# Novel Curcumin Analogs, GO-Y030 and GO-Y078, Are Multi-targeted Agents with Enhanced Abilities for Multiple Myeloma

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**Abstract.** *Background: Multiple myeloma remains an incurable malignancy despite of the recent approval of new molecular-targeted agents. The complex molecular mechanism, composed of various signal networks, including nuclear factor-κB (NF-κB), phosphoinositide 3-kinase (PI3K)/AKT, Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3), and interferon regulatory factor 4 (IRF4) pathways, is a major reason for treatment failure. Curcumin can regulate these molecules, but its low bioavailability prevents its clinical application. Materials and Methods: Growth-suppressive abilities of newly synthesized analogs, GO-Y030 and GO-Y078 were analyzed. Molecular-targeted abilities of the analogs for NF-κB, PI3K/AKT, JAK/STAT3, IRF4 pathways, as well as inhibition of interleukin-6 (IL-6) production, were also examined. Results: GO-Y030 and GO-Y078 were 7 to 12-fold more potent growth suppressors for myeloma cells, and 6- to 15-fold stronger inhibitors of NF-κB, PI3K/AKT, JAK/STAT3, and IRF4 pathways than curcumin. GO-Y78 also 14-fold more potently inhibited IL-6 production. Conclusion: GO-Y030 and GO-Y078 are potential therapeutic candidates with enhanced abilities for multiple myeloma.*

Multiple myeloma (MM) is generally a B-cell malignancy characterized by profound immunoglobulin (Ig) gene hypermutation. MM ranks as the second most frequent hematological malignancy in the U.S. (1). Despite high-dose chemotherapy and the recent introduction of agents such as thalidomide and bortezomib, MM remains incurable, with a median survival of 44.8 months (2).

MM cells adhere to bone marrow stromal cells (BMCs), which then triggers the secretion of cytokines such as interleukin-6 (IL-6) to activate the mitogen-activated protein kinase (MAPK), Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3), phosphoinositde 3-kinase (PI3K)/AKT and nuclear factor κB (NF-κB) pathways (1). Recently, it was shown that interferon regulatory factor 4 (IRF4), playing an important role in differentiation and Ig class switching in plasma cells (3), works as a master regulator in MM cells (4). IRF4 controls metabolism, membrane biogenesis, cell cycle progression, and cell death of MM cells. MYC is a direct target of IRF4, and vice versa, thus forming an autoregulatory loop (5). Knockdown of IRF4 induced effective cell death, therefore IRF4 is considered as an Achilles heel for MM (5). These understandings of MM biology have assisted the identification of targeted molecules that are critical for treatment of MM.

Curcumin is a dietary constituent of turmeric. It has the ability to suppress tumor growth (6). Curcumin interferes with the transactivation of NF-κB (7) and β-catenin (8), resulting in the negative regulation of various oncogenes such as *c-MYC* and *cyclin D1*. Curcumin also blocks signaling of various growth factors (9-11), and has anti-invasive, antimetastatic, and antiangiogenic properties (12, 13). Therefore, curcumin is considered as a multi-targeted drug. Curcumin suppresses NF-κB activation, thus, reducing MM cell growth, and induces apoptosis (14). A phase I/II study of curcumin for MM is currently ongoing. However, the reduced bioavailability of curcumin prevents its clinical application. To ameliorate these limitations, we recently synthesized curcumin analogs, GO-Y030 and GO-Y078. In this study, we analyzed the potency of new analogs against MM.

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*Key Words:* Multiple myeloma, curcumin analog, NF-κB, PI3K/AKT, JAK/STAT3, IRF4.

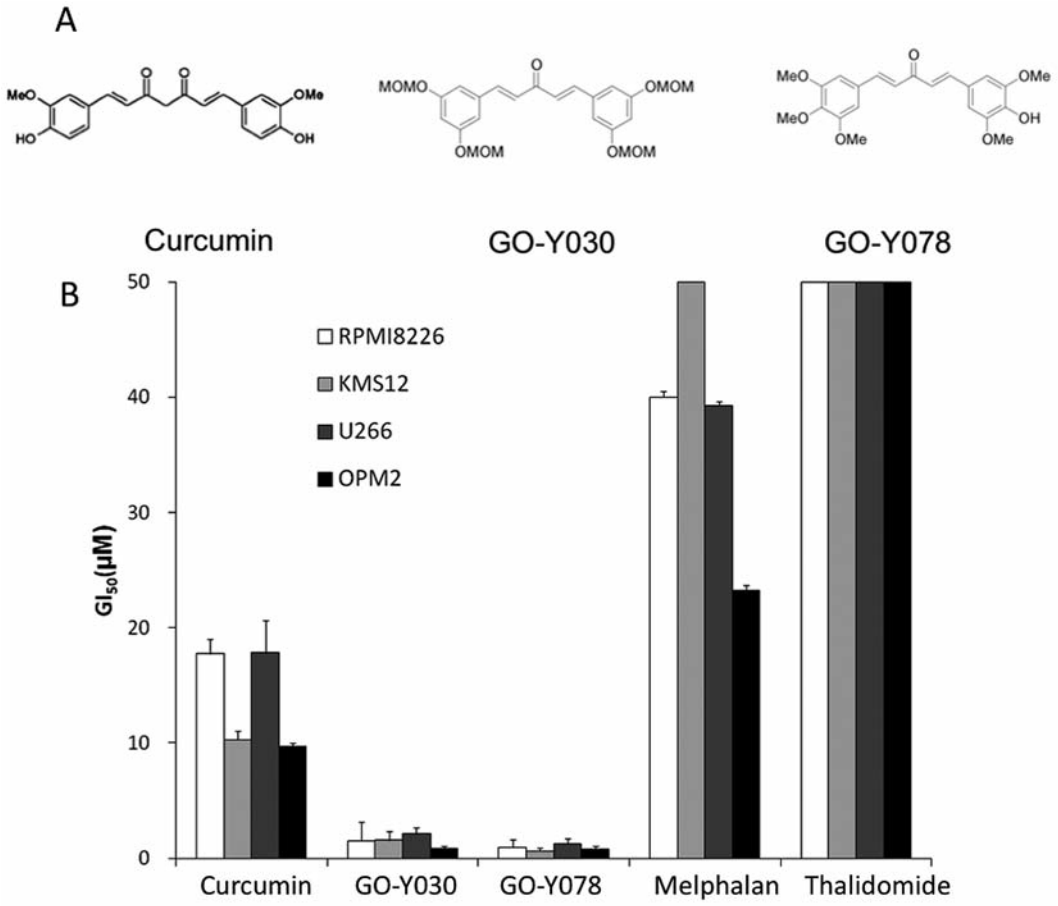


Figure 1. *Growth inhibitory effect of curcuminoids. A: Chemical structure. The methoxy group is indicated as MeO, and the methoxymethoxy group as MOMO. B: The $GI_{50}$ values of curcumin, its analogs, melphalan, and thalidomide against MM cell lines.*

## Materials and Methods

*Chemicals*. Chemical synthesis of the new analogs has been previously described (15, 16). Curcumin (Sigma–Aldrich, St Louis, MO, USA) and its analogs were dissolved in dimethyl sulfoxide (DMSO) at 10-50 mM as a stock solution. Working concentrations ranged from 1 to 20 μM, indicating that over one thousand-fold dilution was conducted. DMSO was used as a control at 1%. Melphalan and thalidomide were purchased from Sigma-Aldrich.

*Cell lines*. RPMI8226 cells were obtained from the Cell Resource Center for Biomedical Research (Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan). U266, OPM2, and KMS12-BM cells were obtained from the American Type Culture Collection (Manassas, VA, USA), Deutsche Sammlung von Mikroorganismen und Zelkulturen (Braunschweig, Germany), and Health Science Researches Bank, Japan Health Science Foundation (Osaka, Japan) respectively. Cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum at 37˚C with 5% $CO_2$.

*Growth suppression analysis*. Growth-suppressive effects of analogs were measured for 72 h, compared with the control, which was treated with 1% DMSO alone. Cell viability was assayed as previously described (15). The data were obtained from three independent experiments.

*Cell cycle analysis*. The cell cycle distribution was determined by fluorescence-activated cell sorting (FACS) analysis as previously described (15, 16). Briefly, cells were inoculated into 6-well plates at a density of $5\times10^5$ cells per well, exposed to the analogs at 2 μM, cultured for 18 h, and then assayed. The data were obtained from the triplicate wells.

*Western blotting*. Anti-actin monoclonal antibody (MoAb) was purchased from Sigma-Aldrich. Anti-NF-κB p65 (p65), anti-phospho-IκBα, and anti-TATA-box-binding protein MoAbs were purchased from Abcam (Cambridge, UK). Anti-caspase3, anti-pro-caspase3, anti-poly ADP-ribose polymerase (PARP), anti-inhibitors of NF-κBα (IκBα) and anti-c-MYC MoAbs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The Western blotting procedure was carried out as

described previously (15). Nuclear extracts were prepared using NE-PER™ nuclear extraction reagents (Pierce Corp., Rockford, IL, USA), according to the manufacturer's instructions. Anti-AKT, anti-phospho-AKT, anti-STAT3, and anti-phospho-STAT3 MoAbs were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The 50% growth inhibition concentration ($GI_{50}$) values were estimated from the band intensity.

*Immunofluorescence microscopic observation of p65*. RPMI8226 cells were inoculated at a density of $2\times10^4$ cells per well on glass chamber slides (NalgeNunc International, Rochester, NY, USA) as adherent cells for 24 h. Immunofluorescence observation was conducted as previously described with anti-p65 MoAb (Abcam) and goat anti-rabbit IgG-FITC (Beckman Coulter K.K., Tokyo, Japan) (17). Cells were also stained with 1 mM Hoechst 33258 dye (Sigma) and photographed using a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan).

*Quantitative analyses of transcripts*. Total RNA was extracted using the RNeasy mini kit (QIAGEN Corporation Japan, Tokyo, Japan) and converted to cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems Inc., Foster City, CA, USA). The number of transcripts was measured by Taq-Man gene expression assay with commercially available primers (Applied Biosystems Inc.) using an ABI 7500 real-time PCR System. The number of β-actin transcripts was used as an internal control.

*Quantification of IL-6 production*. U266 cells ($1\times10^6$ cells/ml) were treated with/without curcuminoids for 24 h. Cell-free supernatants were collected and the protein level of IL-6 was determined by Human IL-6 ELISA kit (R&D Systems, Minneapolis, MN, USA).

*Statistical analysis*. Each experiment was conducted three times, unless otherwise specified. The values shown are means±SDs. Student's *t*-test (two-sided) was used to test for significant differences between the groups.

## Results

*GO-Y030 and GO-Y078 are more effective suppressors of myeloma*. The growth suppressive effects of GO-Y030 and GO-Y078 on four MM cell lines with different genetic background: RPMI8226, KMS12-BM, U266, and OPM2 (18) (Figure 1). The $GI_{50}$ values of curcumin for RPMI8226, KMS12-BM, U266, and OPM2 cells were 17.7, 10.3, 17.8, and 9.7 μM, respectively. The corresponding $GI_{50}$ values of GO-Y030 were 1.5, 1.5, 2.1, and 0.8 μM (Figure 1B), which were 11.6-, 6.7-, 8.3-, and 11.5-fold lower than those of curcumin. For GO-Y078, the corresponding $GI_{50}$ values were 1.0, 0.6, 1.2, and 0.8 μM. For comparison, the $GI_{50}$ values of melphalan for these four MM cell lines ranged from 23.3 μM to >50 μM. The $GI_{50}$ values of thalidomide were over 50 μM. The results indicate that GO-Y030 and GO-Y078 are stronger growth suppressors than curcumin.

*GO-Y030 and GO-Y078 are more effective inducers of apoptosis*. We examined the apoptosis-inducing ability of GO-Y030 and GO-Y078 against MM cells using FACS analysis. As representatively shown in Figure 2A, compared with the control, in KMS12-BM cells, only a 1.5-fold higher sub G1 fraction (6.0% versus 4%) was induced with 20 μM of curcumin, whereas a 10.4- and 9.1-fold higher sub $G_1$ fraction (41.6% and 36.2%) was induced with 2 μM of GO-Y030 and GO-Y078, respectively. Thereafter, we examined caspase-3 conversion and PARP cleavage (Figure 2B). In curcumin-treated cells, conversion of pro-caspase 3 to caspase 3, as well as PARP cleavage, was not observed at 10 μM, but was observed at 20 μM of curcumin. In the GO-Y030 and GO-Y078 treated cells, caspase-3 conversion and PARP cleavage were observed at 2 μM. These results indicate that GO-Y030 and GO-Y078 can induce apoptosis at a concentration at least 10 times lower than that of curcumin.

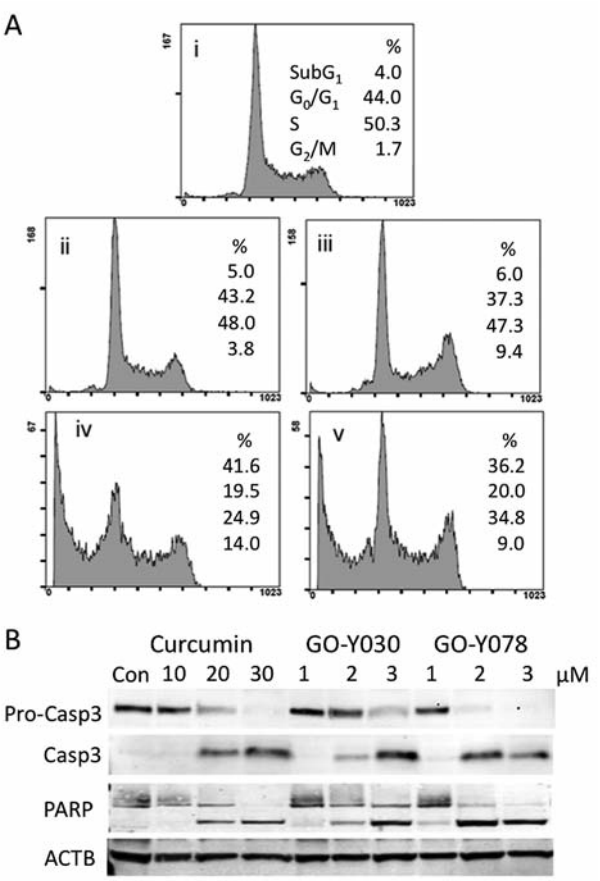


Figure 2. *Apoptosis-inducing ability of curcumin analogs. A: FACS analysis of KMS12-BM cells treated with analogs: (i) control (DMSO alone), (ii) 2 μM curcumin, (iii) 20 μM curcumin, (iv) 2 μM GO-Y030, and (v) 2 μM GO-Y078. B: Caspase-3 induction by analogs. Pro-caspase-3 (pro-Casp3), caspase-3 (Casp3), and poly ADP-ribose polymerase (PARP) cleavage were assayed. Con: Control (DMSO alone), ACTB: β-actin.*

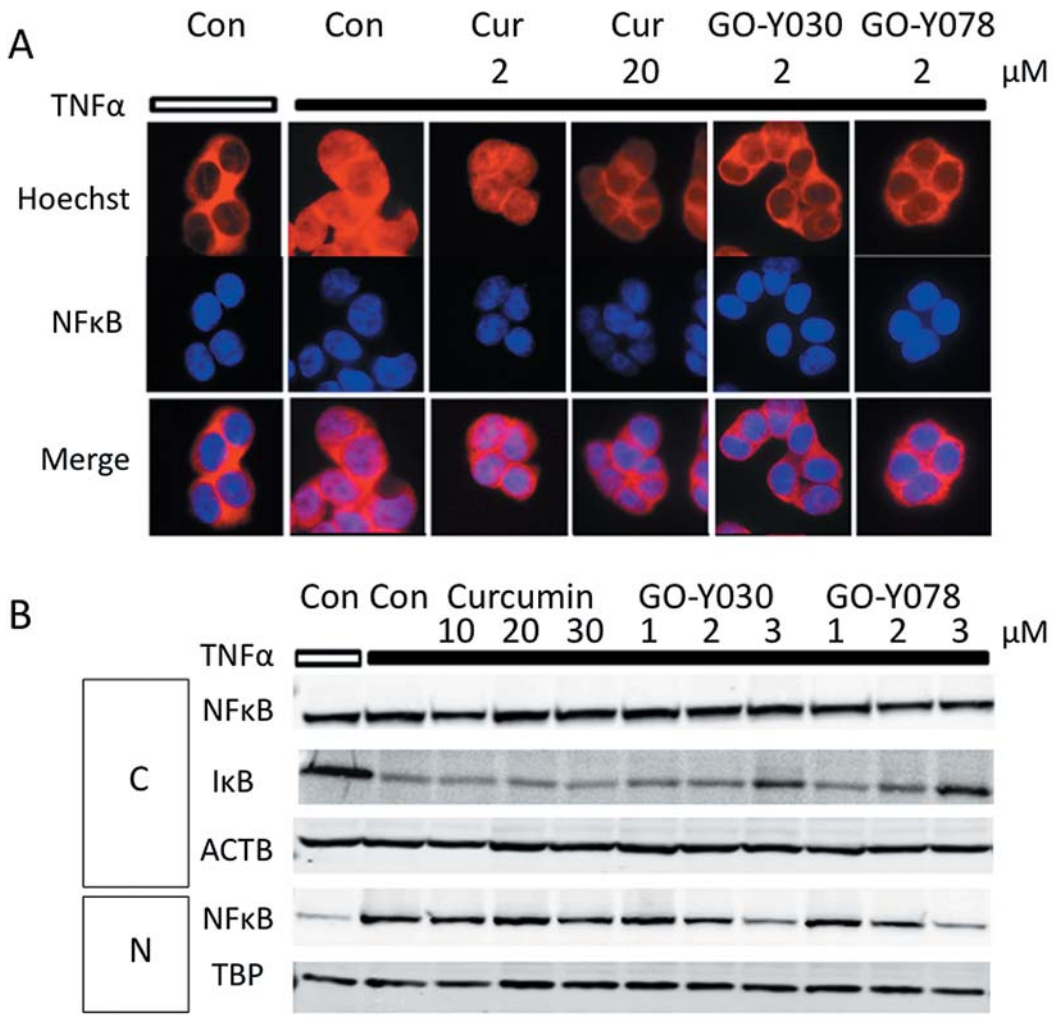


Figure 3. *Effects on NF-κB transactivation. RPMI8226 cells were pretreated with curcuminoids with (indicated by open bar) or without tumor necrosis factor-α (TNF-α) (solid bars). A: Inhibition of nuclear translocation of p65. Con: Control (DMSO alone); Cur: curcumin. B: Western analysis of p65 and inhibitors of NF-κBα (IκB) in the cytosol (C) and the nucleus (N). ACTB: β-Actin, TBP: TATA-box-binding protein.*

*Effects on NF-κB transactivation.* Immunomodulatory analogs and bortezomib (19) are known to inhibit NF-κB transactivation, and are approved therapeutic agents for MM. In response to various signals, NF-κB released from IκB anchoring, migrates into the nucleus and activates target genes. Nuclear translocation of p65 was examined using immunofluorescence microscopy of RPMI8226 cells (Figure 3A). p65 was translocated to the nucleus in the presence of tumor necrosis factor α (TNFα) (10 ng/ml). However, 2 μM of GO-Y030 and GO-Y078, as well as 20 μM of curcumin, inhibited the translocation. In the absence of TNFα, IκBα was expressed in the cytosol, but in its presence, the amount of IκBα in the cytosol decreased to 21.5% (Figure 3B). Treatment with curcumin analogs inhibited the decrease in IκBα expression dose-dependently: 10 μM GO-Y030 recovered the expression to 36.9% that of the control, and 10 μM GO-Y078 to 63.8%. Curcumin did not restore expression, even at 30 μM.

In the nucleus, a 36.0-fold higher amount of p65 (p65nuc) was detected in the presence of TNFα. In the presence of TNFα, the analogs suppressed p65nuc expression dose-dependently, 10 μM GO-Y030 reduced p65nuc expression to 31.6% that of the control, and 10 μM GO-Y078 reduced it to 34.4%. On the other hand, curcumin did not reduce p65nuc expression even at 30 μM. The $IC_{50}$ of GO-Y030 for p65nuc was 1.72 μM, and that of GO-Y078 was 4.71 μM. However, inhibition with curcumin did not reach 50% of the control, even at 30 μM. These observations indicate that GO-Y030 and GO-Y078 are at least 6.4-fold stronger inhibitors of NF-κB than curcumin.

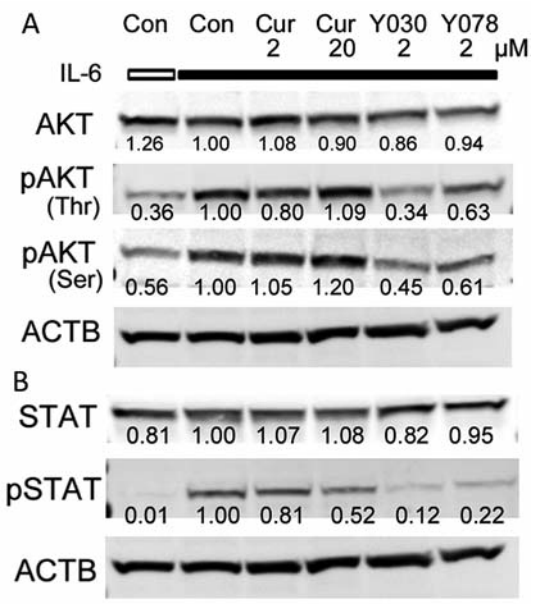


Figure 4. *Effects of curcumin analogs on the phosphoinositide 3-kinase/AKT (PI3K/AKT) and Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathways. OPM2 cells were pretreated with curcuminoids, and then interleukin-6 (IL-6) was added (solid bar) or not (open bar). After 10 min treatment, cells were harvested. The relative band intensity is indicated below the band. Con: Control (DMSO alone), Cur: curcumin, Y030: GO-Y030, Y078: GO-Y078. A: PI3K/AKT pathway. B: JAK/STAT3 pathway.*

*Effects on the PI3K/AKT pathway*. Phosphorylation of the threonine residue at codon 308 (pAKT (Thr 308)) and the serine residue at codon 473 (pAKT (Ser 473)) are essential to activate AKT (20). IL-6 can activate AKT in OPM2 cells. In the presence of IL-6, 2.8-fold higher pAKT (Thr 308) and 1.8-fold higher pAKT (Ser 473) expressions were observed compared to in the absence of IL-6 (Figure 4A). Curcumin did not suppress pAKTs at either residue at 20 μM, whereas GO-Y030 suppressed the levels of both pAKT (Thr 308) and pAKT (Ser 473) to 33.8% and 45.3% that of the control, respectively, at 2 μM (Figure 4). GO-Y078 suppressed the levels of pAKT (Thr 308) to 62.9% and pAKT (Ser 473) to 61.2% of the control, respectively, at 2 μM (Figure 4A). GO-Y030 and GO-Y078 are at least 10-fold stronger inhibitors of pAKTs than curcumin.

*Effects on the JAK/STAT3 pathway*. IL-6 can activate JAK, which subsequently phosphorylates STAT3 at tyrosine residue 705 (pSTAT3) and thus, activates it (21). The presence of IL-6 induced a 172.8-fold increase of pSTAT3 in OPM2 cells, compared to in the absence of IL-6 (Figure 4B). Curcumin dose-dependently suppressed the level of pSTAT3 to 81.4% and 52.4% of the control at 2 μM and 20 μM, respectively. GO-Y030 and GO-Y078 suppressed the level of pSTAT3 to 12.5% and 21.6% of the control at 2 μM, respectively (Figure 4B). GO-Y030 and GO-Y078 are considerably 10-fold stronger inhibitors of pSTAT3 than curcumin.

*Effects on the IRF4 pathway*. The effects of curcuminoids on the IRF4 pathway were examined. Treatment with 10 μM curcumin reduced the expression of IRF4 to 34.6% that of the control in KMS12-BM cells (Figure 5A). GO-Y030 and GO-Y078 also reduced the expression of IRF4 dose-dependently, but more efficiently than did curcumin (Figure 5A). The $IC_{50}$ of IRF4 expression was 9.69 μM with curcumin treatment, but was 0.65 μM with GO-Y030, and 0.51 μM with GO-Y078. MYC is transcriptionally regulated by IRF4 (4), and curcumin reduced MYC expression dose-dependently, and its $IC_{50}$ value was 9.87 μM (Figure 5A). The $IC_{90}$ values for MYC expression after GO-Y030 and GO-Y078 treatments were 1.05 μM and 0.71 μM, respectively. B lymphocyte-induced maturation protein 1 (BLIMP1) is also transcriptionally regulated by IRF4 (22). Curcumin reduced BLIMP1 expression dose-dependently; the $IC_{50}$ value was 9.64 μM (Figure 5A). The $IC_{50}$ values after GO-Y030 and GO-Y078 treatments were 1.63 μM and 0.85 μM, respectively.

Thereafter, we examined the effect of GO-Y030 and GO-Y078 on the expression of other target genes of IRF4 (5), such as cyclin-dependent kinase 6 (*CDK6*), stromal antigen 2 (*STAG2*), cyclin C (*CCNC*), *SUB2* gene, homolog of the human splicing factor hUAP56, squalene epoxidase (*SQLE*), and stearoyl-CoA desaturase (*SCD*) genes as well as *c-MYC* and *BLIMP1* by reverse transcription-PCR (RT-PCR) of KMS12-BM cells. Curcumin at 2 μM did not have any effect on the number of transcripts; however, 20 μM curcumin suppressed the transcripts for *IRF4, BLIMP1, c-MYC, CDK6, STAG2, CCNC, SUB2, SQLE* and *SCD* to 66.7, 65.8, 61.5, 67.9, 87.3, 71.3, 74.7, 68.8, and 62.6% that of the control, respectively. At 2 μM, GO-Y030 suppressed the number of transcripts to 53.1, 53.6, 47.2, 42.9, 77.6, 55.5, 67.0, 74.3, and 60.2% that of the control, respectively, while GO-Y078 suppressed them to 36.5, 62.9, 59.1, 62.7, 111.6, 78.9, 81.2, 64.5, and 37.8% that of the control, respectively. The transcriptional levels of *IRF4, BLIMP1, c-MYC, CDK6*, and *SCD* were suppressed with 20 μM curcumin, as well as with 2 μM GO-Y030 and GO-Y078 (Figure 5B).

*Effect on IL-6 production*. IL-6 is secreted from BMCs, and a major growth factor of MM cells (22). A minority of MM cell lines, including U266, produces IL-6 by an autocrine mechanism (Figure 6). In U266 cells, the basal level of IL-6 production in the medium was 564.05±15.19 mg/ml, whereas the amount decreased dose-dependently following curcumin treatment. The $IC_{50}$ value of curcumin for IL-6 production was 10.21 μM. GO-Y030 did not considerably reduce IL-6 production, however, the $IC_{50}$ value of GO-Y078 for IL-6 production was 0.73 μM. Thus, GO-Y078 is a more potent inhibitor of IL-6 production.

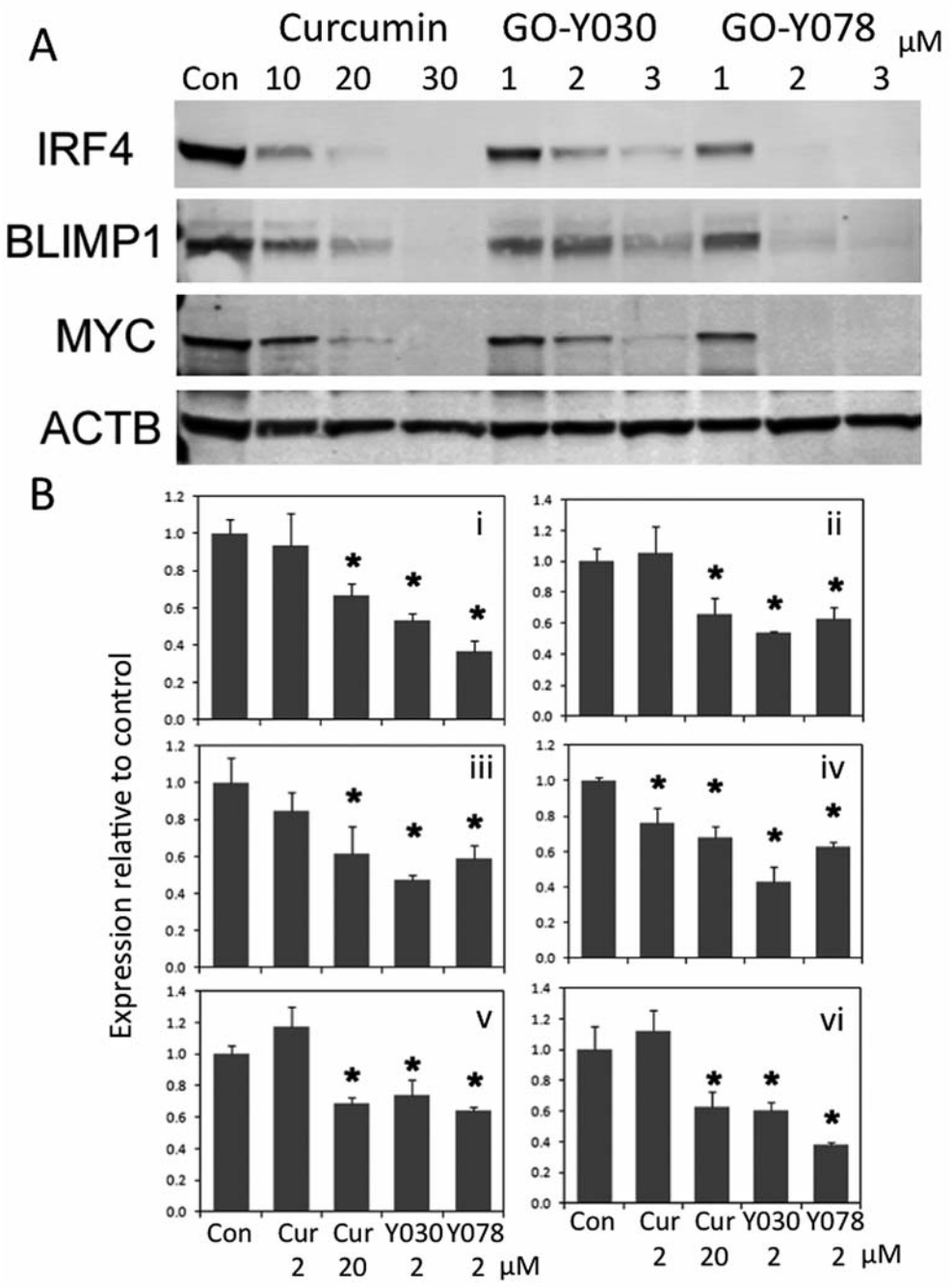


Figure 5. *Effects of curcumin analogs on the IRF4 pathway. A: Western blot analysis of interferon regulatory factor 4 (IRF4), B lymphocyte-induced maturation protein 1 (BLIMP1), and c-MYC. B: Quantitative RT-PCR analyses of IRF4 and its transcripts: (i) IRF4, (ii) BLIMP1, (iii) c-MYC, (iv) cyclin-dependent kinase 6 (CDK6), (v) squalene epoxidase (SQLE), (vi) stearoyl-CoA desaturase (SCD). The asterisk indicates a significant difference between the control (Con, DMSO alone) and each treatment.*

## Discussion

The results of clinical trials determining the clinical applicability of curcumin for MM seem to indicate it is not useful. This is mainly attributed to the low bioavailability of curcumin. We have developed 69 species of diarylpentanoid analogs, and assayed their antitumor abilities; *in silico* absorption, distribution, metabolism and elimination (ADME) analysis was conducted simultaneously (23). GO-Y030 was one of the most effective growth suppressants, and GO-Y078

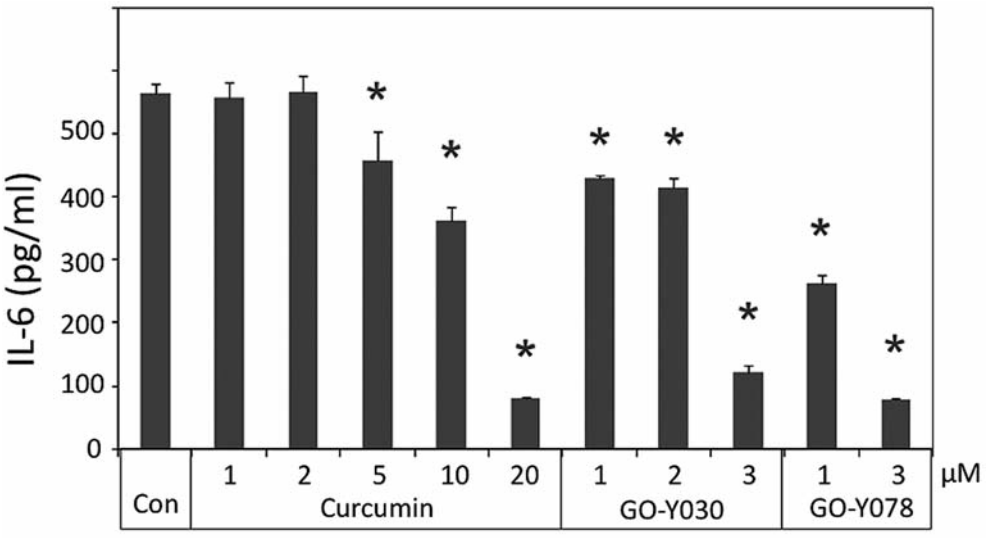


Figure 6. *Effect of curcumin analogs on interleukin-6 (IL-6) production. The asterisk indicates a significant difference between the control (Con, DMSO alone) and each treatment.*

had the additional characteristics of improved solubility and effectiveness in a mouse model of gastric cancer (23). Single-targeted agents cannot overcome the redundancy of signaling networks. Therefore the multitargeted potency of curcumin cannot be readily abandoned. In this study, multitarget inhibition of the analogs was shown by their enhanced activities in MM cells. Down-regulation of *IRF4* expression is also promising because knockdown of as low as 50% of *IRF4* alone can kill MM cells effectively (5); complete suppression of IRF4 expression may result in severe immunodeficiency. While the amount of IRF4 in heterozygous $IRF4^{+/-}$ mouse was half of that of the wild type littermates, the amount of Ig was comparable to the wild type (24). Although 50% knockdown of IRF4 is lethal to myeloma cells, it has no adverse effect on immunity. Moreover, as the toxicity of GO-Y030 and GO-Y078 against normal hepatocytes is low (23), there is room for combination therapy with the cytotoxic agents. Although the efficacy and safety of GO-Y030 and GO-Y078 *in vivo* remain to be elucidated, the present study suggests that the new curcumin analogs GO-Y030 and GO-Y078 can offer potential leads for the development of therapeutic agents against MM. As the attachment group modification of each aromatic ring could improve both physical and pharmacological properties of analogs, it is worth exploring to synthesize the analogs carrying various attached groups.

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## References

1 Munshi NC and Anderson KC: Plasma cell neoplasms. *In*: DeVita Cancer, 8th edition. Vol 2. DeVita VT Jr., Lawrence TS, Rosenberg SA (eds.). Philadelphia, Lippincott Williams & Wilkins, pp. 2305-2342, 2008.

2 Kumar SK, Rajkumar SV, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, Zeldenrust SR, Dingli D, Russell SJ, Lust JA, Greipp PR, Kyle RA and Gertz MA: Improved survival in multiple myeloma and the impact of novel therapies. Blood *111*: 2516-2520, 2008.

3 Klein U, Casola S, Cattoretti G. Shen Q, Lia M, Mo T, Ludwig T, Rajewsky K and Dalla-Favera R: Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. Nat Immunol *7*: 773-782, 2006.

4 Shaffer AL, Emre NC, Romesser PB and Staudt LM: IRF4: Immunity. Malignancy! Therapy? Clin Cancer Res *15*: 2954-2961, 2009.

5 Shaffer AL, Emre NC, Lamy L, Ngo VN, Wright G, Xiao W, Powell J, Dave S, Yu X, Zhao H, Zeng Y, Chen B, Epstein J and Staudt LM: IRF4 addiction in multiple myeloma. Nature *454*: 226-231, 2008.

6 Goel A, Kunnumakkara AB and Aggarwal BB: Curcumin as 'Curecumin': from kitchen to clinic. Biochem Pharmacol *75*: 787-809, 2008.

7 Singh S and Aggarwal BB: Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane). J Biol Chem *270*: 24995-25000. 1995.

8 Jaiswal AS, Marlow BP, Gupta N and Narayan S: Beta-catenin-mediated transactivation and cell–cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells. Oncogene *21*: 8414-8127, 2002.

9 Hong RL, Spohn WH and Hung MC: Curcumin inhibits tyrosine kinase activity of p185neu and also depletes p185neu. Clin Cancer Res *5*: 1884-1891, 1999.

10 Yang X, Thomas DP, Zhang X, Culver BW, Alexander BM, Murdoch WJ, Rao MN, Tulis DA, Ren J and Sreejayan N: Curcumin inhibits platelet-derived growth factor-stimulated vascular smooth muscle cell function and injury-induced neointima formation. Arterioscler Thromb Vasc Biol *26*: 85-90, 2006.

11 Mohan R, Sivak J, Ashton P, Russo LA, Pham BQ, Kasahara N, Raizman MB and Fini ME: Curcuminoids inhibit the angiogenic response stimulated by fibroblast growth factor-2, including expression of matrix metalloproteinase gelatinase B. J Biol Chem *275*: 10405-10412, 2000.

12 Menon LG, Kuttan R and Kuttan G: Anti-metastatic activity of curcumin and catechin. Cancer Lett *141*: 159-165, 1999.

13 Hahm ER, Gho YS, Park S, Park C, Kim KW and Yang CH: Synthetic curcumin analogs inhibit activator protein-1 transcription and tumor-induced angiogenesis. Biochem Biophys Res Commun *321*: 337-344, 2004.

14 Bharti AC, Donato N, Singh S and Aggarwal BB: Curcumin (diferuloylmethane) down-regulates the constitutive activation of nuclear factor-kappa B and Ikappa B alpha kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis. Blood *101*: 1053-1062, 2003.

15 Ohori H, Yamakoshi H, Tomizawa M, Shibuya M, Kakudo Y, Takahashi A, Takahashi S, Kato S, Suzuki T, Ishioka C, Iwabuchi Y and Shibata H: Synthesis and biological analysis of new curcumin analogues bearing an enhanced potential for the medicinal treatment of cancer. Mol Cancer Ther *5*: 2563-2571, 2006.

16 Yamakoshi H, Ohori H, Kudo C, Sato A, Kanoh N, Ishioka C, Shibata H and Iwabuchi Y: Structure–activity relationship of C5-curcuminoids and synthesis of their molecular probes thereof. Bioorg Med Chem *18*: 1083-1092, 2010.

17 Sato A, Kudo C, Yamakoshi H, Uehara Y, Ohori H, Ishioka C, Iwabuchi Y and Shibata H: Curcumin analog GO-Y030 is a novel inhibitor of IKKβ that suppresses NF-κB signaling and induces apoptosis. Cancer Sci *102*: 1045-1051, 2011.

18 Lombardi L, Poretti G, Mattioli M, Fabris S, Agnelli L, Bicciato S, Kwee I, Rinaldi A, Ronchetti D, Verdelli D, Lambertenghi-Deliliers G, Bertoni F and Neri A: Molecular characterization of human multiple myeloma cell lines by integrative genomics: insights into the biology of the disease. Genes Chromosomes Cancer *46*: 226-238, 2007.

19 Hideshima T, Chauhan D, Richardson P, Mitsiades C, Mitsiades N, Hayashi T, Munshi N, Dang L, Castro A, Palombella V, Adams J and Anderson KC: NF-kappa B as a therapeutic target in multiple myeloma. J Biol Chem *277*: 16639-16647, 2002.

20 Cicenas J: The potential role of AKT phosphorylation in human cancers. Int J Biol Markers *23*: 1-9, 2008.

21 Aggarwal BB, Kunnumakkara AB, Harikumar KB, Gupta SR, Tharakan ST, Koca C, Dey S and Sung B: Signal transducer and activator of transcription-3, inflammation, and cancer: How intimate is the relationship?. Ann NY Acad Sci *1171*: 59-76, 2009.

22 Lauta VM: A review of the cytokine network in multiple myeloma: diagnostic, prognostic, and therapeutic implications. Cancer *97*: 2440-2452, 2003.

23 Kudo C, Yamakoshi H, Sato A, Nanjo H, Ohori H, Ishioka C, Iwabuchi Y and Shibata H: Synthesis of 86 species of 1,5-diaryl-3-oxo-1,4-pentadienes analogs of curcumin can yield a good lead *in vivo*. MC Pharmacol *11*: 4, 2011.

24 Mittrücker HW, Matsuyama T, Grossman A, Kündig TM, Potter J, Shahinian A, Wakeham A, Patterson B, Ohashi PS and Mak TW: Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. Science *275*: 540-543, 1997.

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