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Molecular modeling of the interaction protein-ligand using molecular docking: natural products MEGx database screening-PBPa1

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ABSTRACT

The principal aim of this study was to discover, through virtual screening, new inhibitors retrieved from MEGx database for the Penicillin Binding Protein a1 (PBPa1), a target of β-lactams, which responsible of actual resistance. Using PyRx and AutodockVina 500 molecules were docked and only 4 molecules gave a good results NP-000205,NP-000206, NP-000122, NP-000251: -11.2 Kcal/mol,-11.2 Kcal/mol,-11.2 Kcal/mol,-11.2 Kcal/mol respectively, comparing to the original inhibitor MES -5.6Kcal/mol.The druggability of these molecules were tested, the application of the lipinski's rule of five gave positive information on the pharmacokinetics properties of NP-000122,NP-000251 which are inhibitor of PBP a1.

The permeability properties for NP-000205, NP-000206were found within the limit range stated for Lipinski's rule of five.The approach of molecular Docking with Autodock program is considered among the best technics which is used nowadays to develop more effective protein.

Key words: AutoDock VINA,PyRx,PBPa1, docking, Inhibitor, MEGx, β-lactams, Pharmacokinetic, druggability, Lipinski's rule of five.

In the memory of our brothers

MAHDI & ISLAM

Dedication

To our beloved parents

Acknowledgements

Thanks first and foremost to ALLAH; without his grace this thesis would never have been finished on time.

We would like to thank everyone who has accompanied and supported us and made his direct and indirect contributions to this dissertation.

This work would not have been possible without the expert guidance of our extraordinarily talented and great educator,**Dr. RAHAL Khaled**. His expertise and continuous guidance provided us the opportunity to develop skills in learning and working, his approachable character and ever-present enthusiasm led to a lot of discussions with him, from which we got patient answers and fresh insights that covered various areas, also his oral and written comments were extremely perceptive and his constructive criticisms at different stages of our research were thought-provoking and very helpful.We are deeply thankful to him.

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ABSTRACT

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Resumé

L'objectif principal de cette étude était de découvrir, grâce au criblage virtuel, de nouveaux inhibiteurs de Penicillin Binding Protein a1 (PBPa1), à partir de la base de données MEGx, cette protéine est la cible naturelle des β-lactamines est responsables de la résistance actuelle accrue. L’amarrage moléculaire a été réalisé en utilisant PyRx et Autodock Vina, pour étudier les interactions de liaison de 500 composés avec les cibles, seulement 4 molécules ont donné de bons résultats NP-000205, NP-000206, NP-000122, NP-000251: -11,2 Kcal / mol, -11,2 Kcal / mol, -11,2 Kcal / mol , -11,2 Kcal / mol respectivement, par rapport à l'inhibiteur original MES -5,6Kcal / mol. La pharmacocinétique de ces molécules a été testée, l'application de la règle de cinq de Lipinski a donné des informations positives sur les propriétés pharmacocinétiques de NP-000122, NP-000251 qui sont des inhibiteurs de la PBP a1.Les propriétés de perméabilité pour NP-000205, NP-000206 ont été trouvées dans la limite indiquée pour la règle de cinq de Lipinski. Le programme d'approche de l’amarragemoléculaire avec Autodock est considéré parmi les meilleures techniques utilisées aujourd'hui pour développer des protéines plus efficaces.

Mots clés:AutoDock VINA, PyRx, PBPa1, l’amarrage moléculaire, Inhibiteur, MEGx, β-lactamines, Pharmacocinétique, la règle de Lipinski de cinq.

الملخص

إن الهدف الرئيسيمن هذه الدراسة كان معرفة مثبطات جديدة للبروتين رابط للبنيسيلين ا1 (pBpa1), يعتبر هذا البروتين المستهدف الطبيعي المسؤول عن المقاومة الحالية للبتالاكتامين وذلكمن خلال الفحص الافتراضي للمركبات الطبيعية لقاعدة البيانات MEGx. لقد تم إجراء الالتحام الجزيئي لخمسمائة 500 مركب طبيعي بواسطة البيركس ;اتودوك , لدراسة تفاعلات الربط لهذه المركبات مع البروتين , والنتيجة كانت أربع جزيئات ناجحة . NP-000122NP-000206 .
-11,2 Kcal / mol, -11,2 Kcal / mol, -9,2 Kcal / mol , -11,2 Kcal / . NP-000251 . NP-000205.
molعلى التوالي مقارنة بالمثبط الأصلي .MES = -5,6Kcal / mol

ولقد تم العثور على القابلية الدوائيةل : NP-000251 .NP-000122ضمن المجال المسوحلقاعدة الخمس لبنسكي أما NP-000205. NP-000206فهي لا تملك القابليةالدوائية لعدم خضوعها لقاعدة الخمس للبنسكي

طريقة الالتحام الجزيئيتعتبر من أفضل التقنيات المستعملة حاليا لتطوير و عر ض مثبطات أكثر فعالية لبروتين معين.

الكلمات المفتاحية : الالتحام الجزيئي- مثبطات- للبروتين رابط للبنيسيلين ا1- بيركس-اوتودوك- لقاعدة 5 للبنسكي.
قاعدة البيانات للمركبات الطبيعية

Table of content

Abbreviations

1-D:	one-dimensional
2-D:	two-dimensional
3-D:	three-dimensional
A°:	Ångström
ASPRE:	Active site. Serine Penicillin Recognizing Enzymes
GT:	Transglycosylase
GLN:	Glutamine
GLU:	Glutamate
H:	Hydrogen
HIA:	Human Intestinal Absorption
HIV:	Human Immunodeficiency Virus
HTVS:	High throughput virtual screening
KDA:	Kilo Dalton
Log P:	Partition coefficient water octanol
MR:	Molar refractivity
N:	Nitrogen
NCBI:	National Center for Biotechnology Information
NIH:	National Institutes of Health
nOHNH:	Hydrogen bonds donors
nOH:	Hydrogen bonds acceptor
O:	Oxygen
PBPa1:	Penecillin binding protein a1
PDB:	Protein data bank
PHE:	Phenylalanine
PyMOL:	Python-enhanced Molecular
QSAR:	Quantitative Structure Activity Relationship
SMILE:	Simplified molecular input line entry
SBVS:	Structure-based virtual screening
SER:	Serine
TP:	Transpeptidase
TPSA:	Topological polar surface area

THR: Threonine

TYR: Tyrosine

vdW: van-der-walls

VS : virtual screening

Figure Caption

Table Caption

Introduction

I- Introduction

Today virtual screening (vs) is becoming a standard procedure in drug development. The time and the cost required to design a new drug are immense and at an unacceptable level. So in order to bring down the cost and time required in the drug discovery process, in recent years, there has been an increasing interest in virtual screening of natural compounds extracted from plants **[1]**;there has been a push for alternative methods and technologies for molecular discovery, one alternative being computational methods (*In silico).*

In silico is one ofwidely used bioinformatics method for drug discovery, and it's an expression used to mean « performed on computer or via computer simulation » **[2]**;the term *in silico* indicates the use of individualized computer simulation in the development or regulatory evaluation of a medicinal product, medical device, or medical intervention **[3]**;*In silico*studies provide a good platform to estimate the applicability of various virtual screening methods in the assessment of a desired biological activity.

In silico studies are based on the **QSAR** concept (Quantitative Structure Activity Relationship) that can then be utilized to help guide chemical synthesis. QSAR is a technique that quantifies the relationship between structure and biological data and is useful for optimizing the groups that modulate the potency of the molecule. It can provide an estimate of the highest potency expected of a molecule in series or can provide information on whether all parts of the molecule are in close contact with the binding site. In addition the concept of druglikenesshas been introduced to determine the characteristics necessary for a drug to be successful. It helps to optimizepharmacokinetic and pharmaceutical properties **[5]**.

One of virtual database of commercially available natural products is (MEGx library) supplied by AnalytiCon Discovery GmbH (Potsdam, Germany) consisting of 4803 natural compounds (5) served as the basis for the identification of new stimulators or inhibitors of PenecillinBnding Protein a1 (PBPa1), which isresponsible of bacterial cell vitality **[6]**. This enzyme is one of the classical target enzymes for b-lactam antibiotics.

MEGx has been studied by many researchers using many integrated web based *insilico* tools, with several proteins, but the first time with **PBPa1**.

The aim of our dissertation is to

- To screen out the biologically active phytochemical compounds available in the database.
- *In silico* docking studies of the compounds with **PBPa1.**

- To analyze the druggable properties of the compounds using bioinformatics tools.

In order to get fixed aim and objectives, the overall structure of the study takes the form of three chapters, including:

- Chapter one that begins by laying out the theoretical dimensions of the research, in order to understand different molecular interactions, what Docking is, the approaches used in our thesis;
- Chapter two is concerned with the material and methodology used for this study;
- Finally, the third chapter presents the findings and discussion of the research.

Chapter I

Literature review / Virtual screening

A drug is a substance that is used to treat or prevent a disease. A key protein that is involved in a metabolic or signaling pathway affected by the disease usually mediates the actions of a drug molecule. Proteins form anessential group of macromolecules that participate in many important processesinside the cells. Examples of proteins include enzymes that catalyze chemical lreactions, receptors that mediate signals, or structural proteins that providestructural support. Because of the versatility of proteins, the drug molecules canalso produce their effects in different ways. A drug can for example work as anantagonist and inhibit enzymes or receptors by binding to the active site andthereby preventing the action of the natural substrate. Conversely, a drug can bean agonist by promoting the action of the target protein.

I.1 Drug discovery:

The drug discovery is the process by which drugs are discovered it involves many process, the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. Drug design is the approach of finding drugs by design, based on their biological targets **[7]**.

The research and development process often used in the pharmaceuticalindustry to develop new drugs are illustrated in (**Figure 01)**.

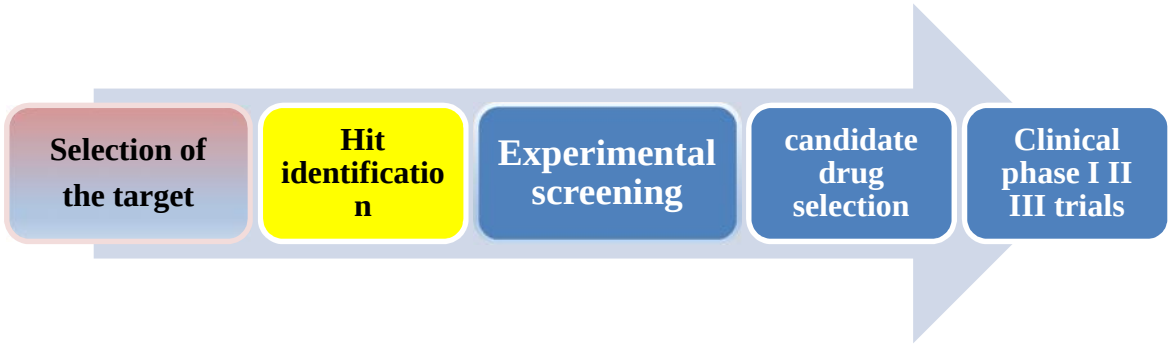

Figure 01: New drug discovery process**[8].**

The process cost of a new drug is between $ 500 million and $ 2 billion**[9]**, and the time from a potential drug candidate is discovered to it reaches thepharmacy shelves can be more than 10 years **[10]**.

In order to minimize the cost and the money an alternative approach is used which is virtual screening (VS).

I.2. Virtual screening:

Virtual screening or sometimes, high throughput virtual screening (HTVS)is becoming a standard procedure in drug development; it has its roots in computational chemistry and in

structural biology. In the1970s,the development of structural biology and the growing availability of atomic structures of diverse proteins, led to the hope that it would be possible to identify new medicines by first solving the structure of the potential drug target at the atomic level and then using this information to design small molecules that had the required effect**[11]**. There are already drugs on the market for which the initial discovery can betraced to a successful virtual screening campaign, such as the anticoagulantAggrastat (tirofiban), which was found with a pharmacophore model, and the HIV-integrase inhibitor Isentress (raltegravir)**[12]**.

Virtual screening, is a computationally approach that screens large libraries of chemicals for compounds that complement targetsof known structure, and experimentally test those that are predicted to bind well.Even with its current limitations, virtualscreening accesses a large number of possible new ligands, most of which may then besimply purchased and tested. For those who can tolerate its falsepositiveand falsenegativepredictions, virtual screening offers a practical route to discovering new reagents andleads for pharmaceutical research **[12, 13]**.

Virtualscreening can roughly be divided into two distinct areas of research: ligand-based andreceptor-based. In the ligand-based approach pharmacophores can be used. These areessential features of a known natural ligand, e.g. charge distribution, shape orhydrophobicity. The compound database is then searched for compounds that have apharmacophore close to the natural ligand. In the receptor-based approach a moleculardocking program is used to dock all the compounds in a database to a specific known target protein (with known 3D-structure)**[14].(Figure 02)**

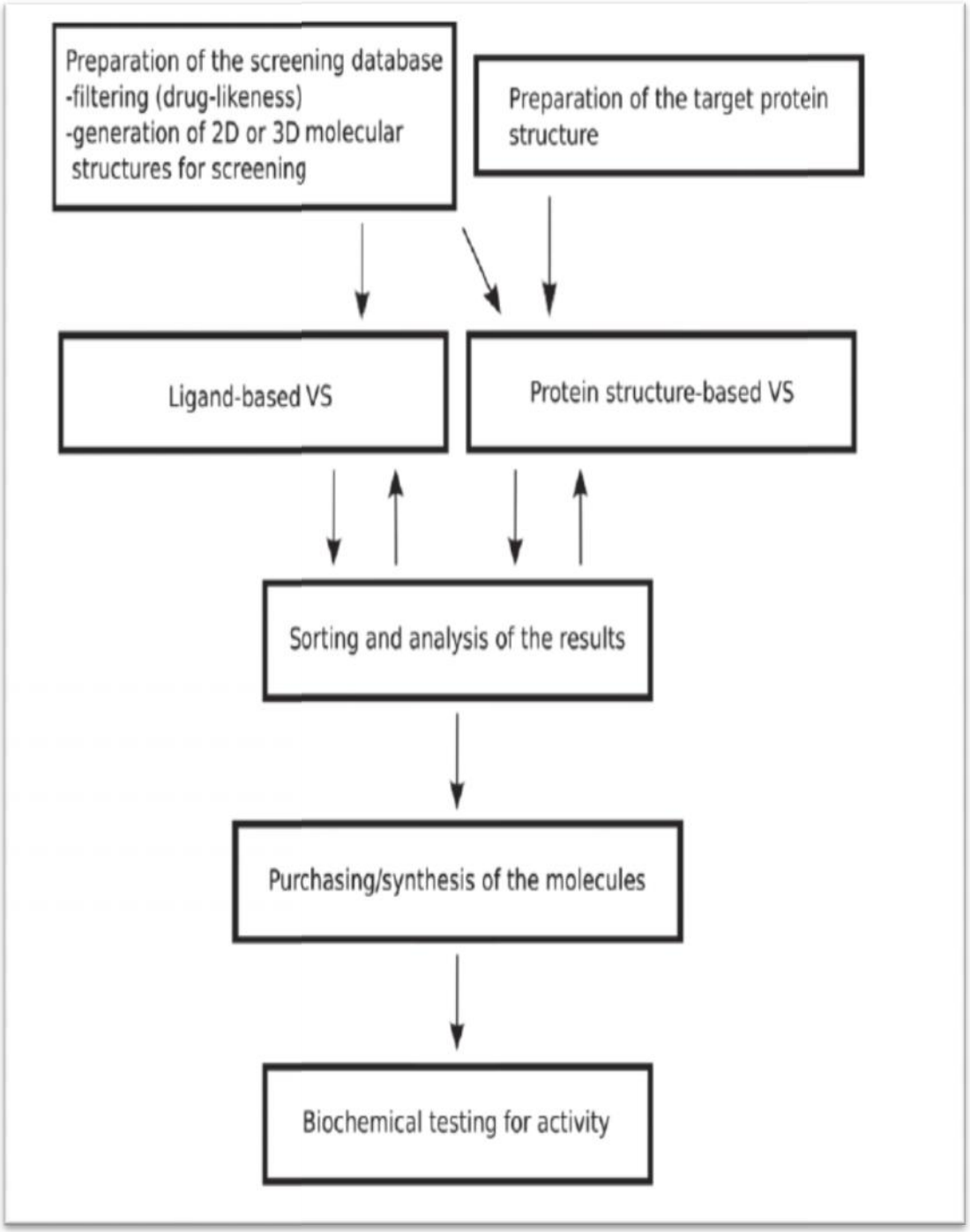

Figure 02: An overview of virtual screening **[14].**

I.3.1 Structure-based virtual screening:

Structure-based virtual screening (SBVS) is the prediction of binderstotarget proteins through computational methods, using the known 3Dstructure of these targets. Alexander Crum-Brown wrote in 1869, “There can be no reasonable doubtthat a relation exists between the physiological action of a substance and its chemicalconstitution, understanding by the

latter the mutual relations of the atoms in the substance**[15]**.The basic approach in SBVS is to predict thebinding pose of each small molecule in a test library (docking), and fromthat predict the free energy of binding of that molecule (scoring) **[08]**. Itemploys the idea of 'similar property principle which states that similar compounds should have similar biological activity **[16]**. The similarity between molecules is oftenevaluated with different kinds of molecular descriptors, which define theproperties of the molecules. These descriptors can be divided into categories based on their dimensionality: D1, D2 and D3.

- Dimensional (1D) descriptors can be calculated from the chemical formula of the molecule. These descriptors include such bulk properties as molecular weight and the number of specific atoms in the molecules.
- Dimensional (2D) chemical structures of the molecules is used to calculate many properties such as substructure and connectivity information.
- Dimensional (3D) is molecular descriptors of solvent-accessible surface area or 3D pharmacophore properties.

The 1D cannot be used alone in VS but in combination with 2D and 3D. The most common VS application uses 2D and 3D fingerprints **[17]**.

Many retrospective virtual screening studies have shown that ligand-basedmethods can often identify the known active molecules with high precision**[18]**. However, the results dependheavily on the available template molecule(s) **[19]**. The 2Dfingerprint methods by default tend to select molecules that have similartopology as the molecules used as targets **[18]**. Therefore, 2D fingerprints might not be the best choice for screening ifscaffold hopping is the primary goal. 3D similarity methods, however, areexpected to perform better in recognizing active molecules with diversescaffolds, because they consider the volume of the molecules, not the topology **[20]**.

The receptor based virtual screening faces several fundamental challenges, including sampling the various conformations of flexible molecules and calculating absolute binding energies in an aqueous environment. Nevertheless, the field has recently had important successes: new ligands have been predicted along with their receptor bound structures in several cases with hit rates (ligands discovered per molecules tested) significantly greater than with high throughput screening **[12]**.

I.3.2 Structure-based methods:

The most widely used method for structure-based VS is molecular docking. In docking, the ligands are fitted into the binding site of the targetprotein, and the

complementarity of steric and chemical properties is evaluated.The ligands can be treated either rigidly or flexibly, whereas the protein structureis usually static. The prediction of the best binding mode and the binding affinityto the protein is accomplished with scoring functions. There are three typesofscoring functions: force field based, empirical, and knowledge based. Force fieldbased scoring functions employ molecular mechanics force fields. In thesescoring functions, the Van-der-Waals (VdW) and electrostatic interactions arecalculated between the ligand and the protein, and additionally intramolecularenergies can be included in the scores. Empirical scoring functions areparameterized to reproduce experimental data, such as binding affinity orconformations. These functions frequently include individual terms for hydrogenbonds, ionic interactions, hydrophobic effects, and entropy. Knowledge based scoring functions have been developed by statistical analysis of protein-ligandstructural data. The assumption is that atoms or functional groups foundfrequently in close proximity to each other are energetically favorable, andtherefore contribute favorably to the binding affinity. Some scoring functions usea combination of the scoring function types described above **[20]**.

Structure-based virtual screening has evolved over the past decade,andmany different variations of the basic methodology have been proposed.Literally hundreds of reviews have been published on virtual screening. Oneof the more recent ones, from March 2008,6 covers the current state of the artand many of the practical aspects of SBVS.

The difficulty lies in how completely we describe thegeometric arrangement, the properties and the interactions between the atoms in the virtual model **[08,12]**.

I.3.3. Pharmacophore modeling:

A pharmacophore model includes the features required for molecular recognition between the small molecule and the macromolecular target.These features include steric properties and chemical properties, such ashydrogen bond donors or acceptors, hydrophobic interactions and ring structures. The pharmacophore model can be derived either from known ligands,where several different known active molecules are used to identify the commonimportant features, or from the target protein structure. However, the creation ofa reliable pharmacophore model can be challenging and time-consuming **[21]**. To overcome the problem of complex pharmacophore models, PHASE**[22,23]**; offers a ligand-based pharmacophore modeling method inwhich the biological activities of the molecules can be incorporated into thegeneration of the model. A threshold activity value can be set to affect thecomplexity of the resulting pharmacophore model. This approach can also createa 3D

quantitative structure-activity relationship (3D-QSAR) model for theprotein system under study, which can be usedto find correlation betweenstructural differences in the molecules and their biological activities **[12]**.

I.4. 3D quantitative structure-activity relationship:

3D-QSAR is a statistical method to find correlations between experimentalbinding data and structural properties of molecules. Common uses for 3D-QSARmodels include the prediction of binding affinities for new structures, thedetection of regions that may have significant effects on activity, and providing information of the important interactions between the small molecule and theprotein. For the generation of a 3D-QSAR model, active molecules with knownaffinitiesare needed. Some methods require that the molecules are aligned witheach other. The alignment can be made for example by superimposing themolecules in order to find the best common 3D alignment, or the alignment canbe made by using information from protein structures, such as by using multipleprotein crystal structures or by docking the molecules into the binding site. Thevalidity of the model can be tested by predicting the activities of a set of ligandsnot present in the training set.

I.5. Molecular docking:

Molecular docking is a computational tool commonly applied in drug discovery project and fundamental biological studies of protein-ligand interactions. Traditionally, molecular docking is used to address one of the three following questions:

- Given a ligand molecule and a protein receptor;
- Predict the binding mode (pose) of the ligand within the context of a receptor;
- Screen a collection (library) of small-molecules against a receptor and identify potential active ligands, and given a ligand molecule and a target receptor;
- Predict the binding affinity of the two **[24]**.

In essence, this is equivalent to finding the global free energyminimum of the system consisting of the ligand and the target.

Kuntz et al published the first algorithm developed to dock small molecules into the binding pocket of the macromolecule, the DOCK algorithm; in 1982 and in 2007 more than 60 algorithm programs and 30 scoring functions were listed**[08]**.

I.6. Scoring functions:

Scoring functions are currently the bottleneck of docking algorithms. A good scoring function should be able to dierentiate active and non-active ligands, identify correct poses, and estimate binding energy. Free energy calculations using molecular dynamics simulations

may provide better accuracy but they are extremely slow and thus inadequate for evaluating large molecular libraries. Scoring functions implemented in docking algorithms tend to simplify or ignore complex physical and chemical terms such as entropy or hydrophobicity. Nevertheless, physical descriptors that correlate with these complexterms may be used instead (e.g., number of rotational bonds instead of entropic loss).Scoring functions can be divided into the following three categories:

I.6.1. Force Field:

Force field based functions approximate the strength of interaction by accounting for the electrostatic and Van-der-Waals interactions between all pairs of atoms.

I.6.2. Empirical:

Empirical functions use a set of physical and chemical descriptors to accountfor the interaction between a ligand and a receptor. Terms such as numberofhydrogen-bonds, hydrophobic contact, and number of rotational bonds may be used to approximate entrapic and entropic contributions. The coefficients of thesetermsare usually determined by multiple linear regressions using a dataset of bindingenergies for protein-ligand complexes**[25]**.

Chapter II

Materials and Methods

II.1.Material

II.1.1. The macromolecule:

PBPs are divided into three classes called A, B and C. Those belonging to class A and B arehigh molecular mass (mass > 60 kDa) PBPs, while class C PBPs have lower molecular mass. Only the isoform of PBPa1 in ***Streptococcus pneumonia***is the subject of the present study **[06].**

II.1. 2. Websites:

II.1. 2.1. Molecular databases:

A virtual database of commercially available natural products (MEGx library) supplied by AnalytiCon Discovery GmbH (Potsdam, Germany) consisting ofnatural compounds served as the basis for the identification of new bioactive (inhibitorsor stimulators) of PBP1. The database is available on https://ac-discovery.com/megx-purified-natural-product-screening-compounds**[26]**.

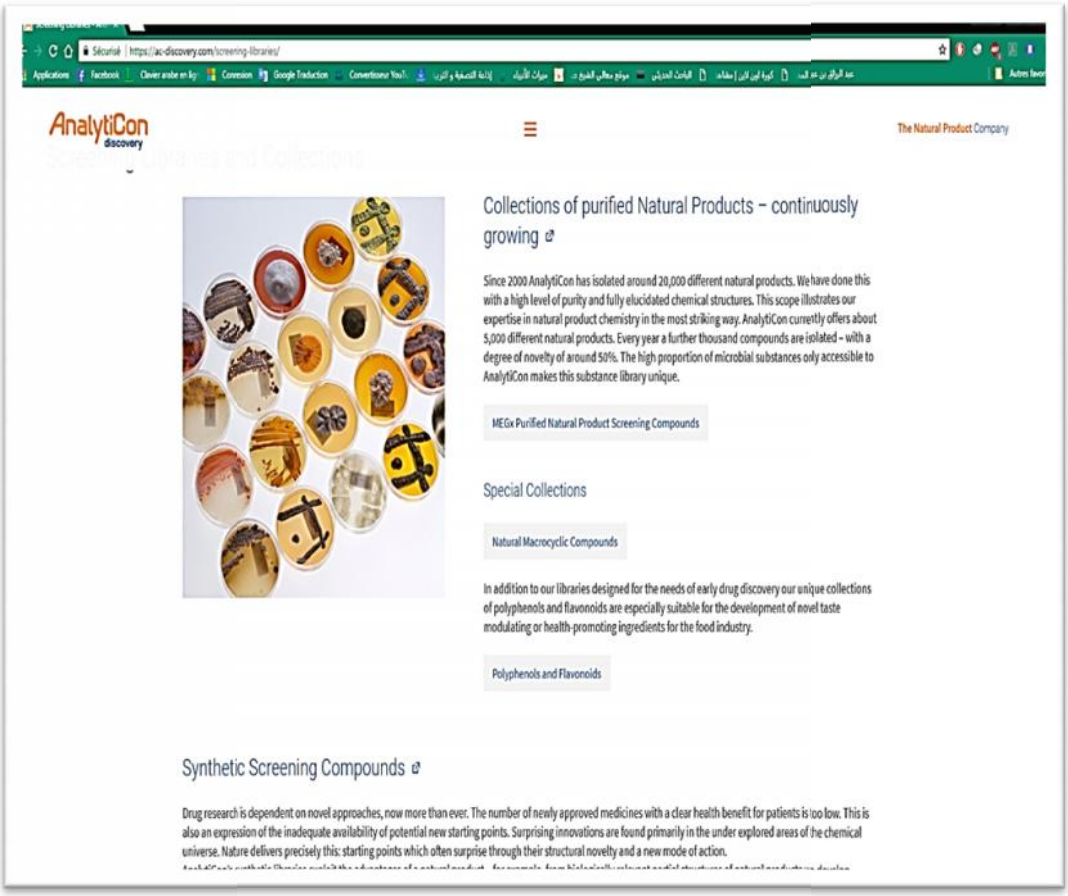

Figure 03: Home page of AnalyticonMEGx database**[26]**.

II.1. 2.2. PDB:

The Protein data bank is free to access on: http://www.rcsb.org/pdb/home/home.do**[27]**. It is a key resource in areas of structural biology, and it is a fundamental repository for3D structure data of large molecules.

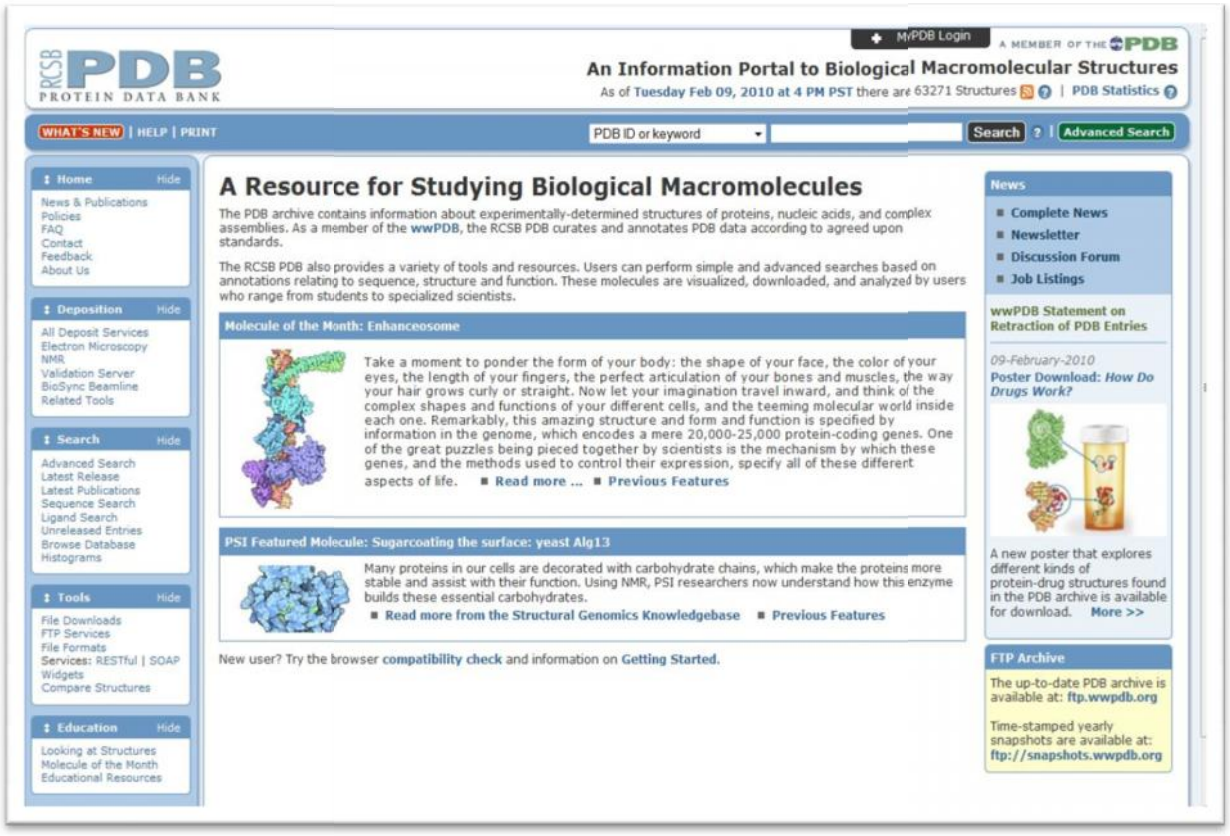

Figure 04: Home page of Protein Data base**[27]**.

II.1. 2.3. Molinspiration:

Molinspiration is a chemical informatics online server available on http://www.molinspiration.com**[28]**. It was founded in 1986 as a spin-off of Bratislava University, it offers broad range of cheminformatics software tools supporting molecule manipulation and processing in the present study Molinspiration was used to estimate molecular properties and druglikeness (bioactivity).

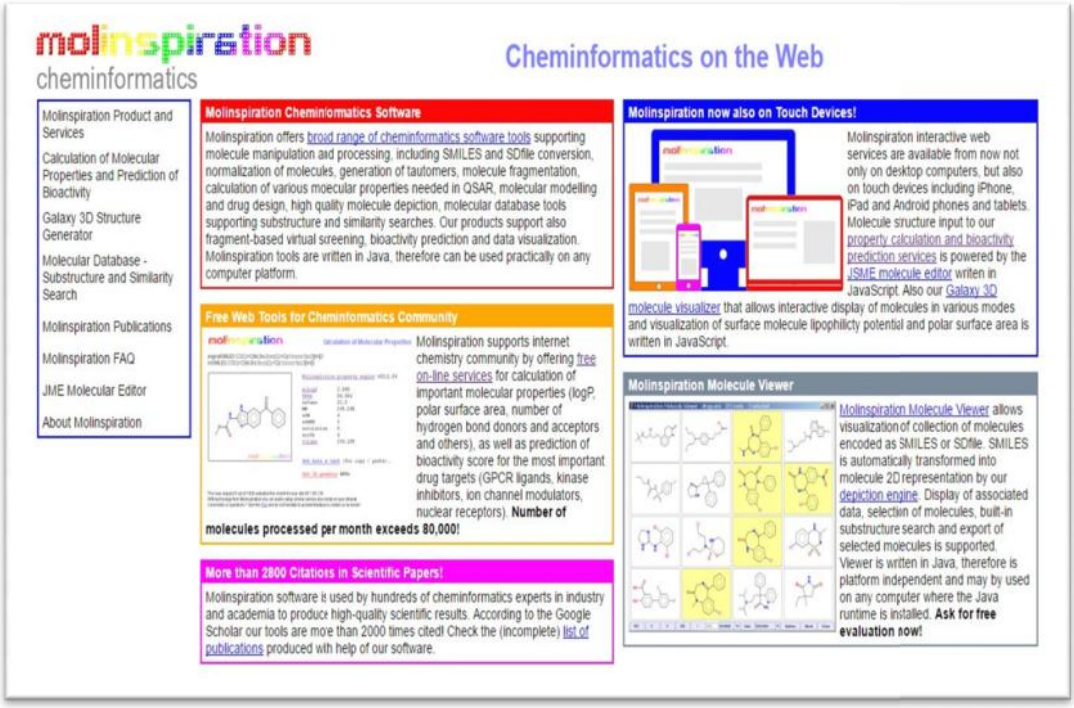

Figure 05: Molinspiration interface**[28]**.

II.1. 3.Software:

II.1. 3.1. PyRx:

Virtual screening of the first 500natural compounds retrieved from MEGx database with PBP1 is carry out using PyRx tool.

PyRx is open source software to performvirtualscreeningfor Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables Medicinal Chemists to run Virtual Screening from any platform and helps users in every step of this process from data preparation to job submission and analysis of the results. It is a combination of several softwares such as AutoDockVina, AutoDock 4.2,Mayavi, Open Babel, etc. PyRx uses Vina and AutoDock 4.2 as docking softwares **[29]**.

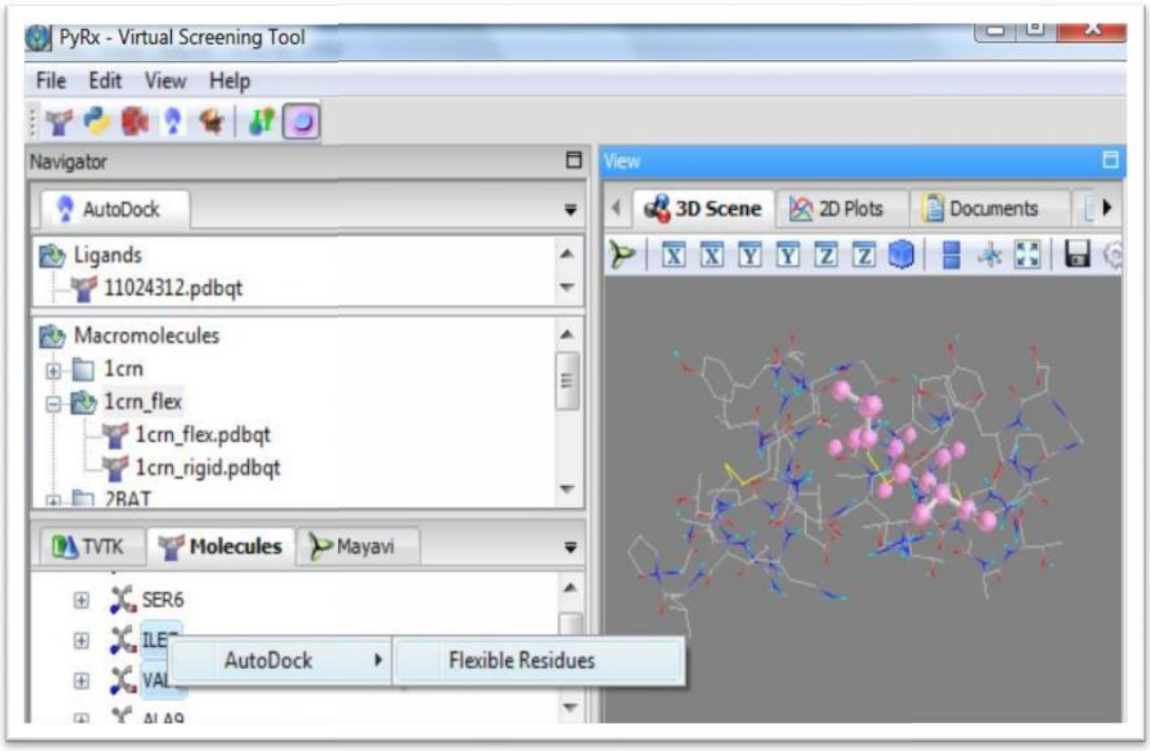

Figure 06: The plat form of PyRx.

II.1. 3.2. Open babel:

Open babel is a chemical toolbox designed to speak the many languages of chemical data. It's an open, collaborative project allowing anyone to search, convert, analyze, or store data from molecular modeling, chemistry, solid-state materials, biochemistry, or related areas.

II.1. 3.3. AUTODOK VINA:

Autodock is a suite of automated docking tools. It is designed to predict the binding of small molecules, such as substrates or drug candidates, to a receptor of known 3D structure. It performs the docking of the ligand to a set of grids describing the target protein; autogrid pre-calculates these grids**[30]**.

II.1.3.4. PyMOL:

PyMOL is a molecular visualization software, created by Warren Delano. In addition to offering many possibilities of 3D display, the PyMOL software allows to perform animations, alignments of structure, generate structures ... etc. It is one of the most used tools in scientific publications. This is a free and open source software. A free access is available on: http://www.pymol.org**[31]**.

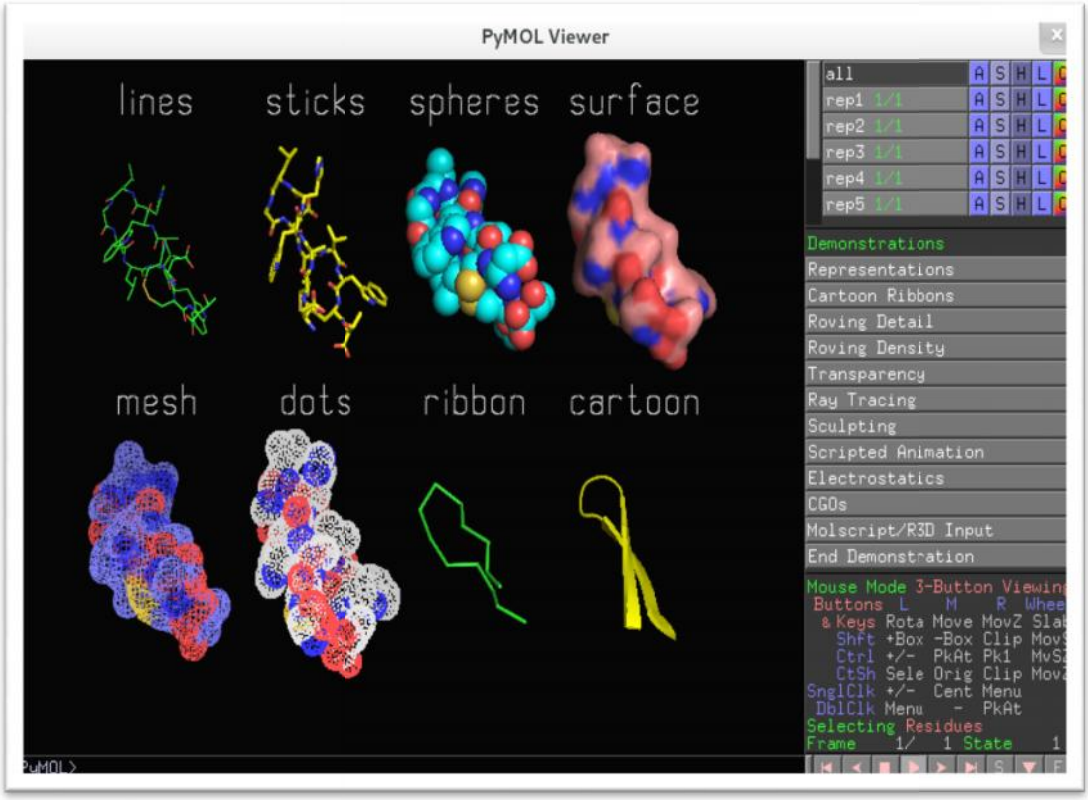

Figure 07: Pymol visualization interface

II.2.Methods:

II.2.1. Downloading the protein:

The download of the penicillin binding protein was made from the protein data bank **pdb** (access code 2v2f) it is co-crystallized with the inhibitor **MES** (C_6 H_{13} N O_4 S).

The three-dimensional structure of penicillin binding protein- 1A was obtained by diffraction in X-ray with a resolution (1.9 Å).The co-crystallization data are summarized in the **table 01**

Table 01:PBPa1 crystallographic data

Classification	**Resolution**	**chain**	**ligand**
Transferase	1.9 Å	A	**No Ligand**
		F	1-Ba^{2+} :**(BARIUM ION)** 2-MES: **(2-(N-MORPHOLINO)-ETHANESULFONIC ACID)**

Only the chain F contains the active pocket, where the MES founded as inhibitor and for this reason the chain A is eliminated in this study.

II.2.2. VS molecular docking:

II.2.2.1.Creating groups:

The first 500 molecules are devised into groups, each group contain 50 molecules.

II.2.2.2. Preparing *.pdbq to ligand files using Openbabel:

The *.pdb file of each lead compound downloaded from MEGx was repared using Openbabel. After that all lead compounds of each group are minimizedand converted into *.pdbqt, this necessary minimization step avoids that an energy the molecular assembly from the very beginning of the molecular dynamics production; the format*.pdbqtis needed for AUTODOCK input after wards. Prior to docking.

AUTODOCK automatically assigned the type of each atom and detected the root (the rigid part of the ligand) also, the number of rotatable bonds that move was assigned via torsions option in the software.

II.2.2.3. Preparation of the Grid Parameter File:

The receptor file was retrieved from the Protein Data Bank (PDB) database in (*.pdb) format. Water molecules were deleted in addition to any odd molecule or a chemical entity found on the surface of our macromolecules, all hydrogen atoms were added to before the Gastiger partial charges were assigned and then saved. Finally, the macromolecule file was saved in (*.pdbqt) file format.

II.2.2.4.Preparation of the Docking Parameter File:

The grid box was prepared with pre-calculated dimensions; where the suitable grid size was determined, according to flexible residues, the grid spacing was modified depending on the size of the old inhibitor of PBPa1 already downloaded from PDB, this space refer to the active pocket of the enzyme. The grid file was located in a place on the receptor surface where the expected ligand- receptor interaction occurs.

II.2.2.5. Preparation of the Macromolecule File:

II.2.2.5.A.Docking with the exhaustiveness 8:

After preparation of the input files (ligand and protein) and the calculation of the affinity maps, also setting the exhaustiveness 8, docking runs were conducted using AUTODOCK VINA software using the Lamarckian Genetic Algorithm docking with the resultant structure files of AUTODOCK VINA **[4]**.

Docking can be carried out by various methods. But, the most efficient method is Lamarckian genetic algorithm. AUTODOCK was run several times to get various docked

conformations, and used to analyze the predicted docking energy. The binding sites for these molecules were selected based on the ligand-binding pocket of the templates **[32]**.

As a sum up, the working steps are:

The work starts with adding the enzyme file in a *.pdb format, then all the odd molecules and artifacts that can be found on the enzyme (from former research) were deleted in addition to water molecules, hydrogen atoms were added automatically and saved in (*.pdbqt) file format.

For the charges, Kollman charges were added and Gasteiger charges were computed, afterwards the ligand file was added in a pdb after converting it using Openbabel then saved as it is without any modifications in a *.pdbqt format.

The macromolecule and the ligand were chosen in the grid, and both were saved in a *.pdbqt format, the grid box was selected with the maximum surface that takes in all the parts of the enzyme which is the AUTODOCK's work and prediction space, to find the most stable complex, the grid box is where AUTODOCK computes the possible interaction points in the binding site of the protein prior to exploring the conformations, finally the grid file was saved in *.gpf file.

Same for the docking, the mocromolecule and the ligand were selected, the research parameters were set as default as genetic algorithm, then a *.dpf file was saved for Lamarkian parameter.

The final operation was launching autogrid and when it finishes, AUTODOCK was launched.

The conformations results were ranked based on the highest docking scores with energy minimization values of the above drug –target interactions and virtual screening results of the PyRx tool, the phytochemicals, were screened outfor molecular docking analysis with respective target proteins.

II.2.2.5.B. Docking with the exhaustiveness 24:

After finishing the work with exhaustiveness 8 to screen lowest energies, the four molecules, in addition to the original ligand downloaded with PBPa1 are the subject of another screening, launched with exhaustiveness 24 for more precision, using AUTODOCK VINA following the previous steps.

II.2.2.6.Finding molecular properties and druglikeness:

Smiles notations of the selected compounds submitted were fed in the online Molinspiration in order to calculate molecular properties (Log P, Total polar surface area, number of hydrogen bond donors and acceptors, molecular weight, number of atoms, number of

rotatable bonds etc.) and prediction of bioactivity score for drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors).

Chapter III

Results and discussion

This study attempts to screen out the biologically active phytochemical compounds available in the MEGx database and to analyze the druggablilityof the natural compounds.In this chapter results and discussions are developed.

III.1.Penecillin Binding Protein a1 (PBPa1):

Streptococcus pneumoniae is responsible for a high proportion of cases of pneumonia, acute otitis media, acute sinusitis, bacteremia, and meningitis, which lead to more than 1 million deaths per year, mostly of young children in developing countries.

This gram-positive pathogen possesses six Penicillin-binding proteins PBPs.This class of enzyme are the classical targetenzymes for b-lactam antibiotics **[33]**.

The penicillin-binding domains of PBPs are transpeptidases or carboxypeptidases involved in peptidoglycan metabolism **[34]**.

They belong to the protein superfamily called ASPRE for Activesite. Serine Penicillin Recognizing Enzymes have the ability to break the amide bond of the cycle B-lactam of penicillins by establishing an ester bond between the catalytic serine of their active site and the antibiotic, resulting in a stable penicilloyl-enzyme complex In the active site of these proteins are present threepreserved catalytic motifs which constitute the characteristic signature of the function oflactam binding**[35]**. the classification of PBPa1 three class A (PBP1a, 1b, and 2a) and two class B (PBP2x and 2b) molecules as well as a d,d-carboxypeptidase, PBP 3. Individual deletion of the pbp2x or pbp2b genes is lethal for S. pneumoniae. However, neither the PBPa1, PBPb1, nor PBPa2gene is required for growth when deleted individually, but the presence of at least pbp1a or pbp2a is essential for cell viability**[36]**.

PBPa1 was the subject of the present study**.(Figure 08)**

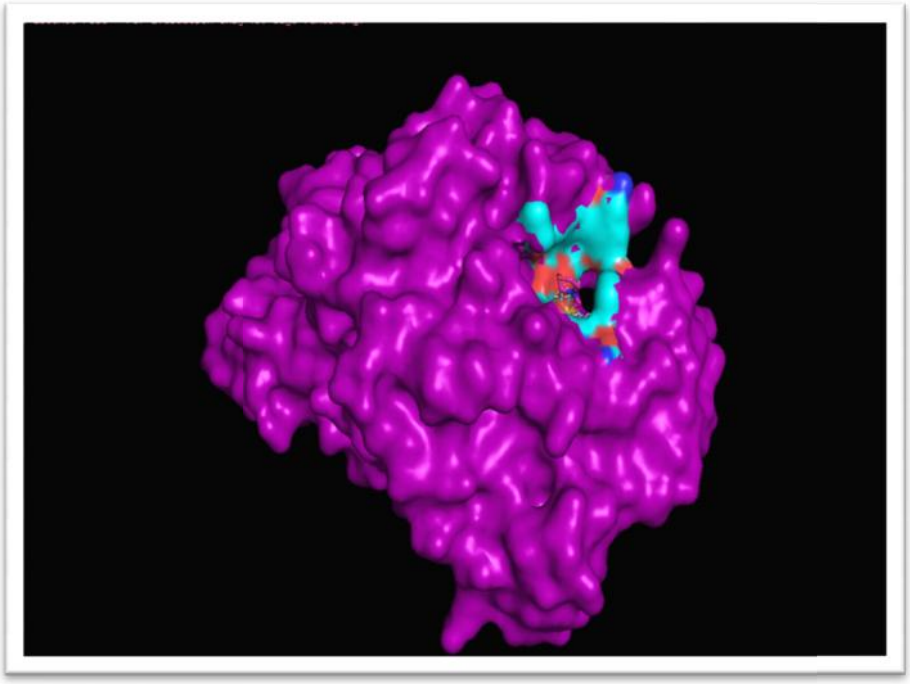

Figure 08: 3D structure of **PBPa1**protein.

III.2. *In SILICO* molecular docking:

III. 2.1. First screening: exhaustiveness 8:

The first screening results using Pyrx with the exhaustiveness 8, of **PBPa1** with the first 500 natural products show that this protein is well docked with the following lead compounds:

- 1 NP-000205
- 2 NP-000206
- 3 NP-000122
- 4 NP-000251

The results are shown in table:

Table 02: Docking results of **PBPa1** with exhaustiveness 8

Ligand	**Formula**	**Binding energy (kcal/mol)**
NP-000205	C30H22O11	-10.5
NP-000206	C30H20O11	-9.8

NP-000122	C22H25N3O3	-9.2
NP-000251	C19H18O5	-8.8

All the results will be discussed in the next step.

III.2.2.Second screening: exhaustiveness 24:

Another level of exhaustiveness was used with the four lead compounds and the original ligand MES downloaded with the protein, in order to get more precision. The second screening was launched using AUTODOCK VINA. Table summarizes the results of docking of 4 lead compounds and original ligand MES. The results are reported in the table below.

Table 03: Docking results (Hydrogen bonds, binding energy, involved amino acids)

Ligand	**The residues in interaction**	**Number of hydrogen bonds**	**Binding energy (Kcal/mol)**
MES	SER 370 SER 428 THR 560 THR 558	4	-5.6
NP-000205	TYR 577 THR 560 PHE 611 GLU 582 GLN 427	5	-11.2
NP-000206	THR 558 THR 560 GLN 427 PHE 611	4	-11.2
NP-000122	TYR 577	1	-9.2
NP-000251	SER 370 THR 560	2	-9.2

III.2.2.1. Docking of PBPa1 with MES:

MES it is chemical formula is $C_6 H_{13} N O_4 S$ corresponding to 2-(N-MORPHOLINO)-ETHANESULFONIC ACID. It is a zwitterionic N-substituted aminosulfonic acid known as a Good's buffer, active in the pH range 5.5-6.7 with a pKa of 6.10 at 25°C. Its chemical structure contains a morpholine ring**[36]**. The MES is foundas a good inhibitor for the PBPa. **(Figure 09)**

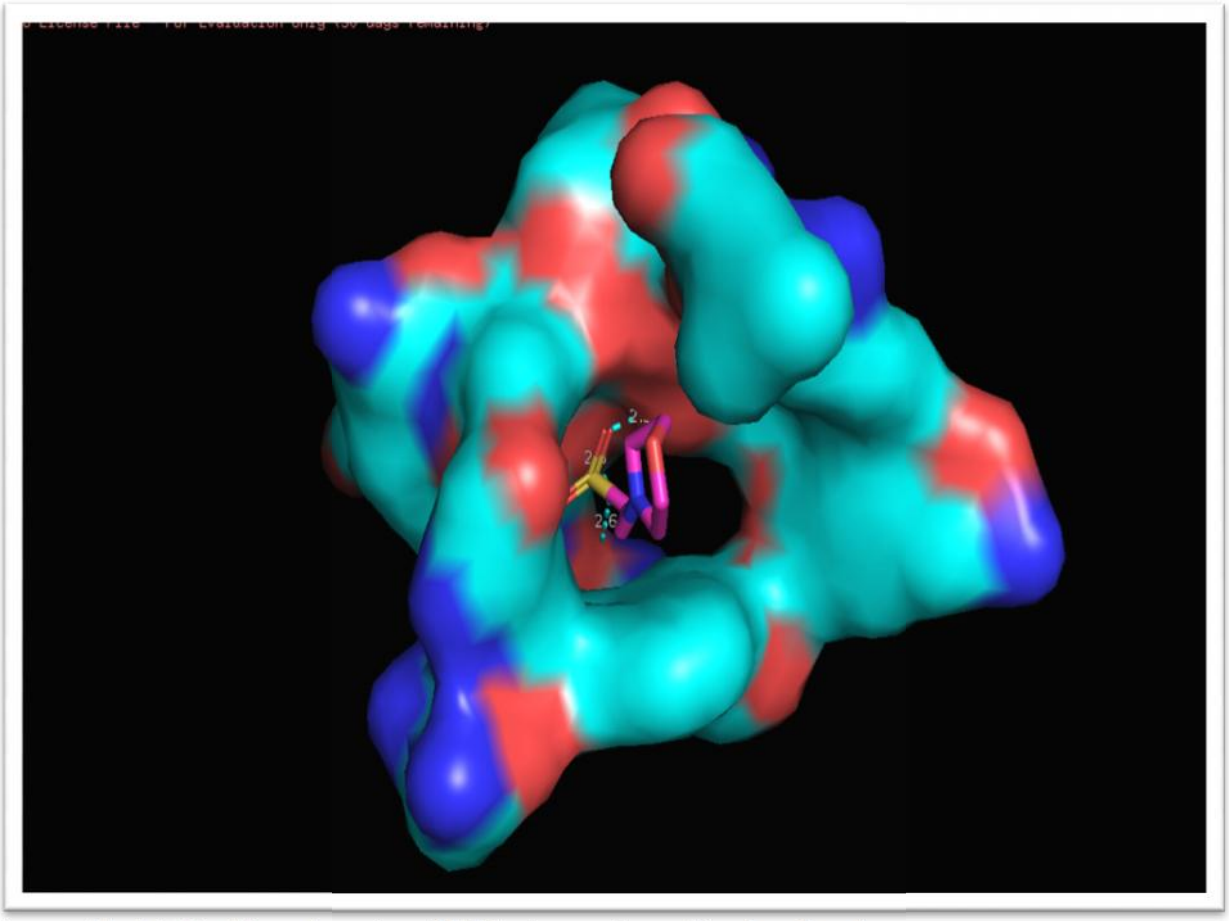

Figure 09: MES with active site of **PBPa1** complex with clear bond

When docked MES donates 4 hydrogen bonds to the backbone carbonyl group of SER 370, SER 428, THR 560, THR 558of 2V2F via4hydrogen bonds. To the **PBPa1** with -5.6 **Kcal/mol**. In the present study, MES will be compared to other molecules docked to 2V2F. **(Figure 10)**

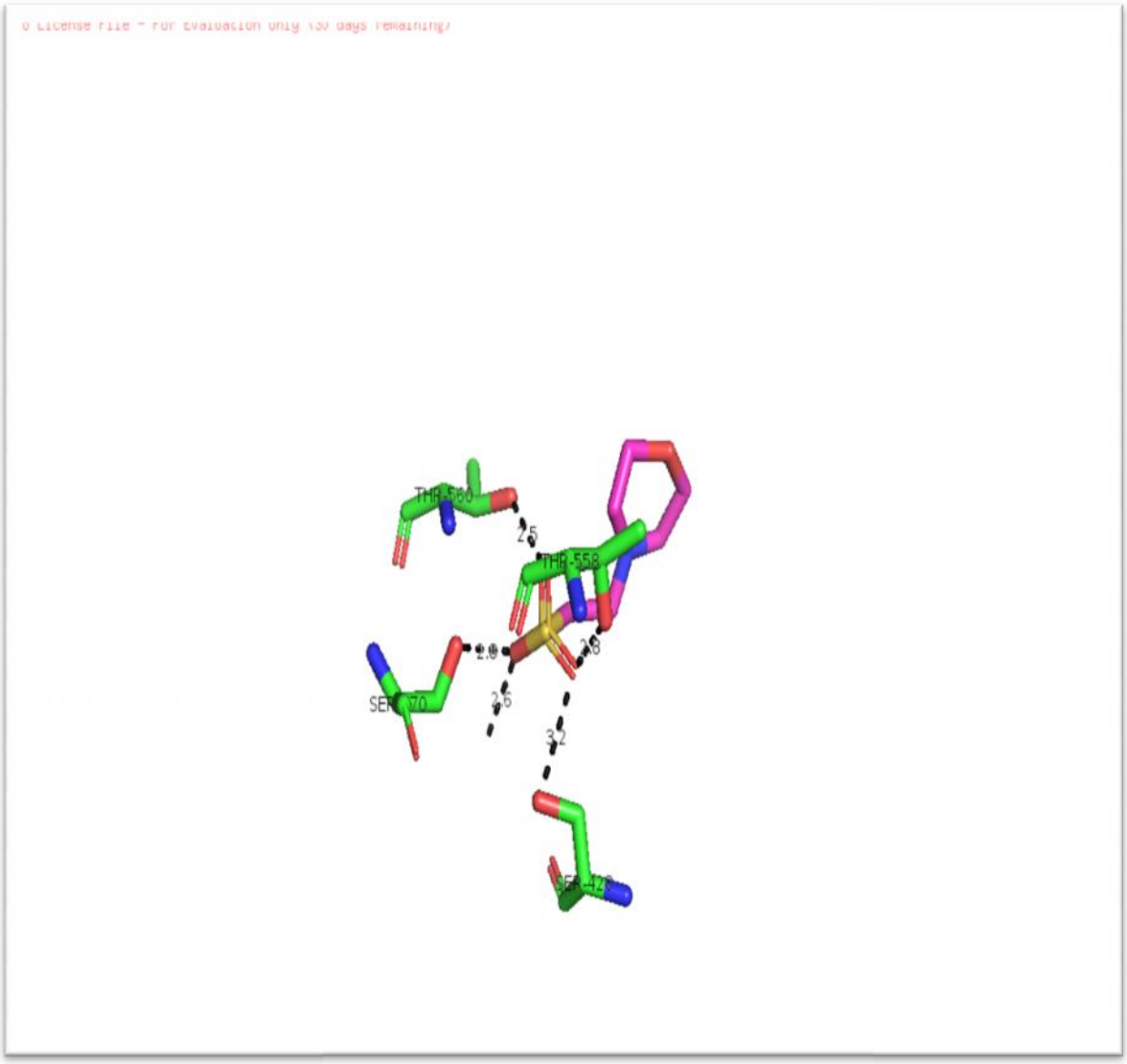

Figure 10: Hydrogen binding mode of MESwith **PBPa1** protein

III.2.2.2. Docking of PBPa1 with NP-000205:

NP-000205=$C_{30}H_{22}O_{11}$, commonly known asBTRRHXATSDHYNW-UHFFFAOYSA-N, which correspond to the 8-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-2,3-dihydrochromen-3-yl]-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one. No much data on line available for this molecule, In this study NP-000205is foundas a good inhibitor for the PBPa1, Using AUTODOCK VINA, NP-000205 is well docked with 2V2F with a high energy value -11.2 **Kcal/mol**. **(Figure 11)**

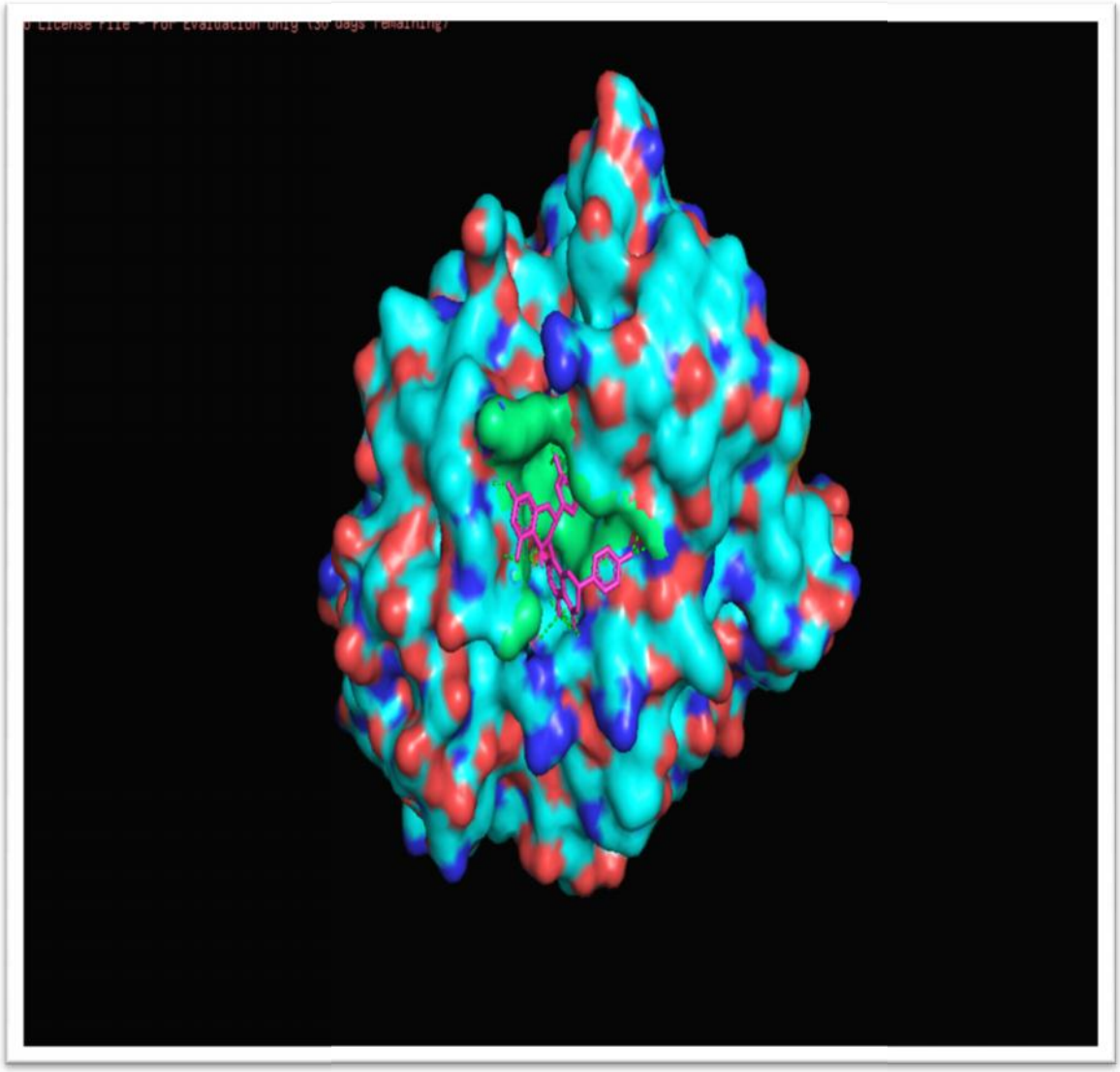

Figure 11: MES with **PBPa1** complex with clear bond

Hydrogen bonds were formed with five carbonyl Os of NP-000205and backbone Ns of the five amino-acids TYR 577, THR 560, PHE 611, GLU 582, GLN 427that belong to which belong to the active pocket. The result is the inhibition of **PBPa1**.**(Figure 12)**

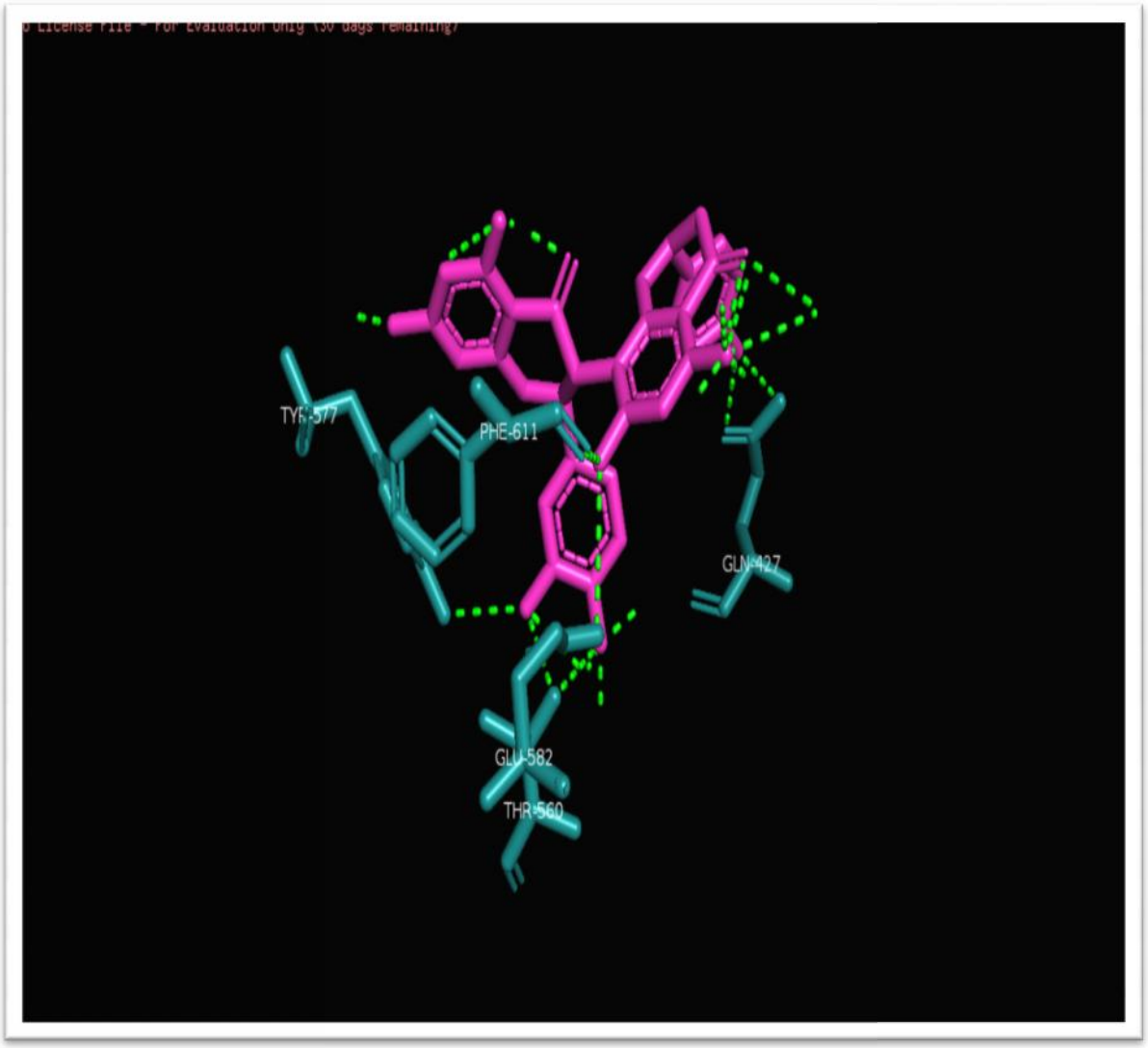

Figure 12: Hydrogen binding mode of NP-000205with **PBPa1.**

III.2.2.3 Docking of PBPa1 with NP-000206:

NP-000206=C30H20O11known as 3'-Hydroxy-Volkensiflavon**[37,38]**.

As the previous molecule no much data founded, the same, it is found that this molecule can inhibit 2V2Fwith a high-energy value -11.2 **Kcal/mol**. **(Figure 13)**

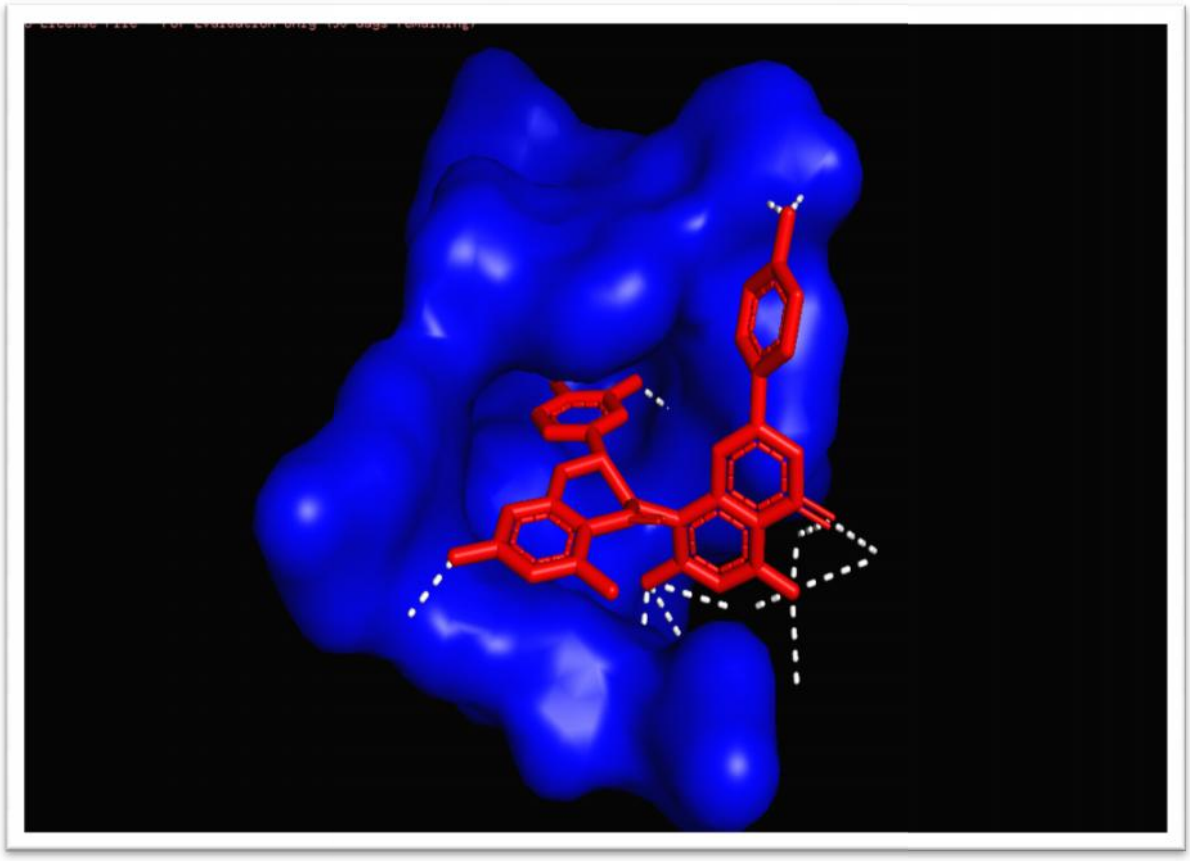

Figure 13: NP-000206 with active site of **PBPa1** protein complex with clear bond.

In additionHydrogen bonds were formed with four carbonyl Os of NP-000206 and backbone Ns of the four amino-acids TYR 577, THR 560, PHE 611, GLN 427that belong to which belong to the active pocket, excluding GLU 582 this result can be explained by the similarity of two molecules.**(Figure 14)**

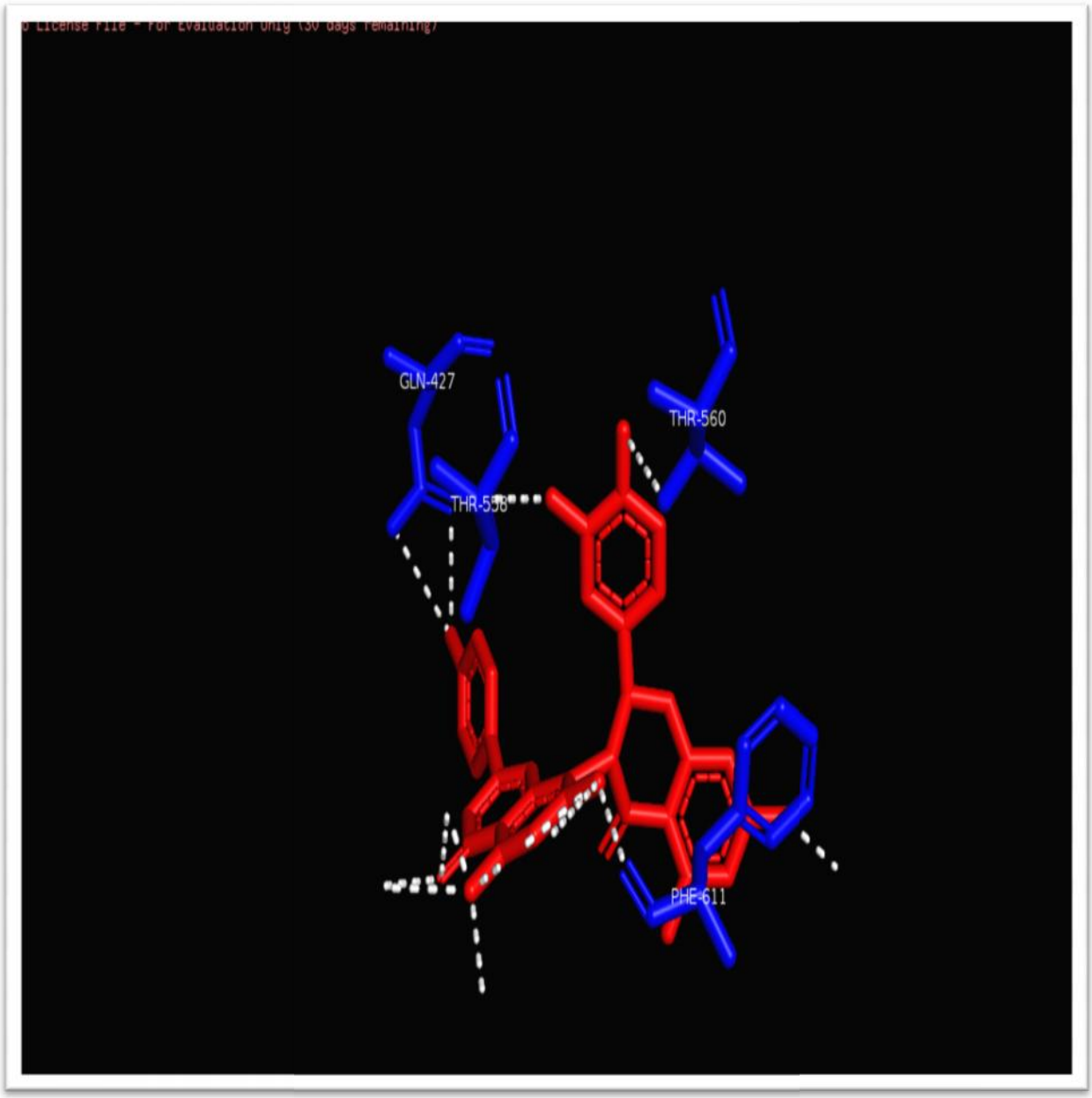

Figure 14: Hydrogen binding mode of NP-000206 with **PBPa1** protein

III.2.2.4. Docking of PBPa1 with NP-000122:

NP-000122 = $C_{22}H_{25}N_3O_3$corresponding to 9-Methoxy-12-(2-methyl-1-propen-1-yl)-1,2,3,5a,6,11,12,14a-octahydro-5H,14H-pyrrolo[1",2":4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione =Fumitremorgin B, it is isolated from *Aspergillus fumigatus* and *Penicilliumpiscarium*this molecule can cause severe tremors and convulsion in experimental animals **[39.40]**.

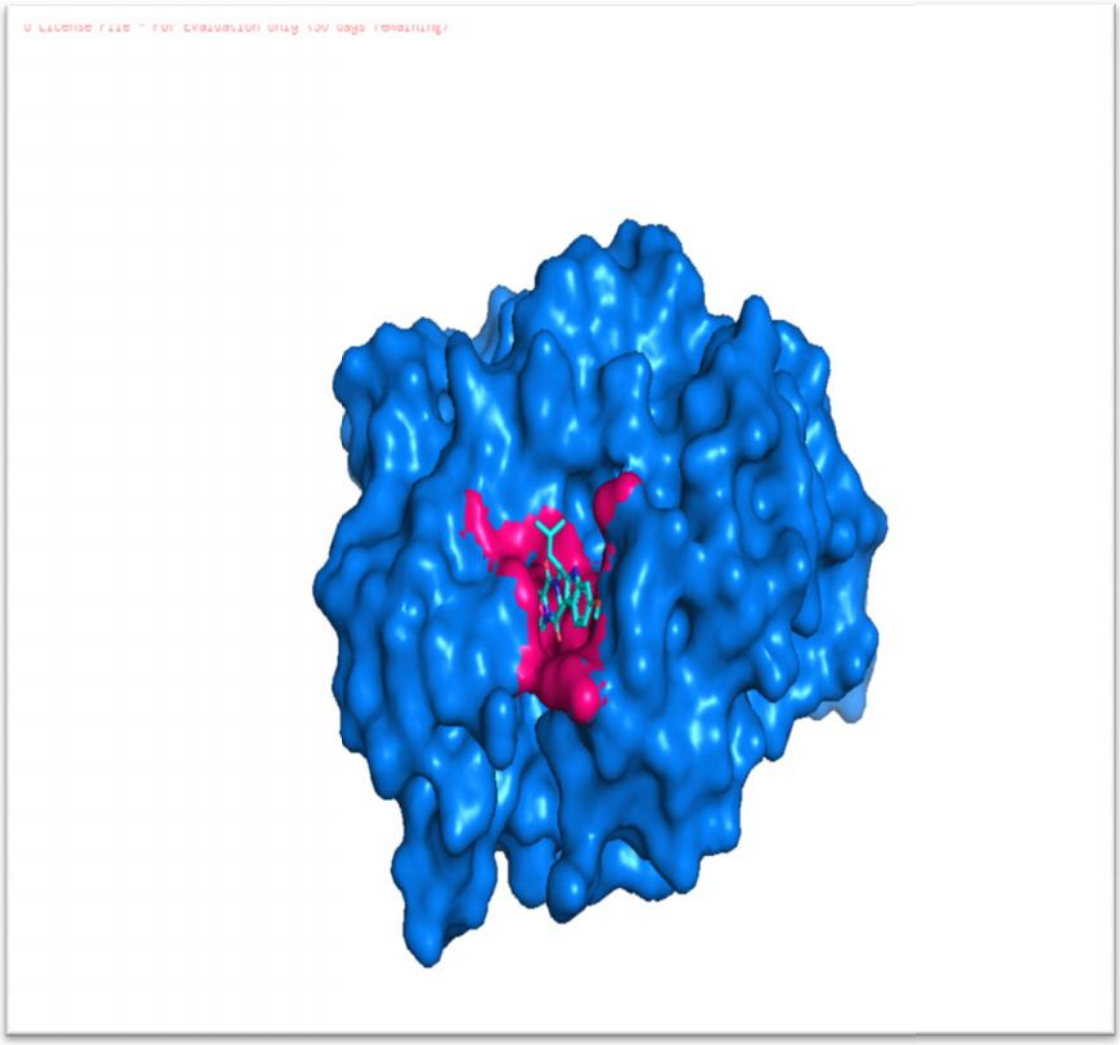

Figure 15:NP-000122 with **PBPa1** protein complex with clear bond

Molecular docking studies revealed an interesting ligand interaction of 2V2F withNP-000122; the binding energy is -9.2 **Kcal/mol**. Three hydrogen bonds were formed onehydrogen bond formed: TYR 577. This result is not found with corresponding amino-acid, Further *in-vivo* work is required to explain this outcome.

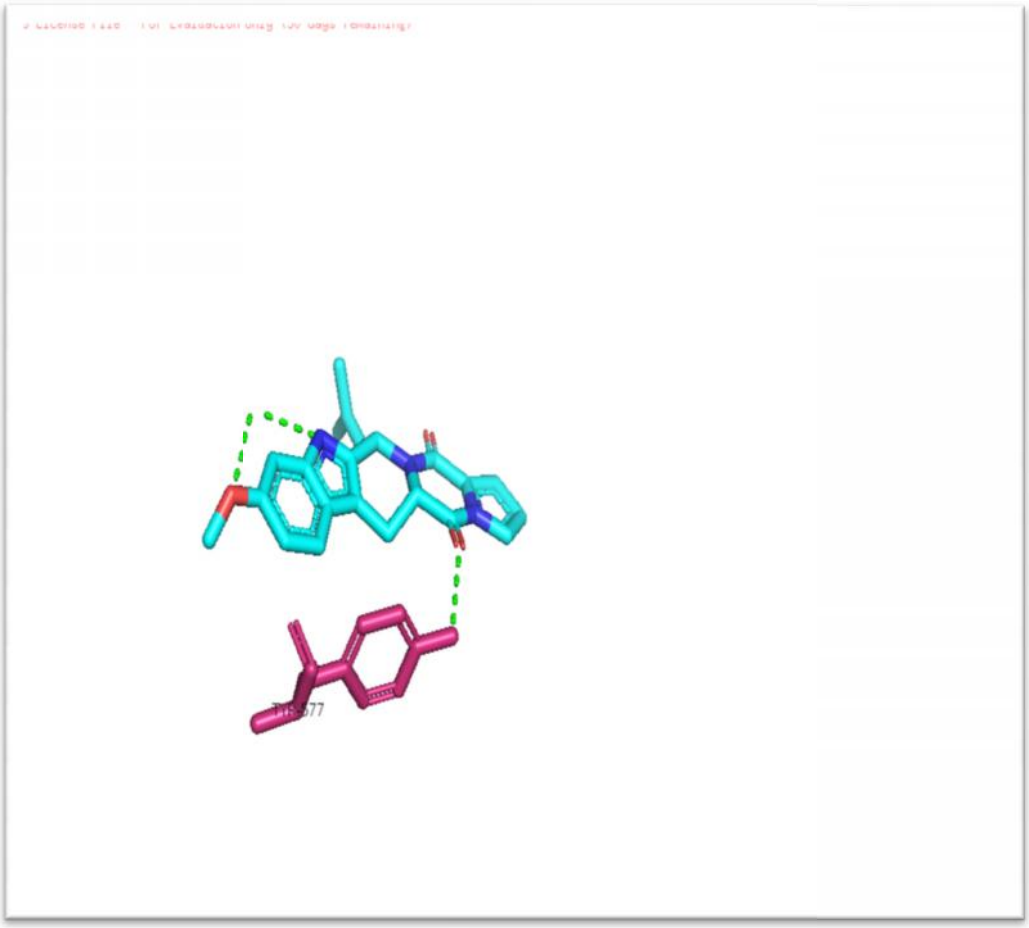

Figure 16: Hydrogen binding mode of NP-000122 with **PBPa1**

III.2.2.5. Docking of PBPa1 with NP-000251:

XIUOIRCLYAZEGD-UHFFFAOYSA: 4-HYDROXY-3-(4'-METHOXYPHENYL)-5-[(4''-METHOXYPHENYL)-METHYL]-FURAN-2(5H)-ONE; this Dibenzylacyloinsis a natural pigment of fungi, its formula is $C_{19}H_{18}O_5$**[41]**.

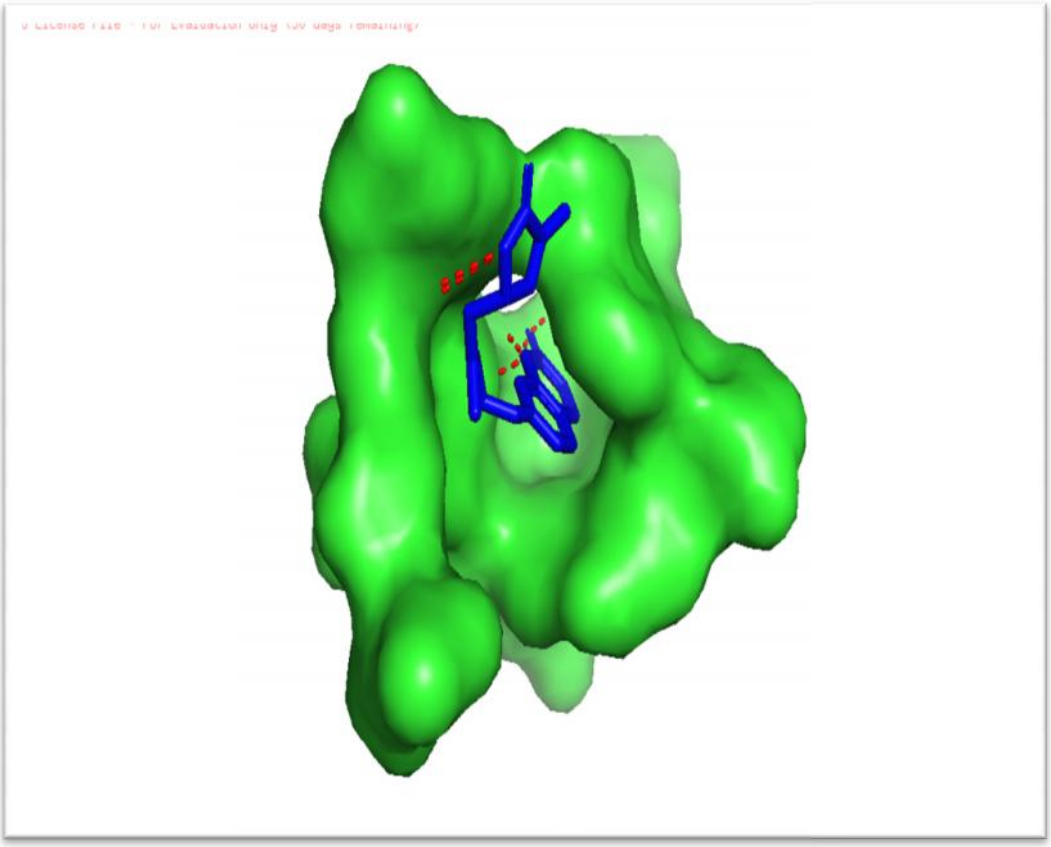

Figure 17: NP-000251 with active site of **PBPa1**complex

NP-000251 binds via 2hydrogen bonds to the key residuesSER 370 and THR 560of **PBPa1**.

These two residues belong to the active pocket. NP-000251 can in consequence inhibit the **PBPa1**. In this study, NP-000251is docked well to PBPa1 with a value of -9.2 **Kcal/mol**,

according to the reference and its confirmation **(6)**.

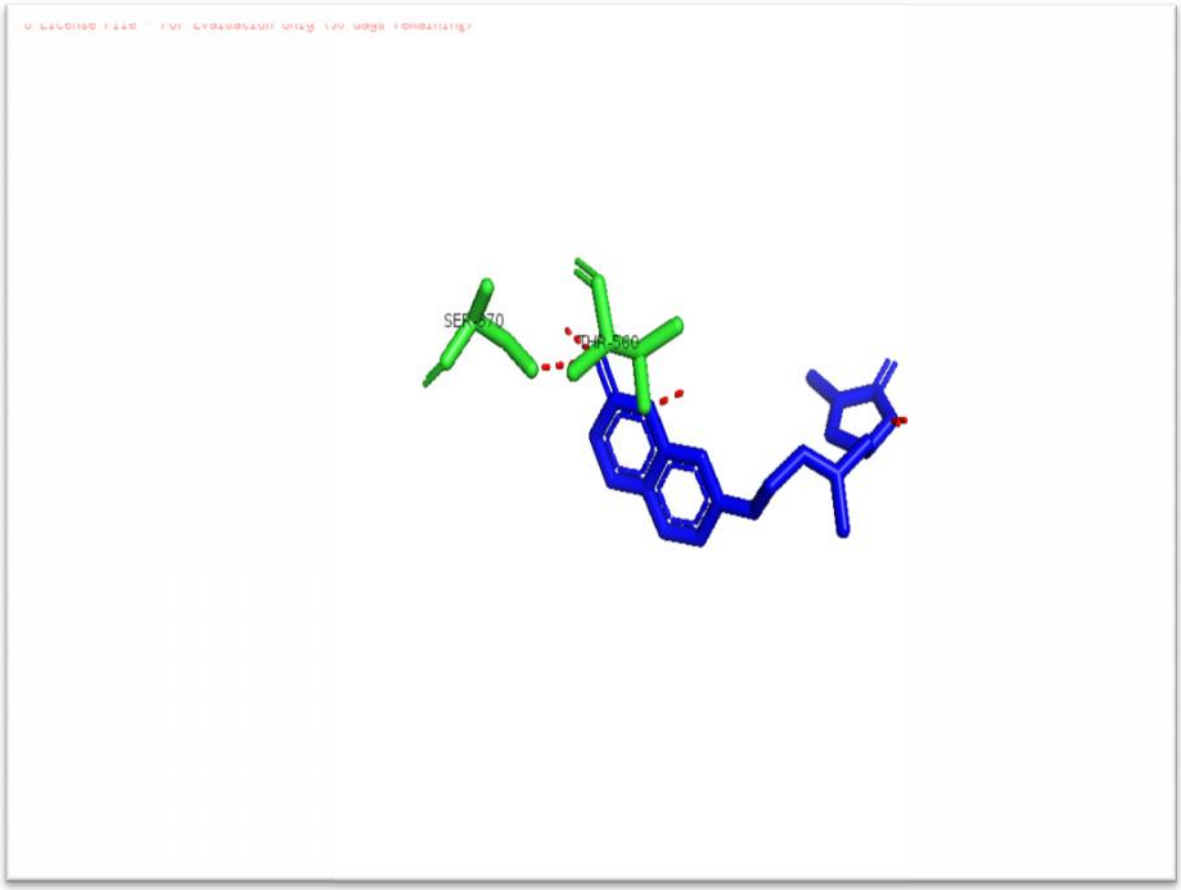

Figure 18: Hydrogen binding mode of NP-000251 with **PBPa1** protein

III.3.Finding chemical structures format:

PYRx has the ability to switch the format of the lead compounds using open babel by their corresponding canonical simplified molecular input line entry system (S.M.I.L.E) which is a linear text format indicates that the string that identifies the molecule does not contain information about chirality or isomerism **[42]**.

III.4.Finding molecular properties and druglikeness:

Predictions of drug properties were calculated in Molinspiration, a cheminformatics tool. It calculates lipinski's rule of five, a key to evaluate drug likeness or determine the chemical compounds pharmacological and biological properties, which concur with the oral prescription drugs for human.

III.4.1.Predict molecular proprieties:

The rule of five values includes cLog P, molecular weight, hydrogen bond donors and hydrogen bond acceptor for the drugs, itpredicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight is greater than 500 Da and the calculated LogP (cLogP) is not greater than 5 (or miLogP<4.15). Moreover, good bioavailability is more likely for compounds with ≤10 rotatable bonds (nrotb) and total polar surface area TPSA ≤140 $Å^2$.

These parameters are defined in the following passage:

III.4.1.1. LogP:

LogP is a partition coefficient between n-octanol and water. It indicates the hydrophobicity of drug molecules and influences the absorption, bioavailability, metabolism and toxicity risks of a drug.

LogP is a key parameter in drug discovery and in the environmental toxicity studies. The calculated value of LogP for >3000 drugs in the market are indicating that the high logP value causes poor absorption or permeation. The logP value must not be greater than 5.0 **[43]**.

III.4.1.2. Molecular weight:

As the absorption of a drug molecule is linked with molecular weight, increasing the molecular weight will decrease the absorption. Keeping less molecular weight is essential in the drug development process. Analysis of molecular weight of theavailable drugs has pointed out that the 80% of drugs have <450 Da**[44]**.

III.4.1.3. Surface area (TPSA):

TPSA belonging to polar atoms, calculated from the surface areas that are occupied by oxygen and nitrogen atoms and by hydrogen atoms attached to them.

Molecular polar surface area (TPSA) is a very useful parameter to predict the transport properties of drugs like intestinal absorption (TPSA ≤140 $Å^2$) **[51, 52]**and blood-brain barrier penetration (TPSA ≤60 $Å^2$). TPSA and molecular volume is inversely proportional to percentage absorption (%ABS).

TPSA is used to calculate the percentage of absorption (%ABS) using the equation:

Percentage of absorbance = 109 − 0.345 × TPSA **[53]**.

III.4.1.4. Hydrogen bonding (HB) descriptors:

These includes the count of the number of potential HB donors and acceptors. Hydrogen bond donors were taken as any heteroatom with at least one hydrogen bond, and hydrogen bond acceptors were taken as any heteroatom without a formal positive charge,

excluding halogens, pyrrole nitrogen, heteroaromatic oxygen and sulfur, and higher oxidation states of nitrogen, phosphorus, and sulfur but including the oxygen bonded to them **[45]**.

III.4.1.5. Number of rotatable bonds (nrotb):

It measures molecular flexibility and proved to be a very good descriptor of absorption and bioavailability of drugs, the rotatable bond count increases with molecular weight **[46]**.

In this study, 2 molecules: NP-000122 and NP-000251 were predicted to have good oral bioavailability, where the calculated LogP with values ranging between 2.97 and 3.46 agreed with Lipinski's rule of five; these results (lipophilicity) indicate for good lipid solubility that will help the drug to interact with the membranes. In addition, TPSA is recognized in literature, as a good indicator of drug absorption in the intestine (TPSA less than 140 $Å^2$) and brain (TPSA less than 60 $Å^2$). The compoundsNP-000122 and NP-000251 exhibit computational TPSA values between 65.00and 65.64.However, the lead compounds do not have adequate blood-brain barrier penetration, as the TPSA values are more than 60 $Å^2$. Furthermore, the number of hydrogen bond acceptors (nON) and hydrogen bond donors (nOHNH) for each lead were found to be within Lipinski's limit range from 5–6 and 1, i.e less than 10 and 5, respectively.

Drug molecules of molecular weight less than 500 Da are easily transported, diffuses and to be absorbed compared to heavy molecules. Fortunately enough, the two leads have molecular weights (MW) in the range of 326.35–379.46Da,so these compounds are expected to present good bioavailability.

Whereas NP-000205 and NP-000205 violated more than one Lipinski’s rule, molecular weights (MW) in the range of 558.50–556.48Darespectively, the number of hydrogen bond acceptors (nON) and hydrogen bond donors (nOHNH) for each lead were found to be within Lipinski's limit range from 11–7 which is less than 10 and 5, respectively.

All of the Molinspirtion results of druglikenessare presented in **table 05**.

Table 05: Ligand parameters predicted by Molinspiration

Ligand	miLogP	TPSA	Natoms	MW	nON	nOHNH	nviol	nrotb	interpretation
NP-000205	**3.53**	**194.21**	**41**	**558.50**	**11**	**7**	**3**	**3**	Not Drug like
NP-000206	**3.87**	**198.11**	**41**	**556.48**	**11**	**7**	**3**	**3**	Not Drug like
NP-000122	**2.97**	**65.64**	**28**	**379.46**	**6**	**1**	**0**	**2**	Drug like
NP-000251	**3.46**	**65.00**	**24**	**326.35**	**5**	**1**	**0**	**5**	Drug like

In bleu: Obedience of Lipinski's rule of 5

In RED: Violation of the rule of 5.

Ro5 provides good overall guidancetowards criteria for solubility and availabledrugs, it is revealed that Ro5 is applicable to medicinalcompounds originate from HTS assays, Such molecules almost certainly are absorbedvia passive diffusion. However, the Ro5 is irrelevant to those compounds that may be morereadily absorbed through the actions of active transport**[47]**.

Conclusion

Conclusion:

Drug discovery is a fierce, lengthy and an incorporative strive. Drug discovery is mostly described as a linear, succeeding process and mainly should be followed by *in vitro* and *in vivo* studies to find out if such compounds gratify certain criteria. A new approach which is *in silico*techniques that can help in recognizing drug targets using bioinformatics tools, studying the pharmacokinetics profiles, the chemical properties, the bioactivity and many other profiles and information.

This study is a section of *in silico* study, it shows the virtual screening techniques, using bioinformatics tools (2 software PyRX and AUTODOCK VINA) to predict the binding of the studied target 2V2F (Penicillin Binding Protein a1 founded in *Streptococcus pneumoniae*) involved in peptidoglycan metabolismwhich is the major component of bacterial cell walls. Bacterial cell wall synthesis is essential to growth, cell division (thus reproduction) and maintaining the cellular structure in bacteria. Inhibition of PBPs produces an imbalance in cell wall metabolism, resulting in growth inhibition or lysis. PBPs bind to β-lactam antibiotics because they are similar in chemical structure to the modular pieces that form the peptidoglycan,and because of development of high level β-lactam resistance in the pneumococcus requires discovery of new inhibitors of PBP1a.

This work results can be summed up in 2 groups of molecules, the first one dockedand activemolecules:

- NP-000122
- NP-000251

These two molecules were actually docked withPBPa1, giving bonds with a strong binding energy and they gave significant results in the docking part -9.2 **Kcal/mol,** and can be a drug like.

The second group contains molecules that are docked but inactive:

- NP-000205
- NP-000206

The moleculeswere docked with PBPa1, giving bonds with a strong binding energy and they gave significant results in the docking part -11.2 **Kcal/mol,** NP-000205; NP-000206Cannot be drug likedue to their disobedience of Lipinski's rule of 5.

Therefore and as mentionned above Virtual screening that belong to the *in silico* studies are of a great interest and a strong complimentarity between the*in silico* and *in vitro*

and*in vivo* should be assured for a good complete drug discovery, without neglecting any given result with each method.

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