were placed on a horizontal wire ring 5.5 cm in diameter, which was attached to a 16-cm vertical rod. The hind paws and fore paws were placed at opposite sides of the ring. It is important that the ambient temperature is maintained at 30 °C and that the environment be free of auditory stimuli and bright lights. The criteria for immobility are detailed in ref 22. The response is calculated as the fraction of time the mouse is immobile over a 5-min test period. Measurements were always done between 2 and 4 p.m. and the animals were used only once.

Acknowledgment. We thank NIDA for partial support (Grants DA02052, DA06481 and a Research Scientist Award to SHB) and Prof. M. A. Tius, University of Hawaii, for helpful correspondence.

Registry No. **1b**, 113418-02-3; **3a**, 137945-48-3; **3b**, 137945-49-4; **3c**, 137945-50-7; **3d**, 137945-51-8; **3e**, 137945-52-9; **4a**, 137945-53-0; **4b**, 137945-54-1; **4c**, 137945-55-2; **5a**, 137945-56-3; **5b**, 137945-57-4; **5c**, 137945-58-5; **6a**, 137945-59-6; **6b**, 137945-60-9.

Synthesis and Cholinergic Properties of *N*-Aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamino Analogs of Ranitidine

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A series of *N*-aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamino analogs of the H_2-antagonist, ranitidine, was synthesized and the abilities of the compounds to alleviate the cholinergic deficit characteristic of Alzheimer's disease evaluated. The compounds were initially tested for their ability to inhibit human erythrocyte acetylcholinesterase activity in vitro. Selected compounds were further evaluated for butyrylcholinesterase inhibition, M_1 and M_2 cholinergic receptor binding, potentiation of ileal contractions, and the ability to elevate brain acetylcholine levels in mice. The analogs were compared to tetrahydroaminoacridine and to a recently reported series of bis[[(dimethylamino)methyl]furans]. The *N*-aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine derivatives were generally comparable to tetrahydroaminoacridine and the bis[[(dimethylamino)methyl]furans] in acetylcholinesterase inhibition, M_1/M_2 receptor binding, and the potentiation of ileal contractions, while being more potent inhibitors of acetylcholinesterase than butyrylcholinesterase. The 4-nitro-3-pyridazinyl analog, **26**, was notable in demonstrating a potent and selective binding to the M_2 receptor, with an M_2 IC_{50}/M_1 IC_{50} of 0.060. Compounds in which the substituents on the dinitro-*N*-aryl moiety were relatively small were the best at inhibiting acetylcholinesterase in vitro. The *N*-aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamines in general, and those with small *N*-aryl substituents in particular, were superior to the bis[[(dimethylamino)methyl]furans] in elevating brain ACh levels in mice, probably due to enhanced distribution into the CNS. The 1,5-difluoro-2,4-dinitrophenyl analog, **8**, resulted in the largest elevation in brain acetylcholine levels, affording a 53% increase at 88 mg/kg.

In a previous communication, we described the synthesis and cholinergic properties of a series of bis[[(dimethylamino)methyl]furan] analogs of ranitidine.[1] These compounds possessed the general structure shown in Figure 1, and demonstrated potent acetylcholinesterase (AChE) inhibitory activity in vitro for a wide variety of substituents, "Z". Compound 1 (IC_{50} = 0.03 μM) was the most potent AChE inhibitor in the series and was found to be approximately 6 times more potent than tetrahydro-9-aminoacridine (THA), which is currently undergoing extensive clinical investigation in the treatment of Alzheimer's disease (AD). A number of these analogs also exhibited an enhanced selectivity for AChE inhibition vs butyrylcholinesterase (BChE) inhibition, possessed M_1/M_2 muscarinic receptor affinities similar to THA, and potentiated acetylcholine-induced contractions of isolated rat ileum. The bis[[(dimethylamino)methyl]furans], however, showed little ability to elevate mouse brain acetylcholine levels in vivo, with the most potent compound, **2**, demonstrating a 22% increase at 80% of its approximate lethal dose. The relatively high molecular weights of these compounds and the presence of two very basic tertiary, aliphatic amino groups probably limited distribution into the central nervous system (CNS).[1]

Utilizing compound 1 as a prototype, we have therefore synthesized a series of *N*-aryl derivatives of 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine with the general structure shown in Figure 2. We have also synthesized N-substituted analogs of 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine, containing 2-cyano-3-fluorophenyl, 2-nitro-4-fluorophenyl and 4-nitro-3-pyridazinyl moieties.

In comparison to compound 1, the series of compounds in the present work had lower molecular weights and generally possessed a single, tertiary, aliphatic amino group. It was postulated that these changes would augment distribution across the blood–brain barrier, thus allowing these compounds to display greater cholinergic effects within the CNS.

Chemistry

N-Arylation of the primary amine[2] **3** with either 2,4-dinitrofluorobenzene, 1,5-difluoro-2,4-dinitrobenzene, 2,6-difluorobenzonitrile, or 2,5-difluoronitrobenzene in acetonitrile in the presence of anhydrous sodium carbonate yielded compounds **4–7**, respectively. These products were

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‡ Shanxi Medical College.
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(1) Sowell, J. W., Sr.; Tang, Yunzhao; Valli, M. J.; Chapman, J. M., Jr.; Usher, L. A.; Vaughn, C. M.; Kosh, J. W. Synthesis and cholinergic properties of bis[[(dimethylamino)methyl]furanyl] analogs of ranitidine. *J. Med. Chem.* **1992**, *35*, 1102–1108.
(2) Price, B. J.; Clitherow, J. W.; Bradshaw, J. Patentschrift (Switz.) 647, 517. Furanalkanamide derivatives. *Chem. Abstr.* **1986**, *104*, P 168352f.

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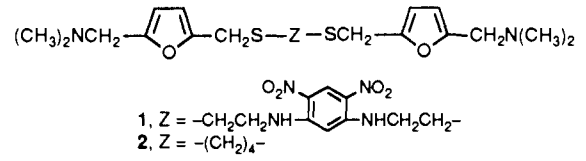


1, Z = $-CH_2CH_2NH-$ (2,4-dinitrophenyl) $-NHCH_2CH_2-$
2, Z = $-(CH_2)_4-$

Figure 1.

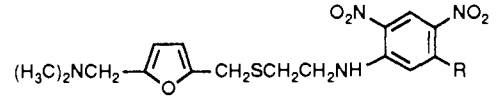
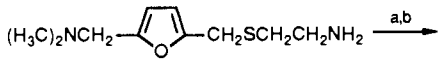
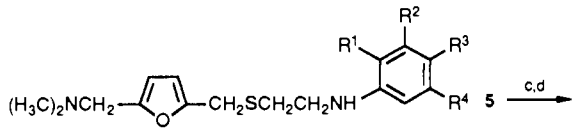


Figure 2.

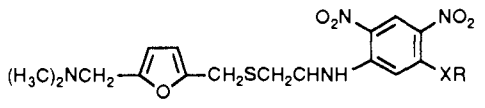
Scheme I[a]



3



4–7



8–23

comp	XR	comp	XR	comp	R^1	R^2	R^3	R^4
8	NH_2	16	$NHCH_2C_6H_5$	4	NO_2	H	NO_2	H
9	$NHCH_3$	17	$NHNH_2$	5	NO_2	H	NO_2	F
10	NHC_3H_7n	18	$NHN{=}C(CH_3)_2$	6	CN	F	H	H
11	$NHC_5H_{11}n$	19	NH_6H_5	7	NO_2	H	F	H
12	$NHCH_2CH_2N(CH_3)_2$	20	NHC_6H_4-*p*-F					
		21	NHC_6H_4-*o*-CN					
13	$NHCH_2CH_2CH_2$-morpholino	22	NH-(2-nitro-3-pyridyl)					
14	$NHCH_2$-(3-pyridyl)	23	OC_6H_4-*p*-NO_2					
15	$NHCH_2$-(4-pyridyl)							

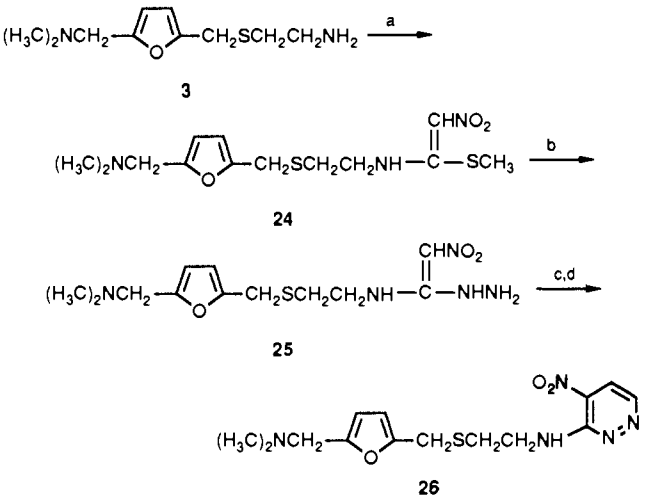
[a] Reagents: (a) Na_2CO_3, CH_3CN; (b) 2,4-dinitrofluorobenzene, 1,5-difluoro-2,4-dinitrobenzene, 2,6-difluorobenzonitrile, or 2,5-difluoronitrobenzene; (c) Na_2CO_3 or NaH; (d) primary amine or phenol.

obtained in yields ranging from 38 to 90% (Scheme I).

Compound **5** served as the immediate precursor to compounds **8–23**. The remaining fluorine atom of compound **5** was displaced by either ammonia, primary aliphatic amines, hydrazine, aniline, substituted anilines, or *p*-nitrophenol to yield the desired products. Typically, these compounds were easily purified by flash chromatography and yields were generally greater than 75%.

Compound **24**[3] was prepared by condensation of the primary amine **3** with 1,1-bis(methylthio)-2-nitroethylene in refluxing acetonitrile under an inert atmosphere (Scheme II). Displacement of methyl mercaptan in compound **24** by hydrazine was facile, yielding compound **25**. Utilizing the procedure of Hamberger, et al.,[4] the pyridazine **26** was obtained in an excellent yield by the condensation of the hydrazine **25** with glyoxal.

Scheme II[a]



[a] Reagents: (a) 1,1-bis(methylthio)-2-nitroethylene, CH_3CN; (b) H_2NNH_2, absolute C_2H_5OH; (c) 40% aqueous glyoxal; (d) triton B, toluene, water.

Results and Discussion

With several significant exceptions, compounds **4–26** denote a novel series of potent acetylcholinesterase inhibitors which are pharmacologically similar to both THA and the previously reported[1] bis[[(dimethylamino)-methyl]furans] **1** and **2**. All of the agents were initially tested for in vitro AChE (human erythrocyte) inhibitory activity to determine their potential to reverse the cholinergic deficit characteristic of AD. Compounds that demonstrated AChE inhibitory concentrations (IC_{50}'s) of 10^{-6} M or less were further examined for additional cholinergic properties.

AChE Inhibition. With the exception of compound **6**, all the analogs presented in Table I were more potent in vitro inhibitors of AChE than ranitidine (IC_{50} = 2.3 μM).[1] In addition, the most active compounds, **5**, **8**, **9**, and **17** were slightly more potent inhibitors of the enzyme than THA (IC_{50} = 0.18 μM). Furthermore, compounds **8** and **17** possessed inhibitory activities similar to those of the more potent bis[[(dimethylamino)methyl]furanyl] derivatives previously reported, **1** and **2**.[1] It is interesting to note that the most active agents, **5** (R = F), **8** (R = NH_2), **9** (R = $NHCH_3$), and **17** (R = $NHNH_2$) all contained small R substituents (Table I). Activity, in fact, decreased as the size of the alkyl substituent on nitrogen, R, increased in compounds **8–11**. Compounds possessing aromatic R substituents were not generally as active as those containing small alkyl substituents, although the 3-(pyridylmethyl) analog **15** (IC_{50} = 0.31 μM) and the *p*-nitrophenyl ether **23** (IC_{50} = 0.27 μM) demonstrated moderate AChE inhibitory activity. Deviation from the 1,3-diamino-4,6-dinitrophenyl moiety resulted in the least active compound, **6**, although the 4-fluoro-2-nitrophenyl analog **7** (IC_{50} = 0.65 μM) and the 4-nitro-3-pyridazinyl analog **26** (IC_{50} = 0.47 μM) showed moderate activity.

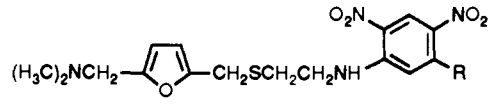
Finally, it should be noted that compound **5** retains an aromatic fluorine capable of undergoing nucleophilic displacement in this in vitro assay as well as the other pharmacological assays detailed below. Therefore, compound **5** may deviate significantly in mechanism from the other compounds presented herein.

Butyrylcholinesterase Inhibition. The ability of the compounds to selectively inhibit AChE rather than BChE was assessed in vitro. Compounds **4–26** were all much less

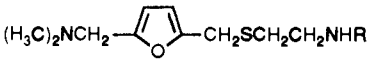
(3) Martin-Smith, M.; Price, B. J.; Bradshaw, J.; Clitherow, J. W. Eur. Patent Appl. 2,930. Amine derivatives and pharmaceutical compositions containing them. *Chem. Abstr.* **1980**, *92*, P76145w.

(4) Hamberger, H.; Reinshagen, H.; Schulz, G.; Sigmund, G. Polar Ethylene I: The synthesis of 4-nitropyridazine. *Tetrahedron Lett.* **1977**, *41*, 3619–3622.

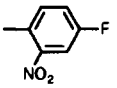
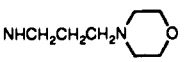
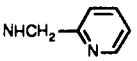
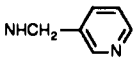
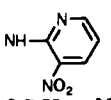
Table I. Physical and Biological Data of *N*-Aryl Derivatives of 2-[[[5-[(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine



compounds **4**, **5**, **8–23**



compounds **6**, **7**, **26**

compd	R	AChE-I[a] IC_{50} (μM)	yield (%)	recryst solvent	mp (°C)	anal.[b]
physostigmine		0.043	–	–	–	–
tetrahydro-9-aminoacridine (THA)		0.18	–	–	–	–
4	H	0.20	84	–	oil	C,H,N,S
5	F	0.12	90	ether[c]	68–69	C,H,N,S
6		2.1	38	ether[c]	37–38	C,H,N,S
7		0.65	68	ether[c]	49–51	C,H,N,S
8	NH_2	0.083	95	ether[c]	142–144	C,H,N,S
9	$NHCH_3$	0.16	69	ether[c]	104–106	C,H,N,S
10	NHC_3H_7n	0.26	90	ether[c]	63–64	C,H,N,S
11	$NHC_5H_{11}n$	1.2	87	ether[c]	49–51	C,H,N,S
12	$NHCH_2CH_2N(CH_3)_2$	0.52	73	ether[c]	70–71	C,H,N,S
13		0.37	78	ether[c]	97–99	C,H,N,S
14		1.3	70	ether[c]	124–126	C,H,N,S
15		0.31	41	ether[c]	107–108	C,H,N,S
16	$NHCH_2C_6H_5$	0.69	53	ether[c]	91–93	C,H,N,S
17	$NHNH_2$	0.076	29	MeOH	146–148	C,H,N,S
18	$NHN{=}C(CH_3)_2$	0.44	41	EtOH	101–103	C,H,N,S
19	NHC_6H_5	0.64	89	ether[c]	104–106	C,H,N,S
20	NHC_6H_4-*p*-F	0.68	93	ether[c]	118–120	C,H,N,S
21	NHC_6H_4-o-CN	0.63	24	ether[c]	119–121	C,H,N,S
22		0.65	13	ether[c]	110–112	C,H,N,S
23[d]	OC_6H_4-*p*-NO_2	0.27	40	EtOH	58 dec	C,H,N,S
26		0.47	80	–	oil	C,H,N,S

[a] Human acetylcholinesterase (Sigma Type XIII) was used and the procedure of Ellman[9] was followed. Experiments were conducted at room temperature and substrate (acetylthiocholine) concentration was 5.0×10^{-5} M. [b] Carbon, hydrogen, nitrogen, and sulfur analyses were within ±0.4% of theoretical. [c] Product obtained after flash chromatography was triturated with ether. [d] Isolated, characterized, and evaluated as the *p*-toluenesulfonic acid salt.

active inhibitors of BChE than THA or physostigmine and also generally possessed significantly more favorable BChE IC_{50}/AChE IC_{50} ratios (Table II). In fact, 11 of the 19 compounds tested had ratios greater than 10. The *p*-fluorophenyl analog, **20**, with a ratio of 1.6, was the least favorable, whereas the hydrazino derivative, **17**, with a ratio of 67, was the most favorable.

Ileal Activity. The effect of the compounds on acetylcholine-induced contractions in isolated rat ileum was determined in order to initially assess their distribution and overall cholinergic activity in an intact tissue.

Nine of the compounds tested potentiated acetylcholine-induced rat ileal contraction by at least 20% at a concentration of 0.1 μM or less (Table II). Compounds **5**, **12**, and **20** were the most active in this assay and were comparable to THA, possessing activity at a concentration of 0.01 μM or less.

Compounds **7**, **9**, **15**, **16**, **19**, and **21** were inactive, possibly reflecting poor distribution of these compounds into the intact ileal tissue. Furthermore, no contractions were observed immediately following the addition of any of the test compounds, thereby denoting the absence of significant direct agonist activity.

Muscarinic M_1 and M_2 Receptor Binding Studies. Whereas, agonist activity at M_1-cholinergic receptor sites is believed to be of benefit in alleviating the cognitive deficit characteristic of AD, similar activity at M_2-receptor sites may inhibit ACh release.[5,6,7] Therefore, the binding affinities of the test compounds were determined for these two muscarinic receptors (Table II).

(5) Mash, D. C.; Flynn, D. D.; Potter, L. T. Loss of M_2 muscarine receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. *Science* **1985**, *228*, 1115–1117.

(6) Potter, L. T. Muscarine receptors in the cortex and hippocampus in relation to the treatment of Alzheimer's disease. In *International Symposium on Muscarinic Cholinergic Mechanisms*; Cohen, S., Sokolousky, M., Eds.; Freund Publishing Ltd.: London, 1987; pp 294–301.

(7) McCormick, D. A.; Prince, D. A. Two types of muscarinic response to acetylcholine in mammalian cortical neurons. *Proc. Nat. Acad. Sci. U.S.A.* **1985**, *82*, 6344–6348.

Table II. Cholinergic-Related Activities of Selected *N*-Aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine Analogs of Ranitidine

compd	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[e] activity (μM)	[ACh][f,g] % change from control	approx[b] lethal dose
physostigmine	0.035	0.80	37	160	4.3	<0.005	144.4***	0.8
THA	0.023	0.10	2.0	2.1	1.1	<0.01	140.8***	>20 <25
4	1.8	9.2	1.4	5.7	4.1	<0.02	128.4***	>160 <180
5	3.8	33	1.8	7.0	3.9	<0.01	115.2+++	>140 <150
7	3.3	5.1	4.0	2.4	0.60	I	101.6	>140
8	2.4	28	3.8	6.8	1.8	<0.1	153.2***	>100 <120
9	3.3	21	0.43	3.1	7.2	I	119.2+++	>100 <120
10	3.8	15	3.2	3.5	1.1	<1	87.2	>100 <110
12	1.4	2.8	0.67	0.38	0.57	<0.01	103.2	>150
13	4.7	13	0.53	0.30	0.57	<0.1	113.6+	>150
14	5.2	4.1	1.5	2.4	1.6	<1	103.6	>170
15	6.1	19	0.38	0.40	1.1	I	-	>150
16	1.3	18	2.0	3.3	1.7	I	82.0*	>110 <130
17	5.1	67	3.8	4.7	1.2	<0.1	126.8***	>170
18	8.4	19	3.1	3.5	1.1	<0.1	102.0	>110 <130
19	1.6	2.5	6.4	6.4	1.0	I	89.6	>170
20	1.1	1.6	2.1	4.4	2.1	<0.01	107.6	>170
21	1.8	2.8	3.3	3.7	1.1	I	-	>170
22	18	28	-	-	-	-	-	-
23	1.1	4.1	-	-	-	-	-	-
26	10	22	0.97	0.060	0.060	<0.1	-	>160 <180

[a] BChE activity was determined using human butyrylcholinesterase at 37 °C (pH 7.9), with butyrylthiocholine as substrate (5×10^{-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]-*N*-methylscopolamine. [e] Concentration of compound required to potentiate the contraction of isolated rat ileum produced by 1×10^{-7} M ACh by 20%. I = inactive. [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 nmoles/g). [g] (***) $p \leq 0.001$, (+++) $p \leq 0.002$, (*) $p \leq 0.01$, (+) $p \leq 0.05$. [h] 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, or the maximum dose tested if the compound was nontoxic.

Table III. Effect of Varying Doses of Selected *N*-Aryl-2-[[[5-(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine Analogs of Ranitidine on Brain Acetylcholine Concentration in Mice

	lethal dose of analog								
	80%			60%			40%		
compd	mg/kg	*N*	[ACh]:[a,b] percent of control	mg/kg	*N*	[ACh]:[a,b] percent of control	mg/kg	*N*	[ACh]:[a,b] percent of control
THA	20	6	140.8***	13.5	6	130.0***	9	6	113.2+++
4	140	6	128.4***	102	6	120.4**	68	6	110.8+
8	80	6	153.2***	66	7	126.8***	-	-	-
17	170	4	126.8***	102	5	128.8***	68	6	110.0+

[a] Mouse brain ACh concentrations were determined using GC/MS methodology. Mice were sacrificed by microwave irradiation 15 min after intraperitoneal administration of either 80%, 60%, or 40% of the estimated lethal dose (Table II) for each respective compound. ACh values are expressed as a percent of the ACh control value (25.0 nmol/g). [b] (***) $p \leq 0.001$, (**) $p \leq 0.005$, (+++) $p \leq 0.002$, (+) $p \leq 0.05$.

The majority of the compounds were similar to THA (M_2 IC_{50} = 2.1 μM; M_1 IC_{50} = 2.0 μM; M_2 IC_{50}/M_1 IC_{50} = 1.1) demonstrating approximately micromolar IC_{50} values with little receptor selectivity. Compounds **9**, **12**, **13**, **15**, and **26**, however, inhibited M_1 receptor binding of [^{3}H]-pirenzepine at submicromolar levels (0.38–0.97 μM). With the exception of compound **9**, the same agents also inhibited M_2 receptor binding of [^{3}H]-*N*-methylscopolamine at submicromolar levels with compound **26**, the 4-nitro-3-pyridazinyl analog, possessing an M_2 IC_{50} of 0.060 μM. Compound **9** demonstrated the most selectivity for the M_1 receptor with an M_2 IC_{50}/M_1 IC_{50} of 7.2. More interestingly, compound **26** exhibited the most selectivity for the M_2 receptor with a ratio of 0.060, thus making it both a potent and selective agent at this receptor site.

Brain Acetylcholine Levels. In a preliminary effort to evaluate the ability of the more potent AChE inhibitors to elevate brain levels of ACh, mice were initially administered each compound at 80% of its approximate lethal dosage (Table II). Six compounds, **4**, **5**, **8**, **9**, **13**, and **17**, produced significant increases in brain ACh concentrations. Compound **8** resulted in the highest elevation in brain ACh levels, affording a 53% increase at 88 mg/kg, relative to THA at 18 mg/kg (80% of approximate lethal dose) which yielded a 41% increase. Compounds **4** and **17** produced 28% and 27% increases, respectively, under similar conditions. It is noteworthy, that as previously demonstrated with the bis[(dimethylamino)furans],[1] the compounds containing two aliphatic, tertiary, amino groups, **12** and **13**, did not significantly alter brain ACh levels. The benzyl analog **16** actually caused a significant decrease.

The three compounds causing the largest increases in brain ACh concentrations, **4**, **8**, and **17**, were further tested at 60% and 40% of their approximate lethal doses and compared to THA (Table III). All the test compounds elevated brain levels of ACh by 20–29% at 60% of the approximate lethal dose, compared to THA which afforded a 30% increase. Similarly, compounds **4** and **17** increased brain ACh concentration by 11% and 10%, respectively, at 40% of the approximate lethal dose, again relative to THA which yielded a 13% increase.

It should be noted that although compounds **4**, **8**, and **17** required higher dosages than THA, all increased brain ACh levels more than the most active bis[[(dimethylamino)methyl]furanyl] analog,[1] **2**, at 80% of its approximate lethal dose. Furthermore, the most active in vitro

Table IV. Differentiation of Agonist–Antagonist Binding

compd	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
4	12	0.87	14
8	9.5	0.46	21
17	12	1.1	11

[a] Displacement data was obtained from mouse cerebral cortex tissue using the method of Freedman et al.[8] Apparent K_i values were obtained from IC_{50} values using the Cheng–Prusoff[13] 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 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 inhibitory bis[[(dimethylamino)methyl]furan], 1, was completely inactive in elevating brain ACh levels in mice.[1] The lower molecular weights of compounds 4, 8, and 17, as well as possibly their less basic character, probably allowed better distribution of these compounds across the blood–brain barrier, resulting in increased levels of brain ACh. Statistical analysis demonstrated that brain ACh levels positively correlated with acetylcholinesterase inhibition ($r = 0.57$), calculated log P ($r = 0.73$) and calculated molar refractivity ($r = 0.52$), as determined by the MedChem Computer Program, V3.54—Pomona College.

Differentiation of Agonist–Antagonist Binding. Because antagonist activity at M_1 receptor sites may mitigate the beneficial effects of AChE inhibition in the CNS, agonist–antagonist activity was examined for those compounds most effective in elevating brain ACh levels: 4, 8, and 17 (Table IV). In order to cover the spectrum of muscarinic receptor binding, atropine (antagonist), oxotremorine (partial agonist), and carbachol (agonist) were also assayed as reference compounds, with the results for atropine and oxotremorine being in general agreement with those of Freedman et al.[8]

Compounds 4, 8, and 17 afforded NMS/OXO-M ratios of 14, 21, and 11, respectively, and according to the classification of Freedman et al.,[8] may be categorized as possessing binding activities that more closely resemble an antagonist rather than a partial agonist. Therefore, compounds 4, 8, and 17 resemble not only THA which had an NMS/OXO-M ratio of 26, but also the bis[[(dimethylamino)methyl]furan] analog 2, which had an NMS/OXO-M ratio of 17.2.[1]

Toxicity Comparisons. All of the compounds were found to be at least one-fifth as toxic as THA. The toxicity data was in contrast to the previously reported toxicity of the bis[(dimethylamino)furan] analogs[1] which were generally more toxic.

Summary

The *N*-aryl-2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamino analogs 4–26 comprise a novel group of AChE inhibitors, which resembled the previously reported bis[[(dimethylamino)methyl]furans] and THA in potency.[1] Distinct similarities also existed in M_1 and M_2 receptor binding, agonist–antagonist profiles, and potentiation of ACh-induced ileal contractions. Compounds 4–26, in conjunction with the bis[[(dimethylamino)methyl]furans], differed from THA in generally being more selective inhibitors of AChE than BChE. More significantly, compounds 4–26 generally resulted in larger increases in brain ACh levels than the bis[[(dimethylamino)methyl]furans]. Compounds 4, 8, and 17 which contained small substituents on the dinitroaniline ring system were the most potent in producing elevations in brain ACh levels, probably due to enhanced distribution into the CNS.

Experimental Section

Chemistry. Melting points were determined on an Electrothemal melting point apparatus (capillary method) and are uncorrected. The NMR spectra were determined on a Varian EM 360A or 390 NMR spectrometer 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, GA. Thin-layer chromatography was performed on Eastman Chromatogram sheets, type 6060 (silica gel).

Compounds 4–7. The procedure given for the synthesis of 5 was utilized for the synthesis of compounds 4, 6, and 7.

***N*-[2-[[[5-[(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-2,4-dinitro-5-fluoroaniline (5).** A solution of 2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethylamine (3) (0.42 g, 2 mmol) in dry acetonitrile (10 mL) was added dropwise to a cold solution of 1,5-difluoro-2,4-dinitrobenzene (0.41 g, 2 mmol) and anhydrous sodium carbonate (0.21 g, 2 mmol) in dry acetonitrile (35 mL). The solution was allowed to warm to room temperature as it stirred overnight. The solvent was removed in vacuo, and the resulting oil was partitioned between CH_2Cl_2 and water. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and filtered. The solvent was removed in vacuo to yield a crude product which was purifiied by column 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 Et_2O and solidified overnight in the freezer to yield a bright yellow solid which was collected (0.72 g, 90%) via vacuum filtration and air-dried: mp 68–69 °C; IR (KBr) 3360, 3100, 2970, 2800, 2760, 1620, 1580, 1410, 1360, 1280, 1240, 1130, 1010, 800, 670 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.21 (s, 6 H, $N(CH_3)_2$), 2.88 (t, 2 H, SCH_2CH_2), 3.37 (s, 2 H, $CH_2N(CH_3)_2$), 3.55 (q, 2 H, CH_2CH_2NH), 3.75 (s, 2 H, furan-CH_2S), 6.15 (s, 2 H, furan protons), 6.66 (d, 1 H, ArH), 8.70 (br s, 1 H, NH), 8.98 (s, 1 H, ArH) ppm. Anal. ($C_{16}H_{19}FN_4O_5S$) C, H, N, S.

Compounds 8–22. The procedure given for the synthesis of 16 was utilized for the synthesis of compounds 8–22.

***N*-[2-[[[5-[(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-*N'*-benzyl-4,6-dinitro-1,3-benzenediamine (16).** A solution of compound 5 (0.8 g, 2 mmol) and anhydrous sodium carbonate (0.21 g, 2 mmol) in dry acetonitrile (35 mL) was treated with benzylamine (0.25 g, 2.3 mmol) in dry acetonitrile (10 mL) and refluxed overnight. The solvent was removed in vacuo, and the resulting yellow oil was partitioned between CH_2Cl_2 and water. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and filtered. The solvent was removed in vacuo to yield a crude product which was purified by column 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 Et_2O and stored in a freezer overnight. The bright yellow solid was collected (0.51 g, 53%) via vacuum filtration and air-dried: mp 91–93 °C; IR (KBr) 3380, 3080, 2940, 2800, 1620, 1550, 1410, 1370, 1300, 1200, 1170, 1020, 785, 730, 680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.18 (s, 6 H, $N(CH_3)_2$), 2.55 (t, 2 H, SCH_2CH_2), 3.23 (q, 2 H, CH_2CH_2NH), 3.32 (s, 2 H,

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$CH_2N(CH_3)_2$), 3.63 (s, 2 H, furan-CH_2S), 4.45 (d, 2 H, $CH_2C_6H_5$), 5.50 (s, 1 H, ArH), 6.00 (s, 2 H, furan protons), 7.25 (s, 5 H, ArH), 8.28–8.72 (br s, 2 H, NH), 9.15 (s, 1 H, ArH) ppm. Anal. ($C_{23}H_{27}N_5O_5S$) C, H, N, S.

***N*-[2-[[[5-[(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-2,4-dinitro-5-(4-nitrophenoxy)aniline Tosylate (23).** A solution of compound **5** (0.60 g, 1.5 mmol) and anhydrous sodium carbonate (0.32 g, 2 mmol) in dry acetonitrile (35 mL) was treated with *p*-nitrophenol (0.21 g, 1.5 mmol) and stirred overnight at room temperature. The solvent was removed in vacuo, and the resulting yellow oil was partitioned between CH_2Cl_2 and water. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and filtered. The solvent was removed in vacuo to yield a crude product which was purified by column chromatography (230–400 mesh silica gel) using acetone as the eluting solvent. After removal of the acetone under reduced pressure, the resulting oil was dried at 65 °C for 2.5 h in a drying pistol under P_2O_5. The resulting product was dissolved in acetone and an equivalent of *p*-toluenesulfonic acid monohydrate was stirred into the solution. The acetone was removed in vacuo, and the resulting yellow solid was recrystallized from absolute EtOH and collected (0.22 g, 40%) via vacuum filtration and air-dried: mp 58 °C dec; ^{1}H-NMR (DMSO-d_6/$CDCl_3$) δ 2.31 (s, 6 H, N-$(CH_3)_2$), 2.95 (s, 3 H, CH_3Ts), 3.35–3.75 (m, 4 H, SCH_2CH_2, CH_2CH_2NH), 3.85 (s, 2 H, $CH_2N(CH_3)_2$), 4.35 (s, 2 H, furan-CH_2S), 6.30 (m, 1 H, furan proton), 6.65 (m, 1 H, furan proton), 6.95–7.55 (m, 7 H, ArH), 8.25 (s, 1 H, ArH), 8.35 (s, 1 H, ArH), 9.00 (s, 1 H, ArH) ppm. Anal. ($C_{29}H_{31}N_5O_{11}S_2$) C, H, N, S.

5-[[[2-[[1-(Hydrazino)-2-nitroethenyl]amino]ethyl]thio]methyl]-*N*,*N*-dimethyl-2-furanmethanamine (25). Compound **24**[3] (1.66 g, 5 mmol) was treated with 12.5 mL of 1.0 M hydrazine in absolute EtOH. The pale yellow solution was stirred at ambient temperature for 19 h, diluted with 2 volumes of Et_2O, and stored in a freezer for 1 h. The off-white solid was collected, washed with Et_2O, and air-dried. The crude solid (1.47 g, 93%) was recrystallized from EtOH/Et_2O (1:2) to yield an off-white powder: mp 89–90 °C; IR (KBr) 3340, 3200, 1570, 1410, 1230, 1210, 1045 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.13 (s, 6 H, $(CH_3)_2N$), 2.87 (t, 2 H, SCH_2CH_2), 3.33 (s, 2 H, $CH_2NCH_3)_2$ 3.70 (s, 2 H, furan-CH_2S), 3.90 (t, 2 H, CH_2CH_2NH), 5.90 (s, 2 H, furan protons), 6.30 (s, 2 H, NH_2), 7.0 (s, 1 H, =$CHNO_2$) ppm. Anal. ($C_{12}H_{21}N_5O_3S$) C, H, N, S.

3-[[2-[[[5-[(Dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]amino]-4-nitropyridazine (26). To a solution of compound **25** (0.315 g, 1 mmol) in toluene (50 mL) and water (50 mL) was added 1.45 g of a 40 wt % solution of glyoxal in water. Triton B (1.0 mL) was added and the mixture stirred rapidly at room temperature for 18 h. The organic layer was separated, extracted with water (3 × 20 mL) and brine, and then dried over anhydrous Na_2SO_4. the toluene was removed under reduced pressure, and the dark oil (0.27 g, 80%) was purified by flash chromatography (230–400 mesh silica gel) using acetone as the eluting solvent. Removal of the acetone under reduced pressure yielded a dark yellow oil (0.22 g, 65%): ^{1}H-NMR ($CDCl_3$) δ 2.20 (s, 6 H, $(CH_3)_2N$), 2.85 (t, 2 H, SCH_2CH_2), 3.35 (s, 2 H, $CH_2N(CH_3)_2$), 3.70 (s, 2 H, furan-CH_2S), 3.90 (q, 2 H, CH_2CH_2NH), 6.0 (s, 2 H, furan protons), 7.80–8.5 (m, 3 H, NH and pyridazine protons) ppm. Anal. ($C_{14}H_{19}N_5O_3S$) C, H, N, S.

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 analogs was determined at 37 °C and a pH of 7.9 using the method of Ellman et al.[9] 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 5×10^{-5} M. The concentration of analog 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 analogs for M_1 and M_2 muscarinic receptors was examined using a filtration assay[10] as modified by Patterson et al.[11] IC_{50} values are determined from the ability of the analog to inhibit by 50% the binding of the radioactive ligand to the receptor. Nonspecific binding was determined with 1 μM atropine. Data analysis was performed using the EBDA program contained in the software package LIGAND (Elsevier-BIOSOFT). Protein determinations were obtained using the Bradford assay.[12]

The affinity of the analogs 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 μg protein, 100 μM DFP, 3 nM [^{3}H]pirenzepine, analog, and sodium potassium phosphate buffer. Analog 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 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 μg protein, 100 μM DFP, 0.3 nM [^{3}H]-*N*-methylscopolamine, analog, 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.[8] was used to determine whether the observed analog binding represented muscarinic agonist or antagonist affinity. The procedure required determination of the binding affinity of the analog 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 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 μg of protein, 2.0 nM [^{3}H]oxotremorine-M, and analog. After 40 min of incubation, the tubes were filtered on Whatman GF/C filters soaked in 0.05% polyethyleneimine using a Brandel Cell Harvester. The filters were then washed three times with ice-cold saline (0.9%), dried,

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and counted after 12 h. Nonspecific binding was determined with 1 μM 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 μg of protein, 0.3 nM [^{3}H]-*N*-methylscopolamine, and analog. After incubating for 60 min at 30 °C, the tubes were filtered and 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[13] $K_i = IC_{50}/(1 + C/K_d)$ where IC_{50} is the concentration of the analog 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 nM and 0.3 nM, respectively.

Determination of agonist or antagonist activity for the analog 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, respectively, 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 1×10^{-7} M acetylcholine. Varying concentrations of analog were then added to the bath 30 s prior to the addition of acetylcholine. The minimum concentration of analog required to potentiate the acetylcholine-induced contraction by 20% was the endpoint of the procedure.

Toxicity Determination. Mice were administered, intraperitoneally, varying doses of analog dissolved in 0.05 M sodium acetate buffer (pH 4.0). The analog 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 Analogs on Brain Acetylcholine Concentrations. Mice were administered selected compounds by intraperitoneal injection at 80% of the approximate lethal dose of the analog 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.[14] 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 Corp.) 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 (J.K.). 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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