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Synthesis, Biological Evaluation and Molecular Modeling of (E)-3-Propargylene-1, 3-Dihydro-2H-Indol-2-Ones as Acetyl- and Butyrylcholinesterase Inhibitors for the Treatment of Alzheimer's Disease

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Abstract

The synthesis, pharmacological evaluation and molecular modeling of (*E*)-3-propargylene-1,3-dihydro-2*H*-indol-2-ones, targeting both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), are described. *In vitro* inhibition experiments of AChE and BuChE showed that compound **2**, **5** and **12** are able to inhibit the two forms of cholinesterases in the submicromolar range. The most selective inhibitor of *Ee*AChE (acetylcholinesterase, E.C. 3.1.1.7, from Electrophorus electricus) and *eq*BuChE (butylcholinesterase, E.C. 3.1.1.8, from equine serum) in this series are compound **9** (IC_{50}=0.011 ± 0.018 μM) and compound **14** (IC_{50}=0.12 ± 0.22 μM) respectively. But the substitution at 5- or 6- position of indolones is not generally favored for *eq*BuChE inhibition. Kinetic studies of the BuChE inhibition suggested that compound **1** and **5** produce a mixed inhibition pattern. The molecular modeling investigation confirmed the result and indicated that π-π stacking interaction is a main contributor to the increase of inhibition efficiency.

Keywords: Indolones; Acetylcholinesterase; Butyrylcholinesterase; Kinetic analysis; Molecular modeling; Alzheimer's disease

Introduction

Alzheimer's disease (AD) is currently recognized as a complex neurodegenerative disorder which is the most common cause of late life dementia [1]. The typical pathological features of AD include cell loss, senile plaques, and neurofibrillary tangles in the neocortex, hippocampus, amygdala and the basal nucleus of Meynert [2]. The most severe and consistent biochemical change in AD is cholinergic deficit. It is seen as decreased levels of acetylcholine (ACh), choline acetyltransferase (CAT), and acetylcholinesterase (AChE). The reduced activity of both CAT and AChE could often be observed [2,3].

Since the 1970s, a great deal of research has been conducted to develop the cholinesterase inhibitors (ChEIs) for symptoms of this disease. The common mechanism of action for ChEIs is an increase in available synaptic ACh through inhibition of the catabolic cholinesterase. Clinically, approximately 50% of the AD patients show therapeutic effect of ChEIs which is to stabilize cognitive function at a steady level during 12 months of treatment as compared to placebo, this cognitive stabilizing effect can be prolonged up to 24 months in approximately 20% of the patients [4]. Meaningful symptomatic benefit supports ChEIs as the mainstay of pharmacotherapy in AD. AChE and butyrylcholinesterase (BuChE) are two major forms of cholinesterases but differ significantly in substrate specificity, enzyme kinetics, expression and activity in different regions of the brain, both of them can catalyze the hydrolysis of choline esters including ACh and play a collaborative role in cholinergic transmission [5-7]. It was pointed out that the activity of BuChE rises while the activity of AChE remains unchanged or declines in the AD brain of more severe cases [8], so dual or selective inhibitors of AChE and BuChE may hold particular benefits for the patients with different neurobiological characteristics in various stages of AD. In addition, growing evidence suggests that cholinesterase may have many non-cholinergic effects separate from its 'classical' function of ACh hydrolysis, which include the regulation of the activity of other proteins, regional cerebral blood flow, tau phosphorylation, and the amyloid cascade [9]. This mechanism may contribute to the patient long-term cognitive stabilization seen during ChEIs treatment [10].

Here we report the synthesis, biological evaluation, investigation on the mode of action, and the molecular modeling studies of (*E*)-3-propargylene-1, 3-dihydro-2*H*-indol-2-ones as a new class of dual or selective inhibitors of AChE and BuChE. The preliminary structure–activity relationships (SARs) are also discussed.

Results and Discussion

Chemistry

Various indolones (**1**~**16**, Table 1) were prepared by the route outlined in Scheme 1 [11,12]. The initial strategy was to synthesis *N*-substituted indolin-2-ones **v** [13]. Isonitrosoacetanilides **ii** were prepared by the reaction of appropriate anilines **i** with oxammonium hydrochloride and chloral hydrate in the yield of 55-90%. The subsequent cyclization of **ii** in the presence of concentrated sulfuric acid gave isatins **iii** in 60-85% yield. Isatins **iii** were subsequently alkylated with various alkyl halides to give the corresponding *N*-substituted isatins **iv** in the yield of 85-95%. Reduction of **iv** by using hydrazine hydrate afforded the *N*-substituted indolin-2-ones **v** with the yield of 50-91%. Then the alkyne aldehydes **viii** were prepared from corresponding aldehydes through a Corey–Fuchs reaction in 47-91% yield [14]. The target products (E)-3-propargylene-1, 3-dihydro-2*H*-indol-2-ones **ix** (compounds **1**~**16**) were obtained through the condensation of *N*-substituted indolin-2-

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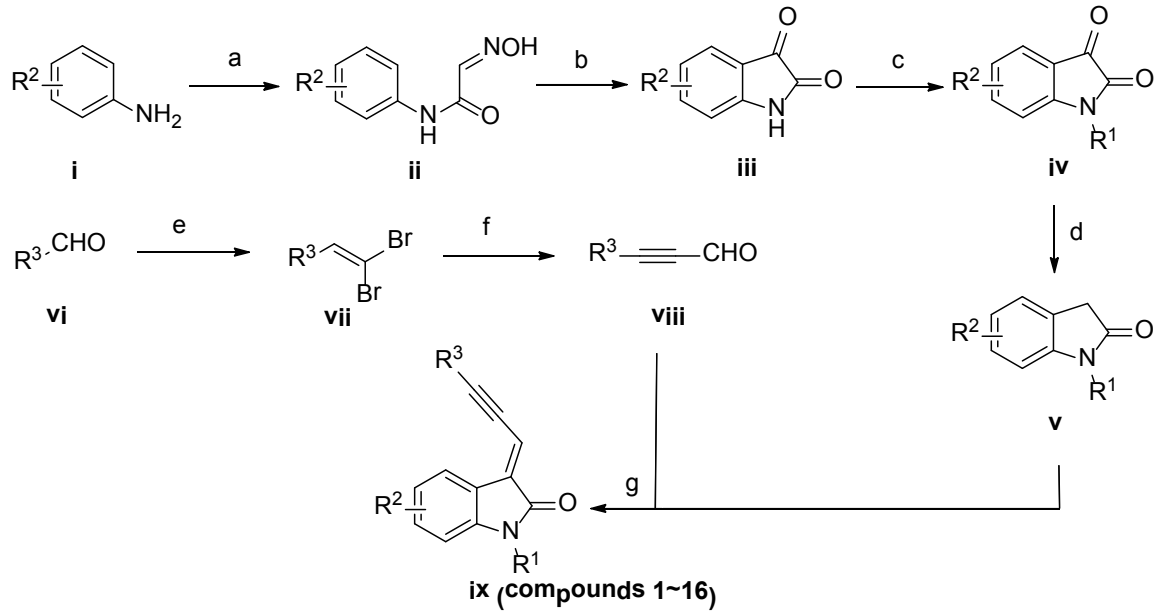
Compds.	R^1	R^2	R^3	IC_{50} (μM) *Ee*AChE[b]	IC_{50} (μM) *eq*BuChE[b]	Selectivity BuChE/AChE
1	-Me	-H	-Ph	0.074 ± 0.016	1.12 ± 0.73	15.14
2	-Bn	-H	-Ph	0.036 ± 0.018	0.29 ± 0.24	8.06
3	-Bn	5-Me	-Ph	>7.5	>16	
4	-H	-H	*p*-Me-Ph	>7.5	>16	
5	-Bn	5-F	-Ph	0.25 ± 0.21	0.13 ± 0.25	0.52
6	-Bn	5-Cl	-Ph	0.44 ± 0.30	>16	>36
7	-Bn	6-Cl	-Ph	>7.5	>16	
8	-Bn	-H	naphthalen-2-yl	>7.5	>16	
9	-Bn	-H	3,4-dimethoxy-Ph	0.011 ± 0.018	>16	>1455
10	-Bn	-H	phenylethyl	0.026 ± 0.017	>16	>615
11	-Bn	-H	*p*-Me-Ph	>7.5	>16	
12	-Bn	-H	*p*-OMe-Ph	0.97 ± 0.19	0.39 ± 0.46	0.40
13	-Bn	-H	*p*-F-Ph	>7.5	>16	
14	-Bn	-H	*p*-Cl-Ph	6.95 ± 1.7	0.12 ± 0.22	0.017
15	-Bn	-H	*m*-Cl-Ph	2.66 ± 0.027	0.36 ± 0.86	0.14
16	-Bn	-H	*o*-Cl-Ph	>7.5	>16	
Galantamine				0.362 ± 0.31	1.42 ± 1.0	3.92
Tacrine				0.045 ± 0.028	0.0055 ± 0.0023	0.12

[a]All reactions were carried out with **v** (5 mmol) and **viii** (6 mmol) in the presence of Et_3N (50.5 mg, 5 mmol) in Et_2O (10.0 mL) at r.t for 6 hours under nitrogen atomosphere (see Scheme 1). [b]Values are expressed as mean ± Standard error of the mean. [c]Isolated yield based on **v**.

Table 1: Yields and inhibitory activities on *Ee*AChE and *eq*BuChE of compounds **1~16**[a].



Scheme 1: The synthetic pathway of (*E*)-3-propargylene-1,3-dihydro-2*H*-indol-2-ones **ix** (compounds **1~16**). Reagents and conditions: (a) Cl_3CCHO, $NH_2OH{\cdot}HCl$, Na_2SO_4, HCl, H_2O, 40-75°C; (b) concd. H_2SO_4, 75-80°C; (c) K_2CO_3, R^1Cl, DMF, room temperature; (d) 85% hydrazine hydrate, reflux; (e) Ph_3P, CBr_4, CH_2Cl_2, 0°C; (f) n-BuLi, THF, DMF, KH_2PO_4, -60°C; (g) Et_3N, **viii**, Et_2O, room temperature.

ones **v** with alkyne aldehydes **viii** in the presence of triethylamine in diethyl ether at room temperature with good to high yields. All these compounds gave analytical and spectroscopic data in agreement with the structures assigned. All derivatives, except **1** and **4**, have three-aromatic-ring backbone.

Pharmacology

The inhibition of AChE and BuChE: The *in vitro* *Ee*AChE (acetylcholinesterase, E.C. 3.1.1.7, from Electrophorus electricus) and *eq*BuChE (butylcholinesterase, E.C. 3.1.1.8, from equine serum) inhibitory potential of these compounds were evaluated based on the Ellman's method [15] using galantamine and tacrine as reference compounds. From Table 1, it can be seen that compound **9** exhibits a significant and selective inhibition of *Ee*AChE (IC_{50}=0.011 ± 0.018 μM) which is more potent than the reference compounds. Compound **14** is the most selective inhibitor of *eq*BuChE (IC_{50}=0.12 ± 0.22 μM) in this series. In addition, compound **2**, **5** and **12** were able to inhibit the two forms of cholinesterases in the submicromolar range.

Kinetic analysis of the BuChE inhibition: In order to explore the mechanism involved in the BuChE inhibition by these indolones a kinetic analysis of the BuChE inhibition by compound **1** and **5** was performed. The K_i, K_m, and V_{max} values and inhibition types were determined by fitting the kinetic data to four general models of enzyme inhibition (competitive, noncompetitive, uncompetitive and mixed

models) by nonlinear regression analysis using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). The K_i and K_m values represent the mean ± S.E. Statistically significant difference (F test, $p<0.05$) was observed between mixed inhibition and other types of inhibitions. The Lineweaver-Burk plot (Figure 1) shows increased slopes and intercepts while inhibition, indicating that the two compounds induced a mixed type of inhibition.

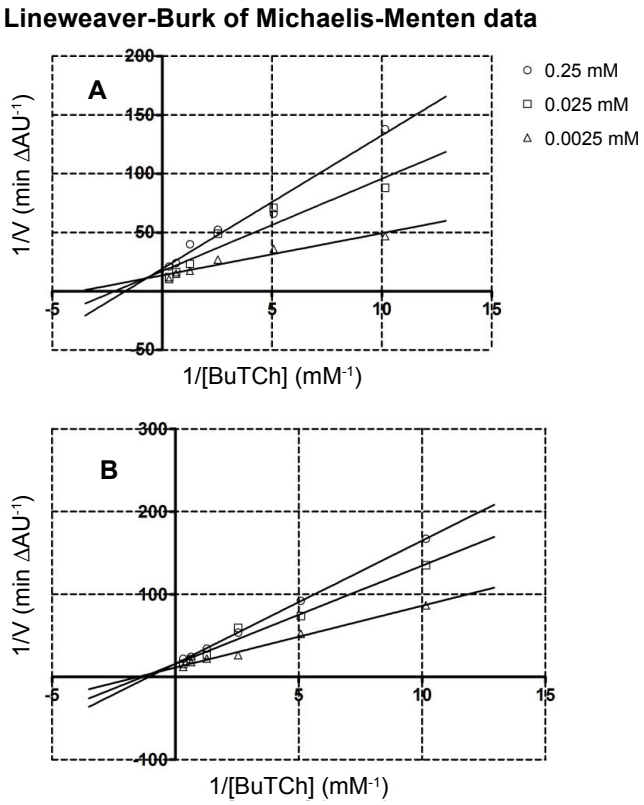


Figure 1: BuChE inhibition kinetic profile by compound **1** (A) and **5** (B). Reciprocal plot of velocity versus butyrylthiocholine (BTCh) concentration (0.03-1.0 mg/mL). All points represent the mean of three determinations. Compound **1**: K_i=0.26 ± 0.21 mM, K_m=1.00 ± 0.23 mM, V_{max}=0.12 ± 0.01 mmol/min. Compound **5**: K_i=0.65 ± 0.90 mM, K_m=1.01 ± 0.26 mM, V_{max}=0.10 ± 0.01 mmol/min.

Molecular modeling: As it has suggested that the similar series of derivatives binds to the enzyme in different ways despite the high sequence homology among species, molecular modeling was used to investigate the preferred binding mode adopted by the indolone ligands (**1**, **2**, **5**, **9** and **14**).

The *Ee*AChE has a narrow, deeply buried active-site gorge. The result of docking **2** and **9** into *Ee*AChE indicates that the binding site of the two compounds might be the peripheral anionic site (PAS) rather than the catalytic binding site (CAS) where the hydrolysis reaction takes place. The PAS is located at the entry to the active gorge and it is a very important structural element responsible for binding of many inhibitors [15]. Stabilization of the ligand-AChE complex may better account for the hydrophobic contacts and π-π interactions. No interaction with the amino acid residues of the catalytic triad (Ser203, His447 and Glu334) was detected. Figure 3 shows that the indolone scaffold of compound **2** is stacked against the aromatic rings of Trp286, and the characteristic π–π stacking for one benzene ring of compound **2** and the residue of Tyr341 is observed. The indolone scaffold of compound **9** is stacked against the aromatic rings of Trp286 and Tyr341 which located at the PAS. Moreover, additional hydrogen bond is observed between compound **9** and Phe295 (Figure 2). All these characters, together with the specific double bond configuration, contribute to the higher AChE inhibitory activity for compound **2** and **9**.

As depicted in Figure 3, the best ranked docking solutions revealed that BuChE can effectively accommodate the indolone ligands deeply inside the active-site gorge. Here Trp82 was found to have π-π stacking interaction with the benzene moiety of the ligands (**1**, **2** and **5**). Tyr332 was found to allow for further π-π stacking interaction with the indolone scaffold of the ligands (**2**, **5** and **14**). The ligand-BuChE complex was found to be stabilized mainly by the π-π stacking and hydrophobic interactions with the amino acid residues of the enzyme.

Comparing with the active site of AChE, the active-site gorge of BuChE is larger because the residues of acyl pocket (Phe295, Phe297) in AChE, which responsible for substrate specificity, are replaced by smaller aliphatic residues (Leu286, Val288) [16]. It enables the access

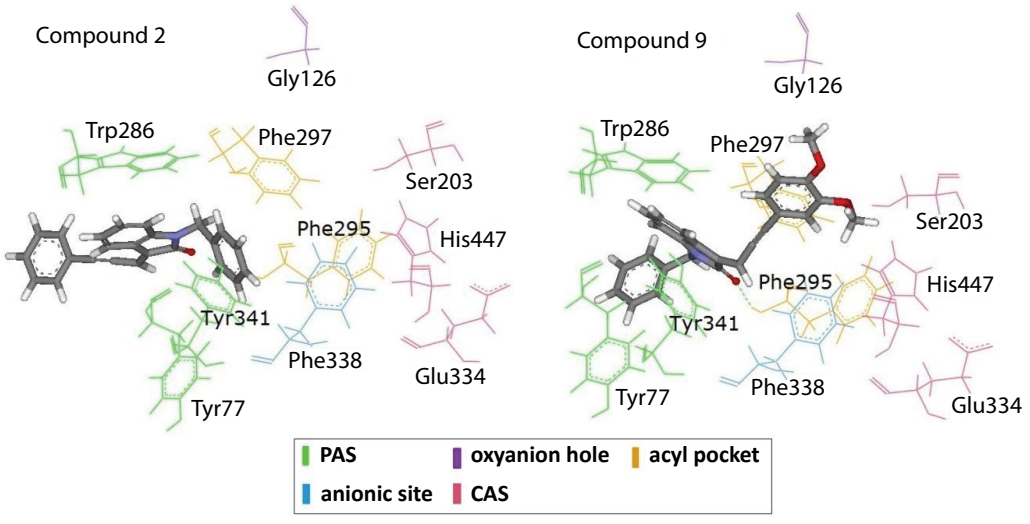


Figure 2: The binding mode of inhibitors (Compound **2** and **9**) at the *Ee*AChE gorge predicted by the docking simulation. The hydrogen bond is represented in dashed green line.

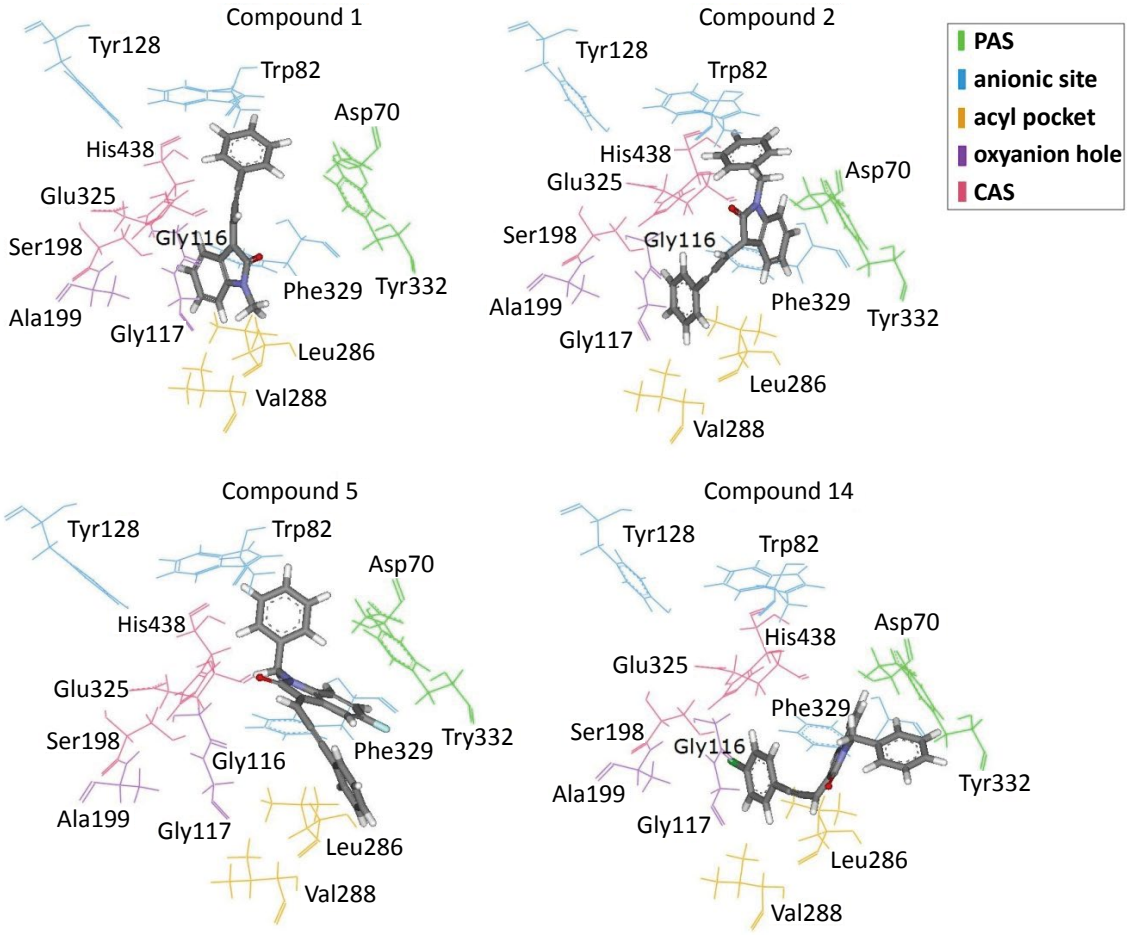


Figure 3: The binding mode of inhibitors (compound **1**, **2**, **5** and **14**) at the *eq*BuChE gorge predicted by the docking simulation.

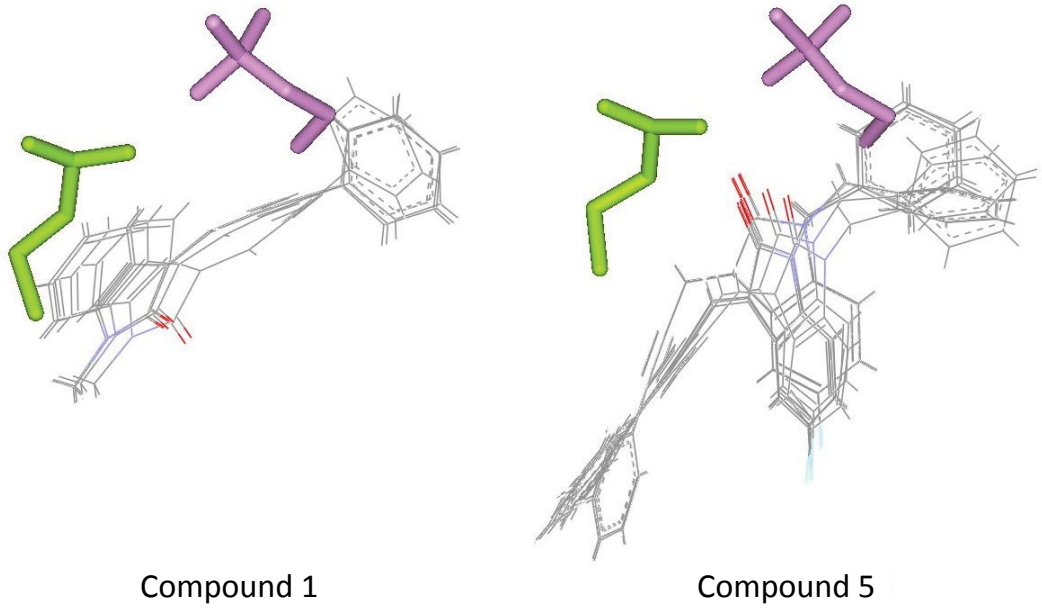


Figure 4: Superposition of the top-score cluster of the ligands with butyrate (green stick) and choline (pink stick). A series of snapshots of compound **1** and **5** at the active site of *eq*BuChE (PDB: 1C2B), and choline, butyrate at the active site of *h*BuChE (PDB: 1P0M, 1P0I).

of larger molecules to the catalytic center, but could not provide equivalent residues to establish adequately favorable π-π stacking, hydrogen bond or simple hydrophobic interactions that can stabilize the complex. It might be the reason why the molecule with increased width was found to have reduced inhibition of *eq*BuChE, especially for the 5- or 6-substituted indolones.

As Figure 4 shows, the top-score cluster of compound **1** and **5** that had a significantly lower FullFitness than the others is located near the central region of the active site gorge, especially near the choline binding sites, but not overlapping. According to the kinetic studies, compound **1** and **5** produce a mixed inhibition pattern which means it has a general equation that includes competitive, uncompetitive and noncompetitive inhibition as special cases. That is to say the inhibitor may bind to the *eq*BuChE whether or not the enzyme has already bound the substrate. As expected, there is a concordance between the results of docking and kinetic studies.

Conclusions

Sixteen derivatives of (E)-3-propargylene-1,3-dihydro-2*H*-indol-2-ones were synthesized. These compounds were subjected to pharmacological evaluation as multipotent inhibitors of AChE and BuChE. Three compounds (**2**, **5** a nd **12**) are able to inhibit the two forms of cholinesterases in the submicromolar range. *Ee*AChE affinity and selectivity are maximal for compound **9** (IC_{50}=0.011 ± 0.018 μM). Compound **14** is the most selective inhibitor of *eq*BuChE (IC_{50}=0.12 ± 0.22 μM) in this series. Kinetic studies of the BuChE inhibition suggested that compound **1** and **5** produce a mixed inhibition pattern. The molecular modeling investigation confirmed the result and indicated that π-π stacking interaction is a main contributor to the increase of inhibition efficiency. Along with further research, the dual and selective inhibitors could be considered as potential drug candidates for the treatment of AD. Moreover, it can be concluded that, regardless of the electronic features, substitution at 5- or 6- position of indolones is not generally favored for *eq*BuChE inhibition.

Acknowledgements

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