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Hydroxyl radical scavenging by edaravone derivatives: Efficient scavenging by 3-methyl-1-(pyridin-2-yl)-5-pyrazolone with an intramolecular base

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Abstract—We synthesized various 3-methyl-1-phenyl-5-pyrazolone (edaravone) derivatives and evaluated their oxidation potential and hydroxyl radical scavenging activity. It was found 3-methyl-1-(pyridin-2-yl)-5-pyrazolone had a much higher ability to scavenge the radical than did edaravone itself. Its efficient radical scavenging activity was assumed to be due to the increase of its anion form, an active form, by a hydrogen-bonded intramolecular base.

Reactive oxygen species are involved in many pathological conditions such as ischemic-reperfusion injury,[1,2] cellular aging,[3] and progression of arteriosclerosis.[4]

Recently, a new pyrazolin compound, edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, also known as MCI-186, Fig. 1) has been developed as a medical drug for brain ischemia,[5,6] and has been reported to be effective for myocardial ischemia as well.[7] Edaravone (**A**) is known to be an efficient antioxidant, which is considered to be the basis of its protective effect against ischemia. Its enolate form (**B**) can interact with both peroxyl ($LOO^{\bullet}$) and hydroxyl radicals ($^{\bullet}OH$), followed by the formation of a stable oxidation product (OPB: 2-oxo-3-(phenylhydrazono)-butanoic acid) through a radical intermediate[8,9] (Fig. 1).

We were encouraged to study the structure–activity relationship (SAR) as a means of characterizing the structural features of edaravone and optimizing the structure with regard to its radical scavenging activity in an aqueous solution. For this purpose, we synthesized edaravone derivatives with various substituents such as electron-withdrawing groups (EWG), electron-donating groups (EDG), and π-conjugated groups at the 1-, 3-, or 4-positions of the pyrazolone ring (see supporting information).[10] Oxidation potentials of the synthesized pyrazolone derivatives were measured by cyclic voltammetry (CV) in an aqueous solution, and their hydroxyl radical scavenging activity was evaluated using the electron spin resonance (ESR) spin-trapping method.

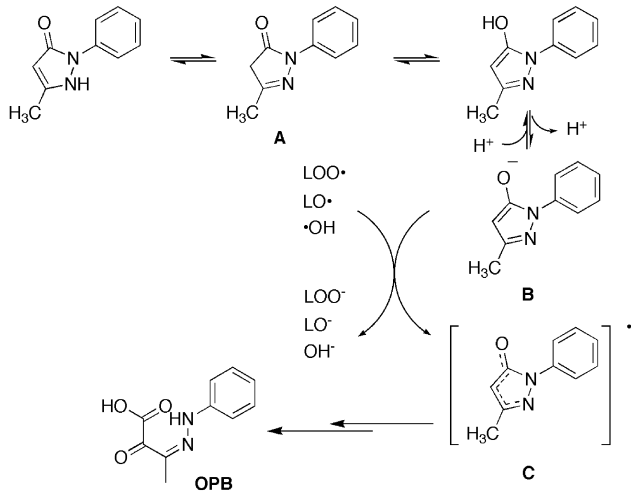

Figure. 1. Reaction mechanisms of edaravone (**A**) with free radicals. OPB; 2-oxo-3-(phenylhydrazono)-1-butanoic acid.

Keywords: Pyrazolone derivatives; Antioxidant; Anion form amount.
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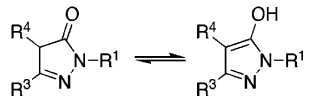
Table 1. Oxidation potentials (E_{pa}) of the edaravone derivatives



Compound	R^1	R^3	R^4	E_{pa}[a] (mV)	pH
A (edaravone)	Ph–	CH_3–	H	483	7.0
A (edaravone)	Ph–	CH_3–	H	480	7.8
1	4-CH_3OPh–	CH_3–	H	678	7.8
2	4-ClPh–	CH_3–	H	473	7.4
3	Cyclohexyl–	CH_3–	H	549	7.4
4	2-Pyridinyl–	CH_3–	H	483	7.0
5	Ph–	CF_3–	H	673	7.6
6	Ph–	Ph–	H	397	7.6
7	Ph–	4-NO_2Ph–	H	419	7.4
8	Ph–	4-CH_3OPh–	H	397	7.8
9	Ph–	CH_3OCONH–	H	454	7.8
10	Ph–	PhOCONH–	H	397	7.0
11	Ph–	CyclopentylNHCONH–	H	372	7.8
12	Ph–	Isopropenyl–	H	387	7.4
13	Ph–	Bn–	H	269	>8.0
14	Ph–	CH_3–	Isobutyl–	262	>8.0
15	Ph–	CH_3–	Ph–	227	7.6
16	Ph–	CH_3–	Cyclopropyl–	275	7.8
17	Ph–	CH_3–	PhCO–	640	7.0
18	4-NO_2Ph–	CH_3–	H	525	7.6

Conditions for measurement: 10 mM sample in 50 mM NaCl. Working electrode; Pt, reference electrode; Ag^+/AgCl, counter electrode; Pt, scan speed; 50 mV/s, Scan range −0.2 to 1.0 V.

[a] The oxidation potentials were expressed versus Ag^+/AgCl.

One-electron oxidation potentials (E_{pa}) of all the synthesized derivatives were measured in a 50 mM NaCl solution (Table 1). Oxidation currents were observed with all the tested compounds, but were irreversible, probably because the one-electron oxidation products were unstable and converted to degraded compounds as reported.[8,9] Because of the poor solubility of several derivatives in the neutral aqueous solution, the solutions were slightly basified using aqueous NaOH to solubilize these compounds.

Although the derivatives with strong EWGs, such as compound **5** with a trifluoromethyl group and **17** with a benzoyl group, had relatively higher oxidation potentials as expected, the other derivatives showed a wide variety of oxidation potentials regardless of the electronic properties of the substituents (Table 1). It is possible that the oxidation potentials were not only affected by the electron density on the pyrazolone ring but also by the stability of the resulting radical species (**C**) with conjugated π-orbitals on the substituent. In comparison, among the positions of the substituents, the substitution at position 1 did not positively affect the reduction of the oxidation potential, whereas that at the position 4 seemed to be more effective (Fig. 2).

The radical scavenging activity was evaluated by the ESR spin-trapping method with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as a spin trap. Hydroxyl radicals were generated by UV irradiation (2000 J/cm^2) of the hydrogen peroxide solution containing DMPO and edaravone derivatives.[11,12] The inhibitory effect of the derivatives on the formation of hydroxyl radical adducts of DMPO was used as a measure of the radical scavenging activity. The IC_{50} values were determined for seven of nineteen derivatives with diverse oxidation potentials (**1A**, **5**, **10**, **15**, **16**, **17**, and **18**). The relationship between the IC_{50} value and the oxidation potential was not simply proportional, but showed a V-shaped correlation (Fig. 3). Edaravone (**A**), which had an oxidation potential of 483 mV (vs Ag^+/AgCl), showed the lowest IC_{50} value among the seven derivatives tested.

The edaravone anion (**B**) is reported to be an active form in scavenging free radicals by a one-electron-transferring mechanism.[13,14] Actually, in the case of one-electron oxidation of edaravone with 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN), a radical initiator, the oxidation rate was increased in a pH-dependent manner in methanol/buffer solutions.[8] Therefore, the amount of the anionic form of the synthesized derivatives is important for scavenging activities. On the other hand, the electron density of the pyrazolone moiety is also important for one-electron oxidation reactivity. The relatively electron-rich substituents on the pyrazolone ring may lower the oxidation potential, but concomitantly decrease the amount of their anionic form by protonation due to the increasing partial negative charges. In contrast, electron-poor substituents may increase the amount of the anionic form but enlarge the oxidation potential. As shown in Table 2 and Figure 3, edaravone had almost the best oxidation potential for hydroxyl radical scavenging under our experimental conditions, an activity in which the oxidation potential and the amount of the anion form may be well balanced.

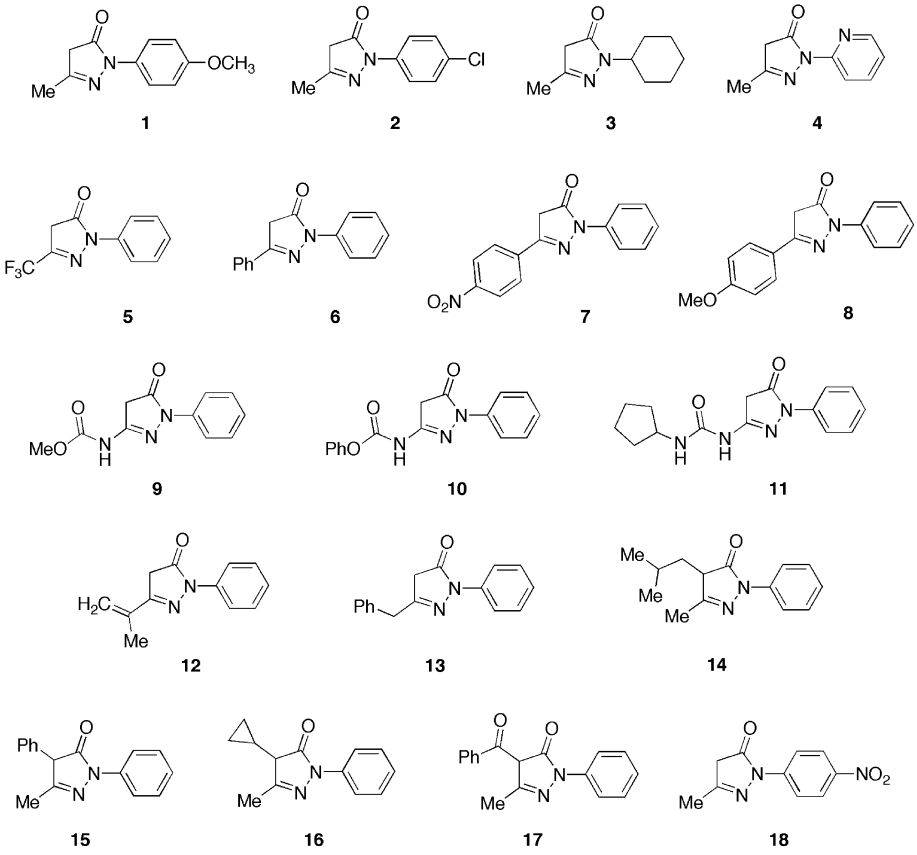


Figure. 2. Structures of edaravone derivatives synthesized in this study.

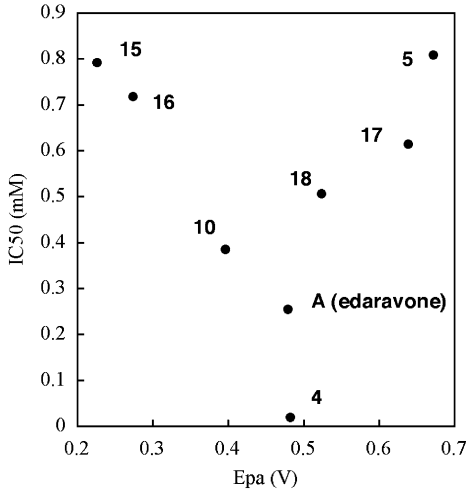


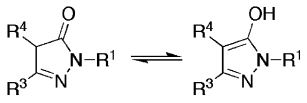
Figure. 3. The relationship between oxidation potentials (E_{pa}) and IC_{50} values. Each data point represents a specific derivative with its number along side.

As reported,[10] edaravone derivatives with lipophilic substituents on the phenyl group at position 1 of the pyrazolone ring showed higher inhibitory activity against the lipid peroxidation, which was likely due to the increasing concentrations of the derivatives in the lipid phase. With aqueous solutions, our results suggested that the increase of the anionic form of the derivatives appeared to be an important requirement for efficient radical scavenging.

Since the pK_a values of the derivatives in the aqueous solutions were hardly evaluated due to the poor solubility of the derivatives in neutral and acidic solutions, we referred to the CAS database and only used the data for the comparison of the relative stability of the deprotonated form. For novel compounds, pK_a values were estimated by calculating free energy changes in their deprotonation with density functional theory (B3LYP/6-31G*) on Spartan 02 or 04 software (Wavefunction, Inc. Irvine, CA, USA) (see supporting information). The calculated pK_a values were found to be roughly higher for derivatives with lower oxidation potentials than that of edaravone, implying that the amount of the anionic form may be related to the radical scavenging activity of derivatives with lower oxidation potentials than that of edaravone.

In the light of the properties clarified above, the increase in the anionic form without concomitant positive shift of the oxidation potential may be effective for efficient one-electron radical reduction. Although one-electron oxidation of a derivative apparently occurs in its anion form, deprotonation of a derivative does not affect its potential of one-electron oxidation, but does affect its oxidation current. We next focused on compound **4**, which has a pyridin-2-yl moiety as an intramolecular base at position 1 of the pyrazolone ring. This basic function may facilitate the partial deprotonation from the pyrazolone ring moiety. As expected, its IC_{50} value was 13.9 times smaller than that of edaravone with almost the same oxidation

Table 2. Hydroxyl radical scavenging activity (IC_{50}) of the edaravone derivatives



Compound	R^1	R^3	R^4	IC_{50} (mM)
A (edaravone)	Ph–	CH_3–	H	0.25
4	2-Pyridinyl–	CH_3–	H	0.018
5	Ph–	CF_3–	H	0.81
10	Ph–	PhOCONH–	H	0.38
15	Ph–	CH_3–	Ph–	0.79
16	Ph–	CH_3–	Cyclopropyl–	0.72
17	Ph–	CH_3–	PhCO–	0.61
18	4-NO_2Ph–	CH_3–	H	0.50

Conditions for measurement: a mixture of 25 mM H_2O_2, 25 mM DMPO, and a compound was irradiated with UV. ESR spectrometer parameters were: microwave power, 10 mW; modulation width, 0.063 mT; time constant, 0.03 s; sweep width, 7.5 mT; sweep time, 1 min; gain, 320.

potential (Table 2 and Fig. 3). This implies that the pyridin-2-yl function partially deprotonated from the pyrazolone ring by forming an intramolecular hydrogen-bond without a marked decrease in the electron density of the pyrazolone ring. Supporting this idea, it was confirmed by ^1H NMR analysis that **4** was in its enol form in $CDCl_3$ solution, in which a singlet methyne proton (δ 5.4) and a broadened enol proton (δ 12.7) were observed instead of two methylene protons (δ 3–4) of the pyrazolone ring (see supporting information).

In conclusion, we determined the oxidation potential and hydroxyl radical scavenging activity of various edaravone-related derivatives in an aqueous solution, and analyzed their characteristics as free radical scavengers under aqueous conditions. Finally, we found the derivative that was the most efficient at radical scavenging in aqueous solutions was one that stabilized the active anionic form with an intramolecular base.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2006.09.005.

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