Inhibition of Mitochondrial Hydrogen Peroxide Production by Lipophilic Metalloporphyrins

Pablo R. Castello,† Derek A. Drechsel, Brian J. Day, and Manisha Patel

Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, Colorado (P.R.C., D.A.D., M.P.); and Department of Medicine, National Jewish Medical and Research Center, Denver, Colorado (B.J.D.)

Received September 27, 2007; accepted December 5, 2007

ABSTRACT

Many studies have established a role for oxidative stress and mitochondrial dysfunction as an important mechanism in the pathogenesis of neuronal disorders. Metalloporphyrins are a class of catalytic antioxidants that are capable of detoxifying a wide range of reactive oxygen species. The AEOL112 series of glyoxylate metalloporphyrins were designed with increased lipid solubility for better oral bioavailability and penetration of the blood-brain barrier. The goal of this study was to develop an in vitro assay using rat brain mitochondria to reliably detect endogenously released hydrogen peroxide (H$_2$O$_2$) and identify glyoxylate metalloporphyrins based on rank order of potency for removal of physiologically relevant H$_2$O$_2$. A polarographic method was established for the sensitive, accurate, and reproducible detection of low levels of H$_2$O$_2$. The assay identified several potent glyoxylate metalloporphyrins with H$_2$O$_2$ scavenging potencies (IC$_{50}$) in the nanomolar range. These results provide a simplified in vitro model system to detect physiologically generated mitochondrial H$_2$O$_2$ as a screening tool to predict the biological efficacy of potential therapeutic entities.

Oxidative stress is strongly implicated as a mediator of neuronal damage in diverse acute and chronic neuronal disorders (Lin and Beal, 2006). Metalloporphyrins are a class of catalytic antioxidants that are capable of detoxifying a wide range of reactive oxygen species (ROS), such as superoxide (O$_2^-$), H$_2$O$_2$, peroxynitrite, and lipid peroxyl radicals (Patel and Day, 1999). Several water-soluble metalloporphyrin compounds, including manganese (III) meso-tetrakis (4-carboxyphenyl or benzoic acid) porphyrin (MnTBAP), AEOL10150, and AEOL10113, have been shown to be efficacious in animal models of central nervous system disorders, including status epilepticus, (Liang et al., 2007). Previous work has also demonstrated that manganic porphyrins can protect mature neuronal cultures from excitotoxic injury by scavenging intracellular O$_2^-$ (Patel et al., 1996; Li et al., 2001). These compounds contain a manganese center that catalytically dismutes both O$_2^-$ and H$_2$O$_2$ (Pasternack and Skowronek, 1979; Day et al., 1997) Previous meso-substituted porphyrin rings contained positively charged pyridyl (AEOL10113) or imidazole (AEOL10150) groups to electrostatically facilitate reaction with negatively charged O$_2^-$ (Batinic-Haberle et al., 1998; Kachadourian et al., 2004). However, the charged nature of the water-soluble pyridine- and imidazole-substituted metalloporphyrins makes them less efficient in crossing lipid membranes. To overcome these issues, a series of novel glyoxylate metalloporphyrins (AEOL112 series) with improved lipid solubility have been developed and chemically characterized (Trova et al., 2003) to improve the potential for in vivo therapeutic use in neurological disorders characterized by increased ROS levels and oxidative stress. This newly developed glyoxylate series of metalloporphyrins (Fig. 1) have been shown to dismute H$_2$O$_2$ in a catalase-like reaction to generate O$_2$ and inhibit lipid peroxidation in cell-free systems (Kachadourian et al., 2003, 2004; Trova et al., 2003; Liang et al., 2007).

A major initial step toward determining the in vivo efficacy of glyoxylate (AEOL112 series) metalloporphyrins is the pre-

This work was supported by NINDS, National Institutes of Health Grants RO1 NS045748, RO1 NS039587, and R21 NS053548 (to M.P.) and Aeolus Pharmaceuticals (to M.P. and B.J.D.). B.J.D. is a consultant for and holds equity in Aeolus Pharmaceuticals, which is developing catalytic antioxidants as therapeutic agents.

† Current affiliation: Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

do:10.1124/jpet.107.132134.

ABBREVIATIONS: ROS, reactive oxygen species; MnTBAP, manganese (III) meso-tetrakis (4-carboxyphenyl or benzoic acid) porphyrin; AEOL10150, manganese (III) meso-tetrakis (N,N'-diethyldiaminol-2-yl) porphyrin; AEOL10113, manganese (III) meso-tetrakis (N-ethyl pyridinium-2-yl) porphyrin; PQ$^-$, paraquat; LDH, lactate dehydrogenase; COX, cytochrome c oxidase; DMSO, dimethyl sulfoxide; HRP, horseradish peroxidase; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive species; MDA, malondialdehyde; MnTE-2-PyP, manganese tetakis-(N-ethyl-2-pyridyl) porphyrin.
Fig. 1. Chemical structure of AEOL compounds. The structure of hydrophobic glyoxylate manganoporphyrins with the indicated side groups (R1–R4) is shown. a, AEOL11249, Mnsup(2+) was substituted by Znsup(2+); b, AEOL11250, Mnsup(2+) was substituted by Fesup(2+); c, AEOL11251, Mnsup(2+) was substituted by Cosp(2+).
selection of lead compounds in a simple yet physiologically relevant in vitro system. Cell-free antioxidant assays can be used for this purpose and have the advantage of allowing accurate assessment of antioxidant potencies without interference from cellular components. However, the lack of endogenous factors renders these systems less predictive of in vivo efficacy. These issues may be overcome by using simplified in vitro model systems that recapitulate more physiologically relevant conditions and therefore serve as better screening tools to predict the biological efficacy of potential therapeutic entities. We have recently demonstrated the mechanism of net ROS production from purified rat brain mitochondria by the redox-cycling agent parquat (PQ$^{2+}$) using a polarographic assay (Castello et al., 2007). The goals of this study were to 1) develop an in vitro assay that generated physiologically relevant H$_2$O$_2$ levels and 2) identify lead metalloporphyrin compounds based on rank order of potency for scavenging endogenously generated H$_2$O$_2$.

**Materials and Methods**

**Materials.** Metalloporphyrins with >97% purity were provided by Aeolus Pharmaceuticals (Laguna Niguel, CA). With the exceptions indicated, all the other drugs used in these studies were obtained from Sigma-Aldrich (St. Louis, MO).

**Isolation of Purified Rat Brain Mitochondria.** Animal housing was conducted in compliance with University of Colorado at Denver and Health Sciences Center (Denver, CO) procedures. Mitochondria were isolated from adult male Sprague-Dawley rats using Percoll gradient density centrifugation as described previously (Anderson and Sims, 2000) with minor modifications (Castello et al., 2007). The purity of mitochondrial fractions was assessed using Western blotting techniques. In brief, denatured protein fractions of cytosol, mitochondrial, and whole-cell homogenate were separated by electrophoresis on a 10% polyacrylamide gel (Bio-Rad, Hercules, CA) and transferred to nitrocellulose membrane. Membrane blots were incubated with primary antibodies against lactate dehydrogenase (LDH) (1:1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) or cytochrome c oxidase subunit IV (COX); (1:1000; Molecular Probes, Eugene, OR). LDH and COX membranes were incubated with horse-radish peroxidase-conjugated anti-goat or anti-mouse secondary antibodies, respectively. Membranes were developed using an ECL Western blotting detection reagent (GE Healthcare, Buckinghamshire, UK). Figure 2 shows that COX was undetectable in cytosolic fractions and robustly expressed in mitochondrial fractions.

**Purity of brain mitochondria fraction.** Cytosolic and mitochondrial fractions of rat brain were isolated as described under **Materials and Methods** and subjected to Western blot analysis for cytosolic and mitochondrial protein markers, LDH and COX, respectively. Mitochondria samples robustly expressed COX, whereas LDH was undetectable, indicating highly purified mitochondrial fractions.

**Fig. 2.** Purity of brain mitochondria fraction. Cytosolic and mitochondrial fractions of rat brain were isolated as described under **Materials and Methods** and subjected to Western blot analysis for cytosolic and mitochondrial protein markers, LDH and COX, respectively. Mitochondria samples robustly expressed COX, whereas LDH was undetectable, indicating highly purified mitochondrial fractions.
A Polarographic Assay for Evaluation of H$_2$O$_2$-Scavenging Activity. To develop a biologically relevant in vitro assay for detection of H$_2$O$_2$-scavenging activities, a polarographic method of H$_2$O$_2$ detection was used. Unlike other methods of H$_2$O$_2$ detection based on endpoint readings, the use of polarographic sensors allows for the real-time measurement of steady-state H$_2$O$_2$ concentrations that takes into account the contributions of both production and consumption of H$_2$O$_2$ in the system under study. We have previously shown that the addition of PQ$^{2+}$ to isolated rat brain mitochondria supplemented with respiration substrates results in a rapid and robust net production/increase of H$_2$O$_2$ (Castello et al., 2007). The consistent and stable increase in H$_2$O$_2$ allows for the analysis of antioxidant activity of added compounds over a short period of time. To validate this method as a tool for identifying antioxidant molecules, we analyzed the effect of externally added endogenous antioxidant enzymes, catalase, and SOD. SOD had no effect on net H$_2$O$_2$ production at a saturating concentration of 500 U/ml, whereas catalase produced a concentration-dependent inhibition of PQ$^{2+}$-induced mitochondrial H$_2$O$_2$ (Figs. 3, A and B, and 4).

Because polarographic measurement of H$_2$O$_2$ is a novel, electrode-based methodology, a series of experiments were performed with the aim of identifying possible interferences. Control studies with the following treatments showed no changes over baseline H$_2$O$_2$ signal: 1) reaction buffer, 2) reaction buffer + mitochondria, 3) reaction buffer + mitochondria + PQ$^{2+}$, 4) reaction buffer + mitochondria + malate + pyruvate, and 5) reaction buffer + PQ$^{2+}$ + malate + pyruvate. Upon addition of PQ$^{2+}$ to reaction buffer + mitochondria + malate + pyruvate, H$_2$O$_2$ was generated at the rate of 0.75 to 1 nmol/mg/min. Overall, these controls indicate that the polarographic measurement using an H$_2$O$_2$ electrode is not affected by the majority of the components used in the assays.

Inhibition of PQ$^{2+}$-Induced Mitochondrial H$_2$O$_2$ by AEOL112 Compounds. Using the polarographic method described above, we were able to measure the H$_2$O$_2$-scavenging activities of metalloporphyrin compounds. Figure 4 shows an example of the results using AEOL11207. After the addition of PQ$^{2+}$, H$_2$O$_2$ levels increased at a steady rate for several minutes at which point compounds were added to determine inhibitory effects. Catalase at a concentration of 12 nM was chosen as a positive control and was considered to represent 100% inhibition of the H$_2$O$_2$ net increase. This concentration of catalase caused no significant change in levels of H$_2$O$_2$, indicating a balance between the production and removal of H$_2$O$_2$ in the system. Increasing concentrations of catalase (125 nM) led to a decrease in H$_2$O$_2$ signal corresponding with the enzymatic removal of endogenously generated H$_2$O$_2$ in the system. Addition of SOD at saturating concentrations had no effect on the rate of H$_2$O$_2$ net production. A full range of inhibition curves was determined for different concentrations of AEOL112 compounds (Fig. 5). Figure 5 represents the percentage of inhibition of steady state of PQ$^{2+}$-induced mitochondrial H$_2$O$_2$ as a function of the logarithm concentration of each compound. Using non-linear regression, the best fit for each concentration-response curve was obtained to determine IC$_{50}$ values for each compound. Compounds were divided into two groups based on their observed H$_2$O$_2$-scavenging activities: 1) those compounds exhibiting a strong concentration-response relationship (Fig. 5; Table 1) and 2) those compounds exhibiting a...
The inhibition of H2O2 net increase and/or have an IC50 value obtained after nonlinear regression of the activity data. The ability of glyoxylate metalloporphyrins to inhibit PQ2+-induced mitochondrial H2O2 net production was evaluated. Compounds that demonstrated a strong concentration-response relationship are shown. Compounds that exhibited a less optimal concentration-response relationship are indicated in Table 1. Each point represents the mean values from independent duplicate experiments.

### Table 1

Inhibition of PQ2+-induced H2O2 and catalase activity of selected glyoxylate metalloporphyrins

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM)</th>
<th>% Catalase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Catalase</td>
<td>5.5</td>
<td>100</td>
</tr>
<tr>
<td>AEOL11050</td>
<td>17 (3000)*</td>
<td>(0.2)*</td>
</tr>
<tr>
<td>AEOL11209a</td>
<td>93 (3000)*</td>
<td>32.4</td>
</tr>
<tr>
<td>AEOL11216</td>
<td>3 (30)*</td>
<td>5.3 (18.3)*</td>
</tr>
<tr>
<td>AEOL11215</td>
<td>206</td>
<td>2.2</td>
</tr>
<tr>
<td>AEOL11223</td>
<td>408</td>
<td>1.3</td>
</tr>
<tr>
<td>AEOL11210</td>
<td>725</td>
<td>0.8</td>
</tr>
<tr>
<td>AEOL11202b</td>
<td>1642</td>
<td>0.3</td>
</tr>
<tr>
<td>AEOL11203</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11204</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11206</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11219</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11227</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11238</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11239</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11243</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11244</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11249</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11250</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>AEOL11251</td>
<td>&gt;3000</td>
<td>&gt;0.2</td>
</tr>
</tbody>
</table>

N.A., not applicable.

a Activity determined using Amplex Red assay.

b AEOL112 compounds in italics exhibit a less optimal concentration-response relationship.

## Fig. 5

Concentration-response curves of the inhibition of PQ2+-induced mitochondrial H2O2 by glyoxylate metalloporphyrins. The ability of glyoxylate metalloporphyrins to inhibit PQ2+-induced mitochondrial H2O2 net production was evaluated. Compounds that demonstrated a strong concentration-response relationship are shown. Compounds that exhibited a less optimal concentration-response relationship are indicated in Table 1. Each point represents the mean values from independent duplicate experiments.

## Fig. 6

Concentration-response curves of inhibition of PQ2+-induced mitochondrial H2O2 by AEOL11050 and AEOL112 Series. To validate the polarographic method and overcome its interference with AEOL10150, a HRP-linked fluorometric method (Amplex Red assay) was used. The values obtained were used to construct the concentration-response curve shown in Fig. 6. To compare the IC50 values obtained using the fluorometric assay with the polarographic method, AEOL11207 was used as a positive control. AEOL11207 showed a concentration-response relationship comparable to that obtained using the polarographic method. The IC50 values of AEOL11207 using the fluorometric and polarographic assays were 30 and 104 nM, respectively. AEOL10150 showed a concentration-response relationship with an IC50 value of 3 μM, using the fluorometric assay.

### Structure Activity Relationships of the AEOL112 Series with Their Ability to Inhibit Net Production of H2O2

The most potent inhibitors of H2O2 net production in the AEOL112 series were meso-substituted with electron-withdrawing groups such as aldehydes, as seen in the bis-substituted AEOL11209 compound and trifluoromethyl groups in the bis-substituted AEOL11207 and AEOL11216 compounds. It was interesting to note that n-alkyl-substituted ester groups also correlated with the ability of the compounds to inhibit the net production of H2O2 as seen with AEOL11215 that has a meso-tetrakis propyl ester substituion, but activity drops off substantially as one lengthens the alkyl chain as seen in the meso-tetrakis hexyl ester-substituted compound AEOL11238.

To determine whether inhibition of PQ2+-induced H2O2 by metalloporphyrins showing an optimal concentration-response relationship was due to the manganese moiety, the effects of metal-substituted analogs of AEOL11215 (AEOL11249, Zn2+/H9262)

The effect of exogenously added H2O2 was determined. No change in signal was observed after the addition of the AEOL112 series compounds listed in Table 1 to the reaction buffer + mitochondria + malate + pyruvate + 2 μM H2O2. The most potent metalloporphyrins identified with IC50 < 1 μM are as follows: AEOL11209 (IC50 = 17 nM) > AEOL11216 (IC50 = 93 nM) > AEOL11207 (IC50 = 104 nM) > AEOL11215 (IC50 = 206 nM) > AEOL11223 (IC50 = 408) > AEOL11210 (IC50 = 725 nM) > AEOL11202 (IC50 = 1642 nM).

## Comparison of the Inhibition of PQ2+-Induced Mitochondrial H2O2 by AEOL10150 and AEOL112 Series

To determine whether inhibition of PQ2+-induced H2O2 by metalloporphyrins showing an optimal concentration-response relationship was due to the manganese moiety, the effects of metal-substituted analogs of AEOL11215 (AEOL11249, Zn2+/H9262)
In this study, we developed a novel in vitro screening assay using polarographic detection of H$_2$O$_2$ endogenously generated in isolated rat brain mitochondria treated with PQ$^{2+}$. Using this assay, we identified several potent antioxidant compounds belonging to a novel class of lipophilic glyoxylate metalloporphyrins.

A polarographic method was established for the sensitive, accurate, and reproducible detection of H$_2$O$_2$ scavenging by AEOl compounds in a physiologically relevant in vitro model involving rat brain mitochondrial H$_2$O$_2$. The method is rapid and sensitive, with low level of interferences, and has the potential for high-throughput analysis. The assay uses respiring mitochondria and PQ$^{2+}$ to generate endogenous H$_2$O$_2$. The system is specific for H$_2$O$_2$ as shown by the high sensitivity to the addition of catalase but not SOD (Fig. 4). The ability of catalase to inhibit the H$_2$O$_2$ signal is due to the ability of intramitochondrially generated H$_2$O$_2$ to cross mitochondrial membranes, which then can be readily dismutated by catalase. The lack of effect with SOD is probably due to the inability of this large protein to penetrate mitochondrial membranes to dismute intramitochondrial O$_2^-$, which is short-lived and not very permeable to biological membranes. Once the steady-state net increase of H$_2$O$_2$ was induced by PQ$^{2+}$ in mitochondria, the addition of either several metalloporphyrins or catalase (but not SOD) changed the steady-state net production of H$_2$O$_2$. The velocity of H$_2$O$_2$ net production in the lower steady state was used as an indicator of compound potency. This approach has the advantage of using the initial steady state as a control, eliminating the random differences between different measurements and preparations in which the initial steady state may vary.

It is worth mentioning that in vitro H$_2$O$_2$ concentrations previously used in the measurement of catalase activity of AEOl compounds was 1 mM (Day et al., 1997). This concentration is orders of magnitude higher than H$_2$O$_2$ steady-state concentrations in physiological systems that are in the nanomolar range (Chance et al., 1979). Because catalase activity is assumed to follow pseudo-first-order kinetics, the use of such high amounts of H$_2$O$_2$ can result in an overestimation of the pseudo-first-order rate constant and, therefore, of catalase activity. Moreover, it has been reported that high amounts of H$_2$O$_2$ can inactivate the metalloporphyrins (Day et al., 1997). This new assay presents several advantages over the established ones. First, it uses concentrations of H$_2$O$_2$ (~1–100 nM) that may be achieved physiologically. Steady-state concentrations of H$_2$O$_2$ are estimated to $10^{-8}$ to $10^{-7}$ M (Chance et al., 1979; Gardner et al., 2006). Second, it uses H$_2$O$_2$ produced by brain mitochondria, which are an important cellular source of ROS contributing to neurodegeneration and aging. Third, it is based in an in vitro system using the redox-cycling agent PQ$^{2+}$, an environmental toxin implicated in the etiology of Parkinson’s disease (Di Monte, 2003).

The results presented in Table 1 reveal the following order of potencies of the metalloporphyrins tested in this study that showed an IC$_{50}$ < 1 μM: AEOl11209 (IC$_{50}$ = 17 nM) > AEOl11216 (IC$_{50}$ = 93 nM) > AEOl11207 (IC$_{50}$ = 104 nM) > AEOl11215 (IC$_{50}$ = 206 nM) > AEOl11223 (IC$_{50}$ = 408) > AEOl11210 (IC$_{50}$ = 725 nM) > AEOl11202 (IC$_{50}$ = 1642 nM). The potencies of the compounds obtained in our in vitro assay have been validated in the in vivo setting by the dem-
onstration that orally administered AEOL11207 achieving brain concentrations of ~200 nM inhibited oxidative stress indices and neuronal damage in a mouse model of mitochondrial oxidative stress (Liang et al., 2007).

The ability of manganese-substituted, but not zinc-, cobalt-, and iron-substituted, metalloporphyrins to inhibit the net production of \( \text{H}_2\text{O}_2 \) illustrates the importance of manganese as the optimal metal in the \( \text{H}_2\text{O}_2 \)-scavenging effects of the compounds. Although the glyoxylate metalloporphyrins are lipid-soluble, the control studies described under Results suggest that most AEOL compounds do not inhibit the net production of \( \text{H}_2\text{O}_2 \) by interfering with the redox-cycling mechanism of \( \text{PQ}^+ \) in the mitochondria. Their ability to remove \( \text{H}_2\text{O}_2 \) is probably not caused by scavenging of intramitochondrial \( \text{O}_2^- \). This observation is based on their low SOD activity in cell-free assays (Trova et al., 2003), which renders the compounds less suitable as SOD mimetics.

Comparison of the data obtained in this study (Table 1) with previously published values for the AEOL112 series (Gauuan et al., 2002; Trova et al., 2003) demonstrates that compounds exhibiting a strong concentration-response relationship (Fig. 5; Table 1) have an average catalase activity ~145% higher than the average activity of the compounds exhibiting a less optimal concentration-response relationship (Table 1). On the other hand, the compounds demonstrating a strong concentration-response relationship (Fig. 5; Table 1) display average TBARS levels that are eight times lower than the average of the compounds exhibiting a less optimal concentration-response relationship, indicating a greater ability to remove lipid peroxides (Table 2). Together, these results conclude that the grouping of compounds according to their antioxidant properties was reflected not only by the current method but also by other previously screening methods.

One interesting finding that has emerged from several in vitro models of neuronal injury is a discrepancy between antioxidant potency and neuroprotective efficacy of metalloporphyrins. For example, although the water-soluble metalloporphyrin MnTE-2-PyP (AEOL10113) has at least 20 times more SOD activity in cell-free assays compared with MnTBAP, it was only two to three times more potent in its efficacy as a neuroprotective agent in Sod2-/- cultures (Patel, 2003). This paradoxical difference between antioxidant activities and neuroprotective efficacy has also been observed in other in vitro models involving glutamate excitotoxicity and oxygen-glucose deprivation injury (Li et al., 2001). These observations suggest that high SOD activity in a cell-free assay per se may not be sufficient to predict neuroprotection in vivo (cells or animals) and provides the rationale for the development of metalloporphyrins with broad antioxidant properties other than antioxidant activities derived from cell-free assays. The glyoxylate (AEOL112) series of metalloporphyrins have modest SOD activity but show high potencies as inhibitors of lipid peroxidation and cell and tissue injury (Choudhary et al., 2001; Kachadourian et al., 2003, 2004; Trova et al., 2003; Liang et al., 2007). The ability of metalloporphyrins to scavenge mitochondrial generated oxidative stress (Schriner et al., 2005). Although the overexpression of mitochondrial catalase is difficult to achieve, the particular scavenging of mitochondrial \( \text{H}_2\text{O}_2 \) by AEOL compounds opens a new paradigm in the therapeutic treatment of neuronal diseases in which mitochondrial oxidative stress is a major contributor.

Acknowledgments

We gratefully acknowledge Wenfang (Wendy) Ji for technical assistance.

References


Dr Manisha Patel, Department of Pharma-


Li QY, Pedersen C, Day BJ, and Patel M (2001) Dependence of excitotoxic neurode-


