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Phenolic thiazoles as novel orally-active neuroprotective agents

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Abstract—Novel phenolic thiazoles compounds were prepared which demonstrated potent antioxidant activity and potent in vivo neuroprotection in mitochondrial toxin models and also possess good oral bioavailability.

1. Introduction

Neurodegenerative disorders, Alzheimer's, Huntington's and Parkinson's disease as well as amyotrophic lateral sclerosis are characterised by a selective or generalised neuronal dysfunction. The cascade of events that leads to neuronal dysfunction and ultimately to cell death is multifactorial involving mitochondrial dysfunction, neuro-inflammatory processes and oxidative stress.[1]

Cells exposed to oxidative stress suffer DNA and lipid damage which may overwhelm the cellular antioxidant capacity resulting in cell death by apoptosis or necrosis.[2] Free radicals are mainly produced during mitochondrial respiration and mitochondrial dysfunction has been proposed as a cause of oxidative damage.[3]

Neuro-inflammatory processes have also been implicated in the toxicity cycle leading to neuronal dysfunction.[4] Inflammatory processes in the central nervous system (CNS) lead to increased formation of proinflammatory cytokines, formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Recently in our laboratories we have discovered novel orally-active thiazole containing compounds (**1** and **2**) that demonstrate potent in vitro antioxidant activity and remarkable neuroprotection in in vivo models of mitochondrial poisoning induced by different toxins.[5]

This report describes the chemical synthesis of these compounds, their in vitro antioxidant activities and preliminary in vivo biological results.

2. Chemistry

The thiazole compounds **1** and **2** were prepared according to the synthetic pathway shown in Scheme 1.

Readily available *N*-Cbz or *N*-Boc protected α-amino amides **1a** and **2b** were treated with phosphorous pentasulphide in dimethoxyethane under basic conditions to yield the corresponding thioamides **1b** and **2c**. The use of the Lawesson's reagent led to lower yields of thioamides. The *N*-Boc protected intermediate **2b** was prepared from sarcosinamide **2a** and **1a** is commercially available. The thioamides **1b** and **2c** in toluene were then coupled to 3,5-di-*tert*-butyl-4-hydroxyphenacyl bromide **3**, to afford the thiazoles **1c** and **2d**. The carbamate protecting groups of **1c** and **2d** were removed under acidic conditions to afford the primary and secondary free amines **1d** and **2e**.

Removal of the *N*-Cbz protecting group from intermediate **1c** under catalytic palladium-hydrogenation proved problematic and basic hydrolysis (alcoholic

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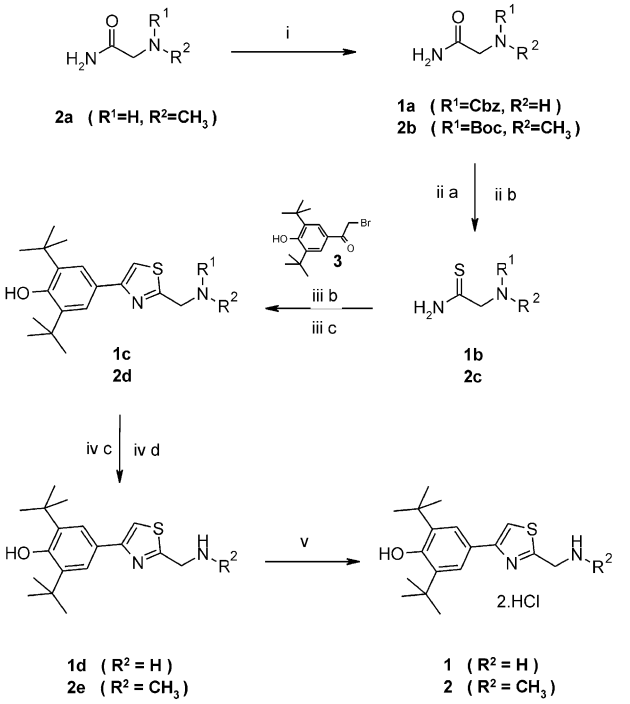


Scheme 1. (i) BocOBoc, diisopropylethylamine, dichloromethane, 0–20 °C, 72%; (ii) (a) P_2S_5, dimethoxyethane, solid $NaHCO_3$, 0–20 °C, 48 h, 75%; (ii) (b) 65%; (iii) (b) toluene, 90 °C, 3 h, 72%; (iii) (c) 28%; (iv) (c) concentrated HCl, glacial acetic acid, 100 °C, 1 h, basic workup, 88%; (iv) (d) trifluoroacetic acid, dichloromethane, triethylsilane, 0 °C–rt, 1 h, basic workup, 73%; (v) 1 N HCl in anhydrous diethyl-ether, diethyl-ether as solvent, quantitative yields.

potassium or sodium hydroxide solutions) afforded low yields. The free amine were then transformed into their respective dihydrochloride salts using hydrochloric acid in anhydrous diethyl ether to provide **1** and **2** as readily water-soluble compounds.

3. Results and discussion

The butylated hydroxy toluene (BHT) (2,6-di-*tert*-butyl-4-methylphenol) moiety of compounds **1** and **2** is a feature common to many antioxidants. The antioxidant activity of BHT is believed to be partly due to its capacity to scavenge free radicals and by doing so ultimately terminate the free-radical chain oxidation cycle. The capacity of **1** and **2** to act as antioxidants was measured by their ability to inhibit iron-induced lipid peroxidation (LPO) in rat brain microsomes.[6] LPO induced by Fe^{2+} is due to the generation of free radicals where the antilipoperoxidant activity of **1** and **2** is measured by their ability to reduce the concentration of malondialdehyde, a product of unsaturated fatty acid peroxidation.

The compounds **1** and **2** were expected to have improved antioxidant activity compared to BHT. This may be partly attributed to the extra stabilisation of the phenoxy radical (product of free radical scavenging) provided by the π-conjugated thiazole system in **1** and **2** compared to the simple aromatic nucleus of the BHT compound. As can be seen from Table 1, the compounds are potent inhibitors of LPO. As antioxidants they are approximately 30–60 times more potent than BHT and the antioxidant drug edaravone,[7] a recently launched neuroprotective agent.

The mitochondrial protecting activities of the compounds were evaluated in specific in-vivo mitochondrial toxin models. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and potassium cyanide (KCN) are neurotoxins that block ATP production at different sites of the mitochondrial respiratory chain.[8] The toxins inhibit respectively complex I (NADH dehydrogenase) and IV (cytochrome c oxidase) of the respiratory chain causing free radical generation and ultimately leading to neuronal cell death.

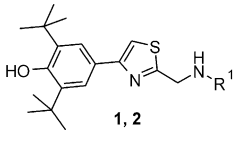
The compounds were evaluated for their ability to protect against KCN induced toxicity in mice as a measure of their anti-anoxic effect.[9] Compounds were administrated orally 1 h before the KCN challenge and the time between KCN injection and the last gasp (the survival time) was determined.

The antioxidants BHT and edaravone orally administrated were inactive while the thiazole derivatives were found to dramatically delay death (see Fig. 1A). The compounds **1** and **2** were found to have ED_{50}'s of 9.3 and 9.7 mg/kg.

The remarkable protection observed for these compounds in this acute model of intoxication was shown where the mice treated with the highest dose of 30 mg/kg were still alive 24 h after cyanide intoxication.

MPTP administration to rodents induces a selective and extensive destruction of dopaminergic neurons in the substantia nigra pars compacta and provokes the depletion of brain dopamine thus providing a striking pathological analogy to Parkinson's disease.[10] Monoamine oxidase-B (MAO-B) mediates the conversion of MPTP to 1-methyl-4-phenylpyridinium (MPP^+), its

Table 1. The effect of compounds **1**, **2**, BHT and Edaravone on the inhibition of lipid peroxidation and protection against potassium cyanide induced lethality and the neuroprotective effect of **1** and **2** on MPTP-mediated loss of dopamine.



Compd	R^1	LPO inhibition IC_{50}, μM[a]	KCN induced lethality ED_{50}, mg/kg[b]	MPTP mediated dopamine loss ED_{50}, mg/kg[b]
1	H	0.54	9.3	6.5
2	CH_3	0.93	9.7	4.3
BHT		30.00	NS[c]	—
Edaravone		32.00	NS[d]	—

[a] Values are from one experiment in quadruplicate.
[b] Dose producing 50% activity, $n = 9$–10, see Figure 1.
[c] NS, non significant effect when tested at 30 mg/kg (po).
[d] NS, non significant effect when tested up to 100 mg/kg (po).

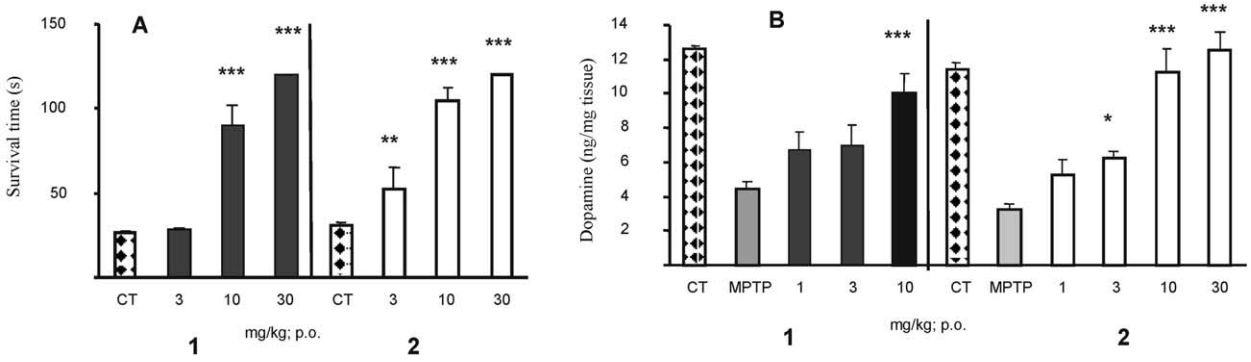


Figure 1. In vivo mitochondria protecting models: (A) the neuroprotective effect of **1** and **2** (po) on the cyanide intoxication in mice. *Significance, compared with control group (CT), **$p<0.01$ and ***$=p<0.001$ (Student and Dunnett tests), $n=9$–10; (B) the neuroprotective effect of **1** and **2** (po) on MPTP-mediated loss of dopamine in the mouse striatum. *Significance, compared with MPTP group, *$p<0.05$ and ***$p<0.001$ (Student and Dunnett tests), $n=5$–6.

toxic metabolite accumulating in the mitochondria where it disrupts cellular respiration.

Oral administration of **1** at 1, 3, 10 mg/kg and **2** at 1, 3, 10, 30 mg/kg antagonised, in a dose-dependent manner, dopamine depletion in the mouse striatum[11] induced by MPTP (see Fig. 1B). The ED_{50} for **1** was found to be 6.5 mg/kg and for **2** was 4.3 mg/kg. The remarkable protection afforded by these compounds in this model can be seen where **1** provided a 68% protection against brain dopamine depletion at 10 mg/kg and **2** at the same dose provided 100% protection.

In conclusion, we have synthesised new phenolic thiazoles **1** and **2** which are potent antioxidants. They are orally bioavailable and afford potent in vivo neuroprotection in models of mitochondrial toxin administration. Since the neurotoxins administrated act at different levels of the mitochondrial respiratory chain it may be considered that these compounds are acting as mitochondrial protecting agents.[5b] Moreover, it has recently been shown that **1** provided increased survival and neuroprotective effects in a transgenic mouse model of Huntington's disease.[12]

In the light of these promising results, compounds **1** and **2** merit further study.

Acknowledgements

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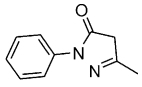
References and notes

1. (a) Alexi, T.; Borlongan, C. V.; Faull, R. L.; Williams, C. E.; Clark, R. G.; Gluckman, P. D.; Hughes, P. E. *Prog. Neurobiol.* **2000**, *60*, 409. (b) Albers, D. S.; Beal, M. F. *J. Neural. Transm.* **2000**, *59*, 133. (c) Beal, M. F. *Biochim. Biophys. Acta* **1998**, *1366*, 211.
2. Powis, G.; Briehl, M. *J. Pharmacol. Ther.* **1995**, *68*, 149.
3. Hosokawa, M.; Ueno, M. *Neurobiol. Aging* **1999**, *2*, 117.
4. (a) Floyd, R. *Free. Radical. Biol. Med.* **1999**, *26*, 1346. (b) Gonzalez-Scarano, F.; Baltuch, G. *Annu. Rev. Neurosci.* **1999**, *22*, 219. (c) Hirsch, E. C.; Hunot, S.; Damier, P.; Faucheux, B. *Ann. Neurol.* **1998**, *44*, S115. (d) McGeer, P. L.; McGeer, E. G. *Alzheimer. Dis. Assoc. Disord.* **1998**, *12* (Suppl. 2), S1.
5. (a) Harnett, J. J.; Auguet, M.; Viossat, I.; Dolo, C.; Bigg, D.; Chabrier, P.-E. *Biorg. Med. Chem. Lett.* **2002**, *12*, 1439. (b) Chabrier, P. E.; Roubert. V.; Harnett, J. J.; Cornet, C.; Delaflotte, S.; Charnet, C.; Spinnewyn. B.; Auguet, M. *New Neuroprotective Agents Are Potent Inhibitors of Mitochondrial Toxins: In Vivo and In Vitro Studies*; 31st Annual Meeting of the Society for Neuroscience, San Diego, CA, Nov. 10–15, 2001. (c) Spinnewyn, B.; Cornet, S.; Auguet, M.; Chabrier, P.-E. *J. Cereb. Blood Flow Metab.* **1999**, *19*, 139.
6. Esterbauer, H.; Cheeseman, K. H. *Methods Enzymol.* **1990**, *186*, 407.
7. (a) Edaravone (MCI-186, Radicut™): Graul, A.; Castane, J. *Drugs Future* **1996**, *21*, 1014. (b) Tabrizchi, R. *Curr. Opin. Investig. Drugs* **2000**, *1*, 347.



Edaravone

8. (a) Nicklas, W. J.; Vyas, I.; Heikkila, R. E. *Life. Sci.* **1985**, *36*, 2503. (b) Webb. J. L. In *Enzymatic and Metabolic Inhibitors*; Academic: New York, 1966. (c) Araki, H.; Karasawa, Y.; Nojiri, M.; Aihara, H. *Methods Find. Exp. Clin. Pharmacol.* **1988**, *10*, 349.
9. CD1 male mice were used, weighing 23–27 g (Charles River, France). The test drugs were administrated orally (20 mL/kg) 1 h before a bolus injection of KCN (4 mg/kg, iv, the LD_{100}). The mice were then observed for 120 s, noting the survival time, which represented the time between KCN injection and the last gasp.
10. (a) Burns, R. S.; Chiueh, C. C.; Markey, S. P.; Ebert, M. H.; Jacobowitz, D. M.; Kopin, I. J. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4546. (b) Langston, J. W.; Forno, L. S.; Rebert, C. S.; Irwin, I. *Brain. Res.* **1984**, *292*, 390. (c) Tetrud, J. W.; Langston, J. W. *Science* **1989**, *245*, 519.
11. The study was performed on C57bl6 mice. Striatal dopamine levels were measured by HPLC one day after treatment with MPTP (three intraperitoneal injections of 20 mg/kg at 2-h intervals). Compounds **1** and **2** were dissolved in water (10 mL/kg) and administered orally 90 min before each MPTP injection, 30 min after the last MPTP injection and 24 h after the beginning of the experiment.

24 h after the last MPTP injection the whole brain was removed, the striatum dissected, frozen in solid CO_2 and stored at −80 °C. Dopamine was measured by adding homogenisation solution containing L-cysteine, $Na_2S_2O_5$ and $HClO_4$ to the striatum (40 mg/mL). The striatum was then homogenised with Ultraturax 30 s in ice and the homogenate centrifuged for 10 min at 2000*g* at 4 °C. The supernatant was re-centrifuged at 4000*g* for 1 min to ensure removal of solid matter. Aliquots (20 μL) of the striatum extracts were then analysed by HPLC. A HPLC apparatus with electrochemical detection was used to measure striatal levels of dopamine. Dopamine was separated using a refrigerated Waters 717 plus Ultrawisp autoinjector at 6 °C; a Beckman Ultrasphere ODS C18 reverse phase, 5-μ column (4.5 × 150 mm) at 24 °C, using a ANTEC Leyden electrochemical detector set at a potential of 770 mV. Samples were eluted at 1 mL/min. The system was recalibrated after every six samples. The mobile phase consisted of 90% of 70 mM KH_2PO_4, 0.1 mM EDTA, 2.1 mM TEA and 1.25 mM sodium octane sulfate pH = 3.7 and 10% methanol. Methanol was added after pH adjustment.

12. Klivenyi, P.; Ferrante, R. J.; Gardian, G.; Browne, S.; Chabrier, P.-E.; Beal, M. F. *J. Neurochem.* **2003**, *86*, 267.