Full Paper

Design, Synthesis and Evaluation of Novel 2-(Aminoalkyl)-isoindoline-1,3-dione Derivatives as Dual-Binding Site Acetylcholinesterase Inhibitors

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A new series of 2-(diethylaminoalkyl)-isoindoline-1,3-dione derivatives intended as dual binding site cholinesterase inhibitors were designed using molecular modeling and evaluated as inhibitors of acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and the formation of the β-amyloid (Aβ) plaques. For AChE inhibitory activity, the spectrophotometric method of Ellman and the electrophoretically mediated microanalysis assay were used, giving good results. Most of the synthesized compounds had AChE inhibitory activity with IC_{50} values ranging from $IC_{50} = 0.9$ to 19.5 μM and weak Aβ anti-aggregation inhibitory activity. These results support the outcome of docking studies which tested compounds targeting both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE. The most promising selective AChE inhibitors are compounds **10** ($IC_{50} = 1.2$ μM) and **11** ($IC_{50} = 1.1$ μM), with 6–7 methylene chains, which also inhibit Aβ fibril formation.

Keywords: 2-(Aminoalkyl)-isoindoline-1,3-dione derivatives / Aβ aggregation / Acetylcholinesterase inhibitors / Electrophoretically mediated microanalysis (EMMA) / Molecular modeling

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Introduction

Alzheimer's disease (AD) is the most common neurodegenerative brain disorder of the 21st century [1]. AD is characterized by progressive dementia caused by the deficits in the cholinergic system in the brain areas related to memory and learning, brain deposits of amyloid beta (Aβ) peptide and neurofibrillary tangles [2]. Treatment of AD currently focuses on increasing cholinergic neurotransmission in the brain by cholinesterase inhibitors: donepezil, rivastigmine, and galantamine [3]. Clinical experience has shown that all these medications represent forms of symptomatic therapy; however there is reason to suspect their involvement in certain disease-modifying effects including β-amyloid formation and neuroprotection [4, 5]. Acetylcholinesterase (AChE) inhibitors can interact at the active catalytic site of the enzyme as well as at its peripheral anionic binding site (PAS), the latter of which is thought to possess the ability to bind to Aβ peptides thus promoting fibrillogenesis. Dual binding site inhibitors of cholinesterases influence acetylcholine hydrolysis and PAS-dependent β-amyloid aggregation [6, 7]. Compounds with these properties can be defined as multi-target ligands. The multi-target-directed ligand design strategy is an attractive approach to seeking novel effective drugs for the treatment of disorders with complex pathological mechanisms such as AD [8–10]. Thus, there is reason to develop novel dual binding site cholinesterase inhibitors as multipotent anti-Alzheimer's agents. In recent years many dual binding site cholinesterase inhibitors with β-amyloid anti-aggregation properties have been described and collected into review articles [11–13]. Structures of selected compounds are presented in Fig. 1 (see [14] for *bis*-tacrine).

Our research group has been involved in the development of cholinesterase inhibitors as potential means of treating AD. The recently described series of carbamate derivatives of *N*-benzylpiperidine displayed non-selective BuChE/AChE inhibitory activities [15]. Results of molecular modeling

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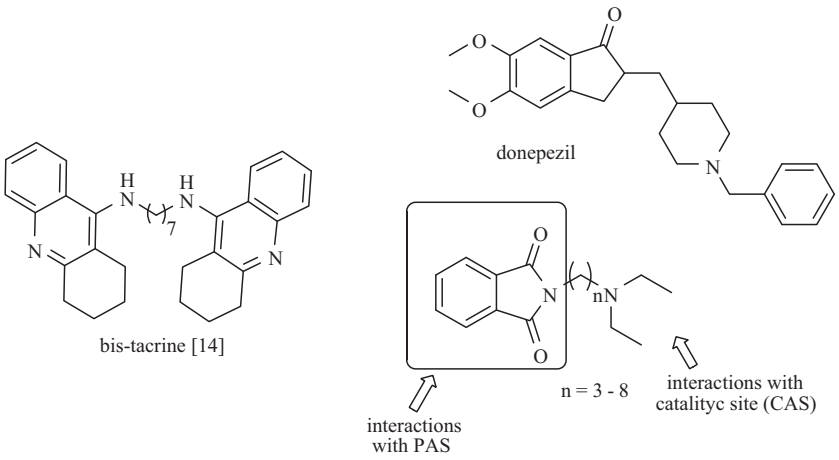


Figure 1. Structures of dual binding site cholinesterase inhibitors and the designed compounds.

studies showed that these inhibitors can bind to the active and the peripheral site of the AChE and like donepezil its *N*-benzylpiperidine fragment is oriented toward the catalytic center. In the present study a novel series of isoindoline-1,3-dione derivatives intended as cholinesterase inhibitors were designed using molecular modeling. Target compounds are designed as dual binding site inhibitors with an alkylamine moiety responsible for the interactions with the catalytic binding site, connected by an alkyl chain *N*-phthalimido fragment. The latter fragment, similarly to the indan-1-one fragment of donepezil may be able to bind at the peripheral active site of AChE and therefore prevent AChE-induced Aβ-aggregation (Fig. 1). Herein, we describe the synthesis, pharmacological evaluation (AChE and BuChE, inhibition and Aβ-anti-aggregation effect) and molecular modeling on a new series of 2-(diethylaminoalkyl)-isoindoline-1,3-dione derivatives.

Results and discussion

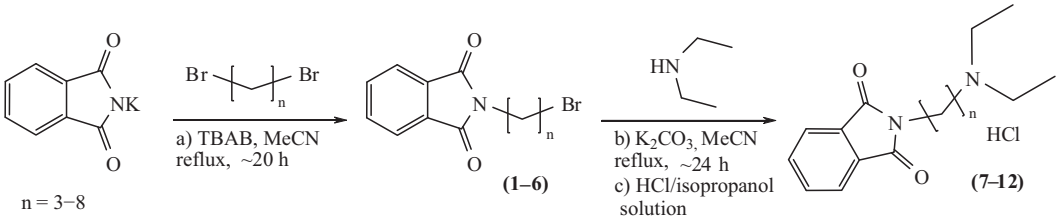
Chemistry

New isoindoline-1,3-dione derivatives (**7**–**12**) were synthesized via the route outlined in Scheme 1. At the first stage phthalimide potassium was alkylated with α,ω-dibromoalkane using tetra-*n*-butylammonium bromide (TBAB) as a catalyst. The reactions were carried out in acetonitrile for 20 h under reflux. Purification by silica gel column chromatography produced bromoalkyl derivatives of isoindoline-1,3-dione (**1**–**6**) with good yields (51–94%). The bromoalkyl derivatives of isoindoline-1,3-dione were then used as alkylating agents in reaction with diethylamine. Reactions were carried out in acetonitrile in the presence of potassium carbonate for 24 h under reflux. Following purification by silica gel column chromatography, the final 2-(3-diethylaminoalkyl)-isoindoline-1,3-diones (**7**–**12**) were isolated as yellow-colored oils with satisfactory yields (35–71%) and then converted into hydrochloride salts.

Biological activity

The inhibitory potency of the target compounds against AChE (from electric eels) and BuChE (from horse serum) were evaluated via the classical method established by Ellman et al. [16] and by electrophoretically mediated microanalysis (EMMA, AChE) [17, 18]. For the Aβ-anti-aggregating assay, a modified thioflavine T test was applied.

The method commonly used for testing anti-cholinesterase activity is a spectrometric procedure developed by Ellman et al. [16]. Ellman's test is based on the reaction of 5,5′-dithio-*bis*-(2-nitrobenzoic) acid (DTNB, also called Ellman's reagent) with the thiocholine (product of hydrolysis of acetylthio-



Scheme 1. Synthesis of 2-(3-diethylaminoalkyl)-isoindoline-1,3-dione derivatives **7**–**12**.

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Table 1. Inhibitory activity of isoindoline-1,3-dione derivatives **7–12** and reference compounds against AChE and BuChE, determined by Ellman's method

Compound	*n*	AChE		BuChE	
		IC_{50} [μM]	% of inhibition at 100 μM	IC_{50} [μM]	% of inhibition at 100 μM
7	3	–	31.5	–	3.5
8	4	19.5		–	8.9
9	5	3.4		–	11.0
10	6	1.2		–	26.8
11	7	1.1		–	47.0
12	8	0.9		67.5	62.6
Donepezil		0.03		2.8	
Tacrine		0.02		0.005	
Rivastigmine[a)]		0.04–0.8		0.9–4.2	

[a)] Data from ref. [19].

choline or butyrylthiocholine) which results in generation of a yellow-colored product, i.e. 2-nitro-5-thiobenzoic acid. Changes in absorbance determine the activity of the evaluated compounds. Experiments were carried out at 100 μM concentrations of potential inhibitors. The activity measured is given as a percentage of enzyme inhibition and as IC_{50} values, which were only determined for compounds with better than 50% inhibitory activity at 100 μM concentrations (Table 1). Donepezil and tacrine were used as reference compounds.

Target compounds **7–12** displayed moderate or good inhibitory activity against AChE and weak activity against BuChE. Their anti-AChE activity ranged from $IC_{50} = 0.9$ to 19.5 μM with only compound **7** showing inhibitory activity lower than 50%. Inhibitory potency against BuChE ranged from 3.49 to 46.99% at 100 μM concentrations, with only compound **12** showing activity >50% (the corresponding IC_{50} value being equal to 67.5 μM). These compounds proved less active than reference substances (donepezil and tacrine), however they were comparable with rivastigmine in terms of inhibitory potential. Results indicate that 2-(3-diethylaminoalkyl)-isoindoline-1,3-dione derivatives could be described as selective AChE inhibitors. Structure–activity relationship analysis showed that elongation of the alkyl chain from 3 to 8 methylene groups resulted in an increase of inhibitory activity against both enzymes. The strongest activity was observed for compound **12**, with an 8-methylene alkyl chain. It appears that the optimal length of the tether is between 5 and 8 groups.

Preliminary steps in the development of new drugs typically include *in vitro* evaluation of the biological activity of the candidate compounds – hence efficient and rapid methods for estimating such activity are needed. Quite recently, new approaches to testing anti-cholinesterase activity by means of capillary electrophoresis have been described: this includes EMMA [17, 20] and the immobilized capillary enzyme reactor method [21]. Capillary electrophoresis has a number of advantages over other methods, including short analysis time and high-efficiency separation; moreover, it does not require large samples. In our previous studies, EMMA has been adopted for rapid AChE inhibition assay [18]. In the presented study the EMMA method was optimized, enabling us to measure the activity of four of the most active 2-(3-diethylaminoalkyl)-isoindoline-1,3-dione derivatives (**9–12**) and then compare it with reference inhibitors – tacrine and donepezil, whose activity was assessed by Ellman's method.

In the scope of the EMMA method, solutions of enzymes, potential inhibitors, and acetylthiocholine (enzyme substrate) were injected into a capillary and mixed electrophoretically by applying voltage for a short time. Following 1 min of incubation, voltage was reapplied to separate the product from any substrate that did not undergo reaction, as well as from any potential inhibitor. Detection was performed at UV 230 nm wavelength, with the area bounded by the product curve peak assumed to represent the enzymatic activity. The inhibition percentage and IC_{50} values were then calculated using nonlinear regression (Table 2). Figure 2 shows the

Table 2. Inhibitory potency (IC_{50}) against AChE obtained by Ellman's method and by EMMA

Compound	IC_{50} [μM] Ellman's method	IC_{50} [μM] EMMA
Donepezil	0.03	0.04
Tacrine	0.16	0.6
9	3.4	33.0
10	1.2	9.6
11	1.1	7.23
12	0.9	5.8

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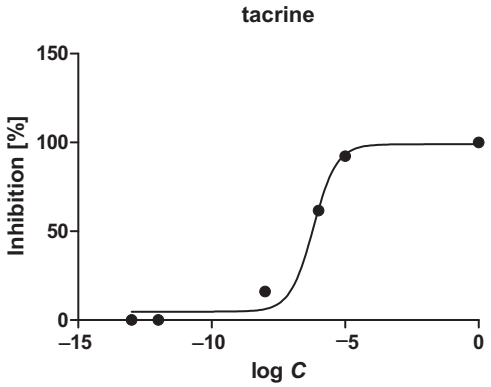

Figure 2. Dose–response plot for tacrine (EMMA method).

inhibition curve for tacrine obtained by plotting the inhibition percentage versus the logarithm of inhibitory concentration in the capillary.

In our experiments the IC_{50} values obtained using the EMMA method were higher than in Ellman's test. This discrepancy was probably caused by different concentration of reagents used in the assay and different reaction conditions in the capillary. Although the temperature in both methods was the same (25°C), there were dissimilarities in the time of enzymatic reaction and the volume of mixture. In Ellman's method the reaction lasted about 5 min and in EMMA about 1 min. Volume in which reaction took place in EMMA was much smaller than in colorimetric test (nL vs. mL). Correlation between both methods is shown in Fig. 3. The EMMA method is fast and repeatable (RSD area <2%); moreover, unlike Ellman's method, it does not require indirect detection. The migration time for thiocholine was 1.25 min, with RSD under 2%. In summary, the EMMA method can be used for rapid screening of new compounds for anticholinesterase inhibitory activity.

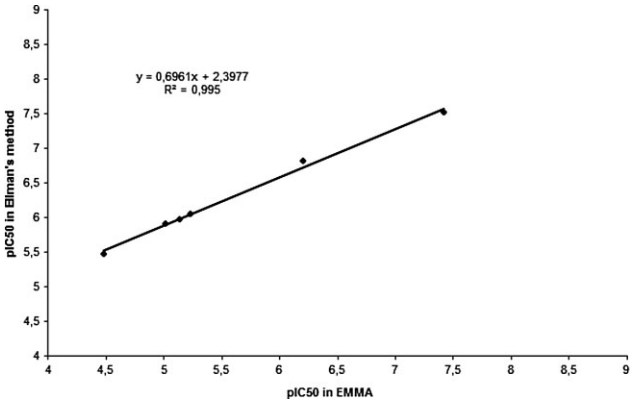

Figure 3. Relation between IC_{50} values in both methods.

Thioflavin T fluorescence assay

The thioflavin T (ThT) assay is a well-known test for inhibition of amyloid β fibrillogenesis as it can be used to visualize fibril formation. Compounds **7**–**12** were tested for their ability to inhibit Aβ aggregation in a modified test, using a smaller peptide composed of eleven amino acids (HHQKLVFFAED) [22]. The short peptide containing five highly conserved amino acids (KLVFF) has been described as a core peptide responsible for fibril formation [23]. The test was validated using 4-aminophenol as the reference compound, whose IC_{50} value of blocking the Aβ fibril formation was reported to be 83 μM. Donepezil – dual binding site AChE inhibitor was found to inhibit AChE-induced $A\beta_{1-42}$ aggregation by 22% at 100 μM [24] while in the test against $A\beta_{1-40}$ self-aggregation was not active (at 100 μM) [25]. In our assays, at 500 μM concentration six of the tested compounds exhibited anti-Aβ aggregation effects with percentage of inhibition ranging from 19 to 72%. The most active compounds, **10** and **11**, inhibited Aβ aggregation by about 34 and 40%, respectively, even at 80 μM concentration (Table 3). Their activity was higher than that of the described reference donepezil and promising for further studies.

Molecular modeling

The presented series of novel isoindoline-1,3-dione derivatives was designed from structural fragments. Some simple moieties were chosen for docking into the active site of AChE and BuChE to locate preferable binding areas. Among them, phthalimide and diethylamine were selected as promising fragments. It was noted that phthalimide could interact by π–π stacking and CH–π interactions with aromatic amino acids at the anionic subsite (Phe330, Phe331, and Trp84) and/or with residues at the PAS (Trp279, Tyr334) of AChE. A similar situation occurs with diethylamine, which binds the same amino acids through cation–π interactions (due to its protonated form). It can also form H-bonds with Tyr121. In case of BuChE, both fragments interacted mainly with Trp82 and Trp430 at the anionic subsite of the catalytic center. Docking results showed that there were

Table 3. Inhibition of Aβ aggregation by isoindoline-1,3-dione derivatives **7**–**12**

Compound	Inhibition (%) at 80 μM	Inhibition (%) at 500 μM
7	5.7	19.8
8	22.9	56.6
9	5.0	54.7
10	33.8	72.3
11	39.4	45.1
12	7.9	30.1

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two interaction sites in AChE but only one in BuChE for such fragments. Thus, we decided to connect both moieties with an oligomethylene spacer, thereby obtaining a new series of isoindoline-1,3-dione derivatives. The structures of these compounds were then docked into the active gorge of both cholinesterases and it was noted that they interacted with AChE but not with BuChE. This confirmed our expectations: the new compounds could not bind BuChE because the PAS in this enzyme is extremely reduced, while interactions with only one site (anionic subsite in catalytic active site, CAS) are insufficient to produce activity. The presented compounds are therefore selective AChE inhibitors. Linker length analysis showed that the optimal number of methylene groups is 7–8, ensuring the best arrangement of diethylamine and phthalimide in the AChE active gorge. The binding potency of novel inhibitors was assessed by the ChemScore function. The most active compound (**12**) reached a score of 40.54, in comparison with 49.48 for the reference inhibitor – donepezil. This suggests that the presented compounds are less active than reference substances. The binding mode of compound **12** is presented in Fig. 4. The inhibitor assumes an extended conformation, lining up with the gorge. Its protonated amine group produces cation–π interactions with Trp84 while also integrating itself into the H-bond network (amine group – water molecule – Tyr121). The tether is located in the middle of the active gorge, near aromatic amino acids Phe330, Phe331, and Tyr334, where it initiates hydrophobic interactions. The phthalimide moiety is involved in π–π stacking with Trp279 and in CH–π interactions with Tyr70. Both carbonyl groups produce H-bonds: one with Tyr121 and the other one with water. Results of biological assays confirm that compound **12** is the most active selective AChE inhibitor in this group.

Conclusion

A series of new 2-(diethylaminoalkyl)-isoindoline-1,3-dione derivatives were designed using molecular modeling. Our goal was to produce dual binding site cholinesterase inhibitors. The synthesized compounds were evaluated in terms of their inhibitory potency against AChE and BuChE, as well as against the formation of Aβ plaque. For AChE inhibitory activity, the spectrophotometric methods of Ellman's and EMMA assay were used. Both methods produced comparable results, which lends support for EMMA as a convenient tool for rapid screening of novel cholinesterase inhibitors. All synthesized compounds turned out to be moderately potent, selective inhibitors of AChE, with IC_{50} values ranging from 0.9 to 19.5, and weak inhibitors of Aβ plaque formation. These results agree with the conclusions of docking studies aimed at compounds which target both the CAS and PAS of AChE. The most promising selective AChE inhibitors are compounds **10** ($IC_{50} = 1.2$ μM) and **11** ($IC_{50} = 1.1$ μM) with 6–7 methylene spacers, which also inhibit Aβ fibril formation. The presented study shows that these compounds are of interest as prospective multipotent agents.

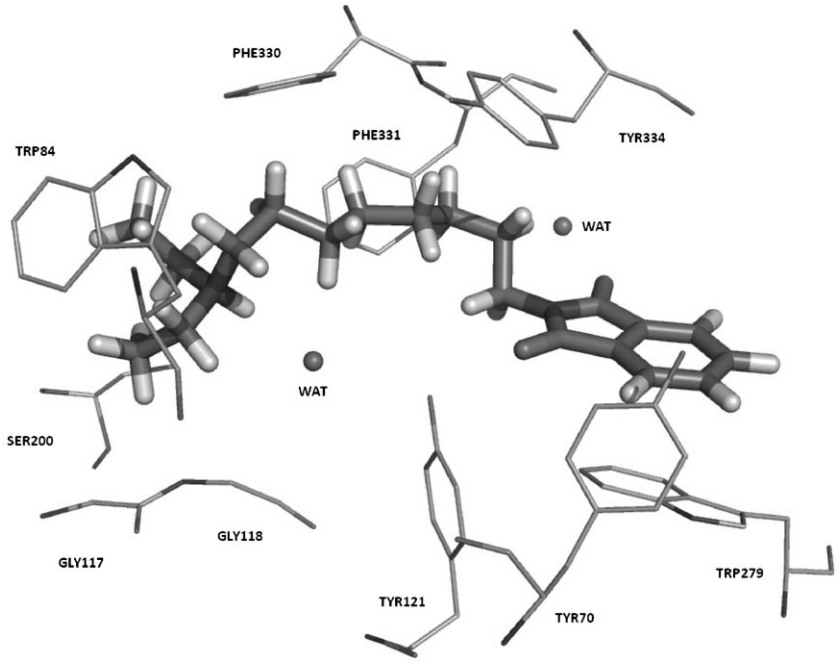

Figure 4. Binding mode of compound **12** at the active site of AChE.

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Experimental

Chemistry

*General procedure for the synthesis of compounds (**1–6**)*

A mixture of potassium phthalimide (15 mmol, 2.78 g) with 2.5-fold excess of suitable α,ω-dibromoalkane (37.5 mmol) and catalytic amount of TBAB (0.56 mmol, 0.18 g) was stirred in 150 mL of acetonitrile under reflux for 20 h. Subsequently, the reaction mixture was filtered and the filtrate concentrated. The resulting yellow oil was purified by column chromatography (*n*-hexane/ethyl acetate; 1:1).

*2-(3-Bromopropyl)-isoindoline-1,3-dione (**1**)*

White solid; yield: 51%; R_f (*n*-hexane/ethyl acetate; 1:1) 0.74; mp = 77°C (mp lit. = 74–76°C [26]); ^{1}HNMR ($CDCl_3$) δ ppm: 7.95–7.67 (m, 4H, phthalimide); 3.9–3.79 (t, 2H, J = 6.85 Hz, $-CH_2-C_2H_2Br-$); 3.45–3.38 (t, 2H, J = 6.75 Hz, $-C_2H_4-CH_2-Br$); 2.31–2.21 (p, 2H, J = 6.78 Hz, $-CH_2-CH_2-CH_2Br$).

*2-(4-Bromobutyl)-isoindoline-1,3-dione (**2**)*

White solid; yield: 79%; R_f (*n*-hexane/ethyl acetate; 1:1) 0.85; mp = 74°C (mp lit. = 72.5–74°C [27]); ^{1}HNMR ($CDCl_3$) δ ppm: 7.96–7.63 (m, 4H, phthalimide); 3.79–3.68 (dd, 2H, J = 6.85, $-CH_2-C_3H_8Br$); 3.45–3.38 (t, 2H, J = 6.75, $-C_3H_8-CH_2-Br$); 2.31–2.21 (p, 4H, J = 6.78, $-CH_2-C_2H_4-CH_2Br$).

*2-(5-Bromopentyl)-isoindoline-1,3-dione (**3**)*

White solid; yield: 76%; R_f (*n*-hexane/ethyl acetate; 1:1) 0.82; mp = 61.5°C; ^{1}HNMR ($CDCl_3$) δ ppm: 7.89–7.65 (m, 4H, phthalimide); 3.68 (t, 2H, $-CH_2-C_4H_8Br$); 3.38 (t, 2H, $-C_4H_8-CH_2-Br$); 1.94–1.83 (m, 2H, $-C_3H_6-CH_2-CH_2Br$); 1.75–1.64 (m, 2H, $-C_2H_4-CH_2-C_2H_4Br$); 1.54–1.40 (m, 2H, $-C_2H_4-CH_2-C_2H_4Br$).

*2-(6-Bromohexyl)-isoindoline-1,3-dione (**4**)*

White solid; yield: 53%; R_f (*n*-hexane/ethyl acetate; 1:1) 0.59; mp = 60°C; ^{1}HNMR ($CDCl_3$) δ ppm: 7.89–7.62 (m, 4H, phthalimide); 3.67 (t, 2H, $N-CH_2-C_6H_{12}Br$); 3.40 (t, 2H, $C_6H_{12}-CH_2-Br$); 1.91–1.79 (m, 2H, $-CH_2-CH_2-C_4H_8Br$); 1.73–1.62 (m, 2H, $C_4H_8-CH_2-CH_2Br$); 1.50–1.42 (m, 4H, $-C_2H_4-C_2H_4-C_2H_4Br$).

*2-(7-Bromoheptyl)-isoindoline-1,3-dione (**5**)*

White solid; yield: 66%; R_f (*n*-hexane/ethyl acetate; 1:1) 0.85; mp = 58.5°C; ^{1}HNMR ($CDCl_3$) δ ppm: 7.89–7.63 (m, 4H, phthalimide); 3.70–3.61 (m, 2H, $-CH_2-C_6H_{12}Br$); 3.45–3.28 (dt, 2H, $-C_6H_{12}-CH_2-Br$); 1.90–1.76 (m, 2H, $-C_5H_{10}-CH_2-CH_2Br$); 1.70–1.63 (m, 2H, $-CH_2-CH_2-C_5H_{10}Br$); 1.40–1.30 (m, 6H, $-C_2H_4-C_3H_6-C_2H_4Br$).

*2-(8-Bromooctyl)-isoindoline-1,3-dione (**6**)*

White solid; yield: 94%; R_f (*n*-hexane/ethyl acetate; 1:1) 0.80; mp = 55.5°C (mp lit. = 54–55°C [28]); ^{1}HNMR ($CDCl_3$) δ ppm: 7.89–7.67 (m, 4H, phthalimide); 3.72–3.66 (m, 2H, $-CH_2-C_7H_{14}Br$); 3.43–3.36 (t, 2H, $-CH_2-Br$); 1.91–1.80 (m, 2H, $C_6H_{12}-CH_2-CH_2Br$); 1.75–1.64 (m, 2H, $-CH_2-CH_2-C_6H_{12}Br$); 1.54–1.30 (m, 8H, $-C_2H_4-C_4H_8-C_2H_4Br$).

*General procedure for synthesis of compounds (**7–12**)*

2-(Bromoalkyl)-isoindoline-1,3-dione (6 mmol) with fourfold excess of diethylamine (24 mmol, 1.76 g) in the presence of K_2CO_3 (18 mmol, 2.49 g) was stirred in 100 mL acetonitrile under reflux for 24 h. Subsequently, the solvent was evaporated in vacuum, producing a residue which was further dissolved in 40 mL of sodium bicarbonate and extracted with ethyl acetate (3 × 30 mL). The organic layer was dried with Na_2SO_4. The solvent was then evaporated and the residue purified by column chromatography (*n*-hexane/ethyl acetate/TEA, 5:5:1) to afford the pure product. The final product was obtained in the form of hydrochloride salts.

*2-(3-Diethylaminopropyl)-isoindoline-1,3-dione (**7**)*

Yellow oil; yield 70%. R_f (*n*-hexane/ethyl acetate/TEA; 5:5:1) 0.84; ^{1}HNMR ($CDCl_3$) δ ppm: 7.82–7.62 (m, 4H, phthalimide); 3.71–3.64 (m, 2H, $-CH_2-C_2H_4-N-(C_2H_5)_2$); 2.50–2.39 (m, 2H, $-C_2H_4-CH_2-N-(CH_2-CH_3)_2$), 1.88–1.70 (m, 2H, $-CH_2-CH_2-CH_2-N-(C_2H_5)_2$), 0.9 (t, 6H, $-N-(CH_2-CH_3)_2$. As the HCl salt: mp = 158°C; anal. calc. for $C_{15}H_{20}N_2O_2$ HCl: C-60.7%; N-9.44%; H-7.13%, found C-60.64%; N-9.41%; H-7.50%.

*2-(4-Diethylaminobutyl)-isoindoline-1,3-dione (**8**)*

Yellow oil; yield 70%. R_f (*n*-hexane/ethyl acetate/TEA; 5:5:1) 0.56; ^{1}HNMR ($CDCl_3$) δ ppm: 7.91–7.60 (m, 4H, phthalimide); 3.71–3.65 (t, 2H, $-CH_2-C_3H_{12}-$); 2.54–2.38 (qd, 6H, $-CH_2-N-(CH_2-CH_3)_2$); 1.73–1.60 (m, 2H, $-C_2H_4-CH_2-CH_2-$); 1.53–1.40 (m, 2H, $-CH_2-CH_2-C_2H_4-$); 1.05–0.91 (m, 6H, $(-CH_2-CH_3)_2$). As the HCl salt: mp = 170°C; anal. calc. for $C_{16}H_{22}N_2O_2$ HCl: C-61.83%; N-9.01%; H-7.46%, found C-61%.92; N-9.04%; H-7.76%.

*2-(5-Diethylamiopentyl)-isoindoline-1,3-dione (**9**)*

Yellow oil; yield 64%. R_f (*n*-hexane/ethyl acetate/TEA; 5:5:1) 0.54; ^{1}HNMR ($CDCl_3$) δ ppm: 7.88–7.61 (m, 4H, phthalimide); 3.71–3.65 (t, 2H, $-CH_2-C_4H_{14}-$); 2.52–2.41 (q, 4H, J = 7.13 Hz, $-N-(CH_2-CH_3)_2$); 2.39–2.33 (m, 2H, $-CH_2-N-$); 1.73–1.60 (m, 2H, $-C_3H_6-CH_2-CH_2-$); 1.51–1.40 (m, 2H, $-CH_2-CH_2-C_3H_6-$); 1.36–1.26 (m, 2H, $-C_2H_4-CH_2-C_2H_4-$); 1.00–0.93 (t, 6H, J = 7.16 Hz, $(-CH_2-CH_3)_2$). As the HCl salt: mp = 165°C; anal. calc. for $C_{17}H_{24}N_2O_2$ HCl: C-62.86%; N-8.62%; H-7.76%, found C-62.73%; N-8.61%; H-8.10%.

*2-(3-Diethylaminohexyl)-isoindoline-1,3-dione (**10**)*

Yellow oil; yield 35%. R_f (*n*-hexane/ethyl acetate/TEA; 5:5:1) 0.54; ^{1}HNMR ($CDCl_3$) δ ppm: 7.87–7.61 (m, 4H, phthalimide); 3.81–3.50 (m, 2H, $-CH_2-C_5H_{10}-$); 2.53–2.42 (m, 4H, $-(CH_2-CH_3)_2$); 2.40–2.31 (m, 2H, $-C_5H_{10}-CH_2-N$); 1.73–1.57 (m, 2H, $-CH_2-CH_2-C_4H_8-$); 1.48–1.19 (m, 2H, $-C_2H_4-C_3H_6-CH_2-$); 1.00–0.94 (m, 6H, $(-CH_2-CH_3)_2$). As the HCl salt: mp = 172°C anal. calc. for $C_{18}H_{26}N_2O_2$ HCl: C-63.8%; N-8.27%; H-8.03%, found C-63.58%; N-8.15%; H-8.25%.

*2-(3-Diethylaminoheptyl)-isoindoline-1,3-dione (**11**)*

Yellow oil; yield 56%. R_f (*n*-hexane/ethyl acetate/TEA; 5:5:1) 0.75; ^{1}HNMR ($CDCl_3$) δ ppm: 7.85–7.64 (m, 4H, phthalimide); 3.73–3.56 (m, 2H, $-CH_2-C_6H_{12}-$); 2.52–2.42 (m, 4H, $-(CH_2-CH_3)_2$); 2.38–2.31 (m, 2H, $-C_6H_{12}-CH_2-N$); 1.69–1.59 (m, 2H, $-CH_2-CH_2-C_3H_6-$); 1.45–1.17 (m, 6H, $-C_2H_4-C_3H_6-C_2H_4-$); 1.02–0.93 (m, 6H, $(-CH_2-CH_3)_2$). As the HCl salt: mp = 132.5°C; anal. calc. for $C_{19}H_{28}N_2O_2$ HCl: C-64.83%; N-8.13%; H-8.48%, found C-64.67%; N-7.94%; H-8.28%.

*2-(3-Diethylaminooctyl)-isoindoline-1,3-dione (**12**)*

Yellow oil; yield 71%. R_f (*n*-hexane/ethyl acetate/TEA; 5:5:1) 0.67; ^{1}HNMR ($CDCl_3$) δ ppm: 7.82–7.53 (m, 4H, phthalimide); 3.61–3.53 (m, 2H, $-CH_2-C_7H_{14}-$); 2.47–2.34 (m, 4H, $-(CH_2-CH_3)_2$); 2.31–2.27

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(m, 2H, $-C_7H_{14}-CH_2-N$); 1.67–1.46 (m, 2H, $-CH_2-CH_2-C_6H_{12}-$); 1.33–1.17 (m, 10H, $-C_2H_4-C_5H_{10}-CH_2-$); 0.93–0.89 (m, 6H, $(-CH_2-CH_3)_2$). As the HCl salt: mp = 117°C; anal. calc. for $C_{20}H_{30}N_2O_2$ HCl: C-65.47%; N-7.63%; H-8.52%, found C-65.84%; N-7.83%; H-8.72%.

Biology

Ellman's method AChE/BuChE inhibition activity

Reagents and chemicals: DNTB, acetylthiocholine iodide (ATCh), butyrylthiocholine iodide (BTCh), AChE from *Electrophorus electricus* (425.96 U/mg solid), and BuChE from horse serum (2.5 units/1 mL) – were purchased from Sigma–Aldrich. All assays were performed using the Perkin Elmer Lambda 12 device. Detection was performed at 412 nm. The reaction took place in 100 mM phosphate buffer, pH 8.0, containing 0.25 units of AChE or BuChE, 0.3 mmol 5,5′-dithio-*bis*-(2-nitrobenzoic) acid (DTNB) and 0.45 mmol acetylthiocholine or butyrylthiocholine as substrates. The tested compounds were incubated with the enzyme for 5 min at 25°C prior to starting the reaction by adding the substrate. Enzymatic activity was determined by measuring absorbance over a period of 5 min. Two kinds of assays were conducted: the peak activity of the enzyme was determined by using a blank sample, followed by reference measurements (for tacrine and donepezil) and measurements for the synthesized compounds. Data from concentration–inhibition experiments was integrated through nonlinear regression analysis using the GraphPad Prism program (GraphPad Prism Software Inc. 2005), producing estimates of IC_{50}.

EMMA AChE inhibition activity

Reagents and chemicals: AChE from *Electrophorus electricus* (425.96 U/mg solid), ATCh, boric acid, and sodium phosphate monobasic – were purchased from Sigma–Aldrich. A solution of AChE (0.4 mg/mL) was prepared with 30 mM borate-phosphate buffer at pH 8.0. A solution of ATCh (10 mM) and solutions of inhibitors were prepared with 30 mM borate-phosphate buffer at pH 8.0. All solutions were filtered through 0.45 μm Millex filters. All separations were performed on a Beckman CE system (P/ACE MDQ) equipped with a diode-array detector (DAD) and controlled by 32 Karat software version 8.0. An uncoated fused-silica capillary with a total length of 30 cm (20.2 cm to detection window) and internal diameter 50 μm (external diameter 375 μm) was purchased from Beckman. The capillary was thermostatted at 25°C and samples at 10°C. Detection was performed at UV 230 nm.

Procedures: A new capillary was pretreated with 0.1 M NaOH solution for 10 min, followed by water for 10 min and running buffer for 10 min. The same rinsing sequence was performed every day prior to starting separations. Between runs, the capillary was rinsed with 0.1 M hydrochloric acid, 0.1 M sodium hydroxide, water and borate-phosphate buffer (50 psi, 2 min each). For every rinsing and separation cycle 30 mM borate-phosphate buffer pH 8.0 was used. Two kinds of assays were conducted: the peak activity of the enzyme was determined by using a blank sample, followed by measurements for inhibitors at different concentrations. The procedure was repeated three times for each sample. The plug–plug method was used with the following injection sequence: enzyme solution 0.5 psi, 10 s, inhibitor or buffer (in blank) 0.5 psi, 15 s, substrate 0.5 psi, 10 s, 1 kV for 15 s, incubation for 1 min, separation at 12 kV for 4 min.

Thioflavin T assay fibril formation

Inhibitors were diluted in buffer (25 mM NaH_2PO_4, 120 mM NaCl, 3 μM ThT, 0.02% NaN_3, pH of 7.4) to reach concentrations between 16 and 500 μM. A 2 mM stock solution of the peptide HHQKLVFFAED in DMSO was added to reach a final peptide concentration of 100 μM. Incubations were performed on 96-well fluorescence microtiter plates (Nunc GmbH, Wiesbaden, Germany). Fluorescence was measured every 10 min over a period of 12 h (excitation wavelength 450 nm, emission wavelength 482 nm) on a Cary Eclipse fluorescence spectrophotometer (Varian, Darmstadt, Germany).

Molecular modeling

Three-dimensional representations of ligands were created using Corina online tool (Molecular Networks) and saved as pdb files. Using Sybyl 8.0 (Tripos) Gasteiger-Marsili charges were assigned following checks of atom types and protonation of the compounds. Finally, ligand structures were saved in the mol2 format. Dockings were performed to AChE from 1EVE and to BuChE from the 1P0I crystal complex using Gold 4.1 (CCDC) program. In the preparatory phase all histidine residues were protonated at Nε, hydrogen atoms added, ligand molecules removed, and binding sites defined as all amino acid residues within 10 Å from donepezil (for AChE) and within 20 Å from the glycerol molecule present in the active center of BuChE. The presence of some water molecules was also taken into account. A standard set of genetic algorithms with population size 100, number of operations 100 000 and clustering tolerance of 1 Å were applied. As a result, 10 ligand conformations were obtained and sorted according to ChemScore function values. Results were visualized by PyMOL.

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