Chapter 4
Exploring the Potential of Herbal Ligands Toward Multidrug-Resistant Bacterial Pathogens by Computational Drug Discovery

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Abstract The emergence of multidrug resistance (MDR), extensive drug resistance (XDR), and pan-drug resistance (PDR) has become a critical issue worldwide. The available drugs are no longer effective therapeutic remedy against such bacterial pathogens. This necessitates alternative therapy remedies. Computational drug discovery plays a central role in designing novel phytotherapeutics against drug-resistant bacterial pathogens. This chapter initially describes the recent issues and concerns associated with bacterial extreme resistance. Further, it demonstrates the utility of herbal-based compounds as probable lead molecules against various drug targets of multidrug-resistant bacteria by molecular docking approaches.

Keywords Multidrug resistance • Probable lead molecules • Therapeutic remedy • Molecular docking • Phytotherapeutics • Probable lead molecules • Extreme resistance • Drug targets

4.1 Introduction

The development of multidrug resistance is a major healthcare burden in the treatment of pathogenic bacteria by distinct antimicrobial agents. Moreover, it is not just an issue confined only to bacteria but all microorganisms that have the efficiency to mutate and deliver the new drugs unsuccessful (Carlet 2014). Most of the pathogenic strains have become drug resistant, and some have become resistant to multiple conventionally used antibiotics and chemotherapeutic agents; they emerged as multidrug-resistant (MDR) strains or superbugs (Nikaido 2009; Carlet 2014). Recent studies revealed that antibiotics have lost their status as the "miracle

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drug," and "treatment failure" is a new and often observed situation (Schjørring and Krogfelt 2011; Gowrishankar et al. 2013). Since the bacteria became resistant to many conventional antibiotics, there is a necessity to identify probable drug targets and screen for alternative therapeutic substances. One promising method is to prevent such drug-resistant pathogens by novel therapeutic compounds that are not based on existing synthetic antimicrobial agents. There is also a need for a deeper understanding of the mechanisms by which bacteria gain resistance to antibiotics which will aid in identifying novel targets for drugs or treatment. There are reports suggesting that several herbs produce bioactive compounds which are effective therapeutic agents (Nair et al. 2005). These medicinal plants are well studied and their bioactive compounds have been separated (Briskin 2000). Moreover, the bioactivity assay, modes of action, and inhibitory properties against various drug targets for many herbal-derived compounds are studied. Molecular docking-based studies pave new insight to screen natural herbal ligands which have ideal drug likeliness and pharmacokinetic properties (Bharath et al. 2011).

Computational drug discovery is the fundamental concept of structure-based drug design that uses a variety of computational methods to screen novel lead molecules with selectivity, efficacy, and safety (Lionta et al. 2014). The study of receptor-ligand interaction is the main focus of rational drug design, and the prediction of such interactions by computational approaches has profound scope and applications. Molecular docking is the prime component in computer-assisted molecular design. Molecular docking plays a vital role to understand the binding mechanism of herbal ligands toward various drug targets and inhibition of the pathways or any other means. Both rigid-body docking and flexible-body docking are playing vital roles in this dimension. The utility of best docking program, simulations and scoring, ranking, and docked conformations helps to hypothesize the probable mechanism. This provides profound scope and insight to further experimental analysis and screening of novel natural therapeutic substances (Lionta et al. 2014).

This chapter focuses the recent concerns and issues associated with multidrug resistance of bacterial pathogens and scope of molecular docking-based approaches for the discovery of novel herbal therapeutics against multidrug-resistant strains. The main strategy to achieve application for phytomedicine toward MDR is molecular docking-based studies and further in vitro and in vivo evaluation for the proposed approach.

4.2 Recent Issues Associated with MDR Bacteria

The increase in multidrug resistance poses a foremost healthcare threat. In the context of an almost complete absence of new chemotherapeutic drugs in progress, antibiotic resistance (ABR) has become one of the main healthcare implications (Boucher et al. 2009). According to Margret Chan, director general of World Health Organization, *Post antibiotic era is almost upon us*. Similarly, David Cameron,

prime minister, UK, recently called for *a global action to tackle the growing threat of resistance to antibiotics*. Antibiotics are a unique class of therapeutic remedy because of their major impact in society. The application of an antibiotic in a person can select for ABR that can spread across human populations, animals, and the environment, making an antibacterial used in one person unproductive for many others. As bacteria acquire resistance mechanisms, the altered bacterial genetic material coding for resistance can be transferred between bacterial populations, expanding the reach and coverage of bacterial resistance. Treatment failures because of multidrug-resistant (MDR) bacteria arise very commonly in hospitals, in particular in the intensive care unit, and increasingly spreading in the other areas such as food, water, and air. *Methicillin-resistant Staphylococcus aureus* (MRSA) infections, especially due to community-acquired MRSA (DeLeo et al. 2010), are tremendously widespread in many European countries (European Center for Diseases Control and Prevention, EARSS-Net database. http://www.ecdc.europa.eu), the USA, South America, and Asia (Morcillo et al. 2015). MRSA infection accounts for 44% of all hospital-associated infections in the USA, and as many as 92% of persons hospitalized for MRSA have community-acquired MRSA (CA-MRSA) (Gould et al. 2008). There are newly developed agents that are active against vancomycin-resistant MRSA, such as linezolid and quinupristin/dalfopristin known as vancomycin-resistant enterococci (VRE). These bacteria are also very common, with large variations between countries ranging from 1 to >50% (Mutters et al. 2015). The predominance of *Escherichia coli* and *Klebsiella pneumoniae* harboring extended-spectrum β-lactamases is widespread across the world reaching 50–70% for *E. coli* in some European or Asian countries (Lowe et al. 2012). One of the study revealed that prevalence of *K. pneumoniae* with carbapenemases was going from 1 to >50% (Nordmann et al. 2009). Furthermore, a serious threat may be the emergence of Gram-negative bacteria that are resistant to all classes of the available chemotherapeutic agents referred to as pan resistance (Enani 2015). The emergence of "pan-resistant strains," mainly belonging to *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, occurred in the recent past, after most of the major pharmaceutical industries stopped the development of new chemotherapeutic agents against bacterial infections (Nikaido 2009). One of the main global health concerns is the emergence and spread of drug-resistant tubercle bacilli across the world. The high burden of multidrug-resistant tuberculosis (MDR-TB) and the emergence and rise of advanced forms of drug resistance such as extensively drug-resistant TB (XDR-TB) and extremely drug-resistant TB (XXDR-TB) are some of the major concerns in the global healthcare sectors (Dalal et al. 2015).

In addition to clinical and hospital-associated cases, the multidrug resistance is spread across the environmental sectors. The lake, river, water storage tanks, etc. have become a cesspool of antibiotic-resistant bacteria (Thevenon et al. 2012). Due to massive accumulation of organic and industrial effluents especially sewage from hospitals and pharmaceutical industries, the natural status of the water bodies changed in terms of nutritional contents, dissolved oxygen, temperature, pH, and other physiochemical parameters. These create an ideal environment for the

growth, survival, adaptation, and rapid proliferation of many pathogenic microorganisms especially bacterial coliforms. Along with the rapid multiplication, bacteria acquire many additional features due to the sudden changes in their chromosomes; an important concern is the acquisition of drug-resistant genes. These ingested coliforms are able to transfer drug resistance to other sensitive coliforms or enteric pathogens (Truman et al. 2014). The prevalence of carbapenem-resistant *E. coli* that harbored *NDM-1* gene in drinking water and sewage samples in New Delhi, India, was recently reported (Walsh et al. 2012). The superbugs carried various drug resistance genes in tap and springwaters in coastal region of Turkey (Ozgumus et al. 2007), and drinking water biofilms in Mainz, Germany, were also reported (Schwartz et al. 2003). Further, the prevalence of many pathogenic bacteria and their genes responsible for multidrug resistance toward β-lactam, amoxicillin/ampicillin (bla_{TEM}), streptomycin/spectinomycin (*aadA*), tetracycline (*tet*), chloramphenicol (*cmlA*), methicillin (*mec*), and vancomycin (*van*) in various aquatic ecosystems was also reported (Thevenon et al. 2012). Similarly, the prevalence of sulfonamide resistance genes in many aquatic environments in Tianjin, China (Gao et al. 2012), and cefotaxime and ciprofloxacin resistance genes in hospital-associated wastewater samples in Madhya Pradesh, India, were also reported (Diwan et al. 2012). A multidrug-resistant strain of *Salmonella* serovar *typhimurium* definitive type 104 (DT104) (resistant to sulfamethoxazole, tetracycline, streptomycin, chloramphenicol, and ampicillin) emerged across the USA during the 1990s (Glynn et al. 1998). In 2000, the Center for Disease Control and Prevention and several state health departments have identified a surge in the incidence of *Salmonella* serovar *Newport* (known as Newport-MDRAmpC), particularly multiple drug-resistant strains. These strains were also resistant to sulfamethoxazole, tetracycline, streptomycin, chloramphenicol, and ampicillin. Moreover, Newport-MDRAmpC isolates were resistant to cefoxitin, amoxicillin/clavulanic acid, ceftiofur, and cephalothin and showed decreased sensitivity to ceftriaxone (Gupta et al. 2003).

The infections due to MDR pathogens require very complex associations of high doses of old and new antibiotics, and mortality rate is very high. It is expected that at a minimum 25,000 patients in Europe and 23,000 in the USA die each year from infections caused by resistant bacteria (CDC, ECDC). The cost of ABR is incredible, whether measured as the personal and societal burden of illness, death rates, or healthcare costs. The WHO theme for the year 2011 was antimicrobial resistance (AMR), prioritizing the enhanced threat of a return to the pre-antibiotic era, when millions of lives were lost annually due to the MDR pathogens. In the European Union (EU), drug-resistant infections are estimated to generate healthcare costs of 1.5 billion euros per annum. In 2009, the EU has declared November 18 as "European Antibiotic Awareness Day," on each year to promote the cautious use of antimicrobial drugs (Gyles 2011).

4.3 Mechanism of Antibiotic Resistance: Recent Perspective

The expansion of bacterial resistance to antibiotics that had been available in nature prior to antibiotics was considered in chemotherapy. It has been reported that most pathogenic bacteria acquire resistance genes from the natural environments especially soils and water. The entire molecular and genetics cascade responsible for multidrug resistance (antibiotic resistome) has been superior to provide the basic framework for understanding the ecology of resistance. The antibiotic resistome comprises a set of all antibiotic resistance genes including those distributing in pathogenic bacteria, antibiotic producers, and benign nonpathogenic organisms found either free living or commensals of other organisms (Tavares et al. 2013). Most of the antibiotic producers live in soils and water, and as an ecological consequence, most of the susceptible bacteria in their locality, including human and animal pathogens, vanish, but some build up resistance to these natural habitats thought to manage the microbial population (Cox and Wright 2013).

The bacteria have become multidrug resistant by natural means or by acquired resistance. The natural resistance (intrinsic resistance) is due to some genes responsible for resistance to its own antibiotics. Acquired resistance is due to the mutation in bacterial chromosomes or the acquisition of mobile genetic elements (plasmid or transposons) which harbor the drug resistance genes (Martinez 2008). The resistance can be transferred between bacteria by horizontal gene transfer via transformation, transduction, or conjugation. Many drug resistance genes present in plasmids, facilitating their transfer, and develop multidrug-resistant bacteria. Thus, antibiotic resistance genes may be shared among different bacteria. Common biochemical and genetic aspects of antibiotic resistance mechanism are illustrated in Fig. 4.1. Further in detail, the probable mechanisms of antibiotic resistance that are reviewed by Nikaido (2009) are explained below.

4.3.1 Alteration of the Target Protein by Mutation

The bacteria can become resistant through mutations that make the target protein less susceptible to antibiotics. In the case of fluoroquinolone, the resistance is probably due to mutations in DNA topoisomerases, one of the target enzymes (Hooper 2000). The resistance of this antibiotic that is easily transferred to other cells on plasmids depends on the mode of action of the drug. The transfer of the drug-resistant enzyme gene is unable to make the bacteria completely resistant, and the mutated target gene will be transferred. This will be more prevalent in the presence of selective pressure by clonal selection. Similarly, the resistance acquired from target modification is conferred by the *erm* gene, which is responsible for the resistance toward macrolide (such as erythromycin), lincosamide, and streptogramin B. The *erm* gene is a plasmid-encoded gene which produces the

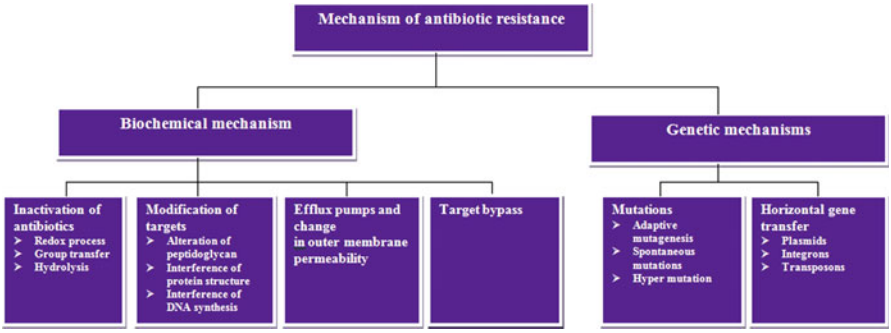

Fig. 4.1 Biochemical and genetic mechanism behind the evolution of drug resistance in bacteria

methylation of adenine at position 2058 of the 50S rRNA (Weisblum 1995). Furthermore, the sulfa drugs select drug-resistant mutants of the respective enzymes. The production of drug-resistant target enzymes from plasmids can make the bacteria resistant, and the resistant genes is widespread on plasmids in the case of sulfa drugs (Huovinen et al. 1995).

4.3.2 Inactivation of the Drug by Various Enzymes

This is the most common mechanism for natural resistance by bacteria. The antibiotic groups such as β-lactams (penicillins, cephalosporins, and carbapenems such as imipenem) inactivated via enzymatic hydrolysis by β-lactamases and aminoglycosides (amikacin, kanamycin, tobramycin, etc.) by enzymatic phosphorylation by aminoglycoside phosphoryltransferase (APH), adenylation by aminoglycoside adenyltransferase or nucleotidyltransferase, and acetylation by aminoglycoside acetyltransferase (AAC). The encoded genes for these inactivating enzymes can easily produce resistance as additional genetic components on plasmids.

4.3.3 Gene Acquisition for Less Susceptible Target Proteins from Other Species

This concept is based on the sequence data of penicillin-binding proteins (PBPs) or DD-transpeptidase, major penicillin target, which revealed that penicillin resistance observed in *Streptococcus pneumoniae* and *Neisseria meningitidis* was due to the production of mosaic proteins, parts of which came from other bacterial species (Spratt 1994, pp. 388–393). Similarly, methicillin-resistant *Staphylococcus aureus* contains a methicillin-resistant penicillin-binding protein (PBP), called PBP-2A or

2′, whose expression is induced by methicillin and other semisynthetic penicillin. The gene for this new PBP is located in a 30–60-kb large segment of DNA, which apparently came from other bacterial species and also contains other antibiotic resistance genes (de Lencastre et al. 2007).

4.3.4 Target Bypassing

The antibiotic vancomycin has an unusual mode of action. Instead of inhibiting an enzyme, vancomycin binds to a substrate, the lipid-linked disaccharide pentapeptide, a precursor of cell wall peptidoglycan. Studies revealed that the end of the pentapeptide, D-Ala-D-Ala, where vancomycin binds, was replaced in the resistant strain by an ester structure, D-Ala-D-lactic acid, which is not bound by vancomycin (Courvalin 2006). Production of this altered structure requires the involvement of many imported genes. Vancomycin resistance is common among enterococci. Since the enterococci are naturally resistant to aminoglycosides, β-lactams, tetracycline, and macrolides, these vancomycin-resistant strains of enterococci become predominant in a hospital environment, colonize the patients, and cause infections that are difficult to treat.

4.3.5 Declining Drug Access to Targets

The drug entrances can be reduced by an active efflux process especially by decreasing the influx across the outer membrane barrier. The main mechanisms are (i) local inhibition of drug access, (ii) drug-specific efflux pumps, and (iii) nonspecific inhibition of drug access.

4.3.6 Local Inhibition of Drug Access

Tet(S) or Tet(M) proteins, produced by Gram-positive bacteria, bind to ribosomes with high affinity and change the conformation of ribosomes, thereby preventing the association of tetracyclines to ribosomes (Connell et al. 2003). Similarly, Qnr proteins are thought to protect DNA topoisomerases from fluoroquinolones (Robicsek et al. 2006).

4.3.6.1 Drug-Specific Efflux Pumps

Drug resistance due to active efflux was discovered with TetA, the tetracycline resistance protein in Gram-negative bacteria. This protein catalyzes a proton-

motive-force-dependent outward pumping of Mg-tetracycline complex (Tamura et al. 2003).

4.3.6.2 Nonspecific Inhibition of Drug Access

Reports suggested that porin, a membrane protein, mutants are found in some of the bacteria as a means of last-line resistance to the recent version of β-lactams that withstand inactivation by β-lactamases. Mutations within the coding sequences of the porin probably reduce the permeation rates of β-lactams without disturbing those of smaller molecules in the nutrient medium (Achouak et al. 2001).

4.4 Need for an Alternative Therapy

The antibiotic resistance became sustainable in the environment as already resistant bacteria emerged as new dominant population and evolved as superbugs (Schjørring and Krogfelt 2011; Gowrishankar et al. 2013). Since the bacteria became resistant to many conventional antibiotics, there is a necessity to identify probable drug targets and screen for alternative therapeutic substances. One promising method is to prevent such drug-resistant pathogens by novel therapeutic compounds that are not based on existing synthetic antimicrobial agents (Chah et al. 2006). The new approaches which have to be implemented include identification of novel molecular markers, screening of novel lead molecules for drug development, identification of novel treatment methods, and identification of a sample bacteria and its susceptibility to antibiotic treatment. There is also a need for a deeper understanding of the mechanisms by which bacteria gain resistance to antibiotics which will aid in identifying novel targets for drugs or treatment (Daniels 2011). Studying the genetic variation among plasmids from different bacterial species or strains is a key step toward understanding the mechanism of virulence and their evolution. Understanding their virulence helps in designing more effective drugs against the antibiotic-resistant microorganisms. The recent availability of new sequencing technologies provides the capability for rapid and cost-effective sequencing of small genomes (Siegel et al. 2006). Drug discovery and development are complex, laborious, and interdisciplinary approaches. For the pharmaceutical industry, the time span required to introduce a new drug to market is approximately 12–14 years and costing up to $1.2–$1.4 billion. For every 10,000 compounds that are tested in animal models, around 10 will qualify for clinical trials in order to get one drug on the market (Pandey et al. 2010).

By considering all the socio-environmental issues, there is a pressing need for screening novel lead molecules. The new approaches which have to be implemented include identification of new molecular markers, identification of novel lead molecules for drug development, identification of novel treatment methods, and identification of a sample bacteria and its susceptibility to antibiotic

treatment. There is also a need for a deeper understanding of the mechanisms by which bacteria gain resistance to antibiotics which will aid in identifying novel targets for drugs or treatment (Daniels 2011). Advanced drug discovery process has been revolutionized with the advent of computational biology, genomics, proteomics, combinatorial chemistry, high-throughput screening, and structure-based design. The important aspects of computation in drug developments are virtual screening, de novo design, in silico ADMET prediction, and determination of receptor-ligand interactions. In silico ADMET prediction, screening is performed alongside of the in vitro data generated, for analyzing the target structures for possible binding conformation, generating bioactive conformation, checking the drug likeliness of ligands, docking these molecules with the target, ranking them according to their binding affinities, and further optimizing the molecules to improve the binding characteristics. Computational biology tools provide the advantage of delivering new therapeutic agents with ideal drug likeliness and pharmacophoric properties. High-performance computing and data management tools are enabling the access of large amount of complex biological data into executable knowledge in advanced drug discovery process.

4.5 Scope of Computer-Assisted or Structure-Based Drug Discovery

The screening and characterization of lead molecule showing therapeutic property against a biological target and standardization of the druggish properties and efficiency of these molecules are the initial stages of drug screening. For this purpose, many pharmaceutical industries have adopted the experimental screening of large chemical libraries against a therapeutically appropriate target (high-throughput screening or HTS) to discover new lead compounds. Through HTS, bioactive compounds, drug-resistant genes, or toxins, which amend a particular metabolic pathway, can be identified; these provide an initial insight for drug discovery and for knowing the role of a particular biochemical process in biological sciences. Even though HTS remains as the main attraction for drug discovery in the pharmaceutical industry, the various demerits of this approach that include the high capital cost, time, and the ambiguity of the mode of action of the bioactive lead molecules have turned to the increasing service of rational, structure-based drug design (SBDD) with the use of computational biology approaches. The important stages of structure-based drug discovery are illustrated in Fig. 4.2 (Lionta et al. 2014).

Computer-assisted drug discovery (CADD) is now being used for the identification of active drug candidates and selection and optimization of lead molecules which transform biologically active compounds into suitable drugs by improving their pharmacokinetic and drug likeliness properties. Computer-aided virtual screening is used to screen novel lead molecules from various chemical scaffolds

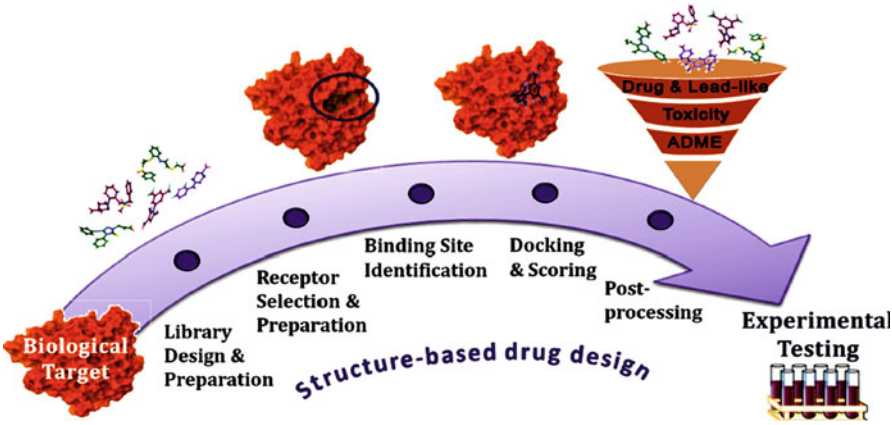

Fig. 4.2 Various stages of structure-based virtual screening ranging from receptor and library preprocessing to docking, scoring, and post-processing of top-scoring hits (Lionta et al. 2014)

by searching chemical structure databases and other resources (Kapetanovic 2008). CADD is the fundamental concept of structure-based drug design that uses a variety of computational methods to screen novel lead molecules with selectivity, efficacy, and safety. The study of receptor-ligand interaction is the main focus of rational drug design, and the prediction of such interactions by computational approaches has profound scope and applications (Lyskov and Gray 2008).

At present, structure-based drug discovery (SBDD) is the vital approach to the resourceful development of various therapeutic leads and to the understanding of metabolic processes especially the molecular-level mechanisms. SBDD is a well-established approach than the traditional way of drug discovery process to demonstrate the molecular mechanisms of a disease and utilizes the understanding of the three-dimensional (3D) structure of the biological target in the process. By the application of various bioinformatics approaches and the 3D structural information of the target protein, it is possible to explore the molecular interactions concerned with the protein-ligand binding and thus deduce the experimental results in molecular level. The utility of computer science and information technology in drug discovery provides the additional benefit of delivering novel drug candidates cost-effectively and quickly (Lionta et al. 2014).

The main concepts behind structure-based drug design methods are virtual screening (VS) and de novo drug design; these approaches serve as an alternative efficient approach to HTS. The main concept of virtual screening includes large libraries of drug-like molecules that are commercially obtainable and are screened computationally against probable targets of known structure, and those that are predicted to have better binding potential are validated experimentally (Lavecchia and Di Giovanni 2013). However, virtual screening does not offer molecules that are structurally "novel" as these molecules have been previously synthesized by various medicinal chemists. In the de novo drug design process, the information obtained from the 3D cavity of the receptor is used to design structurally relevant

molecules that have not been synthesized previously by chemical intuition or any other methods (Jorgensen 2004).

Computer-assisted drug screening has recently had an important accomplishment: novel biologically active molecules have been predicted along with their receptor-bound conformation, and in quite a few cases, the success rates have been greater than with conventional high-throughput screening (Lavecchia and Di Giovanni 2013; Benod et al. 2013). Furthermore, though it is unusual to deliver lead molecules in the nanomolar (nM) concentration through virtual screening, several recent studies have demonstrated that the identification of nM leads from virtual screening approaches (Heifetz et al. 2013). Hence, computational biology methods play a vital role in the drug discovery and development process in the pharmaceutical sectors.

Computational biology became increasingly important in various areas such as gene and protein prediction, comparative or homology modeling, functional site location, characterization of active site for binding, docking of lead molecules into receptor-binding sites, protein-protein interactions, and molecular simulations. The outcome of computational studies yields information that is sometimes beyond current experimental possibilities and can be used to guide and improve a vast array of experiments (Gago 2004). Studies emphasize that the recognition of remote protein homologies is a major aspect of the structural and functional annotation of newly determined antibiotic resistance genes. PSI-BLAST is used for genome annotation using the widely used homology-searching program (Muller et al. 1999).

The primary necessity of computer-aided drug design is the three-dimensional structure of the resistant gene products or other drug targets such as toxins. However, the three-dimensional structures of most of the targets are not available in native forms. Hence, there is a need for an accurate three-dimensional model. This can be achieved by comparative modeling or homology modeling. Comparative modeling of proteins is a predictive technique to build high-resolution atomic model for a given amino acid sequence based on the structures of templates that have been experimentally determined. The ultimate goal of this modeling is to predict a structure from its sequence with an accuracy that is comparable to the best results achieved experimentally (Marti-Renom et al. 2000).

4.5.1 Scope of Molecular Docking Studies

A lead molecule is usually a small organic molecule, also known as ligand that binds to the target protein or receptors and changes the physiological function of the receptor, thus, leading to a therapeutic impact. Molecular docking or computer-assisted docking is an exceptionally useful means to achieve the understanding of receptor-ligand interactions which is a fundamental concept behind structure-based drug discovery. Computational docking is the method of computationally predicting the interaction and binding affinity of the lead molecule or inhibitor in the binding cavity of the protein. Molecular docking methods depend on search

algorithms which determine the interaction of ligand in the binding cavity and a scoring function which calculates the binding efficiency, how perfectly the ligand interacts with the receptor (Dhanik and Kavraki 2012). The main forces that stabilized the receptor-ligand interactions are weak interactions such as hydrogen bonds, hydrophobic interactions, van der Waals forces, and electrostatic interaction. Hence, the main parameters required to evaluate a stable docked complexes are number of hydrogen bonding, extent of electrostatic interactions, and negative binding energy (kcal/mol). There are various methods that have been developed to explain the principles and concepts behind computational docking problems; some of the main concepts of molecular docking are:

- Molecular docking methods play a vital role in the drug discovery and development process.
- The docking methods identify the interaction of a ligand molecule in the binding cavity of receptor and determine the binding efficiency.
- There are two main important approaches for docking studies: (i) rigid-body docking and (ii) flexible body docking. The rigid-body docking approaches consider both the receptor and ligand as rigid bodies. However, flexible-body docking approaches consider the ligand as a flexible molecule, and flexible receptor approaches consider both the ligand and the protein as flexible molecules. In most of the cases, the docking programs consider the ligand as a flexible molecule and protein as a rigid molecule.
- The fundamental concepts involved in the docking studies are conformation search (by algorithm) and a scoring function that evaluate the binding capacity and efficiency.
- The flexibility of the protein is an essential component to determine the accuracy of various docking programs.
- There are various efforts that have been made to demonstrate the flexibility of protein in molecular docking studies; however, more studies need to be carried out.

Molecular docking has been an ideal option for the modeling of three-dimensional structure of the protein-ligand complex and evaluating the stability that estimates the specific biological recognition. However, there are few issues associated with these approaches: primarily, investigating the conformational space of ligands that interact with the receptor, and, secondly, ranking the conformations according to their estimated binding affinities (scoring) (Koehler and Villar 2000). More clearly, with the help of scoring function, the conformation of ligand is generated and compared to the previous conformations. The present conformation is further considered or discarded on the basis of the total score for that conformation. Furthermore, a new conformation is generated, and the search process continues until it covers all possible conformations. Hence, searching conformation and scoring can be coupled in docking process (Shoichet et al. 2002). Hence, it is very essential to identify better scoring functions so that the maximum rank ordered conformation would have higher experimental binding affinity with the receptor.

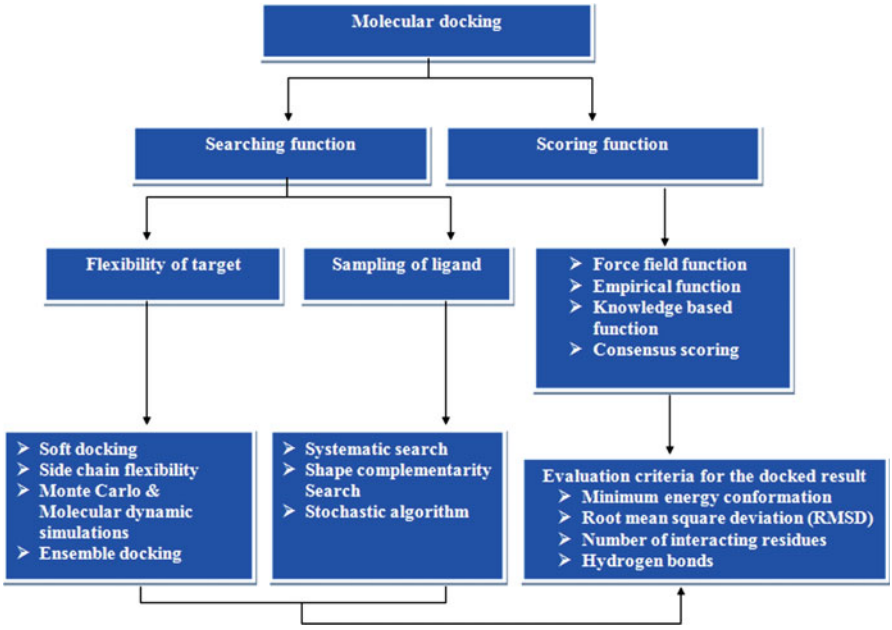

Fig. 4.3 Overview of molecular docking shows the steps involved in searching function and scoring function

The overview of molecular docking is illustrated in Fig. 4.3. The docking algorithm utilizes various approaches for conformational search in order to search conformational space of the ligand. The main approaches are:

(a) Systematic torsion exploration, which places the small molecules in the predicted binding pocket after considering the possible degrees of freedom
(b) Stochastic or random torsion exploration about rotatable bonds, such as genetic algorithms or Monte Carlo method to "achieve" new minimum energy conformers
(c) Molecular dynamics simulation approach and energy minimization for exploring the stable energy landscape of a compound (Lionta et al. 2014)

Scoring function is another critical step in docking process. The estimation of binding affinity between the receptor and ligands is the main logic of scoring function. The scoring functions have two main responsibilities. First, these functions serve as an objective function to distinguish between various poses of a single ligand in the receptor-binding pocket. Second, the scoring functions are essential to determine binding capabilities of various receptor-ligand complexes and to rank them as per the binding energies. The main factors that influence the receptor-ligand interactions are hydrogen bonding, van der Waals and dispersion interactions, hydrophobic effects, steric and electrostatic interactions, and solvation effects

which are directed by various kinetic and thermodynamic principles (Reddy et al. 2007). The various approaches of scoring functions include shape and chemical complementary scoring, force field scoring, empirical scoring functions, and knowledge-based scoring functions. These methods are more or less combinations of ensemble-averaged terms and comprise a compromise between real and computational effort. The most effective search algorithm stops functioning in the absence of an ideal scoring function. The popular scoring functions currently available are grouped as (a) force field-based, (b) empirical-based, (c) knowledge-based, and (d) consensus-based scoring functions (Perola et al. 2004). A comprehensive list of various docking software available for public domains for the effective protein-ligand docking studies is reviewed in Table 4.1.

Table 4.1 List of the most popular protein-ligand docking programs available as of the middle of 2015

Docking software/ program	Year of establishment	Country of origin	References
DOCK	1988	USA	Ewing et al. (2001)
AutoDock	1990	USA	Morris et al. (1998)
SOFTDocking	1991	USA	Jiang and Kim (1991)
DockVision	1992	Canada	Hart and Read (1992)
LUDI	1992	Germany	Bohm (1992, pp. 61–78)
ADAM	1994	Japan	Mizutani et al. (1994)
FLOG	1994	USA	Miller et al. (1994)
SYSDOC	1994	USA	Luty et al. (1995)
DIVALI	1995	USA	Clark (1995, pp. 1210–1226)
GOLD	1995	UK	Jones et al. (1997)
FlexX	1996	Germany	Kramer et al. (1999)
Hammerhead	1996	USA	Welch et al. (1996)
LIGIN	1996	Israel/Germany	Sobolev et al. (1996)
FTDOCK	1997	UK	Gabb et al. (1997)
ICM-Dock	1997	USA	Totrov and Abagyan (1997)
QXP	1997	USA	McMartin and Bohacek (1997)
PRO LEADS	1998	UK	Baxter et al. (1998)
SANDOCK	1998	UK	Burkhard et al. (1998)
MCDOCK	1999	USA	Liu and Wang (1999)
PRODOCK	1999	USA	Trosset and Scheraga (1999)
SFDOCK	1999	China	Rodinger and Pomes (2000)
DARWIN	2000	USA	Taylor and Burnett (2000)
EUDOC	2001	USA	Pang et al. (2001)
FLEXE	2001	Germany	Claussen et al. (2001)
FDS	2003	UK	Taylor et al. (2003)
FRED	2003	USA/UK	McGann et al. (2003)
LigandFit	2003	USA	Venkatachalam et al. (2003)

(continued)

Table 4.1 (continued)

Docking software/ program	Year of establishment	Country of origin	References
PhDOCK	2003	USA	Joseph-McCarthy et al. (2003)
Surflex	2003	USA	Jain (2003, pp. 499–511)
iGEMDOCK	2004	Taiwan	Yang and Chen (2004)
Glide	2004	USA	Halgren et al. (2004)
ProPose	2004	Germany	Seifert et al. (2004)
YUCCA	2005	USA	Choi (2005, pp. 1517–1524)
eHiTS	2006	Canada/UK	Zsoldos et al. (2007)
MolDock	2006	Denmark	Thomsen and Christensen (2006)
PLANTS	2006	Belgium/ Germany	Korb et al. (2006)
PSI-DOCK	2006	China	Pei et al. (2006)
EADock	2007	Switzerland	Grosdidier et al. (2007)
FLIPDock	2007	USA	Zhao and Sanner (2007)
MDock	2007	USA	Huang and Zou (2007)
ParDOCK	2007	India	Gupta et al. (2007)
PSO@AUTODOCK	2007	Germany	Namasivayam and Gunther (2007)
SODOCK	2007	Taiwan	Chen et al. (2008)
Lead finder	2008	Russia/Canada	Stroganov et al. (2008)
MS-DOCK	2008	France	Sauton et al. (2008)
Q-Dock	2008	USA	Brylinski and Skolnick (2008)
MADAMM	2009	Portugal	Cerqueira et al. (2009)
AutoDock Vina	2010	USA	Trott and Olson (2010)
AADS	2011	India	Singh et al. (2011)
BetaDock	2011	South Korea	Kim et al. (2011)
LigDockCSA	2011	South Korea	Shin et al. (2011)
PythDock	2011	South Korea	Chung et al. (2011)
VoteDock	2011	Poland	Plewczynski et al. (2011)
idTarget	2012	Taiwan	Wang et al. (2012)
EpiDOCK	2013		Atanasova et al. (2013)
rDock	2013	UK	Ruiz-Carmona et al. (2014)
FIPSDock	2013	China	Liu et al. (2013)
DINC	2013	USA	Dhanik et al. (2013)
iStar	2014	UK	Li et al. (2014)
PharmDock	2014	USA	Hu and Lill (2014)
MoDock	2015	China	Gu et al. (2015)

4.6 Herbal Bioactive Compounds as Novel Therapeutics Against MDR Bacteria

There are reports suggesting that several herbs produce bioactive compounds which are effective therapeutic agents (Nair et al. 2005). These medicinal plants are well studied and their bioactive compounds have been separated. Their structural and functional mechanisms have also been established. Moreover, the bioactivity assay, modes of action, and inhibitory properties against various drug targets for many herbal-derived compounds are well studied (Briskin 2000). Computer-aided drug design (CADD) is an effective platform to screen several herbal lead molecules with better pharmacokinetic features and bioavailability (Bharath et al. 2011).

There are many databases which host the complete information of various lead molecules. The three-dimensional structures of most of the ligands are elucidated experimentally and can be retrieved from various databases. The most popular small molecule databases are ZINC (Irwin and Shoichet 2005), NCBI PubChem (Wang et al. 2012), Chemspider (Little et al. 2012), Drug Bank (Wishart et al. 2008), KEGG (Kanehisa 2002), etc.

There are many reports revealing the utility of computer-aided virtual screening toward the screening of novel therapeutic agents with better pharmacokinetic properties. Recent reports revealed the inhibitory properties of bioactive compounds screened from essential oils toward various drug targets of *Streptococcus mutans* (Galvão et al. 2012) by computational virtual screening. Similar reports showed that phytochemical compounds screened from few medicinal plants have significant inhibitory properties against various drug targets of multidrug-resistant clinical isolates (Dahiya and Purkayastha 2012). Similarly, the inhibitory activity of kurarinone, a bioactive flavonoid isolated from *Sophora flavescens*, against drug targets of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Streptococcus* sps., and *Streptococcus mutans* was also reported (Chen et al. 2005). Recently, it has been suggested that novel herbal inhibitors screened by computational virtual screening demonstrated good inhibitory properties against streptolysin-O of MDR *Streptococcus pyogenes* (Skariyachan et al. 2014). Similarly, a study also suggested that the herbal leads screened by in silico approach were found to have better inhibitory activities against the MDR gene products of *Vibrio cholerae*, *Salmonella typhi*, and *Staphylococcus aureus* (Skariyachan et al. 2013). Furthermore, previous studies have identified many novel lead molecules against virulent toxins of many superbugs (Skariyachan et al. 2012).

The computational redesign of bacterial biotin carboxylase inhibitors using structure-based virtual screening was recently reported (Brylinski and Waldrop 2014). Further, the identification of novel inhibitors of the glyoxylate shunt in MDR Gram-negative pathogens was also reported (Fahnoe et al. 2012). In silico discovery and virtual screening of multi-target inhibitors for various drug targets in *Mycobacterium tuberculosis* were recently reported (Chung et al. 2013). Similarly, another report revealed the utility of polyphosphate kinase (PPK) as a novel antimicrobial drug target and its high-throughput virtual screening toward MDR

E. coli isolates (Saha and Verma 2013). The inhibitory properties of novel lead candidates toward bacterial serine protease from various MDR isolates by computational virtual screening were also recently reported (Mandal et al. 2014). Furthermore, recent study demonstrated that herbal-based compounds such as nimbolide and isomargololone showed an appreciable IC50 value and significant binding properties toward New Delhi metallo-beta-lactamase 1 (bla_{NDM}) in comparison with 14 β-lactam antibiotics. The docking result of the antibacterial herbal compounds demonstrated that nimbolide (1.34 μM), isomargolonone (1.25 μM), margolone (5.25 μM), margolonone (5.34 μM), acetyl aleuritolic acid (0.2772 μM), and harmane (4.32 μM) had IC50 value lower than β-lactam antibiotics; this implies the therapeutic potential of herbal-based ligands over conventional drugs (Thakur et al. 2013). Similarly, lanatoside C and daidzein, two natural herbal leads, were identified as natural compound inhibitors against multidrug efflux pumps of *Escherichia coli* and *Pseudomonas aeruginosa* using computer-assisted virtual screening and in vitro validation (Aparna et al. 2014).

4.6.1 Relevance of Computational Discovery of Novel Herbal Therapeutics Toward MDR Bacterial Targets

The study of receptor-ligand interactions plays a vital role in understanding the screen novel therapeutic agents against multidrug-resistant pathogens. Molecular docking is the fundamental approach to study such kind of interactions for structure-based drug discovery. The following sections will explain how the docking studies are useful screen novel herbal therapeutic agents against various MDR pathogens.

The binding properties of various phytoligands toward the probable drug targets of multidrug-resistant *Salmonella typhi* and *Vibrio cholerae* and methicillin- and vancomycin-resistant *Staphylococcus aureus* were explored by molecular docking studies (Skariyachan et al. 2013). The genes responsible for multidrug properties of these organisms were screened. The selection of the genes was based on literature studies (Chen et al. 2010; Martínez 2012; Reimer et al. 2011; Hiramatsu et al. 1992; Weigel et al. 2003). Aminoglycoside phosphotransferase (aph; Uniprot ID: E2D0Y8), virulent protein for kanamycin resistance (Chen et al. 2010), and dihydrofolate reductase (dhfr; Uniprot ID: A7DY50), responsible factor for trimethoprim resistance of *Salmonella typhi* (Martínez 2012), were selected. Similarly, dihydrofolate reductase type I (dfrA1; Uniprot ID: G7TU76) and virulent factor for trimethoprim resistance from *Vibrio cholerae* (Reimer et al. 2011) were selected. Methicillin-resistant gene (Mec1; Uniprot ID: P68261) (Hiramatsu et al. 1992) and vancomycin-resistant gene (VanH; Uniprot ID: Q7BWD8) from *Staphylococcus aureus* (Weigel et al. 2003) were also selected.

Molecular docking studies suggested that baicalein, a type of flavonoid, commonly present in the root of *Scutellaria baicalensis*, and luteolin, another flavonoid

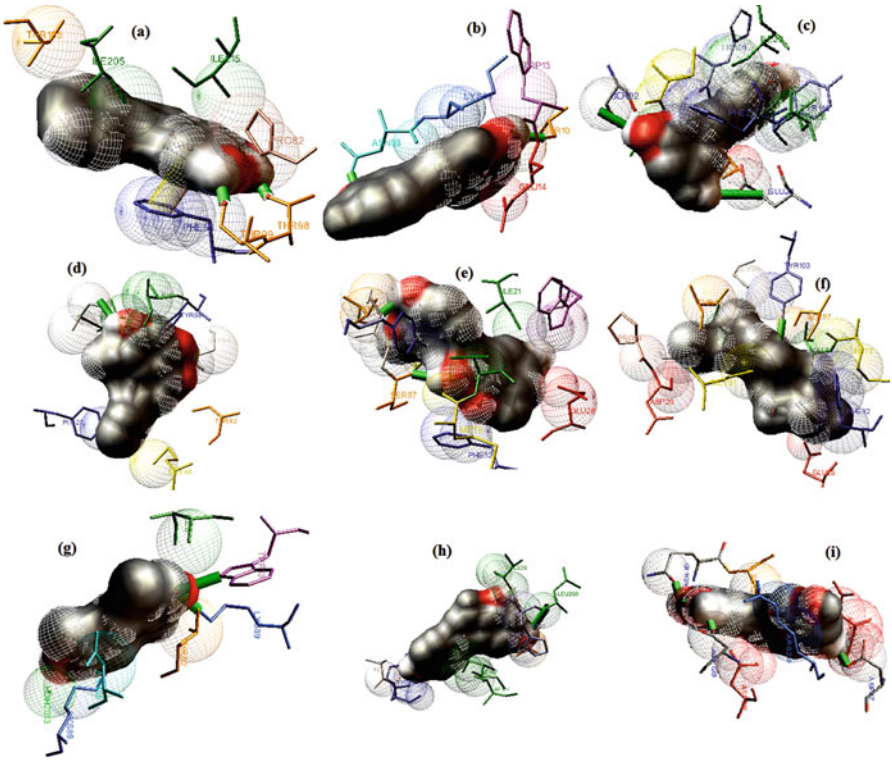

Fig. 4.4 Binding efficiency of phytoligand toward various drug targets of MDR pathogenic bacteria. Interaction between *aph* of *Salmonella typhi* and (**a**) baicalein and (**b**) luteolin. Interaction between *dhfr* of *Salmonella typhi* and (**c**) resveratrol and (**d**) wogonin. Interaction between dfrA1 of *Vibrio cholerae* and (**e**) herniarin and (**f**) pyrocide. Interaction between *mec1* of methicillin *Staphylococcus aureus* and taraxacin (**g**). Interaction between *vanH* of vancomycin-resistant *Staphylococcus aureus* and (**h**) apigenin and (**i**) luteolin

present in *Terminalia chebula*, showed the best interactions with aminoglycoside phosphotransferase (*aph*, drug-resistant gene for kanamycin resistance) of *Salmonella typhi*. The binding energy of *aph*-baicalein docked complex was estimated to be −6.39 kcal/mol, and the interactions were stabilized by two hydrogen bonds (Thr 98, Thr 99) (Fig. 4.4a). Similarly, the binding energy of *aph*-luteolin was estimated to be −6.42 kcal/mol, and the interactions were stabilized by two hydrogen bonds (Asn 88 and Ser 10) (Fig. 4.4b). From this study, it is clear that these phytoligands have significant binding and inhibitory properties toward kanamycin-resistant protein. Resveratrol, a natural phytoalexin commonly present in *Vitis vinifera* (grape), and wogonin, an O-methylated flavone found in *Scutellaria baicalensis* (baikal skullcap), showed significant inhibitory activities against dihydrofolate reductase (*dhfr*, gene product responsible for trimethoprim resistance) of *Salmonella typhi*. From the docking studies, it is clear that the binding energy of resveratrol toward *dhfr* was identified as −7.58 kcal/mol, and the

interaction was stabilized by two hydrogen bonds (Glu 23, Ser 92) (Fig. 4.4c). The binding energy of wogonin against *dhfr* was estimated to be −7.28 kcal/mol (Fig. 4.4d). The interactions were stabilized by two hydrogen bonds (Ala 3, Gly 93). The antimicrobial effects of resveratrol and wogonin against various bacterial pathogens and their toxins have been studied (Schrader 2010, pp. 1676–1689; Chan 2002, pp. 99–104), which revealed that these phytochemicals have significant inhibitory properties toward virulent factors of many MDR pathogens. Herniarin, a natural chemical compound found in *Herniaria glabra* (smooth rupturewort), and pyrocide, a common flavone present in *Daucus carota* (carrot), showed the best binding activity toward dihydrofolate reductase (*dfrA1*, trimethoprim-resistant protein) of *Vibrio cholerae*. The docking studies suggested that the docked complex of *dfrA1* herniarin was stabilized by two hydrogen bonds (Ser 97, Gly 98) with binding energy of −8.06 kcal/mol (Fig. 4.4e). Studies on the antifungal and antibacterial activities of various herniarin derivatives revealed that these phytoligands showed good inhibitory activities against various enteric bacterial pathogens (Céspedes et al. 2006). Similarly, the interaction between pyrocide and *dfrA1* was stabilized by a hydrogen bond (Tyr 103) with binding energy of −8.93 kcal/mol (Fig. 4.4f). Though pyrocide exhibits better binding energies (−8.93 kcal/mol), the number of interactions with receptor is only Tyr 103 residue. Hence, better simulation studies are essential to screen this ligand, and present data pave significant insight for such studies. Luteolin and taraxacin, a sesquiterpene guaianolide present in *Taraxacum officinale* (weber), showed better binding properties toward *mecI* protein (gene code for methicillin resistance) of *Staphylococcus aureus*. The molecular docking studies revealed that the docked complex of *mecI* and luteolin were stabilized by two hydrogen bonds (Ala 101, Tyr 102) with binding energy of −7.58 kcal/mol. Similarly, taraxacin binds with *mecI* by the formation of two hydrogen bonds (Trp 13 and Lys 89) with the binding energy of −7.28 kcal/mol (Fig. 4.4g). This study depicts that Gly, Lys, His, and Thr are the main conserved residues which play a major functional role in the receptor (Kahlon et al. 2012). From this study, it is clear that these phytochemicals have significant inhibitory properties toward probable drug targets of MDR pathogens. Many studies revealed the inhibitory properties of taraxacin and luteolin (Ahmad et al. 2000) against various pathogenic microorganisms by different mechanisms. Apigenin, a flavone found in *Coffea arabica* (coffee), and luteolin were found to interact against *vanH* (gene responsible for vancomycin resistance) protein. The docked complex of *vanH*–apigenin was stabilized by two hydrogen bonds (Tyr 102, Leu 200; binding energy −6.07 kcal/mol) (Fig. 4.4h). Similarly, luteolin interacted with *vanH* by three hydrogen bonds (Gln 35, Asp 198, and Asp 64; binding energy −6.32 kcal/mol), and the main residues present in the active sites are Gln 35, Ser 36, Asp 64, Asp 67, Asp 198, Asp 216, and Arg 219 (Fig. 4.4i) (Skariyachan et al. 2013). The antimicrobial activities of all these lead molecules are well studied. A significant inhibitory property of apigenin toward drug-resistant *Enterobacter cloacae* was recently reported in comparison with the known chemotherapeutic agent, ceftazidime (Eumkeb and Chukrathok 2013). The current study identified various phytoligands which showed effective binding and conformational changes in drug targets. The binding

efficiency of these phytoligands toward various drug-resistant proteins paves better understanding of the inhibitory mechanism of herbal leads, and such studies have high relevance in clinical and preclinical trials.

In another study, the author suggested that afzelin and gallocatechin, two important herbal ligands, demonstrated good binding affinities toward bla_{TEM} (gene products responsible for β-lactam resistance) of multidrug-resistant bacteria. Afzelin is a flavonol glycoside commonly present in the medicinal herb *Nymphaea odorata* (fragrant water lily). The molecular docking studies suggested that afzelin binds to bla_{TEM} with an energy of −7.44 kcal/mol, and the interaction is stabilized with three hydrogen bonds (Fig. 4.5a). Similarly, gallocatechol or gallocatechin is a flavanol commonly present in *Camellia sinensis* (green tea). It was found to be a noncarcinogenic compound to both rat and mouse models. The molecular docking suggested that the phytoligand binds bla_{TEM} with three hydrogen bonds by the binding energy of −6.36 kcal/mol (Fig. 4.5b). The antibacterial potential of Azelin (azelaic acid) against various clinical pathogens is reported (Fluhr and Degitz 2010). Similarly, the inhibitory potential of gallocatechin against drug targets of various multidrug-resistant isolates was also reported (Radji et al. 2013).

The in silico data provides significant insights for further experimental validation of novel inhibitors against the drug targets of MDR pathogens. A recent study reported by Wang et al. (2015) revealed the discovery of novel New Delhi metallo-β-lactamase-1 inhibitors by multistep virtual screening and docking studies (Wang et al. 2015). The *NDM-1* enzyme provides bacterial resistance against the β-lactam ring of antibiotics by its hydrolytic activity. Inhibition of *NDM-1* may stop the hydrolysis of β-lactam ring and plays a vital role against antibacterial resistance. The study focused the screening of potential *NDM-1* inhibitors by multistep virtual screening and molecular docking simulations. The study demonstrated that they have screened 2,800,000 lead-like molecules from the ZINC database and generated 298 compounds, and the binding efficiency was studied by molecular docking

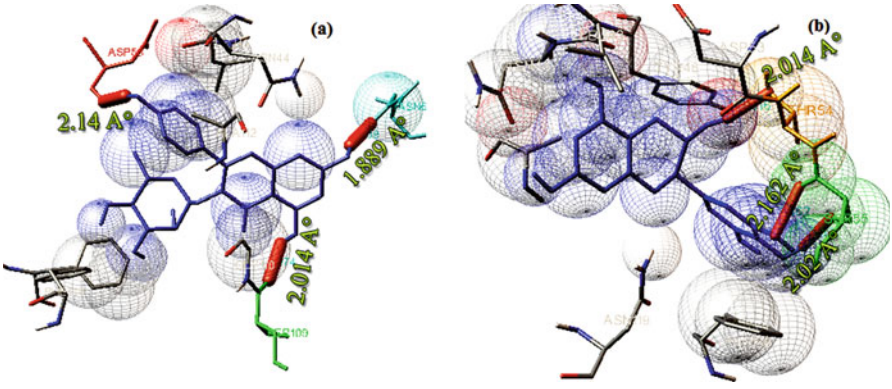

Fig. 4.5 Receptor-ligand interaction between (**a**) afzelin and bla_{TEM} and (**b**) gallocatechin and bla_{TEM} studied by molecular docking

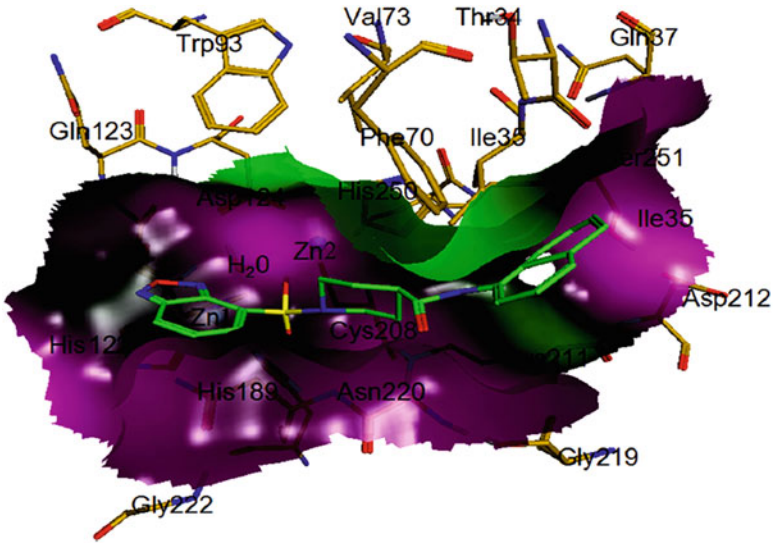

Fig. 4.6 The docked conformation showing the interaction between the active site of *NDM-1* and VNI-41. VNI-41 and adjacent *NDM-1* residues shown in stick representation, and the binding cavity is shown as molecular surface (Wang et al. 2015)

with *NDM-1*. The best lead molecules obtained from virtual screening and docking analysis were experimentally validated. Three novel *NDM-1* inhibitors with IC50 μM values were validated. Among the tested molecules, VNI-41 showed better inhibitory properties against *NDM-1* with an IC50 of 29.6 ± 1.3 μM. Molecular dynamic simulation based on the docking studies revealed that VNI-41 interacted with the active site (Fig. 4.6) (Wang et al. 2015). This study clearly demonstrated the possibility of applying virtual screening especially molecular docking studies in discovering novel and potential inhibitors against *NDM-1*, a metallo-β-lactamase of various multidrug-resistant bacterial pathogens.

Similar studies conducted by Thakur et al. (2013) suggested that molecular docking studies pave significant insight to design novel natural compounds against *NDM-1* gene products of various MDR pathogens. They have used molecular docking approach to design novel natural inhibitors against *NDM-1* receptor of MDR pathogens. The study suggested that lead molecules from botanicals such as nimbolide and isomargololone, bioactive compounds derived from *Azadirachta indica* (Neem tree), demonstrated good IC50 value as well as significant binding potential toward *NDM-1*. The study further suggested that the natural compounds expressed better binding affinity to *NDM-1* in comparison with conventional *β*-lactam antibiotics (Thakur et al. 2013).

4.7 Conclusion

As many bacteria emerged as extreme drug-resistant strains, designing of alternative remedies has prime scope and therapeutic relevance. Thus, there is a priority to screen new leads. The exploration of phytomedicine through molecular docking-based approaches serves as ultimate platforms to discover novel inhibitors against these drug targets, and present concepts offer outstanding landmarks for further in vitro and in vivo studies.

4.8 Future Perspectives

Molecular docking approaches are an effective platform to discover novel lead molecules against various drug targets of multidrug-resistant bacteria when conventional therapies seem to have failed. Molecular docking provides a comprehensive profile of the receptor-ligand interaction which is the fundamental concept of structure-based drug discovery. However, further experimental analysis is required to appreciate the hypothesis. Hence, the herbal bioactive compounds hypothesized needed to be isolated and characterized. The purified lead molecules need to be tested in vitro and in vivo to validate the proposed hypothesis-based molecular docking studies. The current approach has profound scope and applications in the development of future therapies against multidrug-resistant pathogens.

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