An Amide Analog of (−)-Epigallocatechin Gallate Shows Preferential Cytotoxicity toward Triple-Negative Breast Cancer Cells

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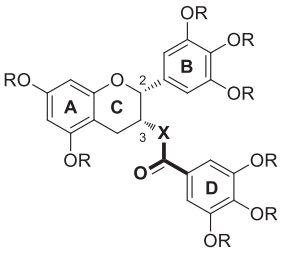
MDA-MB-231 is a highly aggressive, invasive and poorly differentiated triple-negative breast cancer (TNBC) cell line as it lacks estrogen receptor and progesterone receptor expression, as well as HER2 (human epidermal growth factor receptor 2) amplification.[1,2] Similar to other invasive cancers, there are only limited treatment options for TNBC and, as a result, there is an urgent need to discover cytotoxic agents against the MDA-MB-231 cells.

(−)-Epigallocatechin gallate [EGCG (**1**), Figure 1], the most abundant tea catechin with a broad-spectrum bioactivity,[3] is also known as a cytotoxic agent against MDA-MB-231 cells.[4] More interestingly, a prodrug of EGCG [AcEGCG (**2**), Figure 1] showed significantly improved antiproliferative effect against MDA-MB-231 cells compared with EGCG,[5,6] which prompted structure–activity relationship study of EGCG esters.[7–9] Glycosylation[10,11] as well as bioisosteric replacement of the D-ring phenolic hydroxyl groups with a fluorine atom have also been attempted.[12] Nevertheless, the ester functionality bridging C- and D-ring (bold lines in **1**, Figure 1) has rarely been targeted for structural modification of the EGCG scaffold. From a synthetic point of view, replacement of the bridging ester with other functionalities is more challenging than substitution at the peripheral phenolic hydroxyl groups (OR, Figure 1). In addition, substitution at the epimerization-susceptible 3-position provides additional consideration.[13] In this study, we devised a synthetic route to an amide analog of EGCG [EGCG-Amide (**3**), Figure 1], which showed preferential cytotoxicity toward triple-negative breast cancer cell.

Synthesis of EGCG-amide (**3**) was performed by using the stereospecific reductive amination as the key step (Scheme 1). Starting from EGCG (**1**), global protection of the phenolic hydroxyls with *tert*-butyldimethylsilyl chloride followed by removal of the 3-gallate moiety by $LiAlH_4$ reduction provided TBS-protected epigallocatechin **4** in 78% yield. The substrate for the reductive amination, 3-keto epigallocatechin (**5**, Scheme 1), was prepared only under mild oxidation conditions, and Dess-Martin oxidation of **4** provided clean conversion to the desired ketone **5**. Upon reductive amination with benzylamine followed by debenzylation, **5** was converted into 3-amino epigallocatechin (**6**) in 64% combined yields. The reductive amination proceeded in a stereospecific manner to give only a single stereoisomer, which was unequivocally confirmed to have (2*R*, 3*R*) configuration by comparison of its spectroscopic data with those of the known compound.[14] Condensation of **6** with methyl 3,4,5-tribenzyloxybenzoate in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and 4-dimethylaminopyridine provided the protected EGCG-amide, which was converted into **7** after removal of the TBS protecting group upon treatment with HF-pyridine (65% yield). Debenzylation of **7** provided the EGCG-amide (**3**) in 90% yield.

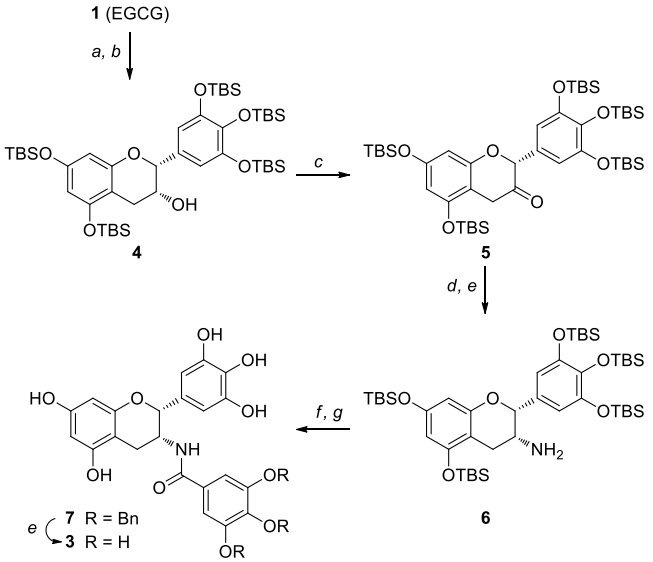
Antioxidative activity[15] and cytotoxicity against tumor cells,[16] the representative bioactivity of EGCG (**1**), were evaluated for EGCG-amide (**3**), but no significantly different activity was observed compared with EGCG (**1**) (data not shown). Thus, EGCG-amide (**3**) was equipotent to EGCG (**1**) in 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity and MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay in various cancer cells (data not shown). As such, the replacement of the ester linkage with an amide does not seem to affect the bioactivity of EGCG (**1**).

However, to our surprise, EGCG-amide (**3**) was significantly more potent than EGCG (**1**) in reducing the viability of MDA-MB-231 cells (Figure 2(b)). MDA-MB-231 was found to be less susceptible than MCF-7 to the cytotoxic activity of EGCG (**1**) (Figure 2), and **1** showed twofold increase in IC_{50} value against MDA-MB-231 compared to that against MCF-7 cells (EC_{50} = 22.7 μM and 45.6 μM for MCF-7 and MDA-MB-231, respectively). A decrease in cytotoxic effect against MDA-MB-231 cells is not unprecedented, but a general trend is observed in anticancer drug discovery.[17,18] However, EGCG-amide (**3**) was more potent as an inhibitor of cell proliferation in MDA-MB-231 cells than in MCF-7 cells (IC_{50} = 34.7 μM and 27.4 μM for MCF-7 and MDA-MB-231, respectively).



	R	X
1 (EGCG)	H	O
2 (AcEGCG)	$COCH_3$	O
3 (EGCG-Amide)	H	NH

Figure 1. Structures of the EGCG derivatives.



Reagents and Conditions: (a) TBSCl, Et_3N, DMF, rt; (b) $LiAlH_4$, THF, 0 °C; (c) Dess-Martin periodinane, CH_2Cl_2, rt; (d) Bezylamine, HOAc, $NaBH_3CN$, THF, rt; (e) H_2, 10% Pd/C, CH_2Cl_2, MeOH, rt; (f) 3,4,5-Tribenzyloxybenzoic acid, EDC, DMAP, CH_2Cl_2, rt; (g) HF-Py, pyridine, THF, rt.

SCHEME 1 Synthesis of EGCG-amide (**3**).

Considering the minimal structural difference between EGCG (**1**) and EGCG-amide (**3**) resulting from the isosteric replacement of an ester (**1**) with an amide (**3**) functionality, the peculiar cytotoxicity profile of the EGCG-amide (**3**) against MDA-MB-231 draws special attention. From a structural point of view, the most notable difference between an ester and an amide is the presence of a hydrogen bond donor on the amide functionality. Thus, one might assume that the amide group on EGCG-amide (**3**) is involved in a specific interaction with the characteristic target molecule in MDA-MB-231 cells. Identification of the MDA-MB-231-specific target by using EGCG-amide (**3**) as a probe molecule would provide valuable information for development of anticancer agents for treatment of the chemotherapy-resistant TNBC.

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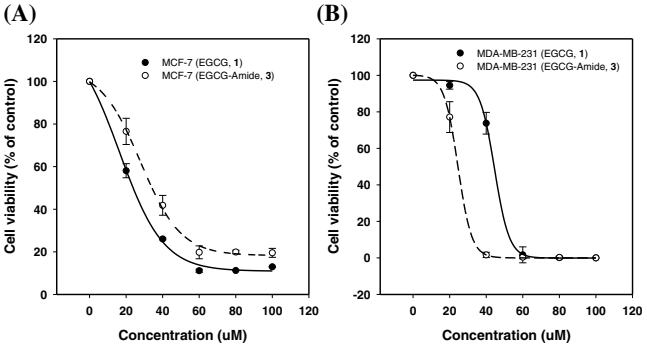

Figure 2. Comparison of anticancer effects of EGCG (**1**) and EGCG-amide (**3**) against (a) MCF-7 and (b) MDA-MB-231 cells.

Supporting Information. Additional supporting information may be found online in the Supporting Information section at the end of the article.

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