Flexible 1-[(2-Aminoethoxy)alkyl]-3-ar(o)yl(thio)ureas as Novel Acetylcholinesterase Inhibitors. Synthesis and Biochemical Evaluation

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A series of flexible 1-(2-aminoethoxy)-3-ar(o)yl(thio)ureas was synthesized and assessed for antiacetylcholinesterase activity. This series was designed in order to optimize the spacer length linking the two pharmacophoric moieties, i.e., the basic nitrogen and the ar(o)yl(thio)-urea unit, and to test compounds with greater conformational flexibility. Thus, the replacement of the previously described spacer, 4-piperidinylethyl, by a linear ethoxyethyl chain gave compounds of slightly comparable potency, providing that they were correctly substituted. The results show that this new flexible spacer is compatible with high inhibitory activities. The optimal chain length corresponds to five methylene groups, allowing an efficient interaction between the two pharmacophoric units and the two reported hypothetical enzyme hydrophobic binding sites. Moreover, the initially optimized benzyl group, attached to the basic nitrogen, was found to be advantageously replaced by a cyclohexyl group, showing that an aromatic residue does not represent a prerequisite for activity.

Introduction

Alzheimer's disease has become a major health care problem, affecting mainly the industrialized nations with aging populations. The NIH projections (see ref 17) for the year 2000 reveals that 5–8 million Americans could be affected, corresponding to a worldwide patient population of about 24 million people. The market consequences could reach $2 billion for the treatment, and $325 million for the diagnosis of the disease, the whole being estimated to at least $6 billion by the year 2007.

The rational treatment of this challenging neurodegenerative disease must take into account at least four main problems: (1) efficient diagnosis during a postulated reversible phase of the disease, (2) the understanding of the relevant underlying biochemical processes, (3) the definition and choice of appropriate targets, and (4) the design of potent and selective molecules directed to these targets.

Acetylcholinesterase (AChE) inhibition belongs to the cholinergic replacement strategy designed for compensation of the primary selective degeneration of cholinergic neurons.[1] This target requires functional cholinergic neurons and is thus only applicable to the early stages of the disease. The observed clinical efficiency of AChE inhibitors such as physostigmine, tacrine,[2] and, more recently, Huperzine A[3] and E-2020,[4] despite some controversial results, strengthens this hypothesis.

Structural polymorphism of AChE, associated with its noncholinergic actions, were recently suggested[5] and then demonstrated.[6] In certain pathological conditions, including Alzheimer's disease, AChE was found to exist as several isoforms, some of which have been reported to act as proteases.[7]

The intense research devoted to the understanding the biochemical processing of APP and to the accumula-

Chart 1

Physostigmine

Tacrine

Huperzine A

Galanthamine

E-2020

8a
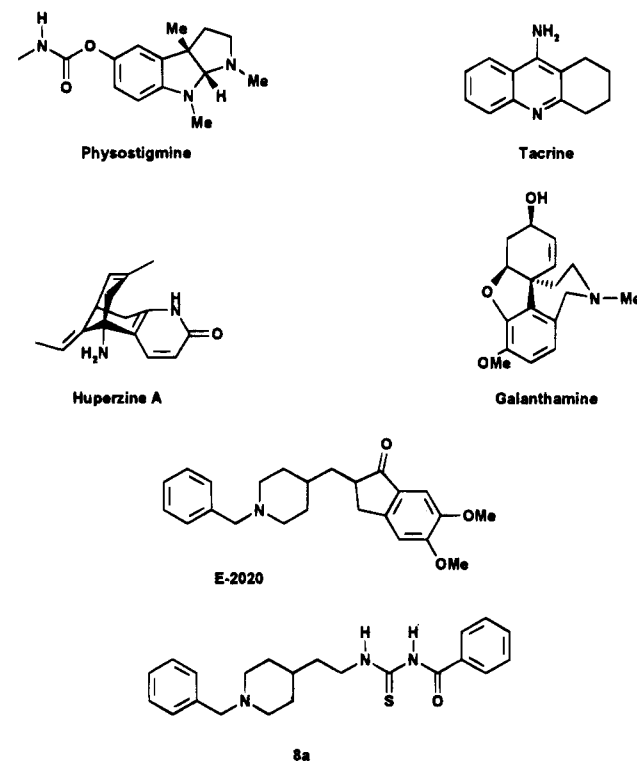
tion of βA4-amyloid protein (βAP) in the cerebral cortex suggested the action of an unknown protease of the chymotrypsin-like type.[8] Moreover, the evidence of a colocalization[9a] of AChE and βAP led to the hypothesis that the "abnormal"[9b] processing of APP could be due to the proteolytic action of at least one of the pathological isoforms of AChE. A unifying hypothesis was recently reported[9c] as a model for β-amyloid aggregation and neurotoxicity based on free radical generation by the peptide. Thus, possibly, AChE inhibitors could act in a convergent manner: first, as a classical replacement strategy in providing a normal level of acetylcholine in the synaptic cleft; second, as potential inhibitors

Scheme 1[a]

Method A

Method B
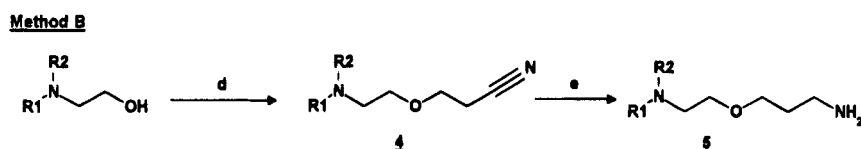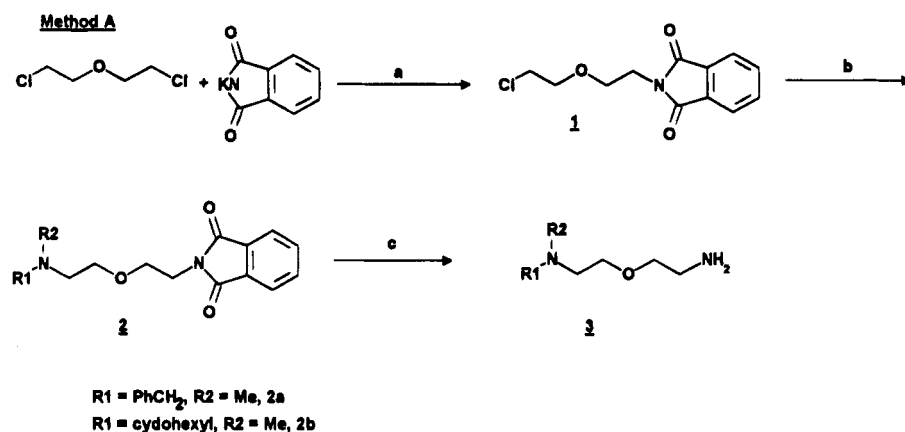
[a] (a) KI, DMF, 100 °C; (b) R1R2NH, DMF, 100 °C, 8 h; (c) NH₂NH₂·H₂O, EtOH, reflux, 3 h; (d) H₂C=CHCN, EtONa, 80 °C, 7 h; (e) H₂, Raney Ni, EtOH−NH₄OH.

Scheme 2
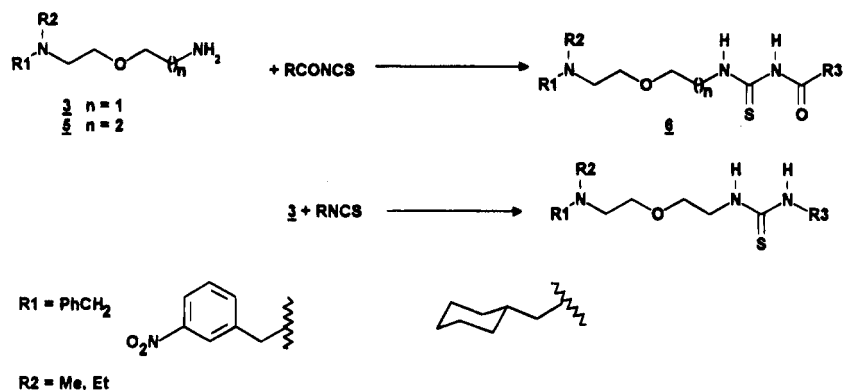
of the production of βAP which leads to the formation and accumulation of the senile plaques.

Most known and newly reported[10a] AChE inhibitors are rigid, as illustrated by tacrine, physostigmine, huperzine A, and galanthamine (see Chart 1), or at least conformationally restrained as represented by E-2020 and compounds that we recently described.[10c] Flexible compounds still remain a rare structural class among the AChE inhibitors. However, a number of flexible molecules have been reported to behave as "molecular chameleons", i.e., to adapt their polarity to that of the medium.[11] The resulting different conformations could display variable ability to cross hydrophobic cell membranes, thus generating compounds with potential centrally selective bioavailability.

In order to assess the importance of the previously reported conformationally restrained piperidine[12] linker on AChE inhibitory activity, we now describe the synthesis and biochemical evaluation of a series of flexible new 1-[(2-aminoethoxy)alkyl]-3-ar(o)yl(thio)-ureas derivatives related to compounds 8[10b,c] (Chart 1) as acetylcholinesterase inhibitors.

Chemistry

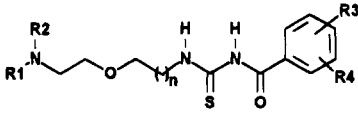The described structures were prepared by classical methods as shown in Schemes 1 and 2. 2-Aminoethoxy

ethers 3 were synthesized by a standard Gabriel sequence starting from the commercially available bis(2-chloroethyl) ether (method A). In the case of aminopropoxy ethers 5, a base-catalyzed Michael addition of 2-aminoethanols to acrylonitrile was used, followed by catalytic hydrogenation of the resulting β-cyano ethers 4 (method B). Compounds 6 and 7 were obtained, as described in our previous paper,[10c] by reaction of the above amino ethers 3 and 5 with the appropriate ar(o)-yliso(thio)cyanates (Scheme 2).
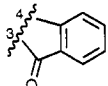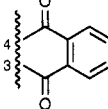
Structure−Activity Relationships

This series was designed to test the opportunity of inserting a linear spacer between a basic nitrogen and the pharmacophoric ar(o)yl(thio)urea moiety in order to enhance molecular flexibility. The direct comparison of **8a** (see Chart 1) and its flexible analog **6a** showed a lower affinity for the latter (see Table 1). In most cases classical substitution of the aromatic ring with polar R3 and R4 groups led to enhanced inhibitory activities for the substituted compounds versus the parent compound **6a**, as shown in Table 1. The highest activities were observed for the 3-nitro derivative **6e** and for **6j** as was reported for the piperidine series.[10c]

A comparison of **6c** (or **6d**) and **6a** showed that a 4-methoxy (or 4-chloro) substitution resulted in a 4-fold
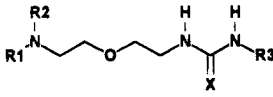
Table 1. Aroylthiourea Derivatives

no.	R1	R2	n	R3	R4	yield,[e] %	mp,[a] °C	formula	IC$_{50}$ of AChE inh, nM[d]
6a	C$_6$H$_5$CH$_2$	Me	1	H	H	44	116–8	C$_{20}$H$_{26}$ClN$_3$O$_2$S	120 [13][g]
6b	C$_6$H$_5$CH$_2$	Me	1	4-Me	H	48	152–3[b]	C$_{25}$H$_{31}$N$_3$O$_6$S	77
6c	C$_6$H$_5$CH$_2$	Me	1	4-OMe	H	39	140–2	C$_{21}$H$_{28}$ClN$_3$O$_3$S	26 [10][g]
6d	C$_6$H$_5$CH$_2$	Me	1	4-Cl	H	40	150–1[b]	C$_{24}$H$_{28}$ClN$_3$O$_6$S	30 [20][g]
6e	C$_6$H$_5$CH$_2$	Me	1	3-NO$_2$	H	35	94–6[b]	C$_{24}$H$_{28}$N$_4$O$_8$S	15 [1.5][g]
6f	C$_6$H$_5$CH$_2$	Me	1	3-Cl	4-Cl	52	123–4[b]	C$_{24}$H$_{27}$Cl$_2$N$_3$O$_6$S	55
6g	C$_6$H$_5$CH$_2$	Me	1	4-Ph	H	35	130–2	C$_{26}$H$_{30}$ClN$_3$O$_2$S	230
6h	C$_6$H$_5$CH$_2$	Me	1	3,4-benzo		45	202–4	C$_{24}$H$_{28}$ClN$_3$O$_2$S	100
6i	C$_6$H$_5$CH$_2$	Me	1			22	205–10	C$_{27}$H$_{28}$ClN$_3$O$_2$S	30 [80][g]
6j	C$_6$H$_5$CH$_2$	Me	1			21	132–4[b]	C$_{32}$H$_{31}$N$_3$O$_8$S	6 [2][g]
6k	C$_6$H$_5$CH$_2$	Me	2	H	H	26	98–100[b]	C$_{25}$H$_{31}$N$_3$O$_6$S	1100
6l	c-C$_6$H$_{11}$	Me	1	H	H	44	141–2[c]	C$_{21}$H$_{31}$N$_3$O$_4$S[4]f	13

^{a} All melting points refer to hydrochlorides unless otherwise indicated. ^{b} Fumarate. ^{c} Hemifumarate. ^{d} The IC$_{50}$ values for AChE inhibition were estimated graphically from log concentration/% inhibition curves of 6–15 values. ^{e} Yields refer to isolated, analytically pure bases. ^{f} Slightly off value of carbon analysis. ^{g} Values of the corresponding piperidine analogues[10c] are shown in brackets. (Compound **8a** [13 nM], shown in Chart 1, is the corresponding piperidine analog of **6a**.)

Table 2. Aryl(thio)urea Derivative

no.	R1	R2	X	R3	yield,[e] %	mp, °C	formula	IC$_{50}$ of AChE inh, nM[d]
7a	C$_6$H$_5$CH$_2$	Me	S	4-chlorophenyl	50	134–5[a]	C$_{19}$H$_{25}$Cl$_2$N$_3$OS[f]	1800
7b	C$_6$H$_5$CH$_2$	Me	s	1-naphthyl	64	116–7[c]	C$_{23}$H$_{27}$N$_3$OS	>10000
7c	3-NO$_2$-PhCH$_2$	Me	S	3,4-dimethoxyphenyl	61	118–25[a]	C$_{21}$H$_{29}$ClN$_4$O$_5$S	1500
7d	C$_6$H$_5$CH$_2$	Me	S	4-(trifluoromethoxy)phenyl	33	142–3[a]	C$_{20}$H$_{25}$F$_3$ClN$_3$OS	1000
7e	C$_6$H$_5$CH$_2$	Me	S	3-pyridyl	56	70–90[b]	C$_{22}$H$_{27}$N$_5$O$_7$S	4000 [500][g]
7f	C$_6$H$_5$CH$_2$	Me	S	cyclohexyl	64	132–4[b]	C$_{19}$H$_{32}$ClN$_3$OS	1400
7g	c-C$_6$H$_{11}$CH$_2$	Et	O	C$_6$H$_5$CH$_2$	49	66–7[c]	C$_{20}$H$_{33}$N$_3$O$_2$	>10000
7h	C$_6$H$_5$CH$_2$	Me	O	4-nitrophenyl	82	176–7[a]	C$_{19}$H$_{25}$ClN$_4$O$_4$	2300

^{a} Hydrochloride. ^{b} Fumarate. ^{c} Free base. ^{d} See note d of Table 1. ^{e} Yields refer to isolated, analytically pure bases. ^{f} Slightly off value of carbon analysis. ^{g} Value of the corresponding piperidine analog.

increase in affinity; this fact was not observed in the piperidine series. In contrast, the 8-fold increase in potency of **6e** versus **6a** is also observed by comparing the piperidine analogs (see Table 1). The potential interest in the flexible compounds was especially shown by compound **6i** which displayed a significantly better potency than its more strained analog.

The above results may be explained in terms of a different (better in the case of **6i**) ability of flexible compounds to adapt their molecular shape to that of the active site of the enzyme.

The elongation by one carbon atom of the chain of **6a** resulted in a 10-fold decrease in potency as shown by compound **6k**. The optimum spacer was observed for n = 1, which was similar in length to that reported in the piperidine series.

Interestingly, the replacement of the standard benzyl group in **6a** by a cyclohexyl unit in **6l** resulted in a substantially higher activity which contrasts with data reported elsewhere.[13a–f] Thus, the initial view of the necessity of a benzyl substituent at the basic nitrogen appeared to be unfounded.

The series of the aryl(thio)urea derivatives (compounds **7**, Table 2) showed a 10-fold global loss of potency compared to the aroyl(thio)ureas. No real interest was raised by classical substitution of the phenyl ring.

In conclusion, the flexible aroylthiourea derivatives **6**, with a linear (2-aminoethoxy)ethyl spacer, proved to be as potent AChE inhibitors as the conformationally more restrained reference compounds such as E-2020 or **8a** (**8a** corresponds to compound **7a** of our previous study[10c]); they can show even better inhibitory activities than the highly strained reference compounds (Table 3). Replacement of a benzyl group by a cyclohexyl group on the basic nitrogen resulted in a 10-fold better activity, showing that an aromatic residue does not represent a prerequisite for efficiency. In contrast, the linear (thio)-urea derivatives **7** were 10-fold less active than their piperidine analogs.

Experimental Section

All melting points were determined on a Kofler apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a

Table 3. Reference Values in AChE Inhibition

	IC$_{50}$ of AChE inh, nM	
reference compounds	observed	reported
physostigmine	80 ± 3.4^a	0.68 ± 0.02^{d,12d}
galanthamine	538 ± 60^a	360^{16}
tacrine	63 ± 1.3^a	80.6 ± 2.5^{12d}
huperzine A	nd^b	100^{3e}
E-2020	17 ± 5.0^a	5.7 ± 0.2^{12d}
8a^e	13^{c,10}	

^{a} These values represent the mean ± SE from four dose–response curves for each reference compound. ^{b} nd = not determined. ^{c} This value was estimated graphically from log $C/\%$ inhibition curves of five experiments. ^{d} Differences frequently observed in the reported values for physostigmine are a consequence of its short half-life and variations in the time of observation. ^{e} This compound **8a** (see Chart 1) corresponds to compound **7a** of our previous paper.^{10c} (It corresponds to the piperidine analog of **6a** of the present paper.)

Bruker AC-200 spectrometer using Me$_4$Si 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 unless otherwise noted.

2-[2-(2-Chloroethoxy)ethyl]-1H-isoindole-1,3(2H)-dione (1). This versatile compound was first synthetized using a procedure described by Cretcher and collaborators and recently modified by Calderon and collaborators.^{14} We report here our conditions involving an excess of bis(2-chloroethyl) ether in hot DMF which led to better yields. To a solution of bis(2-chloroethyl) ether (23 g, 0.161 mol) in DMF (100 mL) at 100 °C were successively added KI (50.0 mg, 0.30 mmol) and potassium phthalimide (10.0 g, 54.0 mmol) portionwise over 1 h. The resulting brown-yellow suspension was stirred for a further 2 h, allowed to cool to room temperature, poured into 200 mL of H$_2$O, and extracted with CH$_2$Cl$_2$. The organic phase was washed, dried over Na$_2$SO$_4$, and evaporated to leave an orange oil. This was purified by silica gel column chromatography (hexane–AcOEt, 1:1) to give 11.0 g (80.3%) of white crystals of **1**: mp 69–70 °C; ^{1}H NMR (CDCl$_3$) δ 3.50–3.56 (t, 2H), 3.68–3.77 (m, 4H), 3.85–3.91 (t, 2H), 7.66–7.71 (m, 2H), 7.78–7.84 (m, 2H).

2-{2-[2-(N-Benzyl-N-methylamino)ethoxy]ethyl}-1H-isoindole-1,3(2H)-dione (2a). To a stirred solution of N-methylbenzylamine (6.45 mL, 50.0 mmol) in 15 mL of DMF was added dropwise a solution of **1** (6.34 g, 25.0 mmol) in 20 mL of DMF. The mixture was stirred at 100 °C for 8 h, poured into 300 mL of H$_2$O, and extracted with AcOEt. The organic phase was washed with water and brine, dried over Na$_2$SO$_4$, and evaporated to give a yellow oil which was purified by silica gel column chromatography (CHCl$_3$, then CHCl$_3$–MeOH, 95:5) to give 7.4 g (87.5%) of **2a** as a yellow oil: ^{1}H NMR (CDCl$_3$) δ 2.15 (s, 3H), 2.52–2.58 (t, 2H), 3.46 (s, 2H), 3.55–3.69 (m, 4H), 3.83–3.89 (t, 2H), 7.15–7.27 (m, 5H), 7.62–7.69 (m, 2H), 7.73–7.79 (m, 2H).

2-[2-(N-Benzyl-N-methylamino)ethoxy]ethanamine (3a). To a stirred solution of **2a** (4.90 g, 14.5 mmol) in 50 mL of EtOH was added hydrazine hydrate (1.40 mL, 28.8 mmol). The mixture was heated at reflux with stirring for 3 h (after around 30 min, white crystals formed). The mixture was filtered, the precipitate rinsed with EtOH, and the filtrate evaporated to dryness. The resulting yellow oily residue was taken up in 2 N HCl, stirred for 5 min at 50 °C, then basified with 2 N NaOH, and extracted with CHCl$_3$. The organic phase was washed with H$_2$O and brine, dried over Na$_2$SO$_4$, and evaporated to dryness to leave 2.02 g (67.0%) of **3a** as a yellow oil which was used for the next step without further purification; ^{1}H NMR (CDCl$_3$) δ 2.26 (s, 3H), 2.58–2.64 (t, 2H), 2.78–2.87 (m, 4H), 3.45–3.61 (m, 6H), 7.23–7.32 (m, 5H).

2-[2-(N-Benzyl-N-methylamino)ethoxy]propionitrile (4a). A suspension of N-benzyl-N-methyl-2-ethanolamine (16.52 g, 10 mmol), acrylonitrile (5.57 g, 10.5 mmol), and sodium ethoxide (0.55 g, 10.2 mmol) was heated at 80 °C with stirring for 7 h. After cooling to room temperature, the dark brown mixture was poured into H$_2$O and extracted with CH$_2$-

Cl$_2$. The organic phase was washed with water and brine, dried over Na$_2$SO$_4$, and filtered and the solvent evaporated. The resulting brown oily residue was purified by silica gel column chromatography (AcOEt, then AcOEt–MeOH, 95:5) to leave 16.75 g (76.7%) of **4a** as a brown oil: ^{1}H NMR (CDCl$_3$) δ 2.30 (s, 3H), 2.56–2.67 (m, 4H), 3.58 (s, 2H), 3.60–3.68 (m, 4H), 7.25–7.34 (m, 5H).

3-[2-(N-Benzyl-N-methylamino)ethoxy]propanamine (5a). To a solution of **4a** in EtOH (50 mL), H$_2$O (40 mL), and NH$_4$OH (20 mL) was added Raney Ni catalyst (1.5 g). The atmosphere above the mixture was successively purged with nitrogen and hydrogen and then stirred vigorously at room temperature until 2.8 L of H$_2$ was adsorbed. The catalyst was removed by filtration using a Whatman glass microfibre filter and the filtrate evaporated to dryness. The residual oil was treated with brine and extracted with AcOEt; the organic phase was dried over Na$_2$SO$_4$ and evaporated to dryness to leave 15.7 g of an oily yellow residue which was purified by silica gel column chromatography (CHCl$_3$–MeOH–NH$_4$OH, 90:10:1) to give 10.0 g (70.4%) of a pale oil of **5a**: ^{1}H NMR (CDCl$_3$) δ 1.61 (s, 2H), 1.63–1.76 (q, 2H), 2.25 (s, 3H), 2.56–2.62 (t, 2H), 2.74–2.81 (t, 2H), 3.45–3.57 (m, 6H), 7.22–7.31 (m, 5H).

1-Benzoyl-3-{1-[2-[2-(N-benzyl-N-methylamino)ethoxy]ethyl}thiourea (6a). To a well-stirred suspension of finely powdered KSCN (0.9 g, 9.26 mmol) in 20 mL of dry acetone under nitrogen was added dropwise benzoyl chloride (1.2 mL, 10.34 mmol) in 5 mL of acetone. The immediately obtained white suspension was stirred under reflux for 5 min. Then a solution of **3a** (2.0 g, in 5 mL) in CH$_2$Cl$_2$ was added, and the resulting orange suspension was allowed to cool to room temperature for 1 h. The solvents were evaporated, H$_2$O was added to the residue, and the mixture was extracted with CHCl$_3$. The organic phase was washed, dried over Na$_2$SO$_4$, and evaporated to leave an orange gummy residue which was purified by silica gel column chromatograpihy (CHCl$_3$, then CHCl$_3$–MeOH, 95:5) to give 1.55 g (43.4%) of **6a** as an orange gum: ^{1}H NMR (CDCl$_3$) δ 2.31 (s, 3H), 2.69–2.75 (t, 2H), 3.63 (s, 2H), 3.67–3.74 (m, 4H), 3.90–3.97 (q, 2H), 7.21–7.37 (m, 5H), 7.47–7.67 (m, 3H), 7.79–7.84 (d, 2H), 9.02 (bs, 1H), 10.94 (bs, 1H). Anal. (C$_{20}$H$_{26}$ClN$_3$O$_2$S) C, H, N, Cl, S (hydrochloride).

1-Cyclohexyl-3-{1-[2-[2-(N-benzyl-N-methylamino)ethoxy]ethyl}thiourea (7f). To a stirred solution **3a** (3.6 g, 17.3 mmol) in 50 mL of CH$_2$Cl$_2$ was added dropwise a solution of cyclohexyl isothiocyanate (2.43 g, 17.3 mmol) in 10 mL of CH$_2$-Cl$_2$. The mixture was allowed to stir at room temperature for 1 h. The solvent was removed and the resulting yellow oily residue purified by silica gel column chromatography (AcOEt–MeOH, 95:5) to give 3.87 g (64.1%) of the title product as an oily base which was converted to its hydrochloride with HCl–EtOH in Et$_2$O to give 2.35 g (39.0%) of **7f** as white crystals: mp 132–4 °C; ^{1}H NMR (CDCl$_3$) δ 1.10–1.71 (m, 9H), 1.99–2.01 (m, 2H), 2.87–2.89 (ds, 3H), 2.90–3.01 (m, 1H), 3.28–3.38 (m, 1H), 3.59–3.71 (m, 6H), 3.88–3.96 (m, 1H), 3.98–4.15 (m, 2H), 4.49–4.57 (dd, 1H), 7.45–7.50 (m, 3H), 7.58–7.63 (m, 2H), 11.08 (bs, 1H). Anal. (C$_{19}$H$_{32}$ClN$_3$OS) C, H, N, Cl, S.

Materials and Methods. Determination of Acetylcholinesterase Activity. The in vitro inhibitory effects of the various compounds on acetylcholinesterase activity were determined spectrophotometrically by the method of Ellman et al.^{15} using homogenates of rat forebrain.

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