termined as A_{660nm}) had reached a value of 0.22, the culture was used for inoculation purposes. The remaining part was stored at -20 °C.

Determination of Antimycoplasmal Activity. The antimycoplasmal activity of all compounds was determined in the presence or the absence of copper and expressed as the minimal inhibitory concentration (MIC). In the former case the final concentration of $CuSO_4$ in the test tube was 40 μM. Tylosin and compound 1 were included as controls in every test. All compounds were dissolved in dimethyl sulfoxide whereas tylosin was dissolved in water. It was established that DMSO in the final concentration in the Adler medium (1.25%) has no effect on mycoplasmal growth. Serial 2-fold dilutions (in duplicate) of test compounds were made in Adler medium. Each tube, containing 3 mL of medium, was inoculated with 1 mL of a fresh culture of *Mycoplasma gallisepticum* K514, and these mixtures were incubated at 37 °C for 24 h. Mycoplasmal growth was indicated by a change in color of the indicator present in the medium. The minimal inhibitory concentration was determined as the lowest concentration that did not cause a change in color.

Data Processing. Statistical correlations were performed by using a commercial multiple linear regression program (Statworks, Cricket Software Inc., Philadelphia, PA). The figures in parentheses are the standard errors of regression coefficients. The parameters included in each equation are significant on a 1% level. For a given equation, n is the number of compounds, r if the multiple correlation coefficient, s is the standard error of estimate, and F represents the value of the F test.

Acknowledgment. This research was supported by the Netherlands Technology Foundation (STW).

Registry No. 1, 37989-04-1; **2a**, 118112-02-0; **2b**, 118112-03-1; **2b** (amide), 112575-49-2; **2c**, 118112-04-2; **2c** (amide), 112575-50-5; **2d**, 118112-05-3; **2e**, 118112-06-4; **2f**, 118112-07-5; **2f** (amide), 112575-53-8; **2g**, 118112-08-6; **2h**, 118112-09-7; **2h** (amide), 112575-55-0; **3a**, 118112-10-0; **3b**, 118112-11-1; **3c**, 118112-12-2; **3d**, 118112-13-3; **3e**, 118112-14-4; **3f**, 118112-15-5; **3g**, 118112-16-6; **4**, 79240-63-4; **5**, 118112-17-7; benzonitrile, 100-47-0; 4-chlorobenzonitrile, 623-03-0; 3,4-dichlorobenzonitrile, 6574-99-8; 3-methoxybenzonitrile, 1527-89-5; 2-pyridinecarbonitrile, 100-70-9; 4-pyridinecarbonitrile, 100-48-1; 2-pyrimidinecarbonitrile, 14080-23-0; 2-pyrazinecarbonitrile, 19847-12-2; 4,6-dimethyl-2-pyrimidinecarbonitrile, 22126-16-5; 6-methyl-2-pyridinecarbonitrile, 1620-75-3; 6-methyl-4-pyridinecarbonitrile, 2214-53-1; 2-aminopyridine, 504-29-0; 1-amino-5-methyl-3-phenylisoquinoline, 58814-44-1; *p*-toluoyl chloride, 874-60-2; *p*-methoxybenzoyl chloride, 100-07-2; *m*-toluoyl chloride, 1711-06-4; *m*-chlorobenzoyl chloride, 618-46-2.

Quaternary Salts of 2-[(Hydroxyimino)methyl]imidazole. 2. Preparation and in Vitro and in Vivo Evaluation of 1-(Alkoxymethyl)-2-[(hydroxyimino)methyl]-3-methylimidazolium Halides for Reactivation of Organophosphorus-Inhibited Acetylcholinesterases

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A series of structurally related mono- and bis-1,3-disubstituted 2-[(hydroxyimino)methyl]imidazolium halides were evaluated in vitro for their ability to reactivate electric eel, bovine, and human erythrocyte (RBC) acetylcholinesterases (AChE) inhibited by ethyl *p*-nitrophenyl methylphosphonate (EPMP) and 3,3-dimethyl-2-butyl methylphosphonofluoridate (soman, GD). All new compounds were characterized for (hydroxyimino)methyl acid dissociation constant, nucleophilicity, octanol–buffer partition coefficient, reversible AChE inhibition, and kinetics of reactivation of EPMP-inhibited AChEs. For GD-inhibited AChEs, maximal reactivation was used to compare compounds since rapid phosphonyl enzyme dealkylation "aging" complicated interpretation of kinetic constants. For comparison, we also evaluated three known pyridinium therapeutics, 2-PAM, HI-6, and toxogonin. In vivo evaluation in mice revealed that when selected imidazolium compounds were coadministered with atropine sulfate, they were effective in providing lifesaving protection against both GD and EPMP challenges. This was a major accomplishment in the search for effective anticholinesterase therapeutics—the synthesis and preliminary evaluation of the first new monoquaternary soman antidotes with potencies superior to 2-PAM. Significantly, there was an apparent inverse relationship between in vitro and in vivo results; the most potent in vivo compounds proved to be the poorest in vitro reactivators. These results suggested that an alternative and possibly novel antidotal mechanism of protective action may be applicable for the imidazolium aldoximes. Selected compounds were also evaluated for their inhibition of AChE phosphonylation by GD and antimuscarinic and antinicotinic receptor blocking effects.

Organophosphorus (OP) pesticides and chemical warfare (CW) nerve agents are powerful inhibitors of synaptic acetylcholinesterase (AChE).[1-7] Though pyridinium oximes effectively reverse intoxication symptoms in cases of accidental pesticide or nerve agent poisoning by many OP compounds, they are ineffective in preventing or treating

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§ U.S. Army Medical Research Institute of Chemical Defense.

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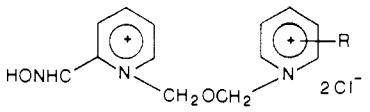
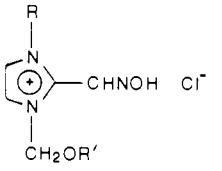
intoxication by 3,3-dimethyl-2-butyl methylphosphonofluoridate (soman, GD) when GD is administered in quantities exceeding approximately 1.5 times the LD_{50}.[8-10]

In 1970, Oldiges and Schoene[8] reported that certain unsymmetrically substituted bis(pyridinium) dimethyl ether derivatives constitute effective therapy for GD poisoning in mice under conditions where 2-PAM, toxogonin, and TMB-4 have insignificant therapeutic efficacy. The findings of Oldiges and Schoene evoked considerable interest[8-26] in the synthesis and evaluation of bis(pyridinium) dimethyl ether derivatives. The reactivators that are effective against GD conform to the general structure 1, where R = $C(O)NH_2$, $C(O)C_6H_5$, or $C(O)C_6H_{11}$ in the 3- or 4-position of the indicated pyridinium ring.[27]



1

Our own research[28-32] has focused on the preparation of reactivator analogues of 1 that differ not in the substitution pattern or groups on the pyridinium ring but rather in the nature of the alkyl/aralkyl ether moiety and in the nature of the heteroaromatic ring system. In search of improved reactivators of organophosphones-inhibited AChE, a series of 2-[(hydroxyimino)methyl]-3-methyl-1-(alkoxy/aralkoxymethyl)-substituted imidazolium halides 2 were prepared and evaluated for activity in vitro versus electric eel AChE inhibited by ethyl *p*-nitrophenyl methylphosphonate (EPMP) and GD.[32] These studies revealed particularly high reactivation potencies of type 2 compounds toward phosphonated AChE with only moderate competitive reversible inhibition of the enzyme. These findings suggested the imidazole ring system as a likely target for structural "fine tuning" to improve maximal activity. Accordingly, 31 new 3-alkyl-1-alkoxymethyl quaternary type 2 compounds and derivatives representing the 2-[(hydroxyimino)methyl]imidazolium ring system were synthesized.



2

All compounds were evaluated for (hydroxyimino)methyl acid dissociation constant, nucleophilicity, lipophilicity, reversible inhibition of AChE, and kinetics of reactivating EPMP- and GD-inhibited electric eel AChE. The present study was extended to test the in vitro activity of type 2 compounds versus EPMP- and GD-inhibited bovine and human erythrocyte (RBC) AChE. Additionally, the new imidazolium oximes were examined in vivo in mice against both EPMP and GD challenges. The remarkable ability of selected type 2 compounds to offer lifesaving protection against a challenge of 2×10 LD_{50} of GD while demonstrating only moderate to low in vitro reactivation potencies prompted an examination of nonreactivator modes of protective action. Antinicotinic and antimuscarinic receptor blocking effects of type 2 compounds were examined in an effort to elucidate the protective action of these new GD therapeutics. For comparison, the previously prepared imidazolium compounds[32] and the pyridinium reactivators 2-PAM, toxogonin, and 4-carbamoyl-2'-[(hydroxyimino)methyl]-1,1'-oxydimethylenebis(pyridinium chloride) (HI-6) were examined.

Results and Discussion

Synthesis, Structure, and Acidity. The 1-methyl-3-alkoxymethyl imidazolium compounds 2 (Table I), benzimidazolium compounds 3 (Table II), and the nonoxime 1-alkylimidazolium compound 4 (Table II) were prepared by the general synthesis route shown below, as previously described.[32] Quaternization of the imidazole ring with selected chloromethyl ethers, prepared from their respective alcohols[33,34,47] or with alkyl iodides followed by chloride ion exchange, provided the desired quaternary salts 2, 3, and 4. Table I provides structures and selected physical data for the new type 2 compounds, as well as previously reported derivatives.[32]

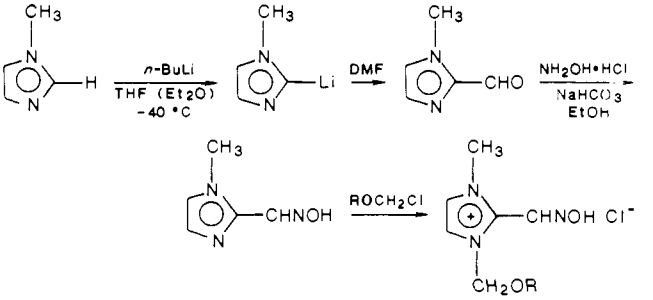


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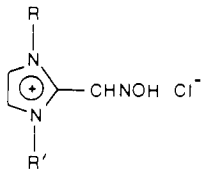
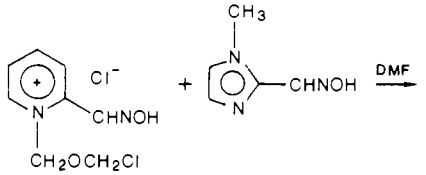
Table I. Selected Physicochemical Data for 1-Methyl-3-(alkoxymethyl)imidazolium Aldoximes

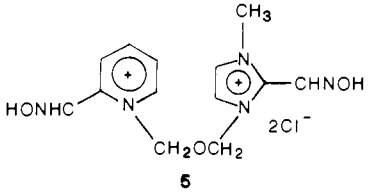


compd[a]	structure R	structure R′	mp, °C	% yield[b]	pK_a[c]	log P[d]	formula[e]
			Linear Alkyl				
2a[f]	CH_3	CH_2OCH_3	168–172	79	7.93	−3.0	$C_7H_{12}N_3O_2Cl$
2b	CH_3	$CH_2OCH_2CH_3$	125–128	87	8.07	−2.70	$C_8H_{14}N_3O_2Cl$
2c[f]	CH_3	$CH_2OCH_2CH_2CH_2CH_3$	102–105	38	8.04	−1.39	$C_{10}H_{18}N_3O_2Cl$
2d	CH_3	$CH_2O(CH_2)_5CH_3$	123–125	84	8.03	−0.11	$C_{12}H_{22}N_3O_2Cl$
2e[f]	CH_3	$CH_2O(CH_2)_7CH_3$	132–134	77	8.01	+0.88	$C_{14}H_{26}N_3O_2Cl$
			Branched Alkyl				
2f[f]	CH_3	$CH_2OCH(CH_3)_2$	128–130	94	7.93	−2.17	$C_9H_{16}N_3O_2Cl$
2g[f]	CH_3	$CH_2OCH_2C(CH_3)_3$	167–168	50	8.04	−0.91	$C_{11}H_{20}N_3O_2Cl$
2h[f]	CH_3	$CH_2OCH(CH_3)C(CH_3)_3$	169–170	64	8.01	−0.68	$C_{12}H_{22}N_3O_2Cl$
2i	CH_3	$CH_2OCH(C_2H_5)C(CH_3)_3$	149–151	43	7.94	−0.36	$C_{13}H_{24}N_3O_2Cl$
2j	CH_3	$CH_2OCH(CH_3)CH_2C(CH_3)_3$	154–156	62	8.00	−0.28	$C_{13}H_{24}N_3O_2Cl$
2k	CH_3	$CH_2OCH_2CH_2CH(CH_3)_2$	133–134	93	8.06	−1.08	$C_{11}H_{20}N_3O_2Cl$
2l	CH_3	$CH_2OCH(CH_3)CH_2CH_2CH_3$	135–137	87	8.02	−1.12	$C_{11}H_{20}N_3O_2Cl$
2m	CH_3	$CH_2OCH(C_2H_5)CH_2CH_2CH_3$	150–152	47	7.97	−0.86	$C_{12}H_{22}N_3O_2Cl$
2n	CH_3	$CH_2OCH(CH_3)CH(CH_3)CH_2CH_3$	150–152	48	7.97	−0.76	$C_{12}H_{22}N_3O_2Cl$
2o	CH_3	$CH_2OC(CH_3)_2C(CH_3)_3$	161	42	8.02	−0.25	$C_{13}H_{24}N_3O_2Cl$
			Alicyclic				
2p	CH_3	$CH_2OC_4H_7$	130–132	51	7.99	−2.10	$C_{10}H_{16}N_3O_2Cl$
2q	CH_3	$CH_2OC_5H_9$	122–126	86	8.04	−1.46	$C_{11}H_{18}N_3O_2Cl$
2r	CH_3	$CH_2OC_6H_{11}$	144–145	37	8.01	−1.20	$C_{12}H_{20}N_3O_2Cl$
2s	CH_3	$CH_2OC_7H_{13}$	155–157	55	8.03	−0.56	$C_{13}H_{22}N_3O_2Cl$
2t	CH_3	$CH_2OC_8H_{15}$	154	44	8.03	*g*	$C_{14}H_{24}N_3O_2Cl$
2u	CH_3	$CH_2OC_{12}H_{23}$	160–161	45	8.03	+1.48	$C_{18}H_{32}N_3O_2Cl$
2v	CH_3	$CH_2OCH_2C_3H_5$	142–143	66	8.01	*g*	$C_{10}H_{16}N_3O_2Cl$
2w	CH_3	$CH_2OCH_2C_6H_{11}$	145–148	67	8.03	−0.12	$C_{13}H_{22}N_3O_2Cl$
2x	CH_3	$CH_2OCH(CH_3)C_6H_{11}$	156–158	53	8.05	+0.22	$C_{14}H_{24}N_3O_2Cl$
2y	CH_3	$CH_2OCH(CH_2CH_2CH_3)C_5H_9$	130–140	57	7.97	+0.22	$C_{15}H_{26}N_3O_2Cl$
			Aromatic				
2z[f]	CH_3	$CH_2OCH_2C_6H_5$	124–127	79	7.94	−1.30	$C_{13}H_{16}N_3O_2Cl$
2aa[f]	CH_3	$CH_2OCH_2C_{10}H_7$	125–128	60	8.11	−0.36	$C_{17}H_{18}N_3O_2Cl$
2ab	CH_3	$CH_2OCH_2C_{10}H_{11}$	109–111	55	8.13	+0.07	$C_{17}H_{22}N_3O_2Cl$
2ac[f]	CH_3	$CH_2OCH_2CH_2CH_2C_6H_5$	130–131	82	8.06	−0.45	$C_{15}H_{20}N_3O_2Cl$

[a] See text for description of general synthesis route. [b] Yield for production of target compounds from immediate precursor. [c] Determined spectrometrically in 0.1 M phosphate buffer. [d] Log P is the octanol buffer partition coefficient for 0.1 M, pH 7.6 phosphate buffer. [e] All compounds were analyzed for C, H, N, and Cl; analytical results were within ±0.4% of the theoretical values. [f] See ref 32 for preparation. [g] Not determined.

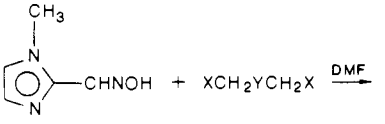
In addition to the 21 monoimidazolium compounds, five bisquaternary compounds were synthesized. Compound **5** was synthesized by treating 2-[(hydroxyimino)-methyl]-1-[(chloromethoxy)methyl]pyridinium chloride, the precursor to numerous bis-(pyridinium) oximes **1**,[11] with 2-[(hydroxyimino)methyl]-1-methylimidazole.

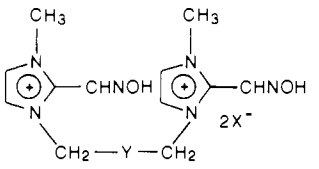




5

Compound **5** incorporates both the 2-[(hydroxyimino)-methyl]pyridinium moiety and the 2-[(hydroxyimino)-methyl]imidazolium, moiety thus combining the essential antidotal features of both reactivator types. Two other bis(quaternary) heteroaromatic reactivators, compounds **6** and **7**, were prepared by treating bis(chloromethyl) ether or ethylene (bis-chloromethyl) ether with the appropriate heteroaromatic aldoxime. The methylene analogues of **6**, compounds **8** and **9**, were prepared by treating the appropriate α,ω-diiodoalkane with 2-[(hydroxyimino)-methyl]-1-methylimidazole followed by ion exchange.

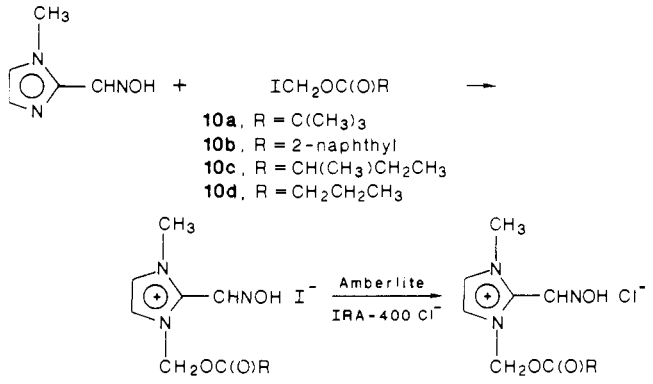




6: Y = O; X = Cl
7: Y = OCH_2CH_2O; X = Cl
8: Y = CH_2; X = Cl
9: Y = CH_2CH_2; X = Cl

Finally, the series of imidazolium analogues **10a** through

10d was prepared by treating the 2-[(hydroxyimino)-methyl]-1-methylimidazole with selected iodomethyl esters followed by ion exchange. Substitution of the "soft"[35,36] ester linkage in place of the "hard" ether linkage in analogues **10a–d** may provide compounds with improved therapeutic value (i.e. compounds with reduced toxicity while maintaining high therapeutic activity). Compounds **10a–d** can be considered as a prodrug form of the non-



quaternized imidazole oxime. Ester hydrolysis would yield the unstable imidazolium-3-formate, which spontaneously decomposes to form the nonquaternary imidazolium oxime plus formaldehyde. Table II provides structures and selected physical data for new quaternary compounds **3** through **10**.

All of the alkoxymethyl substituted mono-imidazolium oximes (**2a** through **2ac**) exhibited acid dissociation constants (pK_a) near 8.0 in contrast to the value of 8.2 to 8.4 for the dialkyl-substituted derivatives.[37] In contrast, the benzimidazolium oximes **3a** and **3b** along with the bis-(quaternary) oximes **5**, **6**, and **7** exhibited lower pK_a's of 7.5, 7.3, 7.5, 7.7, and 8.0, respectively. The additional stabilization of the oximate anion with these compounds can be attributed to increased charge delocalization through the benzo system and increased ring electronegativity. Since the oximate anion serves as the nucleophile during reactivation,[38,39] it would be expected that the increased oxime acidity of the alkoxymethyl derivatives **2** relative to the 1,3-dialkyl derivatives[37] will lead to a higher oximate concentration in vivo and therefore provide more efficient reactivation. As noted previously,[28-32] the increased oxime dissociation at physiological pH is offset by a decrease in inherent nucleophilicity of the oximate anion.

All oximes were obtained configurationally pure as evidenced by ^1H NMR spectra (taken in DMSO-d_6). They all exhibited chemical shift values for the oxime hydroxyl proton between δ 12.50 to 13.70. By analogy with literature results,[37] the *E* configuration is assigned to these derivatives.

Reversible Acetylcholinesterase Inhibition. Suitable control experiments were performed with the EPMP-inhibited bovine and human erythrocyte AChE, which showed that the previously derived equations and relationships[32] also extend to these AChE systems. The degree to which type **2** compounds reversibly inhibit the enzyme was determined to probe possible AChE binding interactions (and also as a control in our enzyme assays). Following the general procedure described earlier,[28] enzyme activities in the presence of each test compound were determined. In the absence of EPMP, test compounds inhibited AChE in a reversible, time-independent fashion. Therefore, we express the reactivator–AChE inhibition as the IC_{50}, i.e., the concentration of test compound that contributes to 50% AChE activity (Table III). Type **2** compounds are moderate to strong reversible inhibitors, highly dependent upon structural features.

Reactivation of EPMP-Phosphonylated Eel, Bovine, and Human AChE. The kinetics of AChE reactivation for EPMP-inhibited eel AChE or bovine and human erythrocyte (RBC) AChE were examined. Pseudo-first-order reactivation kinetics were revealed when the test compound was added in excess over EPMP-inhibited enzyme. Following the previously derived kinetic treatment,[28,32] it is convenient to express reactivation potency as k_{OX} ($= k_r/K_r$, where K_r = the binding of reactivator to inhibited enzyme and k_r = the transformation rate of [reactivator/inhibited enzyme] complex to active enzyme), the bimolecular rate constant for reactivation in the limit of low reactivator concentration ([OX]) $\ll K_r$). For all compounds tested, k_r and K_r were determined (Table A, see paragraph at end of paper about supplementary material). Because various imidazolium compounds ionize to different extents at pH 7.6, the effective rate constant for reactivating inhibited AChE, k_{HOX}, was defined as the product of k_{OX} and the fraction of added test compounds present as oximate at pH 7.6; $k_{OX}[1 + \text{antilog } (pK_a - 7.6)]$. Table III summarizes data for reactivation of ethyl methylphosphonyl-inhibited eel, bovine, and human AChE by selected imidazolium test compounds. For comparison, the table also gives data for 2-PAM and HI-6.

Comparing the reactivation kinetics (k_{HOX}) for EPMP-inhibited AChE) of the three enzyme systems revealed striking differences and similarities. The table demonstrates that against EPMP type **2** compounds are potent reactivators, highly dependent upon the alkoxymethyl side chain: k_{HOX} values varied from a low of 230 M^{-1} min^{-1} (**2h**) to a high of 2800 M^{-1} min^{-1} (**2e**) for eel AChE; for bovine AChE k_{HOX} values varied from a low of 22 M^{-1} min^{-1} (**2h**) to a high of 401 M^{-1} min^{-1} (**2ac**); with similar values for the k_{HOX} of human AChE ranging from a low 33 M^{-1} min^{-1} (**2z**) to a high of 482 M^{-1} min^{-1} (**2x**). An order of magnitude difference in the reactivation kinetics is observed between eel and either bovine or human AChEs.

One can infer several general trends from Table III. First, increased side-chain steric bulk (i.e. neopentyl (**2g**) or pinacolyl (**2h**) substituents) significantly lowers the relative effective reactivation rate constant, k_{HOX}. Secondly, aryl-substituted type **2** compounds (i.e. **2z**, **2aa**, and **2ac**) have high relative reactivation constants, as do the linear substituted (i.e. **2a**, **2c**, **2d**, and **2e**) and the alicylic type **2** compounds (**2w** and **2x**). Third, significant in vitro reactivation potencies were observed with all bis(quaternary) systems studied, compounds **5**, **6**, and **7**. Finally, as would be expected, removal of the oxime moiety, compound **4**, resulted in the complete loss of in vitro reactivation potency.

Comparing the relative k_{HOX} values for the new imidazolium derivatives indicated the broad range of potencies displayed by the new mono- and bis(quaternary) imidazolium oximes toward EPMP-inhibited AChEs, Table III. The relative k_{HOX} potencies remained consistent within each subgroup of reactivators. Linear alkoxymethyl substituents (**2a–e**) generally demonstrated high k_{HOX} values for all enzyme sources, whereas highly branched alkoxy-

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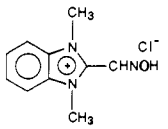
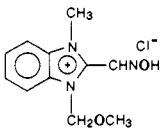
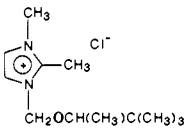
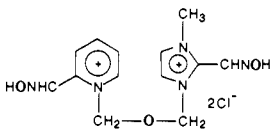
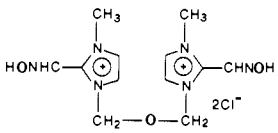
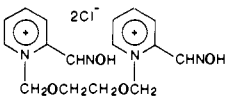
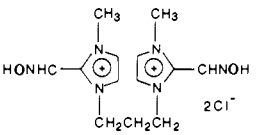
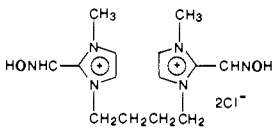
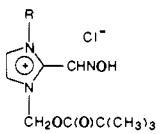
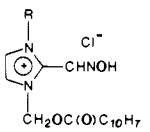
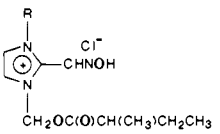
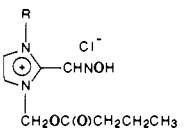
Table II. Selected Physicochemical Data for Miscellaneous Imidazolium Compounds

compd[a]	structure	mp, °C	% yield[b]	pK_a[c]	log P[d]	formula[e]
3a		223–224	55	7.54	−1.13	$C_{10}H_{12}H_3OCl$
3b		143–146	41	7.33	−0.97	$C_{11}H_{14}N_3O_2Cl$
4		150–160	89		−1.71	$C_{12}H_{23}N_2OCl$
5		196–198	11	7.56	<−3.0	$C_{13}H_{17}N_5O_3Cl_2$
6		232–233	90	7.71	<−3.0	$C_{12}H_{18}N_6O_3Cl_2$
7		176–179	76	8.02	<−3.0	$C_{16}H_{20}N_4O_4Cl_2$
8		260	4.5	8.13	<−3.0	$C_{13}H_{20}N_6O_2Cl_2$
9		236–238	30	8.02	<−3.0	$C_{14}H_{22}N_6O_2Cl_2$
10a		194–196	46	8.30	−1.36	$C_{11}H_{18}N_3O_3Cl$
10b		203–205	36	8.21	+0.52	$C_{17}H_{16}N_3O_3Cl$
10c		148–153	27	8.24	−1.21	$C_{11}H_{18}N_3O_3Cl$
10d		135–136	31	8.13	−1.53	$C_{10}H_{16}N_3O_3Cl$

[a] See text for description of general synthesis route. [b] Yield for production of target compounds from immediate precursor. [c] Determined spectrometrically in 0.1 M phosphate buffer. [d] log P is the octanol buffer coefficient for 0.1 M, pH 7.6 phosphate buffer. [e] All compounds were analyzed for C, H, N, or Cl; analytical results were within ±0.4% of the theoretical values.

methyl substituents (**2f–j**) displayed moderate to low reactivation potencies, while aromatic substituted ethers (**2z–ac**) showed high in vitro reactivation potencies independent of AChE source. None of the imidazolium reactivators tested approach HI-6 in potency toward reactivation of RBC AChEs, although numerous test compounds were superior in reactivating EPMP-inhibited eel AChE.

For the imidazolium class of compounds, reactivation potentcy of these drugs can vary greatly with AChE source. No clearcut extrapolation to the in vivo mouse studies (vide

Table III. Rate of EPMP-Inhibited Eel, Bovine, and Human AChE Reactivation by Selected Quaternary Imidazolium Halides

	eel AChE			bovine erythrocyte (RBC)			human erythrocyte (RBC)		
compd[a]	IC_{50},[b] μM	k_{OX},[c] 10^{-3} M^{-1} min^{-1}	k_{HOX},[d] 10^{-3} M^{-1} min^{-1}	IC_{50},[b] μM	k_{OX},[c] M^{-1} min^{-1}	k_{HOX},[d] M^{-1} min^{-1}	IC_{50},[b] μM	k_{OX},[c] M^{-1} min^{-1}	k_{HOX},[d] M^{-1} min^{-1}
2a	290.0	7.6	2.2	139.0	710	208	134.0	916	260
2c	20.0	3.8	1.0	42.0	490	130	27.5	423	121
2d	e	e	e	e	e	e	25.0	1328	378
2e	7.0	9.8	2.8	11.0	670	189	9.5	226	64
2f	80.0	1.6	0.52	48.0	170	52	49.0	274	78
2g	12.0	2.0	0.54	9.4	270	73	8.4	457	130
2h	16.0	0.83	0.23	19.0	80	22	11.5	317	90
2i	e	e	e	e	e	e	24.0	242	69
2j	e	e	e	e	e	e	32.5	273	78
2w	e	e	e	e	e	e	42.0	2056	585
2x	e	e	e	e	e	e	26.0	1692	482
2z	50.0	8.2	2.6	62.0	555	175	56.0	115	33
2aa	7.0	7.3	1.7	4.2	870	206	3.8	1188	480
2ab	e	e	e	e	e	e	56.0	1154	328
2ac	15.0	8.4	2.2	30.0	1530	401	21.2	899	256
3a	45.0	2.6	0.74	e	e	e	123.0	400	114
3b	180.0	1.7	0.48	e	e	e	198.0	198	56
4	e	e	e	e	e	e	33.0	0	0
5	12.0	5.1	1.44	e	e	e	38.0	1405	400
6	3.0	11.5	3.3	e	e	e	7.8	2202	627
7	120.0	15.5	4.4	e	e	e	339.6	6835	1946
2-PAM	185.0	9.6	2.8	346	2930	857	366	1695	482
HI-6	800.0	0.61	0.46	126	2300	1650	89	11500	8680

[a] See Tables I and II for structures. [b] IC_{50} is the concentration of test compound required to inhibit 50% active AChE. [c] k_{OX}, the bimolecular reactivation rate constant, was calculated as previously described; see ref 28–32. [d] k_{HOX}, the effective rate constant for reactivation, adjusts for the differences in oxime ionization at pH 7.6. [e] Not determined.

infra) is possible with the available data, and the "best" model system for in vitro studies is still unknown. Additional data on relevent test systems will be necessary before an understanding of these variations can be established.

Reactivation of GD-Phosphonylated Human AChE. GD-inhibited AChE differs from EPMP-inhibited enzyme in several important respects. Spontaneous dealkylation of the phosphonyl moiety is faster for GD-inhibited AChE than for EPMP-inhibited enzyme. The presence of four different GD isomers (vs two for EPMP) and potential rapid reinhibition by the phosphonyl oximes further complicate reaction kinetics. Additionally, the pinacolyl moiety of GD-inhibited AChE effectively covers the enzyme active site,[40,41] retarding interactions between inhibited enzyme and reactivator. Although it is possible in principle to design experiments that permit determination of all kinetic parameters for reactivation and dealkylation of GD-inhibited enzyme (e.g., see the excellent papers of DeJong and Wolring),[42,43] in practice such determinations are extremely difficult. Thus the precent reactivation at various times, R_t, was determined in this investigation.

The compounds clearly showed the expected time-dependent increase in R_t. The R_t values approached a maximum, R_{max} at about 30 min but never approached the theoretical limit of $R_{max} = 100$. This behavior is consistent with rapid dealkylation concurrent with reactivation of phosphonylated enzyme.

A comparison of the R_{max} values for GD-inhibited eel, bovine, and human AChEs is shown in Table IV. 2-PAM, HI-6, and toxogonin were included as the base line for this

Table IV. Percentage of Maximum Reactivation (% R_{max}) for GD-Inhibited Eel, Bovine, and Human AChE by Imidazolium Oximes[a]

compd[b]	% R_{max}[c] eel AChE	% R_{max}[c] bovine erythrocyte AChE	% R_{max}[c] human erythrocyte AChE
2a	15.0	11.2	9.8
2c	17.4	5.7	6.8
2d	e	e	13.4
2e	9.3	19.6	19.5
2f	9.8	5.0	5.8
2g	5.4	9.0	8.5
2h	5.4	9.0	8.5
2i	e	e	3.7
2j	e	e	2.8
2w	e	e	16.2
2x	e	e	9.4
2z	25.5	13.6	12.0
2aa	20.4	13.4	14.4
2ab	e	e	4.8
2ac	19.3	25.2	20.4
4	0.0	e	0.0
5	58.8	e	58.2
6	51.6	34.1	54.2
7	28.7	37.2	34.3
2-PAM	23.3	29.0	26.1
toxogonin	36.5	50.6	48.6
HI-6	48.7	88.4	(100)[d]

[a] A 1.0 mM concentration was used for all test oximes; incubation with oxime was conducted in buffer at pH 7.6 and 25 °C. [b] See text for compound synthesis. [c] Maximum reactivation for all test oximes was generally achieved by 30-min incubation; the values given represent the average of % R_{max} from 30 to 90 min. [d] HI-6 was incubated at 1.0×10^{-4} M. [e] Not determined.

inhibitor/enzyme system. In comparing the R_{max} values for the standard therapeutics, 2-PAM is essentially equipotent for all enzyme systems. A similar effect was observed for toxogonin. However, HI-6 displayed remarkable species dependency with R_{max} values of 48.7, 88.4, and essentially 100% for eel, bovine, human enzymes, respectively. In this regard, HI-6 follows the same pattern observed with EPMP reactivation. It should be kept in mind that changes in R_{max} are not linear functions and that a change from 48.7 for eel to 88.4 for bovine is considerably

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Table V. Survival of Mouse against 2 × LD_{50} of Soman[a]

compd[b]	LD_{50},[c] im, mM/kg	number of surviving mice (dose level of test compound + 11.2 mg/kg atropine)[d] $^1/_4$ LD_{50} test drug	$^1/_8$ LD_{50} test drug	$^1/_{16}$ LD_{50} test drug
		Linear Alkyl		
2a	0.620	0	0	*e*
2b	0.612	0	0	0
2c	0.705	4	1	*e*
2d	0.288	1	0	0
2e	0.094	0	0	*e*
		Branched Alkyl		
2f	0.417	0	0	*e*
2g	0.219	4	3	*e*
2h	0.492	6	4	*e*
2i	0.222	2	1	0
2j	0.205	3	0	1
2k	0.392	2	1	0
2l	0.651	7	4	0
2m	0.321	3	0	0
2n	0.167	2	0	1
		Alicyclic		
2p	0.495	4	0	0
2q	0.712	0	0	0
2r	0.261	0	2	0
2s	0.193	2	0	0
2t	0.631	1	1	0
2u	0.161	0	0	0
2v	0.621	4	0	0
2w	0.391	0	0	0
2x	0.521	3	1	0
2y	0.184	0	0	0
		Aromatic		
2z	0.646	0	0	*e*
2aa	0.043	1	1	*e*
2ab	0.199	0	0	0
2ac	0.585	0	0	*e*
3a	1.40	3	0	1
3b	0.451	0	0	*e*
5	0.342	0	0	*e*
6	0.255	5	0	0
7	0.278	0	0	*e*
8	0.124	1	1	0
9	0.372	0	0	0
10a	0.372	5	4	0
10b	0.177	2	0	1
10c	0.333	0	0	0
10d	0.511	0	0	1

[a] LD_{50} GD (plus 11.2 mg/kg atropine) ≅ 130 μg/kg in ~20 to 30 g mouse; without atropine, GD LD_{50} ≅ 98 μg/kg. [b] See Tables I and II for structures. [c] The 24 h LD_{50}s were determined intramuscularly (im) using 5 to 7 dose groups with 5 animals per dose. The test oxime generally range in toxicity between 2-PAMCl (853 μmol/kg, im) and TMB-4 (224 μmol/kg, im). [d] 2-PAMCl was used as a base line. No mice survived after intoxication with 2 × LD_{50} GD followed immediately by coadministration of 25 mg/kg 2-PAM (~$^1/_4$ LD_{50}) plus 11.4 mg/kg atropine sulfate. The number recorded represents the number of surviving out of 10 animals. Mice were challenged im with 2 × LD_{50} of soman and treated 10 s later with the indicated dose level of oxime plus atropine sulfate (11.2 mg/kg). [e] Not determined.

greater than a doubling of the reactivation potency. For the type **2** compounds, the reactivation of GD-inhibited AChEs was somewhat comparable for all three enzymes.

The linear alkoxymethyl-substituted imidazolium compounds (**2a–e**) generally demonstrated moderate R_{max} values irrespective of enzyme source. The highly branched type **2** compounds (**2f–j**) exhibited poor R_{max} values, whereas the alicyclic and aralkoxymethyl reactivators (compounds **2w–ac**) demonstrated moderate reactivating potencies. The bis(quaternary) compounds reported in Table IV showed the highest R_{max} of any of the compounds studied. Only HI-6, the most effective reactivator of GD-inhibited AChE both in vivo and in vitro,[24] was superior to the imidazolium compounds **5** and **6**. For the imidazolium class of compounds, reactivation potency varied greatly, depending on the side-chain substituent, but appeared to be rather consistent for both GD- and EPMP-inhibited AChE systems. Only the absolute magnitude of the reactivation potency changed for EPMP-inhibited eel enzyme system.

Table VI. Protection of Mice against EPMP by Oximes plus Atropine (20 mg/kg)[a]

compd[b]	EPMP dose (μg/kg) (mice live/total)			PI[c]
2a	2000	3000	3500	
$^1/_8$ LD_{50}, 16 mg/kg	(7/7)	(5/9)	(4/9)	3.0
2g	1000	1250	2000	
$^1/_8$ LD_{50}, 7.2 mg/kg	(7/8)	(7/10)	(3/7)	1.5
2h	1000	2000	3000	
$^1/_8$ LD_{50}, 17 mg/kg	(9/10)	(4/9)	(0/5)	2.0
2ac	600	800	1000	
$^1/_8$ LD_{50}, 33 mg/kg	(5/9)	(0/9)	(0/9)	0
2-PAM	2000	2700	5000	
$^1/_4$ LD_{50}, 25 mg/kg	(5/10)	(5/10)	(1/7)	2.5
HI-6	3000	4000	5000	
$^1/_4$ LD_{50}, 77 mg/kg	(7/8)	(7/8)	(5/8)	5.0

[a] LD_{50} EPMP (plus 20 mg/kg atropine sulfate) ≃ 1000 μg/kg in ~35-g mouse; without atropine, EPMP LD_{50} ≃ 600–700 μg/kg. [b] See Table I for structures. [c] PI is the protective index = LD_{50} with oxime/LD_{50} without oxime.

Protective Effects in Vivo against Soman and EPMP. The results of initial in vivo mouse evaluations against GD and EPMP for the compounds prepared in this study are shown in Tables V and VI, respectively. As Table V demonstrates, numerous type **2** compounds afford significant protection against the lethal effects of 2 × LD_{50} of soman. These compounds are the first monoquaternary oximes to provide life-saving protection against GD at this dose level. In all cases 2-PAM was used as the base-line standard. At this dose level, 2 × LD_{50} of GD, all mice treated with $^1/_4$ the LD_{50} of 2-PAM (approximately 25 mg/kg) expire. Similar results are obtained with lower 2-PAM dose levels.

It is apparent from Table V that the most effective in vivo therapeutics are the highly branched alkoxymethyl compounds (**2f–n**) with potencies approaching 60 to 70% survival rates for compounds **2h** and **2l**, respectively. This was unexpected, since several of these compounds (**2f–j**) showed only modest or low in vitro reactivating ability irrespective of enzyme or inhibitor. Additionally, compounds that demonstrated high in vitro potencies showed little or no ability to protect mice against the lethal effects of GD (the linear alkoxymethyl, alicyclic, and aralkyloxymethyl type **2** compounds).

The bis(quaternary) compounds **5** through **9** showed only modest protective abilities in contrast to the high in vitro reactivation potencies measured in the above enzyme model systems. None of the compounds tested to date work in the absence of the anticholinergic agent atropine, which must be coadministered with the test reactivator.

Several type **2** compounds, 2-PAM, and HI-6 were also evaluated against the toxic effects of EPMP, Table VI. Interestingly, the protection afforded by the compounds did not directly correlate with the GD results. The primary difference is that compound **2a** is the most protective of the compounds tested against EPMP, yet it showed no efficacy against GD. The compounds **2g** and **2h**, which were quite effective against GD (40% and 60%, respectively), also protected against EPMP, indicating that their action is not GD-specific. Compound **2ac**, which has high

Table VII. Potency of Selected Oximes for Inhibition of Phosphonylation of Eel AChE by GD

compd[a]	IC_{50},[b] mM	K_i,[c] mM
2a	0.2900	0.0480
2aa	0.0070	0.0035
2h	0.0160	0.0076
2ac	0.0150	0.0040
2-PAM	0.1847	0.1265
HI-6	0.8027	0.2288

[a] See text for compound structures. [b] Competitive inhibition of AChE; taken from Table III. [c] Inhibition of phosphonylation.

in vitro reactivation potency, appeared to enhance the toxicity of EPMP.

Nonreactivation Evaluation of Imidazolium Oximes. The type **2** compounds were designed to have a high affinity for the active site(s) on AChE and could provide protection against the effects of GD by several mechanisms of action other than reactivation of inhibited AChE. Such mechanisms of action include (1) inhibition of AChE phosphonylation by GD, (2) a decrease in the aging rate of the GD-inhibited AChE, (3) antinicotinic or antimuscarinic receptor blocking effects, and (4) direct reaction of the antidote with GD to form a nontoxic adduct or degradation product.

Four type **2** compounds (**2a**, **2h**, **2aa**, and **2ac**), which varied from highly protective to nonprotective against GD in mice and from moderately to highly toxic, 2-PAM, and HI-6 were evaluated for direct inhibition of phosphonylation of eel AChE. None of these compounds are particularly potent in protecting eel AChE, as revealed by the K_i, a measure of phosphonylation inhibitory potency (Table VII). The reversible inhibitory potency of these compounds (IC_{50}) is similar to the K_i for all compounds studied except **2a**. However, since direct reversible inhibition of AChE by these test compounds was independent of the AChE source (see Table III), inhibition of phosphonylation should also be invariant with AChE source. If this principle is valid, then neither competitive inhibition of AChE or inhibition of phosphonylation explains the antidotal efficacy of type **2** compounds.

The same four type **2** compounds were tested for muscarinic and nicotinic receptor blocking effects, Table VIII. The weak potency of the compounds in competition for quinuclidinyl benzylate (QNB) binding sites (antimuscarinic efficacy) and for α-bungarotoxin binding sites (antinicotinic efficacy) makes it extremely unlikely that these compounds could be working (in their tested form) as muscarinic and nicotinic antagonists. We have not evaluated the rate at which these compounds directly react with GD or other organophosphonates because previous work[38,44] has demonstrated that this factor is related simply to the nucleophilicity of the oximes.

Conclusions

A variety of monquaternary alkyl, alicyclic, and arylalkoxymethyl imidazolium aldoximes were prepared and evaluated as reactivators of ethyl methylphosphonylated and 3,3-dimethyl-2-butyl methyl phosphonylated eel, bovine, and human AChE. In the study, it was found that incorporation of linear, alicyclic, and arylalkoxy moieties on the imidazole ring enhance activity toward EPMP- and GD-inhibited AChEs. Branching on the alkyl substituent signifcantly reduces in vitro reactivating potency toward both EPMP- and GD-inhibited AChEs.

Table VIII. Antimuscarinic and Antinicotinic Potencies for Selected Oximes

	IC_{50}, mM	
compd[a]	muscarinic[b]	nicotinic[c]
2a	1.0	$\gg$1.0
2aa	0.035	0.7
2h	0.080	0.6
2ac	0.022	0.2
HI-6	0.16	$\gg$1.0
2-PAM	0.17	$\sim$1.2

[a] See text for compound structures. [b] The method of Yamaura and Snyder, ref 49, was used to evaluate muscarinic receptor binding. Mouse brain membranes were incubated at 25 °C with ^{3}H-QNB and various concentrations of the test compound. IC_{50} for inhibition was determined via scintillation number after correction for ^{3}H-QNB nonspecific binding. [c] The method of Moore and Brandy, ref 50, was used to evaluate nicotinic receptor binding.

A major milestone was achieved with the synthesis and evaluation of the first effective non-bis(pyridinium) soman antidotes. In an effort to characterize more accurately the parameters that influence antidotal efficacies, toxicities, and mechanisms of action of the type **2** compounds, an extensive series of structurally related imidazolium oximes and non-oximes are being prepared (see the accompanying paper). The poor correlation between in vitro and in vivo evaluation in mice suggests that the imidazolium compounds (designed to have a high affinity for the AChE active site) might provide protection against the effects of GD by several mechanisms in addition to reactivation of inhibited AChE. This anomalous behavior of the imidazolium oximes points to a significant shortcoming in our understanding of the reactivation/protection process.

The following observations emerged from this study on the development of new imidazolium antidotes: (1) Quaternary derivatives of 2-[(hydroxyimino)methyl]-1-methylimidazolium salts provide significant life-saving protection in the mouse against both GD and EPMP when used in conjunction with atropine. (2) The protective efficacy of imidazolium compounds against EPMP in the mouse generally parallels that observed against GD in the mouse. (3) Highly branched bulky substituents in **2** appear to be detrimental to in vitro reactivation potency for EPMP- and GD-inhibited eel, bovine, and human AChE enzymes while being favorable for in vivo protection against GD intoxication in the mouse. (4) The observed protective efficacy of the imidazolium compounds against organophosphorus agents in the mouse is apparently not due to muscarinic and/or nicotinic binding.

Experimental Details[45]

Physical Measurements. Reactivator pK_a values were determined spectrophotometrically in 0.1 M phosphate buffer by the method of Albert and Sargeant.[51] Octanol/water partition coefficients were determined spectrophotometrically by the method of Fujita et al.[46] The aqueous phase for all log P determinations was pH 7.4, 0.1 M phosphate buffer. Competitive inhibition of eel, bovine, and human AChE and reactivation of AChE after inhibition with GD and EPMP were performed as described previously.[31,32]

General Procedure for Preparing Alkyl/Aryl Chloromethyl Ethers. The preparation of selected chloromethyl ethers required in this study have been reported elsewhere.[47] The

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(45) Instrumentation and materials used in the current study were described in detail in the Experimental Section of ref 32.

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Table IX. Selected Physicochemical Data for New Chloromethyl Ethers, $ClCH_2OR$

chloromethyl ether	bp, °C (mmHg)	yield, %	^1H NMR[a] $ClCH_2O$	^1H NMR[a] OR
chloromethoxycyclohexane	90–91 (4.0)	76	5.62	1.00–2.33 (br m, 10 H, cyclohexyl), 3.35–4.11 (m, 1 H, CH)
1-(chloromethoxy)-1-cyclopentylbutane	65–67 (0.4)	60	5.62	0.95 (br t, 3 H, CH_3), 1.00–2.30 (m, 13 H, cyclopentyl, CH_2), 3.65 (m, 1 H, CH)
1-(chloromethoxy)-1-cyclohexylethane	86–87 (5.0)	38	5.50	1.12 (d, 3 H, J = 6.0 Hz, CH_3), 1.0–2.0 (br m, 11 H, cyclohexyl), 3.58 (m, 1 H, CH)
3-(chloromethoxy)-2,2-dimethylpentane	64–65 (5.0)	58	5.50	0.87 (s, 9 H, CH_3), 1.0–1.8 (br m, 5 H, CH_2, CH_3), 3.22 (dd, 1 H, J = 7.0 and 4.0 Hz, CH)
chloromethoxy(1,2,3,4-tetrahydronaphth-1-yl)methane	119–121 (0.25)	76	5.53	1.80 (m, 4 H, CH_2), 2.73 (t, 2 H, CH_2), 3.10 (m, 1 H, CH), 3.83 (d, 2 H, J = 6.0 Hz, CH_2), 7.15 (AB q, 4 H, J = 5.0 Hz, aryl)
4-(chloromethoxy)-2,2-dimethylpentane	62–63 (7.0)	60	5.43	0.95 (s, 9 H, CH_3), 1.22 (d, 3 H, J = 6.0 Hz, CH_3), 1.37 (m, 2 H, CH_2), 4.07 (m, 1 H, CH)
1-(chloromethoxy)-3-methylbutane	60–61 (2.5)	41	5.53	0.92 (d, 6 H, CH_3), 1.53 (t/m, 3 H, CH_2/CH), 3.65 (m, 2 H, CH_2)
2-chloromethoxypentane	64–69 (4.5)	71	5.62	0.94 (t, 3 H, CH_3), 1.21 (d, 3 H, CH_3), 1.47 (m, 4 H, CH_2), 3.92 (m, 1 H, CH)
(chloromethoxy)cyclopentane	79–80 (42)	69	5.56	1.67 (br s, 8 H, cyclopentyl), 4.40 (m, 1 H, CH)
2-(chloromethoxy)-3-methylpentane	89–91 (60)	77	5.60	0.97 (br t, 3 H, CH_3), 1.17 (d, 3 H, CH_3), 1.1–2.0 (m, 2 H, CH_2), 3.80 (m, 1 H, CH)
3-(chloromethoxy)hexane	85–88 (54)	65	5.60	0.93 (t, 6 H, CH_3), 1.1–1.8 (m, 6 H, CH_2), 3.70 (q, 1 H, CH)
(chloromethoxy)cyclododecane	122–125 (0.25)	74	5.60	1.1–1.9 (m, 22 H, CH_2), 3.97 (m, 1 H, CH)
(chloromethoxy)cycloheptane	112–115 (45)	75	5.55	1.1–2.2 (m, 12 H, cycloheptyl), 3.94 (m, 1 H, CH)

[a] 60 MHz in $CDCl_3$ solvent. Shift values are reported in δ units relative to tetramethylsilane.

remaining chloromethyl ethers were prepared by using the following general procedure.[33,34]

Dry HCl gas was bubbled into an ice-cooled mixture of the appropriate alcohol, 1 equiv of *s*-trioxane, and benzene (5 mL/g of alcohol) at a rate that maintained the reaction temperature below 15 °C. The reactions were generally complete within 2.5 h, at which time a second phase had formed. After the aqueous layer was separated, the benzene layer was dried over calcium chloride, nitrogen gas was bubbled through the mixture to remove HCl, and the mixture was distilled at atmospheric pressure to remove benzene. The remaining residue was distilled under reduced pressure to provide the pure chloromethyl ethers. The properties of all new chloromethyl ethers prepared by the above procedure are presented in Table IX.

General Procedure for Preparing Quaternary Salts. The imidazolium salts **2** were prepared by dissolving 2-[(hydroxyimino)methyl]-1-methylimidazole[32] in THF–DMF (5:1) and adding 1.2–1.5 equiv of the appropriate alkyl halide. The reactions with chloromethyl ethers were generally complete after being stirred overnight. The reactions with iodomethyl esters and alkyl iodides required up to a week at room temperature. The solid precipitates were filtered, washed with ether, dried in vacuo, and recrystallized. In certain cases, the iodide salts did not precipitate from the reaction mixture. In those cases, it was necessary to dilute with ether to induce precipitation. The iodide salts were then ion-exchanged to the chloride salts using Amberlite IRA-400 Cl^- exchange resin. The following type **2** compounds were prepared by this method. Spectral data are available in Table B (see paragraph at end of manuscript about supplementary material).

1-(Ethoxymethyl)-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2b) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl ethyl ether (Aldrich) and obtained as a hygroscopic crystalline solid from EtOAc/EtOH.

1-[(1′-Hexyloxy)methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2d) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl 1-hexyl ether and obtained as white crystals from 2-propanol/EtOAc.

1-[[(4′,4′-Dimethyl-3′-pentyl)oxy]methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2i) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl 2,2-dimethyl-3-pentyl ether and obtained as colorless crystals from MeOH/Et_2O.

1-[[(4′,4′-Dimethyl-2′-pentyl)oxy]methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2j) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl 4,4-dimethyl-2-pentyl ether and obtained as white crystals from 2-propanol.

1-[(3′-Hexyloxy)methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2m) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl 3-hexyl ether and recrystallized from EtOH/EtOAc.

2-[(Hydroxyimino)methyl]-3-methyl-1-[[(3′-methyl-2′-pentyl)oxy]methyl]imidazolium chloride (2n) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl 3-methyl-2-pentyl ether and recrystallized from EtOH/EtOAc.

2-[(Hydroxyimino)methyl]-3-methyl-1-[[(2′,3′,3′-trimethyl-2′-butyl)oxy]methyl]imidazolium chloride (2o) was prepared from chloromethyl 2,3,3-trimethyl-2-butyl ether and 2-[(hydroxyimino)methyl]-1-methylimidazole and recrystallized from EtOAc/EtOH.

1-[(Cyclobutyloxy)methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2p) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl cyclobutyl ether and recrystallized from EtOH/EtOAc.

1-[(Cyclopentyloxy)methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2q) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl cyclopentyl ether and obtained as white crystals from EtOAc/EtOH.

1-[(Cyclohexyloxy)methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2r) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl cyclohexyl ether and recrystallized from 2-propanol.

1-[(Cycloheptyloxy)methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2s) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl cycloheptyl ether and recrystallized from EtOH/EtOAc.

1-[(Cyclooctyloxy)methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2t) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl cyclooctyl ether and obtained as white crystals from EtOAc/EtOH.

1-[(Cyclododecyloxy)methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2u) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl cyclododecyl ether and recrystallized from EtOH/EtOAc.

1-[[(Cyclopropylmethyl)oxy]methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2v) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl cyclopropylmethyl ether and obtained as white crystals from EtOAc/EtOH.

1-[[(Cyclohexylmethyl)oxy]methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2w) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl cyclohexylmethyl ether and obtained as white crystals from 2-propanol.

1-[[(1′-Cyclopentyl-1′-butyl)oxy]methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride (2y) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and

1-(chloromethoxy)-1-cyclopentylbutane and recrystallized from EtOH/EtOAc.

2-[(Hydroxyimino)methyl]-3-methyl-1-[(1′,2′,3′,4′-tetrahydronaphth-1-yl)methoxy]methylimidazolium chloride (2ab) was prepared from the 2-[(hydroxyimino)methyl]-1-methylimidazole and chloromethyl 1-(1,2,3,4-tetrahydronaphthyl)methyl ether and obtained as white crystals of the hemihydrate from acetone/water (20:1).

1,3-Dimethyl-2-[(hydroxyimino)methyl]benzimidazolium Chloride (3a). The iodide salt of **3a** was prepared by treating 5 g (0.0286 mol) of 2-[(hydroxyimino)methyl]-1-methylbenzimidazole[48] with iodomethane (10 mL) all in 30 mL of DMF and 125 mL of THF. After being stirred in the dark for 5 days, the mixture was filtered to give 8.65 g of pale yellow powder. This material was dissolved in 850 mL of deionized water and ion-exchanged with Amberlite IRA-Cl^- exchange resin. After concentrating, the solid was recrystallized from EtOH and then a second time from water.

2-[(Hydroxyimino)methyl]-3-methyl-1-(methoxymethyl)-benzimidazolium Chloride (3b). Compound **3b** was prepared by treating 2-[(hydroxyimino)methyl]-1-methylbenzimidazole with chloromethyl methyl ether, yielding an off-white powder. Recrystallization from DMSO/acetone (3:1) gave colorless prisms.

2,3-Dimethyl-1-[(pinacolyloxy)methyl]imidazolium Chloride (4). Compound **4** was prepared by adding 8.0 mL of chloromethyl pinacolyl ether[33] dropwise to a cold solution of 5.0 g of 1,2-dimethylimidazole (Aldrich) in 150 mL of dry THF. After the mixture was stirred for 60 h, the solid material was filtered, washed repeatedly with diethyl ether, and vacuum dried, yielding white crystals.

2-[(Hydroxyimino)methyl]-1-[[[2-[(hydroxyimino)-methyl]-3-methyl-1*H*-imidazolium-1-yl]methoxy]methyl]-pyridinium Dichloride (5). The intermediate 2-[(hydroxyimino)methyl]-1-[(chloromethoxy)methyl]pyridinium chloride (0.45 g, 1.99 mmol), dissolved in 20 mL of freshly distilled DMF at 45 °C, was treated with 0.2 g of 2-[(hydroxyimino)methyl]-1-methylimidazole (1.6 mmol) in 5 mL of DMF. The resulting solution was stirred at 45 °C for 5 h and then stored at room temperature overnight. The resulting precipitate was filtered, washed first with acetone and then ether, and dried, yielding an off-white powder. Recrystallization from EtOH/H_2O (3:1) yielded white microfine crystals.

1,1′-[Oxybis(methylene)]bis[2-[(hydroxyimino)methyl]-3-methyl-1*H*-imidazolium chloride] (6). Compound **6** was prepared by adding 3.0 g (0.024 mol) of 2-[(hydroxyimino)-methyl]-1-methylimidazole in DMF/THF (1:15, 80 mL) to excess bis(chloromethyl) ether in THF to give 4.5 g of white powder. Recrystallization from EtOH/H_2O (3:1) gave 0.61 g of white crystals.

1,1′-[Ethylenebis(oxymethylene)]bis[4-[(hydroxyimino)-methyl]-3-methyl-1*H*-imidazolium chloride] (7). Compound **7** was prepared by treating 24.4 g (0.2 mol) of 2-[(hydroxyimino)methyl]-1-methylimidazole in 100 mL of dry DMF with excess 1,2-bis(chloromethoxy)ethane to provide 30.5 g of off-white crystals.

1,3-[Trimethylene]bis[2-[(hydroxyimino)methyl]-3-methyl-1*H*-imidazolium chloride] (8). To a solution of 2-[(hydroxyimino)methyl]-1-methylimidazole (1 g) in 8 mL of warm DMF was added 1,3-diiodopropane (2.3 g) in 8 mL of THF. The mixture was stirred in the dark for 8 days and filtered, and the pale-yellow solid was washed with Et_2O. The solid was then dissolved in water and passed through a column of Amberlite IRA-400 Cl^- exchange resin in the usual manner. The eluant was evaporated in vacuo to give a solid residue, which was recrystallized from 2-PrOH/H_2O to yield a white solid.

1,4-[Tetramethylene]bis[2-[(hydroxyimino)methyl]-3-methyl-1*H*-imidazolium chloride] (9). Compound **9** was prepared in similar fashion and yield to **8** by substituting 1,4-diiodobutane in place of 1,3-diiodopropane; the product was obtained in similar yield as a white solid.

General Procedure for Preparing Iodomethyl Esters. The preparation of salts **10a–d** required certain iodomethyl esters. These materials were prepared by the following general procedure.

The respective acid chloride (0.21 mol) in 300 mL of benzene was treated with paraformaldehyde (0.23 mol) and $SnCl_4$ (5–10 drops), and the resulting mixture was heated at 70–80 °C overnight while being stirred. The benzene was then removed by rotary evaporation to give the crude chloromethyl ester, which was purified by vacuum distillation. The chloromethyl ester (0.074 mol) was then treated with NaI (0.13 mol) in acetone (100 mL), and the resulting mixture was stirred under N_2 with exclusion of light for a minimum of 4 h. After filtering the mixture and concentrating the filtrate, the remaining residue was flash chromatographed over silica and eluted with hexanes–EtOAc (9:1) to give the iodomethyl ester. The esters exhibited air and light sensitivity and therefore were used immediately. The following iodomethyl esters were prepared.

Iodomethyl 2,2-dimethylpropanoate (iodomethyl pivalate) was isolated as a pale yellow, light-sensitive oil in quantitative yield from chloromethyl pivalate (Aldrich): ^1H NMR ($CDCl_3$) δ 0.95 (s, 9 H, CH_3), 4.93 (s, 2 H, CH_2I).

Iodomethyl 2-methylbutanoate was prepared from 2-methylbutanoyl chloride via chloromethyl 2-methylbutanoate [bp 75–78 °C (40 mmHg)] and obtained in 31% overall yield as a light- and air-sensitive pale-yellow oil: ^1H NMR ($CDCl_3$) δ 5.95 (s, 2 H, CH_2I), 2.40 (m, 1 H, CH), 1.57 (m, 2 H, CH_2), 1.11 (d, 3 H, CH_3), 0.90 (t, 3 H, CH_3).

Iodomethyl butanoate was prepared from butanoyl chloride via chloromethyl butanoate [bp 65–67 °C (42 mmHg)] and obtained in 40% overall yield as a light- and air-sensitive pale yellow oil: ^1H NMR ($CDCl_3$) δ 5.97 (s, 2 H, CH_2I), 2.37 (t, 2 H, CH_2), 1.70 (m, 2 H, CH_2), 0.97 (t, 3 H, CH_3).

Iodomethyl 2-naphthoate was prepared from 2-naphthoyl chloride via chloromethyl 2-naphthoate (mp 76–77 °C) and obtained in quantitative yield as a light-sensitive pale yellow solid: ^1H NMR ($CDCl_3$) δ 6.30 (s, 2 H, CH_2I), 7.43–8.40 (m, 6 H, aryl), 9.13 (dd, 1 H, aryl).

1-[[(2′,2′-Dimethylpropanoyl)oxy]methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium Chloride (10a). A mixture of 2-[(hydroxyimino)methyl]-1-methylimidazole (3.3 g) and iodomethyl pivalate (6.63 g) in 15 mL of dry THF was stirred in the dark for 3 days, poured into water, and extracted with Et_2O. The aqueous layer was then passed through a column of Amberlite IRA-400 (Cl^- form) ion-exchange resin and the eluant was evaporated to dryness to give a solid residue. The residue, after recrystallization from 2-propanol, provided 3.2 g of **10a** as a white solid.

2-[(Hydroxyimino)methyl]-3-methyl-2-[(2′-naphthoyl-oxy)methyl]imidazolium chloride (10b) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and iodomethyl 2-naphthoate in a manner similar to the preparation of **10a** above. Ion-exchange provided the chloride salt as a white crystalline solid recrystallized from DMSO.

2-[(Hydroxyimino)methyl]-3-methyl-1-[[(2′-methylbutanoyl)oxy]methyl]imidazolium chloride (10c) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and iodomethyl 2-methylbutanoate and ion-exchanged to provide the chloride salt recrystallized from EtOH/EtOAc.

1-[(Butanoyloxy)methyl]-2-[(hydroxyimino)methyl]-3-methylimidazolium choride (10d) was prepared from 2-[(hydroxyimino)methyl]-1-methylimidazole and iodomethyl butanoate and crystallized from EtOH/EtOAc after ion-exchange to give the imidazolium salt in 31% yield as a colorless solid.

Muscarinic and Nicotinic Receptor Binding. Muscarinic receptor binding was evaluated in the mouse (male Charles River ICR) brain membrane fraction essentially as described by Yamaura and Snyder.[49] Brains were homogenized in 40 volumes of 50 mM Tris·HCl buffer, pH 7.7, and centrifuged at 40000*g* for 15 min. The pellets were rehomogenized in 40 times the original tissue volume, recentrifuged as above, and suspended in the Tris buffer at a concentration of 5 mg original weight/mL for the binding assay.

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Membranes were incubated at 25 °C for 60 min with 0.2 nM ^{3}H-QNB (quinuclidinyl benzylate) and various concentrations of the test compounds (in triplicate). Atropine (1.0 μm) was used to correct for nonspecific binding; micromoles of eserine was used to inhibit AChE in the preparation when compounds were AChE substrates. The incubated samples were filtered over Whatman GF/B glass fiber filters and washed 3 times with Tris buffer. The filteres were then assayed for retained ^{3}H-QNB by scintillation counting, and the results were calculated, after subtraction of the portion of ^{3}H due to nonspecific binding, as the percent inhibition of QNB bound. Nonspecific binding is about 5% of total binding in control conditions. Compounds were normally evaluated at seven different concentrations between mM and nM, and results are reported as the IC_{50} for inhibition of QNB binding.

Nicotinic receptor binding, adapted from the method of Moore and Brady,[50] was evaluated in mouse brain tissue prepared as above for the muscarinic assay. The final pellet was homogenized in 20 mM Na_3PO_4 buffer, 0.9% NaCl, pH 7.4 (phosphate buffer), to a final concentration of 10 mg original tissue/mL.

For the binding reaction, 0.8 mL of the tissue homogenate was incubated with 0.1 mL (^{125}I) α-bungarotoxin (usually 200000 CPM, ~0.75 nM) and 0.1 mL of competing drug in PBS in glass centrifuge tubes. The solution was incubated 60 min at 25 °C and then centrifuged at 4 °C three times, removing the supernatant and resuspending the pellet each time in 1 mL of PBS. After the third spin (each 10 min at 40000*g*), the resulting pellets were counted in a gamma counter. Incubations were performed with and without 10 mM carbachol to calculate the fraction of nonspecific binding, which is about 40% of total binding in control tubes under these conditions. Drugs were evaluated at five or more concentrations from mM to nM, with each concentration run in triplicate. Results are reported as the IC_{50} for inhibition of α-bungarotoxin binding.

In Vivo Evaluation against GD and EPMP Intoxication. Male ICR Swift mice from Charles River (20 to 30 g average weight) were challenged with two LD_{50}'s of soman (aqueous solution, containing 0.9% NaCl) intramuscularly (im). Within 10 s after the soman challenge, the imidazolium test compound and 11.2 mg/kg of atropine sulfate were coadministered im in aqueous solution at the three different dose levels as noted in Table V. Collectives of ten mice were used in each experiment, and survivors in each group were noted after 24 h. As an internal check, a set of ten mice were treated with 25 mg/kg of 2-PAM and 11.5 mg/kg atropine sulfate. Up to a 10% survival rate was considered acceptable after intoxication by two LD_{50}'s soman although in general, zero mice survived.

For experiments with EPMP male mice from Charles River were used. EPMP was injected at the nape of the neck; atropine sulfate (20 mg/kg) and an oxime (usually at $^1/_8$ LD_{50}) were injected 1 min later in the muscles of the right and left thighs, respectively. In mice weighing 35 ± 5 g, the LD_{50} of EPMP along was about 600 μg/kg, and with EPMP plus atropine, the LD_{50} was about 1000 μg/kg. The amount of EPMP necessary to kill mice treated with atropine and selected imidazolium oximes, 2-PAM, or HI-6 was estimated by using at least three different doses of EPMP.

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Registry No. **2a**, 91900-09-3; **2aa**, 91900-17-3; **2ab**, 117941-64-7; **2ac**, 91900-16-2; **2b**, 117941-45-4; **2c**, 91900-13-9; **2d**, 117941-46-5; **2e**, 91900-14-0; **2f**, 91900-10-6; **2g**, 91900-11-7; **2h**, 91900-12-8; **2i**, 117941-47-6; **2j**, 117941-48-7; **2k**, 117941-49-8; **2l**, 117941-50-1; **2m**, 117941-51-2; **2n**, 117941-52-3; **2o**, 117941-53-4; **2p**, 117941-54-5; **2q**, 117941-55-6; **2r**, 117941-56-7; **2s**, 117941-57-8; **2t**, 117941-58-9; **2u**, 117941-59-0; **2v**, 117941-60-3; **2w**, 117941-61-4; **2x**, 117941-62-5; **2y**, 117941-63-6; **2z**, 91900-15-1; **3a**, 21749-66-6; **3b**, 117941-65-8; **4**, 117941-40-9; **5**, 93490-44-9; **6**, 53035-14-6; **7**, 117941-41-0; **8**, 117941-42-1; **9**, 117941-43-2; **10a**, 117941-44-3; **10b**, 117941-66-9; **10c**, 117941-67-0; **10d**, 117941-68-1; chloromethoxycyclohexane, 3587-62-0; 1-(chloromethoxy)-1-cyclopentylbutane, 117941-69-2; 1-(chloromethoxy)-1-cyclohexylethane, 117941-70-5; 3-(chloromethoxy)-2,2-dimethylpentane, 117941-71-6; chloromethoxy-(1,2,3,4-tetrahydronaphth-1-yl)methane, 117941-72-7; 4-(chloromethoxy)-2,2-dimethylpentane, 117941-73-8; 1-(chloromethoxy)-3-methylbutane, 41965-70-2; 2-chloromethoxypentane, 86442-65-1; (chloromethoxy)cyclopentane, 68060-41-3; 2-(chloromethoxy)-3-methylpentane, 117941-74-9; 3-(chloromethoxy)-hexane, 117941-75-0; (chloromethoxy)cyclododecane, 58567-10-5; (chloromethoxy)cycloheptane, 117941-76-1; trioxane, 110-88-3; cyclohexanol, 108-93-0; 1-cyclopentyl-1-butanol, 117941-77-2; 1-cyclohexylethanol, 1193-81-3; 2,2-dimethyl-3-pentanol, 3970-62-5; 1,2,3,4-tetrahydronaphthalen-1-ol, 66377-63-7; 2,2-dimethyl-4-pentanol, 6144-93-0; 3-methyl-1-butanol, 123-51-3; 2-pentanol, 71-41-0; cyclopentanol, 96-41-3; 3-methyl-2-pentanol, 565-60-6; hexanol, 111-27-3; cyclododecanol, 1724-39-6; cycloheptanol, 502-41-0; 2-[(hydroxyimino)methyl]-1-methylimidazole, 20062-62-8; chloromethyl ethyl ether, 3188-13-4; chloromethyl 1-hexyl ether, 39979-92-5; chloromethyl 2,3,3-trimethylbutyl ether, 117941-78-3; chloromethyl cyclobutyl ether, 104620-74-8; chloromethyl cyclooctyl ether, 58567-17-2; chloromethyl cyclopropylmethyl ether, 105688-12-8; chloromethyl cyclohexylmethyl ether, 1625-60-1; 2-[(hydroxyimino)methyl]-1-methylbenzimidazole, 3013-07-8; chloromethyl methyl ether, 107-30-2; chloromethyl pinacolyl ether, 91900-18-4; 1,2-dimethylimidazole, 1739-84-0; 2-[(hydroxyimino)methyl]-1-[(chloromethoxy)-methyl]pyridinium chloride, 27123-11-1; bis(chloromethyl) ether, 542-88-1; 4-pyridine aldoxime, 696-54-8; 1,2-bis(chloromethoxy)ethane, 13483-18-6; 1,3-diiodopropane, 627-13-6; 1,4-diiodobutane, 628-21-7; iodomethyl 2,2-dimethylpropanoate, 53064-79-2; iodomethyl 2-methylbutanoate, 111013-40-2; iodomethyl butanoate, 63379-59-9; iodomethyl 2-naphthoate, 117941-79-4; chloromethyl pivalate, 18997-19-8; 2-methylbutanoyl chloride, 5856-79-1; chloromethyl 2-methylbutanoate, 82504-44-7; butanoyl chloride, 141-75-3; chloromethyl butanoate, 33657-49-7; 2-naphthoyl chloride, 2243-83-6; chloromethyl 2-naphthoate, 49598-76-7.

Supplementary Material Available: Kinetic values K_r (the binding equilibrium of reactivator to inhibited enzyme) and k_r (the transformation rate of [reactivator/inhibited enzyme] complex to active enzyme) for in vitro reactivation (Table A) and the proton NMR spectral data for all new imidazolium compounds prepared in this study (Table B) (4 pages). Ordering information is given on any current masthead page.