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Ugi Multicomponent Reaction Product: The Inhibitive Effect on DNA Oxidation Depends upon the Isocyanide Moiety

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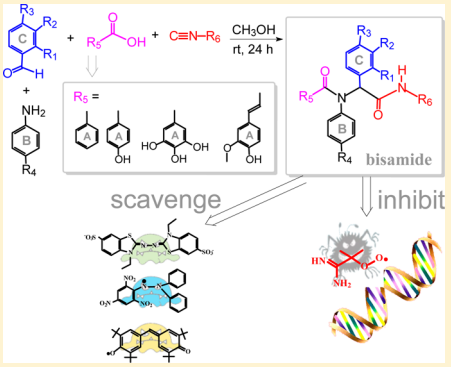
Supporting Information

ABSTRACT: The hydroxyl-substituted benzoic acid (as phenyl group A in the product), aniline (as phenyl group B in the product), benzaldehyde (as phenyl group C in the product), and four isocyanides are employed to synthesize bis-amide via an Ugi four-component reaction. The effects of the obtained 20 bis-amides on quenching radicals and inhibiting DNA oxidation are estimated. It is found that the antioxidant effectiveness of bis-amide generated by hydroxyl groups is markedly influenced by the structural feature derived from isocyanide. The phenolic hydroxyl group attaching to phenyl group A plays a major role in scavenging radicals, and the radical-scavenging property is reinforced by the structural moiety introduced from ferrocenylmethyl isocyanide. The same conclusion is also obtained when bis-amides are used to inhibit DNA oxidation. It is still found that the ferrocenylmethyl moiety enhances the antioxidant effect of hydroxyl group at phenyl group A in protecting DNA against the oxidation. Moreover, when the bis-amide is prepared by the same isocyanide, e.g. ethyl isocyanoacetate, it is found that the hydroxyl group at phenyl group C plays the major role in inhibiting DNA oxidation, followed by the hydroxyl groups attaching to phenyl groups B and A.



INTRODUCTION

A fast-growing interest in multicomponent reactions (MCRs) has been witnessed in recent years, because MCRs provide a highly efficient approach for constructing polyfunctional molecules from simple materials.[1] The Ugi four-component reaction (Ugi-4CR) is a convenient access to bis-amides with a carboxylic acid, an amine, an aldehyde, and an isocyanide being the starting materials.[2] At present, the research of the Ugi-4CR mainly follows three directions. Screening feasible substrates to replace traditional reagents may be the first aspect in the research of Ugi-4CR. The use of diamine,[3] secondary amine,[4] β-ketoamide,[5] phenol,[6] or enol[7] to replace the primary amine or carboxylic acid enlarge the applicability of the Ugi-4CR. Also, elaborately selecting substrates and accurately controlling reaction conditions permit even more than four components to form a domino system[8] or at least a cascade operation[9] for constructing complicated structures via a simple operation.[10] The Ugi-4CR is found to be accelerated in water.[11] A newly theoretical calculation illustrates an overall downward trend for the activation energy when the Ugi-4CR is carried out in methanol and toluene.[12] Thus, the Ugi-4CR is a self-catalyzed reaction, in which catalysts seem to be not a necessary factor. However, some catalysts are still applied to promote the post-Ugi-4CR. For example, gold salt[13] and palladium or copper acetate[14] trigger the annulation of Ugi-4CR products. Also, asymmetric catalysts are used to promote enantioselective Ugi-4CR,[15] and an enzyme as catalyst agitates some inert reagents to perform the Ugi-4CR.[16] Hence, screening catalysts is the second aspect in the investigation on the Ugi-4CR. Finally, the application of Ugi-4CR for preparing molecules with various properties is the largest field in the study of this method. Special isocyanides are adopted in sequential Ugi-4CR for producing peptides with biological[17] or chemical activities[18] and for producing novel topological structures.[19] The combination of various carboxylic acid, aldehyde, amine, and isocyanide in the Ugi-4CR allows hundreds of bis-amides to be formed for the sake of combinatorial screening drugs.[20,21]

The phenolic −OH is a well-known functional group for generating antioxidant effectiveness.[22] If hydroxyl-substituted benzoic acid, benzaldehyde, aniline, and some isocyanides are employed to carry out Ugi-4CR, the produced bis-amide is suitable for being explored the mutual antioxidant effects among phenolic hydroxyl groups at different positions. Reports on the application of Ugi-4CR to construct antioxidants are not usually found,[23] and the influence of the isocyanide moiety on antioxidant effectiveness remains unclear. As shown in Scheme 1, 20 bis-amides have been synthesized by using Ugi-4CR, and the radical-scavenging properties have been compared by quenching 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{\bullet+}$), 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), and galvinoxyl radical, respectively.[24] The antioxidant effect has been evaluated by inhibiting the oxidation of DNA mediated by 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH). Mutual antioxidant effects among hydroxyl

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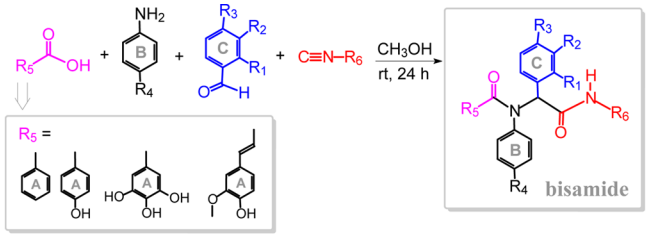
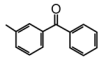
Scheme 1. Structures of Bis-Amides Employed in the Present Work[a]



Compound	R_1	R_2	R_3	R_4	R_5	R_6	Yield (%)[a]
1	H	H	H	H	A	$-CH_2COOC_2H_5$	75
2	H	H	OH	H	A	$-CH_2COOC_2H_5$	83
3	H	H	H	H	A-OH	$-CH_2COOC_2H_5$	87
4	H	H	H	OH	A	$-CH_2COOC_2H_5$	80
5	H	H	OH	OH	A-OH	$-CH_2COOC_2H_5$	74
6	OH	H	OH	H	A	$-CH_2COOC_2H_5$	51
7	H	OCH_3	OH	H	A	$-CH_2COOC_2H_5$	89
8	H	OCH_3	OH	H	A-OH	$-CH_2COOC_2H_5$	67
9	H	OCH_3	OH	OH	A	$-CH_2COOC_2H_5$	63
10	H	OCH_3	OH	OH	A-OH	$-CH_2COOC_2H_5$	54
11	H	H	H	H	A-HO	$-CH_2COOC_2H_5$	49
12	H	H	H	H	A-HO-OH	$-CH_2COOC_2H_5$	33
13	H	OCH_3	OH	OH	A-HO	$-CH_2COOC_2H_5$	42
14	H	H	H	H	A-HO		33
15	H	OCH_3	OH	OH	A-OH	$-C(CH_3)_3$	63
16	H	H	H	H	A-HO-OH	$-C(CH_3)_3$	36
17	H	H	H	H	A-HO	$-C(CH_3)_3$	51
18	H	H	H	H	A-HO-OH		56
19	H	H	H	H	A-HO		80
20	H	OCH_3	OH	OH	A-HO		56

[a]The isolation yields.

groups attaching to different benzenes as well as the influence of isocyanide on the antioxidant effect have been investigated.

RESULTS AND DISCUSSION

Scavenging Radicals. Scavenging radicals is regarded as the primary property of an antioxidant.[25] $ABTS^{\bullet+}$, DPPH, and galvinoxyl radical are usually applied as a way to evaluate the antioxidant ability to scavenge radicals. The reaction of an antioxidant with $ABTS^{\bullet+}$ reveals the ability of the antioxidant to reduce the radical, and reactions of the antioxidant with DPPH and galvinoxyl radical exhibit the ability of the antioxidant to donate its hydrogen atom to N- and O-centered radicals, respectively. All bis-amides have been applied to quench $ABTS^{\bullet+}$, DPPH, and galvinoxyl radical, respectively. The decays of the concentrations for these radicals are included in the Supporting Information (Figures 1S–3S). Some bis-amides that do not reaction with the aforementioned radicals are indicated by the symbol of "–" in Table 1.

All bis-amides used herein (except compound **1**) decrease the concentration of $ABTS^{\bullet+}$ at longer reaction times, indicating that a phenolic hydroxyl group rather than N–H is the active group reducing $ABTS^{\bullet+}$. Figure 2S (Supporting Information) shows that only compounds **6**–**20** are able to quench DPPH, while compounds **2**–**5** do not show any activity in this case. Every phenyl group bearing one hydroxyl group and the whole molecule containing three hydroxyl groups in compound **5** still do not donate the hydrogen atom in –OH to the N-centered radical. Figure 3S (Supporting Information) illustrates that compounds **11**–**20** (except for compound **15**) can quench galvinoxyl radical. The common structural feature in these compounds is an *o*-methoxyhydroxyl or 1,2,3-trihydroxyl group at phenyl group A. However, in compounds **10** and **15** the *o*-methoxyhydroxyl group attaching to phenyl group C cannot donate the hydrogen atom in –OH to the O-centered radical. Thus, only the *o*-methoxyhydroxyl group attaching to phenyl group A can be the active quenching the O-centered radical. On the other hand, it is found that the selectivity of radical resources employed to screen the radical-scavenging properties of the bis-amides is galvinoxyl radical > DPPH > $ABTS^{\bullet+}$.

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Table 1. Rate Constants (k) for Bis-Amides in Scavenging ABTS$^{+\cdot}$, DPPH, and Galvinoxyl Radical

	rate constant for bis-amides to scavenge radicals, k (M^{-1} s^{-1})		
compd	ABTS$^{\bullet+}$	DPPH	galvinoxyl radical
1	_	_	_
2	13.0	_	_
3	16.7	_	_
4	1.08×10^2	_	_
5	67.3	_	_
6	9.86×10^2	0.297	_
7	2.97×10^3	1.73	_
8	3.27×10^3	4.18	_
9	3.26×10^3	2.74	_
10	2.86×10^3	1.59	_
11	9.77×10^3	79.7	15.5
12	1.84×10^4	5.04×10^3	2.54×10^3
13	8.38×10^3	97.0	59.4
14	2.09×10^3	17.2	7.85
15	4.00×10^3	2.52	_
16	1.63×10^4	4.40×10^3	1.10×10^3
17	1.09×10^4	78.1	18.0
18	1.76×10^4	1.07×10^4	6.93×10^3
19	1.59×10^4	1.04×10^2	51.5
20	2.18×10^4	1.01×10^2	1.15×10^2

We have suggested a method for treating data obtained from the interaction of a radical with an antioxidant.[26] The reaction between an antioxidant and ABTS$^{\bullet+}$ is taken as an example. The rate constant (k) can be calculated following the chemical kinetics equation (1) in the case of [ABTS$^{\bullet+}$], [antioxidant],

$$-\frac{\mathrm{d}[\mathrm{ABTS}^{\bullet+}]}{\mathrm{d}t} = r = k[\mathrm{ABTS}^{\bullet+}][\mathrm{antioxidant}] \quad (1)$$

and the corresponding reaction rate (r) at a certain time-point being measured. Equation 1 is based on a previous report, in which the elementary reaction between an antioxidant and radical is regarded as a first-order reaction in every reagent.[27] $t = 0$ may be an appropriate time point because at $[\mathrm{ABTS}^{\bullet+}]_{t=0}$ and $[\mathrm{antioxidant}]_{t=0}$, the concentrations of the antioxidant and ABTS$^{\bullet+}$ at the beginning of the reaction are known. As a result, eq 1 becomes eq 2. The key problem focuses on how the

$$r_0 = k[\mathrm{ABTS}^{\bullet+}]_{t=0}[\mathrm{antioxidant}]_{t=0} \quad (2)$$

reaction rate at $t = 0$ (r_0) can be obtained. Thus, the variation of the concentration of ABTS$^{\bullet+}$ with the reaction time (t) should be expressed. The measured concentration of ABTS$^{\bullet+}$ ([ABTS$^{\bullet+}$]) and the corresponding time point (t) are input into statistical software in order to find a function for expressing the relationship of [ABTS$^{\bullet+}$] ~ t. Consequently, the exponential function (3) is found to be the suitable one.

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

Although other functions may also be perfectly fitted for the experimental data, the double-exponential function as shown in eq 3 implies that every item (either the antioxidant or the radical) is expressed by a single-exponential function because the antioxidant and the radical are first order in the reaction, respectively, and the antioxidant and the radical are simultaneously expressed by a double-exponential function. The equations of [ABTS$^{\bullet+}$] ~ t for every compound are given in Table 1S (Supporting Information). Then, the differential operation is performed with eq 3 in order to reveal the variation of the reaction rate (r) with the reaction time (t), $-\mathrm{d}[\mathrm{ABTS}^{\bullet+}]/\mathrm{d}t \sim t$ (eq 4).

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

The reaction rate at $t = 0$ (r_0) can be calculated following eq 4 when the reaction time (t) is assigned as 0. The rate constant (k) is subsequently calculated by using eq 2. This method is also used to treat data obtained from bis-amides quenching other radicals, and the results are given in Table 1. Sometimes the method of a pseudo-first-order reaction is a convenient way to simplify the operation of the measurement of k. However, if the concentration of either antioxidant or radical is high enough, the reaction between the antioxidant and radical is so fast that the variation for the compound with low concentration is difficult to follow. The present method takes the concentrations of both antioxidant and radical into consideration simultaneously and thus avoids the inconvenient operation derived from a pseudo-first-order reaction.

By the comparison of the rate constant (k) in quenching ABTS$^{\bullet+}$, it is also found that *ortho*-methoxyhydroxyl group or 1,2,3-trihydroxyl groups attaching to phenyl group A increases k values of compounds **11**−**13** and **16**−**20** to 10^4 magnitude, while the *o*-methoxyhydroxyl group attached to phenyl group C does not play the same role for compounds **7**−**10**. For quenching DPPH and galvinoxyl radical, k values of compounds **12**, **16**, and **18** are higher than those of other compounds, indicating that 1,2,3-trihydroxyl groups attaching to phenyl group A are the major functional groups for contributing the hydrogen atom in −OH to N- and O-centered radicals. Furthermore, by the comparison of k values of compounds **12**, **16**, and **18**, it is found that the ferrocenylmethyl group enhances the k value of compound **18** markedly when these compounds are used to donate hydrogen atoms to N- and O-centered radicals (quenching DPPH and galvinoxyl radical). However, in a comparison of abilities of these compounds to reduce radical (quenching ABTS$^{\bullet+}$), the ferrocenylmethyl group enhances the k value of compound **18** not very remarkably. Hence, the hydrogen atom donating ability is mainly due to the hydroxyl group attached to phenyl group A and is reinforced by the ferrocenylmethyl group derived from the corresponding isocyanide.

Inhibiting AAPH-Induced Oxidation of DNA. The decomposition of AAPH in the presence of oxygen at 37 °C generates peroxyl radical for oxidizing the guanine bases in DNA.[28] The products from AAPH-induced oxidation of DNA can be colorized after the reaction with thiobarbituric acid, and thus the determination of thiobarbituric acid reactive species (TBARS, λ_{max} 535 nm, ε_{TBATS} = 1.56×10^5 M^{-1} cm^{-1}) becomes a convenient way to follow the process of DNA oxidation.[29] As shown in Figure 1, a continual increase of the absorbance in the blank experiment ([bis-amide] = 0 μM) indicates that much more TBARS is generated for longer reaction times. Thus, the variation of the concentration of TBARS with the reaction period (t) can be linearly fitted by eq 5. The differential operation on eq 5, $\mathrm{d}[\mathrm{TBARS}]/\mathrm{d}t$ = 6.8 nM·

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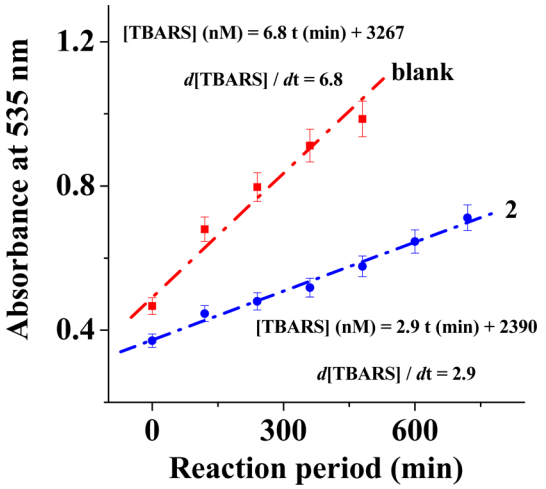

Figure 1. Increase of TBARS absorbance in a mixture of 40 mM AAPH and 2.24 mg mL^{-1} DNA with the reaction period in the presence of 200 μM compound **2**.

min^{-1}, expresses the formation rate of TBARS in the blank experiment of AAPH-induced oxidation of DNA.

$$[\text{TBARS (nM)}] = [6.8(\pm 0.3)]t\ (\text{min}) + 3267(\pm 173) \quad (5)$$

The addition of compound **1** (200 μM) does not affect the increase of TBARS (see Figure 4S in the Supporting Information) because d[TBARS]/dt in the presence of compound **1** is 7.0 nM min^{-1}, similar to that in the blank experiment (d[TBARS]/dt = 6.8 nM min^{-1}). Hence, bis-amide cannot inhibit the DNA oxidation without the aid of the phenolic hydroxyl group. Moreover, additions of compounds **2**–**4**, **5**, **11**, and **14** (200 μM) obviously slow down the increase of TBARS (the linear relationships of [TBARS] ~ t are given in Table 4S in the Supporting Information), demonstrating that these compounds can protect DNA against AAPH-induced oxidation. Moreover, d[TBARS]/dt values in the presence of these compounds are collected in Table 2. The lowest value of

Table 2. Formation Rate of TBARS (d[TBARS]/dt)

compd	d[TBARS]/dt (nM min^{-1})
blank	6.8
1	7.0
2	2.9
3	4.7
4	3.6
5	0.6
11	4.8
14	5.3

d[TBARS]/dt of compound **5** indicates that hydroxyl groups at different benzenes exhibit relatively high activity in inhibiting DNA oxidation. This is quite different from the result of trapping DPPH and galvinoxyl radical, in which compound **5** does not show any activity. The values of d[TBARS]/dt of compound **2** (hydroxyl group at phenyl group C), **3** (hydroxyl group at phenyl group A), and **4** (hydroxyl group at phenyl group B) follows the order **2** < **4** < **3**, implying that the position of hydroxyl group influences antioxidant effectiveness follows the order of phenyl groups C > B > A.

As shown in Figure 2, the antioxidant effects of other bis-amides are higher than those of the aforementioned species

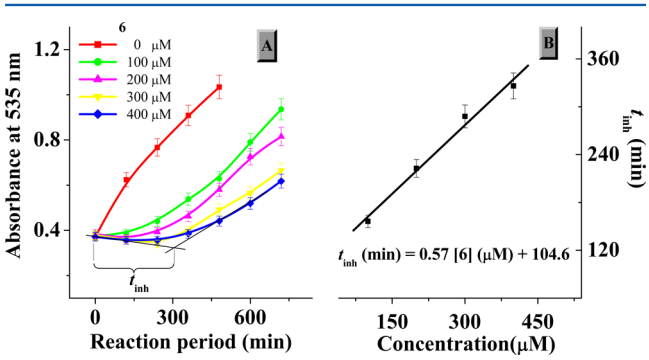

Figure 2. Increase of TBARS absorbance in a mixture of 40 mM AAPH and 2.24 mg mL^{-1} DNA with the reaction period in the presence of various concentrations of compound **6** (A) and the relationship between t_{inh} and the concentration of compound **6** (B).

because these bis-amides can inhibit the formation of TBARS for a period. When compound **6** is taken as an example, it is found that the *inhibition period* (t_{inh}) can be measured from the beginning of the reaction to the cross point of tangents for the inhibition and oxidation periods. This method for obtaining t_{inh} is derived from the measurement of the same parameter in recording the oxygen-exhausting process of a radical-induced oxidation of polyunsaturated fatty acid. The cross point of tangents for the inhibition and the oxidation period is assigned to t_{inh}.[30] Herein the experimental data from the measurement of TBARS are individual points and not a successive line as in the recording of the oxygen-exhausting process. The data of TBARS concentration are fitted by the Boltzmann function to form a successive line, and then the t_{inh} value can be obtained from the cross point of tangents.[31] As shown in Figure 2A, t_{inh} increases with the concentration of compound **6** employed, and the linear relationship is illustrated in Figure 2B. As shown in Figure 5S and 6S (Supporting Information), t_{inh} generated by compounds **7**–**10**, **12**, **13**, and **15**–**20** in inhibiting DNA oxidation correlates linearly with concentrations of these compounds, and the linear equations of t_{inh} ~ [bis-amide] are given in Table 3.

It has been proved that t_{inh} correlates proportionally with concentration of the antioxidant, as shown in eq 6.[32]

$$t_{inh} = (n/R_i)[\text{antioxidant}] \quad (6)$$

In this equation, R_i is the initiation rate of the radical-induced reaction, and the stoichiometric factor (n) refers to the number of radicals trapped by one molecule of the antioxidant. It is assumed that R_i is equal to the generation rate (R_g) of radicals ($R_g = ((1.4 \pm 0.2) \times 10^{-6})[\text{AAPH}]\ s^{-1}$)[32] because AAPH and the sodium salt of DNA are both water-soluble substances, and radicals produced by AAPH attacked DNA at the same phase. Hence, R_i can be calculated by $R_i = R_g = (1.4 \times 10^{-6}) \times 40$ mM s^{-1} = 3.36 μM·min^{-1}. The n value is a quantitative index for estimating the antioxidant effectiveness.[33] Accordingly, the n values of bis-amides are calculated and given in Table 3. In order to explore the structure–activity relationship, the n values and the corresponding structure of bis-amides are illustrated in Figure 3.

The first panel in Figure 3 shows the results from compounds **14**, **11**, **17**, and **19**, which have the same structures

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Table 3. Linear Equations between Inhibition Period (t_{inh}) and Concentrations of Bis-Amides in AAPH-Induced Oxidation of DNA[a]

compd	t_{inh} (min) =(n/R_i)[concentration (μM)]	n
6	t_{inh} = 0.57(±0.03) [**6**] + 104.6(±5.3)	1.93(±0.09)
7	t_{inh} = 0.48(±0.02) [**7**] + 108.4(±5.4)	1.61(±0.08)
8	t_{inh} = 0.79(±0.04) [**8**] + 57.4(±2.9)	2.87(±0.14)
9	t_{inh} = 1.31(±0.06) [**9**] + 57.4(±3.3)	4.40(±0.22)
10	t_{inh} = 1.38(±0.07) [**10**] + 24.6(±1.2)	4.64(±0.23)
12	t_{inh} = 0.46(±0.02) [**12**] + 20.4(±1.0)	1.56(±0.08)
13	t_{inh} = 1.16(±0.06) [**13**] + 59.0(±2.9)	3.90(±0.19)
15	t_{inh} = 1.38(±0.07) [**15**] + 42.2 (±2.1)	4.64(±0.23)
16	t_{inh} = 0.48(±0.02) [**16**] + 27.7(±1.4)	1.61(±0.08)
17	t_{inh} = 0.47(±0.02) [**17**] + 36.8(±1.8)	1.58(±0.08)
18	t_{inh} = 4.24(±0.21) [**18**] − 26.2(±1.3)	14.24(±0.71)
19	t_{inh} = 3.44(±0.17) [**19**] − 5.67(±0.3)	11.56(±0.58)
20	t_{inh} = 2.56(±0.13) [**20**] + 53.9(±2.7)	8.60(±0.43)

[a]The value of n is the product of the coefficient of t_{inh} ~ [bis-amides] and R_i = (1.4 × 10^{-6}) × 40 mM s^{-1} = 3.36 μM min^{-1} when 40 mM AAPH is employed.

at phenyl groups A−C. Compounds **14** and **11** do not generate an n value, while the n value of compound **17** is 1.58 and that of compound **19** promptly increases to 11.56. Thus, the structural moieties derived from benzophenonyl and ethoxycarbonyl isocyanides are not able to improve the antioxidant effectiveness derived for the phenyl group A, a ferulic acid moiety. In contrast, it can be regarded that the structural moiety derived from *tert*-butyl isocyanide causes compound **17** to generate an n value of 1.58, and the use of ferrocenylmethyl isocyanide remarkably enhances the n value of compound **19** to 11.56. Moreover, the same conclusion can be drawn by observing the second panel in Figure 3. The n values of compounds **12**, **16**, and **18** are 1.56, 1.61, and 14.24, respectively. The antioxidant effects of these compounds are definitely generated by 1,2,3-trihydroxyl groups attached to phenyl group A. The highest n value for compound **18** (14.24) indicates that this compound possesses the highest antioxidant ability that undoubtedly correlates with the ferrocenylmethyl group. As can be seen from the last panel in Figure 3, the n value of compound **20** (8.60) is higher than that of compound **13** (3.90), while compounds **10** and **15** have the same n value (both 4.64), and compounds **12** and **16** possess similar n values (1.56 and 1.61, respectively). The aforementioned results reveal that the increasing effect of the isocyanide moiety on the antioxidant property follows the sequence ferrocenylmethyl group > *tert*-butyl group > ethoxycarbonyl group > benzophenonyl group. Furthermore, as shown as the last panel in Figure 3, a comparison of the n values of compounds **7**−**9** reveals the influence of hydroxyl groups at phenyl groups B and C on the antioxidant effectiveness, because the structures of phenyl group A and the ethoxycarbonyl group are the same in these compounds. In compound **7** the phenyl group C is a vanillylidene group, in which the 4-hydroxyl-3-methoxyl group is the functional group for generating antioxidant effectiveness. Hence, the n value of compound **7** (1.61) is attributed to the vanillylidene group. The n value of compound **6** (1.93) is higher than that of compound **7** (1.61), indicating that the ability of the 4-hydroxyl-3-methoxyl group to generate an antioxidant effect is lower than that of 2,4-dihydroxyl groups at the same position (phenyl group C). The addition of a hydroxyl group to the phenyl group A of compound **7**, giving compound **8**, increases the n value to 2.87. However, the addition of a hydroxyl group to phenyl group B of compound **7**, giving compound **9**, increases the n value to 4.40. If another hydroxyl group attaches to phenyl group A of compound **9**, giving compound **10**, the n value of compound **10** only increases to 4.64. Thus, hydroxyl groups at phenyl group C and then B play the major roles in protecting DNA against AAPH-induced oxidation. As shown in Scheme 2, the conformations of compounds **11**, **14**, and **17** are calculated at the B3LYP/6-31g(d) level at the same zero vibration energy. The carbon chain exhibits a linear structure in the molecule of bis-amide, where phenyl groups B and C form a dihedral angle, and phenyl group A and the structural moiety from isocyanide are located at the two tips of the molecule. Hence, hydroxyl groups attaching to phenyl group A and the isocyanide moiety play a mutual antioxidant role over a long distance, while hydroxyl groups attaching to phenyl groups B and C play the same role over a dihedral angle.

CONCLUSION

Ugi-4CR provides a powerful tool for integrating phenolic hydroxyl groups within one bis-amide molecule, which is suitable for exploring the mutual antioxidant effects among phenolic hydroxyl groups attached to different parts of the molecule. It is actually found that the antioxidant property of the phenolic hydroxyl group at one tip of the molecule depends upon the isocyanide moiety at another tip of the molecule, in which ferrocenylmethyl isocyanide enhances the antioxidant effect markedly. Thus, the isocyanide moiety influences the antioxidant effect of the hydroxyl group via a long distance within a molecule. Mutual antioxidant effectiveness is also found among phenolic hydroxyl groups at adjacent benzenes. The present finding provides novel information on the structure−activity relationship for antioxidant design.

EXPERIMENTAL SECTION

Materials and Instrumentation. ABTS, DPPH, and galvinoxyl radical were purchased from Fluka Chemie GmbH, Switzerland, and AAPH and naked DNA sodium salt were purchased from Acros Organics, Belgium. Ferrocenylmethyl isocyanide was synthesized by following a literature procedure.[34] Other reagents were of analytical grade and were used directly. The structures of the obtained products were identified by ^{1}H and ^{13}C NMR, and the spectral data are included in the Supporting Information.

General Synthetic Procedure. The bis-amides were synthesized following a literature procedure.[35] Briefly, a methanol solution (3 mL) of aldehyde (2 mmol) and amine (2 mmol) was stirred at room temperature for 1 h, followed by adding carboxylic acid (2 mmol) and isocyanide (2 mmol). The reaction mixture was stirred for 24 h at room temperature, and the solvent was removed under vacuum. The residual solid was purified by recrystallization or column chromatography on silica gel to afford products.

*Ethyl 2-[2-Phenyl-2-(N-phenylbenzamido)acetamido]acetate (**1**).* Purified by column chromatography with petroleum ether and ethyl acetate as eluent. Yield: 582.4 mg, 75%. Mp: 132−133 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.19 (t, 3H, CH_3 of Et), 3.80−4.02 (m, 2H, CH_2 of Et), 4.11 (d, J = 7.2 Hz, 2H, CH_2), 6.42 (s, 1H, CH), 6.91 (s, 3H, aromatic), 7.00 (s, 2H, aromatic), 7.15−7.21 (m, 10H, aromatic), 8.63 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.1, 41.1, 60.4, 63.9, 126.7, 127.5, 127.7, 127.7, 127.9, 129.0, 130.5, 130.8, 134.8, 136.7, 140.2, 169.6, 169.9, 170.0.

*Ethyl 2-[2-(4-Hydroxyphenyl)-2-(N-phenylbenzamido)-acetamido]acetate (**2**).* Recrystallization from C_2H_5OH/H_2O. Yield: 717 mg, 83%. Mp: 190−193 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.19 (t, 3H, CH_3 of Et), 3.76−4.05 (m, 2H, CH_2 of Et), 4.06−4.13 (q, J = 6.9 Hz, 2H, CH_2), 6.30 (s, 1H, CH), 6.51 (d, J = 8.4, 2H,

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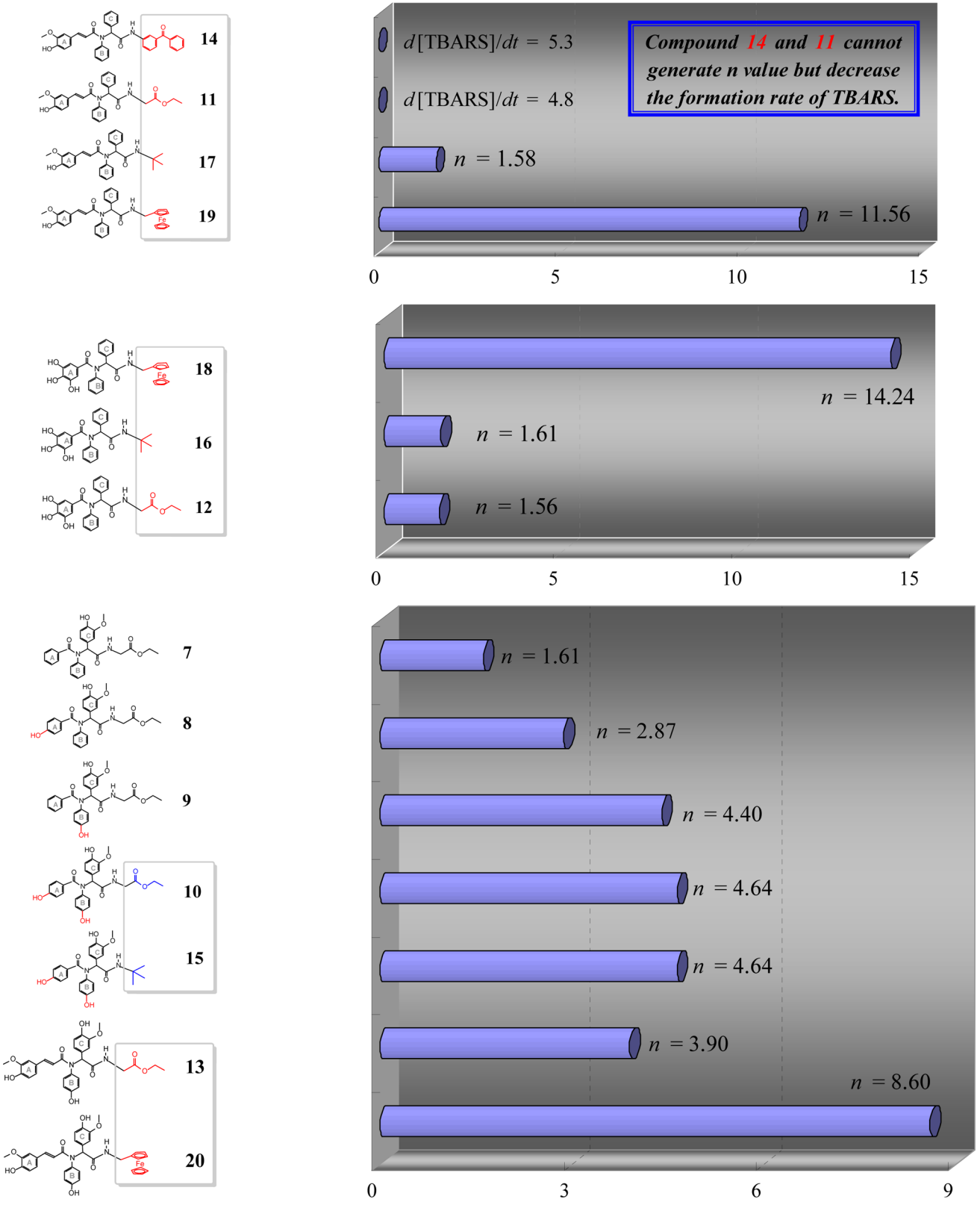

Figure 3. Relationship between the structure of bis-amides and stoichiometric factor (n).

aromatic), 6.94−7.20 (m, 12H, aromatic), 8.50 (s, 1H, NH), 9.33 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.1, 41.1, 60.4, 63.5, 114.6, 124.9, 126.6, 127.5, 127.7, 127.9, 128.9, 131.0, 131.8, 140.3, 169.7, 169.8, 170.5.

*Ethyl 2-[2-(4-Hydroxy-N-phenylbenzamido)-2-phenylacetamido]acetate (**3**).* Purified by column chromatography with petroleum ether and ethyl acetate as eluent. Yield: 752 mg, 87%. Mp: 145−147 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.19 (t, 3H, CH_3 of Et), 3.79−3.99 (m, 2H, CH_2 of Et), 4.07−4.14 (q, J = 6.9 Hz, 2H, CH_2), 6.41 (s, 1H, CH), 6.49 (d, J = 7.2, 2H, aromatic), 6.30 (s, 5H, aromatic), 7.07 (d, J = 6.9, 2H, aromatic), 7.16 (s, 5H, aromatic), 8.60 (t, 1H, NH), 9.70 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ

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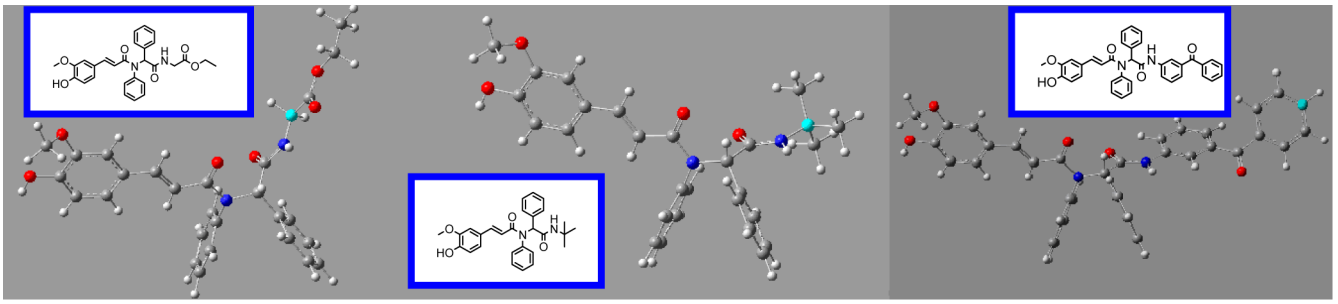
Scheme 2. Conformations of Compounds 11, 14, and 17 Calculated at the B3LYP/6-31g(d) Level



14.1, 41.1, 60.4, 64.1, 114.2, 126.4, 126.8, 127.6, 127.8, 127.9, 128.0, 130.5, 130.6, 135.1, 141.0, 158.3, 169.7, 169.7, 170.2.

*Ethyl 2-[2-(N-(4-Hydroxyphenyl)benzamido)-2-phenylacetamido]acetate (**4**).* Purified by column chromatography with petroleum ether and ethyl acetate as eluent. Yield: 691 mg, 80%. Mp: 165−166 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.19 (t, 3H, CH_3 of Et), 3.78−4.00 (m, 2H, CH_2 of Et), 4.06−4.14 (q, J = 6.9 Hz, 2H, CH_2), 6.27 (d, J = 8.1, 2H, aromatic), 6.34 (s, 1H, CH), 6.77 (s, 1H, aromatic), 7.19 (d, J = 7.2, 11H, aromatic), 8.53 (t, 1H, NH), 9.23 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.1, 41.1, 60.4, 63.9, 114.3, 127.4, 127.6, 127.7, 127.9, 128.8, 130.6, 131.3, 131.9, 135.0, 137.0, 155.6, 169.7, 170.1, 171.0.

*Ethyl 2-[2-(4-Hydroxy-N-(4-hydroxyphenyl)benzamido)-2-(4-hydroxyphenyl)acetamido]acetate (**5**).* Recrystallization from C_2H_5OH/H_2O. Yield: 687 mg, 74%. Mp: 212−214 °C dec. ^{1}H NMR (400 MHz, DMSO-d_6): δ1.18 (s, 3H, CH_3 of Et), 3.76−3.98 (m, 2H, CH_2 of Et), 4.09 (s, 2H, CH_2), 6.19 (s, 1H, CH), 6.33 (s, 2H, aromatic), 6.51 (t, 5H, aromatic), 6.71 (s, 1H, aromatic), 6.90 (d, J = 5.2 Hz, 2H, aromatic), 7.04 (d, J = 5.2 Hz, 2H, aromatic), 8.37 (s, 1H, NH), 9.23 (s, 1H, OH), 9.31 (s, 1H, OH), 9.64 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.1, 41.1, 60.4, 63.9, 114.1, 114.4, 114.6, 125.5, 127.3, 130.4, 131.8, 131.9, 132.2, 155.5, 156.7, 158.1, 169.8, 170.7.

*Ethyl 2-[2-(2,4-Dihydroxyphenyl)-2-(N-phenylbenzamido)-acetamido]acetate (**6**).* Recrystallization from C_2H_5OH/H_2O. Yield: 457 mg, 51%. Mp: 144−146 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.20 (t, 3H, CH_3 of Et), 3.72−4.05 (m, 2H, CH_2 of Et), 4.07−4.14 (q, J = 6.9 Hz, 2H, CH_2), 5.86 (d, J = 8.4, 1H, aromatic) 6.14 (s, 1H, CH), 6.45 (s, 1H, aromatic), 6.65 (d, J = 8.4, 1H, aromatic), 6.93−7.16 (m, 10H, aromatic), 8.46 (s, 1H, NH), 9.12 (s, 1H, OH), 9.58 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.5, 46.6, 64.1, 65.8, 107.1, 111.1, 117.4, 132.0, 132.9, 132.9, 133.2, 134.2, 135.8, 137.1, 142.7, 145.9, 162.1, 163.5, 175.2, 175.3, 176.6.

*Ethyl 2-[2-(4-Hydroxy-3-methoxyphenyl)-2-(N-phenylbenzamido)acetamido]acetate (**7**).* Purified by column chromatography with petroleum ether and ethyl acetate as eluent. Yield: 822 mg, 89%. Mp: 169−171 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, 3H, CH_3 of Et), 3.67 (s, 3H, OCH_3), 4.11 (t, 2H, CH_2 of Et), 4.16−4.23 (q, J = 7.2 Hz, 2H, CH_2), 5.62 (s, 1H, CH), 6.26 (s, 1H, NH), 6.71−7.18 (m, 12H, aromatic), 7.31 (d, J = 7.8, 1H, aromatic). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.0, 41.1, 55.4, 60.4, 63.8, 114.7, 114.9, 123.5, 125.2, 126.6, 127.5, 127.6, 127.9, 128.9, 131.0, 136.9, 140.3, 146.1, 146.7, 169.7, 169.8, 170.4.

*Ethyl 2-[2-(4-Hydroxy-3-methoxyphenyl)-2-(4-hydroxy-N-phenylbenzamido)acetamido]acetate (**8**).* Recrystallization from C_2H_5OH/H_2O. Yield: 640 mg, 67%. Mp: 134−136 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.18 (t, 3H, CH_3 of Et), 3.53 (s, 3H, OCH_3), 3.74−4.04 (m, 2H, CH_2 of Et), 4.05−4.12 (q, J = 7.2 Hz, 2H, CH_2), 6.27 (s, 1H, CH) 6.46−6.63 (m, 5H, aromatic), 6.98−7.07 (m, 7H, aromatic), 8.46 (t, 1H, NH), 8.90 (s, 1H, OH), 9.68 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9, 40.9, 55.2, 60.2, 63.7, 114.0, 114.6, 114.7, 123.3, 125.4, 126.3, 126.9, 127.5, 130.3, 130.7, 140.9, 145.9, 146.6, 158.0, 169.4, 169.6, 170.5.

*Ethyl 2-[2-(4-Hydroxy-3-methoxyphenyl)-2-(N-(4-hydroxyphenyl)benzamido)acetamido]acetate (**9**).* Recrystallization from C_2H_5OH/H_2O. Yield: 602 mg, 63%. Mp: 203−205 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.18 (t, 3H, CH_3 of Et), 3.57 (s, 3H, OCH_3), 3.74−4.04 (m, 2H, CH_2 of Et), 4.05−4.12 (m, 2H, CH_2), 6.21 (s, 1H, CH), 6.31 (d, J = 7.8, 2H, aromatic), 6.55−6.63 (m, 4H, aromatic), 7.18 (d, J = 3.0, 6H, aromatic), 8.42 (t, 1H, NH), 8.91 (s, 1H, OH), 9.24 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9, 40.9, 55.2, 60.2, 63.6, 114.1, 114.5, 114.9, 123.4, 125.3, 127.3, 127.7, 128.6, 131.3, 132.0, 137.1, 145.9, 146.6, 155.5, 169.6, 169.8, 170.4.

*Ethyl 2-[2-(4-Hydroxy-3-methoxyphenyl)-2-(4-hydroxy-N-(4-hydroxyphenyl)benzamido)acetamido]acetate (**10**).* Recrystallization from C_2H_5OH/H_2O. Yield: 533 mg, 54%. Mp: 165−167 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.18 (t, 3H, CH_3 of Et), 3.54 (s, 3H, OCH_3), 3.74−4.05 (m, 2H, CH_2 of Et), 4.06−4.13 (m, 2H, CH_2), 6.27 (s, 1H, CH), 6.47−6.64 (m, 5H, aromatic), 6.98−7.04 (d, 6H, aromatic), 7.01 (s, 1H, OH), 8.45 (t, 1H, NH), 8.89 (s, 1H, OH), 9.67 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.5, 46.5, 60.8, 65.8, 69.3, 119.6, 120.1, 120.3, 128.9, 130.1, 131.8, 132.5, 133.1, 135.9, 136.3, 146.5, 151.5, 152.2, 163.6, 175.0, 175.2, 176.1.

*(E)-Ethyl 2-[2-(3-(4-Hydroxy-3-methoxyphenyl)-N-phenylacrylamido)-2-phenylacetamido]acetate (**11**).* Purified by column chromatography with petroleum ether and ethyl acetate as eluent. Yield: 478 mg, 49%. Mp: 195−197 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.18 (t, 3H, CH_3 of Et), 3.68 (s, 3H, OCH_3), 3.77−4.02(m, 2H, CH_2 of Et), 4.05−4.13 (q, J = 6.9, 2H, CH_2), 5.93 (d, J = 15.0, 1H, CH═CH), 6.34 (s, 1H, CH), 6.71 (s, 2H, aromatic), 6.86 (s, 1H, aromatic), 7.13 (s, 6H, aromatic), 7.18 (s, 4H, aromatic), 7.50 (d, J = 15.3, 1H, CH═CH), 8.59 (t, 1H, NH), 9.46 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.1, 41.1, 55.6, 60.4, 63.5, 112.0, 115.9, 116.2, 120.8, 126.2, 127.6, 127.7, 127.8, 128.4, 130.5, 131.0, 135.1, 139.4, 141.6, 147.7, 148.6, 165.5, 169.7, 170.3.

*Ethyl 2-[2-Phenyl-2-(3,4,5-trihydroxy-N-phenylbenzamido)-acetamido]acetate (**12**).* Recrystallization from petroleum ether and ethyl acetate. Yield: 306 mg, 33%. Mp: 193−194 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.18 (t, 3H, CH_3 of Et), 3.76−3.99 (m, 2H, CH_2 of Et), 4.06−4.13 (q, J = 6.9 Hz, 2H, CH_2), 6.21 (s, 2H, aromatic), 6.36 (s, 1H,CH), 6.95 (s, 5H), 7.15 (s, 5H, aromatic), 8.34 (s, 1H, OH), 8.56 (t, 1H, NH), 8.80 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9, 41.0, 60.3, 63.9, 108.0, 126.1, 126.2, 127.5, 127.6, 130.2, 130.3, 134.3, 135.1, 140.9, 144.7, 169.6, 170.0, 170.1.

*(E)-Ethyl 2-[2-(4-Hydroxy-3-methoxyphenyl)-2-(3-(4-hydroxy-3-methoxyphenyl)-N-(4-hydroxyphenyl)acrylamido)acetamido]-acetate (**13**).* Purified by column chromatography with petroleum ether and ethyl acetate as eluent. Yield: 462 mg, 42%. Mp: 150−154 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.17 (t, 3H, CH_3 of Et), 3.56 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.77−4.00 (m, 2H, CH_2 of Et), 4.05−4.11 (q, J = 6.9, 2H, CH_2), 6.00 (d, J = 15.6, 1H, CH═CH), 6.15 (s, 1H, CH), 6.55 (s, 6H, aromatic), 6.70−6.76 (m, 3H, aromatic), 6.88 (s, 1H, aromatic), 7.39 (d, J = 15.6, 1H, CH═CH), 8.37 (t, 1H, NH), 8.90 (s, 1H, OH), 9.44 (s, 1H, OH), 9.47 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.1, 41.0, 55.4, 55.6, 60.4, 63.2, 112.3, 114.7, 114.8, 114.9, 115.9, 116.6, 120.4, 123.5, 125.8, 126.4, 130.5, 132.1, 141.0, 146.0, 146.7, 147.7, 148.5, 156.4, 165.7, 169.8, 170.7.

*(E)-N-[2-(3-Benzoylphenylamino)-2-oxo-1-phenylethyl)-3-(4-hydroxy-3-methoxyphenyl)-N-phenylacrylamide (**14**).* Purified by column chromatography with petroleum ether and ethyl acetate as eluent. Yield: 384 mg, 33%. Mp: 233−235 dec. ^{1}H NMR (400 MHz,

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DMSO-d_6): δ 3.68 (s, 3H, OCH_3), 5.96 (d, J = 15.6, 1H, CH═CH), 6.36 (s, 1H, CH), 6.69−6.75 (q, J = 10.4, 2H, aromatic), 6.86 (s, 1H, aromatic), 7.11−7.27 (m, 9H, aromatic), 7.46 (d, J = 15.6, 1H, CH═CH), 7.54−7.84 (m, 11H, aromatic), 9.47 (s, 1H, NH), 10.72 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.5, 64.8, 112.1, 115.8, 115.8, 118.3, 120.8, 126.1, 127.7, 128.1, 128.1, 128.4, 129.3, 130.3, 130.7, 131.1, 131.2,131.2, 131.4, 131.5, 132.2, 134.0, 137.5, 139.0, 141.8, 143.2, 147.6, 148.7, 165.6, 169.6, 194.5.

*N-(2-(tert-Butylamino)-1-(4-hydroxy-3-methoxyphenyl)-2-oxoethyl)-4-hydroxy-N-(4-hydroxyphenyl)benzamide (**15**).* Purified by column chromatography with petroleum ether and ethyl acetate as eluent. Yield: 584 mg, 63%. Mp: 259−261 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25 (s, 9H, $C(CH_3)_3$), 3.54 (s, 3H, OCH_3), 6.01 (s, 1H, CH), 6.32 (d, J = 7.5, 2H, aromatic), 6.48−6.53 (m, 6H, aromatic), 6.78 (s, 1H, aromatic), 7.03 (d, J = 8.7, 2H, aromatic), 7.57 (s, 1H, OH), 8.86 (s, 1H, NH) 9.20 (s, 1H, OH), 9.63 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.5, 50.2, 55.4, 64.4, 114.0, 114.1, 114.4, 114.7, 123.1, 126.7, 127.6, 130.3, 132.0, 132.4, 145.7, 146.7, 155.3, 157.9, 169.4, 169.8.

*N-(2-(tert-Butylamino)-2-oxo-1-phenylethyl)-3,4,5-trihydroxy-N-phenylbenzamide (**16**).* Recrystallization from petroleum ether and ethyl acetate. Yield: 312 mg, 36%. Mp: 238−239 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.24 (s, 9H, $C(CH_3)_3$), 6.21 (s, 3H, CH and aromatic), 6.93−7.13 (m, 10H, aromatic), 7.74 (s, 1H, OH), 8.31 (s, 1H, OH), 8.56 (t, 1H, NH), 8.78 (s, 2H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.4, 50.3, 64.7, 108.1, 126.1, 126.7, 127.3, 127.5, 127.7, 129.8, 130.4, 134.3, 136.2, 141.2, 144.8, 168.9, 169.9.

*(E)-N-(2-(tert-Butylamino)-2-oxo-1-phenylethyl)-3-(4-hydroxy-3-methoxyphenyl)-N-phenylacrylamide (**17**).* Purified by column chromatography with petroleum ether and ethyl acetate as eluent. Yield: 467 mg, 51%. Mp: 224−225 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25 (s, 9H, $C(CH_3)_3$), 3.68 (s, 3H, OCH_3), 5.93 (d, J = 14.7, 1H, CH═CH), 6.18 (s, 1H, CH), 6.72 (s, 2H, aromatic), 6.87 (s, 1H, aromatic), 7.01−7.17 (m, 10H, aromatic), 7.43 (d, J = 15.6, 1H, CH═CH), 7.78 (t, 1H, NH), 9.44 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.5, 50.3, 55.5, 63.9, 112.0, 115.8, 116.3, 120.6, 126.2, 127.4, 127.4, 127.8, 128.2, 130.0, 131.1, 135.9, 139.6, 141.3, 147.6, 148.5, 165.3, 169.2.

*N-(2-Ferrocenylmethylamino)-2-oxo-1-phenylethyl)-3,4,5-trihydroxy-N-phenylbenzamide (**18**).* Recrystallization from C_2H_5OH/H_2O. Yield: 645 mg, 56%. Mp: 155−158 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.98−4.16 (m, 11H, ferrocenyl and CH_2), 5.75 (s, 1H, CH), 6.22 (s, 2H, aromatic), 6.98 (s, 5H, aromatic), 7.15 (s, 5H, aromatic), 8.17 (s, 1H, OH), 8.31 (s, 1H, NH), 8.78 (s, 2H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.8, 64.7, 67.1, 67.2, 67.5, 67.6, 68.3, 86.1, 108.1, 126.3, 126.5, 127.6, 127.8, 130.1, 130.4, 134.4, 135.6, 141.2, 144.8, 169.3, 170.0.

*(E)-N-(2-(Ferrocenylmethylamino)-2-oxo-1-phenylethyl)-3-(4-hydroxy-3-methoxyphenyl)-N-phenylacrylamide (**19**).* Recrystallization from C_2H_5OH/H_2O. Yield: 960 mg, 80%. Mp: 183−184 °C dec. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.68 (s, 3H, OCH_3), 3.98−4.16 (m, 11H, ferrocenyl and CH_2), 5.94 (d, J = 15.2, 1H, CH═CH), 6.24 (s, 1H, CH), 6.69−6.74 (q, J = 4.0, 2H, aromatic), 6.86 (s, 1H, aromatic), 7.13 (s, 6H, aromatic), 7.20 (s, 4H, aromatic), 7.44 (d, J = 15.6, 1H, CH═CH), 8.26 (s, 1H, NH), 9.44 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.8, 55.5, 63.9, 67.1, 67.2, 67.6, 67.7, 68.3, 86.0, 112.0, 115.8, 116.2, 120.6, 126.2, 127.5, 127.7, 127.8, 128.3, 130.2, 131.0, 135.3, 139.5, 141.4, 147.6, 148.5, 165.4, 169.3.

*(E)-N-(2-(Ferrocenylmethylamino)-1-(4-hydroxy-3-methoxyphenyl)-2-oxoethyl)-3-(4-hydroxy-3-methoxyphenyl)-N-(4-hydroxyphenyl)acrylamide (**20**).* Recrystallization from C_2H_5OH/H_2O. Yield: 741 mg, 56%. Mp: 170−172 °C dec. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.51 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.88−4.06 (m, 11H, ferrocenyl and CH_2), 5.99 (s, 1H, CH), 6.04 (s, 2H, aromatic and CH═CH), 6.51 (s, 2H, aromatic), 6.55 (s, 3H, aromatic), 6.70−6.76 (m, 3H, aromatic), 6.89 (s, 1H, aromatic), 7.39 (d, J = 15.6, 1H, CH═CH), 8.1 (s, 1H, NH), 8.89 (s, 1H, OH), 9.42 (s, 1H, OH), 9.47 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.6, 55.3, 55.7, 63.5, 67.1, 67.1, 67.5, 67.6, 68.3, 86.3, 112.2, 114.5, 114.8, 115.9, 116.6, 120.3, 123.3, 126.1, 126.3, 130.7, 132.1, 140.8, 146.0, 146.7, 147.6, 148.4, 156.3, 165.6, 169.9.

Scavenging Radicals. The tests for scavenging ABTS$^{\bullet+}$, DPPH, and galvinoxyl radical were performed by following a previous description.[26] ABTS salt and $K_2S_2O_8$ were dissolved in 2.0 mL of water, in which the final concentrations of ABTS salt and $K_2S_2O_8$ were 4.0 and 1.41 mM, respectively. The aforementioned solution was kept for 16 h and then diluted with 100 mL of ethanol to give an ABTS$^{\bullet+}$ solution, whose absorbance was around 0.80 at 734 nm ($\varepsilon_{ABTS^{\bullet+}}$ = 1.6 × 10^4 M^{-1} cm^{-1}). DPPH and galvinoxyl radical were dissolved in 50 mL of ethanol, and the absorbances were around 1.00 at 517 nm (ε_{DPPH} = 4.09 × 10^3 M^{-1} cm^{-1}) and 428 nm ($\varepsilon_{galvinoxy}$ = 1.4 × 10^5 M^{-1} cm^{-1}), respectively. Dimethyl sulfoxide (DMSO) solutions of bis-amides (0.1 mL) were added to 1.9 mL of ABTS$^{\bullet+}$ solution, with the final concentrations being 300.0 μM for compounds **1**−**5**, 25.0 μM for **6**, 10.0 μM for **7**−**10** and **15**, 5.0 μM for **11**, **13**, **14**, **17**, **19**, and **20**, and 2.5 μM for **12**, **16**, and **18**. The DMSO solutions (0.1 mL) of bis-amides were added to 1.9 mL of DPPH solution, with the final concentrations being 100.0 μM for compounds **13** and **20**, 10.0 μM for **12**, **16**, and **18**, and 300.0 μM for the other compounds. The DMSO solutions of bis-amides (0.1 mL) were added to 1.9 mL of galvinoxyl radical solution, with the final concentrations being 100.0 μM for compounds **13** and **20**, 5.0 μM for **18**, 2.5 μM for **12** and **16**, and 300.0 μM for the other compounds. The decrease in the absorbance of these radicals was recorded at 25 °C within a certain time interval.

Inhibiting AAPH-Induced Oxidation of DNA. AAPH-induced oxidation of DNA was performed following a previous description.[36] DNA sodium salt and AAPH were dissolved in phosphate-buffered solution (PBS: 8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, 10.0 mM EDTA), with the final concentrations being 2.24 mg/mL and 40 mM, respectively. The aforementioned solution also contained various concentrations of bis-amides (dissolved in 0.1 mL of DMSO) except in the blank experiment, which also contained the same volume of DMSO as the control. The solution was then dispatched into test tubes, with each one containing 2.0 mL. These tubes were heated to 37 °C in a water bath to initiate the oxidation of DNA. Three of them were taken out at every 2 h and cooled to room temperature, 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) and 1.0 mL of 3.0% trichloroacetic acid aqueous solution and then heating in a boiling water bath for 15 min. After the tubes were cooled to room temperature immediately, 1.5 mL of *n*-butanol was added and the mixture shaken vigorously to extract thiobarbituric acid reactive species (TBARS). Finally, the tubes were centrifuged for few minutes to obtain an *n*-butanol layer for measuring the absorbance at 535 nm (ε_{TBARS} = 1.56 × 10^5 M^{-1} cm^{-1}).[37]

Statistical Analysis. All data were average values from at least three independent measurements, with the experimental error being within 10%. The equations in the present work were fitted by using Origin 7.5 professional software, and $p < 0.001$ indicated a significant difference.

■ ASSOCIATED CONTENT

Supporting Information

Figures and tables giving ^{1}H and ^{13}C NMR spectra for compounds **1**−**20**, the decay of the radical concentrations, the increase of the absorbance of TBARS in AAPH-induced oxidation of DNA, and equations of [radical] vs reaction period and its differential style together with the reaction rate at t = 0 (r_0). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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