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Original article

Feruloylacetone as the model compound of half-curcumin: Synthesis and antioxidant properties

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ABSTRACT

In order to clarify the contribution of phenolic and enolic hydroxyl group to the antioxidant capacity of feruloylacetone, a model compound of half-curcumin, 6-(*p*-hydroxy-*m*-methoxyphenyl)-5-hexene-2,4-dione (FT), 6-(*p*-benzyloxy-*m*-methoxyphenyl)-5-hexene-2,4-dione (BMFT), 6-(*m*,*p*-dihydroxyphenyl)-5-hexene-2,4-dione (DDFT), 6-(*p*-hydroxy-*m*-methoxyphenyl)hexane-2,4-dione (DHFT), 6-(*p*-hydroxy-*m*-methoxyphenyl)-5-hexene-2,4-diol (THFT), and ethyl 2-(*p*-hydroxy-*m*-methoxybenzylidene)-3-oxobutanoate (EOFT) were synthesized. The radical-scavenging abilities of these compounds were tested by trapping 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\bullet}$), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), and galvinoxyl radicals. The reductive capacities were screened by quenching singlet oxygen and by inhibiting the oxidation of linoleic acid. They were also employed to inhibit the oxidation of DNA mediated by hydroxyl radical and 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH). In addition, they were applied to protect erythrocytes against AAPH- and hemin-induced hemolysis. The obtained results revealed that the antioxidant capacity of half-curcumin was derived from the phenolic-OH and the conjugated linkage between phenolic and enolic-OH. The enolic-OH itself cannot trap radicals.

1. Introduction

Curcumin (1,7-bis(*p*-hydroxy-*m*-methoxyphenyl)-1,6-heptdiene-3,5-dione) is the major component in *Curcuma longa*. Curcumin and its *in vivo* metabolites can prevent inflammatory factor, retard nerval, cardiovascular, and pulmonary degenerations, and even inhibit the occurrence of cancer [1–4]. Recently, much research attention focuses on the synthesis of curcumin-related compounds and the evaluation of their bioactivities [5]. The enolic tautomer of curcumin was believed to mainly contribute to the bioactivities and photophysical and photochemical properties [6,7]. The anti-inflammatory activity of curcumin was enhanced if the structural feature of diketone in curcumin was replaced by cycloketone [8]. Meanwhile, dehydrozingerone whose structure was shown in Scheme 1 was regarded to be the model compound of half-curcumin [9]. The ability of phenolic-OH in dehydrozingerone to trap radicals was estimated in the experimental system of the autoxidation of linoleic acid (LH) [10]. Dehydrozingerone is also usually applied in the design of the antitumor drugs [11,12].

2. Chemistry

Some investigations revealed that hydrogen atom in the enolic-OH other than the methylene of curcumin can be abstracted by radicals in polar solvents [13,14]. Thus, two vanillylidene groups, enol–keto tautomerism, and the conjugated system in the carbon chain play an important role in the bioavailability of curcumin [15,16]. Furthermore, in order to clarify the relationship between the structure of half-curcumin and the antioxidant property, 6-(*p*-hydroxy-*m*-methoxyphenyl)-5-hexene-2,4-dione (feruloylacetone, FT, structure shown in Scheme 1) involves enol–keto tautomerism, the conjugated system, and only one vanillylidene group together with its structural analogs were prepared following Scheme 2. The phenolic-OH in FT was etherified by benzyl group to afford 6-(*p*-benzyloxy-*m*-methoxyphenyl)-5-hexene-2,4-dione (BMFT). The antioxidant capacity of BMFT was only contributed from the enolic-OH. The demethylation of FT produced 6-(*m*,*p*-dihydroxyphenyl)-5-hexene-2,4-dione (DDFT) that contained two phenolic-OHs. The C=C in FT was reduced to generate 6-(*p*-hydroxy-*m*-methoxyphenyl)hexane-2,4-dione (DHFT). The conjugated linkage between phenolic and enolic-OH was cut off, and the antioxidant capacity of DHFT was individually contributed from phenolic and enolic-OH. Two C=O in FT were reduced to form 6-(*p*-hydroxy-*m*-methoxyphenyl)-5-hexene-2,4-diol (THFT). The antioxidant capacity of THFT was only

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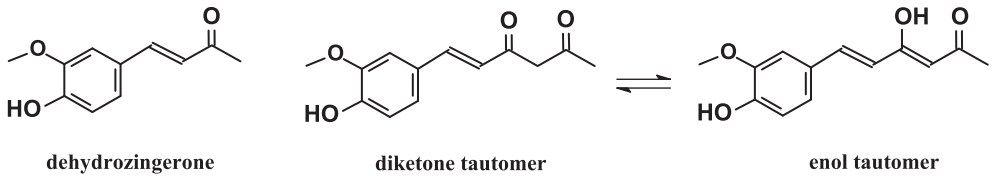


Scheme 1. Structures of dehydrozingerone and tautomers of feruloylacetone.

derived from the phenolic-OH. Finally, ethyl 2-(*p*-hydroxy-*m*-methoxybenzylidene)-3-oxobutanoate (EOFT) was prepared by vanillin and ethyl acetoacetate in order to detect the influence of ester group on the antioxidant capacity.

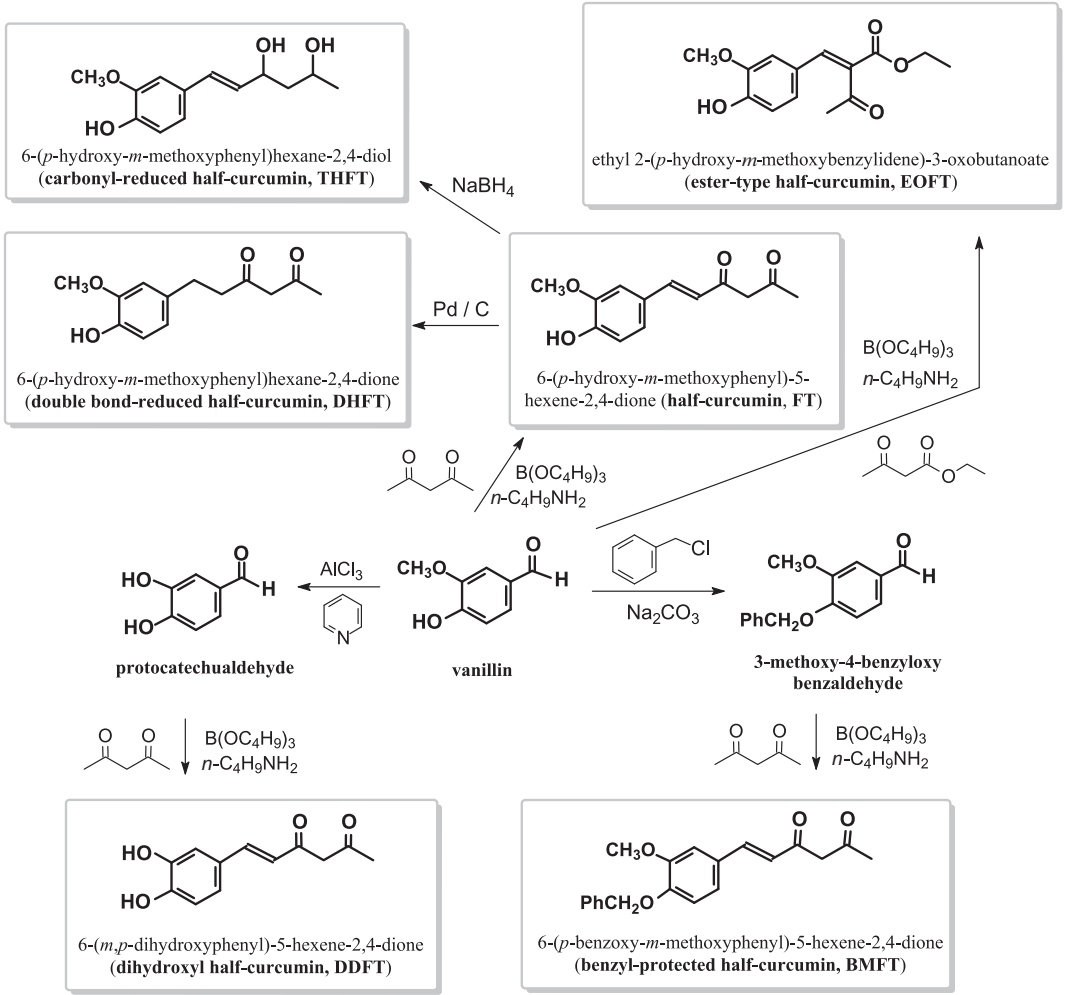
3. Pharmacology

Curcumin and its derivatives are lipophilic antioxidants, but the insolubility in water does not affect the bioavailability *in vivo* [2]. The antioxidant activities of curcumin derivatives were evaluated in chemical and biological experimental systems [17], which were still applied in this work. In the chemical experimental systems, half-curcumin derivatives were applied to trap 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+}$•), 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and galvinoxyl radicals, and to quench singlet oxygen ($^1O_2$). These half-curcumin derivatives were employed to retard the autoxidation of linoleic acid (LH), and to inhibit 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH, R–N=N–R, R = $-CMe_2C(=NH)NH_2$)-induced oxidation of methyl linoleate. In biological experimental systems, half-curcumin derivatives were applied to inhibit AAPH, Cu^{2+}/glutathione (GSH), and hydroxyl radical (•OH)-induced oxidations of DNA. These compounds were also used to inhibit AAPH- and hemin-induced hemolysis of erythrocytes. Therefore, the contribution of phenolic and enolic-OH to the antioxidant capacity of half-curcumin will be clarified by using chemical and biological experimental systems.

4. Results

4.1. Scavenging free radicals and quenching $^1O_2$

The antioxidant abilities of half-curcumin derivatives can be directly characterized by interacting with $ABTS^{+}$•, DPPH and galvinoxyl radicals. Mixing the solutions of half-curcumin derivatives with these radicals decreases the absorbance of radical solution with the reaction period increasing. The decay rates of the concentrations



Scheme 2. Synthetic routine for half-curcumin derivatives in this work.

Table 1
Decay rates of the concentration of free radicals in the presence of half-curcumin derivatives.[a]

Free radicals	d[radical]/dt (μM min^{-1})					
	FT	DDFT	THFT	DHFT	EOFT	BMFT
$ABTS^{+\bullet}$	6.04	7.56	6.26	3.85	1.78	–
DPPH	22.29	41.21	8.57	16.95	7.54	–
Galvinoxyl	0.13	0.08	0.13	0.06	0.02	–

[a] The final concentrations of half-curcumin derivatives were 50.0 μM in the test of trapping $ABTS^{+\bullet}$ and DPPH radicals, and 25.0 μM in the test of trapping galvinoxyl radical.

of these radicals can be calculated by the decay rates of the absorbance multiplying the extinction coefficient (ε) of these radicals, and are listed in Table 1. It can be found that the addition of half-curcumin derivatives lead to fast decrease of DPPH radical, and slow decrease of galvinoxyl radical. BMFT cannot react with these radicals, while DDFT exhibits relative high activity to trap these radicals.

$^1O_2$ is formed in the mixture of histidine, NaClO and H_2O_2, and can decrease the absorbance of 4-nitroso-*N*,*N*-dimethylaniline (NDMA) at 440 nm (A_{440}). The absorbance of NDMA was 1.013 at the beginning of the reaction at 30 °C, and was 0.458 after 40 min since $^1O_2$ generated in the reaction mixture decolorizes NDMA. In the presence of 250 μM EOFT the absorbance of NDMA just decreases to 0.993 after 40 min, implying that EOFT is able to preserve NDMA against $^1O_2$-induced decolorization by quenching $^1O_2$. Hence, the percentage of $^1O_2$ quenched by EOFT can be calculated as $(0.993 - 0.458)/(1.013 - 0.458) \times 100 = 96.4$. Fig. 1 illustrates the relationships between the quenched percentages of $^1O_2$ and the concentrations of half-curcumin derivatives employed. It can be found that THFT cannot quench $^1O_2$, and the quenched percentages of $^1O_2$ increase with the concentrations of FT and DHFT. On the other hand, a maximum percentage of quenched $^1O_2$ is found when 150 μM BMFT is applied. EOFT and DDFT quench $^1O_2$ concentration-dependently. The entire sequence of half-curcumin derivatives to quench $^1O_2$ is EOFT ∼ DDFT > BMFT > FT ∼ DHFT.

4.2. Protecting linoleic acid and methyl linoleate

LH and β-carotene can form water-soluble emulsion in the presence of Triton X-100 or Tween. The oxygen dissolved in water oxidizes LH to form peroxyl radical of LH (LOO•) that can bleach β-carotene. As shown as the blank experiment in Fig. 2, the decay of the absorbance at 460 nm indicates that LOO• in the autoxidation of LH bleaches β-carotene continuously. The addition of 50.0 μM half-curcumin derivatives retards the consumption of β-carotene, indicating that half-curcumin derivatives protect LH by inhibiting the formation of LOO•. FT and THFT can also retard the decay of the absorbance of β-carotene. In particular, DDFT is the most efficient antioxidant to keep the absorbance of β-carotene almost invariable. However, the decays of β-carotene in the presence of BMFT, EOFT and DHFT approach to that of the blank experiment, indicating that the abilities of BMFT, EOFT and DHFT to protect LH are very low.

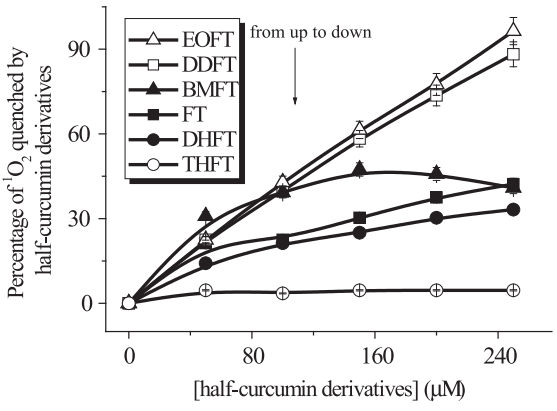


Fig. 1. Percentages of $^1O_2$ quenched by various concentrations of half-curcumin derivatives at 30 °C for 40 min.

The decomposition of AAPH generates radical that combines with oxygen to form a water-soluble peroxyl radical (ROO•), and ROO• is a radical-initiator to oxidize LH. Thus, AAPH-induced oxidation of LH is an *in vitro* experimental system usually employed to mimic polyunsaturated fatty acid (PUFA) undergoing oxidation. Measuring the formation of peroxide of LH (LOOH) [18] or the depletion of LH [19] can follow the oxidative process of LH. Because methyl ester of LH is readily evaporated in GC, methyl linoleate instead of LH is employed if GC is used to follow the oxidation [20]. The panel B and C in Fig. 2 illustrate the decays of the concentration of methyl linoleate in the presence of half-curcumin derivatives. In the blank experiment, the concentration of methyl linoleate decreases continuously, indicating that AAPH exhausts methyl linoleate successively. With BMFT and EOFT added, the decreases of the concentration of methyl linoleate are close to that of the blank experiment, indicating that BMFT and EOFT cannot hinder the oxidation of methyl linoleate. As panel C of Fig. 2 shows, the additions of DDFT, FT, DHFT and THFT retard the decay of the concentration of methyl linoleate for a period, and then, the concentration of methyl linoleate decreases as in the blank experiment. The inhibition periods (t_{inh}) generated by DDFT, FT, DHFT and THFT are shown as the cross-point of the tangents of the inhibitive and the oxidative periods, and listed in Table 2. DDFT and FT generate longer t_{inh} than DHFT and THFT, revealing that DDFT and FT possess higher antioxidant abilities to inhibit AAPH-induced oxidation of methyl linoleate than DHFT and THFT.

4.3. Effects of half-curcumin derivatives on Cu^{2+}/GSH-, •OH- and AAPH-induced oxidation of DNA

The intracellular GSH and Cu(II) oxidize DNA to form carbonyl species [21], which can be measured at 535 nm after reacting with thiobarbituric acid (TBA) [22]. So, carbonyl species deriving from the oxidation of DNA are called TBA reactive substance (TBARS) as well, and are indexed to characterize the oxidative extent of DNA. As an *in vitro* experimental system to mimic DNA destroyed by Cu^{2+}/GSH, the absorbance of TBARS in the blank experiment (A_{ref}) is assigned as 100% when DNA is mixed with Cu^{2+}/GSH for 2 h. Meanwhile, the absorbance of TBARS in the presence of half-curcumin derivatives (A_{detect}) is compared with A_{ref} to express the effects of half-curcumin derivatives on Cu^{2+}/GSH-induced oxidation of DNA. The left columns in Fig. 3 show $A_{detect}/A_{ref} \times 100$ in the presence of half-curcumin derivatives. In the presence of half-curcumin derivatives all the percentage of TBARS are higher than that in the blank experiment, indicating that the additions of half-curcumin derivatives improve Cu^{2+}/GSH-induced oxidation of DNA, and generate much more TBARS. The half-curcumin derivatives behave as prooxidants in this case. Especially, the prooxidant ability of DDFT is much higher than that of FT. The prooxidant abilities of other half-curcumin derivatives are higher than FT, and lower than DDFT.

•OH is conveniently prepared by mixing tetrachlorohydroquinone (TCHQ) with H_2O_2 [23]. The attack of •OH to ribosyl moiety destroys DNA and form TBARS [24], thus, •OH-induced oxidation of DNA is capable of an *in vitro* experimental system to screen the antioxidant ability. TBARS generated in the mixture of DNA, TCHQ and H_2O_2 is measured after 30 min, and the absorbance of TBARS in the blank experiment is assigned as 100%. The absorbance of TBARS in the presence of half-curcumin derivatives is compared with that in the blank experiment, and shown as the right column in Fig. 3. The

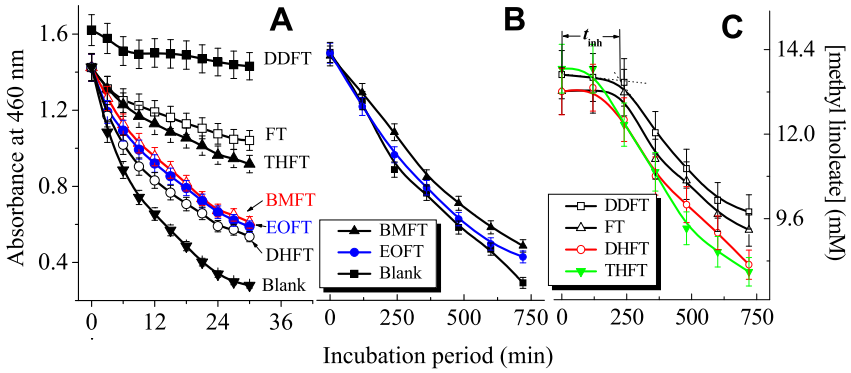


Fig. 2. The decay of the absorbance of β-carotene (panel A), and the decay of the concentration of methyl linoleate (panel B and C) in the presence of half-curcumin derivatives.

percentages of TBARS formed in the presence of half-curcumin derivatives are lower than that in the blank experiment, so, half-curcumin derivatives are active to protect DNA. Especially, BMFT, FT, and DHFT possess relative high efficacy to protect DNA, while the abilities of DDFT, EOFT, and THFT are lower than that of BMFT, FT, and DHFT.

The peroxyl radical (ROO•) deriving from AAPH abstracts an H atom from the C-4′ atom of DNA, leading to strand breaks [25], and generating TBARS eventually [26]. The formation of TBARS increases with the reaction period in the blank experiment [17,19], and the addition of half-curcumin derivatives inhibits the generation of TBARS for a period. In this work the inhibition period (t_{inh}) is measured when different concentrations of half-curcumin derivatives are added. The relationships between t_{inh} and the concentrations of half-curcumin derivatives are illustrated in panel A of Fig. 4. All the half-curcumin derivatives are able to protect DNA against AAPH-induced oxidation since t_{inh} increases with the concentration of half-curcumin derivatives. Moreover, the linear relationships in the panel A of Fig. 4 are quantitatively expressed by the equations of t_{inh} ~ [half-curcumin derivatives], and listed in Table 3.

The linear regressive analysis results in the equations, in which the constants balance the equation, and the coefficients reveal the sensitivity of t_{inh} to the variety of the concentration of half-curcumin derivatives. Higher coefficient in the equation implies that t_{inh} increases more remarkably with the concentration of the half-curcumin, and the half-curcumin possesses higher antioxidant capacity. It can be found in Table 3 that DDFT has the highest activity to protect DNA, while BMFT cannot behave as an antioxidant in this case. Hence, the antioxidant capacity of half-curcumin derivatives is in the sequence of DDFT > DHFT > FT > THFT > EOFT.

4.4. Effects of half-curcumin derivatives on AAPH- and hemin-induced hemolysis of erythrocytes

AAPH-induced hemolysis is a convenient *in vitro* experimental system to mimic erythrocytes undergoing oxidative stress [27]. Hemolysis does not occur immediately when erythrocytes encounter AAPH because of the endogenous antioxidants in the erythrocyte membrane, and takes place after the complete exhaustion of endogenous antioxidants, leading to a lag time (t_{lag}). Adding exogenous antioxidants prolongs the t_{lag}, and the difference of t_{lag} in the presence and absence of the additional antioxidants is designed as inhibition period (t_{inh}) representing the ability of the antioxidant to protect erythrocytes [28]. The t_{inh} generated by adding different concentrations of half-curcumin derivatives are measured and illustrated in panel B of Fig. 4. The addition of 18 and 36 μM BMFT generates −80 and −56 min of t_{inh}, respectively, and till the concentration of BMFT exceeds 54 μM, BMFT generates a positive t_{inh}. This fact implies that low concentration of BMFT plays a prooxidant role to improve the hemolysis. Furthermore, t_{inh} increase with the concentrations of other half-curcumin derivatives, and the equations of t_{inh} ~ [half-curcumin derivatives] are listed in Table 3. The sequence of half-curcumin derivatives to protect erythrocytes against AAPH-induced hemolysis is similar to that in protecting DNA, *viz.*, DDFT > DHFT > BMFT > FT > THFT > EOFT.

Hemin can intercalate into the membrane lipid of erythrocyte, accelerate the potassium leakage, and dissociate skeletal proteins, leading to hemolysis eventually [29]. Thus, if an antioxidant can protect erythrocyte against hemin-induced hemolysis, it can be regarded as a membrane-stabilizer. The absorbance of hemoglobin (535 nm) is measured when different concentrations of half-curcumin derivatives are applied in hemin-induced hemolysis, and outlined in Fig. 5. It can be found that only DDFT protects erythrocytes against hemin-induced hemolysis with the concentration increasing. FT and THFT cannot protect erythrocytes even high concentration applied. EOFT and DHFT improve the hemolysis with their concentrations increasing, and BMFT promotes the hemolysis significantly when high concentration is employed.

Table 2
The inhibition period (t_{inh}) generated by half-curcumin derivatives in protecting methyl linoleate against AAPH-induced oxidation.

Half-curcumin derivatives	DDFT	FT	DHFT	THFT	EOFT	BMFT
t_{inh} (min)	224	221	160	117	–	–

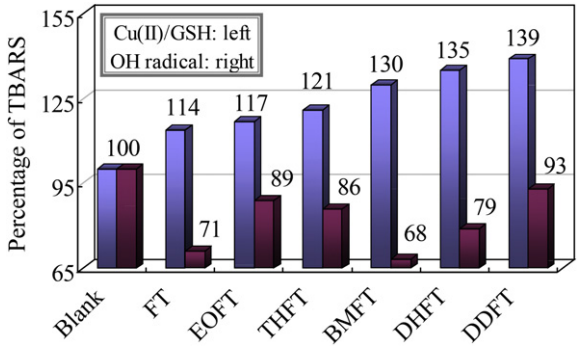


Fig. 3. The percentage of TBARS formed in the mixture of 2.0 mg/mL DNA, 5.0 mM Cu^{2+}, 3.0 mM GSH and 0.2 mM half-curcumin derivatives after incubation for 2 h (left column), and in the mixture of 2.0 mg/mL DNA, 4.0 mM TCHQ, 8.0 mM, H_2O_2, and 0.2 mM half-curcumin derivatives after incubation for 30 min (right column).

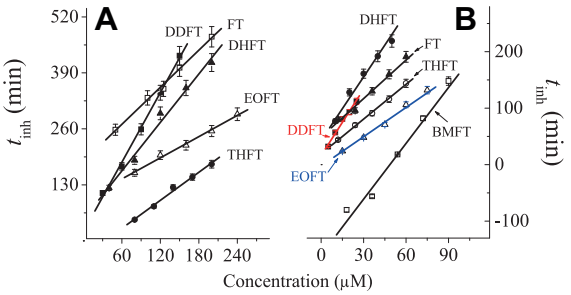


Fig. 4. The relationships between t_{inh} and the concentrations of half-curcumin derivatives in AAPH-induced oxidation of DNA (panel A) and hemolysis of erythrocytes (panel B). The concentration of DNA and AAPH are 2.0 mg/mL and 40 mM, respectively, the concentrations of erythrocytes and AAPH are 3.0% (*v*/*v*) and 20 mM, respectively.

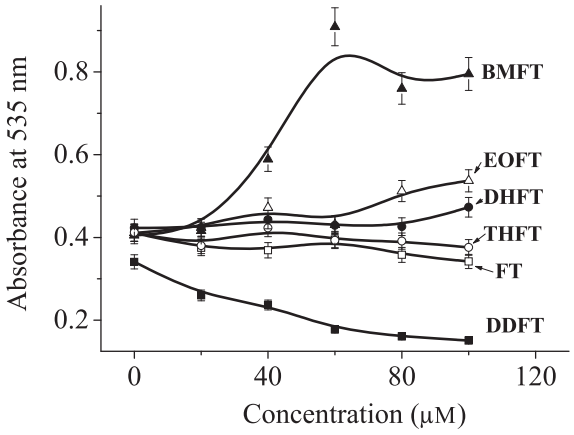


Fig. 5. The correlations of the absorbance at 535 nm and the concentrations of half-curcumin derivatives employed in 20.0 μM hemin-induced hemolysis of 1.0% erythrocytes after incubation for 30 min.

5. Discussion

The multiply methods applied to evaluate the ability of an antioxidant avoid shortcomings from one-dimensional characterization [30,31]. Comparing the antioxidant effectiveness of structural analogs of an antioxidant gives more information on the structure–activity relationship. The treatment of the experimental data obtained from biological samples by chemical kinetics results in a quantitative expression of the antioxidant capacities in biological experimental systems [32]. FT usually acts as the structural feature in the synthesis of curcuminoid molecules [33], and presented here is a comparison of the antioxidant property of FT with its analogs, aiming to clarify the contribution of structural feature in half-curcumin to the antioxidant effectiveness.

The interactions with DPPH and galvinoxyl radicals reveal the ability of an antioxidant to donate hydrogen atom to N- and O-centered radicals [34,35], and the interaction with $ABTS^{+}\bullet$ reveals the ability of the antioxidant to reduce radicals [36]. As shown in Table 1, half-curcumin derivatives are active to decrease the concentrations of DPPH more than galvinoxyl radical, demonstrating that the hydrogen atom in half-curcumin derivatives are more readily to be abstracted by N-centered radical than by O-centered radical. However, BMFT cannot react with these radicals, indicating that radicals cannot abstract hydrogen atom from enolic-OH, and cannot be reduced by enolic-OH. Two C=O bonds are reduced to form alcoholic-OH in THFT, the radical-scavenging activity is just resulted from phenolic-OH. The decrease rate of $ABTS^{+}\bullet$ in the presence of THFT is similar to that in the presence of FT, whereas the rate of THFT to trap DPPH is much lower than that of FT. Hence, phenolic-OH interacts with radical mainly by reducing radicals other than by donating hydrogen atom to radicals. A C–C bond cuts off the conjugated linkage between phenolic and enolic-OH in DHFT, thus, the radical-scavenging activity of DHFT can be regarded as the individual contribution from the phenolic and enolic-OH. The decrease rates of DPPH and $ABTS^{+}\bullet$ in the presence of DHFT are lower than that in the presence of FT. So, the conjugated system between phenolic and enolic-OH is of importance to the radical-scavenging property. Together with the fast rates of DDFT to decrease the concentrations of DPPH and $ABTS^{+}\bullet$, it can be concluded that phenolic-OH linked with enolic-OH by the conjugated system plays the main role in scavenging or reducing radicals. The lowest rates of EOFT to react with these radicals reveal that the enolic-OH from ester group attenuates the radical-scavenging ability of half-curcumin.

The above rules are also valid in protecting methyl linoleate against AAPH-induced oxidation. BMFT almost cannot protect methyl linoleate, thus, enolic-OH is not active in this case. Moreover, no activity of EOFT demonstrates that enolic-OH from ester group cannot prohibit AAPH-induced oxidation of methyl linoleate. Other half-curcumin derivatives protect methyl linoleate against AAPH-induced oxidation and generate t_{inh}, in which t_{inh} of DDFT and FT are much larger than THFT and DHFT. The lowest t_{inh} of THFT (117 min) indicates that phenolic-OH itself cannot enhance the antioxidant ability remarkably. Higher t_{inh} of DHFT (160 min) implies that individual phenolic and enolic-OH devotes to the antioxidant capacity of half-curcumin significantly. Higher t_{inh} of FT reconfirms the importance of the conjugated linkage between phenolic and enolic-OH to reinforce the antioxidant efficacy of half-curcumin. More phenolic-OHs do not enhance the antioxidant efficacy extraordinarily since t_{inh} of DDFT (224 min) is almost the same as that of FT (221 min).

Half-curcumin derivatives exhibit similar activity in β-carotene-bleaching test. BMFT, EOFT and DHFT exhibit low activities in β-carotene-bleaching test, revealing that enolic-OH either itself or tautomerized from an β-keto ester, or unconjugated with phenolic-OH does not make half-curcumin an efficient antioxidant to protect LH against the autoxidation. After C=O is reduced to form alcoholic-OH, the activity of THFT is similar to that of FT, demonstrating that enolic-OH protects LH if only it connects with phenolic-OH by a conjugated system. Furthermore, because DDFT exhibits the most active to protect LH, the antioxidant effectiveness of half-curcumin increases with more phenolic-OHs involved.

The interaction with $^1O_2$ reveals the reductive abilities of half-curcumin derivatives. Low activity of THFT to reduce $^1O_2$ implies that the ability of phenolic-OH itself to reduce $^1O_2$ is very weak. FT and DHFT show similar activity to reduce $^1O_2$, and BMFT has high

Table 3
The equations of t_{inh} ~ [half-curcumin derivatives], and *n* of half-curcumin derivatives in protecting DNA and erythrocytes.

Half-curcumin derivatives	Protect DNA		Protect erythrocytes	
	t_{inh} (min) = (n/R_i) [Half-curcumin derivative (μM)] + constant	*n*	t_{inh} (min) = (n/R_i) [Half-curcumin derivative (μM)] + constant	*n*
DDFT	t_{inh} = 2.57 [DDFT] + 30.4	8.6	t_{inh} = 3.92 [DDFT] + 16.2	6.6
DHFT	t_{inh} = 1.91 [DHFT] + 45.2	6.4	t_{inh} = 3.48 [DHFT] + 51.0	5.9
FT	t_{inh} = 1.42 [FT] + 186.3	4.8	t_{inh} = 2.38 [FT] + 45.6	4.0
THFT	t_{inh} = 1.11 [THFT] − 40.0	3.7	t_{inh} = 2.05 [THFT] + 19.2	3.4
EOFT	t_{inh} = 0.82 [EOFT] + 94.1	2.8	t_{inh} = 1.83 [EOFT] − 6.0	3.1
BMFT	–	–	t_{inh} = 3.3 [BMFT] − 155.8	5.5
curcumin [a]	t_{inh} = 2.43 [curcumin] + 37.9	8.2	t_{inh} = 3.45 [curcumin] + 18.7	5.8

[a] The data of curcumin were cited from Ref. [17].

activity to reduce $^1O_2$, demonstrating that enolic-OH plays a main role in reducing $^1O_2$. Finally, DDFT and EOFT exhibit concentration-dependent abilities to reduce $^1O_2$, indicating that two phenolic-OHs and ester-related enolic-OH are much active to reduce $^1O_2$.

Fig. 3 reveals that the additions of half-curcumin derivatives result in much more TBARS generated in Cu^{2+}/GSH-induced oxidation of DNA, indicating that half-curcumin derivatives play prooxidant role in this case. DDFT possesses the highest prooxidant efficacy whereas FT has the lowest prooxidant efficacy, so, more phenolic-OHs promote the prooxidant effectiveness. Meanwhile, the prooxidant behavior of BMFT reveals that enolic-OH improves Cu^{2+}/GSH-induced oxidation of DNA. Hydroxyl groups reduce Cu (II) to form Cu(I), and Cu(I) can form a complex with DNA, DNA–Cu (I)OOH. Meanwhile, Cu(I) catalyzes O_2 to form O_2^- and H_2O_2 [37]. H_2O_2 improves the cleavage of DNA–Cu(I)OOH, and form TBARS consequently [38]. It was reported that phenolic-OH in hydroxycinnamic acids accelerated the cleavage of DNA by reducing Cu(II) to form Cu(I) [39]. DDFT possesses the highest prooxidant activity because it contains two phenolic-OHs. Hence, the prooxidant effectiveness of half-curcumin derivatives may be due to Cu(I) resulting from reducing Cu(II) by phenolic-OH. The prooxidant behavior of BMFT reveals that enolic-OH can also accelerate the degradation of DNA.

Fig. 3 outlines that half-curcumin derivatives are able to protect DNA against •OH-induced oxidation. In particular, BMFT has the highest effective in this case, demonstrating that enolic-OH plays the main role in inhibiting •OH-induced oxidation of DNA. But the ability of EOFT is lower than that of FT, indicating that enolic-OH from an ester group does not protect DNA with high ability. Furthermore, the lowest activity of DDFT implies that more phenolic-OHs do not enhance the antioxidant effectiveness of half-curcumin to prohibit •OH-induced oxidation of DNA.

From a chemical kinetic viewpoint, t_{inh} generated by an antioxidant (AH) correlates linearly with the concentration as expressed by equation (1) [40].

$$t_{inh} = (n/R_i)[AH] \quad (1)$$

In equation (1), n is designated as *stoichiometric factor* to express the ability of the antioxidant to terminate the radical-chain propagation, and is not related to the concentration of the antioxidant employed. R_i is the initiation rate of the radical-induced reaction. This equation has been applied to calculate n of curcumin derivatives in protecting linoleic acid [41], DNA and erythrocytes against AAPH-induced oxidation [17]. So, n of half-curcumin derivatives is calculated by equation (1), in which the value of n is the product of R_i and the coefficient in equation (1) on the basis of a known R_i. However, R_i should be measured by using trolox (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid) as the reference antioxidant, whose n is assigned as 2 [42]. Therefore, t_{inh} are measured with different concentrations of trolox employed, and then, R_i is obtained from the equation of $t_{inh} \sim$ [trolox]. The addition of trolox to AAPH-induced oxidation of DNA cannot generate t_{inh} (data not shown), thus, it is impossible to obtain R_i by using trolox as reference antioxidant. Because both AAPH and DNA are dissolved in water phase, and radicals generated from the decomposition of AAPH can attack DNA at the same phase, it is safely to assume that R_i is equal to the radical generation rate ($R_g = (1.4 \pm 0.2) \times 10^{-6}$ [AAPH] s^{-1} [42]) in AAPH-induced oxidation of DNA, *viz.*, $R_i = R_g = 1.4 \times 10^{-6} \times 40$ mM $s^{-1} = 3.36$ μM min^{-1} with 40 mM AAPH employed [17,19]. Table 3 lists the n values of half-curcumin derivatives accordingly. The n of half-curcumin derivatives in protecting DNA is DDFT > DHFT > FT > THFT > EOFT.

We have demonstrated that $R_i = R_g$ in AAPH-induced hemolysis, $R_i = R_g = 1.68$ μM min^{-1} when 20 mM AAPH is employed to haemolyze erythrocytes [43]. The n values of half-curcumin derivatives are the products of the coefficients in the equation of $t_{inh} \sim$ [half-curcumin derivatives] and $R_i = R_g = 1.68$ μM min^{-1}, and are listed in Table 3 as well. The n of half-curcumin derivatives in protecting erythrocytes is DDFT > DHFT > BMFT > FT > THFT > EOFT, which is similar to that in protecting DNA, and is largely close to the order of these compounds to react with DPPH. As shown in Scheme 1, the tautomeric structure leads to the formation of a large conjugation system from benzene ring to C=O *via* two C=C bonds. The common characteristics of these half-curcumins are planar molecules. Thus, their antioxidant effects are mainly derived from the different substituents rather than from the molecular configuration. Moreover, it is necessary to interpret the antioxidant activities of half-curcumin derivatives by integrating the results from trapping radical, protecting DNA and erythrocytes, and even from curcumin derivatives in our previous report [17].

BMFT and curcumin with phenolic-OH protected by benzyl group are not active to trap radical and to protect DNA and erythrocytes, indicating that enolic-OH itself does not have the antioxidant activity. As shown in Scheme 1, the enolic-OH is the tautomeric structure of CH_2 in diketone, thus, H atom abstracted by radicals from enolic-OH is equivalent to the H atom from CH_2. The H atom in CH_2 is very difficult to be abstracted by radicals because the bond dissociation energy of C–H is much higher than that of O–H [13]. Hence, the enolic-OH cannot trap radicals directly. The phenolic-OH is the group in THFT to contribute the antioxidant capacity, but low n of THFT reveals that phenolic-OH itself does not contribute largely to the antioxidant effectiveness. The low rate of THFT to trap DPPH also demonstrates that a single phenolic-OH cannot enhance the ability of half-curcumin to trap radicals. The conjugated linkage of phenolic and enolic-OH in DHFT is cut off by C–C. The n values of DHFT are much higher than that of FT, implying that phenolic-OH connected with enolic-OH by a conjugated system as in FT does not benefit for the antioxidant capacity. On the other hand, more phenolic-OHs in DDFT increase the antioxidant capacity significantly, indicating that two adjacent phenolic-OHs enhance the radical-scavenging ability remarkably. This is in agreement with the findings in the research on the antioxidant effectiveness of curcumin derivatives [17]. Furthermore, the low activity of EOFT demonstrates that ester-related enolic-OH decreases the antioxidant property of half-curcumin. The acidity of methylene in β-keto ester ($pK_a = 11$) is lower than that in β-diketone ($pK_a = 9$) [44], meaning that the amount of enolic-OH deriving from the tautomerization of methylene in β-keto ester is lower than that in β-diketone. From the comparison of n values in Table 3, it can be concluded that the antioxidant effects of half-curcumin are generally lower than that of curcumin except two phenolic-OH contained as in DDFT.

Hemin-induced hemolysis is due to the collapse in the microstructure of erythrocyte membrane. The improvement of BMFT to hemolysis indicates that enolic-OH results in the collapse of erythrocyte membrane concentration-dependently. THFT and FT exhibit weak protective effects on erythrocytes with high concentration employed. Contrarily, DHFT and EOFT exhibit weak promotive effects on erythrocytes with high concentration employed. It is worthy to note that more phenolic-OHs enhance the protective effect of DDFT. Thus, half-curcumin with more phenolic-OHs is potential erythrocyte membrane-stabilizer.

6. Conclusion

Taking the results from curcumin derivative into consideration, some conclusions on the antioxidant capacity of curcumin-related compounds can be made. The antioxidant ability is composed of the reductive and radical-scavenging properties resulting from phenolic

and enolic-OH. The enolic-OH itself does not possess any reductive activity and cannot trap radicals, while the phenolic-OH itself does not have marked antioxidant activity either. The phenolic and enolic-OH contained in the same molecule enhances the antioxidant efficacy significantly when they are not connected by a conjugated system, so, the conjugated system does not benefit for the antioxidant effectiveness of half-curcumin. Meanwhile, enolic-OH tautomerized from β-keto ester cannot increase the antioxidant ability of half-curcumin. On the other hand, more phenolic-OHs increase the antioxidant capacity remarkably. The aforementioned information will be useful for the designation of antioxidants involving half-curcumin as structural feature.

7. Experimental section

7.1. Materials and instrumentation

AAPH, diammonium of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), DPPH and galvinoxyl radical, 4-nitroso-*N*,*N*-dimethylaniline (NDMA), LH, methyl linoleate, GSH, hemin and naked DNA sodium salt were purchased from ACROS ORGANICS, Belgium. Other reagents were of analytical grade, and purchased from Beijing Chemical Reagent Co., China. Human erythrocytes were provided by the First Hospital of Jilin University, Changchun, China. The structures of half-curcumin derivatives were identified by ^{1}H and ^{13}C NMR (Varian Mercury 300 NMR spectrometer, USA).

7.2. Synthesis of FT, BMFT and DDFT

Benzaldehyde (0.01 mol vanillin, 3-methoxy-4-benzyloxybenzaldehyde, or protocatechualdehyde), 1.0 g of boric anhydride (0.014 mol) and 2.0 mL of tributyl borate were dissolved in 2 mL of acetylacetone (0.02 mol). The mixture was stirred at 90 °C for 30 min, and cooled to 70 °C. Then, 0.7 mL of butylamine was added dropwisely during 30 min, and stirred at 100 °C for 1.5 h. The mixture was acidified by 20 mL of 0.4 M HCl at 50 °C, stirred at room temperature for 1 h, and extracted by ethyl acetate (3 × 20 mL). The layer of ethyl acetate was washed by distilled water, and dried over Na_2SO_4. After the solvent was evaporated under vacuum pressure, the residue was purified *via* column chromatography (silica gel, petroleum ether/ethyl acetate = 4:1), and feruloylacetone was a pale yellow power, yield 41%, m.p. 142–143 °C ^{1}H NMR (300 MHz, DMSO-*d*6) δ: 2.12 (s, 3H, C*H*$_3$CO–), 3.82 (s, 3H, CH$_3$OC$_6$*H*$_3$–), 5.85 (s, 1H, =C*H*–C), 6.62 (d, J = 15.9 Hz, 1H, CH=C*H*–), 6.79 (d, J = 8.1 Hz, 1H, CH=C*H*– in phenyl), 7.10 (d, J = 8.1 Hz, 1H, –CH=C*H* in phenyl), 7.29 (s, 1H, –C*H*=C in phenyl), 7.46 (d, J = 15.9 Hz, 1H, –C*H*=CH), 9.64 (s, 1H, *H*O–C$_6$H$_3$–); ^{13}C NMR (75 MHz, DMSO-*d*6) δ: 178.3, 149.2, 148.0, 140.2, 126.3, 122.9, 120.0, 115.6, 111.2, 100.4, 55.6, 30.7.

BMFT was a yellow crystal, yield 46%, m.p. 98–99 °C ^{1}H NMR (300 MHz, DMSO-*d*6) δ: 2.13 (s, 3H, C*H*$_3$CO–), 3.83 (s, 3H, CH$_3$OC$_6$*H*$_3$–), 5.14 (s, 2H, –C*H*$_2$C$_6$H$_5$), 5.87 (s, 1H, =CH–C), 6.70 (d, J = 15.9 Hz, 1H, CH=CH–), 7.07 (d, J = 8.1 Hz, 1H, CH=CH– in phenyl), 7.20 (d, J = 8.1 Hz, 1H, –CH=CH in phenyl), 7.35–7.54 (m, 7H, C$_6$H$_5$–, –CH=C in phenyl and –CH=CH); ^{13}C NMR (75 MHz, DMSO-*d*6) δ: 197.1, 177.7, 149.7, 149.3, 139.7, 136.7, 128.4, 127.9, 127.87, 122.8, 122.5, 120.8, 113.2, 110.6, 100.7, 69.8, 55.6, 26.5.

DDFT was a red power, yield 26%, m.p. 216–217 °C ^{1}H NMR (300 MHz, DMSO-*d*6) δ: 2.07 (s, 3H, C*H*$_3$CO–), 6.06 (s, 1H, =CH–C), 6.55 (d, J = 15.6 Hz, 1H, CH=CH–), 6.76 (d, J = 8.1 Hz, 1H, CH=CH– in phenyl), 6.99 (d, J = 8.1 Hz, 1H, –CH=CH in phenyl), 7.06 (s, 1H, –CH=C in phenyl), 7.47 (d, J = 15.6 Hz, 1H, –CH=CH), 9.19 (s, 1H, HO–C$_6$H$_3$), 9.65 (s, 1H, HO–C$_6$H$_3$); ^{13}C NMR (75 MHz, DMSO-*d*6) δ: 183.1, 148.4, 145.7, 140.7, 126.3, 121.6, 120.6, 115.8, 114.7, 101.0, 30.7.

7.3. Synthesis of DHFT

Feruloylacetone (1 mmol, 0.234 g) dissolved in 30 mL of ethyl acetate was hydrogenated over 20 mg of 10% Pd/C for 2 h. After the catalyst was filtered, and the solvent was evaporated under vacuum pressure, the residue was purified *via* column chromatography (silica gel, petroleum ether/ethanol = 50:1), 0.210 g of DHFT (pale yellow power) was obtained, yield 89%, m.p. 40–41 °C ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.03 (s, 3H, C*H*$_3$CO–), 2.55 (t, J = 7.8 Hz, 2H, CH$_2$CH$_2$–), 2.86 (t, J = 7.8 Hz, 2H, CH$_2$CH$_2$–), 3.86 (s, CH$_3$O–), 5.46 (s, 1H, =CH–C), 5.49 (s, 1H, HO– in phenyl), 6.64 (d, J = 8.1 Hz, 1H, CH=CH– in phenyl), 6.68 (s, 1H, –CH=C in phenyl), 6.80 (d, J = 8.1 Hz, 1H, –CH=CH in phenyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 193.2, 191.1, 146.3, 143.9, 132.6, 120.7, 114.3, 110.9, 100.1, 55.8, 40.4, 31.2, 24.8.

7.4. Synthesis of THFT

Feruloylacetone (1 mmol, 0.234 g) was dissolved in 20 mL of methanol, and cooled to 0 °C. $NaBH_4$ (4 mmol, 0.151 g) was added slowly. The mixture was stirred at 0 °C for 0.5 h, and acidified by 0.1 M HCl to pH = 6. After methanol was evaporated under vacuum pressure, the residue was extracted by ethyl acetate (3 × 20 mL). The ethyl acetate layer was washed by saturated saline, and dried over Na_2SO_4. After the solvent was evaporated under vacuum pressure, the residue was recrystallized by petroleum ether/ethanol (10:1), and 0.22 g of THFT (white power) was obtained, yield 92%. ^{1}H NMR (300 MHz, DMSO-*d*6); δ: 1.08 (d, J = 6 Hz, 3H, C*H*$_3$–), 1.41–1.66 (m, 2H, –CH$_2$CH$_3$), 3.79 (s, 3H, CH$_3$OC$_6$H$_3$–), 4.24 (m, 1H, –CH(OH)–CH=), 4.45 (m, 1H, –C*H*(OH)–CH$_3$), 4.77 (s, 1H, HO–C*H*–CH=), 4.86 (s, 1H, HO–C*H*–CH$_3$), 6.10 (m, 1H, CH=CH–), 6.36 (d, J = 15.6 Hz, 1H, –CH=CH), 6.70 (d, J = 8.1 Hz, 1H, –CH=CH in phenyl), 6.79 (d, J = 8.1 Hz, 1H, CH=CH– in phenyl), 7.00 (s, 1H, –CH=C in phenyl), 9.02 (s, 1H, HO–C$_6$H$_3$–); ^{13}C NMR (75 MHz, DMSO-*d*6) δ: 147.7, 146.1, 131.7, 128.4, 127.8, 119.4, 115.4, 109.7, 68.1, 62.9, 55.5, 47.0, 23.9.

7.5. Synthesis of EOFT

Vanillin (1.52 g, 0.01 mol) was dissolved in 2 mL of ethyl acetoacetate (0.0157 mol). Then, 0.7 mL of butylamine was added dropwisely at 60 °C during 30 min. The mixture was stirred at 100 °C for 1.5 h, and 15 mL of 1 M HCl was added at 30 °C and stirred for 1 h at room temperature. The mixture was extracted by ethyl acetate (3 × 20 mL). The layer of ethyl acetate was washed by distilled water, and dried over Na_2SO_4. After the solvent was evaporated under vacuum pressure, the residue was purified *via* column chromatography (silica gel, petroleum ether/ethyl acetate = 4:1), and 1.06 g of EOFT (a pale yellow power) was obtained, yield 40%, m.p. 102–103 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32 (t, J = 6.9 Hz, 3H, CH$_3$CH$_2$–), 2.41 (s, 3H, CH$_3$CO–), 3.90 (s, 3H, CH$_3$O–), 4.34 (m, 2H, –CH$_2$CH$_3$), 5.94 (s, 1H, HO–C$_6$H$_3$–), 6.91 (d, J = 8.4 Hz, 1H, CH=CH– in phenyl), 7.03 (s, 1H, –CH=C in phenyl), 7.05 (d, J = 8.4 Hz, 1H, –CH=CH in phenyl), 7.48 (s, 1H, –CH=C); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 194.7, 164.7, 148.5, 148.1, 143.8, 132.1, 125.0, 123.5, 114.8, 111.3, 61.7, 55.8, 26.4, 14.0.

7.6. Scavenging ABTS^{+}•, DPPH and galvinoxyl radical

The experiments of scavenging ABTS^{+}•, DPPH and galvinoxyl radical were performed following our previous report [17]. DPPH radical (~0.1 mM) and galvinoxyl radical (~2 μM) were dissolved in ethanol to make the absorbance (Abs_{ref}) ~ 1.00 at 517 nm (ε_{DPPH} = 4.09 × 10^3 M^{-1} cm^{-1} [45]) and 428 nm ($\varepsilon_{galvinoxyl}$ = 1.4 × 10^5 M^{-1} cm^{-1} [46]), respectively. Two milliliter of

4.0 mM ABTS aqueous solution was oxidized by 1.41 mM $K_2S_2O_8$ for 16 h, and then, 100 mL of ethanol was added to make the absorbance (Abs_0) ~ 0.70 at 734 nm ($\varepsilon_{ABTS^{+\bullet}} = 1.6 \times 10^4\ M^{-1}\ cm^{-1}$ [36]). The ethanol solutions of half-curcumin derivatives were added to the aforementioned radical solutions at room temperature. The final concentrations of half-curcumin derivatives were 50.0 μM in the test of trapping $ABTS^{+\bullet}$ and DPPH radicals, and 25.0 μM in the test of trapping galvinoxyl radical. The absorbance of the radical solutions was recorded, and the decay rates for DPPH, $ABTS^{+\bullet}$ and galvinoxyl radical were calculated by multiplying the corresponding ε.

7.7. Quenching $^1O_2$

$^1O_2$ was prepared following a literature [47]. Briefly, 10 mM histidine, 20 mM sodium hypochlorite, 20 mM H_2O_2 and 50 μM NDMA were dissolved in 45 mM sodium phosphate buffer (pH = 7.1) to generate $^1O_2$. Various concentrations of half-curcumin derivatives were added to above mixture to a total volume of 2.0 mL, and the mixture was incubated at 30 °C for 40 min to measure the absorbance at 440 nm. The percentage of $^1O_2$ quenched by half-curcumin and its derivatives was calculated by $(A_{detect} - A_{ref})/(A_0 - A_{ref}) \times 100$, where A_0 and A_{ref} were the absorbance before and after the incubation in the control experiment, respectively, and A_{detect} was the absorbance after the incubation in the presence of half-curcumin derivatives.

7.8. β-Carotene-bleaching test and protecting methyl linoleate against AAPH-induced oxidation

An emulsion was formed by dissolving 5.0 mg of β-carotene, 40 mg of LH and 400 mg of Triton X-100 in 5.0 mL of $CHCl_3$. After $CHCl_3$ was evaporated under vacuum pressure, 100 mL of oxygen-saturated water was added and shaken under ultrasonic vibration to form homogeneous β-carotene-LH emulsion (λ_{max} = 460 nm) [48]. The ethanol solutions of half-curcumin derivatives (0.1 mL) were added to 1.9 mL of β-carotene-LH emulsion with the final concentration of half-curcumin derivatives at 50.0 μM. The absorbance of the mixture was determined and plotted *vs* time.

The abilities of half-curcumin derivatives to protect methyl linoleate against AAPH-induced oxidation were tested by measuring the decay of the concentration for methyl linoleate [19]. Methyl linoleate, methyl palmitate, AAPH, and half-curcumin derivatives were dissolved in *tert*-butanol-H_2O (1:1, *v*/*v*) in a test tube with a final concentration at 15.0 mM, 10.0 mM, 80.0 mM, and 0.2 mM, respectively. The test tube was incubated at 37 °C to initiate the oxidation. Aliquots were taken out at every 120 min, and the concentration of methyl linoleate was analyzed by GC (Hewlett–Packard 1890 equipped with an SE-54 30 m × 0.25 mm capillary column, 0.25 μm film thickness, N_2) with methyl palmitate as the internal standard. The temperatures of chromatograph chamber, injector, and hydrogen flame ionization detector were at 260 °C, 280 °C, and 300 °C, respectively.

7.9. Effects of half-curcumin derivatives on Cu^{2+}/GSH-induced oxidation of DNA

The oxidation of DNA mediated by Cu^{2+} and GSH was carried out following a previous report [49] with a little modification. Briefly, DNA, $CuSO_4$, and GSH were dissolved in phosphate buffered solution (PBS_1: 6.1 mM Na_2HPO_4, 3.9 mM NaH_2PO_4) with the final concentration for DNA, Cu^{2+} and GSH at 2.0 mg/mL, 5.0 mM, and 3.0 mM, respectively. Dimethyl sulfoxide (DMSO) solution of half-curcumin derivatives was added with the final concentration at 0.2 mM. The mixture was delivered into test tubes with each one containing 2.0 mL. The test tubes were incubated at 37 °C to initiate the oxidation of DNA. Three tubes were taken out at every 30 min and cooled immediately, to which 1.0 mL of EDTA solution (30.0 mM EDTA in PBS_1 as the stock solution), 1.0 mL of thiobarbituric acid (TBA) solution (dissolving 1.00 g TBA and 0.40 g NaOH in 100 mL PBS_1) and 1.0 mL of 3.0% trichloroacetic acid aqueous solution were added. The tubes were heated in boiling water for 30 min. After the test tubes were cooled to room temperature, 1.5 mL of *n*-butanol was added and shaken vigorously to extract TBA reactive substance (TBARS). The absorbance of *n*-butanol layer was measured at 535 nm.

7.10. Effects of half-curcumin derivatives on •OH-induced oxidation of DNA

•OH was generated by mixing tetrachlorohydroquinone (TCHQ) with H_2O_2 [23]. DNA, H_2O_2 (dissolved in PBS_2: 8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, 10.0 μM EDTA) and TCHQ (dissolved in DMSO) were mixed, and the final concentrations of DNA, H_2O_2 and TCHQ were 2.0 mg/mL, 8.0 mM, and 4.0 mM, respectively. Then, DMSO solutions of half-curcumin derivatives (0.2 mM as the final concentration) were added, and the mixture was delivered into test tubes with each one containing 2.0 mL. The test tubes were incubated at 37 °C for 30 min and cooled immediately, to which 1.0 mL of TBA solution and 1.0 mL of 3.0% trichloroacetic acid aqueous solution were added. The tubes were heated in boiling water for 30 min and cooled to room temperature. Then, 1.5 mL of *n*-butanol was added to extract TBARS. The absorbances of TBARS in the blank experiment and in the presence of half-curcumin derivatives were assigned as A_0 and A_{detect}, respectively. The effects of half-curcumin derivatives on •OH-induced oxidation of DNA were expressed by $A_{detect}/A_0 \times 100$.

7.11. Effects of half-curcumin derivatives on AAPH-induced oxidation of DNA

AAPH-induced oxidation of DNA was carried out following our previous report [50]. DNA and AAPH were dissolved in PBS_2 with the final concentration at 2.0 mg/mL and 40 mM, respectively. After various concentrations of half-curcumin derivatives (dissolved in DMSO) were added, the mixture was delivered into test tubes with each one containing 2.0 mL. All the tubes were incubated in a water bath at 37 °C to initiate the reaction. Three tubes were taken out at every 2 h and cooled immediately. The following operation was the same as •OH-induced oxidation of DNA except the heating period was 15 min after TBA and trichloroacetic acid were added.

7.12. Effects of half-curcumin derivatives on AAPH- and hemin-induced hemolysis of erythrocytes

AAPH- and hemin-induced hemolysis of erythrocytes was performed according to our previous report [17]. Human erythrocytes were washed by phosphate-buffered saline (PBS_3: 150 mM NaCl, 8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, 10 μM EDTA) to remove plasma and centrifuged at 1700 × *g* for exact 10 min to obtain a compacted volume of erythrocytes. Erythrocytes were suspended in PBS_3, to which AAPH and DMSO solutions of half-curcumin derivatives were added. The final concentrations of erythrocytes and AAPH were 3.0% (*v*/*v*) and 20 mM, respectively. The above mixture was incubated at 37 °C to initiate the hemolysis. Aliquots (1.5 mL) were taken out at every 1 h and centrifuged at 1700×*g* to obtain the supernatant, whose absorbance was measured at 535 nm and plotted *vs* incubation time to express the hemolysis process.

Hemin was dissolved in 5 mM NaOH to reach 2.0 mM before usage. Erythrocytes were suspended in PBS_4 (150 mM NaCl, 8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, pH 7.4), to which DMSO solutions of

half-curcumin derivatives were added. The mixture was incubated at 37 °C for 30 min, and then, hemin was added. The final concentrations of erythrocytes and hemin were 1.0% (*v*/*v*) and 20.0 μM, respectively. After incubation for 30 min, the mixture was centrifuged at 1700×*g* to obtain the supernatant. The absorbance of the supernatant was measured at 535 nm and plotted *vs* the concentration of half-curcumin derivatives. The same volume of DMSO (<1.0% to the total volume) was contained in all the control experiment.

7.13. Statistical analysis

The data were the average value from at least three independent measurements with the experimental error within 10%. The linear relationships between inhibition period and concentration were analyzed statistically by one-way ANOVA on Origin 7.5 professional Software, and $p < 0.001$ indicated a significance difference.

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