Cheminformatic Approaches to Hit-Prioritization and Target Prediction of Potential Anti-MRSA Natural Products

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Keywords

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Natural products
Methicillin-Resistant *Staphylococcus aureus* (MRSA)
Profiling
Hit-prioritization
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Pharmacokinetics
Drug-likeness
Desirability score
Target prediction

Declaration

I, Samson Olaitan Oselusi, declare that this written submission represents my work and that it has not been submitted before for any degree or examination at any other higher education institution. Where other's ideas or words have been included, I have adequately cited and referenced the sources. I also declare that I have adhered to all ethics of academic honesty and integrity and have not misrepresented or fabricated any idea/data/source in my submission.

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Signed Date

Abstract

The growing resistance of Methicillin-Resistant *Staphylococcus aureus* (MRSA) to currently prescribed drugs has resulted in the failure of prevention and treatment of different infections caused by the superbug. Therefore, to keep pace with the resistance, there is a pressing need for novel antimicrobial agents, especially from non-conventional sources. Several natural products (NPs) have displayed varying *in vitro* activities against the pathogen but few of these natural compounds have been studied for their prospects to be potential antimicrobial drug candidates. This may be due to the high cost, tedious, and time-consuming process of conducting the important preclinical tests on these compounds. Hence, there is a need for cost-effective strategies for mining the available data on these natural compounds. This would help to get the knowledge that may guide rational prioritization of "likely to succeed" natural compounds to be developed into potential antimicrobial drug candidates. Cheminformatic approaches in drug discovery enable chemical data mining, in conjunction with unsupervised and supervised learning from available bioactivity data that may unlock the full potential of NPs in antimicrobial drug discovery. Therefore, taking advantage of the available NPs with their known *in vitro* activity against MRSA, this study conducted cheminformatic and data mining analysis towards hit profiling, hit-prioritization, hit-optimization, and target prediction of anti-MRSA NPs. Cheminformatic profiling was conducted on the 111 anti-MRSA NPs (AMNPs) retrieved from literature. About 20 current drugs for MRSA (CDs) were used as a reference to identify AMNPs with promising prospects to become drug candidates. This was followed by the prioritization of hits and identification of the liabilities among the AMNPs for possible optimization. Reverse molecular docking was used to predict the possible targets of these natural compounds based on their predicted free binding energy to 34 selected druggable targets in MRSA. The results for the cheminformatics profiling revealed that most of the AMNPs were within the required drug-like space of the investigated properties. The AMNPs (up to 80 %) showed good compliance with the Lipinski, Veber, and Egan predictive rules for oral absorption and permeability. About 30 % of the AMNPs showed prospects to penetrate the blood-brain barrier. Conversely, only 50 to 60 % of the CDs complied with these predictive rules for oral absorption and permeability, and none of the CDs showed the likelihood to pass through the blood-brain barrier. Good oral absorption and permeability are desirable to achieve the desired plasma concentration of the AMNPs, which is a prerequisite to their effectiveness. Regarding the effect on cytochromeP450 (CYP450) enzymes, 16 to 43 % AMNPs were predicted as inhibitors of one or more CYP450 enzymes. CYP450 enzyme inhibitors might be

given less consideration during hit-prioritization and selection because of the potential to interact with other drugs. The analysis of toxicity revealed that 80 and 59 % of the CDs and AMNPs respectively, might have low or no toxicity risks. Hit-prioritization strategy using a novel "desirability scoring function" revealed that the AMNPs with the desired drug-likeness showed the best score. Hit-optimization strategies implemented on AMNPs with poor desirability scores led to the design of two compounds with improved desirability scores and good synthetic accessibility scores. Evaluation of the structural-activity relationship of the AMNPs revealed chemical groups that may be the determinants of the reported bioactivity of the compounds. Regarding druggable target prediction, more than two-thirds of the compounds revealed a sufficient free binding energy (≤ -6 kcal/mol) for all the investigated targets (proteins) involved in fatty acid metabolism. The results also showed that some of the AMNPs might have multiple druggable targets. Prediction of the potential targets of the AMNPs provides a hypothesis for the mechanism of action of the AMNPs. Overall, this study has mined the available bioactivity data and predicted properties of the AMNPs to gain the knowledge for rational AMNPs hit-prioritization and implementation of hit-optimization strategies. This could also be the crucial starting point for the development of drug candidates against MRSA infections from natural compounds.

Dedication

I dedicate this thesis to my Dad, **Mr. Samuel Oselusi**, whose love, trust, unflinching support, and examples over the years have laid the foundation for the commitment and discipline required to complete this work.

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Table of Contents

List of Figures

List of Tables

List of Appendices

Lists of abbreviations

AMR: Antimicrobial resistance

WHO: World Health Organisation

ECDC: European Centre for Disease Prevention and Control

CDC: Centre for Disease Control and Prevention

HIV: Human Immunodeficiency Virus

USA: United States of America

GDP: Gross domestic products

MRSA: Methicillin-Resistance *Staphylococcus aureus*

SCCmec: *Staphylococcal* cassette chromosome *mec*

PBP: Penicillin-binding protein

NPs: Natural products

BBB: Blood-Brain Barrier

CNS: Central nervous system

CARD: Computer-aided rational drug design (CARD)

VS: Virtual screening

HBD: Hydrogen bond donors

HBA: Hydrogen bond acceptors

MW: Molecular weight

PP: Physicochemical parameters

QSAR: Quantitative structure-activity relationships

Ro5: Rule of Five

AMNPs: Anti-MRSA natural products

MIC: Minimum inhibitory concentration

SMILES: Simplified Molecular Input Line Entry System

SA: Significantly active

A: Active

NA: Negligibly active

CD: Current drugs for MRSA

PDB: Protein Data Bank

ANOVA: Analysis of variance

BE: Binding energy

clogP: Calculated octanol/water partition coefficient

TPSA: Total polar surface area

RTB: Rotatable bonds

FDA: Food and Drug Administration

HIA: Human intestinal absorption

CNS: Central nervous system

p-gp: p-glycoproteins

CYP450: Cytochromes P450

PCA: Principal component analysis

SAR: Structure-activity relationship

SALI: Structural-activity landscape index

Chapter one

Introduction

1.1 Background of the study

The advent of antibiotics in the 20th century was a great turning point in the history of medical sciences and mankind (Aslam *et al.*, 2018). Many antibiotics were discovered and developed for human use twenty years after the second world war (Adedeji, 2016). This golden era (the 1940s to 1970s) is remembered for the wonders of antibiotics in transforming human health by saving many lives through the treatment of infectious diseases (Adedeji, 2016; Hutchings *et al.*, 2019). However, the few antibiotics developed after the period were derivatives of the existing antibiotics. The situation was compounded by the sudden emergence of antibiotic-resistant pathogens (Aslam *et al.*, 2018; Silver, 2011). This condition has resulted in a global burden of bacterial infections to a significant threat level especially among those pathogens which cannot be controlled using the old classes of antimicrobial agents (Chokshi *et al.*, 2019; O'neill, 2014).

Inappropriate use, excessive use in livestock feeding, and continuous failure of researchers to discover and develop novel antibiotics are some of the main factors responsible for the emergence of antibiotic resistance (Aslam *et al.*, 2018; Singhai, 2018). The reason for lack of interest in pursuing novel antibiotics among pharmaceutical industries may be due to the low throughput in the antibiotics drug development pipeline with the attendant financial loss as a result of the complexity in balancing efficacy and safety (Jackson *et al.*, 2018; Newman and Cragg, 2020; Tacconelli *et al.*, 2018). Therefore, there is a need for pharmaceutical industries to identify new and more effective strategies for discovering and developing novel antibiotics.

Natural products (NPs) have continued to gain relevance in the battlefront against infectious diseases. Newman and Cragg (2020) studied the use of NPs as sources of novel drugs approved between 1981 and 2019. They concluded that these compounds have prospects for discovering new agents against various infectious diseases (Newman and Cragg, 2020). An earlier study conducted by Seyed (2019) also reported the potentials of NPs as antimicrobial agents against a wide range of human diseases. Therefore, it is anticipated that an efficient exploration of libraries of NPs that are active against bacteria could identify potential antibiotics to defeat drug-resistant bacteria.

Several cheminformatic techniques have been developed and employed in drug discovery, design, and development to reduce the research cycle and minimize the cost of producing new anti-infective agents (Pereira and Aires-de-Sousa, 2018). Generally, the cheminformatics approach to rational drug design involves the estimation of pharmacokinetic and toxicity properties of potential drug candidates, with the prospect of minimizing the risk of future attrition (Obaid *et al.*, 2017; Campillos, 2016; Shivakumar *et al.*, 2018). The following sections describe the research problem, aim, and objectives of this study.

1.2 Research problem

The incidence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) has become a global problem and even with a growing concern among developing countries (Lee *et al.*, 2018; Tacconelli *et al.*, 2018). This pathogen is a common cause of many life-threatening infections especially those associated with catheters, skin, or soft tissue. The continuous failure of the currently prescribed drugs in the treatment of MRSA has called for an urgent need to promote novel antimicrobial agents against MRSA infections (Guo *et al.*, 2020; Tayel *et al.*, 2018).

Researchers have studied cheminformatic analysis of NPs with reported activities against different resistance pathogens (Egieyeh *et al.*, 2016; Seyed, 2019). The *in vitro* activities of hundreds of NPs against MRSA have also been reported (Okwu *et al.*, 2019). Nevertheless, there is a limited study on the pharmacokinetic properties, safety, and potential targets of these anti-MRSA NPs. This may be attributed to the high cost and long time required to conduct these essential preclinical tests. Hence, many of these compounds have not made progress beyond the hit identification stage in the drug development pipeline. This calls for more efficient and cost-effective approaches to screening bioactive compounds for their prospects to become drug candidates. In response to this clarion call, this study provided a framework for profiling bioactive compounds and for data-driven decisions in the transformation of profiled bioactive compounds to potential drug candidates that are optimized for efficacy, safety, and oral administration.

1.3 Significance of the study

This study would provide a framework for the characterization, prioritization, and optimization of anti-MRSA NPs towards becoming drug candidates with desirable efficacy and safety. This could be of great benefit to drug developers by providing insight towards making rational

decisions in optimization towards drug-likeness among known anti-infective compounds against MRSA. Additionally, knowledge about the potential targets of these compounds could be of great importance in the early identification of their potential mechanisms of action. The techniques employed for target identification in this study can identify anti-MRSA NPs that may be simultaneously active on multiple targets thereby minimizing the risk of resistance. Finally, the integration of computational strategies into drug discovery and development as used in this study could minimize the costs and duration of bringing new drugs to the patient bedside.

1.4 Aim

This study was aimed at profiling anti-MRSA NPs for hit-prioritization, optimization and to predict their potential targets in MRSA.

1.5 Research questions

This study was designed to use available cheminformatics approaches in drug discovery to answer the following questions.

i. What are the pharmacokinetic profiles of the selected anti-MRSA NPs?
ii. What is the drug-likeness profiles of the selected anti-MRSA NPs?
iii. Is there a relationship between the *in vitro* activities (MIC) of the anti-MRSA NPs, and their drug-like properties?
iv. Is there any considerable difference in the pharmacokinetic profiles of natural compounds and those of currently prescribed oral drugs against MRSA?
v. What are the potential targets for the anti-MRSA NPs in MRSA?

1.6 Objectives

The objectives of this study are;

I. To conduct a literature search for retrieval of anti-MRSA NPs with their minimum inhibitory concentration (MIC).
II. To perform cheminformatics data mining and analysis of the anti-MRSA NPs toward hit profiling, hit-prioritization, and hit-to-lead optimization using different cheminformatics software.
III. To predict the binding affinity of the compounds within the sites of MRSA proteins using molecular docking.

1.7 Thesis outline

In total, there are six chapters in this thesis. Chapter one presents the rationale for the study and a general overview of this thesis. It starts with brief background information on the global incidence of bacteria resistance to currently prescribed drugs and the need for novel antibiotics. This is followed by a brief description of the use of cheminformatic techniques in drug discovery. Furthermore, the statement of the problem, the aim, and objectives of this study are also provided. The concluding part of the chapter presents the outline of the subsequent chapters of this thesis. Four more chapters follow. Chapter two provides a comprehensive review of the literature on the global prevalence of antimicrobial resistance. It also discusses the prospects of NPs in drug discovery and their limitations. This chapter further identifies some of the molecular descriptors that are crucial for hit identification and hit-to-lead optimization process. In addition, some basic computational target prediction approaches, their advantages, and limitations in drug discovery research are also presented here. Chapter three is the methodology section. This chapter starts with the data collection process and the different software that was used in the study. It further describes the specific details of how the study was carried out. The remaining two chapters describe the actual findings of this study. Chapter four is the results and discussion section. It starts by providing the findings on the cheminformatic profiling, prioritization, and optimization of NPs that have shown *in vitro* activities against MRSA for drug-likeness. The latter part of this chapter provides the findings on the target prediction and the implications. Chapter 5 presents a summary of the major findings based on the objectives highlighted in chapter one. It also gives recommendations for future studies. Finally, the full lists of the cited works and supplemental information from this study are presented in chapter 6.

Chapter two

Literature review

This chapter provides a brief survey of previous studies that have been reported on the antimicrobial resistance of bacteria. The global prevalence of drug-resistant pathogens, their impact on public health and the economy is described. Information is also provided on the urgent need to avert the impending danger of antimicrobial resistance. Furthermore, the prospects of natural products over synthetic compounds in drug discovery and development are highlighted. The approaches and application of cheminformatic strategies in hit profiling and hit-to-lead optimization process of drug discovery are also discussed in this review.

2.1 Overview of antimicrobial resistance

Antimicrobial resistance (AMR) is a genetic alteration in microorganisms, that causes them to resist eradication by previously effective antibiotics (Aslam *et al.,* 2018; O'neill, 2014). These microbes tend to prevent drugs from inhibiting, destroying, or killing them, causing persistent infections with increased risk of transmission (Smith *et al.,* 2015; Wallinga *et al.,* 2015). Resistance is developed by most pathogenic microbes (bacteria, viruses, fungi, protozoans, etc.) to the drugs used in their treatments (Dadgostar, 2019). The emergence of novel mechanisms of resistance in some pathogens accounts for their ability to simultaneously resist several classes of antibiotics (Ferri *et al.,* 2017). These multidrug-resistant microbes are commonly called "superbugs'' (Wallinga *et al.,* 2015; WHO, 2014).

The risk for inefficient treatment of infectious diseases as a result of multidrug resistance is becoming more problematic globally (McAdam *et al.,* 2012). Researchers have unambiguously used AMR to describe public health associated problems. Of all the pathogenic microbes studied, the focus on bacteria has gained momentum due to the rapid loss in potency of various antibiotics that are used in the treatment of infections caused by them (McAdams *et al.,* 2012; Wallinga *et al.,* 2015). Some strains of bacteria are resistant to all groups of antibiotics (Krishnamoorthy *et al.,* 2018). For instance, Methicillin-Resistant *Staphylococcus aureus* (MRSA) is not only resistant to β-lactam antibiotics but also aminoglycosides, chloramphenicol, macrolides, and tetracycline (Krishnamoorthy *et al.,* 2018). Similarly, *Klebsiella pneumoniae* has shown resistance to third-generation cephalosporins and carbapenems (WHO, 2014). Therefore, there is a need for infection control and interventions aimed at preventing the spread of these highly resistant pathogens.

2.1.1 Contributing factors to antimicrobial resistance

After the discovery of the first antibiotic, AMR was observed as a natural process. However, the genes responsible for resistance in some bacterial strains were shown to have existed millions of years before the discovery of antibiotics (McAdam *et al.*, 2012; O'neill *et al.*, 2014). Some factors have been reported to contribute to the exacerbation of AMR. These include the inappropriate use of antibiotics, patient non-compliance, and transfer of resistance within or from one strain to another (Borges *et al.*, 2013; McAdam *et al.*, 2012; Ventola, 2015; Wikaningtyas and Sukandar, 2016). Lastly, the extensive use of antibiotics as growth additives in livestock feeding in most parts of the world has also contributed to the menace of AMR (Dadgostar, 2019; Ventola, 2015). The alteration in the genetic makeup of resistant bacteria because of these factors is prompting the potency of conventional drugs to fail within a very short period (Chandra *et al.*, 2017).

2.1.2 The worldwide prevalence of antimicrobial resistance to conventional drugs

The global rates and spread of drug-resistant bacteria have been reported by various health agencies. The World Health Organisation (WHO), the European Centre for Disease Prevention and Control (ECDC), and the Centre for Disease Control and Prevention (CDC) have identified AMR pathogens as one of the main threats to public health (Ferri *et al.*, 2017). The CDC recently identified 220 pathogenic bacterial strains with unusual antibiotic-resistant genes in about 27 regions of the United States of America (USA) (CDC, 2018). It further reported that about 25 % of all the identified pathogens can transfer resistant genes to non-resistant bacteria (CDC, 2018). In another study, high-risk clones such as *Klebsiella pneumoniae* ST258, *Pseudomonas aeruginosa* ST255, *Enterococcus faecium* CC17, and *Escherichia coli* ST 131 were shown to transfer highly antibiotic-resistant phenotypes, thereby causing almost untreatable infections (Friedman *et al.*, 2016). Additionally, based on the emergence of vancomycin-resistant *Staphylococcus aureus* strains, the failure of vancomycin which is considered as the mainstay of treatment for MRSA-caused infections has also been reported (Escobar *et al.*, 2020).

Furthermore, to investigate the current AMR worldwide surveillance, WHO has studied the resistance pattern of more than 30 bacteria isolates from each continent. One of the crucial findings of this investigation was the high rate of resistance among the isolated pathogens such as *Staphylococcus aureus, Enterococcus spp, K. pneumonia, Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* species commonly associated with

hospital-acquired infections (Founou *et al.,* 2017; Santajit and Indrawattana, 2016; WHO, 2014). In addition, Wang *et al.* (2020) studied multidrug resistance in patients with urinary infections and noticed a high rate of resistance to ampicillin, 3rd generation classes of cephalosporins, and fluoroquinolones in *Escherichia coli*, *Enterococcus faecalis*, *Proteus mirabilis*, and *Klebsiella pneumoniae*. Furthermore, the increased use of antibiotics as a regimen in the fight against the COVID-19 pandemic can also increase the prevalence of AMR globally (Nieuwlaat *et al.,* 2020). Thus this problem could eventually have a negative impact on the economy and public health (Dadgostar, 2019; McGowan, 2001).

2.1.3 Clinical and economic impacts of antimicrobial resistance

The impact of AMR bacteria on global health and economy has been widely studied (Founou *et al.,* 2017; Sharland *et al.,* 2015; WHO, 2014). Presently, about 700,000 reported cases of mortality are annually attributed to AMR (Dadgostar, 2019; Ghosh *et al.,* 2019). Various studies have also projected a rise above 100 million cases of untimely death by 2030, and an increase to 10 million per annum by 2050 (Founou *et al.,* 2017; Ghanbar *et al.,* 2018; O'neill *et al.,* 2014). Similarly, it was recently mentioned that AMR would aggravate the rate of poverty in developing countries compared to the rest of the world (Dadgostar, 2019). Furthermore, bearing in mind that the high use of antibiotics in COVID-19 patients may shift gains in short-term COVID-19 mortality to an increase in long-term deaths caused by AMR, one may infer that AMR would be a worse global enemy to manage than the current pandemic (Nieuwlaat *et al.*, 2020). Regarding the global economic impact, it was estimated that the AMR threat may cause a 1.4 and 2.5 % reduction in gross domestic products (GDP) by 2030 and 2050 respectively (de Kraker *et al.,* 2016; Ghosh *et al.,* 2019; O'neill *et al.,* 2014). Therefore, the current approaches to fight COVID-19 such as the development of new therapies, and vaccines may also be required to avert the impending danger of AMR (Nieuwlaat *et al.,* 2020).

2.1.4 Approaches to combat antimicrobial resistance

The two crucial strategies to address the challenges associated with drug-resistant pathogens are preventive and remedial approaches (Singhai, 2018). The goal of preventive measures is to combat the rate at which AMR develops as a result of human-related factors such as the inappropriate use of antimicrobial therapies. On the other hand, remedial approaches are targeted towards the development of novel treatment options (Abreu *et al.,* 2012; Singhai, 2018). The development of new antibiotics is crucial in eradicating AMR as it is the only means to ensure the level of infection control (Hughes and Karlén, 2014).

2.2 Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a bacteria that has developed resistance to β-lactam therapies and several first-line drugs (Barrett, 2005; Hampton, 2016). This resistance is due to a *mecA* gene located on the *Staphylococcal* cassette chromosome *mec* (SCC*mec*) that codes for a 78-kDa penicillin-binding protein (PBP2a). This causes the MRSA to have a decreased affinity for methicillin and all other β-lactam drugs (Abdulgader *et al.,* 2015; Amoako *et al.,* 2019; Boswihi *et al.,* 2018; Catteau *et al.,* 2018).

2.2.1 MRSA: A serious challenge to public health

MRSA is a serious threat to the public health of many countries. It is the main aetiological agent of nosocomial and community-acquired infections (Abubakar and Sulaiman, 2018; Abdulgader *et al.,* 2015; Lee *et al.,* 2018; Lee *et al.,* 2010). During the last 4 decades, MRSA caused infections has worsened globally. This is evidenced by a rapid increase in reported cases from an average of 3 % in the mid-1980s to about 65 % in 2018 (Dong *et al.,* 2018). Clinical conditions commonly associated with MRSA include bacteremia, bone, and joint infections, endocarditis, meningitis, osteomyelitis, pneumonia or respiratory infections, skin and soft-tissue infections, surgical site infections, toxic shock syndrome, and urinary tract infections (Abubakar and Sulaiman, 2018; Amoako *et al.,* 2019; Dong *et al.,* 2018). Some of these infections if left untreated can result in serious morbidity, high economic burden, and eventually death (Abubakar and Sulaiman, 2018).

2.2.2 The global prevalence of MRSA

There has been an increasing concern about MRSA since the 1960s in many countries. Ventola, (2015) investigated the crises of antibiotic resistance and reported that MRSA kills more Americans annually than the combination of HIV/AIDS, Parkinson's disease, homicide, and emphysema. Similarly, Lee *et al.* (2018) examined the global prevalence of MRSA infections and reported the highest rate of prevalence (above 50 %) in South America. Intermediate rates (between 25 and 50 %) are common in Africa, Australia, and some European countries such as Portugal and Italy. Scandinavia and Netherlands are some of the European nations with a very low burden (less than 5%) of MRSA (Craft *et al.,* 2019; Ferri *et al.,* 2017; Lee *et al.,* 2018). The global rate of this prevalence is described in Figure 2.1 below.

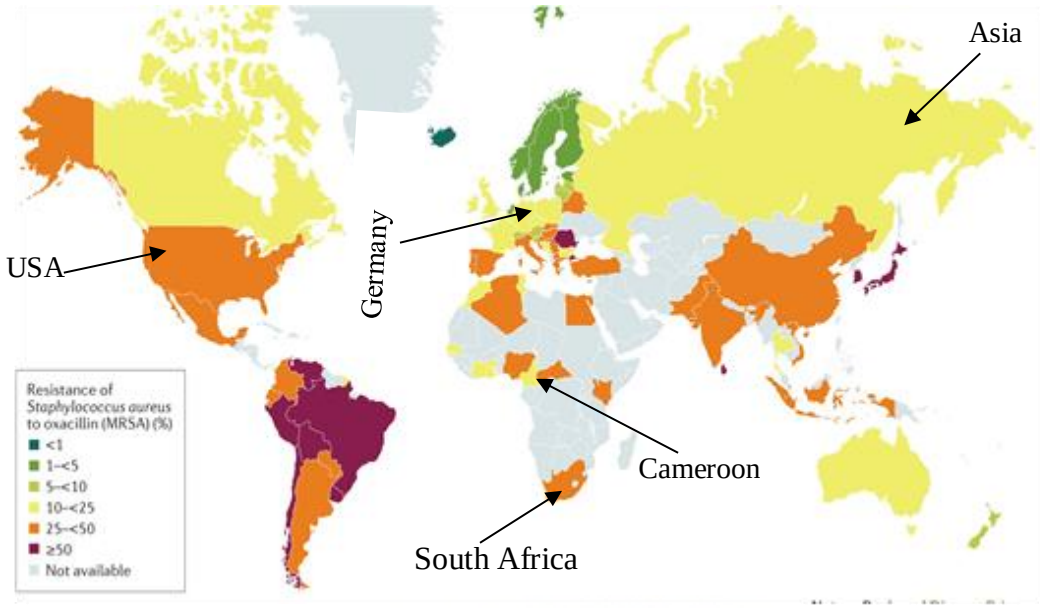


Figure 2.1. The global prevalence of MRSA (Adapted from Lee *et al.*, 2018).

Other studies have compared the prevalence of MRSA in developed and developing countries. For instance, an improved condition of MRSA burden in some parts of developed countries such as Asia, Germany, Europe, the United States, and Canada was reported in the literature (Lee *et al.*, 2018; Abubakar and Sulaiman, 2018). This improvement was credited to the execution of control intervention programs (Abubakar and Sulaiman, 2018; Ferri *et al.*, 2017; Lee *et al.*, 2018). Although there is a limited record of cases in many developing countries (Fig. 2.1), the available data has shown that the incidence of MRSA in sub-Saharan African countries has increased since the year 2000 (Abubakar and Sulaiman, 2018; Falagas *et al.*, 2013; Lee *et al.*, 2018). The differences in drug availability and usage as well as the risk factors such as weakened immune systems have contributed to the reported rate of MRSA prevalence in Africa (Lee *et al.*, 2018). Therefore, MRSA prevalence in Africa needs to be given more attention while addressing antimicrobial resistance globally (Falagas *et al.*, 2013).

2.2.3 Mechanisms of resistance in MRSA

Methicillin and other β-lactam families are known for targeting and disrupting the bacterial cell wall (Berger-Bächi, 2002; Stapleton and Taylo, 2002). Strains of MRSA can resist these antibacterial substances via the activities of penicillin-binding proteins (PBPs) such as PBP(2a) (Peacock and Paterson, 2015; Stapleton and Taylo, 2002). The primary role of PBP(2a) is to synthesize the bacteria-peptidoglycan layer. Therefore, to possess transpeptidase activity,

PBP(2a) is characterized by a negligible affinity for β-lactam drugs (Berger-Bächi, 2002; Peacock and Paterson, 2015; Stapleton and Taylo, 2002). This accounts for the ability to maintain structural integrity of the bacterial cell wall even in the presence of β-lactam drugs (Berger-Bächi, 2002; French, 2010). Thus, the mechanism of MRSA is through the expression of a PBP(2a) which is resistant to the activities of methicillin and its families (Stapleton and Taylo, 2002).

2.2.4 Health and economic impact of MRSA

Different studies have described the worldwide health and economic burden of the MRSA threat. Nosocomial infections caused by MRSA in Europe were reported to affect more than 150,000 patients annually resulting in an additional health care cost of approximately £ 400 million (Abubakar and Sulaiman, 2018; Abdulgader *et al.,* 2015). Similarly, in the USA, about 11,300 MRSA-associated deaths are reported annually. This has resulted in an economic burden of up to US$ 13.8 billion on the society, depending on the prevalence of the associated infections (Abdulgader *et al.,* 2015; Lee *et al*., 2013). Moreover, in South-eastern China, the average cost of treating MRSA infections was estimated at US$ 10.565 per patient (You *et al.,* 2017). Currently, there is no available report for the health and economic burden of MRSA-associated infections in developing countries. Nevertheless, the increased isolation rates in the healthcare settings of these less developed countries have led to the expectation of similar or higher effects than those of the advanced countries (Abubakar and Sulaiman, 2018; Founou *et al.,* 2017).

2.3 Natural products in drug discovery

2.3.1 Natural products and their inherent medicinal values

Compounds sourced from natural products (NPs) have proved to be promising in the discovery and development of novel anti-MRSA drugs (Abreu *et al.,* 2012; Dong *et al.,* 2018). These compounds are obtained from living organisms such as bacteria, fungi, plants, and marine microorganisms (Chen *et al.,* 2015; Özakin and Bostanci, 2019). It was recently mentioned that four-fifths of the population in most developing nations are living on trado-medical practices as the main source of treatment in basic healthcare services (Wright, 2019; Zengin *et al.,* 2017). In addition, a previous study has extensively described the approval of some NP-based therapies against a range of diseases such as cancer, diabetes, and other infections (Harvey, 2008). Furthermore, three out of the five newly developed drugs by the United States Food and Drug Administration (FDA), representing novel classes of antibiotics between 1981 and

2010 were also sourced from NPs (Harvey *et al.*, 2015). Therefore, there is an increased interest in exploring or pursuing NPs as promising lead compounds in combating multidrug-resistant bacteria (Jaradat *et al.*, 2017; Zengin *et al.*, 2017).

2.3.2 *In vitro* antimicrobial activity of extracts from natural products

Generally, the identification and evaluation of potential hits among NPs are essential towards the achievement of a set goal in drug development. This is because any biologically active compound could provide selective ligands for the target of disease-causing organisms eventually disrupting the disease pathways (Gu *et al.*, 2013). The antimicrobial potential of crude extracts and pure NPs has been studied by observing the growth response of pathogens to samples. The selection criteria of potential antimicrobial compounds relate to minimum inhibitory concentration (MIC) values of not more than 100 μg/mL and 25 μM for crude extract and pure compounds respectively (Bueno, 2012).

2.3.3 Natural products have more prospects than synthetic compounds in drug discovery.

Nature has been described as the most inspiring source of new and efficient pharmacological molecules (Fang *et al.*, 2018; Shen, 2015). The poly-pharmacological profiles of NPs are more than those found in synthetic drugs. Synthetic compounds have less complex stereochemical properties and are sometimes characterized by unacceptable side effects. Natural products however are known with broad chemical diversities, fewer aromatic rings, increased oxygen but lower nitrogen or halogen constituents, sp3-hybridization chiral centers, and larger macro-cyclic aliphatic rings (Davison and Brimble, 2019; Guo, 2017; Wright, 2019). These properties enable the molecules to efficiently interact with biological targets (Wright, 2019). Thus, these compounds have privileged structures and they have remained essential components in the search for and development of novel, cheap, and safe drug candidates.

2.3.4 Limitations of natural products in drug discovery projects

Despite the enormous potential and previous accomplishments in drug developments, modern pharmaceutical industries have favored synthetic compounds as a more tractable replacement (Wright, 2019). This is due to the chemical complexity, toxicity, and poor pharmacokinetic properties that are often associated with NPs (Davison and Brimble, 2019; Harvey, 2008). Moreover, shifting in drug discovery strategies to biochemical and high throughput screening of large quantities of active molecules has also limited the use of NPs in drug development. This is because the screening process only permits large libraries of molecules to be explored

using modern synthetic approaches such as combinatorial chemistry which is not suitable for NPs (Davison and Brimble, 2019).

Furthermore, since they are usually obtained in small amounts from the original organism, it is slow and difficult to work with NPs especially in terms of their purification and identification from complex mixtures. Finally, more than 200,000 NPs have been described in the literature. The consequence of this is that evaluation of the biological activities of extract from natural substances aimed at discovering novel molecules may lead to the identification of already known compounds repeatedly. All these challenges have limited the therapeutic potentials of NPs for modern drug designers. However, modifying the structure of these products can help in their optimization for drug candidates (Davison and Brimble, 2019).

2.4 Challenges in drug discovery and development

The process of drug discovery and development is difficult, broad, risky, costly, and time-intensive (Pereira and Aires-de-Sousa, 2018; Prada-Graci *et al.*, 2016). The probability of a bioactive compound reaching the clinical trial stage and eventually making it to the market was estimated at 12 % (Nicolaou, 2014; Pereira and Aires-de-Sousa, 2018). Additionally, it takes more than 10 years, and an average cost of US$ 2.5 billion to transform a bioactive compound into a commercialized drug. Furthermore, the ratio of drugs approved per annum to the resources used in their discovery and development has remained relatively unchanged over the last decade (Jayasundara *et al.*, 2019; Mullard *et al.*, 2014; Pereira and Aires-de-Sousa, 2018; Prada-Graci *et al.*, 2016; Shivakumar *et al.*, 2018). Therefore, it has become crucial to embrace the available knowledge in the quest for faster and more effective approaches to drug discovery and development.

2.5 Modern techniques in drug discovery and development

Modern drug developers have employed different strategies targeted at overcoming the aforementioned challenges. Some of these techniques rely on previously described methods (Nicolaou, 2014; Zhang *et al.*, 2017). For instance, the use of traditional techniques such as combinatorial chemistry and high-throughput screening approaches have led to a large increase in the available volume of structural and biological data to steer rational decision making in pharmaceutical industries. This has given rise to a technique called cheminformatics (Gillet, 2019).

2.5.1 Overview of cheminformatics

Cheminformatic is a data mining technique that uses computer and information strategies to solve chemical problems by processing raw data into information and information into knowledge (Egieyeh *et al.*, 2016; Gillet, 2019; Medina-Franco, 2013). Chemical data processing in this context involves working with chemical structures (Xu and Hagler, 2002). Therefore, the goal of this strategy for drug developers is to provide better and faster decision-making processes in terms of discovery and lead optimization (Egieyeh *et al.*, 2016; Gillet, 2019). Cheminformatics is gaining much acceptance in the field of computational chemistry. It has great potential especially in the retrieval and extraction of chemical information, database search for compounds, interactive data mining for molecular graph, and analyses of chemical diversity (Gillet, 2019; Jónsdóttir, 2005; Medina-Franco, 2013; Xu and Haggler, 2002). Since these techniques employ computer-based modeling, they are sometimes called computer-aided rational drug design (CARD) (Wang *et al.*, 2015).

2.5.2 Computer-aided rational drug design

Computer-aided rational design (CARD) has been used as a key tool in drug development to explore collections of small molecular compounds for potential lead (Pereira and Aires-de-Sousa, 2018; Zhang *et al.*, 2017;). Compounds with the most possibility of binding to an enzyme or other related drug targets are identified with CARD techniques and it enhances a more reliable hit rate than when only the traditional experimental screening technique is used (Zhang *et al.*, 2017). The use of CARD by drug developers have been described as of great advantage over traditional techniques. The traditional technique is commonly characterized by a high cost of resources or time, and a high attrition rate (Chen *et al.*, 2017; Živković *et al.*, 2019). In contrast, CARD can substantially save the cost of developing new drugs by up to 50 %. This is because this tool can be employed to guide the focus of drug developers only on potential drug candidates instead of the large chemical libraries of small molecules (Chen *et al.*, 2017; Wang *et al.*, 2015).

Corresponding CARD techniques involved in identifying hit or hit-to-lead compounds include pharmacophore modeling, QSAR models, and molecular docking. In addition, CARD has been successfully used to predict drug characteristics such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) of drug candidates (Chen *et al.*, 2017; Hassan *et al.*, 2016). This technique is also relevant in predicting the binding affinity of active molecules to putative drug targets (Chen *et al.*, 2017; Hassan *et al.*, 2016). Although to make relevant predictions

using these strategies for NPs, it is vital to have access to chemical structure with a well-defined spatial arrangement of the atoms. In another way, there is a propensity that computer-aided prediction favours synthetic molecules over natural compounds for physicochemical parameters. This is due to the more abundance and less structural complexity of the synthetic compounds. Nevertheless, CARD has proven significantly important in the evaluation of novel drug candidates from NPs (Chen *et al.*, 2017). Some of the studies that have successfully applied CARD techniques towards identifying potential lead from NPs against MRSA are described in Table 2.1 below.

Table 2.1. Cases where CARD techniques were employed to identify potential lead compounds from NPs against MRSA.

Investigated NPs	Source of data	Approaches used	References
Flavonoids	In-house library of phenolic compounds	Docking, molecular dynamic simulation and structure-activity relationship (SAR) analysis	Alhadrami *et al.*, 2020
Melantriol		ADMET and docking studies	Skariyachan *et al.*, 2011
β- Sitosterol		ADMET and docking studies	Skariyachan *et al.*, 2011
Anthraquinone		Docking studies	Wang *et al.*, 2018
Diflunisal	Commercial databse screening-drug repurposing	Virtual screening and similarity search	Khodaverdian *et al.*, 2013
Marinopyrrole A, AGN-PC-07NF8H, Azalomycin, Methylsulfomycin I, a10255, GE37468, Tallysomycin, Cleomycin B2, Bleomycin z, Bottromycin A2, Berninamycin C and Cyclothiazomycin.	StreptomeDB 2.0 library	Machine learning through quantitative structure–activity relationship (QSAR) studies	Dias *et al.*, 2019
Sesamin, pellitorine, uineesine, brachystamide B and pipataline	Plant (piper longum)	Pharmacokinetics and docking studies	Alluraiah *et al.*, 2019
Oxadiazoles	ZINC database	Virtual sreening and docking	O'Daniel *et al.*, 2014
Quinazolinone	Plants	Docking and ADMET prediction studies	Qureshi *et al.*, 2019
Phenolic compounds	Algerian Sahara plant (Forssk)	Docking studies	Ziani,*et al.*, 2020
Phenolic compounds (Protocatechuic, *p*-coumaric acid, and 2,4-dihydroxybenzoic, and)	Wild mushroom	SAR analysis and docking studies	Alves *et al.*, 2013
Aspermerodione	Fungus (*Aspergillus* sp.)	Docking and virtual screening	Qiao *et al.*, 2018
Cannabinoid compounds	*Cannabis sativa*	Drug-likenesss prediction, QSAR and docking studies	Cortes *et al.*, 2020
Hamamelitannin derivative compounds	PubChem database	Ligand-Based Pharmacophore Modeling and Virtual Screening	Johari *et al.*, 2013

2.5.3 Virtual screening for hit identification.

Computational strategies enable the prediction of biological targets of active compounds at the early stage of drug discovery by using information from the chemical database (Banegas-Luna *et al.*, 2018; Hassan *et al.*, 2016). This technique commonly known as virtual screening (VS) is employed to identify promising molecules from a large chemical scaffold by exploring commercial or freely available chemical structure databases (Tomar *et al.*, 2018). In a previous study, 11 compounds were discovered after VS analysis was conducted on about 4000 phytochemicals against estrogen receptors (Medina-Franco, 2013). Apart from facilitating the querying of active molecules for their targets, this technique also tends to identify active compounds that require optimization (Prada-Gracia *et al.*, 2016). Compounds that meet up with the desired filtering criteria in VS are called hit compounds. Nevertheless, hit molecules from VS are recommended for experimental validation of their predicted activities (Gimeno *et al.*, 2019). Furthermore, based on the type of information available about the system under inspection, VS techniques can be classified as either structure-based or ligand-based screening (Fig. 2.2) (Banegas-Luna *et al.*, 2018; Vyas *et al.*, 2008; Wermuth *et al.*, 2015).

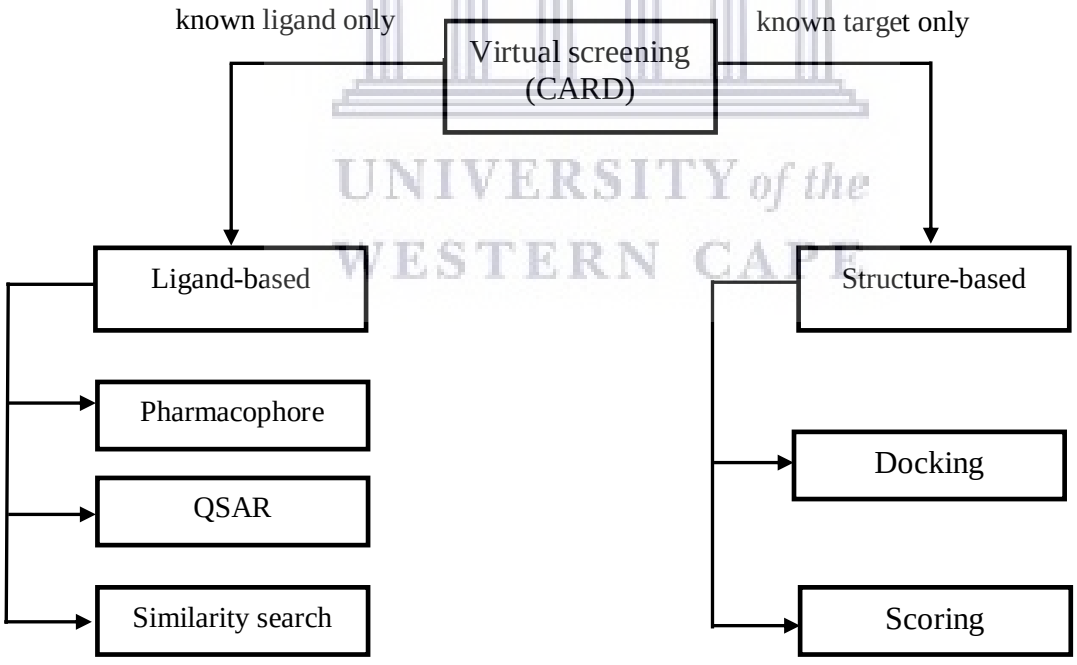


Figure 2.2. A schematic representation of virtual screening techniques in drug discovery.

Structure-based virtual screening (Fig. 2.2) is employed during lead identification and optimization process to identify potential drugs by using the three-dimensional structural information of the target protein (Banegas-Luna *et al.*, 2018). The ligand-based approach (Fig.

2.2), however, uses the available information in known active ligands to predict the unknown targets (Banegas-Luna *et al.*, 2018; Hamza *et al.*, 2012). The ligand-based approach is popularly known for similarity comparisons between molecules (Pereira *et al*., 2020). Common examples of this technique include chemical similarity searching, pharmacophore modeling, and quantitative structure-activity relationship (QSAR) (Moumbock *et al.*, 2019; Pereira *et al*., 2020). The applications of some of these screening methods in drug discovery research are discussed below.

2.5.3.1 Docking and scoring in structural-based screening.

2.5.3.1.1 Docking

Docking is a structure-based VS technique that is employed in the identification and optimization of prospective drug candidates through molecular modeling and investigation of ligand-target interactions (Sethi *et al.*, 2019; Tomar *et al.*, 2018). These interactions could generate many ligand conformations and orientations of which the most suitable ones are considered (Prada-Gracia *et al.*, 2016). Additionally, the effectiveness of any promising drug candidate is a function of how appropriate the ligand is positioned in the receptor (Jónsdóttir *et al.*, 2005). Docking has proved effective in the investigation of a large collection of chemical substances, narrowing them into a more reasonable subset that can be enhanced for the interacting targets (Prada-Gracia *et al.*, 2016). Different software including Automated docking, AutoDock Vina, Gold, SURFLEX, DOCK, and GLIDE has been identified as programs available for docking (Katsila *et al.*, 2016). Different studies have also reported the successful application of docking strategy in drug discovery (Olğaç *et al.*, 2017; Rout *et al.*, 2017; Sethi *et al.*, 2019). Nevertheless, Sethi *et al.* (2019) studied the principles and applications of docking in modern drug discovery and reported poor scoring functions as the major limitation.

2.5.3.1.2 Scoring

Scoring is employed in VS to estimate and rank the free energy binding of a ligand at their different conformation to the target (Prada-Gracia *et al.*, 2016; Sethi *et al.*, 2019). Scoring evaluates the ligand-target interaction energy through a regression of two or more variable quantities of various properties to get a likely or actual binding energy in a short time (Tomar *et al.*, 2018). These properties are lipophilicity, ionic interactions, and the number of hydrogen bonds, etc. The major classes of scoring functions include force field, statistical-based

potentials, machine learning, consensus-based and empirical-based score. SURFLEX software as an example employs an empirical-based function that relies on the counted numbers of existing ligand-target interactions. Similarly, DOCK employs a force field to estimate the strength of the intermolecular interactions between existing atoms of the two interacting partners (Katsila *et al.,* 2016).

2.5.3.2 Ligand-based screening methods in drug discovery

2.5.3.2.1 Pharmacophore modeling

Huang *et al.* (2018) have studied several methods of searching for the unknown targets of chemo-preventive molecules. They reported that the basic principle of pharmacophore modeling is the spatial arrangement of the features that are necessary for the binding of a ligand to its target. The pharmacophore model is described by the chemical features and spatial arrangement of features such as partial charge, aromatic and aliphatic hydrophobic moieties of the active site, hydrogen bonds, acidic, and basic side chains. The use of these descriptors in VS can provide a guide towards identifying prospective binding partners. The generated pharmacophore model can be used to query a database for potential hits or targets (Huang *et al.,* 2018; Moumbock *et al.,* 2019). Furthermore, commonly used software programs for pharmacophore modeling include Discovery studio, MOE, Schrodinger maestro, and LigandScout (Moumbock *et al.,* 2019; Prada-Gracia *et al.,* 2016). Kirchweger *et al.* (2018) recently employed LigandScout to identify G protein-coupled receptors (GPBAR1) as the target for two NPs; arnesiferol B and microlobidene. Nevertheless, there is no direct process for generating a pharmacophore query (Hassan *et al.,* 2016).

2.5.3.2.2 Quantitative structure-activity relationship (QSAR)

The basis of this model is that physicochemical features and biological activities of a compound are embedded in its chemical structure (Tomar *et al.,* 2018). Therefore, QSAR tends to link the chemical structure of a molecule to the physicochemical (including lipophilicity, molecular weight, aqueous solubility, geometry, atom types, molar refractivity, electronegativity, etc.) or therapeutic attributes (such as binding sites affinities of ligands and inhibition constants, toxicity, etc.) within the library of congeneric molecules (Ekins *et al.,* 2007; Prada-Gracia *et al.,* 2016; Tomar *et al.,* 2018). In other words, if a considerable connection is generated for a group of compounds in the library with the robustness of biological data, informatics approaches can be employed in predicting the biological activities for other compounds.

Nevertheless, a successful prediction of QSAR is a function of choosing the appropriate descriptors as well as the capability to establish a suitable mathematical connection between different compounds (Ekins *et al.,* 2007; Tomar *et al.,* 2018).

Finally, VS methods are preferred to the traditional techniques of screening because millions of substances can be screened within a very short period and at a very lower cost (Prada-Gracia *et al.,* 2016; Pereira *et al.,* 2020; Tomar *et al.,* 2018). Nevertheless, considering the strength and weaknesses of each of the VS methods, an *in silico* project workflow that combines the different techniques is highly recommended to minimize false-positive result that is common to a single method (Pereira *et al*., 2020).

2.6 Basic descriptors for evaluating physicochemical and pharmacokinetic properties.

Evaluation of the physicochemical parameters (PP) of potential drug candidates is very crucial in drug development as it helps in the early identification of molecules that may fail at a later stage (Wenlock and Barton, 2013). The absorption or therapeutic action elicited by a drug depends largely on the interaction between the various physical and chemical properties of the drug and the targets (Chandrasekaran, 2018). Therefore, the physical and chemical properties of any compound are crucial to evaluate the drug-likeness (Medina-Franco, 2015). Furthermore, for a better drug-receptor relationship, PP can be manipulated to an optimized condition using computer-aided strategies (Chandrasekaran, 2018). A few of these PP that are key to determining the biological activity of any drug candidate are discussed below (Chandrasekaran, 2018; Wenlock and Barton, 2013).

2.6.1 Partition coefficient (logP)

The partition coefficient (logP) is the ability of an uncharged molecule to dissolve in a nonhomogeneous two-phase system of lipid and water (Bhal, 2007). It measures the amount of solute that mixes in the water against that which dissolves in a lipophilic portion. This property is used to evaluate how a molecule travels to the target from the site of administration (Bhal, 2007; Chandrasekaran, 2018). This implies that the values of logP are significant indicators of the fate of an administered drug in the target organism. A negative logP indicates that the molecule is more hydrophilic, and a positive logP shows that the molecule has a higher affinity for the lipophilic phase. Similarly, zero logP means that the substance is equally partitioned between the bi-phasic system (Bhal, 2007). To achieve the desired antibiotic efficiency, it is

therefore important to design high lipophilic drug candidates since the biological target is a lipid layer.

2.6.2 Hydrogen bonding

Hydrogen bonding refers to the affinity between an atom of hydrogen from a given compound (known as the donor) and a hydrogen atom from different compounds (known as acceptor). This is evidence by bond formation (Schwöbel *et al.,* 2011; Yunta, 2017). Hydrogen bonds (HBs) are significantly important in evaluating the specificity of the binding of a ligand substance to a receptor. Studies have established the impact of HBs in the analysis of the quantitative structure-activity relationships (QSAR) model (Chandrasekaran, 2018; Schwöbel *et al.,* 2011). Hence, the role of quantifying HBs is significant in the process of designing and optimization of lead compounds (Schwöbel *et al.,* 2011). Furthermore, the addition of a properly positioned HBA side chain (to form an intramolecular HB) may be logical when HBDs are required for target activity (Rankovic, 2015).

2.6.3 Permeability of drugs

The propensity of a druglike substance to successfully move across the membrane of living organisms is highly essential. Knowledge about the permeability capacity of a drug is required in understanding the movement from sites of administration to the bloodstream (Bohnert and Prakash, 2011). A permeable drug is expected to pass through semi-permeable barriers, intestinal epithelial, and blood-brain barriers (BBB) by the pathway of passive diffusion. A poorly permeable drug may, however, be due to various structural characteristics and or efflux pathways connected with the membrane of the target. Additionally, drug permeability is influenced by other properties. For instance, physicochemical properties such as high lipophilicity, low molecular size, polarity, and hydrogen bond have been established to have a great influence on the prediction and optimization for drug permeability (Bohnert and Prakash, 2011; Rankovic, 2015).

2.7 Concept of drug-likeness

Molecular techniques are preferred by drug developers than the experimental design because the former has the potential of enhancing the prospects of seeking novel drug candidates (Meanwell, 2011). Bickerton *et al.* (2012) reported that the distribution of some key PPs (such as molecular weight, lipophilicity, polarity, numbers of hydrogen bond acceptors, and donors)

of approved drugs confirms that they are relatively found within a definite range of possible values. Molecules within this range are called drug-like (Bickerton *et al.,* 2012). The idea behind predicting the drug-likeness of a substance is that some properties can be more desirable in developing a drug candidate. For instance, some sets of criteria have been used by drug developers to evaluate the prospects of hits to become successful drugs (Krämer *et al.,* 2016; Medina-Franco, 2013). In addition, by determining the pharmacokinetics and toxicity of the potential drug candidate, one could rather increase *in vivo* efficacy instead of attrition that is linked to drug toxicity (Boufridi and Quinn, 2018).

2.7.1 Lipinski's rule of five (Ro5)

The Ro5 is a collection of some important PP that needs to be prioritized in determining the success of orally administered drugs (Chandrasekaran, 2018; Lipinski, 2004; Tian *et al.*, 2015). There is a likelihood for poor absorption and permeability for drug candidates whose logP, hydrogen bond donors (HBDs), hydrogen bond acceptors (HBAs), and molecular weight (MW) are above 5, 5, 10, and 500, respectively (Krämer *et al.,* 2016; Lipinski, 2004; Mignani *et al.,* 2018; Tian *et al.,* 2015). The digit 5 in Ro5 indicates the limit of the parameters which are multiples of 5 (Chandrasekaran, 2018). The goal of this strategy is to use a drug-likeness filter to quickly identify for; removal or optimization of poor pharmacokinetic compounds at an earlier stage of drug discovery (Krämer *et al.,* 2016; Mignani *et al.,* 2018; Tian *et al.*, 2015).

Several authors have explained successful cases where Ro5 has been employed to evaluate the drug-likeness of hundreds and thousands of NPs (Boufridi and Quinn, 2018; Lipinski *et al.*, 2012; Tian *et al.*, 2015). Zhang and Wilkinson, (2007) also reported that about two-thirds of the FDA-approved drugs are both administered orally and passed the Ro5. However, some drawbacks have been identified with the use of Lipinski's rule. For example, approved drugs such as atorvastatin, bromocriptine, and everolimus are notable violators of the Ro5 (Abad-Zapatero, 2007; Benet *et al.,* 2016). Similarly, Zhang and Wilkinson (2007) have reported that 20 % of all orally administered drugs failed at least one of the parameters of Lipinski's rule. Furthermore, the harsh cut-off that is used in Lipinski's parameters has failed to distinguish between molecules with similar properties (Bickerton *et al.,* 2012; Segall, 2014). In another way, a compound with a MW of 501 Da is considered to have a considerably lower likelihood of success than one with a MW of 499 Da (Wager *et al.,* 2010). These constraints can result in significant missed opportunities (Segall, 2014; Wager *et al.,* 2010). Therefore, the Ro5 alone

may not evaluate the pharmacological and drug-likeness prospects of any molecule (Mignani *et al.*, 2018).

2.7.2 Pharmacokinetics and toxicity parameters

Pharmacokinetic descriptors such as absorption, distribution, metabolism, and excretion (ADME), and toxicity (T) are commonly used properties for profiling or predicting the fate of any drug candidate in the site of action (Vrbanac and Slauter, 2017). The concept of investigating the ADMET is of interest in early drug discovery because over 70 % of clinical failures have been connected to these properties (Sharma *et al.*, 2018; Wang and Urban, 2004). In addition to potency, a successful drug candidate is expected to have favourable ADMET properties (Vrbanac and Slauter, 2017; Wang and Urban, 2004).

The use of technologies in determining these parameters has significantly contributed to recent advancements in the discovery and development of new drug candidates (Chandrasekaran, 2018). For instance, *in silico* approaches to evaluate ADME properties of drug candidates could guide computational chemists towards an effective structure-activity relationship (SAR) based optimization for good absorption, high bioavailability, metabolic stability, and the required distribution in the body (Ekins *et al.*, 2007; Wang and Urban, 2004).

2.8 Techniques for optimization of NPs

The aim of structurally optimizing NPs is to enhance the development of a potential drug candidate. In this process, the physicochemical and pharmacokinetic properties of a potential drug candidate can be selectively modified based on the limitations of their structure or activity (Chen *et al.*, 2015; Guo, 2017; Xiao *et al.*, 2016). In general, the required technique for this process is to improve the efficacy of the molecule. Additionally, this strategy also tends to optimize the molecules toward reducing toxicity, adjusting the violation of Lipinski's criteria and in general, raising their ADMET properties for maintaining balanced structural features with potency (Chen *et al.*, 2015; Guo, 2017). Finally, optimization process tends to enhance the synthetic accessibility of a drug-like compound.

Structural optimization in drug design can be carried out through a combination of different approaches (Xiao *et al.*, 2016). The simplest of these strategies is the direct chemical modification of functional groups through isosteric replacement, addition, and alteration of the

ring systems (Harrold *et al.*, 2013). This strategy is based on the chemical similarity principle, which states that chemically similar structures will have similar bioactivity. Another optimization approach is through SAR and subsequent SAR-directed optimization. At this stage, the combination of chemical and biological information of the compound is used to generate a SAR, for rational optimization of hit compounds. These two approaches describe the case of more than 30 % of anti-cancer drugs that are analogues of natural products (Sharifi-Rad *et al.*, 2019*;* Xiao *et al.*, 2016). Optimization of natural hit also uses a molecular design based on the core structures to generate pharmacophore-oriented molecular design. Examples of this strategy include the elimination of redundant chiral centers and scaffold hopping, commonly used to identify novel hits with intellectual properties. Unlike the first two approaches, the core structures of the original compound may change significantly during the last approach (Xiao *et al.*, 2016).

2.9 Conclusion

The known catastrophe from the familiar enemies called AMR bacteria is inescapable. Nevertheless, going by the lessons learned from the COVID-19 pandemic, it has become imperative for researchers to stay ahead of another global pandemic by developing newer and more potent antibiotics. Although many NPs have proven to have the potential of filling this gap, the high financial implications, cost in time, and attrition rates commonly associated with drug discovery and development may not encourage this move. In contrast, the potential of computational strategies to speed up the process of resurrecting many valuable NPs from the graveyard to become the solution to AMR has been reviewed in this chapter. Researchers have used some of the different computational approaches discussed in this chapter towards lead identification in drug discovery. For instance, prediction of drug-likeness, pharmacokinetic properties, and structural-based virtual screening of selected NPs against viral targets was used recently to identify potential inhibitors of matrix protein (VP40) in the Ebola virus (Pereira and Aires-de-Sousa, 2018). Nevertheless, the success of combining different drug approaches for the final identification of informatic leads is significantly greater than that which is done either *in silico* or *in vitro* (Neves *et al.*, 2016). That said, the use of *in vitro* or *in vivo* techniques is crucial in the validation of *in silico* predictions as this could guide drug developers against false-positive results.

Chapter three

Materials and methods

This chapter describes the research methodology. It begins with an overview of the materials used for the research. This is followed by a detailed description of the methods used in the two phases of the study. Phase one contains a detailed overview of data collection and data mining procedures for drug-likeness and pharmacokinetic profiling of the ligands. Phase two describes an overview of the procedures for target prediction and analysis.

3.1 Hardware and software

All the computational studies were carried out on a Windows 10 ultimate PC with Intel Core i5-7200U processor, 8 GB memory, and 64-bit operating system. Biological databases: ChEMBL, Drug bank, PubChem, and PDB (Protein Data Bank). Software: Cytoscape version 3.7.0, OSIRIS DataWarrior, MOE program (2019.01), StarDrop™, GraphPad Prism, KNIME, OpenBabel, PyRx version 0.8, and UCCF Chimera were all used in the study.

3.2 Phase 1: Procedures for cheminformatics profiling of the AMNPs

3.2.1 Data collection and preparation

An electronic search was conducted in May 2019 to identify relevant studies by employing freely available public databases (Google Scholar, Science Direct, Scopus, and PubMed). The keywords: "Marine OR natural products AND MRSA'', "Phytochemicals AND MRSA'', and "MIC of phytochemicals AND MRSA'' were used. The last search date was 20th May 2019. The reference lists of some of those eligible studies were also checked for related studies. Studies that reported the susceptibility of clinical isolates of MRSA to NPs, as determined by the reported minimum inhibitory concentration (MIC) were also included in this study. The search was customized and limited to reported publications from January 2009 to May 2019.

A sum of 111 anti-MRSA natural products (AMNPs) (**Appendix A**) was retrieved based on the search strategy described above. The "Simplified Molecular Input Line Entry System (SMILES)" structures of the AMNPs and their respective bioactivity data were stored as a text file. The dataset was divided into three categories based on the *in-vitro* bioactivities as reported by the MIC of the different AMNPs (which ranged from 0.01 to 1600 μg/mL), using a modification of previously described methods (Catteau *et al.,* 2018; Ndjateu *et al.,* 2014). The

bioactivity categories include significantly active (SA), active (A), and negligibly or less active (NA) for MIC values ≤ 10, 10 < MIC ≤ 100, and MIC >100, respectively. The 111 AMNPs were made up of 55 % SA, 34.2 % A, 10.8 % NA. In addition, current drugs for MRSA (CD) that are mostly derivatives of natural products were also retrieved from literature and used as the reference compounds (**Appendix B**).

3.2.2 Estimation of molecular descriptors and physicochemical properties of the datasets.

The SMILES structures of the datasets, retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov) (Hähnke *et al.*, 2018), were uploaded onto the SwissADME webserver (Daina *et al.*, 2017) to estimate the physicochemical properties of the AMNPs. Key molecular descriptors such as molecular weight, hydrogen bonds, partition coefficient between n-octanol and water, rotatable bonds, and polar surface area were also predicted with the MOE program (2019.01) (CCGI, 2016). The mean values of these properties were calculated for the different bioactivity categories of AMNPs and compared with that of the CDs. The SwissADME web tool was used to predict the potential of each AMNP to inhibit the cytochrome P450 (CYP450) enzymes. Biotransformation processes of the compounds were predicted by using a freely available web service at *www.biotransformer.ca* (Djoumbou-Feunang *et al.*, 2019). The rules proposed by Lipinski, Veber, and Egan were used to predict the drug-likeness of the AMNPs and CDs (Daina *et al.*, 2017). The absorption and bioavailability properties of AMNPs were also predicted as described by Daina and Zoete (2016). Toxicity properties such as mutagenic, tumorigenic, reproductive, and irritant effects were assessed using OSIRIS DataWarrior software (Sander *et al.*, 2015).

3.2.3 Exploration of chemical space (Assessment of chemical diversity)

ChemGPS-NP Web was used to explore the chemical space occupied by AMNPs relative to CDs. The SMILES structure and identifier of the datasets were submitted in the space provided on the ChemGPS-NP Web service (http://chemgps.bmc.uu.se) (Rosén *et al.*, 2009). The output with eight principal components added for each structure was retrieved as a text file. The text file was visualized on a 3D scatter plot in OSIRIS DataWarrior software (Sander *et al.*, 2015) using the first three principal components. Markers were coloured according to the categories of bioactivity, and the fourth principal component was used to size the markers.

3.2.4 Data analysis and visualization

Scatter plots, box plots, and bar charts of the molecular descriptors, physicochemical properties, and other parameters estimated or predicted were plotted for AMNPs (and the SA, A, and NA categories) and CD using OSIRIS DataWarrior (Sander *et al.*, 2015) and Prism GraphPad 6.0 (GraphPad Software). The mean of the molecular descriptors and physicochemical properties for AMNPs (and the SA, A, and NA categories) and CD were compared, and statistical differences were assessed using analysis of variance (ANOVA), with significance set at $p < 0.05$. Furthermore, the association between *in vitro* activities (MIC) of AMNPs and the molecular descriptors or physicochemical properties were determined using the Bravais-Pearson correlation coefficient (r).

3.3 Phase 2: Procedures for target prediction by reverse docking.

3.3.1 Protein selection

The protein-ligand interaction is a significant part of computer-aided drug design. Therefore, native protein structures were collected as drug targets. The keyword ''multidrug-resistant *Staphylococcus aureus''* was used to search for putative biological target information of *S. aureus* in ChEMBL (www.ebi.ac.uk/chembl/) and drug bank (www.drugbank.ca). The search result was filtered to targets in *S. aureus* only. About 57 hits were obtained in this process. The crystal structure of the four-letter code complexes with the lowest crystallographic resolution was downloaded from the RCSB protein data bank (https://www.rcsb.org/). The hits were further refined by removing all entries that were not related to *S. aureus*. Finally, about 34 putative proteins were retained and used for the experiment (**Appendix C**). The search was conducted between March and June 2020.

3.3.2 Ligand preparation

The combined sdf structure of the AMNPs was imported onto PyRx (freely downloaded from http://pyrx.sourceforge.net/downloads) using the OpenBabel plugin tool (Dallakyan and Olson, 2015). The energies of the ligands were minimized using the uff geometry optimization force field, other parameters were left as default, and the minimized ligands were converted to a ready-to-dock PDBQT file (Shaker *et al.*, 2020).

3.3.3 Protein preparation

Each of the downloaded protein structures (in their PDB format) was prepared with UCSF Chimera (https://www.cgl.ucsf.edu/chimera/) by removing molecules such as water, assigning

charges, and conducting energy minimization. The resultant protein was saved in PDB format, loaded onto PyRx, and converted to a ready-to-dock macromolecule PDBQT format (Shaker *et al.*, 2020).

3.3.4 Docking

In this study, the PyRx version 0.8 software was selected over every other available resource for docking because it has a user-friendly graphical interface (Shaker *et al.*, 2020). In addition, the scoring function in this tool provides for high efficiency, reliability, and accuracy of results (Attique *et al.*, 2019; Chen and Ren, 2014). Molecular docking simulation was performed on this tool using the Autodock vina option inbuilt in the workspace (Dallakyan and Olson, 2015; Trott and Olson, 2010). The grid box was adjusted where necessary to enclose the residues of the active sites and their surroundings. Docking was run at exhaustiveness of 8 and all other parameters were left as default.

3.3.5 Analysis

The results from the docking were binding free energy values given in kcal/mol. The higher negative binding free energy (BE) values indicate better ligand-protein interaction (Dallakyan and Olson, 2015; Shaker *et al.*, 2020). The summary result consists of the best binding free energy values of each AMNPs to the 34 putative proteins from MRSA. This was visualized as networks on Cytoscape software. Finally, the individual ligand-protein complexes were stored for further analysis of the amino acid interactions.

3.4 Summary of methodology

The Figure below (Fig. 3.1) provides the synopsis of all the major procedures explained in this chapter. It also highlights the major steps taken to conduct the current research project.

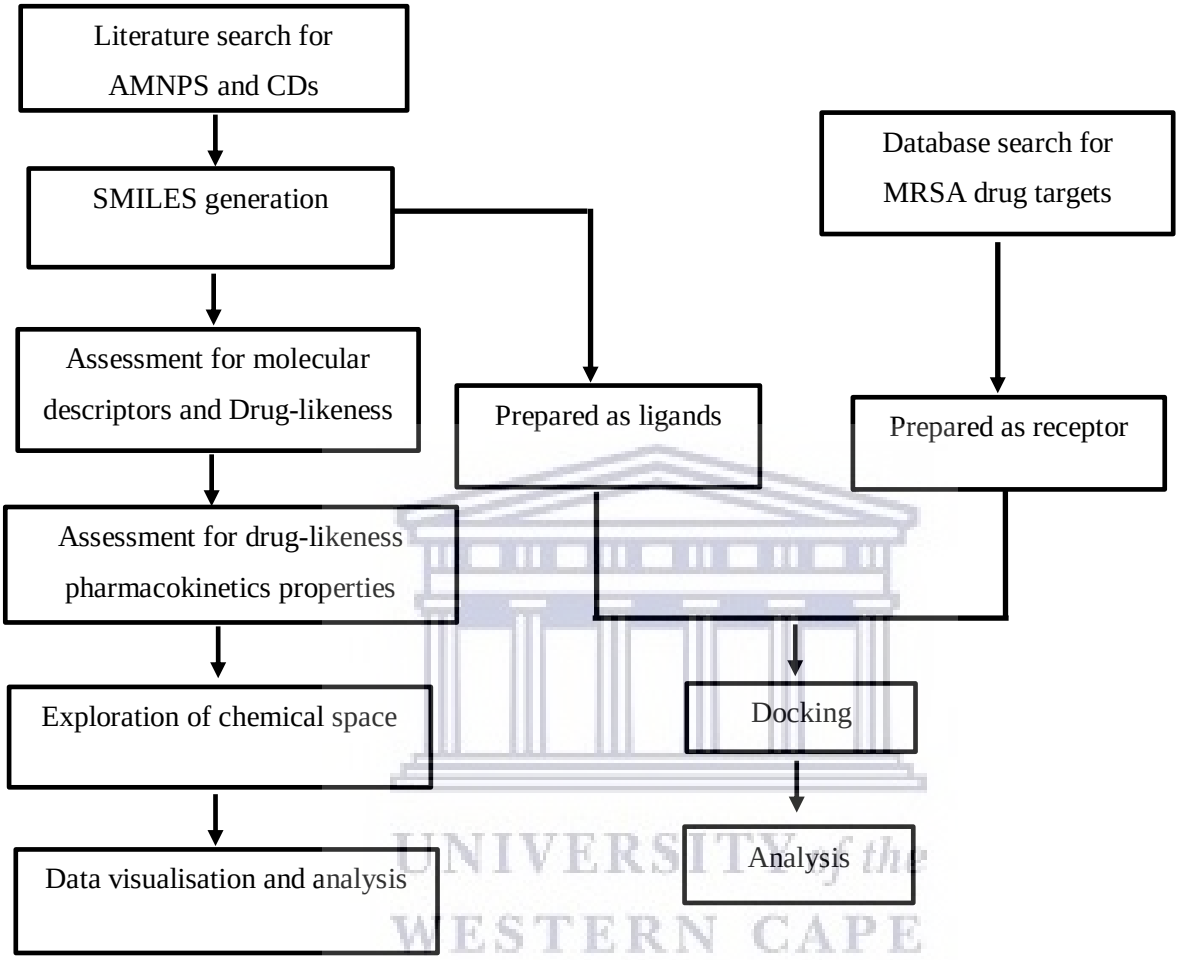


Figure 3.1. A flow chart of the method employed in the current research.

Chapter four

Results and discussion

This chapter is in two phases. The first phase provides detailed results from and implications of the cheminformatics profiling of the anti-MRSA natural products (AMNPs) for drug-likeness and their pharmacokinetic properties. It also identifies and discusses their potentials for "hit" to "lead" optimization. The second phase of this chapter provides the findings on target prediction analysis and the implications. Finally, this chapter would be concluded with a summary of the key findings.

4.1 Phase 1- Cheminformatics profiling for hit-prioritization and optimization of AMNPs

The goal of this section was to use different cheminformatics software to perform data mining analysis of the datasets for drug-likeness profiling, hit-prioritization, and hit-to-lead optimization. The datasets were made up of the 111 AMNPs obtained from literature search. These compounds were predominantly phytochemicals and marine microbes, the range of reported bioactivity (MIC) was between 0.01 μg/mL to 1600 μg/mL. Drug-likeness profiling was done using 20 current drugs for MRSA (CDs) as reference compounds. The major results of these analyses are summarized below.

4.1.1 Molecular descriptors and physicochemical properties of AMNPs and CDs

The key molecular descriptors and physicochemical properties of the overall AMNPs and the different categories (significant active; SA, active; A and less active; NA) were profiled for drug-likeness using the current drugs for MRSA (CDs) as reference. The distribution of these properties and their implications are described below.

4.1.1.1 Molecular weight (MW)

Molecular weight (MW) is one of the key parameters required for oral bioavailability (Veber *et al.*, 2002). Compounds with MW above 500 Da have been suggested to have a higher tendency for absorption problems, though natural products (NPs) may be an exception to this rule (Lipinski, 2004; Rosén *et al.*, 2009). The results from this study depict that more than 86 % of the AMNPs, as against 65 % of CDs were found less than 500 Da (Fig. 4.1a), and the mean of both datasets (AMNPs and CDs) were 381.1 and 733.5 Da, respectively. This implies that most of the AMNPs will be bioavailable via the oral route.

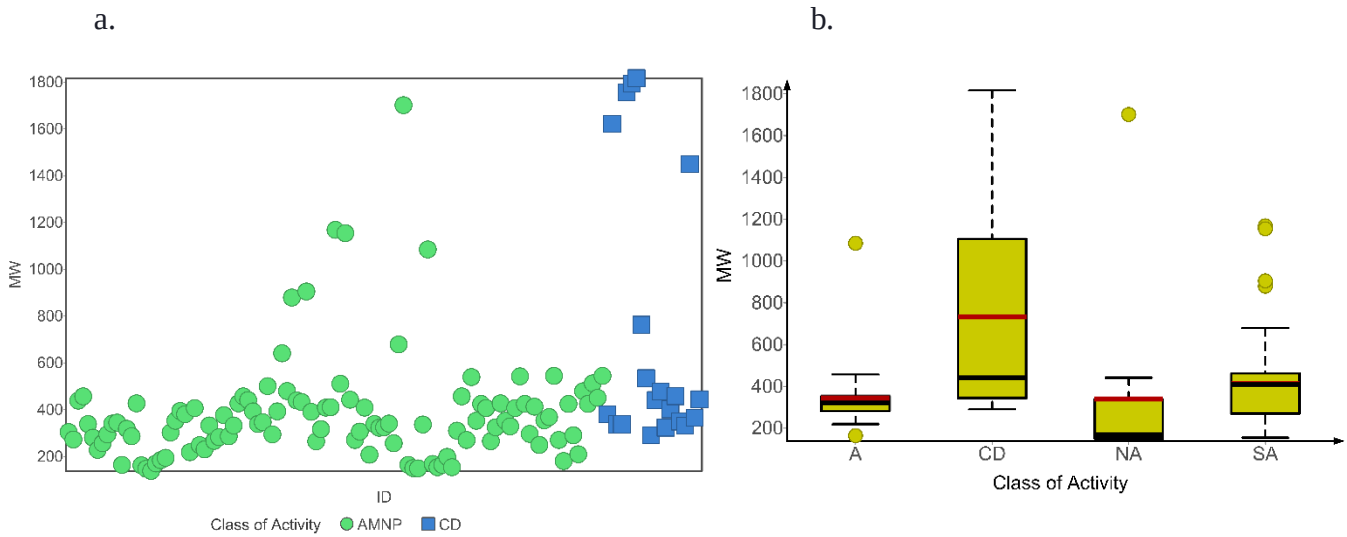


Figure 4.1. Distribution of MW for both AMNPs and CDs. Figure 4.1a presents the distribution of AMNP (green markers) and the CDs (blue markers) on a scatter plot. Figure 4.1b shows the box plot of the different categories of AMNPs with CDs. The statistical mean and median of each category are represented by red and black lines, respectively. The average MW of SA, A, and NA are significantly lower ($P < 0.05$) than CDs.

Additionally, most of the AMNPs could also have "room" for the addition of required bioisosteres towards improving certain drug-like properties during the hit-to-lead optimization process. The complex compounds in CDs, that are sourced from fungus or derivative of such natural products, may explain the higher MW observed in the CDs. The result for the average MW of the AMNPs categories is presented in Figure 4.1b. The A and NA categories showed similar mean MW (342.4 Da and 340.0 Da respectively), and they are significantly lower ($p < 0.05$) than that of SA (mean = 413.3) (Fig. 4.1a). This implies that the categories of AMNPs with the best bioactivity (SA) revealed the highest average MW. High MW has been associated with greater bioactivity because of the propensity of big compounds to encumber binding pockets of drug targets to bring about efficacy (Veber *et al.*, 2002; Yunta, 2016). Furthermore, the high average MW observed for the CDs agrees with literature where the property space of antibacterial substances was characterized by larger MW (O'Shea and Moser, 2008; Reck *et al.*, 2019). This is also consistent with the study by Doak *et al.* (2014) who observed that the average MW of small molecules approved in the past decades is above 500 Da. In contrast to this study, Egieyeh *et al.* (2016), Feher and Schmidt (2003), and Stratton *et al.* (2015) reported a higher mean value for MW in favour of NPs over approved drugs.

4.1.1.2 Calculated octanol/water partition coefficient (clogP)

The clogP is a well-known measure of hydrophilicity of a compound (Nalini *et al.*, 2011). This descriptor also contributes to drug-receptor interaction as well as the solubility and absorption of bioactive compounds. Figure 4.2a illustrates the distribution of clogP for the AMNPs in comparison to CDs. All categories of AMNPs have clogP values that are significantly higher than CDs ($p < 0.05$) (Fig. 4.2b).

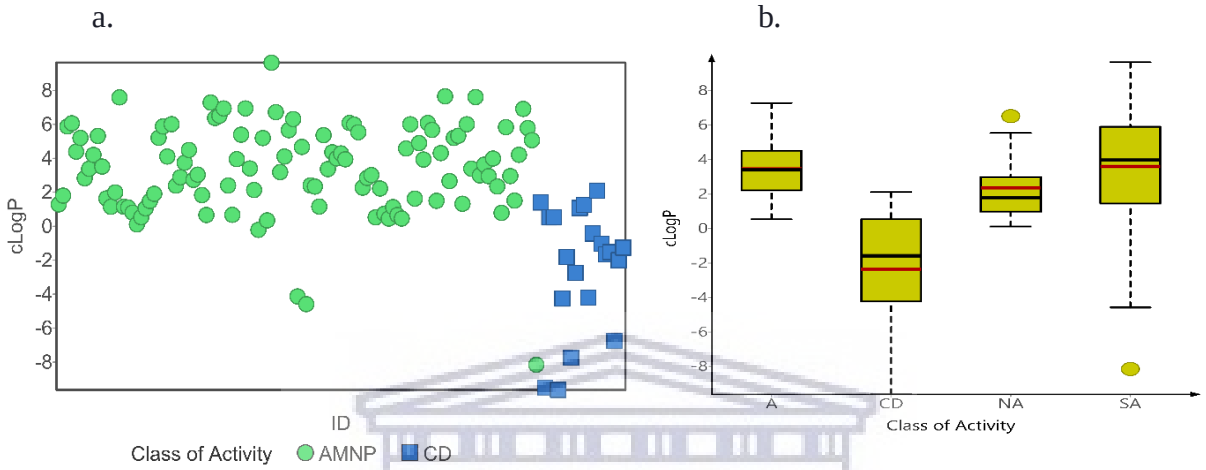


Figure 4.2. Distribution of clogP for both AMNPs and CDs. Figure 4.2a is a scatter plot showing the clogP of AMNP (green markers) and the CDs (blue markers). Figure 4.2b shows the box plot of the different categories of AMNPs and CDs. The statistical mean and median of each distribution are represented by red and black lines, respectively. The average clogP for all the categories of the AMNPs is significantly higher ($P < 0.001$) than CDs.

Compounds with clogP values above 5 are not likely to be well absorbed. This is because high logP tends to compromise the bioavailability of an active molecule (Arnott and Planey, 2012; Bhal, 2007). It was observed that the means of both datasets (AMNPS and CDs) were lower than 5. In addition, 69 % of the AMNPs were found below 5, and mostly positive values (Fig. 4.2a). Similarly, all the CDs have clogP values below 5, and about 70 % of these compounds showed negative clogP (Fig. 4.2a). This can be an indication that the CDs are more hydrophilic and with poor membrane permeability than the AMNPs. On the other hand, the more positive clogP values observed for the AMNPs indicate that they are more hydrophobic compared to most of the CDs. This finding is consistent with the study by Chen *et al.* (2018) who reported a higher positive clogP value in favour of NPs. Furthermore, this result agrees with the use of Lipinski's rule in drug discovery (Lipinski, 2004; Stratton *et al.*, 2015). Arnott *et al.*(2013) also reported that compounds with clogP values below 4 could stand a higher chance of success

in the discovery pipelines. Therefore most of the AMNPs may be desirable to follow as potential anti-MRSA drug candidates.

The more hydrophobic a molecule is the more likely it is to bind to a target and thus resulting in greater bioactivity. Therefore, the correlation between the clogP values of the AMNPs and the reported bioactivity (MIC) was explored. Surprisingly, there was a very weak linear correlation (r = -0.178) between clogP and the bioactivity of AMNPs (**Appendix D1**). This suggests that other molecular descriptors may have contributed to the observed differences in the *in vitro* activities of the AMNPs.

4.1.1.3 Hydrogen bond acceptors and donors

The hydrogen bond is a crucial property in drug-receptor interaction that may lead to pharmacological action. It also plays an important role in membrane transport and drug distribution in a biological system (Loureiro *et al.*, 2019). In this study, the average hydrogen bonds of AMNPs (HBAs = 5.523 and HBDs =3.135) was generally lower than that of CDs (HBAs =12.65 and HBDs = 7.9) (Fig. 4.3a and b).

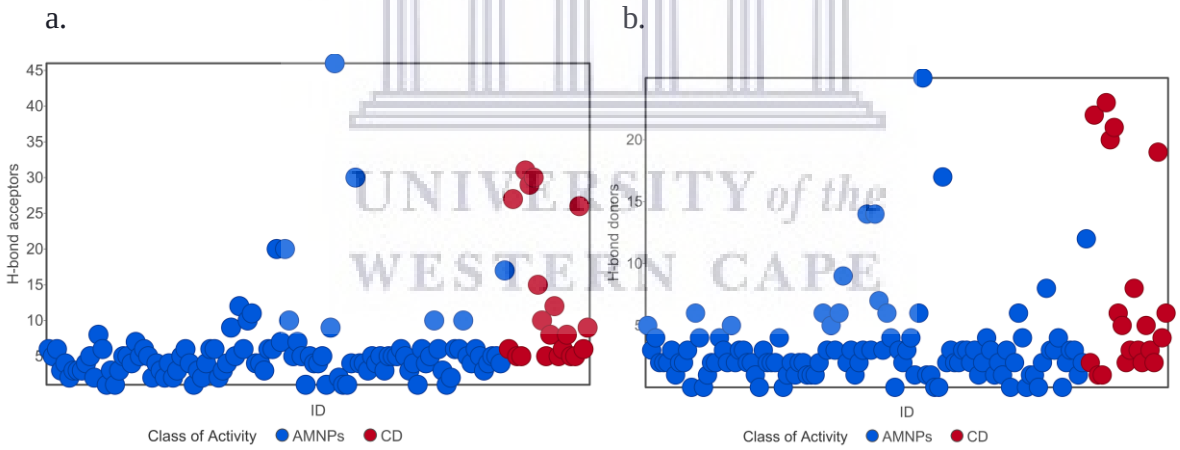


Figure 4.3. Distribution of hydrogen bonds for both AMNPs and CDs. Figures 4.1.3a and b are scatter plots showing the distribution of HBAs and HBDs respectively for both datasets. Blue and red markers represent AMNPs and CDs, respectively.

The hydrogen bond acceptors (HBAs) (Fig. 4.4a) and hydrogen bond donors (HBDs) (Fig. 4.4b) for the NA categories of AMNPs was not statistically different from the CDs ($p > 0.05$). Furthermore, for both HBAs (SA = 5.508; A = 5.158; NA = 6.75; CD = 12.65) and HBDs (SA = 3.131; A = 2.895; NA = 3.917; CD = 7.9), the average value for SA and A categories were

significantly lower than CDs ($p < 0.05$). Among the categories of AMNPs, the result, however, showed no statistical difference ($p > 0.05$) for both descriptors (Fig. 4.4a and b).

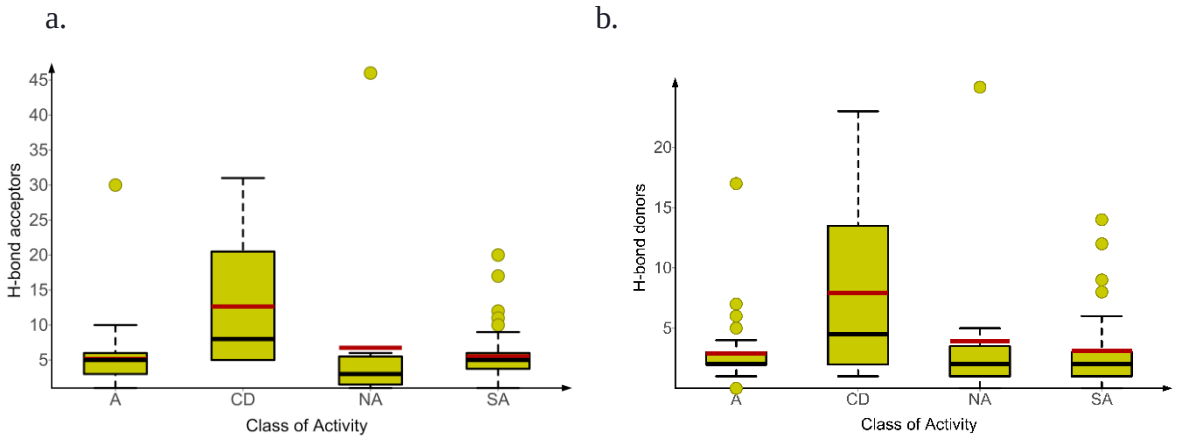


Figure 4.4. Box plots of HBAs (a) and HBDs (b) against class of activity. The statistical mean and median of each distribution are represented by red and black lines, respectively. Only the NA category of AMNPs was not statistically different ($p > 0.05$) from the CDs.

This implies that most of the AMNPs (especially A and SA categories) are likely to have more promising bioavailability compared to the CDs. This can be an indication that these compounds may not necessarily require optimization. This observation corroborates the use of Lipinski's rule in drug discovery (Stratton *et al.*, 2015). However, the observed distribution of AMNPs and CDs in this study are not consistent with cheminformatics studies which demonstrated that NPs have more HBDs and HBAs counts than approved drugs (Bade *et al.* 2010; Stratton *et al.*, 2015).

4.1.1.4 Total polar surface area (TPSA)

Studies have shown that molecules with TPSA above 140 $Å^2$ are not likely to penetrate through the intestinal membrane (Nalini *et al.*, 2011; Veber *et al.*, 2002; Whitty *et al.*, 2016). Figure 4.5a indicates that there was no significant difference in the TPSA of AMNPs categories.

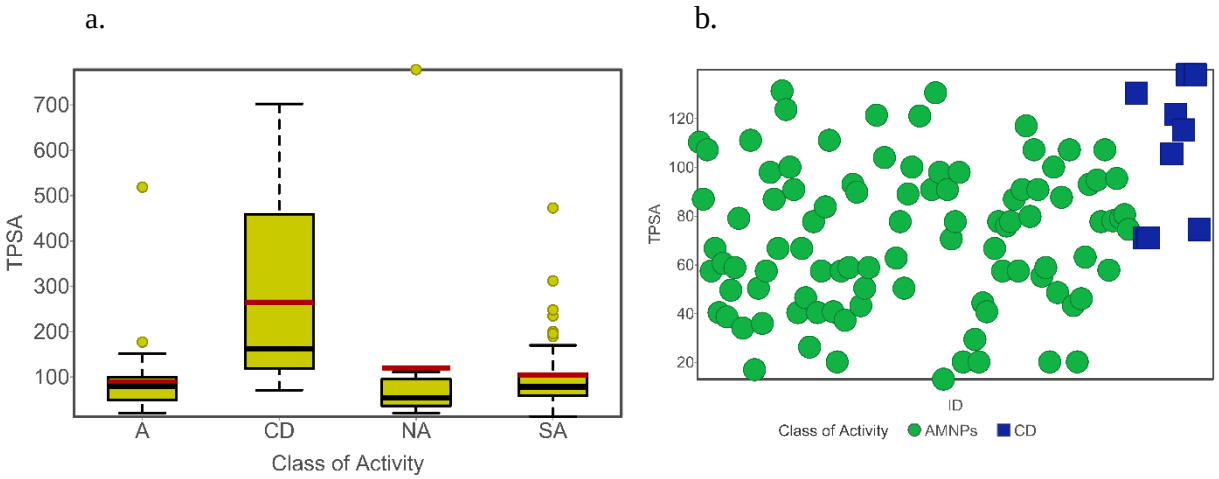


Figure 4.5. Distribution of TPSA for both AMNPs and CDs. Figure 4.5a shows box plots of TPSA against the class of activity. The average value was significantly higher ($p < 0.05$) in CDs than the A and SA categories of AMNPs. Figure 4.5b is a scatter plot displaying the distribution of the TPSA for AMNPs (green markers) and the CDs (blue markers).

The TPSA was significantly higher ($p < 0.05$) for CDs than the A and SA categories. Overall, 87 % of the AMNPs were found below 140 $Å^2$ while less than 50 % of the CD were found within this limit (Fig. 4.5b). Therefore, it can be inferred that most of the AMNPs have prospects for good intestinal epithelial permeability, and they may be pursued in the development of anti-MRSA drug candidates.

The higher TPSA values observed for the CDs may be connected with their large MW because there is a strong and positive ($r = 0.966$) correlation between these two parameters (MW and TPSA) (**Appendix D2**). According to Whitty *et al.* (2016), oral drugs with high MWs mostly tend to have TPSA above the recommended range (≤ 140 Å) Therefore, these chameleonic properties may have helped the CDs to achieve oral bioavailability as approved drugs. However, the observed distribution of TPSA is contrary to the findings of Egieyeh *et al.* (2016) who reported a significantly higher TPSA value in favour of NPs.

4.1.1.5 Rotatable bonds (RTBs) count

The number of RTBs has a direct effect on the flexibility of a molecule. It is used to predict how compounds transverses the membrane. Therefore, it is a key determinant of the bioavailability of a molecule via the oral route (Craciun *et al.*, 2015). Based on the obtained results, the average RTBs for CDs were significantly higher ($p > 0.05$) than the A and SA categories of AMNPs (Fig. 4.6a). The NA however, showed no difference ($p > 0.05$) from the

CDs. Similarly, there was no significant difference (P > 0.05) observed between the three categories of AMNPs.

An RTBs value, not more than 10 has been reported as one of the selection criteria for determining oral bioavailability (Craciun *et al.*, 2015). This study reveals that 70 % of the CDs, and more than 90 % of AMNPs were found within this limit (Fig. 4.6b).

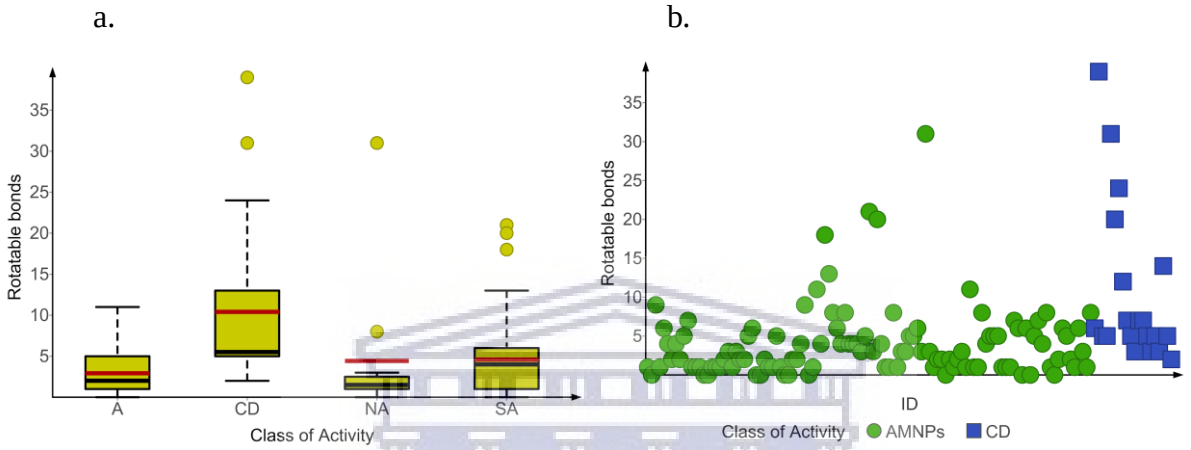


Figure 4.6. Distribution of RTBs for both AMNPs and CDs. Figure 4.6a displays the box plots of RTBs for CDs and the categories of AMNPs. The NA showed no statistical difference (p > 0.05) from the CDs. Figure 4.6b is a scatter plot showing the RTBs of AMNPs (green markers) and the CDs (blue markers).

Therefore, most of the AMNPs could have a higher chance of good absorption. The estimated average value for both datasets (AMNPs = 4 and CD = 10.4) is consistent with a study by Chen *et al.* (2018). They reported that the mean of RTBs for readily obtainable NPs is smaller than approved drugs. Similarly, this study also corroborates the findings of Bade *et al.* (2010) who observed that more than 70 % of NPs have RTBs below the established value.

4.1.2 Profiling drug-likeness of AMNPs

4.1.2.1 Prediction of absorption and distribution based on drug-likeness rules

Some predictive models have been developed to efficiently evaluate the drug-likeness of a molecule (Di and Kerns, 2015). These rules provide guidelines for the early identification of compounds with an increased chance of high oral absorption. In this study, the predictive rules of Lipinski, Veber, and Egan were employed to investigate the consistency of drug-likeness among AMNPs with known anti-MRSA drugs (CDs).

Lipinski's rule describes molecules with MW < 500, clogP < 5, HBDs < 5, and HBAs < 10 as more likely to have prospects for good oral absorption and permeation (Lipinski, 2004; Loureiro *et al.*, 2019). Figure 4.7 displays the results for compliance of the datasets with this rule. Overall, it was observed that 55 % of both AMNPs and CDs passed the rule without any violation (Fig. 4.7). Similarly, for the AMNPs categories, 43 %, 68 %, and 83 % of SA, A, and NA, respectively, obeyed the rule of five (Fig. 4.7).

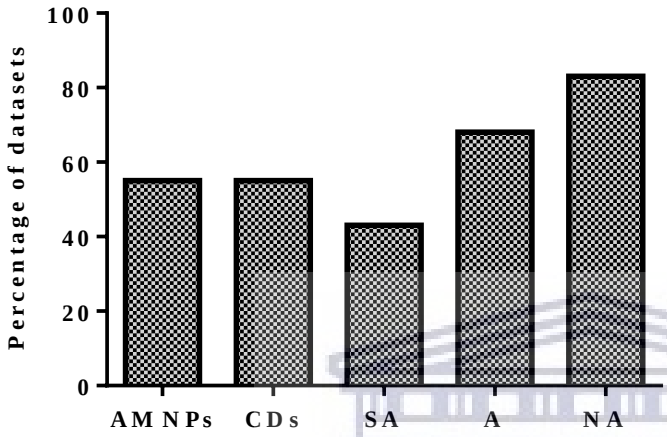


Figure 4.7. Histograms of Lipinski's rule compliance for both AMNPs and CDs. Up to 55 % of both AMNPs and CD complied with the rule without any violation. About 43, 68, and 83 % of SA, A, and NA respectively also infringed none of the rules.

Lipinski mentioned that a compound that violates any one of the limits can still be considered drug-like (Craciun *et al.*, 2015). When the rule was relaxed to exceed any one of the cut off values, AMNPs (55 to 83 %) and CDs (55 to 60 %) passed this rule (Table 4.1).

Table 4.1. Lipinski's rule compliance based on the principle that drug-like compounds can break up to 1 of the rules.

Class of activity	MW relaxed	clogP relaxed	HBDs relaxed	HBAs relaxed
AMNPs (n = 111)	64	92	65	61
CD (n = 20)	12	11	12	11
SA (n = 61)	28	47	26	25
A (n = 38)	26	34	29	26
NA (n= 12)	10	11	10	10

Among the categories of AMNPs, almost all (83 to 91.7 %) of the NA molecules were within the space of this rule (Table 4.1). This can be an indication that the less active category of AMNPs (NA) has the highest prospect to become orally active drugs. In another way, it can be inferred from this result that bioactivity may not suggest drug-likeness. A similar distribution was reported for subgroups of NPs with activity against plasmodium in the literature (Egieyeh *et al.*, 2016). The observed compliance of CDs agrees with the study by Nazarbahjat *et al.* (2016) who reported that 30 % of FDA-approved drugs violate Lipinski's rule. Similarly, Zhang and Wilkinson (2007) reported that 20 % of all orally administered drugs failed at least one of the parameters of Lipinski's rule. Therefore, the yardstick employed by Lipinski may not measure the actual absorption or permeability of NPs and the derivatives (Lipinski, 2004; Loureiro *et al.*, 2019).

The obtained result for oral absorption based on Veber's rule is presented in Figure 4.8. Approximately, 85 % of AMNPs and 45 % of CDs were found within the space of the rule (RTB ≤ 10 and TPSA ≤ 140 Å^2).

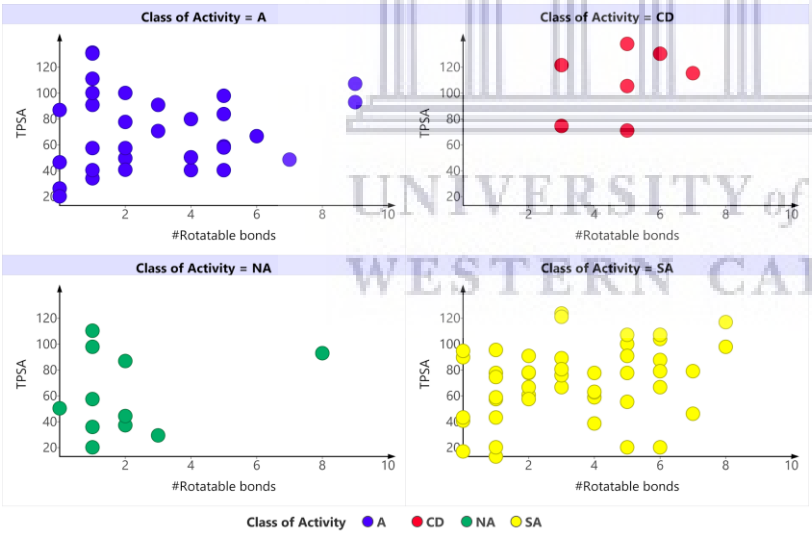


Figure 4.8. Prediction of oral absorption for both AMNPs and CDs based on Veber's rule. Each of the panels represents a class of activities (CDs and categories of AMNPs). More than 80 % of each of the AMNPs, and 45 % of CDs obeyed Veber's rule without any violation.

For the AMNPs categories, all compounds in the NA, except tannic acid (TPSA = 777.98 and RTB = 31) were found within the rule. Additionally, more than 80 % of both A and SA categories complied. Hence, there are greater chances for most of the AMNPs to achieve permeability at the Veber's limits.

The human intestinal absorption (HIA), permeation through the blood-brain barrier (BBB), inhibition of cytochrome P450 isozymes, and drug assessment for substrates of p-glycoproteins (p-gp) are crucial pharmacokinetic properties. They can be used in the early discovery process to determine the extent of intestinal absorption of a bioactive compound in humans (Dahlgren and Lennernäs, 2019; Daina and Zoete, 2016; Daina *et al.*, 2017; Nazarbahjat *et al.*, 2016). The result presented in Figure 4.9 revealed that more than 77 % of the AMNPs (compounds found within the white region) exhibited "high'' HIA.

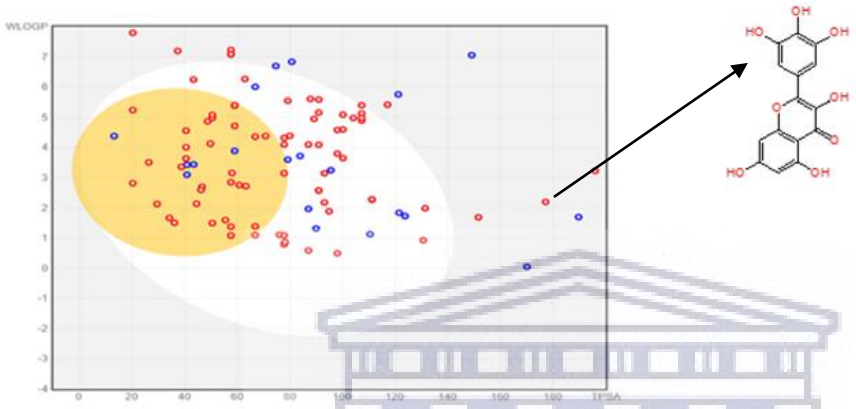


Figure 4.9. BOILED-Egg predictive model for absorption, and bioavailability of AMNPs. The markers (markers representing AMNPs) within the white region are occupied by molecules (77 % of AMNPs) that are most likely to be absorbed by the gastrointestinal tract and those within the yellow region are for molecules (30 % of AMNPs) that are likely to pass through the BBB. The blue and red markers respectively represent substrates and non-p-gp substrates.

The result further revealed that 80 % (red markers) of the AMNPs are non-p-gp substrates (i.e may not efflux from cells). Conversely, for the reference data (CDs), 45 % were predicted to have high HIA, and 25 % were predicted as non-p-gp substrates (**Appendix E**). One of the crucial screenings during the early stage of the drug discovery process is to check whether the biologically active molecules are substrates of p-gp. This is because p-gp functions to decrease cellular uptake, absorption, oral bioavailability, distribution, and retention time of drugs in the body through unidirectional lipid flippase pathway (Prachayasittikul and Prachayasittikul, 2016). The p-gp can limit the effective concentration of bioactive molecules at the desired cellular sites leading to the rapid development of resistance, especially for anti-infective compounds. Therefore, more than 80 % AMNPs (red markers) that were predicted as non-substrates of p-gp in this study could have prospects for well absorption and bioavailability. For the reference compounds, linezolid, tedizolid, and ciprofloxacin are few examples of these

compounds that are known for good absorption and bioavailability as contained in the drug bank (www.drugbank.ca). On the other hand, vancomycin, ceftaroline, and ampicillin among others, are also known for poor oral absorption (www.drugbank.ca). Therefore, the present study is consistent with the established pharmacokinetic profile of the CDs.

Furthermore, it was also observed that 30 % of the AMNPs (compounds found within the yellow region) can permeate the BBB (Figure 4.9). In contrast, none of the CDs showed a propensity for permeation of this barrier (**Appendix E**). The poor BBB permeability of the CDs could be as a result of their negative average clogP values. The BBB is a major hindrance in the development of drugs for the central nervous system (CNS) (Wen *et al.,* 2015). This has made the CNS infections that are caused by multidrug-resistant organisms such as multidrug-resistant gram-negative aerobic bacilli, MRSA, penicillin-resistant pneumococci, and other organisms continually result in serious health threat (Nau *et al.,* 2010; Wen *et al.,* 2015) Therefore, the AMNPs with predicted BBB in this study are desirable for these CNS infections.

4.1.2.2 Predicted metabolism of the AMNPs and identification of their metabolites

Orally administered drugs are prone to extensive biotransformation in the liver such that their bioavailability and efficacy are extremely reduced (Mannan and Unnisa, 2019). Therefore, the propensity for the metabolism of AMNPs by phase 1 and phase 2 enzymes was evaluated. The results summarised in Table 4.2 revealed that 59 and 71 % of AMNPs are likely to be metabolized by phase 1 and 2 enzymes respectively, while more than 50 % of AMNPs may be metabolized by both phase 1 and phase 2 enzymes. A total of 20 % AMNPs are not likely to be biotransformed by both phase 1 and phase 2 enzymes.

Table 4.2. Prediction of phase 1 and phase 2 biotransformation of AMNPs

Phase of metabolism	No of AMNPs that formed or without metabolites (n = 111)
Phase 1	66
Phase 2	79
Both phase 1 and 2	56
Without metabolites	22

The obtained result for phase 1 reveals that 60 % of the AMNPs may suffer the first-pass biotransformation while passing through the liver. This could consequentially reduce the

bioavailability of these compounds before reaching their targets. In contrast, the result for phase 2 shows that 71 % of AMNPs may produce metabolites at this stage, implying that most of the AMNPs could be readily excreted out of the body. The structural representation of the biotransformation result for one of the AMNPs (juncuenin D) is presented in Figure 4.10 below. Juncuenin D was metabolized to produce 2 and 1 metabolites in phase 1 and phase 2, respectively.

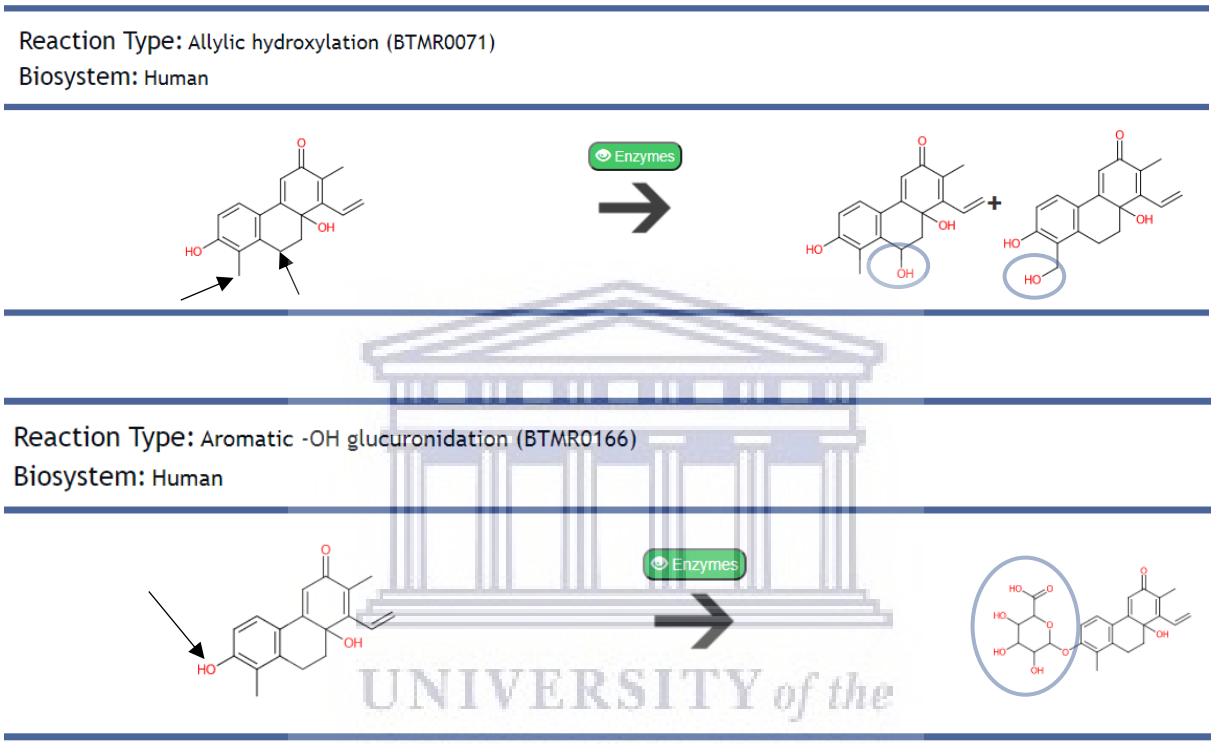


Figure 4.10. HTML document containing the results for biotransformation predicted for Juncuenin D. The first line illustrates the structure of the parent compound (left side of the reaction), the enzymes (Cytochrome P450) that may likely act on it, and the products of the reactions (right side of the reaction) during phase 1 metabolism. The second line illustrates the parent structure (left side of the reaction), the enzymes (UDP-glucuronosyltransferase) that may likely act on it, and the predicted metabolite (right side of the reaction) during phase 2. Each of the circles represents the points of transformation while the arrow points to the atom that is transformed.

It has been reported that metabolites may play significant roles in the pharmacology of the parent molecules. Metabolites can cause adverse effects or become active products (Zhang and Tang, 2018). Therefore, biologically active categories of AMNPs (SA and A) with pharmacologically active metabolites might be a prospect for prolonged action of these

compounds in the body. Similarly, active metabolites formed by less active categories of AMNPs (NA) may be considered further for advancement rather than the parent compounds. Hence, recognizing the pharmacological prospects of the AMNPs metabolite can be necessary to prevent their efficacy from being compromised at a later stage of drug development (Kang *et al.*, 2010; Zhang and Tang, 2018).

4.1.2.3 Predicted CYP450 inhibitory potential of the AMNPs

Potent inhibitors of CYP450 isozymes are not desirable in drug discovery as it may result in drug-drug interactions (Sychev *et al.*, 2018). The results from this study revealed that between 16 and 43 % of the AMNPs returned " YES'' for inhibition of one or more of the isozymes (Fig. 4.11a). A closer look at the different categories of AMNPs revealed that 36, 21, and 50 % of the SA, A, and NA respectively, might not inhibit any of the isozymes (Fig. 4.11b).

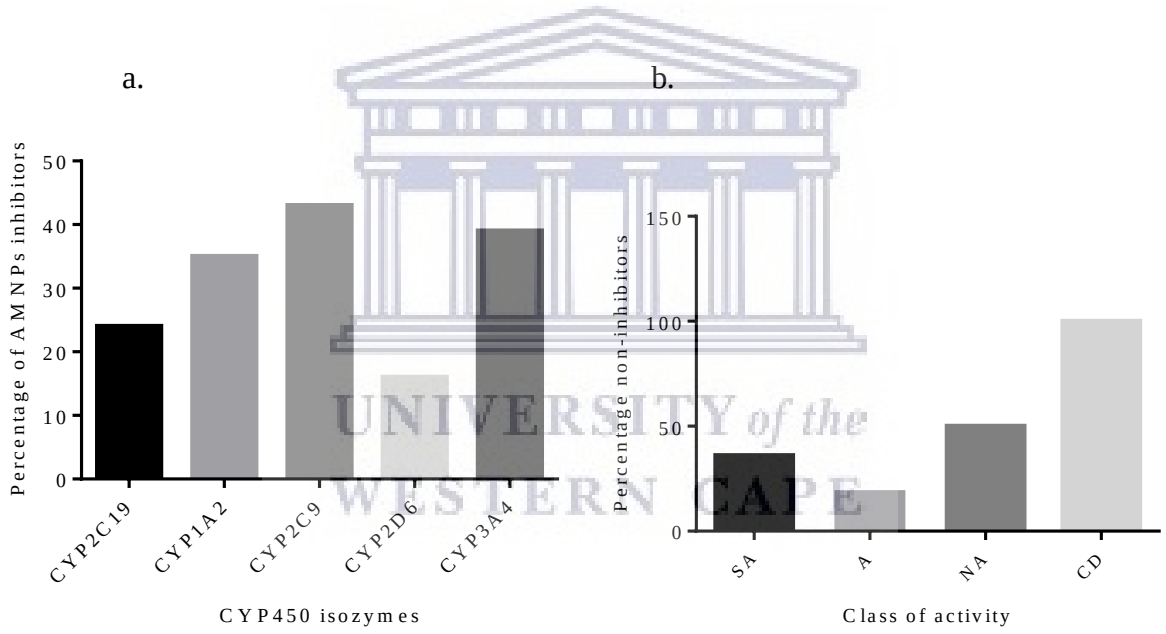


Figure 4.11. The percentage of inhibitors and non-inhibitors of CYP450 isozymes. Figure 4.11a shows the percentage of the AMNPs that are inhibitors of one or more CYP450 isozymes. Figure 4.11b shows the percentage of CDs and AMNPs categories that are non-inhibitors of any CYP450 isozymes.

Similarly, none of the CDs showed a tendency to inhibit any of the CYP450 isozymes (Fig. 4.11b). Among the various CDs, chloramphenicol has been established to have no inhibitory effect on CYP450 isozymes (Živković *et al.*, 2019). Inhibition of CYP450 isozymes has led to the market withdrawal of many drugs, causing loss of valuable time, resources as well as drug lag (Kumar *et al.*, 2012). Therefore, *in silico* prediction of CYP450 enzyme inhibition potential

for AMNPs at the early stage of drug discovery is desirable. This can help to prevent the colossal waste that may come from the withdrawal of drug candidates at a later stage of development. Hence, the AMNPs predicted to inhibit CYP450 enzymes in this study should be given less consideration during hit selection irrespective of their bioactivity.

4.1.2.4 Toxicity profiling of AMNPs

The prediction of the toxicity of potential drug candidates is one of the important components of modern drug discovery. In this study, the toxicity of AMNPs was predicted by OSIRIS DataWarrior software. The result revealed that 59 % AMNPs and 80 % CDs may likely have negligible or no mutagenic, tumorigenic, reproductive, and irritant effects (**Appendix F**). For the categories of AMNPs, at least 50, 66, and 60 % of NA, A, and SA respectively returned "none'' for all the toxicological parameters (Table 4.3).

Table 4.3. Estimated toxicological properties of AMNPs and CDs

Class of activity	Mutagenicity	Tumorigenicity	Reproductive effects	Irritant effects
SA (n = 61)	53	55	43	52
A (n = 38)	33	35	32	34
NA (n = 12)	6	9	8	9
CD (n = 20)	18	19	17	18

This observation is consistent with the toxicity assessment conducted by Kumar *et al.* (2017). They reported that about 57 % of the compounds identified from a 3D-QSAR study showed none for tumorigenic, mutagenic, irritant, and reproductive risks. Similarly, chloramphenicol (DB224) was one of the CDs that showed high toxicity risk for all these toxicological properties. This observation builds on existing evidence that chloramphenicol is likely to have all the four risks (Živković *et al.,* 2019). Toxicity has been identified as the cause of attrition of approximately 33 % of drug candidates especially at the late stage of drug development. However, chemical manipulation through the replacement or removal of functional groups during the optimization stage can avert this major issue (Xiao *et al.,* 2016). Therefore, early identification of potentially toxic chemotypes can help to circumvent safety liabilities (Kramer *et al.,* 2007).

4.1.2.5 Synthetic accessibility score

It is assumed that molecular fragments that frequently occur among easily obtainable compounds would be synthesized easily (Daina *et al.*, 2017). Hence, the synthetic accessibility value of AMNPs was predicted to know their ease of being produced. The result with the value from 1 (easy to synthesize) to 10 (not easy to synthesize) is presented below (Fig.4.12).

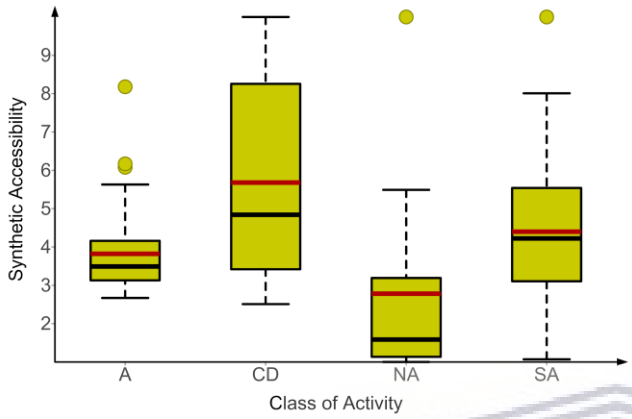


Figure 4.12. Box plots of synthetic accessibility against class of activity. The statistical mean and median of each distribution are represented by red and black lines, respectively. The mean value of both A and NA subgroups of AMNPs were both significantly ($p < 0.05$) lower than CDs.

It was observed that only the most active categories of the AMNP (SA) may be similar ($p > 0.05$) to CDs (Fig. 4.12). Similarly, there was no difference ($p > 0.05$) between the average synthetic accessibility score of the AMNPs categories. Overall, about 77 % of the AMNPs were observed to have synthetic accessibility values below 5. This can be an indication that most of the AMNPs have frequently occurring chemical moieties than CDs.

4.1.3 Hit-prioritization of the AMNPs

A "drug-likeness profile" provides a qualitative visualization of the various predicted "drug-like" properties and parameters (Bickerton *et al.*, 2012; Manallack *et al.*, 2013; Mignani *et al.*, 2018; Yusof and Segall, 2013). This visualization allows for the prioritization of compounds with desirable or ideal drug-likeness. Secondly, it highlights properties and/or parameters that need to be optimized to get the compounds with the desired drug-likeness profile. In another way, a balanced prioritization strategy that considers the physicochemical and pharmacokinetic attributes of bioactive compounds is important for early identification of molecules with the prospect to truly become drug candidates (Bickerton *et al.*, 2012; Lobell *et al.*, 2006; Mignani *et al.*, 2018; Wunberg *et al.*, 2006).

In this study, the probability scoring function described in Segall (2014) was used to combine the desirability of key ADMET and physicochemical properties of each of the AMNPs into a single number, ranging between 0 and 1. These properties include MW, TPSA, HBA, HBD, logP, RTBs, aromatic rings, and the toxicity risks of the compounds. A score of 1 in this context describes any compound that has all the physicochemical and pharmacokinetic properties within the ideal drug-like profile. On the other hand, a score of 0 represents compounds with undesired properties. Those compounds with a desirability score close to 1 may have prospects for success during the preclinical drug discovery stage and might be prioritized for further development.

The comprehensive prioritized list of the AMNPs is shown in **Appendix G**. Based on their desirability score, the distribution of the prioritized list for the three categories of AMNPs is described in Figure 4.13a.

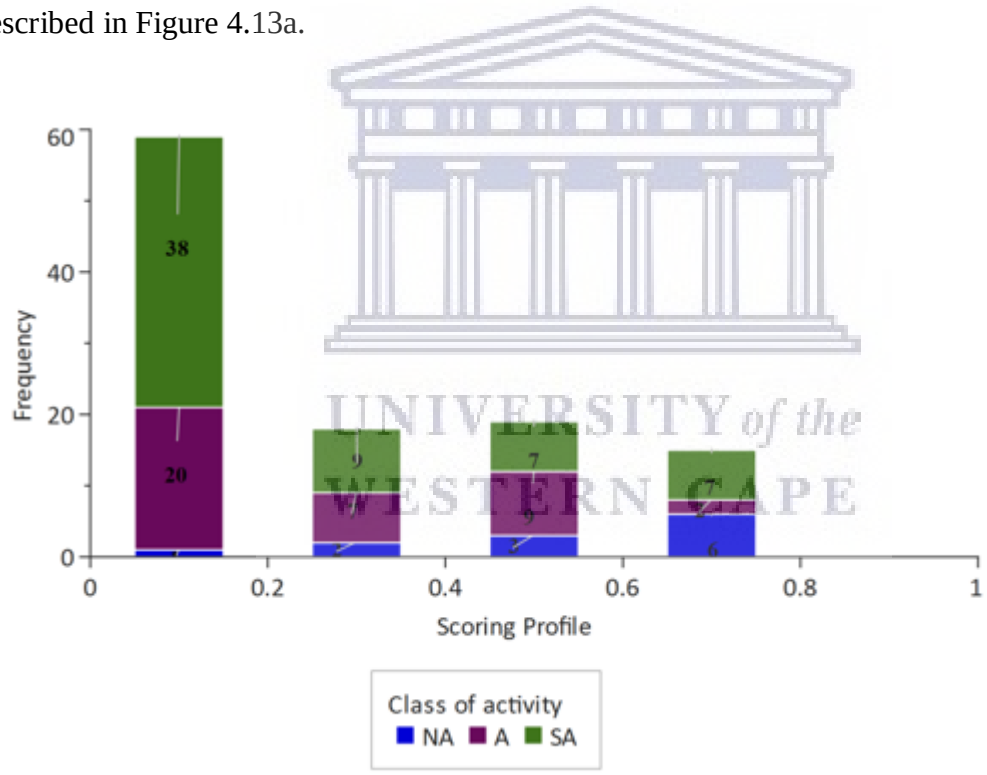


Figure 4.13a. Histogram showing the distribution of AMNPs for prioritization. The numbers of AMNPs were plotted against the overall desirability score. The bars were coloured by the class of activity. The score for each compound suggests the likelihood of the compound to achieve the ideal property criteria.

Most of the compounds in the active categories of AMNPs (SA and A) have desirability scores distributed closer to 0 compared to the less active (NA categories) which were found closer to

1. This can be an indication that most of the NA compounds are more likely to become successful in the discovery and development pipelines. Further analysis of the level of confidence in this score revealed that the best 40 (36 %) of the AMNPs are not significantly different from one another (Fig. 4.13b).

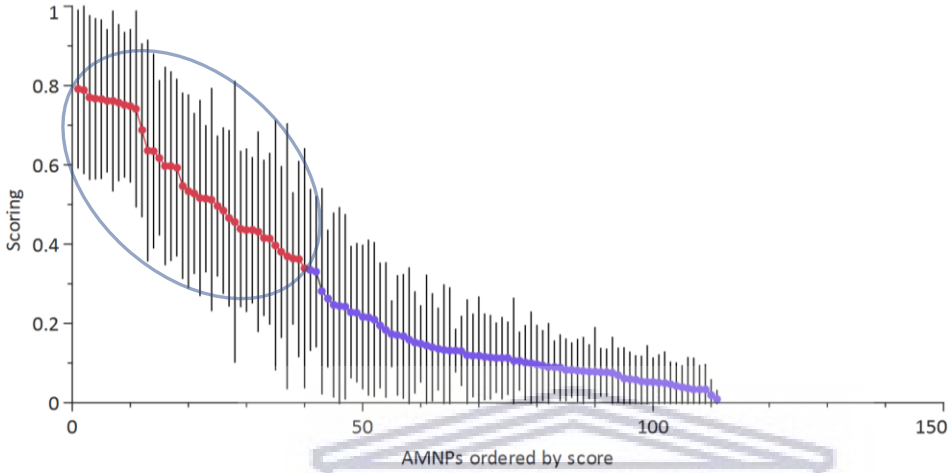


Figure 4.13b. Snake plot showing the statistical distribution of the scoring function. The plots on the X-axis describe each of the AMNPs ordered from left to right by score while the Y-axis presents the overall score for the individual compound. The AMNPs indicated with red markers are those whose error bars revealed no statistical difference from the best scoring compound.

Interestingly, 10 out of the 12 compounds in the NA category were among these compounds, implying that there might be a need to only optimize for potency in this group of compounds. On the contrary, the few compounds that showed a score that is close to 1 among the active categories (especially SA) may have more prospects for success during the preclinical drug discovery stage. However, compounds among the SA or A categories that revealed a low score can be optimized to increase drug-likeness order than potency. This result is consistent with a previous study by Egieyeh *et al.* (2016). They reported a high and low average desirability score respectively, for the less active and active categories of antimalarial NPs (Egieyeh *et al.*, 2016). Wunberg *et al.* (2006) conducted a data-driven screening of hits for drug-like and lead-like. They established that the most promising molecules are those having good potency, and with liabilities that can easily be addressed. Therefore, some of the compounds with a poor balance of properties in the active categories might be a promising starting point for discovery and design since most of them have room for optimization. Nevertheless, the identified liabilities of some of the AMNPs might vary in severity, and thus complicating the optimization process.

4.1.4 Desirability scoring function allows *in silico* hit-optimization strategies

The goal of preclinical drug discovery is to maintain desirable properties with sufficient safety as well as to improve on the identified liabilities in the lead compounds (Hughes *et al.*, 2011). Therefore, molecules with deficiencies in their physicochemical, pharmacokinetic, or toxicological properties can be modified structurally for improvement (Harrold and Zavod, 2013). In this study, two compounds from the SA categories identified with liabilities were optimized for a good balance of properties. The 2-dimensional structure of these compounds was drawn and modified using Chemdraw software (version 12.0) and their molecular descriptors were estimated using the MOE program (2019.01). The details of the selected compounds and the results are described below.

Cryptotanshinon (DB196) is one of the AMNPs with a good potency (0.5 µg/mL) but low desirability score of (0.1589). Poor permeability, inhibitory effects on drug metabolic enzymes (CYP3A4, CYP2C9, CYP2C19, and CYP1A2), and high reproductive risk were some of the liabilities identified with this compound. These red flags were improved by changing some of its atoms or functional groups (Fig. 4.14a).

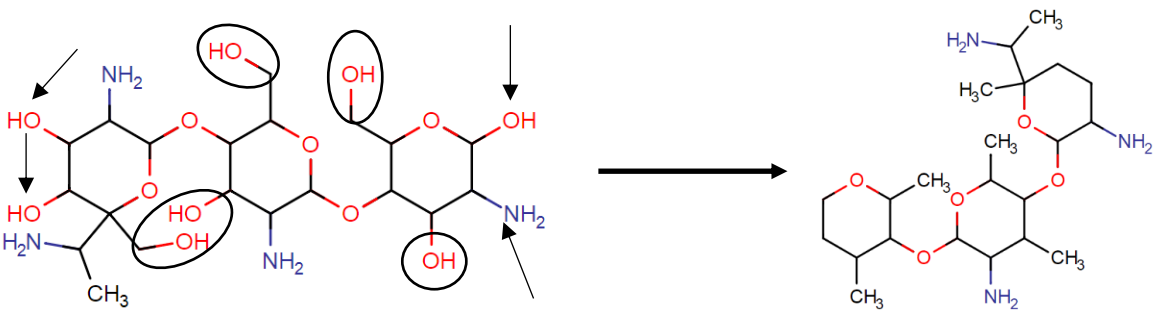
Compound DB196 Compound ANA196

Figure 4. 14a. Optimization of cryptotanshinon evolved the compound ANA196. The round circles highlight the atoms (CH_3) that were replaced in DB196 with polar functional groups (OH and OH) during optimization.

The product of this modification is compound ANA196 which exhibited a well-improved physicochemical property, and a desirability score (0.3427) not statistically different from the best scoring compound (**Appendix H**).

Similarly, aminoethyl-chitosan (DB211) is another compound that showed good potency (0.5 µg/mL) but one of the lowest desirability scores (0.04693). The poor pharmacokinetics and drug-likeness properties of this molecule were, however, improved by removal or replacement of some of its polar functional groups (OH and NH_2). The details of this process are illustrated

in Fig. 4.14b. The desirability score of the new compound; ANA211 (0.667) showed an improvement and with no significant difference from the best scoring AMNPs (**Appendix H**).



Compound DB211 Compound ANA211

Figure 4.14b. Optimization of DB211 (aminoethyl-chitosan) produced compound ANA211. The round circles highlight the atoms that were replaced with non-polar functional group (CH_3), and the arrows are pointing to the atoms that were removed during optimization.

In addition to their well-improved property, each of the two analogues revealed improved chemical accessibility and safety. A search through the chemical database further revealed no result for both compounds, implying that they might be representing novel chemotypes.

The quest for minimizing high attrition rates at the latter stages of drug development has necessitated the need to balance the efficacy of hit molecules with pharmacokinetic and toxicological properties through optimization (Joubert *et al.*, 2017; Lipinski, 2003; Miao *et al.*, 2019). The characterization of the drug-likeness profiling of AMNPs has allowed for the design of multi-parameter hit-optimization strategies for compounds with undesired properties. This process is essential for building up activity against undesired effects, and at the same time, keeping the physicochemical properties in the drug-like range (Xiao *et al.*, 2016). However, the bioactivity of the novel compounds obtained in this study was not assessed. Structural-activity relationship (SAR) or activity cliff could reveal which functional group is required for such bioactivity. Therefore, optimization should be done with a knowledge of SAR.

4.1.5 Exploration of the molecular similarity/diversity within the AMNPs

To visualize the chemical space occupied by the ANMPs relative to CDs, principal component analysis (PCA) was conducted on the structural and physicochemical properties of the datasets. The PCA is a statistical approach to visualize molecular similarity/diversity within a molecule set (Begam and Kumar, 2014). It allows for the visualization of multidimensional data on two

or three-dimensional plots with reduced loss of information (Rosén *et al.*, 2009; Stratton *et al.*, 2015). The result of the PCA analysis for ANMPs relative to CDs in this study is shown in Figure 4.15.

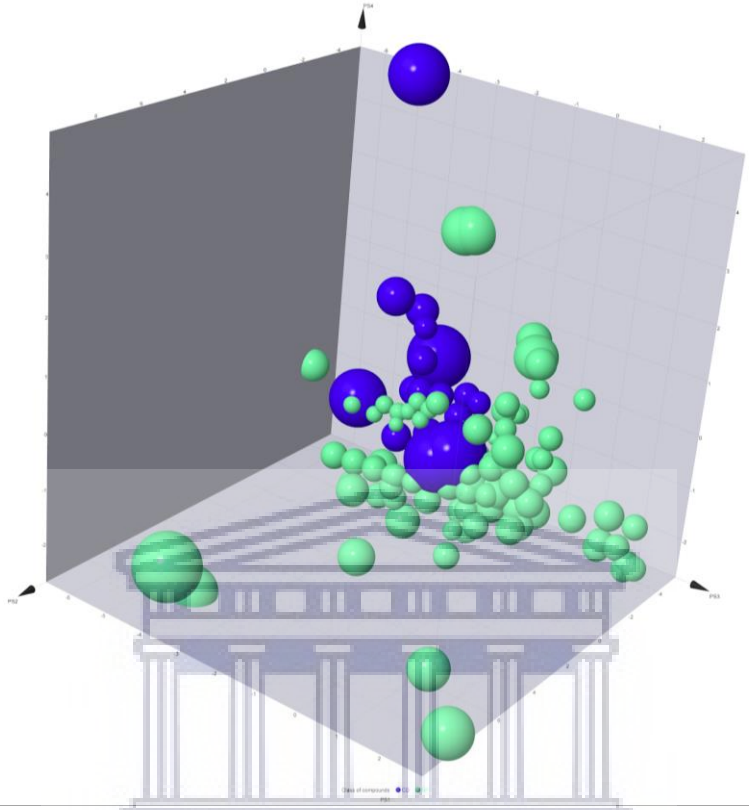


Figure 4.15. Distribution of AMNPs and CDs in chemical space. Green and blue markers represent AMNPs and CDs, respectively. The coordinates were generated from ChemGPS-NP with dimensions PS1, PS2, PS3, and PS4 for size (of the marker), aromaticity, lipophilicity, and flexibility of the compounds, respectively. The AMNPs showed higher diversity in comparison with the CDs.

The first four components of the ChemGPS-NP Web map are interpreted as size, aromaticity, lipophilicity, and flexibility for PS1, PS2, PS3, and PS4, respectively. The X, Y, and Z axes of the 3-dimensional plots are respectively PS2, PS3, and PS4 while PS1 represents the size of the markers. Although CDs are distributed across the chemical space, the AMNPs showed a higher diversity (Fig. 4.15). Furthermore, the plot displayed that CDs are bigger than AMNPs (PS1) while a closer look at the PS2 revealed that AMNPs are more aromatic than CDs. Similarly, the plot on the Y-axis reveals higher lipophilicity in favour of AMNPs while the diversity of AMNPs tends towards less flexibility (PS4) than CDs. In respect to bioactivity, the significantly active category of the AMNPs (SA) was the most diverse. Stratton *et al.* (2015)

used PCA to compare the chemical diversity of NPs and synthetic drugs that were approved between 1981 to 2010. They reported that NPs revealed larger diversity in chemical space than approved drugs (Stratton *et al.*, 2015). Similarly, Calixto (2019) studied the role of NPs in modern drug discovery and reported that the chemical diversity of these compounds are unmatched by their synthetic counterparts. These reports are in agreement with the present study, which confirmed that NPs are more structurally diverse than CDs.

The AMNPs that are highly far apart from the reference compounds (CDs) may indicate novel mechanisms of action. In the light that most CDs are now known to be ineffective in the treatment of MRSA infections, those AMNPs with prospects for novel biological targets are highly desirable for anti-MRSA drug development. Nevertheless, the AMNPs situated close to CDs in the chemical space may indicate similar pharmacokinetic or drug-likeness prospects. Hence, some of the AMNPs may have satisfactory pharmacokinetic properties and thus pass through preclinical screening for anti-MRSA drug development. The concepts of diversity and similarity of molecules are widely used in quantitative methods for designing and selecting a representative set of molecules (Egieyeh *et al.*, 2016; Rosén *et al.*, 2009). This is also important for analyzing the relationship between chemical structure and biological activity (Rosén *et al.*, 2009). Therefore, the relationship between the chemical structure and biological activity of the AMNPs was further explored in this study.

4.1.6 Structural-activity relationship (SAR) landscape

The analysis of structure-activity relationships (SAR) is one of the fundamental tasks in medicinal chemistry. The underlying goal of exploration of the SAR landscape is to identify structural differences between molecules in a large dataset that lead to differences in their bioactivities (Stumpfe *et al.*, 2020). Therefore, given a pair of structurally similar molecules that showed activity cliff, structural-activity landscape index (SALI) calculates how much potency is gained or lost while Delta Activity represents the difference between the activity of a pair of similar molecules (Egieyeh *et al.*, 2016). The results of the exploration of SAR for the AMNPs are summarised in Figure 4.16.

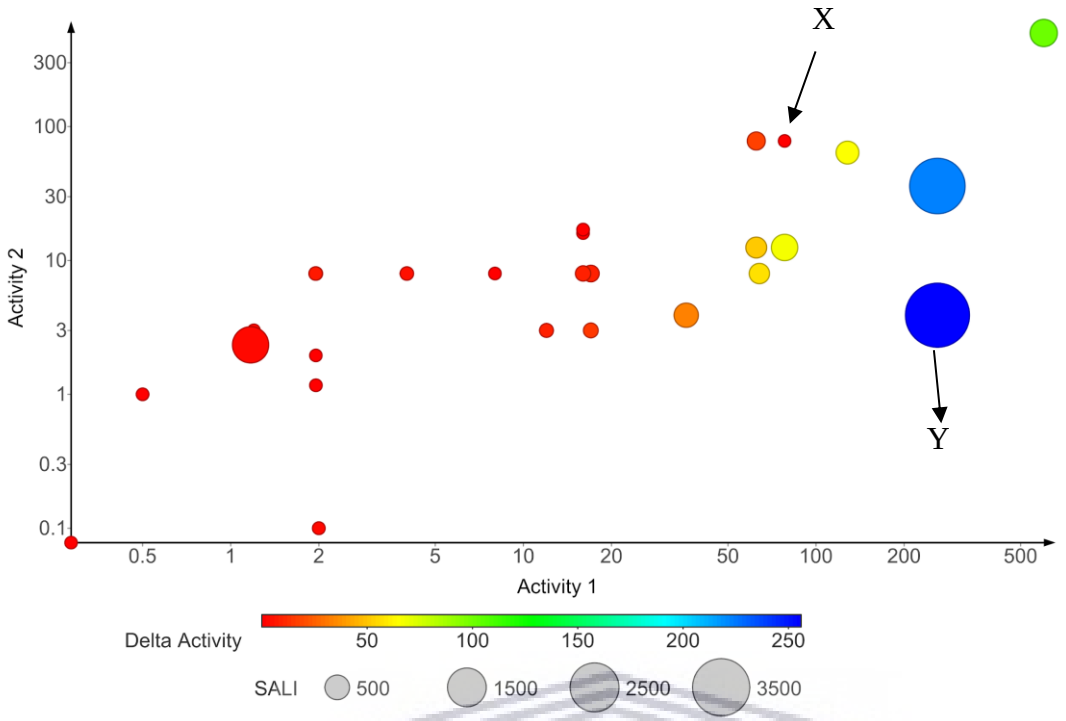


Figure 4.16. Scatter plot of the structural-activity relationship landscape of AMNPs. Markers are coloured by Delta Activity (the difference between bioactivity) and sized by SALI (structural-activity landscape index). Red markers represent smooth regions of the SAR landscape. Blue, green, and yellow markers represent compounds that exhibited an activity cliff.

Red coloured markers represent pairs of similar compounds with little or no difference in bioactivity (see example in Fig. 4.17a). This is often referred to as the smooth region (X) of the SAR landscape.

i. ii.

MIC = 78.1 µg/mL MIC = 78.1 µg/mL

Figure 4.17a. A pair of similar AMNPs (X) with little or no difference in bioactivity. The addition of hydroxyl (the small circle) in structure (ii) to the 1,4-benzopyron ring structure (i) did not cause any changes in bioactivity (MIC values). Hence, the two structures have about 87 % structural similarity and the same bioactivity (Delta Activity = 0 µg/mL).

Markers with blue, green, and yellow colours signify a pair of similar molecules with widely different bioactivity. These regions (e.g Y) are often called activity cliff of the SAR landscape (see example in Fig. 4.17b).

i. ii.

MIC = 260 µg/mL MIC = 3.9 µg/mL

Figure 4.17b. A pair of AMNPs (Y) showing an activity cliff. The two structures have about 94 % similarity but are very widely different in bioactivity (Delta Activity = 256.1µg/mL). An aldehyde group in structure (i) (the small circle) replaced by a methyl group in structure (ii) (the small circle) caused a large difference in bioactivity.

The concept of activity cliff is significant in identifying small structural modifications associated with large changes in potency (Kang *et al.*, 2010; Stumpfe *et al.*, 2020). Therefore, those AMNPs identified with blue, green, and yellow colour (Figure 4.16) provide insight into potential functional groups in the AMNPs that can modulate bioactivities. On the other hand, the smooth region of the SAR landscape contains compounds that are good input data to build QSAR models (Ekins *et al.*, 2007). The predicted SAR between the AMNPs is provided in **Appendix I**.

4.2 Phase 2- Target prediction of the AMNPs

This phase was conducted to assess the binding affinity of AMNPs within the sites of MRSA proteins and to identify potential inhibitors using reverse molecular docking. Reverse molecular docking is a computational technique that is aimed at identifying the unknown target(s) of a bioactive compound. It involves docking of a ligand to a set of drug targets (Huang *et al.*, 2018; Kulkarni *et al.*, 2020). Docking and scoring can be used during the early stage of drug discovery to screen a library of active compounds for the identification of possible drug candidates against a given protein target (Huang *et al.*, 2018; Kulkarni *et al.*, 2020; Park and Cho, 2017). Vital components of bacterial cells can be explored as potential targets in the discovery of antimicrobial agents. Few of these targets are associated with cell wall

biosynthesis, biofilms formation, DNA replication, fatty acid, protein synthesis, etc (Bandyopadhyay and Muthuirulan, 2018; Hooper, 2001; Khameneh *et al.*, 2019). The AMNPs were docked against each of the 34 MRSA targets that perform any of these functions in the bacteria. A comprehensive list of binding affinity for the AMNPs-target interactions obtained in this phase is presented in **Appendix J**. The summary of the observed affinity is presented below.

4.2.1 Inhibitors of the cell wall and membrane synthesis targets

The cell wall synthesis pathway is a common pharmacological target for the development of new antibiotics. This is because the inhibition of this target can lead to cell lysis and death (Mahasenan *et al.*, 2017). Methicillin-resistant *S. aureus* is, however, resistant to most of the classes of antibiotics that are linked to the disruption of this mechanism. Therefore, there is a need for new classes of compounds with the ability to circumvent their resistance pathways. In this study, eleven (11) proteins among the metabolic pathways used by *S. aureus* for cell wall biosynthesis were investigated for their potential inhibitors among the AMNPs (Table 4.4).

Table 4.4. Summary of the AMNPs with a profound affinity for cell wall biosynthesis pathway, as measured by binding energy (BE).

PDB	Nos of ligands with strong BE (≤ -6 kcal/mol)	Comments (n = no of ligands)	Best ligand with their BE (kcal/mol)
4YWZ	96	Nil	DB184: 4-(((Z)-5-((4-((E)-3-(2-chlorophenyl)-3-oxoprop-1-en-1-yl) benzylidene)-2,4-dioxothiazolidin-3-yl) methyl) benzoic acid (-11.8)
1NG5	20	Nil	DB129: Atractylenolide I and DB163: Anthocyanin (-6.0)
4BL2	1	Nil	DB175 Punicalagin (-6.5)
3Q81	0	Generally low to mild affinity	DB185: Gancaonin G (-5.4) DB187: 8-(γ,γ-dimethylallyl)-wighteone (-5.4)
5M19	1	Nil	DB125: 12b-Hydroxy-des-D-garcigerrin A (-6.3)
1MWS	38	Nil	DB162: Bartericin A, and DB209: Acetyl-11-keto-b-boswellic acid (-7.0)
1HSK	70	27 showed BE ≤ -7	DB210: Celastrol (-8.5)
2OLV	85	55 showed BE ≤ -7	DB194: Rugulosin A (-9.8)
1ALQ	73	21 showed BE ≤ -7	DB210: Celastrol (-8.0)
1VQQ	19	2 showed BE ≤ -7	DB 194: Rugulosin A (-7.3)
3HUN	49	Nil	DB199: Hinokinin (-8.0)

About 86 % of the AMNPs (comprising DB101-136, 139-146, 148, 150-155, 159-168, 171,172, 174, 176-181, 183-189, 191-208, and 211) showed a significant affinity for the sensor protein kinase WalK receptor (4WYZ). The compound 4-(((Z)-5-((4-((E)-3-(2-chlorophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl) methyl (DB184) revealed the highest binding energy (BE) of -11.8 kcal/mol for this protein (Table 4.4). As a novel drug target in bacteria, inhibitors of sensor protein kinase WalK have been evaluated and described in the literature (Bem *et al.,* 2015). However, no structural similarity was observed between the already established inhibitors, and the promising anti-4WYZ identified in this study. Therefore, this can be an indication that some of the identified anti-4WYZ may be novel chemical classes with the ability to inhibit the enzyme through novel mechanisms of action.

Similarly, only atractylenolide I (DB129) and anthocyanin (DB163), both with BE of -6.0 kcal/mol showed a considerable affinity for the sortase A (1NG5) target. This result is consistent with previous studies where the activity of anthocyanin was reported to damage the integrity of the cytoplasmic membrane of *S. aureus* (Sivamaruthi *et al.,* 2018; Sun *et al.*, 2018). Additionally, punicalagin (DB175) with the BE of -6.8 kcal/mol was found to have the strongest molecular interaction with the 4BL2: β-lactam-inducible penicillin-binding protein (PBP2a). Except for the 4-benzoic acid (DB184) which also revealed a BE of -5.7 kcal/mol, all other AMNPs showed a weak to mild binding score for 4BL2, as revealed by their docking score (> -5). The high binding score observed of punicalagin agrees with Mun *et al.* (2018) who reported the therapeutic role of punicalagin as a potential mediator in the inhibition of PBP2a protein in *S. aureus.* Since most of the AMNPs showed a low affinity for the 4BL2, DB184 might also be a promising drug candidate against this target.

The target, regulatory protein blar1(3Q81) also showed a similar trend. The highest but mild affinity was exhibited by both gancaoninG (DB185) and 8-(γ,γ-dimethylallyl)-wighteone (DB187) for this protein. Both compounds had a docking score of -5.4 kcal/mol. This finding is consistent with a study by Hatano *et al.* (2005) who presumed that both DB185 and DB187 were targeting the cell membrane of *S. aureus*.

Furthermore, only 12b-hydroxy-des-D-garcigerrin A (DB125) with BE of -6.3 kcal/mol revealed a strong affinity for mecA (5M19) (Table 4.4). β-lactam antibiotics are designed against this target. The analysis of the structural similarity between β-lactam antibiotics and

DB125, however, revealed low relationships. Therefore, DB125 may have the potential to circumvent β-lactamase resistance through a different mechanism from the known antibiotics (Fuda *et al.*, 2005; Mahasenan *et al.*, 2017).

Another part of the results also revealed an energetically favourable interaction of the AMNPs with penicillin-binding proteins (PBPs). It was observed that DB113, 114, 122, 124, 135, 141, 159, 160, 162, 175, 183, 184, 186, 191, 194, 195, 200, 201, and 210 revealed high binding energy scores for the penicillin-binding protein 2a (1VQQ) (Figure 4.18). This observation was based on their docking scores which were found less than -6 kcal/mol. Rugulosin A (DB194; yellow) with BE of -7.3 kcal/mol, however, showed the highest affinity for this target (Fig 4.18).

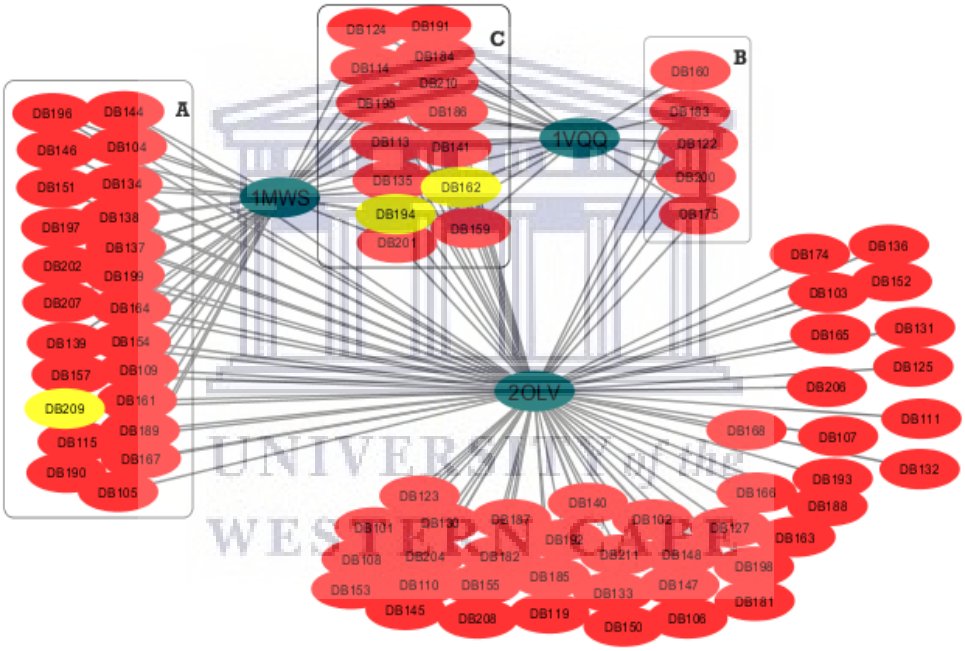


Figure 4.18. Visualization map showing the AMNPs with strong affinities (BE ≤ -6 kcal/mol) for PBPs proteins. The proteins (2OLV, 1VQQ, and 1MWS) are presented as colour green, yellow represents compounds with the strongest affinity for the proteins (DB194 for 1VQQ and 2OLV; D162 and DB209 for 1MWS). A represents AMNPs inhibitors of both 1MWS and 2OLV, B represents inhibitors of both 1VQQ and 2OLV while C represents inhibitors of the three enzymes.

Moreover, bartericin A (DB162) and acetyl-11-keto-b-boswellic acid (DB209) (yellow) with BE of -7.0 kcal/mol had the strongest binding affinity for mecA PBP2 (1MWS) (Fig. 4.18). Other compounds comprising DB104, 105, 109, 113-115, 124, 134, 135, 137-139, 141, 144,

146, 151, 154, 157, 159, 161, 164, 167, 184, 186, 189-191, 194-197, 199, 201, 202, 207, and 210) also showed promising affinity for this enzyme (≤ -6 kcal/mol). Similarly, rugulosin A (DB194) with BE of -9.8 kcal/mol exhibited the highest docking score for PBP2; penicillin-binding protein 2 (2OLV). Overall, more than 85 % of the AMNPs (such as DB101-111, 113-115, 119, 122-125, 127, 130-141, 144-148, 150-168, 174-175, 181-202, 204, and 206-211) demonstrated promising affinity for the target. This observation was based on their energetically favourable interaction with the 2OLV. Those compounds that revealed a high affinity for 1VQQ also showed a profound affinity for the other two targets (2OLV and 1MWS) (Fig. 4.18). Turk *et al.* (2011) conducted a screening for an in-house bank of compounds and identified 3-(Quinoline-8-sulfonamido) benzamide and 5-Bromo-2-(3-propoxybenzamido) benzoic acid as inhibitors of two PBPs enzymes. Lahiri and Alm (2016) also reported that ceftaroline can disrupt the pathways involving PBPs and PBP2a. These reports corroborate the present study. Therefore, it can be inferred that many of the AMNPs are multi-target compounds with the prospect of binding to related proteins (Gray and Wenzel, 2020).

Celastrol (DB210) exhibited profound molecular interactions with the UDP-N-acetylenolpyruvoylglucosamine reductase (1HSK) target (BE of -8.5 kcal/mol). Other compounds (DB101-107, 109-111, 113-115, 122, 124, 125, 129-141, 144, 146, 151, 153-155, 157, 159-162, 164-168, 174, 177, 182-184, 186-187, 189-197, 199-202, 204, and 207-209) also showed high docking score (BE ≤ -6 kcal/mol) for this protein (Fig. 4.19).

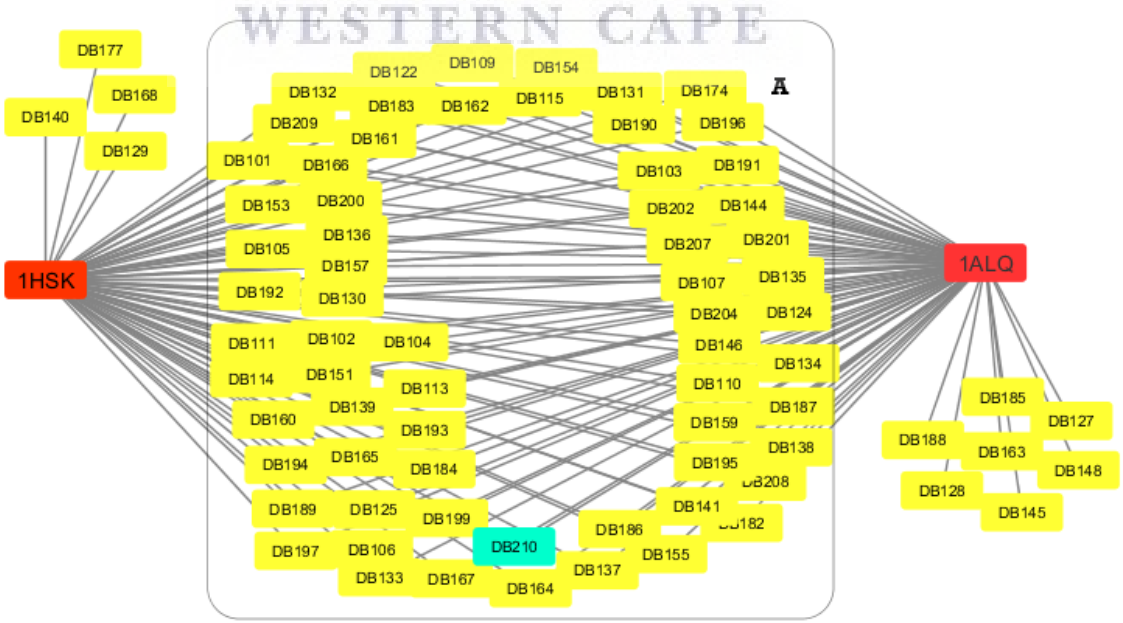


Figure 4.19. Visualization map showing the AMNPs with a strong affinity (BE ≤ -6 kcal/mol) for 1HSK and 1ALQ proteins. The proteins are presented as red, each of the yellow represents compounds with a profound affinity for the proteins. A represents AMNPs inhibitors of both proteins while green represents the strongest inhibitor of the two enzymes.

Similarly, celastrol (DB210) demonstrated the strongest affinity for the β-lactamase (1ALQ) by showing the highest docking score (-8.0 kcal/mol) (Fig. 4.19). The compounds DB101-107, 109-111, 113-115, 122, 124-125, 127-128, 130-139, 141, 144-146, 148, 151, 153-155, 157, 159-167, 174, 182-197, 199-202, 204, and 207-210 also showed BE below -6 kcal/mol for the target (Fig. 4.19). A closer look at the results of 1HSK and 1ALQ targets revealed that they almost have the same AMNPs inhibitors (Fig 4.19). This can be an indication that many of the AMNPs are multi-target compounds with the prospect of binding to related proteins (Gray and Wenzel, 2020). The role of DB210 in biofilm eradication has been established (Kim *et al.*, 2018; Woo *et al.*, 2016). Nevertheless, the observed significant binding affinity of this compound for the proteins involved in cell wall biosynthesis implies that celastrol could bind to proteins involved in independent cellular processes (Gray and Wenzel, 2020).

For the penicillin-binding protein 4 (PBP4); 3HUN, 44 % of AMNPs (DB103, 106-107, 111, 113-114, 116, 118-122, 124, 130, 135, 139, 141, 145, 151, 152, 159-162, 164-167, 176-181, 183, 184, 186, 187, 191, 192, 195, 197, 199, 200, 203, 205, 206, 208, 210) can potentially bind to this protein. This is based on their strong docking scores (BE ≤ -6 kcal/mol). These compounds, especially hinokinin (DB199) which had the BE of -8.0 kcal/mol might actively alter the role of penicillin-binding protein 4 (PBP4) in MRSA.

4.2.2 Inhibitors of growth and cell division related proteins

The mechanism of growth and cell division in bacteria play a pivotal role in cell survival. The proteins associated with this process have been widely studied as promising targets for various antimicrobial agents (Eswara *et al.*, 2018; Wagstaff *et al.*, 2017). In this study, 2 proteins in MRSA that are crucial for cell survival were studied for their possibility of being inhibited by the AMNPs. The results depicts that 19.8 % of the AMNPs comprising DB103, 109, 111, 135, 139, 145-146, 150-151, 159, 164, 166-167, 175, 184, 189, 191, 194-195, 199, 201, and 207 had high affinity (BE ≤ -6 kcal/mol) for 5MN4 (cell division protein ftsZ) (Fig. 4.20).

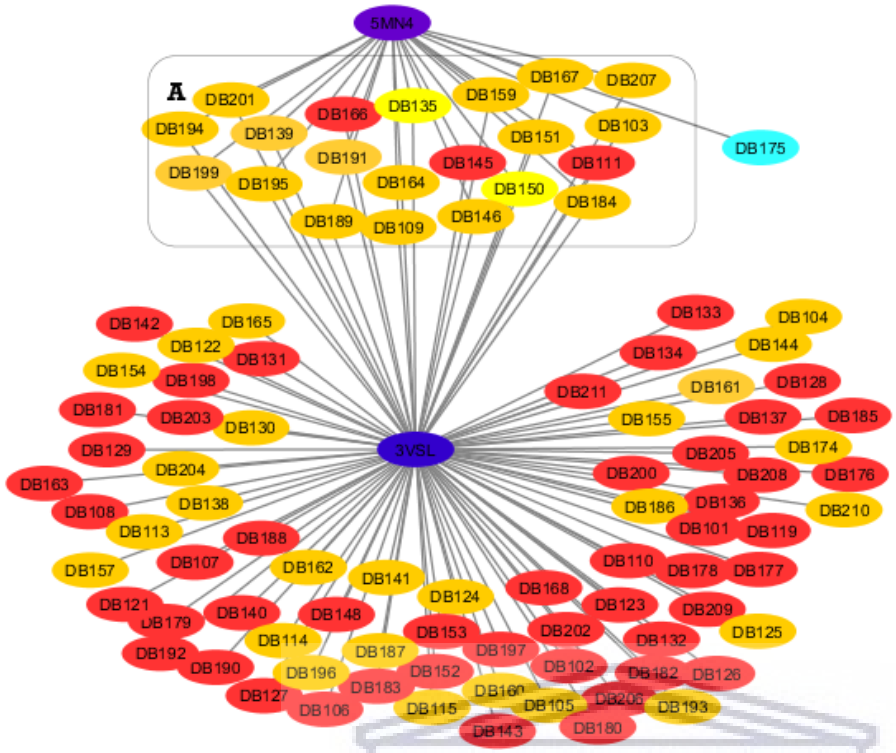


Figure 4.20. Visualization map showing the AMNPs with a profound affinity (BE ≤ -6 kcal/mol) for growth and cell division proteins. The proteins (5MN4 and 3VSL) are presented as blue markers, yellow represents compounds with the strongest affinity for the proteins (DB150 for 5MN4; D135 for 3VSL) while brown (including DB135) represents AMNPs with a stronger affinity for 3VSL than curcumin. The groups annotated with A represent AMNPs inhibitors of both proteins while the green marker represents the compound that showed a strong affinity for 5MN4 only.

Similarly, about 86 % of AMNPs comprising DB101-111, 113-115, 119, 121-146, 148, 150-155, 157, 159-168, 174, and 176-211 demonstrated BE below -6 kcal/mol for the 3VSL (penicillin-binding protein 3) target. Notably, all the compounds that showed a strong affinity for 5MN4 also revealed considerable BE for 3VSL, except DB175 (Fig. 4.20; green). Among these compounds, heyneanol A (DB150; yellow) exhibited the highest binding affinity (-7.1 kcal/mol) for the 5MN4 protein while sanguinarine (DB135; yellow) with the BE of -9.3 kcal/mol demonstrated the highest affinity for the 3VSL protein (Fig. 4.20). Curcumin (DB200) also had a docking score of -7.5 kcal/mol and a strong affinity for the protein. However, more than 40 other compounds showed a stronger affinity (< -7.5 kcal/mol) for 3VSL than DB200. This implies that more than 40 AMNPs could be prioritized in the search for new inhibitors of penicillin-binding protein 3 target.

The antibacterial activity of DB200 has been identified with the blockage of cell proliferation (Teow *et al.*, 2016). However, the observed BE of DB200 confirms that some of the AMNPs could potentially disrupt the pathway involving cell division and growth better than some of the already established molecules. In another study, heyneanol A sourced from the root of wild grape was reported to have an anti-infective mechanism identified with the inhibition of growth (Peng *et al.*, 2008). Similarly, the antimicrobial activity of sanguinarine (DB135) has also been linked to the disruption of the pathway leading to cell division (Beuria *et al.*, 2005; Obiang-Obounou *et al.*, 2011; Opperman *et al.*, 2016). Therefore, these reports are in agreement with the observations of this study.

4.2.3 Inhibitors of enzymes involved in protein biosynthesis

Protein biosynthesis plays a key role in the regulation of gene expression and it has been studied as an important pathway that aids bacteria cell survival (Bandyopadhyay and Muthuirulan, 2018). Therefore, it is a promising strategy to target protein biosynthesis in drug discovery (Bandyopadhyay and Muthuirulan, 2018). In this study, 5 different proteins (1NYR, 1FFY, 4QRE, 1KNY, and 1LM4) that are involved in protein biosynthesis in *S. aureus* were explored for the possibility of the AMNPs to inhibit their functions. The summary AMNPs that showed a strong affinity for these proteins is presented in Table 4.5.

Table 4.5. The summary of AMNPs that showed a promising affinity for protein synthesis, as measured by binding energy (BE).

PDB	Nos of ligands with BE ≤ -6	Comments (n = no of ligands)	Best ligand with their BE (kcal/mol)
1NYR	91	77 showed BE ≤ -7	DB150: Heyneanol A (-10.1)
4QRE	80	49 showed BE ≤ -7	DB199: Hinokinin (-9.0)
1KNY	16	Nil	DB131: Juncusol (-6.9)
1FFY	74	36 showed BE ≤ -7	DB150: Heyneanol A, and DB184: 4-(((Z)-5-((4-((E)-3-(2-chlorophenyl)-3-oxoprop-1-en-1-yl) benzylidene)-2,4- dioxothiazolidin-3-yl) methyl) benzoic acid (-8.2)
1LM4	12	Nil	DB164: Corylifol C (-7.3)

Although heyneanol A (DB150) demonstrated the highest affinity (BE of -10.1 kcal/mol) for threonine-tRNA ligase (1NYR), 82 % of the AMNPs (comprising DB101-115, 122-148, 151-168, 174-175, 181-202, 204-211) could potentially inhibit this protein. This observation was based on their docking scores which were found below -6 kcal/mol. The heyneanol A (DB150) and 4-(((Z)-5-((4-((E)-3-(2-chlorophenyl)-3-oxoprop-1-en-1-yl)benzylidene)-2,4-dioxothiazolidin-3-yl) methyl) benzoic acid (DB184) showed the strongest affinity for isoleucine-tRNA ligase (1FFY) as indicated by their docking score (-8.2 kcal/mol). Other compounds including DB101-102, 104-111, 113-115, 122, 124, 125, 127, 129-139, 141, 144-146, 148, 151, 153-155, 157, 159-167, 174, 175, 182-183, 185-187, 189-197, 199-202, 204, and 207-210 also exhibited strong binding affinity for this protein.

Another part of the results showed that most of the AMNPs showed favourable interaction with the methionine-tRNA ligase (4QRE). In this complex, the best binding affinity was demonstrated by hinokinin (DB199) and BE of -9.0 kcal/mol (Table 4.5). Other compounds such as DB101-114, 116, 119-125, 127-135, 139-146, 148, 151-153, 155, 159-168, 172, 174, 178, 181, 183-189, 191-193, 195-198, 200, 202-206, 208, and 211 also demonstrated strong binding affinities (BE ≤ -6 kcal/mol) for the protein. Additionally, the complexes formed by AMNPs and 1KNY showed that DB113, 114, 122, 131, 159, 160, 174, 183, 184, 192, 195, and 199-202 have a greater chance of targeting this enzyme as their major mechanism of destroying the MRSA. Juncusol (DB131) with a molecular docking score of -6.9 kcal/mol, however, exhibited the best affinity for this target.

Likewise, for the peptide deformylase (1LM4); DB101-102, 109-110, 114, 122, 161, 164, 167, 192, 199, and 204 were found to reveal a notable affinity for this enzyme. Among these compounds, protosappanin B (DB101) had a docking score of -6.2 kcal/mol. DB101 has been identified in a virtual screening study as one of the few potential inhibitors of peptide deformylase (Liang *et al.*, 2018). Nevertheless, 7 other molecules (Fig. 4.21) including corylifol C (DB164) revealed a higher docking score (< -6.2 kcal/mol) against 1LM4. This can be an indication that the compounds may be more valuable candidates against the 1LM4 target in MRSA.

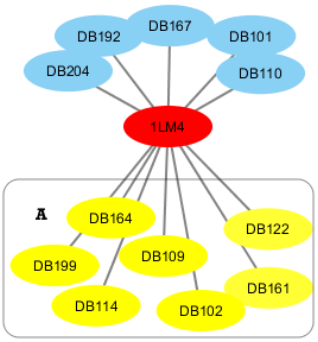


Figure 4.21. Visualization map showing the AMNPs with a profound affinity (BE ≤ -6 kcal/mol) for peptide deformylase. The protein (1LM4) is presented as a red marker. Each of the other markers represents compounds with a strong affinity for the 1LM4. A (yellow markers) represents AMNPs with a stronger affinity compared with protosappanin B (DB101).

Many of the AMNPs bind strongly to at least two proteins. Berberine (DB174) and sanguinarine (DB135) showed a strong affinity for most of the enzymes. It implies that both compounds could be potential antimicrobial agents with broad-spectrum activity. This observation corroborates the study of Khameneh *et al.* (2019) who described both DB135 and DB174 as potential antimicrobial agents with broad-spectrum activity. Similarly, protosappanin_A (DB102) and curcumin (DB200) demonstrated a high affinity for nearly all the proteins. The antimicrobial effect of curcumin has been identified with the disruption of protein synthesis (Teow and Ali, 2015). Therefore, this report is consistent with the predicted interaction of curcumin (DB200) in this study. However, the effects of some of the AMNPs (especially, DB131, 150, 164, 184, and 199) on protein synthesis machinery in *S aureus* has not been reported. Therefore, the strong affinity of these compounds for the different proteins (Table 4.5) strongly indicates that they could be prioritized in the search for new antimicrobial agents against MRSA. In addition, the AMNPs that showed a strong affinity for more than one protein might be an indication of their ability to destroy the pathogen through multi-target strategies (Gray and Wenzel, 2020).

4.2.4 Inhibitors of DNA biosynthesis proteins

Bacteria cells generally depend on DNA biosynthesis for their growth. Drug candidates that inhibit this target may result in the loss of cell viability (Bandyopadhyay and Muthuirulan, 2018; Dastidar *et al.*, 2000). In this study, 5 proteins (3E2I, 1EYA, 1RRI, 2INR, and 3JSL) used by *S. aureus* for the biosynthesis of DNA were investigated for their potential inhibitors

among the AMNPs. Figure 4.22 represents the AMNPs that demonstrated a profound interaction based on their binding affinities (BE ≤ -6 kcal/mol) for the proteins.

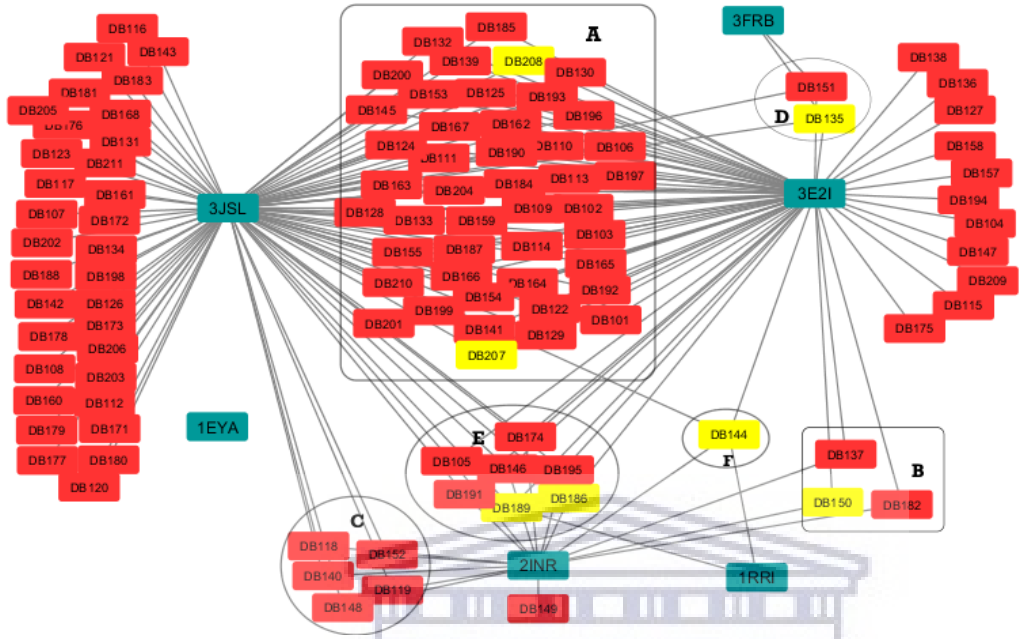


Figure 4.22. Visualization map showing the AMNPs with a strong affinity (BE ≤ -6 kcal/mol) for targets related to DNA synthesis. The proteins are presented as green markers, yellow represents compounds with the strongest affinity for the proteins (DB150 for 3E2I; DB144 and DB186 for 2INR, DB144 and DB189 for 1RRI; DB208 for 3JSL, and D135 for 3FRB). The groups annotated with: A represents AMNPs with a strong affinity for both 3JSL and 3E2I; B represents AMNPs with a strong affinity for both 3E2I and 2INR; C represents AMNPs with a strong affinity for both 2INR and 3JSL; D represents AMNPs with a strong affinity for 3JSL, 3E2I, and 3FRB; E represents AMNPs with a strong affinity for 3JSL, 3E2I, and 2INR while F represents AMNPs with a strong affinity for all the proteins except 1EYA. The absence of connections to the 1EYA node means that the AMNPs generally have BEs weaker than -6 kcal/mol.

Overall, 62 % of AMNPs showed a considerable affinity for the thymidine kinase (3E2I) target (Fig. 4.22). One of these compounds is heyneanol A (DB150) (Fig. 4.22; yellow marker) which demonstrated the highest BE (-9.5 kcal/mol) for this enzyme (Fig. 4.22). On the contrary, low to moderate binding affinity was revealed across the AMNPs for thermonuclease (1EYA). Ikarugamycin (DB207) with the BE of -5.6 kcal/mol showed the strongest affinity for this enzyme. The low binding affinity of AMNPs for 1EYA is further illustrated in Figure 4.22, where the node (1EYA) has no edge (AMNPs) connected to it. Similarly, only erybraedin A

(DB144) and glabridin (DB189) both with BE of -6 kcal/mol showed a promising affinity for the dihydroneopterin aldolase (1RRI) enzyme (Fig. 4.22).

The observed low affinity for these proteins implies that the reported *in vitro* activities of AMNPs may not be through interference with the mechanisms involving thermonuclease or dihydroneopterin aldolase in MRSA. On the contrary, few of the AMNPs (DB105, 118, 119, 137, 140, 146, 148-150, 152, 174, 182, 191, and 195) including erybraedin A (DB144) and 3-kievitone (DB186) both with BE of -6.4 kcal/mol showed a strong affinity (≤ -6 kcal/mol) for the DNA topoisomerase 4 subunit A enzyme (2INR) (Fig. 4.22). The result also revealed that 83 % of the compounds, comprising DB101-103, 105-114, 116-126, 128-135, 139-146, 148, 151-155, 159-168, 171-174, 176-181, 183-193, 195-208 and 210-211 showed profound molecular interactions with the protein target; 3JSL. One of these compounds, licoricidin (DB208) with BE -9.7 kcal/mol had the strongest affinity for the protein (Fig. 4.22; yellow marker). As shown in Figure 4.22, some of the AMNPs (A, B, C, D, E, F) may potentially inhibit at least two of these proteins. This can be an indication that many of the AMNPs are multi-target compounds. This could also imply that they may have the prospect of inhibiting the pathogen by binding to related proteins (Gray and Wenzel, 2020).

4.2.5 Inhibitors of fatty acid synthesis (FASII) proteins

The pathway of fatty acid synthesis (FASII) is essential for its role in bacteria cell membrane structure (Parsons and Rock, 2011). Therefore, the enzymes associated with this process have been prioritized in the present-day drug development against MRSA (Kénanian *et al.*, 2019; Parsons and Rock, 2011). In this study, the potentials of AMNPs to disrupt the activities of some of the proteins (1XPM, 4ALM, 4FS3, 3IM9) involved in FASII were explored. The result shows that about 96 % of the AMNPs were in the pocket of the 1XPM (HMG-CoA synthase), each exhibiting a strong binding affinity (BE ≤ -7.3 kcal/mol). One of these compounds is erythrabyssin II (DB139) which demonstrated the overall strongest binding affinity (BE = -12.1 kcal/mol) for 1XPM (Fig. 4.23).

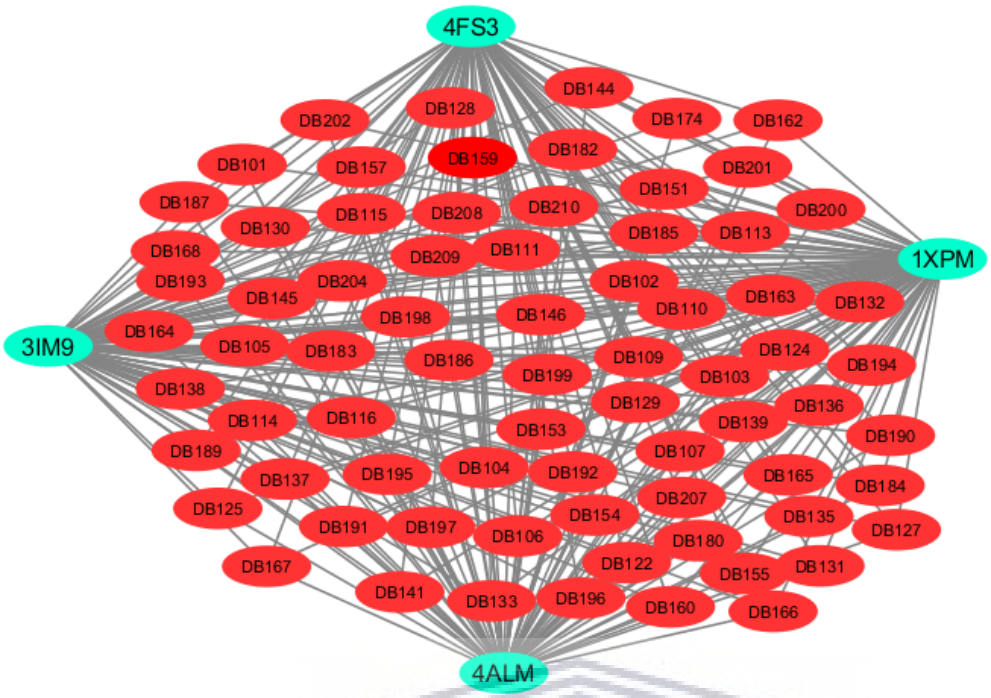


Figure 4.23. Visualization map showing multi-target inhibitors (BE ≤ -6 kcal/mol) of FASII proteins. The proteins (4ALM, 3IM9, 4FS3, and 1XPM) are presented as green markers while red markers represent the AMNPs.

Similarly, DB101-111, 113-116, 122-125, 127-142, 144-146, 151-155, 157, 159-168, 174, 177, 180-211 were compounds that showed strong affinity (BE ≤ -6 kcal/mol) for the malonyl CoA-acyl carrier protein transacylase (3IM9). Among these compounds, sanguinarine (DB135) had a docking score of -9.1 kcal/mol, the highest affinity for the 3IM9 target. About 73.8 % of the AMNPs (comprising of DB101-107, 109-111, 113-116, 118-122, 124-125, 127-133, 135-139, 141, 144-147, 150, 151, 153-155, 157, 159, 160, 162-167, 174, 175, 177, 178, 180, 182-197, 199-204, 207-210) also showed profound interaction with the enoyl-[acyl-carrier-protein] reductase [NADPH] (4ALM) target. This observation was based on their docking scores which were found below -6 kcal/mol. The results also revealed a favourable interaction between the AMNPs and threonine-tRNA ligase (4FS3) target. As shown in Figure 4.23, rugulosin A (DB 194) revealed the strongest BE (-8.6 kcal/mol) for 4FS3. The compounds DB101-111, 113-116, 122-125, 127-146, 148, 151-155, 157, 159-168, 174, 182-197, 199-202, and 204-210 also showed BE below -6 kcal/mol for the same protein. Among these compounds, dihydrokaempferol (DB134), andrimid (DB146), epicatechin gallate (DB159), and hinokinin (DB199) exhibited the highest binding affinity (BE of -8.1 kcal/mol) (Fig. 4.23). Furthermore, about 60 % of the AMNPs could potentially inhibit all of the four enzymes (Fig. 4.23). Among

these compounds are DB135, 146, 159, and 174 whose antimicrobial activity has been identified with the disruption of fatty acid biosynthesis (Chow and Sato 2013; Ishikawa *et al.*, 2016; Wang *et al.*, 2003; Zhang *et al.*, 2016). On the other hand, erythrabyssin II (DB139) and a few other AMNPs have no previous reports on the effect that they disrupt FASII enzymes. Hence, these compounds could be novel and promising potential drug candidates against MRSA.

4.2.6 Inhibitors of hemolysins associated proteins

The hemolysins are classes of four different exotoxins produced by *S. aureus* causing pathogenesis through lysis of red blood cells (Otto, 2014; Mohan and Venugopal, 2013). These proteins include α, β, and γ-hemolysins which function through the receptor-mediated process and δ which is a non-receptor mediated hemolytic toxin (Kong *et al.*, 2016; Mohan and Venugopal, 2013). Inhibitors of these enzymes have been widely described as potential anti-virulence agents (Bandyopadhyay and Muthuirulan, 2018; Escajadillo and Nize, 2018). In this study, the potential AMNPs inhibitors of the γ and δ-hemolysin were investigated and the result is summarised in Table 4.6.

Table 4.6. Summary of the AMNPs that showed a strong affinity for hemolysin proteins, as measured by binding energy (BE).

PDB	Nos of ligands with BE ≤ -6	Comments (n = no of ligands)	Best ligand with their BE (kcal/mol)
1DHL	0	Low binding affinity (less negative BE) was observed across all the ligands	DB207: Ikarugamycin and DB210: Celastrol (-4.8)
2ERN	71	22 showed BE ≤ -7	DB194: Rugulosin A (-9.4)

Generally, the AMNPs revealed a low to mild binding affinity for delta-hemolysin (1DHL), where ikarugamycin (DB207) and celastrol (DB210) both with BE of -4.8 kcal/mol demonstrated the strongest affinity for the target. On the contrary, about 64 % of the AMNPs comprising DB101-105, 108-111, 113-115, 122, 124-125, 127, 130-135, 137-139, 141, 144-148, 150-151, 153-155, 157, 159-162, 164-167, 174, 182-191, 193-197, 199-202, 204, and 207-211 exhibited binding energy below -6 kcal/mol for the gamma-hemolysin component B protein (2ERN).

Among these compounds, rugulosin A (DB194) with the binding energy of -9.4 kcal/mol revealed the highest affinity for this protein and thus could be the most promising molecule with the ability to inhibit the role of gamma-hemolysin component B in MRSA. The low binding affinity observed across the AMNPs for 1DHL however, implies that most of the AMNPs are more likely to inhibit the pathogen by neutralizing the receptor-mediated process rather than the δ-hemolysin machinery.

4.2.7 Inhibitors of quorum-sensing associated proteins

The quorum-sensing (QS) system in bacteria aids their communication with each other through the activation of some specific arsenals of virulence behaviours (Bandyopadhyay and Muthuirulan, 2018; Jiang *et al.*, 2019). It regulates various kinds of biological processes including oxidative stress responses, pathogenicity, and antibiotic resistance (Bandyopadhyay and Muthuirulan, 2018). Therefore, the disruption of this system can result in neutralized bacterial virulence; a promising approach to overcome drug-resistant pathogens (Fleitas-Martínez *et al.*, 2019; Kalia and Purohit, 2011). The possible interactions between AMNPs and the enzymes (1WCZ, 4G4K, 4GCM, and 1MJT) associated with the pathways leading to the QS system were explored in this study. The result showed that AMNPs demonstrated a low to a mild affinity for glutamyl endopeptidase (1WCZ) target (Table 4.7).

Table 4.7. Summary of the AMNPs that showed a potent affinity for the quorum-sensing system, as measured by binding energy (BE).

PDB	Nos of ligands with BE ≤ -6	Comments (n = no of ligands)	Best ligand with their BE
1CWZ	2	Nil	DB199: Hinokinin (-7.1)
4G4K	6	Nil	DB191: Licoisoflavone B (-6.8)
4GCM	74	26 showed BE ≤ -7	DB135: Sanguinarine (-8.1)
1MJT	95	77 showed BE ≤ -7; 48 showed BE ≤ -8 while 32 showed BE ≤ -9	DB151: Erycristagallin (-11.1)

Only DB161 and 199 showed strong affinity (BE < -6 kcal/mol) for this receptor. This is evidenced by the score of -6.6 kcal/mol observed for epigallocatechin (DB161) and the hinokinin (DB199) having the best score of -7.1 kcal/mol. This result implies that DB161 and DB199 might be prioritized in the search for AMNPs with the prospect of disrupting the activities of glutamyl endopeptidase.

The potentials of epigallocatechin to alter biofilm formation by interference with the QS has been reported (Matsunaga *et al.*, 2010; Zhu *et al.*, 2015). These findings agree with the observation of the present study, where epigallocatechin (DB161) showed a strong affinity for 1WCZ. In another study, carvacrol was suggested to alter the formation of bacterial biofilms and QS only at a sub-lethal concentration (Burt *et al.*, 2014). This could be the cause of the mild affinity (-4.3 kcal/mol) demonstrated by this compound (DB172) as observed in the present study. Nevertheless, there is no report on the anti-biofilms nor QS related effect of hinokinin (DB199) on any bacteria. This implies that DB199 could be a new and promising compound in quenching the biofilms and QS compared to some of the already known compounds.

As part of this study, it was observed that licoisoflavone B (DB191) with BE of -6.8 kcal/mol had the strongest affinity among the 5 % AMNPs (DB113, 125, 184, 191, 196, and 201) that exhibited a high binding affinity (BE ≤ -6 kcal/mol) for accessory gene regulator protein A (4G4K) (Table 4.7). Similarly, sanguinarine (DB135) was best docked with the thioredoxin reductase (4GCM) target. This is evidenced by the BE of -8.5 kcal/mol observed for the ligand (Table 4.7). Additionally, 60 % of AMNPs (comprising DB101-111, 113-115, 122-125, 127, 129-135, 138-141, 144-146, 151, 153-155, 157, 159-168, 174, 181, 183-197, 199, 200, 202, 204, and 207-211) were found to potentially alter the role of this enzyme in the pathogen.

Energetically favourable interaction was observed between the nitric oxide synthase oxygenase (1MJT) enzyme and most of AMNPs. This was evidenced by the 85 % of AMNPs (comprising DB101-114, 116-137, 139-146, 148, 151-155, 159-168,171-174,176-189, 191-193,195-206, 208 and 211) that showed strong affinity (BE ≤ -6 kcal/mol) for this target. One of these compounds, erycristagallin (DB151) with BE of -11.1 kcal/mol showed the most profound molecular interaction (Table 4.2.4). These results imply that most of the AMNPs may potentially block the activity of 1MJT in MRSA. Resveratrol (DB107), myricetin (DB113), and glabridin (DB189) were among the compounds identified with a promising affinity (BE ≤ -7 kcal/mol) for the 1MJT protein. This result is consistent with previous reports where antimicrobial activities of the three compounds; resveratrol, myricetin and glabridin were identified with alteration of the pathways related to oxidative stress response in *S. aureus* (Ma *et al.*, 2018; Silva *et al.*, 2017; Singh *et al.*, 2015). The compounds DB151, DB191, and DB199 demonstrated more promising affinities for this target. However, none of these compounds has been linked to oxidative stress response in the literature. Therefore, it can be inferred that some

of the AMNPs may potentially disrupt the stress response pathway in MRSA better than some of the already known compounds.

4.3 Summary of the chapter

This chapter has presented, discussed, and interpreted the results for the cheminformatic characterization, prioritization, and optimization of AMNPs towards becoming safe orally administered drugs. The results and implications of the predicted putative drug targets for the AMNPs in MRSA were also discussed. Most of the investigated properties were found within the drug-likeness space of more AMNPs compared with the CDs. In addition, some AMNPs might be more desirable in the synthesis of novel anti-MRSA drugs. Interestingly, AMNPs with the least bioactivity showed the greatest potential to become oral drugs. Optimization for drug-likeness deficiencies among the SA category also led to the identification of two promising and novel chemotypes with better safety and synthetic accessibility scores. For target prediction analysis, AMNPs with promising affinity were identified based on their molecular docking score for the different putative drug targets in MRSA. Most of the AMNPs showed strong affinities for related or different independent targets. It was also established that some of the AMNPs could be prioritized above most of the already known inhibitors in the search for novel anti-MRSA drugs with broad-spectrum.

Chapter five

Conclusion and recommendations

This chapter presents a summary of the major findings and conclusions from the objectives set out in this study. It also defines the limitations of the study as well as provides recommendations for future work.

Computational strategies are valuable and essential in the drug discovery and development process. They have the benefits of providing a cost-saving and efficient output within a very short time (Pereira and Aires-de-Sousa, 2018). Additionally, continuous failure of the currently prescribed drugs against Methicillin-Resistant *Staphylococcus aureus* (MRSA) necessitates an urgent need for novel antimicrobial agents, especially from non-conventional sources (Tayel *et al.*, 2018). Therefore, to stay ahead of the impending danger of MRSA, it is crucial to embrace computational strategies in the search for potential drug candidates that can combat the pathogen. Taking advantage of the available natural products that have shown *in vitro* activity against MRSA (AMNPs), this study was set out to conduct cheminformatic and pharmacokinetic profiling of these compounds as well as to predict their potential targets in the bacteria. The major findings from this study are outlined below.

5.1 Summary of key findings

The main results for each of the objectives of this study are succinctly summarised below:

I. To conduct a literature search to retrieve anti-MRSA natural products with their minimum inhibitory concentration (MIC).

Relevant keywords were used on freely available public databases to identify recent studies that reported AMNPs with their bioactivity. A sum of 111 AMNPs was retrieved based on the conducted search, and the MIC of these compounds ranged from 0.01 μg/mL to 1600 μg/mL.

II. To perform cheminformatics and data mining analysis of the AMNPs toward hit profiling, hit-prioritization, and hit-to-lead optimization using different cheminformatics software.

Cheminformatics profiling was conducted on the AMNPs. A few approved anti-MRSA drugs (CDs) were used as a reference to identify compounds with prospects to become drug candidates. This was followed by prioritization of the AMNPs and identification of compounds for possible optimization. Profiling of molecular descriptors and physicochemical properties

of the AMNPs and CDs revealed that the AMNPs could be more drug-like compared to most of the CDs. This is because most of the AMNPs were found within the required drug-like limits of the investigated properties. In addition, most of the AMNPs were identified to have "room" for the potential addition of required chemical entities towards improving certain drug-like properties during the hit-to-lead optimization stage. Among the bioactivity categories of AMNPs, there were no significant differences in some of the properties evaluated. Furthermore, it was also observed that some crucial properties that determine drug-likeness (including hydrogen-bond acceptors, donors, and flexibility) were within the required limit for the less-active (NA) category. However, these properties were outside the limits for most of the active categories (A and SA), implying that strong bioactivity may not determine drug-likeness.

Absorption and permeability profiles were based on the rules defined by Lipinski, Veber, and Egan. The result revealed that more than 80 % of the AMNPs could have the chance of achieving good absorption. About 30 % of these compounds were also predicted to pass through the blood-brain barrier and up to 80 % were non-substrates of efflux transporters. Among the categories of AMNPs, the NA exhibited the highest prospect for good oral absorption. The CDs however, revealed a lower absorption. This is based on 60 % of these compounds that were found within the space of Lipinski's rule. In addition, less than 50 % of the CDs revealed the chances of achieving high permeability at the Veber and Egan's limits. The predicted permeability results for the CDs also reinforced their low permeability potentials. This is based on 25 % of the compounds that were confirmed as none p-gp substrates. None of the CDs was also predicted as a CNS drug. Above all, the absorption profiles revealed that the AMNPs exhibited the potential to become more orally active drugs than most of the CDs.

The CDs did not show a tendency to inhibit any of the CYP450 isozymes. On the contrary, about 16 to 43 % of the AMNPs could potentially inhibit one or more of the isozymes. These inhibitors might be given less consideration during hit selection regardless of their *in vitro* activity. Additionally, the result predicted for phase 2 revealed that 71 % may produce metabolites at this stage. This implies that most of the AMNPs could be readily excreted out of the body. The assessment of toxicity also established that 80 and 59 % of the CDs and AMNPs respectively, might have negligible or no toxicity risks. The synthetic accessibility score of the AMNPs indicated that most of these compounds might be easier to synthesize than the CDs.

The hit-prioritization strategy led to the design of a visualization that allows easy identification of AMNPs that met all the desirable drug-likeness criteria and the drug-likeness deficiencies of other AMNPs. The drug-likeness deficiencies of two AMNPs were addressed by hit-optimization to evolve novel compounds (ANA196 and ANA 211) that are druglike. Furthermore, visualization of the chemical space revealed that AMNPs displayed higher diversity but lesser structural complexity in comparison with the CDs. Exploration of the structural-activity relationship also revealed activity cliffs (i.e. similar compounds with significantly diverse activities). Chemical groups responsible for enhanced anti-MRSA bioactivity were identified.

III. To predicts the binding affinity of the compounds within the sites of MRSA proteins using molecular docking.

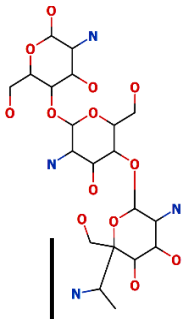
In the second phase of this study, the potential targets of the 111 AMNPs in MRSA were successfully predicted using reverse molecular docking. The analysis of the docking interactions of AMNPs with the different putative protein targets in MRSA revealed that most of the AMNPs had an exceptionally strong binding affinity for some of the important protein targets.

Overall, the AMNPs showed the strongest and most promising prospect for fatty acid metabolism associated proteins. This was evidenced by their high percentage of inhibitors for all the investigated fatty acid metabolism targets; 1XPM (96 %), 3IM9 (73.8 %), 4ALM (73.8 %), and 4FS3 (76 %). Among the cell wall and membrane synthesis proteins, 88 and 77 % of the datasets showed a strong affinity for the 4WYZ and 2OLV, respectively. Additionally, most of the ligands interfere with growth and cell division related proteins. This is evidenced by the strong binding affinity of about 88 % AMNPs obtained for the penicillin-binding protein 3 (3VSL). Most of the compounds were also predicted to interact with protein synthesis related pathways, based on the inhibition of 1NYR protein by 82 % of the ligands. Similarly, 83 % of the AMNPs revealed higher chances of blocking the DNA synthesis pathway as their therapeutic target through the inhibition of 3JSL protein. Furthermore, about 64 % of AMNPs showed promising prospects in disrupting the pathway involving hemolysins by targeting the gamma-hemolysin component B (2ERN). Additionally, 67 and 86 % of the AMNPs might strongly interfere with the 4GCM and 1MJT targets, respectively to cause inhibition of quorum sensing in the pathogen.

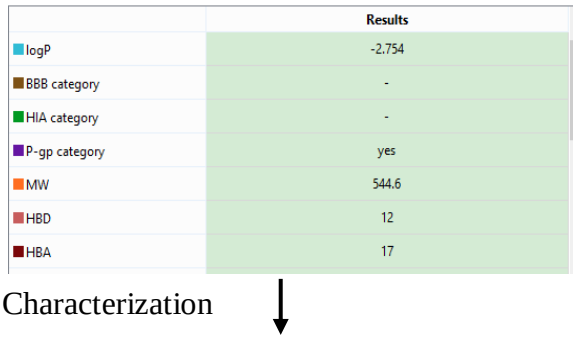
Some of the AMNPs were predicted to have the best binding scores for 2 or more related and unrelated targets. These compounds include celastrol (DB210) and sanguinarine (DB135) that showed the strongest affinity for three different proteins. Heyneanol A (DB150), and hinokinin (DB199) also revealed the best docking score and affinity for about four different proteins while rugulosin (DB194) had the best and significant binding affinity for about five proteins. This can be an indication that many of the AMNPs might be multi-target compounds with the prospect of destroying the pathogen by binding to different proteins. Additionally, most of these AMNPs were structurally diverse from established inhibitors in the literature. This structural diversity could be an indication of their potentials to inhibit the same or similar targets by different mechanisms.

5.2 Overall goal and conclusion

This work has provided a framework for the characterization, prioritization, optimization, and target prediction of AMNPs towards the discovery of orally active anti-MRSA lead compounds. The findings strongly indicate that some of the top prioritized AMNPs are safer and have greater potential than most currently administered drugs for MRSA. This study has also shown that most of the AMNPs have desired drug-like properties, and those with liabilities can be optimized for better performance. Furthermore, the study identified the prospective targets for the AMNPs thus giving insight into the potential mechanisms of the compounds. Therefore, the AMNPs with good drug-like properties and binding affinity for the MRSA drug targets are suggested to establish ideal lead candidates to be developed into new generation of drugs against MRSA-caused infections. Finally, the knowledge gained through this study could help realize the full prospects of available data in drug development chains, thereby reducing the attrition rates. The schematic representation of the main achievements of this study is shown in Figure 5.1 below.

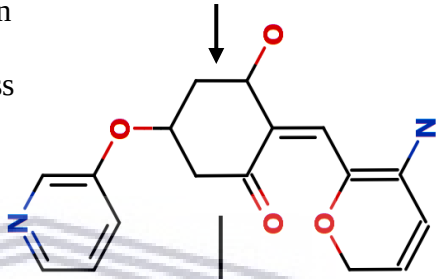


AMNPs obtained from literature

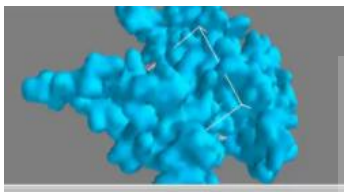


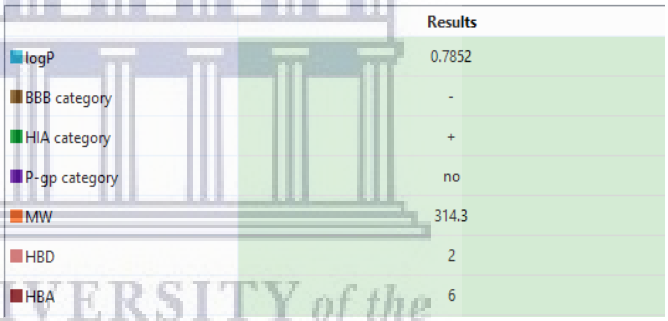
Characterization

Prioritization revealed drug-likeness prospects



Compounds obtained at optimization



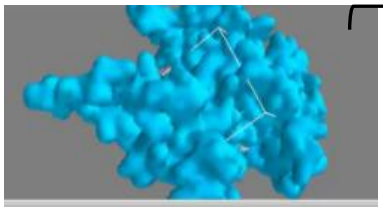


Characterization of the new compounds

In vitro validation (future studies)

Improved properties of the new compounds

Suggestions for future studies



Docking analysis of the new compounds

In vitro and *in vivo* validation

Figure 5.1 Schematic representation of the main achievements of this study.

5.3 Limitations of the study and recommendations for future work

The generalizability of the predicted drug-likeness and targets of the AMNPs is limited by the lack of *in vitro* validation which is beyond the scope of this study. Additionally, it was not ascertained whether the two novel compounds identified in this study exhibited any antimicrobial activity. Therefore, further *in vitro* and *in vivo* studies are needed to substantiate the *in silico* predictions.

Chapter six

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