Resurrecting the Dead: Recovery of Organophosphorus Poisoned Acetylcholinesterase using Quinone Methide Precursors

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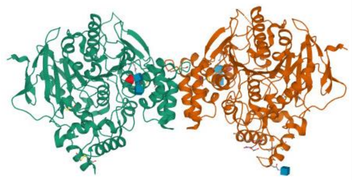
Introduction

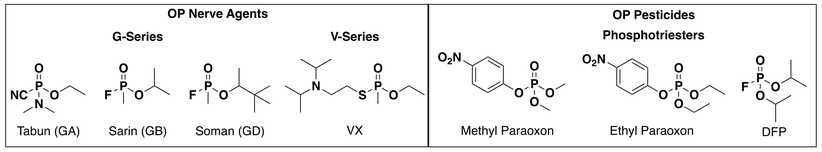
Acetylcholinesterase (AChE):

- A vital enzyme located in the blood, as well as the central and peripheral nervous system
- Activity due to a Ser-His-Glu catalytic triad with multiple amino acid residues working together



Organophosphorus Nerve Agents and Pesticides:

- Responsible for inhibition of AChE by phosphylation of the active site serine residue
- Pesticides estimated to cause 3 million hospitalizations & 220,000 deaths annually[1]



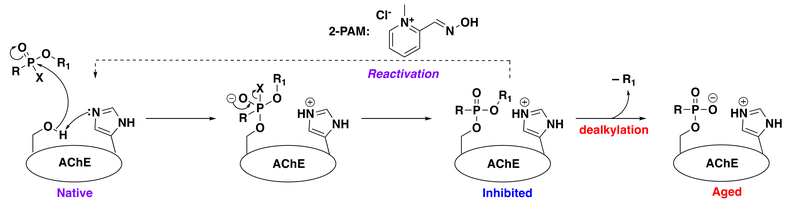
OP Inhibition and Aging of AChE:

Inhibition of AChE can result in death by respiratory failure.

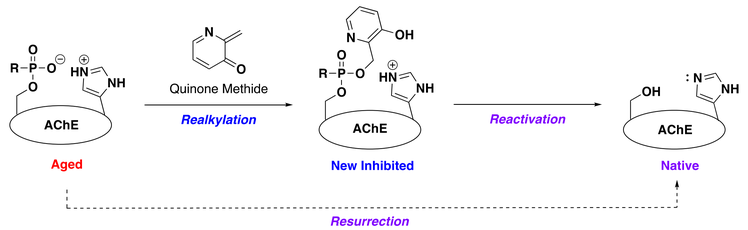
- OP-inhibited form can be reactivated to its native state with pyridinium oximes, like FDA-approved pralidoxime chloride (2-PAM).

OP-inhibited enzyme can dealkylate to the "aged" form of AChE (shown in red below), that forms an oxyanion at the phosphylated serine residue.

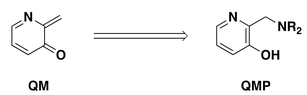
- The aged form of AChE is resistant to pyridinium oxime therapeutics.
- Currently there are no approved therapeutics for the OP-aged form.



Hypothesis: "resurrect" the OP-aged form to the native state with a quinone methide to re-alkylate the aged form of AChE,[2] along with subsequent reactivation:



A Quinone Methide (QM) must be disguised as a Quinone Methide Precursor (QMP)[3] for drug-like utility:

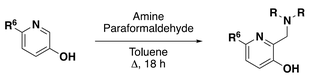


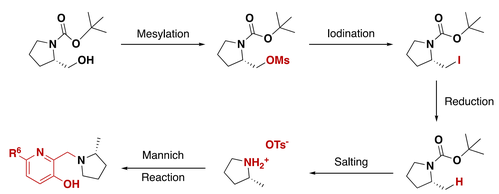
1. Nosseir, O.; Hadad, C. *Chemical Warfare Agents & Treatments*; ACS In Focus; American Chemical Society: Washington, DC, USA, 2021.
2. Zhuang, Q.; et al. *J. Med. Chem.* **2018**, *61*, 7034–7042.
3. Weinert, E.: et al. *J. Am. Chem. Soc.* **2006**, *128*, 11940–11947.

Methodology

QMP Synthesis:

- QMPs typically synthesized with a Mannich reaction

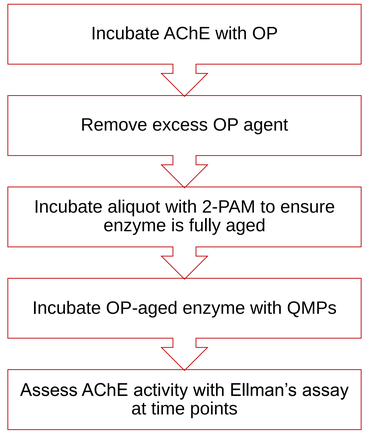




(*R*)-2-methylpyrrolidine QMP Synthesis

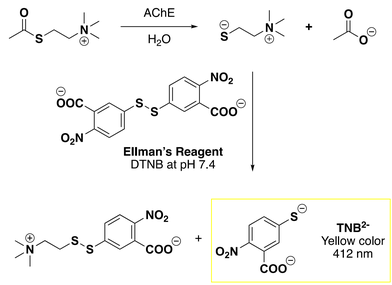
- Cost from Millipore-Sigma is $179/g
- This salt is efficient for the Mannich reaction

Mid-Throughput Biochemical Assay Preparation



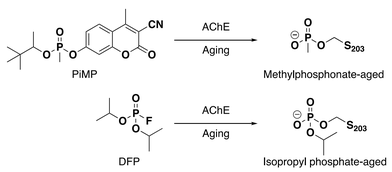
Ellman's Assay:

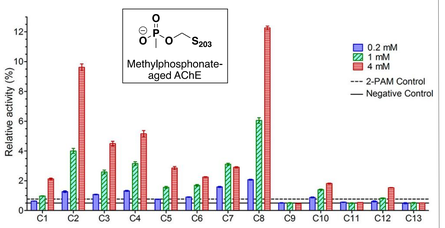
- Indirect colorimetric assay to detect native AChE
- Assay can be completed after QMP incubation with OP-inhibited or OP-aged enzyme



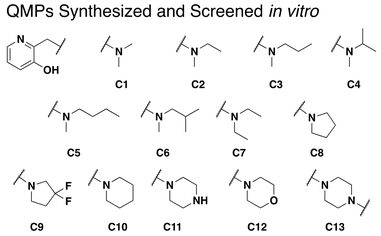
Initial Resurrection QMP Family[2] - Electric eel AChE (24-hour QMP incubation)

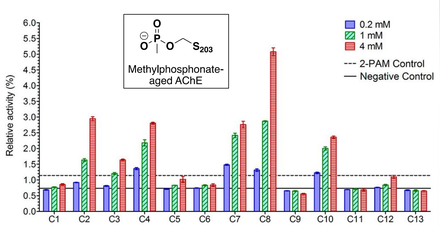
OP Agents and OP-aged forms of AChE





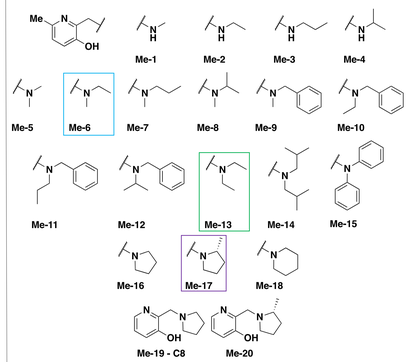
QMPs Synthesized and Screened *in vitro*





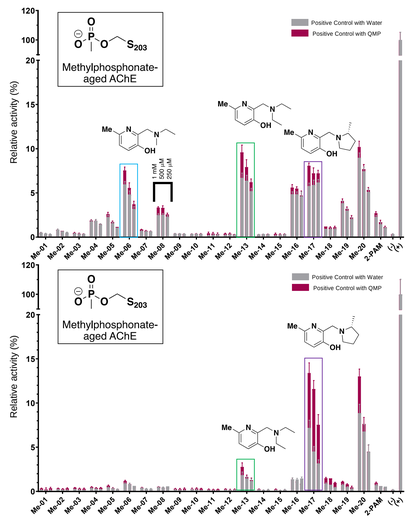
6-methylpyridin-3-ol based QMPs – *manuscript in preparation*

- *C. perl* AChE – monomeric human AChE – hypothesized to be a better model for *in vitro* screening
- Screening at 1 mM, 500 μM, 250 μM
- 24-hour QMP incubation



Observed increased resurrection:

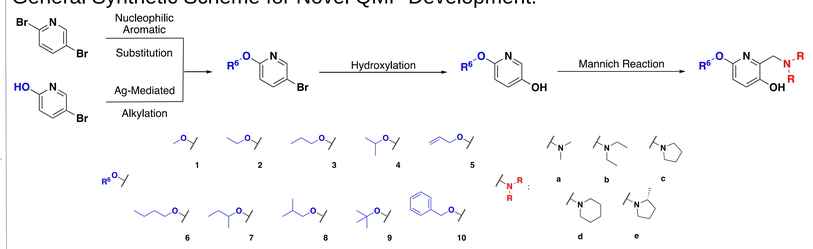
- Me-6
- Me-13
- Me-17



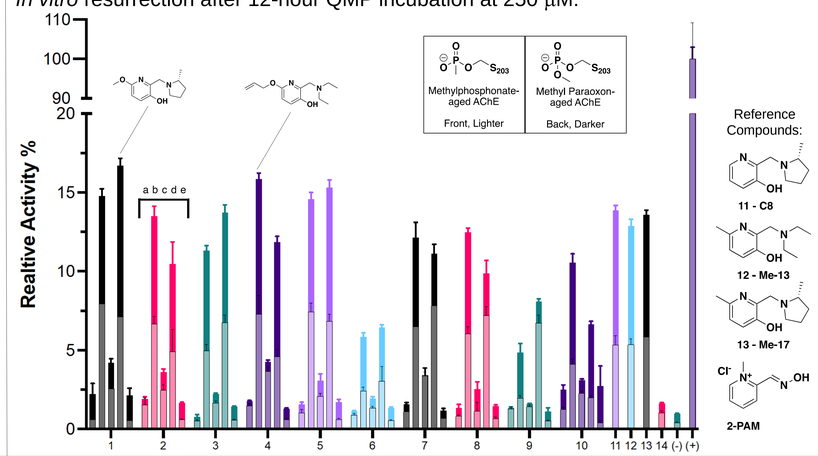
6-alkoxypyridin-3-ol based QMPs – *manuscript in preparation*

- *C. perl* AChE – monomeric human AChE

General Synthetic Scheme for Novel QMP Development:



In vitro resurrection after 12-hour QMP incubation at 250 μM:



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