Chapter 4
Recent Updates on In Silico Screening of Natural Products as Potential Inhibitors of Enzymes of Biomedical and Pharmaceutical Importance

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Abstract Natural products from medicinal plants have been increasingly used in modern medicine due to their safety, efficacy, and lesser toxicity. World over, a large number of natural compounds are evaluated for the desired bioactivity. A wide range of phytoconstituents such as alkaloids, terpenoids, tannins, steroids, etc. have been recognized for their varying biological activities. However, obtaining the natural products with the desired bioactivity is a time-consuming and commercially difficult process. Molecular docking is used for screening known as well as novel drugs to identify novel compounds by predicting their binding mode and affinity. Moreover, in silico molecular docking can be performed to analyze their binding capabilities into the 3D structure of proteins. AutoDock and AutoDockTools are open-source techniques that have been extensively cited in the literature as essential tools in structure-based drug design. These methods are fast enough to permit the virtual screening of ligand libraries containing tens of thousands of compounds. This article highlights the recent developments in the virtual screening of enzyme inhibitors using various docking tools and their significant applications in designing potent inhibitors for the management of various metabolic and infectious diseases.

Keywords Natural products · Virtual screening · Molecular docking · In silico · AutoDock and AutoDockTools

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4.1 Introduction

Natural products have been a valuable source of new drugs. Secondary metabolites are naturally derived substances and/or by-products from plants, animals, and microorganisms (Baker et al. 2000). According to the World Health Organization (WHO) estimates, 80% of the people of developing countries rely on traditional medicines, mostly plant-derived drugs, for their primary health needs. Over 49% of the new medicines registered by the United States Food and Drug Administration (USFDA) are derived from natural products or their derivatives. The Indian subcontinent has been one of the rich hotspots with over 2500 plant species being used in indigenous medicines. The use of medicinal plant resources for health management is an imperative part of tribal culture. It is evident that these people have vast traditional knowledge about medicinal plants and use them for a wide range of health-related applications. In the old traditions, local tribal communities have discovered the medicinal uses of hundreds of plants and plant-based substances. Medicinal plants contain a variety of bioactive constituents known as phytochemicals that make them effective in the treatment of a wide range of diseases (Twilley et al. 2017; Anigboro et al. 2020), and they are becoming more popular as therapeutic agents due to their high safety profile, low cost, and widespread availability (Govindappa 2015; Limanaqi et al. 2020). Natural oxyprenylated ferulic, umbelliferone derivatives and coumarins, flavonoids, glycosides make up a large portion of these natural phytochemicals.

Enzyme inhibitors are mostly plant or microbial bioactive secondary metabolites that bind to enzymes and inhibit/reduce their activity. The use of natural products as enzyme inhibitors is well documented (Rauf and Jehan 2017). The binding of inhibitors to the enzymes alters the conformation of the active site, thereby halting the chemical process. Inhibition of enzymes can be both irreversible and reversible. The inhibitory effects of plant extracts and the isolated compounds on the activity of various enzymes, viz. α-amylase, α-glucosidase, and pancreatic lipase have been studied to discover potential therapeutic candidates. Various reports have demonstrated the in vitro, in vivo, and in silico enzyme inhibitory potential of natural products of plant and microbial origin (Rauf and Jehan 2017).

The discovery of new, safe, and effective enzyme inhibitors is a long and costly process. Conventionally, a trial-and-error method is used in screening vast libraries of chemical constituents against a known enzyme in the hopes of finding some useful lead drugs. Combinatorial chemistry approaches were found effective as they offered rapid and high-throughput screening capabilities to quickly screen these enormous chemical libraries for novel and potent inhibitors.

Molecular docking is important in silico technique normally used to predict the interaction between the receptor and the ligand. This technique is employed in drug discovery because it is inexpensive and time-saving (Miners et al. 2004). For these reasons, the development of in vitro and in silico approaches is important to predict drug interactions, to possibly identify the pharmacophore, to reduce time and costs (FitzGerald et al. 2020). Using in silico docking approach, the most effective

inhibitors of different enzymes can be screened for further investigation to understand the structures of proteins and molecular interaction of ligands to exert their inhibitory activity. Therefore, to understand such possible molecular interactions between the enzyme and inhibitor, a combined in vitro and in silico method is required to screen for bioactive molecules.

Several approaches are available for determining whether a bioactive compound is interacting with a biological macromolecule; for example, kinetic investigations and in silico molecular docking study (Mentes et al. 2018; Moreno et al. 2020). Prediction of chemical compounds' reactivity, chemical hardness, stability, nucleophilic or electrophilic nature, as well as drug-like properties (such as absorption, distribution, metabolism, and excretion) with plausible precision using in silico analytical methods such as semi-empirical quantum mechanics (frontier molecular orbital (FMO) theory calculations) and pre-ADME or adsorption, distribution, metabolism, and excretion cheminformatics tools have also been reported (Bondzic et al. 2020; Siahaan et al. 2021). These methods have been used to investigate molecular interactions between chemical substances or ligands and proteins, including acetylcholinesterase (Bondzic et al. 2020), epoxide hydrolase (Jo et al. 2016), 3-hydroxysteroid dehydrogenase (Oyebamiji et al. 2020), and pancreatic lipase (Hou et al. 2020). The use of molecular docking analysis, as one of the biophysical/biocomputational techniques for probing ligand–protein binding interactions, has gained more recognition and relevance over the years in the fields of biochemistry and biophysics (Almasri 2020).

Molecular Docking for Screening of Enzyme Inhibitors

Molecular docking of protein–ligand interaction is an effective and competent tool for in silico screening of natural products as inhibitors. It plays the most important and ever-increasing role in rational drug design. Molecular docking is a computational program of virtual screening for an appropriate ligand (enzyme inhibitor) that fits both energetically and geometrically the proteins' binding pocket. Consensus molecular docking is one of the approaches to increase detection of real actives within virtual screening campaigns. With molecular docking, it is possible to find the most favorable position, orientation, and conformation for the binding of a molecule to, a protein target, assigning a score that is the estimate of the likelihood of binding of each molecule and conformation (Cavasotto 2015). There are different open-source molecular docking programs, Autodock Vina, Smina, LeDock, and rDock, available for this purpose. Here we propose/present the protocol for Autodock Vina. AutoDock and AutoDockTools are open-source techniques that have been used as essential tools in structure-based drug design. Moreover, these methods are fast enough to permit the virtual screening of ligand (inhibitors) libraries containing thousands of natural compounds. The protocol can be implemented in any virtual screening campaign where proteins (enzymes) are used as molecular targets. In

addition, we introduce a scoring strategy based on the average RMSD value obtained from comparing the different confirmations to predict the best fit.

4.2 Molecular Docking with AutoDock

AutoDock suite is molecular modeling simulation software that attempts to predict the noncovalent binding of the macromolecule (receptor) and ligand (small molecule). It is one of the most accurate and effective tools that is capable of predicting the bound conformations and binding energies of the ligands with macromolecules targets. Furthermore, it is open-source software, and it includes several complementary tools like AutoDock Vina, Autodock, Raccoon2, AutoDockTools, and AutoLigand. AutoDockTool combines precision in identifying the binding pose of a tiny molecule in corresponding to the receptor pocket for computational docking researchers.

There are four basic steps in a typical docking process. First, prepare the ligand and target protein (depending on the nature of the ligand and/or target, this may necessitate more complex processes). Second, prepare to dock and scoring parameters (for Autodock to run, the following files should be created: grid parameter file, map files, docking parameter files). Third, using a graphical interface or a command-line interface terminal to launch the docking program (e.g., AutoDock). The fourth step is to analyze and evaluate the docking data (by comparing docking poses to crystalline ligand). Here we are providing easy step-by-step and practical docking protocols using AutoDock and AutoDockTools. In nutshell, the AutoDock engine uses a proprietary format known as PDBQT. For both ligand and protein, this file contains all information on atom kinds and charges. This file is created when a PDB file (http://www.wwpdb.org/documentation/file-format) is converted. It can be made with AutoDockTools, the preferred graphical user interface for AutoDock. A Grid calculating approach is used to evaluate the binding site's energy (AutoGrid). The ligand's energetics is then compared to values calculated from the interaction terms assigned by the affinity grids computations. In the last step, the ligand is docked utilizing a variety of search techniques. The Lamarckian genetic algorithm (LGA), which is implemented in AutoDock, is one of the most widely used and effective approaches for determining optimal ligand binding conformation with predicted free energies of association.

A landmark enzyme, Pancreatic Lipase (PL) was identified as a major target for binding of inhibitors in the treatment of obesity. Human pancreatic lipase consists of 449 amino acids with a coated catalytic center of the N-terminal domain including Ser-152, his-263, and Asp-176. The catalytic triad (S152–D176–H263) and the lid (peptide stretch C237–C261) are found in the large N-terminal domain (residues 1–335) of 2OXE, which has the typical/-hydrolase fold of lipases and is dominated by a central parallel sheet (Winkler et al. 1990; Van Tilbeurgh et al. 1993). In the present review, PL will be used as a model for docking using AutoDock as it is a promising target for binding of inhibitors in the treatment of obesity.

4.2.1 Computational Tools Required for Docking

4.2.1.1 Starting with a Set of Preliminary and Important Requirements

This would require user-friendly tools such as protein data bank (https://www.rcsb.org/) and Swiss-PdbViever (https://spdbv.vital-it.ch/) in order to understand and deduce the structural alignment, finding the active site, amino acid mutations, H-bond, angles, and distance between the atoms of the protein–ligand complexes. These tools offer a great help that explains all you need to know about manipulating protein–ligand complexes, removing the solvent, fixing structure, and more.

4.2.1.2 Download the PDB (Protein Data Bank) File

The X-ray diffraction crystallographic structure (3D) of human pancreatic lipase-related protein (PDB ID, 2OXE) is downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (https://www.rcsb.org/structure/2OXE). The current crystal structure has 466 amino acid residues and the monomer has a resolution of 2.8 Å (Fig. 4.2). The 3D structure of the ligand can be obtained from https://pubchem.ncbi.nlm.nih.gov/. The structure of both target protein and corresponding ligand were extracted separately from the original PDB files. Users are encouraged to regenerate the target.pdb and ligand.pdb files using Swiss-PDB viewer http://spdbv.vital-it.ch/.

4.2.1.3 Download the Computational Tools

AutoDock 4.2.6

The latest version of Autodock should be downloaded from http://autodock.scripps.edu/downloads/autodock-registration/autodock-4-2-download-page/. This new version of Autodock runs natively under Windows, see instructions for installation (http://autodock.scripps.edu/downloads/autodock-4-2-x-installation-on-windows). The main files, AutoDock4 and AutoGrid4 are necessary to run the pre-docking (energy maps), docking, and scoring calculation.

MGL/AutoDockTools

AutoDockTools can be downloaded from http://mgltools.scripps.edu/downloads. It is a Graphical User Interface for preparing input, running, and analyzing Autodock dockings (adding atomic charges, fixing bonds, adding hydrogens, preparing the ligand and target in Autodock-compatible PDBQT format, creating grids and docking parameter files, and visualizing interactively docking results).

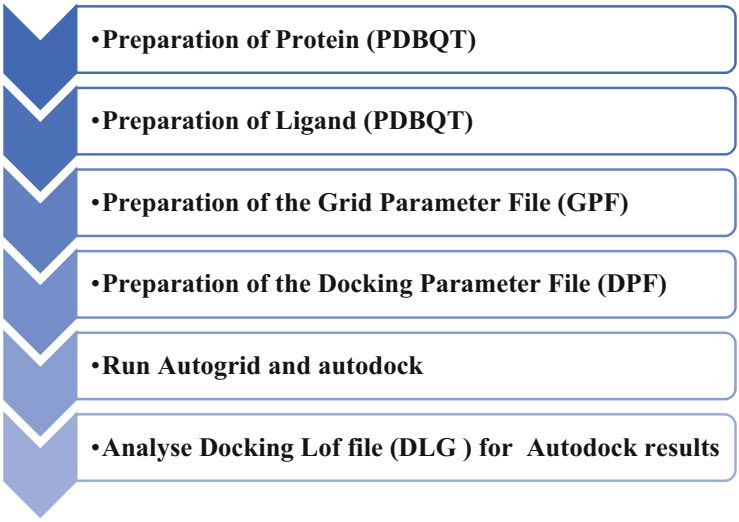

Fig. 4.1 Step-wise docking approach for the molecular docking of protein–ligand interaction

Method-Docking Approach

The following steps are crucial since they specify how to execute AutoGrid and AutoDock and offer accurate docking parameters. The coordinated files and associated information should be created in the PDBQT format, which includes atom/bond types, partial atomic charges, and other information. These data types are prepared typically using AutoDockTools (ADT). The steps in the docking method are represented in Fig. 4.1.

4.2.2 Preparation of Protein for Autodock

The following steps represent the preparation of the crystal structure of PL protein (2OXE Fig. 4.2) PDB file into the PDBQT file format.

- First, open AutoDockTools and MGL tools from desktop or program files. Before preparing protein, make sure that protein.pdb and ligand.pdb files are in the same folder, e.g., Desktop/autodock.
- Click on File > Preferences > Set: change the startup directory > set file path.
- From File menu > open > read molecule > select target protein.pdb from same folder: Desktop/autodock.
- The crystal 3D structure of 2OXE will appear on screen, then Click on Edit > Delete water > Hydrogen > Add (polar Hydrogen's) > Click on Charges

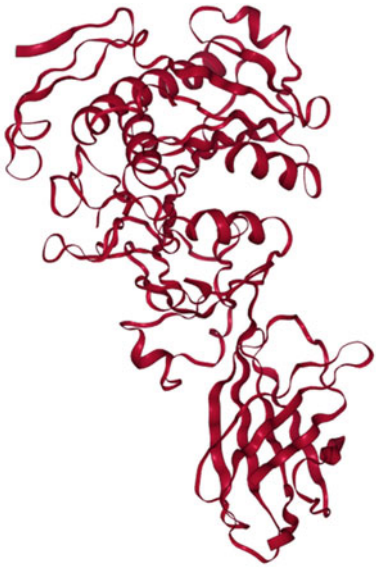

Fig. 4.2 Three-dimensional crystal structure of the generated 2OXE model of pancreatic lipase

> Add Kollman charges > Compute Gasteiger charges. This will add partial charges to protein.

- Click on Grid > Macromolecule > Open: Open and select target protein pdb file, and ADT tool will prompt a new window to save our protein into pdbqt file> save the PDBQT file in the same folder where we created target pdb. This will store the partial charges and Autodock atom types that are compatible with autodock grid computing.

4.2.3 Preparation of Ligand for Autodock

The process of creating ligand PDBQT from a PDB file from the crystal structure of ligand.pdb consists of the following steps.

- Click on Ligand > Input > open: open ligand.pdb file from the same folder, the crystal 3D structure will appear on the screen.
- Click on Edit > Delete water > Hydrogen > Add (polar Hydrogen's) > Click on Charges > Add Kollman charges > Compute Gasteiger charges.
- Click on Ligand > Torsion Tree > Detect Root > Ligand > Torsion Tree > Choose Torsions: decide on the rotatable bonds to be considered and set the number of torsions.
- Select Ligand > Output > Save as PDBQT: save the ligand file with "pdbqt" extension (e.g., ligand.pdbqt). Type in the extension manually and save it in the same folder.

4.2.4 Docking Approach

4.2.4.1 Preparation of Grid Parameter File (GPF)

- For the preparation of the grid parameter file to run with Autogrid4.exe, open the Grid menu in AutoDockTools to prepare the parameters for Autogrid calculations. Autogrid calculates grid maps of interaction energies for various atom types. This is important to calibrate the docking procedure.
- Click on Grid > Set Map Types > Choose Ligand: select the ligand molecule from the list or open the ligand. pdbqt saved previously. Select Grid > Grid Box: Select the number of grid points in *x*, *y*, and *z* directions. Usually, the default 40 is taken. However, we can increase or decrease that number in any direction. From the "Center" menu, select "Center on ligand." Leave other parameters on default.
- The number of grid points in each dimension: only give even numbers (from $2 \times 2 \times 2$ to $126 \times 126 \times 126$). Grid Maps depend on the orientation of the macromolecule.
- Click on File > Close saving current: save the settings before closing. Otherwise, settings will be lost if the program is closed. Select Grid > Output > Save GPF: save our gpf file as docking.gpf. Type in the extension manually and save it in the same directory. This will create one grid map for each atom type in the ligand plus electrostatics and a desolvation map. Check out the content of Grid Parameter File, docking.gpf (by Notepad).

4.2.4.2 Preparation of Docking Parameter File (DPF)

In AutoDockTools, open the docking menu to prepare the docking parameters for Autodock.

- Click on Docking > Macromolecule > Set Rigid Filename: Select target macromolecule protein file (e.g., protein.pdbqt). Again select Docking > Ligand > choose: select ligand in pdbqt format. Leave everything on default and select Accept.
- Select again docking > Search Parameters > Genetic Algorithm: set the number of GA Runs as 10. Leave other parameters on default and select Accept.
- Click on Docking > Docking Parameters: Leave on default parameters. Again click on Docking > Output > Lamarckian GA: save dpf file (e.g., docking.dpf) in the same directory. Type in the extension manually.
- Now we have all the required files for docking; target protein.pdbqt, ligand.pdbqt, docking.gpf, docking.dpf files in the same folder.

4.2.4.3 Running AutoGrid4 and AutoDock4

After downloading and installing AutoDock 4.2.6 from http://autodock.scripps.edu/downloads/autodock-registration/autodock-4-2-download-page/, for Windows users, Start > Run and type "cmd.exe," then type the command: "C:\Program Files\ The Scripps Research Institute\ Autodock\autodock4.exe".

For running Autogrid4, Start > Run and type "cmd.exe," change the working directory to ~Desktop\autodock (using the cd command). Type in the console: autogrid4.exe -p docking.gpf -l docking.glg. For running autodock4, type in the console: autodock4.exe -p docking.dpf -l docking.dlg & . This will take some time depending on your CPU and memory capacity.

All information about the docking runs, the calculated binding energy in Kcal/mol, as well as other data RMSD versus crystal binding pose is contained in the dlg file. Figure 4.3 represents different conformations of protein–ligand interactions.

4.2.4.4 Analyzing Docking Results

Open the analyze menu in AutoDockTools to analyze the docking results. Docking results can be found in the docking.dlg log file in the same directory.

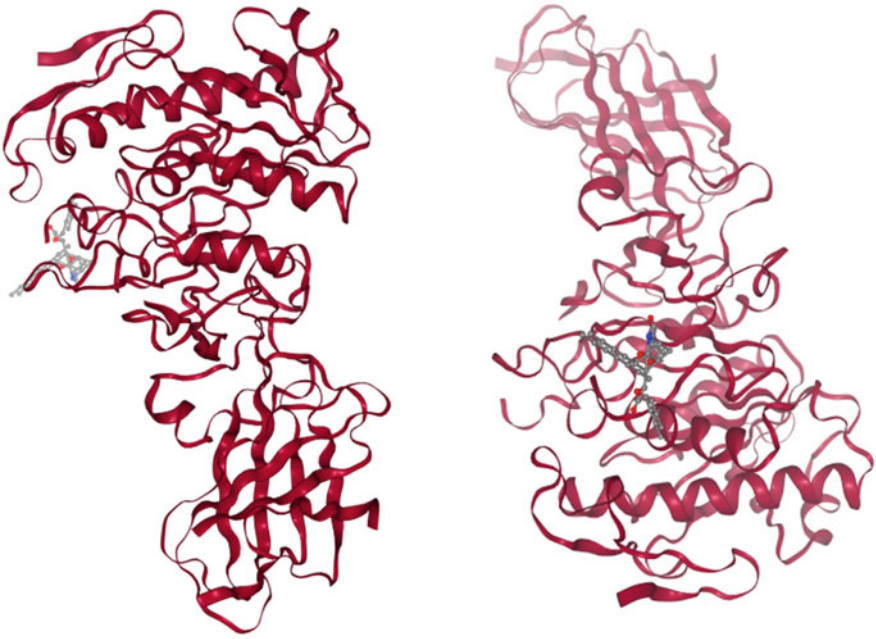

Fig. 4.3 Predicted active sites for binding of compounds (ligands) in the pancreatic lipase enzyme in the molecular docking

- Select the Analyze > Docking> open > Docking.dlg. file> open analyze > select Conformations> play. This will show the conformation from 1 to 10 of the ligand bound to PL protein.
- The best conformation shows binding energy (G) of −10.59 kcal/mol and inhibition constant (K_i) of 17.17 nM (nanomolar) and a RMSD (root-mean-square deviation of atomic positions) from a reference structure of 1.22 Å.
- This demonstrates that Autodock's results are accurate and dependable (in the nanomolar range for a recognized inhibitor). Docking and virtual screening would be extremely useful in the search for novel PL inhibitors.

4.3 Significance of Molecular Docking in Inhibitor Screening for Biomedical Applications

Molecular docking is a widely used platform to understand drug biomolecular interactions that have applications in rational drug design and discovery. It is also used for mechanistic studies binding studies of ligand into the preferred binding site of the target protein/DNA through the noncovalent interactions. The values of the binding energy, free energy, and stability of complexes help predict the binding affinity of the molecule under study. There are numerous examples where the docking approach successfully identifies the target sites for several different receptors/targets. For example, using docking technology, Schames et al. (2004) discovered HIV 1 integrase as a new binding site for drugs treating AIDS. Using AutoDock, this receptor was effectively used for the inhibition of HIV integrase. The most striking discoveries involving docking approaches include the discovery of novel type I TGF-beta receptor kinase inhibitor (Singh et al. 2003), aurora kinase A inhibitor (Park et al. 2018), dopamine D3 receptor for anticancer molecules (Varady et al. 2003), etc. Table 4.1 provides a detailed outlook of various target proteins/receptors that are inhibited using a ligand with its PDB ID, binding sites, software used in the study, and the biological significance of the study.

4.4 Limitations of Molecular Docking

The lack of confidence in scoring functions' ability to provide correct binding energies is a key constraint of molecular docking. This is because some intermolecular interaction terms, such as solvation effect and entropy change, are difficult to anticipate precisely. Furthermore, several intermolecular interactions that have been proved to be significant are rarely taken into account in scoring methods. For example, halogen bonding and guanidine–arginine interactions have been shown to contribute to protein–ligand binding affinity, although they are not taken into account. Secondly, the water molecules that are present in the binding pocket

Table 4.1 Recent studies on screening of protease/enzyme inhibitors using molecular docking and their biological significance

Sr. no	Target enzyme/ protein	Inhibitor/ligand	PDB ID	Binding sites/residues	Biological significance	Software used in the study	References
1.	Acetylcholinesterase inhibitors (AChEIs)	Compound D	NR	Peripheral anionic subsite	In the treatment of Alzheimer's disease	AutoDock Vina 1.1.1	Pablo et al. (2015)
2.	Butyrylcholinesterase	Compound D	NR	Peripheral anionic subsite	In the treatment of Alzheimer's disease	AutoDock Vina 1.1.1	Pablo et al. (2015)
3.	ERK2 (protein kinase)	Hypo1	NA	Glycine rich loop	Inhibition of cell proliferation and oncogenesis	AutoDock	Sofiene et al. (2014)
4.	Glycoprotein receptor-binding domain	Solanine, acetoside, and rutin	6M0J	Arg403 (6.34 Å) and π-π stacking with Tyr505 (4.75 Å)	In the treatment of COVID-19	Schrödinger	Teli et al. (2021)
5.	COVID-19 main protease (Mpro)	Solanine, acetoside, and rutin	6 LU7	Leu141 (1.81 Å), His164 (2.06 Å), and Thr190 (1.69 Å) residues	In the treatment of COVID-19	Schrödinger	Teli et al. (2021)
6.	Hemoglobinase Falcipain-2	Nonpeptidic inhibitors	6JW9	His114 and Thr120	To develop anti-malarial drugs to treat resistant strains of *Plasmodium*	PyRx virtual screening software	Rajguru et al. (2022)
7.	Cyclophilin D	ZINC161011368	1ZKC	π-Sulfur interaction with a methoxy group	For the treatment of acute pancreatitis	Schrodinger	Chen et al. (2022)
8.	Aldehyde dehydrogenases (ALDHs)		1KOL	Thr26, Gly123, Cys145, Glu146, Glu189	In the treatment of cyclophosphamide and cisplatin resistance cancer	Schrodinger	Narendra et al. (2021)
9.	COVID-19 main protease	Bieckol	5R82	Thr24, Thr26, Gly143, and Glu189	In the treatment of COVID-19	MOE dock program	Rauf et al. (2021)
10.	COVID-19 main protease	7-Hydroxyeckol hepta-acetate	5R82	Thr26, Gly143, Cys145, Glu166, Glu189	In the treatment of COVID-19	MOE dock program	Rauf et al. (2021)

(continued)

Table 4.1 (continued)

Sr. no	Target enzyme/ protein	Inhibitor/ligand	PDB ID	Binding sites/residues	Biological significance	Software used in the study	References
11.	COVID-19 main protease	Met165	5R82	π-Sulfur interaction with a methoxy group	In the treatment of COVID-19	MOE dock program	Rauf et al. (2021)
12.	COVID-19 main protease	5-Hydroxy-cystofurano-quinol	5R82	Thr24, Thr26, Ser46	In the treatment of COVID-19	MOE dock program	Rauf et al. (2021)
13.	COVID-19 main protease	Sargaquinoic acid	5R82	Pro168, Gly143, and Gln189	In the treatment of COVID-19	MOE dock program	Rauf et al. (2021)
14.	COVID-19 main protease	Methoxybifurcarenone	5R82	Thr24, Ser46, Met165, Glu166, and Arg188	In the treatment of COVID-19	MOE dock program	Rauf et al. (2021)
15.	COVID-19 main protease	Rutin	5R82	Thr24, Thr26, Ser46	In the treatment of COVID-19	MOE dock program	Rauf et al. (2021)
16.	Monoacylglycerol lipase	Rutin	4CDD		To attenuate the inflammatory response	MOE dock program	
17.	Dipeptidyl-peptidase-4	Butirosin	4CDD	s740(H), Tyr631(H)	In the treatment of type II diabetes mellitus	Schrodinger	Xiong et al. (2021)
18.	Dipeptidyl-peptidase-4	Alogliptin	4CDD	yr631(H), Tyr662(π), Tyr666(π), Glu205 (H),	In the treatment of type II diabetes mellitus	Schrodinger	Xiong et al. (2021)
19.	Dipeptidyl-peptidase-4	Rutin	4CDD	Phe357(π), Tyr585(H), Ser630(H)	In the treatment of type II diabetes mellitus	Schrodinger	Xiong et al. (2021)
20.	Dipeptidyl-peptidase-4	Homatropine	4CDD	Glu206(H), Arg125(H and π-cationic)	In the treatment of type II diabetes mellitus	Schrodinger	Xiong et al. (2021)
21.	Mycobacterial InhA	Enamine 57340	5G0T	Tyr158	Therapeutic applications against TB	GRO-MACS (Groningen Machine for Chemical Simulations) 5.0.2package	Jayaraman et al. (2021)

22.	Mycobacterial InhA	Enamine 57294	5G0T	Phe149 and Tyr158	Therapeutic applications against TB	GRO-MACS (Groningen Machine for Chemical Simulations) 5.0.2package	Jayaraman et al. (2021)
23.	Histone acetyltransferase	Chlorogenic acid (CA)	2RNZ	CA can be buried to the bromine domain of p300	Anti-non-alcoholic fatty liver disease	Autodock, Autogrid, autotors	Xu et al. (2021)
24.	Stearyl coenzyme A desaturase enzyme 1	Stearoyl-coenzyme A	4ZYO	ASN71 ASN144ARG151ASP152ARG184LYS185	To alter the metabolism of tumor cells	Schrödinger software	Huang et al. (2020)
25.	Stearyl coenzyme A desaturase enzyme 1	Aramchol	4ZYO	ASN71ASN144GLH148ARG151ARG21	To alter the metabolism of tumor cells	Schrödinger software	Huang et al. (2020)
26.	Protein tyrosine phosphatase 1B	H1 to H20	1PA1	Arg114andArg222	In the management of diabetes	Schrödinger software	Yang et al. (2020)
27.	Phosphodiesterase 10A	MA8	3HR1	GLN716A	Alleviating the negative and cognitive symptoms of schizophrenia	Autodock Vina 1.1	Mayasah et al. (2018)
28.	Phosphodiesterase 10A	MA98	3HR1	GLN716A	Alleviating the negative and cognitive symptoms of schizophrenia	Autodock Vina 1.1	Mayasah et al. (2018)
29.	Lactate dehydrogenase	Quercetin	1I0Z	Arg111, Gly96	To arrest tumor invasion and metastasis	Autodock Vina 1.1	Li et al. (2021)
30.	Lactate dehydrogenase	Luteolin	1I0Z	Asp51 Thr247	To arrest tumor invasion and metastasis	Autodock Vina 1.1	Li et al. (2021)

(continued)

Table 4.1 (continued)

Sr. no	Target enzyme/ protein	Inhibitor/ligand	PDB ID	Binding sites/residues	Biological significance	Software used in the study	References
31.	Lactate dehydrogenase	Ursolic acid	1I0Z	Arg111, Gly96	To arrest tumor invasion and metastasis	Autodock Vina 1.1	Li et al. (2021)
32.	COVID-19 main protease	Penciclovir	6M0J	HIS 41 LEU141	To develop effective therapeutics to deal with the COVID-19 pandemic	Libdock	Padhi et al. (2021)
33.	COVID-19 main protease	Jasmonic acid	6M0J	MET 165 GLU 16 HIS41	To develop effective therapeutics to deal with the COVID-19 pandemic	Libdock	Padhi et al. (2021)
34.	COVID-19 main protease	Jasmonic acid methyl ester	6M0J	HIS41 MET49 GLY143	To develop effective therapeutics to deal with the COVID-19 pandemic	Libdock	Padhi et al. (2021)
35.	COVID-19 main protease	Putaminoxin B	6M0J	HIS 41 ASN 142 CYS 145	To develop effective therapeutics to deal with the COVID-19 pandemic	Libdock	Padhi et al. (2021)
36.	COVID-19 main protease	Putaminoxin D	6M0J	HIS 41 LEU141 2.20 SER 144 CYS 145	To develop effective therapeutics to deal with the COVID-19 pandemic	Libdock	Padhi et al. (2021)

37.	SARS-CoV-2 3-chymotrypsin-like protease	Chicoric acid	6Y84	CYS^{145} CYS^{148}	Preventive nutraceuticals and/or antiviral drugs in COVID-19	AutoDock Vina in PyRx 0.8	Gyebi et al. (2021)
38.	SARS-CoV-2 3-chymotrypsin-like protease	Rosmarinic acid	6Y84	HIS^{41} LEU^{49}	Preventive nutraceuticals and/or antiviral drugs in COVID-19	AutoDock Vina in PyRx 0.8	Gyebi et al. (2021)
39.	SARS-CoV-2 3-chymotrypsin-like protease	Myricetin	6Y84	CYS^{148} LEU^{49} HIS^{41}	Preventive nutraceuticals and/or antiviral drugs in COVID-19	AutoDock Vina in PyRx 0.8	Gyebi et al. (2021)
40.	SARS-CoV-2 3-chymotrypsin-like protease	Rosmarinic acid	6Y84	GLY^{192} MET^{168}	Preventive nutraceuticals and/or antiviral drugs in COVID-19	AutoDock Vina in PyRx 0.8	Gyebi et al. (2021)
41.	SARS-CoV-2 3-chymotrypsin-like protease	Isorhamnetin	6Y84	MET^{168} CYS^{145}	Preventive nutraceuticals and/or antiviral drugs in COVID-19	AutoDock Vina in PyRx 0.8	Gyebi et al. (2021)
42.	SARS-CoV-2 3-chymotrypsin-like protease	Luteolin	6Y84	SER^{144}, GLU^{166}(2), and GLN^{189}	Preventive nutraceuticals and/or antiviral drugs in COVID-19	AutoDock Vina in PyRx 0.8	Gyebi et al. (2021)

(continued)

Table 4.1 (continued)

Sr. no	Target enzyme/ protein	Inhibitor/ligand	PDB ID	Binding sites/residues	Biological significance	Software used in the study	References
43.	SARS-CoV-2 3-chymotrypsin-like protease	Vernomenin	6Y84	GLY^{143}, SER^{144}, and CYS^{145}	Preventive nutraceuticals and/or antiviral drugs in COVID-19	AutoDock Vina in PyRx 0.8	Gyebi et al. (2021)
44.	Main protease (M^{pro}) of SARS-CoV-2	Kazinol T	6Y7M	GLY^{143}, SER^{144}	Lead compound against the main protease of SARS-CoV-2	AutoDock	Ijaz et al. (2021) and Avwioroko et al. (2020)

create lots of difficulties during the docking process. The major challenges faced in the docking experiments are that of rigid receptors. Most importantly, molecular docking does not provide insights into the spectrum of action against non-target proteins/receptors. One has to depend on animal and human trials to get this information.

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