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Enantioselective synthesis and antioxidant activity of 3,4,5-substituted piperidine derivatives

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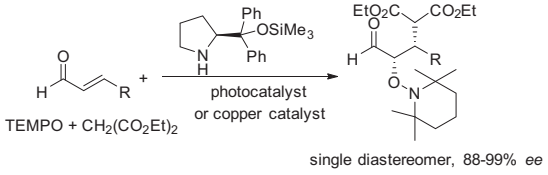
ABSTRACT

In this study, 3,4,5-trisubstituted piperidines were synthesized enantioselectively, and their antioxidant activity was evaluated. The 3,4,5-trisubstituted piperidines containing TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) and a spatially proximal hydroxy group showed good antioxidant activity. Some of these compounds showed IC_{50} values in a nanomolar range, comparable to that of TEMPO. Probably the TEMPO generated from the homolysis of the C—ON bond of 3,4,5-trisubstituted piperidines functions as a radical-scavenging entity, and the hydroxy group of piperidines has a synergistic effect to the antioxidant activity.

Reactive oxygen species (ROS) encompass both the oxygen radicals such as superoxide anion radicals ($O_2^{\cdot-}$) and hydroxyl radicals (·OH), and some nonradical derivatives of oxygen such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$).[1] ROS are generated during the routine metabolic processes of normal cells such as energy-generating catabolism or external stimuli such as radiation. Although ROS play some beneficial roles in diverse physiological processes, e.g., bactericidal action,[2] detoxification,[3] prostaglandin production and certain cell-signaling processes,[4] they are infamous for their negative roles such as cellular damage by inducing lipid and protein peroxidation and damage of nucleic acids to trigger inflammation and mutations.[5,6] Therefore, if these ROS are not scavenged by antioxidants such as vitamin C and resveratrol,[7] they can cause rapid ageing and various diseases that originate from oxidative stress.[8] Moreover, the use of antioxidants has shown to reduce the accumulation of amyloid-β protein in the mouse Alzheimer's disease (AD) model, resulting in a slow functional decline in clinical studies.[9]

Synthesis of piperidine derivatives has attracted much interest because of their biological activities and the importance of piperidines in natural product synthesis.[10–14] Depending on the regio- and stereochemistry of substituents, different synthetic routes to control the regio- and stereoselectivity have been proposed.[15–18] For example, lithiation of piperidines renders the substitution at the 2-position, and the carbonyl ene and Prins cyclizations afford the corresponding 3,4-disubstituted piperidines.[19–24] Although numerous reactions of piperidines including enantioselective reactions have been reported,[25–33] the regio- and stereoselective reactions can still be improved, affording diverse pharmaceuticals containing piperidine units. Recently, we have reported a highly enantioselective and diastereoselective synthesis of α,β-substituted aldehydes in the presence of transition-metal and chiral cyclic amine catalysts (Scheme 1).[34–36]

In this study, we report the stereoselective synthesis of various 3,4,5-trisubstituted piperidines and their antioxidant activity. It would be interesting to study the antioxidant activity of 3,4,5-substituted piperidines containing TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) as a substituent. To the best of our knowledge, this is the first synthesis of nonradical TEMPO-bearing



Scheme 1. Enantioselective reactions for the synthesis of α,β-substituted aldehydes.

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piperidines that exhibit antioxidant activity, although the radical-scavenging activity of TEMPO and related compounds has been reported.[37–41] The antioxidant activity of these piperidines was evaluated by the ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) test, a free-radical scavenging assay.

Diethyl 2-((1*R*,2*S*)-3-oxo-1-phenyl-2-(2,2,6,6-tetramethylpiperidin-1-yloxy)propyl)malonate **1a** was synthesized using (*S*)-2-(diphenyl(trimethylsilyloxy)methyl)pyrrolidine (20 mol %) and CuCl (10 mol%) with 99% enantiomeric excess (ee) in a single diastereomeric form. Following the Jørgensen's protocol for cyclization,[42] **1a** was subjected to the reductive amination conditions involving benzyl amine and sodium cyanoborohydride, affording (3*S*,4*R*,5*S*)-ethyl 1-benzyl-2-oxo-4-phenyl-5-(2,2,6,6-tetramethylpiperidin-1-yloxy)piperidine-3-carboxylate **1b** in 92% yield (Scheme 2). The subsequent reduction of **1b** using lithium aluminum hydride (LAH) afforded ((3*S*,4*R*,5*S*)-1-benzyl-4-phenyl-5-(2,2,6,6-tetramethylpiperidin-1-yloxy)piperidin-3-yl) methanol **1c** in 57% yield. To cleave the O—N bond of **1c**, zinc dust was used, affording (3*S*,4*R*,5*S*)-1-benzyl-5-(hydroxymethyl)-4-phenyl-piperidin-3-ol **1d** in 80% yield. (3*S*,4*R*,5*S*)-Ethyl 1-benzyl-5-hydroxy-2-oxo-4-phenylpiperidine-3-carboxylate **1e** was synthesized from **1b** via O—N bond cleavage in 34% yield. The fluoro-, and methyl-substituted compounds were subjected to the same conditions, affording diverse piperidine derivatives in moderate-to-good yield as shown in Scheme 2.

The IC_{50} values of compounds **1b**–**3e**, ascorbic acid, quercetin, and TEMPO obtained from ABTS assay are shown in Table 1. The majority of **b** and **c** series compounds (**1b**–**3b** and **1c**–**3c**) showed superior antioxidant activity than ascorbic acid, one of the reference compounds. In contrast, most of the **d** and **e** series compounds except **2d**, did not show significant activity. In **b** and **c** series, there is a C—ON connected TEMPO group. Therefore, the radical-scavenging activity of **b** and **c** series compounds can be attributed to the in situ generation of TEMPO radical by the homolytic cleavage of C—ON bond during the ABTS assay. Although the homolysis of C—ON bond occurs at 130 °C affording TEMPO radicals,[43,44] in this study, the TEMPO radical may have been produced by homolytic cleavage at room temperature to some extent. Previously reported TEMPO N—O· radical intact compounds showed radical-scavenging activity.[45,46] However, the originally nonradical **c** series compounds showed a similar antioxidant activity with TEMPO, indicating the release of TEMPO radicals from these compounds. Based on the results of previous studies on the cleavage of CO—N versus C—ON bonds of simple alkoxyamines, C—ON homolysis is usually easier than CO—N homolysis, as shown by bond-dissociation energies (BDEs).[47–50] Therefore, the observed radical-scavenging activity can be attributed to the dissociation of TEMPO radicals from nonradical **b** and **c** series compounds.

By comparing the antioxidant activity between **b** and **c** series compounds, clearly **c** series compounds possessing both a hydroxy group and TEMPO showed better IC_{50} values (60–95 nM), much better than that of quercetin, and comparable to that of TEMPO (26 nM). To the best of our knowledge, the IC_{50} values of **c** series compounds are the best values reported for nonradical compounds. Compared to **b** series piperidines, **c** series piperidines have a hydroxy group in close proximity to the TEMPO group; this may be responsible for the higher activity of **c** series piperidines than **b** series piperidines. The presence of a hydroxy group near TEMPO may accelerate the homolytic cleavage of C—ON bond, generating TEMPO radicals.

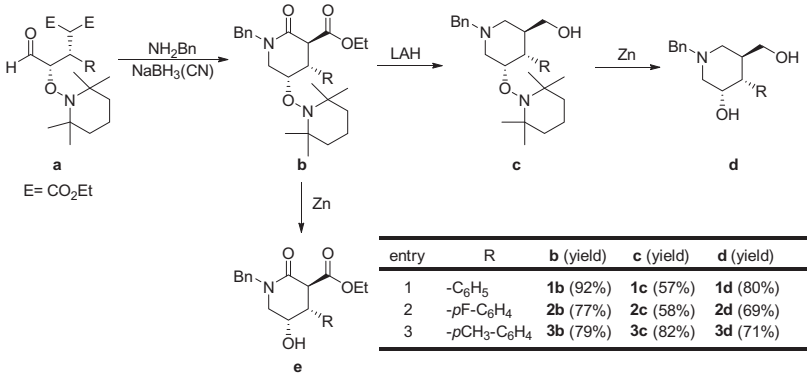
Table 1
IC_{50} values of compounds **1b**–**3e**, ascorbic acid, quercetin, and TEMPO by ABTS assay[a]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1b	12.25 ± 2.499	**3b**	98.35 ± 5.687
1c	0.093 ± 0.008	**3c**	0.090 ± 0.013
1d	>100	**3d**	85.34 ± 7.517
1e	>100	**3e**	>100
2b	15.93 ± 2.258	Ascorbic acid	25.63 ± 0.838
2c	0.095 ± 0.003	Quercetin	5.57 ± 0.365
2d	9.61 ± 0.507	TEMPO	0.026 ± 0.005
2e	>100		

[a] The assay were performed in triplicate.

To confirm the importance of TEMPO for the antioxidant activity, compounds **1f** and **1g** without TEMPO (Fig. 1) were synthesized, and their antioxidant activity was evaluated. As expected, these compounds did not show significant antioxidant activity (Table 2). Thus, regardless of other substituents such as an ester or a hydroxy group, the compounds without TEMPO do not show antioxidant activity. Because the dissociation of TEMPO from **1b** and **1c** could form **1f** and **1g**, respectively, during the assay involving C—ON bond cleavage, the ABTS assay mixtures of **1b** and **1c** were analyzed. Compounds **1f** and **1g** were not detected by NMR and GC–MS analysis. Presumably, after the homolysis of **1b** and **1c**, the resulting radical species are degraded.

Because **c** series compounds bearing a hydroxy group showed much better antioxidant activity than the corresponding **b** series compounds bearing an ethyl ester group, the role of the hydroxy group in influencing the antioxidant activity was investigated. Compound **1h** in which the hydroxy group of **1c** was protected by an acetyl group (Fig. 1) was synthesized, and the antioxidant activity was evaluated. Although this compound showed some activity, it was less active than **1c** in terms of the IC_{50} values (Table 2). Therefore, it can be concluded that the hydroxy group enhances the antioxidant activity by assisting the homolytic cleavage of the C—ON bond generating TEMPO radicals, the major contributor to the antioxidant activity. The actual radical-scavenging entity can be attributed to the TEMPO dissociated from the parent compounds.



entry	R	**b** (yield)	**c** (yield)	**d** (yield)	**e** (yield)
1	-C_6H_5	**1b** (92%)	**1c** (57%)	**1d** (80%)	**1e** (34%)
2	-*p*F-C_6H_4	**2b** (77%)	**2c** (58%)	**2d** (69%)	**2e** (61%)
3	-*p*CH_3-C_6H_4	**3b** (79%)	**3c** (82%)	**3d** (71%)	**3e** (55%)

Scheme 2. Synthesis of 3,4,5-trisubstituted piperidine derivatives.

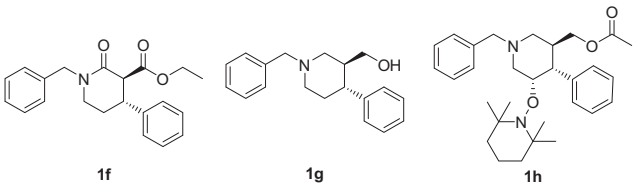


Figure 1. Structures of **1f**, **1g** and **1h** used for control experiments.

Table 2
IC_{50} values of compounds **1f**, **1g**, and **1h** by ABTS assay[a]

Compound	**1f**	**1g**	**1h**
IC_{50} (μM)	>100	>100	0.573 ± 0.123

[a] The assay were performed in triplicate.

In conclusion, novel 3,4,5-trisubstituted piperidines were successfully synthesized enantioselectively from enantiomerically enriched α,β-substituted aldehydes, and their antioxidant activity was evaluated. Among these compounds, those containing both TEMPO and a hydroxy group showed the best IC_{50} values, comparable to that of TEMPO. This confirms that the hydroxy group-assisted homolysis of C—ON bond accelerates the release of TEMPO. These results may pave the way to develop nonradical antioxidant moieties.

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Supplementary data

Supplementary data (experimental procedures, characterization data and antioxidant assay data for all compounds, as well as copies of ^{1}H and ^{13}C NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.092.

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