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In Silico Comparison of Synthetic and Natural Molecules Bindings with Acetylcholinesterase Enzyme using Molecular Docking

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Abstract. Inhibition of Acetylcholinesterase (AChE) is an important approach for Alzheimer's disease (AD) treatment. Different synthetic and natural inhibitors are used for Acetylcholinesterase inhibition. Synthetic inhibitors Polyphenols (Tacrine, Donepezil, Rivastigmine, Galantamine) and Natural molecules from Green tea which mainly contains catechins (Epicatechin, Epicatechin Gallate, Epigallocatechin, Epigallocatechin Gallate) are used to inhibit Acetylcholinesterase. In this work we use molecular docking methods to identify the ligand which has the best interaction energy with AChE among synthetic and natural products, and also descript binding affinity in purpose to design new inhibitor ligands. Obtained results from Docking and analyze of complexes parameters showed that the best affinity binding was observed for both (Galantamie and Epicatechin Gallete).This latter leads to same conclusion with experimentation inhibition study. We observed also that bulky group causes conformational rearrangement in the active pocket, which will probably give better interactions.

Keywords: Alzheimer; acetylcholinesterase enzyme; polyphenol; green tea; molecular docking.

1 Introduction

Alzheimer's disease (AD), also known as just Alzheimer's, is a chronic neurodegenerative disease that usually starts slowly and gets worse over time. It is the cause of 60% to 70% of cases of dementia. The most common early symptom is difficulty in remembering recent events (short-term memory loss). As a person's condition declines, they often withdraw from family and society. Gradually, bodily functions are lost, ultimately leading to death. Although the speed of progression can vary, the average life expectancy following diagnosis is three to nine years [1-2].

Several studies indicated that the Green tea is useful for the people suffering Alzheimer's disease (AD). The work of Katergaris et al. has proved that Green Tea Catechins GTC extracts or Epigallocatechin gallate EGCG in its pure form may serve as nootropic options in the prevention or treatment of neurodegeneration associated diseases such as AD [3]. The use of Acetylcholinesterase (AChE) inhibitors to treat symptoms caused by cholinergic imbalances in Alzheimer disease (AD) represented a rational approach[4].

Recently, Green Tea Catechins (GTC) have been related to a variety of different beneficial health effects particularly with respect to their potential for preventing and treating different cancers [5] cardiovascular diseases [6], and some of the neurodegenerative diseases [7] in humans. Accounting for at least half of the total GTC [8], the most predominant catechin found in green tea is Epigallocatechin gallate (EGCG), which has been ascribed numerous beneficial properties including antioxidant [9], anti-inflammatory [10], anti-microbial [11] and anticancer effects [12]. With the development of theoretical methods in drug design it is nowadays possible to elucidate and compare between different inhibitors of the same enzyme, and get conclusions about the inhibition power of each inhibitor.

In this work our objective is to evaluate theoretically the AChE inhibition power of two series of

inhibitors naturals (molecules of Green tea (GT) which mainly contains Catechins (Epicatechin, Epicatechin Gallate, Epigallocatechin, Epigallocatechin Gallate) and synthetics (tacrine, donepezil, rivastigmine, galantamine). By molecular docking we can study the complex (enzyme/inhibitor) formation and consequently delay its progression, in order to determine interaction of the complex (Natural and Synthetic) to the enzyme.

2 Materials and Methods

2.1 Acetylcholinesterase Enzyme

Acetylcholinesterase (HGNC symbol AChE), also known as AChE or acetylhydrolase, is the primary cholinesterase in the body. It is an enzyme that catalyzes the breakdown of acetylcholine and of some other choline esters that function as neurotransmitters. AChE is found at mainly neuromuscular junctions and in chemical synapses of the cholinergic type, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes. It is the primary target of inhibition by organo-phosphorus compounds such as nerve agents and pesticides. The structure and mechanism of action of AChE have been elucidated from the crystal structure of the enzyme [14].

2.2 Synthetic Inhibitors of AChE

An acetylcholinesterase inhibitor (often abbreviated AChEI) or anti-cholinesterase is a chemical or a drug that inhibits the acetylcholinesterase enzyme from breaking down acetylcholine, thereby increasing both the level and duration of action of the neurotransmitter acetylcholine. Acetylcholinesterase inhibitors are classified as reversible, irreversible, or quasi-irreversible (also called pseudo-irreversible) [15]. Acetylcholinesterase inhibitors (tacrine, donepezil, rivastigmine, galantamine), in the cholinergenic family, act to slow acetylcholinesterase activity in order to maintain high levels of acetylcholine, which is greatly reduced in patients with cholinergenic disease. 'Alzheimer's. Acetylcholine is a neurotransmitter of the brain involved in the transmission of messages to the centers of memory, reasoning and other processes of thought [16].

Donepezil is a specifically designed piperidine derivative with reversible acetylcholinesterase inhibitor activity. It has a much higher specificity for AChE inhibition compared with tacrine [17] and its CNS selectivity is highlighted by the lack of activity in peripheral tissue such as cardiac tissue or gut smooth muscle.

Rivastigmine is a brain selective carbamate AChe inhibitor. It is known as a ‘pseudo-irreversible’ inhibitor because it mimics ACh by binding with the enzyme AChe forming a carbamylated complex. This prevents further enzyme-catalysed hydrolysis of ACh for several hours after the drug has been eliminated from the plasma. Thus, despite a half- life of only 1 h, rivastigmine has a duration of action of about 10 h [18].

Galantamine (Nivalin, Razadyne, Razadyne ER, Reminyl, Lycoremine) is used for the treatment of mild to moderate Alzheimer's disease and various other memory impairments, in particular those of vascular origin [19].

Tacrine, an aminoacridine, has several actions such as monoamine oxidase inhibition, potassium channel blockade and interaction with subtypes of muscarinic and nicotinic receptors. However the most prominent action is as a centrally active reversible cholinesterase inhibitor. Tacrine is rapidly absorbed and cleared by the liver during a first pass metabolism [20].

2.3 Natural Inhibitors of AChE

Tea is an aromatic beverage commonly prepared by pouring hot or boiling water over cured leaves of the camellia sinensis native to Asia. After water, it is the most widely consumed drink in the world. Tea is also a source of antioxidants in the form of polyphenols of different natures depending on the type and method of manufacture. Green tea contains mainly catechins (Epicatechin, Epicatechin Gallate, Epigallocatechin, Epigallocatechin Gallate), and its fermentation transforms them into theaflavins and thearubigines [21]. Gallocatechol or gallocatechin (GC) is a flavan-3-ol known as one of the antioxidants

present in food. Epicatechin gallate (ECG) is a flavan-3-ol, one of the flavonoides, consequent in green tea. Epigallocatechin gallate (EGCG), is the most plentiful catechin in tea, is a polyphenol with a potential affection on the human health and disease. Indeed it is used in many dietary supplements.
Natural and synthetic inhibitors chosen for for studying inhibition of Acetylcholinesterase (AChE) are given in table1.

Table1: Synthetic and Natural for AChE

Ligand	Name	IUPAC name	PubChem CID	Molar mass (g/mol)
1	Donepezil (Aricept)	2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one	3152	379,492
2	Rivastigmine (Exelon)	[3-[(1S)-1-(dimethylamino)ethyl]phenyl]N-ethyl-N-methylcarbamate	77991	250,3367
3	Galantamie (Reminyl)	(4aS,6R,8aS)-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6H-benzo[2,3]benzofuro[4,3-cd]azepin-6-ol	9651	287,3535
4	Tacrine(Cognex)	1,2,3,4-tetrahydroacridin-9-amine	1935	198,2637
5	Epicatechin	(2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	72276	290.271
6	Epicatechin Gallate	[(2R,3R)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate	107905	442.37
7	Epigallocatechine	(2R,3S)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	65084	306.27
8	Epigallocatechine Gallate	[(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate	65064	458.372

2.4 Preparation and Optimization of both Enzyme and Inhibitors Natural and Synthetic

Download of Acetylcholinesterase was done from PROTEIN DATA BANK (code 4TVK) with three-dimensional structure obtained by X-ray diffraction (resolution 2.3 Å) (Figure 1). Note that the Acetylcholinesterase crystallizes as a monomer (Figure 1) with 534 residues and 8377 atoms. Compounds of inhibitors were downloading from Pub Chem data base. Using MOE software (Molecular operating environment) [22]. The active site in the enzyme and we minimize the energy of both enzyme and molecules (a, b, c). Energy minimizing was done under following conditions: Temperature = 300 °K, pH = 7, the geometry was performed using the field strengths in the MMFF94x implanted in MOE and Hamiltonian AM1. Docking was performed using London dG force and refinement of the results was done using Force field energy. Figure 2 shows the active site of the enzyme with molecule of co-crystallization. Minimized energy of ligands and their toxicity are obtained by MOE software (table 4).

Table 2. Synthetic polyphenols inhibitors of AChE

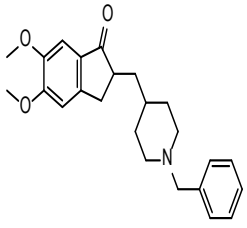
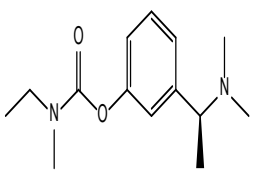
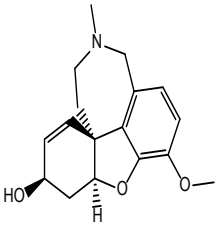
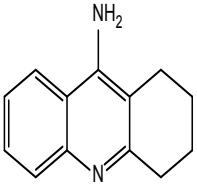
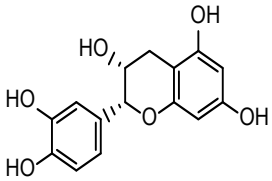
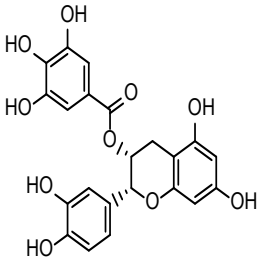
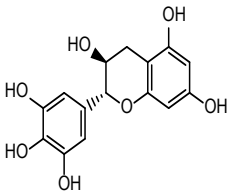
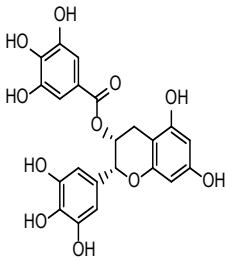
			
Ligand1 (CID 3152)	**Ligand2 (CID 77991)**	**Ligand3 (CID 9651)**	**Ligand 4(CID 1935)**

Table 3. Natural inhibitors of AChE

			
Ligand5 (CID72276)	**Ligand6 (CID107905)**	**Ligand7 (CID65084)**	**Ligand8 (CID 65064)**

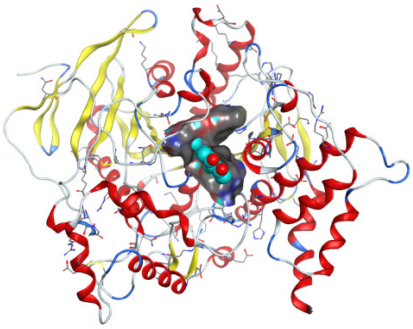


Figure 1: 3D structure of (AChE)

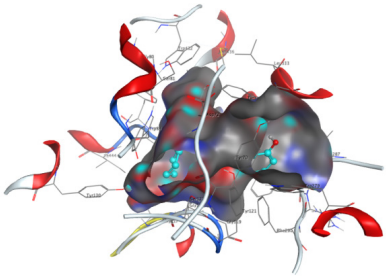


Figure 2: Isolated enzyme active site.

Table 4. Energy minimization results of synthetic and natural ligands

Ligand	Molecules	Energies(Kcal/mol)	LogP	LogS	Toxcicity
1	Donepezil	8.70672e+001	3.21	-4.20	No
2	Rivastigmine	3.17161e+001	1.44	-1.07	No
3	Galantamie	6.76170e+001	0.70	-2.27	No
4	Tacrine	4.82338e+001	2.70	-2.78	No
5	Epicatechin	5.77003e+001	1.64	-1.74	No
6	Epicatechin gallate	7.73960e+001	2.62	-3.03	No
7	Epigallocatechine	5.56122e+001	1.35	-1.37	No
8	Epigallocatechine Gallate	7.64465e+001	2.33	-2.67	No

2.5 Docking and Building Complexes

The next step consists of positioning of ligands in AChE active site. For this, we used the Molecular

Docking Module using MOE software. Once the ligand -receptor complex is formed, the most stable conformation is adapted and will show the lowest energy. The purpose of the Dock application is looking at favorable conformational binding between medium size ligands and a not so soft macromolecular target, which is usually a protein [24]. For each compound a number of conformations called poses were generated for the first approach to identify favorable binding modes [25]. The search for binding modes is generally constrained to a small specific region of the receptor called the active site.

3 Results and Discussion

Table 5. Energy Balance of complexes formed between ligands and AChE(Kcal/mol).

Complex	Score	Rmsd-refine	E-Conf	E-PLACE	E-SCORE1	E-REFINE	E-SCORE2
Ligand ref	-18.208	2.0142	137.1829	-72.5964	-13.2105	19.2969	-18.2089
Complexe-1	-14.080	1.72080147	28.4046	-71.1366	-12.4092	0.7917	-14.0808
Complexe-2	-12.199	1.3913	-59.1072	-46.4278	-11.3380	-11.80745	-12.1994
Complexe-3	-15.890	1.0627	4.5286	-56.8647	-12.7848	34.1320	-15.8909
Complexe-4	-9.4663	0.9120	38.2057	-62.1670	-9.9031	-12.7840	-9.4663
Complexe-5	-16.1771	0.7566	25.6229	-89.2038	-15.3782	3.0038	-16.1771
Complexe-6	-21.1324	2.3473	45.5496	-94.0829	-16.7406	-1.7876	-21.1324
Complexe-7	-16.5014	1.7588	27.0696	-120.7888	-14.7346	-6.9466	-16.5014
Complexe-8	-19.2184	1.2014	23.7697	-142.832	-17.0954	-26.1940	-19.2184

S: the final score; is the score of the last step, rmsd_refine: the mean square deviation between the l aying before refinement and after refinement pose, E_conf: energy conformer, E_place: score of the placement phase, E_scor1: score the first step of notation, E_refine: score refinement step and number of conformations generated by ligand E_scor2: score the first step notation, number of poses: Number of conformations [22].

Table 6. Interactions patterns observed in the determined complexes between the ligands and the corresponding amino acid in AChE.

Ligand	Name	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
1	Donepezil	O6 6	O TYR 70	(A)H-donor	2.97	-2.3
		O8 8	OE1 GLN 69	(A) H-donor	2.44	1.2
		O11 11	OG SER 200	(A) H-donor	2.57	-1.4
2	Rivastigmine	O2 2	OH TYR 121	(A) H-acceptor	2.77	-3.4
		N3 3	OE1 GLU 199	(A) ionic	3.52	-3.1
		N3 3	OE2 GLU 199	(A) ionic	3.95	-1.5
		C10 11	6-ring PHE 330	(A) H-pi	4.76	-0.6
3	Galantamie	N4 4	OE1 GLU 199	(A) H-donor	2.63	-15.8
		N4 4	OE1 GLU 199	(A) ionic	2.63	-7.5
		N4 4	OE2 GLU 199	(A) ionic	3.54	-1.7
4	Tacrine	No interaction				
5	Epicatechin	O3 4	OE1 GLU 199	(A) H-donor	2.77	-4.7
6	Epicatechin gallate	O4 4	OE1 GLU 199	(A) H-donor	2.70	-4.3
		O8 8	O SER 81	(A) pi-pi	3.05	-1.6
		6-ring	6-ring PHE 330	(A) H-donor	3.58	-0.0
7	Epigallocatechine	O7 8	O TYR 70	(A) H-donor	3.00	-2.1
		6-ring	OH TYR 121	(A) pi-H	4.51	-0.6
8	Epigallocatechine Gallate	O6 6	O TYR 70	(A) H-donor	2.97	-2.3
		O8 8	OE1 GLN 69	(A) H-donor	2.44	1.2
		O11 11	OG SER 200	(A) H-donor	2.57	-1.5

Results presented in tables 5 and 6 show that the orientation of the ligands plays a significant role for the positioning of the ligands in the active site of the enzyme, one can conclude that the introduction of bulky groups causes a rearrangement of conformation inside the cavity of the active site, which will be probably the complementarity and consequently the activity [26]. 2D molecular method of the screen has been attributed to the MOE (Molecular Operating Environment) software, which is designed to visualize the active sites of the complex (protein-ligand). The ligand is prepared and made with an improved 2D depiction layout algorithm, and protein residues version are arranged around it to indicate links spatial proximity. Residues are marked with their amino acid code of 3 letters, and job classification [27-28]. If there are multiple channels in the system, the positions are prefixed by the letters of the alphabet. Interactions between 2.5 Å and 3.1 Å are considered high and those between 3.1Å and 3.55Å are average. Greater than 3.55Å interactions are weak [29].

Table 6 also shows that for the tow best inhibitors interaction with active site is assured by interaction with the GLU 199 amino acid.

3.1 Synthetic Compounds

These results show that the complex- 3 has the lowest energy (-15.8909054 Kcal/mol) and is more active than complex - 1 (-14.0808125 Kcal/mol) which is more active than complex -2 (Figure 3c) (-12.1994009 Kcal/mole).

For complex 3 (Figure 3a): *Galantamie* interacts with the amino acid [GLU 199 (A) H-donor; GLU 199 (A) ionic (OE1, OE2)] at a distance of 2.63; 2.63; 3.54 Å, respectively (for the 1st and 2nd strong interaction, 3rd average interaction), with the existence of two electric forces PHE 330 and TRP 84 which suggest that *Galantamie* has important binding affinity with Acetylcholinesterase and interferes with [GLU 199 (A) H-donor; GLU 199 (A) ionic (OE1, OE2)] [30].

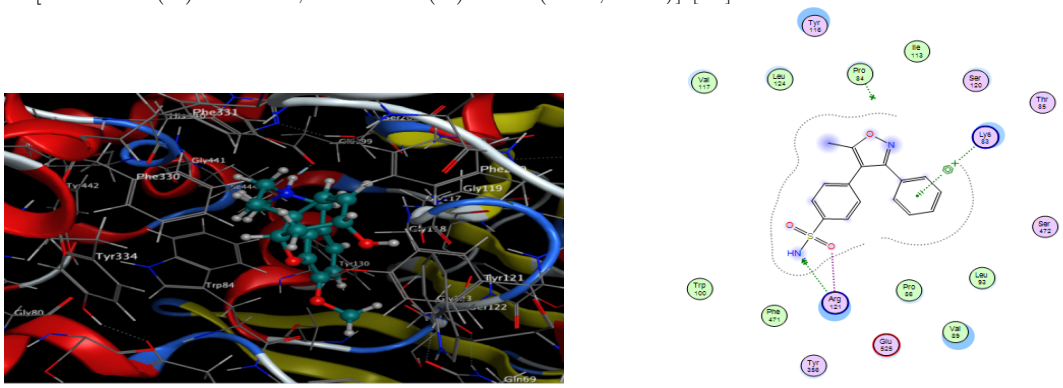


Figure 3 (a). Diagram interaction of complex-3 (AChE+*Galantamie*)

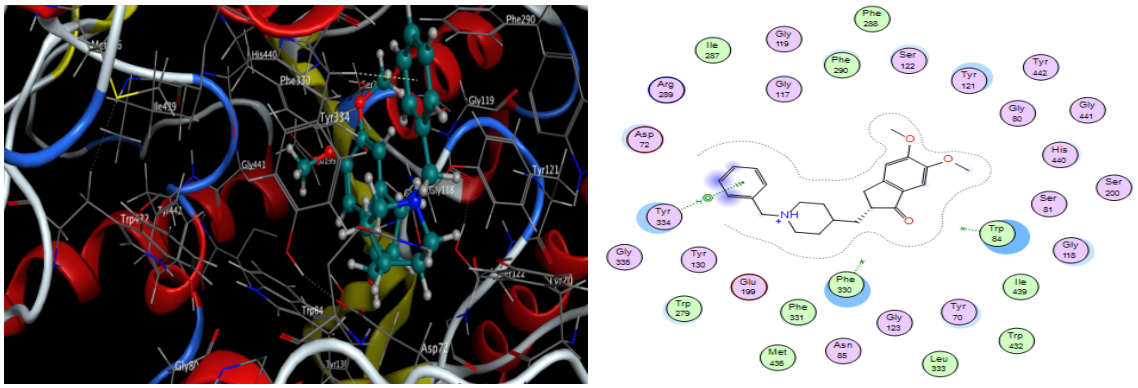


Figure 3 (b). Diagram interaction of complex-1(AChE+*Donepezil*)

For complex 1 (Figure 3b): *Donepezil* interacts with the amino acid [TYR 334 (A) pi-H] at a distance of 4.03 Å (for the 1st weak interaction), with the existence of two electric forces PHE 330; TRP 84

which suggest that *Donepezil* can have good affinity interaction with Acetylcholinesterase and interfere with [TYR 334 (A) pi-H] [30].

For complex 2 (Figure 3c): *Rivastigmine* interacts with the amino acid [TYR 121 (A) H-acceptor; GLU 199 (A) ionic (OE1, OE2)]; PHE 330 (A) H-pi] at a distance of 2.77; 3.52.

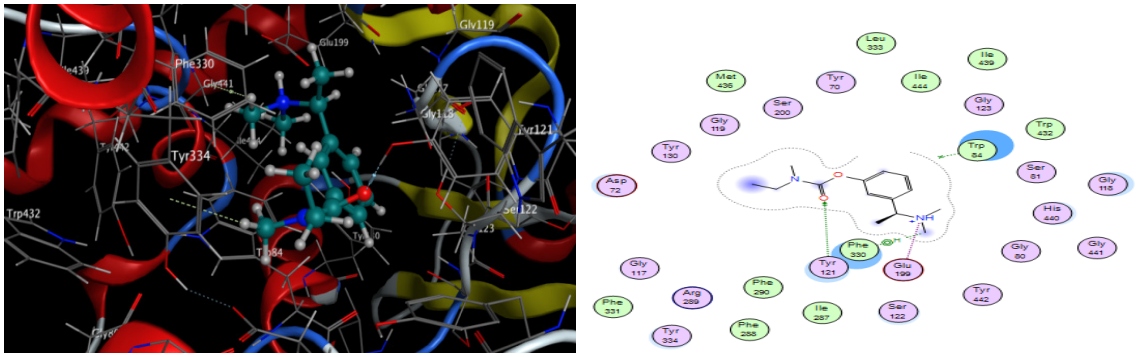


Figure 3 (c). Diagram interaction of complex-2 (AChE+*Rivastigmine*)

3.2 Natural Compounds

These results show that the complex-6 has the lowest energy (-21.1324406 Kcal/mol) and is more active than complex -8 (-19.218462 Kcal/mol) which is more active than complex -3 (-16.5014267 Kcal/mole).

The energy of the reference ligand is important in comparison with that obtained by the Natural ligands Epicatechin gallate and Epigallocatechine Gallate. Therefore, we can validate Epicatechin Gallate as a reference inhibitor (Figure 4a).

Energy complex (Ref: -18.2089138Kcal/mol, Epigallocatechine Gallate -19.218462Kcal/mol and Epicatechin Gallate -21.1324406 Kcal/mol).

For complex 6 (Figure 4b): Epicatechin gallate interacts with the amino acid [GLU 199 (A) H-donor (OE1; O); PHE 330 (A) pi-pi] at a distance of 2.70; 3.05; 3.58 Å, respectively (for the 1st and 2nd strong interaction, 3rd weak interaction), with the existence of one electric force TRP 84 which suggests that Epicatechin gallate can inhibit Acetylcholinesterase and interfere with [GLU 199 (A) H-donor (OE1; O) ; PHE 330 (A& [30].

For complex 8 (Figure 4c): Epigallocatechine Gallate interacts with the amino acid [TYR 70 (A) H-donor; GLN 69 (A) H-donor; SER 200 (A) H-donor] at a distance of 2.97: 2.44; 2.57 Å, respectively (for the 1 st , 2nd and 3 rd strong interaction), with the existence of two electric forces PHE 330; TRP 84 which suggest that Epigallocatechine Gallate can inhibit Acetylcholinesterase and interfere with [TYR 70 (A) H-donor; GLN 69 (A) H-donor; SER 200 (A) H-donor] [30].

For complex 7 (Figure 4d): Epigallocatechine interacts with the amino acid [TYR 70 (A) H-donor; TYR 121 (A) pi-H] at a distance of 3.00; 4.51 Å, respectively (for the 1st strong interaction; and 2nd weak interaction) with the exixtence of two electric forces PHE 330; TRP 84 which suggest that Epigallocatechine can inhibit Acetylcholinesterase and interfere with [TYR 70 (A) H-donor; TYR 121 (A) pi-H] [30].

The inhibitor of the co-cristalisation 2-(2-[(6-chloro-1, 2, 3, 4-tetrahydroacridin-9-yl) amino]-5-hydroxynaphthalene-1; 4-dione Chain A $C_{25}H_{22}ClN_3O_3$ (TJH).

Energy (*Galantamie* -15.8909054 Kcal/mol<*Donepezil* -14.0808125 Kcal/mol<*Rivastigmine* -12.1994009 Kcal/mol).

Energy (Epicatechin Gallate -21.1324406Kcal/mol<Epigallocatechine Gallate -19.218462Kcal/mol<Epigallocatechine -16.5014267 Kcal/mol).

Galantamie and Epicatechin Gallate would be the best to slow down the evolution of studied pathology (Alzheimer's disease (AD).

The examination of the enzymatic cavity confirms that the structure of natural inhibitor Epicatechin gallate with the groupings of atoms (O4, O8) presents a strong interaction hydrogen bond with [GLU 199 (A) H-donor (OE1; O)] and the structure of inhibitor synthetic *Galantamie* with the atom (N4) presents a strong interaction hydrogen bond with [GLU 199 (A) H-donor; GLU 199 (A) ionic (OE1)] and one better complementarity with Acetylcholinesterase (AChE).

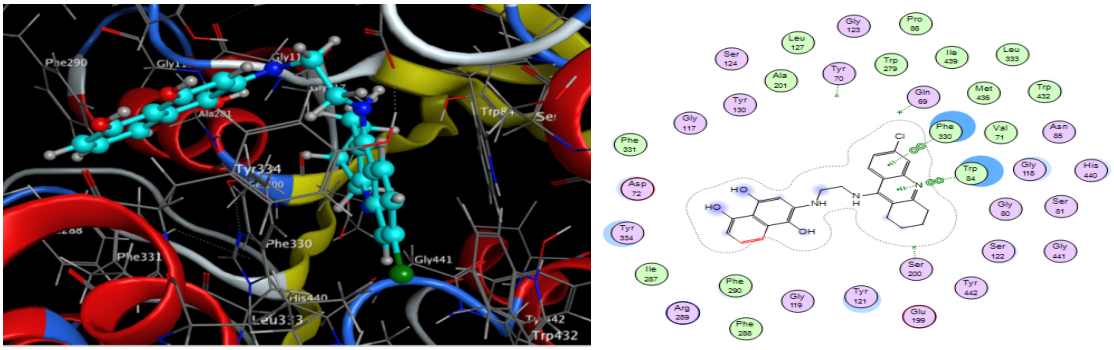


Figure 4 (a). Diagram interaction of complex-Lig Ref (AChE +TJH)

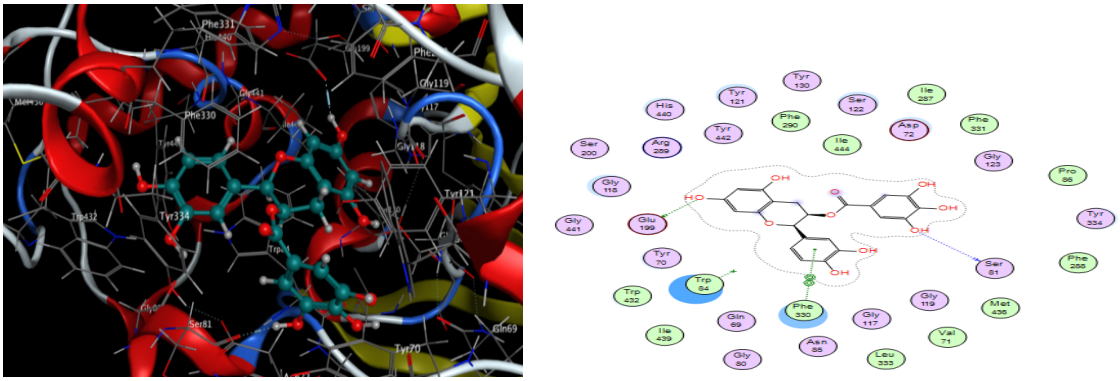


Figure 4 (b). Diagram interaction of complex-2 (AChE + Epicatechin Gallate)

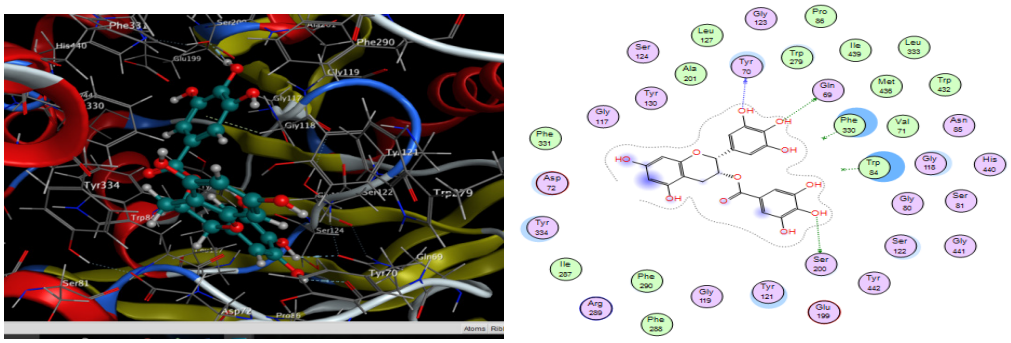


Figure 4 (c). Diagram interaction of complex-4 (AChE + Epigallocatechine Gallate)

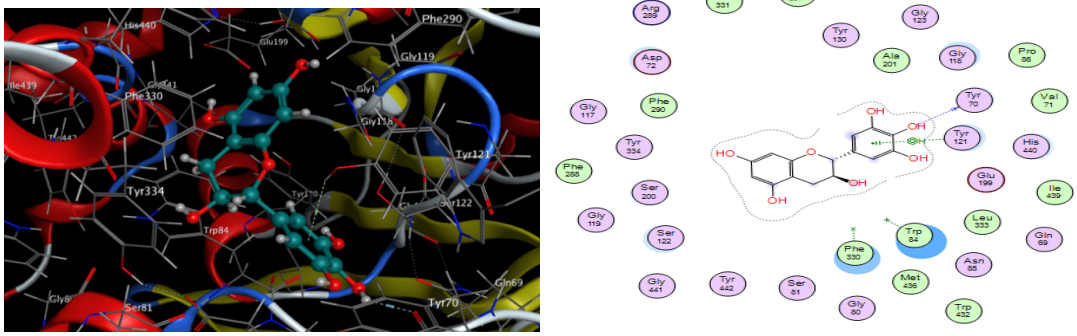


Figure 4 (d). Diagram interaction of complex-3 (AChE + Epigallocatechine)

4 Conclusion

Acetylcholinesterase (AChE) inhibition analyses by studying interactions and binding with different synthetic and natural ligands has been investigated. Study result showed Polyphenol (*Galantamie-ligand 3*) has the best binding with enzyme and may present the best inhibition activity of AChE, whereas, from green tea *Epicatechin Gallate* has the best interaction with AChE which may be the best natural inhibitor. Natural compounds are in accordance with Lipinski rules for drug orally administration [31-32]. On the other hand one comparing between those two inhibitors we conclude that the natural inhibitor present better activity compared to synthetic one and it can contribute as an efficient treatment of the Alzheimer disease. Because of none signaled side effect of Epicatechin Gallate on human health, this latter has to be more investigated to perform his activity and power inhibition using bulky group. Also these results are very significant in field of phototherapy and Alzheimer diseases prevention.

Conflict of Interest
The authors declare no conflict of interest.
Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.
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