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Synthesis and biological activity of derivatives of tetrahydroacridine as acetylcholinesterase inhibitors

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ABSTRACT

Current state of medical sciences does not allow to treatment neurodegenerative diseases such as Alzheimer's disease (AD). At present treatment of AD is severely restricted. The main class of medicines which are applied in AD is acetylcholinesterase inhibitors (AChEIs) like tacrine, donepezil, galantamine and rivastigmine that do not contribute to significant and long-term improvement in cognitive and behavioural functions.

In this work, we report synthesis and biological evaluation of new hybrids of tacrine-6-hydrazinonicotinamide. The synthesis was based on the condensation reaction between tacrine derivatives and the hydrazine nicotinate moiety (HYNIC). All obtained compounds present affinity for both cholinesterases and are characterized by high selectivity in relation to butyrylcholinesterase (BChE).

1. Introduction

AD is the most common ailment contributing to decline of cognitive function. It accounts for 50–60% of all reasons of dementia. The chances of developing AD increase in conjunction with advanced age, rising from 1% in people below 65 years old to more than 24% in those aged 85 years [1]. Beside advanced age there are other risk factors for acquiring AD. One of them is a diminished reserve capacity of the brain which may be a result of low educational background or decreased mental or physical activity during the lifetime. Another cause confirmed by several epidemiological researches is head injury [1].

The most degenerated by AD are the cortex and hippocampus, regions in brain which are associated with the highest and more complex functions [2]. Progressive development of forgetfulness, deterioration of cognitive functions such as language, memory and behavioural disturbances are characteristic features of patients suffering from AD [3,4].

Pathogenic background of AD is highly complicated. Scientists have proposed several theories explaining the mechanism of AD development. Among them there are: loss of cholinergic function (known as cholinergic hypothesis), the amyloid cascade (amyloid hypothesis), oxidative stress, decrease of steroid hormone concentration and inflammation process [2,3]. Cholinergic hypothesis has received plentiful verification and has been widely approved.

The most characteristic abnormality associated with AD is a decrease in central cholinergic neurotransmission. This pathology is a result of decreased activity of choline acetyltransferase (ChAT), the enzyme that synthesizes acetylcholine (Ach). There is a correlation between reduction of ChAT activity in AD and severity of cognitive disturbances [5,6].

AChE inhibitors are at present the one of two groups of drugs used for the clinical treatment of AD. The symptomatic efficacy of AChEIs is attained through their increase of acetylcholine-mediated transmission between neurons. However, these agents do not reverse the progression of the disease and contribute only to modest improvement in cognitive function in the mild to moderate stages AD.

Advances in knowledge of the pathogenesis of AD have led to numerous studies conducting investigations of new potential cholinergic drugs for the treatment of AD [2]. Recent endeavours have been focused on increasing cholinergic neurotransmission, utilizing cholinergic receptor agonists or AChEIs [2,6].

In this article we describe the synthesis and biological evaluation of a series of tetrahydroacridine derivatives with hydrazine nicotinate (HYNIC) moiety as bifunctional acetylcholinesterase inhibitors. The fragment of tetrahydroacridine has possibility to inhibit the enzymes and HYNIC moiety could be used by standard as a co-ligand to radiolabeling. These new compounds can be new potential drugs for treatment of AD or a good ligand to radioisotopes as a marker in neurological process.

2. Material and methods

2.1. Chemistry

Reactions were monitored by TLC using 25 DC-Alufolien Kieselgel 60F_{254} (Merck), and detection was done by UV Lamp (254 nm). Melting points were measured on an electrothermal apparatus in

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open capillaries and are uncorrected. Anhydrous Na_2SO_4 was used to dry organic solutions during work-up and the removal of solvents was carried out under vacuum with a rotary evaporator. Column chromatography was performed using silica gel 60 (200–400 mesh, Merck). IR spectra were recorded in KBr using a Mattson Infinity Series FT-IR spectrophotometer. ^{1}H NMR spectra were recorded with a Varian Mercury 300 MHz spectrometer, using tetramethylsilane as internal standard. Mass spectra were performed by the Centre of Molecular and Macromolecular Studies in Lodz (Polish Academy of Sciences).

2.2. N-(1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine (**4a**)

9-Chloro-1,2,3,4-tetrahydroacridine (**3**) (0.75 g, 3.5 mmol), 1,4-diaminobutane (0.70 ml, 7 mmol), phenol (1.5 g), and NaI (0.07 g) were carefully heated at 180 °C for 2 h and then cooled at room temperature. The mixture was diluted with ethyl acetate (50 ml) and made basic with 10% KOH solution (30 ml). The organic layer was washed with water (20 ml) and brine (20 ml – twice), and then dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified on silica gel chromatography (CH_2Cl_2:CH_3OH:NH_3 = 10:4.6:0.5) to afford **4a** as an oil. Compound **4a**: yield 72%; IR (KBr) ν (cm^{-1}): 1580.3, 2859.3, 2931.8, 3060.8, 3350.3; ^{1}H NMR ($CDCl_3$) (δ ppm): 8.0 (2H, t, J = 7.7 Hz, ArH), 7.6 (1H, t, J = 5.5 Hz, ArH), 7.4 (1H, t, J = 8.1 Hz, ArH), 4.2 (1H, br, NH), 3.5 (2H, t, J = 7.1 Hz, $NHCH_2$), 3.1 (2H, s, CH_2), 2.8–2.7 (4H, m, CH_2, CH_2NH), 1.9–1.8 (6H, m, CH_2CH_2, NH_2), 1.9 (2H, k, J = 7.3 Hz, CH_2), 1.9 (2H, k, J = 7.1 Hz, CH_2); MS (FAB) m/z (M + 1) 270.2, 199.0; MS-HR (FAB) Calcd for $C_{17}H_{23}N_3$: 269.18920. Found: 270.19637 (M + 1).

2.3. N-(1,2,3,4-tetrahydroacridin-9-yl)pentane-1,5-diamine (**4b**)

A mixture of **3** (0.75 g, 3.5 mmol), 1,5-diaminopentane (0.82 ml, 7 mmol), phenol (1.5 g), and NaI (0.07 g) were combined as above to afford **4b** as an oil. Compound **4b**: yield 74%; IR (KBr) ν (cm^{-1}): 1562.8, 2854.7, 2930.6, 3065.2, 3275.1; ^{1}H NMR ($CDCl_3$) (δ ppm): 7.8 (2H, t, J = 7.7 Hz, ArH), 7.5 (1H, t, J = 1.4 Hz, ArH), 7.3 (1H, t, J = 1.6 Hz, ArH), 3.9 (1H, br, NH), 3.4 (2H, t, J = 7.1 Hz, $NHCH_2$), 3.0 (2H, s, CH_2), 2.7–2.6 (4H, m, CH_2, CH_2NH), 1.9–1.8 (6H, m, CH_2CH_2, NH_2), 1.6 (2H, k, J = 6.7 Hz, CH_2), 1.5–1.3 (4H, m, CH_2); MS (FAB) m/z (M + 1) 284.2, 199.0; MS-HR (FAB) Calcd for $C_{18}H_{25}N_3$: 283.20485. Found: 284.21288 (M + 1).

2.4. N-(1,2,3,4-tetrahydroacridin-9-yl)heptane-1,7-diamine (**4c**)

A mixture of **3** (0.75 g, 3.5 mmol), 1,7-diaminoheptane (0.97 g, 7 mmol), phenol (1.5 g), and NaI (0.07 g) were combined as above to afford **4c** as an oil. Compound **4c**: yield 70%; IR (KBr) ν (cm^{-1}): 1580.3, 2854.9, 2927.2, 3059.5, 3307.4; ^{1}H NMR ($CDCl_3$) (δ ppm): 7.9 (2H, dd, J = 8.3, 1.6 Hz, ArH), 7.5 (1H, t, J = 6.9 Hz, ArH), 7.3 (1H, t, J = 7.1 Hz, ArH), 3.9 (1H, s, NH), 3.4 (2H, t, J = 7.1 Hz, $NHCH_2$), 3.0 (2H, s, CH_2), 2.7 (4H, d, J = 7.0 Hz, CH_2NH_2), 2.6 (2H, s, CH_2), 2.0 (2H, s, NH_2), 1.8 1.9 (4H, m, CH_2 CH_2), 1.5–1.6 (2H, m, CH_2CH_2), 1.2–1.4 (6H, br, CH_2CH_2); MS (FAB) m/z (M + 1) 312.3, 199.0; MS-HR (FAB) Calcd for $C_{20}H_{29}N_3$: 311.23615. Found: 312.24328 (M + 1).

2.5. N-(1,2,3,4-tetrahydroacridin-9-yl)nonane-1,9-diamine (**4d**)

A mixture of **3** (0.75 g, 3.5 mmol), 1,9-diaminononane (1.10 g, 7 mmol), phenol (1.5 g), and NaI (0.07 g) were combined as above to afford **4d** as an oil. Compound **4d**: yield 68%; IR (KBr) ν (cm^{-1}): 1562.4, 2853.3, 2925.9, 3059.8, 3289.1; ^{1}H NMR ($CDCl_3$) (δ ppm): 7.9 (2H, dd, J = 8.3, 0.6 Hz, ArH), 7.5 (1H, t, J = 6.9 Hz, ArH), 7.3 (1H, t, J = 6.9 Hz, ArH), 3.9 (1H, s, NH), 3.4 (2H, t, J = 7.3 Hz, $NHCH_2$), 3.0 (2H, s, CH_2), 2.6 (4H, d, J = 6.7 Hz, CH_2NH_2), 2.5 (2H, s, CH_2), 2.1 (2H, s, NH_2), 1.9 (4H, m, CH_2 CH_2), 1.5–1.6 (2H, m, CH_2CH_2), 1.4–1.1 (10H, br, CH_2CH_2); MS (FAB) m/z (M + 1) 340.4, 199.1; MS-HR (FAB) Calcd for $C_{22}H_{33}N_3$: 339.26745. Found: 340.27668 (M + 1).

2.6. N-{5-[4-(1,2,3,4-tetrahydroacridin-9-ylamino)butylcarbamoyl] pyridin-2-yl}hydrazinecarboxylic acid tert-butyl ester (**5a**)

To the 2-chloro-4,6-dimethyl-1,3,5-triazine (CDMT) (1.76 g, 10 mmol) and **2** (2.53 g, 10 mmol) in THF (10 ml), N-methylmorpholine (1.1 ml, 10 mmol) was added dropwise at such a rate as to keep temperature at −5 °C to 0 °C. Stirring have been continued at 0 °C for 1–4 h until all CDMT has been consumed. Then to the crude mixture, obtained as described above, **4a** (2.69 g, 10 mmol) in THF (8 ml), at −5 °C to 0 °C, was added. Stirring have been continued at 0 °C for 2 h, and then for 12 h at room temperature. Precipitate had formed and was isolated by filtration. Recrystallization from ethyl acetate afforded the desired product **5a** as yellow solid. Compound **5a**: mp 173–175 °C; yield 36%; IR (KBr) ν (cm^{-1}): 1522.3, 1636.4, 1718.8, 2866.7, 2935.2, 3250.4, ^{1}H NMR (CD_3OD) (δ ppm): 8.4 (2H, d, J = 8.1 Hz, ArH), 7.7–7.9 (2H, m, ArH), 7.6 (1H, d, J = 5.8 Hz, CCHC), 7.5 (1H, t, J = 5.7 Hz, ArH), 6.6 (1H, d, J = 8.7 Hz, CCHC), 4.6 (2H, s, NH), 3.9–4.0 (2H, m, $NHCH_2$), 3.7 (1H, m, NH), 3.3–3.4 (2H, m, CH_2N), 2.9 (2H, m, CH_2), 2.7 (2H, m, CH_2), 2.6 (1H, m, NH), 1.8–2.0 (6H, m, CH_2), 1.7 (2H, p, J = 7.3, 7.9, Hz, CH_2), 1.4–1.5 (9H, m, BOC); MS (FAB) m/z (M + 1) 505.4, 405.2; 199.0; MS-HR (FAB) Calcd for $C_{27}H_{36}N_6O_3$: 504.28489. Found: 505.29345 (M + 1).

2.7. N-{5-[5-(1,2,3,4-tetrahydroacridin-9-ylamino)pentylcarbamoyl] pyridin-2-yl}hydrazinecarboxylic acid tert-butyl ester (**5b**)

A mixture of 2-chloro-4,6-dimethyl-1,3,5-triazine (CDMT) (1.76 g, 10 mmol), **2** (2.53 g, 10 mmol) in THF (10 ml), and N-methylmorpholine (1.1 ml, 10 mmol) and after 4 h **4b** (2.83 g, 10 mmol) in THF were combined as above to afford **5b** as yellow solid. Compound **5b**: mp 188–190 °C; yield 38%; IR (KBr) ν (cm^{-1}): 1522.0, 1636.2, 1717.8, 2862.6, 2934.4, 3242.6; ^{1}H NMR (CD_3OD) (δ ppm): 8.4 (2H, d, J = 8.1 Hz, ArH), 7.7–7.9 (2H, m, ArH), 7.6 (1H, d, J = 5.8 Hz, CCHC), 7.5 (1H, t, J = 5.7 Hz, ArH), 6.6 (1H, d, J = 8.7 Hz, CCHC), 4.6 (2H, s, NH), 3.9–4.0 (2H, m, $NHCH_2$), 3.7 (1H, m, NH), 3.3–3.4 (2H, m, CH_2N), 3.0 (2H, m, CH_2), 2.7 (2H, m, CH_2), 2.5 (1H, m, NH), 1.8–2.0 (8H, m, CH_2), 1.7 (2H, m, CH_2), 1.4–1.5 (9H, m, BOC); MS (FAB) m/z (M + 1) 519.4, 419.3, 199.1; MS-HR (FAB) Calcd for $C_{28}H_{38}N_6O_3$: 518.30054. Found: 519.30688 (M + 1).

2.8. N-{5-[7-(1,2,3,4-tetrahydroacridin-9-ylamino)heptylcarbamoyl] pyridin-2-yl}hydrazinecarboxylic acid tert-butyl ester (**5c**)

A mixture of 2-chloro-4,6-dimethyl-1,3,5-triazine (CDMT) (1.76 g, 10 mmol), **2** (2.53 g, 10 mmol) in THF (10 ml), and N-methylmorpholine (1.1 ml, 10 mmol) and after 4 h **4c** (3.12 g, 10 mmol) in THF were combined as above to afford **5c** as yellow solid. Compound **5c**: mp 179–181 °C; yield 39%; IR (KBr) ν (cm^{-1}): 1522.2, 1636.1, 1717.1, 2855.2, 2929.0, 3261.7; ^{1}H NMR (CD_3OD) (δ ppm): 8.4 (2H, d, J = 8.7 Hz, ArH), 7.9–8.0 (1H, m, ArH), 7.6–7.7 (2H, m, ArH), 7.5 (1H, t, J = 5.4 Hz, ArH), 6.7 (1H, d, J = 8.7 Hz, CCHC), 4.6 (2H, s, NH), 3.9–4.0 (2H, m, $NHCH_2$), 3.3–3.4 (3H, m, NH,CH_2), 3.0 (2H, m, CH_2), 2.7 (2H, m, CH_2), 2.3 (1H, m, NH), 1.9 (4H, m, CH_2), 1.8 (2H, m, CH_2), 1.6 (8H, m, CH_2), 1.2–1.5 (9H, m, BOC); MS (FAB) m/z (M + 1) 547.3, 447.2, 199.1; MS-HR (FAB) Calcd for $C_{31}H_{42}N_6O_3$: 546.33184. Found: 547.34212 (M + 1).

*2.9. N-{5-[9-(1,2,3,4-tetrahydroacridin-9-ylamino)nonylcarbamoyl] pyridin-2-yl}hydrazinecarboxylic acid tert-butyl ester (**5d**)*

A mixture of 2-chloro-4,6-dimethyl-1,3,5-triazine (CDMT) (1.76 g, 10 mmol), **2** (2.53 g, 10 mmol) in THF (10 ml), and N-methylmorpholine (1.1 ml, 10 mmol) and after 4 h **4d** (3.40 g, 10 mmol) in THF were combined as above to afford **5d** as yellow solid. Compound **5d**: mp 176–178 °C; yield 36%; IR (KBr) ν (cm^{-1}): 1522.7, 1636.0, 1719.2, 2853.6, 2926.7, 3251.6; ^1H NMR (CD_3OD) (δ ppm): 8.5 (1H, m, ArH), 8.3 (1H, d, J = 8.7 Hz, ArH), 8.0 (1H, m, ArH), 7.7 (1H, m, ArH), 7.6 (1H, m, ArH), 7.5 (1H, t, J = 5.3 Hz, ArH), 6.7 (1H, d, J = 8.9 Hz, CCHC), 4.6 (2H, s, NH), 3.9–4.0 (2H, m, $NHCH_2$), 3.3 (3H, m, NH,CH_2), 3.0 (2H, m, CH_2), 2.7 (2H, m, CH_2), 2.3 (1H, m, NH), 1.9 (4H, m, CH_2), 1.8 (2H, m, CH_2), 1.6 (4H, m, CH_2), 1.3–1.4 (9H, s, BOC), 1.3 (8H, br, CH2); MS (FAB) m/z (M + 1) 575.4, 475.4, 197.1; MS-HR (FAB) Calcd for $C_{33}H_{46}N_6O_3$: 574.36314. Found: 575.36921 (M + 1).

*2.10. 6-Hydrazino-N-[4-(1,2,3,4-tetrahydroacridin-9-ylamino)butyl] nicotinamide hydrochloride (**6a**)*

Compound **5a** (0.20 g, 0.39 mmol) was dissolved in ether (2 ml), HCl/ether (4 ml) was added, and the reaction mixture was stirred at room temperature. After 24 h, the solution became cloudy and precipitate had formed. The precipitate was isolated by filtration and the solid was washed with ether and dried. Compound **6a**: mp 124–126 °C; yield 66%;IR (KBr) ν (cm^{-1}): 1576.3, 1637.7, 2854.1, 2923.9, 3388.3; ^1H NMR (DMSO-d6) (δ ppm): 14.0 (1H, s, HCl), 8.4 (1H, d, J = 8.5 Hz, NHC), 8.0 (1H, d, J = 8.9 Hz, CHC), 7.8–7.9 (3H, m, ArH), 7.5 (1H, t, J = 6.2 Hz, ArH), 6.8 (1H, d, J = 8.3 Hz, CCHC), 3.9 (2H, m, NH), 3.2–3.5 (5H, m, NH,CH_2) 2.9 (2H, m, CH_2), 2.5 (3H, m, NH,CH_2), 1.7–1.9 (4H, m, CH_2), 1.5 (2H, m, CH_2); MS (FAB) m/z (M + 1) 405.3, 390.3; 199.1; MS-HR (FAB) Calcd for $C_{23}H_{28}N_6O$: 404.23246. Found: 405.23920 (M + 1).

*2.11. 6-Hydrazino-N-[5-(1,2,3,4-tetrahydroacridin-9-ylamino)pentyl] nicotinamide hydrochloride (**6b**)*

A **5d** (0.20 g, 0.38 mmol) were combined as above to afford **6d** as brown solid. Compound **6d**: mp 189–192 °C; yield 60%; IR (KBr) ν (cm^{-1}): 1524.3, 1644.0, 2853.6, 2921.6, 3406.3; ^1H NMR (DMSO-d6) (δ ppm): 13.6 (1H, s, HCl), 8.4 (1H, d, J = 8.3 Hz, NHC), 8.1 (1H, d, J = 11.1 Hz, CHC), 7.8–7.9 (3H, m, ArH), 7.6 (1H, m, ArH), 6.8 (1H, d, J = 8.5 Hz, CCHC), 3.9 (2H, m, NH), 3.1–3.4 (5H, m, NH,CH_2) 2.9 (2H, m, CH_2), 2.6 (3H, m, NH,CH_2), 1.7–1.9 (4H, m, CH_2), 1.5 (2H, m, CH_2) 1.5 (2H, m, CH_2); MS (FAB) m/z (M + 1) 419.4, 404.4;199.1 MS-HR (FAB) Calcd for $C_{24}H_{30}N_6O$: 418.24811. Found: 419.25625 (M + 1).

*2.12. 6-Hydrazino-N-[7-(1,2,3,4-tetrahydroacridin-9-ylamino)heptyl] nicotinamide hydrochloride (**6c**)*

A **5b** (0.20 g, 0.37 mmol) were combined as above to afford **6b** as yellow solid. Compound **6b**: mp 173–176 °C; yield 59%; IR (KBr) ν (cm^{-1}): 1530.5, 1648.5, 2854.8, 2927.7, 3262.6, 3408.4; ^1H NMR (DMSO-d6) (δ ppm): 13.9 (1H, s, HCl), 8.4 (1H, d, J = 8.5 Hz, NHC), 8.1 (1H, d, J = 6.7 Hz, CCHC), 7.8–7.9 (3H, m, ArH) 7.5 (1H, t, J = 7.7 Hz, ArH), 6.9 (1H, d, J = 8.9 Hz, CCHC), 3.9 (2H, m, CH_2NH_2), 3.2 (2H, m, CH_2), 3.0 (2H, m, CH_2NH), 2.6 (2H, m, CH_2), 1.9 (4H, m, CH_2), 1.7 (2H, m, CH_2), 1.5 (8H, m, CH_2); MS (FAB) m/z (M + 1) 447.3, 432.3, 199.1; MS-HR (FAB) Calcd for $C_{26}H_{34}N_6O$: 446.27941. Found: 447.28818 (M + 1).

*2.13. 6-Hydrazino-N-[9-(1,2,3,4-tetrahydroacridin-9-ylamino)nonyl] nicotinamide hydrochloride (**6d**)*

A **5d** (0.20 g, 0.35 mmol) were combined as above to afford **6d** as brown solid. Compound **6d**: mp 171–173 °C; yield 54%; IR (KBr) ν (cm^{-1}): 1531.5, 1647.6, 2852.6, 2924.8, 3285.5, 3425.3; ^1H NMR (DMSO-d6) (δ ppm): 13.7 (1H, s, HCl), 8.6 (1H, m, ArH), 8.4 (1H, d, J = 10.1 Hz, ArH), 8.4 (1H, d, J = 9.1 ArH), 7.7–7.8 (2H, m, ArH), 6.9 (1H, d, J = 8.9 Hz, ArH), 3.9 (2H, m, CH_2NH_2), 3.2 (2H, m, CH_2), 3.0 (2H, m, CH_2NH), 2.6 (2H, m, CH_2), 1.8 (4H, m, CH_2), 1.7 (2H, m, CH_2), 1.5 (4H, m, CH_2), 1.3 (8H, m, CH2); MS (FAB) m/z (M + 1) 475.3, 460.3, 199.0; MS-HR (FAB) Calcd for $C_{28}H_{38}N_6O$: 474.31071. Found: 475.31748 (M + 1).

2.14. Biochemical studies

2.14.1. Materials

Acetylthiocholine iodide (ATChI) and 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co. (St. Louise, MO). Sodium hydrogen phosphate anhydride and sodium dihydrogen phosphate were from J.T. Baker (Europe). All other chemicals used were of analytical grade and the highest chromatographic purity available.

2.14.2. Enzymes

Acetylcholinesterase from Electrophorus electricus (Electric eel) – Type III and butyrylcholinesterase from equine serum were obtained from Sigma Chemical Co. (St. Louise, MO).

2.14.3. Enzyme assays

The activity of acetylcholinesterase (AChE) inhibitors was measured according to the method of Ellman et al. [7] using ATChI as substrate. The compounds **6a–6d** activities of the cholinoesterases were assayed as described earlier [8].

2.14.4. Fluorescence spectroscopy

Acetylthiocholine iodide at 7 concentrations in phosphatate-buffered solution (0.1 M, pH 8.0) and solution of 5,5′-dithiobisnitrobenzoic acid (DTNB, 0.05 ml, 0.5 M) in the absence and presence of compounds **6a–6d** were mixed. Then AChE from Electrophorus electricus at concentration 5 units/ml was added to the samples to a final volume of 3 ml and placed at 37 °C in the cuvette holder of a PerkinElmer fluorescence spectrophotometer. The emission spectra were recorded with wavelength set at 412 nm after 1 min. Butyrylcholinesterase (BChE) inhibitory activity determination were carried out similarly using 5 units/ml of BChE instead of AChE in a final volume 3 ml.

2.14.5. Statistical analysis

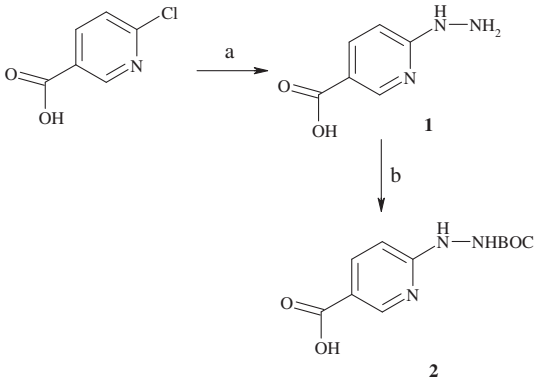
The drug concentration producing the 50% AChE and BChE activity inhibition (IC_{50}) was calculated by non-linear and linear regression.

3. Results and discussion

In the present article we describe synthesis and biological evaluation of a series of derivatives where the aliphatic link between hydrazine nicotinic acid and nitrogen atom in position 9 of tetrahydroacridine comprises of 4, 5, 7 or 9 carbon atoms.

In the design of new acetylcholinesterase inhibitors we focused on coupling 9-chloro-1,2,3,4-tetrahydroacridine with diamine and moiety of 6-BOC-hydrazinopyridine-3-carboxylic acid.

Firstly, compound **1** was obtained as previously described by Abrams et al. [9]. 6-Chloronicotinic acid and hydrazine hydrate was refluxed at 100 °C with a good yield (Scheme 1). In the next



Scheme 1. Synthesis of 6-BOC-hydrazinopyridine-3-carboxylic acid. Reagents: (a) 85% NH_2NH_2; (b) $(t\text{-BuOCO})_2O$, triethyl amine, DMF.

step 6-hydrazinopyridine-3-carboxylic acid was treated by di-*tert*-butyl dicarbonate and triethylamine in dimethylformamide and afforded compound **2** (66% yield) [9].

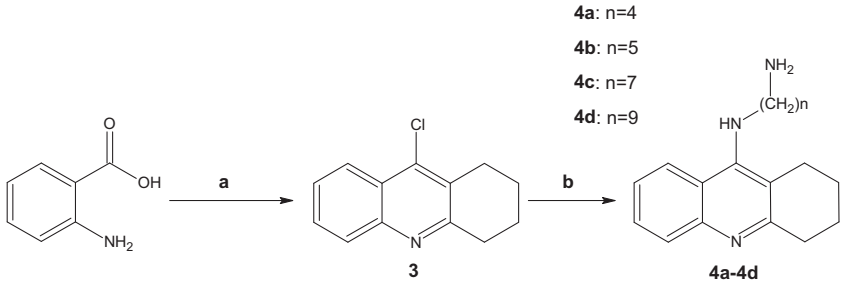
Secondly, the synthesis of 3 was made by treatment of anthranilic acid with cyclohexanone (Scheme 2). We disclosed that **3** could be concisely synthesized with high efficiency (71% yields) by direct heating the mixture of anthranilic acid and cyclohexanone in fresh $POCl_3$. Combination of 9-chloro-1,2,3,4-tetrahydroacridine (**3**) with 2 equivalent of the appropriate ω-diamine and catalytic amounts of sodium iodide in the presence of phenol at 180 °C provided **4a–4d** in a good yield (83–91%). [10–15] Chemical studies of compounds **1–3** were presented in our publication from 2006 [16]. The preparation of **5a–5d**, in 51–78% yield, was conducted by using 2-chloro-4,6-dimethyl-1,3,5-triazine (CDMT) (Scheme 3). CDMT, a coupling reagent for peptide synthesis, presents several advantages above carbodiimides which are generally approved, efficient condensing reagents. One of them is physicochemical features such as stable, crystalline form and good solubility in organic solvents. [17] The next one is easiness of elimination of the side products and the excess of condensing reagent due to weakly basic character of the triazine ring. Furthermore, CDMT allows synthesizing di-, tri-, and pentapeptides in yield above 75% under mild reaction conditions and without accompanying racemization [18,19].

The procedure of acquiring of amides by means of CDMT is highly effective for a wide variety of reagents, including chiral amino acids without alteration of configuration. The reaction's mechanism involves activation of the carboxylic acid by CDMT and the base (tertiary amine) which frequently is N-methylmorpholine. The best results were achieved by dropwise addition of N-methylmorpholine to the solution of compound **2** in an appropriate inert solvent, such as dichloromethane, acetonitrile, dioxane or tetrahydrofuran at −5 °C. Monitoring of the reaction by TLC showed that the reaction was usually completed after 1 h. It led to active ester which afterwards reacted with the appropriate amine **4a–4d** which was added in the same pot. [17] In this way, compounds **5a–5d** were obtained with a good yield (about 80%). Compounds **6a–6d** as hydrochlorides were acquired by crystallization from HCl in ether.

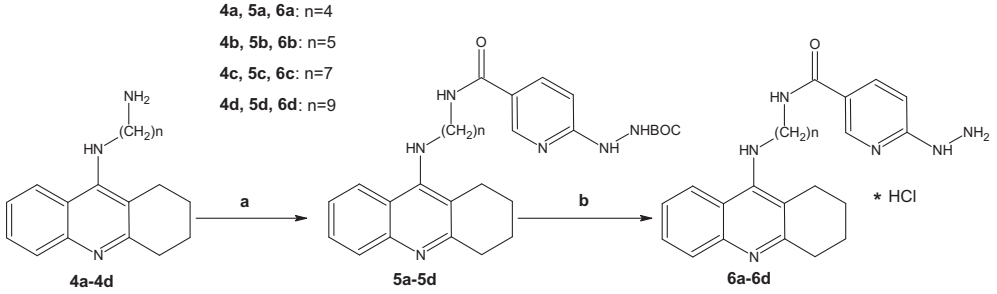
Newly synthesized compounds were assayed for acetylcholinesterase (*Electrophorus electricus*) and butyrylcholinesterase (equine serum) inhibition potency by the Ellman's method.

The linear Lineweaver–Burk equation which is a double reciprocal form of the Michaelis–Menten equation was converted to evaluate the type of inhibition. Linear regression of the reaction rate depending on concentration of substrate was used to calculate constants K_m, V_{max}.

Subsequently, apparent K_i constants were estimated by means of nonlinear regression using the Cheng–Prusoff equation [20]. In Table 1 there are shown statistical parameters and values of K_m, and V_{max} for cholinesterases (AChE and BChE). Table 2 presents obtained data of activities of compounds **6a–6d** towards AChE and BChE inhibition. In comparison with tacrine all synthesized constituents are less active towards inhibition of AChE. Among them the most active appears to be particle **6b**. The highest value of IC_{50} presents molecule **6c**. Apart from evaluating of activity



Scheme 2. Synthesis of compounds **3**, **4a–4d**. Reagents: (a) cyclohexanone, $POCl_3$, reflux; (b) diamine, phenol, NaI, reflux.



Scheme 3. Synthesis of compounds **5a–5d** and **6a–6d**. Reagents: (a) **2**, CDMT, methylmorpholine, THF; (b) HCl/ether.

Table 1
Statistical parameters values of K_m and V_{max} for AChE and BChE.

Parameters	AChE	BChE
K_m	0.092683 μM	0.100176 μM
V_{max}	2.29645 μM/min/ml	2.526429 μM/min/ml
r^2	0.9954	0.9858
Standard error	0098	0.021

Table 2
IC_{50} values for activities towards AChE and BChE.

Compounds	AChE inhibition IC_{50}, μM	BChE inhibition IC_{50}, μM	Selectivity index (SI) for AChE[a]
6a	4.40×10^{-7}	1.45×10^{-9}	0.0033
6b	3.08×10^{-7}	6.49×10^{-10}	0.0021
6c	7.21×10^{-7}	1.13×10^{-9}	0.0016
6d	3.70×10^{-7}	8.11×10^{-10}	0.0022
Tacrine	5.46×10^{-9}	2.44×10^{-11}	0.0044

[a] Selectivity for AChE is defined as IC_{50}(BChE)/IC_{50}(AChE).

towards AChE inhibition all molecules were tested to estimate their potential as BChE inhibitors. All synthesized particles showed stronger BChE inhibition in comparison with tacrine. Afterwards, we calculated selectivity index of every component for AChE inhibition (ratio of IC_{50} BChE/AChE). All compounds are less selective towards AChE than tacrine.

Our structural modifications focused on the amino group of tacrine: different aliphatic chains were inserted and coupling with 6-hydrazinonicotinamide moiety to assess the importance change in this part of molecules. Compounds **6a–6d** showed a remarkable increase in activity towards BChE, especially the compound **6c** containing the aliphatic chain with 7 carbon atoms. This displays us that it was good way to introduce such modification. Generally it was shown that after coupling tacrine with Hynic, we obtained compounds more selective towards BChE. This is extremely important because it is known that in Alzheimer's disease there is observed higher level of this enzyme in central nervous system. It is established that AD is associated with alterations of cholinergic neurotransmission that is manifested by decrease of the cholinergic markers such as choline acetyltransferase (ChAT) and acetylcholinesterase (AChE). A reduction of the level of AChE and increased BChE level in patients with AD constitute a biochemical marker for AD that might be utilized in diagnosis of this disorder or could be strong advantage as a new potential AChEIs. Designed and synthesized compounds of a series of tacrine analogues led to the design of potent AChE inhibitors simultaneously being more active and selective for BChE. Treatment of AD, the most common form all kinds of dementia, still remains a challenge for scientists. In the recent years, there has been an accelerating general effort to determine the risk factors and causes of AD. Studies have also been conducted to find better ways of treating or diagnosis this illness and delaying its onset. AChEI like tacrine, donepezil, galantamine and rivastigmine are well-know drugs in the treatment of AD. These medicines are only administered for mild- to moderate-AD treatment. However, the use of these drugs with tacrine as an example is frequently limited because of their side effects. Thus, there is still very important to search for new compounds with acetylcholinestrase inhibitory activity. A drug design allowed us to synthesize a new series of AChEIs as analogues of tacrine with hynic moiety. The obtained compounds **6a–6d** are characterised by higher activity towards BChE inhibition than the reference tacrine compound. Different structural modifications carried out on tacrine especially on amino group demonstrated improvement of AChE inhibitory activity. This finding is very significant, especially in the context of AD therapy, because this illness is associated with AChE deficiency and these compounds could be potential new acetylcholinesterase inhibitors.

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