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Synthesis of physostigmine analogues and evaluation of their anticholinesterase activities

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ABSTRACT

A series of physostigmine analogues were prepared and evaluated for cholinesterase inhibition activities, including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Most of them showed potent inhibition activities against AChE, in which compound **17** especially exhibited significantly higher selectivity over BChE than phenserine, a compound currently on clinical trial. Discussion about the relationships between structure and activity of these derivatives was also presented.

Alzheimer's disease (AD) is believed to be the most common cause of dementia among the elderly. It is a disorder with degeneration of the central nervous system, and the major hallmarks are the decline of progressive cognition and loss of memory.[1] The enzyme acetylcholinesterase, one of the well-known enzymes and catalyzes the cleavage of acetylcholine in the synaptic cleft after depolarization, plays an important role in the central nervous system.[2] Several medicines such as donepezil and galantamine (Fig. 1) approved for AD treatment are acetylcholinesterase inhibitors (AChEIs). They can enhance cholinergic neurotransmission by increasing acetylcholine (ACh) availability in the synaptic cleft.

The vast array of secondary metabolites found in nature provide valuable guidance for the discovery of lead compounds or medicines to cure AD.[3] Physostigmine (**1**), also known as eserine, an indole alkaloid isolated from the seeds of *Physostigma venenosum*, is the prototype of acetylcholinesterase inhibitor.[4,5] It drew much of attention from medicinal[6] and synthetic groups[7] due to its unique structural feature and potent biological activity.

One modification to the structure of physostigmine, **1** was the replacement of methyl group by aliphatic alkyl groups or phenyl groups. Among these derivatives of physostigmine, the most attractive compound, phenserine (**3**), is a dual AChE and β-amyloid precursor protein (β-APP) inhibitor being developed to treat mild to moderate Alzheimer's disease.[8] None of the AChE inhibitors currently on the market reduce the level of β-APP, therefore phenserine may represent an important new catalog of compounds for treatment of AD. With the goal of developing potential Alzheimer's pharmacotherapeutics, we synthesized a series of analogues of phenserine (**3**) and physostigmine (**1**). These derivatives mainly contain electron-withdrawn substituent in each position of the phenyl group of the phenylcarbamoyl moieties and they have been not studied in depth previously. We also quantified the inhibitory action of these compounds against cholinesterase. An analysis of the structure/anticholinesterase–activity relationship of these compounds was also presented in the Letter.

The analogues of physostigmine were synthesized by the general method shown as in Scheme 1.[6] Except naphthalenyl and cyclohexyl isocyanate (**16** and **17**) which were prepared according to a known procedure,[9] other isocyanates were commercially available. The physostigmine was hydrolyzed in *n*-butanol to afford eseroline with excellent yield (**2**, 95%). Eseroline then reacted with corresponding isocyanates to achieve target molecules. Since no product was detected when 2,6-diisopropyl phenyl and naphthalenyl isocyannate reacting with eseroline in ether at room temperature, these reactions were performed in tetrahydrofuran (THF) at higher temperature.

To determine the inhibition potency of AChE and BChE activities and selectivity of these physostigmine derivatives bearing substituted phenyl group (**4**–**15**), their effect on cholinesterase was assayed according to spectroscopic Ellmann's method using physostigmine (**1**) and phenserine (**3**) as positive controls.[10] AChE and BChE were freshly prepared from rats brain hippocampal homogenate and rats plasma, and diluted in 0.1 M phosphate buffer (pH 7.4) with ratio of 1:9 and 1:19, respectively.[11] Inhibition of AChE and BChE activities of these synthesized compounds was shown in Table 1.

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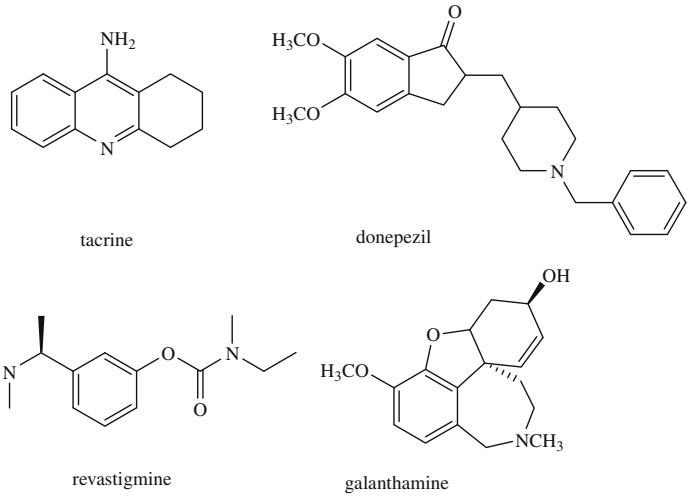
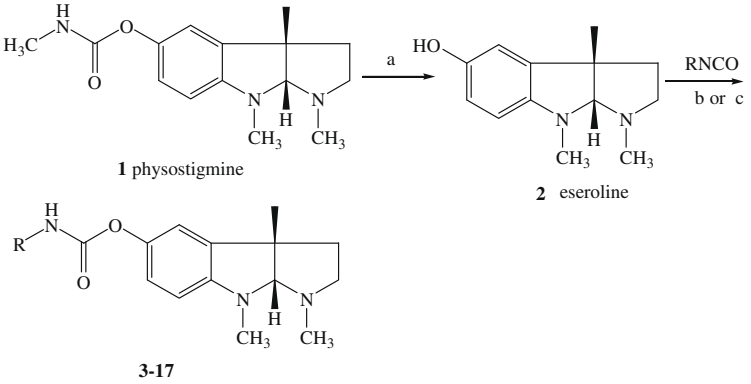
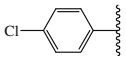
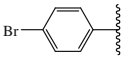
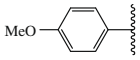
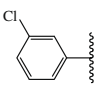
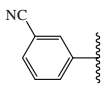
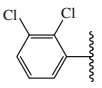
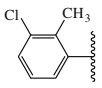
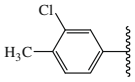
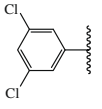
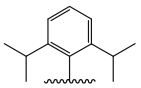
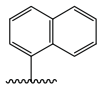


Figure 1. Marketed cholinesterase inhibitors for the treatment of AD.



Compound	R	Compound	R
3		**4**	
5		**6**	
7		**8**	
9		**10**	
11		**12**	
13		**14**	
15		**16**	
17			

Scheme 1. Synthesis of physostigmine analogues **3–17**. Reagent and conditions: (a) *n*-BuOH, Na, rt, 95%; (b) Et_2O, Na, rt, 55–87%; (c) THF, Na, 40 °C, compounds **15** and **16** were prepared under this condition (40% and 51% yield, respectively).

Table 1
50% Inhibitory concentration of compounds against AChE and BChE

No.	IC_{50} (nM)		Selectivity
	AChE	BChE	
1[a]	1.0	7.6	Sevenfold AChE
3[a]	11.9	103.7	10-fold AChE
4	914.3	61.3	15-fold BChE
5	168.1	14.9	Sevenfold BChE
6	29.9	5.8	Fivefold BChE
7	38.7	105.4	Threefold AChE
8	441.8	1021.6	Twofold AChE
9	264.3	88.7	Threefold BChE
10	66.5	39.6	—
11	66.2	516.8	Eightfold AChE
12	6.4	113.5	18-fold AChE
13	7.3	8.5	—
14	113.0	935.3	Eightfold AChE
15	735.0	829.9	—
16	4.2	14.1	Threefold AChE
17	8.5	1397.5	174-fold AChE

[a] Physostigmine and phenserine were used as positive controls.

As shown in Table 1, most of physostigmine derivatives showed potent inhibition against AChE, especially **12** and **13** exhibiting the same level of activities as physostigmine. The reason could be that all these derivatives retained the carbamoyl group, the pharmacophore of physostigmine.[6] Apparently these derivatives only containing electron-withdrawn substituent (Cl, Br, CN) reduced their AChE potencies (**4**, 5, **8**, and **9**) comparing to phenserine (**3**) and its analogues,[6g] In contrast, methyl/methoxyl groups, electron-donor substituent maintained or improved their AChE potencies. In particular, compound **12** displayed nearly twofold improvement in AChE potency and only a marginal decline in BChE activity to afford an AChE selectivity of 18-fold. On the other hand, compound **15**, an analogue with larger size electron-donor substitution (isopropyl) in 2′ and 6′ position of phenyl, almost lost its inhibition against AChE. Furthermore, those analogues with substitution in *para*-position of phenyl reduced their AChE potencies (**4**, **5**, and **6**) comparing to phenserine, even if the substitution was electron-donor. These results clearly showed that small electron-donor substituents in *meta*- or *ortho*-position of phenyl are better choices for the retention of AChE inhibition potency.

To determine whether the phenyl group in the carbamate moiety of physostigmine derivatives is an important factor for the retention of bioactivity and selectivity, compounds **16**–**17** were synthesized and assayed against cholinesterase. The preparation of **17** was reported before,[12] but its bioactivity against cholinesterase was not tested. Very excitingly, both compounds showed higher potent inhibition activity against AChE than phenserine (**3**) (Table 1). Especially, compound **17** exhibited extremely higher selectivity inhibiting AChE over BChE (174-fold BChE) comparing to only 10-fold BChE selectivity seen with phenserine. The analogues of physostigmine, in which the methyl carbamate group substituted by aliphatic alkyl carbamate group, showed potent bioactivity but low selectivity.[6a] Herein, the cyclic alkyl carbamate may be the key moiety for the acquisition of high selectivity plus the retention of bioactivity. Further investigations on this kind of substrates are undergoing and the details will be reported in due course.

In summary, a synthetic physostigmine library was designed and constructed in the study. The anticholinesterase evaluation of these compounds revealed that most of compounds bearing electron-withdrawn substituents in each position of the phenyl group, showed less inhibition against AChE and BChE than phenserine. Compound **17**, a cyclic alkyl carbamate of eseroline, was vetted as a strong inhibitor of AChE with significantly high selectivity over BChE. BChE is a serine hydrolase, produced in the liver and enriched in the circulation. In addition, it is also present in adipose tissue, intestine, smooth muscle cells, white matter of the brain, and many other tissues.[13] Unlike AChE, which plays a vital role in the central and peripheral nervous systems, the physiological function of BChE remains unclear.[14,15] Despite having no identified endogenous substrate, BChE plays a key role in detoxification, by degrading esters such as succinylcholine and cocaine.[16,17] Herein, Compound **17** can be used as a lead compound for further optimization for the potential use of treatment of AD.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.097.

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