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Theoretical Study of the Inhibition of β-Secretase

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The brain of the human being suffering of the Alzheimer disease undergoes modifications caused by enzymes *e.g.*, β-secretase and γ-secretase. These enzymes produce fragments of amyloid β. The inhibition of the cleavage of this amyloïd β by enzymes β and γ-secretase prevents the amyloïd forming deposits plates and can improve the efficiency of the system of the elimination. Our research objective is to study the inhibition of the β-secretase by the methods of molecular modelling.

Key Words: Alzheimer disease, β-Secretase inhibitors, Molecular modelling.

INTRODUCTION

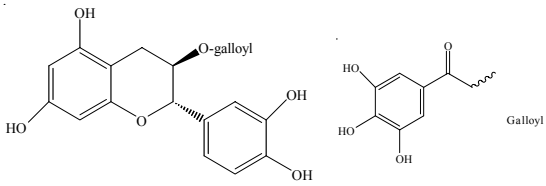
Alzheimer disease is a degenerative disease of the brain from which there is no recovery. The disease attacks nerve cells in all parts of the cortex of the brain, as well as some surrounding structures, thereby impairing a person's abilities to govern emotions, recognize errors and patterns, coordinate movement and memory. At the last, an affected person loses all memory and mental functioning[1].

β-Secretase is an enzyme that appears to be directly involved in the early development of Alzheimer disease. β-Secretase[2,3] is a protease (an enzyme that catalyzes the splitting of interior peptide bonds in a protein).

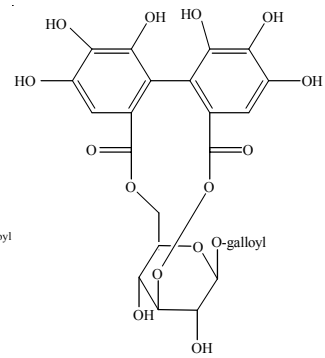
The discovery of this enzyme was made in 1999[4-7] and may lead to the development of drugs designed (inhibitors) to block this enzyme and hopefully block the progression of Alzheimer disease.

The inhibitors used in present work were divided into 2 categories A and B on the basis of their geometries and their nature.

Category A: The inhibitors of this category are natural substances extracted the green tea[8], their structure is represented below:

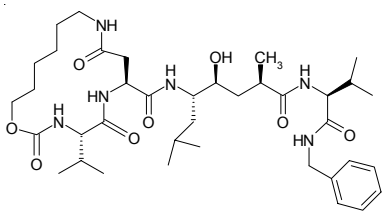


Inhibitor 1: (Catechin gallate)

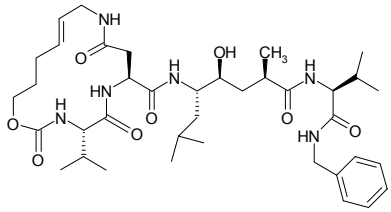


Inhibitor 2: (Corilagin)

Category B: The inhibitors of this category represent the cycloamide-urethane derivatives[9], they are obtained by synthesis. They have the following structures:



Inhibitor 3



Inhibitor 4

THEORETICAL APPROACH

The downloading of β-secretase was made from the data base Bookhaven Protein Data Bank (access code 1FKN) it is co-crystallized with inhibitor OM99-2.

The three-dimensional structure of β-secretase was obtained by diffraction in X-ray with a resolution (1.90 Å). It is noticed that β-secretase crystallizes in the dimer forms (Fig. 1).

Fig. 1. The dimerous form of β-secretase

With the reducing effect of molecular modelling, we simplified the model of the enzyme and retained only one monomer (Fig. 2). This monomer comprises the amino acids forming the active site (Fig. 3).

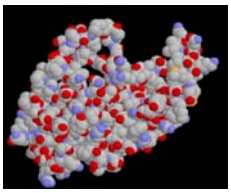


Fig. 2. Simplified model of β-secretase

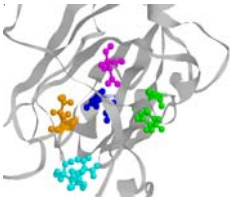


Fig. 3. Amino acids of the active site (Asp32, Tyr198, Asp228, Thr232, Arg235)

The optimization of the geometry[10] of the β-secretase was carried out using the field of forces Amber99.

The main chain was maintained rigid, whereas the lateral chains remain flexible. This approximation allows the lateral chains of proteins to more easily find the position in which the interactions are the most favourable.

The value of the energy of optimization is: E_{Opt} = -1933.20 Kcal/mol.

The optimization of all the inhibitors was made by program EMO[11-14]. The results obtained are collected in Table-1.

TABLE-1
RESULTS OBTAINED USING PROGRAM EMO

Steric energy (KJ/mol)	$E_{Stretching}$	$E_{Bending}$	$E_{Torsion}$	E_{Vdw}	$E_{Electrostatic}$	E_{Steric}
Inhib-1	7.59	44.14	-76.12	108.33	-55.73	27.61
Inhib-2	27.38	119.49	159.12	252.69	-102.46	456.22
Inhib-3	22.65	134.49	7.79	169.29	-65.09	269.15
Inhib-4	22.28	162.54	65.69	205.46	-109.28	346.70

After optimization of all the inhibitors, we made a calculation of load to attribute to each atom an electronic partial load, for it we used the software AMPAC.

RESULTS AND DISCUSSION

Molecular dynamics of the β-secretase (Fig. 4): This stage is necessary to eliminate conformations the least favourable and to lead to the most stable conformation.

We began dynamics with an initialization of the system: with t = 0, we have R (T) = 0, *i.e.* the initial structure, previously minimized. Then we

heated the system up to 300 K during 1000 steps with a step of integrations of 1fs.

In 300 K, there is an equilibration. Speeds are straightened to keep the constant temperature (there is exchange between the kinetic energy and the potential energy). Then, there is production of conformations. The time of simulation of the molecular dynamics is 100 picoseconds.

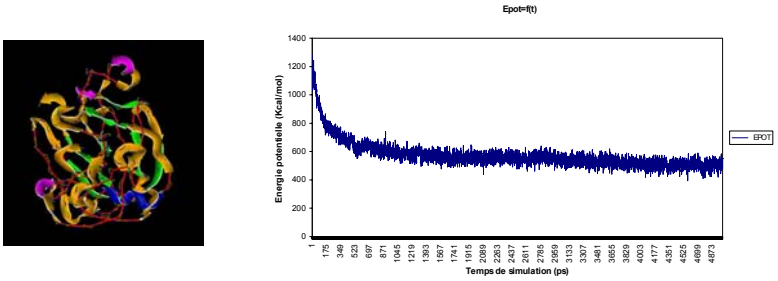


Fig. 4. Variation of the potential energy of the 1FKN only according to time

Molecular dynamics of the inhibitors:

A) Inhib-1:

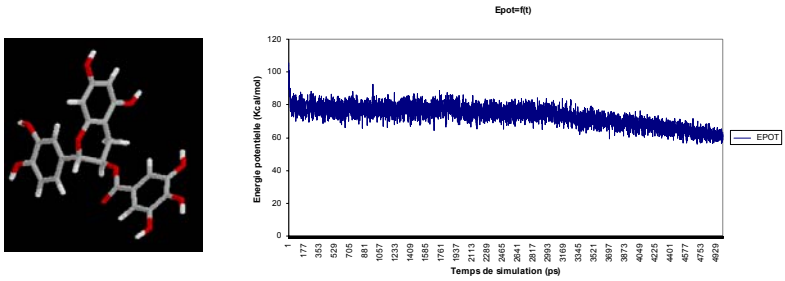


Fig. 5. Variation of the potential energy of the inhib-1 only according to time

B) Inhib-2:

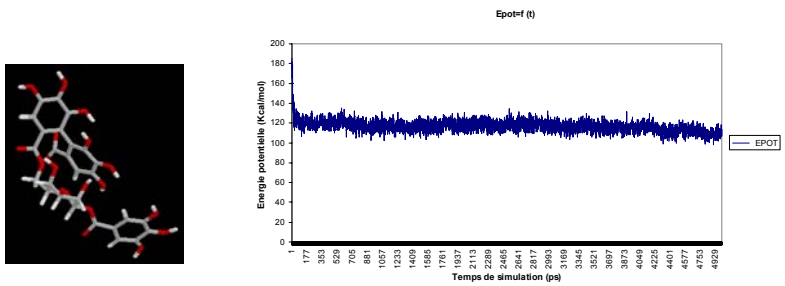


Fig. 6. Variation of the potential energy of the inhib-2 only according to time

C) Inhib-3:

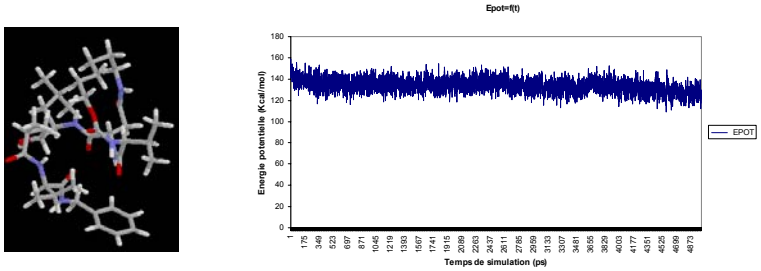


Fig. 7. Variation of the potential energy of the inhib-3 only according to time

D) Inhib-4:

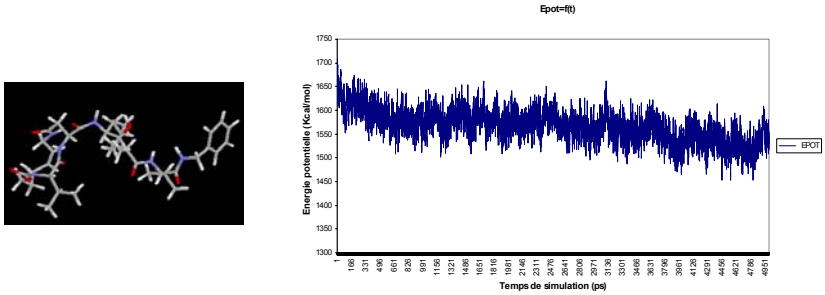


Fig. 8. Variation of the potential energy of the inhib-4 only according to time

Docking molecular: This step consists moved closer to two entities molecular that is the location of the inhibitors in the active site of β-secretase (forming of the complexes). We used the Dock program of the software ChemOffice (2000).

Once all the complexes were formed, we are going to make an optimization of the geometry and a calculation of molecular dynamics to look for the most stable conformation.

a) Complex 1FKN-Inhib-1:

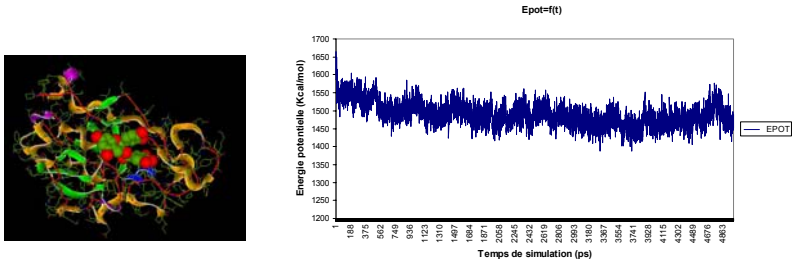


Fig. 9. Variation of the potential energy of complex 1 according to time

b) Complex 1FKN-Inhib-2:

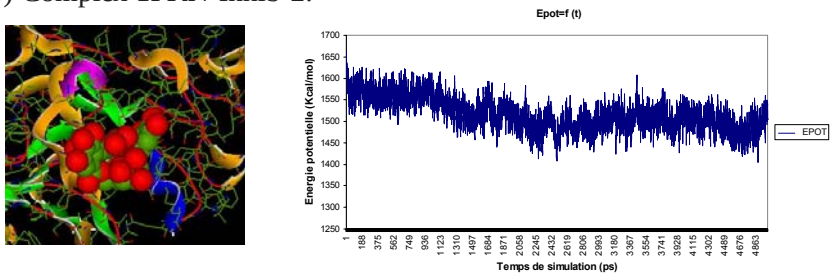


Fig. 10. Variation of the potential energy of complex 2 according to time

c) Complex 1FKN-Inhib-3:

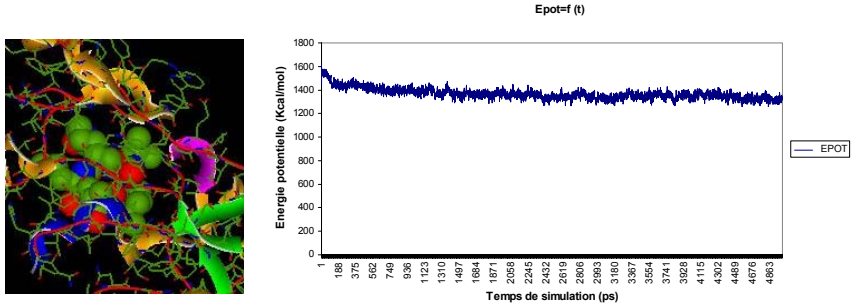


Fig. 11. Variation of the potential energy of complex 3 according to time

d) Complex 4 1FKN-Inhib-4:

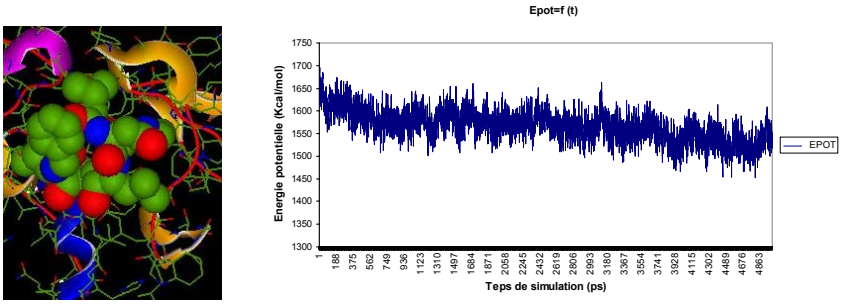


Fig. 12. Variation of the potential energy of complex 4 according to time

Distances separating the amino acids from the active site and the groupings of inhibitors:

TABLE-2
DISTANCE IN Å MEASURED BETWEEN THE GROUPINGS OF INHIB1 AND THE GROUPINGS OF THE SIDE CHAINS OF AMINO ACIDS RESPONSIBLE FOR INTERACTION

	Asp32	Tyr198	Asp228	Thr232	Arg235
Inhib1	6.10	9.62	6.02	8.75	11.94

TABLE-3
DISTANCE IN Å MEASURED BETWEEN THE GROUPINGS OF INHIB-2 AND THE GROUPINGS OF THE SIDE CHAINS OF AMINO ACIDS RESPONSIBLE FOR INTERACTION

	Asp32	Tyr198	Asp228	Thr232	Arg235
Inhib-2	6.94	7.63	12.37	2.99	2.42

TABLE-4
DISTANCE IN Å MEASURED BETWEEN THE GROUPINGS OF INHIB-3 AND THE GROUPINGS OF THE SIDE CHAINS OF AMINO ACIDS RESPONSIBLE FOR INTERACTION

	Asp32	Tyr198	Asp228	Thr232	Arg235
Inhib-3	3.73	24.21	10.05	8.46	8.15

TABLE-5
DISTANCE IN Å MEASURED BETWEEN THE GROUPINGS OF INHIB-4 AND THE GROUPINGS OF THE SIDE CHAINS OF AMINO ACIDS RESPONSIBLE FOR INTERACTION

	Asp32	Tyr198	Asp228	Thr232	Arg235
Inhib-4	3.02	14.04	6.84	8.54	2.86

The energies of interactions between the β-secretase and the various studied inhibitors are calculated from the following relation:

E (interaction) = [E pot (complex enzyme - inhibitor)] - [E pot (enzyme) + E pot (inhibitor)]

It is necessary to take into account also Van der Walls's interactions because it is the interactions between nondependent atoms that stabilize complex enzyme-inhibitor.

TABLE-6
RESULTS OF INTERACTIONS ENERGIES (Kcal/mol)

Inhibitors	(Kcal/mol)						Category
	$E_{total.\ pot\ of\ the\ complex\ E\text{-}I}$	$E_{total.\ Pot\ of\ the\ inhibitor\ I}$	$E_{vdw\ (complex\text{-}inhibitor)}$	$E_{Vdw\ (inhibitor)}$	$E_{Vdw\ inter.}$	$E_{total\ inter}$	
Inhib-1	1474.05	60.56	-679.25	25.58	-39.72	867.90	A
Inhib-2	1541.54	111.26	-703.89	29.09	-67.87	884.78	A
Inhib-3	1336.00	118.77	-1048.2	21.40	-420.30	671.73	B
Inhib-4	1552.51	134.45	-738.10	31.21	-104.21	872.57	B

Potential energy of the β-secretase only is: Epot = 545.48 (Kcal/mol).
Potential energy of the β-secretase only is: E_{Vdw} = -665.11 (Kcal/mol).

The values of K_i and IC_{50} are given below:

TABLE-7
VALUES OF K_i AND IC_{50}

Category A		Category B	
Inhibitors	IC_{50}	Inhibitors	K_i
Inhib-1	6.0×10^{-6} M	Inhib-3	25.1 nM
Inhib-2	1.6×10^{-4} M	Inhib-4	14.2 nM

In the light of the results obtained during present work, it emerges that the values obtained relating to energies of total interaction are of the same order of magnitude for both categories with the exception of the inhibitor 3.

The inhibitor 3 belonging in the category B constituted by 114 atoms, synthetic product possesses an important number of doublet and being able to interact with the residues of the active site, possesses an energy of (671.73 Kcal/mol) who can engender a conformation favourable to an important complementarity which from a consequent activity.

It is noted a peculiarity between the inhibitor 3 and the inhibitor 4, on the level of this last the presence of a double connection inserted in the cycle into 17 links which confers a relatively stable conformation of energy less low than the inhibitor 3; thus an interaction of atom nondependent is less important.

This structure presents a relatively cumbersome conformation from where a steric gene influencing the stability of the complex.

Distances measured between the groupings of the inhibitors and those of the side chains, by not considering that the most brought closer groupings show according to the inhibitor considered of the interactions variable of 2.42 to 24.21 Å.

The interactions ranging between 2.5 and 3.1 Å are regarded as strong and those ranging between 3.1 and 3.55 Å are supposed to be average. The interactions higher than 3.55 Å are weak[15].

The examination of the cavity of the active site (Fig. 10) presents geometry of 8.65 Å of depth, an opening of 16.76 and 9 Å, this narrowed pocket reaching a width of 12.61 Å.

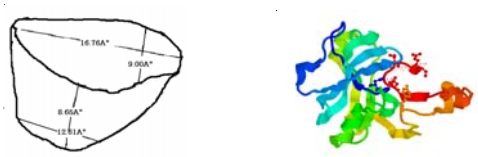


Fig. 13. Dimension of the cavity enzymatique

The category A, natural products extracts of the green tea, presents a geometry of association of cycle at 6 links and a less number of electronic doublet being able to interact with the residues of the active site.

By taking account of the various geometrical constraints, the approach according to inhibitors' considered raises difficulties being able to influence the complementarity and consequently the activity.

The exploitation of some experimental data in occurrence[8] the IC_{50} for the structures of category A and while being based on energy of total interaction, shows an agreement acceptable for inhibitors 1 and 2. Indeed, inhibitor 1 (867.90 Kcal/mol) is more active ($IC_{50} = 6 \times 10^{-6}$ M) that inhibitor 2 (884.76 Kcal/mol) with $IC_{50} = 1.6 \times 10^{-4}$ M.

Concerning the category B, Ki (constants of inhibition)[9] available and total energies of interaction, show the following order: inhibitor 3 (671.73 Kcal/mol) with Ki = 25.1 nM is more active than inhibitor 4 (872.57 Kcal/mol) with Ki = 14.2 nM.

The examination of the values of the energy of Van der Waal's interaction which is generally retained to explain the interactions between biological systems, show that in certain structures; mainly the category A whose geometry and characterized by a steric obstruction, the order of stability is relatively disturbed.

Inhibitor 1 (-39.72 Kcal/mol, $IC_{50} = 6 \times 10^{-6}$ M) normally more stable than inhibitor 2 (-67.87 Kcal/mol, $IC_{50} = 6 \times 10^{-4}$ M), is observed destabilizing taking into account the values of the energy of van der Waals. The same order is obtained by examining the values of the IC_{50}. Allotting this disorder in the classification to us to a competitive inhibition of with the geometry of the inhibitors.

This phenomenon is generated by a change of conformation of the enzyme (active site) which prevents the mutual connection of the substrate.

Another probable approach is that the connection of the inhibitor to the free enzyme with a site meadow of the active site can prevent the connection of the substrate by steric obstruction which is probably the present case.

Conclusions

This modest work consists in studying the inhibition of β-secretase which is an enzyme involved in the Alzheimer's disease by method of molecular modelling.

Within sight of the results obtained and experimental values recorded in the bibliography, it arises:

1st classification: while basing itself on the energy of total interaction.

Inhib-3 > Inhib-1 > Inhib-4 > Inhib-2

2nd classification: while basing itself on the energy of van der Waals's interaction.

Inhib-3 > Inhib-4 > Inhib-2 > Inhib-1

Thus according to these 2 classifications, we can say that the inhibitor 3 would be probably the best to slow down the evolution of studied pathology (Alzheimer's disease).

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