Methyl Analogues of the Experimental Alzheimer Drug Phenserine: Synthesis and Structure/Activity Relationships for Acetyl- and Butyrylcholinesterase Inhibitory Action

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With the goal of developing potential Alzheimer's pharmacotherapeutics, we have synthesized a series of novel analogues of the potent anticholinesterases phenserine (**2**) and physostigmine (**1**). These derivatives contain methyl (**3**, **4**, **6**), dimethyl (**5**, **7**, **8**, **10**, **11**) and trimethyl (**14**) substituents in each position of the phenyl group of the phenylcarbamoyl moieties, and with *N*-methyl and 6-methyl substituents (**12**, **13**, **31**, **33**). We also quantified the inhibitory action of these compounds against human acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). An analysis of the structure/anticholinesterase activity relationship of the described compounds, together with molecular modeling, confirmed the catalytic triad mechanism of the binding of this class of carabamate analogues within AChE and BChE and defined structural requirements for their differential inhibition.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder that leads to dementia and accounts for up to two-thirds of all such cases. It is, additionally, the fourth leading cause of death in the United States of America and afflicts some 4 million adults. Although several neurotransmitter systems are affected in AD, in light of the early and dramatic cholinergic cell loss in the AD brain,[1−3] agents that augment the cholinergic system have received the greatest attention with regard to drug development and intervention.[4,5] In this regard, three strategies have been investigated to increase cholinergic neurotransmission. These have involved the use of (i) precursors, such as choline, to augment neurotransmitter synthesis at presynaptic terminals,[6] (ii) direct agonists to stimulate postsynaptic muscarinic and/or nicotinic cholinergic receptors,[7,8] and (iii) anticholinesterases (anti-ChE) to inhibit the enzymes that metabolize naturally released acetylcholine (ACh) and thereby amplify its postsynaptic action.[4,5,9,10] The development of anti-ChEs has thus far proved to be the most productive approach, with four drugs currently approved for clinical use: specifically, tacrine (Cognex, originally Pfizer/Warner-Lambert, MI, currently: Horizon Pharm, GE), donepezil (Aricept, Pfizer, NY, Eisai, Japan), rivastigmine (Exelon, Novartis, Switzerland), and galantamine (Reminyl, Janssen, NJ).

In large part, the successful development of current anti-ChEs has benefited from the extensive information presently available that defines the relationship between the chemical structure of compounds and their ChE inhibitory action.[11,12] During recent years, detailed X-ray diffraction studies of the two ChE enzymes, acetyl- and butyrylcholinesterase (AChE: EC 3.1.1.7 and BChE: EC 3.1.1.8; respectively), as well as of inhibitors, together with extensive biochemical research has elucidated the three-dimensional fit of different types of inhibitors within AChE and BChE.[13−21] This has provided us the opportunity to study the structure/activity relationship of anti-ChEs in a new manner to develop even more selective and potent inhibitors.

Phenserine (**2**), the unsubstituted phenylcarbamate analogue of the natural product and classical anti-ChE, physostigmine (**1**) (Figure 1),[9,22−27] is a potent and selective inhibitor of AChE (70-fold versus BChE action). Phenserine (**2**) proved to be well tolerated in phase I clinical trials for the treatment of Alzheimer's disease and is currently in phase II efficacy trials. We have previously demonstrated[9] that substitution into the phenyl ring of its carbamate modifies the selectivity of the resulting compounds, with 2′-substitution providing increased AChE selectivity and 4′-substitution negating this to provide moderate BChE selectivity. There are, however, only a few reports in the literature in which a systematic approach has been taken to define the structure/activity relationship of each important substitution on a compound that bears the potential to inhibit two structurally similar but separate critical enzymes, such as AChE and BChE. We, herein, report such a study.

Results

Chemistry. The commercially unavailable isocyanates **23** and **25** were prepared from anilines **19** and **20**

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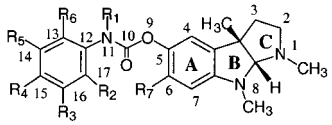


2, Phenserine, R_1- R_7 = H.

3, Tolserine, $R_2 = CH_3$, R_1, R_3 - R_7 = H.

4, 4'-Methylphenserine, $R_4 = CH_3$, R_1- R_3, R_5 - R_7 = H.

5, 2', 4'-Dimethylphenserine, R_2, $R_4 = CH_3$, R_1, R_3, R_5 - R_7 = H.

6, 3'-Methylphenserine, $R_3 = CH_3$, R_1, R_2, R_4 - R_7 = H.

7, 2',3' - Dimethylphenserine, R_2, $R_3 = CH_3$, R_1, R_4 - R_7 = H.

8, 3',4'-Dimethylphenserine, R_3, $R_4 = CH_3$, R_1, R_2, R_5 - R_7 = H.

9, 3',5'-Dimethylphenserine, R_3, $R_5 = CH_3$, R_1, R_2, R_4, R_6, R_7 = H.

10, 2',5'-Dimethylphenserine, R_2, $R_5 = CH_3$, R_1, R_3, R_4, R_6, R_7 = H.

11, 2',6'-Dimethylphenserine, R_2, $R_6 = CH_3$, R_1, R_3 - R_5, R_7 = H.

12 *N*-Methylphenserine, $R_1 = CH_3$, R_2 - R_7 = H.

13, 6-Methylphenserine, $R_7 = CH_3$, R_1 - R_6 = H.

14, 2',4',6'-Trimethylphenserine, R_2, R_4, $R_6 = CH_3$, R_1, R_3, R_5, R_7 = H.

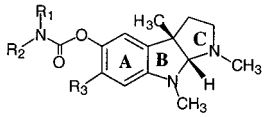
15, 2'-Ethylphenserine, $R_2 = CH_3CH_2$, R_1, R_2 - R_7 = H.

16, 2',6'-Diethylphenserine, R_2, $R_6 = CH_3CH_2$, R_1, R_3 - R_5, R_7 = H.

17, 2'- Isopropylphenserine, $R_2 = (CH_3)_2CH$, R_1, R_3 - R_7 = H.

18, 4'-Isopropylphenserine, $R_4 = (CH_3)_2CH$, R_1-R_3, R_5 - R_7 = H.

32, 6-Dimethylaminomethylenphenserine, $R_7 = (CH_3)_2NCH_2$, R_1 - R_6 = H.



1, Physostigmine, R_1, R_3 = H, R_2=CH_3

31, 6-Methylphysostigmine, R_1 = H, R_2 , $R_3 = CH_3$.

33, N-Methylphysostigmine, $R_1 = CH_3$, R_2, R_3 = H.

Figure 1.

with phosgene by a general method, shown in Scheme 1.[28] In this method, it proved important to use the hydrochlorides of the anilines as the starting materials and to undertake the reaction in a sealed vessel. Consequently, highly volatile phosgene could not escape from the reaction mixture, even at high temperature.

The aniline hydrochlorides proved to be poorly soluble in the reaction solvent, toluene. Hence, after the reaction, unreacted material was removed by filtration, and the remaining phosgene, together with the solvent, toluene, was readily removed by evaporation to give the isocyanates as brownish oils. This procedure minimized the handling of these irritant and moisture sensitive materials and, additionally, avoided chromatography or distillation.

Eseroline (**27**) then was reacted with isocyanates (**21**−**26**) in ether to afford phenylcarbamates (**6**−**11**) (Figure 1) according to a known procedure[23] (Scheme 2). Usually, after the completion of the reaction, evaporation of solvent provides a residue that invariably contains a small quantity of unreacted isocyanate. This then reacts with the carbamate reaction-product to form *N*-phenyl-*N*-phenylallophanyl eseroline.[29] Thereby the yield is decreased, and purification is required. The purification is complex as the R_f values of the allophanyl and eseroline carbamate are similar. The original reaction procedure was therefore improved by adding H_2O to destroy any trace of isocyanate, and the ether solution was washed with dilute NaOH to remove unreacted eseroline to give a highly pure product without the use of chromatography, a notable improvement on the synthetic scheme.

Chloride (**28**) was reacted with eseroline (**27**) at 90 °C for 56 h to give the desired product, **12**, in 25% yield (Scheme 3). The Mannich reaction of eseroline (**27**) with dimethylamine hydrochloride and formaldehyde gave 6-dimethylaminomethylene-eseroline (**29**) in a yield of 89%. Qualitative assessment of this product demonstrated that no substitution in the 4′-position occurred. Compound **29** was converted to 6-methyl eseroline (**31**) quantitatively, by catalytic hydrogenation. Compound **30** then was reacted with methylisocyanate and phenylisocyanate to give 6-methylphysostigmine (**31**) and 6-methylphenserine (**13**), respectively. Compound **29** also was reacted with phenylisocyanate to afford 6-dimethylaminomethylene-phenserine (**32**). Compound **32** then was deaminated to give the desired 6-methylphenserine (**13**) by catalytic hydrogenation in a low yield (Scheme 4). We have previously reported our procedures for synthesizing known compounds **14**−**18**[23] and **33**.[29]

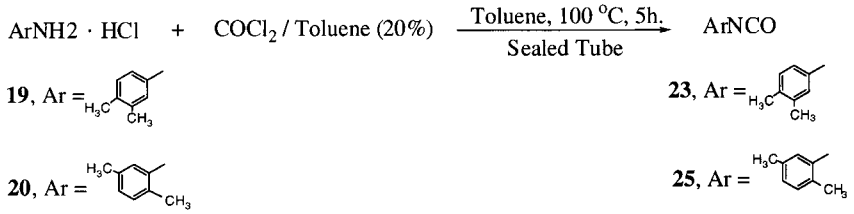
Phenserine (**2**) and the fumarate of 2′,4′,6′-trimethylphenserine (**14**) were subjected to X-ray analysis to define their configuration and conformation in their crystalline state (Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C.). Figure 2 illustrates their absolute configuration. Aligning the tricyclic ring structures of both compounds to the same plane demonstrates that substitution onto the phenyl ring of the carbamate function causes its rotation in relation to the rest of the molecule; torsion angles are shown.

Biological Evaluation. Table 1 illustrates the biological activity of compounds **3**−**18** and **31**−**33** against freshly prepared human AChE and BChE, in comparison to physostigmine (**1**) and phenserine (**2**), whose measured values are in accord with our previous reports.[9,22−27]

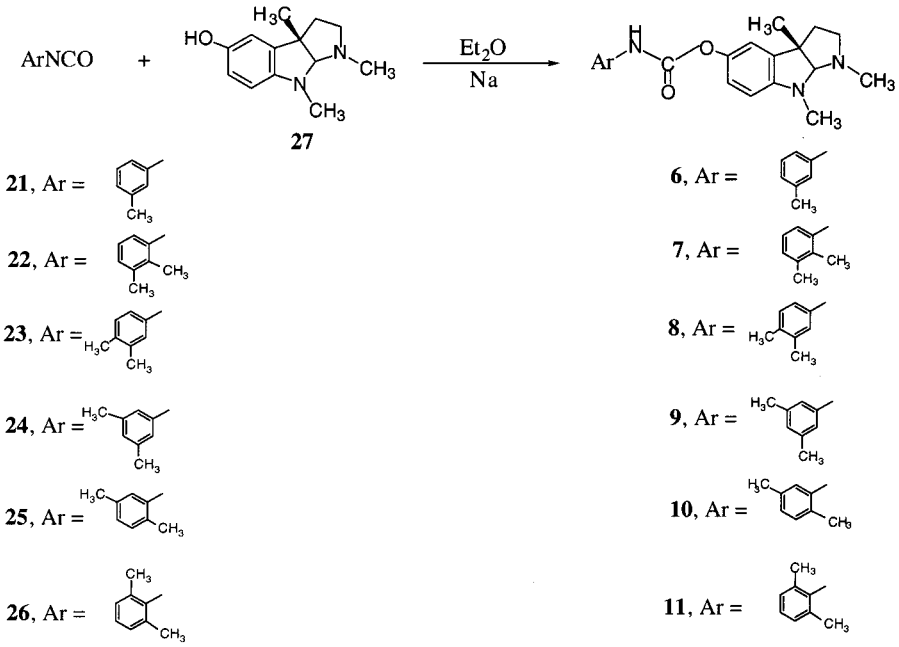
(a) Phenylcarbamate modification, monosubstitution: In comparison to unsubstituted phenserine (**2**), which has a selectivity for AChE inhibition of 70-fold, methyl substitution in the 2′ position, **3**, provided a 2-fold improvement in AChE potency and a marginal decline in BChE activity to afford an AChE selectivity of 195-fold. The 2′-ethyl substitution, **15**, rendered the compound less BChE active still, with an AChE selectivity of 290-fold. In contrast, methyl substitution in the 3′ position, **6**, maintained AChE potency, compared to phenserine (**2**), and increased BChE potency to afford a 12-fold AChE selective compound. The 4′-methyl substitution, **4**, reduced AChE and increased BChE potency, compared to **2**, to afford a compound that was unselective. Larger substitution in the 4′ position, such as with an isopropyl, **18**, further reduced AChE and increased BChE potency to render a 15-fold BChE selective agent. In contrast, similar substitution in the 2′ position, **17**, provided a 43-fold AChE selective compound.

(b) Phenylcarbamate modification, disubstitution: Compounds with dimethyl substituents in the 2′,3′ (**7**), 2′,4′ (**5**), and 2′,5′ positions (**10**) possessed a similar AChE inhibitory activity to that of the lead compounds physostigmine (**1**) and unsubstituted phenserine (**2**). All these compounds, similar to **2**, were AChE selective. However, this was reduced for **7** and **10** as a consequence of improved BChE potency. Dimethyl substitution in the 3′,4′-position, **8**, likewise maintained AChE activity but provided significant potency against BChE,

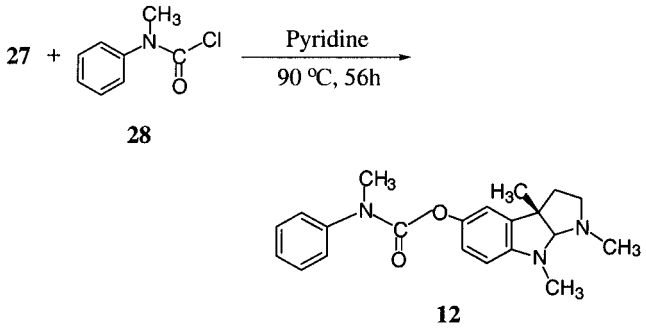
Scheme 1



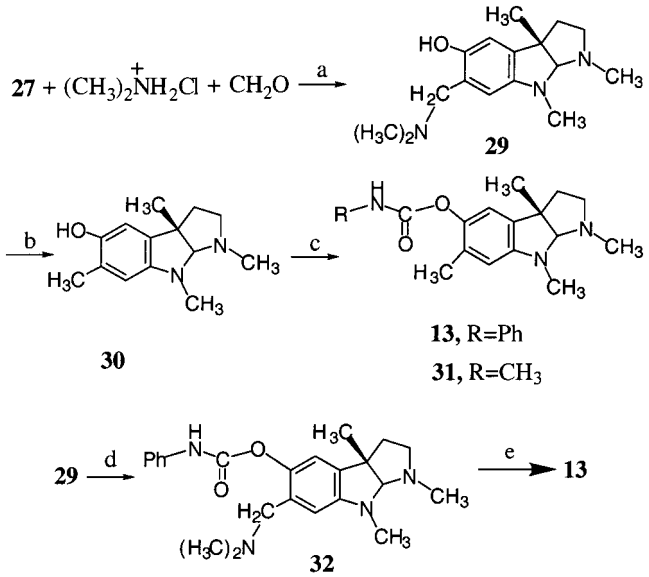
Scheme 2



Scheme 3



Scheme 4[a]



[a] Reagents: (a) HCl (pH 3), EtOH, 80 °C, 5 h; (b) $Pd(HO)_2/C$, MeOH, H_2, 1 h; (c) PhNCO or CH_3NCO, Et_2O, Na; (d) PhNCO, Et_2O, Na; (e) $Pd(HO)_2/C$, MeOH, H_2.

rendering an unselective inhibitor. In contrast, 2′,6′- (**11**) and 3′,5′- (**9**) dimethyl substitutions reduced inhibitory action against both enzyme subtypes, with the former primarily affecting AChE and the latter BChE activity. This was heightened by diethyl substitution (**16**).

(c) Phenylcarbamate modification, trisubstitution: The 2′,4′,6′-trimethyl substitution (**14**) was not tolerated and resulted in a lack of inhibitory action against both enzyme subtypes.

In summary, substitution into the 2′ position provided improved AChE and reduced BChE inhibitory action, compared to phenserine (**2**). In contrast, 4′ substitution provided the reverse: a reduced AChE and improved BChE activity. Substitution into the 3′ position maintained AChE activity and improved BChE action, yielding a less selective compound. In general, disubstitution was well tolerated when involving the 2′ position, with the exception of 2′,6′-dimethylphenserine (**11**), and resulted in improved BChE activity, compared to monosubstitution, with a corresponding reduction in AChE selectivity. Disubstitution was well tolerated when involving the 4′ position for AChE inhibition, with improved BChE action being derived from the 3′,4′ position. Disubstitutions involving the 3′ position were well tolerated and provided compounds with activity

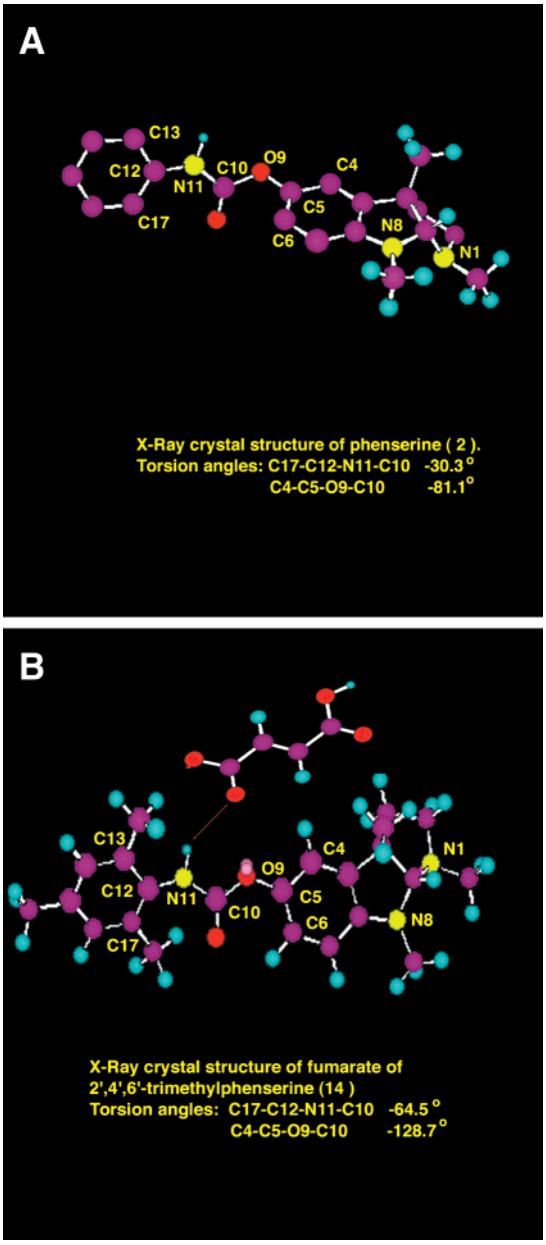


Figure 2.

intermediate between 2′ and 4′ substitution. Trimethyl substitution in the 2′,4′,6′ position resulted in a lack of activity.

(d) C6 position modification: Substitution in the C6 position was not tolerated for either phenserine or physostigmine (**13**, **31**, **32**). AChE and BChE inhibitory activities were substantially reduced.

(e) N17 position modification: Substitution in the N17 position was not tolerated for either phenserine or physostigmine. *N*-Methylphenserine (**12**) and *N*-methylphysostigmine (**33**) were dramatically less active than **2** and **1**, respectively.

X-ray Crystallography. Single-crystal X-ray analysis of 2 and 14: The results of the X-ray studies are illustrated in Figure 2. Compound **14** crystallized as a fumarate salt. Except for the rotation of the phenyl ring of the carbamate function, both compounds exhibit the same overall conformation. The terminal five-membered rings have an envelope conformation with C2 being the out of plane atom (+0.61 Å in **2** and −0.65 Å in **14**). The central five-membered ring is coplanar with its adjoining phenyl ring (±0.05 Å in **2** and ± 0.02 Å in **14**). The fused ring system is folded at the bond in common to the two five-membered rings (C3a−C8a). In **2** there is only one weak intermolecular interaction between N1 and N11 at 3.16 Å. The presence of the fumarate in **14** provides stronger intermolecular hydrogen bonding with both N1 and N11, interacting with the fumarate at 2.75 and 2.82 Å, respectively. There is also a strong hydrogen bond between neighboring fumarate ions (O···O = 2.56 Å).

Discussion

Figure 3 shows a diagram of mammalian AChE into which phenserine has been placed in its assumed binding site. The binding domain for phenserine is inside a 20 Å deep gorge that intrudes into the surface of the enzyme.[16,18,20] This is the very same domain that binds and rapidly metabolizes ACh. Within the gorge of AChE or BChE, three primary binding domains exist. These include (i) an acyl pocket that defines the active center involved in the catalysis of ACh and is centered around an active serine residue, Ser_{203}, in human enzyme; (ii) an active center choline subsite involved in the attraction and binding of the quaternary ammonium of the choline moiety of ACh; and (iii) a peripheral anionic site that is uninvolved in ACh hydrolysis but is the binding site of the ChEIs, tacrine, and donepezil and lies at the mouth of the gorge. Binding to this latter domain blocks access of ACh to the former binding sites and produces a conformational change such that the binding domains cannot align sufficiently for enzyme activity.[20,21]

Within the acyl binding domain of AChE, attack of the carbonyl function of phenserine (**2**) and analogues or ACh occurs through Ser_{203}, via a charge relay system within a catalytic triad of amino acids that involves the imidazole ring of His_{447} and carboxylic group of Glu_{334}.[16,18,20] The phenserine-AChE intermediate, like physostigmine (**1**), likely exists in a tetrahedral conformation that then collapses to a carbamylated drug-enzyme complex. This complex is far more stable than the acetylenzyme observed as a result of nucleophilic attack on the carbonyl group of ACh, which rapidly hydrolyzes to regenerate active enzyme.[19] In contrast, the carbamylated enzyme only slowly hydrolyzes at rate that is dependent on the structure of the carbamate moiety.[19,30] For phenserine (**2**), this complex likely is further stabilized by both hydrophobic and π electron interactions, due to the $\pi-\pi$ stacking of the phenyl group of the phenylcarbamate between the flanking phenyl moieties of Phe_{295} and Phe_{297} (Figure 3). These amino acids are lacking within BChE, wherein they are replaced by valine and leucine, respectively.[18,20,21] These latter residues that delineate the limit of the acyl domain within BChE are smaller than phenyalanine and thus create a pocket within BChE that allows the additional steric bulk of 4′-substituted phenylcarbamate analogues, **4** and **18**. These same compounds are not well tolerated within AChE and hence 4′ substitution attentuates the selectivity of the analogues to favor BChE inhibitory activity. This is in accord with site-directed mutagenesis studies in both human[31,32] and *Torpedo*[33] AChE, which illustrated that mutation of

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

no.	compounds	IC_{50} (nM) AChE	IC_{50} (nM) BChE	selectivity
1	physostigmine	28 ± 2	16 ± 3	2-fold BChE
2	phenserine	22 ± 1	1560 ± 270	70-fold AChE
3	2′-methylphenserine	10 ± 2	1950 ± 240	195-fold AChE
15	2′-ethylphenserine	10 ± 1	2915 ± 535	290-fold AChE
17	2′-isopropylphenserine	15 ± 1	650 ± 45	43-fold AChE
6	3′-methylphenserine	28 ± 4	165 ± 41	12-fold AChE
4	4′-methylphenserine	140 ± 4	250 ± 8	2-fold AChE
18	4′-isopropylphenserine	760 ± 20	50 ± 1	15-fold BChE
7	2′,3′-dimethylphenserine	23 ± 6	170 ± 32	13-fold AChE
5	2′,4′-dimethylphenserine	14 ± 1	1820 ± 560	130-fold AChE
10	2′,5′-dimethylphenserine	26 ± 1	490 ± 79	19-fold AChE
11	2′,6′-dimethylphenserine	785 ± 140	290 ± 50	3-fold BChE
16	2′,6′-diethylphenserine	1500 ± 50	1100 ± 50	none
8	3′,4′-dimethylphenserine	31 ± 7	66 ± 7	2-fold AChE
9	3′,5′-dimethylphenserine	78 ± 17	798 ± 147	10-fold AChE
14	2′,4′,6′-trimethylphenserine	1300 ± 75	3290 ± 885	3-fold AChE
33	*N*-methylphysostigmine	210 ± 40	420 ± 120	2-fold AChE
12	*N*-methylphenserine	690 ± 50	>10000	15-fold AChE
31	6-methylphysostigmine	>10000	8530 ± 235	none
13	6-methylphenserine	260 ± 130	5020 ± 180	19-fold AChE
32	6-dimethylaminoethylenphysostigmine	2500 ± 100	3890 ± 1500	none

[a] The IC_{50} data of compounds **1**−**5** and **14**−**18** were cited from reference 23. The IC_{50} data of compound **33** is from reference 43.

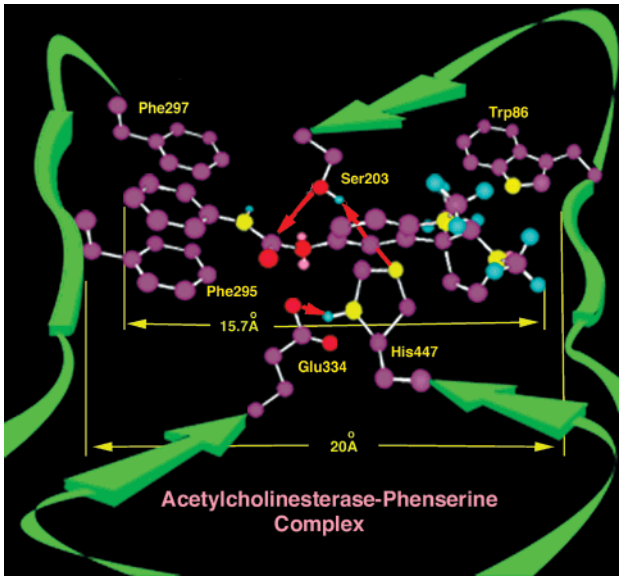


Figure 3.

Phe_{295} and Phe_{297} to their corresponding amino acids for BChE provided the wide substrate specificity associated with BChE.

As shown by X-ray crystallography (Figure 2), the hydrogen bonding between the N11−H of **14** and the carbonyl group of fumaric acid, together with substitution onto the phenylcarbamate, causes rotation of the phenyl moiety of the carbamate in relation to the 'A' ring of the remaining tricyclic structure, when compared to **2**. The torsion angles are indicated by dihedral angles C17−C12−N11−C10 and C10−O9−C5−C4.

Computer aided molecular modeling was undertaken, using MM2 force field, [34] as available in CS Chem 3D. When comparing target compounds, all parameters were kept constant throughout. Initially, the three-dimensional structure of each compound was built, then conformational analysis was performed on each to provide the energy minimized conformation of all substituted phenserines, **2**−**18** and **32**, in their noncrystalline state, together with their corresponding minimum steric energy E_1 (Table 2); which is a combination of bend, stretch−bend, torsion, van der Waals, and dipole/dipole interactions. According to our biological evaluation (Table 1), 2′-methylphenserine (**3**) possesses the highest anti-AChE activity. We therefore chose its conformation as the most favored for AChE interaction and binding of the phenserine series. Our enzyme kinetics studies support this choice, confirming the greater potency of **3** over phenserine (**2**) and physostigmine (**1**) with respective inhibition constants, K_i, of 0.0047 μM (**3**), 0.048 μM (**2**), and 0.19 μM (**1**).[35,36]

Figure 4 shows the computed energy minimized conformation of compounds **2**, **3**, **12**−**14**, and **18**, with their critical dihedral angles, C17−C12−N11−C10 and C10−O9−C5−C4, which for 2′-methylphenserine (**3**) are 3.4° and 159.8°, respectively. E_1 values, as determined by MM2,[34] are likewise shown. The predicted conformation of **3** has the two phenyl groups on either side of its carbonyl in less of an orthogonal relationship than compounds **2**, **12**−**14**, and **18**. To obtain a 2′-methylphenserine-like conformation, the resulting torsion angles for **2**, **12**−**14**, and **18** would change the energy to E_2, with ΔE ($E_2 - E_1$) being the energy difference between the two conformations and representing the conformational barrier.[34] Values for ΔE for **2**−**18** and **32** versus AChE inhibitory activity are shown in Table 2. The differences in measured versus computed values for **2** and **14** between Figures 2 and 4 likely are a consequence of (i) the compounds assuming a different low energy conformation in the noncrystalline versus crystalline state and (ii) the assessment of 2′,4′,6′-trimethylphenserine as a fumarate salt to obtain a crystalline form for X-ray analysis.

For compounds **12** and **13**, unlike **3**, the C17−C12−N11−C10 and C10−O9−C5−C4 computed torsion angles are dramatically different (by 19.9° and 0.98°, and by −3.33° and −14.1°, respectively) from 2′-methylphenserine (**3**) (Figure 4). In addition, their ΔE values (0.58 and 0.68 kcal/mol, respectively) are substantial compared to compounds **2** and **3** (Figure 4, Table 2). For example, they are equivalent to the free energy contri-

Table 2. Relationship between IC_{50} for AChE and Conformational Energy Barrier ΔE ($E_2 - E_1$) for Substituted Phenserines

no.	compound	IC_{50} AChE (nM)	ΔE (kcal/mol)	E_1 (kcal/mol)	E_2 (kcal/mol)
2	phenserine	22 ± 2	0.01	15.20	15.21
		Methyl Substituted Phenserine			
3	2′-methylphenserine	10 ± 2	0	15.16	15.16
5	2′,4′-dimethylphenserine	14 ± 1	0.02	14.82	14.84
7	2′,3′-dimethylphenserine	23 ± 6	0.01	16.34	16.35
10	2′,5′-dimethylphenserine	26 ± 1	0.01	14.86	14.87
6	3′-methylphenserine	28 ± 4	0	14.89	14.89
8	3′,4′-dimethylphrnserine	31 ± 7	0	15.75	15.75
9	3′,5′-dimethylphenserine	78 ± 17	0.01	14.49	14.50
4	4′-methylphenserine	140 ± 4	0.01	14.83	14.84
13	6-methylphenserine	210 ± 130	0.58	16.12	16.70
12	*N*-methylphenserine	690 ± 50	0.68	29.13	29.81
11	2′,6′-dimethylphenserine	785 ± 140	0.14	20.29	20.43
14	2′,4′,6′-trimethylphenserine	1300 ± 75	0.05	19.83	19.88
		Other Substituted Phenserine			
15	2′-ethylphenserine	10 ± 1	0.01	16.44	16.45
17	2′-isopropylphenserine	15 ± 1	0.07	18.03	18.10
18	4′-isopropylphenserine	760 ± 20	0	17.38	17.38
16	2′,6′-diethylphenserine	1500 ± 50	0.53	24.22	24.75
32	6-dimethylaminomethylenphenserine	2500 + 100	0.66	24.16	24.82

butions associated with the force of a weak van der Waals or a hydrophobic methylene−methylene interaction[37] and are sufficient to potentially alter the binding equilibrium constant, K_i, by some 2-fold ($\Delta E° = -RT \ln K_i$).[38] However, interactions between the enzyme binding domains and an inhibitor may compensate for or, vice versa, may increase the conformational barrier. The reduced AChE inhibitory activities associated with compounds **12** and **13**, compared to **3** (Table 2), suggest that a methyl group substituted in either the N11 or C6 positions seriously impedes the free rotation of the phenyl group of the phenylcarbamate and the 'A' ring (Figure 1) around the carbonyl group. In contrast, when substituted onto the phenylcarbamate (**4**−**10**), this does not occur and ΔE values are comparatively low. Interaction with the active center choline binding domain of AChE and BChE likely occurs via the basic N1 position of physostigmine (**1**), phenserine (**2**), and analogues.[19,39] This choline binding site exists as a region of 14 conserved aromatic amino acids centered around Trp_{86} in humans.[20,31] With required binding within the two relatively fixed enzyme domains, rotation of the phenylcarbamate in relation to the tricyclic moiety, bearing the N1 group in the 'C' ring (Figure 1), can therefore favor or disfavor the alignment of the carbonyl function for attack by Ser_{203}. Poor alignment, as a consequence of a different conformation and impeded rotation, likely explains the reduced activity of **12** and **13** compared to **1**−**3**.

In contrast, this dynamic analysis cannot explain the reduced AChE inhibitory activity of **18** compared to **3**, as its conformational barrier, ΔE, was zero, and its conformation was similar to **3** (Figure 4 and Table 2); indicating that 4′-substitutions elongate the molecule without impeding free rotation around the carbonyl moiety. It is this elongation within the size-restricted gorge of AChE that perturbs the AChE versus BChE inhibitory action of **18**. Similarly, the reduced anti-AChE action of 2′,4′,6′-trimethylphenserine (**14**) and 2′,6′-dimethylphenserine (**11**) also cannot be explained by impeded rotation. Their low ΔE value suggests that both compounds, similar to **2**−**10**, should be capable of aligning to the binding domains of the enzyme. Rather, the reduced biological activity of **14** and **11** likely results from their methyl substitutions obstructing the approach of Ser_{203} to the carbonyl moiety. For enzyme−drug binding, the oxygen anion of Ser_{203} requires free access to the positive carbon of the carbonyl group of the inhibitor.[19,21] The direction of approach of this nucleophilic attack has been the subject of intensive study. In general, and specifically for those cases that proceed by the described tetrahedral mechanism, there is no single definable and preferred transition state, but rather a "cone" of trajectories exists.[40] All approaches within this cone then lead to reaction at comparable rates. It is only when an approach comes from outside the cone that the reaction rate falls, resulting in reduced biological action.

It is additionally possible that the *N*-methyl of **12** and C6 substitutions of **13** and **32** are likewise located within the 'cone' area of the carbonyl group to hinder the approach of Ser_{203}, and, for these as well as for **16**, a combination of factors may reduce their final biological activity.

In summary, for optimal interaction, binding, and inhibition of AChE and BChE by phenserine (**2**) and analogues, the active center choline subsite and acyl pocket of the enzymes require holding the compounds' tricyclic π-system and carbamate structures, respectively, in an appropriate conformation to allow the attack of Ser_{203} to the carbonyl group of the inhibitor to form a tetrahedral intermediate and drug−enzyme complex.[19] We predict that amino acid differences that discriminate AChE from BChE[20,21] can then be utilized to differentially stabilize either drug−AChE or drug−BChE complexes by alkyl substitution into specific positions on the phenylcarbamate (i.e., 2′-methylphenserine (**3**) and 4′-isopropylphenserine (**18**), respectively) or the tricyclic moiety.[27] Particular substitutions can, however, sterically impede drug−enzyme interactions and/or disfavor the required alignment between the drug and enzyme for optimal binding and thus reduce their inhibitory activity. In addition, hydrophobic as well as π electron interactions, between the phenylcarbamate of phenserine (**2**) and analogues and either the phenyl moieties of Phe_{295} and Phe_{297} in AChE or flanking

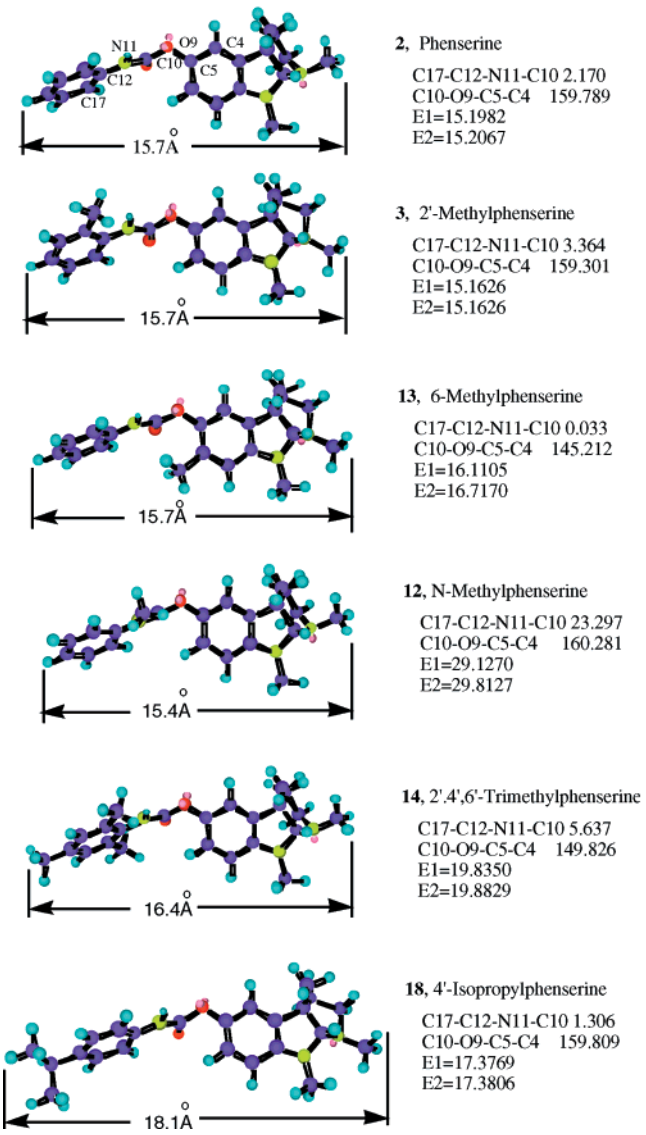


Figure 4. Conformation with minimum steric energy (E_1) and minimized energy in 2′-methylphenserine-like conformation (E_2).

aromatic amino acids in BChE, likely stabilize the drug−enzyme complex in a manner that cannot occur for physostigmine (**1**). Hydrolysis to regenerate active enzyme then would require a higher free energy and account for the long in vivo duration of enzyme inhibition of phenserine (**2**) and analogues (half-life 8 h) compared to physostigmine (**1**) (half-life 30 min).[9] Currently, X-ray crystallographic studies are being undertaken on phenserine (**2**) and analogues within AChE from *Torpedo californica* (Dr. Doriano Lamba, Istituto di Strutturistica Chimica, Trieste, Italy) to further elucidate the mechanism of action of the compounds and the validity of our computer modeling.

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.

3,4-Methylphenylisocyanate (23). 3,4-Dimethylaniline (1.58 g, 10 mmol) was added to 10 mL of toluene in a sealed tube with a small magnetic stirring bar. After addition of phosgene (in toluene, 20%) (11 mL, approximately 21 mmol), the sealed tube was closed tightly and inserted into a small oil bath, maintained at 100 °C, that was set on top of a stir plate. Following 6 h of reaction with vigorous stirring, the suspended hydrochloride of aniline almost disappeared and the reaction was stopped. Cooling to room temperature provided a reaction mixture that was filtered to give a clear, transparent filtrate that was evaporated by vacuum to obtain compound **23** (1.03 g, 70%) as a slight brownish oil: ^{1}H NMR ($CDCl_3$) δ 7.75−6.82 (m, 3 H, Ph-H), 2.28 (s, 6H, 3,4-dimethyl); EI-MS, *m*/*z*: 147 (M^+).

2,5-Dimethylphenylisocyanate (25). According to the procedure for the synthesis of **23**, compound **25** was obtained as slightly brown oil: ^{1}H NMR ($CDCl_3$) δ 7.10−6.05 (m, 3 H, Ph-H), 2.38 (s, 3H, 2-CH_3), 2.28 (s, 3H, 5-CH_3); EI-MS, *m*/*z*: 147 (M^+).

(−)-(3a*S*)-1,3a,8-Trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-(3′-Methylphenyl)carbamate (6). Eseroline (**27**) (108 mg, 0.5 mmol) was dissolved in anhydrous ether (5 mL), and a piece of Na metal (approximately 1 mg) was added. The mixture was stirred at room temperature for 1 min, then 3′-methylphenylisocyanate (**21**) (66 mg, 0.5 mmol) was added. The reaction mixture was continuously stirred at room temperature and monitored by TLC. When compound **27** had virtually disappeared, 1 mL of H_2O was added to destroy any remaining trace of unreacted isocyanate. The reaction mixture was diluted into 20 mL of Et_2O and washed with NaOH aqueous solution (approximately 5% concentration) followed by brine. The ether solution was dried over $MgSO_4$, with stirring, and then filtered to obtain a clear ether solution of the product. Evaporation of ether provided compound **5** (158 mg, 90%) as a foam: $[\alpha]_D^{20}$ −66.9° (*c* = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.25−6.70 (m, 6H, Ar-H), 6.32 (d, *J* = 8.5 Hz, 1H, C7-H), 4.05 (s, 1H, C8a-H), 2.88 (s, 3H, CH_3-N8), 2.80−2.50 (m, 2H, C2-H_2), 2.48 (s, 3H, CH_3-N1), 2.28 (s, 3H, 3′-CH_3), 1.90 (m, 2H, C3-H_2), 1.40 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 352 (MH^+). The base of compound **5** was dissolved in MeOH and then was added to a methanol solution containing an equivalent of L-(+)-tartaric acid. This was concentrated under vacuum until it became syrup-like and then was diluted with ether. A precipitated gum was separated by decanting off the ether solution, and, thereafter, a further batch of fresh ether was added. After scraping, the gum was crystallized from the ether solution to give the tartrate of compound **5**: mp 96−98 °C. Anal. ($C_{21}H_{25}N_3O_2 \cdot C_4H_6O_6$) C, H, N.

Compounds **7**−**11** were synthesized from their corresponding isocyanates (**22**−**26**) according to the above procedure for synthesis of compound **6**.

(−)-(3a*S*)-1,3a,8-Trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-(2′,3′-Dimethylphenyl)carbamate (7): $[\alpha]_D^{20}$ 62.5° (*c* = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.20−6.50 (m, 5 H, Ar-H), 6.28 (d, *J* = 8.5 Hz, 1H, C7-H), 4.10 (s, 1H, C8a-H), 2.88 (s, 3H, CH_3-N8), 2.75−2.50 (m, 2H, C2-H_2), 2.45 (s, 3H, CH_3-N1), 2.25 (s, 3H, 2′-CH_3), 2.15 (s, 3H, 3′-CH_3), 1.90 (m, 2H, C3-H_2), 1.35 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 366 (MH^+). Tartrate of compound **7**: mp 108−110 °C. Anal. ($C_{22}H_{27}N_3O_2 \cdot C_4H_6O_6$) C, H, N.

(−)-(3a*S*)-1,3a,8-Trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-(3′,4′-Dimethylphenyl)carbamate (8): $[\alpha]_D^{20}$ 62.0° (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.15−6.25 (m, 6 H, Ar-H), 4.15 (s, 1H, C8a-H), 2.88 (s, 3H, CH_3-N8), 2.75−2.50 (m, 2H, C2-H_2), 2.48 (s, 3H, CH_3-N1), 2.22 (s, 3H, 3′-CH_3), 2.20 (s, 3H, 4′-CH_3), 1.99 (m, 2H, C3-H_2), 1.40 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 366 (MH^+). Tartrate of compound **8**: mp 96−98 °C. Anal. ($C_{22}H_{27}N_3O_2 \cdot C_4H_6O_6$) C, H, N.

(−)-(3a*S*)-1,3a,8-Trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-(3′,5′-Dimethylphenyl)carbamate (9): $[\alpha]_D^{20}$ −67.8° (*c* = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.02−6.65 (m, 5 H, Ar-H), 6.30 (d, *J* = 8.5 Hz, 1H, C7-H), 4.10 (s, 1H, C8a-H), 2.89 (s, 3H, CH_3-N8), 2.75−2.55 (m, 2H, C2-H_2), 2.52 (s, 3H, CH_3-N1), 2.28 (s, 6H, 3′-CH_3 and 5′-CH_3), 1.85−2.00 (m, 2H, C3-H_2), 1.40 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 366 (MH^+). Tartrate of compound **9**: mp 96−98 °C. Anal. ($C_{22}H_{27}N_3O_2 \cdot C_4H_6O_6$) C, H, N.

(−)-(3a*S*)-1,3a,8-Trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-(2′,5′-Dimethylphenyl)carbamate (10): $[\alpha]_D^{20}$ −61.0° (*c* = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.10−6.30 (m, 6 H, Ar-H), 4.13 (s, 1H, C8a-H), 2.95 (s, 3H, CH_3-N8), 2.80−2.55 (m, 2H, C2-H_2), 2.55 (s, 3H, CH_3-N1), 2.32 (s, 6H, 2′-CH_3), 2.27 (s, 3H, 5′-CH_3), 2.00−1.90 (m, 2H, C3-H_2), 1.42 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 366 (MH^+). Tartrate of compound **10**: mp 108−110 °C. Anal. ($C_{22}H_{27}N_3O_2 \cdot C_4H_6O_6$) C, H, N.

(−)-(3a*S*)-1,3a,8-Trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-(2′,6′-Dimethylphenyl)carbamate (10): $[\alpha]_D^{20}$ −60.8° (*c* = 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.10−6.15 (m, 6 H, Ar-H), 4.10 (s, 1H, C8a-H), 2.83 (s, 3H, CH_3-N8), 2.80−2.55 (m, 2H, C2-H_2), 2.48 (s, 3H, CH_3-N1), 2.30 (s, 6H, 2′-CH_3 and 6′-CH_3), 2.00−1.80 (m, 2H, C3-H_2), 1.42 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 366 (MH^+). Tartrate of compound **10**: mp 128−130 °C. Anal. ($C_{22}H_{27}N_3O_2 \cdot C_4H_6O_6$) C, H, N.

(−)-(3a*S*)-1,3a,8-Trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-(Methyl)-phenylcarbamate (12). Eseroline **27** (218 mg, 1 mmol) was dissolved in pyridine (5 mL), and *N*-methyl-phenylcarbamoyl chloride (**28**) (678 mg, 4 mmol) was added. The reaction mixture was heated in an oil bath at 90 °C and stirred for 56 h. Evaporation of pyridine by vacuum gave a residue which was chromatographed on a silica gel (CH_2Cl_2/MeOH = 20:1) to give compound **12** (90 mg, 25.6%) as a gum: $[\alpha]_D^{20}$ −32.5° (*c* = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40−7.10 (m, 5 H, Ar-H), 6.80−6.50 (m, 2H, C4-H and C6-H), 6.22 (d, *J* = 8.5 Hz, 1H, C7-H), 4.08 (s, 1H, C8a-H), 3.37 (s, 3H, CH_3-N), 2.87 (s, 3H, CH_3-N8), 2.75−2.45 (m, 2H, C2-H_2), 2.47 (s, 3H, CH_3-N1), 1.85 (m, 2H, C3-H_2), 1.35 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 352 (MH^+); EI-MS, *m*/*z* (relative intensity): 218 (MH^+ − $PhNCH_3CO^-$, 15), 134 ($PhNCH_3O$, 100), 106 ($PhNCH_3$, 50), 77 (Ph, 60). The base of compound **12** was dissolved in MeOH, and then was added to a methanol solution containing an equivalent of L-(+)-tartaric acid. The methanol solution was concentrated under vacuum until it became syrup-like and then was diluted with ether. A precipitated gum was separated by decanting off the ether solution, and, thereafter, a further batch of fresh ether was added. After scraping, the gum was solidified from the ether solution to provide the tartrate of compound **12**: Anal. ($C_{21}H_{25}N_3O_2 \cdot C_4H_6O_6$) C, H, N.

(−)-(3a*S*)-1,3a,8-Trimethyl-6-(dimethylamino)methylene-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol (29). Eseroline **27** (334 mg, 1 mmol), hydrochloride of dimethylamine (405 mg, 6 mmol), and formaldehyde (37.9%) aqueous solution (1 mL, about 10 mmol) were added into 10 mL of EtOH in a sealed tube. The reaction mixture was stirred for 5 h at 80 °C in an oil bath. After cooling, the reaction mixture was concentrated to provide a residue which was directly chromatographed on silica gel (CH_2Cl_2/MeOH = 10:1) to give compound **29** (245 mg, 89.0%) as a gum: $[\alpha]_D^{20}$ −3.4° (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.55 (s, 1H, C4-H), 6.12 (s, 1H, C7-H), 4.12 (s, 1H, C8a-H), 3.50 (s, 2H, Ar-CH_2-N), 2.88 (s, 3H, CH_3-N8), 2.85−2.55 (m, 2H, C2-H_2), 2.54 (s, 3H, CH_3-N1), 2.28 (s, 6H, Me_2N-Ar), 2.10−1.80 (m, 2H, C3-H_2), 1.45 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 275 (MH^+). HR-MS *m*/*z*: Calcd for $C_{16}H_{25}N_3O$: 275.19995; Found: 275.1990.

(−)-(3a*S*)-1,3a,6,8-Tetramethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol (30). Compound **29** (215 mg, 0.78 mmol) was dissolved in MeOH (5 mL), and $Pd(OH)_2$/C (10 mg) was added. The reaction mixture was stirred under hydrogen at atmospheric pressure and room temperature for 1 h. Thereafter, the catalyst was removed by filtration. Evaporation of solvent gave a residue that was chromatographed on silica gel (CH_2Cl_2/MeOH = 10:1) to give compound **30** (163 mg, 90.0%) as a gum: $[\alpha]_D^{20}$ −82.8° (*c* = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.60 (s, 1H, C4-H), 6.34 (s, 1H, C7-H), 4.80 (s, 1H, C8a-H), 2.95 (s, 3H, CH_3-N8), 2.70−2.50 (m, 2H, C2-H_2), 2.65 (s, 3H, CH_3-N1), 2.15 (s, 3H, CH_3-Ar), 2.05−1.95 (m, 2H, C3-H_2), 1.45 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 232 (MH^+). According to the procedure of making tartrate of compound **12**, tartrate of compound **30** was obtained as solid: Anal. ($C_{14}H_{20}N_2O \cdot 1.5C_4H_6O_6 \cdot 0.5H_2O$) C, H, N.

(−)-(3a*S*)-1,3a,6,8-Tetramethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Phenylcarbamate (13). (i) Compound **30** (55 mg, 0.2 mmol) was dissolved in anhydrous ether (2 mL), and a piece of Na metal (approximately 1 mg) was added. The mixture was stirred under nitrogen at room temperature for 5 min, then phenylisocyanate (23.6 mg, 0.2 mmol) was added. The reaction mixture was continuously stirred at room temperature and monitored by TLC. When compound **30** had almost disappeared, 0.5 mL of H_2O was added to destroy any trace of remaining unreacted phenylisocyanate. The reaction mixture was diluted into 10 mL of ether and washed with diluted NaOH aqueous solution (approximately 5% concentration), followed by brine. The ether solution was dried over $MgSO_4$, with stirring, and then filtered to obtain a clear ether solution of the product. Evaporation of solvent gave compound **13** (54 mg, 77.6%) as a foam: $[\alpha]_D^{20}$ −80.0° (*c* = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.45−6.80 (m 5H, Ar-H), 6.72 (s,1H, C4-H), 6.20 (s, 1H, C7-H), 4.20 (s, 1H, C8a-H), 2.88 (s, 3H, CH_3-N8), 2.75−2.55 (m, 2H, C2-H_2), 2.50 (s, 3H, CH_3-N1), 2.23 (s, 3H, 6-CH_3), 2.00−1.85 (m, 2H, C3-H_2), 1.45 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 351(MH^+). In accord with the procedure for preparing a tartrate of compound **12**, the tartrate of compound **13** was obtained as crystals: mp 127−130 °C. Anal. ($C_{21}H_{25}N_3O_2 \cdot C_4H_6O_6$.) C, H, N.

(ii) Compound **32** (45 mg, 0.12 mmol) was dissolved in MeOH (2 mL), and $Pd(OH)_2$/C (2 mg) was added. The reaction mixture then was stirred under hydrogen at atmospheric pressure (room temperature, 1 h). Following removal of the catalyst by filtration, evaporation of the solvent gave a residue that was chromatographed on silica gel (CH_2Cl_2/MeOH = 10:1) to give compound **13** (2 mg, 5%)

(−)-(3a*S*)-1,3a,6,8-Tetramethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Methylcarbamate (31). In accord with the described procedure for making compound **11**, reaction of compound **30** with methylisocyanate gave compound **31** (in 83.0% yield) as a foam: $[\alpha]_D^{20}$ −63.4° (*c* = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.65 (s, 1H, C4-H), 6.15 (s, 1H, C7-H), 4.15 (s, 1H, C8a-H), 2.88 (s, 3H, CH_3-N8), 2.70 (d, *J* = 5 Hz, 3H,CH_3-NH-), 2.70−2.50 (m, 2H, C2-H_2), 2.48 (s, 3H, CH_3-N1), 2.08 (s, 3H, C6-CH_3), 2.00−1.80 (m, 2H, C3-H_2), 1.40 (s, 3H, C3a-CH_3); CI-MS (NH_3), *m*/*z*: 290 (MH^+). Similar to the procedure for preparing the tartrate of compound **12**, the tartrate of compound **31** was obtained as a solid: Anal. ($C_{16}H_{23}N_3O_2 \cdot C_4H_6O_6$.) C, H, N.

(−)-(3a*S*)-1,3a,8-Trimethyl-6-(dimethylamino)methylene-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl *N*-Phenylcarbamate (32). Reaction of compound **29** with phenylisocyanate, according to the procedure for preparing compound **13**, provided compound **32** (in 90.0% yield) as a foam: $[\alpha]_D^{20}$ −48.7° (*c* = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40−6.90 (m, 5H, Ar-H), 6.72 (s, 1H, C4-H), 6.25 (s, 1H, C7-H), 4.05 (s, 1H, C8a-H), 3.30 (s, 2H, Ar-CH_2-N), 2.90 (s, 3H, CH_3-N8), 2.70−2.50 (m, 2H, C2-H_2), 2.47 (s, 3H, CH_3-N1), 2.22 (s, 6H, Me_2N-Ar), 1.95−1.80 (m, 2H, C3-H_2), 1.40 (s, 3H, C3a-CH_3); EI-MS, *m*/*z*: 394 (M^+). HR-MS *m*/*z*: Calcd for $C_{23}H_{30}N_4O_2$: 394.2371; Found: 394.2369.

Single-Crystal X-ray Analysis of 2 and 14. Data for compound **2** were collected on a Bruker P4 serial automatic diffractometer with a graphite monochromator in the incident beam. The crystal is orthorhombic in space group $P2_12_12_1$ with *a* = 7.689(1), *b* = 14.459 (1), and *c* = 16.636(3) Å. Data for compound **14** were collected on a Bruker SMART 1K CCD system mounted on a 6 Kw Cu rotating anode using Gobels mirrors to focus the beam. This crystal was also orthorhombic

in space group $P2_12_12_1$ with $a = 8.632(1)$, $b = 12.571(1)$, and $c = 12.495(1)$ Å. The structures were solved by direct methods and refined by full-matrix least-squares on F^2 values using programs in the SHELXTL-PLUS package.[41] The parameters refined included the coordinates and anisotropic thermal parameters for all non-hydrogen atoms and coordinates only for the hydrogen atoms on the fumarate hydroxyl. All other hydrogen atoms were included using a riding model in which the coordinate shifts of their covalently bonded atoms were applied to the attached hydrogens with C−H = 0.96 Å and N−H = 0.86 Å. H angles were idealized and Uiso(H) set at fixed ratios of Uiso values of bonded atoms. Final *R*-factors were 0.054 for 1822 observed reflections for **2** and 0.060 for 1468 observed reflections for **14**. The coordinates for both compounds have been deposited as Supporting Information and are also available from the Cambridge Crystallographic Database.[42]

Quantitation of Anticholinesterase Activity. The action of compounds **1**−**18** and **31**−**33** 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. Lois, MO) were quantified.[23−27,43] 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 concentration range that spanned 0.3 nM to 30 mM. Tween 80/EtOH was diluted to in excess of 1 in 5000 and no inhibitory action on either AChE or BChE was detected in separate prior experiments.

For the preparation of AChE, freshly collected blood was centrifuged (10000g, 10 min, 4 °C), and plasma was removed and diluted 1:125 with 0.1 M Na_3PO_4 buffer (pH 7.4). For BChE preparation, 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 were 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 (**1**)), and the associated alteration in absorbance was subtracted from that observed through the concentration range of each test compound. Each agent was analyzed on four separate occasions and assayed alongside phenserine (**2**) and physostigmine (**1**), as control and external standards whose activity we have previously reported.[23−27]

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 was 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.

Computer Aided Molecular Modeling. Computer aided molecular modeling was undertaken using MM2 force field[34] and the default parameters. A PDB entry for AChE was used (Figure 3) in which phenserine was docked manually; thereafter molecular mechanics were applied to optimize the geometries between it and the active site.

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Supporting Information Available: Crystallographic information for compounds **2** and **14** and tables of the parameters used in MM2 for the comformational analysis of cabamates. This material is available free of charge via the Internet at http://pubs.acs.org.

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