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Contribution à la modélisation des biomolécules et leurs interactions : Cas des inhibiteurs de la monoamine oxydase B dans la maladie de Parkinson

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Contribution to the modeling of biomolecules and their interactions: Case of monoamine oxidase B inhibitors in Parkinson's disease

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Dedication

I dedicate this thesis and everything I do with my mother "Naziha" and my father "Hcene " for all the sacrifices they make each day and there is a source of encouragement, inspiration, and help to me throughout my life, with all the members of my family, a special feeling of gratitude for their support.

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List of abbreviations

A

Å: Angchtrom

Ala: alanine

AM1: Austin Model 1

AMBER: Assisted Model Building with Energy Refinement

AMPAC: Advanced Materials Processing and Analysis Center

Arg : Arginine

Asn : Asparagine

Asp : Aspartic

C

C-C : carbon-carbon

CHARMM: Chemistry at Harvard Macromolecular Mechanics

COMT: Catechol-O-methyl transferase

CNDO: Complete Neglect of Differential Overlap

Cys : cysteine

cZI : caudal zona incerta

D

DA: dopamine

DBS: Deep Brain Simulation

DFP: di-isopropylfluorophosphate

DFT: Density functional theory

DNA: Deoxyribonucleic acids

DoF: Degrees of Freedom

3D: 3 Dimensional

$\mathcal{E}$

E: Energy

EMO: Energy Of Molecule

$\mathcal{F}$

FAD: Flavin Adenine Dinucleotide

$\mathcal{G}$

GROMOS: Groningen Molecular Simulation Program Package

Glu: Glutamine

Gly: Glycine

$\mathcal{H}$

HF: Hartree-Fock

His: Histidine

hMAO: human monoamine oxidase

HOMO: Highest Occupied Molecular Orbital

$\mathcal{I}$

ICOMTs: Inhibitors of Catechol-O-methyl transferase

Ile: Isoleucine

INDO: Intermediate Neglect of Differential Overlap

$\mathcal{K}$

KDa: kilodalton

$\mathcal{L}$

Leu: leucine

L-DOPA:L-dihydroxyphenylalanine

LUMO: Lowest Unoccupied Molecular Orbital

Lys: Lysine

$\mathcal{M}$

MAOs: Monoamine oxidases

MD: Molecular Dynamic

Met: Methionine

MM: Molecular mechanics

MNDO: Modified Neglected of Differential Overlap

MO-LCAO: Linear Combination of Atomics Orbitals

MO: Molecular Orbital

MOPAC: Molecular Orbital PACkage

$\mathcal{N}$

NDDO: Neglect of Diatomic Differential Overlap

NMR: Nuclear magnetic resonance

$\mathcal{O}$

OPLS: Optimized Potentials for Liquid Simulations

$\mathcal{P}$

Phe: phenylalanine

PI: Postural instability

PM3: Parametric Method 3

Pro: proline

Q

QM: Quantum-Mechanical

R

REM: Rapid Eye Movement

RNA: Ribonucleic Acids

S

SCF: Self-Consistent Field

Ser: Serine

SN: Substantia nigra

SPASIBA: Spectroscopic Potential Algorithm for Simulating biomolecular conformational Adaptability

STN: Subthalamic nucleus

T

Thr: Threonine

Tyr: Tyrosine

Trp: Tryptophan

TRAP: Tremor at rest, Rigidity, Akinesia, Postural instability

V

VdW: Van der Walls

General Introduction

Neurodegenerative disorders are common diseases that afflict our society with tremendous medical and financial burdens. As a whole, neurodegeneration strikes individuals of all ages, but becomes increasingly frequent with age, coming to affect a very large share of our elderly population. Due to the very complex nature of these diseases, which often result from combined genetic and environmental pathogenic factors, the scientific community that researches the causes and the therapy of neurodegeneration faces remarkable challenges, requiring constant technological advancements**[1]**.

Alzheimer's (AD) and Parkinson's diseases (PD) are the most common neurodegenerative disturbances. Several biochemical and molecular mechanisms are involved in the AD and PD pathogenesis, such as synaptic dysfunction, energetic deficit triggered by mitochondrial disorder, oxidative stress and neuro-inflammation.

AD is characterized by a progressive cognitive decline due to a variety of pathological changes in the brain, mainly in the basal forebrain cholinergic neurons. On the other hand, PD is classically known as a chronic and progressive movement disorder related to dopaminergic neurodegeneration of the substantia nigra pars compacta. In both illnesses, neurotrophic factors play an essential role for the survival of neurons affected by degenerative processes **[2]**.

Parkinson's disease is the second most common neurodegenerative disorder, after Alzheimer's disease. It is characterized clinically by parkinsonism (resting tremor, bradykinesia, rigidity, and postural instability) **[3]** pathologically by the loss of neurons in the substantia nigra and elsewhere in association with the presence of ubiquitinated protein deposits in the cytoplasm of neurons (Lewy bodies) **[4]**.

The monoamine oxidases (MAO-A and MAO-B) are FAD (flavin adenine dinucleotide)-dependent enzymes that are responsible for the metabolism of neurotransmitters, such as dopamine (DA), serotonin, adrenaline, and noradrenaline, and for the oxidative deamination of exogenous arylalkyl amines. Due to their central role in neurotransmitter metabolism, these enzymes represent attractive drug targets in the pharmacological therapy of neurodegenerative diseases and neurological disorders. Recent efforts toward the development of MAO inhibitors have focused on selective MAO-A or MAO-B inhibitors. Selective MAO-A inhibitors are effective in the treatment of depression and anxiety, whereas the MAO-B inhibitors are useful for

treatment Parkinson's disease **[5]**.

Catechol-O-methyltransferases (COMT) are another important class of conjugative enzymes of Parkinson's disease, which play a key role in the metabolism and inactivation of catechol neurotransmitters, catechol estrogens and a wide range of endobiotics and xenobiotics that bear the catechol group**[6].**

During the1960s, L-DOPA, a direct precursor of DA, which is synthesized in vivo from tyrosine in DA neurons by tyrosine hydroxylase and is converted to DA by aromatic L-amino acid decarboxylase, was introduced to treat this DA deficiency in the striatum. In addition to L-DOPA as a treatment, MAO-B inhibitors (MAO-B-Is) have been used since the 1970s, first selegiline (L-(-)-deprenyl), then rasagiline, and more recently safinamide, as an effective therapy for PD by preventing the degradation of DA. Furthermore, monotherapy with MAO-B-I, selegiline, rasagiline, or safinamide has been proved to be effective in the case of early PD. Accumulating data suggest that MAOB-Is may also have neuroprotective efficacy due to several mechanisms that may or may not be related to MAO inhibition **[7]**.In addition, COMT inhibition can block metabolism of catecholamines including DA. Since the increase in DA bioavailability is dependent on the inhibition of DA metabolism at the periphery, the development of COMT inhibitors as adjuvants to levodopa/aromatic amino acid decarboxylase (AADC) inhibitor treatment improves the clinical benefits of PD symptomatic treatment significantly **[8]**.

The aim of this work is to use three methods, namely; molecular docking, molecular dynamics and ADME properties for study the interactions between a newly synthesized series of molecules with the enzyme (4a79) involved in Parkinson's disease, as well as the stability of the complexes formed during these interactions.

The work of this thesis is presented in three chapters:

✓ ***A General Introduction***: In which we gave an overview of PD and clarified the objective of our work.

✓ ***The first chapter***: It encompasses all the main approaches and different methods of molecular modeling.

✓ ***The second chapter***: It is devoted to two parts:

1. Part (A) : Proteins, enzymes and amino acids.
2. Part (B) : Parkinson's disease.

✓ ***In the third chapter***: We have analyzed and discussed the results obtained.

✓ ***A General Conclusion***: summarizes and concludes all of the work carried out.

References

[1] Kawamata, Hibiki, and Giovanni Manfredi. "Introduction to neurodegenerative diseases and related techniques." Neurodegeneration. Humana Press, Totowa, NJ, **(2011)**: 3-8.

[2] Sampaio, TuaneBazanella, et al. "Neurotrophic factors in Alzheimer's and Parkinson's diseases: implications for pathogenesis and therapy." Neural regeneration research 12.4 **(2017)**: 549.

[3] Mm, Hoehn. "Yahr MD. Parkinsonism: onset, progression and mortality." Neurology 17.5 **(1967)**: 427-442.

[4] Pollanen, Michael S., Dennis W. Dickson, and Catherine Bergeron. "Pathology and biology of the Lewy body." Journal of Neuropathology & Experimental Neurology 52.3 **(1993)**: 183-191.

[5] Chaurasiya, Narayan D., et al. "Selective inhibition of human monoamine oxidase B by Acacetin 7-methyl ether isolated from Turneradiffusa (Damiana)." Molecules 24.4 **(2019)**: 810.

[6] Wang, Fang-Yuan, et al. "Analytical methodologies for sensing catechol-O-methyltransferase activity and their applications." Journal of Pharmaceutical Analysis **(2020)**.

[7] Nagatsu, Toshiharu, and Akira Nakashima. "Monoamine Oxidase Inhibitor (MAO-I)-Mediated Neuroprotection for Treating Parkinson's Disease." NeuroPsychopharmacotherapy **(2020)**: 1-21.

[8] Moschovou, Kalliopi, et al. "Cheminformatics and virtual screening studies of COMT inhibitors as potential Parkinson's disease therapeutics." Expert Opinion on Drug Discovery 15.1 **(2020)**: 53-62.

Chapter I: Molecular Modeling

1. Introduction

Computational techniques in advanced chemistry mimic atomistic systems to study their behavior and properties mathematically. It is a theoretical approach of simplifying graphical models that has long inspired the molecular graphics in published works, books and, more recently, on computers. In other words, molecular modeling has revolutionized the understanding of chemistry visually and quantitatively.

Molecular modeling is used as a computational tool for interpreting, elucidating, and investigating existing and novel phenomena in several areas of chemistry such as molecular structure determination by NMR spectroscopy, catalysis, biometrics, protein mutagenesis, and nucleotides/ protein interaction studies. It has changed the face of pharmaceuticals by contributing in drug design studies and, consequently, creating incredible opportunities for advanced therapeutic research in the studies of drug-drug as well as drug-living organism interactions at the molecular level **[1]**.

2. Molecular Modeling Methods

The definition currently accepted of what molecular modeling is can be stated as this: "molecular modeling is anything that required the use of computer to paint describe or evaluate any aspect of the properties of the structure of a molecule **[2]**.

Molecular modeling involves the use of theoretical calculation methods (molecular mechanics, molecular dynamics, ab-initio or semi-empirical quantum mechanics…) **[3]**.

2.1. Quantum Methods (QM)

Quantum mechanical methods are based on the solution of the Schrodinger equation **[4**,**5]**. This fundamental approach is attractive since 3D structures, molecular energies, and many associated properties can be calculated on the basis of fundamental physical principles, namely electronic and nuclear structures of atoms and molecules. Indeed, the quantum mechanics pioneer Paul Dirac is believed to have expressed the sentiment that the Schrödinger equation reduces theoretical chemistry to applied mathematics **[6]**.

Although historically quantum calculations were practical only for very small systems, exciting developments in both software and hardware (computer speed as well as memory) have made quantum-mechanical calculations feasible for larger systems, including bimolecular, with various approximations **[7]**.

The Schrödinger equation is perhaps the most important equation in the entire field of quantum mechanics. The time-dependent non-relativistic one-particle variant is:

$$\left[-\frac{1}{2}\nabla^2 + \hat{\mathcal{V}}\right]\Psi(\mathbf{x},t) = i\hbar\frac{\partial}{\partial t}\Psi(\mathbf{x},t) \qquad \textbf{(1)}$$

Where $\hat{v}$is a possibly time-dependent, possibly position dependent potential. The time independent equation is:

$$\left[-\frac{1}{2}\nabla^2 + \hat{\mathcal{V}}\right]\Psi(\mathbf{x}) = E\Psi(\mathbf{x}) \qquad \textbf{(2)}$$

Where E: is the energy of the system. These equations can be expanded to match a multi-particle system **[8]**.

2.1.1. ab initio Methods (Hartree-Fock, Roothaan)

'*ab initio*' quantum chemistry has emerged as a viable and powerful approach to address the issues and problems related to the chemical systems. Quantum chemical calculations offer the real promise of being able to complement experiment as a means to uncover and explore new chemistry. It is used for predicting the properties of new materials even those which are not synthesized in the laboratory, using computer simulation technique **[9]**.

Hartree-Fock-Roothaan method

The Hartree method is a single electron approximation technique used in multielectron systems. The molecular Hamiltonian is split up into individual single electron Hamiltonians. Consider a molecular system with N-electrons, each with degrees of freedomr_i. The wave function (Hartree function) $\boldsymbol{\psi_h(r_1, r_2, \ldots r_N)}$ is given by the Hartree product as shown in Eq. 3: **[10]**.

$$\boldsymbol{\psi_h(r_1, r_2, \ldots r_N) = \varphi_1(r_1).\varphi_2(r_2).\varphi_N(r_N)} \qquad \textbf{(3)}$$

For the n-electron system, the Hamiltonian is given by:

$$\hat{H}_e = \hat{T}_e + \hat{v}_{ne} + \hat{v}_{ee} + \hat{v}_{nn} \qquad (4)$$

The Hartree-Fock (HF) model is a standard tool for computing an approximation of the ground state of a molecular system within the Born-Oppenheimer setting. From a mathematical viewpoint, the HF model gives rise to a nonquadratic constrained minimization problem for the numerical solution of which iterative procedures are needed; such procedures are referred to as Self-Consistent Field (SCF) algorithms **[11]**.

It is always possible to decompose ψ (i) on a complete basis of known functions. The problem of determining ψ (i) comes down to that of calculating the numerical coefficients of the development of ψ (i) on the complete basis. It was Roothaan who used theMO-LCAO technique to build the MO, he unblocked the crisis of ab initio methods. This method consists in expressing the molecular orbital ψ (i) by a linear combination of atomic orbitals ϕμ **[12]**.

$$\phi_i = \sum_{\mu=1}^{N} C_{i\mu}\, \varphi_\mu \qquad (5)$$

$\mathbf{C}_{i\mu}$: are the coefficients to be varied. (Method of variations), N: is the number of OAs combined.

2.1.2. Density Functional Theory

Density functional theory (DFT) is a quantum-mechanical (QM) method used in chemistry and physics to calculate the electronic structure of atoms, molecules and solids. It has been very popular in computational solid-state physics since the 1970s. However, it was not until the 1990s that improvements to the method made it acceptably accurate for quantum-chemical applications, resulting in a surge of applications.

The real forte of DFT is its favorable price/performance ratio compared with electron-correlated wave function-based methods such as Møller–Plesset perturbation theory or coupled cluster. Thus, larger (and often more relevant) molecular systems can be studied with sufficient accuracy,

thereby expanding the predictive power inherent in electronic structure theory. As a result, DFT is now by far the most widely used electronic structure method. The huge importance of DFT in physics and chemistry is evidenced by the 1998 award of the Nobel Prize to Walter Kohn 'for his development of the density-functional theory' **[12]**.

2.2. Semi Empirical methods

Semiempirical calculations are set up with the same general structure as a HF calculation. Within this framework, certain pieces of information, such as two electron integrals, are approximated or completely omitted. In order to correct for the errors introduced by omitting part of the calculation, the method is parameterized, by curve fitting in a few parameters or numbers, in order to give the best possible agreement with experimental data. The merit of semiempirical calculations is that they are much faster than the ab initio calculations. The demerit of semiempirical calculations is that the results can be slightly defective. If the molecule being computed is similar to molecules in the database used to parameterize the method, then the results may be very good. If the molecule being computed is significantly different from anything in the parameterization set, the answers may be very poor. Semiempirical calculations have been very successful in the description of organic chemistry, where there are only a few elements used extensively and the molecules are of moderate size. However, semiempirical methods have been devised specifically for the description of inorganic chemistry as well **[13]**.

In the various semi-empirical methods such as CNDO (Complete Neglect of Differential Overlap), INDO (Intermediate Neglect of Differential Overlap), NDDO (Neglect of Diatomic Differential Overlap), MNDO (Modified Neglected of Differential Overlap), AM1 (Austin Model 1), PM3 (Parametric Method 3), only the valence electrons are taken into account. Through these methods, we have access to different molecular properties such as atomic charges, orbital energies (HOMO, LUMO) among others, the ionization potential, the enthalpy of formation and the electronic distribution **[14]**.

2.3. Non Quantum Methods

Empirical methods are methods of molecular mechanics based on concepts of classical mechanics where atoms and their electrons are merged into a collection of material points. The latter act on each other by means of an empirical potential also called field of forces depending only on the relative position of the atoms in space. Within the computer, the representation of a molecule therefore consists of a set of atomic coordinates, a list of chemical bonds and a set of functions and parameters constituting the interaction potential. The empirical potential which determines the energy conformation it of the molecule is made up of two types of terms representing, respectively, the interactions between the bonded atoms (bond length, valence angle, dihedral angle) and the unbound atoms (Van der Waals, electrostatic) **[15]**.

2.3.1. Molecular Mechanics

The MM appeared in 1930 **[16]**, but developed from the sixties when computers were no longer accessible and more efficient. The MM is based on Born Oppenheimer's approximation that electrons are much faster than nuclei **[17]**.

Molecular mechanics (MM) is the simplest and fastest way to evaluate molecular systems. These methods rely on classical potential functions, and quantum-mechanical properties of systems, such as bond breaking or forming, are entirely neglected. Molecular mechanics can be used to study small molecules as well as large biological systems or material assemblies with many thousands of atoms, but only in their equilibrium states. In all-atomistic molecular mechanics methods, each atom is represented as a single particle, and each particle is assigned a radius (typically the van der Waals radius), polarizability, and a constant net charge, which is derived from quantum-mechanical calculations and/or experiment. Bond interactions are treated as “springs” with an equilibrium distance equal to the experimental or calculated bond length. The collection of potential functions made to describe a molecular system is referred to as a force field, which can be used to calculate molecular energy based on bond-stretching, valence bond bending, torsions, and non-bonded interactions **[18]**.

Molecular mechanics methods are based on the following principles: Nuclei and electrons are lumped into atom-like particles; Atom-like particles are spherical (radii obtained from measurements or theory) and have a net charge (obtained from theory); Interactions are based on springs and classical potentials; Interactions must be preassigned to specific sets of atoms; Interactions determine the spatial distribution of atom-like particles and their energies; Note how these principles differ from those of quantum mechanics. In short, the goal of molecular mechanics is to predict the detailed structure and physical properties of molecules. Examples of physical properties that can be calculated include enthalpies of formation, entropies, dipole moments, and strain energies.... Molecular mechanics calculate the energy of a molecule and then adjusts the energy through changes in bond lengths and angles to obtain the minimum energy structure. Steric Energy A molecule can possess different kinds of energy such as bonds and thermal energy. Molecular mechanics calculate the steric energy of a molecule–the energy due to the geometry or conformation of a molecule **[19]**.

Molecular mechanics uses the following approximations:

- Each atom constitutes a particle.
- The atom is considered as a rigid sphere having a radius and a specific charge.
- Energies are calculated by formulas derived from the classical mechanics **[20]**.

a. Term of the force field

The mathematical model representing the potential energy is called the force field of a molecule in molecular mechanics.

The "Force Field", which represents as well as possible the variations of the potential energy with the molecular geometry. Its purpose is to calculate the potential energy of a molecule (or of a molecule system) according to the coordinates of the atoms:

$$Ep = f(r1, r2\ldots., Rn)$$

Or: ri: represents the position vector of atom i.

Ep: Potential energy

But a better idea may be obtained by considering the situation physically. Consider a molecule as a collection of atoms held together by elastic forces. (If you want to get even simpler then one could consider a molecule to be a collection of point masses connected by elastic springs). Now the forces can be written in terms of potential energy functions of various structural features such as bond lengths, bond angle, non-bonded interactions etc. The force field is the combination of these potential energy terms. Hence force fields are also sometimes referred to as potentials. Thus, the energy, E, of a molecule in a force field arises from the deviations from the ideal structural features.

The mechanical molecular model considers atoms as spheres and bonds as springs. The mathematics of spring deformation can be used to describe the ability of bonds to stretch, bend, and twist:

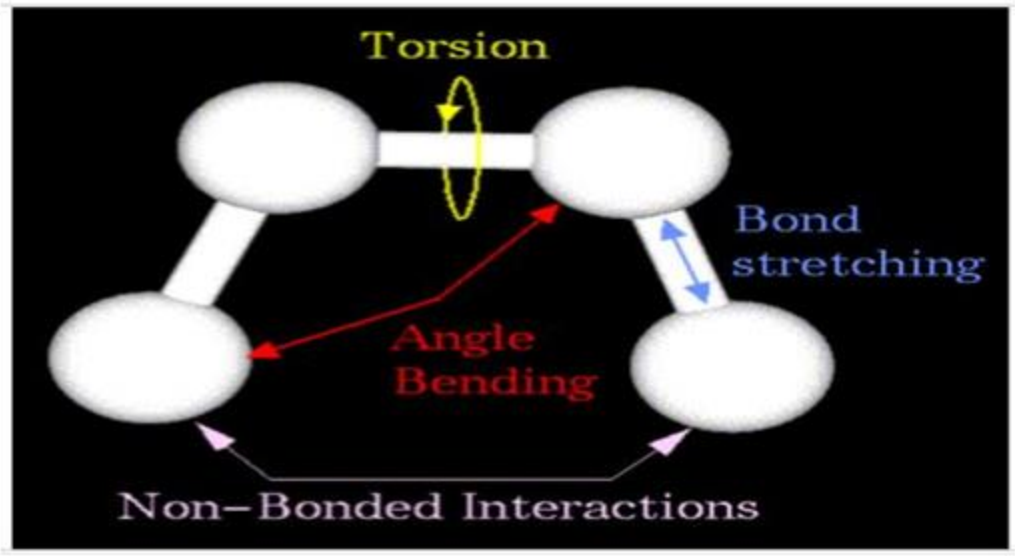


Figure 1: *Intramolecular interactions between bound and unbound atom*

Non-bonded atoms (greater than two bonds apart) interact through van der Waals attraction, steric repulsion, and electrostatic attraction/repulsion. These properties are easiest to describe mathematically when atoms are considered as spheres of characteristic radii.

The object of molecular mechanics is to predict the energy associated with a given conformation of a molecule. However, molecular mechanics energies have no meaning as absolute quantities. Only differences in energy between two or more conformations have meaning. A simple molecular mechanics energy equation is given by:

Energy = Stretching Energy + Bending Energy + Torsion Energy + Non-Bonded Interaction Energy

These equations together with the data (parameters) required to describe the behavior of different kinds of atoms and bonds, is called a force-field. Many different kinds of force-fields have been developed over the years. Some include additional energy terms that describe other kinds of deformations. Some force-fields account for coupling between bending and stretching in adjacent bonds in order to improve the accuracy of the mechanical model **[21,22]**.

Steric energy is expressed by the following equation:

$$E = E_{stretching} + E_{bending} + E_{torsion} + E_{vdw} + E_{elec} + E_{Hydrogen}$$

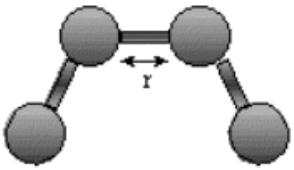
The term **"Stretching"** represents the elongation of bonds.
The term **"Bending"** represents the variation of angles.
The term **"Torsion"** refers to the torsional energy of dihedral angles.
The term **"Vdw"** accounts for the non-covalent interaction energies between atoms unrelated.
The term **"Elec"** describes the electrostatic interaction energies between atoms unrelated.
The term **"Hydrogen"** describes hydrogen bonds **[23]**.

b. Term of linked atoms

Intermolecular interactions only depend on coordinates internal molecules that is to say, bonds, valence angles, and torsions. In fact, to refine the expression of the potential term is to make the description of the system; terms of couplings between different atoms have been introduced.

The mathematical form of the energy terms varies from force-field to force-field. The more common forms will be described.

- ***Stretching Energy:***



$$E_{stretch} = \sum_{bonds} k_b (r - r_o)^2$$

The stretching energy equation is based on Hook's law. The kb parameter defines the stiffness of the bond spring. R_0 is the equilibrium distance between the two atoms. It should make sense that deviations from the equilibrium length would be associated with higher energy.
Obviously only small changes in r are allowed as to large an r value would lead to bond breaking.

- ***Bending Energy:***

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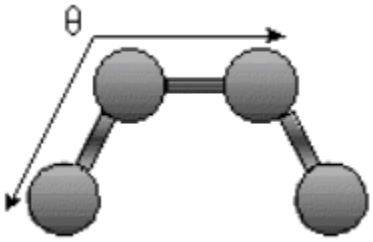
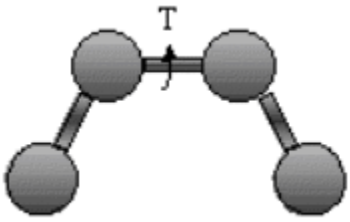


$$E_{bending} = \sum_{angles} k_\theta (\theta - \theta_o)^2$$

The bending energy equation is also based on Hook's law. The kΘ parameter controls the stiffness of the angle spring, while the Θ_0 is the equilibrium angle.

- ***Torsion Energy:***



$$E_{torsion} = \sum_{torsions} A[1+\cos(n\theta)]$$

The torsion energy is modeled by a periodic function, much as you have seen with energy plots associated with Newman projections sighting down C-C bonds for example **[24]**.

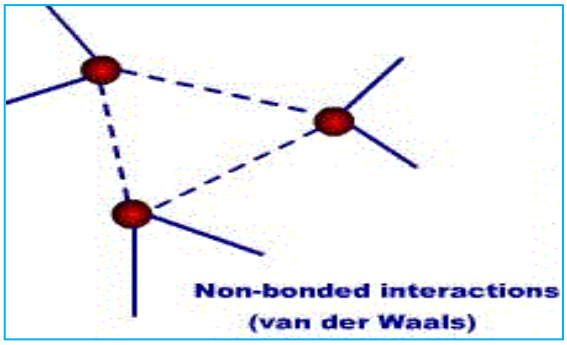
c. Interaction energy between unbound atoms

Intermolecular interactions take into account interactions that do not interact by terms of bond, angle of curvature and angle of torsion. The non-binding potential is expressed in two terms: a Van der Walls term and an electrostatic energy term.

- ***Van der Waals Interaction***

Van Der Waals interactions are non-permanent dipoles with a small range of action. They are numerous and essentially contribute to the search for steric agreement between the ligand and the receptor protein **[25]**.

It is generally expressed in the form of a John Lennard-Jones potential (dispersion and repulsion or a Buckingham potential) **[26]**.



$$E_{\text{van der Waals}} = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \left[\frac{A_{i,j}}{d_{i,j}^{12}} - \frac{B_{i,j}}{d_{i,j}^{6}} \right]$$

di, j: distance between unbound atoms i and j

Ai, j: parameters of the force field linked to the repulsion between the atoms i and j

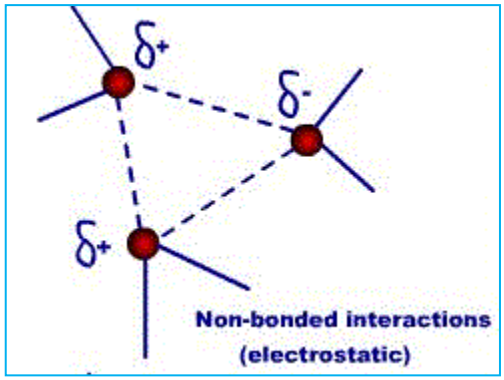
Bi, j: parameters of the force field linked to the attraction between atoms i and j

The radius of van der Waals corresponds to the minimum distance between the 2 atoms

- ***Interactions electrostatics***

Energy of electrostatic interactions between atoms not covalently linked. It is expressed using a Coulomb potential.

This term increases with the polarity of the chemical bonds and can be particularly important, for example in the case of molecules which contain heteroatom **[27]**.



$$E_{\text{électrostatique}} = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \frac{q_i q_j}{4\pi\epsilon d_{i,j}}$$

di, j: distance between atoms i and j

qi and qj: partial charge of unbound atoms i and j. Atomic partial charges can be calculated for small molecules using an ab initio or semi-empirical quantum method (example: MOPAC and AMPAC)

ε : permittivity of the medium = dielectric constant of the environment (the solvent or the molecule itself)

- ***Energy of hydrogen bonds***

Hydrogen bonds are the result of electrostatic (70%) and van der Waals (30%) interactions between an electronegative atom (usually an oxygen or nitrogen atom) carrying a free electron doublet and an atom of hydrogen carried by an electronegative atom **[28]**.

$$E_{Liaison\text{-}hydrogene} = \sum \left[\frac{A'}{r_{AD}^{12}} - \frac{B'}{r_{AD}^{10}} \right] \cos^{m} \theta_{A\text{-}H\text{-}D} \cos^{n} \theta_{AA\text{-}H\text{-}D}$$

A', B': parameters depending on the nature of the donor and the acceptor of H bonds, distant from r AD.

θA-H-D: angle formed by the acceptor (A), the hydrogen (H) and the donor (D).

θAA-A-H: angle formed by the history of the acceptor (AA), A and H.

m, n: exponents given by the type of D and A, m = 0, 2, 4; n = 0, 2.

d. <u>Different force fields in molecular mechanics</u>

Different force fields use the same type of energy terms but different Parameters. Force fields in MM can be grouped into three main classes **[29]:**

- Fields of force containing only the harmonic terms.
- Force fields using higher order terms (cubic, quadratic…).
- Fields of force suggested by Allinger et al. **[30]** not only considering the terms of classical molecular mechanics but also chemical effects such as electronegativity.

- **MM2 / MM3 / MM4:**

MM2 is the first force field developed by Allinger et al. **[31,32].** It was initially designed for simple molecules (alkanes, alkenes, unconjugated alkynes, amines, etc.), but its improved versions MM3 (1989) **[33]** and MM4 (1996) **[34]** allow it to process more organic molecules and more complex.

- **OPLS:**

The Optimized Potentials for Liquid Simulations (OPLS) program, as its name suggests, is designed to optimize the potential for describing solvation properties. It is written by W. L Jorgensen and J. Tirado Rives **[35].**

- **GROMOS:**

GROMOS (Groningen Molecular Simulation Program Package), is written by VanGusteren**[36]** and designed specifically for biomolecules in an aqueous medium for the study of interactions between water molecules and polar groups of proteins.

- **CHARM (Bio +):**

Developed by Karplus et al **[37-38]**, for the calculation of biomolecules. Its concept is similar to that of AMBER. Although initially this force field was designed for amino acids and proteins, now it deals with other biomolecules.

- **SPASIBA:**

(Spectroscopic Potential Algorithm for Simulating biomolecular conformational Adaptability), developed by Gérard Vergoten et al. (1995).

It combines the Urey-Bradly-Shimanouchi modified spectroscopic force field **[39]** and the AMBER force field. It makes it possible to find the structures, the conformational energies and the vibrational frequencies at the minimum energy of a molecule **[40]**.

- **EMO:**

The EMO program (Energy Of Molecule), is developed by B. Blaive**[41-44]**, it is based on the force field MM2

- **AMBER:**

AMBER (Assisted Model Building with Energy Refinement), was written by Kollman **[45]**. The field is configured for proteins and nucleic acids (UCSF, 1994).

e. Minimization of steric energy

Minimization of a model is done in two steps. First, the energy expression (an equation describing the energy of the system as a function of its coordinates) must be defined and evaluated for a given conformation. Energy expressions may be defined that include external restraining terms to bias the minimization, in addition to the energy terms

Next, the conformation is adjusted to lower the value of the energy expression. A minimum may be found after one adjustment or may require many thousands of iterations, depending on the nature of the algorithm, the form of the energy expression, and the size of the model.

The efficiency of the minimization is therefore judged by both the time needed to evaluate the energy expression and the number of structural adjustments (iterations) needed to converge to the minimum **[46]**.

2.3.2. Molecular Dynamics

Molecular dynamics simulations are important tools for understanding the physical basis of the structure and function of biological macromolecules. The early view of proteins as relatively rigid structures has been replaced by a dynamic model in which the internal motions and resulting conformational changes play an essential role in their function **[47]**. Molecular dynamics is the study of how molecules move, deform, and interact over time. Predicting or interpreting these changes is essential in chemistry, physics, biology, engineering, and other fields **[48]**.

a. Molecular Dynamics calculation

Molecular dynamics are generally simulated in these stages:**[49]**

- ***Minimization***

This step involves finding the global minimum energy with respect to the position of side chains atoms that represents the geometry of the particular arrangements of atoms in which the net attractive force on each atom reaches a maximum

- ***Heating the system***

In heating phase, initial velocities (at 0 K) are assigned to each atom of the system during energy minimization and Newton's equations of motion that represent the time evolution of system are numerically integrated. At short predefined intervals, new velocities are assigned corresponding to a slightly higher temperature and the simulation is allowed to continue until desired temperature is achieved.

- ***Equilibration***

Equilibration stage is used to equilibrate kinetic and potential energies means distribute the kinetic energy "pumped" into the system. In explicit solvent simulation, protein positions are fixed and waters move accordingly. Once the solvent is equilibrated, the constraints on the protein can be removed and the whole system (protein solvent) can evolve in time.

- ***Production phase***

Production phase is the last step of the simulation methodology to remove constraints on protein

❖ ***Analysis***

In this step, stored coordinates and velocities of the system are used for further analysis.

b. <u>Applications of Dynamic molecular</u>

Molecular dynamics can now be routinely applied in the investigation of a wide range of dynamic properties and processes by researchers in numerous fields, including structural biochemistry, biophysics, enzymology, molecular biology, pharmaceutical chemistry, and biotechnology. Using MD simulations, one is able to study thermodynamic properties and time-dependent (i.e., kinetic) phenomena. This enables an understanding to be developed of various dynamic aspects of biomolecular structure, recognition, and function. However, when used alone, MD is of limited utility. An MD trajectory (i.e., the progress of simulated structure with respect to time) generally provides data only at the level of atomic positions, velocities, and single-point energies. To obtain the macroscopic properties in which one is usually interested requires the application of statistical mechanics, which connects microscopic simulations and macroscopic observables **[50]**.

2.3.3. Molecular Docking

Molecular docking is the process that involves placing molecules in appropriate configurations to interact with a receptor. Molecular docking is a natural process which occurs within seconds in a cell when bound to each other to form a stable complex.

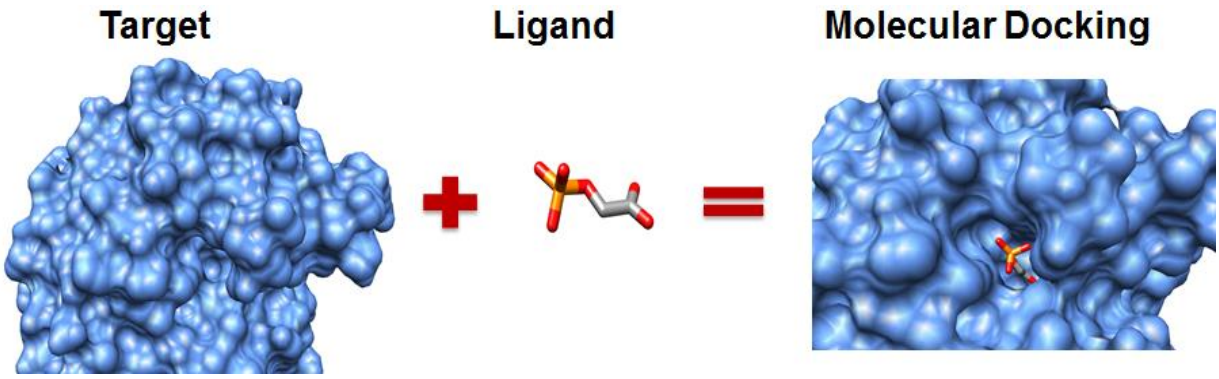


Figure 2: *Molecular Docking process*

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in rational drug design **[51]**.

Molecular docking programs use scoring functions to estimate the binding energetics of the predicted ligand-receptor complexes. Scoring functions are categorized in the three following groups: force-field-based, empirical, and knowledge-based functions **[52]**.

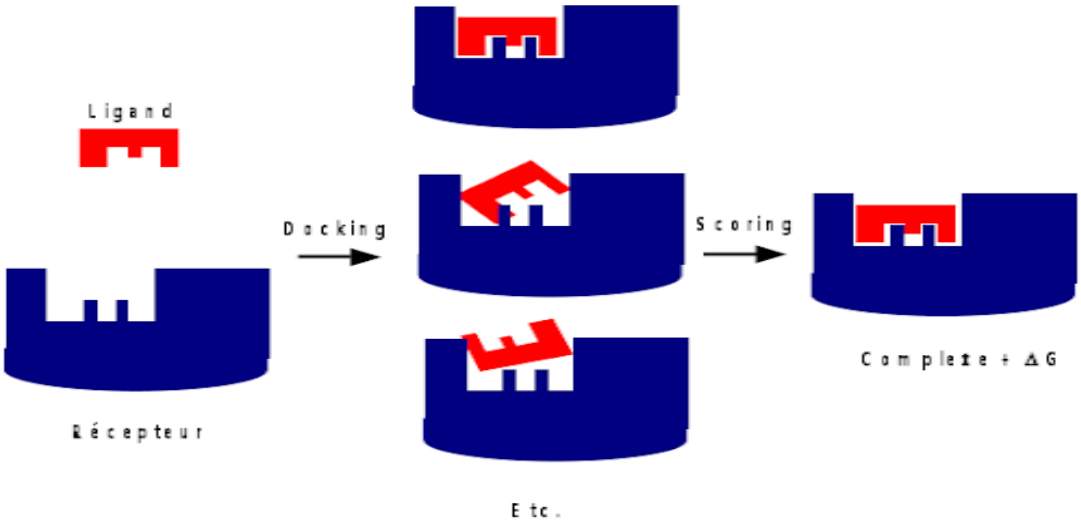


Figure 3: *Docking and Scoring function*

a. Different types of Interactions

Interactions between particles can be defined as a consequence of forces between the molecules contained by the particles. These forces are divided into four categories.

❖ Electrostatic forces: Forces with electrostatic origin due to the charges residing in the matter. The most common interactions are charge-charge, charge-dipole and dipole-dipole.

❖ Electrodynamics forces: The most widely known is the Van der Waals interactions.

❖ Steric forces: Steric forces are generated when atoms in different molecules come into very close contact with one another and start affecting the reactivity of each other. The resulting forces can affect chemical reactions and the free energy of a system.

❖ Solvent-related forces: These are forces generated due to chemical reactions between the solvent and the protein or ligand. Examples are Hydrogen bonds (hydrophilic interactions) and hydrophobic interactions.

❖ Other physical factors: Conformational changes in the protein and the ligand are often necessary for successful docking **[53]**.

b. General protocol of docking

The approaches currently used are exclusively calculative and evaluated by visualization tools. These approaches can be broken down into four to five successive phases: **[54]**.

(1) Choice of the mode of representation of proteins (all atoms, pseudo-atoms, grid, etc.).

(2) Conformational exploration (rigid body position / orientation of the ligand and / or flexible position / orientation / shape of the ligand).

(3) Minimization of the interaction energy evaluation function (or score function) of the conformations resulting from the exploration.

(4) Grouping by similarities and classification by evaluation or finer re-evaluation of the score, accompanied by a non-automatic step of visual evaluation of the results when the score does not allow discriminating the native conformation from the different conformations generated.

(5) An optional step of refining the complexes selected by minimization or molecular dynamics.

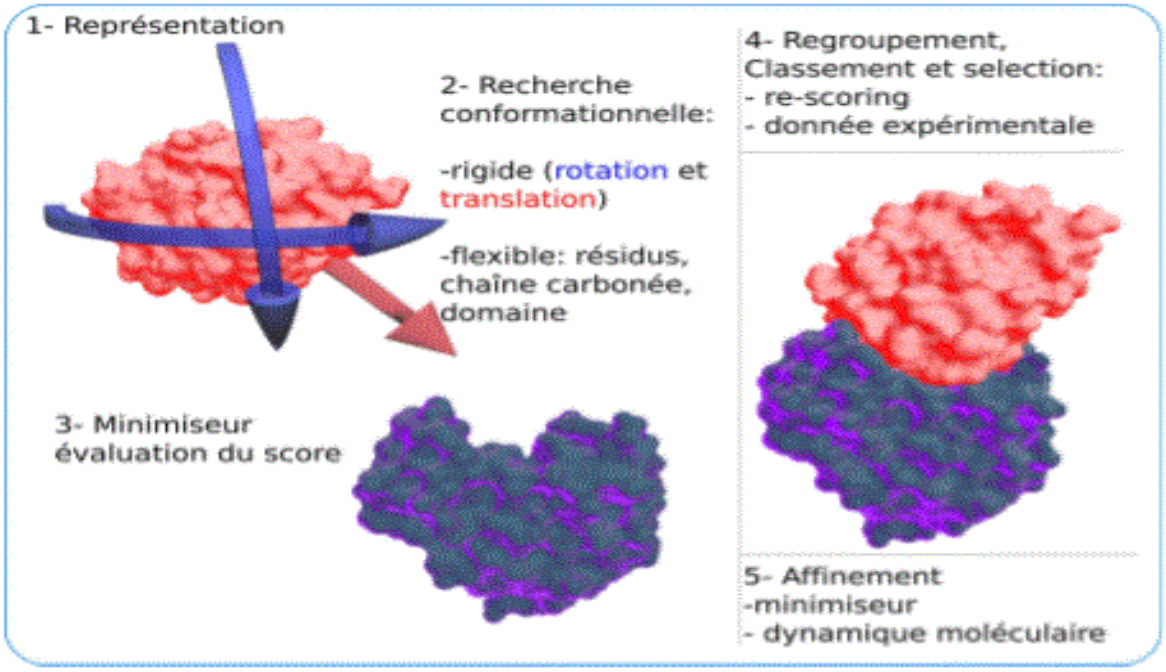


Figure 4: *The general docking protocol*

c. ***Molecular docking programs***

There are three main docking simulations types, depending on considered degrees of freedom (DoF):**[55]**.

- ❖ Rigid docking (target and ligand are considered rigid)
- ❖ Remi-flexible docking (flexible ligand and rigid target)
- ❖ Flexible docking (flexible ligand and partly flexible target)

Rigid docking programs are very fast but often inaccurate since the required bioactive conformation of the ligand is rarely known. The program FRED **[56]** behaves as a fully rigid tool during the sampling step, but it uses a pool of conformers for each ligand FlexX **[57]**, Glide **[58]**, DOCK **[59]** and Surflex **[60]** are widely used software of the semi-flexible category.

Eventually, programs like Autodock **[61,62]** and Gold **[63, 64]** may explicitly sample target DoF local flexibility, from rearranging polar hydrogens to sidechain rotations. Programs like RosettaLigand **[65]**,FlipDock **[66]** allow for both backbone and sidechain flexibility.

Over the last two decades, more than 60 different docking tools and programs have been developed for both academic and commercial, use such as GOLD **[67]**,MOE **[68]**, MOE-Dock **[69**],AutoDock Vina **[70]**,and many others.

3. ADME

ADME is the four-letter acronym for absorption, distribution, metabolism and excretion that has described pharmacokinetics for 50 years. These terms were first presented together in English by Nelson in 1961, rephrasing resorption, distribution, consumption and elimination used by Teorell in 1937. Other relevant seminal works include Widmark's description of first-order elimination in 1919 and Dost's 1953 treatise defining the term pharmacokinetics. ADME (T) has become a standard term, widely used in the literature, in teaching, in drug regulation and in clinical practice **[71]**.

To be effective as a drug, a powerful molecule must reach its target in the body in sufficient concentration, and remain there in a bioactive form long enough for the expected biological events to occur. Drug development involves the evaluation of absorption, distribution, metabolism and excretion (ADME) earlier and earlier in the discovery process, at a stage where the compounds considered are numerous but access to physical samples is limited. In this context, computer models are valid alternatives to experiences.

A large variety of in silico methods share the objective of predicting ADME parameters from the molecular structure **[72]**. Of note, the pioneering work of Lipinski et al examined oral active compounds to define physicochemical intervals for a high probability of being an oral medication. (That is, the drug resemblance) **[73]**. this is called Rule-of-delve which defines the relationship between pharmacokinetics and physicochemical parameters.

ADME and toxicological studies are critical parts of any drug development program, and essential for compliance with regulatory guidelines **[74]**.

Early ADME provides the necessary data for selecting preclinical candidates, appropriate dosage forms, formulation and accelerates the timeline for investigational new drug applications and subsequently new drug application submission to the FDA **[75,76]**.

a. Pharmacokinetics

Pharmacokinetics may be simply defined as what the body does to the drug**[77]**.

Pharmacokinetics is essentially the study of the absorption, distribution, metabolism andexcretion (ADME) of drugs ; how the body affects the drug Pharmacokinetics doesnot limit its scope to healthy or normal subjects but rather it includes variations in bioavailability, physiological or pathological conditions, disease related dose adjustment, and drug interactions **[78]**.

Pharmacokinetics is explain The movement of drug molecules from the site of application to the systemic circulation, through various barriers, their conversion into another chemical form and finally their exit out of the body can be expressed **[79]**.

b. Absorption, Distribution, Metabolism, Elimination (ADME)

Understanding these processes is extremely important for prescribers because they form the basis on which the optimal dose regimen is chosen and explain the majority of the inter-individual variation in the response to drug therapy.

The main processes involved in pharmacokinetics are absorption, distribution, and the two routes of drug elimination, metabolism and excretion. Together they are sometimes known by the acronym 'ADME'. Distribution, metabolism and excretion are sometimes referred to collectively as drug disposition.

- absorption is the process by which drugs enter the body.
- Distribution is the process by which drugs move around the body.
- Metabolism is the process by which drugs are chemically altered to make them sufficiently water-soluble for excretion in urine or faces (via the biliary tract).
- Excretion is the process by which drugs leave the body **[80]**. 4 steps in the pharmacokinetics of a drug are: Absorption - Distribution - Metabolism – Elimination (ADME).

❖ **Absorption**

The intent of drug administration is for the active component of the drug to reach the site of action and initiate a series of events that will culminate in a drug effect. The sum of all the processes that are entailed in the movement of the drug from the site of administration to the inside of the body is considered to be absorption. For most practical purposes, administration is

considered to be complete when all of the drug has entered the circulatory system and is thus being distributed to the rest of the body **[81]**.

Routes of administration can be considered in two categories:

Enteral: Drugs given by mouth are normally swallowed before being absorbed in the stomach or small bowel, after which they enter the portal venous system and pass through the liver before gaining access to the systemic circulation. Some drugs introduced into the alimentary tract are absorbed directly into the systemic circulation without passing through the liver (e.g. via the buccal, sublingual or rectal routes), thereby avoiding the potential hazards of gastric acid, binding to food, and metabolism by gut wall or liver enzymes (first-pass metabolism).

Parenteral: This includes any route that avoids absorption via the gastrointestinal tract such as administration by injection, inhalation or by application to the skin **[82]**.

❖ Distribution

Distribution of the drug to its site of action is the description of the differential distribution of the drug within the body. Distribution is easier to consider after bioavailability and clearance have defined steady-state concentration in the systemic circulation. Importantly, the concentration gradient between the systemic circulation and the site of action is often determined by transport processes. Although drug transport is important to bioavailability and clearance, it sits more comfortably under the heading of distribution **[83]**.

Some drugs leave the bloodstream very slowly because they bind tightly to proteins circulating in the blood. Others quickly leave the bloodstream and enter other tissues because they are less tightly bound to blood proteins. Some or virtually all molecules of a drug in the blood may be bound to blood proteins. The protein-bound part is generally inactive. As unbound drug is distributed to tissues and its level in the bloodstream decreases, blood proteins gradually release the drug bound to them. Thus, the bound drug in the bloodstream may act as a reservoir for the drug **[84]**.

❖ Metabolism

Metabolism is the process by which drugs are chemically changed from a lipid-soluble form suitable for absorption and distribution to a more water-soluble form that is suitable for excretion.

The process effectively eliminates the parent drug.

Drug metabolism occurs in two phases:

Phase I: in which drug molecules are altered chemically (by oxidation, reduction or hydrolysis) to make them suitable for Phase II reactions or for excretion. Oxidation is much the commonest form of Phase 1 reaction and involves chiefly members of the cytochrome P450 family of membrane-bound enzymes in the smooth endoplasmic reticulum of the liver cells. Most products of Phase 1 metabolism are pharmacologically inactive, although some retain activity to a greater or lesser degree, while others have activity that the parent drug did not possess.

Phase II: in which molecules of Phase I metabolite (or in some cases, unchanged drug) combine with an endogenous substrate to form an inactive conjugate that is much more water-soluble than the Phase I metabolite. Phase II reactions include synthesis of glucuronide or sulphate products, acetylation or methylation, and conjugation with glutathione **[85]**. The substances that result from metabolism (metabolites) may be inactive, or they may be similar to or different from the original drug in therapeutic activity or toxicity. Some drugs, called prodrugs, are administered in an inactive form, which is metabolized into an active form. Metabolites may be metabolized further instead of being excreted from the body. The subsequent metabolites are then excreted. Excretion involves elimination of the drug from the body, for example, in the urine or bile **[86]**.

❖ **Elimination**

All drugs are eventually eliminated from the body. They may be eliminated after being chemically altered (metabolized), or they may be eliminated intact. Most drugs, particularly water-soluble drugs and their metabolites are eliminated largely by the kidneys in urine. Therefore, drug dosing depends largely on kidney function. Some drugs are eliminated by excretion in the bile (a greenish yellow fluid secreted by the liver and stored in the gallbladder). Some drugs are excreted in saliva, sweat, breast milk, and even exhaled air. Most are excreted in small amounts. The excretion of drugs in breast milk is significant only because the drug may affect the breastfeeding infant. Excretion in exhaled air is the main way that inhaled anesthetics are eliminated **[87]**.

References

[1] Asiri, Yazeed. "Ab Initio and Semi-Empirical Calculations of Cyanoligated Rhodium Dimer Complexs." **(2017)**.

[2] Pensak, David A. "Molecular modelling: scientific and technological boundaries." Pure and Applied Chemistry 61.3 **(1989)**: 601-603.

[3] MOSTEFAOUI, Larbi. "Contribution à la description et à la compréhension de la solvatation des biomolécules." **(2011)**.

[4] Levine, I. N. "Quantum Chemistry, 4a edizione, **1991**.

[5] Pauling, Linus, and E. Bright Wilson. Introduction to quantum mechanics with applications to chemistry. Courier Corporation, **2012**.

[6] Lifson, S. "THEORETICAL FOUNDATION FOR THE EMPIRICAL FORCE-FIELD METHOD." GazzettaChimicaItaliana 116.12 **(1986)**: 687-692.

[7] Gogonea, Valentin, et al. "New developments in applying quantum mechanics to proteins." Current opinion in structural biology 11.2 **(2001)**: 217-223.

[8] Sachan, Alok Kumar. Applications of ab initio quantum chemistry to small organic molecules. Diss. Ph. D. thesis, University of Lucknow, India, **2015**.

[9] Austad, Jon. Møller-Plesset theory to second order for the study of molecuar systems in finite magnetic fields. MS thesis. **2013**.

[10] Ramachandran, K. I., Gopakumar Deepa, and Krishnan Namboori. Computational chemistry and molecular modeling: principles and applications. Springer Science & Business Media, **2008**.

[11] Cancès, Eric. "SCF algorithms for HF electronic calculations." Mathematical models and methods for ab initio quantum chemistry. Springer, Berlin, Heidelberg, **2000**. 17-43.

[12] Kohn, Walter. "Nobel Lecture: Electronic structure of matter—wave functions and density functionals." Reviews of Modern Physics 71.5 **(1999)**: 1253.

[13] Van Mourik, Tanja, Michael Bühl, and Marie-Pierre Gaigeot. "Density functional theory across chemistry, physics and biology." **(2014)**: 20120488.

[14] Christensen, Anders S., et al. "Semiempirical quantum mechanical methods for noncovalent interactions for chemical and biochemical applications." Chemical reviews 116.9 **(2016)**: 5301-5337.

[15] Karzazi, Yasser. Structure électronique et champ de forces moléculaire des cycloimmonium ylures. Diss. Lille 1, **1996**.

[16] Andrews, Donald H. "The relation between the Raman spectra and the structure of organic molecules." Physical Review 36.3 **(1930)**: 544.

[17] Nicotra, Francesco, ed. Organic and Bio-molecular Chemistry-Volume II. eolss Publications, **2009**.

[18] Bendix, J. "Ligfield In Comprehensive Coordination Chemistry II, Vol. 2: Fundamentals: Physical Methods, Theoretical Analysis, and Case Studies." **(2003)**: 673-676.

[19] Solomon, K. Anand. Molecular modelling and drug design. MJP Publisher, **2019**.

[20] J. Debord, Introduction à la modélisation moléculaire, **(2004)** : 37-41.

[21] Allinger, N. L. "Calculation of molecular structure and energy by force-field methods." Advances in physical organic chemistry. Vol. 13. Academic Press, **(1976):** 1-82.

[22] Guha, Rajarshi, and Rajesh Sardar. "Force Fields in Molecular Mechanics." **2001**.

[23] Brooks, B R et al. "CHARMM: the biomolecular simulation program." Journal of computational chemistry vol. 30,10 **(2009)**: 1545-614.

[24] Shattuck, Thomas W. "Colby College Molecular Mechanics Tutorial." Department of Chemistry, Colby College, Waterville, Maine 4901, **2008**.

[25] Navizet, I. Soury-Lavergne. thèse de doctorat "Modélisation et analyse des propriétés mécaniques des protéines." CHIMIE (Matière Condensée), **2004**.

[26] Dugas, Hermann. "Principes de base en modélisation moléculaire." Aspects théoriques et pratiques, quatrième édition, Librairie de l'Université de Montréal **1996**.

[27] Allinger, Janet, and Norman L. Allinger. "The conformers of 2-bromocyclohexanone." Tetrahedron 2.1-2 **(1958)**: 64-74.

[28]Richard, Philippe. "Introduction à la modélisation moléculaire." Mécanique Moléculaire, chapitre II,(mastercmpp. u-bourgogne. fr/Modelisation_Notes_Cours2_2009. pdf) **2010.**

[29] Dinur, Uri, and Arnold T. Hagler. "New approaches to empirical force fields." Reviews in computational chemistry 2 **(1991)**: 99-164.

[30] Allinger, Norman L., Kuohsiang Chen, and Jenn-HueiLii. "An improved force field(MM4) for saturated hydrocarbons." Journal of computational chemistry 17.5-6 **(1996)**: 642-668.

[31] Allinger, N. L. "Calculation of molecular structure and energy by force-field methods." Advances in physical organic chemistry. Vol. 13. Academic Press, **(1976):** 1-82.

[32] Allinger, Norman L. "Conformational analysis. 130. MM2. A hydrocarbon force field utilizing V1 and V2 torsional terms." Journal of the American Chemical Society 99.25 **(1977)**:8127-8134.

[33] Allinger, Norman L., Young H. Yuh, and Jenn HueiLii. "Molecular mechanics. The MM3 force field for hydrocarbons. 1." Journal of the American Chemical Society 111.23 **(1989)**: 8551-8566.

[34] Allinger, Norman L., Kuohsiang Chen, and Jenn-HueiLii. "An improved force field (MM4) for saturated hydrocarbons." Journal of computational chemistry 17.5-6 **(1996)**: 642-668.

[35] Jorgensen, William L., and Julian Tirado-Rives. "The OPLS [optimized potentials for liquid simulations] potential functions for proteins, energy minimizations for crystals of cyclic peptides and crambin." Journal of the American Chemical Society 110.6 **(1988)**: 1657-1666.

[36] Van Gunsteren, Wilfred F., and Martin Karplus. "Effect of constraints on the dynamics of macromolecules." Macromolecules 15.6 **(1982)**: 1528-1544.

[37] Brooks, Bernard R., et al. "CHARMM: a program for macromolecular energy, minimization, and dynamics calculations." Journal of computational chemistry 4.2 **(1983)**: 187-217.

[38] Smith, Jeremy C., and Martin Karplus. "Empirical force field study of geometries and conformational transitions of some organic molecules." Journal of the American Chemical Society 114.3 **(1992)**: 801-812.

[39] Shimanouchi, T. "Force constants of small molecules." Pure and Applied Chemistry 7.1 **(1963)**: 131-146.

[40] Aguilar-Parrilla, F., et al. "ELSEVIER Journal of Molecular Structure" 378 **(1996)** 259.

[41] Bouraoui, Ali, et al. "Design and molecular mechanics calculations of new iron chelates." Journal of the Chemical Society, Perkin Transactions 2 7 **(1990)**: 1211-1214.

[42] A. Bouraoui, M. Fathallah, F. M'Henni, B. Blaive, R. Gallo, Studies in Physical and Theoritical chemistry (Modeling of Molecular Structures and Properties. Proceeding of an International Meeting Nancy, France), J.L. Rivail., **(1989):** 381.

[43] Zinelabidine, A., et al. "Molecular mechanics modelling of siderophores." Journal of Molecular Structure: THEOCHEM 286 **(1993)**: 267-274.

[44] Blaive, B., G. Legsai, and R. Lai. "Utilization of d0, d1, d2 electron configurations to obtain parameters for transition metals in the molecular mechanics of dioxo-or diimido-tetrahedral complexes (Cr, Mo, Re, Os)." Journal of molecular structure 354.3 **(1995)**: 245-250.

[45] Weiner, Scott J., et al. "An all atom force field for simulations of proteins and nucleic acids." Journal of computational chemistry 7.2 **(1986)**: 230-252.

[46] Auerbach, Scott M., Kathleen A. Carrado, and Prabir K. Dutta. Handbook of layered materials. CRC press, **2004**.

[47] Karplus, Martin, and J. Andrew McCammon. "Molecular dynamics simulations of biomolecules." Nature structural biology 9.9 **(2002)**: 646-652.

[48] Leimkuhler, Ben, and Charles Matthews. Molecular Dynamics. Springer International PU, **2016**.

[49]Patodia, Sachin, Ashima Bagaria, and Deepak Chopra. "Molecular dynamics simulation of proteins: A brief overview." Journal of Physical Chemistry & Biophysics 4.6 **(2014):** 1.

[50] Hollingsworth, Scott A., and Ron O. Dror. "Molecular dynamics simulation for all." Neuron 99.6 **(2018)**: 1129-1143.

[51] Adcock, Stewart A., and J. Andrew McCammon. "Molecular dynamics: survey of methods for simulating the activity of proteins." Chemical reviews 106.5 **(2006)**: 1589-1615.

[52] Supriya, T., et al. "An overview on molecular docking." American Journal of Biological and Pharmaceutical Research 3 **(2016)**: 83-89.

[53] Ferreira, Leonardo G., et al. "Molecular docking and structure-based drug design strategies." Molecules 20.7 **(2015)**: 13384-13421.

[54] Lam, Heidi, et al. "Empirical studies in information visualization: Seven scenarios." IEEE transactions on visualization and computer graphics 18.9 **(2011)**: 1520-1536.

[55] Férey, Nicolas, et al. "Docking de protéines en réalité virtuelle. Une approche hybride et multimodale." **(2009).**

[56] Hoffer, Laurent, et al. "S4MPLE—sampler for multiple protein-ligand entities: methodology and rigid-site docking benchmarking." Molecules 20.5 **(2015)**: 8997-9028.

[57] McGann, Mark. "FRED pose prediction and virtual screening accuracy." Journal of chemical information and modeling 51.3 **(2011)**: 578-596.

[58] Rarey, Matthias, et al. "A fast flexible docking method using an incremental construction algorithm." Journal of molecular biology 261.3 **(1996)**: 470-489.

[59] Friesner, Richard A., et al. "Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy." Journal of medicinal chemistry 47.7 **(2004)**: 1739-1749.

[60] Ewing, Todd JA, et al. "DOCK 4.0: search strategies for automated molecular docking of flexible molecule databases." Journal of computer-aided molecular design 15.5 **(2001)**: 411-428.

[61] Jain, Ajay N. "Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine." Journal of medicinal chemistry 46.4 **(2003)**: 499-511.

[62] Goodsell, David S., Garrett M. Morris, and Arthur J. Olson. "Automated docking of flexible ligands: applications of AutoDock." Journal of molecular recognition 9.1 **(1996)**: 1-5.

[63] Morris, Garrett M., et al. "Distributed automated docking of flexible ligands to proteins: parallel applications of AutoDock 2.4." Journal of computer-aided molecular design 10.4 **(1996)**: 293-304.

[64] Jones, Gareth, et al. "Development and validation of a genetic algorithm for flexible docking." Journal of molecular biology 267.3 **(1997)**: 727-748.

[65] Verdonk, Marcel L., et al. "Improved protein–ligand docking using GOLD." Proteins: Structure, Function, and Bioinformatics 52.4 **(2003)**: 609-623.

[66] Meiler, Jens, and David Baker. "ROSETTALIGAND: Protein–small molecule docking with full side-chain flexibility." Proteins: Structure, Function, and Bioinformatics 65.3 **(2006)**: 538-548.

[67] Zhao, Yong, and Michel F. Sanner. "FLIPDock: docking flexible ligands into flexible receptors." Proteins: Structure, Function, and Bioinformatics 68.3 **(2007)**: 726-737.

[68] Jones, Gareth, et al. "Development and validation of a genetic algorithm for flexible docking." Journal of molecular biology 267.3 **(1997)**: 727-748.

[69] Vilar, Santiago, Giorgio Cozza, and Stefano Moro. "Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery." Current topics in medicinal chemistry 8.18 **(2008):** 1555-1572.

[70] Corbeil, Christopher R., Christopher I. Williams, and Paul Labute. "Variability in docking success rates due to dataset preparation." Journal of computer-aided molecular design 26.6 **(2012)**: 775-786.

[71] Doogue, Matthew P., and Thomas M. Polasek. "The ABCD of clinical pharmacokinetics." **(2013)**: 5-7.

[72] Tian, Sheng, et al. "The application of in silico drug-likeness predictions in pharmaceutical research." Advanced drug delivery reviews 86 **(2015)**: 2-10.

[73] Lipinski, Christopher A., et al. "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings." Advanced drug delivery reviews 23.1-3 **(1997)**: 3-25.

[74] Reichel, Andreas. "The role of blood-brain barrier studies in the pharmaceutical industry." Current drug metabolism 7.2 **(2006)**: 183-203.

[75] Baumann, Andreas. "Nonclinical development of biopharmaceuticals." Drug discovery today 14.23-24 **(2009)**: 1112-1122.

[76] Gannu, Ramesh, et al. "Optimization of matrix diffusion mediated transdermal therapeutic system for buspirone: effect of variables on in vitro release, ex vivo permeation and pharmacotechnical properties." ACTA PharmaceuticaSciencia 52.3 **(2010)**.

[77] Peters, Sheila Annie. Physiologically-based pharmacokinetic (PBPK) modeling and simulations: principles, methods, and applications in the pharmaceutical industry. John Wiley & Sons, **2012**.

[78] Currie, Geoffrey M. "Pharmacology, part 2: introduction to pharmacokinetics." Journal of nuclear medicine technology 46.3 **(2018)**: 221-230.

[79] Talevi, Alan, and Pablo AM Quiroga, eds. ADME Processes in Pharmaceutical Sciences: Dosage, Design, and Pharmacotherapy Success. Springer, **2018**.

[80] Mangoni, Arduino A., and Stephen HD Jackson. "Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications." British journal of clinical pharmacology 57.1 **(2004)**: 6-14.

[81] Martinez, Marilyn N., and Gordon L. Amidon. "A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals." The Journal of Clinical Pharmacology 42.6 **(2002)**: 620-643.

[82] Shekhawat, Prachi B., and Varsha B. Pokharkar. "Understanding peroral absorption: regulatory aspects and contemporary approaches to tackling solubility and permeability hurdles." Acta pharmaceutica sinica B 7.3 **(2017)**: 260-280.

[83] Doogue, Matthew P., and Thomas M. Polasek. "The ABCD of clinical pharmacokinetics." **(2013)**: 5-7.

[84] Shargel, Leon, B. C. Andrew, and Susanna Wu-Pong. Applied biopharmaceutics & pharmacokinetics. Stamford: Appleton & Lange, **1999**.

[85] Benet, Leslie Z. "The drug transporter− metabolism alliance: uncovering and defining the interplay." Molecular pharmaceutics 6.6 **(2009):** 1631-1643.

[86] Salvo, Susan G. Mosby's Pathology for Massage Therapists-E-Book. Elsevier Health Sciences, **2017**.

[87] Seng kok-Yong, Lee Lawrence «Drug Distribution and Elimination» Nov **2015**.

Chapter II : Proteins, Enzymes, Amino Acids

PART (A)

Proteins, enzymes and amino acids

1. Introduction

The basic elements of the functioning of any living system, proteins and nucleic acids (DNA - deoxyribonucleic acids or RNA - ribonucleic acids) are long chains of polymers made up of hundreds or thousands of atoms, mainly carbon, oxygen, nitrogen and hydrogen.

Ribosomes are nanometric-sized structures (or organelles). Present in the cytoplasm of living cells, they serve to assemble amino acids to form proteins **[1]**.

A human organism is a huge "machine": biochemical reactions take place continuously. Proteins are essential actors in these reactions and cellular mechanisms. These long chains made up of thousands of atoms have an astonishing property: to be active, they must be folded correctly if not, their function is disturbed. The shape of a protein is imposed by the interactions between atoms **[2]**.

Protein-protein interactions are an essential aspect of biological processes. They are strongly involved in the formation of macromolecular structures, in signaling, in regulation and in the various metabolic pathways.

Protein interaction, major goal in the study of biological systems. Protein-protein interactions play a significant role in the induction of many disease states and in the processes important for the pathogenesis of bacterial and viral infections **[3]**.

The high specificity of these interactions makes them ideal targets for therapeutic agents **[4]**.

Therefore, their study has enormous importance in understanding the functioning of the human body and in the fight against deadly diseases. Because of the very great complexity of proteins, (the average size of a protein is several thousand atoms), computer processing is necessary to analyze their structure **[5]**.

In this chapter we will give some biological concepts concerning the structures of proteins, and their interactions.

2. The proteins

Proteins discovered by Dutch chemist Gerhard Mulder (1802-1880). The term protein comes from the ancient Greek prôtos which means first, essential. This probably refers to the fact that proteins are essential for life and that they often constitute the majority (≈60%) of the dry weight of cells. Proteins take many forms and perform multiple functions. But this was not discovered until much later, during the 20th century **[6]**.

Proteins are remarkable and mysterious molecules. Not only they are the most abundant molecules in biology (besides water) but they are also responsible for every chemical process that makes the existence of living systems possible. Proteins are synthesized as polymeric sequences of amino acid residues but, in order to be functional, most proteins need to acquire a specific three-dimensional structure. The folding process is usually extremely efficient, leading to the formation of highly specific structures that grant proteins selective and diverse functions **[7]**.
Proteins are the main building blocks of all cells in the human body. These are chains of amino acids that can enter into the composition of muscles, skin, nails, hair, blood, etc. These are also the basis of many hormones, enzymes and antibodies and are necessary for the growth, repair and defense of human body tissue.

- Protein characteristics:
  - ❖ Macro nutrients essential for life
  - ❖ Made up of amino acids, essential or not, which define the quality of the protein
  - ❖ In the diet we find animal proteins and vegetable proteins
  - ❖ They have many roles in the body (enzyme, transport, tissue structure, etc…)
  - ❖ Protein requirements change over a lifetime **[8]**.

2.1.Definition of proteins

A protein is a biological polymer comprising numerous amino acids linked recursively through peptide bonds between a carboxyl group and an amino group of adjacent amino acids to form a long chain with the defining side group of each amino acid protruding from it. The sequence of amino acids in a protein is defined by a gene and encoded in the genetic code, which selects protein components from a set of 20 "standard" amino acids.

Some proteins function as separate entities while others associate together to form stable functional complexes, such as the ribosomes, which comprise more than 50 proteins. Along with polysaccharides, lipids, and nucleic acids, proteins are one of the major classes of macromolecules that make up the primary constituents of biological organisms **[9]**.in that they contain not only carbon, hydrogen and oxygen, but also nitrogen. This composition exists both in small molecules, amino acids and, in macromolecules, proteins. These are in fact formed by the chain of amino acids attached to each other by so-called peptide bonds (–CO-NH-) associating

the acid function of an amino acid with the amine function of the next amino acid **[10]**.

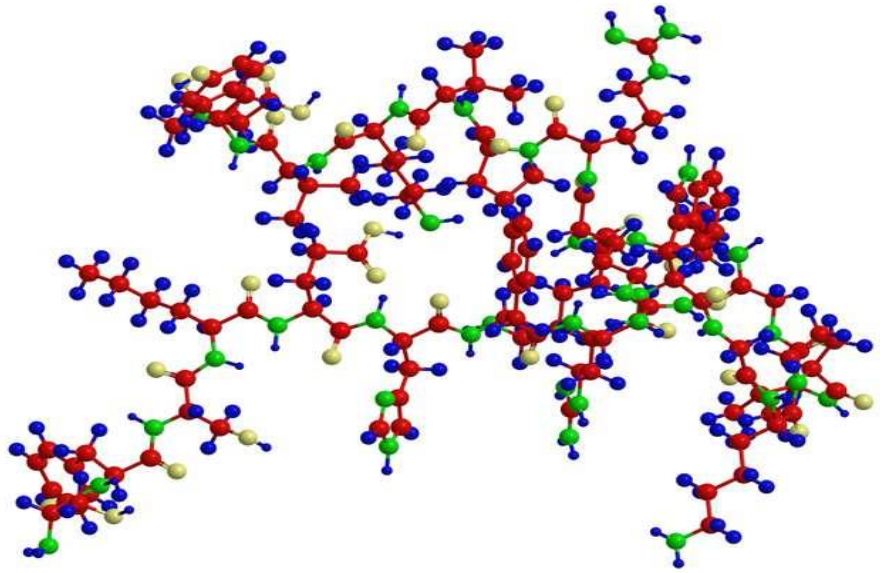


Figure 1: *The molecular structure of a peptide (a small protein) consists of a sequence of amino acids.*

Proteins are the essential elements of the life of the cell: they can play a structural role, a role in mobility, a catalytic role (enzymes), a role in regulating DNA compaction or gene expression, etc. In fact, the vast majority of cellular functions are carried out by proteins. Proteins are essential elements for tissue growth and repair, the proper functioning of living cells and their structure **[11]**.

2.2.The peptide bond

The common property of all proteins is that they consist of long chains of α-amino (alpha amino) acids. The general structure of α-amino acids is shown in Figure 2 . The α-amino acids are so called because the α-carbon atom in the molecule carries an amino group (—NH_2); the α-carbon atom also carries a carboxyl group (—COOH**) [12]**.

Amino acids can be linked by a condensation reaction in which an —OH is lost from the carboxyl group of one amino acid along with a hydrogen from the amino group of a second, forming a molecule of water and leaving the two amino acids linked via an amide called, in this case, a peptide bond. At the turn of the 20th century, German chemist Emil Fischer first proposed this linking together of amino acids. Note that when individual amino acids are combined to form

proteins, their carboxyl and amino groups are no longer able to act as acids or bases, since they have reacted to form the peptide bond.

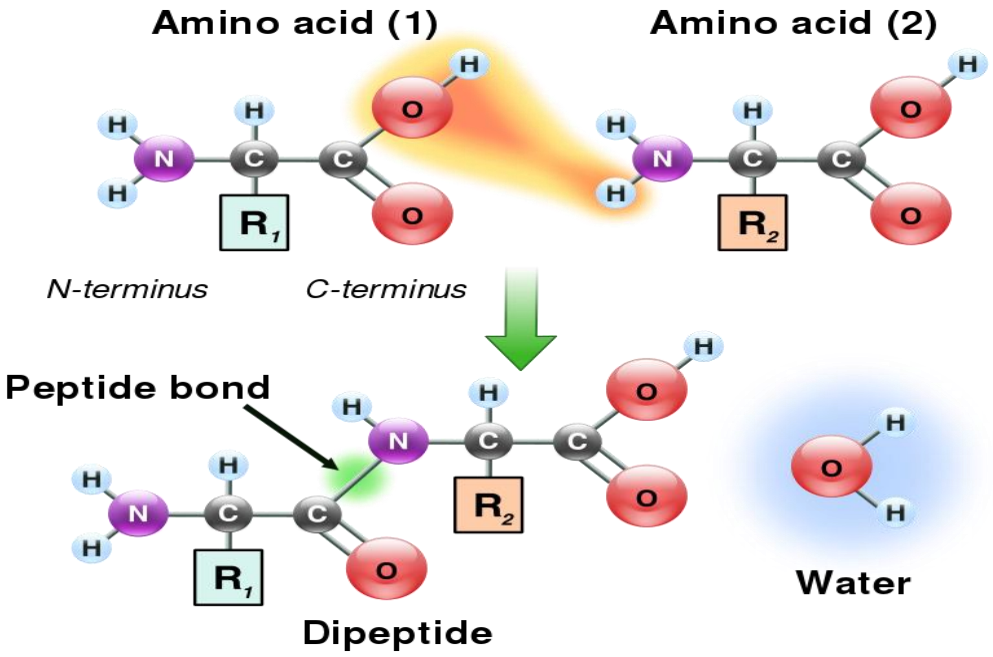


Figure 2: *Formation of a peptide bond*

Amino acids joined by a series of peptide bonds are said to constitute a peptide. After they are incorporated into a peptide, the individual amino acids are referred to as amino acid residues. Small polymers of amino acids (fewer than 50) are called oligo-peptides, while larger ones (more than 50) are referred to as polypeptides. Hence, a protein molecule is a polypeptide chain composed of many amino acid residues, with each residue joined to the next by a peptide bond. The lengths for different proteins range from a few dozen to thousands of amino acids, and each protein contains different relative proportions of the 20 standard amino acids **[13]**.

2.3.Protein structure

Proteins have different levels of structure within the body. These levels vary according to the type of protein (i.e. according to the amino acid sequence of the protein defining its functionality), the level of maturation of a protein, or according to the medium in which the protein is found **[14]**.

There are 4 levels of structure:

- ✓ Primary structure
- ✓ Secondary structure
- ✓ Tertiary structure
- ✓ Quaternary structure

a. Primary structure

A protein's primary structure is the unique sequence of amino acids in each polypeptide chain that makes up the protein. Really, this is just a list of which amino acids appear in which order in a polypeptide chain, not really a structure. But, because the final protein structure ultimately depends on this sequence, this was called the primary structure of the polypeptide chain **[15]**.

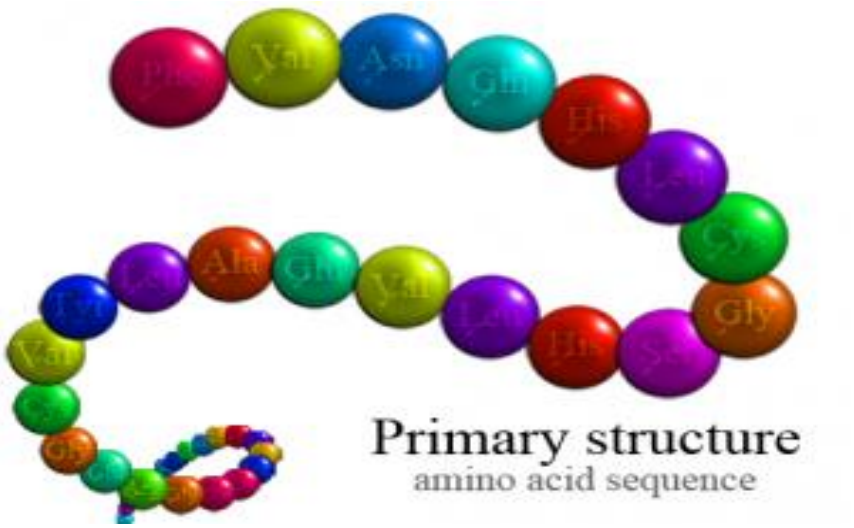


Figure 3: *Primary structure of a protein.*

b. Secondary structure

The nitrogen and carbon atoms of a peptide chain cannot lie on a straight line, because of the magnitude of the bond angles between adjacent atoms of the chain; the bond angle is about 110°. Each of the nitrogen and carbon atoms can rotate to a certain extent, however, so that the chain has a limited flexibility. Because all of the amino acids, except glycine, are asymmetric L-amino acids, the peptide chain tends to assume an asymmetric helical shape; some of the fibrous proteins consist of elongated helices around a straight screw axis. Such structural features result from properties common to all peptide chains. The product of their effects is the secondary structure of the protein **[16]**.

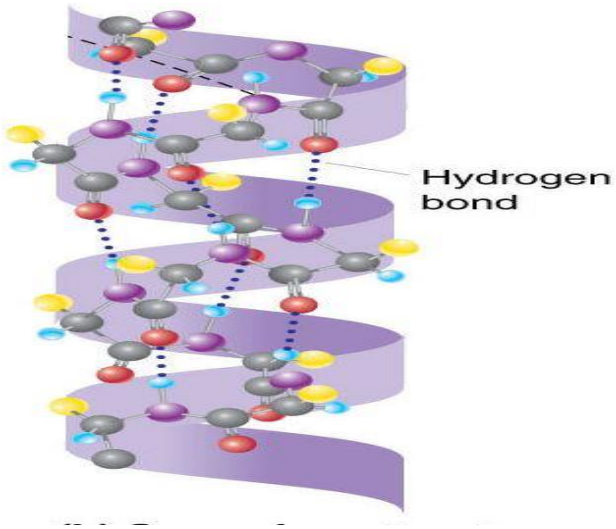


Figure 4 : *Secondary structure*

The two main types of secondary structure are the α-helix and the ß-sheet:

The α-Helix: The α-helix is a common secondary structure encountered in proteins of the globular class. The formation of the α-helix is spontaneous and is stabilized by H-bonding between amide nitrogens and carbonyl carbons of peptide bonds spaced four residues apart. This orientation of H-bonding produces a helical coiling of the peptide backbone such that the R-groups lie on the exterior of the helix and perpendicular to its axis.

Not all amino acids favor the formation of the (α-helix due to steric constraints of the R-groups. Amino acids such as A, D, E, I, L and M favor the formation of α-helices, whereas, G and P favor disruption of the helix. This is particularly true for P since it is a pyrrolidine based imino acid (HN=) whose structure significantly restricts movement about the peptide bond in which it is present, thereby, interfering with extension of the helix. The disruption of the helix is important as it introduces additional folding of the polypeptide backbone to allow the formation of globular proteins.

The β-Sheets: Whereas a α-helix is composed of a single linear array of helically disposed amino acids, β-sheets are composed of 2 or more different regions of stretches of at least 5-10 amino acids. The folding and alignment of stretches of the polypeptide backbone aside one another to form β-sheets is stabilized by H-bonding between amide nitrogens and carbonyl carbons. However, the H-bonding residues are present in adjacently opposed stretches of the polypetide

backbone as opposed to a linearly contiguous region of the backbone in the α-helix. β-sheets are said to be pleated. This is due to positioning of the α-carbons of the peptide bond which alternates above and below the plane of the sheet. β-sheets are either parallel or antiparallel. In parallel sheets adjacent peptide chains proceed in the same direction (i.e. the direction of N-terminal to C-terminal ends is the same), whereas, in antiparallel sheets adjacent chains are aligned in opposite directions. β-sheets can be depicted in ball and stick format or as ribbons in certain protein formats **[17].**

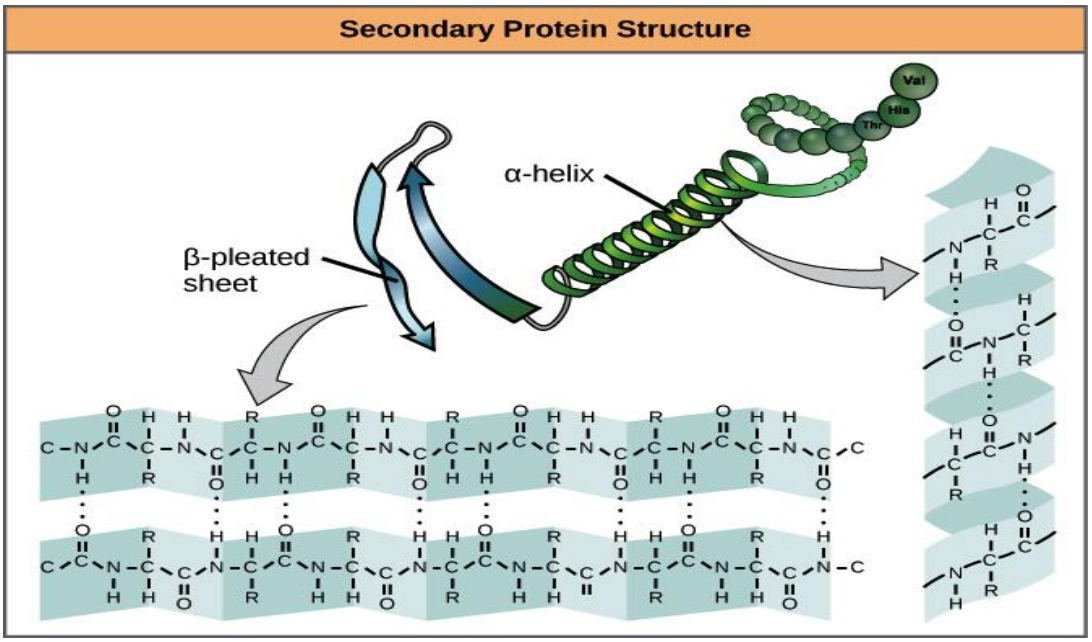


Figure 5:*α and β conformations of the secondary structures of a protein*

c. Tertiary structure

The tertiary structure of a polypeptide chain is its overall three-dimensional shape, once all the secondary structure elements have folded together among each other. Interactions between polar, nonpolar, acidic, and basic R group within the polypeptide chain create the complex three-dimensional tertiary structure of a protein. When protein folding takes place in the aqueous environment of the body, the hydrophobic R groups of nonpolar amino acids mostly lie in the interior of the protein, while the hydrophilic R groups lie mostly on the outside. Cysteine side chains form disulfide linkages in the presence of oxygen, the only covalent bond forming during protein folding. All of these interactions, weak and strong, determine the final three-dimensional

shape of the protein. When a protein loses its three-dimensional shape, it will no longer be functional **[18]**.

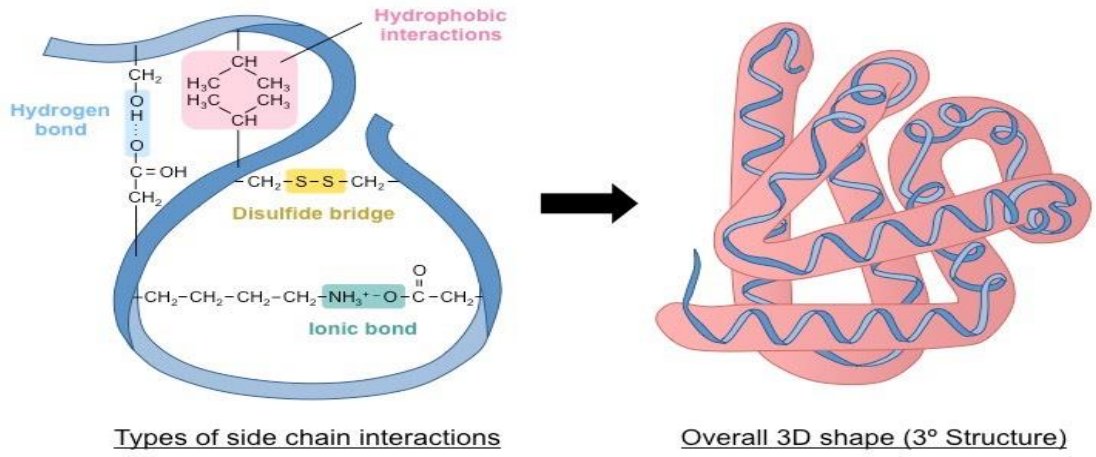


Figure 6: *Tertiary structure of a protein*

d. Quaternary structure

Quaternary Structure refers to the structure of a protein macromolecule formed by interactions between multiple polypeptide chains. Each polypeptide chain is referred to as a subunit. Proteins with quaternary structure may consist of more than one of the same type of protein subunit. They may also be composed of different subunits. **[19]**

Each of the subunits has its own primary, secondary, and tertiary structure. The subunits are held together by disulfide-bridges and hydrogen bonds and, van der Waals forces between non polar side chains **[20]**.

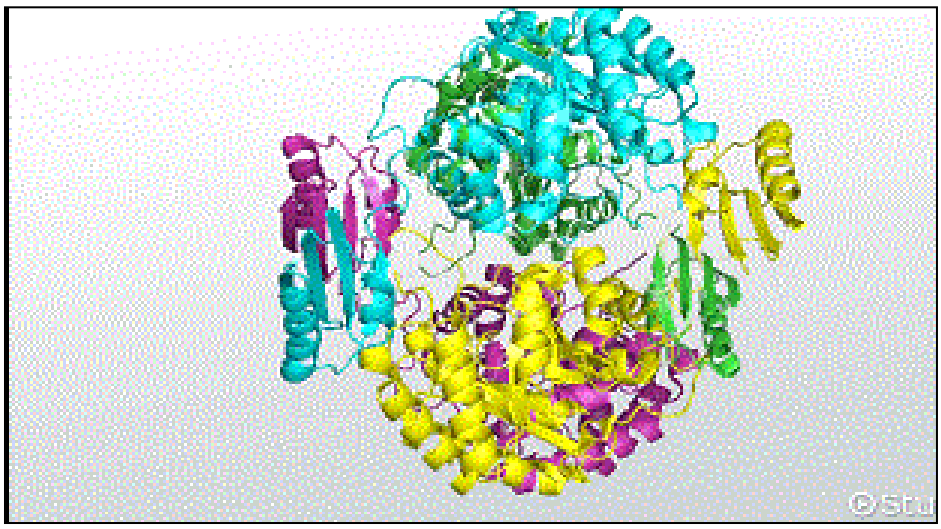


Figure 7: *Quaternary structure of a protein*

2.4.Protein functions

Proteins are large, complex molecules that play many critical roles in the body. They do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs.

Proteins can be described according to their large range of functions in the body: **[21]**.

- ***Antibodies***

Protein forms antibodies that help prevent infection, illness and disease. These proteins identify and assist in destroying antigens such as bacteria and viruses. They often work in conjunction with the other immune system cells. For example, these antibodies identify and then surround antigens in order to keep them contained until they can be destroyed by white blood cells **[22]**.

- ***Growth and Maintenance***

Protein is required for the growth and maintenance of tissues. Your body's protein needs are dependent upon your health and activity level.

- ***Causes Biochemical Reactions***

Enzymes are proteins that aid the thousands of biochemical reactions that take place within and outside of your cells

The structure of enzymes allows them to combine with other molecules inside the cell called substrates, which catalyze reactions that are essential to your metabolism

Enzymes may also function outside the cell, such as digestive enzymes like lactase and sucrase, which help digest sugar.

Some enzymes require other molecules, such as vitamins or minerals, for a reaction to take place.

Bodily functions that depend on enzymes include:

-Digestion -Energy production -Muscle contraction

- ***Acts as a Messenger***

Amino acid chains of various lengths form protein and peptides, which make up several of your body's hormones and transmit information between your cells, tissues and organs.

- ***Provides Structure***

A class of proteins known as fibrous proteins provides various parts of your body with structure, strength and elasticity.

- ***Maintains Proper pH***

Proteins act as a buffer system, helping your body maintain proper pH values of the blood and other bodily fluids.

- ***Balances Fluids***

Proteins regulate body processes to maintain fluid balance.

Proteins in your blood maintain the fluid balance between your blood and the surrounding tissues.

- ***Bolsters Immune Health***

Proteins form antibodies to protect your body from foreign invaders, such as disease-causing bacteria and viruses.

- ***Transports and Stores Nutrients***

Some proteins transport nutrients throughout your entire body, while others store them.

- ***Provides Energy***

Protein can serve as a valuable energy source but only in situations of fasting, exhaustive exercise or inadequate calorie intake **[23]**.

3. The enzymes

the word ‘enzyme’ was first used by the German physiol-ogist Wilhelm Kühne in 1878, when he was describing the ability of yeast to produce alcohol from sugars, and it is derived from the Greek words en (meaning ‘within’) and zume (meaning ‘yeast’).In the late nineteenth century and early twentieth century, signifi cant advances were made in the extraction, characterization and commercial exploitation of many enzymes, but it was not until the 1920s that enzymes were crystallized, revealing that catalytic activity is associated with protein molecules **[24]**.

Enzymes are proteins that act as catalysts within living cells. Catalysts increase the rate at which chemical reactions occur without being consumed or permanently altered themselves. A chemical reaction is a process that converts one or more substances (known as reagents, reactants, or substrates) to another type of substance (the product). As a catalyst, an enzyme can facilitate the same chemical reaction over and over again.

On the surface of each enzyme is a special cleft called the **active site**, which provides a place where reagents can 'meet' and interact. Much like a lock and its key, an enzyme's active site will only accommodate certain reagents, and only one type of chemical reaction can be catalyzed by a given enzyme **[25]**.

3.1. Classification of enzymes

According to the type of reactions that the enzymes catalyze, enzymes are classified into six categories, which are oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Oxidoreductases, transferases and hydrolases are the most abundant forms of enzymes. Individual enzyme classes are further classified systematically based on the chemical name of the substrate and its reaction mechanism **[26, 27]**.

Table 1: *Represents classifications of enzymes*

Enzyme class	Reaction type	Description
EC 1 Oxidoreductases	$A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$	Catalyze redox reaction and can be categorized into oxidase and reductase.
EC 2 Transferases	$A\text{-}B + C \longrightarrow A + B\text{-}C$	Catalyze the transfer or exchange of certain groups among some substrates
EC 3 Hydrolases	$A\text{-}B + H_2O \longrightarrow A\text{-}H + B\text{-}OH$	Accelerate the hydrolysis of substrates
EC 4 Lyases	$A\text{-}B \rightleftharpoons A + B$ (reverse reaction: synthase)	Promote the removal of a group from the substrate to leave a double bond reaction or catalyze its reverse reaction
EC 5 Isomerases	$A\text{-}B\text{-}C \rightleftharpoons A\text{-}C\text{-}B$	Facilitate the conversion of isomers, geometric isomers or optical isomers.
EC 6 Ligases	$A + B + ATP \longrightarrow A\text{-}B + ADP + P_i$	Catalyze the synthesis of two molecular substrates into one molecular compound with the release energy

3.2.Nomenclature

- Before 1961, enzymes were named after the name of the S on which they act by adding the suffix "ase".
- Then there was a functional nomenclature, where the name of the enzyme indicates both:
  - ✓ The name of the substrate
  - ✓ The catalyzed reaction

- ✓ Followed by the suffix "ase"

- The Enzyme Commission has established a classification that assigns each enzyme a number with four figures E.C. X1.X2.X3.X.4.

This nomenclature specifies and supplements the functional nomenclature.

The four digits of the EC nomenclature of enzymes each designate a characteristic of the enzyme which makes it possible to identify it according to the type of catalyzed reaction:

• X1: The first number which can vary from 1 to 6 indicates the type of reactions

• X2: The second designates the subclass of the enzyme which is defined according to its mechanism of action.

• X3: The 3rd number designates the nature of the molecule that serves as an acceptor, when it is a transfer of electrons.

• X4: The 4th number is a serial number in the group and in the subgroup **[28]**.

3.3. Notions of specificity

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity, they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity :

- ❖ Absolute specificity - the enzyme will catalyze only one reaction.
- ❖ Group specificity - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
- ❖ Linkage specificity - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.

- ❖ Stereo-chemical specificity - the enzyme will act on a particular steric or optical isomer **[29]**.

The specificity is manifested, on the one hand with respect to the reaction catalyzed by the enzyme, on the other hand with respect to the substrate of the reaction

The specificity of the enzyme is due to the presence of a three-dimensional region called "active site" which recognizes and fixes the substrate. Enzymes therefore have the ability to target reactions or even prevent other undesirable ones **[30].**

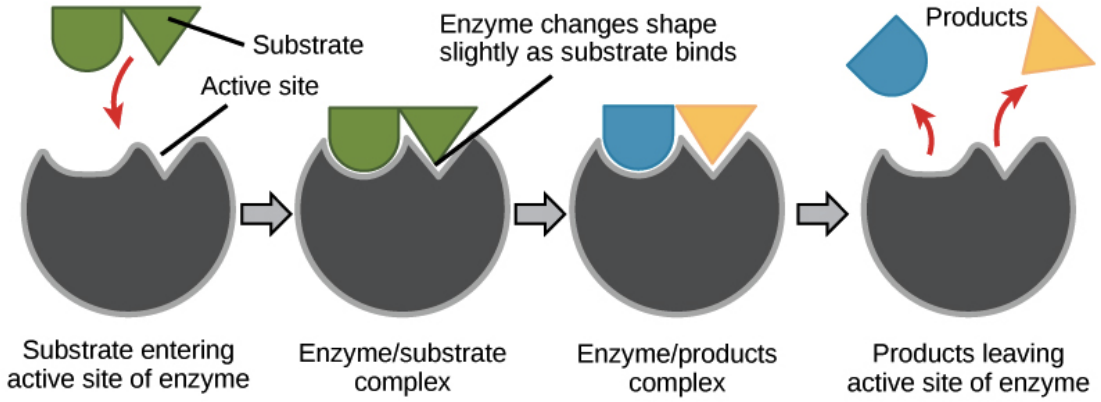


Figure 8 : *Action of an enzyme*

3.4.The Active Site

The active site of an enzyme is the region where specific substrates bind to the enzyme, catalyzing the chemical reaction. Substrate binding site along with the catalytic site form the active site of the enzyme. The enzyme binds with a specific substrate in order to catalyze a chemical reaction that changes the substrate in some way. The substrate is smaller in size than its enzyme. The substrate is perfectly oriented inside the enzyme by the active site. One or more substrate binding sites can be found in an enzyme. The catalytic site occurs next to the binding site, carrying out the catalysis. It is composed of around two to four amino acids, involved in the catalysis. The amino acids that form the active site are located in distinct parts of the amino acid sequence of the enzyme. Therefore, the primary structure the enzyme should fold into its 3D structure; it is a spatial structure enabling the active site to come together **[31]**.

Two models have been proposed to elucidate this specificity:

- *Fisher model (1890):* key and lock model the form of substrate (key) is complementary to that of the active site of the enzyme (the lock)
- *Koshland model (1985):* induced adjustment model the enzyme is not rigid, but flexible, the enzyme and the substrate mutually adapt their respective forms, which are complementary only within the substrate enzyme complex **[32]**.

3.5.Cofactors

The proper functioning of enzymes requires the presence of a molecule, called a cofactor. The cofactor is a chemical body necessarily involved in an enzymatic reaction to transport or

complete a substrate, to accept a product and as participating in the structure of the enzyme **[33]**.

A cofactor is a non-protein chemical compound that is required for the protein's biological activity. Many enzymes require cofactors to function properly. Cofactors can be considered "helper molecules" that assist enzymes in their action. Cofactors can be ions or organic molecules (called coenzymes). They can bind temporarily or more permanently depending on their action. Inactive enzymes without a cofactor are called apo-enzymes and when the cofactor is attached to them and the enzyme is functional, they are called holo-enzymes **[34]**.

3.6.Enzyme-Substrate Complex (E-S)

The enzyme substrate complex is a temporary molecule formed when an enzyme comes into perfect contact with its substrate. Without its substrate an enzyme is a slightly different shape. The substrate causes a conformational change, or shape change, when the substrate enters the active site. The active site is the area of the enzyme capable of forming weak bonds with the substrate. This shape change can force two or more substrate molecules together, or split individual molecules into smaller parts. Most reactions that cells use to stay alive require the actions of enzymes to happen fast enough to be useful. These enzymes are directly coded for in the DNA of the organism.

The enzyme substrate complex is extremely important for a number of reasons. First, the enzyme substrate complex is only temporary. This means that once the substrate has changed, it can no longer bind to the enzyme. The products are released and the enzyme is ready for another substrate molecule. A single enzyme can operate repeatedly millions of times, meaning only a small amount of enzyme is needed in each cell **[35]**.

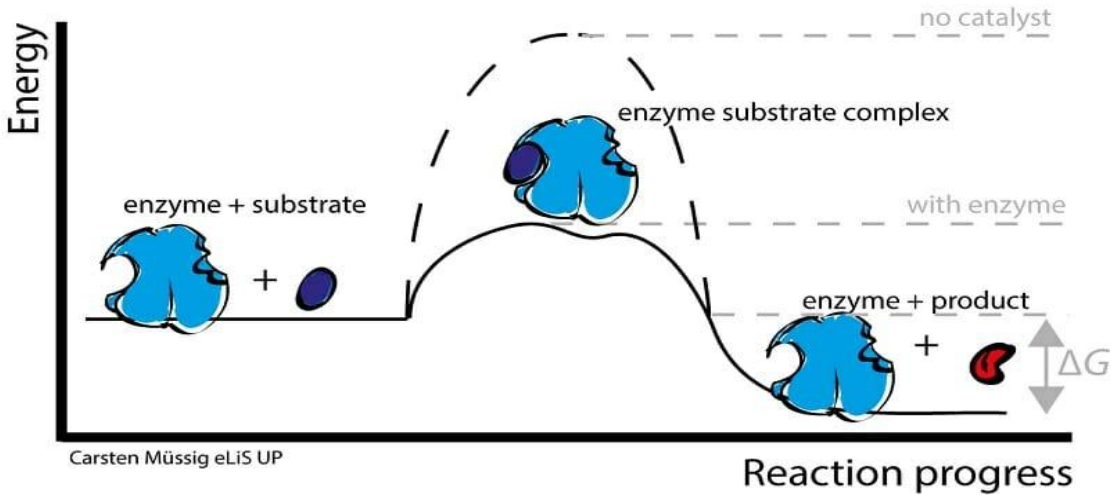


Figure 9: *Enzyme-Substrate Complex*

3.7.Enzymatic Inhibition

An enzyme inhibitor is a molecule that disrupts the normal reaction pathway between an enzyme and a substrate and it prevent the formation of an enzyme-substrate complex and hence prevent the formation of product.

An enzyme inhibitor is a substance that binds to an enzyme and decreases its activity. It can prevent the fixation of the substrate on the active site by fixing itself in its place and make the enzyme less active. The organization of the enzymatic activity can be ensured by compounds called effectors (activators or inhibitors) which are generally of low molecular mass. They act directly or indirectly on the active site of the enzyme. Positive effectors (activators) stabilize the active catalytic configuration of the enzyme and thus increase its activity. Negative effectors (inhibitors) act on the contrary by binding to the enzyme to decrease its catalytic activity **[36]**. Natural inhibitors can take many forms: antibiotics, toxins, drugs, poison **[37]**.

3.8.Different kinetic types of enzyme inhibitors

If the speed of an enzymatic reaction decreases under conditions where the enzyme is not denatured, this means that the enzyme is inhibited. Substances that decrease the activity of an enzyme in this way are called inhibitors **[38]**.

Enzyme inhibitors are classified as reversible or irreversible. Reversible inhibitors bind non covalently with the enzyme; irreversible inhibitors commonly form stable covalent bonds with the enzyme.

Reversible inhibitors associate with enzymes through non-covalent bonds such as hydrogen bonds, hydrophobic interactions, and ionic bonds. Many low energy bonds between inhibitor and active site combine to produce a strong and specific bond. Unlike irreversible substrates and inhibitors, reversible inhibitors do not undergo a chemical reaction when they bind to the enzyme and can be easily removed by dilution or dialysis **[39]**.

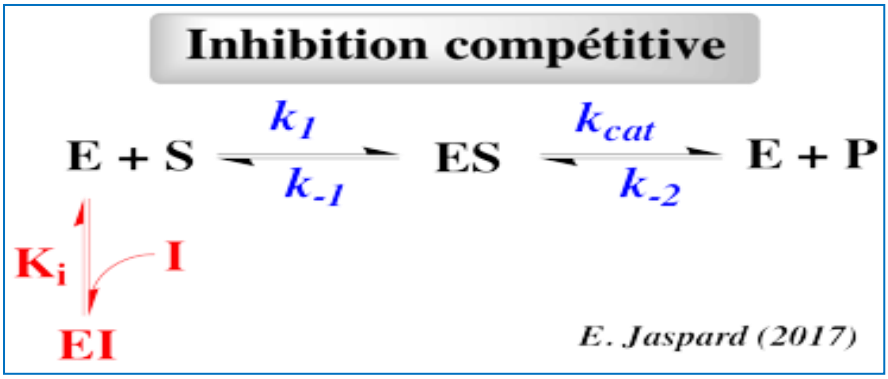
3.8.1. Reversible inhibitors

In reversible inhibition, which is further subdivided into competitive, noncompetitive, and uncompetitive, types, the activity of the enzyme is fully restored when the inhibitor is removed from the system in which the enzyme functions **[40]**.

a. Competitive inhibition

Competitive inhibition involves a molecule, other than the substrate, binding to the enzyme's active site but without giving a reaction. The molecule (inhibitor) is structurally and chemically similar to the substrate (hence able to bind to the active site). T he inhibitor (I) compete directly with the substrate (S) for an active site of the enzyme (E). The fixation of the inhibitor prevents that of the substrate and vice versa. Their bindings are mutually exclusive. There is therefore the formation of two complexes (EI) and (ES). As the inhibitor is in competition with the substrate, its effects can be reduced by increasing substrate concentration **[41]**.

We schematize the reaction by:



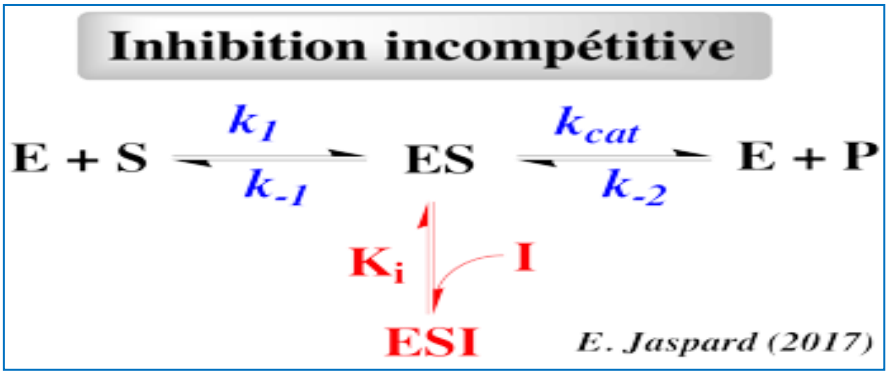
b. In competitive (or anti-competitive) inhibition

This type of inhibition is also called inhibition by blocking of the intermediate complex. This designation better describes the mechanism: the enzyme and the substrate first form the enzyme-

substrate complex (the intermediate complex), then the inhibitor binds to this complex.

An inactive ESI ternary complex is formed. Binding of the substrate (S) to the enzyme, at the active site, induces a change in conformation, thus forming the binding site of the inhibitor (I) **[42]**.

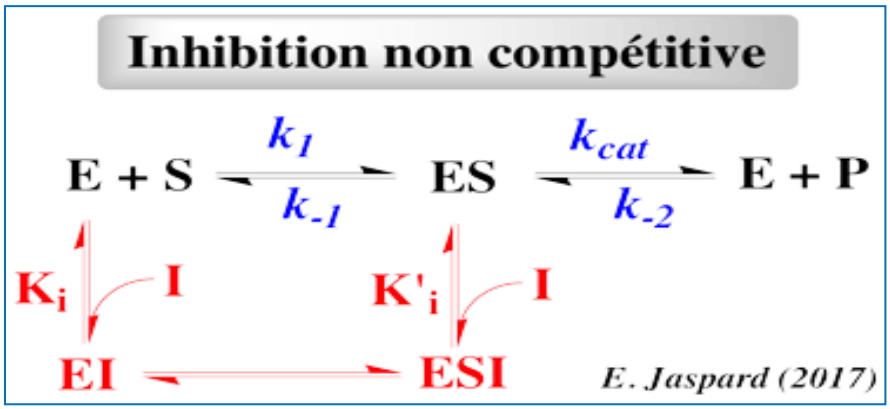
We schematize the reaction by:



c. ***Non-competitive or mixed inhibition***

Non-competitive inhibition is observed when inhibitor can bind with both enzymes and enzyme-substrate complex. The inhibitor bears no structural resemblance to substrate and bind to distinct site than the substrate. There is no competition between substrate and inhibitor for the active site of the enzymes. This type of inhibition cannot be overcome by increasing the substrate concentration. This may bind to enzyme or enzyme-substrate complex making both of them catalytically inactive **[43]**.

We schematize the reaction by:



There are two types of non-competitive inhibition: pure non-competitive inhibition and mixed non-competitive inhibition.

We call "pure", non-competitive inhibition where the inhibitor binds with the same affinity to the free enzyme E and to the enzyme-ES substrate complex (KI = K'I); we call "mixed", non-competitive inhibition where the inhibitor is fixed with different affinities to E and ES (different KI K'I).

Non-competitive inhibition is rare and examples are found primarily in the case of allosterically regulated enzymes **[44]**.

3.8.2. Irreversible inhibitors

Irreversible inhibition occurs, when inhibitor molecule bind with enzyme so strongly that it does not dissociate from the enzyme. This kind of inhibitor binds rapidly with the enzyme and deactivated the enzyme completely **[45]**.

The action of an inhibitor is irreversible when a covalent bond is formed between the enzyme and the inhibitor: it is called an in-activator. And also called suicide inhibitors

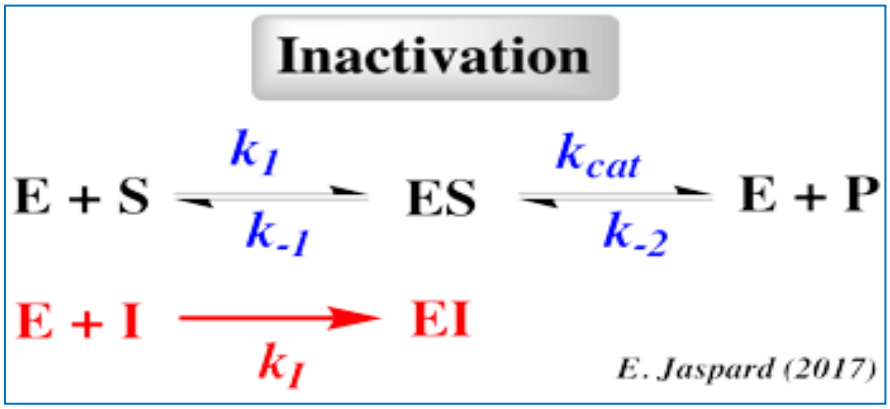
The study of the effect of irreversible inhibitors is often used to determine the active groups of the catalytic site.

A classic example of an in-activator is di-isopropylfluorophosphate or DFP. The study of the effect of this compound on the activity of serine proteases has made it possible to identify serine 195 as one of the two residues involved in catalysis (the second being histidine 57).

The complex is inactive

e, but depending on the relative concentration of the enzyme and the inhibitor, any free enzyme molecule is obviously fully active **[46]**.

We schematize the reaction by:



4. Amino acids

Amino acids are the monomeric building blocks of proteins. The α carbon atom (C_α) of amino acids, which is adjacent to the carboxyl group, is bonded to four different chemical groups: an amino (NH_2) group, a carboxyl (COOH) group, a hydrogen (H) atom, and one variable group, called a side chain or R group. All 20 different amino acids have this same general structure (see figure 10), but their side-chain groups vary in size, shape, charge, hydrophobicity, and reactivity **[47]**.

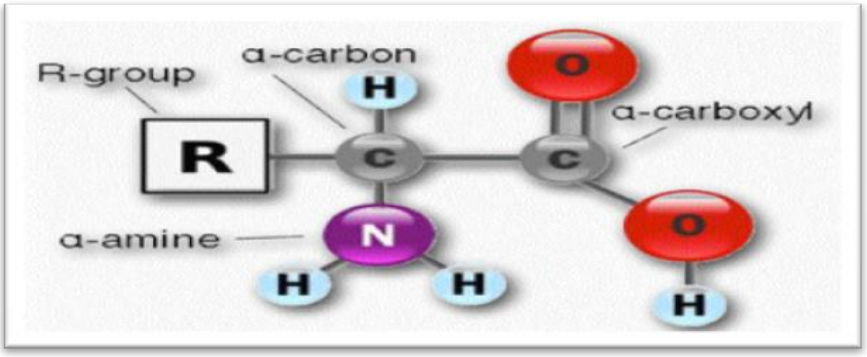


Figure 10: *General structure of amino acid*

Amino acids are building blocks of protein. More than 300 amino acids have been described, but only 20 amino acids take part in protein synthesis. All twenty amino acids did not appear simultaneously in nature. Instead some of them appeared early, while others were added into the genetic code later **[48]**.

4.1.Classification

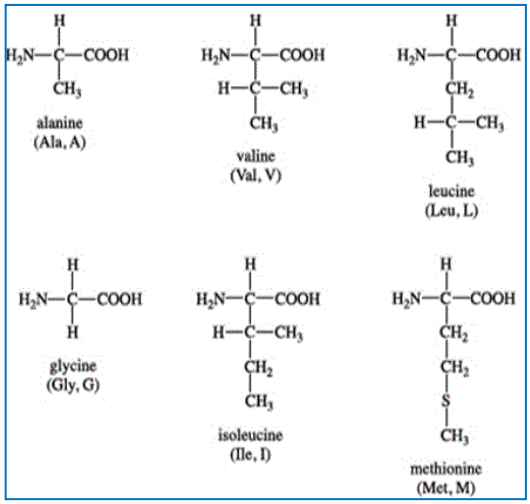
There are many possibilities to classify amino acids, according to the criteria selected to this aim**[49]**.

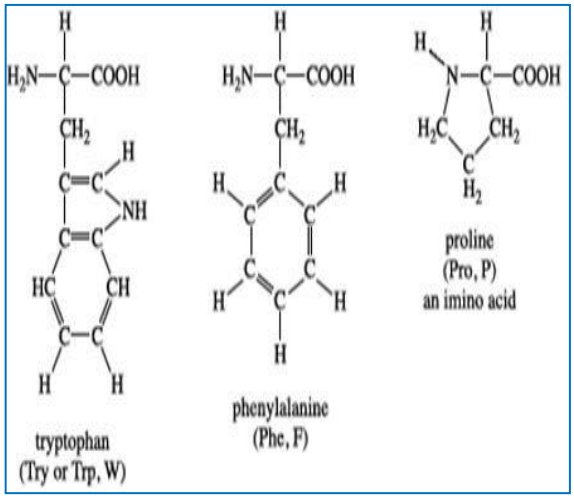
- ❖ Based on their chemical structure
- ❖ A classification based on structure and physical and chemical properties
- ❖ The same amino acid can be assigned to more different classification groups based on the property considered:
  - ✓ Non polar / hydrophobic (Gly, Ala, Leu, Tyr, Phe, Trp, Met …).
  - ✓ Polar / hydrophilic (Asn, Gln, Ser, Thr, Lys, Arg, His, Asp, Glu, [Cys, Tyr])

a. Non-polar amino acids

The side chain of these acids is a non-polar group. We distinguish those whose side chain is an alkyl (alanine, valine, leucine and isoleucine), those containing a heteroatom (methionine, which contains a sulfur atom) and those whose chain contains one ring (proline, phenylalanine and tryptophan) **[50]**.

The chemical structures of these amino acids are:

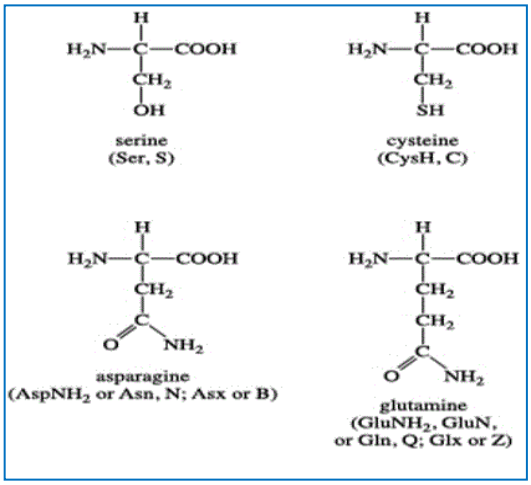


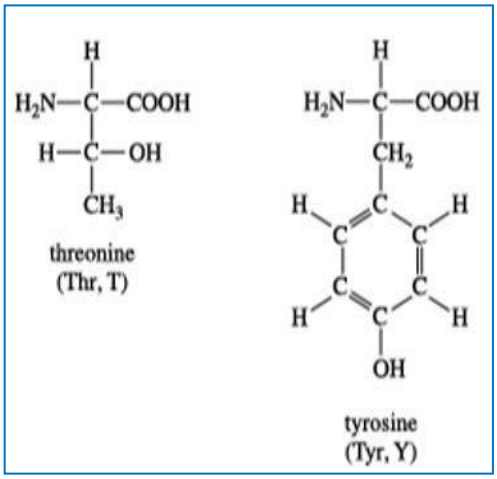


b. Polar, uncharged amino acids

Amino acids are serine, cysteine, threonine, tyrosine, asparagine, and glutamine. The side chains in this group possess a spectrum of functional groups. However, most have at least one atom (nitrogen, oxygen, or sulfur) with electron pairs available for hydrogen bonding to water and other molecules **[51]**.

The chemical structures of Group II amino acids are:





PART (B)

Parkinson's disease

5. Parkinson's disease

5.1.History of Parkinson's disease

The history of Parkinson's disease begins in 1817, with the publication of a 66-page book entitled "An Essay on the Shaking Palsy" under the signature of James Parkinson, Member of the Royal College of Surgeons. The author thus defined agitating paralysis: "involuntary trembling movement, associated with a decrease in muscular strength, occurring in parts of the body at rest (not in action) and even sustained, with a propensity to curve the trunk forward and go from walking to running, the senses and the intellect remaining intact. " **[52]**.

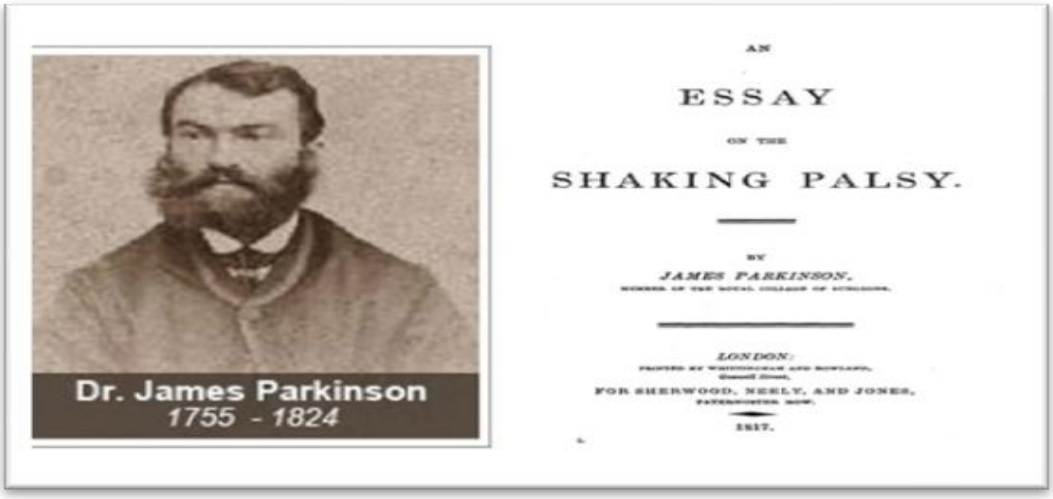


Figure 11: *James Parkinson with his short monograph which is the first clear medical document dealing with Parkinson's disease*

5.2.Causes of Parkinson's disease

Although the exact cause of Parkinson's disease is not yet known, it is considered to be potentiated by the interaction of environmental and genetic factors **[53]**:

a. Environmental factors

Although many studies were conducted to determine the environmental influencers of PD, they have all been deemed to yield inconclusive results. The focus has been on the effects of pesticide exposure and water-borne risk factors. The GeoParkinson study explored these associations in five European countries and found that a large percentage of pesticides containing manganese

were found in the brains of patients after a necropsy study. It was suggested that even the slightest amount of pesticide exposure can greatly increase the risk of PD. Though this study was able to show that pesticides do in fact have an influence on the development of PD, it was limited due to its inability to determine the specific agent within the pesticide that induced this risk.

b. Genetic factors

Though the genetically induced PD constitutes a small portion of the cases, it can provide a strong and clear understanding of the underlying mechanisms. A study by Kurosinski et al. aimed to find the exact genetic mutations that can be attributed to the onset of PD using transgenetic mice and fly models. Another study, by Abramov et al. looked at the human disease model. Findings from the study suggested that the manifestation of PD can be attributed to different genes, since different protein products relate to PD.

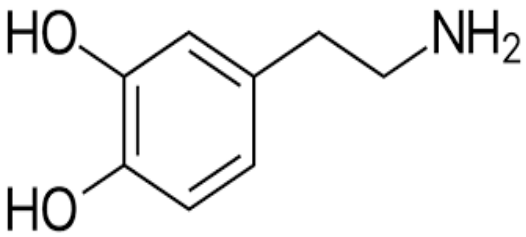
5.3.Pathophysiology of the disease

The substantia nigra (SN) is a midbrain dopaminergic nucleus which has a critical role in modulating motor movement and reward functions as part of the basal ganglia circuitry. Projections from the SN to the putamen, called the nigrostriatal pathway, are critically involved in the motor deficits observed in Parkinson's disease. These dopaminergic neural projections leave the SN via the medial forebrain bundle and forming synapses on multiple neuronal populations throughout the basal ganglia, but especially in the putamen. The basal ganglia are a grouping of interconnected subcortical nuclei that mitigate and control functions ranging from voluntary movement, cognitive planning, emotions and reward functions, and even cognition and learning. The substantia nigra is classically considered to be the primary input into the basal ganglia circuitry and a critical element to these functions. When these subcortical nuclei are damaged such as in stroke or during neurodegeneration, a multitude of neurological conditions can result, including Parkinson disease, Huntington disease, Tourette syndrome, schizophrenia, attention-deficit hyperactivity disorder, and obsessive-compulsive disorder **[54]**.

The PD is characterized by two major pathologic processes **[55]**:

a. Premature selective loss of dopamine neurons

Dopamine is a neurotransmitter in the brain which has an important role in the functioning of the different brain activities such as motor coordination, memory etc. It also works as signaling molecule and act is a signaling pathway for the brain cells to communicate with each other.
The chemical structure of Dopamine is as follows:



The loss of the dopamine neurons in the midbrain of the human brain is the main feature of Parkinson's disease. Approximately 50%-60% of the dopamine neurons are damaged, when a person is detected with Parkinson's disease **[56]**.

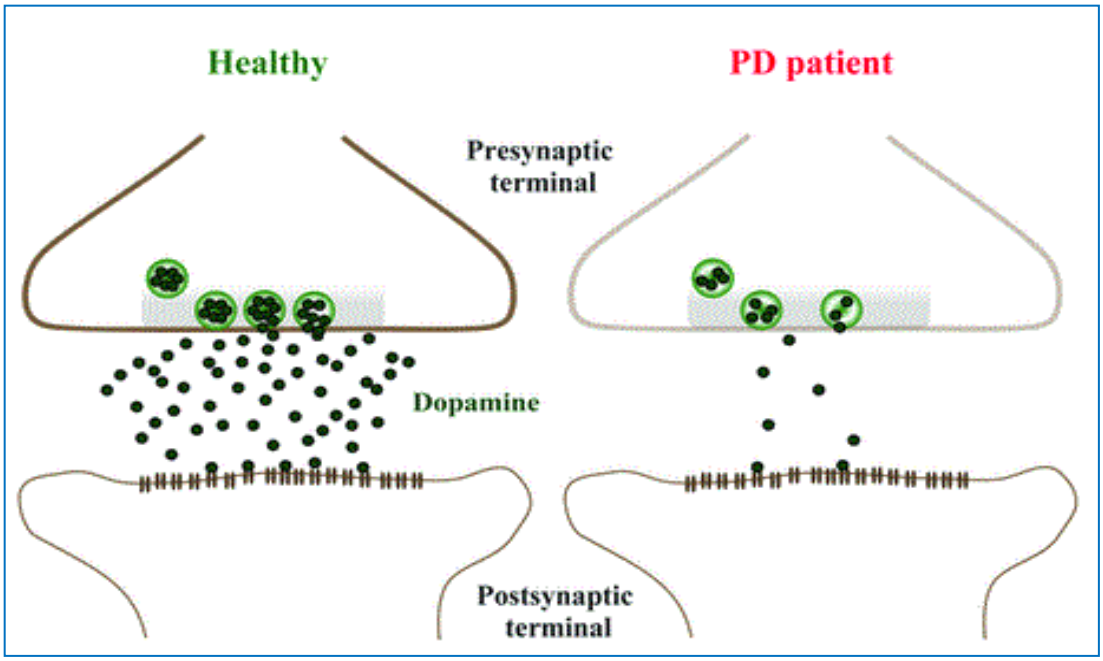


Figure 12: *Healthy and PD patient synaptics*

b. <u>The accumulation of Lewy bodies, composed of α-synuclein</u>

Lewy bodies are largely the result of intracellular aggregations composed of the α-synuclein protein, which in this case is present in an insoluble form and in an abnormal conformation. Lewy's bodies gradually accumulate in neurons, slowly leading to their degeneration **[57]**.

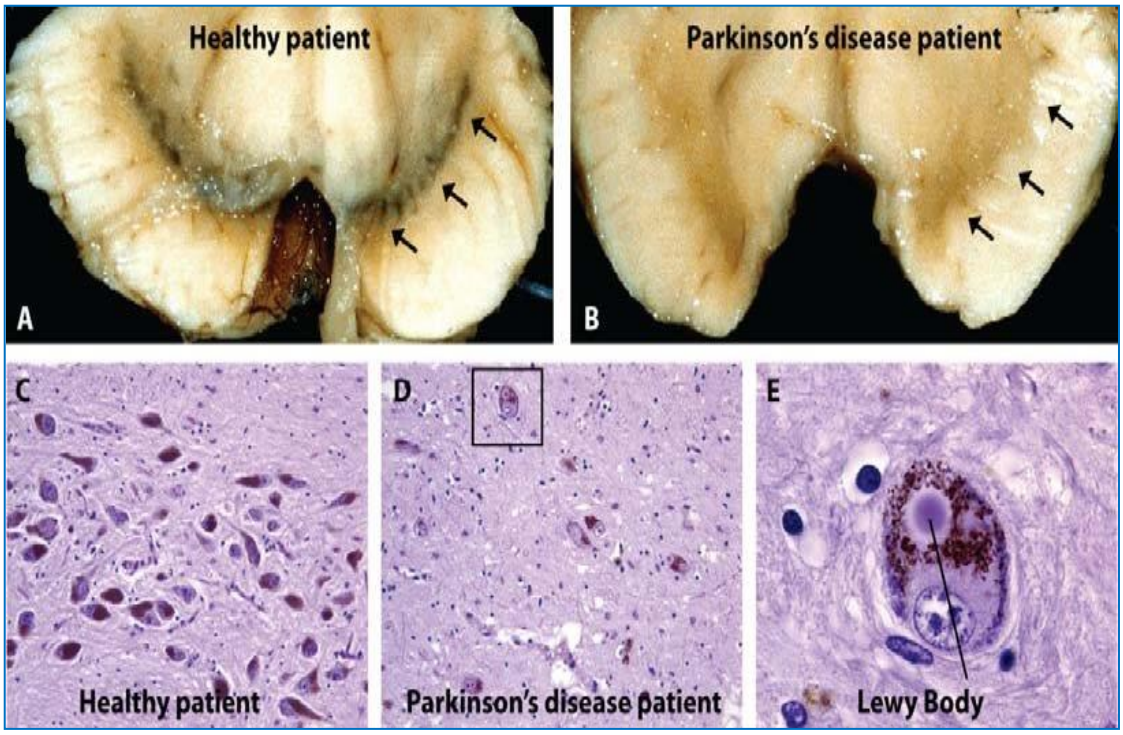


Figure 13 : *Lewy bodies*

Loss of substantia nigra (SN) neurons causes Parkinson's disease. Pathological examination of a healthy patient **(A)** reveals typical pigmented DA neurons in the SN (arrows); in contrast, loss of SN neurons leads to pigment disappearance in the PD brain (**B**, arrows). Magnification of the SN area reveals a dense network of melanin-pigmented SN neurons in the healthy brain **(C)** while most of SN neurons are lost in PD **(D)**. Some of the remaining neurons in PD contain insoluble cytoplasmic protein aggregates (Lewy Bodies, **E**) that are made of aggregated alpha-synuclein and other proteins. The melanin-containing granules have a red-brown hue and are distributed in

the cytosol of all SN neurons **(C-E).** The picture in E is the higher magnification of the dark-boxed area in D.

5.4.Monoamine oxidase

Monoamine oxidases (MAOs) are among a family of flavin adenine dinucleotide (FAD)-dependent enzymes that play a key role in the breakdown of endogenous and exogenous amines **[58]**. These enzymes are located on the mitochondrial outer membrane, mostly in the brain, and are also present in the liver, gut, intestine, skin, placenta, lymphocytes, and platelets **[59]**.
MAOs are responsible for catalyzing the oxidative deamination of various biogenic amine neurotransmitters and a variety of xenobiotic amines, and modulate their concentrations in the brain and peripheral tissues **[60]**.

a. Isoforms

The MAO enzymes exist as two isoforms, MAO-A and MAO-B, with a sequence similarity of 73% but with different inhibitor selectivity, substrate specificity, and tissue distribution **[61]**. The MAO-A isoform predominantly deaminates serotonin, norepinephrine, and epinephrine, whereas MAO-B has substrate specificity for benzylamine and b-phenylethylamine. Tyramine and dopamine are common substrates for both isoforms **[62]**.

b. MAO-A

MAO-A is the predominant isoform in the intestinal tract. It deactivates circulating catecholamines and dietary vasopressors, and also plays a role in the breakdown of serotonin and catecholamines in the brain. MAO-A inhibitors are used in the treatment of psychiatric **[63]**.

Although the chain-fold of hMAO A is similar to that human MAO B (hMAO B), hMAO A is unique in that it crystallizes as a monomer and exhibits the solution hydrodynamic behavior of a monomeric form rather than the dimeric form of hMAO B . hMAO A's active site consists of a single hydrophobic cavity of ≈550 $Å^3$, which is smaller than that determined from the structure of deprenyl-inhibited hMAO B (≈700 $Å^3$)**[64]**.

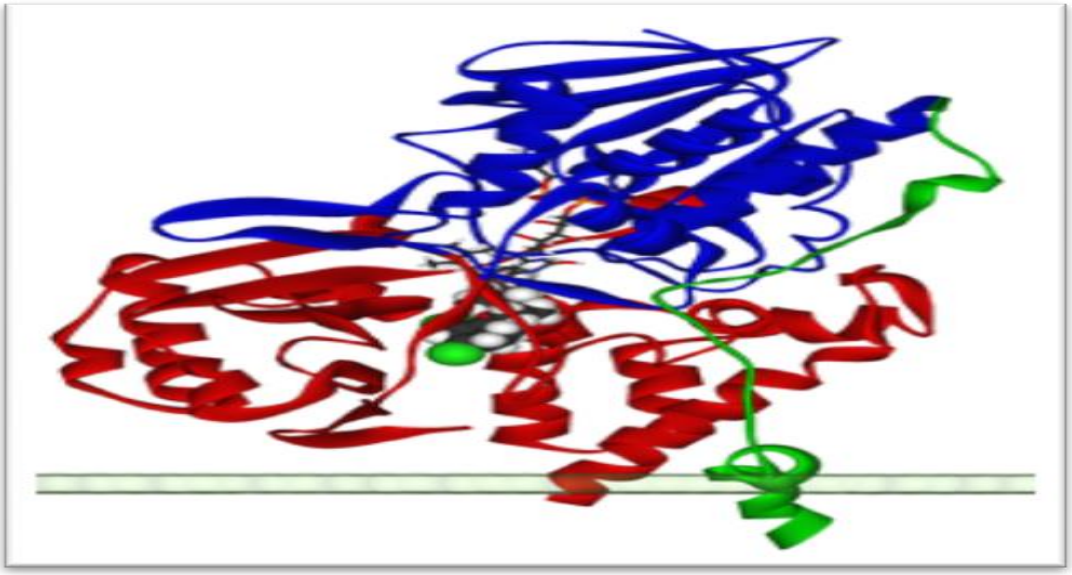


Figure 14: *Three Dimensional structure of MAO-A*

c. MAO-B

MAO-B is an enzyme with a molecular weight of approximately 63 KDa which constitutes a dimer of 520 amino acids.

MAO-B forms a dimer in which each subunit (59000 daltons) contains one covalently-bound FAD linked to cysteine 397. Each monomer has one helix buried in the mitochondrial outer membrane and further surface area thought to associate with the membrane.

In MAO-B, two amino acid side chains (Ile199 and Tyr326) form a "gate" between the entrance cavity and the catalytic site. The gate is easily opened by movement of these side chains, to accommodate large ligands **[65]**.So the crystal structure of human MAO-B shows that the enzyme is composed of three domains which are also, the "FAD-binding" domain (in blue), the substrate domain "substrate-binding" (in red), and the "membrane-binding" domain (in green) (Fig. 15). MAOB supports some locations hydrophobic Pro109, Ile110, Trp157 which are close to the C-terminal, likely to be involved in the attachment of the membrane **[66]**.

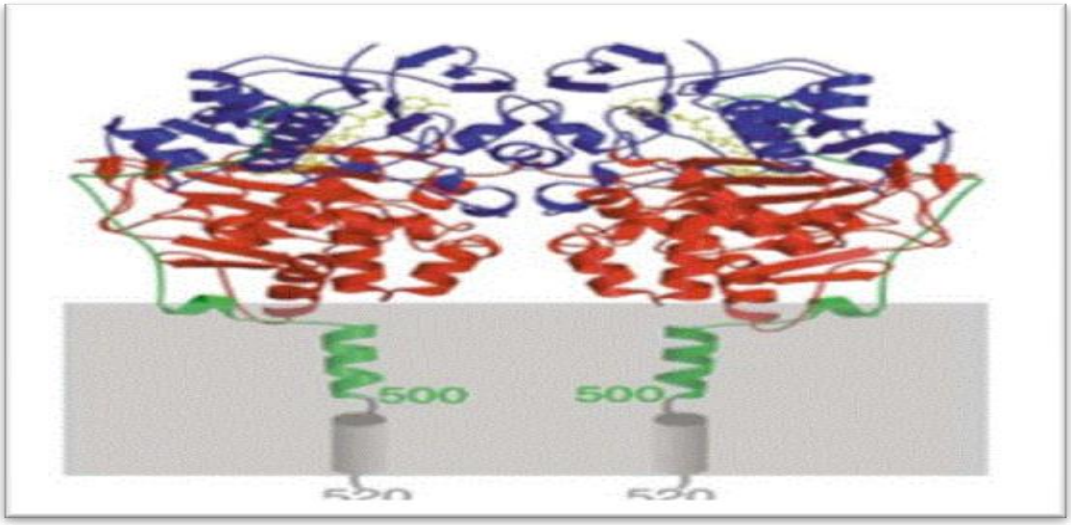


Figure 15: *Three dimensional structure of MAO-B*

5.5.Symptoms of Parkinson's disease

a. <u>The motor symptoms</u>

There are four cardinal features of PD that can be grouped under the acronym TRAP: Tremor at rest, Rigidity, Akinesia (or bradykinesia) and Postural instability. In addition, flexed posture and freezing (motor blocks) have been included among classic features of Parkinsonism **[67]**.

❖ *<u>Tremor at rest</u>:* There are a number of tremors that may affect patients with Parkinson's disease, but the classic is tremor-at-rest. The tremor is also seen during postural action after a short pause, and is often called re-emergent tremor **[68]**.

❖ *<u>Rigidity</u>:* Rigidity is defined by increased resistance during passive mobilization of an extremity, independent of direction and velocity of movement. The underlying mechanism of rigidity in PD is poorly understood, and no direct relationship exists between dopamine deficiency and rigidity, making it difficult to explain through the classic model of basal ganglia pathophysiology **[69]**.

❖ *Bradykinesia:* The term bradykinesia was first used by James Parkinson to describe one of the cardinal features of the disease that now bears his name. Bradykinesia is often used synonymously with two other terms: akinesia and hypokinesia. Strictly speaking, bradykinesia describes the slowness of a performed movement, whereas akinesia refers to a poverty spontaneous movement or associated movement **[70]**.

❖ *Postural instability:* Postural instability (PI) is one of the most debili-tating motor symptoms of Parkinson's disease (PD), as it is associated with an increased risk of falls and subsequent medical complications (e.g. fractures), fear of falling, decreased mobility, self-restricted physical activity, social isolation, and decreased quality of life. PI is more common among PD patients with rapid disease progression as well as in advanced rather than early stages of PD **[71]**.

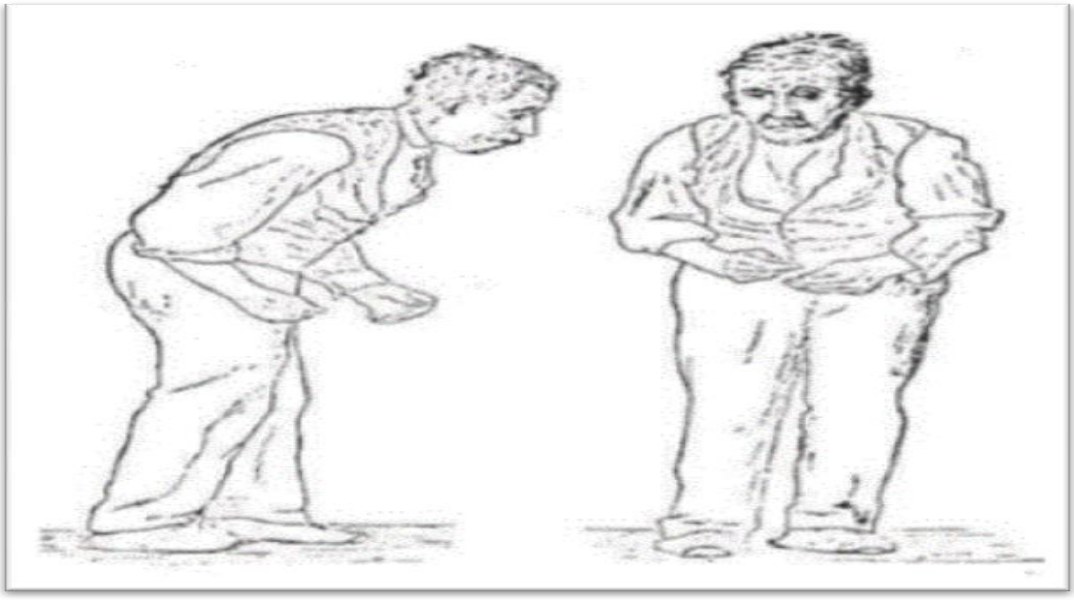


Figure 16: *The etching of an individual with Parkinson's disease included in William Gowers' A Manual of Diseases of the Nervous System*

b. The non-motor symptoms

Before motor symptoms appear and the diagnosis is made, patients may have a variety of pre-motor symptoms. These may start as early as 10 or more years before the diagnosis and presentation with non-motor symptoms may delay the diagnosis .One study of 109 recently diagnosed patients who had not yet started treatment showed that symptoms such as a lack of emotional involvement and interest (apathy), excessive daytime sleepiness, sleep problems and constipation may occur in up to 60–70% of patients prior to the diagnosis and these symptoms were more common than in normal controls. Other pre-motor symptoms included inability to experience pleasure from activities usually found enjoyable (anhedonia) memory complaints, loss of smell and taste, mood disturbances, excessive sweating, fatigue and pain. Constipation, dream-enacting behavior (REM behavior sleep disorder), frequent nightmares, daytime drowsiness and postprandial fullness were often reported to occur more than 10 years before onset of motor symptoms. Depression and anxiety may also occur long before the diagnosis is made.

The premotor symptoms vary from patient to patient, but they continue while other motor or non-motor symptoms of PD may appear in the clinical course. With advancing disease, the non-motor symptoms generally become more troublesome for the patients than the motor symptoms. The non-motor symptoms are categorized here into disturbances in autonomic function, sleep disturbances, cognitive and psychiatric disturbances and sensory symptoms **[72]**.

5.6.Treatment of Parkinson's disease

Many current therapies of PD can only address the symptoms and not the underlying neurodegeneration of PD **[73].** The treatment of Parkinson's disease is symptomatic with the use of dopaminergic medications: levodopa, dopaminergic agonists and enzymatic inhibitors **[74]**.

a. Levodopa (L-dopa)

Levodopa (3,4-dihydroxy-L-phenylalanine), a naturally occurring amino acid, is an intermediate in the pathway of dopamine synthesis **[75]**.

Levodopa is the most effective drug available for the symptomatic treatment of Parkinson's disease (PD) and is the gold standard with which other therapies must be compared. Levodopa

improves most parkinsonian symptoms and is associated with an apparent decrease in mortality rate **[76]**.

In vitro studies with high doses of L-dopa and absent glia had shown that it may be neurotoxic, but other tissue culture studies show L-dopa to be neuroprotective. Most studies in animal models and clinico-pathological and mortality studies in humans failed to show evidence in favour of accelerated dopaminergic neuronal loss with long-term L-dopa therapy **[77]**.

b. Dopaminergic agonists

An agonist is a molecule capable of binding to a membrane receptor, thus mimicking the effect of its endogenous ligand. Dopaminergic agonists (Figure 10) mimic the effect of DA at the synapse by acting directly on post-synaptic DA receptors. However, they have a more moderate effect on motor skills than the DA itself (eg Apomorphine, Bromocriptine and Piribedil) **[78]**.

c. Enzyme inhibitors

These molecules have the effect of prolonging the effect of L-DOPA by inhibiting its degradation by two enzymes: COMT and MAO-B.

COMT inhibitors (ICOMTs) are competitive inhibitors (they attach to the active site of the enzyme). They are always administered in combination with the L-DOPA, which they extend the duration of action, saving 20% of the total daily dose. Currently, only entacapone (Comtan) is marketed, the most potent inhibitor tolcapone (Tasmar) of COMT was available in 1998 but quickly its commercialization was discontinued as it was implicated in three cases of fatal hepatitis (it remains available in Switzerland and the United States).

MAO inhibitors (MAO) have been known for many years. Selegiline (Deprényl) is a non-competitive MAO-B inhibitor (it attaches not to the active site but to another part of the molecule and distorts the active site). Combined with dopatherapy, it increases the effect by 20 to 30%. The beneficial effects are prolonged but also the side effects. It can be administered on its own, even if its effectiveness is more modest **[79]**.

Inhibitors of MAOB (e.g. selegiline, rasagiline) are used clinically to provide symptomatic benefit in Parkinson's disease because of the elevation of brain dopamine by inhibiting its breakdown **[80]**.

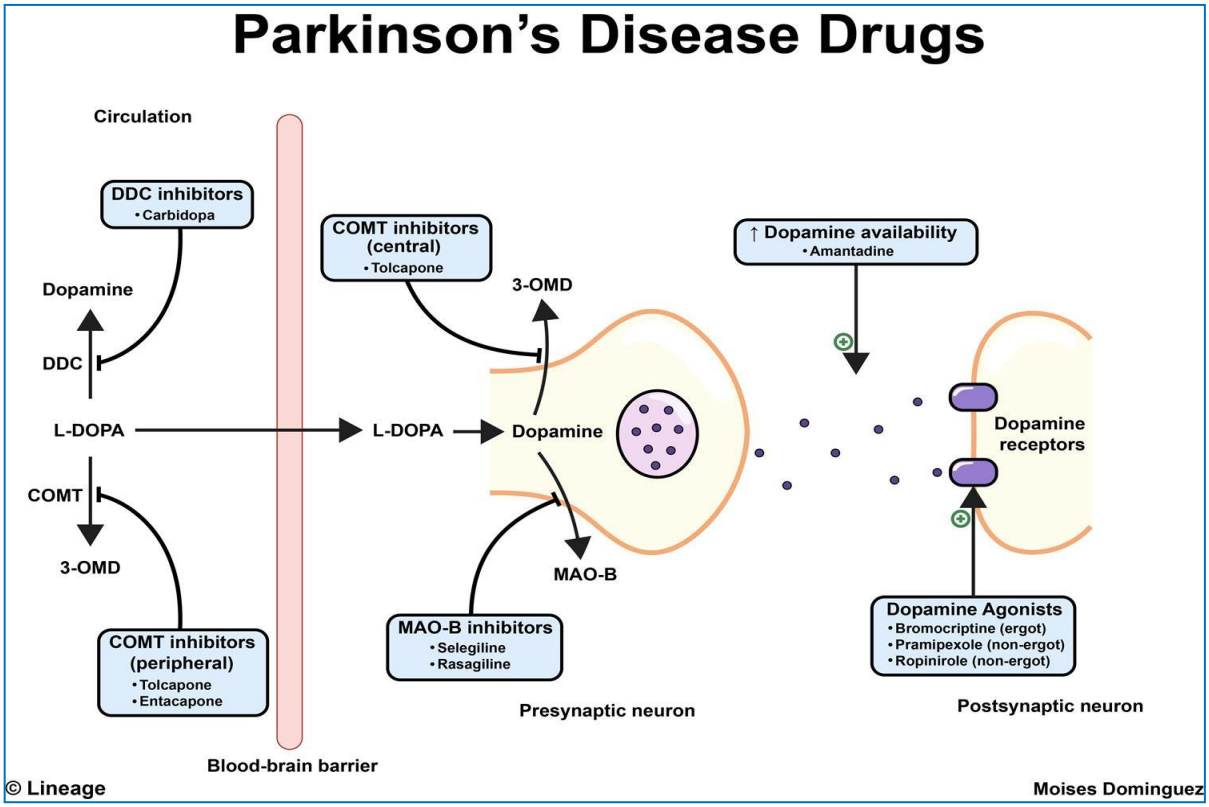


Figure 17: *Parkinson's disease drugs*

d. <u>Surgery</u>

Deep brain stimulation therapy is rarely used for certain types of brain-related disorders including PD, dystonia, obsessive compulsive disorder and treatment resistant depression. When PD symptoms are very severe and medications cannot moderate them, surgery and DBS can be considered as the final options for the treatment. It involves sending electrical impulses to certain parts of the brain (usually SN or globus pallidus, which communicate with the SN) by a neurostimulator device that is a brain implant known as a 'brain pacemaker.' The target area of DBS is usually the subthalamic nucleus (STN). The stimulation of the dorsolateral STN border alongside the surgery can improve its efficiency. Later it was found that stimulation of caudal zona incerta (cZI) can be more effective with fewer complications after the surgery. Stimulation of neurons may also lead to neurogenesis and neuroplasticity and thus can improve for long-time motor problems, such as dyskinesia and tremor, and all other levodopa-responsive symptoms, for

a long time. However, there are two problems with DBS, namely a 3- to 6-month waiting period required for optimal results and the possibility of brain infection **[81]**.

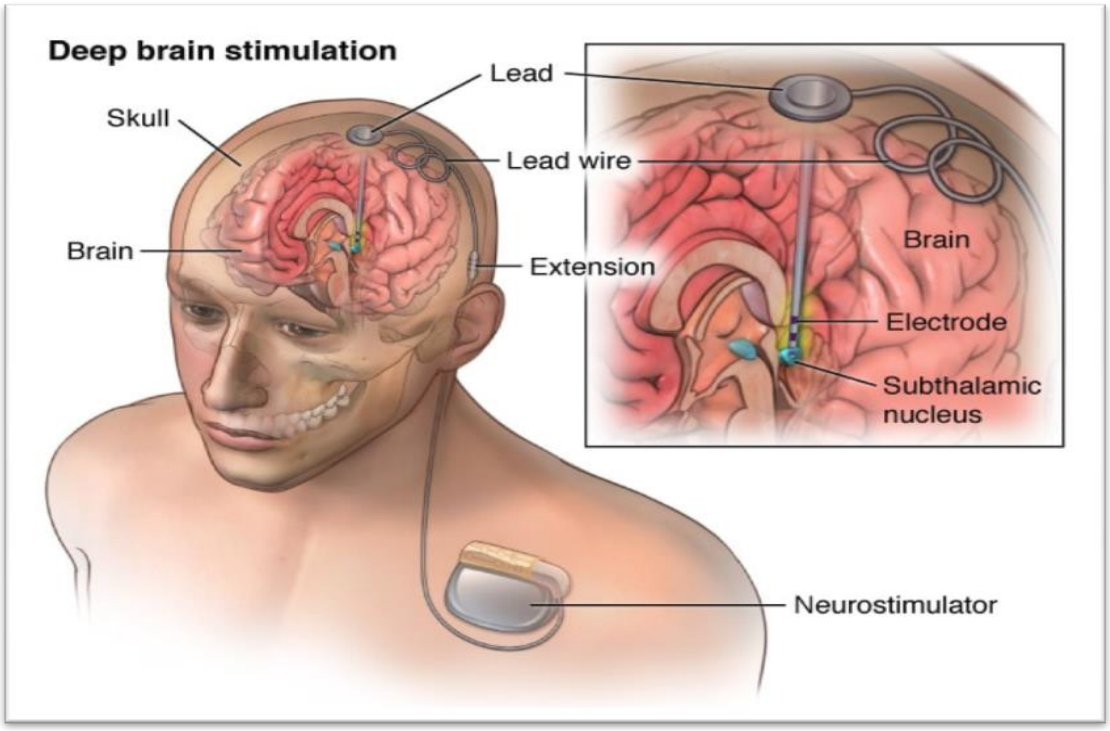


Figure 18 : *Deep Brain Simulation (DBS)*

References

[1] Urry, L. A. "The Structure and Function of Large Biological Molecules." REECE, JB et al. Campbell Biology 9 **(2009)**: 68-91.

[2] Fair, Richard J., and Yitzhak Tor. "Antibiotics and bacterial resistance in the 21st century." Perspectives in medicinal chemistry 6 **(2014):** PMC-S14459.

[3] Karimova, Gouzel, Daniel Ladant, and Agnes Ullmann. "Two-hybrid systems and their usage in infection biology." International journal of medical microbiology 292.1 **(2002):** 17-25.

[4] Park, Sang-Hyun, and Ronald T. Raines. "Genetic selection for dissociative inhibitors of designated protein–protein interactions." Nature biotechnology 18.8 **(2000)** : 847-851.

[5] Lodish, Mr Harvey, et al. Biologie moléculaire de la cellule. De Boeck Supérieur, **2005**.

[6]Ronsin, Christophe. L'histoire de la biologie moléculaire : pionniers & héros. De BoeckSupérieur, **2005**.

[7] Correia, Ana Raquel Viegas. "PROTEIN FOLDING AND DISEASE THE MITOCHONDRIAL PROTEIN FRATAXIN." **(2010).**

[8] Vasudevan, Damodaran M., S. Sreekumari, and Kannan Vaidyanathan. Textbook of biochemistry for medical students. JP Medical Ltd, **2013**.

[9] Cooper, Melanie M., et al. "Organic chemistry, life, the universe and everything (OCLUE): a transformed organic chemistry curriculum." Journal of Chemical Education 96.9 **(2019)**: 1858-1872.

[10] JM, TJ Berg, and L. Stryer. "Biochemistry: Section 3.2: Primary Structure: Amino Acids Are Linked by Peptide Bonds to Form Polypeptide Chains." **(2002).**

[11] Hudson, William H., and Eric A. Ortlund. "The structure, function and evolution of proteins that bind DNA and RNA." Nature reviews Molecular cell biology 15.11 **(2014):** 749-760.

[12] Donovan, Sam, Carl Stiefbold, and Karen Sprague. "Chemical properties of amino acids and in dentification of unknown amino acids." Association for Biology Laboratory Education, **1996**.

[13] Rogers, Kara, ed. The components of life: from nucleic acids to carbohydrates. Britannica Educational Publishing, **2010**.

[14] Adrien Melquionde, Thése de Doctorat de l'université -Paris 7 - Denis Diderot,**2007**.

[15] Solomon, Eldra P., Linda R. Berg, and Diana W. Martin. "Biology (9th edn)." Brooks/Cole, Cengage Learning: USA, **2011.**

[16] Louise Eaton, Kara Rogers Examining Basic Chemical Molecules, Encyclopaedia Britannica, 2017

[17] Tseng, Ampere A. Nanofabrication: fundamentals and applications. World Scientific, **2008**.

[18] Jeffrey Norton, Philip S. Barie, Ralph R. Bollinger, Alfred E. Chang, Stephen Lowry, Sean J. Mulvihill, Harvey I. Pass, Robert W. Thompson « Basic Science and Clinical Evidence » Springer Science & Business Media, **2009**.

[19] Petsko, Gregory A., and Dagmar Ringe. Protein structure and function. New Science Press, **2004**.

[20] Alberts, Bruce, et al. "The shape and structure of proteins." Molecular Biology of the Cell. 4th edition. Garland Science, **2002**.

[21] Abder-Rahim Biad "Debugging Human DNA" ,illustrée; Lulu.com, 2016

[22] Nicholson, Lindsay B. "The immune system." Essays in biochemistry 60.3 **(2016)**: 275-301.

[23] Rose, George D., and Richard Wolfenden. "Hydrogen bonding, hydrophobicity, packing, and protein folding." Annual review of biophysics and biomolecular structure 22.1 **(1993)**: 381-415.

[24] Robinson, Peter K. "Enzymes: principles and biotechnological applications." Essays in biochemistry 59 **(2015):** 1-41.

[25] Cooper, Geoffrey M., and R. E. Hausman. "A molecular approach." The Cell. 2nd ed. Sunderland, MA: Sinauer Associates, **2000.**

[26] Copeland, Robert A. Enzymes: a practical introduction to structure, mechanism, and data analysis. John Wiley & Sons, **2004**.

[27] Arpigny, Jean Louis, and Karl-Erich JAEGER. "Bacterial lipolytic enzymes: classification and properties." Biochemical journal 343.1 **(1999):** 177-183.

[28] Roberts, Richard J., et al. "A nomenclature for restriction enzymes, DNA methyltransferases, homing endonucleases and their genes." Nucleic acids research 31.7 **(2003):** 1805-1812.

[29] Bhatt, S. M. Enzymology and enzyme technology. S. Chand Publishing, **2011**.

[30] Kraut, Joseph. "How do enzymes work?." Science 242.4878 **(1988)**: 533-540.

[31] Lieberman, Michael, et al. Marks' essential medical biochemistry. Lippincott Williams & Wilkins, **2007**.

[32] Dr KASSOUL.A, Cours d'ENZYMOLOGIE, faculté médecine, **(2016)** : 4-5.

[33] Senge, Mathias O., Stuart A. MacGowan, and Jessica M. O'Brien. "Conformational control of cofactors in nature–the influence of protein-induced macrocycle distortion on the biological function of tetrapyrroles." Chemical Communications 51.96 **(2015)**: 17031-17063.

[34] Xie, Yubing, ed. The nanobiotechnology handbook. CRC Press, **(2012):** 348.

[35] Jones, Mary, et al. Cambridge International AS and A Level Biology Coursebook with CD-ROM. Cambridge University Press, **2014**.

[36] Law, Ruby HP, et al. "An overview of the serpin superfamily." Genome biology 7.5 **(2006):** 1-11.

[37] Van Ryn, J., and M. Pairet. "Clinical experience with cyclooxygenase-2 inhibitors." Inflammation Research 48.5 **(1999):** 247-254.

[38] Bjelaković, Gordana, et al. "Competitive inhibitors of enzymes and their therapeutic application." Med Biol 9 **(2002):** 201-206.

[39] Ouertani, A., M. Neifar, and R. Ouertani. "Effectiveness of enzyme inhibitors in biomedicine and pharmacotherapy." Adv Tissue Eng Regen Med Open Access 5.2 **(2019):** 85-90.

[40] Bhagavan, Nadhipuram V. Medical biochemistry. Academic press, **2002**.

[41] Adugna, Solomon, et al. "Medical Biochemistry." Lecture Notes For Health Science Students. In collaboration with the Ethiopia Public Health Training Initiative, The Carter Center, the Ethiopia Ministry of Health, and the Ethio-pia Ministry of Education **2004**.

[42] Auletta, Jeffrey T., Joseph L. Johnson, and Terrone L. Rosenberry. "Molecular basis of inhibition of substrate hydrolysis by a ligand bound to the peripheral site of acetylcholinesterase." Chemico-biological interactions 187.1-3 **(2010)**: 135-141.

[43] Yadav, Ganapati D., and Deepali B. Magadum. "Kinetic Modelling of Enzyme Catalyzed Biotransformation Involving Activations and Inhibitions." Enzyme Inhibitors and Activators **(2017)**: 73.

[44] Delaune, Keith P., and Khalid Alsayouri. "Physiology, Noncompetitive Inhibitor." **2019.**

[45] Cisse, A., et al. "Antidiabetic activity of Zizyphusmauritiana Lam (Rhamnaceae)." Dakar medical 45.2 **(2000)**: 105-107.

[46] Colovic, Mirjana B., et al. "Acetylcholinesterase inhibitors: pharmacology and toxicology." Current neuropharmacology 11.3 **(2013)**: 315-335.

[47] Lodish, Harvey, et al. "Hierarchical structure of proteins." Molecular Cell Biology. 4th edition. WH Freeman, **2000**.

[48] Akram, M., et al. "Amino acids: A review article." Journal of medicinal plants research 5.17 **(2011)**: 3997-4000.

[49] Horovitz, Ossi, and ROXANA-DIANA PAŞCA. "Classification of Amino Acids by Multivariate Data Analysis, Based on Thermodynamic and Structural Characteristics." Studia Universitatis Babes-Bolyai, Chemia 62, **2017**.

[50] Yahiaoui, Sofiane, Kamel Zitouni, and Assia Sid. "Synthèse et Caractérisation par UV et IR de quelques benzamides chiraux par la réaction de L-acides aminés avec l'acide gallique." **(2018).**

[51] Azad, Sabina. "Amino Acids: Its types and uses." Int. J. Clin. Diagnost. Pathol. 1.1 **(2018) :** 13-16.

[52] Khalil, Richard. "Histoire de la maladie de Parkinson." Histoire des sciences médicales 30 **(1996)**: 215-220.

[53] Shafique, Hassan, et al. "Causes of Parkinson's disease: literature review." Journal of Parkinsonism & Restless Legs Syndrome 1 **(2011)**: 5.

[54] Sonne, James, and Morris R. Beato. "Neuroanatomy, substantia nigra." Stat Pearls [Internet]. Stat Pearls Publishing, **2018**.

[55] Rizek, Philippe, Niraj Kumar, and Mandar S. Jog. "An update on the diagnosis and treatment of Parkinson disease." Cmaj 188.16 **(2016)**: 1157-1165.

[56] Saikia, A., P. Bhattacharya, and S. Paul. "Importance of dopamine in Parkinson's disease." Adv. Tissue Eng. Regen. Med 4 **(2018)**: 47-48.

[57] Eger, Antoine-Flavien, Christophe Gaudet-Blavignac, and Arthur Hammer. "La Maladie de Parkinson." Université de Genève. **(2009):** 5.

[58] Dezsi, Livia, and Laszlo Vecsei. "Monoamine oxidase B inhibitors in Parkinson's disease." CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders) 16.4 **(2017)**: 425-439.

[59] Rodríguez, Manuel J., et al. "Cellular localization of monoamine oxidase A and B in human tissues outside of the central nervous system." Cell and Tissue Research 304.2 **(2001)**: 215-220.

[60] Tipton, Keith F., et al. "Monoamine oxidases: certainties and uncertainties." Current medicinal chemistry 11.15 **(2004)**: 1965-1982.

[61] Geha, Rani Maurice, et al. "Substrate and inhibitor specificities for human monoamine oxidase A and B are influenced by a single amino acid." Journal of Biological Chemistry 276.13 **(2001)**: 9877-9882.

[62] Youdim, Moussa BH, Dale Edmondson, and Keith F. Tipton. "The therapeutic potential of monoamine oxidase inhibitors." Nature reviews neuroscience 7.4 **(2006)**: 295-309.

[63] Dezsi, Livia, and Laszlo Vecsei. "Monoamine oxidase B inhibitors in Parkinson's disease." CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders) 16.4 **(2017)**: 425-439.

[64] De Colibus, Luigi, et al. "Three-dimensional structure of human monoamine oxidase A (MAO A): relation to the structures of rat MAO A and human MAO B." Proceedings of the National Academy of Sciences 102.36 **(2005)**: 12684-12689.

[65] Ramsay, Rona R. "Molecular aspects of monoamine oxidase B." Progress in neuro-psychopharmacology and biological Psychiatry 69, **(2016)**: 81-89.

[66] R. Benarab, M. Khenoussi , Thème : Les inhibiteurs de la Monoamine oxydase en psychopharmacologie. Option: Biochimie **(2011-2012)** :3.

[67] Jankovic, Joseph. "Parkinson's disease: clinical features and diagnosis." Journal of neurology, neurosurgery & psychiatry 79.4 **(2008)**: 368-376.

[68] Hallett, Mark. "Parkinson's disease tremor: pathophysiology." Parkinsonism & related disorders 18 **(2012):** S85-S86.

[69] Baradaran, Nazanin, et al. "Parkinson's disease rigidity: relation to brain connectivity and motor performance." Frontiers in neurology 4 **(2013)**: 67.

[70] Berardelli, Alfredo, et al. "Pathophysiology of bradykinesia in Parkinson's disease." Brain 124.11 **(2001):** 2131-2146.

[71] Crouse, Jacob J., et al. "Postural instability and falls in Parkinson's disease." Reviews in the Neurosciences 27.5 **(2016):** 549-555.

[72] Sveinbjornsdottir, Sigurlaug. "The clinical symptoms of Parkinson's disease." Journal of neurochemistry 139 **(2016):** 318-324.

[73] Stoddard-Bennett, Theo, and Renee Reijo Pera. "Treatment of Parkinson's disease through personalized medicine and induced pluripotent stem cells." Cells 8.1 **(2019):** 26.

[74] Defebvre, Luc, and Caroline Moreau. "Traitements médical et chirurgical de la maladie de Parkinson." La Presse Médicale 46.2 **(2017):** 218-224.

[75] Le Witt, Peter A. "Levodopa for the treatment of Parkinson's disease." New England Journal of Medicine 359.23 **(2008):** 2468-2476.

[76] Koller, William C. "Levodopa in the treatment of Parkinson's disease." Neurology 55.11 Suppl 4 **(2000):** S2.

[77] Katzenschlager, Regina, and Andrew J. Lees. "Treatment of Parkinson's disease: levodopa as the first choice." Journal of neurology 249.2 **(2002):** ii19-ii24.

[78] Zeghbib, Narimane. Synthèse de nouveaux inducteurs chimiques de la maladie de Parkinson: étude relation structure/activité. Diss. Paris Est, **2016**.

[79] HENRION, Laure. Thèse d'exercice. Diss. Université de Limoges, **1992**.

[80] Tong, Junchao, et al. "Brain monoamine oxidase B and A in human parkinsonian dopamine deficiency disorders." Brain 140.9 **(2017):** 2460-2474.

[81] Emamzadeh, Fatemeh N., and Andrei Surguchov. "Parkinson's disease: biomarkers, treatment, and risk factors." Frontiers in neuroscience 12 **(2018)**: 612.

Chapter III: Results and discussion

1. Introduction

Computational prediction of drug-target interactions (DTIs) has become an important step in the drug discovery or repositioning process, aiming to identify putative new drugs or novel targets for existing drugs **[1]**.

The interaction between a protein and its substrate (frequently: Van der Waals, hydrogen bond, hydrophobic effect ...) is the first step in most biological reactions. Understanding how it works means defining what residues are involved:

- The affinity between two molecules.
- The distances between the amino acids of the active site of the enzyme and those of the inhibitors.
- The energy of interaction.

Although enzymes are absolutely essential for life, abnormally high enzyme activity can lead to disease conditions. Hence, overactive enzymes are attractive targets for development of inhibitor molecules to alleviate disease conditions. Manipulation of enzyme catalysis with inhibitors is critical for prevention of diseases **[2].** For this purpose, those molecular modeling techniques have been developed.

In recent years researchers have been trying to combine different methods of molecular modeling, namely: molecular dynamics and molecular docking. Molecular docking is used to predict the structure of the inter-molecular complex resulting from the association between at least two molecules **[3]**, knowing that molecular dynamics is used as a method of validating the results of molecular docking. The use of these methods has led to many successes in the field of discovery of new bioactive molecules (drugs) **[4, 5]**.

This work consists in studying the interactions between the different inhibitors with enzyme by the methods of molecular modeling.

In this chapter we have included all the calculation results carried out in this study and the discussion that we are going to engage is based on the interaction energies and the distances between certain groupings of the side chain of the enzyme and those of inhibitors.

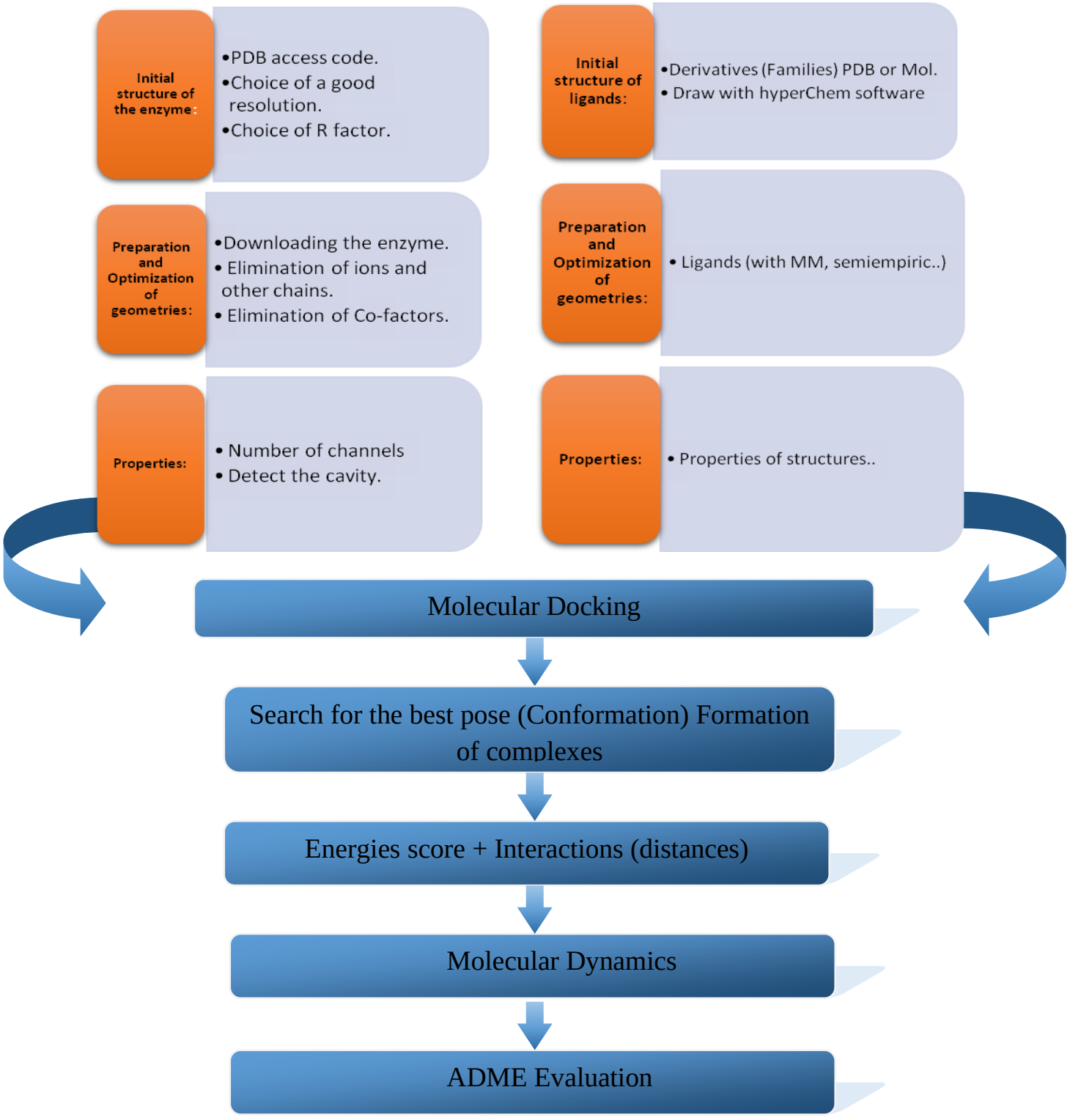


Figure 1: *Simulation protocol*

2. Materials and methods

2.1. Target and Compounds preparations

2.1.1. Target preparation

The enzyme was downloaded from the Bookhaven Protein Data Bank database (www.rcsb.org/pdb). Crystal structureof Human Monoamine Oxidase B (MAO-B) (PDB ID: 4a79) is complexed with pioglitazone (RS)-5-(4-[2-(5-éthylpyridin-2-yl)éthoxy] benzyl)-1,3-thiazolidine-2,4-dione ($C_{19}H_{20}N_2O_3S$) **[6]**.

The 3D structure of our enzyme was obtained by X-ray diffraction with a resolution of 1.89 Å and R-Value Free: 0.208, R-Value Work: 0.178.

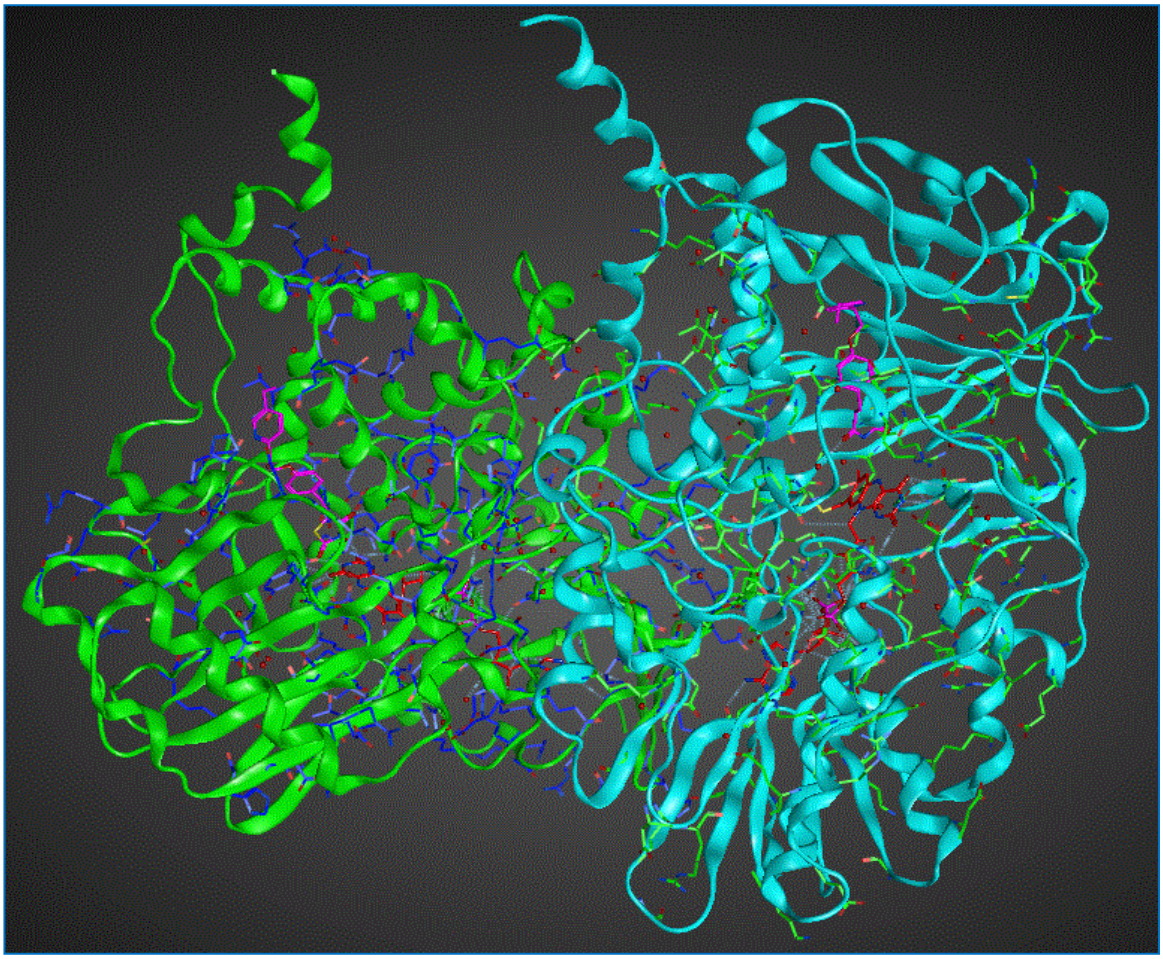


Figure 2: *The dimeric form of MAO-B*

All MAO-B's information (PDB: 4A79) are grouped in the following table:

Table 1: *The atomic composition of the 4a79 complex*

Compounds	Chain	Residues	Atoms				
			Total	**O**	**N**	**C**	**S**
MAO-B (PDB : 4a79)	A	499	3974	728	681	2541	24
	B	494	3940	721	676	2519	24
P1B	A	1	25	3	2	19	1
	B	1	25	3	2	19	1
			Total	**O**	**N**	**C**	**P**
FAD	A	1	53	15	9	27	2
	B	1	53	15	9	27	2
Water	A	348	348	348	/	/	/
	B	147	347	347	/	/	/

FAD: FLAVIN-ADENINE DINUCLEOTIDE**, P1B:** (5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione.

Clément, G. et *al* **[7]** and Didierjean, C et *al* **[8]** demonstrate that the protein structure with a resolution between 1.5 and 2.5 Å has a good quality for further studies, whereas, the resolution values of MAO-B target belong to this interval. In addition, we note that the R-value of all enzymes belongs to the range of typical values according to Kleywegt, G. J. et *al* **[9]**.

For simplify the structure of this enzyme, all ions and Co-crystal ligand molecules were deleted from the structure and the PDB, but the water molecules are kept because Klebe, G **[10]** shows that the presence of water is sometimes essential to ensure a relay between the compound and the active site and thus create networks of hydrogen bonds. On the other hand, Marechal.Y **[11]** confirmed that water molecules in the cavities of proteins can sometimes be a fundamental element some algorithms are able to simulate the presence of water molecules in the cavities of proteins.

Since all PDB structure has its crystal structure in a state that represents the pharmacological targets for the development of new drugs to PD, this PDB was selected for modeling studies.

2.1.2. Compounds preparation

In our work, we study a series of 4-(benzyloxy)phenyl and biphenyl-4-yl derivatives by modifying the benzyloxyphenyl moiety **[12]** , a common structure of potent, selective, and reversible MAO-B inhibitors such as safinamide and sembragiline.

First, the structures of all the 48 compounds were drawn by ChemDraw Ultra 11.0 **[13]**, then, the three-dimensional structures of all compounds tested (Tables.2,3)were pre-optimized by means of the Molecular Mechanics using Force Field MM+. After that, the resulted minimized structures were further refined using the semi-empirical method AM1 **[14]** with default parameters such as: the Polak-Ribiere conjugate gradient algorithm of 0.01 kcal/(Åmol). All methods are implemented in Hyperchem 8.0.8 software **[15]**. In the end, the database was created in which all the compounds were converted into their 3D structures and this database was used as input MOE-docking **[16]**.

Table 2: *Basic structure table*

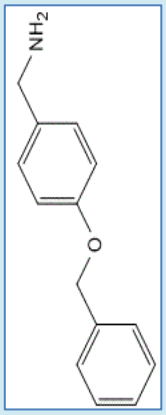
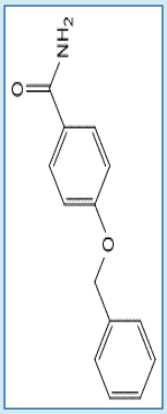
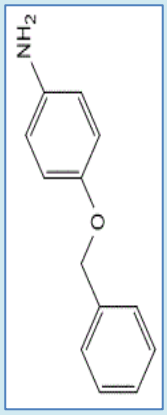
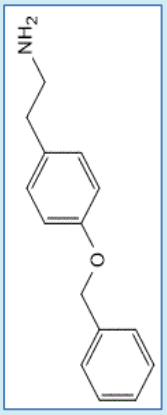
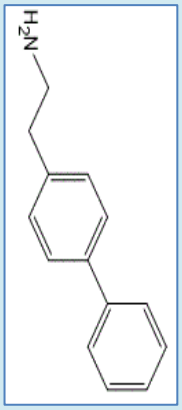
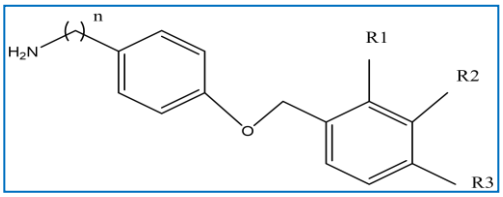
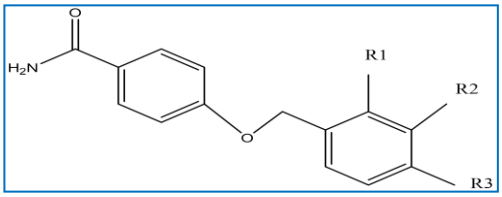
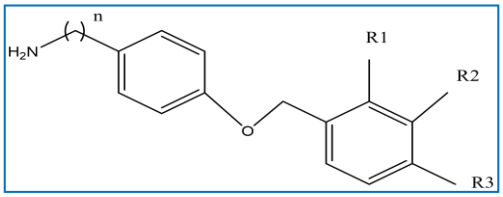
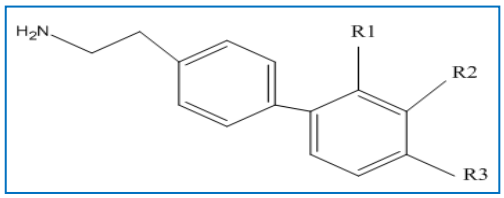
Ligand	**L1**	**L2**	**L3**	**L4**	**L5**
Basic structure					

Table 3: *Chemical structures of compounds*

Structures	Compounds	Substituent			
		n	R1	R2	R3
	L6	1	H	H	H
	L7	1	CF_3	H	H
	L8	1	H	CF_3	H
	L9	1	H	H	CF3
	L10	1	F	H	H
	L11	1	H	F	H
	L12	1	H	H	F
	L13	1	H	Cl	H
	L14	1	H	H	Cl
	L15	1	H	OMe	H
	L16	1	H	H	OMe
	L17	/	CF_3	H	H
	L18	/	H	CF_3	H
	L19	/	H	H	CF_3
	L20	/	F	H	H
	L21	/	H	F	H
	L22	/	H	H	F
	L23	0	CF_3	H	H
	L24	0	H	CF_3	H
	L25	0	H	H	CF_3
	L26	0	F	H	H
	L27	0	H	F	H
	L28	0	H	H	F
	L29	2	CF_3	H	H
	L30	2	H	CF_3	H
	L31	2	H	H	CF_3
	L32	2	F	H	H
	L33	2	H	F	H
	L34	2	H	H	F
	L35	2	Cl	H	H
	L36	2	H	Cl	H
	L37	2	H	H	Cl
	L38	2	H	OMe	H
	L39	/	H	CF_3	H
	L40	/	H	H	CF_3
	L41	/	F	H	H
	L42	/	H	F	H
	L43	/	H	H	F
	L44	/	Cl	H	H
	L45	/	H	Cl	H
	L46	/	H	H	Cl
	L47	/	H	OMe	H
	L48	/	H	H	OMe

The information of all compounds was obtained from MOE **[16]** software and MVD software **[17, 18]** given in table 4.

Table 4: *Some properties of the compounds studied*

Compds	*Toxic*	*Rsynth (%)*	*Weight (g/mol)*	*TPSA Å²*	*Number of Torsion*
L1	Yes (aromatic methylamine)	100.00	213.28	21.26 (don :1,acc :1)	4 (out of 4)
L2	No	100.00	227.26	52.32 (don :1,acc :2)	4 (out of 4)
L3	Yes (aromatic amine)	100.00	199.25	35.25 (don :1,acc :1)	3 (out of 3)
L4	No	100.00	227.31	35.25 (don :1,acc :2)	5 (out of 5)
L5	No	0.00	197.28	26.02 (don :1,acc :1)	3 (out of 3)
L6	Yes (aromatic methylamine)	100.00	213.28	21.26 (don :1,acc :1)	4 (out of 4)
L7	Yes (aromatic methylamine)	100.00	281.28	21.26 (don :1,acc :1)	5 (out of 5)
L8	Yes (aromatic methylamine)	100.00	281.28	21.26 (don :1,acc :1)	5 (out of 5)
L9	Yes (aromatic methylamine)	100.00	281.28	21.26 (don :1,acc :1)	5 (out of 5)
L10	No	100.00	231.27	35.25 (don :1,acc :2)	4 (out of 4)
L11	No	100.00	231.27	35.25 (don :1,acc :2)	4 (out of 4)
L12	No	100.00	231.27	35.25 (don :1,acc :2)	4 (out of 4)
L13	No	100.00	247.72	35.25 (don :1,acc :2)	4 (out of 4)
L14	No	100.00	247.72	35.25 (don :1,acc :2)	4 (out of 4)
L15	Yes (aromatic methylamine)	100.00	243.31	30.49 (don :1,acc :2)	5 (out of 5)
L16	Yes (aromatic methylamine)	100.00	243.31	30.49 (don :1,acc :2)	5 (out of 5)
L17	No	100.00	295.26	52.32 (don :1,acc :2)	5 (out of 5)
L18	No	100.00	295.26	52.32 (don :1,acc :2)	5 (out of 5)
L19	No	100.00	295.26	52.32 (don :1,acc :2)	5 (out of 5)
L20	No	100.00	245.25	52.32 (don :1,acc :2)	4 (out of 4)
L21	No	100.00	245.25	52.32 (don :1,acc :2)	4 (out of 4)
L22	No	100.00	245.25	52.32 (don :1,acc :2)	4 (out of 4)
L23	Yes (aromatic amine)	100.00	267.25	35.25 (don :1,acc :1)	4 (out of 4)
L24	Yes (aromatic amine)	100.00	267.25	35.25 (don :1,acc :1)	4 (out of 4)
L25	Yes (aromatic amine)	100.00	267.25	35.25 (don :1,acc :1)	4 (out of 4)
L26	Yes (aromatic amine)	100.00	217.24	35.25 (don :1,acc :1)	3 (out of 3)
L27	Yes (aromatic amine)	100.00	217.24	35.25 (don :1,acc :1)	3 (out of 3)
L28	Yes (aromatic amine)	100.00	217.24	35.25 (don :1,acc :1)	3 (out of 3)
L29	No	100.00	295.30	35.25 (don :1,acc :2)	6 (out of 6)
L30	No	100.00	295.30	35.25 (don :1,acc :2)	6 (out of 6)
L31	No	100.00	295.30	35.25 (don :1,acc :2)	6 (out of 6)
L32	No	100.00	245.30	35.25 (don :1,acc :2)	5 (out of 5)
L33	No	100.00	245.30	35.25 (don :1,acc :2)	5 (out of 5)
L34	No	100.00	245.30	35.25 (don :1,acc :2)	5 (out of 5)
L35	No	100.00	261.75	35.25 (don :1,acc :2)	5 (out of 5)
L36	No	100.00	261.75	35.25 (don :1,acc :2)	5 (out of 5)
L37	No	100.00	261.75	35.25 (don :1,acc :2)	6 (out of 6)
L38	No	100.00	257.33	44.48 (don :1,acc :3)	6 (out of 6)
L39	No	0.00	265.28	26.02 (don :1,acc :1)	4 (out of 4)
L40	No	0.00	265.28	26.02 (don :1,acc :1)	4 (out of 4)
L41	No	0.00	215.27	26.02 (don :1,acc :1)	3 (out of 3)

L42	No	0.00	215.27	26.02 (don :1,acc :1)	3 (out of 3)
L43	No	100.00	215.27	26.02 (don :1,acc :1)	3 (out of 3)
L44	No	0.00	231.73	26.02 (don :1,acc :1)	3 (out of 3)
L45	No	0.00	231.73	26.02 (don :1,acc :1)	3 (out of 3)
L46	No	100.00	231.73	26.02 (don :1,acc :1)	3 (out of 3)
L47	No	5.88	227.31	35.25 (don :1,acc :2)	4 (out of 4)
L48	No	100.00	227.31	35.25 (don :1,acc :2)	4 (out of 4)

We note that compounds L1, L3, L6, L7, L8, L9, L15, L16, L23, L24, L25, L26, L27, and L28 are toxic. Also, the compounds L29, L30, L31, L37, and L38 have a high flexibility (table 4) with 6 (out of 6) torsion. In the end, compound L38 has high acceptor.

3. Computational approach

3.1. Molecular Docking Protocol

3.1.1. Default parameters and steps of Molecular Docking

Molecular docking is one of the key computational chemistry technics that are routinely applied to drug discovery **[19]**. Docking methods fit a ligand into a binding site by combining and optimizing variables like steric, hydrophobic and electrostatic complementarity and also estimating the free energy of binding (scoring)**[20]**.

The identification of the most likely binding conformations requires two steps:

a) Exploration of a large conformational space representing various potential binding modes;

b) Accurate prediction of the interaction energy associated with each of the predicted binding conformations.

Molecular docking software performs these tasks through a cyclical process, in which the ligand conformation is evaluated by specific scoring functions. This process is carried out recursively until converging to a solution of minimum energy **[21]**.

The MOE program was used for our study because:

1st: it is easy to handle.

2nd: it has proven its effectiveness in several studies, we can cite some example of work: Eman K.A. Abdelall et al **[22]**, Matteo Aldeghi et al **[23]**, Sarah T. A. Al-Rashood et al **[24]**, Khac-Minh Thai et al **[25]**,Somaia S. Abd El-Karim et al **[26]**, Sheng Liu et al **[27]**,Tamer Nasr et al **[28]**,Jiansong Fang et al **[29]** and Sally S. El-Nakkady et al **[30]**.

During the interaction, the flexibility of the ligand (often) and the target protein (rarely) is engaged. In addition, the ligand has too much degree of freedom to be taken into account directly in an optimization algorithm.

3.2. Molecular Dynamics (MD) Simulation

In order to find the best inhibitor, MD simulations were carried out to evaluate the conformational changes and to understand the stability of complexes formed between receptors and inhibitor **[31, 32]**. The Compounds-MAO-B complexes with the best docking scores were considered as input files for 1000 ps (1ns) molecular dynamic (MD) simulations.

The enzyme topology file was prepared by Chimera.1.8.1. Software **[33]**. Then, the compounds-protein interactions were studied by the MOE package **[16]**. The procedure was given on our previous study **[34]**.

3.3. ADME predictions

For in silico ADME (Adsorption, Distribution, Metabolism and Excretion) evaluation, and estimation of drug-likeness with physicochemical parameters. All these properties were computed by the freely offered web server Swiss ADME online (http://www.swissadme.ch/) **[35, 36]** by entering the chemical structure followed by SMILES format.

In particular, our study based on the analysis of relevant pharmaceutical properties, including: blood–brain barrier (BBB) partitioning **[37]**, human intestinal absorption **[38-40]**, oral bioavailability **[38]**, Caco-2 permeability **[41,42]**, and including solubility **[43]**, Topological Polar Surface Area (TPSA), Lipinski **[44]**, Veber **[45]** and Egan **[46]** rules violations.

4. Results and Discussion

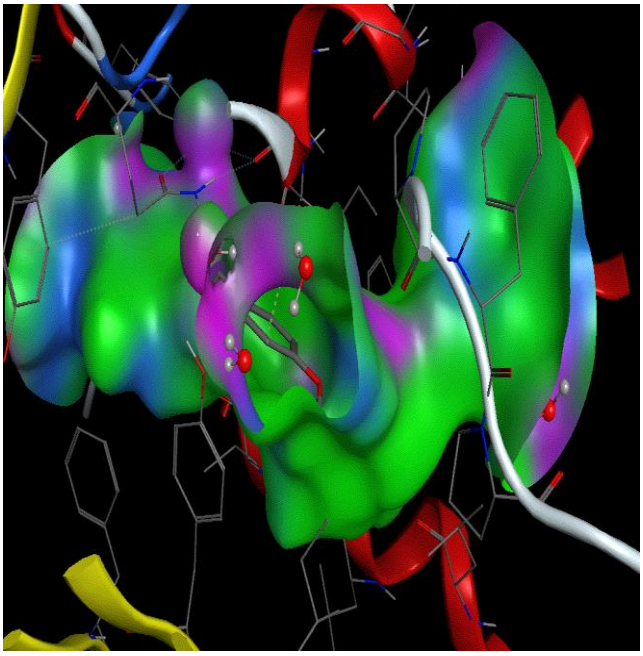
4.1. Docking and Pose Analysis

4.1.1. Active site residues identifications of target

Enzymes are made up of several active sites. The MOE 2014 software allowed us to identify and present the residues that form the active sites (the cavities) using the "Site Finder" module **[47]**.

The Site Finder methodology is based upon Alpha Shapes which are a generalization of convex hulls developed in **[48-50]**.

The properties of the first cavity detected by MOE are listed in the table below:



Residues
1: (VAL10 GLY11 GLY12 GLY13 ILE14 SER15 GLY16 LEU33 GLU34 ALA35ARG36 GLY40 GLY41ARG42 THR43 LEU56 GLY57 GLY58 SER59 TYR60 GLN65 GLU84 LEU88 PRO102 PHE103 PRO104 TRP119 LEU164 LEU167 PHE168 LEU171 CYS172 TYR188 THR195 ILE198 ILE199 SER200 THR201 GLY205 GLN206 ARG233 PRO234 VAL235 ALA 263 ILE264 PRO65 LEU268 LYS271 ILE272 VAL294 LYS296 THR314 ILE316 TYR326 LEU328 LEU326 PHE343)

***Figure 3**: Simplified model and the selected 4A79 cavity.*

This method of detecting active site residues is validated by the both previous studies of Krueger MJ et al **[51]** and Veselovsky AV et al **[52]** which are confirmed that the active site of MAO-B is formed by the following residues: Tyr60, Phe168, Leu171, Cys172, Tyr188, Ile198, Ile199, Gln206, Tyr326, Phe343, Tyr398 and Tyr435.

4.1.2. Interactions of compounds with MAO-B target and calculation of binding free energy

The docking results of compounds with **MAO-B** target were obtained by using MOE 2014 Software **[16]**, and the corresponding docking poses were visualized in workspace (Ligand Interactions). Subsequently, the binding free energy values calculate for each pose and the distances of Interactions of compounds with MAO-B target were listed in table 5.

Table 5: *S-score, IC50 and the interactions between the residues of the active site and the atoms of the compounds*

Compds	Pose	S-score (kcal/mol)	IC_{50} value (µM)	Bonds between atoms of compounds and active site residues					
				Atom of compound	Involved receptor atoms	Involved receptor residues	Type of interaction bond	Distance (Å)	Energies (kcal/mol)
L1	8	-7.230	/	N-3 O-4 O-4 6-ring	O OH O CA	HOH2348 TYR435 HOH2183 ILE 199	H-don H-acc H-acc pi-H	2.78 3.13 2.90 3.78	-3.0 -1.3 -1.9 -0.70
L2	5	-7.561	/	N-17	O	HOH2348	H-don	3.30	-0.40
L3	6	-6.279	/	6-ring	CG	LEU171	Pi-H	4.14	-0.60
L4	7	-7.626	/	N-32	O	HOH2183	H-acc	3.08	-1.80
L5	8	-6.740	/	N-23 N-23	OH O	TYR435 HOH2183	H-acc H-acc	3.22 3.48	-1.80 -0.50
L6	9	-7.526	3.225	N-16 6-ring	OH CG	TYR435 LEU171	H-acc Pi-H	3.18 3.79	-1.20 -0.70
L7	8	-7.183	3.146	/	/	/	/	/	/
L8	7	-8.083	0.387	6-ring	CG	LEU171	Pi-H	3.77	-0.70
L9	8	-7.813	0.136	ARJ3ILHA	/	/	/	/	/
L10	9	-7.479	1.462	C-8	6-ring	TYR326	H-Pi	4.44	-0.08
L11	7	-6.868	0.926	/	/	/	/	/	/
L12	9	-6.945	0.452	/	/	/	/	/	/
L13	9	-7.941	0.257	N-8 6-ring	OH CG	TYR435 LEU171	H-acc Pi-H	3.13 3.78	-1.40 -0.70
L14	6	-7.926	0.440	N-16 6-ring	OH CG	TYR435 LEU171	H-acc Pi-H	3.16 3.80	-1.30 -0.70
L15	10	-7.750	0.175	C-8	6-ring	TYR326	H-Pi	4.52	-0.80
L16	8	-7.573	0.283	/	/	/	/	/	/
L17	9	-7.916	2.023	O-21 O-21 6-ring	OH O CG	TYR435 HOH2183 LEU 171	H-acc H-acc Pi-H	3.07 3.33 3.81	-2.1 -0.7 -0.6
L18	**9**	**-8.454**	**1.108**	**N-16 O-17 O-17**	**O OH O**	**HOH2348 TYR435 HOH2183**	**H-don H-acc H-acc**	**3.22 2.92 3.03**	**-0.7 -2.6 -1.6**
L19	**6**	**-8.315**	**0.642**	**N-16 O-17 O-17**	**O OH O**	**HOH2348 TYR 435 HOH2183**	**H-don H-acc H-acc**	**3.07 2.96 2.94**	**-0.5 -2.7 -1.8**
L20	9	-7.629	1.055	N-16 O-18 O-18	O OH O	HOH2348 TYR435 HOH2183	H-don H-acc H-acc	3.27 2.95 3.11	-0.5 -2.6 -1.3
L21	8	-6.843	-1.550	6-ring 6-ring	CG CB	LEU171 ILE199	Pi-H Pi-H	3.79 3.91	-0.6 -0.8
L22	8	-7.758	0.271	N-16 O-17 O-17	O OH O	HOH2348 TYR435 HOH2183	H-don H-acc H-acc	3.26 2.95 3.08	-0.5 -2.6 -1.4
L23	8	-7.213	>10	6-ring	CG	LEU171	Pi-H	3.81	-0.6
L24	9	-7.535	1.507	C	6-ring	TYR326	H-Pi	4.37	-0.6

L25	9	-7.470	0.402	/	/	/	/	/	/
L26	7	-6.111	>10	N-14 6-ring	SG CA	CYS172 ILE199	H-don Pi-H	3.73 3.81	-1.4 0.7
L27	7	-7.024	2.483	C-6	6-ring	TYR326	H-Pi	4.38	-0.6
L28	6	-7.176	3.324	C-6	6-ring	TYR326	H-Pi	4.38	-0.6
L29	8	-7.664	1.803	/	/	/	/	/	/
L30	**6**	**-8.481**	**0.110**	**N-17**	**OH**	**TYR435**	**H-acc**	**3.02**	**-1.06**
L31	8	-7.875	0.009	N-17 N-17	OH O	TYR435 HOH2183	H-acc H-acc	3.32 3.27	-1.2 -1.2
L32	9	-8.127	0.396	/	/	/	/	/	/
L33	9	-7.757	0.193	6-ring	CG	LEU171	Pi-H	3.82	-0.7
L34	8	-7.751	0.109	6-ring	CG	LEU171	Pi-H	3.81	-0.7
L35	9	-8.007	2.849	6-ring	CG	LEU171	Pi-H	3.78	-0.7
L36	8	-7.821	0.062	N-17 N-17	OH O	TYR435 HOH2183	H-acc H-acc	3.11 2.92	-1.2 -1.6
L37	9	-8.302	0.059	/	/	/	/	/	/
L38	**9**	**-8.548**	**0.305**	**C-7**	**6-ring**	**TYR326**	**H-Pi**	**4.44**	**-0.70**
L39	4	-7.720	1.568	6-ring 6-ring	CG CB	LEU171 ILE199	Pi-H Pi-H	3.78 3.68	-0.7 -0.6
L40	6	-7.466	0.041	/	/	/	/	/	/
L41	6	-6.874	>10	/	/	/	/	/	/
L42	6	-6.835	>10	N-14 N-14	OH O	TYR435 HOH2183	H-acc H-acc	3.23 3.50	-1.80 -0.50
L43	4	-6.868	6.806	N-14 6-ring	O CA	HOH2183 ILE199	H-acc Pi-H	3.36 3.85	-0.90 -0.60
L44	7	-6.889	8.340	/	/	/	/	/	/
L45	7	-7.166	2.110	6-ring	CA	ILE199	Pi-H	4.21	-0.60
L46	3	-6.948	0.625	6-ring	CA	ILE199	Pi-H	4.30	-0.60
L47	8	-7.540	5.003	/	/	/	/	/	/
L48	6	-7.554	5.757	/	/	/	/	/	/
Pioglitazone		-10.125	/	N3-3 O4-1 O4-1 6-ring	O OH O CA	HOH2348 TYR 435 HOH2183 ILE199	H-don H-acc H-acc Pi-H	2.78 3.13 2.90 3.78	-3.0 -1.3 -1.9 -0.7

The results obtained from docking simulation show that the binding free energy values of complexes formed between compounds and MAO-B target were included in the interval -6.111 and -8.548 Kcal/mol, and we note also, that compounds: L18, L19, L30, and L38 forms complexes which have the lowest score of binding energy compared to the other complexes.

These complexes give the best docking scores, based on the binding free energy, citing here:

-8.454, -8.315, -8.481 and -8.548 respectively. This justifies that these complexes are more stable than others.

The binding free energy of complex formed by compound L38 has a score value very close (slightly higher) to the value of the native Ligand (Pioglitazone) which binding free energy was -10.125 Kcal/mol.

Complex 4A79-L38 gives the lowest score energy **-8.548** kcal/mol (see Table 5). We observed that compound L38 establishes one interaction with the pocket of the receptor, this type interaction H-Pi does appear in Figure 4, it is between atom C-7 of a compound L38 and 6-ring of TYR326 with a distance 4.44 Å. Furthermore, recently published research**[53-55]**confirmed that TYR326 is responsible for the formation of different interactions Pi-Pi or H-Pi in the pocket of the MAO-B target.

Compound **L30** forms one interaction with active site residue of MAO-B target, an H–acceptor interaction was observed between atom N-17 of a compound and aromatic residue TYR435 with distance equal 3.02 Å. This distance is considered strong according to A. Imberty et al **[56]**. Additionally, recent evidence **[57-60]**suggests that TYR435 is responsible for the formation of different interactions H-bond (H-acc and H-don) with the active site residues of MAO-B target.

Similarly compound L18 forms complex with 4A79 which has a low score energy -8.454 kcal/mol (see Table 5) and establish three interactions with the active site residues of the MAO-B target (Figure. 4). The first one is H-donor with a distance 3.22 Å; it is between atom N-16 of a compound and O of HOH2348. Both other interactions are of types H-acceptor formed; the first one is making between atom O-17 of a compound and OH of TYR435 with a distance 2.92 Å. The second interaction is formed between atom O-17 of a compound and OH of HOH2183 with a distance 3.03 Å. In addition, H-donor is considered weak and both interactions H-acceptor are considered strong according to A. Imberty et al **[56]**.

We note also that, a complex formed by compound L19 has low score energy values -8.315 kcal/mol. Compound L19 is involved in making three interactions with the active site residues of the target are shown in (Table 5). That interacts with one amino acid and two Water molecules (TYR435, HOH2348 and HOH2183).

The first one is H-donor with a distance 3.07 Å; it is between atom N-16 of a compound and OH of TYR435. Both other interactions are of types H-acceptor formed; the first one is making between atom O-17 of a compound and O of HOH2348 with a distance 2.96 Å. The second interaction is formed between atom O-17 of a compound and O of HOH2183 with distance 2.94Å. Furthermore, according to A. Imberty et al **[56]**, all these distances belong to the interval between 2.5 and 3.1 which are considered as strong interactions.

Besides, the results of other bonds formed between atoms of compounds and active site residues of the target are given in (See Table 5 and Annex).

According to Yeon, Seul Ki, et al **[12]** the compound L31(12c) has the most potent inhibitory activity (IC_{50}=0.009 µM) against MAO-B and they are considered it a potent and selective MAO-B inhibitor with structural features that can be a good starting point for the development of therapeutic agents for PD. Moreover, we can note also that L37(12i) has a low value of IC_{50}= 0.062 µM, with high potency against MAO-B.

Turning now to the result of molecular docking, it can seem that both compounds L31 and L37 have a high score energy value: -7.875 and -8.302 kcal/mol respectively (See Table 5), and compound L31 established two Hydrogen bonds (H-acc) with the pocket of MAO-B, the first one with TYR435 (3.32 Å) and the second with HOH2183 (3.27 Å). (See Table 5 and Annex), also, it was observed that no one interaction formed between a compound L37 and the active site cavity of MAO-B. (See Table 5 and Annex).

On the other hand, several recently published papers **[61-65]** have confirmed that it has a relationship between activity and affinity. On the contrary, we find that M.H.J. Seifert **[66]** have demonstrated that it has no relationship between these two parameters because the score is an estimate of the affinity between the macromolecule and the compound (molecular docking). Also, a score therefore in no way predicts an activity but rather an affinity.

A comparison of the results obtained from molecular docking, it can seem that the four best compounds L38, L30, L18 and L19 have average values of IC_{50} given here: 0.305, 0.110, 1.108 and 0.642 µM respectively, and complexes formed by these compounds are given low score energy with justifying by formation the many interactions between these compounds and catalytic site of MAO-B.

Overall, this finding indicates that the results obtained by the molecular docking study are in good agreement with those obtained with the experimental study, knowing that the four best compounds obtained at docking have a good inhibitory activity (IC_{50}) against MAO-B compared to the other compounds.

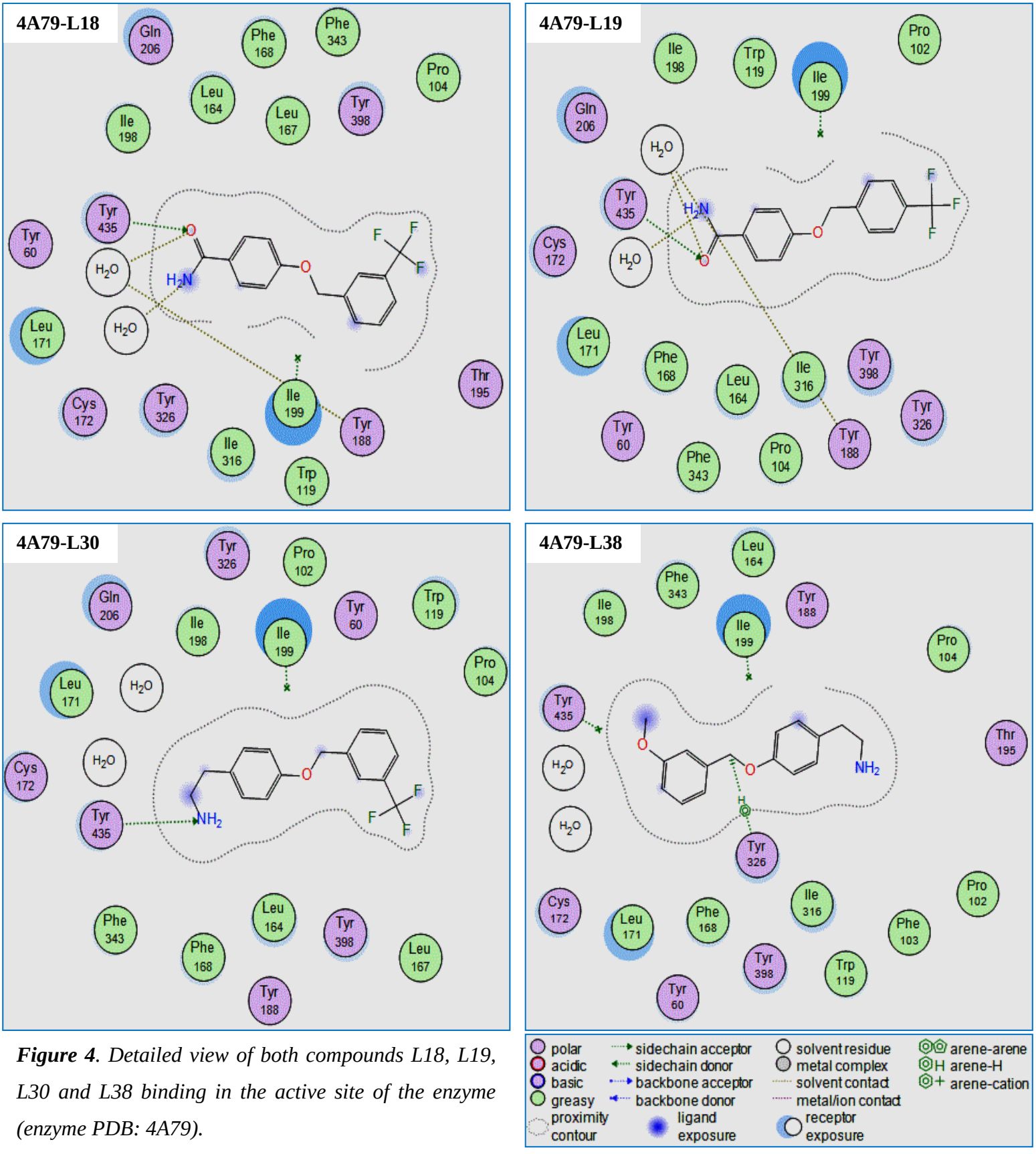


Figure 4. *Detailed view of both compounds L18, L19, L30 and L38 binding in the active site of the enzyme (enzyme PDB: 4A79).*

4.2. Analysis on molecular dynamic simulations

4.2.1. Stabilities and flexibilities of the four simulation systems

Molecular dynamics simulations performed on best docked conformations of compounds L18, L19, L30 and L38 binding with MAO-B target obtained from molecular docking in order to evaluate their structural stability. The Potential energy (U) was used to check the stability of complex protein-compounds after molecular docking during the MD simulations. The plot of Potential energy (U) as a function of simulation time (during 1000ps = 1ns) is reported in (Figure 5, 6, 7 and 8).

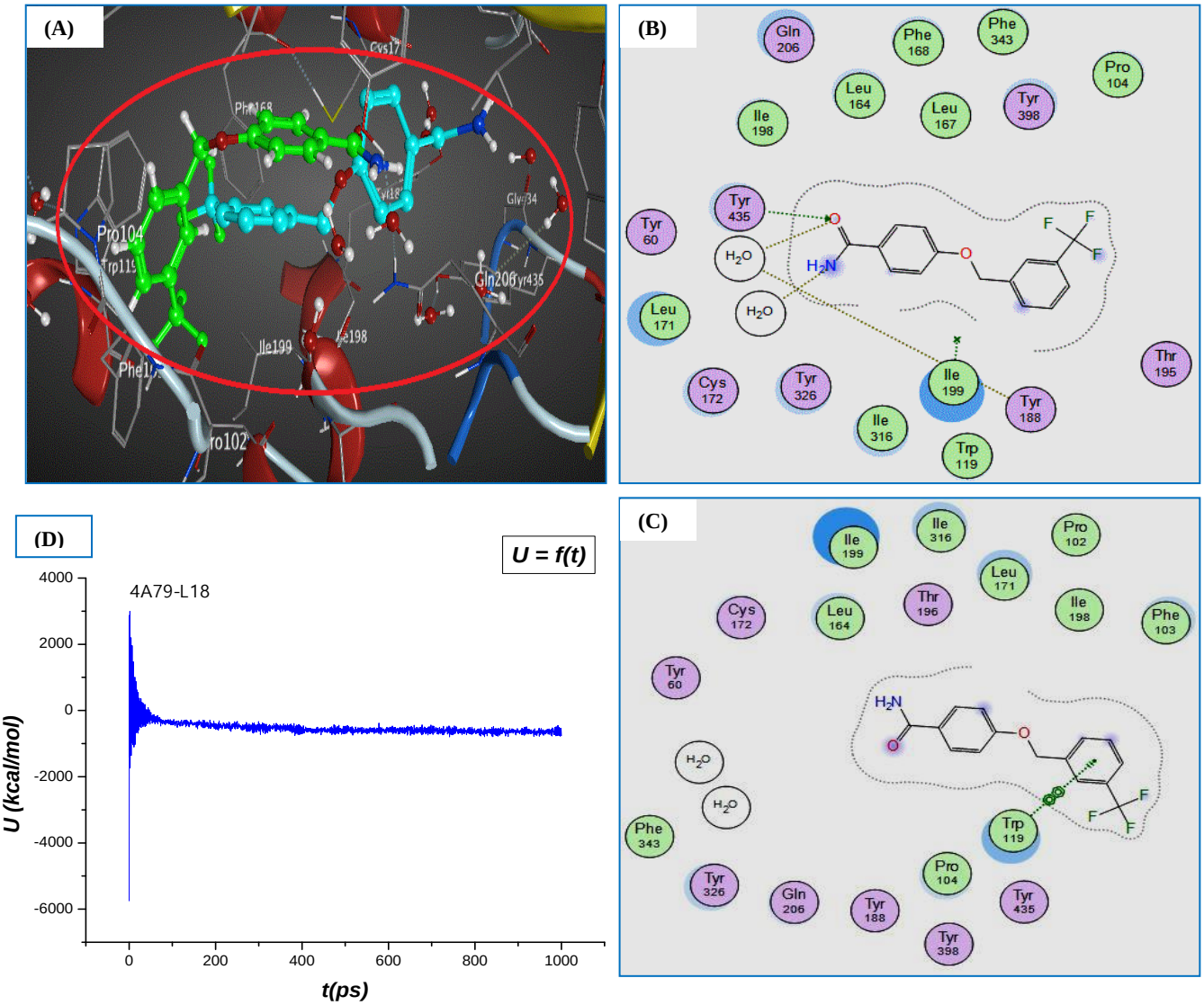


Figure 5 :(A) *: 3D diagram of interaction between L18 with MOA-B using docking (green color) and dynamics (blue color) simulations,* ***(B)****: 2D diagram of interaction between L18 with MOA-B using molecular docking,* ***(C)****: 2D diagram of interaction between L18 with MOA-B using molecular dynamics,* ***(D)****: Potential energy (U) as a function of simulation time (during 1000ps = 1ns*).

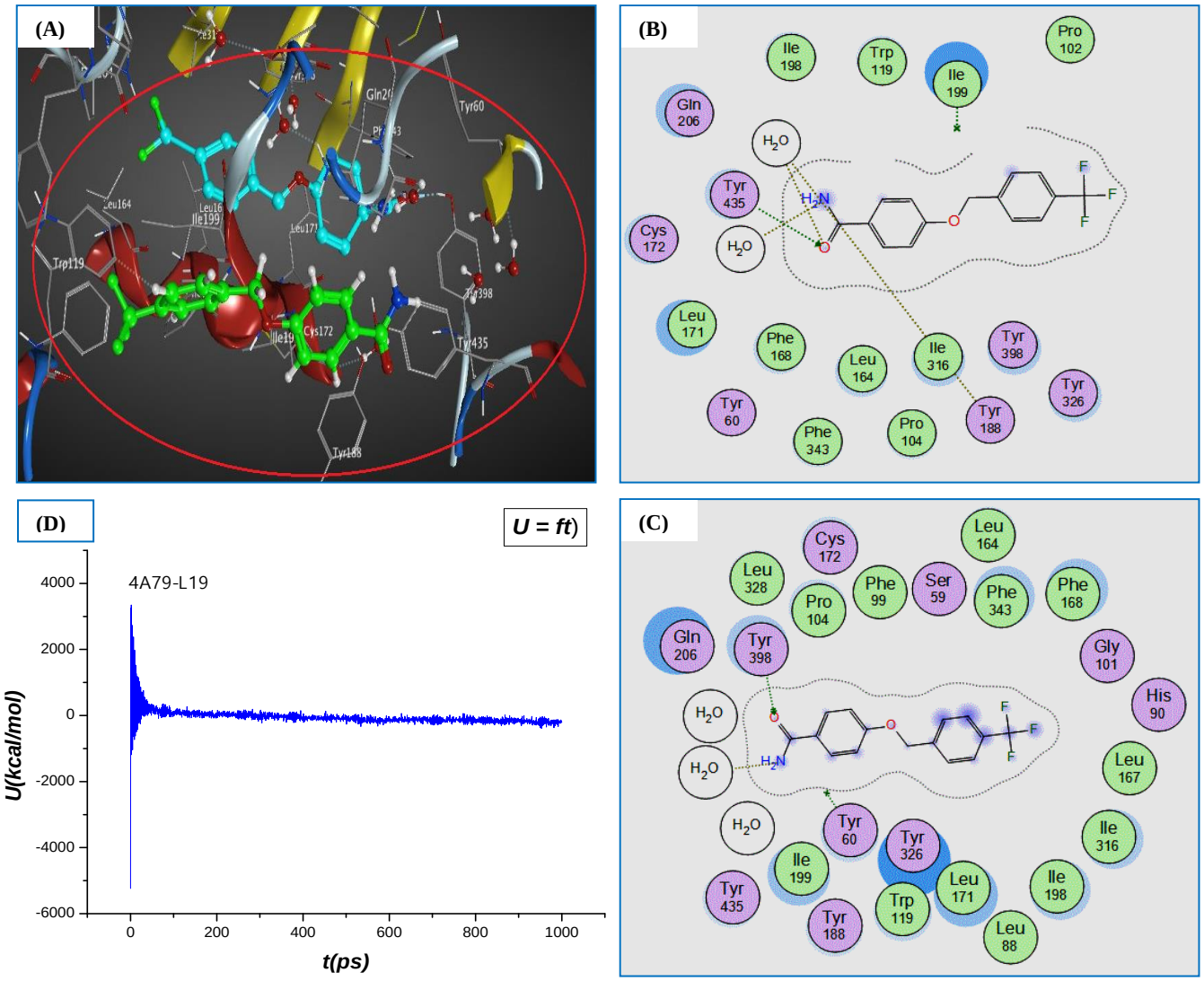


Figure 6 :(A) *: 3D diagram of interaction between L19 with MOA-B using docking (green color) and dynamics (blue color) simulations,* ***(B)****: 2D diagram of interaction between L19 with MOA-B using molecular docking,* ***(C)****: 2D diagram of interaction between L19 with MOA-B using molecular dynamics,* ***(D)****: Potential energy (U) as a function of simulation time (during 1000ps = 1ns).*

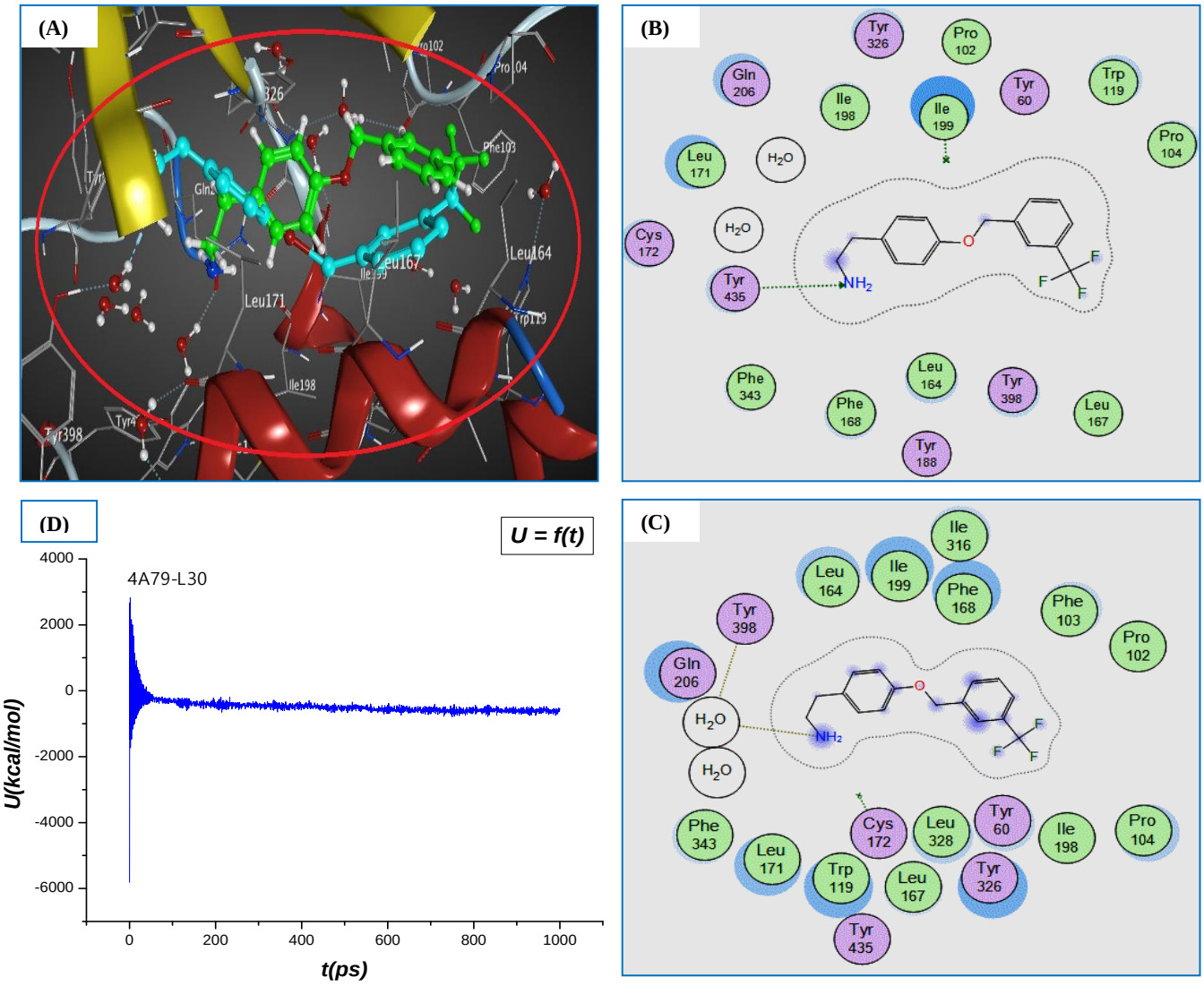


Figure 7 :(A) *: 3D diagram of interaction between L30 with MOA-B using docking (green color) and dynamics (blue color) simulations,* ***(B)****: 2D diagram of interaction between L30 with MOA-B using molecular docking,* ***(C)****: 2D diagram of interaction between L30 with MOA-B using molecular dynamics,* ***(D)****: Potential energy (U) as a function of simulation time (during 1000ps = 1ns).*

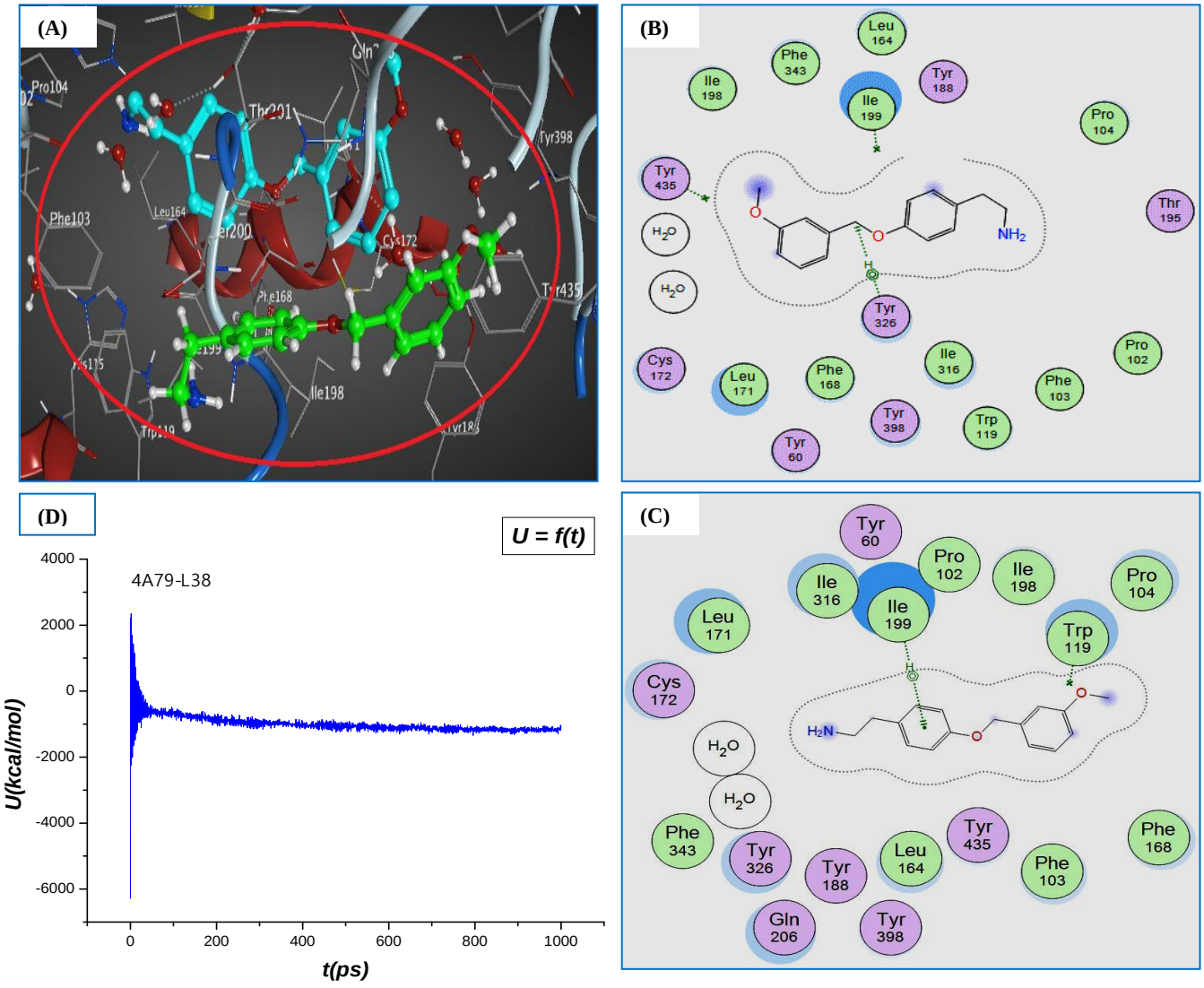


Figure 8 :(A) *: 3D diagram of interaction between L38 with MOA-B using docking (green color) and dynamics (blue color) simulations,* ***(B)****: 2D diagram of interaction between L38 with MOA-B using molecular docking,* ***(C)****: 2D diagram of interaction between L38 with MOA-B using molecular dynamics,* ***(D)****: Potential energy (U) as a function of simulation time (during 1000ps = 1ns).*

The plot of Potential energy (U) as a function of simulation time during 1000 ps of four complexes 4A79-L18, 4A79-L19, 4A79-L30 and 4A79-L38 is reported in four Figures above: Fig. 5(D), 6(D), 7(D) and 8(D) respectively.

Fig. 5(D), 6(D), 7(D) and 8(D), during first 100 picoseconds, the backbone fluctuation has been observed initially as indicated by Potential energy value of MAO-B protein with compounds (L18, L19, L30 and L38).

The curve for complex 4A79-L18 (Fig. 5(D)) showed a potential energy change from -412,981 to -645.845 kcal/mol between 100 and 400 ps, we also notice a slight change in the potential energy: -645.845to -663.348 kcal/mol in the second interval between 400 and 600 ps. Finally, we can clearly see that the last interval (between 600 and 1000 ps) indicating that the complex 4A79-L18 is retaining full stability.

For the curve of the second complex 4A79-L19 (Fig. 6(D)), it showed that we found two intervals of time, the first one: it is between 100 and 400 ps with a slight change of potential energy between -35.129 to -37.216 kcal/mol. The values of potential energy in the last interval clearly indicate that compound 19 is retaining the stability of the protein complexation.

In the curve of third complex 4A79-L30 (Fig. 7(D)), we note also that is three intervals of time. The first between 100 and 400 ps with a variation of potential energy: -314.499 and -451.200 kcal/mol, we also notice a slight change in the potential energy: -451.200to -439.259 kcal/mol, in the second interval between 400 and 600 ps. Finally, we can clearly see that the last interval (between 600 and 1000 ps) indicating that the complex 4A79-L30 is retaining full stability.

The last curve of complex 4A79-L38(Fig. 8(D)), it showed that potential energy variation from -628.508 to -993.054 kcal/mol in an interval between100and 400 ps, the second interval between 400 and 700 ps with a slight change in the potential energy: -993.054 and -1240.138 kcal/mol. Finally, we can clearly see the stability of the 4A79-L18 complex from 7000 ps up to 1000 ps.

Table 6, gives the new different interactions formed between the active site residues of MAO-B and the four best compounds (L18, L19, L30 and L38) according to the calculation of molecular dynamics.

Table 6: *Molecular dynamics results of the four best poses obtained by molecular docking*

Compds	Pose	S-score (kcal/mol)	Bonds between atoms of compounds and residues of active site					
			Atom of compound	Involved receptor atoms	Involved receptor residues	Type of interaction bond	Distance (Å)	Energies (kcal/mol)
L18	9	-8.454	6-ring	5-ring	TRP119	Pi-Pi	3.84	-0.00
			6-ring	6-ring	TRP119	Pi-Pi	3.97	-0.00
L19	6	-8.315	N-16	O	HOH2060	H-don	2.83	-0.90
			O-17	OH	TYR398	H-acc	3.15	-2.40
L30	6	-8.481	N-17	O	HOH2205	H-acc	2.89	-1.40
L38	9	-8.548	6-ring	CA	ILE199	Pi-H	3.94	-0.7

From the table above, we easily found that, according to the results of molecular dynamics, the four compounds formed new types of interactions with the residues of the active site of MAO-B comparing with the results of molecular docking, except in the case of the compounds L30 and L38 formed the same interactions with the active site residues of the target. On the other hand, compounds L18 and L19 both form three interactions in the docking molecular simulation and both form two interactions in the case of dynamics molecular simulations. At the end, both complexes 4A79-L30 and 4A79-L38 are better stable in molecular dynamics because they keep the same types of interactions which are obtained through the simulation of molecular docking.

4.3. In silico evaluation of the ADME properties and drug-likeness

The prediction of the ADMET properties plays an important role in the drug design process because these properties account for the failure of about 50-60% of all drugs in the clinical phases. In addition, it is estimated that close to 50% of drug candidates fail because of unacceptable efficacy**[67]**and that up to 40% of drug candidates have failed in the past due to toxicity **[68]**. A computational study of four top scoring lead compounds was performed for assessment of ADME properties and the obtained value is depicted in table **7**.

Table 7: *ADME properties for the top four compounds*

Entry	ABS	BBB Permeability	n-ROTB	MW	MLog P	n-ON acceptors	n-OHNH donors	Lipinski's Violations	Veber violations	Egan Violations
Rule	**-**	**-**	**-**	**<500**	**≤5**	**<10**	**<5**	**≤1**	**≤1**	**≤1**
L18	High	yes	5	295.26	3.29	5	1	Yes	yes	Yes
L19	High	yes	5	295.26	3.29	5	1	Yes	yes	Yes
L30	High	yes	6	295.30	3.82	5	1	Yes	yes	Yes
L39	High	yes	6	257.33	2.57	3	1	Yes	yes	Yes

ABS: Absorption**, BBB**: blood–brain barrier**, n-ROTB:** Number Of Rotatable Bonds**, MW:** Molecular Weight**, MLog *P*:** logarithm of partition coefficient of compound between n-octanol and water**, n-ON acceptors:** Number of hydrogen bond acceptors**, n-OHNH donors:** Number of hydrogen bonds donors**.**

The results presented in table 7 revealed that the four compounds have high absorption. In addition, the authors report that if exists a good correlation between logP and BBB permeation compounds able to cross the BBB **[69,70]**.In our study, we found that LogP values are ranging from 2.57 – 3.82 (Table7), indicating that the five compounds are likely able to cross the BBB. Also, we can be observed that all compounds are respected the Lipinski's rule of 5, Veber and Egan, where log*P* values ranged between: 2.57 – 3.82 (<5), MW range 257.33 – 295.30 (<500), HBA range 3 – 5 (≤ 10) and HBD is equal to 1 (<5). In the end, we can suggest that these compounds would not be expected to cause problems with oral bioavailability and thus showing possible utility for developing the news compounds with a good drug-like properties against MAO-B.

References

[1] Luo, Yunan, et al. "A network integration approach for drug-target interaction prediction and computational drug repositioning from heterogeneous information." Nature communications 8.1 **(2017):** 1-13.

[2] K.L. Chandra Mohan, "(PDF) Introduction to Inhibitors," ResearchGate. https://www.researchgate.net/publication/280494256_Introduction_to_Inhibitors (accessed Apr. 14, **2020**).

[3] Méndez, Raúl, et al. "Assessment of CAPRI predictions in rounds 3–5 shows progress in docking procedures." Proteins: Structure, Function, and Bioinformatics 60.2 **(2005):** 150-169.

[4] Alvarez, Juan C. "High-throughput docking as a source of novel drug leads." Current opinion in chemical biology 8.4 **(2004):** 365-370.

[5] Ghosh, Sutapa, et al. "Structure-based virtual screening of chemical libraries for drug discovery." Current opinion in chemical biology 10.3 **(2006):** 194-202.

[6] Binda, Claudia, et al. "Molecular insights into human monoamine oxidase B inhibition by the glitazone antidiabetes drugs." ACS medicinal chemistry letters 3.1 **(2012):** 39-42.

[7] Clément, Gilles, and Klaus Slenzka, eds. Fundamentals of space biology: research on cells, animals, and plants in space. Vol. 18. Springer Science & Business Media, **2006**.

[8] Didierjean, Claude, and Frédérique Tête-Favier. "Introduction to Protein Science. Architecture, Function and Genomics. By Arthur M. Lesk. Oxford University Press, 2016. Pp. 466. Paperback. Price GBP 39.99. ISBN 9780198716846." Acta Crystallographica Section D: Structural Biology 72.12 **(2016):** 1308-1309.

[9] Kleywegt, Gerard J., and T. Alwyn Jones. "[11] Model building and refinement practice." Methods in enzymology. Vol. 277. Academic Press, **(1997):** 208-230.

[10] Klebe, Gerhard. "Virtual ligand screening: strategies, perspectives and limitations." Drug discovery today 11.13-14 **(2006):** 580-594.

[11] Maréchal, Yves. The hydrogen bond and the water molecule: The physics and chemistry of water, aqueous and bio-media. Elsevier, **2006**.

[12] Yeon, Seul Ki, et al. "Synthesis and evaluation of biaryl derivatives for structural characterization of selective monoamine oxidase B inhibitors toward Parkinson's disease therapy." Bioorganic & medicinal chemistry 26.1 **(2018):** 232-244.

[13] Cousins, Kimberley R. "ChemDraw Ultra 9.0. CambridgeSoft, 100 CambridgePark Drive, Cambridge, MA 02140. www. cambridgesoft. com. See Web site for pricing options." **(2005):** 4115-4116.

[14] Stewart, James JP. "Optimization of parameters for semiempirical methods V: modification of NDDO approximations and application to 70 elements." Journal of Molecular modeling 13.12 **(2007):** 1173-1213.

[15] HyperChem v8. Molecular Modelling System, Hypercube Inc., 1115 NW 4th Street, Gainesville, FL 32601, USA **(2009).**

[16] Molecular Operating Environment (MOE), 2013.08; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, **2019**.

[17] http://www.molegro.com/mvd-product.php

[18] Thomsen, René, and Mikael H. Christensen. "MolDock: a new technique for high-accuracy molecular docking." Journal of medicinal chemistry 49.11 **(2006):** 3315-3321.

[19] Novikov, Fedor N., and Ghermes G. Chilov. "Molecular docking: theoretical background, practical applications and perspectives." Mendeleev Communications 5.19 **(2009):** 237-242.

[20] Sethi, Aaftaab, et al. "Molecular docking in modern drug discovery: Principles and recent applications." Drug Discovery and Development-New Advances. IntechOpen, **2019**.

[21] Ferreira, Leonardo G., et al. "Molecular docking and structure-based drug design strategies." Molecules 20.7 **(2015):** 13384-13421.

[22] Abdelall, Eman KA, Phoebe F. Lamie, and Waleed AM Ali. "Cyclooxygenase-2 and 15-lipoxygenase inhibition, synthesis, anti-inflammatory activity and ulcer liability of new celecoxib analogues: determination of region-specific pyrazole ring formation by noesy." Bioorganic & Medicinal Chemistry Letters 26.12 **(2016):** 2893-2899.

[23] Aldeghi, Matteo, et al. "Predictions of ligand selectivity from absolute binding free energy calculations." Journal of the American Chemical Society 139.2 **(2017):** 946-957.

[24] Al-Rashood, Sarah TA, et al. "Synthesis, biological evaluation and molecular modeling study of some new thiazolodiazepine analogs as CNS active agents." Bioorganic & Medicinal Chemistry Letters 26.2 **(2016):** 445-453.

[25] Thai, Khac-Minh, et al. "Computational assay of Zanamivir binding affinity with original and mutant influenza neuraminidase 9 using molecular docking." Journal of theoretical biology 385 **(2015):** 31-39.

[26] Abd El-Karim, Somaia S., et al. "Design, synthesis, biological evaluation and molecular docking studies of novel benzofuran–pyrazole derivatives as anticancer agents." Bioorganic Chemistry 63 **(2015):** 1-12.

[27] Liu, Sheng, et al. "Design, synthesis, biological evaluation and molecular docking studies of phenylpropanoid derivatives as potent anti-hepatitis B virus agents." European Journal of Medicinal Chemistry 95 **(2015):** 473-482.

[28] Nasr, Tamer, Samir Bondock, and Sameh Eid. "Design, synthesis, antimicrobial evaluation and molecular docking studies of some new thiophene, pyrazole and pyridone derivatives bearing sulfisoxazole moiety." European journal of medicinal chemistry 84 **(2014):** 491-504.

[29] Fang, Jiansong, et al. "Inhibition of acetylcholinesterase by two genistein derivatives: kinetic analysis, molecular docking and molecular dynamics simulation." Acta Pharmaceutica Sinica B 4.6 **(2014):** 430-437.

[30] El-Nakkady, Sally S., et al. "Synthesis, molecular docking study and antitumor activity of novel 2-phenylindole derivatives." European journal of medicinal chemistry 47 **(2012):** 387-398.

[31] Aier, Imlimaong, Pritish Kumar Varadwaj, and Utkarsh Raj. "Structural insights into conformational stability of both wild-type and mutant EZH2 receptor." Scientific reports 6.1 (2016): 1-10.

[32] Jacob K, Sony, et al. "Homology model, molecular dynamics simulation and novel pyrazole analogs design of Candida albicans CYP450 lanosterol 14 α-demethylase, a target enzyme for antifungal therapy." Journal of Biomolecular Structure and Dynamics 35.7 **(2017):** 1446-1463.

[33] Pettersen, Eric F., et al. "UCSF Chimera—a visualization system for exploratory research and analysis." Journal of computational chemistry 25.13 **(2004):** 1605-1612.

[34] Daoud, Ismail, et al. "Combined QSAR, molecular docking and molecular dynamics study on new Acetylcholinesterase and Butyrylcholinesterase inhibitors." Computational biology and chemistry 74 **(2018):** 304-326.

[35] SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7:42717.**2017**.

[36] Daina, Antoine, and Vincent Zoete. "A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules." ChemMedChem 11.11 **(2016):** 1117.

[37] Norinder, Ulf, and Markus Haeberlein. "Computational approaches to the prediction of the blood–brain distribution." Advanced drug delivery reviews 54.3 **(2002):** 291-313.

[38] Johnson, Stephen R., and Weifan Zheng. "Recent progress in the computational prediction of aqueous solubility and absorption." The AAPS journal 8.1 **(2006):** E27-E40.

[39] Hou, Tingjun, et al. "Recent developments of in silico predictions of intestinal absorption and oral bioavailability." Combinatorial chemistry & high throughput screening 12.5 **(2009):** 497-506.

[40] Fagerholm, Urban. "Prediction of human pharmacokinetics—gastrointestinal absorption." Journal of pharmacy and pharmacology 59.7 **(2007):** 905-916.

[41] Norinder, Ulf, and Christel AS Bergström. "Prediction of ADMET properties." ChemMedChem: Chemistry Enabling Drug Discovery 1.9 **(2006):** 920-937.

[42] Hou, Tingjun, et al. "Recent advances in computational prediction of drug absorption and permeability in drug discovery." Current medicinal chemistry 13.22 **(2006):** 2653-2667.

[43] Delaney, John S. "Predicting aqueous solubility from structure." Drug Discovery Today 10.4 **(2005):** 289-295.

[44] Lipinski, Christopher A., et al. "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings." Advanced drug delivery reviews 23.1-3 **(1997):** 3-25.

[45] Veber DF1, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. Jun 6;45(12):2615-23. **2002**.

[46] Egan, William J., Kenneth M. Merz, and John J. Baldwin. "Prediction of drug absorption using multivariate statistics." Journal of medicinal chemistry 43.21 **(2000):** 3867-3877.

[47] Le Guilloux, Vincent, Peter Schmidtke, and Pierre Tuffery. "Fpocket: an open source platform for ligand pocket detection." BMC bioinformatics 10.1 **(2009):** 1-11.

[48] Edelsbrunner, H., Facello, M., Fu, R., Liang, J.; Measuring Proteins and Voids in Proteins; Proceedings of the 28th Hawaii International Conference on Systems Science (**1995**): 256–264.

[49] Soga, Shinji, et al. "Use of amino acid composition to predict ligand-binding sites." Journal of chemical information and modeling 47.2 **(2007):** 400-406.

[50] Volkamer, A., Griewel, A., Grombacher, T., Rarey, M.; Analyzing the Toplogy of Active Sites: On the Prediction of Pockets and Subpockets; J. Chem. Inf. Model. 50 **(2010)**: 2041-2052.

[51] Krueger, Matthew J., et al. "Dramatic species differences in the susceptibility of monoamine oxidase B to a group of powerful inhibitors." Biochemical and biophysical research communications 206.2 **(1995):** 556-562.

[52] Veselovsky, Alexander V., Alexey S. Ivanov, and Alexei E. Medvedev. "Computer modelling and visualization of active site of monoamine oxidases." Neurotoxicology 25.1-2 **(2004):** 37-46.

[53] Kuder, Kamil J., et al. "Novel, dual target-directed annelated xanthine derivatives acting on adenosine receptors and monoamine oxidase B." ChemMedChem **(2020)**.

[54] Sawant, V. S., Park, H., Baek, S. Y., Lee, J., Choi, J. W., Park, K. D., ... & Choo, H. Benzoxazoles as Selective Monoamine Oxidase B (MAO-B) Inhibitors. Bulletin of the Korean Chemical Society, 40.5**(2019)**: 457-460.

[55] Jaiteh, Mariama, et al. "Docking screens for dual inhibitors of disparate drug targets for Parkinson's disease." Journal of medicinal chemistry 61.12 **(2018):** 5269-5278.

[56] Imberty, Anne, et al. "Molecular modelling of protein-carbohydrate interactions. Docking of monosaccharides in the binding site of concanavalin A." Glycobiology 1.6 **(1991):** 631-642.

[57] Azam, Faizul, et al. "Rutin as promising drug for the treatment of Parkinson's disease: an assessment of MAO-B inhibitory potential by docking, molecular dynamics and DFT studies." Molecular Simulation 45.18 **(2019):** 1563-1571.

[58] Załuski, Michał, et al. "Novel multi-target directed ligands based on annelated xanthine scaffold with aromatic substituents acting on adenosine receptor and monoamine oxidase B. Synthesis, in vitro and in silico studies." Bioorganic & medicinal chemistry 27.7 **(2019):** 1195-1210.

[59] Chaurasiya, Narayan D., et al. "Selective inhibition of human monoamine oxidase B by Acacetin 7-methyl ether isolated from Turneradiffusa (Damiana)." Molecules 24.4 **(2019):** 810.

[60] Mellado, Marco, et al. "Design, Synthesis and Docking Calculations of Prenylated Chalcones as Selective Monoamine Oxidase B Inhibitors with Antioxidant Activity." ChemistrySelect 4.26 **(2019):** 7698-7703.

[61] Alagesan, Venkatesan, et al. "Antioxidant activity guided isolation of a coumarin compound from Ipomoea pes-caprea (Convolvulaceae) leaves acetone extract and its biological and molecular docking studies." European Journal of Integrative Medicine 32 **(2019):** 100984.

[62] Simon, Lalitha, et al. "In silico drug-designing studies on flavanoids as anticolon cancer agents: pharmacophore mapping, molecular docking, and monte carlo method-based QSAR modeling." Interdisciplinary Sciences: Computational Life Sciences 9.3 **(2017):** 445-458.

[63] Dhara, Lena, and Anusri Tripathi. "Antimicrobial activity of eugenol and cinnamaldehyde against extended spectrum beta lactamase producing enterobacteriaceae by in vitro and molecular docking analysis." European Journal of Integrative Medicine 5.6 **(2013):** 527-536.

[64] Zengin, Gokhan, et al. "In vitro and in silico insights of Cupressus sempervirens, Artemisia absinthium and Lippiatriphylla: Bridging traditional knowledge and scientific validation." European Journal of Integrative Medicine 12 **(2017):** 135-141.

[65] Abdelli, Imane, et al. "In silico evaluation of phenolic compounds as inhibitors of α-amylase and α-glucosidase." Journal of Biomolecular Structure and Dynamics **(2020):** 1-7.

[66] Seifert, Markus HJ. "Assessing the discriminatory power of scoring functions for virtual screening." Journal of chemical information and modeling 46.3 **(2006):** 1456-1465.

[67] Kennedy, Tony. "Managing the drug discovery/development interface." Drug discovery today 2.10 **(1997):** 436-444.

[68] DiMasi, Joseph A. "Success rates for new drugs entering clinical testing in the United States." Clinical Pharmacology & Therapeutics 58.1 **(1995):** 1-14.

[69] Levin, Victor A. "Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability." Journal of medicinal chemistry 23.6 **(1980):** 682-684.

[70] Goodwin, Jay T., and David E. Clark. "In silico predictions of blood-brain barrier penetration: considerations to "keep in mind"." Journal of Pharmacology and Experimental Therapeutics 315.2 **(2005):** 477-483.

General Conclusion

In this study, we used three methods: molecular docking analyzes, MD simulations and ADME properties to investigate the potential of new compounds such as: 4-(benzyloxy)phenyl and biphenyl-4-yl derivatives with MAO-B target witch that can be new inhibitors against Parkinson disease (PD).

The results of molecular docking simulation show that the top four compounds L18, L19, L30 and L38 of 4-(benzyloxy)phenyl and biphenyl-4-yl derivatives form complexes which have a lowest score energy compared to other compounds, this is justified, that these compounds have ahigh affinity with the MAO-B pocket and they established many types of interactions with active site residues of the target.

However, to validate the results of docking simulation; molecular dynamic calculation is used to confirm the stability of the complexes formed between these four compounds and of the active site residues of MAO-B.

The potential energy (U) was used to verify the stability of the complexes protein-compounds during 1000 ps which obtained by molecular dynamic simulation and according to the type of interactions, we found that both compounds L30 and L38 maintain the same type of interactions, which were H-acc type in the case of complex 4a79-L30 with a distance of 2.89 Å and Pi-H type with the complex 4a79-L38- with a distance of 3.94 Å.

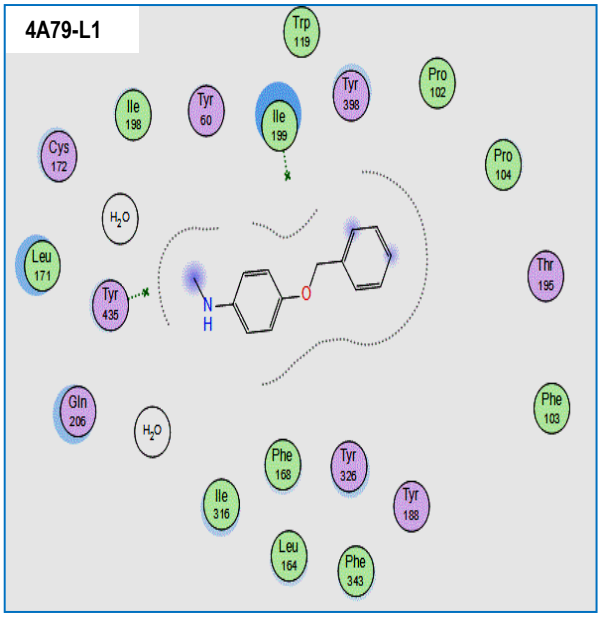
A comparison of the results obtained from molecular docking and molecular dynamic simulations, it can seem that both top 4-(benzyloxy)phenyl and biphenyl-4-yl derivatives candidates, compound L30 and compound L38 have weak values of IC_{50} given here: 0.110 and 0.305 µM respectively, and complexes formed by these compounds are given low score energy justify by thee formation the many bonds between these compounds and catalytic site of MAO-B.

Moreover, these both compounds respect the Lipinski, Veber and Egan rules, they are able to cross the BBB, in addition, these findings are in good agreement with the experimental results. In conclusion, we can be concluded that the three methods used in this study have proven to be able to discover a new class of MAO-B inhibitors and both compounds L30 and L38 are considered a potent MAO-B inhibitor with structural features that can be a good starting point for the development of therapeutic agents for PD.

Annex

Interactions between ligands
and active site residues for the 4A79 enzyme

4A79-L1



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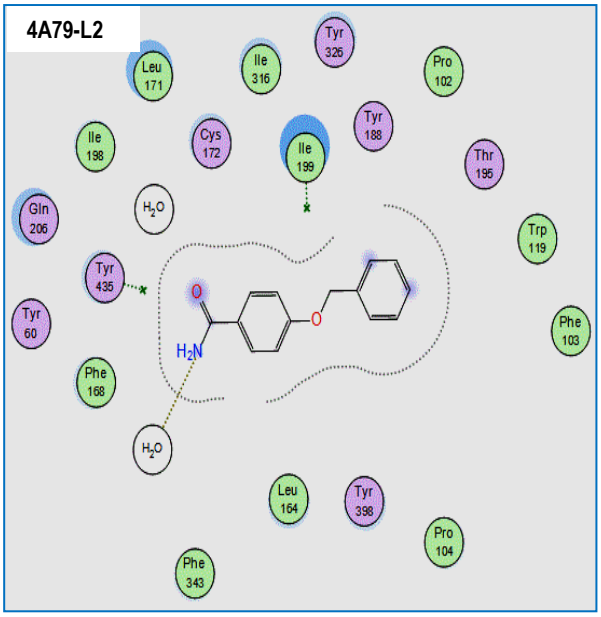

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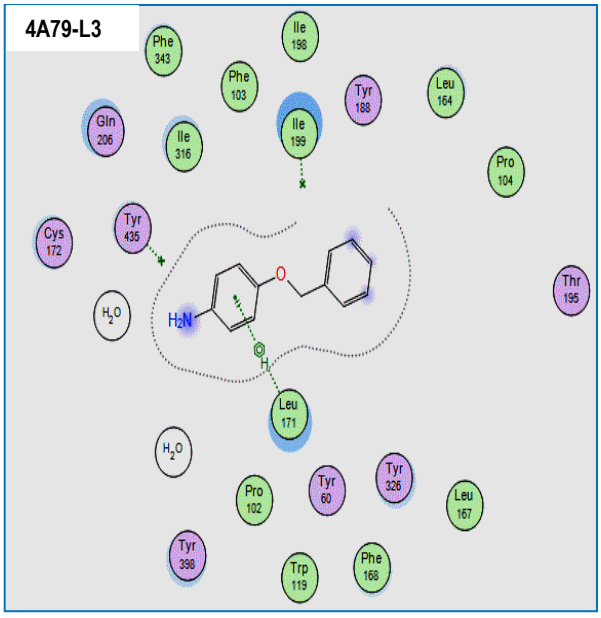
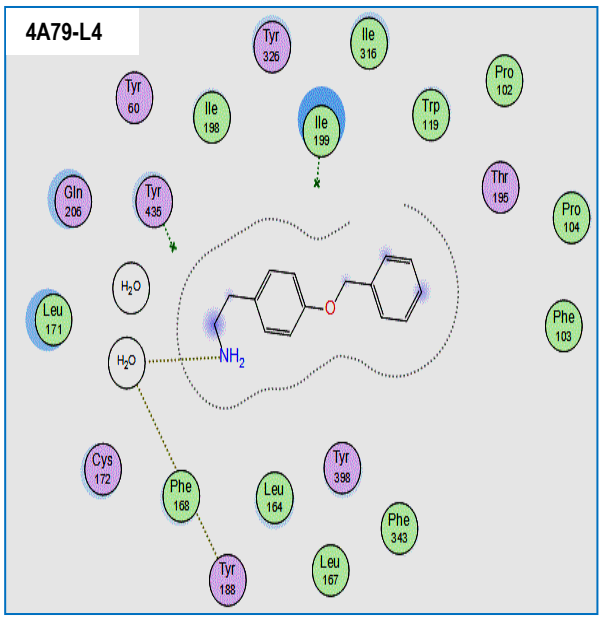
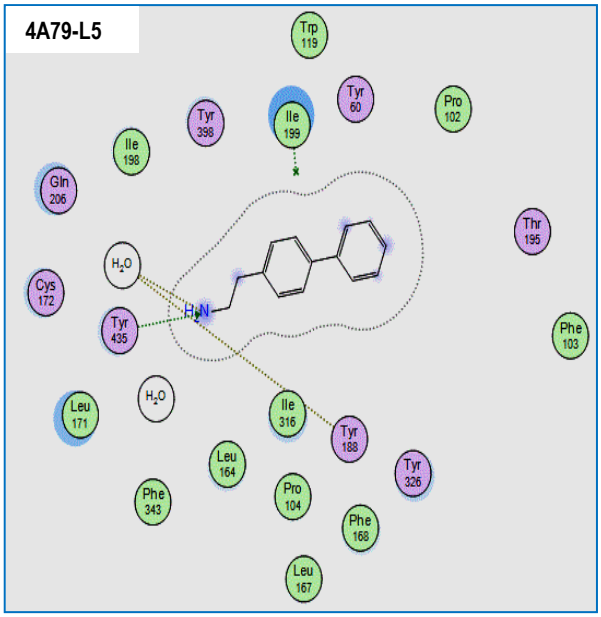


4A79-L4



4A79-L5



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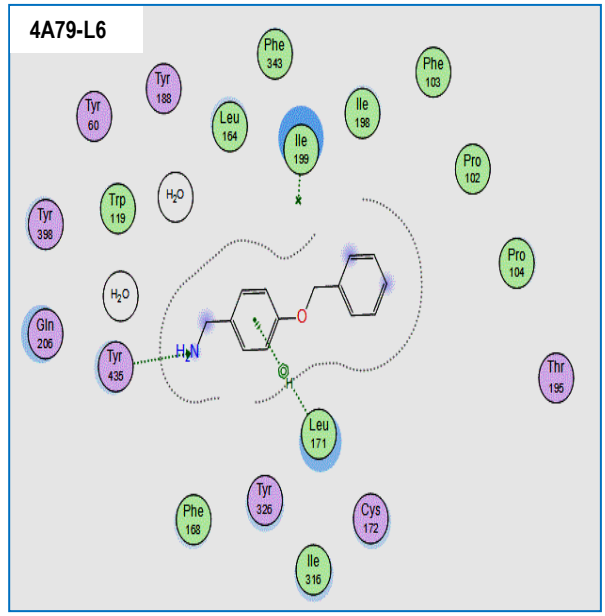

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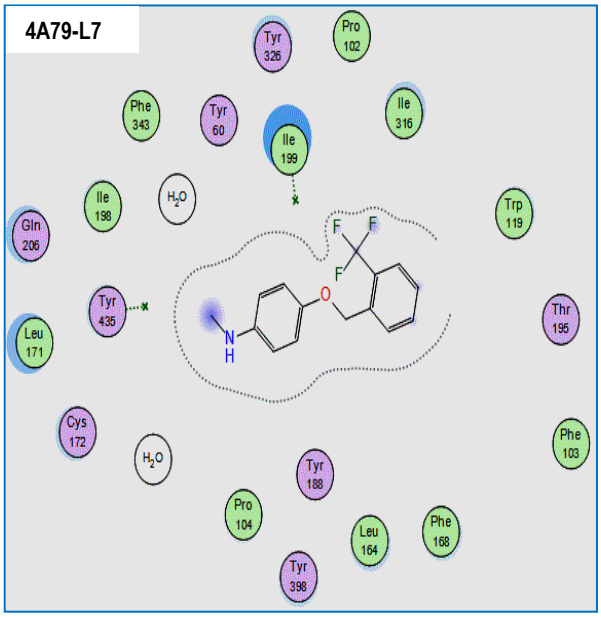
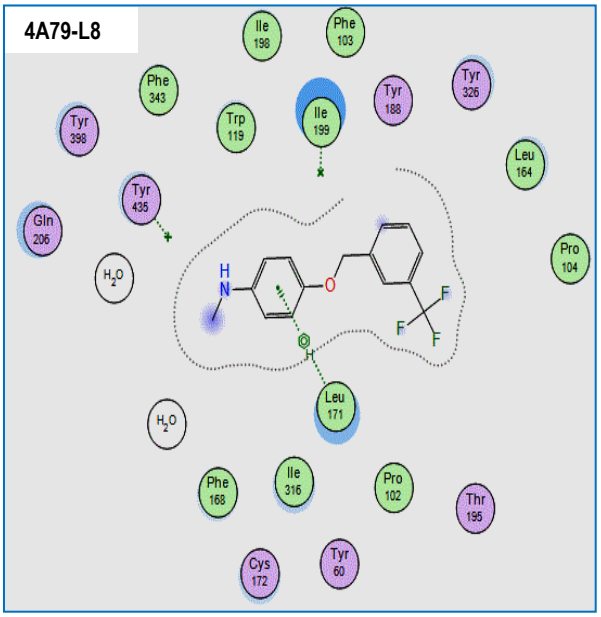
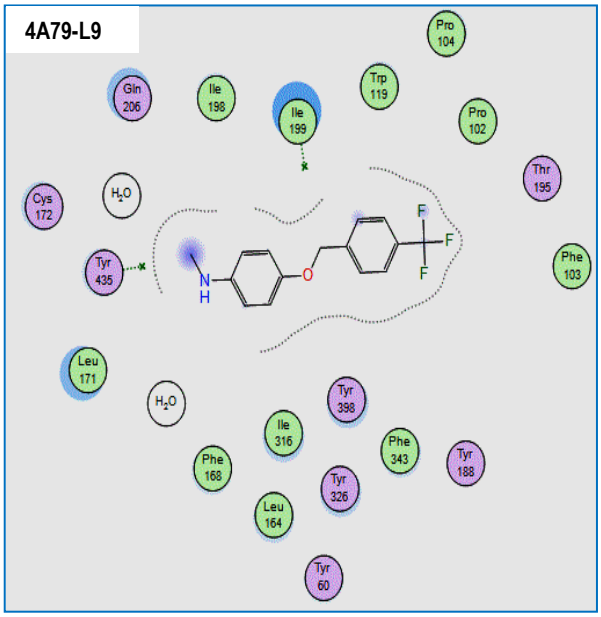


4A79-L8



4A79-L9



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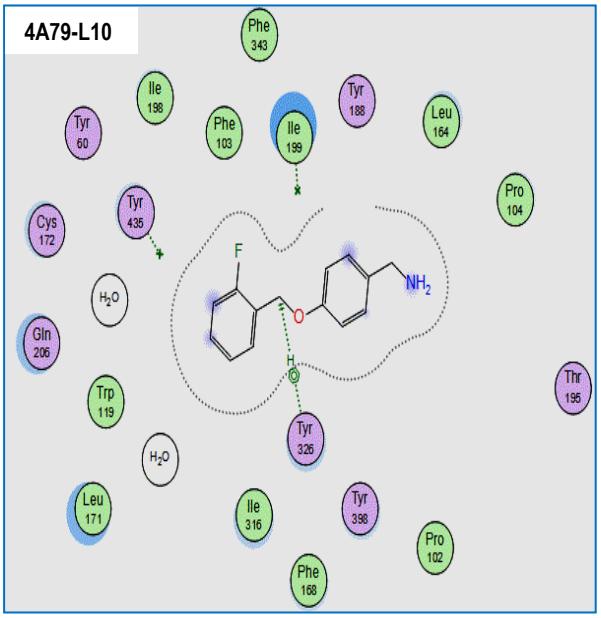

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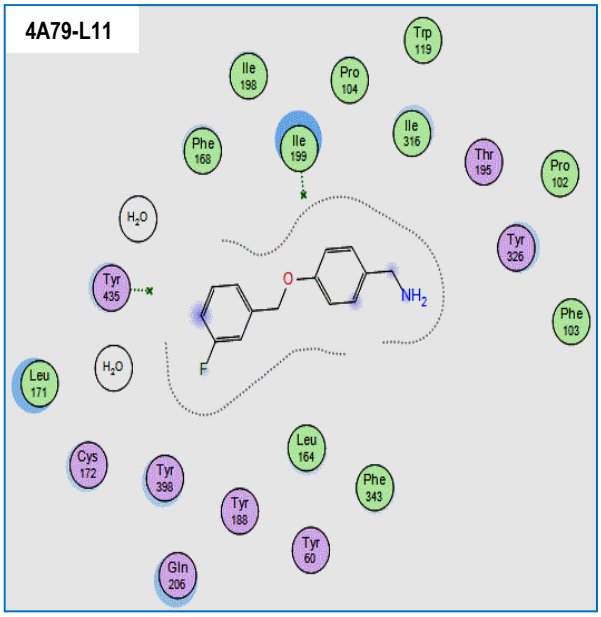

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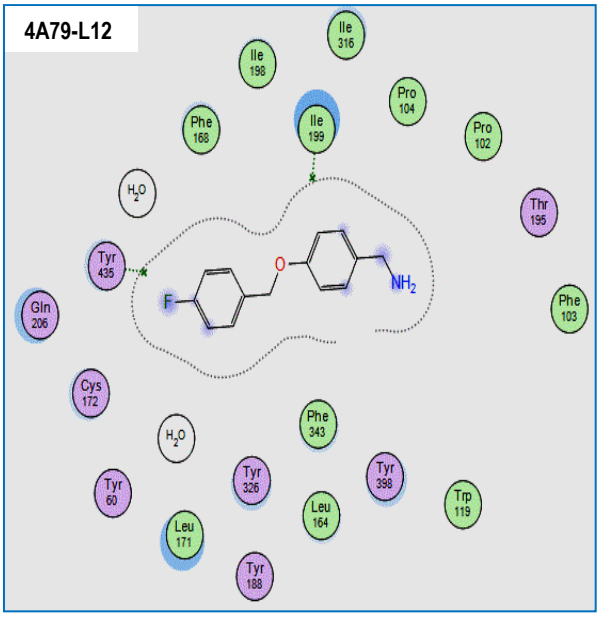

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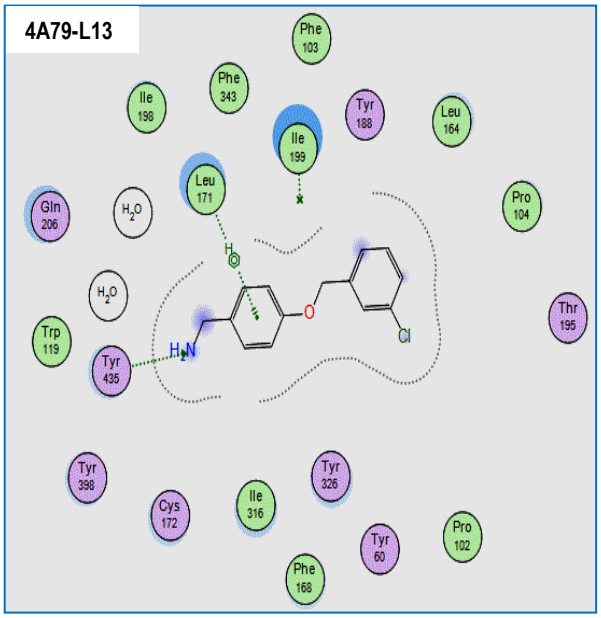

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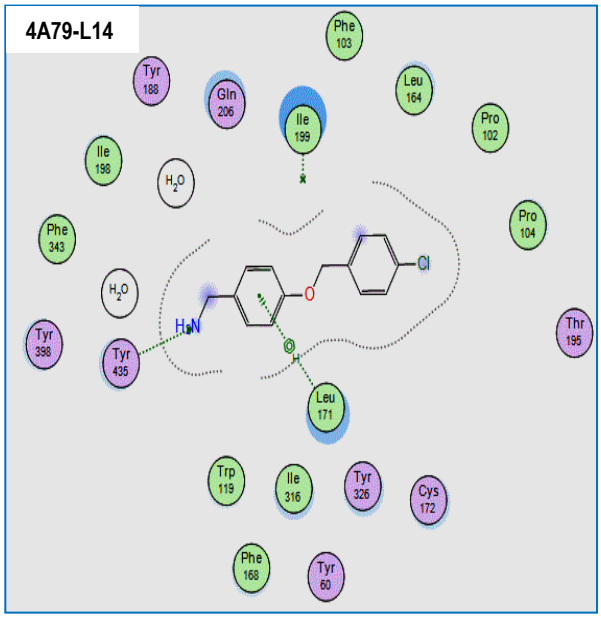

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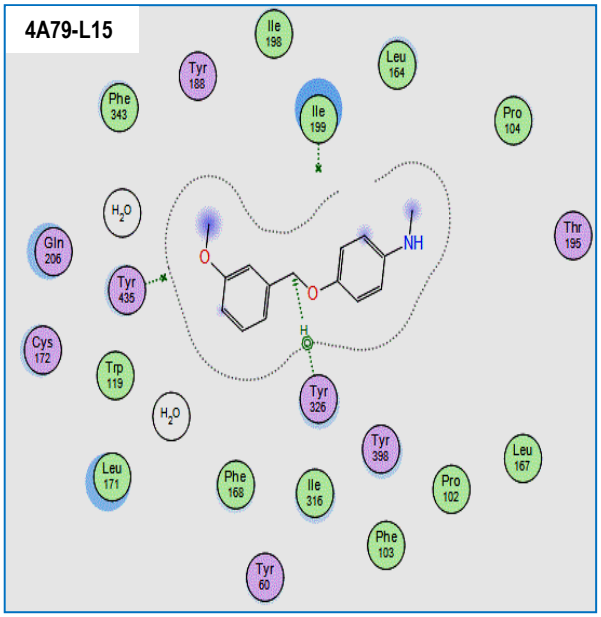

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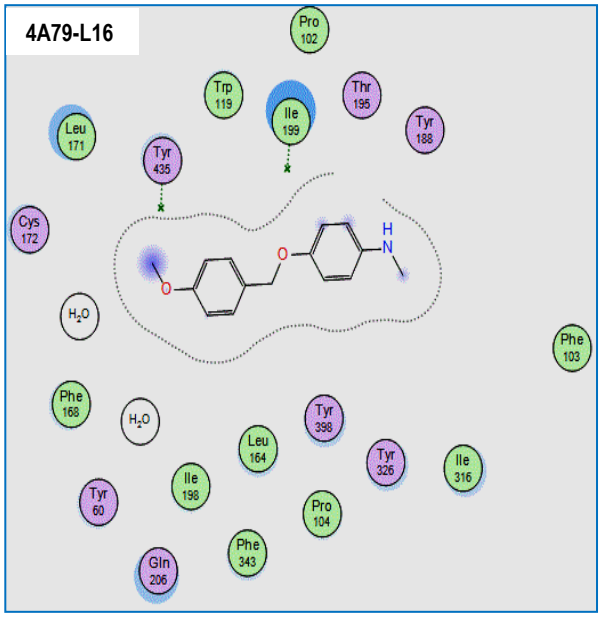

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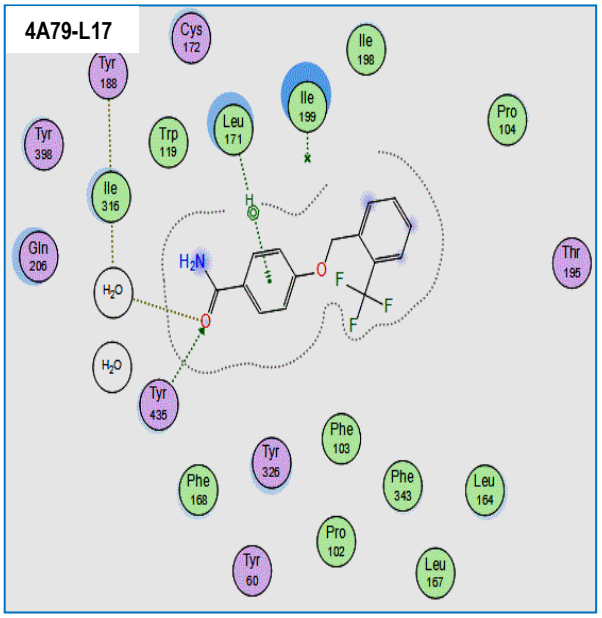

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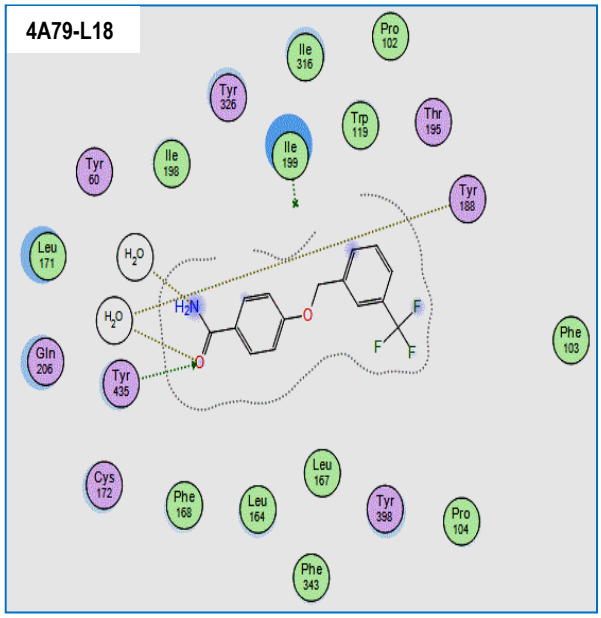

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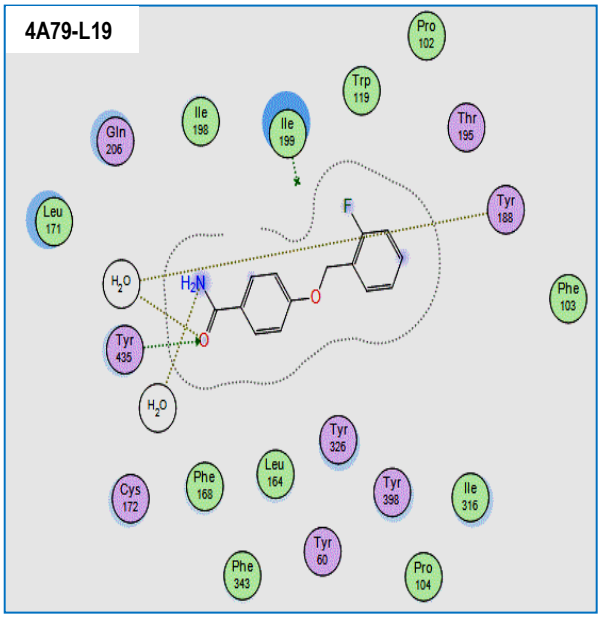

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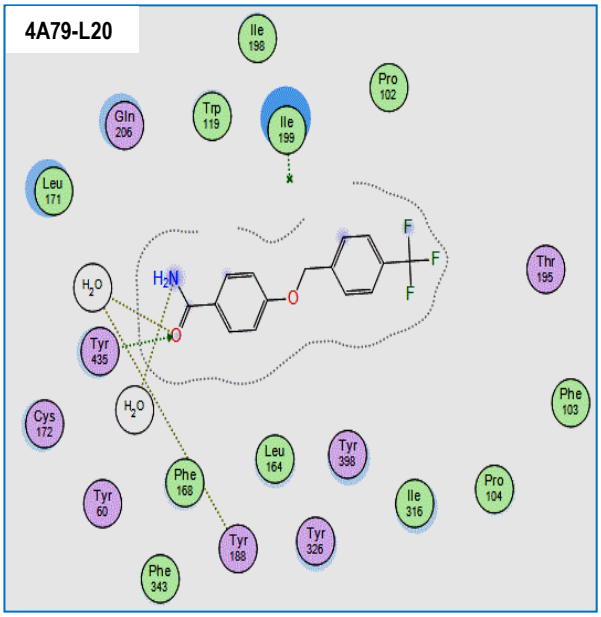

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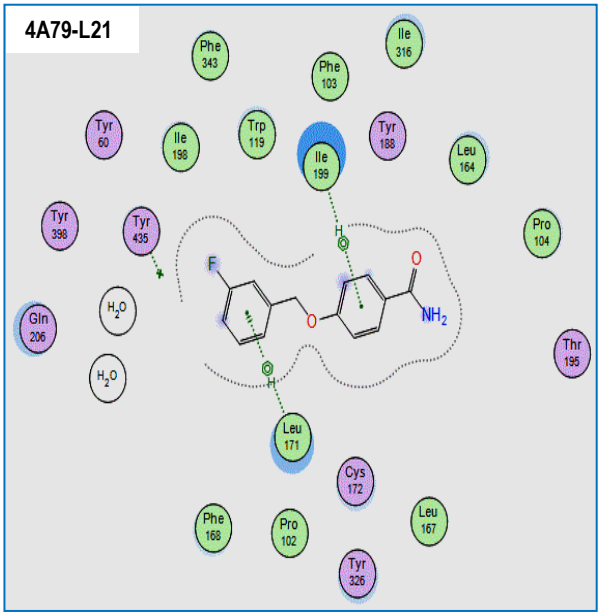

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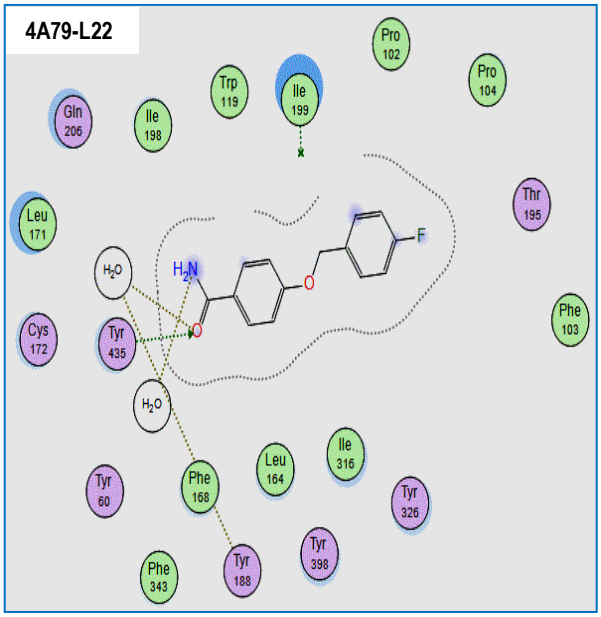

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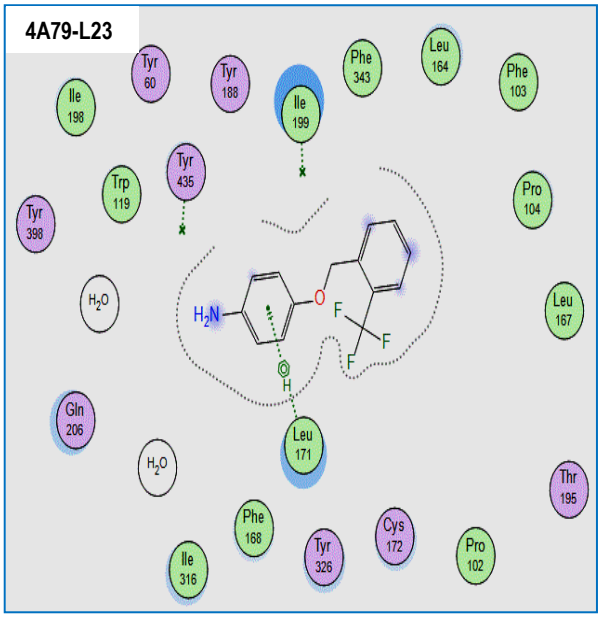

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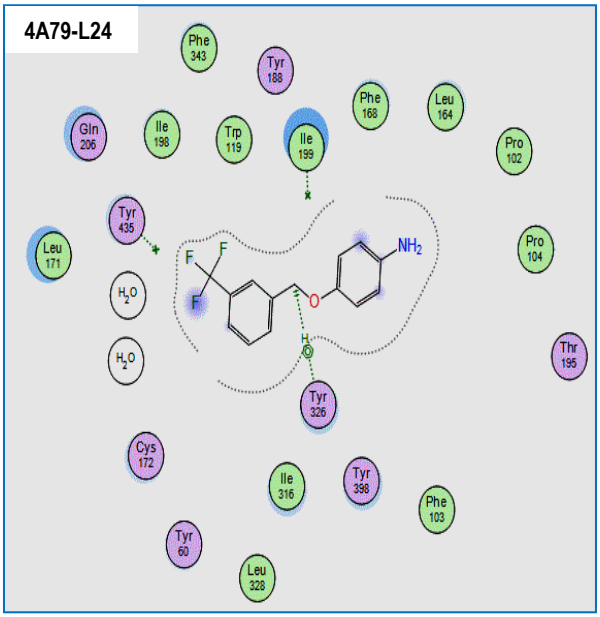

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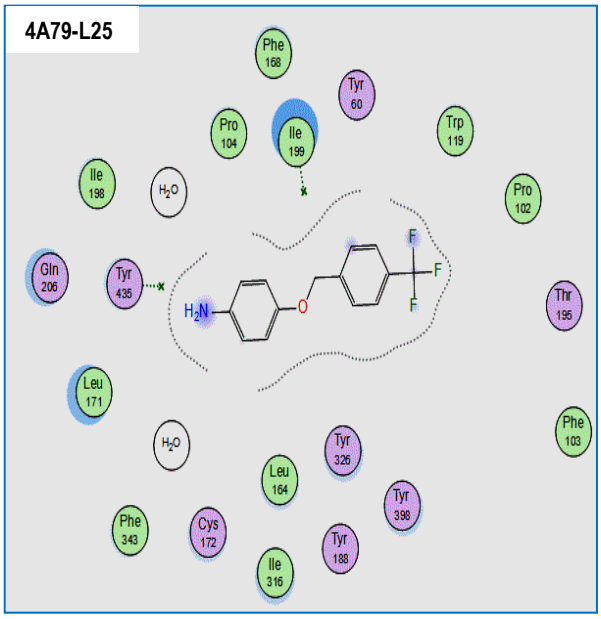

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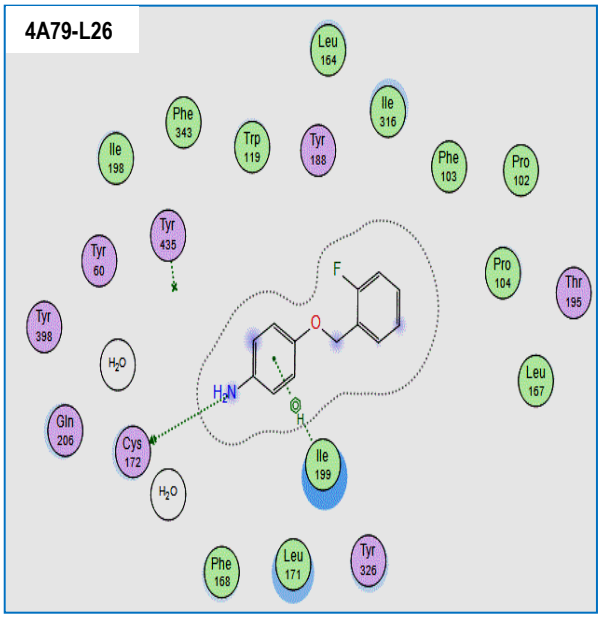

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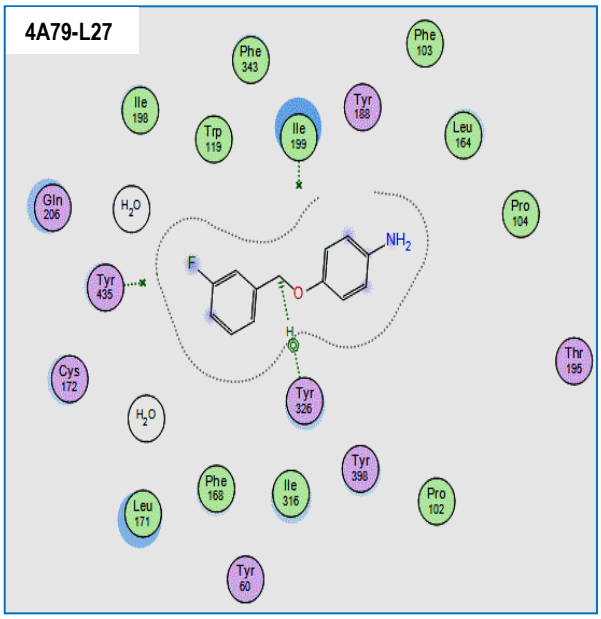

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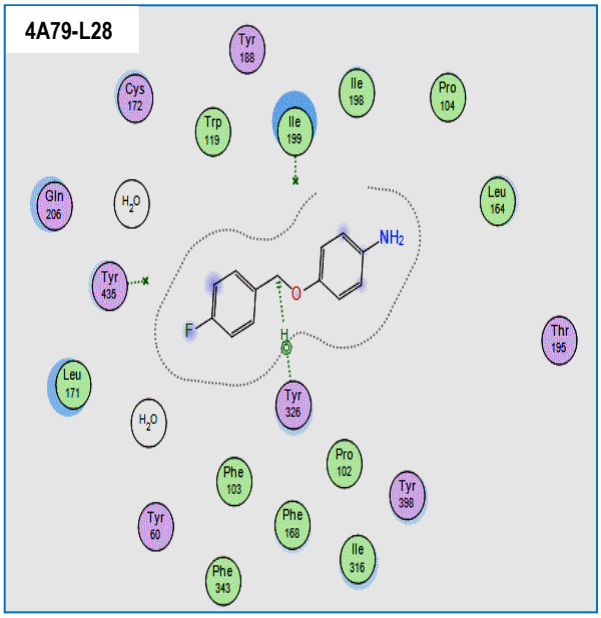

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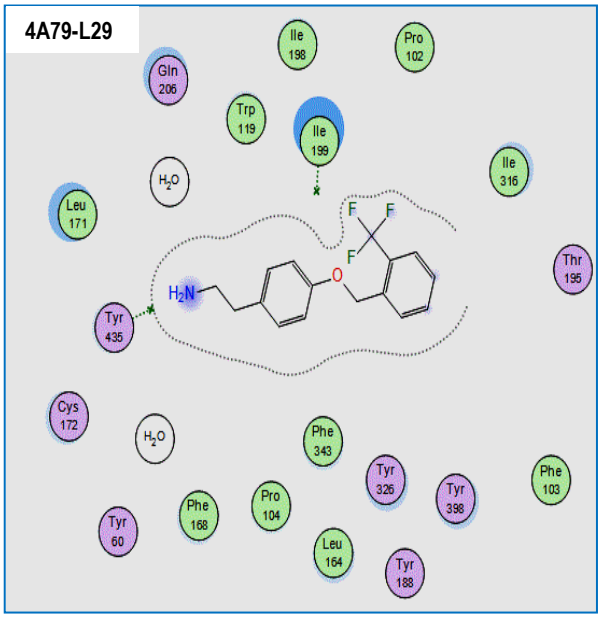

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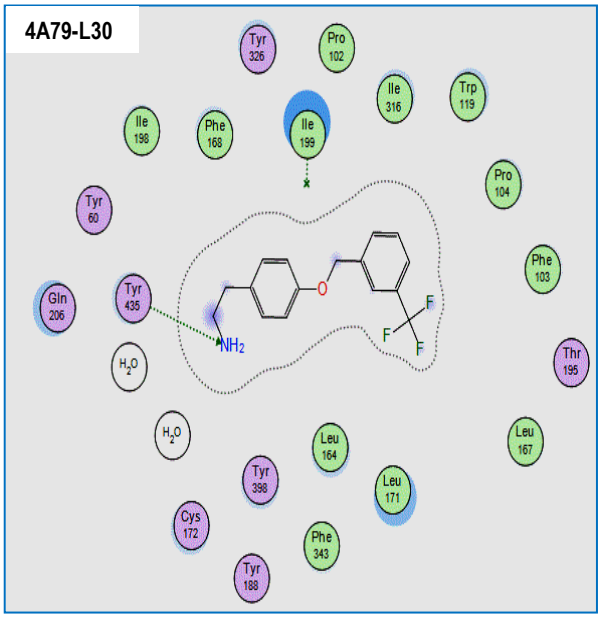

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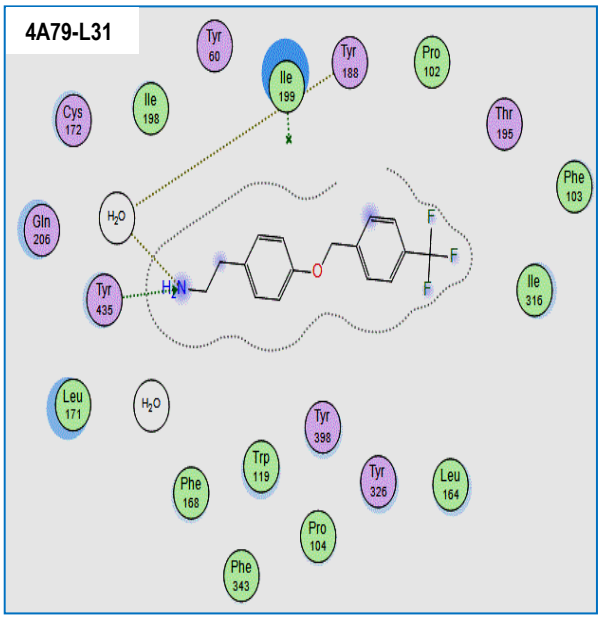

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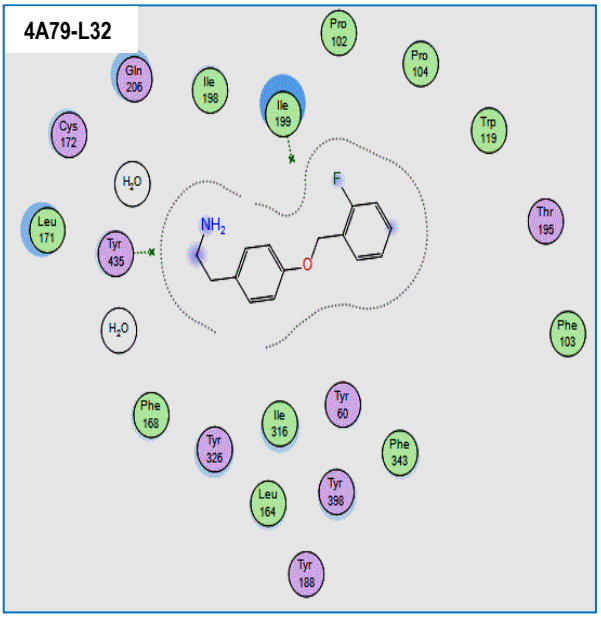

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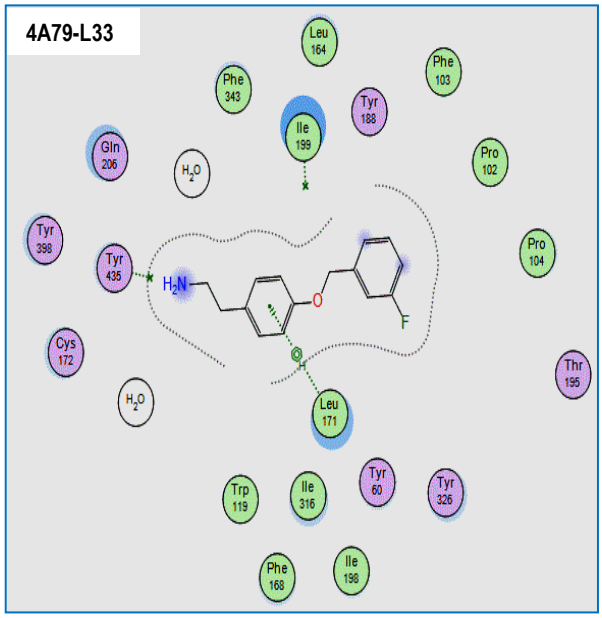

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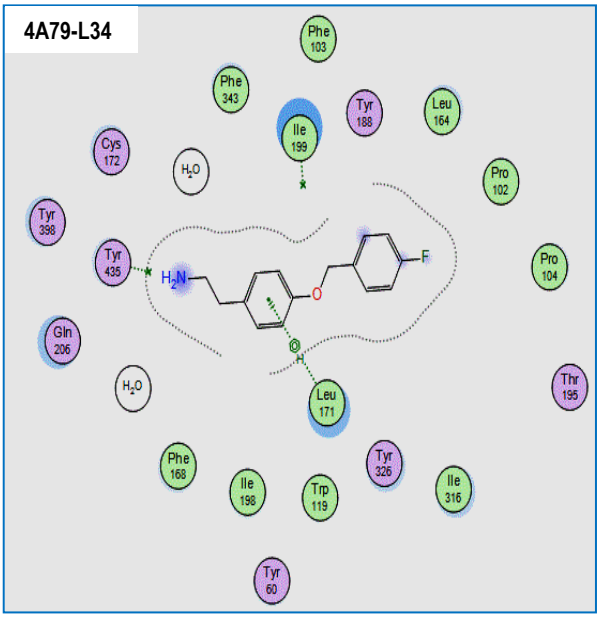

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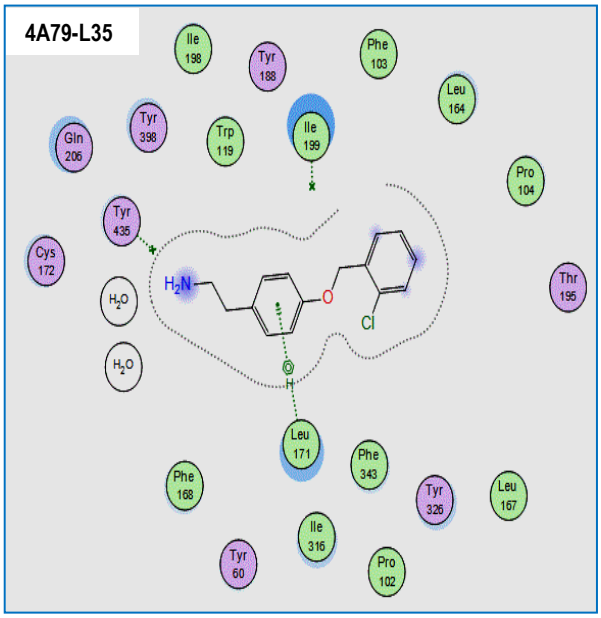

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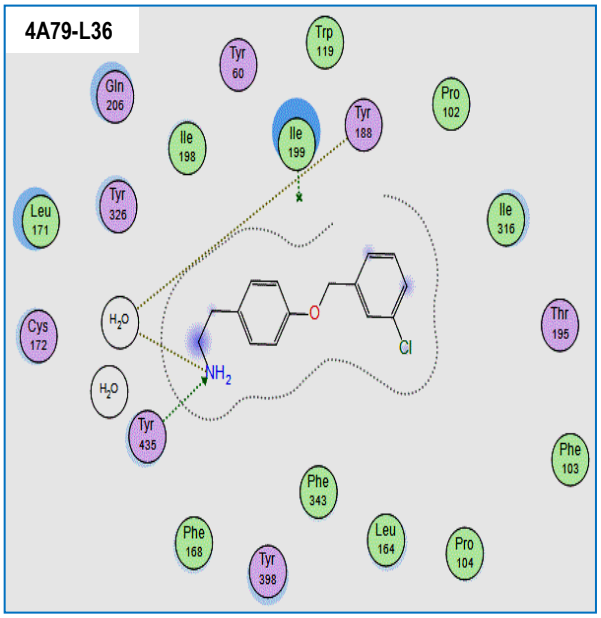

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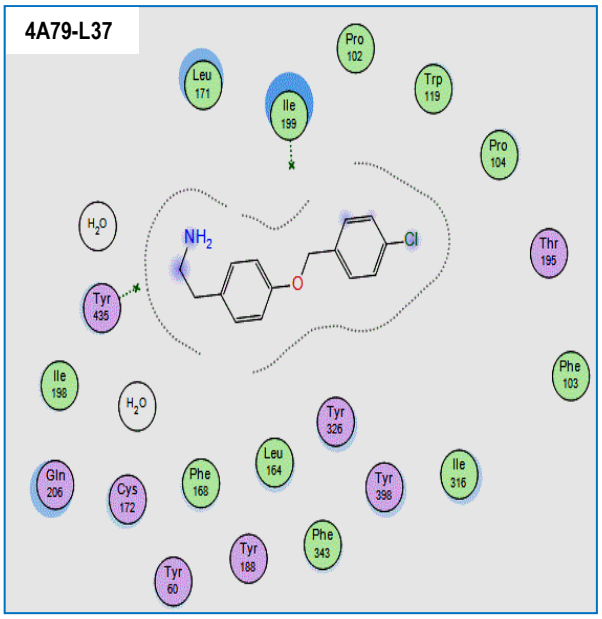

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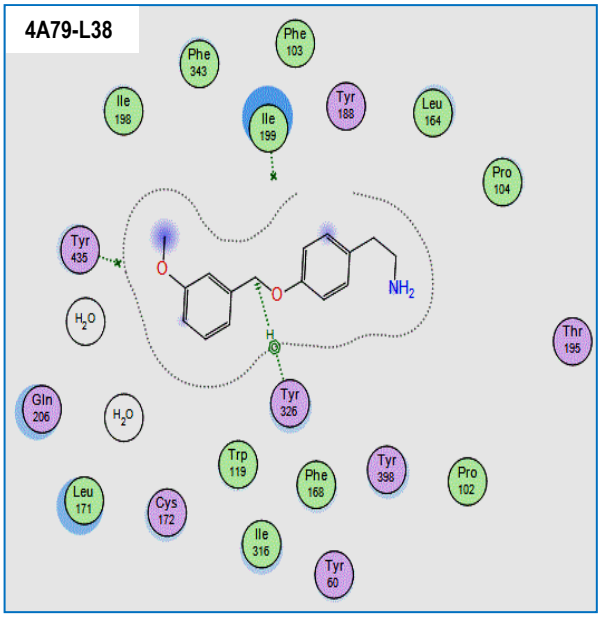

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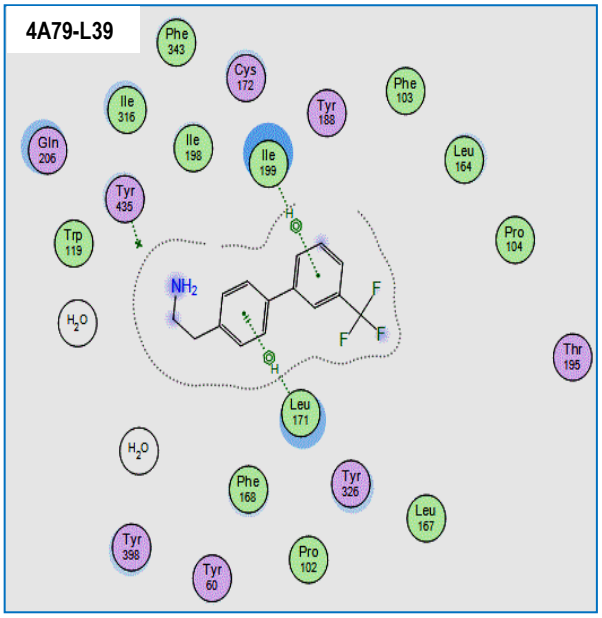

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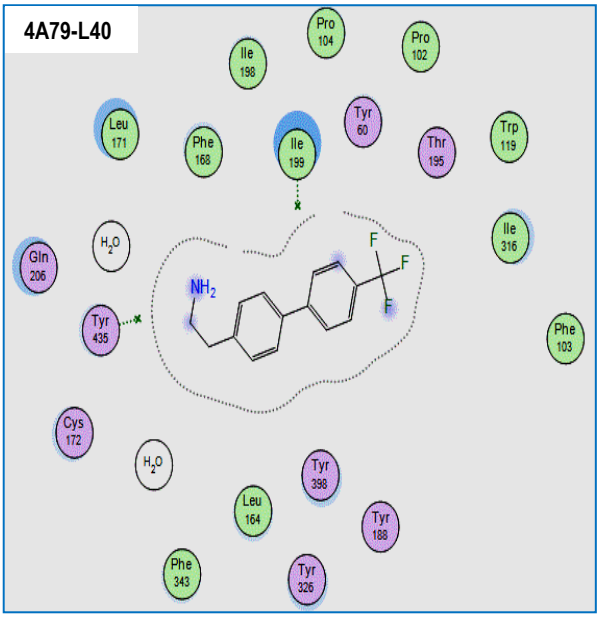

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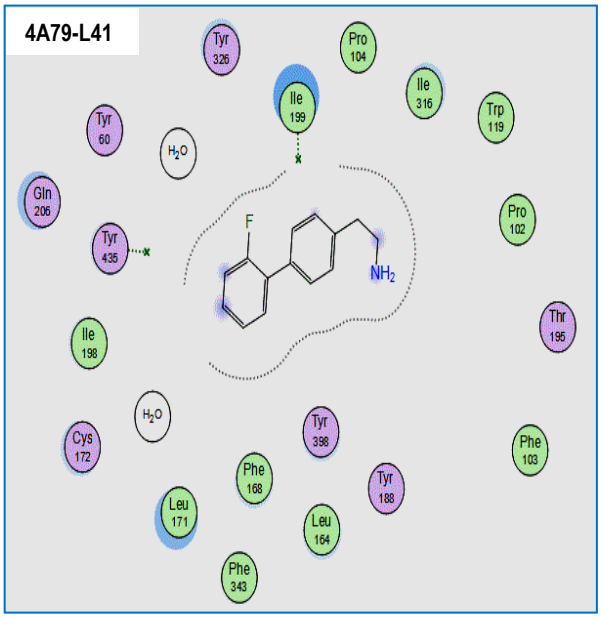

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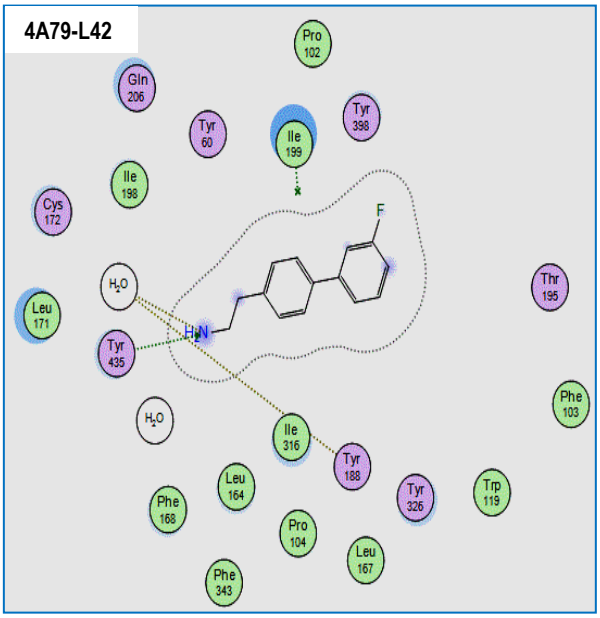
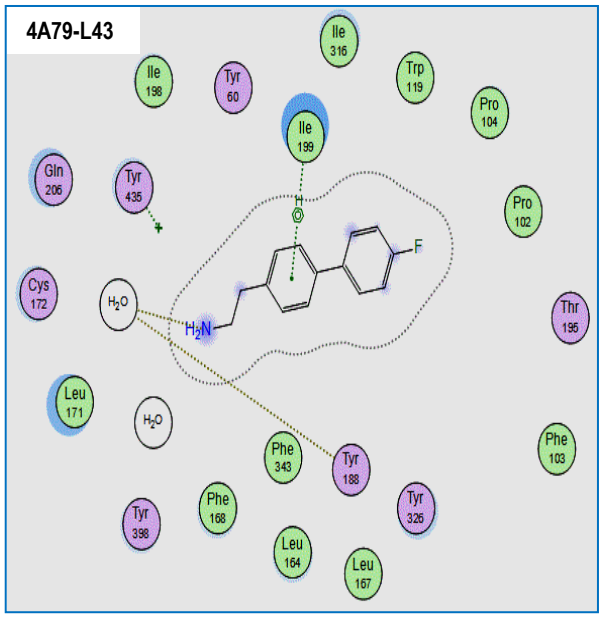
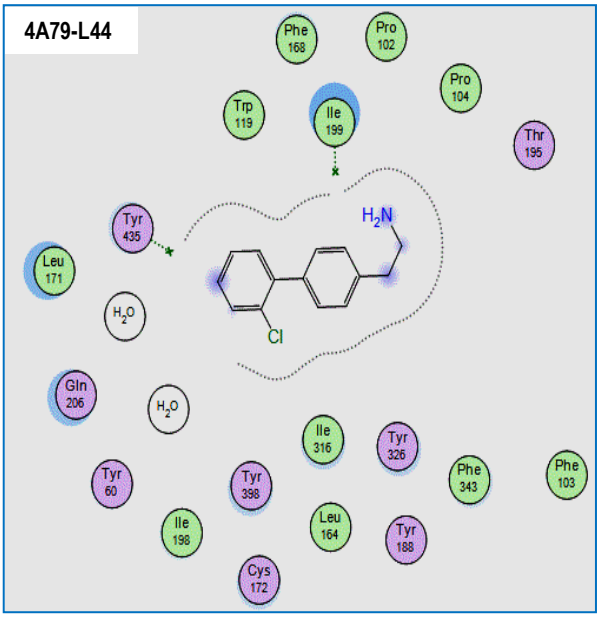
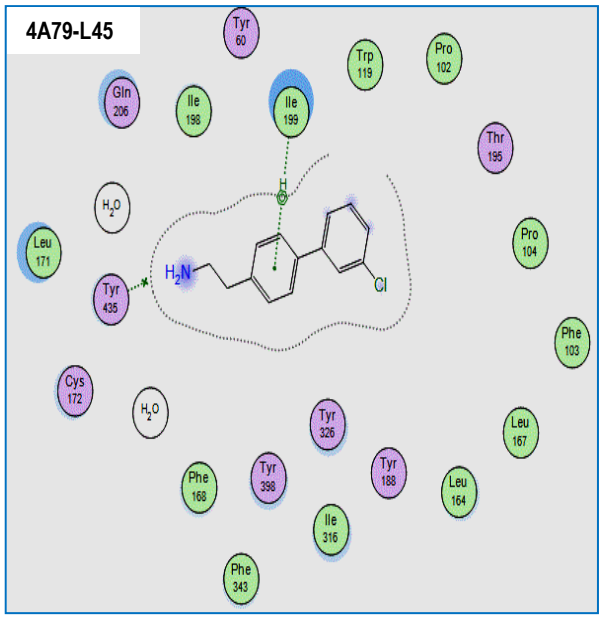



4A79-L43
Ile 316
Ile 198
Tyr 60
Trp 119
Pro 104
Ile 199
Gln 206
Tyr 435
Pro 102
Cys 172
H_2O
H_2N
F
Thr 195
Leu 171
H_2O
Phe 343
Tyr 188
Phe 103
Tyr 326
Phe 168
Tyr 398
Leu 164
Leu 167


4A79-L44
Phe 168
Pro 102
Pro 104
Trp 119
Ile 199
Thr 195
Tyr 435
H_2N
Leu 171
H_2O
Cl
Gln 206
H_2O
Ile 316
Tyr 326
Phe 343
Phe 103
Tyr 60
Tyr 398
Leu 164
Ile 198
Tyr 188
Cys 172


4A79-L45
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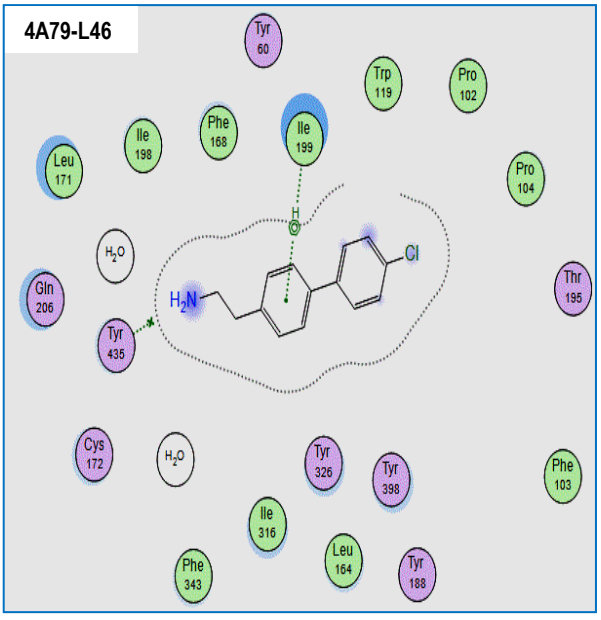
Trp 119
Pro 102
Gln 206
Ile 198
Ile 199
Thr 195
H_2O
Leu 171
H_2N
Cl
Tyr 435
Pro 104
Phe 103
Cys 172
H_2O
Tyr 326
Leu 167
Tyr 188
Phe 168
Tyr 398
Leu 164
Ile 316
Phe 343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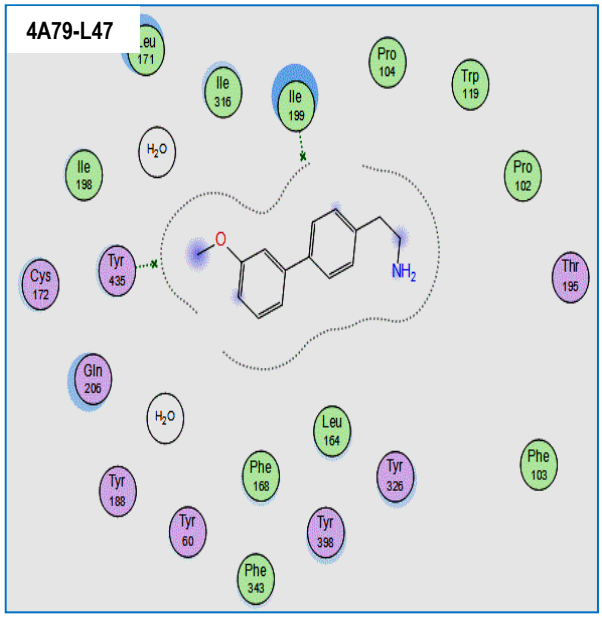
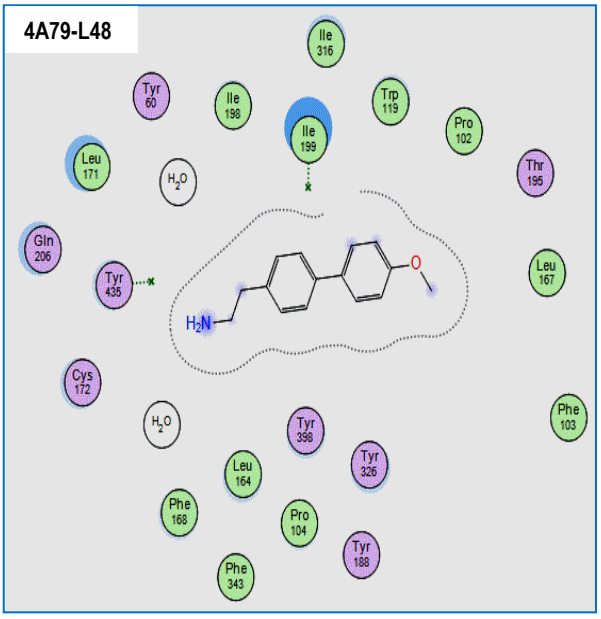
Pro 102
Ile 198
Phe 168
Ile 199
Leu 171
Pro 104
H_2O
Cl
H_2N
Gln 206
Thr 195
Tyr 435
Cys 172
H_2O
Tyr 326
Tyr 398
Phe 103
Ile 316
Leu 164
Phe 343
Tyr 188


4A79-L47
Leu 171
Ile 316
Pro 104
Trp 119
Ile 199
H_2O
Ile 198
Pro 102
O
Tyr 435
NH_2
Cys 172
Thr 195
Gln 206
H_2O
Leu 164
Phe 168
Tyr 326
Phe 103
Tyr 188
Tyr 60
Tyr 398
Phe 343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Tyr 60
Ile 198
Trp 119
Ile 199
Pro 102
Leu 171
H_2O
Thr 195
Gln 206
Tyr 435
O
Leu 167
H_2N
Cys 172
H_2O
Tyr 398
Phe 103
Tyr 326
Leu 164
Phe 168
Pro 104
Tyr 188
Phe 343

Abstract:

Parkinson's disease (PD) is the second most common neurodegenerative disorder, after Alzheimer's disease. Several studies have been conducted to discover new and effective drugs for this rapidly spreading disease, knowing that, to this day, no medicine has been discovered to treat this disease, but there have been attempts to reduce its severity.

In order to study the inhibition of enzymes involved in this disease and to identify novel inhibitors, our work focused to use molecular modeling methods based on molecular docking and molecular dynamics using MOE software, as well as by an estimation of the ADME properties.

Our discussion is based on two parameters: energy score and distances of interactions between active site residues of MAO B and a series of 4-(benzyloxy) phenyl and biphenyl-4-yl derivatives.

After the simulations and according to the comparison between the results of the two previous methods, the compounds L30 and L38 selected to be the best inhibitors of MAO-B and more, both compounds respect the Lipinski, Veber, and Egan rules, they are able to cross the BBB, and they may behave the potential to be used in the development of novel pharmacological agents for the treatment of patients with PD.

Keywords: Parkinson's disease, Molecular Docking, Molecular Dynamic, ADME, Interaction.

Résumé :

La maladie de Parkinson (MP) est le deuxième trouble neuro-dégénératif le plus courant, après la maladie d'Alzheimer. Plusieurs études ont été menées pour découvrir de nouveaux médicaments efficaces contre cette maladie à propagation rapide, sachant qu'à ce jour, aucun médicament n'a été découvert pour traiter cette maladie, mais des tentatives ont été faites pour en réduire la gravité.

Afin d'étudier l'inhibition des enzymes impliquées dans cette maladie et d'identifier de nouveaux inhibiteurs, nos travaux se sont concentrés sur l'utilisation de méthodes de modélisation moléculaire basées sur le docking moléculaire et la dynamique moléculaire à l'aide du logiciel MOE, ainsi que par une estimation des propriétés ADME.

Notre discussion est basée sur deux paramètres : le score énergétique et les distances d'interactions entre les résidus de site actif de MAO-B et une série de dérivés 4-(benzyloxy) phényl et biphényl-4-yle.

Après les simulations et selon la comparaison entre les résultats des deux méthodes précédentes, les composés L30 et L38 sélectionnés pour être les meilleurs inhibiteurs de MAO-B et plus, les deux composés respectent les règles de Lipinski, Veber et Egan, ils sont capables de traversent le BBB, et ils pourraient avoir le potentiel d'être utilisés dans le développement de nouveaux agents pharmacologiques pour le traitement des patients atteints de MP.

Mots clés : Maladie de Parkinson, Docking moléculaire, Dynamique moléculaire, ADME, Interaction.