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Faculty of Exact Sciences and Natural and Life Sciences  
Department of Material Sciences

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Pharmaceutical chemistry

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Presented and supported by:  
Younes Djihene

## Molecular docking studies on small molecule inhibitors targeting Covid-19 receptor

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### Jury :

Mr	Belaidi Salah	M.C.A	Mohamed Kheider University of Biskra	President
Mrs	Harkati Dalal	M.C.A	Mohamed Kheider University of Biskra	Supervisor
Mrs	Zekri Afaf	M.C.A	Mohamed Kheider University of Biskra	Examiner

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Faculté des Sciences Exactes et Sciences de la Nature et de la Vie  
Département des Sciences de la Matière

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Présenté et soutenu par :

Younes Djihene

Le : Juin 2022

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Mme	Harkati Dalal	M.C.A	Université Med Kheider Biskra	Superviseuse
Mme	Zekri Afaf	M.C.A	Université Med Kheider Biskra	Examinatrice

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## Index

Table list .....	
List of figures .....	
List of abbreviations and acronyms.....	
General introduction .....	1
References .....	4
Chapter I : Molecular docking .....	6
1. Introduction .....	7
2. Molecular docking.....	8
3. Types of molecular docking.....	10
4. Applications of molecular docking .....	11
5. Molecular docking Approaches.....	11
6. Mechanism of docking .....	12
7. Available softwares for docking.....	13
8. Theory of molecular docking .....	14
9. Molecular dynamics simulation .....	15
10. Major steps involved in mechanics of molecular docking .....	15
11. Conclusion.....	17
References .....	18
Chapter II: Coronavirus disease (COVID-19) .....	19
1. Introduction .....	20
2. Coronavirus origins .....	21
3. SARS-COV-2 (COVID-19).....	22
4. ACE2 receptor.....	24
5. Therapeutics and Drugs.....	26
6. Conclusion.....	29
References .....	30
Chapter III : Materials and methods .....	34
1. Introduction .....	35
2. Materials and methods .....	36
3. Geometric optimization of different molecules.....	40
4. Molecular docking.....	42
References .....	43

Chapter VI : Results and discussion.....	46
Results and discussion .....	47
1. The binding affinities of four curcumin derivatives into [SARS-CoV- 2/ACE2] complex active sites.....	47
2. Conclusion.....	57
General Conclusion.....	58

## Table list

### Chapter I

Table I-1: Pros and Cons of Docking tools .....	13
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### Chapter III

Table III. 1: Binding sites residues used as input for receptor grid generation during Induced Fit Docking .....	37
Table III. 2: Binding sites residues used as input for receptor grid generation during Induced Fit Docking .....	38

### Chapter VI

Table VI-1: After optimization energy calculated for curcuminoids.....	47
Table VI.2: The results obtained from docking of Curcumin (enolate)(1) with 6M0J in site1 .....	48
Table VI.3 : The results obtained from docking of Curcumin (enol)(2) with 6M0J in site1 .....	49
Table VI-4 : The results obtained from docking of Demetoxycurcumin(3) with 6M0J in site1 .....	49
Table VI-5: The results obtained from docking of Bisdemetoxycurcumin(4) with 6M0J in site1.....	50
Table VI.6. The results obtained from docking of Curcumin (enolate)(1) with 6M0J in site 2.....	52
Table VI.7:The results obtained from docking of Curcumin (enol)(2) with 6M0J in site 2 .....	51
Table VI.8:The results obtained from docking of Demethoxycurcumin(3) with 6M0J in site 2.....	54
Table VI.9: The results obtained from docking of Bisdemethoxycurcumin(4) with 6M0J in site 2.....	55

## List of figures

### Chapter I

Figure I-1: Molecular docking flow chart .....	8
Figure I-2: Molecular Docking process .....	8
Figure I-3: The computer aided drug design and discovery (CADD) procedure .....	9
Figure I-4: Rigid and Flexible Docking .....	10
Figure I-5: Molecular Docking Approach .....	12
Figure I-6: Mechanisms of Docking.....	12
Figure I-7: The induced -Fit theory .....	14

### Chapter II

Figure II-1: The mechanism of SARS-CoV-2 binding on human host cells.....	21
Figure II-2: Potential transmission routes for SARS-CoV .....	21
Figure II-3 SARS-CoV-2 virus structure.....	23
Figure II-4: Binding of the S-protein receptor (RBD) with the angiotensin-converting enzyme 2 (ACE2) receptor. ....	24
Figure II-5: A. Schematic representation of the regulation of ACE2. ....	25
Figure II-5: B. Schematic representation (left) of the ACE2.....	26
Figure II-6 Structure of (a) Chloroquine and (b) Hydroxychloroquine .....	27
Figure II-7 Intracellular activation of remdesivir (GS-5734) and inhibition of coronavirus replication. ....	28

### Chapter III

Figure III-1 Schematic representation of the docking procedure, analysis of drugs and reactivity .....	35
Figure III-2: Crystal structure of [SARS-CoV-2/ACE2] complex (PDB ID: 6M0J). ....	36
Figure III-3: isolated active site 1 of Sars-coronavirus-2 related with ACE2 enzyme Pdb ID: 6m0j.....	37
Figure III-4: isolated active site 2 of Sars-coronavirus-2 related with ACE2 enzyme Pdb ID: 6m0j.....	38
Figure III.5: 2D structures of Curcumin (enolate) (1) .....	39
Figure III.6: 2D structures of Curcumin (enol) (2).....	39
Figure III.7: 2D structures of Demetoxycurcumin (3).....	39
Figure III.8: 2D structures of Bisdemetoxycurcumin (4) .....	40
Figure III.9: Curcumin structure after optimization (enolate) .....	40

Figure III.10: Curcumin structure after optimization (enol).....	41
Figure III.11: Demetoxycurcumin structure after optimization.....	41
Figure III.12: Bisdemetoxycurcumin structure after optimization .....	42

### **Chapter VI**

Figure VI-1: 2D diagram interaction between Curcumin (enolate) (1) and site 1 of 6M0J	48
Figure VI-2: 2D diagram interaction between Curcumin (enol) (2) and site 1 of 6M0J .....	49
Figure VI-3: 2D diagram interaction between Demetoxycurcumin (3) and site 1 of 6M0J	50
Figure VI.4: 2D diagram interaction between Bisdemetoxycurcumin (4) and site 1 of 6M0J .....	51
Figure VI.5: 2D diagram interaction between Curcumin (enolate) (1) and site 2 of 6M0J	52
Figure VI.6: 2D diagram interaction between Curcumin (enol) (2) and site 2 of 6M0J.....	53
Figure VI.7: 2D diagram interaction between Demethoxycurcumin (3) and site 2 of 6M0J .....	54
Figure VI.8: 2D diagram interaction between Bisdemethoxycurcumin (4) and site 2 of 6M0J .....	55

## List of abbreviations and acronyms

### A

**Å:** Angstrom

**ACE2:** Angiotensin converting enzyme 2

**ADMET:** Absorption, Distribution, Metabolism, Excretion and Toxicity

**ALA:** alanine

**AM1:** Austin Model 1

**ARDS:** acute respiratory distress syndrome

**ARG :** Arginine

**ASN :** Asparagine

**ASP:** Aspartic

### C

**CADD: :** computer aided drug design

**CADDD:** computer aided drug design and discovery

**COPD:** Chronic obstructive pulmonary disease

**CoV:** Coronavirus

**CVDs:** Cardiovascular disease

**CT:** computed tomography

**CYS :** cysteine

### D

**DFT:** Density functional theory

**DNA:** Deoxyribonucleic acids

**DoF:** degrees of freedom

**2D:** 2 Dimensional

**3D:** 3 Dimensional

### E

**E:** Energy



**EVD:** Ebola virus disease

## **G**

**GLU:** Glutamate or acid glutamique

**GLN:** Glutamine

**Gly:** Glycine

## **H**

**HIS:** Histidine

## **I**

**ICU:** Intensive Care Unit

**ILE:** Isoleucine

## **L**

**Leu:** leucine

## **M**

**MET:** Méthionine

**MOE:** Molecular operating environment

## **N**

**NMR:** Nuclear magnetic resonance

## **P**

**PHE:** phenylalanine

**PD:** peptidase domain

**PDB:** protein data bank

**PRO:** proline

## **R**

**RAAS:** renin-angioten-aldosterone system

**RBD:** receptor-binding domain

**RMS:** root mean square

**RMSD:** Root mean square deviation

**RNA:** Ribonucleic acid

## **S**

**S:** score

**SER:** Serine

**SBDD:** structure-based drug design

**SBVS:** structure based virtual screening

**SSCP:** System Services Control Point

**S1:** site 1

**S2:** site 2

## **T**

**THR:** Threonine

**TYR:** Tyrosine

## **V**

**VdW:** Van der Waals

## **W**

**WHO:** World Health Organization

## **Z**

**ZN:** zinc

*General introduction*

Molecular Docking is a structure based virtual screening (SBVS) that is used to place the computer generated three dimensional Structures of small molecules into a target structure in a variety of positions, conformations and orientations. Protein-ligand docking is a new concept with a variety of applications. It acts as a vivacious explore domain because of its significance to structure based drug design (SBDD), Lead Optimization, Evaluation of Biochemical pathways and in De Novo drug design. Through Molecular Docking the Binding mode and affinity of the complex so formed is estimated and this helps in the Molecular Recognition Process docking towards discovery of new drug leads. Molecular docking is an inexpensive, safe and easy to use tool, helps in investigating, interpreting, explaining and identification of molecular properties using three-dimensional structures.

Computer aided drug design facilitate the use of structural knowledge of both the target (structure-based) and bioactivity ligands (ligand-based) by determining promising candidate drugs. Over the last 20 years methods for molecular docking have been improved, yielding accurate results on pose prediction.

The field of computer aided drug design and discovery (CADD) is a rapidly growing area that has seen many successes in the last few years. Many giant pharmaceutical companies and academia adopt CADD for drug lead discovery. The explosion of structural informatics, genomics and proteomic plays a major role in leading the efforts towards modern era drug discovery and development.

Corona virus causes serve acute respiratory syndrome, this virus has so far infected more than twenty thousand people, mainly in China. Coronavirus is interspecific, it transmitted from person to person, the incubation period ranges from one to 14 days. Human coronavirus infection can cause not only mild to severe respiratory illness, but also inflammation, high fever, cough, acute respiratory infection, and dysfunction of internal organs that can lead to death. The virus are located in the submucosal layer of the respiratory tract and in the nasal cavity which represent a barrier protect against microorganisms. Human CoVs, COVID-19 shows less severe pathogenesis but higher transmission competence, Compared to other emerging viruses, such as Ebola virus, avian H7N9, SARS-CoV, and Middle East respiratory syndrome coronavirus (MERS-CoV), Codon usage studies suggest that this novel virus has been transferred from an animal source, such as bats. Early diagnosis by real-time PCR and next generation sequencing has facilitated the identification of the pathogen at an early stage. Since no antiviral drug or vaccine exists to treat or prevent SARS- CoV-2, potential therapeutic strategies that are currently being evaluated pre-dominantly stem from previous experience with treating SARS-CoV, MERS-CoV and other emerging viral diseases. The virus spread rapidly around the world and was declared a pandemic by the World Health Organization (WHO) with many countries adopting unprecedented public health measures to curb its spread

Social distancing policies have been widely adopted in many countries to limit spread of SARS-CoV-2 at great economic and social cost. Social-distancing policies that apply to children such as school closures may have an important role in mitigating the spread of pandemics; for many infectious diseases, such as influenza, children are known to drive

transmission in households and communities. However, early reports of SARS-CoV-2, as well as MERS-CoV and SARS-CoV, suggest that children are less likely to be infected and to develop serious disease compared with adults.

The rapid spread of SARS coronavirus 2 (SARS-CoV-2) demands an immediate public health emergency, and are currently available. SARS-CoV-2 Spike (S) protein (1267 amino acids) is essential for virus entry through binding with the host receptor angiotensin converting enzyme II (ACE2) and mediating virus-host membrane fusion. The S protein contains two functional domains (S1 and S2). The S1 (residues 14-685) domain performs the function of virion attachment with human ACE2 receptor on epithelial membrane cell surface, followed by its internalization, hence initiating the infection. This binding induces certain conformational changes in the S protein, which results the S2 (residue 686-1273) to mediate fusion with cellular membrane. The receptor binding domain (RBD) of the SARS-CoV-2 S protein are highly conserved and directly involve in binding to human ACE2. Since, ACE2 is not mutated/evolved to recognize S protein of SARS-CoV-2; therefore, using alternative of ACE2 with more binding affinity for S protein than the wild type receptor, may inhibit entry of SARS-CoV-1& 2 into human cells. This strategy can play important role in devising therapeutics of SARS-CoV-2. Several studies have proposed small compounds based inhibitors as therapeutic agents for Covid-19

In a recent study, it was suggested that the 2019-nCoV binds to the human ACE2 receptor via densely glycosylated spike (S) protein as the initiation step of the entry mechanism to human cells. The entry of the virus depends on its binding with the cell surface units at site 1 and site 2 S1/S2 that contains Zn<sup>2+</sup>, an important cofactor for numerous viral proteins as well. Existence of this metallic ion facilitates the viral attachment to the surface of target cells. It is well known that zinc ions serve as intracellular second messenger and may trigger apoptosis or efficiently impair replication of a number of viruses and this effect may be based on direct inhibition.

Recently, chloroquine, a medication used primarily to treat malaria, is being studied to treat coronavirus. Its putative anti-viral effects have been hypothesized to be related to the elevation of endosomal and lysosomal pH in addition to its angiotensin converting enzyme 2 inhibitory potentials

The binding of SARS-CoV (CoV) spike protein (S-Protein) Receptor Binding Domain (RBD) to Angiotensin converting enzyme 2 (ACE2) receptor initiates the entry of corona virus into the host cells leading to the infection. The nCoV RBD was found binding to ACE2 receptor with 11 hydrogen bonds and 1 salt bridge. The major hot spot amino acids involved in the binding identified by interaction analysis after simulations includes Glu 35, Tyr 83, Asp 38, Lys 31, Glu 37, His 34 amino acid residues of ACE2 receptor and Gln 493, Gln 498, Asn 487, Tyr 505 and Lys 417 residues in nCoV S-protein RBD. Based on the hydrogen bonding, RMSD and RMSF, total and potential energies, the nCoV was found binding to ACE2 receptor with higher stability and rigidity.

There is growing interest in peptide based therapeutics for Covid-19 treatment and approximately 140 peptide based drugs have been evaluated in clinical trials. Peptide based drugs have little side effects and little drug tolerance compared with chemical drugs.

In this study, molecular docking was applied for four curcumin derivatives, the potential inhibitors to angiotensin converting enzyme (ACE2). Those curcumin derivatives were selected from literature. The molecular docking was performed for [SARS-CoV-2/ACE2] complex receptor in two active sites to find a ligand, which may inhibit COVID-19 . [1-16].

The work of this thesis is presented in four chapters:

✓ **The first chapter:** Includes most of the key information and different molecular docking methods.

✓ **The second chapter:** Informations about the Coronavirus pandemic and the spread of this phenomenon in the world and how to limit its spread.

✓ **In the third chapter:** Materiels and methods then we applied molecular docking and interaction of fourcurcumin derivatives, potential inhibitors of angiotensin converting enzyme (ACE2).

✓ **The fourth chapter:** We have analyzed and discussed the results obtained.

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# Chapter 1 : Molecular docking

## 1. Introduction

Molecular docking is a computational method used to predict the interaction of two molecules generating a binding model. In many drug discovery applications, docking is done between a small molecule and a macromolecule for example, protein-ligand docking. Molecular docking of small molecules to a biological target includes imaginative sampling of possible poses of the ligand in the specified groove or pocket of target candidate in an order to establish the optimal binding geometry. This can be performed using user defined fitness or scoring function of docking software. Docking is frequently used to predict the binding orientation of 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 the rational design of drugs. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand so that the free energy of the overall system is minimized.

Computer-aided have been widely used in pharmaceutical research to improve the efficiency of the drug discovery and development. To identify and design small molecules as clinically effective therapeutics, various computational methods have been evaluated as promising strategies, depending on the purpose and systems of interest. Both ligand and structure-based drug design approaches are powerful technologies, which can be applied to virtual screening for lead identification and optimization.

Computer Aided Drug Discovery entails:

- A. Advantage of chemical and biological information about ligands and/or targets to discover and optimize novel drugs.
- B. Designing of in-silico filters to get rid of chemical compound with unwanted properties (poor activity and/ or poor Absorption, Distribution, Metabolism, Excretion and Toxicity, (ADMET)) and select the most promising candidates.
- C. Identification of novel drug targets and retrieval through database of target protein structures like the protein data bank (PDB) [www.pdb.org](http://www.pdb.org). CADD (Figure 2) is being used to discover hits (drug candidates).
- D. Virtual screening is applied to find out novel drug candidates from various chemical scaffolds by exploring databases.
- E. Use of computational ability to streamline drug discovery and development process (Figure 3).

The process of discovery of a new drug is a very difficult task. Modern drug discovery is mainly based In-silico–chemico biological approach. Use of computer aided techniques in drug discovery and development process is rapidly gaining popularity, implementation and appreciation ( Figure 1) [1,2,3,4,5].

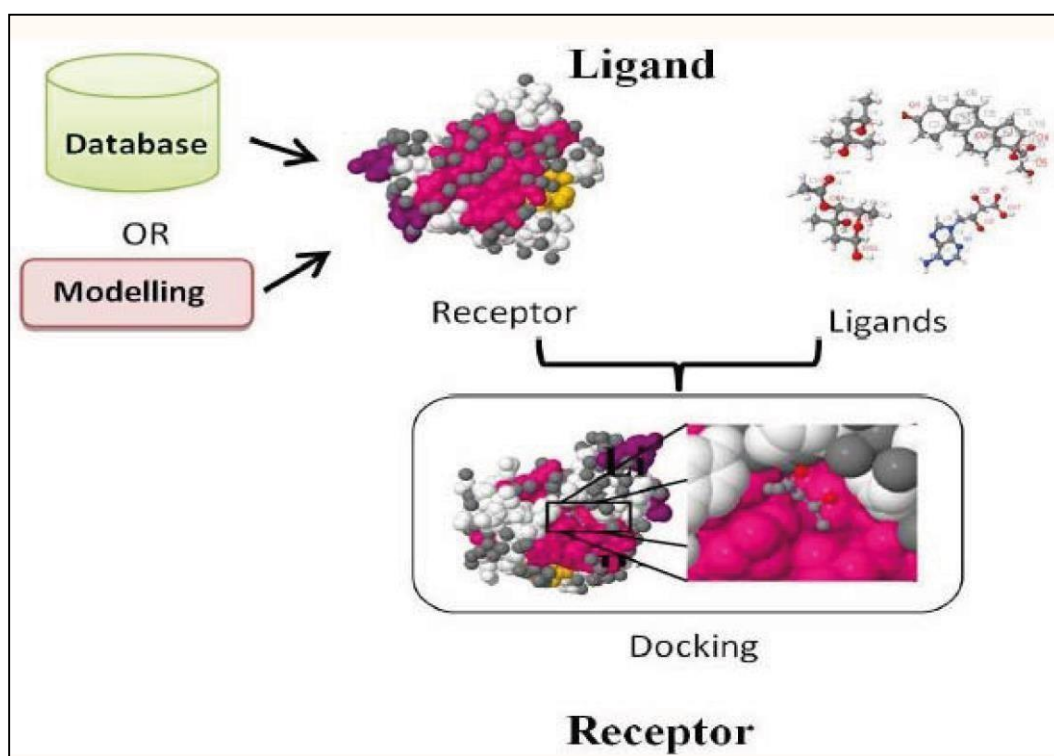


Figure I.1: Molecular docking flow chart.

## 2. Molecular docking

Molecular docking is a kind of computational modeling, which facilitates the prediction of preferred binding orientation of one molecule (ex. **ligand**) to another (ex. **Receptor**), when both interact each other to form a stable complex. 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) [1,2,4].

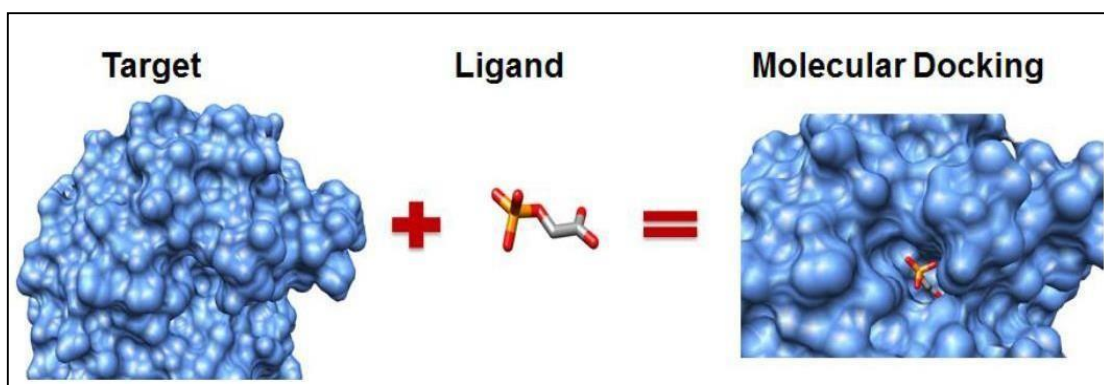


Figure I.2: Molecular Docking process

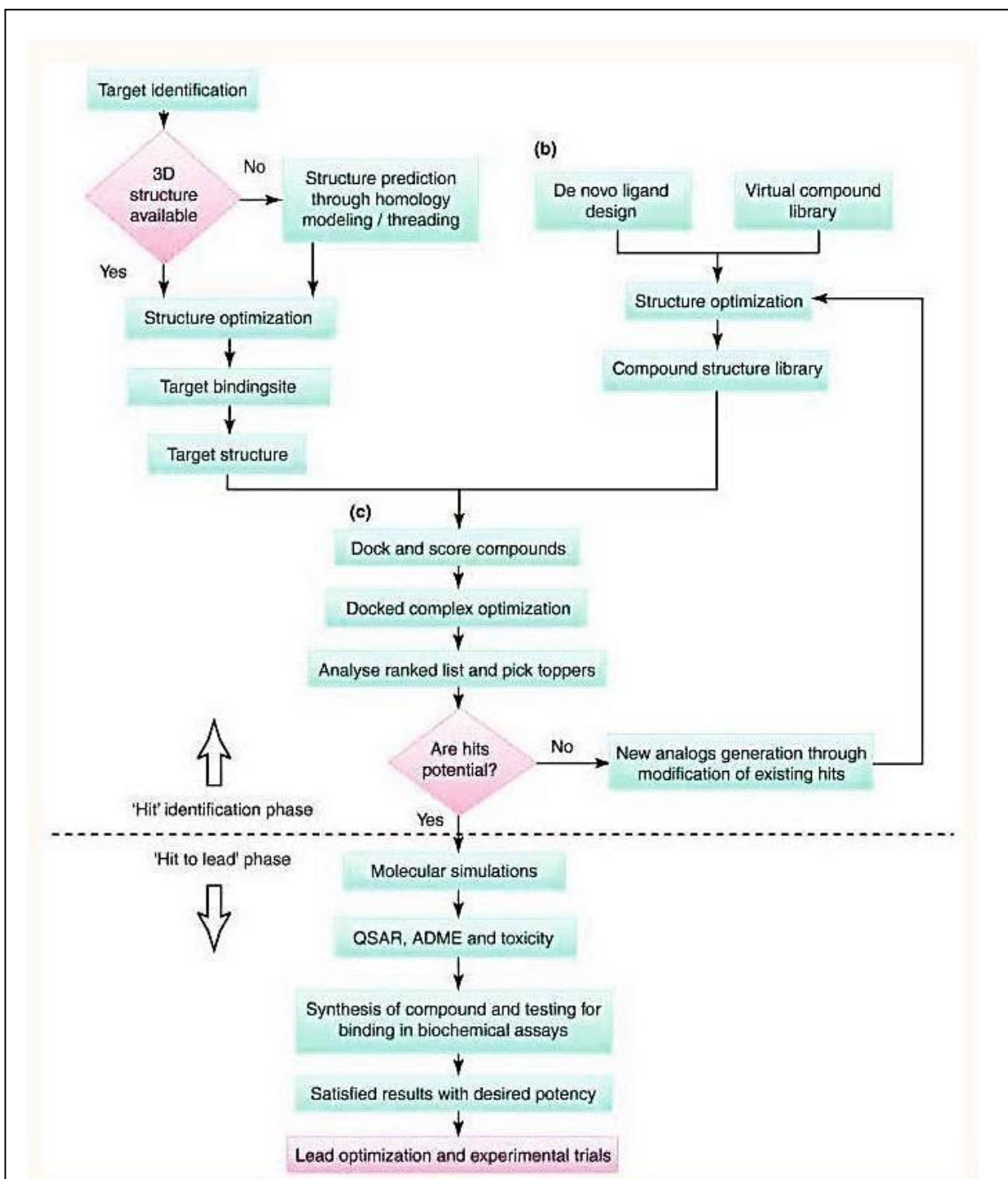


Figure I.3: The computer aided drug design and discovery (CADD) procedure.

### 3. Types of molecular docking

There are three main docking simulations types, depending on considered degrees of freedom (DoF):

- Rigid docking (target and ligand are considered rigid):

If we assume that the molecules are rigid, then we are looking for a transformation in 3D space of one of the molecules which brings it to an optimal fit with the other molecules in terms of a scoring function.

- Semi-flexible docking (flexible ligand and rigid target)
- Flexible docking (flexible ligand and partly flexible target):

We consider molecule as flexible then the transformation, our aim to find the confirmations of the receptor and the ligand molecules, as they appear in complex (figure 4) [3, 4, 5].

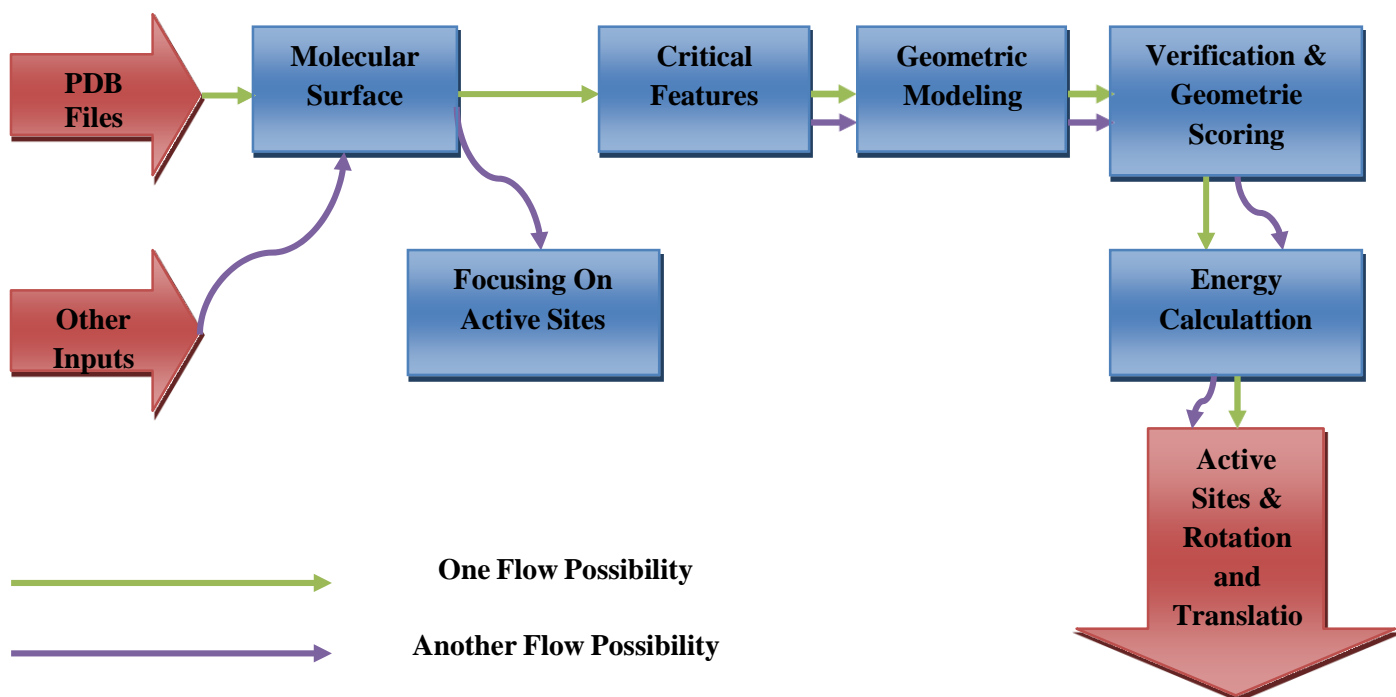


Figure I.4: Rigid and Flexible Docking

## 4. Applications of molecular docking

There are three main applications to the docking:

**a-Hit identification:** Molecular docking in combination with scoring function can be used to screen huge databases for finding out potent drug candidates in silico, which can target the molecule of interest.

**b-Lead optimization:** Molecular docking can predict an optimized orientation of small molecule or ligand on its target. It can predict different binding modes of ligand in the groove of target molecule. Knowledge gained from such type of investigations may be employed to develop more potent, selective and efficient analogs.

**c-Bioremediation:** Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes.

- Identification of target site.
- Selection of best drug (based on scoring function).
- Enzymes and its mechanisms
- Protein interactions
- Virtual Screening of compounds.[2,4,6]

## 5. Molecular docking Approaches

There are number of approaches exist for docking as follows: (figure 5)

**a-Monte Carlo Approach:** It generates an initial configuration of a ligand in an active site consisting of random conformation, translation & rotation.

**b-Distance Geometry:** Many types of structural information can be expressed as intra or intermolecular distances.

**c-Matching approach:** These approach focus on complimentarity. Ligand atom is placed at the “best” position in the site, generating a ligand receptor configuration that may require optimization.

**d-Inverse Docking:** In this use of a computer method for finding toxicity & side effect protein targets of a small molecule. [4,8]

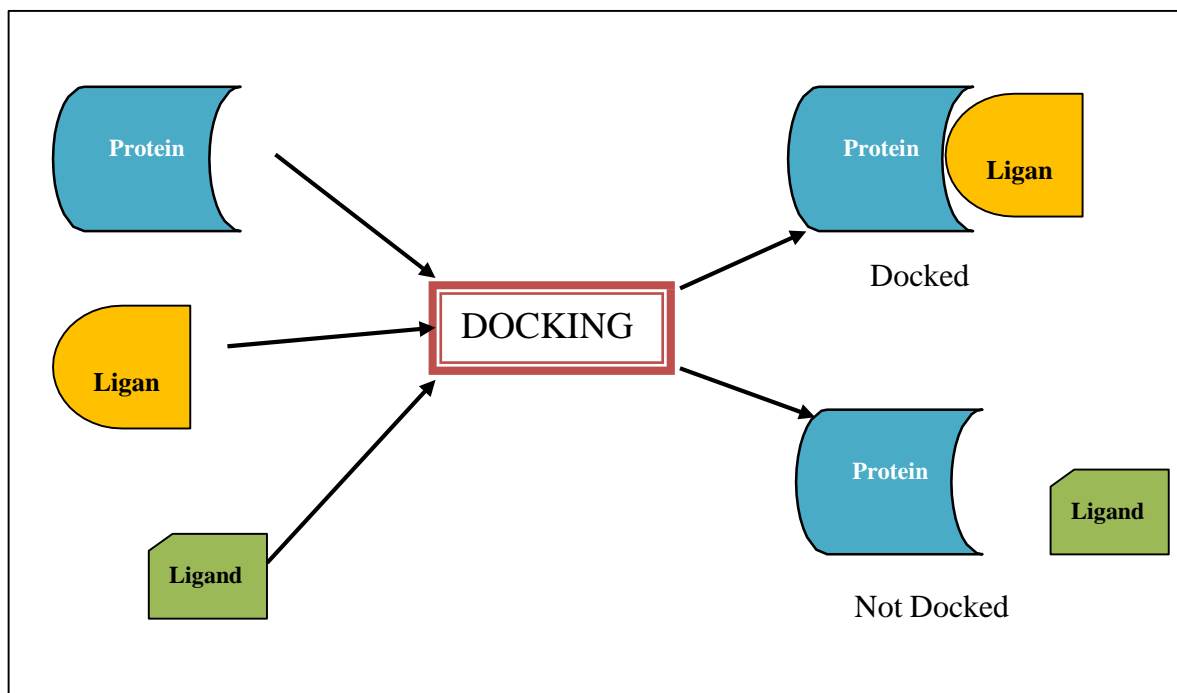


Figure I.5: Molecular Docking Approach

## 6. Mechanism of docking:

To perform a docking screen, the first requirement is a structure of the protein of interest. Usually the structure has been determined using a biophysical technique such as x-ray crystallography, or less often, NMR spectroscopy. This protein structure and a database of ligands serve as inputs to a docking program. The success of a docking program depends on two components such as search algorithm and scoring function. Searching Conformational Space The search space consists of all possible orientations and conformations of the protein paired with ligand.in (figure 6) [4,7,9].

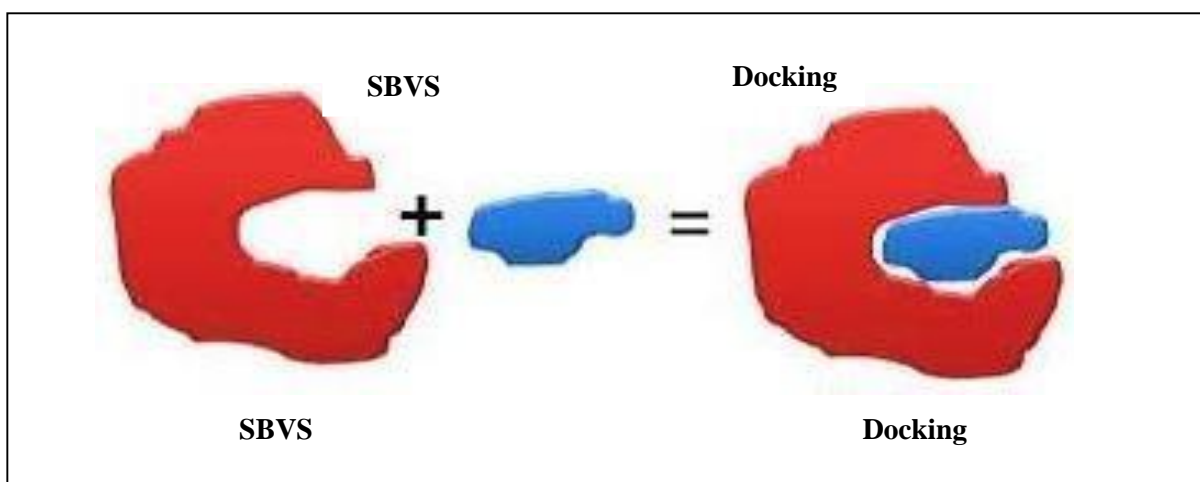


Figure I.6: Mechanisms of Docking

## 7. Available softwares for docking:

At present, more than 30 docking programs molecular (commercial or free) are available.

Among all molecular docking programs that have already proven their effectiveness, we can cite:

**Ex :**

- DOCK
- Flex
- Hammerhead
- Surflex
- SLIDE
- AutoDock
- ICM
- MCDock
- GOLD
- GemDock
- PLANTS
- Glide
- MOE
- VINA
- Fred
- QXP [4,11,12]

**Table I-1: Pros and Cons of Docking tools**

Program	Pros	Cons
DOCK	Small binding sites Opened cavities Small hydrophobic ligands	Flexible ligands Highly polar ligands
FLEXX	Small binding sites Small hydrophobic ligands	Very flexible ligands
FRED	Large binding sites Flexible ligands Small hydrophobic ligands High speed	Small polar buried ligands
GLIDE	Flexible ligands Small hydrophobic ligands	Ranking very polar ligand Slow speed
GOLD	Small binding sites Small hydrophobic ligands	Ranking very polar ligands Ranking ligands in large cavities
SLIDE	Side chain flexibility	Sensitivity to input coordinates
SURFLEX	Large and opened cavities Small binding sites Very flexible ligands	Low speed for large ligands
QXP	Optimizing known binding modes	Sensitivity to input coordinates



## 8. Theory of molecular docking:

The docking process may be divided on three main parts:

**preparing the ligand and macromolecule:** This is made based on force fields allowing for surface representation and cavities as potential ligand sites .

**defining the docking type:** rigid or flexible.

**setting the search strategy for ligand conformations:** systematic or stochastic . Each part is further elaborated on next sections [2,3].

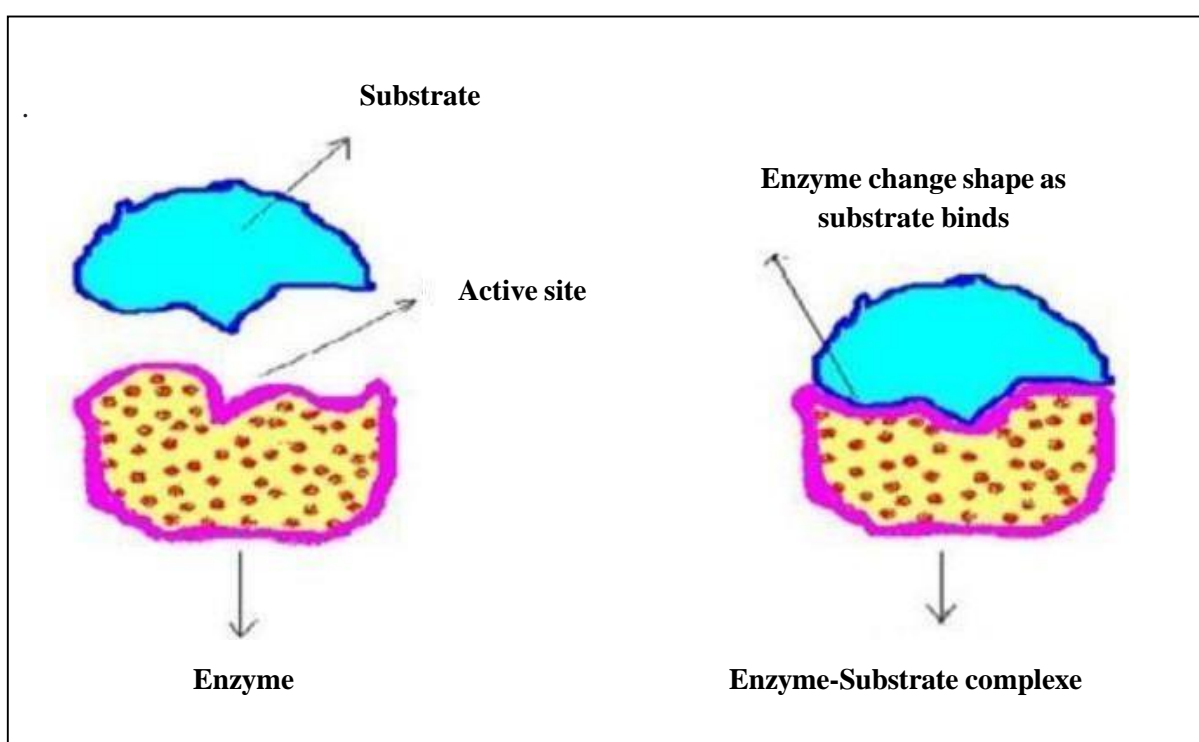


Figure I.7: The induced -Fit theory

## 9. Intermolecular [drug-target] interactions

### A. 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

### A. Interactions electrostatics :

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

### B. 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 [3].

## 10. Molecular dynamics simulation

Molecular dynamics simulation addresses the problem of numerically solving the classical equations of motion for a system of N atoms in an effort to sample a thermodynamic ensemble, or trajectory, under specified thermodynamic conditions (e.g. constant temperature or constant pressure).

Such a trajectory is important for two reasons:

- It gives configurational and momentum information for each atom, from which thermodynamic properties of the system can be calculated.
- The trajectory represents an exploration, or search, of the conformation space available to a particular system. The principle underlying conformation search using dynamics is that atoms in a dynamics simulation will eventually search their entire conformation space. The obvious problem with this approach is that the search space may be huge, and thus, it may take many thousands, millions, or even billions of steps to adequately and accurately sample the space.

Molecular dynamics simulations are often a sequence of simulation stages each of which is run under different temperature or pressure conditions; e.g. when heating or cooling a system. These sequences of stages are called protocols and are specified with a special protocol language. The protocol language is a sequence of stages, each with a name and parameters enclosed in braces [6].

## 11. Major steps involved in mechanics of molecular docking

### Step I: preparation of protein:

Three dimensional structure of the Protein should be retrieved from Protein data bank (PDB); afterward the retrieved structure should be pre-processed. This should admit removal of the water molecules from the cavity, stabilizing the charges, filling the missing residues, generation the side chains etc. according to the parameters available.

- Dependent on docking program used
- Structure selection
- Site selection

- Often have to add hydrogens, some programs more sensitive to positions than other
- Remove/include waters, cofactors, metals
- Pre-docking remember to consider missing residues or atoms.

**Step II: active site prediction:**

After the preparation of protein, the active site of protein should be predicted. The receptor might possess lots of active sites merely the one of the concern should be picked out. Mostly the water molecules and hetero atoms are removed if present.

**Step III: Preparation of ligand:**

Ligands can be retrieved from several databases such as ZINC, Pub Chem or can be sketched applying Chem sketch tool. While picking out the ligand

Ligand preparation involves similar considerations, while the first step often involves its extraction from the protein structure. In virtual screening, ligands may come from sources different from PDB (e.g. Public repositories like PubChem, organic synthesis or virtual compounds).

**Step IV: docking:**

Ligand is docked against the protein and the interactions are analyzed. The scoring function gives score on the basis of best docked ligand complex is picked out. [2,4,5,8,9]

## 12. Conclusion

This chapter has enlightened us on some basic notions about **Molecular docking**. Molecular docking is an inexpensive, safe and easy to use tool, helps in investigating, interpreting, explaining and identification of molecular properties using three-dimensional structures. Molecular docking is a useful technique in structure-based drug design, virtual screening, and optimization of lead compounds. To date, there are several standalone and online docking tools to assist the computational and medicinal chemist to help explain the activity of compounds at the molecular level or to filter compounds for further studies. The increasing number of publications associated with molecular docking reflects the interest of the scientific community to continue developing or refining docking tools, and/or using such tools in drug discovery projects. In pharmaceutical industries, the impact of molecular docking is well recognized and established. Nowadays computational docking simulations are routinely employed at different stages of the drug discovery and rational drug designing procedures [1,2,4,7,10].

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# Chapter 11: Coronavirus disease

(COVID-19)

## 1. Introduction

On December 2019, the China Health Authority alerted the World Health Organization (WHO) to several cases of pneumonia of unknown aetiology in Wuhan City in Hubei Province in central China. The cases had been reported since December 8, 2019, and many patients worked at or lived around the local Huanan Seafood Wholesale Market although other early cases had no exposure to this market. On January 7, a novel coronavirus, originally abbreviated as 2019-nCoV by WHO, was identified from the throat swab sample of a patient .

Coronavirus (CoV) is a genus of the Coronavirus family named for the crown like spikes found on their surface. They are a huge family of viruses containing a genome composed of a long RNA strands which is the largest of all RNA viruses, and this genome acts like a messenger RNA when it infects a cell, and directs the synthesis of two long poly-proteins that include the machinery that the virus needs to replicate new viruses. the order Nidovirales These are divided into four genera **a**, **b**, **c**, and **d**. The SARS-CoV-2 belongs to the **b** genus that contain at least four structural proteins: Spike (S-protein), envelope (E-protein), membrane (M-protein), and nucleocapsid (N-protein).

A novel betacoronavirus from the subgenus Sarbecovirus has been isolated from human airway epithelial cells. Coronaviruses are large, lipid enveloped, single stranded RNA viruses found in avian and mammalian species.

The most common symptoms associated with COVID-19 are fever, cough, dyspnea, expectoration, headache and myalgia or fatigue. Less common signs at the time of hospital admission include diarrhea, hemoptysis, and shortness of breath .

Due to the severity of SARS-CoV infection, the potential for rapid spread of the disease, and the absence of proven effective and safe in vivo inhibitors of the virus, it is important to identify drugs that can effectively be used to treat or prevent potential SARS-CoV infections .

From large datasets in China, COVID-19 has been observed in children and young people at a low rate relative to the adult population. Limited data on attack rate suggest that children under **10** are infected with SARS-CoV-2 at approximately the same rates as adults. Children may be asymptomatic or too mildly infected to draw medical attention and be tested and counted in observed cases of COVID-19.

There are several reports of asymptomatic infection in children, which would appear to be consistent with emerging data relating to infection rates. There are, as yet, inadequate data on transmissibility of SARS-CoV-2 from children to other children. More evidence is required on all aspects of COVID-19 in pediatric populations including seroprevalence studies when an assay is available and long-term follow-up of silent radiographic abnormalities. [1-6]

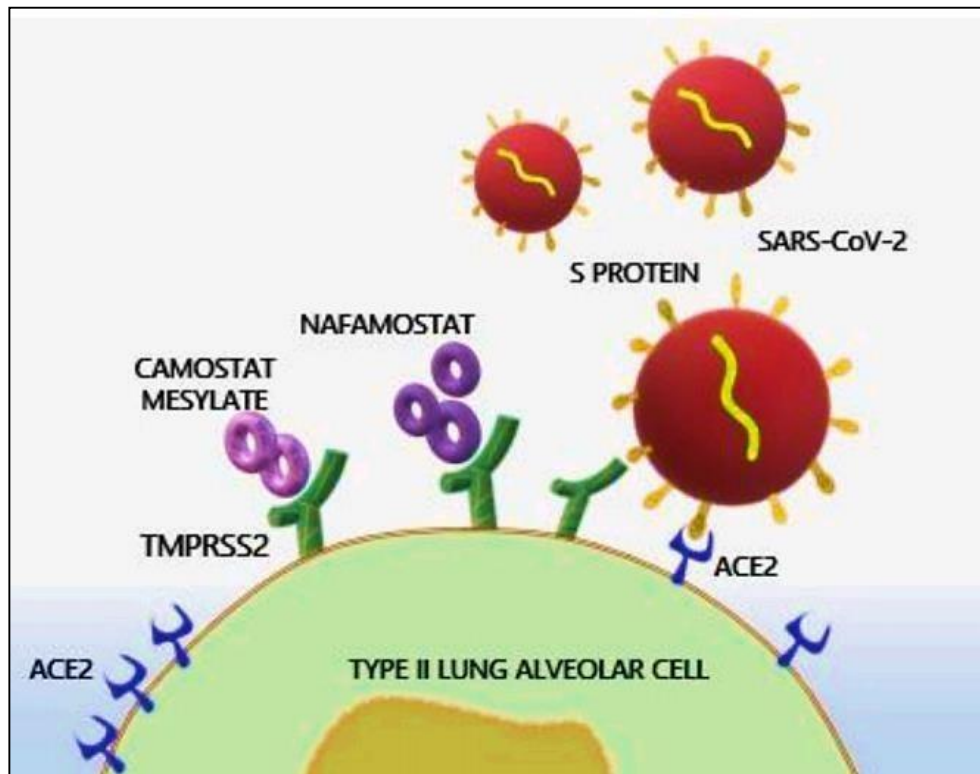


Figure II.1: The mechanism of SARS-CoV-2 binding on human host cells.

## 2. Coronavirus origins:

Coronavirus is the most prominent example of an emerging virus that has crossed the species barrier from wild animals to humans, like SARS and MERS. The origin of SARS-CoV-2 is also suspected to be from an intermediate animal host. The possibility of crossing the species barrier again for the fourth time cannot be ruled out (figure 2).[6,7,8]

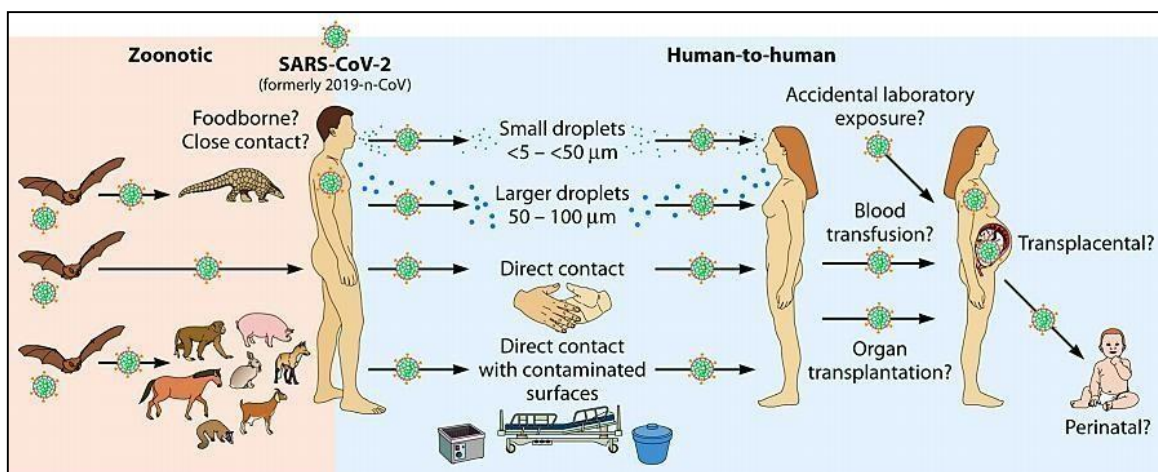


Figure II.2: Potential transmission routes for SARS-CoV.



### 3. SARS-COV-2 (COVID-19):

The disease caused by SARS-CoV-2 is also named severe specific contagious pneumonia (SSCP), Wuhan pneumonia, and, recently, COVID-19. Compared to SARS-CoV, SARS-CoV-2 has less severe pathogenesis but has superior transmission capability, as evidenced by the rapidly increasing number of COVID-19 cases. The incubation period of SARS-CoV-2 in familial clusters was found to be 3 to 6 days. The mean incubation period of COVID-19 was found to be 6.4 days, ranging from 2.1 to 11.1 days. Among an early affected group of 425 patients, 59 years was the median age, of which more males were affected. Similar to SARS and MERS, the severity of this nCoV is high in age groups above 50 years. Symptoms of COVID-19 include fever, cough, myalgia or fatigue, and, less commonly, headache, hemoptysis, and diarrhea. Compared to the SARS-CoV-2 infected patients in Wuhan during the initial stages of the outbreak, only mild symptoms were noticed in those patients that are infected by human-to-human transmission.

The initial trends suggested that the mortality associated with COVID-19 was less than that of previous outbreaks of SARS. The updates obtained from countries like **China, Japan, Thailand, and South Korea** indicated that the COVID-19 patients had relatively mild manifestations compared to those with SARS and MERS. Regardless of the coronavirus type, immune cells, like mast cells, that are present in the submucosa of the respiratory tract and nasal cavity are considered the primary barrier against this virus. Advanced in-depth analysis of the genome has identified 380 amino acid substitutions between the amino acid sequences of SARS-CoV-2 and the SARS/SARS-like coronaviruses. These differences in the amino acid sequences might have contributed to the difference in the pathogenic divergence of SARS-CoV-2. Further research is required to evaluate the possible differences in tropism, pathogenesis, and transmission of this novel agent associated with this change in the amino acid sequence. With the current outbreak of COVID-19, there is an expectancy of a significant increase in the number of published studies about this emerging coronavirus, as occurred with SARS and MERS. SARS-CoV-2 invades the lung parenchyma, resulting in severe interstitial inflammation of the lungs. This is evident on computed tomography (CT) images as ground-glass opacity in the lungs. This lesion initially involves a single lobe but later expands to multiple lung lobes.

The histological assessment of lung biopsy samples obtained from COVID-19-infected patients revealed diffuse alveolar damage, cellular fibromyxoid exudates, hyaline membrane formation, and desquamation of pneumocytes, indicative of acute respiratory distress syndrome. It was also found that the SARS-CoV-2-infected patients often have lymphocytopenia with or without leukocyte abnormalities. The degree of lymphocytopenia gives an idea about disease prognosis, as it is found to be positively correlated with disease severity. Pregnant women are considered to have a higher risk of getting infected by COVID-19. The coronaviruses can cause adverse outcomes for the fetus, such as intrauterine growth restriction, spontaneous abortion, preterm delivery, and perinatal death.

Nevertheless, the possibility of intrauterine maternal-fetal transmission (vertical transmission) of CoVs is low and was not seen during either the SARS- or MERS-CoV

outbreak. However, there has been concern regarding the impact of SARS-CoV-2/COVID-19 on pregnancy. Researchers have mentioned the probability of in utero transmission of novel SARS-CoV-2 from COVID-19-infected mothers to their neonates in **China** based upon the rise in IgM and IgG antibody levels and cytokine values in the blood obtained from newborn infants immediately postbirth; however, RT-PCR failed to confirm the presence of SARS-CoV-2 genetic material in the infants. Recent studies show that at least in some cases, preterm delivery and its consequences are associated with the virus. Nonetheless, some cases have raised doubts for the likelihood of vertical transmission. COVID-19 infection was associated with pneumonia, and some developed acute respiratory distress syndrome (ARDS). The blood biochemistry indexes, such as albumin, lactate dehydrogenase, C-reactive protein, lymphocytes (percent) and neutrophils (percent) give an idea about the disease severity in COVID-19 infection. During COVID-19, patients may present leukocytosis, leukopenia with lymphopenia, hypoalbuminemia and an increase of lactate dehydrogenase, aspartate transaminase, alanine aminotransferase, bilirubin and especially, D-dimer. Middle-aged and elderly patients with primary chronic diseases, especially high blood pressure and diabetes were found to be more susceptible to respiratory failure. Therefore, it had poorer prognoses. Providing respiratory support at early stages improved the disease prognosis and facilitated recovery. The ARDS in COVID-19 due to the occurrence of cytokine storms that results in exaggerated immune response, immune regulatory network imbalance and multiple-organ failure. In addition to the exaggerated inflammatory response seen in patients with COVID-19 pneumonia, the bileduct epithelial cell-derived hepatocytes upregulate ACE2 expression in liver tissue by compensatory proliferation that might result in hepatic tissue injury. In addition to the exaggerated inflammatory response seen in patients with COVID-19 pneumonia, the bileduct epithelial cell-derived hepatocytes upregulate ACE2 expression in liver tissue by compensatory proliferation that might result in hepatic tissue injury. [9-30]

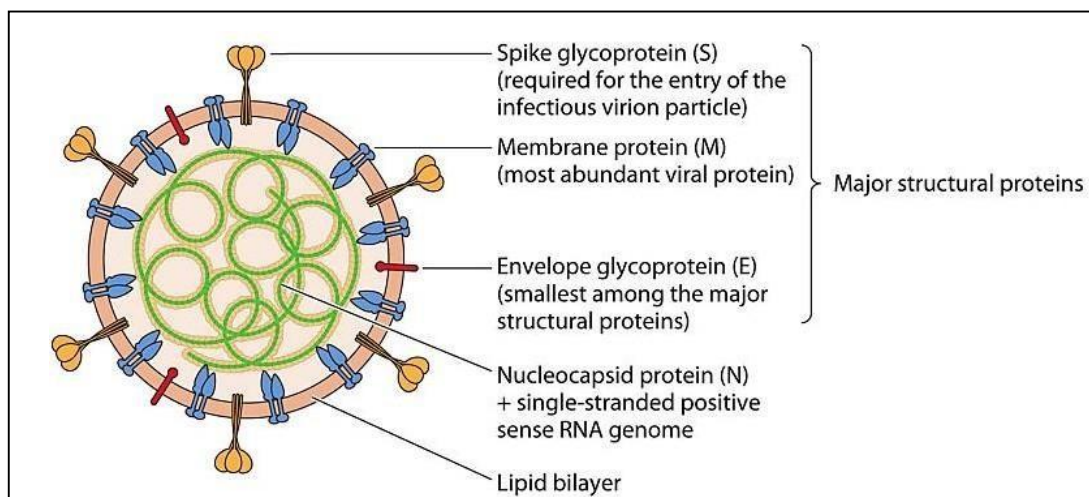


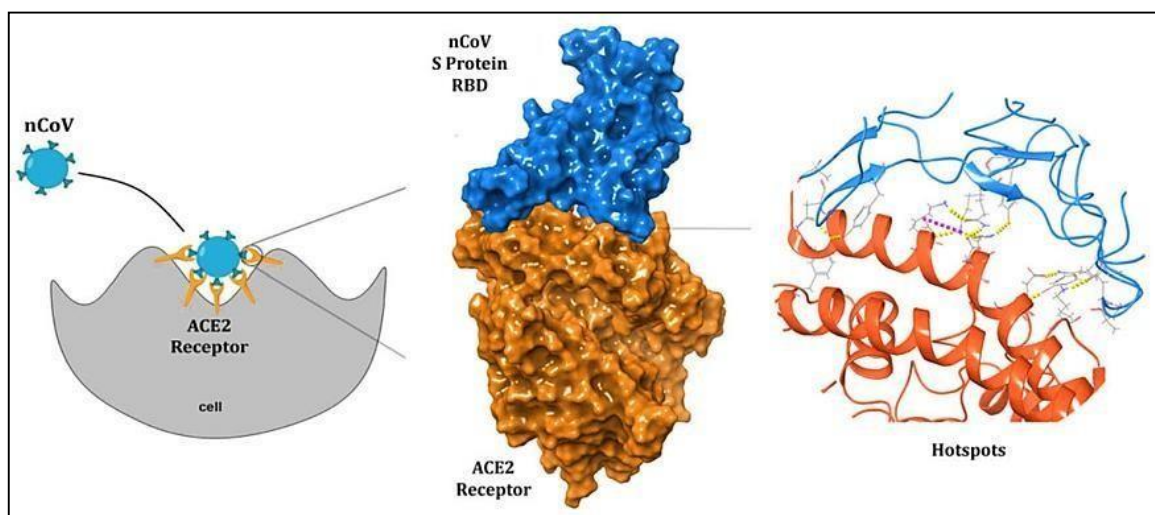
Figure II.3: SARS-CoV-2 virus structure.

#### 4. ACE2 receptor:

Angiotensin-converting enzyme 2 (ACE2) is the cellular receptor for severe acute respiratory syndrome–coronavirus (SARS-CoV) and the new coronavirus (SARS-CoV-2) that is causing the serious coronavirus disease 2019 (COVID-19) epidemic. Here, we present cryo–electron microscopy structures of full-length human ACE2 in the presence of the neutral amino acid transporter B0AT1 with or without the receptor binding domain (RBD) of the surface spike glycoprotein (S-protein) of SARS-CoV-2, both at an overall resolution of **2.9** angstroms with a local resolution of **3.5** angstroms at the ACE2-RBD interface. The ACE2-B0AT1 complex is assembled as a dimer of heterodimers with the collectrin-like domain of ACE2 mediating homodimerization. The RBD is recognized by the extracellular peptidase domain of ACE2 mainly through polar residues. These findings provide important insights into the molecular basis for coronavirus recognition and infection.

ACE2, is a type I integral membrane protein, which it consists of 805 amino acid residues with one Zn<sup>2+</sup> essential for enzyme activity. ACE2 was implicated in the regulation of heart function and as a functional receptor for the coronavirus, which is linked to the severe acute respiratory syndrome (SARS). ACE2 is the cellular receptor for the new coronavirus (SARS- CoV-2) which is causing the serious pandemic COVID-19.

Recently, SARS-CoV-2 was reported to be a human angiotensin I converting enzyme 2 (ACE2)-tropic virus able to bind the alveolar pneumocytes which express ACE2 at their surface. Yet, in humans the ACE2 mRNAs were found expressed in virtually all organs including the heart, blood vessels, kidney and testis, opening the possibility for this virus to infect other tissues beside lung. ACE2 is a known peptidase that regulates the renin-angioten-aldosterone system (RAAS), thus controlling blood pressure. [3,16,17,31]



**Figure II.4: Binding of the S-protein receptor (RBD) with the angiotensin-converting enzyme 2 (ACE2) receptor.**

### A. ACE2 structure and function

The ACE2 gene spans **39.98 kb** of genomic DNA and contains **18 exons**. It maps to chromosome **X** at position **Xp22**. It encodes a type I cell-surface glycoprotein of about **100 kDa**, composed by **805 amino acids** and characterized by a N-terminal signal peptide of **17 amino acid residues**, a peptidase domain (PD) (residues **19e615**) with its **HEXXH** zinc binding metalloprotease motif, a C-terminal Collectrin (a regulator of renal amino acid transport and insulin) like domain (CLD) (residues **616e768**) that includes a ferredoxin like fold “**Neck**” domain (**615e726**), that ends with an hydrophobic transmembrane hydrophobic helix region of **22 amino acid residues** followed by an intracellular segment of **43 amino acid residues**. The histidine motif **HEXXH** identified as an important component in a wide variety of zinc-dependent metalloproteases consists of five residues, the first histidine followed by glutamic acid being conserved, then the two variable amino acids and a final histidine. Crystal structure analysis has suggested the presence of several hinge regions and N-glycosylations.

ACE2 belongs to the family of ACE members which have a wider tissue distribution. The juxtamembrane, trans-membrane and cytoplasmic tail of ACE2 do not resemble ACE but these two proteins share the CLD region, a **220 amino-acid domain**. Angiotensin converting enzymes (ACE) are zinc metalloproteases. ACE is a widely distributed protein of **170 kDa** encoded by a **21 kb** gene located on chromosome **17 (17q23)**. That converts the inactive decapeptide, angiotensin (Ang) I to an active vasoconstrictor octapeptide Ang II [Asp-Arg-Val-Tyr-Ile-His-Pro-Phe] that controls the blood pressure, and through inactivation of bradykinin vasodilator. AngII also triggers the release of aldosterone that regulates the capacity of kidney to absorb sodium and water.[31-38]

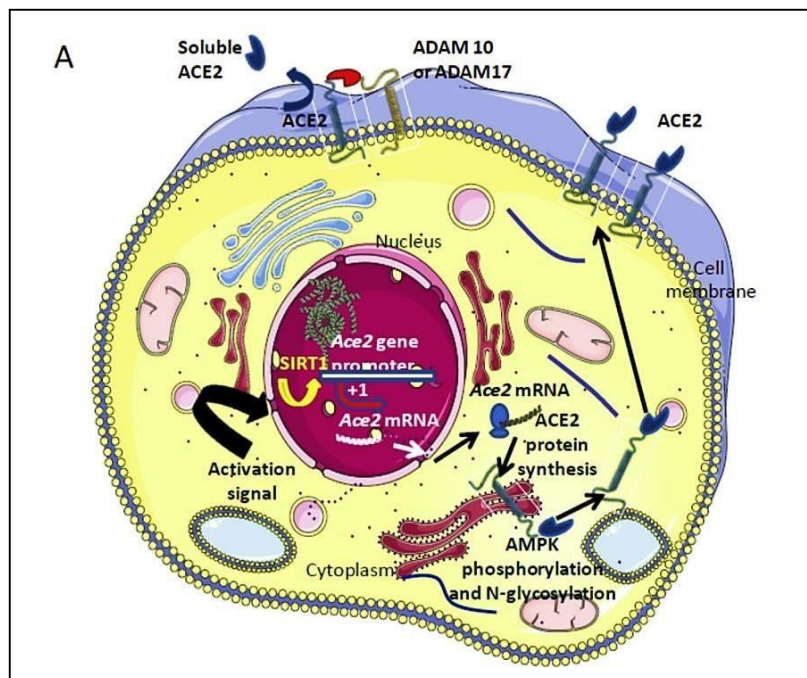


Figure II.5: A. Schematic representation of the regulation of ACE2.





SARS-CoV spike glycoprotein/ACE-2 complex and viral proteases. The pharmacodynamic and pharmacokinetic properties were predicted through the pkCSM server while the corresponding binding affinity of the selected drugs towards the proteins was computed using AutodockVina Screening tool. The ADMET properties revealed all the drugs possess drug like properties. [39,40,41,42,43]

#### a) Chloroquine :

Chloroquine, a relatively safe, effective and cheap drug used for treating many human diseases including malaria, amoebiasis and human immunodeficiency virus is effective in inhibiting the infection and spread of SARS CoV in cell culture. The fact that the drug has significant inhibitory antiviral effect when the susceptible cells were treated either prior to or after infection suggests a possible pro-phylactic and therapeutic use.

Chloroquine, a medication used primarily to treat malaria, is being studied to treat coronavirus. Its putative antiviral effects have been hypothesized to be related to the elevation of endosomal and lysosomal pH in addition to its angiotensin-converting enzyme 2 inhibitory potentials, Unlike viruses such as human immunodeficiency virus and herpes simplex virus, Sendai virus that can fuse the plasma membrane to success fully infect the host, enveloped viruses such as coronaviruses are endocytosed in the endosome and lysosome before fusion aiding its entry into cells.

The role of Chloroquine and Hydroxychloroquine as potential treatments for is still under debate globally because of some side effects associated with it. This study involves the In silico interactions of Chloroquine and Hydroxychloroquine with the NTD-N-protein of SARS-CoV-2. With the help of various computational methods, we have explored the potential role of both of these antiviral drugs for the treatment of patients by comparing the efficacy of both drugs to bind to NTD-N-protein. [8,9,44-46].

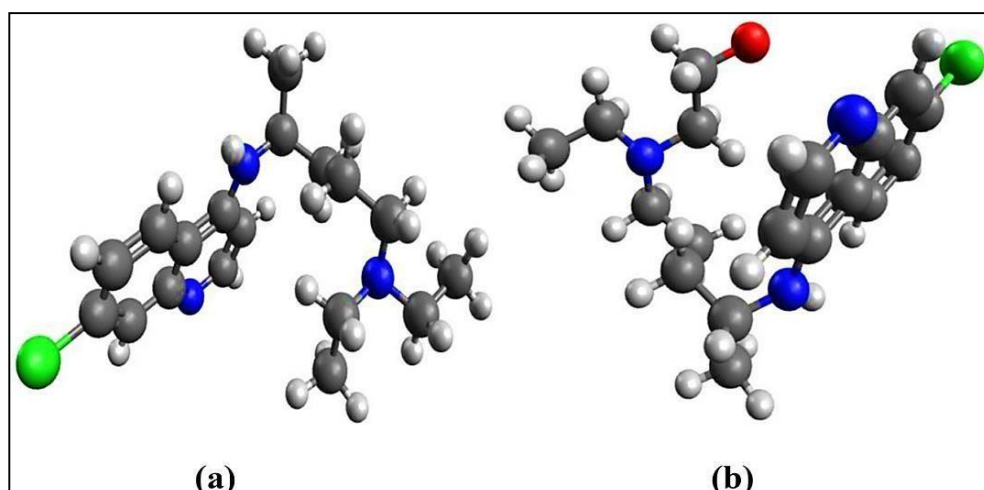
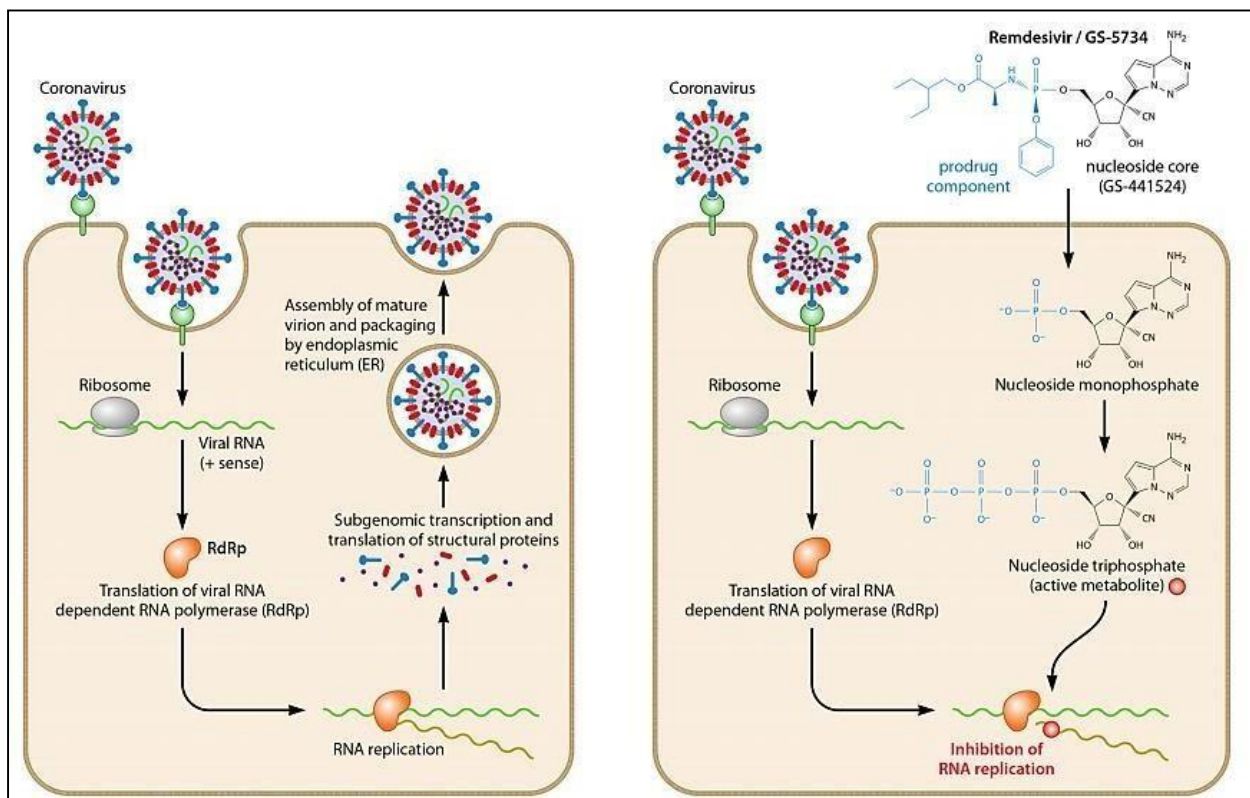


Figure II.6 Structure of (a) Chloroquine and (b) Hydroxychloroquine

## b) Remdesivir :

A novel nucleotide analog prodrug was developed for treating Ebola virus disease (EVD) and it was also found to inhibit the replication of SARS-CoV and MERS-CoV in primary human airway epithelial cell culture systems. Recently, in vitro study has proven that remdesivir has better antiviral activity than lopinavir and ritonavir. Further, in vivo studies conducted in mice, also identified that treatment with remdesivir improved pulmonary function and reduced viral loads and lung pathology both in prophylactic and therapeutic regimens compared to lopinavir/ritonavir-IFN- treatment in MERS-CoV infection. Remdesivir also inhibits a diverse range of coronaviruses, including circulating human CoV, zoonotic bat CoV and pre-pandemic zoonotic CoV. Remdesivir is also considered the only therapeutic drug that significantly reduces pulmonary pathology. All these findings indicate that remdesivir has to be further evaluated for its efficacy in the treatment of COVID-19 infection in humans. The broad-spectrum activity exhibited by remdesivir will help control the spread of disease in the event of a new coronavirus outbreak (figure II-7) [8,43,47].



**Figure II.7 Intracellular activation of remdesivir (GS-5734) and inhibition of coronavirus replication.**

## 6. Conclusion:

The current COVID-19 pandemic is clearly an international public health problem. There have been rapid advances in what we know about the pathogen, how it infects cells and causes disease and clinical characteristics of disease.

Older age (**65 years old**), male gender, hypertension, CVDs, diabetes, COPD and cancer were associated with greater risk of death from COVID-19 infection. Coronaviruses can also result in adverse outcomes for the fetus and infant including intrauterine growth restriction, preterm delivery, admission to the ICU, spontaneous abortion and perinatal death.

Currently, no effective drug treatment or vaccine exists. It is necessary for monitoring of the virus to be strengthened and drugs and vaccines to be developed against SARS-Cov-2 infection as soon as possible. [1,10,16,47,48,49].



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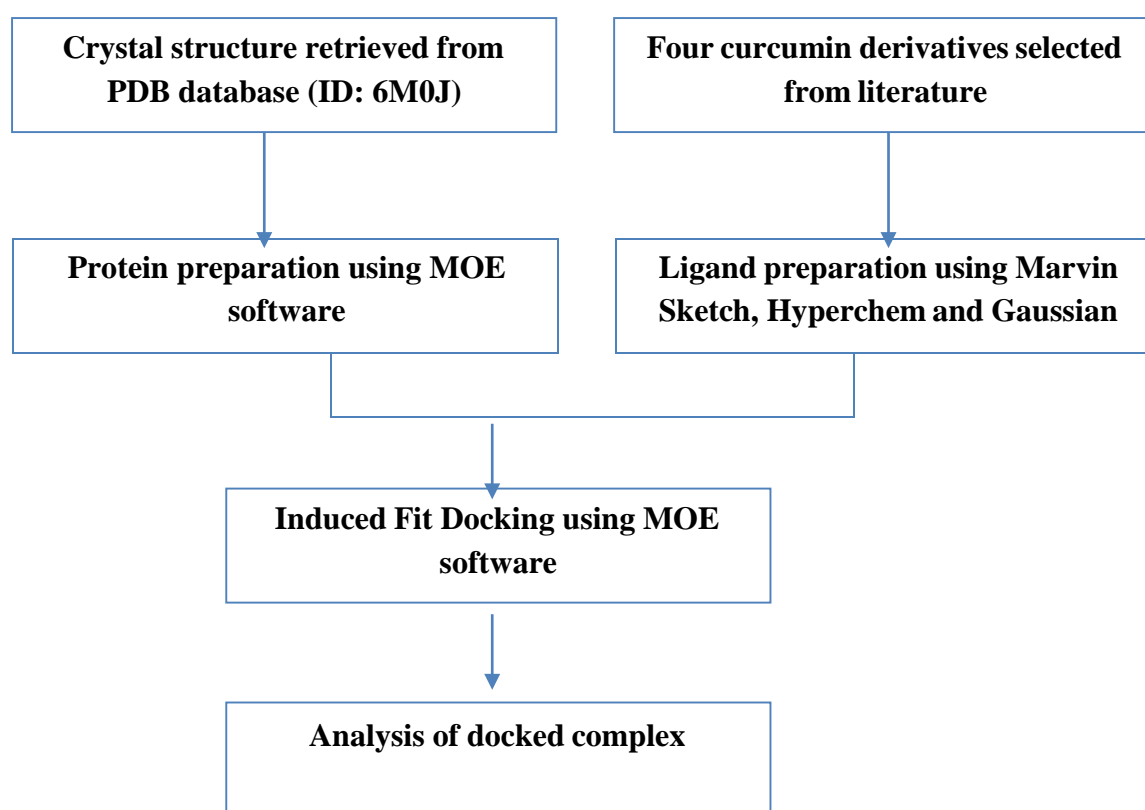
Chapter III : Materials and  
methods

## 1. Introduction

Recently, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19) emerged in late 2019, and it has posed a global health threat, causing an ongoing pandemic in many countries and territories, COVID-19 shows less severe pathogenesis but higher transmission competence.

Automated molecular docking aims at predicting the possible interactions between two molecules. This method has proven useful in medicinal chemistry and drug discovery and design providing atomistic insights into molecular recognition.

Human infections by the SARS coronavirus to be closely associated with interactions between the viral spike protein (S-protein) which has favorable binding affinity for the human Angiotensin-Converting Enzyme 2 (ACE2). [1,2,3,4,5]



**Figure III.1 Schematic representation of the docking procedure, analysis of drugs.**

## 2. Materials and methods

Molecular docking was applied to the four curcumin derivatives to study their affinity with the crystal structure of [SARS-CoV-2/ACE2] (PDB identification number: **6M0J**) to select the most active four curcumin derivatives that inhibit **COVID-19**. The methodology of this work is illustrated in (Figure 1). [6,-9].

### A) Molecule library preparation :

The chemical structure of four curcumin derivatives were extracted from the literature. The structures were sketched in **2D** format and converted to **3D** format using **Mervin Sketch**. The structures were pre-optimized with semi-empirical **AM1** method using **Hyperchem** software. The structures were optimized using density functional theory **DFT** method by employing the **B3LYP/6-31G** basis set to obtain the most stable conformation through **Gaussian 09**. The convergent value of maximum force, root-mean-square (**RMS**) force, maximum displacement and RMS displacement are set by default and achieved “**YES**”. All values are positive after calculation vibrational frequencies to curcumin derivatives, those results indicate that the curcumin derivatives are stable. The optimized structures were combined in one database on **MOE** software in order to study the affinity of ligands. [10-14].

### B) Receptor preparation :

The crystal structure of the angiotensin-converting enzyme related with coronavirus-2 [SARS-CoV-2/ACE2] complex (PDB ID: **6M0J**) were found in the Protein Data Bank [15-16].

The enzyme was prepared by removing the **N-acetyl-D-glucosamine** in sequence editor. Because the water molecule in the active site of the target enzyme plays an important role, it was inserted in the active sites tonsure making a hydrogen bond between the ligand and the target [17].

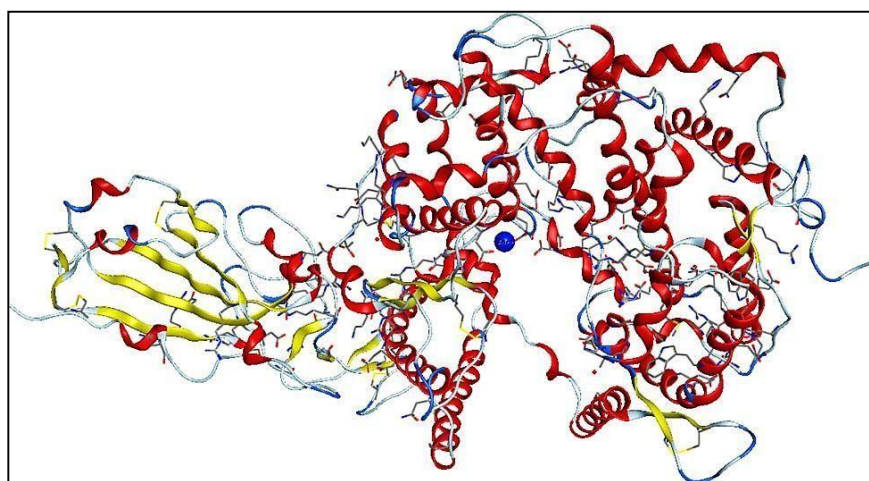
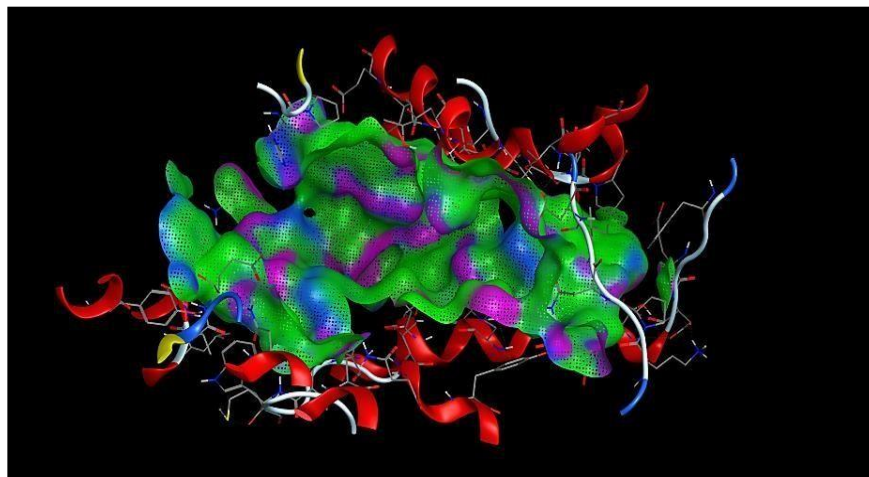


Figure III.2: Crystal structure of [SARS-CoV-2/ACE2] complex (PDB ID: **6M0J**).



Since  $Zn^{2+}$  is an important cofactor for many viral proteins,  $Zn^{2+}$  can inhibit the replication of ARN polymerase, a zinc-containing active site ( $Zn^{2+}$ ) in **6M0J** enzyme was selected as shown in (**Figure III-3 and III-4**) . Then, the protein structure was prepared by correcting the missing bonds, which were broken in X-ray diffraction, and then hydrogen atoms were added (table 1 and 2) . [18,19,20,21,22]



**Figure III-3: isolated active site 1 of Sars-coronavirus-2 related with ACE2 enzyme  
Pdb ID: 6m0j**

**Table III. 1: Binding sites residues used as input for receptor grid generation during Induced Fit Docking.**

Receptor	Site	Residues
<b>6M0J</b>	Site 1	(Tyr127, Asn149, Asp269, Trp271, Arg273, Phe274, Thr276, Tyr279, Lys288, Pro289, Asn290, Ile291, Asp292, Thr294, His345, Pro346, Thr365, Met366, Asp367, Leu370, Thr371, His374, Glu375, Glu402, Glu406, Ser409, Leu410, Ala413, Thr414, Pro415, Leu418 Phe428, Glu430, Asp431, Thr434, Glu435, Asn437, Phe438, Lys441, Gln442, Thr445, Ile446, Thr449, Leu503, Phe504, His505, Tyr515, Arg518, Thr519, Gln522, Phe523, His540) 3 :(Zn901)



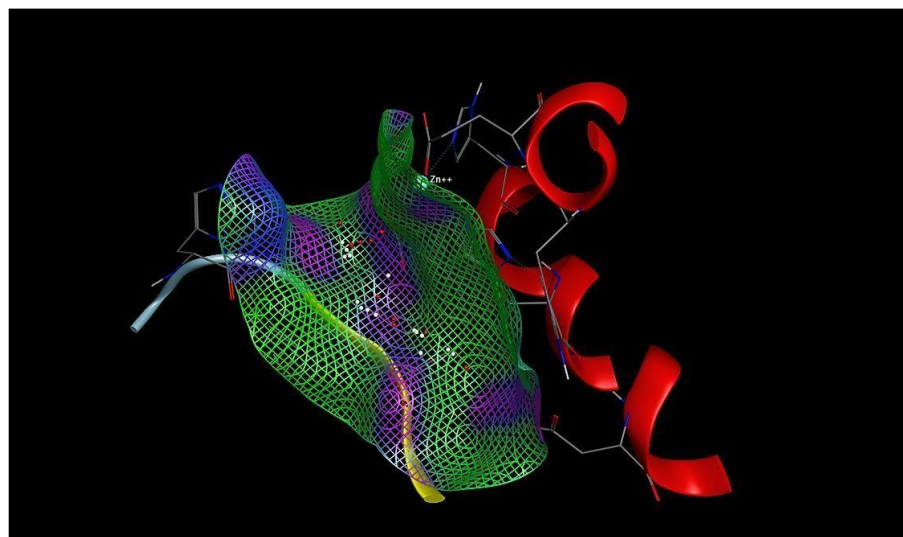


Figure III.4: isolated active site 2 of Sars-coronavirus-2 related with ACE2 enzyme  
Pdb ID: 6m0j

Table III. 2: Binding sites residues used as input for receptor grid generation during Induced Fit Docking.

Receptor	Site	Residues
6M0J	Site 2	(His345, Pro346, Thr347, Ala348, Glu375, His378, Asp382, His401, Glu402) 3 :(Zn901)

- Molecule 1 is a protein called Angiotensin-converting enzyme 2

Molecule	Chains	Sequence Length	Organism	Details	Image
Angiotensin-converting enzyme 2	A	603	Homo sapiens	Mutation(s): 0 Gene Names: ACE2, UNQ868/PRO1885 EC: 3.4.17.23 (PDB Primary Data), 3.4.17 (PDB Primary Data)	

- Molecule 2 is a protein called Spike protein S1

Molecule	Chains	Sequence Length	Organism	Details	Image
Spike protein S1	B [auth E]	229	Severe acute respiratory syndrome coronavirus 2	Mutation(s): 0 Gene Names: S, 2	

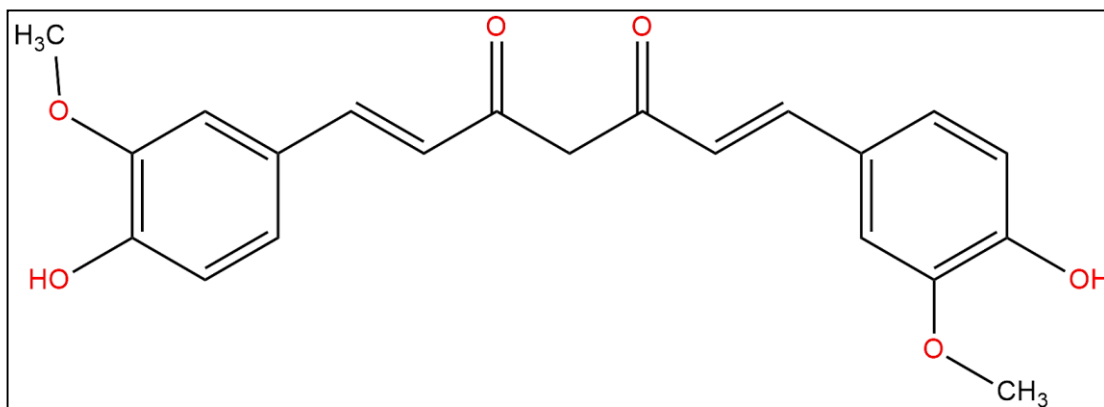
### 1. Structure of the different molecules

Figures bellow represents the **2D** structure of the studied curcuminoids:

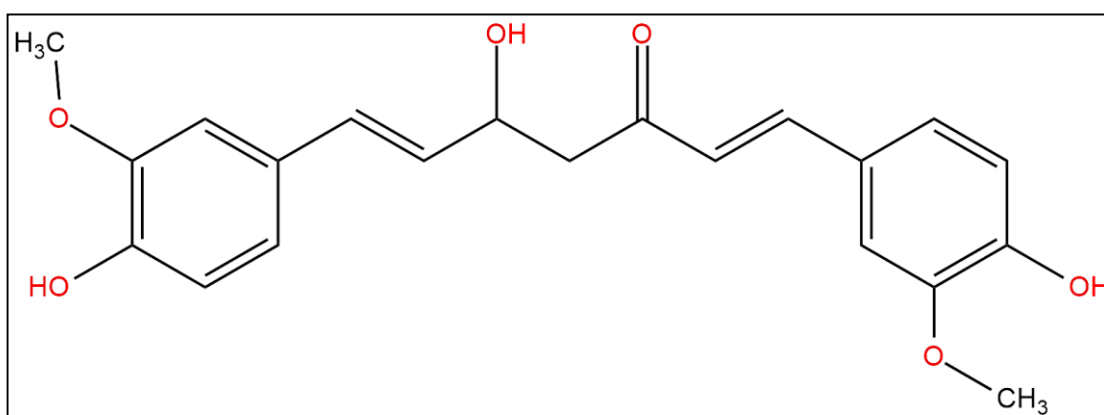
- Curcumin with both enolate form (1)
- Curcumin enol form (2)
- Demetoxycurcumin (3)

- Bisdemetoxycurcumin (4)

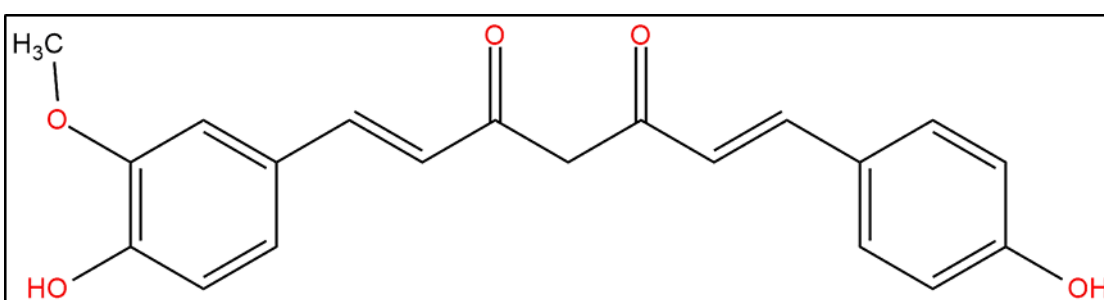
Which were created and visualised using **MarvinSketch** and **MarvinView**



**Figure III.5: 2D structures of Curcumin (enolate) (1)**



**Figure III.6: 2D structures of Curcumin (enol) (2)**



**Figure III.7: 2D structures of Demetoxycurcumin (3)**

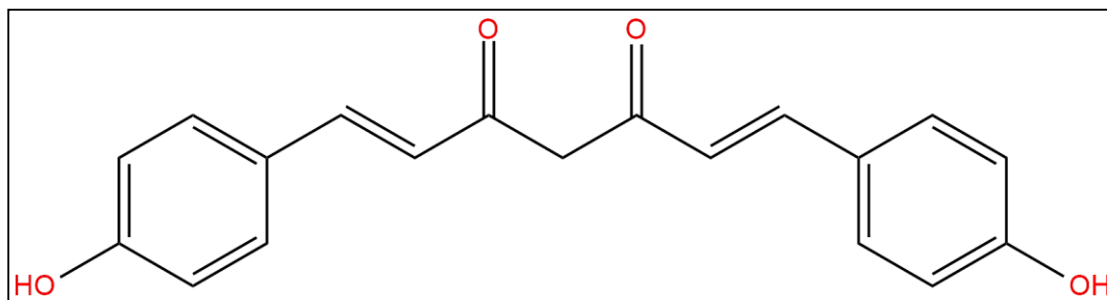


Figure III.8: 2D structures of Bisdemetoxycurcumin (4)

### 3. Geometric optimization of different molecules

The theoretical study begins after creating the **3D** conformation of the previous structures using **HyperChem 8.0.10** by adding the Hydrogen to structures then perform a semi-empirical optimization using **AM1** method, the obtained conformations were saved as **MDL MOL**. The next step was optimizing the previous molecules using **Gaussian 09** software, **DFT** as a calculation method with **B3LYP** as correlation and **6-31G** as a basis set. The best obtained structure with the least energy values recorded after optimization are shown in figures below: [23-28]

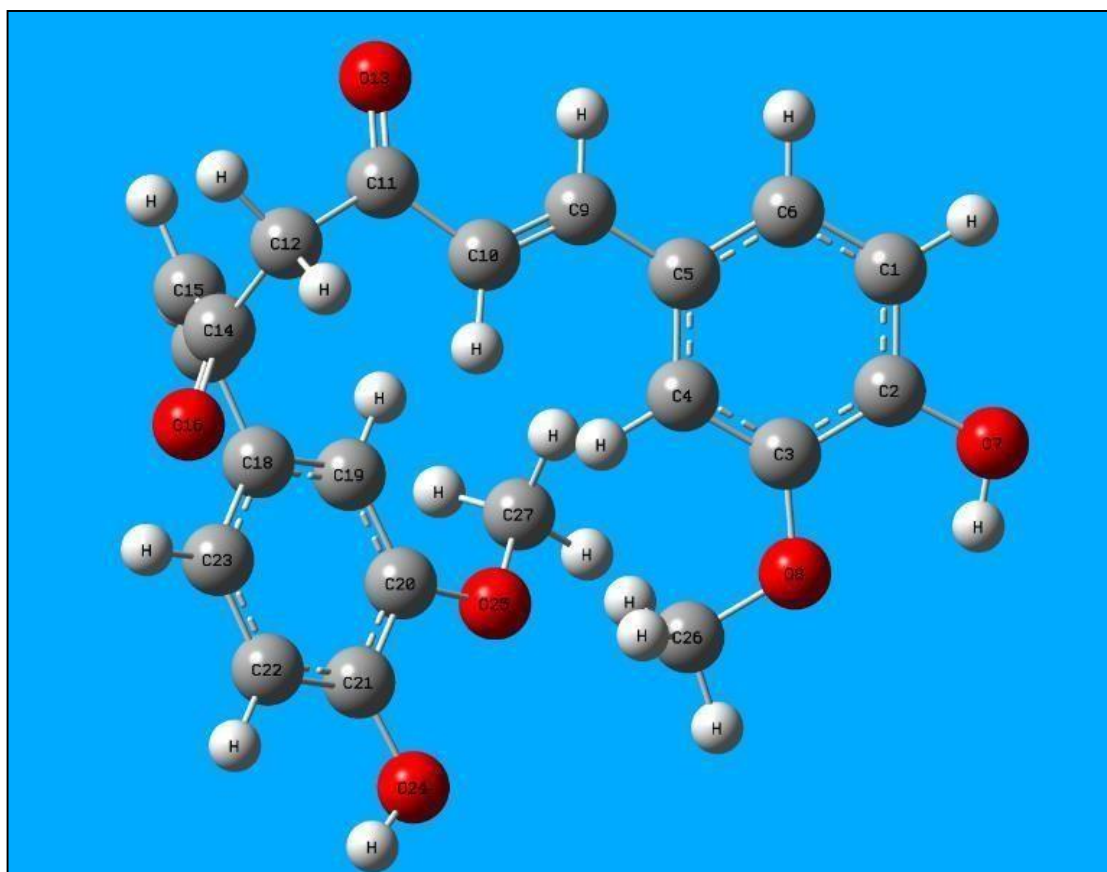
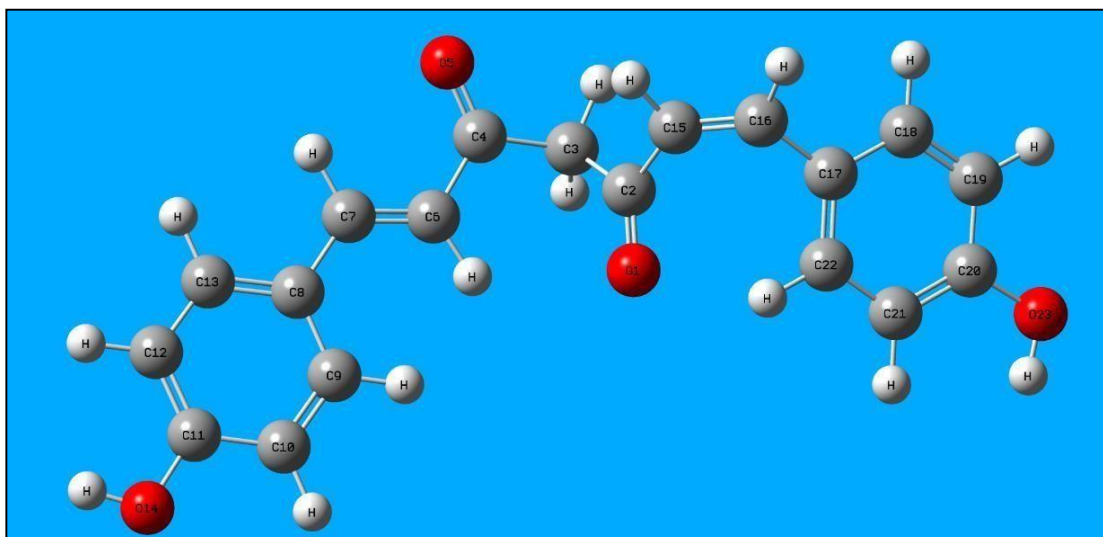


Figure III.9: Curcumin structure after optimization (enolate)





**Figure III.12: Bisdemetoxycurcumin structure after optimization**

#### **4. Molecular docking**

All the docking and scoring calculations were performed using the molecular operation environment software (MOE). The crystal structure of [SARS-CoV-2/ACE2] complex (PDB entry: **6M0J**) at a resolution of **2.45 Å** were obtained from the Protein literature. A resolution between **1.5** and **2.5 Å** is considered as a good quality for docking studies. It is known that the best score of **RMSD** values should be near to **2 Å** with an energy score less or equal to **7 Kcal/mol**. These two values are often used as criterion to validate the result of the molecular docking. [29-38].

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Chapter VI : Results and  
discussion

## Results and discussion

After creating the molecules and performing a semi empirical **AM1** optimization to prepare the molecular system, **Gaussian** software was the best distribution to handle the Quantum mechanics calculations with the best accurate results.

The table below summaries the after optimization energy:

**Table VI-1: optimization energy calculated for curcuminoids**

Molecule	Calculated Energy (U.A)
<b>Curcumin (enolate)</b>	-1260.528211
<b>Curcumin (enol)</b>	-1261.800977
<b>Demethoxycurcumin</b>	-1139.357355
<b>bisdemethoxycurcumin</b>	-1033.808455

### 1. The binding affinities of four curcumin derivatives into [SARS-CoV- 2/ACE2] complex active sites:

The tables below show the results of docking of four curcumin derivatives in **6M0J** at two selected pockets **S1** and **S2** respectively.

#### a- Site 1:

The results in pocket **S1** revealed that **Demethoxycurcumin(3)** had the lowest docking score (**-7.7786 Kcal/mol**) and RMSD (**1.7225 Å**) compared with **Curcumin (enolate)(1)** and **Curcumin (enol)(2)**, which they had energy scores and RMSD values of (**-7.3734 Kcal/ mol, 2.0116 Å**) and (**-7.1983 Kcal/mol, 2.3831 Å**) respectively.

In spite of **Curcumin (enolate)(1)** and **Curcumin (enol)(2)** did not have the lowest score, they have the best RMSD values. **Bisdemethoxycurcumin(4)** had RMSD value less than (**1.8477 Å**). The results shown in tables below (2,3,4,5,).

The results of the binding of four curcumin derivatives with **6M0J** in site **1** are shown in the figures below (1,2,3,4).

From the Figures, It is apparent that **Curcumin (enolate)(1)** had **H-donor** and **Pi-H** interactions with amino acids **GLU406** and **PHE438** respectively, whereas **Demethoxycurcumin(3)** had **H-donor** and **pi-pi** interactions with amino acids **PRO289** and **PHE438** respectively. **Curcumin (enol)(2)** had numerous interactions; **H-donor** interaction with amino acid **GLU375**, and metallic interactions with **zinc, HIS374, HIS378** and **GLU402** and ionic interactions with amino acids **HIS378** and **GLU402**.

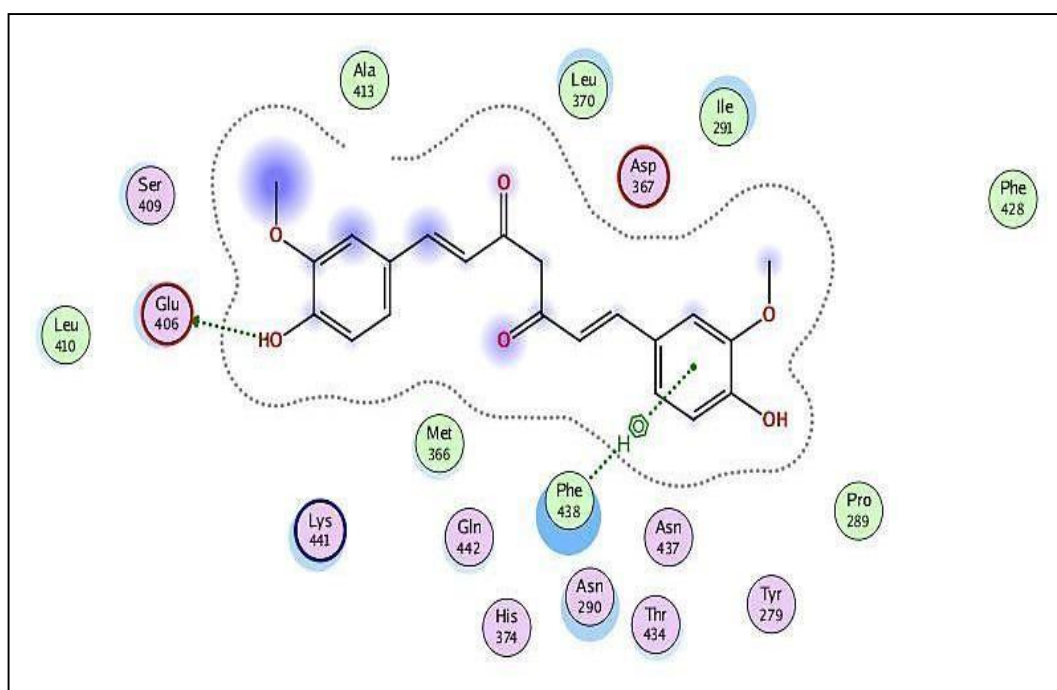
**Bisdemethoxycurcumin(4)**, had **H-donor** interaction with amino acid **GLU406** and **Pi-H** interaction with amino acids **ASN290, ILE291, PHE438** and **GLN442**.

The interaction of hydroxyl group in **Curcumin (enol)(2)** with **zinc** motivates the **zinc** to interact with **ZN901** by metallic interaction and with **HIS378** and

**HIS374** by metallic and ionic interactions respectively. As mentioned above, **zinc** had an antiviral activity and this type of interaction may inhibit the COVID-19.

**Table VI.2: The results obtained from docking of Curcumin (enolate)(1) with 6M0J in site1**

Four curcumin derivatives	(S) Score Kcal/mol	RMSD (Å)	Atom of compound	Involved Receptor Residues	Type of interaction bond	Distance (Å)	E Kcal/mol
<b>Curcumin (enolate)</b>	-7.3734	2.0116	0 11	OE1 GLU 406	H-donor	2.83	-4.9
			6-ring	CB PHE 438	Pi-H	3.86	-0.8



**Figure VI.1: 2D diagram interaction between Curcumin (enolate) (1) and site 1 of 6M0J**

Table VI.3 : The results obtained from docking of Curcumin (enol)(2) with 6M0J in site1

four curcumin derivatives	S Score Kcal/mol	RMSD (Å)	Atom of compound	Involved Receptor Residues	Type of interaction bond	Distance (Å)	E Kcal/mol
Curcumin (enol)	-7.1983	2.383	0 26	OE2 GLU 375	H-donor	2.76	-3.3
			0 26	ZN ZN 901	Metal	2.02	-2.5
			ZN 901	NE2 HIS 374	Metal	2.40	-3.2
			ZN 901	NE2 HIS 378	Metal	2.27	-5.7
			ZN 901	OE1 GLU 402	Metal	2.10	-5.6
			ZN 901	NE2 HIS 378	Ionic	2.27	-11.7
			ZN 901	OE1 GLU 402	Ionic	2.10	-14.4
			ZN 901	OE2 GLU 402	Ionic	3.13	-3.7

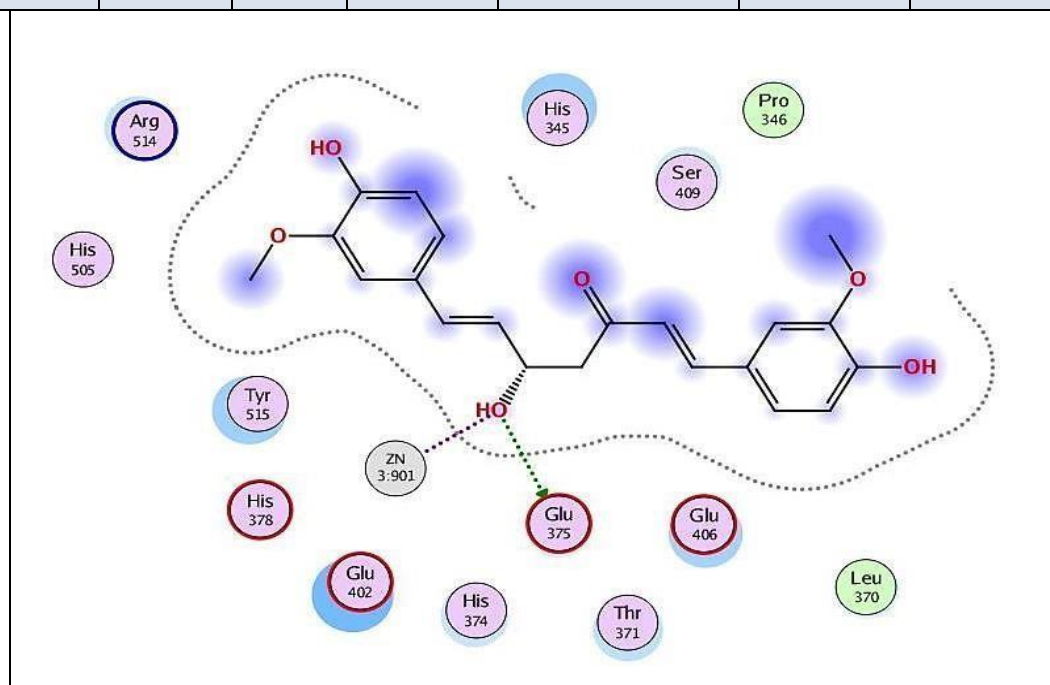


Figure VI.2: 2D diagram interaction between Curcumin (enol) (2) and site 1 of 6M0J

Table VI.4 : The results obtained from docking of Demetoxycurcumin(3) with 6M0J in site1

four curcumin derivatives	S Score Kcal/mol	RMSD (Å)	Atom of compound	Involved Receptor Residues	Type of interaction bond	Distance (Å)	E Kcal/mol
Demetoxy-curcumin	-7.7786	1.7225	0 42	0 PRO 289	H-donor	2.70	-1.0
			6-ring	6-ring PHE 438	Pi-pi	3.76	-0.0

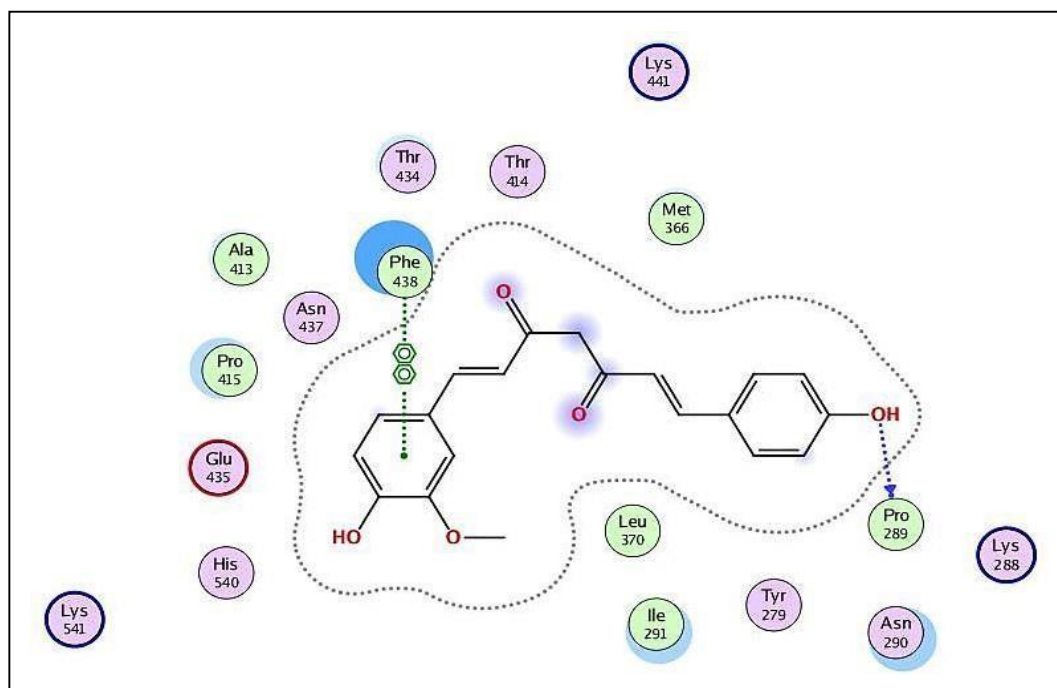
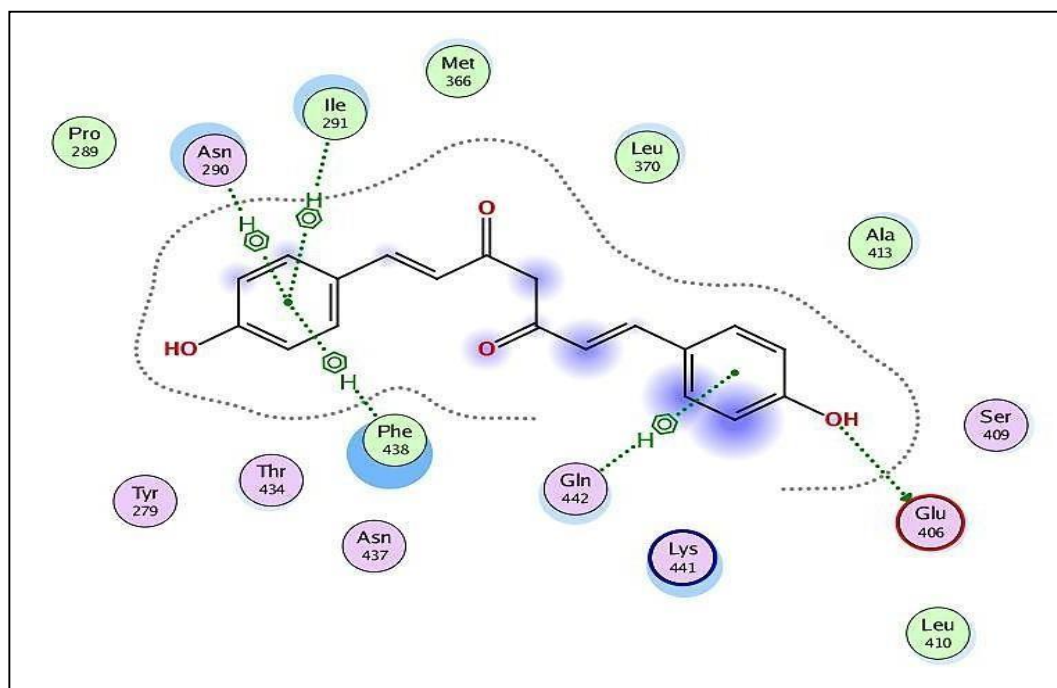


Figure VI.3: 2D diagram interaction between Demetoxycurcumin (3) and site 1 of 6M0J

Table VI.5: The results obtained from docking of Bisdemetoxycurcumin(4) with 6M0J in site1

four curcumin derivatives	S Score Kcal/mol	RMSD (Å)	Atom of compound	Involved Receptor Residues	Type of interaction bond	Distance (Å)	E Kcal/mol
Bisdemetoxycurcumin	-6.6551	1.8477	0 38	OE1 GLU 406	H-donor	3.33	-0.9
			6-ring	CA ASN 290	Pi-H	4.32	-0.7
			6-ring	N ILE 291	Pi-H	4.00	-1.3
			6-ring	CB PHE 438	Pi-H	3.87	-0.7
			6-ring	NE2 GLN 442	Pi-H	3.89	-0.6



**Figure VI.4: 2D diagram interaction between Bisdemetoxycurcumin (4) and site 1 of 6M0J**

**b- Site 2:**

The results of docking of four curcumin derivatives with **6M0J** in site **2** are shown in Tables below (6,7,8,9). According to the results in this site **2**, almost all curcumin derivatives make interacted in pocket **S2** via **zinc**. **Curcumin (enol)(2)** showed excellent docking score (**-6.7862 Kcal/mol**), and had RMSD (**2.0628 Å**) compared with **Curcumin (enolate)(1)**, **Demetoxycurcumin(3)** with energy scores and RMSD values of (**-5.9258 Kcal/mol, 2.0418 Å**) and (**-6.3554 Kcal/mol, 2.0712 Å**) respectively. **Bisdemethoxycurcumin(4)** had the highest score and the best RMSD values (**- 5.5799 Kcal/mol, 1.6650 Å**).

Table VI.6. The results obtained from docking of Curcumin (enolate)(1) with 6M0J in site 2

four curcumin derivatives	S Score Kcal/mol	RMSD (Å)	Atom of compound	Involved Receptor Residues	Type of interaction bond	Distance (Å)	E Kcal/mol
<b>Curcumin (enolate)</b>	-5.9258	2.0418	0 11	O HOH 1004	H-donor	2.78	-1.9
			0 46	NE2 HIS 345	H-donor	3.32	-1.2
			0 25	NE ARG 514	H-acceptor	3.16	-1.7
			0 25	NH2 ARG 514	H-acceptor	2.87	-2.3
			ZN 901	NE2 HIS 374	Metal	2.40	-3.2
			ZN 901	NE2 HIS 378	Metal	2.27	-5.7
			ZN 901	OE1 GLU 402	Metal	2.10	-5.6
			ZN 901	NE2 HIS 378	Ionic	2.27	-11.7
			ZN 901	OE1 GLU 402	Ionic	2.10	-14.4
			ZN 901	OE2 GLU 402	Ionic	2.13	-3.7

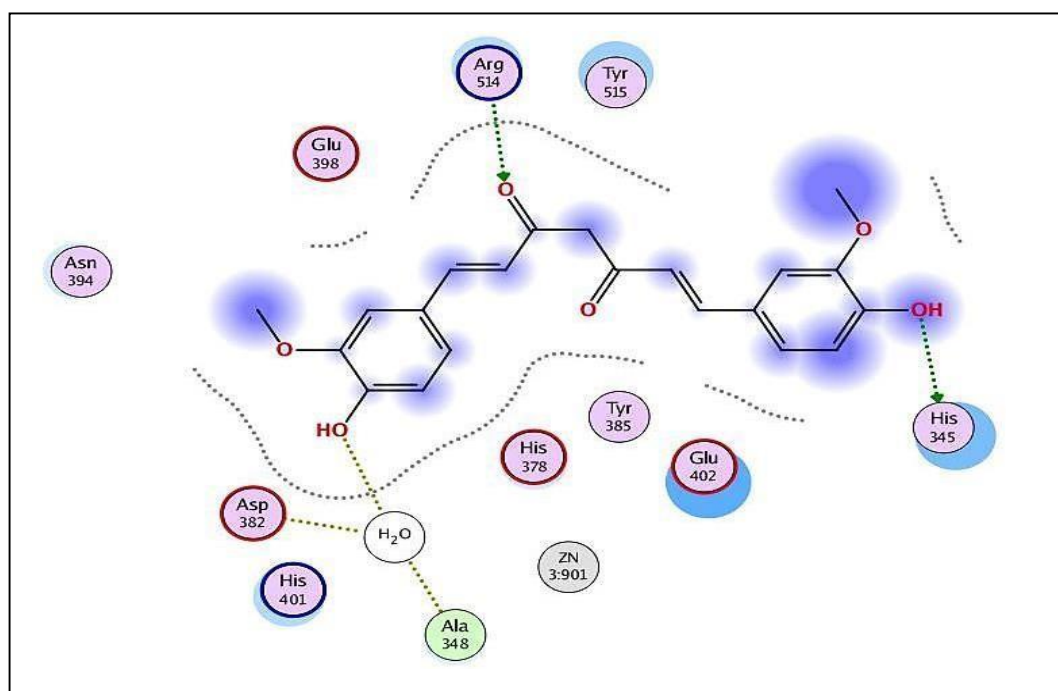


Figure VI.5: 2D diagram interaction between Curcumin (enolate) (1) and site 2 of 6M0J

Table VI.7: The results obtained from docking of Curcumin (enol)(2) with 6M0J in site 2

four curcumin derivatives	S Score Kcal/mol	RMSD (Å)	Atom of compound	Involved Receptor Residues	Type of interaction bond	Distance (Å)	E Kcal/mol
Curcumin (enol)	-6.7862	2.0628	0 11	OE2 GLU 375	H-donor	2.82	-2.3
			0 48	O HOH 1004	H-donor	3.03	-1.2
			0 31	NE ARG 514	H-acceptor	3.15	-2.2
			0 31	NH2 ARG 514	H-acceptor	2.84	-2.3
			ZN 901	NE2 HIS 374	Metal	2.40	-3.2
			ZN 901	NE2 HIS 378	Metal	2.27	-5.7
			ZN 901	OE1 GLU 402	Metal	2.10	-5.6
			ZN 901	NE2 HIS 378	Ionic	2.27	-11.7
			ZN 901	OE1 GLU 402	Ionic	2.10	-14.4
			ZN 901	OE2 GLU 402	Ionic	3.13	-3.7
			6-ring	CE1 HIS 345	Pi-H	4.49	-0.6
			6-ring	5-ring HIS 401	Pi-pi	3.99	-0.0

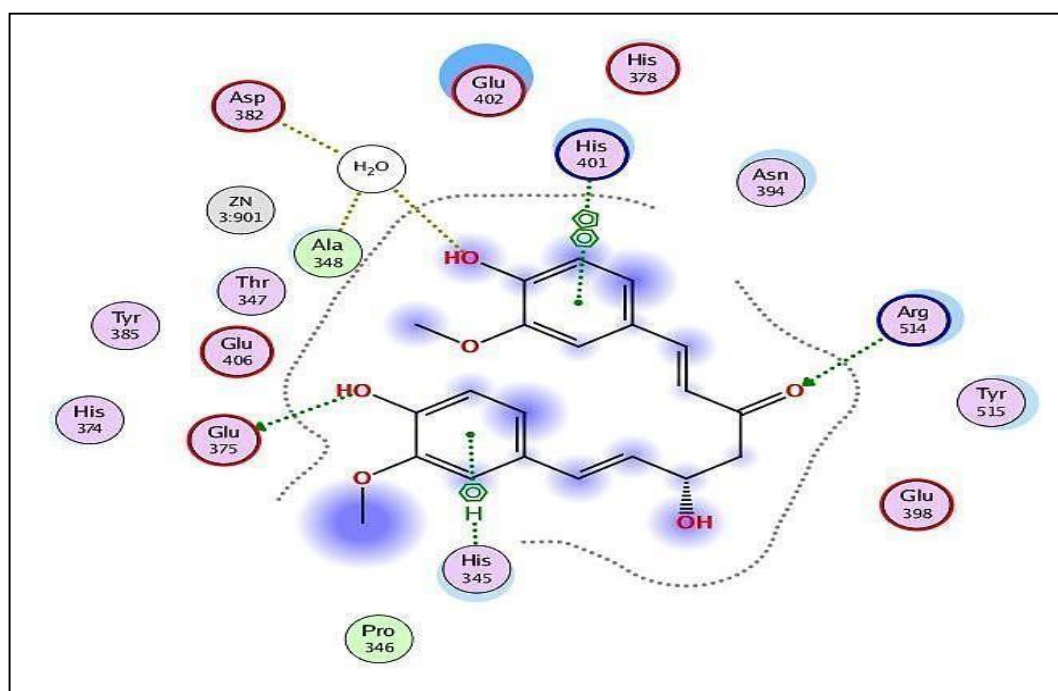


Figure VI.6: 2D diagram interaction between Curcumin (enol) (2) and site 2 of 6M0J



Table VI.8: The results obtained from docking of Demethoxycurcumin(3) with 6M0J in site 2

four curcumin derivatives	S Score Kcal/mol	RMSD (Å)	Atom of compound	Involved Receptor Residues	Type of interaction bond	Distance (Å)	E Kcal/mol
Demethoxy-curcumin	-6.3554	2.0712	0 25	ZN ZN 901	Metal	2.04	-1.3
			ZN 901	NE2 HIS 374	Metal	2.40	-3.2
			ZN 901	NE2 HIS 378	Metal	2.27	-5.7
			ZN 901	OE1 GLU 402	Metal	2.10	-5.6
			ZN 901	NE2 HIS 378	Ionic	2.27	-11.7
			ZN 901	OE1 GLU 402	Ionic	2.10	-14.4
			ZN 901	OE2 GLU 402	Ionic	3.13	-3.7
			6-ring	5-ring HIS 374	Pi-pi	3.52	-0.0

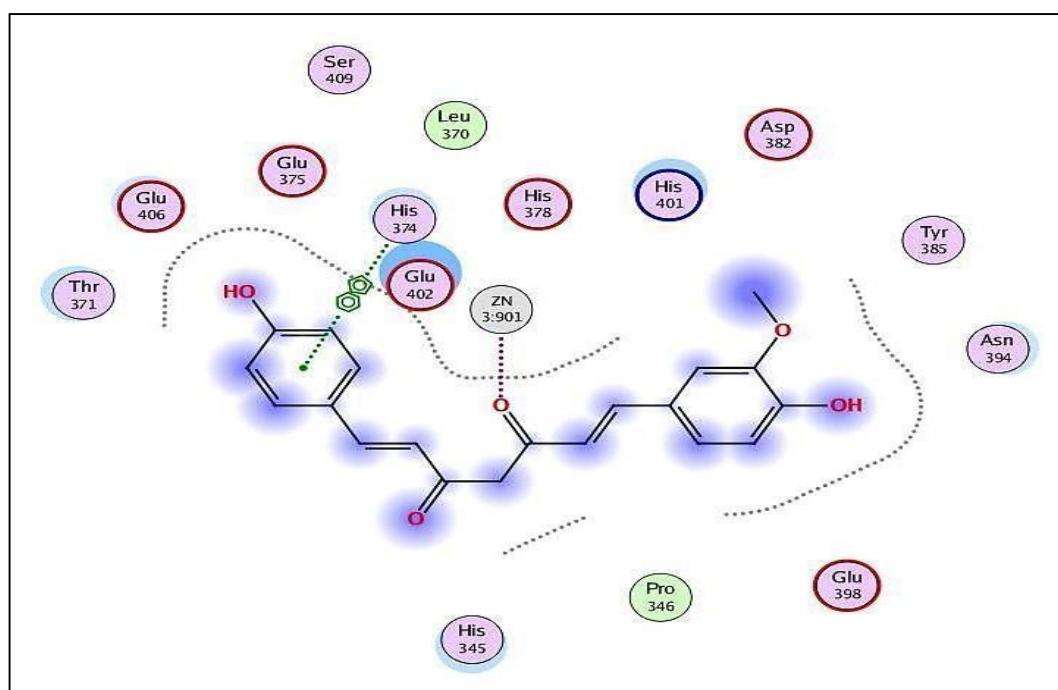


Figure VI.7: 2D diagram interaction between Demethoxycurcumin (3) and site 2 of 6M0J

Table VI.9: The results obtained from docking of Bisdemethoxycurcumin(4) with 6M0J in site 2

four curcumin derivatives	S Score Kcal/mol	RMSD (Å)	Atom of compound	Involved Receptor Residues	Type of interaction bond	Distance (Å)	E Kcal/mol
Bisdemethoxycurcumin	-5.5799	1.6650	0 11	O HOH 1004	H-donor	2.91	-1.5
			0 38	OE2 GLU 375	H-donor	2.80	-4.5
			0 21	NE ARG 514	H-acceptor	3.01	-3.1
			0 21	NH2 ARG 514	H-acceptor	2.96	-1.9
			ZN 901	NE2 HIS 374	Metal	2.40	-3.2
			ZN 901	NE2 HIS 378	Metal	2.27	-5.7
			ZN 901	OE1 GLU 402	Metal	2.10	-5.6
			ZN 901	NE2 HIS 378	Ionic	2.27	-11.7
			ZN 901	OE1 GLU 402	Ionic	2.10	-14.4
			ZN 901	OE2 GLU 402	Ionic	3.13	-3.7
			6-ring	5-ring HIS 401	Pi-pi	3.94	-0.0

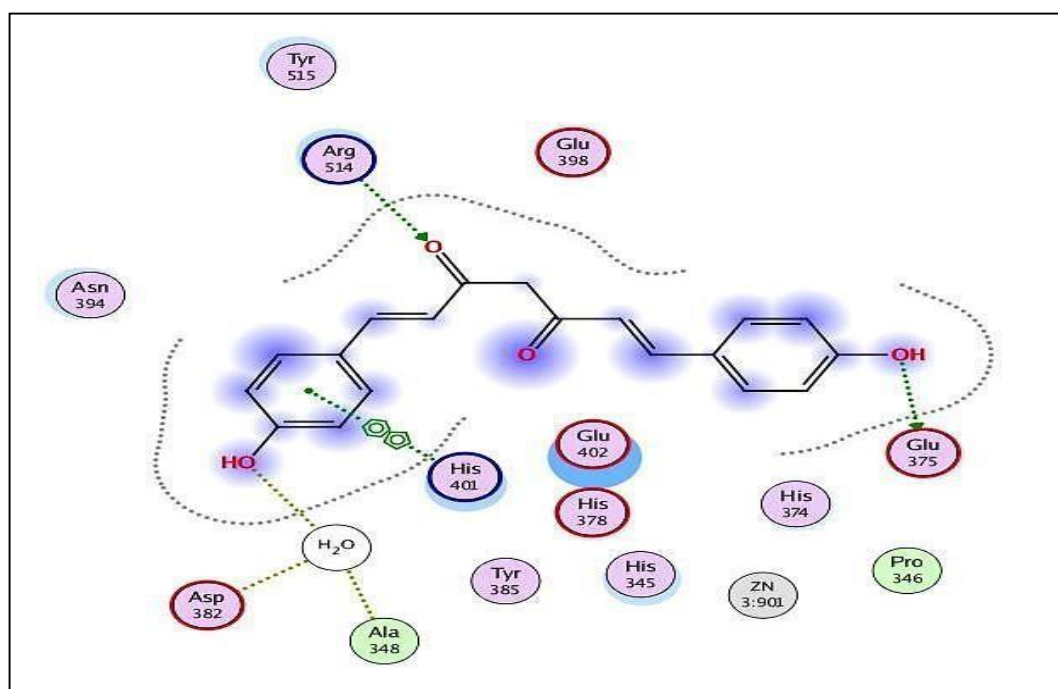


Figure VI.8: 2D diagram interaction between Bisdemethoxycurcumin (4) and site 2 of 6M0J

The figures above (5,6,7,8) presents the interactions of four curcumin derivatives with 6M0J in site2.

From the figures, it can be seen that **Demethoxycurcumin(3)** had a **metallic** interaction with **Zn**, three **metallic** interactions with the amino acids **HIS374**, **HIS378** and **GLU402** respectively. Three **ionic** interactions with the amino acids **HIS378** and **GLU402** respectively. In addition, this compound formed **pi- pi** interaction with the amino acid **HIS374**.

**Curcumin (enolate)(1)** had two **H-donor** interactions with amino acids **HOH1004** and **HIS345**, two **H-acceptor** interactions with amino acid **ARG514**, **metallic** interactions with amino acids **HIS374**, **HIS378** and **GLU402**, **ionic** interaction with amino acids **HIS378** and **GLU402**.

**Curcumin (enol)(2)** had numerous interactions **H-donor**, **H-acceptor**, **metallic** interactions, **ionic** interactions, **Pi-H** and **Pi-pi** with amino acids **GLU375**, **HOH1004**, **ARG 514**, **ARG514**, **HIS374**, **HIS378**, **GLU402**, **HIS378**, **GLU402**, **GLU402**, **HIS345** and **HIS401** respectively.

**Bisdemetoxycurcumin(4)** had four hydrogen bonds with the amino acids **water**, **GLU375** and **ARG514**, three **metallic** interactions with amino acids **HIS374**, **HIS378** and **GLU402**, three **ionic** interactions with the amino acids **HIS378** and **GLU402**, and **pi-pi** interaction with amino acid **HIS401**.

## 2. Conclusion

The aim of the present research was to examine the binding of four curcumin derivatives with [SARS- CoV-2/ACE2] complex using docking analysis. The docking ranking results in this study showed that some of these ligands might have the ability to inhibit SARS-CoV-2.

The results of docking these ligands with [SARS- CoV-2/ACE2] complex enzyme in two pockets **S1** and **S2** ; in site1 **Demethoxycurcumin(3)** gave the lowest energy score and good RMSD value followed by **Curcumin (enolate)(1)** . In addition, the docking results showed that only **Curcumin (enol)(2)** interacted with **Zn** in site 1.

While in site 2 **Curcumin (enol)(2)** gave the best energy score followed by **Demethoxycurcumin(3)**. The curcumin derivatives mentioned above presented good results with the two chosen enzymes compared with **Curcumin (enolate)(1)** and **Bisdemethoxycurcumin(4)**, only **Demethoxycurcumin(3)** interacted with **Zn** in site 2 according to the results above.

The most obvious finding to emerge from this study is that **Curcumin (enol) (2)**, **Demethoxycurcumin(3)** gave good docking results. Further investigation and experimentation into **Curcumin (enol) (2)**, **Demethoxycurcumin(3)**, which they are promising candidate drugs for COVID-19 patients, is strongly recommended.

General Conclusion

The goal of this study was to use docking analysis to look at the binding of four curcumin derivatives to the ACE2 enzyme and the [SARS-CoV-2/ACE2] complex.

In this research we focused on studying and investigating curcumin and some curcumin analogues biological activity. We also focused on types and approaches of molecular docking in brief. The main objective of molecular docking simulations is to identify new lead candidates.

Computer aided drug design is a powerful tool in the search of promising drug candidates, particularly when used in tandem with current chemical biology screening techniques. Despite the fact that CADD makes use of several restrictions and approximations, this knowledge driven approach has become an essential part in the drug design process due to its ability to fast-track drug discovery by utilizing existing knowledge and theories on receptor-ligand

The theoretical approach on the other hand, highlights curcuminoids as a potential ACE2 inhibitors using docking study as an investigation tool to validate this theory. The optimization using Gaussian shows that there might be a difference between both curcumin tautomers and that can influence the way each molecule interacts. The docking study as well, shows that the interactions can be achieved between both curcuminoids and the [SARS-CoV-2/ACE2] complex. All the studied structures were show very impressive results.

So as a result we can say that the investigated curcuminoids can play a high role inhibiting the [SARS-CoV-2/ACE2] complex and might be a very promising future drug towards this specific target.

## **Abstract**

The recent new disease coronavirus 2019 (COVID-19) is a new generation of severe acute respiratory syndrome coronavirus-2 SARS-CoV-2 that has infected millions of confirmed cases and killed hundreds of thousands of people worldwide. One of the most significant tools in drug discovery and design is molecular docking, which is used to analyze the kind of interaction between the ligand and its protein enzyme.

Four curcumin derivatives, were studied using molecular docking. Curcumin derivatives were chosen from the literature. To discover a ligand that might inhibit COVID-19, molecular docking were done for [SARS-CoV-2/ACE2] complex receptor in two active sites.

The findings of this investigation revealed that Curcumin (enol)(2) and Demetoxycurcumin (3) may bind the [SARS-CoV-2/ACE2] complex.

## **Résumé :**

La récente nouvelle maladie coronavirus 2019 (COVID-19) est une nouvelle génération de coronavirus-2 du syndrome respiratoire aigu sévère SARS-CoV-2 qui a infecté des millions de cas confirmés et tué des centaines de milliers de personnes dans le monde. L'un des outils les plus importants dans la découverte et la conception de médicaments est l'amarrage moléculaire est utilisé pour analyser le type d'interaction entre le ligand et son enzyme protéique.

Quatre dérivés de la curcumine, ont été étudiés en utilisant le docking moléculaire. Les dérivés de la curcumine ont été choisis dans la littérature. Pour découvrir un ligand qui pourrait inhiber la COVID-19, le docking moléculaire ont été effectués pour le récepteur [SARS-CoV-2/ACE2] dans deux sites actifs.

Les résultats de cette étude ont révélé que la curcumine (énol) (2) et la déméttoxycurcumine (3) peuvent se lier au récepteur ACE2 ainsi qu'au complexe [SARS-CoV-2/ACE2].