Hemicholinium-3 Congeners as Potential Antagonists to Organophosphate-Induced Toxicity

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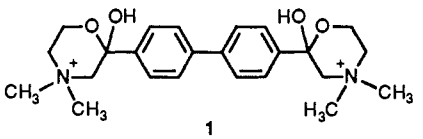
A series of congeners of hemicholinium-3, in which the 1,4-oxazinium rings of hemicholinium are replaced by pyrrolidine, piperidine, 1,3-dioxane, or 1,4-oxazine rings, is described. Several of the target compounds produced blockade of neuromuscular transmission in the rabbit, and three heterocyclic derivatives, **10**, **11**, and **13**, significantly antagonized paraoxon-induced lethality in mice. 1,3-Dioxane derivative **11** was an extremely potent antagonist of paraoxon-induced toxicity in mice, compared with prototypical protective agents physostigmine and pyridostigmine. Compound **11** exhibited a much more favorable therapeutic ratio than the reference drugs. The mechanism of action of **11** has not been elucidated, although it is concluded that it differs from that of hemicholinium-3 (inhibition of high-affinity, sodium-dependent uptake of choline into nerve terminals).

Prior papers[1,2] described potent hemicholinium-like activity of some congeners of hemicholinium-3 (**1**) containing 4-methylpiperidine rings (e.g., **2a**) or noncyclic diethyl acetal moieties (e.g., **2b**) instead of oxazinium rings as in hemicholinium itself. Most of these compounds



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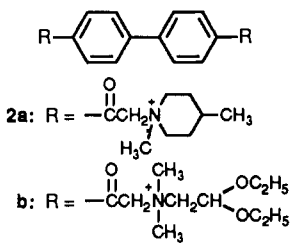
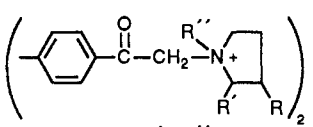


2a: R = —CCH$_2$N(CH$_3$)(H$_3$C)

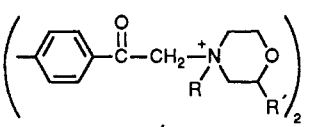
b: R = —CCH$_2$N(CH$_3$)CH$_2$CH(OC$_2$H$_5$)$_2$

exhibited some degree of hemicholinium-3-like pharmacologic effects: inhibition of high-affinity, sodium-dependent uptake of choline into nerve terminals with resulting lowering of acetylcholine levels in nerve terminals.

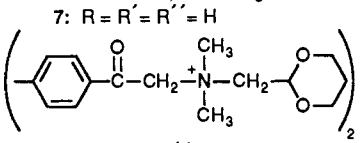
We have speculated that this type of pharmacologic action might provide some degree of protective effect against systemic effects of organophosphate intoxicants such as the agricultural insecticide paraoxon (**3**). The present paper describes extension of the series of hemicholinium-3 congeners to include some pyrrolidine systems (**4–7**); 1,4-oxazine systems (**8–10**); a 1,3-dioxane system (**11**),



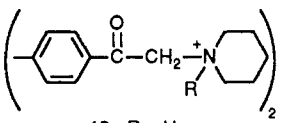
4: R = H; R′ = R″ = CH$_3$
5: R = R″ = CH$_3$; R′ = H
6: R = R′ = H; R″ = CH$_3$
7: R = R′ = R″ = H



8: R = R′ = H
9: R = CH$_3$; R′ = H
10: R = R′ = CH$_3$



11



12: R = H
13: R = CH$_3$

Table I. Inhibition of Rabbit Neuromuscular Transmission

no.		remarks
4	0.057 (0.04–0.08)[a]	
5	0.021 (0.02–0.03)[a]	
6	0.07 (0.03–0.3)[a]	potentiates AcCh
7	inactive to 5.6	
8	inactive to 2.08	
9	0.58 (0.26–2.5)	
10	inactive to 0.16[b]	potentiates AcCh
11	0.34 (0.14–4.8)[a]	tears, salivation, potentiates AcCh
12	inactive to 1.8	
13	0.11 (0.03–0.4)[a]	tears, potentiates AcCh

[a] Reversed by choline chloride, 5 mg/kg. [b] Gradually decreased blood pressure, which probably decreased stimulation responses.

a cyclic acetal congener of **2b**; and some piperidine derivatives (**12**, **13**) and presents pharmacologic data on their protective effects against paraoxon. Target compounds **7**, **8**, and **12** are tertiary amines rather than quaternary ammonium systems which have been addressed in past studies.

Chemistry. Target compounds were prepared by standard procedures from 4,4′-bis(bromoacetyl)biphenyl and the appropriate amine. 2-(Dimethylamino)methyl-1,3-dioxane (**14**) was conveniently prepared in high yield in a one-step synthesis by acid-catalyzed transetherification of (*N*,*N*-dimethylamino)acetaldehyde diethyl acetal with propane-1,3-diol. Spectral (IR, NMR, MS) data on all intermediates and final compounds were consistent with the proposed structures.

Pharmacology. Derivatives of hemicholinium-3 may antagonize organophosphate-induced toxicity by at least three mechanisms, including the following: (1) inhibition of choline transport, thus suppressing synthesis of acetylcholine and decreasing acetylcholine concentration within the synapse; (2) binding in the region of the esteratic site on acetylcholinesterase, resulting in diminished inhibition of the enzyme; or (3) alteration of postjunctional membranes and the ionic channels, resulting in antagonism of acetylcholine and/or the organophosphate. Table I shows the activity of the subject compounds, evaluated for their ability to inhibit neuromuscular transmission. Compounds **4** and **6** are potent inhibitors of neuromuscular transmission, and since choline is an effective antagonist, the mechanism of their action probably involves inhibition of choline transport, similar to that of hemicholinium-3.

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(1) Part I: Cannon, J. G.; Lee, T. M.-L.; Chang, Y.-a.; Nyanda, A. M.; Bhattacharyya, B.; Flynn, J. R.; Chatterjee, T.; Bhatnagar, R. K.; Long, J. P. *Pharm. Res.* **1988**, *5*, 359.
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Table II. Toxicity and Antagonistic Activity against Paraoxon in Mice

compd	LD_{50}, μmol/kg	% of mice surviving after injection of a fraction of LD_{50} dose											
		0.5 h[a]								2.0 h[a]			
		1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/4	1/8	1/16	1/32
4	0.67	0								0			
5	0.84	25											
6	0.74	0								0			
7	743.5	0								0			
8	no death to 2079 μmol/kg[b]												
9	3.8	25								12			
10	13.0	63	88	0	0					0			
11	7.3	63	100	100	88	100	100	63	25	88	100	38	25
12	397.5	37								0			
13	1.6	75	50	0						0			

[a] Pretreatment time. [b] Not tested for protective action against paraoxon.

Table III. Relative Activity of Three Potent Antagonists of Paraoxon-Induced Toxicity in Mice

compound	LD_{50}, μmol/kg	protection vs paraoxon: ED_{50}, μmol/kg	therapeutic index
physostigmine	1.2	0.02	60
pyridostigmine	10.3	1.3	8
11	7.3	0.02	360

Several of the agents (**6**, **9**, **10**, **11**, **13**) produced "cholinergic" responses, probably by inhibition of acetylcholinesterase.

Table II shows the LD_{50} values of the compounds and their ability to protect mice against paraoxon-induced lethality. Only compounds **10**, **11**, and **13** were capable of producing at least 50% survival. Compounds **10** and **13** were only moderately effective. Compound **11** is a very effective antagonist of paraoxon-induced toxicity, with an ED_{50} of 13 μg/kg.

Table III summarizes the activity of **11** and two reference compounds for protection against paraoxon-induced toxicity. Compound **11** shows dramatic antagonistic actions against paraoxon; however, its mechanism of antagonistic action is unknown. Studies on rabbits suggest that **11** is a weak inhibitor of choline transport, and it is possibly an inhibitor of acetylcholinesterase. Postjunctional activity of **11** has yet to be described. It is possible that optimal efficacy against organophosphate-induced toxicity will be realized with compounds exhibiting multimechanisms.

Experimental Section

Pharmacology. Methods. Inhibition of Sciatic Nerve—Gastrocnemius Muscle Preparation. Inhibition of neuromuscular transmission was determined as described by Benz and Long.[3] Dutch rabbits weighing 1.5–2.0 kg were anesthetized with 250 mg/kg of phenobarbital sodium administered iv. The trachea was isolated and respiration was supported by a Harvard respiration pump. The jugular vein was cannulated for iv administration of drugs. One of the sciatic nerves was isolated, sectioned centrally, and bipolar Ag electrodes were placed on the distal end of the sciatic nerve and were attached to a Grass S4C stimulator. The ankle was attached to a solid mount and the tendon of Achilles was isolated and sectioned. A 10-g resting tension was applied to the tendon and contractions were recorded with a Beckman R-611 recorder. The following parameters of stimulations were used: every 10 s interrupted tetanic stimulation was delivered for 0.2 s at 200 Hz. The pulse duration was 0.2 ms, and maximal voltage was applied (usually 20 V). Antagonistic properties of intravenous choline chloride (5 mg/kg) were evaluated.

Antagonism of Paraoxon-Induced Toxicity. Mice weighing 18–22 g were used to evaluate the protective efficacy of the compounds. First, the LD_{50} dose (im) of an experimental compound was determined, then fractional doses (varied by 0.3 log unit) of the LD_{50} were assayed. The assay for protective activity was conducted as follows: (1) the dose-ratio of the LD_{50} was administered im into the left hindlimb; (2) either 30 or 120 min later, a 3 × LD_{50} dose of paraoxon was administered sc, followed immediately by 11.2 mg/kg of atropine sulfate administered im into the right hindlimb. The mice were observed for 24 h. In this experimental procedure using saline solution, the dose of paraoxon was regarded as LD_{99}. With this experimental procedure, information was obtained concerning behavioral responses of experimental compounds alone and concerning interactive responses with paraoxon, duration of action, 50% protective dose (PD_{50}), and therapeutic index (TI). The TI was calculated by the formula LD_{50}/PD_{50}.

Statistics. Fifty percent response levels (PD_{50}, LD_{50}, ED_{50}) were calculated by probit analysis as outlined by Goldstein.[4] Relative potencies were calculated as described by Finney.[5]

Chemistry. Melting points were determined in open glass capillaries with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are indicated by the symbols of the elements, analytical results were within ±0.4% of the theoretical values. NMR spectra were recorded on a Varian Associates EM360A spectrometer and on a Bruker-IBM NR 80 instrument using Me_4Si as the internal standard. Mass spectra were recorded with a Ribermag R-10-10C mass spectrometer.

4,4′-Bis[(2-methylpyrrolidino)acetyl]biphenyl Dimethiobromide (4). 4,4′-Bis(bromoacetyl)biphenyl[6] (1.32 g, 0.0033 mol) and 0.99 g (0.01 mol) of 1,2-dimethylpyrrolidine[7] in 70 mL of THF and 30 mL of MeOH were heated under reflux until all of the insoluble material dissolved; soon after, a yellowish white solid began to separate from the refluxing solvent. At this point, volatiles were removed from the reaction mixture under reduced pressure, and the solid residue was recrystallized from MeOH–Me_2CO (1:2) to afford 1.6 g (81%) of a crystalline solid, mp 241 °C. Anal. ($C_{28}H_{38}Br_2N_2O_2$) C, H, N. Karl Fischer H_2O 0.96%.

4,4′-Bis[(3-methylpyrrolidino)acetyl]biphenyl Dimethiobromide (5). This was prepared in 86% yield with 1,3-dimethylpyrrolidine[8] by the method described for **4**, mp 245–246 °C. Anal. ($C_{28}H_{38}Br_2N_2O_2$) C, H, N. Karl Fischer H_2O 2.15%.

2-[(Dimethylamino)methyl]-1,3-dioxane (14). A mixture of 16.1 g (0.1 mol) of (*N*,*N*-dimethylamino)acetaldehyde diethyl acetal, 8.36 g (0.11 mol) of propane-1,3-diol, and 12 mL of concentrated HCl was subjected to slow distillation until the theoretical volume of EtOH was collected. The reaction mixture was neutralized with saturated Na_2CO_3 and then was extracted with three 30-mL portions of Et_2O. The pooled extracts were dried (Na_2SO_4) and filtered, and the volatiles were removed from the filtrate under reduced pressure. The liquid residue was distilled,

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bp 40 °C (2.5 mm) to afford 10.15 g (70%) of product. This was characterized as its HCl salt, mp 168–169 °C (from MeOH). Anal. ($C_7H_{16}ClNO_2$) C, H, N. Karl Fischer H_2O 0.70%.

4,4'-Bis[[(1,3-dioxan-2-ylmethyl)methylamino]acetyl]biphenyl Dimethiobromide (11). Compound 14 (1.45 g, 0.01 mol) and 1.32 g (0.0033 mol) of 4,4'-bis(bromoacetyl)biphenyl[6] were stirred at room temperature in 50 mL of MeCN–H_2O (4:1) until all of the insoluble material dissolved. Volatiles were then removed under reduced pressure and the solid residue was recrystallized from 2-PrOH–H_2O to yield 1.75 g (77%) of product, mp 289 °C dec. Anal. ($C_{30}H_{42}Br_2N_2O_8$) C, H, N. Karl Fischer H_2O 0.32%.

4,4'-Bis(2,3,5,6-tetrahydro-1,4-oxazin-4-ylacetyl)biphenyl Dihydrochloride (8). 4,4'-Bis(bromoacetyl)biphenyl[6] (1.32 g, 0.0033 mol) and 0.87 g (0.01 mol) of tetrahydro-1,4-oxazine were stirred in 50 mL of MeCN until all insoluble material dissolved. Volatiles were removed under reduced pressure, and the residue was taken up in 25 mL of H_2O and this solution was treated with excess Na_2CO_3. The resulting yellow solid was collected on a filter and air-dried. A solution in MeOH was treated with anhydrous HCl and the resulting solid was recrystallized from 2-PrOH–H_2O (1:1) to yield 1.4 g (83%) of a white solid, mp 289 °C dec. Anal. ($C_{24}H_{30}Cl_2N_2O_4$) C, H, N. Karl Fischer H_2O 9.37%.

4,4'-Bis(2,3,5,6-tetrahydro-1,4-oxazin-4-ylacetyl)biphenyl Dimethiobromide (9). 4,4'-Bisbromoacetylbiphenyl[6] (1.31 g, 0.0033 mol) and 1.01 g (0.01 mol) of 4-methyltetrahydro-1,4-oxazine were treated as described for 11. The product (1.7 g, 86%) was recrystallized from 2-PrOH-H_2O, mp 261 °C dec. Anal. ($C_{26}H_{34}Br_2N_2O_4$) C, H, N. Karl Fischer H_2O 0.72%.

4,4'-Bis[(2-methyl-2,3,5,6-tetrahydro-1,4-oxazine-4-yl)-acetyl]biphenyl Dimethiobromide (10). To an ice–H_2O-chilled solution of 10.1 g (0.1 mol) of 2-methyl-2,3,5,6-tetrahydro-1,4-oxazine[9] in 11 mL of 37% aqueous formaldehyde solution (0.135 mol) was added dropwise and with stirring 8.5 mL of 95% formic acid (0.25 mol). After addition was complete, the reaction mixture was heated under reflux for 24 h. It was then brought to room temperature and 15 mL of concentrated HCl was added. The resulting mixture was extracted with Et_2O, and the pH of the aqueous phase was adjusted to 12 (pH paper) with 20% NaOH. It was then extracted with 500 mL of Et_2O for 24 h in a liquid–liquid extractor. The ethereal extract was dried (Na_2SO_4) and the volatiles were removed under reduced pressure to afford 7.8 g (68%) of 2,4-dimethyl-2,3,5,6-tetrahydro-1,4-oxazine, which was used in the next step without further purification. A 1.5 g (0.01 mol) portion of this tertiary amine and 1.32 g (0.0033 mol) of 4,4'-bis(bromoacetyl)biphenyl[6] were stirred at room temperature in 50 mL of MeCN–H_2O (4:1) until all solid material dissolved (ca. 4 h). Volatiles were then removed under reduced pressure, and the solid residue was recrystallized from EtOH to yield 1.83 g (89%) of product, mp 217 °C dec. Anal. ($C_{28}H_{38}Br_2N_2O_4$) C, H, N.

4,4'-Bis(pyrrolidinoacetyl)biphenyl Dimethiobromide (6). *N*-Methylpyrrolidine (0.85 g, 0.01 mol) and 1.32 g (0.0033 mol) of 4,4'-bis(bromoacetyl)biphenyl[6] in 70 mL of THF and 30 mL of MeOH were treated as described for **4**. The crude product was recrystallized from EtOH to yield 1.56 g (84%) of material, mp 288–289 °C. Anal. ($C_{26}H_{34}Br_2N_2O_2$) C, H, N. Karl Fischer H_2O 0.35%.

4,4'-Bis(piperidinoacetyl)biphenyl Dihydrobromide (12). 4,4'-Bis(bromoacetyl)biphenyl[6] (1.32 g, 0.0033 mol) and 1.13 g (0.013 mol) of piperidine were stirred at room temperature in 70 mL of THF and 30 mL of MeOH until a clear solution resulted (ca. 30 min). Anhydrous HBr was passed through this solution until a solid separated. This material was collected on a filter and was recrystallized from EtOH to yield 1.52 g (82%) of product, mp 301 °C dec. Anal. ($C_{26}H_{34}Br_2N_2O_2$) C, H, N. Karl Fischer H_2O 0.30%.

4,4'-Bis(piperidinoacetyl)biphenyl Dimethiobromide (13). A mixture of 2.64 g (0.0067 mol) of 4,4'-bis(bromoacetyl)biphenyl[6] and 1.98 g (0.02 mol) of *N*-methylpiperidine in 70 mL of THF and 30 mL of MeOH was stirred at room temperature until all suspended solid material dissolved. Volatiles were then evaporated under reduced pressure, and the residue was recrystallized from EtOH to afford 3.6 g (90%) of product, mp 246 °C dec. Anal. ($C_{28}H_{38}Br_2N_2O_2$) C, H, N. Karl Fischer H_2O 2.94%.

4,4'-Bis(pyrrolidinoacetyl)biphenyl Dihydrobromide (7). A mixture of 1.32 g (0.0033 mol) of 4,4'-bis(bromoacetyl)biphenyl[6] and 0.52 g (0.0073 mol) of pyrrolidine in 70 mL of THF and 30 mL of MeOH was stirred at room temperature until all the solid material dissolved (ca. 0.5 h). Anhydrous HBr was bubbled through the reaction solution; the resulting mixure was cooled in an ice bath, and the white solid which separated was collected on a filter. This was crystallized twice from EtOH to afford 1.48 g (83%) of product, mp 306 °C dec. Anal. ($C_{24}H_{30}Br_2N_2O_2$) C, H, N. Karl Fischer H_2O 6.44%.

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Registry No. 1, 312-45-8; **3**, 311-45-5; **4**, 123489-62-3; **5**, 123489-63-4; **6**, 123489-64-5; **7**, 123489-65-6; **8**, 123489-66-7; **9**, 123489-67-8; **10**, 123489-68-9; 11, 123489-69-0; **12**, 123489-70-3; **13**, 15172-85-7; 14, 4740-66-3; $BrCH_2CO(p\text{-}C_6H_4)_2COCH_2Br$, 4072-67-7; 1,2-dimethylpyrrolidine, 765-48-0; 1,3-dimethylpyrrolidine, 45470-22-2; morpholine, 110-91-8; 4-methylmorpholine, 109-02-4; *N*-methylpyrrolidine, 120-94-5; piperidine, 110-89-4; *N*-methylpiperidine, 626-67-5; pyrrolidine, 123-75-1; 2,4-dimethylmorpholine, 59229-58-2; 2-methylmorpholine, 27550-90-9.

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