# Synthesis and Cholinergic Properties of Bis[[(dimethylamino)methyl]furanyl] Analogues of Ranitidine

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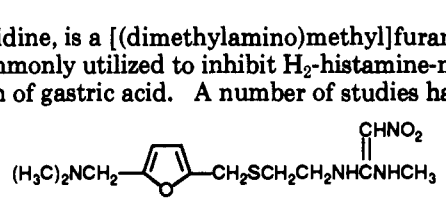
The histaminergic $H_2$ antagonist, ranitidine, has also been found to significantly inhibit acetylcholinesterase (AChE) in vitro. In an effort to develop novel, nonquaternary AChE inhibitors capable of penetrating into the CNS and alleviating the cholinergic deficit characteristic of Alzheimer's disease, a series of bis[[(dimethylamino)methyl]furanyl] analogues of ranitidine has been synthesized. All compounds were evaluated for human erythrocyte AChE inhibitory activity and compared to ranitidine, physostigmine, and tetrahydro-9-aminoacridine (THA). The most active AChE inhibitors were N,N'-disubstituted derivatives of 2-nitro-1,1-ethenediamine and 4,6-dinitro-1,3-benzenediamine, with compound 8 demonstrating activity greater than physostigmine. Deletion of the diaminonitroethene group in a series of alkyl and aryl bis-thioethers, yielded a number of slightly less active compounds, comparable in potency to THA. The 13 most active AChE inhibitors all demonstrated a more selective inhibition of AChE, as opposed to butyrylcholinesterase inhibition, than did THA. Compounds 3 and 22 were equally active to THA in potentiating rat ileal contractions. Binding studies demonstrated $M_1$ and $M_2$ cholinergic receptor affinities slightly greater than or equal to THA. Differential receptor binding studies showed compound 12 resembled THA in agonist/antagonist activity. Compounds 11-13 significantly elevated mouse brain acetylcholine levels, when administered at 80% of their approximate lethal doses, but were less active than THA or physostigmine.

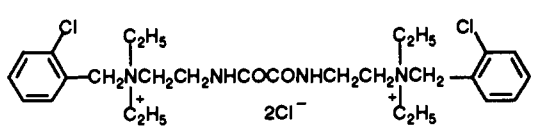
## Introduction

Alzheimer's disease (AD) is a progressive dementia, currently estimated to affect up to 15% of the population over 65 years of age.[1] The decreased choline acetyltransferase activity in the cortex and hippocampus of brains from AD patients, as well as corroborating evidence from a number of other areas, has resulted in a cholinergic deficit theory regarding the disease.[2,3] As cortical $M_1$-muscarinic receptors have been shown to remain relatively unaffected, a great deal of research has been undertaken to provide therapeutically viable cholinergic agonists or acetylcholinesterase (AChE) inhibitors capable of penetrating into the CNS.[4,5] Previously, the AChE inhibitors, tetrahydro-9-aminoacridine (THA) and physostigmine have yielded controversial but hopeful results in clinical trials.[6,7]

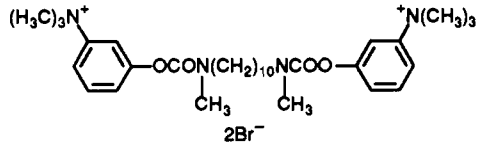
Ranitidine, is a [(dimethylamino)methyl]furan derivative, commonly utilized to inhibit $H_2$-histamine-mediated secretion of gastric acid. A number of studies have demonstrated that this compound significantly inhibits AChE in vitro yet is poorly distributed across the blood–brain barrier.[8,9] As several bisquaternary compounds, such as ambenonium chloride and demecarium bromide, have been found to be extremely potent inhibitors of AChE, we have synthesized a number of analogous nonquaternary, bis-[[(dimethylamino)methyl]furanyl] derivatives of ranitidine, in order to increase its AChE inhibitory activity.[10,11] In the general structure in Table I, modification of Z was undertaken to include not only the *N,N'*-diethyl-1,1-diamino-2-nitroethene moiety, but also to provide the analogous cyanoguanidine, amidine, urea, amide, and 1,3-diamino-4,6-dinitrophenyl derivatives. In order to further explore the effects of functionality and chain length, Z was also replaced by a series of alkylene and aryl groups. It was postulated that the enhanced lipophilicity of these agents would also allow sufficient penetration of these inhibitors into the CNS in order to alleviate the cholinergic deficit characteristic of AD patients.




ranitidine




ambenonium chloride




demecarium bromide

## Chemistry

*N,N'*-Bis[2-[[[5-[(dimethylamino)methyl]-2-furanyl]-

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(1) Collerton, D. Cholinergic function and intellectual decline in Alzheimer's disease. *Neuroscience* **1986**, *19* (1), 1.
(2) Perry, E. K. The cholinergic hypothesis--ten years on. *Br. Med. Bull.* **1986**, *42* (1), 63.
(3) Roberts, F.; Lazareno, S. Cholinergic treatments for Alzheimer's disease. *Biochem. Soc. Trans.* **1989**, *17*, 76.
(4) Sauerberg, P.; Falch, E.; Meier, E.; Lembol, H. L.; Krogsgaard-Larsen, P. Heterocyclic muscarinic agonists. Synthesis and biological activity of some bicyclic sulfonium arecoline bioisosteres. *J. Med. Chem.* **1988**, *31*, 1312.
(5) Hershenson, F. M.; Moos, W. H. Drug development for senile cognitive decline. *J. Med. Chem.* **1986**, *29*, 1125.
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(8) Gwee, M. C. E.; Cheah, L. S. Actions of cimetidine and ranitidine at some cholinergic sites: implications in toxicity and anesthesia. *Life Sci.* **1986**, *39*, 383.
(9) Kagevi, I.; Throhallsson, E.; Wahlby, L. CSF concentrations of famotidine. *Br. J. Clin. Pharmacol.* **1987**, *24*, 849.
(10) Kirchner, F. K. N,N'-Bis(dialkylaminoalkyl)dicarboxylic acid amides. U.S. Patent Appl. 3,096,372, Apr 30, 1956; *Chem. Abstr.* **1964**, *60*, 453b.
(11) Joshi, L. D.; Parmar, S. S. Structural consideration in the inhibition of rat brain acetylcholinesterase. *J. Pharm. Pharmacol.* **1964**, *16*, 763.

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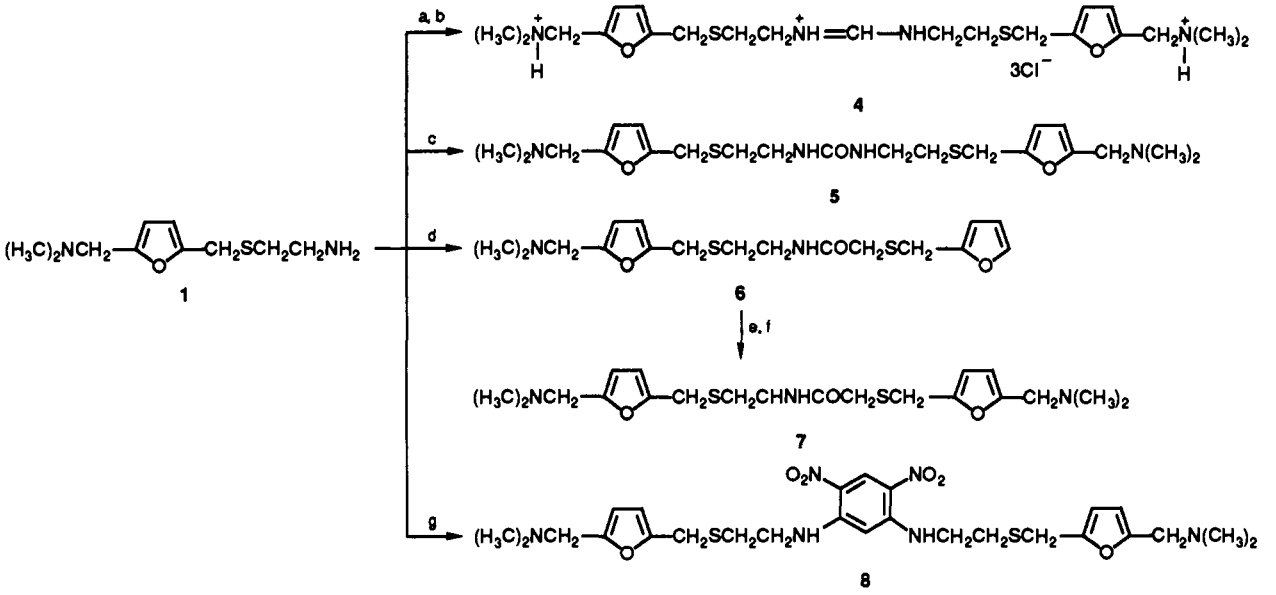
**Scheme I**[a]



[a] Reagents: (a) *s*-triazine, THF; (b) HCl, THF; (c) bis(trichloromethyl) carbonate, triethylamine, $CH_3CN$; (d) 2-[(2-furanylmethyl)thio]-acetyl chloride, $CH_3CN$; (e) $H_2C{=}N^+(CH_3)_2$ $Cl^-$, $CH_3CN$; (f) NaOH, water; (g) 1,5-difluoro-2,4-dinitrobenzene, $Na_2CO_3$, $CH_3CN$.

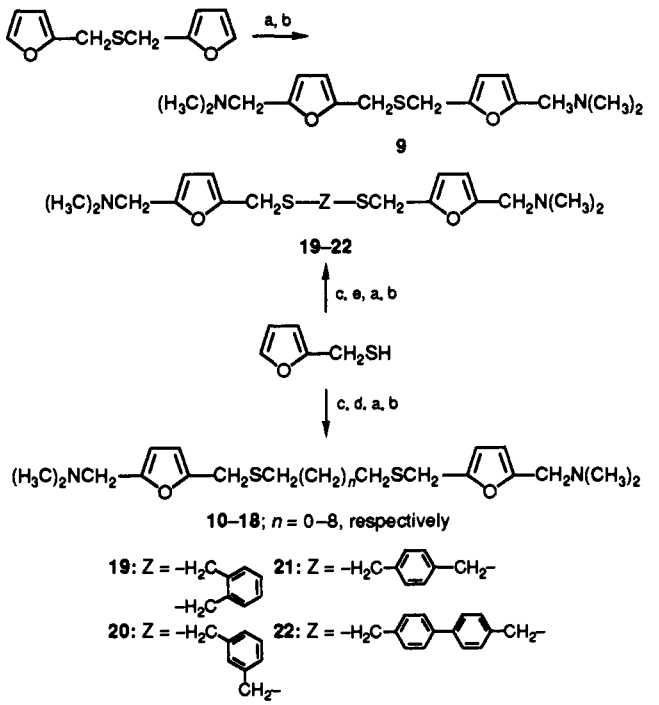
methyl]thio]ethyl]-2-nitro-1,1-ethenediamine (**2**) and the corresponding *N*-cyanoguanidine analogue (**3**) were synthesized utilizing the method of Borchers et al.,[12] by the reaction of 2 equiv of the primary amine, 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine[13] (**1**) with 1,1-bis(methylthio)-2-nitroethylene or dimethyl *N*-cyanodithioiminocarbonate, respectively.

By utilizing the procedure of Grundmann and Kreutzberger,[14] a solution of compound **1** in THF was refluxed with *s*-triazine to yield the N,N′-bis-substituted formamidine **4** (Scheme I). The formamidine was isolated and characterized as the trihydrochloride salt. Similarly, compound **1** was reacted with bis(trichloromethyl)-carbonate (triphosgene) in the presence of triethylamine to yield the N,N′-bis-substituted urea **5** (Scheme I).

Condensation of compound **1** with 2-[(2-furanylmethyl)thio]acetyl chloride[15] gave the amide **6**. Electrophilic substitution of the monosubstituted furan ring of amide **6** with dimethylmethyleneammonium chloride according to the procedure described by Dowle et al.[16] gave compound **7**.

N-Arylation of compound **1** with 1,5-difluoro-2,4-dinitrobenzene in acetonitrile in the presence of anhydrous sodium carbonate gave the N,N′-bis-heteroaryl-substituted 1,3-benzenediamine **8** (Scheme I).

**Scheme II**[a]



[a] Reagents: (a) $H_2C{=}N^+(CH_3)_2$ $Cl^-$, $CH_3CN$; (b) NaOH, water; (c) potassium *tert*-butoxide, THF; (d) $BrCH_2(CH_2)_nCH_2Br$; (e) $\alpha,\alpha'$-dibromo-*o*-xylene, $\alpha,\alpha'$-dibromo-*m*-xylene, $\alpha,\alpha'$-dibromo-*p*-xylene, or 4,4′-bis(bromomethyl)-1,1′-biphenyl.

Electrophilic substitution of the furan rings of commercially available furfuryl sulfide with dimethylmethyleneammonium chloride yielded compound **9** (Scheme II). A homologous series of $\alpha,\omega$-bis[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]alkanes (**10–18**) were synthesized by alkylating furfuryl mercaptan with $\alpha,\omega$-dibromoalkanes followed by electrophilic substitution of the furan rings with dimethylmethyleneammonium chloride (Scheme II). In an analogous manner, furfuryl mercaptan was alkylated with either $\alpha,\alpha'$-dibromo-*o*-xylene, $\alpha,\alpha'$-dibromo-*m*-xylene, $\alpha,\alpha'$-dibromo-*p*-xylene, or 4,4′-bis(bromomethyl)-1,1′-biphenyl followed by electrophilic substitution as previously described to yield

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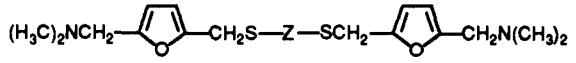
(14) Grundmann, C.; Kreutzberger, A. Triazines. XIII. The ring cleavage of s-triazine by primary amines. A new method for the synthesis of heterocycles. *J. Am. Chem. Soc.* **1955**, *77*, 6559.

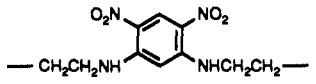
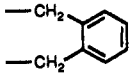
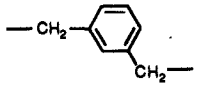
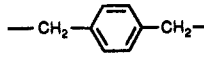
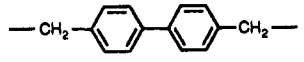
(15) Gol'dfarb, Ya. L.; Tarasova, L. D. A new path for the synthesis of 2,3-disubstituted furans. *Dokl. Akad. Nauk.* **1962**, *142*, 358; *Chem. Abstr.* **1962**, *57*, 789d.

(16) Dowle, M. D.; Hayes, R.; Judd, D. B.; Williams, C. N. A convenient synthesis of Mannich bases of thiophene and substituted thiophene. *Synthesis* **1983**, 73.

(17) Gol'dfarb, Ya. L.; Yabukou, A. P.; Belen'kii, L. I. Dimethylaminomethylation of furans. *Izy. Akad. Nauk SSSR, Sec. Khim.* **1967** *11*, 2509–12; *Chem. Abstr.* **1968**, *69*, 59004g.

Table I. Physical and Biological Data of Bis[[(dimethylamino)methyl]furanyl] Analogues



| compd no. | Z or name | AChE-I:[a] $IC_{50}$, μM | yield, % | recrystallization solvent | mp, °C | anal.[b] |
|---|---|---|---|---|---|---|
| | physostigmine | 0.043 | | | | |
| | tetrahydro-9-aminoacridine (THA) | 0.18 | | | | |
| | ranitidine | 2.3 | | | | |
| 2 | $-CH_2CH_2NHCH{=}C(NO_2)NHCH_2CH_2-$ | 0.074 | 66 | ether[d] | 61–63[e] | |
| 3 | $-CH_2CH_2NHC{=}N(CN)NHCH_2CH_2-$ | 0.29 | 29 | | oil[f] | |
| 4[c] | $-CH_2CH_2NH-CH{=}NCH_2CH_2-$ | 3.0 | 40 | 2-propanol/acetone (2:1) | 145–148 | C,H,N,S |
| 5 | $-CH_2CH_2NHCONHCH_2CH_2-$ | 48 | 13 | ether[d] | 44–46 | C,H,N,S |
| 7 | $-CH_2CONHCH_2CH_2-$ | 2.5 | 75 | | oil | C,H,N,S |
| 8 |  | 0.028 | 76 | ether[d] | 56–57 | C,H,N,S |
| 9 | *h* | 44 | 95 | | 61–62[g] | |
| 10 | $-(CH_2)_2-$ | 0.50 | 73 | | oil | C,H,N,S |
| 11 | $-(CH_2)_3-$ | 0.33 | 74 | | oil | C,H,N,S |
| 12 | $-(CH_2)_4-$ | 0.21 | 96 | hexanes | 30–31 | C,H,N,S |
| 13 | $-(CH_2)_5-$ | 0.90 | 73 | | oil | C,H,N,S |
| 14 | $-(CH_2)_6-$ | 0.74 | 86 | hexanes | 32–33 | C,H,N,S |
| 15 | $-(CH_2)_7-$ | 0.61 | 78 | hexanes | 30–31 | C,H,N,S |
| 16 | $-(CH_2)_8-$ | 0.47 | 73 | hexanes | 39.5–40 | C,H,N,S |
| 17 | $-(CH_2)_9-$ | 0.46 | 91 | hexanes | 39–40 | C,H,N,S |
| 18 | $-(CH_2)_{10}-$ | 1.3 | 80 | hexanes | 47–48 | C,H,N,S |
| 19 |  | 9.0 | 70 | | oil | C,H,N,S |
| 20 |  | 1.5 | 99 | | oil | C,H,N,S |
| 21 |  | 0.26 | 83 | hexanes | 59.5–60.0 | C,H,N,S |
| 22 |  | 0.18 | 68 | hexanes | 76–77 | C,H,N,S |

[a] Human acetylcholinesterase (Sigma type XIII) was used and the procedure of Ellman[19] was followed. Experiments were conducted at room temperature and substrate (acetylthiocholine) concentration was $5.0 \times 10^{-5}$ molar. [b] Carbon, hydrogen, nitrogen, and sulfur analyses were within ±0.4% of the theoretical. [c] Compound 4 was isolated and characterized as the trihydrochloride salt. [d] Oily product obtained after flash chromatography was triturated with ether. [e] Reference 12, mp = 63–65 °C. [f] Reference 12, reported as an oil. [g] Reference 17, mp = 58–59 °C. [h] See Scheme II for structure 9.

compounds 19–22, respectively (Scheme II).

## Results and Discussion

Compounds 2–22 represent a novel class of nonquaternary cholinergic agents. Because AD is characterized by a central deficiency of acetylcholine (ACh), the ability to inhibit human acetylcholinesterase (AChE) in vitro was initially assessed for all compounds, and the results compared to ranitidine, THA, and physostigmine. Compounds that possessed an AChE inhibitory activity ($IC_{50}$) of $10^{-6}$ M or lower were further examined for additional pharmacological properties.

**AChE Inhibition.** As presented in Table I, 18 of the 21 synthesized compounds inhibited AChE, possessing $IC_{50}$ values of $10^{-6}$ M or lower. Only compounds 4, 5, 7, 9, and 19 failed to demonstrate enhanced AChE inhibitory activity relative to their precursor, ranitidine ($IC_{50}$ of 2.3 μM, Table I). In addition, compounds 2, 8, and 22 displayed AChE inhibitory activities greater than or equal to the investigational agent THA ($IC_{50}$ of 0.18 μM). Furthermore, compound 8 demonstrated an AChE inhibitory activity greater than physostigmine ($IC_{50}$ of 0.043 μM). Relative to ranitidine, the inhibitory activity was increased more than 30-fold in the analogous diaminonitroethene dimer 2 ($IC_{50}$ of 0.074 μM). Substitution of a cyanoguanidine group for the diaminonitroethene functionality of compound 2 resulting in compound 3, afforded only a slight decrease in inhibitory activity. Similar substitution of amidine and urea groups, however, resulted in a much more pronounced decrease in inhibitory activity, with the amidine analogue 4 possessing an $IC_{50}$ of 3.0 μM and the urea derivative 5 possessing an $IC_{50}$ of 48 μM. The amide derivative 7 also displayed a significant decrease in activity relative to compound 2 with an $IC_{50}$ of 2.5 μM.

Interestingly, the replacement of the diaminonitroethene group in compound 2 by a 1,3-diamino-4,6-dinitrophenyl substituent in compound 8 led to an increase in inhibitory activity. It is therefore tempting to speculate that either a nonaromatic or aromatic nitroenamine structure is necessary for optimal activity.

In an effort to enhance lipophilicity in this series, the diaminonitroethene group was replaced by an alkylene chain in compounds 10–18. It is interesting to note that there was a relatively small but random effect on AChE inhibitory activity caused by this variation in chain length, with $IC_{50}$ values ranging from 0.21–1.3 μM (Figure 1). This is in contrast to the results of Joshi and Parmar in which analogues of demecarium bromide demonstrated a continuous increase in rat brain homogenate AChE inhibition with increasing alkylene chain length.[1] Compound 9, however, which contained only one thioether group, was relatively inactive, suggesting there may indeed be a minimum chain length necessary for separation of the furan nuclei, or a requirement for two thioether groups.

A degree of structural specificity was also indicated in the relative activities of the ortho, meta, and para phenyl

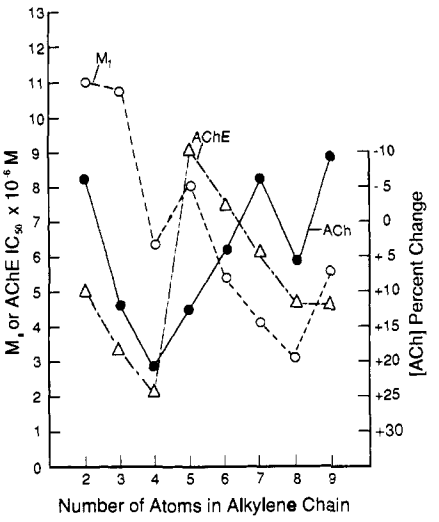



**Figure 1.** Comparison of $M_1$ receptor binding, AChE inhibitory activity, and brain ACh concentration changes versus chain length ($-S(CH_2)_nS-$) in compounds **10–17**. $M_1$ binding was performed using mouse cortex tissue, and AChE inhibitory activity was determined using human AChE. ACh levels were determined in mouse brain using a GC/MS procedure. ($M_1$ = muscarinic receptor subtype, AChE = acetylcholine esterase, and ACh = acetylcholine.)

analogues, compounds **19**, **20**, and **21**, respectively. The para isomer **21** displayed the most potent inhibitory activity with an $IC_{50}$ of 0.26 $\mu$M. AChE inhibitory activity decreased in the meta isomer **20** to 1.5 $\mu$M and further decreased in the ortho isomer **19** to an $IC_{50}$ of 9.0 $\mu$M.

The para-substituted biphenyl analogue **22** resulted in further evidence of the aforementioned trends, with an $IC_{50}$ of 0.18 $\mu$M. This, once again, suggests that appropriate para substitution can result in very active compounds and that chain length, even in the case of aromatic substitution, is usually of minimal consequence.

**Butyrylcholinesterase (BChE) Inhibition.** The ability to inhibit AChE at a lower concentration than is required to inhibit BChE implies a degree of selectivity necessary to avoid selected drug interactions. All the compounds tested possessed a favorable BChE/AChE inhibitory ratio relative to THA and physostigmine. In addition, all the compounds tested, with the exception of **8**, inhibited AChE more readily than BChE. Compound **8** ($IC_{50}$ of $2.5 \times 10^{-8}$ M) was approximately 40 times more active than any of the synthesized compounds and was intermediate in potency between physostigmine and THA. Interestingly, the two most potent AChE inhibitors, compounds **2** and **8**, yielded both the most favorable ratio (18) in the case of **2** and the least favorable ratio (0.89) in the case of **8**.

**Ileal Activity.** The majority of the compounds tested potentiated acetylcholine at a concentration of 0.1 $\mu$M or less, and compounds **3** and **22** possessed activity similar to that of physostigmine and THA. Compounds **14**, **15**, and **21** were inactive in the ileal assay. All the compounds tested demonstrated a poor correlation ($r = 0.529$) between ileal activity and AChE inhibition. Obviously, other parameters such as partition coefficient, size, and possibly other pharmacological activities may strongly influence these results. Finally, no immediate contractions were observed with any of the compounds tested following addition, thereby denoting a lack of direct agonist activity.

**Muscarinic $M_1$ and $M_2$ Receptor Binding Studies.** Because cholinergic agonist activity at $M_1$ receptor sites is thought to be beneficial in AD patients in contrast to presynaptic $M_2$ receptor agonist activity which may inhibit acetylcholine release, the selectivity for these receptors was determined for the most active compounds. In addition, antagonist activity at $M_1$ receptor sites may mitigate the beneficial effects of AChE inhibition in the CNS.

The range of $M_2/M_1$ ratios was relatively narrow, varying approximately 7-fold, with compound **12** being the most $M_1$ selective agent possessing an $M_2/M_1$ ratio = 3.5. Compound **2** demonstrated the least $M_1$ selectivity with an $M_2/M_1$ ratio of 0.55. THA demonstrated no selectivity, while physostigmine displayed a relatively high $M_2/M_1$ ratio, although possessing a much weaker affinity for either receptor as evidenced by its $M_1$ $IC_{50}$ of 37 $\mu$M and its $M_2$ $IC_{50}$ of 160 $\mu$M. Interestingly, $M_1$ binding affinity in the homologous series **10–17** seems to somewhat parallel AChE inhibitory activity (Figure 1), possibly indicating a degree of homology at these receptor sites.

**Brain Acetylcholine (ACh) Levels.** In an initial attempt to evaluate the ability of compounds **2–22** to elevate brain ACh levels, physostigmine, THA, and the synthesized compounds were administered to mice at 80% of their approximate lethal doses. Only the homologous series of compounds **11–13** caused significant elevations. Compound **12**, the most potent agent in this series, increased brain ACh concentrations by 21% relative to control. In general, compounds **2–22** were all much less active than physostigmine and THA which increased brain ACh levels by 44.4% and 40.8%, respectively at much lower dosages.

Interestingly, two of the more potent AChE inhibitors, compounds **2** and **3**, significantly decreased brain ACh levels, suggesting additional pharmacological effects may be involved. It is also noteworthy that the changes in brain ACh levels roughly parallelled the AChE inhibitory activity in the homologous series **10–17** (Figure 1) although there was once again a very weak statistical correlation ($r = 0.21$) between these activities in this series and in the 13 synthetic compounds listed in Table II ($r = 0.38$).

**Differentiation of Agonist–Antagonist Binding.** As a check on the present method, three reference compounds were included to cover the spectrum of muscarinic receptor binding: atropine (antagonist), oxotremorine (partial agonist), and carbachol (agonist). In general, the present results for atropine and oxotremorine are in good agreement with the data of Freedman et al.[18] The *N*-methylscopolamine/oxotremorine-M (NMS/OXO-M) ratio for carbachol was higher in the present assay (8300) compared to the data of Freedman et al.[18] (4100). One obvious explanation is the substitution of mouse tissue in the present method for rat tissue as the source of receptor material.

As shown in Table III, the degree of agonist–antagonist binding was examined for compound **12**, which also demonstrated the greatest increase in brain acetylcholine levels at 80% of its approximate lethal dose. Compound **12** possessed an NMS/OXO-M ratio of 17 and therefore, according to the classification of Freedman et al.,[18] may be ranked between a pure antagonist (atropine) and a partial agonist (oxotremorine). In this respect, compound **12** resembles THA which had an NMS/OXO-M ratio of 26.

## Summary

The bis[[(dimethylamino)methyl]furanyl] analogues **2–22** represent a previously unreported class of potent

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Table II. Cholinergic Related Activity of Selected Bis[[(dimethylamino)methyl]furanyl] Analogues

| compd no. or name | BChE-I:[a] $IC_{50}$, μM | BChE-I/AChE-I[b] | $M_1$:[c] $IC_{50}$, μM | $M_2$:[d] $IC_{50}$, μM | $M_2$-$IC_{50}$/$M_1$-$IC_{50}$ | ileal activity,[e] μM | [ACh] % change from control[f] | approximate lethal dose,[g] mg/kg |
|---|---|---|---|---|---|---|---|---|
| physostigmine | 0.035 | 0.81 | 37 | 160 | 4.4 | <0.005 | 144***[h] | 0.8 |
| THA | 0.023 | 0.13 | 2.0 | 2.1 | 1.0 | <0.01 | 140*** | >20, <25 |
| 2 | 1.3 | 18 | 0.90 | 0.50 | 0.55 | <0.1 | 84+++ | >5, <12.5 |
| 3 | 2.4 | 8.3 | 0.66 | 0.47 | 0.70 | <0.01 | 86++ | >50, <60.0 |
| 8 | 0.025 | 0.89 | 0.49 | 0.59 | 1.2 | <0.05 | 99 | >70, <80 |
| 10 | 4.3 | 8.6 | 1.1 | 1.4 | 1.3 | <0.1 | 94 | >100, <110 |
| 11 | 4.1 | 12 | 1.1 | 1.5 | 1.4 | <0.1 | 113+ | >90, <100 |
| 12 | 3.1 | 15 | 0.63 | 2.2 | 3.5 | <1 | 121+++ | >60, <80 |
| 13 | 3.6 | 4.0 | 0.81 | 0.52 | 0.64 | <0.1 | 113+++ | >70, <80 |
| 14 | 2.9 | 3.9 | 0.54 | 0.58 | 1.1 | inactive | 104 | >60, <70 |
| 15 | 1.9 | 3.1 | 0.41 | 0.71 | 1.7 | inactive | 93 | >100, <110 |
| 16 | 1.9 | 4.0 | 0.31 | 0.54 | 1.8 | <0.1 | 106 | >110, <120 |
| 17 | 1.0 | 2.2 | 0.56 | 0.40 | 0.71 | <0.1 | 90 | >100, <110 |
| 21 | 2.7 | 10 | 0.54 | 0.55 | 1.0 | inactive | 104 | >80, <90 |
| 22 | 1.3 | 7.2 | 0.68 | 1.6 | 2.4 | <0.01 | 93 | >100, <120 |

[a] BChE activity was determined using human butyrylcholinesterase at 37 °C (pH 7.9), with butyrylthiocholine as substrate ($5 \times 10^{-5}$ M). [b] Ratio of $IC_{50}$ values obtained for BChE and AChE. [c] Mouse $M_1$ muscarinic receptor binding assay using cerebral cortex and [$^3$H]pirenzepine. [d] Rat $M_2$ muscarinic receptor binding assay using heart tissue and [$^3$H]methylscopolamine. [e] Concentration of compound required to potentiate the contraction of isolated rat ileum produced by $1 \times 10^{-7}$ M ACh by 20 percent. [f] Mouse brain ACh concentrations were determined using GC/MS methodology. Mice were sacrificed by microwave irradiation 15 min after intraperitoneal administration of 80% of the estimated lethal dose for each respective compound. Values are expressed as a percent of the ACh control value (25.0 nmol/g). [g] Mice were administered increasing or decreasing doses of compound intraperitoneally until one fatality was observed ($N = 1$). The middle of the approximate lethal dose range was used as the approximate lethal dose. [h] *** $p \leq 0.001$; +++ $p \leq 0.02$; ++ $p \leq 0.025$; + $p \leq 0.05$.

Table III. Differentiation of Agonist–Antagonist Binding

| agent | $K_i$[a] [$^3$H]NMS,[b] μM | $K_i$[a] [$^3$H]OXO-M,[c] μM | NMS/OXO-M[d] |
|---|---|---|---|
| atropine | 0.00070 | 0.00035 | 2.0 |
| oxotremorine | 1.5 | 0.0017 | 880 |
| carbachol | 66 | 0.0080 | 8300 |
| THA | 5.8 | 0.22 | 26 |
| 12 | 20 | 1.2 | 17 |

[a] Displacement data was obtained from mouse cerebral cortex tissue using the method of Freedman et al.[18] Apparent $K_i$ values were obtained from $IC_{50}$ values using the Cheng–Prusoff[24] equation. Each apparent $K_i$ value represents the mean of 2–3 determinations. Each determination was obtained from 7–9 concentrations performed in duplicate. [b] $IC_{50}$ values were obtained using 0.3 nM [$^3$H]methylscopolamine ([$^3$H]NMS) with 50 μg of protein in a 1-mL volume and mathematically converted to $K_i$ values. [c] $IC_{50}$ values were obtained using 2 nM [$^3$H]oxotremorine-M ([$^3$H]OXO-M) with 200 μg of protein in a 1-mL volume and mathematically converted to $K_i$ values. [d] NMS/OXO-M values are calculated from the ratio of the respective apparent $K_i$ values.

AChE inhibitors. Evidence of the distribution of these nonquaternary compounds into the CNS is empirically suggested by the ability of compounds 11–13 to significantly elevate brain acetylcholine (ACh) levels. However, the fact that only these three agents increased brain ACh levels in the homologous series 10–17, suggests that distribution into the CNS is limited and that an optimum lipophilicity and molecular weight range are necessary for blood–brain barrier penetration. This is further corroborated by the enhanced abilities of the relatively low molecular weight compounds, THA and physostigmine, to increase brain ACh levels, although these compounds possess AChE $IC_{50}$ values similar to those of the series 2–22. Therefore, work is continuing on lower molecular weight analogues containing one [(dimethylamino)methyl]furan nucleus in an effort to optimize distribution into the CNS.

## Experimental Section

**Chemistry.** Melting points were determined on an Electrothermal melting point apparatus (capillary method) and are uncorrected. The NMR spectra were determined on a Varian EM 360A or 390 NMR spectrophotometer using tetramethylsilane as an internal standard and deuteriochloroform as the solvent. Infrared spectra were determined on a Beckman Acculab 4 spectrophotometer using the potassium bromide technique when applicable. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Georgia. Thin-layer chromatography was performed on Eastman Chromatogram sheets, type 6060 (silica gel).

***N,N'*-Bis[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]formamidine Trihydrochloride (4).** A solution of 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine (1.07 g, 5.0 mmol) and *s*-triazine (0.075 g, 0.92 mmol) in dry THF (35 mL) was refluxed for 3 h. The solvent was removed in vacuo, and the resulting oil was added to a solution of 1.0 M hydrogen chloride in diethyl ether while stirring in an ice bath for 20 min. Additional diethyl ether was added until a precipitate formed. The solvent was removed under reduced pressure, and the resulting white crystals were recrystallized from 2-propanol/acetone (2:1). The product was dried in a drying pistol under $P_2O_5$ at 78 °C (refluxing EtOH), and the crystals were collected (1.09 g, 40%): mp 145–148 °C; IR (KBr) 3250, 2950, 2860, 2820, 2780, 1650, 1450, 1250, 1135, 1010, 840, 780 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 2.72 (s, 12 H, $N(CH_3)_2$), 2.83 (m, 4 H, $-SCH_2CH_2-$), 3.60 (broad s, 4 H, $-NCH_2CH_2-$), 3.95 (d, 4 H, furan-$CH_2$-N), 4.42 (s, 4 H, furan-$CH_2$-S), 6.42 (d, 2 H, furan protons) 6.73 (d, 4 H, furan protons) ppm. Anal. ($C_{21}H_{37}Cl_3N_4O_2S_2$) C, H, N, S.

***N,N'*-Bis[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]urea (5).** A solution of 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine (2.14 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) in dry acetonitrile (35 mL) was stirred in an ice bath for 15 min as bis(trichloromethyl) carbonate (triphosgene) (0.495 g, 1.67 mmol) in dry acetonitrile (10 mL) was added dropwise. The solution was allowed to warm to room temperature as it stirred overnight. The solvent was removed in vacuo, and the resulting oil was added to a solution of 1.0 N NaOH (25 mL) and ice. The mixture was extracted with EtOAc (3 × 30 mL), the organic layer was washed with water, and then extracted with 0.1 N HCl. The aqueous layer was alkalinized with 1.0 N NaOH and extracted with $CH_2Cl_2$. The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, and filtered. The solvent was removed in vacuo, to yield a crude product which was purified by flash chromatography (230–400 mesh silica gel) using acetone/methanol (3:1) as the eluting solvent. After removal of the solvent under reduced pressure, the resulting product was triturated in anhydrous diethyl ether and solidified overnight in the freezer to yield a white solid which was collected (0.60 g, 13%) via vacuum filtration and air dried: mp 44–46 °C; IR (KBr) 3200, 2940, 2840, 1665, 1550, 1380, 1350, 1245, 1220, 1000, 780 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 2.21 (s, 12

H, $N(CH_3)_2$), 2.62 (t, 4 H, $SCH_2CH_2$), 3.32 (m, 4 H, $CH_2CH_2NH$), 3.42 (s, 4 H, $CH_2N(CH_3)_2$), 3.72 (s, 4 H, furan-$CH_2$-S), 6.02 (s, 4 H, furan protons), 8.02 (s, 2 H, NH) ppm. Anal. ($C_{21}H_{34}N_4O_3S_2 \cdot 1.0H_2O$) C, H, N, S.

**N-[2-[[[5-[(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-2-[(2-furanylmethyl)thio]acetamide (6).** 2-[(2-Furanylmethyl)thio]acetyl chloride[15] (2.28 g, 12 mmol) in 10 mL dry acetonitrile, was added dropwise to a cold solution of 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine (2.14 g, 10 mmol) in dry acetonitrile (35 mL). The solution was allowed to warm to room temperature and was stirred for 24 h. The solvent was removed in vacuo, and the resulting crude product was partitioned between $CH_2Cl_2$ and water. The aqueous layer was discarded and the organic layer was extracted with 10% $NaHCO_3$. The combined organic layers were washed with brine, dried over $Na_2SO_4$, and filtered. The solvent was removed in vacuo to yield a crude product which was purified by flash chromatography (230–400 mesh silica gel) using acetone as the mobile phase. Removal of the acetone under reduced pressure yielded a yellow-orange oil (2.11 g, 58%): IR (KBr) 3300, 3110, 2940, 2810, 2770, 1650, 1515, 1350, 1210, 1140, 1000, 925, 780, 725 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.23 (s, 6 H, $N(CH_3)_2$), 2.63 (t, 2 H, 2-$CH_2CH_2$), 3.16 (s, 2 H, $SCH_2CO$), 3.34 (m, 4 H, $CH_2CH_2NH$ and $CH_2N(CH_3)_2$), 3.68–3.88 (d, 4 H, furan-$CH_2$-S-), 6.05 (s, 2 H, furan protons), 6.18 (m, 2 H, furan protons), 7.26 (m, 1 H, ArH) ppm. Anal. ($C_{17}H_{24}N_2O_3S_2$) C, H, N, S.

**N-[2-[[[5-[(Dimethylamino)methyl]-2-furanyl]-2-methyl]thio]ethyl]-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]acetamide (7).** N-[2-[[[5-[(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-2-[(2-furanylmethyl)thio]acetamide (6) (1.47 g, 4.0 mmol) in dry acetonitrile (10 mL) was added dropwise to a solution of dimethylmethyleneammonium chloride (0.43 g, 4.6 mmol) in dry acetonitrile (35 mL). The solution was stirred at room temperature for 72 h. The solvent was removed in vacuo, and the resulting crude product was partitioned between $CH_2Cl_2$ and water. The organic layer was discarded and the aqueous layer was alkalinized with 1 N NaOH and extracted with $CH_2Cl_2$ (3 × 30 mL). The organic layers were combined, washed with brine, dried over anhydrous $Na_2SO_4$, and filtered. The solvent was removed in vacuo to yield a crude product which was purified by flash chromatography (230–400 mesh silica gel) using acetone/methanol (4:1) as the mobile phase. Removal of the solvents under reduced pressure yielded a light yellow oil (1.28 g, 75%): $^1H$ NMR ($CDCl_3$) $\delta$ 2.25 (s, 12 H, $N(CH_3)_2$), 2.60 (t, 2 H, $SCH_2CH_2$), 3.10 (s, 2 H, $-SCH_2-$), 3.10 (s, 2 H, $-SCH_2CO-$), 3.30 (q, 2 H, $CH_2CH_2NH$), 3.40 (s, 4 H, $CH_2N(CH_3)_2$), 3.70 (s, 4 H, furan-$CH_2$-S-), 6.05 (s, 4 H, furan protons), 7.10 (broad s, 1 H, NH) ppm. Anal. ($C_{20}H_{31}N_3O_2S_2$) C, H, N, S.

**N,N'-Bis[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-4,6-dinitro-1,3-benzenediamine (8).** A solution of 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine (2.14 g, 10 mmol) and anhydrous $Na_2CO_3$ (1.06 g, 10 mmol) in dry acetonitrile (35 mL) was treated with 1,5-difluoro-2,4-dinitrobenzene (1.02 g, 5.0 mmol) in dry acetonitrile (10 mL) and refluxed for 3 h. The solvent was removed in vacuo, and the resulting oil was partitioned between $CH_2Cl_2$ and water. The aqueous layer was extracted with methylene chloride (3 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, and filtered. The solvent was removed in vacuo to yield a crude product which was purified by flash chromatography (230–400 mesh silica gel) using acetone as the eluting solvent. After removal of the acetone under reduced pressure, the resulting product was triturated in anhydrous diethyl ether and solidified overnight in the freezer to yield a bright yellow solid which was collected (2.26 g, 76%) via vacuum filtration and air dried: mp 56–57 °C; IR (KBr) 3360, 2920, 2750, 1600, 1570, 1400, 1240, 1190, 1070, 1000, 770, 680 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.20 (s, 12 H, $N(CH_3)_2$), 2.82 (t, 4 H, $SCH_2CH_2$), 3.40 (s, 4 H, $CH_2N(CH_3)_2$), 3.45 (q, 4 H, $CH_2CH_2NH$), 3.73 (s, 4 H, furan-$CH_2$-S), 5.60 (s, 1 H, ArH), 6.16 (d, 4 H, furan protons), 8.50 (t, 2 H, NH), 9.18 (s, 1 H, ArH) ppm. Anal. ($C_{26}H_{36}N_6O_6S_2 \cdot 0.5H_2O$) C, H, N, S.

**Bis[[5-[(dimethylamino)methyl]-2-furanyl]methyl] Sulfide (9).** Furfuryl sulfide, (1.94 g, 10 mmol) was dissolved in 15 mL of dry acetonitrile. N,N-Dimethylmethyleneammonium chloride, (2.06 g, 22 mmol) was added, and the resulting solution was allowed to stir overnight at room temperature. The solvent was removed in vacuo, and the resulting residue dissolved in 50 mL of $H_2O$. Aqueous sodium hydroxide solution (10%) was added to alkalinity, and the solution extracted with $CH_2Cl_2$. The organic layer was dried over $MgSO_4$ to yield 2.92 g (95%) of slightly impure 9. Flash chromatography of 1.50 g of this material yielded 0.150 g of pure 9: mp 61–62 °C (lit.[17] mp 58–59 °C); $^1H$ NMR (CDCl) $\delta$ 6.03 (s, 4 H, furan), 3.62 (s, 4 H, $CH_2S$), 3.40 (s, 4 H, $CH_2N$), 2.26 (s, 12 H, $(CH_3)_2$) ppm.

**1,2-Bis[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethane (10).** A solution of furfuryl mercaptan (5.02 g, 44 mmol) in dry THF (50 mL) was treated with potassium *tert*-butoxide (4.94 g, 44 mmol) while stirring in an ice bath. After 10 min, 1,2-dibromoethane (3.76 g, 20 mmol) was added and the mixture stirred at room temperature for 7 days. The solvent was removed in vacuo and the residue partitioned between EtOAc (200 mL) and 1.0 N aqueous NaOH (100 mL). The organic layer was extracted again with 1.0 N aqueous NaOH and brine and then dried over anhydrous $Na_2SO_4$. The solvent was removed in vacuo to yield the desired product (5.08 g, 100%) as a light yellow oil. A solution of the product, 1,2-bis[(2-furanylmethyl)thio]ethane (1.27 g, 5.0 mmol), in dry acetonitrile (10 mL) was treated with N,N-dimethylmethyleneammonium chloride (1.17 g, 12.5 mmol) and the resulting suspension stirred at room temperature for 24 h. Water (50 mL) was added, and the aqueous solution was extracted with $CH_2Cl_2$ (25 mL). The aqueous layer was alkalinized with 1.0 N aqueous NaOH and the free base extracted into $CH_2Cl_2$. The organic layer was dried over anhydrous $Na_2SO_4$, the solvent was removed in vacuo, and the crude product was purified by column chromatography (230–400 mesh silica gel) using acetone as the eluting solvent. The pale yellow oil (1.35 g, 73%) was crystallized from hexanes (freezer); however, the crystals melted at ambient temperature: $^1H$ NMR ($CDCl_3$) $\delta$ 2.24 (s, 12 H, $N(CH_3)_2$), 2.67 (s, 4 H, $-CH_2CH_2-$), 3.38 (s, 4 H, $=NCH_2-$), 3.67 (s, 4 H, $-CH_2S-$), 6.05 (s, 4 H, furan protons) ppm. Anal. ($C_{18}H_{28}N_2O_2S$) C, H, N, S.

**Compounds 11–22.** The procedure given for the synthesis of 10 was utilized for the synthesis of compounds 11–22.

**Animal Species.** Male albino mice (Harland/ICR) weighing 20–30 g and male albino rats (Harlan Sprague-Dawley) weighing 175–300 g were used in all procedures requiring animal tissue sources.

**Cholinesterase Assays.** Acetylcholinesterase and butyrylcholinesterase inhibitory activity of the analogues was determined at 37 °C and a pH of 7.9 using the method of Ellman et al.[19] Enzyme prepared from human red blood cells was used as the source of acetylcholinesterase (Sigma Type XIII) and butyrylcholinesterase (Sigma). Test compounds were incubated for 2 min prior to the addition of substrate, and enzyme velocity was determined during the initial 1–2 min after substrate addition. The final substrate concentration (acetylthiocholine) was $5 \times 10^{-5}$ M. The concentration of analogue that reduced enzyme activity by 50% ($IC_{50}$) was determined from a semilog plot of the percentage inhibition of enzyme velocity versus the substrate concentration.

**$M_1$ and $M_2$ Receptor Binding Assays.** The affinity of the analogues for $M_1$ and $M_2$ muscarinic receptors was examined using a filtration assay[20] as modified by Patterson et al.[21] $IC_{50}$ values were determined from the ability of the analogue to inhibit by 50% the binding of the radioactive ligand to the receptor. Nonspecific binding was determined with 1 $\mu$M atropine. Data analysis was performed using the EBDA program contained in the software package LIGAND (Elsevier-BIOSOFT). Protein deter-

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minations were obtained using the Bradford assay.[22]

The affinity of the analogues for the muscarinic $M_1$ receptor was examined in mouse brain cerebral cortex tissue using [$^3$H]-pirenzepine. Cerebral cortex tissue was homogenized in 10 volumes of sodium potassium phosphate buffer (pH 7.4, 0.05 M) in an Elvehjem apparatus with a Teflon pestle. The homogenate was then centrifuged at 1000$g$ for 10 min, the supernatant recentrifuged at 40000$g$ for 1 h, and the pellet resuspended in buffer. The reaction volume was 1 mL and contained 200 $\mu$g of protein, 100 $\mu$M DFP, 3 nM [$^3$H]pirenzepine, analogue, and sodium potassium phosphate buffer. Analogue and ligand were added 20 min after the addition of DFP and incubated an additional 40 min at 20 °C. The assay was terminated by filtration through Whatman GF/B filters using a Brandel Cell Harvester. The filters were washed three times with 2 mL of cold buffer, air dried, and counted for radioactivity after 12 h.

Rat heart was the tissue source for $M_2$ muscarinic receptor material. After rinsing in buffer, the tissue was minced with scissors, and homogenized in 10 volumes of sodium potassium phosphate buffer, as above, in a Polytron (2 × 30 s, speed 6), and further homogenized in a Elvehjem apparatus with a Teflon pestle. The homogenate was then centrifuged at 1000$g$ for 10 min, and then the pellet rehomogenized with fresh buffer and centrifuged at 1000$g$. The 1000$g$ supernatants were pooled and centrifuged at 40000$g$ for 35 min, and the pellet was resuspended in buffer. The reaction volume used was 2 mL and contained 500 $\mu$g of protein, 100 $\mu$M DFP, 0.3 nM [$^3$H]$N$-methylscopolamine, analogue, and sodium potassium phosphate buffer. The remaining assay procedure was the same as for the $M_1$ receptor assay.

**Differentiation of Agonist–Antagonist Activity.** The method of Freedman et al.[18] was used to determine whether the observed analogue binding represented muscarinic agonist or antagonist affinity. The procedure requires determination of the binding affinity of the analogue to receptors in the presence of either [$^3$H]oxotremorine-M or [$^3$H]$N$-methylscopolamine. Mouse, instead of rat, cerebral cortex was used as the tissue source for the receptor material.

Briefly, for determination of agonist affinity, tissue was homogenized in ice-cold 0.32 M sucrose (1:10 w/v) using a Teflon–glass homogenizer and centrifuged at 1000$g$ for 10 min, and the supernatant was recentrifuged at 17000$g$ for 20 min. The crude synaptosomal pellet ($P_2$) was resuspended in 20 mM HEPES buffer (pH 7.4) and centrifuged at 17000$g$ for 15 min. The resulting pellet was then resuspended in 20 mM HEPES buffer and used in the binding protocol. Binding studies were performed in polystyrene tubes at 30 °C in a 1-mL volume using 200 $\mu$g of protein, 2.0 nM [H]oxotremorine-M, and analogue. After 40 min of incubation, the tubes were filtered on Whatman GF/C filters soaked in 0.5% polyethyleneimine using a Brandel Cell Harvester. The filters were then washed three times with ice-cold saline (0.9%), dried, and counted after 12 h. Nonspecific binding was determined with 1 $\mu$M atropine.

For determination of antagonist affinity, tissue was prepared as above except that the $P_2$ pellet was resuspended in Krebs–HEPES buffer (pH 7.4) and used without further treatment. Binding studies utilized a 1-mL final volume with 50 $\mu$g of protein, 0.3 nM [$^3$H]$N$-methylscopolamine, and analogue. After incubating for 60 min at 30 °C the tubes were filtered, washed as above using Whatman GF/B filters, and counted after 12 h.

Data from both procedures were analyzed using the EBDA program from LIGAND (Elsevier-BIOSOFT). Apparent $K_i$ values were also calculated by the program using the Cheng–Prusoff equation[23] $K_i = IC_{50}/(1 + C/K_d)$ where $IC_{50}$ is the concentration of the analogue which inhibits specific radioligand binding by 50%; $K_d$ is the dissociation constant of the radioligand receptor complex, and $C$ is the concentration of free radiolabeled ligand. The $K_d$ values used in $K_i$ calculations for [$^3$H]oxotremorine-M and [$^3$H]$N$-methylscopolamine were 0.7 and 0.3 nM, respectively.

Determination of agonist or antagonist activity for the analogue was then obtained from the ratio of the apparent $K_i$ values for oxotremorine-M and $N$-methylscopolamine. Atropine, oxotremorine, and carbachol were used for comparison purposes as examples of agents that exhibited antagonistic, partial agonistic, and full agonistic activity at the muscarinic receptor.

**Potentiation of ACh-Induced Contraction.** Rat ileal tissue was placed in Tyrode's solution at 37 °C and prepared for recording using a Narco-BioSystems's DMP4-A and an isometric transducer to monitor contractions. Muscle contractions were induced with a bath concentration of $1 \times 10^{-7}$ M acetylcholine. Varying concentrations of analogue were then added to the bath 30 s prior to the addition of acetylcholine. The minimum concentration of analogue required to potentiate the acetylcholine-induced contraction by 20% was the endpoint of the procedure.

**Toxicity Determination.** Mice were administered intraperitoneally, varying doses of analogue dissolved in 0.05 M sodium acetate buffer (pH 4.0). The analogue dosage was increased until one fatality was observed. The data is only a crude estimate of toxicity since only one animal was tested at each dosage level. The approximate lethal dose is therefore, presented as the midpoint of the dosage range, represented by the highest dose that was not lethal, and the lowest dose that was lethal.

**Effect of Analogues on Brain Acetylcholine Concentrations.** Mice were administered selected compounds by intraperitoneal injection at 80% of the approximate lethal dose of the analogue as determined above. After 15 min, mice were sacrificed by microwave irradiation and brain tissue (minus the cerebellum) was processed for acetylcholine content.

Quantitation of acetylcholine was accomplished using a modification of a gas chromatographic–mass spectrometric assay.[23] Briefly, tissue was homogenized in formic acid/acetonitrile together with 5.0 nmol [$^2H_9$]acetylcholine as internal standard. The tissue was then ion-pair extracted with dipicrylamine, demethylated with sodium thiophenolate, washed, and extracted into chloroform. The chloroform extract was then injected into a Hewlett-Packard gas chromatograph–mass spectrometer (Model 5890/5970). The compounds of interest were separated on a Stabilwax (Restek Corporation) column using an initial temperature of 50 °C, a ramp of 40 °C/min to 110 °C, followed by a second ramp of 10 °C/min, and a final temperature of 150 °C. [$^2H_0$]Acetylcholine and [$^2H_9$]acetylcholine were analyzed in the selected ion monitoring (SIM) mode at $m/e$ 58 and $m/e$ 64, respectively. Areas were corrected for spillover using a Pascal-based program written by the author (JK). Quantitation was accomplished from the ratio of the corrected areas of these two ions (58/64) and referenced to a standard curve.

**Statistical Evaluations.** All statistical calculations were performed using multiple regression or "$t$-test" procedures from the software package ABSTAT marketed by Anderson Bell.

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**Registry No.** 1, 66356-53-4; 2, 72126-78-4; 3, 72148-12-0; 4, 138878-39-4; 4·free base, 138878-40-7; 5, 72126-82-0; 6, 108498-90-4; 7, 138878-41-8; 8, 138878-42-9; 9, 19260-90-3; 10, 117846-01-2; 11, 138878-43-0; 12, 138878-44-1; 13, 138878-45-2; 14, 138878-46-3; 15, 138878-47-4; 16, 138878-48-5; 17, 138878-49-6; 18, 138878-50-9; 19, 138878-51-0; 20, 138878-52-1; 21, 138878-53-2; 22, 138878-54-3; $s$-triazine, 290-87-9; bis(trichloromethyl)carbonate, 32315-10-9; 2-[(2-furanylmethyl)thio]acetyl chloride, 89793-50-0; dimethylmethyleneammonium chloride, 30354-18-8; 1,5-difluoro-2,4-dinitrobenzene, 327-92-4; furfuryl sulfide, 13678-67-6; furfuryl mercaptan, 98-02-2; 1,2-dibromoethane, 106-93-4.

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