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Activity of coumarin–oxadiazole-appended phenol in inhibiting DNA oxidation and scavenging radical

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ABSTRACT

Fourteen coumarin–oxadiazole-appended phenols were synthesized, while halogen atom, methoxyl, and hydroxyl groups acted as the functional groups for testing the ability to trap 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\cdot}$) and to inhibit the oxidation of DNA caused by 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH) and Cu^{2+}/glutathione (GSH). The coumarin–oxadiazole-appended phenols containing the hydroxyl group were able to inhibit AAPH-induced oxidation of DNA and to quench $ABTS^{+\cdot}$, and the antioxidant effectiveness depended upon the number of hydroxyl groups. Moreover, the coumarin–oxadiazole-appended phenols used herein were able to inhibit Cu^{2+}/GSH-induced oxidation of DNA, and the inhibitory effect generated by bromide was similar to that of the hydroxyl group. In particular, the antioxidant effectiveness of *para*-bromide at the benzene ring even approached to that of Trolox.

Coumarin is composed of a benzopyran-δ-lactone skeleton[1] and exhibits a large number of bioactivities.[2,3] When the coumarin acts as a substituent, it can also increase the bioactivity of the whole molecule.[4,5] The coumarin moiety can form a conjugation system with other structural features[6] or just act as a single substituent.[7,8] On the other hand, oxadiazole is a five-membered heterocycle with double nitrogen atoms and an oxygen atom contained.[9] It is usually employed as the central skeleton to combine with other structural features.[10] For example, the hybrid of 1,3,4-oxadiazole and 1,4-benzodioxan inhibits DNA strand breakage and lipid peroxidation caused by 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH, R-N=N-R, R=C-Me_2C(=NH)NH_2).[11] The hybrid of 1,3,4-oxadiazole and thieno[2,3-*d*]pyrimidine can trap radicals efficiently.[12] As an isomer of 1,3,4-oxadiazole, the bioactivities of 1,2,4-oxadiazole are not usually reported.[13,14] We have reported that 1,2,4-oxadiazole increased the inhibitory effect of the phenolic hydroxyl group on AAPH-induced DNA oxidation,[15] and coumarin moiety forming a hybrid with dihydropyrazole exhibited a high inhibitory effect on DNA oxidation.[16] The aforementioned background motivates us to test the antioxidant effect of the hybrid formed by coumarin, 1,2,4-oxadiazole, and phenol in order to find novel antioxidant effectiveness. As shown in Scheme 1, 14 coumarin–oxadiazole-appended phenols are synthesized, and the inhibitory effects on AAPH- and Cu^{2+}/glutathione (GSH)-induced oxidation of DNA together with the ability to quench 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\cdot}$) are investigated.

The coumarin moiety is usually found in natural compounds,[17] and some methods are developed to construct coumarin skeleton with different substituents involved.[18] Some functional groups are allocated at the 3-position of coumarin in order to connect the coumarin moiety with other structural features.[19] We herein employed 2,2-dimethyl-1,3-dioxane-4,6-dione to react with salicylaldehyde for introducing —COOH into the 3-position of coumarin. Then, carboxylic acid was converted into chloride by $SOCl_2$ because acyl chloride should be applied during cyclization with amidoxime for producing 1,2,4-oxadiazole eventually.[15] Thus, the acyl chloride in coumarin can be used to bridge with amidoxime for forming coumarin-appended 1,2,4-oxadiazole. It was reported that the antioxidant effects of formyl-pyrazoles[20] and dihydropyrazoles[16] were enhanced by the intramolecular coumarin moiety. This encourages us to test whether the coumarin moiety can play the same role in 1,2,4-oxadiazole derivatives.

The ability to trap radicals is an important property of an antioxidant, while $ABTS^{+\cdot}$, a *N*-centered radical, is usually used in the radical-scavenging test.[21,22] The rate constant (***k***) of the antioxidant in trapping $ABTS^{+\cdot}$ can be measured by the second-order kinetic Eq. 1.[23]

$$-\frac{d[ABTS^{+\cdot}]}{dt} = \boldsymbol{r} = \boldsymbol{k}[ABTS^{+\cdot}]\,[\text{antioxidant}] \quad (1)$$

Eq. 1 is valid for every time-point during the reaction between $ABTS^{+\cdot}$ and the antioxidant. So, in the case of $t = 0$ min, Eq. 1

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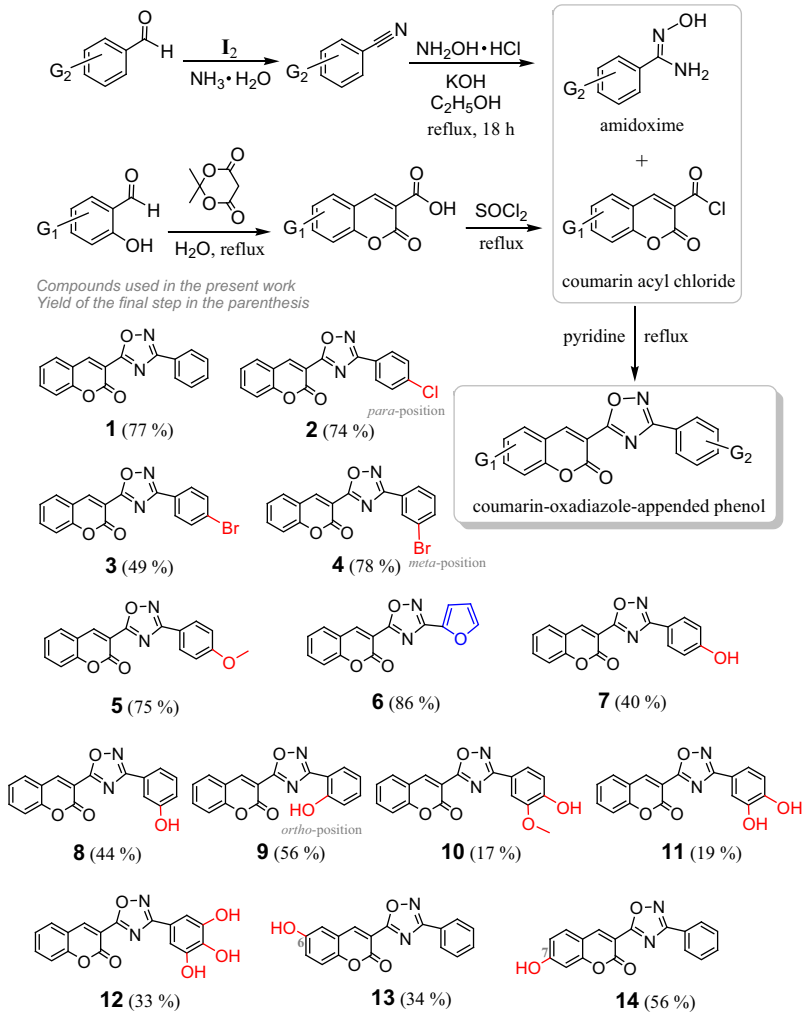

Scheme 1. Synthetic routine of coumarin–oxadiazole-appended phenols.

converts into Eq. 2, in which $[ABTS^{+\cdot}]_{t=0}$ and $[antioxidant]_{t=0}$ refer to the concentrations of $ABTS^{+\cdot}$ and the antioxidant at the beginning of the reaction.

$$\boldsymbol{r}_0 = \boldsymbol{k}[ABTS^{+\cdot}]_{t=0}\,[antioxidant]_{t=0} \quad (2)$$

As we have pointed out in a previous report,[24] the decay of the concentration of $ABTS^{+\cdot}$ (shown in Fig. 1S) follows the double exponential function (Eq. 3).

$$[ABTS^{+\cdot}] = A\,e^{-(t/a)} + B\,e^{-(t/b)} + C \quad (3)$$

Eq. 3 is performed by the differential operation to the relationship between the reaction rate ($\boldsymbol{r}$) and the time (t) (see Eq. 4), and Eq. 4 can be used to calculate the reaction rate at $t = 0$ min ($\boldsymbol{r}_0$).

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

The rate constant ($\boldsymbol{k}$) obtained for every compound is collected in Table 1.

The rate constant ($\boldsymbol{k}$) in Table 1 shows that only **4**, **7**, **8**, **9**, **10**, **11**, **12**, and **13** are able to scavenge $ABTS^{+\cdot}$. The $\boldsymbol{k}$ values of **11** and **12** (12,100 and 20,000 $M^{-1}\,s^{-1}$, respectively) approach to that of Trolox (29,200 $M^{-1}\,s^{-1}$),[25] indicating that catechol and pyrogallol moieties increase the radical-scavenging property of coumarin–oxadiazole-appended phenol remarkably. However, comparing the $\boldsymbol{k}$ value of **11** (12,100 $M^{-1}\,s^{-1}$) with that of catechol (2580 $M^{-1}\,s^{-1}$),[25] it may conclude that the coumarin and oxadiazole moieties enhance the radical-scavenging ability of the whole molecule although the catechol moiety plays the major role in trapping $ABTS^{+\cdot}$. The $\boldsymbol{k}$ values of **7**, **8**, and **9** are around 10–20 $M^{-1}\,s^{-1}$, indicating that a single hydroxyl group at the benzene ring possesses weak activity in trapping $ABTS^{+\cdot}$. A methoxyl group at the adjacent position of the hydroxyl group increases the $\boldsymbol{k}$ value to 166.3 $M^{-1}\,s^{-1}$ for **10**. Thus, the *ortho*-methoxyl group and the number of hydroxyl group still play a key role in trapping radical. On the other hand, the single hydroxyl group at coumarin moiety exhibits different properties. The hydroxyl group at the 6-position in the coumarin moiety generates 66.5 $M^{-1}\,s^{-1}$ of the $\boldsymbol{k}$ value for **13**, higher than that of the hydroxyl group at the benzene ring (see the $\boldsymbol{k}$ values of **7**, **8**, and **9**). But the hydroxyl group at the 7-position in the coumarin moiety (as in **14**) cannot trap $ABTS^{+\cdot}$. In particular, **4**, *meta*-bromobenzene-contained compound, can scavenge $ABTS^{+\cdot}$ although the $\boldsymbol{k}$ value is only 3.08 $M^{-1}\,s^{-1}$, revealing that *meta*-bromide in the hybrid of coumarin and oxadiazole can generate the ability to quench $ABTS^{+\cdot}$, and thus, may suppress the oxidation occurring in biological systems.

The peroxyl radical ($ROO^{\cdot}$) is generated at a stable rate (R_g, $R_g = (1.4 \pm 0.2) \times 10^{-6}$ [AAPH] s^{-1}) via the decomposition of AAPH,[26] and the guanine bases in DNA can be oxidized by $ROO^{\cdot}$ to form carbonyl species.[27] The carbonyl species react with thiobarbituric acid (TBA) to form thiobarbituric acid reactive substances (TBARS), whose absorbance can be detected at 535 nm.[28] As shown in Figure 2S, the absorbance of TBARS increases with the reaction period in the control experiment. But the additions of **1**–**6**, **9**, **13**, and **14** decrease the absorbance of TBARS when