A NON-TOXIC ISONIAZID-DERIVED HYDRAZONE EFFECTIVELY DISRUPTS COPPER AND ZINC INTERACTIONS WITH THE AMYLOID-β PEPTIDE: IMPLICATIONS FOR AD TREATMENT

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“If you think that biochemistry is the organic chemistry of living systems, then you are misled; *biochemistry is the coordination chemistry of living systems.*”

J. M. Wood

*(Naturwissenschaften 1975, 62, 357)*
Review Article

Metallostasis in Alzheimer's disease
Scott Ayton¹, Peng Lei¹, Ashley I. Bush* 
Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, VIC 3010, Australia

Role of metal dyshomeostasis in Alzheimer's disease†
David J. Bondader, Hyung-gon Lee,a Jeffrey A. Blair,a Xiongwei Zhu,a George Perryab and Mark A. Smithd 

The Metal Theory of Alzheimer’s Disease
Ashley I. Bush* 
Oxidation Biology Laboratory, Mental Health Research Institute, The University of Melbourne, Parkville, VIC, Australia
In 2004: Prof. Ashley I. Bush and co-workers introduce the concept of MPAC (Metal-Protein Attenuating Compound).

“\textit{The vast majority of effort in Alzheimer science and drug making has been focused on the perils of amyloid. Clearly, this focus is struggling, possibly because failed drug trials have targeted the disease too late in its natural history, but also possibly because the toxic amyloid hypothesis is too simplistic.}”


Literature Background

Our MPAC (INHHQ)

- One-step synthesis;
- Good yields and high atom economy;
- Environmentally friendly process;
- INH: cheap and FDA-approved anti-TB drug.


National Patent Deposit (BR 10 2013 033006 0)

C₁₆H₁₂O₂N₄Cl₂Zn
428.6 g mol⁻¹
%Experim.(%Calcd.): C, 45.3(44.8); H, 2.7(2.8); N, 13.3(13.1)

C₁₆H₁₁O₂N₄ClCu·3H₂O
444.4 g mol⁻¹
%Experim.(%Calcd.): C, 42.7(43.2); H, 3.3(3.9); N, 12.4(12.6)
This Work...

In silico pharmacological analyses

In vitro INHHQ/Cu-Aβ and INHHQ/Zn-Aβ interaction experiments

In vivo acute toxicity studies

In silico pharmacological analyses
Results and Discussion

Interaction of INHHQ with Aβ? $^1$H Nuclear Magnetic Resonance spectroscopy

INHHQ does not interact directly with the amyloid-β peptide
Results and Discussion

Interaction with the Aβ-Cu(II) and the Aβ-Zn(II) systems:

Disruption of Metal-Protein interactions

NMR (\textsuperscript{1}H-\textsuperscript{15}N HSQC) spectroscopy

Upon addition of 5 eq of INHHQ, signal recovery is almost complete!
**In silico** pharmacological analyses: ABSORPTION

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PBT1 (clioquinol)</th>
<th>PBT2</th>
<th>INHHQ</th>
<th>DFO (desferrioxamine)</th>
<th>Deferiprone</th>
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<tr>
<td>HBD</td>
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<td>1</td>
<td>2</td>
<td>5</td>
<td>2</td>
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<tr>
<td>HBA</td>
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<td>3</td>
<td>6</td>
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<tr>
<td>MW</td>
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<td>243.9</td>
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<td>log P</td>
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<td>log S</td>
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<td>PSA (Å²)</td>
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<td>Rotatable bonds</td>
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<tr>
<td>Drug score</td>
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<td>81%</td>
<td>68%</td>
<td>12%</td>
<td>97%</td>
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</table>

1D-QSAR (log P, log S, pKₐ) and the Lipinski rule of five

Theoretical pKₐ values: 3.12 (py), 9.00 (−OH) and 9.76 (−NH)
**In silico** pharmacological analyses: **METABOLISM**

90% of drug metabolism occurs through oxidation by the cytochrome P450 superfamily of enzymes

**In silico** pharmacological analyses: **TOXICITY** (comparison to 3,300 drugs and 15,000 chemicals)

**Completely ATOXIC!!!**
In vivo acute toxicity studies: MALE WISTAR RATS

$N = 15$ (total)

• 04 controls

• 04 injected only with the Vehicle
  [10% DMSO in NaCl (0.9%)]

• 07 injected with 300 mg kg$^{-1}$ INHHQ
  (1.0 mL 100 g$^{-1}$ corporal weight)

Conditions

• 72 hours of experiment

• Ad libitum feed

• 12-hour photoperiod

• Removal of brain, liver, kidneys and heart

No animals died within 72 h
Normal behavior
No macroscopic irregularities observed during dissection
In vivo acute toxicity studies: Metallothionein (MT) levels in the brain by spectrophotometry

MTs are thermo-stable proteins involved in biometal homeostasis and protection against oxidative stress...

No statistically significant differences were observed between the groups!
Results and Discussion

*In vivo* acute toxicity studies: **Biometal levels in the brain** by ICP-MS

The only statistically significant difference observed was a slight reduction in Cu levels in the **Vehicle + INHHQ** group, when compared to the controls (p<0.05)
Conclusions

• INHHQ inhibits the interaction of Aβ with biometals, \textit{in vitro}, by a mechanism that probably involves metal ion sequestering;

• Theoretical calculations show ideal values for Lipinski parameters and that the compound is able to pass the hematoencephalic barrier;

• INHHQ is not toxic in concentrations up to 300 mg kg$^{-1}$ for a mammal model system, as predicted by the \textit{in silico} pharmacological analyses;

• No significant differences were observed between brain MT levels when comparing the group injected with the compound to the controls;

• Brain metal levels were not different between the groups, except for a slight reduction in Cu concentrations in the INHHQ-injected animals;

• The present results indicate that this hydrazone is an excellent candidate for further pharmaceutical trials.