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Design, synthesis, evaluation and QSAR analysis of N^1-substituted norcymserine derivatives as selective butyrylcholinesterase inhibitors

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ABSTRACT

We synthesized a series of N^1-substituted norcymserine derivatives **7a**–**p** and evaluated their anti-cholinesterase activities. In vitro evaluation showed that the pyridinylethyl derivatives **7m**–**o** and the piperidinylethyl derivative **7p** improved the anti-butyrylcholinesterase activity by approximately threefold compared to N^1-phenethylnorcymserine (PEC, **2**). A quantitative structure-activity relationship (QSAR) study indicated that log*S* might be a key feature of the improved compounds.

Alzheimer's disease (AD) is one of the primary neurodegenerative diseases that develops in the elderly, and its prevalence is expected to increase substantially in the future.[1] While AD is definitively characterized by its pathological hallmarks, such as senile plaques and neurofibrillary tangles, in the postmortem brain, AD is diagnosed by the progressive cognitive impairment observed in living patients. Since the decline of cholinergic neurotransmission is responsible for cognitive dysfunction, considerable effort has been devoted to developing effective cholinesterase inhibitors (ChEIs).

Acetylcholinesterase (AChE) is the main cholinesterase (ChE) in the brain, and hence most works aimed at developing ChEIs have targeted AChE. To date, some AChE inhibitors have been clinically approved, and use of these inhibitors has been successful in the treatment of AD. However, in the AD brain, cholinergic neurons are lost along with the progression of the disease and AChE is also reduced.[2]

Vertebrates have another acetylcholine (ACh) hydrolase known as butyrylcholinesterase (BuChE). In contrast to AChE, BuChE is mainly derived from glia in the brain.[3] BuChE is therefore not reduced, and its contribution in degrading ACh is thought to be increased in the AD brain.[2] Interestingly, it has been reported that selective BuChE inhibitors (BuChEIs) increase the brain ACh,[4] and BuChE knockout mice and silent mutants in humans showed no physiological disadvantage,[5,6] inspiring the hypothesis that BuChE may be a promising target for developing anti-AD drugs with no adverse effect.

Physostigmine (**1**), an alkaloid isolated from the Calabar bean, the seed of *Physostigma venenosum*, is the most classical ChEI, and still used in the treatment of glaucoma. Although many mechanistic studies have been carried out, clarification of three-dimensional structures of AChE[7] and BuChE[8] have enabled us to investigate the detailed enzyme-inhibitor interaction. For instance, regarding the N^1-position of the physostigmine derivatives, the bulkiness of substituents at this position affects the enantioselectivity at **3a**, and the phenethyl group renders the compounds more selective for BuChE than their corresponding N^1-H and N^1-Me analogs.[9–11] It has also been reported that N^1-phenethylnorcymserine (PEC, **2**) exhibits the property of a potent and selective BuChEI.[12]

Although these findings were intriguing, these studies did not investigate a large variety of substituents and, overall, they were limited to relatively simple compounds. Hence, previous report studying the quantitative structure–activity relationship (QSAR) has pointed out that large polar and large hydrophobic substituents would have to be synthesized and tested.[13] An investigation

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using more diverse derivatives may increase our understanding and help improve the development of better compounds. In light of this, we investigated the structure-activity relationship of the N^1 moiety of norcymserine derivatives **1**, **2**, **3** and **7a**–**p** in this report. Furthermore, for a detailed understanding, we carried out the QSAR study. For all compounds, the carbamate moiety was fixed in the shape of 4-isopropylphenylcarbamate, because this structural arrangement increases the potency and the selectivity for BuChE.[12]

PEC (**2**) and cymserine (**3**) were prepared according to procedures described previously.[9,14] A series of N^1-substituted norcymserine derivatives **7a**–**p** were synthesized via intermediates **5a**–**p** as indicated in Scheme 1. Ammonium iodide (**4**), prepared from physostigmine (**1**) using four steps,[14] was treated with different R–NH_2 compounds (**b**, **d**–**l** and **o**, Scheme 1),[9,11,15] followed by demethylation with BBr_3[9–12,16] to generate N^1-substituted noreserolines (**5b**, **5d**–**l** and **5o**). Alternatively, the intermediate **5** was prepared using only one step in a convenient one-pot procedure.[17] Physostigmine (**1**) was reacted with KOH and MeI in DMSO at room temperature for 1 h, before the addition of different R–NH_2 compounds (**a**, **c**, **m**, **n** and **p**, Scheme 1). After the reaction mixtures were stirred at 120 °C for 3 h, N^1-substituted noreserolines **5a**, **5c**, **5m**, **5n** and **5p** were obtained at 10–21% yield, accompanied by the corresponding methyl ethers **6a**, **6c**, **6m**, **6n** and **6p** at 0–10% yield, respectively. Finally, the N^1-substituted norcymserine derivatives **7a**–**p** were prepared from **5a**–**p** using previously reported methods.[9–12,16]

Anti-ChE activities were measured using a modified Ellman's colorimetric method.[18] Briefly, we used the extracts from mice brain and mice serum as sources of AChE and BuChE, respectively, and all compounds were preincubated with enzymes for 1 h at 37 °C before adding acetylthiocholine or butyrylthiocholine in combination with the coloring reagent 5,5′-dithiobis-(2-nitrobenzoic acid).

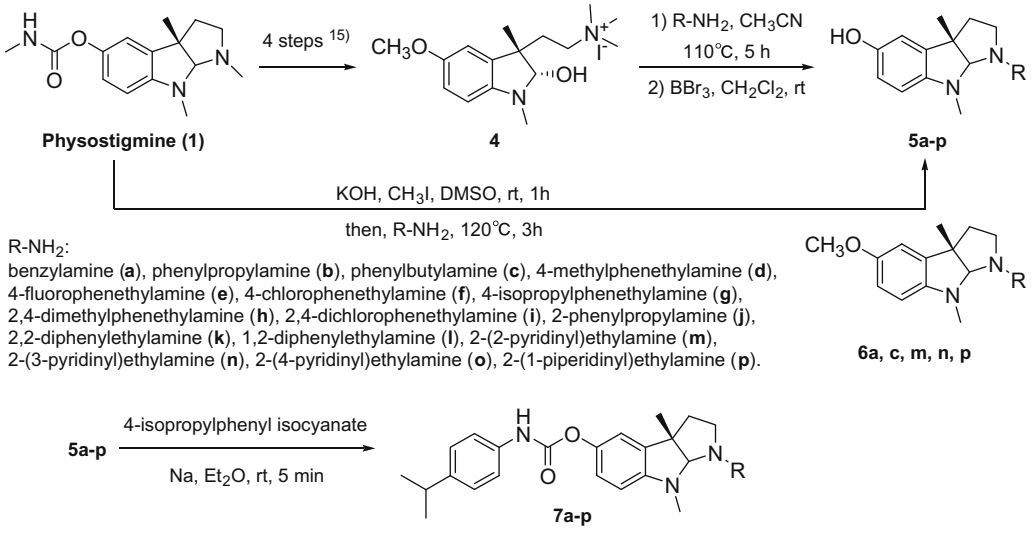
The IC_{50} values for AChE and BuChE are summarized in Table 1. We first assessed whether the length of the alkyl chain had an influence on anti-BuChE activity, and we found that the activity did not differ between the N^1-benzyl, -phenetyl, -phenylpropyl and -phenylbutyl derivatives (**2** and **7a**–**c**). Since the length of the alkyl chain did not influence the activity, we subsequently investigated the effects of various substituents on the phenethyl group. Neither electron-donating nor electron-withdrawing group at the 4-position of the phenyl group influenced the activity (**7d**–**f**). On the other hand, bulky substituent at the same position was shown to reduce the activity (**7g**). Disubstituents at the 2 and 4-position of the phenyl group also lowered the potency of the compounds (**7h and 7i**). The increased bulkiness of the alkyl chain of the phenethyl group also decreased the anti-BuChE activity (**7j**–**l**). Most physostigmine derivatives reported previously did not include a heteroatom in its N^1-substituents. Therefore, we also examined the effects of the substitution of heterocycles for the phenyl ring of the phenethyl group. Substitution of pyridine for the phenyl group increased the anti-BuChE activity by approximately threefold regardless of the position of the nitrogen on the pyridine, and the 1-piperidinylethyl group substitution exhibited a similar activity (**7m–p**).

The QSAR study probing the N^1-position of physostigmine derivatives has ever been conducted using a small dataset, and was used to evaluate for the anti-AChE activity.[19] Therefore, in order to rationalize the observed anti-BuChE activities, we carried out the QSAR study.

For the QSAR study dataset, the structures of all compounds excluding physostigmine, and their corresponding pIC_{50} values for BuChE were used. The chemical structures were drawn using CHEMDRAW Std 7.0 (Chembridge Soft. com) and the database was exported as an sdf file and imported into the MOE software (version 2008.10, Chemical Computing Group). Notably, the calculations were performed using the appropriately ionized form. The QSAR model generation was done using AutoQSAR packaged in MOE, and the QSAR models were constructed based on the partial least square method using 327 descriptors built in MOE. The equation we obtained was shown below:

$$\begin{aligned} pIC_{50} &= 0.452(0.044)\log S - 0.051(0.009)\text{weinerPol} \\ &\quad + 0.010(0.002)\text{PEOE\_VSA-0} \\ &\quad + 0.327(0.049)\text{opr\_violation} + 10.556(0.413) \\ n &= 18, \quad RMSE = 0.080, \quad R^2 = 0.980, \\ &\text{cross-validated } RMSE = 0.187, \\ Q^2 &= 0.909, \quad S = 0.094, \quad F_{4,13} = 160.2 \end{aligned}$$

In the QSAR equation, log*S* is the log of the aqueous solubility, weinerPol is the Wiener polarity number,[20] PEOE_VSA-0 is the sum of the van der Waals surface area of atoms where the atomic partial charges are ranging from −0,05 to 0, opr_violation is the number of violations of Oprea's lead-like test,[21] n is the number of compounds, *RMSE* is the root mean square error, R^2 is the goodness



Scheme 1.

Table 1
Anti-ChE activity and enzyme selectivity

Compounds	R	AChE[a] (nM)	BuChE[a] (nM)	Selectivity[b]
1	Methyl (methylcarbamate, physostigmine)	40	280	0.14
7a	Benzyl	>100,000	510	>196
2	Phenethyl	>100,000	540	>185
7b	Phenylpropyl	>100,000	510	>196
7c	Phenylbutyl	>100,000	550	>182
7d	4-Methylphenethyl	>100,000	730	>137
7e	4-Fluorophenethyl	>100,000	500	>200
7f	4-Chlorophenethyl	>100,000	500	>200
3	Methyl	8400	240	35
7g	4-Isopropylphenethyl	>100,000	3300	>30
7h	2,4-Dimethylphenethyl	>100,000	1100	>91
7i	2,4-Dichlorophenethyl	>100,000	2600	>38
7j	2-Phenylpropyl	>100,000	720	>139
7k	2,2-Diphenylethyl	>100,000	10,000	>10
7l	1,2-Diphenylethyl	>100,000	15,000	>7
7m	2-(2-Pyridinyl)ethyl	4200	160	26
7n	2-(3-Pyridinyl)ethyl	>100,000	180	>556
7o	2-(4-Pyridinyl)ethyl	23,000	170	135
7p	2-(1-Piperidinyl)ethyl	3500	170	21

[a] In order to estimate the enzyme activity, changes in the absorbance at 2 min intervals were measured at 415 nm using a spectrophotometer. The enzyme activity is expressed as a percent of the activity of the solvent, DMSO. Seven different concentrations of each compound were used and the IC_{50} values of each compound were calculated by nonlinear regression of the sigmoidal dose-response curve using GraphPad Prism version 4.03. Only the results with correlation coefficients of $r^2 \geqslant 0.95$ were accepted. The results are represented as the mean of the IC_{50} obtained from at least four independent measurements.

[b] The selectivity was calculated as follows; selectivity = IC_{50} AChE/IC_{50} BuChE.

of fit, Q^2 is the cross-validated R^2, S is the standard error, and F is the ratio of the variance of the calculated values to that of the observed values. Among the descriptors, log$S$ was the relatively most important factor and was positively correlated to the anti-BuChE activity. This descriptor might be greatly influenced by the result that the four most active compounds had an ionizable nitrogen in their N^1-substituents. Interestingly, a report previously described that less hydrophobic substituents are favorable,[13] consistent with our result, although the former report focused on the anti-AChE activity. Since it is considered that a part of the tricyclic moiety itself interacts with Trp82 in the choline binding site (or anionic site), it is not known how the ionizable nitrogen of pyridine or piperidine interacts with BuChE, but it appears to be beneficial to have the polar atom in the N^1 substituent. Moreover, weinerPol descriptor was negatively correlated with the anti-BuChE activity. Since this value is defined as the number of carbon atoms pairs that are separated by three carbon–carbon bonds, its value is generally larger in the branched alkane than in the linear alkane. Namely, this descriptor indicates that branched or spatially bulky substituents might be unfavorable.

In summary, by synthesizing various norcymserine derivatives substituted on the N^1 moiety, and by performing the QSAR study, we found that pyridinylethyl and piperidinylethyl group substitution increased the anti-BuChE activity, and that the ionizable nitrogen of the substituent might contribute to this improvement.

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