J. Microbiol. Biotechnol. (2017), 27(9), 1639–1648
https://doi.org/10.4014/jmb.1701.01054

jmb

Synthesis of Curcumin Glycosides with Enhanced Anticancer Properties Using One-Pot Multienzyme Glycosylation Technique S

Rit Bahadur Gurung[1], So Youn Gong[2], Dipesh Dhakal[1], Tuoi Thi Le[1], Na Rae Jung[1], Hye Jin Jung[1,2], Tae Jin Oh[1,2], and Jae Kyung Sohng[1,2]*

[1]*Department of Life Science and Biochemical Engineering, Sun Moon University, Asan 31460, Republic of Korea*
[2]*Department of BT-Convergent Pharmaceutical Engineering, Sun Moon University, Asan 31460, Republic of Korea*

Received: January 19, 2017
Revised: May 30, 2017
Accepted: June 19, 2017

First published online
June 21, 2017

**Corresponding author*
Phone: +82-41-530-2246;
Fax: +82-41-530-8229;
E-mail: sohng@sunmoon.ac.kr

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

Copyright© 2017 by
The Korean Society for Microbiology and Biotechnology

Curcumin is a natural polyphenolic compound, widely acclaimed for its antioxidant, anti-inflammatory, antibacterial, and anticancerous properties. However, its use has been limited due to its low-aqueous solubility and poor bioavailability, rapid clearance, and low cellular uptake. In order to assess the effect of glycosylation on the pharmacological properties of curcumin, one-pot multienzyme (OPME) chemoenzymatic glycosylation reactions with UDP-α-D-glucose or UDP-α-D-2-deoxyglucose as donor substrate were employed. The result indicated significant conversion of curcumin to its glycosylated derivatives: curcumin 4′-*O*-β-glucoside, curcumin 4′,4′′-di-*O*-β-glucoside, curcumin 4′-*O*-β-2-deoxyglucoside, and curcumin 4′,4′′-di-*O*-β-2-deoxyglucoside. The products were characterized by ultra-fast performance liquid chromatography, high-resolution quadruple-time-of-flight electrospray ionization-mass spectrometry, and NMR analyses. All the products showed improved water solubility and comparable antibacterial activities. Additionally, the curcumin 4′-*O*-β-glucoside and curcumin 4′-*O*-β-2-deoxyglucoside showed enhanced anticancer activities compared with the parent aglycone and diglycoside derivatives. This result indicates that glycosylation can be an effective approach for enhancing the pharmaceutical properties of different natural products, such as curcumin.

Keywords: Curcumin glucosides, one-pot multienzyme glycosylation, biological activity

Introduction

Curcumin (diferuloylmethane) and related compounds are bioactive polyphenolic phytochemical compounds generally isolated from the rhizome of *Curcuma longa* L. (turmeric). It has been used as a spice, food additive, Ayurvedic, and Chinese traditional medicine in Southeast Asia for thousands of years [1]. Several studies have revealed that curcumin has promising pharmacological activities, such as antioxidant [2], antibacterial [3], antimalarial [4], anti-inflammatory [5], antiviral [6], antitumor [7, 8], antiallergic [9], hepatoprotective [10], renoprotective [11], hypocholesterolemic [12], and other effects [1]. Recently, the synergistic effects of curcumin in combination with other molecules (drugs) were also studied [13–16]. Much attention has been directed towards the study of the therapeutic applications of curcumin in the treatment of human cancers, since curcumin was shown to have encouraging antitumor and apoptotic activities in a wide range of human cancer cell lines [7, 8, 17–19, 23]. In particular, methoxy groups on the phenyl rings have been reported to play a critical role as the relative efficacy for suppression of tumor necrosis factor (TNF)-induced nuclear factor-kappaB (NF-κB) activation. This activity was found to be in the order of curcumin > demethoxycurcumin > bisdemethoxycurcumin [20]. Reports also suggested that the number of *ortho*-methoxy substitutions and the level of hydrogenation of the heptadiene moiety of curcumin is directly associated with the radical scavenging potential of cucuminoids [20, 21]. Moreover, the high anti-inflammatory and antitumor potentials of curcuminoids are associated with their low level of hydrogenation and high level of

methoxylation, and also to the high level of unsaturation of the diketone moiety [22].

Although curcumin possesses an exceptional pharmacodynamic profile, its use has been limited by an extremely low aqueous solubility and poor bioavailability, rapid clearance, and low cellular uptake [23]. Besides this, curcuminoids are unstable at physiological and alkaline pH values [24]. Several approaches were undertaken to overcome these limitations [25, 26]. The new curcumin analogs were synthesized to improve the pharmacological profile of the natural compound [27]. Among several available techniques, glycosylation is one approach that generates compounds with greater water solubilities and/or enhanced biological activities [28, 29]. Kaminaga *et al.* [30] reported an enhancement in water solubility by 2×10^7 times when curcumin was glycosylated. The one-pot multienzyme (OPME) approaches are often employed for the synthesis of diverse kinds of glycoconjugates. Our group has successfully developed and applied the efficient OPME systems for the synthesis of glycosides of α-mangostin [28], nargenicin A(1) [31], and resvera-A [32]. Such methods are more efficient and cheaper than the chemical methods, which involve several protection/deprotection steps before any product can be obtained. In this case, the target products can be achieved without isolation or purification of intermediates. In this study, we have applied OPME systems of two different types, catalyzed by six different enzymes [28], for the synthesis of curcumin glycosides (Fig. 1). The literature review showed that enzymatic synthesis of curcumin glucosides had been carried out by utilizing various other genes [30, 33].

The synthesized curcumin glycosides were characterized and tested for antibacterial activities against four representative bacteria. The same compounds were also tested against selected cancer cell lines using the standard MTT assay method.

Materials and Methods

Chemicals and Reagents

2-Deoxy-D-glucose was purchased from Carbosynth Ltd. (UK). Glucose 1-phosphate, acetyl phosphate, uridine monophosphate (UMP), adenine triphosphate (ATP), and isopropyl-β-D-thiogalactopyranoside (IPTG) were obtained from GeneChem Inc. (Korea). Standard curcumin (98% purity) was purchased from Tokyo Chemical Industry Co. Ltd., Japan. Ampicillin, kanamycin, and dimethyl sulfoxide-d6 (DMSO-d_6) were procured from Sigma-Aldrich (USA). High-performance liquid chromatography (HPLC)-grade water, methanol, and acetonitrile were acquired from Avantor Performance Materials (USA).

Enzymatic Synthesis of Curcumin Glycosides

Two OPME systems were applied with 3–5 mM of curcumin for the synthesis of glycosides. The procedure for preparation and components of the reactions are referenced by Le *et al.* [28].

Analytical Methods

The reaction samples were analyzed on an ultra-fast performance liquid chromatography-diode array detector (UFLC-DAD) (CBM-20A; Shimadzu Systems, Japan) equipped with a reversed-phase column (Mightysil RP-18 GP 250 × 4.6 mm, (5 μm); Kanto Chemical,

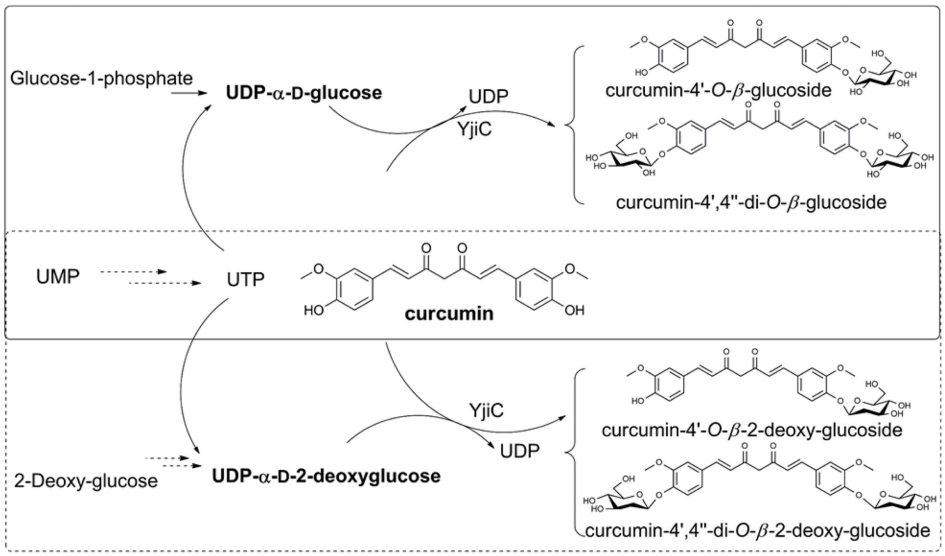

Fig. 1. Schematic diagram representing one-pot multienzyme reactions for the synthesis of curcumin glucosides (rectangle in solid line) and curcumin 2-deoxyglucosides (rectangle in dotted line).

Japan) and a diode array detector (SPD-M20A; Shimadzu Systems). The column was eluted at a flow rate of 1.0 ml/min by water (0.01% TFA) as eluent A and 100% acetonitrile as eluent B at 30°C. The effluent was monitored with a UV detector at 425 nm. The high-resolution quadruple-time-of-flight electrospray ionization-mass spectrometry (HR-QTOF ESI/MS) analysis was performed in positive-ion mode using an ACQUITY system (UPLC, Waters Corp., USA) coupled with an SYNAPT G2-Si (Water Corp.) column. The purification of compounds was performed by a preparative HPLC (UltiMate 3000 UHPLC; Thermo Scientific, USA) system coupled with ODS column (YMC-Pack-ODS-AQ-HG; 250 × 20 mm I.D., S-10 μm, 12 nm) and RS variable wavelength detector (Dionex UltiMate 3000; Thermo Scientific). The purified curcumin analogs were evaporated, lyophilized, and dissolved in DMSO-d_6 and further subjected to nuclear magnetic resonance (NMR) analysis (Bruker Biospin, USA) for complete characterization.

Solubility Test

In order to assess the solubility of curcumin and its glycosides, 100 mg of test compounds was dissolved in 1 ml of phosphate-buffered saline (PBS) solution at pH 7.4 followed by vortexing for 30 min and centrifuging at 11,400 ×*g* for 15 min at room temperature. Then, aliquots were filtered through a 0.45 μm syringe filter, diluted 5 times with methanol, and analyzed by UFLC at 425 nm. The concentration of curcumin and its glycosides in PBS solution was determined by the regression equation.

Antibacterial Test

The minimal inhibitory concentration (MIC) test of curcumin and its glycosylated derivatives was recorded against gram-positive bacteria *Staphylococcus aureus* subsp. *aureus* KCTC 1916 and *Bacillus subtilis* KACC17047, and gram-negative bacteria *Pseudomonas aeruginosa* KACC10232 and *Enterobacter cloaceae* subsp. *disolvens* KACC 13002, by using the microdilution broth method with some modifications in Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard: ninth edition," CLSI Documents M07-A9. CLSI, Wayne, PA, USA, 2012). Each of the compounds was serially diluted by 2-fold in 96-well sterile microplates containing Mueller-Hinton broth (MHB). Each of the test bacterial suspension was inoculated in each well to give a final concentration of 10^4 CFU. Each of the tested compounds for antibacterial activities ranged from 2,500 to 19.52 μg/ml adjusted by a series of 2-fold dilutions. The inhibition of growth was demonstrated by optical density at 600 nm using a microplate reader (Bio-Rad, USA) after 24h incubation at 37°C. Considering the total growth (100%) in the control well (MHB + bacteria), the percentage of growth reduction was attributed on the remaining wells. A control solution containing DMSO and sterile water that was used for dissolving curcumin and its glycosides, respectively, was included in this experiment to exclude the possibility of toxic effects on the microorganisms. Each experiment was conducted in triplicates and the MIC was reported as the lowest concentration of curcumin and its glycosides that inhibited the bacterial growth after 24 h of incubation at 37°C.

Evaluation of Anticancer Properties

AGS (gastric carcinoma) and HCT116 (colon carcinoma) cells were grown in RPMI 1640 medium (Invitrogen, USA; Catalog # 11875093) supplemented with 10% fetal bovine serum (FBS; Gibco, USA; Catalog no # 26140079). HepG2 (hepatocarcinoma), HeLa (cervical carcinoma), and B16F10 (melanoma) cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen; Catalog # 11965092) supplemented with 10% FBS. U87MG (glioblastoma) cells were grown in minimum essential medium (Invitrogen; Catalog # 11095080) containing 10% FBS. All cells were maintained at 37°C in a humidified 5% CO_2 incubator. For cell growth assay, cells seeded at 2×10^3 cells/well onto 96-well plates (SPL Lifesciences, Korea) were treated with each compound at various concentrations (a 2-fold series of serial dilution with 100 mM initial concentration of each compounds) for 72 h. Cell growth was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. The control sample contained DMSO that was included in this experiment to exclude the possibility of background effects on tumor cells. The data are reported as the mean ± standard deviation of three independent experiments. They were evaluated by Student's *t*-test where values of $p < 0.05$ were considered to be statistically significant.

Results and Discussion

Glycosylation of Curcumin

Curcumin is a member of the curcuminoids, which consist of curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%) [34]. Structurally, it is a symmetric molecule with three chemical entities in its structure: two aromatic ring systems containing *O*-methoxy and phenolic groups, connected by a seven-carbon linker consisting of an α, β-unsaturated β-diketone moiety [35]. It has been associated with diverse biological activities with pharmaceutical values. Hence, glycosylation can be a promising approach for altering physical properties such as water solubilities and/or enhanced biological activities [28, 29] for bioactive chemicals like curcumin. Hence, OPME chemoenzymatic synthesis of curcumin glycosides was carried out by applying systems of two types; one producing UDP-α-D-glucose and the other producing UDP-α-D-2-deoxyglucose (Fig. 1), in which the reactions start with all the enzymes necessary for the reaction and simpler starting materials in a single vessel [28].

First, the OPME chemoenzymatic reaction that generates glucoside products was carried out and the products were first analyzed by UFLC-DAD, which revealed the formation

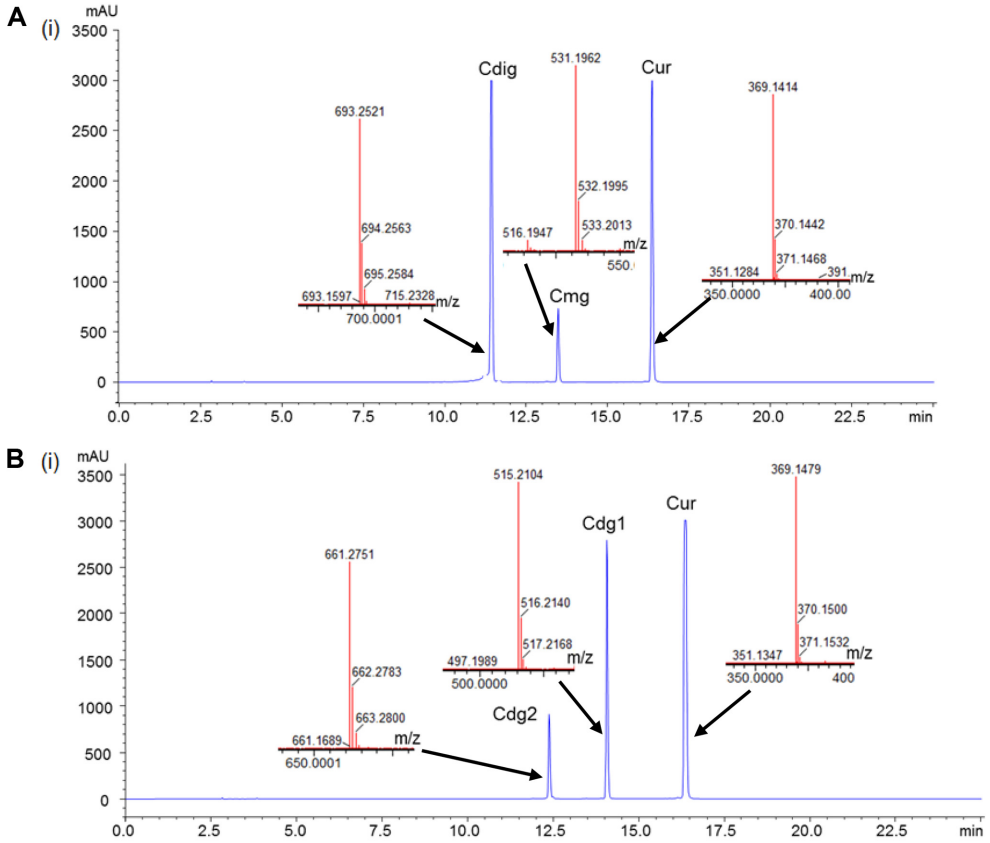

Fig. 2. Characteristic UFLC chromatogram for the glucosylation reaction of curcumin with *m/z* values of corresponding peaks for curcumin glucosides (**A**) and curcumin 2-deoxyglucosides (**B**).

Cur, curcumin; Cmg, curcumin 4′-*O*-glucoside; Cdig, curcumin 4′,4′′-di-*O*-glucoside; Cdg1, curcumin 4′-*O*-2-deoxygluoside; Cdg2, curcumin 4′,4′′-di-*O*-2-deoxyglucoside.

of two products (Fig. 2A). The first product (Cdig, t_R 11.4 min) and the second product (Cmg, t_R 13.5 min) had their own characteristic UV absorbance maxima when compared with the substrate peak (Cur, t_R 16.4 min).

ESI/MS analysis was performed to further confirm the identity of the new peaks. The $[M+H]^+$, *m/z* = 531.1962 was obtained for Cmg, which is the exact mass of curcumin monoglucoside (calculated: 531.1866), and $[M+H]^+$, *m/z* = 693.2521 was obtained for Cdig, which is the exact mass of curcumin diglucoside (calculated: *m/z* = 693.2395) in positive-ion mode (Fig. 2A). Therefore, the reaction was successful in attaching one and two glucosyl moieties to curcumin aglycone and, as a result, a monoglucoside and a diglucoside were synthesized. The products were then purified for structural elucidation.

Similar to the synthesis of glucosides, the OPME chemoenzymatic reaction was performed for the synthesis of 2-deoxyglucosides of curcumin. Then, the reaction mixture was analyzed by UFLC-DAD (Fig. 2B). Analysis of the reaction mixture revealed the formation of two products, which seemed obvious since the substrate molecule has two hydroxyl groups. The identity of the products formed was further confirmed by ESI/MS analysis. The product (Cdg1, t_R 14.1 min) had observed molecular mass $[M+H]^+$, *m/z* = 515.2104, which is the same as that for the mono-2-deoxyglucoside of curcumin (calculated: *m/z* = 515.1917) in positive-ion mode. Another peak at t_R 12.40 min (Cdg2) showed the molecular mass $[M+H]^+$, *m/z* = 661.2751 in positive-ion mode, which is the same to that of curcumin di-2-deoxyglucoside (calculated: *m/z* = 661.2496). Both products showed characteristic UV absorbance maxima different from that of parent substrate curcumin (data not shown). Furthermore, a large-scale reaction was performed in order to collect products for structural elucidation and other assessments.

Both the systems recycle UDP generated during the course of the enzymatic reaction, as a result of which UDP-glycosyltransferase (UGT) can function well without

inhibition [36, 37]. Similarly, ATP is regenerated, which makes the systems cost efficient. This work integrated both systems, UDP recycling and ATP regeneration, with a flexible UGT (YjiC) from *Bacillus licheniformis*, belonging to family GT1 type, for the successive glycosylation. Furthermore, the formation of product in OPME reaction systems could be controlled by adjusting the concentration of substrate molecule and starting materials for sugar nucleotide donors. The analyses of OPME reactions with curcumin revealed that the formation of monoglycosides was favored at higher concentration of substrate, whereas the lower concentration of substrate was favorable for diglycosides. These OPME systems have proven their efficiency by successful glycosylation of α-mangostin [28] and nargenicin A(1) [31].

Structural Elucidation of Curcumin Glycosides

The purified products of the OPME chemoenzymatic reaction were subjected to ^{1}H and ^{13}C NMR analyses. The ^{1}H and ^{13}C NMR analyses of curcumin standard were also performed for comparison (Figs. S1A and S1B). As the samples were prepared in DMSO-d_6, the signal for phenolic hydroxyl groups at δ 9.68 (2H, s, OH-4′,4′′) was visible for curcumin molecule accompanied by the two methoxy group signals at δ 3.85 (6H, s, OCH_3-3′,3′′) and other signals for aromatic protons and linker protons in ^{1}H NMR analysis. Similarly, ^{13}C NMR analysis showed characteristic signals at δ 183.68 for the two ketonic groups (C3, C5), δ 149.82 for two phenolic hydroxyl groups (C4′, C4′′), and δ 56.15 for two methoxy groups (OCH_3-3′, 3′′) (Figs. S1 and S2).

When the ^{1}H NMR spectrum of the single glucosyl moiety appended product (Cmg) was analyzed, signals at δ 9.70 (1H, s), δ 5.01 (1H, d, J = 6.8 Hz), and δ 3.19-3.69 (6H) were obtained, which confirmed the attachment of one glucosyl moiety with β-configuration and one hydroxyl group still remaining free. Furthermore, the signal for H5′ (δ 7.25) shifted downfield, indicating the attachment at OH-4′. Comparison of ^{13}C NMR of monoglucosylated product (Cmg) with curcumin standard showed additional signals of carbon for the glucose moiety, and a *para* effect was observed between C1′ (δ 129.143) and C4′ (δ 149.607), whereas the chemical shift value for C1′′ (δ 126.76) remained unaffected. Besides this, changes in chemical shift values of the other carbons near to attachment of sugar were also observed (Figs. S2A and S2B). With all these evidences and comparison of values from the literature, the monoglucosylated compound was characterized as curcumin 4′-*O*-β-glucoside [30, 38].

In the same way, when the ^{1}H NMR spectrum of two glucosyl moieties conjugated product (Cdig) was compared and analyzed, no signals were obtained for the phenolic hydroxyl groups, which indicated the attachment of glucosyl groups to those two hydroxyl groups. A signal at δ 5.02 (2H, d, J = 6.8) confirmed β-configuration for both glucosyl groups attached. No such significant change in chemical shift values was observed for other protons in contrast to glucosylation at a single point. The signals from δ 3.21 to 3.78 (12H) further confirmed the existence of two glucosyl moieties (Fig. S3A). The comparison of ^{13}C NMR spectrum with curcumin showed the downfield shift of the chemical shift value for C1′ and C1′′ by δ 2.94. The signal at δ 100.037 for the anomeric carbons (C1′′′, C1′′′) further confirmed the β-configuration of attached sugars (Fig. S3B). Owing to attachment of glucose to both hydroxyl groups, the symmetry of the substrate was maintained and hence no such significant changes were observed in chemical shift values when compared with the ^{13}C NMR spectrum of curcumin (Fig. S1B). Therefore, based on this evidence and the available literature [30, 39], the name of the compound was assigned as curcumin 4′,4′′-di-*O*-β-glucoside.

Similarly, ^{1}H and ^{13}C NMR analyses were performed for the 2-deoxyglucosides of curcumin. When comparison of the ^{1}H NMR spectrum of Cdg1 and curcumin was made, new signals at δ 5.28 (1H, dd, J = 10.0, 2.1 Hz) and δ 1.62 to 3.72 (7H) were observed, whereas a signal at δ 9.71 (1H, s) was also observed for one hydroxyl group. The signal for H5′ was found shifted downfield by δ 0.41. The coupling constant value indicated a β-configuration for the sugar. Similarly, signals at δ 1.62 (1H, m) and δ 2.20 (1H, m) were observed, which were the characteristic signals of two protons of C2′′ of a sugar moiety (Fig. S4A). The rest of the signals in the ^{1}H NMR spectrum were comparable to those of curcumin.

The effect of attachment of the glucosyl moiety was clearly observed in the ^{13}C NMR spectrum (Fig. S4B). The signal at δ 97.21 for anomeric carbon was in favor of the β-configuration of the sugar, since for an α-configuration of sugar, it appears below δ 92.0 [40]. Therefore, with the help of these proofs and references published [28, 41–43], Cdg1 was designated as curcumin 4′-*O*-β-2-deoxyglucoside.

The comparisons were also made between the ^{1}H NMR spectra of curcumin, Cdg1, and Cdg2 for structure elucidation. Owing to the attachment of the sugar on either side of the molecule, the signals for protons in the linker and aromatic rings remained almost unaltered for Cdg2. A signal at δ 5.27 (2H, dd) was observed with the coupling constant value (J = 9.9, 2.2 Hz) favoring the β-configuration. The signals for phenolic hydroxyl groups were missing and the

characteristic signals for the protons H2‴ and H2⁗ were observed at δ 1.62 (dd) and δ 2.20 (dd). 2-Deoxysugars have a hydroxyl group at the C-2 position replaced by a hydrogen atom. Here, two anomeric protons, 12 protons from δ 1.62 to δ 3.72, and the signals at δ 1.62 (dd) and δ 2.20 (dd) confirmed two attached glucosyl moieties as two units of 2-deoxyglucose (Fig. S5A).

Furthermore, the anomeric carbon signal was observed at δ 97.18, the other carbons of sugar moieties appeared upfield to the anomeric carbon signal (δ 77.76- δ 40.02), and a signal at δ 56.16 was also detected for two methoxy groups at C3′ and C3′′ in the ^{13}C NMR spectrum of Cdg2 (Fig. S5B). Because of the attachment of two sugar moieties, there was no significant change in the chemical shift values for signals, which was observed in the case of curcumin 4′-*O*-β-2-deoxyglucoside. All the evidence mentioned above suggested that Cdg2 was curcumin 4′,4′′-di-*O*-β-2-deoxyglucoside. To the best of our knowledge, curcumin 4′-*O*-β-2-deoxyglucoside and curcumin 4′,4′′-di-*O*-β-2-deoxyglucoside were synthesized for the first time.

The NMR spectra with chemical shift values of all the compounds mentioned above are available in the Supplementary file.

Solubility Test

As mentioned in Materials and Methods, the experiments were performed to analyze the solubility of curcumin and its four glycosides. The glycosylation of both types has increased the solubility of the compound (Fig. 3). The results indicated that the number of sugar moieties directly affects the solubility of a compound; the more the number of sugar, the better will be the solubility. Surprisingly, the deoxy-sugar derivatives with one hydroxyl group less did show comparable solubility with their oxy derivatives. The order of solubility in PBS solution was curcumin < curcumin 4′-*O*-β-2-deoxyglucoside < curcumin 4′-*O*-β-glucoside< curcumin 4′,4′′-di-*O*-β-2-deoxyglucoside < curcumin 4′,4′′-di-*O*-β-glucoside. Kaminaga *et al.* [30] reported an increased water solubility of about 20-million fold when curcumin was converted to curcumin-4′,4′′-*O*- β-digentiobioside.

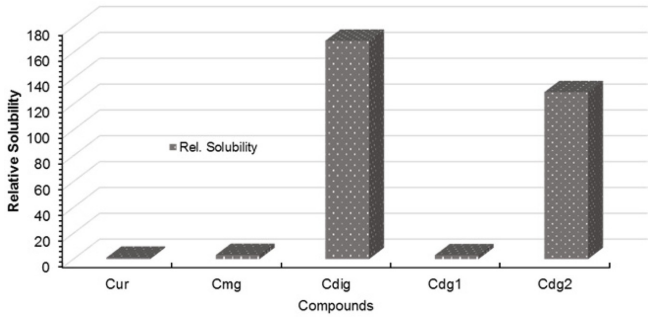

Fig. 3. Relative solubility of curcumin and its glycosides.
Cur, curcumin; Cmg, curcumin 4′-*O*-glucoside; Cdig, curcumin 4′,4′′-di-*O*-glucoside; Cdg1, curcumin 4′-*O*-2-deoxygluoside; Cdg2, curcumin 4′,4′′-di-*O*-2-deoxyglucoside.

The increase in solubility associated with glycosylation has been reported for different compounds [28, 29, 31]. Moreover, glycosides are considered like pro-drugs, which dramatically improve the solubility of aglycones by linking with sugar moieties. It has been predominantly observed that glycosylation has a significant role in enhancing the adsorption and metabolism of plant-based natural products such as quercetin and phloretin in the human body [44]. In this prospect, the dramatic increase in solubility of all the derivatives of curcumin upon glycosylation accounts for prominent importance in increasing the pharmaceutical value of those products.

Antibacterial Test

For assessment of the effect of curcumin and its glycosides against different gram-positive and gram-negative bacteria, the microdilution method was employed. The evaluation of antimicrobial susceptibility of different pathogenic bacteria against the tested compounds showed that the MIC ranged from 39.0 to 312.2 μg/ml (Table 1). It was evident that the antimicrobial activity was enhanced for the curcumin glycosides; the deoxyglucose conjugate showed better potency than the parental compound and other glycosides. The values of MIC are different from previously reported for curcumin and/or its glucosides [45–48], which might be due to the different laboratory and handling conditions. The enhancement in antibacterial activity for glycosides can be accredited to better cellular uptake, increased cellular concentration, and better receptor binding [49, 50].

It was observed that the antibacterial activity of curcumin is promising against gram positive bacteria, where the activity is greater in *B. subtilis* than *S. aureus*. It is established that curcumin suppresses cytokinesis in *B. subtilis* through induction of filamentation and without significantly affecting the segregation and organization of the nucleoids markedly suppressed the cytokinetic Z-ring formation. The stability and assembly of FtsZ protofilaments as a crucial factor for bacterial cytokinesis are introduced as a possible drug target for antibacterial agents [51]. Similarly, it exhibits antibacterial activity against gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *etc*., but the mechanism remained unclear. Recently, curcumin-induced membrane damage was reported at relatively high

Table 1. Antibacterial test for curcumin and its glycosides by the broth microdilution method.

Compounds	Antibacterial test by broth microdilution method[a]			
	Bacteria			
	S. aureus MIC (μg/ml)	*B. subtilis* MIC (μg/ml)	*P. aeruginosa* MIC (μg/ml)	*E. cloaceae* MIC (μg/ml)
Curcumin	>156.13	156.06	>156.13	>156.13
Curcumin 4′-*O*-glucoside	156.13	78.06	156.13	>156.13
Curcumin 4′-*O*-2-deoxygluoside	156.13	39.05	78.06	>156.13
Curcumin 4′,4′′-di-*O*-glucoside	>156.13	156.13	156.13	>156.13
Curcumin 4′,4′′-di-*O*-2-deoxyglucoside	>156.13	156.13	156.13	>156.13

[a]The results are from experiments performed in triplicates.

concentrations, but there was no effect at the MIC. At the MIC, curcumin-treated cells displayed various apoptotic markers such as reactive oxygen species accumulation, membrane depolarization, and Ca^{2+} influx. Based on the results, it was concluded that curcumin induces an apoptosis-like response through overexpression of the RecA protein [52].

Both oxy- and deoxyglucose moieties have been associated with the antimicrobial potential of different compounds such as doxorubicin and epirubicin, erythromycin, vancomycin, nystatin, *etc*. [53]. The glycosylated curcumin derivative, particularly monoglucoside, exhibited better antimicrobial potential than aglycon. However, there is a reduction in the potential for diglucoside, which may be due to greater bulkiness with the attachment of two sugars. There is a need of detailed mechanistic study for deducing the contributing factors for enhancement of activity in monoglucoside but reduction for diglucosides. However, whatever remains the mechanism of action, curcumin glycosides potentiate themselves as an effective antibacterial against both gram-positive and -negative bacteria.

Assessment of Anticancer Activity

Curcumin has effect on a variety of biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis, and metastasis. It shows antiproliferative effect in multiple cancers, and acts as an inhibitor of the transcription factor NF-κB and downstream gene products (including c-myc, Bcl-2, COX-2, NOS, Cyclin D1, TNF-α, interleukins, and MMP-9). In addition, it affects a variety of growth factor receptors and cell adhesion molecules involved in tumor growth, angiogenesis, and metastasis [54]. Thus, it exhibits cellular and molecular mechanisms pertaining to antiproliferative and anticarcinogenic properties in a wide variety of cell lines and animal models [55].

We conducted the cell viability test by an MTT colorimetric assay for curcumin and its four different glycosides at concentrations of 3.125, 6.25, 12.5, 25.0, 50.0, and 100.0 μM. The cytotoxicity of curcumin and its analogs was tested against gastric carcinoma (AGS), colon carcinoma (HCT116), hepatocarcinoma (HepG2), cervical carcinoma (HeLa), glioblastoma (U87MG), and melanoma (B16F10) cell lines (Table 2). The IC_{50} values, which are defined as the concentration of an inhibitor where the response (or binding) is reduced by half, were determined using a method described by Monks *et al*. [56]. Each IC_{50} test was performed in triplicate. The results showed that the curcumin glycosides effectively inhibit the growth of various cancer cells in a dose- and time-dependent manner. It was found that curcumin 4′-*O*-β-glucoside (Cmg) was most effective against AGS (IC_{50} = 7.11 μM) and HCT116 (IC_{50} = 5.27 μM) cell lines as compared with curcumin, which had IC_{50} = 9.77 μM against AGS and IC_{50} = 5.51 μM against HCT116 cell lines. Similarly, curcumin 4′-*O*-β-2-deoxyglucoside (Cdg1) more effectively inhibited the growth of AGS (IC_{50} = 5.86 μM), HCT (IC_{50} = 5.40 μM), HepG2 (IC_{50} = 18.61 μM), and HeLa (IC_{50} = 12.47 μM) cell lines when compared with curcumin. Furthermore, the effectiveness of curcumin 4′,4′′-di-*O*-β-glucoside (Cdig) in inhibiting the growth of various cancer cells was the lowest among the tested compounds.

However, curcumin 4′,4′′-di-*O*-β-2-deoxyglucoside (Cdig2) was found to inhibit AGS (IC_{50} = 6.90 μM), HepG2 (IC_{50} = 13.04 μM), and HeLa (IC_{50} = 13.49 μM) more effectively than curcumin (Table 2). In brief, the monoglycosides had better anticancer activity compared with the diglycosides. Among the monoglycosides, 2-deoxyglucoside exhibited greater anticancer activity than their corresponding oxy-forms. Generally, glucosylation has also been associated with enhanced cytotoxicity and modification of specificity of compounds on its targets [57, 58]. In our study as well, it

Table 2. Assessment of the anticancer activity of curcumin and its glycosides by MTT assay.

Cell lines	IC_{50} (μM)				
	Cur	Cmg	Cdig	Cdg1	Cdg2
AGS	9.77 ± 0.77	7.11 ± 0.17	18.09 ± 0.63	5.86 ± 0.02	6.90 ± 0.24
HCT116	5.51 ± 0.18	5.27 ± 0.16	17.30 ± 0.52	5.40 ± 0.11	10.35 ± 0.52
HepG2	36.77 ± 1.68	41.94 ± 1.67	>100	18.61 ± 0.47	13.04 ± 1.48
HeLa	22.25 ± 0.29	25.07 ± 0.63	93.24 ± 2.94	12.47 ± 0.75	13.49 ± 0.47
U87MG	7.00 ± 0.98	23.32 ± 1.71	99.10 ± 4.46	19.90 ± 0.50	10.77 ± 0.27
B16F10	7.49 ± 0.37	21.60 ± 0.86	66.57 ± 3.09	18.60 ± 0.65	20.86 ± 0.94

Cur, curcumin; Cmg, curcumin 4′-*O*-glucoside; Cdig, curcumin 4′,4′′-di-*O*-glucoside; Cdg1, curcumin 4′-*O*-2-deoxygluoside; Cdg2, curcumin 4′,4′′-di-*O*-2-deoxyglucoside (IC_{50}, μM; Mean ± SD, n = 3).

was observed that that there was enhanced activity in monoglycosides. However, there was a reduction in activity for diglycosides, which may be attributed to the bulkiness of the compound due to the attachment of multiple sugars.

The discovery of compounds or drug candidates with novel or better bioactivity is very limited. Moreover, the identified bioactive compounds may contain undesirable biological and chemical properties as side-effects, unspecific cytotoxicity, or lack of solubility. Hence, the targeted modification of already-identified bioactive compounds for enhancing their utility and potency is the most rational approach for fulfilling the commercial requirement of drugs. The rational integration of biological and chemical approaches is accelerating such drug development processes [59, 60]. Curcumin has been used extensively in Ayurvedic medicine for centuries, as it is a nontoxic phytomolecule. Recently, it has been extensively studied for its antioxidant, anti-inflammatory, antimicrobial, antiparasitic, antimutagen, and anticancer properties. In this study, we successfully modified curcumin by utilizing a simple glycosylation technique, and generated two novel glycosides and assessed the antibacterial and anticancer activities of all synthesized glycosides. All the modified derivatives had increased solubility in water compared with their aglycone counterpart. Meanwhile, all of the derivatives significantly retained their antibacterial potency; nevertheless, curcumin 4′-*O*-β-glucoside and curcumin 4′-*O*-β-2-deoxyglucoside showed better anticancer activities than the parent aglycone and diglycoside derivatives. Hence, it can be concluded that glycosylation is an effective approach for not only structurally diversifying curcumin but also generating analogs with better physical properties (water solubility) and bioactivity (anticancer). It provides evidential basis that the similar glycosylation reaction can be utilized for generating more diverse and potent derivatives of different natural products using this simple and cost-effective approach.

Acknowledgments

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project title: National Agricultural Genome Program, Project No. PJ01045705)," Rural Development Administration, Republic of Korea. We would like to thank the Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Chungbuk, Korea for NMR analyses.

References

1. Gupta SC, Sung B, Kim JH, Prasad S, Li S, Aggarwal BB. 2013. Multitargetting by tumeric, the golden spice: from kitchen to clinic. *Mol. Food Res*. **57:** 1510-1528.
2. Ak T, Gülçin İ. 2008. Antioxidant and radical scavenging properties of curcumin. *Chem. Biol. Interact*. **174:** 27-37.
3. Lal J, Gupta SK, Thavaselvam D, Agarwal DD. 2012. Design, synthesis, synergistic antimicrobial activity and cytotoxicity of 4-aryl substituted 3,4-dihydropyrimidinones of curcumin. *Bioorg. Med. Chem. Lett*. **22:** 2872-2876.
4. Mishra S, Karmodiya K, Surolia N, Surolia A. 2008. Synthesis and exploration of novel curcumin analogues as anti-malarial agents. *Bioorg. Med. Chem*. **16:** 2894-2902.
5. Chainani-Wu N. 2003. Safety and anti-inflammatory activity of curcumin: a component of turmeric (*Curcuma longa*). *Altern. Complement. Med*. **9:** 161-168.
6. Singh RK, Rai D, Yadav D, Bhargava A, Balzarini J, De Clercq E. 2010. Synthesis, antibacterial and antiviral properties of curcumin bioconjugates bearing dipeptide, fatty acids and folic acid. *Eur. J. Med. Chem*. **45:** 1078-1086.
7. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. 1995. Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer Lett*. **94:** 79-83.
8. Aggarwal S, Takada Y, Singh S, Myers JN, Aggarwal BB. 2004. Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor-κB signaling. *Int. J. Cancer* **111:**

679-692.
9. Yano S, Terai M, Shimizu KL, Futagami Y, Horie S, Tsuchiya S, *et al*. 2000. Antiallergic activity of *Curcuma longa*. (II). Features of inhibitory actions on histamine release from mast cells. *Nat. Med. (Tokyo)* **54:** 325-329.
10. Girish C, Pradhan SC. 2012. Hepatoprotective activities of picroliv, curcumin, and ellagic acid compared to silymarin on carbon-tetrachloride-induced liver toxicity in mice. *J. Pharmacol. Pharmacother*. **3:** 149-155.
11. Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, Tapia E, Pedraza-Chaverrí J. 2013. Renoprotective effect of the antioxidant curcumin: recent findings. *Redox Biol*. **1:** 448-456.
12. Kim M, Kim Y. 2010. Hypocholesterolemic effects of curcumin via up-regulation of cholesterol 7a-hydroxylase in rats fed a high fat diet. *Nutr. Res. Pract*. **4:** 191-195.
13. Altenburg JD, Bieberich AA, Terry C, Harvey KA, Vanhorn JF, Xu Z, *et al*. 2011. A synergistic antiproliferation effect of curcumin and docosahexaenoic acid in SK-BR-3 breast cancer cells: unique signaling not explained by the effects of either compound alone. *BMC Cancer* **11:** 149.
14. Du Q, Hu B, An HM, Shen KP, Xu L, Deng S, *et al*. 2013. Synergistic anticancer effects of curcumin and resveratrol in Hepa1-6 hepatocellular carcinoma cells. *Oncol. Rep*. **29:** 1851-1858.
15. Sreenivasan S, Krishnakumar S. 2015. Synergistic effect of curcumin in combination with anticancer agents in human retinoblastoma cancer cells lines. *Curr. Eye Res*. **40:** 1153-1165.
16. Guzzarlamudi S, Singh PK, Pawar VK, Singh Y, Sharma K, Paliwal SK, *et al*. 2016. Synergistic chemotherapeutic activity of curcumin bearing methoxypolyethylene glycol-g-linoleic acid based micelles on breast cancer cells. *Nanosci. Nanotechnol*. **16:** 4180-4190.
17. Zlotogorski A, Dayan A, Dayan D, Chaushu G, Salo T, Vered M. 2013. Nutraceuticals as new treatment approaches for oral cancer – I: curcumin. *Oral Oncol*. **49:** 187-191.
18. Shehzad A, Lee J, Lee JS. 2013. Curcumin in various cancers. *Biofactors* **39:** 56-68.
19. Perrone D, Ardito F, Giannatempo G, Dioguardi M, Troiano G, Lo Russo L, *et al*. 2015. Biological and therapeutic activities, and anticancer properties of curcumin. *Exp. Ther. Med*. **10:** 1615-1623.
20. Sandur SK, Pandey MK, Sung B, Ahn KS, Murakami A, Sethi G, *et al*. 2007. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. *Carcinogenesis* **28:** 1765-1773.
21. Somparn P, Phisalaphong C, Nakornchai S, Unchern S, Morales NP. Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. *Biol. Pharm. Bull*. **30:** 74-78.
22. Sugiyama Y, Kawakishi S, Osawa T. 1996. Involvement of the beta-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. *Biochem. Pharmacol*. **52:** 519-525.
23. Ahmad MZ, Alkahtani SA, Akhter S, Ahmad FJ, Ahmad J, Akhtar MS, *et al*. 2015. Progress in nanotechnology-based drug carrier in designing of curcumin nanomedicines for cancer therapy: current state-of-the-art. *J. Drug Target*. **11:** 1-21.
24. Wang YJ, Pan MH, Cheng AL, Lin LI, Ho YS, Hsieh CY, *et al*. 1997. Stability of curcumin in buffer solutions and characterization of its degradation products. *J. Pharm. Biomed. Anal*. **15:** 1867-1876.
25. Anand P, Nair HB, Sung B, Kunnumakkara AB, Yadav VR, Tekmal RR, *et al*. 2010. Bioavailability of curcumin: problems and promises. *Biochem. Pharmacol*. **79:** 330-338.
26. Lian T, Peng M, Vermorken AJ, Jin Y, Luo Z, Van de Ven WJ, *et al*. 2016. Synthesis and characterization of curcumin-functionalized HP-β-CD-modified GoldMag nanoparticles as drug delivery agents. *J. Nanosci. Nanotechnol*. **16:** 6258-6264.
27. Liang G, Shao L, Wang Y, Zhao C, Chu Y, Xiao J, *et al*. 2009. Exploration and synthesis of curcumin analogues with improved structural stability both in vitro and in vivo as cytotoxic agents. *Bioorg. Med. Chem*. **17:** 2623-2631.
28. Le TT, Pandey RP, Gurung RB, Dhakal D, Sohng JK. 2014. Efficient enzymatic systems for synthesis of novel α-mangostin glycosides exhibiting antibacterial activity against gram-positive bacteria. *Appl. Microbiol. Biotechnol*. **98:** 8527-8538.
29. Lepak A, Gutmann A, Kulmer ST, Nidetzky B. 2015. Creating a water-soluble resveratrol-based antioxidant by site-selective enzymatic glucosylation. *ChemBioChem* **16:** 1870-1874.
30. Kaminaga Y, Nagatsu A, Akiyama T, Sugimoto N, Yamazaki T, Maitani T, *et al*. 2003. Molecular cloning and characterization of a glucosyltransferase catalyzing glucosylation of curcumin in cultured *Catharanthus roseus* cells. *FEBS Lett*. **555:** 311-316.
31. Dhakal D, Le TT, Pandey RP, Jha AK, Gurung RB, Parajuli P, *et al*. 2015. Enhanced production of nargenicin A(1) and generation of novel glycosylated derivatives. *Appl. Biochem. Biotechnol*. **175:** 2934-2949.
32. Shin JY, Pandey RP, Jung HY, Chu LL, Park YI, Sohng JK. 2016. In vitro single-vessel enzymatic synthesis of novel Resvera-A glucosides. *Carbohydr. Res*. **424:** 8-14.
33. Vijayakumar GR, Divakar S. 2005. Amyloglucosidase-catalysed synthesis of curcumin-*bis*-alpha-D-glucoside, a response surface methodology study. *Biotechnol. Lett*. **27:** 1411-1415.
34. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. 2007. Bioavailability of curcumin: problems and promises. *Mol. Pharm.* **4:** 807-818.
35. Priyadarsini KI. 2013. Chemical and structural features influencing the biological activity of curcumin. *Curr. Pharm. Des*. **19:** 2093-2100.
36. Masada S, Kawase Y, Nagatoshi M, Oguchi Y, Terasaka K, Mizukami H. 2007. An efficient chemoenzymatic production of small molecule glucosides with in situ UDP-glucose recycling. *FEBS Lett*. **581:** 2562-2566.
37. Terasaka K, Misutani Y, Nagatsu A, Mizukami H. 2012. In situ UDP-glucose regeneration unravels diverse functions of

plant secondary product glycosyltransferases. *FEBS Lett*. **586:** 4344-4350.
38. Mohri K, Watanabe Y, Yoshida Y, Satoh M, Isobe K, Suqimoto N, *et al*. 2003. Synthesis of glycosylcurcuminoids. *Chem. Pharm. Bull. (Tokyo)* **51:** 1268-1272.
39. Masada S, Kawase Y, Nagatoshi M, Oguchi Y, Terasaka K, Mizukami H. 2007. An efficient chemoenzymatic production of small molecule glucosides with in situ UDP-glucose recycling. *FEBS Lett*. **581:** 2562-2566.
40. Bubb WA. 2003. NMR spectroscopy in the study of carbohydrates: characterizing the structural complexity, pp. 1-19. *In* Traficante DD (ed.), *Concepts in Magnetic Resonance Part A*, Vol. 19A. John Wiley & Sons, Inc., New Jersey.
41. Amann S, Drager G, Rupprath C, Kirschning A, Elling L. 2001. (Chemo)enzymatic synthesis of dTDP-activated 2,6-dideoxysugars as building blocks of polyketide antibiotics. *Carbohydr. Res*. **335:** 23-32.
42. Oh J, Lee SG, Kim BG, Sohng JK, Liou KK, Lee HC. 2003. One-pot enzymatic production of dTDP-4-keto-6-deoxy-D-glucose from dTMP and glucose-1-phosphate. *Biotechnol. Bioeng*. **84:** 452-458.
43. Kaminaga Y, Sahin FP, Mizukami H. 2004. Molecular cloning and characterization of a glucosyltransferase catalyzing glucosylation of curcumin in cultured *Catharanthus roseus* cells. *FEBS Lett*. **567:** 197-202.
44. Crespy V, Morand C, Besson C, Manach C, Démigné C, Rémésy C. 2001. Comparison of the intestinal absorption of quercetin, phloretin and their glucosides in rats. *J. Nutr*. **131:** 2109-2114.
45. Parvathy KS, Negi PS, Srinivas P. 2009. Antioxidant, antimutagenic and antibacterial activities of curcumin-β-diglucoside. *Food Chem*. **115:** 265-271.
46. Gunes H, Gulen D, Mutlu R, Gumus A, Tas T, Topkaya AE. 2013. Antibacterial effects of curcumin: an in vitro minimum inhibitory concentration study. *Toxicol. Ind. Health* **32:** 246-250.
47. Mun SH, Joung DK, Kim SH, Kang OH, Kim SB, Seo YS, *et al*. 2013. Synergistic antibacterial effect of curcumin against methicillin-resistant *Staphylococcus aureus*. *Phytomedicine* **20:** 714-718.
48. Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, Zandi K. 2014. A review on antibacterial, antiviral, and antifungal activity of curcumin. *Biomed. Res. Int*. **2014:** 12.
49. Kapoor N, Narain U, Misra K. 2007. Bioactive conjugates of curcumin having ester, peptide, thiol and disulphide links. *J. Sci. Ind. Res*. **66:** 647-650.
50. Prasad E, Hameeda B, Rao AB, Reddy G. 2014. Biotransformation of curcumin for improved biological activity and antiproliferative activity on acute HT-29 human cell lines. *Indian J. Biotechnol*. **13:** 324-329.
51. Rai D, Singh JK, Roy N, Panda D. 2008. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. *Biochem. J*. **410:** 147-155.
52. Yun DG, Lee DG. Antibacterial activity of curcumin via apoptosis-like response in *Escherichia coli*. *Appl. Microbiol. Biotechnol*. **100:** 5505-5514.
53. Lombó F, Olano C, Salas JA, Méndez C. 2009. Sugar biosynthesis and modification, pp. 277-308. *In* Abelson JN, Simon MI (eds.), *Methods in Enzymology*. Academic Press, San Diego.
54. Wilken R, Veena MS, Wang MB, Srivatsan ES. 2011. Curcumin: a review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Mol. Cancer* **10:** 12.
55. Chen CC, Sureshbabul M, Chen HW, Lin YS, Lee JY, Hong QS, *et al*. 2013. Curcumin suppresses metastasis via Sp-1, FAK inhibition, and E-cadherin upregulation in colorectal cancer. *Evid. Based Complement. Alternat. Med*. **2013:** 541695.
56. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, *et al*. 1991. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst*. **83:** 757-766.
57. Langenhan JM, Peters NR, Guzei IA, Hoffmann FM, Thorson JS. 2005. Enhancing the anticancer properties of cardiac glycosides by neoglycorandomization. *Proc. Natl. Acad. Sci. USA* **102:** 12305-12310.
58. Ahmed A, Peters NR, Fitzgerald MK, Watson JA, Hoffmann FM, Thorson JS. 2006. Colchicine glycorandomization influences cytotoxicity and mechanism of action. *J. Am. Chem. Soc*. **128:** 14224-14225.
59. Dhakal D, Sohng JK. 2015. Commentary: toward a new focus in antibiotic and drug discovery from the *Streptomyces* arsenal. *Front. Microbiol*. **6:** 727.
60. Dhakal D, Sohng JK. 2017. Coalition of biology and chemistry for ameliorating antimicrobial drug discovery. *Front. Microbiol*. **8:** 734.