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Original article

Synthesis of methyl-substituted xanthotoxol to clarify prooxidant effect of methyl on radical-induced oxidation of DNA

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ABSTRACT

4-Methyl-8-hydroxylpsoralen (MXan) and 4,9-dimethyl-8-hydroxylpsoralen (DMXan) were synthesized in order to clarify the effect of methyl on the antioxidant effectiveness of xanthotoxol (8-hydroxylpsoralen, Xan), which were assessed by bleaching β-carotene in linoleic acid–Triton emulsion, by interacting with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\bullet}$), 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), and galvinoxyl radical, and by protecting DNA against the oxidation induced by Cu^{2+}/glutathione (GSH) and 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH). Methyl attaching to xanthotoxol did not affect its ability to protect linoleic acid against autoxidation and to inhibit Cu^{2+}/GSH-induced oxidation DNA, but decreased its ability to scavenge $ABTS^{+\bullet}$ and DPPH, and to protect DNA against AAPH-induced oxidation. Therefore, methyl attenuated the antioxidant effectiveness of xanthotoxol in radical-induced oxidation of DNA.

1. Introduction

Xanthotoxol (8-hydroxylpsoralen, Xan) was a natural psoralen [1] with high ability to treat Alzheimer disease [2], to inhibit human cytochrome P450 [3], and to retard the formation of fibril and β-amyloid [4]. Because the hydroxyl at 8-position made xanthotoxol a free-radical-scavenger obviously [5,6], a large amount of research dealt with the isolation of xanthotoxol derivatives from plants [7] and the investigation of antioxidant effectiveness. However, 8-methoxypsoralen increased the amount of 8-hydroxy-2′-deoxyguanosine (8-OHdG) when calf thymus DNA was irradiated by ultraviolet A (UVA) [8]. The cytotoxicity of 8-methoxypsoralen was due to induce the formation of interstrand cross-link of DNA under irradiation of UVA [9], in which the conversion among various excited states of 8-methoxypsoralen was confirmed by quantum calculation [10].

2. Chemistry

In addition to many contributions to the total synthesis of psoralen derivatives, some works devoted to investigate the effect of substituents on the bioactivity of psoralen. For example, 5-hydroxylpsoralen was synthesized to detect the ability of hydroxyl to trap radicals [11], and 5-amino-8-methoxypsoralen was applied to condense with anhydride for preparing antitumor drugs [12]. As an alkyl group, the effect of methyl on the bioactivity of psoralens was not often reported. We have reported that methyl attaching to 4-position in coumarin played a prooxidant role in the oxidation of low-density lipoprotein (LDL) induced by 2,2′-azobis (2-amidinopropane hydrochloride) (AAPH, R–N=N–R, $R = -CMe_2C(=NH)NH_2$) [13]. So, presented here was a study to test whether methyl also played prooxidant role in xanthotoxol. As shown in Scheme 1, 4-methyl-8-hydroxylpsoralen (MXan) and 4,9-dimethyl-8-hydroxylpsoralen (DMXan) were chosen to explore the effect of methyl at furan ring and/or lactone ring on the antioxidant effectiveness of xanthotoxol, the routines to synthesize MXan and DMXan were outlined in Scheme 2 [14].

3. Pharmacology

Because a large body of evidence has revealed the correlation of the *in vivo* oxidative stress with ageing [15] and some fatal diseases [16,17], many works focused on the synthesis of antioxidants and the evaluation of their activities in various experimental systems [18]. Cu^{2+}/glutathione (GSH)-mediated and AAPH-induced oxidation of DNA were usually employed as the experimental systems to investigate the antioxidant capacity because Cu^{2+} may cause the damage of DNA in the presence of similar concentration of GSH *in vivo* [19], and peroxyl radical ($ROO^{\bullet}$) generated from the decomposition of AAPH can mimic the *in vivo* oxidative stress of DNA [20]. The effects of DMXan, MXan and Xan were evaluated on Cu^{2+}/GSH- and AAPH-induced oxidation of DNA. In addition, their antioxidant

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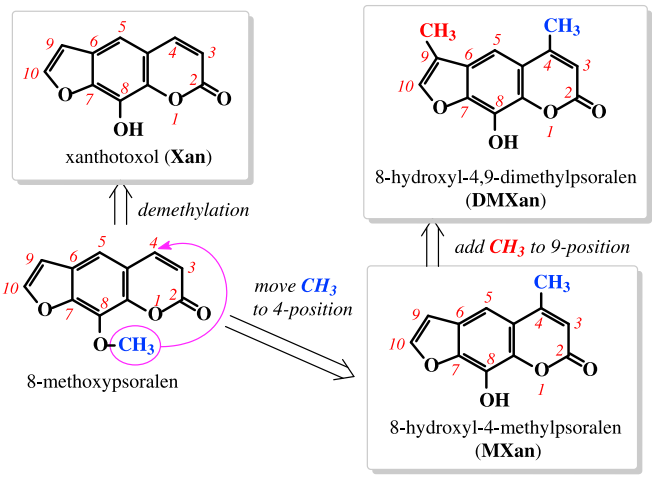


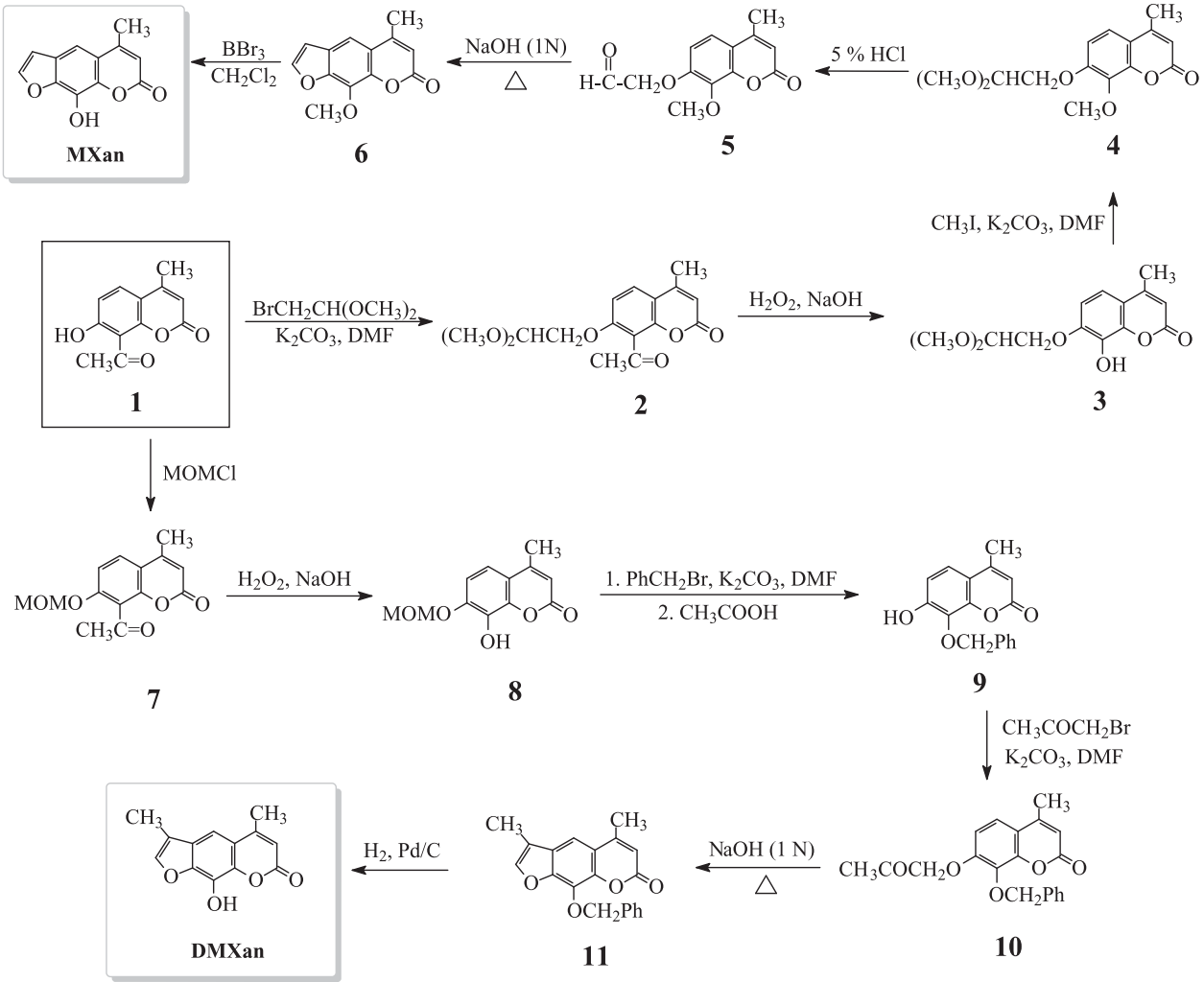
Scheme 1. Structures of psoralen derivatives employed in this work.

capacities were screened by bleaching β-carotene in the emulsion of linoleic acid (LH) and Triton [21], and by interacting with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\bullet}$) [11], 2,2′-diphenyl-1-picrylhydrazyl (DPPH) [11], and galvinoxyl radical [22], respectively.

4. Results and discussion

4.1. Synthesis of methyl-substituted xanthotoxol

Methyl at 4-position in methyl-substituted xanthotoxol was derived from compound **1** employed as the reagent herein. So, the synthesis protocol mainly focused on the construction of furan ring in this work. The furan ring was formed by the condensation between C=O in formacylmethoxy or acetylmethoxy and H atom at 6-position in coumarin under basic condition. DMXan was derived from the coumarin with acetylmethoxy at 7-position, while MXan was derived from the coumarin with formacylmethoxy at the same position. The coumarin containing acetylmethoxy at 7-position, **10**, was prepared by using CH_3COCH_2Br to react with the hydroxyl in compound **9**, and the reaction between $(CH_3O)_2CHCH_2Br$ and compound **1** formed the coumarin containing formacylmethoxy at the same position, **2**. However, the difficulty in the synthesis of MXan and DMXan was to preserve the hydroxyl at 8-position when the anion of hydroxyl at 7-position reacted with CH_3COCH_2Br or $(CH_3O)_2CHCH_2Br$. Williamson reaction can take place between hydroxyl at both 7- and 8-position with CH_3COCH_2Br or $(CH_3O)_2CHCH_2Br$ without selectivity, thus, protecting hydroxyl at 8-position to make hydroxyl at 7-position react with CH_3COCH_2Br or $(CH_3O)_2CHCH_2Br$ was the key strategy in the synthesis of methyl-substituted xanthotoxol. As shown in Scheme 2, compound



Scheme 2. Synthesis routines of MXan and DMXan.

1 was chosen to be reagent because acetyl can be converted into hydroxyl group *via* Baeyer–Villiger oxidation and hydrolysis reaction [23]. Furthermore, we have attempted to simplify the synthetic routines as shown in Scheme 3. After 7-formacylmethoxy and 7-acetylmethoxycoumarin, **12** and **13**, were prepared by the reaction between compound **1** and $(CH_3O)_2CHCH_2Br$ or CH_3COCH_2Br, they were treated by NaOH in order to construct furan ring directly. But this operation did not generate target product, **14** and **15**.

As shown in Scheme 2, compound **1** reacted with $(CH_3O)_2CHCH_2Br$ to form compound **2**, and then, the acetyl converted into hydroxyl group to form compound **3** by Baeyer–Villiger oxidation and hydrolysis reaction. After the hydroxyl in compound **3** was methylated to form compound **4**, and ketal in $(CH_3O)_2CHCH_2O-$ was hydrolyzed to form formacylmethoxy. The cyclization reaction occurring in compound **5** formed furan ring in compound **6**. On the other hand, the aforementioned routine was not suitable to synthesize DMXan because Baeyer–Villiger oxidation will take place at 8-acetyl and 7-acetylmethoxy simultaneously as shown in Scheme 4. Thus, hydroxyl at 7-position in compound **1** was protected by MOMCl, and then, 8-acetyl was converted into hydroxyl to generate compound **8**. After the hydroxyl at 8-position in compound **8** was etherized by $PhCH_2Br$, the protective group of 7-OH (methoxymethoxy) was hydrolyzed to form hydroxyl under acidic condition. Thus, the furan ring in DMXan was constructed on the basis of the hydroxyl at 7-position in compound **9**. Finally, benzyloxy at 8-position in compound **11** was removed by hydrogenation.

4.2. Abilities of DMXan, MXan and Xan to protect linoleic acid and to scavenge radicals

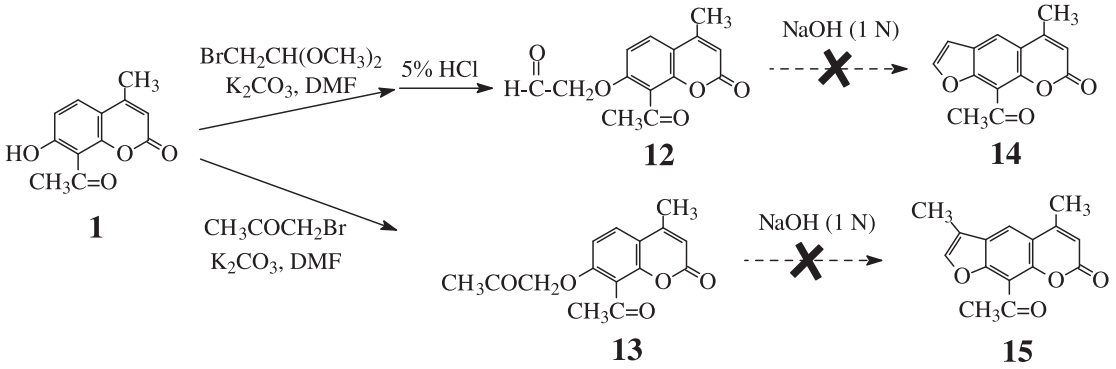
As shown in Fig. 1, the antioxidant abilities of DMXan, MXan and Xan were screened by bleaching β-carotene in LH emulsion, and by scavenging $ABTS^{+}\bullet$, DPPH, and galvinoxyl radicals.

LH and β-carotene can form a water-soluble emulsion in the presence of Triton X-100 or Tween [21]. Oxygen in atmosphere oxidized LH to form peroxyl radical ($LOO\bullet$), which can be trapped by β-carotene (λ_{max} = 460 nm). So, as shown as the line from the control experiment in panel A of Fig. 1, the decay of the absorbance at 460 nm indicated that more $LOO\bullet$ generated from the autoxidation of LH were eliminated by β-carotene with the incubation period increasing. As shown in panel A, the decay of the absorbance was retarded by the addition of 13.3 μM DMXan, MXan or Xan, indicating that DMXan, MXan and Xan were able to protect LH against the autoxidation. The lines in the presence of DMXan and MXan were almost superposition to that of Xan, revealing that the abilities of DMXan and MXan to protect LH were the same as that of Xan. So, methyl attaching to xanthotoxol did not affect the antioxidant capacity in this case. On the other hand, as shown in panel **B** of Fig. 1, the addition of 40.0 μM Xan decreased the percentage of $ABTS^{+}\bullet$ rapidly, while the same concentration of DMXan and MXan cannot decrease the percentage of $ABTS^{+}\bullet$ as fast as Xan, indicating that Xan possessed stronger ability to reduce $ABTS^{+}\bullet$. The decay of the percentage for $ABTS^{+}\bullet$ in the presence of DMXan was even slower than MXan, revealing that methyl at 4- and 9-position in xanthotoxol was not beneficial to reduce $ABTS^{+}\bullet$. As shown in panel **C** of Fig. 1, the additions of 200 μM DMXan, MXan, Xan decreased the percentage of DPPH markedly. In particular, MXan and Xan decreased the percentage of DPPH even faster than DMXan, indicating that methyl at 4- and 9-position in xanthotoxol was not beneficial for hydrogen atom in hydroxyl to donate to N-centered radical. As shown in panel **D** of Fig. 1, both DMXan, MXan and Xan (200 μM) were able to decrease the percentage of galvinoxyl radical. The line of DMXan was almost superposition to that of MXan in this case, revealing that DMXan and MXan possessed the similar ability to donate hydrogen atom in hydroxyl to O-centered radical. But Xan decreased the percentage of galvinoxyl radical even slower than DMXan and MXan, indicating that methyl was beneficial for hydrogen atom in hydroxyl to donate to O-centered radical. Moreover, the concentrations of DMXan, MXan and Xan to scavenge 50% $ABTS^{+}\bullet$, DPPH and galvinoxyl radical (IC_{50}) were measured and listed in Table 1.

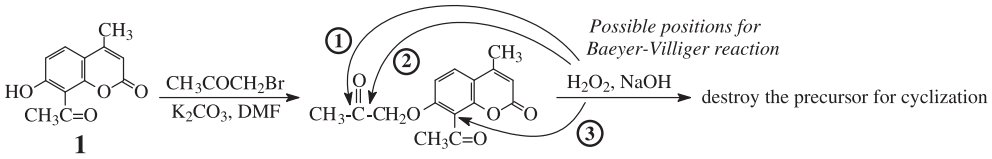
A low value of IC_{50} implicated that the antioxidant possessed relative high effectiveness to scavenge the corresponding radical [24]. It can be found that DMXan, MXan and Xan were effective scavengers to trap $ABTS^{+}\bullet$ and DPPH. The lowest value of IC_{50} was found in Xan rather than methyl-substituted xanthotoxol, implying that introducing methyl to xanthotoxol cannot enhance the free-radical-scavenging ability to trap $ABTS^{+}\bullet$ and DPPH. Finally, high value of IC_{50} for Xan to trap galvinoxyl radical revealed a completely reverse result, which was worthy to study in the future work.

4.3. Abilities of DMXan, MXan and Xan to protect DNA against the oxidation induced by Cu^{2+}/GSH or AAPH

The oxidation of Cu(II)-binding DNA catalyzed by intracellular GSH increased the amount of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) [25] because of the generation of GSH radical ($GS\bullet$) *via* $Cu(II) + GSH \rightarrow Cu(I) + GS\bullet$ [26]. The further oxidation of DNA mediated by Cu^{2+}/GSH broke the strand of DNA to form propenals, which can be detected after treated by thiobarbituric acid (TBA), thus, propenals were also called TBA reactive substance (TBARS, λ_{max} = 535 nm) [19]. In addition, AAPH usually acted as peroxyl radical ($ROO\bullet$) resource to mimic LDL [27], erythrocytes [28], and DNA [20] under oxidative stress *in vitro*. The oxidation of



Scheme 3. Furan rings in MXan and DMXan cannot be constructed from **12** and **13**.



Scheme 4. Possible positions for Baeyer–Villiger oxidation occurring in 8-acetyl-7-acetylmethoxycoumarin.

aforementioned biological samples can also be detected by measuring TBARS [8]. Hence, we herein applied above *in vitro* experimental systems to assess the effect of DMXan, MXan and Xan on DNA. Fig. 2 illustrated the variety of the absorbance for TBARS in the process of the oxidation of DNA.

The line from blank experiment in Fig. 2 exhibited an increase of the absorbance of TBARS, indicating that more propenals were generated in the presence of Cu^{2+}/GSH or AAPH with the incubation period increasing. Comparing with the line from the control experiment, the addition of DMXan, MXan and Xan moved down the lines of the formation of TBARS, revealing that DMXan, MXan and Xan were able to protect DNA against Cu^{2+}/GSH-induced damage. Moreover, in order to compare the ability of DMXan, MXan and Xan to protect DNA, the absorbance at 150 min in the control experiment of Cu^{2+}/GSH-induced oxidation of DNA was designated as A_0, and the absorbance at the same time point in the presence of DMXan, MXan and Xan was designated as A_x. Thus, the oxidative percentage of DNA in the presence of DMXan, MXan and Xan can be calculated by $(A_x/A_0) \times 100$. A low percentage of the oxidative DNA indicated that the antioxidant possessed high ability to protect DNA against the oxidation. Meanwhile, 360 min was chosen as the time point to calculate the oxidative percentage in AAPH-induced oxidation of DNA. The results were illustrated in Fig. 3.

The percentages of oxidative DNA in the presence of DMXan, MXan and Xan were around 85% without significant difference in Cu^{2+}/GSH-induced oxidation of DNA, implying that methyl did not affect the protective effects of xanthotoxol obviously. On the other hand, in AAPH-induced oxidation of DNA, the addition of Xan and MXan decreased the oxidative percentage of DNA to 60% and 68%, respectively, while DMXan can only decrease the oxidative percentage of DNA to 80%, indicating that methyl attenuated the antioxidant capacity of xanthotoxol in this case. This finding was in agreement with our previous report on the prooxidant effect of 4-methylcoumarin in AAPH-induced oxidation of LDL [13], in which the prooxidant effect of methyl at 4-position of coumarin was illustrated by Scheme 5.

The hydrogen atom of methyl in 4-methylcoumarin was abstracted by ROO• to form a benzyl radical, which was able to initiate the oxidation of LDL [13]. Similarly, hydrogen atom in methyl at 4- and 9-position in xanthotoxol was possibly abstracted by ROO• to form corresponding radical, which was able to improve the oxidation of DNA. But the hydroxyl at 8-position in xanthotoxol functioned as an antioxidative group to protect DNA against AAPH-induced oxidation. So, the observed property of methyl-substituted xanthotoxol was the overall effect from the antioxidant property of hydroxyl and the prooxidant property of methyl.

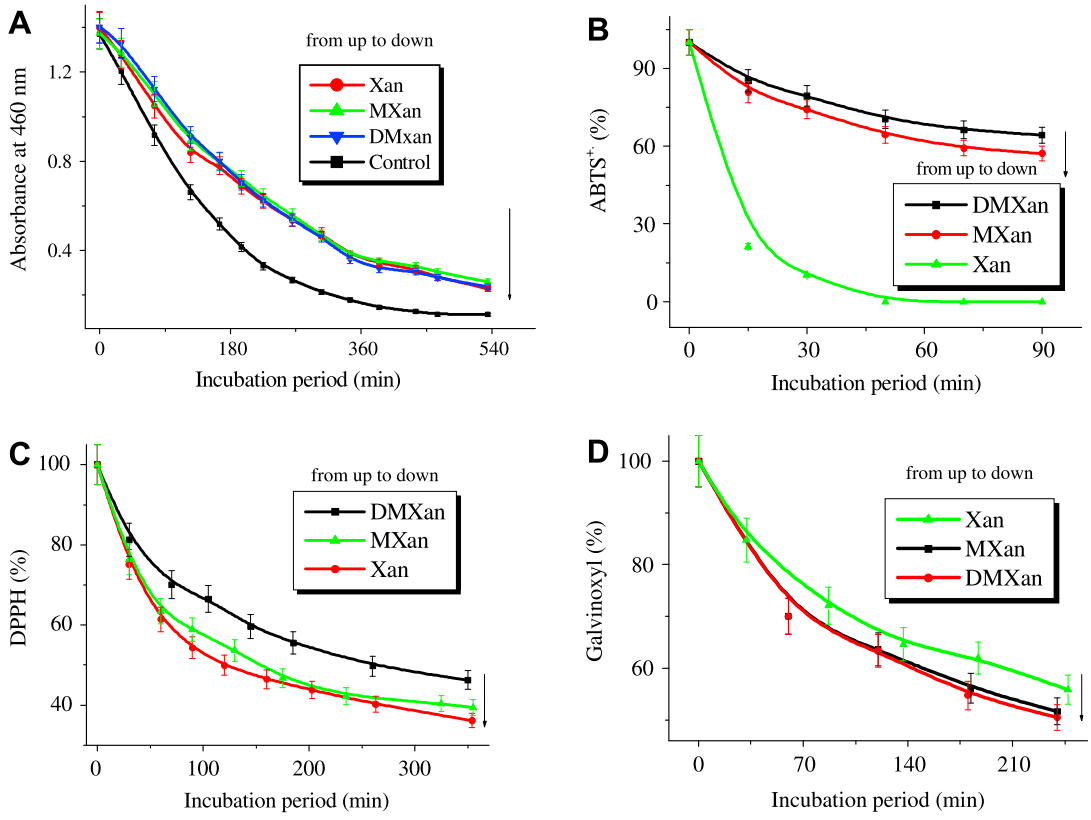


Fig. 1. Decay of the absorbance of β-carotene-LH emulsion in the presence of 13.3 μM DMXan, MXan and Xan (A), percentage of $ABTS^{+\bullet}$ scavenged by 40.0 μM DMXan, MXan and Xan (B), percentage of DPPH scavenged by 200 μM DMXan, MXan and Xan (C), and percentage of galvinoxyl radical scavenged by 200 μM DMXan, MXan and Xan (D).

Table 1
IC_{50} of DMXan, MXan and Xan in scavenging $ABTS^{+\bullet}$, DPPH and galvinoxyl radical.

Antioxidants	IC_{50} (μM)		
	$ABTS^{+\bullet}$	DPPH	Galvinoxyl
DMXan	18.0	118.8	170.1
MXan	15.8	81.5	173.2
Xan	10.3	69.6	188.3

5. Conclusion

Methyl attaching to xanthotoxol did not influence the protective effect on the autoxidation of LH and on the oxidation of DNA induced by Cu^{2+}/GSH, but decreased the ability to scavenge $ABTS^{+\bullet}$ and DPPH radicals, and to protect DNA against AAPH-induced oxidation. So, methyl attenuated the antioxidant effectiveness of hydroxyl in xanthotoxol, and the prooxidant mechanism of methyl in xanthotoxol will be explored experimentally in the further research. The obtained results give a useful caution for the designation of antioxidant because benzyl-type methyl has the potential to initiate additional radical propagation.

6. Experimental protocols

6.1. Materials

AAPH, diammonium salt of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), DPPH, naked DNA sodium salt, GSH, LH, β-carotene, and 8-methoxypsoralen were purchased from ACROS ORGANICS, Belgium, and used as received. Xanthotoxol was derived from the demethylation of 8-methoxypsoralen [29], and ^{13}C NMR (DMSO-d_6, 75 MHz) of Xan were found at 159.6, 147.0, 145.3, 145.0, 139.7, 129.8, 124.9, 116.0, 113.5, 109.9, 106.7. 4-Methyl-7-hydroxyl-8-acetylcoumarin (**1**) was synthesized following literature [30]. Other reagents were of analytical grade, and purchased from Beijing Chemical Reagent Co., China. Flash column chromatography was performed on a silica gel (300–400 mesh) and thin layer chromatography (TLC) inspections were carried out on a silica gel GF_{254} plates. The structures of the obtained compounds were identified by ^1H NMR (Varian Mercury 300 NMR spectrometer). The synthetic compounds were analyzed by high performance liquid chromatography, and the purities of these compounds were larger than 98.0%.

6.2. Synthesis of 4-methyl-8-hydroxylpsoralen (MXan)

6.2.1. 4-Methyl-7-(2′,2′-dimethoxyethoxy)-8-acetylcoumarin (**2**)

Compound **1** (19.2 g, 88 mmol) and K_2CO_3 (22.6 g, 164 mmol) was dissolved in 100 mL of *N*,*N*-dimethylformamide (DMF), to which $BrCH_2CH(OCH_3)_2$ (14.8 mL, 98.4 mmol) was added dropwisely at room temperature. Then, the mixture was heated to 140 °C and stirred for 4 h. After cooled to room temperature, the mixture was diluted by water, and then, extracted by ethyl acetate. The organic phases were washed by brine, dried over Na_2SO_4. After the organic solvent was evaporated, the crude product was purified by flash chromatograph column with petroleum ether and ethyl acetate (1:1, *v*:*v*) as eluent. Compound **2** was obtained as a white solid, yield 86%. ^1H NMR (300 MHz, $CDCl_3$): 7.56 (d, 1H, $J = 9.0$ Hz, *H* at 5-position), 6.89 (d, 1H, $J = 9.0$ Hz, *H* at 6-position), 6.17 (s, 1H, *H* at 3-position), 4.68 (s, 1H, $(CH_3O)_2CHCH_2O-$), 4.10 (d, 2H, $J = 5.1$ Hz, $(CH_3O)_2CHCH_2O-$), 3.46 (s, 6H, $(CH_3O)_2CHCH_2O-$), 2.61 (s, 3H, $CH_3-C{=}O$), 2.40 (s, 3H, $-CH_3$ at 4-position).

6.2.2. 4-Methyl-7-(2′,2′-dimethoxyethoxy)-8-hydroxylcoumarin (**3**)

Compound **2** (21.4 g, 70 mmol) was heated in 88 mL of 2 N NaOH aqueous solution for 45 min, then cooled to 0 °C, to which hydrogen peroxide (90 mL, 30 wt%) was added dropwisely under stirring. The mixture was stirred for 2 h, and acidified by 250 mL of 2 N HCl aqueous solution to pH = 1 at 0 °C. Compound **3** was precipitated and yield 72%. ^1H NMR (300 MHz, $CDCl_3$): 7.08 (d, 1H, $J = 8.7$ Hz, *H* at 5-position), 6.90 (d, 1H, $J = 8.7$ Hz, *H* at 6-position), 6.18 (s, 1H, *H* at 3-position), 4.78 (s, 1H, $(CH_3O)_2CHCH_2O-$), 4.14 (d, 2H, $J = 5.1$ Hz, $(CH_3O)_2CHCH_2O-$), 3.48 (s, 6H, $(CH_3O)_2CHCH_2O-$), 2.39 (s, 3 H, $-CH_3$ at 4-position).

6.2.3. 4-Methyl-7-(2′,2′-dimethoxyethoxy)-8-methoxycoumarin (**4**)

Methyl iodide (0.75 mL, 12 mmol) and K_2CO_3 (2.76 mg, 20 mmol) was dissolved in 150 mL of DMF, to which compound **3** (2.8 g, 10 mmol) was added. The mixture was stirred for 6 h at room temperature, then $CHCl_3$ and water were added. The aqueous phase was extracted by $CHCl_3$. The organic phase was combined and washed by water for three times, and dried over Na_2SO_4. After the organic solvent was evaporated, the crude product was purified by flash chromatograph column with petroleum ether and ethyl acetate (3:1, *v*:*v*) as eluent. Compound **4** was obtained as a white solid powder, yield 95%. ^1H NMR (300 MHz, $CDCl_3$): 7.27 (d, 1H, $J = 7.5$ Hz, *H* at 5-position), 6.89 (d, 1H, $J = 7.5$ Hz, *H* at 6-position), 6.16 (s, 1H, *H* at 3-position), 4.77 (s, 1H, $(CH_3O)_2CHCH_2O-$), 4.12 (d, 2H, $J = 5.1$ Hz, $(CH_3O)_2CHCH_2O-$), 3.98 (s, 3H, $-OCH_3$ at 8-position), 3.48 (s, 6H, $(CH_3O)_2CHCH_2O-$), 2.39 (s, 3H, $-CH_3$ at 4-position).

6.2.4. 4-Methyl-7-formylmethyl-8-methoxycoumarin (**5**)

Compound **4** (0.88 g, 3 mmol) was refluxed in 4 mL of 5% HCl for 10 min under nitrogen atmosphere. After the mixture was cooled, the precipitate was purified by flash chromatography column with petroleum ether and ethyl acetate (1:2, *v*:*v*) as eluent.

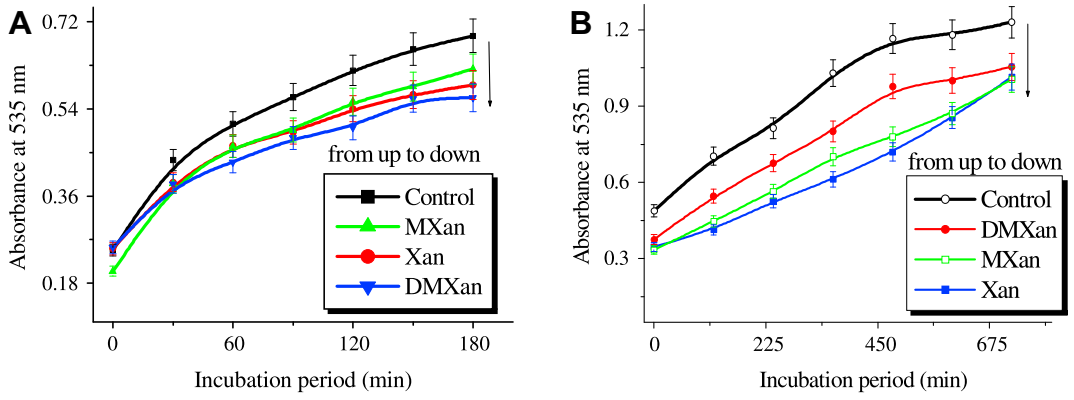


Fig. 2. The variety of the absorbance of TBARS in the oxidation of DNA (2.0 mg/mL) induced by 5.0 mM Cu^{2+} and 3.0 mM GSH in the presence of 0.2 mM DMXan, MXan or Xan (A), and by 40 mM AAPH and in the presence of 80.0 μM DMXan, MXan or Xan (B).

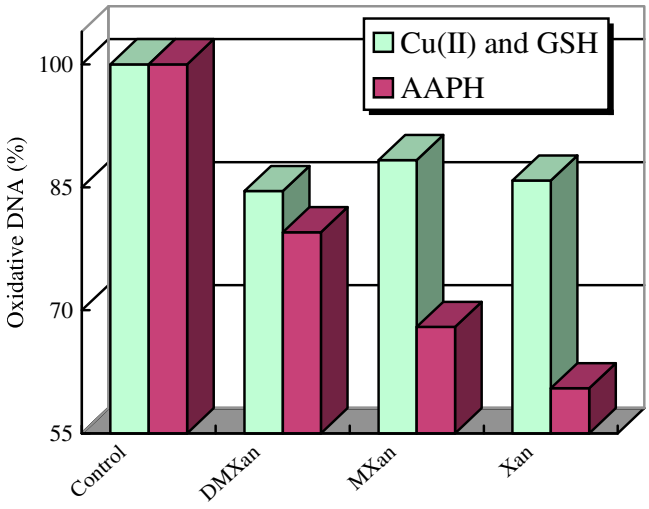


Fig. 3. Comparison of the protective effects of DMXan, MXan and Xan on DNA against the oxidation induced by Cu^{2+}/GSH at 150 min, and by AAPH at 360 min.

Compound **5** was obtained, yield 80%. ^{1}H NMR (300 MHz, $CDCl_3$): 9.90 (s, 1H, −C*H*O), 7.29 (d, 1H, *J* = 9.0 Hz, *H* at 5-position), 6.80 (d, 1H, *J* = 9.0 Hz, *H* at 6-position), 6.21 (s, 1H, *H* at 3-position), 4.75 (s, 2H, −OC*H*$_2$CHO), 4.04 (s, 3H, −OC*H*$_3$), 2.41 (s, 3H, −C*H*$_3$ at 4-position).

*6.2.5. 4-Methyl-8-methoxypsoralen (**6**)*

Compound **5** (0.496 g, 2 mmol) was refluxed in 50 mL of 1 N NaOH aqueous solution for 80 min under nitrogen. After the mixture was cooled and acidified by 1 N HCl aqueous solution, the crude product was precipitated, and purified by flash chromatography column with petroleum ether and ethyl acetate (2:1, *v*:*v*) as eluent to obtain compound **6**, yield 50%. ^{1}H NMR (300 MHz, $CDCl_3$): 7.67 (d, 1H, *J* = 1.2 Hz, *H* at 10-position), 7.48 (s, 1H, *H* at 5-position), 6.82 (d, 1H, *J* = 1.5 Hz, *H* at 9-position), 6.25 (s, 1H, *H* at 3-position), 4.28 (s, 3H, −OC*H*$_3$), 2.48 (s, 3H, −C*H*$_3$ at 4-position).

6.2.6. 4-Methyl-8-hydroxylpsoralen (MXan)

Compound **5** (0.23 g, 1 mmol) was dissolved in 50 mL of CH_2Cl_2 and cooled to −10 °C, to which 0.48 mL of 4.2 N CH_2Cl_2 solution of BBr_3 was added dropwisely. The mixture was stirred for 12 h at 0 °C. Then, 50 mL of water was added dropwisely, and stirred vigorously for 1 h. The precipitate was purified by flash chromatography column with petroleum ether and ethyl acetate (1:1, *v*:*v*) as eluent. MXan was obtained and yield 93%. m. p. >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 10.58 (s, 1H, −O*H*), 8.07 (d, 1H, *J* = 2.1 Hz, *H* at 10-position), 7.52 (s, 1H, *H* at 5-position), 7.03 (d, 1H, *J* = 2.1 Hz, *H* at 9-position), 6.34 (s, 1H, *H* at 3-position), 2.48 (s, 3H, -C*H*$_3$ at 4-position); ^{13}C NMR (DMSO-d_6, 75 MHz): 159.4, 153.5, 146.9, 145.1, 139.4, 129.8, 124.6, 116.8, 112.2, 106.8, 106.6, 18.3. MS: *m*/*z* 217.7 ($M^+ + 1$).

6.3. Synthesis of 4,9-dimethyl-8-hydroxylpsoralen (DMXan)

*6.3.1. 4-Methyl-7-methoxymethoxy-8-acetylcoumarin (**7**)*

Compound **1** (19.2 g, 88 mmol), MOMCl (12.0 mL, 132 mmol) and K_2CO_3 (24.3 g, 176 mmol) were dissolved in 200 mL of DMF, and stirred at 40 °C till compound **1** cannot be detected by TLC. Then, the mixture was poured into ice-water with stirring. Compound **7** was precipitated and yield 85%. ^{1}H NMR (300 MHz, $CDCl_3$): 7.55 (d, *J* = 8.7 Hz, 1H, *H* at 5-position), 7.12 (d, *J* = 8.7 Hz, 1H, *H* at 6-position), 6.18 (s, 1H, *H* at 3-position), 5.26 (s, 2H, CH_3OCH_2O−), 3.49 (s, 3H, CH_3OCH_2O−), 2.62 (s, 3H, CH_3C═O), 2.41 (s, 3H, −C*H*$_3$ at 4-position).

*6.3.2. 4-Methyl-7-methoxymethoxy-8-hydroxycoumarin (**8**)*

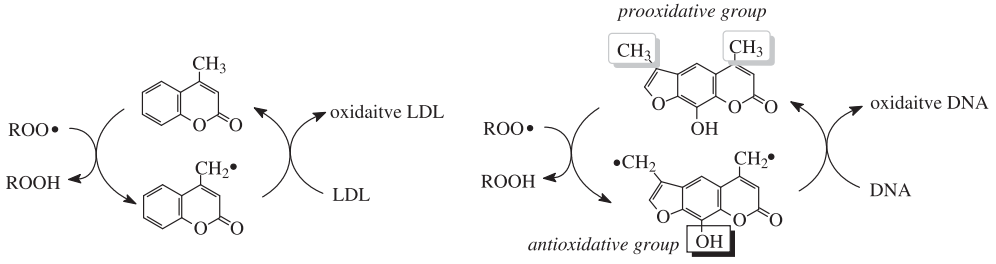
Compound **7** (18.3 g, 70 mmol) was stirred in 88 mL of 2 N NaOH aqueous solution at 0 °C, to which hydrogen peroxide (90 mL, 30 wt %) was added dropwisely. The mixture was stirred for 2 h, and then, acidified by 250 mL of 2 N HCl aqueous solution to pH = 1 at 0 °C. Compound **8** was precipitated and yield 73%. ^{1}H NMR (300 MHz, $CDCl_3$): 7.11 (s, 2H, *H* at 5- and 6-position), 6.19 (s, 1H, *H* at 3-position), 5.32 (s, 2H, CH_3OCH_2O−), 3.55 (s, 3H, CH_3OCH_2O−), 2.41 (s, 3H, −C*H*$_3$ at 4-position).

*6.3.3. 4-Methyl-7-hydroxy-8-benzyloxycoumarin (**9**)*

Benzylbromide (75 mmol) and K_2CO_3 (13.8 g, 100 mmol) were dissolved in 150 mL of DMF, to which compound **8** (11.8 mg, 50 mmol) was added. The mixture was stirred for 6 h at room temperature, then $CHCl_3$ and water were added. The aqueous phase was extracted by $CHCl_3$, and the combined organic phase was washed by water for three times, and dried over Na_2SO_4. After the organic solvent was removed, the crude product was purified by flash chromatography column with petroleum ether and ethyl acetate (3:1, *v*:*v*) as eluent to obtain 4-methyl-7-methoxymethoxy-8-benzyloxycoumarin, yield 95%. Then, 4-methyl-7-methoxymethoxy-8-benzyloxycoumarin (2.77 g, 8.5 mmol) was dissolved in 250 mL of 2 N CH_3COOH aqueous solution, and heated at 90 °C for 40 h. After the mixture was cooled to room temperature, pH of the mixture was adjusted to 7 by adding $NaHCO_3$. Then, the mixture was extracted by ethyl acetate (50 mL × 4). The organic phase was washed by brine, and dried over Na_2SO_4. After the organic solvent was evaporated, compound **9** was obtained as a yellow solid powder, yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$): 7.46–7.37 (m, 5H, *H* at Ph in $PhCH_2O$−), 7.25 (d, 1H, *J* = 9.9 Hz, *H* at 5-positon), 6.87 (d, 1H, *J* = 8.7 Hz, *H* at 6-positon), 6.16 (s, 1H, *H* at 3-positon), 6.07 (s, 1H, −O*H*), 5.30 (s, 2H, $PhCH_2O$−), 2.41 (s, 3H, −C*H*$_3$ at 4-position).

*6.3.4. 4-Methyl-7-acetylmethoxy-8-benzyloxycoumarin (**10**)*

Compound **10** was prepared by using compound **9** (1.13 g, 4 mmol) and bromoacetone (0.46 mL, 6 mmol) as reagents, and following the procedure of the synthesis of compound **7**, yield 86%. ^{1}H NMR (300 MHz, $CDCl_3$): 7.54 (d, 1H, *J* = 6.3 Hz, *H* at 5-position), 7.37–7.27 (m, 5H, *H* at Ph in $PhCH_2O$−), 6.75 (d, 1H,



Scheme 5. Methyl as prooxidative group, while hydroxyl group as antioxidative group in xanthotoxol.

J = 9.0 Hz, *H* at 6-position), 6.19 (s, 1 H, *H* at 3-position), 5.23 (s, 2H, PhC*H*$_2$O–), 4.63 (s, 2H, CH$_3$COC*H*$_2$O–), 2.40 (s, 3H, C*H*$_3$COCH$_2$O–), 2.23 (s, 3 H, –C*H*$_3$ at 4-position).

6.3.5. 8-Benzyloxy-4,9-dimethylpsoralen (**11**)

Compound **10** (0.68 g, 2 mmol) was refluxed in 100 mL of 1 N NaOH aqueous solution for 2 h under nitrogen. Then, the mixture was cooled and acidified by 1 N HCl aqueous solution. The precipitate was purified by flash chromatography column with petroleum ether and ethyl acetate (2:1, *v*:*v*) as eluent. Compound **11** was obtained and yield 72%. ^{1}H NMR (300 MHz, CDCl$_3$): 7.54 (s, 1H, *H* at 10-position), 7.46 (s, 1H, *H* at 5-position), 7.35–7.26 (m, 5H, *H* at Ph in PhCH$_2$O–), 6.25 (s, 1H, *H* at 3-position), 5.52 (s, 2H, PhC*H*$_2$O–), 2.49 (s, 3H, –C*H*$_3$ at 9-position), 2.26 (s, 3 H, –C*H*$_3$ at 4-position).

6.3.6. 4,9-Dimethyl-8-hydroxylpsoralen (DMXan)

Compound **11** (0.32 g, 1 mmol) and 5% Pd/C (29 mg) were suspended in 30 mL of ethyl acetate, and stirred overnight under H_2 atmosphere. The mixture was filtered to remove catalyst, and the organic phase was evaporated. The crude product was purified by flash chromatography column with petroleum ether and ethyl acetate (1:1, *v*:*v*) as eluent to obtain DMXan, yield 90%. m. p. >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 10.51 (s, 1H, –O*H*), 7.85 (s, 1H, *H* at 10-position), 7.44 (s, 1H, *H* at 5-position), 6.33 (s, 1H, *H* at 3-position), 2.50 (s, 3H, –C*H*$_3$ at 9-position), 2.24 (s, 3H, –C*H*$_3$ at 4-position). ^{13}C NMR (DMSO-d_6, 75 MHz): 159.5, 153.6, 145.2, 143.0, 139.6, 129.7, 126.1, 116.5, 115.5, 112.0, 105.0, 18.4, 7.0. MS: *m*/*z* 231.7 ($M^+ + 1$).

6.4. Antioxidant effectiveness of DMXan, MXan and Xan in chemical experimental systems

An emulsion was prepared by mixing 5.0 mg of β-carotene, 40 mg of LH and 400 mg of Triton X-100 in 100 mL of double distilled water under ultrasonic vibration [21]. The ethanol solutions of DMXan, MXan and Xan (0.04 mL) were mixed with 2.96 mL of β-carotene-LH emulsion, of which the final concentration of DMXan, MXan and Xan was 13.3 μM. The absorbance of the mixture was measured at 460 nm, and plotted *versus* time.

The experiments of DMXan, MXan and Xan to trap $ABTS^{+}$•, DPPH, and galvinoxyl radical were carried out following the description in literatures [22,24]. Briefly, ABTS (2.00 mL, 4.0 mM) was oxidized by 1.41 mM $K_2S_2O_8$ for 16 h to generate $ABTS^{+}$•, to which 100 mL of ethanol was added to make the absorbance (Abs_{ref}) around 0.70 at 734 nm. DPPH and galvinoxyl radical were dissolved in ethanol to make the absorbance (Abs_{ref}) around 1.00 at 517 nm and 428 nm, respectively, to which ethanol solution of DMXan, MXan or Xan was added with 200 μM as the final concentration in the radical solutions. The decay of the absorbance for DPPH and galvinoxyl radical solution (Abs_{detect}) was recorded. The same operation was performed to $ABTS^{+}$• with 40 μM as the final concentration for DMXan, MXan or Xan. The percentages of these radicals trapped by DMXan, MXan and Xan were calculated by $(1-Abs_{detect}/Abs_{ref}) \times 100$, and plotted *versus* incubation time.

6.5. Effects of DMXan, MXan and Xan on the oxidation of DNA mediated by Cu^{2+}/GSH

Cu^{2+}/GSH-mediated oxidation of DNA was carried out according to the reference [19] with a little modification. Briefly, $CuSO_4$ aqueous solution was mixed with the phosphate buffered solutions (PBS_1: 6.1 mM Na_2HPO_4, 3.9 mM NaH_2PO_4) of DNA and GSH, in which the final concentration for DNA, Cu^{2+} and GSH were 2.0 mg/mL, 5.0 mM, and 3.0 mM, respectively. Then, dimethyl sulfoxide (DMSO) solution of DMXan, MXan or Xan was added with the final concentration at 0.2 mM, respectively. The above mixture was dispensed into test tubes, each one contained 2.0 mL. The test tubes were incubated in a water bath (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 PBS_1 solution of EDTA (30.0 mM as the final concentration) was added to chelate Cu^{2+}. The tubes were heated in boiling water for 30 min after 1.0 mL of thiobarbituric acid (TBA) solution (1.00 g TBA and 0.40 g NaOH dissolved in 100 mL of PBS_1) and 1.0 mL of 3.0% trichloroacetic acid aqueous solution were added. After the mixture in the test tube was 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, and plotted *versus* incubation time.

6.6. Effects of DMXan, MXan and Xan on AAPH-induced oxidation of DNA

The oxidation of DNA induced by AAPH was carried out according to the reference [20] with a little modification. Briefly, the phosphate buffered solutions (PBS_2: 8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, 10.0 μM EDTA) of DNA and AAPH were mixed with DMSO solutions of DMXan, MXan and Xan, respectively, in which the final concentration for DNA, AAPH and DMXan, MXan or Xan were 2.0 mg/mL, 40.0 mM, and 80.0 μM, respectively. The following operation was the same as that in the oxidation of DNA mediated by Cu^{2+}/GSH except that the heating period was 15 min after TBA and trichloroacetic acid aqueous solution were added to the mixture.

6.7. Statistical analysis

All the data were the average values from at least three independent measurements with the experimental error within 10%. The data were analyzed by one-way ANOVA on Origin 6.0 professional Software, and $p < 0.001$ indicated a significance difference.

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