chemistry proceedings

MDPI

Proceeding Paper

Hybrids of Methylxanthines and Azoderivatives as Acetylcholinesterase Inhibitors: Structure-Activity Relationship Analysis †

Brunella Biscussi *, Santiago Gundin, Martina Donozo and Ana Paula Murray

Instituto de Química del Sur (INQUISUR-CONICET-UNS), Departamento de Química, Universidad Nacional del Sur, Av. Alem 1253, Bahía Blanca 8000, Argentina; san.jo.r@hotmail.com (S.G.); martinadonozo@gmail.com (M.D.); apmurray@uns.edu.ar (A.P.M.)

* Correspondence: brunella.biscussi@uns.edu.ar

† Presented at the 28th International Electronic Conference on Synthetic Organic Chemistry (ECSOC 2024), 15–30 November 2024; Available online: https://sciforum.net/event/ecsoc-28.

Abstract: In this work, we synthesized methylxanthine and azobenzene derivatives, linked to secondary amines via a seven-carbon chain, to evaluate their acetylcholinesterase (AChE) inhibitory activity. Among the azobenzene compounds, **3a** exhibited the highest activity with an IC_{50} of 1.1 μM. Meanwhile, the theobromine derivative **2a**, was the most potent inhibitor among the methylxanthines, with an IC_{50} of 0.19 μM. These results highlight the importance of structure-activity relationship analysis to optimize AChE inhibition by modifying pharmacophore fragments and secondary amines.

Keywords: acetylcholinesterase inhibitors; microwave-assisted synthesis; caffeine derivatives; azobenzene derivatives

Citation: Biscussi, B.; Gundin, S.; Donozo, M.; Murray, A.P. Hybrids of Methylxanthines and Azoderivatives as Acetylcholinesterase Inhibitors: Structure-Activity Relationship Analysis. *Chem. Proc.* **2024**, *6*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2024

Copyright: © 2024 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder, primarily affecting the elderly and characterized by a gradual decline in cognitive functions. A key feature of AD pathology is the degeneration of neurons and brain atrophy, leading to impairments in neurotransmission, particularly involving acetylcholine (ACh) [1]. Acetylcholinesterase (AChE) is an enzyme that regulates ACh levels, and its inhibition has been widely studied as a strategy to alleviate AD symptoms by enhancing cholinergic transmission.

Currently available AChE inhibitors (AChEIs) such as donepezil, rivastigmine, and galantamine temporarily improve cognitive symptoms in AD patients but are not capable of halting disease progression. This has prompted ongoing research to develop new compounds that target multiple biological pathways simultaneously [2]. Our previous work demonstrated that hybrids of caffeine and pyrrolidine were effective AChE inhibitors and could also activate nicotinic acetylcholine receptors (nAChRs), suggesting the potential for multifunctional therapeutic agents [3–5].

In this study, we expanded on this concept by synthesizing new derivatives based on methylxanthines, such as theobromine and theophylline, linked to secondary amines via a seven-carbon chain. Additionally, inspired by the resveratrol scaffold—due to its interesting biological properties and previous findings from our group—we synthesized new azobenzene derivatives, replacing the methylxanthine fragment with azobenzene (Ph-N=N-Ph), known for its photo-modulable properties [6,7]. The primary aim was to perform a structure-activity relationship analysis to evaluate the impact of the pharmacophore (azobenzene or methylxanthine) and the type of amino group (diethylamine or N-

benzylmethylamine) on the inhibitory activity against AChE. This comparison allowed us to assess which combination of these structural features results in more potent AChE inhibitors.

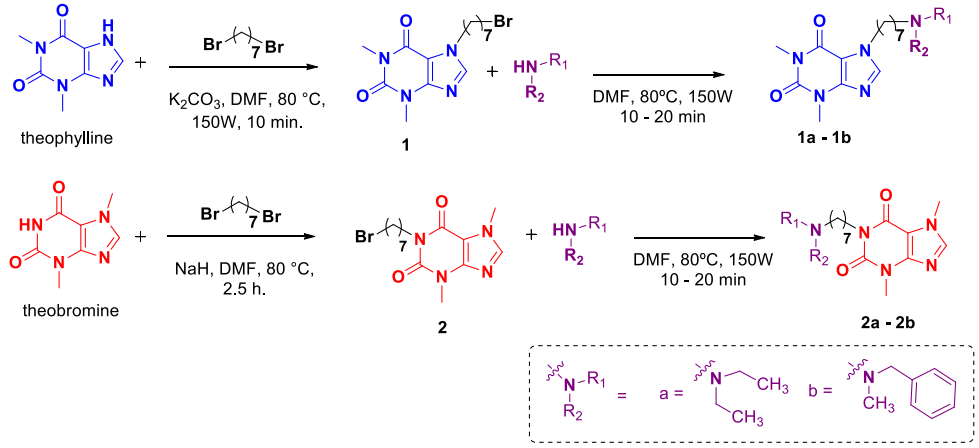
2. Materials and Methods

All the solvents used were purified by distillation and dried over a specific agent (previously activated by heating in an oven). Dimethylformamide (DMF) was distilled and kept over 4A molecular sieves under a nitrogen atmosphere. Column chromatography was carried out with Merck silica gel 60 (0.2–0.63 mm, 240–400 mesh). The progress of the reactions was controlled by using silica gel 60 F 254 chromatofoils (Merck). The development of thin layer chromatograms was performed by visualization with ultraviolet light of wavelengths 254 nm. The alumina (mm) for the column chromatography of azobenzene derivatives was acquired from Merck, Argentina. The TLC detection was carried out using p-anisaldehydeacetic acid spray reagent (Mallinckrodt, New York, NY, USA) and 254 and 366 nm UV light.

Microwave-assisted reactions were performed in a microwave reactor CEM Discover Benchmate oven, CEM Corp, Matthews, NC, USA. All derivatives were rigorously characterized by NMR spectroscopy. ^{1}H and ^{13}C NMR spectra, including COSY, HSQC, and HMBC experiments, were recorded on a Bruker Avance ARX-300 spectrophotometer at room temperature in $CDCl_3$. Chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane (TMS, δ = 0.00 ppm).

2.1. Preparation of Alkyl Brominated Intermediates **1**, **2** *and* **3**

Alkylbrominated intermediates were obtained using theophylline or 4-(phenyldiazenyl)phenol as the starting reagent, as previously reported [4,7]. The same methodology was used to synthesize intermediate **2** by reacting or theobromine with 1,7-dibromoheptane (Scheme 1). Synthesis of 1-(7-bromoheptyl)-3,7-dimethyl-1H-purine-2,6(3H,7H)-dione (**2**): To a solution of theobromine (1.0 mmol) and NaH (1 mmol) in dry DMF (10 mL), 7-dibromoheptane was added (2 mmol). The solution was placed in a 25 mL round-bottom flask equipped with a magnetic stirrer and heated conventionally at 80 °C for 2.5 h with continuous stirring. The solvent was subsequently removed by the addition of distilled H_2O (3 mL) and extraction with dichlomethane (3 × 2 mL). The organic phase was dried over anh. Na_2SO_4, filtered, and the solvent was evaporated to obtain the desired product. The residue was purified by column chromatography on silica gel 60 (70–230 mesh) with dichlomethane/methanol (95:5) to obtain the desired ether (yields of 33%).



Scheme 1. Synthesis of methylxanthine derivatives **1a–1b** and **2a–2b**.

2.2. Preparation of Compounds **1a–1b** *and* **2a–2b**

In a microwave reaction vessel, a solution of compound **1**–**2** (0.1 mmol) in dry DMF (1 mL) was prepared, followed by the addition of diethylamine or benzylmethylamine (0.3 mmol). The glass vial was placed in the microwave reactor at 150 W and 100 °C for 10–20 min until complete conversion was observed by TLC. The mixture was then partitioned between water and CH_2Cl_2. The separated organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was evaporated under reduced pressure, and the crude reaction product was purified by column chromatography using silica gel 60 (0.2–0.63 mm, 240–400 mesh) as the stationary phase and mixtures of CH_2Cl_2/methanol (90:10 and 80:20).

2.3. Preparation of Compounds **3a–3b**

To a solution containing 0.1 mmol of **3** in 1 mL of dry DMF placed in a microwave reaction glass tube containing a magnetic stirrer, it was added 0.3 mmol of diethylamine or N-methylbenzylamine. The glass tube was placed in the microwave reactor at 150 W, 80 °C, for 10 to 40 min until observing total conversion by TLC. The solvent was subsequently removed by the addition of distilled H_2O (3 mL) and extraction with ethyl acetate (3 × 2 mL). The separated organic layer was dried over anhydrous $NaSO_4$ and filtered. The solvent was evaporated under reduced pressure, and the crude reaction product was purified by column chromatography by column chromatography on neutral aluminum oxide (Fluka AG, Bursch SG), using as eluents hexane: ethyl acetate (70:30).

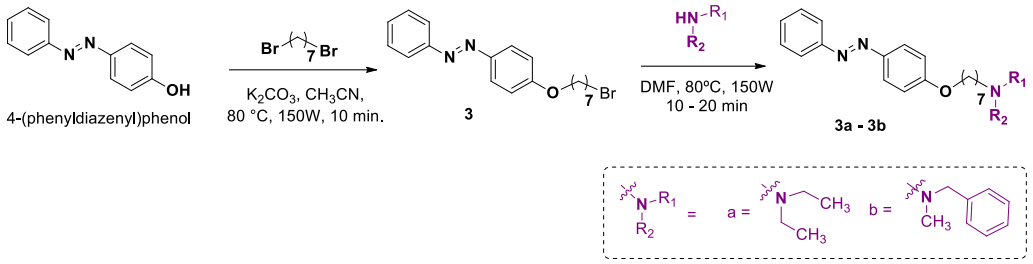
2.4. Cholinesterase Inhibition Assay

AChE from electric eels (500 U, Sigma, Buenos Aires, Argentina) was used as a source of acetylcholinesterase. The inhibitory activity of AChE was determined in vitro using Ellman's spectrophotometric method with minor modifications [8]. The absorbance was recorded at 405 nm for 120 s at 25 °C. Enzymatic activity was calculated by comparing the reaction rates between the sample and the blank. The sample concentration reflecting 50% inhibition (IC_{50}) was calculated by nonlinear rgression of the response curve versus log (concentration) using GraphPadPrism 5. Tacrine was used as the reference inhibitor.

3. Results and Discussion

Based on our group's previous experience, we decided to synthesize new caffeine hybrids by replacing the pyrrolidine fragment with other amino groups. This strategy has proven successful when applied to various molecular scaffolds in prior studies [4–7]. Furthermore, to evaluate the impact of substitution positions in the methylxanthine core (N1 or N7) on biological activity, the natural alkaloids theobromine and theophylline were used as starting materials. The preparation of derivatives was carried out using the procedures shown in Scheme 1. In the first step, the natural alkaloid was reacted with 1,7-dibromoheptane and subsequently with a secondary amine (diethylamine and benzylmethylamine). The length of the linker (n = 7) was chosen based on previous reports, which demonstrated that a seven-carbon linker provides the highest inhibition potency against AChE in caffeine-pyrrolidine hybrids [4]. All derivatives were obtained in very short reaction times using a microwave reactor, achieving good to very good yields. Enzymatic inhibition against AChE was evaluated for compounds **1a–b** and **2a–b** and compared with the activity observed for caffeine and the previously reported caffeine-C7-pyrrolidine hybrid (Table 1). The results show that the potency of the new compounds was higher than caffeine, with derivative **2a** (IC_{50} = 0.188 μM) displaying a significantly higher inhibition potency than the previously reported caffeine-pyrrolidine hybrids. Moreover, the results highlight the importance of the methylxanthine fragment for inhibition potency, as hybrids synthesized from theobromine were more active than their theophylline counterparts.

In addition to these derivatives, two analogs were synthesized by replacing the methylxanthine scaffold with azobenzene, aiming to obtain resveratrol-like derivatives with photo-modulable properties. The synthesis and evaluation of these azobenzene derivatives are illustrated in Scheme 2. The enzymatic inhibition assays revealed that all compounds demonstrated AChE inhibitory capacity. Among the azobenzene derivatives, **3a** ((*E*)-N,N-diethyl-7-(4-(phenyldiazenyl)phenoxy)heptan-1-amine) was the most active, with an IC_{50} of 1.1 μM.



Scheme 2. Synthesis of azobenzene derivatives **3a**–**3b**.

Table 1. Inhibition of cholinesterase activity by theophylline derivatives **1a**–**1b**, theobromine derivatives **2a**–**2b** and azobenzene derivatives **3a**–**3b**.

Compound	Amine	EeAChE IC_{50} (μM) (95% CI)
1a	diethylamine	0.33
2a		0.19
3a		1.1
1b	benzylmethylamine	0.29
2b		0.24
3b		10.2
Caffeine [1]		87.0 [1]
Theophylline [2]		473.02 [2]
Caffeine-C7-pirrolidine [2]		0.22 [2]
Tacrine		0.029

[1] Ref [3]. [2] Ref [4].

4. Conclusions

In summary, the structure-activity relationship study revealed that among the synthesized derivatives, the theobromine-diethylamine hybrid, **2a**, (7-(7-(benzyl(methyl)amino)heptyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione) emerged as the most potent AChE inhibitor, with an IC_{50} of 0.19 μM. This study underscores the superiority of the theobromine pharmacophore over theophylline and azobenzene, as well as the diethylamine group over benzylamine, in the development of potent AChE inhibitors. The findings demonstrate that both the scaffold and the nature of the amino substituents play a crucial role in enhancing inhibitory potency, making theobromine derivatives promising candidates for further optimization in the search for new cholinesterase inhibitors.

Author Contributions:

Funding: This work was supported by CONICET (National Scientific and Technical Research Council), ANPCyT (National Agency for Promotion of Science and Technology) and UNS (Universidad Nacional del Sur).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hampel, H.; Mesulam, M.M.; Cuello, A.C.; Farlow, M.R.; Giacobini, E.; Grossberg, G.T.; Khachaturian, A.S.; Vergallo, A.; Cavedo, E.; Snyder, P.J.; et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain* **2018**, *141*, 1917–1933. https://doi.org/10.1093/brain/awy132.
2. Nasb, M.; Tao, W.; Chen, N. Alzheimer's Disease Puzzle: Delving into Pathogenesis Hypotheses. *Aging Dis.* **2024**, *15*, 43–73. https://doi.org/10.14336/AD.2023.0608.
3. Fabiani, C.; Murray, A.P.; Corradi, J.; Antollini, S.S. A novel pharmacological activity of caffeine in the cholinergic system. *Neuropharmacology* **2018**, *135*, 464–473. https://doi.org/10.1016/j.neuropharm.2018.03.041.
4. Fabiani, C.; Biscussi, B.; Munafó, J.P.; Murray, A.P.; Corradi, J.; Antollini, S.S. New Synthetic Caffeine Analogs as Modulators of the Cholinergic System. *Mol. Pharmacol*. **2022**, *101*, 154–167. https://doi.org/10.1124/molpharm.121.000415.
5. Munafó, J.P.; Biscussi, B.; Obiol, D.; Costabel, M.; Bouzat, C.; Murray, A.P.; Antollini, S. New Multitarget Molecules Derived from Caffeine as Potentiators of the Cholinergic System. *ACS Chem. Neurosci*. **2024**, *15*, 994–1009. https://doi.org/10.1021/acschemneuro.3c00710.
6. Biscussi, B.; Richmond, V.; Javier, C.J.; Arroyo, P.; Murray, A.P. Design and Microwave-Assisted Synthesis of Aza-Resveratrol Analogues with Potent Cholinesterase Inhibition. *CNS Neurol. Disord. Drug Targets* **2020**, *19*, 630–641. https://doi.org/10.2174/1871527319666200905121536.
7. Biscussi, B.; Sequeira, M.A.; Richmond, V.; Mañez, P.A.; Murray, A.P. New photochromic azoderivatives with potent acetylcholinesterase inhibition. *J. Photochem. Photobiol. A Chem*. **2021**, *418*, 113375. https://doi.org/10.1016/j.jphotochem.2021.113375.
8. Ellman, G.; Courtney, K.; Andres, V.; Featherstone, R. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.***1961**, *7*, 88–95. https://doi.org/10.1016/0006-2952(61)90145-9.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.