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Increased Water Solubility of the Curcumin Derivatives *via* Substitution with an Acetoxy Group at the Central Methylene Moiety

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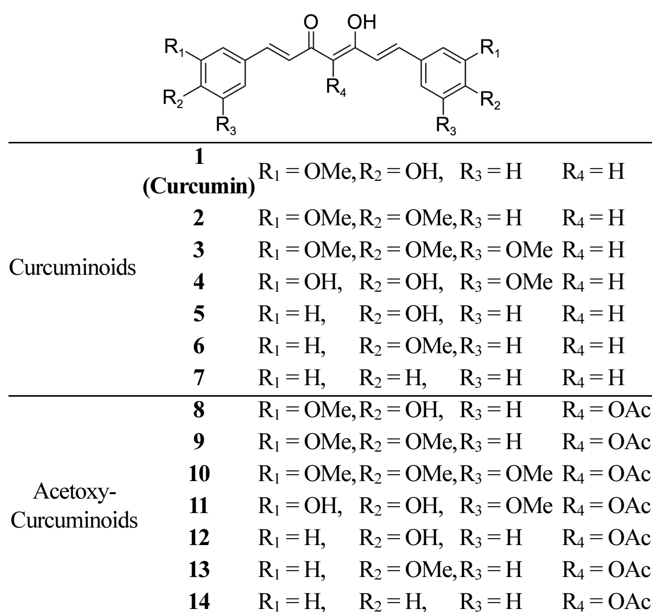
Curcumin (diferuloyl methane), a natural yellow pigment in the roots of turmeric, has been considered as one of the most promising chemopreventive agents against a variety of human cancers.[1] Curcumin is known to exhibit its antiproliferative effect against various cancer cells through cell cycle arrest and induction of apoptosis.[2] Although not as potent as many other cytotoxic agents, curcumin has been demonstrated to be safe in humans at relatively high doses (10 grams/day),[3] making it an attractive target for chemotherapeutic drug discovery efforts. Unfortunately, in spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent due to its low *in vivo* activity resulting from poor bioavailability.[4] In particular, low aqueous solubility is the key issue limiting the bioactivity of curcumin in living organism.[5] Therefore, design and preparation of a water-soluble curcumin analogue are highly desirable. Previously, as a part of our ongoing efforts to derivatize the curcumin scaffold, we prepared novel curcuminoids with acetoxy group attached to the central methylene moiety (Fig. 1), which showed significantly increased antioxidant activity.[6]

Prompted by this interesting activity of the novel curcuminoids, we attempted to estimate their solubility as well as various health-promoting effects. Herein, we report the potential of the curcumin derivatives as soluble anticancer agents through evaluation of their solubility as well as antiproliferative effect against several cancer cell lines.

Solubility of the acetoxy-curcuminoids was determined by the following methods.[7] Stock solutions of the curcuminoids were prepared at 0.5 mM, 2.5 mM, 5 mM, 10 mM, 15 mM, and 20 mM in 1% DMSO (dimethyl sulfoxide), and then diluted in 99% phosphate buffered saline (PBS, pH 7.4) buffer. As a result, the diluted compounds had final concentrations of 5 μM, 25 μM, 50 μM, 100 μM, 150 μM and 200 μM, respectively. The volume of the test compound in each 96 well plate was set to be 250 μL, and the solubility was measured by the NEPHELOstar laser based microplate nephelometer which checks the solubility of compounds by measuring forward light scattering in microplates. All raw data were processed using the BMG LABTECH NEPHELOstar Galaxy Evaluation software.

The solubility of the acetoxy-curcuminoids in PBS is summarized in Figure 2. As light scattering is caused by insoluble particles in solution, low light scattering intensity indicates high solubility. In general, the acetoxy-curcuminoids



		R1	R2	R3	R4
Curcuminoids	1 (Curcumin)	R_1 = OMe,	R_2 = OH,	R_3 = H	R_4 = H
	2	R_1 = OMe,	R_2 = OMe,	R_3 = H	R_4 = H
	3	R_1 = OMe,	R_2 = OMe,	R_3 = OMe	R_4 = H
	4	R_1 = OH,	R_2 = OH,	R_3 = OMe	R_4 = H
	5	R_1 = H,	R_2 = OH,	R_3 = H	R_4 = H
	6	R_1 = H,	R_2 = OMe,	R_3 = H	R_4 = H
	7	R_1 = H,	R_2 = H,	R_3 = H	R_4 = H
Acetoxy-Curcuminoids	8	R_1 = OMe,	R_2 = OH,	R_3 = H	R_4 = OAc
	9	R_1 = OMe,	R_2 = OMe,	R_3 = H	R_4 = OAc
	10	R_1 = OMe,	R_2 = OMe,	R_3 = OMe	R_4 = OAc
	11	R_1 = OH,	R_2 = OH,	R_3 = OMe	R_4 = OAc
	12	R_1 = H,	R_2 = OH,	R_3 = H	R_4 = OAc
	13	R_1 = H,	R_2 = OMe,	R_3 = H	R_4 = OAc
	14	R_1 = H,	R_2 = H,	R_3 = H	R_4 = OAc

Figure 1. Structures of the curcuminoids used in the present study.

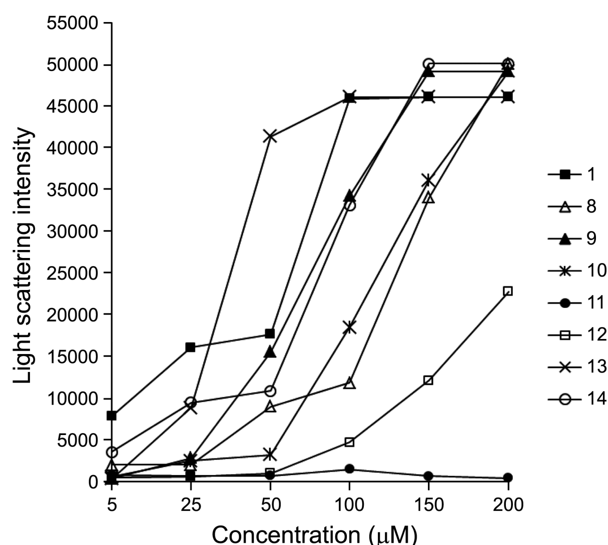


Figure 2. Aqueous solubility of the curcuminoids measured by forward light scattering intensity.

showed increases in water solubility compared with the curcumin. Among the series, the acetoxy-curcuminoids with free phenolic hydroxyl groups, **11** and **12**, showed remarkable increases in water solubility (Fig. 2). In particular, the curcuminoid **11** was completely soluble in PBS up to 200 μM.

Various cancer cell lines such as colon cancer (HCT116), prostate cancer (LNCap) and hepatocarcinoma (Huh-7) were treated with curcumin (**1**), curcuminoids (**2-7**) or actoxy-curcuminoids (**8-14**), and their viabilities were measured. The number of viable cells remaining after appropriate treatment was determined by using the modified tetrazolium salt (MTT) assay.[8] Briefly, cells (HCT116, LNCap and Huh-7) were seeded (5×10^3 cells/well) in tissue-cultured COSTAR clear-bottom 96-well plate in complete DMEM (Dulbecco's Modified Eagle Media) and incubated for 24 h at 37 °C under CO_2. Prepared curcuminoids which were dissolved in DMSO were diluted into 6 different concentrations (1, 5, 10, 25, 50 and 100 μM) and added to the media. After 24 h of incubation, cell medium was removed. New cell culture medium was added to each well (180 μL/well), and MTT solution (5 mg/mL in PBS) was added (20 μL/well). After 4 h of incubation at 37 °C, DMSO (100 μL) was added to each plate. After 30 min of incubation with shaking at room temperature, optical densities at 570 nm were measured by ELISA reader, with the extraction buffer serving as a blank. Antiproliferative effects of curcuminoids were estimated and the results are summarized in Table 1.

Throughout the series of the curcuminoids with various aromatic substituents (**2-7**, Table 1), no significant enhancement of the antiproliferative effect was observed. Rather, complete (**6** and **7**) or cell-specific (**4** and **5**) loss of activity was observed depending on the type and location of the aromatic substituents. Among the series, only two compounds with *meta*-methoxy substituents (**2** and **3**) maintained comparable antiproliferative activity with curcumin (**1**).

In contrast, the acetoxy-curcuminoids (**8-14**) showed moderate to potent activity against all three cancer cell lines tested (Table 1). In particular, the colon cancer cell (HCT116) was most susceptible to the acetoxy-curcuminoids (**8-12**, Table 1) to show 2-2.5 times increase in EC_{50} values compared with that of curcumin (**1**, Table 1). In this series, like the simple curcuminoids (**2-7**), the aromatic *meta*-methoxy substituent turned out to be critical for the antiproliferative effect, and the corresponding acetoxy-curcuminoids **10** and **11** showed the most potent activity against HCT116 with EC_{50} values of 18.5 μM and 16.9 μM, respectively. Also noteworthy is the broad spectrum antiproliferative effect of the acetoxy-curcuminoid **11** with a free catechol moiety, which exhibited almost similar antiproliferative activity against all three cancer cell lines tested.

Taken together, through evaluation of solubility as well as antiproliferative effect of the acetoxy-curcuminoids, we figured out that the acetoxy group substituted at the central methylene unit which served to enhance the solubility of the corresponding curcuminoids also played a key role in potentiating their antiproliferative effect. Thus, upon combination of the methylenyl acetoxy group and the aromatic *meta*-methoxy group on the curcumin framework, we could come up with a novel soluble curcuminoids with potent antiproliferative effect.

Table 1. Antiproliferative effect of Curcumin (**1**), Curcuminoids (**2-7**), and Acetoxy-curcuminoids (**8-14**) on Three Different Cancer Cell Lines HCT116, LNCap and Huh-7

Compounds		EC_{50} (μM)[a]		
		HCT116	LNCap	Huh-7
	1 (Curcumin)	42.2 ± 2.5	38.0 ± 1.7	33.0 ± 0.7
Curcuminoids	**2**	45.3 ± 1.4	54.4 ± 2.2	25.2 ± 1.6
	3	60.2 ± 2.8	34.6 ± 1.8	16.5 ± 1.0
	4	>100	57.4 ± 2.0	31.7 ± 2.4
	5	>100	49.2 ± 1.4	36.8 ± 2.0
	6	>100	>100	>100
	7	>100	>100	>100
Acetoxy-Curcuminoids	**8**	26.5 ± 1.3	37.8 ± 1.2	31.2 ± 1.5
	9	23.5 ± 0.9	38.8 ± 1.6	84.1 ± 0.8
	10	18.5 ± 1.1	59.8 ± 1.5	60.5 ± 1.9
	11	16.9 ± 0.8	19.3 ± 1.3	21.1 ± 0.7
	12	23.8 ± 1.5	32.3 ± 1.5	86.3 ± 0.5
	13	41.5 ± 1.2	26.2 ± 1.0	72.7 ± 1.4
	14	45.3 ± 1.3	41.5 ± 2.5	78.6 ± 1.3

[a]Concentration of the curcuminoids required to reduce cell viability to 50%. Data are presented as mean ± S.D. of three separated experiments on each cell line.

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