Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

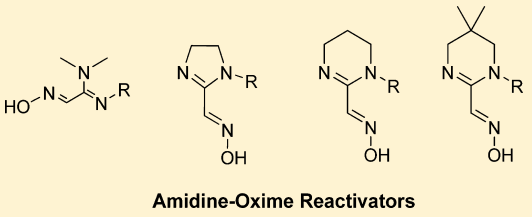
Nonquaternary Reactivators for Organophosphate-Inhibited Cholinesterases

Jarosław Kalisiak, Erik C. Ralph, and John R. Cashman*

Human BioMolecular Research Institute, 5310 Eastgate Mall, San Diego, California 92121, United States

Supporting Information

ABSTRACT: A new class of amidine-oxime reactivators of organophosphate (OP)-inhibited cholinesterases (ChE) was synthesized and tested in vitro and in vivo. Compared with 2-PAM, the most promising cyclic amidine-oxime (i.e., **12e**) showed comparable or greater reactivation of OP-inactivated AChE and OP-inactivated BChE. To the best of our knowledge, this is the first report of a nonquaternary oxime that has, comparable to 2-PAM, in vitro potency for reactivation of Sarin (GB)-inhibited AChE and BChE. Amidine-oximes were tested in vitro, and reactivation rates for OP-inactivated butyrylcholinesterase (BChE) were greater than those for 2-PAM or MINA. Amidine-oxime reactivation rates for OP-inactivated acetylcholinesterase (AChE) were lower compared to 2-PAM but greater compared with MINA. Amidine-oximes were tested in vivo for protection against the toxicity of nerve agent model compounds. (i.e., a model of Sarin). Post-treatment (i.e., 5 min after OP exposure, i.p,) with amidine oximes **7a−c** and **12a**, **12c**, **12e**, **12f**, and **15b** (145 μmol/kg, i.p.) protected 100% of the mice challenged with the sarin model compound. Even at 25% of the initial dose of amidine-oxime (i.e., a dose of 36 μmol/kg, i.p.), **7b** and **12e** protected 100% of the animals challenged with the sarin nerve agent model compound that caused lethality in 6/11 animals without amidine-oxime.



INTRODUCTION

Organophosphate (OP) nerve agents (e.g., Sarin, Cyclosarin, VX) and pesticides (e.g., Parathion, Chlorpyrifos) are extremely toxic compounds that could be used as weapons of mass destruction and represent a potential threat to humans worldwide. Toxic effects of OPs are primarily related to their covalent binding to acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), critical detoxication enzymes in the blood and in the central nervous system (CNS).[1] The main role of cholinesterases (ChEs) includes the hydrolysis of the neurotransmitter acetylcholine (ACh) and other esters. After OP exposure and irreversible inhibition of ChE, accumulation of ACh overstimulates autonomic receptors and blocks neuromuscular junction receptors resulting in convulsions and other CNS toxicity.[2] Current clinically available drugs for acute OP-poisoning include oxime reactivators, a muscarinic receptor antagonist (i.e., atropine), and an anticonvulsant (i.e., diazepam). Oximes currently in use for treatment of OP-poisoning are functionalized quaternary pyridinium salts (Figure 1).

However, quaternary pyridinium oximes have a limited ability to enter the brain via the blood−brain barrier (BBB) because they are positively charged. Because the brain is the key target for toxic action of OPs, cationic reactivators possess significant deficiencies in reactivating brain ChE's. Consequently, lack of CNS protection from OPs leads to neuronal death and chronic neurological side effects (e.g., flashbacks, headaches, depressive feelings, and lack of concentration).[3] The limitations of pyridinium oximes can be overcome by nonquaternary oximes with improved lipophilicity and BBB permeability.[4,5] Development of uncharged oximes that reactivate ChE is one of major goals in the field of OP antidotes, but few alternative approaches have been reported. Mercey et al.[6] designed reactivators containing the oxime function linked to 6,7-dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline, a compound that binds to the peripheral site of AChE.[7] Introduction of a peripheral site-binding inhibitor moiety into the structure of an OP reactivator significantly increases its binding to OP-inactivated AChE and an optimal linker between the tetrahydroisoquinoline motif and an oxime functionality enables the oxime group to reach the vicinity of the OP-labeled active site residue. Another elegant approach to uncharged oximes takes advantage of copper-catalyzed azide alkyne cycloaddition (i.e., "Click Chemistry") and has been reported by Sit et al.[8] This approach allowed for synthesis of a large number of oximes including the lead structure RS41A, an

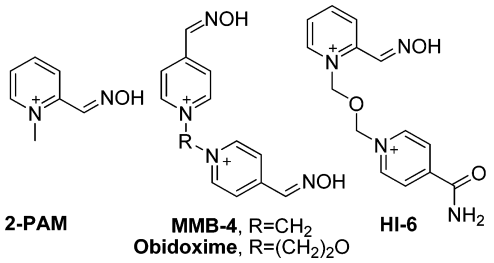


Figure 1. Chemical structures of currently used pyridinium oximes.

Received: October 10, 2011
Published: December 29, 2011

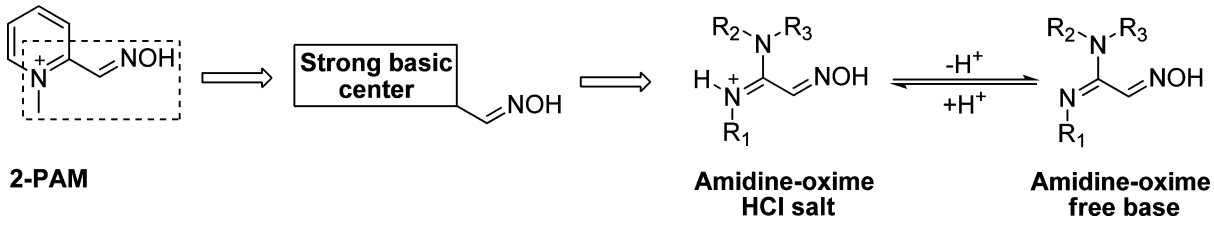


Figure 2. Retro-structural analysis of 2-PAM led to the structure of an amidine-oxime.

analogue of glyoxalate oxime containing an amide side chain with a tertiary cyclopentylamine.

The oxime drug discovery effort described herein is focused on overcoming the limitations of cationic pyridinium oximes and the development of nonquaternary oximes with significantly increased lipophilicity and BBB permeability. Recently, we reported the general structure of nonquaternary oximes containing a strongly basic amidine center adjacent to the oxime functionality (Figure 2).[9]

The strongly basic amidine group supplies a pseudopositive charge that mimics the quaternary center of a pyridinium oxime and is responsible for efficient binding to OP-inhibited ChEs, while the proximal oxime group is a powerful nucleophile responsible for reactivation of the phosphylated enzyme. The electron-withdrawing character of the protonated amidine group enhances ionization of the oxime group and increases the concentration of the nucleophilic oxime anion (Figure 3).

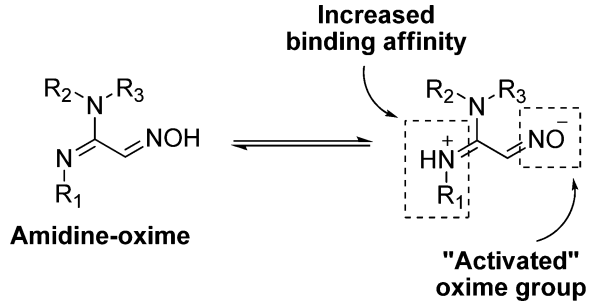


Figure 3. Amidine functional group plays a dual role of providing a positive charge and increasing the ionization of the oxime oxygen atom.

Herein, we describe the further development of a new class of amidine-oximes, nonquaternary ChE reactivators with superior in vitro reactivation of OP-BChE and similar efficacy in the reactivation of OP-AChE compared to 2-PAM. In addition, several highly potent amidine oxime reactivators were shown to be 100% effective in protecting small animals from lethality in an in vivo mouse model of exposure to a compound that mimics the pharmacology of sarin.

RESULTS

The amidine-oximes **1** described herein were obtained using recently established synthetic methodology.[9] The retrosynthetic approach outlined in Figure 4 shows that glyoxalate oxime ester **3** was converted to amide **2** containing a *p*-methoxybenzyl (PMB) protected oxime functionality. Amide **2** was subsequently modified into a thioamide, the starting material for amidine **1** synthesis through a thioimidate intermediate.[10,11] A significant advantage of the synthetic approach is that amidine preparation and oxime group deprotection take place in one-pot step without isolation or purification of the intermediates.

On the basis of our previous findings, amidine-oximes containing the most lipophilic (i.e., propyl and butyl) substituents on the amidine function had the greatest reactivation rate of OP-ChE.[9] Herein we explored the effect of more bulky substituents on the amidine functionality. The PMB-protected ethyl glyoxylate oxime **4**[9] was readily converted to amides **5a**–**j** using aliphatic (**a**–**f**), cycloalkyl (**g**, **h**), or aromatic (**i**, **j**) amines (Table 1). Thioamides **6a**–**j** were obtained in good yield using a standard protocol employing Lawesson's reagent.[9] The final step of the synthesis was an activation of the carbonyl group via *S*-methylation of thioamides **6a**–**j**. This step was carried out using 1.2 equivalents of MeOTf. Previously, we reported that traces of water in the reaction mixture caused hydrolysis of MeOTf and formed triflic acid in situ and that under these acidic conditions the oxime protecting group (i.e., PMB) was efficiently cleaved. Without any purification, the crude mixture was used in the synthesis of amidine hydrochlorides **7a**–**j** after treatment with the dimethylamine solution and salt exchange. This synthetic methodology was successfully used to prepare over 10 g of amidine **7b**.

After successful synthesis of oximes **7a**–**j**, we explored the synthesis of cyclic amidine-oxime analogues, because there are literature examples of compounds that potently interact with AChE and contain a cyclic amidine scaffold.[12,13] The synthesis of cyclic amidine-oximes was accomplished using a reported method[14] that started with 1,1-*bis*(methylthio)-2-nitroethylene **8**. Subsequent treatment of **8** with the requisite diamine afforded the 5-member (**9**) or 6-member (**10**) nitro vinyl intermediate (Table 2). Monoalkylation of **9** or **10** was accomplished using alkyl iodides (**a**, **e**, **f**) or benzyl chlorides (**b**,

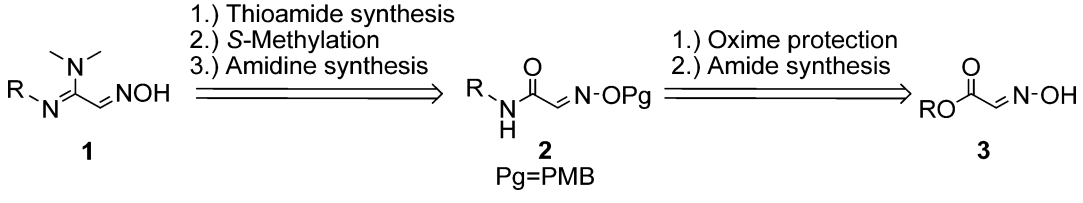
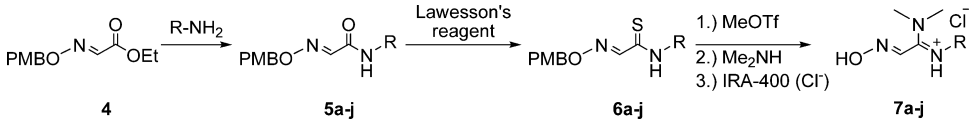
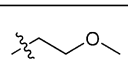
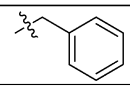
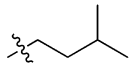
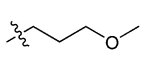
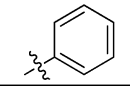


Figure 4. Retro-synthetic strategy for synthesizing amidine-oxime **1**.

dx.doi.org/10.1021/jm201364d | J. Med. Chem. 2012, 55, 465–474

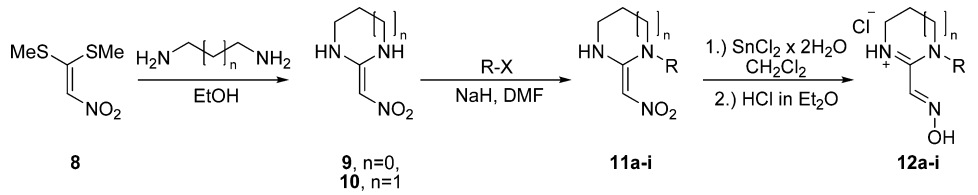
Table 1. Synthesis of Amidine-Oximes 7a–j[a]

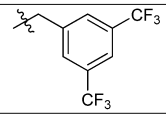
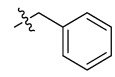
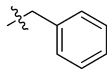
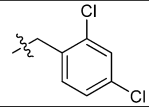
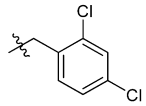
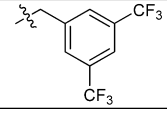


	R		R		R		R		R
a	*n*-Pr	**c**	*n*-Pentyl	**e**		**g**		**i**	
b	*n*-Bu	**d**		**f**		**h**		**j**	

[a]Oximes **7a** and **7b** have been reported before.[9].

Table 2. Synthesis of Cyclic Amidine-Oximes 12a–i[a]



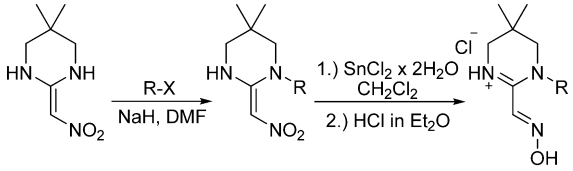
	R		R		R
a, n=0	n-Bu	**d, n=0**		**g, n=1**	
b, n=0		**e, n=1**	n-Bu	**h, n=1**	
c, n=0		**f, n=1**	n-Pe	**i, n=1**	

[a]Oximes **12b** and **12g** have been reported before.[14].

c, **d**, **g–i**). The final step of the synthesis of **12a–i** was two electron reduction of **11a–i** employing $SnCl_2 \times 2H_2O$ (Table 2).

Additional analogues **15a,b** containing two geminal methyl groups in the 6-member ring structure were synthesized to examine whether an increase in lipophilicity improved the overall pharmaceutical properties of the reactivators (Scheme 1).

Scheme 1. Synthesis of Cyclic Amidine-Oximes 15a,b



In Vitro Biochemical Studies. Amidine-oximes synthesized as described herein were tested for their competitive inhibition of hAChE and hBChE. For the competitive inhibition experiments, ChE enzyme samples were titrated with an oxime over a range of concentrations from 3 to 200 μM, and esterase activity was measured using the Ellman assay in incubations in the presence of 1 mM substrate (i.e., acetylthiocholine (ATC) for AChE and butyrylthiocholine (BTC) for BChE), as previously described.[9] The observed IC_{50} values were recorded (Table 3). With the exception of compounds **7i**, **12b**, **12c**, **12g**, and **15b**, in most cases examined, more potent binding was observed for AChE (e.g., **7b–d**) compared with that of BChE (Table 3). In addition, more lipophilic amidine-oximes apparently had more potent binding to both enzymes. For example, oxime **7a** with a propyl substituent showed an $IC_{50} > 200$ μM (for AChE and BChE), whereas increasing the size of the side chain from propyl (**7a**) to butyl (**7b**) to pentyl (**7c**) or isopentyl (**7d**) significantly increased the inhibition potency for both AChE and BChE. (i.e., for **7d**, the IC_{50} was 17 ± 1 μM (hAChE) and the IC_{50} was 66 ± 8 μM for hBChE). No detectable inhibition of AChE was observed for oximes **7a**, **7e–j**, and **12a** containing aliphatic side chains (**7a**), aliphatic side chains with oxygen atoms (**7e–f**), cycloalkyl groups (**7g–h**, **12a**) or aryl substituents (**7i–j**). In this series, with the exception of **7i** a similar profile of ChE inhibition was observed for BChE.

In the case of the aryl-substituted cyclic amidine-oximes examined (i.e., **12b–c** and **12g–h**), significantly more potent inhibition was observed for hBChE compared to that for hAChE. Compounds **12d** and **12i** (both of which had a 3,5-

dx.doi.org/10.1021/jm201364d | J. Med. Chem. 2012, 55, 465–474

Table 3. IC_{50} Values of Amidine-Oximes for the Inhibition of hAChE or hBChE

oxime	IC_{50} AChE [μM], (N)[a]	IC_{50} BChE [μM], (N)	oxime	IC_{50} AChE [μM], (N)	IC_{50} BChE [μM], (N)
7a	>200[b]	>200	**12a**	>200	>200
7b	54 ± 8 (2)	>200	**12b**	44 ± 5 (2)	10 ± 5 (3)
7c	13 ± 5 (2)	180 ± 30 (1)	**12c**	24 ± 10 (2)	3.9 ± 0.4 (1)
7d	17 ± 1 (1)	66 ± 8 (1)	**12d**	85 ± 3 (1)	>200
7e	>200	>200	**12e**	157 ± 3 (1)	>200
7f	>200	>200	**12f**	21 ± 5 (1)	>200
7g	>200	>200	**12g**	28 ± 12 (2)	11 ± 6 (3)
7h	>200	>200	**12h**	9 ± 4 (3)	6.3 ± 0.6 (1)
7i	>200	140 ± 14 (1)	**12i**	45 ± 2 (1)	>200
7j	>200	>200	**15a**	93 ± 48 (1)	114 ± 10 (1)
			15b	89 ± 19 (1)	68 ± 5 (1)

[a] *N*, the number of independent IC_{50} value determinations. [b] Compounds showing less than 10% inhibition at 200 μM were assigned a value of >200 (N = 1).

bis(trifluoromethyl)benzyl group) did not inhibit hBChE but did inhibit hAChE (Table 3). For the aryl-substituted 5-membered cyclic amidine oximes examined (i.e., **12b,c**), the potency for inhibition of hAChE was greater than 4-fold less than that for hBChE. Compared to the corresponding 6-membered analogues (i.e., **12g−h**), the potency for inhibition of hAChE for the aryl-substituted 5-membered cyclic amidine oximes examined (i.e., **12b−d**) was 2-fold less for aryl-substituted 6-membered analogues **12g−i** (i.e., **12b** (44 ± 5 μM) vs **12g** (28 ± 12 μM); **12c** (24 ± 10 μM) vs **12h** (9 ± 4 μM); **12d** (85 ± 3 μM) vs **12i** (45 ± 2 μM). In this series, the most potent inhibitors of either hAChE or hBChE were observed for the 5- or 6-member cyclic amidine oximes **12c** and **12h** containing a lipophilic 2,4-dichlorobenzyl group. Introduction of additional geminal dimethyl groups into the ring structure (i.e., **15a,b**) decreased the IC_{50} for hBChE compared to **12e,f** (i.e., **15a** (114 ± 10 μM) vs **12e** (>200 μM) and **15b** (68 ± 5 μM) vs **12f** (>200 μM), but the effect on inhibition of hAChE was less marked.

Reactivation of OP-inhibited ChEs was investigated to ascertain if inhibition potency as determined by IC_{50} values paralleled reactivation rate constants. The ability of oximes **7**, **12**, and **15** to reactivate hAChE and hBChE inhibited by nerve agent model compounds (i.e., model compounds of sarin, cyclosarin, tabun)[15,16] and a pesticide (i.e., echothiophate) (Figure 5) was evaluated. Incubation of OP-inhibited ChE enzyme with each oxime was examined for reactivation (i.e., 60 min for AChE or 20 min for BChE) at 37 °C and was compared to the amount of ChE inhibition in a vehicle-treated control sample without oxime or the positive control, 2-PAM. Observed esterase functional activity was expressed as the ratio of relative reactivation rates with a value from 0 to 1.0, where 1.0 was complete reactivation of the functional activity of ChE by an oxime (i.e., a ratio of 1.0 was equivalent to an oxime reactivation rate giving a noninhibited rate divided by a vehicle-treated control rate indicative of 100% esterase activity).

Noncyclic amidine-oximes **7a−j** provided additional information to the previously reported results[9] where compounds **7a** and **7b** containing lipophilic substituents (i.e., propyl and butyl moieties, respectively) showed the greatest overall OP reactivation of OP-inactivated AChE and BChE (Figure 6).

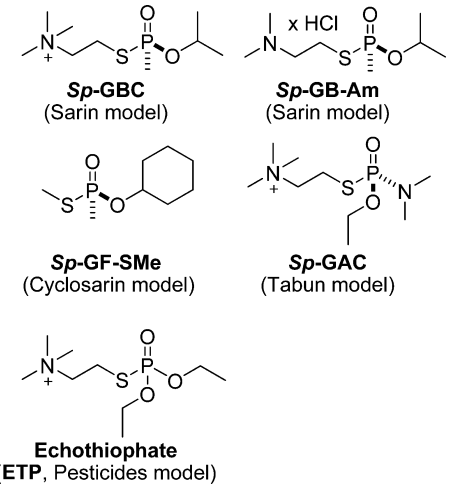


Figure 5. Chemical structures of nerve agent model compounds used in these studies.[15,16].

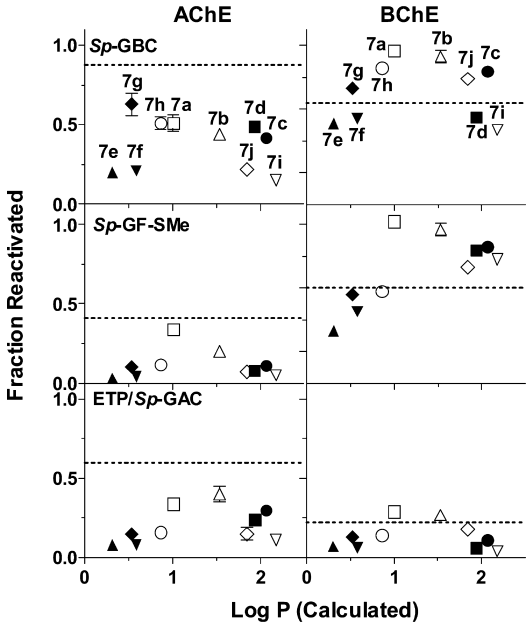


Figure 6. Relative reactivation of OP-inhibited hAChE and hBChE by noncyclic amidine-oximes **7a−j**. Data represent the mean ± standard deviation for the ratio of observed reactivation rate divided by the control (noninhibited) rate. Results for the reactivation of **ETP**- and ***Sp*-GAC**-inhibited ChE were identical and presented on the same panel. Dotted lines represent the relative reactivation for 2-PAM. Symbols without apparent error bars have errors less than standard deviation of the size of the symbol. Numeric values and the number of determinations are given in Supporting Information.

Oximes **7c−j** were designed to possess comparable or greater lipophilicity on the amidine functionality compared to previously synthesized compounds **7a** and **7b**. Of the new compounds synthesized in this series (i.e., **7c−j**), the most potent oxime reactivation of **GB**-AChE was observed for **7g** (i.e., containing a cyclopropyl group); however, reactivation mediated by **7g** was only slightly greater than **7h** (i.e., cyclobutyl group), **7a** (i.e., propyl group), or **7b** (i.e., butyl group) (Figure 6).

Amidine-oximes **7c** (i.e., pentyl group) and **7d** (i.e., isopentyl group) with more lipophilic substituents also possessed comparable efficacy for reactivation of **GB**-AChE. In contrast to

dx.doi.org/10.1021/jm201364d | J. Med. Chem. 2012, 55, 465−474

amidine-oximes **7a−h**, oximes with aryl (i.e., **7i−j**) or heteroalkyl substituents (i.e., **7e−f**) were significantly less potent at the reactivation of **GB**-AChE. In the case of **GF-SMe**-inhibited AChE, functional reactivation using oximes **7a−j** was significantly lower, and only compounds **7b−c** and **7g−h** showed reactivation within 10−20% of a noninhibited control. A similar trend was observed for the reactivation of **ETP**- and ***Sp*-GAC**-inhibited AChE where the most efficient oximes were **7a** and **7b** on the basis of reactivation rates. In the case of the reactivation of **GBC**- and **GF-SMe**-inhibited BChE, generally, amidine-oximes **7a−j** were significantly more potent than 2-PAM, and compounds **7a** and **7b** showed almost complete reactivation within 20 min of initiating the experiment. From this series of compounds, in general, oximes **7a** and **7b** gave the greatest relative reactivation for the OP inhibitors examined in this study for both AChE and BChE with the except for the reactivation of **GB**-AChE where **7g** was slightly more potent.

Modification of previously reported lead structures **7a** and **7b** and introduction of more lipophilic, cycloalkyl, or aryl substituents in the amidine function did not significantly improve reactivation potency. To investigate whether the same trend was observed for cyclic amidine-oximes, compounds **12a−i** and **15a−b** were examined for reactivation of OP-inhibited ChEs (Figure 7). Direct comparison of 5-membered

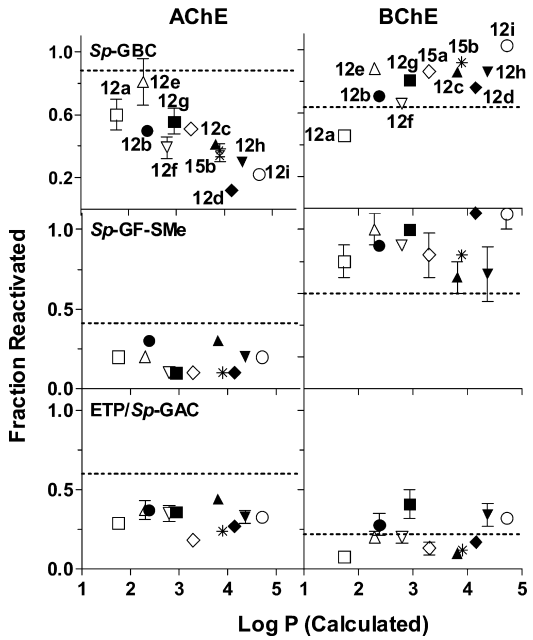


Figure 7. Relative reactivation of OP-inhibited hAChE and hBChE by cyclic amidine-oximes **12a−i** and **15a,b**. Data represent the mean ± standard deviation for the ratio of observed reactivation rate divided by the control (noninhibited) rate. Results for the reactivation of **ETP**- and ***Sp*-GAC**-inhibited ChEs were identical and presented on the same panel. Dotted line represents relative reactivation for 2-PAM. Symbols without apparent error bars have standard deviation less than the size of the symbol. Numeric values and the number of determinations are given in Supporting Information.

cyclic amidine-oximes (i.e., **12a−d**) and 6-member analogues (i.e., **12e−i**) showed that in most cases the 6-membered compounds gave greater relative reactivation than the 5-membered analogues (Figure 7). The most potent oxime for the reactivation of **GBC**-inhibited AChE was **12e** and possessed comparable potency for that of 2-PAM. It is notable that **12e** has an amidine substituted with a butyl group, and this is the same as that in **7b** (i.e., the lead structures from noncyclic amidine-oximes **7a−j**). Increasing the size of the side-chain group decreased the relative reactivation for **GB**-AChE, but the opposite trend was observed for the reactivation of **GB**-BChE, where the greatest reactivation was observed for compound **12i** (1.02 ± 0.02). Increasing the size of the side-chain from an alkyl group (i.e., **12a** and **12e−f**) to one with an aryl substituent (i.e., **12b−d** and **12g−i**) decreased the relative reactivation for **GB**-AChE. However, the opposite trend was observed for the reactivation of **GB**-BChE with **12**. Comparison of **12e** vs **7b** and **12g** vs **7i** clearly confirmed an advantage of cyclic-amidine oximes over noncyclic analogues for reactivation of **GBC**-inhibited AChE. Generally, cyclic amidine-oximes were more potent for the reactivation of OP-inhibited AChE than the corresponding noncyclic oxime analogues **7a−j**. However, in the case of reactivation of OP-inhibited BChE, comparable reactivation was observed for both 5- and 6-membered classes of amidine-oximes.

On the basis of the results of reactivation studies for cyclic amidine-oximes, excellent relative reactivation values were observed for **GBC**- and **GF-SMe**-inhibited BChE. In most of the cases examined, amidine oximes **12a−i** were significantly more potent than the quaternary oxime 2-PAM at the reactivation of BChE. Compound **12e** showed comparable reactivation to 2-PAM for **GBC**-inhibited AChE, and to the best of our knowledge, this is the first report of a nonquaternary oxime that has comparable in vitro potency for the reactivation of **GBC**-inhibited hAChE and hBChE. To investigate the effect of the additional lipophilicity of compound **12e** on ChE reactivation, two geminal methyl groups were introduced into the 6-member ring to produce **15a**. This modification did not markedly change the potency of compound **15a** compared to that for **12e** for most of the OP-inhibited BChEs. In the case of AChE, however, for the reactivation of **GBC**-inhibited AChE, the reactivation potency was significantly lower for **15a** than that for **12e**.

In Vivo Studies. On the basis of the results of in vitro studies and the potency of reactivation of **GBC**-inhibited AChE (i.e., approximately 50%; Figures 6 and 7) and **GB**-BChE (i.e., approximately 80%; Figures 6 and 7), a select group of amidine oximes was studied in nerve agent model compound protection experiments in small animals. The protection of mice from nerve agent model compounds was done using a nerve agent model compound of sarin (i.e., ***Sp*-GB-Am**, Figure 5). In the case of a real life situation, the OP-exposed individual will likely be treated with an oxime antidote after OP exposure, and therefore, a compound that protects from OP postexposure is desirable. To investigate whether amidine-oximes were able to protect animals from an OP postexposure treatment situation, mice were exposed to the sarin model compound (i.e., ***Sp*-GB-Am**, Figure 5), and 5 min later, the vehicle, MINA (12.6 mg/kg, 145 μmol/kg, i.p.), 2-PAM (25.0 mg/kg, 145 μmol/kg, i.p.), or amidine-oxime **7a−c**, **12a−f**, or **15a−b** (145 μmol/kg, i.p.) was administered. In the in vivo OP protection studies, we used equimolar amounts (i.e., 145 μmol/kg) of amidine-oxime, MINA, or 2-PAM so we could compare and verify whether in vitro−in vivo correlates were apparent. After treatment, animals were observed for neurological toxicity symptoms (i.e., seizures, convulsions, muscle twitching, salivation, and rhinorrhea) and after 24 h, survival was recorded (Table 4). MINA and 2-PAM were used as positive controls because MINA is currently the

dx.doi.org/10.1021/jm201364d | J. Med. Chem. 2012, 55, 465−474

Table 4. Effect of Vehicle, MINA, 2-PAM, or Amidine-Oximes on the Lethality of *Sp*-GB-Am

	treatment[a]		*Sp*-GB-Am		
experiment	vehicle	μmol/mg	mg/kg	μmol/kg	survival (24 h)
1		145	0.08	0.305	6/11
	MINA (12.6 mg/kg)				3/6
	2-PAM (25.0 mg/kg)				6/6
	7a (28.0 mg/kg)				6/6
	7b (30.1 mg/kg)				6/6
	7c (32.1 mg/kg)				6/6
	12a (29.6 mg/kg)				6/6
	12b (34.6 mg/kg)				4/6
	12c (44.6 mg/kg)				6/6
	12d (54.3 mg/kg)				5/6
	12e (31.7 mg/kg)				6/6
	12f (33.7 mg/kg)				6/6
	15a (35.7 mg/kg)				2/6
	15b (37.8 mg/kg)				6/6
2	**2-PAM** (6.2 mg/kg)	36.2			6/6
	7b (7.5 mg/kg)				6/6
	12a (7.4 mg/kg)				5/6
	12e (7.9 mg/kg)				6/6

[a]Mice were treated (i.p.) with ***Sp*-GB-Am**, and 5 min later, vehicle or amidine-oxime or MINA or 2-PAM was administered. All oximes were administered as a hydrochloride salt in isotonic saline.

only known BBB-penetrating oxime commercially available, and 2-PAM serves as the benchmark for quaternary oximes.

As shown in Table 4, for ***Sp*-GB-Am**-pretreatment, postexposure administration of vehicle protected only 6/11 animals for survival to 24 h. Mice experienced significant CNS toxicity including seizures, lack of spontaneous activity, and no interest in food consumption. Similar symptoms were observed for the MINA-treated group, which only protected 3/6 animals from lethality. At a dose of 145 μmol/kg, noncyclic amidine-oximes **7a−c**, and cyclic amidine oximes **12a**, **12c**, **12e**, and **12f** protected all animals challenged with a lethal dose of ***Sp*-GB-Am**. After 24 h from OP exposure, the amidine-oxime treated animals behaved in a manner similar to that of non-OP treated animals and showed no significant symptoms of toxicity. Likewise, after pretreatment of animals with OP, postadministration of amidine-oxime **15b** protected all OP-treated mice and the mice showed behavioral activity comparable with that of healthy, untreated animals. Next, a lower dose (i.e., 36 μmol/kg) of oxime was shown to protect mice from OP pretreatment. As shown in Table 4, amidine oxime **7b** and **12e** fully protected from preadministration of ***Sp*-GB-Am** in a dose-dependent fashion comparable to that of an equimolar dose of 2-PAM.

DISCUSSION AND CONCLUSIONS

In summary, two series of novel cyclic and acyclic amidine-oximes were designed and prepared using thioimidate intermediates. Amidine oximes **7a−j**, **12a−j**, and **15a,b** were tested in vitro for the reactivation of hAChE and hBChE inhibited in the presence of nerve agent model compounds and the pesticide **ETP**. On the basis of the results of in vitro reactivation studies, the amidine-oximes examined had superior functional activity compared with that of MINA for the reactivation of OP-inhibited AChE and BChE.[9] In general, an increase in the lipophilic character of the substituent on the amidine group of the amidine-oxime increased the reactivation potency for ***Sp*-GBC**- or ***Sp*-GF-SMe**-inhibited BChE. The most lipophilic compounds examined (i.e., **7b**, **7j**, and **7c** with an increase in CLogP from calc. +1.5 to +2.1) had reactivation potency for ***Sp*-GBC**- or ***Sp*-GF-SMe**-inhibited BChE comparable to or greater than that of 2-PAM (CLogP calc. −3.7). However, lipophilicity is not the only physiochemical property that is required to reactivate OP-inhibited ChEs. As described herein, size of the reactivator and nucleophilicity of the oxime undoubtedly makes an important contribution to reactivation efficiency. Despite the fact that the amidine-oximes examined did not afford significantly greater reactivation rates than 2-PAM for OP-inhibited AChE in vitro, two amidine-oximes were efficacious in vivo in protecting mice from lethal doses of nerve agent model compound toxicity. For example, post-treatment with amidine-oximes **7b** or **12e** (i.e., at a dose of 7.4 and 7.9 mg/kg, respectively, 5 min after OP administration, i.p.) was able to protect mice from ***Sp*-GB-Am** toxicity. Groups of mice treated with amidine-oximes **7a−c** and cyclic amidine oximes **12a**, **12c**, **12e**, and **12f** survived 24 h and had only minor signs of CNS toxicity, whereas in the case of MINA or vehicle post-treatment, only 3/6 or 6/11 animals, respectively, survived. In the case of post-treatment with MINA, similar neurotoxicity symptoms as vehicle-treated animals were observed. We hypothesize that even though amidine-oximes are less potent than 2-PAM in the reactivation of OP-AChEs in vitro, they are orders of magnitude more efficient at entering the brain. We have administered amidine-oximes to animals and have examined the blood to brain ratios. Compared to the blood, the amount of amidine oxime in the brain for an i.v. infusion was quite great (data not shown), but this is the subject of another manuscript. The large BBB-penetrating properties of amidine-oximes may be related to lipophilicity or possibly to very efficient uptake by transporters. Regardless, amidine-oximes achieve much greater concentrations in the brain than 2-PAM, and amidine-oximes effectively protect against post-treatment of toxic OPs. In addition, because both enzymes, AChE and BChE are present in the brain,[17] efficient reactivation of BChE by amidine-oximes **7b** and **12e** even at low doses also contributes to the protection of OP-pretreated animals. We conclude that post-treatment of mice with amidine-oximes **7b** or **12e** rapidly delivers the compound to the plasma and to the CNS and protects animals from CNS toxicity. The concept of using lipophilic amidine-oximes as an alternative to quaternary oximes (e.g., 2-PAM) to more efficiently protect the brain from toxicity of OPs holds much promise as an OP toxicity countermeasure.

EXPERIMENTAL SECTION

General. All reagents and solvents were used as received from commercial sources. Buffers and reagents were purchased from VWR Scientific, Inc. (San Diego, CA) in the highest purity commercially available. ETP was a generous gift from Professor O. Lockridge of the University of Nebraska Medical Center. Synthetic products were isolated using a flash column chromatography system (Teledyne ISCO, CombiFlash Rf) with UV detection at 254 nm. NMR spectra were recorded at 300 MHz (^{1}H) on a Varian Mercury 300 or at 500 MHz (^{1}H) and 125 MHz (^{13}C) on a Bruker AMX-500 II (NuMega

dx.doi.org/10.1021/jm201364d | J. Med. Chem. 2012, 55, 465−474

Resonance Lab, San Diego) in $CDCl_3$ or d_6-DMSO, respectively. Chemical shifts were reported as ppm (δ) relative to $CDCl_3$ at 7.26 ppm and d_6-DMSO at 2.5 ppm or 39.52 ppm. Low resolution mass spectra were obtained using a Hitachi M-8000 mass spectrometer with an ESI source. Compounds **5a−j** and **6a−j** containing the *p*-methoxybenzyl (PMB) protected oxime group undergo decomposition during the mass spectrometry experiment, and the correct mass could not be obtained. Microwave assisted syntheses were carried out using Biotage Initiator Microwave Synthesizer (Charlotte, NC). Calculated ClogP values were determined using ChemDraw Ultra 11.0.

Purity Determination. Purity of products was determined by a Hitachi 8000 LC-MS (Hitachi, San Jose, CA) using reverse phase chromatography (C18 column, 50 × 4.6 mm, 5 μm, Thomson Instrument Co., Oceanside, CA). Compounds were eluted using a gradient elution of 95/5 to 5/95A/B over 10 min at a flow rate of 1.5 mL/min, where solvent A was aqueous 0.05% TFA, and solvent B was acetonitrile (0.05% TFA). For purity data, peak area percent for the TIC, at 210 or 254 nm, and retention time (t_R in minutes) are provided (see Supporting Information). Purity of products was ≥95%.

Caution! Nerve agent model compounds used in these studies are toxic and must be handled with extreme care by well-trained personnel. Use of these materials has been approved by NIH and DOD. After reactivation studies, biochemical samples were neutralized by stirring with 2 M NaOH/EtOH for 12 h. Remaining solutions were brought back to pH∼7 and disposed in chemical waste.

*2-(4-Methoxybenzyloxyimino)-N-pentylacetamide (**5c**).* A mixture of **4** (5.0 g, 21.1 mmol) and *n*-pentylNH_2 (4.9 mL, 3.7 g, 42.2 mmol, 2 equiv) in anhydrous EtOH (75 mL) was stirred at 60 °C for 2 days and then evaporated. The residue was purified by column chromatography (silica gel, hexanes → 7:3, hexanes/EtOAc) to give a yellow solid (4.6 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.43 (s, 1H), 7.31−7.27 (m, 2H), 6.92−6.88 (m, 2H), 6.46 (brs, 1H), 5.11 (s, 2H), 3.82 (s, 3H), 3.34−3.27 (m, 2H), 1.55−1.50 (m, 2H), 1.39−1.24 (m, 4H), 0.93−0.88 (m, 3H).

*N-Isopentyl-2-(4-methoxybenzyloxyimino)acetamide (**5d**).* The title compound was obtained in a manner similar to that used for **5c** as a yellow solid (75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.43 (s, 1H), 7.31−7.27 (m, 2H), 6.93−6.88 (m, 2H), 6.42 (br s, 1H), 5.11 (s, 2H), 3.82 (s, 3H), 3.37−3.30 (m, 2H), 1.68−1.57 (m, 1H), 1.48−1.41 (m, 2H), 0.93 (d, J = 6.6 Hz, 6H).

*2-(4-Methoxybenzyloxyimino)-N-(2-methoxyethyl)acetamide (**5e**).* A mixture of **4** (5.0 g, 21.1 mmol) and 2-methoxyethylamine (3.6 mL, 3.2 g, 42.2 mmol, 2 equiv) in anhydrous EtOH (10 mL) was placed in a 20 mL microwave vial. The reaction was carried out in the microwave synthesizer at 170 °C for 25 min and then evaporated. The residue was purified by column chromatography (silica gel, hexanes → 1:1, hexanes/EtOAc) to give a yellow solid (1.6 g, 28%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.43 (s, 1H), 7.32−7.27 (m, 2H), 6.93−6.87 (m, 2H), 6.81 (br s, 1H), 5.12 (s, 2H), 3.81 (s, 3H), 3.54−3.48 (m, 4H), 3.38 (s, 3H).

*2-(4-Methoxybenzyloxyimino)-N-(3-methoxypropyl)acetamide (**5f**).* The title compound was obtained in a manner similar to that used for **5e** as a yellow solid (36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.42 (s, 1H), 7.32−7.27 (m, 2H), 6.98 (br s, 1H), 6.92−6.87 (m, 2H), 3.81 (s, 3H), 3.50−3.40 (m, 4H), 3.34 (s, 3H), 1.85−1.77 (m, 2H).

*N-Cyclopropyl-2-(4-methoxybenzyloxyimino)acetamide (**5g**).* The title compound was obtained in a manner similar to that used for **5c** as a yellow solid (54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.41 (s, 1H), 7.30−7.27 (m, 2H), 6.93−6.89 (m, 2H), 6.54 (br s, 1H), 5.09 (s, 2H), 3.82 (s, 3H), 2.77−2.71 (m, 1H), 0.85−0.78 (m, 2H), 0.60−0.55 (m, 2H).

*N-Cyclobutyl-2-(4-methoxybenzyloxyimino)acetamide (**5h**).* The title compound was obtained in a manner similar to that used for **5c** as a yellow solid (84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.40 (s, 1H), 7.32−7.27 (m, 2H), 6.93−6.88 (m, 2H), 6.57 (br s, 1H), 5.12 (s, 2H), 4.50−4.37 (m, 1H), 3.82 (s, 3H), 2.41−2.31 (m, 2H), 2.01−1.87 (m, 2H), 1.80−1.67 (m, 2H).

*N-Benzyl-2-(4-methoxybenzyloxyimino)acetamide (**5i**).* The title compound was obtained in a manner similar to that used for **5c** as a white solid (84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.49 (s, 1H), 7.39−7.25 (m, 7H), 6.92−6.87 (m, 2H), 6.78 (br s, 1H), 5.09 (s, 2H), 4.52 (d, J = 6.0 Hz, 2H), 3.81 (s, 3H).

*2-(4-Methoxybenzyloxyimino)-N-phenylacetamide (**5j**).* To a mixture of **4** (3.4 g, 14.3 mmol) and aniline (1.3 mL, 1.3 g, 14.3 mmol) in anhydrous toluene (100 mL), $AlMe_3$ (2.3 M in hexanes, 6.2 mL, 14.3 mmol) was added under an atmosphere of N_2. The reaction was carried out at rt, and after 1 h, an additional portion of $AlMe_3$ solution (1.9 mL, 4.3 mmol, 0.3 equiv) was added. The reaction was continued for overnight and then carefully diluted with 10% citric acid in H_2O (200 mL) and EtOAc (100 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes → 9:1, hexanes/EtOAc) to give a yellow solid (3.4 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.22 (br s, 1H), 7.61−7.55 (m, 2H), 7.52 (s, 1H), 7.39−7.30 (m, 4H), 7.17−7.11 (m, 1H), 6.95−6.90 (m, 2H), 5.19 (s, 2H), 3.83 (s, 3H).

*2-(4-Methoxybenzyloxyimino)-N-pentylethanethioamide (**6c**).* A mixture of **5c** (4.0 g, 14.4 mmol) and Lawesson's reagent (3.5 g, 8.6 mmol, 0.6 equiv) in anhydrous THF (100 mL) was stirred at 60 °C for 1 h and then evaporated. The crude material was purified by column chromatography (silica gel, hexanes → 8:2, hexanes/EtOAc) to give a yellow oil (3.4 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.17 (br s, 1H), 7.77 (s, 1H), 7.34−7.27 (m, 2H), 6.94−6.86 (m, 2H), 5.11 (s, 2H), 3.82 (s, 3H), 3.71−3.64 (m, 2H), 1.77−1.65 (m, 2H), 1.42−1.31 (m, 4H), 0.95−0.87 (m, 3H).

*N-Isopentyl-2-(4-methoxybenzyloxyimino)ethanethioamide (**6d**).* The title compound was obtained in a manner similar to that used for **6c**, as a yellow solid (80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.13 (br s, 1H), 7.77 (s, 1H), 7.32−7.27 (m, 2H), 6.93−6.86 (m, 2H), 5.11 (s, 2H), 3.82 (s, 3H), 3.73−3.67 (m, 2H), 1.74−1.56 (m, 3H), 0.96 (d, J = 6.3 Hz, 6H).

*2-(4-Methoxybenzyloxyimino)-N-(2-methoxyethyl)-ethanethioamide (**6e**).* The title compound was obtained in a manner similar to that used for **6c**, as a yellow oil (87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.51 (br s, 1H), 7.78 (s, 1H), 7.34−7.27 (m, 2H), 6.92−6.88 (m, 2H), 5.13 (s, 2H), 3.92−3.87 (m, 2H), 3.83 (s, 3H), 3.64−3.61 (m, 2H), 3.41 (s, 3H).

*2-(4-Methoxybenzyloxyimino)-N-(3-methoxypropyl)-ethanethioamide (**6f**).* The title compound was obtained in a manner similar to that used for **6c**, as a yellow solid (92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 9.02 (br s, 1H), 7.77 (s, 1H), 7.32−7.27 (m, 2H), 6.94−6.86 (m, 2H), 3.82−3.75 (m, 5H), 3.55 (t, J = 5.5 Hz, 2H), 3.35 (s, 3H), 1.99−1.91 (m, 2H).

*N-Cyclopropyl-2-(4-methoxybenzyloxyimino)ethanethioamide (**6g**).* The title compound was obtained in a manner similar to that used for **6c**, as a yellow oil (45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.15 (br s, 1H), 7.76 (s, 1H), 7.31−7.24 (m, 2H), 6.92−6.88 (m, 2H), 5.10 (s, 2H), 3.82 (s, 3H), 3.24−3.14 (m, 1H), 1.00−0.93 (m, 2H), 0.75−0.70 (m, 2H).

*N-Cyclobutyl-2-(4-methoxybenzyloxyimino)ethanethioamide (**6h**).* The title compound was obtained in a manner similar to that used for **6c**, as a yellow oil (72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.26 (br s, 1H), 7.74 (s, 1H), 7.32−7.27 (m, 2H), 6.93−6.89 (m, 2H), 5.13 (s, 2H), 4.85−4.73 (m, 1H), 3.82 (s, 3H), 2.54−2.44 (m, 2H), 2.08−1.95 (m, 2H), 1.90−1.77 (m, 2H).

*N-Benzyl-2-(4-methoxybenzyloxyimino)ethanethioamide (**6i**).* The title compound was obtained in a manner similar to that used for **6c**, as a yellow solid (95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.39 (br s, 1H), 7.83 (s, 1H), 7.44−7.31 (m, 5H), 7.30−7.24 (m, 2H), 6.91−6.86 (m, 2H), 5.09 (s, 2H), 4.89 (d, J = 5.4 Hz, 2H), 3.81 (s, 3H).

*2-(4-Methoxybenzyloxyimino)-N-phenylethanethioamide (**6j**).* The title compound was obtained in a manner similar to that used for **6c**, as an orange solid (85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 9.78 (br s, 1H), 7.86−7.80 (m, 2H), 7.45−7.24 (m, 6H), 6.96−6.90 (m, 2H), 5.19 (s, 2H), 3.83 (s, 3H).

*2-(Hydroxyimino)-N,N-dimethyl-N′-pentylacetimidamide Hydrochloride Salt (**7c**).* To a solution of **6c** (3.0 g, 10.2 mmol) in $MeNO_2$ (20 mL) at rt, MeOTf (1.4 mL, 2.0 g, 12.2 mmol, 1.2 equiv) was

dx.doi.org/10.1021/jm201364d | J. Med. Chem. 2012, 55, 465−474

added. The reaction mixture was stirred at rt for 3 days and then evaporated. The crude product was dissolved in anhydrous THF (100 mL), and Me_2NH (2 M in THF, 10.2 mL, 20.4 mmol, 2 equiv) was added. The reaction was carried out at rt 1 h and then evaporated. The residue was purified by column chromatography (silica gel, CH_2Cl_2 → 9:1, CH_2Cl_2/MeOH) to give **7c** as the triflate salt. The chloride salt was obtained using an IRA-400 (Cl^-) ion-exchange resin (yellow oil, 1.0 g, 44%). ^1H NMR (300 MHz, d_6-DMSO) δ = 12.92 (s, 1H), 9.95 (br s, 1H), 8.08 (s, 1H), 3.31−3.23 (m, 2H), 3.17 (br s, 3H), 3.13 (br s, 3H), 1.56−1.46 (m, 2H), 1.30−1.18 (m, 4H), 0.84 (t, J = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 157.0, 138.8, 44.6, 41.0, 33.9, 29.1, 27.9, 21.6, 13.7. ESI MS for $[M + H]^+$ = 185.9 Da.

*2-(Hydroxyimino)-N′-isopentyl-N,N-dimethylacetimidamide Hydrochloride Salt (**7d**).* The title compound was obtained in a manner similar to that used for **7c**, as a yellow oil (48%). ^1H NMR (300 MHz, d_6-DMSO) δ = 12.93 (s, 1H), 9.53 (br s, 1H), 8.09 (s, 1H), 3.33−3.24 (m, 2H), 3.17 (br s, 3H), 3.13 (br s, 3H), 1.58−1.38 (m, 3H), 0.84 (d, J = 6.6 Hz, 6H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 157.0, 138.8, 43.0, 41.4, 38.4, 24.9, 22.2. ESI MS for $[M + H]^+$ = 185.9 Da.

*2-(Hydroxyimino)-N′-(2-methoxyethyl)-N,N-dimethylacetimidamide Hydrochloride Salt (**7e**).* The title compound was obtained in a manner similar to that used for **7c**, as a yellow oil (53%). ^1H NMR (300 MHz, d_6-DMSO) δ = 12.95 (s, 1H), 9.70 (br s, 1H), 8.05 (s, 1H), 3.59−3.36 (m, 4H), 3.25−3.22 (m, 3H), 3.19−3.11 (m, 6H). ESI MS for $[M + H]^+$ = 173.8 Da.

*2-(Hydroxyimino)-N′-(3-methoxypropyl)-N,N-dimethylacetimidamide Hydrochloride Salt (**7f**).* The title compound was obtained in a manner similar to that used for **7c**, as a yellow oil (34%). ^1H NMR (300 MHz, d_6-DMSO) δ = 12.94 (s, 1H), 9.67 (br s, 1H), 8.05 (s, 1H), 3.39−3.26 (m, 4H), 3.21−3.11 (m, 9H), 1.87−1.72 (m, 2H). ESI MS for $[M + H]^+$ = 187.9 Da.

*N′-Cyclopropyl-2-(hydroxyimino)-N,N-dimethylacetimidamide Hydrochloride Salt (**7g**).* The title compound was obtained in a manner similar to that used for **7c**, as a yellow solid (22%). ^1H NMR (300 MHz, d_6-DMSO) δ = 12.94 (br s, 1H), 9.62 (br s, 1H), 8.10 (s, 1H), 3.16−3.09 (m, 6H), 2.82−2.58 (m, 1H), 0.89−0.70 (m, 4H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 159.2, 139.4, 42.0, 33.9, 26.6, 7.5. ESI MS for $[M + H]^+$ = 155.9 Da.

*N′-Cyclobutyl-2-(hydroxyimino)-N,N-dimethylacetimidamide Hydrochloride Salt (**7h**).* The title compound was obtained in a manner similar to that used for **7c**, as a yellow oil (23%). ^1H NMR (500 MHz, d_6-DMSO) δ = 12.95 (s, 1H), 9.31 (br s, 1H), 8.04 (s, 1H), 4.16−4.07 (m, 1H), 3.25−3.12 (m, 6H), 2.30−2.21 (m, 2H), 2.19−2.12 (m, 2H), 1.68−1.60 (m, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 156.1, 139.0, 50.2, 41.4, 33.9, 29.9, 14.1. ESI MS for $[M + H]^+$ = 169.9 Da.

*N′-Benzyl-2-(hydroxyimino)-N,N-dimethylacetimidamide Hydrochloride Salt (**7i**).* The title compound was obtained in a manner similar to that used for **7c**, as a yellow solid (48%). ^1H NMR (300 MHz, d_6-DMSO) δ = 10.20 (br s, 1H), 9.18 (br s, 1H), 8.82 (br s, 1H), 7.41−7.29 (m, 5H), 4.47 (s, 2H), 3.21−3.13 (m, 6H). ESI MS for $[M + H]^+$ = 205.9 Da.

*2-(Hydroxyimino)-N,N-dimethyl-N′-phenylacetimidamide Hydrochloride Salt (**7j**).* The title compound was obtained in a manner similar to that used for **7c**, as a yellow solid (10%). ^1H NMR (300 MHz, d_6-DMSO) δ = 12.85 (br s, 1H), 11.48 (br s, 1H), 7.79 (s, 1H), 7.44−7.38 (m, 2H), 7.31−7.25 (m, 1H), 7.22−7.19 (m, 2H), 3.32 (br s, 6H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 156.4, 139.9, 137.0, 129.2, 126.9, 125.4, 41.7, 33.9. ESI MS for $[M + H]^+$ = 191.9 Da.

*2-(Nitromethylene)imidazolidine (**9**).* The mixture of 1,1-*bis*(methylthio)-2-nitroethylene (20.0 g, 121.2 mmol) and 1,2-diaminoethane (8.1 mL, 7.3 g, 121.2 mmol) in anhydrous EtOH (250 mL) was stirred for overnight at reflux. The mixture was cooled to 4 °C and the product collected by filtration as a yellow solid (15.3 g, 98%). ^1H NMR (300 MHz, d_6-DMSO) δ = 8.26 (br s, 2H), 6.31 (s, 1H), 3.56 (s, 4H). ESI MS for $[M + H]^+$ = 129.8 Da.

*2-(Nitromethylene)hexahydropyrimidine (**10**).* The title compound was obtained in a manner similar to that used for **9** using 1,3-diaminopropane, as a yellow solid (85%). ^1H NMR (300 MHz, d_6-DMSO) δ = 8.84 (br s, 2H), 6.23 (s, 1H), 3.29−3.24 (m, 4H), 1.84−1.76 (m, 2H). ESI MS for $[M + H]^+$ = 143.8 Da.

*1-Butyl-2-(nitromethylene)imidazolidine (**11a**).* To a mixture of **9** (6.0 g, 46.5 mmol) in anhydrous DMF (90 mL) cooled to 0−5 °C (ice-bath), solid NaH (60% in oil, 2.0 g, 51.1 mmol, 1.1 equiv) was added in small portions. After addition, the reaction mixture was stirred at 5 °C for 40 min and then 1-iodobutane (5.3 mL, 8.5 g, 46.5 mmol) was added. The reaction was carried out overnight at rt and then evaporated. The residue was dissolved in H_2O (100 mL) and EtOAc (100 mL). The aqueous layer was extracted using EtOAc (2 × 100 mL), and the combined organic fractions were dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography (silica gel, CH_2Cl_2 → 9:1, CH_2Cl_2/MeOH) to give the product as a yellow solid (3.5 g, 41%). ^1H NMR (300 MHz, d_6-DMSO) δ = 8.73 (br s, 1H), 6.54 (s, 1H), 3.71−3.51 (m, 4H), 3.16−3.10 (m, 2H), 1.50−1.39 (m, 2H), 1.32−1.19 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H). ESI MS for $[M + H]^+$ = 185.7 Da.

*1-(2,4-Dichlorobenzyl)-2-(nitromethylene)imidazolidine (**11c**).* To a mixture of **9** (5.0 g, 38.7 mmol) in anhydrous DMF (80 mL) cooled to 0−5 °C (ice-bath), solid NaH (60% in oil, 1.7 g, 42.6 mmol, 1.1 equiv) was added in small portions. After addition, the reaction mixture was stirred at 5 °C for 40 min and then 2,4-dichloro-1-(chloromethyl)benzene (5.4 mL, 7.6 g, 38.7 mmol) was added. The reaction was carried out overnight at rt and then diluted with H_2O (150 mL). The product was separated as a yellow solid (11.1 g, 99%). ^1H NMR (300 MHz, $CDCl_3$) δ = 8.71 (br s, 1H), 7.45 (d, J = 2.1 Hz, 1H), 7.30−7.27 (m, 1H), 7.15 (d, J = 8.1 Hz, 1H), 6.59 (s, 1H), 4.38 (s, 2H), 3.85−3.78 (m, 2H), 3.64−3.57 (m, 2H). ESI MS for $[M + H]^+$ = 287.7 Da.

*1-(3,5-Bis(trifluoromethyl)benzyl)-2-(nitromethylene)-imidazolidine (**11d**).* The title compound was obtained in a manner similar to that used for **11c** using 1-(chloromethyl)-3,5-bis-(trifluoromethyl)benzene, as a yellow solid (77%). ^1H NMR (300 MHz, d_6-DMSO) δ = 8.93 (br s, 1H), 8.08−7.96 (m, 3H), 6.76 (s, 1H), 4.62 (s, 2H), 3.67−3.50 (m, 4H). ESI MS for $[M + H]^+$ = 355.7 Da.

*1-Butyl-2-(nitromethylene)hexahydropyrimidine (**11e**).* The title compound was obtained in a manner similar to that used for **11a**, as a yellow solid (50%). ^1H NMR (300 MHz, d_6-DMSO) δ = 10.37 (br s, 1H), 6.54 (s, 1H), 3.35−3.29 (m, 4H), 3.21−3.16 (m, 2H), 1.88−1.81 (m, 2H), 1.51−1.41 (m, 2H), 1.33−1.21 (m, 2H), 0.88 (t, J = 7.2 Hz, 3H). ESI MS for $[M + H]^+$ = 199.8 Da.

*2-(Nitromethylene)-1-pentylhexahydropyrimidine (**11f**).* The title compound was obtained in a manner similar to that used for **11a** using 1-iodopentane, as a brown solid (58%). ^1H NMR (500 MHz, $CDCl_3$) δ = 10.76 (br s, 1H), 6.62 (s, 1H), 3.43−3.39 (m, 2H), 3.38−3.36 (m, 2H), 3.16−3.13 (m, 2H), 2.05−1.99 (m, 2H), 1.62−1.56 (m, 2H), 1.36−1.22 (m, 4H), 0.90 (t, J = 7.3 Hz, 3H). ESI MS for $[M + H]^+$ = 213.7 Da.

*1-(2,4-Dichlorobenzyl)-2-(nitromethylene)hexahydropyrimidine (**11h**).* The title compound was obtained in a manner similar to that used for **11c**, as a yellow solid (74%). ^1H NMR (300 MHz, $CDCl_3$) δ = 10.84 (br s, 1H), 7.44 (d, J = 1.8 Hz, 1H), 7.29−7.25 (m, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.46 (s, 1H), 4.44 (s, 2H), 3.55−3.50 (m, 2H), 3.443.40 (m, 2H), 2.16−2.08 (m, 2H). ESI MS for $[M + H]^+$ = 301.7 Da.

*1-(3,5-Bis(trifluoromethyl)benzyl)-2-(nitromethylene)-hexahydropyrimidine (**11i**).* The title compound was obtained similar to **11c** using 1−(chloromethyl)−3,5−bis(trifluoromethyl)benzene, as a yellow solid (35%). ^1H NMR (300 MHz, d_6-DMSO) δ = 10.45 (br s, 1H), 8.06 (br s, 1H), 7.87 (br s, 2H), 6.54 (s, 1H), 4.74 (s, 2H), 3.48−3.40 (m, 4H), 1.97−1.90 (m, 2H). ESI MS for $[M + H]^+$ = 369.6 Da.

*1-Butyl-4,5-dihydro-1H-imidazole-2-carbaldehyde oxime hydrochloride (**12a**).* The title compound was obtained according to a modified literature procedure. To a solution of **11a** (2.2 g, 11.9 mmol) in anhydrous CH_2Cl_2 (100 mL) was added solid $SnCl_2 \times 2\ H_2O$ (14.7 g, 65.4 mmol, 5.5 equiv). The reaction mixture was stirred overnight at rt, and then solid $NaHCO_3$ (17.0 g, 202.3 mmol, 17.0 equiv) was carefully added in small portions. After addition, the mixture was stirred for 1 day at rt and then diluted with MeOH (100 mL). The suspension was filtered through Celite, washed with MeOH (3 × 100 mL), and evaporated. The crude product was purified by column

chromatography (silica gel, CH_2Cl_2 → 8:2, CH_2Cl_2/MeOH). The isolated product, in free base form, was dissolved in CH_2Cl_2/MeOH (9:1) and an excess of 2 M HCl in Et_2O was added, and the mixture was evaporated to give a yellow oil (0.46 g, 19%). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 13.50 (s, 1H), 10.13 (br s, 1H), 8.27 (s, 1H), 4.00−3.76 (m, 4H), 3.56 (t, J = 7.2 Hz, 2H), 1.58−1.48 (m, 2H), 1.32−1.19 (m, 2H), 0.88 (t, J = 7.2 Hz, 3H). ESI MS for $[M + H]^+$ = 169.9 Da.

*1-(2,4-Dichlorobenzyl)-4,5-dihydro-1H-imidazole-2-carbaldehyde Oxime Hydrochloride (**12c**).* The title compound was obtained in a manner similar to that used for **12a**, as a yellow solid (30%). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 13.59 (s, 1H), 10.65 (br s, 1H), 8.40 (s, 1H), 7.72 (d, J = 1.8 Hz, 1H), 7.55−7.47 (m, 2H), 4.93 (s, 2H), 3.88−3.76 (m, 4H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 159.0, 135.1, 133.8, 133.7, 131.4, 130.8, 129.2, 127.8, 49.6, 47.3, 42.8. ESI MS for $[M + H]^+$ = 271.9 Da.

*1-(3,5-bis(trifluoromethyl)benzyl)-4,5-dihydro-1H-imidazole-2-carbaldehyde oxime hydrochloride (**12d**).* The title compound was obtained in a manner similar to that used for **12a**, as a yellow solid (41%). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 13.53 (s, 1H), 10.58 (br s, 1H), 8.42 (s, 1H), 8.18 (br s, 2H), 8.12 (br s, 1H), 5.00 (s, 2H), 3.82 (br s, 4H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 159.2, 137.9, 135.4, 130.5 (q, J = 32.9 Hz), 129.2, 123.2 (q, J = 271.2 Hz), 121.9, 49.7, 48.8, 42.8. ESI MS for $[M + H]^+$ = 340.0 Da.

*1-Butyl-1,4,5,6-tetrahydropyrimidine-2-carbaldehyde Oxime Hydrochloride (**12e**).* The title compound was obtained in a manner similar to that used for **12a**, as a pink solid (28%). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 13.06 (s, 1H), 9.69 (br s, 1H), 8.27 (s, 1H), 3.56−3.48 (m, 4H), 3.30−3.36 (m, 2H), 1.96−1.88 (m, 2H), 1.58−1.48 (m, 2H), 1.31−1.19 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 153.0, 138.5, 51.3, 46.3, 38.1, 29.6, 18.9, 18.3, 13.5. ESI MS for $[M + H]^+$ = 184.1 Da.

*1-Pentyl-1,4,5,6-tetrahydropyrimidine-2-carbaldehyde Oxime Hydrochloride (**12f**).* The title compound was obtained in a manner similar to that used for **12a**, as a brown oil (42%). ^{1}H NMR (500 MHz, d_6-DMSO) δ = 13.20 (s, 1H), 9.90 (br s, 1H), 8.27 (s, 1H), 3.553.50 (m, 4H), 3.35−3.30 (m, 2H), 1.96−1.90 (m, 2H), 1.59−1.53 (m, 2H), 1.31−1.17 (m, 4H), 0.86 (t, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 152.9, 138.4, 51.5, 46.3, 38.1, 27.7, 27.2, 21.7, 18.3, 13.7. ESI MS for $[M + H]^+$ = 198.0 Da.

*1-(2,4-Dichlorobenzyl)-1,4,5,6-tetrahydropyrimidine-2-carbaldehyde Oxime Hydrochloride (**12h**).* The title compound was obtained in a manner similar to that used for **12a**, as a yellow solid (21%). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 13.10 (s, 1H), 10.19 (br s, 1H), 8.26 (s, 1H), 7.72 (d, J = 2.1 Hz, 1H), 7.52−7.40 (m, 2H), 4.90 (s, 2H), 3.48−3.35 (m, 4H), 2.02−1.92 (m, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 154.2, 138.8, 133.5, 133.3, 131.1, 130.4, 129.2, 127.8, 52.5, 46.6, 38.3, 18.1. ESI MS for $[M + H]^+$ = 285.9 Da.

*1-(3,5-Bis(trifluoromethyl)benzyl)-1,4,5,6-tetrahydropyrimidine-2-carbaldehyde Oxime Hydrochloride (**12i**).* The title compound was obtained in a manner similar to that used for **12a**, as a yellow solid (63%). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 13.05 (s, 1H), 10.11 (br s, 1H), 8.35 (s, 1H), 8.11−8.06 (m, 3H), 5.02 (s, 2H), 3.49−3.32 (m, 4H), 1.99−1.85 (m, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 154.3, 139.2, 138.5, 130.6 (q, J = 32.9 Hz), 128.8, 123.2 (q, J = 271.3 Hz), 121.8, 53.7, 46.5, 38.3, 18.1. ESI MS for $[M + H]^+$ = 354.0 Da.

*5,5-Dimethyl-2-(nitromethylene)hexahydropyrimidine (**13**).* The title compound was obtained in a manner similar to that used for **9** using 2,2-dimethylpropane-1,3-diamine, as a yellow solid (92%). ^{1}H NMR (500 MHz, d_6-DMSO) δ = 8.91 (br s, 2H), 6.27 (s, 1H), 2.99−2.98 (m, 4H), 0.95 (s, 6H). ESI MS for $[M + H]^+$ = 171.7 Da.

*1-Butyl-5,5-dimethyl-2-(nitromethylene)hexahydropyrimidine (**14a**).* The title compound was obtained in a manner similar to that used for **11a**, as an orange solid (47%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 10.80 (br s, 1H), 6.66 (s, 1H), 3.15−3.11 (m, 2H), 3.07−3.06 (m, 2H), 3.02 (br s, 2H), 1.59−1.53 (m, 2H), 1.36−1.27 (m, 2H), 1.06 (s, 6H), 0.94 (t, J = 7.3 Hz, 3H). ESI MS for $[M + H]^+$ = 227.7 Da.

*5,5-Dimethyl-2-(nitromethylene)-1-pentylhexahydropyrimidine (**14b**).* The title compound was obtained in a manner similar to that used for **11a** using 1-iodopentane, as a yellow solid (71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 10.79 (br s, 1H), 6.65 (s, 1H), 3.14−3.11 (m, 2H), 3.06 (br s, 2H), 3.01 (br s, 2H), 1.60−1.54 (m, 2H), 1.36−1.22 (m, 4H), 1.05 (s, 6H), 0.90 (t, J = 7.2 Hz, 3H). ESI MS for $[M + H]^+$ = 241.8 Da.

*1-Butyl-5,5-dimethyl-1,4,5,6-tetrahydropyrimidine-2-carbaldehyde Oxime Hydrochloride (**15a**).* The title compound was obtained in a manner similar to that used for **12a**, as a yellow solid (37%). ^{1}H NMR (500 MHz, d_6-DMSO) δ = 13.22 (br s, 1H), 9.98 (br s, 1H), 8.29 (s, 1H), 3.54−3.51 (m, 2H), 3.29 (br s, 2H), 3.06 (br s, 2H), 1.57−1.51 (m, 2H), 1.31−1.22 (m, 2H), 0.97 (s, 6H), 0.88 (t, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 152.3, 138.2, 56.7, 51.2, 48.9, 29.6, 25.9, 23.1, 18.9, 13.5. ESI MS for $[M + H]^+$ = 212.1 Da.

*5,5-Dimethyl-1-pentyl-1,4,5,6-tetrahydropyrimidine-2-carbaldehyde Oxime Hydrochloride (**15b**).* The title compound was obtained in a manner similar to that used for **12a**, as a yellow solid (35%). ^{1}H NMR (500 MHz, d_6-DMSO) δ = 13.22 (br s, 1H), 9.98 (br s, 1H), 8.29 (s, 1H), 3.54−3.50 (m, 2H), 3.27 (br s, 2H), 3.06 (br s, 2H), 1.59−1.52 (m, 2H), 1.31−1.20 (m, 4H), 0.98 (s, 6H), 0.86 (t,J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, d_6-DMSO) δ = 152.2, 138.2, 56.7, 51.4, 48.9, 27.7, 27.2, 25.9, 23.1, 21.6, 13.7. ESI MS for $[M + H]^+$ = 226.2 Da.

Enzyme Studies. Recombinant AChE, β-lactoglobulin from bovine milk (BLG), acetylthiocholine (ATC), butyrylthiocholine (BTC), and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO). Recombinant monomeric hBChE was expressed and purified from mammalian cells as previously described.[18] Syntheses and purification methods of OP model compounds were previously reported.[15,16] Reactivation studies were conducted using the same method for both AChE and BChE enzymes. ChE enzyme was diluted in PBS/BLG (pH 7.4), and a sample was taken and set aside for noninhibitor-treated controls. The remaining portion was incubated with a nerve agent model compound or ETP (Figure 7) for sufficient time to achieve 90% or greater inhibition (i.e., ca. 15 min). The reaction of the enzyme with nerve agent model compounds afforded covalent modification of the enzyme identical to that obtained with authentic nerve agents.[15,16] An excess of OP was removed from the inhibited enzyme by filtration through a 10 kDa MWCO filter with a modified PES membrane (VWR Scientific, Inc., San Diego, CA), followed by two washes prior to the final resuspension in PBS/BLG (pH 7.4). A 1000-fold excess of dilution of the OP was thus achieved. ChE enzyme was then added to PBS/BLG (pH 7.4) containing amidine-oxime, MINA, or 2-PAM (100 μM oxime with 2% DMSO after addition of enzyme) or vehicle. Oxime samples were equilibrated at 37 °C prior to the addition of enzyme. Enzyme was allowed to reactivate at 37 °C for 20 min (BChE) or 1 h (AChE), at which time the catalytic activity was determined using a modified Ellman's assay[19] in the presence of 1 mM substrate in PBS/BLG (pH 7.4). The Ellman assay utilized a 20-fold dilution of enzyme from the oxime incubation, yielding incubation concentrations in the range of 10 to 50 units L^{-1} (where 1 unit cleaves 1 μmol of substrate per min in PBS pH 7.4, room temperature) and 5 μM oxime. Two reactivation samples were prepared per oxime. The reported data show the average esterase activity for each oxime divided by the average functional activity of noninhibited control samples, and the propagated errors of the average functional activities.

In Vivo Studies. Adult Swiss Webster female mice (from Taconic) were housed in groups of four and maintained in a temperature-controlled environment on a 12 h/12 h light cycle (0600 h on−1800 h off) upon arrival to the laboratory. Animals were given free access to food and water during a one-week habituation period to the laboratory. Animals used in the research studies were handled, housed, and euthanized in accordance with a current HBRI IACUC protocol and NIH guidelines regarding the use and care of laboratory animals, and all applicable local, state, and federal regulations and guidelines. Each test compound, MINA, and 2-PAM were dissolved in isotonic saline and administered to separate groups of mice in a volume of 0.25 mL/mouse. The survival rate was recorded after 24 h from inititating an experiment.

***Sp*-GB-Am Studies.** At the 0 min time point, mice received ***Sp*-GB-Am hydrochloride** (0.08 mg/kg in 0.25 mL/mouse in saline, i.p.).

dx.doi.org/10.1021/jm201364d | J. Med. Chem. 2012, 55, 465−474

Five minutes later, an appropriate amidine-oxime (145 μmol/kg or 36 μmol/kg), MINA, or 2-PAM (145 μmol/kg) or vehicle was administered i.p. in isotonic saline (0.25 mL/mouse). The survival was recorded after 24 h. Animals that survived 24 h were then immediately euthanized by cervical dislocation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table with purity data for all compounds examined and reactivation data of OP-inhibited ChEs with oximes **7a−j**, **12a−i**, **15a−b**, and **2-PAM**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 001-(858) 458-9305. Fax: 001-(858) 458-9311. E-mail: JCashman@hbri.org.

■ ACKNOWLEDGMENTS

The financial support from HBRI is gratefully acknowledged. We acknowledge the helpful support of Professor Oksana Lockridge (University of Nebraska Medical Center).

■ ABBREVIATIONS USED

ACh, acetylcholine; AChE, acetylcholinesterase; ATC, acetylthiocholine; BBB, blood−brain barrier; BChE, butyrylcholinesterase; BTC, butyrylthiocholine; CNS, central nervous system; ChE, cholinesterase enzymes; DAM, diacetylmonooxime; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; BLG, β-lactoglobulin; PMB, *p*-methoxybenzyl protecting group; MeOTf, methyl trifluoromethanesulfonate; MINA, monoisonitrosoacetone; OP, organophosphate; 2-PAM, pralidoxime

■ REFERENCES

(1) Marrs, T. C. Organophosphate poisoning. *Pharmacol. Ther.* **1993**, *58*, 51−66.

(2) Taylor, P. Anticholinesterase Agents. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 10th ed.; Hardman, J. G., Limbird, L. E., Gilman, A. G., Eds.; McGraw-Hill: New York, 2001; pp 175−191.

(3) Okumura, T.; Hisaoka, T.; Yamada, A.; Naito, T.; Isonuma, H.; Okumura, S.; Miura, K.; Sakurada, M.; Maekawa, H.; Ishimatsu, S.; Takasu, N.; Suzuki, K. The Tokyo subway sarin attack-lessons learned. *Toxicol. Appl. Pharmacol.* **2005**, *207*, 471−476.

(4) Demar, J. C.; Clarkson, E. D.; Ratcliffe, R. H.; Campbell, A. J.; Thangavelu, S. G.; Herdman, C. A.; Leader, H.; Schulz, S. M.; Marek, E.; Medynets, M. A.; Ku, T. C.; Evans, S. A.; Khan, F. A.; Owens, R. R.; Nambiar, M. P.; Gordon, R. K. Pro-2-PAM therapy for central and peripheral cholinesterases. *Chem.-Biol. Interact.* **2010**, *187*, 191−198.

(5) Skovira, J. W.; O'Donnell, J. C.; Koplovitz, I.; Kan, R. K.; McDonough, J. H.; Shih, T. M. Reactivation of brain acetylcholinesterase by monoisonitrosoacetone increases the therapeutic efficacy against nerve agents in guinea pigs. *Chem.-Biol. Interact.* **2010**, *187*, 318−324.

(6) Mercey, G.; Verdelet, T.; Saint-Andre, G.; Gillon, E.; Wagner, A.; Baati, R.; Jean, L.; Nachon, F.; Renard, P. Y. First efficient uncharged reactivators for the dephosphylation of poisoned human acetylcholinesterase. *Chem. Commun. (Cambridge, U.K.)* **2011**, *47*, 5295−5297.

(7) Krasinski, A.; Radic, Z.; Manetsch, R.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. In situ selection of lead compounds by click chemistry: target-guided optimization of acetylcholinesterase inhibitors. *J. Am. Chem. Soc.* **2005**, *127*, 6686−6692.

(8) Sit, R. K.; Radic, Z.; Gerardi, V.; Zhang, L.; Garcia, E.; Katalinic, M.; Amitai, G.; Kovarik, Z.; Fokin, V. V.; Sharpless, K. B.; Taylor, P. New structural scaffolds for centrally acting oxime reactivators of phosphylated cholinesterases. *J. Biol. Chem.* **2011**, *286*, 19422−19430.

(9) Kalisiak, J.; Ralph, E. C.; Zhang, J.; Cashman, J. R. Amidine-oximes: reactivators for organophosphate exposure. *J. Med. Chem.* **2011**, *54*, 3319−3330.

(10) Dijkink, J.; Eriksen, K.; Goubitz, K.; van Zanden, M. N. A.; Hiemstra, H. Synthesis and X-ray crystal structure of (S)-9-hydroxymethyl-1,5-diazabicyclo[4.3.0]non-5-ene, an enantiopure DBN-analogue. *Tetrahedron: Asymmetry* **1996**, *7*, 515−524.

(11) Ostendorf, M.; van der Neut, S.; Rutjes, F. P. J. T.; Hiemstra, H. Enantioselective synthesis of hydroxy-substituted DBN-type amidines as potential chiral catalysts. *Eur. J. Org. Chem.* **2000**, *2000*, 105−113.

(12) Jaen, J. C.; Gregor, V. E.; Lee, C.; Davis, R.; Emmerling, M. Acetylcholinesterase inhibition by fused dihydroquinazoline compounds. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 737−742.

(13) Sauvaitre, T.; Barlier, M.; Herlem, D.; Gresh, N.; Chiaroni, A.; Guenard, D.; Guillou, C. New potent acetylcholinesterase inhibitors in the tetracyclic triterpene series. *J. Med. Chem.* **2007**, *50*, 5311−5323.

(14) Wang, K.; Qian, X.; Cui, J. One step from nitro to oxime: a convenient preparation of unsaturated oximes by the reduction of the corresponding vinylnitro compounds. *Tetrahedron* **2009**, *65*, 10377−10382.

(15) Barakat, N. H.; Zheng, X.; Gilley, C. B.; MacDonald, M.; Okolotowicz, K.; Cashman, J. R.; Vyas, S.; Beck, J. M.; Hadad, C. M.; Zhang, J. Chemical synthesis of two series of nerve agent model compounds and their stereoselective interaction with human acetylcholinesterase and human butyrylcholinesterase. *Chem. Res. Toxicol.* **2009**, *22*, 1669−1679.

(16) Gilley, C.; MacDonald, M.; Nachon, F.; Schopfer, L. M.; Zhang, J.; Cashman, J. R.; Lockridge, O. Nerve agent analogues that produce authentic soman, sarin, tabun, and cyclohexyl methylphosphonate-modified human butyrylcholinesterase. *Chem. Res. Toxicol.* **2009**, *22*, 1680−1688.

(17) Brimijoin, S.; Hammond, P. Butyrylcholinesterase in human brain and acetylcholinesterase in human plasma: trace enzymes measured by two-site immunoassay. *J. Neurochem.* **1988**, *51*, 1227−1231.

(18) Ralph, E. C.; Xiang, L.; Cashman, J. R.; Zhang, J. His-tag truncated butyrylcholinesterase as a useful construct for in vitro characterization of wild-type and variant butyrylcholinesterases. *Protein Expression Purif.* **2011**, *80*, 22−27.

(19) Ellman, G. L.; Courtney, K. D.; Andres, V. Jr.; Feather-Stone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88−95.