Approaches to Protection against Nerve Agent Poisoning. (Naphthylvinyl)pyridine Derivatives as Potential Antidotes[1]

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Analogues of the potent inhibitor of choline acetyltransferase (CAT) (*E*)-4-(1-naphthylvinyl)pyridine methiodide were synthesized and evaluated for their ability to inhibit CAT and protect against nerve agent intoxication. Several compounds, notably (*E*)-1-(2-hydroxyethyl)-(1-naphthylvinyl)pyridinium bromide (**3**), (*E*)-1-methyl-4-(1-naphthylvinyl)-1,2,3,6-tetrahydropyridine hydrochloride (**22**), and (*E*)-1-methyl-4-(1-naphthylvinyl)piperidine hydrochloride (**23**), were found to afford significant protection against sarin in the mouse and against soman in the guinea pig. However, protection was apparently not related to CAT inhibition. Compound **23**, our most effective compound in protecting against nerve agent, was without CAT inhibitory activity. Compound **22**, which proved to be a potent CAT inhibitor, most likely owed this activity to being dehydrogenated back to the pyridinium quaternary salt by oxidative enzymes. Several of the (naphthylvinyl)pyridine quaternary salts, but not their tertiary amine analogues, were found to be effective in slowing the rate of aging of soman-inhibited acetylcholinesterase. Ability to slow the rate of aging was enhanced by introduction of methoxy substituents on the aryl moiety whereas the aging rate was actually accelerated by chloro substituents. To date, our most effective compound in slowing the rate of aging, (*E*)-4-[(4-methoxy-1-naphthyl)vinyl]pyridine methochloride (**6**), did not provide significant protection against soman in the mouse.

In continuing our investigations[2] directed toward potential antidotes to organophosphorus nerve agents, we have been focusing particularly on possible approaches to compounds able to protect against soman (GD, 3,3-dimethyl-2-butyl methylphosphonofluoridate) where "aging" of inhibited acetylcholinesterase (AChE) prevents reactivation of the enzyme.[2,3] This paper reports on two approaches we have been exploring. One has involved inhibitors of choline acetyltransferase (CAT), specifically compounds related to the (naphthylvinyl)pyridine quaternary salts studied by Cavallito and associates,[4-6] the idea being that inhibition of the synthesis of acetylcholine (ACh) could counter the elevation of ACh levels caused by soman and thereby ameliorate the effects of the nerve agent. The second approach has involved compounds with the ability to slow the rate of aging, with the view that slowing the rate of aging would give a reactivator like 2-(hydroximinomethyl)-1-methylpyridinium chloride (pralidoxime chloride, 2-PAM) more time to dephosphonylate the enzyme.

Some work dealing with the potential utility of CAT inhibition for protecting against nerve agent poisoning had been reported previously.[3,7,8] The hydrochloride salt of 4-(1-naphthylvinyl)pyridine, a relatively weak CAT inhibitor that would be largely in the free-base form at physiological pH and thus able to penetrate into the CNS,[6] was found to provide significant protection against soman.[7] On the other hand, quaternary pyridinium salt analogues, which were far more potent inhibitors of CAT in vitro but would have difficulty passing through the blood–brain barrier, were reported to be ineffective.[7]

Our original objective was to design potent CAT inhibitors that would be able to pass freely into the CNS. To this end, we investigated tertiary amine analogues of the (naphthylvinyl)pyridine quaternary salts, analogues that would be more basic than (naphthylvinyl)pyridine itself and thus exist primarily in cationic form at physiological pH. Although it would not have been predicted on the basis of hypothesized structure–activity requirements,[6] (*E*)-1-methyl-4-(1-naphthylvinyl)-1,2,3,6-tetrahydropyridine hydrochloride (**22**) proved to be a potent CAT inhibitor. The corresponding piperidine, (*E*)-1-methyl-4-(1-naphthylvinyl)piperidine hydrochloride (**23**), did not inhibit CAT; however, both compounds as well as (*E*)-1-(2-hydroxyethyl)-4-(1-naphthylvinyl)pyridinium bromide[10] (**3**) proved to be effective against 2-propyl methylphosphonofluoridate (sarin) in the mouse and against soman in the guinea pig. In agreement with the conclusions of others,[7,8] our results indicate that the protective efficacy is not related to CAT inhibition.

Our interest in the potential utility of compounds able to slow the rate of aging stemmed from our previously reported finding of two compounds, (2-pyridylethyl)- and (4-pyridylethyl)diethylmethylammonium iodide, having the ability to effect a significant slowing of the aging rate in vitro.[2] Intriguingly, many of the (arylvinyl)pyridine quaternary salts, but not tertiary amine analogues, were discovered to be appreciably more effective in slowing the rate of aging. To date, our most effective compound in this series is (*E*)-4-[(4-methoxynaphthyl)vinyl]pyridine methochloride (**6**).

Chemistry

Compounds prepared are shown in Tables I–III. (Arylvinyl)pyridine quaternary salts (Table I) were synthesized as described earlier[4,11] by base-catalyzed con-

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(1) A portion of this work was presented at the FASEB meeting, Washington, DC, April 1987. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1987**, *46*, 859.
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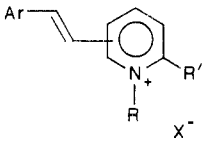
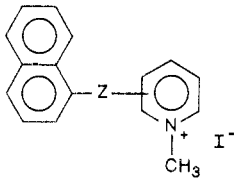
Table I. (Arylvinyl)pyridinium Salts



no.	isomer	Ar	R	R′	X	mol formula	mp, °C
1[a]	4	phenyl	CH_3	H	I	$C_{14}H_{14}IN$	208–210
2[b]	4	1-naphthyl	CH_3	H	Cl	$C_{18}H_{16}ClN$	252–253
3[c]	4	1-naphthyl	CH_2CH_2OH	H	Br	$C_{19}H_{18}BrNO$	246–248
4	4	1-naphthyl	$(CH_2)_5CH_3$	H	Br	$C_{23}H_{26}BrN$	199–200
5[d]	2	1-naphthyl	CH_3	H	Cl	$C_{18}H_{16}ClN\cdot H_2O$	268–270
6	4	4-methoxy-1-naphthyl	CH_3	H	Cl	$C_{19}H_{18}ClNO\cdot H_2O$	247
7	4	2-methoxy-1-naphthyl	CH_3	H	Cl	$C_{19}H_{18}ClNO\cdot{}^1/_2H_2O$	114–116
8	2	4-methoxy-1-naphthyl	CH_3	H	Cl	$C_{19}H_{18}ClNO$	246
9	4	4-methoxy-1-naphthyl	CH_2CH_2OH	H	Br	$C_{20}H_{20}BrNO_2$	247
10	4	4-(benzyloxy)phenyl	CH_3	H	Cl	$C_{21}H_{20}ClNO\cdot{}^1/_2H_2O$	204–206
11	4	9-anthryl	CH_3	H	I	$C_{22}H_{18}IN$	252–255
12[e]	4	9-phenanthryl	CH_3	H	I	$C_{22}H_{18}IN$	295–298
13[f]	4	1-naphthyl	CH_3	NH_2	I	$C_{18}H_{17}IN_2$	290–291
14	4	1-naphthyl	CH_3	NHAc	Cl	$C_{20}H_{19}ClN_2O$	217–218
15	4	1-naphthyl	OH	H	Cl	$C_{17}H_{14}ClNO$	231–232
16	4	3,4-dimethoxyphenyl	CH_3	H	Cl	$C_{16}H_{18}ClNO_2\cdot H_2O$	224–225
17[g]	4	3,4-dichlorophenyl	CH_3	H	Cl	$C_{14}H_{12}Cl_3N$	260

[a] Previously prepared.[4,5] [b] Prepared from the iodide salt.[4,5] [c] Literature[10] reported mp 252–255 °C. [d] Prepared from the iodide salt.[6] [e] Literature[6] reported mp 300–305 °C. [f] Literature[24] reported mp 278–281 °C. [g] Prepared from the iodide salt.[10]

Table II. Naphthyl-Substituted Pyridinium Salts



no.	isomer	Z	mol formula	mp, °C
18[a]	4	C≡C	$C_{18}H_{14}IN$	255–257
19[b]	4	CH_2CH_2	$C_{18}H_{18}IN$	200–201
20	4	NHC(=O)NH	$C_{17}H_{16}IN_3O$	233–234
21	3	NHC(=O)NH	$C_{17}H_{16}IN_3O$	237–238

[a] Literature[6] reported mp 248–251 °C. [b] Literature[24] reported mp 193–195 °C for this compound prepared by another method.

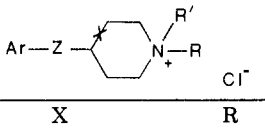
densation of the appropriate 2- or 4-picolinium salt with an arenecarboxaldehyde. (*E*)-4-(1-Naphthylvinyl)-2-(acetylamino)pyridine methochloride (**14**) was prepared by acetylation of the pyridonimine base derived from the 2-aminopyridine salt (**13**). (*E*)-4-(1-Naphthylvinyl)-pyridinium *N*-oxide hydrochloride (**15**) was prepared by the base-catalyzed condensation of 4-picoline *N*-oxide with 1-naphthaldehyde. A strong base (sodium methoxide) was required for this condensation whereas corresponding condensations with picoline quaternary salts were effected in good yield with a secondary amine such as piperidine or diethylamine as catalyst.

An ethynyl analogue of our (arylvinyl)pyridinium salts, 4-(1-naphthylethynyl)pyridine methiodide (**18**, Table II), was synthesized essentially as described by Cavallito et al.,[11] via the bromination and dehydrobromination of 4-(1-naphthylvinyl)pyridine. The corresponding compound with the exocyclic double bond saturated, 4-(1-naphthylethyl)pyridine methiodide (**19**), was prepared via the hydrogenation of the (naphthylvinyl)pyridine over a palladium catalyst. The compounds with the ureyl linking group (**20** and **21**) were prepared via reaction of 1,1′-carbonyldiimidazole successively with an aminopyridine and 1-naphthylamine (for **21**) or via reaction of the aminopyridine with 1-naphthyl isocyanate (for **20**).

The (naphthylvinyl)-1,2,3,6-tetrahydropyridine derivatives in Table III (**22**, **25**, and **27**), as well as 1-methyl-4-phenyltetrahydropyridine (MPTP, **28**), were prepared by sodium borohydride reduction of the corresponding pyridine quaternary salts. NMR spectral data, particularly the vinyl proton coupling constants, indicated retention of the trans configuration for the exocyclic double bond. The saturated (naphthylethyl)piperidine analogues **24** and **26** were obtained by platinum-catalyzed hydrogenation of the quaternary salts.

After considerable experimentation, the (naphthylvinyl)piperidine derivative **23** was finally prepared in two steps as follows. Under carefully controlled conditions at below −70 °C, methyl 1-methylisonipecotate was treated with 1 equiv of diisobutylaluminum hydride (DIBAL-H)

Table III. Tetrahydropyridine and Piperidine Salts



no.	Ar	Z[a]	X	R	R′	mol formula	mp, °C
22	1-naphthyl	CH=CH	double bond	CH_3	H	$C_{18}H_{20}ClN$	262–264
23	1-naphthyl	CH=CH	single bond	CH_3	H	$C_{18}H_{22}ClN\cdot{}^1/_2H_2O$	195–197
24	1-naphthyl	CH_2CH_2	single bond	CH_3	H	$C_{18}H_{24}ClN$	187–189
25	1-naphthyl	CH=CH	double bond	$(CH_2)_5CH_3$	H	$C_{23}H_{30}ClN\cdot{}^1/_2H_2O$	178–180
26	1-naphthyl	CH_2CH_2	single bond	CH_2CH_2OH	H	$C_{19}H_{26}ClNO$	169–171
27	4-methoxynaphthyl	CH=CH	double bond	CH_3	H	$C_{19}H_{22}ClNO$	254–256
28[b]	phenyl	single bond	double bond	CH_3	H	$C_{12}H_{16}ClN$	254
29	1-naphthyl	CH=CH	double bond	CH_3	CH_3	$C_{19}H_{22}ClN\cdot{}^1/_2H_2O$	262

[a] When Z represents a double bond, it is trans. [b] MPTP; Aldrich catalog gives mp 254–257 °C.

Table IV. In Vitro Effects of Compounds

no.	enzyme inhibitory activity: choline acetyltransferase: I_{50}, μM	enzyme inhibitory activity: acetylcholinesterase: I_{50}, μM	AChE/CAT	effect on rate of aging,[a] ratio control/treated[b]: 0.1 mM	1.0 mM
1	12.00	1200	100.0		2.6
2	0.34	120	352.9	1.4	5.2
3	3.00	170	56.7	1.0	3.0
4	4.20	32	7.6	1.2	
5	2.20	64	29.1	1.5	
6	360	69	0.2	3.4	
7	120	135	1.1	1.9	
8	118	33	0.3	1.3	
9	90	143	1.6	1.6	
10	1500	52	0.0		
11	800	120	0.2	1.6	
12	3000	20	0.0		0.8
13	5.30	32	6.0		1.9
14	3.70	900	243.2		1.4
15	1390	>2500	>1.8	0.7	
16	140	260	1.8	1.6	
17	4.5	24	5.3	0.5	
18	1.1	43	39.0	0.9	3.1
19	168	217	1.3	1.0	
20	800	230	0.3	1.1	
21	800	320	0.4		
22	4.3	480	112.0	0.9	1.0
23	2000	480	0.2	1.0	0.8
24	2500	890	0.4		1.1
25	1000	1000	1.0	1.1	
26	1400	1260	0.9		1.0
28	1600	1390	0.9		
29	>1000	190	<0.19	1.0	

[a] Effect on rate of aging of soman-inhibited AChE. [b] Ratios of first-order rate constants. Rate constant determined by slope of regression line (time vs percent reactivation). Aging rate in absence of test compound determined for each assay run. Average control rate K_a = (5.6 ± 0.8) × 10^{-2} min^{-1}. Assays were performed in duplicate or triplicate; the standard deviation never exceeded 10% of the mean.

in hexane to yield 1-methylpiperidinecarboxaldehyde. A Wittig reaction of this with (1-naphthylmethyl)triphenylphosphonium chloride and sodium methoxide gave **23**. With sodium methoxide as the condensing agent, the pure trans isomer of **23** was obtained; use of *n*-butyllithium in place of sodium methoxide afforded a product contaminated by about 12% of the isomer with the exocyclic double bond in the cis configuration.

Results

Biochemistry. The effects of our compounds in vitro on the activities of the enzymes CAT and AChE and their effect on the rate of aging of soman-inhibited AChE are shown in Table IV. In agreement with the findings of Cavallito et al.,[6] most of the (naphthylvinyl)pyridinium salts were potent CAT inhibitors, (*E*)-4-(1-naphthylvinyl)pyridine methochloride (**2**), with a CAT I_{50} concentration of 0.34 μM, being the most potent. Compound **2** also had the highest ratio of AChE/CAT I_{50} values, making it the most selective CAT inhibitor in our experience. The next most selective CAT inhibitor was the 2-acetamidopyridinium salt **14**. The *N*-oxide analogue **15** was essentially without activity. Compound **2** appeared to have an optimal balance of lipophilic to hydrophilic properties for CAT inhibitory activity, with less lipophilic compounds, e.g., the phenyl analogue **1**, and more lipophilic compounds, e.g., **4**, **6**, and **12**, being less potent to virtually inactive. On the other hand, increased lipophilicity tended to enhance AChE inhibitory activity.

As noted by Cavallito,[6] a replacement of the vinylene linking group by ethynylene (**16**) was not deleterious to CAT inhibitory activity whereas replacement by the saturated ethylene group (**17**) or a ureylene group (**18**, **19**) caused a profound reduction in activity.

Of particular interest was the high CAT inhibitory potency of (*E*)-1-methyl-4-(1-naphthylvinyl)-1,2,3,6-tetrahydropyridine hydrochloride (**20**). With a CAT I_{50} value of 4.3 μM and an AChE/CAT I_{50} ratio of 112, this tertiary amine salt was comparable to our pyridine quaternary salts in CAT inhibitory potency and more selective than most. The activity of **20** would not have been predicted from earlier advanced suggestions[6] as to structural requirements for activity. It does, however, appear to fit with the recent report of the potent CAT inhibitory activity of certain (arylvinyl)oxazine and -oxazoline hydrochloride salts.[12]

A more lipophilic (naphthylvinyl)tetrahydropyridine (**23**), with *n*-hexyl in place of methyl as the N-substituent, showed markedly reduced CAT inhibitory activity, as did MPTP (**26**), a compound with a phenyl ring attached directly to the tetrahydropyridine nucleus. Owing to its apparent instability in solution, the enzyme inhibitory ability of compound **27** (the 4-methoxynaphthyl analogue of **20**) could not be determined. It is difficult to account for the instability of **25** since its IR and NMR spectra as well as microanalytical data all accord with the assigned structure.

Saturation of the remaining ring double bond to give the piperidine derivatives **21**, **22**, and **24** resulted in loss of CAT inhibitory activity. Particularly noteworthy is the lack of activity of the direct analogue of **20**, (*E*)-1-methyl-4-(1-naphthylvinyl)piperidine (**21**). Thus, inhibitory activity appears to require retention of at least one double bond in the nitrogen-containing ring.

Most of the (naphthylvinyl)pyridine quaternary salts, but not their tertiary amine analogues, showed the ability to slow the rate of aging of soman-inhibited AChE in vitro. As was to be expected, the compounds were more effective at a concentration of 1 mM than at 0.1 mM. Some of the more lipophilic pyridine quaternary salts, i.e., **4**, **10**, 11, and **12**, caused hemolysis at 1 mM, and meaningful results

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Table V. Effect of Other Compounds on Nerve Agent Lethality

no.	ip dose, μm/kg	treatment[a]	species	protective ratio (95% confidence interval)[b] sarin	soman
		AS + 2-PAM[c]	mouse	1.2 (1.18–1.27)	1.4 (1.28–1.44)
5	50.0	AS + 2-PAM	mouse	1.7* (1.34–2.17)	ND
6	54.5	AS + 2-PAM	mouse	1.9* (1.59–2.15)	1.4 (1.25–1.60)
9	51.8	AS + 2-PAM	mouse	1.4* (1.27–1.59)	ND
15	387	AS + 2-PAM	mouse	1.4* (1.27–1.46)	ND
		AS + 2-PAM + PY[d]	guinea pig	ND	7.6 (6.59–8.81)
2	92	AS + 2-PAM + PY	guinea pig	ND	6.5 (4.37–9.70)
24	131	AS + 2-PAM + PY	guinea pig	ND	8.6 (5.67–13.07)
26	94	AS + 2-PAM + PY	guinea pig	ND	14.2* (9.07–22.1)

[a] AS = atropine sulfate, 2-PAM = pralidoxime chloride, PY = pyridostigmine bromide. [b] Protective ratios were determined by the quotient of the LD_{50} of the treated groups over that of nerve agent alone. ND = not determined. [c] AS 3.0 μm/kg, 2-PAM 57.8 μm/kg. [d] AS 24 μm/kg, 2-PAM 144 μm/kg, PY 0.5 μm/kg. * $p < 0.05$ on comparison with AS + 2-PAM + PY treated groups by Litchfield and Wilcoxon t test.[21]

could only be obtained at 0.1 mM. The most effective compound in this series, and the most effective compound of which we are aware, was (*E*)-4-[(4-methoxy-1-naphthyl)vinyl]pyridine methochloride (**6**), which slowed the rate of aging by a factor of 3.4 at 0.1 mM. Compound **7**, the 2-pyridyl isomer of **6**, was less active.

Our interest in compounds with a ureylene linking group (**18**, **19**) stemmed from the work of Schoene,[13] who tested a series of mostly bispyridinium salts for their effect on the rate of aging and reported the most potent, slowing the rate of aging by a factor of 5 at 2 mM, to be 1,3-bis-(3-pyridyl)urea dimethiodide. However, a urea analogue of our compounds, 1-(1-naphthyl)-3-(4-pyridyl)urea methiodide (**18**), had no significant effect at 0.1 mM. In fact, in our hands, Schoene's compound had no effect at 0.1 mM.

Protective Efficacy of 3, 22, and 23. Initial studies focused on determining the effectiveness of CAT inhibitors as potential prophylactics against nerve agent toxicity. As has been noted, compound **2** was the most potent CAT inhibitor of those evaluated and also had the highest AChE/CAT ratio. Preliminary in vivo testing of **2** was done (Table V), but the compound was abandoned in favor of the hydroxyethyl derivative, compound **3**, which was more water soluble. Compounds **22** and **23** were selected for comparison with **3** because (1) as tertiary amine analogues of **3**, they would be better able to reach the CNS and (2) they would aid in the evaluation of the relevance of CAT inhibition to protective efficacy since **22** had CAT inhibitory potency comparable to that of **3** and a higher AChE I_{50}/CAT I_{50} ratio whereas **23** was devoid of CAT inhibitory activity.

Efficacy against Soman and Sarin (GB, Isopropyl Methylphosphonofluoridate) Poisoning in Mice. The results of experiments with compounds **3**, **22**, and **23** are shown in Figure 1. The left side of the figure depicts the levels of protection seen against soman lethality when the test compounds were administered as pretreatments with the support of a standard treatment regimen of atropine sulfate (AS) and 2-PAM. The LD_{50} of soman alone was 89 (83–96) μg/kg. Treatment with AS + 2-PAM increased the LD_{50} to 121 (114–128) μg/kg corresponding to a protective ratio (PR) of 1.36. None of the test compounds provided any additional protection to that seen with AS + 2-PAM alone.

The right side of Figure 1 shows the effectiveness of these treatments against sarin lethality. The LD_{50} of sarin alone was 164 (155–174) μg/kg and was increased to 201 (193–209) μg/kg, a PR of 1.22, by treatment with AS + 2-PAM. When compounds **3** and **23** were added to the standard treatment regimen, the LD_{50} of sarin was significantly increased to 273 (250–298) and 285 (257–316) μg/kg, respectively, corresponding to PRs of 1.66 and 1.74. Compound **22** did not improve the protection afforded by AS + 2-PAM treatment alone.

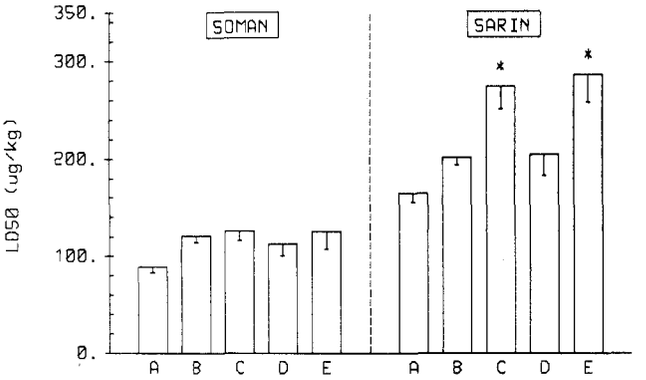

Figure 1. The effects of **3**, **22**, and **23** on the LD_{50} of soman and sarin in mice. Mice were treated with atropine sulfate (AS) (3.0 μm/kg) and pralidoxime chloride (2-PAM) (57.8 μm/kg), im, 10 min before nerve agent. The test compounds were administered ip 10 min before nerve agent at the following doses: **3** (140 μm/kg), **22** (157 μm/kg), **23** (118 μm/kg). Both soman and sarin were administered im. Data shown are the mean 24-h LD_{50} values of soman and sarin with and without treatment. The error bars represent the lower portion of the 95% confidence interval. (*) $p < 0.05$ t test comparison to group B (Ltichfield and Wilcoxon[21]).

Efficacy against Soman Poisoning in Guinea Pigs. Compounds **3**, **22**, and **23** were all found to be efficacious against soman lethality in guinea pigs, but only in the presence of pyridostigmine (PY). The left side of Figure 2 depicts the levels of protection seen when the compounds were given as pretreatments with the support of AS, 2-PAM, and PY. The LD_{50} of soman alone was 27 (22–33) μg/kg. Pretreatment with AS + 2-PAM + PY increased the LD_{50} to 206 (178–238) (PR = 7.63) μg/kg. Addition of compounds **3**, **22**, and **23** significantly enhanced the LD_{50} to values of 382 (307–476) (PR = 14), 522 (312–872) (PR = 19), and 854 (546–1332) (PR = 31) μg/kg, respectively. Compounds **3** and **22** were not significantly different from each other, but compound **23** provided significantly more protection than compound **3**. In the absence of PY, neither compound **3** nor **23** were found to be more efficacious than AS + 2-PAM alone (Figure 2).

Protective Efficacy of Selected Additional Compounds. Data on the protective efficacy of several additional (naphthylvinyl)pyridine derivatives are presented in Table V, expressed in terms of protective ratio. The (2-naphthylvinyl)pyridine derivative **5** and the 4-[(4-

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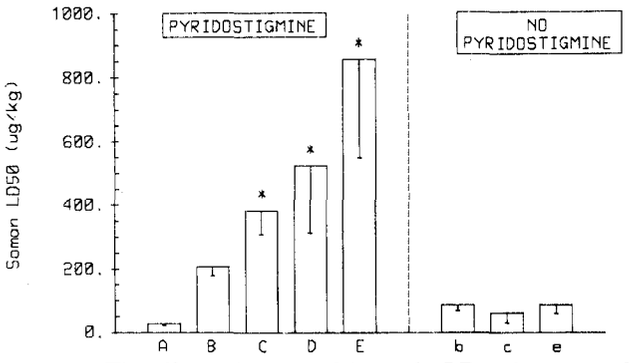

Figure 2. The effects of **3**, **22**, and **23** on the LD_{50} of soman and sarin in the guinea pig. Guinea pigs were treated with atropine sulfate (AS) (24 μm/kg) and pralidoxime chloride (2-PAM) (144 μm/kg), im, 10 min before soman. Pyridostigmine bromide (PY) (0.5 μm/kg) was given, where indicated, im 30 min before soman. The test compounds were administered ip, 30 min before soman, at the following doses: **3** (98 μm/kg), **22** (157 μm/kg), **23** (118 μm/kg). Soman was administered sc. Data shown are the mean 24-h LD_{50} values of soman with and without treatment. The error bars represent the lower portion of the 95% confidence interval. (*) $p < 0.05$ *t* test comparison to group B (or b) (Litchfield and Wilcoxon[21]).

methoxynaphthyl)vinyl]pyridine **6** proved to be comparable to **3** and **23** in protecting against sarin in the mouse; **6** was ineffectie against soman in the mouse. Of the compounds tested against soman in the guinea pig, only *N*-(hydroxyethyl)-4-(naphthylethyl)piperidine hydrochloride (**26**) showed any activity, with a protective ratio comparable to that of **3**.

Discussion

The unprecedented CAT inhibitory potency of the tetrahydropyridine derivative **22** appears at first glance to be in line with the recently reported activity of certain (arylvinyl)oxazine and -oxazoline hydrochlorides.[12] It is, however, most likely that **22** owes its ability to inhibit CAT to a process not open to those latter compounds, namely rearomatization of the heterocyclic ring by oxidative enzymes to re-form an aromatic heterocyclic quaternary ion. Aromatization of the tetrahydropyridine ring of **22** would give the potent CAT inhibitor **2**, the former thus serving as a depot form of **2**. Such an aromatization has been demonstrated with the tetrahydropyridine MPTP (**28**),[14] but not with its ring-saturated piperidine analogue.[15] In this connection, it is noteworthy that **23**, the piperidine analogue of **22**, is virutally devoid of activity. Strong support for this explanation of the CAT inhibitory potency of **22** is provided by the observed lack of activity of its methiodide **29**. Conversion of **29** to **2** would require enzymatic N-demethylation as well as aromatization of the tetrahydropyridine ring.

The pathway(s) through which **3** and **23** act to protect against sarin in the mouse and **3**, **22**, and **23** act to protect against soman in the guinea pig require(s) clarification, and investigation is continuing. Their protective efficacies cannot be ascribed to inhibition of CAT. As already noted, **23**, the most effective of the three, was devoid of CAT inhibitory activity. Also, the protective ratios against sarin in the mouse shown by the compounds in Table V do not correlate with CAT inhibitory activity. Further, although CAT, but not AChE, activity in the rat brain could be markedly reduced in vivo by intracerebroventricular administration of **3** or by ip administration of **22**, this reduction was not accompanied by a corresponding reduction in brain ACh levels or by a countering of the elevation of those levels elicted by nerve agent.[1,9] The compounds also show no anticholinergic activity[1] and therefore are not acting as atropine surrogates.

The fact that none of the compounds protected against soman in the mouse is in agreement with other studies in which it has been shown that it is much more difficult to protect mice than guinea pigs against soman toxicity.[16] Our finding that **3**, **22**, and **23** afford protection against soman in the guinea pig only in the presence of pyridostigmine (PY) is in agreement with the work of Inns and Leadbeater[17] who found certain of the bispyridinium derivative studied by Schoene and associates[13,18,19] to be able to protect the guinea pig only when PY was added to the treatment regimen. Neither they nor we can explain this phenomenon.

We have previously discussed the aging process and how compounds may act to retard it.[2,3,22] In the (arylvinyl)-pyridinium series, replacing *N*-methyl by hydroxyethyl reduces the ability to slow the rate of aging (**3** vs **2** and **9** vs **6**) as does replacing the vinylene link by ethynylene (**18**) or ethylene (**19**). It is particularly intriguing that introduction of electropositive (electron-releasing) methoxy substituents on the aryl moiety (**6**, **7**, **16**) markedly increased the ability to slow the aging rate whereas introduction of electronegative (electron-withdrawing) chloro substituents (**17**) had an opposite effect, with **17** actually causing a marked acceleration of the rate. The greatest retarding effect on the aging rate was achieved with a methoxy substituent in the 4-position of the naphthalene ring and the naphthylvinyl moiety attached at the 4-pyridyl position as in **6**. Although by far our best compound in slowing the rate of aging, **6** did not show a significant ability to aid 2-PAM in protecting against soman in the mouse. It may be that compounds with greater potency in slowing the rate of aging will be needed. We are investigating the possibility that this may be achieved by introducing substituents with greater electron-releasing ability.

Compound **6** did prove to have significant efficacy against sarin in the mouse; this, however, cannot be ascribed to its ability to reduce the aging rate since sarin-inhibited AChE does not age at a rate sufficient to interfere with reactivation by 2-PAM. Further, **6** is a relatively weak AChE inhibitor (Table IV) so its efficacy cannot be ascribed to reversible inhibition of AChE.

Experimental Section

Chemistry. Reagents and solvents were used as obtained commercially without purification. Temperatures were reported in degrees Celcius. Silica gel GF (Analtech) plates scored 10 × 20, 250 μm, were used for analytical thin-layer chromatography (TLC). Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton (^1H)

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nuclear magnetic resonance (NMR) spectra were determined in deuteriated methanol (CD_3OD), in deuteriated chloroform ($CDCl_3$), or in deuteriated dimethyl sulfoxide (DMSO-d_6), containing tetramethylsilane (TMS) with a Varian 220 NMR spectrometer. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. For multiplets either the center or the range of the multiplet are reported. Coupling constants (J) are reported in hertz (Hz). Infrared spectra (IR) were determined with KBr disks with a Perkin-Elmer 1320 infrared spectrophotometer or Beckman IR-4230 infrared spectrophotometer. The spectra are reported in cm^{-1}. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. All samples were analyzed for C, H, and N; results were acceptable within 0.4% of calculated.

(*E*)-4-[(4-Methoxy-1-naphthyl)vinyl]pyridine Methiodide and Methochloride (6). The iodide and bromide salts 1–13 listed in Table I were prepared by the general method illustrated here, condensation of the appropriate aldehyde with the appropriate picolinium salts (cf. ref 4, 11). Conversion of iodide salts to the corresponding chlorides was achieved by ion exchange.

A solution of 4-methoxy-1-naphthaldehyde (0.05 mol, 9.3 g) in 100 mL of methanol was treated with 4-picoline methiodide (0.05 mol, 11.75 g) and 1 mL of piperidine as catalyst. The mixture was stirred at 50 °C for 5 days. The orange precipitate was collected by filtration to give 17.1 g (85%) of 4-[(4-methoxy-1-naphthyl)vinyl]pyridine methiodide: mp 258–260 °C dec; NMR (CD_3OD–DMSO-d_6) δ 4.09 (s, 3 H), 4.30 (s, 3 H), 7.09 (d, 1 H), 7.41 (d, vinyl, 1 H), 7.57 (t, 1 H), 7.68 (t, 1 H), 8.09 (d, 1 H), 8.20–8.34 (m, 3 H), 8.42 (d, 1 H), 8.64–8.73 (m, 3 H); J(vinyl protons) = 15 Hz (trans form). Anal. ($C_{19}H_{18}NOI$) C, H, N.

A suspension of the iodide salt (17.1 g) in 900 mL of methanol was stirred at ambient temperature with 80 g of Amberlyst A-21 ion-exchange resin, chloride form, until the methanol solution gave a negative iodine test (aliquot diluted with water and treated with 1% ammonium cerium(IV) nitrate). The mixture was filtered through a bed of Celite and evaporated to dryness. The resulting residue was recrystallized from methanol to give 8.6 g (65%) of **6** as an orange solid: mp 247 °C dec; NMR (CD_3OD) same as the iodide.

(*E*)-4-(1-Naphthylvinyl)-2-(acetylamino)pyridine Methochloride (14). An aqueous solution of 4-(1-naphthylvinyl)-2-aminopyridine methiodide (**13**) (1.16 g, 3 mmol) was treated with excess 10% aqueous sodium hydroxide to precipitate the base as a yellow solid (yield quantitative): mp 232–235 °C dec; IR 3450, 3030, 2960, 1640, 1575, 1510, 1330, 1210, 1175, 1105, 1050, 980, 870, 840, 800, and 760 cm^{-1}; NMR ($CDCl_3$) δ 3.43 (s, 3 H), and 6.00–8.30 (m, 14 H). The solid was dissolved in acetic anhydride, and the solution was stirred at room temperature for 16 h. Dilution with hexanes and cooling at −20 °C gave 1-methyl-4-(1-naphthylvinyl)-2-(acetylimino)pyridine as needles: mp 178–180 °C; IR 3450, 3040, 3000, 2920, 1630, 1600, 1540, 1500, 1480, 1360, 1300, 1270, 1190, 1130, 1015, 960, 920, 850, 780, 765, 720, and 650 cm^{-1}; NMR ($CDCl_3$) δ 2.20 (s, 3 H), 3.63 (s, 3 H), 6.55–7.03 (dd, 2 H), and 7.30–8.20 (m, 10 H). An ether solution of the product was treated with ethereal hydrogen chloride. The precipitate was recrystallized from 2-propanol/ether to give 0.53 g (52%) of **14**: mp 217–218 °C; IR 3400, 3120, 3020, 2920, 2830, 1720, 1630, 1570, 1540, 1520, 1450, 1440, 1380, 1360, 1310, 1300, 1230, 1220, 1150, 1030, 960, 890, 830, 790, and 720 cm^{-1}; NMR (DMSO-d_6) δ 2.30 (s, 3 H), 4.18 (s, 3 H), and 7.33–8.93 (m, 13 H).

(*E*)-4-(1-Naphthylvinyl)pyridine *N*-Oxide Hydrochloride (15). A solution of 1-naphthaldehyde (0.02 mol, 3.1 g) in 8 mL of methanol was treated with 4-picoline *N*-oxide (0.02 mol, 2.2 g) and 2 mL of 25% sodium methoxide in methanol as catalyst. The mixture was stirred at reflux for 2 days. The reaction mixture was evaporated, and the residual semisolid was suspended in 20 mL of water. The resulting solid was collected by filtration and recrystallized from methanol to give 3 g (61%) of 4-(1-naphthylvinyl)pyridine *N*-oxide: mp 192–193 °C; NMR ($CDCl_3$) δ 7.03 (d, vinyl, 1 H), 7.45 (d, 2 H), 7.48–7.61 (m, 4 H), 7.75 (d, 1 H), 7.87 (d, 1 H), 7.97 (d, vinyl, 1 H), 8.16 (d, 1 H), 8.20 (d, 2 H), J(vinyl) = 16 Hz; IR 3060, 3040, 1480, 1450, 1250, 1170, 1030, 960, 810, and 780 cm^{-1}.

A suspension of 2.47 g (0.01 mol) of the *N*-oxide in 100 mL of methanol was treated with 2 mL of concentrated hydrochloric acid and heated on a steam bath until the solid had dissolved. The precipitate of yellow needles that formed as the solution cooled to room temperature was collected by filtration to give 2.0 g (70.5%) of **15**: mp 231–232 °C; IR 3110, 3050, 1610, 1500, 1450, 1370, 1360, 1300, 1210, 1200, 970, 860, 830, 790, and 770 cm^{-1}.

(*E*)-1-Methyl-4-(1-naphthylvinyl)-1,2,3,6-tetrahydropyridine Hydrochloride (22). To a solution of reflux of (naphthylvinyl)pyridine methiodide (0.01 mol, 3.73 g) in 250 mL of methanol was added, within 30 min, 3.78 g (0.1 mol) of sodium borohydride pellets. The reaction mixture was heated under reflux for 1 h and evaporated to dryness. The residue was dissolved in water (150 mL), and the solution was extracted with ether (4 × 50 mL). The ether solution was dried and evaporated. The residual syrup was crystallized from hexane to yield 1-methyl-4-(1-naphthylvinyl)-1,2,3,6-tetrahydropyridine as colorless needles (2.34 g, 94%): mp 59–60 °C; IR 3370, 2980, 2960, 2940, 2860, 2800, 2780, 1640, 1590, 1510, 1460, 1400, 1380, 1290, 1270, 1210, 1140, 1080, 980, 790, and 775 cm^{-1}; NMR ($CDCl_3$) δ 2.52 (s, 3 H), 2.77 (m, 4 H), 3.27 (m, 2 H), 5.80 (m, 1 H), 6.53–7.17 (m, 2 H), and 7.37–8.30 (m, 7 H).

The hydrochloride salt (**22**) was recrystallized from ethanol: mp 262–264 °C; NMR (CD_3OD) δ 2.93 (br, 2 H), 3.02 (s, 3 H), 3.70–4.14 (m, 4 H), 5.94 (br, 1 H), 6.95 (d, vinyl, 1 H), 7.48 (d, vinyl, 1 H), 7.48–7.59 (m, 3 H), 7.72 (d, 1 H), 7.84 (d, 1 H), 7.89 (d, 1 H), 8.20 (d, 1 H), J(vinyl protons) = 16 Hz (trans form); IR 3020, 2900, 2640, 2440, 2400, 1570, 1440, 1400, 1290, 1240, 1180, 1110, 1040, 990, 940, 850, and 780 cm^{-1}.

23, 25 and 26 were similarly prepared from the corresponding pyridinium quaternary salts.

(*E*)-1-Methyl-4-(1-naphthylvinyl)piperidine Hydrochloride (23). To a stirred solution of 100 g (0.76 mol) of isonipecotic acid in 210 mL of formic acid at ambient temperature was added, dropwise over 30 min, 135 mL of 37% formaldehyde solution. The solution was stirred for 16 h at reflux and evaporated to give a syrup. The syrup was dissolved in 50 mL of concentrated hydrochloric acid, and the solution was again evaporated to give a semisolid. The semisolid was washed with 300 mL of acetone to give 130.4 g (94%) of 1-methylisonipecotic acid hydrochloride as white crystals: mp 229–230 °C; IR 3460, 2960, 2600, 1720, 1465, 1400, 1365, 1300, 1270, 1250, 1205, 1180, 1160, 1040, 980, 955, 920, 875, 830, 720, and 630 cm^{-1}; NMR spectrum showed NCH_3 group at δ 2.87.

To a stirred solution of 1-methylisonipecotic acid hydrochloride (178.6 g, 1 mol) in 350 mL of methanol (8 equiv) was added, dropwise with stirring and cooling (ice–salt bath) to −10 °C, 113 mL (1.55 equiv) of thionyl chloride. After completion of the addition (1 h), the ice–salt bath was removed, and the temperature was allowed to rise to 40 °C and held at this point for 2 h. The solution was brought to about pH 8 with sodium carbonate and extracted with methylene chloride. The methylene chloride solution was dried and evaporated to give 136.9 g (87%) of methyl 1-methylisonipecotate as a clear liquid: NMR ($CDCl_3$) δ 1.64–2.05 (m, 6 H), 2.18–2.34 (m, 1 H), 2.25 (s, 3 H), 2.73–2.84 (m, 2 H), 3.66 (s, 3 H).

To a cold (below −70 °C) solution of methyl 1-methylisonipecotate (15.7 g, 0.1 mol) in 500 mL of hexane was added, dropwise over 1 h, 100 mL of 1 N diisobutylaluminum hydride (DIBAL-H) in hexane. The solution was stirred for 1.5 h, and 20 mL of saturated aqueous ammonium chloride was very slowly added. The reaction mixture was gradually allowed to come to ambient temperature, and stirring was continued for an additional hour. To the reaction mixture was added 10 mL of saturated aqueous sodium bicarbonate, and the organic layer was decanted. The aqueous layer was extracted with methylene chloride (200 mL × 3) and then with ether (200 mL × 3). The combined extracts were dried with magnesium sulfate and evaporated to give 10 g (79%) of crude 1-methyl-4-piperidinecarboxaldehyde as a pale yellow oil: IR (neat) 2940, 2850, 2790, 2740, 2680, 1725, 1440, 1450, 1380, 1280, 1145, 1090, 1070, 1040, 970, 930, and 760 cm^{-1}; NMR ($CDCl_3$) δ 1.85 (m, 5 H), 2.05 (m, 2 H), 2.22 (s, 3 H), 1.65 (m, 2 H), and 9.67 (s, 1 H).

A mixture of 5.3 g (0.03 mol) of 1-(chloromethyl)naphthalene and 8.65 g (0.033 mol) of triphenylphosphine in 150 mL of dimethylformamide was heated at reflux with stirring for 5 h. A copious white precipitate formed. The precipitate was filtered and washed with 100 mL of dimethylformamide and 100 mL of

ether. The product was dried in vacuo to yield 11.97 g (91%) of (1-naphthylmethyl)triphenylphosphonium chloride as white crystals: mp 285–288 °C; IR 3050, 3010, 2880, 2790, 1665, 1590, 1510, 1485, 1440, 1385, 1335, 1275, 1155, 1110, 1095, 875, 810, 785, 730, and 690 cm^{-1}; NMR (DMSO) δ 5.67 (d, J = 15 Hz, 2 H), 7.60 (m, 15 H), and 7.73 (m, 7 H).

To a mixture of 10 g of crude 1-methylpiperidinecarboxaldehyde and 34.5 g (0.079 mol) of (1-naphthylmethyl)triphenylphosphonium chloride in 300 mL of ether was added, under nitrogen, 22.8 mL (0.1 mol) of 25% sodium methoxide solution in methanol. The orange mixture was maintained in a nitrogen atmosphere and stirred for 3 days at ambient temperature. The mixture was evaporated, and the residue was extracted with 800 mL of hexane. The extract was washed with water (100 mL), dried with magnesium sulfate, and evaporated to give 1-methyl-4-(1-naphthylvinyl)piperidine as a pale yellow semisolid: IR 3060, 3040, 3000, 2940, 2850, 2780, 2740, 2680, 1920 (w), 1800 (w), 1725 (w), 1640 (w), 1590, 1510, 1465, 1445, 1380, 1280, 1200, 1140, 1120, 1070, 970, 850, 780, 720, and 700 cm^{-1}; NMR ($CDCl_3$) δ 1.70 (m, 5 H), 1.33 (m, 2 H), 2.20 (s, 3 H), 2.68 (m, 2 H), 6.13 (dd, J = 16 Hz, 2 H), and 7.40 (m, 7 H).

The hydrochloride salt (**23**), recrystallized from ethanol–ether, was obtained in 28% overall yield (from methyl 1-methylisonipecotate): mp 195–197 °C; NMR (CD_3OD) δ 1.75–1.91 (m, 2 H), 2.09–2.30 (m, 2 H), 2.55–2.70 (br, 1 H), 2.91 (s, 3 H), 3.00–3.20 (m, 2 H), 3.48–3.66 (m, 2 H), 6.20 (dd, 1 H, J = 7 Hz and 16 Hz), 7.28 (d, 1 H, J = 16 Hz), 7.36–7.61 (m, 4 H), 7.73–7.89 (m, 2 H), 8.07–8.16 (m, 1 H). IR 2950, 2680, 2550, 1740, 1460, 1410, 1250, 1040, 950, and 780 cm^{-1}. The NMR spectrum indicated the product to be the trans isomer.

1-Methyl-4-(1-naphthylethyl)piperidine Hydrochloride (24). To a solution of **2** (1.965 g, 7 mmol) in 100 mL of ethanol contained in a 250-mL round-bottomed flask was added 0.23 g of platinum oxide (Adams catalyst). The flask was connected to a volumetric hydrogenation apparatus incorporating a 100-mL gas buret, and the system was repeatedly evaucated and filled with hydrogen, and finally filled with hydrogen. The reaction mixture was magnetically stirred at room temperature and atmospheric pressure for 2 h during which 684 mL (4 equiv) of hydrogen was absorbed. The catalyst was filtered off, and the filtrate was evaporated to dryness. The residue was crystallized from ethanol to give **24** as white needles (1.86 g, 92%): mp 187–189 °C; IR 3040, 3000, 2940, 2860, 2630, 2500, 1600, 1505, 1460, 1430, 1400, 1250, 1050, and 980 cm^{-1}.

4-(1-Naphthylethyl)pyridine Methiodide (19). A mixture of 9.3 g (0.1 mol) of 4-picoline, 15.6 g (0.1 mol) of 1-naphthaldehyde, and 50 mL of acetic anhydride was heated at reflux for 3 days. The mixture was poured into 100 mL of 2 N hydrochloric acid, and the resultant solution was washed with 100 mL of ether. The aqueous layer was made basic with 50% sodium hydroxide and extracted with 500 mL of ethyl acetate. The extract was dried over sodium sulfate and passed through a short silica gel column. The eluate was evaporated to give 10 g of yellowish brown syrup.

A solution of the syrup in 150 mL of methanol containing 0.5 g of 5% Pd/C was shaken overnight under 25–30 psi of hydrogen. The filtered solution was evaporated. The NMR spectrum of the crude reduction product in $CDCl_3$ showed no vinyl protons: δ 3.09 (t, 2 H, J = 9 Hz), 3.39 (t, 2 H, J = 9 Hz), 7.11–7.18 (m, 2 H), 7.30–7.39 (m, 1 H), 7.41–7.57 (m, 3 H), 7.68–8.02 (m, 3 H), 8.45–8.50 (m, 2 H).

The methiodide (**19**), recrystallized from methanol, was obtained in 5% overall yield: mp 200–201 °C (lit.[18] mp 193–195 °C); IR 3040, 1640, 1600, 1570, 1510, 1470, 1400, 1340, 1300, 1270, 1230, 1190, 1020, 820, 800, and 780 cm^{-1}.

1-Naphth-1-yl-3-pyridin-4-ylurea Methiodide (20). A solution of 4-aminopyridine (0.055 mol, 5.2 g) in 30 mL of dry methylene chloride was stirred with 8.45 g (0.05 mol) of 1-naphthyl isocyanate at ambient temperature for 2 days. Addition of 100 mL of hexane gave a white precipitate (13 g, 98%).

The methiodide (**20**), recrystallized from methanol, was obtained in 42% yield: mp 233–234 °C; IR 3250, 3150, 3080, 2980, 1740, 1680, 1600, 1520, 1400, 1340, 1290, 1250, 1190, 1000, 840, 800, and 780 cm^{-1}.

1-Naphth-1-yl-3-pyridin-3-ylurea Methiodide (21). A solution of 3-aminopyridine (0.05 mol, 4.7 g) in 30 mL of dry tetrahydrofuran was stirred with 8.1 g (0.05 mol) of 1,1′-carbonyldiimidazole at 50 °C for 1.5 h. To the reaction mixture was added 7.2 g (0.05 mol) of α-naphthylamine. The solution was stirred overnight at ambient temperature and gave a white precipitate (13 g, 98%).

The methiodide (**21**), recrystallized from methanol, was obtained in 62% yield: mp 237–238 °C; NMR (CD_3OD–DMSO-d_6) δ 4.64 (s, 3 H), 7.48–7.64 (m, 3 H), 7.73–8.14 (m, 4 H), 8.30–8.39 (m, 1 H), 8.45–8.52 (m, 1 H), 9.34 (s, 1 H); IR 3080, 1710, 1600, 1550, 1500, 1400, 1340, 1290, 1260, 1200, and 770 cm^{-1}.

Biochemistry. Materials. Dilute (2 mg/mL) solutions of soman and sarin in normal saline were obtained from the Army (USAMRICD). Atropine sulfate (AS), pralidoxime chloride (2-PAM), and the reagents used in the enzyme assays were obtained commercially. Pyridostigmine bromide (PY) was obtained as a gift from Hoffman-La Roche, Inc. Male Sprague–Dawley rats (180–220 g) and male Swiss mice (25–30 g) were obtained from Charles River Laboratories. Male Hartley guinea pigs (280–320 g) were obtained from Hilltop Lab Animals, Inc.

Choline Acetyltransferase. CAT activity was measured by the method of Fonnum.[19] [1-^{14}C]Acetyl coenzyme A, diluted with unlabeled compound, served as the acetyl donor and choline chloride was used as the acceptor. The reaction takes place in the presence of rat brain homogenate during a 15-min incubation at 37 °C in a neutral pH buffer. Labeled acetylcholine is isolated by liquid cation exchange with sodium tetraphenylboron in heptanone and quantified by liquid scintillation counting. Acetyl-CoA remains in the aqueous phase and is not counted.

Acetylcholinesterase Assay. AChE activity was measured by using the radiochemical method of Siakotos et al.[20] Enzymatic hydrolysis of the labeled acetylcholine substrate (acetyl[1-^{14}C]-choline iodide) takes place in the presence of rat erythrocyte lysate during a 30-min incubation at 37 °C. The reaction is stopped by adding 5 mL of a mixture of dioxane and Amberlite CG-120. Unhydrolyzed acetylcholine is adsorbed on the Amberlite resin and removed by centrifugation; the dioxane supernatant contains the radioactive acetate hydrolysis product, which is measured by liquid scintillation spectrometry.

Rate of Aging Determinations. The effect of each compound on the rate of aging of soman-inhibited red blood cell acetylcholinesterase was determined by the method previously described in detail.[2] AChE is phosphonylated by soman and undergoes rapid dealkylation (aging). The aged fraction of the inhibited enzyme cannot be reactivated by conventional oximes such as 2-PAM. The inability of 2-PAM to reactivate the dealkylated phosphonyl-AChE can be used as a measure of the fraction of aged enzyme and thus as a way of following the time course of the aging reaction.

LD_{50} Determinations. All reported LD_{50} determinations (Figure 1 and 2 and Table V) were done at least in triplicate. All animals were fasted for 16 h with free access to water prior to testing. AS and 2-PAM were mixed together in saline and administered as a single im injection, at a volume of 1 mL/kg, 10 min prior to nerve agent exposure. Treated animals received either a high dose of 24 μm/kg AS with 144 μm/kg 2-PAM or a low dose of 3.0 μm/kg AS with 58 μm/kg 2-PAM as indicated. In experiments with guinea pigs, a separate im injection of PY (0.5 μm/kg) given 30 min before soman was included in the pretreatment regimen. The test compounds were given ip in 2 mL/kg of freshly prepared solution in saline or 10% dimethyl sulfoxide (DMSO) in saline 30 min prior to nerve agent in the guinea pig and 10 min prior to nerve agent in the mouse. The dose of each compound was equivalent to $^1/_4$ the respective LD_{50} in each species. The nerve agents were diluted with saline and administered im in the mouse and sc in the guinea pig.

The general procedure was as follows. Animals were divided into three treatment groups. The first group received nerve agent alone. The second group was pretreated with AS and 2-PAM and, where appropriate, PY prior to receiving nerve agent. The third group received the same pretreatment as the second with the addition of the test compound. Each of the treatment groups was further divided into four or more subgroups. Each subgroup received four or more progressively larger doses of sarin or soman so that the expected LD_{50} would fall in the middle of the range. The number of animals in each subgroup was 10 for mice and six for guinea pigs. Animals were observed for 24 h after treat-

ment. The LD_{50} values, 95% confidence interval, and a test of significance ($p \leq 0.05$) were calculated by the method of Litchfield and Wilcoxon.[21] A protective ratio was determined as the quotient of the LD_{50} value of the treated groups over the LD_{50} of the nerve agent alone.

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Chemistry and Positive Inotropic Effect of Pelrinone and Related Derivatives. A Novel Class of 2-Methylpyrimidones as Inotropic Agents

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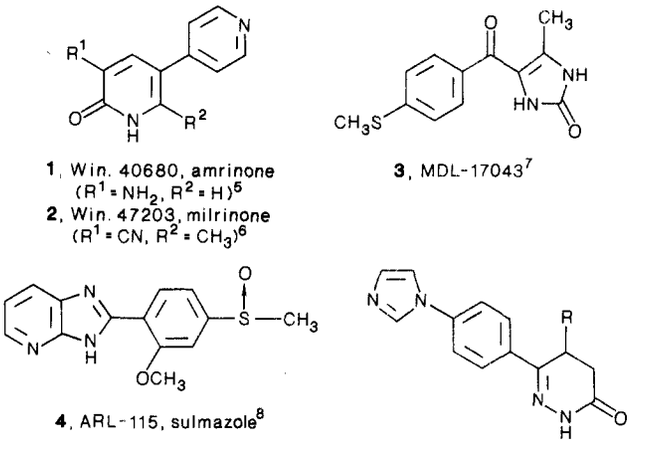
A novel series of pyrimidine derivatives was synthesized and evaluated for positive inotropic activity. Inotropic and chronotropic effects were determined in vitro in cat papillary muscle and right atrium, respectively. Selected compounds were then evaluated in vivo in a dog heart failure model. Changes in ventricular dP/dt, heart rate, and blood pressure were monitored. Several of these agents produced relatively minor changes in heart rate. This class of agents demonstrated a varying degree of vasodilator effects concomitant with increases in ventricular contractility. The most potent analogues, **9**, **48**, and **49**, were evaluated orally in conscious dogs with implanted Konisberg pressure transducers, and their effect on left ventricular dP/dt was compared with that of milrinone. Mechanistically, the agents of this novel class appear not to mediate their effect either via β-receptors or inhibition of Na^+/K^+-ATPase. A major component of their inotropic effect is mediated by the inhibition of cardiac phosphodiesterase (PDE)-Fr. III. This was clearly demonstrated by **9**, **48**, and **49**. Compound **48** was found to be the most potent inhibitor of PDE-Fr. III from among the compounds tested in this assay.

There are several classes of compounds known to exert positive inotropic effect. Of these, there are two that have found utility in the treatment of congestive heart failure in humans, namely, the cardiac glycosides and the catecholamines, both of which suffer from serious disadvantages. The cardiac glycosides, in spite of their long-standing reputation in therapy, have a very narrow therapeutic ratio, are potentially arrhythmogenic, and can cause digitalis intoxication.[1] Serious limitations of the catecholamines[2] include the potential to cause tachycardia concomitant with an increase in contractility, which can lead to cardiac arrhythmia and increased myocardial oxygen consumption. Furthermore, the catacholamines have a short duration of action and often cannot be administered orally.[3]

In recent years, attention has been directed toward the development of orally active, nonsteroidal, noncatechol cardiotonic agents. An ideal agent of this kind would exert direct positive inotropic effect on the heart, without increasing heart rate or myocardial oxygen consumption and without causing vasodilation in the capacitance vessels; moreover, it would possess a high toxic to therapeutic ratio. The attention of several groups has been directed toward generating agents that modulate intracellular levels of cyclic nucleotides. This has led to a variety of phosphodiesterase inhibitors,[4] some of which are shown in Chart I.

The pyrimidine moiety represents an integral part of a number of phosphodiesterase inhibitors.[4] We report herein on some novel derivatives of pyrimidine, identified as potent, positive inotropic agents, which also possess potent phosphodiesterase inhibitory activity.

Chart I



Chemistry. Appropriately functionalized ketene acetals have been reported[10] to yield a variety of heterocyclic

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