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Anticancer activity of 3-*O*-acyl and alkyl-(−)-epicatechin derivatives

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Abstract—By changing the structure or replacing the gallate group of (−)-ECG, 3-*O*-acyl and alkyl-(−)-epicatechin derivatives were synthesized to be screen as anticancer agents using the MTT assay in vitro against cancer cell lines (PC3, SKOV3, U373MG). 3-*O*-Acyl and alkyl-(−)-epicatechin derivatives (**4**–**25**) exhibited better anticancer activity than (−)-ECG and specially, compounds **6**–**8**, **17**–**19**, which were modified aliphatic chains with moderate sizes (C8–C12) showed strong anticancer activity (IC_{50} = 6.4–31.2 μM). The introduction of an alkyloxy group on 3-*O*-hydroxyl instead of an acyloxy group significantly enhanced inhibitory activity. Consequently, the compound that showed the most potency as anticancer agents were 3-*O*-decyl-(−)-epicatechin (**18**) (IC_{50} = 8.9, 7.9, 6.4 μM against PC3, SKOV3, U373MG, respectively), which modified the appropriate lipophilic group on the C-3 hydroxyl as an alkyloxy group.

Recently, much attention has been paid to tea, *Camellia sinensis*, for the beneficial biological activities of its compounds, catechins, which have been reported to possess antimutagenic, antibacterial, antioxidant, antitumor and cancer preventive properties.[1–4] The constituents of the catechins of green tea are epigallocatechin-3 gallate (EGCG), epigallocatechin (EGC), epicatechin-3 gallate (ECG) (**1**), epicatechin (EC) (**2**) (Fig. 1).[5] Among catechins, (−)-EGCG and (−)-ECG exhibited better anticancer activity than (−)-EGC and (−)-EC.[1,6] Because (−)-EGCG and (−)-ECG was postulated to prevent human cancer by inhibiting enzymes, such as urokinase and 5α-reductase, that are crucial for cancer growth and development, though it might be one of many ways of cancer inhibition by green tea. [7–9] However, Hiipakka et al. discovered that (−)-EGCG and (−)-ECG exerted potent inhibition of human 5α-reductase in cell-free but not in whole-cell assays. The lack of activity in whole cells may be due to an inability of these catechins to cross the cell membrane or to enzymatic or nonenzymatic changes in the structure of these catechins in assay using whole-cell culture.[9]

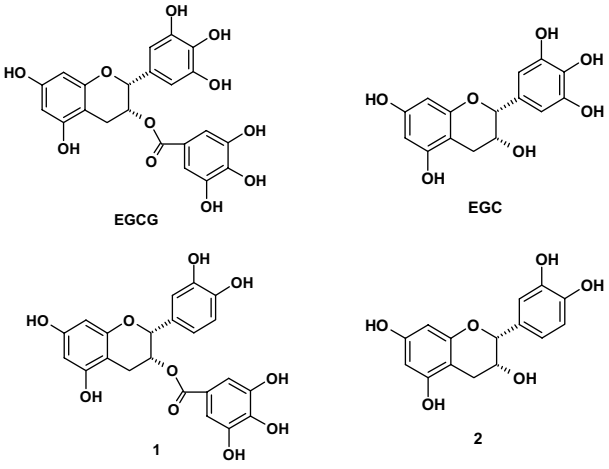

Figure 1. Chemical structures of major catechins of tea.

Keywords: 3-*O*-Acyl-(−)-epicatechin; 3-*O*-Alkyl-(−)-epicatechin; Anticancer activity.
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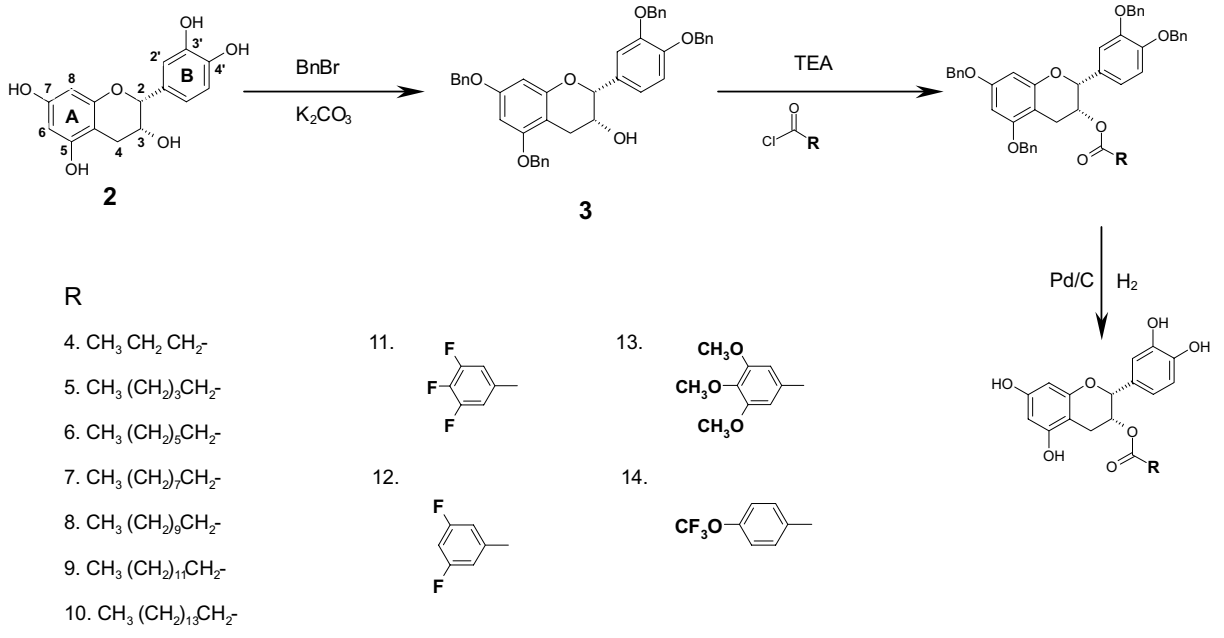
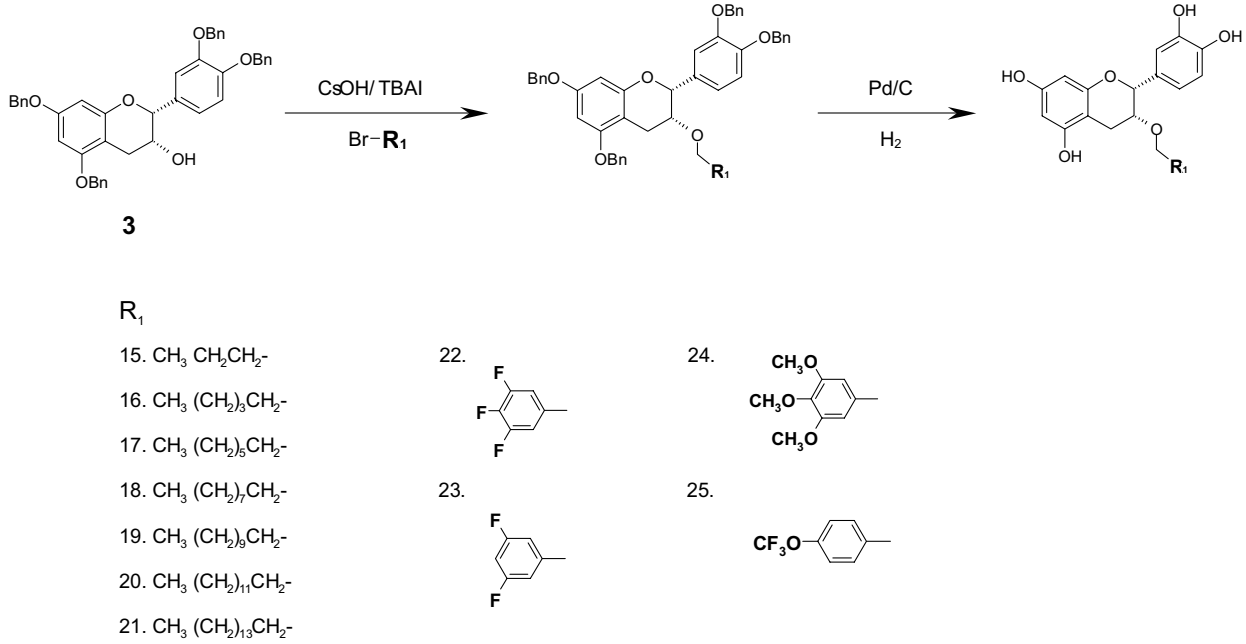
The purpose of the present study was to synthesize 3-*O*-acyl-(−)-epicatechin derivatives, which might enhance anticancer activities on whole cell assay. By replacing the gallate group of (−)-ECG with lipophilic group, various aromatic group and aliphatic chains, for increasing the lipophilicity, 3-*O*-acyl-(−)-epicatechin derivatives were synthesized (**4**–**14**). In order to prevent the acyloxy group at the C-3 hydroxyl group from enzymatic or non-enzymatic cleavage, the synthesized compounds were modified with an alkyloxy group instead of an acyloxy group (**15**–**25**).

For the synthesis of 3-*O*-acyl-(−)-epicatechin derivatives, the phenolic hydroxyl group of **2** was benzylated by treatment with benzyl bromide and K_2CO_3 to give 5,7,3′,4′-tetra-*O*-benzyl-(−)-epicatechin (**3**) in 74% yield.[10] The treatment of **3** with various acyl chloride and triethylamine (TEA) gave the 5,7,3′,4′-tetra-*O*-benzyl-3-*O*-acyl-(−)-epicatechins. The debenzylation of 5,7,3′,4′-tetra-*O*-benzyl-3-*O*-acyl-(−)-epicatechins was carried out with Pd/C in presence of H_2 to give compounds **4**–**14** (Scheme 1). To obtain the 3-*O*-alkyl-(−)-epicatechin derivatives, **3** was treated with cesium



Scheme 1. Synthesis of 3-*O*-acyl epicatechins.



Scheme 2. Synthesis of 3-*O*-alkyl epicatechins.

hydroxide (CsOH), *tert*-butyl ammonium iodide (TBAI) and various alkyl bromide to give 5,7,3′,4′-tetra-*O*-benzyl-3-*O*-alkyl-(−)-epicatechins, as shown in Scheme 2. Compounds **15**–**25** were obtained with the deprotection of the benzyl group in the same way as Scheme 1.

All the above compounds were tested for their in vitro anticancer activity against PC3, SKOV3, U373MG cells using the MTT assay, which was performed in 96-well plates essentially as described by Mosmann.[11] The IC_{50} concentration represents the concentration, which results in a 50% decrease in cell growth after 3 days incubation. The given values are the mean values of the three experiments.

The pharmacological activities against the PC3, SKOV3, U373MG cells are summarized in Table 1. In effect, 3-*O*-acyl and alkyl-(−)-epicatechin derivatives (**4**–**25**) were synthesized to support the assumption that increasing their lipophilicity and the permeability of the cell membrane would enhance the anticancer action of ECG. Among the new compounds (**4**–**25**), the replacement of aliphatic chains with moderate sizes (C10–C12) (**7**–**8**, **18**–**19**)[12] exhibited great enhancement of anticancer activity and too short (C4) or too long (C16) displayed little increase of anticancer effect. These results suggests that the presence of lipophilic substituents with moderate sizes might be crucial for the optimal anticancer activity. The most significant structural change leading to enhance activity was the introduction of an alkyl group at the C-3 hydroxyl in place of an acyloxy group. 3-*O*-(4-Trifluoromethoxy benzyl)-(−)-epicatechin (**25**)[12] showed three times the activity than 3-*O*-(4-trifluoromethoxy benzoyl)-(−)-epicatechin (**14**).[12] This modification is likely to prevent the compounds from enzymatic or nonenzymatic cleavage, which in turn may make them more stable in whole-cell culture. The compound **18** exerted the strongest anticancer activities with a IC_{50} values of 8.9, 7.9 and 6.4 μM against PC3, SKOV3 and U373MG, respectively. This result indicates that the introduction of moderate sized aliphatic chain at the C-3 hydroxyl with an alkyloxy group can lead to increasing of permeability and stability, which significantly improve inhibitory effect of cancer cell growth.

Table 1. In vitro anticancer effects against the PC3, SKOV3 and U373MG cell lines

Compounds	IC_{50} (μM)		
	PC3	SKOV3	U373MG
1	168.2	185.4	157
2	>500	>500	>500
4	95.6	103.5	107.3
5	67.2	68.2	71.2
6	24.2	31.2	29.7
7	14.6	20.8	19.2
8	23.1	22.4	24.3
9	33.7	34.7	36.7
10	43.1	48.1	42.9
11	71.9	61	59.2
12	58.7	78.2	64.5
13	51.3	50.1	57.2
14	45.2	47.3	50.3
15	98.3	78.3	77.2
16	35.6	27.8	23.4
17	14.3	15.5	12.3
18	8.9	7.9	6.4
19	9.3	8.6	7.1
20	17.5	18.2	16.9
21	29.1	30.3	22.5
22	21.8	27.2	22.3
23	33.2	41.7	25.2
24	32.5	38.2	29.8
25	14.6	19.1	11.2

Acknowledgements

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References and notes

1. Paschka, A. G.; Butler, R.; Young, C. Y. F. *Cancer Lett.* **1998**, *130*, 1.
2. Kondo, K.; Kurihara, M.; Miyata, N.; Suzuki, T.; Toyoda, M. *Free Radical Biol. Med.* **1999**, *27*, 855.
3. Hu, Z. Q.; Zhao, W. H.; Hara, Y.; Shimamura, T. *J. Antimicrob. Chemother.* **2001**, *48*, 361.
4. Kohri, T.; Matsumoto, N.; Yamakawa, M.; Nanjo, F.; Oku, N.; Hara, Y. *Abstracts of Papers, p98 Chemistry and Health Promotion*, 2nd International Conference on Food Factors, Kyoto, Japan, Dec 1999; 12–17.
5. Kuroda, Y.; Hara, Y. *Mutation Res.* **1999**, *436*, 69.
6. Uesato, S.; Kitagawa, Y.; Hara, Y.; Tokuda, H.; Okuda, M.; Mou, M. O.; Mou, X. Y.; Mukainaka, T.; Nishino, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1673.
7. Jankun, J.; Selman, S. H.; Swiercz, R.; Skrzypczak-jankun, E. *Nature* **1997**, *387*, 561.
8. Yang, C. S. *Nature* **1997**, *389*, 134.
9. Hiipakka, R. A.; Zhang, H. Z.; Dai, W.; Dai, Q.; Liao, S. *Biochem. Pharm.* **2002**, *63*, 1165.
10. Typical procedure: to a stirred solution of (−)-epicatechin (2.63 g, 9.1 mmol) in 13 mL of DMF, benzyl bromide (4.30 mL, 36.3 mmol) and potassium carbonate (7.50 g, 54.4 mmol) were added and the resulting solution was stirred at room temperature for 20 h. The reaction mixture was diluted with ethyl acetate and washed successively with water and brine. The organic layer was dried over $MgSO_4$, and the solvent was evaporated in vacuo to yield a brown oil, which was applied on a silica gel short column (CH_2Cl_2) to remove high polar products. Compound **3** was crystallized from MeOH/ether to give colorless crystals. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.20–7.60 (m, 20H), 6.95, 7.01 (2s, 3H, aromatic proton (B-ring)), 6.20, 7.01 (2d, 2H, aromatic proton (A-ring)), 5.16 (s, 4H, –Bzl), 4.99, 5.02 (2s, 4H, –Bzl), 4.62 (d, 1H, C_2–H), 3.97 (m, 1H, C_3–H), 3.10 (dd, 1H C_4–$H_{equatorial}$), 2.61 (dd, 1H, C_4–H_{axial}); MS (positive ESI mode) *m/z*: 650.3.
11. Mosmann, T. J. *Immunol. Meth.* **1983**, *65*, 55.
12. Spectral data for compound **4**–**14**; **7**; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.89–9.35 (m, 4H, OH (A,B-ring)), 6.73, 6.892 (2s, 3H, aromatic proton (B-ring)), 5.76, 5.88 (2d, 2H, aromatic proton (A-ring)), 5.36 (d, 1H, C_2–H), 5.05 (m, 1H, C_3–H), 2.77 (dd, 1H C_4–$H_{equatorial}$), 2.62 (dd, 1H, C_4–H_{axial}), 2.50 (2H, t, $CO\mathit{CH}_2(CH_2)_7CH_3$ at C_3–O), 1.38 (14H, m, $COCH_2(\mathit{CH}_2)_7CH_3$ at C_3–O), 0.85 (3H, t, $COCH_2(CH_2)_7\mathit{CH}_3$ at C_3–O); MS (positive ESI mode) *m/z*: 444.3. **8**; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.84–9.35 (m, 4H, OH (A,B-ring)), 6.62, 6.84 (2s, 3H, aromatic proton (B-ring)), 5.83, 5.92 (2d, 2H, aromatic proton (A-ring)), 5.37 (d, 1H, C_2–H), 5.08 (m, 1H, C_3–H), 2.83 (dd, 1H

C_4–$H_{equatorial}$), 2.62 (dd, 1H, C_4–H_{axial}), 2.50 (2H, t, $COCH_2$-$(CH_2)_7CH_3$ at C_3–O), 1.35 (14H, m, $COCH_2(CH_2)_9CH_3$ at C_3–O), 0.85 (3H, t, $COCH_2(CH_2)_9CH_3$ at C_3–O); MS (positive ESI mode) *m*/*z*: 472.2. **14**; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.83–9.32 (m, 4H, OH (A,B-ring)), 7.46–7.68 (m, 4H aromatic proton (*Ar*–CO– at C_3–O)), 6.66, 6.89 (2s, 3H, aromatic proton (B-ring)), 5.86, 5.95 (2d, 2H, aromatic proton (A-ring)), 5.31 (d, 1H, C_2–H), 5.05 (m, 1H, C_3–H), 2.82 (dd, 1H C_4–$H_{equatorial}$), 2.69 (dd, 1H, C_4–H_{axial}); MS (positive ESI mode) *m*/*z*: 478.1. **18**; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.85–9.36 (m, 4H, OH (A,B-ring)), 6.71, 6.86 (2s, 3H, aromatic proton (B-ring)), 5.85, 5.95 (2d, 2H, aromatic proton (A-ring)), 4.68 (d, 1H, C_2–H), 3.67 (m, 1H, C_3–H), 3.08, 3.28 (–$CH_2(CH_2)_8CH_3$ at C_3–O), 2.82 (dd, 1H C_4–$H_{equatorial}$), 2.67 (dd, 1H, C_4–H_{axial}), 1.31 (16H, m, (–$CH_2(CH_2)_8CH_3$ at C_3–O)), 0.84 (3H, t, (–$CH_2(CH_2)_8CH_3$ at C_3–O)); MS (positive ESI mode) *m*/*z*: 430.3. **19**; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.85–9.34 (m, 4H, OH (A,B-ring)), 6.71, 6.84 (2s, 3H, aromatic proton (B-ring)), 5.84, 5.93 (2d, 2H, aromatic proton (A-ring)), 4.64 (d, 1H, C_2–H), 3.68 (m, 1H, C_3–H), 3.03, 3.26 (–$CH_2(CH_2)_{10}CH_3$ at C_3–O), 2.86 (dd, 1H C_4–$H_{equatorial}$), 2.68 (dd, 1H, C_4–H_{axial}), 1.29 (16H, m, (–$CH_2(CH_2)_{10}CH_3$ at C_3–O)), 0.85 (3H, t, (–$CH_2(CH_2)_{10}CH_3$ at C_3–O)); MS (positive ESI mode) *m*/*z*: 458.2. **25**; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.84–9.24 (m, 4H, OH (A,B-ring)), 6.9–7.33 (m, 4H aromatic proton (*Ar*–CH_2– at C_3–O)), 6.62, 6.72 (2s, 3H, aromatic proton (B-ring)), 5.72, 5.91 (2d, 2H, aromatic proton (A-ring)), 4.76 (d, 1H, C_2–H), 4.35, 4.5 (dd, 2H Ar–CH_2– at C_3–O), 3.79 (m, 1H, C_3–H), 2.89 (dd, 1H C_4–$H_{equatorial}$), 2.65 (dd, 1H, C_4–H_{axial}); MS (positive ESI mode) *m*/*z*: 464.1.