Ethyl adenosine-5′-uronate (5 g, 16.2 mmol) in a 50-mL flask was cooled in an ice bath and treated with 3 mL (46 mmol) of anhydrous $C_2H_5NH_2$. The thick slurry was stirred overnight, at which time the product had solidified. Product was suspended in dry $(C_2H_5O)_2O$, filtered, and crystallized from C_2H_5OH; yield 4 g (80%).

2′,3′-*O*-Isopropylideneinosine-5′-uronic Acid (3). A solution of 25 g (81.1 mmol) of 2′,3′-*O*-isopropylideneinosine (**2**) in 300 mL of glacial acetic acid was vigorously stirred in a 500-mL flask containing a large magnetic stirring bar. When cooling in an ice bath reduced the temperature to just above the freezing point of the acetic acid (ca. 17 °C), 8.9 g (89 mmol) of CrO_3 was added portionwise and the dark mixture was stirred at room temperature for 48 h. Product was filtered off and washed with acetic acid. Repeated crystallizations from boiling acetic acid freed the product of traces of Cr salts: yield 15.7 g (60%) of an amorphous white powder; mp 272–274 °C (lit.[13] mp 252 °C).

2′,3′-*O*-Isopropylidene-*N*-ethyl-6-chloropurine-5′-uronamide (5). A mixture of 6.5 g (20 mmol) of **3**, 3 mL (40 mmol) of $SOCl_2$, 1.5 mL (19.4 mmol) of *N,N*-dimethylformamide, and 250 mL of dry $CHCl_3$ was heated at reflux with the exclusion of moisture for 5–6 h. Vacuum evaporation of solvents yielded a syrup that was disolved in 80 mL of dry $CHCl_3$. This solution was added to an ice-cold solution of 14 mL of anhydrous $C_2H_5NH_2$ in 150 mL of dry $CHCl_3$. After 20 min at <10 °C this solution was extracted with 3 × 250 mL of dilute HCl, once with 250 mL of saturated $NaHCO_3$, and with 2 × 50 mL of water. Drying ($MgSO_4$) and evaporation gave a pale yellow syrup that was used directly in the next step.

***N*-Ethyl-N^6-cyclohexyladenosine-5′-uronamide (13).** A solution of **5** (7.36 g, 20 mmol), cyclohexylamine (2.1 g, 21.2 mmol), and $(C_2H_5)_3N$ (5.5 mL, 40 mmol) in 200 mL of absolute C_2H_5OH was heated as reflux with exclusion of moisture. After 48 h the solvent was evaporated to leave a syrupy residue. The addition of dry $(C_2H_5)_2O$ precipitated $(C_2H_5)_3$·HCl, which was filtered off. The residue after evaporation was purified by LPLC on a 3 × 30 cm column of C-18 silica gel equilibrated with CH_3OH/H_2O (3:2, v/v). The column was eluted with a linear gradient of CH_3OH/H_2O generated by pumping CH_3OH into CH_3OH/H_2O (3:2, v/v). Fractions containing the product was pooled and evaporated, and the residue was heated in 1 N HCl for 1 h at 60 °C. Cooling and neutralization with $NaHCO_3$ precipitated a white solid that was purified by preparative reversed-phase LPLC employing a linear gradient formed from two solutions of CH_3OH/H_2O (3:2, v/v; 4:1, v/v). Evaporation of appropriate fractions yielded 3.5 g (47%) of white powder.

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Registry No. 1, 35920-39-9; 2, 2140-11-6; 3, 28440-13-3; 5, 103201-21-4; 6, 50908-62-8; 7, 35788-27-3; 8, 35788-21-7; 9, 72209-31-5; 10, 103201-22-5; 11, 103201-23-6; 12, 103201-24-7; 13, 103201-25-8; 14, 103201-26-9; 15, 103201-27-0; 16, 103201-28-1; 17, 103201-29-2; 18, 103201-30-5; 19, 103201-31-6; 20, 103201-32-7; 21, 103201-33-8; 22, 103201-34-9; 23, 103201-35-0; 24, 103201-36-1; 25, 103201-37-2; 26, 103201-38-3; 27, 103201-39-4; 28, 103224-48-2; $C_2H_5NH_2$, 75-04-7; 2′,3′-*O*-isopropylideneadenosine, 362-75-4; adenosine-5′-uronic acid, 3415-09-6; ethyl adenosine-5′-uronate hydrochloride, 50663-70-2; ethyl adenosine-5′-uronate, 35803-57-7; cyclohexylamine, 108-91-8.

Nonquaternary Cholinesterase Reactivators. 4. Dialkylaminoalkyl Thioesters of α-Keto Thiohydroximic Acids as Reactivators of Ethyl Methylphosphonyl- and 1,2,2-Trimethylpropyl Methylphosphonyl-acetylcholinesterase in Vitro

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In the search for improved lipophilic centrally active acetylcholinesterase (AChE) antidotes, a series of α-keto thiohydroximates were prepared and evaluated for their ability to reactivate AChEs inhibited by ethyl *p*-nitrophenyl methylphosphonate (EPMP) and soman (GD). The compounds conformed to the general structure 4-RC_6H_5C-(O)C(NOH)S$(CH_2)_nN^+R'R''\cdot X^-$ where R = H, CH_3, F, Br, Cl, OCH_3, CN; R′ = CH_3, C_2H_5, *i*-C_3H_7; R″ = H, CH_3; X = Cl, I; and *n* = 2, 3. In this series, varying R substituents on the aryl ring produced compounds with oxime pK_a values from 6.8 to 8.0, optimum for an AChE reactivator. Increasing lipophilicity of the amine segment correlated with reactivator potency, as did electron-withdrawing groups on the aryl moiety, presumably due to increased binding to hydrophobic sites surrounding the AChE active site. The in vitro reactivation potency of the α-keto thiohydroximates approaches and even surpasses that of 2-PAM and toxogonin for GD-inhibited AChE. These initial findings point to additional structure–activity relationships to assist in the design of improved antidotal compounds.

Although the highly toxic nature of organophosphorus compounds has been known for many years,[1-10] there still exists serious limitations in the antidotal therapy available against poisoning of these compounds. Most toxic organophosphorus esters are irreversible inhibitors of acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7; AChE).[1,11-13] Conventional therapy against organo-

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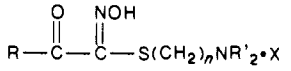
phosphorus ester intoxication entails coadministration of atropine that antagonizes the effects of accumulated acetylcholine and AChE "reactivators" that restore activity to the enzyme.[14-17]

Currently, pyridinium aldoximes are the only clinically used reactivators. This conventional treatment is effective for general organophosphorus esters including the chemical warfare agents tabun (GA) and sarin (GB) but is unsuccessful in cases of soman (GD) intoxication. It is generally assumed that the failure of oxime therapy against GD is due to the rapid transformation of the GD-inhibited AChE into a nonreactivatable dealkylated or "aged" enzyme.[18-20]

The pyridinium aldoximes reactivate inhibited AChE because of two factors: an oxime acid dissociation constant (pK_a) in the range of 7–8 and a cationic moiety (the alkylated pyridinium ion) at a distance from the oxime to give the reactivator a structural similarity to acetylcholine. The oximate anion displaces the organophosphorus moiety from the phosphylated[21] serine hydroxyl group of the AChE active site. The cationic moiety interacts electrostatically at the "anionic" region (presumably as aspartic acid carboxylate residue) near the enzyme active site.

Although the pyridinium aldoximes are useful therapeutics, the hydrophilic pyridinium cations limit membrane penetration. This causes a disproportionately high serum concentration of the pyridinium reactivators, rapid renal elimination, and marginal activity in regions (such as the central nervous system) where organophosphorus esters elicit pronounced physiological responses.[12]

On the basis of our earlier work on the α-keto thiohydroximic acid S-esters (1), we concluded that, in principle, it is possible to design nonquaternary and quaternary AChE reactivators that would equal the inherent activity of the pyridinium oximates toward phosphorylated AChE in general and GD-inhibited AChE in particular but would exhibit improved tissue distribution more like the lipophilic organophosphorus esters.[22-24] In view of the foregoing, we investigated a series of type 1 compounds where R was chosen to "fine tune" the oxime pK_a and adjust the compound lipophilicity; the tertiary amine functional group was incorporated to facilitate penetration into lipophilic tissues while providing Coulombic or electrostatic interactions with the anionic region of the enzyme; n was varied to optimize separation between cationic and nucleophilic moieties of the reactivators; and X was varied to give quaternary or nonquaternary test compounds. We evaluated selected type 1 compounds as reactivators of human erythrocyte (RBC) AChE inhibited by ethyl p-nitrophenyl methylphosphonate (EPMP) and by 1,2,2-trimethylpropyl methylphosphonofluoridate (GD). For comparison we also examined 2-[(hydroxyimino)methyl]-1-methylpyridinium (2-PAM), 1,3-bis[4-[(hydroxyimino)methyl]-1-pyridinio]-2-oxapropane (toxogonin), and 1-[2-[(hydroxyimino)methyl]-1-pyridinio]-3-[4-(aminocarbonyl)pyridinio]-2-oxapropane (HI-6).



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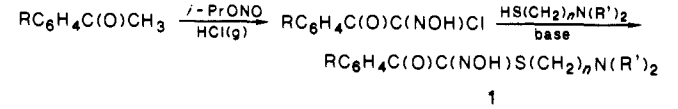
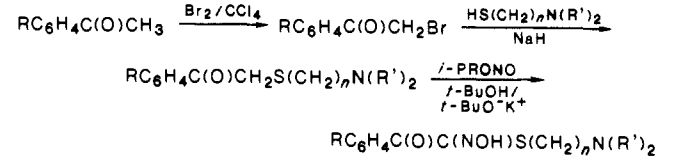
Scheme I. Synthesis of α-Keto Thiohydroximates

Method A:

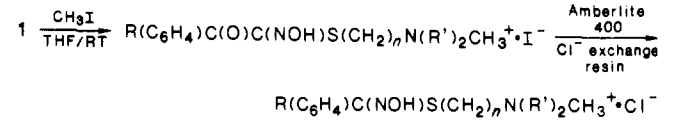


Method B:



R = H, p-CH_3, p-Cl, p-Br, p-F, p-OCH_3, p-CN; n = 2,3; R' = CH_3, C_2H_5, $CH(CH_3)_2$

Scheme II. Quaternization of α-Keto Thiohydroximates



Results and Discussion

Synthesis, Structure, and Acidity. The α-keto thiohydroximates 1 were prepared by the two general synthesis routes shown in Scheme I. Substituted acetophenones were converted to the α-chloro hydroximic acid by the method of Brachwitz.[25] The hydroximoyl chlorides were then converted to the α-keto thiohydroximate derivatives 1 in standard fashion.[22] Alternatively, bromination of the substituted acetophenone gives the α'-bromoacetophenone intermediate. Subsequent thiolation with the (*N*,*N*-dialkylamino)alkanethiol followed by nitrosation with an alkyl nitrite in 2-methyl-2-propanol yields the desired α-keto thiohydroximates 1. Treatment of the amino-substituted α-keto thiohydroximate with methyl iodide followed by elution over a chloride ion-exchange resin column provided the quaternary salts of type 1 compounds (Scheme II). Generally, the synthetic approaches gave type 1 compounds in a 20–50% overall yield. Table I shows structures and pertinent physical data for type 1 compounds.

The possibility of *E* and *Z* isomerization in the α-keto thiohydroximates was considered insofar as the absolute oxime configuration can profoundly influence reactivation of inhibited AChE.[16] The observations of Exner and coworkers that benzohydroxamoyl chlorides[26] and methyl benzothiohydroximates adopt the *E* configuration in solution, and our earlier observations on the α-keto thiohydroximates,[22] suggested that type 1 compounds adopt the *Z* configuration in solution with a six-centered intra-

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Table I. Selected Data for Benzoylthioformohydroximates

compd[a]	structure	mp, °C	yield,[b] %	formula[c]	method of prep
1a	$C_6H_5C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2 \cdot HCl$	139–140	32	$C_{14}H_{21}N_2O_2SCl$	A
1b	$4\text{-}BrC_6H_4C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2 \cdot HCl$	164–165	29	$C_{14}H_{20}N_2O_2SBrCl$	A
1c	$4\text{-}BrC_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_2 \cdot HCl$	149–150	62	$C_{12}H_{16}N_2O_2SBrCl$	A
1d	$4\text{-}BrC_6H_4C(O)C(NOH)SCH_2CH_2CH_3$	84–85	15	$C_{11}H_{12}NO_2SBr$	A
1e	$4\text{-}BrC_6H_4C(O)C(NOH)S(CH_2)_3N(CH_3)_2 \cdot (CO_2H)_2$	104–106	32	$C_{15}H_{19}N_2O_6SBr$	A
1f	$4\text{-}CH_3OC_6H_4C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2$	103–104	60	$C_{15}H_{22}N_2O_3S$	A
1g	$4\text{-}CH_3OC_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_2$	113–114	73	$C_{13}H_{18}N_2O_3S$	A
1h	$4\text{-}BrC_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_3{}^+I^-$	144–147	91	$C_{13}H_{18}N_2O_2SBrI$	
1i	$4\text{-}CH_3OC_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_3{}^+Cl^-$	175–178	85	$C_{14}H_{21}N_2O_3SCl$	
1j	$4\text{-}CH_3OC_6H_4C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2CH_3{}^+Cl^-$	164–165	65	$C_{16}H_{25}N_2O_3SCl$	
1k	$4\text{-}CH_3OC_6H_4C(O)C(NOH)S(CH_2)_3N(CH_3)_2 \cdot (CO_2H)_2$	151–153.5	29	$C_{16}H_{22}N_2O_7S$	A
1l	$4\text{-}ClC_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_2 \cdot HCl$	149–150	70	$C_{12}H_{16}N_2O_2SCl_2$	B
1m	$4\text{-}ClC_6H_4C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2 \cdot HCl$	181–183	18	$C_{14}H_{20}N_2O_2SCl_2$	B
1n	$4\text{-}ClC_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_3{}^+Cl^-$	196–197	64	$C_{13}H_{18}N_2O_2SCl_2$	
1o	$4\text{-}ClC_6H_4C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2CH_3{}^+Cl^-$	168–170	53	$C_{15}H_{22}N_2O_2SCl_2$	
1p	$C_{10}H_7C(O)C(NOH)S(CH_2)_2N(CH_3)_2 \cdot HCl$	160–162	50	$C_{16}H_{19}N_2O_2SCl$	B
1q	$4\text{-}FC_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_2$	135–136	33	$C_{12}H_{15}N_2O_2SF$	B
1r	$4\text{-}FC_6H_4C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2$	130–131	94	$C_{14}H_{19}N_2O_2SF$	B
1s	$4\text{-}FC_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_3{}^+Cl^-$	193–194	88	$C_{13}H_{18}N_2O_2SClF$	
1t	$4\text{-}FC_6H_4C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2CH_3{}^+Cl^-$	152–153	56	$C_{15}H_{22}N_2O_2SClF$	
1u	$4\text{-}CH_3C_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_2 \cdot HCl$	143–145	40	$C_{13}H_{19}N_2O_2SCl$	A
1v	$4\text{-}CH_3C_6H_4C(O)C(NOH)S(CH_2)_2N(CH_3)_3{}^+Cl^-$	190–193	59	$C_{14}H_{21}N_2O_2SCl$	
1w	$4\text{-}NCC_6H_4C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2 \cdot HCl$	175	58	$C_{15}H_{20}N_3O_2SCl$	A
1x	$4\text{-}NCC_6H_4C(O)C(NOH)S(CH_2)_2N(C_2H_5)_2CH_3{}^+Cl^- \cdot H_2O$	134–136	35	$C_{16}H_{22}N_3O_2SCl \cdot H_2O$	
1y	$4\text{-}ClC_6H_4C(O)C(NOH)S(CH_2)_2N(CH(CH_3)_2)_2 \cdot HCl$	168–169	67	$C_{16}H_{24}N_2O_2SCl_2$	B

[a] See text and ref 22 for synthesis methods. [b] Yield for production of target compound from immediate precursor. [c] All compounds were analyzed for C, H, N, S, and Cl, Br, or I; analytical results were within ±0.5% of the theoretical values.

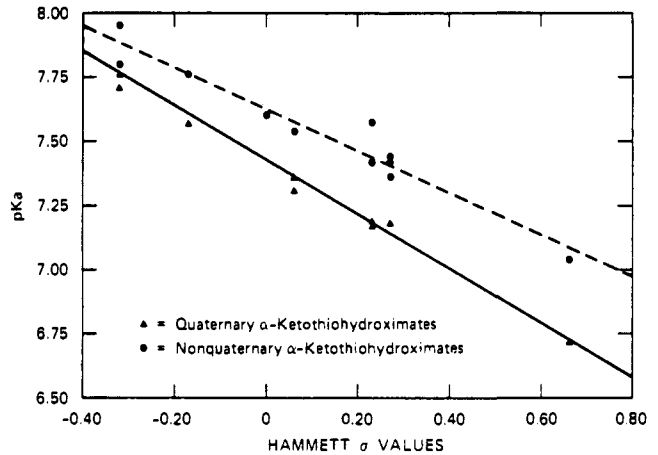

Figure 1. Correlation between the α-keto thiohydroximate acid dissociation constants (pK_a) vs. Hammett values (σ_p).

molecular hydrogen bonding of oximino proton to the α-carbonyl group.

Oxime acid dissociation constants (pK_a) (Table II) were measured by spectrophotometric determination of oximate concentration in buffers of various pH values.[27] For the quaternary aryl thiohydroximates a plot of pK_a vs. Hammett acid substituent constant (σ_p)[28] is linear and conforms to eq 1a, whereas the nonquaternary aryl thiohydroximates

$$pK_a = 7.40\ (\pm 0.01) - 1.01\ (\pm 0.03)\sigma_p \qquad (1a)$$

conformed to eq 1b; see Figure 1. These data provide a

$$pK_a = 7.62\ (\pm 0.02) - 0.81\ (\pm 0.06)\sigma_p \qquad (1b)$$

general indication of the validity of the pK_a values, an important consideration because the α-keto thiohydroximate reactivity is directly related to the population of dissociated oximate form at a given pH and hence to the acidity of the oxime functionality. Quaternization of the amino moiety reduces the oxime pK_a approximately 0.2 unit. Although the oxime pK_a strongly influences the α-keto thiohydroximate's reactivating potency, increased

Table II. Characteristic Physical Constants for α-Keto Thiohydroximates: Bimolecular Rate Constants (k_n), AChE Reversible Inhibition Potency (IC_{50}), and Other Selected Physical Data

compd[a]	pK_a[b]	k_n,[c,d] $M^{-1}\ min^{-1}$	IC_{50},[e] mM	log P[f]
1a	7.60	6.3	>1.0	g
1b	7.20	12.3	0.21	2.41
1c	7.36	25.2	0.71	1.70
1d	8.45	g	g	3.38
1e	7.41	46.6	>1.0	g
1f	7.65	75.0	0.26	1.43
1g	7.88	69.8	>1.0	1.07
1h	7.18	19.9	>1.0	−0.55
1i	7.71	52.6	>1.0	−1.96
1j	7.70	59.6	>1.0	−1.60
1k	8.42	g	g	0.49
1l	7.57	9.46	>1.0	1.74
1m	7.42	15.18	0.52	1.97
1n	7.19	9.29	g	−0.80
1o	7.17	15.18	>1.0	−0.42
1p	7.10	g	g	2.32
1q	7.54	g	g	1.15
1r	g	g	g	g
1s	7.36	15.76	>1.0	−1.55
1t	7.31	15.36	0.72	−1.17
1u	7.76	17.70	>1.0	1.52
1v	7.57	23.19	>1.0	−1.44
1w	7.02	4.43	0.18	g
1x	6.72	4.38	>1.0	g
1y	7.20	12.28	0.33	g
2-PAM	7.99	50.4	0.34	>−3.0
HI-6	7.27	16.3	0.46	>−3.0

[a] See Table I for structures. [b] Determined spectrophotometrically in 0.1 M phosphate buffer. [c] Calculated according to the equation $-d[SCh]/dt = k_0 + [OX]k_n$. See ref 22 and 23 for details. [d] k_n is the bimolecular rate constant for reaction of oximate with 7.5×10^{-4} M AcSCh in 0.1 M phosphate buffer. [e] IC_{50} is the concentration of test compound required to inhibit 50% of active AChE. [f] log P is the octanol/buffer partition coefficient for 0.1 M, pH 7.6, phosphate buffer. [g] Not determined.

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Coulombic interactions between phosphonylated enzyme and type 1 compounds caused by quaternization appears to dominate the in vitro reactivation kinetics (see discussion below).

Nucleophilicity. As a measure of inherent nucleophilicities (and as a control in our in vitro assay) of type 1 compounds, we determined bimolecular rate constants, k_n, for reaction of the anionic (oximate) form with acetylthiocholine, AcSCh. The methodology used was described earlier.[22,23] We previously demonstrated that type 1 compounds as well as heteroaromatic oximes are nucleophilic toward trigonal carbon and tetrahedral phosphorus.[24] Table II summarizes k_n values for AcSCh reaction with type 1 compounds.

Linear least-squares regression of the data in Table II gave the following Brønsted relationship:

$$\log k_n = -3.75\ (\pm 0.96) + 0.71\ (\pm 0.12)\mathrm{p}K_a \qquad (2)$$

For comparison,[22] similar relationships were previously established for type 1 compounds reacting with *p*-nitrophenyl acetate and for a series of heteroaromatic aldoximes and their thiohydroximate derivatives reacting with acetylthiocholine[24] (eq 3 and 4, respectively). The slope (β)

$$\log k_n = -3.04\ (\pm 0.34) + 0.69\ (\pm 0.08)\mathrm{p}K_a \qquad (3)$$

$$\log k_n = -3.02\ (\pm 0.38) \pm 0.58\ (\pm 0.05)\mathrm{p}K_a \qquad (4)$$

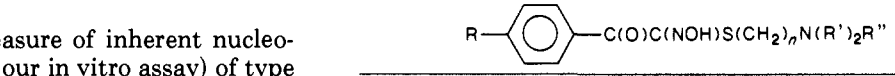
values of the above Brønsted relationship are typical[27,29,30] for reactions of (hydroxyimino)methyl compounds with various esters and demonstrate that type 1 compounds behave as nucleophiles in the anticipated fashion.

Reversible Inhibition of Human AChE. To probe possible AChE binding interactions (and also as a control in our enzyme assays), we determined the degree to which type 1 compounds reversibly inhibit the enzyme. Following the general procedure described earlier,[24] we determined enzyme activities in the presence of each test compound. Table II summarizes data for AChE inhibition and shows that type 1 compounds are weak competitive inhibitors, causing little AChE inhibition in our reactivation studies. Interestingly, the quaternary reactivators were also weakly inhibitory of human AChE. This suggests that both the quaternary and nonquaternary α-keto thiohydroximates will not significantly interact with the enzyme active site of uninhibited AChE at relevant concentrations in vivo or in vitro. Thus, if significant reactivation occurs, it must be related to specific alterations of the enzyme structure caused by binding of a particular inhibitor.

Reactivation of EPMP-Phosphonylated Human AChE. We inhibit human erythrocyte (RBC) AChE with ethyl *p*-nitrophenyl methylphosphate (EPMP) and investigated the kinetics of AChE reactivation as a function of the concentration of added test compound. The experimental methods and kinetic derivations were as previously described.[22-24] Definitions of relevant kinetic and experimental constants follow: [HOX] = concentration of added test compound; [OX] = concentration of added test compound present as deprotonated (oximate) form = $[\mathrm{HOX}][1 + \text{antilog}(\mathrm{p}K_a - 7.6)]^{-1}$; k_{OX} = bimolecular rate constant for reactivation in the limit of low concentration of oximate; k_{HOX} = effective bimolecular rate constant corrected for fraction of added test compound present as oximate = $k_{OX}[1 + \text{antilog}(\mathrm{p}K_a - 7.6)]^{-1}$.

Table III summarizes kinetic constants for reactivation of EPMP-inhibited human AChE by selected type 1 compounds. For comparison, the table also gives data for 2-PAM, toxogonin, and HI-6.

Table III. Rate of EPMP-Inhibited Human Erythrocyte (RBC) AChE Reactivation by Quaternary and Nonquaternary α-Keto Thiohydroximates



compd[a]	R	R′	R″	k_{OX},[b] M^{-1} min^{-1}	k_{HOX},[c] M^{-1} min^{-1}
		Nonquaternary			
1b	Br	CH_2CH_3	HCl	71.9	58.7
1g	OCH_3	CH_3		35.7	11.9
1f	OCH_3	CH_2CH_3		77.0	36.3
1l	Cl	CH_3	HCl	75.4	38.8
1m	Cl	CH_2CH_3	HCl	74.8	45.0
1u	CH_3	CH_3	HCl	93.7	38.3
1w	CN	CH_2CH_3	HCl	57.8	45.8
1y	Cl	$CH(CH_3)_2$	HCl	68.2	48.8
		Quaternary			
1h	Br	CH_3	CH_3I	78.0	51.8
1l	OCH_3	CH_3	CH_3Cl	53.7	23.4
1j	OCH_3	CH_2CH_3	CH_3Cl	73.2	32.4
1o	Cl	CH_2CH_3	CH_3Cl	203	148
1s	F	CH_3	CH_3Cl	134	57.1
1t	F	CH_2CH_3	CH_3Cl	251.2	166
1v	CH_3	CH_3	CH_3Cl	79.3	41.0
1x	CN	CH_2CH_3	CH_3Cl	94.3	83.3
2-PAM				2477	717
toxogonin				2637	1400
HI-6				7242	4930

[a] See Table I for structures. [b] k_{OX}, the bimolecular reactivation rate constant, was calculated as previously described; see ref 22-24. [c] k_{HOX}, the effective rate constant for reactivation, adjusts for the differences in oxime ionization at pH 7.6.

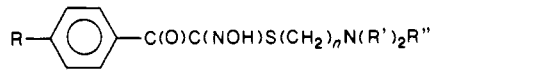
The table demonstrates that, against EPMP, type 1 compounds are only moderate reactivators: k_{HOX} values varied from a low of 11.9 M^{-1} min^{-1} (**1g**) to a high of 166 M^{-1} min^{-1} (**1t**). All values fall far below the standard therapeutics 2-PAM, toxogonin, and HI-6. The individual k_{HOX} values varied little with respect to oximate structure. For example, in the nonquaternary series, compounds **1g** and **1w** exhibited the greatest difference in pK_a values, and the two oximates feature a 16-fold difference in Brønsted nucleophilicities. However, the k_{HOX} values only differed by a factor of 4.

Surprisingly, quaternization of the α-keto thiohydroximates did little to increase the overall inherent reactivity against EPMP-inhibited AChE. For the five reactivator pairs studied (**1g**/**1i**; **1f**/**1j**; **1m**/**1o**; **1u**/**1v**; **1w**/**1x**), the effective bimolecular rate constant (k_{HOX}) varied only slightly: 1.96, 0.89, 3.28, 1.07, and 1.81, respectively. Interestingly, the reactivator pair **1f**/**1j** actually decreased with quaternization.

As a first approximation, increased steric hinderance on the amino functional group appears to increase reactivation potency. The (diethylamino)ethyl and (diisopropylamino)ethyl moieties had consistently higher reactivities than the (dimethylamino)ethyl analogues. Increasing the steric bulk and effective charge of the Coulombic portion presumably enhances interactions with the inhibited enzyme. This should increase the relative effectiveness of the reactivator by increasing the probability that the oximate will be in an appropriate geometry for reaction. In this series, quaternization appears to be more effective than steric bulk in optimizing reactivation of EPMP-inhibited AChE. Furthermore, although the oxime pK_a strongly influences α-keto thiohydroximate reactivating potency, increased Coulombic interactions between phosphonylated enzyme and quaternized type 1 com-

(29) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622.
(30) Guillot-Edelheit, G.; Laloi-Diard, M.; Eisenstein, O. *Tetrahedron* **1978**, *34*, 523.

Table IV. Percentage of Maximum Reactivation (% R_{max}) for GD-Inhibited Human Erythrocyte (RBC) AChE by Oximes[a]



compd	R	R′	R″	n	% R_{max}[b]
		Nonquaternary			
1a	H	CH_2CH_3	HCl	2	2.2
1b	Br	CH_2CH_3	HCl	2	35.0
1c	Br	CH_3	HCl	2	14.6
1e	OCH_3	CH_3	$(CO_2H)_2$	3	4.6
1f	OCH_3	CH_2CH_3		2	*c*
1g	OCH_3	CH_3		2	*c*
1l	Cl	CH_3	HCl	2	8.0
1m	Cl	CH_2CH_3	HCl	2	28.6
		Quaternary			
1h	Br	CH_3	CH_3I	2	51.8
1i	OCH_3	CH_3	CH_3Cl	2	5.6
1j	OCH_3	CH_2CH_3	CH_3Cl	2	8.5
1o	Cl	CH_2CH_3	CH_3Cl	2	59.4
2-PAM					28.2
toxogonin					48.6
HI-6					79.6[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] Maximum reactivation for all test oximes was achieved by 30-min incubation; the values given represent the average of percent R_{max} from 30 to 90 min. [c] No reactivation was observed. [d] HI-6 was incubated at 1.0×10^{-4} M.

pounds appear to dominate the in vitro reactivation kinetics.

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 anionic binding site, retarding Coulombic 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),[19,20] in practice such determinations are extremely difficult, and we have not yet achieved satisfactory results. Thus, we determined the percent reactivation at various times, R_t.

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.

Comparing values of R_{max}, the nonquaternary compounds **1b** and **1m** equal 2-PAM as reactivators of GD-inhibited AChE but are considerably less potent than HI-6 or toxogonin (see Table IV). The reactivation potencies of the quaternary derivatives **1h** and **1o** are remarkable, surpassed only by HI-6.

Unlike EPMP-inhibited human AChE, GD-inhibited human AChE manifests a broad range of activities for type 1 compounds investigated: percent R_{max} values varied from negligible for the nonquaternary aryl methoxy compounds **1f** and **1g** to a high of 59.4 for **1o**.

For GD-inhibited human AChE halide substitution on the benzoyl ring significantly enhances the percent reactivation. Additionally, the (diethylamino)ethyl moiety also favors reactivation over the dimethyl analogue. Increases in the steric bulk of the reactivator enhanced GD-inhibited AChE reactivation similar to that observed with EPMP. The potency of the nonquaternary compounds **1b** and **1m** was particularly interesting, since their high lipophilicity (log P = 2.41 and 1.97, respectively) should allow them access to the central nervous system. Use as a pretreatment or postreatment in combination with the more peripherally potent HI-6 or 2-PAM could be desirable.

Quaternization of type 1 compounds significantly increases their affinity for the GD-inhibited enzyme. As such, **1h**, the quaternary analogue of **1c**, was 3.5 times more effective in reactivating GD-inhibited human AChE. Although Jarv[31] demonstrated that organophosphorus ester alkyl groups can effectively shield the enzyme anionic regions of phosphonylated AChE, it appears that the potency of this shielding is very dependent on reactivator, inhibitor, and AChE source. For type 1 compounds, the quaternary (*N*,*N*-dialkylamino)alkyl moiety presumably binds to some anionic site by means of increased Coulombic interactions despite the presence of the bulky pinacolyl group. The particularly high efficacy of the bis quaternary Hagedorn oximes may be related to this ability to bind to multiple anionic sites. For type 1 compounds we also assume that additional binding intereactions between the substituted aryl moiety and lipophilic enzyme binding sites near the catalytic center may play a significant role in the reactivation process.

Conclusions

The search for AChE reactivators for treating organophosphorus ester poisoning in general and GD (soman) intoxication in particular has continued for over 25 years. With the exception of the Hagedorn oximes,[26] no effective treatment for GD-inhibited AChE in vivo has been demonstrated by oximate reactivators. The thiohydroximates reported here proved to be modest reactivators of human AChE inhibited by EPMP, but against GD-inhibited human AChE, type 1 compounds (particularly the 4′-halo-substituted materials **1h** and **1o**) demonstrated high potencies. We presume that they should also demonstrate some activity against GD in vivo.

For EPMP- and GD-inhibited AChE, steric interactions between oxime reactivators and enzyme dictate the inherent in vitro reactivity of α-keto thiohydroximate reactivators. Substituents [(dialkylamino)alkyl] can be extensively varied and still provide reactivators with pK_a values in the useful range while tailoring the lipophilicity of the system. Increased steric hindrance on the (dialkylamino)alkyl moiety favors increased reactivation potency. Furthermore, quaternization of the (dialkylamino)alkyl group enhances reactivation of inhibited AChE.

The best type 1 compounds were approximately equal to 2-PAM and toxogonin in their ability to reactivate GD-inhibited AChE but inferior to HI-6. Their ease of synthesis and apparent stability in solution make the quaternary and nonquaternary α-keto thiohydroximate family of reactivators an attractive alternative to existing therapeutic oximes. Additional research will be required to develop the optimal interactions between GD-inhibited AChE and type 1 compounds. Moreover, other organophosphonate/AChE systems should be examined against type 1 compounds both in vivo and in vitro. Results with this series of α-keto thiohydroximates as well as with the imidazole reactivators, described earlier,[24] demonstrate that the structure–activity principles developed for the qua-

(31) Jarv, J.; Aaviksaar, A.; Godovikov, N.; Labanov, P. *Biochem. J.* **1967**, *167*, 823.

ternary pyridinium oximes apply equally well to other families of compounds. Our results clearly demonstrate that the quaternary pyridinium nucleus is not required for high reactivity against organophosphonate-inhibited AChE.

Experimental Details

Materials. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Associates EM-360 spectrometer. Chemical shifts are reported in parts per million (δ) from an internal tetramethylsilane standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Infrared (IR) spectra were obtained on a Perkin-Elmer Model 1420 spectrophotometer. Melting points were determined on a calibrated Fisher-Johns melting point apparatus. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Analytical thin-layer chromatography (TLC) was performed on Analtech Uniplate silica gel GF (scored 10 × 20 cm plates, 250 μm); preparative TLC was performed on Uniplate silica gel GF (20 × 20 cm plates, 2000 μm). Column chromatography was done on reagent silica gel (90–200 mesh) obtained from Accurate Chemical and Scientific Corp. Ethyl *p*-nitrophenyl methylphosphonate (EPMP) was prepared by conversion of diethyl methylphosphonate to ethyl methyl phosphonylchloridate followed by reaction with *p*-nitrophenol.[32] *Caution*! EPMP and GD (supplied by the U.S. Army Medical Research and Development Command) are extremely toxic anticholinesterase agents. They must be handled with gloves and in a fume hood or at high dilutions at all times.

Thiolation of α-Keto Hydroximoyl Chlorides. The α-aroylhydroximoyl chlorides 4-$RC_6H_4C(O)C(NOH)Cl$, where R = H, Br, OCH_3, CH_3, and CN, were prepared by treating the corresponding acetophenones with isopropyl nitrite and gaseous HCl, as described by Brachwitz.[25] These compounds were converted to the corresponding α-keto thiohydroximates without further purification.

Method A: General Procedure for Preparing *S*-(*N*,*N*-Dialkylamino)alkyl α-Keto Thiohydroximates (1). The compounds listed below were prepared from the corresponding hydroximoyl chlorides by the same general procedure. Sodium methoxide (2.1 mol equiv) was prepared by dissolving sodium metal in dry methanol. The mixture was cooled (0 °C), and the appropriate (*N*,*N*-dialkylamino)alkanethiol hydrochloride salt was added to the alkoxide solution in several portions. To this solution was added the oximino chloride (50 mmol) dissolved in 2-propanol (100 mL) dropwise, and the resulting mixture was stirred at room temperature overnight.

The product precipitated from solution and was separated from salt byproducts by recrystallization. In cases where no precipitate formed, the solvent was removed in vacuo and the oil extracted with several portions of ethyl acetate. The combined extracts were washed with several portions of water and dried over anhydrous Na_2SO_4, and the solvent was concentrated. Purification was generally accomplished on a silica gel column using 100% CH_2Cl_2 up to 5% MeOH in CH_2Cl_2. The following test compounds were prepared by this procedure.

***S*-2-(*N*,*N*-Diethylamino)ethyl Benzoylthioformohydroximate Hydrochloride (1a).** Recrystallization from absolute EtOH yielded analytically pure **1a** as colorless needles: mp 139–140 °C; ^{1}H NMR ($CDCl_3$) δ 11.78 (s, 1 H, NOH), 8.05 (m, 4 H, aryl), 3.10 (m, 2 H, CH_2), 2.65 (m, 6 H, CH_2), 0.98 (t, 6 H, CH_3).

***S*-2-(*N*,*N*-Diethylamino)ethyl (4′-Bromobenzoyl)thioformohydroximate Hydrochloride (1b).** The purified free base (recrystallized from a methylene chloride/ether mixture) was converted to the hydrochloride salt by suspending the free amine in 100 mL of ether and slowly bubbling through excess HCl gas, yielding analytically pure **1b** as pale yellow crystals: mp 161–165 °C; ^{1}H NMR (Me_2SO-d_6) δ 13.00 (br s, NOH), 10.64 (br s, 1 H, NH^+), 7.80 (m, 4 H, aryl), 3.4–2.9 (m, 8 H, CH_2), 1.18 (t, 6 H, CH_3).

***S*-2-(*N*,*N*-Dimethylamino)ethyl (4′-Bromobenzoyl)thioformohydroximate Hydrochloride (1c).** Recrystallization from an ether/methanol mixture (1:4) yielded analytically pure **1c** as white crystals: mp 149–150 °C; ^{1}H NMR (Me_2SO-d_6) δ 13.40 (s, 1 H, NOH), 11.31 (br s, 1 H, NH^+), 7.10 (s, 4 H, aryl), 3.35 (br s, 4 H, CH_2), 2.73 (s, 6 H, CH_3).

***S*-*n*-Propyl (4′-Bromobenzoyl)thioformohydroximate (1d).** Compound **1d** was chromatographed on thin-layer silica gel plates with a chloroform/methanol eluent (20:1): mp 67–69 °C; ^{1}H NMR (Me_2SO-d_6) δ 12.60 (s, 1 H, NOH), 7.81 (m, 4 H, aryl), 2.83 (t, 2 H, CH_2), 1.47 (m, 2 H, CH_2), 0.86 (t, 3 H, CH_3).

***S*-2-(*N*,*N*-Dimethylamino)propyl (4′-Bromobenzoyl)thioformohydroximate Oxalate (1e).** Recrystallization from an ether/methanol mixture yielded analytically pure **1e** as a white amorphous solid: mp 104–106 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.80 (m, 7 H, aryl, NOH), 2.98 (m, 4 H, CH_2), 2.65 (s, 6 H, CH_3), 1.7–2.0 (m, 2 H, CH_2); free base of **1e**; ^{1}H NMR ($CDCl_3$) δ 11.40 (br s, 1 H, NOH), 7.73 (q, 4 H, aryl), 2.93 (m, 2 H, CH_2), 2.52 (m, 2 H, CH_2), 2.18 (s, 6 H, CH_3), 1.6–1.9 (m, 2 H, CH_2).

***S*-2-(*N*,*N*-Diethylamino)ethyl (4′-Methoxybenzoyl)thioformohydroximate (1f).** Recrystallization from absolute ethanol yielded analytically pure **1f** as white crystals: mp 103–104 °C; ^{1}H NMR ($CDCl_3$) δ 12.49 (s, 1 H, NOH), 7.93 (d, 2 H, aryl), 7.10 (d, 2 H, aryl), 3.90 (s, 3 H, CH_3), 3.10 (m, 2 H, CH_2), 2.65 (m, 6 H, CH_2), 1.13 (t, 6 H, CH_3).

***S*-2-(*N*,*N*-Dimethylamino)ethyl (4′-Methoxybenzoyl)thioformohydroximate (1g).** Recrystallization from methanol yielded analytically pure **1g** as white crystals: mp 113–114 °C; ^{1}H NMR ($CDCl_3$) δ 12.01 (s, 1 H, NOH), 8.03 (d, 2 H, aryl), 6.98 (d, 2 H, aryl), 3.85 (s, 3 H, CH_3), 3.00 (m, 2 H, CH_2), 2.50 (m, 2 H, CH_2), 2.22 (s, 6 H, CH_3).

***S*-2-(*N*,*N*-Dimethylamino)ethyl (4′-Bromobenzoyl)thioformohydroximate Methiodide (1h).** Quaternization of **1c** with methyl iodide in dry THF and recrystallization from absolute ethanol yielded analytically pure **1h** as yellow plates: mp 144–147 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.85 (s, 4 H, aryl), 4.48 (br s, 2 H, CH_2), 3.30 (s, 2 H, CH_2), 3.05 (s, 9 H, CH_3).

***S*-2-(*N*,*N*-Dimethylamino)ethyl (4′-Methoxybenzoyl)thioformohydroximate Methchloride (1i).** Quaternization of **1g** with methyl iodide was followed by Cl^- ion exchange. Recrystallization from absolute ethanol yielded analytically pure **1i** as a white solid: mp 175–178 °C; ^{1}H NMR ($CDCl_3$/CD_3OD) δ 8.10 (d, 2 H, aryl), 7.03 (d, 2 H, aryl), 3.96 (s, 3 H, CH_3), 3.72 (m, 2 H, CH_2), 3.40 (m, 2 H, CH_2), 3.25 (s, 9 H, CH_3).

***S*-2-(*N*,*N*-Diethylamino)ethyl (4′-Methoxybenzoyl)thioformohydroximate Methchloride (1j).** Compound **1j** was prepared by quaternization of **1f** with methyl iodide followed by ion exchange with Amberlite IRA 400: mp 164–165 °C; ^{1}H NMR ($CDCl_3$/CD_3OD) δ 8.17 (d, 2 H, aryl), 7.05 (d, 2 H, aryl), 3.93 (s, 3 H, CH_3), 3.40 (m, 4 H, CH_2), 3.10 (s, 3 H, CH_3), 1.30 (t, 6 H, CH_3).

***S*-2-(*N*,*N*-Dimethylamino)propyl (4′-Methoxybenzoyl)thioformohydroximate Oxalate (1k).** Recrystallization from methanol/ether yielded analytically pure **1k** as white crystals: mp 151–153.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 12.74 (s, 1 H, NOH), 7.73 (d, 2 H, aryl), 7.07 (d, 2 H, aryl), 3.95 (s, 3 H, CH_3), 3.00 (m, 2 H, CH_2), 2.50 (m, 2 H, CH_2), 2.20 (s, 6 H, CH_3), 1.85 (m, 2 H, CH_2).

***S*-2-(*N*,*N*-Dimethylamino)ethyl (4′-Methylbenzoyl)thioformohydroximate Hydrochloride (1u).** Recrystallization from methanol/ether yielded analytically pure **1u** as a white powder: mp 143–145 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.90 (d, 2 H, aryl), 7.44 (d, 2 H, aryl), 3.28 (s, 4 H, CH_2), 2.73 (s, 6 H, CH_3), 2.44 (s, 3 H, CH_3).

***S*-2-(*N*,*N*-Dimethylamino)ethyl (4′-Methylbenzoyl)thioformohydroximate Methchloride (1v).** Quaternization of **1u** with methyl iodide was followed by Cl^- ion exchange on Amberlite IRA 400. Recrystallization from methanol/ether yielded analytically pure **1v** as a white powder: mp 193–194 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.96 (d, 2 H, aryl), 7.47 (d, 2 H, aryl), 3.50 (m, 4 H, CH_2), 3.10 (s, 9 H, CH_3), 2.43 (s, 3 H, CH_3).

***S*-2-(*N*,*N*-Diethylamino)ethyl (4′-Cyanobenzoyl)thioformohydroximate (1w).** Compound **1w** was obtained as yellow crystals in 17% yield: mp 118–120 °C; ^{1}H NMR ($CDCl_3$) δ 10.34 (s, 1 H, NOH), 8.10 (d, 2 H, aryl), 7.80 (d, 2 H, aryl), 2.77 (s, 4 H, CH_2), 2.60 (q, 2 H, CH_2), 2.58 (q, 2 H, CH_2), 1.02 (t, 3 H, CH_3), 0.98 (t, 3 H, CH_3).

(32) Fukuto, T. R.; Metcalf, R. L. *J. Am. Chem. Soc.* **1959**, *80*, 372.

The hydrochloride salt was recrystallized from MeOH/Et_2O in 58% yield: mp 174–176 °C; ^1H NMR (Me_2SO-d_6) δ 11.30 (s, 1 H, NOH), 8.10 (d, 2 H, aryl), 7.74 (d, 2 H, aryl), 3.40–3.04 (m, 4 H, CH_2), 2.66 (q, 4 H, CH_2), 1.00 (t, 6 H, CH_3).

S-2-(N,N-Diethylamino)ethyl (4'-Cyanobenzoyl)thioformohydroximate Methchloride (1x). Quaternization of **1w** with methyl iodide was followed by Cl^- ion exchange. Recrystallization from MeOH/Et_2O yielded pale yellow crystals: mp 134–136 °C; ^1H NMR (Me_2SO-d_6) δ 13.40 (s, 1 H, NOH), 8.12 (s, 4 H, aryl), 3.61 (s, 4 H, CH_2), 3.52 (q, 4 H, CH_2), 3.10 (s, 3 H, CH_3), 1.30 (t, 6 H, CH_3).

Method B: Oxidation of α-Thio-β-ketoalkanes. The compounds listed below were prepared by oxidation of the corresponding α-keto sulfides with base and isopropyl nitrate. The α-keto sulfides were prepared by treating the appropriately substituted α-bromoacetophenone with 1 equiv of a (dialkylamino)alkane thiol. The general procedures are detailed below.

General Procedure for α-Bromoacetophenones. The starting acetophenone was dissolved in dry CCl_4. To this was added 1 equiv of 2.0 M Br_2/CCl_4 dropwise under nitrogen(g). The reaction was monitored by NMR by the disappearance of the acetyl peak. The solvent was removed and the product (generally a white solid) washed with cold hexane. This material was used in subsequent steps without further purification.

General Procedure for the Synthesis of α-Keto Sulfides. To a stirred ethereal solution (100 mL) cooled to 0 °C and under nitrogen gas was added 1 equiv of a 60% dispersion of NaH. The appropriate (dialkylamino)alkanethiol (1 equiv) was then added dropwise. To the resulting mixture was added the α-bromoacetophenone (described above) dissolved in 100 mL of diethyl ether dropwise while the reaction temperature was maintained at 0 °C (generally 45 min was required for the addition on a 0.20-mol scale). The resulting mixture was stirred for 1 h at room temperature, and the reaction was monitored by TLC. The crude mixture was washed with 3 × 100-mL portions of water and dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo. The products were purified by flash chromatography on silica gel with a 0–5% methanol/dichloromethane eluent. No further purification was necessary, and these sulfides were used directly in subsequent reactions. Individual characteristics of selected α-keto sulfides are described below.

2-[[2-(N,N-Dimethylamino)ethyl]thio]-4'-chloroacetophenone: isolated as a brown oil in 87% yield; ^1H NMR ($CDCl_3$) δ 7.96 (d, 2 H, aryl), 7.42 (d, 2 H, aryl), 3.80 (s, 2 H, CH_2), 2.60 (t, 2 H, CH_2), 2.57 (t, 2 H, CH_2), 2.30 (s, 6 H, CH_3).

2-[[2-(N,N-Diethylamino)ethyl]thio]-4'-chloroacetophenone: isolated as a brown oil in 94% yield; ^1H NMR ($CDCl_3$) δ 8.01 (d, 2 H, aryl), 7.48 (d, 2 H, aryl), 3.80 (s, 2 H, CH_2), 2.68 (s, 4 H, CH_2), 2.53 (q, 4 H, CH_2), 1.00 (t, 6 H, CH_3).

2-[[2-(N,N-Dimethylamino)ethyl]thio]acetonaphthone: isolated as a white solid in 100% yield; ^1H NMR (CCl_4) δ 8.00 (m, 7 H, aryl), 3.80 (s, 2 H, CH_2), 2.60 (m, 4 H, CH_2), 2.20 (s, 6 H, CH_3).

2-[[2-(N,N-Diethylamino)ethyl]thio]-4'-fluoroacetophenone: isolated as a pale yellow solid in 45% yield; ^1H NMR ($CDCl_3$) δ 8.13 (q, 2 H, aryl), 7.22 (t, 2 H, aryl), 3.92 (s, 2 H, CH_2), 2.78 (s, 4 H, CH_2), 2.50 (q, 4 H, CH_2), 1.42 (t, 6 H, CH_3).

2-[[2-(N,N-Diisopropylamino)ethyl]thio]-4'-chloroacetophenone: isolated as a brown oil in 21% yield; ^1H NMR ($CDCl_3$) δ 8.00 (d, 2 H, aryl), 7.50 (d, 2 H, aryl), 3.81 (s, 2 H, CH_2), 3.04 (m, 2 H, CH), 2.66 (s, 4 H, CH_2), 1.01 (d, 6 H, CH_3).

General Procedure for Preparing S-(N,N-Dialkylamino)alkyl α-Keto Thiohydroximates (1). Sodium hydride (1.1 equiv) was added to 150 mL of dry 2-methyl-2-propanol under argon and stirred until hydrogen evolution ceased, at which point the solution was clear. The alkoxide solution was cooled to 0 °C and 1 equiv of the appropriate thioacetophenone added dropwise followed immediately by 1 equiv of isopropyl nitrite. The reaction mixture was stirred for 1 h at 0 °C and monitored by TLC to ensure completion. The crude reaction mixture was treated with 1.1 equiv of acetic acid and concentrated. The crude concentrate was partitioned between ethyl acetate (300 mL) and brine. The organic layer was washed twice more with brine, dried (Na_2SO_4), concentrated in vacuo, and chromatographed with a 0–5% methanol/dichloromethane eluent to purify the target compound. The following test compounds were prepared by this procedure.

S-2-(N,N-Dimethylamino)ethyl (4'-Chlorobenzoyl)thioformohydroximate (1l). Recrystallization from methylene chloride/hexane yielded analytically pure **1l** as a white powder: mp 110–111 °C; ^1H NMR (Me_2SO-d_6) δ 11.03 (br s, 1 H, NOH), 8.08 (d, 2 H, aryl), 7.51 (d, 2 H, aryl), 3.26 (t, 2 H, CH_2), 2.58 (t, 2 H, CH_2), 2.21 (s, 6 H, CH_3). The hydrochloride salt of **1l** was prepared in the usual manner and exhibited the following properties: mp 149–150 °C; ^1H NMR (Me_2SO-d_6) δ 12.80 (br s, 1 H, NOH), 8.04 (d, 2 H, aryl), 7.60 (d, 2 H, aryl), 3.44 (s, 4 H, CH_2), 2.90 (s, 6 H, CH_3).

S-2-(N,N-Diethylamino)ethyl (4'-Chlorobenzoyl)thioformohydroximate (1m). Recrystallization from methylene chloride/hexane yielded analytically pure **1m** in 18% yield: mp 92–93 °C; ^1H NMR ($CDCl_3$) δ 11.40 (br s, 1 H, NOH), 7.96 (d, 2 H, aryl), 7.42 (d, 2 H, aryl), 3.03 (m, 2 H, CH_2), 2.70 (m, 2 H, CH_2), 2.51 (q, 4 H, CH_2), 0.95 (t, 6 H, CH_3). The hydrochloride salt was prepared and exhibited the following properties: mp 181–183 °C; ^1H NMR (Me_2SO-d_6) δ 12.87 (br s, 1 H, NOH), 8.08 (d, 2 H, aryl), 7.62 (d, 2 H, aryl), 3.47 (s, 4 H, CH_2), 3.22 (q, 4 H, CH_2), 1.32 (t, 6 H, CH_3).

S-2-(N,N-Dimethylamino)ethyl (4'-Chlorobenzoyl)thioformohydroximate Methchloride (1n). Compound **1n** was prepared by quaternization of **1l** with methyl iodide followed by Cl^- ion exchange with Amberlite 400: mp 196–197 °C; ^1H NMR (Me_2SO-d_6) δ 8.03 (d, 2 H, aryl), 7.71 (d, 2 H, aryl), 3.58 (br s, 4 H, CH_2), 3.12 (s, 9 H, CH_3).

S-2-(N,N-Diethylamino)ethyl (4'-Chlorobenzoyl)thioformohydroximate Methchloride (1o). Compound **1o** was prepared by quaternization of **1m** with methyl iodide followed by ion exchange with Amberlite 400, Cl^- form: mp 168–170 °C; ^1H NMR (Me_2SO-d_6) δ 12.90 (s, 1 H, NOH), 8.10 (d, 2 H, aryl), 7.63 (d, 2 H, aryl), 3.7–3.3 (m, 8 H, CH_2), 3.14 (s, 3 H, CH_3), 1.42 (t, 6 H, CH_3).

S-2-(N,N-Dimethylamino)ethyl 2-Naphthoylthioformohydroximate (1p). Recrystallization from ethyl acetate yielded analytically pure **1p** as pale yellow cubes: mp 139–141 °C; ^1H NMR ($CDCl_3$) δ 8.80 (s, 1 H, NOH), 7.80 (m, 7 H, aryl), 3.20 (t, 2 H, CH_2), 2.51 (t, 2 H, CH_2), 2.10 (s, 6 H, CH_3).

S-2-(N,N-Dimethylamino)ethyl (4'-Fluorobenzoyl)thioformohydroximate (1q). Recrystallization from methanol/ether mixture yielded analytically pure **1q** as white plates: mp 135–136 °C; ^1H NMR (Me_2SO-d_6) δ 8.13 (dd, 2 H, aryl), 7.49 (t, 2 H, aryl), 3.06 (t, 2 H, CH_2), 2.47 (t, 2 H, CH_2), 1.97 (s, 6 H, CH_3).

S-2-(N,N-Diethylamino)ethyl (4'-Fluorobenzoyl)thioformohydroximate (1r). Recrystallization from an ether/hexane mixture yielded analytically pure **1r** as a white powder: mp 130–131 °C; ^1H NMR ($CDCl_3$) δ 10.90 (br s, 1 H, NOH), 8.08 (dd, 2 H, aryl), 7.10 (t, 2 H, aryl), 3.35–3.03 (m, 2 H, CH_2), 2.90–2.60 (m, 2 H, CH_2), 2.56 (q, 4 H, CH_2), 0.97 (t, 6 H, CH_3).

S-2-(N,N-Dimethylamino)ethyl (4'-Fluorobenzoyl)thioformohydroximate Methchloride (1s). Compound **1s** was prepared by quaternization of **1q** with methyl iodide followed by ion exchange with Amberlite 400, Cl^- form: mp 193–194 °C; ^1H NMR (Me_2SO-d_6) δ 8.10 (dd, 2 H, aryl), 7.47 (t, 2 H, aryl), 3.57 (m, 4 H, CH_2), 3.17 (s, 9 H, CH_3).

S-2-(N,N-Diethylamino)ethyl (4'-Fluorobenzoyl)thioformohydroximate Methchloride (1t). Compound **1t** was prepared by quaternization of **1r** with methyl iodide followed by ion exchange with Amberlite 400, Cl^- form: mp 152–153 °C; ^1H NMR (Me_2SO-d_6) δ 13.12 (br s, 1 H, NOH), 8.12 (dd, 2 H, aryl), 7.50 (t, 2 H, aryl), 3.47 (br s, 4 H, CH_2), 3.40 (q, 4 H, CH_3), 2.98 (s, 3 H, CH_3), 1.17 (t, 6 H, CH_3).

S-2-(N,N-Diisopropylamino)ethyl (4'-Chlorobenzoyl)-thioformohydroximate Hydrochloride (1y): recrystallized from MeOH/Et_2O as white crystals; mp 168–169 °C; ^1H NMR (Me_2SO-d_6) δ 10.30 (s, 1 H, NOH), 8.00 (d, 2 H, aryl), 7.67 (d, 2 H, aryl), 4.00–3.17 (m, 6 H, CH_2 and CH), 1.28 (d, 6 H, CH_3).

Physical Measurements. Reactivator pK_a values were determined spectrophotometrically in 0.1 M phosphate buffer by the method of Albert and Sergeant.[27] Octanol/buffer partition coefficients were determined spectrometrically by the method of Fujita et al.[33] The aqueous phase for all log P determinations

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was pH 7.4, 0.1 M phosphate buffer. Competitive inhibition of human AChE as well as reactivation of AChE after inhibition with GD and EPMP were performed as described previously.[22-24]

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Registry No. **1a**, 102941-46-8; **1a**·HCl, 102941-27-5; **1b**, 102941-47-9; **1b**·HCl, 90507-14-5; **1c**, 102976-77-2; **1c**·HCl, 99481-60-4; **1d**, 99481-58-0; **1e**, 99481-61-5; **1e**·oxalate, 99481-62-6; **1f**, 102941-28-6; **1g**, 102941-29-7; **1h**, 99481-59-1; **1i**, 102976-75-0; **1j**, 102976-76-1; **1k**, 102941-30-0; **1k**·oxalate, 102941-31-1; **1l**, 102941-48-0; **1l**·HCl, 102941-32-2; **1m**, 102941-49-1; **1m**·HCl, 102941-33-3; **1n**, 102941-34-4; **1o**, 102941-35-5; **1p**, 102941-50-4; **1p**·HCl, 102941-36-6; **1q**, 102941-37-7; **1r**, 102941-38-8; **1s**, 102941-39-9; **1t**, 102941-40-2; **1u**, 102941-51-5; **1u**·HCl, 102941-41-3; **1v**, 102941-42-4; **1w**, 102941-52-6; **1w**·HCl, 102941-43-5; **1x**, 102941-44-6; **1y**, 102941-55-9; **1y**·HCl, 102941-45-7; $C_6H_5COCH_3$, 98-86-2; $4\text{-}BrC_6H_4COCH_3$, 99-90-1; $4\text{-}H_3COC_6H_4COCH_3$, 100-06-1; $4\text{-}H_3CC_6H_4COCH_3$, 122-00-9; $4\text{-}NCC_6H_4COCH_3$, 1443-80-7; $C_6H_5COCl{=}NOH$, 4937-87-5; $4\text{-}BrC_6H_4COCl{=}NOH$, 7733-43-9; $4\text{-}H_3COC_6H_4COCl{=}NOH$, 33108-93-9; $4\text{-}H_3CC_6H_4COCl{=}NOH$, 33108-89-3; $4\text{-}NCC_6H_4COCl{=}NOH$, 102941-53-7; $(C_2H_5)_2N(CH_2)_2SH$·HCl, 1942-52-5; $(CH_3)_2N(CH_2)_2SH$·HCl, 13242-44-9; $(CH_3)_2N(CH_2)_3SH$·HCl, 55778-17-1; $4\text{-}ClC_6H_4COCH_3$, 99-91-2; $4\text{-}FC_6H_4COCH_3$, 403-42-9; $4\text{-}(2\text{-}C_{10}H_7)C_6H_4COCH_3$, 93-08-3; $(C_2H_5)_2N(CH_2)_2SH$, 100-38-9; $(CH_3)_2N(CH_2)_2SH$, 108-02-1; $((CH_3)_2CH)_2N(CH_2)_2SH$, 5842-07-9; $4\text{-}ClC_6H_4COCH_2Br$, 536-38-9; $4\text{-}FC_6H_4COCH_2Br$, 403-29-2; $4\text{-}(2\text{-}C_{10}H_7)C_6H_4COCH_2Br$, 102941-54-8; $4\text{-}(CH_3)_2N(CH_2)_2SCH_2COC_6H_4Cl$, 102941-56-0; $4\text{-}(C_2H_5)_2N(CH_2)_2SCH_2COC_6H_4Cl$, 102941-57-1; $(CH_3)_2N(CH_2)_2SCH_2COC_{10}H_7$, 102941-58-2; $4\text{-}(C_2H_5)_2N(CH_2)_2SCH_2COC_6H_4F$, 102941-59-3; $4\text{-}((CH_3)_2CH)_2N(CH_2)_2SCH_2COC_6H_4Cl$, 102976-78-3; $4\text{-}(CH_3)_2N(CH_2)_2SCH_2COC_6H_4F$, 102941-60-6; acetylcholine esterase, 9000-81-1.

Long-Acting Dihydropyridine Calcium Antagonists. 1. 2-Alkoxymethyl Derivatives Incorporating Basic Substituents

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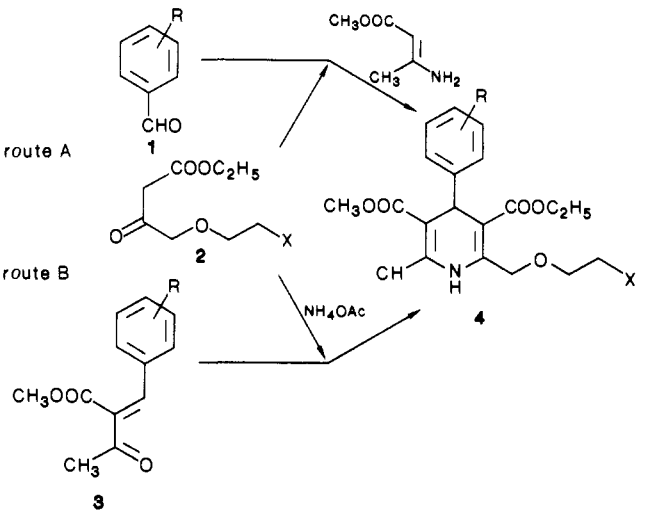
A series of dihydropyridines substituted at the 2-position by basic side chains are described and their potencies as calcium antagonists listed. One compound, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (**17**, amlodipine) was found to be comparable in potency to nifedipine and to have an elimination half-life of 30 h in dogs. Oral bioavailability approached 100%, and hemodynamic responses were gradual in onset and long-lasting in effect. The two enantiomers have been prepared, and the bulk of the activity was found to reside with the (-) isomer, **18**. X-ray crystallographic studies, carried out on a close analogue of **17**, suggest the existence of a weak hydrogen bond between the side-chain oxygen and the proton on the ring nitrogen.

Because of their vasodilator properties calcium channel blockers are important drugs in the treatment of angina[1] and hypertension.[2] Thus, in the heart, coronary dilatation increases the supply of oxygen and nutrients and coronary vasospasm may be prevented. Peripheral vasodilatation lowers the oxygen demand of the heart via a reduction in cardiac work and also accounts for the antihypertensive properties of these drugs.

Three structurally distinct compounds, nifedipine, diltiazem, and verapamil, have led the way toward defining the overall biological profile of this new class of drugs. Our research program on calcium blockers focused on the 1,4-dihydropyridines (DHPs) typified by nifedipine, since this chemical class offered both high potency and scope for wide structural variation. The aim of our work was to produce a drug comparable in overall pharmacological profile to nifedipine, but with superior bioavailability and, most importantly, with a duration of action allowing a once-a-day dosage regimen in man.

In commencing our program we noted that nearly all the DHP drugs then under investigation were essentially neutral molecules with low aqueous solubilities. The exception was nicardipine,[3,4] which differed from the other DHPs in possessing a basic side chain attached to the ester moiety at the 3-position of the DHP ring. However, despite improved aqueous solubility and good absorption, the systemic availability of nicardipine after oral administration was low due to a marked first-pass effect in the liver,[5] as appears to be common for the DHP series.

Scheme I



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