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Original article

Synthesis and antimicrobial activities of 3-*O*-alkyl analogues of (+)-catechin: Improvement of stability and proposed action mechanism

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ABSTRACT

We report here the synthesis and biological properties of 3-*O*-alkyl analogues of (+)-catechin (**5**), which itself is one of the major natural polyphenols found in green tea and has several physiological activities. Starting from **5**, a series of 3-*O*-alkyl-(+)-catechin derivatives were investigated as potent antimicrobial agents. The presence of an alkyl chain rather than acyl on 3-*O*- showed an increase in antimicrobial activity which may be due to stability in standard culture condition. The most promising compound is **8e**, 3-*O*-decyl analogue, with the MIC of 0.5–2, 32–128 and 2–4 μg/mL against Gram-positive bacteria, Gram-negative bacteria and human pathogenic fungi, respectively. Regarding action mechanism, the antimicrobial activity is possibly due to the lipophilicity and disrupting ability of the analogues to the liposome membrane.

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1. Introduction

Green tea, made solely with the leaves of *Camellia sinensis*, is one of the most popular and commonly consumed beverages next to water in the world [1]. It has been paid much attention over last decade with respect to the beneficial biological activities of its components, catechins, which have been reported to have antimutagenic [2], antioxidant [3], antibacterial [4,5], antitumor and cancer preventive [6] properties. Green tea contains primarily catechins such as (−)-epigallocatechin-3-gallate (EGCG) (**1**), (−)-epigallocatechin (EGC) (**2**), (−)-epicatechin-3-gallate (ECG) (**3**), (−)-epicatechin (EC) (**4**) and (+)-catechin (C) (**5**) (Fig. 1) [7]. (−)-EGCG (**1**) is the most abundant and biologically promising polyphenol in green tea, making up more than 40% of the total polyphenolic mixture [8], and there has been extensive investigation into the ways by which (−)-EGCG (**1**) might act [9–11]. Several studies have shown that the presence of the 3-galloyl moiety in catechins led to higher biological activities [12–14]. For example, (−)-EGCG (**1**) and (−)-ECG (**3**) showed stronger anticancer activities than (−)-EGC (**2**) and (−)-EC (**4**) [2,6]. However, it causes the major limitations of low bioavailability which could be related to their low stability in neutral or slightly alkaline solutions and their inability to cross cellular membranes [15]. In addition, catechin gallates such as (−)-ECG (**3**) and (−)-EGCG (**1**) are susceptible to hydrolysis by bacterial and possibly host esterases. To prevent the esterase-mediated removal of the galloyl moiety from catechin gallates, Anderson and coworkers have synthesized (−)-ECG (**3**) derivatives through the replacement of hydrolytically susceptible ester bond with a more stable amide bond [16]. Landis-Piwowar and coworkers developed acetyl-protected (−)-EGCG (**1**) analogues as putative pro-drugs which exhibited more potent proteasome inhibitory activity than (−)-EGCG (**1**) in cultured tumor cell [17]. However, stability problems still remain after deacetylation in the cell.

It is of great importance to understand the use of individual catechins because this can provide the information of intrinsic metabolism and bioavailability in the absence of the drug–drug interactions from other catechins and impurities. Another promising direction is to generate more chemically stable analogues of green tea catechins.

In our previous study, we thus reported that the synthesized 3-*O*-acyl analogues of (−)-EC (**4**) and (+)-C (**5**) with the lipophilic substituents enhanced antimicrobial activities [18]. Furthermore, we found that 3-*O*-alkyl and acyl analogues of (−)-EC (**4**) showed potent anticancer activities against various cancer cell line and alkyl analogues exerted better activities than acyl analogues [19].

In this study, we synthesized a series of 3-*O*-alkyl-(+)-C derivatives (**8a–8l**), evaluated their antimicrobial activities, and examined stability of 3-*O*-acyl and alkyl analogues in culture media (pH 7.4) using HPLC analysis. Furthermore, the interaction between those analogues and the lipid membranes was investigated using liposome as a model membrane to propose the possible mechanism of the activity.

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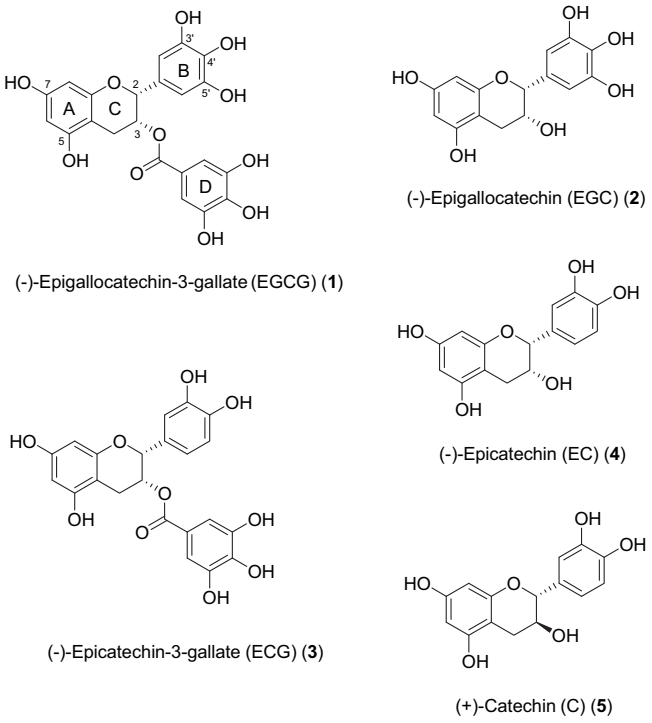


Fig. 1. Structures of major tea catechins.

2. Chemistry

The synthesis of the 3-*O*-alkyl-(+)-C derivatives is outlined in Scheme 1. For the introduction of an alkyl group at 3-hydroxy group, the phenolic groups of (+)-C (**5**) were benzylated by treatment with benzyl bromide and K_2CO_3 to give 5,7,3′,4′-tetra-*O*-benzyl-(+)-catechin (**6**) in 74% yield. The treatment of the benzylated intermediate (**6**) with cesium hydroxide (CsOH), tetrabutylammonium iodide (TBAI) and various substituted benzyl bromide or various alkyl iodide gave 5,7,3′,4′-tetra-*O*-benzyl-3-*O*-alkyl-(+)-catechins (**7a–7l**) in 46–72% yields. The debenzylation of the alkylated compounds (**7a–7l**) was carried out with Pd/C in the presence of H_2 to give the corresponding target compounds **8a–8l** in 78–89% yields.

3. Results

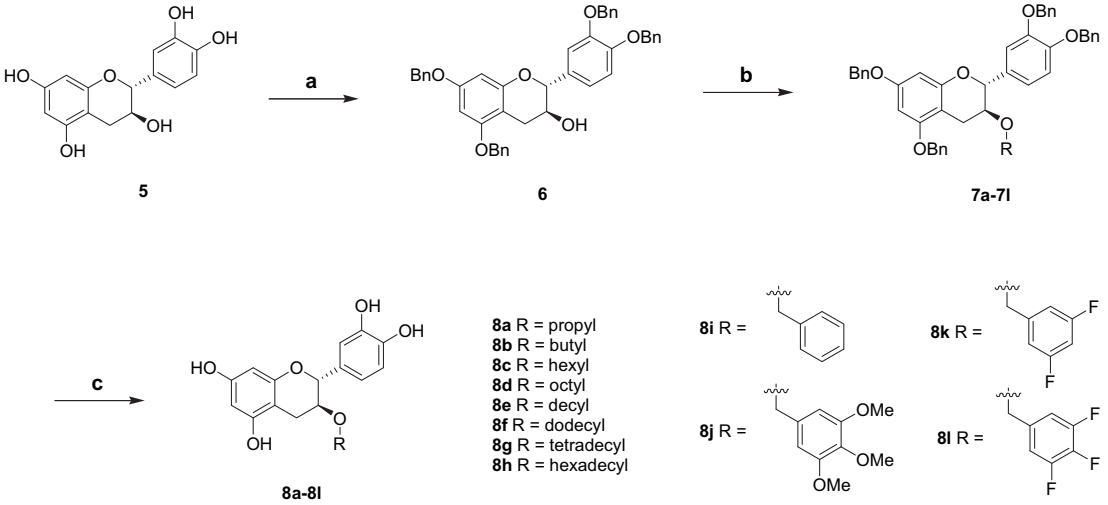
3.1. Antimicrobial activities of 3-O-alkyl-(+)-catechin derivatives

The antimicrobial activities of the compounds **8a–8l** against Gram-positive bacteria, Gram-negative bacteria and human pathogenic fungi are summarized in Tables 1 and 2. The antimicrobial activities of synthesized compounds **8a–8l** were compared with those of major catechins, 3-*O*-decanoyl-(+)-catechin (**9**) [18] and the positive controls, kanamycin sulfate and ciprofloxacin for antibacterial and amphotericin B and itraconazole for antifungal.

In the previous study, the introduction of various aromatic ring and aliphatic chain as an acyl group at the 3-*O* position of (−)-EC (**4**) or (+)-C (**5**) exhibited mild antibacterial activities against Gram-positive bacteria and antifungal activities [18]. In this study, we tested whether introducing an alkyl group at the 3-*O* position instead of an acyl group to (+)-C (**5**) enhances the antimicrobial activity from preventing of enzymatic or non-enzymatic cleavage of the acyl group in assay [20]. We found that 3-*O*-alkyl-(+)-C derivatives showed significantly enhanced antimicrobial activities, 2 to 4-folds more active than 3-*O*-acyl-(+)-C derivatives in general. Among them, (+)-C derivatives bearing an alkyl chain of carbon atoms in the close vicinity of C_8 to C_{10} showed strong antimicrobial activities. Especially, the 3-*O*-decyl-(+)-C (**8e**) exerted the strongest antimicrobial activities (MIC = 0.5–2 μg/mL and 2–4 μg/mL against Gram-positive bacteria and fungi, respectively) and showed 4–8 times the activities than the previous acyloxy analogue, 3-*O*-decanoyl-(+)-C (**9**). For Gram-negative bacteria, the synthesized compounds have shown less antibacterial activities compared to kanamycin sulfate (MIC = 32–128 μg/mL) and ciprofloxacin (MIC = 0–0.125 μg/mL). It has been observed that the introduction of a lipophilic substituent with appropriate sizes (**8d–8f**) on the 3-*O* maximized the antimicrobial activities in the series of aliphatic chain analogues. Also, structural change in the linker of an ester to ether on the 3-*O* position significantly enhanced antimicrobial activities.

3.2. Stability of compounds

We investigated the stability of 3-*O*-decyl-(+)-C (**8e**) compared with 3-*O*-decanoyl-(+)-C (**9**) and (−)-EGCG (**1**) in aqueous PBS buffer (pH 7.4). We observed that both compounds **8e** and **9** were considerably much more stable than (−)-EGCG (**1**) (Fig. 2). The data



Scheme 1. Synthesis of 3-*O*-alkyl-(+)-C analogues (**8a–8l**). Reagents and conditions: (a) benzyl bromide, K_2CO_3, DMF, rt, 20 h; (b) R–X, cesium hydroxide, tetrabutylammonium iodide, DMF, −20 °C, 20 h; (c) Pd/C, H_2, MeOH, rt, 5 h.

Table 1
Antibacterial activities of major catechins (**1**–**5**) and 3-*O*-alkyl-(+)-C derivatives (**8a**–**8l**).

Test organisms	MIC (μg/mL)															
	1	**8a**	**8b**	**8c**	**8d**	**8e**	**8f**	**8g**	**8h**	**8i**	**8j**	**8k**	**8l**	**9**[a]	**Km**	**Cpfx**
Enterococcus faecalis ATCC 29212	>128	64	32	8	4	2	2	16	32	32	64	8	8	16	32	0.5
Staphylococcus aureus ATCC 25923	>128	64	16	4	2	1	1	8	16	64	32	16	16	8	16	0.25
Micrococcus luteus ATCC 10240	32	32	8	2	1	0.5	1	4	16	32	16	8	8	2	8	<0.06
Staphylococcus epidermidis ATCC 0155	128	64	16	8	2	1	2	8	16	64	32	16	16	4	4	0.25
Bacillus Subtilis ATCC 6633	>128	128	64	16	4	2	2	8	32	64	32	32	32	32	2	<0.06
Escherichia coli ATCC 25922	>128	>128	>128	>128	64	64	64	>128	>128	>128	>128	128	128	>128	8	0.125
Escherichia coli ATCC 10536	>128	>128	>128	>128	>128	128	>128	>128	>128	>128	64	64	64	>128	8	0.06
Proteus mirabilis ATCC 27853	>128	>128	128	128	64	32	128	>128	>128	128	128	16	16	32	32	0.125
Klebsiella pneumonia ATCC 10031	>128	>128	128	128	64	32	128	>128	>128	128	128	128	128	32	64	<0.06

Compounds **2**–**5** (MIC > 128) were not shown. Determined after 24 h of incubation at 37 °C for the bacteria. All experiments were run in triplicates. Km = Kanamycin sulfate. Cpfx = Ciprofloxacin.
[a] 3-*O*-decanoyl-(+)-C [18].

showed that more than 90% and 75% of the initial concentration of compounds **8e** and **9**, respectively, remained in the culture medium after an incubation period of 16 h while only 15% of the initial concentration of (−)-EGCG (**1**) remained (Fig. 2). Certain studies have indicated the most active tea polyphenols are unstable in neutral or alkaline medium [21]. Several possible mechanisms have been reported for explaining their instability such as ester cleavage of the gallate moiety and oxidation at the phenolic groups on B and D rings [22]. As such, introduction of an aliphatic chain instead of the reactive phenolic groups on D ring and absence of the pyrogalloyl B-ring led to enhanced stability. Consistent with antimicrobial activities of both compounds, we observed that introduction of alkyl group facilitated stability compared with acyl group and this modification is likely to prevent the compounds from not only enzymatic but also non-enzymatic cleavage in culture medium.

3.3. Proposed action mechanism

The antimicrobial activities of (+)-C derivatives increased with elongation of an alkyl chain of suitable length (Table 1). Thus, we presumed that the antibacterial activities of the (+)-C derivatives and their interaction with the bacterial membrane are closely correlated.

Indeed, the percent incorporation of the (+)-C derivatives (**8a**–**8l**) into liposomes increased with elongation of the alkyl chain lengths of the derivatives (Fig. 3). While *c* Log *P* value was significantly increased (3.5–9) according to the increment of alkyl chain length or the introduction of lipophilic substituents, their incorporative abilities were shown over 90% as long as *c* Log *P* value of compounds is more than 4 in the series. Besides, (−)-EGCG (**1**) and (+)-C (**5**) were not incorporated well into the liposome membrane out of proportion to the corresponding *c* Log *P* values possibly due to many polar hydroxy groups on the molecule.

The percent incorporation of compounds **8c**–**8h** was almost 100%, but the antimicrobial activities of compounds **8g** and **8h** including much longer aliphatic chain (C_{14}–C_{16}) were generally weaker than those of compounds **8d**–**8f** (C_8–C_{12}). It was supplemented in Fig. 4 that only compounds **8d**–**8f** including a suitable length of alkyl chain caused more calcein leakage than compounds including shorter (**8c**) or longer chain (**8g** and **8h**).

4. Discussion

Although (−)-EGCG (**1**) and (−)-ECG (**3**), major catechins of green tea, have been shown various biological activities including antioxidant, antimicrobial and anticancer, their instability due to enzymatic or non-enzymatic cleavage of the 3-galloyl group has hampered their clinical use. In this study, we synthesized a series of catechin analogues to enhance not only antimicrobial activities but also stability at pH 7.4. The synthesized compounds showed increased levels of antimicrobial activities. Among them, 3-*O*-decyl-(+)-C (**8e**) was examined stability at pH 7.4 compared with 3-*O*-decanoyl-(+)-C (**9**) and EGCG (**1**). We found that the replacement of the 3-galloyl group led to significant increased stability and the introduction of an ether bond led to higher stability (more than 10%) than an ester bond at pH 7.4. These results indicate that ether bond prevent from non-enzymatic cleavage more than ester bond. Accordingly, 3-*O*-alkyl-(+)-catechins are likely to minimize both enzymatic and non-enzymatic cleavage *in vivo* assay.

To propose action mechanism of the synthesized compounds, we prepared model membrane using liposome. The synthesized compounds showed significant ability of incorporation into liposomes upon length of aliphatic chain. Despite almost 100% incorporation ability, compounds **8g** and **8h** including much longer aliphatic chain (C_{14}–C_{16}) exhibited weaker activities than compounds **8d**–**8f** (C_8–C_{12}) (Fig. 3 and Tables 1 and 2). Consistent with these findings, compounds including a suitable length of alkyl chain (**8d**–**8f**) caused more calcein leakage than compounds including shorter (**8c**) or longer chain (**8g** and **8h**) (Fig. 4). Thus, it is likely that the activity disrupting membrane structure in addition to the amount incorporated into the lipid bilayers should affect the antibacterial activities of (+)-C derivatives such as compounds **8e** and **8f** because the presence of the alkyl chain accelerated the adsorption on the surface of the membrane and the disruption of the membrane by increasing the lipophilicity.

Table 2
Antifungal activities of major catechins (**1**–**5**) and 3-*O*-alkyl-(+)-C derivatives (**8a**–**8l**).

Test organisms	MIC (μg/ml)												
	8b	**8c**	**8d**	**8e**	**8f**	**8g**	**8h**	**8j**	**8k**	**8l**	**9**[a]	AmpB	Itrc
Candida krusei IFO 1664	128	32	8	4	8	16	64	128	64	64	>128	0.5	0.25
Candida lusitaniae ATCC 42720	32	16	8	4	4	16	32	64	32	32	64	0.5	0.125
Candida albicans ATCC 10231	32	8	2	2	4	8	32	64	32	16	32	0.25	0.06
Candida tropicalis IFO 10241	64	32	16	4	8	32	32	128	64	64	16	0.5	0.06

Compounds **1**–**5**, **8a** and **8i** (MIC > 128) were not shown. Determined after 24–72 h of incubation at 28–30 °C for the fungi. All experiments were run in triplicates. AmpB = Amphotericin B. Itrc = Itraconazole.
[a] 3-*O*-decanoyl-(+)-C [18].

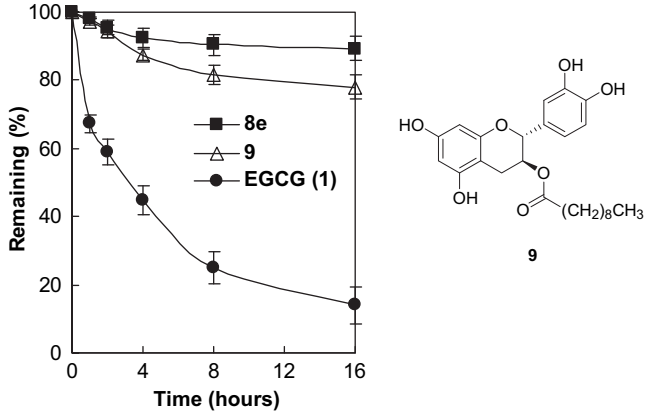


Fig. 2. Stability of (−)-EGCG (1), **8e** and **9** in standard culture condition in the absence of cells.

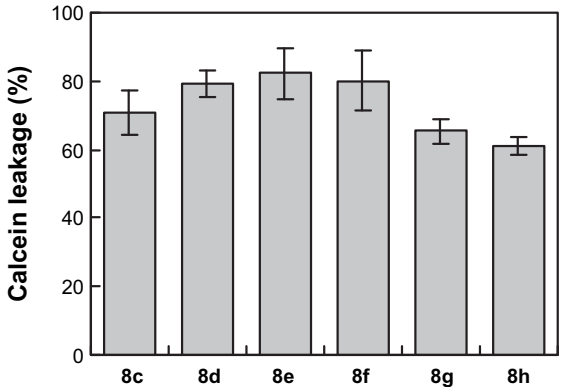


Fig. 4. Effect of 3-*O*-alkyl analogues of (+)-C (**8c**–**8h**) on calcein leakage.

5. Conclusion

It has been paid much attention over last decade with respect to the beneficial biological activities of its components, catechins. The present work reports the chemical synthesis of 12 new 3-*O*-alkyl analogues of (+)-C (**5**), the antimicrobial activities, the stability in pH 7.4, and possible action mechanism of activities using model membrane. We showed that introducing of alkyl group instead of acyl group enhanced activities and stability in pH 7.4. Using model membrane, we suggested that the synthesized compounds showed potent activities may effectively adsorb and disrupt the surface of the membrane in microsome.

6. Experimental

6.1. Chemistry

^{1}H and ^{13}C NMR spectra were obtained with a Varian Mercury spectrometer at 300 and 75 MHz, respectively. ^{1}H chemical shifts (δ) were reported in ppm downfield from internal Me_4Si. Mass spectra were measured by Fisons-VG platform in positive mode electrospray ionization (ESI).

6.1.1. General procedure for the preparation of 3-*O*-alkyl-(+)-catechins (**8a**–**8l**)

*6.1.1.1. 5,7,3′,4′-Tetra-O-benzyl-(+)-catechin (**6**).* (+)-Catechin hydrate (2 g, 6.89 mmol) dissolved in *N*,*N*-dimethylformamide (DMF) was reacted with K_2CO_3 (5.7 g, 41.34 mmol) and benzyl bromide (3.8 ml, 27.56 mmol) for 20 h at rt. To the resulting mixture were added water and Et_2O. The organic layer was separated, and the water layer was extracted with Et_2O (×2). The combined organic layers were dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (CH_2Cl_2) and recrystallized from ether/MeOH to afford the title compound as an off-white solid (3.32 g, 74% yield): mp 125–126.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.23 (m, 20 H), 7.03 (s, 1 H), 6.94 (s, 2 H), 6.27 (m, 1 H), 6.20 (m, 1 H), 5.17 (s, 2 H), 5.16 (m, 2 H), 5.03 (s, 2 H), 4.98 (s, 2 H), 4.63 (d, J = 8.5 Hz, 1 H), 4.00 (m, 1 H), 3.10 (m, 1 H), 2.65 (m, 1 H), 1.58 (d, J = 3.5 Hz, 1 H).

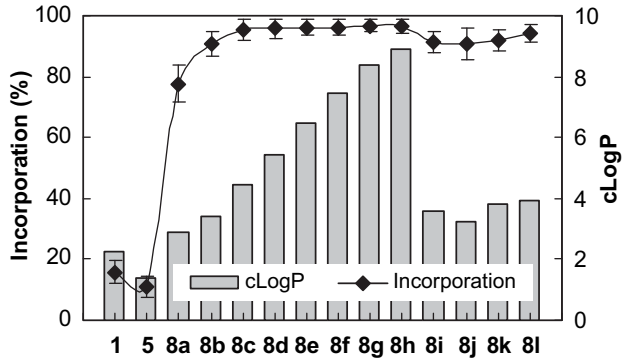


Fig. 3. Percent incorporation of (−)-EGCG (1), (+)-C (5) and 3-*O*-alkyl analogues of (+)-C (**8a**–**8l**).

*6.1.1.2. 5,7,3′,4′-Tetra-O-benzyl-3-O-alkyl-(+)-catechins (**7a**–**7l**).* Compound **6** (2 g, 3.08 mmol) was reacted with tetrabutylammonium iodide (TBAI) (1.5 equiv), cesium hydroxide (CsOH) (1.5 equiv), and various substituted benzyl bromides or aliphatic alkyl iodides (1.5 equiv) for 24 h at room temperature. The reaction mixture was diluted with ethyl ether and washed successively with water. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography ($MeOH/CH_2Cl_2$ 1:90) to afford the title compound as off-white solids in 46–72% yields.

*6.1.1.3. 3-O-Alkyl-(+)-catechins (**8a**–**8l**).* The debenzylation of compounds **7a**–**7l** was carried out with Pd/C in presence of H_2 to afford the title compounds **8a**–**8l** (78–89% yields) as white sticky foams.

8a: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.30–8.84 (m, 4H, OH (A,B-ring)), 6.77–6.60 (m, 3H, aromatic proton (B-ring)), 5.91, 5.75 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.63 (d, J = 7.5 Hz, 1H, C_2-H), 3.65 (m, 1H, C_3-H), 3.34, 3.13 (2m, 2H, $\underline{CH_2}$– at C_3-O), 2.71 (dd, J = 16.4, 5.0 Hz, 1H, C_4-$H_{equatorial}$), 2.40 (dd, J = 15.5, 7.5 Hz, 1H, C_4-H_{axial}), 1.28 (m, 2H, C_3–O–$CH_2\underline{CH_2}CH_3$), 0.74 (t, J = 7.3 Hz, 3H, C_3–O–$CH_2CH_2\underline{CH_3}$); $[\alpha]_D^{27}$ +5.1° (c 0.70; DMSO); MS (positive ESI mode) m/z: 332.3.

8b: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.24–8.86 (m, 4H, OH (A,B-ring)), 6.73–6.57 (m, 3H, aromatic proton (B-ring)), 5.89, 5.70 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.65 (d, J = 7.2 Hz, 1H, C_2-H), 3.65 (m, 1H, C_3-H), 3.39, 3.18 (2m, 2H, $\underline{CH_2}$- at C_3-O), 2.67 (dd, J = 16.5, 5.1 Hz, 1H, C_4-$H_{equatorial}$), 2.38 (dd, J = 15.6, 7.5 Hz, 1H, C_4-H_{axial}), 1.36–1.09 (m, 4H, C_3–O–$CH_2(\underline{CH_2})_2CH_3$), 0.77 (t, J = 7.2 Hz, 3H, C_3–O–$CH_2(CH_2)_2\underline{CH_3}$); $[\alpha]_D^{27}$ −62.5° (c 1.04; DMSO); MS (positive ESI mode) m/z: 346.4.

8c: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.25–8.84 (m, 4H, OH (A,B-ring)), 6.74–6.56 (m, 3H, aromatic proton (B-ring)), 5.89, 5.71 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.64 (d, J = 7.1 Hz, 1H, C_2-H), 3.65 (m, 1H, C_3-H), 3.39, 3.17 (2m, 2H, $\underline{CH_2}$- at C_3-O), 2.66 (dd, J = 16.6, 5.3 Hz, 1H, C_4-$H_{equatorial}$), 2.38 (dd, J = 15.7, 7.5 Hz, 1H, C_4-H_{axial}), 1.36–1.10 (m, 8H, C_3–O–$CH_2(\underline{CH_2})_4CH_3$), 0.80 (t, J = 7.0 Hz, 3H, C_3–O–$CH_2(CH_2)_4\underline{CH_3}$); $[\alpha]_D^{27}$ +12.4° (c 0.87; DMSO); MS (positive ESI mode) m/z: 374.1.

8d: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.24–8.83 (m, 4H, OH (A,B-ring)), 6.74–6.55 (m, 3H, aromatic proton (B-ring)), 5.89, 5.70 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.65 (d, J = 6.9 Hz, 1H, C_2-H), 3.64 (m, 1H, C_3-H), 3.38, 3.17 (2m, 2H, $\underline{CH_2}$– at C_3-O), 2.65 (dd, J = 16.7, 5.5 Hz, 1H, C_4-$H_{equatorial}$), 2.38 (dd, J = 15.8, 7.5 Hz, 1H, C_4-H_{axial}), 1.38–1.08 (m, 12H, C_3–O–$CH_2(\underline{CH_2})_6CH_3$), 0.80 (t, J = 7.0 Hz, 3H, C_3–O–$CH_2(CH_2)_6\underline{CH_3}$); $[\alpha]_D^{27}$ +6.5° (c 1.15; DMSO); MS (positive ESI mode) m/z: 402.4.

8e: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.23–8.83 (m, 4H, OH (A,B-ring)), 6.73–6.55 (m, 3H, aromatic proton (B-ring)), 5.90, 5.70 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.65 (d, J = 6.8 Hz, 1H, C_2-H), 3.64 (m, 1H, C_3-H), 3.37, 3.16 (2m, 2H, $\underline{CH_2}$- at C_3-O), 2.65 (dd, J = 16.7, 5.6 Hz, 1H, C_4-$H_{equatorial}$), 2.37 (dd, J = 15.8, 7.5 Hz, 1H, C_4-H_{axial}), 1.37–1.05 (m, 16H, C_3–O–$CH_2(\underline{CH_2})_8CH_3$), 0.85 (t, J = 6.6 Hz, 3H, C_3–O–$CH_2(CH_2)_8\underline{CH_3}$); $[\alpha]_D^{27}$ +14.5° (c 1.00; DMSO); MS (positive ESI mode) m/z: 430.3.

8f: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.23–8.84 (m, 4H, OH (A,B-ring)), 6.72–6.56 (m, 3H, aromatic proton (B-ring)), 5.89, 5.70 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.65 (d, J = 6.6 Hz, 1H, C_2-H), 3.64 (m, 1H, C_3-H), 3.36, 3.15 (2m, 2H, $\underline{CH_2}$- at C_3-O), 2.65 (dd, J = 16.8, 5.7 Hz, 1H, C_4-$H_{equatorial}$), 2.37 (dd, J = 15.9, 7.5 Hz, 1H, C_4-H_{axial}), 1.38–1.10 (m, 20H, C_3–O–$CH_2(\underline{CH_2})_{10}CH_3$), 0.85 (t, J = 6.3 Hz, 3H, C_3–O–$CH_2(CH_2)_{10}\underline{CH_3}$); $[\alpha]_D^{27}$ +48.2° (c 0.74; DMSO); MS (positive ESI mode) m/z: 458.2.

8g: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.22–8.84 (m, 4H, OH (A,B-ring)), 6.71–6.56 (m, 3H, aromatic proton (B-ring)), 5.89, 5.69 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.64 (d, J = 6.6 Hz, 1H, C_2-H), 3.64 (m, 1H, C_3-H), 3.36, 3.15 (2m, 2H, $\underline{CH_2}$– at C_3-O), 2.65 (dd, J = 16.8, 5.7 Hz, 1H, C_4-$H_{equatorial}$), 2.36 (dd, J = 15.9, 7.5 Hz, 1H, C_4-H_{axial}), 1.39–1.08 (m, 24H, C_3–O–$CH_2(\underline{CH_2})_{12}CH_3$), 0.86 (t, J = 6.3 Hz, 3H, C_3–O–$CH_2(CH_2)_{12}\underline{CH_3}$); $[\alpha]_D^{27}$ +47.1° (c 1.02; DMSO); MS (positive ESI mode) m/z: 486.4.

8h: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.22–8.85 (m, 4H, OH (A,B-ring)), 6.72–6.55 (m, 3H, aromatic proton (B-ring)), 5.90, 5.69 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.64 (d, J = 6.5 Hz, 1H, C_2-H), 3.65 (m, 1H, C_3-H), 3.36, 3.14 (2m, 2H, $\underline{CH_2}$– at C_3-O), 2.64 (dd, J = 16.9, 5.7 Hz, 1H, C_4-$H_{equatorial}$), 2.35 (dd, J = 15.9, 7.5 Hz, 1H, C_4-H_{axial}), 1.40–1.10 (m, 28H, C_3–O–$CH_2(\underline{CH_2})_{14}CH_3$), 0.86 (t, J = 6.2 Hz, 3H, C_3–O–$CH_2(CH_2)_{14}\underline{CH_3}$); $[\alpha]_D^{27}$ +4.9° (c 1.04; DMSO); MS (positive ESI mode) m/z: 514.4.

8i: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.30–8.79 (m, 4H, OH (A,B-ring)), 7.46–6.84 (m, 5H, aromatic proton ($\underline{Ar}$-CH_2– at C_3-O)), 6.74–6.60 (m, 3H, aromatic proton (B-ring)), 5.90, 5.73 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.68 (d, J = 6.6 Hz, 1H, C_2-H), 4.53–4.41 (m, 2H, Ar-$\underline{CH_2}$– at C_3-O), 3.85 (m, 1H, C_3-H), 2.94 (dd, J = 17.0, 5.7 Hz, 1H, C_4-$H_{equatorial}$), 2.50 (dd, J = 16.2, 7.5 Hz, 1H, C_4-H_{axial}); $[\alpha]_D^{27}$ +9.8° (c 1.12; DMSO); MS (positive ESI mode) m/z: 380.2.

8j: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.42–8.87 (m, 4H, OH (A,B-ring)), 7.43–6.92 (m, 2H, aromatic proton ($\underline{Ar}$-CH_2– at C_3-O)), 6.77–6.60 (m, 3H, aromatic proton (B-ring)), 5.88, 5.63 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.74 (d, J = 6.6 Hz, 1H, C_2-H), 4.55–4.34 (m, 2H, Ar-$\underline{CH_2}$– at C_3-O), 3.85 (m, 1H, C_3-H), 3.73 (m, 9H, $\underline{CH_3}$–O–), 2.83 (dd, J = 17.2, 5.5 Hz, 1H, C_4-$H_{equatorial}$), 2.48 (dd, J = 16.3, 7.4 Hz, 1H, C_4-H_{axial}); $[\alpha]_D^{27}$ +87.0° (c 1.00; DMSO); MS (positive ESI mode) m/z: 470.4.

8k: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.34–8.85 (m, 4H, OH (A,B-ring)), 7.41–6.91 (m, 3H, aromatic proton ($\underline{Ar}$-CH_2– at C_3-O)), 6.63–6.56 (m, 3H, aromatic proton (B-ring)), 5.93, 5.69 (2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.74 (d, J = 6.7 Hz, 1H, C_2-H), 4.45–4.25 (m, 2H, Ar-$\underline{CH_2}$- at C_3-O), 3.75 (m, 1H, C_3-H), 2.83 (dd, J = 17.2, 5.4 Hz, 1H, C_4-$H_{equatorial}$), 2.64 (dd, J = 16.2, 7.4 Hz, 1H, C_4-H_{axial}); $[\alpha]_D^{27}$ +35.8° (c 1.37; DMSO); MS (positive ESI mode) m/z: 416.3.

8l: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.37–8.97 (m, 4H, OH (A,B-ring)), 7.31–6.98 (m, 2H, aromatic proton ($\underline{Ar}$-CH_2– at C_3-O)), 6.73–6.54 (m, 3H, aromatic proton (B-ring)), 5.93, 5.72(2d, J = 2.4, 2.1 Hz, 2H, aromatic proton (A-ring)), 4.81 (d, J = 6.6 Hz, 1H, C_2-H), 4.54–4.33 (m, 2H, Ar-$\underline{CH_2}$– at C_3-O), 3.75 (m, 1H, C_3-H), 2.83 (dd, J = 17.3, 5.3 Hz, 1H, C_4-$H_{equatorial}$), 2.64 (dd, J = 16.4, 7.3 Hz, 1H, C_4-H_{axial}); $[\alpha]_D^{27}$ +60.0° (c 0.6; DMSO); MS (positive ESI mode) m/z: 434.3.

6.2. Antimicrobial activity

The MIC is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation [23]. MIC values of the synthesized compounds against Gram-positive and Gram-negative test bacteria were determined by method of NCCLS [24]. Muller Hinton agar (MHA) was used for MIC determination. All the test cultures were streaked on the soybean casein digest agar (SCDA) and incubated overnight at 37 °C. Turbidity of all the bacterial cultures was adjusted to 0.5 McFarland standard by preparing bacterial suspension of four to six well isolated colonies. The cultures were further diluted 10-fold to get inoculum size of 1.2×10^7 CFU/mL. Stock solution of 4 mg/mL was prepared in DMSO and was diluted to get final concentration of 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125 μg/mL 320 μL of each dilution was added to 20 mL cooled 45 °C molten MHA (separate flask was taken for each dilution). After thorough mixing, the medium was poured in sterilized petri plates. The test bacterial cultures were spotted in a predefined pattern by ascetically transferring 10 μL of each culture on the surface of presolidified agar plates. The spotted plates were incubated at 35 °C for 24 h.

MIC values of the synthesized compounds against fungi were determined by method of NCCLS [25]. RPMI-1640 broth was used for MIC determination. All the test cultures were streaked of the Sabouraud dextrose agar (SDA) and incubated overnight at 35 °C. Cell density adjusted with a spectrophotometer by adding sufficient sterile saline to increase the transmittance to that produced by a 0.5 McFarland standard. This procedure yielded a yeast stock suspension of 1.0×10^6 to 5.0×10^6 cell/mL. A working suspension is made by a 1:100 dilution followed by a 1:20 dilution of the stock suspension with RPMI-1640 broth medium, which results in 5.0×10^2 to 2.5×10^3 cell/mL. Tubes are incubated at 35 °C for 48 h in ambient air.

6.3. Stability of 3-O-decyl- and decanoyl-(+)-catechins (*8e* and *9*) at pH 7.4

To examine the stability of catechin derivatives in culture medium, a 1% DMSO/aqueous 50 mM PBS buffer (pH 7.4, 10 mL) containing either compound **8e** (0.86 mg, 2 μmol, 200 μM) or **9** (0.89 mg, 2 μmol, 200 μM) was incubated at 37 °C. The pH of the solution was measured both before and after each reaction and was found to be within 0.1 pH unit of the listed pH value. The solutions were analyzed by HPLC using a photodiode array detector (210–340 nm). Samples (50 μL) were injected onto a μBondapak C-18 column (3.9 × 300 mm, Waters Corp. Cat. No. WAT027324). A mobile phase (35/65 CH_3CN/H_2O) was employed for 30 min using a flow rate of 1 mL/min. The column was maintained at 37 °C. The relative percentage of catechin derivatives remaining was determined by integration of the HPLC peak compared with the internal standard at 254 nm.

6.4. Incorporation into liposome

The affinities of the synthesized compounds for lipid bilayers were measured as previously reported with slight modification [26]. Briefly, egg PC was dissolved in a small amount of chloroform and solvent evaporated off with a rotary evaporator. The thin film of egg PC was dried with a vacuum pump. An aqueous glucose solution (300 mM) was then poured into the flask, and the mixture was

sonicated using an ultrasonicator. The resulting solution of multilamellar vesicles was then sonicated in a cup-horn type of sonicator in order to change the multilamellar vesicles to small unilamellar vesicles. The liposomal solution was diluted 10 times with PBS, and untrapped glucose was removed by centrifugation at 130,000*g* for 5 min at 20 °C. The final concentration of egg PC in the liposomal solution was adjusted to 1 mg/mL. Each compound solution in ethanol was added to the liposomal solution. The final concentration was 50 μM containing 10% ethanol. The proportion (%) of the compound incorporated into the lipid bilayers was determined by integration of the HPLC peak of remaining compound in the solution.

6.5. Analysis of calcein leakage from calcein-trapped liposomes

The membrane injury action of (+)-C derivatives (**8c**–**8h**) was examined with model membranes using calcein-trapped liposomes. The enclosed fluorescence substance, calcein, leaks out by the destruction of membrane structure. The leakage of calcein was measured as previously reported [26]. Briefly, the liposome with a dense internal aqueous phase containing calcein was prepared. The designated amount of a (+)-C derivative was added to the liposomal solution, and the resulting solution was incubated for 1 h at 20 °C. Fluorescence of calcein leaking from the internal aqueous phase to the external medium was measured with excitation at 480 nm and emission at 520 nm. The degree of calcein leakage was calculated as the percent of the fluorescence intensity of completely released calcein from the liposomes after treatment with 1% Triton X-100.

6.6. Parameters calculation

c Log *P* value was calculated using Molinspiration online property calculation toolkit [27].

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