Modular mimics of neuroactive alkaloids – design, synthesis, and cholinesterase inhibitory activity of novel rivastigmine analogs

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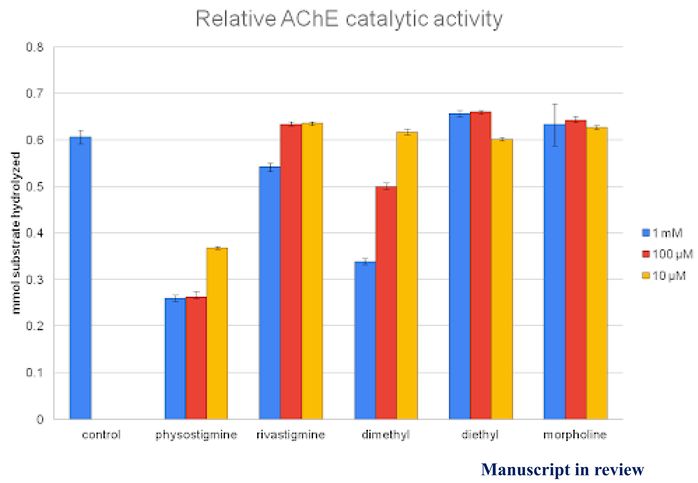
Abstract

For centuries, neuroactive alkaloids isolated from naturally occurring phytochemical sources have been crucial in the identification and optimization of small molecules with potency in treating neurological disorders. While some of these compounds have gone on to clinical use themselves, others have inspired the development of synthetic analogs, which might possess greater potency or better pharmacological features than the natural product itself. One such naturally occurring alkaloid, physostigmine, which is found in the calabar bean plant *Physostigma venenosum*, has been demonstrated to be a potent cholinesterase inhibitor. However, some of physostigmine's characteristics limit its therapeutic potential, prompting the development of its synthetic counterpart, rivastigmine. The research in our group focused on the synthetic optimization of rivastigmine and its analogs, utilizing computer modeling and biological assays to determine the most favorable analog for inhibition of acetylcholinesterase (AChE). Through such studies, it was determined that rivastigmine and its analogs were less effective at inhibiting AChE than physostigmine. This discovery prompted us to pursue two routes: the synthesis of S-enantiopure versions of our analogs with the goal of making more potent analogs and the study of biological activity for all analogs on both cholinesterase enzymes to determine enzyme selectivity.

Results

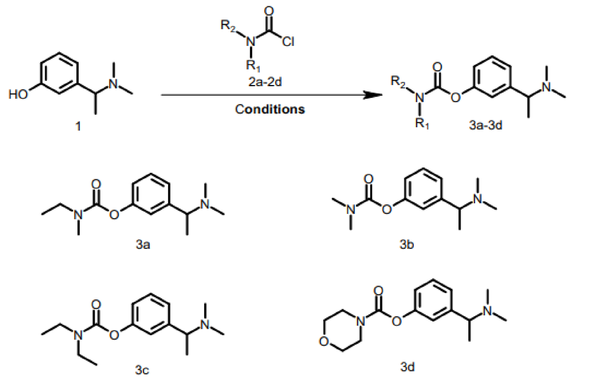


Physostigmine was shown to be the most effective inhibitor. Of the analogs, compound 3b exhibited the highest level of inhibition. Compounds 3a, 3c, and 3d were observed to have minimal effect on the inhibition of acetylcholinesterase, as the results were not significantly different from the control absorbance values.

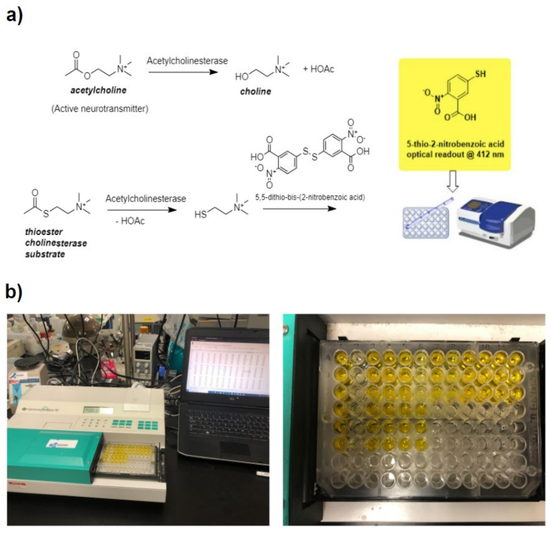
Conclusion

In this study, we synthesized and evaluated a library of rivastigmine analogs for acetylcholinesterase inhibitory activity. Namely, we explored the role of alkyl substitution at the carbamate fragments. Through our experiments, it was determined that physostigmine outperformed rivastigmine and all of our analogs, and that our analog with the smallest alkyl substituents (compound 3b, with an N,N-dimethyl carbamate moiety) performed better than those with bulkier alkyl substituents, and this was consistent with our initial hypothesis, although all of our compounds performed unexpectedly poorly in comparison to physostigmine. Within the 15-minute compound exposure window, the inhibitory activity of rivastigmine at 1 mM was minimal, and almost zero inhibition of AchE was observed at 100 µM and 10 µM of rivastigmine. Similarly, our diethyl analog (compound 3c) and morpholine analog (compound 3d) did not result in any meaningful enzymatic inhibition even at 1 mM. This suggests that the efficacy of these compounds in inhibiting acetylcholinesterase is governed by a steric factor introduced by the alkyl chains attached to the nitrogen of the carbamate. Physostigmine, which only has a n-monomethyl, was the most potent of all the compounds, and this might be either attributed to greater binding affinity, less steric encumbrance at the reaction center, or a combination of these factors.

Methodology



Rivastigmine (3a) and analogs (3b, 3c, 3d) were synthesized through one step acylation of 3-(1-(dimethylamino)ethyl)phenol with the corresponding n, n-dialkyl carbamoyl chloride while avoiding the use of pyrophoric hydride metals. S-enantiopure Rivastigmine and analogs were synthesized using the same methods, but with the use of (S)-3-(1-(dimethylamino)ethyl)phenol.



(a) AChE catalyzes the hydrolysis of acetylcholine into choline and acetic acid. Similarly, AChE catalyzes the hydrolysis of acetylthiocholine into an intermediate that results in the breakdown of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) into 5-thio-2-nitrobenzoic acid, which was used to colorimetrically determine the activity of AChE.
(b) On the left, a plate reader is pictured, which was used to obtain quantitative data regarding absorbance. On the right, a well plate is pictured, containing compounds after DTNB has been added, which results in varying intensities of pigmentation.

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