medical sciences forum

MDPI

Proceeding Paper

New Resveratrol Derivatives Exhibit Acetylcholinesterase Inhibitory Properties and Decrease Reactive Oxygen Species Production in the SH-SY5Y Human Neuroblastoma Cell Line †

Martina Donozo [1], Valeria Cavallaro [1], Carlos Javier Baier [2,*] and Ana Paula Murray [1,*]

[1] Instituto de Química del Sur (INQUISUR-CONICET-UNS), Departamento de Química, Universidad Nacional del Sur, Av. Alem 1253, Bahía Blanca, Argentina; email1@email.com (M.D.); email2@email.com (V.C.)

[2] Laboratorio de Toxicología, Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR-CONICET-UNS), Departamento de Biología, Bioquímica y Farmacia (DBByF), San Juan 670, Bahía Blanca B8000ICN, Argentina

* Correspondence: cjbaier@criba.edu.ar (C.J.B.); apmurray@uns.edu.ar (A.P.M.)

† Presented at the 2nd International Electronic Conference on Biomedicines, 1–30 March 2023; Available online: https://ecb2023.sciforum.net/.

Abstract: Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder. Acetylcholinesterase (AChE) inhibitors constitute a pharmacotherapeutic strategy for AD treatment. Resveratrol has anti-inflammatory, neuroprotective, anticarcinogenic, and antioxidant properties. This study aimed to evaluate the AChE inhibitory properties and the protective role against oxidative stress damage of 5 resveratrol analogs (M1 to M5) in SH-SY5Y cells. The studied compounds were not cytotoxic in a wide range of concentrations. Treatment of SH-SY5Y cells at the AChE's IC50s concentration significantly decreased the AChE enzyme activity in live cells. Through the dichlorofluorescein (DCF) assay, 3 compounds decreased the endogenous production of reactive oxygen species (ROS). These results demonstrate that, in addition to their action as biologically active AChE inhibitors, some resveratrol derivatives exhibit neuroprotective effects against endogenous ROS production.

Keywords: resveratrol; Alzheimer's disease; acetylcholinesterase; SH-SY5Y cells; reactive oxygen species

Citation: Donozo, M.; Cavallaro, V.; Baier, C.J.; Murray, A.P.; Gudumac, V.; Tagadiuc, O. New Resveratrol Derivatives Exhibit Acetylcholinesterase Inhibitory Properties and Decrease Reactive Oxygen Species Production in the SH-SY5Y Human Neuroblastoma Cell Line. *Med. Sci. Forum* **2023**, *3*, x. https://doi.org/10.3390/xxxxx

Published: 1 March 2023

1. Introduction

AChE inhibitors (iAChE) improve AD symptoms by inhibiting AChE, raising the levels of acetylcholine (ACh) in the synaptic cleft, constituting a pharmacotherapeutic strategy for reducing AD symptoms [1,2]. Resveratrol (3,5,4′-trihydroxystilbene) is a natural polyphenol, which is widely found in foods and medicinal plants. It exerts a variety of promising biological activities, including anti-inflammatory, neuroprotective, anticancer, and antioxidant properties [3,4]. However, resveratrol has limited pharmacokinetic parameters [5]. This limitation has led to the development multiple strategies aiming to increase its bioavailability and/or solubility through synthesizing resveratrol analogs. In this work, using a simple, rapid, and efficient microwave synthesis, a series of new resveratrol derivatives were synthesized, incorporating secondary amines to increase structural diversity.

2. Materials and Methods

All the chemicals were from Aldrich-Merck and were used without further purification. AChE (from electric eel, type VI-S), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATChI), and tacrine were obtained from Sigma Aldrich.

NMR spectra were recorded on a Bruker Avance ARX-300 spectrophotometer, in CDCl3. A CEM Discover reactor (CEM Corp, Matthews, North Carolina, USA) was employed in microwave-assisted reactions. Alumina (mm) for column chromatography, was acquired from Merck, Argentina. TLC detection was carried out with p-anisaldehyde-acetic acid spray reagent (Mallinckrodt, New York, NY, USA) and 254 and 366 nm UV light.

SH-SY5Y cells were grown in a DMEM/Ham F12 medium (1:1) (Gibco) supplemented with 10% fetal bovine serum (Internegocios), 100 U/mL penicillin (Gibco), and 100 µg/mL streptomycin (Gibco) at 37 °C in a 5% CO_2. For biochemical determinations, 2×10^5 SH-SY5Y cells were seeded in 24 well culture plates (Gibco) and maintained in the conditions described above [7].

Cellular damage/death was analyzed through the activity of the cytoplasmic enzyme lactate dehydrogenase (LDH), which is released by cells with damaged plasma membranes. SH-SY5Y cells, grown as described above for 72 h, were treated with M1–M5 in the growth medium for 24–48h. Then, 100 µL of the medium was collected, and LDH activity was spectrophotometrically determined using a kinetic assay, according to the manufacturer's instructions (Wiener LDH-P UV). LDH release was compared to 100% LDH release (complete lysis) obtained by treating the cells with Triton X-100 0.1% in phosphate-buffered saline (PBS; 150 mM NaCl, 10 mM Na2HPO4, 10 mM, NaH2PO4, pH 7.4) [7,8].

AChE activity was determined according to Ellman´s method [6] with minor modifications [30]. Briefly, SH-SY5Y cells grown in 24 well culture plates for 72h were washed three times with PBS and pre-incubated for 15 min with M1–M5. Then, the cells were maintained for 30 min in the presence of DTNB (final concentration of 0.31 mM in PBS) and ATChI (final concentration of 0.9 mM in PBS). DMSO was used as a vehicle and was kept below 0.1% in all cases. Control cells were incubated in the presence of the vehicle. After 30 min, 0.9 mL of solution was collected from each condition, and absorbance was measured at 412nm. Results are expressed as a percentage of the control.

All results are presented as mean ± SEM from at least three independent experiments. LDH and AChE activity determinations were analyzed using one-way ANOVA followed by Tukey's test for multiple comparisons. The differences were considered to be statistically significant when $p < 0.05$. Analysis of data was performed using GraphPad Prism software.

3. Results

3.1. Chemistry

To obtain a series of resveratrol derivatives, azobenzene was first reacted with the corresponding dibromo alkane (6–8 carbon atoms) and then, the intermediate was reacted with the secondary amine. Five compounds (M1–M5) were synthesized in moderate yields. These compounds were purified by column chromatography and identified by 1H and 13C NMR. The derivatives were evaluated as iAChE by Ellman's method [6].

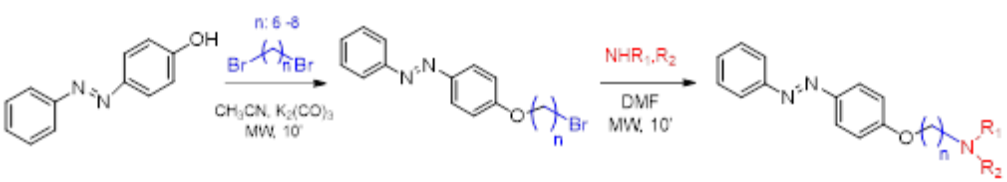
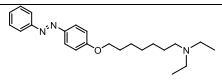
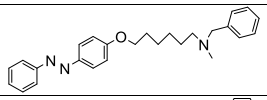
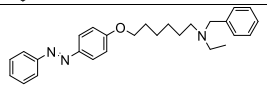
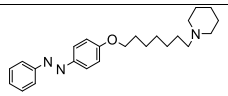
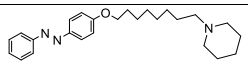


Figure 1.

3.2. In Vitro AChE-Inhibition Studies

Table 1 shows the obtained IC50 ± SD values of the different resveratrol analogs. All of them were found to be active against AChE. The most active, with an IC50 of 0.27 μM, was the derivative with an 8 carbon atoms spacer and a piperidine ring, compound M5.

Table 1. AChE inhibitory activity values for compounds M1–M5 expressed in IC_{50} ± SD (μM).

Comp.	Spacer	Amine	AChE IC_{50} (μM)	Estructure
M1	7	Diethylamine	1.1 ± 0.01	
M2	6	methylbenzylamine	1.4 ± 0.001	
M3	6	ethylbenzylamine	0.9 ± 0.001	
M4	7	Piperidine	0.96 ± 0.001	
M5	8	Piperidine	0.27 ± 0.1	

3.3. Cytotoxicity Assay

The cytotoxic effect of resveratrol analogs was evaluated in the human neuroblastoma cell line, SH-SY5Y [8]. Treatment of SH-SY5Y cells with a concentration equal to 6 times the IC50 (previously determined in vitro enzyme assays) of the tested compounds was not cytotoxic during 24–48h of treatment (data not shown).

3.3.1. Cellular Assay for the Inhibition of Acetylcholinesterase

We used SH-SY5Y cells to evaluate the effects of M1–M5 analogs on AChE activity. For this purpose, SH-SY5Y cells were treated for 24 h with the aforementioned compounds, and AChE activity was determined by Elman's method [40]. All tested compounds significantly inhibited AChE activity in the cell assay (Figure 2). The drug concentration used in this assay corresponded to the IC50 previously determined in vitro [7,8].

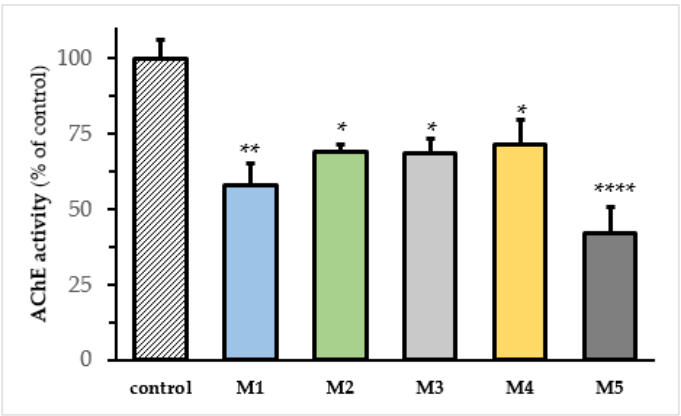


Figure 2. AChE activity in SH-SY5Y cells treated with resveratrol derivatives. AChE activity in SH-SY5Y cells treated for 24h with vehicle (DMSO, control) M1 (1.1 μM), M2 (1.4 μM), M3 (0.9 μM), M4 (0.96 μM) and M5 (0.27 μM), respectively. AChE activity was determined according to Ellman's method in live cells. The activities of the enzyme are expressed as % of control (AChE activity) ±

SEM of at least 7 independent experiments. One-way ANOVA followed by Turkey's test; (*), (***), and (****) denote p values <0.05, <0.001, and <0.0001, respectively.

3.3.2. Antioxidant Activity

The antioxidant activity of M1, M2, M3, M4, and M5, was evaluated in SH-SY5Y cells exposed to H_2O_2 100 μM for 24 h. M1–M5 compounds were administered 24 h before incubation of the cells with H_2O_2 and maintained throughout the experiment [8]. The results are shown in Figure 3. None of the administered compounds showed antioxidant activity when SH-SY5Y cells were exposed to H_2O_2.

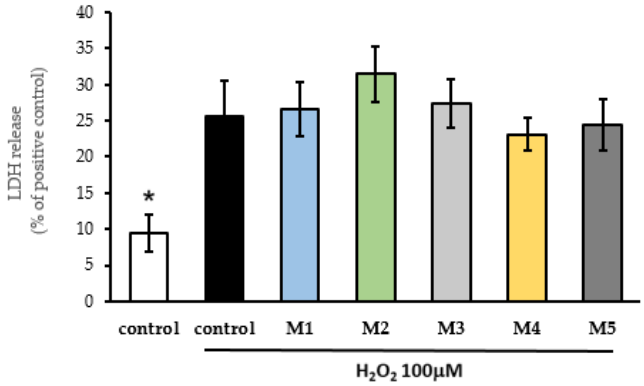


Figure 3. Evaluation of antioxidants properties of resveratrol derivatives against exposure of SH-SY5Y cells to 100 μM H_2O_2. SH-SY5Y cells were pretreated M1 (1.1 μM), M2 (1.4 μM), M3 (0.9 μM), M4 (0.96 μM), and M5 (0.27 μM) for 24 h before the oxidative insult. Afterward, 100 μM H_2O_2 was added to the cell culture and incubated for 24h, in the presence of resveratrol derivatives. Cellular damage was analyzed by an LDH release assay. Data are expressed as a percentage of LDH released compared with the positive control. Data are expressed as the means ± SEM of at least four independent experiments. (*) denote $p < 0.05$ concerning control H_2O_2.

3.3.3. Cellular Protection

We investigated the potential protective activity of M1–M5 against Ca^{2+} overload in SH-SY5Y cells as in Cavallaro et al. [8]. For this purpose, SH-SY5Y cells, previously treated with the aforementioned compounds, were subsequently exposed for 24 h to depolarizing medium containing 70 mM KCl, which induced Ca^{2+} overload and cell death. The M1–M5 were administered 24 h before incubation of the cells with high K^+ and maintained throughout the experiment. Subsequently, LDH release was measured as a marker of cell damage. As shown in Figure 4, only compound M1 was shown to exhibit cytoprotective effects against 70 mM KCl treatment.

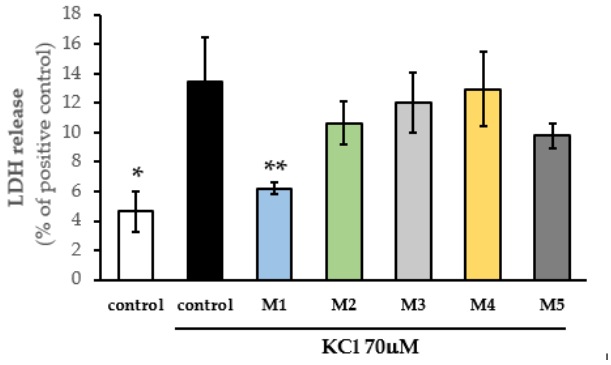


Figure 4. Evaluation of cellular protective effects of resveratrol derivatives against depolarizing concentration of KCl (70 mM) in SH-SY5Y cells. SH-SY5Y cells were pretreated with M1 (1.1 μM), M2

(1.4 μM), M3 (0.9 μM), M4 (0.96 μM), and M5 (0.27 μM) for 24 h before the 70 mM KCl insult. Afterward, a depolarizing concentration of KCl (70 mM) was added to the culture medium and the cells were incubated for 24 additional h, in the presence of resveratrol derivatives. Cellular damage was analyzed by an LDH release assay. Data are expressed as the means ± SEM of at least four independent experiments. (*) and (**) denote $p < 0.05$ and $p < 0.01$ respect of control KCl.

3.3.4. Resveratrol Analogs on Intracellular ROS Production

The influence of M1–M5 was analyzed on the intracellular production of ROS in SH-SY5Y cells. This study was performed using fluorogenic 2′,7′-Dichlorodihydrofluorescein diacetate (H2DCFDA) dye. After the passive diffusion of H2DCFDA into SH-SY5Y cells, it is deacetylated by intracellular esterases to a non-fluorescent compound. In the presence of cellular ROS, it is oxidized into highly fluorescent 2′,7′-dichlorofluorescein (DCF) [9]. Compounds M2, M4, and M5 can reduce ROS production in SH-SY5Y cells. (Figure 5).

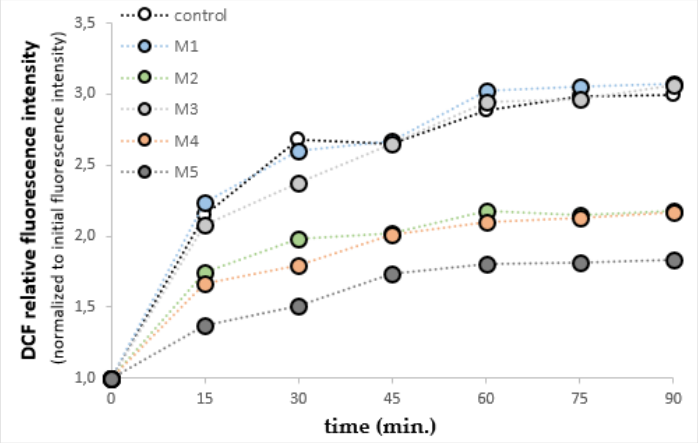


Figure 5. Resveratrol derivatives effects on DCF fluorescence in SH-SY5Y cells. SH-SY5Y cells were treated with M1 (1.1 μM), M2 (1.4 μM), M3 (0.9 μM), M4 (0.96 μM), and M5 (0.27 μM), for 48 h. Then, the cell culture medium was removed by aspiration. After rinsing twice with a warm RPMI medium, the cells were incubated in DCFD 100 μM in RPMI at 37 °C for 1 h. Following the incubation period, the DCFDA-containing medium was replaced with RPMI containing either DMSO or resveratrol derivatives. Fluorescence readings (492 nm excitation and 515 nm emission) were taken at 15-min intervals for 1 h 30 min at 37 °C. Fluorescence at each point was normalized to the initial fluorescence intensity for each experimental condition.

4. Conclusions

The synthesis of 5 new resveratrol analogs, with different spacer lengths and terminal amine, was performed. All the analogs were found to be active against the AChE enzyme (in vitro) in the micromolar range.

These results prompted us to perform several biological assays in SH SY5Y cells. Treatments of SH-SY5Y cells did not affect cell viability. All the compounds tested significantly inhibited AChE activity in cellular assays. In addition to acting as an AChE inhibitor, compound M1 exhibited neuroprotective effects against KCl-induced Ca^{2+} overload. Furthermore, we observed that compounds M2, M4, and M5 have the ability to decrease ROS production in SH-SY5Y cells. These findings indicate that these new resveratrol derivatives could be considered as interesting entities with potential therapeutic applications for AD treatment.

Author Contributions: Conceptualization, C.J.B. and A.P.M.; methodology, M.D., V.C. and C.J.B.; formal analysis, M.D. and C.J.B.; investigation, C.J.B. and A.P.M.; resources, C.J.B. and A.P.M.; data curation, C.J.B. and A.P.M.; writing—original draft preparation, M.D.; writing—review and editing, M.D., C.J.B. and A.P.M.; visualization, M.D. and C.J.B.; supervision, C.J.B. and A.P.M.; project administration, C.J.B. and A.P.M.; funding acquisition, C.J.B. and A.P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by grants from the Secretaría General de Ciencia y Tecnología of Universidad Nacional del Sur PGI 24/B278 (CJB), PGI 24/B297 (CJB), CONICET Grant PIP 11220200102660CO (CJB), PGI 24/Q105 (APM), CONICET Grant PIP 11220200100834CO (APM), FONCYT Grant PICT-2017-1443 N (APM),

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

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

References

1. León, R.; Garcia, A.G.; Marco-Contelles, J. Recent advances in the multitarget-directed ligands approach for the treatment of Alzheimer's disease. *Med. Res. Rev.* **2013**, *33*, 139–189.. https://doi.org/. https://doi.org/10.1002/med.20248. PMID: 21793014.
2. Selkoe, D.J. Preventing Alzheimer's disease. *Science* **2012**, *337*, 1488–1492.
3. Yáñez, M.; Fraiz, N.; Cano, E.; Orallo, F. Inhibitory effects of cis- and trans-resveratrol on noradrenaline and 5-hydroxytryptamine uptake and on monoamine oxidase activity. *Biochem. Biophys. Res. Commun.* **2006**, *344*, 688–695.
4. Baur, J.A.; Sinclair, D.A. Therapeutic potential of resveratrol: The in vivo evidence. *Nat. Rev. Drug Discov.* **2006**, *5*, 493–506.
5. Ahmed, T.; Javed, S.; Javed, S.; Tariq, A.; Šamec, D.; Tejada, S.; Nabavi, S.F.; Braidy, N.; Nabavi, S.M. Resveratrol and Alzheimer's Disease: Mechanistic Insights. *Mol. Neurobiol.* **2017**, *54*, 2622–2635.
6. Ellman, G.L.; Courtney, K.D.; Andres, V., Jr.; Feather-Stone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95.
7. Alza, N.P.; Richmond, V.; Baier, C.J.; Freire, E.; Baggio, R.; Murray, A.P. Synthesis and cholinesterase inhibition of cativic acid derivatives. *Bioorg. Med. Chem.* **2014**, *22*, 3838–3849.. https://doi.org/. https://doi.org/10.1016/j.bmc.2014.06.030. PMID: 25017625.
8. Cavallaro, V.; Baier, C.J.; Murray, M.G.; Estévez-Braun, A.; Murray, A.P. Neuroprotective effects of *Flaveria bidentis* and *Lippia salsa* extracts on SH-SY5Y cells. *S. Afr. J. Bot.* **2018**, *119*, 318–324.. https://doi.org/. https://doi.org/10.1016/j.sajb.2018.10.006.
9. Tetz, L.M.; Kamau, P.W.; Cheng, A.A.; Meeker, J.D.; Loch-Caruso, R. Troubleshooting the dichlorofluorescein assay to avoid artifacts in measurement of 3 toxicant-stimulated cellular production of reactive oxidant species. *J. Pharmacol. Toxicol. Methods* **2013**, *67*, 56–60.