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Synthesis of diethylamino-curcumin mimics with substituted triazolyl groups and their sensitization effect of TRAIL against brain cancer cells

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Abstract— A newly designed curcumin mimic library (**11a**-**11k**) with 2-ethylamino groups in a chalcone structure and variously substituted triazole groups as side chains was synthesized using the Huisgen 1,3-cycloaddition reaction between various alkynes (**a**–**k**) and an intermediate (**10**), with $CuSO_4$ and sodium ascorbate in a solution mixture of chloroform, ethanol, and water (5:3:1) at room temperature for 5h. In the lactate dehydrogenase (LDH) release assay involving co-treatment with tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and/or synthetic curcumin derivatives using TRAIL-resistant human CRT-MG astroglioma cells, the novel curcumin mimic library was found to effectively stimulate the cytotoxicity of TRAIL, causing mild cytotoxicity when administered alone. In particular, **11a** and **11j** are promising candidates for TRAIL-sensitizers with potential use in combination chemotherapy for brain tumors.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily of cytokines. Members of the TNF family contain highly conserved carboxyl-terminal domains and induce receptor trimerization to transduce intracellular signaling.[1] TRAIL can induce apoptotic cell death through caspase-dependent mechanisms.[1,2] TRAIL binds to four different receptors, two of which, death receptor 4 and 5 (DR4 and DR5

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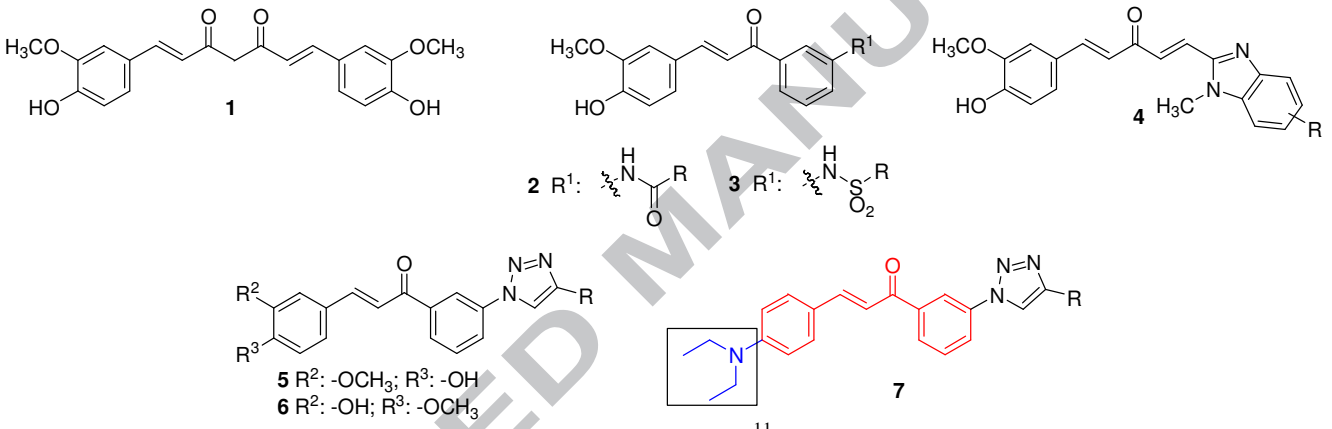
respectively), induce apoptosis. However, decoy receptors for TRAIL, DcR1 and DcR2, which lack the cytoplasmic death domain for transducing apoptotic death signals, protect cells from TRAIL-induced cell death by interfering with signaling through DR4 and DR5.[2-4] Transformed tumor cells are generally believed to be more susceptible to TRAIL-mediated cell death owing to the selective loss of decoy receptors.[5]

Glioblastoma multiforme (GBM) is one of the most aggressive forms of human malignant brain tumors and has a high mortality rate.[6,7] It is characterized by rapid growth, extensive invasiveness and robust neo-angiogenesis. GBM is refractory to all of the current therapeutic approaches including surgery, radiotherapy, and chemotherapy with drugs such as temozolomide (TMZ). Therefore, there is an urgent need for new chemo-therapeutic strategies to effectively treat GBM. In this respect, TRAIL is a promising drug target. TRAIL has been shown to exert strong antitumor activity on intracranial malignant glioma xenografts in athymic mice.[8] It showed synergistic cytotoxicity against human astrocytoma or neuroblastoma *in vitro* in combination with chemotherapeutic agents such as silibinin,[9] bortezomib,[10] and the anti-diabetic drug troglitazone.[11] Co-treatment with a chemo-sensitizer and TRAIL is more efficient to treat against GBM than is treatment with TRAIL alone.[11]



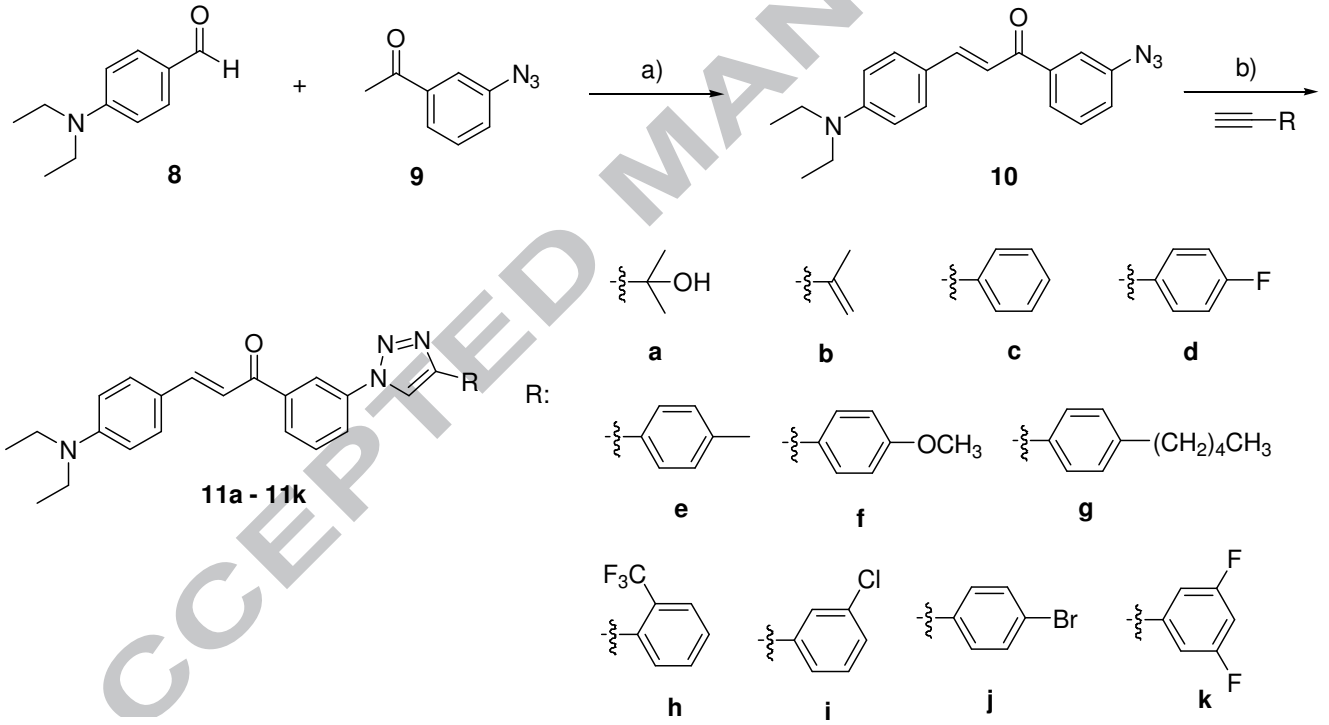
Throughout our decade of efforts to discover drug candidates based on natural products and structural mimics libraries, curcumin (diferuloyl methane, **1**) has consistently provided better-than-expected results. Curcumin (**1**), an important constituent of the root of *Curcuma longa* L., has versatile and useful biological properties. It shows anti-inflammatory,[12] antioxidant,[13] antiviral,[14] chemopreventive,[15] anti-infective,[16] and wound-healing properties.[17] Based on the basic biological properties of curcumin, we previously synthesized a curcumin mimic library and reported improved biomedical properties. For example, curcumin mimics (**2**) possessing alkyl amide and aryl amide functional groups inhibit angiogenesis[18] and reverse multidrug resistance (MDR).[19,20] Sulfonyl amide-linked curcumin derivatives (**3**) exhibit a vasodilatation effect on the basilar artery After K^+-induced contraction.[21]

Figure 1. Structures of curcumin and synthetic curcumin mimic derivatives

The structure-activity relationships of the curcumin mimics indicate that the diversification of biological properties comes from the right side functionality attached to the feruloyl scaffold. For example, amide- and sulfonyl amide-linked compounds (**2** and **3**) showed weak cytotoxicity whereas curcumin mimics with substituted benzimidazole groups (**4**) exhibited strong cytotoxicity against various cancer cells[22] and multidrug-resistant cancer cells.[23] In

addition, we reported that substituted triazolyl curcumin mimics (**5** and **6**) synthesized through Cu(I)-catalyzed Huisgen 1,3-cycloaddition exhibited moderate to strong inhibitory activity against the osteoclastogenesis induced by the receptor activator of NF-κB ligand (RANKL)[24] (Fig. 1).

When considering our previous reports for the structure-activity relationship of diverse curcumin mimic libraries, it is obvious that curcumin (**1**) can be used as a lead compound for discovering TRAIL sensitizers for use in combination therapy with TRAIL. Gautam *et al* reported that co-treatment of curcumin (**1**. 10 μM) and TRAIL (20 ng/ml) enhanced cytotoxicity against LNCaP cells (prostate cancer cells) 2- to 3-fold, by activating caspase-3.[25] Kwon *et al* also discovered that curcumin increases the sensitivity of human renal cancer cells to TRAIL, inducing DR5 expression and generating reactive oxygen species.[26] Moreover, Gautam *et al* reported that co-treatment with curcumin (20 μM) and TRAIL (5 ng/ml) induced cytotoxicity by 60% in U251MG cells and 45% in U87MG cells via cleavage of procaspases-3 and -8.[27] Although its potency needs to be further improved for use in clinical treatment, we can postulate that curcumin-derived derivatives might be novel TRAIL sensitizers in the combination chemotherapy against human brain tumors. Based on previous reports, we synthesized a novel curcumin mimic library and investigated its usefulness for combination therapy with TRAIL in the treatment of GBM.



Scheme 1. *Reagents and conditions*: (a) 40% KOH, EtOH, RT, 10 h; (b) Sodium ascorbate (2.5 eq.), $CuSO_4$ (1 eq.), chloroform:EtOH:H_2O = 5:3:1, RT, 5 h.; isolated yields for **10**, 58%; **11a**, 30%; **11b**, 80%; **11c**, 94%; **11d**, 45%; **11e**, 61%; **11f**, 48%; **11g**, 55%; **11h**, 88%; **11i**, 74%; **11j**, 92%; **11k**, 56%.

Unlike the previous curcumin mimic library (**2**-**6**) shown in Fig 1, we introduced a diethylamino group at the 4-position of the chalcone structure, in place of the methoxy and hydroxyl group of curcumin to add bulkiness and an electronic effect. To obtain a synthetic intermediate (**10**) for the curcumin mimic library (**11a-11k**), we started with the aldol reaction of commercially available 4-(diethylamino)benzaldehyde (**8**) with 3-azidoacetophenone (**9**) synthesized by the previously shown method[24] in the presence of a basic catalyst (40% KOH) in ethanol at room

temperature for 10h. The α,β-unsaturated double bond in the intermediate (**10**) was confirmed to the *trans* configuration because of a large coupling constant (J = 15.7 Hz) between its two protons. To construct a curcumin mimic library with substituted triazolyl groups (**11a-11k**), we attempted the Huisgen 1,3-cycloaddition reaction between various alkynes (**a**–**k**) and the intermediate (**10**), with $CuSO_4$ and sodium ascorbate in a solution mixture of chloroform, ethanol, and water (5:3:1) at room temperature for 5h.[24, 28] The yields of triazole products are summarized in Scheme 1.

As shown in previous reports,[25-27] curcumin (**1**) can stimulate the cytotoxic potency of TRAIL against various cancer cells. Based on the those backgrounds, we sought to determine whether the curcumin mimic library (**11a-11k**) could enhance TRAIL-mediated cell death against human CRT-MG astroglioma cells by using the lactate dehydrogenase (LDH) release assay.[29] The results are summarized in Fig 2.

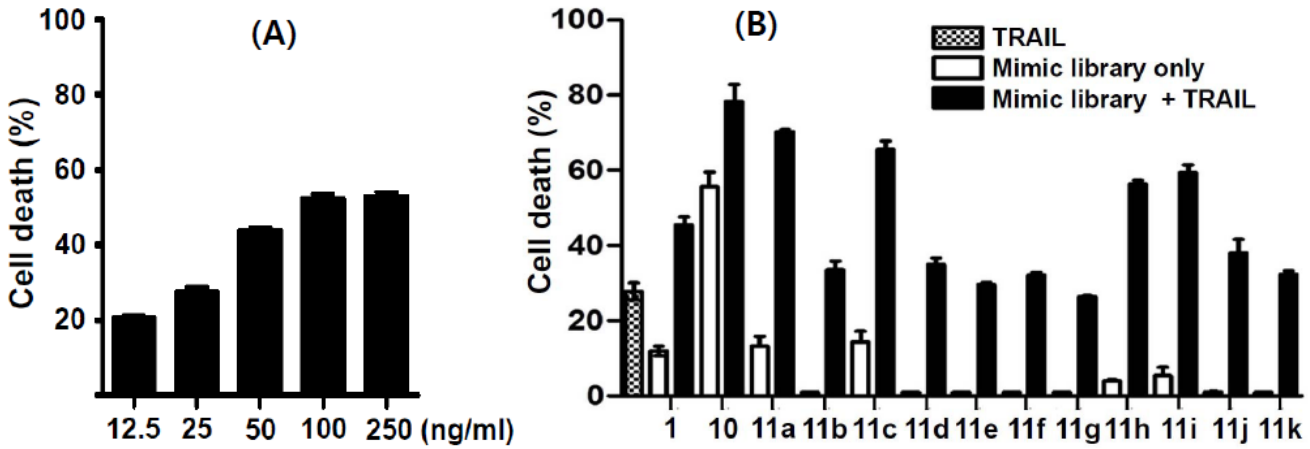


Figure 2. The cytotoxic effect of TRAIL and/or curcumin (**1**) and curcumin mimics library (**10**, **11a**-**11k**). (A) The cell death percentage of human CRT-MG astroglioma cells is due to treatment with TRAIL concentration of 12.5 ng/ml to 250 ng/ml for 24h. (B) Human CRT-MG astroglioma cells were treated with TRAIL (25 ng/ml) and/or all tested drugs (10 μM). Data are presented as means ± SD. Levels of significance for comparisons between samples were determined using Student's t-test distribution.

First, we tested the cytotoxicity of various concentrations of TRAIL to determine whether human CRT-MG astroglioma cells are resistant to TRAIL treatment. Although TRAIL induced cell death in a dose-dependent manner within the range of 12.5 ng/ml to 100 ng/ml, the cytotoxic effect did not increase further with TRAIL concentration above 100 ng/ml, which means that human CRT-MG astroglioma cells are resistant to treatment with TRAIL as an antitumor agent (Fig, 2A). Secondly, before evaluating the TRAIL sensitization effect of the synthesized curcumin mimic derivatives, their cell cytotoxicity was measured by applying a concentration of 10 μM to CRT-MG cells for 24 h. As shown in Fig. 2B (white bar), curcumin and other tested compounds except synthetic intermediate (**10**), showed low toxicity, less than 20%. Several compounds (**11b**, **11d**-**11g**, **11j**, and **11k**) did not induce apoptosis at all at the tested concentration. Considering their low cytotoxicity, if co-treatment with the curcumin mimics and TRAIL increase apoptosis of TRAIL-resistant cancer cells, we can postulate that the increase activity is due to sensitization effect of the curcumin derivatives, and not from their direct cytotoxicity. For example, synthetic intermediate (**10**), a concentration of 10 μM, enhanced TRAIL-induced cell death from 27.8% to 78.3%. In this case, the enhancement was attributable to the cytotoxicity of **10,** which inhibited CRT cell proliferation to 55.6%. However, curcumin mimic

library showed a sensitization effect of TRAIL without or with only slight cytotoxicity. 2-Hydroxypropantriazolyl chalcone (**11a**) and benzenetriazolyl chalcone (**11c**) similarly increased the sensitivity of TRAIL about 2.5-fold, but their cell cytotoxicity was very low (13.3% and 14.3%, respectively). We can conclude that they sensitize human CRT-MG astroglioma cells to TRAIL. Moreover, *o*-trimethylphenyltriazolyl chalcone (**11h**) and *m*-chlorophenyltriazolyl chalcone (**11i**) effectively increased the anticancer activity of TRAIL without producing cell cytotoxicity themselves (**11h** and **11i**). The other derivatives (**11b**, **11d**-**11g**, **11j**, and **11k**) showed no inhibitory activity against cancer cells and slightly sensitized the cells to TRAIL. This suggests that they cannot interact with the receptors that influence the sensitization to TRAIL.

Based on a preliminary analysis of structure-activity relationships, curcumin mimics possessing an electron donating group cannot influence cell death and the sensitization of TRAIL simultaneously. Interaction of active compounds with cancer cells can influence the sensitization of cancer cells to TRAIL, as well as cell viability.

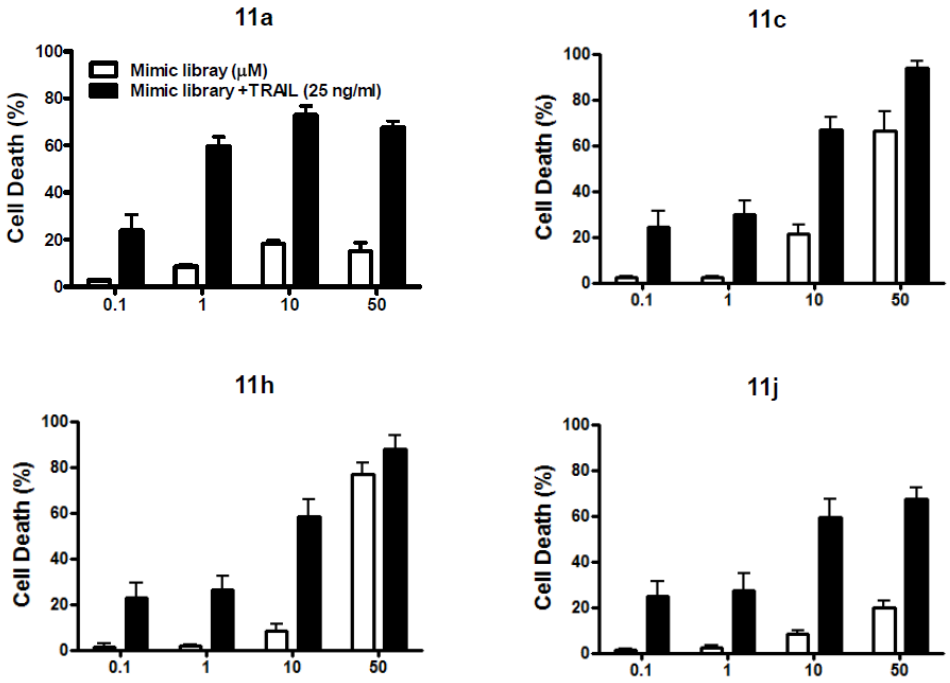


Figure 3. Dose-dependent cytotoxic effects of combination treatment of TRAIL and selected curcumin mimics (**11a**, **11c**, **11h**, and **11j**). Data are presented as means ± SD. Levels of significance for comparisons between samples were determined using Student′s t-test distribution.

Finally, we tested the cytotoxicity of TRAIL (25 ng/ml) against CRT-MG cells with co-treatment of selected curcumin mimics (**11a**, **11c**, **11h**, and **11j**) at variable concentrations. The results showed that **11a** and **11j** were the most promising TRAIL sensitizers among in curcumin mimic library because they showed low cell toxicity (under 20% in cell death percentage) at a high concentration (50 μM). Although combination treatment of TRAIL with high concentrations (50 μM) of **11c** and **11h** showed maximum cell death, it is predicted that most of the increment in cytotoxicity comes from the increasing cytotoxicity of curcumin derivatives (**11c** and **11h**). However, **11c** and **11h** are potential candidates for TRAIL sensitizers if they are used at concentrations under 10 μM.

In conclusion, we synthesized a novel curcumin mimic library (**11a**-**11k**) by using the Huisgen 1,3-cycloaddition

reaction between azido chalcone intermediate (**10**) having 2-ethylamino group and various alkynes (**a**-**k**) with the intention of discovering new TRAIL sensitizer candidates. Based on the LDH release assay of co-treatment TRAIL and/or synthetic curcumin derivatives by using TRAIL-resistance human CRT-MG astroglioma cells, we discovered that a 2-ethylamino-curcumin mimic library possessing various substituted triazole moieties exhibited a sensitization effect of TRAIL without or with slight cytotoxicity. Four compounds (**11a**, **11c**, **11h**, and **11j**) were promising TRAIL-sensitizers with the potential for application in combination chemotherapy of brain tumors. Based on the preliminary structure–activity relationships, the curcumin mimic library having 2-ethylamino and substituted triazol groups will be a promising template for developing novel TRAIL sensitizers as anticancer agents.

Acknowledgement

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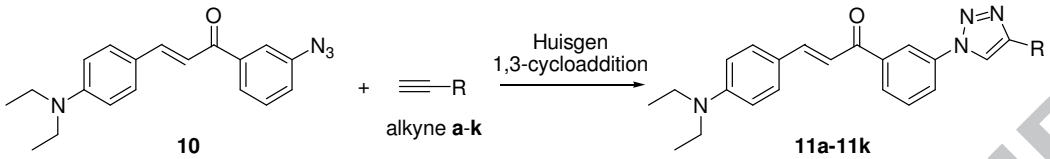
28. NMR data for **11a**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.27 (t, *J* = 1.7 Hz, 1H), 8.01 (m, 3H), 7.83 (d, *J* = 15.4 Hz, 1H), 7.61 (t, *J* = 7.9 Hz, 1H), 7.53 (d, *J* = 8.9 Hz, 2H), 7.30 (d, *J* = 15.3 Hz, 1H), 6.65 (d, *J* = 9.0 Hz, 2H), 3.42 (q, *J* = 7.1 Hz, 4H), 1.73 (s, 6H), 1.20 (t, *J* = 7.0 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 188.9, 150.0, 147.2, 140.7, 137.3, 131.1, 129.9, 128.2, 123.8, 121.4, 119.8, 115.1, 111.2, 68.6, 44.5, 30.4, 12.5 ppm.; **11b**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.28 (t, *J* = 1.7 Hz, 1H), 8.04 (m, 3H), 7.85 (d, *J* = 15.4 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.32 (d, *J* = 15.3 Hz, 1H), 6.67 (d, *J* = 8.9 Hz, 2H), 5.87 (s, 1H), 5.20 (t, *J* = 1.6 Hz, 1H), 3.43 (q, *J* = 7.1 Hz, 4H), 2.21 (s, 3H), 1.21 (t, *J* = 7.1 Hz, 6H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 188.9, 150.0, 149.5, 147.2, 140.7, 137.3, 133.0, 131.1, 129.9, 128.1, 123.7, 121.5, 119.6, 117.6, 115.2, 113.4, 111.3, 44.5, 20.6, 12.6 ppm.; **11c**: ^{1}H-NMR (300 MHz, $CDCl_3$): 8.35 (t, *J* = 1.7 Hz, 1H), 8.33 (s, 1H), 8.07 (m, 2H), 7.93 (m, 2H), 7.87 (m, 1H), 7.67 (t, *J* = 7.9 Hz, 1H), 7.56 (d, *J* = 8.9 Hz, 2H), 7.45 (m, 4H), 6.68 (d, *J* = 9.0 Hz, 2H), 3.43 (q, *J* = 7.1 Hz, 4H), 1.21 (t, *J* = 7.1 Hz, 6H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 188.9, 150.2, 147.2, 140.8, 137.8, 131.1, 130.1, 130.0, 128.9, 128.5, 128.3, 125.9, 123.8, 121.7, 119.7, 115.2, 111.3, 44.5, 12.6 ppm.; **11d**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.33 (t, *J* = 1.7 Hz, 1H), 8.28 (s, 1H), 8.07 (dd, *J* = 1.7, 8.3 Hz, 2H), 7.90 (m, 3H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 9.0 Hz, 2H), 7.32 (d, *J* = 15.4 Hz, 1H), 7.15 (t, *J* = 8.7 Hz, 2H), 6.65 (d, *J* = 9.0 Hz, 2H), 3.42 (q, *J* = 7.1 Hz, 4H), 1.20 (t, *J* = 7.1 Hz, 6H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ188.7, 164.4 (d, *J* = 246.2 Hz), 150.0, 147.6, 147.2, 140.7, 137.2, 131.1, 129.9, 128.2, 127.6, 127.5, 126.3 (d, *J* = 2.9 Hz), 123.6, 121.4, 119.6, 117.4, 116.0, 115.7, 115.1, 111.2, 44.5, 12.5 ppm.; **11e**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.33 (t, *J* = 1.8 Hz, 1H), 8.28 (s, 1H), 8.08 (dd, *J* = 2.0, 8.0 Hz, 2H), 7.91 (m, 3H), 7.67 (t, *J* = 8.1 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.32 (d, *J* = 15.4 Hz, 1H), 7.16 (t, *J* = 8.6 Hz, 2H), 6.68 (d, *J* = 8.9 Hz, 2H), 3.42 (q, *J* = 7.1 Hz, 4H), 2.36 (s, 3H), 1.21 (t, *J* = 7.1 Hz, 6H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ188.7, 161.2, 150.1, 147.7, 147.2, 140.8, 137.2, 131.1, 130.0, 128.3, 127.7, 127.6, 126.3, 123.7, 121.5, 119.7, 117.4, 116.1, 115.8, 115.1, 111.3, 44.5, 29.7, 12.6 ppm.; **11f**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.33 (t, *J* = 1.8 Hz, 1H), 8.23 (s, 1H), 8.04 (dd, *J* = 2.0, 7.4 Hz, 2H), 7.84 (m, 3H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 8.9 Hz, 2H), 7.31 (d, *J* = 15.3 Hz, 1H), 6.98 (d, *J* = 8.9 Hz, 2H), 6.64 (d, *J* = 9.0 Hz, 2H), 3.84 (s, 3H), 3.40 (q, *J* = 7.1 Hz, 4H), 1.97 (t, *J* = 7.1 Hz, 3H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ188.8, 159.8, 150.0, 148.4, 147.1, 140.7, 137.3, 131.1, 129.9, 128.1, 127.1, 123.6, 122.7, 121.5, 119.6, 116.8, 115.1, 114.3, 111.2, 55.3, 44.5, 12.5 ppm.; **11g**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.34 (s, 1H), 8.28 (s, 1H), 8.06 (d, *J* = 7.9 Hz, 2H), 7.84 (m, 3H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 8.9 Hz, 2H), 7.29 (m, 3H), 6.66 (d, *J* = 8.9 Hz, 2H), 3.42 (q, *J* = 7.0 Hz, 4H), 2.64 (t, *J* = 7.5 Hz, 2H), 1.65 (m, 2H), 1.34 (m, 4H), 1.20 (t, *J* = 7.1 Hz, 6H), 0.91 (t, *J* = 7.0 Hz, 3H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 188.9, 150.0, 147.1, 143.5, 140.7, 137.3, 131.1, 129.9, 128.9, 128.1,

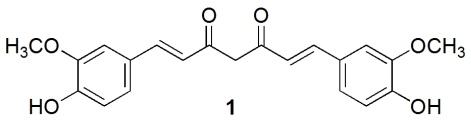
127.4, 125.8, 123.7, 121.5, 119.6, 117.2, 115.2, 111.3, 44.5, 35.7, 31.4, 31.0, 22.5, 14.0, 12.6 ppm.; **11h**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.38 (t, *J* = 1.8 Hz, 1H), 8.29 (s, 1H), 8.05 (m, 3H), 7.84 (d, *J* = 15.3 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.68 (t, *J* = 7.9 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 2H), 7.32 (d, *J* = 15.3 Hz, 2H), 6.66 (d, *J* = 9.0 Hz, 2H), 3.42 (q, *J* = 7.1 Hz, 4H), 1.20 (t, *J* = 7.1 Hz, 6H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 188.8, 150.0, 147.2, 145.1, 140.9, 137.1, 132.1, 131.8, 131.1, 130.0, 128.6, 128.4, 126.3 (q, J = 5.9 Hz), 125.2, 123.9, 121.5, 121.0 (q, *J* = 5.3 Hz), 120.0, 115.2, 111.3, 44.5, 29.7, 12.5 ppm.; **11i**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.34 (s, 2H), 8.06 (m, 2H), 7.93 (t, *J* = 1.8 Hz, 1H), 7.86 (d, *J* = 15.4 Hz, 1H), 7.80 (m, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 8.9 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.34 (m, 2H), 7.27 (d, *J* = 9.2 Hz, 1H), 6.66 (d, *J* = 8.9 Hz, 2H), 3.42 (q, *J* = 7.1 Hz, 4H), 1.20 (t, *J* = 7.1 Hz, 6H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 188.7, 150.1, 147.3, 140.8, 137.1, 134.9, 131.8, 131.1, 130.2, 130.0, 128.5, 128.4, 125.9, 123.9, 123.7, 121.4, 119.7, 118.0, 115.0, 111.3, 44.5, 12.6 ppm.; **11j**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.32 (s, 2H), 8.06 (m, 2H), 7.85 (d, *J* = 15.5 Hz, 1H), 7.80 (m, 2H), 7.67 (t, *J* = 7.9 Hz, 1H), 7.60 (d, *J* = 8.5 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.32 (d, *J* = 15.3 Hz, 1H), 6.66 (d, *J* = 8.9 Hz, 2H), 3.42 (q, *J* = 7.1 Hz, 4H), 1.21 (t, *J* = 7.1 Hz, 6H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$) δ 188.8, 150.1, 147.5, 147.3, 140.8, 137.2, 132.1, 131.1, 130.0, 129.0, 128.4, 127.4, 123.7, 122.4, 119.7, 117.7, 115.1, 111.3, 44.5, 12.6 ppm.; **11k**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.33 (m, 2H), 8.07 (m, 2H), 7.84 (d, *J* = 15.4 Hz, 1H), 7.68 (t, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 8.9 Hz, 2H), 7.47 (m, 2H), 7.32 (d, *J* = 15.3 Hz, 1H), 6.82 (m, 1H), 6.67 (d, *J* = 9.0 Hz, 2H), 3.43 (q, *J* = 7.1 Hz, 4H), 1.21 (t, *J* = 7.1 Hz, 6H) ppm.; ^{13}C-NMR (75 MHz, $CDCl_3$) δ 188.7, 165.1 (d, *J* = 246.2 Hz), 161.9 (d, *J* = 248.0 Hz), 150.1, 147.3, 140.9, 137.0, 133.2, 131.1, 130.1, 128.5, 123.8, 121.4, 119.7, 118.4, 115.0, 111.3, 108.9, 108.8(d, *J* = 9.0 Hz), 108.5, 103.7 (d, *J* = 25.4 Hz), 44.5, 12.6 ppm.

29. Kwon, D.; Choi, C.; Lee, J.; Kim, K. O.; Kim, S. J.; Kim, J. D.; Choi, I. H. *J. Neuroimmunol.* **2001**, *113*, 1.; LDH (lactic dehydrogenase) release assay: Cell cytotoxicity was measured using the LDH cytotoxicity detection kit (Takara, Otsu, Shiga, Japan). 100 µl of cell-free supernatant was used for LDH cytotoxicity assay by incubation with 22.9 mM pyruvate and 1 mM β-NADH in 0.2 M LDH buffer, pH 7.4. The soluble reaction product was quantified spectrophotometrically at 490 nm using an ELISA reader VERSAmax (Molecular Devices, Sunnyvale, CA). Total LDH activity was determined by lysing control cells with 1% Triton X-100 for 30 min. LDH release was expressed as the percentage of total LDH activity.

Synthesis of diethylamino-curcumin mimics with substituted triazolyl groups and their sensitization effect of TRAIL against brain cancer cells

Youngchel Ahn, Sangtae Oh, Seong Jun Lee, Byong-Gon Park, Yoon-Sun Park, Woon-Seob Shin, Hyuk-Jai Jang, Jin-Hoon Park, Dae Ho Kwon*, and Seokjoon Lee*





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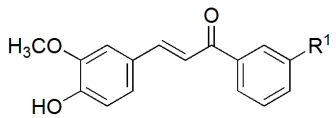

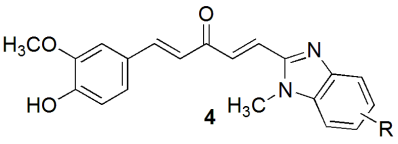

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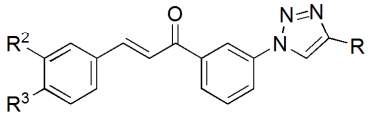
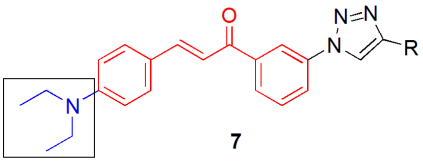
2 R^1: 

3 R^1: 



5 R^2: -OCH_3; R^3: -OH
6 R^2: -OH; R^3: -OCH_3



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