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New tacrine-hydrazinonicotinamide hybrids as acetylcholinesterase inhibitors of potential interest for the early diagnostics of Alzheimer's disease

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The syntheses and the preliminary results of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition by an affinity series of tacrine-hydrazinonicotinamide hybrids are described. These molecules were prepared by condensation of tacrine analogues with the hydrazine nicotinate moiety (HYNIC). Derivatives **6a** and **6b** showed lower activity than the model tacrine, while compounds **6c** and **6d** showed the strongest affinity to AChE. All the tested compounds exhibited lower affinity for BChE than tacrine. Alzheimer disease (AD) is characterised by a deficit of acetylcholinesterase, and these new compounds, as ligands for ^{99m}Tc complexes, are potential radiopharmaceuticals for an early diagnosis of Alzheimer's disease.

1. Introduction

Emil Kraepelin, head of the Psychiatry Department of the University of Munich, called Alois Alzheimer to Munich in 1903. In 1907 Alzheimer described in the medical literature the case of his patient Auguste D. We have now passed the millennium, nearly 100 years, after Alzheimer described the disease bearing his name. Typical symptoms of AD are characterized by gross and progressive impairment of the cognitive functions, often accompanied by behavioral disturbances such as aggression, depression, and wandering. Acetylcholinesterase inhibitors (AChEI) are the first line of treatment for AD. One well-characterized effect of AChEI is the inhibition of acetylcholinesterase, the enzyme that regulates synaptic availability of the neurotransmitter acetylcholine. Unfortunately, there is no single, simple test for AD, and it is clear that research into the etiology and treatment of AD need more diagnostic criteria than those in use. Considerable data exist to support the use of neuroimaging scanning as a biomarkers for diagnosis of Alzheimer disease. Neuroimaging is traditionally divided into structural and functional imaging. Functional techniques seek to examine physiological functioning, either at rest or during activity, and include single photon emission computed tomography (SPECT) organ mapping.

A lower level of AChE and higher level of BChE are observed in AD patients. This observation encouraged us to seek selective inhibitors of AChE with a moiety capable of binding the radiotracer.

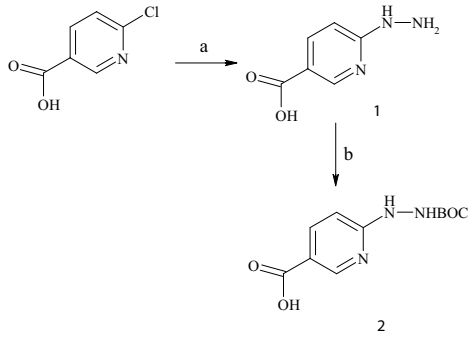
This work combines knowledge of the acetylcholinesterase inhibitors and neuroimaging, as the first step in research into new bifunctional biomarkers for AD diagnosis. One part of a new molecule should be responsible for binding with acetylcholinesterase and the second part for binding the radiotracer, which is technetium-99m (^{99m}Tc). The prototype for the centrally acting AChE inhibitor was tacrine (Cognex), the first drug to be approved in the United States for the treatment of AD. Tacrine derivatives are the principal structures of the new molecules that are responsible for binding with aceylcholinesterase. The second part of these potential biomarkers is hydrazine nicotinate (HYNIC). The synthesis of HYNIC has been reported previously by Abrams et al. (1990).

In the present study, we describe the synthesis of a series of tetrahydroacridine analogues coupled with the HYNIC moiety. All the bifunctional compounds obtained were tested for their affinity and selectivity for AChE and BChE. The preliminary radiolabeling of the new compounds was performed in the Department of Nuclear Medicine of the Medical University in Lodz. All the compounds were radiolabeled with ^{99m}Tc with a good yield and these results will be described in another paper.

2. Investigations and results

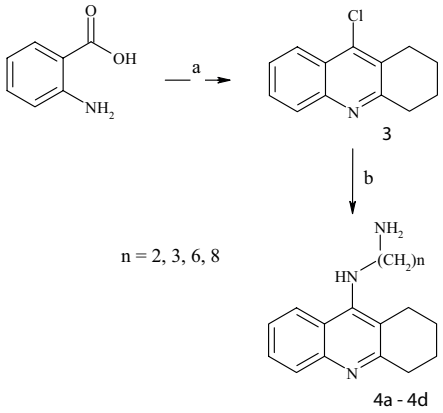
Compound **1** was obtained from 6-chloronicotinic acid and hydrazine hydrate (reflux at 100 °C) (85% yield), as shown in Scheme 1. In the next step 6-hydrazinopyridine-3-carboxylic acid in DMF was treated by di-*tert*-butyl dicarbonate and triethyl amine as catalyst and afforded **2** (66% yield). These syntheses have been described before by Abrams and co-workers. Ming-Kuan Hu reported the synthesis of **3** by treatment of anthranilic acid with cyclohexanone. Here (Scheme 2), we disclose that **3** could be concisely synthesized with high efficiency (71% yield) by directly heating the mixture of anthranilic acid and cyclohexanone in fresh $POCl_3$. Combination of 9-chloro-1,2,3,4-tetrahydroacridine (**3**) with 2 equivalents of the appropriate ω-diamine and catalytic amounts of sodium iodide in the presence of phenol at 180 °C provided **4a**–**4d** in good yield (83%–91%). The preparation of **5a**–**5d**, in

Scheme 1



(a) 85% NH_2NH_2; (b) $(t\text{-}BuOCO)_2O$, triethyl amine, DMF

Scheme 2



(a) cyclohexanone, $POCl_3$, reflux; (b) diamine, phenol, NaI, reflux

51%–78% yields, using 2-chloro-4,6-dimethyl-1,3,5-triazine (CDMT) proceeds as a sequence of two independent steps in a one-pot synthesis (Scheme 3). In the first step, 6-BOC-hydrazinopyridine-3-carboxylic acid (**2**) is activated by treatment with stoichiometric amounts of CDMT and *N*-methylmorpholine at low temperature. The best results in this reaction were achieved by dropwise addition of *N*-methylmorpholine to the solution in an appropriate inert solvent, such as dichloromethane, acetonitrile, dioxane or tetrahydrofuran at −5 °C. Monitoring of the reaction by TLC showed that the reactive intermediate was usually completed within 1–4 h. In the second step, a mixture of the appropriate **4a**–**4d** in the respective solvent is added to the crude solution obtained as described above at −5 °C. The final compounds **6a**–**6d**, were obtained from compounds **5a**–**5d**, in the presence of hydrochloric acid, by crystallization from HCl in ether. In this step the BOC group falls off and the precipitate of **6a**–**6d** is formed as hydrochloride.

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

Parameters	AChE	BChE
K_m	0.062058 μM	0.060378 μM
V_{max}	0.099877 μM/min/ml	0.075895 μM/min/ml
r^2	0.9943	0.9938
Standard error	0.0153	0.0015

Table 2: IC_{50} values for activities on acetylcholinesterase and butyrylcholinesterase

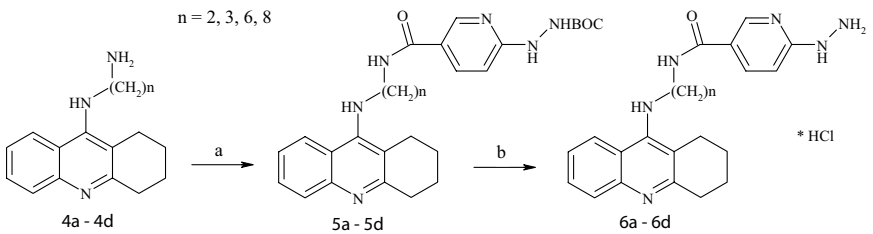
Compounds	AChE inhibition (IC_{50}, μM)	BChE inhibition (IC_{50}, μM)	Selectivity for AChE[a]	Selectivity for BChE[b]
6a	5.63×10^{-5}	2.25×10^{-5}	0.40	2.50
6b	3.38×10^{-5}	3.53×10^{-5}	1.04	0.95
6c	3.38×10^{-6}	9.60×10^{-6}	2.84	0.35
6d	2.65×10^{-6}	1.76×10^{-5}	6.64	0.15
Tacrine	2.38×10^{-5}	1.16×10^{-6}	0.05	20.51

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

To determine the type of inhibition, the Michaelis-Menten equation was fitted using linear transformation: Lineweaver-Burk (1/v vs. 1/[S]) plot. Michaelis-Menten constants (K_m, V_{max}) were obtained by linear regression of the reaction rate as a function of substrate concentration. Apparent K_i constants were then calculated using nonlinear regression. Statistical parameters and values of K_m, and V_{max} for AChE and BChE are given in Table 1. According to the standard methodology, we obtained the following data for the acetylcholinesterase inhibition activity (Table 2). Compared with tacrine, we can see that the more active compounds are **6c** and **6d** (about 4 to 9 times more active, respectively). These compounds have a long carbon chain between the tetrahydroacridine and hydrazinopyridine moieties. Compounds **6a** and **6b** showed similar IC_{50} activity compared with tacrine. All the compounds were tested to determine their activity on butyrylcholinesterase and the pertinent data are shown in Table 2. All the tested compounds exhibit lower inhibition of BChE compared with tacrine. These results are very interesting, as it is known that **6c** and **6d** inhibit mainly AChE.

The most active and representative compounds, as well as the reference inhibitor tacrine, were then compared in or-

Scheme 3



(a) **2**, CDMT, methylmorpholine, THF; (b) HCl/ether

der to determine their relative inhibitory effects (Table 2) towards acetyl and butyrylcholinesterase (ratio of IC_{50} BChE/AChE), and toward butyryl and acetylcholinesterase (ratio of IC_{50} AChE/BChE). Compounds **6c** and **6d**, which were the most active derivatives on AChE, were found to be respectively 57- and 133-fold more selective for AChE than the reference tacrine. The derivatives **6a** and **6b** present a weak selectivity profile for AChE. All the compounds tested showed less selectivity for BChE compared to tacrine.

3. Discussion

The synthesis and biological evaluation of a series of tacrine analogues led to the design of inhibitors which were potent and selective for AChE and less selective for BChE. These molecules were prepared from tacrine analogues and hydrazine nicotinate. Compounds **6c** and **6d** have more selectivity for AChE and less selectivity for BChE compared to tacrine. This is very important in SPECT because Alzheimer disease is characterized by deficits of acetylcholinesterase. All the findings open the way for the modification of these molecules. The data presented give support to our initial hypothesis, and encourage us to continue this project. Work is now in progress, and in the next step these compounds will be radiolabeled with ^{99m}Tc.

4. Experimental

4.1. Chemistry

Reactions were monitored by TLC using 25 DC-Alufolien Kieselgel $60F_{254}$ plates (Merck), with detection by UV lamp (254 nm). Melting points were measured on an Electrothermal apparatus in 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 (230–400 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 and elemental analyses were performed by the Centre of Molecular and Macromolecular Studies in Lodz (Polish Academy of Sciences). All the results of elemental analyses were in an acceptable range.

4.1.1. 6-Hydrazinopyridine-3-carboxylic acid (**1**)

6-Chloronicotinic acid (8.0 g, 50.77 mmol) was dissolved in 80% hydrazine hydrate (35 ml, 930.0 mmol) and placed in a 100 °C oil bath for 4 h. The homogeneous reaction mixture was cooled to room temperature and concentrated to dryness to give a white solid. The solid was dissolved in water and on acidification to pH 5.5 with concentrated hydrochloric acid a precipitate was formed. The precipitate was isolated by filtration, the solid was washed with 95% ethanol and ether, and dried in vacuum. Compound **1**: m.p. 292–293 °C; yield 85%; IR (KBr) ν (cm^{-1}): 1333.0, 3080.1, 3308.2; ^{1}H NMR (DMSO) (δ ppm.): 8.5 (1 H, s, COOH), 8.3 (1 H, s, CHN), 7.8 (1 H, dd, J = 8.9, 2.4 Hz, CCHC), 6.7 (1 H, d, J = 8.9 Hz, CCHC), 3.2 (1 H, s, CNH), 2.5 (2 H, d, J = 1.8 Hz, NH_2).
MS (FAB) m/z (M + 1) 154.1
$C_6H_7N_3O_2$

4.1.2. 6-BOC-hydrazinopyridine-3-carboxylic acid (**2**)

To a solution of **1** (1.4 g, 9.8 mmol) and triethyl amine (1.2 ml, 11.8 mmol) in DMF (10 ml) was added di-*tert*-butyl dicarbonate (2.13 g, 9.8 mmol). The reaction mixture became homogeneous over 1 h and stirring was continued for 16 h at room temperature. The reaction mixture was concentrated to dryness under reduced pressure to give a brown solid. Recrystallization from ethyl acetate gave the desired product **2** as a white solid. Compound **2**: m.p. 282–285 °C; yield 66%; IR (KBr) ν (cm^{-1}): 1608.8, 1706.4, 3253.7; ^{1}H NMR (DMSO) (δ ppm.): 12.5 (1 H, s, COOH), 8.9 (1 H, d, J = 25.6 Hz, NHC), 8.6 (1 H, s, CHN), 7.9 (1 H, dd, J = 8.7, 6.9 Hz, CCHC), 6.5 (1 H, d, J = 8.7 Hz, CCHC), 3.3 (1 H, d, J = 6.9 Hz, CNH), 1.4–1.5 (9 H, m, BOC).
MS (FAB) m/z (M + 1) 254.2, 198.0
$C_{11}H_{15}N_3O_4$

4.1.3. 9-Chloro-1,2,3,4-tetrahydroacridine (**3**)

To a mixture of anthranilic acid (7.4 g, 53.9 mmol) and cyclohexanone (5.36 ml, 51.7 mmol) was carefully added 30 ml of $POCl_3$ in an ice bath. The mixture was heated under reflux for 2 h, then cooled at room temperature, and concentrated under reduced pressure to give a slurry. The residue was diluted with ethyl acetate (50 ml), neutralized with aqueous K_2CO_3 (30 ml), and washed with brine (2 × 20 ml). The organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure to give a yellow solid. Recrystallization from acetone yielded the desired product **3** as a yellow solid. Compound **3**: m.p. 66–68 °C; yield 71%; IR (KBr) ν (cm^{-1}): 1638.9, 2851.1, 2920.1, 3432.7; ^{1}H NMR ($CDCl_3$) (δ ppm.): 8.1 (1 H, d, J = 7.1 Hz, ArH), 7.9 (1 H, d, J = 7.1 Hz, ArH), 7.6 (1 H, t, J = 1.6 Hz, ArH), 7.4 (1 H, t, J = 6.9 Hz, ArH), 3.0 (2 H, t, J = 4.6 Hz, CH_2), 2.9 (2 H, t, J = 6.5 Hz, CH_2), 1.8–2.0 (4 H, m, CH_2CH_2).
MS (FAB) m/z (M + 1) 218.0, 184.9
$C_{13}H_{12}ClN$

4.1.4. N-(1,2,3,4-Tetrahydroacridin-9-yl)ethane-1,2-diamine (**4a**)

A mixture of **3** (0.75 g, 3.5 mmol), 1,2-diaminoethane (0.47 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 (2 × 20 ml), 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 91%; IR (KBr) ν (cm^{-1}): 1562.5, 2854.1, 2930.8, 3058.1, 3358.8; ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.9 (2 H, t, J = 8.6 Hz, ArH), 7.5 (1 H, t, J = 7.6 Hz, ArH), 7.3 (1 H, t, J = 7.5 Hz, ArH), 5.1 (1 H, s, NH), 3.5 (2 H, t, J = 5.5 Hz, $NHCH_2$), 3.0 (2 H, s, CH_2), 2.8–2.9 (2 H, m, CH_2NH_2), 2.6 (2 H, s, CH_2), 2.0 (2 H, s, NH_2), 1.8 (4 H, t, J = 3.0 Hz, CH_2CH_2).
MS (FAB) m/z (M + 1) 242.2, 199.0, 183.0
$C_{15}H_{19}N_3$

4.1.5. N-(1,2,3,4-Tetrahydroacridin-9-yl)propane-1,3-diamine (**4b**)

A mixture of **3** (0.75 g, 3.5 mmol), 1,3-diaminopropane (0.58 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 87%; IR (KBr) ν (cm^{-1}): 1579.1, 2856.1, 2928.0, 3059.5, 3313.1; ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.9 (1 H, d, J = 4.3 Hz, ArH), 7.8 (1 H, d, J = 4.3 Hz, ArH), 7.5 (1 H, t, J = 7.0 Hz, ArH), 7.3 (1 H, t, J = 7.6 Hz, ArH), 5.0 (1 H, s, NH), 3.5 (2 H, t, J = 6.6 Hz, $NHCH_2$), 2.9 (2 H, s, CH_2), 2.6–2.8 (2 H, m, CH_2NH_2), 2.6 (2 H, s, CH_2), 2.0 (2 H, s, NH_2), 1.8 (4 H, t, J = 3.3 Hz, CH_2CH_2), 1.6–1.7 (6 H, m, $CH_2CH_2CH_2$).
MS (FAB) m/z (M + 1) 256.2, 199.0
$C_{16}H_{21}N_3$

4.1.6. N-(1,2,3,4-Tetrahydroacridin-9-yl)hexane-1,6-diamine (**4c**)

A mixture of **3** (0.75 g, 3.5 mmol), 1,6-diaminohexane (0.81 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 84%; IR (KBr) ν (cm^{-1}): 1563.0, 2856.0, 2929.0, 3063.0, 3354.0; ^{1}H NMR ($CDCl_3$) (δ ppm.): 8.0 (2 H, t, J = 8.4 Hz, ArH), 7.6 (1 H, t, J = 6.9 Hz, ArH), 7.4 (1 H, t, J = 7.1 Hz, ArH), 4.1 (1 H, s, NH), 3.6 (2 H, t, J = 7.8 Hz, $NHCH_2$), 3.1 (2 H, s, CH_2), 2.7 (2 H, d, J = 6.5 Hz, CH_2NH), 2.6 (2 H, s, CH_2), 2.1 (2 H, s, NH_2), 1.9 (4 H, t, J = 3.2 Hz, CH_2CH_2), 1.5–1.7 (8 H, m, $CH_2CH_2CH_2$), 1.2–1.4 (8 H, br, $CH_2CH_2CH_2$); MS (FAB) m/z (M + 1) 298.3, 199.1.
MS-HR (FAB) 297.22050 Found: 298.22959 (M + 1).
$C_{19}H_{27}N_3$

4.1.7. N-(1,2,3,4-Tetrahydroacridin-9-yl)octane-1,8-diamine (**4d**)

A mixture of **3** (0.75 g, 3.5 mmol), 1,3-diaminooctane (1.01 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 84%; IR (KBr) ν (cm^{-1}): 1562.0, 2854.0, 2926.0, 3061.0, 3347.0; ^{1}H NMR ($CDCl_3$) (δ ppm.): 7.9 (2 H, dd, J = 13.9, 8.3 Hz, ArH), 7.5 (1 H, t, J = 7.1 Hz, ArH), 7.3 (1 H, t, J = 7.1 Hz, ArH), 3.9 (1 H, s, NH), 3.4 (2 H, t, J = 6.7 Hz, $NHCH_2$), 3.0 (2 H, s, CH_2), 2.6 (4 H, d, J = 6.9 Hz, CH_2NH_2), 2.5 (2 H, s, CH_2), 2.1 (2 H, s, NH_2), 1.8 (4 H, p, CH_2 CH_2), 1.5–1.6 (2 H, m, CH_2CH_2), 1.2–1.3 (8 H, br, CH_2CH_2); MS (FAB) m/z (M + 1) 326.3, 197.1.
MS-HR (FAB) 325.25180 Found: 326.26099 (M + 1).
$C_{21}H_{31}N_3$

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

To a stirred mixture of 2-chloro-4,6-dimethyl-1,3,5-triazine (CDMT) (1.76 g, 10 mmol) and **4a** (2.46 g, 10.2 mmol) in THF (10 ml), *N*-methylmorpholine (1.12 ml, 10.2 mmol) was added dropwise at such a rate as to keep temperature at −5 °C to 0 °C. Stirring was continued at 0 °C for 1–4 h

until all CDMT had been consumed. Then to the crude mixture, obtained as described above, **2** (2.56 g, 10.2 mmol) in THF (8 ml), at −5 °C to 0 °C, was added. Stirring was continued at 0 °C for 2 h, and then for 12 h at room temperature. A precipitate formed and was isolated by filtration. Recrystallization from ethyl acetate afforded the desired product **5a** as a yellow solid. Compound **5a**: m.p. 225–227 °C; yield 51%; IR (KBr) ν (cm^{-1}): 1521.5, 1635.4, 1718.0, 2871.5, 2932.0, 3245.8; ^{1}H NMR (CD_3OD) (δ ppm.): 8.5 (1 H, d, J = 8.7 Hz, NHC), 8.4 (1 H, s, CHC), 7.9 (1 H, d, J = 8.3 Hz, CCHC), 7.6–7.8 (3 H, m, ArH), 7.5 (1 H, t, J = 1.6 Hz, ArH), 6.7 (1 H, d, J = 6.7 Hz, CCHC), 4.6 (1 H, s, NH), 4.2 (2 H, t, J = 5.4 Hz, $NHCH_2$), 3.7 (2 H, t, J = 5.3 Hz, CH_2), 3.3 (1 H, s, CHNH), 2.9 (2 H, s, CH_2NH), 2.7 (2 H, t, J = 5.4 Hz, CH_2), 1.9 (4 H, d, J = 5.7 Hz, CH_2), 1.4–1.5 (9 H, m, BOC); MS (FAB) m/z (M + 1) 477.3, 377.2.
MS-HR (FAB) 476.25359 Found: 477.25975 (M + 1).
$C_{26}H_{32}N_6O_3$

4.1.9. N-{5-[3-(1,2,3,4-Tetrahydroacridin-9-ylamino)propylcarbamoyl]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), **4b** (2.60 g, 10.2 mmol) in THF (10 ml), and *N*-methylmorpholine (1.12 ml, 10.2 mmol) were combined as above to afford **5b** as a yellow solid. Compound **5b**: m.p. 194–196 °C; yield 54%; IR (KBr) ν (cm^{-1}): 1562.8, 1637.4, 1720.5, 2852.8, 2925.4, 3292.4; ^{1}H NMR (CD_3OD) (δ ppm.): 8.4 (1 H, s, CHN), 8.3 (1 H, d, J = 8.3 Hz, NHC), 7.9 (1 H, d, J = 8.3 Hz, CCHC), 7.6–7.8 (3 H, m, ArH), 7.5 (1 H, t, J = 7.2 Hz, ArH), 6.7 (1 H, d, J = 8.7 Hz, CCHC), 4.6 (1 H, s, NH), 4.0 (2 H, t, J = 6.5 Hz, $NHCH_2$), 3.5 (2 H, t, J = 6.2 Hz, CH_2NH_2), 3.3 (1 H, s, CHNH), 3.0 (2 H, s, CH_2NH), 2.7 (2 H, s, CH_2), 2.1 (2 H, s, CH_2), 1.9 (4 H, d, J = 3.1 Hz, CH_2), 1.2–1.5 (9 H, m, BOC); MS (FAB) m/z (M + 1) 491.3, 391.2.
MS-HR (FAB) 490.26924 Found: 491.27693 (M + 1).
$C_{27}H_{34}N_6O_3$

4.1.10. N-{5-[6-(1,2,3,4-Tetrahydroacridin-9-ylamino)hexylcarbamoyl]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), **4c** (3.03 g, 10.2 mmol) in THF (10 ml), and *N*-methylmorpholine (1.12 ml, 10.2 mmol) were combined as above to afford **5c** as a yellow solid. Compound **5c**: m.p. 144–145 °C; yield 79%; IR (KBr) ν (cm^{-1}): 1523.9, 1637.1, 1717.8, 2858.2, 2929.8, 3270.5, 3404.9; ^{1}H NMR (CD_3OD) (δ ppm.): 8.4 (1 H, s, NHC), 8.3 (1 H, s, CHC), 7.9 (1 H, d, J = 7.9 Hz, CCHC), 7.6–7.8 (3 H, m, ArH) 7.5 (1 H, t, J = 7.7 Hz, ArH), 6.7 (1 H, d, J = 8.7 Hz, CCHC), 3.9 (1 H, s, NH), 3.7–3.8 (4 H, m, $NHCH_2$), 3.4 (2 H, t, J = 4.9 Hz, CH_2), 3.2 (2 H, t, J = 9.6 Hz, CH_2), 2.9 (1 H, d, J = 6.5 Hz, CNH), 2.6 (2 H, s, CH_2), 1.9 (7 H, d, J = 2.8 Hz, CH_2), 1.8 (4 H, t, J = 3.3 Hz, CH_2), 1.6 (2 H, t, J = 7.0 Hz, CH_2), 1.4–1.5 (9 H, m, BOC); MS (FAB) m/z (M + 1) 533.4, 433.3.
MS-HR (FAB) 532.31619 Found: 533.32517 (M + 1).
$C_{30}H_{40}N_6O_3$

4.1.11. N-{5-[8-(1,2,3,4-Tetrahydroacridin-9-ylamino)octylcarbamoyl]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), **4d** (3.32 g, 10.2 mmol) in THF (10 ml), and N-methylmorpholine (1.12 ml, 10.2 mmol) were combined as above to afford **5d** as a yellow solid. Compound **5d**: m.p. 87–89 °C; yield 76%; IR (KBr) ν (cm^{-1}): 1524.7, 1637.3, 1719.0, 2855.0, 2928.0, 3289.9, 3412.0; ^{1}H NMR (CD_3OD) (δ ppm.): 8.5 (1 H, s, NHC), 8.4 (1 H, d, J = 8.6 Hz, CHC), 7.9 (1 H, d, J = 8.6 Hz, CCHC), 7.6–7.8 (3 H, m, ArH), 7.5 (1 H, dd, J = 8.4, 7.3 Hz, ArH), 6.7 (1 H, d, J = 8.8 Hz, CCHC), 4.0 (1 H, s, NH), 3.9 (2 H, t, J = 7.2 Hz, $NHCH_2$), 3.7 (2 H, t, J = 6.5 Hz, CH_2), 3.2 (1 H, d, J = 7.3 Hz, CHNH), 2.9–3.1 (2 H, m, CH_2NH), 2.6–2.7 (3 H, m, CH_2), 1.8–1.9 (6 H, m, CH_2CH_2), 1.6 (2 H, br, CH_2), 1.3–1.4 (9 H, s, BOC), 1.3 (8 H, br, CH_2); MS (FAB) m/z (M + 1) 561.5, 461.4.
MS-HR (FAB) 560.34749 Found: 561.35480 (M + 1).
$C_{32}H_{44}N_6O_3$

4.1.12. 6-Hydrazino-N-[2-(1,2,3,4-tetrahydroacridin-9-ylamino)ethyl]nicotinamide hydrochloride (**6a**)

A solution of HCl in ether was prepared by bubbling HCl into ether (50 ml) at a moderate rate for 20 min. **5a** (0.20 g, 0.4199 mmol) was dissolved in ether (2 ml), HCl/ether (4 ml) was added, and the reaction mixture was stirred at room temperature. After 2 min, the solution became cloudy and a precipitate formed. The precipitate was isolated by filtration and the solid was washed with ether and dried in vacuum. Compound **6a**: m.p. 260–262 °C; yield 52%; IR (KBr) ν (cm^{-1}): 1528.1, 1683.6, 2858.3, 2937.0, 3055.4, 3267.3; ^{1}H NMR (DMSO) (δ ppm.): 13.8 (1 H, s, HCl), 9.9 (1 H, s, NHC), 9.0 (1 H, s, CHC), 8.5 (1 H, d, J = 1.9 Hz, CCHC), 7.8–8.1 (3 H, m, ArH), 7.5 (1 H, t, J = 7.8 Hz, ArH), 6.8 (1 H, d, J = 8.3 Hz, CCHC), 4.1–4.5 (3 H, br, CH_2NH), 4.0 (2 H, d, J = 5.7 Hz, CH_2), 3.6 (1 H, d, J = 5.5 Hz, CHNH), 3.4 (2 H, d, J = 7.1 Hz, $NHCH_2$), 3.0 (2 H, s, CH_2), 2.7 (2 H, s, CH_2NH), 1.8 (4 H, s, CH_2); MS (FAB) m/z (M + 1) 377.3, 242.1, 199.0.
MS-HR (FAB) 376.20116 Found: 377.20820 (M + 1).
$C_{21}H_{24}N_6O$

4.1.13. 6-Hydrazino-N-[3-(1,2,3,4-tetrahydroacridin-9-ylamino)propyl]-nicotinamide hydrochloride (**6b**)

5b (0.20 g, 0.4079 mmol) was combined as above to afford **6b** as a yellow solid. Compound **6b**: m.p. 256–258 °C; yield 46%; IR (KBr) ν (cm^{-1}): 1525.1, 1680.3, 2870.3, 2932.3, 3071.8, 3267.4; ^{1}H NMR (DMSO) (δ ppm.): 14.0 (1 H, s, HCl), 8.6 (1 H, s, CHN), 8.4 (1 H, d, J = 8.5 Hz, NHC), 8.1 (1 H, d, J = 10.9 Hz, CCHC), 7.5–7.9 (3 H, m, ArH) 7.4 (1 H, t, J = 7.9 Hz, ArH), 6.9 (1 H, d, J = 8.7 Hz, CCHC), 5.4 (1 H, s, NH), 3.9 (2 H, s, CH_2NH_2), 3.3 (3 H, s, CHNH), 2.9 (2 H, s, CH_2NH), 2.7 (2 H, s, CH_2), 1.9 (2 H, s, CH_2), 1.7 (4 H, s, CH_2), 1.6 (2 H, s, CH_2); MS (FAB) m/z (M + 1) 391.2, 376.3, 199.0.
MS-HR (FAB) 390.21681 Found: 391.22295 (M + 1).
$C_{22}H_{26}N_6O$

4.1.14. 6-Hydrazino-N-[6-(1,2,3,4-tetrahydroacridin-9-ylamino)hexyl]nicotinamide hydrochloride (**6c**)

5c (0.20 g, 0.3757 mmol) was combined as above to afford **6c** as a brown solid. Compound **6c**: m.p. 154–156 °C; yield 43%; IR (KBr) ν (cm^{-1}): 1528.9, 1638.9, 2876.0, 2976.0, 2933.7, 3380.4; ^{1}H NMR (DMSO) (δ ppm.): 14.1 (1 H, s, HCl), 8.7 (1 H, d, J = 5.3 Hz, NHC), 8.6 (1 H, d, J = 1.7 Hz, CHC), 8.4 (1 H, d, J = 8.3 Hz, CCHC), 7.8–8.1 (3 H, m, ArH), 7.5 (1 H, t, J = 8.0 Hz, ArH), 6.9 (1 H, d, J = 8.7 Hz, CCHC), 5.7 (1 H, d, NH), 3.9 (4 H, m, $NHCH_2$), 3.3 (1 H, s, CH_2), 3.2 (2 H, d, J = 5.1 Hz, CH_2NH), 3.0 (2 H, s, CH_2), 2.6 (2 H, s, CH_2), 1.7–1.8 (4 H, m, CH_2), 1.5 (4 H, d, J = 24.3 Hz, CH_2), 1.3 (4 H, s, CH_2); MS (FAB) m/z (M + 1) 433.4, 340.3.
MS-HR (FAB) 432.26376 Found: 433.27153 (M + 1).
$C_{25}H_{32}N_6O$

4.1.15. 6-Hydrazino-N-[8-(1,2,3,4-tetrahydroacridin-9-ylamino)octyl]nicotinamide hydrochloride (**6d**)

5d (0.20 g, 0.3569 mmol) was combined as above to afford **6d** as a brown solid. Compound **6d**: m.p. 94–97 °C; yield 59%; IR (KBr) ν (cm^{-1}): 1527.0, 1638.0, 2854.8, 2930.9, 3099.2, 3263.5; ^{1}H NMR (DMSO) (δ ppm.): 13.9 (1 H, s, HCl), 8.6 (1 H, s, NHC), 8.4 (1 H, d, J = 7.7 Hz, CHC), 8.3 (1 H, s, CCHC), 7.8–7.9 (3 H, m, ArH), 7.5–7.6 (1 H, m, ArH), 6.8 (1 H, d, J = 9.1 Hz, CCHC), 3.8–4.2 (7 H, br, $NHCH_2$), 3.2 (1 H, br, CHNH), 3.0 (2 H, s, CH_2NH), 2.6 (2 H, s, CH_2), 1.7–1.9 (6 H, m, CH_2CH_2), 1.5 (2 H, br, CH_2), 1.2 (8 H, s, CH_2); MS (FAB) m/z (M + 1) 461.4, 326.4.
MS-HR (FAB) 460.29506 Found: 461.30091 (M + 1).
$C_{27}H_{36}N_6O$

4.2. Biochemical studies

The activity of the acetylcholinesterase (AChE) inhibitors was measured spectrophotometricaly according to the method of Ellman (1961) with some modification. The reaction took place in a final volume of 3 ml of phosphate-buffered solution (0.1 M, pH 8.0) at 37 °C, containing 5 units/ml AChE and a solution of 5,5′-dithiobisnitrobenzoic acid (DTNB, 0.05 ml, 0.5 M) and the appropriate inhibitor. Inhibition curves with the different derivatives were obtained using 7 concentrations of acetylthiocholine iodide for 1 min. Enzyme activity was determined by measuring the absorbance at 412 nm after 1 min. One triplicate sample without inhibitor was always present to give 100% of AChE activity.
Butyrylcholinesterase (BChE) inhibitory activity determination, was carried out similarly using 5 units/ml of BChE instead of AChE in a final volume of 3 ml.
The drug concentration producing 50% inhibition of AChE or BChE activity (IC_{50}) was calculated by non-linear and linear regression.
DTNB, enzymes (C2629 and C4290) and acetylthiocholine iodide were purchased from Sigma-Aldrich.

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