Journal of Sciences, Islamic Republic of Iran 30(1): 5 - 12 (2019) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Cholinesterase Inhibition Activity and Docking Simulation Study of Coumarin Mannich Base Derivatives

A. Kiani[1], L. Jalili-baleh[2], Z. Abdolahi[3], H. Nadri[3], A. Foroumadi[2,4], S. E. S. Ebrahimi[*1,4], M. Khoobi[**2,5]

[1] *Department of Medical Chemistry, School of Pharmacy, International Campus, Tehran University of Medical Science (IC-TUMS),Tehran, Iran*
[2] *Biomaterials Group, The Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences, Tehran 1417614411, Iran*
[3] *Department of Medicinal Chemistry, Faculty of pharmacy and Pharmaceutical Sciences Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[4] *Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[5] *Department of Pharmaceutical Biomaterials, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*

Received: 25 April 2018 / Revised: 13 November 2018 / Accepted: 9 December 2018

Abstract

Inhibition of acetylcholinesterase and butyrylcholinesterase (AChE and BuChE) as two major forms of cholinesterases (ChEs) is considered as the common approach for the treatment of Alzheimer's disease (AD). The present study was done to explore the anticholinesterase inhibition property of coumarin Mannich base derivatives. A series of cumarin Manich bases were synthesized (4a-h) through one-pot tri-component reaction in an environmentally friendly condition and evaluated against AChE and BuChE by Ellman's assay. Ligand-protein docking simulation was also performed for the most active compound 4a. Additionally, the criteria of drug likeness of the target compounds was predicted using SwissADME web service. All compounds exhibited weak to moderate inhibitory activity against both AChE and BuChE enzymes. Compound 4a containing p-tolyl piperazin group showed the best activity against AChE (42.4 % at 32 µM), while compound 4g bearing phenylpiperazine moiety was the best BuChE inhibitor (43.9% at 32 µM). Ligand-protein docking simulation also exhibited that the main part of compound 4a in ChE inhibitory activity is amine moiety. Moreover, the prediction of "Lipinski's rule of five" showed that most target compounds can cross the BBB and have properties that would make them likely orally active compounds in humans. This study suggested that the synthesized cumarin Manich bases with some more structural modifications may be considered as a potential compound to target AChE and BuChE.

Keywords: Alzheimer’s disease; Acetylcholinesterase; Butyrylcholinesterse; 4-Hydroxycoumarin; Mannich base.

[*] Corresponding author: Tel: +982166959090; Email: sesebrahimi@sina.tums.ac.ir
**Corresponding author: Tel: +982164121510; Email: mehdi.khoobi@gmail.com

Introduction

Alzheimer's disease (AD) is neurodegenerative disorder cusing memory deterioration. Two major pathological cause of AD are the progressive loss of cholinergic neural activity and formation of intra- and extracellular plaques of beta-amyloid (Aβ) causing senile plaques and neurofibrillary tangles of hyperphosphorylated tau protein [1-2]. Acetylcholinesterase (AChE) through acetylcholine (ACh) hydrolysis and cognitive impairment is one of the important factor in alleviation of AD's symptoms [3-5]. AChE inhibitors not only delay the decay of ACh but it also promotes the amyloid-beta fibril formation through its interactions with peripheral anionic site (PAS). In addition, growing evidence suggested that, along with decreased level of ACh in certain brain regions during progression of AD, level of butyrylcholinesterase (BuChE) as other form of cholinesterase increases in the brain of mammals [6-8]. Since BuChE inhibition can raise ACh levels [9-10], dual inhibition of AChE and BuChE can control AD symptoms with no notable side effects [11].

Coumarin has been utilized as a potent anticholinesterase natural based scaffold, due to its ability to bind peripheral binding site of the enzyme [12,13], antioxidant activity [14] and an excellent therapeutic properties in the management of cognition disorders [15-18]. Among the AChE inhibitors belong to coumarin scaffold, AP2238 was the first dual binding site AChE inhibitor [19]. Moreover, amino moiety is the key functional group to improve the activity of the target compounds through interactions with catalytic site of AChE [20].

The Mannich reaction is one of the simple multi-component reactions and versatile green approaches to produce β-amino-carbonyl compounds [21]. Coumarins containing active hydrogen atoms placed between two carbonyl functional groups can reach to Mannich bases in the presence of secondary amines and aldehydes [22].

Hence, in continuation of our previous works on coumarin derivatives [23,24] and based on the important principles of "Green Chemistry" such as one-pot reaction and usage of green solvents [25], we report herein successful preparation, cholinesterase evaluation and in silico studies of a series of novel coumarin Mannich-base derivatives (**4a-h**) for AD therapy (Figure 1).

Materials and Methods

All chemical reagents were commercially. Reactions were monitored by TLC on silica gel. Kofler hot stage

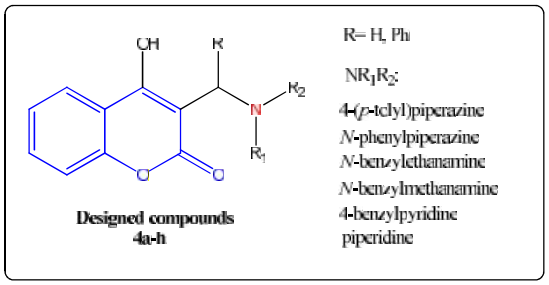


Figure 1. Designed compounds **4a-h**.

was utilized to measure melting points. The IR spectra were acquired using PerkinElmer Spectrum version 10.03.06 (KBr discs). ^{1}H and ^{13}C NMR spectra were recorded on Brucker 500 MHz. The coupling constants (*J*) and chemical shifts (δ) were expressed in Hertz and parts per million, respectively. The CHN elemental analyses were performed using a Rapid Heraeus elemental analyzer and the results were within ±0.5% of the calculated values.

Preparation of Mannich bases of coumarin 4a-h

Mannich bases (**4a-h**) were synthesized according to the method reported before [26, 27]. 4-hydroxycoumarin (1 mmol) in EtOH (5 mL) was added dropwise to ethanolic solution of formaldehyde 37% (1 mmol) and appropriate amine (1.2 mmol). The solution was refluxed for 1 hr. After completion of the reaction (monitored by TLC), the reaction was cooled and allowed to stand in a refrigerator for 24 h. The resulting precipitate was filtered off and washed with ethanol. If no precipitation was produced, the resulting mixture was evaporated under vacuo and the crude product was purified by crystallization from ethylacetate/*n*-hexan (50:50) to yield the desired compounds **4a-h** (yields 75-90 %).

4-hydroxy-3-((4-(p-tolyl)piperazin-1-yl)methyl)-2H-chromen-2-one (4a):

White solid; Yield (80%) mp 194-196 °C; IR (KBr, cm^{-1}): 3490 (O-H), 2930 (C-H), 1665 (C=O). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 16.9 (s, 1H, OH), 7.87 (d, 1H, *J* = 7.5 Hz, H_5 coumarin), 7.48 (t, 1H, *J* = 7.5 Hz, H_7 coumarin), 7.27-7.22 (m, 2H, $H_{6,8}$ coumarin), 7.09 (d, 2H, *J* = 8.0 Hz, Phenyl), 6.85 (d, 2H, *J* = 8.0 Hz, phenyl), 4.06 (s, 2H, CH_2), 2.87-3.60 (m, 8H, piperazine), 2.28 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 173.5, 164.8, 153.5, 147.2, 130.9, 130.3, 129.4, 123.8, 122.6, 120.0, 116.6, 115.9, 88.7, 54.0, 51.1, 47.3, 20.0. Anal. Calcd for: $C_{21}H_{22}N_2O_3$ (350.42): C, 71.98; H, 6.33; N, 7.99. Found: C, 71.65; H, 5.97; N, 7.83.

3-((4-benzylpiperidin-1-yl)methyl)-4-hydroxy-2H-chromen-2-one (4b) [28]:

White solid; Yield (90%) mp 189-191 °C; IR (KBr, cm^{-1}): 3426 (O-H), 2932 (C-H), 1640 (C=O). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 17.36 (s, 1H, OH), 7.90 (dd, 1H, *J* = 7.5 Hz, *J* = 1.5 Hz, H_5 coumarin), 7.47-7.44 (m, H_7 coumarin), 7.30-7.20 (m, 5H, $H_{6,8}$ coumarin and 3H Phenyl), 7.12 (d, 2H, *J* = 7.5 Hz, Phenyl), 4.11 (s, 2H, CH_2), 3.54-3.51 (m, 2H, CH_2 piperidine), 2.61-2.57 (m, 4H, CH_2 piperidine), 1.93-1.79 (m, 3H, CH piperidine and CH_2 benzyl), 1.58.1.53 (m, 2H, CH_2 piperidine). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 175.7, 165.7, 153.8, 138.7, 131.1, 128.7, 126.2, 124.1, 122.8, 121.0, 116.0, 88.1, 54.9, 52.6, 42.0, 36.2, 29.6. Anal. Calcd for: $C_{22}H_{23}NO_3$ (349.42): C, 75.62; H, 6.63; N, 4.01. Found: C, 75.31; H, 6.23; N, 4.12.

3-((4-benzylpiperidin-1-yl)(phenyl)methyl)-4-hydroxy-2H-chromen-2-one (4c):

White solid; Yield (90%) mp 184-186 °C; IR (KBr, cm^{-1}): 3426 (O-H), 2932 (C-H), 1640 (C=O). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 16.70 (s, 1H, OH), 8.06 (d, 1H, J = 7.0 Hz, H_5 coumarin), 7.48-7.46 (m, 1H, H_7 coumarin), 7.42-7.19 (m, 10H, $H_{6,8}$ coumarin and 2×4H Phenyl), 7.08-7.07 (m, 2H, Phenyl), 5.26 (s, 1H, CH), 3.47 (m, 2H CH_2 piperidin), 2.79-2.74 (m, 2H, CH_2 piperidin), 2.53 (d, 2H, *J* = 6.5 Hz, CH_2 benzyl), 1.78-1.57 (m, 5H, CH and 2×CH_2 piperidin). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 167.7, 164.5, 152.5, 142.3, 129.0, 128.7, 127.6, 126.6, 125.9, 124.7, 124.1, 122.8, 119.9, 115.4, 103.3, 69.7, 59.7, 31.5, 28.9, 14.0. Anal. Calcd for: $C_{28}H_{27}NO_3$ (349.42): C, 79.03; H, 6.40; N, 3.29. Found: C, 79.21; H, 6.13; N, 3.12.

3((benzyl(methyl)amino)(phenyl)methyl)-4-hydroxy2H-chromen-2-one (4d):

White solid; Yield (70%) mp 196-198 °C; IR (KBr, cm^{-1}): 3500 (O-H), 2930 (C-H), 1672 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 16.77 (s, 1H, OH), 7.82 (d, 1H, *J* = 7.5 Hz, H_5 coumarin), 7.51-7.41 (m, 5H coumarin and 2H phenyl), 7.26-7.06 (m, 8H, 2×4H Phenyl), 6.28 (s, 1H, CH), 4.13-4.10 (m, 2H, CH_2 benzyl), 2.57-2.53 (m, 3H, N-CH_3), ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 167.5, 164.5, 152.4, 142.2, 131.9, 130.9, 129.8, 129.0, 127.6, 126.6, 124.8, 124.1, 122.9, 119.7, 115.5, 103.4, 51.3, 36.1, 32.2. Anal. Calcd for: $C_{24}H_{21}NO_3$ (371.44): C, 77.61; H, 5.70; N, 3.77. Found: C, 77.34; H, 5.53; N, 4.01.

3-((benzyl(methyl)amino)methyl)-4-hydroxy-2H-chromen-2-one (4e) [29]:

White solid; Yield (80%) mp 198-200 °C; IR (KBr, cm^{-1}): 3500 (O-H), 2930 (C-H), 1672 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 16.77 (s, 1H, OH), 7.84 (d, 1H, *J* = 7.5 Hz, H_5 coumarin), 7.61-7.51 (m, 1H, H_7 coumarin), 7.46-7.43 (m, 2H, $H_{6,8}$ coumarin), 7.22-7.14 (m, 6H, Phenyl), 4.28 (s, 2H, N-CH_2), 4.01 (s, 2H, CH_2 benzyl), 2.57 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 174.4, 167.6, 164.4, 163.2, 153.9, 131.9, 129.0, 124.8, 122.7, 122.2, 119.4, 115.8, 115.3, 101.6, 51.3, 36.1, 32.2. Anal. Calcd for: $C_{24}H_{21}NO_3$ (371.44): C, 77.61; H, 5.70; N, 3.77. Found: C, 77.58; H, 5.43; N, 3.46.

3-((benzyl(ethyl)amino)(phenyl)methyl)-4-hydroxy-2H-chromen-2-one (4f):

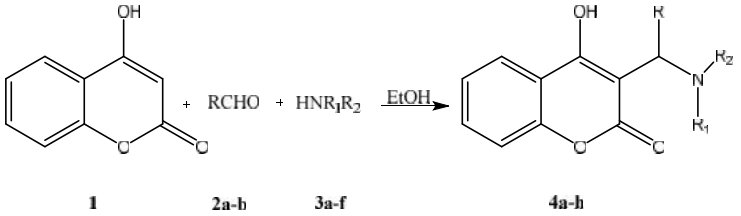
White solid; Yield (75%) mp 218-220 °C; IR (KBr, cm^{-1}): 3450 (O-H), 2910 (C-H), 1676 (C=O). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 17.35 (s, 1H, OH), 8.09 (bs, 1H, H_5 coumarin), 7.48-6.99 (m, 13H, 3H coumarin and 10H phenyl), 6.18 (s, 1H, CH), 4.11 (s, 2H, CH_2 benzyl), 3.10 (bs, 2H, CH_2), 1.24 (bs, 3H, CH_3), ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.3, 167.3, 152.5, 130.8, 130.5, 129.6, 129.0, 128.6, 127.8, 126.5, 124.9, 123.1, 120.1, 115.3, 103.5, 51.1, 42.6, 39.9, 11.1. Anal. Calcd for: $C_{25}H_{23}NO_3$ (385.46): C, 77.90; H, 6.01; N, 3.63. Found: C, 77.69; H, 5.86; N, 3.86.

4-hydroxy-3-((4-phenylpiperazin-1-yl)methyl)-2H-chromen-2-one (4g) [30]:

White solid; Yield (80%); mp 185-187 °C; IR (KBr, cm^{-1}): 3500 (O-H), 2890(C-H), 1661(C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 16.60 (s, 1H, OH), 7.84 (d, 1H, *J* = 8.0 Hz, H_5 coumarin), 7.45 (t, 1H, *J* = 7.5 Hz, H_7 coumarin), 7.24-7.18 (m, 4H, $H_{6,8}$ coumarin and 2H Phenyl), 6.95-6.93 (m, 2H, *J* = 8.0 Hz, Phenyl), 6.79-6.82 (t, 1H, *J* = 7.5 Hz, Phenyl), 3.64 (s, 2H, CH_2), 3.22 (bs, 4H, piperazine), 3.11 (bs, 4H, piperazine). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 167.6, 163.2, 152.5, 150.7, 130.5, 130.3, 129.0, 123.6, 122.7, 119.4, 119.1, 115.7, 101.6, 47.3, 44.0. Anal. Calcd for: $C_{20}H_{20}N_2O_3$ (336.36): C, 71.41; H, 5.99; N, 8.33. Found: C, 71.27; H, 6.18; N, 8.09.

4-hydroxy-3-(piperidin-1-ylmethyl)-2H-chromen-2-one (4h):

White solid; Yield (90%); mp 183-185 °C (Ref [27] 182 °C); IR (KBr, cm^{-1}): 3425 (O-H), 2937 (C-H), 1667 (C=O). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 16.6 (s, 1H, OH), 7.91 (d, 1H, *J* = 8.0 Hz , H_5 coumarin), 7.41-7.44 (m, 1H, H_7 coumarin), 7.16-7.20 (m, 2H, $H_{6,8}$ coumarin), 4.19 (s, 2H CH_2), 3.59-3.56 (m, 2H piperidine), 2.82-2.78 (m, 2H piperidine), 1.98-1.88 (m, 6H piperidine), ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 175.8, 165.4, 154.0, 131.3, 124.1, 123.0, 116.4, 96.0, 87.7, 65.5, 23.5. Anal. Calcd for: $C_{15}H_{17}NO_3$ (259.30): C,



Scheme 1. Synthesis of the target compounds **4a-h**

69.48; H, 6.61; N, 5.40. Found: C, 69.23; H, 6.99; N, 5.12.

In vitro AChE/BuChE inhibition assay

Based on the spectrophotometric method of Ellman [31], anticholinesterase activity of the target compounds (**4a-h**) were evaluated against horse serum BuCh and AChE from *electrophorus electricus* (AChE, *eel*). Five different concentrations from each compound were tested to obtain a range of 20 to 80% enzyme inhibition. A mixture containing phosphate buffer (0.1 M, pH=8.0, 2 mL), AChE/BuChE (20 µL), compounds solution (30 µL), and 5,5-dithio-bis-2-nitrobemzoic acid (DTNB, 60 µL) were incubated for five minutes and then acetylcholine iodide or butyrylcholine iodide (20 µL) was added as substrate and the absorbance was measured (412 nm) by a Synergy Biothech® multiplate reader. The IC_{50} values were obtained from Log concentration vs. percent of inhibition curves. To find the optimum result, each experiment was done in triplicate.

Drug Likeness Prediction

To predict the drug likeness of the synthesized molecules, SwissADME web service at http://www.swissadme.ch/ was used. The obtained data was summarized in Table 2.

Docking simulations

Ligand-protein docking was performed using Autodock Vina (1.1.2) [32]. The 3D coordinate of the AChE and BuChE was retrieved from Protein Data Bank (PDB) at http://www.rcsb.org/pdb/home/home.do. The crystal structure of AChE (PDB ID: 1eve) and BuChE (PDB ID: 6esy) were selected. For enzyme protein preparation, all of the non-protein atoms were detached and then minimized using OPLS3 force field (RMSD = 0.3 Å). After docking the best poses were selected for further analysis.

Results and Discussion

Among methods which was reported for preparation benzylamino coumarin derivatives via one pot Mannich reaction under green condition, we examined reported methods using Triton X-100 as the surfactant in water [33]. Unfortunately, the products were obtained in poor yields. Therefore, target compounds (**4a-h**) were synthesized via one pot Mannich reaction outlined in scheme 1 according to the previously reported procedure [26-30]. Ethanolic solution of 4-hydroxycoumarin (**1**) was added dropwise to the refluxing solution of appropriate secondary amine (**3a-f**) and formaldehyd (**2a**) or benzaldehyd (**2b**) in ethanol. On cooling, the precipitates was filtered and washed with ethanol. Target compounds were obtained in 50-80 % isolation yields.

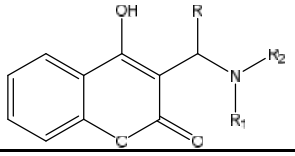
Anti-cholinesterase activity

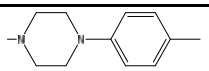
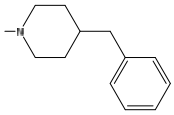
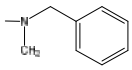
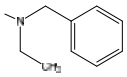
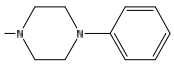
All compounds **4a-h** were evaluated against AChE and BuChE and compared with donepezil as standard ChE inhibitor. The activities were summarized in Table 1 as inhibitory percentage values. Compound **4a** with methyl group on phenyl piperazine moiety exhibited the most potent inhibitory activity against AChE (42.4 %). Elimination of methyl group in compound **4g** led to the diminishing in AChE activity, while the BuChE activity increased by 43.9 %. The amines bearing benzyl substitution (**4f** and **4d**) possessed greater AChE inhibitory activity than the cyclic amine compounds (**4h** with piperidine moiety). However, with addition of benzyl group to piperidine moiety (**4b-c**) a significant increase of AChE inhibitory activity was seen rather than **4h**. Also, changing the methylene group to phenyl group by using benzaldehyde in compounds **4c** and **4d** instead of formaldehyde in compounds **4b** and **4e** increased the BuChE activity. All compounds revealed less activity against BuChE compared to AChE, except for compound **4h** and **4g**. Compound **4g** was the most potent compound for inhibition of BuChE (43.9 %).

Calculation of ADME properties

To predict some physicochemical properties of the molecules that are essential to meet the criteria of drug likeness, the SwissADME web service was used. The calculate data was summarized in table 2. All

Table 1. Inhibitory activity of the target compounds 4a-h against (AChE) and (BuChE)



Compound	-NR_1R_2	R	% of AChE inhibition at 32 µM	% of BuChE inhibition at 32 µM	Ref.
4a		H	42.4	5.2	
4b		H	33.7	9.0	
4c		Ph	29.0	12.2	
4d		Ph	31.7	10.1	
4e		H	6.4	NA[a]	
4f		Ph	29.2	19.0	
4g		H	16.2	43.9	
4h		H	6.7	17.7	
Donepezil			100	78	

[a] NA (Not active)

Table 2. Calculated (ADME) properties of the target compounds 4a-h

Compound	Num. H-bond acceptors	Num. H-bond donors	TPSA (Å^2)	XLOGP3	BBB permeation	Lipinski
4a	4	1	56.92	3.08	yes	No violation
4b	4	1	53.68	3.82	yes	No violation
4c	4	1	53.68	5.51	yes	1 violation XLOGP>4.5
4d	4	1	53.68	4.67	yes	No violation
4e	4	1	53.68	2.61	yes	No violation
4f	4	1	53.68	4.67	yes	No violation
4g	4	1	56.92	2.71	yes	No violation
4h	4	1	53.68	1.96	yes	No violation
Donepezil	4	0	38.77	4.28	yes	No violation

compounds satisfy the requirements for drug-likeness except compound **4c** due to large partition coefficient.

Ligand-protein docking simulation

To get better insight into the ligand-enzyme interactions, the most active compound **4a** was subjected for docking studies as representative compound. The docking results are represented in Figures 2 and 3 (Figures was prepared using Discovery studio visualizer 4.5 client) [34]. According to the docking results it was revealed that the hydrophobic and π-stacking interactions were the predominant forces contributing in ligand-AChE interaction. Furthermore, the stability of the ligand-AChE complex got benefit from additional conventional hydrogen bond and salt bridge while in the case of BuChE the π-stacking and hydrophobic interactions were predominant. The molecule is composed of two different segments including 4-hydroxycoumarin and 4-tolylpiperazine. The 4-tolyl moiety is involved in hydrophobic interaction with Phe330 and Trp432 of AChE and Phe329 and Trp231 of BuChE. Moreover, there was two π-stacking between phenyl ring and Trp84 and Phe330. The positively charged nitrogen atom of piperazine ring formed a salt bridge with Glu199 at the distance of 4.66 Å; but in BuChE, such interaction was not observed. The 4-hydroxycoumarin segment was just involved in a hydrogen bond with Tyr121 of AChE; while in BuChE stacked against Trp82. Accordingly, it is concluded that the 4-hydroxycoumarin is not involved in modulation of

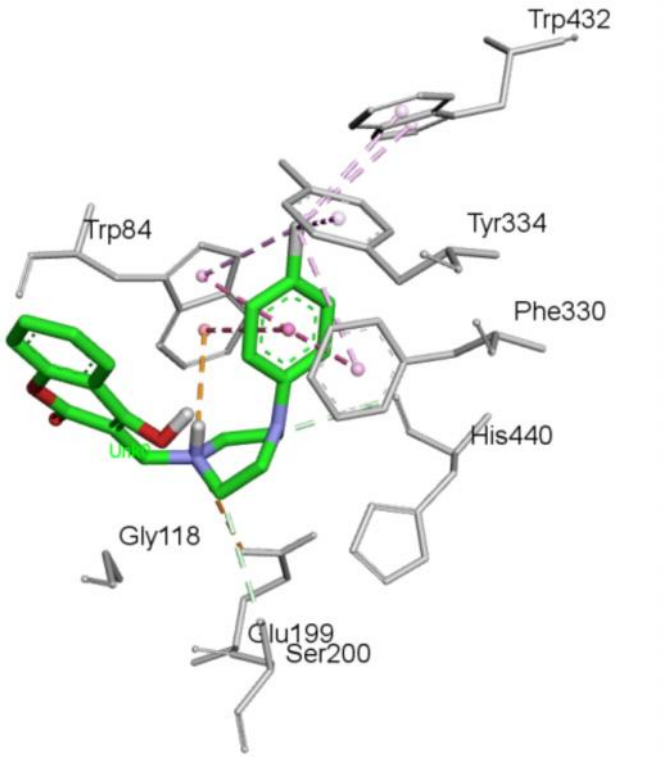


Figure 2. The orientation of **4a** in the active site of (AChE)

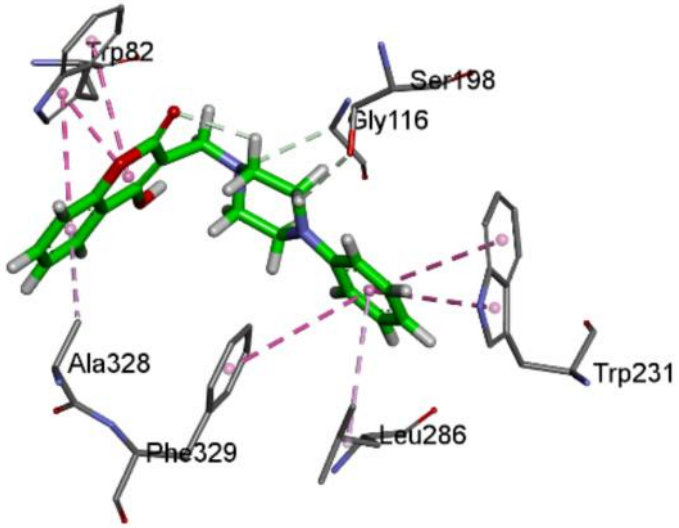


Figure 3. The predicted binding mode of **4a** in interaction with (BuChE)

the anticholinesterase activity while the amine counterpart plays an important role in the activity.

Conclusion

In this work, we prepared a novel series of coumrin Mannich base derivatives through environmentally friendly chemical condition with controlled formation of biscoumarin as byproduct. Target compounds were evaluated against AChE and BuChE. Compound **4a** containing *N*-(4-pheny)piperazine moiety showed the best activity against AChE with 42.4 % of inhibition. The docking study revealed that the amine moieties of

the compound **4a** play an important role in both ACh- and BuChE activity. Eventually, we suggested compound **4a** with some more structural modifications may be consider as a useful central nervous system (CNS)-active template to promote more effective cholinesterase inhibitors in future.

References

1. Tang H., Zhao H.T., Zhong S.M., Wang Z.Y., Chen Z.F., Liang H. Novel oxoisoaporphine-based inhibitors of acetyl- and butyrylcholinesterase and acetylcholinesterase-induced beta-amyloid aggregation. *Bioorg. Med. Chem*. **22**:2257-2261(2012).
2. Querfurth H.W., La Ferla F.M. Mechanism of disease Alzheimer'disease. *N. Engl. J. Med*. **362**:329-344(2010).
3. Pepeu G., Giovannini M.G. Cholinesterase inhibitors and beyond. *Curr. Alzheimer Res*. **6**:86-96(2009) .
4. Mohammadi-Farani A., Abdi N., Moradi A.R., Aliabadi A.R. 2-(2-(4-Benzoylpiperazin-1-yl) ethyl)isoindoline-1,3-dione derivatives: Synthesis, docking and acetylcholinesterase inhibitory evaluation as anti-alzheimer agents. *Iran J. Basic Med Sci*. **20**:59-66(2017).
5. Giacobini E. Cholinesterase inhibitors: new roles and therapeutic alternatives. *Pharmacol. Res*. **50**:433-440(2004).
6. Darvesh S., Hopkins D.A., Geula C. Neurobiology of butyrylcholinesterase. *Nat. Rev. Neurosci*. **4**:131-8(2003).
7. Decker M., Krauth F., Lehmann J. Design, synthesis and pharmacological evaluation of hybrid molecules out of quinazolinimines and lipoic acid lead to highly potent and selective butyrylcholinesterase inhibitors with antioxidant properties. *J. Bioorg. Med. Chem.* **16**:4252-61(2008).
8. Eskander M.F., Nagykery N.G., Leung E.Y., Khelghati B., Geula C. Rivastigmine is a potent inhibitor of acetyl- and butyrylcholinesterase in Alzheimer's plaques and tangles. *Brain Res*. **1060**:144-52(2005).
9. Greig N.H., Utsuki T., Ingram D.K., Wang Y., Pepeu G., Scali C., *et al.* Selective butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer beta-amyloid peptide in rodent. *Proc. Natl. Acad. Sci.* U.S.A **102**:17213-8 (2005).
10. Marcelo F., Silva F.V., Goulart M., Justino J., Sinay P., Bleriot Y., Rauter A.P. Synthesis of novel purine nucleosides towards a selective inhibition of human butyrylcholinesterase. *Bioorg. Med. Chem*. **17**:5106-5116(2009).
11. Lane R.M., Potkin S.G., Enz A. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. *Int. J. Neuropsychopharmacol*. **9**:101-24(2006).
12. Alipour M., Khoobi M., Foroumadi A., Nadri H., Moradi A., Sakhteman A., *et al.* Novel coumarin derivatives bearing N-benzyl pyridinium moiety: Potent and dual binding site acetylcholinesterase inhibitors. *Bioorg. Med. Chem*. **20**:7214-7222(2012).
13. Ghanei-Nasab S., Khoobi M., Hadizadeh F., Marjani A., Moradi A., Nadri H., *et al*. Synthesis and anticholinesterase activity of coumarin-3-carboxamides bearing tryptamine moiety. *Eur. J. Med. Chem.* **121**:40–46(2016).
14. Razavi S.F., Khoobi M., Nadri H., Sakhteman A., Moradi A., Emami S., *et al.* Synthesis and evaluation of 4-substituted coumarins as novel acetylcholinesterase inhibitors. *Eur. J. Med. Chem*. **64**:252–259(2013).
15. Zhou X., Wang X.B., Wang T., Kong L.Y. Design, synthesis, and acetylcholinesterase inhibitory activity of novel coumarin analogues. *Bioorg. Med. Chem*. **16**:8011-8021(2008).
16. Fallarero A., Oinonen P., Gupta S., Blom P., Galkin A., Mohan C.G., *et al.* Inhibition of acetylcholinesterase by coumarins: the case of coumarin 106. *Pharmacol. Res*. **58**:215-21(2008).
17. Shen Q., Peng Q., Shao J., Liu X., Huang Z., Pu X., *et al.* Synthesis and biological evaluation of functionalized coumarins as acetylcholinesterase inhibitors. *Eur. J. Med. Chem*. **40**:1307-15(2005).
18. Soto-Ortega D.D., Murphy B.P., Gonzalez-Velasquez F.J., Wilson K.A., Xie F., Wang Q., *et al.* Inhibition of amyloid-β aggregation by coumarin analogs can be manipulated by functionalization of the aromatic center. *Bioorg. Med. Chem*. **19**:2596-2602(2011).
19. Piazzi L., Rampa A., Bisi A., Gobbi S., Belluti F., Cavalli A., *et al.* 3-(4-{[Benzyl(methyl)amino]methyl}phenyl)-6,7-dimethoxy-2*H*-2-chromenone (AP2238) Inhibits Both Acetylcholinesterase and Acetylcholinesterase-Induced β-Amyloid Aggregation: A Dual Function Lead for Alzheimer's Disease Therapy. *J. Med. Chem*. **46**:2279-2282(2003).
20. Alipour M., Khoobi M., moradi M., Nadri H., Homayouni Moghadam F., Emami S., *et al*. Synthesis and anti-cholinesterase activity of new 7-hydroxycoumarin derivatives. *Eur. J Med. Chem.* **82**:536–544(2014).
21. Mannich C. Eine Synthese von β-Ketonic bases. *Arch. Pharm.* **255**: 261276(1917).
22. Montagut-Romans A., Boulven M., Lemaire M., Popowycz F. 3-Methylene-2,4-chromandione in situ trapping : Introducing molecular diversity on 4-hydroxycoumarin. *RSC Adv*. **6**:4540-4544(2016).
23. Asadipour A., Alipour M., Jafari M., Khoobi M., Emami S., Nadri H., *et al*. Novel coumarin-3-carboxamides bearing N-benzylpiperidine moiety as potent acetylcholinesterase inhibitors. *Eur. J. Med. Chem*. **70**:623-630(2013).
24. Hirbod K., Jalili-baleh L., Nadri H., Sadat Ebrahimi S.E., Moradi A., Pakseresht B., *et al*. Coumarin derivatives bearing benzoheterocycle moiety: synthesis, cholinesterase inhibitory, and docking simulation study. *Iran J. Basic. Med. Sci*. **20**:631-638(2017).
25. Sreevalli W., Ramachandran G., Madhuri W., Sathiyanarayanan K.I. Green Trends in Mannich Reaction. *Mini-Reviews in Org. Chem*. **11**:97-115(2014).
26. Savel'ev V.L., Artamonova O.S., Troitskaya V.S., *et al*. Synthesis and study of the properties of 4-substituted 3-aminomethylcoumarins. *Chem. Heterocycl. Compd*. **18**(7):676-679(1982).
27. Robertson D.N., Link K.P. Studies on 4-Hydroxycoumarins. XII. 3-Substituted-aminomethyl-4-hydroxycoumarin Derivatives by the Mannich Reaction. *J. Am. Chem. Soc*.**75**:1883-1885(1953).
28. National Center for Biotechnology Information. PubChem

Compound Database; CID=54693757, https://pubchem.ncbi.nlm.nih.gov/compound/54693757 (accessed Nov. 18, 2018).
29. National Center for Biotechnology Information. PubChem Compound Database; CID=54687524, https://pubchem.ncbi.nlm.nih.gov/compound/54687524 (accessed Nov. 18, 2018).
30. National Center for Biotechnology Information. PubChem Compound Database; CID=54721006, https://pubchem.ncbi.nlm.nih.gov/compound/54721006 (accessed Nov. 18, 2018).
31. Ellman G.L., Courtney KD., Andres V., Featherstone R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol*. **7**:88-95(1961).
32. Trott O., Olson A.J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem*. **31**:455-461(2010).
33. Atul K., Maneesh K.G., Mukesh K. An efficient non-ionic surfactant catalyzed multicomponent synthesis of novel benzylamino coumarin derivative via Mannich type reaction in aqueous media. *Tetrahedron Lett*. **52**:4521-4525(2011).
34. Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 4.5, San Diego: Dassault Systèmes, (2015).