# Trigonelline and homarine alkyl esters derivatives: synthesis and effects on acetylcholinesterase activity

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## Background

Acetylcholinesterase (AChE), a serine hydrolase enzyme with a key role on acetylcholine-mediated neurotransmission, is considered an important target for the treatment of pathologic conditions associated with a deficit of cholinergic activity. The number of reversible AChE inhibitors with approval for therapeutic application are very limited, and their long-time use is associated with significant side effects. Thus, the demand for new AChE inhibitors has scientific and medical relevance. Trigonelline (1-methylpyridin-1-ium-3-carboxylate) and homarine (1-methylpyridin-1-ium-2-carboxylate) are quaternary ammonium-containing alkaloids. These molecules are synthetized by several marine invertebrates, and experimental data suggest their involvement in defense mechanisms against predators[1], [2], [3] with activation of cholinergic neuronal networks. Thus, trigonelline and homarine were considered suitable molecules templates to obtain new synthetic AChE inhibitors. In this work, four alkyl esters derivatives of trigonelline (TGN-$C_x$) or homarine (HO-$C_x$) were synthesized by a two-step reactional synthesis procedure. Subsequently, their potential to inhibit AChE was assessed using pure enzyme from *Electrophorus electricus*. Cell viability studies were also performed using human neuronal SH-SY5Y cells.

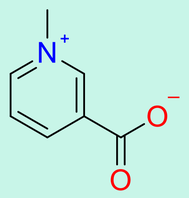

**Fig. 1:** Chemical structure of trigonelline (TGN).

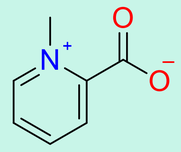

**Fig. 2:** Chemical structure of homarine (HO).

## Methods

### Synthesis of trigonelline derivatives

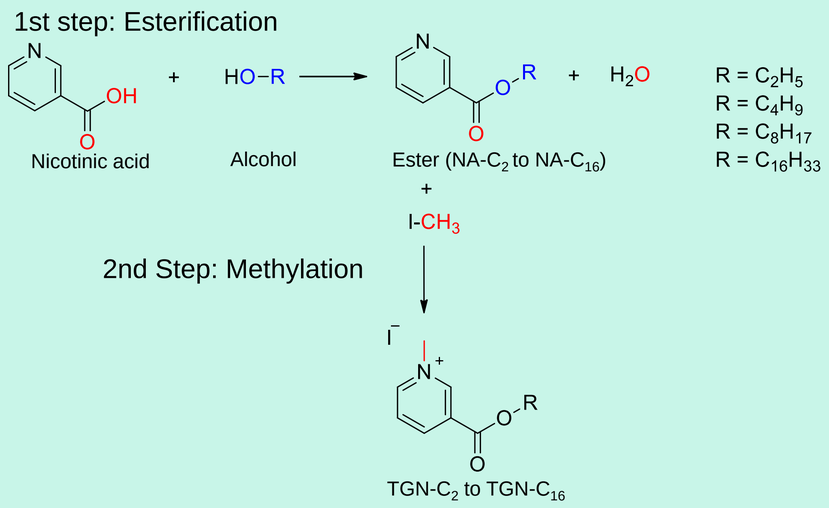


**Fig. 3:** Synthesis of NA- and TGN-derived molecules with different alkyl chain lengths.

### Synthesis of homarine derivatives

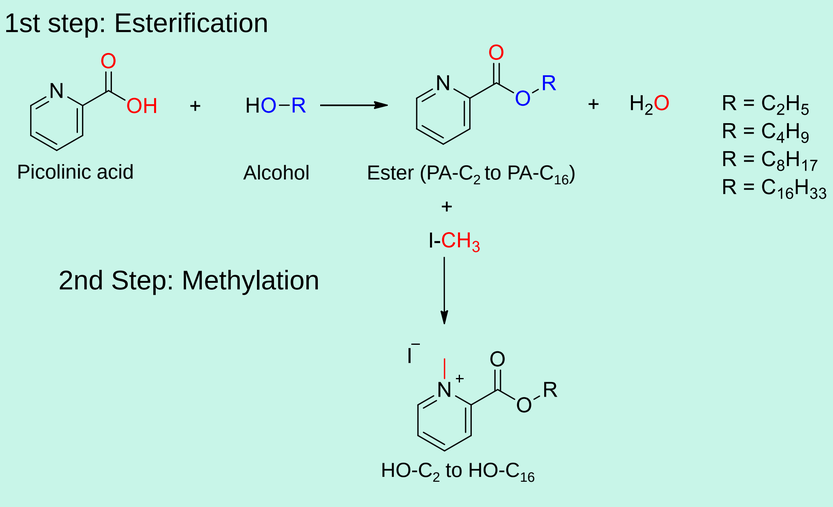


**Fig. 4:** Synthesis of PA- and HO-derived molecules with different alkyl chain lengths.

### AChE inhibition assay

Impact on the activity of AChE from *E. electricus* was assessed using the Ellman's method[4], [5], with slight modifications.

### Cellular assays

**Cellular viability assay:**

Human neuronal SH-SY5Y cytotoxicity was evaluated by the MTT reduction assay[6].

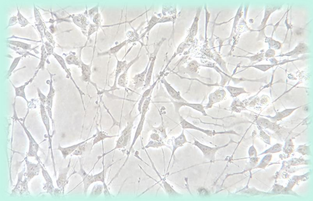

**Fig. 5:** Human neuronal SH-SY5Y cell line.

## Results

### Effects on AChE activity

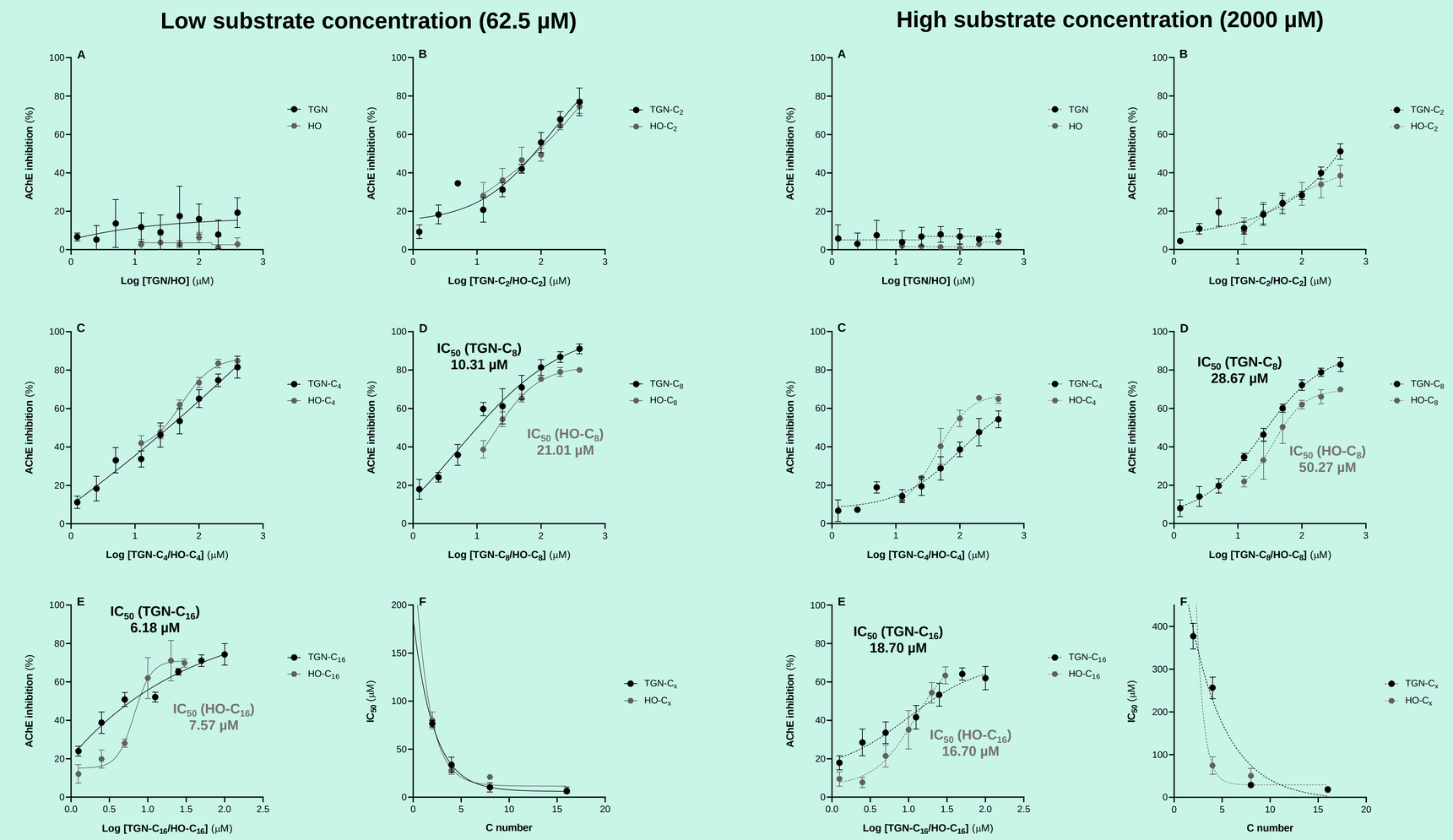


**Fig. 6:** Pure AChE inhibitory activity as function of log concentration of **(A)** trigonelline (TGN) or homarine (HO) and **(B – E)** trigonelline derivatives (TGN-$C_x$) or homarine derivatives (HO-$C_x$) obtained in the presence of the substrate acetylthiocholine iodide (ATCh) at 62.5 µM. **(F)** Relation between the $IC_{50}$ values and the alkyl side chain length of the synthesized compounds.

**Fig. 7:** Pure AChE inhibitory activity as function of log concentration of **(A)** trigonelline (TGN) or homarine (HO) and **(B – E)** trigonelline derivatives (TGN-$C_x$) or homarine derivatives (HO-$C_x$) obtained in the presence of the substrate acetylthiocholine iodide (ATCh) at 2000 µM. **(F)** Relation between the $IC_{50}$ values and the alkyl side chain length of the synthesized compounds.

### Cytotoxicity studies in SH-SY5Y cells

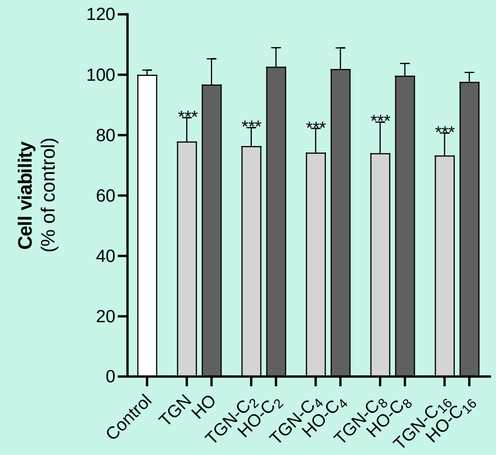


**Fig. 8:** Cellular viability of SH-SY5Y cells in the presence of TGN-$C_x$ or HO-$C_x$ molecules at 50 µM after a 24h incubation period.

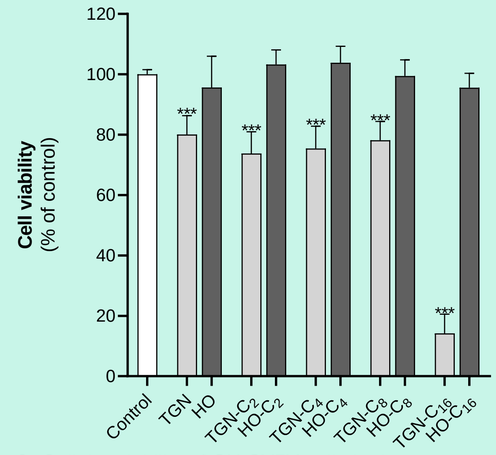


**Fig. 9:** Cellular viability of SH-SY5Y cells in the presence of TGN-$C_x$ or HO-$C_x$ molecules at 100 µM after a 24h of incubation period.

## Conclusions

- ✓ Trigonelline and homarine do not show ability to inhibit pure AChE;
- ✓ In contrast, all synthetic alkyl esters derivatives of trigonelline or homarine significantly inhibit pure AChE at the µM range. The inhibition effectiveness is dependent on the alkyl chain length. Therefore, the alkyl ester chain connected to a quaternary ammonium-containing alkaloid acts as a structural requirement for AChE inhibition;
- ✓ Homarine and all alkyl esters derivatives do not present any detectable cytotoxicity against SH-SY5Y cells;
- ✓ Trigonelline and its shorter alkyl esters show low cytotoxicity for SH-SY5Y cells. However, TGN-$C_{16}$ can only be used at concentrations lower than 50 µM.

**TGN-$C_8$, HO-$C_8$ and HO-$C_{16}$ are the most promising molecules and have potential to be considered in the development of pharmacological approaches to target AChE, with high relevance in neurodegenerative diseases!**

**References**

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**Acknowledgments**

This work was supported through the project UIDB/50006/2020, funded by FCT/MCTES through national funds. Karen G. João thanks FCT (Fundação para a Ciência e Tecnologia) and ESF (European Social Fund) through POCH (Programa Operacional Capital Humano) for her PhD grant PD/BD/135084/2017.