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Original article

Solvent-free Povarov reaction for synthesizing ferrocenyl quinolines: Antioxidant abilities deriving from ferrocene moiety

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ABSTRACT

Twenty-two 2-phenyl-4-ferrocenylquinolines are synthesized by Povarov three-component-reaction (3CR) among the substituted anilines, benzaldehydes, and ferrocenylacetylene with $Ce(OTf)_3$ being catalyst in the absence of solvents. The antioxidative effects of the obtained quinolines are estimated by quenching 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\bullet}$), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), and galvinoxyl radicals, and by inhibiting Cu^{2+}/glutathione (GSH)-, hydroxyl radical (•OH)–, and 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH)-induced oxidations of DNA. It is found that the ferrocenyl group instead of hydroxyl group generates the antioxidative effect for quinoline to quench radicals and to protect DNA against radical-induced oxidations. The antioxidative effect generated by ferrocenyl group can be further increased by the electron-donating moieties such as furan, $-N(CH_3)_2$, $-OCH_3$, and ferrocenyl group, while the electron-withdrawing groups such as $-NO_2$ and –Cl are not beneficial for quinolines to be antioxidants. The ferrocenyl group in quinoline exhibits higher antioxidant activity than hydroxyl group in Trolox.

1. Introduction

As an old compound prepared by Skraup method initially [1], quinoline attracts much research attentions because of a variety of biological properties such as anticancer [2], antimalarial [3], antifungal [4], antileishmanial [5], and antioxidative activities [6]. The wide applications of quinoline motivate researchers to explore the synthetic methods. The recent developments on constructing the pyridine moiety for quinoline can be generally cataloged as Friedländer annulation [7] and Povarov reaction [8]. In Friedländer annulation, an *ortho*-aminobenzoyl ketone is used to condense with a carbonyl or an alkynyl compound to afford 2,4-disubstituted quinoline. On the other hand, Povarov reaction, a three-component-reaction (3CR), takes place among amine, aldehyde, and alkyne to form quinoline scaffold, during which a Diels–Alder reaction between imino (produced by the condensation of amine with aldehyde) and alkyne acts as the key step [9].

2. Chemistry

The investigation on the synthesis of quinoline mainly focuses on screening catalysts employed in the aforementioned reactions. As an acid-catalyzed reaction, Friedländer annulation can be carried out in the presence of solid acids such as Chitosan-SO_3H [10], MCM-41 [11] and SBA-15 molecular sieves [12], CuO nanoparticles [13], and of metallic salts such as LiOTf [14], $Zr(OTf)_4$ [15], and $InCl_3$ [16]. On the other hand, oxidants are needed in the Povarov 3CR to activate the alkynyl bond for the following imino-involved Diels–Alder reaction. Thus, $Ce(OTf)_3$ [17], $FeCl_3$ [18], I_2 [19], YCl_3 [20], $SbCl_3$ [21], $SnCl_2$ [22], and $Fe(OTf)_2$ with TEMPO oxoammonium salt [23] are usually applied to catalyze the Povarov 3CR. Moreover, some operation skills including microwave irradiation [24], ultrasonic vibration [25], UV-irradiation [26], and ionic liquid [27] are also employed in the synthesis of quinoline. As shown in Scheme 1, we herein follow the method reported in a literature [17] to prepare 22 ferrocenyl-substituted quinolines with $Ce(OTf)_3$ being the catalyst. Comparing with the report [17], the major innovation in the present work is due to a solvent-free condition applied, decreasing the reaction period in the case of a similar yield obtained and simplifying the purification procedure.

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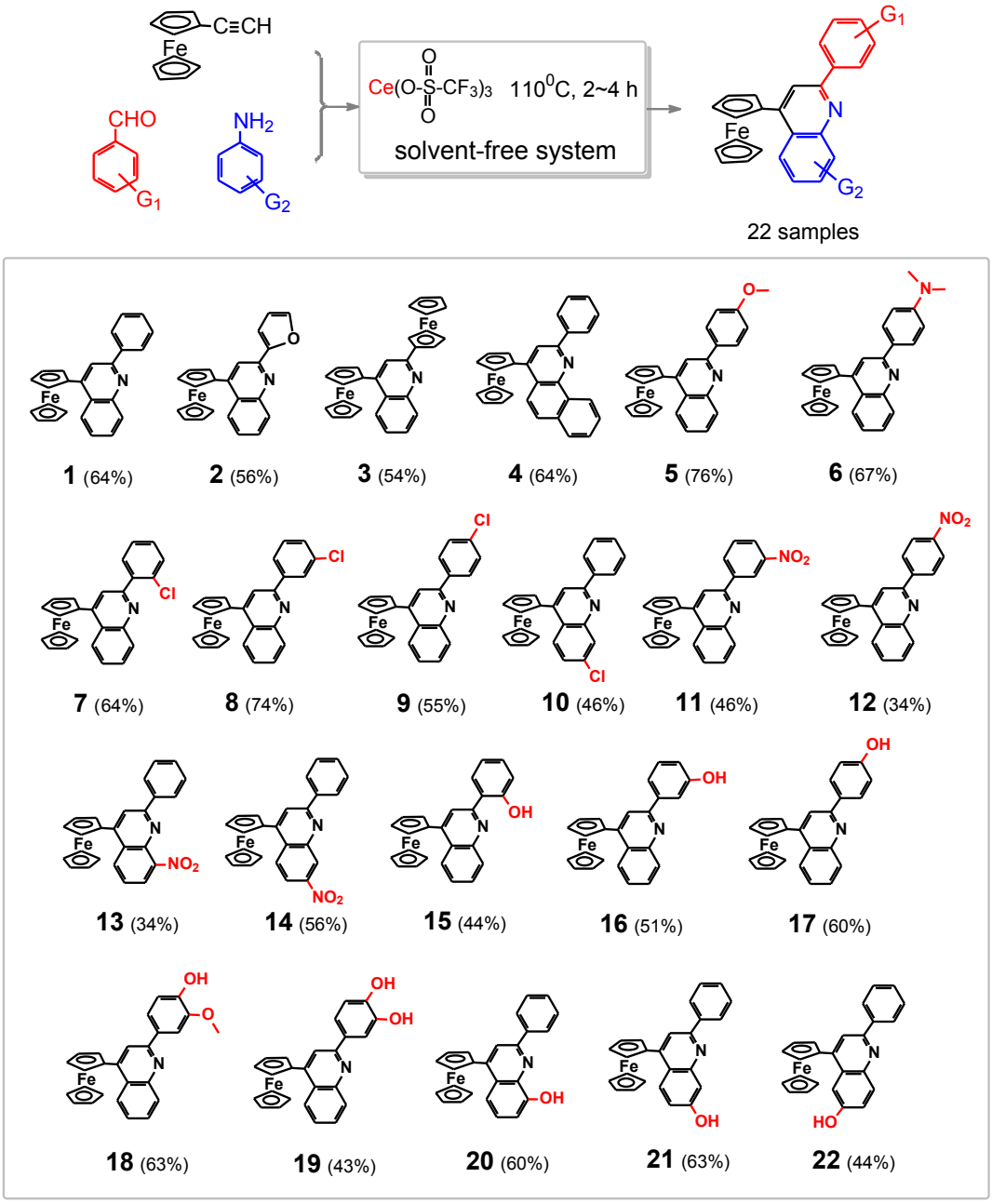


Scheme 1. Synthetic routine of ferrocenylquinolines.

3. Pharmacology

The reason for selecting ferrocenylquinolines in this work is owing to the electron-abundant ferrocene moiety may change the interaction modes between the ferrocenyl compounds and biological species [28], and especially, may improve the radical-scavenging and antioxidative properties of ferrocenyl compounds as we have reported previously [29]. Nevertheless, we have synthesized some quinolines including fluoro- or chloro-substituted quinolinols and furoquinolines [30] for exploring the antioxidant properties, but the hydroxyl group in quinoline only exhibits relative low antioxidant effectiveness. The developments of ferrocene-appended drugs such as ferrocenic steroids [31] and ferrocenyl hydrazones [32] encourage us to investigate whether ferrocene can be a substituent to ameliorate the antioxidative effectiveness of quinolines. Thus, presented here is a study on the abilities of ferrocenylquinolines to scavenge radicals and to inhibit DNA oxidations. The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\bullet}$), 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), and galvinoxyl radical act as the radical resources, and 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH, R–N=N–R, R = $-CMe_2C(=NH)NH_2$), Cu^{2+}/glutathione (GSH), and hydroxyl radical (•OH)-induced oxidations of DNA act as the oxidative modes for biological species.

4. Results and discussion

4.1. Synthesis and radical-scavenging abilities of ferrocenylquinolines

The catalyst used in Povarov 3CR possesses Lewis acidic and oxidative properties because it should catalyze the reaction between $-NH_2$ and $-CHO$ to form imino and then activate C≡C for

taking part in the following Diels–Alder reaction with the produced imino. As pointed out in the Introduction, many oxidants are able to carry out the Povarov 3CR, while those catalysts with strong oxidative abilities (such as I_2) may oxidize iron atom in ferrocene moiety, and weak oxidants (such as $FeCl_3$) cannot activate C≡C in ferrocenylacetylene. Following the method in the literature [17], we select a mild oxidant, $Ce(OTf)_3$, as the catalyst. In addition, following the direction in a review on the synthesis of heterocycles [33], we attempt to avoid solvent to be used in the synthetic process. It is found that the reaction period is decreased from ~6 h as reported in the literature [17] to ~3 h in our work. The solvent-free condition simplifies the purification procedure because the pure products can be readily obtained by a silica chromatography.

The antioxidant effectiveness mainly involves scavenging radicals and inhibiting oxidations of biological species. As stable radicals at ambient temperature, $ABTS^{+\bullet}$, DPPH, and galvinoxyl radical are usually used to test the ability of an antioxidant to reduce radical and to donate its hydrogen atom or electron to *N*-centered and *O*-centered radicals, respectively [34]. As shown in Fig. 1S, mixing ferrocenylquinolines with the solutions of the aforementioned radicals leads to decreases of the concentrations of these radicals for longer the reaction period, indicating that quinolines used herein are able to quench these radicals except that compounds from **1** to **14** cannot react with galvinoxyl radical. So, it can be deduced that the ferrocenyl is an active group for compounds from **1** to **14** to quench $ABTS^{+\bullet}$ and DPPH but cannot provide electrons to quench *O*-centered radical (galvinoxyl radical).

The thermodynamic method can express the number of radicals trapped by an antioxidant [35], but the kinetic mode can reveal the reaction rate (r) of the antioxidant to quench radical. It has been pointed out that the reaction between an antioxidant and DPPH follows second-order kinetics as shown as Equation (1) [36],

$$-\frac{d[\mathrm{DPPH}]}{dt} = r = k[\mathrm{DPPH}][\mathrm{antioxidant}] \quad (1)$$

where r correlates proportionally with the concentrations of DPPH and the antioxidant in the process of the reaction. Thus, Equation (1) is also available at the reaction time being 0 min, and $[\mathrm{DPPH}]_{t=0}$ and $[\mathrm{antioxidant}]_{t=0}$ are the concentrations of DPPH and the antioxidant at the beginning of the reaction. So, if the reaction rate at the beginning of the reaction (r_0) is measured, the rate constant (k) can be calculated by Equation (2).

$$r_0 = k[\mathrm{DPPH}]_{t=0}[\mathrm{antioxidant}]_{t=0} \quad (2)$$

The r_0 is the slope of the tangent of the decay curves in Fig. 1S. As we have suggested a method for estimating r_0 [37], the double exponential function (Equation (3)) is applied to fit for the data in Table 1S because the reaction rate (r) is respectively related to the concentrations of two reagents (see Equation (1)).

$$[\mathrm{radical}] = Ae^{-(t/a)} + Be^{-(t/b)} + C \quad (3)$$

Then, a differential operation is carried out on Equation (3) to obtain Equation (4) for expressing the variation of the reaction rate (r) with the reaction time (t).

$$-d[\mathrm{radical}]/dt = \boldsymbol{r} = (\mathrm{A}/\mathrm{a})e^{-(t/a)} + (\mathrm{B}/\mathrm{b})e^{-(t/b)} \quad (4)$$

The reaction rate at $t = 0$ (r_0) can be calculated by Equation (4), and thus, the rate constant (k) are obtained by Equation (2). The Equations (3) and (4) together with r_0 in the case of quinolines quenching $ABTS^{+\bullet}$, DPPH, and galvinoxyl radical are listed in Table 1S. The rate constants are collected in Table 1 for comparing the abilities of quinolines to quench these radicals.

We have synthesized 7-chloro-4-hydroxylquinoline, a popular anticancer drug [30], which is selected to be the reference compound herein. As shown in Table 1, 7-chloro-4-hydroxylquinoline can only quench $ABTS^{+\bullet}$ with the lowest k value (0.009 $mM^{-1}\ s^{-1}$) among quinolines used herein and cannot react with other two radicals. This result indicates that the radical-scavenging ability of ferrocenyl group at 4-position in quinoline is much higher than a hydroxyl group at the same position. But it can be found that the k values of all the quinolines used herein are lower than that of Trolox, indicating that the abilities of ferrocenylquinolines either containing hydroxyl group (as compounds from 15 to 22) or not (as other compounds) to reduce $ABTS^{+\bullet}$ are lower than that of Trolox. However, the k value of compound 3 (25.99 $mM^{-1}\ s^{-1}$), 2,4-diferrocenyl-substituted quinoline, approaches to that of Trolox (29.2 $mM^{-1}\ s^{-1}$), again demonstrating that more ferrocenyl-substitution may enhance the ability of quinoline to reduce radical. Furthermore, comparing with compound 1, it can be found that both electron-withdrawing group ($-NO_2$, $-Cl$) and electron-donating group ($-OCH_3$, $-N(CH_3)_2$) do not markedly vary the k values of the corresponding quinolines, but the hydroxyl group changes the k values obviously. This result reveals that the hydroxyl group in combination with ferrocenyl group can improve the ability of quinolines to reduce $ABTS^{+\bullet}$ as well as to quench DPPH. As the reference compound, 7-chloro-4-hydroxylquinoline cannot react with DPPH, and thus the reaction between compound 1 and DPPH is owing to the ferrocenyl group providing electrons for quenching DPPH. With the increase of the amount of ferrocenyl group in quinoline, the ability of compound 3 to quench DPPH is enhanced significantly. This is in agreement with our previous observation that more ferrocenyl group can improve the radical-scavenging properties of chalcones [38]. The k values of compounds from 15 to 22 are higher than that of Trolox (0.353 $mM^{-1}\ s^{-1}$), thus the introduction of hydroxyl groups into ferrocenylquinolines improves the abilities to quench DPPH remarkably because both hydrogen atom in hydroxyl group and electron in ferrocenyl group can quench *N*-centered radical

Table 1
The rate constants (k) for ferrocenylquinolines in scavenging $ABTS^{+\bullet}$, DPPH, and galvinoxyl radicals.

Compound	Rate constants for ferrocenylquinolines to scavenge radicals, k ($mM^{-1}\ s^{-1}$)		
	$ABTS^{+\bullet}$	DPPH	Galvinoxyl radical
1	3.08	0.16	–
2	2.08	0.15	–
3	25.99	1.63	–
4	2.19	0.26	–
5	2.40	0.15	–
6	2.21	0.57	–
7	1.65	0.33	–
8	2.45	0.32	–
9	3.24	0.26	–
10	3.32	0.19	–
11	2.36	0.12	–
12	1.99	0.24	–
13	1.93	0.35	–
14	1.55	0.20	–
15	5.15	1.18	0.54
16	5.53	0.97	0.89
17	5.45	1.33	1.62
18	7.30	1.19	1.07
19	13.69	1.71	3.34
20	7.15	2.95	0.89
21	16.38	2.35	2.82
22	11.58	1.83	1.63
7-Chloro-4-hydroxylquinoline	0.009	–	–
Trolox	29.2	0.353	1.70

simultaneously. On the other hand, only hydroxyl-substituted quinolines (compounds from 15 to 22) can react with galvinoxyl radical, indicating that galvinoxyl radical can only accept hydrogen atom in hydroxyl group, and more hydroxyl groups (in compound 19) increases the k value (3.34 mM^{-1} s^{-1}) even higher than that of Trolox (1.70 mM^{-1} s^{-1}). The hydroxyl group at 4-position as 7-chloro-4-hydroxylquinoline cannot react with galvinoxyl radical, while the hydroxyl group at 6- and 8-position (compounds 22 and 20, respectively), especially at 7-position (compound 21), is active to contribute hydrogen atom to *O*-centered radical. The above results indicate that ferrocenyl group can be an electron-donator for quenching radicals and therefore encourage us to test whether ferrocenylquinolines can inhibit radical-induced oxidation of biological species.

4.2. Effects of ferrocenylquinolines on AAPH-induced oxidation of DNA

The guanine bases in DNA are susceptible to the peroxyl radical ($•OOCMe_2C(=NH)NH_2$) generated from the decomposition of AAPH [39]. The oxidative products can react with thiobarbituric acid (TBA) to form colorful adducts with λ_{max} at 535 nm and is thereby called as thiobarbituric acid reactive species (TBARS) [40]. Fig. 2S shows that the absorbance in the blank experiment increases with the reaction period, indicating that more TBARS are produced in the process of the DNA oxidation. But the addition of quinolines retards the oxidation of DNA for a period, and the *inhibition period* (t_{inh}) can be measured from the beginning of the reaction ($t = 0$ min) to the cross-point of the tangents for the inhibition and oxidation period. Fig. 3S outlines the relationships between t_{inh} and the concentrations of quinolines, and the lines in Fig. 3S are quantitatively expressed by the equations of t_{inh}~[ferrocenylquinoline] and listed in Table 2.

Although the slope in the equation of t_{inh}~[ferrocenylquinoline] indicates the sensitivity of t_{inh} with the variation of the concentrations of ferrocenylquinoline, chemical kinetic deduction reveals that t_{inh} is proportionally related to the concentration of an antioxidant as shown as Equation (5), in which n stands for the stoichiometric factor, and R_i refers to the initiation rate of the radical-induced oxidation [41].

$$t_{inh} = (n/R_i)[\text{antioxidant}] \quad (5)$$

The n value can be used to characterize the antioxidant effectiveness because this parameter is not related to the experimental conditions such as the concentrations of antioxidants and radical-initiators but just correlates with the structure of the antioxidant. Meanwhile, it is safely to assume that the R_i is equal to the generation rate (R_g) of radicals ($R_g = (1.4 \pm 0.2) \times 10^{-6}$ [AAPH] s^{-1}) [41] because sodium salt of DNA and AAPH are dissolved in water, and radicals generated from AAPH attack DNA at the same phase [42]. Thus, the n values of ferrocenylquinolines are the product of the coefficients in the equation of t_{inh}~[ferrocenylquinoline] and $R_i = R_g = 1.4 \times 10^{-6} \times 40$ mM $s^{-1} = 3.36$ μM min^{-1}. The obtained n values are listed in Table 2 as well. Unfortunately, 7-chloro-4-hydroxylquinoline and Trolox just decrease the formation rate of TBARS but cannot generate inhibition period, resulting in that the n values of these two compounds cannot be obtained. This result again proves that the ferrocenyl group makes quinoline a stronger antioxidant than hydroxyl group. The functions of other substituents on the n values of ferrocenylquinolines are illustrated in Scheme 2.

As shown in the first panel of Scheme 2 (Fc represents ferrocenyl group), when the n value of compound **1** ($n = 6.2$) acts as the reference, revealing the antioxidant effectiveness generated by the skeleton of quinoline (2-phenyl-4-ferrocenylquinoline). An electron-donating group attaching to the *para*-position in the 2-phenyl group, for example, $-OCH_3$ in compound **5** ($n = 9.6$) and $-N(CH_3)_2$ in compound **6** ($n = 30.0$), or an electron-abundant moiety, for example, furan ring in compound **2** ($n = 10.3$) and another ferrocenyl group in compound **3** ($n = 20.4$), can significantly enhance the abilities of quinolines to protect DNA against AAPH-induced oxidation. Contrarily, as can be seen in the second panel of Scheme 2, when an electron-withdrawing group ($-NO_2$) attaches to compounds from **11** to **14**, the n values of the corresponding compounds are lower than that of compound **1**. Moreover, as can be seen in the third panel of Scheme 2, a hydroxyl group at *meta*-position of ring C (compound **16**, $n = 10.2$), especially at *para*-position of ring C (compound **17**, $n = 16.9$), increases the abilities of the corresponding quinolines to inhibit AAPH-induced oxidation. But a methoxyl or another hydroxyl group at the adjacent position of the hydroxyl group (compound **18**, $n = 9.9$, and compound **19**, $n = 17.6$) does not affect the antioxidant effectiveness of the corresponding quinolines markedly. On the other hand, the hydroxyl group at ring A increases the n value remarkably. In particular, the hydroxyl group at 7-position increases the n value of compound **21**–15.6. To sum up the aforementioned results, it can be concluded that electron-donating moiety as ring C or a single hydroxyl group at *para*-position of ring C or at 7-positon is beneficial for quinolines to inhibit AAPH-induced oxidation of DNA. As can be seen in the forth panel of Scheme 2, the antioxidant effectiveness in the case of chloro as the substituent also follows the above rule, in which the chloride atom at *ortho*- or *meta*-position of ring C (compound **7**, $n = 6.4$, and compound **8**, $n = 7.0$) does not affect the n values, while the antioxidant effectiveness is obviously enhanced by the chloride at *para*-position in ring C or at 7-position (compound **9**, $n = 9.6$, and compound **10**, $n = 9.3$).

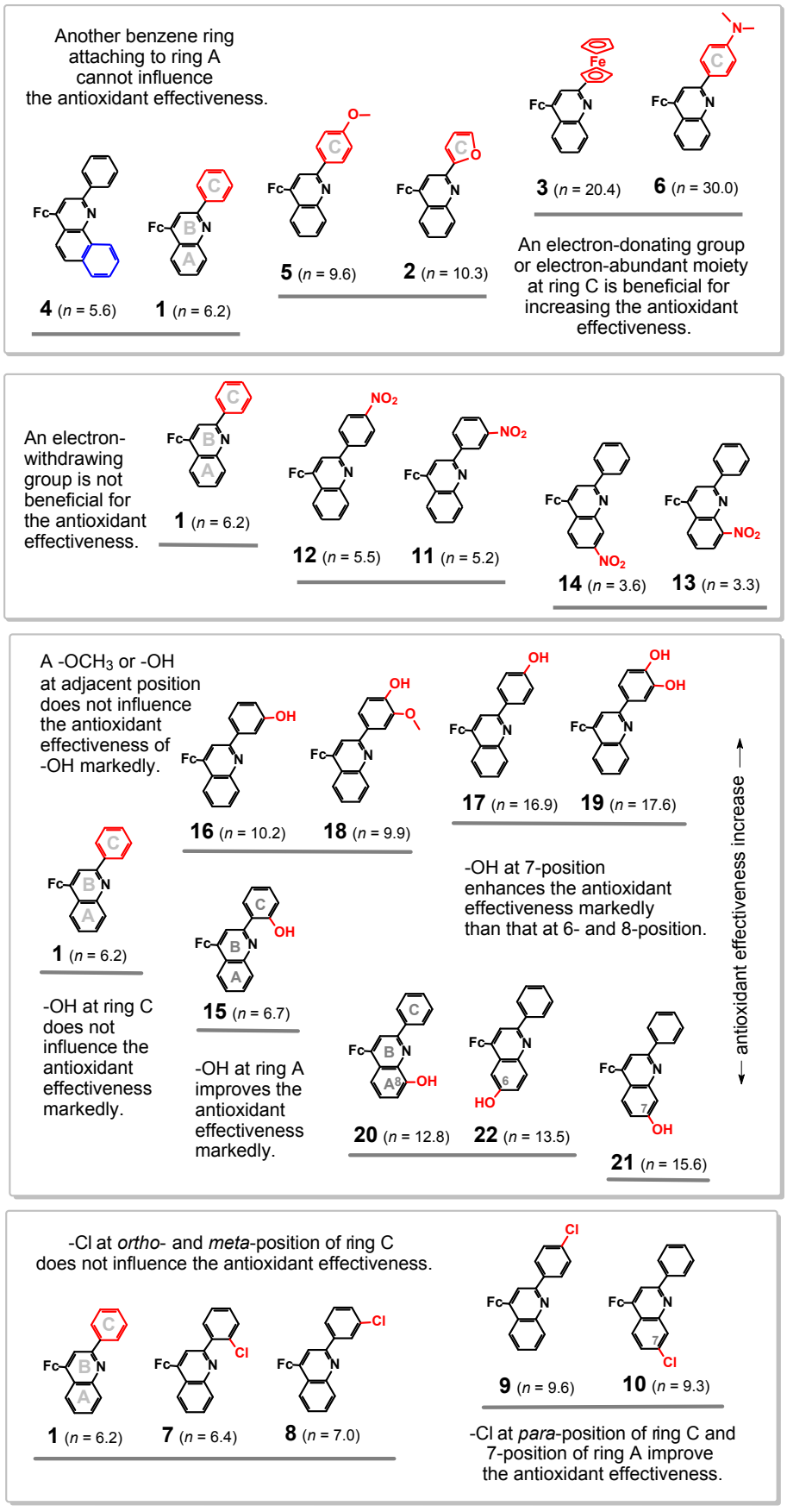
Table 2
The equations of t_{inh}~[ferrocenylquinolines] and n of ferrocenylquinolines in protecting DNA against AAPH-induced oxidation.[a]

Compound	t_{inh} (min) = (n/R_i) [ferrocenylquinolines (μM)] + constant[b]	n
1	$t_{inh} = 1.85$ (±0.09) [**1**] + 20.0 (±1.00)	6.2 (±0.3)
2	$t_{inh} = 3.06$ (±0.15) [**2**] + 110.8 (±5.54)	10.3 (±0.5)
3	$t_{inh} = 6.06$ (±0.30) [**3**] + 123.6 (±6.18)	20.4 (±1.0)
4	$t_{inh} = 1.66$ (±0.08) [**4**] + 12.2 (±0.61)	5.6 (±0.3)
5	$t_{inh} = 2.85$ (±0.14) [**5**] − 4.5 (±0.23)	9.6 (±0.5)
6	$t_{inh} = 8.94$ (±0.45) [**6**] + 19.3 (±0.97)	30.0 (±1.5)
7	$t_{inh} = 1.90$ (±0.10) [**7**] − 3.9 (±0.20)	6.4 (±0.3)
8	$t_{inh} = 2.07$ (±0.10) [**8**] + 2.8 (±0.14)	7.0 (±0.4)
9	$t_{inh} = 2.85$ (±0.14) [**9**] + 6.4 (±0.32)	9.6 (±0.5)
10	$t_{inh} = 2.78$ (±0.14) [**10**] + 1.6 (±0.08)	9.3 (±0.5)
11	$t_{inh} = 1.56$ (±0.08) [**11**] + 64.0 (±3.20)	5.2 (±0.3)
12	$t_{inh} = 1.65$ (±0.08) [**12**] + 24.7 (±1.24)	5.5 (±0.3)
13	$t_{inh} = 0.99$ (±0.05) [**13**] + 18.5 (±0.93)	3.3 (±0.2)
14	$t_{inh} = 1.08$ (±0.05) [**14**] + 61.2 (±3.06)	3.6 (±0.2)
15	$t_{inh} = 1.98$ (±0.10) [**15**] + 85.7 (±4.29)	6.7 (±0.3)
16	$t_{inh} = 3.02$ (±0.15) [**16**] − 2.0 (±0.10)	10.2 (±0.5)
17	$t_{inh} = 5.04$ (±0.25) [**17**] − 19.9 (±1.00)	16.9 (±0.9)
18	$t_{inh} = 2.94$ (±0.15) [**18**] + 8.7 (±0.44)	9.9 (±0.5)
19	$t_{inh} = 5.23$ (±0.26) [**19**] − 6.7 (±0.34)	17.6 (±0.9)
20	$t_{inh} = 3.82$ (±0.19) [**20**] + 3.5 (±0.18)	12.8 (±0.6)
21	$t_{inh} = 4.63$ (±0.23) [**21**] + 33.5 (±1.68)	15.6 (±0.8)
22	$t_{inh} = 4.02$ (±0.20) [**22**] + 3.1 (±0.16)	13.5 (±0.7)

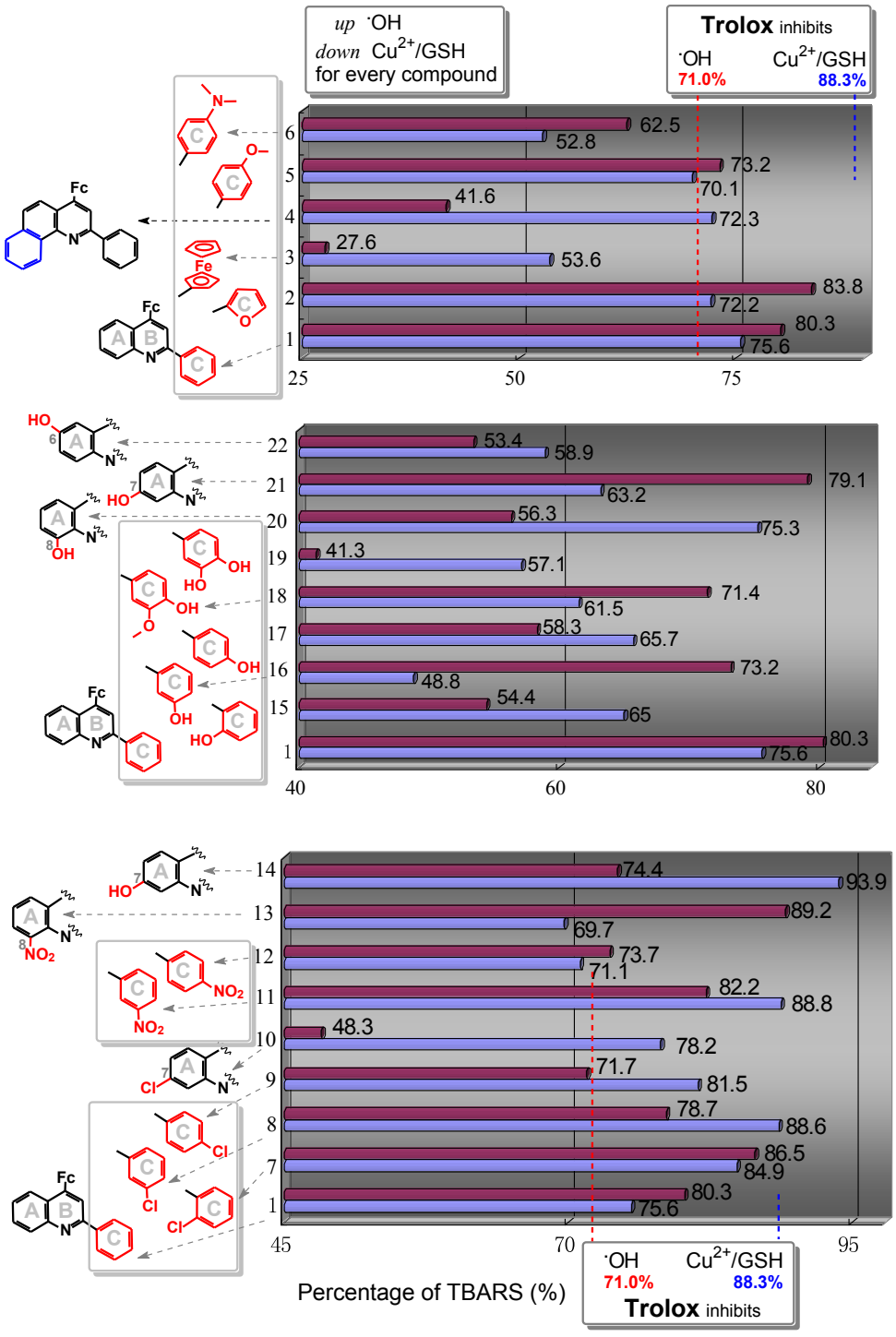
[a] $R_i = R_g = 1.4 \times 10^{-6}$ [AAPH] $s^{-1} = 3.36$ μM min^{-1} when 40 mM AAPH was employed, thus, n = coefficient × 3.36 μM min^{-1}.
[b] The constant was generated from the linear regression analysis.

4.3. Effects of ferrocenylquinolines on Cu^{2+}/GSH- and •OH-induced oxidations of DNA

The above result from ferrocenylquinoline inhibiting AAPH-induced oxidation of DNA motivates us to explore whether these



Scheme 2. The relationships between the *n* values and the structures of ferrocenylquinolines.



Scheme 3. The relationships between the percentage of TBARS and structures of ferrocenylquinolines.

quinolines can also protect DNA against the oxidation caused by other radicals. The glutathione (GSH) can be converted into a radical (GS•) *in vivo* in the presence of copper ions [43]. •OH is a metabolic-generating radical and can be produced by mixing H_2O_2 with tetrachlorohydroquinone (TCHQ) [44]. So, Cu^{2+}/GSH- and •OH-induced oxidations of DNA can be the experimental systems used to evaluate the antioxidant activity. After a certain reaction period (90 min for Cu^{2+}/GSH-induced oxidation of DNA and 30 min for •OH-induced oxidation of DNA), the generated TBARS is measured as the control. Then, a certain concentration of ferrocenylquinolines (50 μM) is added to the experimental system, the measured TBARS is compared with that in the blank experiment. A low percentage of TBARS indicates a high antioxidant effectiveness of quinolines. The TBARS percentages generated by 7-chloro-4-hydroxylquinoline in Cu^{2+}/GSH- and •OH-induced oxidations of DNA are 104% and 115%, respectively, indicating that hydroxyl group at 4-position in quinoline cannot protect DNA against GS- and •OH-induced oxidations. The TBARS percentages generated by

compound **1** are 75.6% and 80.3% in inhibiting Cu^{2+}/GSH- and •OH-induced oxidations of DNA, respectively, and thus, ferrocenyl group at 4-position makes quinoline an antioxidant in this case. The TBARS percentages of Trolox are 88.3% and 71.0% in inhibiting Cu^{2+}/GSH- and •OH-induced oxidations of DNA, respectively. The ability of compound **1** to inhibit Cu^{2+}/GSH-induced oxidation of DNA is higher than that of Trolox, but a contrary result is obtained in inhibiting •OH-induced oxidations of DNA. Scheme 3 outlines the percentages of TBARS in the presence of ferrocenylquinolines together with their structures for revealing structure–activity relationships.

As shown in Scheme 3, it can be found that the TBARS percentages in the presence of all the ferrocenylquinolines are lower than that generated by Trolox (88.3%), indicating that the ferrocenyl group exhibits higher antioxidant effectiveness than hydroxyl group in Trolox. Moreover, the percentages of TBARS generated by the compounds from **2** to **6** and from **15** to **22** are lower than those of compound **1** (75.6% and 80.3%), indicating that electron-abundant moiety together with hydroxyl group increase the abilities of quinolines to inhibit Cu^{2+}/GSH- and •OH-induced oxidations of DNA. In particular, the percentage of compound **3** in inhibiting Cu^{2+}/GSH- and •OH-induced oxidations of DNA (53.6% and 27.6%, respectively) are much lower than those of compound **1**, again revealing that more ferrocenyl groups actually ameliorate the antioxidative effectiveness of quinoline. In addition, the percentages of compound **19** (57.1% and 41.3%, respectively) are also lower than that of compound **1**, owing to the function of the traditionally structural feature for an antioxidant, *ortho*-dihydroxyl groups. On the other hand, as shown in the third panel of Scheme 3, the electron-withdrawing group makes the circumstance much complicated. The introductions of $-NO_2$ and $-Cl$ generally decrease the antioxidant effects of ferrocenylquinolines on Cu^{2+}/GSH- and •OH-induced oxidations of DNA because the percentages of compounds from **7** to **14** are close or even higher than that of compound **1**. However, the percentage of compound **10** in inhibiting •OH-induced oxidation of DNA (48.3%) is lower than that of Trolox and compound **1**, indicating that the chloride at 7-position is beneficial for the quinoline to protect DNA against •OH-induced oxidation. This finding is in agreement with our previous study on 4-(5-(*o*-chlorophenyl)-1,2,4-oxadiazol-3-yl)-2-methoxyphenol (CHOP) at the same experimental system [45]. Thus, the chloride may be an antioxidative group in some appropriate structures.

5. Conclusion

The solvent-free Povarov 3CR takes place among the substituted anilines, benzaldehydes, and ferrocenylacetylene to conveniently afford 2-phenyl-4-ferrocenylquinolines with various substituents. The purification of the final products is simplified in the absence of solvents, and reaction period is decreased markedly. The mild catalyst, $Ce(OTf)_3$, drives the annulation between imino and ferrocenylacetylene and cannot oxidize ferrocenyl group. The most important finding in this work is that the antioxidative effect of quinoline can be derived from ferrocenyl group other than hydroxyl group. The antioxidative effect generated by ferrocenyl group can be further increased by the electron-donating moieties such as furan, $-N(CH_3)_2$, $-OCH_3$, and ferrocenyl group. In particular, $-N(CH_3)_2$ exhibits the strongest ability to enhance the inhibiting function on radical-induced oxidation of DNA. However, the electron-withdrawing groups are not beneficial for the antioxidant effectiveness of ferrocenylquinolines. Therefore, ferrocene is a novel antioxidative group for quinolines, and it is worthy to further explore the mutual effectiveness between ferrocenyl group and other substituents within the same molecule.

6. Experimental section

6.1. Materials and instrumentation

Diammonium salt of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS salt), DPPH, and galvinoxyl radical were purchased from Fluka Chemie GmbH, Buchs, Switzerland. AAPH and the naked DNA sodium salt were purchased from Acros Organics, Geel, Belgium. Other agents were of analytical grade and used directly. The structures of ferrocenylquinolines were identified by ^1H and ^{13}C NMR (Bruker Avance III 400 MHz spectrometer), and the spectra were included in Supporting information.

6.2. Synthesis and structural identification of ferrocenylquinolines

6.2.1. A general synthetic operation

Three reagents including ferrocenylacetylene (0.42 g, 2.0 mmol), substituted benzaldehydes (2.4 mmol), and substituted anilines (2.4 mmol) together with $Ce(OTf)_3$ (0.12 g, 0.2 mmol) were mixed in a flask (25 mL) and heated at 110 °C for 2–4 h. After ferrocenylacetylene was not detected, the reaction mixture was cooled to the room temperature and purified by silica chromatography with ethyl acetate and petroleum ether being eluent to afford product.

6.2.2. The NMR data of ferrocenylquinolines

*6.2.2.1. 2-Phenyl-4-ferrocenylquinoline (**1**).* $R_f = 0.36$ (ethyl acetate: petroleum ether = 1:20, *v*:*v*), 0.50 g red product, yield 64%. m.p.: 150–152 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.58 (d, J = 8.4 Hz, 1H), 8.24 (d, J = 7.2 Hz, 1H), 8.19 (d, J = 7.6 Hz, 2H), 8.14 (s, 1H), 7.71 (t, J = 7.6 Hz, 1H), 7.56 (t, J = 7.6 Hz, 3H), 7.49 (t, J = 7.6 Hz, 1H), 4.81 (s, 2H), 4.52 (s, 2H), 4.21 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.6, 148.9, 147.0, 139.9, 130.4, 129.3, 128.9, 127.6, 126.1, 125.7, 119.7, 83.8, 70.6, 70.0, 69.4.

*6.2.2.2. 2-(Furan-2′-yl)-4-ferrocenylquinoline (**2**).* R_f = 0.21 (ethyl acetate: petroleum ether = 1:25, *v*:*v*), 0.42 g red product, yield 56%. m.p.: 184–186 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.52 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 7.6 Hz, 1H), 8.11 (s, 1H), 7.65–7.70 (m, 2H), 7.47–7.51 (m, 1H), 7.26 (s, 1H), 6.61–6.62 (m, 1H), 4.80 (s, 2H), 4.51 (s, 2H), 4.21 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.9, 148.7, 148.3, 147.0, 144.0, 130.0, 129.4, 126.1, 125.8, 125.6, 118.0, 112.3, 109.9, 83.5, 70.6, 70.0, 69.4.

*6.2.2.3. 2,4-Diferrocenylquinoline (**3**).* R_f = 0.23 (ethyl acetate: petroleum ether = 1:20, *v*:*v*), 0.54 g red product, yield 54%. m.p.: 192–194 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.45 (d, J = 8.4 Hz, 1H), 8.09 (s, 1H), 7.86 (s, 1H), 7.64 (t, J = 7.6 Hz, 1H), 5.13 (s, 2H), 4.78 (s, 2H), 4.51 (s, 4H), 4.24 (s, 5H), 4.11 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.6, 149.0, 145.3, 129.7, 129.1, 125.8, 124.9, 120.1, 84.3, 84.0, 70.7, 70.5, 70.1, 69.8, 69.3, 68.0.

*6.2.2.4. 2-Phenyl-4-ferrocenylbenzo[h]quinoline (**4**).* $R_f = 0.35$ (ethyl acetate: petroleum ether = 1:40, *v*:*v*), 0.56 g yellow product, yield 64%. m.p.: 201–203 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 9.58 (d, J = 8.0 Hz, 1H), 8.47 (d, J = 8.8 Hz, 1H), 8.37 (d, J = 7.6 Hz, 2H), 8.29 (s, 1H), 7.90 (d, J = 7.6 Hz, 1H), 7.68–7.81 (m, 3H), 7.60 (t, J = 7.6 Hz, 2H), 7.51 (d, J = 7.2 Hz, 1H), 4.83 (s, 2H), 4.53 (s, 2H), 4.23 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.4, 146.7, 139.9, 133.5, 132.2, 129.2, 128.9, 128.2, 127.5, 126.8, 126.6, 125.4, 123.7, 123.2, 120.0, 84.2, 70.8, 70.0, 69.4.

*6.2.2.5. 2-(4′-Methoxyphenyl)-4-ferrocenylquinoline (**5**).* $R_f = 0.19$ (ethyl acetate: petroleum ether = 1:20, *v*:*v*), 0.64 g yellow product, yield 76%. m.p.: 170–172 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.56 (d, J = 8.4 Hz, 1H), 8.09–8.18 (m, 4H), 7.69 (t, J = 7.6 Hz, 1H),

7.51 (t, J = 7.6 Hz, 1H), 7.08 (d, J = 8.4 Hz, 2H), 4.80 (s, 2H), 4.51 (s, 2H), 4.21 (s, 5H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.8, 156.1, 148.9, 146.7, 132.4, 130.1, 129.2, 128.8, 125.8, 125.3, 119.3, 114.3, 83.9, 70.5, 70.0, 69.4, 55.5.

*6.2.2.6. N,N-Dimethyl-4-(4′-ferrocenylquinolin-2′-yl)benzenaniline (**6**).* R_f = 0.38 (ethyl acetate: petroleum ether = 1:5, *v*:*v*), 0.59 g red product, yield 67%. m.p.: 164–166 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.50 (d, J = 7.2 Hz, 1H), 8.11–8.16 (m, 4H), 7.66 (s, 1H), 7.46 (s, 1H), 6.87 (d, J = 7.2 Hz, 2H), 4.79 (s, 2H), 4.49 (s, 2H), 4.21 (s, 5H), 3.06 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.6, 151.4, 149.1, 146.1, 130.0, 129.1, 128.5, 127.6, 125.7, 124.9, 119.2, 112.4, 84.3, 70.6, 70.0, 69.3, 40.5.

*6.2.2.7. 2-(2′-Chlorophenyl)-4-ferrocenylquinoline (**7**).* R_f = 0.24 (ethyl acetate: petroleum ether = 1:20, *v*:*v*), 0.54 g yellow product, yield 64%. m.p.: 181–183 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.56 (d, J = 8.8 Hz, 1H), 8.23 (s, 1H), 8.05 (s, 1H), 7.71–7.76 (m, 2H), 7.54–7.59 (m, 2H), 7.39–7.46 (m, 2H), 4.80 (s, 2H), 4.51 (s, 2H), 4.23 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.5, 148.7, 146.2, 139.9, 132.4, 131.9, 130.3, 129.9, 127.3, 126.2, 125.8, 123.3, 83.3, 70.7, 70.0, 69.4.

*6.2.2.8. 2-(3′-Chlorophenyl)-4-ferrocenylquinoline (**8**).* R_f = 0.34 (ethyl acetate: petroleum ether = 1:10, *v*:*v*), 0.63 g red product, yield 74%. m.p.: 148–150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.63 (d, J = 8.8 Hz, 1H), 8.21 (s, 2H), 8.05–8.07 (m, 2H), 7.73 (t, J = 7.6 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.44–7.51 (m, 2H), 4.82 (s, 2H), 4.54 (s, 2H), 4.21 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.0, 149.0, 141.8, 135.1, 130.5, 129.6, 127.8, 126.1, 125.9, 119.4, 113.0, 83.6, 70.6, 69.6.

*6.2.2.9. 2-(4′-Chlorophenyl)-4-ferrocenylquinoline (**9**).* R_f = 0.37 (ethyl acetate: petroleum ether = 1:10, *v*:*v*), 0.47 g yellow product yield 55%. m.p.: 96–98 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.58 (d, J = 6.4 Hz, 1H), 8.12–8.18 (m, 3H), 8.06 (s, 1H), 7.70 (s, 1H), 7.50–7.51 (m, 3H), 4.78 (s, 2H), 4.50 (s, 2H), 4.18 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.3, 149.0, 147.3, 138.4, 135.6, 130.4, 129.6, 128.9, 126.1, 125.8, 119.3, 83.7, 70.6, 69.6.

*6.2.2.10. 7-Chloro-4-ferrocenyl-2-phenylquinoline (**10**).* R_f = 0.58 (ethyl acetate: petroleum ether = 1:10, *v*:*v*), 0.39 g yellow product, yield 46%. m.p.: 213–215 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.72 (s, 1H), 8.26 (d, J = 7.2 Hz, 2H), 8.17 (s, 1H), 7.59 (t, J = 7.6 Hz, 2H), 7.50–7.54 (m, 2H), 7.46 (d, J = 7.6 Hz, 1H), 4.63 (s, 2H), 4.41 (s, 2H), 4.23 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.9, 150.1, 146.1, 138.9, 130.8, 129.8, 128.8, 127.4, 125.5, 90.2, 72.9, 69.7, 67.8.

*6.2.2.11. 2-(3′-Nitrophenyl)-4-ferrocenylquinoline (**11**).* R_f = 0.30 (ethyl acetate: petroleum ether = 1:10, *v*:*v*), 0.40 g red product, yield 46%. m.p.: 160–162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.09 (t, J = 1.6 Hz, 1H), 8.71 (d, J = 8.4 Hz, 1H), 8.61 (d, J = 7.6 Hz, 1H), 8.36 (d, J = 7.6 Hz, 1H), 8.27 (s, 1H), 8.16 (t, 1H), 7.74–7.81 (m, 2H), 7.63 (t, J = 7.6 Hz, 1H), 4.87 (s, 2H), 4.59 (s, 2H), 4.25 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.6, 148.9, 141.5, 133.3, 130.5, 129.8, 126.4, 125.9, 123.8, 122.4, 118.9, 113.0, 83.2, 70.5, 69.8.

*6.2.2.12. 2-(4′-Nitrophenyl)-4-ferrocenylquinoline (**12**).* R_f = 0.34 (ethyl acetate: petroleum ether = 1:10, *v*:*v*), 0.30 g red product, yield 34%. m.p.: 178–180 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.66 (d, J = 8.4 Hz, 1H), 8.36–8.41 (m, 4H), 8.23 (d, J = 8.0 Hz, 1H), 8.13 (s, 1H), 7.76 (t, J = 7.2 Hz, 1H), 7.60 (t, J = 7.2 Hz, 1H), 4.83 (s, 2H), 4.56 (s, 2H), 4.22 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.7, 148.9, 147.9, 145.7, 130.6, 129.8, 128.3, 126.6, 125.9, 124.1, 119.3, 83.2, 70.5, 69.8.

*6.2.2.13. 8-Nitro-2-phenyl-4-ferrocenylquinoline (**13**).* R_f = 0.35 (ethyl acetate: petroleum ether = 1:10, *v*:*v*), 0.30 g red product, yield 34%. m.p.: 150–152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.76 (d, J = 8.4 Hz, 1H), 8.25–8.29 (m, 3H), 7.95 (d, J = 7.2 Hz, 1H), 7.50–7.56 (m, 4H), 4.80 (s, 2H), 4.56 (s, 2H), 4.21 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.6, 148.8, 147.8, 145.7, 130.5, 129.7, 128.2, 126.5, 125.8, 124.0, 119.2, 83.1, 70.4, 69.7.

*6.2.2.14. 7-Nitro-2-phenyl-4-ferrocenylquinoline (**14**).* R_f = 0.44 (ethyl acetate: petroleum ether = 1:5, *v*:*v*), 0.49 g red product, yield 56%. m.p.: 185–187 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.59 (s, 1H), 8.41 (d, J = 8.4 Hz, 1H), 8.26 (d, J = 8.4 Hz, 2H), 7.86 (dd, J = 1.2 Hz, J = 7.6 Hz, 1H), 7.69 (t, J = 8.0 Hz, 1H), 7.55–7.63 (m, 3H), 4.65 (s, 2H), 4.44 (s, 2H), 4.10 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.3, 149.4, 148.7, 146.2, 138.5, 135.1, 130.2, 129.2, 127.5, 124.4, 123.7, 119.5, 87.4, 70.2, 69.8, 69.0.

*6.2.2.15. 2-(4′-Ferrocenylquinoline-2′-yl)phenol (**15**).* R_f = 0.25 (ethyl acetate: petroleum ether = 1:20, *v*:*v*), 0.36 g red product, yield 44%. m.p.: 192–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.86 (s, 1H), 8.71 (d, J = 8.4 Hz, 1H), 8.42 (s, 1H), 8.30 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.84 (t, J = 7.6 Hz, 1H), 7.72 (t, J = 7.6 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 8.0 Hz, 2H), 5.07 (s, 2H), 4.64 (s, 2H), 4.24 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.7, 148.4, 145.3, 131.8, 130.1, 128.2, 126.6, 125.9, 119.0, 117.8, 83.3, 70.7, 69.7.

*6.2.2.16. 3-(4′-Ferrocenylquinoline-2′-yl)phenol (**16**).* R_f = 0.23 (ethyl acetate: petroleum ether = 1:5, *v*:*v*), 0.41 g red product, yield 51%. m.p.: 230–232 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.65 (s, 1H), 8.62 (d, J = 8.4 Hz, 1H), 8.20 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 1.6 Hz, 2H), 7.73 (d, J = 8.0 Hz, 1H), 7.64 (t, J = 7.6 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 4.98 (s, 2H), 4.60 (s, 2H), 4.23 (s, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.3, 155.7, 148.7, 147.0, 140.6, 130.4, 126.5, 125.8, 119.3, 118.5, 117.1, 114.3, 83.2, 70.8, 70.0.

*6.2.2.17. 4-(4′-Ferrocenylquinoline-2′-yl)phenol (**17**).* R_f = 0.27 (ethyl acetate: petroleum ether = 1:5, *v*:*v*), 0.49 g yellow product, yield 60%. m.p.: 188–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.85 (s, 1H), 8.62 (d, J = 8.4 Hz, 1H), 8.19 (t, J = 7.6 Hz, 3H), 8.04 (d, J = 8.4 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H), 6.96 (d, J = 8.0 Hz, 2H), 4.97 (s, 2H), 4.59 (s, 2H), 4.23 (s, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.5, 155.7, 148.8, 146.7, 130.1, 129.8, 126.1, 125.9, 118.6, 116.1, 83.4, 70.7, 70.0.

*6.2.2.18. 4-(4′-Ferrocenylquinoline-2′-yl)-2-methoxyphenol (**18**).* R_f = 0.21 (ethyl acetate: petroleum ether = 1:5, *v*:*v*), 0.55 g red product, yield 63%. m.p.: 96–98 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.44 (s, 1H), 8.62 (d, J = 8.4 Hz, 1H), 8.18 (s, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.89 (s, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 4.98 (s, 2H), 4.59 (s, 2H), 4.23 (s, 5H), 3.94 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.7, 149.0, 148.7, 146.6, 130.6, 129.8, 126.1, 125.9, 120.9, 118.9, 116.2, 111.4, 83.4, 70.8, 69.9, 56.2.

*6.2.2.19. 4-(4′-Ferrocenylquinoline-2′-yl)benzene-1,2-diol (**19**).* R_f = 0.22 (ethyl acetate: petroleum ether = 1:2, *v*:*v*), 0.36 g violet product, yield 43%. m.p.: 117–119 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.31 (s, 1H), 9.23 (s, 1H), 8.56 (d, J = 8.4 Hz, 1H), 8.13 (s, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.82 (s, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.63 (t, J = 8.4 Hz, 1H), 7.58 (t, J = 7.6 Hz, 1H), 6.91 (t, J = 8.4 Hz, 1H), 4.94 (s, 2H), 4.58 (s, 2H), 4.23 (s, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.7, 148.8, 147.9, 146.5, 130.6, 129.8, 126.1, 125.8, 119.3, 118.7, 116.3, 114.8, 83.5, 70.8, 69.9.

*6.2.2.20. 2-Phenyl-4-ferrocenylquinolin-8-ol (**20**).* R_f = 0.52 (ethyl acetate: petroleum ether = 1:10, *v*:*v*), 0.49 g red product, yield 60%. m.p.: 155–157 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.73 (s, 1H), 8.18 (d, J = 8.4 Hz, 2H), 8.15 (s, 1H), 8.05 (d, J = 8.8 Hz, 1H), 7.55–7.59 (m, 2H), 7.52 (d, J = 7.2 Hz, 1H), 7.45 (t, J = 8.0 Hz, 1H), 7.19 (d, J = 7.6 Hz, 1H), 4.83 (s, 2H), 4.52 (s, 2H), 4.20 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.9, 152.6, 147.9, 139.0, 138.7, 129.6, 127.4, 126.7, 120.0, 116.2, 109.7, 83.4, 70.5, 69.6.

*6.2.2.21. 2-Phenyl-4-ferrocenylquinolin-7-ol (**21**).* R_f = 0.35 (ethyl acetate: petroleum ether = 1:5, *v*:*v*), 0.51 g red product, yield 63%. m.p.: 173–175 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40 (s, 1H), 8.20 (d, J = 7.2 Hz, 2H), 7.80 (d, J = 8.0 Hz, 1H), 7.48–7.59 (m, 4H), 6.88 (d, J = 7.6 Hz, 1H), 6.15 (s, 1H), 4.68 (s, 2H), 4.55 (s, 2H), 4.39 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.9, 153.1, 150.0, 142.7, 139.4, 130.1, 129.5, 127.4, 123.1, 122.5, 117.3, 111.8, 89.0, 71.5, 69.4.

*6.2.2.22. 2-Phenyl-4-ferrocenylquinolin-6-ol (**22**).* R_f = 0.24 (ethyl acetate: petroleum ether = 1:5, *v*:*v*), 0.36 g red product, yield 44%. m.p.: 197–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.07 (s, 1H), 8.26 (d, J = 7.2 Hz, 2H), 8.14 (s, 1H), 7.93–7.97 (m, 2H), 7.56 (t, J = 7.6 Hz, 2H), 7.48 (t, J = 7.2 Hz, 1H), 7.33 (dd, J = 2.4 Hz, J = 8.8 Hz, 1H), 4.94 (s, 2H), 4.58 (s, 2H), 4.25 (s, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.8, 152.6, 144.7, 139.6, 132.0, 129.4, 127.3, 122.4, 119.1, 107.3, 83.8, 70.4, 69.8.

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

$ABTS^{+}$• was generated in a 2.0 mL of aqueous solution containing 4.0 mM ABTS salt and 1.41 mM $K_2S_2O_8$ for 20 h and then diluted by 100 mL of ethanol. The absorbance of $ABTS^{+}$• was around 1.00 at 734 nm ($\varepsilon^{+}_{ABTS•} = 1.6 \times 10^4\ M^{-1}\ cm^{-1}$). DPPH and galvinoxyl radical were dissolved in ethanol directly, and the absorbance was around 1.00 at 517 nm ($\varepsilon_{DPPH} = 4.09 \times 10^3\ M^{-1}\ cm^{-1}$) and 428 nm ($\varepsilon_{galvinoxyl} = 1.4 \times 10^5\ M^{-1}\ cm^{-1}$), respectively. A 0.1 mL of a certain concentration of ferrocenylquinolines (dissolved in dimethyl sulfoxide (DMSO) as the stock solutions) was added to 1.9 mL of $ABTS^{+}$•, DPPH, or galvinoxyl radical solution. The decreases of the absorbance of these radicals were recorded at 25 °C with a certain time interval.

6.4. Inhibiting AAPH-induced oxidation of DNA

AAPH and DNA sodium salt were dissolved in PBS_1 (8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, 10.0 μM EDTA) as the stock solution. A mixture containing 2.0 mg/mL DNA, 40 mM AAPH, and a certain concentration of ferrocenylquinolines (dissolved in DMSO as the stock solution) was poured into test tubes, and each test tube contained 2.0 mL. The test tubes were incubated at 37 °C to initiate the oxidation of DNA, and three of them were taken out at every 2 h and cooled immediately, followed by adding 1.0 mL of thiobarbituric acid (TBA) solution (1.00 g of TBA and 0.40 g of NaOH dissolved in 100 mL of PBS_1) and 1.0 mL of 3.0% trichloroacetic acid aqueous solution. The test tubes were heated in boiling water for 15 min and cooled to room temperature, 1.5 mL of *n*-butanol was added and shaken vigorously to extract thiobarbituric acid reactive species (TBARS) whose absorbance was measured at 535 nm. The absorbance of TBARS was plotted *vs* the incubation period.

6.5. Inhibiting Cu^{2+}/GSH- and •OH-induced oxidations of DNA

Cu^{2+}/GSH-induced oxidation of DNA was carried out as following description. Briefly, DNA, $CuSO_4$, and GSH were dissolved in PBS_2 (8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4), and ferrocenylquinolines were dissolved in DMSO as the stock solution. A mixture containing 2.0 mg/mL DNA, 5.0 mM Cu^{2+}, 3.0 mM GSH, and 50.0 μM ferrocenylquinolines was poured into test tubes, and each test tube contained 2.0 mL. The test tubes were incubated at 37 °C to initiate the oxidation of DNA, and three of them were taken out at 90 min and cooled immediately. PBS_2 solution of EDTA (1.0 mL, 30.0 mM) was added to chelate Cu^{2+}, followed by adding 1.0 mL of TBA solution and 1.0 mL of 3.0% trichloroacetic acid aqueous solution. The test tubes were heated in boiling water for 30 min and 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.

•OH-induced oxidation of DNA was performed as the following description. DNA and H_2O_2 were dissolved in PBS_1, and tetrachlorohydroquinone (TCHQ) and ferrocenylquinolines were dissolved in DMSO as the stock solution. A mixture containing 2.0 mg/mL DNA, 4.0 mM TCHQ, 2.0 mM H_2O_2, and 50.0 μM ferrocenylquinolines was poured into test tubes, and each test tube contained 2.0 mL. The test tubes were incubated at 37 °C for 30 min and cooled immediately. The following operation was the same as in Cu^{2+}/GSH-induced oxidation of DNA except EDTA was not added. The absorbances in the control experiment and in the presence of ferrocenylquinolines were assigned as A_0 and A_{detect}, respectively. The effects of ferrocenylquinolines on Cu^{2+}/GSH- and •OH-induced oxidations of DNA were expressed by $A_{detect}/A_0 \times 100$.

6.6. Statistical analysis

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

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.044.

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