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Quantitative structure–activity relationship analyses of antioxidant and free radical scavenging activities for hydroxybenzalacetones

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Abstract—Antioxidant activities for a series of hydroxybenzalacetones, **OH-BZ**, were evaluated by measuring inhibitory potencies of **OH-BZ** against lipid peroxidation induced by *t*-BuOOH or γ-irradiation. Their quantitative structure–activity relationship (QSAR) studies indicated that the activities are mainly governed by electronic and steric factors. To rationalize these results, we also performed QSAR analyses for DPPH radical scavenging activities of **OH-BZ**, which indicated that antioxidant and radical scavenging activities could be expressed by the same physicochemical parameters but the hydrogen bonding behavior of phenolic OH varies with the reaction medium.
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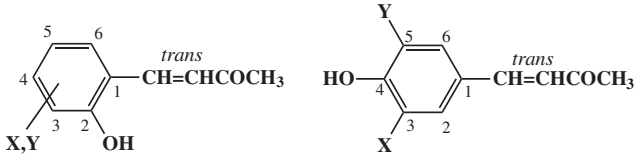
1. Introduction

In the course of our continuing studies on bioactivities for dehydrozingerone (4-hydroxy-3-methoxybenzalacetone), regarded as 'half-curcumin', and its structurally related compounds,[1] we have taken much interest in the antioxidant activity produced by hydroxylbenzalacetones, **OH-BZ**, including dehydrozingerone. Although some of these compounds are known to have potent antioxidant activity and qualitative structure–activity relationships have been discussed,[2] few investigators have performed quantitative structure–activity relationship (QSAR) analyses. In the present study, we measured inhibitory potencies of variously substituted hydroxybenzalacetones (**2OH-BZ** and **4OH-BZ**) against lipid peroxidation induced by *t*-BuOOH (*tert*-butyl hydroperoxide) or γ-irradiation, and the relationship between their structure and activity was analyzed quantitatively by using free-energy related substituent parameters. To interpret the resultant correlations, we further measured their DPPH free radical (1,1-diphenyl-2-picrylhydrazil radical) scavenging activities and performed the QSAR analysis.



2OH-BZ **4OH-BZ**

2. Activities

2.1. Antioxidant activity (RBC system)

Antioxidant activities were assessed by measuring the inhibitory effect of **OH-BZ** against peroxidation of red blood cell (RBC) membrane ghost, prepared from commercially available rabbit blood, induced by *t*-BuOOH or γ-irradiation. The peroxidation was initiated by adding 100 μL of 10 mM *t*-BuOOH to a reaction mixture consisting of 100 μL of RBC membrane ghost suspension (2.0 mg protein/mL), 20 μL of dimethyl sulfoxide

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(DMSO) solution containing various concentrations of test compound and 280 μL of phosphate buffer (pH 6.8, 0.067 mM) and the mixture was incubated at 37 °C for 30 min. For peroxidation by γ-irradiation of 96 Gy, the reaction mixture containing 380 μL (instead of 280 μL) of the phosphate buffer was irradiated at room temperature for 3 h using a ^{137}Cs-source of 3.7 TBq (32 Gy/h). After appropriate treatments the lipid peroxidation was monitored by the TBA method:[3] the amount of TBA-reactive substances produced in the RBC membrane ghost was measured at 535 nm. The IC_{50} value was derived from the dose–activity curves and was used to represent the antioxidant activity.

2.2. Radical scavenging activity (DPPH system)

To 3.00 mL of 5.0×10^{-5} M DPPH methanolic solution was added 0.300 mL methanolic solution containing various concentrations of **OH-BZ**. After 15 min, decrease in the DPPH concentration was determined spectrophotometrically at 513 nm (25 °C).

3. Results and discussion

Phenolic antioxidants, ArOH, are thought to exert their antioxidant activities by breaking the radical chain reaction of the peroxidized lipid radical, LOO•, according to

$$LOO^{\bullet} + ArOH \rightarrow LOOH + ArO^{\bullet}$$

The effects of ring substituents on the antioxidant activity have been discussed qualitatively and quantitatively,[2,4,5] showing that introduction of electron-donating substituent(s) into the phenol ring and/or steric crowding around the phenolic OH group tend to enhance the activity. As to the electronic effects, Hansch and co-workers have shown that the electronic substituent, σ^+,[6] which involves through-resonance effects is often useful in describing the radical reactions of organic compounds rather than σ or other special radical parameters.[7] With these in mind, we first analyzed $\log(1/IC_{50})$ values for **2OH-BZ** (Table 1) by regression analyses using various substituent parameters. Among parameters tested, σ^+ was the most useful parameter, providing $r = 0.75$ and 0.85 (r: correlation coefficient) for oxidation by *t*-BuOOH and γ-irradiation, respectively, where σ^+ represents the electronic effects of the substituent on the phenolic OH group and the effect of the *ortho* substituent is supposed to be equivalent with that of the corresponding *para* substituent; $\sigma^+_{ortho} = \sigma^+_{para}$.[8] To represent the steric effect of the *ortho* substituent, we further applied the steric parameter E_s[9] only to the *ortho* substituent (the substituent at the 3-position), otherwise $E_s = 0$. Addition of E_s improved the correlations, yielding excellent regressions as shown by Eqs. 1 and 2.

t-BuOOH

$$\log(1/IC_{50}) = -0.745\sigma^+ - 0.276E_s + 3.763 \quad (1)$$
$$(0.255) \quad (0.124) \quad (0.151)$$

Table 1. Activities and physicochemical parameters for hydroxybenzalacetones

No.	Substituent(s)	$\log(1/IC_{50})$[a]			σ^{+d} $(\Sigma\sigma^+)^*$	E_s^e $(\Sigma E_s)^*$	HB	I_p
		t-BuOOH[b]	γ-irradiation[c]	DPPH				
2OH-BZ								
1	H	3.824	4.423	2.407	0.00	0.00	0	0
2	3-Me	4.274	5.440	3.684	−0.31	−1.24	0	0
3	3-*t*-Bu	4.793	5.662	4.225	−0.26	−2.78	0	0
4	3-F	3.690	4.291	1.883	−0.07	−0.46	0	0
5	3-OMe	4.604	5.488	2.649	−0.78	−0.55	1	0
6	3-OEt	4.434	5.479	2.736	−0.81	−0.55	1	0
7	3-OH	4.573	5.708	5.341	−0.92	−0.55	0	0
8	4-OMe	3.542	4.449	2.486	0.05	0.00	0	0
9	5-Me	4.102	5.097	3.056	−0.31	0.00	0	0
10	5-*t*-Bu	4.205	5.070	2.725	−0.26	0.00	0	0
11	5-Cl	3.790	4.220	1.649	0.11	0.00	0	0
12	5-OMe	4.212	5.400	4.691	−0.78	0.00	0	0
13	5-OH	4.487	5.845	5.111	−0.92	0.00	0	0
14	3,5-Di-Cl	—[f]	—[f]	2.478	0.22*	−0.97	0	0
15	3,5-Di-*t*-Bu	—[f]	—[f]	4.544	−0.52*	−2.78	0	0
4OH-BZ								
16	H	3.256	4.180	1.970	0.00	0.00	0	1
17	3-OMe	4.301	4.939	4.534	−0.78	−0.55	0	1
18	3-OH	4.439	5.631	5.123	−0.92	−0.55	0	1
19	3,5-Di-Me	4.582	5.199	4.600	−0.62*	−2.48*	0	1
20	3,5-Di-OMe	4.764	5.599	4.628	−1.56*	−1.10*	1	1

[a] IC_{50}: M.
[b] Trolox: $\log(1/IC_{50}) = 4.105$.
[c] Trolox: $\log(1/IC_{50}) = 4.963$.
[d] $\Sigma\sigma^+ = \sigma^+(X) + \sigma^+(Y)$.
[e] $\Sigma E_s = E_s(X) + E_s(Y)$.
[f] Not tested because of low solubility of the compounds.

$n = 13, r = 0.933, s = 0.153, F = 33.8, q^2 = 0.751$

γ-Irradiation

$$\log(1/IC_{50}) = -1.279\sigma^+ - 0.292E_s + 4.466 \quad (2)$$
$$(0.357) \quad (0.174) \quad (0.212)$$

$n = 13, r = 0.942, s = 0.214, F = 39.1, q^2 = 0.825$

In these equations and throughout this paper, n is the number of compounds used for calculations, r the correlation coefficient, and s the standard deviation. F is the value of the F-ratio between the variances of the observed and calculated values, and q^2 represents the correlation coefficient obtained from the leave-one-out cross-validation. The figures in parentheses are the 95% confidence intervals of the regression coefficients and the intercept.

Analyses for the combined data set, **2OH-BZ** and **4OH-BZ**, were performed by introducing an indicator variable I_p, where $I_p = 1$ for **4OH-BZ** and 0 for others. Where multiple substituents were present (**19**, **20**) it was assumed that the effects were additive: the electronic term was replaced by $\Sigma\sigma^+ = \sigma^+(X) + \sigma^+(Y)$, and the steric term by $\Sigma E_s = E_s(X) + E_s(Y)$. This allows Eqs. 3 and 4 to be applied to all the compounds tested.

t-BuOOH

$$\log(1/IC_{50}) = -0.763\Sigma\sigma^+ - 0.277\Sigma E_s - 0.338I_p + 3.755 \quad (3)$$
$$(0.189) \quad (0.099) \quad (0.191) \quad (0.125)$$

$n = 18, r = 0.948, s = 0.153, F = 41.2, q^2 = 0.829$

γ-Irradiation

$$\log(1/IC_{50}) = -1.091\Sigma\sigma^+ - 0.229\Sigma E_s - 0.523I_p + 4.571 \quad (4)$$
$$(0.322) \quad (0.169) \quad (0.325) \quad (0.213)$$

$n = 18, r = 0.909, s = 0.261, F = 22.1, q^2 = 0.678$

In Eqs. 3 and 4, $\Sigma\sigma^+ = \sigma^+$ and $\Sigma E_s = E_s$ except for **19** and **20**. Although the steric term for **2OH-BZ** is given by the sum of E_s for the 3-X-substituent and the fixed substituent, $\Sigma E_s = E_s$(3-X or H) + E_s(CH=CHCOMe), the contribution from the latter term is expected to remain constant, and hence be incorporated in the I_p term. Similarly the electronic contribution from the CH=CHCOMe substituent, located *ortho* and *para* to the phenolic OH group in **2OH-BZ** and **4OH-BZ**, respectively, is expected to be included in the constant term according to our assumption ($\sigma^+_{ortho} = \sigma^+_{para}$). Although these treatments may be oversimplified, stability of the correlation between Eq. 1 for **2OH-BZ** and Eq. 3 for the combined set (**2OH-BZ**+**4OH-BZ**) is excellent, suggesting that correlations thus obtained are reliable in view of the number of compounds used for correlations. For γ-irradiation, extension of the correlation by inclusion of the **4OH-BZ** derivatives leads to a slightly less precise correlation, albeit one of acceptable statistical significance. This is possibly because a number of additional factors may tend to lower the activity of compounds in the group **16**–**20**. In the case of di-hydroxy derivatives (**7**, **13**, and **18**), 2-OH or 4-OH group is regarded as the reaction center, with the second OH being regarded as a substituent. This treatment could be justified by the fact that the quality of the fit of the equations remained largely unchanged by excluding each di-hydroxy derivative from the analyses. Calculated values by Eqs. 3 and 4 are plotted with the observed values in Figure 1. Addition of a log P term to Eqs. 3 and 4 did not improve the correlation, although more lipophilic compounds are expected to play more important role in the highly lipophilic RBC membrane ghost.

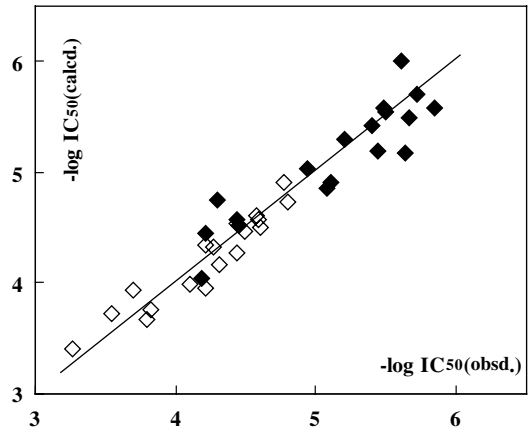

Figure 1. Plot of log(1/IC_{50}) observed and calculated by Eqs. 3 and 4. Open symbols: Oxidation by *t*-BuOOH (Eq. 3). Closed symbols: Oxidation by γ-irradiation (Eq. 4). The line is drawn with unit gradient.

The above results demonstrate that more electron-donating substituents and bulkier *ortho* substituents are required to increase the activity. Similar results have been reported by Nakao et al. who analyzed quantitatively the inhibitory activity of variously substituted hydroxyphenylureas against lipid peroxidation of rat-blain homogenate induced by aeration and found that the antioxidant activity was mostly governed by the electronic and steric effects of substituents on the phenolic hydroxyl group.[5] Finding that antioxidant activities derived from different types of phenolic compounds by different screening procedures yield correlations of similar quality seems to imply that the critical process for antioxidant activity is something common to both systems; it is possible that this could be the process of formation and stabilization of phenoxy radicals.

To examine this possibility, we measured the free radical scavenging activity for these compounds by using the stable DPPH radical (denoted as DPPH·).[10] With this radical, phenols react as shown by

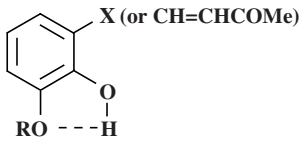

Figure 2. Intra-molecular hydrogen bonding.

$$\mathrm{ArOH} + \mathrm{DPPH}^{\bullet} \rightarrow \mathrm{ArO}^{\bullet} + \mathrm{DPPH:H}$$

When $\log(1/IC_{50})$ values for 20 compounds including **14** and **15** (Table 1) were analyzed with the same parameters used in Eqs. 3 and 4, the results show a remarkable enhancement of activity for **5**, **6**, and **20**, $\log(1/IC_{50})_{calcd} - \log(1/IC_{50})_{obsd} = 1.1–1.4$, where the hydroxyl group is sandwiched between two *ortho* substituents at least one of which is alkoxy and is capable of forming an intra-molecular hydrogen bond (Fig. 2). To accommodate this in the model, an additional indicator variable, HB, representing intra-molecular H-bonding (HB = 1 for **5**, **6**, and **20**, and HB = 0 for others) was introduced. This yielded improved correlation shown in the results with Eq. 5.

$$\begin{aligned}\log(1/IC_{50}) = &-2.979\Sigma\sigma^{+} - 0.350\Sigma E_s - 2.220\mathrm{HB} \\ &\quad (0.432) \qquad (0.166) \qquad (0.501) \\ &-0.297 I_p + 2.272 \\ &\quad (0.378) \quad (0.238)\end{aligned} \tag{5}$$

$$n = 20, r = 0.975, s = 0.311, F = 71.4, q^2 = 0.926$$

For this regression, HB = 0 was taken for compound **7**. This may be indicative that the 2-hydroxyl group of **7** preferentially hydrogen bonds to the solvent molecule (MeOH), which is more basic than the 3-OH group. Predictive power of Eq. 5 is shown in Figure 3. The calculated $\log(1/IC_{50})$ value for **4** from Eq. 5 was found to be larger than the observed value by 0.76. This can be ascribed to the electronic ability of the fluorine substituent that allows it to be that is slightly electron donating by resonance ($\sigma^{+} < 0$) but inductively electron withdrawing ($\sigma_I > 0$, σ_I: inductive electronic constant) and thereby decreasing the activity. Since the electronic factor plays an important role in governing the radical scavenging potency, the calculated value for **4** with a larger σ_I value by Eq. 5 with no inductive effect term can be expected to show a more positive deviation from the observed value. Addition of an inductive term to Eq. 5 could provide a more precise correlation if the range of data justified a more generalized analysis.

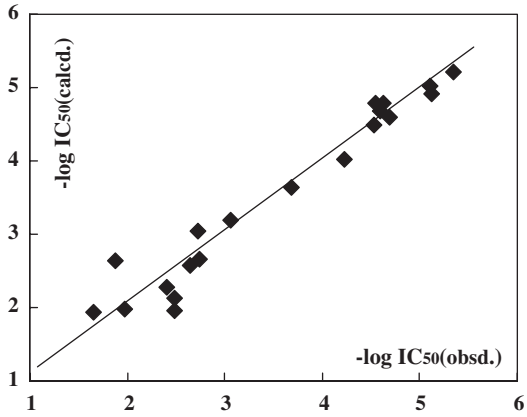

Figure 3. Plot of $\log(1/IC_{50})$ observed and calculated by Eq. 5. The line is drawn with unit gradient.

It is of interest to compare the correlations derived from the RBC and DPPH systems. The fact that the HB parameter is significant only for DPPH but not for RBC system can be rationalized by considering the difference in hydrogen-bonding abilities of the reaction medium; under the conditions of the RBC system, the phenolic OH of **OH-BZ** preferentially hydrogen bonds to DMSO present in the reaction mixtures, which reduces the extent of intra-molecular-hydrogen bonding as shown in Figure 2. Although direct correlation between the corresponding activities for DPPH and RBC was not so high ($r = 0.73$ and $r = 0.80$ for *t*-BuOOH and γ-irradiation oxidations, respectively), determining factors for the activity are the same in both systems except for the HB term, suggesting that hydrogen abstraction from **OH-BZ** by peroxidized lipid radicals is the critical process for producing inhibitory activity against lipid peroxidation of the RBC ghost membrane. The negative coefficient of the $\Sigma\sigma^{+}$ term (ρ^{+}) indicate that an electron-deficient radical-type transition state for radical formation from **OH-BZ** would be stabilized by electron-donating substituent(s), similarly, a negative coefficient for ΣE_s suggests that steric shielding by bulkier *ortho* substituents(s) stabilizes the resultant phenoxy radicals. It is important to notice that the $|\rho^{+}|$ value is much larger for DPPH than for RBC. One reason for this would be that more stable DPPH radical is more selective than peroxidized lipid radicals. A similar trend that is, the more reactive the radical, the smaller the $|\rho^{+}|$, has been reported by Hansch and Gao.[7]

In conclusion, inhibitory potencies of **OH-BZ** against lipid peroxidation of RBC membrane ghost were shown to be governed mainly by the process of phenoxy radical formation; more electron-donating substituents contribute to facilitating phenoxy radical formations; and *ortho* substituent(s) are effective in stabilizing the generated phenoxy radicals. Although correlations for inhibitory effects against RBC-lipid peroxidation and DPPH radical scavenging activities caused by **OH-BZ** are of similar quality, it was observed that solvent effects markedly modify hydrogen-bonding properties of phenols. Therefore, care must be taken in selecting the solvent system, when the DPPH assay is employed as a first screening test for antioxidant activity.

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