

## Synthesis of Curcumin Mimics Library with $\alpha,\beta$ -Unsaturated Carbonyl Aromatic Group and their Inhibitory Effect against Adipocyte Differentiation of 3T3-L1

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Received August 19, 2013, Accepted September 30, 2013

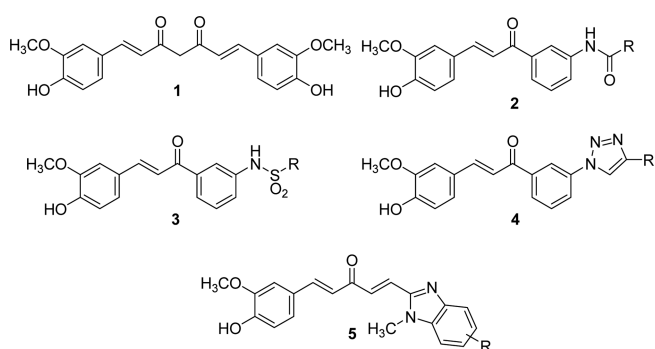
**Key Words :** Curcumin, Curcumin mimics, Anti-adipogenesis, Adipocyte differentiation, Anti-obesity

Curcumin (diferuloyl methane, **1**) of the root of *Curcuma longa* L. has useful biological properties such as antiinflammatory,<sup>1</sup> antioxidant,<sup>2</sup> antiviral,<sup>3</sup> chemopreventive,<sup>4</sup> and anti-infective activity,<sup>5</sup> as well as wound-healing properties.<sup>6</sup> Based on the diverse biological properties of curcumin, we synthesized and tested curcumin mimics libraries by using several screening systems to identify more enhanced effects and undiscovered activities. For example, amide linked curcumin mimics (**2**) showed an angiogenesis inhibition effect<sup>7</sup> and sulfonyl amide-linked curcumin derivatives (**3**) exhibit a vasodilatation effect on the basilar artery of white rabbit induced by high K<sup>+</sup> ion.<sup>8</sup> In addition, we found that substituted triazolyl curcumin derivatives (**4**) exhibited moderate to strong inhibitory activity against osteoclastogenesis induced by the receptor activator of nuclear factor (NF)- $\kappa$ B ligand (RANKL).<sup>9</sup> Curcumin derivatives (**5**) with benzimidazole groups exhibited cytotoxicity against various cancer cells<sup>10</sup> and, particularly, strongly inhibited multi-drug resistant cancer cells.<sup>11</sup> Our results and those of previous studies show that the relationship between structural modification and biological properties, strongly depends on the identity of the functional groups attached to the feruloyl structure.

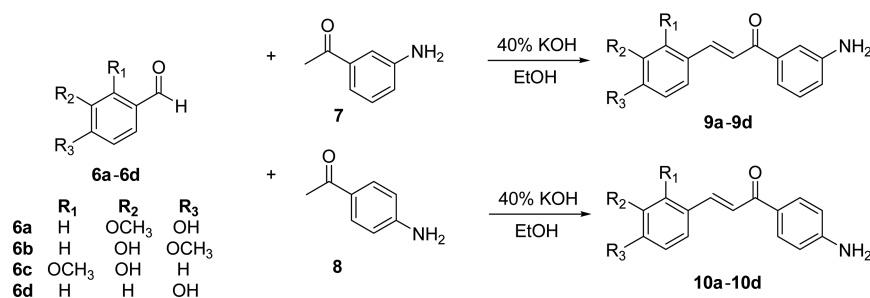
Among the useful biological properties of curcumin, the inhibitory property against adipocyte proliferation and differentiation has received significant attention because these processes are critical in the development of obesity, which is a main cause of chronic diseases such as type II diabetes and cardiovascular disease.<sup>12</sup> Ha *et al.* suggested that curcumin suppresses adipogenesis of 3T3-L1 cells through the Wnt

signaling pathway.<sup>13</sup> Park *et al.* also reported that curcumin regulates several targets such as peroxisome proliferator-activated receptor (PPAR)- $\gamma$ , Map kinases, and cyclooxygenase (COX)-2 by stimulating AMP-activated protein kinase (AMPK).<sup>14</sup> Although the usefulness and mechanistic pathway of curcumin for inhibiting the differentiation of adipocytes has been determined in several studies,<sup>12-14</sup> potential drug candidates for controlling obesity and its associated chronic diseases remain unknown. Here, we describe the design and synthesis of a novel curcumin mimics library and their anti-differentiation properties toward adipocyte.

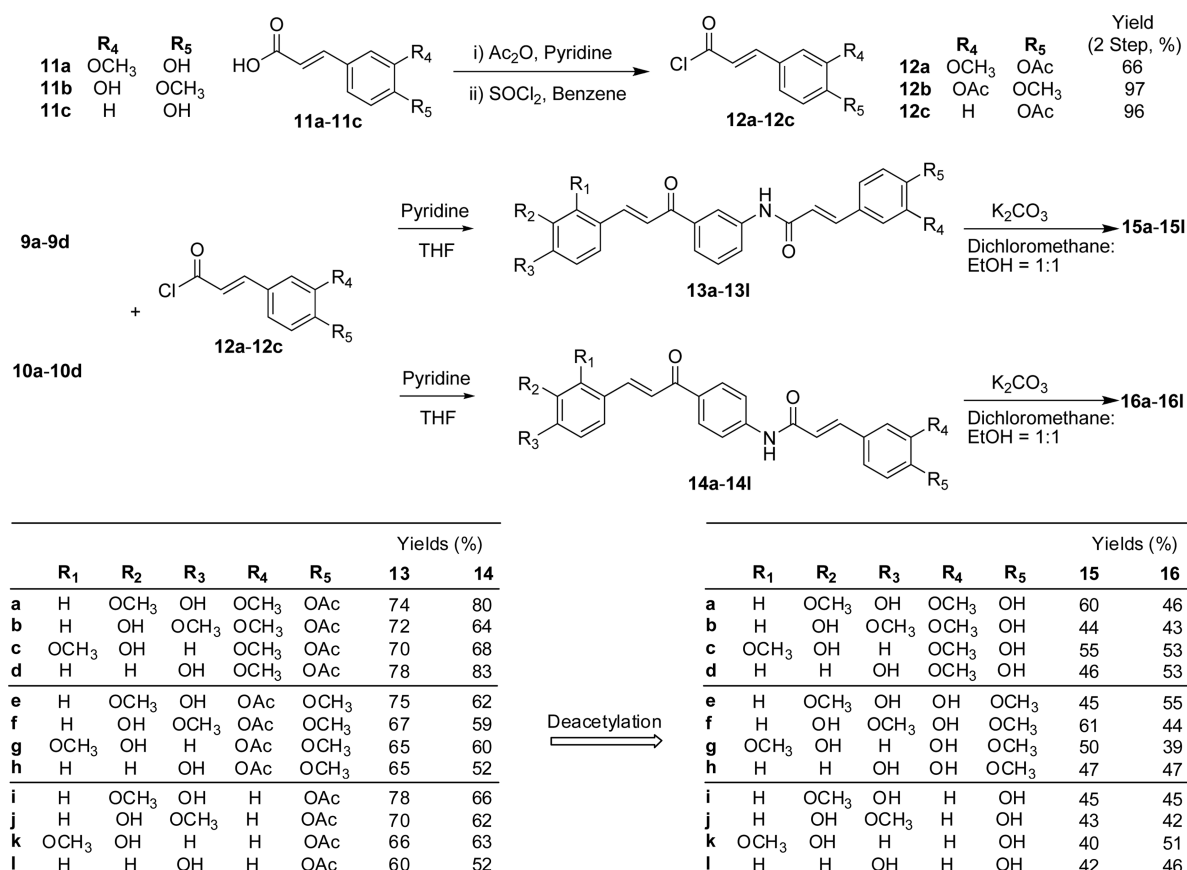
To accurately determine the structure of curcumin mimics and obtain their structure-activity relationships, we selected 4 types of benzaldehyde (**6a-6d**) and 2 aminoacetophenones (**7** and **8**) as shown in Scheme 1. Two groups of important



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



**Scheme 1.** Synthesis of 3'-aminochalcone (**9a-9d**) and 4'-aminochalcone (**10a-10d**) intermediates. The yields for each intermediates: **9a** (35%); **9b** (32%); **9c** (35%); **9d** (36%); **10a** (34%); **10b** (32%); **10c** (31%); **10d** (29%).



**Scheme 2.** Synthesis of asymmetric curcumin mimics library, **13a-13l** and **15a-15l**, from 3'-aminochalcone (**9a-9d**) and **14a-14l** and **16a-16l**, from 4'-aminochalcone (**10a-10d**) intermediates.

synthetic intermediate (**9a-9d** and **10a-10d**) were obtained from the aldol reaction of various benzaldehydes (**6a-6d**) with 2 aminoacetophenones (**7** and **8**) in the presence of a basic catalyst (40% KOH) in ethanol at room temperature for 10 h.<sup>15</sup> These intermediates were used to synthesize curcumin mimics such as **2**, shown in Figure 1, which is classified as an asymmetric curcumin mimic with additional binding groups different from those of curcumin (**1**). Particularly, we obtained the mimics from these intermediates (**9a-9d** and **10a-10d**) with variability at the left benzene ring and the *meta*- and *para*-position of additional amide linkages.

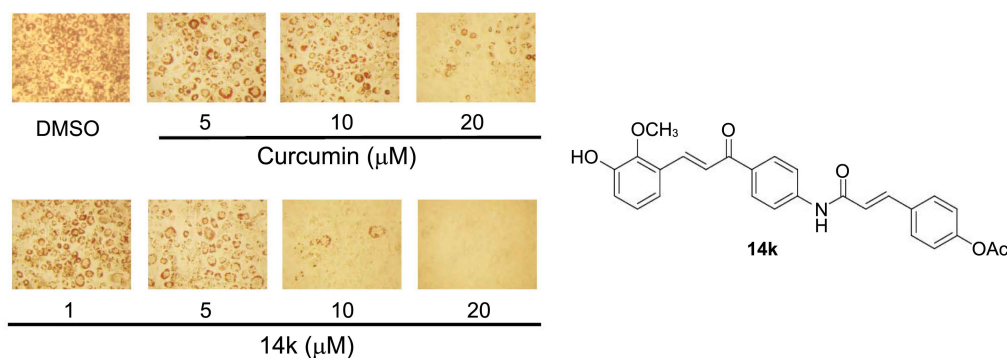
In order to add a diverse functional group to 3'-aminochalcone (**9a-9d**) and 4'-aminochalcone (**10a-10d**) to synthesize the amide-linked curcumin mimics library, 3 types of

acryloyl chloride with a 3,4-substituted phenyl group (**12a-12c**) were synthesized *via* acetylation with acetic anhydride in pyridine,<sup>16</sup> which is needed to avoid self-condensation between the phenol group and acyl chloride of **12a-12c**.<sup>17</sup> Chlorination with thionyl chloride to carboxylic acid groups in benzene was successively conducted from each starting material, ferulic acid (**11a**), isoferulic acid (**11b**), and *p*-coumaric acid (**11c**). The substitution reaction of acyl chloride compounds, **12a-12c**, with 2 aminochalcones, **9a-9d** and **10a-10d**, in pyridine and tetrahydrofuran (THF) gives structurally diversified asymmetric amide-linked curcumin mimics (**13a-13l** and **14a-14l**), which were converted into **15a-15l** and **16a-16l** by deprotection of the acetyl group using K<sub>2</sub>CO<sub>3</sub> in dichloromethane/ethanol (1:1).<sup>16</sup> Experi-

**Table 1.** Inhibitory concentration of curcumin mimic library against differentiation of 3T3-L1 preadipocyte

	IC <sub>50</sub> (μM) <sup>a</sup>											
	a	b	c	d	e	f	g	h	i	j	k	l
<b>13</b>	3.5	3.6	- <sup>b</sup>	6.6	-	-	-	5.8	3.8	5.3	-	3.5
<b>14</b>	4.5	-	-	3.4	-	3.4	-	6.8	3.3	6.8	3.3	5.7
<b>15</b>	12.4	8.8	-	15.2	-	27.0	-	14.7	15.7	8.7	-	7.6
<b>16</b>	5.5	57.4	-	28.7	-	8.4	-	15.0	38.5	17.4	8.0	15.1
<b>1<sup>c</sup></b>	9.8						Res. <sup>c</sup>	31.8				

<sup>a</sup>IC<sub>50</sub> was calculated from nonlinear regression by Graphpad Prism software. <sup>b</sup>These compounds were ruled out for the test because of cytotoxicity against 3T3L1 adipocyte. <sup>c</sup>These are positive controls, curcumin (**1**) and Resveratrol (Res.).



**Figure 2.** Oil red O staining pictures of differentiation of 3T3-L1 cells with DMSO and various concentration of Curcumin (**1**) and **14k**.

mental detail for synthesizing the curcumin mimics library shown in Scheme 2 is included in the supporting information.

Based on previous reports<sup>12-14</sup> stating that curcumin inhibits the proliferation and differentiation of 3T3-L1 preadipocytes, we tested the effect of our in-house curcumin mimics library on adipocyte differentiation.<sup>18</sup> After evaluation of cell viability using the MTT assay of the asymmetrical curcumin mimic library against 3T3-L1 at a single concentration of 10  $\mu\text{M}$ , 16 compounds showing less than 70% cell viability were ruled out (data for cytotoxicity not shown). The tested compounds generally showed stronger inhibitory concentration or somewhat weak activity compared to the lead compound, curcumin (**1**).

However, the most of the components in the tested library exhibited stronger activity than other positive control, resveratrol (Res.), as shown in Table 1. When considering the strength of inhibition against the proliferation and differentiation of 3T3-L1 adipocyte, some differences were observed that depend on their structure. For example, deacetylated curcumin mimics (**15a-15l** and **16a-16l**) showed weaker inhibitory activity than the acetylated curcumin mimics (**13a-13l** and **14a-14l**), indicating that the hydrogen bond donor of the free phenol group in **15a-15l** and **16a-16l** may give a negative effect to improve an inhibition against adipocyte differentiation. Interestingly, compounds **c**, **e**, and **g** of **13-16** compounds showed strong cell cytotoxicity and was excluded from the test. Additionally, the *meta*- or *para*-position of the amide group did not correlate with the potency of inhibition. Results of microscopy studies of Oil red O stained curcumin (**1**) and **14k**, the most potent inhibitor among in the library, are shown in Figure 2. Curcumin (**1**) effectively inhibited differentiation of 3T3-L1 cells at the concentration of 20  $\mu\text{M}$ , but showed minimal inhibition at 10 and 5  $\mu\text{M}$ . However, **14k** strongly inhibited adipocyte differentiation at 10 and 5  $\mu\text{M}$  without cell cytotoxicity, indicating **14k** showed inhibitory activity against the differentiation of 3T3-L1 adipocyte in a dose-dependent manner. The other active compounds also exhibited a similar tendency for inhibition.

In conclusion, a curcumin mimics library (**13a-13l**, **14a-14l**) created through the substitution reaction of  $\alpha,\beta$ -unsaturated carbonyl chlorides (**12a-12c**) with 3'-aminochalcone (**9a-9d**) and 4'-aminochalcone (**10a-10d**) and their

deacetylated library (**15a-15l**, and **16a-16l**) were synthesized to identify novel anti-obesity drug candidates that inhibit the proliferation and differentiation of 3T3-L1 adipocyte. Based on adipogenesis tests using Oil Red O staining, we confirmed that our curcumin mimics library contains stronger inhibitors than 2 natural products, curcumin and resveratrol although several compounds showed cell cytotoxicity. In the tested library, 8 compounds (**13a**, **13b**, **13i**, **13l**, **14d**, **14f**, **14i**, and **14k**) were found to be strong candidates for inhibiting preadipocyte differentiation, which will be examined in mechanistic studies.

**Acknowledgments.** This work was supported by a research grant from Yonsei University Wonju College of Medicine (YUWCM 2012-25).

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**Cell culture and differentiation.** 3T3-L1 mouse embryo fibroblasts were obtained from American Type Culture Collection (Manassas, VA). 3T3-L1 cells were grown in DMEM (Gibco, Rockville, MD, USA) supplemented with 10% new born serum (Gibco) and 1% Pen/Strep mix(Gibco) at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>/95% air. After 3T3-L1 cells reached confluence for 2 days (referred as day 0), the cells were differentiated into adipocytes with DMEM containing 10% fetal bovine serum (FBS, Gibco), 10 µg/mL insulin, 0.5 mM isobutylmethylxanthine (IBMX), and 1 µM dexamethasone for 2 days. The cells were then maintained in the medium containing 10% FBS and 10 µg/mL

insulin for 2 days and replenished with the DMEM containing 10% FBS for an additional two days, at which time > 90% of cells were mature adipocytes with accumulated fat droplets.; **Oil Red O staining.** Adipogenesis was evaluated by Oil Red O staining (a specific lipid staining, Ref. 1) and quantitated by determining the amount of extracted Oil Red O stain as measured by the optimal absorbance at 540 nm. Briefly, differentiated cells were washed twice in cold PBS and fixed with 4% paraformaldehyde for 1 h. After a single wash in water, cells were stained for 30 min with Oil Red O solution, prepared by diluting stock solution (0.6 g of Oil Red O (Sigma) in 100 mL of isopropanol) with H<sub>2</sub>O (3:2) followed by filtration. Cells were washed three times with H<sub>2</sub>O and images were photographed and then Oil Red O was eluted with isopropanol. The extent of extracted Oil Red O was quantitated by measuring the optimal absorbance at 540 nm.

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