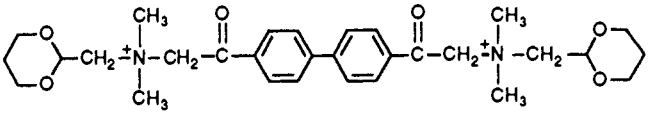
Structure–Activity Studies on a Potent Antagonist to Organophosphate-Induced Toxicity

Joseph G. Cannon,*,† M. Fethi Sahin,†,§ Ranbir K. Bhatnagar,‡ Jan R. Flynn,‡ and John Paul Long‡

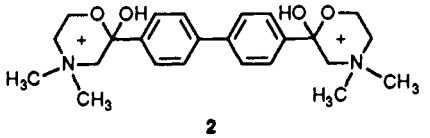
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, and Department of Pharmacology, College of Medicine, The University of Iowa, Iowa City, Iowa 52242. *Received October 12, 1990*

Molecular modifications have been made on a highly potent, active antagonist to organophosphate-induced toxicity, 4,4′-bis[1,3-dioxan-2-ylmethyl)methylamino]acetyl]biphenyl dimethobromide (1). Stepwise removal of the oxygen atoms from the dioxane rings, as well as changing the position of attachment of substituents on the 1,3-dioxane rings and decreasing the ring size from six-membered to five-membered caused drastic or complete loss of pharmacological effect. Partial structures of 1 were all inactive. Thus, the structure of 1 seems to be remarkably specific. Additional pharmacological data are reported for 1.

A prior communication[1] described the high potency and biological activity and low acute toxicity of 4,4′-bis[[(1,3-dioxan-2-ylmethyl)methylamino]acetyl]biphenyl dimethobromide (1) (an analogue of hemicholinium-3 (2) as an antagonist of paraoxon-induced toxicity in mice, as compared with the efficacy of the prototypical protective agents physostigmine and pyridostigmine.



1



2

The present communication describes synthesis and pharmacological studies of a variety of molecular variations of 1 and also reports some additional pharmacological studies on 1. Compounds 4 and 5 illustrate stepwise removal of the oxygen atoms from the 1,3-dioxane rings of 1, and in 6 the position of attachment to the 1,3-dioxane ring has been moved to carbon 4. In 3, the six-membered 1,3-dioxane rings of 1 have been diminished to five-membered 1,3-dioxolane rings. Compounds 7–9 illustrate various monoquaternary fragments of 1; compound 8 is the "half molecule" of 1.

Chemistry

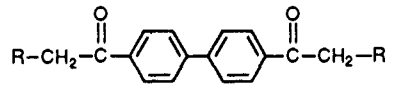
Compounds 3–8 were prepared by standard reactions, involving treatment of an α-halo ketone with the appropriate *N,N*-dimethyl tertiary amine. 4-[(Dimethylamino)methyl]-1,3-dioxane (13) is mentioned in the literature.[2] However, experimental details for its preparation are not readily accessible, and characterization data seem not to have been reported. Therefore, preparation of 13 is described in detail. Spectral (NMR, MS) data on all intermediates and final compounds in this study were consistent with the proposed structures.

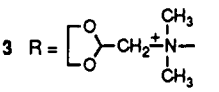
Results and Discussion

Pharmacology. Possible modes of action of hemicholinium-3 analogues for protection against organophosphate-induced (O–P) toxicity include:

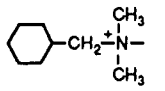
Decreased synthesis or release of acetylcholine from cholinergic nerve terminals.

2. Decrease in responsiveness of post-junctional membranes to acetylcholine or to the O–P. Electrophysiological

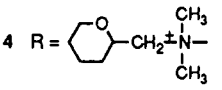




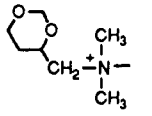
3



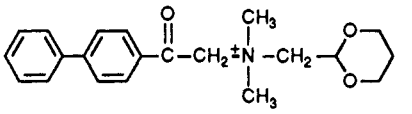
5



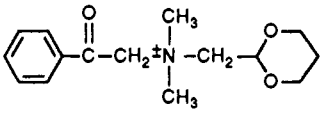
4



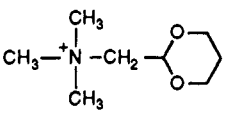
6



7



8



9

studies with 1 using frog skeletal muscle demonstrate protective action against several O–P's at post-junctional membranes.[3]

3. Interference with attachment of the O–P to the esteratic site(s) of acetylcholinesterase such that the hydrolytic activity of the enzyme is retained. The protective compound should not inhibit acetylcholinesterase in concentrations needed to protect the acetylcholine binding site against the O–P, lest efficacy be diminished. It is generally assumed that the protective effects of carbamate derivatives (e.g., physostigmine and pyridostigmine) relate to a reversible interaction (perhaps acylation) with the serine hydroxyl group of the catalytic surface of the enzyme, thereby interfering with attachment of the O–P. It does not seem likely that 1 would form a covalent bond with the serine hydroxyl group, and it is concluded that some other (unknown) chemical mechanism(s) is (are) involved. Whether combining dosage of 1 with a carbamate derivative enhances the protective effect has not yet been established.

Table I shows results obtained when compounds 1 and 3–9 were evaluated for their ability to antagonize paraoxon-induced lethality in mice and for their ability to

† College of Pharmacy.

‡ College of Medicine.

§ Visiting scientist from Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Hipodrom, Ankara, Turkey.

(1) Cannon, J. G.; Sahin, M. F.; Long, J. P.; Flynn, J. R.; Bhatnagar, R. K. *J. Med. Chem.* **1990**, *33*, 577.

(2) Zhu, Y.-Q.; Ghen, F.-X.; Li, Z.-Y.; Wu, Y.-Z. *Yao Hsueh Hsueh Pao.* **1980**, 15, 656; *Chem. Abstr.* **1981**, *95*, 115347x.

(3) Bhattacharyya, B.; Sokoll, M. D.; Flynn, J. R.; Nyanda, A. M.; Lee, T.; Cannon, J. G.; Long, J. P. *Arch. Intern. Pharmacodyn. Ther.* **1990**, *308*, 149.

0022-2623/91/1834-1582$02.50/0

Table I. Biological Activity of Antagonists of Paraoxon-Induced (LD_{99}) Toxicity

compd no.	PD_{50}[a] vs paraoxon, μmol/kg (95% CI)	therapeutic index LD_{50}/ED_{50}	inhibition of AcChE: IC_{50},[b] μM (95% CI)
physostigmine	0.02 (0.008–0.05)	40	0.03 (0.02–0.05)
pyridostigmine	0.66 (0.3–4.8)	16	0.05 (0.03–0.07)
1	0.019 (0.01–0.03)	380	0.003 (0.001–0.03)
3	inactive[c]		9.9 (4.8–15.4)
4	0.7 (0.5–0.9)	10	6.90 (0.2–34.3)
5	inactive		ca. 25
6	inactive		14.2 (11.0–17.1)
7	inactive		20.8 (14.2–32.9)
8	inactive		>100
9	inactive		>100

[a] PD_{50} = μmol/kg required to protect 50% of the mice for 24 h vs a LD_{99} dose of paraoxon. [b] IC_{50} = concentration required to inhibit hydrolytic activity of bovine erythrocyte AcChE by 50%. [c] Compounds which failed to protect at least 40% of the mice with doses ranging from 1/4 to 1/16 of the LD_{50} dose were regarded as inactive.

Table II. Antagonism in Mice of Paraoxon (1.0 mg/kg sc) Induced Motor Incoordination with 1, Physostigmine, and Pyridostigmine

compd	inclined screen assay: ED_{50}, nmol/kg (95% CI)	relative potency
1	3.1 (1.3–4.5)	1.0
physostigmine	7.6 (3.7–10.5)	0.37 (0.2–0.7)
pyridostigmine	13.6 (12.4–15.6)	0.21 (0.1–0.3)

inhibit the catalytic ability of acetylcholinesterase (AcChE). Compound 1 has a much higher therapeutic index than either physostigmine or pyridostigmine. Also, 1 produces 100% protection over a wide dose range, at either 30- or 120-min pretreatment time before administration of paraoxon.[1] Pyridostigmine did not afford 100% protection at any dose at either pretreatment time period (30 or 120 min), but it demonstrated some effectiveness for 2 h after administration. Physostigmine produced 100% protection over a wide dose range at 30-min pretreatment time, but it was ineffective at 120-min pretreatment time. The high potency of 1 (compared with physostigmine and pyridostigmine) in inhibiting AcChE is striking, as is the dramatic structural specificity of the molecule, compared with the congeners 3–9. It is appealing to speculate that at least a portion of the protective action of 1 against O–P is due to this effect upon AcChE.

Physostigmine, pyridostigmine, and 1 are very active in antagonizing skeletal muscle paralysis and loss of muscular coordination induced by paraoxon (Table II). Compound 1 was significantly more active than physostigmine or pyridostigmine, and in this assay 1 exhibited an amazing therapeutic index of >2000 (TI = LD_{50}/ED_{50} = 7.2 μmol/kg/0.0031 μmol/kg).

Table III lists results obtained by using 5-fold LD_{50} doses of paraoxon. With this extremely challenging dose of the O–P, the mice died within 2 min. Neither physostigmine nor pyridostigmine was capable of increasing survival time, but 1 exhibited marked antagonism to paraoxon-induced toxicity, which further demonstrates the efficacy of 1.

Structure–Activity Considerations. All modifications of the heterocyclic rings of 1 resulted in great diminution or (usually) complete loss of O–P protective activity. As illustrated in Table I, there is a stepwise loss in activity as the number of oxygen atoms in the ring is diminished (cf. 1 vs 4 vs 5). It may be inferred that both oxygen atoms of the 1,3-dioxane rings of 1 participate in receptor binding. This hypothesis is reinforced by the

Table III. Protection in Mice Using Five Times LD_{50} Dose of Paraoxon[a]

compound combination	protective agent fraction of LD_{50}	survival time
paraoxon + physostigmine	1/4, 1/8, 1/16	<2 min
paraoxon + pyridostigmine	1/4, 1/8, 1/16	<2 min
paraoxon + 1	1/4	38%[b] at 24 h[c]
	1/8	50%[b] at 24 h[c]
	1/16	0.0% at 24 h

[a] All control mice treated with paraoxon + saline died < 2 min. [b] All mice lived ≫ 2 min. [c] All survivors at 24 h appeared normal.

inactivity of 6, in which the spatial relationship of the methylammonium substituent on the 1,3-dioxane ring relative to the ring oxygen atoms differs from that in 1. The inactivity of the 1,3-dioxolane congener 3 is inexplicable. There are no obvious major differences in valence angles or interatomic (N to O) distances in comparing the molecular architecture of 1 and 3. Study of molecular models suggests that in the chair conformation of the 1,3-dioxane rings of 1, the methylammonium substituents can assume an equatorial disposition. In contrast, due to the near planarity of the 1,3-dioxolane rings in 3, the methylammonium side chains are more nearly in a pseudoaxial disposition. Future molecular modeling studies may permit assessment of the significance of this conformational difference.

The inactivity of the partial structures 7–9 suggests that both of the quaternary heads of 1 are necessary for a productive interaction with the receptor(s). This same observation has been reported for other hemicholinium-3 partial structures with respect (inter alia) to inhibition of high affinity, sodium-dependent choline uptake[4] and antinicotinic activity.[5]

Experimental Section

Pharmacology. Methods. Antagonism of Paraoxon-Induced Toxicity. The 24-h LD_{50} dose for each compound was determined by using Sprague-Dawley male mice weighing 18–24 g. To determine 24-h protective efficacy against a 2-fold LD_{50} (LD_{99}) of paraoxon, experimental compounds were administered im into the left hindlimb 30 min prior to administration of 2× LD_{50} of paraoxon (sc) and atropine sulfate (11.2 mg/kg im) into the right hindlimb. The protective agents were administered to groups of 10 mice in ratios of their LD_{50} dose, i.e., 1/4, 1/8, 1/16.

Motor Nerve Coordination. A wire mesh screen was elevated to 80° from the horizontal; naive mice placed on the upper third of this screen grip the mesh and remain in position for at least 5 min. Mice receiving paraoxon (1.0 mg/kg sc) are unable to grip the wire mesh, and they fall. Physostigmine, pyridostigmine, and 1 were assayed for their ability to antagonize motor incoordination induced by paraoxon. In the assay, the experimental compound or saline (control) was administered im into the right hindlimb. Doses of O–P antagonists were varied by 0.3 $\log_{10}$ intervals. After 30 min, paraoxon (1.0 mg/kg sc) was administered, followed at once by atropine sulfate (11.2 mg/kg im into the right hindlimb). After 15 min, the mice were placed upon the wire mesh and their individual abilities to remain on the screen were recorded.

Inhibition of Acetylcholinesterase. Bovine erythrocyte acetylcholinesterase (Type XII-S, lyophilized powder, Sigma Chemical Co.) was reconstituted in phosphate buffer at pH 8 (5 units/mL stock). Acetylthiocholine iodide was used as a substrate for the acetylcholinesterase. Enzyme inhibition was measured according to the method of Ellman et al.[6] The enzyme (0.05 mg/mL) and the inhibitor were incubated for 5 min before the substrate was added (2 mM final concentration). The reaction

(4) Chatterjee, T. K.; Long, J. P.; Cannon, J. G.; Bhatnagar, R. K. *Eur. J. Pharmacol.* **1988**, *149*, 241.
(5) Peterson, L. A.; Sharabi, F. M.; Long, J. P.; Taylor, C. A.; Barfknecht, C. F. *Eur. J. Pharmacol.* **1976**, *37*, 303.
(6) Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.

mixture contained the thiol reagent 5,5′-dithiobis(2-nitrobenzoic acid) (0.33 mM final concentration). The absorbance was read by using a Gilford spectrophotometer at 412 nm against a blank. Protein was estimated by the method of Lowry et al.[7]

Statistical Analysis. Response levels of 50% (PD_{50}, LD_{50}, IC_{50}, ED_{50}) were calculated by probit analysis as outlined by Goldstein.[8] Relative potencies were calculated as described by Finney.[9]

Chemistry. Melting points were determined in open glass capillaries with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses (C, H, N) were performed by Galbraith Laboratories, Knoxville, TN. Analyses for ionic halogen were performed by a titrimetric Volhard method. 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 Bruker-IBM NR 80 instrument with Me_4Si used as the internal standard. Mass spectra were recorded with a Ribermag R-10-10C mass spectrometer.

4,4′-Bis[[(1,3-dioxolan-2-ylmethyl)methylamino]acetyl]biphenyl Dimethobromide (3). 2-[(Dimethylamino)methyl]-1,3-dioxolane[10] (2.88 g, 0.022 mol) and 3.96 g (0.01 mol) of 4,4′-bis(bromoacetyl)biphenyl[11] were stirred in 75 mL of MeCN and 25 mL of H_2O at ambient temperature for 8 h. Volatiles were then removed under reduced pressure and the solid residue was recrystallized from 2-PrOH–H_2O to afford 5.57 g (85%) of product: mp 290 °C dec. Anal. ($C_{28}H_{38}Br_2N_2O_6$) C, H, N (Karl Fischer H_2O 1.06%).

2-[(Dimethylamino)methyl]tetrahydropyran (11). 2-(Bromomethyl)tetrahydropyran (19.1 g, 0.1 mol) and 105 g of 30% dimethylamine in EtOH (0.7 mol) were heated in a Parr bomb at 130 °C for 4 h. Volatiles were removed from the reaction mixture under reduced pressure. The residue was taken up in 50 mL of 2-PrOH and 200 mL of Et_2O was added. The dimethylamine hydrobromide which separated was removed by filtration and the filtrate was evaporated under reduced pressure. The liquid residue was distilled, bp 166 °C (760 mm), to provide 13 g (84%) of product which was used in the next step without further treatment. The compound was characterized as its picrate salt, mp 164–166 °C (from EtOH). Anal. ($C_{14}H_{20}N_4O_8$) C, H, N.

4,4′-Bis[[(2-tetrahydropyranylmethyl)methylamino]acetyl]biphenyl Dimethobromide (4). Compound 11 (3.41 g, 0.022 mol) and 1.98 g (0.005 mol) of 4,4′-bis(bromoacetyl)biphenyl[11] in 70 mL of THF and 30 mL of MeOH were stirred at room temperature for 6 h. Volatiles were evaporated and the residue was recrystallized from 2-PrOH–Et_2O to afford 3.2 g (94%) of product, mp 260–261 °C. Elemental analysis was obtained for the dipicrate salt, mp 209–210 °C (from EtOH). Anal. ($C_{44}H_{50}N_8O_{18}$) C, H, N.

4,4′-Bis[[(cyclohexylmethyl)methylamino]acetyl]biphenyl Dimethobromide (5). Dimethyl(cyclohexylmethyl)amine[12] (1.55 g, 0.011 mol) and 1.98 g (0.005 mol) of 4,4′-bis(bromoacetyl)biphenyl[11] were stirred in 70 mL of THF and 30 mL of MeOH for 6 h at ambient temperature. Volatiles were removed under reduced pressure and the solid residue was recrystallized from Me_2CO–MeOH (20:1) to afford 2.85 g (87%) of light yellow crystals, mp 240 °C dec. Anal. ($C_{34}H_{50}Br_2N_2O_2$) C, H, N.

4-(Bromomethyl)-1,3-dioxane (12). This compound was prepared by a method utilized by Price and Krishnamurti[13] for preparation of 4-(chloromethyl)-1,3-dioxane. Allyl bromide (60.5 g, 0.5 mol) was added over 1.5 h to an ice–H_2O chilled, stirred mixture of 30 g (1.0 mol) of paraformaldehyde and 50 mL of concentrated H_2SO_4. The resulting mixture was stirred for 0.5 h more and then it was poured over excess ice–H_2O. The organic layer was extracted with 200 mL of Et_2O and this extract was washed with 5% $NaHCO_3$ and then with H_2O. The Et_2O was evaporated and the liquid residue was distilled. The fraction boiling at 80 °C (3 mm) was collected, yield 38 g (41%). Anal. ($C_5H_9BrO_2$) C, H.

4-[(Dimethylamino)methyl]-1,3-dioxane (13). Compound 12 (19.1 g, 0.1 mol) and 105 mL of 30% Me_2NH in EtOH (0.7 mol) were heated in a Parr bomb at 130 °C for 4 h. Volatiles were removed from the reaction mixture and the residue was taken up in 50 mL of 2-PrOH. This solution was treated with 250 mL of Et_2O. The precipitated dimethylamine hydrobromide was removed by filtration, and the filtrate was evaporated under reduced pressure. The liquid residue was distilled, bp 70 °C (3 mm), to afford 13 g (89%) of product. For characterization, an ethereal solution of the product was treated with ethereal HCl, and the insoluble salt was recrystallized from EtOH–Et_2O, mp 154–155 °C. Anal. ($C_7H_{16}ClNO_2$) C, H, N. (Karl Fischer H_2O 1.07%).

4,4′-Bis[[(1,3-dioxan-4-ylmethyl)methylamino]acetyl]biphenyl Dimethobromide (6). Compound 13 (2.9 g, 0.02 mol) and 1.98 g (0.005 mol) of 4,4′-bis(bromoacetyl)biphenyl[11] were stirred in 75 mL of MeCN and 25 mL of H_2O for 4 h at room temperature. Volatiles were removed under reduced pressure, and the residue was recrystallized from 2-PrOH-H_2O to afford 3 g (87%) of product, mp 218-219 °C dec. Anal. ($C_{30}H_{42}Br_2N_2O_6$) C, H, Br, N

4-[[(1,3-Dioxan-2-ylmethyl)methylamino]acetyl]biphenyl Methobromide (7). 4-Phenylphenacyl bromide (2.75 g, 0.01 mol) and 1.45 g (0.01 mol) of 2-[(dimethylamino)methyl]-1,3-dioxane[1] were heated under reflux in 75 mL of MeOH for 2 h. Volatiles were removed and the oily residue was induced to crystallize by treatment with 50 mL of Et_2O. The resulting solid was recrystallized from 2-PrOH–Et_2O to afford 4 g (95%) of product, mp 211–212 °C. Anal. ($C_{21}H_{26}BrNO_3$) C, H, N. (Karl Fischer H_2O 0.17%).

[*N*-Methyl-*N*-(1,3-dioxan-2-ylmethyl)amino]acetophenone Methochloride (8). Phenacyl chloride (1.45 g, 0.01 mol) and 1.45 g (0.01 mol) of 2-[(dimethylamino)methyl]-1,3-dioxane[1] were heated under reflux in 75 mL of MeOH for 2 h. Volatiles were removed and the gummy residue was induced to solidify by treatment with Et_2O. The solid was recrystallized from EtOH to yield 2.8 g (93%) of a solid, mp 200–201 °C. Anal. ($C_{15}H_{22}ClNO_3$) C, H, N.

2-[(Dimethylamino)methyl]-1,3-dioxane Methiodide (9). To 0.725 g (0.005 mol) of 2-[(dimethylamino)methyl]-1,3-dioxane[1] in 50 mL of Et_2O was added dropwise and with stirring 0.78 g (0.0055 mol) of MeI. The resulting mixture was stirred at room temperature for 3 h. The solid which formed was collected on a filter and was recrystallized from EtOH to yield 1.2 g (84%) of product, mp 182–183 °C. Anal. ($C_8H_{18}INO_2$) C, H, N. (Karl Fischer H_2O 0.16%).

Acknowledgment. This research was supported in part by the U.S. Army Medical Research Acquisition Activity, Contract DAMD17-87-C-7113.

(7) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1954**, *193*, 265.
(8) Goldstein, A. *Biostatistics: An Introductory Text*; Macmillan: New York, 1964.
(9) Finney, D. J. *Probit Analysis*; Cambridge University: New York, 1962; p 20.
(10) Fourneau, J. P.; Chantalou, S. *Bull. Soc. Chim. Fr.* **1945**, *12*, 845.
(11) Long, J. P.; Schueler, F. W. *J. Am. Pharm. Assoc. Sci. Ed.* **1954**, *43*, 79.
(12) Cope, A. C.; Ciganek, E. *Organic Syntheses*; Wiley: New York 1963; Collect Vol. 4, p 339.
(13) Price, C. C.; Krishnamurti, I. V. *J. Am. Chem. Soc.* **1950**, *72*, 5335.