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# Preparation and antioxidant activity of α-pyridoin and its derivatives

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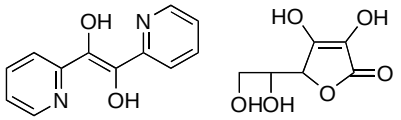
**Abstract**—Focusing on α-pyridoin (**1**, 1,2-di(2-pyridyl)-1,2-ethenediol) as the lead compound of the novel antioxidative enediol, we synthesized 5,5′- or 6,6′-bis-substituted derivatives of **1** from disubstituted pyridines. The antioxidant activity of **1** and its synthetic derivatives **2-7** was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl radical) scavenging assay and inhibition of lipid peroxidation. In the DPPH assay, **1** exhibited an activity stronger than that of ascorbic acid, and 5,5′-dimethyl-(**5**) or 5,5′-dimethoxy-substituted derivatives (**6**) exhibited more potent activity than **1**. The DPPH scavenging activities of α-pyridoins were correlated with their oxidation potential and thus the electron density of enediol. **5** and **6** effectively inhibited lipid peroxidation in the rat liver microsome/*tert*-butyl hydroperoxide system. Therefore, **5** and **6** serve as good candidates for a pharmacologically useful enediol antioxidant.


## 1. Introduction

Reactive oxygen species (ROS) and free radicals are considered to be implicated in a variety of pathological events, such as cancer and aging.[1–3] ROS, including superoxide anion, hydrogen peroxide, and hydroxyl radical, are thought to be generated subsequent to the reduction of molecular oxygen in aerobic organisms.[4,5] Under normal conditions, cells and tissues are protected against ROS by an array of enzyme defense systems, such as superoxide dismutase, catalase, and glutathione peroxidase or free radical scavengers.[6] Among these radical scavengers, ascorbic acid (AsA, Fig. 1) shows very effective activity[7] and has often served as a lead compound for the design and synthesis of pharmacologically effective antioxidants.[8] The active site of AsA is the 2,3-enediol moiety conjugated with the carbonyl group of a five-membered lactone. The anionic form of AsA is very stable and has a strong electron-donating ability to act as an effective radical scavenger.[9] Many *O*-acyl, *O*-alkyl, and imine analogues of AsA have been synthesized and their antioxidant activity been studied.[8] Almost all these AsA derivatives have a γ-lactone formula, and a few papers have reported the antioxidant activities of even simpler enediols, such as reductic acid (2,3-dihydroxy-2-cycropentenone)[10] or a kind of sugar analogue.[11]




**1** AsA

**Figure 1.** Structure of α-pyridoin (**1**) and ascorbic acid (AsA).

In the current study, we focused on α-pyridoin (**1**, Fig. 1), having a coplanar *E*-enediol form, as a candidate for enediol antioxidant. The enediol form is stabilized by the intramolecular hydrogen bonding of pyridine nitrogen and hydroxyl group, and no α-hydroxyketone form exists.[12,13] The enediol form is conjugated with the electron-withdrawing pyridine ring. Actually, the p*K*a value of **1** is approximately 0.5. Therefore, it is thought that the anion form (i, Fig. 2) is easily formed, such as AsA, under physiological conditions. Furthermore, the electron density of the enediol can be easily controlled by substitution on a pyridine ring. The physical and chemical properties of **1** have been well-studied since the 1950s.[14–16] The inhibition of mild steel corrosion in hydrochloric acid by **1** has been reported.[17] However, the biological activities of **1** have been

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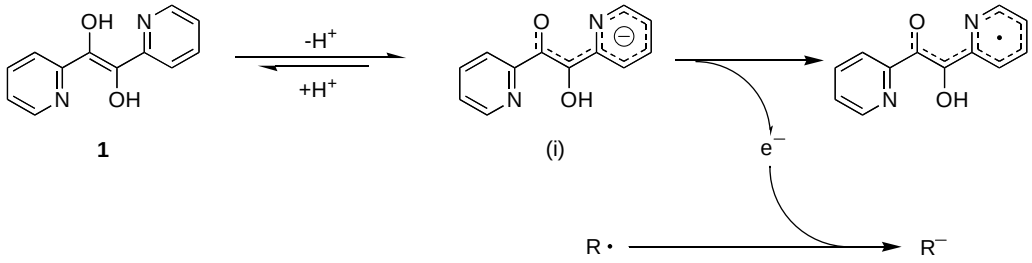


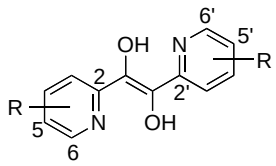

**Figure 2.** Proposed mechanism of free radical scavenging by α-pyridoin (**1**).

reported only with regard to the inhibition activity of aldehyde dehydrogenase as an analogue of metyrapone.[18] In this paper, we report the preparation of α-pyridoin derivatives and the antioxidant activity of these α-pyridoins.

## 2. Results and discussion

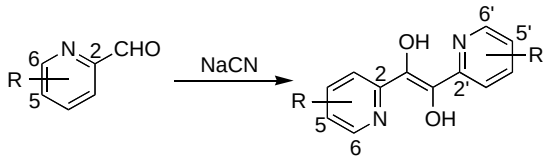
### 2.1. Chemistry

**2.1.1. Synthesis of α-pyridoin derivatives.** The structures of synthetic α-pyridoin derivatives **2**–**7** are illustrated in Figure 3. α-Pyridoin derivatives were prepared in a modification of α-pyridoin (**1**) synthesis using the benzoin condensation (Scheme 1).[19] 6-Methoxy-2-pyridinecarboxaldehyde (**9**) was prepared by monomethoxylation of 2,6-dibromopyridine (**14**), followed by formylation of the remaining bromine (Scheme 2).[20] 6-Acetyl-2-pyridinecarboxaldehyde (**10**) was prepared in four steps (Scheme 3). 2,6-Dibromopyridine (**14**) was treated with *n*-butyllitium and *N*,*N*-dimethylacetamide to give 6-acetyl-2-bromopyridine (**16**).[21] The acetyl carbonyl of **16** was protected to form acetal **17**.[22] Formylation of **17** and subsequent deprotection of acetal gave **10**. 5-Acetyl-2-pyridinecarboxaldehyde (**13**) was prepared by the same procedure as that followed for **10** using 2,5-dibromopyridine (**18**) as a starting material (Scheme 3). Monoacetylation of **18** predominantly gave 5-acetyl-2-bromopyridine (**19**).[23] 5-Methyl-2-pyridinecarboxaldehyde (**11**) was prepared by the formylation of 6-bromo-3-picoline (**23**, Scheme 4). 5-Methoxy-2-pyridinecarboxaldehyde (**12**) was prepared from 5-hydroxy-2-picoline (**24**) in five steps (Scheme 5). The *O*-methylation and subsequent oxidation of **24** afforded 5-methoxy-2-picoline-*N*-oxide (**26**).[24] *N*-Oxide **26** was converted to pyridylmethanol **28** via trifluoroacetate




**2**: R=6-, 6'-$CH_3$
**3**: R=6-, 6'-$OCH_3$
**4**: R=6-, 6'-$COCH_3$
**5**: R=5-, 5'-$CH_3$
**6**: R=5-, 5'-$OCH_3$
**7**: R=5-, 5'-$COCH_3$

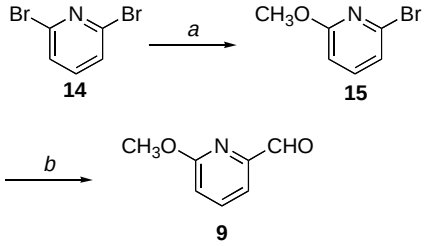
**Figure 3.** Structure of α-pyridoin derivatives **2**–**7**.




**8**: R=6-$CH_3$
**9**: R=6-$OCH_3$
**10**: R=6-$COCH_3$
**11**: R=5-$CH_3$
**12**: R=5-$OCH_3$
**13**: R=5-$COCH_3$

**2**: R=6-, 6'-$CH_3$
**3**: R=6-, 6'-$OCH_3$
**4**: R=6-, 6'-$COCH_3$
**5**: R=5-, 5'-$CH_3$
**6**: R=5-, 5'-$OCH_3$
**7**: R=5-, 5'-$COCH_3$

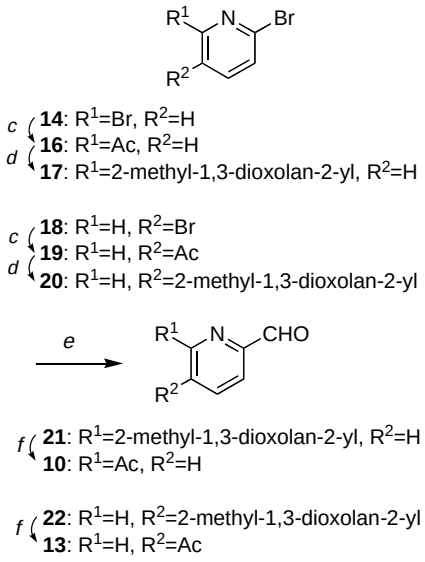
**Scheme 1.** Benzoin condensation of substituted 2-pyridinecarboxaldehydes **8**–**13**.



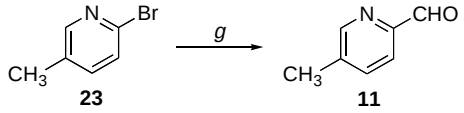

**Scheme 2.** Synthesis of aldehyde **9**. Reagents and conditions: (a) $CH_3ONa$/DMF, 80 °C, 2 h, 50%; (b) *n*-BuLi, DMF/THF, −78 °C to rt, 53%.

intermediate **27**, and then **28** was oxidized to aldehyde **12** by manganase dioxide.[25] Finally, benzoin condensation of these substituted pyridinecarboxaldehydes **8**–**13** was carried out to give pyridoins **2**–**7**. $^1$**H** and $^{13}$C NMR spectra in DMSO-$d_6$ revealed that all α-pyridoin derivatives, except for **4**, existed in an *E*-enediol form and 4 was a mixture of *E*-enediol and *Z*-enediol forms (7:3).

**2.1.2. Lipophilicity of α-pyridoins.** The biomembrane consists largely of lipids, one of the main components of which are unsaturated fatty acids, and it is easily peroxidized with ROS. Thus, it is thought that the lipophilic antioxidant acts effectively. To elucidate the ability to penetrate a biomembrane, the lipophilic property of α-pyridoins **1**–**7** was measured. The log $k_w$ value is nearly in agreement with the partition coefficient log $P$ and can be calculated by measuring the retention time on the reverse-phase HPLC using methanol/3-(*N*-morpholino) propanesulfonate (MOPS) buffer (pH 7.4).[26–28] The log $k_w$ value, which is considered to be the lipophilic property of **1**–**7**, is shown in Table 1. α-Pyridoin (**1**)




**Scheme 3.** Synthesis of aldehydes **10** and **13**. Reagents and conditions: (c) *n*-BuLi, $AcN(CH_3)_2/Et_2O$, −78 °C to rt, **17**: 76%, **18**: 50%; (d) ethylene glycol, *p*-TsOH/toluene, reflux, **19**: 4 h, 79%, **20**: 6 h, 87%; (e): *n*-BuLi, DMF/$Et_2O$, −78 °C to rt, **21**: 54%, **22**: 53%; (f) HCl, reflux, **10**: 2 h, 82%, **13**: 10 min, 48%.




**Scheme 4.** Synthesis of aldehyde **11**. Reagents and conditions: (g) *n*-BuLi, DMF/$Et_2O$, −78 °C to rt, 31%.

had high lipophilicity because it contains two internal hydrogen bonds between pyridine nitrogen and hydroxyl group. A methyl and a methoxy substituent augmented lipophilicity by 1.3- to 1.6-fold. In particular, 6, 6′-dimethoxy derivative **3** showed an extremely high lipophilicity. On the other hand, acetyl substitution showed little effect on lipophilicity.

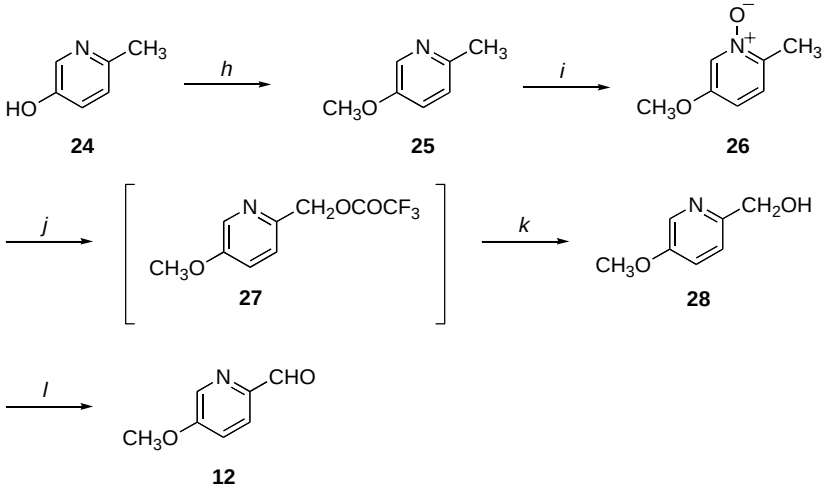
**Table 1.** Lipophilic property of α-pyridoins

| Compound | log $k_w$ |
|---|---|
| **1** | 3.15 |
| **2** | 3.94 |
| **3** | 5.10 |
| **4** | 3.51 |
| **5** | 4.13 |
| **6** | 4.68 |
| **7** | 3.33 |

**2.1.3. Oxidation potential.** The reducing ability of enediol is related to its electron density. Thus, as an index of the electron density, oxidation potential of α-pyridoins was measured by cyclic voltammetry (Table 2) in a mixture of DMF and 2-(*N*-morpholino)ethanesulfonate (MES) buffer (3:2 (v/v), pH 7.4). **1**, **2**, and **5**–**7** had greater negative potential than AsA. These data indicated that **1**, **2**, and **5**–**7** could be oxidized easily, as their reducing abilities are higher than that of AsA. It is thought that a methyl or a methoxy group at the 5-position of the pyridine ring increased the electron density of enediol due to its electron-donating effects. On the other hand, **3** and **4** had more positive potential than **1**. This result suggested that the electron density on the enediol of **3** and **4** was lower than that of **1** and the methoxy group of **3** worked as an electron-withdrawing group. C-6 of α-pyridoin is in

**Table 2.** DPPH radical scavenging activity and oxidation potential of α-pyridoins

| Compound | DPPH radical scavenging rate $k$ ($10^3$ $M^{-1}$ $s^{-1}$) | Oxidation potential $E_{1/2}$ (mV vs Ag/AgCl) |
|---|---|---|
| **1** | 45.1 | −10 |
| **2** | 94.2 | 40 |
| **3** | 9.37 | 190 |
| **4** | 6.99 | 240 |
| **5** | 130 | 5 |
| **6** | 108 | −20 |
| **7** | 43.7 | 125 |
| AsA | 15.1 | 180 |




**Scheme 5.** Synthesis of aldehyde **12**. Reagents and conditions: (h) NaH, $CH_3I$/DMF, 180 °C, 2 h, 75%; (i) $H_2O_2$/AcOH, 100 °C, 5 h, 35%; (j) $(CF_3CO)_2O/CH_2Cl_2$, 0 °C to rt, 24 h; (k) MeOH, reflux, 5 h; (l) $MnO_2/CH_2Cl_2$, rt, 30 min.

a meta position to the enediol-binding site. Thus, it was thought that the methoxy group showed its electron-withdrawing inductive effect rather than its electron-donating resonance effect, which can be accounted for by Hammett's substituent constant ($\sigma_{m-OCH_3} = 0.115$, $\sigma_{p-OCH_3} = -0.268$).[29]

### 2.2. Antioxidant activity

**2.2.1. DPPH radical scavenging activity and oxidation potential.** DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is the simplest method to measure the ability of antioxidants to quench free radicals.[30] The DPPH radical scavenging activities of α-pyridoins were evaluated by the second-order rate constant with DPPH obtained by pseudo first-order rate constant in the mixture of ethanol and MES buffer (3:2 (v/v), pH 7.4). As shown in Table 2, all α-pyridoins **1**–**7** showed DPPH radical scavenging activity. In particular, the activity of **1** was three times greater than that of AsA, and the activities of **5**, and **6** were 8.6- and 7.2-fold greater than that of AsA, respectively. On the other hand, **3** and **4** showed activity lower than that of AsA.

Figure 4 shows the relation between the oxidation potential and DPPH radical scavenging rate. Though the correlation coefficient was not satisfactory, compounds showing a negative oxidation potential tended to show a higher radical scavenging activity. This result indicated that one-electron transfer reaction step, but not the deprotonation, was the rate-determining step of radical scavenging by α-pyridoins. This kind of correlation is also demonstrated by the reaction of α -tocopherol analogues with peroxyradical species.[31]

**2.2.2. Inhibition of lipid peroxidation.** To investigate the antioxidant activity of biomembrane, **1**–**7** and AsA were evaluated for the inhibition of lipid peroxidation in rat liver microsomes, initiated by cytochrome P450 and *tert*-butyl hydroperoxide.[32] Lipid peroxidation was measured by the formation of thiobarbituric acid reactive substances (TBARS, Fig. 5). The most potent DPPH radical scavengers, **5**, and **6**, inhibited lipid peroxidation effectively in a dose-dependent manner. Similarly, **3** inhibited lipid peroxidation effectively; nevertheless, **3** showed a DPPH radical scavenging activity lower than that of AsA. The high activity of **3** may have been due to its high lipophilicity. On the other hand, **1**, **2**, **4**, **7**, and AsA exerted little inhibition on lipid peroxidation. These results indicated that some modification on the pyridine ring is the important factor in antioxidant activity under the heterogeneous system and they demonstrated that **5** and **6** had excellent antioxidant activity.

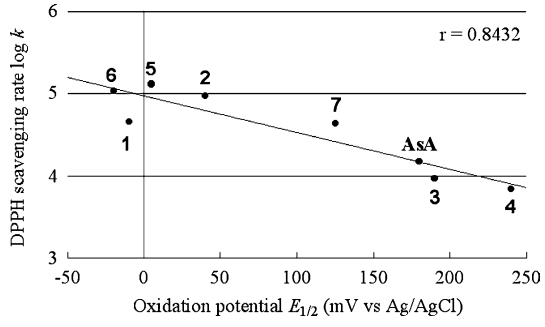



**Figure 4.** Correlation between DPPH scavenging activity and oxidation potential.

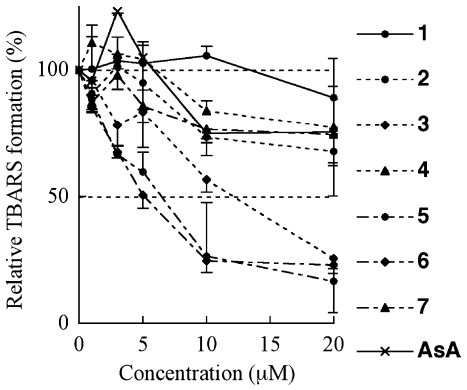



**Figure 5.** Inhibition activity on microsomal lipid peroxidation by *t*-BuOOH. Values are means ± SEM.

## 3. Conclusion

To find novel antioxidative enediols, we have investigated the antioxidant activity of α-pyridoin (**1**) and its synthetic derivatives **2**–**7**. The DPPH radical scavenging activities of **1** and its derivatives **2** and **5**–**7** were more potent than the activity of AsA. In the *tert*-butyl hydroperoxide/rat liver microsome system, **5** and **6** effectively inhibited lipid peroxidation. Therefore, we propose that **5** and **6** are good candidates for the novel antioxidative enediols discussed here.

## 4. Experimental

### 4.1. Preparation of α-pyridoin and its derivatives

**4.1.1. General.** $^1$H NMR spectra (500 MHz) were measured on a JEOL JNM-A500 FT-NMR spectrometer with tetramethylsilane as an internal standard ($\delta = 0.00$) in $CDCl_3$ or DMSO-$d_6$. $^{13}$C NMR spectra (125 MHz) were obtained on the same spectrometer and the chemical shifts were referenced to the signals of $CDCl_3$ ($\delta = 77.0$) or DMSO-$d_6$ ($\delta = 39.5$). Mass spectra were recorded on a JEOL AUTOMASS SUN200 quadrupole mass spectrometer or a JEOL JMS-700 mass spectrometer. Melting points were determined with a Yanagimoto MP-J3 micro-melting point apparatus and are uncorrected. Column chromatography was performed using Merck Silica gel 60. α-Pyridoin (**1**), 2,5-dibromopyridine (**18**), and 6-bromo-3-picoline (**23**) were purchased from Aldrich Chemical Co. 6-Methyl-2-pyridinecarboxaldehyde (**8**) and 2,6-dibromopyridine (**14**) were purchased from Tokyo Kasei Kogyo Co., Ltd. 5-Hydroxy-2-picoline (**24**) was purchased from Acros Organics.

**4.1.2. Preparation of 6,6′-dimethyl-2,2′-pyridoin (2).** Sodium cyanide (196 mg, 4 mmol in $H_2O$, 2 mL) was added to a stirred mixture of 6-methyl-2-pyridinecarboxaldehyde (**8**, 1.0 g, 8.0 mmol) and methanol (5 mL). The mixture was refluxed at 80 °C for 10 min. The resulting orange solid was collected by filtration, washed with methanol and $H_2O$ successively, and dried under reduced pressure to afford **2** (946 mg, 95%). $^1$H NMR (DMSO-$d_6$): $\delta$ 2.55 (s, 6H, –$CH_3$), 7.26 (d, 4H, $J$ = 7.9 Hz, 5 and 5′-H), 7.61 (d, 4H, $J$ = 7.9 Hz, 3 and 3′-H), 7.92 (t, 4H, $J$ = 7.9 Hz, 4 and 4′-H), 13.3 (s, 2H, –OH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 23.36, 116.11, 121.55, 135.01, 138.63, 154.87, 155.08; MS: $m/z$ 242 ($M^+$), 122, 93, 66; mp: 189–191 °C (dec) (pyridine).

**4.1.3. Preparation of 6,6′-dimethoxy-2,2′-pyridoin (3)**

**4.1.3.1. 2-Bromo-6-methoxypyridine (15).** Under $N_2$ atmosphere, sodium methoxide (6.5 mL, 8.4 mmol, 28% in methanol) was added to a mixture of 2,6-dibromopyridine (**14**, 2.0 g, 8.4 mmol) and DMF (33 mL). The mixture was stirred at 80 °C for 2 h. After cooling to ambient temperature, the reaction mixture was poured into $H_2O$ (100 mL) and then extracted with $Et_2O$ (3× 100 mL). The combined organic layer was washed with brine, dried, and concentrated. The crude product was chromatographed on silica-gel column using a mixture of *n*-hexane/AcOEt (30:1) to give 788 mg of **15** as a colorless oil (50%). $^1$H NMR ($CDCl_3$): $\delta$ 3.94 (s, 3H, –$OCH_3$), 6.68 (d, 1H, $J$ = 7.9 Hz, 5-H), 7.05 (d, 1H, $J$ = 7.9 Hz, 3-H), 7.41 (t, 1H, $J$ = 7.9 Hz, 4-H); $^{13}$C NMR ($CDCl_3$): $\delta$ 54.07, 109.40, 120.19, 138.63, 140.32, 163.77.

**4.1.3.2. 6-Methoxy-2-pyridinecarboxaldehyde (9).** Under $N_2$ atmosphere, *n*-BuLi (2.9 mmol, 1.9 mL, 1.6 M in *n*-hexane) was added to a mixture of **15** (536 mg, 2.9 mmol) and anhydrous THF (15 mL) at −78 °C. The reaction mixture was stirred for 30 min. Then DMF (0.25 mL) was added, and the mixture was warmed to ambient temperature within 1 h. The reaction mixture was quenched by adding 5% $NaHCO_3$, and then the whole mixture was extracted with $Et_2O$ (3× 50 mL). The combined organic layer was washed with brine, dried, and concentrated. The crude product was chromatographed on silica-gel column using a mixture of *n*-hexane/AcOEt (20:1) to give 206 mg of **9** as a yellow oil (53%). $^1$H NMR ($CDCl_3$): $\delta$ 4.03 (s, 3H, –$OCH_3$), 6.98 (d, 1H, $J$ = 8.2 Hz, 5-H), 7.05 (d, 1H, $J$ = 7.3 Hz, 3-H), 7.41 (t, 1H, $J$ = 7.6 Hz, 4-H), 9.96 (s, 1H, –CHO); $^{13}$C NMR ($CDCl_3$): $\delta$ 53.64, 115.51, 116.33, 139.06, 150.44, 164.42, 193.12.

**4.1.3.3. 6,6′-Dimethoxy-2,2′-pyridoin (3).** Sodium cyanide (196 mg, 4 mmol in $H_2O$, 2 mL) was added to a stirred mixture of **9** (232 mg, 1.7 mmol) and methanol (2 mL). The mixture was refluxed at 80 °C for 10 min. The resulting yellow solid was collected by filtration, washed with methanol and $H_2O$ successively, and dried under reduced pressure to afford **3** (65 mg, 28%). $^1$H NMR (DMSO-$d_6$): $\delta$ 3.94 (s, 6H, –$OCH_3$), 6.86 (d, 2H, $J$ = 8.2 Hz, 5 and 5′-H), 7.40 (d, 2H, $J$ = 7.6 Hz, 3 and 3′-H), 7.90 (t, 2H, $J$ = 7.9 Hz, 4 and 4′-H), 12.3 (s, 2H, –OH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 53.64, 108.93, 112.00, 134.53, 141.09, 152.45, 161.30; MS: $m/z$ 274 ($M^+$), 259, 231, 138, 109, 94; HRMS calcd for $C_{14}H_{14}N_2O_4$, 274.0954; found: 274.0933 ($M^+$); mp: 183–184 °C (pyridine).

**4.1.4. Preparation of 6,6′-diacetyl-2,2′-pyridoin (4)**

**4.1.4.1. 2-Acetyl-6-bromopyridine (16).** Under $N_2$ atmosphere, *n*-BuLi (10.6 mL, 17 mmol, 1.6 M in *n*-hexane) was added to a mixture of 2,6-dibromopyridine (**14**, 4.0 g, 17 mmol) and anhydrous $Et_2O$ (40 mL) at –78 °C. The reaction mixture was stirred for 30 min. Then, *N*,*N*-dimethylacetamide (2.4 mL, 28 mmol) was added, and the mixture was warmed to ambient temperature within 1 h. The reaction mixture was quenched by adding saturated $NH_4Cl$, and then the whole mixture was extracted with $Et_2O$ (3× 150 mL). The combined organic layer was washed with brine, dried, and concentrated. The crude product was chromatographed on silica-gel column using *n*-hexane/AcOEt (25:1) to give 2.6 g of **16** as a colorless solid (76%). $^1$H NMR ($CDCl_3$): $\delta$ 2.70 (s, 3H, –$COCH_3$), 7.64–7.72 (m, 2H, 4 and 5-H), 7.99 (d, 1H, $J$ = 7.0 Hz, 3-H); $^{13}$C NMR ($CDCl_3$): $\delta$ 25.71, 120.45, 131.75, 139.11, 141.34, 154.30, 198.57; MS: $m/z$ 201 ($M^+$+2), 199 ($M^+$), 173, 171, 158, 156, 131, 129, 106, 76.

**4.1.4.2. 2-Bromo-6-(2-methyl-1,3-dioxlane-2-yl)pyridine (17).** A mixture of **16** (1.5 g, 7.5 mmol), ethylene glycol (0.88 mL, 1.6 mmol), *p*-TsOH–$H_2O$ (428 mg, 2.3 mmol), and toluene (30 mL) was stirred for 4 h at 140 °C with a Dean–Stark apparatus. The reaction mixture was washed with saturated $Na_2CO_3$ and with brine successively, dried, and concentrated. The crude product was chromatographed on silica-gel column using *n*-hexane/AcOEt (12:1) to give 1.45 g of **17** as a colorless oil (79%). $^1$H NMR ($CDCl_3$): $\delta$ 1.72 (s, 3H, $CH_3$), 3.84–3.94 (m, 2H, –$OCH_2$–), 4.04–4.14 (m, 2H, –$OCH_2$–), 7.41 (d, 1H, $J$ = 7.3 Hz, 5-H), 7.48–7.56 (m, 2H, 3 and 4-H); $^{13}$C NMR ($CDCl_3$): $\delta$ 25.11, 65.08, 108.02, 118.21, 127.51, 138.75, 142.04, 162.51; MS: $m/z$ 230 ([$M^+$+2]–$CH_3$), 228 ([$M^+$]–$CH_3$), 214, 212, 202, 200, 186, 184, 173, 171, 158, 156, 87, 76.

**4.1.4.3. 6-(2-Methyl-1,3-dioxolan-2-yl)-2-pyridineacarboxaldehyde (21).** Under argon atmosphere, *n*-BuLi (3.7 mL, 6.0 mmol, 1.6 M in *n*-hexane) was added to a stirred mixture of **17** (1.45 g, 5.9 mmol) and anhydrous $Et_2O$ (45 mL) at −78 °C and stirred for 30 min. Then, DMF (0.5 mL, 7.1 mmol) was added and the whole mixture was stirred at −78 °C to ambient temperature within 1 h. The whole mixture was poured into 5% $NaHCO_3$ and extracted with $Et_2O$ (3× 50 mL). The combined organic layer was washed with brine, dried, and concentrated. The crude product was chromatographed on silica-gel column using *n*-hexane/AcOEt (4:1) to give 625 mg of **21** as a colorless oil (54%). $^1$H NMR ($CDCl_3$): $\delta$ 1.79 (s, 3H, $CH_3$), 3.88–4.02 (m, 2H, –$OCH_2$–), 4.10–4.20 (m, 2H, –$OCH_2$–), 7.80 (d, 1H, $J$ = 7.3 Hz, 5-H), 7.86–7.95 (m, 2H, 3 and 4-H), 10.13 (s, 1H, –CHO); $^{13}$C NMR ($CDCl_3$): $\delta$ 25.18, 65.18, 108.39, 120.56, 123.59, 137.66, 152.65, 161.92, 193.79; MS: $m/z$ 178 ($M^+$–$CH_3$), 150, 134, 107, 87, 78.

**4.1.4.4. 6-Acetyl-2-pyridinecarboxaldehyde (10).** Under $N_2$ atmosphere, a mixture of **21** (625 mg, 3.2 mmol) and HCl (3 M, 20 mL) was stirred at 100 °C for 2 h. After cooling to ambient temperature, the reaction mixture was alkalified with saturated $NaHCO_3$ and extracted with $Et_2O$ (3× 50 mL). The combined organic layer was washed with brine, dried, and concentrated to give 397 mg of **10** as a colorless solid (82%). $^1H$ NMR ($CDCl_3$): $\delta$ 2.81 (s, 3H, $-COCH_3$), 8.03 (t, 1H, $J$ = 7.6 Hz, 4-H), 8.14 (d, 1H, $J$ = 7.6 Hz, 3-H), 8.26 (d, 1H, $J$ = 7.6 Hz, 5-H), 10.13 (s, 1H, –CHO); $^{13}C$ NMR ($CDCl_3$): $\delta$ 25.63, 124.51, 125.49, 138.10, 152.13, 153.82, 192.86, 199.16; MS: *m*/*z* 149 ($M^+$), 121, 107, 79.

**4.1.4.5. 6,6′-Diacetyl-2,2′-pyridoin (4).** Sodium cyanide (196 mg, 4 mmol in $H_2O$, 2 mL) was added to a stirred mixture of **10** (300 mg, 2 mmol) and methanol (3 mL). The mixture was refluxed at 80 °C for 20 min. The resulting yellow solid was collected by filtration, washed with methanol and $H_2O$ successively, and dried under reduced pressure to afford **4** (300 mg, 100%). The $^1H$ NMR spectra of product indicated that the afforded **4** existed as a mixture of *E*-enediol and *Z*-enediol (70% and 30%, respectively). $^1H$ NMR (DMSO-$d_6$): *E*-enediol $\delta$ 2.71 (s, 6H, $-COCH_3$), 8.00 (d, 2H, $J$ = 7.6 Hz, 3 and 3′-H), 8.12 (d, 2H, $J$ = 7.6 Hz, 5 and 5′-H), 8.26 (t, 2H, $J$ = 7.9 Hz, 4 and 4′-H), 12.9 (s, 2H, –OH), *Z*-enediol $\delta$ 2.14 (s, 6H, $-COCH_3$), 8.19 (d, 2H, $J$ = 7.6 Hz, 3 and 3′-H), 8.35 (t, 2H, $J$ = 7.9 Hz, 4 and 4′-H), 8.45 (t, 2H, $J$ = 7.9 Hz, 5 and 5′-H), 10.2 (s, 2H, –OH); $^{13}C$ NMR (DMSO-$d_6$): as a mixture of *E*-enediol and *Z*-enediol $\delta$ 24.78, 26.08, 120.46, 122.83, 124.81, 125.62, 135.14, 139.93, 139.96, 149.42, 150.02, 150.19, 152.26, 154.63, 196.22, 197.74; MS: *m*/*z* 298 ($M^+$), 269, 255, 225, 150, 122, 121, 106, 93, 78; HRMS calcd for $C_{16}H_{14}N_2O_4$, 298.0954; found: 298.0966 ($M^+$); mp: 183–185 °C (dec) (pyridine).

### 4.1.5. Preparation of 5,5′-dimethyl-2,2′-pyridoin (5)

**4.1.5.1. 5-Methyl-2-pyridinecarboxaldehyde (11).** Under argon atmosphere, *n*-BuLi (5.4 mL, 8.7 mmol, 1.6 M in *n*-hexane) was added to a stirred mixture of 6-bromo-3-picoline (**23**, 1.5 g, 8.7 mmol) and anhydrous $Et_2O$ (35 mL) at –78 °C and stirred for 30 min. Then DMF (0.8 mL, 11 mmol) was added to the mixture, which was stirred at –78 °C to ambient temperature within 1 h. The whole mixture was poured into 5% $K_2CO_3$ and extracted with $Et_2O$ (4× 50 mL). The combined organic layer was washed with brine, dried, and concentrated. The crude product was chromatographed on silica-gel column using *n*-hexane/AcOEt (7:1 to 4:1) to give 324 mg of **11** as a yellow oil (31%). $^1H$ NMR ($CDCl_3$): $\delta$ 2.45 (s, 3H, $-CH_3$), 7.68 (d, 1H, $J$ = 7.6 Hz, 4-H), 7.88 (d, 2H, $J$ = 7.9 Hz 3-H), 8.62 (s, 1H, 6-H), 10.05 (s, 1H, –CHO); $^{13}C$ NMR ($CDCl_3$): $\delta$ 18.81, 121.43, 137.34, 138.61, 150.65, 150.74, 193.17; MS: *m*/*z* 121 ($M^+$), 93, 65.

**4.1.5.2. 5,5′-Dimethyl-2,2′-pyridoin (5).** Sodium cyanide (196 mg, 4 mmol in $H_2O$, 2 mL) was added to a mixture of **11** (324 mg, 2.7 mmol) and methanol (3 mL). The mixture was refluxed at 80 °C for 20 min. The resulting orange solid was collected by filtration, washed with methanol and $H_2O$ successively, and dried under reduced pressure to afford **5** (238 mg, 73%). $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.36 (s, 6H, $-CH_3$), 7.69 (d, 2H, $J$ = 8.2 Hz, 3 and 3′-H), 7.85 (d, 2H, $J$ = 7.9 Hz, 4 and 4′-H), 8.44 (s, 2H, 6 and 6′-H), 12.9 (s, 2H, –OH); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 17.83, 118.45, 131.35, 134.43, 138.82, 146.13, 153.00; MS: *m*/*z* 242 ($M^+$), 183, 138, 122, 92, 65; HRMS calcd for $C_{14}H_{14}N_2O_2$, 242.1055; found: 242.1043 ($M^+$); mp: 203–205 °C (pyridine).

### 4.1.6. Preparation of 5,5′-dimethoxy-2,2′-pyridoin (6)

**4.1.6.1. 5-Methoxy-2-picoline (25).** Under argon atmosphere, 5-hydroxy-2-picoline (**24**, 5.0 g in 60 mL DMF) was added to a stirred mixture of sodium hydride (1.8 g, 60% dispersed in oil, 45 mmol) and DMF (60 mL), and the mixture was heated to 180 °C for 2 h. After cooling to ambient temperature, methyl iodide (2.9 mL, 46 mmol) was added to the mixture, which was stirred for 4 h. The reaction mixture was diluted with isopropyl alcohol (20 mL) and $H_2O$ (20 mL), and then was extracted with AcOEt (3× 100 mL). The combined organic layer was washed with saturated $NaHCO_3$ and brine successively, dried, and concentrated. The crude product was chromatographed on silica-gel column using *n*-hexane/AcOEt (3:2) to give 4.2 g of **25** as a yellow oil (75%). $^1H$ NMR ($CDCl_3$): $\delta$ 2.49 (s, 3H, $-CH_3$), 3.83 (s, 3H, $-OCH_3$), 7.06 (d, 1H, $J$ = 8.5 Hz, 3-H), 7.11 (dd, 1H, $J$ = 8.5 Hz, 2.4 Hz, 4-H), 8.19 (d, 1H, $J$ = 2.4 Hz, 6-H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 23.25, 55.54, 121.31, 123.20, 136.13, 150.30, 153.63.

**4.1.6.2. 5-Methoxy-2-picoline-*N*-Oxide (26).** Thirty percent of aqueous $H_2O_2$ (1.8 mL, 16 mmol) was added to a stirred mixture of **25** (2.0 g, 16 mmol) and AcOH (12 mL). After being stirred for 5 h at 100 °C, the reaction mixture was alkalified with 10% NaOH and extracted with $CHCl_3$ (3× 100 mL). The combined organic layer was dried and concentrated to give 787 mg of **26** as a yellow semi-solid (35%). No further purification was performed. $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.27 (s, 3H, $-CH_3$), 3.79 (s, 3H, $-OCH_3$), 6.97 (dd, 1H, $J$ = 8.5 Hz, 1.5 Hz, 4-H), 7.36 (d, 1H, 3-H), 8.08 (d, 1H, $J$ = 1.5 Hz, 6-H); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 16.28, 56.14, 112.37, 125.94, 126.63, 140.49, 155.91.

**4.1.6.3. 5-Methoxy-2-pyridylmetanol (28).** Trifluoroacetic anhydride (4.5 mL, 32 mmol) was added to a stirred mixture of **26** (894 mg, 6.4 mmol) and $CH_2Cl_2$ (30 mL) in two portions at 0 °C. After being stirred for 30 min, the mixture was stirred at ambient temperature for 24 h. Then, the whole mixture was diluted with methanol (12 mL), refluxed for 5 h, alkalified with saturated $NaHCO_3$, and extracted with $CH_2Cl_2$ (3× 50 mL). The combined organic layer was washed with brine, dried, and concentrated. The crude product was chromatographed on silica-gel column using AcOEt/methanol (20:1) to give 573 mg of **28** as a yellow paste (64%). $^1H$ NMR ($CDCl_3$): $\delta$ 3.45 (br s, 1H, $-CH_2-OH$) 3.87 (s, 3H, $-OCH_3$), 4.70 (s, 2H, $-CH_2-OH$), 7.18 (d, 1H, $J$ = 8.5 Hz, 3-H), 7.22 (dd, 1H, $J$ = 8.5 Hz, 2.4 Hz, 4-H), 8.25 (d, 1H, $J$ = 2.4 Hz, 6-H); $^{13}C$ NMR

($CDCl_3$): $\delta$ 55.16, 63.79, 121.11, 121.24, 135.40, 152.08, 154.35; MS: *m*/*z* 139 ($M^+$), 138, 124, 110, 95, 78, 68.

**4.1.6.4. 5-Methoxy-2-pyridinecarboxaldehyde (12).** Under argon atmosphere, $MnO_2$ (2.1 g, 24 mmol) was added to a stirred mixture of **28** (258 mg 1.9 mmol) and $CH_2Cl_2$ (20 mL). After being stirred for 30 min at ambient temperature, the whole mixture was filtered through a Celite pad, and the filtrate was concentrated. The crude product was chromatographed on silica-gel column using *n*-hexane/AcOEt (3:1) to give 153 mg of **12** as a colorless solid (60%). $^1H$ NMR ($CDCl_3$): $\delta$ 3.96 (s, 3H, –$OCH_3$), 7.31 (dd, 1H, $J$ = 8.5 Hz, 2.4 Hz, 4-H), 7.97 (d, 1H, $J$ = 8.5 Hz, 3-H), 8.44 (d, 1H, $J$ = 2.4 Hz, 6-H), 10.00 (s, 1H, –CHO); $^{13}C$ NMR ($CDCl_3$): $\delta$ 55.92, 119.97, 123.33, 138.53, 146.39, 158.98, 192.05; MS: *m*/*z* 137 ($M^+$), 109, 93, 79, 66

**4.1.6.5. 5,5′-Dimethoxy-2,2′-pyridoin (6).** Sodium cyanide (196 mg, 4 mmol in $H_2O$, 2 mL) was added to a stirred mixture of **12** (300 mg, 2.2 mmol) and methanol (3 mL). The mixture was refluxed for 20 min at 80 °C. The resulting yellow solid was collected by filtration, washed with methanol and $H_2O$ successively, and dried under reduced pressure to afford **6** (190 mg, 63%). $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.90 (s, 6H, –$OCH_3$), 7.64 (dd, 2H, $J$ = 8.8 Hz, 2.5 Hz, 4 and 4′-H), 7.72 (d, 2H, $J$ = 8.8 Hz, 3 and 3′-H), 8.33 (d, 2H, $J$ = 2.5 Hz, 6 and 6′-H), 12.5 (s, 2H, –OH); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 55.89, 119.63, 123.40, 133.13, 133.52, 148.46, 153.69; MS: *m*/*z* 274 ($M^+$), 259, 215, 138, 136, 109, 108, 93; HRMS calcd for $C_{14}H_{14}N_2O_4$, 274.0954; found: 274.0918 ($M^+$); mp: 158–159 °C (pyridine).

### 4.1.7. Preparation of 5,5′-diacetyl-2,2′-pyridoin (7)

**4.1.7.1. 5-Acetyl-2-bromopyridine (19).** Under $N_2$ atmosphere, *n*-BuLi (7.9 mL, 13 mmol, 1.6 M in *n*-hexane) was added to a mixture of 2,5-dibromopyridine (**18**, 3.0 g, 13 mmol) and anhydrous $Et_2O$ (50 mL) at −78 °C. The reaction mixture was stirred for 30 min and then *N*,*N*-dimethylacetamide (1.3 mL, 15 mmol) was added and stirred at –78 °C to ambient temperature within 1 h. The whole mixture was poured into saturated $NH_4Cl$ and then extracted with $Et_2O$ (3× 150 mL). The combined organic layer was washed with brine, dried, and concentrated. The crude product was chromatographed on silica-gel column using *n*-hexane/AcOEt (7.5:1) to give 1.3 g of **19** as a colorless solid (50%). $^1H$ NMR ($CDCl_3$): $\delta$ 2.63 (s, 3H, –$CH_3$), 7.62 (d, 1H, $J$ = 8.5 Hz, 3-H), 8.08 (dd, 1H, $J$ = 8,5 Hz, 2.4 Hz, 4-H), 8.90 (d, 1H, $J$ = 2.4 Hz, 6-H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 26.72, 128.43, 131.45, 137.65, 146.97, 150.40, 195.57; MS: *m*/*z* 201 ($M^+$+2), 199 ($M^+$), 158, 156, 131, 129, 76.

**4.1.7.2. 2-Bromo-5-(2-methyl-1.3-dioxolan-2-yl)pyridine (20).** A mixture of **19** (1.3 g, 6.3 mmol), ethylene glycol (1.1 mL, 20 mmol), and *p*-TsOH–$H_2O$ (364 mg, 1.9 mmol), and toluene (25 mL) was stirred for 6 h at 140 °C with a Dean–Stark apparatus. The reaction mixture was washed with saturated $Na_2CO_3$ and brine successively and concentrated. The crude product was chromatographed on silica-gel column using *n*-hexane/AcOEt (7:1) to give 1.4 g of **20** as a colorless solid (87%). $^1H$ NMR ($CDCl_3$): $\delta$ 1.65 (s, 3H, –$CH_3$), 3.74–3.81 (m, 2H, –$OCH_2$–), 4.03–4.10 (m, 2H, –$OCH_2$–), 7.46 (d, 1H, $J$ = 7.9 Hz, 3-H), 7.64 (dd, 2H, $J$ = 8.2 Hz, 2.1 Hz, 4-H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 27.52, 64.65, 107.25, 127.57, 135.97, 138.25, 141.62, 147.74; MS: *m*/*z* 230 ([$M^+$+2]–$CH_3$), 228 ([$M^+$]–$CH_3$), 186, 184, 158, 156, 149, 134, 104, 87, 77.

**4.1.7.3. 5-(2-Methyl-1,3-dioxolan-2-yl)-2-pyridinecarboxaldehyde (22).** Under argon atmosphere, *n*-BuLi (1.6 M in *n*-hexane, 3.5 mL, 5.7 mmol) was added to a stirred mixture of **20** (1.4 g, 5.7 mmol) and anhydrous $Et_2O$ (30 mL) at −78 °C and stirred for 30 min. Then, DMF (0.5 mL, 15 mmol) was added to the mixture and stirred at –78 °C to ambient temperature within 1 h. The reaction mixture was poured into 5% $NaHCO_3$ and extracted with $Et_2O$ (3× 50 mL). The combined organic layer was washed with brine, dried, and concentrated. The crude product was chromatographed on silica-gel column using *n*-hexane/AcOEt (6:1) to give 568 mg of **22** as a yellow oil (53%). $^1H$ NMR ($CDCl_3$): $\delta$ 1.69 (s, 3H, –$CH_3$), 3.77–3.83 (m, 2H, –$OCH_2$–), 4.07–4.14 (m, 2H, –$OCH_2$–), 7.93–7.98 (m, 2H, 3 and 4-H), 8.91 (s, 1H, 6-H), 10.09 (s, 1H, –CHO); $^{13}C$ NMR ($CDCl_3$) $\delta$ 27.55, 64.83, 107.42, 121.30, 134.23, 143.43, 147.85, 152.45, 193.06.

**4.1.7.4. 5-Acetyl-2-pyridinecarboxaldehyde (13).** Under $N_2$ atmosphere, a mixture of **22** (314 mg, 1.6 mmol) and HCl (3 M, 20 mL) was stirred at 100 °C for 10 min. After cooling to ambient temperature, the reaction mixture was alkalified with saturated $NaHCO_3$ and extracted with $Et_2O$ (3× 50 mL). The combined organic layers were washed with brine, dried, and concentrated to give 117 mg of **13** as a yellow solid (48%). $^1H$ NMR ($CDCl_3$): $\delta$ 2.70 (s, 3H, –$COCH_3$), 8.07 (d, 1H, $J$ = 8.2 Hz, 3-H), 8.39 (d, 1H, $J$ = 8.2 Hz, 4-H), 9.31 (s, 1H, 6-H), 10.15 (s, 1H, –CHO); $^{13}C$ NMR ($CDCl_3$): $\delta$ 27.07, 121.42, 134.76, 136.74, 150.13, 154.85, 192.51, 195.95; MS: *m*/*z* 149 ($M^+$), 134, 121, 106, 78.

**4.1.7.5. 5,5′-Diacetyl-2,2′-pyridoin (7).** Sodium cyanide (98 mg, 2 mmol in $H_2O$, 1 mL) was added to a mixture of **13** (117 mg, 0.78 mmol) and methanol (3 mL). The mixture was refluxed for 20 min at 80 °C, and the resulting red-orange solid was collected by filtration, washed with methanol and $H_2O$ successively, and dried under reduced pressure to afford **7** (15 mg, 13%). $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.67 (s, 6H, –$COCH_3$), 7.97 (d, 2H, $J$ = 8.4 Hz, 3 and 3′-H), 8.51 (d, 2H, $J$ = 8.4 Hz, 4 and 4′-H), 9.20 (s, 2H, 6 and 6′-H), 13.3 (s, 2H, –OH); $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 26.87, 119.26, 130.11, 136.78, 137.64, 147.21, 157.87, 195.98; MS: *m*/*z* 298 ($M^+$), 296, 269, 255, 225, 212, 198, 150, 121, 106, 78; HRMS calcd for $C_{16}H_{14}N_2O_4$, 298.0954; found: 298.0912 ($M^+$); mp: 198–201 °C (dec) (pyridine).

## 4.2. Measurement of log $k_w$

The measurement procedure is a modification of the method of Tsantili-Kakoulidou et al.[26] Sample solution contained **1**–**7** (3 mM) and acetone (0.1 μM) as an unretained substance in $CH_3CN$. The mobile phase was

made up volumetrically using 41.8 mM MES buffer (pH 7.4), instead of a MOPS buffer, and different proportions of methanol in the range of 85–65%. Two microliters of the sample was injected on an octadecyl-silica-gel-prepacked column Prodigy 5 ODS (4.6 * 150 mm, Phenomenex Inc.), followed by development with a flow rate of 1.2 mL/min. **1**–**7** and acetone were detected with a Shimadzu SPD-10A UV–vis detector at 254 nm. Isocratic capacity factors, defined as $\log[(t_r - t_0)/t_0]$, were linearly extrapolated to 0% methanol to yield $\log k_w$ ($t_r$: retention time of **1**–**7**, $t_0$: retention time of acetone).

### 4.3. Oxidation potential

Oxidation potential was measured by a Hokuto HA-301 electrochemical apparatus equipped with glassy carbon as a working electrode, platinum as a counter electrode, and Ag/AgCl as a reference electrode. The sample mixture contained **1**–**7** or AsA (0.5 mM) and tetra-*n*-butylammonium hexafluorophosphate (60 mM) as a supporting electrolyte in the mixture of DMF and MES buffer, pH 7.4 (3:2, v/v), and it was scanned from −300 to 900 mV at 50 mV/s to gain a voltammogram.

### 4.4. DPPH radical scavenging activity

The measurement procedure is a modification of the method of Yamaji et al.[11] Sample **1**–**7** in DMSO solution (1 μM) was added to DPPH (16.7–50 μM) in a mixture of MES buffer (pH 7.4) and ethanol (3:2, v/v) at 25 °C. The decrease in absorbance at 517 nm was recorded on a JASCO V-560 UV–vis spectrometer for 60 s to gain the pseudo-first order reaction rate constant. The resulting pseudo first-order reaction rate constant was converted to a second-order reaction rate constant by plotting against the initial DPPH concentration.

### 4.5. Inhibition of lipid peroxidation

Rat liver microsomes were prepared from a phenobarbital-treated Wistar rat, as previously described.[33] The incubation mixtures contained microsomes (0.56 mg protein) and the indicated amount of sample in a mixture of 680 μL of 0.1 M sodium phosphate buffer (pH 7.4) and 300 μL ethanol. Peroxidation was initiated by the addition of *tert*-butyl hydroperoxide (1 mM) and continued for 15 min at 37 °C. The peroxidation was terminated by an addition of 100 mL of 2,6-di-*tert*-butyl-*p*-cresol in ethanol (2%). The solution was then mixed with 2.0 mL of 15% trichloroacetic acid and 0.375% thiobarbituric acid in 0.25 M HCl. After incubation at 80 °C for 15 min, the precipitate was removed by centrifugation (3000 rpm, 20 °C, 20 min), and the difference between the absorbance at 535 nm and 600 nm was measured to gain a relative TBARS formation.[34]

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