Bridging The Gap: Providing Selectivity And Specificity For Potential Therapeutics Against Organophosphorus Intoxication By Connecting Quinone Methide Precursors To Peripheral Binding Site Linkers

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INTRODUCTION

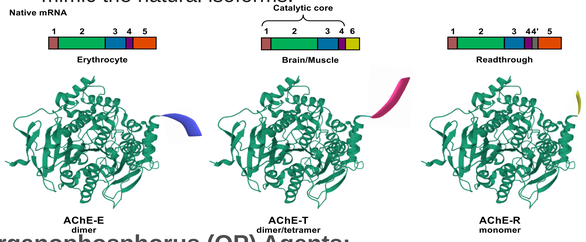
Acetylcholinesterase (AChE):

Hydrolyzes the neurotransmitter acetylcholine at the catalytic triad: Serine 203, Histidine 447, Glutamate 334

- 25,000 acetylcholine (ACh) molecules per second

Three human isoforms are found in the blood as well as the peripheral and central nervous systems.

- Many recombinant human variants can be expressed to mimic the natural isoforms.



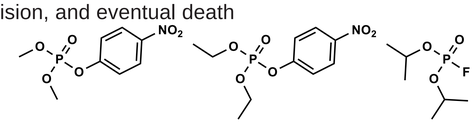
Organophosphorus (OP) Agents:

Two varieties: chemical warfare agents and pesticides

Initially developed around World War II

Covalently binds to AChE, preventing hydrolysis of ACh

- Symptoms include muscle spasms, convulsions, reduced vision, and eventual death



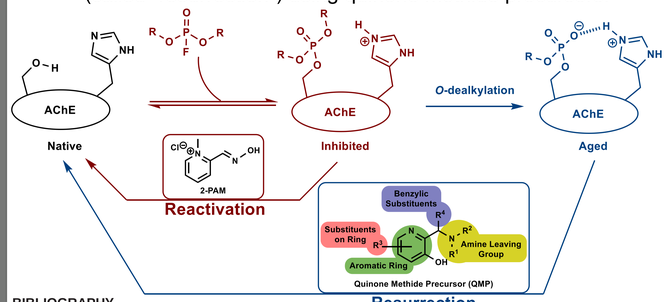
Stages of OP Intoxication and Ways to Revive AChE

Stage One: Inhibition of catalytic serine of AChE by OP

- Treated with known, approved nucleophilic oximes (2-PAM)
- Reversal of inhibited state is deemed reactivation.

Stage Two: Spontaneous *O*-dealkylation of the phosphylated serine residue

- Dealkylated state, or the aged form of AChE, is inactive (considered “dead”).
- No approved treatments exist.
- We have demonstrated the reversal of the OP-aged state (called “resurrection”) using quinone methide precursors.



BIBLIOGRAPHY

(1) Franjesevic, A. J., *et al. Chem. Eur. J.* **2019**, 25 (*21*), 5337–5371.

INSPIRATION

Noncovalent inhibition of native AChE by Donepezil

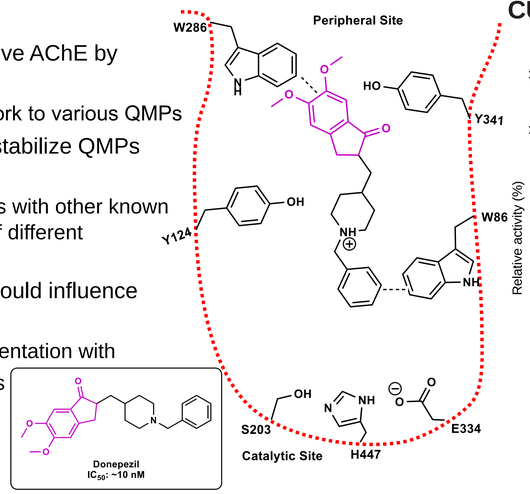
- “Link” indanone framework to various QMPs

Utilize the peripheral site to stabilize QMPs within the active site.

- Need to continue studies with other known noncovalent inhibitors of different frameworks

Presence of diastereomers could influence QMP efficiency.

- Separation and experimentation with individual diastereomers is in progress.



METHODS

Pesticide metabolites (MP, EP) and pesticide (DFP) were purchased through MilliporeSigma to produce the aged or inhibited form of recombinant human AChE.

Aging is confirmed by attempted reactivation by 2-PAM before QMPs are introduced.

Size-exclusion columns were utilized to remove excess QMP to prevent the inhibition of native AChE post-reactivation or resurrection.

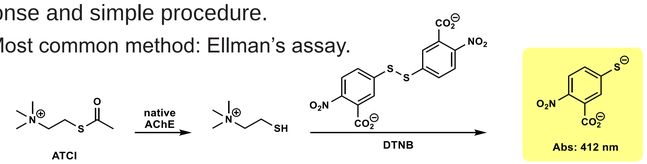
Donepezil was added to native AChE to demonstrate the effectiveness of the size exclusion columns.

Cross-correlation between experiments is possible through reference compounds and (-)/(+) controls.

- 2-PAM with (-) control, Donepezil HCl with (+) control
- References are constantly added to the list as more screening results are obtained.

To determine whether QMPs can reactivate or resurrect OP-exposed AChE, colorimetric or photometric methods are typically used due to their quick response and simple procedure.

Most common method: Ellman’s assay.



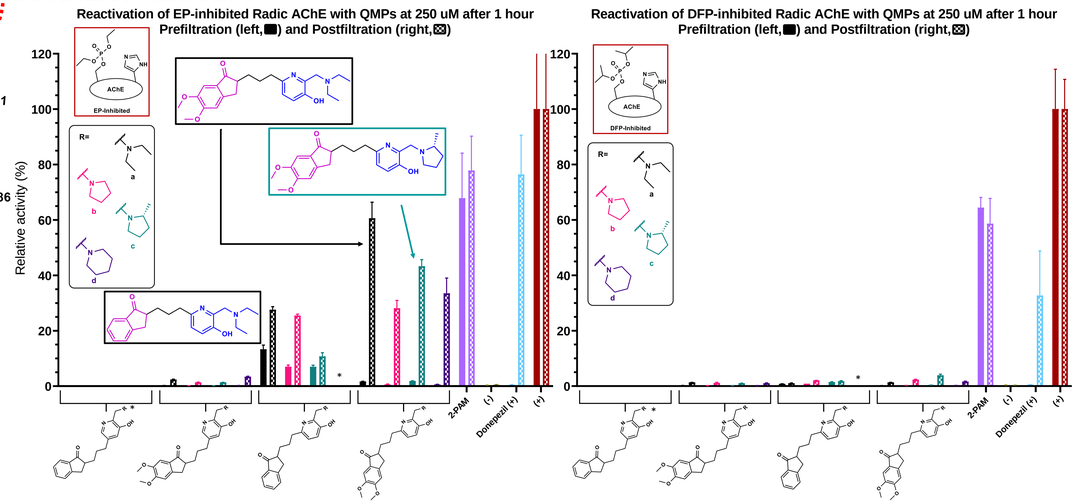
By measuring the initial change of absorbance over time, a relative percent of reactivation or resurrection can be obtained by comparing the originally poisoned enzyme to a positive control (AChE which was never exposed to OP).

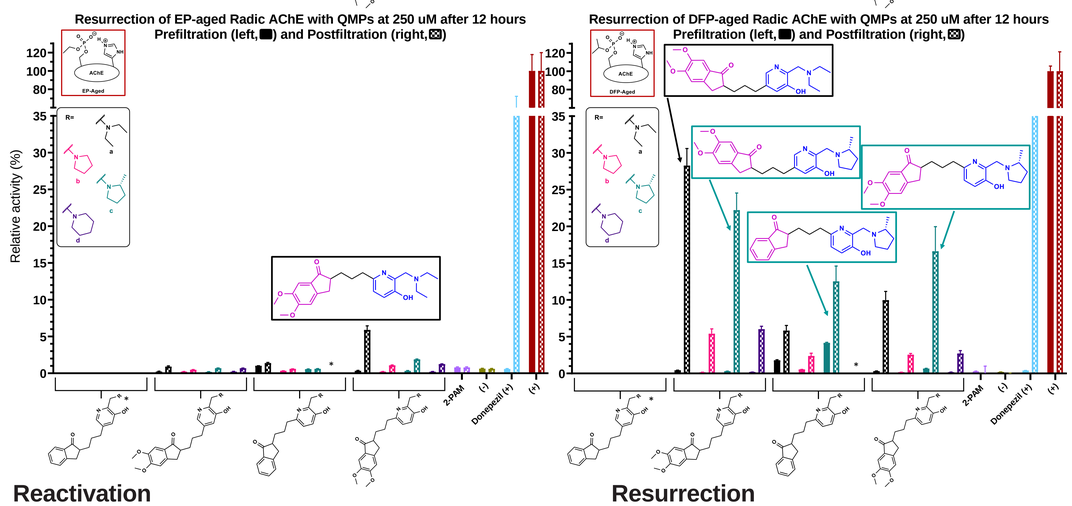
*No data are available for the 5-substituted QMPs containing the indanone without methoxy groups and the 6-substituted QMP containing a piperidine leaving group and the indanone without methoxy groups.

Both resurrection and reactivation experiments.

Each QMP showed increased AChE activity post-filtration, indicating noncovalent binding to native enzyme.

CURRENT RESULTS





Reactivation

MP was excluded due to its shorter aging half-life.

Linker QMPs ineffective against DFP-inhibited AChE.

Less than 10% reactivated after 1 hour.

6-substituted QMPs are better at reactivating EP-inhibited AChE than 5-substituted.

Opposite trend from resurrection experiments.

Diethylamine leaving group outperformed all other amine leaving groups.

Pyrrolidine, (*R*)-2-methylpyrrolidine, and piperidine groups behaved similarly.

No Linker QMP is better than 2-PAM at reactivating DFP or EP-inhibited AChE.

Six QMPs can reactivate >20% EP-inhibited AChE after 1 hour.

Resurrection

Less than 10% EP-aged or MP-aged (not shown) AChE was resurrected.

The most efficient were 6-substituted QMPs with either a diethylamine or (*R*)-2-methylpyrrolidine leaving group.

5-substituted QMPs were better at resurrecting DFP-aged AChE than 6-substituted.

Methoxy group must be present on the indanone group.

Diethylamine or (*R*)-2-methylpyrrolidine leaving groups outperformed pyrrolidine and piperidine in all cases.

Two QMPs can resurrect >20% of DFP-aged AChE after 12 hours.

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