All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates, which were visualized using ultraviolet light (254 nm) and/or 10% phosphomolybdic acid/EtOH. All cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) were obtained from a Shanghai cell bank in China.

Synthesis of 2,3,4,6-tetra-O-butyryl-1-azido-α-D-glucopyranose (7)

D-glucose (1.8 g, 10 mmol) was suspended in *n*-butyric anhydride (3.5 mL, 20 mmol) and stirred at 0 ºC. Iodine (100 mg) was added to this stirred suspension and the stirring was continued for 1 h. The resulting reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed successively with aqueous saturated $Na_2S_2O_3$ and aqueous saturated $NaHCO_3$. The resulting organic layer was then dried over Na_2SO_4 and concentrated under vacuo to give the crude per-butyrylated product (5.2 g, 99%). This crude product (5.2 g, 10 mmol) was dissolved in CH_2Cl_2 (30 mL), and hydrobromic acid (5 mL) was slowly added dropwise to this solution while the resulting mixture was slowly and stirred at 0 ºC. The mixture was stirred for 8 h until no starting material was detected using the TLC analysis. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with aqueous saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, concentrated, and dried in vacuo to afford crude 2,3,4,6-tetra-O-butyryl-α-D-glucopyranosyl bromide **6** (3.7 g, 70%). The crude butyryl-glucopyranosyl bromide **6** (3.7 g, 7.1 mmol) was dissolved in DMF (5 mL) and sodium azide (690 mg, 10.6 mmol) was added to the resulting solution. The resulting mixture was stirred at 50 ºC for 12 hours until no starting material was detected using TLC analysis. The reaction mixture was diluted with CH_2Cl_2 (20 mL), and then washed with aqueous saturated $NaHCO_3$. The organic layer was dried using Na_2SO_4 and evaporated, and the resulting residue was purified by column chromatography in silica gel (petroleum ether 60°C–90°C: ethyl acetate = 9:1) to afford 2,3,4,6-tetra-O-butyryl-1-azido-α-D-glucopyranose **7** (2.1 g, 60%).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.25 (t, 1H, *J* = 9.6 Hz), 5.12 (t, 1H, *J* = 9.6 Hz), 4.97 (t, 1H, *J* = 9.6 Hz), 4.62 (d, 1H, *J* = 8.6 Hz, C^{1}-H), 4.21–4.19 (m, 2H), 3.76–3.72 (m, 1H), 2.35–2.19 (m, 8H, 4 × CO$\underline{CH_2}$), 1.68–1.53 (m, 8H, 4 × $\underline{CH_2}CH_3$), 0.96–0.87 (m, 12H, 4 × $CH_2\underline{CH_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.3 (C=O), 172.6 (C=O), 171.9 (C=O), 171.9 (C=O), 88.0, 74.2, 72.2, 70.4, 67.7, 61.5, 35.8 (CO$\underline{CH_2}$), 35.8 (CO$\underline{CH_2}$), 35.7 (CO$\underline{CH_2}$), 35.7 (CO$\underline{CH_2}$), 18.2 ($\underline{CH_2}CH_3$), 18.2 ($\underline{CH_2}CH_3$), 18.2 ($\underline{CH_2}CH_3$), 18.1 ($\underline{CH_2}CH_3$), 13.6 ($CH_2\underline{CH_3}$), 13.5 ($CH_2\underline{CH_3}$), 13.5 ($CH_2\underline{CH_3}$), 13.5 ($CH_2\underline{CH_3}$); ESIMS *m/z* 508 $[M + Na]^+$.

Synthesis of O-alkylated (-)-epigallocatechin-3-gallate conjugates 8 and 9

A mixture of EGCG (2.3 g, 5 mmol), DMF (15 mL) and K_2CO_3 (1.0 g, 7.5 mmol) was made at room temperature under nitrogen and stirred for 0.5 h. Propargyl bromide (0.3 mL, 7.5 mmol) was quickly added to the resulting mixture, which was then stirred at room temperature for 12 h until no starting material was detected according to TLC analysis. The solvent of the resulting mixture was evaporated under vacuum and the residue was purified using column chromatography with silica gel ($CHCl_3$/CH_3OH, 9:1→4:1) to afford the EGCG conjugates **8** (1.1 g, 45%) and **9** (0.8 g, 30%).

5,7-Dihydroxy-2-(3',4',5'-trihydroxyphenyl)chroman-3-yl 3",5"-dihydroxy-4"-

(prop-2-yn-1-yloxy)benzoate (8)

^{1}H-NMR (CD_3OD, 500 MHz) δ: 6.90 (s, 2H, $C^{2"}$-H, $C^{6"}$-H), 6.50 (s, 2H, $C^{2'}$-H, $C^{6'}$-H), 5.96 (s, 2H, C^6-H, C^8-H), 5.53 (brs, 1H, C^3-H), 4.97 (s, 1H, C^2-H), 4.78 (d, 2H *J* = 2.4 Hz, C^{12}-CH_2), 3.29 (t, 1H, *J* = 1.6 Hz, C^{14}-H), 2.96 (dd, 1H, *J* = 4.6 Hz, 12.0 Hz, C^4-CH_a), 2.85 (dd, 1H, *J* = 4.6 Hz, 12.0 Hz, C^4-CH_b); ^{13}CNMR (CD_3OD, 125 MHz) δ: 167.0 (C=O), 157.9 (C-7), 157.8 (C-5), 157.2 (C-9), 151.9 (C-3", C-5"), 146.7 (C-3', C-5'), 138.4 (C-4"), 133.8 (C-4'), 130.7 (C-1'), 127.1 (C-1"), 110.1 (C-2", C-6"), 106.8 (C-2', C-6'), 99.3 (C-10), 96.5 (C-6), 95.9 (C-8), 80.4 (C-2), 79.5 (C≡CH), 78.5 (C≡CH), 70.3 (C-3), 60.0 (C-14), 26.8 (C-4); ESIMS *m/z* 495 $[M - H]^-$.

5,7-Dihydroxy-2-(3',5'-dihydroxy-4'-(prop-2-yn-1-yloxy)phenyl)-3",5"-dihydroxy-4"-(prop-2-yn-1-yloxy)benzoate (9)

^{1}H-NMR (CD_3OD, 500 MHz) δ: 6.90 (s, 2H, $C^{2"}$-H, $C^{6"}$-H), 6.52 (s, 2H, $C^{2'}$-H, $C^{6'}$-H), 5.96-5.95 (m, 2H, C^6-H, C^8-H), 5.56-5.55 (m, 1H, C^3-H), 5.00 (s, 1H, C^2-H), 4.77 (d, 2H, *J* = 2.4 Hz, OCH_2), 4.67 (d, 2H, *J* = 2.4 Hz, OCH_2), 3.33 (s, 2H, 2 × C≡CH), 2.98 (dd, 1H, *J* = 4.6 Hz, 12.0 Hz, C^4-CH_a), 2.88 (m, 1H, C^4-CH_b); ^{13}CNMR (CD_3OD, 125 MHz) δ: 167.0 (C=O), 157.9 (C-7), 157.8 (C-5), 157.0 (C-9), 151.9 (C-3", C-5"), 151.7 (C-3', C-5'), 138.4 (C-4"), 136.3 (C-4'), 133.7 (C-1'), 127.0 (C-1"), 110.1 (C-2", C-6"), 106.8 (C-2', C-6'), 99.3 (C-10), 96.6 (C-6), 95.9 (C-8), 80.4 (C≡CH), 80.0 (C≡CH), 78.2 (C-2), 76.7 (C≡CH), 76.4 (C≡CH), 70.2 (C-3), 60.2 (OCH_2), 60.0 (OCH_2), 26.8 (C-4); ESIMS *m/z* 533 $[M - H]^-$.

General procedure for the synthesis of 2,3,4,6-tetra-O-butyryl-α-D-glucopyranosyloxy-1,2,3-triazol-1-yl-(-)-epigallocatechin-3-gallates (10 and 11)

For each of the two target compounds, a solution of compound **7** (0.1 mmol) and *O*-alkylated (-)-epigallocatechin-3-gallate conjugates **8** or **9** (0.1 mmol) in THF (1.0 mL) and ^{t}BuOH-H_2O (1.0 mL, 1:1) was prepared, and copper(II) acetate (0.01 mmol) and sodium ascorbate (0.01 mmol) were added to this solution. This reaction mixture was stirred at room temperature for 2 h until no starting material was detected according to TLC analysis. The resulting mixture was evaporated under vacuum and the residue was purified using column chromatography with silica gel ($CHCl_3$/CH_3OH, 9:1) to afford the target cycloaddition product.

4"-O-[(2"",3"",4"",6""-tetra-O-butyryl-α-D-glucopyranosyloxy)-1,2,3-triazol-1-yl)tetrahydro-2H-pyran]-(-)-epigallocatechin-3-gallate (10)

White amorphous powder; Yield: 80%; MP. 98–100 °C; [α]24.1 D: +14.2 (c 0.22, CH_3OH); ^{1}H-NMR (CD_3OD, 500 MHz) δ: 8.19 (s, 1H, $C^{14'}$-H), 6.90 (s, 2H, $C^{2"}$-H, $C^{6"}$-H), 6.50 (s, 2H, $C^{2'}$-H, $C^{6'}$-H), 6.10 (d, 1H, *J* = 2.3 Hz, $C^{1""}$-H), 5.94 (s, 2H, C^6-H, C^8-H), 5.61 (t, 1H, *J* = 9.4 Hz), 5.53–5.50 (m, 2H), 5.27–5.24 (m, 1H), 5.23 (s, 2H, $C^{12'}$-CH_2), 4.96 (s, 1H, C^2-H), 4.36–4.18 (m, 3H), 2.98 (dd, 1H, *J* = 4.6 Hz, 12.1 Hz, C^4-H_a), 2.88–2.86 (m, 1H, C^4-H_b), 2.23–1.94 (m, 8H, 4 × CO$\underline{CH_2}$), 1.60–1.26 (m, 8H, 4 × $\underline{CH_2}CH_3$), 0.93–0.62 (m, 12H, 4 × $CH_2\underline{CH_3}$); ^{13}CNMR (CD_3OD, 125 MHz) δ: 174.7 (C=O), 173.8 (C=O), 173.5 (C=O), 172.9 (C=O), 167.0 (C-11), 157.9 (C-7), 157.8 (C-5), 157.2 (C-9), 151.6 (C-3", C-5"), 146.6 (C-3', C-5'), 145.8 (C-14'), 138.8 (C-4"), 133.7 (C-4'), 130.7 (C-1'), 126.9 (C-13'), 124.8 (C-1"), 110.3 (C-2", C-6"), 106.7 (C-2', C-6'), 99.2 (C-10), 96.5 (C-6), 95.8 (C-8), 86.4 (C-1""), 78.5 (C-2), 75.9, 74.0, 71.4, 70.3 (C-12'), 68.9, 65.3 (C-3), 62.6, 36.7 (CO$\underline{CH_2}$), 36.6 (CO$\underline{CH_2}$), 36.6 (CO$\underline{CH_2}$), 36.2 (CO$\underline{CH_2}$), 26.8 (C-4), 19.2 ($\underline{CH_2}CH_3$), 19.2 ($\underline{CH_2}CH_3$), 19.2 ($\underline{CH_2}CH_3$), 19.0 ($\underline{CH_2}CH_3$), 13.9 ($CH_2\underline{CH_3}$), 13.9 ($CH_2\underline{CH_3}$), 13.9 ($CH_2\underline{CH_3}$), 13.6 ($CH_2\underline{CH_3}$); ESIMS *m/z* 980 $[M - H]^-$, HRESIMS was calculated for $C_{47}H_{55}N_3O_{20}$ $[M - H]^-$ 980.3306 and was found to be 980.3306.

{4'-O-[(2"",3"",4"",6""-tetra-O-butyryl-α-D-glucopyranosyloxy)-1,2,3-triazol-1-yl)tetrahydro-2H-pyran]-4"-O-[(2"",3"",4"",6""-tetra-O-butyryl-α-D-glucopyranosyloxy)-1,2,3-triazol-1-yl)tetrahydro-2H-pyran]}-(-)-epigallocatechin-3-gallate (11)

White amorphous powder; Yield: 77%; MP: 92–93 °C; [α]24.1 D: +19.3 (c 0.14, CH_3OH); ^{1}H-NMR (CD_3OD, 500 MHz) δ: 8.19 (s, 2H, C^{14}-H, $C^{14'}$-H), 6.91 (s, 2H, $C^{2"}$-H, $C^{6"}$-H), 6.50 (s, 2H, $C^{2'}$-H, $C^{6'}$-H), 6.15 (d, 1H, *J* = 2.3 Hz, $C^{1""}$-H), 6.10 (d, 1H, *J* = 9.3 Hz, $C^{1""}$-H), 5.94 (s, 2H, C^6-H, C^8-H), 5.61 (t, 2H, *J* = 9.4 Hz), 5.56–5.53 (m, 3H), 5.29–5.26 (m, 2H), 5.20–5.15 (m, 4H), 4.96 (s, 1H, C^2-H), 4.38–4.36 (m, 2H), 4.22–4.18 (m, 2H), 3.10–3.06 (m, 2H, C^4-CH_2), 2.24–2.00 (m, 16H, 8 × CO$\underline{CH_2}$), 1.70–1.49 (m, 16H, 8 × $\underline{CH_2}CH_3$), 1.01–0.87 (m, 24H, 8 × $CH_2\underline{CH_3}$); ^{13}C-NMR (CD_3OD, 125 MHz) δ: 174.3 (C=O), 174.2 (C=O), 174.1 (C=O), 174.1 (C=O), 174.0 (C=O), 174.0 (C=O), 173.9 (C=O), 173.7 (C=O), 167.0 (C-11), 157.9 (C-7), 157.8 (C-5), 157.2 (C-9), 151.6 (C-3", C-5"), 146.6 (C-3', C-5'), 145.8 (C-14), 145.8 (C-14'), 138.8 (C-4"), 133.7 (C-4'), 130.7 (C-1'), 126.9 (C-13), 126.9 (C-13'), 124.8 (C-1"), 110.3 (C-2", C-6"), 106.7 (C-2', C-6'), 99.2 (C-10), 96.5 (C-6), 95.8 (C-8), 86.6 (C-1""), 86.4 (C-1""), 78.5 (C-2), 77.0, 74.1, 73.6, 73.1, 72.8, 72.5, 71.8, 71.5, 69.8 (C-12), 69.5 (C-12'), 66.9 (C-3), 62.6, 62.6, 36.9 (CO$\underline{CH_2}$), 36.9 (CO$\underline{CH_2}$), 36.8 (CO$\underline{CH_2}$), 36.8 (CO$\underline{CH_2}$), 36.7 (CO$\underline{CH_2}$), 36.7 (CO$\underline{CH_2}$), 36.6 (CO$\underline{CH_2}$), 36.6 (CO$\underline{CH_2}$), 26.8 (C-4), 19.5 ($\underline{CH_2}CH_3$), 19.5 ($\underline{CH_2}CH_3$), 19.4 ($\underline{CH_2}CH_3$), 19.4 ($\underline{CH_2}CH_3$), 19.3 ($\underline{CH_2}CH_3$), 19.2 ($\underline{CH_2}CH_3$), 19.2 ($\underline{CH_2}CH_3$), 19.1 ($\underline{CH_2}CH_3$), 14.2 ($CH_2\underline{CH_3}$), 14.1 ($CH_2\underline{CH_3}$), 14.0 ($CH_2\underline{CH_3}$), 14.0 ($CH_2\underline{CH_3}$), 13.9 ($CH_2\underline{CH_3}$), 13.9 ($CH_2\underline{CH_3}$), 13.9 ($CH_2\underline{CH_3}$), 13.7 ($CH_2\underline{CH_3}$); ESIMS *m/z* 1503 $[M - H]^-$, HRESIMS was calculated for $C_{72}H_{91}N_6O_{29}$ $[M - H]^-$ 1503.5946 and was found to be 1503.5939.

General procedure for the syntheses of α-D-glucopyranosyloxy-1,2,3-triazol-1-yl-(-)-epigallocatechin-3-gallates (12 and 13)

For each of the target compounds, a solution of *2,3,4,6-tetra-O-butyryl-α-D-glucopyranosyloxy*-1,2,3-triazol-1-yl-*(-)-epigallocatechin-3-gallate (**10**or**11**)* (0.05 mmol) in CH_3OH (1 mL) was prepared, and to this solution was added a KOH solution (0.15 mmol, dissolved in CH_3OH). This mixture was stirred at 0 ºC for 72 h, and then neutralized with Dowex 50WX4-400 ion-exchange resin to pH = 7. The solvent of the resulting mixture was evaporated in vacuum and resulting the residue was purified using column chromatography with silica gel ($CHCl_3/CH_3OH$, 4:1) to afford the product.

4"-O-[(1""-α-D-glucopyranosyloxy)-1,2,3-triazol-1-yl)tetrahydro-2H-pyran]-(-)-epigallocatechin-3-gallate (12)

White amorphous powder; Yield: 55%; MP: 125–126 °C; [α]23.8 D: +14.4 (c 0.23, CH_3OH); ^{1}HNMR (CD_3OD, 500 MHz) δ: 8.20 (s, 1H, $C^{14'}$-H), 6.91 (s, 2H, $C^{2"}$-H, $C^{6"}$-H), 6.53 (s, 2H, $C^{2'}$-H, $C^{6'}$-H), 5.96-5.95 (m, 2H, C^6-H, C^8-H), 5.58 (d, 1H, *J* = 2.3 Hz, $C^{1""}$-H), 5.21 (s, 1H, C^3-H), 5.15 (s, 1H, C^2-H), 5.11 (d, 2H, *J* = 2.4 Hz, $C^{12'}$-CH_2), 3.89–3.86 (m, 2H), 3.72–3.69 (m, 1H), 3.57–3.53 (m, 2H), 3.51–3.48 (m, 1H), 2.99–2.87 (m, 1H, C^4-CH_a), 2.85–2.82 (m, 1H, C^4-CH_b); ^{13}CNMR (CD_3OD, 125 MHz) δ: 167.1 (C-11), 157.9 (C-5), 157.8 (C-7), 157.0 (C-9), 151.9 (C-3", C-5"), 151.7 (C-3', C-5'), 146.7 (C-14'), 139.4 (C-4"), 136.3 (C-4'), 127.0 (C-1'), 124.9 (C-1"), 124.9 (C-13'), 110.4 (C-2", C-6"), 106.9 (C-2', C-6'), 99.3 (C-10), 96.6 (C-6), 95.9 (C-8), 89.6 (C-1""), 81.1 (C-2), 78.4, 74.1, 70.8, 70.2 (C-12'), 66.0, 62.4 (C-3), 60.0, 26.7 (C-4); ESIMS: *m/z* = 702 $[M + H]^+$, HRESIMS was calculated for $C_{31}H_{31}N_3O_{16}$ $[M - H]^-$ 700.1632 and was found to be 700.1636.

{4'-O-[(1""-α-D-glucopyranosyloxy)-1,2,3-triazol-1-yl)tetrahydro-2H-pyran]-4"-O-[(1""-α-D-glucopyranosyloxy)-1,2,3-triazol-1-yl)tetrahydro-2H-pyran]}-(-)-epigallocatechin-3-gallate (13)

White amorphous powder; Yield: 52%; MP: 120–121 °C; [α]25.0 D: +22.4 (c 0.24, CH_3OH); ^{1}HNMR (CD_3OD, 500 MHz) δ: 8.19 (s, 2H, C^{14}-H, $C^{14'}$-H), 6.91 (s, 2H, $C^{2"}$-H, $C^{6"}$-H), 6.52 (s, 2H, $C^{2'}$-H, $C^{6'}$-H), 5.96 (m, 2H, C^6-H, C^8-H), 5.59 (d, 1H, *J* = 1.6 Hz, $C^{1""}$-H), 5.57 (d, 1H, *J* = 1.6 Hz, $C^{1"}$-H), 5.58 (s, 1H, C^3-H), 5.21 (s, 2H, C^{12}-CH_2), 5.11 (s, 2H, $C^{12'}$-CH_2), 5.00 (s, 1H, C^2-H), 3.90–3.85 (m, 4H), 3.71–3.68 (m, 2H), 3.57–3.54 (m, 4H), 3.53–3.52 (m, 2H), 2.98 (dd, 1H, *J* = 4.6 Hz, 12.1 Hz, C^4-H_a), 2.88–2.86 (m, 1H, C^4-H_b); ^{13}CNMR (CD_3OD, 125 MHz) δ: 166.9 (C-11), 157.9 (C-7), 157.8 (C-5), 157.0 (C-9), 151.7 (C-3", C-5"), 151.5 (C-3', C-5'), 145.5 (C-13), 145.4 (C-14'), 139.4 (C-4"), 136.3 (C-4'), 134.5 (C-1'), 126.9 (C-1"), 124.8 (C-14), 124.7 (C-13'), 110.4 (C-2", C-6"), 107.1 (C-2', C-6'), 99.2 (C-10), 96.6 (C-6), 95.8 (C-8), 89.6 (C-1""), 89.5 (C-1"), 81.1 (C-2), 78.4, 78.3, 78.3, 78.2, 74.1, 74.0, 70.8 (C-12), 70.8 (C-12'), 70.2 (C-3), 66.0, 65.8, 62.4, 62.3, 26.7 (C-4); ESIMS: *m/z* = 945 $[M + H]^+$, HRESIMS was calculated for $C_{40}H_{44}N_6O_{21}$ $[M - H]^-$ 943.2487 and was found to be 943.2460.

Cytotoxicity Assay

MTT assays were conducted to evaluate the cell viabilities of the triazole-linked glucose-(-)-epigallocatechin-3-gallate derivatives. Cells of five human cancer lines, namely HL-60, SMMC-7721, A-549, MCF-7 and SW480, were seeded in 96-well plates and then exposed to the test compound at various concentrations in triplicate for 48 h. After the incubation, MTT (100 μg) was added to each well, and the incubation was continued for 4 h at 37 °C. After removal of the culture medium, the produced MTT formazan crystals were dissolved in DMSO (150 μL) and the OD of the resulting solution was measured at a wavelength of 492 nm using a microplate reader. The percent inhibition was calculated using the formula inhibition ratio (IR, %) = (1 − OD(sample)/OD(control)) × 100%. The experiments were carried out in triplicate, and the IC_{50} (the concentration of drug that inhibits cell growth by 50%) values were determined.

Cell Apoptosis Assay

An annexin V/propidium iodide (PI) detection kit (BD Biosciences, PA, USA) was employed to quantify apoptosis using flow cytometry. For each experiment, HL-60 cells were seeded into each well of a 6-well plate at 5×10^5 cells/well and then treated with one of the EGCG derivatives. After the treatment, the collected cells were incubated in 100 μL of binding buffer, and then into the resulting suspension were added 5 μL of FITC annexin V and 10 μL of PI. Each mixture was gently vortexed and then incubated for 15 min at room temperature in the dark before taking the flow cytometry measurements (BD FACSCalibur) within 1 h.

Calculated Partition Coefficient

All structures of EGCG derivatives were built and energy minimized by applying the Tripos force field. The Gasteiger–Huchel method was used to calculate charges. Energy minimization was performed by applying the Powell method with 2000 iterations. The calculated partition coefficient (ClogP) was obtained from MarvinSketch version 5.3.8. (www.chemaxon.org) [29].

Water Solubility Analysis

All compounds were subjected to water solubility analyses. Each compound was mixed in 200 μL of water in an Eppendorf tube at room temperature. An ultrasonic cleaner was used to maximize the amount of the compound that became dissolved. After 1 h of sonication, each sample was diluted and then filtered through a 0.45 μm MFS membrane for HPLC analysis to determine the concentrations of the tested compound [21]. Analytical HPLC was performed using an Agilent 1260 liquid chromatograph equipped with a ZORBAX SB-C18 (4.6 × 250 mm) column. Here, the detection wavelength was 280 nm, the injection volume was 10 μL. The flow rate was 1 mL/min.

Declarations

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Technology Department (Grant No. 2017ZF003, 2017FD084, 2017FG001-046); and the Yunnan Provincial Key Programs of Yunnan Eco-friendly Food International Cooperation Research Center Project (Grant No. 2019ZG00904, 2019ZG00909).

Compliance with ethical standards

Conflict of Interests All authors declare that: (i) except for National Natural Science Foundation of China, the Yunnan Provincial Science and Technology Department and the Yunnan Provincial Key Programs of Yunnan Eco-friendly Food International Cooperation Research Center Project, no support, financial or otherwise, has been received from any other organization that may have an interest in the submitted work; and (ii) there are no other relationships or activities that could appear to have influenced the submitted work.

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Tables

Table 1 *In vitro* anticancer activity (IC_{50}, μM) of triazole-linked gucose-(-)-epigallocatechin-3-gallate derivatives 10 – 13.

Compounds	IC_{50} (μM)					
	HL-60	SMMC-7721	A-549	MCF-7	SW480	BEAS-2B
10	4.56	23.32	38.48	36.47	38.21	38.66
11	3.78	21.26	30.12	28.24	31.00	31.58
12	13.47	>40	>40	35.60	>40	NT
13	15.30	>40	>40	39.89	>40	NT
4	>40	>40	>40	>40	>40	>40
cisplatin	1.17	6.43	9.24	15.86	13.42	12.87

Abbreviation: NT = not tested.

Table 2 The selectivity index of compounds 10, 11 and cisplatin to cancer cells as compared with BEAS-2B normal cell Line.

Compounds	Selectivity index (SI[a])				
	HL-60	SMMC-7721	A-549	MCF-7	SW480
10	8.4	1.7	1.0	1.1	1.0
11	8.4	1.5	1.0	1.1	1.0
Cisplatin	11.0	2.0	1.4	0.8	1.0

Note: [a]Selectivity index (SI) = IC_{50} of the compound in BEAS-2B cell line/IC_{50} of the compound in cancer cee line.

Table 3 The ClogP values and PBA of triazole-linked glucose-(-)-epigallocatechin-3-gallate derivatives 10 – 13.

Compounds	Molecular formula	m.p.(°C)	Yield (%)	ClogP[a]
10	$C_{47}H_{55}N_3O_{20}$	98–100	80	6.84
11	$C_{72}H_{92}N_6O_{29}$	92–93	77	10.61
12	$C_{31}H_{31}N_3O_{16}$	125–126	55	0.50
13	$C_{40}H_{44}N_6O_{21}$	120–121	52	-2.08
4	$C_{22}H_{18}O_{11}$	—	—	3.08

[a]ClogP = calculated partition coefficient.

Table 4 Solubilities of EGCG and its derivatives.

Compounds	Solubility in water[a] (mM)	Relative solubility
10	5.71 ± 0.73	0.3
11	1.05 ± 0.73	0.06
12	281.17 ± 0.09	17
13	512.88 ± 1.01	31
4	16.40 ± 0.73	1

[a] Mean ± standard deviation (n = 3).

Figures

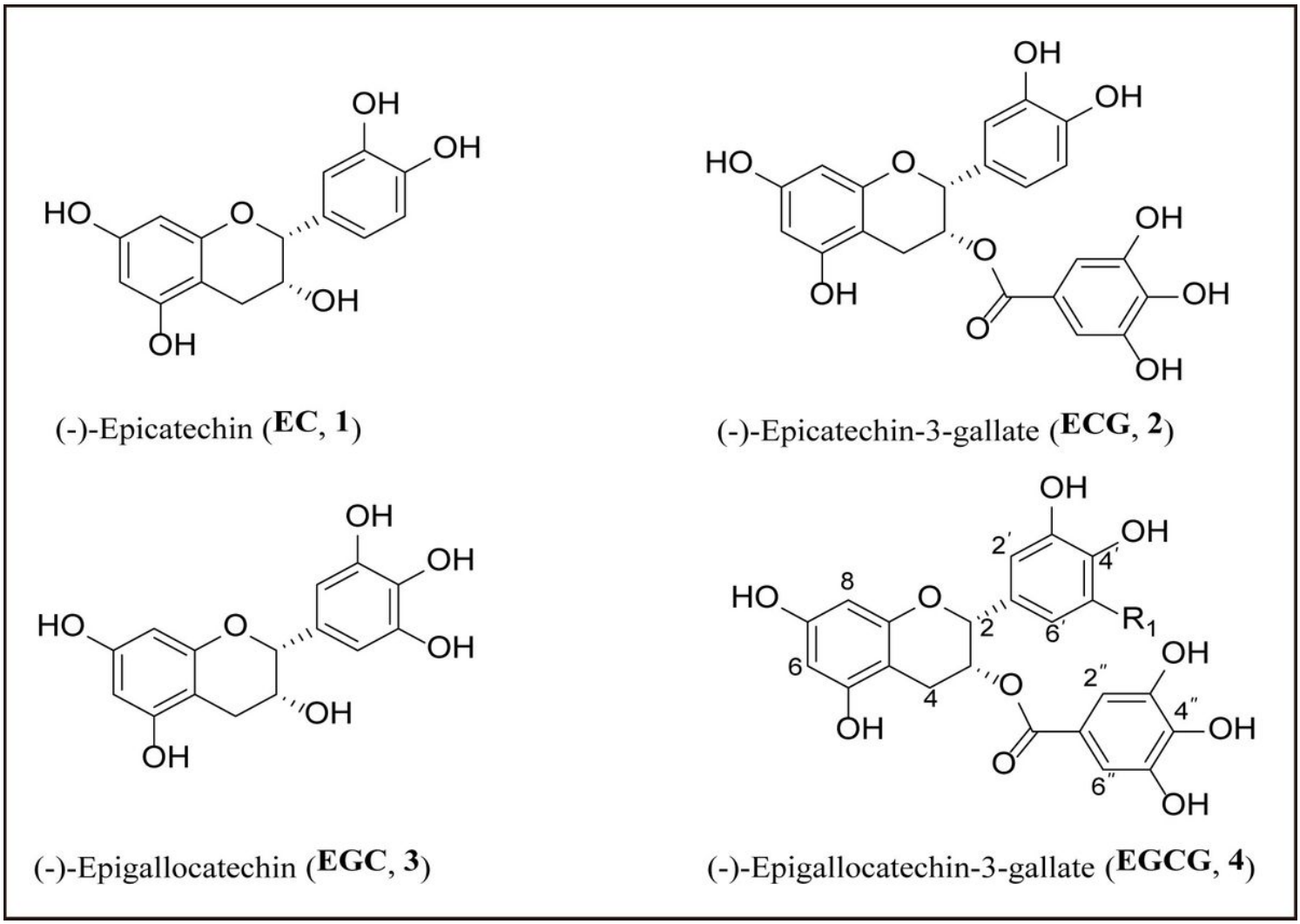

Figure 1

Structures of (-)-epicatechin (EC, 1), (-)-epicatechin-3-gallate (ECG, 2), (-)-epigallocatechin (EGC, 3), (-)-epigallocatechin-3-gallate (EGCG, 4).

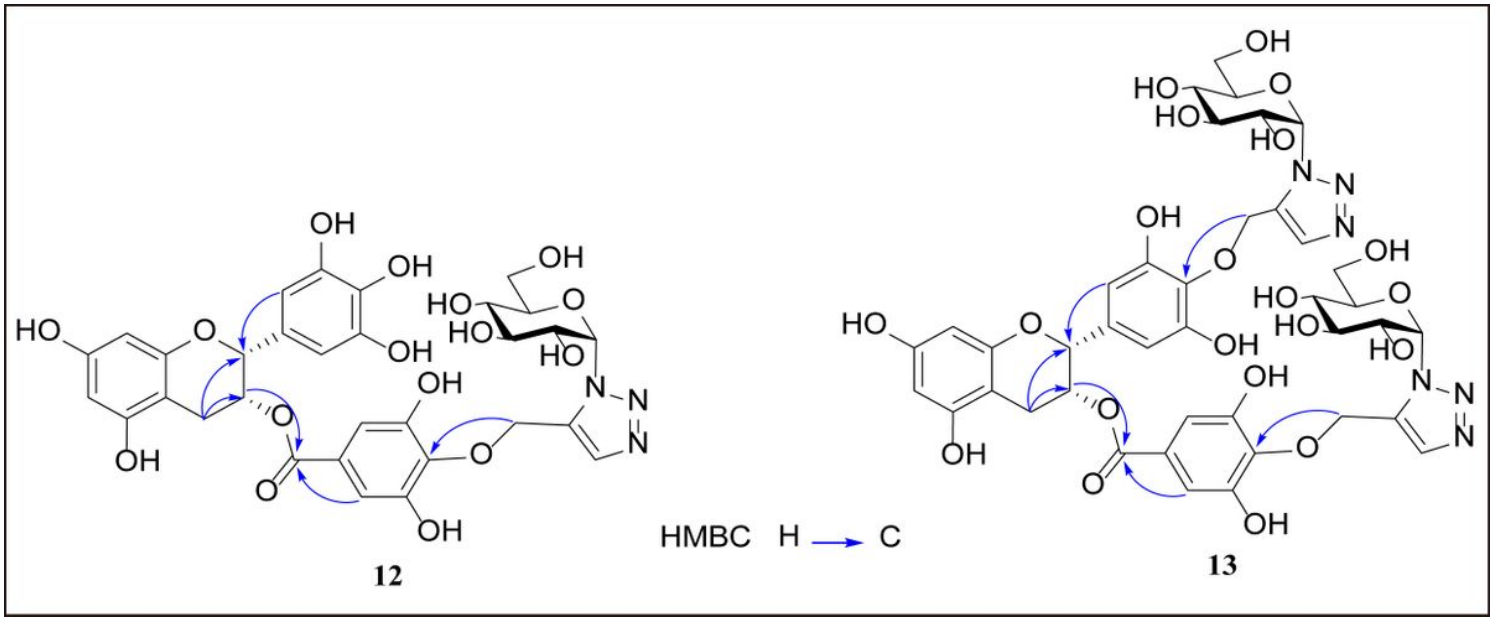

Figure 2

HMBC correlations in compounds 12 and 13.

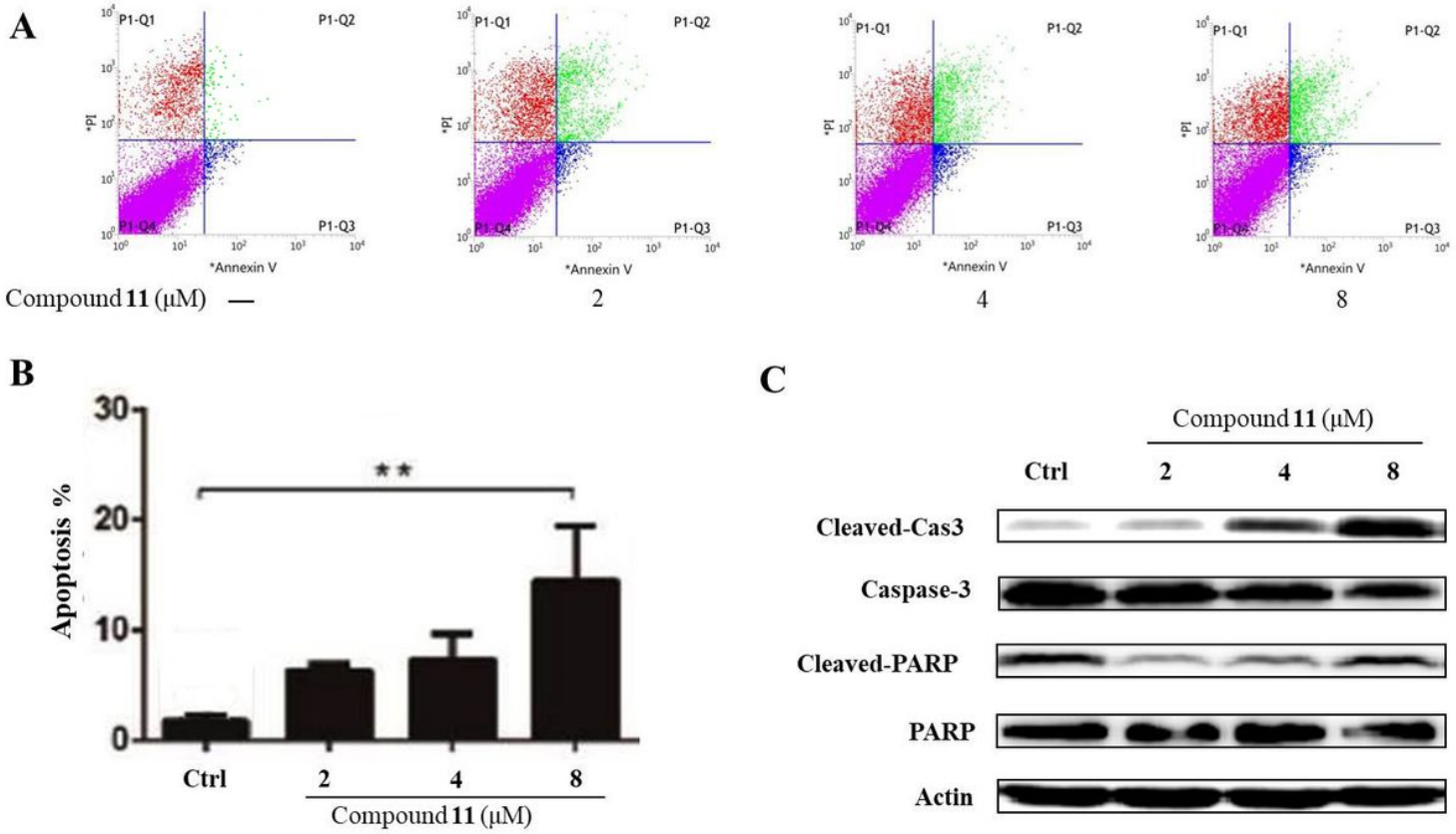

Figure 3

Effect of compound 11 on the induction of cell apoptosis and the expression of relevant proteins in HL-60 cells.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.jpg