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Multi-target strategy to address Alzheimer's disease: Design, synthesis and biological evaluation of new tacrine-based dimers

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ABSTRACT

The multifactorial nature of Alzheimer's disease (AD) offers us a textbook example where parental compounds, mostly marketed, are modified with the aim of improving and/or conferring two or even more biological activities to contrast or less frequently revert the disease's symptoms. This is the case of tacrine and its dimeric derivative *bis*(7)-tacrine which, for instance, paved the way for the development of a broad collection of very interesting homo- and heterodimeric structures, conceived in light of the emerging multi-target approach for AD-related drug discovery. As a contribution to the topic, we report here the design, synthesis and biological evaluation of **12** compounds referable to *bis*(7)-tacrine. In addition to the cholinesterase activity, some of the selected compounds (**7**–**9** and **12**) were capable of inhibiting the non-enzymatic function of AChE and/or showed a remarkable activity against BACE1. Thus, the present study outlines a series of newly synthesized molecules, structurally related to *bis*(7)-tacrine, endowed with extended biological profile in agreement with the emerging multi-target paradigm.

1. Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder of the central nervous system (CNS) causing neuronal death. As AD advances, a progressive loss of brain's structure and functions is observed and a series of symptoms arise which include confusion, language breakdown, mood swings as well as loss of memory and bodily functions [1]. The etiopathogenesis of this multifactorial disease still remains unknown although, in the last decades, several involved factors have been identified and found consistent with its onset. Among them is the impairment of the cholinergic system, as indicated by the presence of altered cholinergic markers in AD patients, resulting in a pronounced acetylcholine (ACh) deficiency which translates into a generalized withdrawal of the cholinergic tone [2,3]. Other involved factors appear to be the presence of intracellular neurofibrillary tangles [4,5], containing hyperphosphorylated tau protein, and extracellular senile plaques [6], mostly composed of aggregated β-amyloid peptide (Aβ). Despite the lack of a firmly established causal link between plaques formation and AD, increasing evidences suggest that amyloid polymerization may play a critical role in the neurodegenerative process by disrupting the cell's calcium homeostasis, and inducing neuronal apoptosis [7]. The formation of senile plaques involves a cascade of events triggered by the abnormal catabolism of a transmembrane glycoprotein of undetermined function known as the amyloid precursor protein (APP), which is consecutively processed by two proteolytic enzymes, namely the β-secretase or beta-site APP cleavage enzyme (BACE1), and the γ-secretase to generate soluble amyloid-beta peptides [8,9]. Monomeric Aβ units can organize themselves, either spontaneously or in a pathological chaperone-induced fashion, into aggregates of increasing complexity, eventually forming neurotoxic senile plaques.

Most of the currently available therapies on the market are intended to treat AD by compensating for ACh deficiency so as to enhance ACh-mediated transmission [10,11]. This strategy consists in inhibiting cholinesterases (ChEs) [12], a family of enzymes that catalyze the breakdown of ACh, such as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) [13]. Examples of this ChE inhibitors are tacrine, donepezil, rivastigmine and galanthamine, which lead to symptomatic cognitive, functional and behavioral benefits although none of them has proven effective against the progression of the disease. Aware of the multifactorial nature of AD, researchers are nowadays focusing on the development of new

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therapeutic strategies that could arrest and revert its progress. This is the case of the multi-target-directed ligand (MTDL) approach [14,15], where drugs are designed to address selected activities toward selected targets involved in the disease. To this regard, a lot of interest has recently regained the so-called *bis*(7)-tacrine (Fig. 1), an heptylene-tethered dimer of tacrine able to simultaneously contact both the central anionic site and the peripheral anionic site (PAS), and endowed with AD-related multi-target activity [16,17]. Indeed, besides its cholinesterase function, AChE can also act as a promoter of Aβ fibril formation. This activity is thought to take place involving the PAS [18a,b] of the enzyme, located at the entrance of its "catalytic gorge" and characterized by the presence of the key aminoacid Trp^{286}. Consequently, new dimeric families of compounds, containing two identical or different structural units, connected by a linker of suitable length, have been developed, with tacrine being the active site acetylcholinesterase inhibitor (AChEI) most extensively incorporated in their structure [19,20]. The purpose of the homo- and heterodimers is to improve and enlarge their biological profile beyond their ability to act as AChEI. Our research group has been involved for many years in the development of potential drugs for AD, including the design of dual binding site AChEI [21]. In this regard, AP2238 was the first published compound [21a] designed to bind to both anionic sites of the human AChE (hAChE), for which the simultaneous inhibition of the catalytic and the amyloid-β pro-aggregating activities of AChE was verified. As a follow-up of our studies, we turn here the attention to *bis*(7)-tacrine designing dimeric compounds as potential MTDL (Fig. 1). With this in mind, a 9-amino-1,2,3,4-tetrahydroacridine (THA) core, for interaction with the central anionic site, and an amino group, for π-cation interaction with the PAS, were linked through alkyl tethers of diverse lengths. While a previous series of derivatives carrying a diethylamino group was reported in literature [22] as AChE inhibitors, in the present paper the morpholino group was chosen since its pKa was expected to improve the pharmacokinetic properties of the compounds maintaining the basicity of the inhibitors low enough to allow a sufficient rate of penetration of the blood–brain barrier [23]. This series of compounds (**1**–**5**) was followed by a second one (**6**–**12**) in which morpholine was replaced by different moieties, such as azaxanthone [24], indenoquinoline [25], and benzofuran [26]. These heterocyclic scaffolds, previously reported by our research group, could allow us to evaluate the impact of structural diversification upon the π-π interactions with the PAS. As a first modification to this second series of molecules, a chlorine atom was introduced at position 6 of the THA resulting in the tacrine-indenoquinoline heterodimer **8**. This small structural change, as previously shown [27,28], is thought to enhance the compound's binding affinity through favorable interactions with AChE. A further modification of **8** led to compound **9**, where an additional chlorine was added at position **7** of the indenoquinoline ring. In fact, the chloro-substituted indenoquinoline ring loses the ability of penetrating the enzymatic gorge of the hAChE, due to steric hindrance [25b], therefore addressing the 6-chloro-THA moiety toward the central anionic site of the enzyme. An indenoquinoline homodimer **12** was prepared as well. In summary, **12** new derivatives were synthesized and their structures are collected in Fig. 1 and Table 1. All compounds were tested for their biological activities toward hAChE and hBuChE. **5** compounds, properly selected, were also tested for their ability to prevent the AChE-induced Aβ aggregation, and were evaluated for their ability to inhibit $Aβ_{42}$ production through the inhibition of the aspartyl protease BACE1, which catalyzes the rate-limiting step in the production of Aβ.

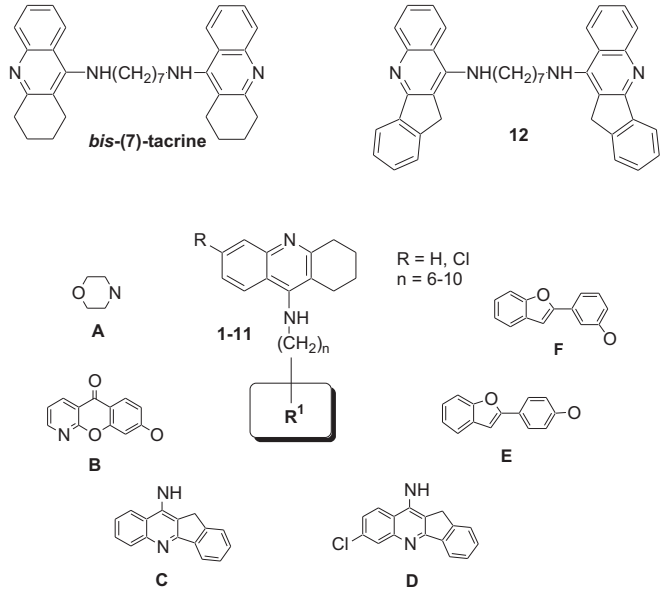
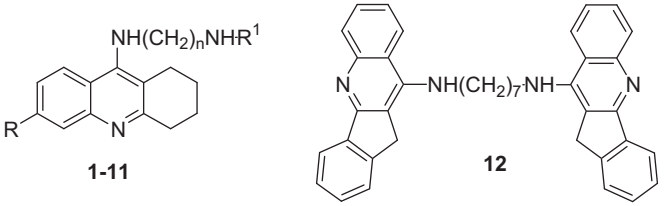


Fig. 1. *Bis*-(7)-tacrine and design of target molecules.

2. Chemistry

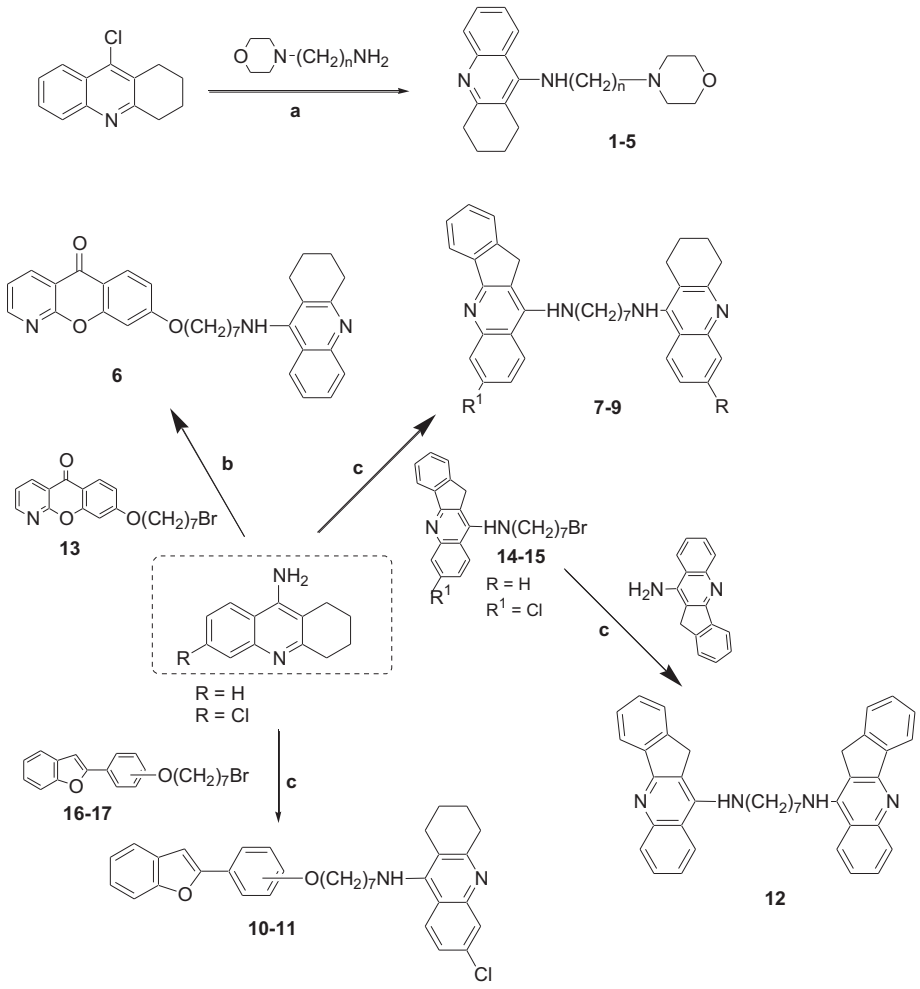
The synthesis of the studied compounds was accomplished as shown in Scheme 1. 9-Chloro-1,2,3,4-tetrahydroacridine [29] was heated with the selected morpholin-ω-alkylamine [23] in phenol to afford the final compounds **1**–**5**. For the synthesis of derivative **6**, 9-amino-1,2,3,4-tetrahydroacridine (THA) x [27] was reacted with **13** using a phase transfer catalyst in a heterogeneous medium. Similarly, when THA or 6-Cl-THA were reacted with **14**–**15** , and **16**–**17** (**16** [26], for *meta* derivative **17**) in DMSO, using KOH as the base, compounds **7**–**9** and **10**–**11** were respectively obtained. The intermediate **14** was converted to the desired homodimer **12** upon bromine displacement with 11*H*-indeno [1,2-b]quinolin-10-amine [25a].

Table 1
Inhibitory activity on human AChE and BuChE, and IC_{50} Ratio of the studied compounds.



Comp.	n	R	R^1	IC_{50} (nM) ± SEM		Ratio IC_{50} BuChE/AChE
				hAChE	hBuChE	
1	6	H	A	68.9 ± 3.8	101 ± 4	1.46
2	7	H	A	44.5 ± 3.1	90.5 ± 4.0	2.03
3	8	H	A	16.5 ± 0.8	42.8 ± 1.5	2.59
4	9	H	A	34.0 ± 1.6	38.1 ± 0.9	1.12
5	10	H	A	28.4 ± 1.8	14.7 ± 1.5	0.52
6	7	H	B	283 ± 6	101 ± 6	0.35
7	7	H	C	13.9 ± 2.1	24.8 ± 0.8	1.78
8	7	Cl	C	1.05 ± 0.08	63.7 ± 3.5	60.7
9	7	Cl	D	12.1 ± 1.6	1660 ± 80	137
10	7	Cl	E	310 ± 13	7280 ± 550	23.5
11	7	Cl	F	258 ± 6	41.4 ± 3.8	0.16
12	–	–	–	455 ± 26	28.6 ± 1.5	0.06
THA	–	–	–	250 ± 10	50 ± 2	0.20
Bis(**7**)-**THA**[a]	–	–	–	0.81 ± 0.09	5.66 ± 0.15	6.99

[a] from Ref. [40].



Scheme 1. Synthesis of the studied compounds. Reagents and conditions: a) PhOH, 130 °C; b) tetra-*n*-butyl ammonium hydrogen sulfate, 50% NaOH sol./DCM, r.t; c) KOH, DMSO, r.t.

3. Enzyme inhibition

The inhibitory activities against AChE of the newly synthesized compounds were investigated using the method described by Ellman [30]. The selectivity of the compounds was also tested by determining their inhibitory activities against hBuChE. Some compounds, rationally selected, were also tested for their abilities to prevent the hAChE-induced Aβ aggregation by a ThT-based fluorescence assay. Moreover, in view of enlarging their spectrum of action, some compounds were evaluated for their capabilities of inhibiting BACE1, using a fluorescence resonance energy transfer (FRET) assay.

4. Results and discussion

The inhibitory activities against both human recombinant AChE and BuChE from human serum of new derivatives, together with those of tacrine and *bis*(7)-tacrine taken as references, are reported in Table 1, and are expressed as IC_{50} values.

With regard to the first series (compounds **1**–**5**), it appears that variations of the chain length (n in the general formula) had a stronger influence on BuChE with respect to AChE inhibition. Indeed, the behavior of this series is rather uniform for AChE inhibition and the most potent compound is **3** (n = 8; IC_{50} = 16.5 nM). On the contrary, for BuChE the inhibitory activity increases with increasing chain length and the most potent compound is **5** (n = 10; IC_{50} = 14.7 nM).

In the second series (compounds **6**–**12**), where morpholine was replaced by different heterocyclic scaffolds to test the effect on π–π interactions with the PAS, the chain length was fixed to seven methylene, as in *bis*(7)-tacrine. With regard to AChE inhibition, switching the heterocycle to an azaxanthone (Fig. 1B), and to a benzofuran (E-F), resulted in new molecules (compounds **6** and **10**–**11**, respectively) with lower activity compared to the positive reference compound THA, although compound **11** showed a satisfactory BuChE inhibition (IC_{50} = 41.4 nM). Much to our delight, switching the heterocycle to an indenoquinoline (C) and to a 7-Cl-indenoquinoline (D) afforded the most active heterodimers of the series (compounds **7**–**9**). Remarkably, compound **8**, a THA-indenoquinoline heterodimer, showed an activity in the low nanomolar range, comparable to that of the standard *bis*(7)-tacrine. It could reasonably been assumed that four-ring heterocycles such as C and D are best suited for stacking interactions, mostly with aromatic residues at the PAS (i.e. Trp^{286} of hAChE). Furthermore, the 7-Cl-indenoquinoline scaffold (D) exerted a noteworthy effect on the selectivity of the inhibitor, as compound **9** showed a higher inhibitory activity on AChE than on BuChE (ratio IC_{50} BuChE/AChE = 137, Table 1). Finally, the indenoquinoline homodimer **12** was a potent BuChE inhibitor having IC_{50} = 28.6 nM. Considering that in this latter enzyme, Lys^{286} and Val^{288} line the acyl pocket, and that these aminoacid side chains are smaller, compared to the side chains of the corresponding Phe residues of AChE, BuChE could better tolerate the binding of larger compounds with respect to AChE.

Concerning the potential therapeutic interest on BuChEI, it must be mentioned that a possible role for BuChE inhibition in contributing to disease modification has been recently proposed [31]. Furthermore, BuChE activity progressively increases as the severity of dementia advances, while AChE activity declines. Therefore, the inhibition of BuChE may become an increasingly important therapeutic target over time [13,32].

The ability of new cholinesterase inhibitors to interact with the AChE peripheral binding site has gained interest in the last two decades in view of developing a new disease-modifying drug able to interfere with the pro-aggregating action of AChE on amyloid-peptides, likely exerted through the interaction of Aβ with PAS [18a,b]. Therefore, the mechanism of action of the most active compound in the series was investigated. As shown in Fig. 2, binding of **8** to hAChE changed both v_{max} and K_m values, a trend that is generally ascribed to mixed-type inhibition. In particular, a decreased v_{max} at increasing inhibitor concentrations and increasing intercepts (higher K_m) with higher inhibitor concentration were observed. *Ki* value for compound **8** resulted 1.01 ± 0.01 nM.

On the basis of these promising results, compound **8** and other properly selected tacrine-derivatives were also tested for their ability to prevent AChE-induced Aβ aggregation. A rational selection was made on the basis of the lowest IC_{50} values obtained in Ellman's assay since AChE inhibition remains one of the desired feature of the new MTDLs. Furthermore, it must be considered that AChE inhibition and AChE-induced β-amyloid aggregation do not represent independent phenomena, but can both be modulated by inhibitor binding to PAS. The ability of interfering with the triggering activity of AChE on amyloid aggregation, expressed as % inhibition, is reported in Table 2. Compound **3**, carrying a morpholino group, showed to act as a weak but significant inhibitor of AChE-induced Aβ aggregation (% inhibition = 27.7). With the introduction of indenoquinoline (C, compounds **7**–**8**) or 7-Cl-indenoquinoline (D, compound **9**), the compounds showed a significant increase of the ability to inhibit the Aβ aggregation induced by AChE. The most potent agents are **7** and **8**, heterodimers of a THA or a 6-Cl-THA nucleus with indenoquinoline. As expected, the compounds showed the same ability to block the AChE-induced Aβ aggregation, as they only differ for a chlorine atom on the nucleus of tacrine. Indeed, the chlorine atom in position 6 of the THA core is thought to enhance affinity toward AChE, possibly by

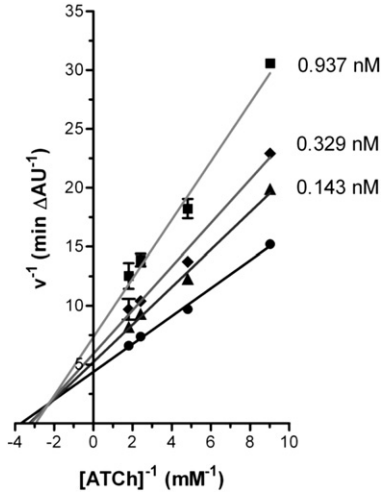


Fig. 2. Steady-state inhibition by **8** of the hydrolysis of acetylthiocholine (ATCh) catalyzed by hAChE. Lineweaver–Burk reciprocal plots of initial velocity vs substrate concentrations in the presence and absence of **8** (three concentrations) are presented. Lines were derived from a weighted least-squares analysis of the data points.

Table 2
Inhibitory activity of AChE-mediated Aβ aggregation and Aβ production of the selected compounds.

Comp.	Inhibition of AChE-induced Aβ_{40} aggregation[a] (%)±SEM	BACE1 inhibition[b] (%)±SEM	BACE1 IC_{50}μM ± SEM
3	27.7 ± 1.5	9.3 ± 2.6	
7	44.7 ± 7.4	36.1 ± 3.8	
8	46.1 ± 0.3	78.2 ± 1.4	1.0 ± 0.1
9	30.5 ± 0.3	4.7 ± 1.3	
12	30.2 ± 5.7	92.8 ± 1.7	0.4 ± 0.1
THA	<5[c]	n.a.	–
***Bis*-(7)-THA**	68.0 ± 3.5[d]	–	7.5 ± 0.4[e]

SEM = standard error of the mean; n.a. = not active.
[a] Determined at [inhibitor]c = 100 μM, [Aβ_{40}] = 230 μM and [hAChE] = 2.3 μM.
[b] Determined at [inhibitor] = 5 μM.
[c] From Ref. [38].
[d] From Ref. [40].
[e] From Ref. [17].

means of hydrophobic interactions with some residues within the enzyme gorge [27,28], and this is confirmed by the finding that **8** showed AChE inhibiting activity 10 fold higher than **7**.

With regard to BACE1 inhibition, compound **3**, the most potent AChE inhibitor of the first series, was shown to be ineffective, as well as compound **9**, a THA-7-Cl-indenoquinoline dimer.

All the other compounds tested (**7**–**8**, **12**) were found more active than the standard *bis*(7)-tacrine, which displayed a 27% inhibition when tested at 10 μM. This figure is consistent with the previously published IC_{50} value of 7.5 μM [17]. The most potent compounds were **8** and **12**, which inhibited enzyme activity by 78 and 93%, respectively, while the indenoquinoline fragment alone was inactive (data not shown).

It is noteworthy that the presence of a chlorine atom on the indenoquinoline nucleus leads to a complete loss in activity (compare compounds **8,** which has no chlorine, and **9,** which carries a Cl atom at position 7, Table 2). For **8** and **12**, which displayed at 5 μM a percentage of inhibition higher than 50%, the corresponding IC_{50} values were also calculated. **12** exhibited a considerable activity with a submicromolar IC_{50} value of 0.4 μM and it was found to be more potent than **8** (IC_{50} value of 1.0 μM). Thus, the indenoquinoline fragment was found to be the most suitable substituent to obtain a submicromolar BACE1 inhibition.

It has to be noted that these compounds show a lower potency toward BACE1 compared to AChE and BuChE inhibition. Nevertheless, it is known that BACE1 is a more challenging target and our compounds are in line with non peptidic inhibitors reported in literature showing activity in the micromolar range, among these the non competitive inhibitor *bis*(7)-tacrine [17], myricetin [33] or other tacrine-based dual binding site acetylcholinesterase inhibitors [34].

Interestingly, recent studies have determined that BACE1 levels and activity are approximately twofold elevated in AD brain, suggesting the possibility that BACE1 increase might initiate or accelerate AD pathogenesis [35]. Moreover, it has recently been demonstrated that *bis*(7)-tacrine may reduce the generation of Aβ by inhibiting BACE1 and simultaneously activating α-secretase activity, the enzyme responsible for the physiological cleavage of APP, through the direct activation of protein kinase C [36]. From the results obtained for BACE1 inhibition and due to the structural similarity with *bis*(7)-tacrine, a comparable antiamyloidogenic profile could also be envisaged for derivatives **8** and **12**.

5. Conclusions

In conclusion, we report the design, synthesis and biological evaluation of 12 novel homo- and heterodimer molecules referable

to *bis*(7)-tacrine-derivatives. The potential of these analogs as gateway molecules' for the development of multi-target drugs was demonstrated by evaluating their biological profile toward a series of AD-related targets such as cholinesterases, Aβ aggregation and BACE1. In agreement with the trend observed for *bis*(7)-tacrine, the majority of the newly synthesized derivatives were found to be active in the nanomolar range against AChE. In addition to the cholinesterase activity, some of the selected compounds (**7**–**9**, and **12**) were capable of inhibiting the non-enzymatic function of AChE and/or showed a remarkable activity against BACE1, with a slightly (compound **7**) or significantly (compounds **8** and **12**) higher potency compared to *bis*(7)-tacrine. Thus, our study outlines a series of newly synthesized molecules, structurally related to *bis*(7)-tacrine, endowed with extended biological profile. Particularly interesting proved to be compound **8**, which resulted the most potent AChE inhibitor of the series, equaling the activity of *bis*(7)-tacrine, and showed a good activity against BuChE, useful in advanced stages of the disease. Even if only a slight reduction of Aβ aggregation induced by AChE was observed with **8**, it also proved to be remarkably more potent than the reference compound as BACE1 inhibitor, which may represent the most direct approach in anti-amyloid therapy.

Moreover, our selected compounds **7**–**9** and **12** could serve as a platform for further studies aimed at fine-tuning their multifaceted activities for the identification of new molecular entities, in which the activities on the different targets would be appropriately balanced, endowed with broader biological profile in awareness of the emerging multi-target paradigm for drug discovery.

6. Experimental section

6.1. Chemistry. General methods

All melting points were determined in open glass capillaries using a Büchi apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Varian Gemini 300/400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and multiplicities are given as s (singlet), d (doublet), t (triplet), m (multiplet) or br (broad). Mass spectra were recorded on a Waters ZQ 4000 apparatus operating in electrospray (ES) mode. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm; Merck) by flash chromatography. Compounds' names were obtained using AUTONOM, PC software for nomenclature in organic chemistry, Beilstein-Institut and Springer.

6.1.1. (6-Morpholin-4-ylhexyl)-(1,2,3,4-tetrahydroacridin-9-yl) amine (**1**)

A stirred suspension of 9-chloro-1,2,3,4-tetrahydroacridine [29] (0.35 g, 2.76 mmol) and phenol (1.50 mL) was heated at 85–90 °C until a solution was obtained. 6-Morpholin-4-ylhexylamine [23] (0.6 g, 5.53 mmol) was then added and the reaction mixture was heated to 130 °C for 4 h. The crude was washed with NaOH 2 N solution and extracted with EtOAc, dried over Na_2SO_4 and evaporated. Flash chromatography (DCM/MeOH/NH_4OH 70:30:0.7) afforded **1** as a brownish oil (0.26 g, 26%). ^{1}H NMR δ: 1.20–1.58 (m, 6H), 1.60–1.75 (m, 2H), 1.80–1.99 (m, 4H), 2.30 (t, 2H, J = 18 Hz), 2.39–2.45 (m, 4H), 2.60–2.68 (m, 2H), 2.98–3.17 (m, 2H), 3.50 (t, 2H, J = 18 Hz), 3.65–3.79 (m, 4H), 7.35 (t, 1H, J = 18 Hz), 7.55 (t, 1H, J = 18 Hz), 7.85–7.90 (m, 2H). ^{13}C NMR δ : 22.4, 22.8, 24.5, 26.3, 26.7, 27.1, 31.6, 32.9, 49.2, 53.7, 58.9, 66.9, 114.9, 119.4, 122.5, 123.8, 127.9, 128.9, 139.3, 151.4, 157.1. MS (ES) *m/z*: 368 (M + H$^+$). Anal. calcd for $C_{23}H_{33}N_3O$: C 75.16, H 9.05, N 11.43, found: C 75.28, H 9.03, N 11.48.

6.1.2. (7-Morpholin-4-yl-heptyl)-(1,2,3,4-tetrahydroacridin-9-yl) amine (**2**)

Starting from 7-morpholin-4-yl-heptylamine and following the previous procedure, **2** was obtained as a brownish oil. ^{1}H NMR δ : 1.20–1.50 (m, 8H), 1.60–1.78 (m, 2H), 1.85–1.99 (m, 4H), 2.30 (t, 2H, J = 18 Hz), 2.39–2.48 (m, 4H), 2.65–2.75 (m, 2H), 3.01–3.17 (m, 2H), 3.50 (t, 2H, J = 18 Hz), 3.65–3.79 (m, 4H), 7.30–7.40 (m, 1H), 7.58 (t, 1H, J = 18 Hz), 7.88–7.97 (m, 2H). ^{13}C NMR δ : 21.4, 22.6, 24.0, 26.0, 26.7, 27.1, 29.0, 31.2, 32.8, 49.1, 53.7, 58.9, 66.5, 115.1, 120.1, 122.7, 123.8, 127.9, 129.1, 139.8, 151.5, 156.9. MS (ES) *m/z*: 382 (M + H$^+$). Anal. calcd for $C_{24}H_{35}N_3O$: C 75.55, H 9.25, N 11.01, found: C 75.48, H 9.23, N 11.08.

6.1.3. (8-Morpholin-4-yloctyl)-(1,2,3,4-tetrahydroacridin-9-yl) amine (**3**)

Starting from 8-morpholin-4-yloctylamine and following the previous procedure, **3** was obtained as a brownish oil. ^{1}H NMR δ : 1.20–1.58 (m, 10H), 1.60–1.75 (m, 2H), 1.80–1.99 (m, 4H), 2.35 (t, 2H, J = 18 Hz), 2.39–2.48 (m, 4H), 2.65–2.78 (m, 2H), 3.05–3.17 (m, 2H), 3.52 (t, 2H, J = 18 Hz), 3.68–3.79 (m, 4H), 7.35 (t, 1H, J = 18 Hz), 7.58 (t, 1H, J = 18 Hz), 7.80–7.88 (m, 2H). ^{13}C NMR δ: 22.1, 22.8, 24.4, 26.3, 26.7, 27.5, 29.1, 29.3, 31.3, 32.7, 49.2, 53.6, 58.9, 66.4, 115.0, 118.5, 121.9, 123.8, 128.1, 129.1, 139.3, 151.6, 157.3. MS (ES) *m/z*: 396 (M + H$^+$). Anal. calcd for $C_{25}H_{37}N_3O$: C 75.91, H 9.43, N 10.62, found: C 75.88, H 9.40, N 10.68.

6.1.4. (9-Morpholin-4-ylnonyl)-(1,2,3,4-tetrahydroacridin-9-yl) amine (**4**)

Starting from 9-morpholin-4-ylnonylamine and following the previous procedure, **4** was obtained as a brownish oil. ^{1}H NMR δ: 1.20–1.52 (m, 12H), 1.60–1.75 (m, 2H), 1.84–1.98 (m, 4H), 2.30 (t, 2H, J = 19 Hz), 2.39–2.48 (m, 4H), 2.65–2.78 (m, 2H), 3.00–3.11 (m, 2H), 3.48 (t, 2H, J = 19 Hz), 3.65–3.79 (m, 4H), 7.35 (t, 1H, J = 18 Hz), 7.55 (t, 1H, J = 18 Hz), 7.91–7.99 (m, 2H). ^{13}C NMR δ : 21.9, 22.6, 24.8, 26.3, 26.7, 27.1, 28.8, 29.1, 29.3, 31.6, 32.6, 49.5, 53.3, 58.9, 66.5, 115.0, 119.5, 122.6, 123.8, 127.9, 128.9, 139.4, 151.5, 157.2. MS (ES) *m/z*: 410 (M + H$^+$). Anal. calcd for $C_{26}H_{39}N_3O$: C 76.24, H 9.60, N 10.26, found: C 76.29, H 9.59, N 10.28.

6.1.5. (10-Morpholin-4-yldecyl)-(1,2,3,4-tetrahydroacridin-9-yl) amine (**5**)

Starting from 10-morpholin-4-yldecylamine and following the previous procedure, **5** was obtained as a brownish oil. ^{1}H NMR δ: 1.20–1.58 (m, 14H), 1.60–1.75 (m, 2H), 1.85–1.99 (m, 4H), 2.30 (t, 2H, J = 18 Hz), 2.39–2.45 (m, 4H), 2.68–2.75 (m, 2H), 3.02–3.12 (m, 2H), 3.50 (t, 2H, J = 18 Hz), 3.65–3.79 (m, 4H), 7.35 (t, 1H, J = 18 Hz), 7.58 (t, 1H, J = 18 Hz), 7.90–8.05 (m, 2H). ^{13}C NMR δ: 20.7, 21.9, 23.7, 25.9, 26.6, 27.3, 28.6, 29.1, 29.3, 29.3, 31.1, 48.6, 53.4, 58.9, 66.4, 110.9, 116.0, 121.3, 124.2, 125.0, 132.0, 139.3, 151.7, 155.2. MS (ES) *m/z*: 424 (M + H$^+$). Anal. calcd for $C_{27}H_{41}N_3O$: C 76.55, H 9.76, N 9.92, found: C 76.49, H 9.79, N 9.94.

6.1.6. 7-[7-(1,2,3,4-Tetrahydroacridin-9-ylamino)heptyloxy]-9-oxa-1-azaanthracen-10-one (**6**)

A mixture of 9-amino-1,2,3,4-tetrahydroacridine (0.078 g, 0.39 mmol), **13** (0.31 g, 0.79 mmol) and a catalytic amount of tetra-*n*-butyl ammonium hydrogensulphate (0.0195 g) was stirred at room temperature for 4 h in a biphasic mixture composed of DCM/NaOH 50% aq. sol. (7.5 mL/5 mL). The organic layer was separated and washed with water, then dried and evaporated. The crude was purified by flash chromatography (DCM/MeOH 97:3) affording **6** as a brownish oil (0.015 g, 7.6%). ^{1}H NMR δ:1.42–1.65 (m, 6H), 1.77–2.05 (m, 8H), 2.60 (t, 2H, J = 16 Hz), 3.30 (t, 2H, J = 16 Hz), 3.90 (t, 2H, J = 16 Hz), 4.15 (t, 2H, J = 18 Hz), 5.80 (br, 1H), 7.05–7.71 (m, 8H), 8.00–8.17 (m, 2H). ^{13}C NMR δ: 23.1, 26.6, 27.7, 29.9, 30.6, 31.8, 31.9, 34.7, 52.3, 72.3, 106.9, 110.2, 115.7 (2C), 117.4, 118.1, 119.3, 121.1,

124.2, 127.7, 128.4, 131.1, 140.7, 146.7, 147.6, 151.5, 155.5, 155.9, 163.7, 164.8. ES-MS *m/z*: 508 ($M + H^+$). Anal. calcd for $C_{32}H_{33}N_3O_3$: C 75.71, H 6.55, N 8.28, found: C 75.69, H 6.52, N 8.24.

*6.1.7. N-(11H-Indeno[1,2-b]quinolin-10-yl)-N'-(1,2,3,4-tetrahydroacridin-9-yl)heptane-1,7-diamine (**7**)*

Following the previous procedure and starting from **14** (0.1 g, 0.24 mmol) and 9-amino-1,2,3,4-tetrahydroacridine (0.048 g, 0.24 mmol), **7** was obtained as a pale brown oil (0.03 g, 24%). ^{1}H NMR δ: 1.20–1.60 (m, 8H), 1.62–1.97 (m, 6H), 2.59–2.67 (m, 2H), 3.02–3.18 (m, 2H), 3.60 (t, 2H, $J = 12$ Hz), 3.80 (t, 2H, $J = 12$ Hz), 4.08 (s, 2H), 5.60 (br, 1H), 7.20–7.60 (m, 7H), 7.85–8.17 (m, 4H), 8.32–8.41 (m, 1H). ^{13}C NMR δ: 21.4, 21.7, 23.8, 26.1, 28.1, 27.9, 29.9, 30.1, 31.3, 35.8, 44.1, 53.7, 61.1, 111.6, 111.9, 118.0, 119.0, 120.2, 120.8, 125.3, 127.8, 128.1, 128.9, 129.5, 130.2, 130.3, 132.1, 138.9, 145.8, 150.9, 156.2, 158.1, 161.8. ES-MS *m/z*: 527 ($M + H^+$). Anal. calcd for $C_{36}H_{38}N_4$: C 82.09, H 7.27, N 10.64, found: C 82.19, H 7.28, N 10.61.

*6.1.8. N-(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)-N'-(11H-indeno [1,2-b]quinolin-10-yl)heptane-1,7-diamine **(8)***

Following the previous procedure and starting from **14** (0.1 g, 0.24 mmol) and 6-chloro-9-amino-1,2,3,4-tetrahydroacridine (0.055 g, 0.24 mmol), **8** was obtained as a pale brown oil (0.07 g, 51%). ^{1}H NMR δ: 1.27–1.58 (m, 6H), 1.59–1.80 (m, 4H), 1.81–1.96 (m, 4H), 2.57–2.65 (m, 2H), 3.00–3.11 (m, 2H), 3.55 (t, 2H, $J = 17$ Hz), 3.70 (t, 2H, $J = 17$ Hz), 3.98 (s, 2H), 7.20–7.60 (m, 6H), 7.85–8.17 (m, 4H), 8.32–8.41 (m, 1H). ^{13}C NMR δ: 21.4, 21.7, 23.7, 26.1, 28.1, 27.9, 29.9, 30.1, 31.3, 35.8, 44.1, 53.7, 61.1, 111.6, 111.8, 118.0, 120.2, 120.5, 120.8, 126.1, 128.0, 128.9, 129.5, 130.2, 130.3, 132.1, 134.1, 138.9, 146.7, 150.9, 156.2, 158.1, 162.4. ES-MS *m/z*: 561 ($M + H^+$). Anal. calcd for $C_{36}H_{37}ClN_4$: C 77.05, H 6.65, N 9.98, found: C 77.09, H 6.67, N 9.96.

*6.1.9. N-(7-Chloro-11H-indeno[1,2-b]quinolin-10-yl)-N'-(3-chloro-1,2,3,4-tetrahydroacridin-9-yl)heptane-1,7-diamine (**9**)*

Following the previous procedure and starting from **15** (0.15 g, 0.34 mmol) and 6-chloro-9-amino-1,2,3,4-tetrahydroacridine (0.078 g, 0.34 mmol), **9** was obtained as a pale brown oil (0.03 g, 15%). ^{1}H NMR δ: 1.27–1.58 (m, 6H), 1.59–1.80 (m, 4H), 1.81–1.96 (m, 4H), 2.57–2.65 (m, 2H), 3.00–3.11 (m, 2H), 3.55 (t, 2H, $J = 18$ Hz), 3.70 (t, 2H, $J = 18$ Hz), 3.98 (s, 2H), 7.20–7.60 (m, 6H), 7.85–8.17 (m, 4H). ^{13}C NMR δ: 21.4, 21.7, 23.8, 26.1, 28.1, 28.0, 29.9, 30.1, 31.3, 35.8, 44.1, 53.7, 61.1, 111.6, 111.9, 118.7, 119.9120.5, 120.8, 127.9, 129.1, 129.9, 130.2, 130.3, 132.1, 132.3, 134.1, 138.1, 146.7, 150.9, 156.2, 158.1, 162.4. ES-MS *m/z*: 595 ($M + H^+$). Anal. calcd for $C_{36}H_{36}Cl_2N_4$: C 72.60, H 6.09, N 9.41, found: C 72.59, H 6.08, N 9.41.

*6.1.10. N,N'-Bis-(11H-indeno[1,2-b]quinolin-10-yl)heptane-1,7-diamine (**12**)*

Following the previous procedure and starting from **14** (0.1 g, 0.24 mmol) and 11*H*-indeno [1,2-*b*]quinolin-10-ylamine (0.056 g, 0.24 mmol), **12** was obtained as a pale brown oil (0.05 g, 37%). ^{1}H NMR δ: 1.35–1.59 (m, 6H), 1.60–1.77 (m, 4H), 3.59–3.72 (m, 4H), 4.11 (s, 2H), 4.80 (br, 2H), 7.05–7.71 (m, 12H), 8.00–8.17 (m, 2H), 8.20–8.32 (m, 2H). ^{13}C NMR δ: 26.1, 28.1, 30.1, 35.8, 44.1, 53.7, 61.1, 111.9, 119.0, 120.8, 123.9, 128.1, 129.5, 130.3, 138.9, 145.8, 147.4, 150.9, 158.7, 159.8. ES-MS *m/z*: 561 ($M + H^+$). Anal. calcd for $C_{39}H_{36}N_4$: C 83.54, H 6.47, N 9.99, found: C 83.59, H 6.48, N 10.01.

*6.1.11. [7-(4-Benzofuran-2-yl-phenoxy)heptyl]-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amine (**10**)*

A solution of 6-chloro-9-amino-1,2,3,4-tetrahydroacridine (0.15 g, 0.65 mmol), **16** [25] (0.3 g, 0.78 mmol) and KOH (0.06 g, 1.04 mmol) in dry DMSO (8 mL) was stirred for 16 at room temperature under nitrogen in the presence of freshly activated 4 Å molecular sieves (100 mg). The mixture was filtered through Celite® and the filtrate diluted with water (300 mL), extracted with EtOAc (3 25 mL), anhydrified over Na_2SO_4 and concentrated to dryness giving 0.26 g of crude. Flash chromatography (DCM/MeOH 98:2) afforded **10** as a yellowish oil (0.18 g, 51%). ^{1}H NMR δ: 1.28–1.52 (m, 6H), 1.54–1.92 (m, 8H), 2.52–2.68 (m, 2H), 2.92–3.12 (m, 2H), 3.41 (t, 2H, $J = 18$ Hz), 3.92 (t, 2H, $J = 18$ Hz), 5.24 (s, 1H), 6.82 (s, 1H), 6.90 (d, 2H, J 8.8 Hz), 7.18–7.25 (m, 3H), 7.45–7.49 (m, 2H), 7.73 (d, 2H, J 8.8 Hz,), 7.86 (d, 2H, J 10.6 Hz). ^{13}C NMR ($CDCl_3$) δ: 22.5, 22.7, 24.4, 25.8, 26.7, 28.9, 31.5, 33.9, 40.8, 49.3, 67.7, 99.4, 110.8, 114.6, 115.5, 118.2, 120.4, 122.7, 122.9, 123.5, 123.9, 124.5, 126.2, 127.4, 129.3, 133.7, 148.0, 150.6, 150.8, 154.5, 155.9, 159.3. ES-MS *m/z*: 539 ($M + H^+$). Anal. calcd for $C_{34}H_{35}ClN_2O_2$: C 75.75, H 6.54, N 5.20, found: C 75.79, H 6.56, N 5.18.

*6.1.12. [7-(3-Benzofuran-2-yl-phenoxy)heptyl]-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amine (**11**)*

Following the previous procedure and starting from 6-chloro-9-amino-1,2,3,4-tetrahydroacridine (0.15 g, 0.65 mmol) and **17** (0.3 g, 0.78 mmol), **11** was obtained as a yellowish oil (0.05 g, 14%). ^{1}H NMR δ: 1.38–1.58 (m, 6H), 1.60–1.93 (m, 8H), 2.60–2.65 (m, 2H), 2.98–3.10 (m, 2H), 3.48 (t, 2H, $J = 18$ Hz), 4.03 (t, 2H, $J = 18$ Hz), 5.28 (s, 1H), 6.81–6.92 (m, 1H), 7.00 (s, 1H), 7.21–7.59 (m, 8H), 7.87–7.91 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 22.4, 22.8, 24.4, 25.9, 26.8, 29.0, 29.1, 29.6, 31.6, 33.6, 49.4, 67.7, 101.6, 110.8, 113.9, 114.8, 114.9, 115.3, 117.4, 120.9, 122.9, 124.3, 124.6, 124.7, 126.9, 129.1, 129.8, 131.7, 134.3, 151.0, 154.8, 155.7, 158.9, 159.4. ES-MS *m/z*: 539 ($M H^+$). Anal. calcd for $C_{34}H_{35}ClN_2O_2$: C 75.75, H 6.54, N 5.20, found: C 75.77, H 6.53, N 5.19.

*6.1.13. 7-(7-Bromoheptyloxy)-9-oxa-1-azaanthracen-10-one (**13**)*

A stirred suspension of 7-hydroxy-9-azaanthracen-10-one [24] (0.8 g, 3.7 mmol), 1,7-dibromoheptane (1.2 mL, 7.5 mmol) and K_2CO_3 (0.97 mg) in acetone (30 mL) was refluxed for 20 h. The reaction mixture was hot filtered and the filtrate evaporated. The residue was purified by flash chromatography (toluene/acetone 90:10), affording **13** as an oil (0.65 g, 45%). ^{1}H NMR ($CDCl_3$): δ 1.35–1.57 (m, 6H), 1.79–1.97 (m, 4H), 3.43 (t, 2H), 4.10 (t, 2H), 6.91–7.03 (m, 2H), 7.39–7.45 (m, 1H), 8.16–8.23 (m, 1H), 8.60–8.75 (m, 2H).

*6.1.14. (7-Bromoheptyl)-(11H-indeno [1,2-b]quinolin-10-yl)amine (**14**)*

A suspension of 11*H*-indeno [1,2-b]quinolin-10-ylamine [25a] (0.65 g, 2.8 mmol), 1,7-dibromoheptane (0.57 mL, 3.4 mmol) and KOH (0.25 g, 4.5 mmol) in dry DMSO (10 mL) was stirred for 16 at room temperature under nitrogen in the presence of freshly activated 4 Å molecular sieves (100 mg). The mixture was filtered through Celite® and the filtrate diluted with water (250 mL), extracted with EtOAc (3 x 30 mL), dried over Na_2SO_4 and the solvent was evaporated. Flash chromatography (DCM/MeOH 98:2) afforded 0.11 g of **14** as a yellowish oil (10%). ^{1}H NMR ($CDCl_3$): δ 1.38–1.60 (m, 6H), 1.62–1.83 (m, 4H), 3.60–3.78 (m, 4H), 4.12 (s, 2H), 7.40–7.61 (m, 4H), 7.75 (t, 1H), 8.16–8.29 (m, 3H).

*6.1.15. (7-Bromoheptyl)-(7-chloro-11H-indeno [1,2-b]quinolin-10-yl)amine (**15**)*

Following the previous procedure and starting from 7-chloro-11*H*-indeno [1,2-b]quinolin-10-ylamine [25b] (0.5 g, 1.88 mmol), **15** was obtained as a brownish oil (0.18 g, 22%). ^{1}H NMR ($CDCl_3$): δ 1.37–1.59 (m, 6H), 1.63–1.85 (m, 4H), 3.58–3.76 (m, 4H), 4.11 (s, 2H), 7.42–7.63 (m, 4H), 7.77 (t, 1H), 8.15–8.32 (m, 2H).

6.1.16. Synthesis of 3-Benzofuran-2-yl-phenol: 2-(3-Methoxyphenyl)benzofuran

A stirred suspension of 3-methoxybenzoylchloride (5 g, 29 mmol), 2-hydroxybenzyltriphenyl phosphonium bromide (12 g, 27 mmol) and Et_3N (11.1 mL, 80 mmol) in toluene (125 mL) was

refluxed for 10 . The reaction mixture was filtered under vacuum and evaporated to dryness. EtOH (150 mL) was added to the oily residue and the flask kept in the freezer overnight. A yellowish solid formed which was filtered off and purified by flash chromatography on silica gel (petroleum ether/ethyl acetate 95:5), affording the compound as a colorless oil (3.58 g, 55%). ^{1}H NMR ($CDCl_3$) δ: 3.87(s,3H), 6.82–6.95 (m, 1H), 7.00 (s, 1H), 7.18–7.60 (m, 6H). 3-Benzofuran-2-yl-phenol. To a cold (0 °C) solution of 2-(3-methoxyphenyl)benzofuran (3.0 g, 13 mmol) in anhydrous DCM (300 mL), BBr_3 (18 mL of 1 M solution in DCM) was added slowly. The resulting mixture was stirred overnight at room temperature. The reaction was quenched with ice/water and stirred for 30 min. The organic layer was washed with water (3 x 50 mL), then with brine (3 x 25 mL) and dried over Na_2SO_4. The solvent was removed affording the compound (2.65 g, 97%) as a white solid, mp 123 °C ^{1}H NMR ($CDCl_3$) δ: 4.92 (br, 1H), 6.83–6.97 (m, 3H), 7.18–7.28 (m, 2H), 7.43–7.58 (m, 2H), 7.70–7.79 (m, 2H).

6.1.17. 2-[3-(7-Bromoheptyloxy)phenyl]benzofuran (**17**)

A stirred mixture of 3-benzofuran-2-yl-phenol (1.00 g, 4.7 mmol), 1,7-dibromoheptane (0.57 mL, 3.3 mmol) and K_2CO_3 (1.2 g) was refluxed in acetone (100 mL) for 20 . The suspension was hot filtered and the solvent was removed. After adding petroleum ether, the residue was kept in the freezer overnight and the white solid that formed was filtered off, affording **17**, mp 145 °C ^{1}H NMR ($CDCl_3$) δ: 1.23–1.58 (m, 6H), 1.69–1.98 (m, 4H), 3.40 (t, 2H), 4.02 (t, 2H), 6.89 (s, 1H), 6.87–6.95 (m, 2H), 7.24–7.31 (m, 2H), 7.50–7.56 (m, 2H), 7.717.83 (m, 2H).

6.2. Biology

6.2.1. Inhibition of AChE and BuChE

The method of Ellman et al. was followed [30]. Five different concentrations of each compound were selected in order to obtain inhibition of AChE or BuChE activity comprised between 20% and 80%. The assay solution consisted of a 0.1 M potassium phosphate buffer pH 8.0, with the addition of 340 μM 5,5′-dithio-bis(2-nitrobenzoic acid), 0.02 unit/mL of human recombinant AChE or BuChE derived from human serum (Sigma Chemical), and 550 μM of substrate (acetylthiocholine iodide or butyrylthiocholine iodide, respectively). Test compounds were added to the assay solution and preincubated at 37 °C with the enzyme for 20 min followed by the addition of substrate. Assays were carried out with a blank containing all components except AChE or BuChE in order to account for non-enzymatic reaction. The reaction rates were compared, and the percent inhibition due to the presence of test compounds was calculated. Each concentration was analyzed in triplicate, and IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration *vs* percent inhibition, GraphPad Prism 4.0, GraphPad Software Inc).

6.2.2. Determination of steady-state inhibition constant

To obtain graphical estimates of the mechanism of inhibition, reciprocal plots of 1/V versus 1/[S] were constructed in the presence of 3 different concentrations of **8** ranging from 0.143 to 0.937 nM and increasing concentration of substrate (below 0.56 mM). The plots were assessed by a weighted least square analysis that assumed the variance of *v* to be a constant percentage of *v* for the entire data set. Data analysis was performed with GraphPad Prism 4.03 software (GraphPad Software Inc.).

6.2.3. Inhibition of AChE-induced amyloid-β peptide aggregation

Aliquots of 2 μL $A\beta_{40}$ peptide (Bachem AG, Germany), lyophilized from a 2 mg mL^{-1} HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) solution and dissolved in DMSO, were incubated for 24 h at room temperature in 0.215 M sodium phosphate buffer (pH 8.0) at a final concentration of 230 μM. For co-incubation experiments aliquots (16 μL) of human recombinant AChE (final concentration 2.30 μM, Aβ/AChE molar ratio 100:1) and AChE in the presence of 2 μL of tested inhibitor (final concentration = 100 μM) were added. Blanks containing Aβ, AChE, and Aβ plus inhibitors, in 0.215 M sodium phosphate buffer (pH 8.0) were prepared. The final volume of each vial was 20 μL. Each assay was run in duplicate. To quantify amyloid fibril formation, the thioflavin T (ThT)-based fluorescence method was applied [37–39].

After incubation, the samples containing Aβ, or Aβ plus AChE, or Aβ plus AChE in the presence of inhibitors were diluted with 50 mM glycine-NaOH buffer (pH 8.5) containing 1.5 μM thioflavin T to a final volume of 2.0 mL. A 300 s-time scan of the emitted fluorescence (λ_{exc} = 446 nm, λ_{em} = 490 nm) was performed and the intensity values at the plateau were averaged after subtracting the background fluorescence of 1.5 μM thioflavin T and AChE.

The fluorescence intensities in the presence and in the absence of inhibitor were compared and the percentage of inhibition was calculated by comparing the fluorescence intensities obtained for Aβ plus AChE in the presence and in the absence of inhibitor [37].

6.2.4. Inhibition of BACE1

Purified Baculovirus-expressed BACE1 (β-secretase) in 50 mM Tris (pH 7.5), 10% glycerol and rhodamine derivative substrate (Panvera peptide) were purchased from Invitrogen (Milan, Italy). Sodium acetate and DMSO were obtained from Sigma–Aldrich (Milan, Italy). Purified water from Milli-RX system (Millipore, Milford, USA) was used to prepare buffers and standard solutions. Spectrofluorometric analyses were carried out on a Fluoroskan Ascent multiwell spectrofluorometer (excitation: 544 nm; emission: 590 nm) using black microwell (96 wells) Corning plates (Sigma–Aldrich, Milan, Italy). Stock solutions of the test compounds were prepared in DMSO and diluted with 50 mM sodium acetate buffer (pH 4.5). Specifically, 20 μL of BACE1 enzyme (11.7 nM, final concentration) were incubated with 20 μL of test compound for 60 min. To start the reaction, 20 μL of Panvera peptide (0.25 μM, final concentration) were added to each well. The mixture was incubated at 37 °C for 60 min. To stop the reaction, 20 μL of BACE1 stop solution (sodium acetate, 2.5 M) were added to each well. The spectrofluorometric assay was subsequently performed by reading the fluorescence signal at 590 nm. The DMSO concentration in the final mixture was maintained below 5% (v/v) to guarantee no significant loss of enzyme activity. The fluorescence intensities with and without inhibitor were compared, and the percent inhibition due to the presence of test compounds was calculated. The background signal was measured in control wells containing all the reagents except BACE1 and subtracted. The inhibition (%) due to the presence of five or six increasing concentrations of test compound was calculated by the following expression: 100-(IFi/IFo × 100) where IFi and IFo are the fluorescence intensities obtained for BACE1 in the presence and in the absence of inhibitor, respectively. Inhibition curves were obtained by plotting the percent inhibition versus the logarithm of inhibitor concentration in the assay sample, when possible. The linear regression parameters were determined and the IC_{50} value extrapolated (GraphPad Prism 4.0, GraphPad Software Inc.). To demonstrate inhibition of BACE1 activity, a peptidomimetic inhibitor (β-secretase inhibitor IV, Calbiochem, Merck; Nottingham, UK) was serially diluted into the reaction wells (IC_{50} = 0.013 μM).

Additional measurements were performed for *bis*(7)-tacrine, **8** and **12** in the presence of a detergent (CHAPS, 0.1% w/v) to check for non specific effects.

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