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The TRAIL Sensitization Effect of Substituted Triazolyl Curcumin Mimics Against Brain Cancer Cells

Daeho Kwon,[a] Sangtae Oh,[†,a] Ji-Hye Park,[‡] Seoul Lee,[‡,*] and Seokjoon Lee[§,*]

Department of Microbiology, Catholic Kwandong University College of Medicine, Gangneung 210-701, Korea
[†]Department of Basic Science, Catholic Kwandong University College of Medicine, Gangneung 210-701, Korea
[‡]Department of Pharmacology, Wonkwang University School of Medicine, Iksan 570-749, Korea. [*]E-mail: stress@wku.ac.kr
[§]Department of Pharmacology, Catholic Kwandong University College of Medicine, Gangneung 210-701, Korea
[*]E-mail: sjlee@cku.ac.kr
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Glioblastoma multiforme (GBM) is one of the most aggressive forms of human malignant brain tumor and has a high mortality rate.[1,2] GBM is characterized by a highly heterogeneous and infiltrative phenotype. Treatment for GBM is surgery, followed by radiotherapy and/or chemotherapy with drugs such as temozolomide (TMZ). The median survival of GBM patients is around one year.[3,4] Therefore, there is an urgent need for new chemo-therapeutic strategies to effectively treat GBM. In this respect, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) seems to be a promising drug.[5] 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.[6] TRAIL is an attractive and promising anticancer agent that induces apoptosis of tumor cells without causing harm to normal cells.[7,8] The activity of TRAIL is caused from the expression of cell membrane TRAIL receptors and activation of caspase-8.[9] There are five receptors that can recognize TRAIL, including death receptor 4 (DR4) and death receptor 5 (DR5), which have functional cytoplasmic death domains (DD). DR4 and DR5 trigger a TRAIL-induced apoptotic signal by forming a death-inducing signal complex (DISC) that activates caspase-8.[10] Specifically, TRAIL induces apoptosis in cancer cells without inducing the apoptosis of normal cells, and shows synergistic cytotoxicity against human astrocytoma and neuroblastoma *in vitro* when combined with chemotherapeutic agents such as silibinin,[11] bortezomib,[12] and the anti-diabetic drug, troglitazone.[13] Thus, co-treatment with a TRAIL and its sensitizer is a more efficient treatment against GBM than treatment with TRAIL alone. At present, when comparing the potential efficacy of TRAIL with its sensitizer and the usefulness of clinical drugs such as TMZ, it is more effective to discover a novel TRAIL-sensitizer for combination chemotherapy to treat human brain tumors.

Throughout our decade of efforts to discover drug candidates based on the synthesis of a curcumin mimic library simplifying symmetric curcumin structure (**1**), we concluded that curcumin (**1**) is a useful lead natural compound in novel drug candidate synthesis. Curcumin (diferuloyl methane, **1**) from the root of *Curcuma longa* L., has versatile biological properties such as antiinflammatory,[14] antioxidant,[15] antiviral,[16] chemopreventive,[17] and anti-infective activities,[18] as well as wound-healing properties.[19] When considering previous reports for the biological properties of curcumin (**1**), the feruloyl structure was expected to be essential to its diverse biological usefulness. Based on that postulation, we synthesized a simplified curcumin mimic library having only one feruloyl structure and discovered novel biological properties.

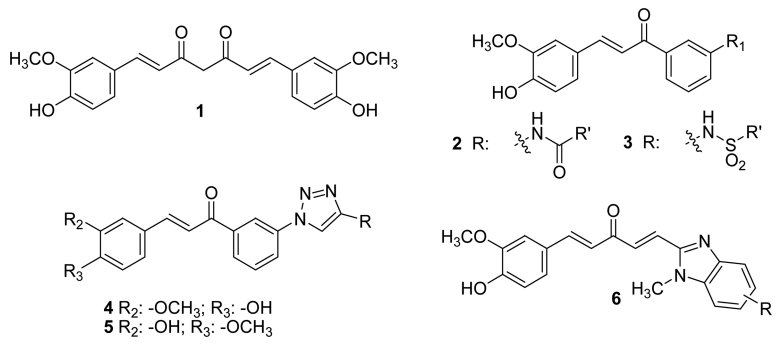


Figure 1. Structures of curcumin and synthetic curcumin mimic derivatives.

[a]These authors contributed equally to this work.

For example, alkyl amide- and aryl amide-linked curucmin mimic derivatives (**2**) showed anti-angiogenesis[20] and recovered the anticancer activity of vincristine and paclitaxel against multidrug resistance (MDR) cancer cells by inhibiting the drug efflux function of P-gp.[21,22] Similarly, the curcumin mimic library, having variously substituted sulfonyl amide groups (**3**), exhibited a vasodilatation effect on the basilar artery after K^+-induced contraction.[23] In addition, we reported that substituted triazolyl curcumin mimics (**4** and **5**) 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] Because those curcumin mimics libraries were obtained by changing the right feruloyl group to diverse aromatic functionalities resulting in weak cell cytotoxicity, they are very promising as potential novel drugs for many diseases which do not depend on cell death. Interestingly, we found curcumin derivatives with right-side benimidazole moieties (**6**) exhibited strong cytotoxicity. The structure-activity relationship of the curcumin mimics indicates that the diversification of biological properties comes from the right side functionality attached to the feruloyl scaffold. Therefore, we can expect to find a novel TRAIL sensitizer for use in combination therapy with TRAIL among the curcumin mimic library. Especially, there are previous reports that curcumin has the sensitization effect of an anticancer agent. Gautam *et al.* reported that co-treatment of curcumin and TRAIL increased anticancer activity against prostate cancer cells (LNCaP cells) 2- to 3-fold by activating caspase-3[25] and induced cytotoxicity against U251MG cells and U87MG cells, brain tumor cell lines, *via* cleavage of procaspases-3 and -8.[26] 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 (ROS).[27] Based on previous reports and postulation, we decided to test whether the curcumin mimic library (library type-**2**, -**3**, -**4**, and -**5**) obtained from our previous research could enhance TRAIL-mediated cancer cell death, especially against human CRT-MG astroglioma cells. Because library type-**6** showed cytotoxicity, we excluded it. After screening the remaining library, we discovered library type-**4** and -**5** showed promises as TRAIL sensitizer for TRAIL combination therapy. Among the tested library, the chemistry of curcumin mimic libraries type-**4** and -**5** that strongly sensitize the anticancer activity of TRAIL are summarized in Scheme 1.

The aldol reaction of commercially available 4-hydroxy-3-methoxybenzaldehyde (**5**) and 3-hydroxy-4-methoxybenzaldehyde (**6**) with 3-azidoacetophenone (**9**) in the presence of a basic catalyst (40% KOH) in ethanol at room temperature for 10 h yields two important synthetic intermediates, (*E*)-1-(3-azidophenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (**10**) and (*E*)-1-(3-azidophenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one (**11**), which are transformed to the curcumin mimic library with substituted triazolyl functionalities (**4a-4k** and **5a-5k**) by the Huisgen 1,3-cyclo-

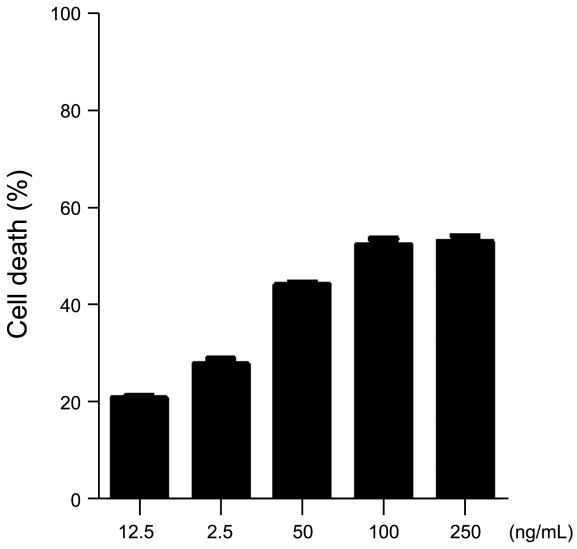
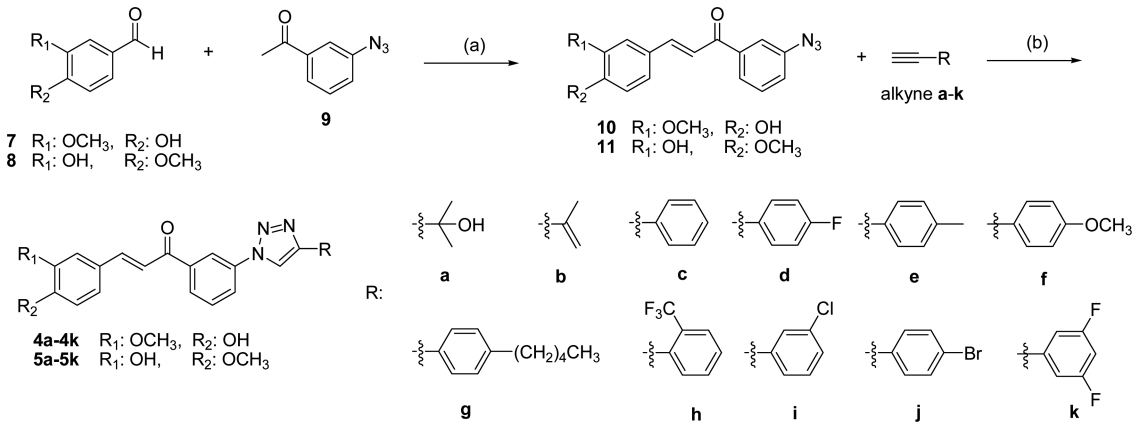


Figure 2. The cytotoxic effect of TRAIL. The cell death percentage of human CRT-MG astroglioma cells is due to treatment with TRAIL concentration of 12.5 ng/mL to 250 ng/mL for 24 h. Data are presented as mean ± SD. Levels of significance for comparisons between samples were determined using Student's t-test distribution.



Scheme 1. *Reagents and conditions*: (a) 40% KOH, EtOH, RT, 10 h, **10**, 28%; **11**, 40%; (b) Sodium ascorbate (2.5 eq.), $CuSO_4$ (1 eq.), chloroform:EtOH:H_2O = 5:3:1, RT, 5 h.; isolated yields for **4a**, 43%; **4b**, 64%; **4c**, 74%; **4d**, 73%; **4e**, 80%; **4f**, 45%; **4g**, 90%; **4h**, 95%; **4i**, 63%; **4j**, 63%; **4k**, 92%; **5a**, 25%; **5b**, 52%; **5c**, 52%; **5d**, 49%; **5e**, 60%; **5f**, 50%; **5g**, 58%; **5h**, 46%; **5i**, 55%; **5j**, 54%; **5k**, 30%.

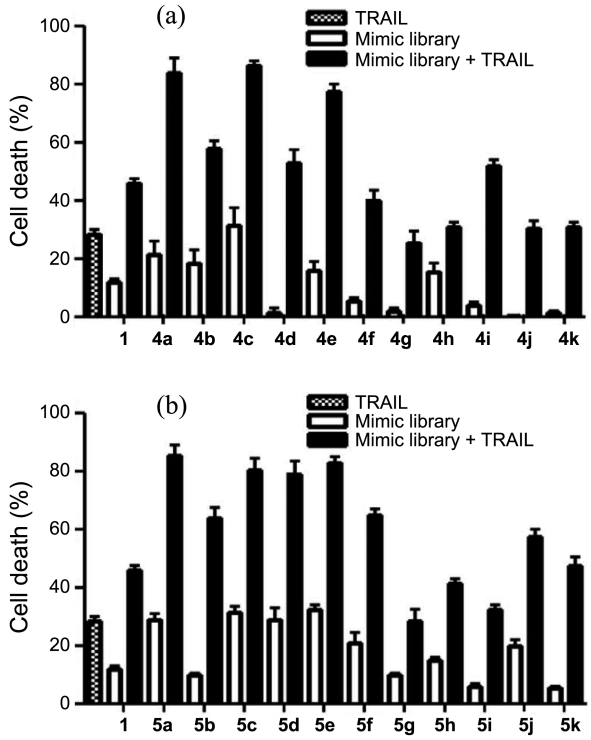


Figure 3. The cytotoxic effect of TRAIL and/or curcumin (**1**) and curcumin mimics library (**4a-4k**, **5a-5k**). Human CRT-MG astroglioma cells were treated with TRAIL (25 ng/mL) and/or all tested drugs (10 μM). Data are presented as mean ± SD. Level of significance for comparison between samples was determined using Student's t-test distribution.

addition reaction[28,29] with various alkynes (**a-k**) in a solution mixture of chloroform, ethanol, and water (5:3:1) in presence of $CuSO_4$ and sodium ascorbate.[24] All spectral information for the structure of library is previously published in reference 24.

When considering several reports that curcumin (**1**) can stimulate the cytotoxic potency of TRAIL against various cancer cells,[25-27] we expected the synthetic library (**4a-4k** and **5a-5k**) could have a sensitizing effect on TRAIL against cancer cells, especially brain tumor cells (human CRT-MG astroglioma). In order to confirm our postulation, we carried out the lactate dehydrogenase (LDH) release assay.[30] First, we determined whether human CRT-MG astroglioma cells were resistant to TRAIL treatment by testing the cytotoxicity of TRAIL in various concentrations as shown in Figure 2. We found TRAIL-induced cell death in a dose-dependent manner within the range of 12.5 ng/mL to 100 ng/mL. However, the cytotoxic effect of TRAIL did not further increased in concentrations above 100 ng/mL, which means that human CRT-MG astroglioma cells have a resistance to treatment with TRAIL. Secondly, the cell toxicity of every synthetic curcumin mimic library (**4a-4k** and **5a-5k**) was measured to determine whether the increased anticancer effect of TRAIL was caused by the cytotoxicity of curcumin derivatives in combination treatment. As shown in Figure 3 (white bar), curcumin (**1**) and other library compounds showed low toxicity, less than 30%, at 10 μM concentration. Considering their low cytotoxicity, if co-treatment with curcumin mimics and TRAIL increase apoptosis of TRAIL-resistant cancer cells, we can postulate that the increased activity is due to the sensitization effect of the curcumin derivatives, and not from their direct cytotoxicity. Thirdly, after co-treating TRAIL-resistant human CRT-MG astroglioma cells with TRAIL (25 ng/mL) and the curcumin mimics (10 μM), the TRAIL sensitization effect of curcumin derivatives was determined as shown in Figure 3 (black bar). Curcumin (**1**) showed a weak sensitization effect but our synthetic curcumin library showed strong TRAIL-sensitization effects. In particular, **4a**, **4c**, **4e**, and **5a** strongly increased the apoptosis of CRT-MG astroglioma cells by approximately 2.5 fold. The other derivatives (**4b**, **4d**, and **5b-5f**) also moderately sensitized the cells to TRAIL. Although we cannot find a general tendency of increased sensitization from a preliminary analysis of structure-activity relationships, we can conclude that the feruloyl structure of curcumin is important in increasing the sensitivity of TRAIL-resistant human brain tumor cell to TRAIL. Lastly, by co-treating with TRAIL (25 ng/mL) and variable concentrations of selected curcumin mimics (**4a**, **4c**, **4e** and **5a**) against CRT-MG cells, we can confirm the TRAIL-sensitization effect of curcumin derivatives as shown in Figure 4. Although **5a** showed weak dose-dependency, the others (**4a**, **4c**, and **4e**) enhanced the cytotoxicity of TRAIL in a dose-dependent manner. As a result, the cell death percentage of the four tested 4 compounds (**4a**, **4c**, **4e** and **5a**) in 50 μM concentrations increased to almost 50%, while the cytotoxicity with TRAIL did not significantly increase. When considering the result of Figure 4, the curcumin mimic library effectively sensitizes the cytotoxicity of TRAIL in the range of 10 μM without toxicity. If the co-treatment of curucmin sensitizer is limited to 10 μM, the results were very promising as because there was low cell toxicity (under 30% in cell death percentage). In particular, **4a** and **4e** are the best potential candidates for TRAIL sensitizers.

In conclusion, we synthesized a novel curcumin mimic

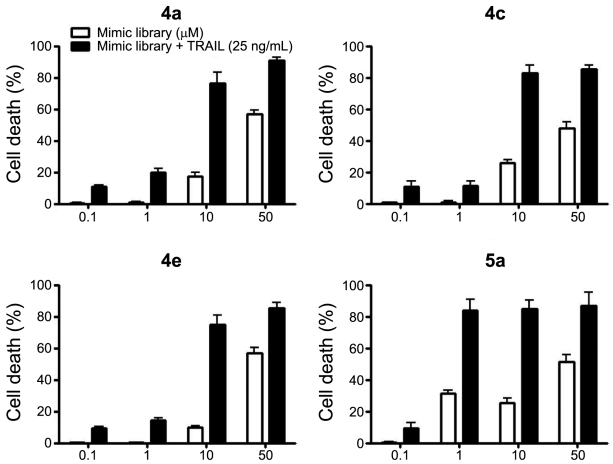


Figure 4. Dose-dependent cytotoxic effects of combination treatment of TRAIL and selected curcumin mimics (**4a**, **4c**, **4e** and **5a**). Data are presented as mean ± SD. Level of significance for comparison between samples was determined using Student's t-test distribution.

library (**4a-4k** and **5a-5k**) by using the Huisgen 1,3-cycloaddition reaction between two azido intermediates (**10** and **11**) 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 curcumin mimic library possessing various substituted triazole moieties exhibited a sensitization effect on TRAIL without any or with only slight cytotoxicity. Four compounds (**4a**, **4c**, **4e** and **5a**) were promising TRAIL-sensitizers, with the potential to be used in combination chemotherapy for brain tumors. Based on the preliminary structure–activity relationships, the curcumin mimic library substituted triazol groups will be a promising template for developing novel TRAIL sensitizers as anticancer agents in the future.

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