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REVIEW ARTICLE

DUAL USE RESEARCH OF CONCERN: DERIVATIVES OF 3-QUINUCLIDINYL BENZILATE (BZ)

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Summary

Dual Use Research of Concern (DURC) deals with the unintended consequences of research and development and is particularly acute in the area of drug development. The United States National Science Advisory Board for Biosecurity (NSABB) has defined DURC as "research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health, agriculture, plants, animals, the environment, or materiel" (1). One particular receptor antagonist and glycolate anticholinergic compound (BZ, QNB or 3-quinuclidinyl benzilate) was stockpiled by the United States as a non-lethal chemical weapon that incapacitates and severely degrades the capability of exposed individuals (2). Given time and medical treatment, combatants and non-combatants exposed to such incapacitating agents could recover without any long-term effects. The purpose of this review is to identify potential new and more potent incapacitating agents based on the structures of BZ or atropine using peer reviewed publications of new pharmaceutical agents. A number of peer reviewed studies have reported on compounds with effects observed at lower concentrations than or comparable to BZ suggesting that these compounds could also be developed as potential chemical incapacitating chemical warfare agents and represent good examples of the principal of Dual Use Research of Concern.

Key words: Dual Use Research of Concern; BZ; QNB; 3-Quinuclidinyl Benzilate; incapacitating agents; chemical warfare; muscarinic acetylcholine receptor antagonists

INTRODUCTION

Dual Use Research of Concern (DURC) is a recent principle that is concerned with the unintended consequences of research and development in almost all areas of science. The issue is particularly acute in the areas of biotechnology (e.g. bioengineering and gene modification) and drug development. The United States National Science Advisory Board for Biosecurity (NSABB) has defined DURC as "research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health, agriculture, plants, animals, the environment, or materiel" (1).

While biotechnology research can have clear beneficial applications there are potentially less obvious military applications. Recently, the advent of sophisticated biotechnology research has led to the possibility that DURC could be used to generate bioweapons

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based on adapting well known pathogenic organisms, primarily viruses (3). Another less well known area of DURC is in the area of drug development. This paper will focus on one aspect of new drug development. Specifically, new drugs are being investigated for their ability to be antagonists to different neurological receptor sites thereby blocking the normal function of these receptors sites for therapeutic reasons. One particular receptor antagonist and glycolate anticholinergic compound (3-quinuclidinyl benzilate) was stockpiled by the United States as a non-lethal chemical weapon that incapacitates and severely degrades the capability of exposed individuals (2). Given time and medical treatment, combatants and non-combatants exposed to such incapacitating agents could recover without any long-term effects. This agent is included in the Chemical Weapons Convention (4,5) and has been or is in the process of being destroyed by those countries that have stockpiled the agent and signed the treaty.

Quinuclidinyl benzilate

Quinuclidinyl benzilate (Figure 1), abbreviated as BZ (used in this paper) by the chemical weapons community or QNB by the drug-development community, is a compound originally developed to be an antispasmodic agent or to treat gastrointestinal spasms (6). BZ was further developed into a non-lethal incapacitating agent designed to be used against opposing forces in a modern-day battle. BZ can be absorbed from inhaling an aerosol of the compound or can be ingested. If the compound is dissolved in a suitable solvent, there is also the possibility that BZ could be absorbed through the skin. The onset of symptoms from BZ exposure is dose dependent and typically take hours for the full effects to be manifested. A typical exposure of ~ 7 µg/kg (~0.56 mg for an 80 kg male) to BZ leads to the following effects (7-9):

- Early central nervous system (CNS) effects include slurring of speech, dizziness, headache, nausea, ataxia (failure of muscle coordination), and weakness in the legs.
- During the first 4 hours an exposed individual will feel discomfort and subjective feelings of apprehension, restlessness with occasional clonic spasms. Some individuals will start to "flap" their arms in a bird-like fashion. Individuals will show errors in speech and will be confused.
- Physiological symptoms include: dryness of mouth, tachycardia (irregular heart beat) at rest, elevated temperature, flushed face, blurred vision, and dilated pupils (papillary dilation).
- From 4-12 hours, the exposed individual becomes sedate, shows signs of stupor or may develop semi-comatose symptoms. The individual may appear to sleep and can be aroused with only strong stimulation. The subject may mutter incoherently, grope or crawl and sometimes the individual will attempt to move through obstacles known as "obstinate progression."
- After 12 hours, exposed individuals begin to hallucinate to the point that real objects and persons are ignored. Individuals are sensitive to touch and continuously grope and explore clothes, walls, beds and in general, their local environment.
- Symptoms generally subside over time and the individual begins to accept food, water, and will follow simple instructions. The individual is generally quite tractable at this stage but may show aggressive behavior if annoyed.
- During the recovery phase, the exposed individual will begin to appear rational and coherent and may deny that he is impaired in any fashion. The subject may feel paranoid. Finally, if the symptoms last more than a day, the subject may have a period of deep sleep. Subjects fully recover from the exposure to BZ.

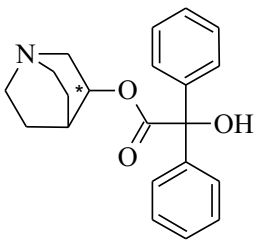


Figure 1. 3-Quinuclidinyl Benzilate (BZ or QNB)
(1-azabicyclo[2.2.2]oct-3-yl 2-hydroxy-2,2-diphenylacetate; * = chiral carbon)

BZ was first synthesized and described by chemists from the laboratories of Hoffman-La Roche in 1952 (10-12). The pharmacologic activities of several compounds were measured using isolated rabbit intestine by measuring the relaxation produced by the compound in question from a spasm induced by a known concentration of acetylcholine. Potency was estimated by comparison to the dose of atropine (Figure 2) which caused a relaxation response. The racemic mixture (the 3rd carbon on the quinuclidinol moiety is a chiral carbon, noted by a star in Figure 1) of the benzilic acid ester of 3-quinuclidinol (BZ) was twice as potent as atropine in relaxing the spasm induced by acetylcholine (11).

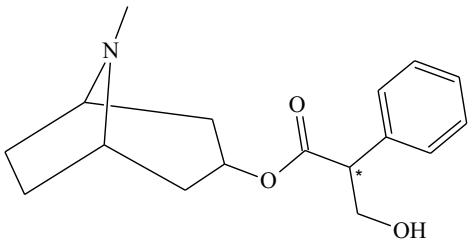


Figure 2. Atropine
(RS)-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl) 3-hydroxy-2-phenylpropanoate; * = chiral carbon)

BZ and similar compounds were investigated for their potential as incapacitating agents for deployment as chemical weapons. The United States stockpiled thousands of tons of BZ for chemical warfare usage, but most if not all of the stockpile has been destroyed (2,13). It is not clear from the open literature if BZ or other similar incapacitating agents were ever developed for use as chemical weapons by other governments. After further research with US Army volunteers, BZ was not considered a viable incapacitating agent due to a wide variability in effects, a long onset time and an inefficient method of delivering the chemical (2,7,13). However, other muscarinic antagonists were deemed more suitable as incapacitating agents, but required further research and development (7,13). There are no publically available reports suggesting that BZ has ever been used by the United States nor has the military of United States been exposed to this or similar agents.

Background on Muscarinic Acetylcholine Receptors

Physiology of Muscarinic Receptors

A brief, but not comprehensive, review of the physiology and biochemistry of muscarinic receptors is discussed below. Muscarinic receptors are widely distributed throughout the human body and mediate distinct physiological functions according to location and receptor subtype (14). Muscarinic receptors are located primarily in the parasympathetic autonomically innervated visceral organs, piloerector muscles, parasympathetic nervous system and post and pre-synaptically in the CNS (15). Specifically, muscarinic receptors associated with the parasympathetic nervous system, a subsystem of the peripheral nervous system, innervates the following organs systems: the eye, heart, respiratory system, skin, gastrointestinal tract, and bladder. In the CNS, muscarinic receptors are involved in motor control, temperature and cardiovascular regulation, and memory.

Five different muscarinic receptor subtypes (M1–M5) are well known. However, the precise location and function of these receptor subtypes is incomplete and not yet completely characterized. The following organ systems have been studied for the presence and distribution of subtypes of muscarinic receptors:

Bladder - Under normal conditions, human detrusor contractility is predominantly under the control of the parasympathetic nervous system, where the primary input is via acetylcholine acting on the M3 muscarinic receptors with a limited involvement of the M2 receptor (16).

Salivary Glands - Studies report that M1 and M3 receptors are present in the salivary glands in humans and rodents. The parotid glands, however, are dominated primarily by the M3 receptors (17-19).

Gastrointestinal Tract - The primary receptors in the smooth muscle of the human GI tract are the M2 and M3 receptors. In the guinea pig, all five receptors have been identified (20).

Brain - All five muscarinic receptors are found in the human brain and activate a number of signaling pathways important in the regulation of neuronal activity. The muscarinic receptor subtypes are not uniformly distributed among the different brain regions and include the following distributions (21):

1. M1 receptors are primarily found in the neocortex, hippocampus and neostriatum.
2. M2 receptors are found throughout the brain.
3. M3 receptors are generally low in numbers throughout the brain.
4. M4 receptors are the primary muscarinic receptor found in the neostriatum.
5. M5 receptors are primary muscarinic receptors found in the substantia nigra, pars compacta, ventral tegmental area and the hippocampus.

The Eye - All five muscarinic receptor subtypes are found in the human eye but the M3 receptor predominates in ciliary processes and the iris sphincter. M3 receptors mediate contractile responses in the iris sphincter of humans (22,23). The role of the M3 muscarinic receptor in controlling the contraction of the iris sphincter has been documented in M3 muscarinic receptor knockout mice (24,25).

The Heart - The activation of the M2 muscarinic subtype in the mammalian heart reduces the force of contraction and a decrease in the rate of beating. As expected, the use of M2 antagonists will increase the force of the contraction and stimulate the heart beating rate. (14,26,27).

Biochemistry of Muscarinic Receptors.

Muscarinic receptors are the largest class of membrane proteins in humans and are well known as either G protein coupled receptors or 7 transmembrane receptors and are found primarily in parasympathetically innervated visceral organs. The 7 transmembrane receptor is composed of seven hydrophobic transmembrane segments with an extracellular amino terminus and an intracellular carboxylic acid terminus. The extracellular amino acid terminus and the intracellular carboxyl terminus are regions of greatest diversity among 7 transmembrane receptors (28,29). Acetylcholine or other muscarinic agonists bind to the receptor inducing a conformational change in the associated GTP-binding protein (G protein). The G protein is made up of the three subunits α, β and γ. In response to the altered conformation of the muscarinic receptor, the α subunit of the G protein releases bound guanosine diphosphate (GDP) while simultaneously binding guanosine triphosphate (GTP). The binding of GTP causes the dissociation of the α subunit from the trimeric complex. The α subunit interacts with other biochemical systems to generate specific responses. The G protein also catalyzes the hydrolysis of GTP thus ending the action of the G protein. The rate of this enzymatic reaction determines how long the G protein will be active. There are three primary responses of the G protein: the inhibition of adenylate cyclase, the stimulation of phospholipase C and the activation of K+ channels (14,30).

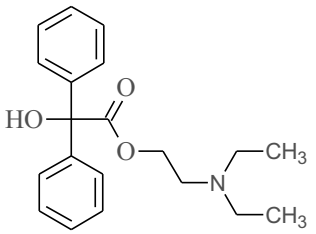
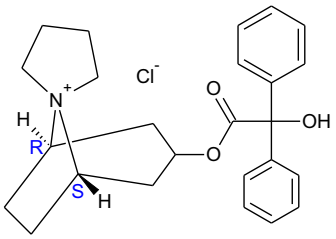
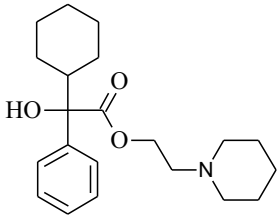
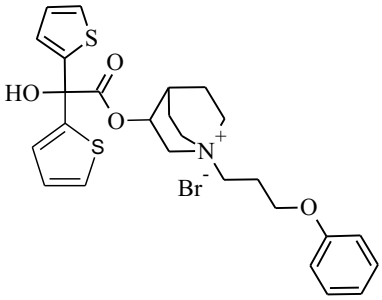
Clinical Research

Muscarinic receptor agonists and antagonists are currently being developed as potential therapeutic agents in the treatment of Alzheimer's disease, Parkinson's disease, asthma, chronic obstructive pulmonary disease, analgesia, cardiac function, disorders of intestinal motility, urinary bladder function and irritable bowel syndrome. It is beyond the scope of this paper to review all of the therapeutic drugs that have been developed based on muscarinic antagonists. The development of new muscarinic antagonists is an active field of research and some antagonists may not be suitable as therapeutic agents because they are extremely potent and demonstrate non-selective binding to the five muscarinic receptors subtypes. While not effective clinically, these compounds could be developed into incapacitating agents more powerful that BZ. Table 1 shows some representative drugs that have modified either the benzilic acid moiety (Figure 3) or the quinuclidinol portion (Figure 4) of BZ. Some drugs have combined the benzilic acid (or a derivative) and the nitrogen heterocyclic ring (tropanol) moiety of atropine (or a derivative; Figure 2) or scopolamine (Figure 5).

These examples demonstrate a wide-spread interest and research into new and novel muscarinic acetylcholine antagonists with therapeutic benefits. Because of the potential for significant side effects of these compounds, research is focused on muscarinic antagonists that are selective for one subtype of muscarinic receptors or can be modified to minimize the side effects of these drugs (e.g. quaternization of the heterocyclic amine to prevent

the drug from penetrating the blood-brain membrane barrier). It is possible that in the process of discovering new muscarinic antagonists, new and more potent incapacitating agents could also be developed.

Table 1. Examples of drugs developed from the basic structure of 3-quinuclidinyl benzilate (BZ)

Drug Name (IUPAC Name)	Structure	Comments	Reference
Benactyzine 2-(diethylamino)ethyl hydroxy(diphenyl)acetate		Retains the benzilic acid character of BZ This compound was originally developed for treatment of neurosis and depression. This compound has been proposed to treat human exposures to organophosphate nerve agents.	(31-35)
Trospium Chloride ([(1R,5S)-spiro[8-azoniabicyclo[3.2.1]octane-8,1'-azolidin-1-ium]-3-yl] 2-hydroxy-2,2-diphenylacetate;chloride)		Retains the benzilic acid character of BZ, but modifies the structure of the heterocyclic amine of atropine to a quaternary amine that cannot cross the blood-brain membrane barrier. In tissues other than the CNS, this drug binds tightly to all of the muscarinic receptor subtypes; it is not selective. This drug is prescribed for the treatment of an overactive bladder.	(36)
Trihexyphenidyl (R,S)-1-cyclohexyl-1-phenyl-3-(1-piperidyl)propan-1-ol		Changes to both the benzilic acid and the 3-quinuclinol of BZ. This drug was developed for treatment of Parkinson's disease and cerebral palsy.	(37,38)
Aclidinium Bromide [(3 R)-3-[Hydroxy (di-2-thienyl) acetyl] oxy-1-(3-phenoxypropyl)-1-azoniabi cyclo [2.2.2] octane bromide]		Benzene rings in the benzilic acid replaced with 2-thienyl moieties; the amine in 3-quinuclinol is alkylated to form a quaternary amine. Cannot penetrate the blood-brain membrane barrier. Developed as a long-acting bronchodilator used in the treatment of chronic obstructive pulmonary disease.	(39,40)

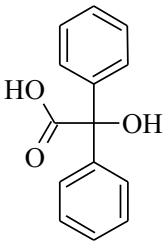


Figure 3. Benzilic acid (hydroxy(diphenyl)acetic acid)

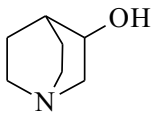


Figure 4. 3-Quinuclidinol
(1-Azabicyclo[2.2.2]octan-3-ol)

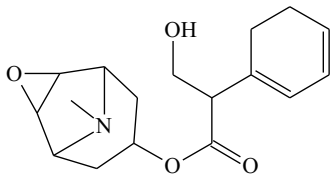


Figure 5. Scopolamine
[(–)-(*S*)-3-Hydroxy-2-phenylpropionic acid (1*R*,2*R*,4*S*,7*S*,9*S*)-9-methyl-3-oxa-9-azatricyclo[3.3.1.0]non-7-yl ester]

The purpose of this review is to identify potentially new and more potent incapacitating agents based on the structures of BZ or atropine using open source studies of new pharmaceutical agents. The scope of this review will be limited to those agents structurally similar to the two main components of BZ or atropine. For example, the derivatives of benzylic acid (Figure 3) or tropic acid (Figure 6) and their respective esters, and derivatives of 3-quinuclidinol (Figure 4) or tropanol (Figure 7) will be considered. This review will focus on tertiary amines that are able to cross the blood-brain membrane barrier, bind to muscarinic receptors and induce effects on behavior of affected individuals. This review serves as an example of the principal of Dual Use Research of Concern and will briefly discuss key papers that demonstrate the synthesis of compounds, developed for other purposes, that could have similar or greater potency than the incapacitating agent developed as a chemical weapon, BZ. This review has identified 14 papers useful to demonstrate the DURC nature in the development of muscarinic acetylcholine receptor antagonists; a summary of each of these papers is presented.

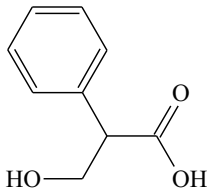


Figure 6. Tropic Acid
(3-Hydroxy-2-phenylpropanoic acid)

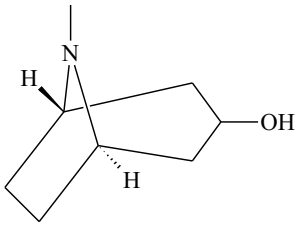


Figure 7. Tropanol
[(3-endo)-8-Methyl-8-azabicyclo[3.2.1]octan-3-ol)]

Literature Search Strategy

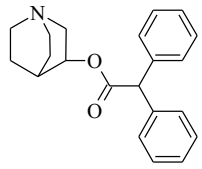
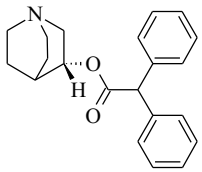
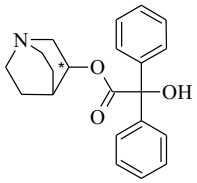
Publically available online databases (e.g. PubMed, Toxline and Google Scholar) were searched for the following words, phrases and abbreviations: 3-quninuclidinol benzilate, BZ, QNB, quinuclidinol, atropine, benzylic acid, tropanol, muscarinic antagonists, and incapacitating agents. References cited in key papers and reports were also used to identify additional studies. Retrieved papers were used in a forward search of the literature; reviewing papers that cited the paper in question. There were an overwhelming number of publications identified in this search strategy (> 5000 publications) and due to space limitations, papers included in this review were carefully selected using three primary criteria: 1) the paper contained information on: (a) the biological effects of the compounds presented, (b) measurements of the relative binding of compounds to muscarinic receptors subtypes (M1-M5), or (c) measurements of a physiologically appropriate response in tissues (e. g. guinea pig ileum), 2) compounds studied needed to be compared to one of three muscarinic antagonists: BZ, atropine, or scopolamine (preference was given to studies using BZ as the reference compound), and 3) the relative biding or the physiological responses of muscarinic antagonists had to be at least as effective (e.g. a lower concentration) as BZ, atropine or scopolamine.

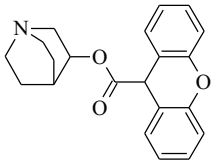
Papers are presented in chronological order starting with the earliest papers on the pharmacology of BZ. The discussion of each paper includes a table of the results using the metrics described in the paper; compounds in the table use the numbering system and nomenclature used in the paper being reviewed. The structures of BZ, atropine and scopolamine in these tables refer to Figures 1, 2, and 5, respectively.

Sternbach & Kaiser (1952)

This is the original paper that first described the synthesis and some physiological properties of BZ. The pharmacologic activities of these compounds were measured using isolated rabbit intestine by measuring the relaxation produced by the compound from a spasm induced by a known concentration of acetylcholine. Potency was estimated by comparison to the dose of atropine which caused a relaxation response. They isolated two optically active forms of the diphenyl acetic acid ester of 3-quinuclidinol and demonstrated that one isomer is much more potent. Several compounds were as potent as BZ by the assay used in this study (Table 2).

Table 2. Structure and pharmacological activity of 3-quinuclidinol esters[a]

Structure, Compound name, number	Pharmacological activity relative to atropine (1)
 Compound 3202, (±), diphenyl acetate ester	1
 Compound 4030, levorotatory, diphenyl acetate ester	2
 Compound 3308, (±), BZ, quinuclidinyl benzilate ester	2

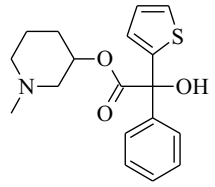
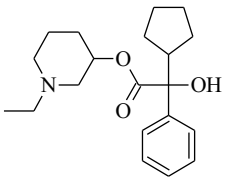
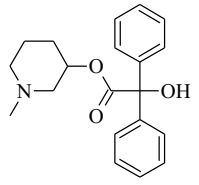
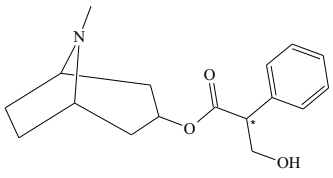
Structure, Compound name, number	Pharmacological activity relative to atropine (1)
 Compound 3208, (±), 9-fluorenecarboxylate ester	2

[a] Adapted from Table 1, Sternbach and Kaiser (11); higher values suggest more potent effects.

Biel et al. (1955)

The purpose of this paper was to synthesize new derivatives of N-substituted-2-piperidines due to the recent availability of this compound (Table 3). The present paper presents compounds and preliminary pharmacology on 3-piperidyl compounds. It is clear from this study, that cyclic alkanes and aromatic cyclic thiol ether derivatives of the benzilic acid portion of the BZ molecule can impart potencies similar to or greater than atropine.

Table 3. Most active antispasmodics N-alkyl piperidnyl derivates[a]

Structure Compound number	Antispasmodic activity of compounds to acetylcholine induced spasms of the guinea pig ileum relative to atropine
 21	2.0
 18	0.75
 13	0.60
 Atropine	1.0

[a] Adapted from Table I, Biel et al. (6); higher values suggest more potent effects.

Abood et al. (1959)

The authors initiated extensive structure-activity studies of psychotogenic compounds (n-alkyl-3-piperidyl benzilate esters) for consideration as candidate drugs for psychiatric treatments. This study attempted to correlate the anticholinergic properties of these compounds with their psychotogenic effects in humans – a rare study. Over 100 hundred subjects participated in this study comprised of 25% clinical and ambulatory patients with the remainder being medical students, laboratory and staff personnel, and nurses. Each subject was reviewed just prior to the initiation of the experiment. Subjects were screened for current or past psychiatric disease and these were eliminated from the study. No other information on consent or review by an appropriate Institutional Review Board was presented, but the study appears to follow ethical guidelines in place at the time of the study.

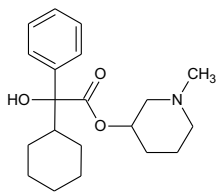
This class of compounds has psychotogenic properties including visual and auditory hallucinations. The psychotogenic effects are noted in Table 4 along with other symptoms associated with muscarinic antagonists (e.g. muscular and autonomic effects). Interestingly, compound 457 caused the greatest autonomic effects with essentially no psychotogenic effects. Compound 336 was the most effective compound at causing effects on muscular control and coordination compared to atropine. In comparison to the mydriatic effects of atropine in mice, compound 336 was the most active compound studied (Table 5).

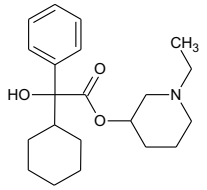
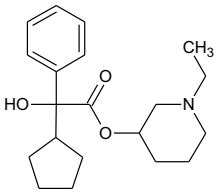
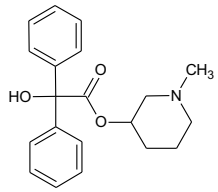
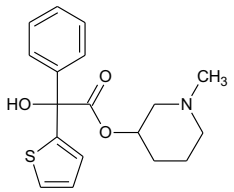
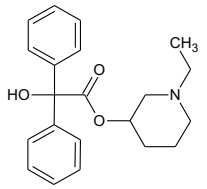
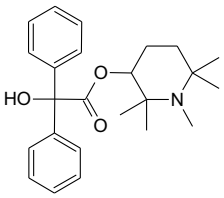
They also measured pharmacological properties in rats. All of the compounds examined in this study were considered potent anticholinergic agents. The LD_{50} and ED_{50} for these compounds are shown below (Table 5). Compound 344 (2-thienyl derivative) was the most potent anticholinergic compound based on the ED_{50} for inhibiting spasms induced by acetylcholine (Table 5). Compound 457 is 1.5 times more active than atropine in causing mydriasis in mice and is the most toxic (i.e. has the lowest LD_{50}) compound studied in rats.

Rats pretreated with physostigmine prevented mydriasis, tachycardia and hyperemia from compounds 336 and 329. This pretreatment temporarily blocks the hydrolysis of acetylcholine by inhibiting acetylcholinesterase presumably increasing the concentration of acetylcholine at muscarinic receptor sites. This suggests that acetylcholine can effectively compete for muscarinic receptor sites. However, not all symptoms are removed using physostigmine and the effects are complicated. The effect of pretreatment by physostigmine is not simple and the paper does not speculate on its effects.

The psychotogenic properties do not correlate with toxicity, antispasmodic activity, or mydriasis. The paper does not discuss this lack of correlation. The authors hypothesized that different receptor sites may have different affinities – well established at the present time. However, it is currently unknown which muscarinic subtypes (M1-5) are responsible for these psychotogenic effects. There are no papers that have specifically addressed the psychotogenic effects and relative affinity of binding of muscarinic antagonists to specific muscarinic receptors in the brain.

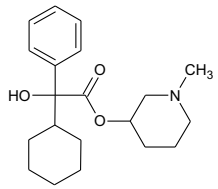
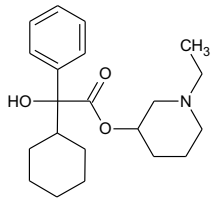
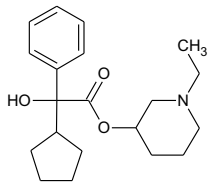
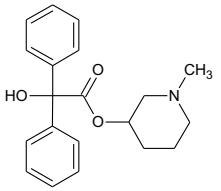
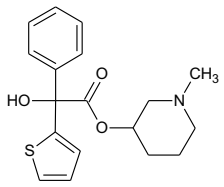
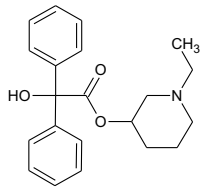
Table 4. Effectiveness of Compounds in Human Participants[a]

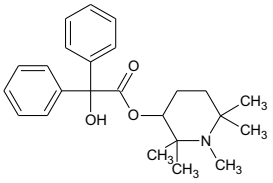
Structure, Compound Number	Dose (mg, p.o.)	Autonomic Effects[b]		Muscular Effects[c]		Psychotogenic Effects[d]	
		Magnitude	Duration (hours)	Magnitude	Duration (hours)	Magnitude	Duration (hours)
 Compound 840	10	+++	28	++	15	+++++	12

Structure, Compound Number	Dose (mg, p.o.)	Autonomic Effects[b]		Muscular Effects[c]		Psychotogenic Effects[d]	
		Magnitude	Duration (hours)	Magnitude	Duration (hours)	Magnitude	Duration (hours)
 Compound 328	5	++	8-16	+	3	+++	1-4
	10	+++	25	++	14	+++++	12
 Compound 329	10	+++	25-28	++	14	+++++	11-14
 Compound 336	5	++	6-10	++	8-12	+++	1-2
	10	+++	12-24	+++	12-24	++++	4-7
 Compound 344	10	+++	2-6	++	2-3	+++++	1
 Compound 318	10	+++	14-30	++	12-24	+++	1-5
	20	+++	16-36	+++	16-36	++++	1-10
 Compound 457	5	++++	50	++	12-14	+ –	1-2

[a] Adapted from Table II, Abood et al.(1959) (41).
[b] Autonomic Effects - mydriasis, dryness of mouth, flushing of skin, tachycardia
[c] Muscular Effects - weakness, ataxia, feeling of heaviness and lethargy
[d] Psychotogenic Effects - auditory, visual hallucinations, mood changes, disorientation, hypochondriacal and paranoid delusions

Table 5. Pharmacological properties of 3-piperidyl benzilate esters[a]

Structure, Compound Number	LD_{50}[b]		ED_{50}[c]		Mydriasis	Hyperactivity[d]
	Mice	Rats	Acetylcholine (μg/mL)	Histamine (μg/mL)	Atropine (1) Mice	Rats
 Compound 840	32	18	0.01	0.5	1/5	++++
 Compound 328	37	20	0.01	1.0	1/5	++++
 Compound 329	44	19	0.003	0.4	1/4	+++
 Compound 336	40	22	0.003	0.3	2/5	++++
 Compound 344	47	20	0.001	0.03	1/10	++++
 Compound 318	34	25	0.006	0.85	1/4	+++

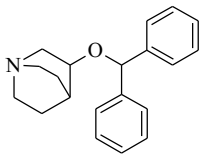
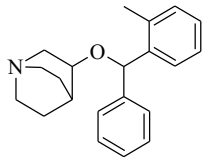
Structure, Compound Number	LD_{50}[b]		ED_{50}[c]		Mydriasis	Hyperactivity[d]
	Mice	Rats	Acetylcholine (μg/mL)	Histamine (μg/mL)	Atropine (1) Mice	Rats
 Compound 457	ND[e]	14	0.02	ND	1.5	+++

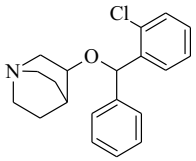
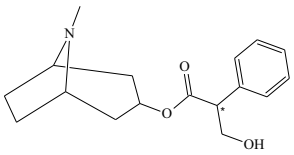
[a] Adapted from Table III, Abood et al.(1959) (41)
[b] LD_{50}, lethal dose causing an estimated 50% mortality from i.v. injection.
[c] ED_{50}, effective dose causing 50% blockage of contraction of the guinea pig ileum from 0.1 μg/mL of acetylcholine or histamine.
[d] Hyperactivity – increased in frequency and amplitude of vertical head movements.
[e] ND – not determined.

Lars et al. (1969) (42)

Lars et al. (1969) (42) investigated a series of quinuclidinyl benzhydryl ethers for potential use in the treatment of Parkinson's disease. Benzhydryl amino alcohols are a well established class of anti-histamine compounds such as diphenhydramine or for use in treatment of Parkinson's syndrome. The compounds tested in this study (Table 6) were examined for their anticholinergic effects using an assay to detect the mydriatic and tremorolytic dose; none of these compounds were more potent than atropine. The effects of these compounds on the acetylcholine induced spasms in guinea pig ileum suggest that compounds 1-3 were comparable to the antagonistic effects of atropine.

Table 6. Quinuclidinyl benzhydryl ethers[a]

Compound	Mydriatic dose in mice compared to atropine[b,c]	Tremorolytic dose in mice compared to atropine[b,d]	Effect of compound on acetylcholine induced spasmolysis from isolated guinea pig ileum compared to atropine[e]
 Compound 1	5.3	3.2	0.5
 Compound 2	6.7	2.6	0.8

Compound	Mydriatic dose in mice compared to atropine[b,c]	Tremorolytic dose in mice compared to atropine[b,d]	Effect of compound on acetylcholine induced spasmolysis from isolated guinea pig ileum compared to atropinee
 Compound 3	7.3	3.7	1
 Atropine		1	

[a] Adapted from Tables II and III from Lars et al. (1969) (42).
[b] Lower value suggests more potent effects.
[c] Mydriatic assay measured the amount of compound needed to double the size of the pupil in mice.
[d] The tremorolytic assay measured the amount of compound giving 2-fold protection against oxotremorine.
[e] Higher value suggests more potent effects

Albanus (1970) (43)

This is one of the few studies that investigated the behavioral effects of muscarinic acetylcholine antagonists in animals; most studies measured effects on receptors isolated from tissues, or in later studies, the binding of these compounds to cells that have incorporated specific subtype receptors. All of the compounds (Table 7) showed similar behavioral effects after s.c. injection of the compound, but at different doses and different lengths of time required for the onset of the first effect. The symptoms for atropine injections were given as representative of all 13 compounds (Table 7). Within 40 minutes of injection, the dogs gait lost its normal elasticity and the hind legs became stiff (ataxia). In active dogs, their movements became slow. Later on, quick ear movement and head jerks became apparent. Some dogs started chewing and stroked their nose with a paw or forearm. They became interested in details of their surroundings and sometimes would try to catch invisible flies or appear to step over unseen objects. Their response to their human handlers was notably diminished. These symptoms became more severe with time and as the intoxication reached its peak. All dogs vomited 1-4 times (possibly reflecting effects on the GI tract). All of these compounds induced a marked decreased salivation and light reflex to a standardized light stimulus. Scopolamine elicited similar effects as atropine but at a concentration about 10 fold less.

These compounds can be divided into 3 classes based on their effects on behavior (Table 7). The most active compounds were the quinuclidinyl esters, compounds 8, 10, 11 and 12. The author grouped compounds 2,7 and 9 as being as potent as the first group but of shorter duration of effects. The third group, compounds 1, 3, 4, 5, and 6 did not cause the full anticholinergic syndrome at a dose of 0.05 mg/kg but did so at a dose 10 times higher (0.5 mg/kg).

The effect of these compounds on heart rate was also measured. Injection of atropine (0.5 mg/kg), scopolamine (0.05 mg/kg) or compound 11 (0.01 mg/kg) caused an immediate increase in heart rate (90%, 55% and 98%, respectively). The heart rate increase declined over time; atropine and scopolamine declined below base line levels after 280 and 200 minutes, respectively. Compound 11, however, remained high (35%) even after 320 minutes. Thus, compound 11 is more active, stays active longer and

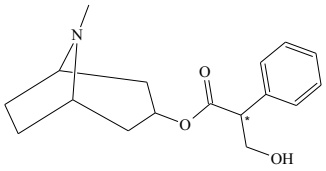
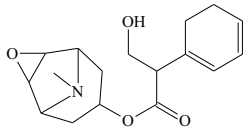
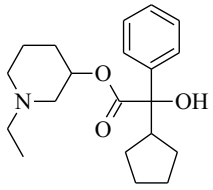
is effective at a lower dose than either atropine or scopolamine.

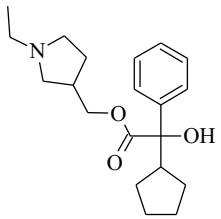
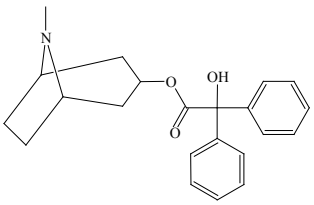
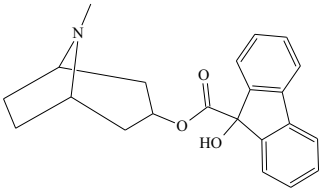
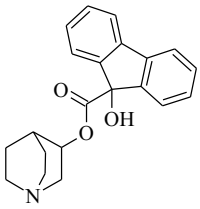
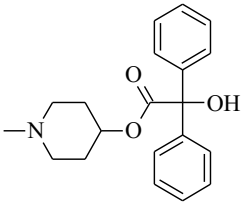
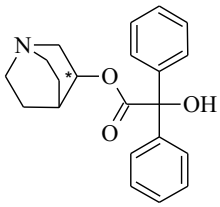
The dose-response effects of compound 10 and atropine are shown in Table 8. Compound 10 at a dose of 0.01 mg/kg is as effective as 0.5 mg/kg atropine. By this criteria, compound 10 is 50 times more potent than atropine. This relative potency is highly variable and depends on the metric used to calculate the potency.

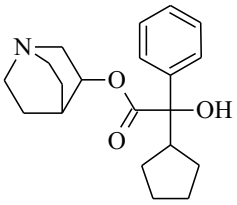
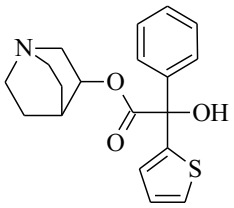
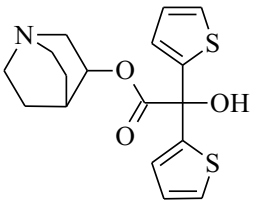
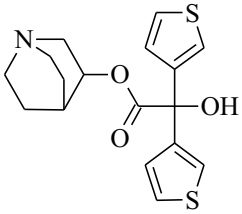
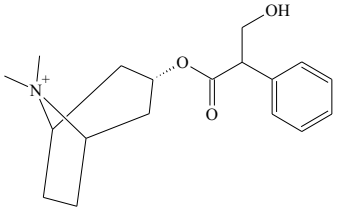
These compounds had measurable effects on salivation, mydriasis and blood pressure. All treated dogs showed depressed salivation, the degree to which is highly variable. All compounds showed mydriatic effects in dogs with compounds 8, 10, 11 and 12 being most active at low doses. The slope of the dose response curve for these compounds were similar to each other and much steeper than the other compounds with the exception of compound 9. Compound 9 showed mydriatic effects at higher concentrations that the other class 1 compounds. Compounds 8, 10, 11, and 12 continued to demonstrate mydriasis over a 6 hour time period post injection in mice. The other compounds showed increasing mydriatic effects initially, but then mydriasis quickly decreased with time.

Acetylcholine injections (i.v.) causes a marked increase in blood pressure. These compounds were tested for their ability to inhibit this increase in blood pressure. Compounds 8, 10, and 11 almost obliterated this increase in blood pressure. These results suggest that the varied effects of these compounds among different physiological endpoints is due to compounds with different receptor subtype specificities. The M1-M5 receptor subtypes were not known at the time of this study.

Table 7. Structures of compounds and behavioral effects[a]

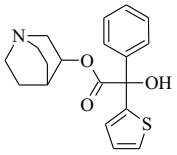
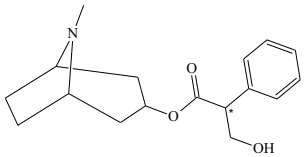
Structure, number	Name	Ataxia, max. dose injected, mg/kg; time for effect to appear (min)	No-retreating behavior, max. dose injected, mg/kg; time for effect to appear (min)
 1	Atropine	2.5 (16)	2.5 (31)
 2	Scopolamine	0.50 (11)	0.50 (16)
 3	Ditran: N-ethyl-3-piperidyl cyclopentylphenyl glycolate, 30%	0.50 (22)	0.50 (31)

Structure, number	Name	Ataxia, max. dose injected, mg/kg; time for effect to appear (min)	No-retreating behavior, max. dose injected, mg/kg; time for effect to appear (min)
 3	N-ethyl-2-pyrrolidyl-methyl cyclopentylphenyl glycolate, 70 %	0.50 (22)	0.50 (31)
 4	3-tropyl benzilate	0.50 (16)	0.50 (19)
 5	3-tropyl-9-hydroxyl-9-fluorene carboxylate	0.50 (31)	0.50 (56)
 6	3-quinuclidinyl-9-hydroxy-9-fluorene carboxylate	0.50 (30)	0.50 (41)
 7	1-methyl-4-piperidyl benzilate	0.50 (16)	0.50 (34)
 8	QNB, BZ, 2-quinuclidinyl benzilate	0.05 (36)	0.50 (42)

Structure, number	Name	Ataxia, max. dose injected, mg/kg; time for effect to appear (min)	No-retreating behavior, max. dose injected, mg/kg; time for effect to appear (min)
 9	3-quinuclidinyl-cyclopentylphenyl glycolate	0.50 (13)	0.50 (16)
 10	3-quinuclidinyl-2-thienylphenyl glycolate	0.05 (19)	0.05 (27)
 11	3-quinuclidinyl-2,2-bis (2,2'-thienyl) glycolate	0.55 (9)	0.55 (17)
 12	3-quinuclidinyl-2,2 bis (3,3'-thienyl) glycolate	0.05 (25)	0.05 (30)
 13	N-methyl atropine	20 (72)	20 (84)

[a] Adapted from Table 1, Albanus (1970)(43).

Table 8. Dose-response effects of atropine and compound 10 on heart rate and behavior[a]

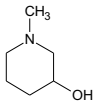
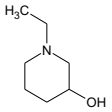
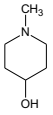
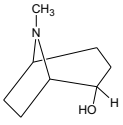
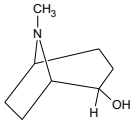
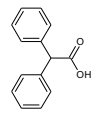
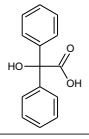
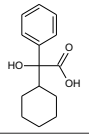
Compound	Dose, s.c. inj. (mg/kg)	Maximal heart rate (% increase over baseline)	Behavior
 Compound 10 3-quinuclidinyl-2-thienylphenyl glycolate	0.005	20	Slight ataxia
	0.01	96	Ataxia and non-retreating
	0.05	147	Ataxia and non-retreating
 Compound 1 Atropine	0.1	61	No ataxia
	0.3	76	Ataxia and some non-retreating
	0.5	89	Ataxia and non-retreating
	2.5	110	Ataxia and non-retreating

[a] Adapted from Table 4, Albanus(1970) (43).

Abramson et al. (1974) (44).

In this paper, the authors examined a range of tertiary amines and their effects on postganglionic acetylcholine receptors of the guinea pig ileum (Table 9). The goal of this study was to be able to predict the binding of new compounds to muscarinic acetylcholine receptors. The procedure for measuring the affinity constant, K_a, was described earlier (45). Several compounds were very potent in this assay including BZ and the cyclohexylphenyl glycolate ester of N-methyl-4-piperidinol.

Table 9. Mean log of the binding constant to receptors in the guinea pig ileum, K_a

	Tertiary ammonium group Log Binding Constant					
Acetyl Derivative				 Tropine	 Pseduotropine	 (±)Quinuclidin-3-ol
	6.76	ND	8.36	8.11	7.51	9.29
	8.70	8.22	9.93	9.49	8.79	10-11? (BZ; Figure 1)
	ND	ND	10.6-11?	ND	ND	ND

[a] Adapted from Table 2(b), Abramson et al. (1974) (44).

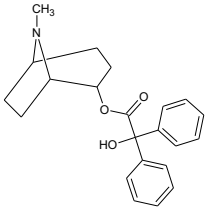
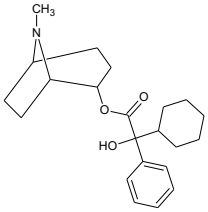
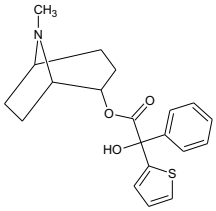
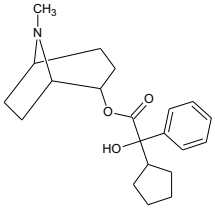
[?] = a progressive antagonism that never became steady.

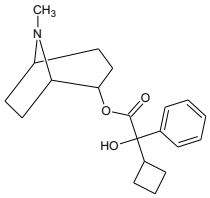
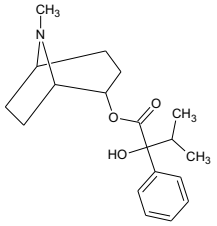
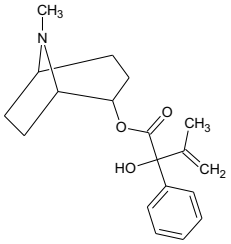
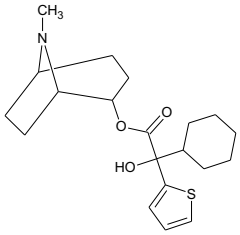
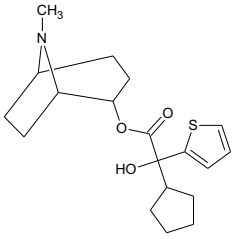
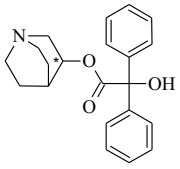
Atkinson et al.(1977) (46).

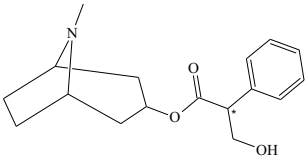
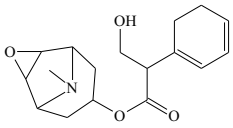
The purpose of this study was to synthesize new anticholinergic drugs for the potential treatment of Parkinson's Disease. The study focused on glycolate esters of 2-tropanol. The most active of these compounds are based on the (+)-2-tropanol isomer and these will be reviewed here. The paper measured mydriatic effects of a compound by comparing the dilation of the pupil compared to the dilation seen with 10 mg/kg atropine – the maximal dilation effect observed. The effective dose (ED_{50}) was a calculated concentration that caused 50% of the maximum dilation. Anti-tremorine effects were measured by injections of 10 mg/kg tremorine and noting the number of times the mouse crossed a beam in 30 minutes. Tremorine caused an 80% decrease in the unmediated rat crossing of the beam (normally 260 beam crossings in 30 minutes). The test compound would restore the normal level of beam crossings. The normalizing dose required to completely reverse the effect of tremorine was estimated from 3 logarithmically spaced doses.

The paper examined several optical isomers of different glycolate esters (Table 10). The (-) isomer was always more potent than the (+) isomer with the racemic mixture showing potencies between these isomers. Many of the compounds showed mydriatic and inhibition of tremors at lower doses than BZ, suggesting that some of these compounds could be more potent incapacitating agents than BZ.

Table 10. Potential anticholinergic effects of (+)-2-tropanyl glyxolate esters[a]

Compound number and structure using the (+)-2-Tropanol isomer	Optical isomer of the glycolic acid	Mydriasis, ED_{50}, sc, mouse (mg/kg)	Anti-tremorine, sc, mouse, normalizing dose (mg/kg)	LD_{50}, sc, mouse, (mg/kg)
 Compound 1 (+)	NA[b]	0.05	0.03	14
 Compound 2 (+/-), 3 (-), 4 (+)	(+/-)	0.17	0.12	ND[c]
	(-)	0.07	0.03	50
	(+)	1.1	0.77	40
 Compound 5 (-), 6 (+)	(-)	0.02	0.02	20
	(+)	0.03	0.03	20
 Compound 7 (+/-), 8 (-), 9 (+)	(+/-)	0.04	0.05	71
	(-)	0.016	0.03	50
	(+)	0.76	0.35	36

Compound number and structure using the (+)-2-Tropanol isomer	Optical isomer of the glycolic acid	Mydriasis, ED_{50}, sc, mouse (mg/kg)	Anti-tremorine, sc, mouse, normalizing dose (mg/kg)	LD_{50}, sc, mouse, (mg/kg)
 Compound 10	(+/-)	0.04	ND	56
 Compound 11 (+/-), 12 (-)	(+/-)	0.04	ND	56
	(-)	0.023	0.027	63
 Compound 13 (+/-, enriched in +), 14 (-), 15 (+/-)	(+/-, enriched in +)	0.02	0.04	50
	(-)	0.016	0.029	63
	(+/-)	0.077	0.16	50
 Compound 18 (-), 19 (+)	(-)	0.05	0.03	56
	(+)	0.18	0.20	56
 Compound 20 (+/-), 21 (-)	(+/-)	0.04	0.03	56
	(-)	0.015	0.02	71
 (+/-) BZ	(+/-)	0.05	0.16	25

Compound number and structure using the (+)-2-Tropanol isomer	Optical isomer of the glycolic acid	Mydriasis, ED_{50}, sc, mouse (mg/kg)	Anti-tremorine, sc, mouse, normalizing dose (mg/kg)	LD_{50}, sc, mouse, (mg/kg)
 Atropine	(+/-)	0.061	2.2	32
 Scopolamine	(-)	0.012	1.3	100

[a] Adapted from Table III, Atkinson et al. (1977) (46).
[b] Not applicable
[c] Not determined

NRC (1982)(47)

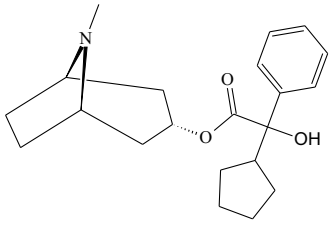
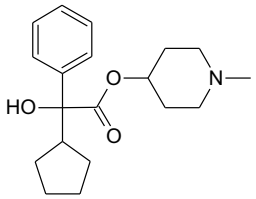
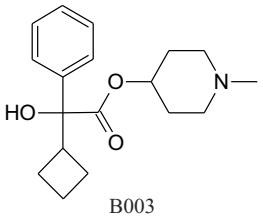
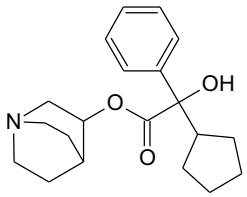
The purpose of this report is to evaluate the long-term or delayed adverse health effects of chemical agents, including BZ, tested on military volunteers from 1961 - 1971. The Board on Toxicology and Environmental Health Hazards of the National Research Council provided reports that are related to the administration of anticholinergic compounds to human volunteers. The 17 esters represented in the 58 reports cited in the NRC (1982) study belong to seven series of compounds including esters of the following acids: tropic acid, benzylic acid, phenyliso-propylglycolic acid, phenylcyclopentyl-glycolic acid, and the following alcohols: 3-quinuclidinol, N-methyl-4-piperidinol, and N-diethylaminoethanol. Compounds with chiral carbons showed effects that suggest one of the enantiomers is significantly more potent that the other enantiomer; the L isomers of esters are more potent than the D isomers. The acute physiological and psychological effects of intoxicating doses of these muscarinic antagonists will be summa-rized here (Table 11 and Table 12).

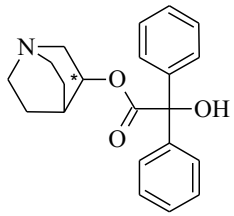
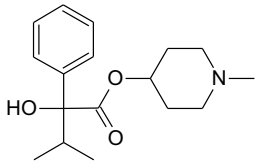
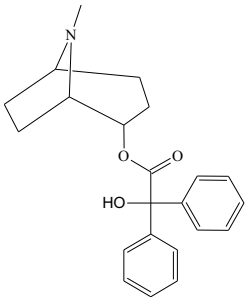
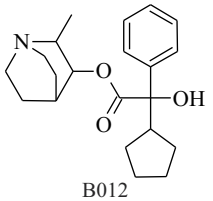
The NRC (1982) (47) descriptions of the effects induced by 3-quinuclidinyl benzilate in human volunteers are described here. At low doses, the effects include a dry mouth, decreased gastric mobility, inhibition of sweating, an increase in heart rate, pupillary dilatation and loss of accommodation, mild sedation and mental slowing. At high doses these effects are intensified. There are marked disturbances of function at all levels of the central nervous system; motor coordination, attentiveness and control of thought and learning processes all decline. Confusion, restlessness, impairment in perception, interpretation, memory span, poor judgment and deficient insight are all features of this syndrome. True hallucinations are present. If the dose is quite high the subject may demonstrate pupillary dilatation or even become comatose for several hours. An analysis of changes to human personality suggest that the effects of atropine, scopolamine and five other anticholinergic compounds on the performance of the Number Facility Test is positively related to the lie, hypochondriasis, and mania scales of the Minnesota Multiphasic Personality Index (MMPI). The greatest hazard to life arises from suppression of the ability to secrete sweat, which can give rise to fatal hyperthermia. In addition, an accel-erating heart rate may result in serious arrhythmias up to and including ventricular fibrillation.

Estimates of the doses of BZ required to be inhaled [expressed as concentration x time (C x t), $mg.min/m^3$] to produce various effects were as follows: mild incapacitation (moderate dilatation of pupils and slight blurring of vision, minimal incoordination, some slowing of thought), 66–124 $mg.min/m^3$; moderate incapacitation (hallucination, confusion, unorganized hyperactivity, incoherent speech, shortening of memory and

attention span), 102–152 mg.min/m^3; and severe incapacitation (stupor or coma, possibly preceded by a period of agitation, followed after 10–15 h by prolonged hallucination and inappropriate behavior), 110–165 mg.min/m^3. The LD_{50} for a man was estimated to be 5.7–6.7 µg/kg.

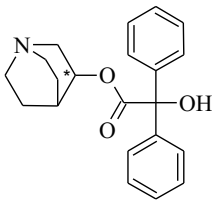
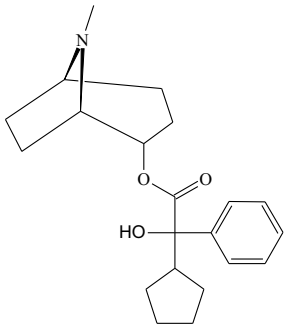
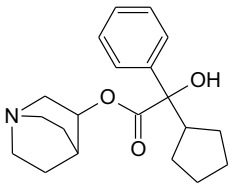
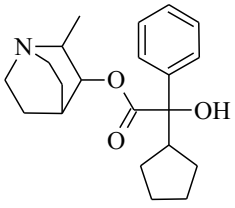
Table 11. Effects of glycolate esters on human volunteers from Edgewood Arsenal studies, 1960 – 1971.[a]

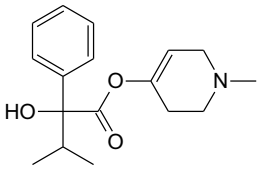
Compound, Study number, Name, Tox Number, CAS number	MED[b]	ID_{50}[b]	Relative Potency	
	µg/kg (relative to BZ; BZ=1)[e]		Peripheral Effects[d] (BZ=1)[e]	Central Effects[d] (BZ=1)[e]
 B010 L-2-α-tropinyl L-cyclopentylphenylglycolate 226086 CAS 64474-85-8	1.5 (0.65)	2.0 (0.12)	0.37 — 0.74	2.5
 B002 N-methyl-4-piperidyl cyclopentylphenylglycolate EA 3443 CAS 37830-21-0	1.2 (0.52)	3.4 (0.65)	0.43	1.6
 B003 N-methyl-4-piperidyl cyclobutylphenylglycolate EA 3580 CAS 54390-94-2	1.4 (0.61)	3.9 (0.75)	0.48	1.5
 B006 3-Quinuclidinyl cyclopentylphenylglycolate EA 3167 CAS 54390-94-2	2.8 (1.2)	4.1 (0.78)	0.37–0.74	1.4

Compound, Study number, Name, Tox Number, CAS number	MED[b]	ID_{50}[b]	Relative Potency	
	µg/kg (relative to BZ; BZ=1)[e]		Peripheral Effects[d] (BZ=1)[e]	Central Effects[d] (BZ=1)[e]
 B001 Quinuclidinyl benzilate EA 2277 CAS 6581-06-2	2.3 (1.0)	5.2 (1.0)	1.0	1.0
 B023 N-methyl-4-piperidyl isopropylphenylglycolate EA 3834 CAS (none)	2.0 (0.86)	5.7 (1.1)	0.19	0.92
 B009 L-2-α-tropinyl benzilate CS 27349, 219758 CAS 64520-33-8	3.0 (1.3)	6.0 (1.2)	0.37	0.85
 B012 Cis-2-methyl-3-quinuclidnyl cyclopentylphenylglyocolate 301060 CAS (none)	3.0 (1.3)	6.0 (1.2)	0.74	0.85

[a] NRC (1982) (47); adapted from Chapter 3, Table 3.
[b] Mean effective dose (MED) required to product 25% decrement in number facility score in 50% of volunteers; doses administer i.v. or i.m..
[c] Incapicitating dose (ID_{50}) required to product 90% decrement in number facility score in 50% of volunteers.
[d] Peripheral effects are measured as dose required to reach at least 100 heart beats/min 50% of volunteers.
[e] BZ = Quinuclidinyl benzilate (EA 2277); smaller values are more potent.

Table 12. Peripheral and Central Nervous System Effects of selected glycolate esters in human volunteers from Edgewood Arsenal studies, 1960-1971[a].

Compound, Study number, Name, Tox Number, CAS number	Dose (µg/kg)	Duration of effects (hours)	Peripheral effects[b] ; potency relative to BZ[d] (BZ=1)	Central nervous system effects[c]; potency relative to BZ[d] (BZ=1)
 B001 Quinuclidinyl benzilate EA 2277 CAS 6581-06-2	5–8	48–96	1	1
 B010 L-2-α-tropinyl L-cyclopentylphenylglycolate 226086 CAS 64474-85-8	1–2	48–72	1	1
 B006 3-Quinuclidinyl cyclopentylphenylglycolate EA 3167 CAS 54390-94-2	3–4	48–120	1	1
 B012 Cis-2-methyl-3-quinuclidnyl cyclopentylphenylglyocolate 301060 CAS (NA)[e]	3–5	48–120	1	1.11

Compound, Study number, Name, Tox Number, CAS number	Dose (µg/kg)	Duration of effects (hours)	Peripheral effects[b] ; potency relative to BZ[d] (BZ=1)	Central nervous system effects[c]; potency relative to BZ[d] (BZ=1)
 B016 4-(1-methyl-1,2,3,6-tetrahydropyridyl)-Methyl-Isopropylphenylglycolate 302668 CAS (NA)	13-18	12–48	1.25	1.25

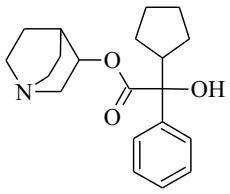
[a] NRC (1982) (47); adapted from Appendix L, Table 1
[b] Peripheral effects include mydriasis, dryness of mouth, decreased secretion of saliva; increase in heart rate; dryness and flushing of the skin.
[c] Central nervous system effects include confusion, hallucinations, memory loss and delirium.
[d] BZ =Quinuclidinyl benzilate; smaller values are more potent.
[e] NA = Not available

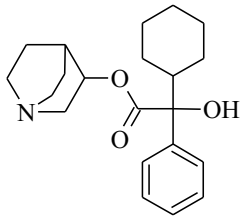
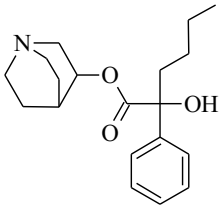
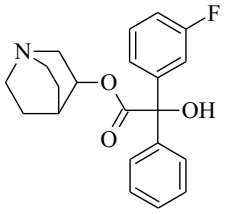
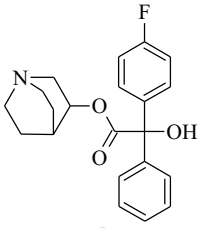
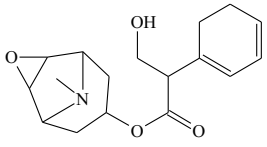
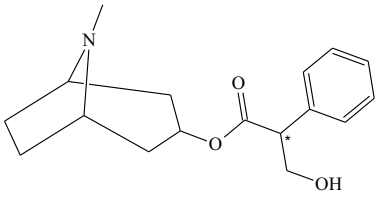
Eckelman et al. (1984) (48); Gibson et al. (1983) (49); Rzeszotarski et al.(1982) (50)

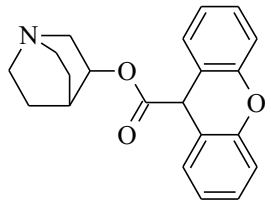
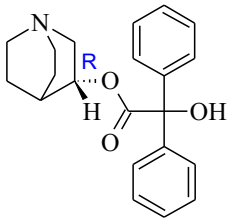
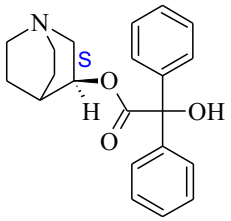
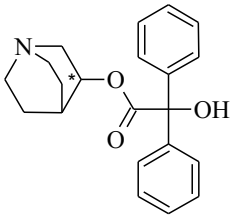
The purpose of these papers was to study analogs of BZ to differentiate the relative binding of these compounds to heart muscle and brain tissue. The relative binding of BZ analogs to dog ventricular muscle muscarinic receptors were measured (Table 13) and ranked by the relative binding index starting from compounds with the highest binding index: compounds 1, 4 > 2, 3, 5 > (±)-QNX, atropine, scopolamine with BZ overlapping the first two groups. For the relative binding of BZ analogs to the muscarinic receptors of the caudate/putamen of rabbits, compounds 1-5, (±)-QNX, BZ > scopolamine, atropine (Table 13). (±)-QNX, a compound where the ester moiety is planar, shows a 16-fold preference for brain tissue over heart tissue. One might reasonably hypothesize that impaired cognitive function is an important property of a militarily useful incapacitating agent. It is possible that (±)-QNX is much more effective than BZ because this compound would be less cardiotoxic than BZ at the same concentration. Thus, higher, non-lethal doses of (±)-QNX could be used as an incapacitating agent. It should be pointed out that both (±)-BZ and (±)-QNX are racemic mixtures and it is highly probable that one isomer of QNX is more active than the other. This has been demonstrated for the optical isomers of BZ.

It is not known which receptor subtypes are responsible for the confusion and incapacitating properties of BZ in humans but the brain receptors are likely key targets for this compound. The authors conclude that the heart and brain receptors represent two distinct classes of muscarinic receptors.

Table 13. Relative equilibrium association constants of (±)-quinuclidinyl derivatives to ventricular muscle and caudate/putamen muscarinic receptors[1, 2, 3]

Structure, compound	Relative binding index (BZ = 1)	
	Ventricular muscle from dogs (M1)	Caudate/ putamen from rabbits (M2)
 1	1.55[c]	0.99[a]

Structure, compound	Relative binding index (BZ = 1)	
	Ventricular muscle from dogs (M1)	Caudate/ putamen from rabbits (M2)
 2	0.66[d]	0.70[a]
 3	0.55[d]	0.95[a]
 4	0.95[c,d]	1.16[a]
 5	0.57[d]	1.10[a]
 Scopolamine	0.026[e]	0.22[b]
 Atropine	0.032[e]	0.21[b]

Structure, compound	Relative binding index (BZ = 1)	
	Ventricular muscle from dogs (M1)	Caudate/ putamen from rabbits (M2)
 (±)-QNX	0.043[e]	0.98[a]
 (R)-BZ[4]	184	230
 (S)-BZ[4]	24	44
 (R,S)-BZ	1.00[c,d]	1.00[a]

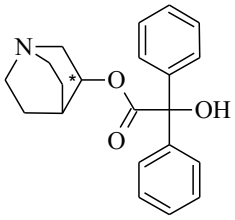
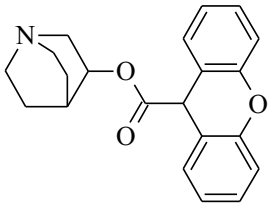
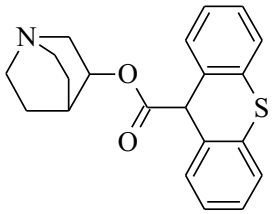
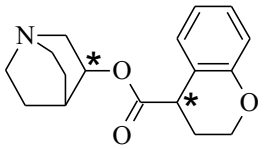
[1] The larger the number the more tightly the compound binds to the receptor.
[2] Adapted from Table I, Gibson et al.(1983) (49).
[3] Relative constants with the same superscript are within the 95% confidence interval of each other.
[4] Adapted from Table II, Eckelman et al. (1984) (48).

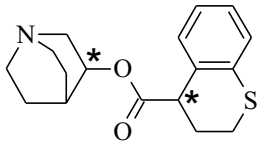
Cohen et al.(1987) (51)

The purpose of this paper was to synthesize analogues of BZ that showed selectivity for different muscarinic acetylcholine receptors (M1-M5). One compound QNX [(R,S)-3-quinuclidinyl xanthene-9-carboxylate] (Table 14) showed ~14x greater affinity for the corpus striatum from the rat brain compared to the ventricular muscle from the heart of a dog. Using R-3-quinuclidinol, the authors synthesized esters of chormane-4-carboxlic acid and thiochromane-4-carboxylic acid. These two compounds were several thousand times more potent than BZ at binding to either ventricular muscle (predominantly M2 receptors sites) or the corpus striatum (predominately M1 receptor sites). These compounds have two chiral centers (noted with an * in Table 14). Previous studies on chiral BZ analogs have shown

that enantiomers and diastereomers can have markedly different binding properties. Therefore, it highly probable that one of the two possible isomers of R-3-quinuclidinol (R,S)-chormane-4-carboxlate and R-3-quinuclidinol (R,S)-thiochromane-4-carboxylate would bind tighter to the muscarinic receptors than the racemic mixture. It is likely that these compounds could have physiological and psychological effects at doses much lower than BZ and could be very potent incapacitating agents.

Table 14. Binding affinities of selected compounds for the muscarinic acetylcholine receptors in dog heart muscle and rat brain[a]

Compound	Ventricular Muscle		Corpus Striatum	
	K_a (x $10^9 M^{-1}$)	Relative binding to BZ	Ka (x $10^9 M^{-1}$)	Relative binding to BZ
 BZ	3.62	1	5.28	1
 QNX R-3-quinuclidinyl xanthene-9-carboxylate	5.43	0.67	0.391	13
 R-3-quinuclidinyl thioxanthene-9-carboxylate	0.193	19	0.0226	230
 R-3-quinuclidinyl (R,S)-chromane-4-carboxylate	0.0227	160	0.00437	1200

Compound	Ventricular Muscle		Corpus Striatum	
	K_a (x $10^9 M^{-1}$)	Relative binding to BZ	Ka (x $10^9 M^{-1}$)	Relative binding to BZ
 R-3-quinuclidinyl (R,S)-thiochromane-4-carboxylate	0.0124	290	0.00247	2100

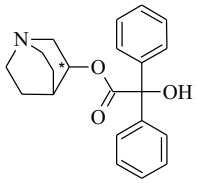
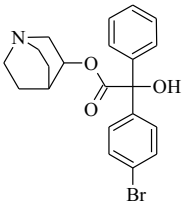
[a] Adapted from Table I, Cohen et al. (1987) (51).
[*] Denotes a chiral carbon

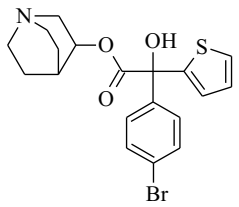
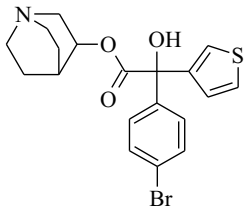
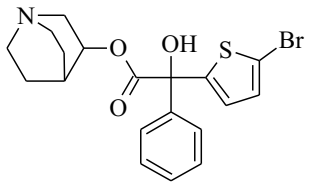
Baumgold et al. (1991) (52)

The purpose of this paper was to investigate the binding of BZ derivatives to the five muscarinic receptor subtypes based on competition of drugs with known receptor binding affinities such as: pirenzepine for the M1 receptor subtype; AF-DX 116, methoctramine, and 4-DAMP for M2 receptor subtype; pHHSiD for M3 receptor subtype; and tritiated N-methylscopolamine ([^{3}H]NMS) for general binding to muscarinic receptors. The identification of five distinct muscarinic receptor subtypes has raised the possibility of developing subtype-selective antimuscarinic agents. This paper synthesized several BZ derivatives with the goal of developing centrally-active subtype-selective antimuscarinic agents. The authors replaced one of the phenyl groups of BZ with a thiophene functional groups and studied the receptor subtype selectivity of the resulting BZ derivative.

Four compounds (3, 6-8; Table 15) are equal to or more potent than BZ for binding to the M3 receptor. The M3 receptor is found in many organs including the brain, lungs, etc. Compounds 6 and 8 are similar in potency to BZ for the M1 receptors which are found primarily in the CNS. The binding of compounds 6 and 8 to heart muscle muscarinic receptors (M2) are greater than four times less effective than BZ. Compounds 6 - 8 may mimic the incapacitating properties of BZ whereas compound 3 binds considerably less tightly to the M2 receptors and would likely have a mixed response compared to BZ.

Table 15. Comparative inhibition constants of selected heterocyclic BZ derivatives[a,b]

Selected Compound	Inhibition constants (K_i) at muscarinic receptor subtypes (relative to BZ)[b]			
	M1	M2	M3	M4
 Compound 1, BZ	44 (1)	60 (1)	241 (1)	117 (1)
 Compound 3	88 (2)	1080 (18)	237 (0.98)	497 (4.2)

Selected Compound	Inhibition constants (K_i) at muscarinic receptor subtypes (relative to BZ)[b]			
	M1	M2	M3	M4
 Compound 6	38 (0.86)	261 (4.4)	118 (0.49)	185 (1.6)
 Compound 7	82 (1.9)	322 (5.4)	214 (0.89)	326 (2.8)
 Compound 8	46 (1.05)	264 (4.4)	173 (0.72)	322 (2.8)

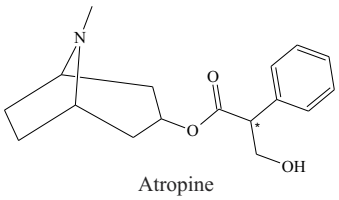
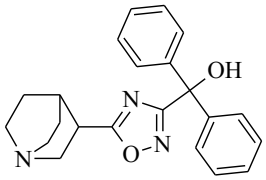
[a] Adapted from Table I, Baumgold et al. (1991) (52).
[b] Competition experiments were carried out with [^{3}H]N-methylscopolamine. The smaller the value the tighter the binding to the muscarinic receptor.

Saunders et al. (1990) (53)

The purpose of this paper is to develop quinuclidinyl-based acetylcholine muscarinic agonists that can penetrate the blood-brain membrane barrier and activate M1 muscarinic receptors. The authors state that M1 muscarinic receptors are responsible for cognitive deficits, as noted previously. The goal of this research is to enhance cortical cholinergic transmission through the use of muscarinic agonists that could be clinically effective with Alzheimer's patients. Agonistic compounds bind to a high affinity site on the M1 receptor that is competitive with oxotremorine while the low affinity binding site is responsible for antagonistic binding and is competitive with N-methylscopolamine. The ratio of the binding to these two sites gives information on the relative efficacy of impacting the cortical receptors. Thus, a ratio close to 1 is antagonistic while a ratio > 1000 is indicative of an agonist.

The paper reports on the synthesis of one compound that is a strong muscarinic receptor antagonist, compound 17. Table 16 shows a relative binding ratio of 1.8 for compound 17, the lowest relative binding ratio of the compounds tested in this paper. Compound 17 binds more tightly (low K_{app}) than atropine, a good muscarinic receptor antagonist, to sites sensitive to scopolamine or oxotremorine. This compound is not an ester and would not be susceptible to hydrolysis (enzymatic or spontaneous) compared to other benzilate esters. It is not known how this would affect the physiological half-life of this compound but suggests that the compound would not be metabolized as quickly as ester-based glycolate antagonists. This paper is a classic example where research into potential drugs effective for Alzheimer's patients results in the synthesis of a potent muscarinic antagonist that could be developed for chemical warfare purposes.

Table 16. Potential antagonistic compound with binding (K_{app}) greater than atropine using N-methylscopolamine/oxotremorine[a]

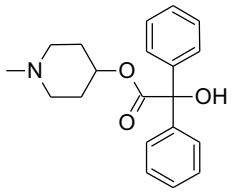
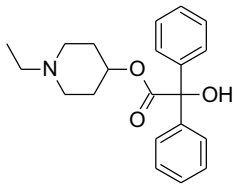
Compound (ranked from lowest K_{app}, NMS binding constant)	Binding data (Kapp, μM)		Ratio of [^{3}H]NMS/[^{3}H]OXO (relative to atropine)
	[^{3}H]NMS[b] (relative to atropine)	[^{3}H]OXO[c] (relative to atropine)	
Reference Compound:  Atropine	0.0010 (1)	0.00048 (1)	2.1 (1)
 Compound 17	0.00012 (0.12)	0.000067 (0.14)	1.8 (0.86)

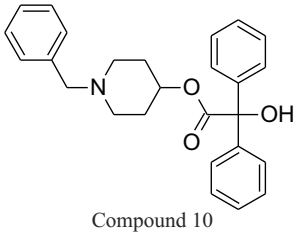
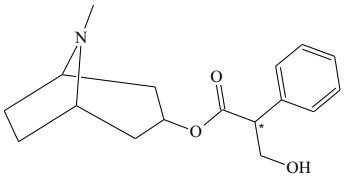
[a] Adapted from Table II, Saunders et al. (1990) (53)
[b] NMS = N-methylscopolamine
[c] OXO = oxotremorine

Tejani-Butt et al. (1990) (54).

The purpose of this paper was to systematically study the alkyl and aromatic structural modifications on the nitrogen atom on the cyclic alcohol group of benzilate esters. This paper focused on the binding affinities of muscarinic antagonists based on the parent compound, N-methyl-4-piperidinyl benzilate ester (Compound 6, Table 17), which possesses high affinity for muscarinic receptors. These piperidinyl derivatives also lack a chiral carbon in contrast to the 3-quinuclidinyl esters. Several piperidinyl benzilates (Table 17) are potent muscarinic receptor antagonists in the rat brain.

Table 17. In vitro binding of piperidinyl benzilate derivatives to rat brain muscarinic receptors[a]

Compound Structure, Number	Inhibition of binding of [^{3}H]-Quinuclidinyl benzilate to muscarinic receptors in rat cortex and cerebellum relative to atropine[b]
 Compound 6	170
 Compound 7	100

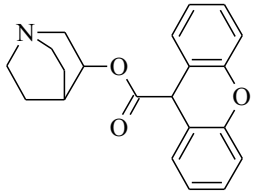
Compound Structure, Number	Inhibition of binding of [^{3}H]-Quinuclidinyl benzilate to muscarinic receptors in rat cortex and cerebellum relative to atropine[b]
 Compound 10	250
 Atropine	100

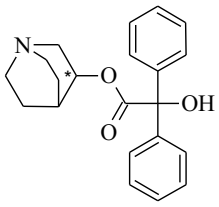
[a] Adapted from Table 2, Tejani-Butt et al. (1990) (54).
[b] The higher the value the more effective the compound blocks binding of [^{3}H]-BZ to muscarinic receptors.

Gibson et al. (1994) (55)

The purpose of this paper was to measure the relative binding of (R)-3-quinuclidinyl 8-xanthene carboxylate or (R)-QNX to muscarinic acetylcholine receptors (M_1-M_5) reported in 1988 (56). The nomenclature for this compound has changed; the revised name is (R)-3-quinuclidinyl-9-xanthene carboxylate (57). The paper reports the binding of (R)-QNX to different brain sections: cortex, hippocampus, caudate/putamen, thalamus, Pons, and colliculate bodies. BZ essentially binds equally to the caudate/putamen, cortex and thalamus of rat brains. (R)-QNX binding to brain tissues is not significantly different among the following brain structures: caudate/putamen, cortex, hippocampus, thalamus, colliculate bodies or the Pons/medulla. In cloned receptors, the dissociation constants for BZ for the M_1-M_4 receptors are 32, 35, 43, 49 pmole, respectively suggesting that BZ binds across all receptor sites approximately equally. (R)-QNX binds to the M_1 , M_3, and M_4 receptors about equally with much less binding to the M2 receptors (ventricular muscle, Table 18). Since the M_1 receptors reside in human brain tissues it is not clear how effective (R)-QNX would be as an incapacitating agent in humans. This study supports the previous finding that QNX is less cardiotoxic (see study summary of Eckelman et al. (1984) (48)) than BZ and thus, could be used at higher doses, which could act more quickly than BZ on cognitive functions.

Table 18. Relative binding index of (R)-3-quinuclidinyl-9-xanthene carboxylate (QNX) to cloned muscarinic receptor subtypes[a]

Compound	Relative binding index Muscarinic receptor subtype			
	M_1	M_2	M_3	M_4
 (R)-QNX	1.07	0.041	0.60	0.34

Compound	Relative binding index Muscarinic receptor subtype			
	M_1	M_2	M_3	M_4
 BZ[b]		1		

[a] Adapted from Table 3, Gibson et al. (1994) (55).
[b] It was not clear from the paper if the R enantiomer or the racemic mixture was used. A 1982 paper by the same group used (±) BZ; Rzeszotarski et al. (50).

Lee et al.(1995) (58)

The purpose of this paper was to develop selective muscarinic subtype receptor (M1, M2, etc) derivatives that can be used as fluorinated radiotracers to map these receptors in the brain using positive emission tomography (PET). The M1 receptor is associated primarily with the cerebral cortex, hippocampus and the corpus striatum in the rat brain. The M2 receptor is the dominant receptor in the Pons, medulla and cerebellum. The M3 receptor is not abundant in brain tissues of the rat. The density of M2 receptor sites was reported to be low in the cortical and hippocampus areas of the human brain from post-mortem analysis of the brain in Alzheimer's disease.

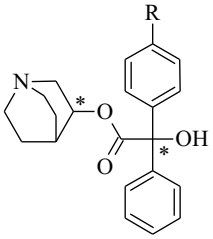
The relative binding index of the eight stereoisomers identified in this study (Table 19) suggest that the stereochemistry of BZ derivatives plays an important role in the binding of these compound to the muscarinic acetylcholine receptor. There are two chiral centers in the derivatives studied here. One chiral center is found on the quinuclidinol moiety and the second chiral carbon is the benzilic center. The largest increases in binding were found using the (R,S) diastereomers for the M2 receptor subtype with increases in binding of 32, 14 and 11 times that of the 4-iodophenyl BZ derivative of BZ. None of the compounds were more effective at binding to the M3 receptor than the 4-iodophenyl BZ derivative. The effect of stereochemistry at the M1 receptor was mixed with the CH_3F derivative showing an increase of 3-fold with the (R,R) diastereomer that was not replicated in the CH_2CH_2F derivative. The effect of stereochemistry on the incapacitating properties of BZ as a chemical weapon has not been reported. It seems likely that BZ derivatives with the ideal stereochemistry could be much more effective as a chemical incapacitating agent.

Ketchum (2012) (7)

This book is unique among the references cited for effects of incapacitating agents in human volunteers. Many of the references cited in this book were declassified studies or reports; this book reports on studies that are not generally available to the public. To the knowledge of this author, this book has not been peer-reviewed, but nevertheless it is a critical source of information on the effects of these compound in humans. The goal of this US Army research group was to better understand the physiological and psychological properties of BZ and derivatives of this compound in humans with the goal of developing an effective, yet safe (with post-exposure treatment), incapacitating agents for use in chemical warfare. They investigated the following aspects of incapacitating agents: measures of potency, time to onset of effects, duration of physiological and cognitive effects, dose/response relationships, and methods of post-exposure treatment.

They used 6700 healthy male volunteers between 1955 and 1975. Slightly less than half of these were exposed to chemical agents. The 1982 National Research Council (47) review of long-term effects of exposure to these agents found fewer deaths than expected, probably because of the selection process that recruited only healthy subjects. The number of deaths were higher than expected for subjects exposed to atropine and scopolamine – two drugs approved by the FDA.

Table 19. Relative binding index of 4-phenyl BZ derivatives[a]



R Substituent	Stereoisomer configuration (* = chiral carbon centers)	Relative binding index[b] for muscarinic acetylcholine receptor subtypes		
		M1	M2	M3
CH_3F	R,R	3.09	5.00	0.088
	R,S	0.38	32.3	0.048
	S,R	0.02	0.36	0.0008
	S,S	0.12	0.25	0.85
CH_2CH_2F	R,R	0.40	0.55	0.0008
	R,S	1.31	13.6	0.096
$CH_2CH_2CH_2F$	R,S	1.7	11.1	0.14
I (Iodine)	R,R		1.00	

[a] Adapted from Table 1, Lee et al. (1995) (58).
[b] Relative binding index was calculated by dividing the affinity constant, K_a, of the compound by the K_a for the (R,R)-4-iodophenyl BZ derivative. The larger the number the better the compound bound to the muscarinic receptor.

Doses were typically logarithmic with progressive increases of 40%. Data were analyzed using a Probit analysis where the fraction of subjects that reached a specific endpoint was plotted against the logarithm of the dose. To alleviate stress they attempted to administer doses no higher than the ID_{50} (dose that caused incapacitation of 50% of the subjects); this did not always happen.

The book reported several methods for measuring effects. The ID_{50} was defined as the incapacitating dose required to decrease the Number Facility Test to ≤ 10% of baseline in 50% of the subjects tested. Peripheral effects were measured as changes in heart rate; HR_{50} is the dose required to increase the heart rate to ≥ 100 bpm. The MED_{50} is the minimal effective dose (oral) required to produce 3 of 5 successive Number Facility score below 75% of baseline. The D_{50} was a measurement of duration; the length of time between the time for the first Number Facility score that falls below 25% and the time during recovery that the Number Facility Test score reaches 75%. The researchers also estimated an LD_{50} (lethal dose that results in 50% fatalities in humans) based on historical atropine data.

Cognitive function was measured using the Number Facility Test and the Speed of Closure Test. The problem is that participants got better at the test every time they took one of these tests. Therefore, each subject took the test about 20 times (practice tests) ensuring they reached a plateau on the test performance. Another qualitative test was to have the subject draw a picture of a man. In subjects treated with a high enough dose, this picture became markedly unrecognizable or degraded during the incapacitation phase of the testing.

A number of observations were noted. In general, the higher the dose, the quicker the subject reached incapacitation and the longer the subject remained incapacitated. Doubling the ID_{50} dose significantly increased the D_{50}. The researchers concluded that BZ and other glycolate esters showed a multiple compartment distribution with differing elimination rates. The rate of elimination was between a zero-order (linear) and a first-order (logarithmic) rate.

Responses to oral ingestion of BZ was 80% of the i.v. dose suggesting incomplete absorption or greater first-pass though the liver. The inhalation route of exposure was 65% of the i.v. exposure due

to 80% retention by the lung. Dermal exposure, in a suitable solvent, was only 5-10% as effective as the i.v. route.

Tetrahydroaminacridine (THA) is an effective antidote for glycolate esters but there is possible reversible liver damage with multiple injections of THA. A double blind, cross over study was used to demonstrate that physostigmine was a good, but short lived antidote to BZ after the peak effect had been reached. This compound was not effective until the peak effect had been observed. As long as the subject is treated with physostigmine, the subject is cognitively normal. If the treatments are not maintained the subject relapses back into incapacitation. Physostigmine is ~50% more effective by injection than by oral administration. Small, non-lethal doses of VX nerve agent are effective and long lasting antidotes for BZ, presumably because VX inactivates acetylcholinesterases thereby increasing the concentration of acetylcholine available to compete for muscarinic acetylcholine receptors sites.

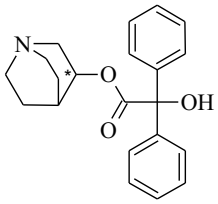
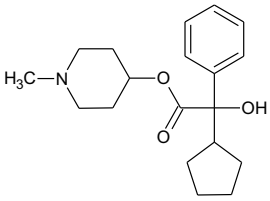
Compound specific information is shown in Table 20 and additional notes for selected compounds are shown below. EA 3443 has a much reduced effect on heart rate at the ID_{50} compared to BZ. This suggests that different glycolates bind differently to different muscarinic receptor sites. EA 3443 did not cause pupil dilation at or below the ID_{50}. Cutaneous absorption is not effective with EA 3443. It takes up to 3 days to achieve maximal effects after cutaneous absorption. Physostigmine and VX were also effective in treating the symptoms from this compound.

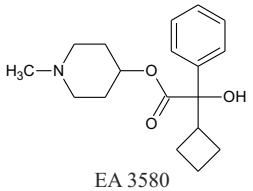
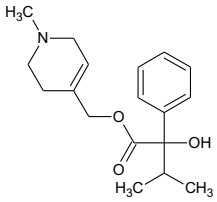
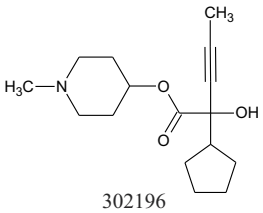
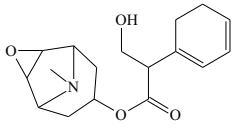
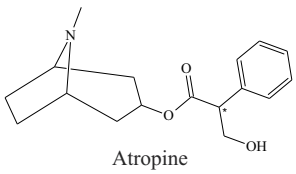
EA 3580 showed a lower ID_{50} compared to BZ but comparable to EA 3443. This compound had a lower duration of effects than BZ.

EA 3834 showed rapid onset of effects compared to the other compounds. Peripheral effects such as heart rate and pupil size were not greatly affected at the ID_{50} concentration.

EA 302196 showed maximal effects after 15 minutes using either i.m. or i.v. injections. Partial recovery occurred after 4 hours and completely after 10 hours. Pupil dilation was observed and followed recovery. This compound was not as potent as BZ as an incapacitating agent but its effects presented faster than BZ.

Table 20. Relative Central and Peripheral Potency of Selected Glycolate Compounds[a]

Compound	Central Potency[a,b] ID_{50} (µg/kg i.m. injection)	Peripheral Potency[a,c] HR_{50} (µg/kg i.m. injection)	Central Potency Relative to BZ[d]	Peripheral Potency Relative to BZ[d]	MED_{50}[e] (µg/kg i.m. injection)	LD_{50}[f] (µg/kg i.m. injection)	D_{50}[g] (hr)
 BZ 3-Quinuclidinyl benzilate	6.2	2.5	1	1	2.3	~250	48-72
 EA 3443 N-methyl-4-piperidinyl-α-cyclopentylphenylglycolate	3.4	7.1	2.8	0.55	1.2	~350	16-24

Compound	Central Potency[a,b] ID_{50} (µg/kg i.m. injection)	Peripheral Potency[a,c] HR_{50} (µg/kg i.m. injection)	Central Potency Relative to BZ[d]	Peripheral Potency Relative to BZ[d]	MED_{50}[e] (µg/kg i.m. injection)	LD_{50}[f] (µg/kg i.m. injection)	D_{50}[g] (hr)
 EA 3580 N-methyl-4-piperidinyl-α-cyclobutylphenylglycolate	3.9	8.1	1.6	0.31	1.4	~400	16-24
 EA 3834 N-methyl-4-piperidinyl-α-isopropylphenylglycolate	12	5	0.52	0.5	5.0	~400	12-15
 302196 N-methyl-4-piperidinyl-α-propynyl-cyclopentylglycolate	29	20	0.21	0.12	12	~2000	20-28
 Scopolamine	20.2	16	0.31	0.16	9.4	~4000	5-10
 Atropine	152	70	0.041	0.036	63	~6000	8-12

[a] Data were extracted from the Appendix, pages324-326 and Figure 74 of Ketchum (2012)(7).
[b] Central effects include cognitive function as measured by the Number Facility test, ID_{50} is the incapacitating dose required to decrease the Number Facility test to ≤10% of baseline.
[c] Peripheral effects as measured by changes in heart rate; HR50 is the dose required to increase the heart rate to ≥ 100 bpm.
[d] The higher the number the greater the potency.
[e] MED_{50} = Minimal effective dose (oral) required to produce 3 of 5 successive Number Facility score below 75% of baseline.
[f] LD_{50} estimated lethal dose that results in 50% fatalities humans based on historical atropine data.
[g] D_{50} - measurement of duration; the length of time between the time for the first Number Facility score that falls below 25% and the time during recovery that the Number Facility score reaches 75%.

SUMMARY AND CONCLUSIONS

The purpose of this review is to identify potentially new and more potent incapacitating agents than BZ that had been developed in the 1960s. The identification of new compounds was based on the structures of BZ, atropine or scopolamine using peer-reviewed, pharmaceutical studies of new muscarinic acetylcholine antagonists. A number of published studies reported on compounds with effects observed at lower concentrations than or comparable to BZ (Table 21). This review suggests several conclusions:

1. The pharmacological effects of many of the compounds identified in this review suggest that nitrogen heterocyclic alcohols other than 3-quinucludinol (e.g. N-methyl-4-piperidinol or tropanol) could be used to synthesize potent esters that could be developed into incapacitating agents (44,46).

Table 21. Compounds with potential incapacitating properties equal to or more potent (effects at lower concentrations) than ± BZ.

Compound, name or number	Table Number	Reference
Compound 840	Table 4	(41)
Compound 328		
Compound 329		
3-quinuclidinyl-2-thienylphenyl glycolate	Table 7	(43)
3-quinuclidinyl-2,2-bis(2,2'-thienyl) glycolate		
3-quinuclidinyl-2,2 bis(3,3'-thienyl) glycolate		
N-methy-4-piperidyl cyclohexylphenyl glycolate	Table 9	(45)
Compound 1 (+)	Table 10	(46,47)
Compound 3 (-)		
Compound 5 (-)		
Compound 8 (-)		
Compound 11 (+/-)		
Compound 12 (-)		
Compound 14 (-)		
Compound 18 (+/-)		
Compound 20 (+/-)		
Compound 21 (-)		
(R)-BZ	Table 13	(48)
R-3-quinuclidinyl (R,S)-thioxanthene-9-carboxylate	Table 14	(51)
R-3-quinuclidinyl (R,S)-thiochromane-4-carboxylate		
R-3-quinuclidinyl (R,S)- chromane-4-carboxylate		
Compound 17	Table 16	(53)
3-quinuclidnyl (R)-9-fluorenecarboxylate (R-QNX)	Table 18	(55)

2. One compound (Compound 17, Table 16) was synthesized that was a potent muscarinic acetylcholine antagonist but was not an ester. It is possible that this compound would be resistant to esterases found in the blood or liver and thus, could have a substantially longer in vivo half-life (53).

3. Reported effects of many of these compounds include psychotogenic effects (e.g. visual and auditory hallucinations) in animals and humans. The psychotogenic properties do not correlate with toxicity, antispasmodic activity, or mydriasis. It would be interesting to see if the more recent, potent compounds such as quinuclidinyl thiochromane carboxylate have similar effects. In addition, further research may elucidate which muscarinic receptors or combination of receptors are responsible for the psychotogenic effects of these compounds (7,41,43,47).

4. The effects of stereoisomers was observed early in the research on muscarinic antagonists. Sternbach and Kaiser (11) observed that one enantiomer of 3-quinucludinol diphenyl acetate was much more potent than the other enantiomer. Other studies identified enantiomeric or diastereomeric esters that were much more potent that the complimentary stereoisomers and included (+)-2-tropanol benzilate, (+)-2-tropanol cyclohexylphenyl glycolate, (+)-2-tropanol 2-thienylphenyl glycolate, (+)-2-tropanol cyclopentylphenyl glycolate, and diastereomers of para-substituted phenyl derivatives of BZ (Tables 10,11,12, 19) (46,47,58).

5. The most potent compounds identified in this review were quinuclidinyl thiochromane and chromane carboxylates (Table 14)(51). These two compounds were up to several thousand times more potent than BZ at binding to muscarinic receptors in either ventricular muscle or the corpus striatum. As discussed above, these compounds have two chiral centers (noted with an * in Table 14) suggesting the possibility that diastereomers of quinuclidinyl chromane or thiochromane carboxylates could enhance the potency of already potent compounds.

The compounds identified in this review were developed for reasons other than the their potential as chemical warfare agents. Thus, this review suggests that research into muscarinic acetylcholine receptor antagonists represents a good example of research with the potential for unintended consequences and falls within the concept of Dual Use Research of Concern.

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