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Microwave-assisted synthesis and butyrylcholinesterase inhibitory activity of new azobenzene derivatives

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pharmaceuticals MDPI

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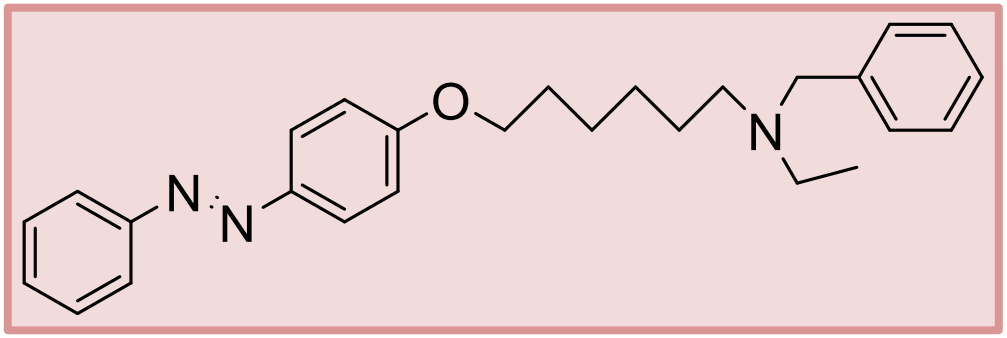
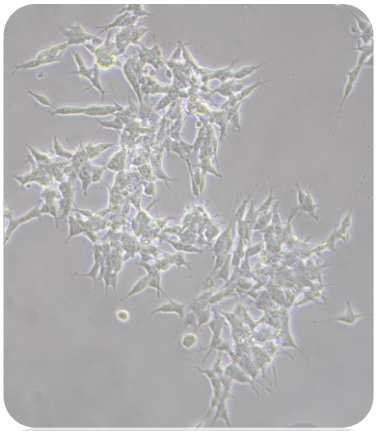


Compound 6: increased inhibition of BChE *in-vitro*



No cytotoxic effect SH-SY5Y cells.

Alzheimer Disease

Abstract:

Cholinesterase inhibitors (ChEis) play an important role enhancing cholinergic synaptic activity and, consequently, have therapeutic applications in the treatment of neurodegenerative diseases like Alzheimer's disease (AD). The inhibition of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) avoids the degradation of the neurotransmitter acetylcholine and constitutes a pharmacotherapeutic strategy that has shown important efficacy reducing AD symptoms.
Based on previous results obtained by our group and to obtain diverse and more effective compounds, a series of new azobenzene derivatives were designed and synthesized. Additionally, considering the powerful AChE inhibition displayed by this type of compound it was decided to expand the research by testing BChE inhibitory activity in this work. Nine azobenzene derivatives, with different spacer lengths (4-8 carbons) and terminal tertiary amines, were synthesized by microwave-assisted synthesis and tested for biological activity *in vitro*. The synthesis was carried out using a microwave oven (in two steps) with a reaction time of around 20 min and moderate to good yields. Also cytotoxic properties of the compounds in SH-SY5Y human neuroblastoma cells were tested.
The inhibitory activity of BChE was determined using Ellman's method. All the compounds synthesized were active against BChE, being the most effective the one with a six-carbon atom spacer and ethylbenzylamine moiety (IC_{50}: 6.621 µM ± 0.001). From these results, we could establish that the optimum length for the spacer was six carbons and that diamines were less active than monoamines.

Keywords: Alzheimer's disease; azobenzene; butyrylcholinesterase; cytotoxic**;** microwave.

Introduction

Alzheimer's disease → Cholinergic deficit → Progressive loss of cognitive functions

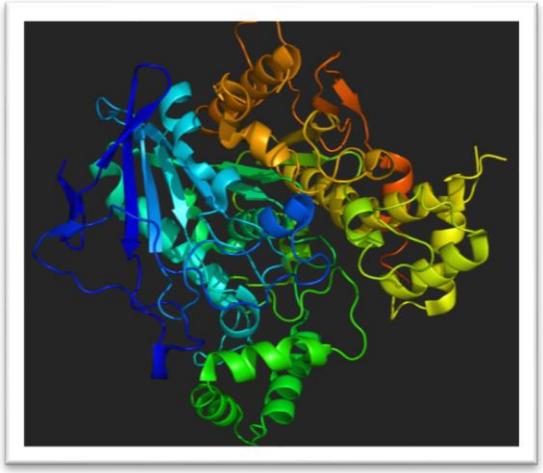
↓

The most effective treatment found to date are cholinesterase inhibitors

↓

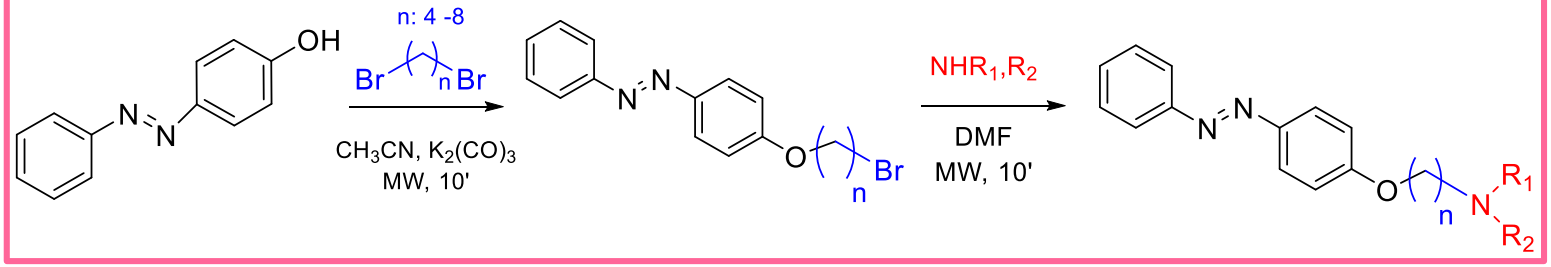
BChE inhibition affects the formation and maturation of the amyloid protein, preventing the formation of neuritic plaques

Based on the results obtained by Biscussi et al. (2021) and in order to obtain diverse and more effective compounds, a series of new azobenzene derivatives with different spacer lengths and terminal amines were synthesized.



Results and discussion

Synthesis strategy



The synthesis was carried out in a microwave oven (in two steps). The derivatives obtained were evaluated as inhibitors of butyrylcholinesterase by the Ellman's method.

Table 1: BChE inhibitory activity values for compounds 1-9 expressed in IC_{50} ± SD (µM)

Comp.	Spacer	Amine	IC_{50} BChE (µM)
1	4	Diethylamine	14.5 ± 1.4
2	5	Diethylamine	9.0 ± 0.7
3	6	Diethylamine	8.2 ± 0.6
4	7	Diethylamine	11.5 ± 1.7
5	8	Diethylamine	11.0 ± 0.8
6	6	methylbenzylamine	6.621 ± 0.001
7	6	methylpiperazine	36.2 ± 0.001
8	6	piperazine	45.8 ± 0.001
9	6	pyrrolidine	7.420 ± 0.001

Compounds were identified and characterized by their ^{1}H and ^{13}C NMR spectra. All the synthesized compounds were active against BChE. The most active turned out to be the derivative of azobenzene with a spacer of 6 carbon atoms and ethylbenzylamine, compound **6** (**Figure 1**).

Enzymatic activity

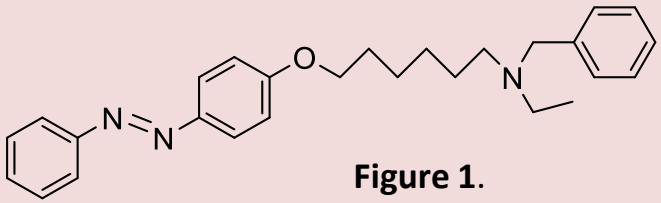
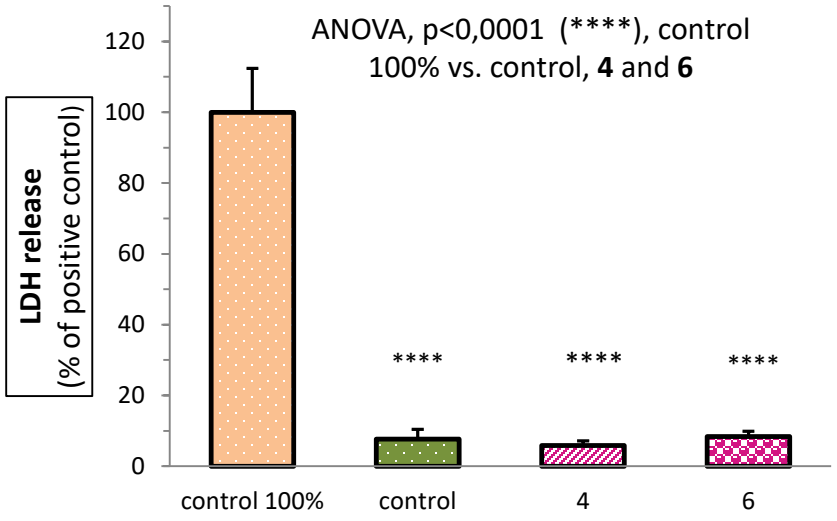


Figure 1.

Figure 2: Cytotoxic effect of **4** and **6** on SH-SY5Y cells.



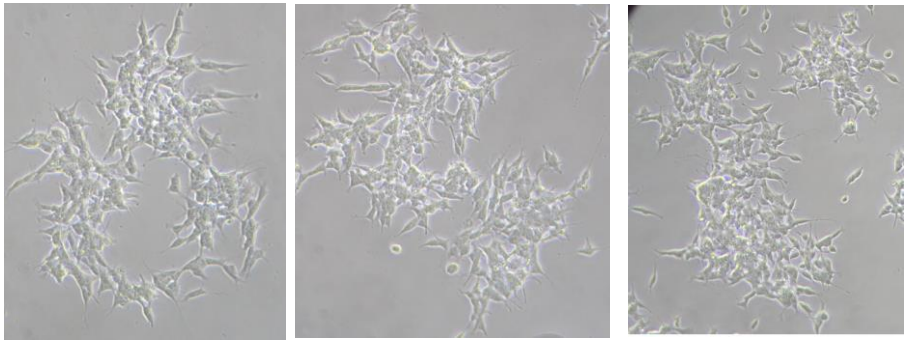
LDH is a soluble enzyme located in the cell cytoplasm, and its release into the cell culture medium is an accepted marker of cell death.

Treatment of SH-SY5Y cells with **4** and **6** for 24 hours did not affect cell morphology (Figure 3)

Cytotoxic activity

In Figure 2, the cell viability of SH-SY5Y cells treated for 24 h with vehicle (DMSO, control), **4** (1.1 µM), and **6** (0.9 µM) was determined. Cell viability was plotted against 100% LDH release (orange bar). Data are expressed as means ± SEM of at least 3 independent experiments. (****) denotes p<0.0001 with respect 100% LDH release (orange bar).

Figure 3: Phase contrast images of SH-SY5Y cells treated for 24 h with vehicle (control), **4**, and **6.**



Control **4** (1,1 µM) **6** (0,9 µM)

Conclusion

The **synthesis of 9 new azobenzene derivatives** with different spacer length and terminal amine was performed. All compounds were active against the BChE enzyme in the micromolar range. **The length of 6-carbon atoms with ethylbenzylamine** was found to be the **most active** combination.

The **cytotoxic effect** of **4** (1.1 µM) and **6** (0.9 µM) was analyzed in SH-SY5Y cells. Treatments of SH-SY5Y cells with **6** and **4**, **did not affect cell viability**. In addition, treatment of SH-SY5Y cells with **4** and **6** for 24 hours did **not affect cell morphology.**

The results obtained offer a starting point for the synthesis of other derivatives with greater activity and potential application in **Alzheimer's therapy.**

Acknowledgments

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