

Evaluation of aromatics guanylhydrazones as inhibitors of acetylcholinesterase by NMR test

Elaine C. Petronilho¹ (PG), Angelo C. Pinto¹ (PQ), José Daniel Figueroa-Villar^{1*} (PQ)

¹ Medicinal Chemistry Group, Department of Chemistry, Military Institute of Engineering, Praça General Tibúrcio, 80, 22290-270, Praia Vermelha, Rio de Janeiro, Brazil.

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Introduction

Alzheimer's disease (AD) is one of the main problems of dementia the world.^{1,2} This disorder leads to decreased activity of cholinergic neurons, a problem that can be treated by increasing the amount of the neurotransmitter acetylcholine (ACh).² The best way to increase the concentration of ACh is by inhibition of the enzyme acetylcholinesterase (AChE). AChE inhibitors used in the treatment of AD are donepezil, galantamine, rivastigmine, and tacrine, but have major side effects. AChE is strongly inhibited by organophosphate and can be reactivated by cationic oximes.³ A molecular modeling study by ab initio method suggest the evaluation of different nucleophiles such as hydrazones.⁴ According to these results, we planned, synthesized and evaluated a new family of guanylhydrazones as potential agents for the treatment of AD by RMN method.

Results and Discussion

The tacrine (**1**) used with standard was obtained commercially and the aromatics guanylhydrazones (**2** to **7**) was prepared by direct reaction of respective aldehyde with aminoguanidine hydrochloride using ethanol and reflux. The structures of compounds tested are shown in figure 1.

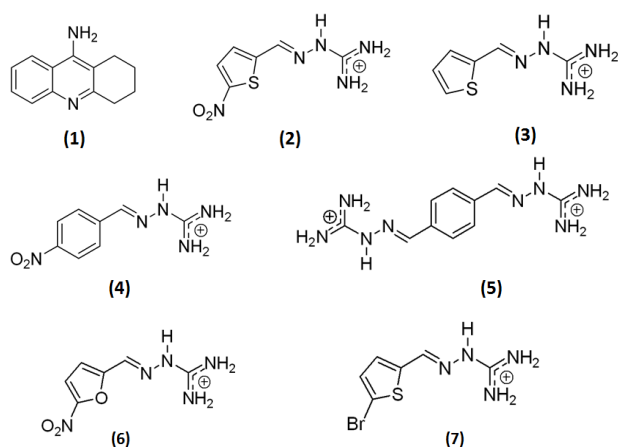


Figure 1. Structure of compounds tested.

The NMR tests were performed by monitoring the methyl signal intensity of ACh (δ 2.24) and the

formed acetate (δ 2.16) along the reaction time. The samples had a 0.2 μ M concentration of *Electrophorus electricus* AChE with 1% of bovine serum albumin (BSA) as a stabilizer, 2.5 mM ACh and 5.0 μ M of the inhibitors (3, 4 or 5), dissolved in a phosphate buffer solution (0.1 M, pH 7.4) in D₂O. The experiments were conducted um a Varian UNITY-600 NMR spectrometer with the temperature controlled as 25.0 \pm 0.1 $^{\circ}$ C. The results are shown in Table 1, indicating that the monitoring by NMR test is efficient.

Table 1. NMR test in presence of guanylhydrazones.

Compound	% of inhibition
1	94.4 \pm 3.5
2	48.6 \pm 1.5
3	12.7 \pm 1.3
4	60.8 \pm 3.2
5	25.0 \pm 3.0
6	35.5 \pm 1.2
7	24.9 \pm 2.2

Conclusions

It was shown that the guanylhydrazones proved to be inhibitors of AChE, especially the compounds 4 potential substances to be used in the treatment of Alzheimer's disease.

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