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REVIEW ARTICLE

BINDING OF QUATERNARY AMMONIUM SALTS TO ACETYLCHOLINE RECEPTORS: POSSIBLE CHEMICAL WARFARE NERVE AGENTS

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Summary

Classical chemical nerve agents are organophosphate based compounds such as Sarin, Soman, Tabun, VX and others. These compounds inhibit acetylcholinesterases in the synapses of nerve junctions. Instead of inactivating acetylcholinesterases, compounds, quaternary amines, can block nerve transmission by binding primarily to the acetylcholine receptor sites. The U.S. Patent Office has published 23 unique patents or invention registrations on the synthesis of compounds that can bind to muscarinic and nicotinic acetylcholine receptors. It is likely that some of these compounds could serve as either polarizing or nonpolarizing nerve agents that bind to the acetylcholine receptor and block binding of acetylcholine. This paper has systematically reviewed a series of bisquaternary amines that could be potentially deadly nerve agents. Analogous tertiary amines, based on the structure of the parent bisquaternary amines, have been proposed as compounds that might be more soluble in membranes making them more bioavailable and toxic. Finally, methods have been discussed that could identify this class of compounds using a bioassay for binding to the acetylcholine receptor. A tool in the identification of these compounds, the Hofmann elimination reaction, has been proposed as a novel method for helping to establish that quaternary or tertiary amines are functional groups of the nerve agent.

Key words: Quaternary ammonium salts; nerve agents; chemical warfare; acetylcholine receptor

INTRODUCTION AND BACKGROUND

Classical chemical nerve agents are organophosphate based compounds such as Sarin, Soman, Tabun, VX and others. These agents have properties that make them uniquely suitable as chemical warfare agents including high toxicity by all routes of exposure. The hydrophobic nature of these chemicals makes them susceptible to absorption through the skin as well as through inhalation and oral exposure. These compounds act on acetylcholinesterases in the synapses of nerve junctions. The mode of action of these agents involves phosphorylation of the active site of acetylcholinesterase followed by "aging" in some compounds, a chemical reaction involving the spontaneous formation of a stable covalent bond in the active site of the enzyme. In a typical nerve conduction scenario, acetylcholine is released from the nerve terminus of the synaptic junction and rapidly diffuses across the synaptic cleft to the postjunctional membrane where it binds to acetylcholine receptors. When two acetylcholine molecules bind to their respective sites within a re-

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ceptor, a conformational change occurs in the receptor proteins allowing the opening of a channel with the influx of Na^+/Ca^{2+} and efflux of K^+ [1]. The movement of cations through the channel causes the nerve to become depolarized; the nerve repolarizes once the acetylcholine has diffused away from the receptor causing the channel to close. Acetylcholine is prevented from continually binding to the receptor by rapid hydrolysis in a reaction catalyzed by acetylcholinesterase. If this enzyme is inactivated, as happens with the binding of the phosphorus-based nerve agents, the levels of acetylcholine do not diminish, the channel remains open and the nerve remains polarized; nerve conduction ceases to exist because the nerve cannot repolarize. Nicotinic acetylcholine receptors are cholinergic receptors (responsive to acetylcholine) found in the central nervous system, peripheral nervous systems and skeletal muscles.

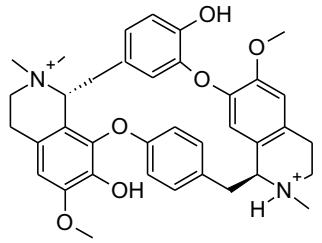


Figure 1. d-Tubocurarine 6,6'-dimethoxy-2,2,2',2'-tetramethyltubocuraran-2,2'-diium-7',12'-diol

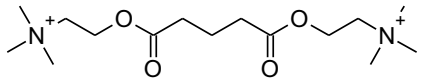


Figure 2. Succinylcholine Cation 2,2'-[(1,4-dioxobutane-1,4-diyl)bis(oxy)]bis(*N,N,N*-trimethylethanaminium)

Tubocurarine was the first compound identified to cause muscle paralysis via binding to the nicotinic acetylcholine receptor. This compound was identified from curare, an extract of a South American plant Pareira, *Chondrodendron tomentosum* [2]. Tubocurarine is a compound with two quaternary ammonium functional groups (Figure 1) that binds to the nicotinic acetylcholine receptor and blocks the binding of acetylcholine. The binding of this compound does not open the membrane channel. Succinylcholine (Figure 2, basically two acetylcholine molecules joined back to back) binds to both acetylcholine binding sites in the acetylcholine receptors and opens the channel for the migration of cations. Thus, succinylcholine acts to depolarize the neuromuscular endplate. Succinylcholine is not hydrolyzed by the usual acetylcholinesterase but is hydrolyzed by butyrylcholinesterase – a slower enzyme. The development of nondepolarizing compounds as an adjunct to anesthesia and used as a tool to relax muscles during surgery has resulted in the identification of several bisquaternary ammonium compounds such as pancuronium (Figure 3), vercuronium (Figure 4) and tubocurarine (Figure 1) [3].

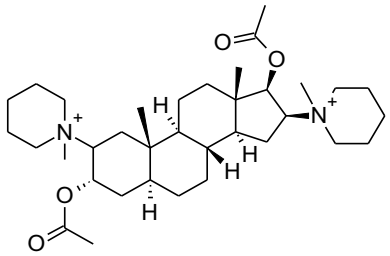


Figure 3. Pancuronium Cation [(2*S*,3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,16*S*,17*R*)- 17-acetyloxy-10,13-dimethyl-2,16-bis(1-methyl-3,4,5,6-tetrahydro-2*H*-pyridin-1-yl)-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl] acetate

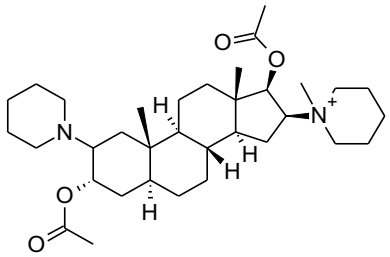


Figure 4. Vercuronium Cation [(2*S*,3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,16*S*,17*S*)-17-acetyloxy-10,13-dimethyl-16-(1-methyl-3,4,5,6-tetrahydro-2*H*-pyridin-1-yl)-2-(1-piperidyl)-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yl] acetate

A key feature of nondepolarizing compounds is that they form a rigid molecular framework. Decamethonium (Figure 5), with two quaternary ammonium centers separated by 10 methylene ($-CH_2-$) groups, is sufficiently flexible that both sites for acetylcholine binding are bound resulting in the nerve becoming depolarized [4, 5]. Two broad classes of compounds that bind to acetylcholine receptors are the competitive inhibitors that block binding of the acetylcholine neurotransmitters, but

do not activate the ion channel and compounds that bind tightly to both sites in the acetylcholine receptor causing depolarization of the nerve. Depolarization of the nerve allows initial transmission of the nerve impulse to the muscle resulting in twitching of the muscles (fasciculations) followed by paralysis of the affected muscles.

Phosphorus-based nerve agents were first synthesized by German chemists prior to and during World War II and were, fortunately, never used in combat [6]. There has been little research published on the development of new nerve agents and it is difficult to determine the extent to which any country has developed new and novel nerve agents. There are a number of motivations why countries would pursue such research. For example, some of the phosphorous-based nerve agents can be counteracted three ways:

1) through the use of agents that reactivate acetylcholinesterase (2-PAM, 2-pyridine aldoxime methyl chloride; Figure 6);

2) by protection of the enzyme from attack (pyridostigmine; Figure 7) [4];

3) or by replacement of inactivated acetylcholinesterase by the intravenous injection (i.v.) of human butyrylcholinesterase [7].

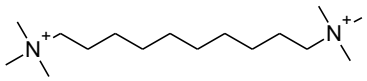


Figure 5. Decamethonium Cation
Trimethyl-(10-trimethylammoniodecyl)ammonium

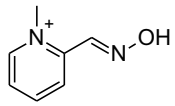


Figure 6. 2-PAM, 2-pyridine aldoxime
2-[(hydroxyimino)methyl]-1-methylpyridin-1-ium

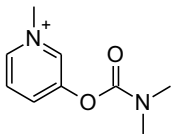


Figure 7. Pyridostigmine
3-[(dimethylcarbamoyl)oxy]-1-methylpyridinium

The development of new agents, which do not depend on the inactivation of acetylcholinesterase, would make the use of these three countermeasures mute. New agents would also significantly delay the forensic identification of the agent and its origin. An example of potential new nerve agents comes from an examination of the U.S. Patent Office which has published 23 unique patents or invention registrations on the synthesis of compounds that can block nerve transmission by binding to muscarinic and nicotinic acetylcholine receptors [8-32]. It is likely that some of these compounds could serve as either polarizing or nonpolarizing nerve agents that bind to the acetylcholine receptor and block binding of acetylcholine.

The primary purpose of this paper is to report on an investigation of the open literature for clues to possible new classes of chemical weapons, or in this case, a new class of nerve agents. This paper will endeavor to review some of the physical properties (e.g. solubility, hydrophobicity) and potential toxicities of a new class of nerve agents and their potential for use in chemical warfare. There are several objectives of this study:

1) to understand the structure, properties and possible modes of action of a "second class" of nerve agents that does not involve the inhibition of acetylcholinesterase, a mechanism common to the phosphorous based nerve agents – the original class of nerve agents,

2) review structural characteristics of these compounds that appear to be important in the structure-toxicity relationship,

3) review characteristics of quaternary ammonium compounds that optimize their binding to the nicotinic or muscarinic acetylcholine receptors,

4) discuss the possibility that more potent nerve agents may be derived from structurally similar compounds with properties that would enhance their lipid solubility and their absorption via inhalation, ingestion or dermal exposure,

5) discuss 3-quinuclidinyl benzilate as a classical example of a tertiary amine that is also an incapacitating agent developed and stock-piled for potential use in a conflict,

6) discuss possible chemical and biochemical methods that could be used to detect and identify

nerve agents discussed in this report, including the following: biochemical methods to detect this class of nerve agents, utilization of the Hoffman elimination reaction of quaternary amines for detection and identification, and analytical chemistry methods to identify these compounds, and

7) to discuss recent results showing similar studies on other published bisquaternary amines.

U.S. Patents Issued to the U.S. Army - Chemical Agents

In the 1970s and 80s, 23 unique U.S. patents (two patents were duplicates) described the synthesis and rudimentary toxicity (intravenous injection, LD_{50} measurements) of chemical nerve agents based on mono and bisquaternary ammonium functional groups (Table 1). The patents were assigned to the U.S. government represented by the United States Army [8-32]. It is not clear why these patents were released as they are potentially deadly nerve agents. They were originally submitted to the Patent Office in the 1960s, but were not published until ~20 years later. Perhaps these were released in preparation for the ratification of the Conventional Weapons Treaty that prohibits the development of new chemical weapons [34]. One may argue that the medicinal research was in the process of identifying nerve blocking agents for muscle relaxants and anesthetic uses. It made sense to lay claim to the hundreds of compounds already investigated that may have some therapeutic benefit. The problem with this argument, however, is that none of the patents claim any potential therapeutic benefits; they all cite the potential for the compounds to be chemical warfare agents. Regardless of the reasoning behind the submission of these patents, they reveal a potentially new class of nerve agents. Each of these patents represents a number of different compounds due to a combination of substituents and anions selected in the synthesis of the compounds, presumably to study their structure-activity relationships. The number of compounds represented by these patents could easily be in the hundreds. For most of these patents, one, two, or three examples of the synthesis of representative compounds are presented. In some cases, the key precursor to the compounds is also shown. There is sufficient detail discussed in these patents that a skilled chemist could easily synthesize and purify these compounds.

General Synthetic Method

A common synthetic and purification scheme is apparent when the syntheses in the all the patents are reviewed and compared. The synthesis and purification of several examples for each patent are presented in adequate detail in the patents. The chemistry used to synthesize these compounds is straight forward and typically involves the mixing of an alkyl bromide with a tertiary amine in the presence of a suitable solvent and allowing the components to combine in a simple nucleophilic displacement reaction. After a suitable time, the volume of the reaction mixture is concentrated, treated with decolorizing charcoal, filtered and allowed to precipitate or crystallize. The compound maybe induced to precipitate or crystallize from solution using a combination of methods including the addition of another less polar solvent and lower temperatures. The combination of solvents/temperature used in the purification of these compounds represents the "art" of the synthesis and varies among the different patents but is similar within one set of compounds represented by one patent. One should note that sophisticated column chromatography (e.g. preparative HPLC or normal phase silica gel column chromatography) is not required to achieve products of reasonable purity. Reasonable purity, in this case, is defined as the ability to obtain an adequate C, H, N elemental analysis, which means that the elemental analyses are with 0.4% of the theoretical values [35]. The rather simple chemistry involved in the syntheses of these compounds allows the syntheses to be carried out in an enclosed environment (e.g. a glove box) minimizing the exposure of the chemist to these lethal compounds.

Toxicology

These patents give no information on the pharmacokinetics of these compounds. There is no information on the absorption, distribution,

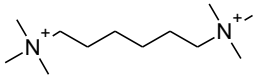
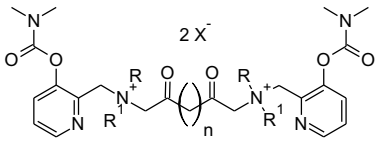
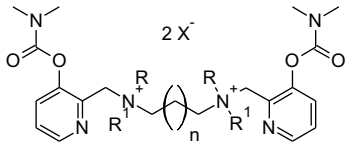
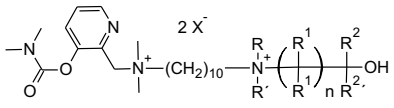
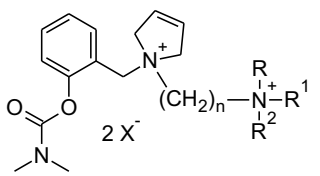
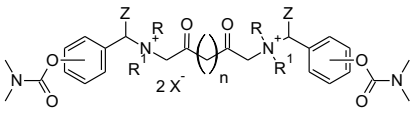
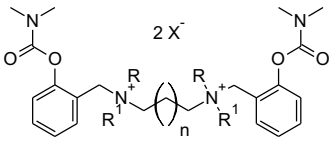
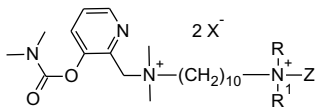
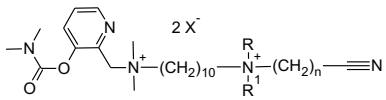
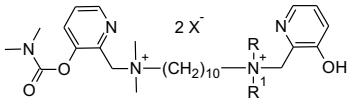
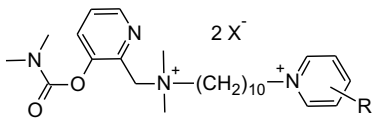


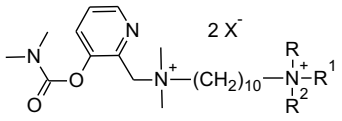
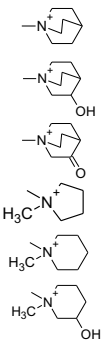
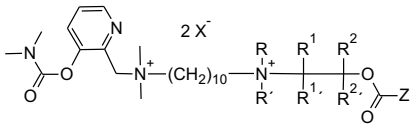
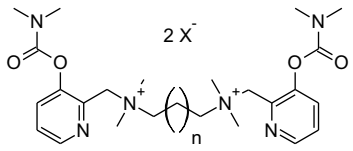
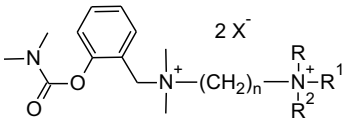
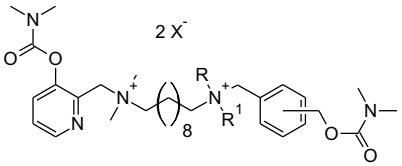
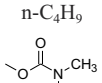
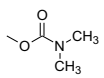
Figure 8. Hexamethonium Cation
N,N,N,N′,N′,N′-hexamethylhexane-1,6-diaminium

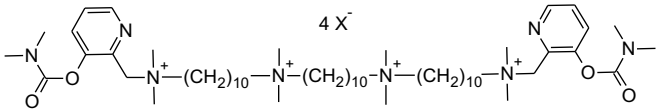
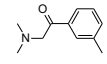
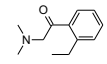
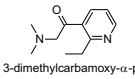
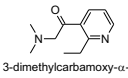
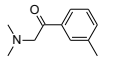
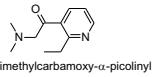
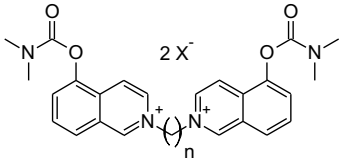
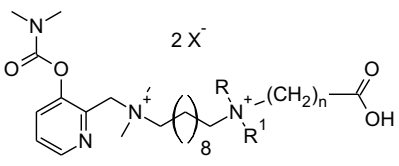
metabolism or elimination via inhalation, oral or dermal exposure. Based on the charged nature of these compounds it seems likely that these compounds would have a difficult time crossing a membrane via dermal contact or though absorption in the GI tract to ultimately enter systemic circulation where they could be transported to muscarinic and/or nicotinic acetylcholine receptors. However, there is one anecdotal report of poisoning by hexamethonium (Figure 8), a doubly charged bisquaternary ammonium salt, via the inhalation route of exposure [37]. Exposure by inhalation to a hexamethonium aerosol resulted in a steady decline in lung function resulting in the death of the subject one month after initial exposure even after hospitalization and respiratory support. Thus, it seems possible that these agents could be toxic via the inhalation route of exposure using an aerosol formulation.

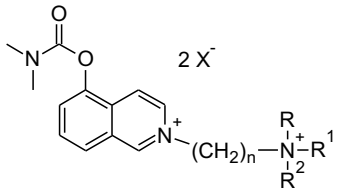
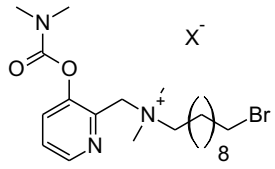
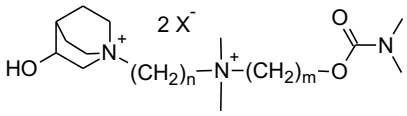
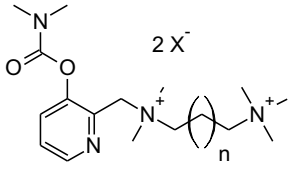
Table 1. Patented potential chemical warfare agents - quaternary ammonium salts

Compound	Compounds Studied	Substituents		Toxicity[a] i.v. injection (μg/kg)		Ref.
		Compound 1	Compound 2	LD_{50} Rabbits	LD_{50} Mice	
	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, t-C_4H_9, n-C_5H_{11}, n-C_6H_{13} n = 2-12 X^- = halides, $HC_2O_4^-$, ClO_4^-, NO_3^-, $(C_6H_6)_4B^-$, HSO_4^-	R, R^1 = CH_3 n = 10 X^- = Br^-	R = CH_3 R^1 = C_2H_5 n = 8 X^- = Br^-	Compound 1		[8]
				2.7 μg/kg	7 μg/kg	
				Compound 2		
				2.7 μg/kg	10 μg/kg	
	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, $C_6H_5CH_2$ n = 3-14 X^- = monovalent, polyvalent anion	R = CH_3 R^1 = C_2H_5 n = 8 X^- = Br^-	R = CH_3 R^1 = C_2H_5 n= 6 X^- = Br^-	Compound 1		[9]
				4 μg/kg	11 μg/kg	
				Compound 2		
				4 μg/kg	6 μg/kg	
	R, R' = CH_3, C_2H_5, n-C_3H_7, C_2H_5OH R^1, $R^{1'}$= H, CH_3, C_2H_5 R^2, $R^{2'}$ = H, OH, CH_3, C_2H_5, n-C_3H_7 n = 1-8 X^- = monovalent, polyvalent anion	R, R' = CH_3 R^1, $R^{1'}$= H R^2, $R^{2'}$ = H n = 1 X^- = Br^-	R, R' = CH_3, C_2H_5OH R^1, $R^{1'}$= H R^2, $R^{2'}$ = H n = 1 X^- = Br^-	Compound 1		[10]
				4.5 μg/kg	9 μg/kg	
				Compound 2		
				5.4 μg/kg	14 μg/kg	
	R^1, R^2, R^3 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 5-16 X^- = monovalent, polyvalent anion	R = CH_3 R^1 = CH_3 R^2 = CH_3 n = 10 X^- = $(C_6H_6)_4B^-$	R = CH_3 R^1 = CH_3 R^2 = CH_3 n = 8 X^- = $(C_6H_6)_4B^-$	Compound 1		[11]
				6 μg/kg	13 μg/kg	
				Compound 2		
				4.6 μg/kg	14 μg/kg	

Compound	Compounds Studied	Substituents		Toxicity[a] i.v. injection (μg/kg)		Ref.
		Compound 1	Compound 2	LD_{50} Rabbits	LD_{50} Mice	
	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}, n-C_6H_{13} n = 2-12 X^- = monovalent, polyvalent anion Z = H, CH_3, C_2H_5 dimethylcarbamoxy group in o, m, p positions	R, R^1 = CH_3 n = 10 X^- = Br^- Z = H dimethylcarbamoxy group in o-position	R, R^1 = CH_3 n = 8 X^- = Br^- Z = H dimethylcarbamoxy group in o-position	Compound 1		[12]
				5 μg/kg	14 μg/kg	
				Compound 2		
				6 μg/kg	18 μg/kg	
	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 3-14 X^- = monovalent, polyvalent anion	R = CH_3 R^1 = C_2H_7 n = 6 X^- = Br^-	R = CH_3 R^1 = n-C_3H_7 n = 8 X^- = Br^-	Compound 1		[13]
				5 μg/kg	7 μg/kg	
				Compound 2		
				10 μg/kg	45 μg/kg	
	R, R^1 = C_2H_5, n-C_3H_7, n-C_4H_9 Z = C_2H_5, n-C_3H_7, n-C_4H_9 When Z = n-C_8H_{17}, n-$C_{12}H_{25}$, C_6H_{11} then R, R^1 = CH_3, C_2H_5 X^- = monovalent, polyvalent anion	R, R^1 = n-C_4H_9 Z = n-C_4H_9 X^- = Br^-	R, R^1 = CH_3 Z = C_6H_{11} (cyclohexane) X^- = Br^-	Compound 1		[14]
				5 μg/kg	28 μg/kg	
				Compound 2		
				10 μg/kg	28 μg/kg	
	R, R^1 = CH_3, C_2H_5, n-C_3H_7, n-C_4H_9 n =1-8 X^- = halides, $HC_2O_4^-$, ClO_4^-, NO_3^-, $(C_6H_6)_4B^-$, HSO_4^-	R, R^1 = CH_3 n = 1 X^- = Br^-	R, R^1 = CH_3 n = 3 X^- = $(C_6H_6)_4B^-$	Compound 1		[15]
				5.6 μg/kg	10 μg/kg	
				Compound 2		
				5.6 μg/kg	11 μg/kg	
	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}, n-C_6H_{13} X^- = monovalent, polyvalent anion	R, R^1 = CH_3 X^- = $(C_6H_6)_4B^-$	ND[b]	Compound 1		[16]
				5,8 μg/kg	22 μg/kg	
	R = H, OH, CH_3, CHNOH in the o, m and p positions X^- = monovalent, polyvalent anion	R = H X^- = Br^-	R = p-CHNOH X^- = Br^-	Compound 1		[17]
				7 μg/kg	13 μg/kg	
				Compound 2		
				5.8 μg/kg	18 μg/kg	

Compound	Compounds Studied	Substituents		Toxicity[a] i.v. injection (μg/kg)		Ref.
		Compound 1	Compound 2	LD_{50} Rabbits	LD_{50} Mice	
	NRR^1R^2 =  X^- = monovalent, polyvalent anion	NRR^1R^2 =  X^- = Br^-	NRR^1R^2 =  X^- = Br^-	Compound 1 5.9 μg/kg Compound 2 6 μg/kg	11 μg/kg 10 μg/kg	[18]
	R, R' = CH_3, C_2H_5 R^1, $R^{1'}$, R^2, $R^{2'}$ = H, CH_3 Z = CH_3, C_2H_5, n-C_3H_7, n-C_4H_9, n-C_5H_{11} X^- = monovalent, polyvalent anion	R, R' = CH_3 R^1, $R^{1'}$ = H R^2, $R^{2'}$ = H, CH_3 Z = CH_3 X^- = Br^-	R, R' = CH_3 R^1, $R^{1'}$, R^2, $R^{2'}$ = H Z = n-C_3H_7 X^- = Br^-	Compound 1 6.3 μg/kg Compound 2 6 μg/kg	13 μg/kg 13 μg/kg	[19, 20]
	X^- = monovalent n = 1-9	n = 1 X^- = Br^-	n = 6 X^- = Br^-	Compound 1[c] >32 μg/kg Compound 2[c] 6.3 μg/kg	>20 μg/kg 2.6 μg/kg	[21]
	R^1, R^2, R^3 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 5-16 X^- = monovalent, polyvalent anion	R, R^1, R^2 = CH_3 n =10 X^- = Br^-	R, R^1, R^2 = CH_3 n = 8 X^- = Br^-	Compound 1 7 μg/kg Compound 2 7 μg/kg	22 μg/kg 14 μg/kg	[22]
	R,R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9  Dimethyl carbamoxy group in o, m. or p X– = monovalent, polyvalent anion	R, R^1 = CH_3  In p position X^- = Br^-	ND	Compound 8 μg/kg	18 μg/kg	[23]

Compound	Compounds Studied	Substituents		Toxicity[a] i.v. injection (μg/kg)		Ref.
		Compound 1	Compound 2	LD_{50} Rabbits	LD_{50} Mice	
	X^- = monovalent, polyvalent anion	$X^- = C_6H_6)_4B^-$		Compound		
			ND			[24]
				8 μg/kg	32 μg/kg	
	R, $R^1 = CH_3$ Z =  3-dimethylcarbamoxyphenyl  2-dimethylcarbamoxybenzyl  3-dimethylcarbamoxy-α-picolinyl When Z =  3-dimethylcarbamoxy-α-picolinyl R,R^1 combine to form  pyrrolidinio  3-pyrrolinio  piperidinio  morpholinio X^- = monovalent, polyvalent anion	R, $R^1 = CH_3$ Z =  3-dimethylcarbamoxyphenyl $X^- = Br^-$	Z =  3-dimethylcarbamoxy-α-picolinyl R,R^1 combine to form  pyrrolidinio $X^- = Br^-$	Compound 1 28 μg/kg 18 μg/kg Compound 2 10 μg/kg 22 μg/kg		[25]
	n = 3-16 X^- = halides, $HC_2O_4^-$, ClO_4^-, NO_3^-, $(C_6H_6)_4B^-$, HSO_4^-	n= 8 $X^- = Br^-$	ND	Compound 16 μg/kg	6 μg/kg	[26]
	R, $R^1 = CH_3$, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 1-9 X^- = monovalent, polyvalent anion	R, $R^1 = CH_3$ n = 1 $X^- = Br^-$	ND	Compound 17 μg/kg	32 μg/kg	[27]

Compound	Compounds Studied	Substituents		Toxicity[a] i.v. injection (μg/kg)		Ref.
		Compound 1	Compound 2	LD_{50} Rabbits	LD_{50} Mice	
	R^1, R^2, R^3 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 5-16 X^- = halides, $HC_2O_4^-$, ClO_4^-, NO_3^-, $(C_6H_6)4B^-$, HSO_4^-	R^1, R^2, R^3 = CH_3 n = 10 X^- = $(C_6H_6)_4B^-$	ND	Compound		[28]
				25 μg/kg	ND	
	X^- = monovalent, polyvalent anion	X^- = Br^-	ND	Compound		[29]
				80 μg/kg	45 μg/kg	
	m =2-6 n = 6-16 X^- = halides, $HC_2O_4^-$, ClO_4^-, NO_3^-, $(C_6H_6)_4B^-$, HSO_4^-	m = 2 n = 10 X^- = Br^-	ND	LD_{50} (i.v., mice)	MED_{50} (i.v., mice)	[30, 31]
				560 μg/kg	56 μg/kg	
	n = 4-14 X^- = halides, $HC_2O_4^-$, ClO_4^-, NO_3^-, HSO_4^-	n = 8 X^- = Br^-	ND	NR[d]	NR	[32]

[a] LD_{50} – Lethal dose producing 50% mortality usually by intravenous injection (i.v.); MED_{50} – minimum effective dose for 50% of the population.

[b] ND = Not determined

[c] The lowest and highest toxicities were selected as examples for this compound as a range of toxicities were reported.

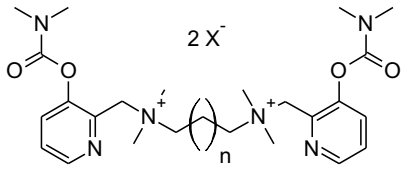
[d] NR = Not reported

Structure-Toxicity Relationships

The mode of action of these compounds is unknown and was not discussed in the patents. The toxicity data in Table 1 are limited to the intravenous injection (i.v.) of the compound and noting the concentration of the compound required to kill 50 % of the animals (LD_{50} in rabbits or mice). There are no data showing how long animals were observed, their symptoms, nor how long it took animals to die. There was no discussion of morbidity effects of these compounds at sub lethal doses. Additional studies that examine clinical effects at sub lethal doses might help inform the mode of action and structure-toxicity relationships more accurately. Even with this limited toxicity data set, one can observe common characteristics of those compounds that were the most toxic in rabbits. The data in Table 1 (and subsequent tables) are ranked by their relative toxicity in rabbits because rabbits are the more

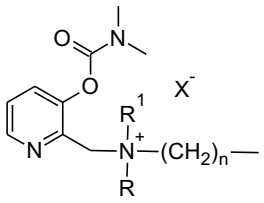
sensitive of the two animal models (rabbits and mice) studied. There are no data to suggest which animal model might be more representative of human exposure, especially since humans would likely be exposed via inhalation or skin contact, not by an intravenous injection. Three structural features are readily apparent from an inspection of Tables 1 and 2. One obvious and well known feature of these compounds are the requirement for two quaternary amines for optimal lethality. This is no surprise given the structure of well known toxic compounds such as decamethonium (Figure 5), succinylcholine (Figure 2), tubocurarine (Figure 1), and pancuronium (Figure 3). The compounds shown in Table 1 appear to be more similar to decamethonium and succinylcholine compared to the curare derived compounds (tubocurarine and pancuronium). The flexibility of these compounds in binding to the receptors is thought to be a key structural feature possibly causing depolarization of the endplate of the neuromuscular junctions [3]. These compounds tend to bind tightly to their receptor keeping the endplate from repolarizing and blocking nerve transmission. The optimal distance between the two charges appears to be 8-10 methylene (or the equivalent) units (see Table 2 for a specific change in toxicity as a function of increasing methylene units) [3]. One additional structural similarity among the more potent compounds is the requirement for an aromatic ring with two substituents in the ortho position. The aromatic ring can be a picolinic quaternary amine or a benzylic quaternary amine with the quaternary amine ortho to a dimethylcarbamoxy functional group (Figure 9). These common structural characteristics are found in the first 16 patents shown in Table 1. The dimethylcarbamoxy functional group is similar to the well known pryidostigmine (Figure 7), a compound used to protect acetylcholinesterase by forming a temporary enzyme-carbamate that subsequently hydrolyzes to yield an active acetylcholinesterase [36]. It is not known if these compounds participate in the transfer of a carbamate residue to an appropriate nucleophile in the receptor or if they participate in hydrogen bonding within the receptor. A second aromatic ring (pyridinyl or phenyl) near the second quaternary amine is not required for enhanced lethality as suggested by structures that do not have this feature and have simple alkyl substituents.

Table 2. LD_{50} toxicity of bis (dimethyl)carbamoxypyridine derivatives with spacing of 1-9 methylene groups.

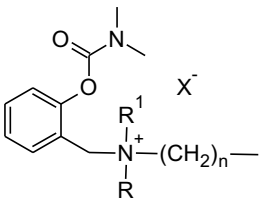
Compound [21]	n, X^-	i.v LD_{50} (µg/kg)	
		Rabbits	Mice
	1, Br^-	>20,000	>32,000
	2, Br^-	5,600	3,200
	3, Br^-	56	63
	4, Br^-	17.6	17.8
	5, Br^-	5.6	13
	6, Br^-	2.6	6.3
	7, Br^-	2.7	11
	8, Br^-	4.2	10
	8, I^-	5	10
	9, Br^-	5	9

A key structural feature of any quaternary ammonium compound that binds to muscarinic or nicotinic receptor sites is the relative spacing between the positively charged ammonium group and an electron-donating functional group (e.g. carbonyl oxygen) that forms a hydrogen bond to the receptor [38]. The optimal distance for compounds binding to nicotinic receptors is about 5.9 Å while the optimal distance between charges for muscarinic receptors is 4.3-4.4 Å. Although molecular models have not made and the optimal geometries have not been estimated for the compounds shown in Tables 1-3, it appears that many of the compounds described by these patents will have the appropriate geometry

and/or flexibility for either the muscarinic or nicotinic binding sites, depending on the number of spacing groups and their rigidity.



Dimethylcarbamoxy functional group ortho to the picolinic quaternary ammonium group



Dimethylcarbamoxy functional group ortho to the benzylic quaternary ammonium group

Figure 9. Picolinic or benzylic ammonium carbamate structures common to agents in Table 1.

Tertiary Amine Derivatives – Possible Lipophilic Analogs

The bisquaternary ammonium structure of these compounds plays an important role in binding of the compounds to acetylcholine receptors. However, these positively charged amines also make the compounds more water soluble which inhibits their movement across membranes. Thus, the absorption of the compounds via inhalation, ingestion or through dermal exposure is confounded by these positively charged amines. Some of the compounds shown in

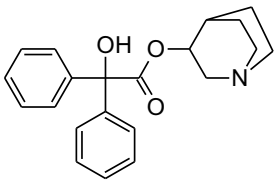


Figure 10. Figure 10. 3-Quinuclidinyl benzilate 1-azabicyclo[2.2.2]oct-3-yl 2-hydroxy-2,2-diphenylacetate

Table 1 potentially have the optimal geometries for binding to nicotinic or muscarinic acetylcholine receptors but have poor lipid solubility characteristics. It is possible that these compounds could be modified to enhance their lipid solubility and their overall absorption while maintaining a structure that binds to acetylcholine receptors. One such set of compounds would be the analogous tertiary amines instead of the positively charged, quaternary amines (Table 3). It is well known that at physiological pH (~pH 7.4) a large fraction of the tertiary amines in Table 3 would be positively charged. These compounds would have the potential to be as toxic as the parent quaternary amino compound because a significant fraction of the tertiary amines would be positively charged. Yet these compounds might have enhanced lipid solubility because a fraction of the tertiary amines would not be charged and, in this form, would be better able to cross membranes, including potentially the blood-brain barrier membrane. The idea that tertiary amines can have profound toxic nerve effects is illustrated by the well known incapacitating agent 3-quinuclidinyl benzilate (Figure 10) and other compounds [39 -41]. The extrapolation of quaternary amines to tertiary amines is a logical extension of the evaluation of quaternary amines and if one assumes quaternary amines are potential chemical weapon threats, one should also make a similar assumption for the analogous tertiary amines.

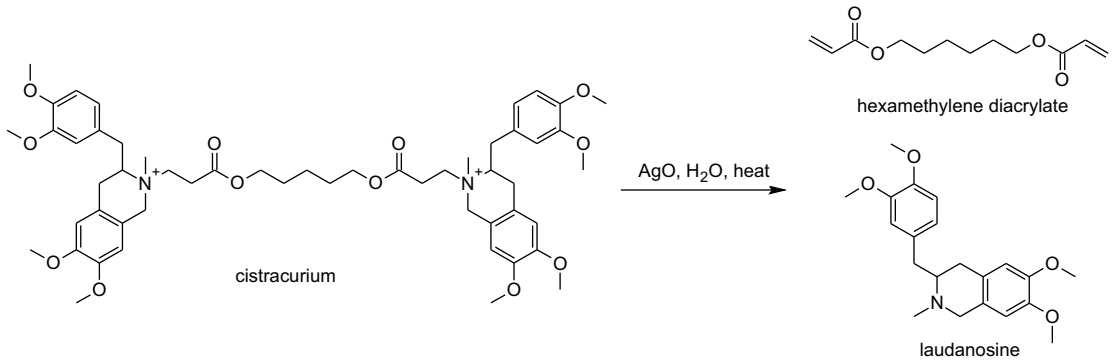


Figure 11. Example of the Hofmann elimination reaction

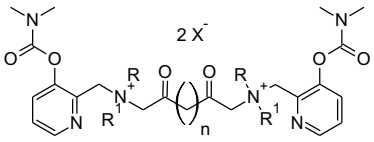
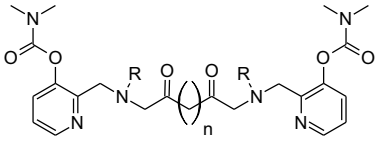
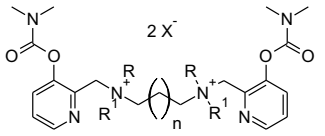
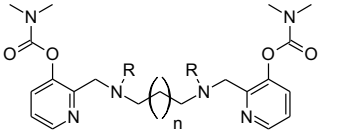
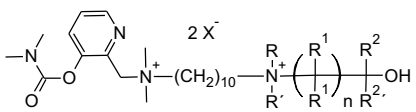
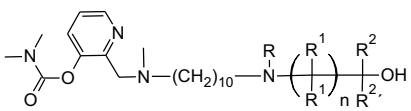
Forensic Identification

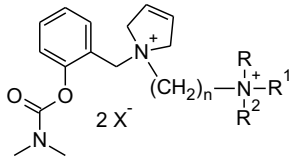
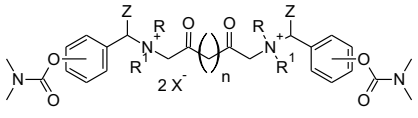
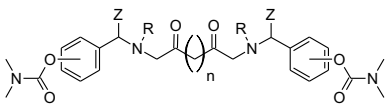
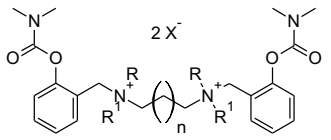
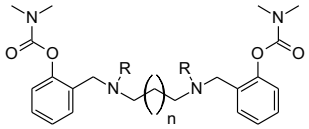
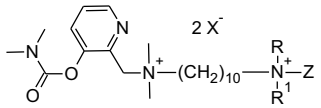
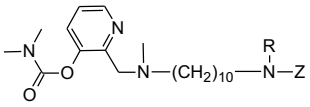
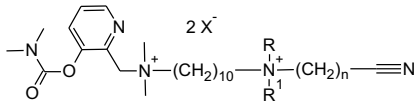
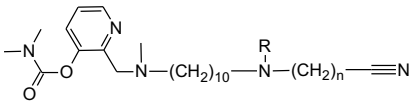
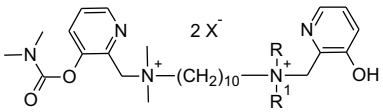
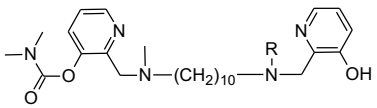
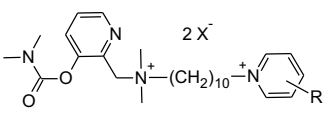
There are many reasons for wanting to identify nerve agents after an attack (or preferably before an attack occurs) including the development of antidotes (if feasible), general management/treatment of the symptoms and the identification of the perpetrators. It is likely that people exposed to sub lethal doses of these nerve agents will require a different treatment protocol than used for active site inhibitors of acetylcholinesterase that is typically found using organophosphate nerve agents.

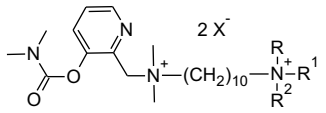
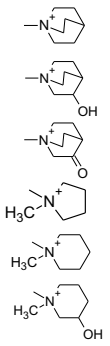
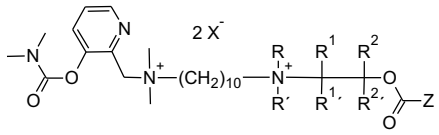
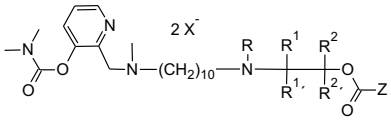
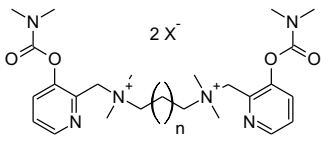
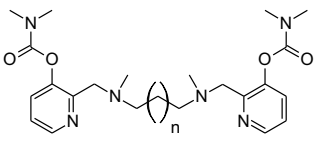
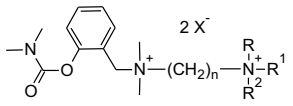
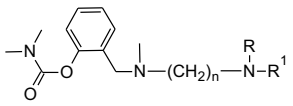
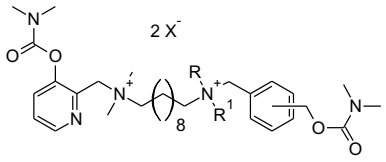
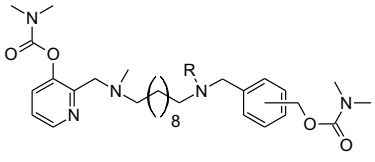
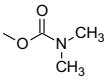
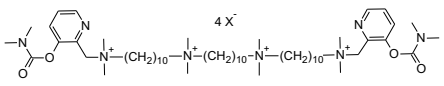
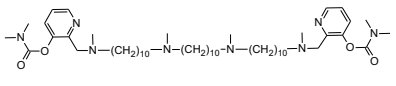
Bioassay for Acetylcholine Receptor Binding

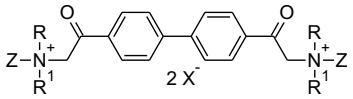
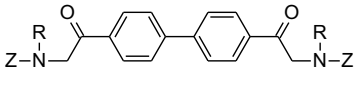
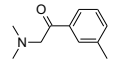
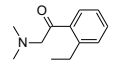
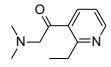
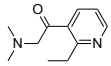
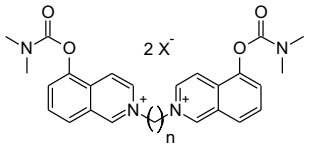
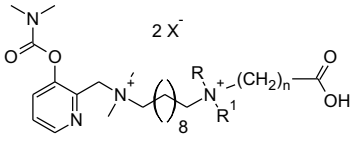
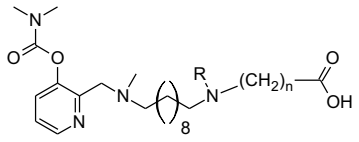
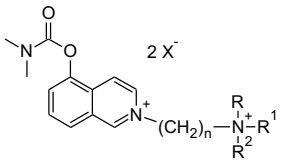
It is evident that there are a very large number of compounds (Tables 1-3 and Table 5) that have the potential to be used as chemical weapons; it will likely be every difficult to narrow down the structure of an agent to a specific compound. There are several possible approaches to identifying compounds of the type presented in this paper. In a typical scenario, only small amounts of sample, perhaps bound to clothing or swabs taken from the scene of attack, will be available for analysis. Given the large number of potential compounds and the complexity of the mass spectra, it is unlikely that GC/MS or GC/LC/MS will be able to identify these compounds. It makes sense then, to develop a method that first identified the biological target of the compound and then tailor the chemical analysis for final identification of the compound. In the case of agents that bind to the acetylcholine receptor, it would be prudent to develop a very sensitive and selective assay for these compounds so that even a small sample would reveal the primary mode of action of the compound. For example, a bioassay using isolated acetylcholine receptors (e.g. the isolation of nicotinic acetylcholine receptors from *Torpedo californica*) [42] and a radioactively labeled compound that binds to the acetylcholine receptor is feasible, sensitive and selective for this class of compounds. Thus, a small amount of sample containing one the compounds in this paper would compete for the binding site on the acetylcholine receptor releasing a radioactively labeled compound that could be detected using conventional methods. Other strategies are also possible, but the development of such assays is beyond the scope of this study. Once the assay suggests that one of the compounds in this paper is being used, one can employ other tools to help identify the compounds.

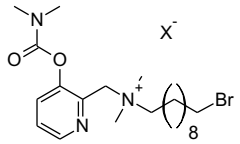
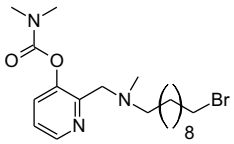
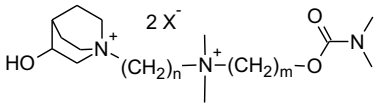
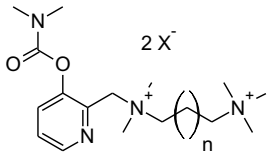
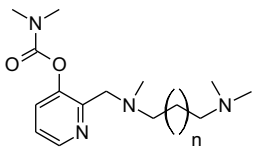
Table 3. Potential tertiary amines

Original Quaternary Amine	Proposed Tertiary Amine (if possible)	Substituents in original quaternary amine proposed for the analogous tertiary amine compound	Ref.
		R = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}, n-C_6H_{13} n = 2-12	[8]
		R = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, $C_6H_5CH_2$ n = 3-14	[9]
		R' = CH_3, C_2H_5, n-C_3H_7, C_2H_5OH R^1, $R^{1'}$= H, CH_3, C_2H_5 R^2, $R^{2'}$ = H, OH, CH_3, C_2H_5, n-C_3H_7 n = 1-8	[10]

Original Quaternary Amine	Proposed Tertiary Amine (if possible)	Substituents in original quaternary amine proposed for the analogous tertiary amine compound	Ref.
	A simple analogous compound is not possible due to heterocyclic amino group	NA[a]	[11]
		R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}, n-C_6H_{13} n = 2-12 Z = H, CH_3, C_2H_5 dimethylcarbamoxy group in O,M,P positions	[12]
		R = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 3-14	[13]
		R = C_2H_5, n-C_3H_7, n-C_4H_9 Z = C_2H_5, n-C_3H_7, n-C_4H_9 When Z = n-C_8H_{17}, n-$C_{12}H_{25}$, C_6H_{11} then R = CH_3, C_2H_5	[14]
		R = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n =1-8	[15]
		R = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}, n-C_6H_{13}	[16]
	Simple analogous compounds are not possible due to heterocyclic amino group	NA	[17]

Original Quaternary Amine	Proposed Tertiary Amine (if possible)	Substituents in original quaternary amine proposed for the analogous tertiary amine compound	Ref.
	Simple analogous compounds are not possible due to heterocyclic amino groups	When NRR^1R^2 = 	[18]
		R = CH_3, C_2H_5 R^1, $R^{1'}$, R^2, $R^{2'}$ = H, CH_3 Z = CH_3, C_2H_5, n-C_3H_7, n-C_4H_9, n-C_5H_{11}	[19, 20]
		n = 1-9	[21]
		R, R^1, = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 5-16	[22]
		R = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9  Dimethyl carbamoxy group in o, m. or p	[23]
		NA	[24]

Original Quaternary Amine	Proposed Tertiary Amine (if possible)	Substituents in original quaternary amine proposed for the analogous tertiary amine compound	Ref.
		R = CH_3 Z =  3-dimethylcarbamoxyphenyl  2-dimethylcarbamoxybenzyl  3-dimethylcarbamoxy-α-picolinyl When Z =  3-dimethylcarbamoxy-α-picolinyl Simple analogous compounds are not possible due to heterocyclic amino groups when R,R^1 combine to form  pyrrolidinio  3-pyrrolinio  piperidinio  morpholinio	[25]
	A simple analogous compound is not possible due to heterocyclic amino groups	NA	[26]
		R = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 1-9	[27]
	A simple analogous compound is not possible due to heterocyclic amino group	NA	[28]

Original Quaternary Amine	Proposed Tertiary Amine (if possible)	Substituents in original quaternary amine proposed for the analogous tertiary amine compound	Ref.
		None	[29]
	A simple analogous compound is not possible due to heterocyclic amino group	NA	[30, 31]
		n = 4-14	[32]

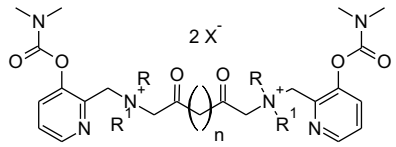
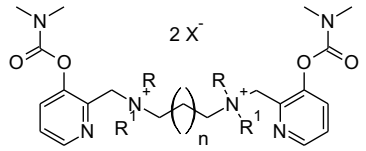
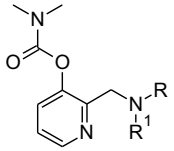
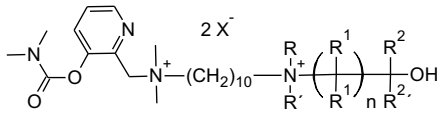
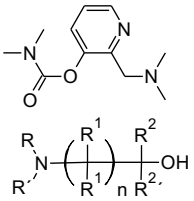
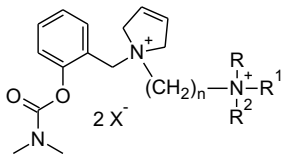
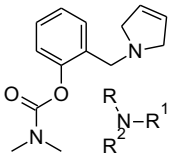
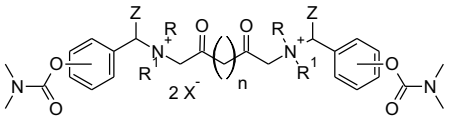
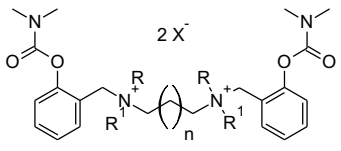
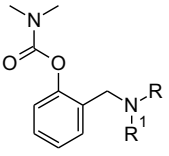
[a] NA = Not applicable

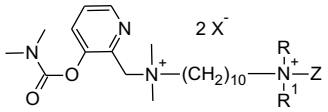
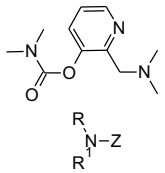
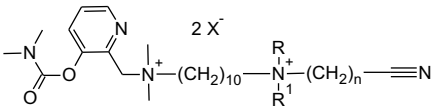
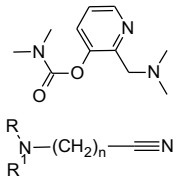
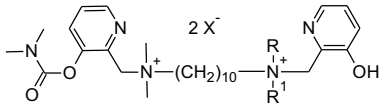
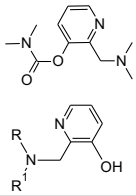
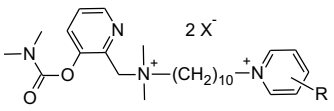
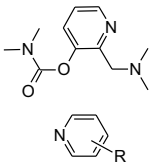
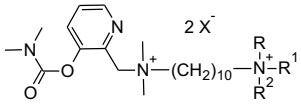
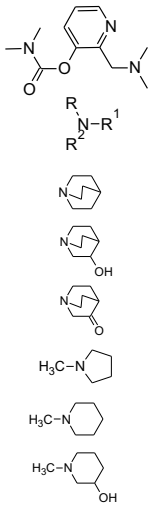
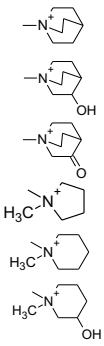
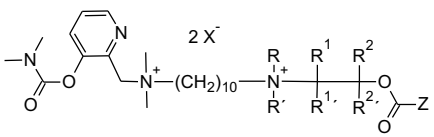
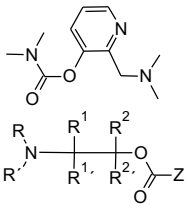
Hofmann Elimination Reaction

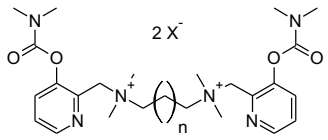
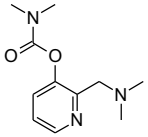
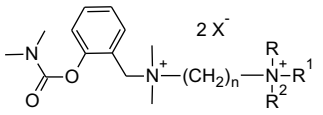
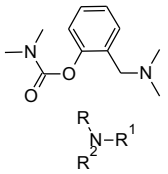
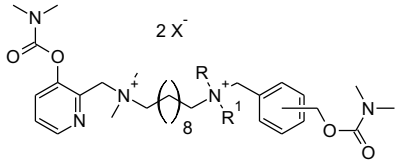
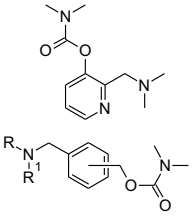
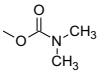
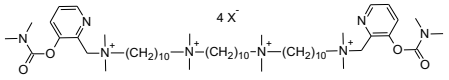
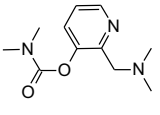
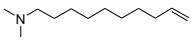
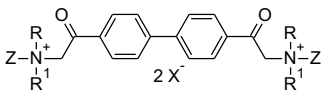
One novel tool for aiding in the identification of quaternary and tertiary amines is through the identification of products from the Hofmann elimination reaction. As discussed previously, the compounds in Table 1 are all quaternary amines and many are susceptible to the Hofmann elimination reaction. The Hofmann elimination reaction of cisatracurium (See Figure 11 for an example of the Hofmann elimination reaction [43]) proceeds spontaneously at physiological pH (pH 7.4) and in vivo. The reaction proceeds by elimination of the quaternary ammonium group yielding a double bond from the carbon containing the fewest substituents (e.g. $CH_3 > CH_2 > CHR$). Hofmann elimination products, in this example, would likely yield hexamethylene diacrylate with physical properties more amenable to analysis by conventional methods such as GC/MS. Some of the more simple Hofmann elimination products of those compounds listed in Table 1 are shown in Table 4. If a sample from a suspected nerve agent attack was subjected to conditions of the Hofmann elimination reaction and the sample was no longer positive in the acetylcholine receptor assay, this would suggest that quaternary amines were the primary class of nerve agents present in the sample. It is also possible that one of the tertiary amines (Table 3) is the nerve agent responsible. Exhaustive methylation of the tertiary amine with methyl iodide followed by the Hoffman elimination reaction would help provide evidence that tertiary amines were the class of nerve agents being used. The use of the Hofmann elimination reaction also has potential forensic benefits in that many of the alkenes, dienes and amines shown in Table 4 would be identifiable using conventional GC/MS with or without derivatization. A conclusive identification of the nerve agent responsible may not be possible because of the wide range of compounds, the expected complex pattern of the mass spectra and the very small amounts of sample available for analyses. However, a conclusive identification may not be required for many of the treatment/preventative objectives once the compound has been detected as a chemical that binds strongly to acetylcholine receptors. On the other hand, it may be prudent to begin developing a mass spectral library of all of the compounds discussed in this

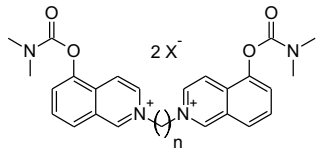
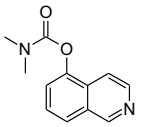
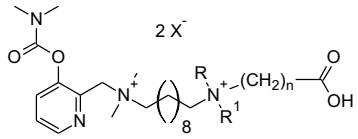
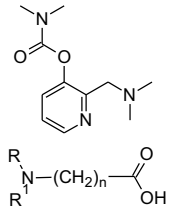
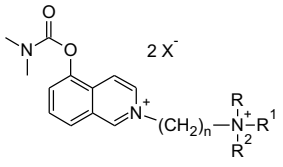
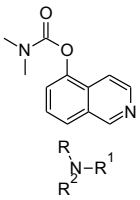
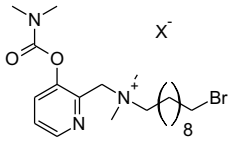
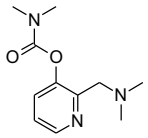
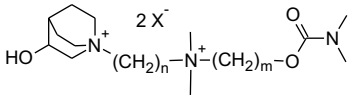
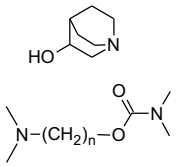
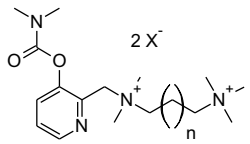
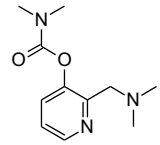
paper. The Chemical Weapons Convention treaty allows the synthesis of small amounts (less than 100 g) of agents that could be used for this purpose. There is also the possibility, however, that a foreign government, doing their own research, would have developed a bisquaternary amine or a tertiary amine different than those in Tables 1-3, 5; a library of mass spectra of known chemicals that bind to acetylcholine receptors would not be helpful in this situation.

Table 4. Possible Hofmann elimination products of quaternary ammonium salts[a]

Compound	Tertiary amine	Alkene	Substituents	Ref.
	Products complex; loss of small chain alkenes	Alkenes	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}, n-C_6H_{13} n = 2-12	[8]
		Dienes n-Alkenes	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, $C_6H_5CH_2$ n = 3-14	[9]
		1,9-Decadiene b.p. 169 °C n-Alkenes	R, R' = CH_3, C_2H_5, n-C_3H_7, C_2H_5OH R^1, $R^{1'}$= H, CH_3, C_2H_5 R^2, $R^{2'}$ = H, OH, CH_3, C_2H_5, n-C_3H_7 n = 1-8	[10, 33]
		Dienes n-Alkenes	R^1, R^2, R^3 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 5-16	[11]
	Products complex; loss of small chain alkenes	Alkenes	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}, n-C_6H_{13} n = 2-12 Z = H, CH_3, C_2H_5 dimethylcarbamoxy group in o, m, p positions	[12]
		Dienes n-Alkenes	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 3-14	[13]

Compound	Tertiary amine	Alkene	Substituents	Ref.
		1,9-Decadiene b.p. 169 °C n-Alkenes	R, R^1 = C_2H_5, n-C_3H_7, n-C_4H_9 Z = C_2H_5, n-C_3H_7, n-C_4H_9 When Z = n-C_8H_{17}, n-$C_{12}H_{25}$, C_6H_{11} then R, R_1 = CH_3, C_2H_5	[14]
		1,9-Decadiene b.p. 169 °C n-Alkene nitriles	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n =1-8	[15]
		1,9-Decadiene b.p. 169 °C n-Alkenes	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, i-C_4H_9, n-C_5H_{11}, n-C_6H_{13}	[16]
		1,9-Decadiene b.p. 169 °C	R = H, OH, CH_3, CHNOH in the o, m and p positions	[17]
		1,9-Decadiene b.p. 169 °C	NRR^{1}R^2 = 	[18]
		1,9-Decadiene b.p. 169 °C n-Alkenes	R, R' = CH_3, C_2H_5 R^1, $R^{1'}$, R^2, $R^{2'}$ = H, CH_3 Z = CH_3, C_2H_5, n-C_3H_7, n-C_4H_9, n-C_5H_{11}	[19,20]

Compound	Tertiary amine	Alkene	Substituents	Ref.
		Dienes	n = 1-9	[21]
		Dienes n-Alkenes	R^1, R^2, R^3 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 5-16	[22]
		 1,9-Decadiene b.p. 169 °C n-Alkenes	R,R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 Dimethyl carbamoxy group in o, m. or p 	[23]
		 1,9-Decadiene b.p. 169 °C  1-dimethylamino-9-decene	NA	[24]
	Products complex if reaction ocurrs at all; when R, R_1 = CH_3 and Z =  3-dimethylcarbamoxyphenyl  2-dimethylcarbamoxybenzyl  3-dimethylcarbamoxy-α-picolinyl Products complex when Z =  3-dimethylcarbamoxy-α-picolinyl and R,R_1 combine to form  pyrrolidinio  3-pyrrolinio  piperidinio  morpholinio	Hofmann elimination products complex – ring-opening of heterocyclic amines is likely when Z =  3-dimethylcarbamoxy-α-picolinyl	ND	[25]

Compound	Tertiary amine	Alkene	Substituents	Ref.
		Dienes	n = 4-16	[26]
		1,9-Decadiene b.p. 169 °C n-Alkene carboxylic acids n-Alkenes	R, R^1 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 2-9	[27]
		Dienes n-Alkenes	R^1, R^2, R^3 = CH_3, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9 n = 5-16	[28]
		Alkene bromide likely to react with AgO	NA	[29]
		Dienes n-Alkene dimethylcarbamates	m =2-6 n = 6-16	[30, 31]
		Dienes	n = 4-14	[32]

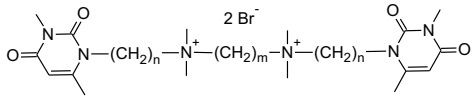
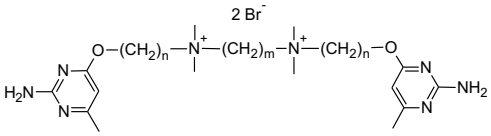
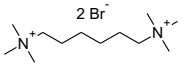
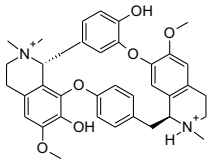
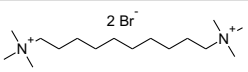
[a] Representative amines possible from the Hofmann eliminate reaction, not a comprehensive list as some of the amines can be quite complex due to elimination of alkenes from substituents bound to the tertiary amine.
ND = Not determined
NA = Not applicable

Other Studies

An update on other quaternary amines similar to those in Table 1 is illustrated in Table 5 [44]. Two oxopyrimidine structures were studied for their therapeutic potential to cause muscle relaxation while attempting to minimize the toxicity of the compounds. It is apparent from inspection that the compounds synthesized are remarkably similar to those in the U.S. Patents. The synthesis of these compounds

basically follows the methods outlined in the U.S. Patents; the synthetic methods are extremely simple with high yields and essentially no purification required. The study also reported chemical characterization using infrared and ^{1}H nuclear magnetic resonance spectra; they were able to verify the structure of their products. This paper reported on biological effects not studied in the U.S. Patents. For example, they measured the binding constant of these compounds to nicotinic acetylcholine receptors and compared their results with well known antagonists of these receptors (tubocurarine, hexamethonium and decamethonium). One set of oxopyrimidines binds almost as effectively as tubocurarine when the spacing between the positively charged ammonium groups is about 10 methylene groups. They measured the relative toxicity of their compounds using intraperitoneal (i.p.) injections compared to intravenous injections (i.v.) in the U.S. Patents. The toxicity of these oxopyrimidines were much less toxic (mg/kg; on the order of 100-1000 times less toxic) than the compounds in the U.S Patents (ug/kg). This maybe due to a lower inherent toxicity or could reflect the route of exposure since i.p. injections would require these compounds to cross membranes to enter systemic circulation where they could reach neuromuscular junctions. The authors also tested to see if these compounds inhibited acetylcholinesterase; there was no inhibition of this enzyme as expected based on the hypothesized mode of action of these compounds.

Table 5. Structure and biological effects of oxopyrimidinyl bisquaternary amines (Zobov et al. 2004).

Compound	Substituent			pK_B[a]	pI_{50}[b]	LD_{50}[c], i.p. Mice (mg/kg)	Myorelaxant Activity ED_{50}[d] (mg/kg)
	n	m	MW (g/mole)				
	3	6	694.5	5.2	3.0	45	15.0
	4	6	722.6	4.7	3.4	20	6.5
	5	6	750.7	<4.0	4.0	20	4.0
	6	6	778.7	5.8	4.1	15	3.0
	7	6	806.8	5.6	4.1	15	2.2
	3	10	750.7	5.8	3.2	5.0	2.0
	4	10	778.7	5.8	4.3	4.5	1.5
	6	10	834.9	6.2	4.9	5.0	1.7
	7	10	862.9	5.9	4.8	7.0	2.2
	2	6	636.5	ND	3.0	7.8	1.6
	3	6	664.5	6.4	4.4	7.0	2.0
	2	10	692.6	8.0	4.1	3.1	0.8
	3	10	720.6	8.1	4.2	4.0	1.2
 Hexamethonium dibromide	NA	NA	362.2	ND	<3.0	120	25.0
 d-Tubocurarine chloride	NA	NA	624.8	8.9	<3.0	0.42	0.2
 Decamethonium bromide	NA	NA	418.3	ND	<3.0	4.0	0.6

[a] pK_b = negative log of the dissociation constant for binding of the compound to nicotinic acetylcholine receptors from frog abdominal muscles.
[b] pI_{50} = negative log of the concentration of the compound causing 50% inhibition of human erythrocyte acetylcholinesterase.
[c] LD_{50} = dose of the compound causing mortality in 50% of the mice tested.
[d] ED_{50} = effective concentration of the compound that causes myorelaxation (inability to perform standard tretbahn test for 30 minutes) in 50% of the mice tested.
ND = not determined.

SUMMARY

This paper has systematically reviewed a number of bisquaternary amines that could be potentially deadly nerve agents. Presumably, these agents would function by blocking the effect of acetylcholine by binding to acetylcholine receptors and stopping nerve transmission. Analogous tertiary amines, based on the structure of the parent bisquaternary amines, have been proposed as compounds that might be more soluble in membranes making them more bioavailable and toxic. Methods have been discussed that could identify this class of compounds using a bioassay for binding to the acetylcholine receptor. A tool in the identification of these compunds, the Hofmann elimination reaction, has been proposed as a novel method for helping to establish that quaternary or tertiary amines are functional groups of the nerve agent. Finally, other studies on the development of similar compounds were presented.

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