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Article

Synthesis and DPPH scavenging assay of reserpine analogues, computational studies and *in silico* docking studies in AChE and BChE responsible for Alzheimer's disease

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Alzheimer's disease (AD) is a fast growing neurodegenerative disorder of the central nervous system and anti-oxidants can be used to help suppress the oxidative stress caused by the free radicals that are responsible for AD. A series of selected synthetic indole derivatives were biologically evaluated to identify potent new antioxidants. Most of the evaluated compounds showed significant to modest antioxidant properties (IC_{50} value 399.07140.0±50 µM). Density Functional Theory (DFT) studies were carried out on the compounds and their corresponding free radicals. Differences in the energy of the parent compounds and their corresponding free radicals provided a good justification for the trend found in their IC_{50} values. *In silico*, docking of compounds into the proteins acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which are well known for contributing in AD disease, was also performed to predict anti-AD potential.

Uniterms: Alzheimer's disease. Antioxidant compounds. Chronic diseases. Density Functional Theory. Molecular docking. Computational studies. DPPH assay. Indole derivatives. Acetylcholinesterase (AChE). Butyrylcholinesterase (BChE).

A doença de Alzheimer (DA) é uma doença neurodegenerativado sistema nervoso central, em rápido crescimento, e antioxidantes ajudam a suprimir o estresse oxidativo causado por radicais livres, responsávies pela DA. Avaliou-se, biologicamente, série de derivados sintéticos de indol selecionados para identificar novos antioxidantes. A maioria dos compostos avaliados apresentou de significativa a boa propriedade antioxidante (valor de IC_{50} 399,07140.0 ± 50 µM). Eftuaram-se estudos de Teoria do Funcional de Densidade (DFT) com os compostos e os seus correspondentes radicais livres. As diferenças de energia entre os compostos protótipos e os radicais livres correspondentes proporcionaram boa justificativa para a tendência encontrada nos seus valores de IC_{50}. O ancoramento *in silico* dos compostos com a acetilcolinesterase (AChE) e com a butirilcolinesterase (BChE), que contribuem para a DA, foi, também, realizado para prever o seu potencial anti-DA.

Unitermos: Doença de Alzheimer. Compostos antioxidantes. Doenças crônicas. Teoria do Funcional de Densidade. Ancoramento molecular. Estudos computacionais. Ensaio de DPPH. Derivados do indol. Acetilcolinesterase (AChE). Butirilcolinesterase (BChE).

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INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative disorder of the central nervous system and the most common form of dementia in elderly people (Scarpini, Scheltens, Feldman, 2003). Affecting over 20 million people worldwide (Suh, Suslick, Suh, 2005), it is characterized by progressive loss of cognitive and memory, speech loss and personality changes and is one of the major causes of admissions to nursing homes (Wilson *et al*., 2007). Moreover, the cholinergic hypothesis postulates that a decrease in acetylcholine (ACh) levels within the brain causes gradual neurodegeneration and leads to AD (Francis *et al*., 1999).

The oxidative stress caused by free radicals has been shown to be a main contributor to the development of Alzheimer's disease (Floyd, Hensley, 2002). Currently drugs designed to treat AD are based on the improvement of cholinergic neurotransmission. This increase in neurotransmission is attained through the inhibition of an enzyme responsible for acetylcholine hydrolysis, AChE, and also by the inhibition of human plasma BChE (Silva *et al*., 2006). The first approved drugs for the management of the disease were the cholinesterase inhibitors tacrine, donepezil, rivastigmine, and galanthamine (Figure 1)**.** These drugs helped increase neurotransmission at cholinergic synapses in the brain and thereby improve cognition (Giacobini, 2004).

The practical efficiency of these acetylcholinesterase inhibiting drugs (AChE-I) for AD treatment remains controversial. Recent AD trials concluded that AChE-I therapies are not cost effective and show detrimental side effects. These problems have led to discontinuation in certain cases, as seen with tacrine (Petersen *et al*., 2005). For these reasons the interest in the development of new and potent cholinesterase inhibitors has increased in the last few years.

A number of studies have shown that in elderly people the antioxidant defense system loses its capacity to neutralize oxidative species, and this oxidative stress can act as a possible reason for the initiation and progression of AD (Floyd, Hensley, 2002). In the brains of patients with AD, according to oxidative and nitrosylative damage hypothesis, reactive nitrogen species (RNS) and reactive oxygen species (ROS) play important roles in the initiation and promotion of neurodegeneration. Clinical studies have shown the beneficial effects of high-dose antioxidants (Sokura *et al*., 2005). Thus, drugs that specifically scavenge oxygen radicals may have a particular therapeutic efficacy (Tan *et al*., 2003), and several antioxidants

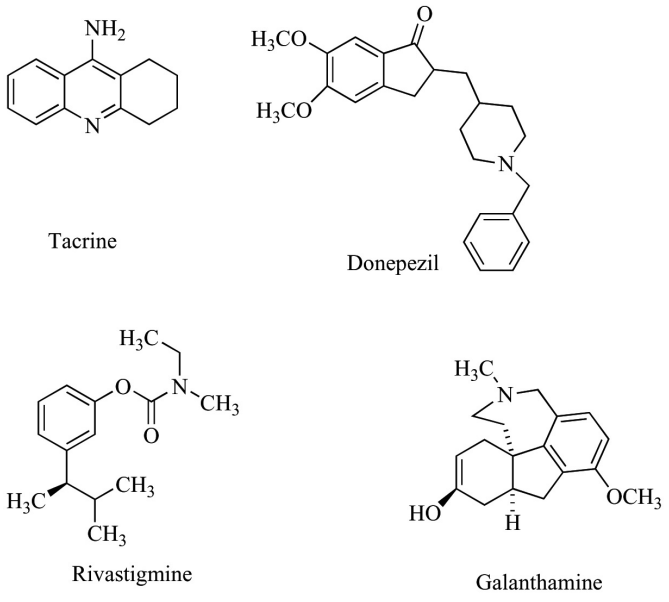


FIGURE 1 - Known drugs as cholinesterase inhibitors.

have been tested in clinical trials (Klatte *et al*., 2003). To design a new anti-AD agent, with potential AChE and BChE inhibitory activities, molecular modeling techniques are of great significance (Sarojini *et al.*, 2010, 2011).

Indole alkaloids are well known due to their extensive biological importance (Inman, Moody, 2013; Yar *et al.,* 2014b) including AChE (Monte-Millán *et al.,* 2006; Ismail *et al.,* 2012; Munoz-Ruiz *et al.,* 2005) and BChE (Boga *et al.,* 2011; Jakubowska *et al.,* 2012; Yar *et al.,* 2014a) inhibition activities. Compounds containing indole skeleton were also found to be the dual binding site AChE inhibitors, which in turn has a potential in disease modifying agents (Munoz-Ruiz *et al.,* 2005) (Figure **2, a** and **b**). Thus, there is a great deal of interest to develop dual binding site AChE inhibitors as a means to control AD (Monte-Millán *et al.,* 2006; Yar *et al.,* 2014a). Tacrine-melatonin hybrids (Figure **2, c**), which were potent inhibitors of human AChE and showed high oxygen radical absorbance capacity, were also synthesized (Franco, Isabel*,* 2006).

Among alkaloids, indole alkaloids represent one of the most interesting class of compounds, especially pentacyclic natural indole alkaloids such as reserpine (Figure 3). Reserpine was isolated from *Rauwolfia serpentina* and is regarded an important pharmacological agent due to its extensive use in the treatment of hypertension and mental disorders (Chatterjee, Pakrashi, Werner, 1956; Woodson *et al*., 1957; Lucas *et al*., 1963; Monachino, 1954).

Such promising biological potential of indole

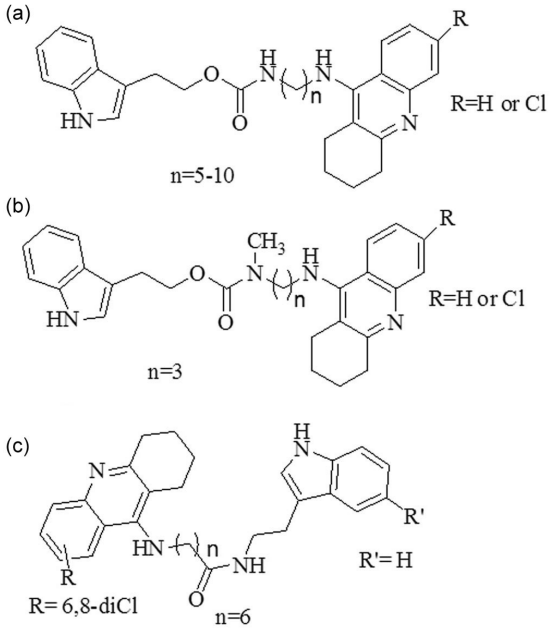


FIGURE 2 - Known indole derivatives as potent AChE inhibitors.

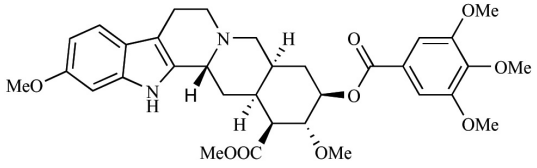


FIGURE 3 -The chemical structure of reserpine.

alkaloids has prompted us to synthesize and biologically evaluate a series of indole derivatives (Table I) to identify potent novel antioxidants. In this paper we report the synthesis of indole derivatives **1-4**, their DPPH scavenging activities and DFT studies based on the calculations of the energy differences of the indole parent molecules and their corresponding free radicals. These energy differences are correlated with the IC_{50} values for the first time ever. In the present study, the binding modes of the newly synthesized indole alkaloid **2** (predicted to have the most anti-oxidant potential in the tested series) with AChE and BChE are also demonstrated.

MATERIAL AND METHODS

Chemistry

Indole derivatives **1-4** were prepared by following our reported procedures (Yar *et al.,* 2012). For detailed synthetic methods and characterization of the compounds **1, 2, 3** and **4,** please see our previous paper (Yar *et al.,* 2012). The structures of the compounds **1-4** are shown in Table I.

Antioxidant activity

DPPH (1, 1-diphenyl-2-picryl hydrazyl radical) free radical scavenging activity: The reaction mixture containing 5 µL of test sample (1 mM in DMSO) and 95 µL of DPPH (sigma, 300 µM) in ethanol was employed. The reaction mixture was taken in a 96-well micro liter plate (molecular devices, USA) and incubated in Elisa at 37 °C for 30 min. At the end of the incubation period the absorbance was measured at 515 nm using a spectrophotometer. Percent radical scavenging activity was determined by comparison with a DMSO treated control (Table I). IC_{50} values showed concentrations of compounds to scavenge 50% DPPH radicals. BHA (3-*t*-butyl-4-hydroxy anisole) was used as positive control. All the chemicals used were of analytical grade purchased from Sigma-Aldrich USA.

Molecular docking

The crystal structure of AChE (PDB Id: 4EYZ) bound to donepezil and BChE (PDB Id: 4B0O) bound to benzyl pyridinium-4-methyltrichloroacetimidate were used for the docking studies. Dock Prep (Lang *et al.,* 2009) module was used in Chimera (Pettersen *et al.,* 2004) to delete solvent water molecules while retaining the water molecules within 5 Å of the bound ligand. Adding hydrogens and repairing the truncated side chains was done by using rotamer libraries (Dunbrack *et al.,* 2002). The prepared protein was saved in pdbqt format using Autodock Tools 1.5.6 (Morris *et al.,* 2009). The ligand coordinates were generated by using the molecular design program TorchV10lite (http://www.cresset-group.com/products/torch/torchlite/) and smiles were converted to 3D structure by using Openbabel (O'Boyle *et al.,* 2011) version (2.3.1). The pdbqt files for ligands were prepared by Autodock Tools 1.5.6 by using default parameters. Kollman charges were added to the standard residues while Gasteiger charges were added to the ligands by using the ADT tools. AUTODOCK 4 (Morris *et al.,* 2009) was used for docking calculations with a grid box size of 40×40×40 and centered on the co-crystallized ligand. For validating the docking protocol, co-crystallized ligand was docked with RMSD from reference ligand as 0.44 and 1.48 for donepezil and 15F, respectively. Docked poses were analyzed by using Autodock Tools 1.5.6 to get the best binding pose of compound **2a** with the lowest binding energy and the best overlap with the co-crystallized ligand.

Computational studies

All calculations were performed with Gaussian 09

(Frisch *et al.,* 2009). The geometries of the structures were optimized at hybrid B3LYP method (Becke, 1993; Lee *et al.,* 1988) with 6-31G* basis set. The method and basis set chosen provided a nice balance between cost and accuracy. The geometries of the optimized structures were visualized using Marvin View (MarvinView 5.9.4. ChemAxon.2012).

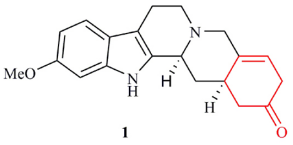
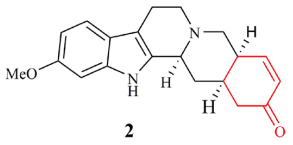
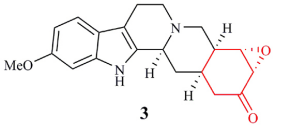
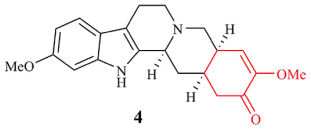
RESULTS AND DISCUSSION

The whole series of compounds (**1-4**) shown in Table I were screened for their antioxidant activity. Among these compounds, **2** presented the highest activity with an IC_{50} value of 140.0 µM, while compound **3,** in which an α, β-unsaturated double bond was converted into *cis*-epoxide, showed the second highest activity with an IC_{50} of 145.8 µM. As compared to the α, β-unsaturated ketone **2**, the activity of the β,γ-unsaturated ketone **1** was curtailed as its IC_{50} was found to be 178 µM. The possible reason for the lower activity in the case of compounds **3** and **1** could be due to the absence of conjugation in the ring E. When compound **2,** bearing the α,β-unsaturated ketone, was converted into compound **4,** which contained a methoxy group at the α-position, its activity was further decreased to IC_{50} 358 µM. The results are summarized in Table I**.**

Computational studies

Computational studies were carried out to correlate the structure-activity relationship of the compounds (**1-4)** as shown in Figure 4. Triggered by the well-established fact that in the DPPH assay scavenging of DPPH free radicals occurs by H-transfer from the radical scavengers, we became interested in correlating the ease of formation of free radicals by the structures **1-4** with their corresponding IC_{50} values. Only the ring E offers the structural variation in all the four structures and hence it is expected to be responsible for the variations of IC_{50} values. The energies of the optimized structures **1-4** and their corresponding proposed free radicals were calculated. We thought that a free radical with higher energy would be more difficult to form. For this purpose we compared the energies of free radicals for each molecule with their parent molecule, Table I. It is noted that their differences justify the trend found in their IC_{50} values. The greater the energy difference between a free radical and its parent molecule the more difficult it is for the molecule to offer a proton to scavenge the DPPH free radical; hence the molecule must exhibit less oxidant potential and have a higher IC_{50} value as a consequence. Indole **4** shows the

TABLE I - The quantitative estimation of antioxidant activity of the indole derivatives IC_{50} values are means of three independent experiments (Mean±SEM, n = 3)

Sr No.	% Scavenging activity of DPPH at 1000 µM	IC_{50} (µM)	Energy of parent molecule (au)	Energy of free radical (au)	Energy difference between parent molecule and radical (kcal/mol)	Structures
1	79.08	178.0	-1035.34	-1034.71	392.70	 **1**
2	79.9	140.0	-1035.31	-1034.71	375.65	 **2**
3	85.7	145.8	-1110.54	-1109.92	386.85	 **3**
4	74.2	358	-1149.86	-1149.24	392.90	 **4**
BHA	**92.1**	**44.2±.02**				

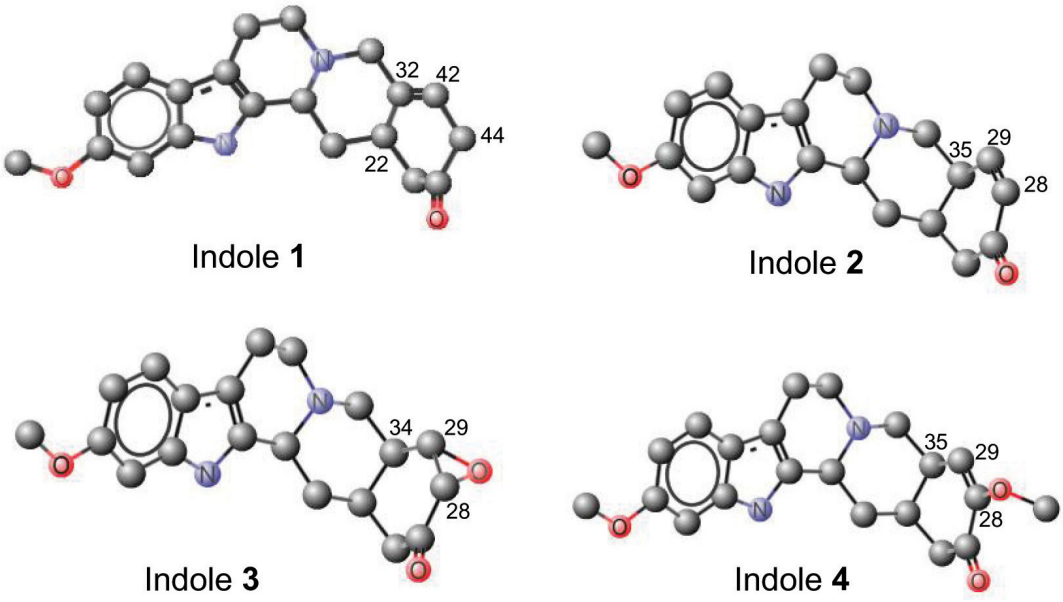


FIGURE 4 - Optimized geometries of the optimized compounds 1-4.

highest IC_{50} value (less potent) due to the highest energy difference. The lowest energy difference and lowest IC_{50} value were observed for the molecule **2,** thus it had the highest antioxidant potential. In the structures **2** and **4**, only one energetically favorable site (C-35) is available from where hydrogen abstraction would take place. In indole **1**, a hydrogen atom abstracted from the position C-44 shows more stability and this is consistent with the IC_{50} value observed experimentally. Similarly, for the indole **3**, hydrogen abstraction is believed to occur from C-28. This ultimately leads to ring opening of the epoxide.

Molecular modelling

AChE docking

All the compounds (**1-4**) were docked in the crystal structure of AChE (PDB Id : 4EYZ) by using AUTODOCK 4 (Morris *et al.,* 2009; Pettersen *et al.,* 2004). The docking conformation of compound **2** overlaps very well with the crystal structure conformation of donepezil bound to Acetylcholinesterase (PDB : 4EYZ) (Figure 5).

The N-H in the indole forms a hydrogen bond with Tyr 124. There are three π-π interactions present such as

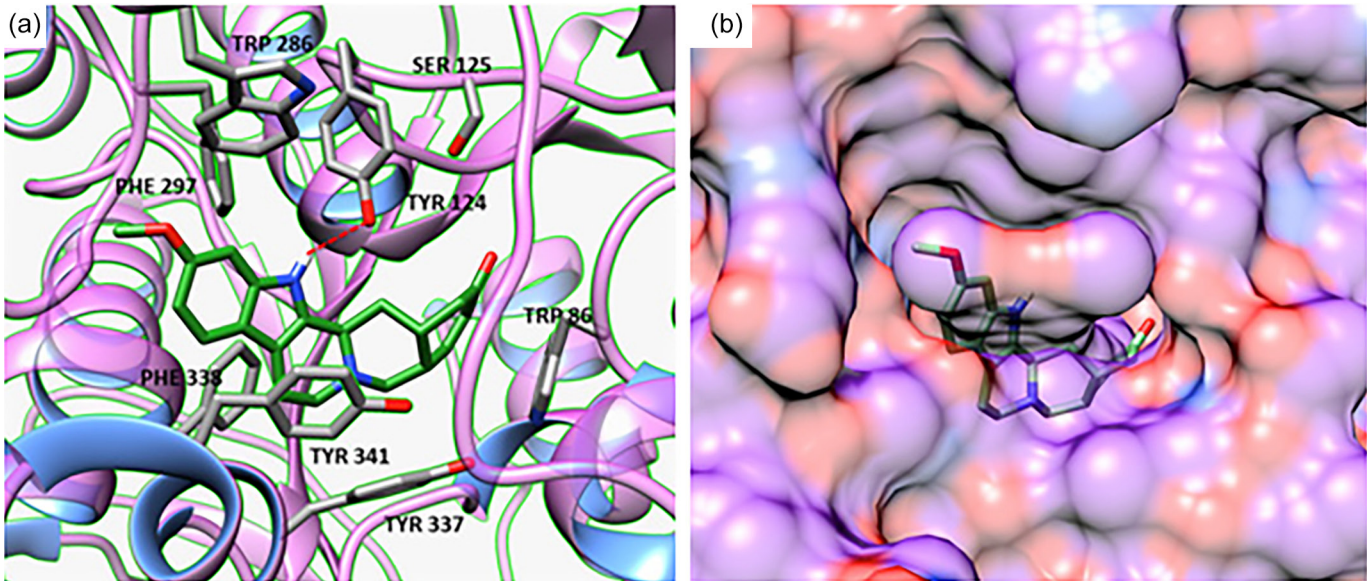


FIGURE 5 - (a) Docked binding mode of indole derivative **2** with AChE (PDB : 4EYZ); (b) The ligand molecule **2** buried in the AChE active pocket.

TABLE II -The docking results for ligand molecule **2** against AChE and BChE

Protein	Compound	H- bonds	Binding Energy (kcal/mole)	Protein	Compound	H- bonds	Binding Energy (kcal/mole)
AChE	1	1	-11.00	BChE	1	2	-8.48
	2	1	- 10.75		2	3	- 8.86
	3	2	- 10.76		3	2	- 8.19
	4	1	- 11.00		4	1	- 8.50
	Donepezil	1	-11.72		Benzyl pyridinium-4-methyltrichloroacetimidate	0	-6.60

with Tyr 341, Phe 338 and Trp 86. Compound **2** mostly forms hydrophobic interactions in addition to 1 hydrogen bond within the active site. The docking binding energy is -10.75 kcal/mol as compared to -11.72 kcal/mol for donepezil.

BChE docking

Similarly, we docked compound **2** in the crystal structure of BChE (PDB Id: 4B0O). The docked pose of **2** overlaps very well with the bound benzyl pyridinium-4-methyltrichloroacetimidate (PDB Id: 15F) (Figure 6). The free energy of binding was estimated to be −8.86 kcal/mol and −6.60 kcal/mol for **2** and pyridinium-4-methyltrichloroacetimidate (15F), respectively. Compound **2** forms 3 hydrogen bonds with Trp 82 and Trp 440 on one side and the $–OCH_3$ functional group forms hydrogen bond with water (W2013) on the other side. A dual binding site was not observed for this compound.

The docking of ligand molecule **2** with AChE (PDB : 4EYZ) revealed that the binding energy is minimum and the inhibitor compound is completely enclosed within the active pocket. Correspondingly the docking of the same ligand molecule with BChE (PDB Id: 4B0O) is also minimum binding energy. Theoretically it is predicted that molecule **2** is docked well in both AChE and BChE.

CONCLUSIONS

In summary, we have synthesized and identified a series of indole derivatives as potent novel antioxidants. Computational studies were also performed. The ease with which the free radicals are formed could suggest a plausible reason for the trend found in their antioxidant potential. Among compounds **1, 2, 3**, and **4**, having IC_{50} 178.0, 140.0, 145.8 and 358.0 µM respectively, compound **2** showed the most appreciable activity. In the future, these compounds may act as potential lead molecules in the field of potent antioxidant research. The theoretical calculations made can be applied to the design of new molecules with

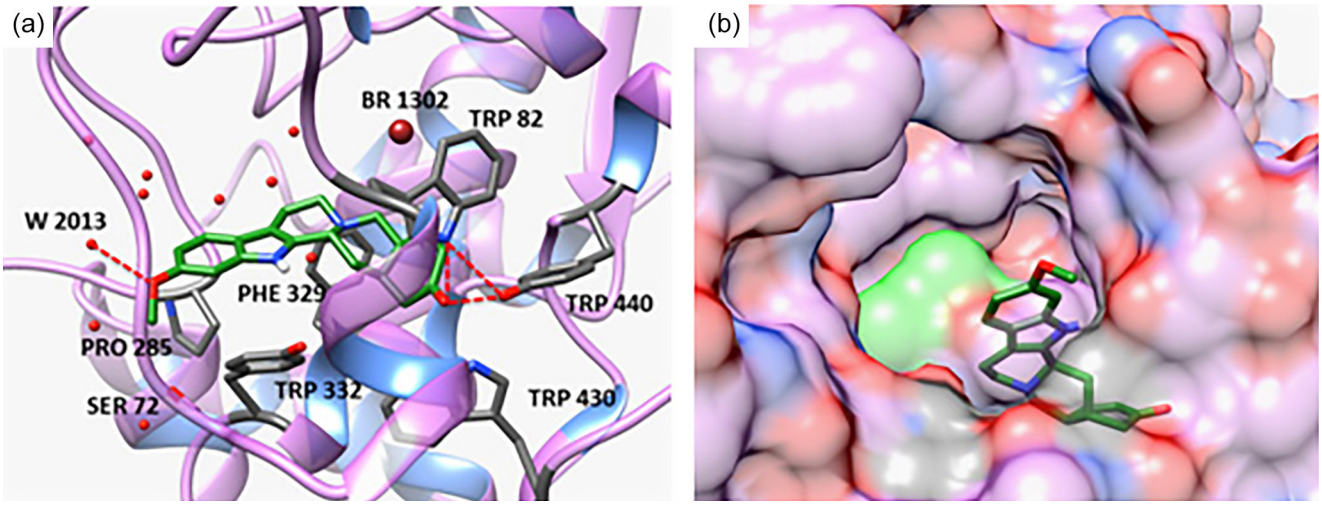


FIGURE 6 -(a) Docked binding mode of indole derivative **2** with BChE (PDB : 4B0O); (b) The ligand molecule **2** buried in the BChE active pocket.

greater potency and we are currently conducting research on this matter. *In silico*, docking of compounds into the proteins (Acetylcholinesterase and Butyrylcholinesterase), which are well known for contributing to AD disease, was also performed to predict their AChE and BChE inhibition potential. Among all of the compounds **(1-4**), compound **2** showed the best inhibition of both AChE and BChE.

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