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SHORT COMMUNICATION

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Synthesis and biological evaluation of benzothiazin-4-ones: a possible new class of acetylcholinesterase inhibitors

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ABSTRACT

A series of nineteen benzothiazin-4-ones from *N*-(3-aminopropyl) piperidine, 4-(2-aminoethyl)morpholine or 1-(2-aminoethyl)piperidine, aliphatic or aromatic aldehyde and thiosalicylic acid, were synthesized in good yields by multicomponent one-pot reactions. The solvent was toluene and this efficient procedure afforded the desired heterocycles in 5 h. Identification and characterization were achieved by NMR and GC–MS techniques. *In vitro* AChE activities of all compounds were evaluated in cerebral cortex and hippocampus of rats and in general, the results in cortex were more promising than hippocampus. The benzothiazinone **5Bd** showed the best AChE inhibition activity IC_{50} 8.48 μM (cortex) and IC_{50} 39.80 μM (hippocampus). The cytotoxicity of seven compounds in MCR-5 human fibroblast cell by SRB test in 24 h were evaluated and **5Bd** suggest preliminary safety, showing no cytotoxicity at 100 μM. Finally, these important findings could be a starting point for the development of new AChE inhibitors agents and will provide the basis for new studies.

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Benzothiazinone; propylpiperidine; acetylcholinesterase; fibroblast cells

Introduction

Alzheimer's disease (AD) is a progressive and neurodegenerative disorder and the main cause of dementia affecting older people. The hallmark pathological abnormalities of AD are the formation of amyloid plaques that are due to accumulation of extracellular Aβ and neurofibrillary tangles formed by Tau protein[1–3]. However, the cognitive dysfunction in this neurodegenerative disease also has been associated with loss of cholinergic neurons in many brain regions[4–6].

Acetylcholinesterase (AChE) is a crucial and the most efficient enzyme of the central nervous system with the active site located near at the bottom of a deep and narrow gorge[7]. The principal biological role of the AChE is termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the acetylcholine neurotransmitter[8]. AChE inhibitors are the most promising approaches for treating the symptoms of AD. These drugs are capable to prevent the degradation of acetylcholine and increase the level of this neurotransmitter in the cholinergic synapses improving cognitive deficits[6,9]. However, the adverse effects, as nausea, vomiting, bradycardia and weight loss, associated with AChE inhibitors therapy have limited their clinical efficacy[10].

In this context, the research in this field is required in order to trigger the synthesis of AChE inhibitors compounds with better pharmacological profile and therapeutic efficacy. The biological potential of heterocycles have been widely reported in the literature. Thiazinones are six-membered heterocycles containing nitrogen, sulfur and carbonyl group. Benzothiazinones has a fusion with benzene at 5 and 6 positions of thiazinone ring[11]. These substances represent a class of compounds that have a great scientific interest due to their chemical and biological properties such as antibacterial, antifungal, anti-hypertensive, anti-inflammatory, antirheumatic, aldose reductase inhibitor, antioxidant, anti-HIV, anti-malarial and anti-helminthic activities[12–17].

Therefore, the aims of this study were the application of the multicomponent one-pot strategy to obtain benzothiazinones, the evaluation of the *in vitro* AChE activity on cerebral cortex and hippocampus of rats and cytotoxicity effect against MCR-5 human fibroblast cells. The compound design was based in structure of the neurotransmitter acetylcholine by mimetic its functional groups, as shown in Figure 1.

Experimental

Chemistry

General

Reagents and solvents were use as obtained from commercial suppliers without further purification. Reaction progress was monitored by thin-layer chromatography (TLC) using hexane:ethyl acetate 3:1 mixture as eluent and/or by Shimadzu Gas Chromatograph GC-2010 (HP-1 column crosslinked methyl siloxane, 30 m × 0.32 mm ×0.25 μm: Column head pressure, 14 psi, program: Ti = 60 °C; ti = 2.0 min; rate 10.0 °C min^{-1}; Tf = 280 °C; tf = 40.0 min; Inj. = 250 °C; Det. = 280 °C). ^{1}H and 13C NMR spectra were recorded on a Bruker DRX 400 spectrometer (^{1}H at 400 MHz

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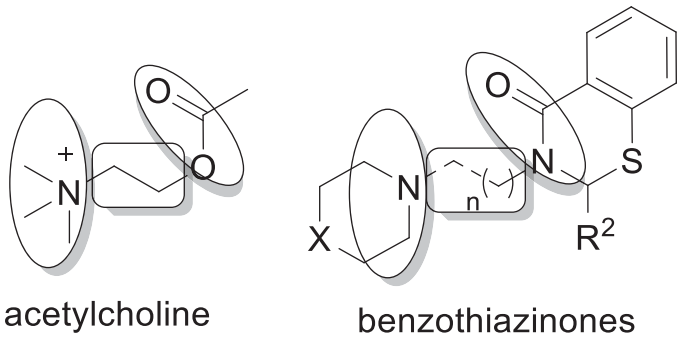


Figure 1. Design of benzothiazinones in comparison with acethylcholine.

and ^{13}C at 100 MHz), on a Bruker Avance 600 spectrometer (^{1}H at 600 MHz and ^{13}C at 150 MHz), or on a Bruker Avance III 600 MHz (^{1}H at 600 MHz and ^{13}C at 150 MHz), in $CDCl_3$ or DMSO containing TMS as an internal standard. The mass spectra were obtained on a Shimadzu GCMS-QP2010SE with a split-splitless injector and equipped with a RDX–SMS capillary column (30 m × 0.25 mm ×0.25 μm); helium was used as the carrier gas (56 kPa).

General procedure for the synthesis of benzothiazinones 5Aa–g, 5Ba–f and 5Ca–f

To a flask with a Dean–Stark apparatus are add 70 ml of toluene, 1 mmol of an aliphatic amine **1A–C** and 1 mmol of corresponding aldehyde (**2a–g**). The 1 mmol thiosalicylic acid **4** is add after 15 min in a preheated (50 °C) reaction mixture due its low solubility. The mixture is maintain in reflux of toluene for 5 h. The organic layer is wash with a saturated solution of NaOH (3 × 30 ml), dry with $MgSO_4$ and the solvent is remove. The crude products are purified by column chromatography using silica and hexane:ethyl acetate (9:1) as eluent.

2-butyl-3-(2-morpholinoethyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Aa

Yield: 77%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H} = Hz): 8.07 (dd, 1H, H7, Ar, 3J = 7.8, ^{4}J = 1.1); 7.34 (td, 1H, H9, Ar, ^{3}J = 7.6, ^{4}J = 1.4); 7.26 (dd, 2H, H8, H10, Ar, ^{2}J = 12.3, ^{3}J = 4.5) 4.53 (dd, 1H, H2, ^{3}J = 9.6, ^{3}J = 5.4); 4.27 (dt, 1H, H11a, ^{2}J = 13.7, ^{3}J = 6.0); 3.22 (dt, 1H, H11b, ^{2}J = 13.7, ^{3}J = 6.8); 3.82–3.66 (m, 4H, H15); 2.69 (dt, 2H, H12a, H12b, ^{2}J = 12.2, ^{3}J = 6.2); 2.62–2.54 (m, 4H, H14); 1.80–1.94 (m, 2H, H17); 1.45–1.38 (m, 2H, H18); 1.30–1.21 (m, 2H, H19); 0.86 (t, 3H, H20, ^{3}J = 7.2). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 162.8 (C4); 134.0 (Ar); 131.8 (Ar); 129.9 (Ar); 129.0 (Ar); 127.8 (Ar); 125.8 (Ar); 66.8 (C15); 61.8 (C2); 57.0 (C12); 53.7 (C14); 45.6 (C11); 34.5 (C17); 29.0 (C18); 21.9 (C19); 13.8 (C20). MS *m/z*: 281 (M^+-53, 0.5%); 248 (0.6%); 220 (1.0%); 113 (47.8%); 100 (100%); 83 (1.6%) 70 (4.5%); 56 (8.9%); 44 (7.5%).

3-(2-morpholinoethyl)-2-phenyl-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Ab

Yield: 81%; oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm, J_{H-H} = Hz): 8.06 (dd, 1H, H7, Ar, ^{3}J = 7.8, ^{4}J = 1.5); 7.23–7.10 (m, 7H, H9, H10, H18, H19, H20, Ar); 7.01 (dd, 1H, H8, Ar, ^{3}J = 7.6, ^{4}J = 1.1) 5.89 (s, 1H, H2,); 4.18 (dt, 1H, H11a, ^{2}J = 13.9, ^{3}J = 5.8); 3.65–3.54 (m, 4H, H15); 3.24 (dt, 1H, H11b, ^{2}J = 13.9, ^{3}J = 6.4); 2.68–2.48 (m, 2H, H12a, H12b); 2.45–2.37 (m, 4H, H14). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.9 (C4); 139.1 (Ar); 132.9 (Ar); 131.9 (Ar); 129.8 (Ar); 129.1 (Ar); 128.4 (Ar); 128.1 (Ar); 127.4 (Ar); 126.2 (Ar); 126.2 (Ar); 67.0 (C15); 62.5 (C2); 57.1 (C12); 53.7 (C14); 45.3 (C11). MS *m/z*: 354 (M^+, 2.9%); 281 (4.6%); 207 (2.4%); 191 (1.6%); 113 (40.9%); 100 (100%); 91 (8.3%) 70 (4.8%); 56 (10.9%); 44 (7.7%).

3-(2-morpholinoethyl)-2-(p-tolyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Ac

Yield: 81%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H} = Hz): 8.12 (dd, 1H, H7, Ar, ^{3}J = 7.8, ^{4}J = 1.3); 7.27 (td, 1H, H9, Ar, ^{3}J = 7.8, ^{4}J = 1.4); 7.21 (td, 1H, H10, Ar, ^{3}J = 7.8, ^{4}J = 1.1); 7.14 (d, 2H, H19, ^{3}J = 8.1); 7.08 (d, 1H, H8, Ar, ^{3}J = 7.7); 7.03 (d, 2H, H18, ^{3}J = 8.0); 5.94 (s, 1H, H2); 4.26 (dt, 1H, H11a, ^{2}J = 13.9, ^{3}J = 5.9); 3.69–3.59 (m, 4H, H15); 3.31 (dt, 1H, H11b, ^{2}J = 13.8, ^{3}J = 6.8); 2.74 (dt, 1H, H12a, ^{2}J = 13.5, ^{3}J = 6.9); 2.64 (dt, 1H, H12b, ^{2}J = 12.8, ^{3}J = 5.8); 2.52 (s, 4H, H14); 2.25 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 164.0 (C4); 138.0 (Ar); 135.9 (Ar); 133.0 (Ar); 131.9 (Ar); 129.7 (Ar); 129.0 (Ar); 129.0 (Ar); 127.4 (Ar); 126.1 (Ar); 126.0 (Ar); 66.7 (C15); 62.4 (C2); 56.9 (C12); 53.6 (C14); 45.2 (C11); 20.9 (CH_3). MS *m/z*: 368 (M^+, 2.9%); 254 (3.3%); 136 (4.0%); 113 (42.7%); 98 (5.2%); 100 (100%); 70 (6.1%); 56 (10.9%); 42 (3.8%).

3-(2-morpholinoethyl)-2-(4-nitrophenyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Ad

Yield: 79%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H} = Hz): 8.13 (dd, 1H, H7, Ar, ^{3}J = 7.8, ^{4}J = 1.4); 8.08 (d, 2H, H19, ^{3}J = 8.8); 7.31 (td, 1H, H9, Ar, ^{3}J = 7.6, ^{4}J = 1.5); 7.25 (td, 1H, H10, Ar, ^{3}J = 7.7, ^{4}J = 1.2); 7.09 (dd, 1H, H8, Ar, ^{3}J = 7.7, ^{4}J = 0.9); 7.46 (d, 2H, H18, ^{3}J = 8.7); 6.11 (s, 1H, H2); 4.27 (dt, 1H, H11a, ^{2}J = 14.1, ^{3}J = 5.5); 3.67 (dtd, 4H, H15, ^{2}J = 15.5, ^{2}J = 10.9, ^{3}J = 4.5); 3.40 (ddd, 1H, H11b, ^{2}J = 13.6, ^{3}J = 7.5, ^{3}J = 5.4); 2.78 (dt, 1H, H12a, ^{2}J = 13.1, ^{3}J = 7.4, ^{3}J = 5.4); 2.68 (dt, 1H, H12b, ^{2}J = 13.2, ^{3}J = 5.4); 2.55 (s, 4H, H14). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 163.7 (C4); 147.4 (C20); 146.7 (Ar); 132.3 (Ar); 131.9 (Ar); 129.9 (Ar); 128.9 (C17); 127.6 (Ar); 127.0 (Ar); 126.7 (Ar); 123.5 (Ar); 66.7 (C15); 61.9 (C2); 57.1 (C12); 53.6 (C14); 45.4 (C11). MS *m/z*: 399 (M^+, 2,6%); 313 (0.4%); 286 (0.5%); 245 (0.1%); 136 (3.6%); 113 (17.4%); 100 (100%); 90 (2.0%); 70 (3.4%); 56 (8.8%); 42 (2.1%).

2-(4-methoxyphenyl)-3-(2-morpholinoethyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Ae

Yield: 50%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.12 (d, 1H, H7, Ar, ^{3}J = 7.8); 7.28 (td, 1H, H9, Ar, ^{3}J = 7.5, ^{4}J = 1.5); 7.21 (t, 1H, H10, Ar, ^{3}J = 7.6); 7.18 (d, 2H, H19, Ar, ^{3}J = 8.7); 7.09 (d, 1H, H8, Ar, ^{3}J = 7.7); 6.75 (d, 2H, H18, Ar, ^{3}J = 8.7); 5.94 (s, 1H, H2); 4.25 (dt, 1H, H11a, ^{2}J = 13.6, ^{3}J = 5.9); 3.72 (s, 3H, OCH_3); 3.72–3.66 (m, 4H, H15); 3.33 (dt, 1H, H11b, ^{2}J = 13.8, ^{3}J = 6.8); 2.74 (dt, 1H, H12a, ^{2}J = 13.4, ^{3}J = 6.9); 2.64 (dt, 1H, H12b, ^{2}J = 12.8, ^{3}J = 5.8); 2.53 (s, 4H, H14). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 164.0 (C4); 159.4 (Ar); 131.9 (Ar); 130.8 (Ar); 129.7 (Ar); 127.4 (Ar); 127.4 (Ar); 126.0 (Ar); 113.7 (Ar); 66.7 (C15); 62.2 (C2); 56.9 (C12); 55.1 (OCH_3); 53.6 (C14); 45.1 (C11). MS *m/z*: 384 (M^+, 4.4%); 351 (0.6%); 270 (6.0%); 245 (0.1%); 113 (38.0%); 121 (11.5%); 100 (100%); 91 (2.2%); 70 (4.5%); 56 (11.1%); 42 (2.2%).

2-(4-fluorophenyl)-3-(2-morpholinoethyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Af

Yield: 88%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.12 (dd, 1H, H7, Ar, ^{3}J = 7.8, ^{4}J = 1.3); 7.28 (td, 1H, H9, Ar, ^{3}J = 7.6, ^{4}J = 1.5); 7.25–7.20 (m, 3H, H10, H19, Ar); 7.09 (dd, 1H, H8, Ar, ^{3}J = 7.7, ^{4}J = 0.9); 6.94–6.87 (m, 2H, H18, Ar); 5.96 (s, 1H, H2); 4.25 (dt, 1H, H11a, ^{2}J = 14.0, ^{3}J = 5.7); 3.68 (dtd, 4H, H15, ^{2}J = 15.7, ^{2}J = 11.1, ^{3}J = 4.6); 3.34 (dt, 1H, H11b, ^{2}J = 13.7, ^{3}J = 6.8); 2.73 (dt, 1H, H12a, ^{2}J = 13.3, ^{3}J = 6.8); 2.63 (dt, 1H, H12b, ^{2}J = 12.9, ^{3}J = 5.9);

2.52 (s, 4H, H14). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm, J_{C-F} =Hz): 163.8 (C4); 162.3 (d, C20, $^1J = 247.8$); 134.9 (d, C17, $^3J = 3.0$); 132.7 (Ar); 132.0 (Ar); 129.8 (Ar); 129.0 (Ar); 127.9 (d, C19, $^3J = 8.3$); 127.5 (Ar); 126.3 (Ar); 115.2 (d, $^2J = 21.8$); 61.9 (C2); 66.8 (C15); 57.0 (C12); 53.6 (C14); 45.3 (C11). MS *m/z*: 372 (M^+, 5,2%); 150 (1.1%); 136 (7.9%); 114 (8.5%); 113 (50.0%); 109 (11.1%); 100 (100%); 86 (6.6%); 70 (6.2%); 56 (15.4%); 42 (3.4%).

2-(4-(Methylsulfonyl)phenyl)-3-(2-morpholinoethyl)-2,3-dihydro-4Hbenzo[e][1,3]thiazin-4-one 5Ag

Yield: 78%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.13 (d, 1H, H7, Ar, $^3J = 7.0$); 7.79 (d, 2H, H19, Ar, $^3J = 8.4$); 7.47 (d, 2H, H18, Ar, $^3J = 8.2$); 7.31 (td, 1H, H9, Ar, $^3J = 7.5$, $^4J = 1.2$); 7.25 (td, 1H, H10, $^3J = 7.3$, $^4J = 0.7$); 7.09 (d, 1H, H8, Ar, $^3J = 7.5$); 6.09 (s, 1H, H2); 4.31 (dt, 1H, H11a, $^2J = 13.8$, $^3J = 5.7$); 3.73–3.64 (m, 4H, H15); 3.37 (dt, 1H, H11b, $^2J = 13.9$, $^3J = 6.7$); 2.99 (s, 3H, H21); 2.80 (dt, 1H, H12a, $^2J = 13.1$, $^3J = 6.7$); 2.71 (dt, 1H, H12b, $^2J = 12.9$, $^3J = 6.2$); 2.57 (s, 4H, H14). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 163.7 (C4); 145.7 (Ar); 140.1 (Ar); 132.3 (Ar); 132.0 (Ar); 129.9 (Ar); 128.7 (Ar); 127.5 (Ar); 127.4 (Ar); 127.0 (Ar); 126.6 (Ar); 66.5 (C15); 61.7 (C2); 56.8 (C12); 53.5 (C14); 45.3 (C11); 44.2 (C21).

2-butyl-3-(3-(piperidin-1-yl)propyl)-2,3-dihydro-4H-benzo[e][1,3]-thiazin-4-one 5Ba

Yield: 50%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.07 (d, 1H, H7, Ar, $^3J = 7.8$); 7.34 (td, 1H, H9, Ar, $^3J = 7.6$, $^4J = 1.3$); 7.23 (t, 2H, H8, H10, Ar, $^3J = 7.6$) 4.58 (dd, 1H, H2, $^3J = 8.4$, $^3J = 6.5$); 4.24 (dt, 1H, H11a, $^2J = 13.5$, $^3J = 6.3$); 3.05 (dt, 1H, H11b, $^2J = 13.5$, $^3J = 7.1$); 2.62 (dt, 1H, H12a, $^2J = 12.6$, $^3J = 7.9$); 2.56–2.34 (m, 5H, H12b, H14); 1.93 (dt, 2H, H13a, H13b, $^2J = 13.5$, $^3J = 6.5$); 1.63 (td, 4H, H15, $^2J = 11.2$, $^3J = 5.6$); 1.88–1.81 (m, 2H, H17); 1.49–1.45 (m, 2H, H18); 1.33–1.22 (m, 2H, H19); 0.86 (t, 3H, H20, $^3J = 7.2$). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 162.7 (C4); 133.9 (Ar); 131.6 (Ar); 129.8 (Ar); 129.2 (Ar); 127.7 (Ar); 125.8 (Ar); 61.3 (C2); 55.3 (C13); 54.1 (C14); 46.6 (C11); 34.5 (C17); 28.8 (C18); 25.5 (C15); 25.0 (C12); 24.1 (C16); 21.8 (C19); 13.7 (C20). MS *m/z*: 346 (M^+, 1.3%); 290 (7.8%); 164 (3.9%); 136 (6.5%); 124 (6.4%); 112 (17.7%); 98 (100%); 84 (19.5%); 70 (6.1%); 56 (3.5%); 41 (8.3%).

2-phenyl-3-(3-(piperidin-1-yl)propyl)-2,3-dihydro-4H-benzo[e][1,3]-thiazin-4-one 5Bb

Yield: 52%; oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.14 (dd, 1H, H7, Ar, $^3J = 7.8$, $^4J = 1.5$); 7.28–7.19 (m, 7H, H9, H10, H18, H19, H20, Ar); 7.08 (dd, 1H, H8, Ar, $^3J = 7.8$, $^4J = 1.0$); 6.05 (s, 1H, H2); 4.32 (ddd, 1H, H11a, $^2J = 13.4$, $^3J = 6.8$, $^3J = 5.3$); 3.04 (dt, 1H, H11b, $^2J = 13.5$, $^3J = 6.2$); 2.64 (dt, 1H, H12a, $^2J = 12.5$, $^3J = 8.0$); 2.51–2.30 (m, 5H, H12b, H14); 2.03–1.84 (m, 2H, H13a, H13b); 1.59 (dt, 4H, H15, $^2J = 11.1$, $^3J = 5.7$); 1.45 (s, 2H, H16). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.9 (C4); 139.3 (Ar); 132.8 (Ar); 131.8 (Ar); 129.7 (Ar); 129.4 (Ar); 128.4 (Ar); 128.0 (Ar); 127.3 (Ar); 126.1 (Ar); 126.0 (Ar); 61.9 (C2); 55.3 (C13); 54.2 (C14); 46.8 (C11); 25.9 (C15); 25.1 (C12); 24.3 (C16). MS *m/z*: 366 (M^+, 12.7%); 347 (3.4%); 289 (4.4%); 136 (5.8%); 124 (7.4%); 118 (9.1%); 112 (12.7%); 98 (100%); 84 (15.6%); 70 (4.3%); 55 (6.1%); 41 (6.0%).

3-(3-(Piperidin-1-yl)propyl)-2-(p-tolyl)-2,3-dihydro-4H-benzo[e][1,3]-thiazin-4-one 5Bc

Yield: 43%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.12 (dd, 1H, H7, Ar, $^3J = 7.9$, $^4J = 1.3$); 7.26 (dt, 1H, H9, Ar, $^3J = 7.6$, $^4J = 2.0$); 7.20 (td, 1H, H10, Ar, $^3J = 7.7$, $^4J = 1.2$); 7.12 (d, 2H, H19, $^3J = 8.1$); 7.08 (dd, 1H, H8, Ar, $^3J = 7.7$, $^4J = 0.7$); 7.03 (d, 2H, H18, $^3J = 8.0$); 5.95 (s, 1H, H2); 4.30 (dt, 1H, H11a, $^2J = 13.3$, $^3J = 6.5$); 3.04 (dt, 1H, H11b, $^2J = 13.6$, $^3J = 6.9$); 2.72 (dt, 1H, H12a, $^2J = 12.5$, $^3J = 7.9$); 2.47–2.56 (m, 5H, H12b, H14); 2.03–1.98 (m, 2H, H13a, H13b); 2.24 (s, 3H, CH_3); 1.67 (dt, 4H, H15, $^2J = 11.2$, $^3J = 5.7$); 1.51–1.48 (m, 2H, H16). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 164.1 (C4); 137.9 (Ar); 135.9 (Ar); 133.0 (Ar); 131.9 (Ar); 129.6 (Ar); 129.1 (Ar); 127.4 (Ar); 126.0 (Ar); 126.0 (Ar); 61.6 (C2); 55.1 (C13); 53.9 (C14); 46.4 (C11); 25.0 (C15); 24.5 (C12); 23.8 (C16); 20.9 (CH_3). MS *m/z*: 380 (M^+, 11.1%); 347 (2.6%); 289 (3.1%); 192 (0.5%); 164 (0.9%); 136 (9.3%); 112 (11.7%); 105 (11.4%); 98 (100%); 84 (13.7%); 70 (6.0%); 55 (6.5%); 41 (10.0%).

2-(4-nitrophenyl)-3-(3-(piperidin-1-yl)propyl)-2,3-dihydro-4H-benzo[e] [1,3]thiazin-4-one 5Bd

Yield: 52%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.12 (d, 1H, H7, Ar, $^3J = 7.9$); 8.06 (d, 2H, H19, Ar $^3J = 8.7$); 7.41 (d, 2H, H18, Ar, $^3J = 8.7$); 7.28 (t, 1H, H9, Ar, $^3J = 7.4$); 7.23 (t, 1H, H10, Ar, $^3J = 7.6$); 7.07 (d, 1H, H8, Ar, $^3J = 7.7$); 6.23 (s, 1H, H2); 4.38 (ddd, 1H, H11a, $^2J = 13.6$, $^3J = 6.7$, $^3J = 6.1$); 3.08 (dt, 1H, H11b, $^2J = 12.7$, $^3J = 7.9$); 2.78 (dt, 1H, H12a, $^2J = 12.7$, $^3J = 7.9$); 2.69–2.43 (m, 5H, H12b, H14); 2.13–1.96 (m, 2H, H13b, H13b); 1.68 (dt, 4H, H15, $^2J = 11.1$, $^3J = 5.5$); 1.51–1.48 (m, 2H, H16). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 163.8 (C4); 146.7 (C20); 132.3 (Ar); 132.0 (Ar); 129.9 (Ar); 129.0 (Ar); 127.5 (Ar); 127.0 (Ar); 126.6 (Ar); 123.6 (Ar); 61.2 (C2); 54.9 (C13); 54.0 (C14); 46.7 (C11); 25.1(C15); 24.5 (C12); 23.8 (C16). MS *m/z*: 411 (M^+, 5.0%); 378 (0.7%); 355 (0.7%); 327 (1.4%); 281 (5.1%); 207 (14.1%); 191 (1.7%); 163 (2.2%); 136 (5.6%); 112 (9.0%); 98 (100%); 84 (11.6%); 70 (4.7%); 55 (5.5%); 41 (6.4%).

2-(4-methoxyphenyl)-3-(3-(piperidin-1-yl)propyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Be

Yield: 47%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.12 (dd, 1H, H7, Ar, $^3J = 7.8$, $^4J = 1.2$); 7.27 (td, 1H, H9, Ar, $^3J = 7.4$, $^4J = 1.4$); 7.20 (t, 1H, H10, Ar, $^3J = 7.6$); 7.15 (d, 2H, H19, Ar, $^3J = 8.8$); 7.08 (d, 1H, H8, Ar, $^3J = 7.7$); 6.75 (d, 2H, H18, Ar, $^3J = 8.7$); 5.96 (s, 1H, H2); 4.28 (dt, 1H, H11a, $^2J = 13.1$, $^3J = 6.4$); 3.79 (t, 1H, H11b, $^3J = 4.8$); 3.72 (s, 3H, OCH_3); 3.05 (dt, 1H, H12a, $^2J = 13.9$, $^3J = 7.1$); 2.65 (dt, 1H, H12b, $^2J = 12.5$, $^3J = 8.0$); 2.01–1.93 (m, 2H, H13a, H13b); 2.50–2.39 (m, 4H, H14); 1.63 (dt, 4H, H15, $^2J = 11.2$, $^3J = 5.6$); 1.46 (s, 2H, H16). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 163.9 (C4); 159.3 (Ar); 133.0 (Ar); 131.8 (Ar); 130.9 (Ar); 129.6 (Ar); 129.1 (Ar); 127.4 (Ar); 127.4 (Ar); 126.0 (Ar); 113.7 (Ar); 61.5 (C2); 55.3 (C13); 55.1 (OCH_3); 54.1 (C14); 46.5 (C11); 25.4 (C15); 24.8 (C12); 24.0 (C16). MS *m/z*: 396 (M^+, 8.9%); 363 (1.9%); 312 (1.0%); 289 (2.1%); 278 (1.2%); 257 (1.5%); 136 (3.9%); 121 (9.0%); 98 (100%); 84 (11.6%); 70 (4.7%); 55 (4.6%); 41 (5.5%).

2-(4-fluorophenyl)-3-(3-(piperidin-1-yl)propyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Bf

Yield: 65%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.12 (dd, 1H, H7, Ar, $^3J = 7.8$, $^4J = 0.9$); 7.27 (td, 1H, H9, Ar, $^3J = 7.4$, $^4J = 1.3$); 7.29–7.19 (m, 3H, H10, H19, Ar); 7.08 (d, 1H, H8, Ar, $^3J = 7.7$); 6.09 (t, 2H, H18, Ar, $^3J = 8.6$); 6.02 (s, 1H, H2); 4.30 (dt, 1H, H11a, $^2J = 12.9$, $^3J = 6.2$); 3.06 (dt, 1H, H11b, $^2J = 13.4$, $^3J = 7.2$); 2.64 (dt, 1H, H12a, $^2J = 12.6$, $^3J = 7.9$); 2.56–2.33 (m, 5H, H12b, H14); 2.00–1.89 (m, 2H, H13a, H13b); 1.61(dt, 4H, H15, $^2J = 11.2$, $^3J = 5.6$); 1.47–1.46 (m, 2H, H16). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm, J_{C-F}=Hz): 163.8 (C4); 162.4 (d, C20, $^1J = 247.6$); 135.1 (d,

C17, $^{3}J = 3.1$); 132.7 (Ar); 131.9 (Ar); 129.7 (Ar); 129.2 (Ar); 127.9 (d, C19, $^{3}J = 8.3$); 127.4 (Ar); 126.2 (Ar); 115.3 (d, $^{2}J = 21.8$); 61.5 (C2); 55.2 (C13); 54.2 (C14); 46.8 (C11); 25.7 (C15); 25.0 (C12); 24.2 (C16). MS *m/z*: 384 (M^{+}, 6.2%); 351 (1.8%); 289 (1.7%); 259 (1.1%); 164 (1.1%); 151 (1.0%); 136 (13.8%); 124 (6.8%); 112(11.6%); 98 (100%); 84 (14.7%); 69 (4.7%); 55 (5.4%); 41 (7.8%).

2-butyl-3-(2-(piperidin-1-yl)ethyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Ca

Yield: 57%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.07 (dd, 1H, H7, Ar, $^{3}J = 8.3$, $^{4}J = 1.4$); 7.36 (td, 1H, H9, Ar, $^{3}J = 7.5$, $^{4}J = 1.4$); 7.26 (dd, 2H, H8, H10, Ar, $^{2}J = 11.2$, $^{3}J = 4.3$); 4.63 (dd, 1H, H2, $^{3}J = 9.6$, $^{3}J = 5.4$); 4.29 (ddd, 1H, H11a, $^{2}J = 13.3$, $^{3}J = 7.4$, $^{3}J = 5.6$); 3.32 (dt, 1H, H11b, $^{2}J = 13.6$, $^{3}J = 6.7$); 2.60 (s, 4H, H14); 2.78–2.68 (m, 2H, H12a, H12b); 1.97–1.79 (m, 2H, H17); 1.72–1.61 (m, 4H, H15); 1.50–1.39 (m, 2H, H18); 1.33–1.22 (m, 2H, H19); 0.86 (t, 3H, H20, $^{3}J = 7.2$). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 162.7 (C4); 134.1 (Ar); 131.7 (Ar); 129.8 (Ar); 129.0 (Ar); 127.8 (Ar); 125.8 (Ar); 61.8 (C2); 54.7 (C14); 57.1(C12); 45.7 (C11); 34.5 (C17); 28.9 (C18); 25.5 (C15); 23.8 (C16); 21.9 (C19); 13.8 (C20). MS *m/z*: 332 (M^{+}, 0.8%); 304 (0.1%); 276 (0.5%); 248 (0.9%); 192 (0.4%); 136 (2.2%); 111 (35.2%); 112 (7.0%); 98 (100%); 84 (3.6%); 70 (4.4%); 55 (7.4%); 42 (7.7%).

2-phenyl-3-(2-(piperidin-1-yl)ethyl)-2,3-dihydro-4H-benzo[e][1,3]-thiazin-4-one 5Cb

Yield: 62%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.15 (dd, 1H, H7, Ar, $^{3}J = 7.8$, $^{4}J = 1.3$); 7.30-7.20 (m, 7H, H9, H10, H18, H19, H20, Ar); 7.10 (d, 1H, H8, Ar, $^{3}J = 7.7$); 6.09 (s, 1H, H2); 4.38 (dt, 1H, H11a, $^{2}J = 13.7$, $^{3}J = 5.8$); 3.28 (dt, 1H, H11b, $^{2}J = 13.9$, $^{3}J = 7.1$); 2.83 (dt, 1H, H12a, $^{2}J = 12.7$, $^{3}J = 6.8$); 2.69 (ddd, 1H, H12b, $^{2}J = 12.5$, $^{3}J = 7.0$, $^{3}J = 5.3$); 2.56 (s, 4H, H14); 1.71–1.52 (m, 4H, H15); 1.55–1.43 (m, 2H, H16). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 163.9 (C4); 139.1 (Ar); 133.0 (Ar); 131.9 (Ar); 129.0 (Ar); 128.3 (Ar); 128.0 (Ar); 127.4 (Ar); 126.1 (Ar); 62.1 (C2); 56.8 (C12); 54.4 (C14); 45.3 (C11); 25.4 (C15); 23.8 (C16). MS *m/z*: 352 (M^{+}, 2.8%); 268 (0.6%); 136 (3.8%); 112 (4.6%); 111 (21.9%); 98 (100%); 84 (7.2%); 70 (4.1%); 55 (5.5%); 44 (6.4%).

3-(2-(Piperidin-1-yl)ethyl)-2-(p-tolyl)-2,3-dihydro-4H-benzo[e][1,3]-thiazin-4-one 5Cc

Yield: 61%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.12 (dd, 1H, H7, Ar, $^{3}J = 7.8$, $^{4}J = 1.3$); 7.26 (dt, 1H, H9, Ar, $^{3}J = 7.6$, $^{4}J = 1.6$); 7.20 (td, 1H, H10, Ar, $^{3}J = 7.7$, $^{4}J = 1.2$); 7.12 (d, 2H, H19, $^{3}J = 8.1$); 7.08 (dd, 1H, H8, Ar, $^{3}J = 7.7$, $^{4}J = 0.8$); 7.03 (d, 2H, H18, $^{3}J = 8.0$); 6.03 (s, 1H, H2); 4.34 (dt, 1H, H11a, $^{2}J = 13.4$, $^{3}J = 7.4$); 3.26 (dt, 1H, H11b, $^{2}J = 14.0$, $^{3}J = 7.1$); 2.80 (dt, 1H, H12a, $^{2}J = 12.7$, $^{3}J = 7.1$); 2.67 (ddd, 1H, H12b, $^{2}J = 12.6$, $^{3}J = 7.2$, $^{3}J = 5.2$); 2.57 (s, 4H, H14); 2.25 (s, 3H, CH_3); 1.67–1.57 (m, 4H, H15); 1.44–1.48 (m, 2H, H16). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 163.9 (C4); 137.9 (Ar); 136.0 (Ar); 133.2 (Ar); 131.9 (Ar); 129.7 (Ar); 129.0 (Ar); 129.0 (Ar); 127.4 (Ar); 126.0 (Ar); 126.0 (Ar); 62.1 (C2); 56.8 (C12); 54.4 (C14); 45.3 (C11); 25.4 (C15); 23.8 (C16); 20.9 (CH_3). MS *m/z*: 366 (M^{+}, 3.1%); 282 (0.6%); 136 (2.3%); 112 (6.6%); 111 (23.7%); 98 (100%); 84 (8.7%); 70 (4.0%); 55 (5.5%); 44 (4.2%).

2-(4-nitrophenyl)-3-(2-(piperidin-1-yl)ethyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Cd

Yield: 93%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.12 (dd, 1H, H7, Ar, $^{3}J = 7.8$, $^{3}J = 1.3$); 8.06 (d, 2H, H19, Ar $^{3}J = 8.8$); 7.42 (d, 2H, H18, Ar, $^{3}J = 8.7$); 7.28 (td, 1H, H9, Ar, $^{3}J = 7.5$, $^{4}J = 1.5$); 7.22 (dd, 1H, H10, Ar, $^{2}J = 11.0$, $^{3}J = 4.1$); 7.07 (d, 1H, H8, Ar, $^{3}J = 7.6$); 6.27 (s, 1H, H2); 4.29 (dt, 1H, H11a, $^{2}J = 14.0$, $^{3}J = 5.5$); 3.28 (dt, 1H, H11b, $^{2}J = 13.9$, $^{3}J = 5.9$); 2.77 (ddd, 1H, H12a, $^{2}J = 13.2$, $^{3}J = 7.3$, $^{3}J = 5.9$); 2.64 (dt, 1H, H12b, $^{2}J = 11.2$, $^{3}J = 5.5$); 2.48 (s, 4H, H14); 1.63–1.47 (m, 4H, H16); 1.44 (m, 2H, H16, $^{2}J = 11.3$, $^{3}J = 5.6$). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 163.7 (C4); 147.5 (C20); 147.07 (Ar); 132.2 (Ar); 132.2 (Ar); 129.9 (Ar); 129.1 (Ar); 127.0 (Ar); 126.6 (Ar); 123.4 (Ar); 61.2 (C2); 54.9 (C13); 54.0 (C14); 46.7 (C11); 25.1 (C15); 24.5 (C12); 23.8 (C16). MS *m/z*: 397 (M^{+}, 1.7%); 267 (0.3%); 163 (1.2%); 136 (3.5%); 111 (7.5%); 98 (100%); 70 (4.6%); 55 (5.3%); 44 (3.5%).

2-(4-methoxyphenyl)-3-(2-(piperidin-1-yl)ethyl)-2,3-dihydro-4Hbenzo[e][1,3]thiazin-4-one 5Ce

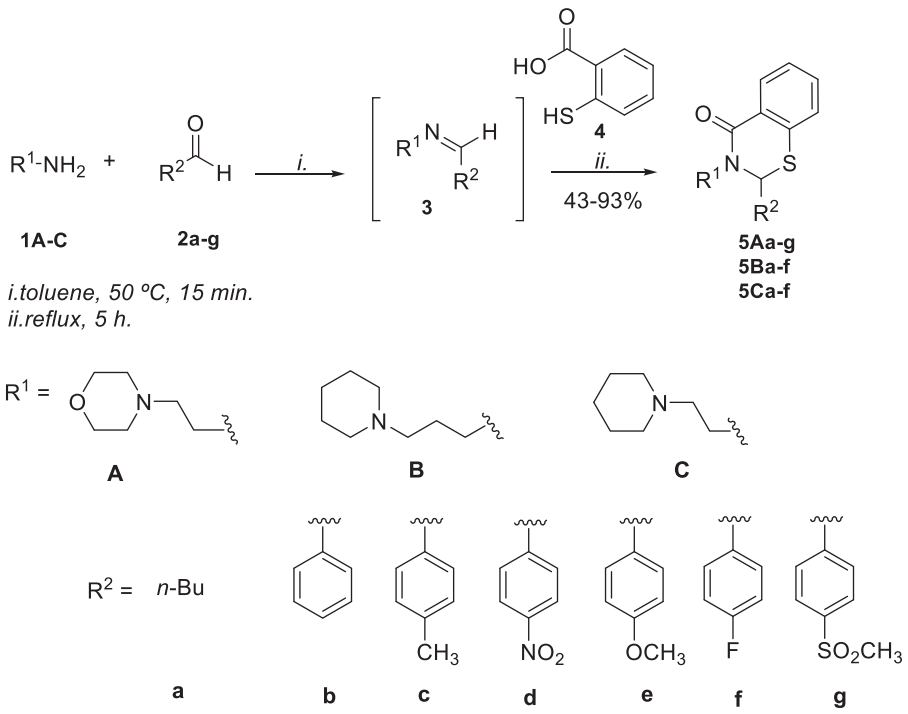
Yield: 72%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.13 (dd, 1H, H7, Ar, $^{3}J = 7.8$, $^{4}J = 1.2$); 7.27 (td, 1H, H9, Ar, $^{3}J = 7.4$, $^{4}J = 1.5$); 7.21 (td, 1H, H10, Ar, $^{3}J = 7.7$, $^{4}J = 1.2$); 7.17 (d, 2H, H19, Ar, $^{3}J = 8.7$); 7.09 (dd, 1H, H8, Ar, $^{3}J = 7.7$, $^{4}J = 0.8$); 6.75 (d, 2H, H18, Ar, $^{3}J = 8.8$); 6.03 (s, 1H, H2); 4.31 (ddd, 1H, H11a, $^{2}J = 13.6$, $^{3}J = 7.0$, $^{3}J = 5.1$); 3.26 (dt, 1H, H11b, $^{2}J = 13.9$, $^{3}J = 7.1$); 3.73 (s, 3H, OCH_3); 2.74 (ddd, 1H, H12a, $^{2}J = 12.9$, $^{3}J = 7.1$, $^{3}J = 5.8$); 2.62 (dt, 1H, H12b, $^{2}J = 12.8$, $^{3}J = 5.8$); 2.49 (s, 4H, H14); 1.67–1.53 (m, 4H, H15); 1.50–1.40 (s, 2H, H16). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 163.9 (C4); 159.3 (Ar); 133.2 (Ar); 131.8 (Ar); 131.0 (Ar); 129.7 (Ar); 129.0 (Ar); 127.4 (Ar); 127.4 (Ar); 126.0 (Ar); 113.7 (Ar); 62.0 (C2); 57.2 (C12); 55.1 (OCH_3); 54.6 (C14); 45.3 (C11); 25.7 (C15); 24.0 (C16). MS *m/z*: 382 (M^{+}, 5.2%); 349 (0.3%); 243 (0.9%); 136 (1.8%); 121 (6.1%); 111 (24.8%); 98 (100%); 84 (10.1%); 70 (3.9%); 55 (6.2%); 44 (3.1%).

2-(4-fluorophenyl)-3-(2-(piperidin-1-yl)ethyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one 5Cf

Yield: 88%; oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm, J_{H-H}=Hz): 8.12 (dd, 1H, H7, Ar, $^{3}J = 7.8$, $^{4}J = 0.9$); 7.28–7.25 (m, 1H, H9, Ar); 7.24–7.17 (m, 3H, H10, H19, Ar); 7.08 (d, 1H, H8, Ar, $^{3}J = 7.7$); 6.89 (t, 2H, H18, Ar, $^{3}J = 8.6$); 6.08 (s, 1H, H2); 4.29 (dt, 1H, H11a, $^{2}J = 13.7$, $^{3}J = 5.7$); 3.26 (dt, 1H, H11b, $^{2}J = 13.8$, $^{3}J = 6.9$); 2.72 (ddd, 1H, H12a, $^{2}J = 12.9$, $^{3}J = 7.2$, $^{3}J = 6.3$); 2.59 (dt, 1H, H12b, $^{2}J = 12.9$, $^{3}J = 5.8$); 1.61 (s, 4H, H14); 1.46–1.39 (m, 2H, H16). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm, J_{C-F}=Hz): 163.8 (C4); 162.3 (d, C20, $^{1}J = 247.6$); 135.1 (d, C17, $^{3}J = 3.2$); 132.9 (Ar); 131.9 (Ar); 129.8 (Ar); 129.2 (Ar); 127.9 (d, C19, $^{3}J = 8.3$); 127.4 (Ar); 126.2 (Ar); 115.2 (d, $^{2}J = 21.8$); 61.8 (C2); 57.5 (C12); 54.7 (C14); 45.5 (C11); 25.9 (C15); 24.1 (C16). MS *m/z*: 370 (M^{+}, 1.8%); 286 (0.5%); 136 (3.3%); 123 (0.8%); 111 (15.8%); 98 (100%); 70 (3.8%); 55 (5.3%); 44 (3.8%).

Effects in vitro of benzothiazinones in the brain AChE activity

Ten rats male Wistar (60 day-old) were obtained from the Central Animal House of Federal University of Pelotas (Brazil). All animals procedures were approved by the Ethics Committee and Animal Experimentation of the institution (protocol number CEEA 9220). The animals were submitted to euthanasia and cerebral cortex and hippocampus were removed and homogenized in the 10 mm Tris–HCl (pH 7.4) buffer. The proteins levels were determined by Bradford method (1976) using bovine albumin as standard. The benzothiazinones were dissolved in methanol and different concentrations (1, 5, 10, 25, 50, 100 and 250 μM) were tested in the enzymatic assay. The AChE activity was determined according to the method of Elmann et al.[18] using acetylthiocholine (AcSCh) as



Scheme 1. Synthetic route to obtain benzothiazin-4-ones.

substrate. The specific AChE activity was expressed in μmol of AcSCh/h/mg of protein.

Cytotoxicity evaluation the benzothiazinones in fibroblast culture

The cultures of MRC-5 (human fibroblast) were treated with benzothiazinones to according to the method of Skehan et al.[19] At first, all compounds were solubilized in Dimethyl Sulfoxide (DMSO) 0,1% and were prepared and tested in DMEM (Dulbecco's Modified Eagles's Medium) at final concentrations of 50, 100 and 250 mm. The MRC-5 were seeded at 5×10^3 cells/well in DMEM/5% FBS (fetal bovine serum) in 96-well plates. Cultures were exposed to compounds for 24 h and the control cells were treated with vehicle.

After 24 h, the cells were washed and added trichloroacetic acid 50% for 45 min in the fridge for cell fixation. After, were carried out five washes with distilled water to full removal of the acid. A solution of B sulfarodamina (SRB) 0.4% acetic acid was added followed by 30 min incubation to stain proteins. Then, washings were made 5 of the wells with 1% acetic acid for complete removal of uncomplexed dye with proteins. Finally, the plates were solubilized and read in a spectrophotometer at 530 nm. B sulforodamina binds to the amino terminal portions of the cells that were fixed with trichloroacetic acid, which is quantified spectrophotometrically. The results are presented in percentage of cell proliferation, considering the proliferation of control group as 100%.

Statistical analysis

Results were analyzed by using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons in the software Graphpad Prism 5. All results were expressed as mean ± standard error (SEM) and the differences between mean values were considered significant when $P < 0.05$.

Results and discussion

Benzothiazinones derivatives have attracted continuing interest over the years because of their diverse biological activities[12]. A literature survey reveals that several benzothiazin-4-ones have been prepared based on different synthetic routes: a) from classic primary amine, aldehyde or ketone and thiosalicylic acid as reported by Zarghi et al.[17] using *p*-toluenesulfonic acid catalyst in toluene reflux or as reported by Kamel et al.[20] using sodium sulfate in dioxane or as reported by Kitsiou et al.[21] and Silverberg et al.[11] using propylphosphonic acid anhydride (T3P) as catalyst; b) from 2-aminothiophenol and chloroacetic acid as reported by Shaikh et al.[16]; c) from aromatic carboxylic acid with ammonium isothiocyanate as reported by Peng et al.[22]

In this work, the compounds were synthesized using the thermal heating methodology and the reaction conditions previously described by our research group[23], however, all reactions were monitored by TLC and/or GC analysis. Novel nineteen 1,3-benzothiazin-4-ones (**5Aa–g**, **5Ba–f** and **5Ca–f**) were synthesized from one-pot reactions between different amines **1A–C**, different aldehydes **2a–g** and thiosalicylic acid **4** (Scheme 1). All reactions were carried out in toluene reflux and a Dean–Stark apparatus was used for water removal.

We observed that is important the complete formation of imine intermediate before addiction of thiosalicylic acid, once when all three reactants were put together in a vessel, a small proportion of by-product oxathiolone was observed in GC (data not shown). The complete formation of benzothiazinone was observed in five hours and it is important to note that this time is lower than generally find in literature for reactions with thiosalyclic acid (20–48 h)[17,20,21].

All compounds were obtained in regular to good yields and have been properly purified and characterized. In general, the yields obtained after purification varied from moderate to excellent and the methodology was considerate efficient once all proposed products were obtained. All compounds were identified and characterized by mass spectrometry (GC–MS) and by nuclear

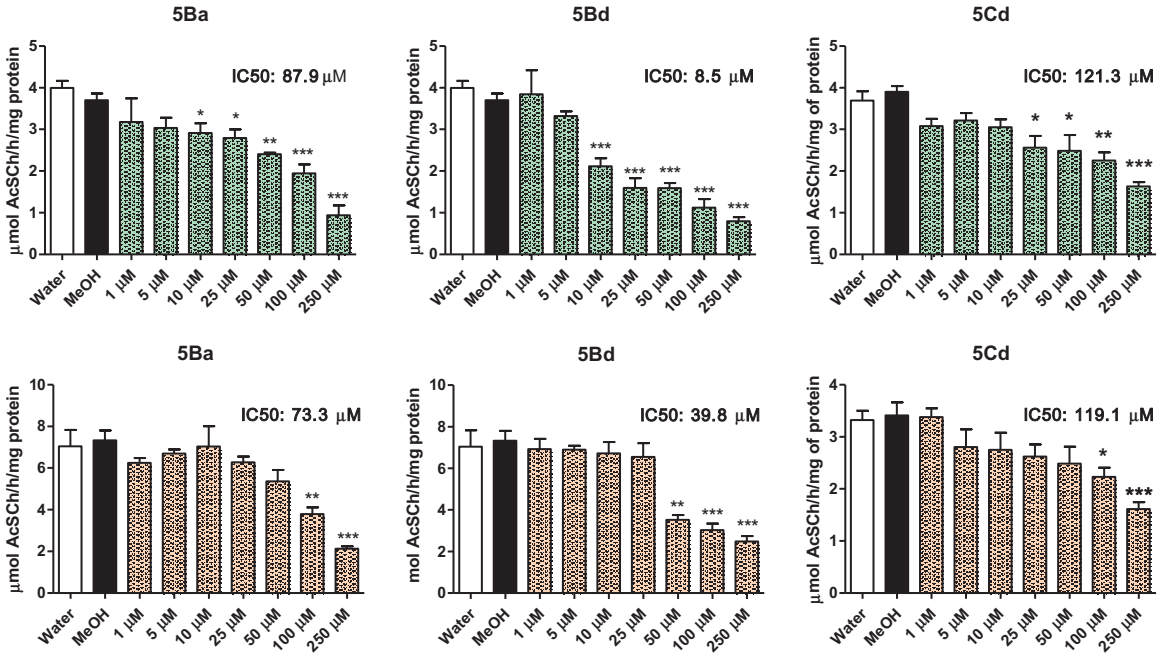


Figure 2. *In vitro* effect of **5Ba, 5Bd** and **5Cd** in the AChE activity in cerebral cortex and hippocampus of rats. One-way ANOVA followed by Tukey's *post hoc* test. $^{*}P< 0.05$, $^{**}P< 0.01$ and $^{***}P< 0.001$ compared to the water control group ($n = 4–5$).

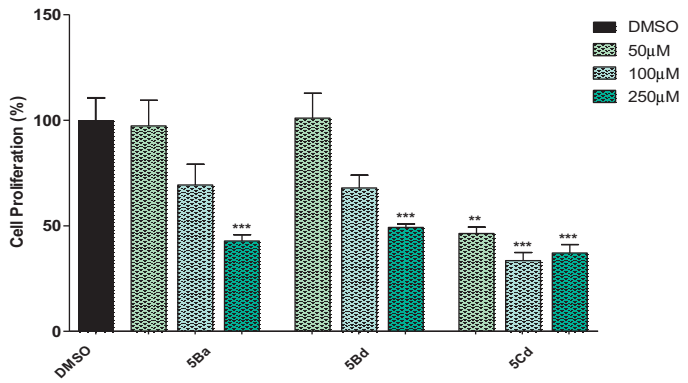


Figure 3. Cytotoxicity of benzothiazinones **5Ba**, **5Bc** and **5Cd** in MCR-5 human fibroblast cell. The results were expressed at percentage of cell proliferation, considering DMSO control group as 100%. $^{***}P< 0.001$ when compared with DMSO group.

magnetic resonance (NMR) of ^{1}H and ^{13}C. The H2/C2 (CH of benzothiazinone ring) and carbonyl group (C4) are the classic signals that confirm the cyclization. The H2 was assigned as a singlet at 5.89–6.27 ppm (aryl substituents, **b–g**) and as a doublet of doublets at 4.53–4.58 ppm (butyl, **a**), while the C4 resonates at typical amide bond at 162.7–164.1 ppm. The other signals agree with the proposal structures.

After the synthesis, the *in vitro* effect of all benzothiazinones was evaluated as AChE activity inhibitors. For this purpose, it was used hippocampus and cerebral cortex of rats, brain structures that play an important role in cognitive functions. Compounds **5Aa–g**, **5Ba–f** and **5Ca–f** were dissolved in methanol and different concentrations were prepared. It is important to note that controls were performed in methanol and water and our results showed no difference between them in the *in vitro* AChE activity, so, no methanol interference was observed. Figure 2 shows the *in vitro* AChE analyses in all concentrations tested for compounds that have IC_{50} values in both cerebral cortex and hippocampus **(5Ba**, **5Bd** and **5Cd)**.

All benzothiazinones **5B** presented inhibitory effect in AChE activity in both cerebral structures. Benzothiazinones **5Ba** inhibited the AChE activity since 10 μM (27.1%) in cerebral cortex (IC_{50} 87.9 μM) and since 100 μM (46.2%) in hippocampus (IC_{50} 73.3 μM). Similar results were obtained for benzothiazinone **5Bb** that inhibited the AChE activity since 10 μM (47.2%) in cerebral cortex (IC_{50} 8.5 μM) and in hippocampus at concentrations of 50 μM (50%) (IC_{50} 39.8 μM) (Figure 2). Additionally, compound **5Bf** showed IC_{50} equal to 61.7 μM in hippocampus, however did not in cerebral cortex (IC_{50}>250 μM) (data not show).

On the other hand, it was verified that in general benzothiazinones **5A** did not demonstrated relevant inhibitory effects in AChE activity in both cerebral structures, except compound **5Ad** that have IC_{50} 70.59 μM and statistical difference since 100 μM (46.4% of inhibition) in hippocampus (data not shown). In general, benzothiazinones **5C** showed better AChE inhibition results than compounds **5A**. From the six tested compounds, **5Cd** showed similar IC_{50} for both cerebral cortex and hippocampus (121.3 and 119.1 μM, respectively).

The *in vitro* effect and IC_{50} values in AChE inhibition in cerebral cortex and hippocampus of rats for all compounds are show in supplementary information section. Compounds that did not exceed the 50% of inhibition at 250 μM (the highest concentration tested), could not be calculate.

The benzothiazinones were prepared from three different aliphatic amines: **1A** 4-aminoethylmorpholine; **1B** *N*-(3-aminopropyl)-piperidine); and **1C** 1-(2-aminoethyl)piperidine). Two heterocycles (morpholine and piperidine) and two carbon link chains (two or three methylenes) were studies and these differences show importance for the activity. Compounds **5A** did not present expressive inhibition rates, when compared with **5B** and **5C** and suggest that the extra oxygen in morpholine ring decrease the inhibitory activity of compounds **5A**. Compounds **5B** demonstrated better activities than compounds **5C**. This fact suggests that the extension of carbon chain link between nitrogen of piperidine and nitrogen of benzothiazinone ring is important once three methylene carbons (propylene moiety) improve the activity of compounds **5B**.

Assessing the different aldehydes varied in the synthesis, the compounds with the best activities (**5Ba**, **5Bd** and **5Cd**) were derivate from valeraldehyde **2a** or 4-nitrobenzaldehyde **2d**. It is important to note that compound **5Ad** also show moderate results in hippocampus suggesting that the NO_2 group (**d**) plays an important role in the AChE inhibition.

Finally, three benzothiazinones with the best AChE inhibition activity were selected to evaluate the cytotoxic effect in MCR-5 human fibroblasts cells compared to DMSO control (Figure 3). The most active compounds in the AChE study **5Ba** and **5Bd** were noncytotoxic at 100 M. Unfortunately compound **5Cd** showed toxicity at the lowest concentration tested 50 μM.

Therefore, these findings encourage the sequence of studies with benzothiazinone **5Bd** exploring chemical changes and trying to improve the AChE inhibition without cytotoxicity.

Conclusions

In summary, novel benzothiazin-4-ones were efficient synthesized by one-pot multicomponent reactions in moderate to excellent yields through conventional heating methodology for 5 h. The novel compounds were fully identified and characterized by ^{1}H, ^{13}C NMR and by GC-MS. In addition, benzothiazinone **5Bd** showed the most significant *in vitro* AChE activity in cerebral cortex of rats (IC_{50} 8.48 μM) and low cytotoxicity in human fibroblast cell. These preliminary results will guide further investigation on heterocyclic benzothiazinones aiming to the development of a new potent AChE inhibitors agent.

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