

HETEROCYCLES, Vol. 61, 2003, pp. 529 - 539

Received, 8th May, 2003, Accepted, 30th June, 2003, Published online, 4th July, 2003

**RACEMIC *N*^l-NORPHENSERINE AND ITS ENANTIOMERS:
UNPREDICTED INHIBITION OF HUMAN ACETYL- AND
BUTYRYLCHOLINESTERASE AND β -AMYLOID
PRECURSOR PROTEIN *IN VITRO***

Qian-sheng Yu,^a Weiming Luo,^a Harold W. Holloway,^a Tada Utsuki,^a TracyAnn Perry,^a Debomoy K. Lahiri,^b Nigel H. Greig,^{a*} and Arnold Brossi^{c@}

a) Drug Design & Development Section, Laboratory of Neurosciences, Intramural Research Program, National Institute on Aging, National Institutes of Health, 5600 Nathan Shock Dr., Baltimore, Maryland 21224-6825.

b) Department of Psychiatry, Psychiatric Research Institute Indiana University School of Medicine, Indianapolis, IN 46202.

c) School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599.

Abstract- The optically pure enantiomers of *N*^l-norpheneserine (**15**, **16**) were synthesized and its racemate **17** was prepared by mixing equal parts of each enantiomer. (-)-*N*^l-Norpheneserine (**15**) was prepared by partial synthesis initiated from the natural product, (-)-physostigmine (**1**). (+)-*N*^l-Norpheneserine (**16**) was prepared by total synthesis using the Julian oxindole route, with modifications. The *in vitro* inhibitory activities of **15-17** were quantified against human erythrocyte AChE and plasma BChE as well as against human neuroblastoma cell β -amyloid precursor protein secretion in cell culture. All were active. Racemic compound (**17**) with a high AChE and β -amyloid precursor protein inhibitory action may warrant further assessment in Alzheimer's disease models.

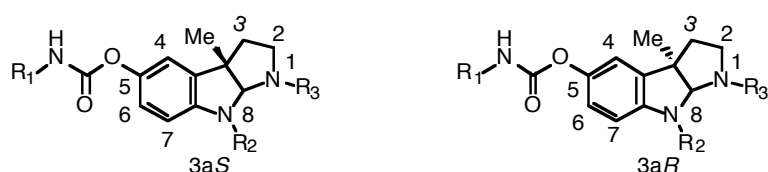
The natural product (-)-physostigmine (**1**), derived from the Calabar bean: the dried ripe seed of the *Physostigma venenosum*, has not only proved to be a useful clinical drug (e.g., *Antilirium*) and pharmacological tool, but has acted as the base pharmacophore in the development of several current drugs (pyridostigmine bromide oral: *Mestinon*, parenteral: *Renenol*; neostigmine methylsulfate:

*Corresponding Author: Phone: 410-558-8278; Fax: 410-558-8323; E-Mail: GreigN@vax.grc.nia.nih.gov

@ In celebration of Arnold Brossi's 80th birthday

Prostigmin) and experimental therapeutics for Alzheimer's disease (e.g., (-)-phenserine tartrate).¹⁻⁴ (-)-Physostigmine (**1**) is a potent and short-acting (half-life 30 min)^{1,3-5} anticholinesterase that co-inhibits the enzymes acetylcholinesterase (AChE: EC 3.1.1.7)^{4,6} and butyrylcholinesterase (BChE: EC 3.1.1.8)^{2,4,6} with equal potency and with a brain/plasma concentration ratio of unity.¹ In contrast, its

Table 1. Our Literature Values of Natural (-)- and Unnatural (+)- Physostigmine and its Analogues Versus Human Erythrocyte AChE and Human Plasma BChE.



No.	Compound	R ₁	R ₂	R ₃	IC ₅₀ * (nM)		Selectivity	Ref
					AChE	BChE		
1	(-)-3aS-Physostigmine	Me	Me	Me	28 ± 2	16 ± 3	2-fold BChE	5,12,13
8	(+)-3aR-Physostigmine	Me	Me	Me	9890 ± 6	2490 ± 290	4-fold BChE	9
3	(-)-3aS-N ^l -Norphysostigmine	Me	Me	Me	21 ± 1	2 ± 1	10-fold BChE	11
10	(+)-3aR-N ^l -Norphysostigmine	Me	Me	Me	193 ± 5	202 ± 16	none	13
4	(-)-3aS-N ⁸ -Norphysostigmine	Me	H	Me	57 ± 4	7 ± 2	9-fold BChE	8
11	(+)-3aR-N ⁸ -Norphysostigmine	Me	H	Me	2190 ± 110	1120 ± 23	2-fold BChE	13
5	(-)-3aS-N ^l N ⁸ -Norphysostigmine	Me	H	H	11 ± 1	2 ± 1	5-fold BChE	9
12	(+)-3aR-N ^l N ⁸ -Norphysostigmine	Me	H	H	1490 ± 120	235 ± 55	6-fold BChE	9
2	(-)-3aS-Phenserine	Ph	Me	Me	22 ± 2	1560 ± 270	70-fold AChE	5,10,12
9	(+)-3aR-Phenserine	Ph	Me	Me	3500 ± 55	23500 ± 300	7-fold AChE	9
6	(-)-3aS-N ⁸ Norphenserine	Ph	H	Me	41 ± 3	516 ± 61	13-fold AChE	8
13	(+)-3aR-N ⁸ Norphenserine	Ph	H	Me	5650 ± 610	1745 ± 325	3-fold BChE	8
7	(-)-3aS-N ^l N ⁸ -Bisnorphenserine	Ph	H	H	22 ± 2	895 ± 104	41-fold AChE	9
14	(+)-3aR-N ^l N ⁸ -Bisnorphenserine	Ph	H	H	230 ± 23	950 ± 107	4-fold AChE	9
15	(-)-3aS-N ^l -Norphenserine	Me	Me	H	14 ± 1	612 ± 1	25-fold AChE	13,14
16	(+)-3aR-N ^l -Norphenserine	Ph	Me	H	64 ± 4	5295 ± 740	88-fold AChE	13
17	(±)-N ^l -Norphenserine	Ph	Me	H	47	1660	35-fold AChE	10

* Concentration to inhibit 50% of enzymatic activity against enzyme obtained from the same individual

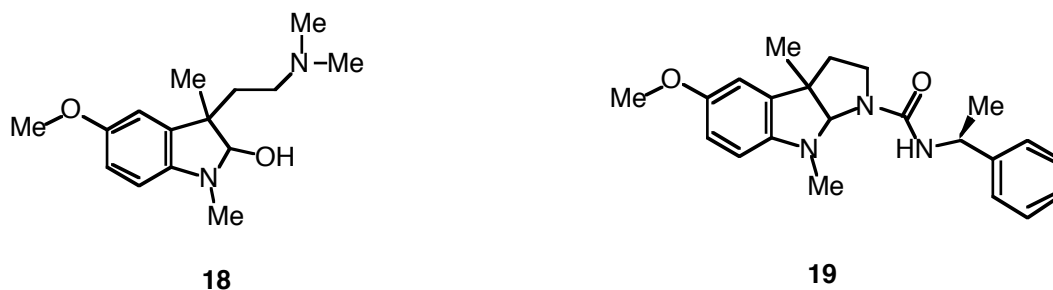
phenylcarbamate analogue, (-)-phenserine (**2**) is similarly a reversible potent anticholinesterase, but is long-acting (half-life ~8 hours), selective for human AChE vs. BChE (70-fold) and has a brain/plasma concentration ratio of 10:1.⁵ Both compounds, (-)-physostigmine (**1**) and (-)-phenserine (**2**), have a N^l-methyl substituent, and their respective (+)-enantiomers (**8,9**) possess minimal anticholinesterase activity (**Table 1**).^{5,7} A similar behavior also is found in the N⁸-nor and N^l,N⁸-bisnor series (**4,5,6,7,11,12,13,14**),^{8,9} wherein the (-)- and (+)-enantiomers are potent and essentially devoid of AChE action, respectively. A

notable exception, however, is the enantiomers of *N*^l-norphysostigmine (**3,10**), a putative metabolite of **1**, that, when prepared earlier, were both found to be potent inhibitors of human AChE and BChE¹⁰ (**Table 1**) as well as eel AChE.¹¹

In assaying enantiomers of biologically active racemates, it is paramount to establish their optical purity as a minor contaminant within a biologically inactive enantiomer by a highly potent optical isomer could falsely mimic a positive biological response.⁷ In this regard, our recent studies with (-)- and (+)-phenserines (**2,9**) have found the (+)-enantiomer to be devoid of anticholinesterase action¹⁵ rather than to possess a minimal, but measurable activity found in earlier studies (**Table 1**).^{8,9} In light of our prior finding that both (-)- and (+)-*N*^l-norphenserine (**15,16**) were potent AChE inhibitors (**Table 1**),¹³ together with our recent finding that both enantiomers of phenserine (**2,9**) possess inhibitory action on β -amyloid precursor protein (APP),¹⁵⁻¹⁸ which is critical in Alzheimer's disease,¹⁹ we decided to revisit **15,16**. In the current study, we report the resynthesis of these compounds with particular emphasis on both their optical as well as chemical purity, together with their evaluation for both cholinesterase and APP inhibitory action.

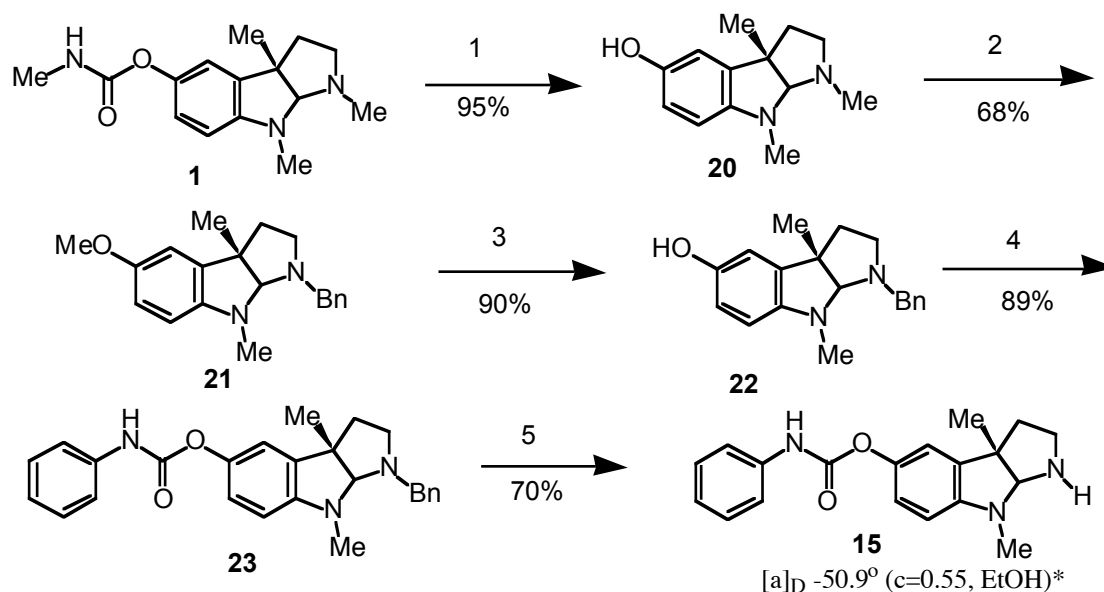
Chemistry: The total synthesis of racemic *N*^l-norphenserine (**17**), together with its enantiomers (**15, 16**), has previously been reported.¹⁰ The precursor in this synthesis is oxindole (**27**), which is similar to Julian's first total synthesis of physostigmine,²⁰ but with additional improvements. Both the (-) and (+) series of analogues were obtained based on the chemical resolution of amino alcohol (**18**) by (+)-2,3-di-*O*-(*p*-toluoyl)-D-tartaric acid. Optically pure intermediates could additionally be obtained by the

Figure 1



chromatographic separation of urea diastereoisomer (**19**) that, after fragmentation, provided optically pure **30** and its enantiomer (**Figure 1**).^{21,22}

Schemes 1



1), BuOH, catalytic amount of Na; 2), a, MeI/DMSO/KOH; b, BnNH₂; 3), BBr₃/CH₂Cl₂; 4), PhNCO/Et₂O/catalytic amount of Na; 5), Pd(OH)₂/C, H₂.

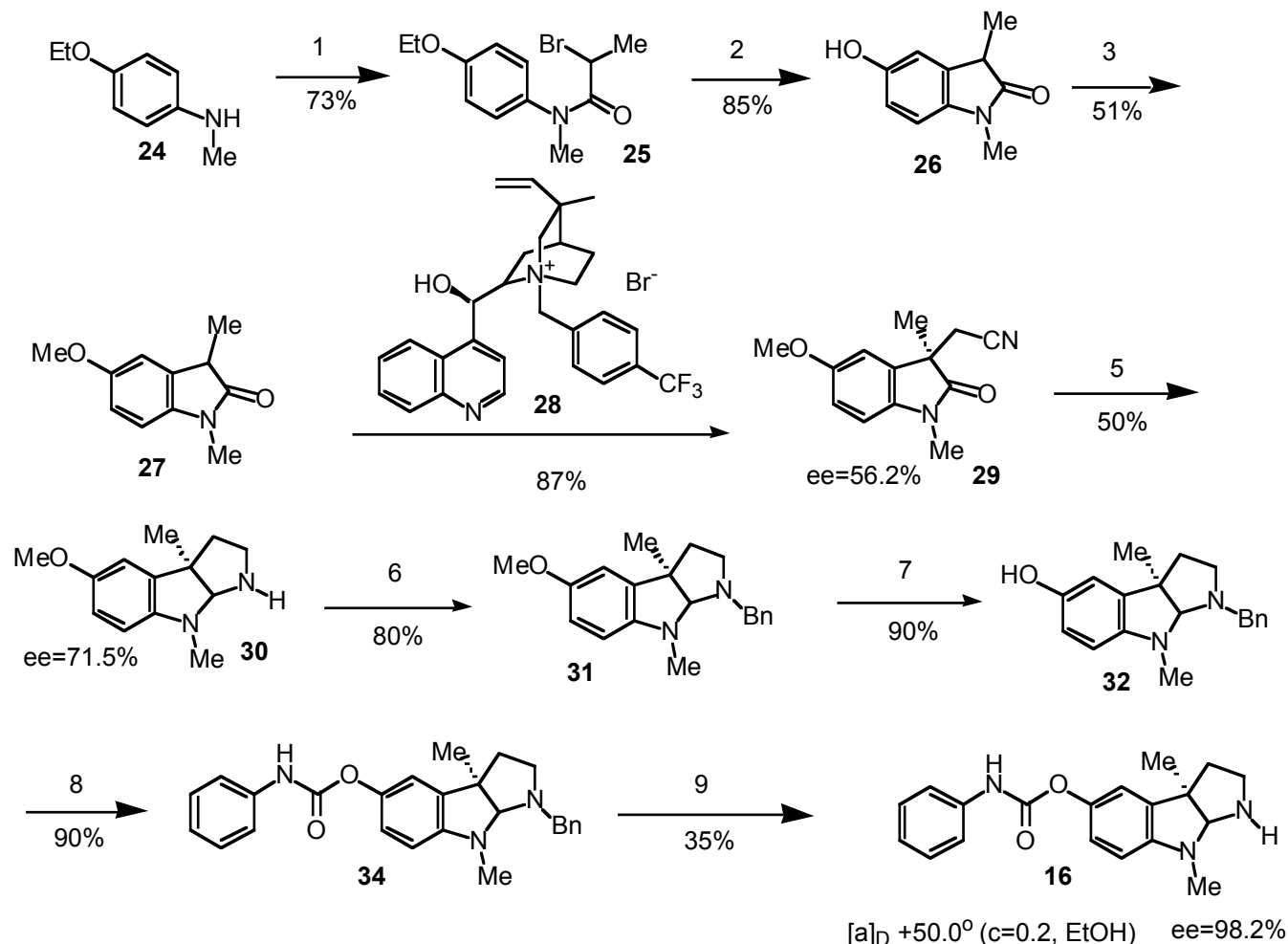
* $[\alpha]_D -50.4^\circ$ (c=0.5, CH₃Cl).¹⁴

In choosing a synthetic route for compounds (**15**, **16**, **17**) with a focus on optical purity, synthesis of (-)-3aS-N'-norpheneserine (**15**) was initiated from natural (-)-physostigmine (**1**) that bears a 3aS configuration. All reactions avoided the 3a chiral center throughout the process (Scheme 1). Specifically, (-)-physostigmine (**1**) was dissolved in BuOH with addition of a catalytic quantity of Na, and was refluxed until **1** disappeared. An equivalent of fumaric acid then was added to this hot solution with stirring. After cooling, the fumarate of **20** was given almost quantitatively.²⁴ Subsequently, **20** was reacted with MeI in the presence of KOH in DMSO, BnNH₂ then was added and the reaction mixture maintained at 100°C for 2 h. It then was partitioned between H₂O and Et₂O. The Et₂O layer contained N-benzyltricyclic methyl ether (**21**) with tiny remainder of BnNH₂ that was easily removed by simple chromatography.^{10,11,23} Demethylation of **21** by BBr₃ in CH₂Cl₂ at room temperature gave the HBr salt of **22** in good yield and quality without further purification.^{10,11} To the ether solution of free base (**22**) in the presence of a catalytic amount of Na, 1 equivalent of phenylisocyanate was added in one portion. After **22** had disappeared, as assessed by TLC, the reaction was quenched with H₂O. The ether layer was dried, evaporated to give crude phenyl carbamate (**23**), which then was directly crystallized from Et₂O without chromatography.^{8,9,14} Debencylation of **23** gave **15** that then was transformed to its fumarate and recrystallized from MeOH.^{8,9,14}

The total synthesis of (+)-3a*R*-*N*^{*l*}-norphenserine (**16**) was initiated from *N*-methylphenetidine (**24**), which was reacted with 2-bromopropionyl bromide to give compound (**25**) in the presence of triethylamine, used as a HBr scavenger, and a catalytic amount of DMAP that proved helpful to increase yield. The intramolecular Friedel-Craft's reaction gave compound (**26**). Oxindole (**27**) was obtained by reaction of **26** with dimethyl sulfate in KOH aqueous solution. Unfortunately, the electro negative active site of the carbonyl α -position caused the occurrence of side reactions; hence the yield of this reaction was not particularly satisfactory. Phase-transfer asymmetric alkylation of oxindole (**27**) gave the 3a*R* configuration enriched compound (**29**). From previous studies²⁵⁻²⁸ the separation of compound (**29**) from its enantiomer has proven to be readily achievable. The absolute optical rotation values of compounds (**29**,²⁵ **30**,^{11,25} **31**,^{11,25} **32**,¹¹ **34**,¹⁴ and **16**¹⁴) have been previously reported. As a consequence, we used the 3a*R* configuration enriched compound (**29**) as the key intermediate in the following known procedure^{10,14,27} to synthesize final product (**16**). Reductive cyclization of **29** by LiAlH₄ gave a crude residue of hexahydropyrroloindole (**30**), which was transformed to its fumarate by adding an equivalent methanol solution of fumaric acid. Further recrystallization provided chemically pure compound (**30**), whose ee% value increased to 71.5%. In the same manner, the final crude product (**16**) was purified by formation and recrystallization of its di-*p*-toluoyl-L-tartrate. Comparing the optical rotation (determined on a JASCO, model DIP-370, Spectroscopic Co., Ltd., Japan) of the free base of compound (**16**) with that of its enantiomer (**15**) provided **16** with an ee% value of 98.2% (from **Schemes 1** and **2**). To secure optical purity prior to biological assessment, we transformed the free base of **16** to its fumarate, recrystallized it again, and then compared its optical rotation with that of the fumarate of its enantiomer (**15**) (**Table 2**). The fumarate of racemic **17** was produced by mixing exact equivalents of fumarates (**15** and **16**) followed by recrystallization from MeOH.

We attempted to measure the optical purity of **15**, **16** and **17** by a chiral HPLC, but streaking occurred and the results proved unsatisfactory, as in the measurement of methyl ether (**29**).²⁶ Taking an alternative approach, we utilized (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol as a chemical shift reagent in the ¹H-NMR spectral measurement of the optical purity of **15**, **16**, and **17** and, as illustrated in **Figure 2**, we determined **15** and **16** to possess an optical purity of 100% based on both optical rotation and ¹H-NMR spectral assessment.²⁹

Scheme 2



1), 2-Bromopropionyl bromide, triethylamine, DMPA; 2), AlCl_3 ; 3), $(\text{MeO})_2\text{SO}_2/\text{KOH}$; 4), ClCH_2CN , (-)-Cinchonidine *p*-trifluorobenzyl bromide/ NaOH ; 5), $\text{LiAlH}_4/\text{THF}$; 6), $\text{BnBr}/\text{K}_2\text{CO}_3/\text{DMF}$; 7), $\text{BBr}_3/\text{CH}_2\text{Cl}_2$; 8), $\text{PhNCO}/\text{Et}_2\text{O}/\text{Na}$; 9), $\text{Pd}(\text{OH})_2/\text{C}$, H_2 ; di-*p*-toluoyl-L-tartaric acid.

Biological evaluation and discussion: Compounds (**15**, **16** and **17**) were tested for *in vitro* inhibitory activity of human erythrocyte AChE and plasma BChE by previously published methodology.^{5,8-15} The results, shown in **Table 2**, in general, are in accord with our studies (**Table 1**, bottom).^{10,13} However, in our current assessment with an emphasis on optical purity, the (-)-enantiomer (**15**) proved to be approximately 2-fold more AChE potent and 10-fold more BChE than our previous data suggested. The AChE selectivity provided by the phenylcarbamate in (-)-phenserine (**2**) (70-fold, **Table 1**, top),^{5,12} having a *N'*-methyl substituent, was dramatically diminished in **15**; which possessed significant and, likely, clinically relevant BChE inhibitory activity when compared to the first approved Alzheimer's disease drug, tacrine (*Cognex*, IC_{50} : AChE 190 ± 40 nM, BChE 47 ± 10 nM).^{1,2,5,12} Particularly interesting are the anticholinesterase activities of (+)-enantiomer (**16**). These are similar to the unpredicted ones found in our prior synthesis, and verify that (+)-*N'*-norphenserine (**16**) is, indeed, a potent inhibitor of AChE with

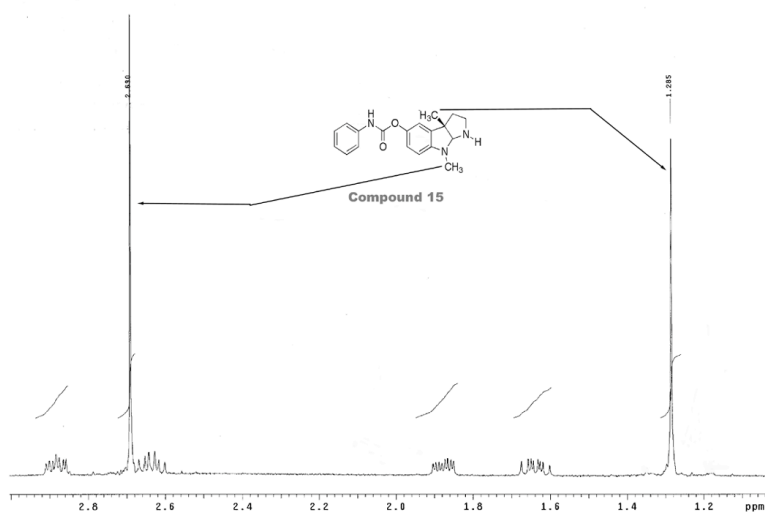
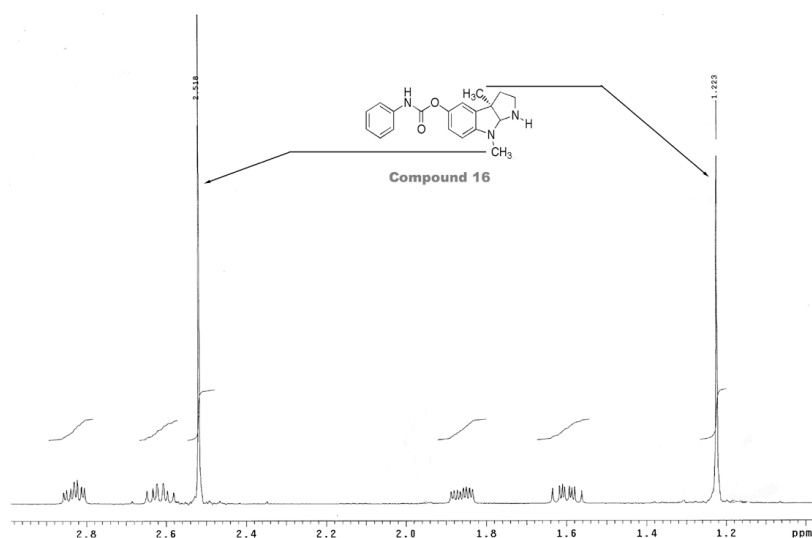
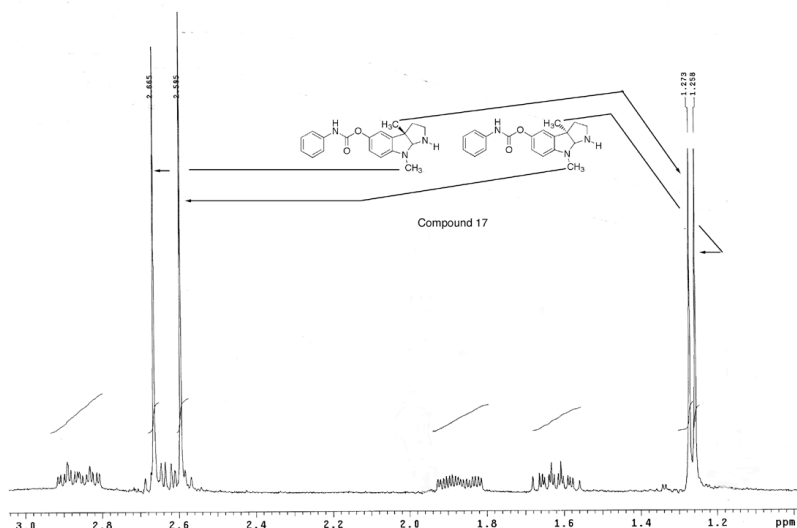


Figure 2: Partially Expanded ^1H -NMR (CDCl_3) (Varian 400) (δ 1.00 - δ 3.00) Spectrum. An equivalent of chemical shift reagent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol was added to each compound.²⁹

(A) (-)-*N'*-Norphenserine (**15**)



(B) (+)-*N'*-Norphenserine (**16**)



(C) (\pm)-*N'*-Norphenserine (**17**).

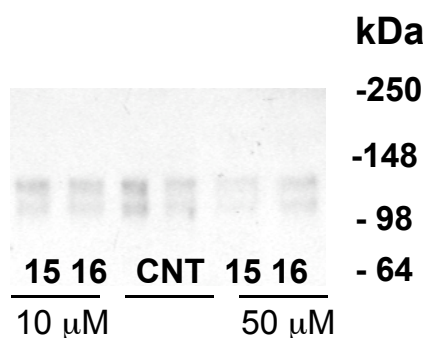
Table 2. (-)-3a*S*-*N*^l-Norphenserine (**15**), (+)-3a*R*-*N*^l-Norphenserine (**16**) and *N*^l-Norphenserine (**17**): Melting Point, Optical Rotation, and Their Inhibitory Activity Versus Human Erythrocyte AChE and Human Plasma BChE.

Compound	mp	[α] _D	IC ₅₀ (nM)AChE	IC ₅₀ (nM)BChE	Selectivity
Fumarate of					
(-)- <i>N</i> ^l -Norphenserine (15)	187-188°C	-44.4° (c=0.3 EtOH) -38.6° (c=0.4 DMSO)	8.1 ± 0.7	54 ± 4.6	7-fold AChE
Fumarate of					
(+)- <i>N</i> ^l -Norphenserine (16)	187-188°C	+44.4° (c=0.3 EtOH) +38.6° (c=0.5 DMSO)	69 ± 24	5895 ± 830	86-fold AChE
Fumarate of					
(±)- <i>N</i> ^l -Norphenserine (17)	191-192°C	none	18.5 ± 0.3	177 ± 37	11-fold AChE

activity comparable to several currently used anticholinesterases (e.g., galantamine, *Reminyl*, IC₅₀: AChE 800 ± 60 nM BChE 7,300 nM; huperzine A AChE 47 ± 22 nM, BChE >10,000 nM; and tacrine: *ibid.*^{1,2,5,12} In light of the AChE activity of (+)-*N*^l-norphenserine, its inactivity against BChE is unexpected, particularly as the (-)-enantiomer (**15**) has reasonable BChE potency. The mechanisms underpinning this are one focus of our current studies and likely relate to the 3-dimensional interaction between the compounds and their binding domains within the cholinesterase proteins.³⁰ This occurs within a narrow gorge of approximately 20 Å depth that extends into the center of these highly homologous (65%) enzymes.^{2,4,6} The *N*^l position of (-)-physostigmine (**1**) and analogues interfaces with the choline binding site within both AChE and BChE and binding likely involves both hydrophobic interactions and hydrogen bonding.² Minor differences in the amino acid sequence that define the differential three-dimensional structures of both enzymes, particularly in the choline binding area, where the binding pocket is known to be physically restrictive,² likely account for the ability of the less size-constrained (-)- and (+)-*N*^l-norphenserine (**15**, **16**) to bind versus active (-)-phenserine (**2**) and inactive (+)- phenserine (**10**). The activity of the racemate (**17**) which demonstrated approximately half the potency of the (-)-enantiomer (**15**), is in accord with expectations. Compounds (**15** and **16**) were additionally assessed for activity to reduce extracellular levels of APP by previously published methodology.^{15,16,31} One of the critical hallmarks of Alzheimer's disease is the presence of extraneuronal senile plaques whose core constituent is amyloid β-peptide,¹⁹ a toxic peptide that is generated from APP processing.^{19,32-34} A reduction in the level of amyloid β-peptide in the brain of afflicted individuals is a

primary focus in the development of drugs to halt or slow the course of neurodegeneration in Alzheimer's disease.³²⁻³⁴ Our previous studies have demonstrated that this can be achieved by lowering APP.¹⁵ As illustrated in **Figure 3**, both **15** and **16**, similar to our prior studies with phenserine (**2**, **10**),^{15,31} decreased secreted APP levels in cultured human neuroblastoma cells; a widely used model of human neural cells.^{15-18,31} We predict that the mechanism underpinning this accomplishment is unrelated to the anticholinesterase action of **15** and **16**, and, as recently demonstrated with phenserine (**2**, **10**),¹⁵ likely relates to interaction in the post-transcriptional events controlling APP synthesis.

Figure 3: Compound (**15**) and (**16**) treatment of SH-SY-5Y human neuroblastoma cells (10 and 50 μ M, 16 h) decreased APP protein levels versus untreated controls (CNT) as assessed by Western blot analysis probed by an *N*-terminal anti-APP antibody (mAb 22C11, Roche Molecular Biochemicals). Two high molecular mass bands were observed that corresponded to alternate forms of APP (100-125 kDa).



In conclusion, the optically pure enantiomers of *N*^{*l*}-norpheneserine (**15**, **16**), together with racemate (**17**), were synthesized and demonstrated potent AChE inhibitory action that was unexpected for the (+)-enantiomer (**16**). Both enantiomers (**15**, **16**) additionally lowered APP levels in a well-established neuronal cell culture model and, together with racemate (**17**) that can be inexpensively synthesized, may warrant further assessment as experimental therapeutics for Alzheimer's disease.

ACKNOWLEDGEMENT

The authors are indebted to Dr. Amy Newman, Medicinal Chemistry Section, National Institute on Drug Abuse, NIH, for use of NMR and polarimeter equipment. D.K.L. is supported in part by NIH grants (AG18379 and AG18884).

REFERENCE

1. N.H. Greig, X.F. Pei, T.T. Soncrant, D.K. Ingram, and A. Brossi, *Med. Chem. Rev.*, 1995, **15**, 3-31.
2. N.H. Greig, K. Sambamurti, Q.S. Yu, T. Perry, H.W. Holloway, F. Haberman, A. Brossi, D.K. Ingram, and D.K. Lahiri, In, *Butyrylcholinesterase: Its Function and Inhibition*, ed. by E. Giacobini, Martin Dunitz Ltd., London, 2003.

3. N.H. Greig, D.K. Lahiri, and K. Sambamurti, *International Psychogeriatrics*, 2002, **14**, Suppl. 1: 77.
4. E. Giacobini, *Cholinesterases and Cholinesterase Inhibitors*, Martin Dunitz, London, 2000.
5. N.H. Greig, E. De Micheli, H.W. Holloway, Q.S. Yu, T. Utsuki, T. Perry, A. Brossi, D.K. Ingram, J. Deutsch, D.K. Lahiri, and T.T. Soncrant, *Acta Neurol Scand*, 2000, **102**, 74.
6. H. Soreq and H. Zakut, *Human Cholinesterases and Anticholinesterases*, Academic Press, New York, NY, 1993.
7. A. Brossi and X. F. Pei, In, *The Alkaloids*, ed. by G.A. Cordell, 1998, **50**, 126.
8. Q.S. Yu, X. F. Pei, H.W. Holloway, N.H. Greig, and A. Brossi, *J. Med. Chem.*, 1997, **40**, 2895.
9. Q.S. Yu, N.H. Greig, H.W. Holloway, and A. Brossi, *J. Med. Chem.*, 1998, **41**, 2371.
10. X.F. Pei, N.H. Greig, J.L. Flippen-Anderson, S. Bi, and A. Brossi, *Helv. Chim. Acta.*, 1994, **77**, 1412.
11. Q.S. Yu, J.R. Attack, S.I. Rapoport, and A. Brossi, *J. Med. Chem.*, 1988, **31**, 2297.
12. Q.S. Yu, X. Zhu, H.W. Holloway, N. Whitaker, A. Brossi, and N.H. Greig, *J. Med. Chem.*, 2002, **45**, 3684.
13. X.F. Pei, N.H. Greig, S. Bi, A. Brossi, and V. Toome, *Med. Chem. Res.*, 1995, **5**, 265.
14. M. Brzostowska, X. S. He, N.H. Greig, and A. Brossi, *Med. Chem. Res.*, 1992, **2**, 238.
15. K.Y.T. Shaw, T. Utsuki, J.R. Rogers, Q.S. Yu, K. Sambamurti, A. Brossi, Y.W. Ge, D.K. Lahiri, and N.H. Greig, *Proc. Natl. Acad. Sc., USA*, 2001, **98**, 7605.
16. D.K. Lahiri, M.R. Farlow, N. Hintz, T. Utsuki, and N.H. Greig, *Acta Neurol Scand*, 2000, **102**, 60.
17. D.K. Lahiri, N.H. Greig, and M.R. Farlow, *Research & Practice in Alzheimer's Disease*, 2001, **5**, 27.
18. D.K. Lahiri, M.R. Farlow, Y.W. Ge, K. Sambamurti, T. Utsuki, and N.H. Greig, In "Mapping the Progress of Alzheimer's and Parkinson's Disease", ed. by Y. Mizuno, A. Fisher, and I. Hanin, Kluwer Academic /Plenum Publishers, 2002, pp. 211-216.
19. K. Sambamurti, N.H. Greig, and D.K. Lahiri, *NeuroMolecular Medicine*, 2002, **1**, 1.
20. P. L. Julian and J. J. Pikl, *J. Am. Chem. Soc.*, 1935, **57**, 755.
21. B. Schönenberger and A. Brossi, *Helv. Chim. Acta.*, 1986, **69**, 1486.
22. Q.S. Yu and A. Brossi, *Heterocycles*, 1988, **27**, 745.
23. Q.S. Yu, C. Liu, M. Brzostowska, L. Chrisey, A. Brossi, N.H. Greig, J.R. Attack, T.T. Soncrant, and H.E. Radunz, *Helv. Chim. Acta.*, 1991, **74**, 761.
24. Q.S. Yu, B. Schönenberger, and A. Brossi, *Heterocycles*, 1987, **26**, 1271.
25. X.F. Pei and A. Brossi, US Patent 5,571,929, 1996 (*Chem. Abstr.*, 1996, 125, 143116).
26. X.F. Pei, Q.S. Yu, B. Y. Lu, N.H. Greig, and A. Brossi, *Heterocycles*, 1996, **42**, 229.
27. X.F. Pei and A. Brossi, *Heterocycles*, 1995, **41**, 2823.
28. T.B.K. Lee and G.S.K. Wong, *J. Org. Chem.*, 1991, **56**, 872.
29. Partially expanded ¹H-NMR (CDCl₃) (Varian 400) (δ 1.00 - δ 3.00) spectrum with an equivalent of chemical shift reagent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol):

Compound (**15**), δ 1.285 (s, 3H, 3a*S*-CH₃), 2.685 (s, 3H, N⁸-CH₃)

Compound (**16**), δ 1.223 (s, 3H, 3a*R*-CH₃), 2.518 (s, 3H, N⁸-CH₃);)

Compound (**17**), δ 1.258 (s, 1 and 1/2H, 3a*R*-CH₃), 1.278 (s, 1 and 1/2 H, 3a*S*-CH₃), 2.595 (s, 1 and 1/2 H, N⁸-CH₃), 2.665 (s, 1 and 1/2 H, N⁸-CH₃).

30. Q.S. Yu, H.W. Holloway, J. Flippen-Anderson, B. Hoffman, A. Brossi, and N.H. Greig, *J. Med. Chem.*, 2001, **44**, 4062.
31. D.K. Lahiri, M.R. Farlow, J.I., Jr. Nurnberger, and N.H. Greig, *Annals of the NY Acad. Sci.*, 1997, **826**, 416.
32. D.K. Lahiri, M.R. Farlow, N.H. Greig, and K. Sambamurti, *Drug Dev. Res.*, 2002, **56**, 267.
33. K. Sambamurti, J. Hardy, L.M. Refolo, and D.K. Lahiri, *Drug Dev. Res.*, 2002, **56**, 211.
34. D.K. Lahiri, M.R. Farlow, K. Sambamurti, N.H. Greig, E. Giacobini, and L.S. Schneider, *Current Drug Targets*, 2003, **4**, 97.