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Comparison of Novel Tacrine and 7-MEOTA Derivatives with Aromatic and Alicyclic Residues: Synthesis, Biological Evaluation and Docking Studies

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Abstract: Cholinesterase inhibitors play an essential role in the treatment of Alzheimer's disease. Since their first introduction over a decade ago, they are an indispensable part of Alzheimer's disease therapy and remain at the forefront of scientific interest worldwide. In this manuscript new analogs of THA and 7-MEOTA were designed, synthesized, and evaluated for their inhibitory activity against both acetylcholinesterase and butyrylcholinesterase. Cholinergic properties were investigated and quantified with respect to their side chain residues (aromatic or alicyclic). All synthesized compounds proved to have potent inhibitory activity at micromolar range. Moreover, compound **4** demonstrated promising efficacy and appears to be an ideal candidate for further testing.

Keywords: 7-methoxytacrine, acetylcholinesterase, Alzheimer`s disease, butyrylcholinesterase, inhibition selectivity, molecular modelling.

INTRODUCTION

Alzheimer`s disease (AD) is a multifactorial impairment and one of the most common forms of neurodegenerative illnesses affecting approximately 10% of the population over the age of 65 years and nearly 50% of those older than 85 years of age. At the moment, two major therapeutic strategies have been developed for the treatment of AD. The first strategy is inspired by the amyloid hypothesis. It basically describes with the aggregation of monomeric peptide (Aβ) into larger oligomeric and fibrillar forms. Aggregation of β-amyloid is seen as a significant step in the AD pathology and cognitive loss. Thereby treatment focuses on prevention of β-amyloid formation and subsequent deposition in the form of plaques. Certain evidence suggests that this can be an early and obligatory event in the pathogenesis of AD [1, 2].

Based on the second strategy called "cholinergic hypothesis", many attempts have been made to reverse cognitive deficits by increasing brain cholinergic activity through the use of cholinomimetics such as cholinesterase inhibitors (ChEIs), acetylcholine (ACh) precursors, and direct cholinergic agonists. Although the decline of cholinergic neurotransmission is most probably a downstream process of AD pathophysiology until now the only therapeutic intervention uses (apart from the N-methyl-D-aspartate (NMDA) antagonist memantine; (Fig. **1**) acetylcholinesterase inhibitors (AChEIs) as the best clinically relevant approach for stabilizing memory and cognitive decline in AD patients [3, 4]. This assumption led to the introduction of 9-amino-1,2,3,4-tetrahydroacridine (tacrine, THA; trade name Cognex®, USP Sciele Pharm Inc) as the first AChEI specifically approved for the treatment of AD in 1993 [5]. THA approval was followed by other AChEIs e.g. donepezil (1997; trade name Aricept®, Eisai Company and Pfizer Inc.), rivastigmine (2000; trade name Exelon®, Novartis Pharmaceuticals) and galantamine (2001; Hoechst Marion Roussel Inc., Shire Pharmaceutical Group, and Janssen Pharmaceutical, trade names Reminyl® and Nivalin®, U.S. trade name Razadyne®) (Fig. **1**).

Focused on THA, large number of patients withdrew during the trials with this drug, many because of THA-

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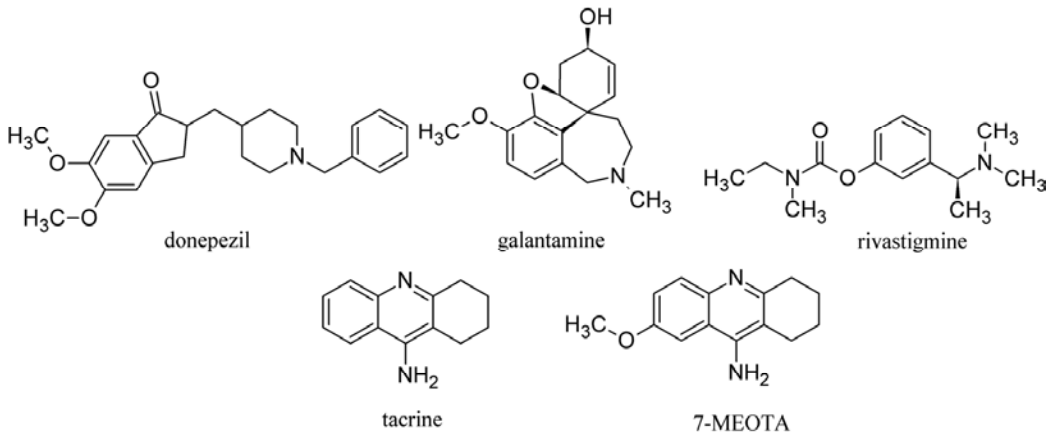
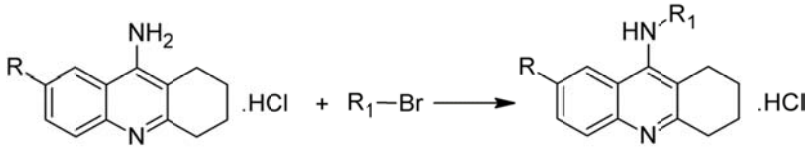


Fig. (1). Structure of cholinesterase inhibitors used and tested for AD treatment.



Scheme 1. Method for the synthesis of **1**-**4**. R = H (**1**, **2**);-O-CH_3 (**3**, **4**); R_1 = *N*-(3-phenylpropyl) (**1**, **3**); *N*-(cyklohexylmethyl) (**2**, **4**); Reagents and conditions: KOH/DMSO/48h; room temperature.

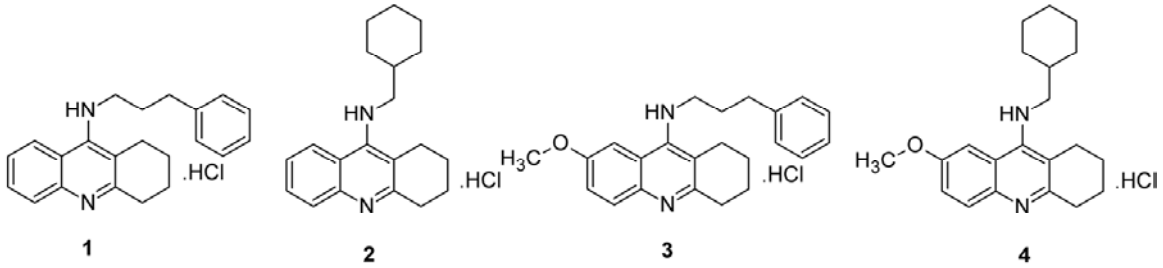


Fig. (2). Structures of newly prepared THA and 7-MEOTA analogues **1**-**4**.

associated increases in transaminase levels that in some cases reached clinical significance. Liver toxicity could be explained by the formation of toxic quinone-type metabolites by the hepatic oxidative metabolism [6-8]. Besides that, THA has a higher incidence of gastrointestinal side effects than the other marketed agents and requires four-times-daily dosing. These side effects and the availability of other less toxic molecules today limit its usefulness in the treatment of AD [9].

We turned our attention to 9-amino-7-methoxy-1,2,3,4-tetrahydroacridine (7-MEOTA) that was formerly found as a less toxic THA derivate (Fig. **1**) [10-11]. 7-MEOTA exhibits inhibitory ability to both cholinesterases, human acetylcholinesterase (*h*AChE; E.C. 3.1.1.7) and human butyrylcholinesterase (*h*BChE; E.C. 3.1.1.8). Normally in the brain of a healthy person *h*AChE is predominant. However, in AD brain *h*BChE activity rises while *h*AChE activity remains unchanged or diminished. Therefore, a drug inhibiting both *h*AChE and *h*BChE like 7-MEOTA and its derivatives could have additive and potential therapeutic benefits in the regimen against AD.

Within this work we report the synthesis, biological evaluation and molecular docking studies of two new analogs of 7-MEOTA and compared them with two new derivatives of THA (Scheme **1**). The main aspect of this study was to investigate the influence of different substituents (aromatic and alicyclic) on THA or 7-MEOTA pharmacophore and their inhibitory activities towards both cholinesterases (Fig. **2**). Subsequently, we studied the interaction of **1**-**4** with *h*AChE and *h*BChE to rationalize findings from *in vitro* experiments and deduced best conformation and orientation of prepared compounds.

MATERIALS AND METHODS

7-MEOTA was prepared at Faculty of Military Health Sciences via earlier described method [12]. Other reagents were obtained from Sigma-Aldrich in reagent grade quality. All experiments were carried out under nitrogen atmosphere. Thin layer chromatography (TLC) was performed on aluminium sheets with pre-coated silica gel 60 F_{254} (Merck). Column chromatography was performed at normal pressure on silica gel 100 (particle size 0.063-0.200 mm, 70-230 mesh ASTM, Fluka). Elemental analysis was measured at Perkin-Elmer CHN Analyzer 2400 Series II apparatus. Mass spectra were recorded using combination of high performance liquid chromatography and mass spectrometry. HP1100 HPLC system was obtained from Agilent Technologies (Wald-

bronn, Germany). It consisted of vacuum degasser G1322A, quaternary pump G1311A, autosampler G1313A and quadrupole mass spectrometer MSD1456 VL equipped with electrospray ionization source. Nitrogen for mass spectrometer was supplied by Whatman 75-720 nitrogen generator. Data were collected in positive ion mode with an ESI probe voltage of 4000 V. The pressure of nebulizer gas was set up to 35 psig. Drying gas temperature was operated at 335 °C and flow at 13 L/min. ^{1}H NMR and ^{13}C NMR spectra were recorded with spectrometer Varian Mercury VX BB 300 operating at 300 and 75 MHz, respectively, using tetramethylsilane (TMS) as reference. Chemical shifts are reported in parts per milion (ppm, δ) relative to TMS. Melting points were measured on micro heating stage PHMK 05 (VEB Kombinant Nagema, Radebeul, Germany) and they were uncorrected.

General Procedure for the Synthesis of the THA and 7-MEOTA Derivatives

Powdered potassium hydroxide (1.27 g, 22.6 mmol) was added to a solution of 7-MEOTA (2.00 g, 7.6 mmol) or THA (2.00 g, 8.5 mmol) in DMSO (30 mL) under nitrogen atmosphere. The mixture was vigorously stirred at room temperature for 2 hours. Subsequently, (3-bromopropyl)benzene (15 mmol) or (bromomethyl)cyclohexane (15 mmol) was added dropwise and the stirring was continued for further 48 hours at the room temperature under nitrogen atmosphere. The end of reaction was determined with triethylamine-saturated TLC, mobile phase hexane/ethyl-acetate (1:1). When the reaction was finished, water (100 mL) was poured into reaction mixture and the water phase was extracted with ethyl-acetate (3×100 mL). The combined ethyl-acetate extracts were dried over anhydrous sodium sulphate, filtered and the solvent was evaporated under reduced pressure. Evaporation of the solvent gave an oily residue that was purified by triethylamine-pretreated silica gel via column chromatography. The mobile phase hexane/ethyl-acetate (1:1) was used for purification. The pure product was isolated as brown crystals [13-16].

N-3-(Phenylpropyl)-1,2,3,4-Tetrahydroacridin-9-Amoniumchloride (1)

Yield: 59.0 %; m.p. 195.6 – 196.8 °C; ^{1}H NMR spectrum (300 MHz, methanol-d_4) δ (ppm): 1.87-1.94 (m, 4H), 2.11-2.20 (m, 2H), 2.54 (t, *J* = 5.87 Hz, 2H), 2.74 (t, *J* = 7.34 Hz, 2H), 2.97 (t, *J* = 6.36 Hz, 2H), 3.93 (t, *J* = 6.85 Hz, 2H), 7.12-7.25 (m, 5H), 7.44 (ddd, *J* = 1.22, 6.85, 8.80 Hz, 1H), 7.74 (dd, *J* = 1.47, 8.56 Hz, 1H), 7.79 (ddd, *J* = 1.22, 6.84, 8.31 Hz, 1H), 8.18 (d, *J* = 8.80 Hz, 1H); ^{13}C NMR spectrum (75 MHz, methanol-d_4) δ (ppm): 21.77, 22.91, 24.76, 29.23, 32.81, 33.90, 48.39, 112.74, 116.84, 120.00, 126.17, 126.37, 127.18, 129.54, 129.55, 133.97, 139.66, 142.14, 151.44, 157.85; ESI-MS: m/z 317.0 $[M]^+$ (calculated for: $[C_{22}H_{25}N_2]^+$ 317.2); EA: calculated 74.88 % C, 7.14 % H, 7.94 % N; found 74.56 % C, 7.34 % H, 7.85 % N logP = 5.49 ± 0.72 (calc. value) [17].

N-(Cyklohexylmethyl)-1,2,3,4-Tetrahydroacridin-9-Amoniumchloride (2)

Yield: 48.1 %; m.p. 204.0 – 206.0 °C; ^{1}H NMR spectrum (300 MHz, methanol-d_4) δ (ppm): 0.93-1.36 (m, 5H), 1.62-1.88 (m, 6H), 1.90-2.00 (m, 4H), 2.71 (t, *J* = 5.91 Hz, 2H), 3.02 (t, *J* = 4.94 Hz, 2H), 3.79 (d, *J* =6.59 Hz, 2H), 7.58 (ddd, *J* = 1.65, 6.73, 8.51 Hz, 1H), 7.77 (dd, *J* = 1.51, 8.38 Hz, 1H), 7.84 (ddd, *J* = 1.10, 6.73, 8.52 Hz, 1H), 8.39 (d, *J* = 8.24 Hz, 1H); ^{13}C NMR spectrum (75 MHz, methanol-d_4) δ (ppm): 21.82, 22.99, 25.02, 26.85, 27.38, 29.31, 31.77, 40.05, 55.29, 112.94, 117.17, 120.11, 126.35, 126.47, 134.09, 139.73, 151.78, 158.14 ESI-MS: m/z 295.0 $[M]^+$ (calculated for: $[C_{20}H_{27}N_2]^+$ 294.21); EA: calculated 72.60 % C, 8.22 % H, 8.47 % N; found 72.91 % C, 8.25 % H, 8.61 % N logP = 5.46 ± 0.85 (calc. value) [17].

7-methoxy-N-(3-phenylpropyl)-1,2,3,4-tetrahydroacridin-9-amoniumchloride (3)

Yield: 12.8 %; m.p. 189.3 – 191.1 °C; ^{1}H NMR spectrum (300 MHz, methanol-d_4) δ (ppm): 1.81-1.95 (m, 4H), 2.07-2.20 (m, 2H), 2.58 (t, *J* = 5.91 Hz, 2H), 2.73 (t, *J* = 7.42 Hz, 2H), 2.98 (t, *J* = 6.18 Hz, 2H), 3.90 (s, 3H), 3.91 (t, *J* = 7.42 Hz, 2H), 7.09-7.17 (m, 5H), 7.47 (dd, *J* = 2.61, 9.20 Hz, 1H), 7.57 (d, *J* = 2.47 Hz, 1H), 7.69 (d, *J* = 9.20 Hz, 1H); ^{13}C NMR spectrum (75 MHz, methanol-d_4) δ (ppm): 21.82, 23.10, 25.52, 29.19, 33.37, 33.98, 56.51, 104.71, 112.86, 118.62, 121.68, 125.51, 127.16, 129.47, 129.53, 134.44, 142.22, 150.60, 157.16, 158.55; ESI-MS: m/z 346.0 $[M]^+$ (calculated for: $[C_{23}H_{27}N_2O]^+$ 346.2); EA: calculated 72.14 % C, 7.11 % H, 7.32 % N; found 72.10 % C, 7.25 % H, 7.28 % N logP = 5.42 ± 0.83 (calc. value) [17].

7-methoxy-N-(cyklohexylmethyl)-1,2,3,4-tetrahydroacridin-9-amoniumchloride (4)

Yield: 6.5 %; m.p. 112.7 – 114.2 °C; ^{1}H NMR spectrum (300 MHz, methanol-d_4) δ (ppm): 0.95-1.38 (m, 5H), 1.52-1.87 (m, 6H), 2.72 (t, *J* = 5.27 Hz, 2H), 3.03 (t, *J* = 5.27 Hz, 2H), 3.22 (m, 2H), 3.90 (s, 3H), 7.20-7.26 (m, 2H), 7.81 (d, *J* = 9.96 Hz, 1H); ^{13}C NMR spectrum (75 MHz, methanol-d_4) δ (ppm): 22.28, 23.09, 24.69, 25.93, 26.44, 31.18, 33.90, 39.88, 55.42, 55.66, 101.69, 117.13, 120.17, 121.22, 130.34, 143.56, 149.99, 156.23, 157.77; ESI-MS: m/z 324.0 $[M]^+$ (calculated for: $[C_{21}H_{29}N_2O]^+$ 324.22); EA: calculated 77.74 % C, 8.70 % H, 8.63 % N; found 77.79 % C, 8.80 % H, 8.55 % N logP = 5.38 ± 0.95 (calc. value)[17].

BIOLOGICAL ACTIVITY

Multichannel spectrophotometer Sunrise (Tecan, Salzburg, Austria) was used for all measurements of cholinesterases activity. Previously optimized Ellman's procedure was slightly adopted in order to estimate anticholinergic properties [18]. 96-wells photometric microplates made from polystyrene (Nunc, Rockilde, Denmark) were used for measuring purposes. Human recombinant AChE or human plasmatic BChE (Aldrich; commercially purified by affinity chromatography) were suspended into phosphate buffer (pH 7.4) up to final activity 0.002 U/µl. Cholinesterase (5 µl), freshly mixed solution of 0.4 mg/ml 5,5´-dithio-bis(2-

Table 1. IC50 Values of Tested Compounds (1-4) and Standards (THA, 7-MEOTA) and Calculated Selectivity Index for hAChE.

Compound	*h*AChE IC_{50} ± SD (μM)	*h*BChE IC_{50} ± SD (μM)	SI[a]
1	0.19±0.04	0.06±0.01	0.31
2	1.09±0.21	0.12±0.02	0.11
3	3.21±0.63	0.17±0.03	0.05
4	1.13±0.22	3.34±0.56	2.96
THA	0.50 ± 0.10	0.023 ± 0.003	0.05
7-MEOTA	10.50 ± 2.0	21.0 ± 3.0	2.0

[a]Selectivity for hAChE is defined as IC50 (hBChE)/IC50 (hAChE).

nitrobenzoic) acid (40 μl), 1 mM acetylthiocholine chloride in phosphate buffer (20 μl) and appropriate inhibitor concentration (1 mM-0.1 nM; 5 μl) were injected per well. Absorbance was measured at 412 nm after 5 minutes incubation using automatic shaking of the microplate.

The obtained data were used to compute percentage of inhibition (I; Equation 1):

$$I = 1 - \frac{\Delta A_i}{\Delta A_0} \quad [\%] \qquad \text{(Eq. 1)}$$

ΔA_i indicates absorbance change provided by cholinesterase exposed to *h*AChE inhibitors and ΔA_0 indicates absorbance change caused by intact cholinesterase (phosphate buffer was applied instead of *h*AChE inhibitor). IC_{50} was calculated using Origin 6.1 (Northampton, MA, USA). Percentage of inhibition for the given anticholinergic compound was overlaid by proper curve chosen according to optimal correlation coefficient. IC_{50} as well as upper limit of inhibition (maximal inhibition provided by given compound) was computed.

MOLECULAR MODELING

Molecular models of the derivatives **1, 3** and **4** were computer-built by the means of building options in the Marvin 5.1.4 2008, ChemAxon [19]. The same software was used to determine the overall protonization of the compounds. Docking simulations were carried out using AUTODOCK ver. 4.2. MGL TOOLS 1.4.5 (revision 30) was used to prepare the input files [20-21]. Molecules of water with other nonenzymatic molecules were removed and hydrogens were added. The united atom representation for the ligands and enzyme were used. Gasteiger partial atomic charges for protein and the ligands were added. The grid for energy was set in the coordinates x=116.4, y=104.3, z=-130.6 within the *h*AChE (PDB ID: 1B41) active site and x= 138.7, y= 116.3, z=41.0 within the *h*BChE (PDB ID: 1P01) with dimensions 80 points x 80 points x 80 points and with spacing 0.375 Å. Autotors was used to define the rotatable bonds in the ligands. Flexible ligand docking was performed for the compounds. Docking runs were performed using the Lamarckian genetic algorithm. Docking began with a population of random ligand conformations in random orientation and at random translation. Each docking experiment was derived from 50 different runs that were set to terminate after a maximum of 5 000 000 energy evaluations or 27 000 generations. The population size was set to 500. The other parameters were used as default. Pictures were prepared using Chimera software [22]. A simple method for displaying the hydropathic character of a protein were used in (Figs. **6** and **8**) [23].

RESULTS AND DISCUSSION

The synthesized compounds **1**-**4** were all tested for their ability to inhibit cholinesterases isolated from human source (*h*AChE, *h*BChE). Results and selectivity index values for *h*AChE (SI) are presented in (Table **1**). THA and 7-MEOTA were used as standards for comparison. Enzyme activity plots of standards and two selected promising compounds (with respect to IC_{50} value) of THA and 7-MEOTA hybrids are displayed (Figs. **3** and **4**). Standard compounds THA and 7-MEOTA showed inhibitory ability in μM range. Surprisingly, THA was a more potent inhibitor of *h*BChE than *h*AChE with two orders of magnitude selectivity. 7-MEOTA was a weaker *h*AChE inhibitor compared to THA and did not show higher selectivity towards this enzyme. The most promising inhibitor of *h*AChE assayed was compound **1** with 2.5-fold more potent inhibition when compared to THA and 55-fold more potent inhibition than 7-MEOTA. Unfortunately, less toxic derivates of 7-MEOTA (**3**, **4)** did not exceed the capability of THA inhibition of *h*AChE. However, when compared to 7-MEOTA greater inhibition of *h*AChE was apparent. Subsequently, none of the prepared compounds exhibited higher *h*BChE inhibition when compared to THA. On the other hand, **1**-**4** were found more effective inhibitors of *h*BChE in comparison with 7-MEOTA. Additionally, derivate **1** really stands out when compared to 7-MEOTA with two orders of magnitude and three orders of magnitude higher inhibitory ability towards *h*AChE and *h*BChE respectively. Regarding the selectivity issues, compound **4** showed best selectivity ratio for hAChE among all tested inhibitors. Comparing THA and 7-MEOTA hybrids, the replacement of methoxy-group resulted in derivates with lower activity against both tested enzymes. Having said that, compound **4** as a derivate of less toxic 7-MEOTA has 3-fold higher selectivity index towards cholinesterases, 9-fold better inhibitory potency towards *h*AChE, and even a 6-fold increase in inhibition of *h*BChE relative to 7-MEOTA. More tests are needed to confirm these findings but it is evident that compound **4** should be in the centre of future research

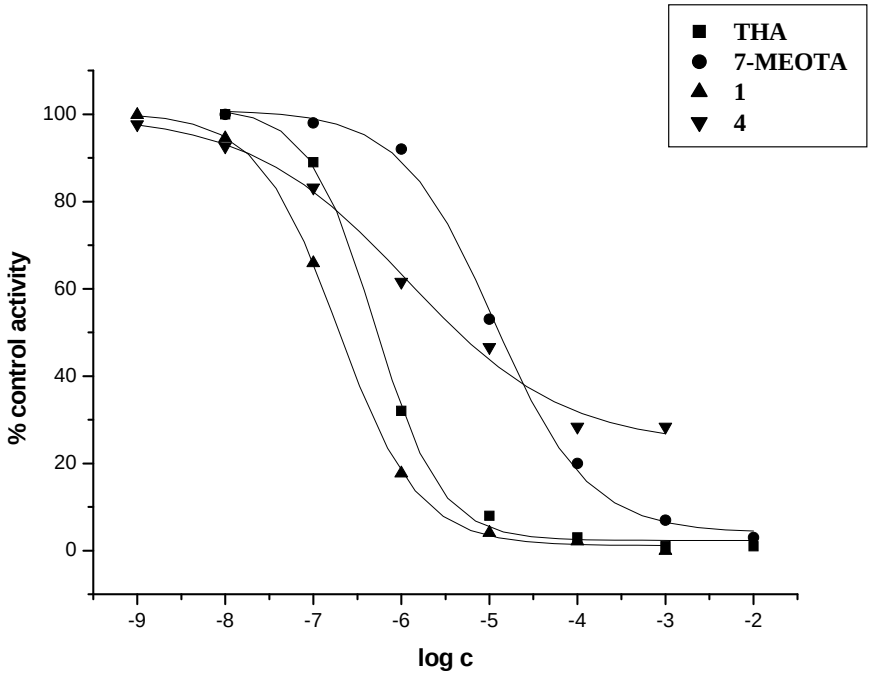


Fig. (3). Enzyme activity plots of tested compounds **1**, **4**, standards THA and 7-MEOTA for *h*AChE.

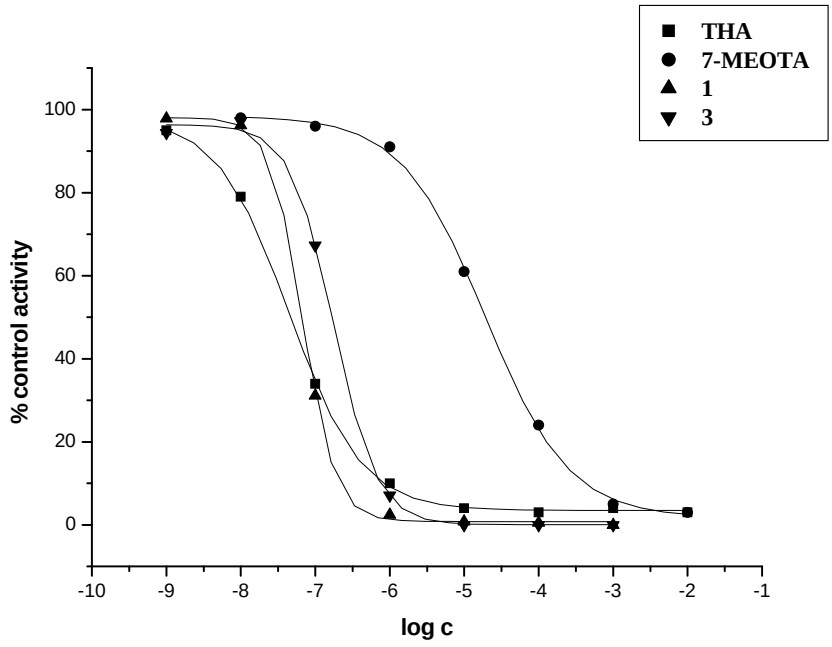


Fig. (4). Enzyme activity plots of tested compounds **1**, **3**, standards THA and 7-MEOTA for *h*BChE.

concentrating on AD. Further toxicity studies of best THA and 7-MEOTA derivatives will be carried out but at this stage it was out of our experimental scope.

Docking studies were carried out to provide better interpretation of the inhibition of two most active derivatives **1** and **4** towards the *h*AChE on the pertinent crystal structure (PDB ID: 1B41). Similar studies were performed for derivatives **1** and **3** within *h*BChE (PDB ID: 1P01).

The proposed binding mode of derivatives **1**, **4** is shown in (Figs. **5** and **6**). THA part of both derivatives **1** and **4** interacts through cation - π interactions with TRP86 in the catalytic site of the *h*AChE but does not interact with catalytic triad. Additionally, the complex of derivative **1** might be stabilized by hydrogen bonds formed between the protonized nitrogen of THA ring and C=O of TRP86 (2.454 Å) as well as NH group at the position nine of THA and OH of TYR337 (2.289 Å).

The different orientation possesses 7-MEOTA derivative **4** forming hydrogen bond 3.322 Å long between methoxy group and NH of ASP74. Additionally week hydrogen bonds could be found between NH of tacrine ring and oxygen

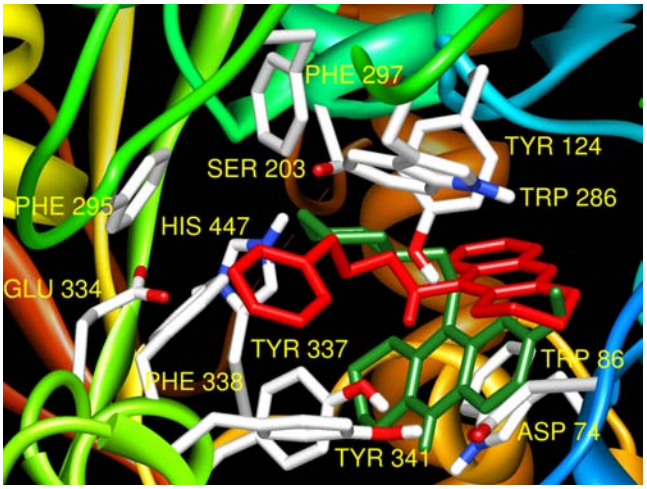


Fig. (5). Top-score docking poses for derivatives **1** (red) and **4** (green) depicted their putative structural orientations in the active-site gorge of the *h*AChE. Enzyme is drawn in a ribbon representation.

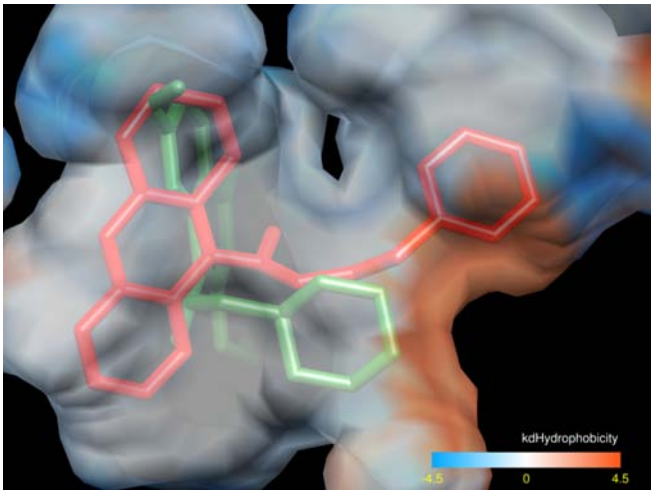


Fig. (6). Top-score docking poses for derivatives **1** (red) and **4** (green) depicted putative structural orientations in the active-site gorge of the *h*AChE. For clarity only enzyme active site is shown as a hydrophobic surface pocket [20].

atoms of TRP83. Putative orientation of derivatives **1** and **3** within *h*BChE catalytic cavity is shown at (Figs. **7** and **8**). There are figured opposite orientations of compounds **1** and **3** in the enzyme active side. Derivative **1** forms hydrogen bond between NH of tacrine ring and C=O of HIS438 (2.078 Å) with possible formation of π-π respectively π-charge interaction between tacrine moiety and TRP82. Compound **3** interacts within active site in the opposite direction, where oxygen atom of 7-MEOTA forms hydrogen bond with OH of TYR128 (2.568 Å). Additionally, tacrine core might interact via electrostatic non-bonded interactions with TRP82, THR120 and GLY121 amino acids residues that surround it. Similar docking studies were done with heterobivalent tacrine derivatives using enzyme *Torpedo californica* (PDB ID: 1ACJ) [24]. Unlike to our compounds, there were predicted putative interactions with amino acids

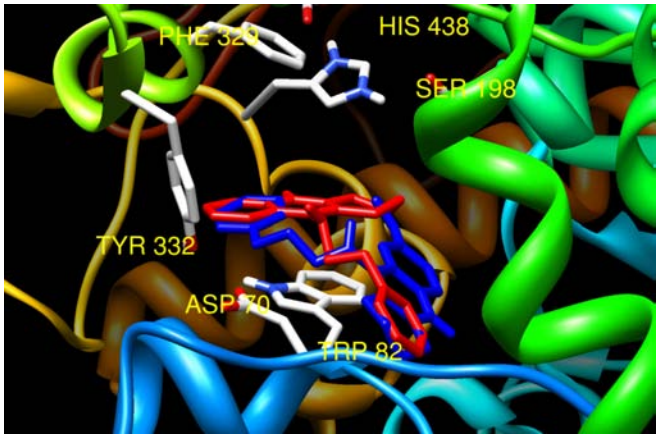


Fig. (7). Top-score docking poses for derivatives **1** (red) and **3** (blue) depicted their putative structural orientations in the active-site gorge of the *h*BChE. Enzyme is drawn in a ribbon style.

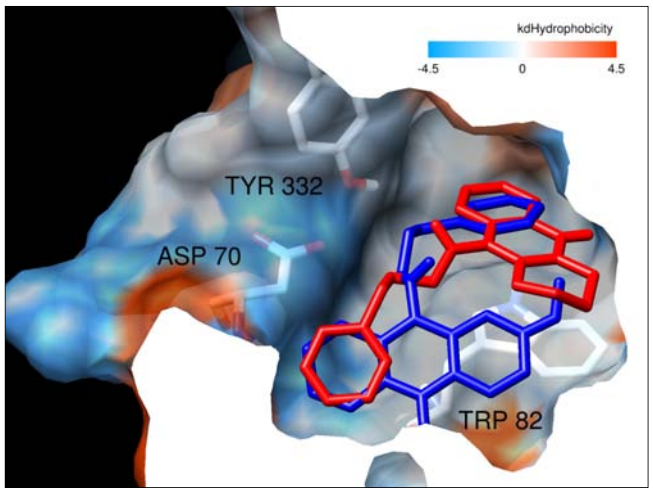


Fig. (8). Top-score docking poses for derivatives **1** (red) and **3** (blue) depicted putative structural orientations in the active-site gorge of the *h*BChE. For clarity the enzyme active site is shown as a cross-section of the hydrophobic surface pocket [20].

near catalytic triad. Addition extensive study could be done to obtain reasonable conclusion for structure-activity relationship of our structure set as it was in the case of tacrine and its derivatives within crystal structure of *Torpedo californica* [25].

CONCLUSION

In summary, this contribution describes two new analogs of 7-MEOTA and two THA derivates. All 7-MEOTA and THA hybrids proved to be promising. 7-MEOTA derivatives are considered to be most probably less toxic cholinesterase inhibitors (according to the low toxicity of the parent molecule 7-MEOTA). However, since no toxicity studies were conducted their therapeutic potential is still unknown. Further work will be aimed generally to confirm their potency as well as studying of other analogs with different functioning but preliminary results showed an immense potential.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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