Syntheses and Anticholinesterase Activities of (3a*S*)-*N*[1],*N*[8]-Bisnorphenserine, (3a*S*)-*N*[1],*N*[8]-Bisnorphysostigmine, Their Antipodal Isomers, and Other Potential Metabolites of Phenserine[†]

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Hydrolysis of the carbamate side chains in phenserine [(−)**1**] and physostigmine [(−)**2**] yields the metabolite (−)-eseroline (**3**), and the red dye rubreserine (**4**) on air oxidation of the former compound. Both compounds lacked anticholinesterase activity in concentrations up to 30 mM, which would be unachievable in vivo. A second group of potential metabolites of **1** and **2** are the *N*[1],*N*[8]-bisnorcarbamates (−)**9** and (−)**10**, prepared from (3aS)-*N*[8]-benzylnoresermethole (−)**12** by the carbinolamine route. These entirely novel compounds proved to be highly potent inhibitors of acetylcholinesterase [(−)**9**] and of acetyl- and butyrylcholinesterase (AChE and BChE) [(−)**10**], respectively. To elucidate further the structure/anticholinesterase activity relationship of the described compounds, the antipodal isomers (3aR)-*N*[1],*N*[8]-bisnorcarbamates (+)**9** and (+)**10** were likewise synthesized from (3a*R*)-*N*[8]-benzylnoresermethole (+)**12** and assessed. The compounds possessed moderate but less potent anticholinesterase activity, with the same selectivity as their 3a*S* enantiomers. Finally, the anticholinesterase activities of intermediates *N*[1],*N*[8]-bisnorbenzylcarbamates (−)**18**, (−) **19**, (+)**18**, and (+)**19**, also novel compounds, were additionally measured. The 3a*S* enantiomers proved to be potent and selective inhibitors of BChE, particularly (−)**19**, whereas the antipodal isomers lacked activity.

Phenserine [(−)**1**], the phenyl carbamate analogue of physostigmine [(−)**2**], is a new and selective inhibitor of acetylcholinesterase (AChE) with minimal butyrylcholinesterase (BChE) action which is about to enter clinical trials for the treatment of Alzheimer's disease.[1,2] The compound is sufficiently long acting for once daily administration and preferentially enters the brain, the site of Alzheimer's disease, reaching and maintaining a brain/plasma ratio of 10:1.[1,3] As a consequence of these incorporated design features, the compound has an unusually wide therapeutic window, a highly favorable toxicological profile in dogs and rodents, and dramatic action in animal cognitive models.[1,3] Additionally, it modulates the molecular events involved in the neuropathology of Alzheimer's disease, reducing the synthesis and secretion of β-amyloid precursor protein, the source of the Alzheimer neurotoxic peptide β-amyloid, both in in vitro and in vivo studies.[3,4]

An attractive and pivotal point in the development of phenserine as a therapeutic is the assessment and synthesis of its potential metabolites and elucidation of their biological properties. Since hydrolysis of carbamyl esters and N-demethylation are well-established metabolic pathways,[5,6] we thought it prudent to prepare the key tricyclic compounds derived from phenserine [(−)**1**]- and physostigmine [(−)**2**] by such action and evaluate their anticholinesterase activities (Figure 1).

On the basis of our recent successful syntheses of the *N*[1]-nor series[7] and *N*[8]-nor series[8] of both phenserine and physostigmine, we accomplished the novel syntheses of (3aS)-*N*[1],*N*[8]-bisnorphenserine [(−)**9**] and (3a*S*)-*N*[1],*N*[8]-bisnorphysostigmine [(−)**10**]. As our earlier studies proved that the 3a*S* enantioselectivity is clearly maintained in the *N*[8]-nor series[8] but not in the *N*[1]-nor series,[9] we additionally synthesized the (3a*R*)-*N*[1],*N*[8]-bisnor compounds (+)**9** and (+)**10** to assess their anticholinesterase activities, and report on them herein. Finally, no report on the synthesis, and chemical and biological evaluation of potential metabolites of phenserine and physostigmine would be complete without the inclusion of (−)-eseroline (**3**), the hydrolysis product of (−)**1** and (−)**2**, and the red dye rubreserine (**4**), which is readily formed on air oxidation of **3**. The evaluation of these compounds is likewise reported herein and was assessed against freshly obtained and prepared human AChE and BChE in vitro, alongside and compared to the activities of (−)- and (+)-phenserine (**1**) and physostigmine (**2**).

Results

Chemistry. Rubreserine (**4**) was obtained on air oxidation of eseroline (**3**),[10] instead of by its isolation from a mixture of degradation products from physo-

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† This paper is dedicated to Dr. Nelson J. Leonard, Faculty Associate in Chemistry, California Institute of Technology, Pasadena, CA, on the occasion of his 80th birthday.

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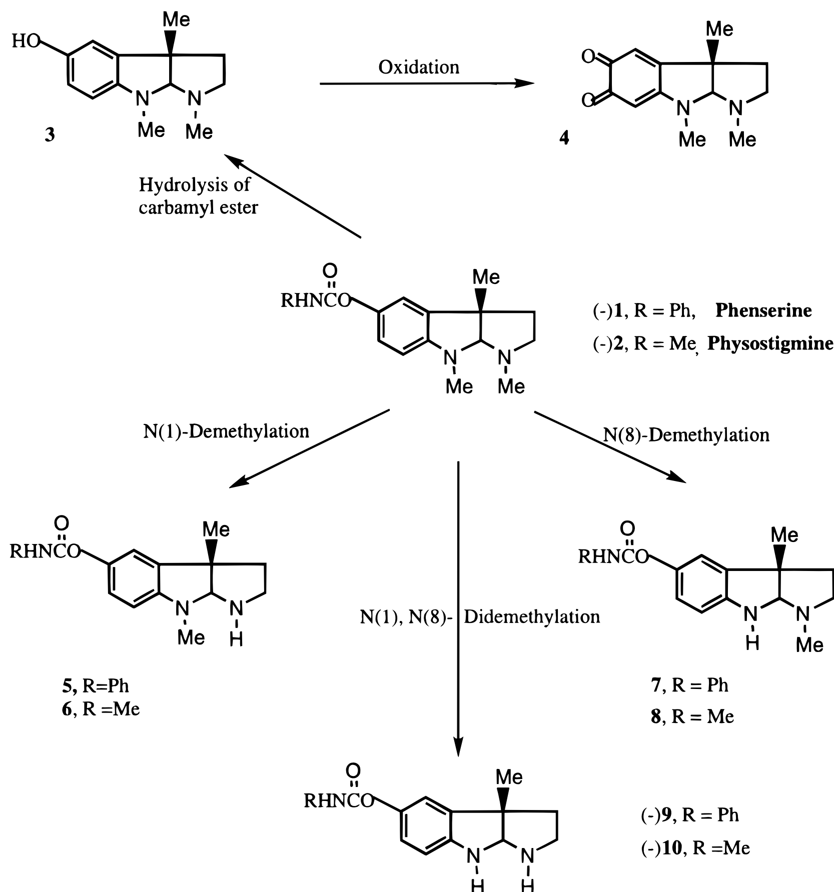
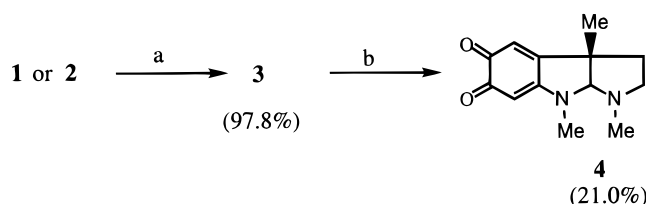


Figure 1.

Scheme 1[a]



[a] Reagents: (a) C_4H_9OH, Na; (b) H_2O, Na_2CO_3, O_2, $CHCl_3$.

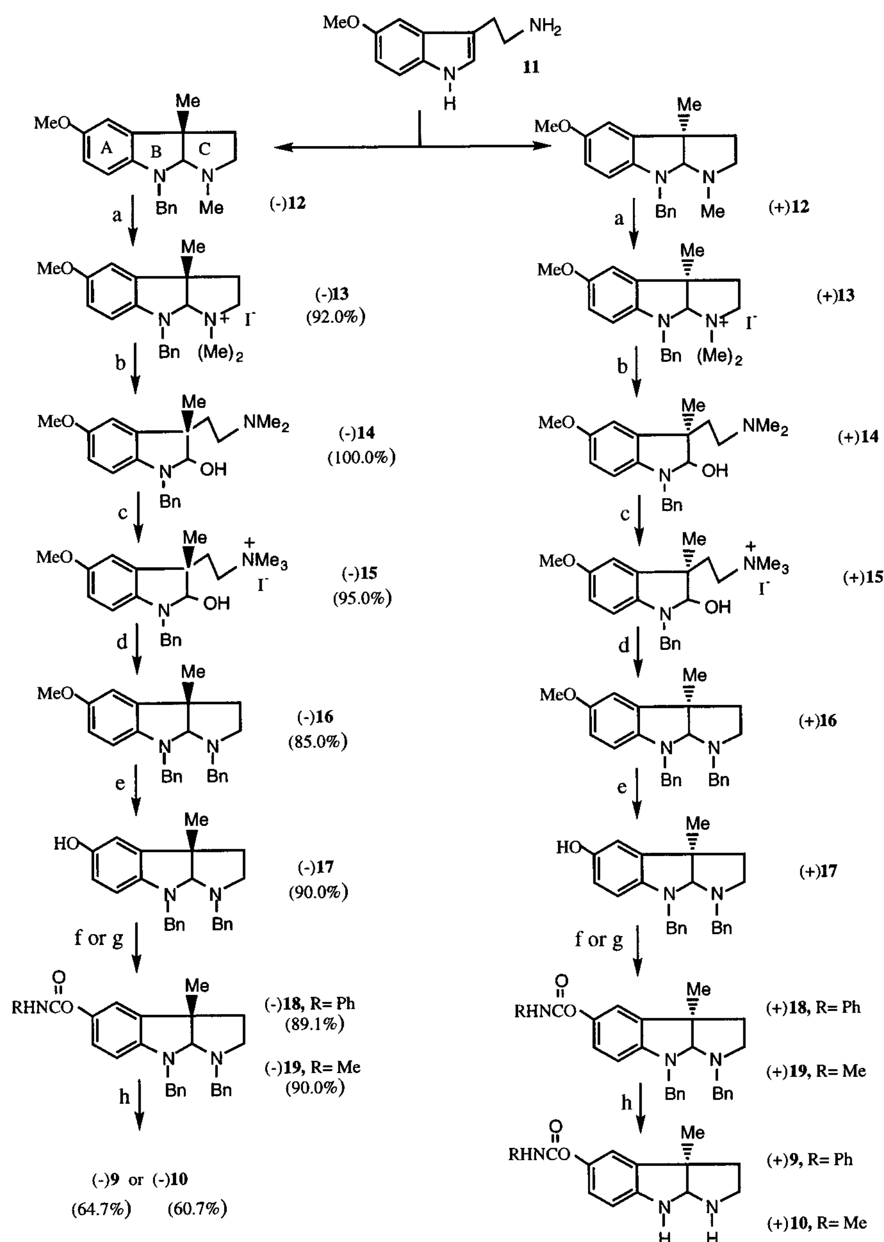
stigmine [(−)**2**].[11−14] Rubreserine (**4**) was initially isolated and identified by Auterhoff.[11] Later, Schönenberger et al. characterized it by X-ray diffraction analysis[13] and, more recently, Cordell provided its ^{1}H and ^{13}C NMR.[14] The synthesis of compound **4** is described in this paper (Scheme 1).

The major task in this project, which had not previously been accomplished, was the synthesis of *N*^{1},*N*^{8}-bisnorphenserine [(−)**9**] and its methylcarbamate analogue (−)**10**, both obtainable from (−)**1** and (−)**2** by N-demethylation. We suspected that (3a*S*)-*N*^{1},*N*^{8}-bisnoresermethole (not shown), obtained from 5-methoxytryptamine (**11**),[15] was not a good substrate to complete our task as the corresponding phenol would be exquisitely sensitive to air oxidation and hence difficult to handle. Furthermore, the lack of selectivity of O−H over N−H for reaction with isocyanate would have resulted in undesired products and hence make the synthesis far more complex than necessary. We have therefore developed an alternative route to prepare the compounds of the *N*^{1},*N*^{8}-bisnor series, and this is shown in Scheme 2.

This route uses the known (−)-*N*^{8}-benzylnoresermethole [(−)**12**], which was made from **11** in six steps including its optical resolution, as has been reported.[8] For introducing a benzyl group into the N^1-position of precious optically pure compound (−)**12**, we adopted, step by step, a procedure reported in the literature.[16] Starting with (−)**12**, it was quaternized with CH_3I, ring-C-opened under basic conditions, and again quaternized with CH_3I, leading via (−)**13** to (−)**14** and (−)**15**. Reaction of (−)**15** with $BnNH_2$ directly provided *N*^{1},*N*^{8}-bisbenzylnoresermethole [(−)**16**] in a total of four steps with a final yield of 74%. Demethylation of (−)**16** gave the phenol [(−)**17**] which, when reacted with either phenyl isocyanate or methyl isocyanate, afforded (−)-*N*^{1},*N*^{8}-bisbenzylnorphenserine [(−)**18**] and (−)-*N*^{1},*N*^{8}-bisbenzylnorphysostigmine [(−)**19**], respectively.

The catalytic debenzylation of (−)**18** and (−)**19** to give the desired bisnor compound (−)**9** and (−)**10**, respectively, was accomplished over $Pd(OH)_2$/C using *i*-PrOH as a solvent. The (3a*R*)-*N*^{1},*N*^{8}-bisnorphenserine [(+)**9**] and (3a*R*)-*N*^{1},*N*^{8}-bisnorphysostigmine [(+)**10**] were produced from (3a*R*)-*N*^{8}-benzylnoresermethole [(+)**12**] in the same manner, as shown in Scheme 2. This debenzylation proved unexpectedly difficult and is summarized in Scheme 3. First, we tried the conditions used in our previous preparation of the *N*^{8}-nor series.[8] The debenzylation of (−)**18** was initially attempted in an acidic medium of $CF_3COOH/CH_3OH/H_2O$ over the catalyst $Pd(OH)_2$/C. After 1.5 h of hydrogenation at atmospheric pressure, only one product, the ring-open *β*-2′-aminoethyl indole, compound **20**, could be isolated from the reaction mixture. This suggests that ring C

Scheme 2[a]



[a] Reagents: (a) CH_3I, ether; (b) EtOH, NaOH (50%); (c) CH_3I, Et_2O; (d) $BnNH_2$; (e) BBr_3, CH_2Cl_2; (f) phenyl isocyanate, Et_2O, Na; (g) methyl isocyanate, Et_2O, Na; (h) $Pd(OH)_2/C$, *i*-PrOH.

opening of the N^1,N^8-bisnor compounds formed during hydrogenation occurs much more readily than does that of the N^8-nor compounds.

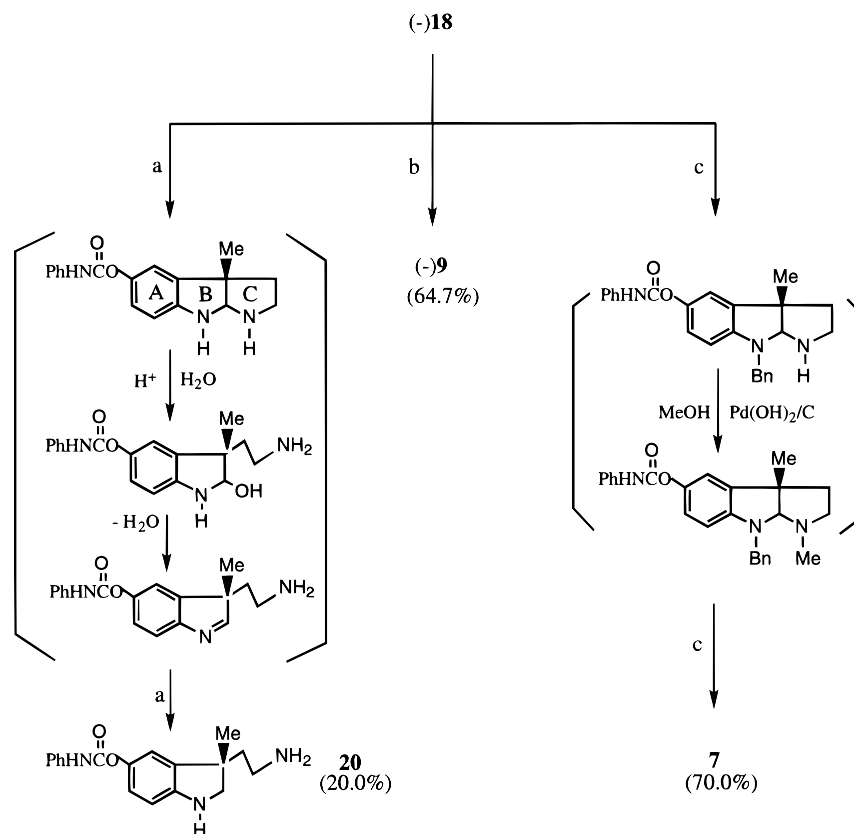
Extensive experimentation culminated in our use of a neutral solvent, initially CH_3OH, which, after 60 h of hydrogenation, provided (−)-N^8-norphenserine **7**, the only product that we were able to isolate from the reaction mixture. This suggests that during the long period of catalytic hydrogenation the initially formed N^1-nor compound was N-methylated, leaving the N^8-benzyl group attached to the aniline nitrogen before finally giving the N^8-nor compound **7**. This result is in accord with studies by Baiker and Richarz[17] and by He and Brossi.[18] They described the alkylation of secondary amines on catalytic hydrogenation using a primary alcohol as the solvent at high temperature and proposed that the key intermediate of this reaction is the aldehyde formed from dehydrogenation of the primary alcohol. We tried numerous solvents other than CH_3OH to avoid N^1-methylation, but these proved unsuccessful. Finally, we utilized *i*-PrOH or *t*-BuOH as the reaction solvents, and both provided positive results (Scheme 3).

Biological Evaluation. Table 1 illustrates the anticholinesterase activity of compounds **3**, **4**, (−)- and (+)**9**, **10**, **18**, and **19** against human AChE and BChE, compared to those of (−)- and (+)-phenserine (**1**) and physostigmine (**2**).

In accord with previous reports, neither (−)-eseroline (**30**) nor (−)-rubreserine (**4**) possessed anticholinesterase action. In contrast, the N^1,N^8-bisnorcarbamates (−)**9** and (−)**10** were highly potent in this regard.

(−)-N^1,N^8-Bisnorphenserine [(−)**9**] demonstrated potent and selective AChE inhibitory action with an IC_{50} value similar to that of (−)-phenserine [(−)**1**]. Like (−)-phenserine, the compound lacked BChE inhibitory potency, and had a selectivity of AChE action of some

Scheme 3[a]



[a] Reagents: (a) $Pd(OH)_2/C$, H_2O, CH_3OH, TFA, 1.5 h; (b) $Pd(OH)_2/C$, *i*-PrOH, 60 h; (c) $Pd(OH)_2/C$, MeOH, 60 h.

Table 1. 50% Inhibitory Concentration (IC_{50}, NM) + SEM[a] of Compounds versus Human Erythrocyte AChE and Plasma BChE

compound	IC_{50} AChE (nM)	IC_{50} BChE (nM)
(−)**1**, (−)-phenserine	24.0 ± 6.0[a]	1300 ± 85[a]
(+)**1**, (+)-phenserine	3500 ± 55	23500 ± 300
(−)**2**, (−)-physostigmine	27.9 ± 2.4	16.0 ± 2.9
(+)**2**, (+)-physostigmine	9890 ± 6	2490 ± 290
3, (−)-eseroline	NDA[b]	NDA
4, (−)-rubreserine	NDA	NDA
(−)**9**, (−)-*N*[1],*N*[8]-bisnorphenserine	22.1 ± 2.3	897 ± 104
(+)**9**, (+)-*N*[1],*N*[8]-bisnorphenserine	231 ± 23	952 ± 107
(−)**10**, (−)-*N*[1],*N*[8]-bisnorphysostigmine	10.9 ± 1.2	2.4 ± 0.8
(+)**10**, (+)-*N*[1],*N*[8]-bisnorphysostigmine	1490 ± 120	237 ± 55
(−)**18**, (−)-*N*[1],*N*[8]-bisbenzylnorphenserine	41130 ± 11760	513 ± 311
(+)**18**, (+)-*N*[1],*N*[8]-bisbenzylnorphenserine	NDA	NDA
(−)**19**, (−)-*N*[1],*N*[8]-bisbenzylnorphysostigmine	1090 ± 123	8.1 ± 2.1
(+)**19**, (+)-*N*[1],*N*[8]-bisbenzylnorphysostigmine	3560 ± 400	776 ± 312
5, (−)-*N*[1]-norphenserine	13.8 ± 0.7	612 ± 1
6, (−)-*N*[1]-norphysostigmine	21.0 ± 1.0	2.0 ± 1.0
7, (−)-*N*[8]-norphenserine	40.8 ± 3.4	516 ± 601
8, (−)-*N*[8]-norphysostigmine	56.7 ± 4.4	6.8 ± 2.2
21, (−)-*N*[8]-benzylnorphysostigmine	330 ± 100	10.4 ± 4.0
22, (−)-*N*[1]-benzylnorphysostigmine	161 ± 41	21.8 ± 10.9

[a] Standard error of the mean. [b] NDA: no detectable activity (at 3×10^{-5} M).

40-fold. In stark contrast to (+)-phenserine [(+)**1**] which lacks anticholinesterase activity, (+)-*N*[1],*N*[8]-bisnorphenserine [(+)**9**] possessed a low but nevertheless significant potency of AChE inhibition. The IC_{50} value of this was 10-fold lower than that of (−)**9** for AChE, whereas the BChE inhibitory action of (+)and (−)**9** were similar and low.

(−)-*N*[1],*N*[8]-Bisnorphysostigmine [(−)**10**] possessed a high potency of inhibitory action against both AChE and BChE, with activity some 3- and 6-fold greater than that of (−)-physostigmine [(−)**2**] against the two enzyme subtypes, respectively. In contrast to (+)-physostigmine [(+)**2**] which entirely lacked anticholinesterase action, (+)-*N*[1],*N*[8]-bisnorphenserine [(+)**9**] proved inactive against AChE but possessed low BChE inhibitory action, quantitatively similar to that of (−)**9**.

The AChE inhibitory activity of the intermediates (−)-*N*[1],*N*[8]-bisbenzylnorphenserine [(−)**18**] and (−)-*N*[1],*N*[8]-bisbenzylnorphysostigmine [(−)**19**] were dramatically reduced compared to (−)**9** and (−)**10**, respectively. However, their respective BChE inhibitory action was maintained, with (−)**19** being particularly potent. In contrast, the (+)-enantiomers were dramatically less active, with (+)-*N*[1],*N*[8]-bisbenzylnorphenserine [(+)**18**]

entirely lacking demonstrable activity in concentrations up to 30 mM.

Discussion

We report, herein, the first synthesis and initial pharmacological evaluation of both natural and unnatural *N*¹,*N*⁸-bisnorphenserine (**9**) and of *N*¹,*N*⁸-bisnorphysostigmine (**10**). Their structures and absolute configurations are secured with the chemical reactions described. Not only were the 3a*S* enantiomers of these novel compounds highly potent as anticholinesterases, but additionally they are potential metabolites of phenserine [(−)**9**] and physostigmine [(−)**10**].

The classical anticholinesterase physostigmine [(−)**2**] has been utilized widely in clinical studies over the last several decades, and it as well as the quaternary carbamates such as neostigmine and pyridostigmine have demonstrated utility and acceptability in treating a variety of disorders, primarily involving an awry cholinergic system. These include paralytic ileus and atony of the bladder, glaucoma, myasthenia gravis, termination of the actions of competitive neuromuscular blocking agents, and overcoming intoxication with atropine, tricyclic antidepressants, or phenothiazines. Physostigmine is available for injection (antilirium), as an ointment, and, more recently, for experimental use in Alzheimer's disease as a slow-release formulation (synapton) that is in phase III clinical trials. As a first-generation drug, its major drawbacks in clinical use are its low therapeutic window and short duration of unselective action on acetyl- and butyrylcholinesterase, with pharmacokinetic and pharmacodynamic half-lives of 30−90 min, respectively.[1]

In contrast, phenserine [(−)**1**] and analogues overcome these disadvantages and possess a long duration of action (half-life 8−10 h) and an unusually wide therapeutic window which results in dramatic cognitive effects.[1,3] Additionally, unlike physostigmine, phenserine possesses the rare characteristic of modulating the processing of β-amyloid precursor protein to limit formation of the Alzheimer toxic peptide, β-amyloid.[3,4] The agent therefore represents a promising drug candidate for the treatment of Alzheimer's disease as it has actions to potentially improve cognition and slow the disease course, through its interaction with the molecular events involved in the disease process, and its true value is about to be assessed in forthcoming clinical trials.

(−)-*N*¹,*N*⁸-Bisnorphenserine [(−)**9**] and (−)-*N*¹,*N*⁸-bisnorphysostigmine [(−)**10**] both possessed potent anticholinesterase action and hence, if produced in vivo as a consequence of metabolism by N-demethylation, likely would potentiate or extend the action of (−)**1** or (−)**2**, respectively. Indeed, whereas (−)**9** is equipotent to (−)**1** versus AChE and BChE, with a slightly more modest AChE selectivity of 41-fold compared to 54-fold for (−)-phenserine [(−)**1**], (−)-*N*¹,*N*⁸-bisnorphysostigmine [(−)**10**] proved to be more potent than (−)-physostigmine [(−)**2**]. Indeed, it was 6-fold more active in inhibiting BChE and 3-fold more potent against AChE, providing it a selectivity for BChE activity of some 5-fold, versus 2-fold for (−)-physostigmine [(−)**2**]. Thus, similar to (−)-N¹ and (−)-N⁸ analogues, also potential metabolites of (−)**1** and (−)**2**, N-demethylation at both the N¹ and N⁸ positions, separately and simultaneously, results in highly potent anticholinesterases with a modest altered selectivity away from AChE (Table 2).

Table 2. Relative Selectivity of Compounds versus Human Erythrocyte AChE and Plasma BChE

compound	AChE selectivity	BChE selectivity
(−)**1**, (−)-phenserine	54-fold	
(−)**2**, (−)-physostigmine		2-fold
5, (−)-*N*¹-norphenserine	44-fold	
6, (−)-*N*¹-norphysostigmine		11-fold
21, (−)-*N*¹-benzylnorphysostigmine		32-fold
7, (−)-*N*⁸-norphenserine	13-fold	
8, (−)-*N*⁸-norphysostigmine		8-fold
22, (−)-*N*⁸-benzylnorphysostigmine		7-fold
(−)**9**, (−)-*N*¹,*N*⁸-bisnorphenserine	41-fold	
(−)**10**, (−)-*N*¹,*N*⁸-bisnorphysostigmine		5-fold
(−)**19**, (−)-*N*¹,*N*⁸-bisbenzylnorphysostigmine		134-fold

We have previously demonstrated that replacement of the methyl carbamate moiety of (−)-physostigmine [(−)-**2**] with a substituted or unsubstituted phenylcarbamate,[1,2,3,19] or with a longer alky carbamate, such as with a butyl, heptyl, or octyl carbamate, incrementally extends the duration of potent anticholinesterase action of the resulting compound.[1,20,21] However, their longer duration of action likely results as a consequence of their higher rates of carbamylation of the cholinesterase enzyme and lower rates of decarbamylation,[1,22,23] compared to (−)-physostigmine [(−)**2**], rather than as a consequence of their in vivo disappearance rates.[1] For example, as discussed, (−)-physostigmine [(−)**2**] has a duration of action of some 30−90 min and an in vivo disappearance half-life of 15 min.[24] The heptyl carbamate (Eptylstigmine, Mediolanum, Italy) in phase II clinical trials for the treatment of Alzheimer's disease, has a duration of action of in excess of 8 h and an in vivo disappearance half-life of 5 h.[20,25] In contrast, (−)-phenserine [(−)**1**], as discussed, likewise has a duration of cholinesterase inhibition of in excess of 8 h, but an in vivo disappearance half-life of only 12 min.[1,3] It is therefore probable that in the event that (−)-*N*¹,*N*⁸-bisnorphenserine [(−)**9**] and (−)-*N*¹,*N*⁸-bisnorphysostigmine [(−)**10**] are generated as metabolic products of (−)-phenserine [(−)**1**] and (−)-physostigmine [(−)**2**] administration in clinical studies, such active metabolites would have a greater affect to potentiate the action of (−)-**2** as its rate of enzyme decarbamylation is far more rapid than for (−)-**1**. Although (−)-eseroline (**3**) has been reported to be pharmacologically active in antinociceptive assays,[13] we reconfirm the lack of anticholinesterase activity of this known metabolite of (−)-physostigmine [(−)**2**] and of (−)-rubreserine (**4**), easily obtained from **3** on air oxidation and synthesized via a novel route.

We have previously demonstrated that inhibition of both AChE and BChE by (−)**1**, (−)**2**, and other *N*¹-methyl-substituted carbamates is highly enantioselective, resting entirely on the 3aS enantiomers (Table 1).[1,9] This, however, does not hold for the *N*¹-nor series[9] or for ring C heterocongeners,[1,2,25,26] but does hold for the *N*⁸-nor series.[8] Similar to the *N*¹-nor series,[9] (+)-*N*(1,8)-bisnorphenserine [(+)**9**] and (+)-*N*(1,8)-bisnorphysostigmine [(+)**10**] retained anticholinesterase action, which was some 10-fold reduced compared to their 3a*S* enantiomers. Nevertheless, their activity is of the same magnitude as several anticholinesterases presently in

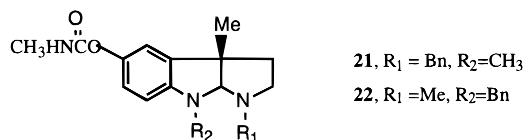


Figure 2.

clinical use: tacrine (Cognex, Warner-Lambert, MI) IC_{50} AChE 190 nM, BChE 47 nM, and galanthamine (Johnson & Johnson, NJ) IC_{50} AChE 800 nM, BChE 7300 nM.

We predicted from our previous studies involving modification of the N^1- and N^8-positions of natural physostigmine [(−)**2**][1,2,8,27] that the intermediate (−)-*N*(1,8)-bisbenzylnorphysostigmine [(−)**19**] would possess anticholinesterase action with a selectivity for BChE, as **21** and **22** (Figure 2) proved to be BChE inhibitors equipotent to (−)**2** but with a 32-fold and 7-fold selectivity (Tables 1 and 2). We were, however, surprised by the remarkable potency and 134-fold BChE selectivity of (−)**19**. Our previous studies have demonstrated that the incorporation of a lipophilic group, such as a phenylethyl group,[28] in the N^1-position imparts a dramatic selectivity that favors BChE inhibitory action. The present studies clearly indicate that BChE inhibitory potency and selectivity can be increased by simultaneous lipophilic substitution in the N^1- and N^8-positions; comparing the activity of (−)**19** to **21** and **22**. Additionally, they further suggest that BChE selectivity and, likely, duration of inhibition could be yet further increased by changing the methyl carbamate of (−)**19** to a 4′-substituted phenyl carbamate. We have previously demonstrated that such carbamates provide an extended duration of action, similar to that of (−)**1**, and a BChE selectivity.[1,2,3,27]

In contrast, (−)-*N*(1,8)-bisbenzylnorphenserine [(−)**18**], which incorporates components to both favor BChE selectivity, in its lipophilic *N*(1,8)-bisbenzyl groups, and favor AChE selectivity, in its unsubstituted phenyl carbamate, possessed a minimal potency compared with (−)**1** and (−)**19** against both AChE and BChE. Likewise, the enantiomers (+)**18** and (+)**19** dramatically lacked activity, clearly indicating that whereas reduced steric factors associated with N^1-methyl substitution in the nor compounds,[9] physovenols,[25] and thiaphysovenols[26] allow potent activity in the antipodal isomers, bulky substitutions do not.

A significant body of work has been published that describes the structures of AChE and BChE and their catalysis of acetylcholine (ACh) and related substrates.[29,30,31] Both proteins have been fully sequenced, and although they are products of different genes on different chromosomes they share a remarkable degree of amino acid homology, particularly around the site involved in ACh hydrolysis.[32] As we have described previously,[8] it is clearly possible to design and synthesize analogues with specific substitutions that exploit critical differences around the active catalytic site that differentiate AChE from BChE to provide cholinesterase subtype inhibitory action for the resulting compounds.

In summary, we have synthesized and characterized the *N*(1,8)-bisnor analogues of both natural, (−)-3a*S*, and unnatural, (+)−3a*R*, physostigmine and phenserine. The 3a*S* enantiomers, potential metabolites of (−)**1** and (−)**2**, proved to be highly active anticholinesterases, as did the intermediate (−)-*N*(1,8)-bisbenzylnorphysostigmine [(−)**19**], which demonstrated a surprising potency and selectivity for BChE. Whereas long-acting and nontoxic selective AChE inhibitors hold potential to augment memory processing and reduce β-amyloid synthesis in the treatment of Alzheimer's disease and age-associated memory deficits,[24,33] recent studies have implicated BChE in the seeding and formation of β-amyloid plaques and its toxicity in Alzheimer's disease,[34,35] providing a potential clinical use for interesting selective nontoxic BChE inhibitors.

Experimental Section

Chemistry. Melting points (uncorrected) were measured with a Fisher-Johns apparatus; ^{1}H NMR were recorded on a Bruker (Bellevica, MA) AC-300 spectrometer; MS (*m/z*) were recorded on a Hewlett-Packard 5890 GC−MS (EI) and on a Finnigan-1015D mass spectrometer. Optical rotations were measured by JASCO, model DIP-370 (Japan, Spectroscopic Co., Ltd.); elemental analyses were performed by Atlantic Microlab, Inc. Unless otherwise indicated, all separations were carried out using flash column chromatography (silica gel 60, 230−400 mesh) with the described solvents. All reactions involving nonaqueous solutions were performed under an inert atmosphere.

Rubreserine (4). The fumarate salt of eserine (**3**)[10] (300 mg, 0.90 mmol) was dissolved in H_2O (10 mL). The solution was mixed with sodium carbonate (95 mg, 0.90 mmol) and $CHCl_3$ (20 mL). The reaction mixture was stirred under constant aeration, by filtering air flow through a small pipet for 20 h, and $CHCl_3$ was occasionally added to maintain a constant volume of $CHCl_3$. The separated $CHCl_3$ solution was washed with brine and dried over $NaSO_4$. After evaporation of solvent, the residue was chromatographed (CH_2Cl_2/MeOH = 20/1) and the visual red fraction was collected. Evaporation of solvent gave a residue which was crystallized from ether to give **4** (44 mg, 21.0%) as red crystals: mp 150−151 °C (lit.[9] mp 152 °C; lit.[12] mp 98−99 °C); $[\alpha]^{20}_D$ −256.3° (c = 0.2, $CHCl_3$); the ^{1}H NMR is identical with that reported in the literature (ref 14).

Methiodide of (−)-(3a*S*)-8-Benzyl-1,3a-dimethyl-1,2,3,3a,8,8a- hexahydro-5-methoxypyrrolo[2,3-*b*]indole [(−)13]. Compound [(−)**12**][8] (180 mg, 0.58 mmol) was dissolved in ether (5 mL), and then CH_3I (0.5 mL) was added. The methiodide salt gradually formed and precipitated from the solution. The mixture was left overnight and filtered to give crystalline (−)**13** (240 mg, 92.0%): mp 140 °C; $[\alpha]^{20}_D$ −109.0° (c = 0.5, EtOH). Anal. ($C_{19}H_{21}N_3O_2$) C, H, N.

(−)-(3*s*)-1-Benzyl-3-methyl-3-[2′-(dimethylamino)ethyl]-5-methoxy-2,3-dihydro-1*H*-indol-2-ol [(−)14]. To a solution of (−)**13** (240 mg, 0.53 mmol) in H_2O (5 mL) and EtOH (1 mL) were added 50% NaOH (0.5 mL) and ether (5 mL). After the mixture was stirred at room temperature for 5 h, the ether layer was separated and the aqueous layer was washed with ether (2 × 5 mL). The combined ether layer then was washed with brine, dried over Na_2SO_4, and evaporated to give (−)**14** (182 mg, 100%) as a gum: $[\alpha]^{20}_D$ −21.9° (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40−7.15 (m, 5 H, Ph-H), 6.50 (m, 2H, C4-H, C6-H), 6.25 (d, J = 8.0 Hz, 1H, C7-H), 4.60 (s, 1H, C2-H), 4.45 and 4.15 (AB, J = 16.6 Hz, 2H, $PhCH_2N$), 3.60 (s, 3H, OCH_3), 2.15 (s, 6H, N-Me_2), 2.00−2.10 (m, 2H, CH_2N), 1.90−1.40 (m, 2H, C3-CH_2), 1.25 (s, 3H, C3-CH_3); EI-MS *m/z* (relative intensity) 322 (M^+ − H_2O, 51), 307 (64), 264 (4.5), 91 (100). Anal. ($C_{33}H_{33}N_3O_3$·$0.3H_2O$) C, H, N.

Methiodide of (−)-(3*s*)-1-Benzyl-3-methyl-3-[2′-(dimethylamino)ethyl]-5-methoxy-2,3-dihydro-1*H*-indol-2-ol [(−)15]. Compound (−)**14** (180 mg, 0.53 mmol) was dissolved in ether (5 mL), and then CH_3I (0.5 mL) was added. The methiodide salt gradually formed and precipitated from solution. The mixture was left overnight and filtered to obtain crystalline (−)**15** (243 mg, 95.0%): mp 172−173 °C; $[\alpha]^{20}_D$ −391.0° (c = 0.2, EtOH). Anal. ($C_{19}H_{21}N_3O_2$·$0.2H_2O$) C, H, N.

(−)-(3aS)-1,8-Dibenzyl-3a-methyl-1,2,3,3a,8,8a-hexahydro-5-methoxypyrrolo[2,3-*b*]indole [(−)16]. Compound (−)**15** (230 mg, 0.48 mmol) was dissolved in CH_3CN (5 mL), and benzylamine (103 mg, 0.96 mmol) was added. The reaction mixture was stirred and refluxed for 6 h. Evaporation of solvent gave a residue which was partitioned between H_2O and Et_2O. The ether layer was evaporated and chromatographed (CH_2Cl_2/MeOH = 20/1) to give (−)**16** (157 mg, 85.0%) as a gum: $[\alpha]^{20}_D$ −72.3° (c = 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.30−7.20 (m, 10 H, Ar-H), 6.65 (d, J = 2.5 Hz, 1H, C4-H), 6.55 (d, J = 8.5 Hz, 1H, C6-H), 6.25 (d, 1H, J = 8.5 Hz, C7-H), 4.45 (s, 1H, C8a-H), 4.40 and 4.20 (AB, J = 16.6 Hz, 2H, $PhCH_2$-N8), 3.80 (m, 2H, $PhCH_2$-N1), 3.60 (s, 3H, OCH_3), 3.70 (s, 3H, CH_3O), 2.75 (t, J = 7 Hz, 2H, C2-H_2), 1.95 (t, J = 7 Hz, 2H, C3-H_2), 1.40 (s, 3H, C3a-CH_3); EI-MS m/z (relative intensity) 294 (MH^+ − benzyl, 48), 279 (8.9), 188 (23), 173 (58), 91 (100). Anal. ($C_{26}H_{28}N_2O$) C, H, N.

(−)-(3a*S*)-1,8-Dibenzyl-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol [(−)17]. Compound (−)**16** (140 mg, 0.36 mmol) was dissolved in CH_2Cl_2 (2 mL), and boron tribromide (456 mg, 1.82 mmol) was added with stirring. The reaction mixture was stirred at room temperature for 1 h, MeOH (1 mL) was added dropwise, and the mixture was stirred for 10 min. After evaporation of solvent in vacuo, the residue was dissolved in H_2O (2 mL), basified with $NaHCO_3$, and extracted with Et_2O (3 × 5 mL). The ether extract was dried over Na_2SO_4 overnight, evaporated, and chromatographed (CH_2Cl_2/MeOH = 10/1) to give (−)**17** (120 mg, 90.0%) as a foam: $[\alpha]^{20}_D$ −76.5° (c = 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.40−7.00 (m, 10 H, Ar-H), 6.70−6.00 (m, 3H, C4-H, C6-H, C7-H), 4.40−3.90 (m, 2H, $PhCH_2$-N8), 3.80−3.60 (m, 2H, $PhCH_2$-N1), 2.65 (m, 2H, C2-H_2), 1.89 (m, 2H, C3-CH_2), 1.30 (s, 3H, C3a-CH_3); EI-MS m/z (relative intensity) 294 (MH^+ − phenyl, 15), 380 (34), 265 (9.3), 250 (2.8), 91 (100). Anal. ($C_{33}H_{33}N_3O_3$·0.9 H_2O) C, H, N.

(−)-(3a*S*)-1,8-Dibenzyl-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrol[2,3-*b*]indol-5-yl *N*-Phenylcarbamate [(−)18]. Compound (−)**17** (40.7 mg, 0.11 mmol) was dissolved in anhydrous ether (2 mL), and a piece of Na metal (approximately 1 mg) was added. The mixture was stirred at room temperature for 1 min, phenyl isocyanate (13.1 mg, 0.11 mmol) was added, and the mixture was stirred for 5 min. Evaporation of solvent gave a crude product which was directly chromatographed to give (−)**18** (48 mg, 89.1%) as a gum: $[\alpha]^{20}_D$ −59.1° (c = 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.40−6.90 (m, 15H, Ar-H), 6.75 (d, J = 2.5 Hz, 1H, C4-H), 6.73 (d, J = 8.5 Hz, 1H, C6-H), 6.17 (d, J = 8.5 Hz, 1H, C7-H), 4.45 (s, 1H, C8a-H), 4.30−4.20 (AB, J = 16.6 Hz, 2H, $PhCH_2$-N8), 3.72 (s, 2H, $PhCH_2$-N1), 2.70 (m, 2H, C2-H_2), 1.90 (m, 2H, C3-H_2), 1.38 (s, 3H, C3a-CH_3); EI-MS m/z (relative intensity) 370 (MH^+ − PhNHCO, 31), 354 (1.0), 279 (8.0), 264 (2.0), 91 (100). Anal. ($C_{27}H_{29}N_3O_3$) C, H, N.

(−)-(3a*S*)-1,8-Dibenzyl-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrol[2,3-*b*]indol-5-yl *N*-Methylcarbamate [(−)19]. Compound (−)**16** (47.5 mg, 0.13 mmol) was dissolved in anhydrous ether (2 mL), and a piece of Na metal (approximately 1 mg) was added. The mixture was stirred at room temperature for 1 min, methyl isocyanate (14.6 mg, 0.26 mmol) was added, and the mixture stirred for 10 min. Evaporation of solvent gave a crude product which was directly chromatographed to give (−)**19** (50.0 mg, 90.0%) as a gum: $[\alpha]^{20}_D$ −58.2° (c = 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.40−7.20 (m, 10H, Ar-H), 6.75 (d, J = 2.2 Hz, 1H, C4-H), 6.64 (d, J = 8.5 Hz, 1H, C6-H), 6.24 (d, J = 8.5 Hz, 1H, C7-H), 4.65 (s, 1H, N-H), 4.40 (s, 1H, C8a-H), 4.35−4.20 (AB, J = 16.6 Hz, 2H, $PhCH_2$-N8), 3.70 (s, 2H, $PhCH_2$-N1), 2.80 (d. J = 3.9 Hz, 3H, $NHCH_3$), 2.70 (m, 2H, C2-H_2), 1.90 (m, 2H, C3-H_2), 1.35 (s, 3H, C3a-CH_3); EI-MS m/z (relative intensity) 370 (MH^+ − CH_3NHCO, 33), 354 (1.5), 279 (8.5), 264 (3.0), 91 (100). Anal. ($C_{27}H_{29}N_3O_3$) C, H, N.

(−)-(3a*S*)-3a-Methyl-1,2,3,3a,8,8a-hexahydropyrrol[2,3-*b*]indol-5-yl *N*-Phenylcarbamate [(−)9]. Compound (−)**18** (29.3 mg, 0.06 mmol) was dissolved in 2-propanol (1 mL), and $Pd(OH)_2$/C (5 mg) was added. The reaction mixture was stirred under hydrogen at atmospheric pressure and room temperature for 60 h, and then the catalyst was filtered. Evaporation of solvent gave a residue which was chromatographed (CH_2Cl_2/MeOH = 10/1) to obtain the most polar component and this was crystallized from petroleum ether to provide compound (−)**9** (12 mg, 64.7%) as crystals: mp 153−155 °C; $[\alpha]^{20}_D$ −73.7° (c = 0.2, EtOH); ^1H NMR ($CDCl_3$/CD_3OD = 8/2) δ 7.50−7.00 (m, 5H, Ph-H), 6.90−6.50 (m, 3H, Ar−H), 4.80 (s, 1H, C8a-H), 3.10−2.75 (m, 2H, C2-H_2), 2.15−1.80 (m, 2H, C3-H_2), 1.45 (s, 3H, C3a-CH_3); EI-MS m/z (relative intensity) 232 (M^+ − phenyl, 67), 217 (38), 190 (11), 174 (100), 160 (73); CI-MS (NH_3) m/z 310 (MH^+). Anal. ($C_{27}H_{29}N_3O_3$) C, H, N.

(−)-(3a*S*)-3a-Methyl-1,2,3,3a,8,8a-hexahydropyrrol[2,3-*b*]indol-5-yl *N*-Methylcarbamate [(−)10]. Compound (−)**19** (34.0 mg, 0.08 mmol) was dissolved in 2-propanol (1 mL), and $Pd(OH)_2$/C (5 mg) was added. The reaction mixture was stirred under hydrogen at atmospheric pressure and room temperature for 48 h, and then the catalyst was filtered. Evaporation of solvent gave a residue which was chromatographed (CH_2Cl_2/MeOH = 10/1) to obtain the most polar component, compound (−)**10** (12 mg, 60.7%), as a gum: $[\alpha]^{20}_D$ −114.3° (c = 0.2, EtOH); ^1H NMR ($CDCl_3$/CD_3OD = 8/2) δ 6.90−6.55 (m, 3H, Ar-H), 5.18 (s, 1H, C8a-H), 2.95−2.70 (m, 2H, C2-H_2), 2.85 (d, J = 4.0 Hz, 3H, NCH_3), 2.10−1.80 (m, 2H, C3-H_2), 1.50 (s, 3H, C3a-CH_3); EI-MS m/z (relative intensity) 232 (M^+ − methyl, 65), 217 (38), 189 (8.3), 174 (100); CI-MS (NH_3) m/z 248 (MH^+). Anal. ($C_{27}H_{29}N_3O_3$) C, H, N.

(+)**9** and (+)**10** were synthesized from (+)**12** according to the same procedures used for making their antipodes. The ^1H NMR and MS of (+)**9** and (+)**10** and the intermediates (+)**14**, (+)**16**, (+)**17**, (+)**18**, and (+)**19** were identical to those of their respective antipodes. The optical rotation values of the plus series compounds were equal and opposite to those of their minus enantiomers.

(−)-*N*[8]-Norphenserine (7) Made from Compound (−)18. Compound (−)**18** (29.3 mg, 0.06 mmol) was dissolved in MeOH (1 mL), and $Pd(OH)_2$/C (5 mg) was added. The reaction mixture was stirred under hydrogen at atmospheric pressure and room temperature for 60 h, and then the catalyst was filtered. Evaporation of solvent gave a residue which was chromatographed (CH_2Cl_2/MeOH = 10/1) to obtain the most polar component and this was crysyallized from petroleum ether to provide compound **7** (13.6 mg, 70.0%) as crystals: The mp, optical rotation, ^1H NMR, and MS were identical with those reported in the literature.[8]

(−)-(3*s*)-3-Methyl-3-[2′-(amino)ethyl]-2,3-dihydro-1*H*-indol-5-yl *N*-Phenylcarbamate (20). Compound (−)**18** (58.6 mg, 0.12 mmol) was dissolved in a mixture of MeOH (2 mL), H_2O (2 mL), and TFA (1 mL), and $Pd(OH)_2$/C (10 mg) was added. The reaction mixture then was stirred under hydrogen at atmospheric pressure and room temperature for 1.5 h, and thereafter the catalyst was filtered. The filtrate was evaporated in vacuo to give a residue which was dissolved in H_2O, basified by Na_2CO_3, extracted with CH_2Cl_2, and then dried over Na_2SO_4. After removal of solvent, the residue was chromatograghed on preparative TLC (silica gel) (CH_2Cl_2/MeOH = 10/1) to give the most polar and also major component **20** (7.4 mg, 20%) as a syrup: $[\alpha]^{20}_D$ −63.2° (c = 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$/CD_3OD = 8/2) δ 7.40−7.00 (m, 5H, Ph-H), 6.85−6.70 (m, 2H, C4-H, C6-H), 6.50 (d, J = 8.5 Hz, 1H, C7-H), 3.75 and 3.65 (AB, J = 12 Hz, 2H, C2-H_2), 3.40−3.20 (AB, J = 12 Hz, CH_2N), 1,65 (m, 2H, C3-CH_2), 1.30 (s, 3H, C3-CH_3); CI-MS (NH_3) m/z 312 (MH^+). Anal. ($C_{13}H_{16}N_2O_3$) C, H, N.

Quantitation of Anticholinesterase Activity. The action of compounds **3**, **4**, and (−)- and (+)**9**, **10**, **18**, and **19** to inhibit the ability of freshly prepared human AChE and BChE, derived from plasma and whole red blood cells, respectively, to enzymatically degrade the specific substrates acetyl(β-methyl)thiocholine and *s*-butyrylthiocholine (0.5 mmol/L) (Sigma Chemical Co., St. Louis, MO) were quantified. Compounds were dissolved in Tween 80/EtOH, 3:1 (v:v; <150 μL total volume), and were diluted in 0.1 M Na_3PO_4 buffer (pH 8.0) in half-log concentrations to provide a final range spanning 0.3

nM to 30 mM. Tween 80/EtOH was diluted in excess of 1 in 5000 and had no inhibitory action on either AChE or BChE.

Freshly collected blood was centrifuged (10000*g*, 10 min, 4 °C), and plasma was removed and diluted 1:125 with 0.1 M Na_3PO_4 buffer (pH 7.4). Whole red blood cells were washed five times in isotonic saline, lysed in 9 volumes of 0.1 M Na_3PO_4 buffer (pH 7.4) containing 0.5% Triton-X (Sigma), and then diluted with an additional 19 volumes of buffer to a final dilution of 1:200. Analysis of anticholinesterase activity, utilizing a 25 μL sample of each enzyme preparation, was undertaken at their optimal working pH, 8.0, in 0.1 M Na_3PO_4 buffer (0.75 mL total volume). Compounds were preincubated with enzymes (30 min, room temperature) and then were incubated with their respective substrates and 5,5′-dithiobis-2-nitrobenzoic acid (25 min, 37 °C). Production of a yellow thionitrobenzoate anion was measured by spectrophotometer at 412 nm λ. To correct for nonspecific substrate hydrolysis, aliquots were co-incubated under conditions of absolute enzyme inhibition (by the addition of 1×10^{-5} M physostigmine [(−)-**2**]), and the associated alteration in absorbance subtracted from that observed through the concentration range of each test compound. Each was analyzed on four separate occasions and assayed alongside (−)-phenserine [(−)-**1**] and (−)-physostigmine [(−)-**2**] as control and external standards whose activity we have previously reported.[1,2,7,8,18,21]

The enzyme activity at each concentration of test compound was expressed as a percent of activity in the absence of compound, transformed into a logit format (logit = %activity/(100 − %activity)) and then plotted as a function of its log concentration. Inhibitory activity was calculated as an IC_{50}, defined as the concentration of compound (nM) required to inhibit 50% of enzymatic activity, and determined from a correlation between log concentration and logit activity.

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