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Tacrine based human cholinesterase inhibitors: Synthesis of peptidic-tethered derivatives and their effect on potency and selectivity

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ABSTRACT

Tacrine based reversible inhibitors of cholinesterases (ChEIs) containing peptidic tethers were synthesized to interact with specific regions at the gorge level, and their potency was determined with human (*h*) acetylcholinesterase and butyrylcholinesterase. Analogues **3i,j** and **3l,m** were identified as promising hits and may pave the way for the development of a new series of tacrine based enzyme selective *h*ChEIs.

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia among older people, it is associated with a loss of basal forebrain cholinergic neurons (particularly in neocortex, hippocampus, and amygdala), with a consequent lack of acetylcholine (ACh) around brain cells showing degenerative changes. Cholinesterases (ChEs) (acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)) are ACh hydrolyzing enzymes and their inhibition represents the currently employed approach for the treatment of AD. AChE inhibitors (AChEIs) used to treat AD patients at present are donepezil, rivastigmine and galantamine, while tacrine (**1**, Fig. 1) was the first AChE inhibitor introduced in therapy.

While AChE has a well established 'classical' esterase activity, the physiologic role of BuChE is still controversial. However, BuChE may have a compensatory role in the hydrolysis of ACh in brains with degenerative changes, thus making it an additional target for increasing the cholinergic tone in AD patients affected by severe symptoms.[1–5] This raised the hypothesis that inhibitory action on both enzymes could lead to an improved therapeutic benefit.[4–6]

AChE and BuChE are multifunctional enzymes characterized by their classical esterase activity, and by non-classical functions that are unrelated to their hydrolytic function (e.g., modulation of glial activation, participation in haematopoiesis, neuritic outgrowth, tau phosphorylation, adhesion protein-like activity, and promotion of amyloid-β aggregation).[7] All these actions involve the peripheral anionic site (PAS) or other AChE surface sites[8] and are related to specific protein conformations, sensitive to the concentration of metal ions.[9–13] Consequently, ChEIs may possibly have a variety

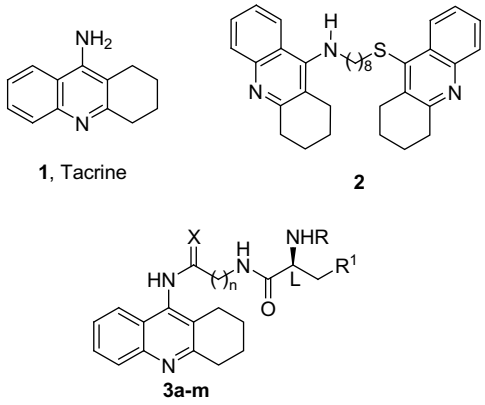

Figure 1. Reference and title compounds.

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of effects in the CNS, modulating classical and non-classical functions of the enzymes. The array of protein conformations present in different structural/functional forms of these enzymes suggests the existence of a high degree of flexibility in ChEs structure, and coherently, multiple AChE motions have been demonstrated.[14]

Very recently we uncovered specific hot spots at the gorge level of both enzymes, and extremely potent tacrine-based heterobivalent ligands were identified through binding to multiple interaction sites in human (*h*) AChE and BuChE.[15]

The relationship between fluctuation in AChE structure and function has been the subject of intense research[7,16] and confirms a mutual allosteric modulation between the catalytic site (CAS) and the PAS through the mid-gorge amino acid residues.

A promising strategy for interfering with biological processes is through the control of intra- and inter-protein interactions by means of small molecules.[17] We recently proposed an integrated approach to the rational design of small molecule enzyme modulators based on the control of protein–protein interactions between ChE substructures (i.e., hot spots) to design novel and extremely potent *h*ChEIs.[16] An in depth study of the amino acid composition of the gorge of AChE drove the rational modification of the tether of the bis-tacrine derivative **2** (Fig. 1) demonstrating that the tether is crucial for potency and selectivity.[16]

We herein report the synthesis of a series of tacrine based *h*ChEIs (**3a**–**m**, Fig. 1 and Table 1) possessing a peptidic tether and one or two tacrine moieties.[18] The natural or unnatural L-amino acid chosen to 'span' the gorge were hydrophobic in nature when coupled to a tacrine monomer (**3a**–**j**), or chosen on the basis of flexibility and adaptability to the gorge, when linked to two tacrine units as in **3k**–**m**, (L-glutamate for **3k**–**l** and L-proline for **3m**). While these compounds were being investigated, similar approaches were published by other authors.[19,20]

For the synthesis of compounds **3a**–**h** (Scheme 1), tacrine (**1**) was reacted with the appropriate acid chloride to afford derivatives **4a**,**b**. Treatment of **4a** with sodium azide followed by hydrogenolysis furnished the 1-aminoacetamido compound **5**, the key intermediate for the synthesis of compounds **3a**–**f**. Compound **5** was coupled with protected amino acids and the resulting intermediates, after cleavage of the protecting group, afforded the desired final compounds. Zinc chloride and sodium borohydride reduction of the cyano group of **4b**, in the presence of Boc anhydride, provided the 3-aminoprotected propionamide **6**. Deprotection of **6**, and coupling with L-BocTrpOH afforded **3g** from which **3h** was obtained after exposure to hydrochloric acid.

Reaction of 9-chlorotetrahydroacridine **7** (Scheme 2) with the appropriate diamines, afforded **8a**,**b**. Compound **8a**[21] was synthesized by applying a new, highly yielding (77%), microwave alkylation protocol, while **8b** was prepared as previously described.[22] Compounds **3i**,**k** were obtained by coupling suitable amino acids to **8a**. Deprotection of **3i**,**k** gave the final compounds **3j**,**l**. By a modification of the literature protocol,[23] 9-chlorotetrahydroacridine **7** was reacted with ethanolamine and the corresponding alcohol was efficiently brominated to **9**. In order to synthesize **3m**, compound **10**, obtained by coupling of L-BocPro-OH with **8b**, was alkylated with the bromo-derivative **9**.

The inhibitory activity of the new tacrine-related compounds was evaluated using purified recombinant *h*AChE and *h*BuChE.[24,16] Results are reported in Table 1. The mono-tacrine derivatives **3a**–**h** and **3i**,**j** show in general a preference for *h*BuChE. Compounds **3a**–**h**, lacking a basic tacrine nitrogen, one of the key determinant for potency, proved to be weak inhibitors of *h*AChE. In particular compounds **3a**–**f**, where a glycine unit was used to space aromatic L-amino acids from the tacrine moiety, exhibited a micromolar affinity for *h*AChE, and submicromolar for *h*BuChE. Compounds **3a**,**b**

Table 1
Dissociation constants for the inhibition of *h*AChE and *h*BuChE by tacrine-related compounds **3a**–**m** and reference compounds

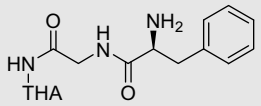
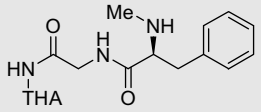
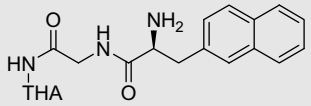
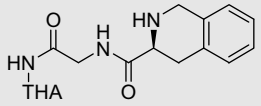
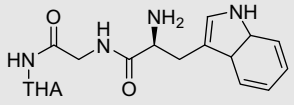
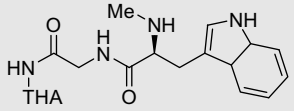
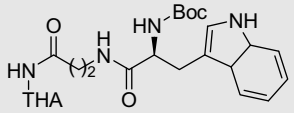
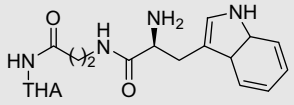
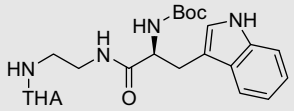
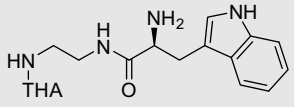
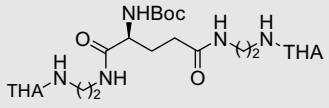
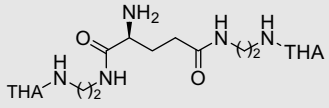
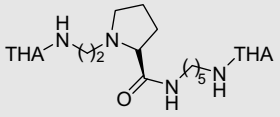
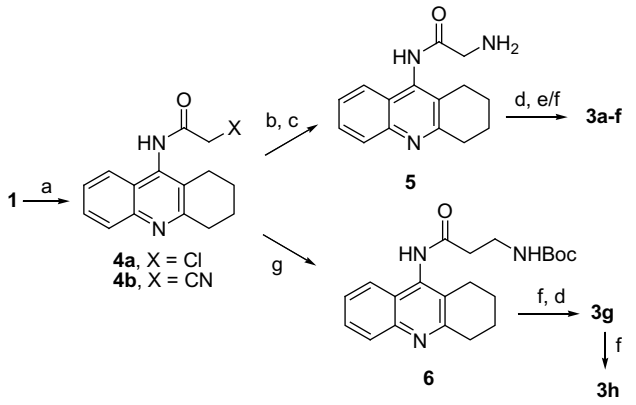
Compound	Structure[a]	K_i[b] (nM) (±SEM)	
		*h*AChE	*h*BChE
3a		1908 (63)	708 (44)
3b		6320 (610)	1198 (53)
3c		11190 (424)	252 (90)
3d		4130 (202)	580 (50)
3e		2214 (414)	251 (14)
3f		3330 (235)	1764 (31)
3g		7970 (1520)	443 (27)
3h		34030 (8160)	173 (55)
3i		130 (20)	1.87 (0.13)
3j		280 (30)	1.33 (0.10)
3k		15.40 (1.43)	12.63 (0.74)
3l		6.21 (0.15)	25.15 (2.45)

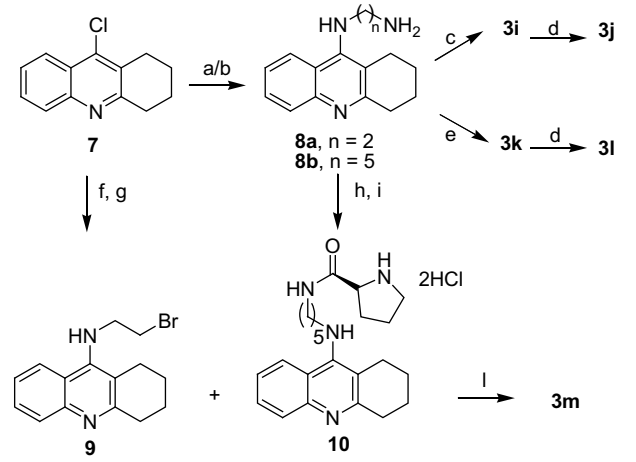
Table 1 (*continued*)

Compound	Structure[a]	K_i[b] (nM) (±SEM)	
		*h*AChE	*h*BChE
3m		6.75 (0.54)	1.77 (0.14)
1	–	36 (1)	7 (2)
2	–	27.90 (2.42)	1.65 (0.1)

[a] THA, 1,2,3,4-tetrahydroacridin-9-yl.
[b] K_i (nM) is the mean of at least three determinations.



Scheme 1. Reagents and conditions: (a) $ClCOCH_2X$, TEA, CH_2Cl_2, rt; (b) (from **4a**) NaN_3, ethanol/water, reflux; (c) H_2, Pd/C 10%, MeOH, rt; (d) Boc or Cbz protected L-amino acid, EDCI, HOBt, dry CH_2Cl_2 or dry DMF, rt (**3c**); (e) H_2, Pd/C 10%, MeOH, rt (**3a**–**c**); (f) CH_3COCl, MeOH, rt (**3d**–**f**); (g) (from **4b**) $ZnCl_2{\cdot}6H_2O$, $NaBH_4$, $(Boc)_2O$, MeOH, rt.



Scheme 2. Reagents and conditions: (a) for **8a** ethylendiamine, MW, 150W, 12 min; (b) for **8b** 1,5-pentandiamine, TEA, 1-pentanol, 160 °C; (c) (from **8a**) (L)-*N*-Boc-tryptophan, EDCI, HOBt, TEA, dry DMF, rt; (d) CH_3COCl, MeOH, rt; (e) (from **8a**, 2 equiv), (L)-*N*-Boc-glutamate EDCI, HOBt, TEA, dry CH_2Cl_2, rt; (f) ethanolamine, 120 °C; (g) CBr_4, PPh_3, CH_2Cl_2, rt; (h) from **8b**, (L)-*N*-Boc-proline EDCI, HOBt, TEA, dry CH_2Cl_2, rt; (i) CH_3COCl, MeOH, rt; (l) TEA, CH_3CN, rt.

differ for the presence of a methyl group on the pendant basic nitrogen which reduced *h*AChE potency of three times (**3a** vs **3b**) while *h*BuChE was less sensitive to the steric hindrance provided by the methylamino group, according to its gorge larger void (6 of the 14 aromatic residues (Y72, Y124, W286, F295, F297 and Y337) of the *h*AChE gorge are replaced by aliphatic residues (N68, Q119, A277, L286, V288, and A328) in *h*BuChE).[15a] The introduction of an extra fused phenyl ring (**3c**) dramatically affected *h*AChE inhibition while *h*BuChE inhibitory activity was increased by almost three times (**3a** vs **3c**). For the conformationally constrained derivative **3d** (**3d** vs **3b**) the same trend of activity was observed, being **3d** two times more potent at *h*BuChE than **3b**. In the L-tryptophan series tethered by glycine (**3e**,**f**), the activity of the analogue **3e** was similar to that of **3a** on *h*AChE, but it was about three times more potent on *h*BuChE (**3e** vs **3a**). While on this latter enzyme **3e** showed a potency similar to that of the naphtyl derivative **3c**, it proved to be five times more potent against *h*AChE. In this case, N-methylation of the tryptophan primary amino group moderately affected *h*AChE activity but significantly reduced *h*BuChE inhibitory potency. The homologation of **3e**, using beta alanine in place of glycine at the tether level, provided a subset of analogues (**3g**,**h**) which were in general much more selective for *h*BuChE. In particular **3h**, bearing the unprotected protonatable function, provided the best *h*AChE/*h*BuChE ratio for the subset (**3h**, *h*AChE/*h*BuChE ratio = 197). In a similar extent, *tert*-butyl carbamolylation of **3h** (**3g**), increased affinity for *h*AChE and reduced potency on *h*BuChE. Considering the larger void of *h*BuChE this data is apparently discordant with the amino acid composition and with the physico-chemical properties of the two enzymes that are due to structural differences in the active site gorge of the two enzymes, leading to a lower electrostatic gradient and a larger void in *h*BuChE.[25]

Substantial improvement of *h*ChE inhibitory activity was obtained with analogues **3i** and **3j** endowed with (i) a more flexible tether, and (ii) a protonatable function at the tacrine level, key determinants for potency improvement. Indeed **3i** and **3j** showed *h*AChE inhibitory potency in the high nanomolar range and displayed excellent *h*BuChE inhibitory properties (**3j** *h*AChE/*h*BuChE ratio = 210). Indeed **3j** was the more potent and selective *h*BuChE inhibitor of the series.

We therefore synthesized bis-tacrine analogues in which two flexible ethylamino moieties were coupled with L-glutamate (**3k**,**l**), reaching a tether length of 11 atoms (improving also tether flexibility) and introducing a protonatable function at the tether level to optimally bind the three interaction points at the gorge level (CAS, mid-gorge and PAS).[15] While the protected analogue **3k**, which lacks the protonatable glutamate function, showed a similar nanomolar potency at both enzymes, the deprotected analogue **3l** proved to be more potent at *h*AChE with respect to *h*BuChE, in agreement with the electrostatic gradient of the gorge of the two enzymes. High potency for *h*ChEs was also found for **3m** in which the ω-glutamate ethylamido moiety of **3l** was replaced by an L-prolinepentylamide (**3m**).

In summary, we described herein the synthesis and biological evaluation of tacrine based *h*ChEIs containing specific peptidic tethers the modification of which determines a substantial modulation of *h*ChEs inhibitory activity. The low potency of glycine containing compounds and beta alanine derivatives (**3a**–**h**) was highly improved enhancing tether length and flexibility. This work allowed the identification of compounds **3i** and **3j** as the more potent and selective *h*BuChE inhibitors of the series, demonstrating the key role played by the protonatable tacrine moiety. Furthermore the three interaction point bis-tacrine derivatives **3k**–**m** proved to be potent *h*ChEIs as well.

Acknowledgments

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ing true views of the Department of the Army or the Department of Defense.

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