Novel [2-(4-Piperidinyl)ethyl](thio)ureas: Synthesis and Antiacetylcholinesterase Activity

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A series of 1-ar(o)yl-3-[2-(1-benzyl-4-piperidinyl)ethyl](thio)urea derivatives was synthesized and evaluated for antiacetylcholinesterase activity. Most aroyl(thio)urea derivatives showed potent inhibitory activity in the sub-micromolar range. A comparable potency was obtained with the aryl(thio)urea analogues by replacing the phenyl with a 2-pyridyl group. The substituted guanidine variations proved to be almost inactive whereas the nitroethylene analogues appeared to be quite efficient. These results were interpreted in terms of the preferential cis–trans conformation of the aroyl(thio)urea and 2-pyridyl(thio)urea moieties involving the existence of hydrogen bonding. In vivo experiments showed that compound **7m** had maximal antiamnestic activity at 0.03 mg/kg with a therapeutic ratio greater than 1000, while cholinergic side effects were only seen at doses 100-fold the maximally effective antiamnestic dose. Compound **7m** represents a potentially interesting antidementia agent.

Introduction

The discovery of a selective degeneration of cholinergic neurons in senile dementia of the Alzheimer's type (SDAT) about 20 years ago[1-3] and the clinical improvement of memory disorders observed with tacrine,[4] a drug with acetylcholinesterase (AChE) inhibitory activity, have stimulated a great deal of research based upon the cholinergic hypothesis of SDAT.[5-14] Interest has been sustained by the finding that a dramatic (up to 90%) and selective loss of the cholinergic marker choline acetyltransferase (ChAT),[1] which catalyzes the synthesis of acetylcholine (ACh) from choline, exists in the hippocampus and cerebral cortex (areas of the brain believed to be associated with learning and memory) of SDAT patients.

The brains of SDAT patients have been found to contain amyloid plaques, the number of which seems to be closely and positively correlated with the degree of dementia.[5,15] This finding has encouraged intense research on the biochemical processes[16] underlying the production of amyloid protein which represents the main component of these senile plaques. The principal conclusion was that this peptide of 39–43 amino acids (4.2 kDa) was produced by an apparently abnormal processing of its presumed precursor (APP) involving a specific proteinase of the chymotrypsin-like type.[17-19] This research led to the design of a number of inhibitors of this type of protease.

Recently, several studies have reported the possibility of noncholinergic actions of AChE by abnormal isoforms of this enzyme which could act as proteases to regulate cell growth and development.[20-22] These apparently pathogenic forms of AChE may be produced during the degenerative process of cholinergic neurons and could be the basis of an acceleration of the production of the amyloid protein and of the evolution of the disease.

In view of the above ideas and the reported clinical activity of physostigmine, tacrine, galanthamine, and more recently, the drug huperzine A (Chart 1), stable, centrally active AChE inhibitors could prove to be a promising approach to the treatment of SDAT.

We describe here the synthesis and structure–activity relationships of novel 1-ar(o)yl-3-[2-(1-benzyl-4-piperidinyl)ethyl](thio)urea derivatives.

Chemistry

Compounds **2a–c** were prepared by standard methods[23-28] as shown in Scheme 1. The replacement of the benzyl group of compound **2a** by substituted benzyl or cycloalkyl groups was achieved by a three-step sequence involving mainly the clean N-debenzylation of **1a** using 1-chloroethyl chloroformate[29-32] (Scheme 1).

Aroyl(thio)ureas **7a–s** were prepared, as shown in Scheme 2, by the direct addition of the diamino compounds **2** on the appropriate aroyliso(thio)cyanates which were either commercial (**4c,q–s**) or obtained in situ (**4a,b,d–p**) by known methods[33-35] (see methods A and B).

The (thio)urea derivatives **8a,b,d–v** were synthesized by the addition of the diamines **2a–e** to the aryliso(thio)-cyanates **5a,b,d–v** (method C) obtained, if necessary, by the condensation of (thio)phosgene or a safer substitute (e.g. triphosgene) with an aniline (method D). In the case of secondary anilines, the intermediate product was an aryl(thio)carbamoyl chloride **6** (methods D and E).[36-42]

Guanidines **9a–c** were prepared, as shown in Scheme 3, either via a sulfonic acid derivative (method F) or by condensation of the appropriate diamine with a carbodiimide intermediate obtained from the corresponding thiourea by the action of PbO or HgO[43] (methods G and H).

Finally, nitromethylene derivatives **10a,b** were prepared by the sequential condensation of 1,1-bis(methylthio)-2-nitroethylene with the appropriate amino fragments[44] (method I).

Structure–Activity Relationships

The aroyl(thio)urea derivatives prepared are shown in Tabe 1 along with the results obtained for the in vitro inhibition of AChE.

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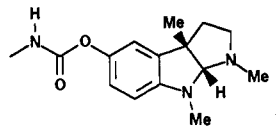
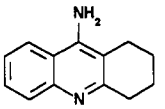
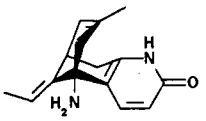
Chart 1



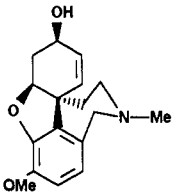
Physostigmine



Tacrine

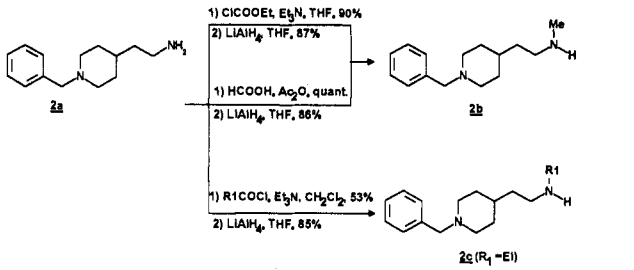


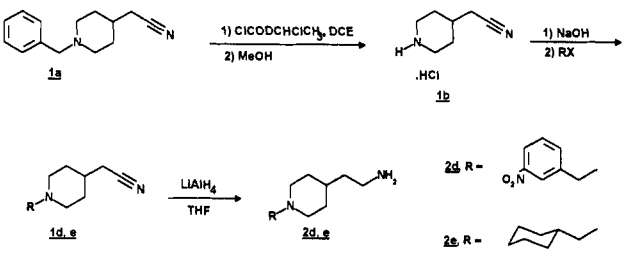
Huperzine A



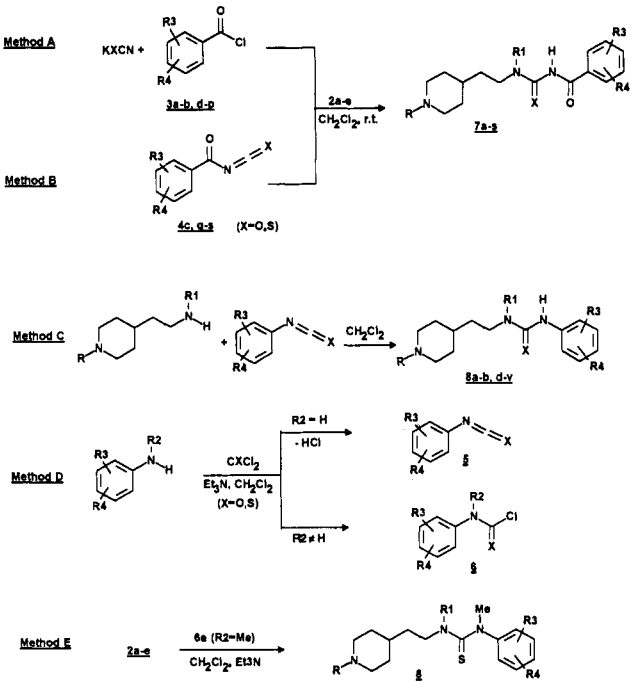
Galanthamine

Scheme 1



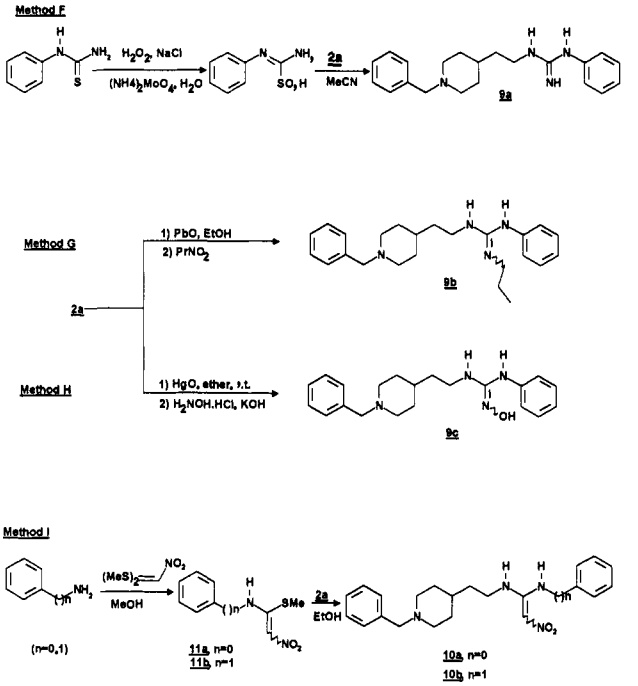


Scheme 2



The 3-nitro derivative **7c** proved to be the most potent compound of the series. The 2-nitro analogue **7b** was 30-fold less active, and the 4-nitro compound proved to be

Scheme 3



unstable.[48] The 4-sulfonyl derivative **7l**, however, showed high activity, as did the 3,4-dimethoxy compound **7g**.

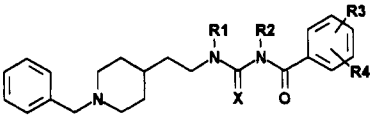
A 70-fold higher activity was seen with the 4-methoxy compound **7e** compared to the 3-methoxy analogue **7d**. Surprisingly, the 3,4-dimethoxy **7g** proved to fit even better with a 140-fold increase in the inhibitory activity, whereas the 3,4-methylenedioxy **7m** confirmed a potency comparable to **7e**. In conclusion, it seemed that an oxygen atom at the meta position was tolerated provided that a substituent at the para position was present; this was in good agreement with the observed activity of **7p** which is a mixed and strained (planar) analogue of **7d** and **7j**.

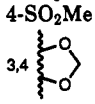
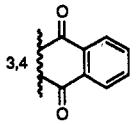
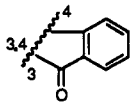
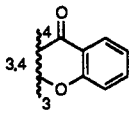
The introduction of a methyl or ethyl group at R1 markedly reduced activity as indicated by a comparison of **7a** with **7q** and **7r**. Replacement of the sulfur atom of **7a** by oxygen to afford **7s** had, however, no effect on activity. The high activity of the tricyclic compound **7n** is of interest in terms of the size of the putative hydrophobic binding region.

The aryl(thio)ureas, shown in Table 2, proved to be considerably less potent than the above series, and the activity was relatively insensitive to substitution in the aromatic ring. Replacement of the phenyl ring by a 3-pyridyl or 4-pyridyl group had little effect. The 2-pyridyl compound **8p** on the other hand was quite potent, and the inhibitory activity was enhanced by the introduction of a 4-nitro group (**8s**) (see Table 3). The benzyl derivatives **8t** and **8u** and the guanidines **9a–c** were weakly active (Table 4) in contrast to the nitroethylene compound **10a** which retained moderate activity.

In conclusion, the most potent AChE inhibitors described above belong to the aroyl(thio)urea and 2-pyridyl(thio)urea series. The higher activity observed in these cases may be related to hydrogen bond formation, as shown in Chart 2, leading to the stabilization of a preferred conformation. This view was confirmed by several studies involving ^{1}H and ^{13}C NMR and IR experiments showing that such hydrogen bonds exist in the case of 2-alkoxybenzamides[49,50] and in 2-pyridyl(thio)ureas.[45–48] Intramolecular hydrogen bonding stabilizes the active con-

Table 1. Aroyl(thio)urea Derivatives

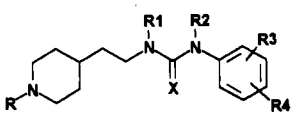


no.	X	R_1	R_2	R_3	R_4	yield, %[e] (method)	mp, °C[a]	formula	AChE inh (IC_{50}, nM)[d]
7a	S	H	H	H	H	62 (A)	183–4	$C_{22}H_{28}ClN_3OS$	13
7b	S	H	H	2-NO_2	H	43 (A)	166–8	$C_{22}H_{27}ClN_4O_3S$	40
7c	S	H	H	3-NO_2	H	59 (B)	160–2	$C_{22}H_{27}ClN_4O_3S$	1,5
7d	S	H	H	3-OMe	H	73 (A)	184–5	$C_{23}H_{30}ClN_3O_2S$	700
7e	S	H	H	4-OMe	H	60 (A)	215–20	$C_{23}H_{30}ClN_3O_2S$	10
7f	S	H	H	3-OPh	H	54 (A)	153–5[b]	$C_{32}H_{35}N_3O_6S$	100
7g	S	H	H	3-OMe	4-OMe	82 (A)	177–8[b]	$C_{28}H_{35}N_3O_7S$	5
7h	S	H	H	4-Cl	H	35 (A)	195–200	$C_{22}H_{27}Cl_2N_3OS$	20
7i	S	H	H	4-COMe	H	10 (A)	202–4[b]	$C_{28}H_{33}N_3O_6S$	7, 5
7j	S	H	H	4-COPh	H	37 (A)	207–8	$C_{29}H_{32}ClN_3O_2S$	14
7k	S	H	H	4-CF_3	H	48 (A)	204–5[b]	$C_{27}H_{30}F_3N_3O_5S$	33
7l	S	H	H	4-SO_2Me	H	38 (A)	194–5[b]	$C_{27}H_{33}O_7S_2$	6
7m	S	H	H		H	92 (A)	176–8	$C_{23}H_{28}ClN_3O_3S$	11
7n	S	H	H		H	13 (A)	218–20	$C_{30}H_{30}ClN_3O_3S$	2
7o	S	H	H		H	48 (A)	248–50	$C_{29}H_{30}ClN_3O_2S$	80
7p	S	H	H		H	6 (A)	149–50[c]	$C_{29}H_{29}N_3O_5S$	11
7q	S	Me	H	H	H	37 (B)	163–5[b]	$C_{27}H_{33}N_3O_5S$	350
7r	S	Et	H	H	H	79 (B)	143–5	$C_{24}H_{32}ClN_3OS$	300
7s	O	H	H	H	H	47 (B)	220–3	$C_{22}H_{28}ClN_3O_2$	15

[a] All melting points refer to hydrochlorides unless otherwise indicated. [b] Fumarate. [c] Free base. [d] The IC_{50} values for AChE inhibition were estimated graphically from log concentration/% inhibition curves of 6–15 values. [e] Yield refers to bases.

Table 2. Aryl(thio)urea Derivatives

IIa



no.	X	R	R_1	R_2	R_3	R_4	yield, %[e] (method)	mp, °C	formula	AChE inh. (IC_{50}, nM)
8a	S	$C_6H_5CH_2$	H	H	H	H	88 (B)	174–5[a]	$C_{21}H_{28}ClN_3S$	250
8b	S	$C_6H_5CH_2$	Me	H	H	H	53 (B)	147–8[b]	$C_{28}H_{33}N_3O_4S$	3000
8c	S	$C_6H_5CH_2$	H	Me	H	H	19 (E)	62–3[c]	$C_{22}H_{29}N_3S$	420
8d	S	$C_6H_5CH_2$	H	H	4-OMe	H	94 (B)	163–4[b]	$C_{28}H_{33}N_3O_5S$	150
8e	S	$C_6H_5CH_2$	H	H	4-COMe	H	99 (B)	190–5[a]	$C_{23}H_{30}ClN_3OS$	400
8f	S	$C_6H_5CH_2$	H	H	3-NO_2	H	97 (B)	158–60[b]	$C_{25}H_{30}N_4O_6S$	700
8g	S	$C_6H_5CH_2$	H	H	3-CF_3	H	88 (B)	160–5[b]	$C_{28}H_{30}F_3N_3O_4S$	210
8h	S	$C_6H_5CH_2$	H	H	2-Me	H	91 (B)	99–100[c]	$C_{22}H_{29}N_3S$	400
8i	S	$C_6H_5CH_2$	H	H	2-F	H	66 (B)	111–2[c]	$C_{21}H_{26}FN_3S$	850
8j	S	$C_6H_5CH_2$	H	H	penta-F		69 (B)	235–40[a]	$C_{21}H_{23}F_5ClN_3S$	3000
8k	O	$C_6H_5CH_2$	H	H	H	H	75 (B)	167–8[b]	$C_{25}H_{31}N_3O_5$	1100
8l	S	4-$NO_2C_6H_4CH_2$	H	H	3-OMe	4-OMe	69 (B)	135–55[b]	$C_{27}H_{34}N_4O_6S$	2100
8m	S	c-$C_6H_{11}CH_2$	H	H	3-CN	H	67 (B)	155–70[b]	$C_{28}H_{29}N_5O_6S$	2200
8n	S	c-$C_6H_{11}CH_2$	H	H	3-OMe	4-OMe	46 (B)	169–70[c]	$C_{28}H_{37}N_3O_2S$	820
8o	S	c-$C_6H_{11}CH_2$	H	H	4-OCF_3	H	99 (B)	125–6[c]	$C_{22}H_{32}F_3N_3OS$	2500

[a] Hydrochloride. [b] Fumarate. [c] Free base. [d] See note *d* of Table 1. [e] Yields refers to bases.

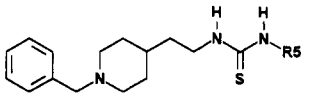
formation, which is characterized by a six-membered pseudocycle and allows an optimal orientation of the heteroaryl ring inside the hydrophobic region of the active site.

In Vivo Results

Tacrine, galanthamine, and eserine inhibited acetylcholinesterase activity in vitro with IC_{50} values of 110, 750, and 190 nM, respectively. All aroyl- and pyridyl(thio)urea derivatives tested had IC_{50} values in the submicromolar range with compounds **7c,g,l,n** and **8s** less than 10 nM (Tables 1 and 3). Other derivatives were, in general, less active.

Ex vivo tacrine, galanthamine, and eserine showed anticholinesterase activity close to the toxic doses so that

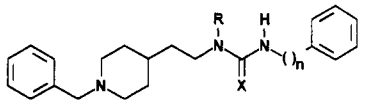
Table 3. Pyridyl(thio)urea Derivatives



no.	R5	yield, % (method)	mp, °C[a]	formula	AChE inh (IC_{50}, nM)[b]
8p	2-pyridyl	36 (D, C)	129–30	$C_{20}H_{26}N_4S$	22
8q	3-pyridyl	89 (B)	136–7	$C_{20}H_{26}N_4S$	500
8r	4-pyridyl	65 (B)	174–5	$C_{20}H_{26}N_4S$	210
8s	3-nitro-6-pyridyl	23 (B)	177–9	$C_{20}H_{25}N_5O_2S$	2, 6

[a] All melting points refer to free bases. [b] See note *d* of Table 1.

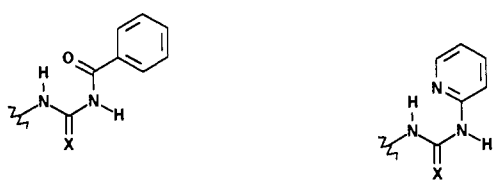
Table 4. Aralkyl(thio)urea, Guanidine, and Nitroethylene Derivatives



no.	*n*	R	X	yield, % (method)	mp, °C	formula	AChE inh (IC_{50}, nM)[d]
8t	1	H	O	94 (B)	135–7[b]	$C_{26}H_{33}N_3O_5$	1400
8u	1	H	S	83[e] (B)	198–200[a]	$C_{22}H_{30}ClN_3S$	6000
8v	1	Et	S	50 (B)	135–40[a]	$C_{24}H_{34}ClN_3S$	260
9a	0	H	NH	67 (F)	137–8[c]	$C_{21}H_{28}N_4$	2500
9b	0	H	NPr	84 (G)	113–6[b]	$C_{28}H_{35}N_4O_4$	>10000
9c	0	H	NOH	24 (H)	146–7[c]	$C_{21}H_{28}N_4O$	>10000
10a	0	H	$CHNO_2$	90 (I)	220–2[a]	$C_{22}H_{29}ClN_4O_2$	80
10b	1	H	$CHNO_2$	68 (I)	193–4[b]	$C_{27}H_{34}N_4O_6$	120

[a] Hydrochloride. [b] Fumarate. [c] Free base. [d] See note *d* of Table 1. [e] This yield refers to the hydrochloride.

Chart 2. Hydrogen Bonding in Aroyl(thio)ureas and 2-Pyridyl(thio)ureas



precise ID_{50} values could not be obtained. Activities in the brain and the periphery (salivary glands) were similar for all three reference compounds. Similarly, for the five compounds shown in Table 5 the maximum selectivity for the brain was only 4-fold (**7a**). For the more active compounds (**7c** and **7n**) there was virtually no selectivity.

The three reference compounds all reversed scopolamine-induced amnesia in the passive avoidance paradigm in rats with a maximal effect of about 300%. In all cases the dose–response relationship was bell-shaped. **7a**,**c**,**m** and **8p** also reversed scopolamine-induced amnesia. Whereas for the reference compounds maximal antiamnesic activity occurred at doses close to the LD_{50} (less than 10-fold less), the maximally active doses of the test compounds (except for **8p**) were separated from the toxic doses by a greater margin. Indeed, the most active compound, **7m**, had maximal antiamnesic activity at 0.03 mg/kg ip whereas the LD_{50} value was greater than 30 mg/kg ip, a therapeutic ratio of greater than 1000. Cholinergic side effects such as salivation and tremor were only seen at doses 100-fold the maximally effective antiamnesic dose. Although potent in vitro and ex vivo at inhibiting acetylcholinesterase activity, **7n** was inactive on the passive avoidance test. The reason for this is obscure. On the basis of these biological results, it would appear that **7m** is a highly effective in vivo antiamnesic agent with low toxicity, making it an interesting candidate for the treatment of Alzheimer's disease.

Experimental Section

All melting points were determined on a Kofler apparatus and are uncorrected. ^1H NMR spectra were recorded using a Bruker AC-200 spectrometer using Me_4Si as an internal standard. Elemental analyses are indicated only by the symbols of the elements; analytical results were within 0.4% of the theoretical values.

4-Piperidineacetonitrile (1b). To a stirred and cooled (ice bath) solution of **1a** (50.0 g, 0.23 mol) in 250 mL of 1,2-dichloroethane (DCE) was added dropwise a solution of 1-chloroethyl chloroformate (28 mL, 0.25 mol) in 50 mL of DCE. After the addition was complete, the mixture was allowed to warm to room temperature with stirring and then refluxed for 4 h. The solvent was removed under reduced pressure, MeOH was added, and the solution was refluxed for a further 2 h. MeOH was evaporated to leave a white solid which was washed with dry acetone and dried under reduced pressure to give 29.8 g (79.5%) of white crystals of **1b** hydrochloride: mp 186–8 °C; ^1H NMR (DMSO-d_6) 1.35–1.56 (m, 2H, piperidine), 1.80–2.01 (m, 3H, piperidine), 2.51–2.56 (d, 2H, CH_2), 2.75–2.93 (q, 2H, piperidine), 3.16–3.26 (d, 2H, piperidine), 8.88 (bs, 1H), 9.32 (bs, 1H). Anal. ($C_7H_{12}N_2$·HCl) C, H, N, Cl.

4-(Cyanomethyl)-1-(cyclohexylmethyl)piperidine (1d). To a stirred suspension of K_2CO_3 (2.45 g, 17.7 mmol) and KI (2.65 g, 16.0 mmol) in 20 mL of MeCN was added **1b** free base (2.0 g, 16.1 mmol). The mixture was refluxed for 30 min, a solution of cyclohexylmethyl bromide (2.85 g, 16.1 mmol) in 10 mL of MeCN was added dropwise, and the resulting mixture was refluxed for 8 h. After the mixture was cooled, the solvent was evaporated, the solid residue was treated with water and extracted with CH_2Cl_2, and the resulting organic phase was washed with water and then brine and dried over Na_2SO_4. The solvent was evaporated to leave a pale yellow oil which was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 98:2) to give 2.82 g (79.5%) of low-melting colorless crystals of **1d**: mp 45–6 °C; ^1H NMR ($CDCl_3$) 0.74–1.95 (m, 18H, cyclohexyl + piperidine), 2.08–2.11 (d, 2H, CH_2N, J = 6.74 Hz), 2.25–2.29 (d, 2H, CH_2CN, J = 6.74 Hz), 2.84–2.90 (bd, 2H, piperidine). Anal. ($C_{14}H_{24}N_2$) C, H, N.

4-(2-Aminoethyl)-1-(cyclohexylmethyl)piperidine (2e). A solution of **1d** (2.75 g, 12.47 mmol) in 5 mL of dry THF was added dropwise under nitrogen to a suspension of $LiAlH_4$ (1.0 g, 26.3 mmol) in dry THF at reflux. After the addition was complete, the mixture was further refluxed with stirring for 2 h. The reaction mixture was cooled with an ice bath, AcOEt was cautiously added, and then 1 N NaOH was added. The mixture was diluted with water and filtered; the organic phase was separated, dried over Na_2SO_4, and evaporated to dryness to give 2.79 g (99.6%) of a crude pale oil of **2e** which was used without further purification: ^1H NMR ($CDCl_3$) 0.76–1.82 (m, 22H), 1.99–2.03 (d, 2H, CH_2, J = 7.14 Hz), 2.62–2.79 (m, 4H). o

1-(3-Nitrobenzoyl)-3-[2-(1-benzyl-4-piperidinyl)ethyl]-thiourea (7c) (Method B). To a stirred suspension of 3-nitrobenzoyl isothiocyanate (2.20 g, 10.0 mmol) in 25 mL of CH_2Cl_2 was added dropwise a solution of **2a** (2.18 g, 10.0 mmol) in 15 mL of CH_2Cl_2. After 1 h the resulting solution was evaporated to dryness and the yellow oily residue purified by silica gel column chromatography ($CHCl_3$–MeOH, 98:2) to give 2.50 g of free base **7c** which was treated with HCl–EtOH in Et_2O, and the resulting crystals were recrystallized from EtOH to give 2.30 g (49.7%) of **7c** hydrochloride: mp 160–2 °C; ^1H NMR ($CDCl_3$) 1.60–2.21 (m, 7H, piperidine), 2.57–2.73 (m, 2H, piperidine), 3.45–3.50 (d, 2H, piperidine), 3.70–3.80 (q, 2H, CH_2), 4.15–4.17 (sd, 2H, benzyl), 7.41–7.65 (m, 5H, arom), 7.70–7.78 (t, 1H, arom), 8.18–8.22 (d, 1H, arom), 8.43–8.48 (d, 1H, arom), 8.70 (s, 1H, arom), 9.43 (s, 1H, NHCO), 10.60 (s, 1H, NHCS), 12.3 (bs, 1H, NH salt). Anal. ($C_{22}H_{26}N_4O_3S$·HCl) C, H, N, S, Cl.

1-[3,4-(Methylenedioxy)benzoyl]-3-[2-(1-benzyl-4-piperidinyl)ethyl]thiourea (7m) (Method A). To a well-stirred suspension of fine powdered KSCN (0.35 g, 3.6 mmol) in 20 mL of acetone under nitrogen was added dropwise 3,4-(methylenedioxy)benzoyl chloride (0.6 g, 3.2 mmol). After the addition was

Table 5. Biological Results[a]

	ex vivo cholinesterase activity		passive avoidance activity		cholinergic activity		
	brain ID50 (mg/kg ip)	salivary gland ID50 (mg/kg ip)	dose (mg/kg ip)	latency % control	salivation ED50 mg/kg ip	tremor ED50 mg/kg ip	toxicity LD50 mg/kg ip
tacrine	>15	>20	0.3	+115	10 [3–30]	5 [3–10]	23 [10–100]
			1.0	+216**			
			3.0	+315***			
			10.0	+176			
eserine	0.2	>1.0	0.01	+40	0.5 [0.1–3]	0.3 [0.1–1]	0.6 [0.1–3]
			0.03	+152**			
			0.10	+286***			
			0.30	+135*			
			1.00	+79			
galanthamine	>30	>30	0.3	+142*	5 [3–10]	3 [1–10]	10 [3–30]
			1.0	+328**			
			3.0	+171*			
7a	12	50	3.0	+211**	>30	>30	75 [30–100]
			10.0	+40*			
7c	1.5	2.5	1.0	+93**	15 [1–30]	1.5 [1–3]	18 [3–30]
7m	>3	>3	0.03	+262***	20 [3–100]	3 [0.3–10]	>30
			0.3	+54**			
8p	>3	>3	3	+98*	>10	5 [1–20]	20 [3–100]
			10	+134*			
7n	0.8	2	0.003–3	inactive	>30	30 [10–100]	>30

[a] The ID50 values for in vivo AChE inhibition were estimated graphically from log dose/% inhibition curves of 6–12 values. The passive avoidance latency is expressed as percent of control for 12 animals per dose. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to vehicle-treated controls using a Kruskal-Wallis nonparametric one-way analysis of variance corrected for ties followed by a 2-tailed Mann-Whitney U-test. The ED50 values for salivation and tremor were calculated graphically from curves of log dose/% occurence of the sign. A minimum of three doses with 10 mice/dose were used. The values for 0% and 100% occurence are given in brackets. The LD50 values were calculated graphically from log dose/% lethality curves. A minimum of three doses with 10 mice/dose were used. The values for 0% and 100% lethality are given in brackets.

complete, the mixture was stirred under reflux for 5 min. Then a solution of **2a** (0.8 g, 3.6 mmol) in 10 mL of CH_2Cl_2 was added at such a rate that the solution gently refluxed. After 4 h the mixture was allowed to cool and stirred at room temperature overnight. The solvents were evaporated, and the residue was diluted with water and extracted with CH_2Cl_2; the organic phase was washed with water, dried with Na_2SO_4, and evaporated to dryness to leave a crude brown oil which was purified by silica gel column chromatography ($CHCl_3$–MeOH, 95:5) to give 1.35 g of free base. It was converted to the hydrochloride with HCl–EtOH in Et_2O, and the resulting crystals were recrystallized from EtOH–Et_2O to give 1.10 g (73.2%) of **7m**: mp 176–8 °C; ^{1}H NMR ($CDCl_3$) 1.59–2.12 (m, 7H, piperidine), 2.60–2.65 (bdd, 2H, piperidine), 3.43–3.49 (bd, 2H, piperidine), 3.70–3.78 (q, 2H, CH_2), 4.13–4.15 (d, 2H, benzoyl), 6.08 (s, 2H, OCH_2O), 6.85–6.89 (d, 1H, aromatics), 7.27 (s, 1H, arom), 7.35–7.44 (m, 4H, arom), 7.61 (bs, 2H, arom), 8.94 (s, 1H, NHCO), 10.72 (bs, 1H, NHCS), 10.29 (bs, 1H, NH salt). Anal. ($C_{22}H_{27}N_3O_3S$·HCl) C, H, N, S, Cl.

1-Phenyl-3-[2-(1-benzyl-4-piperidinyl)ethyl]-3-methylthiourea (8c) (Methods D and E). To a stirred solution of thiophosgene (0.80 mL, 10.0 mmol) in 15 mL of CH_2Cl_2, placed under a nitrogen atmosphere and cooled in an ice bath, was added dropwise a solution of *N*-methylaniline (2.20 mL, 20.0 mmol) in 10 mL of CH_2Cl_2. This mixture was allowed to warm to room temperature for 1 h, added dropwise to a stirred solution of **2a** (2.18 g, 10.0 mmol) and Et_3N (1.5 mL, 10.0 mmol) in 10 mL of CH_2Cl_2, and stirred overnight. The mixture was washed twice with water, dried over Na_2SO_4, and evaporated to leave 2.10 g of a yellow oily residue which was purified by silica gel column chromatography (AcOEt) to give a pale yellow oil. This was crystallized in hexane to give 0.70 g (19.1%) of free base **8c**: mp 62–3 °C; ^{1}H NMR ($CDCl_3$) 1.20–1.42 (m, 5H, piperidine), 1.56–1.60 (bd, 2H, piperidine), 1.82–1.93 (t, 2H, piperidine), 2.80–2.86 (d, 2H, piperidine), 3.47 (s, 2H, benzyl), 3.51–3.61 (m, 2H, CH_2), 3.66 (s, 2H, CH_3), 5.24 (bs, 1H, NH), 7.19–7.53 (m, 10H, arom). Anal. ($C_{22}H_{29}N_3S$) C, H, N, S.

1-(2-Methylphenyl)-3-[2-(1-benzyl-4-piperidinyl)ethyl]thiourea (8h) (Method C). To a stirred solution of 2-methylphenyl isothiocyanate (1.4 mL, 10.5 mmol) in 15 mL of CH_2Cl_2 placed under a dry nitrogen atmosphere was added dropwise a solution of **2a** (2.18 g, 10.0 mmol) in 15 mL of CH_2Cl_2. The mixture was stirred overnight, and the solvent was removed under reduced pressure. The resulting oil was purified by silica gel column chromatography ($CHCl_3$–MeOH, 95:5) to leave a pale yellow oil which was crystallized by the addition of iPr_2O to give 3.35 g (91.2%) of white crystals of pure free base **8h**: mp 99–100 °C; ^{1}H NMR ($CDCl_3$) 1.15–1.96 (m, 9H, piperidine), 2.27 (s, 2H, CH_3), 2.82–2.88 (d, 2H, piperidine), 3.47 (s, 2H, benzyl), 3.57–3.68 (q, 2H, CH_2), 5.56 (bs, 1H, NH), 7.15–7.29 (m, 9H, arom), 7.52 (s, 1H, NH). Anal. ($C_{22}H_{29}N_3S$) C, H, N, S.

1-Phenyl-3-[2-(1-benzyl-4-piperidinyl)ethyl]guanidine (9a) (Method F). To a stirred solution of **2a** (1.10 g, 5.04 mmol) in 6.0 mL of MeCN was added portionwise (*N*-phenylamino)-iminomethanesulfonic acid (0.9 g, 4.50 mmol) at room temperature. The latter was obtained according to the method of Maryanoff and collaborators.[43] The resulting suspension, diluted by an additional 6.0 mL of MeCN, was allowed to stir overnight. After decantation of a gummy residue, 4 N NaOH was added until pH 12–14, and the mixture was extracted rapidly with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and evaporated to give 1.2 g of an oil which was crystallized by the addition of iPr_2O. The product was recrystallized from iPr_2O containing a little CH_2Cl_2 to give 0.80 g (52.9%) of **9a** as a free base: mp 137–8 °C; ^{1}H NMR ($CDCl_3$) 1.21–1.98 (m, 9H, piperidine), 2.84–2.90 (bd, 2H, piperidine), 3.18–3.25 (t, 2H, CH_2), 3.48 (s, 2H, benzyl), 3.75 (bs, 3H, guanidine), 6.88–7.01 (m, 3H, arom), 7.23–7.30 (m, 7H, aromatics). Anal. ($C_{21}H_{28}N_4$) C, H, N.

1-Phenyl-3-[2-(1-benzyl-4-piperidinyl)ethyl]-*N*-propylguanidine (9b) (Method G). **8a** (3.0 g, 8.5 mmol) in 30 mL of absolute EtOH was warmed to 60 °C with stirring and yellow PbO (3.8 g, 17.0 mmol) added to the solution. The resulting black suspension was stirred for 15 min, $PrNH_2$ (3.5 mL, 42.5 mmol) was added, and the mixture was allowed to stir for a further 3 h. The PbS precipitate was filtered off and the solvent evaporated to leave an orange oily residue which was purified by silica gel column chromatography (CH_2Cl_2–MeOH–NH_4OH, 80:20:2) to give 2.7 g of a pale yellow oil which was treated with fumaric acid–EtOH and crystallized from Et_2O to give 2.31 g (74.0%) of white crystals of **9b** fumarate: mp 113–6 °C; ^{1}H NMR (DMSO-d_6) 0.80–0.87 (t, 3H, CH_3), 1.06–1.92 (m, 11H, piperidine + CH_2), 2.72–2.78 (bd, 2H, piperidine), 3.06–3.13 (m, 4H, CH_2), 3.42 (s, 2H, benzyl), 6.43 (s, 2H, fumaric), 7.08–7.42 (m, 10H, arom), 9.1 (bs, 1H, NH salt). Anal. ($C_{24}H_{34}N_4$) C, H, N.

1-Phenyl-3-[2-(1-benzyl-4-piperidinyl)ethyl]-*N*-hydroxyguanidine (9c) (Method H). To a stirred solution of **8a** (5.9 g, 16.7 mmol) in 50 mL of CH_2Cl_2 was added yellow HgO (7.23 g, 33.4 mmol). An orange suspension appeared rapidly at room temperature. A solution containing NH_2OH·HCl (1.45 g, 20.8

mmol) in 50 mL of MeOH and powdered KOH (1.17 g, 20.8 mmol) was prepared and cooled on an ice bath; precipitated KCl was filtered off after about 30 min. The filtrate was added to the solution resulting from the filtration of the previously prepared orange suspension. After 45 min, the cloudy green turbid solution was evaporated and the dirty green gummy residue was diluted with 2 N HCl. This aqueous solution was washed with CH_2Cl_2, rebasified by 2 N NaOH, and extracted with CH_2Cl_2; the organic phase was washed with brine, dried over Na_2SO_4, and evaporated to dryness to leave an oily residue which was purified by silica gel column chromatography (CH_2Cl_2–MeOH–NH_4OH, 90:10:1) to give 1.4 g of a pale yellow oil. This was crystallized with iPr_2O–EtOH to give 1.0 g (17.0%) of the free base **9c**: mp 146–7 °C; ^1H NMR ($CDCl_3$) 1.20–1.95 (m, 9H, piperidine), 2.78–2.84 (bd, 2H, piperidine), 2.97–3.04 (t, 2H, CH_2), 3.45 (s, 2H, benzyl), 4.77 (bs, 3H, NH + OH), 6.94–7.03 (m, 3H, arom), 7.16–7.27 (m, 7H, arom). Anal. ($C_{21}H_{28}N_4O$) C, H, N.

***N*-Benzyl-*N'*-[2-(1-benzyl-4-piperidinyl)ethyl]-1,1-diamino-2-nitroethylene (10a) (Method I).** To a stirred yellow suspension of 1,1-bis(methylthio)-2-nitroethylene (2.0 g, 12.1 mmol) in 20 mL of MeOH was added aniline (1.12 mL, 12.1 mmol), and the resulting mixture was refluxed for 20 h. After the mixture was cooled to room temperature, the suspension was filtered, and the pale yellow precipitate was rinsed with 5 mL of MeOH to give 1.80 g (72.0%) of pale crystals of 1-(methylthio)-1-(phenylamino)-2-nitroethylene **11a**: mp 148–9 °C; ^1H NMR ($CDCl_3$) 2.39 (s, 3H, CH_3), 6.71 (s, 1H, ethylenic), 7.27–7.49 (m, 5H, arom), 11.83 (bs, 1H, NH).

To a solution of **2a** (0.6 g, 2.75 mmol) in 5 mL of absolute EtOH was added **11a** (0.5 g, 2.38 mmol) as a solid, and the mixture was refluxed for 2 h and thereafter allowed to cool and stirred overnight. The precipitate was filtered, washed twice with 10 mL of Et_2O, and dried under reduced pressure to give 0.80 g of pale yellow crystals which were recrystallized from absolute EtOH to give 0.60 g (66.7%) of white crystals of free base **10a**: mp 171–2 °C; ^1H NMR ($CDCl_3$) 1.27–1.95 (bm, 9H, piperidine), 2.85–2.91 (bd, 2H, piperidine), 3.12 (bs, 1H), 3.50 (s, 2H, benzyl), 4.83 (bs, 1H), 6.44–6.64 (m, 1H), 7.20–7.32 (m, 10H, arom), 10.49 (bs, 1H), 11.65 (bs, 1H). Anal. ($C_{22}H_{28}N_4O_2$) C, H, N, Cl.

Materials and Methods. Determination of Acetylcholinesterase Activity. The inhibitory effects of the various compounds on acetylcholinesterase activity were determined in vitro in homogenates of rat forebrain and salivary gland spectrophotometrically by the method of Ellman et al.[51] Ex vivo determinations were made by administering the test drug intraperitoneally to rat 30 min before sacrifice. Homogenates of forebrain and salivary gland were prepared and the acetylcholinesterase activity determined as described above.

Measurement of Passive Avoidance Activity. The effect of test compounds on the amnesia induced in the passive avoidance test in rats by the administration of scopolamine was determined as described previously.[52] Scopolamine (1 mg/kg ip) typically reduced the latency by 70–80%.

Observation of in Vivo Cholinergic Activity. Mice were isolated in groups of three or four and observed for 30 min after intraperitoneal injection with vehicle or the test drug. Salivation and tremor were noted as present or absent for each animal without quantification. Ten mice were observed for each treatment group.

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