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## Chapter VII

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# Challenges in Establishing Potent Cancer Chemotherapy Using Newly Synthesized 1,5-Diaryl-3-Oxo-1,4-Pentadiene Analogs of Curcumin

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## Abstract

Curcumin is a dietary pigment that imparts yellow color to turmeric. It has been used as a traditional medicine, mainly in the South Asian countries. Very recently, curcumin has been revealed to reregulate overexpression of cancer-related molecules, such as NF- $\kappa$ B signaling molecules, molecules controlling tumor cell growth, cell cycle regulators, anti-apoptotic molecules, and molecules associated with invasion, metastasis, and angiogenesis, primarily by their degradation. Low toxicity of curcumin has a potential advantage, particularly in combination with other cytotoxic agents. Multitargeted potential of this compound can overcome problems concerning redundancy of cancer biology. However, the achievements made in clinical studies of curcumin have not been successful and promising. Considering these points, I attempted to improve the potency of curcumin. My approach was to develop 1,5-diaryl-3-oxo-1,4-pentadiene (DOP) analogs of curcumin that possess enhanced potency to control and kill cancer cells at much lower concentrations. This strategy has been implemented since the discovery of GO-035, one of the DOP analogs of curcumin, in the newly synthesized organic compound library of Tohoku University, Department of Organic Chemistry. This compound bears 4 times stronger growth suppressive potential than curcumin. Since then, we began to develop and screen DOP analogs of curcumin. In total, 86 species of DOP analogs have been synthesized as leads. Among them, analogs named GO-Y030 and GO-Y078 have the most enhanced potency to suppress tumor cell growth in a cell panel

composed of 16 types of cancer cells. The most enhanced growth suppressive potency of these analogs reached to >80 times higher than that of curcumin. The growth suppressive potency has been exerted through molecular reregulation including c-Myc, KRAS, cyclin D1, ErbB2,  $\beta$ -catenin, COX-2, NF- $\kappa$ B, IRF-4, BLIMP1, CDK6, IL-6, survivin, Bcl-2, Bcl-XL, Notch1, Notch3, STAT3, and AKT. They also exhibit at least 10 times higher apoptosis induction than curcumin. They can induce apoptosis-related molecules including caspases, PARP, TP53, XIAP, and DR5 toward proapoptosis more efficiently than curcumin. They also exhibit improved potential of angiogenesis inhibition and anti-invasion. One of the reasons for poor bioavailability of curcumin is believed to be its hydrophobicity. Among the new leads, the solubility of GO-Y078 is 2 times higher than that of curcumin, and the solubility of GO-Y038 has been predicted to be approximately 20 times higher than that of curcumin. Enhancement of antitumor ability, enhancement of solubility, or both could improve *in vivo* efficiency. In fact, using GO-Y030 or GO-Y078, *in vivo* efficacies have been confirmed in mouse models of familial adenomatous polyposis and cancerous ascites of gastric malignancy as well as in colonic cancer stem tumors implanted in mice. These results encourage the development of new cancer treatment protocols using newly synthesized DOP analogs. GO-Y038 also has an increased antitrypanosomal activity.

## Introduction

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is a turmeric derivative that is extracted from the ground rhizome of the herb *Curcuma longa*. Turmeric is a spice that gives flavor and color to foods. It is a traditional medicine described in ancient Ayurveda and has been used for hepatobiliary disorders, inflammation, wound healing, anorexia, and other conditions [1]. The pharmacological effects of this compound are diverse and include apoptotic, anti-proliferative, anti-oxidant, and anti-angiogenic effects [2]. It has chemotherapeutic and chemopreventive effect on various cancers. Recent studies indicate that curcumin can inhibit a number of molecules responsible for malignant phenotypes. For example, nuclear factor  $\kappa$ B (NF- $\kappa$ B), which acts as a transcriptional regulator of genes involved in cellular proliferation and survival, is inhibited by curcumin [3]. c-Myc and COX2 are transcriptionally regulated by NF- $\kappa$ B. Bcl-2 and Bcl-XL are anti-apoptotic proteins that are regulated by NF- $\kappa$ B, and curcumin induces growth suppression and apoptosis by downregulating these proteins. Curcumin can inhibit other transcriptional regulators, such as activator protein-1 and  $\beta$ -catenin in Wnt signaling pathways, responsible for cellular growth, carcinogenesis, and anti-apoptosis [4, 5]. Curcumin also suppresses growth factors such as epidermal growth factor receptors and cytokine receptors [6, 7]. Curcumin inhibits signal transducers such as signal transducer and activator of transcription (STAT) and mitogen-activated protein kinase [8, 9]. Curcumin directly inhibits apoptosis-related molecules such as caspase-3 and death receptor 5 (DR5) [10, 11]. Curcumin can arrest cell cycle at G0-G1 and G2-M through the upregulation of the cyclin-dependent kinase inhibitors p21 and p27, and downregulation of Cdc2 and cyclinB1 [12]. Furthermore, curcumin inhibits tumor invasion and angiogenesis by reducing the activities of matrix metalloproteinases (MMPs) and vascular endothelial growth factor [13, 14]. These molecular pharmacological features label curcumin as multi-targeted compound useful for cancer chemotherapy. In addition, curcumin has proven chemopreventive effects against various cancers in model systems, and it also improves the efficacy of chemotherapy and radiotherapy.

Curcumin has a variety of tumor suppressive potencies *in vitro*. Various clinical trials have been planned and conducted for colorectal, oral, pancreatic, gall bladder, cervical cancers, multiple myeloma, osteosarcoma, and the precancerous lesions [15]. Although many of these trials are still ongoing, the outcomes of these studies are far from satisfactory and even disappointing. For example, a phase I/II study of combination therapy with curcumin and gemcitabine for gemcitabine-resistant pancreatic cancer reported a median survival time of 161 days [16]. This result corresponds with other results [17]. In these studies, the response rate ranged from 5 to 9%, and the disease control rate ranged from 30 to 45%. Regarding the safety of curcumin, it was reported that the oral administration of 8 g/day curcumin was tolerable; however, the serum concentration of curcumin was approximately 50 ng/ml, which was observed in other studies as well [16, 17]. This observation indicated that the main cause of the ineffectiveness of curcumin was its low bioavailability. To overcome this problem, many curcumin derivatives have been utilized. Two of these derivatives are nanocurcumin and liposome-encapsulated curcumin. The strategy we adopted was to synthesize a curcumin analog with enhanced potency and improved watery solubility.

## Screening of Newly Synthesized Curcumin Analogs

First, we screened the library in the Department of Organic Chemistry, Tohoku University, which is composed of over 2000 species of newly synthesized organic compounds, and found that GO-035 has a 4-fold higher growth-suppressive ability for colorectal cancer cells than curcumin (Figure 1). GO-035 is a diarylpentanoid type, 1,5-diaryl-3-oxo-1,4-pentadiene (DOP) analog of curcumin [18]. GO-035 has the potential to degrade  $\beta$ -catenin similar to curcumin.

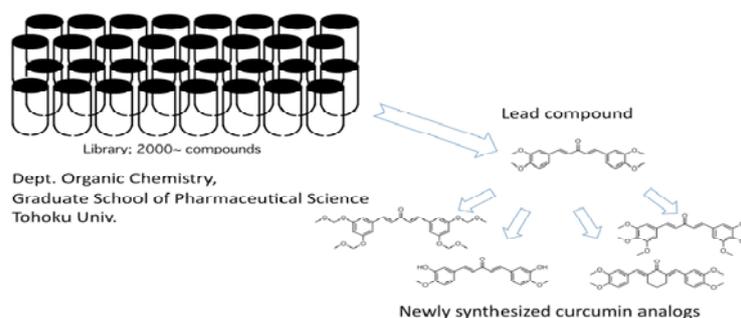


Figure 1. Developmental steps of aDOP analogs. GO-035 (also called GO-Y014) was first screened in the organic compound library of Tohoku University, composed of 2,000 newly synthesized compounds. aDOP analogs are synthesized from GO035.

During earlier investigations, various types of analogs, including curcumin-type analogs, possessing a 7-carbon spacer between the aryl rings (diarylheptanoid type) and cyclic DOP-type analogs possessing a central cyclic ketone structure were also examined. In this process, it was demonstrated that the  $\alpha$ ,  $\beta$ -unsaturated ketone moiety is necessary for the enhancement of growth-suppressive potential. The acyclic DOP (aDOP) type of curcumin analog is

superior to the other analogs in this regard (Figure 1, 2) [18, 19]. We decided to synthesize an aDOP analog to enhance the growth-suppressive ability of curcumin.

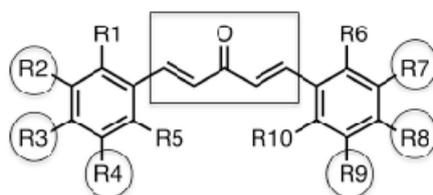


Figure 2. Schematic presentation of acyclic diarylpentanoid type analog (aDOP) of curcumin. Bis(arylmethylidene) acetone structure is depicted in the rectangle.

## Growth-Suppressive Potential of Analogs

The growth-suppressive potential of 88 newly synthesized analogs including 69 DOPs were screened on a cancer cell panel composed of 16 types of cancer cell lines derived from colon, stomach, lung, pancreas, kidney, liver, breast, ovary, gall duct, prostate, thyroid, and skin cancers and melanoma. The half-maximal inhibitory concentration ( $IC_{50}$ ) was calculated in each cell line [20]. According to the clustering analysis, a majority of DOP analogs belonged to the cluster with higher growth-inhibitory ability. Among these analogs, GO-Y030 and GO-Y078 had the highest growth-inhibitory abilities. The  $IC_{50}$  values ranged from 0.19 to 7.71  $\mu$ M, which were at least 10-fold higher compared with that of curcumin, and the maximal  $IC_{50}$  value was 84-fold higher compared with that of curcumin. These analogs were outsourced and analyzed using another cancer cell panel, and our results were reconfirmed.

A comparison of GO-Y030 and GO-Y031 with currently used cytotoxic agents such as 5-fluorouracil (5-FU), cisplatin (CDDP), and irinotecan (CPT-11) was conducted [18]. The  $IC_{50}$  values of the analogs in HCT-116, DLD-1, SW620 (colorectal cancers), GCIY, SH10TC (stomach cancers), MCF7 (breast cancer), OVK18 (ovarian cancer), PC9 (pancreatic cancer), 8505c (thyroid cancer), G361 (melanoma), ACNH (renal cancer), and HepG2 (liver cancer) cells ranged from one-sixth to one-eightieth of that of 5-FU. The  $IC_{50}$  values of the analogs in MCF7, HuCCT1 (cholangiocarcinoma), and 8505c cells ranged from one-eighteenth to one-sixtieth of that of CDDP. The  $IC_{50}$  values of the analogs in DLD-1, SH10TC, MCF7, and 8505c cells ranged from one-tenth to one-thirty-third of that of CPT-11. These analogs have significantly stronger growth-suppressive abilities than commonly used chemotherapeutic drugs.

The structure–activity relationships of the DOP analogs indicated that the structural characters described below could enhance the growth-suppressive potential of compounds (Figure 2) [19]. (1) Bis(arylmethylidene) acetone is the most potent skeleton for eliciting cytotoxicity. (2) The 3-oxo-1,4-pentadiene structure is essential for enhancing cytotoxicity. (3) Hexasubstituted analogs exhibit stronger activities, and in particular 3,4,5-hexasubstitution resulted in the strongest activity. Another study indicated that para positions are allowed to introduce additional functional groups, such as molecular probes or groups conferring watery solubility.

## Abilities of the Analogs to Control the Molecules Responsible for Malignant Phenotypes

### 1. Growth-Related Molecules

As described above, curcumin can control the disorders in growth-related molecules. We examined the effects of curcumin analogs on these molecules, which include transcriptional factors, such as NF- $\kappa$ B,  $\beta$ -catenin, and STAT3, and their targets, such as c-Myc, cyclinD1, and COX2, which confer hyperproliferative ability to malignant cells. The relative values for the inhibition of tumor necrosis factor  $\alpha$ -induced NF- $\kappa$ B transactivation by GO-Y030 and GO-Y078 at 2  $\mu$ M in SW620 cells were 0.16 and 0.26, respectively, compared with 0.32 for curcumin [20]. GO-Y030 can also suppress NF- $\kappa$ B transactivation with at least 10-fold stronger potency than curcumin in PK1 cells, a pancreatic cancer cell line, HuCCT-1 cells, a cholangiocarcinoma cell line, and 8505c cells, a thyroid cancer cell line [21]. NF- $\kappa$ B transactivation is inhibited through its sequestration by inhibitor of NF- $\kappa$ B (I $\kappa$ B) in the cytoplasm. The phosphorylation of I $\kappa$ B by I $\kappa$ B kinase (IKK) complex results in the degradation of I $\kappa$ B, the release of NF- $\kappa$ B from I $\kappa$ B, and its translocation to the nucleus. IKK $\beta$ , a component of the IKK complex, is a major kinase of I $\kappa$ B. It was indicated that curcumin and GO-Y030 directly inhibit the kinase activity of IKK $\beta$  in a cell-free system. The inhibitory potency of GO-Y030 on the kinase activity of IKK $\beta$  is 13.3-fold stronger compared with that of curcumin.

Concerning  $\beta$ -catenin degradation, the effect of 2.5  $\mu$ M GO-Y030 is comparative to that of 20  $\mu$ M curcumin in HCT116 cells.  $\beta$ -catenin degradation by curcumin and GO-Y030 was completely inhibited by the caspase3/8 inhibitor N-CBZ-ASP-GLU-VAL-ASP fluoromethyl ketone (Z-DEVD-fmk) [18]. This reaction is considered to be caspase-3/8-dependent.

STAT3 activation occurs through phosphorylation at tyrosine residue 705 (pSTAT3-Y705) in the SH2 domain contributing to homodimerization [22]. pSTAT3 dimerizes and translocates to the nucleus [23]. Although curcumin does not have any effect on pSTAT3-Y705 at 20  $\mu$ M, 1  $\mu$ M GO-Y030 can reduce the amount of pSTAT3-Y705 to undetectable levels in Panc-1 cells, a pancreatic cancer cell line. Computational binding analysis indicated that GO-Y030 occupies pSTAT3-Y705 more efficiently than curcumin. Furthermore, the transcriptional activity of STAT3 was suppressed below 10% of the control activity by 5  $\mu$ M GO-Y030 in MDA-MB-231 cells, a breast cancer cell line, whereas it was suppressed merely to 80% by curcumin at the same concentration [23].

The resultant effects of curcumin analogs on transcription are described below. c-Myc is transcriptionally regulated by various factors, including NF- $\kappa$ B and  $\beta$ -catenin, and the expression of c-Myc is downregulated to 50% of the control level by 2.5  $\mu$ M GO-Y030 and 20  $\mu$ M curcumin in HCT116 cells [18]. CyclinD1 is transcriptionally regulated by various factors including  $\beta$ -catenin and STAT3; the expression of CyclinD1 is downregulated to 30% of the control level by 2.5  $\mu$ M GO-Y030 in HCT116 cells, whereas it is only downregulated to 80% by 20  $\mu$ M curcumin [18]. COX2 is transcriptionally regulated by NF- $\kappa$ B and other factors. The IC<sub>50</sub> value of GO-Y030 regarding COX2 expression is 1.0  $\mu$ M in PK1 cells, whereas that of curcumin is 20  $\mu$ M [21]. Notch-1 and Notch-3 are also transcriptionally regulated by STAT3. The Notch pathway is involved in cancer stem cell proliferation including hematological and mammary malignancies [22]. The expression of Notch-1 and

Notch-3 was suppressed by GO-Y030 in DLD-1, HCT-116 cells, and SW480 cells, an aldehyde dehydrogenase 1 (ALDH)- and CD133-positive (ALDH<sup>+</sup>/CD133<sup>+</sup>) colorectal cancer cell line. ALDH and CD133 are the markers of cancer stem cells [22]. GO-Y030 can suppress the expression of HER2 to undetectable levels at 2.5  $\mu$ M in HCT-116 cells [18]. It was observed that GO-Y030 reduced the expression of both threonine 308- and serine 473-phosphorylated AKT phosphorylated (pAKT) to below 50% of the control level at 2  $\mu$ M in multiple myeloma cells, whereas 20  $\mu$ M curcumin did not have this effect [24]. Because it is believed that cancer stem cells are developed through Notch, Hedgehog, Wnt, HER2, and AKT pathways, these observations suggest that the curcumin analogs are considered as potential candidates for therapeutic agents against cancer stem cells. The effects of GO-Y078 on NF- $\kappa$ B and pAKT were also observed in myeloma cells [24].

GO-Y030 and GO-Y031, the other aDOP type analog, can affect the cell cycle progression of HCT-116 cells more efficiently than curcumin. Only 15 and 10% of cell fractions progressed into S phase after treatment with 2  $\mu$ M GO-Y030 and 2  $\mu$ M GO-Y031, respectively, whereas approximately 40% of cells progressed into S phase in control and 20  $\mu$ M curcumin-treated cells [18]. These values are reflected by the growth-suppressive potential of the analogs. The fraction arrested at the G<sub>2</sub>/M border was 2-fold higher in GO-Y030- and GO-Y031-treated cells compared with that in control cells, for which the G<sub>2</sub>/M fraction was 17%. Moreover, the sub-G<sub>1</sub> fractions in the cells treated with GO-Y030 and GO-Y031 at 2  $\mu$ M were increased to 20 and 26%, respectively, whereas those in the control and 20  $\mu$ M curcumin-treated cells were 5 and 3%, respectively [18]. GO-Y030 and GO-Y031 appear capable of inducing apoptosis; the details are described in the next section. Regarding the direct effect of the analogs on cell cycle regulators, the expression of cyclin-dependent kinase 6 (CDK6), which participates in G<sub>1</sub> phase progression and G<sub>1</sub>/S transition, was suppressed more efficiently by GO-Y030 and GO-Y078 in multiple myeloma cells than by curcumin [24]. Moreover, phosphorylation of the RB protein is essential for G<sub>1</sub>/S transition, and GO-Y030 could reduce the amount of serine 780-phosphorylated RB to 20 and 5% in HCT-116 and SW48 cells, respectively [22].

We also examined the effects of the analogs on other transcriptional factors, such as interferon regulatory factor 4 (IRF-4), and its targets, such as c-Myc and CDK-6, in multiple myeloma cells [24]. GO-Y030 and GO-Y078 more efficiently suppressed the expression of IRF-4 and its targets than curcumin.

KRAS is a proto-oncogene that is mutated at high rates in colorectal, pancreatic, and lung cancers. KRAS acts as a signal transducer that is located between growth factor receptors and downstream transducers such as c-RAF and PI 3-kinase [25, 26]. GO-Y030 reduced the expression of KRAS in HCT-116 cells, and the addition of Z-DEVD-fmk, an inhibitor of caspase, blocked the  $\beta$ -catenin degradation and KRAS reduction induced by GO-Y030 as well as curcumin. Some of the protein degradation effects of the curcumin analogs appear to be caspase-3/8-dependent [18].

GO-Y078 can inhibit kinase activities of the down-stream molecules of platelet-derived growth factor (PDGF) receptor including phosphorylated AKT, S6 ribosomal protein, Extracellular Signal-regulated kinase (ERK), Phospholipase C $\gamma$ 1, and protein kinase D, and the amount of phosphotyrosine in normal rat kidney fibroblasts (NRK cells) stimulated with PDGF to > 80 %, at 10  $\mu$ M.

As described above, aDOP analogs can reregulate the growth-related molecules (Figure 4).

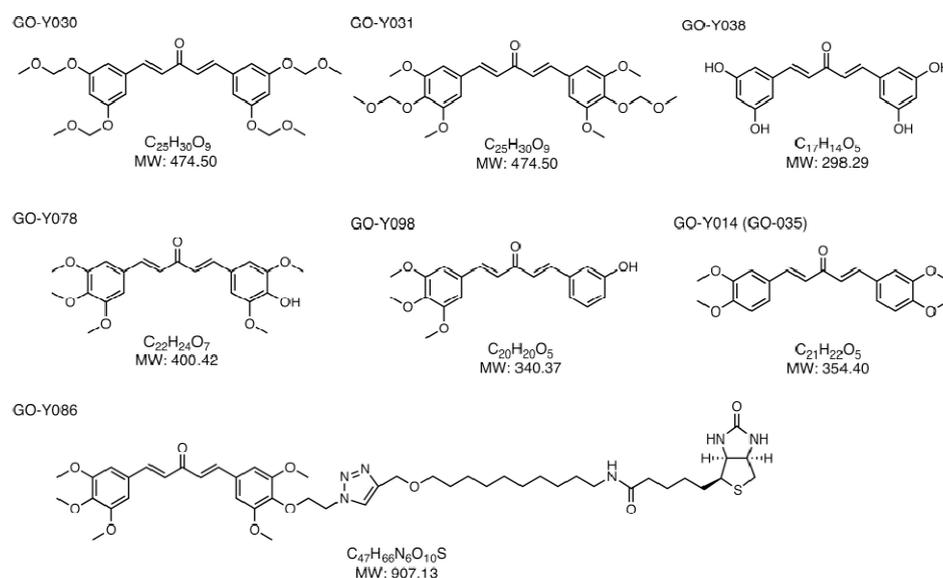


Figure 3. Chemical structures of aDOP analogs. Physical properties, and molecular formulas of the analogs are represented.

## 2. Apoptosis-Related Molecules

Curcumin analogs can induce apoptosis more efficiently than curcumin. From the viewpoint of the sub- $G_1$  fraction, GO-Y030 and GO-Y031 at 2  $\mu$ M increased the sub- $G_1$  fraction to 20 and 26%, respectively, in HCT-116 cells, whereas the sub- $G_1$  fraction in 20  $\mu$ M curcumin-treated cells was only 3%, similar to the background level [18]. In PK-1, HuCCT-1, and 8505c cells, 3  $\mu$ M GO-Y030 increased the sub- $G_1$  fraction to 20.4, 11.7, and 14.5%, respectively, whereas the sub- $G_1$  fraction in 30  $\mu$ M curcumin-treated cells was 4.6, 0.8, and 12.6%, respectively [21]. GO-Y078 and GO-Y098 at 2  $\mu$ M increased the sub- $G_1$  fraction to 17.1 and 19.8% in HCT-116, respectively, whereas 2  $\mu$ M curcumin increased it to 4.5% [20]. GO-Y030 increased the apoptotic fraction from 5 to 39% at 5  $\mu$ M in ALDH<sup>+</sup>/CD133<sup>+</sup> SW480 colon cancer stem cells [22]. As cancer stem cells are generally resistant to chemotherapeutic agents, analogs could improve the efficacy of presently used chemotherapeutics.

After treatment with 30  $\mu$ M curcumin, apoptotic nuclei were observed in 28, 21, and 22% of 8505c, PK-1, and HuCCT-1 cells, respectively, and after treatment with 3  $\mu$ M GO-Y030 in the same three cell lines, apoptotic nuclei were observed with Hoechst 33258 dye in 33, 48, and 30% of cells, respectively [21].

Caspase-3, one of the main executors of the apoptotic process, was apparently induced by GO-Y030 at 2.5  $\mu$ M in HCT-116 cells and at 5.0  $\mu$ M in SW480 and HT-29 cells (human colorectal cancer cell line), respectively [27]. Conversely, caspase-3 was induced in HCT-116 cells by 20  $\mu$ M curcumin and faintly induced in SW480 cells by 20  $\mu$ M curcumin, whereas it was not induced in HT-29 cells even by 20  $\mu$ M curcumin. Caspase-3 activity was measured using a caspase-3 substrate, N-acetyl-ASP-GLU-VAL-ASP-7-amido-4-methylcoumarin. The activities of GO-Y030 and GO-Y031 at 2.5  $\mu$ M were 81.5 and 67.3 arbitrary units/min/mg

protein in HCT-116 cells, respectively, which were 1.6- and 1.3-fold higher than the values observed for 20  $\mu\text{M}$  curcumin [18]. The relative activities of caspase-3/7 activity in the presence of 3  $\mu\text{M}$  GO-Y030 were 8.0, 4.8, and 8.9 in 8505c, PK-1, and HuCCT-1 cells, respectively [21]. These values were 1.0-, 1.5-, and 1.9-fold higher, respectively, than that in the presence of 30  $\mu\text{M}$  curcumin. The caspase activities induced by GO-Y030, GO-Y078, and GO-Y098 at 2  $\mu\text{M}$ , as measured by the cleavage of cytokeratin 18, were 2.1-, 3.5-, and 1.7-fold higher, respectively, than that of curcumin in HCT-116 cells. Caspase activity, as measured by the cleavage of poly (ADP-ribose) polymerase, was detected in the presence of 2.5  $\mu\text{M}$  GO-Y030 in MDA-MB-231, PANC-1, and HPAC cells (human pancreatic cancer cell line); however, no caspase activity was detected in the presence of 20  $\mu\text{M}$  curcumin. Similar results were obtained for BXPC-3 cells, a human pancreatic cancer cell line [23]. The apoptosis-inducing abilities of aDOP analogs, such as GO-Y030, GO-Y031, GO-Y078, and GO-Y098, were at least 2-fold higher than that of curcumin.

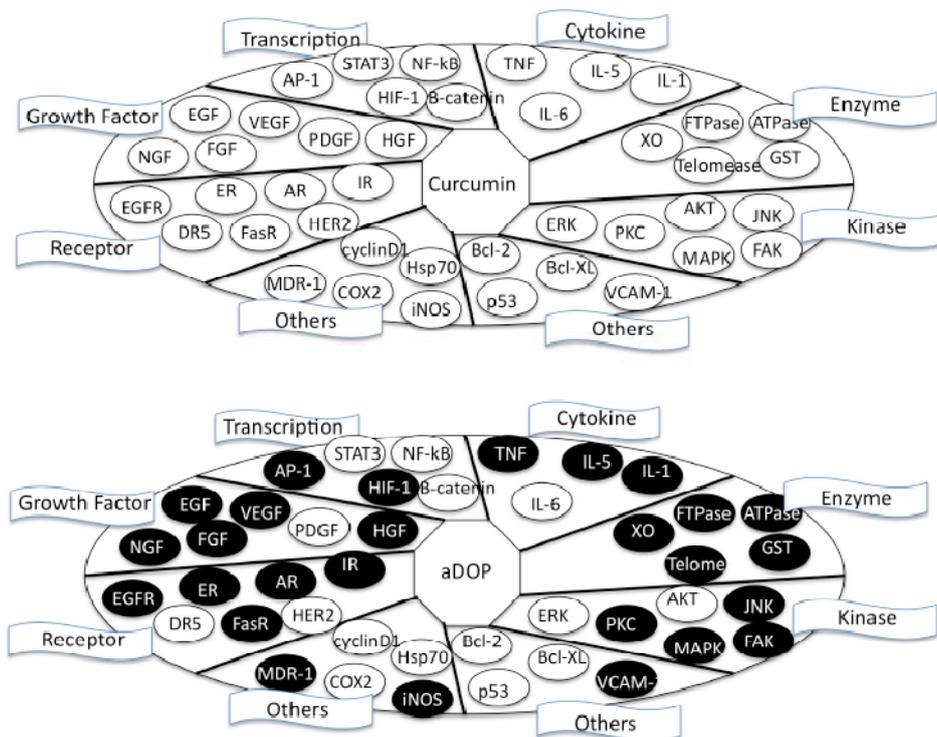


Figure 4. Molecular targets of curcumin and aDOP analogs. The molecules are represented by open circles, those are confirmed to be reregulated with analogs in our experiments.

The underlying mechanisms were examined at the molecular level. Bcl-2 and Bcl-XL are two anti-apoptotic proteins that control mitochondrial membrane permeability [28]. Microarray examination indicated that 2.5  $\mu\text{M}$  GO-Y031 reduced the expression of Bcl-2 to <10 % of the control level in HCT-116 cells [18]. The mRNA expression of Bcl-2 and Bcl-XL was reduced by 5  $\mu\text{M}$  GO-Y030 in ALDH<sup>+</sup>/CD133<sup>+</sup> SW480, HCT-116, and DLD-1 cells [22]. These products are transcriptionally regulated by STAT 3 [29, 30]. X-linked inhibitor of apoptosis (XIAP), which belongs to the inhibitor of apoptosis (IAP) family, acts as an anti-

apoptotic protein that binds and inhibits caspase-3, 7, and 9 [31]. XIAP is transcriptionally regulated by NF- $\kappa$ B as well as Wnt signaling [32, 33]. The IC<sub>50</sub> value of GO-Y030 for XIAP expression was 2  $\mu$ M in PK-1 cells, whereas that of curcumin was >20  $\mu$ M [21]. Survivin is a member of the IAP family that interacts with caspase-3 and 7 and acts as an anti-apoptotic protein [34]. Survivin is transcriptionally regulated by STAT3 and Wnt pathways [35, 36]. The mRNA expression of survivin was suppressed by 5  $\mu$ M GO-Y030 in ALDH<sup>+</sup>/CD133<sup>+</sup> SW480, HCT-116, and DLD-1 cells [22]. The p53 gene is mutated in nearly half of cancers and results in the loss of induction of pro-apoptotic genes [37]. In HCT116 cells, GO-Y030 and GO-Y031 at 2.5  $\mu$ M upregulated TP53 expression by 3.0- and 2.5-fold, respectively, compared with the control expression, whereas 20  $\mu$ M curcumin upregulated TP53 expression by 3.5-fold [18]. In another experiment, GO-Y030, GO-Y078, and GO-Y098 at 2.0  $\mu$ M upregulated TP53 expression in HCT116 cells by 2.3-, 1.5-, and 1.5-fold, respectively, compared to the control expression, whereas TP53 expression was similar to the control level in the presence of 2  $\mu$ M curcumin [20]. The activation of death receptors (DR), particularly DR5, can selectively kill cancer cells through p53-dependent or independent mechanisms [38], and curcumin has been demonstrated to activate DR5 [39]. In DLD-1 cells lacking the normal p53 gene, GO-Y030, GO-Y078, and GO-Y098 at 2.0  $\mu$ M upregulated DR5 expression by 3.6-, 6.2-, and 2.4-fold, respectively, compared to the control expression, whereas DR5 expression was 0.9- and 1.6-fold higher than the control expression in the presence of 2.0  $\mu$ M and 20  $\mu$ M curcumin, respectively. [20] Curcumin analogs have enhanced abilities to downregulate anti-apoptotic proteins and upregulate pro-apoptotic proteins.

As described above, aDOP analogs can reregulate the apoptosis-related molecules (Figure 4).

### 3. Other Functions Related to Malignancy

The colony formation assay is one method of evaluating the malignant phenotypes of cancers. GO-Y030 at 1  $\mu$ M reduced the colony formation of MDA-MB-231 cells to 5% of the control level, whereas 1  $\mu$ M curcumin did not reduce colony formation, although 5  $\mu$ M curcumin reduced colony formation to 41% of the control level [23]. Using GO-Y086, another aDOP analog possessing the biotinylated linker, KSRP/FUBP2, a nuclear protein was screened to bind the aDOP analog [40]. GO-Y086 reduced c-Myc expression, and KSRP/FUBP2 silencing also resulted in the reduction of c-Myc expression. Moreover, KSRP/FUBP2 participates in the regulation of miRNA expression. These findings suggested that the analogs have some regulatory mechanisms through KSRP/FUBP2 and/or miRNA [41]. Preliminarily, we examined the abilities of the analogs to inhibit tumor angiogenesis and tumor invasion. GO-Y078 inhibits the sprouting of human umbilical vein endothelial cells, and its potency is comparable to that of sunitinib, an already approved angiogenesis inhibitor (unpublished data). MMPs play an important role in tissue remodeling processes such as angiogenesis and tumor invasion. GO-Y030 and GO-Y078 can inhibit the expression of MMP2 and MMP9 and their functions in HT1080 cells, a human sarcoma cell line, more efficiently than curcumin (unpublished data). GO-Y030 and GO-Y078 can also suppress the wound healing of HT1080 cells more efficiently than curcumin (unpublished data).

As described above, aDOP analogs can reregulate the other molecules related to malignancy (Figure 4).

## Improvement of the *In Vivo* Efficacy of the Analogs

### 1. Solubility

As described above, curcumin has low bioavailability and efficacy in the clinical setting. The hydrophobicity of curcumin is one of the reasons for this issue. Cancer is a systemic disease and drug delivery through blood flow is very important for medicinal treatment. Although hydrophobicity may be an important characteristic for effective topical use, such as for the treatment of tumors arising from the gastrointestinal lumen or skin, it is not ideal for systemic use. For these reasons, we consider the enhanced solubility of curcumin analogs to be important. The absorption, distribution, metabolism, and excretion (ADME) analysis of 100 curcumin analogs including virtual ones was conducted *in silico* [20]. The predicted solubility of aDOP analogs exhibiting the highest growth-suppressive potential was varied. For example, the predicted solubilities of GO-Y030, GO-Y078, and GO-Y098 were 0.26, 1.07, and 1.58 mg/L, respectively, whereas that of curcumin was 0.54 mg/L [20]. Although GO-Y030 and curcumin dissolved in dimethyl sulfoxide cannot maintain their solubility after serial dilution with phosphate-buffered saline, GO-Y078 and GO-Y098 can do so. These phenomena are compatible with the results of ADME. No survival benefit was observed for GO-Y030 in a peritonitis carcinomatosa (PC) mouse model with gastric cancer, whereas GO-Y078 first exhibited a significant survival benefit in this PC model with only two times administrations (Table) [20]. This result indicated that a simultaneous improvement in both growth-suppressive potential and solubility can improve the bioavailability of curcumin analogs. Other aDOP analogs with both higher growth-suppressive potential and higher solubility were elucidated by the ADME analysis, such as GO-Y022, GO-Y023, and GO-Y026 [20]. Among them, the predicted solubility of GO-Y038 was as high as 11.7 mg/L, which was 10- and 20-fold higher compared with that of GO-Y078 and curcumin, respectively [20]. These analogs may be good candidates to be developed as systemic agents for cancer treatment.

### 2. Confirmation of Efficacy and Safety in Mouse Model

The *in vivo* efficacy of GO-Y030 was examined in immunodeficient mice inoculated with ALDH<sup>+</sup>/CD133<sup>+</sup> SW480 or HCT-116 cells. GO-Y030 was administered daily by intraperitoneal (IP) injection at a tidal dosage of 50 mg/kg. The reduction of tumor volume in GO-Y030-treated mice was significant and the reduction rate was 58% (Table) [22]. As described above, in the PC model inoculated with GCIY cells, GO-Y030 was administered by IP injection once a week for 2 weeks at a tidal dosage of 155 mg/kg [20]. In this model, GO-Y030 also exhibited an anti-tumor effect against PC but a survival benefit was not achieved (Table). The total dosage per week administered to the mice inoculated with colon cancer stem cells was 2-fold higher compared with that in the mice inoculated with GCIY cells. The former mice did not exhibit any adverse events such as weight loss.

**Table. *In vivo* efficacies of aDOP analogs**

Analog	Route	Dose	Tumor Type	Effect (% of control)
GO-Y030	IP	50 mg/kg, D	Colon ca stem	42 %↓ (tumor size)
GO-Y030	IP	155 mg/kg, W	Gastric ca, PC	100 %↓ (ascites)
GO-Y078	IP	133-266 mg/kg, W	Gastric ca, PC	91 %↓ (ascites) 40 %↑ (survival time)
GO-Y030	oral	250 mg/kg, D	Intestinal adenoma	39 %↓ (tumor number) 23 %↑ (survival time)
GO-Y030	oral	1250 mg/kg, D	Intestinal adenoma	93 %↓ (tumor number)

The downwards arrow (↓) indicates shrinkage or reduction, and the upwards arrow (↑) indicates elongation. D; daily, W; weekly administration

We also examined the *in vivo* efficacy of GO-Y030 by using a familial adenomatous polyposis (FAP) mouse model,  $Apc^{580D/+}$  [42]. In this experiment, verification of the chemopreventive potential of GO-Y030 against adenomatous polyposis formation and its therapeutic potential to suppress tumor growth were conducted (Table).  $Apc^{580D/+}$  mice were fed diets supplemented with 0.1% (w/w) curcumin or GO-Y030, and the daily loading dose was estimated to be roughly 5 mg per head, corresponding to 250 mg/kg in a 20-g mouse [41]. The average multiplicity of adenoma in  $Apc^{580D/+}$  mice fed a diet containing 0.1% GO-Y030 was significantly reduced to 60.0 compared with 98.8 in  $Apc^{580D/+}$  mice fed a basal diet. The median survival time of  $Apc^{580D/+}$  fed a diet containing 0.1% GO-Y030 was prolonged to 214.5 days compared with 166.5 days in  $Apc^{580D/+}$  mice fed a basal diet. Oral administration of 0.1% GO-Y030 can prevent intestinal adenoma formation in FAP model mice. Treatment with 0.1% GO-Y030 for 2 months had no adverse effects including liver and renal dysfunction. The ability of 0.5% GO-Y030 (1250 mg/kg) to shrink adenoma was examined (Table). Oral administration of 0.5% GO-Y030 for 10 days reduced the number and size of the adenomas in the proximal jejunum of  $Apc^{580D/+}$  mice without apparent adverse events. The number of adenomas was 21.7 in  $Apc^{580D/+}$  mice fed a diet containing 0.5% GO-Y030, whereas the number was 43 in the mice fed a basal diet. In  $Apc^{580D/+}$  mice fed a diet containing 0.5% GO-Y030, only 5% of adenomas had diameters >2 mm (the number corresponds to 1.1), whereas 38% of adenomas in  $Apc^{580D/+}$  mice (the number corresponds to 16.3) fed a basal diet were >2 mm in diameter. These data indicate that oral administration of 0.5% GO-Y030 can shrink intestinal adenomas *in vivo*.

Although further examination is required, it is obvious that the curcumin analogs exhibiting enhanced anti-tumor potential, such as GO-Y030, may be effective for topical use against surface tumors such as intestinal tumors. Moreover, the analogs exhibiting improved solubility such as GO-Y078 may be effective for systemic use against advanced cancers.

### 3. Safety and Toxicity of Newly Synthesized Analogs

Validation of the safety of the newly synthesized curcumin analogs is still ongoing, as these issues require further examination. Certain issues concerning safety and toxicity are described above and the other observations are described below.

GO-Y030, GO-078, GO-Y098, and GO-035 had no suppressive or harmful effect on a human primary hepatocyte, hNHeps, at 50–100  $\mu\text{M}$  [18, 20]. However, the  $\text{IC}_{50}$  of GO-Y030 for WI-38 cells, a human lung fibroblast line, was rather low at 13.8  $\mu\text{M}$  [27].

The maximum tolerable dose (MTD) was determined by a single IP injection in mice. The MTDs for IP-injected GO-Y030 and GO-Y078 were  $>474.5$  mg/kg and  $>400$  mg/kg, respectively [20]. However, the MTD of GO-Y098 was  $>204$  mg/kg [20]. The toxicities of these analogs may be higher than that of curcumin. These issues must be considered and cautiously examined.

## Antitrypanosomal Activity

Curcumin can suppress the growth of trypanosoma, a unicellular protozoa that causes African trypanosomiasis, also known as sleeping sickness. African trypanosomiasis is transmitted by the tsetse fly and was responsible for 48,000 deaths in 2008 [43]. The  $\text{IC}_{50}$  values of seven curcumin analogs were evaluated using the *Trypanosoma brucei brucei* GUTat 3.1 strain (TBB-3.1) that causes an animal form of African trypanosomiasis known as nagana [44]. Along with this evaluation, the corresponding  $\text{IC}_{50}$  value against MRC-5 cells, a human fetal lung fibroblast line, was estimated, and each selective index (SI) was calculated as a ratio of  $\text{IC}_{50}$  values against MRC-5 cells divided by that of TBB-3.1. The  $\text{IC}_{50}$  values of the analogs against TBB-3.1 ranged from 0.078 to 1.32  $\mu\text{g/ml}$ , whereas that of curcumin was 0.66  $\mu\text{g/ml}$ . The SIs of the curcumin analogs ranged from 16.3 to 65.3, whereas the SI of curcumin was 5.0. The approved drugs for the first and stages of African trypanosomiasis are suramin and eflornithine, respectively. The  $\text{IC}_{50}$  values of suramin and eflornithine were 1.58 and 2.27  $\mu\text{g/ml}$ , respectively, and the SIs values of suramin and eflornithine were  $>63$  and  $>44$ , respectively. All SIs of the analogs ( $>10$ ) met the criteria of Drugs for Neglected Diseases initiative according to the guideline released by the World Health Organization. Among them, GO-Y038, with  $\text{IC}_{50}$  and SI values of 0.078  $\mu\text{g/ml}$  and 65.3, respectively, is the most promising.

In addition, we are examining the protective potential of curcumin analogs against ultraviolet or ionizing radiation as well as their chemosensitizing effects.

## Conclusion

Newly synthesized aDOP curcumin analogs, particularly GO-Y078 and GO-Y038, have promising anti-tumor potential and solubility, indicating their potential systemic use. Using animal models, various *in vivo* examinations concerning the safety, efficacy, pharmacokinetics, and pharmacodynamics of these analogs should be accelerated. Proof of concept research is also required to clarify the complete mechanism of action of curcumin

analogs and to explore the direction of further analog development. In addition to the diseases described above, these analogs could be effective against psoriasis, Crohn's disease, ulcerative colitis, and other diseases. The efficacy of curcumin analogs against these diseases is also worth being explored.

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## References

- [1] Singh S. (2007). From exotic spice to modern drug? *Cell*. 130, 765-768.
- [2] Steward WP., Gescher AJ. (2008). Curcumin in cancer *Mol. Nutr. Food Res.* 52, 1005-1009.
- [3] Singh S., Aggarwal BB. (1995) Activation of transcription factor NF- $\kappa$ B is suppressed by curcumin (diferuloylmethane). *J. Biol. Chem.* 270, 24995–25000.
- [4] Surh YJ., Han SS., Keum YS., Seo HJ., Lee SS. (2000). Inhibitory effects of curcumin and capsaicin on phorbol ester-induced activation of eukaryotic transcription factors, NF- $\kappa$ B and AP-1. *Biofactors*. 1, 107–112.
- [5] Jaiswal AS., Marlow BP., Gupta N., Narayan S. (2002).  $\beta$ -Catenin-mediated transactivation and cell-cell adhesion pathways are important in curcumin (diferuloylmethane)-induced growth arrest and apoptosis in colon cancer cells. *Oncogene*. 21, 8414–8427.
- [6] Lee JY., Lee YM., Chang GC., et al. (2011). Curcumin Induces EGFR Degradation in Lung Adenocarcinoma and Modulates p38 Activation in Intestine: The Versatile Adjuvant for Gefitinib Therapy. *PLoS One*. 6, e23756.
- [7] Ravindran J., Prasad S., Aggarwal BB. (2009). Curcumin and cancer *AAPS J.* 11, 495-510.
- [8] Bill MA., Fuchs JR., Li C., et al. (2010). The small molecule curcumin *Mol. Cancer*. 9, 165.
- [9] Binion DG., Otterson MF., Rafiee P. (2008). Curcumin inhibits VEGF-mediated angiogenesis *Gut*. 57, 1509-1517.
- [10] Lu HF., Lai KC., Hsu SC., et al. (2009). Curcumin induces apoptosis *Oncol. Rep.*, 22, 97-104.
- [11] Jung EM., Lim JH., Lee TJ., et al. (2005). Curcumin sensitizes tumor *Carcinogenesis*. 26, 1905-1913.
- [12] Park MJ, Kim EH, Park IC et al. (2002). Curcumin inhibits cell cycle progression of immortalized human umbilical vein endothelial (ECV304) cells by upregulating cyclin-

- dependent kinase inhibitor, p21WAF1 / CIP1, p27KIP1 and p53. *Int. J. Oncol.*, 21, 379–383.
- [13] Lin HJ., Su CC., Lu HF., et al. (2010) Curcumin blocks migration *Oncol. Rep.*, 23, 665-670.
- [14] Lin SS., Lai KC., Hsu SC., et al. (2009). Curcumin inhibits the migration *Cancer Lett.*, 285, 127-133.
- [15] Shehzad A., Wahid F., Lee YS. (2010) Curcumin in cancer chemoprevention: molecular targets, pharmacokinetics, bioavailability, and clinical trials. *Arch. Pharm. Chem. Life Sci.*, 000, 1-11.
- [16] Kanai M., Yoshimura K., Asada M., et al. (2011). A phase I/II study of gemcitabine-based chemotherapy *Cancer Chemother. Pharmacol.*, 68, 157-164.
- [17] Dhillon N., Aggarwal BB., Newman RA., et al. (2008). Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin. Cancer Res.*, 14, 4491-4499.
- [18] Ohori H., Yamakoshi H., Tomizawa M., et al. (2006). Synthesis and biological analysis of new curcumin analogues bearing an enhanced potential for the medicinal treatment of cancer. *Mol. Cancer Ther.*, 5, 2563–2571.
- [19] Yamakoshi H., Ohori H., Kudo C., et al. (2010). Structure–activity relationship of C5-curcuminoids and synthesis of their molecular probes thereof. *Bioorganic & Medicinal Chemistry*, 18, 1083–1092.
- [20] Kudo C., Yamakoshi H., Sato A., et al. (2011). Synthesis of 86 species of 1,5-diaryl-3-oxo-1,4-pentadienes analogs of curcumin can yield a good lead in vivo. *BMC Pharmacology*, 11, 4
- [21] Sato A., Kudo C., Yamakoshi H., et al. (2011). Curcumin analog GO-Y030 is a novel inhibitor of IKK $\beta$  that suppresses NF- $\kappa$ B signaling and induces apoptosis. *Cancer Sci.*, 102, 1045–1051.
- [22] Lin L., Liu Y., Li H., et al. (2011). Targeting colon *Br. J. Cancer*. 105, 212-220.
- [23] Hutzen B., Friedman L., Sobo M., et al. (2009). Curcumin analogue GO-Y030 inhibits STAT3 activity and cell growth *Int. J. Oncol.*, 35, 867-872.
- [24] Kudo C., Yamakoshi H., Sato A., et al. (2011). GO-Y030 and GO-Y078, are multitargeted agents with enhanced abilities for multiple myeloma. *Anticancer Research*, 31, 3719-3729.
- [25] Takezawa K., Okamoto I., Yonesaka K., et al. (2009). Sorafenib inhibits non-small cell lung cancer *Cancer Res.*, 69, 6515-6521.
- [26] Castellano E., Downward J. (2011). RAS Interaction with PI3K *Genes Cancer.*, 2, 261-274.
- [27] Cen L., Hutzen B., Ball S., et al. (2009). New structural analogues of curcumin exhibit potent growth suppressive activity in human colorectal carcinoma cells. *BMC Cancer*, 9, 99.
- [28] Kim R. (2005). Unknotting the roles of Bcl-2 and Bcl-xL in cell death *Biochem. Biophys. Res. Commun.*, 333, 336-343.
- [29] Choi HJ., Lee JH., Park SY., et al. (2009). STAT3 is involved in phosphatidic acid *Exp. Mol. Med.*, 41, 94-101.
- [30] Grad JM., Zeng XR., Boise LH. (2000). Regulation of Bcl-xL: a little bit of this and a little bit of STAT. *Curr. Opin. Oncol.*, 12, 543-549.
- [31] Gyrd-Hansen M., Meier P. (2010). IAPs: from caspase inhibitors to modulators of NF- $\kappa$ B, inflammation *Nat. Rev. Cancer*. 10, 561-574.

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- [32] Jin HS., Lee DH., Kim DH., et al. (2009). cIAP1, cIAP2, and XIAP act cooperatively via nonredundant pathways *Cancer Res.*, 69, 1782-1791.
- [33] Wang XH, Sun X, Meng XW, et al. (2010).  $\beta$ -catenin siRNA *Oncol. Rep.* 24, 1093-1099.
- [34] Mita AC., Mita MM., Nawrocki ST., et al. (2008). Survivin: key regulator of mitosis *Clin. Cancer Res.*, 14, 5000-5005.
- [35] Aggarwal BB, Kunnammakara AB, Harikumar KB, et al. (2009). Signal transducer *Ann. N. Y. Acad. Sci.* 1171, 59-76.
- [36] Shan BE., Wang MX., Li RQ. (2009). Quercetin inhibit human *Cancer Invest.* 27, 604-612.
- [37] Soussi T, Wiman KG. (2007). Shaping genetic alteration *Cancer Cell.* 12, 30.
- [38] Elrod HA., Sun SY. (2008). Modulation of death receptors by cancer therapeutic agents. *Cancer Biol. Ther.*, 7, 163-173.
- [39] Jung EM., Lim JH., Lee TJ., et al. (2005). Curcumin sensitizes tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis through reactive oxygen species-mediated upregulation of death receptor 5 (DR5). *Carcinogenesis*, 26, 1905-1913.
- [40] Yamakoshi H., Kanoh N., Kudo G., et al. (2010). KSRP/FUBP2 is a binding protein of GO-Y086, a cytotoxic curcumin analogue. *ACS Med. Chem. Lett.* 1, 273-276.
- [41] Trabucchi M., Briata P., Filipowicz W., et al. (2011). KSRP Promotes the Maturation of a Group of miRNA Precursors. *Adv. Exp. Med. Biol.* 700, 36-42.
- [42] Shibata H., Yamakoshi H., Sato A., et al. (2009). Newly synthesized curcumin *Cancer Sci.*, 100, 956-960.
- [43] Boseley S. New treatments raise hope of cutting sleeping sickness deaths. 2009 May 15, Available from <http://www.guardian>
- [44] Newson J., Mahan SM., Black SJ. (1990). Synthesis and secretion *Parasite Immunol.* 12, 125-139.