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Synthesis and in vitro evaluation of *N*-alkyl-7-methoxytacrine hydrochlorides as potential cholinesterase inhibitors in Alzheimer disease

Jan Korabecny [a], Kamil Musilek [b,c,*], Ondrej Holas [a], Jiri Binder [a], Filip Zemek [b], Jan Marek [b], Miroslav Pohanka [d], Veronika Opletalova [a], Vlastimil Dohnal [c], Kamil Kuca [c,d]

[a] Charles University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry and Drug Control, Heyrovskeho 1203, 500 05 Hradec Kralove, Czech Republic
[b] University of Defence, Faculty of Military Health Sciences, Department of Toxicology, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic
[c] University of Jan Evangelista Purkynje, Faculty of Science, Department of Chemistry, Ceske Mladeze 8, 400 96 Usti nad Labem, Czech Republic
[d] University of Defence, Faculty of Military Health Sciences, Centre of Advanced Studies, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic

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ABSTRACT

All approved drugs for Alzheimer disease (AD) in clinical practice ameliorate the symptoms of the disease. Among them, acetylcholinesterase inhibitors (AChEIs) are used to increase the cholinergic activity. Among new AChEI, tacrine compounds were found to be more toxic compared to 7-MEOTA (9-amino-7-methoxy-1,2,3,4-tetrahydroacridine). In this Letter, series of 7-MEOTA analogues (*N*-alkyl-7-methoxytacrine) were synthesized. Their inhibitory ability was evaluated on recombinant human acetylcholinesterase (AChE) and plasmatic human butyrylcholinesterase (BChE). Three novel compounds showed promising results towards hAChE better to THA or 7-MEOTA. Three compounds resulted as potent inhibitors of hBChE. The SAR findings highlighted the C_6–C_7 *N*-alkyl chains for cholinesterase inhibition.

Alzheimer disease (AD) is a progressive neurodegenerative disorder associated with a global impairment of higher mental functions represented by an impairment of memory as the major symptom.[1] It is manifested as selective loss of cholinergic neurons and reduced levels of neurotransmitter acetylcholine in the brain. The extracellular deposition of amyloid β-peptide (Aβ) in senile plaques, the appearance of intracellular neurofibrillary tangles (NFT) containing hyperphosphorylated τ-protein and the extensive synaptic changes in the cerebral cortex, hippocampus or other brain areas essential for cognitive functions are main histopathological signs of the disease.[2]

Based on the cholinergic theory of AD, cholinergic enhancement strategies were developed to pharmacologically avoid the cognitive impairment. Many treatment strategies were used, where only cholinesterase inhibitors (ChEIs) were the first and up-to-date the only compounds that showed some progress in the treatment of AD.[3] Owing to this fact, only ChEIs as AD drugs were approved by Food and Drug Administration (FDA) up-to-date. Thus, various cholinergic drugs such as physostigmine, tacrine (THA), donepezil and more recently galanthamine and huperzine (Fig. 1) were extensively investigated. The only approved AD drug based on different mechanism of action is memantine (NMDA-antagonist) (Fig. 1).[4–6] ChEIs stabilize cognitive functions of patient up to one year and they can result in statistically significant improvement of cognitive and global assessment of AD.[7]

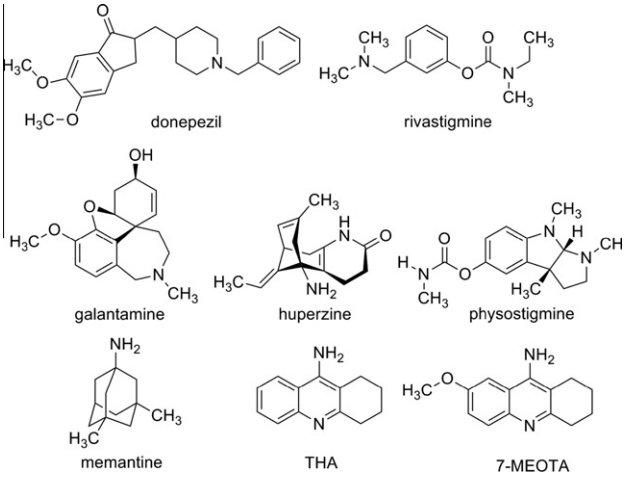

Figure 1. Structures of selected AChEIs and memantine.

* Corresponding author.
E-mail address: kamil.musilek@gmail.com (K. Musilek).

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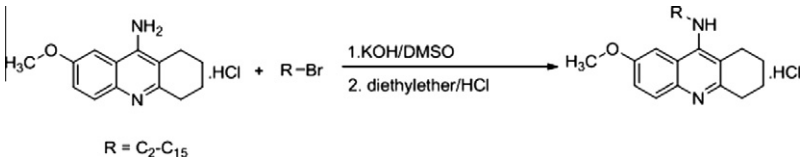

Scheme 1. Synthesis of *N*-alkyl-7-methoxy-1,2,3,4-tetrahydoacridinamine hydrochlorides.

The first AChEI drug approved by FDA for clinical use in 1993 was THA. However, the main issue associated with the use of THA was the toxicity elevation of liver enzymes in AD patients that was dose-dependent and reversible after reduction of dosage or THA withdrawal. The formation of toxic quinone-type metabolites by the hepatic oxidative metabolism was responsible for the hepatotoxicity of THA.[8–10] Even though, considerable amount of THA derivatives and its analogues was synthesized in order to find compounds with reduced side effects. This Letter is focused to analogues of THA, 7-methoxytacrine (7-MEOTA).[11,12] 7-MEOTA was formerly found to be less toxic and pharmacologically equally active to THA.

Herein, the synthesis, in vitro evaluation and molecular docking of fourteen new analogues of 7-MEOTA are reported. The 7-MEOTA (7.6 mmol) was alkylated in KOH/DMSO mixture via the addition of corresponding 1-bromoalkane (15.1 mmol). The oily residue from column chromatography was saturated by hydrochloride gas to give a solid product in 16–63% yield (Scheme 1; Supplementary data available).[13–17]

The inhibitory ability of the newly prepared compounds was evaluated in vitro on the model of human recombinant AChE (hAChE) and human plasmatic BChE (hBChE).[22] The selectivity index (SI) for hAChE was calculated and the novel compounds were compared to standards THA and 7-MEOTA (Table 1). Enzyme activity plots of standards and two selected promising compounds (**5**–**6**) are displayed (Figs. 2 and 3).

The standard compounds (THA, 7-MEOTA) showed inhibitory ability in μM scale. Surprisingly, THA resulted as a more potent inhibitor of hBChE than hAChE with two orders of magnitude selectivity. 7-MEOTA resulted as a weaker hAChE inhibitor compared to THA and did not show higher selectivity.

Several newly prepared compounds showed better inhibitory ability towards hAChE or hBChE compared to standards THA and 7-MEOTA. Three compounds (**5–7**) resulted as better inhibitors of hAChE compared to THA or 7-MEOTA. Particularly, compound **5** was found to be fivefold more potent as an inhibitor of hAChE compared to THA. Additionally, it demonstrated about two orders of magnitude higher inhibitory ability compared to its parent compound 7-MEOTA. On the other hand, some compounds (**9–10, 14**) were found ineffective for hAChE inhibition.

Subsequently, none prepared to be the compound that exhibited increasing inhibition towards hBChE, when compared to THA. Three compounds (**6–8**) were found to have promising inhibitory ability to hBChE on sub-μM scale. From these, compound **6** was found to be a slightly worse inhibitor compared to THA on nM scale and about three orders of magnitude more potent BChE inhibitor to 7-MEOTA. Similar to hAChE results, compounds (**9–14**) were found to be ineffective for hBChE inhibition.

Regarding the selectivity issues, compound **5** showed the best selectivity ratio for AChE among all the tested inhibitors. Additionally, compounds **11**–**13** were found truly AChE inhibitors, where compound **11** might be of potential AD concern with inhibitory

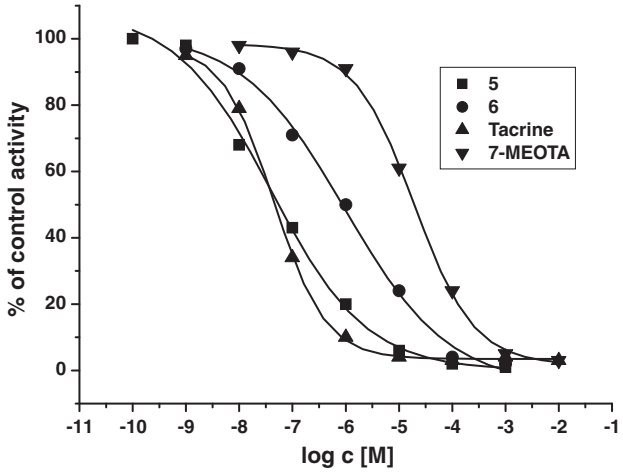

Figure 2. Enzyme activity plot of selected compounds for hAChE.

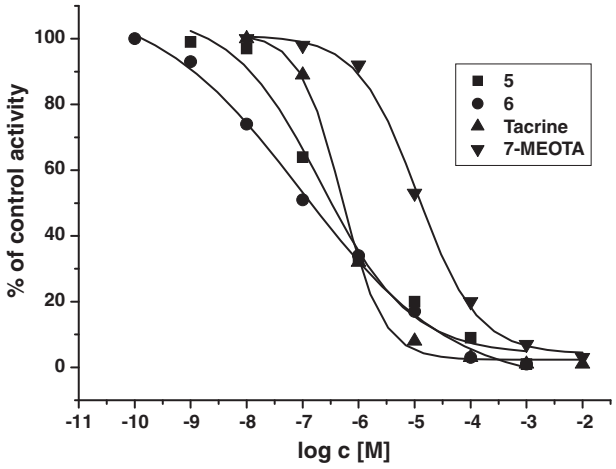

Figure 3. Enzyme activity plot of selected compounds for hBChE.

Table 1
IC_{50} values of tested compounds.

Compound	R	hAChE IC_{50} ± SD (μM)	hBChE IC_{50} ± SD (μM)	SI BChE/AChE
1	C_2	66 ± 13	97±16	1.5
2	C_3	9.2 ± 1.8	70 ± 11	7.6
3	C_4	21 ± 4	64 ± 10	3.0
4	C_5	6.7 ± 1.3	16 ± 3	2.4
5	C_6	0.10 ± 0.02	1.0 ± 0.2	10
6	C_7	0.27 ± 0.05	0.040 ± 0.007	0.15
7	C_8	0.36 ± 0.07	0.12 ± 0.02	0.33
8	C_9	1.6 ± 0.3	0.36 ± 0.06	0.23
9	C_{10}	[a]	[a]	—
10	C_{11}	[a]	[a]	—
11	C_{12}	1.0 ± 0.2	[a]	—
12	C_{13}	100 ± 20	[a]	—
13	C_{14}	46 ± 9	[a]	—
14	C_{15}	[a]	[a]	—
THA		0.5 ± 0.1	0.023 ± 0.003	0.05
7-MEOTA		15 ± 2	21 ± 3	1.4

[a] No significant inhibition in the selected concentration scale.

ability on μM scale. However, the dual hAChE and BChE inhibitors were formerly highlighted to replace selective AChE inhibitors.[23] From this point of view, compound **5** with only 10-fold selectivity, fivefold better inhibitory ability to THA and two orders of magnitude better inhibitory ability to 7-MEOTA may be the matter of further concern.

Importantly, structure–activity relationship (SAR) should be considered. The introduction of 7-methoxy moiety into THA molecule led to be decrease of inhibitory ability, but decreased the toxicity of THA to tolerable limits.[11] Further alkylation of aromatic amino moiety of 7-MEOTA showed interesting results. Whereas the C_2–C_4 and C_{10}–C_{15} alkyl chains were found less effective or almost ineffective cholinesterase inhibitors, compounds bearing C_5–C_9 chains showed results better than their parent compound (7-MEOTA). Among them, compound **5** with hexyl chain was found to be the best inhibitor of hAChE. Compound **6** with heptyl chain resulted as the best inhibitor of hBChE, but worse to THA. Besides C_5–C_9 chains and interestingly, compound **11** with dodecyl alkyl chain showed selective AChE inhibition on μM scale (no inhibition of hBChE) that need not be an advantage concerning other published results.[23]

These structural findings are connected to the accommodation of tested compounds in the AChE or BChE active gorge. Broadening the SAR findings, the docking studies were performed with compound **5** on the model of *Mus musculus* AChE.[18–21] The docking simulations found two higher-populated clusters. The cluster with the lowest energy showed that the 7-MEOTA moiety was bound at the rim of the active gorge between Trp86 and Tyr337 by π–π stacking (Figs. 4 and 5) in the peripheral anionic (aromatic) site. A nitrogen from the amino moiety formed hydrogen bonds with two water molecules. An oxygen from the methoxy moiety formed hydrogen bonds with two water molecules and Glu202. The C_6-chain was oriented inwards the AChE gorge. This may be the plausible explanation of inhibitory ability towards cholinesterases. While the short (C_2–C_4) and long (C_{10}–C_{15}) alkyl chains probably cannot fit the limited cholinesterase gorge space, the C_5–C_9 alkyl chains can spatially accommodate inside and block the cholinesterase native function. The exception of C_{12}–C_{14} chains for hAChE cannot be explained this way and authors supposed other binding of compounds **11**–**13** at the rim of AChE gorge, where the important aromatic amino acid residues in hBChE are missing.

In summary, this Letter describes fourteen novel analogues of 7-MEOTA. These compounds were designed, synthesized and tested in vitro on the source of human cholinesterases. Three novel compounds showed promising results towards hAChE better to THA or 7-MEOTA. Three compounds resulted as potent inhibitors of hBChE. The SAR findings highlighted the C_6–C_7 *N*-alkyl chains for cholinesterase inhibition.

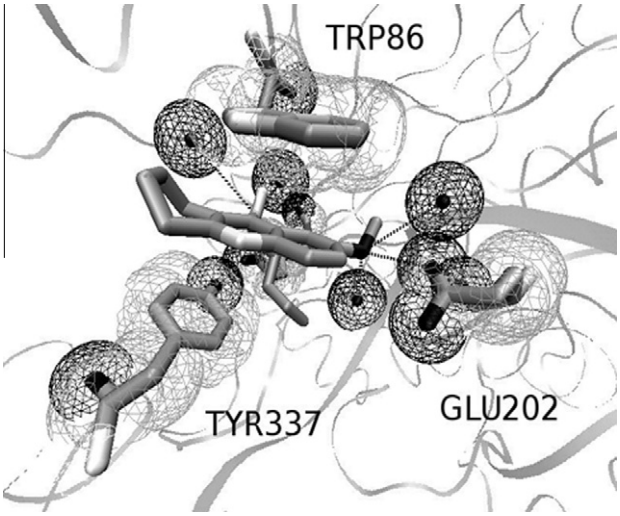

Figure 4. The most populated cluster of compound **5** in the active centre of mAChE.

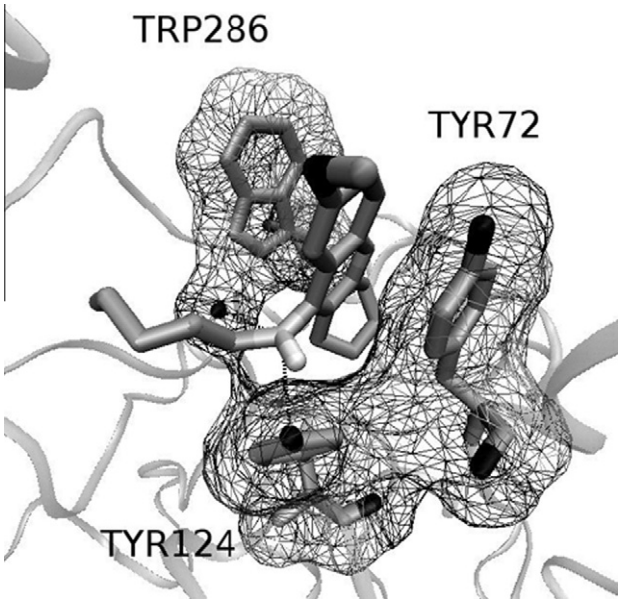

Figure 5. Interaction of compound **5** with Trp286 and Tyr72 by π–π stacking.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.044.

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