

Supplemental material

OXIME-ASSISTED ACETYLCHOLINESTERASE CATALYTIC SCAVENGERS OF ORGANOPHOSPHATES THAT RESIST AGING

Rory Cochran¹, Jaroslaw Kalisiak², Tuba Kucukkilinc¹, Zoran Radić¹, Edzna Garcia¹, Limin Zhang¹, Kwok-Yiu Ho¹, Gabriel Amitai³, Zrinka Kovarik⁴, Valery V. Fokin², K. Barry Sharpless², and Palmer Taylor¹

¹Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, CA 92093-0650

²Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037

³Division of Medicinal Chemistry, Israel Institute for Biological Research, P.O. Box 19, Ness Ziona 74100, Israel

⁴Institute for Medical Research and Occupational Health, P.O. Box 291, HR-10001 Zagreb, Croatia

Running Title: Aging resistant AChE based OP bio-scavengers

Address correspondence to: Palmer Taylor, SSPPS, UCSD, 9500 Gilman Drive, La Jolla, CA 92093-0657; Tel: 858-534-44028; Fax:858-534-8248; E-mail:pwtaylor@ucsd.edu

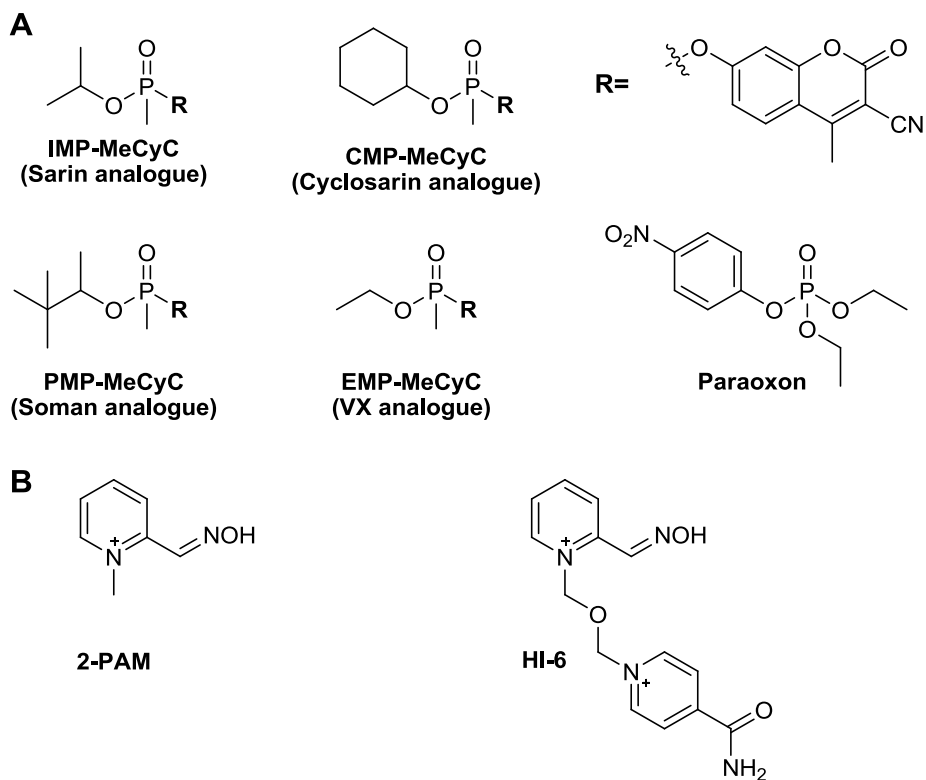
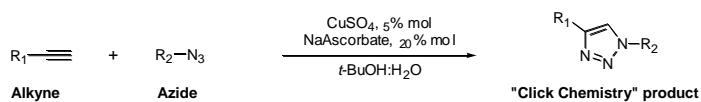
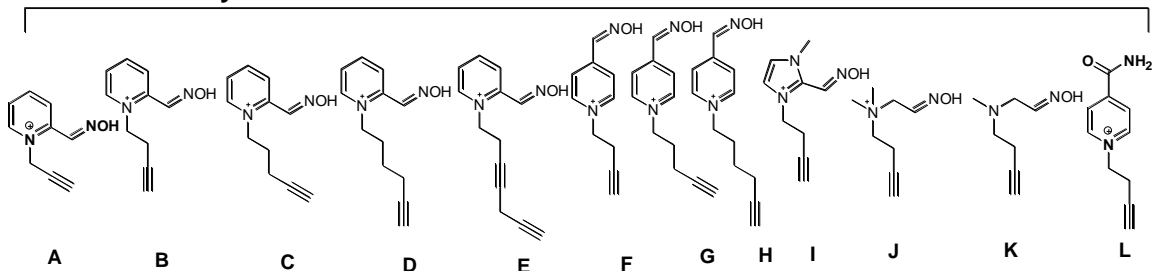


Figure S1. Structures of organophosphate inhibitors and oxime reactivators used in this study.

A) structures of the four Flu-MP nerve agent analogues (sarin, soman, cyclosarin, and VX) and paraoxon, the oxon form of the pesticide, parathion, and B) structures of the oximes 2-PAM and HI-6.



List of used alkynes



A) List of used azides with alkynes B - K

	A	B	C	D	E	F
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

B) List of used azides with alkynes B - G, I, J and L (except combinations LD, LE and LF).

	A	B	C	D	E	F
1						
2						

Figure S2: Library of novel click-chemistry oximes used in this study.

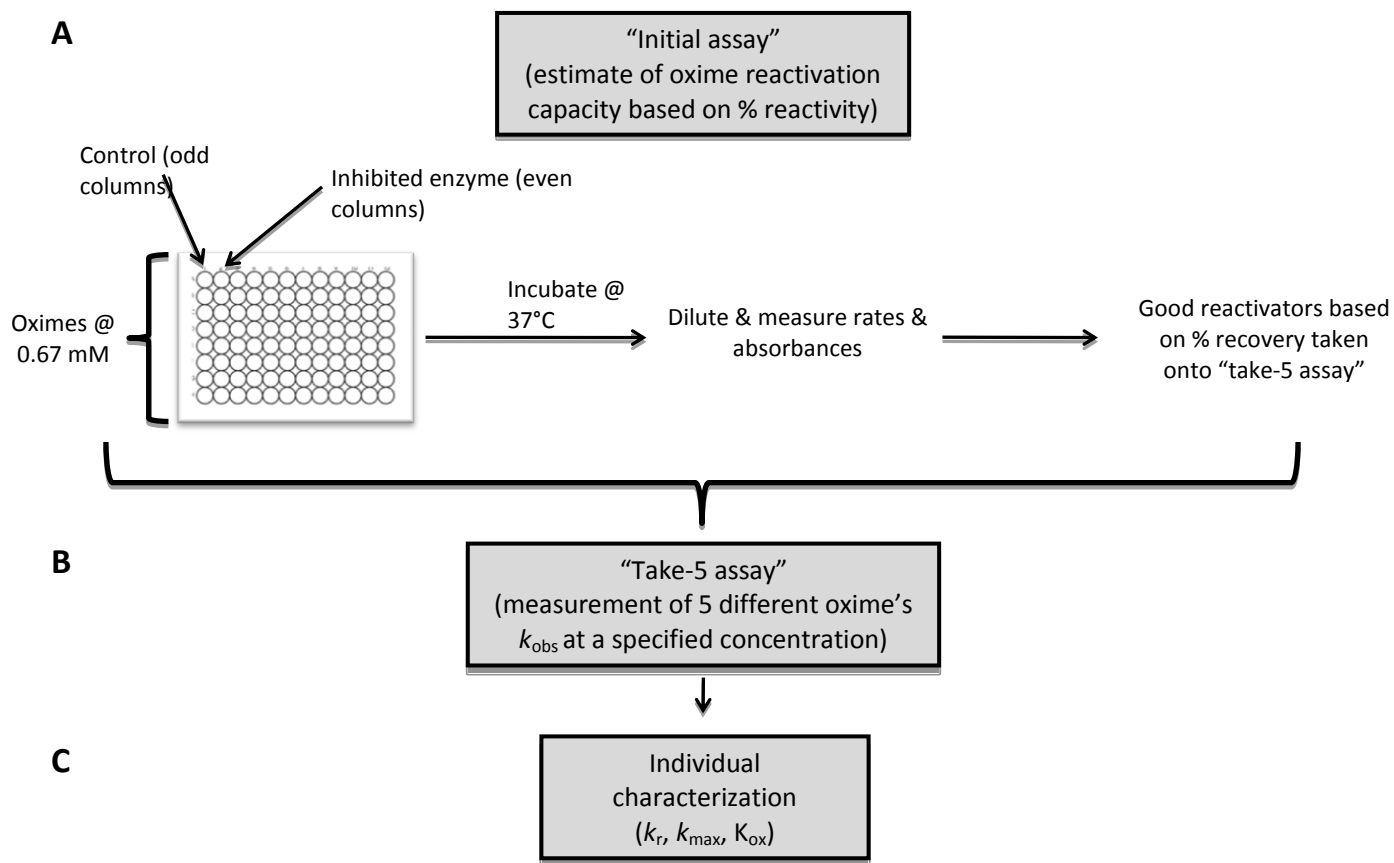


Figure S3. Schematic of the oxime screening procedure. A) oxime capacity for reactivation was assayed initially in a 96-well format. Oximes were aliquoted into two columns (48 oximes screened/96-well plate) for detecting both oxime inhibitory and reactivating properties. B) auspicious oximes were better characterized in a more rigorous format; screening five at a time to obtain k_{obs} . C) best reactivators were characterized to determine their k_r , k_{max} , K_{ox} .

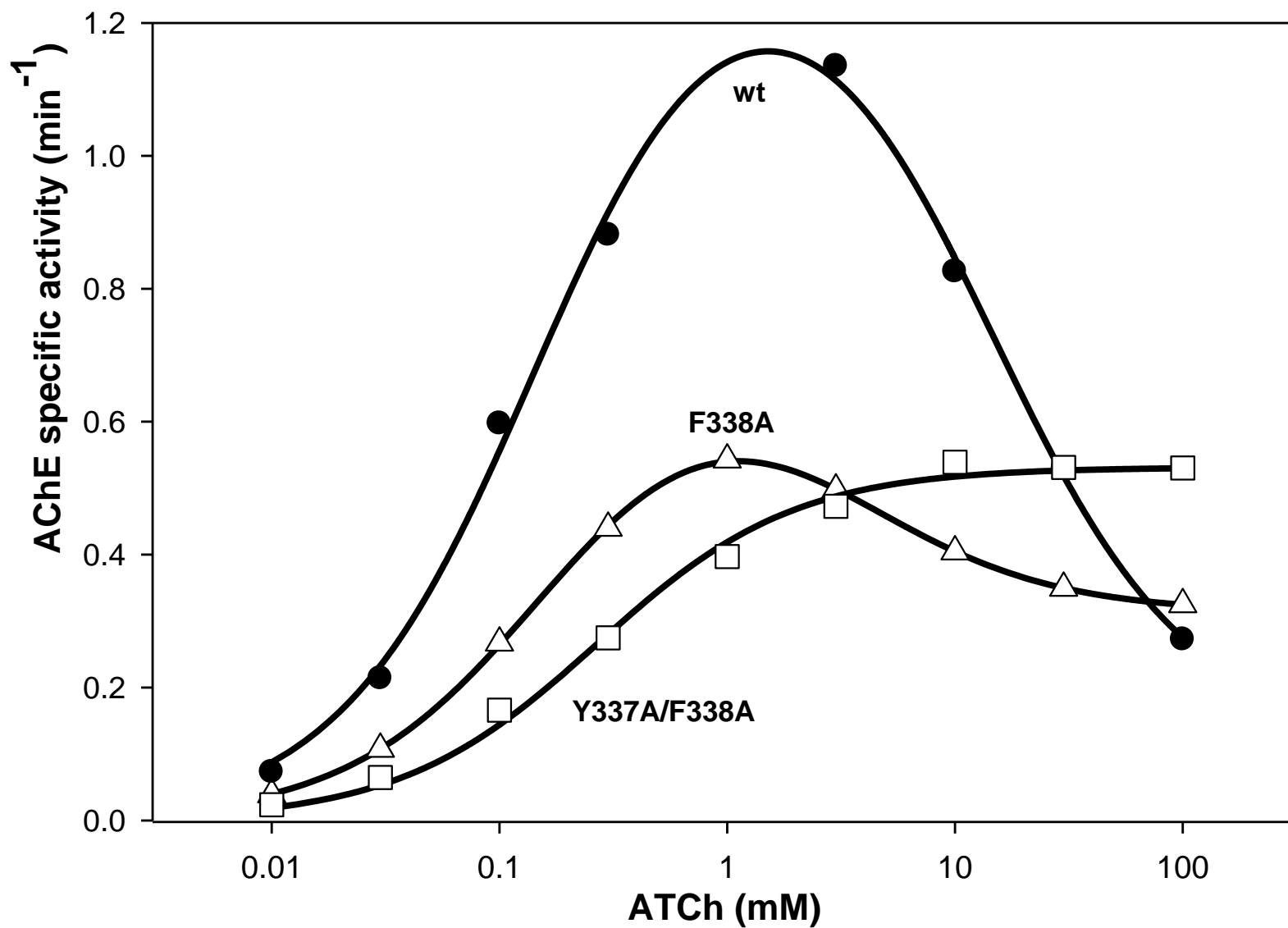


Figure S4. pS curves of enzyme activity for wt hAChE (circles) and mutants F338A (triangles), and Y337A/F338A (squares) in 0.1M phosphate buffer pH 7.4 at 22°C. Curves are obtained by nonlinear regression and resulting constants are given in Table 1.

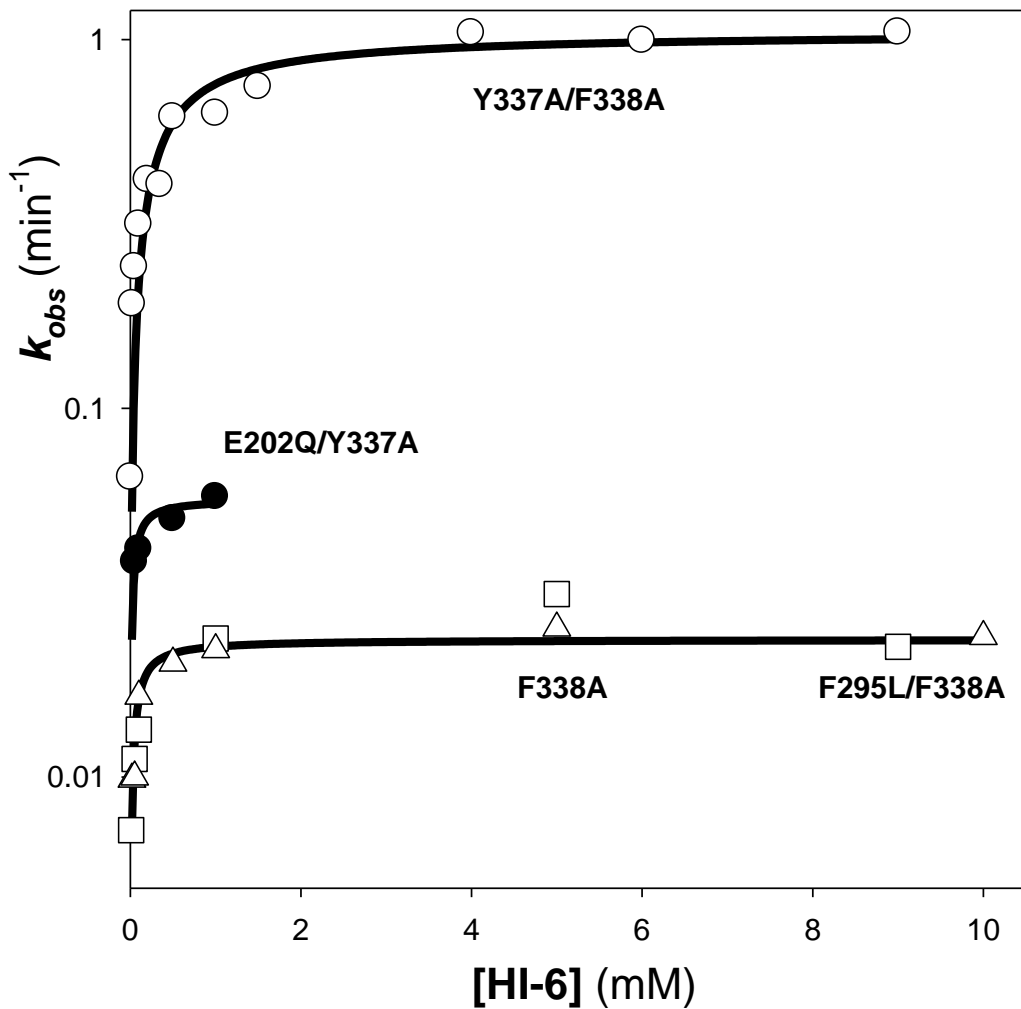


Figure S5. Semi-log representation of reactivation kinetics of soman inhibited human AChE mutants refractory to aging, by HI-6, presented in Figure 2. Reactivation constants determined by nonlinear regression of data for human AChE mutants F338A (squares), E202Q/Y337A (black circles), F295L/F338A (triangles) and Y337A/F338A (white circles) in 0.1M phosphate buffer pH 7.4 at 22°C are given in Table 3.

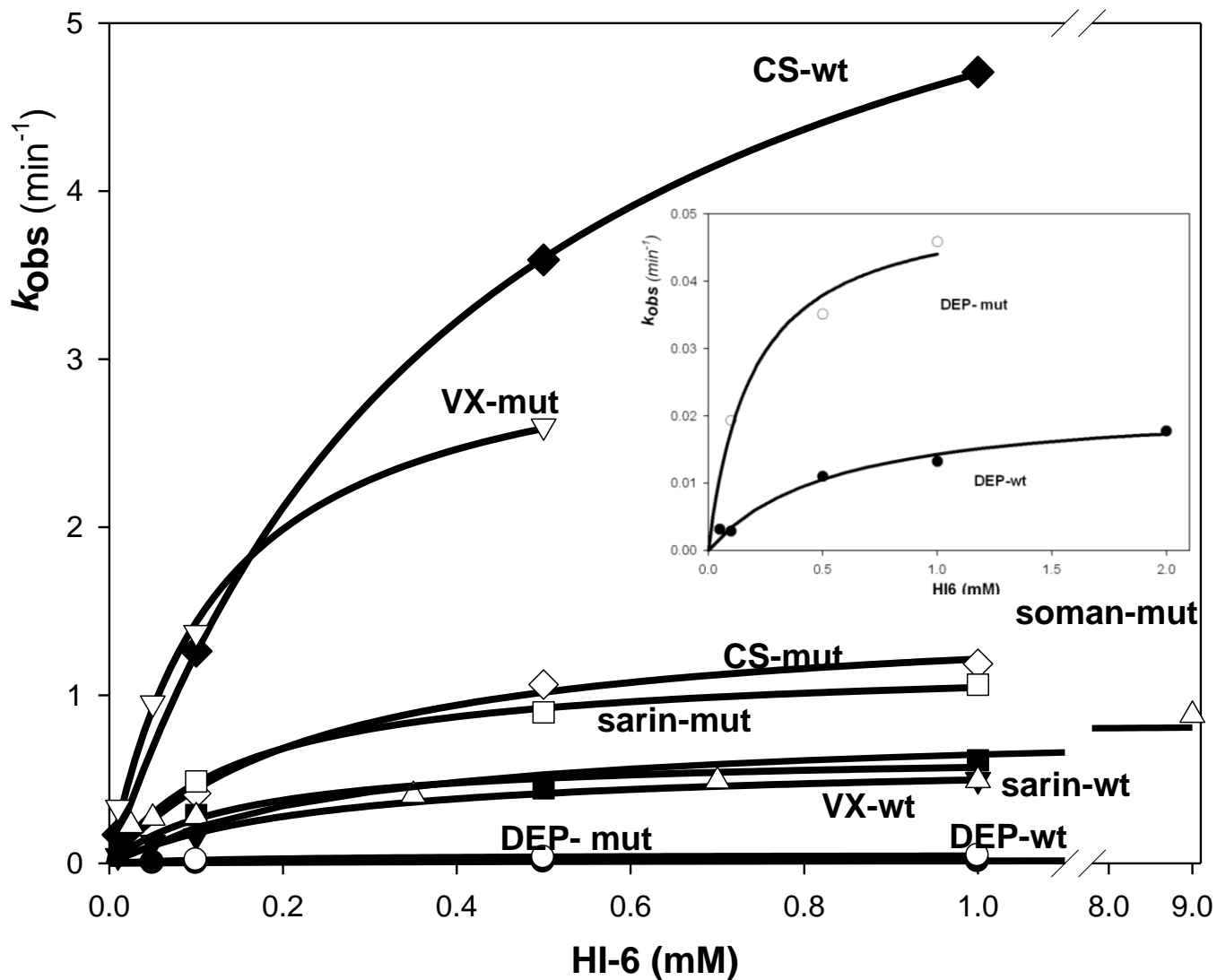
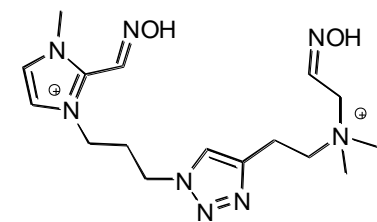
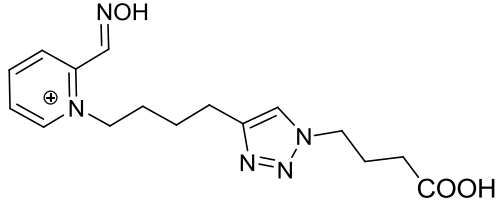
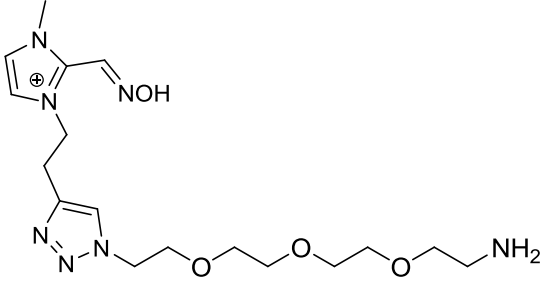


Figure S6. Reactivation kinetics for OP inhibited human AChE wild-type (black symbols) and Y337A/F338A mutant (white symbols) with HI-6 in 0.1M phosphate buffer pH 7.4 at 22°C. Enzymes were inhibited by paraoxon (circles), VX (inverted triangles), sarin (squares), cyclosarin (diamonds) and soman (triangles). Reactivation constants obtained by nonlinear regression are given in Table 4. Inset: Expanded Y-axis representation of paraoxon inhibited hAChE reactivation kinetics.

Table S1. Three top-ranked oxime candidates for reactivation of hAChE Y337A/F338A mutant inhibited with paraoxon. Reactivation was carried out in 0.1 M phosphate buffer pH 7.4 at 37 °C with 0.67 μM oxime concentration.

Oxime	$k_{obs}(\text{min}^{-1})$	Structure
177	0.38	
D-C6	0.29	
I-F4	0.27	
2-PAM	0.18	