Quaternary Salts of 2-[(Hydroxyimino)methyl]imidazole. 4.[1] Effect of Various Side-Chain Substituents on Therapeutic Activity against Anticholinesterase Intoxication

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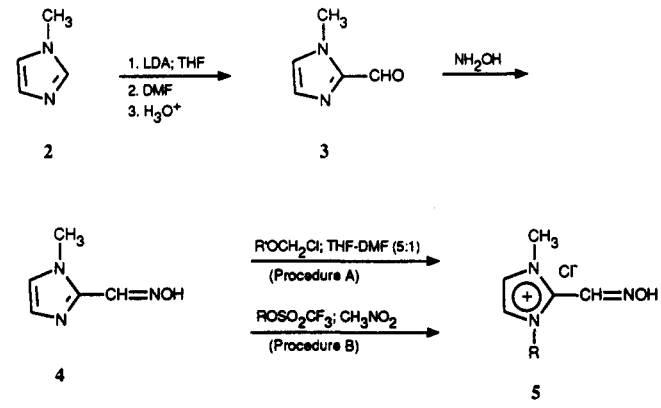
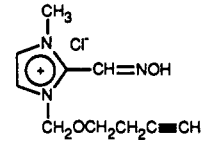
A series of quaternary salt derivatives of 2-[(hydroxyimino)methyl]-1-methylimidazole incorporating various side chains bearing ether, silyl, nitrile, ester, halogen, nitro, sulfone, amino, or aminosulfonyl substituents was prepared and evaluated in vivo for the treatment of anticholinesterase intoxication. Test results in the mouse revealed that the type and location of the side-chain substituent both have a significant influence on the toxicity and antidotal effectiveness of the compounds. Some of the more active examples represent the most potent therapeutics to date against intoxication by the powerful cholinesterase inhibitors soman and tabun. Significantly, the antidotal effectiveness of the compounds was not dependent on the inhibiting agent nor was there any correlation between in vivo efficacy and in vitro reactivation of ethyl (4-nitrophenyl)methylphosphonate inhibited human acetylcholinesterase. These observations suggested that the main mode of antidotal protection by the compounds is something other than enzyme reactivation.

The toxicity of organophosphorus (OP) chemical warfare nerve agents and pesticides is attributed to the ability of these materials to irreversibly inhibit the esteratic site of synaptic acetylcholinesterase[2] (AChE, acetylcholine hydrolase, EC 3.1.1.7), an enzyme that is essential for normal nerve impulse conduction and functions to catalyze the hydrolysis of the neurotransmitter acetylcholine (ACh). Medical treatment for intoxication by AChE inhibitors mainly relies on the coadministration of a cholinergic blocker (e.g., atropine) to counteract the build-up of lethal levels of ACh, and a phosphorophilic nucleophile reactivator that acts to bimolecularly displace enzyme from the covalently bound OP moiety and thereby restore esteratic activity.[2a,3] Although clinical reactivators such as 2-[(hydroxyimino)methyl]-1-methylpyridinium chloride (2-PAM) are known to be effective antidotes in certain cases of accidental pesticide or nerve agent poisoning, in animal models they are ineffective against systemic exposure to greater than 1.2LD_{50} of the nerve agent 3,3-dimethyl-2-butyl methylphosphonofluoridate (soman).[4] Until very recently, the only therapeutic agents reported to be effective against soman in vivo were a series of bispyridinium oximes of which 1-[[(4-carbamoylpyridinio)methoxy]-methyl]-2-[(hydroxyimino)methyl]pyridinium dichloride (HI-6) is the most well-noted example.[4a,5] However, HI-6 and related oximes suffer from being hydrolytically unstable, which creates significant problems in formulating these compounds in an injectable form that can be stored and used for emergency treatment.

In our continuing effort to develop more effective AChE reactivators that would be stable in solution, we recently reported the preparation and biological evaluation of a series of quaternary analogues of 2-[(hydroxyimino)-methyl]imidazole having appended side chains of various length, alkyl branching, and carbon–carbon bond unsaturation.[1] Although these compounds were found to be relatively unimpressive as in vitro reactivators of soman-inhibited AChE, several analogues that had a terminally unsubstituted alkynyl bond in the side chain exhibited significantly enhanced antidotal activity against soman in the mouse relative to our earlier-reported[1a] alkyl-side-chain analogues. Compound 1, when administered intramuscu-

Scheme I





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(1) Preceding papers: (a) Bedford, C. D.; Harris, R. N., III; Howd, R. A.; Miller, A.; Nolen, H. W., III; Kenley, R. A. *J. Med. Chem.* **1984**, *27*, 1431. (b) Bedford, C. D.; Harris, R. N., III; Howd, R. A.; Goff, D. A.; Koolpe, G. A.; Petesch, M.; Miller, A.; Nolen, H. W., III; Musallam, H. A.; Pick, R. O.; Jones, D. E.; Koplovitz, I.; Sultan, W. E. *J. Med. Chem.* **1989**, *32*, 493. (c) Bedford, C. D.; Harris, R. N., III; Howd, R. A.; Goff, D. A.; Koolpe, G. A.; Petesch, M.; Koplovitz, I.; Sultan, W. E.; Musallam, H. A. *J. Med. Chem.* **1989**, *32*, 504.

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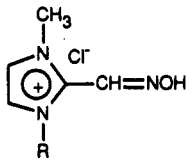
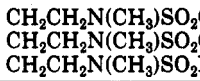
Table I. Physical Data for Quaternary Imidazolium Oximes 5



compd	R	procedure[a]	% yield[b]	mp, °C	log P[c]	formula	anal.[d]
5a	$CH_2OCH_2CH_2OCH_3$	A	53	110–111	−2.81	$C_9H_{10}N_3O_3Cl$	C, H, N
5b	$CH_2OCH_2CH_2CH(OCH_3)CH_3$	A	62	100–101	−2.22	$C_{11}H_{20}N_3O_3Cl$	C, H, N
5c	$CH_2OCH_2CH_2Si(CH_3)_3$	A	72	161–162 dec	−0.34	$C_{11}H_{22}N_3O_2SiCl$	C, H, N
5d	$CH_2OCH_2CH_2CH_2Si(CH_3)_3$	A	40	157–158 dec	+0.43	$C_{12}H_{24}N_3O_2SiCl$	C, H, N
5e	CH_2CH_2CN	B	67	210–211	−3.30	$C_8H_{11}N_4OCl$	C, H, N, Cl
5f	$CH_2CH_2CH_2CN$	B	63	131–132	<−3.00	$C_9H_{13}N_4OCl \cdot {}^1/_2H_2O$	C, H, N, Cl
5g	$CH_2CH_2CH_2CH_2CN$	B	82	131–132	−2.80	$C_{10}H_{15}N_4OCl$	C, H, N, Cl
5h	$CH_2CH_2CH_2CH_2CH_2CO_2CH_3$	B	31	104–105	−2.21	$C_{12}H_{20}N_3O_3Cl$	C, H, N, Cl
5i	$CH_2CH_2CH_2CH_2OC(O)Ph$-2′-OH	B	27	156–159	−0.56	$C_{16}H_{20}N_3O_4Cl$	C, H, N, Cl
5j	CH_2CH_2F	B	87	157–159	−2.58	$C_7H_{11}FN_3OCl$	C, H, N, Cl
5k	$CH_2CH_2OCH_2CH_2F$	B	91	114–115	−2.62	$C_9H_{15}FN_3O_2Cl$	C, H, N, Cl
5l	$CH_2OCH_2CH_2CH_2CH_2Cl$	A	75	85–87	−1.47	$C_{10}H_{17}N_3O_2Cl_2$	C, H, N
5m	$CH_2CH_2OCH_2CH_2Cl$	B	76	124–125	−2.32	$C_9H_{15}N_3O_2Cl_2$	C, H, N, Cl
5n	CH_2CH_2Br	B	57	236–238 dec	−2.44	$C_7H_{11}BrN_3OCl$	C, H, N, Cl
5o	$CH_2OCH_2CH_2CH_2Br$	A	36	109–110	−1.76	$C_9H_{15}BrN_3O_2Cl$	C, H, Br, N, Cl
5p	$CH_2OCH_2C(CH_3)_2CH_2Br$	A	74	172–173 dec	−0.81	$C_{11}H_{19}BrN_3O_2Cl$	C, H, N
5q	$CH_2CH_2NO_2$	B	45	144–146 dec	e	$C_7H_{11}N_4O_3Cl$	C, H, N, Cl
5r	$CH_2OCH_2CH_2NO_2$	A	70	143–145 dec	e	$C_8H_{13}N_4O_4Cl$	C, H, N, Cl
5s	$CH_2OCH_2C(CH_3)_2NO_2$	A	57	172–173 dec	−1.96	$C_{10}H_{17}N_4O_4Cl$	C, H, N
5t	$CH_2CH_2SO_2CH_3$	B	62	226–227	−3.50	$C_8H_{14}N_3O_3SCl$	C, H, N, Cl
5u	$CH_2OCH_2CH_2SO_2CH_3$	A	65	161–162 dec	<−3.30	$C_9H_{16}N_3O_4SCl$	C, H, N, S, Cl
5v	$CH_2CH_2N^+H(CH_3)_2 \cdot Cl^-$	B	37	230–231	−3.06	$C_9H_{17}N_4OCl \cdot HCl$	C, H, N, Cl
5w		B	54	222–224 dec	−2.73	$C_{11}H_{19}N_4OCl \cdot HCl$	C, H, N, Cl
5x	$CH_2CH_2N(CH_3)SO_2CH_3$	B	79	205–206 dec	−3.31	$C_9H_{17}N_4O_3SCl$	C, H, N, S, Cl
5y	$CH_2CH_2N(CH_3)SO_2CF_3$	B	83	177–178	−1.50	$C_9H_{14}F_3N_4O_3SCl$	C, H, N, S, Cl
5z	$CH_2CH_2N(CH_3)SO_2Ph$	B	90	171–172	−1.72	$C_{14}H_{19}N_4O_3SCl$	C, H, N, S, Cl

[a] Synthesis procedures are described in the Experimental Section. [b] Yield from immediate precursor. [c] Octanol–buffer (pH 7.4) partition coefficient. Determined spectrophotometrically. [d] Analysis agrees within ±0.4% of the theoretical values. [e] Not determined.

larly at a dose of 38 mg/kg, in conjunction with atropine sulfate, was particularly effective and provided total test-population survival in mice against a challenge of $2LD_{50}$ of soman.[1c] Concurrent test results for branched-chain and homologous analogues of 1 revealed that a terminally unsubstituted alkynyl bond was essential for activity and that the optimum chain length for placement of the alkynyl bond was four to six chain atoms from the imidazolium ring.[1c] These observations suggested that electron-rich functionality appropriately located in the side chain improved the therapeutic effectiveness of the imidazolium oximes and encouraged us to initiate an investigation to identify other side-chain substituents that could improve antidotal activity. Herein, we report the results of our investigation which has led to the discovery of some of the most potent nerve agent antidotes to date.

Chemistry

All test compounds 5 were prepared by allowing oxime 4 to react with the appropriate chloromethyl ether derivative or trifluoromethanesulfonate (triflate) ester (Scheme I). As previously described,[1a] oxime 4 was prepared from the corresponding aldehyde 3 which in turn was prepared by using a slightly modified and somewhat improved procedure of Iversen and Lund.[6] Thus, we found lithium diisopropylamide (LDA) to be superior to *n*-butyllithium as a base for deprotonating 1-methylimidazole (2) and we were able to consistently obtain 20–30% higher yields of aldehyde 3.

All chloromethyl ether derivatives were prepared by condensation of the appropriate alcohol with formaldehyde in the presence of HCl gas.[7] Triflate esters were all prepared by reacting the appropriate alcohol at ice-bath temperature with trifluoromethanesulfonic anhydride in the presence of triethylamine. Quaternization of 4 to give compounds 5 was accomplished by two general procedures. Procedure A: Oxime 4 in THF–*N*,*N*-dimethylformamide (DMF) solvent mixture was allowed to react with the appropriate chloromethyl ether derivative and the product 5 was collected by filtration and recrystallized from ethanol–ethyl acetate. Procedure B: Oxime 4 in nitromethane was allowed to react with the appropriate triflate ester at 0 °C. After being stirred for 1–2 h at room temperature, the mixture was concentrated and the residue subjected to chloride anion exchange to give 5 as a crude chloride salt that was recrystallized from ethanol–ethyl acetate. As evidenced by ^1H NMR, all of compounds 5 were obtained configurationally pure as the oxime *E* isomer. Structures, yields, and other compound data for 5 are listed in Table I.

Pharmacology

The antidotal effectiveness of compounds 5 against soman and tabun (ethyl *N*,*N*-dimethylphosphoramidocyanidate) was evaluated in male ICR Swiss mice. A group

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Table II. Antidotal Activity of Imidazolium Oximes 5 against Soman and Tabun in the Mouse

compd[b]	LD_{50},[c] mmol/kg	survival[a] soman $^1/_{64}$	soman $^1/_{16}$	soman $^1/_4$	tabun $^1/_{64}$	tabun $^1/_{16}$	tabun $^1/_4$
1	0.62		4	10			
			Ether				
5a	0.96		0	4			
5b	0.44		0	0			
			Silane				
5c	0.20		0	1	1	1	1
5d	0.02		0	0			
			Nitrile				
5e	0.57				0	0	1
5f	0.44		1				
5g	0.73		0	1	0	0	1
			Ester				
5h	0.78		0	0			
5i	1.82	0	1	2	0	1	0
			Halogen				
5j	0.48	0	0	1			
5k	0.66	0	3	9	0	3	8
5l	0.61	0	0	0	0	0	0
5m	0.40	0	6	8	0	1	7
5n	0.16		1	0	1	0	0
5o	0.68		2	4			
5p	0.33	4	8	4			
			Nitro				
5q	0.37		0	2			
5r	0.29		1	2			
5s	0.31		8	6		3	6
			Sulfone				
5t	>1.12		3	9			
5u	2.70		9	6		1	5
			Amine				
5v	>1.48		0	10			
5w	0.87	1	6	6			
			Aminosulfonyl				
5x	0.22	6	10	8	0	7	7
5y	0.21	3	8	6			
5z	0.03	2	10	8	0	3	10
2-PAM	0.85	0	0	0	0	0	0
HI-6	1.67	8				0	2

[a] Refers to the number of survivors noted at 24 h out of a population of 10 mice each exposed im to $2LD_{50}$ of the OP agent. Test compound administered im in doses of $^1/_{64}$, $^1/_{16}$, and $^1/_4LD_{50}$ concurrently with atropine sulfate (11.2 mg/kg) 10 s after nerve-agent challenge. LD_{50} of soman (plus 11.2 mg/kg atropine sulfate) $\simeq$0.13 mg/kg in a 20–30 g mouse; without atropine, LD_{50} soman $\simeq$0.10 mg/kg. [b] See Table I for structures. [c] Determined im in the mouse as previously described.[1b,c]

of 10 mice were each injected intramuscularly (im) in the right hindlimb with $2LD_{50}$ of the OP agent. Ten seconds later, an aqueous solution of atropine sulfate (11.2 mg/kg) and the test drug in doses of $^1/_{64}$, $^1/_{16}$, or $^1/_4$ of the predetermined LD_{50} were administered im in the left hindlimb. As a base reference, a second group of 10 animals was given 2-PAM chloride (25 mg/kg) in place of the test drug, generally under which conditions all animals in the group expired. As a positive reference, a third group of animals was given HI-6 (9.6 mg/kg) in place of the test drug, which generally led to six to eight survivors. The 24-h survival of animals injected with test drug was compared to the 24-h survival observed in the base reference. Differences in the survival rates were compared by using Fisher's exact test ($p < 0.05$, $n = 10$). With this test, a survival difference of at least four is required to identify improved efficacy of the test drug over the base reference. On occasion, as many as two survivors were noted in the base reference. Accordingly, these were subtracted from the number of survivors in the test-drug groups for which the base reference applied.

In vitro reactivation by selected compounds was determined for ethyl (4-nitrophenyl)methylphosphonate (EPMP) inhibited human erythrocyte AChE by using procedures and methods that have all been previously described.[1]

Results and Discussion

Table II shows the LD_{50} values and in vivo antidotal test results in the mouse against soman and tabun for nine subgroups of type 5 imidazolium oximes. The groups are divided according to the type of side-chain substituent. In accordance with previous[1c] structure–activity relationships (SAR) for similar type 5 oximes, most of the compounds have the side-chain substituent located within four to six chain atoms from the imidazolium ring. Compound 1 is included for comparison.

Several SARs are noticeable upon examination of the data in Table II. First, it is clear that the type of substituent in the side chain has a profound influence on both the toxicity and antidotal effectiveness of type 5 oximes. Thus, whereas ether, silyl, nitrile, and ester side-chain

Table III. Kinetic Constants for in Vitro Reactivation of EPMP-Inhibited Human AChE by Selected Type 5 Compounds

compd[a]	IC_{50},[b] μM	k_r,[c] $min^{-1} \times 10^3$	K_r,[d] $M \times 10^6$	k_{HOX},[e] $M^{-1}\ min^{-1}$
5s	10.6 ± 0.33	2.40 ± 0.61	3.34 ± 1.2	205.0 ± 24
5u	26.5 ± 8.9			*f*
5w	17.9 ± 2.1	1.26 ± 0.46	9.37 ● 4.3	38.2 ± 4.4
5x	6.8 ± 0.46			*f*
2-PAM	366.0			482.0

[a] See Table I for structures. [b] IC_{50} is the concentration of test drug HOX that reversibly inhibits 50% of AChE activity. [c] k_r defines the rate of transformation of the [inhibited enzyme/oximate] complex to active enzyme; see eq 13, ref 1a. [d] K_r defines the formation of the inhibited enzyme/oximate complex; see eq 15, ref 1a. [e] k_{HOX} is the effective rate constant for reactivation, adjusted for the differences in oxime ionization at pH 7.6 and was calculated from eq 16, ref 1a. Standard deviations for all parameters were calculated by asymtotic propagation of error methods based on regression models and results. Confidence intervals stated are 95% *t* intervals based on standard error and degrees of freedom. [f] No measurable reactivation observed.

substituents (compounds **5a–i**) impart negligible antidotal protection, halogen, nitro, sulfone, amine, and particularly aminosulfonyl substituents (compounds **5j–z**) in most cases provide significant antidotal activity. Similar to the alkynyl-substituted series,[1c] the activity in the halogen subgroup (compounds **5j–p**) is associated mainly with those analogues having the substituent located four to six chain atoms from the ring which could possibly be an optimum distance for a secondary drug–enzyme interaction. It is noteworthy that the activity in this subgroup does not appear to depend on the type of halogen, suggesting that substituent volume is less critical than other factors for activity. However, examples **5p** and **5s** illustrate that steric bulk, in the form of alkyl branching near the side-chain substituent, is influential and serves to somewhat enhance activity.

As seen in compounds **5t**, **5v**, and **5w**, the sulfone- and amine-substituted analogues offer compounds that are active with only two methylene units separating the imidazolium ring from the side-chain substituent indicating that the four to six atom side-chain spacer is not an absolute requirement for antidotal activity. It is tempting to speculate that this deviation from previous SAR could indicate either a change in antidotal mechanism or a change in the relative orientation of the drug and enzyme during interaction.

The aminosulfonyl-substituted derivatives (**5x–z**) exhibit the highest activity in Table II and on a molar basis represent the most potent nerve agent antidotes presently known. Remarkably, compounds **5x** and **5z** provide total population survival at doses of only 13.8 and 1.9 μmol/kg, respectively. Although it is unclear why the protective ability of compounds **5x–z** (also **5p**, **5s**, and **5u**) against soman decreases on going from $^1/_{16}LD_{50}$ to $^1/_4LD_{50}$, we suspect that this anomalous dose-response behavior could be related to the ability of these compounds to reversibly inhibit AChE.[8]

One final salient feature of the data in Table II is the consistent parallel relationship in antidotal activity against soman and tabun. *Considering that the effectiveness of an AChE reactivator is generally dependent on the inhibiting agent,[2a,3] this result is especially interesting and suggests that reactivation is not the main mode of antidotal action by these compounds.* In previous work on similar type 5 imidazolium oximes, a significant disparity in correlation between in vivo protection in the mouse and in vitro reactivation was observed.[1] To determine if any correlation exists between in vivo efficacy and reactivation, selected type 5 compounds were subjected to in vitro reactivation experiments and the results are shown in Table III. Similar to previous results,[1] the data in Table III reveal that the selected examples are all fairly potent reversible inhibitors of human AChE, but none are as effective as 2-PAM for reactivating EPMP-inhibited enzyme. As illustrated in Figure 1, a double reciprocal plot of v_0 versus substrate concentration for human AChE in the presence of various fixed concentrations of compound **5x** indicates that the reversible inhibition is competitive in nature.

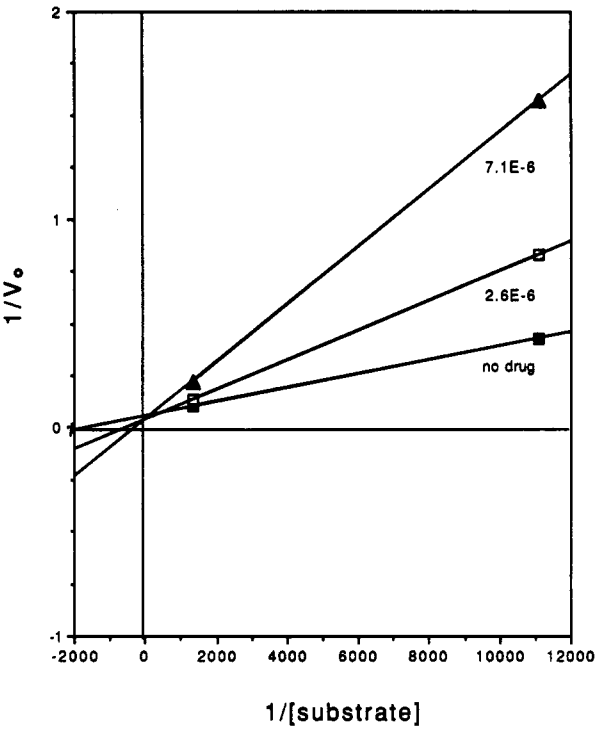

Figure 1. Lineweaver–Burk plot for human AChE in the presence of various fixed concentrations of compound **5x**.

Conclusions

A series of quaternary salts of 2-[(hydroxyimino)methyl]-1-methylimidazole that incorporated side chains bearing various substituents was prepared. Evaluation of these compounds as antidotes for anti-AChE intoxication in the mouse revealed that their effectiveness depends significantly on the type of substituent in the side chain but curiously does not depend on the type of inhibiting agent (soman or tabun). Those derivatives bearing halogen, nitro, sulfone, amine, or aminosulfonyl side-chain substituents provided greatly improved antidotal protection relative to 2-PAM whereas those bearing ether, silyl, nitrile, or ester side-chain substituents were essentially ineffective. In vitro studies of some of the more active derivatives revealed that the compounds are fairly potent reversible inhibitors of human AChE and that the inhibition is apparently competitive in nature. However, although the compounds were designed to be AChE reactivators, their ability to reactivate EPMP-inhibited human AChE is rather poor and does not correlate with in vivo

(8) As seen in the IC_{50} values in Table III, the more active compounds are fairly potent reversible inhibitors of AChE. We suspect that the decline in survival at the higher dose levels for these examples is due to an additive effect of inhibition by the OP agent plus reversible inhibition by the drug, which, in combination, could result in increased lethality. Whatever the case, this anomalous dose–response is apparently characteristic of soman and does not seem to occur with tabun. The exact significance, if any, of these observations eludes us.

survival in the mouse. These observations suggest that reactivation is not the main mode of antidotal action by type 5 compounds.

The main objective of this investigation was to identify side-chain substituents that would improve the effectiveness of the quaternary 2-[(hydroxyimino)methyl]imidazole system as a molecular framework for the treatment of anti-AChE intoxication. We identify nitro, sulfone, amino, and particularly aminosulfonyl as the most effective substituents to date. Continued studies of derivatives of the 1,2,3-(trisubstituted)imidazolium system bearing these substituents will be carried out in order to optimize antidotal activity and hopefully ascertain the protective mechanism(s) involved.

Experimental Section

Melting points were determined in capillary tubes on a Mel-Temp block or Thomas-Hoover Unimelt apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Associates EM-360 or JEOL FX90 spectrometer. Microanalyses were performed by Desert Analytics, Tucson, AZ. Octanol–pH 7.4 phosphate buffer partition coefficients were determined spectrophotometrically by using the method of Hansch.[9] Competitive inhibition and reactivation of human erythrocyte AChE were determined as previously described.[1a] Tetrahydrofuran (THF) was distilled from benzophenone ketyl and used immediately. All other solvents were reagent grade. The chloromethyl ethers required for the preparation of type 5 compounds were prepared by using methods previously described.[1,7] All starting alcohols for the preparation of chloromethyl ethers and triflate esters were commercial materials except for the alcohols required for compounds 5i, 5x, and 5z. The preparations of these alcohols are described as follows.

Preparation of 4-[(2′-Hydroxybenzoyl)oxy]-1-butanol. A mixture of methyl salicylate (1 equiv), and 1,4-butanediol (5 equiv), and *p*-toluenesulfonic acid (0.01 equiv) was heated at 120 °C for 4 days while methanol was allowed to distill from the mixture. Unreacted methyl salicylate was removed by distilling the mixture at 120 °C and 20 mmHg. The remaining residue was taken up in ethyl acetate and washed consecutively with water and brine. After the organic layer was dried ($MgSO_4$), filtered, and concentrated, there was recovered an oil that was flash chromatographed through silica gel and eluted with ethyl acetate–hexane (7:3) to give the pure title compound in 69% yield: ^{1}H NMR ($CDCl_3$) δ 10.90 (br s, 1 H, OH), 8.10–6.80 (m, 4 H, aryl), 4.45 (t, 2 H, J = 6 Hz, CH_2), 3.80 (t, 2 H, J = 6 Hz, CH_2), 2.30 (br s, 1 H, OH), 2.10–1.60 (m, 4 H, CH_2CH_2).

Preparation of 2-[*N*-(Methylsulfonyl)-*N*-methylamino]-ethanol. To a stirred mixture of 2-(methylamino)ethanol (0.25 mol) and triethylamine (0.15 mol) in dichloromethane (DCM, 200 mL) cooled at −78 °C was added methanesulfonyl chloride (0.15 mol) dropwise over 20 min. The mixture was warmed to 0 °C, stirred for an additional 2 h, and then concentrated to give an oily residue. The residue was flash chromatographed through silica gel and eluted with DCM–ethyl acetate (1:2) to give the title compound in 15% yield: ^{1}H NMR ($CDCl_3$) δ 3.78 (t, 2 H, J = 5 Hz, CH_2), 3.35 (t, 2 H, J = 5 Hz, CH_2), 3.13 (br s, 1 H, OH), 2.95 and 2.90 (2 s, 3 H each, 2 CH_3).

Preparation of 2-[*N*-(Phenylsulfonyl)-*N*-methylamino]-ethanol. To a stirred mixture of 2-(methylamino)ethanol (0.13 mol), triethylamine (0.20 mol), and DCM (150 mL) cooled at 0 °C under argon was added benzenesulfonyl chloride (0.13 mol) dropwise over 10 min. The mixture was stirred at room temperature for 18 h, washed with water, dried ($MgSO_4$), filtered, and concentrated to give a colorless oil. The oil was flash chromatographed through silica gel and eluted with ethyl acetate to give the pure title compound in 93% yield: ^{1}H NMR ($CDCl_3$) δ 8.10–7.40 (m, 5 H, aryl), 4.00–3.60 (m, 2 H, CH_2), 3.40–3.05 (m, 2 H, CH_2), 2.86 (s, 3 H, CH_3).

Preparation of 2-Formyl-1-methylimidazole (3). As previously described,[1a] oxime 4 was prepared from 2-formyl-1-methylimidazole (3) which in turn was prepared by the following modified procedure of Iversen and Lund.[6]

Lithium diisopropylamide (2.1 mol) was prepared by adding *n*-butyllithium (210 mL of 10 M solution in hexanes) dropwise over 30 min to a stirred, ice-cooled, nitrogen-blanketed solution of diisopropylamine (2.1 mol) in 2 L of dry THF. After being stirred for 30 min, the mixture was cooled to −60 to −50 °C and there was then added dropwise a solution of 1-methylimidazole (2.05 mol) in THF (400 mL) at a rate such that the temperature of the reaction mixture never exceeded −40 °C. The mixture was stirred at −60 to −50 °C for 3 h, cooled to −78 °C, and there was then added *N*,*N*-dimethylformamide (232 mL) fairly rapidly over 8 min. The mixture was slowly warmed to room temperature, stirred overnight, and then cooled in an ice bath. While the mixture was vigorously stirred, a solution of sodium dihydrogenphosphate (304 g) in 1 L of water was added dropwise, the aqueous layer was separated and filtered, and the filtrate was extracted well with DCM. The combined THF–DCM layers were dried (Na_2SO_4), filtered, and concentrated to give any oily residue. Vacuum distillation of the residue gave aldehyde 3 (89%) as a colorless oil that crystallized on standing: mp 34–36 °C (lit.[6] mp 34–37 °C); bp 70–74 °C (1 mmHg); ^{1}H NMR ($CDCl_3$) δ 9.93 (s, 1 H, CHO), 7.33 (s, 1 H, aryl), 7.22 (s, 1 H, aryl), 4.07 (s, 3 H, CH_3).

General Procedures for Preparing Compounds 5. Compounds 5 were prepared by the two general procedures of reacting oxime 4 with the appropriate chloromethyl ether derivative in THF–DMF (5:1) as previously described[1] (procedure A) or by reacting 4 with the appropriate triflate ester in nitromethane (procedure B). The following examples are illustrative.

Procedure A. Preparation of 2-[(Hydroxyimino)-methyl]-3-methyl-1-[[(2′-methyl-2′-nitropropyl-1′)oxy]-methyl]imidazolium Chloride (5s). Dry HCl gas was gently bubbled into a stirred mixture of 2-methyl-2-nitro-1-propanol (0.17 mol), *s*-trioxane (0.057 mol), and benzene (100 mL) at room temperature over 2.5 h after which time an aqueous second phase had formed. The layers were separated, and the organic layer was dried over $CaCl_2$ and concentrated to give an oil that was vacuum distilled to provide 1-(chloromethoxy)-2-methyl-2-nitropropane in 71% yield: bp 48–49 °C (0.25 mmHg); ^{1}H NMR ($CDCl_3$) δ 5.47 (s, 2 H, OCH_2Cl), 4.00 (s, 2 H, CH_2), 1.62 (s, 6 H, 2 CH_3).

Oxime 4 (0.044 mol) was dissolved in DMF (30 mL) and there was added a solution of 1-(chloromethoxy)-2-methyl-2-nitropropane (0.046 mol) in 150 mL of dry THF. The mixture was stirred overnight and then filtered to give a white solid that was recrystallized from ethanol–ethyl acetate to give compound 5s (57%) as colorless crystals: see Table I; ^{1}H NMR (DMSO-d_6) δ 12.63 (s, 1 H, NOH), 8.62 (s, 1 H, C*H*=NOH), 8.28 and 8.15 (2 d, 1 H each, J = 2 Hz, aryl) 5.93 (s, 2 H, NCH_2O), 4.07 (s, 3 H, NCH_3), 4.06 (s, 2 H, OCH_2), 1.52 (s, 6 H, 2 CH_3).

Procedure B. Preparation of 2-[(Hydroxyimino)-methyl]-1-[2′-[*N*-(methylsulfonyl)-*N*-methylamino]-ethyl]-3-methylimidazolium Chloride (5x). A mixture of 2-[*N*-(methylsulfonyl)-*N*-methylamino]ethanol (0.04 mol) and triethylamine (0.04 mol) in 25 mL of DCM was added dropwise to an ice-cooled, stirred mixture of trifluoromethanesulfonic anhydride (0.04 mol) in 50 mL of DCM. After being stirred for 30 min, the mixture was washed with cold water (2 × 125 mL), dried ($MgSO_4$), filtered, and concentrated to give a tan oil that was passed through a short column of silica gel and eluted with DCM. Evaporation of the solvent provided pure 2-[*N*-(methylsulfonyl)-*N*-methylamino]ethyl triflate as a colorless solid in 86% yield: mp 64–65 °C; ^{1}H NMR ($CDCl_3$) δ 4.73 (t, 2 H, J = 5 Hz, CH_2), 3.67 (t, 2 H, J = 5 Hz, CH_2), 3.03 (s, 3 H, CH_3), 2.92 (s, 3 H, CH_3).

To a stirred suspension of oxime 4 (0.035 mol) in nitromethane (120 mL) cooled at 0–5 °C was added the above triflate ester (0.036 mol). The cooling bath was removed and the mixture was stirred at room temperature for 2 h, after which time all solids had dissolved. The mixture was concentrated to give a viscous residue that was treated with an aqueous suspension of excess Amberlite IRA-400 anion (chloride form) exchange resin and stirred for 1 h. After the mixture was passed through a short column of fresh Cl anion-exchange resin, the eluant was evaporated to give a solid. The last remaining water was removed by azeotropic evaporation with absolute ethanol. There was recovered a white solid that

(9) Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175.

was recrystallized from ethanol–ethyl acetate to give compound **5x** (79%) as colorless crystals: see Table I; ^{1}H NMR (DMSO-d_6) δ 13.41 (s, 1 H, NOH), 8.70 (s, 1 H, C*H*=NOH), 8.00 (s, 2 H, aryl), 4.65 (t, 2 H, J = 5 Hz, NCH_2), 4.00 (s, 3 H, NCH_3), 3.48 (t, 2 H, J = 5 Hz, NCH_2), 2.90 (s, 6 H, 2 CH_3).

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Registry No. **2**, 616-47-7; **3**, 13750-81-7; **5a**, 132540-00-2; **5b**, 132540-01-3; **5c**, 132540-02-4; **5d**, 132540-03-5; **5e**, 132540-04-6; **5f**, 132540-05-7; **5g**, 132540-06-8; **5h**, 132540-07-9; **5i**, 132540-08-0; **5j**, 132540-09-1; **5k**, 132540-10-4; **5l**, 132540-11-5; **5m**, 132540-12-6; **5n**, 132540-13-7; **5o**, 132540-14-8; **5p**, 132540-15-9; **5q**, 132540-16-0; **5r**, 132540-17-1; **5s**, 132540-18-2; **5t**, 132540-19-3; **5u**, 132540-20-6; **5v**, 132540-21-7; **5w**, 132540-22-8; **5x**, 132540-23-9; **5y**, 132540-24-0; **5z**, 132540-25-1; soman, 96-64-0; tabun, 77-81-6; $HO(CH_2)_4OC(O)$Ph-2'-OH, 13461-42-2; $HO(CH_2)_4OH$, 110-63-4; $HO(CH_2)_2NHCH_3$, 109-83-1; $HO(CH_2)_2N(CH_3)SO_2CH_3$, 87113-83-5; $HO(CH_2)_2N(CH_3)SO_2$Ph, 59724-60-6; $ClCH_2O(CH_2)_2OCH_3$, 3970-21-6; $ClCH_2O(CH_2)_2CH(OCH_3)CH_3$, 132539-87-8; $ClCH_2O(CH_2)_2Si(CH_3)_3$, 76513-69-4; $ClCH_2O(CH_2)_3Si(CH_3)_3$, 122100-58-7; $ClCH_2O(CH_2)_4Cl$, 3970-17-0; $ClCH_2O(CH_2)_3Br$, 54314-83-9; $ClCH_2OCH_2C(CH_3)_2CH_2Br$, 132564-31-9; $ClCH_2O(CH_2)_2NO_2$, 54266-58-9; $ClCH_2OCH_2C(CH_3)_2NO_2$, 57039-05-1; $ClCH_2O(CH_2)_2SO_2CH_3$, 129499-56-5; $F_3CSO_2O(CH_2)_2CN$, 132539-88-9; $F_3CSO_2O(CH_2)_3CN$, 132539-89-0; $F_3CSO_2O(CH_2)_4CN$, 87019-99-6; $F_3CSO_2O(CH_2)_5CO_2CH_3$, 132539-90-3; $F_3CSO_2O(CH_2)_4OC(O)$Ph-2'-OH, 132539-91-4; $F_3CSO_2O(CH_2)_2F$, 95353-04-1; $F_3CSO_2O(CH_2)_2O(CH_2)_2F$, 132539-92-5; $F_3CSO_2O(CH_2)_2O(CH_2)_2Cl$, 132539-93-6; $F_3CSO_2O(CH_2)_2Br$, 103935-47-3; $F_3CSO_2O(CH_2)_2NO_2$, 132539-94-7; $F_3CSO_2O(CH_2)_2SO_2CH_3$, 126748-92-3; $F_3CSO_2O(CH_2)_2N(CH_3)_2$·HCl, 132539-95-8; $F_3CSO_2O(CH_2)_2N(CH_3)SO_2CH_3$, 132539-97-0; $F_3CSO_2O(CH_2)_2N(CH_3)SO_2CF_3$, 132539-98-1; $F_3CSO_2O(CH_2)_2N(CH_3)SO_2$Ph, 132539-99-2; $HO(CH_2)_2CN$, 109-78-4; $HO(CH_2)_3CN$, 628-22-8; $HO(CH_2)_4CN$, 2427-16-9; $HO(CH_2)_5C(O)OCH_3$, 4547-43-7; $HO(CH_2)_2F$, 371-62-0; $HO(CH_2)_2O(CH_2)_2F$, 373-22-8; $HO(CH_2)_2O(CH_2)_2Cl$, 628-89-7; $HO(CH_2)_2Br$, 540-51-2; $HO(CH_2)_2NO_2$, 625-48-9; $HO(CH_2)_2SO_2CH_3$, 15205-66-0; $HO(CH_2)_2N(CH_3)_2$·HCl, 2498-25-1; $HO(CH_2)_2N(CH_3)SO_2CF_3$, 40657-06-5; $HO(CH_2)_2OCH_3$, 109-86-4; $HO(CH_2)_2CH(CH_3)OCH_3$, 2517-43-3; $HO(CH_2)_2Si(CH_3)_3$, 2916-68-9; $HO(CH_2)_3Si(CH_3)_3$, 2917-47-7; $HO(CH_2)_4Cl$, 928-51-8; $HO(CH_2)_3Br$, 627-18-9; $HOCH_2C(CH_3)_2CH_2Br$, 40894-00-6; $HO(CH_2)_2NO_2$, 625-48-9; $HOCH_2C(CH_3)_2NO_2$, 76-39-1; $HO(CH_2)_2SO_2CH_3$, 15205-66-0; methyl salicylate, 119-36-8; 1-pyrrolidineethanol (triflate ester)hydrochloric acid, 132539-96-9; 1-pyrrolidineethanol hydrochloric acid, 30727-31-2.

Quaternary Salts of 2-[(Hydroxyimino)methyl]imidazole. 5.[1] Structure–Activity Relationships for Side-Chain Nitro-, Sulfone-, Amino-, and Aminosulfonyl-Substituted Analogues for Therapy against Anticholinesterase Intoxication

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Several quaternary imidazolium oxime derivatives incorporating side chains bearing nitro, sulfone, amino, and aminosulfonyl substituents were prepared and evaluated as treatment therapeutics for anti-AChE intoxication. In vivo test results in the mouse revealed that many of these compounds are highly effective in providing life-saving protection against the extremely toxic cholinesterase inhibitors soman and tabun. Several structure–activity relationships were noted that were characteristic of the side-chain substituent. In vivo test results for additional selected derivatives of some of the more therapeutically active compounds indicated that the quaternary heteroaryl nucleus is essential for activity whereas a nucleophilic moiety (i.e., oxime) is not. In support of previous suspicions, these results afforded additional evidence suggesting that reactivation is not the main mode of antidotal action by the imidazolium oximes. An alternative antidotal mechanism is postulated that is consistent with all data and that involves enzyme protection by the compounds.

There is presently a need in medical defense for effective, stable antidotal drugs that can be stored and used for emergency treatment of poisoning by chemical warfare nerve agents such as 3,3-dimethyl-2-butyl methylphosphonofluoridate (soman), a compound that systemically acts to phosphorylate and thereby irreversibly inhibit the esteratic site of acetylcholinesterase (AChE). Current therapeutics for organophosphorus (OP) inhibited AChE intoxication (e.g., 2-[(hydroxyimino)methyl]-1-methylpyridinium chloride, 2-PAM), which function as reactivators by bimolecularly displacing AChE from OP moieties, are ineffective against soman. This inadequacy presumably arises because of the tendency of the AChE-bound soman residue to rapidly undergo a unimolecular deal-

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