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5-Aryl-imidazolin-2-ones as a scaffold for potent antioxidant and memory-improving activity

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Abstract—A series of 5-phenyl-substituted-*N*-alkyl-imidazolin-2-ones with potent radical-scavenging activity and lipid peroxidation inhibitory activity was synthesized. Many of the compounds showed memory-improving effect in animal models independent of the inhibitory activity on lipid peroxidation.

Free radical species are known to play important roles in maintaining biological systems such as mitochondria, signal transductions, and the immune system. On the other hand chronic overproduction of free radical species and the resulting oxidative/nitrative stress is generally considered to be one of the major contributors to the pathophysiological processes that lead to irreversible cell damage.[1] Free radical production induces the formation of highly reactive oxygen species which readily react with many biological substrates, particularly with the amines and polyunsaturated fatty acids which are the major constituents of the lipid bilayer of the highly vulnerable brain cell membrane. Chronic oxidative stress consequently induces progressive irreversible damage leading to impairment of the normal physiological function of the central nervous system.[2–4] Moreover, oxidative/nitrative stress seems to play a pivotal role in a variety of central nervous system diseases including Alzheimer's disease,[5–12] Parkinson's disease,[9,13–18] amyotrophic lateral sclerosis,[19,20] and stroke.[18,21–23] Free radical-scavenging compounds are therefore looked to as the source of new tools for the treatment of these illnesses, whose prevalence is increasing worldwide.

For many years, our group has been researching free radical-scavenging compounds, one of which, edaravone (**1**, MCI-186) (Fig. 1), shows potent effects against the deterioration caused by edema and infarction in a rat model of cerebral ischemia.[24–26] Edaravone has also proven very effective in the treatment of cerebral ischemia in humans[27] and has been marketed in Japan since 2001 as Radicut™.

The major chemical feature of edaravone is the presence of an aromatic phenol-like hydroxyl group, which in turn is due to the presence of a keto-enol tautomerism

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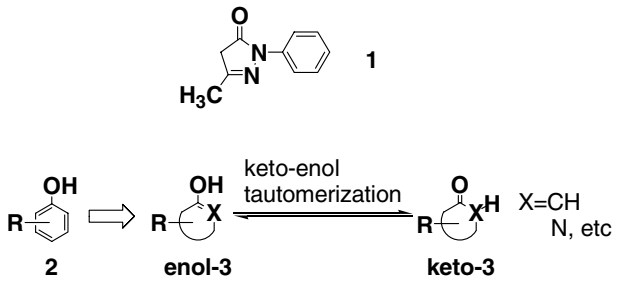
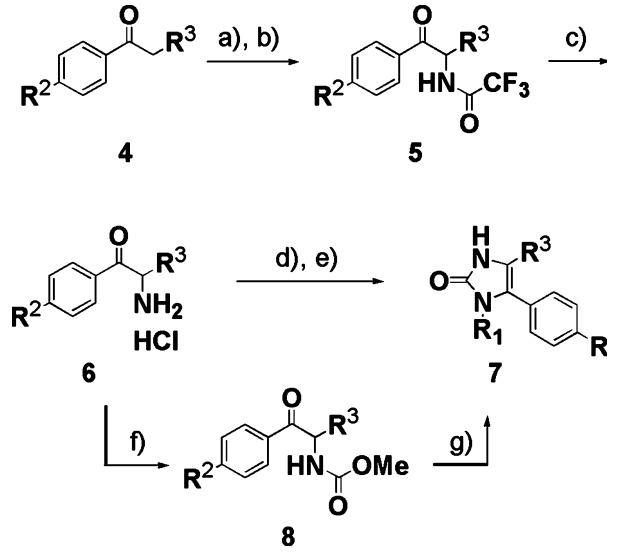


Figure 1. Structure of edaravone **1** and design of phenol-like compound.

(Fig. 1).[28] We predict that this phenol-like function will show good scavenging and antioxidant properties accompanied by a lower intrinsic toxicity than compounds containing pure phenol.

With this aim in mind, we reinvestigated several heteroaromatic scaffolds in which keto-enol tautomerism gave a hydroxy function and synthesized 5-aryl-imidazolin-2-one compounds which showed potent lipid peroxidation inhibitory activity and improvement of memory impairment combined with low toxicity in an animal model.[29]

1,4-Disubstituted-5-aryl-imidazolin-2-ones **7** were prepared from the 2-amino-1-arylketone **6** (Scheme 1). The reaction of bromine with the aryl ketones **4** gave the corresponding 2-bromoketones which were converted to the N-protected aminoketones **5** with trifluoroacetamide under basic conditions. The hydrochloride salt of the aminoketones **6** was obtained by removal of the trifluoroacetyl group by hydrolysis with concentrated hydrochloric acid. The aminoketones **6** were treated with the corresponding isocyanates and intramolecular cyclization of the resulting urea by treatment with trifluoroacetic acid afforded the desired imidazolin-2-ones **7**. Compound **37** (Table 2) was obtained by reaction of the corresponding aminoketone with methyl chloroformate followed by reaction with methylamine and successive cyclization.



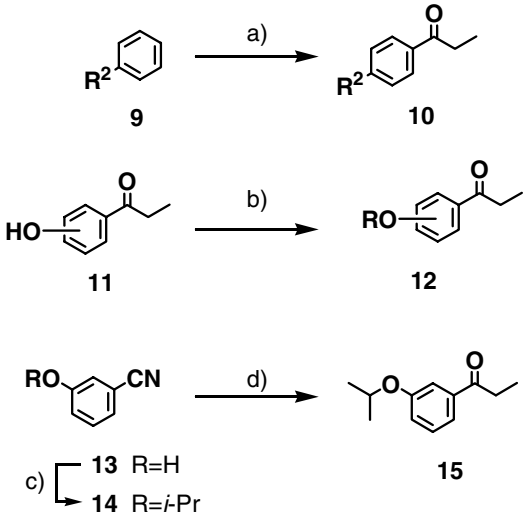
Scheme 1. Reagents and conditions: (a) Br_2, $CHCl_3$ or CH_2Cl_2 or AcOH, 0 °C to room temperature; (b) CF_3CONH_2, K_2CO_3, acetone, reflux; (c) conc. HCl, EtOH, reflux; (d) R_1NCO, Et_3N, acetone, 0 °C; (e) CF_3COOH; (f) ClCOOMe, Et_3N, acetone, 0 °C; (g) $MeNH_2$ HCl, DMF, 170 °C.

The arylketones **10** with alkyl, aralkyl, and aryl groups were prepared by Friedel-Crafts acylation of the corresponding alkyl, aralkyl, and aryl benzene **9**. The 2′- and 4′-alkoxypropiophenones **12** were obtained by alkylation of the corresponding hydroxypropiophenones **11**. The 3′-isopropoxypropiophenone **15** was obtained by alkylation of the 3′-hydroxybenzonitrile **13** followed by reaction with Grignard reagent (Scheme 2).

Radical-scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured by absorbance of solution (OD) at a wavelength of 516.8 nm. An ethanol solution of the test compound was added to an ethanol solution of 100 μM of DPPH (final concentration of test compound 100 μM), and the optical density was measured. The radical-scavenging activity of the compounds was expressed as a percentage of the optical density of a control using an excess of vitamin C.

The lipid peroxidation inhibitory activity was measured by inhibition of the autooxidation of rat brain homogenate in the presence and absence of the test compound according to a modified version of the thiobarbituric acid test (TBARS, thiobarbituric acid reactive substance) of Masugi and Nakamura.[30] The compound was added to brain homogenate prepared from male Wistar rats and the mixture was incubated at 37 °C for 30 min. The IC_{50} values were calculated by non-linear regression.

The effect of the test compound on cycloheximide-induced amnesia was investigated in a step-down passive avoidance task in male mice (ddy, 5–6 weeks old,



Scheme 2. Reagents and conditions: (a) CH_3CH_2COCl, $AlCl_3$, CH_2Cl_2, 0 °C; (b) RBr or RI, K_2CO_3, acetone or 2-butanone, reflux; (c) *i*-PrI, K_2CO_3, 2-butanone, reflux; (d) EtMgBr, THF then aq HCl.

n = 15 in each group).[31] In brief, 30 min after cycloheximide (120 mg/kg, s.c.) or saline treatment, mice kept in a light room were allowed to move freely into a dark room, in which they were given a foot shock (0.5 mA, 3 s) on a grid floor (acquisition trial). One day later, the mice were given a test compound (0.1–30 mg/kg) or vehicle orally 30 min before a retention trial, in which the latent tendency of each subject to move from the light room to the dark room was measured.

Several 1,4-disubstituted 5-phenylimidazolines were prepared and their lipid peroxidation inhibitory activity was measured (Table 1). The unsubstituted phenyl group (R^2 = H) showed no or only low activity regardless of the substituent at the 1-position (**16**, **21**, and **23**). The introduction of a para substituent, such as a 4-*n*-butyl group on the phenyl group, gave compound **17**, which showed good lipid peroxidation inhibitory activity (Table 1), while a 4-*n*-butoxy group afforded a greater increase in activity than the 4-*n*-butyl group (**17** vs **19**, **24** vs **25**). The methyl group in the R^1 position of the imidazolinone structure could be efficiently replaced by other more lipophilic groups such as *n*-butyl, *n*-hexyl, isopropyl, phenyl, and benzyl (**22**, **24**, **20**, **26**, and **27**, respectively). No significant difference in the inhibitory activity was observed when R^3 was methyl or ethyl (**17** and **18**, respectively). Some of the compounds were tested for radical-quenching ability and **17**, **20**, **25**, and **27** showed good DPPH scavenging activity. These results seemed to indicate that lipophilicity could play a role in increasing inhibitory activity against lipid peroxidation and that methyl and cyclohexyl groups were preferable as the R^1 substituent for good free radical-quenching activity and crystallinity.

To confirm the effects of lipophilicity, we also investigated the change in lipid peroxidation inhibitory activity relative to the position of the substituents in the phenyl moiety.

The effect of the position of the substituents on the phenyl group was verified using the isopropoxy group as the fixed substituent. Both *meta* and *para* positions showed similar activity (**57** and **58**), while *ortho*-substitution gave **56** with decreased inhibitory activity (Table 2). Further optimization of the imidazolinone structure was therefore performed with the *para* position fixed because of easier synthetic access to several diverse substituents.

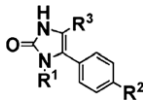
Alkyl groups with various chain lengths were introduced directly and with an oxygen linker. It was clear that, in both the methyl and cyclohexyl series, there were certain alkyl chain lengths suitable for eliciting higher inhibitory activity; shorter alkyl groups such as methyl **28** and methoxy **29**, and longer alkyl groups such as *n*-docecyl **72**, *n*-dodecyloxy **73**, *n*-tetradecyl **48** and **74**, *n*-octadecyl **49** and **75**, *n*-octadecyloxy **50** and **76** had poor activity, while alkyl chains with 4–10 carbon atoms generally showed good inhibitory activity.

The activity did not seem to depend on chain isomerism since no significant difference was observed between linear and branched alkyl groups or linear and ramified alkoxy groups in either the methyl or cyclohexyl series (**17** vs **32** and **33**, **35** vs **36**; **24** vs **59**, and **61** vs **62**). Nor did the change from linear to cyclic structure modify inhibitory activity (**37** vs **39** and **63** vs **65**). The compound group with the highest activity was composed of various aryl, aryloxy, and arylalkoxy substituents (**51**, **52**, **53**, **78**, and **79**) together with substituents with a 4- to 10-carbon alkyl chain moiety.

Furthermore, compounds with a cyclohexyl group as R^1 substituent showed only slightly lower activity than those with a methyl group, which led us to suppose that steric effects did not play an important role in inhibitory activity.

We plotted the IC_{50} of lipid peroxidation inhibitory activity against the calculated *c*log *P* values. The result showed a distribution of the highest inhibitory activity around a *c*log *P* value of 3–9 (Fig. 2). Compounds with *c*log *P* value of below 2.5 or above 9 showed lower inhib-

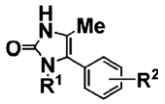
Table 1. SAR of lipid peroxidation inhibitory activity and radical scavenging activity of 1,4-disubstituted-5-aryl-imidazolin-2-one compounds



Compound	R^1	R^2	R^3	Lipid peroxidation inhibitory activity IC_{50} (μM)	DPPH scavenging activity % of control
16	CH_3	H	CH_3	>100	NT
17	CH_3	*n*-C_4H_9	CH_3	10.7	98%
18	CH_3	*n*-C_4H_9	C_2H_5	13.9	NT
19	CH_3	*n*-C_4H_9O	CH_3	5.1	NT
20	*i*-C_3H_7	*n*-C_4H_9	CH_3	10.7	78%
21	*n*-C_4H_9	H	CH_3	>100	NT
22	*n*-C_4H_g	*n*-C_4H_9	CH_3	12.0	NT
23	c-C_6H_{11}	H	CH_3	47.6	NT
24	c-C_6H_{11}	*n*-C_4H_9	CH_3	27.1	NT
25	c-C_6H_{11}	*n*-C_4H_9O	CH_3	13.9	88%
26	C_6H_5	*n*-C_4H_9	CH_3	16.6	NT
27	$C_6H_5CH_2$	*n*-C_4H_9	CH_3	12.4	59%

NT, not tested.

Table 2. Effect of phenyl moiety substituents on lipid peroxidation inhibitory activity



Compound	R^1	R^2	Lipid peroxidation inhibitory activity IC_{50} (μM)
16	CH_3	H	>100
28	CH_3	4-CH_3	85.3
29	CH_3	4-CH_3O	147
30	CH_3	4-*n*-C_3H_7	21.5
31	CH_3	4-*i*-C_3H_7O	20.4
17	CH_3	4-*n*-C_4H_9	10.7
19	CH_3	4-*n*-C_4H_9O	5.1
32	CH_3	4-*i*-C_4H_9	7.8
33	CH_3	4-*t*-C_4H_9	8.4
34	CH_3	4-*n*-C_5H_{11}	5.9
35	CH_3	4-*n*-$C_5H_{11}O$	4.5
36	CH_3	4-$(CH_3)_2CH(CH_2)_2O$	6.6
37	CH_3	4-*n*-C_6H_{13}	9.0
38	CH_3	4-*n*-$C_6H_{13}O$	7.3
39	CH_3	4-c-C_6H_{11}	10.0
40	CH_3	4-*n*-C_7H_{15}	8.4
41	CH_3	4-*n*-$C_7H_{15}O$	5.7
42	CH_3	4-*n*-C_8H_{17}	19.4
43	CH_3	4-*n*-$C_8H_{17}O$	4.0
44	CH_3	4-*n*-$C_{10}H_{21}$	7.9
45	CH_3	4-*n*-$C_{10}H_{21}O$	6.9
46	CH_3	4-*n*-$C_{12}H_{25}$	13.0
47	CH_3	4-*n*-$C_{12}H_{25}O$	9.7
48	CH_3	4-*n*-$C_{14}H_{29}$	43.8
49	CH_3	4-*n*-$C_{18}H_{37}$	>100
50	CH_3	4-*n*-$C_{18}H_{37}O$	>100
51	CH_3	4-Ph	6.3
52	CH_3	4-PhO	8.1
53	CH_3	4-$PhCH_2O$	6.7
23	c-C_6H_{11}	H	47.6
54	c-C_6H_{11}	4-CH_3O	20.4
55	c-C_6H_{11}	4-*n*-C_3H_7	13.9
56	c-C_6H_{11}	2-*i*-C_3H_7O	21.5
57	c-C_6H_{11}	3-*i*-C_3H_7O	10.4
58	c-C_6H_{11}	4-*i*-C_3H_7O	13.0
24	c-C_6H_{11}	4-*n*-C_4H_9	27.1
25	c-C_6H_{11}	4-*n*-C_4H_9O	13.9
59	c-C_6H_{11}	4-*i*-C_4H_9	10.9
60	c-C_6H_{11}	4-*n*-C_5H_{11}	10.6
61	c-C_6H_{11}	4-*n*-$C_5H_{11}O$	9.1
62	c-C_6H_{11}	4-$(CH_3)_2CH(CH_2)_2O$	7.2
63	c-C_6H_{11}	4-*n*-C_6H_{13}	13.7
64	c-C_6H_{11}	4-*n*-$C_6H_{13}O$	8.5
65	c-C_6H_{11}	4-c-C_6H_{11}	28.9
66	c-C_6H_{11}	4-*n*-C_7H_{15}	8.7
67	c-C_6H_{11}	4-*n*-$C_7H_{15}O$	11.6
68	c-C_6H_{11}	4-*n*-C_8H_{17}	10.3
69	c-C_6H_{11}	4-*n*-$C_8H_{17}O$	8.5
70	c-C_6H_{11}	4-*n*-$C_{10}H_{21}$	27.0
71	c-C_6H_{11}	4-*n*-$C_{10}H_{21}O$	10.5
72	c-C_6H_{11}	4-*n*-$C_{12}H_{25}$	>100
73	c-C_6H_{11}	4-*n*-$C_{12}H_{25}O$	52.4
74	c-C_6H_{11}	4-*n*-$C_{14}H_{29}$	>100
75	c-C_6H_{11}	4-*n*-$C_{18}H_{37}$	>100
76	c-C_6H_{11}	4-*n*-$C_{18}H_{37}O$	>100
77	c-C_6H_{11}	4-Ph	10.9
78	c-C_6H_{11}	4-PhO	7.9
79	c-C_6H_{11}	4-$PhCH_2O$	8.3

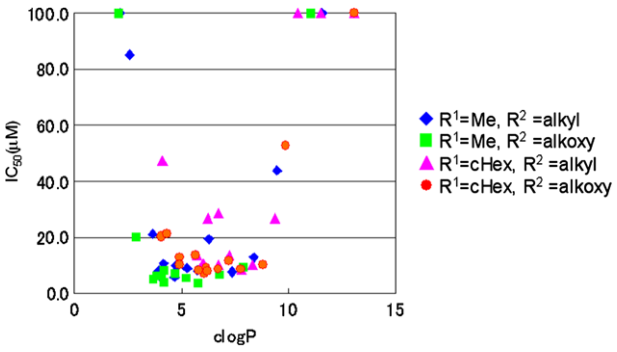


Figure 2. Relationship between *c*log *P* and IC_{50} of lipid peroxidation inhibitory activity. *c*log *P* was calculated by ChemDraw Ultra 7.0.1. IC_{50} values larger than 100 μM were rated 100 μM and plotted.

itory activity. These results seem to indicate that the activity of the compounds was greatly affected by their lipophilicity, which influences the optimal distribution of the compounds in the lipid bilayer of the membrane.

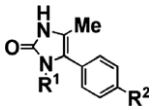
Although scavenging of free radical species is thought to be an effective mechanism for the protection of the central nervous system, it needs to be proven in a relevant animal model that compounds displaying this action are able to cross the blood–brain barrier and show some efficacy in improving the function of an injured central nervous system.

We tested the compounds for pharmacological efficacy in memory improvement by oral administration in a passive avoidance model in rats. Compounds with lipid peroxidation inhibitory activity (IC_{50}) of up to 11 μM were selected for the study and the results listed in Table 3. Many of the substituted imidazolinone compounds had memory-improving activity, some with effect starting from 0.1 mg/kg. These findings showed that there was no obvious correlation with lipid peroxidation inhibitory activity or with lipophilicity. The lack of correlation was not surprising as other physico-chemical properties of the compounds such as solubility, pharmacokinetics, and distribution in the brain need to be known before the results can be explained.

Of the compounds in Table 3, used in acute toxicity studies via oral administration in rats, **17**, **39**, and **51** displayed a lethal dose (LD_{50}) larger than 1000 mg/kg, which indicated a much lower intrinsic toxicity of the phenol-like imidazolinone structure than in the classic phenol containing compounds (LD_{50} 530 mg/kg).[32]

In conclusion, we synthesized free radical scavengers based on the hypothesis that compounds with an aromatic hydroxy group generated by keto-enol tautomerization would show radical-scavenging and antioxidant activities similar to those of phenols but less acute toxicity. Our study resulted in the synthesis of a series of novel 5-aryl-imidazolin-2-ones with good free radical-scavenging activities and potent lipid peroxidation inhibitory activity, many of which combined memory-improving activity with low acute toxicity.

Table 3. Memory-improving activity



Compound	R^1	R^2	Lipid peroxidation inhibitory activity IC_{50} (μM)	Memory-improving activity MED (mg/kg, po)
17	CH_3	*n*-C_4H_9	10.7	0.1
19	CH_3	*n*-C_4H_9O	5.1	0.3
32	CH_3	*i*-C_4H_9	7.8	30
35	CH_3	*n*-$C_5H_{11}O$	4.5	[a]
36	CH_3	$(CH_3)_2CH(CH_2)_2O$	6.6	[a]
37	CH_3	*n*-C_6H_{13}	9.0	0.3
38	CH_3	*n*-$C_6H_{13}O$	7.3	[a]
39	CH_3	c-C_6H_{11}	10.0	0.1
40	CH_3	*n*-C_7H_{15}	8.4	30
41	CH_3	*n*-$C_7H_{15}O$	5.7	[a]
43	CH_3	*n*-$C_8H_{17}O$	4.0	30
44	CH_3	*n*-$C_{10}H_{21}$	7.9	0.3
45	CH_3	*n*-$C_{10}H_{21}O$	6.9	0.1
47	CH_3	*n*-$C_{12}H_{25}O$	9.7	0.1
51	CH_3	Ph	6.3	0.3
52	CH_3	PhO	8.1	[a]
53	CH_3	$PhCH_2O$	6.7	[a]
61	c-C_6H_{11}	*n*-$C_5H_{11}O$	91	[a]
62	c-C_6H_{11}	$(CH_3)_2CH(CH_2)_2O$	7.2	[a]
64	c-C_6H_{11}	*n*-$C_6H_{13}O$	8.5	[a]
69	c-C_6H_{11}	*n*-$C_8H_{17}O$	8.5	30
71	c-C_6H_{11}	*n*-$C_{10}H_{21}O$	10.5	0.1
77	c-C_6H_{11}	Ph	10.9	0.1
78	c-C_6H_{11}	PhO	7.9	0.1
79	c-C_6H_{11}	$PhCH_2O$	8.3	0.1

MED, minimal effective dose.
[a] Not active at 0.1 mg/kg, po.

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