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Original article

Dichloro-4-quinolinol-3-carboxylic acid: Synthesis and antioxidant abilities to scavenge radicals and to protect methyl linoleate and DNA

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ABSTRACT

5,7-, 5,8-, 6,8-, 7,8-Dichloro-4-quinolinol-3-carboxylic acid (5,7-, 5,8-, 6,8-, 7,8-DCQA) together with 7-chloro-4-quinolinol-3-carboxylic acid (7-CQA) and 4-quinolinol-3-carboxylic acid (QA) were synthesized to investigate the antioxidant properties. 5,7-DCQA exhibited the highest ability to scavenge 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\cdot}$), 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and galvinoxyl radicals. 6,8-DCQA possessed the highest efficacy to protect methyl linoleate against 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH)-induced oxidation. 5,7-, 5,8-DCQA and QA were able to retard the β-carotene-bleaching in β-carotene-linoleic acid emulsion. In addition, 5,8- and 6,8-DCQA efficiently protected DNA against hydroxyl radical (·OH)-mediated oxidation, and 5,8-DCQA and 7-CQA were active to protect DNA against AAPH-induced oxidation. Furthermore, only 7-CQA can protect DNA against Cu^{2+}/glutathione (GSH)-mediated oxidation. Dichloro-4-quinolinol-3-carboxylic acids were potent to be antiradical drugs, and were worthy to be researched pharmacologically.

1. Introduction

Many theories are developed to explain the molecular mechanisms of aging [1], among which the oxidative stress is widely admitted [2]. The oxidative stresses of DNA, membrane, lipid and protein vary cellular components, leading to aging and diseases. The free radical-induced *in vivo* oxidation is regarded as the key to the pathogenesis of cardiovascular diseases and cancer [3]. Thus, the use of antioxidant to protect biological species against radical-induced oxidation becomes a novel therapeutic and nutritional idea [4,5]. Some components in diet and medicinal herbs attract much attention because of free-radical-scavenging, antivirus, antiinflammatory, wound healing, and antibacterial activities [6–9]. Meanwhile, design and synthesis of antioxidants with special structural feature are of importance to develop novel drugs and to extend usage of known drugs.

Some novel pharmacological functions are even found in small molecules. 8-Quinolinol used in combination with paclitaxel improves the treatment on breast cancer [10]. Clioquinol (5-chloro-7-iodo-8-quinolinol) is a drug for the treatment of aging and memory impairment [11]. 4-Quinolinol derivatives were found to have various biological activities [12], and chloro-substituted 4-quinolinols were used to prepare novel nucleoside analogues as potential antiviral agents [13]. The antioxidant capacities of 7-chloro (fluoro)-4-quinolinol, a clinic anticancer drug in China, as well as its structural analogues with carboxylic and ester groups at 3-position were investigated in the experimental system of erythrocyte hemolysis induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) [14]. The antioxidant effectiveness of the complex formed between 7-chloro (fluoro)-4-quinolinol-related compounds and β-cyclodextrin was discussed as well [15]. Moreover, 5,7-dichloro-4-quinolinol-3-carboxylic acid was reported to inhibit glycine receptor [16], leading to the present study on the antioxidant effectiveness of a series of dichloro-substituted 4-quinolinol-3-carboxylic acid including 5,7-, 5,8-, 6,8-, and 7,8-dichloro-4-quinolinol-3-carboxylic acid (5,7-, 5,8-, 6,8-, and 7,8-DCQA). 7-Chloro-4-quinolinol-3-carboxylic acid (7-CQA) and 4-quinolinol-3-carboxylic acid (QA) were utilized as reference antioxidants in this work. These compounds were abbreviated as 4-quinolinols.

2. Chemistry

Skraup cyclization is a traditional method to introduce hydroxyl group into benzene ring in quinoline. For example, as shown in Eq. (1), clioquinol, a drug for the treatment of Alzheimer, Parkinson and Huntington diseases [17], can be prepared by the corresponding aminophenol and acrolein formed by the dehydration and oxidation of glycerol in the presence of sulfuric acid.

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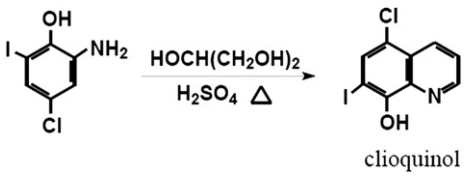
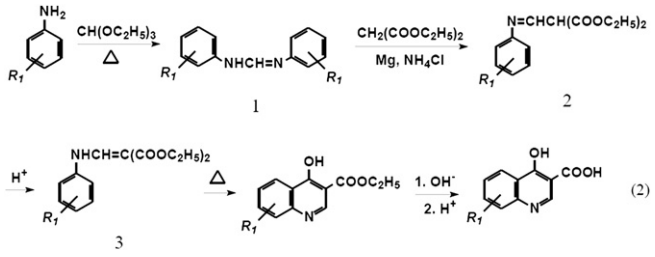


clioquinol

Gould–Jacobs [18] and Niementowski reactions [19], and many other methods [20] have been developed to introduce hydroxyl group into pyridine ring in quinoline. As shown in Eq. (2), the condensation between an amine and triethyl orthoformate generates imine **1** that converts into imine **2** in boiling diethyl malonate. Then, an isomerization transforms C=C in imide **2**, leading to the formation of alkenyl amine **3**. An intramolecular cyclization connects *ortho*-position of benzene ring with C=O in $-COOC_2H_5$ to form ethyl 4-quinolinol-3-carboxylate, the precursor of 4-quinolinol-3-carboxylic acid [21].



As shown in Scheme 1, this method was improved. The imide **3** was formed directly by heating the reactant in diethyl ethoxymethylenemalonate [18]. In our work, dichloro-4-quinolinol-3-carboxylic acids were synthesized following this method. Diphenyl ether was used as a media for the intramolecular cyclization occurring at high temperature. Meanwhile, the surplus diethyl ethoxymethylenemalonate and amine were distilled from the mixture with the temperature increasing. As a result, ethyl dichloro-4-quinolinol-3-carboxylate was precipitated from diphenyl ether. So, the addition of diphenyl ether combined the isolation of surplus reagents with the cyclization reaction in one-pot. Then, hydrolysis under basic condition and acidification afforded dichloro-4-quinolinol-3-carboxylic acid. 7-CQA and QA were prepared following this method as well [22–24].

3. Pharmacology

Free-radical-induced oxidative stress may modify the redox status of tissues and the activation status of redox sensitive transcription factors, and may be an important mechanism for aging [25] and cancer [26]. Thus, the obtained compounds are first screened by trapping 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\cdot}$) [27], 2,2′-diphenyl-1-picrylhydrazyl (DPPH) [28], and galvinoxyl radicals [29].

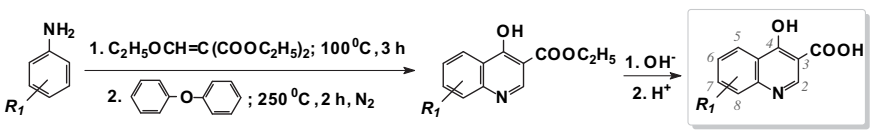
The abundant components of polyunsaturated fatty acids (PUFAs) in lipids and membranes, and the susceptibility to the oxidation make linoleic acid a substrate usually employed to mimic PUFAs undergoing the oxidation chemically [30]. Linoleic acid can be oxidized by the ambient atmosphere automatically, and the autoxidation of linoleic acid can be inhibited by β-carotene, called β-carotene-bleaching test. If a compound can hinder the decrease of the absorbance for β-carotene, it acts as an antioxidant to prohibit the autoxidation of linoleic acid [31]. The oxidation of methyl linoleate initiated by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH, R–N=N–R, R=$-CMe_2C(=NH)NH_2$), a water-soluble azo radical resource, is applied to mimic PUFA undergoing radical-induced oxidation, and can be followed by measuring the concentration of methyl linoleate *via* gas chromatography (GC) [32].

Many experimental systems have been applied to explore the mechanisms of radical-induced DNA oxidation [33]. AAPH- and hydroxyl radical ($\cdot OH$)-induced oxidative damages of DNA are useful *in vitro* systems because the rate of peroxyl radical ($ROO^{\cdot}$) generated from the decomposition of AAPH depends upon the concentration of AAPH [34], and $\cdot OH$ can be readily generated by mixing tetrachlorohydroquinone and H_2O_2 [35]. Supercoiled strand of DNA transforms into open circular and linear forms in the process of radical-induced oxidation [36], and subsequently, generates more than 20 carbonyl species that can be determined spectophotometrically after reacting with thiobarbituric acid (TBA) [37]. Thus, carbonyl species formed in the oxidation of DNA were also called as TBA reactive substance (TBARS). Cu(II) plus glutathione (GSH) generate $GS^{\cdot}$ to oxidize DNA, progressing the formation of 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxodG) [38] and TBARS [39]. The aforementioned experimental systems were *in vitro* methods to evaluate the antioxidant activity to protect DNA against radical-induced oxidation.

4. Results and discussion

4.1. Scavenging $ABTS^{+\cdot}$, DPPH and galvinoxyl radicals

The reaction between an antioxidant and $ABTS^{+\cdot}$ reflects the ability of the antioxidant to reduce radicals since $ABTS^{+\cdot}$ is generated from the oxidation of ABTS salt, and is widely used to assess the antioxidative capacity of phenolics [40]. The reactions between an antioxidant and DPPH and galvinoxyl radicals reveal the abilities of the antioxidant to donate its hydrogen to N- and O-centered radicals [41,29]. Therefore, the reactions with these radicals will give direct evidence for 4-quinolinols to scavenge radicals. Fig. 1 illustrates the residual percentages of these radicals after 1.5 mM 4-quinolinols were incubated with these radicals for 30 min. Low percentage implies that the 4-quinolinol has high free-radical-scavenging activity.



***R*₁** = H
3-chloro
2,3-dichloro
2,4-dichloro
2,5-dichloro
3,5-dichloro

***R*₁** = H; **QA**
7-chloro; **7-CQA**
7,8-dichloro; **7,8-DCQA**
6,8-dichloro; **6,8-DCQA**
5,8-dichloro; **5,8-DCQA**
5,7-dichloro; **5,7-DCQA**

Scheme 1. Synthetic routine for dichloro-4-quinolinol-3-carboxylic acid.

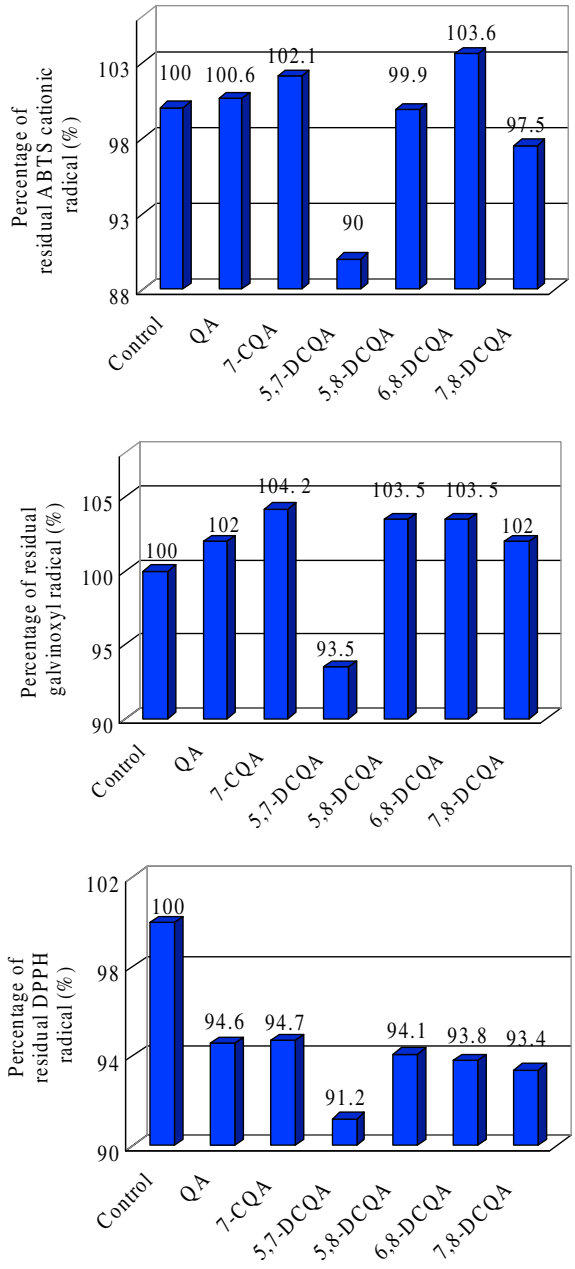
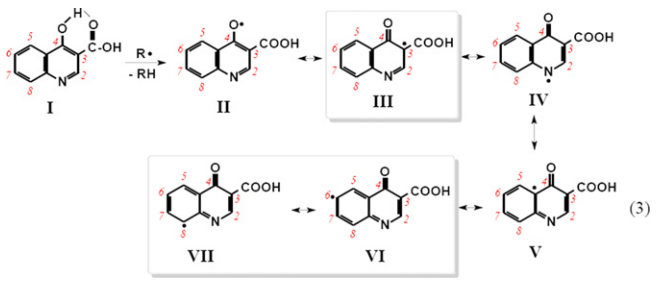
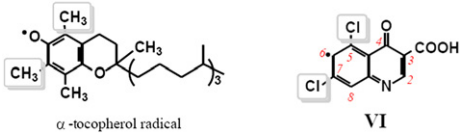


Fig. 1. Percentages of residual $ABTS^{+\cdot}$, DPPH and galvinoxyl radicals in the presence of 1.5 mM 4-quinolinols for 30 min.

In the reaction of 4-quinolinols with $ABTS^{+\cdot}$, only 5,7-DCQA decreases the percentage of $ABTS^{+\cdot}$ to 90%, and other 4-quinolinols almost cannot influence the percentage of $ABTS^{+\cdot}$. Similar phenomenon is also found in the reaction between 4-quinolinols and galvinoxyl radical. 5,7-DCQA is still able to decrease the percentage of galvinoxyl radical to 93.5%, while the percentages of galvinoxyl radical are close to the control experiment in the presence of other 4-quinolinols, indicating that the hydroxyl group in 5,7-DCQA can donate its hydrogen atom to O-centered radical, and can reduce $ABTS^{+\cdot}$ radical. On the other hand, all the 4-quinolinols employed herein are able to decrease the percentage of DPPH radical to ~94%, demonstrating that hydroxyl group in 4-quinolinols are able to donate the hydrogen atom to *N*-centered radical. 5,7-DCQA can decrease the percentage of DPPH radical to 91.2% in this case. The strongest abilities of 5,7-DCQA to trap and to reduce radicals are related to the stabilization of 5,7-DCQA radical. As shown in Eq. (3), the resonance structure of 4-quinolinol radical may help us to understand the effects of two chlorine atoms on the stabilization of 4-quinolinol radical.



The hydrogen atom in the hydroxyl group of 4-quinolinol (**I**) is not very easy to be abstracted by a radical (R·) because of the intramolecular hydrogen bond formed between —OH and —COOH. Once the hydrogen atom in hydroxyl group is abstracted by a radical to form 4-quinolinol radical (**II**), the single electron may transfer to 3-, *N*-, 6-, 8-position *via* resonance structures. As an electron-withdrawing group, —COOH in resonance structure **III** cannot supply the electron to the radical, and is not beneficial for the stabilization of the 4-quinolinol radical. As an electron-withdrawing group, chlorine atom also cannot supply the electron to 4-quinolinol radical when the single electron transfers to 6- and 8-pisition *via* resonance structure. Chlorine atoms at 5- and 7-position avoid direct encountering the single electron locating at 6- and 8-position *via* resonance. In addition, as we all know that the stabilization of α-tocopherol radical makes α-tocopherol an effective antioxidant. Two methyl groups at *ortho*-position (in the framework) stabilizes the single electron in the oxygen-centered radical, *viz*., the steric effects of two methyl groups largely contribute to increase the antioxidant effectiveness of α-tocopherol. Similarly, in the resonance structure **VI** two chlorine atoms locate at *ortho*-position of the radical (in the framework) when the single electron transfers to 6-position *via* resonance structure. The steric effects of chlorine atom at 5- and 7-position may also stabilize the single electron, and consequently, enhance the stabilization of the radical of 5,7-DCQA. Thus, 5,7-DCQA exhibits higher antioxidant effectiveness than other 4-quinolinols.



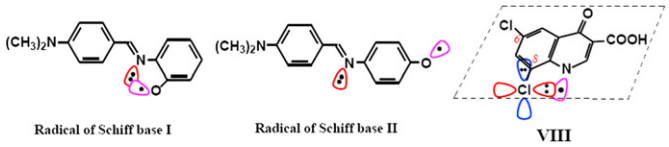
4.2. Protecting methyl linoleate and bleaching β-carotene in linoleic acid emulsion

The abundant composition of polyunsaturated fatty acids (PUFAs) such as linoleic, linolenic, and arachidonic acids makes low-density lipoprotein and membrane phospholipids susceptible to be attacked by ROS, leading to atherothrombotic cardiovascular diseases eventually [42]. Thus, radical-induced oxidation of linoleic acid usually acts as an *in vitro* experimental system to mimic PUFA undergoing ROS-induced oxidative stress. The oxidation of linoleic acid can be followed by measuring the formation of peroxide of linoleic acid *via* high performance liquid chromatography (HPLC) [30]. The decay of the concentration of linoleic acid can also be detected by GC with methyl linoleate applied [32]. In the control experiment, the concentration of methyl linoleate decreases from

13.3 to 11.8 mM after 4 h, indicating that 1.5 mM methyl linoleate was exhausted by 40 mM AAPH-induced oxidation during this period. The concentrations of methyl linoleate were detected at 4 h in the presence of 2.0 mM 4-quinolinols. Thus, the concentration of the exhausted methyl linoleate was calculated and compared with that in the control experiment (1.5 mM). The obtained percentages of the exhausted concentration for methyl linoleate are outlined in Fig. 2. Low percentage indicates that the 4-quinolinol possesses high activity in this case.

Fig. 2 shows that the percentages of the exhausted concentration of methyl linoleate in the presence of 4-quinolinols are even higher than that in the control experiment, indicating that QA, 7-CQA and 5,7-DCQA accelerate the exhaustion of methyl linoleate and function as prooxidants to improve AAPH-induced oxidation of methyl linoleate. On the other hand, the additions of 5,8-, 7,8- and 6,8-DCQA decrease the percentage of the exhausted concentration of methyl linoleate, indicating that these 4-quinolinols are able to protect methyl linoleate against AAPH-induced oxidation. Especially, 6,8-DCQA decreases the percentage of the exhausted concentration of methyl linoleate even to 7.3%, revealing that the antioxidant activity of 6,8-DCQA is much higher than other 4-quinolinols in this case. A common character in the structure of 5,8-, 7,8- and 6,8-DCQA is a chlorine atom attaching to 8-position.

We have compared the antioxidant abilities of 2-((4-*N*,*N*-dimethylaminobenzylidene)amino)phenol (**Schiff base I**) and 4-((4-*N*,*N*-dimethylaminobenzylidene)amino)phenol (**Schiff base II**), and found that the antioxidant effectiveness of **Schiff base I** is higher than that of **Schiff base II** in AAPH-induced hemolysis of erythrocytes. The sp^2 orbital of N atom with electron pair may overlap the *p* orbital of oxygen atom with the single electron, leading to the stabilization of the radical of **Schiff base I** is higher than that of **Schiff base II** [43]. Similarly, in the resonance structure **IV** the sp^2 orbital of N atom with a single electron is overlapped by the *p* orbital of chlorine atom at 8-position. This may contribute to the stabilization of the radical with chlorine atom at 8-position.



Linoleic acid and β-carotene form a water-soluble emulsion with Triton X-100 as the surfactant. The oxygen dissolved in water initiates the autoxidation of linoleic acid and generates peroxyl radical of linoleic acid (LOO·). LOO· is able to bleach β-carotene. In this work the absorbance of the emulsion decreases from 1.00 to 0.58 after 100 min, indicating that β-carotene is bleached by LOO· during this period. The amount of the exhausted β-carotene is related to the decrease of the absorbance, $1.00 - 0.58 = 0.42$. Meanwhile, the decrease of the absorbance in the presence of 0.3 mM 4-quinolinols is also measured and compared with that in the control experiment (0.42). The percentages of the decrease of the absorbance are outlined in Fig. 2. Low percentage indicates that the 4-quinolinol has high ability to protect linoleic acid against the autoxidation. It can be found in Fig. 2 that 7,8- and 6,8-DCQA behave as prooxidants herein. On the contrary, the percentages of the decrease of the absorbance are relative low in the presence of QA, 7-CQA, 5,7- and 5,8-DCQA, indicating that QA, 7-CQA, 5,7- and 5,8-DCQA act as antioxidants to inhibit the autoxidation of linoleic acid.

4.3. Protecting DNA against ·OH-, AAPH-, and Cu^{2+}/GSH-induced oxidation

·OH, one of the *in vivo* ROS, is usually used as a radical resource to evaluate antioxidant activity *in vitro*. Recently, it was reported that ·OH can be generated by Fenton reaction between tetrachlorohydroquinone and H_2O_2 in a metal-free media [35]. Deoxyribose in DNA is the main target to the attack of ·OH, and malondialdehyde is formed as the final product that can be measured after reacting with TBA. Some researchers regarded TBARS as the contribution from the heating in the post-treatment other than from the oxidation of DNA. Actually, in the blank experiment, the absorbance at 535 nm did not vary in the absence of ·OH, AAPH and Cu^{2+}/GSH (data not shown). To take AAPH-induced oxidation of DNA as an example, in the control experiment the absorbance at 535 nm was 0.60 before the oxidation of DNA, and increased to 0.87 after 4 h. The increase of the absorbance is related to the formation of TBARS during the oxidation of DNA. In the presence of 4-quinolinols the absorbance is measured and compared with that in the control experiment (0.87). The percentages of TBARS generated in the oxidation of DNA mediated by various radicals are shown in Fig. 3. Low percentage indicates that the 4-quinolinol has relative high efficacy to protect DNA against radical-induced oxidation. All the 4-quinolinols except 7-CQA can protect DNA against ·OH-induce oxidation. Especially, the additions of 5,8- and 6,8-DCQA even decrease the percentages of TBARS lower than 85%. ·OH is able to react with benzene to form phenol. For example, 2,3- and 2,5-dihydroxylbenzoic acid were detected in ·OH-induced oxidation of salicylic acid [35]. Thus, the antioxidant behaviors of 4-quinolinols may also ascribe to the direct interactions between ·OH and 4-quinolinols.

The decomposition of AAPH generates peroxyl radical (ROO·) to abstract H atom from the C-4′ atom of DNA, causing strand breaks and generating TBARS eventually [44]. The protective effects of 4-quinolinols on DNA against AAPH-induced oxidation are not as

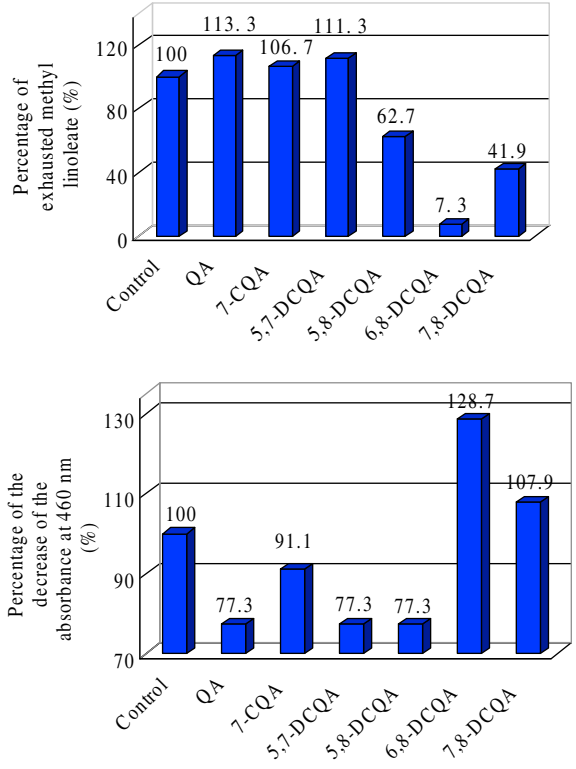


Fig. 2. Percentages of the exhausted methyl linoleate (13.3 mM as the beginning concentration) induced by 35.6 mM AAPH after 4 h in the presence of 2.0 mM 4-quinolinols, and percentages of the decrease of the absorbance at 460 nm after 100 min in the presence of 0.3 mM 4-quinolinols.

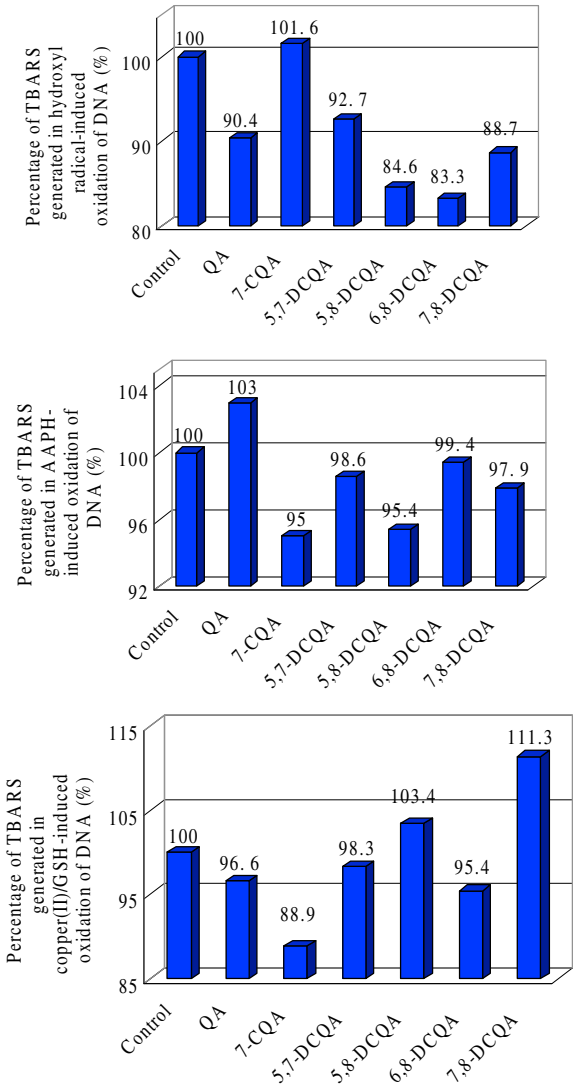


Fig. 3. Percentages of TBARS generated in the oxidation of 2.0 mg/mL DNA induced by 2.0 mM tetrachlorohydroquinone and 4.0 mM H_2O_2 for 30 min in the presence of 400 μM 4-quinolinols; percentages of TBARS generated in the oxidation of 2.0 mg/mL DNA induced by 40 mM AAPH for 4 h in the presence of 200 μM 4-quinolinols; and percentages of TBARS generated in the oxidation of 2.0 mg/mL DNA induced by 5.0 mM Cu^{2+} and 4.0 mM GSH for 4 h in the presence of 200 μM 4-quinolinols.

good as in ·OH-induced oxidation of DNA. Only 7-CQA and 5,8-DCQA can decrease the percentages of TBARS to ~95%. The addition of QA even generates more TBARS than that in the control experiment, indicating that 4-quinolinol without chlorine as substituent even promotes AAPH-induced oxidation of DNA. Thus, introducing chlorine atoms is beneficial for 4-quinolinols to protect DNA against ROO·-induced oxidation. The results for AAPH-induced oxidation of DNA show that 7-CQA and 5,8-DCQA present the best protective effects, and are not in agreement with that found for protecting methyl linoleate. It is difficult to clarify the interaction style between the antioxidants and DNA. But the linkage of the antioxidant with DNA may influence the antioxidant behavior. So, an antioxidant usually gives different results in biological and chemical experimental systems [45].

Cu(II) can react with GSH to form radical (GS·) *via* $Cu(II) + GSH \rightarrow Cu(I) + GS\cdot$, where GS· degrades DNA to produce TBARS [38,39]. Fig. 3 shows that 7-CQA decreases TBARS to 88.9%, and QA, 5,7- and 6,8-DCQA decrease TBARS to 95–98%. The additions of 5,8- and 7,8-DCQA even make the percentage of TBARS higher than that in the control experiment, indicating that 5,8- and 7,8-DCQA behave as prooxidants in this case. The different effects of 4-quinolinols on DNA depend on the radical-generating methods since the oxidation mechanisms of DNA are different in the case of ·OH, ROO· or GS· as initiators. The present findings can be summed up and illustrated in Table 1. 7-CQA, 5,8- and 6,8-DCQA exhibit relative high inhibition activities to radical-induced oxidation of DNA.

It can be also found in Fig. 3 that the percentage of TBARS generated is even higher than 100% when 7-CQA and QA are used in ·OH- and AAPH-induced oxidation of DNA, respectively, and 5,8-DCQA and 7,8-DCQA are applied in Cu^{2+}/GSH-mediated oxidation of DNA. These results imply that much more TBARS is generated in the aforementioned experiments, demonstrating that these compounds act as prooxidants under these experimental conditions. Hence, the antioxidant and prooxidant mechanisms of 4-quinolinols are worthy to be explored in the further research work.

5. Conclusion

The antioxidant properties of the obtained compounds are screened in chemical and biological experimental systems, resulting in complicated results of dichloro-substituted 4-quinolinols to be antioxidants or prooxidants. It can be confirmed that introducing two chlorine atoms into benzene ring greatly changes the antioxidant property of 4-quinolinols in comparison with QA and 7-CQA. 5,7-DCQA has the highest ability to scavenge free radicals. 5,8-, 6,8- and 7,8-DCQA protect methyl linoleate against AAPH-induced oxidation with 6,8-DCQA having the highest ability. 5,7- and 5,8-DCQA together with QA are able to protect linoleic acid against the autoxidation. Except 7-CQA all the 4-quinolinols can protect DNA against ·OH-induced oxidation, while only 7-CQA and 5,8-DCQA play antioxidant role in AAPH-induced oxidation of DNA. In addition, 7-CQA acts as an antioxidant in Cu^{2+}/GSH-induced oxidation of DNA. The present work gives some *in vitro* results of dichloro-4-quinolinol to be antioxidants, and exhibits that it is worthy to screen the pharmacology of these compounds *in vivo*.

6. Experimental protocols

6.1. Materials

Diammonium salt of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), DPPH and galvinoxyl radicals were purchased from Fluka Chemie GmbH, Switzerland. AAPH, the naked DNA sodium salt, methyl linoleate, linoleic acid, diethyl ethoxymethylenemalonate were purchased from ACROS ORGANICS, Belgium. Other agents were of analytical grade and used directly. The structures of the obtained compounds were identified by ^1H and ^{13}C NMR (Varian Mercury 300 NMR spectrometer).

Table 1
A summarization of 4-quinolinol to protect DNA against different radical-induced oxidation.

4-Quinolinol	·OH	ROO·	GS·
QA			
7-CQA		√	√
5,7-DCQA			
5,8-DCQA	√	√	
6,8-DCQA	√		
7,8-DCQA			

√ Indicates the corresponding 4-quinolinol has the relative high ability to protect DNA against the radical-induced oxidation.

6.2. Synthesis of 4-quinolinols and identification of structures

A mixture of a corresponding amine (0.02 mol) and 4.76 g (0.022 mol) of diethyl ethoxymethylenemalonate were heated in a boiling water bath for 3 h and cooled to ambient temperature, followed by 50 mL diphenyl ether added. The mixture was heated at 250 °C in N_2 for 2 h under stirring. A large amount of white deposit were precipitated and cooled to ambient temperature, then diluted with petroleum ether. The deposit was filtered, washed with petroleum ether, and dried at vacuum pressure. Then, the solid was refluxed in 50 mL of 30% KOH aqueous solution with thin layer chromatography (TLC) inspections identifying the end of the hydrolysis. The solution was cooled to ambient temperature and acidified with 10% HCl aqueous solution to pH = 2. The crude product was precipitated and recrystallized with water and acetic acid (10:1) [18].

6.2.1. 4-Quinolinol-3-carboxylic acid (QA) yield 48%

^{1}H NMR (300 MHz, DMSO-*d6*) δ: 8.91 (s, 1H, CH═N), 8.30 (d, J = 8.4 Hz, 1H, CH═CH− in phenyl), 7.90 (t, J = 8.1 Hz and 7.2 Hz, 1H, CH═CH− in phenyl), 7.83 (d, J = 8.1 Hz, 1H, CH═CH− in phenyl), 7.61 (t, J = 7.5 Hz and 7.5 Hz, 1H, CH═CH− in phenyl), 13.43 (s, 1H, −OH), 15.36 (s, 1H, −COOH); ^{13}C NMR (75 MHz, DMSO-*d6*) δ: 178.3, 166.3, 145.2, 139.4, 133.9, 126.2, 125.0, 124.4, 119.6, 107.6. Calculation of elements for $C_{10}H_7NO_3$: C, 63.49; H, 3.73; N, 7.40; found C, 63.60; H, 3.58; N, 7.31.

6.2.2. 7-Chloro-4-quinolinol-3-carboxylic acid (7-CQA) yield 56%

^{1}H NMR (300 MHz, DMSO-*d6*) δ: 8.94 (s, 1H, CH═N), 8.27 (d, J = 8.7 Hz, 1H, CH═CH− in phenyl), 7.84 (s, 1H, CH═CH− in phenyl), 7.60 (d, J = 8.7 Hz, 1H, CH═CH− in phenyl), 15.2 (s, 1H, −COOH); ^{13}C NMR (75 MHz, DMSO-*d6*) δ: 177.5, 166.2, 146.5, 141.0, 138.1, 127.1, 126.2, 123.2, 119.4, 108.0. Calculation of elements for $C_{10}H_6ClNO_3$: C, 53.71; H, 2.70; N, 6.26; found C, 53.83; H, 2.79; N, 6.31.

6.2.3. 5,7-Dichloro-4-quinolinol-3-carboxylic acid (5,7-DCQA) yield 60%

^{1}H NMR (300 MHz, DMSO-*d6*) δ: 8.92 (s, 1H, CH═N), 7.78 (s, 1H, CH═CH− in phenyl), 7.71 (s, 1H, CH═CH− in phenyl), 13.40 (s, 1H, −OH), 15.02 (s, 1H, −COOH); ^{13}C NMR (75 MHz, DMSO-*d6*) δ: 177.9, 165.8, 145.8, 142.3, 137.4, 134.3, 128.3, 119.9, 118.4, 109.3. Calculation of elements for $C_{10}H_5Cl_2NO_3$: C, 46.54; H, 1.95; N, 5.43; found C, 46.69; H, 1.87; N, 5.61.

6.2.4. 5,8-Dichloro-4-quinolinol-3-carboxylic acid (5,8-DCQA) yield 38%

^{1}H NMR (300 MHz, DMSO-*d6*) δ: 8.60 (s, 1H, CH═N), 8.00 (d, J = 8.4 Hz, 1H, CH═CH− in phenyl), 7.58 (d, J = 8.4 Hz, 1H, CH═CH− in phenyl), 12.75 (s, 1H, −OH), 14.82 (s, 1H, −COOH); ^{13}C NMR (75 MHz, DMSO-*d6*) δ: 178.2, 165.5, 145.3, 138.2, 133.5, 131.9, 128.7, 122.4, 122.1, 109.6. Calculation of elements for $C_{10}H_5Cl_2NO_3$: C, 46.54; H, 1.95; N, 5.43; found C, 46.38; H, 2.01; N, 5.55.

6.2.5. 6,8-Dichloro-4-quinolinol-3-carboxylic acid (6,8-DCQA) yield 45%

^{1}H NMR (300 MHz, DMSO-*d6*) δ: 8.60 (s, 1H, CH═N), 8.25 (d, J = 1.5 Hz, 1H, CH═CH− in phenyl), 8.13 (d, J = 1.5 Hz, 1H, CH═CH− in phenyl), 13.04 (s, 1H, −OH), 14.75 (s, 1H, −COOH); ^{13}C NMR (75 MHz, DMSO-*d6*) δ: 176.9, 165.4, 145.9, 135.2, 133.5, 130.5, 126.8, 124.7, 123.5, 108.7. Calculation of elements for $C_{10}H_5Cl_2NO_3$: C, 46.54; H, 1.95; N, 5.43; found C, 46.51; H, 2.05; N, 5.37.

6.2.6. 7,8-Dichloro-4-quinolinol-3-carboxylic acid (7,8-DCQA) yield 40%

^{1}H NMR (300 MHz, DMSO-*d6*) δ: 8.60 (s, 1H, CH═N), 8.23 (d, J = 8.7 Hz, 1H, CH═CH− in phenyl), 7.80 (d, J = 8.7 Hz, 1H, CH═CH− in phenyl), 12.98 (s, 1H, −OH), 14.60 (s, 1H, −COOH); ^{13}C NMR (75 MHz, DMSO-*d6*) δ: 177.7, 165.4, 146.2, 137.4, 137.2, 127.1, 125.3, 124.6, 121.5, 108.7. Calculation of elements for $C_{10}H_5Cl_2NO_3$: C, 46.54; H, 1.95; N, 5.43; found C, 46.44; H, 1.91; N, 5.48.

6.3. Interactions of 4-quinolinols with $ABTS^{+\cdot}$, DPPH and galvinoxyl radicals

The reactions between 4-quinolinols and $ABTS^{+\cdot}$, DPPH and galvinoxyl radicals were carried out as previous descriptions [29,32]. DPPH and galvinoxyl radicals were dissolved in ethanol to make the absorbance around 1.00 at 517 nm and 428 nm, respectively. Two milliliter of 4.0 mM ABTS aqueous solution was oxidized by 1.41 mM $K_2S_2O_8$ for 16 h, then 100 mL of ethanol was added to make the absorbance of $ABTS^{+\cdot}$ around 0.70 at 734 nm. 4-Quinolinols were dissolved in dimethyl sulfoxide (DMSO) as the stock solution, and 0.1 mL was added to 1.9 mL of radical solution to keep the final concentration of 4-quinolinols at 1.5 mM. The absorbance (A_{detect}) was measured after 30 min. The absorbance of the control experiment (A_{ref}) containing 0.1 mL DMSO was also measured at 30 min. The percentages of residual radicals were calculated by $(A_{detect}/A_{ref}) \times 100$.

6.4. Effects of 4-quinolinols on the oxidations of linoleic acid and methyl linoleate

Methyl linoleate, AAPH, and 4-quinolinols were dissolved in *t*-butanol/H_2O (2:1, *v*:*v*) to 13.3 mM, 35.6 mM, and 2.0 mM as the final concentration (C_0), respectively. To quantitate the concentration of methyl linoleate, methyl palmitate was added to a final concentration of 8.9 mM as an internal standard because, as an ester of saturated fatty acid, it cannot be oxidized during the oxidation of methyl linoleate. Then, the above solution was incubated at 37 °C to initiate the oxidation. After 4 h the concentrations of methyl linoleate in the presence of 4-quinolinols and in the control experiment were determined by GC and recorded as C_{detect} and C_{ref}. The percentage of the concentration of the exhausted methyl linoleate was calculated by $(C_0 - C_{detect})/(C_0 - C_{ref}) \times 100$. GC analysis was performed on a Hewlett-Packard 1890 GC equipped with a SE-54 30 m × 0.25 mm capillary column, 0.25 μm film thickness, N_2. The temperature in chamber and injector was 260 °C and 280 °C, respectively, and the temperature in the hydrogen flame ionization detector was 300 °C [32]. The same volume of DMSO was involved in the control experiment.

An emulsion was prepared by dissolving 5.0 mg of β-carotene, 40 mg of linoleic acid 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 then shaken under ultrasonic vibration to form homogeneous β-carotene-linoleic acid emulsion (λ_{max} = 460 nm) with the absorbance recorded as A_0 [31]. DMSO solutions of 4-quinolinols (0.3 mL) were mixed with 2.7 mL of the emulsion with the final concentration of 4-quinolinols being 0.3 mM. The absorbance of the mixture was measured after 100 min and recorded as A_{detect}. The same volume of DMSO was involved in the control experiment, and the absorbance was also measured at 100 min and recorded as A_{ref}. The percentage of the decrease for the absorbance was calculated by $(A_0 - A_{detect})/(A_0 - A_{ref}) \times 100$.

6.5. Effects of 4-quinolinols on the oxidations of DNA

·OH-mediated oxidation of DNA was performed as previous description [37]. DNA sodium salt was dissolved in phosphate buffered solution (PBS: 6.1 mM Na_2HPO_4, 3.9 mM NaH_2PO_4), and 13.4 mL of DNA solution was mixed with 0.1 mL of DMSO solution

of 4-quinolinols and 0.5 mL of 60 mM DMSO solution of tetrachlorohydroquinone. Finally, 1.0 mL of 60 mM PBS solution of H_2O_2 was added. The final concentrations of DNA, tetrachlorohydroquinone, H_2O_2, and 4-quinolinols were 2.0 mg/mL, 2.0 mM, 4.0 mM, and 400 μM, respectively. The solution was dispatched into test-tubes with 2.0 mL contained in each one. The test-tubes were incubated in 37 °C water bath for 30 min to perform the oxidation of DNA. Three test-tubes were taken out, and 1.0 mL of TBA and 1.0 mL of 3.0% trichloroacetic acid aqueous were added. Then, the solution was heated in a boiling water bath for 15 min. After the solution was cooled to room temperature, 1.5 mL of *n*-butanol was added and shaken vigorously to extract TBARS, whose absorbance was measured at 535 nm.

AAPH-induced oxidation of DNA was performed as previous description [46]. AAPH and DNA sodium salt were dissolved in PBS with DMSO solution of 4-quinolinols added. The final concentrations of DNA, AAPH, and 4-quinolinols were 2.0 mg/mL, 40 mM, and 200 μM, respectively. The solution was dispatched into test-tubes with 2.0 mL contained in each one. The test-tubes were incubated in 37 °C water bath for 4 h to perform the oxidation of DNA. The following treatment to measure TBARS was the same as above mentioned.

Cu^{2+}/GSH-induced oxidation of DNA was performed as previous description [38,39]. $CuSO_4$ aqueous solution was mixed with PBS solutions of DNA and GSH with DMSO solution of 4-quinolinols added. The final concentrations for DNA, Cu^{2+}, GSH and 4-quinolinol were 2.0 mg/mL, 5.0 mM, 4.0 mM, and 200 μM, respectively. The mixture was dispatched into test-tubes with 2.0 mL contained in each one. The test-tubes were incubated in 37 °C water bath for 4 h to perform the oxidation of DNA. Three test-tubes were taken out, and 1.0 mL of 30.0 mM PBS solution of EDTA was added to chelate Cu^{2+}. Then, the following treatment to measure TBARS was the same as above mentioned. The same volume DMSO was contained in the control experiment.

6.6. Statistical analysis

The data presented in figures were the average values from three independent measurements with an experimental error within 10%. The results were analyzed statistically by SPSS software (version 10.0) to show a significant difference.

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