Mechanistic Insights into the Alkylation Reactions of Quinone Methide Precursors: Studies Towards the Realkylation of Aged Acetylcholinesterase

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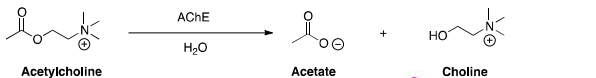
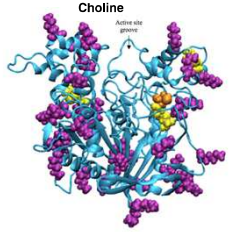
BACKGROUND

The treatment for exposure to chemical warfare agents, such as organophosphorus (OP) nerve agents that inhibit acetylcholinesterase (AChE) causing severe nerve damage, and in some cases death, is an important area of research.



Acetylcholine → Acetate + Choline

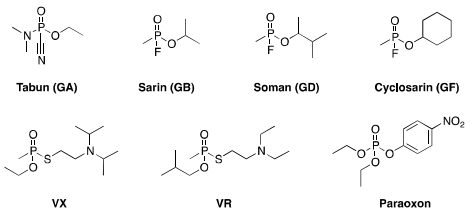
Each AChE degrades 25,000 molecules of acetylcholine per second. The active site is composed of the anionic site and esteratic site. (Ser 203, His 440, Glu 327)



Inhibition

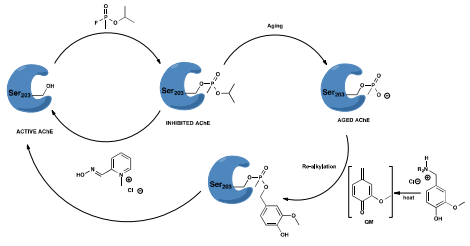
- Bradycardia
- Hypotension
- Hypersecretion
- GI tract hypermotility
- Decrease intraocular pressure
- Bronchoconstriction
- Prolonged muscular contraction

Organophosphorus (OP) Nerve Agents



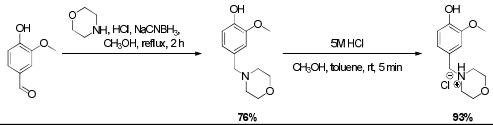
LD_{50} (VX): 20 μg/kg

Organophosphorus Compounds Mode of Action



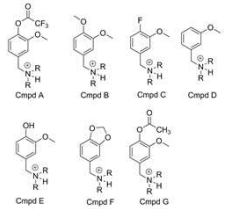
SYNTHESIS

Goal: To successfully synthesize and characterize a library of *para*-quinone methide precursors derived from commercially available materials and to evaluate the nucleophilic reactivity of these precursors with various sulfur, oxygen and nitrogen-based nucleophiles.



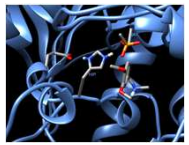
MOLECULAR DOCKING

Goal: To examine the nature of the phenolic oxygen on the QMP scaffold and determine its effects on the specificity and selectivity of the QMP for the dealkylated serine residue on the aged AChE enzyme.

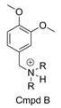


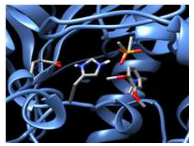
Compound	All Within 3.7A (%)	Within 3.7A, reactive orientation (%)
Cmpd A	36.5	31.9
Cmpd B	53.3	37.4
Cmpd C	60	29.7
Cmpd D	64.1	23.2
Cmpd E	45.9	17.6
Cmpd F	58.9	15.6
Cmpd G	49.9	38.8

- Highest scoring compounds either had an acetyl or methoxy subtituent at the phenolic position
- Lowest score was Compound F, which was a substituted phenol with acetal functional group, but had a high quantity of poses in clusters that earned points



Reactive orientation within 3.7A for Cmpd B



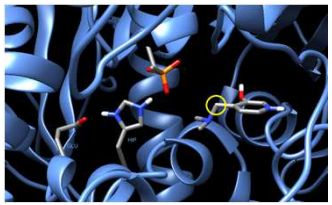


Less reactive orientation within 3.7A for Cmpd B

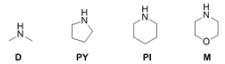
MOLECULAR DYNAMICS

- Distance between benzylic carbon and phosphonylated serine is only variable in zoning
- 3 Zones: Active Site (AS), Bottleneck (BN), Gorge Mouth (GM)
  - AS: 0-5 A
  - Between AS/BN: 5-7 A (open bounds)
  - BN: 7-9.5 A
  - GM: 10-15 A

All bounds are closed unless noted otherwise.



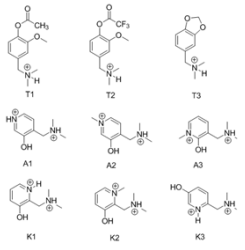
AMINE LEAVING GROUP



- Library of 72 Compounds generated using 9 compounds depicted to the right as base scaffolds
  - 9 Compounds exhibited largest # of poses present in AS during molecular docking
- Variations on base scaffolds were made:
  - LG Variations (4):
    - Dimethylammonia
    - Pyrrolidine
    - Piperidine
    - Morpholine
  - N Protonation States (3):
    - Neutral
    - Protonated
    - Methylated

Protonation State

Neutral, Protonated, Methylated



Above: 9 Best compounds from prior studies

MOLECULAR DYNAMICS

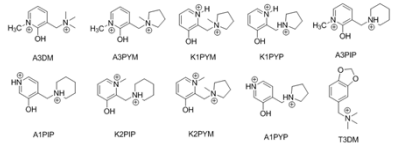
10 Highest % in Active Site

-Majority of compounds had ortho arrangements of the heterocyclic nitrogen to the benzylic carbon

-Majority had pyrrolidine and piperidine leaving groups

-Majority had protonation states of +2

Compound Name	% Active Site	% Between AS and BN	% Bottleneck	% Gorge Mouth	% Other
A2-A3DM	73.7	5.2	3.5	13.5	4.1
A2-A3PYM	71.4	4.6	1.9	17.6	4.5
K2-K1PYM	71.3	5.7	2.4	18.3	2.4
K2-K1PYP	68.6	10.6	2	15.3	3.5
A2-A3PIP	68.5	6.5	4.8	15.3	4.8
A2-A1PIP	67.7	14.1	2.8	8.2	7.3
K2-K2PIP	67.2	8.3	4	15.2	5.3
K2-K2PYM	65.9	12.6	3.4	16.1	2
A2-A1PYP	65.8	16.4	1.8	9.6	6.5
T1-T3DM	63.2	9.5	8.5	14.2	4.5



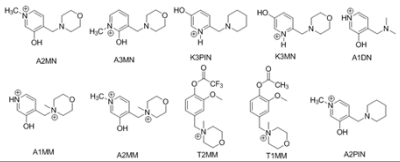
10 Lowest % in Active Site

-Majority of compounds had para arrangements of the heterocyclic nitrogen to the benzylic carbon

-Majority had morpholine leaving group

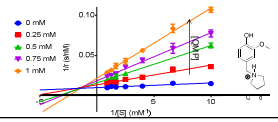
-Majority had protonation states of +1

Compound Name	% Active Site	% Between AS and BN	% Bottleneck	% Gorge Mouth	% Other
A1-A2MN	9.12	29.42	22.55	33.19	5.71
A1-A3MN	11.68	42.33	25.32	17.29	3.38
K1-K3PIN	16.6	30	30.2	19.8	3.4
K1-K3MN	17.8	34.3	25.8	16.9	5.2
A1-A1DN	20.88	35.66	26.87	11.95	4.65
A1-A1MN	21.31	32.82	30.84	12.32	2.72
A2-A2MM	21.5	36.2	11.7	25.1	5.5
T1-T2MM	25.4	15.2	25	31.3	3.2
T1-T1MM	25.73	18.06	23.22	28.99	4
A1-A2PIN	25.71	46.33	15.36	10.69	1.91



FUTURE WORK

-Continued MD simulations on further ligands to correlate with experimental studies
-Examination of ligands in the Native AChE enzyme



ACKNOWLEDGEMENTS AND REFERENCES

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