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Significant enhancement in radical-scavenging activity of curcuminoids conferred by acetoxy substituent at the central methylene carbon

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ABSTRACT

For a compound to be a radical-trapping antioxidant, the antioxidant-derived radical must be sufficiently inert to molecular oxygen as this would generate harmful chain-propagating peroxyl radicals. Curcumin has a unique structure with phenolic hydroxyl group as well as β-diketone moiety in the same molecule, both of which are able to donate electrons to free radicals. However, due to the reactivity toward molecular oxygen, the carbon-centered radical derived from β-diketone moiety do not serve as radical-trapping antioxidants. In this study, we reasoned that stabilization of the carbon-centered radical through substitution with an electron-withdrawing group would enhance the radical-scavenging antioxidative activity of the resulting curcuminoids. Thus, various substituents (methyl, allyl, methoxy, xanthate, and acetoxy) covering broad spectrum of the polar substituent effect were introduced to the central methylene position of both phenolic and non-phenolic curcuminoids. With the free phenolic hydroxyl groups present, the methylene-substituent did not exert significant effect on the antioxidant activity of the curcuminoids (EC_{50} = 23.2–30.3 μM) with the exception of the acetoxy-substituted derivative (EC_{50} = 8.7 μM) which showed more potent activity than curcumin (EC_{50} = 22.6 μM). When substituted to the non-phenolic curcumin scaffold, however, the methylene-substituent enhanced antioxidant activity of the otherwise inactive curcuminoids in the increasing order of methyl < allyl < methoxy < xanthate ≪ acetoxy, which is well correlated with the polar inductive effects of the substituents.

1. Introduction

Free radical-induced chain reaction of oxidation has been implicated to play a major role in progression of pathological disturbances, which eventually leads to cardiovascular disease, cancer, Alzheimer's disease, and Parkinson's disease. Antioxidants break the chain reaction of oxidation by giving up their own electrons to free radicals. Curcumin (diferuloylmethane, **1**; Fig. 1), a natural yellow pigment derived from rhizomes of *Curcuma longa*, is endowed with typical radical-scavenging as well as chain-breaking antioxidant activity by its characteristic structure with both phenol and β-diketone moieties in the same molecule. Phenols and β-diketones are both able to donate electrons to free radicals but, unlike phenoxyl radicals, most carbon-centered radicals including the one derived from β-diketone moiety do not serve as radical-trapping antioxidants due to their instability and reactivity to molecular oxygen to create harmful chain-propagating peroxyl radicals.[1] For this reason, the radical-scavenging as well as chain-breaking antioxidant activity of curcumin is mainly attributed to its free phenoxyl group. Formation of the phenoxy radical is dependent upon the reaction conditions and proceed either through direct hydrogen atom transfer (HAT) of the phenolic hydrogen to a reactive radical source, or by way of more efficient sequential proton loss electron transfer (SPLET) mechanism[2] which includes sequential events of (i) proton loss from the central β-methylene to form an enolate anion, (ii) electron transfer to a reactive radical source to form a carbon-centered radical, (iii) second proton loss from the phenolic hydroxyl group, and finally, (iv) electron transfer from the resulting phenoxide ion to carbon-centered radical to form a stable phenoxy radical.[2] Thus, the free phenolic hydroxyl group is considered to be a prerequisite for the radical-trapping antioxidant activity of the curcuminoids, and this claim has been supported by many studies in which non-phenolic compounds showed no antioxidant activity.[3–7]

However, recently, Weber et al., made unprecedented report on the antioxidant activity of the non-phenolic curcuminoids.[8] The curcumin analogues with no aromatic substituent but an alkyl (methyl or ethyl) group attached to the central methylene moiety (**2** and **3**, Fig. 1) showed significant antioxidant activity, and the authors claimed that formation of a stable tertiary carbon-centered radical was responsible for this unique property.[8] Related to this

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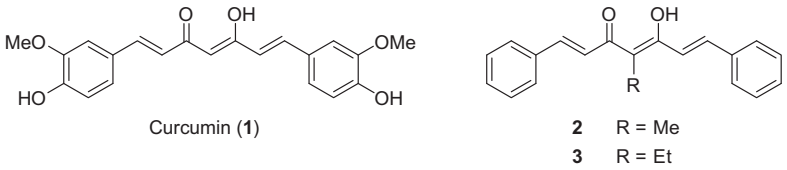


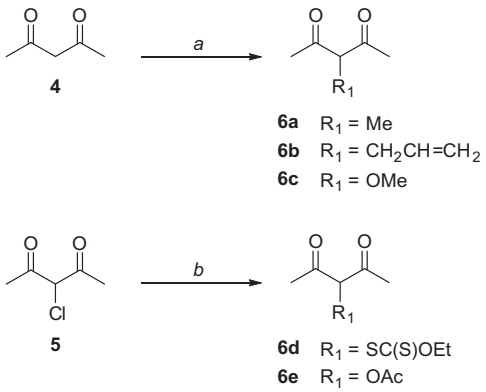
Figure 1. Structures of curcumin (**1**) and non-phenolic curcuminoids with antioxidant activity (**2** and **3**).

issue, it is worth to note that stable carbon-centered radicals, especially the resonance-stabilized ones, exhibit attenuated reactivity toward oxygen.[9–11] Therefore, it can be anticipated that the otherwise unstable reactive radicals derived from β-diketone moiety might serve as a radical-trapping antioxidant by introduction of a substituent stabilizing the carbon-centered radical. Unfortunately, studies on the curcuminoids with a substituent at the central methylene moiety have been focused as anticancer, antiviral, or antibacterial agents but their antioxidant activity has rarely been reported.[12–15] Thus, the lack of systematic study of the curcuminoids with central methylene-substituents prompted us to study the substituents' effect on the antioxidant activity of the corresponding curcuminoids. In this study, various substituents (methyl, allyl, methoxy, xanthate, and acetoxy) covering broad spectrum of the polar substituent effect as well as radical stabilizing effect were introduced to the central methylene position of both phenolic and non-phenolic curcuminoids, and their antioxidant activity was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity.[16]

2. Results and discussion

2.1. Chemistry

As an aldol-type condensation of acetylacetone and vanillin is known to be the most efficient method for preparation of curcumin,[17] substituted acetylacetones (**6**, Scheme 1) are required for preparation of curcuminoids with substituents at the central methylene unit. In this study, in order to study the electronic effect of the methylene-substituents on the antioxidant activity of the curcuminoids, functionalities such as methyl, allyl, methoxy, xanthate, and acetoxy were introduced to the acetylacetone scaffold (Scheme 1). Thus, nucleophilic substitution of the acetylacetone (**4**) provided alkyl- (**6a** and **6b**)[18] and methoxy- (**6c**)[19] substituted acetylacetones, whereas the acetoxy- (**6e**)[20] and xanthate-substituted acetylacetones (**6d**) were obtained by substitution of 3-chloroacetylacetone (**5**) with sodium acetate and potassium xanthate, respectively (Scheme 1).



Scheme 1. Synthesis of substituted acetoacetates. Reagents and conditions: (a) for **6a**, MeI, K_2CO_3, acetone, reflux, 6 h; for **6b**, allyl bromide, K_2CO_3, acetone, reflux, 6 h; for **6c**, $PhI(OAc)_2$, $BF_3{\cdot}OEt_2$, MeOH, rt, 5 h; (b) for **6d**, KSC(S)OEt, acetone, rt, 4 h; for **6e**, NaOAc, DMSO, rt, 3 h.

A series of curcuminoids with no methylene-substituents (**7**) were prepared by condensation of the commercially available acetylacetone (**4**) with various aromatic aldehydes (Scheme 2). Likewise, condensation of the acetylacetone derivatives obtained above (**6**, Scheme 1) with aromatic aldehydes provided the curcuminoids with various methylene-substituent (**8**, Scheme 2).

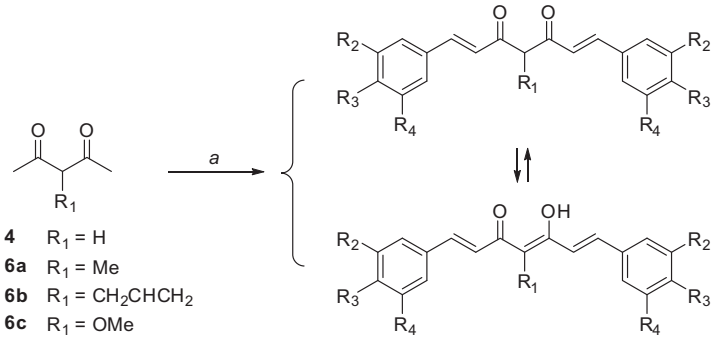
Briefly, the acetylacetones (**4** and **6**) were treated with boric anhydride (B_2O_3) in the presence of tributylborate to provide the corresponding acetylacetone–boron complexes which were allowed to react with variously substituted aromatic aldehydes (ArCHO) using 1,2,3,4-tetrahydroquinoline as a catalyst.[7] The free curcuminoids (**7** and **8**, Scheme 2) were then obtained after decomposition of the resulting boron complex by aqueous AcOH.

2.2. DPPH radical-scavenging activity

The ability to scavenge stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is a widely used and accurate method to evaluate antioxidant activity.[21,22] The radical-scavenging activity of the curcuminoid prepared in this study was evaluated by monitoring reduction of light absorption at 517 nm by DPPH radical upon reaction with curcuminoids in methanol solution (Fig. 2), and, after a plateau has been reached, the radical-scavenging activity of the curcuminoids was characterized by their EC_{50} values (Table 1).

As anticipated, curcumin (**7a**, EC_{50} = 22.6 μM) and a curcuminoid with a 2,3-dihydroxy-4-methoxybenzene moiety (**7d**, EC_{50} = 10.4 μM) showed potent radical-scavenging activity (Fig. 2a, Table 1), whereas the non-phenolic curcuminoids (**7b**–**7c**, **7f**–**7g**) failed to reduce the amount of DPPH radical even at concentration of 100 μM (Fig. 2a, Table 1). Interestingly, the curcuminoid with a monohydroxyphenyl moiety (**7e**) was not an active radical scavenger (Fig. 2a, Table 1), and this result indicates that, in addition to the phenolic hydroxyl group, the adjacent methoxy (**7a**) or hydroxyl (**7d**) group is also required for formation of the stable phenoxy radical which is believed to play the critical role in radical-scavenging activity of the curcuminoids.[3–7] The structure–activity relationship presented above indicates that the phenolic hydroxyl group is prerequisite, but alone insufficient, for radical-scavenging effect of the curcuminoids.

The methylene-substituents such as alkyl (**8a**, **8b**, **8e**, and **8f**), methoxy (**8c**, **8g**), and xanthate (**8d**, **8h**) did not change the nature of the radical-scavenging activity of the curcuminoids, which is highly dependent on the phenolic hydroxyl group (Fig. 2b). Thus, the radical-scavenging activity of the methylene-substituted curcuminoids with dimethoxyphenyl moiety (**8e**–**8h**) was significantly lower than that of the corresponding analogues with free phenolic hydroxyl groups (**8a**–**8d**) (Fig. 2b, Table 1). Nevertheless, slight to moderate increase in radical-scavenging activity of the non-phenolic methylene-substituted curcuminoids (**8e**–**8h**) compared to their unsubstituted counterpart (**7b**) should be noted (Fig. 2b). More interestingly, the radical-scavenging activity was well correlated with the polar inductive effect of the substituent, in which **8g** with electron-withdrawing methoxy group (Hammett constant,



7a	R_1 = H,	R_2 = OMe,	R_3 = OH,	R_4 = H
7b	R_1 = H,	R_2 = OMe,	R_3 = OMe,	R_4 = H
7c	R_1 = H,	R_2 = OMe,	R_3 = OMe,	R_4 = OMe
7d	R_1 = H,	R_2 = OH,	R_3 = OH,	R_4 = OMe
7e	R_1 = H,	R_2 = H,	R_3 = OH,	R_4 = H
7f	R_1 = H,	R_2 = H,	R_3 = OMe,	R_4 = H
7g	R_1 = H,	R_2 = H,	R_3 = H,	R_4 = H
8a	R_1 = Me,	R_2 = OMe,	R_3 = OH,	R_4 = H
8b	R_1 = $CH_2CH{=}CH_2$,	R_2 = OMe,	R_3 = OH,	R_4 = H
8c	R_1 = OMe,	R_2 = OMe,	R_3 = OH,	R_4 = H
8d	R_1 = SC(S)OEt,	R_2 = OMe,	R_3 = OH,	R_4 = H
8e	R_1 = Me,	R_2 = OMe,	R_3 = OMe,	R_4 = H
8f	R_1 = $CH_2CH{=}CH_2$,	R_2 = OMe,	R_3 = OMe,	R_4 = H
8g	R_1 = OMe,	R_2 = OMe,	R_3 = OMe,	R_4 = H
8h	R_1 = SC(S)OEt,	R_2 = OMe,	R_3 = OMe,	R_4 = H
8i	R_1 = OAc,	R_2 = OMe,	R_3 = OH,	R_4 = H
8j	R_1 = OAc,	R_2 = OMe,	R_3 = OMe,	R_4 = H
8k	R_1 = OAc,	R_2 = OMe,	R_3 = OMe,	R_4 = OMe
8l	R_1 = OAc,	R_2 = OH,	R_3 = OH,	R_4 = OMe
8m	R_1 = OAc,	R_2 = H,	R_3 = OH,	R_4 = H
8n	R_1 = OAc,	R_2 = H,	R_3 = OMe,	R_4 = H
8o	R_1 = OAc,	R_2 = H,	R_3 = H,	R_4 = H

Scheme 2. Synthesis of curcuminoids. Reagents and conditions: (a) (i) B_2O_3, $(n\text{BuO})_3B$, DMF, 60 °C, 20 min; (ii) ArCHO, 65 °C, 10 min; (iii) 1,2,3,4,-tetrahydroquinoline, AcOH, 95 °C, 4 h; (iv) aq. AcOH, 70 °C, 1 h.

σ_m = +0.12) showed significantly higher activity compared with **8e** or **8f** with electron-donating methyl (σ_m = −0.07) or allyl substituent (σ_m = −0.11).[23] In line with this observation, the radical-scavenging effect of the acetoxy-substituted curcuminoids (**8i–8o**) is of particular interest (Fig. 2c, Table 1). Acetoxy group has a higher Hammett constant (σ_m = +0.39)[23] than methoxy group and it can be easily introduced to the central methylene position of the curcuminoids by simple substitution of the 3-chloroacetylacetone (**5**) with acetate anion (Scheme 1). To our surprise, even the non-phenolic curcuminoids were endowed with potent radical-scavenging effect (Fig. 2c, Table 1; EC_{50} values of 47.8–22.1 μM) by the acetoxy group substituted at the central methylene position (**8j**, **8k**, **8n**, and **8o**). The inactive curcuminoid with a monohydroxyphenyl moiety (**7e**) also gained radical-scavenging effect upon substitution with the acetoxy group, and the resulting curcuminoid **8m** was as potent (EC_{50} = 26.3 μM) as curcumin (**7a**) (Fig. 2c, Table 1). The influence of the acetoxy group on the radical-scavenging effect of curcuminoids was synergistic with the phenolic hydroxyl group, and the activity of **8i** and **8l** (EC_{50} = 8.7 and 2.9 μM, Table 1) was significantly improved compared with their counterparts with no methylene-substituents, **7a** and **7d** (EC_{50} = 22.6 and 11.3 μM, Table 1), respectively. The SPLET mechanism for antioxidant effect of curcumin (Fig. 3)[2] support these experimental observations regarding the role of the acetoxy group. According to this mechanism, DPPH radical reacts with the curcumin anion (**II**, Fig. 3) formed by proton loss from the central β-methylene.[2] Thus, curcumin ionization is rate-limiting and the reaction rate is dependent upon the equilibrium concentration of the anion.[2] In this context, the remarkable radical-scavenging activity of the acetoxy-substituted curcuminoids (**8h–8o**) might be attributed to the increased anion concentration due to the stabilizing effect of the electron-withdrawing acetoxy group.

Ionization is suppressed in acidic conditions, and at sufficiently high concentration of the acid, the SPLET mechanism is blocked and the residual slow reaction occurs by HAT from a phenolic hydroxyl group of the neutral curcumin to the radical.[2] By the same token, the favored ionization of the curcuminoids due to the stabilization by the methylene-substituted acetoxy group would also be suppressed in acidic conditions resulting in lower level of radical-scavenging effect. For these reasons, antioxidant effect of the acetoxy-substituted curcuminoids (100 μM) was evaluated in methanol solution containing large excess of acetic acid (100 mM),[2] and the relative change in radical-scavenging activity (Fig. 4) was calculated from the equation shown below where E_{acid} and $E_{neutral}$ indicate radical-scavenging effect in acidic and neutral media, respectively.

$$\text{Relative change} = (E_{acid} - E_{neutral})/E_{neutral}$$

It is well known that the main factor controlling the antioxidant activity of ascorbic acid and catechol derivatives such as **8l** is the stabilization by intramolecular hydrogen bonding of the

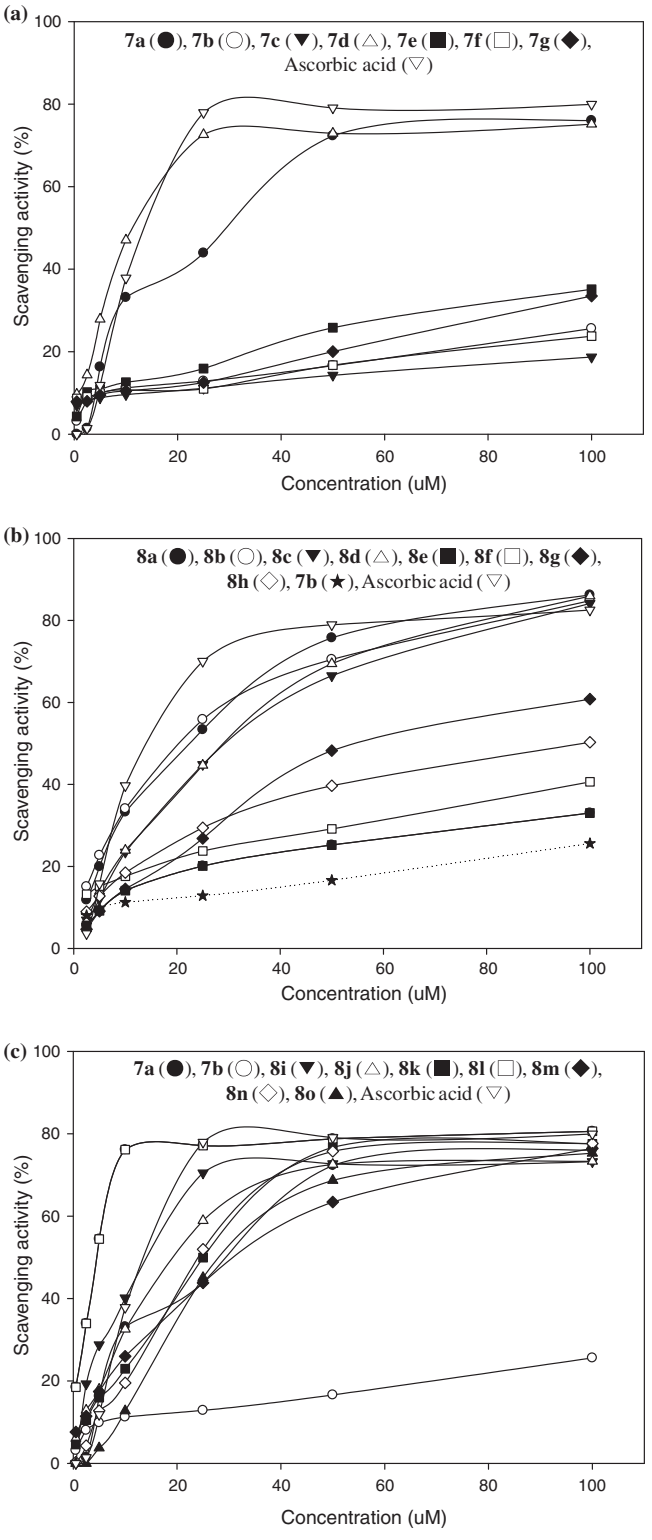


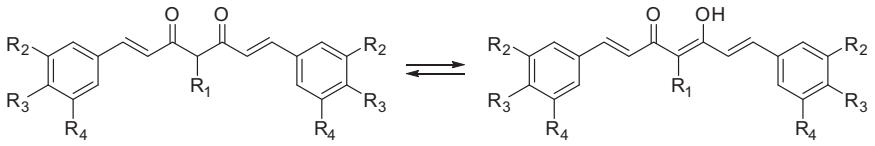
Figure 2. Radical-scavenging activity of curcuminoids with (a) no methylene-substituents (**7a**–**7g**), (b) methylene-substituents of methyl (**8a**, **8e**), allyl (**8b**, **8f**), methoxy (**8c**, **8g**), and xanthate (**8d**, **8h**) group, and (c) methylene-substituents of acetoxy group (**8i**–**8o**).

oxyradicals formed after direct HAT,[24] which explains the acid-independent radical-scavenging effect of ascorbic acid and **8l** (Fig. 4). As anticipated by the SPLET mechanism, the radical-scavenging effects of both curcumin (**7a**) and acetoxy-substituted curcumin (**8i**) were decreased about 10–15% in acidic media (Fig. 4). In contrast, the non-phenolic acetoxy-substituted curcuminoids (**8j**, **8k**, **8n**, and **8o**) showed significant decrease (25–35%) in radical-scavenging activity, and this result clearly indicates that the antioxidant mechanism is anion-dependent and, even in the presence of the stabilizing acetoxy group, the HAT from the β-methylene moiety is less efficient than that from the phenolic hydroxyl group. The monohydroxyl group in **8m** seems to give slight but significant contribution to the HAT mechanism when ionization is suppressed in acidic media showing intermediate decrease (13%) in antioxidant activity (Fig. 4).

3. Conclusion

Autoxidation is a radical chain reaction which occurs in the presence of molecular oxygen to form hydroperoxides, and radical-trapping antioxidants break the chain reaction of oxidation by giving up their own electrons to free radicals. For a compound to be a radical-trapping antioxidant, the antioxidant-derived radical must be sufficiently inert to molecular oxygen as this would generate harmful chain-propagating peroxyl radicals. Phenoxyl radicals are stable enough not to react with molecular oxygen whereas most carbon-centered radicals including the one derived from β-diketone moiety readily react with oxygen to create peroxyl radicals.[1] For this reason, the generally accepted antioxidant mechanism of curcumin includes HAT from phenol rather than from the neutral central methylene unit, which entitles the free phenolic moiety as the prerequisite for antioxidant activity of curcumin (**7a**–**7g**, Table 1).[2] However, it is also known that stable carbon-centered radicals, especially the resonance-stabilized ones, exhibit attenuated reactivity toward oxygen.[9–11] Therefore, it can be anticipated that the otherwise unstable reactive radicals derived from β-diketone moiety might serve as a radical-trapping antioxidant by introduction of a substituent stabilizing the carbon-centered radical. The methylene-substituents used in this study (methyl, allyl, methoxy, xanthate, and acetoxy) cover broad spectrum of the polar substituent effect (σ_m, −0.11 to +0.39), and the observed antioxidant activity in the increasing order of methyl- < allyl- < methoxy- < xanthate- ≪ acetoxy-substituted curcuminoids (Fig. 2b and c) is well correlated with the polar inductive effects (σ_m) of the substituents. In particular, the acetoxy-substituted curcuminoids with free phenolic hydroxyl groups (**8i** and **8l**, Table 1) showed more potent activity (EC_{50} = 8.7 μM for **8i** and 2.9 μM for **8l**) than curcumin (**7a**, EC_{50} = 22.6 μM) and ascorbic acid (EC_{50} = 15.7 μM). More importantly, the acetoxy substituent provided potent antioxidant activity (EC_{50} = 22.1–47.8 μM) even to the non-phenolic curcuminoids (**8j**, **8k**, and **8m**–**8o**), which demonstrated that the carbon-centered radicals are working as radical-trapping antioxidants. In addition, the radical-scavenging effect of the non-phenolic acetoxy-substituted curcuminoids was shown to be substantially diminished in acidic environment, which indicates that the antioxidant mechanism is anion-dependent (Fig. 4). Taken together, the non-phenolic curcuminoids seem to generate the carbon-centered radicals via electron transfer from the enolate anion, which is reminiscent of the initial stage of the SPLET mechanism. According to the SPLET mechanism, the equilibrium formation of the enolate anion is the rate-limiting step in the antioxidant mechanism of the curcuminoids. In non-phenolic curcuminoids in which the β-diketone moiety is the only plausible source of the radical, the anion concentration would be more crucial, and thus, stabilization of the enolate anion by the polar electron-withdrawing substituents such as the acetoxy group is likely to be the key factor for the antioxidant mechanism.

Table 1
EC_{50} values (amount of curcuminoids necessary to decrease the initial DPPH concentration by 50%) of curcuminoids prepared in this study



Curcuminoid	R_1	R_2	R_3	R_4	EC_{50} (μM)
7a	H	OMe	OH	H	22.6 ± 2.3
7b	H	OMe	OMe	H	>100
7c	H	OMe	OMe	OMe	>100
7d	H	OH	OH	OMe	11.3 ± 1.2
7e	H	H	OH	H	>100
7f	H	H	OMe	H	>100
7g	H	H	H	H	>100
8a	Me	OMe	OH	H	23.2 ± 1.7
8b	CH_2CHCH_2				23.2 ± 2.3
8c	OMe				30.3 ± 1.5
8d	SC(S)OEt				29.7 ± 2.0
8e	Me	OMe	OMe	H	>100
8f	CH_2CHCH_2				>100
8g	OMe				75.5 ± 1.7
8h	SC(S)OEt				>100
8i	OAc	OMe	OH	H	8.7 ± 0.6
8j	OAc	OMe	OMe	H	22.1 ± 2.7
8k	OAc	OMe	OMe	OMe	27.4 ± 0.8
8l	OAc	OH	OH	OMe	2.9 ± 0.3
8m	OAc	H	OH	H	26.3 ± 0.6
8n	OAc	H	OMe	H	32.8 ± 1.6
8o	OAc	H	H	H	47.8 ± 2.1
Ascorbic acid					15.7 ± 2.3

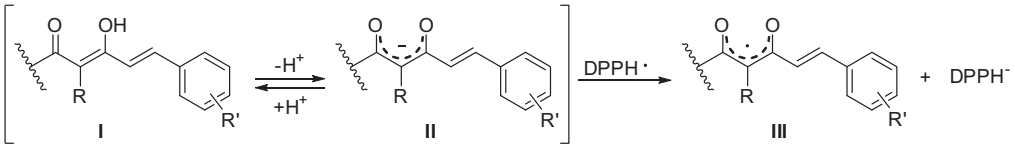


Figure 3. SPLET mechanism.

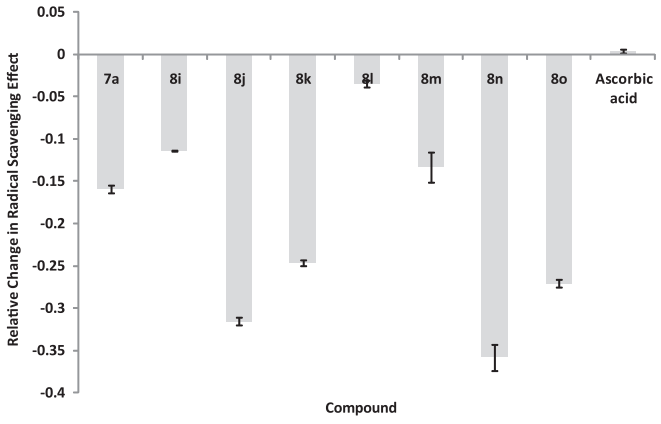


Figure 4. Relative change in radical-scavenging effect of the curcuminoids (100 μM) in the presence of acetic acid (100 mM). Absorbance was measured 30 min after mixing the curcuminoids with DPPH.

4. Experimental

4.1. Materials and general methods

Nuclear magnetic resonance spectra were recorded at 400 MHz for ^1H NMR and at 100 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. Chemical shift are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Coupling constants are reported in hertz (Hz). The chemical shifts are reported as parts per million (δ) relative to the solvent peak. All tested compounds were ⩾95% purity as determined by reverse phase HPLC. HPLC was performed on equipment with variable wavelength (VW) UV detector and C18—A 250 × 4.6 mm column. Analytical conditions were as follows: Gradient used was 20% acetonitrile in water containing 0.1% formic acid (0–8 min), 25–35% acetonitrile in water containing 0.1% formic acid (8–18 min), 35% acetonitrile in water containing 0.1% formic acid (18–25 min), 35–80% acetonitrile in water containing 0.1% formic acid (25–40 min), 80–100% acetonitrile in water containing 0.1% formic acid (40–45 min), and 100% acetonitrile in water

containing 0.1% formic acid (45–50 min). Flow was 1 mL/min. UV was detected at two different wavelengths (340 and 254 nm).

4.2. Synthesis of substituted acetoacetates (6a–6e)

4.2.1. 4-Hydroxy-3-methylpent-3-en-2-one (6a)

A solution of acetylacetone (**5**, 0.45 mL, 4.4 mmol) in acetone (10 mL) was treated with potassium carbonate (608 mg, 4.4 mmol) and iodomethane (0.28 mL, 4.4 mmol) and stirred under reflux for 12 h. After cooling to room temperature, the reaction mixture was filtered and concentrated under reduced pressure to give 4-hydroxy-3-methylpent-3-en-2-one (**6a**) as pale yellow syrup, which was used for the next step without further purification.

4.2.2. 3-(1-Hydroxyethylidene)hex-5-en-2-one (6b)

A solution of acetylacetone (**5**, 0.37 mL, 3.6 mmol) in acetone (10 mL) was treated with potassium carbonate (498 mg, 3.6 mmol) and allyl bromide (0.28 mL, 3.6 mmol) and stirred under reflux for 12 h. After cooling to room temperature, the reaction mixture was filtered and concentrated under reduced pressure to give 3-(1-hydroxyethylidene)hex-5-en-2-one (**6b**) as pale yellow syrup, which was used for the next step without further purification.

4.2.3. 4-Hydroxy-3-methoxypent-3-en-2-one (6c)

Boron trifluoride etherate (1.2 mL, 9.7 mmol) was added to a suspension of (diacetoxyiodo)benzene (3.127 g, 9.7 mmol) in methanol (20 mL). To the resulting mixture was added acetylacetone (**5**, 1 mL, 9.7 mmol). The contents were allowed to stir at room temperature for 5 h. After addition of aqueous sodium hydrogen carbonate solution (60 mL), the reaction mixture was extracted with dichloromethane (100 mL × 3), dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was then purified by column chromatography on silica gel (hexanes/ethyl acetate = 9:1) to afford 4-hydroxy-3-methoxypent-3-en-2-one (**6c**) as clear oil in 85% yield (100% enol-form): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.57 (s, 3H), 2.17 (s, 6H).

4.2.4. *O*-Ethyl-*S*-2-hydroxy-4-oxopent-2-en-3-yl carbonodithioate (6d)

3-Chloroacetylacetone (**4**, 0.3 mL, 2.7 mmol) was treated with potassium ethyl xanthate (426 mg, 2.7 mmol) in acetone (3 mL) and the reaction mixture was stirred at room temperature. After 4 h, the resulting solution was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (hexane/diethyl ether = 15:1) to afford *O*-ethyl-*S*-2-hydroxy-4-oxopent-2-en-3-yl carbonodithioate (**6d**) as clear oil in 75% yield (100% enol-form): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.69 (q, J = 7.1 Hz, 2H), 2.24 (s, 6H), 1.42 (t, J = 7.1 Hz, 3H).

4.2.5. 3-Acetoxy acetylacetone (6e)

3-Chloroacetylacetone (**4**, 0.5 mL, 4.4 mmol) in DMSO (5 mL) was treated with sodium acetate (726 mg, 8.8 mmol), and the resulting mixture was stirred at room temperature for 3 h. After addition of water (20 mL), the reaction mixture was extracted with diethyl ether (30 mL × 3), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was then purified by column chromatography on silica gel (hexanes/ethyl acetate = 8:1) to afford 3-acetoxy acetylacetone (**6e**) as clear oil (60% yield): Inseparable mixture (2.7:1) of the keto–enol tautomers: For keto-form; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.44 (s, 1H), 2.29 (s, 6H), 2.24 (s, 3H); for enol-form; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.26 (s, 3H), 2.02 (s, 6H).

4.3. General procedures for preparation of curcumin analogues (7 and 8)

Tributyl borate (10 mmol) was added to a solution of boric oxide (5.0 mmol) and acetylacetone (**4** or **6**, 5.0 mmol) in DMF (1.0 mL), and the resulting solution was stirred at 60 °C for 20 min. The mixture was treated with aromatic aldehyde (10 mmol) and stirred at 65 °C. After 10 min, a solution of 1,2,3,4-tetrahydroquinoline (0.1 mL) and acetic acid (0.3 mL) in DMF (1 mL) was added dropwise to the mixture, which was allowed to stir at 95 °C for 4 h. After cooling, aqueous acetic acid (20%, 50 mL) was added and the mixture was stirred at 70 °C for 1 h. The reaction mixture were extracted with ethyl acetate three times (200 mL × 3), washed with water, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/ethyl acetate = 2:1) followed by recrystallization from $CHCl_3$ to afford the desired curcuminoids as a yellow solid.

4.3.1. 1,7-Bis(3,4-dimethoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (7b)

(68% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm) 7.62 (d, J = 15.8 Hz, 2H), 7.32 (d, J = 1.9 Hz, 2H), 7.24 (dd, J = 1.9, 8.3 Hz, 2H), 7.01 (d, J = 8.3 Hz, 2H), 6.75 (d, J = 15.8 Hz, 2H), 6.00 (s, 1H), 3.88 (d, J = 6.8 Hz, 12H).

4.3.2. 5-Hydroxy-1,7-bis(3,4,5-trimethoxyphenyl)hepta-1,4,6-trien-3-one (7c)

(72% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.57 (d, J = 15.5 Hz, 2H), 6.78 (br s, 4H), 6.53 (d, J = 15.7 Hz, 2H), 5.86 (d, J = 1.1 Hz, 1H), 3.89–3.94 (m, 18H).

4.3.3. 1,7-Bis(3,4-dihydroxy-5-methoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (7d)

(35% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.52 (d, J = 15.6 Hz, 2H), 6.88 (br s, 2H), 6.62 (br s, 2H), 6.48 (d, J = 15.6 Hz, 2H), 5.80 (s, 1H), 3.91 (s, 6H).

4.3.4. 5-Hydroxy-1,7-bis(4-hydroxyphenyl)hepta-1,4,6-trien-3-one (7e)

(40% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm) 7.55–7.67 (m, 6H), 6.91 (d, J = 8.6 Hz, 4H), 6.65 (d, J = 15.8 Hz, 2H), 5.99 (s, 1H).

4.3.5. 5-Hydroxy-1,7-bis(4-methoxyphenyl)hepta-1,4,6-trien-3-one (7f)

(72% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.93 (d, J = 15.6 Hz, 2H), 7.57 (d, J = 8.8 Hz, 4H), 6.94 (d, J = 8.8 Hz, 4H), 6.58 (d, J = 15.5 Hz, 2H), 6.00 (s, 1H), 3.87 (s, 6H).

4.3.6. 5-Hydroxy-1,7-diphenylhepta-1,4,6-trien-3-one (7g)

(55% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm) 7.68–7.72 (m, 6H), 7.40–7.47 (m, 6H), 6.89 (d, J = 16.0 Hz, 2H), 6.13 (s, 1H).

4.3.7. (1*E*,4*Z*,6*E*)-5-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-methylhepta-1,4,6-trien-3-one (8a)

(35% yield): 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.68 (d, J = 15.4 Hz, 2H), 7.18 (dd, J = 1.5, 8.3 Hz, 2H), 7.06 (d, J = 1.5 Hz, 2H), 6.93–6.99 (m, 4H), 3.96 (s, 6H), 2.21 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 13.6, 57.1 (2C), 107.1, 112.7, 112.9, 117.0 (2C), 119.2, 123.6, 124.7, 125.1, 127.1, 128.1, 142.9, 145.3, 149.3 (2C), 150.7, 151.1, 183.7, 197.7; HRMS (ESI) m/z calcd for $C_{22}H_{23}O_6$ $[M+H]^+$: 383.14946, found: 383.15061.

4.3.8. (1*E*,4*Z*,6*E*)-4-Allyl-5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (8b)

(30% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.69 (d, J = 15.4 Hz, 2H), 7.15 (dd, J = 1.5, 8.2 Hz, 2H), 7.02 (d, J = 1.5 Hz, 2H), 6.93 (d, J = 8.2 Hz, 2H), 6.85 (d, J = 15.4 Hz, 2H), 5.99–6.09 (m, 1H), 5.14–5.18 (m, 2H), 3.94 (s, 6H), 3.33 (d, J = 5.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 30.0, 56.0 (2C), 107.7, 107.7, 110.2 (2C), 114.9 (2C), 116.0, 118.4 (2C), 122.5 (2C), 128.1 (2C), 134.6, 141.6 (2C), 146.7 (2C), 147.8 (2C), 194.7; HRMS (ESI) m/z calcd for $C_{24}H_{25}O_6$ 409.16511 $[M+H]^+$, found 409.16566.

4.3.9. (1*E*,4*E*,6*E*)-5-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-methoxyhepta-1,4,6-trien-3-one (8c)

(55% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm) 7.64 (d, J = 15.8 Hz, 2H), 7.37 (d, J = 1.9 Hz, 2H), 7.26 (dd, J = 1.9, 8.2 Hz, 2H), 7.16 (d, J = 15.8 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 3.94 (s, 6H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 55.9 (2C), 64.21, 112.1 (2C), 115.1 (2C), 115.9 (2C), 123.2 (2C), 126.6 (2C), 139.5, 141.9 (2C), 148.1 (4C), 149.7, 177.7; HRMS (ESI) m/z calcd for $C_{22}H_{23}O_7$ 399.14438 $[M+H]^+$, found 399.14479.

4.3.10. *O*-Ethyl S-(1*E*,3*E*,6*E*)-3-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,3,6-trien-4-yl carbonodithioate (8d)

(53% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm) 7.78 (d, J = 15.5 Hz, 2H), 7.35 (d, J = 15.5 Hz, 2H), 7.31 (br s, 2H), 7.25 (d, J = 8.2 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 4.67 (q, J = 7.1 Hz, 2H), 3.91 (s, 6H), 1.37 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 12.1, 54.2 (4C), 69.8, 100.2 (2C), 110.9 (2C),114.4 (2C),114.9, 121.8 (2C),124.4 (2C), 142.9, 146.4 (4C),148.6, 185.5; HRMS (ESI) m/z calcd for $C_{24}H_{26}O_7S_2$ 489.10417 $[M+H]^+$, found 489.10438.

4.3.11. (1*E*,4*Z*,6*E*)-1,7-Bis(3,4-dimethoxyphenyl)-5-hydroxy-4-methylhepta-1,4,6-trien-3-one (8e)

(45% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.70 (d, J = 15.4 Hz, 2H), 7.19 (dd, J = 1.8, 8.3 Hz, 2H), 7.09 (d, J = 1.8 Hz, 2H), 7.35 (d, J = 15.4 Hz, 2H), 6.89 (d, J = 8.3 Hz, 2H), 3.97 (s, 6H), 3.93 (s, 6H), 2.19 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 11.9, 55.9 (2C), 56.0 (2C), 106.5 (2C), 110.8, 111.0, 111.9, 119.4, 123.5, 123.6, 123.9, 127.3, 128.3, 141.6, 143.9, 149.4 (2C), 151.3, 151.7, 182.8, 196.8; HRMS (ESI) m/z calcd for $C_{24}H_{27}O_6$ 411.18076 $[M+H]^+$, found: 411.18125.

4.3.12. (1*E*,4*Z*,6*E*)-4-Allyl-1,7-bis(3,4-dimethoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (8f)

(42% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.71 (d, J = 15.4 Hz, 2H), 7.17 (dd, J = 1.9, 8.3 Hz, 2H), 7.06 (d, J = 1.9 Hz, 2H), 6.86–6.90 (m, 4H), 6.00–6.08 (m, 1H), 5.19 (d, J = 0.7 Hz, 1H), 5.14–5.19 (m, 1H), 3.94 (s, 6H), 3.93 (s, 6H), 3.34 (d, J = 5.1 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 29.8, 54.8 (4C), 62.7, 109.7, 110.0, 110.9, 122.3, 122.7, 122.9, 126.2, 127.1, 138.1, 140.7, 143.2, 148.2 (4C), 150.2, 162.1, 182.3, 193.8, 228.3; HRMS (ESI) m/z calcd for $C_{26}H_{29}O_6$ 437.19641 $[M+H]^+$, found 437.19690.

4.3.13. (1*E*,4*E*,6*E*)-1,7-Bis(3,4-dimethoxyphenyl)-5-hydroxy-4-methoxyhepta-1,4,6-trien-3-one (8g)

(40% yield): ^{1}H NMR (400 MHz, CD_3COCD_3) δ (ppm) 7.65 (d, J = 15.9 Hz, 2H), 7.37 (d, J = 1.9 Hz, 2H), 7.32 (dd, J = 1.9, 8.3 Hz, 2H), 7.19 (d, J = 15.8 Hz, 2H), 7.03 (d, J = 8.3 Hz, 2H), 3.90 (s, 6H), 3.88 (s, 6H), 3.73 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 56.9 (2C), 57.0 (2C), 65.4, 112.3 (2C), 113.0 (2C), 117.3 (2C), 124.3 (2C), 129.0 (2C), 137.8, 142.8 (2C), 150.4 (4C), 152.5, 179.0; HRMS (ESI) m/z calcd for $C_{24}H_{27}O_7$ 427.17568 $[M+H]^+$, found 427.17605.

4.3.14. *S*-(1*E*,3*E*,6*E*)-1,7-Bis(3,4-dimethoxyphenyl)-3-hydroxy-5-oxohepta-1,3,6-trien-4-yl *O*-ethyl carbonodithioate (8h)

(65% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.79 (d, J = 15.5 Hz, 2H), 7.38 (d, J = 15.5 Hz, 2H), 7.32 (br s, 1H), 7.29 (br s, 3H), 7.04 (d, J = 8.1 Hz, 2H), 4.67 (q, J = 7.1 Hz, 2H), 3.90 (s, 12H), 1.37 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm) 14.0, 55.0, 56.2 (4C), 71.7 (2C), 112.2 (2C), 112.7 (2C), 118.8 (2C), 124.0 (2C), 128.7 (2C), 144.9 (2C), 150.7 (2C), 153.2, 187.8, 188.5, 215.1; HRMS (ESI) m/z calcd for $C_{26}H_{29}O_7S_2$ 517.13547 $[M+H]^+$, found 517.13595.

4.3.15. 3-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,3,6-trien-4-yl acetate (8i)

(65% yield): Inseparable mixture (2.2:1) of keto–enol tautomers; for keto-form, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.73 (d, J = 15.9 Hz, 2H), 7.16 (dd, J = 1.8, 8.2 Hz, 2H), 7.07 (d, J = 1.8 Hz, 2H), 6.92 (d, J = 8.3 Hz, 2H), 6.89 (d, J = 16.0 Hz, 2H), 5.99 (s, 1H), 3.94 (s, 6H), 2.02 (s, 3H); for enol-form, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 15.48 (s, OH), 7.67 (d, J = 15.7 Hz, 2H), 7.16 (dd, J = 1.8, 8.2 Hz, 2H), 7.00 (d, J = 1.7 Hz, 2H), 6.94 (d, J = 8.2 Hz, 2H), 6.62 (d, J = 15.6 Hz, 2H), 3.94 (s, 6H), 2.30 (s, 3H); as a mixture of tautomers, ^{13}C NMR (100 MHz, CD_3COCD_3) δ (ppm) 20.6 (2C), 56.4 (4C), 100.9 (2C), 102.7 (2C), 112.0 (2C), 112.4 (2C), 115.8 (2C), 116.3 (4C), 120.0 (2C), 124.1 (2C), 125.0 (2C), 127.3 (2C), 128.0 (2C), 143.3 (2C), 146.3 (2C), 148.7 (2C), 148.8, 150.5 (2C), 151.0, 168.5, 171.1 (2C), 190.5, 206.2 (2C); HRMS (ESI) m/z calcd for $C_{23}H_{23}O_8$ 427.13929 $[M+H]^+$, found 427.13938.

4.3.16. 1,7-Bis(3,4-dimethoxyphenyl)-3-hydroxy-5-oxohepta-1,3,6-trien-4-yl acetate (8j)

(52% yield): Inseparable mixture (1:4.8) of keto–enol tautomers; for keto-form, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.75 (d, J = 15.9 Hz, 2H), 7.20 (dd, J = 1.6, 8.3 Hz, 2H), 7.09 (d, J = 1.6 Hz, 2H), 6.91 (d, J = 15.9 Hz, 2H), 6.87 (d, J = 8.3 Hz, 2H), 6.00 (s, 1H), 3.92 (s, 12H), 2.37 (s, 3H); for enol-form, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 15.48 (s, OH), 7.69 (d, J = 15.6 Hz, 2H), 7.17 (dd, J = 1.5, 8.3 Hz, 2H), 7.04 (d, J = 1.5 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 6.63 (d, J = 15.6 Hz, 2H), 3.92 (s, 12H), 2.40 (s, 3H); as a mixture of tautomers, ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 22.3 (6C), 57.2 (8C), 83.5, 112.2 (4C), 112.9, 113.2 (4C), 116.9, 121.9, 125.0, 125.8, 128.3, 128.9, 144.1 (2C), 146.7 (2C), 150.5 (4C), 150.6 (4C), 153.0, 153.4 (2C), 171.0, 172.5, 178.3, 191.5 (2C); HRMS (ESI) m/z calcd for $C_{25}H_{27}O_8$ 455.17059 $[M+H]^+$, found 455.17089.

4.3.17. 3-Hydroxy-5-oxo-1,7-bis(3,4,5-trimethoxyphenyl)hepta-1,3,6-trien-4-yl acetate (8k)

(60% yield, 100% enol-form): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 15.34 (s, OH), 7.67 (d, J = 15.6 Hz, 2H), 6.77 (s, 4H), 6.65 (d, J = 15.6 Hz, 2H), 3.89 (s, 18H), 2.40 (s, 3H); as a mixture of tautomers, ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 21.1 (2C), 56.1 (8C), 60.5 (4C), 82.0 (2C), 107.0 (8C), 117.5, 122.5 (4C), 129.8 (4C), 130.3, 140.4, 140.6 (2C), 143.0, 145.4 (2C), 153.5 (8C), 169.8, 171.2, 177.0, 190.4, 207.1 (2C); HRMS (ESI) m/z calcd for $C_{27}H_{31}O_{10}$ 515.19172 $[M+H]^+$, found 515.19135.

4.3.18. 1,7-Bis(3,4-dihydroxy-5-methoxyphenyl)-3-hydroxy-5-oxohepta-1,3,6-trien-4-yl acetate (8l)

(35% yield): Inseparable mixture (1.25:1) of keto–enol tautomers; for keto-form, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.64 (d, J = 15.6 Hz, 2H), 6.90 (br s, 2H), 6.86 (d, J = 15.6 Hz, 2H), 6.70 (br s, 2H), 5.73 (s, 1H), 3.96 (s, 6H), 2.12 (s, 3H); for enol-form, ^{1}H

NMR (400 MHz, $CDCl_3$) δ (ppm) 15.49 (s, OH), 7.51 (d, J = 15.6 Hz, 2H), 6.87 (br s, 2H), 6.60 (br s, 2H), 6.46 (d, J = 15.6 Hz, 2H), 3.91 (s, 6H), 2.12 (s, 3H); as a mixture of tautomers, ^{13}C NMR (100 MHz, CD_3COCD_3) 42.7 (2C), 57.0 (4C), 110.5 (4C), 111.5 (4C), 112.6, 114.9, 115.2 (2C), 120.4 (2C), 126.8 (2C), 127.6 (2C), 127.7 (2C), 138.2, 139.1 (2C), 141.7, 143.0 (2C), 146.7 (2C), 146.9 (2C), 147.0 (2C), 147.4 (2C), 190.9, 191.8, 191.9, 195.4, 200.7 (2C); HRMS (ESI) m/z calcd for $C_{23}H_{23}O_{10}$ 459.12912 $[M+H]^+$, found 459.12875.

4.3.19. 3-Hydroxy-1,7-bis(4-hydroxyphenyl)-5-oxohepta-1,3,6-trien-4-yl acetate (8m)

(52% yield): Inseparable mixture (1:2.3) of keto–enol tautomers; for keto-form, ^1H NMR (400 MHz, CD_3COCD_3) δ (ppm) 7.70 (d, J = 15.9 Hz, 2H), 7.57–7.64 (m, 4H), 7.02 (d, J = 15.9 Hz, 2H), 6.89–6.93 (m, 4H), 6.02 (s, 1H), 2.44 (s, 3H); for enol-form, ^1H NMR (400 MHz, CD_3COCD_3) δ (ppm) 7.68 (d, J = 15.7 Hz, 2H), 7.57–7.64 (m, 4H), 6.89–6.93 (m, 4H), 6.86 (d, J = 15.7 Hz, 2H), 2.44 (s, 3H); as a mixture of tautomers, ^{13}C NMR (100 MHz, CD_3COCD_3) 22.29 (2C), 33.7 (2C), 38.2 (2C), 117.1, 118.4 (4C), 118.5 (4C), 121.1, 128.4, 129.1 (2C), 129.8, 131.8, 133.2 (4C), 133.5 (4C), 144.7 (2C), 147.7, 162.5 (2C), 163.1, 165.2 (2C), 171.6, 172.8, 179.6, 192.3 (2C); HRMS (ESI) m/z calcd for $C_{21}H_{19}O_6$ 367.11816 $[M+H]^+$, found 367.11784.

4.3.20. 3-Hydroxy-1,7-bis(4-methoxyphenyl)-5-oxohepta-1,3,6-trien-4-yl acetate (8n)

(60% yield, 100% enol-form): ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 15.49 (s, OH), 7.70 (d, J = 15.7 Hz, 2H), 7.50 (d, J = 8.6 Hz, 4H), 6.91 (d, J = 8.6 Hz, 4H), 6.65 (d, J = 15.7 Hz, 2H), 3.85 (s, 6H), 2.41 (s, 3H); as a mixture of tautomers, ^{13}C NMR (100 MHz, CD_3COCD_3) 20.4 (2C), 55.7 (4C), 113.5, 113.6, 115.1 (8C), 115.2 (2C), 116.0 (4C), 120.1, 127.7, 127.9, 128.4 (2C), 131.1 (8C), 131.5 (2C), 142.5 (2C), 145.4, 162.6 (2C), 163.1, 170.9, 206.0 (2C); HRMS (ESI) m/z calcd for $C_{23}H_{23}O_6$ 395.14946 $[M+H]^+$, found 395.14985.

4.3.21. 3-Hydroxy-5-oxo-1,7-diphenylhepta-1,3,6-trien-4-yl acetate (8o)

(40% yield): Inseparable mixture (1: 4.3) of keto–enol tautomers; for keto-form, ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.79 (d, J = 16.0 Hz, 2H), 7.58–7.60 (m, 5H), 7.39–7.40 (m, 5H), 7.06 (d, J = 16.0 Hz, 2H), 6.01 (s, 1H), 2.32 (s, 3H); for enol-form, ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 15.27 (s, OH), 7.75 (d, J = 15.6 Hz, 2H), 7.54–7.56 (m, 5H), 7.39–7.40 (m, 5H), 6.78 (d, J = 15.6 Hz, 2H), 2.42 (s, 3H); as a mixture of tautomers, ^{13}C NMR (100 MHz, $CDCl_3$) 20.6 (2C), 106.8, 114.2, 116.4 (2C), 118.2 (2C),121.2 (2C), 127.1 (8C), 128.2 (8C), 131.2, 132.4 (4C), 141.3 (2C), 142.5 (2C), 145.2, 170.3 (2C), 171.5, 178.5, 191.3 (2C); HRMS (ESI) m/z calcd for $C_{21}H_{19}O_4$ 335.12833 $[M+H]^+$, found 335.12885.

4.4. DPPH free radical-scavenging activity assay

Free radical-scavenging activity of curcuminoid was measured using DPPH radical.[16] In a typical procedure, in each well of a 96-well plate, a freshly prepared methanol solution of DPPH (190 μL, 0.1 mM) was mixed with 10 μL of a methanol (1% DMSO) solution of the curcuminoids at different concentrations (0.01, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mM). The absorption spectra at 517 nm were recorded every 2 min. For each curcuminoid concentration tested, the percentage of DPPH remaining reached the steady state after 30 min. Therefore, the percentage of residual DPPH after 30 min was plotted against concentration of the curcuminoids. The values EC_{50} were determined from this plot.

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