MOLECULAR DOCKING STUDY ON 1-(3-(4-BENZYLPIPERAZIN-1-YL)PROPYL)-3,7-DIMETHYL-1H-PURINE-2,6(3H,7H)-DIONE AS AN ACETYLCHOLINESTERASE INHIBITOR

Maria Hristova[1], **Mariyana Atanasova**[2], **Iva Valkova**[3], **Lilya Andonova**[4], **Irini Doytchinova**[5], **Alexander Zlatkov**[6]

Abstract: Acetylcholinesterase (AChE) is a good target in the design of new drugs for the treatment of Alzheimer's disease. The currently known drugs -donepezil, galantamine and rivastignime- act as moderate AChE inhibitors. In the present study, we docked a newly synthesized arylpiperazine derivative 1-(3-(4-benzylpiperazin-1-yl)propyl)-3,7-dimethyl-1H-purine-2,6(3H,7H)-dione (LA1) into *rh*AChE and identified its binding mode. The docking pose of the studied LA1 molecule depends of the protonated state of the nitrogen atom of the piperazine moiety where in the best scored poses, the xanthine moiety of LA1 is bound into the catalytic active site (CAS) of AChE, while the arylpiperazine fragment is placed into the peripheral binding site (PAS). The Ellman's test confirmed the compound binding. LA1 has good permeability through the GIT and BBB assessed by PAMPA. LA1 is a prospective lead for AChE inhibition.

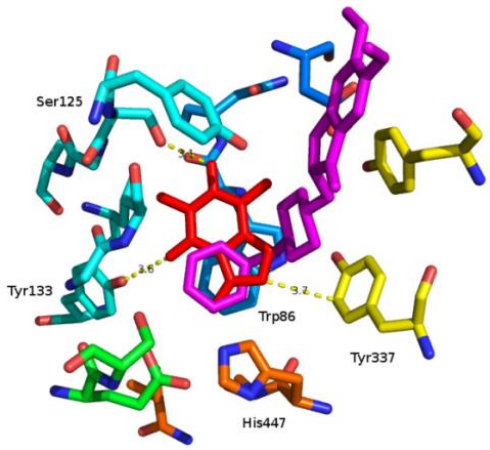
UDC Classification: 615.3; **DOI:** http://dx.doi.org/10.12955/cbup.v6.1268

Keywords: acetylcholinesterase, molecular docking, AChE inhibitors

Introduction

The biological role of acetylcholinesterase (AChE) is termination of impulse transmission at cholinergic synapses by hydrolysis of the neurotransmitter acetylcholine (Barnard, 1974). Many diseases are characterized by degeneration of the cholinergic system. Reversible AChE inhibitors are used in the treatment of various disorders such as Alzheimer's disease, myasthenia gravis, glaucoma, bladder distention, etc. The cognitive, behavioural and motor disabilities that characterize these pathologies are correlated to cholinergic circuit dysfunction (Tata et al., 2014). The most commonly used inhibitors include the natural product galantamine, the semisynthetic rivastigmine and the synthetic donepezil. Organophosphorus compounds act as irreversible AChE inhibitors (Čolović et al., 2013).

Figure 1: Binding of caffeine and donepezil to the active site of AChE



Source: Pohanka et al. (2013)

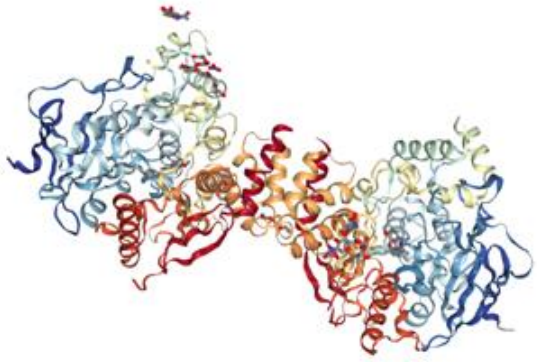
[1] Medical University - Sofia, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sofia, Bulgaria, rumenova_hristova@mail.bg
[2] Medical University - Sofia, Faculty of Pharmacy, Department of Chemistry, Sofia, Bulgaria, mariyanda@gmail.com
[3] Medical University - Sofia, Faculty of Pharmacy, Department of Chemistry, Sofia, Bulgaria, ivalkova@abv.bg
[4] Medical University - Sofia, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sofia, Bulgaria, lilyandonova@gmail.com
[5] Medical University - Sofia, Faculty of Pharmacy, Department of Chemistry, Sofia, Bulgaria, doytchinova@gmail.com
[6] Medical University - Sofia, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sofia, Bulgaria, alexbz2000@yahoo.com

The alkaloid caffeine and its derivatives are known to inhibit human AChE. This pharmacological activity of caffeine is partly responsible for its cognition enhancing properties. Caffeine, pentoxiphylline and propentophylline are less potent than donepezil. They all exhibit selective inhibition of hAChE with no inhibition of hBuChE (Mohamed et al., 2013).

Piperazine derivatives have also been investigated as AChE inhibitors. Several groups such as thiazole (Yurttaş et al., 2013), benzothiazole (Özkay et al., 2016) and coumarin (Modh et al., 2013) derivatives have shown AChE inhibitory activity.

Recombinant human AChE is a dimer. The active site is a deep and narrow gorge, ~ 20 Å long which penetrates halfway into the enzyme and widens out close to its base (Figure 2).

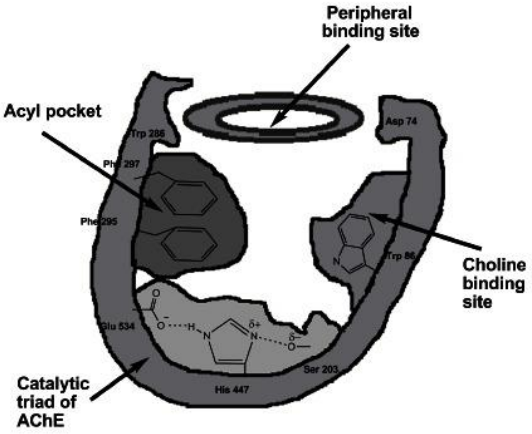
Figure 2: Crystallographic structure of recombinant human AChE



Source: Rose et al.(2016)

The active site of recombinant human AChE contains two subsites - esteratic and anionic. The anionic subsite binds the positive quaternary amine of choline moiety of ACh, as well as quaternary ligands. The cationic substrates are not bound by a negatively-charged amino acid in the anionic site, but by the interaction of 14 aromatic residues that line the gorge leading to the active site. Among the aromatic amino acids, tryptophan 86 is critical and its substitution with alanine causes a 3000-fold decrease in the enzyme activity. The esteratic subsite, where ACh is hydrolysed to acetate and choline, contains the catalytic triad of three amino acids: serine 202, histidine 447 and glutamate 332 (Dvir et al., 2010). In addition to the two subsites of the catalytic center, AChE was shown to possess a peripheral anionic site (PAS) composed of five aromatic residues. PAS allosterically modulates catalysis and is implicated in non-cholinergic functions as amyloid deposition, cell adhesion and neurite outgrowth (Kitz et al., 1970) (Figure 3).

Figure 3: Active site of recombinant human AChE



Source: Čolovićet al. (2013)

Molecular docking is an important tool used in drug discovery because it can model the interaction between small molecules and proteins and can help in lead optimization. The aim of molecular docking is to give a prediction of the ligand-receptor complex structure using computation methods.

Its main application lies in structure-based virtual screening for identification of new active compounds towards a particular target protein (Meng et al., 2011).

The aim of our study is to identify by molecular docking the possible binding conformations of a newly synthesized 1-(3-(4-benzylpiperazin-1-yl)propyl)-3,7-dimethyl-1H-purine-2,6(3H,7H)-dione bound with rhAChE.

Materials and methods

Synthesis:

The evaluated structure was obtained according to a synthetic procedure described in Andonova et al., 2014.

BBT and GIT permeability:

The BBB and GIT permeabilities were measured by a PAMPA Permeability Analyzer (pION Inc.). The settings for BBB were analysed at a wavelength of 250–500 nm, pH 7.4, temperature 25°C, permeation time 4 hr, lipid formulation BBB-1, protocol Iso-pH, no stirring. The settings for GIT were analysed at a wavelength of 250–500 nm, pH 5.0, 6.2 and 7.4, temperature 25°C, permeation time 4 hr, lipid formulation GIT-0, protocol 3-pH, stirring 60 rpm. The permeability was presented as pPe (-logPe), where Pe is the permeability coefficient (10^{-6} cm/s) obtained as an average of 3 parallel measurements. Compounds with pPe< 5 are considered highly permeable, with pPe between 5 and 6 – are considered medium permeable and with pPe> 6 – as low permeable. Theophylline and progesterone were used as negative and positive controls, respectively (Instruction Manual, PAMPA, 2016).

Docking protocol:

The LA1 molecule was modelled in MOE (Molecular Operating Environment, 2018) and was docked into the X-ray structure of human recombinant acetylcholinesterase (*rh*AChE, pdb id: 4EY6, R = 2.15 Å) (Cheung, 2013). The docking simulations were performed by GOLD v. 5.2. (CCDC Ltd., Cambridge, UK) using a protocol previously optimized in terms of scoring function, rigid/flexible ligand and binding site, radius of the binding site, presence/absence of a water molecule (HOH846) within the binding site, number of genetic algorithm (GA) runs (Atanasova et al., 2015a, Atanasova et al., 2015b, Stavrakov et al., 2016, Stavrakov et al., 2017). The docking simulations in the present study were performed at the following settings: scoring function ChemPLP, flexible ligand, rigid protein, radius of the binding site 6Å, no water molecule, 100 GA runs. According to stochastic genetic algorithm search approach implemented in GOLD five independent docking calculations were conducted and a solution with highest the ChemPLP score was selected. AChE inhibitors galantamine and donepezil were used as reference molecules.

In vitro acetylcholinesterase activity:

The *in vitro* acetylcholinesterase activity was evaluated by Ellman's method (Ellman et.al, 1961). The assay is based on the measurement of the change in absorbance at 405 nm. The assay uses thiol ester acetylthiocholine instead of oxy ester acetylcholine. AChE hydrolyses the acetylthiocholine to produce thiocholine and acetate. The thiocholine in turn reduces the dithiobisnitrobenzoic acid liberating nitrobenzoate, which absorbs at 405 nm. In a 96 well microtitre plate reader are placed 15 μL AChE solution, 15 μL of the examined compound in phosphate buffer with pH 7.6 and DTNB. The solutions are incubated for 30 minutes at room temperature and then 15 μL of acetylthiocholine is added. The absorbance at 405 nm is measured. The enzyme activity is determined as percent (%) inhibition compared to solution without inhibitor.

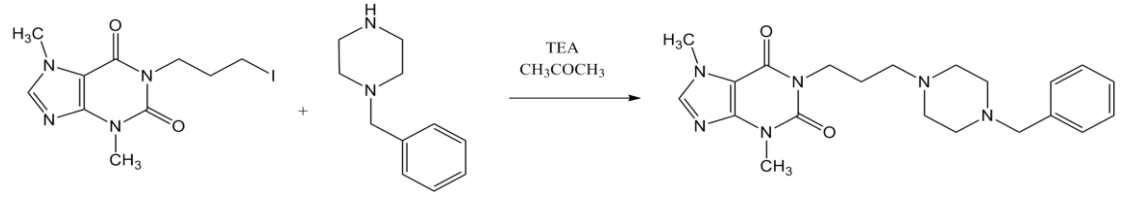
Results and discussion

The evaluated structure was obtained according to a synthetic procedure (Andonova et al., 2014) presented in Figure 4.

In an attempt to obtain preliminary information on some physicochemical properties of the targeted structure, the LA1 molecule was theoretically evaluated by some of the parameters of Lipinski's Rule of Five including molecular weight, miLogP, number of hydrogen bong donators (nOHNH) and hydrogen bond acceptors (nON) calculated by Molinspiration cheminformatics. The method is found to be applied in a high variety of publications as a suitable method for the preliminary identification of

appropriate pharmacologically active structures (Molinspiration Cheminformatics, 2017). The obtained values are presented in Table 1.

Figure 4: Schematic representation of the synthetic procedure



Source: Andonova et al. (2014)

Table 1: Lipinski's Rule of Five parameters

Compound ID	Mw	miLogP	nON	n(OHNH)
LA1	396.50	1.653	8	0

Source: Author

The calculated miLogP by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors was used by us as preliminary information on the permeability of the analysed structure through the blood brain barrier and GIT membranes. Good permeability was confirmed with a PAMPA tests. The results are presented in Table 2:

Table 2: BBB and GIT permeability of the tested compounds measured by PAMPA test. Compounds with p*P*e< 5 are considered as highly permeable, these with p*P*e between 5 and 6 – as medium permeable and these with p*P*e> 6 – aslow permeable

ID	BBB pH 7.4 Pe ± SD 10^{-6}cm/s pPe	GIT pH 5.0Pe ± SD 10^{-6}cm/s pPe	GIT pH 6.2 Pe ± SD 10^{-6}cm/s pPe	GIT pH 7.4 Pe ± SD 10^{-6}cm/s pPe
LA1	14.823 ± 1.965 4.829	10.275 ± 5.775 4.988	32.384 ± 12.156 4.490	57.356 ± 3.629 4.241

Source: Author

The pKa value of 7.06 calculated for the basic nitrogen atom of piperazine ring of LA1 molecule using ACD Labs Software (ACD Inc.) indicates that both protonated and non-protonated forms exist in almost equal ratio at physiological pH. The highest docking score from 5 independent calculations for both forms of LA1, galantamine and donepezil are given in Table3.

Table 3: The highest ChemPLP scores for studied compounds are given.

Compounds	ChemPLP score
LA1	93.16
LA1 protonated	91.93
Galantamine	72.67
Donepezil	85.07

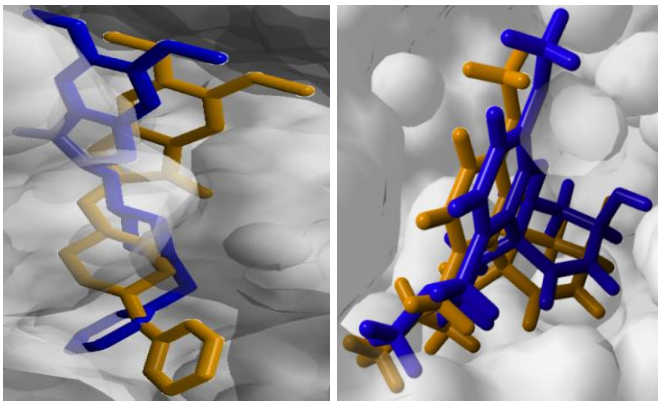
Source: Author

The docking poses of AChE inhibitors donepezil and galantamine superposed with corresponding crystallographic data (pdb codes: 4EY7 and 4EY6) are shown on Figure 5.

The docking pose of the studied LA1 molecule depends of the protonated state of the nitrogen atom of piperazine moiety (Figure 6). The xantine moiety of the non-protonated form takes place deep into the CAT centre and the benzyl residue binds in the PAS. The xantine moiety forms π-π stacking with Trp86 and a hydrogen bond with Ser125. Another hydrogen bond occurs between the N-atom of piperazine and Tyr337. An exactly opposite orientation is observed when the protonated form is docked. The xantine moiety is placed in the PAS forming two hydrogen bonds with Tyr124 and Phe295. The protonated nitrogen atom also forms a hydrogen bond with Tyr124. The benzyl ring binds deeply into the catalytic centre, participating in π-π stacking with Trp86.

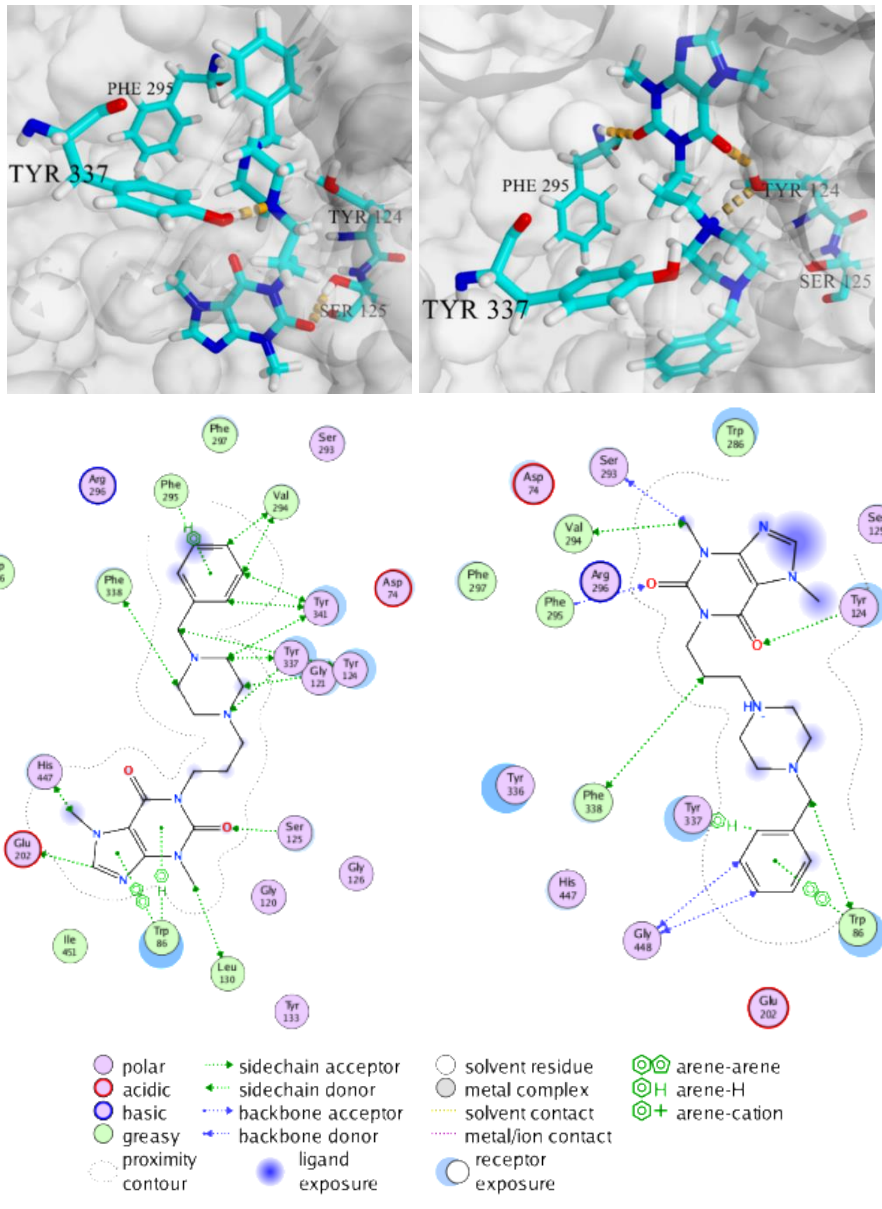
In the preliminary *in vitro* evaluation of the acetylcholinesterase inhibitory activity performed by the Ellman's test (Ellman et.al., 1961) the modelled LA1 showed 50, 70% inhibitory activity, close to the one determined for the reference galantamine.

Figure 5: Superposed docking poses and crystallographic structures of donepezil (left) and galantamine (right) into the AChE binding site. Crystallographic structures are shown in blue.



Source: Author

Figure 6: Docking poses and interactions of LA1 (left) and LA1 protonated (right) within the binding groove of AChE. Hydrogen bonds are shown as orange dashes (up).



Source: Author

Conclusion

In this study the binding mode of the newly synthesized arylpiperazine derivative 1-(3-(4-benzylpiperazin-1-yl)propyl)-3,7-dimethyl-1H-purine-2,6(3H,7H)-dione (LA1) into *rh*AChE was identified by molecular docking. The results show that in the best scored poses in the non-protonated structure the xanthine moiety of LA1 is bound into the catalytic active site (CAS) of AChE, while the arylpiperazine fragment is placed into the peripheral binding site (PAS). On the other hand, exactly the opposite orientation is observed when the protonated form is docked. The binding was confirmed by preliminary results from the Ellman's test. LA1 was found to express good permeability through the GIT and BBB assessed by PAMPA and confirmed by Lipinski's Rule of Five. The obtained results indicate that LA1 as a prospective lead for AChE inhibition.

Acknowledgement

This work was supported by grant from the Medical Science Council of Medical University of Sofia, Contract No.: D-90/2017.

References:

Andonova, L., Zheleva-Dimitrova, D., Georgieva, M., Zlatkov, A. (2014).Synthesis and antioxidant activity of some 1-aryl/ aralkyl piperazine derivatives with xanthine moiety at N4. Biotechnology & Biotechnological Equipment, 28:6, 1165-1171.

Atanasova M, Yordanov N, Dimitrov I, Berkov S, Doytchinova I (2015a) Molecular docking study on galantamine derivatives as cholinesterase inhibitors. MolInf 34: 394-403. PMID: 27490385

Atanasova M, Stavrakov G, Philipova I, Zheleva D, Yordanov N (2015b) Galantamine derivatives with indole moiety: docking, design, synthesis and acetylcholinesterase activity. Bioorg Med Chem 23: 5382-5389. PMID: 26260334

Barnard, E.A. (1974) Neuromuscular transmission - enzymatic destruction of acetylcholine. In: Hubbard J.I., editor.The Peripheral Nervous System.Plenum; New York: pp. 201–224.

Cheung J, Rudolph MJ, Burshteun F, Cassidy MS, Gary EN, et al. (2012) Structures of human acetylcholinesterase in complex with pharmacologically important ligands. J Med Chem 55: 10282-10286. PMID: 23035744

Čolović,M.B., Krstić,D.Z., Lazarević-Pašti,T.D.,Bondžić,A.M., Vasić,V.M. (2013). Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. Curr Neuropharmacol, 11(3), 315–335.

Dvir, H., Silman, I., Harel, M., Rosenberry, T.L., Sussman, J.L. (2010). Acetylcholinesterase: From 3D Structure to Function. Chem Biol Interact, 187(1-3): 10–22.

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

Kitz, R. J., Braswell, L. M., Ginsburg, S. (1970). On the question: is acetylcholinesterase an allosteric protein?.Mol. Pharmacol.,6(2):108-21

Meng, X., Zhang, H., Mezei, M., Cui, M. (2011).Molecular Docking: A powerful approach for structure-based drug discovery.Curr Comput Aided Drug Des**.,** 7(2): 146–157.

Modh, R.P., Kumar, S.P., Jasrai, Y.T., Chikhalia, K.H. (2013). Design, synthesis, biological evaluation, and molecular modeling of coumarin-piperazine derivatives as acetylcholinesterase inhibitors. Arch Pharm (Weinheim), 346(11):793-804

Mohamed, T., Osman, W., Tin, G., Rao, P.P. (2013).Selective inhibition of human acetylcholinesterase by xanthine derivatives: in vitro inhibition and molecular modeling investigations. Bioorg Med Chem Lett, 23(15):4336-41

Molecular Operating Environment (MOE), 2013.08; Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, **2018**.

Molinspiration Cheminformatics, 2017, retrieved from www.molinspiration.com

Özkay, Ü.D., Can, Ö.D., Sağlık, B.N., Çevik, U.A., Levent, S., Özkay,Y., Ilgın, S., Atlı, Ö.(2016). Design, synthesis, and AChE inhibitory activity of new benzothiazole-piperazines**.**Bioorg Med Chem Lett, 26(22):5387-5394

Pohanka, M., Dobes, P. (2013). Caffeine Inhibits Acetylcholinesterase, But Not Butyrylcholinesterase. Int. J. Mol. Sci., 14(5), 9873-9882.

Rose, A.S., Bradley, A.R., Valasatava, Y., Duarte, J.M., Prlić, A., Rose, P.W. (2016) *Web-based molecular graphics for large complexes.* ACM Proceedings of the 21st International Conference on Web3D Technology (Web3D '16): 185-186.

Stavrakov G, Philipova I, Zheleva D, Atanasova M, Konstantinov S (2016) Docking-based design of galantamine derivatives with dual-site binding to acetylcholinesterase. MolInf 35: 278-285. PMID: 27492242

Stavrakov G, Philipova I, Zheleva-Dimitrova D, Valkova I, Konstantinov S (2017).Docking-based design and synthesis of galantamine-camphane hybrids as inhibitors of acetylcholinesterase.ChemBiol Drug Des 90: 709-718. PMID: 28374576

Tata, A.M., Velluto, L., D'Angelo, C., Reale, M. (2014).Cholinergic system dysfunction and neurodegenerative diseases: cause or effect?. CNS NeurolDisord Drug Targets, 13(7):1294-303.

Yurttaş, L., Kaplancıklı, Z.A., Özkay, Y.(2013). Design, synthesis and evaluation of new thiazole-piperazines as acetylcholinesterase inhibitors.J Enzyme Inhib Med Chem, 28(5):1040-7.