

Proceeding Paper

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

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Abstract: In this work we present the microwave-assisted synthesis and in vitro acetylcholinesterase inhibition of a series 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 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 ($IC_{50} = 0.17 \mu M$) 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.

1. Introduction

Alzheimer's Disease (AD), the most prevalent neurodegenerative disorder in the elderly, is mainly described by a progressive cognitive decline. The pathology of AD is characterized by neuronal loss and atrophy of different brain structures that produce a functional impairment of neurotransmitter systems, specifically the lack of acetylcholine (ACh), leading to a progressive cognitive deficit [1]. Cholinesterase (ChE) enzymes regulate ACh levels in the brain. The inhibition of these enzymes increases the ACh level and for that 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 going on, focused on molecules with the ability to act on different targets at the same time. Recently, Antollini et al. 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 activate both muscle and $\alpha 7$ nAChRs with high potency [6]. Based on the studies mentioned, the aim of this work was to obtain more potent caffeine analogs. Applying once again a simple and efficient methodology developed in our research group [7,8], a series of new derivatives were synthesized from theophylline as starting material, which bears similarity to caffeine, and using different secondary amines (Figure 1). Here we demonstrate that the synthesized compounds behave as AChE inhibitors with greater potency than previously reported caffeine-pyrrolidine hybrids.

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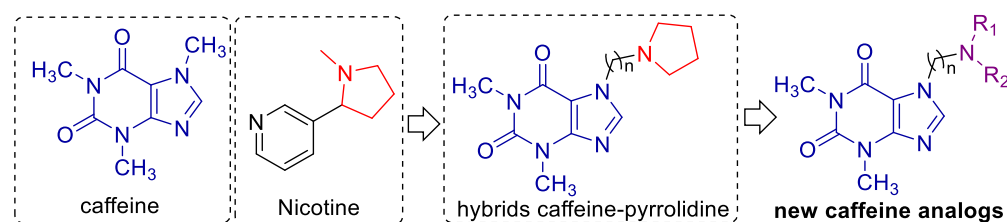


Figure 1. AChE inhibitors, nAChR agonist (nicotine) and caffeine-pyrrolidine hybrids (AChE inhibitors and nAChR modulators) [6], motifs for the design of new multitarget caffeine analogs.

2. Experimental Procedure

2.1. Materials and Method

All 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 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 using silica gel-60 F 254 chromatofolios (Merck). The development of thin layer chromatograms was performed by ultraviolet light $\lambda = 254$ nm.

Microwave assisted reactions were performed in a microwave reactor CEM Discover Benchmate oven, CEM Corp, Matthews, North Carolina, USA. All derivatives were rigorously characterized by NMR spectroscopy. ^1H and ^{13}C NMR spectra including COSY, HSQC, 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, $\delta = 0.00$ ppm).

2.2. Preparation of Compounds 2–3

Alkylbrominated intermediates were obtained using theophylline as starting reagent, as previously reported [6]. The same methodology was used to synthesize intermediate 3 by reacting theophylline with 1,8-dibromooctane (Scheme 1). 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, 150W, 5-minute heating ramp to reach working temperature, medium stirring, max. power off. The solvent was subsequently removed by addition of distilled H_2O (3 mL) and extraction with dichloromethane (3×2 mL). The organic phase was dried over anhydrous Na_2SO_4 , filtered and the solvent was evaporated to afford the desired product. The residue was purified by column chromatography on silica gel 60 (70–230 mesh) with dichloromethane/methanol (90:10) to afford the desired ether (yields of 40%).

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

The appropriate amine (0.3 mmol) was added to a solution of 2–3 (0.1 mmol) in dry DMF (1 mL). The reaction mixture was irradiated (standard method) with 150 W power for 10 min minutes at 80 °C in a microwave reactor until the disappearance of the starting compound was detected by TLC. Then, the solvent was removed by the addition of distilled H_2O (3 mL) and extraction with dichloromethane (3×2 mL). The organic phase was dried over anhydrous Na_2SO_4 , filtered and the solvent was evaporated to afford the desired product (yields 50% to 70%). In some cases, flash column chromatography on silica gel with dichloromethane/methanol (70:30) was necessary.

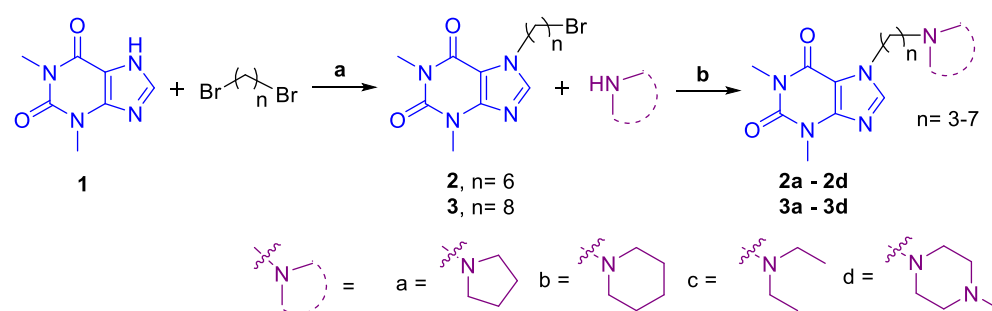
2.4. Cholinesterase Inhibition Assay

AChE from electric eel (500 U, Sigma, Buenos Aires, Argentina) was used as a source of acetylcholinesterase. The inhibitory activity of AChE was determined in vitro using the

Ellman's spectrophotometric method with minor modifications [9,10]. 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].



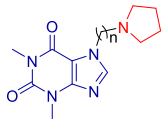
Scheme 1. Synthesis of derivatives **2a–2d**; **3a–3d**. (a) anh K_2CO_3 , dry DMF, MW; (b) dry DMF, MW.

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 inhibitory power [6]. All 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**; **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** ($IC_{50} = 0.14 \mu\text{M}$) 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 analogs reported.

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 IC₅₀ 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; NHR₂ = piperidine) was found to be the most potent AChE inhibitor of the series (IC₅₀ = 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 under progress to evaluate the activity of these derivatives on nAChRs.

Author Contributions:

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