chemistry proceedings

MDPI

Proceeding Paper

New Caffeine Derivatives as Multitarget Agents for the Therapy of Alzheimer's Disease †

Brunella Biscussi * and Ana Paula Murray

Departamento de Química, Instituto de Química del Sur (INQUISUR-CONICET), Universidad Nacional del Sur, Av. Alem 1253, Bahía Blanca 8000, Argentina

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

† Presented at the 26th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2022; Available online: https://sciforum.net/event/ecsoc-26.

Abstract: In this work we present the microwave-assisted synthesis and in vitro acetylcholinesterase inhibition of a series of new caffeine derivatives. The design of these new compounds was inspired by the caffeine–pyrrolidine hybrids that act as AChE inhibitors and nAChR activators, previously reported by our group. All of the new caffeine analogs inhibited AChE. Among them, the compound **2b** (1,3-dimethyl-7-(6-(piperidin-1-yl)hexyl)-3,7-dihydro-1H-purine-2,6-dione) showed the strongest effect (IC_{50} = 0.17 μM) on AChE, with higher potency than caffeine–pyrrolidine hybrids. These preliminary studies suggest that these new compounds might be interesting multifunctional drugs destined to stimulate cholinergic signage.

Keywords: microwave-assisted synthesis; cholinesterase inhibitors; Alzheimer's disease; acetylcholinesterase; caffeine derivatives; caffeine hybrids

Citation: Biscussi, B.; Murray, A.P. New Caffeine Derivatives as Multitarget Agents for the Therapy of Alzheimer's Disease. *Chem. Proc.* **2022**, *12*, 62. https://doi.org/10.3390/ecsoc-26-13578

Academic Editor: Julio A. Seijas

Published: 14 November 2022

1. Introduction

Alzheimer's disease (AD), the most prevalent neurodegenerative disorder in the elderly, is mainly characterized by progressive cognitive decline. The pathology of AD is characterized by neuronal loss and the atrophy of different brain structures, producing the functional impairment of neurotransmitter systems, specifically the lack of acetylcholine (ACh), leading to progressive cognitive deficit [1]. Cholinesterase (ChE) enzymes regulate ACh levels in the brain. The inhibition of these enzymes increases the level of ACh, and for this reason cholinesterase inhibitors (ChEI) play a very important role in the treatment of neurodegenerative diseases, such as AD.

Current drugs for the treatment of AD, such as tacrine, donepezil, rivastigmine, and galantamine, are used to inhibit AChE [2]. Unfortunately, these drugs can alleviate the symptoms of AD but are unable to prevent disease progression [3,4]. For this reason, the search for new drugs is currently ongoing, focusing on molecules with the ability to act on different targets at the same time. Antollini et al. recently demonstrated that caffeine (naturally occurring xanthine) is an agonist of nAChRs and also inhibits AChE activity [5]. Subsequently, our group synthesized a series of caffeine–pyrrolidine hybrids that were potent AChE inhibitors and activated both muscle as well as α7 nAChRs with high potency [6]. Based on the studies mentioned, the aim of this work was to obtain more potent caffeine analogs. Applying a simple and efficient methodology developed in our research group once again [7,8], a series of new derivatives was synthesized from theophylline as the starting material, which bears similarity to caffeine, and by using different secondary amines (Figure 1). Here, we demonstrate that the synthetized compounds behave as AChE inhibitors with greater potency than previously reported caffeine–pyrrolidine hybrids.

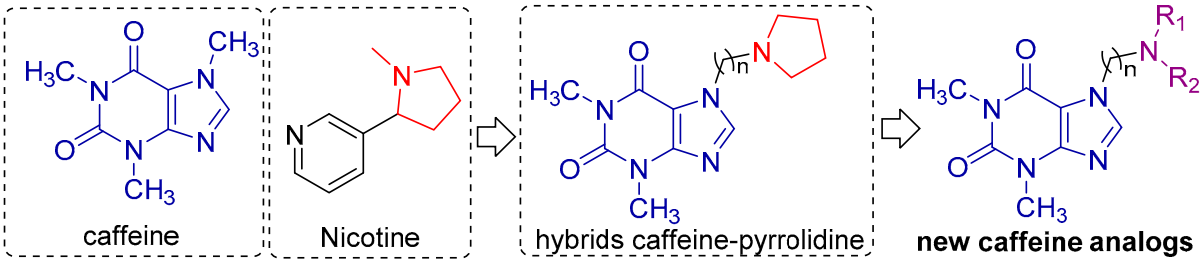


Figure 1. AChE inhibitors, nAChR agonist (nicotine), and caffeine–pyrrolidine hybrids (AChE inhibitors and nAChR modulators) [6], motifs for the design of new multitarget caffeine analogs.

2. Experimental Procedure

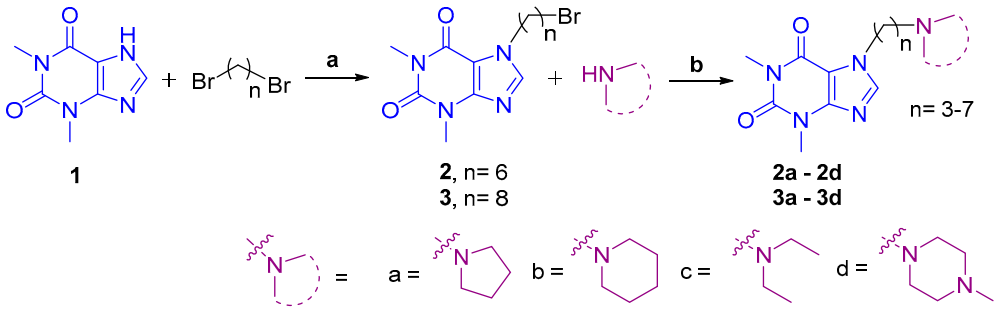
2.1. Materials and Method

All of 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.

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).

2.2. Preparation of Compounds **2**–**3**

Alkylbrominated intermediates were obtained using theophylline as the starting reagent, as previously reported [6]. The same methodology was used to synthesize intermediate 3 by reacting theophylline with 1,8-dibromoctane (Scheme 1). Microwave-assisted synthesis of 7-(8-bromooctyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (**3**): To a solution of theophylline (**1**) (0.1802 g, 1.0 mmol) and anhydrous K_2CO_3 (207 mg, 1.5 mmol) in dry DMF (1 mL), 8-dibromooctane was added (2 mmol). The solution was placed in a 10 mL closed system microwave vessel with a magnetic stirrer and irradiated for 10 min at 80 °C with the following fixed conditions: standard mode, 150 W, 5-minute heating ramp to reach working temperature, medium stirring, and max. power off. 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 (90:10) to obtain the desired ether (yields of 40%).



Scheme 1. Synthesis of derivatives **2a**–**2d** and **3a**–**3d**. (**a**) anh K_2CO_3, dry DMF, and MW; (**b**) dry DMF, MW.

2.3. Preparation of Compounds **2a**–**2d**; **3a**–**3d**

The appropriate amine (0.3 mmol) was added to a solution of compound 2 or 3 (0.1 mmol) in dry DMF (1 mL). The reaction mixture was irradiated (standard method) with 150 W power for 10 minutes at 80 °C in a microwave reactor until the disappearance of the starting compound was detected by TLC. The solvent was then removed via 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 (yields of 50% to 70%). In some cases, flash column chromatography on silica gel with dichloromethane/methanol (70:30) was necessary.

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 [8,9]. 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 regression of the response curve versus log (concentration) using GraphPadPrism 5. Tacrine was used as the reference inhibitor.

3. Results and Discussion

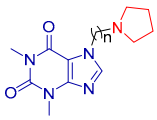
Based on the experience of our group, and with the aim of obtaining more potent caffeine hybrids than those previously reported, we decided to synthesize new analogs by replacing the pyrrolidine fragment with other amino groups. This strategy has proven successful when we have applied it to different molecular scaffolds [7,8].

The preparation of derivatives was carried out using the procedures shown in Scheme 1. In the first step, the natural alkaloid was reacted with the corresponding dibromoalkane (n = 6, 8) and subsequently with a secondary amine (pyrrolidine, piperidine, diethylamine, and 1-methylpiperazine). The length of the linker (n = 6, 8) was chosen based on the previously reported study, where it was shown that the length of the methylene chain of caffeine derivatives influences their inhibition potency on AChE: the greater the length, the higher the inhibitory power [6]. All of the derivatives were obtained in very short reaction times using the microwave reactor and with good to very good yields.

The enzymatic inhibition against AChE was evaluated for compounds **2a**–**2d** as well as **3a**–**3d** and compared to the activity observed for caffeine and caffeine–pyrrolidine hybrids (Table 1).

The results in Table 1 show that the potency of each compound was higher than caffeine, and, more importantly, derivative **2b** (IC_{50} = 0.14 μM) showed a higher inhibition potency than the already reported caffeine–pyrrolidine hybrids. On the other hand, it is noteworthy that the N-methylpiperazine derivatives were the least potent inhibitors of the series.

Table 1. Inhibition of cholinesterase activity by compounds **2a**–**2d**; **3a**–**3d**; and their reported analogs.

Compound	n	Amine	IC_{50} (μM)	Log IC_{50} ± SD
Caffeine			87.0 [1]	1.939 ± 0.0562
	6	Pyrrolidine	6.1 [1]	0.7849 ± 0.0447
	7	Pyrrolidine	0.22 [1]	−0.6655 ± 0.0593
2b	6	Piperidine	0.14	−0.8430 ± 0.02521
2c	6	Diethylamine	1.21	0.08461 ± 0.09466
2d	6	1-methylpiperazine	3.4	0.5325 ± 0.06302
3a	8	Pyrrolidine	0.28	−0.5517 ± 0.04110
3b	8	Piperidine	0.37	−0.4301 ± 0.06577
3c	8	Diethylamine	0.17	−0.7599 ± 0.03920
3d	8	1-methylpiperazine	11.3	1.054 ± 0.02537

[1] IC_{50} values previously determined by our research group [6].

4. Conclusions

A series of new caffeine derivatives was obtained in a sequence of efficient microwave-assisted reactions. The derivative **2b** (n = 6; NHR_2 = piperidine) was found to be the most potent AChE inhibitor of the series (IC_{50} = 0.14 μM), even more than the caffeine–pyrrolidine analogs. These preliminary studies suggest a multifunctional profile for a pharmacophore that may hold promise for the design of new therapies for neurodegenerative diseases. Complementary experiments are currently underway to evaluate the activity of these derivatives on nAChRs.

Author Contributions: Conceptualization, B.B. and A.P.M.; methodology, B.B.; investigation, B.B. and A.P.M.; resources, A.P.M.; writing—original draft preparation, B.B.; writing—review and editing, A.P.M.; project administration, A.P.M.; funding acquisition, A.P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Scientific and Technical Research Council (CONICET, PIP 11220200100834CO), the National Agency for Promotion of Science and Technology (ANPCyT, PICT-2017-1443), and Secretaria General de Ciencia y Técnica de la Universidad Nacional del Sur (SGCyT-UNS, PGI-24/Q105).

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. [CrossRef]
2. Martorana, A.; Esposito, Z.; Koch, G. Beyond the cholinergic hypothesis: Do current drugs work in Alzheimer's disease? *CNS Neurosci. Ther.* **2010**, *16*, 235–245. [CrossRef] [PubMed]
3. Selkoe, D.J. Preventing Alzheimer's disease. *Science* **2012**, *337*, 1488–1492. [CrossRef] [PubMed]
4. Singh, M.; Kaur, M.; Kukreja, H.; Chugh, R.; Silakari, O.; Singh, D. Acetylcholinesterase inhibitors as Alzheimer therapy: From nerve toxins to neuroprotection. *Eur. J. Med. Chem.* **2013**, *70*, 165–188. [CrossRef] [PubMed]
5. 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. [CrossRef] [PubMed]
6. 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. [CrossRef] [PubMed]
7. 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. [CrossRef] [PubMed]

8. 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. [CrossRef]
9. Ellman, G.; Courtney, K.; Andres, V.; Featherstone, R. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95. [CrossRef] [PubMed]