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Heterocyclic inhibitors of AChE acylation and peripheral sites

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Abstract

Notwithstanding the criticism to the so called " cholinergic hypothesis", the therapeutic strategies for the treatment of Alzheimer's disease (AD) have been mainly centered on the restoration of cholinergic functionality and, until the last year, the only drugs licensed for the management of AD were the acetycholinesterase (AChE) inhibitors. Target enzyme AChE consists of a narrow gorge with two separate ligand binding sites: an acylation site at the bottom of the gorge containing the catalytic triad and a peripheral site located at the gorge rim, which encompasses binding sites for allosteric ligands. The aim of this short review is to update the knowledge on heterocyclic AChE inhibitors able to interact with the two sites of enzymes, structurally related to the well known inhibitors physostigmine, rivastigmine and propidium. The therapeutic potential of the dual site inhibithors in inhibiting amyloid-ß aggregatrion and deposition is also briefly summarised.

Keywords: Alzheimer's disease; Acetylcholinesterase; Cholinesterase inhibitors; Rivastigmine; Propidium; Amyloid-β

1. Introduction

Alzheimer's disease (AD), the most common cause of senile dementia, is a complex neurological affection that was described for the first time in 1906 by the Austrian psychiatrist Alois Alzheimer. Today AD affects 13 million people worldwide and, with the aging of Western countries population, represents a major public health issue. It is clinically characterized by a relentless irreversible brain degeneration leading to memory loss, impaired judgment, difficulty in speech, inability to word and finally death. Besides these behavioral manifestations, Alzheimer described neuropathological stigmata in the brain of AD patients that after a century, are still considered the definitive diagnosis of the disease: appearance of amyloid plaques around nerve cells and neurofibrillary tangles within the cells. Another key characteristic is a dramatic atrophy of certain sections of the brain, with a specific degeneration of the cholinergic neurons that appear the most vulnerable cellular population in AD. Even if the primary cause of AD is still speculative, several lines of evidence point toward a central role for the amyloid-β (Aβ) fibrillogenesis in the etiology of AD [1]. In recent years, significant research attention has also been devoted to the role of free radical formation, oxidative cell damage, and inflammation in the pathogenesis of AD, providing new promising targets and validated animal models [2]. To date, however, the therapeutic strategies for the treatment of AD have been mainly centered on the restoration of cholinergic functionality [3,4] and, until the last year, the only drugs licensed for the management of AD were the acetylcholinesterase (AChE) inhibitors (AChEI) tacrine, [5] donepezil, [6] rivastigmine, and galantamine [7]. More recently, the role of the overactivation of glutamate receptors in the neuronal death that characterizes AD has been definitely cleared out, and the antagonist memantine has been approved in the US in the late 2003 (Chart 1) [8].

2. AChE acylation site inhibitors

Target enzyme AChE consists of a narrow gorge with two separate ligand binding sites: an acylation site (A-site) at the bottom of the gorge that contains the catalytic triad (His-447, Glu-334, and Ser-203) as well as Trp-86 which binds the trimethylammonium head of acetylcholine (ACh), and a peri-

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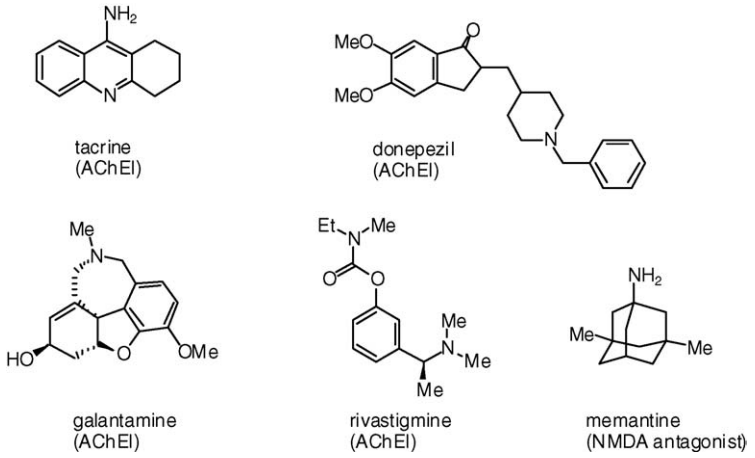


Chart 1. Chemical structures of drugs currently approved for the treatment of AD.

pheral site (P-site) located at the gorge rim, which encompasses binding sites for allosteric ligands.

The aim of this short review is to update the knowledge on heterocyclic AChE inhibitors able to interact with the two sites of the enzyme.

2.1. Physostigmine-related inhibitors

In a previous project, aimed to the development of inhibitors of AChE based on a polyamine backbone, we discovered caproctamine (**1**), a diamine diamide endowed with an interesting affinity profile against AD [9]. In a related study on constrained polyamines, [10] the terminal 2-methoxybenzyl groups were included into a hexahydrochromeno[4,3-*b*]pyrrole system (**2**), to verify whether the spatial relationship of the methoxy moiety relative to the amine function differently affected affinity for cholinergic receptors.

At that time, the new ring system reminded us the hexahydropyrrolo[2,3-*b*]indole system of physostigmine (**3**), the prototype of pseudo-irreversible inhibitors of AChE, which is able to form a carbamoylated complex with the Ser of the A- site [11,12]. In spite of the first encouraging results, **3** was discontinued after the completion of phase III clinical trials for AD, because of a short half-life, variable bioavailability and narrow therapeutic index. All these considerations stimulated our interest in designing new physostigmine-related inhibitors, which showed improved pharmacokinetic properties over physostigmine, with respect to stability, duration of action and oral bioavailability. To verify whether the new ring system may act as a suitable molecular scaffold in AChE recognition, we synthesized carbamates **4**–**6** (see Fig. 1). Furthermore, the enantiomers of the most potent derivative **4** were investigated to verify the importance, if any, of stereochemistry on the affinity for AChE. The quaternary derivative **7** was included in this study to verify further the role of a permanent positive charge on affinity.

Aryl-functionalized tricyclic pyrrolidines have very few synthetic precedents in the literature, [13] so this prompted us to develop an efficient route to target compounds (Scheme 1).

The substituted hexahydrochromeno[4,3-*b*]pyrrole system required for the synthesis of physostigmine-related compounds was synthesized by following a powerful and stereocontrolled procedure found in the literature for related phenyl-unsubstituted compounds [14]. It involved a condensation between an oleifin aldehyde with a secondary amino acid (tri-

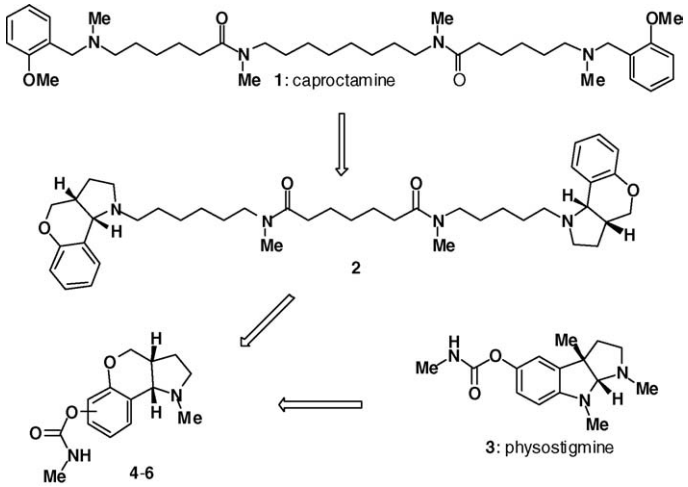
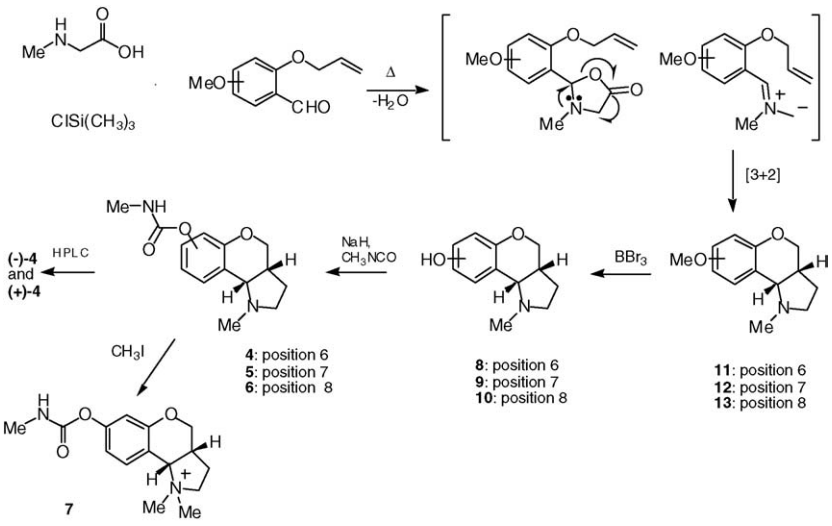


Fig. 1. Design strategy for the synthesis of compounds **4**–**6** by replacing the pyrroline ring of physostigmine (**3**) with the dihydropyran ring of **2**.



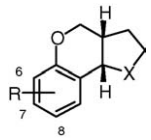
Scheme 1.

methylsylil)ester to give an intermediate, which first decarboxilates and then undergoes a [3 + 2] cycloaddition to the internal oleifin (*cis* fusion only). Thus, starting from *N*-methylglycine, chlorotrimethylsilane, and the appropriate substituted 2-allyloxybenzaldehyde under dehydrating conditions, compounds **11**–**13** were obtained, which, upon treatment with BBr_3, afforded phenols **8**–**10**. The carbamate functionality was introduced by reaction of methyl isocyanate with **8**–**10**, affording **4**–**6**. Quaternary derivative **7** was obtained by treatment of **5** with methyl iodide.

The stereochemistry of the ring-fusion in compounds **11**–**13**, and as a consequence in the compounds that were obtained starting from them, was confirmed from the coupling constant of hydrogens at the ring junction. Enantiomers (+)-**4** and (–)-**4** were obtained by HPLC chromatographic resolution of racemic **4**.

To determine the potential interest of compounds **4**–**8** and enantiomers (+)-**4** and (–)-**4** for the treatment of AD, their AChE inhibitory activity was determined by the method of Ellman et al. [15] in comparison to those of caproctamine (**1**), its constrained analog (**2**), and physostigmine (**3**). Furthermore, to establish the selectivity of **4**–**8**, their butyrylcholinesterase (BChE) inhibitory activity was also calculated by the same method on BChE from human erythrocytes. An analysis of the results shown in Table 1 reveals that the position of the carbamate moiety in the phenyl ring has a pivotal role in determining anticholinesterase activity. The 6-substituted derivative **4** is 60–550-fold more potent than **5** and **6**, which bear the carbamate group at position 7 and 8, respectively, at both AChE and BChE. Substituted hexahydrochromeno[4,3-*b*]pyrroles **4**–**6** did not discriminate, like physostigmine (**3**), between AChE and BChE. As

Table 1
Inhibition of AChE and BChE activities by physostigmine-related compounds



Number	R	X	IC_{50} (nM) ± S.E.M. [a]		k_3 (min^{-1})
			AChE	BChE	
1	caproctamine		170 ± 2	11600 ± 14	nd [b]
2			9740 ± 330	903 ± 55	nd
3	physostigmine	Me	13.4 ± 5.4	26.1 ± 1.8	9.0 ± 0.7
4	6-$OCONHCH_3$	$NHCH_3$	30.0 ± 1.7	59.4 ± 2.3	26.5 ± 1.8
(–)-**4**	6-$OCONHCH_3$	$NHCH_3$	29.8 ± 4.7	53.8 ± 3.9	nd
(+)-**4**	6-$OCONHCH_3$	$NHCH_3$	74.4 ± 7.0	78.1 ± 11.7	nd
5	7-$OCONHCH_3$	$NHCH_3$	1940 ± 100	3560 ± 320	nd
6	8-$OCONHCH_3$	$NHCH_3$	16,200 ± 600	10,200 ± 300	nd
7	7-$OCONHCH_3$	$N^+(CH_3)_2$	1810 ± 70	2970 ± 300	nd
8	6-OH	$NHCH_3$	39,800 ± 2500	788,000 ± 26,000	nd

[a] AChE and BChE from human erythrocytes were used. IC_{50} values represent the concentration of inhibitor required to decrease enzyme activity by 50% and are the mean of two independent measurements, each performed in triplicate.

[b] nd, not determined.

expected, the quaternary derivative **7** was as active as the parent tertiary amino derivative **5**. Similarly, the finding that the phenolic compound **8**, lacking the carbamate function, was more than three orders of magnitude less potent than **4** at both AChE and BChE parallels the result observed following removal of the carbamate group from the physostigmine structure [12].

It is known that inhibition of both AChE and BChE by **3** is highly enantioselective, resting almost exclusively on the 3a*S* enantiomer [12]. However, enantiomers (–)-**4** and (+)-**4** did not follow the same trend as revealed by a comparison of their pIC_{50} values at AChE and BChE. It turned out that (–)-**4** was 3- and 14-fold more potent than enantiomer (+)-**4** at AChE and BChE, respectively. Although the reason for this discrepancy is not clear yet, the low enantioselectivity observed for the enantiomers of **4** is not surprising as other physostigmine derivatives also showed a similar pattern [12].

As previously reported, [16–18] the inhibition of AChE by **3** involves a reversible complex formation followed by carbamylation of the enzyme, yielding a covalent adduct. To compare the mode of action of **4** with that of **3** their equilibrium ($K_c = k_2/k_1$) and rate (k_3) constants were calculated by performing a traditional stopped assay [17]. The rate constant k_3 calculated for **4** were comparable with those found for **3** (Table 1). This would suggest a similar 'time dependent' pattern of inhibition for the two examined inhibitors [16]. Similarly, K_c value calculated for **3** was in agreement with the value reported in literature [18].

In conclusion, the hexahydropyrrolo[2,3-*b*]indole moiety of physostigmine (**3**) can be replaced by a hexahydrochromeno[4,3-*b*]pyrrole, as in **4**, without affecting the affinity for AChE: clearly, compound **4** could form a lead for the development of new AChE inhibitors.

2.2. Rivastigmine-related inhibitors

Rivastigmine (**14**; Fig. 2) represents a second-generation pseudo-irreversible AChE inhibitor, [16] endowed again with a carbamate moiety, able to react covalently with the serine of the A- site. It belongs to a series of miotine (**15**; Fig. 2) derivatives, [17] and shows an inhibitory action toward AChE less marked than physostigmine. However, its superior global pharmacological profile, including a good combination of longer duration of action, good tolerability and lower toxicity, led to its approval by FDA in 2000. In addition, **14** is referred as a 'brain-region' selective cholinesterase inhibitor, [18] since it preferentially inhibits AChE and BChE of the hippocampus and cortex [19]. Recent evidence suggests that in the AD brain, BChE activity rises, while AChE activity remains unchanged or declines. Therefore, both enzymes are likely involved in regulating ACh levels and, consequently, may represent legitimate therapeutic targets for the development of agents such as **14**, which, with the ability to inhibit BChE in addition to AChE, should lead to improved clinical outcomes [20].

On the basis of these findings and in connection with our previous studies [21] on the series of benzopyrano[4,3-*b*]pyrrole carbamates as AChE inhibitors, we set about constructing a series of conformationally restricted analogs of **14** and **15** by including the dimethylamino-ethyl-phenyl moiety in different tricyclic systems related to **4** (Fig. 2) [22]. In order to support this basic idea, a superimposition between the conformation of **14** and the carbon derivative **16**, as obtained from Monte Carlo simulations, was carried out. Low energy conformations of each molecule were selected and fitted, as shown in Fig. 3. Clearly, the overlap was satisfactory (RMSD = 0.28 Å), confirming that the tricyclic derivatives might act as rigid analogs of **14**. Thus, the isosteric

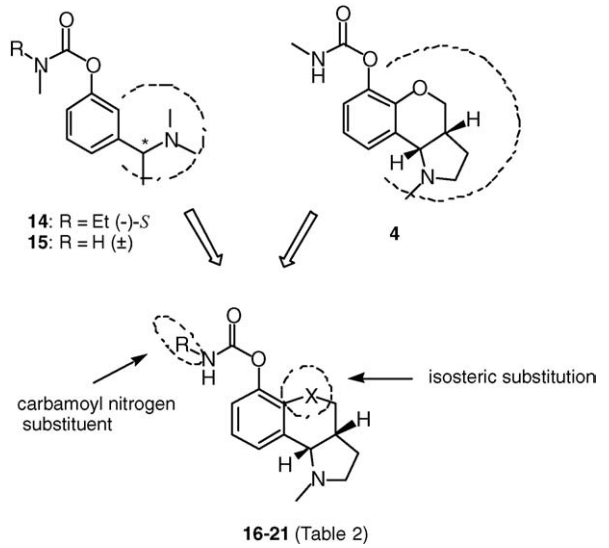


Fig. 2. Design strategy for the synthesis of **16–21** by inserting the dimethylamino-ethyl-phenyl moiety of **14** in different tricyclic systems related to **4**.

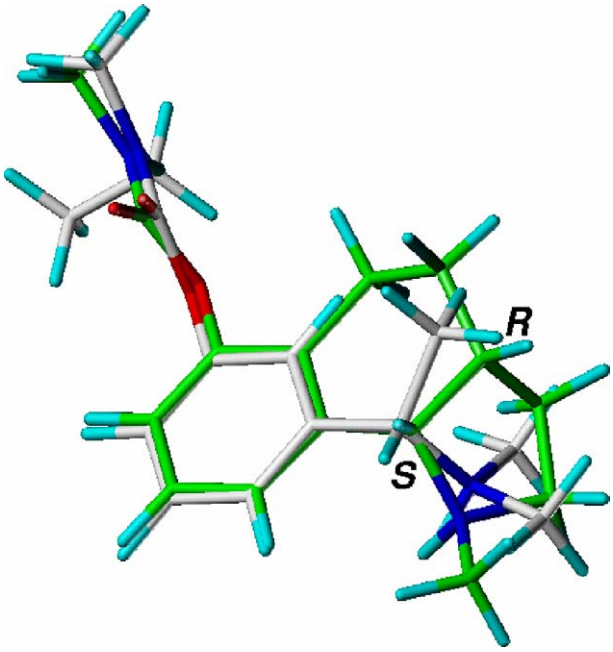
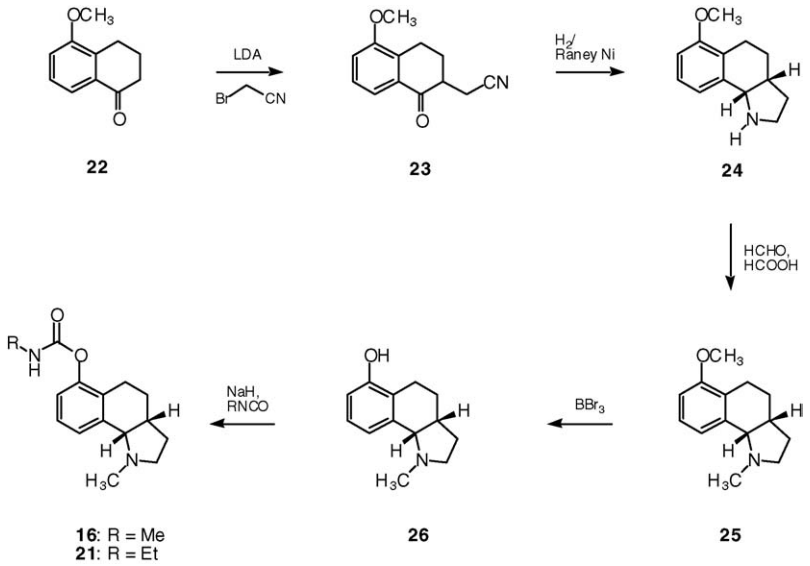


Fig. 3. Superimposition of a PM3 minimized low energy conformation of **16** onto a PM3 minimized low energy conformation of **14**. The pharmacophoric functions of **16** fit very well those of **14**. The rigid analog **16** might indicate the bioactive conformation of rivastigmine. Figure adapted from [22].



Scheme 2.

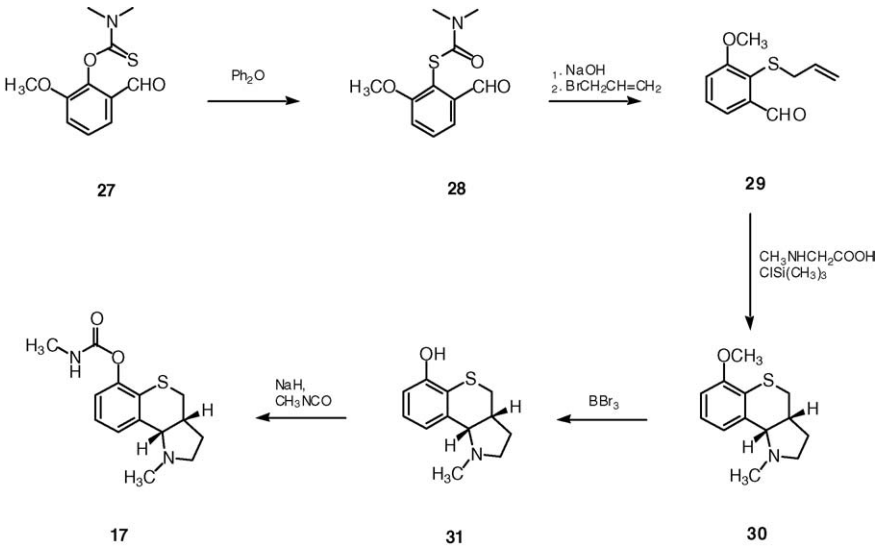
replacement of the endocyclic oxygen of **4** with a sulfur or a carbon atom, leading to **17** and **16**, respectively, might reveal information about the physicochemical requirements of the enzyme binding site, which are still on debate. In addition, the role of the carbamoyl nitrogen substituent of **4** was investigated through the synthesis of different aryl and alkyl carbamates (**18**–**21**).

The previous pathway to **4**, namely the [3 + 2] dipolar cycloaddition of a substituted 2-allyloxibenzaldeyde, was not deemed practical for the synthesis of the carbon analog **16**, since the preparation of the correspondent 2-butenyl-3-methoxy-benzaldheyde was not as feasible. Thus, a different synthetic strategy, starting from the commercially accessible tetralone **22**, was followed (Scheme 2).

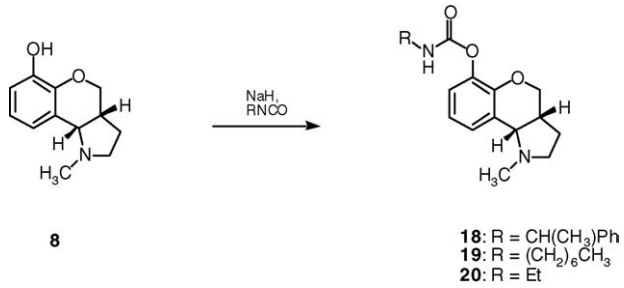
Alkylation of the lithium salt of 5-methoxytetralone with bromoacetonitrile in rigorously anhydrous conditions furnished nitrile **23**, which, in turn, was cyclized to **24** through catalytic hydrogenation over Raney Ni. The coupling constant observed for the ring-fusion protons (J = 6.5 Hz) was consistent with the reported value for *cis*-isomer **4** [21] and the *cis* stereochemistry was further secured from detailed NOE studies. Methylation of the amine function of **24** and subsequent dealkylation of the methoxyl gave phenol **26**, which, upon reaction with the appropriate isocyanate afforded final compounds **16** and **21**.

On the contrary, the synthesis of **17** could be performed using the strategy previously described for **4**, [21] through reaction of the key intermediate 2-allylsulfanyl-3-methoxy-benzaldehyde (**29**) and sarcosine (trimethylsiliyl)ester (Scheme 3).

The preparation of **29** was readily accomplished in one pot by sequential treatment of **28**, [23,24] obtained, in turn, by the Newman–Kwart rearrangement of O-dimethyl-thiocarbamoylated vanillin (**27**), with sodium hydroxide and allyl bromide. After formation of the tricyclic system **30**, the synthesis proceeded as depicted for **4** in Scheme 1 and, in



Scheme 3.



Scheme 4.

agreement with the parent system, the *cis* fusion ring was assigned through the coupling constant of the benzylic methine proton.

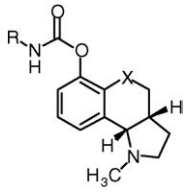
Finally, carbamoylation of **8** to give the different carbamates **18**–**20**, was accomplished as reported for the preparation of **4** (Scheme 4).

It should be mentioned that this time the enantiomer separation of the different racemic mixtures was not performed, since we [21] and others [25] have already noticed that the chirality seems to not strongly affect the inhibitory activity of this class of compounds.

The inhibitory potency, expressed as IC_{50} values, of compounds **16**–**21** of AChE and BChE is reported in Table 2 in comparison with that of the analog **4**, and the two reference compounds **14** and **15**.

The most interesting finding was that the potency towards AChE is generally increased in the rigidified [1]benzopyrano[4,3-*b*]pyrrole derivatives compared to the flexible prototype **14**. In particular, derivatives **16**, **17**, and **19** turned out to be significantly more potent than both rivastigmine (**14**) and the parent derivative miotine (**15**), confirming the rational design carried out. Among the restricted analogs, **17** showed the highest inhibitory activity, being 370 and 12-fold more potent than **14** and **15**, respectively. A reasonable explanation for this phenomenon is that the tricyclic system of the rigid series acts as conformationally restricted mimic of the 3-[1-(dimethylamino)ethy]phenyl fragment of **14**, as revealed by Monte Carlo conformational analyses. In this respect, the conformation of **16**, shown in Fig. 3, might indicate the bioactive conformation of **14**. The restricted compounds have higher affinity because they do not incur the entropic penalty experienced when **14** and **15**, having a freely rotating skeleton, bind.

Table 2
Inhibition of AChE and BChE activities by rivastigmine-related compounds



Number	X	R	IC_{50} (nM) ± S.E.M.[a]		k_{obs} (min^{-1})
			AChE	BChE	
14	rivastigmine		1535± 64	301 ± 14	0.040
15	miotine		100 ± 33	406 ± 13	nd [b]
4	O	Me	30 ± 1.7	59.4 ± 2.3	nd
16	CH_2	Me	17.3 ± 1.2	24.5 ± 1.6	nd
17	S	Me	8.11 ± 0.28	10.5 ± 0.5	0.365
18	O	$CH(CH_3)Ph$	1870 ± 230	209 ± 3	nd
19	O	$(CH_2)_6CH_3$	20.3 ± 3.1	nd	0.047
20	O	Et	420 ± 5	nd	0.049
21	CH_2	Et	393 ± 33	nd	0.052

[a] Human recombinant AChE and BChE from human serum were used. IC_{50} values represent the concentration of inhibitor required to decrease enzyme activity by 50% and are the mean of two independent measurements, each performed in triplicate.

[b] nd, not determined.

However, it is well established that several structural elements concur to determine AChE inhibitory activity of carbamate derivatives. For instance, the alkyl substituent on the carbamoyl nitrogen strongly affects the affinity profile. In our series of rigid compounds, the most potent inhibitors resulted methyl derivatives **4**, **16**–**17**, all endowed with potency in the nanomolar range. Increasing the length of the alkyl chain to ethyl resulted in 40-fold reduction in both the oxygen and carbon series (**4** vs. **20** and **16** vs. **21**). A similar behavior was shown by **18**, carrying a bulky *R*-1-phenylethyl carbamate on the [1]benzopyrano[4,3-*b*]pyrrole scaffold. The potency was restored to a value similar to that of the methyl derivative **4** by lengthening the chain to *n*-heptyl, affording **19**, which displayed an IC_{50} value of 20.3 nM. These results are in good agreement with the higher potency reported for monosubstituted carbamate derivatives of **14** carrying a methyl group instead of an ethyl one [25,26]. The anomalous "ethyl effect" was also observed by Lieske et al. [27], who found in a series of indolinylcarbamates, the diethyl derivative about 7400-fold less potent than the dimethylcarbamoyl analog. Actually, to support these experimental data, the crystal structure of AChE/rivastigmine complex has disclosed a steric hindrance effect between the ethyl group of **1** and His440 in the active site, causing a significant movement of this amminoacid away from its normal partner, Glu327, and resulting in the disruption of the catalytic triad [28].

Moreover, the carbamate moiety also affects the kinetic of AChE carbamoylation, as revealed by the pseudo first order rate constants k_{obs}, determined for **17** and **19**–**21**, following the method reported by Feaster and Quinn [29]. From the data in Table 2, it appears that all of tested compounds showed a time-dependent pattern of inhibition, which is characteristic of pseudo-irreversible inhibitors and which was similar to that of **14**. The time course of the inhibition was characterized by an increase up to a steady state, which was reached after 20 min in the case of **17**, and almost 40 min for **14** and **19**–**21**. This clearly indicates that, once again, the nature of the substituent of the *N*-carbamoyl group of tested molecules plays a role in differentiating the kinetic mechanism of action and the inhibitory potency as well (IC_{50} values). As postulated from the crystallographic study on **14**, the *N*-alkyl carbamic chain of compounds **19**–**21** could increase the bulk of the molecules to an extent where the interaction with the active

site is disturbed. Thus, the nucleophilic attack of the catalytic serine might be hampered, because the steric clash is not experienced by the methyl-carrying derivative **3**. In this regard, compound **17** was the fastest inhibitor of the series, while ethyl (**20** and **21** likewise **14**) and heptyl (**19**) derivatives showed a much slower enzyme inactivation, as revealed by their k_{obs} values (Table 2).

The results at BChE had a potency trend similar to that observed at AChE: rigid carbamates **4**, **16** and **17** were one order of magnitude more potent than flexible compounds **14** and **15**, but, unlike **14**, which displayed a preferential BChE selectivity, showed similar IC_{50} values for AChE and BChE inhibition. Recent evidence suggests that, beyond the regulation of synaptic ACh levels, BChE may also have a role in the etiology and progression of AD. Consequently, the new compounds, which have a dual inhibitory action on both AChE and BChE, might show a better therapeutic profile in AD and related dementia [20]. The unfavorable effect of the carbamic *N*-alkyl chain on AChE inhibition was less striking when considering BChE inhibition, as revealed by comparing the IC_{50} values of the bulky derivative **18** for the two enzymes. A reasonable explanation might be that BChE is characterized by a bigger acyl binding pocket than AChE, [30] thus accounting for the lower clash effect of a wider substituent towards BChE.

Exchanging the oxygen or the carbon atom with a sulfur (**17** vs. **4** and **16**) resulted in a slight increase of both AChE and BChE inhibition, suggesting a favorable short range interaction between the aromatic moiety of Trp86 (AChE) and Trp82 (BChE) of the active sites and the sulfur atom of the inhibitor [31].

3. Peripheral and dual binding site AChE inhibitors

Parallel to the challenges towards validity of cholinergic hypothesis and to the use of AChEI to treat AD, in recent years much research effort has been devoted to identify new roles for AChE in neurodegeneration, other than the regulatory function in terminating ACh-mediated neurotransmission. A unique structural feature of this enzyme is the presence of the peripheral binding site, that was identified in kinetic studies, and that seems to be fundamental for some of its so called "non-classical action". Although our understanding of additional role of AChE is still incomplete, different experimental evidences point towards a remarkable variety of AChE functions. Neuritic plaques from Alzheimer patients include catalitically active AChE [32] and AchE–amyloid-β complexes were shown to be more neurotoxic than amyloid peptides alone, [33] suggesting that AChE imbalances might have implications for AD pathogenesis [34]. Furthermore it has been reported that AChE promotes amyloid fibrils assembly, by binding to Aβ and inducing a conformational transition to the amyloidogenic conformer [35]. The same authors found that this activity was blocked by the peripheral site inhibitor propidium, but not by the active site inhibitor edrophonium, clearly identifying this pro-aggregating activity as a non-classical AChE function. All these results constitute the premise for medicinal chemists to the design of peripheral and dual binding site inhibitors, which able to simultaneously interact with the two sites, might alleviate the cognitive deficit in AD and, what is more important, address the etiology of the disease. A recent crystal structure of phenylphenantridinium ligands complexed with mouse AChE unveiled new structural determinants contributing to P-site ligand interactions, and permitted a detailed topographic delineation of this site. These structure confirmed propidium specific binding to the P-site and definitely highlighted the significant interaction with Trp-286 [36]. Thus, the two Trp of the A- and P-sites represent the target residues for dual inhibitors that, spanning the active center and the peripheral site, might exhibit tighter binding to AChE and a better pharmacological profile in AD.

Following this rationale, several strategies have been employed to design high affinity dual inhibitors with dimeric structure, and all show a marked enhanced potency, compared to the monomer, which interacts with a single site [37–40].

A very interesting piece of work is represented by the approach of Sharpless and coworkers who exploited AChE as a reaction vessel for the selective assembly of building blocks derived from the two known inhibitors tacrine and propidium. The triazole-linked bivalent ligand resulting from the cycloaddition reaction is the most potent noncovalent AChE inhibitor known to date [41].

Based on these findings and aware that AChE gorge is lined with several aromatic amminoacid side chains capable of forming cation-π interaction with a basic counterpart, we planned to synthesize bivalent ligands in which the molecular feature of well known A- and P-site inhibitors were connected through a polyamine chain. In this case, as postulated by the universal template design strategy, [42] the nature and the length of the spacer becomes critical, since it plays an active role in target recognition process. As proof of principle, we choose a triamine backbone to link the structural motif of propidium to the tetrahydroaminoacridine system of tacrine or to the methoxybenzylamino group of caproctamine, affording novel heterobivalent polyamine ligands (Fig. 4). The synthesis and the biological activity of these compounds will be published in due course.

4. Conclusions

A renewed interest in the search of AChEI has occurred, following recent evidences which showed that AChE has secondary "non cholinergic" functions and that peripheral anionic site might be somehow related with aggregation and deposition of Aβ peptide.

Accordingly, novel compounds that might increase the level of ACh and simultaneously interfere with processing of amyloid appears to be a promising strategy to be explored by medicinal chemists.

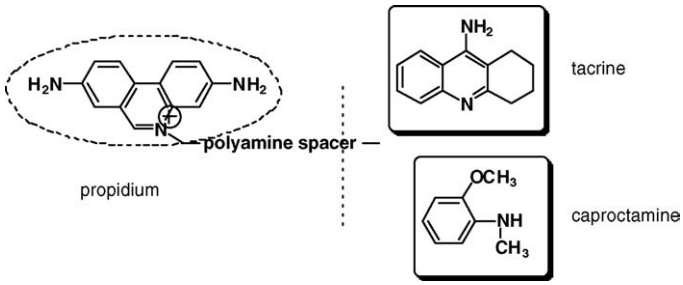


Fig. 4. Universal template approach to the design of dual binding site AChE inhibitors derived from propidium, tacrine and caproctamine.

Moreover, due to the multifaceted pathology of AD, in the field of AChEI pharmacotherapy future trends should point to the development of multipotent drugs, that exploiting inhibition of AChE and hitting different selected targets in the neurodegeneration cascade, could represent a valuable pharmacological treatment.

References

[1] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.

[2] S. Capsoni, G. Ugolini, A. Comparini, F. Ruberti, N. Berardi, A. Cattaneo, Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice, Proc. Natl. Acad. Sci. USA 97 (2000) 6826–6831.

[3] R.T. Bartus, On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis, Exp. Neurol. 163 (2000) 495–529.

[4] A.V. Terry Jr., J.J. Buccafusco, The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development, J. Pharmacol. Exp. Ther. 306 (2003) 821–827.

[5] K.L. Davis, P. Powchik, Tacrine, Lancet 345 (1995) 625–630.

[6] H.M. Bryson, P. Benfield, Donepezil, Drugs Aging 10 (1997) 234–239 (discussion 240–231).

[7] J.J. Sramek, E.J. Frackiewicz, N.R. Cutler, Review of the acetylcholinesterase inhibitor galanthamine, Expert Opin. Investig. Drugs 9 (2000) 2393–2402.

[8] S.H. Ferris, Evaluation of memantine for the treatment of Alzheimer's disease, Expert Opin. Pharmacother. 4 (2003) 2305–2313.

[9] C. Melchiorre, V. Andrisano, M.L. Bolognesi, R. Budriesi, A. Cavalli, V. Cavrini, et al., Acetylcholinesterase noncovalent inhibitors based on a polyamine backbone for potential use against Alzheimer's disease, J. Med. Chem. 41 (1998) 4186–4189.

[10] M. Rosini, R. Budriesi, M.G. Bixel, M.L. Bolognesi, A. Chiarini, F. Hucho, P. Krogsgaard-Larsen, I.R. Mellor, A. Minarini, V. Tumiatti, P.N. Usherwood, C. Melchiorre, Design, synthesis, and biological evaluation of symmetrically and unsymmetrically substituted methoctramine-related polyamines as muscular nicotinic receptor noncompetitive antagonists, J. Med. Chem. 42 (1999) 5212–5223.

[11] N.H. Greig, X.F. Pei, T.T. Soncrant, D.K. Ingram, A. Brossi, Phenserine and ring C hetero-analogues: drug candidates for the treatment of Alzheimer's disease, Med. Res. Rev 15 (1995) 3–31.

[12] Q. Yu, N.H. Greig, H.W. Holloway, A. Brossi, Syntheses and anticholinesterase activities of (3a*S*)-N1, N8-bisnorphenserine, (3a*S*)-N1,N8-bisnorphysostigmine, their antipodal isomers, and other potential metabolites of phenserine, J. Med. Chem 41 (1998) 2371–2379.

[13] S. Hanessian, G. Papeo, M. Angiolini, K. Fettis, M. Beretta, A. Munro, Synthesis of functionally diverse and conformationally constrained polycyclic analogues of proline and prolinol, J. Org. Chem. 68 (2003) 7204–7218.

[14] P.N. Confalone, E.M. Huie, The stabilized iminium ylide-oleifin [3 + 2] cycloaddition reaction. Total synthesis of Sceletium alkaloid A_4, J. Am. Chem. Soc. 106 (1984) 7175–7178.

[15] G.L. Ellman, K.D. Courtney, V. Andres Jr., R.M. Feather-Stone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (1961) 88–95.

[16] M.D. Gottwald, R.I. Rozanski, Rivastigmine, a brain-region selective acetylcholinesterase inhibitor for treating Alzheimer's disease: review and current status, Expert Opin. Investig. Drugs 8 (1999) 1673–1682.

[17] M. Weinstock, M. Razin, M. Chorev, Z. Tashma, Advances in Behavioral Biology, Plenum Press: New York (1986) 539–551.

[18] B.R. Williams, A. Nazarians, M.A. Gill, A review of rivastigmine: a reversible cholinesterase inhibitor, Clin. Ther. 25 (2003) 1634–1653.

[19] R. Bullock, The clinical benefits of rivastigmine may reflect its dual inhibitory mode of action: an hypothesis, Int. J. Clin. Pract. 56 (2002) 206–214.

[20] E. Giacobini, R. Spiegel, A. Enz, A.E. Veroff, N.R. Cutler, Inhibition of acetyl- and butyryl-cholinesterase in the cerebrospinal fluid of patients with Alzheimer's disease by rivastigmine: correlation with cognitive benefit, J. Neural Transm. 109 (2002) 1053–1065.

[21] M.L. Bolognesi, V. Andrisano, M. Bartolini, A. Minarini, M. Rosini, V. Tumiatti, C. Melchiorre, Hexahydrochromeno[4,3-*b*]pyrrole derivatives as acetylcholinesterase inhibitors, J. Med. Chem. 44 (2001) 105–109.

[22] M.L. Bolognesi, M. Bartolini, A. Cavalli, V. Andrisano, M. Rosini, A. Minarini, C. Melchiorre, Design, synthesis, and biological evaluation of conformationally restricted rivastigmine analogues, J. Med. Chem. 47 (2004) 5945–5952.

[23] L.K.A. Rahaman, R.M. Scrowston, 7-Substituted benzo[*b*]thiophenes and 1,2-benzisothiazoles. Hydroxy- or methoxy-derivatives, J. Chem. Soc. Perkin Trans. I 12 (1983) 2973–2978.

[24] L. Yaouancq, M. Anissimova, M.-L. Badet-Denisot, B. Badet, Design and evaluation of mechanism-based inhibitors of D-alanyl-D-alanine dipeptidase van X, Eur. J. Org. Chem. 21 (2002) 3573–3579.

[25] J. Sterling, Y. Herzig, T. Goren, N. Finkelstein, D. Lerner, W. Goldenberg, I. Miskolczi, S. Molnar, F. Rantal, T. Tamas, G. Toth, A. Zagyva, A. Zekany, J. Finberg, G. Lavian, A. Gross, R. Friedman, M. Razin, W. Huang, B. Krais, M. Chorev, M.B. Youdim, M. Weinstock, Novel dual inhibitors of AChE and MAO derived from hydroxy aminoindan and phenethylamine as potential treatment for Alzheimer's disease, J. Med. Chem. 45 (2002) 5260–5279.

[26] M. Weinstock, M. Razin, M. Chorev, A. Enz, Pharmacological evaluation of phenyl-carbamates as CNS-selective acetylcholinesterase inhibitors, J. Neural Transm. Suppl 43 (1994) 219–225.

[27] C.N. Lieske, R.T. Gepp, J.H. Clark, H.G. Meyer, P. Blumbergs, C.C. Tseng, Anticholinesterase activity of potential therapeutic 5-(1,3,3-trimethylindolinyl) carbamates, J. Enzyme Inhib. 5 (1991) 215–223.

[28] P. Bar-On, C.B. Millard, M. Harel, H. Dvir, A. Enz, J.L. Sussman, I. Silman, Kinetic and structural studies on the interaction of cholinesterases with the anti-Alzheimer drug rivastigmine, Biochemistry 41 (2002) 3555–3564.
[29] S.R. Feaster, D.M. Quinn, Mechanism-based inhibitors of mammalian cholesterol esterase, Methods Enzymol. 286 (1997) 231–252.
[30] Y. Nicolet, O. Lockridge, P. Masson, J.C. Fontecilla-Camps, F. Nachon, Crystal structure of human butyrylcholinesterase and of its complexes with substrate and products, J. Biol. Chem. 278 (2003) 41141–41147.
[31] T. Yuan, A.M. Weljie, H.J. Vogel, Tryptophan fluorescence quenching by methionine and selenomethionine residues of calmodulin: orientation of peptide and protein binding, Biochemistry 37 (1998) 3187–3195.
[32] I. Wright, C. Geula, M.M. Mesulam, Neurological cholinesterases in the normal brain and in Alzheimer's disease: relationship to plaques, tangles, and patterns of selective vulnerability, Ann. Neurol 34 (1993) 373–384.
[33] A. Alvarez, R. Alarcon, C. Opazo, E.O. Campos, F.J. Munoz, F.H. Calderon, et al., Stable complexes involving acetylcholinesterase and amyloid-beta peptide change the biochemical properties of the enzyme and increase the neurotoxicity of Alzheimer's fibrils, J. Neurosci. 18 (1998) 3213–3223.
[34] H. Small, S. Michaelson, G. Sberna, Non-classical actions of cholinesterases: role in cellular differentiation, tumorigenesis and Alzheimer's disease, Neurochem. Int. 28 (1996) 453–483.
[35] C. Inestrosa, A. Alvarez, C.A. Perez, R.D. Moreno, M. Vicente, C. Linker, et al., Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme, Neuron 16 (1996) 881–891.
[36] Y. Bourne, P. Taylor, Z. Radic, P. Marchot, Structural insights into ligand interactions at the acetylcholinesterase peripheral anionic site, EMBO J. 22 (2003) 1–12.
[37] P.Y. Pang, P. Quiram, T. Jelacic, F. Hong, S. Brimijoin, Highly potent, selective, and low cost bis-tetrahydroaminacrine inhibitors of acetylcholinesterase. Steps toward novel drugs for treating Alzheimer's disease, J. Biol. Chem 271 (1996) 23646–23649.
[38] L. Savini, A. Gaeta, C. Fattorusso, B. Catalanotti, G. Campiani, L. Chiasserini, C. Pellerano, E. Novellino, D. McKissic, A. Saxena, Specific targeting of acetylcholinesterase and butyrylcholinesterase recognition sites. Rational design of novel, selective, and highly potent cholinesterase inhibitors, J. Med. Chem. 46 (2003) 1–4.
[39] C. Guillou, A. Mary, D.Z. Renko, E. Gras, C. Thal, Potent acetylcholinesterase inhibitors: design, synthesis and structure–activity relationships of alkylene linked bis-galanthamine and galanthamine–galanthaminium salts, Bioorg. Med. Chem. Lett. 10 (2000) 637–639.
[40] P. Camps, B. Cusack, W.D. Mallender, R.E. El Achab, J. Morral, D. Munoz-Torrero, T.L. Rosenberry, Huprine X is a novel high-affinity inhibitor of acetylcholinesterase that is of interest for treatment of Alzheimer's disease, Mol. Pharmacol. 57 (2000) 409–417.
[41] W.G. Lewis, L.G. Green, F. Grynszpan, Z. Radic, P.R. Carlier, P. Taylor, M.G. Finn, K.B. Sharpless, Click chemistry in situ: acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks, Angew. Chem. Int. Ed. Engl. 41 (2002) 1053–1057.
[42] M.L. Bolognesi, A. Minarini, R. Budriesi, S. Cacciaguerra, A. Chiarini, S. Spampinato, et al., Universal template approach to drug design: polyamines as selective muscarinic receptor antagonists, J. Med. Chem 41 (1998) 4150–4160.