

Exploring the binding potential of carbon nanotubes and fullerene towards major drug targets of multidrug resistant bacterial pathogens and their utility as novel therapeutic agents

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1.1 INTRODUCTION

1.1.1 MORTALITY RATE DUE TO GRAM-NEGATIVE BACTERIA

Gram-negative bacteria manifest high level resistance to most classes of antibiotics (Zabawa et al., 2016). The infections caused by multidrug resistant (MDR) gram-negative organisms are increasing in hospitals, particularly in Intensive Care Unit (ICU) and are associated with higher costs, increased morbidity, and lead to high mortality rates (Chelazzi et al., 2015). Several factors that contribute to the increased risk of infection in ICU patients, includes greater severity of illness, overuse of existing antimicrobial agents, underlying conditions, exposure to multiple invasive devices and procedures, increased patient contact with health care personnel, and crowding of patients in a small specialized area (Ivady et al., 2015). Gram-negative bacteria, particularly *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumonia* are causing health problems and especially to the hospitalized patients. There is an increasing demand to come up with alternative therapeutics that target MDR gram-negative infections (MacVane et al., 2015).

The rates of MDR gram-negative bacteria across the world generally suggest an increasing resistance towards the southeast of Europe, Latin America and Asia Pacific and lower resistance in the northwest of Europe, United States, and Canada (Curcio, 2014). In 2010, data from US National Healthcare Safety Network emphasized that gram-negative bacteria account for >30% of hospital-acquired infections, and in ICUs they represent for about 70% of these infections; similar data have reported from different parts of the world (Peleg and Hooper, 2010). In 2010, a systematic review included data from 47 countries and estimated that 13.5 million cases of typhoid fever occurred globally and around 200–300 cases of *Salmonella typhi* are reported each year in the United States (Bula Rudas et al., 2015). One of the effective strategies to combat MDR organisms is the development of novel antimicrobial agents (Cerceo et al., 2016). Fighting MDR bacterial infections with edible plants represents an attractive strategy (Dzotam et al., 2016).

1.1.2 EMERGENCE OF MULTIDRUG RESISTANT AND EXTENSIVELY DRUG RESISTANT

The emergence of multidrug resistance in infectious bacteria has emerged as a global threat to public health. Over last few decades, the consequences of microbial infections have increased dramatically. Constant deployment of novel antimicrobials in treating infections has led to the emergence of resistance amidst different strains of microorganisms (Tanwar et al., 2014). MDR organisms including Vancomycin-resistant *enterococci*, Methicillin-resistant *Staphylococcus aureus* (MRSA), and certain gram-negative bacilli such as *P. aeruginosa* and *A. baumannii* which causes severe and lethal human infections primarily in patients who are critically ill (Izadpanah and Khalili, 2015). Although various novel drugs have been developed commercially, the evolution of resistance among infectious agent is increasing, mainly in patients who undergo prolonged drug exposure (Liao et al., 2015). Nosocomial infections caused by extensively drug-resistant and MDR gram-negative pathogens represent a major threat worldwide (Karaïskos and Giamarellou, 2014).

One of the studies revealed that MDR bacteria isolated from the patients affected with urinary tract infection (UTI) from Jamshedpur city, Jharkhand and Shimoga city, Karnataka, India. This study depicted that *enterobacteriaceae* are the predominant causative organisms to cause UTI and 38.8% were MDR in Jamshedpur city, Jharkhand, India and 55.8% were MDR in Shimoga city, Karnataka, India. A 2013 report by the American Centers for Disease Control estimates that >2 million illnesses and 23,000 deaths are caused by MDR pathogens in US yearly (Gill et al., 2015). A study carried out in Brazil suggested that, out of 237 species of *Salmonella*, 52% of the isolated strains of *Salmonella* were resistant to at least one the tested novel antimicrobials (Rowland et al., 2014). One approach to effectively combat MDR is the use of drug combinations, such as pairing of an antibiotic with a nonantibiotic adjuvant molecule and antibiotic-antibiotic combinations (Worthington and Melander, 2013).

1.2 NEED AND SCOPE OF SCREENING THERAPEUTIC AGENTS

The main problem is that bacteria are showing resistance even to second-line treatments and in order to solve the problems arising from resistant gram-negative bacteria, many researchers have tried to come up with effective novel therapeutic agents. The drug delivery systems (DDS) mediated with nanoparticles have greater potential to combat resistant pathogens (Hosseini et al., 2015). Nanoparticles, usually 0.2–100 nm in size, are used in specific areas, such as superconductors, optical devices, catalysts, fuel cells, gene and drug delivery, and

have a high surface-to-volume ratio which increases their interaction with micro-organisms, which in turn improves their antimicrobial activity (Rudramurthy et al., 2016). The nanoparticles are potential broad-ranging antibacterials because they can suppress a wide range of multidrug-resistant strains of bacteria (Yah and Simate, 2015). Nanoparticle targeted drug delivery can be passively or actively achieved. While passive targeting is through inserting the therapeutic agent into nanoparticle and active targeting can be achieved by conjugating the therapeutic agent to a cell or tissue-specific ligand (Singh and Lillard Jr, 2009). The features of nanoparticulate delivery systems, such as versatility, flexibility, and adaptability have proven their potential to fulfill the need for improved health care and these include polymeric nanoparticles, nanocapsules, solid lipid nanoparticles, and drug nanoparticles (Date and Patravale, 2004).

Polymeric nanoparticles loaded with drug have a larger impact on the clinical approach by increasing the efficacy of pharmaceuticals, high loading capacity for relatively insoluble drugs and controllable drug-release profiles (Hu et al., 2010). Nanocapsules possess features, such as high drug encapsulation efficiency and low polymer content, which help in efficient drug delivery (Mora-Heurtas et al., 2010). Solid lipid nanoparticles are used as an emerging tool in nanotechnology and offer the feasibility to develop new therapeutics and act as an alternate pulmonary drug carrier due to its biocompatibility and small size (Parida et al., 2016). Carbon nanotubes (CNTs) possess several interesting characteristics, such as ultralight weight, high aspect ratio, high thermal conductivity, and tremendous strength, which can be used as efficient DDS (Pandey and Dahiya, 2016). Toxicity studies on CNTs indicate that they exhibit cytotoxicity and pulmonary toxicity to mammalian cells (Li et al., 2008). Fullerenes are large, closed-cage, carbon clusters and are composed entirely of carbon in the form of ellipsoid, hollow sphere, or tube (Venkatesh et al., 2016). The primary property of a fullerene is its high symmetry and it can be used as radical scavenger, antiviral agent, and biological antioxidant (Bakry et al., 2007). Zinc oxide nanoparticles are biodegradable and nontoxic to nature, and are used as bactericidal agents against *E. coli*, *S. aureus*, *Listeria* spp., etc. (Meraat et al., 2016). Silica particles induce oxidative stress through the reduction of glutathione levels, generation of reactive oxygen species, and induction of antioxidant enzymes which ultimately results in the disturbance of cell membrane. The advantages of these several nanoparticles include highly porous structure, biocompatibility, and ease in terms of functionalization (Amato, 2010).

1.2.1 ROLE OF COMPUTATIONAL BIOLOGY

In the current world and the problems with the infectious diseases, it is highly critical to design a drug without the involvement of sophisticated computational modeling approaches. Computational modeling is a process which uses mathematics to represent the components of a biological system and is also used to study

the interactions within a biological system (Mooney et al., 2016). Computational biology greatly helps in the screening of drug molecules against the disease-causing proteins in gram-negative bacteria (Liu et al., 2010). It also plays a primary role in drug identification and prediction of the effect of new lead molecules. The principles of computational biology help in a proper and predictive method of discovering new drugs for a disease (Sylvia et al., 2009). The role of computational biology is crucial because it can provide information about the 3D structure of proteins whose structures are not even determined experimentally. The solution to promote the efficiency of lead identification and optimization is the use of highly refined computational biology approaches (Augen, 2002). Computer-assisted drug discovery has always had a profound role in the establishment of therapeutically significant leads (Sliwoski et al., 2013).

Over recent decades, computer aided drug discovery has a prominent role in the developmental process of drugs and therapeutic agents (Zakharov et al., 2012). Computational drug discovery is a useful method that is effective in enhancement and reduction of the costs involved in the drug development process (Yang et al., 2012). Molecular modeling studies can be used in order to model the therapeutic agents, especially novel antimicrobial in treating the bacterial infections (Yao et al., 2009).

1.2.2 NEED FOR GENE NETWORK AND EXPRESSION ANALYSIS TO IDENTIFY DRUG TARGETS

Genetic interactions are common to understanding the structure and function of molecules, cellular metabolism, and response of an organism to the environments (Segal et al., 2003). The gene network method supports the promise of delivering a conceptual framework for analyzing the abundance of biological data being generated on potential drug targets and providing insights which help to understand the regulatory mechanisms in many diseases, which plays a crucial role in identifying new drug targets (Jiang and Zhou, 2005). Gene network and infection pathway analysis results in the targeting of the bacterial virulence factors and is an alternative approach to identify drug targets that lead to the development of drugs (Cegelski et al., 2008). Studies reported that the *Salmonella* infection network was studied by mRNA expression and the result showed that there were 30,000 genes on an array (Porta et al., 2016). The virulent genes of *Salmonella* were emphasized using gene network analysis and around 46 genes, along with their functions, were identified. Some of the genes are the still life (sifA) which is responsible for endosomal induction, slyA is a regulatory protein, sifB which provides stability to bacteria and mgtC is a virulence protein (Skariyachan et al., 2016).

Expression pathway analysis is also defined as a logical step in any computational experiments that involve high-throughput studies and is also very important

in order to understand the biological phenomenon involved in any infectious network. Pathway analysis software is helpful while studying differential expression of a gene in a disease (Zimmermann et al., 2004). One approach for identifying drug-active pathways involves estimation of the gene network by using a Bayesian network model. The drug-active pathways are identified as the sub networks of the estimated gene network (Lueng et al., 2016). Extracellular ligands, such as hormones or cytokines, bind to the receptors and transmit signals that result in differences in gene expression, which ultimately results in changes in cell differentiation and proliferation (Zanders, 2000). High-throughput proteomics, identifying hundreds to thousands of protein expression changes in the model systems, lends itself particularly well to finding out the targets in drug discovery (Wu et al., 2004).

1.2.3 NEED FOR THREE-DIMENSIONAL (3D) STRUCTURE

Most of the biological functions are mediated by protein structure (Cozzetto and Tramontano, 2008). Three-dimensional structure is an important source of information to better understand the functionalization of a protein, its interactions with the other compounds. 3D structures demonstrate valuable insights into the molecular basis of protein function, allowing an effective design of experiments, such as studies of disease-related mutations or the structure-based design of specific inhibitors or site-directed mutagenesis (Schwede et al., 2003). The role of high-resolution 3D structure of proteins and complexes has been recognized as fundamental for both systems biology and rational drug design (Aloy and Russell, 2006). With recent advancements, 3D printing has been employed as a transformative tool for biomedical applications, especially for tissue engineering (Zhu et al., 2016). Crystal structures and the structures determined by nuclear magnetic resonance (NMR) are the most valuable paths of structural information for drug design (Anderson, 2003).

1.2.4 MAJOR STRUCTURAL DATABASE

The international depository for distribution and processing of protein structures is the Protein Data Bank (PDB). The PDB was renowned in 1971 as a digital access resource with seven X-ray crystal structures. The global PDB archive now contains more than 120,000 experimentally determined 3D structures (Prlic et al., 2016). The structures in PDB were determined experimentally by X-ray crystallography, NMR, etc., and each PDB entry is represented by a PDB ID (Xu, 2004). Research Collaboratory for Structural Bioinformatics is a consortium consisting of three institutions: San Diego Supercomputer Center at University of California, Rutgers University, and the National Institute of Standards and Technology. The PDB distributes coordinate data and structure files in PDB and

mmCIF formats and also provides documentation and derived data (Berman et al., 2000).

The protein model database (PMDb) is a database that collects 3D models of proteins modeled by structure prediction. Currently, PMDb stores the predicted models submitted to all the previous versions. The PMDb is a project of CAPSUR and the Department of Biochemical Sciences, University of Rome (Castrignano et al., 2005). The PMDb is a database dedicated to the deposition of the structures of proteins that are modeled by computational methods such as homology modeling. It is a public database aimed at maintaining manually built 3D models of proteins and a relational repository containing more than 74,000 models for ~240 proteins (Castrignano et al., 2006). The database entry point is protein and the information includes the protein name, length and sequence, organism and whenever required links to the SwissProt sequence database (Boeckmann et al., 2003).

1.2.5 OTHER PROTEIN DATABASES

Molecular Modeling database (MMDB) prescribes a complete set of precomputed and detailed structural alignments and also provides visualization tools for 3D structures and sequence alignment via the molecular graphics viewer, Cn3D (Madej et al., 2012). PDBsum is a web-based database which provides largely pictorial information on each macromolecular structure deposited at the PDB. It incorporates images of the structure, annotated plots of secondary structure of proteins, detailed structural analyses generated by the PROMOTIF program (Laskowski, 2001). The Structural Classification of Proteins database provides detailed description of the relationships of known protein structures. The classification is on hierarchical levels: the first two levels, family and super family, depict distant and near evolutionary relationships; the third, fold, describes geometrical relationships (Conte et al., 2000). Class, architecture, topology, homology (CATH) starts at the class level, defining three major classes of secondary structure content (all α , all β and α/β). The second layer, called architecture, clusters domains with common general features. The topology level group have a similar arrangement and a number of secondary structure elements with the same connectivity. The last (major) level, homologous super family, clusters domains with a high structural and functional similarity (Csaba et al., 2009).

1.2.6 PREDICTION OF 3D STRUCTURE

Techniques such as NMR, Cryo-EM and X-ray crystallography describe the most accurate methods for the characterization of proteins (Dubba, 2016). Prediction of protein structure is of great challenge to biotechnology and medicine, and is one of the most challenging problems in bioinformatics and theoretical chemistry. Protein structure prediction refers to the process of generating 3D models through

amino acid sequences with computational algorithms. Prediction of 3D structures from amino acid sequences demonstrates one of the most important problems in computational structural biology (Zhang, 2008).

3D structure is predicted by theoretical methods, using computational tools such as fold recognition, ab initio method, and homology modeling. The ab initio method constructs a 3D structure based on statistical and physical principles. Fold recognition and homology modeling are both template-based methods, emerging from the concept that proteins with similar sequences have similar folding patterns and structures (Shitaka et al., 2004). PS² an automated protein prediction server utilizes an effective consensus approach both in template selection and target-template alignment. PS² comprises the following four steps: template selection, target-template alignment, model building, and model evaluation and visualization (Chen et al., 2006).

1.2.7 HOMOLOGY MODELING

Homology modeling is the most accurate computational method to create reliable structural models and is commonly used in many biological applications. Homology modeling predicts the 3D structure of a query protein through the sequence alignment of template proteins. Generally, the process of homology modeling involves four steps: target identification, sequence alignment, model building and model refinement (Meier and Soding, 2015).

Modeller 9v16 provides a protein model by comparative modeling between the provided target and template sequences; it calculates the nonhydrogen atoms to generate a model. Modeler is also used for loop modeling and protein optimization (Fiser and Sali, 2003).

PRIMO (Protein Interactive Modeling) is a pipeline for homology modeling of protein monomers. It provides functionality that enables users to model ligands and ions in complex with their protein targets (Hatherley et al., 2016).

I-TASSER is an hierarchical protein structure modeling strategy, based on the secondary structure enhanced profile-profile threading alignment (Wu and Zhang, 2007) and iterative implementation of the threading assembly refinement program (Zhang and Skolnick, 2004).

SWISS-MODEL is a workspace for homology modeling of protein structures; protein models can be built and validated by SWISS-MODEL workspace (Bordoli et al., 2009).

DeepView was designed to incorporate functions for manipulating from sequence to structure with a user-friendly interface, structure visualization of proteins, and analysis (Kopp and Schwede, 2004).

PROCHECK is a program used for the validation of modeled protein which creates a Ramachandran plot and evaluates the torsion angles, surface area, bond angle, and atomic distances (Laskowski et al., 1993).

ERRAT is a protein structure verification algorithm that is utilized to examine the improvement of crystallographic model structure and refinement (Shen and Sali, 2006).

1.2.8 STUDY OF RECEPTOR—LIGAND INTERACTION

Receptor—ligand interactions are key steps in many fundamental biological processes. Binding affinities are one of the important parameters governing these interactions and they promote valuable mechanistic insights into signaling pathways (Dietz et al., 2014). Predicting interactions between small molecules and proteins is a major step to exploring many biological processes, and plays a crucial role in drug discovery (Jacob and Vert, 2008). Traditional computational approaches for identifying drug—target interactions are classified into target-based methods and ligand-based methods. The target-based methods rely on a known 3D structure of targets, whereas ligand-based methods rely on the known interacting ligands of target proteins (Yuan et al., 2016). The interaction of a ligand with its receptor at equilibrium is generally well described by a simple binding isotherm, characterized by the availability of the density of binding sites and the dissociation constant of the ligand for this binding site (Zoelen, 1989). Protein-ligand docking focuses on predicting and ranking the structures arising from the relationship between a ligand and a target protein of a known 3D structure (Sousa et al., 2006).

LigPlot was utilized to create schematic 2D representations of protein-ligand complexes (Wallace et al., 1995); the output provides hydrogen bond and hydrophobic interactions between molecules and their distances in easy and informative representation (Tabassum et al., 2016).

DASPfind is a computational program for calculating reliable new interactions among drugs and proteins and it utilizes simple paths of particular length inferred from a graph that describes drug—target interactions, similarities between drugs, and similarities between the protein targets of drugs (Balawi et al., 2016).

1.2.9 MOLECULAR DOCKING

Computational docking is applied to structure-based drug design and can be utilized to predict bond conformations and free energies of binding for small molecule ligands to macromolecular targets. It is widely used for the study of biomolecular interactions and mechanisms (Forli et al., 2016). Molecular docking can be distinguished as rigid body docking, in which a receptor and small molecule are considered as rigid; and flexible body docking, in which a receptor is considered rigid and the small molecule is held flexible. Molecular docking techniques are designed as an aid to the development of therapeutic agents (Banerjee et al., 2016). Docking analysis was accomplished to have a better understanding about the inhibitory mechanisms as well as the mode of interactions (Emran

et al., 2015). The flexible docking programs such as DOCK, AutoDock, FlexX, and GOLD are able to predict protein-ligand complex structures with reasonable accuracy and speed (Wang et al., 2003).

Auto Dock: Auto Dock 3.0 uses a Lamarckian genetic algorithm, but encompasses also a Monte-Carlo simulated annealing and a traditional genetic algorithm. The program utilizes a five term force field-based function which is dependent on the assisted model building with energy refinement (AMBER) force field (Morris et al., 1998). AutoDockVina is more accurate in exploring the binding potential of protein and ligand (Trott and Olson, 2010).

GOLD: GOLD utilizes a genetic search algorithm and allows for full ligand flexibility, as well as rotational flexibility for the protein-receptor polar hydrogens. The scoring function is force field-based, and includes three terms: a hydrogen bonding term, a 4–8 intermolecular dispersion potential, and 6–12 intramolecular potential (Jones et al., 1997).

FlexX: The fragment-based docking program, FlexX, is rapidly increasing in popularity. The docking of very flexible ligands is one of the weaknesses of FlexX, however this program is considerably faster than Auto Dock and GOLD (Gane and Dean, 2000).

DOCK: The program DOCK incrementally constructs the ligand in the binding site step by step, following the choice and placement of an initial rigid anchor fragment (Moustakas et al., 2006). Additional extensions to DOCK allow the treatment of protein flexibility by considering ensembles of protein structures, and the inclusion of a GB/SA continuum model into the scoring function (Zou et al., 1999). The current release, version 4.0, incorporates an improved matching algorithm for rigid body docking and an algorithm for flexible ligand docking (Ewing et al., 2001). Other popular docking software is listed in Table 1.1.

1.2.10 ROLE OF NANOPARTICLES AS THERAPEUTIC AGENTS AGAINST MULTIDRUG RESISTANT PATHOGENS

Nanoparticles have been considered the latest molecules in DDS because of special characteristic features, mainly surface-to-volume ratio (Shityakov and Forster, 2013). Nanoparticles possessing antimicrobial properties, such as silver oxide, silver, gold, zinc oxide, calcium oxide, copper oxide, titanium dioxide, and silicon dioxide are widely used in prognostic visual monitoring of therapy, targeted drug delivery, and also for detection of tumors (Singla et al., 2016). Recent study stated that Ag nanoparticles are effective against flucanazole-resistant *Candida albicans*, chloroquine-resistant strains of *Plasmodium* spp., and Au nanoparticles against ampicillin-resistant strains of *P. Aeruginosa* and *E. coli* (Zazo et al., 2016). Magnetic nanoparticles are considered biologically and chemically inert and are used in magnetic resonance imaging, drug delivery, tissue engineering and biological catalysis (Xie et al., 2012).

Table 1.1 List of the Most Popular Protein-Ligand Docking Programs Available (as of December 2016)

Docking Software/ Program	Year of Establishment	Country of Origin	References
DOCK	1988	USA	Morris et al. (1998)
AutoDock	1990	USA	
SOFTDocking	1991	USA	
DockVision	1992	Canada	
LUDI	1992	Germany	
ADAM	1994	Japan	
FLOG	1994	USA	
SYSDOC	1994	USA	
DIVALI	1995	USA	
GOLD	1995	UK	
FlexX	1996	Germany	Jones et al. (1997)
Hammerhead	1996	USA	
LIGIN	1996	Israel Germany	
FTDOCK	1997	UK	
ICM-Dock	1997	USA	
QXP	1997	USA	
PRO LEADS	1998	UK	
SANDOCK	1998	UK	
MCDOCK	1999	USA	
PRODOCK	1999	USA	
SFDOCK	1999	China	
DARWIN	2000	USA	
EUDOC	2001	USA	
FlexE	2001	Germany	
FDS	2003	UK	
FRED	2003	USA/UK	
LigandFit	2003	USA	
PhDOCK	2003	USA	
Surflex	2003	USA	
iGEMDOCK	2004	Taiwan	
Glide	2004	USA	
ProPose	2004	Germany	
YUCCA	2005	USA	
eHITS	2006	Canada/UK	
MolDock	2006	Denmark	
PLANTS	2006	Belgium/ Germany	

(Continued)

Table 1.1 List of the Most Popular Protein-Ligand Docking Programs Available (as of December 2016) *Continued*

Docking Software/ Program	Year of Establishment	Country of Origin	References
PSI-DOCK	2006	China	Trott and Olson (2010)
EADock	2007	Switzerland	
FLIPDock	2007	USA	
MDock	2007	USA	
ParDOCK	2007	India	
PSO@AUTODOCK	2007	Germany	
SODOCK	2007	Taiwan	
Lead finder	2008	Russia /Canada	
MS-DOCK	2008	France	
Q-Dock	2008	USA	
MADAMM	2009	Portugal	
AutoDockVina	2010	USA	
AADS	2011	India	
BetaDock	2011	South Korea	
LigDockCSA	2011	South Korea	
PythDock	2011	South Korea	
VoteDock	2011	Poland	
idTarget	2012	Taiwan	
EpiDOCK	2013	Bulgaria	
rDock	2013	UK	
FIPSDock	2013	China	
DINC	2013	USA	
iStar	2014	UK	
PharmDock	2014	USA	
MoDock	2015	China	
NPDock	2015	Poland	
CABS dock	2015	Poland	
GalaxyPepDock	2015	Korea	
MOLS 2.0	2016	Madras	
InterEvDock	2016	France	
SystemsDock	2016	Japan	

Gold nanoparticles (AuNP) are encouraging new drugs for the treatment of rheumatic diseases attributed with lower toxicity and their applications include biomedical imaging, controlled drug delivery and diagnosis, and gene therapy (Carneiro and Barbosa, 2016). Gold nanoparticle conjugates have been attributed

to the greater antibacterial effect, due to their capability to bind and penetrate the cell wall, deliver large number of antibacterials, and are also used to deliver a localized hyperthermic effect because of their plasmon resonance (Dizaj et al., 2014). The Centers for Disease Control and Prevention estimated that approximately 722,000 hospital-acquired infections occur every year and cause around 75,000 annual deaths in US acute care hospitals. One recent study demonstrated that use of copper as an antimicrobial agent in hospital greatly reduced the risk of hospital-acquired infections (Vincent et al., 2016). Another study revealed that silver nanoparticles exhibited clear growth-inhibitory effects on the clinical isolates and silver nitrate has mainly been used for the treatment of bacterial infections (Moezzi et al., 2012). Nanoparticles such as CNTs and fullerenes are comparatively rigid and hence could reserve their geometrical structures while interacting with biomolecules. Carbon nanomaterials possessing unique physiochemical properties such as ultralight weight, high drug-loading capacity, high surface aspect ratio and biocompatibility promote safe and efficacious carrier systems for drug targeting and drug delivery (Mehra and Palakurthi, 2015).

1.2.11 BINDING POTENTIAL OF CARBON FULLERENES AND NANOTUBES

Carbon fullerenes are hollow clusters of carbon atoms with sp^2 -hybridization. They have been assessed as an antibacterial agent, antiinflammatory or antiviral by many researchers and are also presented as a main ingredient in dermatologic and cosmetic products (Mousavi et al., 2016). Fullerenes are comprised of 12 pentagonal rings and 20 hexagons as the basis of icosahedral symmetrical closed-cage structure. The C_{60} molecule has two bond lengths: the 6:6 ring bonds can be considered “double bonds” and are shorter than the 6:5 bonds (Cruz-Silva et al., 2016). Binding affinities of all water soluble C_{60} derivatives were calculated using Generalized Born Volume Integral/Weighted Surface Area method embedded in molecular operating environment (MOE). Generalized born interaction energy includes van der Waals, Coulomb electrostatic interaction, and implicit solvent interaction energies (Junaid et al., 2016).

CNTs are allotropes of carbon that have a nanostructure of length-to-diameter ratio $>1,000,000$ and the aromatic network of CNT surface allows efficient loading. They exist as single-walled nanotubes (SWNTs) and multiwalled nanotubes (MWNTs). SWNTs offer photoluminescence property that could be applied in diagnostics, while MWNTs presents a wider surface that permits a more efficient external functionalization and internal encapsulation with active molecules (Pandey and Dahiya, 2016). CNTs have been explored as one of the most advanced nanovectors for the highly efficient delivery of drugs and biomolecules (Vashist et al., 2011). The harmful effects of the nanoparticles arise due to intrinsic toxicity of surface and high surface area. CNTs, due to their nanoscale

dimensions may induce unexpected toxicological effects upon contact with biological systems (Lacerda et al., 2006). The necessities for new DDS have featured CNTs as one of the main vectors against MDR pathogens to extend the pharmacological profiles, while decreasing the toxicological effects of the delivered drugs (Zhang et al., 2011).

1.2.12 QSAR PROPERTIES OF CARBON NANOTUBES AND FULLERENES

The modified structure of carbon fullerene suggested to act as HIV-1 protease inhibitor [$C_{60}-C_2H_4N-(2,4XCOCH_2OH) C_6H_4$], where the X atom is either O, S or Se. QSAR is useful for expressing the biological activities of the compounds and the outcome of this study concluded that the interaction with oxygen molecule has lowest optimization energy, which makes interaction more stable (Ibrahim et al., 2012). Fullerene and its derivatives were investigated by protein-ligand docking and QSAR methods. This revealed that these approaches are able to predict the binding affinities and interactions with HIV-1 protease and it concluded that they showed better interaction with HIV-1 protease, compared to other nanomaterials (Ahmed et al., 2013). The chemical and physical properties of CNTs and carbon fullerene are illustrated in Tables 1.2 and 1.3.

Fullerenes are extensively explored as excellent electron acceptors for energy storage and photovoltaic energy conversion (Bukovsky et al., 2016). In particular, poly-hydroxylated fullerenes named fullerlenols have shown to be excellent antioxidants, reducing apoptosis in cortical neurons cultures, with their high solubility and their ability to cross the blood–brain barriers. The neuroprotective activity of fullerenes is based on the ability to react with oxygen radical species, such as superoxide and hydroxyl radicals, that attack lipids, proteins, DNA and other macromolecules (Bosi et al., 2003). One study reported that both C_{60} fullerene and $C_{60} (OH)_{24}$ fullerene bind between two of the subunits just outside the tightly constricted region of the pore (Hilder et al., 2014).

1.2.13 MECHANISM OF ANTIBACTERIAL POTENTIAL OF CARBON FULLERENE AND CARBON NANOTUBES

Fullerenes demonstrated antimicrobial activity towards the probable drug targets of various bacteria, such as *Salmonella*, *E. coli* and *Streptococcus* spp. The antibacterial properties of these nanoleads are probably due to inhibition of energy metabolism after internalization of the nanolead in the bacterial cells. Studies have also depicted that it inhibits the bacterial cell by impairing the respiratory chain. Initially, at low concentration of fullerene derivatives, a decline of oxygen uptake and a raise of oxygen uptake occurred, which follows enhancement of hydrogen peroxide production. Furthermore, it is suggested that this nanoparticle

Table 1.2 The Physiochemical Properties of Fullerene C₆₀ That Contribute Probable Drug Like Nature to This Nanolead

Chemical and Physical Properties	Carbon Fullerene (C ₆₀)
Shape	Spherical aromatic molecule with a hollow truncated icosahedron structure
Molecular Formula	C ₆₀
Molecular Weight	720.642 g/mol
Octanol-water partition coefficient (logP)	10.18
Molecular polar surface area (PSA)	0.00
Natom	60
XLogP3	17.8
Hydrogen bond acceptor count	0
Hydrogen bond donor count	0
Rotatable bond count	0
Monoisotopic mass	720 g/mol
Exact mass	720 g/mol
Heavy atom count	60
Formal charge	0
Complexity	2030
Isotope atom count	0
Undefined atom stereocenter count	0
Defined bond stereocenter count	0
Defined atom stereocenter count	0
Undefined bond stereocenter count	0
Covalently bonded unit count	1
Density	3.5 ± 0.1 g/cm ³
Molar refractivity	205.7 ± 0.4 cm ³
Freely rotating bonds	0
Polarizability	81.5 ± 0.5 10 ⁻²⁴ cm ³
Surface tension	481.8 ± 5.0 dyne/cm
Molar volume	207.4 ± 5.0 cm ³
Melting point	280°C

Table 1.3 The Physical Properties of Carbon Nanotubes

Physical and Chemical Properties	Carbon Nanotubes
Shape	Hollow clusters formed by rolling graphene sheets
Diameter	1.9 nm
Young's Modulus	270–950 GPa
Tensile strength	11–63 Gpa
Density	1.33–1.4 g/cm ³
Melting point	3652–3697°C
Thickness	50–150 nm

can cause induction of cell membrane disruption. The hydrophobic surface of the fullerenes can easily interact with membrane lipids and can intercalate, which led to the destruction of cell membrane. It has been believed that the ability of fullerenes to interact with biological membranes is the major reason for its antimicrobials potential (Maleki Dizaj et al., 2015).

There are three different classes of fullerene compounds, namely: positively charged, neutral, and negatively charged fullerenes. Among these, the cationic derivatives demonstrated the highest antibacterial properties towards *E. coli* and *Shewanella oneidensis*. The anionic derivatives of fullerene showed no binding potential to the drug targets of these bacteria. This clearly emphasizes the interactions between negatively charged bacteria and cationic fullerenes (Nakamura and Mashino, 2009; Cataldo and Da Ros, 2008). Studies suggested that fullerenes with protonated amine groups effectively bonded with the probable drug targets of *E. coli*. Fullerene with deprotonated carboxylic (CF) groups are negatively charged which has not showed any antibacterial activity. Therefore, it is clear that watersoluble cationic fullerene derivatives can be used to prepare various fullerene-based disinfectants (Deryabin et al., 2014). The water soluble fullerene, in the presence of certain reducing agents, can generate superoxide, which is more cytotoxic towards bacterial cells than mammalian cells. For example, the antibacterial properties of fulleropyrrolidinium salts after photoirradiation demonstrated 99.9% of bacterial and fungal cells inhibition (Sharma et al., 2011).

Studies demonstrated the antibacterial potential of sulfobutyl fullerene derivatives on probable protein targets of various environmental bacteria, which suggested that photoirradiation completely inhibited the environmental bacteria (Cataldo and Da Ros, 2008). Furthermore, a study revealed that cationic substitutes of fullerene were highly effective in inhibiting a broad spectrum of microbial cells after irradiation with white light. A new group of synthetic fullerene derivatives with basic or quaternary amino groups showed high antibacterial properties towards *S. aureus*, *E. coli* and *C. albicans*. According to this study, the major factors that affect the antimicrobial potential of carbon fullerene are an

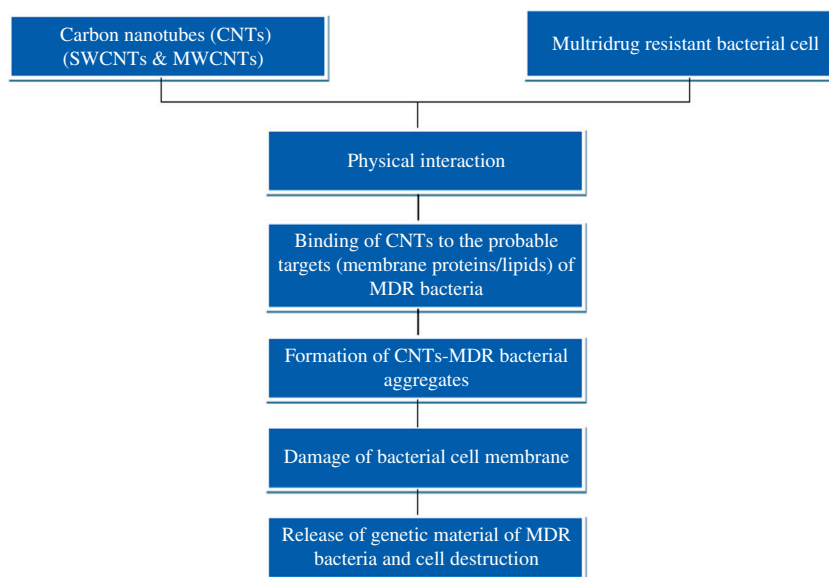
increased number of quaternary cationic groups widely distributed around the fullerene cage. Studies also suggested that quaternized fullerenes can be effectively applied in treatment of various superficial infections, such as wounds and burns (Mizuno et al., 2011).

The antibacterial properties of single-walled CNTs (SWCNTs) towards the probable targets of many MDR bacteria, both gram-positive and gram-negative, have been well established. Studies suggested that one of the major aspects in the antimicrobial activity on *E. coli* is that SWCNTs could damage the cell membrane and cause subsequent cell death. This is probably due to the strong binding affinity of SWCNTs towards various targets present in bacterial cell membranes (Kang et al., 2007). Furthermore, the researchers suggested that direct cell contact with CNTs accelerates the metabolic activities, integrity of the cell membrane, and morphology of *E. coli*. Studies illustrate that the size (diameter) of CNTs is another parameter which affects their antibacterial potential and cytotoxicity mechanism (the cell membrane destruction by direct contact with CNTs). Single-walled CNTs could infiltrate easily into the cell membrane better than multi walled CNTs (MWCNTs), due to the smaller diameter of the CNT. The gene expression result depicted that, in the presence of both MWNTs and SWNTs, *E. coli* expresses greater stress-related gene products; in the presence of SWNTs, the magnitude and quantity of expression is high (Kang et al., 2008).

There are studies suggesting the antimicrobial properties of SWCNTs and MWCNTs with various surface functional groups towards gram-negative and gram-positive bacteria. Single-walled CNTs with the hydroxyl and carboxyl groups demonstrated significant antimicrobial activity against both gram-positive and gram-negative organisms. However, MWCNTs with similar surface functional groups has not exhibited noted antimicrobial activities. This study suggested development of cell-CNTs aggregates which impart damage to the cell membrane of many MDR bacteria and resulted in the release of their genetic content (Arias and Yang, 2009).

In addition to the diameter of CNTs, the length of the CNTs also played a vital role in the antimicrobial activities. Studies pointed out that the longer SWCNTs indicated greater antimicrobial properties, because of their improved aggregation capabilities with bacterial cells (Yang et al., 2010).

Researchers investigated the antibacterial activities of SWCNTs discrete in various surfactant solutions, such as sodium dodecyl benzenesulfonate, sodium dodecyl sulfate, and sodium holate towards MDR *E. coli*, *S. typhi*, and *Streptococcus faecium*. These studies suggested that SWCNTs demonstrated significant antibacterial properties towards both *E. coli* and *S. typhi* which was enhanced by the increased concentrations of the nanotube. The amalgamation of SWCNTs with various surfactants was found to be less toxic to human astrocytoma cells, thus they can be utilized in biomedical purposes, particularly against extreme and multidrug-resistant microorganisms (Dong et al., 2012). The probable mechanism of antimicrobial potential of CNTs is shown in Fig. 1.1.

**FIGURE 1.1**

The proposed mechanism of antimicrobial potential of carbon nanotubes towards multidrug resistant bacteria.

1.2.14 RECENT ACHIEVEMENTS

The misuse of traditional antibiotics led to the antibiotic resistance issue, which made it extremely difficult to treat the bacterial infections. To overcome this, several antibacterial nanoparticles, such as CNTs, metal nanoparticles, and metal oxide nanoparticles have been explored. Recent study proposed graphene as a novel effective antibacterial material against various MDR bacteria (Ji et al., 2016). Study also depicted that silver nanoparticles, because of the safe, nonharmful inorganic nature are equipped for killing around 650 microorganisms that cause infections and are tested for antimicrobial activity against MDR bacteria (Gudikandula and Maringanti, 2016). Another study reported that polymeric nanoparticles can be used to improve bioavailability, permit targeted delivery of drugs, sustain release of drugs, or solubilize drugs for systemic delivery (Singh and Lillard, 2009). The application of functionalized carbon nanomaterials as conveyors for ordinary antibiotics will possibly enhance the bioavailability; decrease the associated resistance, and provide targeted drug delivery against the infections (Dizaj et al., 2015). One of the studies depicted that CNTs are being explored as potent drug carriers for the treatment of cancer and the integration of CNTs with proteins has led to the development of multifunctional nanocomposites, which can be used in the treatment of various MDR infections (Mundra et al., 2014).

Recent studies depicted that the C_{60} derivatives were docked into the binding sites of few drug targets, such as glutamate racemase, inosine monophosphate, dehydrogenase, lumazine synthase, acetylcholinesterase, human estrogen receptor alpha, N-myristoyltransferase and dihydrofolate reductase; the binding affinities were investigated. The results of this study concluded that C_{60} derivatives explored good interactions with the binding sites of these protein targets (Junaid et al., 2016). The study also showed that molecular dynamic simulation provided insights to understand the binding affinity of a CNT to a HIV-1 integrase, and this study concluded that CNT could stably bind to the C-terminal domain of HIV-1 integrase. Further molecular dynamics simulation results also indicated that CNTs serve as a potential dual-functional inhibitor (Zhang et al., 2013). Previous studies also suggested that carbon nanoparticles collected from various natural sources demonstrated high antibacterial potential towards the probable drug targets of MDR gram-negative pathogenic bacteria such as *Proteus refrigere* and *P. aeruginosa*, and gram-positive *S. aureus* and *Streptococcus haemolyticus* (Varghese et al., 2013). The binding potential of carbon fullerene towards the probable drug targets (drug-resistant gene products) of various MDR bacteria is illustrated in Figs. 1.2 and 1.3.

CNTs, especially SWNTs have a significantly higher antibacterial effect than most carbon nanomaterials (Manivasagan et al., 2014; Ji et al., 2010). One of the

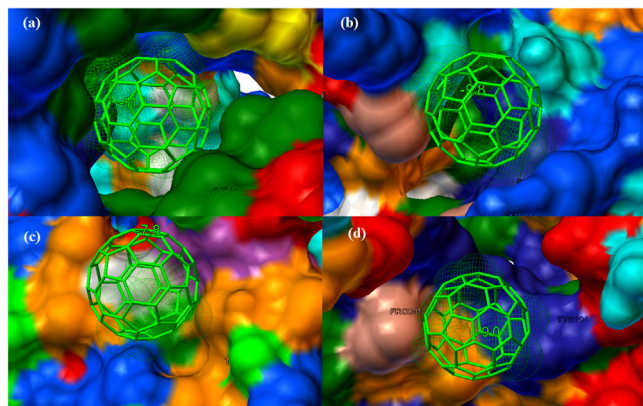
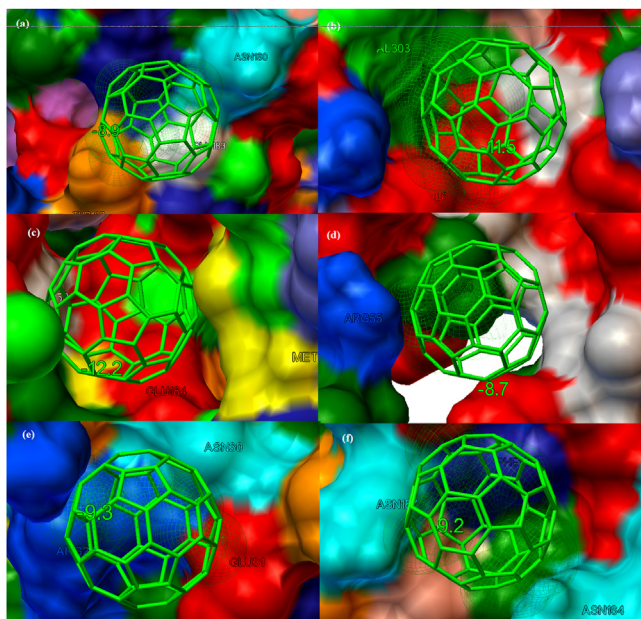


FIGURE 1.2

The binding potential of carbon fullerene towards the probable drug targets (drug-resistant gene products) of various multidrug resistant bacteria. The binding cavity is illustrated as molecular surface display and the carbon fullerene is shown as stick figures. (A) bla_{TEM} of *E. coli* (gene product code for beta lactam antibiotic resistance; theoretical binding energy -11.8 kcal/mol) (B) Dihydrofolate reductase (dhfr) of *Salmonella typhi* (gene product code for trimethoprim resistance); theoretical binding energy -9.8 kcal/mol (C) *mec1* (gene product code for methicillin resistance; theoretical binding energy -7.8 kcal/mol) (D) *vanH* (gene product code for vancomycin resistance; theoretical binding energy -9.0 kcal/mol) of *Staphylococcus aureus*.

**FIGURE 1.3**

The binding potential of carbon fullerene towards the probable drug targets of multidrug resistant *Salmonella typhi* predicted by molecular docking studies. The binding cavity is illustrated as molecular surface display and the carbon fullerene is shown as stick figures. The interaction between fullerene and the crystal structure of the probable drug targets (identified by metabolic pathway analysis) such as (A) SifA (PDB: 3CXB, theoretical binding energy -8.9 kcal/mol) (B) SlyA (PDB: 3DEU, theoretical binding energy -11.5 kcal/mol) (C) PhoQ (PDB: 1YAX, theoretical binding energy -12.2 kcal/mol) (D) hnS (PDB: 1LR1, theoretical binding energy -8.7 kcal/mol) (E) marR (PDB: 4JBA, theoretical binding energy -9.3 kcal/mol) (F) gtgB (PDB: 4G29, theoretical binding energy -9.2 kcal/mol).

recent studies depicted the interaction of CNTs with tau protein as a model of nervous system and also demonstrated a strong correlation between the surfaces tension of CNTs and the ability of nanoparticles to interact with the protein (Zeinabad et al., 2016).

1.2.15 MERITS OF NANOPARTICLES

Graphene nanomaterials possessing excellent biocompatibility is applicable in drug delivery, diagnostics, and therapeutics, and also holds great promise for combating microbial infections (Szunerits and Boukherroub, 2016). The strategy

of using polymer-based nanoparticles has been recognized as a promising prospect for diagnosis and cancer treatment, due to noteworthy properties, including nanosize, excellent biocompatibility, biodegradability, and enhanced drug-loading capacity (Kumari et al., 2016). Protein-based nanoparticles are utilized as DDS. Silk protein was reported as carrier for gene delivery in vitro (Cheng et al., 2016). Dendrimers represent striking applications for the encapsulation and pulmonary delivery of antiTB drugs because of their unique structure (Kaur et al., 2014). Graphene-based nanosheets, including graphenes and graphene oxides, have properties suitable for delivery of various molecules (Shim et al., 2016). CNTs are utilized as versatile biopharmaceutical delivery systems, due to the excellent cell-penetrating ability and high drug-loading capacity (Rallapalli and Smith, 2016).

1.3 CONCLUSION

The incidence of MDR gram-negative infections are one of the emerging problems worldwide and therapeutic remedies have become limited. Over the years, gram-negative bacteria have evolved to become extremely drug resistant against a wide range of antibiotics that includes cephalosporins, fluoroquinolones, and carbapenems, and has resulted in higher mortality rates across the world. The therapy for these infections becomes very tedious because of the resistance activity of bacterial species. Consequently, there is a need to address this issue and identify probable lead molecules to combat these infections. Nanoparticles are identified as effective antimicrobials against these infections. This chapter emphasized the importance of carbon fullerene and carbon nanotubes against MDR infections by computational biology approaches. Computational biology can also be used to model therapeutic agents, especially novel molecules, as an approach in treatment of infections. Computational approaches promise an advance development in drug molecules against targeted genes and complement the work on which drug discovery currently relies. Nanoparticles are considered the latest molecules in drug discovery pipeline, as these lead molecules are less complicated than other molecules and offer effective drug delivery to the target systems. This chapter thus suggests that carbon fullerene and nanotubes can probably act as potential lead molecules against MDR organisms.

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