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Imidazole Aldoximes Effective in Assisting Butyrylcholinesterase Catalysis of Organophosphate Detoxification

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ABSTRACT: Intoxication by organophosphate (OP) nerve agents and pesticides should be addressed by efficient, quickly deployable countermeasures such as antidotes reactivating acetylcholinesterase or scavenging the parent OP. We present here synthesis and initial in vitro characterization of 14 imidazole aldoximes and their structural refinement into three efficient reactivators of human butyrylcholinesterase (hBChE) inhibited covalently by nerve agent OPs, sarin, cyclosarin, VX, and the OP pesticide metabolite, paraoxon. Rapid reactivation of OP−hBChE conjugates by uncharged and nonprotonated tertiary imidazole aldoximes allows the design of a new OP countermeasure by conversion of hBChE from a stoichiometric to catalytic OP bioscavenger with the prospect of oral bioavailability and central nervous system penetration. The enhanced in vitro reactivation efficacy determined for tertiary imidazole aldoximes compared to that of their quaternary N-methyl imidazolium analogues is attributed to ion pairing of the cationic imidazolium with Asp 70, altering a reactive alignment of the aldoxime with the phosphorus in the OP−hBChE conjugate.

INTRODUCTION

The recent massive exposure of Syrian citizens to the nerve gas organophosphate (OP) sarin fatally injured hundreds,† because of the lack of appropriate antidote intervention. It vividly illustrated an immediate need for effective, affordable, and easily administered countermeasures for rapid protection of large populations from OP exposure. Currently approved antidotal therapies for the treatment of OP poisoning in humans encompass intramuscular injections of pyridinium aldoximes,2PAM, HI6, obidoxime, toxogonin, or similar agents2,3 encompass intramuscular injections of pyridinium aldoximes,3 therapies for the treatment of OP poisoning in humans populations from OP exposure. Currently approved antidotal administered countermeasures for rapid protection of large populations from OP exposure. Currently approved antidotal therapies for the treatment of OP poisoning in humans encompass intramuscular injections of pyridinium aldoximes,2PAM, HI6, obidoxime, toxogonin, or similar agents2,3 combined with a muscarinic acetylcholine receptor antagonist (atropine) and an anticonvulsant (benzodiazepine). Alternatively, an intravenously injected highly purified human butyrylcholinesterase (hBChE) can serve as a scavenging agent for the organophosphate in the circulation4−7 that is effective both pre- and post-OP exposure.7,8

Pyridinium aldoxime therapy, developed nearly 60 years ago in the seminal work of Wilson and colleagues,9 is directed toward nucleophilic reactivation of acetylcholinesterase (AChE) covalently inhibited by OPs to restore catalytic hydrolysis of the neurotransmitter acetylcholine (ACh). Protection by pyridinium aldoximes requires parenteral administration and is limited by their rapid elimination and inability to cross the blood−brain barrier. The OPs are lipophilic, become sequestered in lipids where they leach from tissues, and thereby allow residual concentrations to persist. Accordingly, antidotal therapy may require repeated administration to sustain appropriate concentrations in target tissues.

Intravenous injection of purified hBChE affords the potential of covalently conjugating the parent OP molecules distributed in the circulation, thus protecting target tissue AChE from OP inhibition. The molecular weight ratio of hBChE to OP forming the OP−hBChE conjugate can be as high as 500−600, so a large mass of BChE is required for it to be an effective scavenger. While proven to be effective in animal studies, stoichiometric scavenging in plasma by hBChE therapy is limited by cost and the practicalities of wider administration to an exposed or potentially exposed population.

Joint administration of an efficient oxime reactivator of the OP−hBChE conjugate and purified hBChE protein to assist catalysis and turnover of the offending OP in theory should reduce the amount of hBChE needed for efficient protection by establishing a “catalytic bioscavenger” system.10 We recently demonstrated the feasibility, both in vitro and in vivo, of a
catalytic bioscavenger composed of purified hBChE and a cationic non-pyridinium aldoxime, TAB2OH, with selectivity for BChE reactivation. While the catalytic bioscavenger shows a decrease in the size of administered effective hBChE doses, the net protective effects of our catalytic bioscavenger were small because of the relatively low reactivation potency of TAB2OH. Nevertheless, this oxime is to the best of our knowledge the fastest characterized BChE reactivator reported in the literature.

In this study, we follow up on our earlier observation that simple N-alkyl imidazole aldoximes can be good reactivators of hBChE and design more effective hBChE reactivators. Although tertiary imidazole-based aldoximes have not been previously described in the literature as cholinesterase reactivators, quaternary imidazolium aldoximes were extensively studied as mono-oxime OP−AChE reactivators and also as bis-oximes in combination with pyridinium and quinuclidinium oximes. Some of these compounds were characterized as promising for reactivation of tabun- and soman-inhibited AChE, both in vitro and in vivo. Reactivation of OP-inhibited BChE by either tertiary or quaternary imidazole-based aldoximes, on the other hand, had not been described in the literature.

Herein, we thus characterize in vitro reactivation properties of a family of uncharged tertiary imidazole aldoximes and their quaternary methylimidazolium analogues against four different OP−hBChE conjugates resulting from sarin, cyclosarin, VX, and paraoxon inhibition of BChE and AChE and show structural features necessary for efficient OP−hBChE conjugate reactivation. The absence of charge in some of these tertiary imidazole aldoximes bears the promise of reasonable oral bioavailability and retention in tissue as well as potential for central nervous system (CNS) penetration despite some agents having limited solubility. The quaternary analogues are expected to have smaller volumes of distribution and higher initial plasma concentrations.

### RESULTS AND DISCUSSION

**Chemistry.** On the basis of our observation that simple N-alkyl-substituted imidazole aldoximes are good reactivators of OP−hBChE conjugates, we synthesized a series of imidazole-2-aldoxime derivatives substituted at N1. This was achieved by alkylation of formylimidazole (1) with the requisite bromide or mesylate, followed by treatment with hydroxylamine as shown in Scheme 1. Using this protocol, a diverse array of aliphatic, aromatic, and unsaturated substituents were incorporated into the N1 position of the imidazole ring (compounds 3−10).

Next, we sought to improve water solubility and nucleophilicity of oxime derivatives via the preparation of quaternary salts by methylating the N3 atom of the imidazole ring. Thus, the quaternization was conducted by reacting selected corresponding mono-oximes with iodomethane to deliver compounds 11−14.

In addition to the 12 imidazole compounds, two cationic mono-oxime derivatives were synthesized. For the preparation of 17 and 18 (Scheme 2), formylimidazole 1 was first reacted...
with 1,3-dibromopropane in N,N-dimethylformamide (DMF) using K2CO3 as a base while being stirred at room temperature overnight to obtain 1-(3-bromopropyl)-1H-imidazole-2-carboxaldehyde (15). Treatment of 15 with hydroxylamine and Na2CO3 at room temperature afforded 1-(3-bromopropyl)-1H-imidazole-2-carboxaldehyde oxime (16). Heating oxime 16 with pyridine derivatives at 50 °C afforded 17 and isonicotinamide derivative 18.

### Initial Selection of Oxime Structures

Our previously published screen of 135 uncharged oxime reactivators revealed that simple N-alkyl-substituted imidazole aldoximes were good reactivators of OP–hBChE conjugates. That study

![Scheme 2](image)

Reagents: (a) 1,3-Dibromopropane, K2CO3, DMF, rt; (b) NH2OH.HCl, H2O, Na2CO3, rt; (c) CH3NO2, 50 °C

| Oxime   | \( k_{\text{obs}} \) (min\(^{-1}\)) |
|---------|-----------------------------------|
|         | Norm. Avrg | POX | Sarin | CS | VX |
| RS-115C | 75 %       | 0.029 | 0.250 | 0.590 | 0.370 |
| RS-115B | 110 %      | 0.023 | 0.680 | 0.480 | 0.400 |
| RS-115A | 89 %       | 0.054 | 0.220 | 0.650 | 0.480 |
| RS-113B | 160 %      | 0.190 | 0.120 | 0.440 | 1.200 |
| RS-113A | 110 %      | 0.085 | 0.180 | 0.650 | 0.860 |
| 10      | 53 %       | 0.0084 | 0.089 | 1.030 | 0.0280 |

The table shows the dependence of reactivation on the length of the oxime N-alkyl chain, for the five RS oximes, and oxime 10. The normalized average (Norm. Avrg) \( k_{\text{obs}} \) was calculated by averaging four \( k_{\text{obs}} \) values for individual OPs, each expressed as a percentage of the average \( k_{\text{obs}} \) of all (six in this table) different oximes for that single OP. Experiments were performed at 37 °C in 0.1 M phosphate buffer (pH 7.4) in duplicate.
was, however, focused on identifying optimal uncharged reactivators of OP–hAChE conjugates; imidazole aldoximes did not surface as optimal candidates. Herein we revisit OP–hBChE conjugate reactivation by imidazole aldoximes and analyze their potencies for reactivation of four individual OP–hBChE conjugates, three identical to those obtained by nerve agent sarin, cyclosarin, and VX inhibition and the fourth obtained by paraoxon inhibition. The nerve agent OP–hBChE conjugates were prepared using Flu-MPs, low-toxicity nerve agent analogues yielding OP–hBChE covalent conjugates identical with the ones formed upon inhibition with nerve agents. The first-order reactivation rate constants at a single concentration (0.67 mM) of six initial oximes determined under physiological conditions [0.1 M phosphate buffer (pH 7.4) at 37 °C] are listed in Table 1. It appears that the length of the alkyl chain does affect reactivation rates. The N-pentyl derivative RS-113B appeared as the most efficient reactivator of the four conjugates, while shortening or lengthening the alkyl chain oximes generally decreased efficiency. This trend was most evident for VX and paraoxon. Reactivation rate constants for the sarin-derived conjugate peaked at the smaller N-propyl derivative RS-115B, and reactivation of the largest OP–hBChE conjugate, generated by cyclosarin inhibition, was the fastest of all conjugates and for all oximes with a slight preference for the

| Oxime | $k_{obs}$ (min$^{-1}$) | POX | Sarin | CS | VX |
|-------|----------------------|-----|-------|----|----|
|       | Norm. Avrg           |     |       |    |    |
| 3     | 390 %                | 0.37| 0.75  | 6.0| 1.2|
| 4     | 350 %                | 0.86| 0.31  | 1.1| 3.0|
| RS-113B | 110 %                | 0.19| 0.12  | 0.44| 1.2|
| 5     | 66 %                 | 0.15| 0.15  | 0.12| 0.36|
| 6     | 67 %                 | 0.049| 0.35| 0.11| 0.10|
| 11    | 51 %                 | 0.013| 0.033| 1.6| 0.018|
| 17    | 33 %                 | 0.017| 0.057| 0.65| 0.10|
| 8     | 15 %                 | 0.016| 0.032| 0.12| 0.095|
| 7     | 9 %                  | 0.017| 0.034| 0.023| 0.023|
| 18    | 7 %                  | 0.0030| 0.016| 0.095| 0.030|
| 9     | 3 %                  | 0.0084| 0.0038| 0.011| 0.015|
| 2PAM  | 120 %                | 0.050| 0.65 | 0.29| 0.25|

The table shows the dependence of reactivation on the substitution at the terminus of the substituted N-alkyl chain. Oximes are ordered by the normalized average (Norm. Avrg) $k_{obs}$ (for a description and experimental conditions, see Table 1). Values for 2PAM were not included in the evaluation of the $k_{obs}$ average.

The concentration (0.67 mM) of six initial oximes determined under physiological conditions [0.1 M phosphate buffer (pH 7.4) at 37 °C] are listed in Table 1. It appears that the length of the alkyl chain does affect reactivation rates. The N-pentyl derivative RS-113B appeared as the most efficient reactivator of the four conjugates, while shortening or lengthening the alkyl chain oximes generally decreased efficiency. This trend was most evident for VX and paraoxon. Reactivation rate constants for the sarin-derived conjugate peaked at the smaller N-propyl derivative RS-115B, and reactivation of the largest OP–hBChE conjugate, generated by cyclosarin inhibition, was the fastest of all conjugates and for all oximes with a slight preference for the
longest N-alkyl derivative, 10. Because, of six studied imidazole oximes, the N-pentyl imidazole RS-113B appeared to be the most universal efficient reactivator of the four OP–hBChE conjugates, it was selected as a template for further optimization.

**Optimization of Oxime Structures.** On the basis of the RS-113B structure, seven uncharged and three monocationic mono-oxime derivatives with varying substitutions of the alkyl chain were prepared (Table 2). Their reactivation potencies at a concentration of 0.67 mM were compared to the potencies of RS-113B and of a very short N-alkyl derivative, 9 (Table 2). The simple introduction of a double bond at the end of the N-pentyl alkyl chain yielded the most efficient oxime reactivator 3; it was on average 3-fold faster than RS-113B but particularly efficient for reactivation of cyclosarin, sarin, and VX conjugates of hBChE. Equally efficient was the N-dimethylbutyl imidazole 4; its high efficiency was drastically reduced by elimination of a single methyl to yield 6. General trends for 13 tested imidazole oximes listed in Table 2 seem to favor a hydrophobic group positioned four methylenes from the imidazole ring. Inserting

| Oxime | $k_{obs}$ (min⁻¹) |
|-------|------------------|
|       | Norm. Avrg | POX | Sarin | CS | VX |
| 12    | 45 %       | 0.017 | 0.18 | 2.5 | 0.096 |
| 14    | 12 %       | 0.0049 | 0.078 | 0.24 | 0.059 |
| 13    | 81 %       | 0.032 | 0.23 | 5.7 | 0.084 |
| 3     | 200 %      | 0.37 | 0.75 | 6.0 | 1.2 |
| 4     | 220 %      | 0.86 | 0.31 | 1.1 | 3.0 |
| 5     | 41 %       | 0.15 | 0.15 | 0.12 | 0.36 |
| 2PAM  | 73 %       | 0.050 | 0.65 | 0.29 | 0.25 |
| TAB2OH| 60 %       | 0.17 | 0.087 | 1.6 | 0.62 |
| Hi6   | 27 %       | 0.017 | 0.15 | 0.87 | 0.11 |
| MMB4  | 15 %       | 0.021 | 0.11 | 0.13 | 0.056 |
| TMB4  | 18 %       | 0.027 | 0.12 | 0.17 | 0.083 |
| obidoxime | 22 % | 0.025 | 0.12 | 0.64 | 0.069 |

The table shows the dependence of reactivation on the substitution at the end of the oxime N-alkyl chain. For a description of Norm. Avrg and experimental conditions, see Table 1. Values for 2PAM, TAB2OH, Hi6, MMB4, TMB4, and obidoxime were not included in the evaluation of the $k_{obs}$ average.
an azido group to terminate the alkyl chain (compounds 7 and 8) was counterproductive for reactivation for all OPs. Furthermore, introduction of positive charge generally reduced the reactivation efficacy. For example, quaternization of imidazole into the N-methyl imidazolium ring of RS-113B to yield 11 reduced the reactivation efficiency by \( \sim 1 \) order of magnitude, except for that of the more bulky cyclosarin conjugate; its reactivation efficiency was enhanced. Introduc-
tion of pyridinium in place of a phenyl ring of 5, to yield 17, resulted in a similar pattern. Additional small modifi-
cations of the pyridinium ring in 17 to yield 18 decreased the reactivation efficiency.

Of the 10 tested RS-113B analogues from Table 2, the two highest-ranking reactivators 3 and 4 were markedly superior for all OP–hBChE conjugate combinations, on average by 4-fold, and significantly better than common reference oximes, 2PAM, HI6, TMB-4, MMB-4, and toxogonin (Table 3).

**BChE Reactivation Potencies of Three Selected Imidazole Aldoximes and Their N-Methyl Imidazolium Analogues.** Along with two highest-ranking reactivators 3 and 4, oxime 5 was selected for further structural refinement. Although quaternization of RS-113B, our initial lead from Table 1, had generally negative effects on reactivation potency [conversion of RS-113B into 11 (Table 2)], we decided to prepare and investigate quaternized, imidazolium analogues of 3–5 for several reasons. The first is that imidazolium oximes are expected to be more water-soluble than their tertiary counterparts. Second, quaternization of imidazole nitrogen is expected to change the electronic configuration of the imidazole ring, significantly alter the delocalized system, and reduce the level of protonation of the oxime moiety, thus influencing its nucleophilic reactivity. Finally, reactivation of the cyclosarin OP–hBChE conjugate was enhanced significantly by the imidazolium analogue of RS-113B [conversion of RS-113B to 11 (Table 2)].

Reactivation rate constants of three imidazolium derivatives along with their tertiary analogues for reactivation of the OP–
hBChE conjugate are listed in Table 3. It appears that for only one oxime pair and for only cyclosarin-inhibited hBChE was reactivation enhanced for the quaternary imidazolium, albeit significantly, by \( \sim 50 \)-fold [difference between 5 and 13 (Table 3)]. Otherwise, all imidazolium aldoximes exhibited slower reactivation than their tertiary counterparts. More importantly, however, the series of tertiary imidazole compounds were faster reactivators than TAB2OH, to the best of our knowledge the best OP–hBChE conjugate reactivator published to date, \(^1 \) and in our preliminary in vivo experiments less toxic to mice (data not shown). None of the other commonly used pyridinium aldoximes (HI6, MMB4, TMB4, and obidoxime) showed comparable general reactivation potencies for the OP–hBChE conjugates that were tested. The best overall reactivator of all four OP–hBChE conjugates was 4, being most efficient for the VX–BChE conjugate and several-fold better than any other oxime. Its quaternary analogue, 14, was on average \( \sim 70 \)-fold slower and the slowest of all reactivators in Table 3 for each of four OP–hBChE conjugates.

**Modeling the Oxime–BChE Conjugate.** Computational molecular modeling of reversible interactions of 4 and 14 within the active center gorge of the VX–hBChE conjugate reveals a higher frequency of reactive oxime orientations for 4 when criteria of lowest interaction energy, shortest distance between oximate oxygen and conjugated OP phosphorus, and smallest deviation from the ideal in-line attack geometry are considered. It appears that for 4 six to seven conformers (#0, #2, #3, #6, #9, and #5, in improving order) of 10 calculated conformers showed comparatively productive properties (Figure 1) and could be matched by only two or three
conformers of $14$ (#3, #5, and #7) with similar parameters (Figure 1). Conformers of $14$ were consistently found to bind slightly farther from conjugated phosphorus and significantly closer to aspartate 70 of hBChE located in the opposite corner of the hBChE gorge. This is consistent with a Coulombic attraction between the cationic imidazolium ring and anionic Asp 70 of $\sim 4.8$ Å that is not possible for the nonprotonated tertiary imidazole derivative $4$, for which the distance was consequently $\sim 6.4$ Å (Figure 2). Our preliminary computational analysis thus indicates that this electrostatic interaction may reduce the reactivation efficiency of imidazolium aldoxime $14$ by orienting its tertiary butyl side chain deeper into the hydrophobic hBChE gorge (Figure 2).

**AChE Reactivation Potencies.** Reactivation rates of OP-conjugated hAChEs, on the other hand, were relatively slow for all six imidazole aldoximes (Table 4), consistent with the severely restricted size of the OP−hAChE gorge where insertion of hydrophobic side chains of imidazole aldoximes deeper into the gorge likely forces the oximate group into positions farther from conjugated phosphorus. Reactivation rates of imidazoles did not approach those of smaller, cationic 2PAM, except for that of cyclosarin−hAChE conjugate reactivation by imidazolium aldoximes, $12$ and $13$, yielding rates comparable to that of 2PAM. Typically, for AChE, imidazolium aldoximes were several-fold slower reactivators than 2PAM and their tertiary analogues even another order of magnitude slower than 2PAM. Thus, unlike reactivation of hBChE, charged imidazolium aldoximes were better reactivators of OP−hAChE conjugates than tertiary imidazoles (Figure 3). In comparison with TAB2OH, a poor OP−hAChE conjugate reactivator, tertiary imidazoles were similarly poor OP−hAChE conjugate reactivators, but rates were enhanced by an order of magnitude for quaternary imidazolium reactivators with OP−hAChE conjugates.

**Overall Structure−Activity Comparisons.** In our previous study,$^{11}$ we demonstrated both in vitro and in vivo capacities of TAB2OH, an exocyclic cationic, nonpyridinium aldoxime, to turn over nerve agent OPs catalytically in the presence of purified hBChE. The superior in vitro reactivation potency of several imidazole and imidazolium aldoximes against OP−hBChE conjugates, presented in this study in comparison with TAB2OH, positions this series of compounds very favorably for in vivo studies of catalytic OP turnover mediated by hBChE. The greater than an order of magnitude enhancement of reactivation rates allows one to consider that reactivators of this general family or its second-generation cousins could become sufficiently efficient in vivo to support enhanced OP degradation even in the absence of exogeneous administration of purified hBChE. The concentration of naturally occurring hBChE in human plasma is estimated to be $\sim 70$ nM,$^{22}$ and substantial amounts of this enzyme were detected in lung mucosa and intestine, tissue gateways to absorption of initial amounts of toxicant upon nerve gas or pesticide OP exposure.

Furthermore, imidazole aldoximes, as uncharged entities at physiological pH, should be amenable to a more effective distribution across biological membranes resulting in enhanced oral bioavailability compared to those of pyridinium aldoximes. That would allow them to reach OP-exposed tissue rich in BChE and establish catalytic OP degradation *in situ* following a noninvasive oral administration route.

**CONCLUSION**

Imidazole-based aldoximes are identified in this study as a new class of efficient hBChE reactivators. Starting with initial leads
identified from our synthetic library, we refined several highly efficient tertiary imidazole and quaternary imidazolium aldoximes to achieve an order of magnitude or more enhancement of in vitro OP−hBChE conjugate reactivation rates compared to that of TAB2OH, the most efficient hBChE reactivator published to date. The reduction of a cationic species at physiologic pH values and the prospect of good bioavailability and CNS penetration make tertiary imidazole aldoximes candidates well suited for toxicity, pharmacokinetic, and OP exposure efficacy testing in vivo. Thus, we present here a new family of catalytic bioscavengers of nerve agent and pesticide OPs that are dependent on hBChE reactivation.

Unlike existing reactivators, these imidazole-based aldoximes have the potential capacity to enlist endogenous tissue hBChE and establish catalytic OP degradation directly in the exposed tissue before lipophilic OPs are distributed into peripheral and central cholinergic innervated target tissues and cause the sequelae of cholinergic hyperexcitation.

### EXPERIMENTAL SECTION

#### Preparation of Novel Oximes

**General.** All reactions were performed with commercially available ACS grade reagents and solvents. Anhydrous DMF, acetonitrile, and nitromethane were used as received without further purification. All synthesized compounds were determined to possess a purity of more than 95%, as evidenced by high-performance liquid chromatography analysis and 1H nuclear magnetic resonance (NMR). 1H NMR and 13C NMR spectra were recorded on a Varian 400 MHz spectrometer. All chemical shifts were reported in parts per million relative to solvent resonances, as indicated (DMSO-d6, δ 2.49, 1H; δ 39.49, 13C) (CDCl3, δ 7.26, 1H; δ 77.0, 13C). 1H NMR coupling constants (J) are given in hertz.

**General Method A for the Preparation of Imidazole Oximes** 3−10. To a mixture of formylimidazole 1 and K2CO3 in DMF was added the required bromide or mesylate, and the reaction mixture was stirred overnight under an atmosphere of nitrogen at room temperature (rt). The resulting suspension was cooled to rt and filtered. Water was added to the filtrate, and the resulting solution was extracted with Et2O (3 × 25 mL). The organic layer was dried over MgSO4 and evaporated to give the corresponding alkylimidazole-2-carbaldehyde.

Hydroxylamine hydrochloride (1.5 equiv) was dissolved in water and neutralized with Na2CO3 (1.5 equiv). Alkylimidazole-2-carbaldehyde was added to the solution of hydroxylamine, and the reaction mixture was stirred at rt for 1 h. The resulting precipitate of the corresponding oxime was collected by filtration, rinsed with water, and dried over P2O5 under vacuum.

1-(Pent-4-en-1-yl)imidazole-2-carbaldehyde Oxime (3). Prepared according to general method A using formylimidazole 1 (0.50 g, 5.2 mmol), K2CO3 (0.72 g, 5.2 mmol), and 5-bromopent-1-ene (0.93 g, 6.2 mmol) in DMF (20 mL). Yellow oil 2a (0.55 g, 64%).

1-(Pent-4-en-1-yl)imidazole-2-carbaldehyde Oxime (3). Prepared according to general method A using formylimidazole 1 (0.50 g, 5.2 mmol), K2CO3 (0.72 g, 5.2 mmol), and 5-bromopent-1-ene (0.93 g, 6.2 mmol) in DMF (20 mL). Yellow oil 2a (0.55 g, 64%).

Table 4. Reactivation Rate Constants of 0.67 mM N-Alkyl-Substituted Imidazole and Imidazolium Aldoximes for OP−hAChE Conjugates Prepared and Analyzed As Described in Table 3 for OP−hBChE Conjugates

| Oxime   | kobs (min⁻¹) | Norm. Averg | POX | Sarin | CS | VX |
|---------|--------------|-------------|-----|-------|----|----|
| 2PAM    | 5600 %       | 0.20        | 0.73| 0.067 | 0.48|
| TAB2OH  | 670 %        | 0.025       | 0.022| 0.0080| 0.068|
| 12      | 180 %        | ≤ 0.001     | 0.11| 0.073 | 0.11|
| 14      | 110 %        | ≤ 0.001     | 0.057| 0.022 | 0.099|
| 13      | 150 %        | ≤ 0.001     | 0.11| 0.076 | 0.037|
| 3       | 68 %         | ≤ 0.001     | 0.019| 0.026 | 0.035|
| 4       | 40 %         | ≤ 0.001     | 0.0074| 0.0047| 0.019|
| 5       | 54 %         | ≤ 0.001     | 0.012| 0.012 | 0.034|

Values for 2PAM and TAB2OH were not included in the evaluation of the kobs average.
139.6, 137.6, 128.8, 123.7, 115.4, 46.2, 30.0, 29.4; LC−MS (ESI) [M + H]+ calcd for C_{13}H_{16}N_{3}O m/z 196.3, found m/z 196.3.

1-(3,3-Dimethylbutyl)imidazole-2-carbaldehyde Oxime (2b). Prepared according to general method A using formylimidazole (0.50 g, 5.2 mmol), K_{2}CO_{3} (0.72 g, 5.2 mmol), and 3-azidopropylmethanesulfonate (1 g, 6.2 mmol) in DMF (20 mL). Yellow oil 2b (0.57 g, 61%).

1-(3,3-Dimethylbutyl)imidazole-2-carbaldehyde (2b) (0.54 g, 3 mmol), NH_{2}OH·HCl (0.31 g, 4.5 mmol), water (5 mL), Na_{2}CO_{3} (0.48 g, 4.5 mmol): white solid; yield 0.58 g, 84%; 1H NMR (400 MHz, DMSO-d_{6}) δ 11.47 (s, 1H), 8.05 (s, 1H), 7.34 (s, 1H), 7.28 (t, J = 8, 2H), 7.18 (app d, J = 8, 3H), 7.01 (s, 1H), 4.27 (t, J = 8, 2H), 2.55 (t, J = 8, 2H); 13C NMR (400 MHz, DMSO-d_{6}) δ 141.3, 141.0, 139.6, 128.8, 128.4, 128.1, 125.9, 123.6, 46.5, 32.0, 31.9; LC−MS (ESI) [M + H]+ calcd for C_{13}H_{16}N_{3}O m/z 196.3, found m/z 196.3.

1-(3-Phenylpropyl)imidazole-2-carbaldehyde Oxime (5). Prepared according to general method A using formylimidazole (0.50 g, 5.2 mmol), K_{2}CO_{3} (0.72 g, 5.2 mmol), and (3-bromopropyl)methanesulfonate (1 g, 6.2 mmol) in DMF (20 mL). Yellow oil 2e (0.77 g, 69%).

1-(3-Phenylpropyl)imidazole-2-carbaldehyde (2e) (0.64 g, 3 mmol), NH_{2}OH·HCl (0.31 g, 4.5 mmol), water (5 mL), Na_{2}CO_{3} (0.48 g, 4.5 mmol): white solid; yield 0.58 g, 84%; 1H NMR (400 MHz, DMSO-d_{6}) δ 11.47 (s, 1H), 8.05 (s, 1H), 7.34 (s, 1H), 7.28 (t, J = 8, 2H), 7.18 (app d, J = 8, 3H), 7.01 (s, 1H), 4.27 (t, J = 8, 2H), 2.55 (t, J = 8, 2H); 13C NMR (400 MHz, DMSO-d_{6}) δ 141.3, 141.0, 139.6, 128.8, 128.4, 128.1, 125.9, 123.6, 46.5, 32.0, 31.9; LC−MS (ESI) [M + H]+ calcd for C_{13}H_{16}N_{3}O m/z 196.3, found m/z 196.3.

1-Isopentylimidazole-2-carbaldehyde (2d) (0.50 g, 3 mmol), NH_{2}OH·HCl (0.31 g, 4.5 mmol), water (5 mL), Na_{2}CO_{3} (0.48 g, 4.5 mmol): white solid; yield 0.44 g, 81%; 1H NMR (400 MHz, DMSO-d_{6}) δ 11.48 (s, 1H), 8.03 (s, 1H), 7.32 (s, 1H), 6.99 (s, 1H), 4.25 (t, J = 8, 2H), 1.59−1.46 (m, 6H); 13C NMR (400 MHz, DMSO-d_{6}) δ 141.3, 139.5, 128.8, 123.5, 45.1, 25.1, 22.3; LC−MS (ESI) [M + H]+ calcd for C_{9}H_{14}N_{3}O m/z 182.2, found m/z 182.3.

1-(3-Azidopropyl)imidazole-2-carbaldehyde Oxime (7). Prepared according to general method A using formylimidazole (0.50 g, 5.2 mmol), K_{2}CO_{3} (0.72 g, 5.2 mmol), and 3-azidopropylmethanesulfonate (1.1 g, 6.2 mmol) in DMF (20 mL). Yellow oil 2e (0.62 g, 67%).

1-(3-Azidopropyl)imidazole-2-carbaldehyde (2e) (0.54 g, 3 mmol), NH_{2}OH·HCl (0.31 g, 4.5 mmol), water (5 mL), Na_{2}CO_{3} (0.48 g, 4.5 mmol): white solid; yield 0.46 g, 79%; 1H NMR (400 MHz, DMSO-d_{6}) δ 11.48 (s, 1H), 8.05 (s, 1H), 7.32 (s, 1H), 7.01 (s, 1H), 4.29 (t, J = 8, 2H), 3.33 (t, J = 8, 2H), 1.94 (pent, J = 8, 2H); 13C NMR (400 MHz, DMSO-d_{6}) δ 141.3, 139.6, 128.9, 123.7, 47.9, 42.4, 29.4; LC−MS (ESI) [M + H]+ calcd for C_{8}H_{13}N_{6}O m/z 195.2, found m/z 195.3.

1-(3-Azidobutyl)imidazole-2-carbaldehyde Oxime (8). Prepared according to general method A using formylimidazole (0.50 g, 5.2 mmol), K_{2}CO_{3} (0.72 g, 5.2 mmol), and 3-azidobutylmethanesulfonate (1.2 g, 6.2 mmol) in DMF (20 mL). Yellow oil 2f (0.68 g, 68%).

1-(3-Azidobutyl)imidazole-2-carbaldehyde (2f) (0.58 g, 3 mmol), NH_{2}OH·HCl (0.31 g, 4.5 mmol), water (5 mL), Na_{2}CO_{3} (0.48 g, 4.5 mmol): white solid; yield 0.55 g, 88%; 1H NMR (400 MHz, DMSO-d_{6}) δ 11.54 (s, 1H), 8.08 (s, 1H), 7.33 (s, 1H), 7.02 (s, 1H), 4.26 (t, J = 8, 2H), 3.34 (t, J = 8, 2H), 1.73 (pent, J = 8, 2H), 1.47 (pent, J = 8, 2H); 13C NMR (400 MHz, DMSO-d_{6}) δ 141.2, 139.5, 128.6, 123.7, 50.2, 46.1, 27.5, 25.3; LC−MS (ESI) [M + H]+ calcd for C_{9}H_{14}N_{3}O m/z 209.2, found m/z 209.3.

1-(3-Prop-2-yn-1-yl)imidazole-2-carbaldehyde Oxime (9). Prepared according to general method A using formylimidazole (0.50 g, 5.2 mmol), K_{2}CO_{3} (0.72 g, 5.2 mmol), and 3-propynylmethanesulfonate (1 g, 6.2 mmol) in DMF (20 mL). Yellow oil 2g (0.61 g, 71%).
a nitrogen atmosphere at rt. Water (120 mL) was added to the residual solution, and the solution was treated with EtO (3 × 50 mL). The organic layer was dried over MgSO4 and evaporated to give 1-(3-bromopropyl)-1H-imidazole-2-carboxylic acid (15).

Hydroxylamine hydrochloride (3.2 g, 46.8 mmol) was dissolved in water (20 mL) and neutralized with Na2CO3 (5 g, 46.8 mmol). 1-(3-Bromopropyl)-1H-imidazole-2-carboxylic acid (15) was added to the solution of hydroxylamine, and the reaction mixture was stirred at rt for 2 h. The resulting precipitate of the corresponding oxime 16 was filtered out, rinsed with water, and dried over P2O5 under vacuum to yield a white solid: yield 4.3 g, 60%; 1H NMR (400 MHz, DMSO-d6) δ 115.1 (s, 1H), 8.05 (s, 1H), 7.32 (s, 1H), 4.33 (t, J = 8, 2H), 3.44 (t, J = 8, 2H), 2.23 (pent, J = 8, 2H); 13C NMR (400 MHz, DMSO-d6) δ 141.2, 139.6, 129.6, 123.6, 45.3, 33.0, 31.0; LC-MS (ESI) [M + H]+ calcd for C17H21NO5 m/z 323.3, found m/z 323.1; [M + H]+ calcd for C17H21NO6·Br m/z 323.2, found m/z 323.1. General Method C for the Preparation of Imidazole Oximes 17 and 18. To a suspension of imidazole-2-carboxylic acid oxime (16) (0.23 g, 1 mmol) in nitromethane (3 mL) was added the corresponding pyridine derivative (1.5 mmol), and the mixture was stirred for 3 days at 50 °C. The resulting solution was cooled to rt and concentrated. Water (3 mL) was added and the mixture was washed with chloroform (2 × 2 mL). The combined organic layers were discarded, and the water layer was evaporated and purified on a reversed-phase biotage using a water/methanol mixture as the eluent. The solvent was evaporated, and the resulting solid was dried over P2O5 under vacuum to give the corresponding imidazole-2-carboxylic acid oxime quaternary salt.

1-(3-[2-[[Hydroxyimino]methyl]-1H-imidazol-1-yl]propyl)pyridin-1-ium Bromide (17). Prepared according to general method C: brown solid; yield 0.17 g, 54%; 1H NMR (400 MHz, DMSO-d6) δ 114.6 (s, 1H), 8.05 (s, 1H), 7.33 (s, 1H), 7.27 (t, J = 8, 2H), 7.16 (app d, J = 8, 3H), 7.00 (s, 1H), 4.26 (t, J = 8, 2H), 2.54 (t, J = 8, 2H), 1.98 (pent, J = 8, 2H); 13C NMR (400 MHz, DMSO-d6) δ 149.9, 149.5, 141.3, 139.6, 128.8, 123.7, 46.3, 31.2, 30.5; LC-MS (ESI) [M + H]+ calcd for C16H21N5O2Br m/z 274.3, found m/z 274.3.

4-Carbamoyl-1-(3-[2-[[Hydroxyimino]methyl]-1H-imidazol-1-yl]propyl)pyridin-1-ium Bromide (18). Prepared according to general method C: brown solid; yield 0.18 g, 51%; 1H NMR (400 MHz, DMSO-d6) δ 112.5 (s, 1H), 8.60 (s, 1H), 8.08 (s, 1H), 7.84 (2, J = 8, 2H), 7.47–7.31 (m, 4H), 7.07 (s, 1H), 4.39 (t, J = 8, 2H), 4.32 (app s, 2H), 2.34 (app s, 2H); 13C NMR (400 MHz, DMSO-d6) δ 141.3, 141.0, 139.6, 128.7, 128.3, 128.1, 125.9, 125.3, 46.3, 32.0, 31.9; LC-MS (ESI) [M + H]+ calcd for C16H21N5O2Br m/z 274.3, found m/z 274.3

Enzyme. Highly purified recombinant monomeric hACHE (human AChE) was prepared as described previously.23 Purified human BChE isolated from human plasma was a gift from D. Lenz and D. Cerasoli [USARMIDC (U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD). All enzyme concentrations given refer to the concentration of catalytic sites, i.e., monomers. OPs. Low-toxicity nonvolatile Flu-MPs (fluorescent methylphosphonates)24 were used as analogues of nerve agents sarin, cyclosarin, and VX. The Flu-MPs differ from actual nerve agent OPs only by the structure of their respective leaving groups. Inhibition of hACHE and hBChE by Flu-MPs results in OP–hBChE and OP–hACHE covalent conjugates identical with the ones formed upon inhibition with nerve agents. Paraaxon was purchased from Sigma-Aldrich.

Oximes. 2PM (2-pyrimidinylmethoxy methide) and obidoxime [1H-1-oximino-4-carboxylate] dichloride] were purchased from Sigma-Aldrich. TAB20H and RS-113A, RS-113B, RS-115A, RS-115B, and RS-115C were prepared as described previously.11,12 Hi6 was purchased from US Biological. MBB4 and TB4 were gifts from T. Shih and L. Kopoloviz (USARMIDC).

Reactivation Assays. hACHE and hBChE activities were measured using a spectrophotometric assay25 at rt in 0.1 M sodium phosphate buffer (pH 7.4), containing 0.01% BSA and 1.0 mM substrate ACh (acetylcholinol). OP–hBChE and OP–hACHE
conjugates were prepared using Flu-MPs and paraoxon, and oxime reactivation experiments were performed at 37 °C in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.01% BSA, as described previously. The first-order reactivation rate constant \( k_{\text{ox}} \) for each oxime–OP conjugate combination was calculated by nonlinear regression.

**Computational Molecular Modeling.** Molecular models of oximes 4 and 14 were built and prepared using the Insight II modeling suite (Accelrys, San Diego, CA) as described previously for similar oximes.23 The imidazole ring of 4 was not protonated in the calculation, consistent with the determined pK, of 5.6 (data not shown). The crystal structure of the VX–hBChE conjugate (Protein Data Bank entry 2XQK) was prepared for calculation by removing all water molecules and reversibly bound ligands and repairing incomplete amino acid side chains. Oximes were positioned into the VX–hBChE gorge with their oximate oxygens 4 Å from the conjugated phosphoryl atom. A flexible distance constraint was placed between the two atoms at 3 Å, and molecular dynamics (MD) calculations were performed at a series of temperatures starting at 300 K, increasing to 700 K, and decreasing to 300 K in 50 K increments, as calculated to freely rotate. Ten calculations were performed per oxime. Resulting structures were visualized using Discovery Studio Visualizer version 3.5 (Accelrys).

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**Notes**
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**ABBREVIATIONS**

BChE, butyrylcholinesterase; hBChE, human BChE; AChE, acetylcholinesterase; hAChE, human AChE; ATCh, acetylthiocholine; BSA, bovine serum albumin; OP, organophosphate; 2PAM, 2-pyridinedialdoxime methiodide

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