REPUBLIC OF TURKEY
HARRAN UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

MSc. THESIS

INVESTIGATION OF THE ANTIBACTERIAL EFFECTS OF FLUORINATED 5-TERT-BUTYL SALICYLALDEHYDES ON BACTERIA THAT HAVE THE RISK OF DEVELOPING DRUG RESISTANCE

Karzan Hassan HAJI

DEPARTMENT OF BIOLOGY

ŞANLIURFA
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The study entitled **"**Investigatıon of the antibacterial effects of Fluorinated 5-tert-butyl Salicylaldehydes on bacteria that have the risk of developıng drug resistance" prepared by Karzan Hassan HAJI, Under the supervision of Prof. Dr. Faruk SÜZERGÖZ was unanimously accepted as a MASTER of SCIENCE thesis on 20.09.2021 by the following examination committee from the Graduate School of Natural and Applied Science, Department of Biology, Harran University.

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I confirm that this thesis was prepared in the Department of Biology and is organized according to the rules of the Institute.

Assoc. Prof. Dr. İsmail HİLALİ
Head of Institute

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Yüksek Lisans Tezi

İLAÇ DİRENCİ GELİŞTİRME RİSKİNE SAHİP BAKTERİLER ÜZERİNDE FLORLU 5-TERT-BUTIL SALİSİLALDEHİTLERİN ANTİBAKTERİYEL ETKİLERİNİN ARAŞTIRILMASI

Karzan Hassan HAJI

Harran Üniversitesi
Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı

Danışman: Prof. Dr. Faruk SÜZERGÖZ
YIL: 2021, Sayfa: 71

Son yıllarda, çoklu direnç fenotiplerine sahip olanlar da dahil olmak üzere birçok mikroorganizmada antibiyotik dirençleri tanımlanmıştır. Bu, Dünya Sağlık Örgütü'nün de belirttiği gibi endişe verici bir durumdur ve birçok araştırmacı yeni terapötik alternatiflerin geliştirilmesine odaklanmıştır. Çalışmamızda mikroplara karşı direnci çözmek için yeni antimikrobiyal ilaçlar keşfetmeyi ve Schiff bazlarından Florlu 5-tert-butil salisilaldehitlerin antibakteriyel ajan olarak potansiyelini ve etkinliğini değerlendirmeyi amaçlandı. Çalışmada, *Escherichia coli* (ATCC, 25922), *Pseudomonas aeruginosa* (ATCC, 27853) ve *Staphylococcus aureus* (ATCC, 29213) bakteri suşları 50 ml'de Nutrient Broth (NB) içinde 37 °C'de 24 saati inkübe edildi. Seri kültürler için, 1 ml başlangıç kültür numunesi 100 ml NB'de 37 °C'de 24 saat inkübe edildi. Schiff bazı türevlerinden florlu 5-tert-bütil salisilaldehitler (Bileşik 1-5) 1 ila 1000 µM dozlarda triple düzende 96 kuyucuklu kültür plakalarına eklendi. NB'de 0,5 McFarland standartlarına ayarlanan bakteriler, her kültür plakası kuyusuna 100 µl'lik bir dozda eklendi. Kültür plakları 37 °C'de 18 saat inkübe edildi. Kültür periyodundan sonra, her bir kuyucuğa 10 µl MTT solüsyonu (300 µM konsantrasyonunda) eklendi ve 4 saat inkübe edildi. MTT-formazan kristallerini çözmek için her kuyucuğa 100 µl dimetil sülfoksit ilave edildi. Kültür plakaları, bir ELIZA mikroplaka okuyucusunda 570 nm'de okundu ve her bir oyuk için optik yoğunluk (OD) değerleri kaydedildi. Her bileşiğe ait MIC50 değeri, OD değerleri kullanılarak lineer regresyon analizi ile hesaplandı. Gentamisin MIK_{50} değerleri sırasıyla *P. aeruginosa*, *E. coli* ve *S. aureus* için; 1.43, 1.93, 1.98 µM bulundu. Sonuçlar, antibiyotiklerin test edilen suşların büyümesi üzerinde büyük bir engelleyici etkiye sahip olduğunu gösterdi. *P. aeruginosa*'ya karşı en güçlü inhibitör etkiler C2, C3 ve C5 ile elde edilmiştir (MIK_{50} değerleri sırasıyla 9,56, 9,9, 3,46 µM). *S. aureus* üzerindeki en güçlü etki C5 ile ve orta düzeyde etkiler C1, C2, C4 ile elde edildi (MIK_{50} değerleri sırasıyla 5,12, 5.8, 15.1, 12.7 µM). C1, *E. coli* üzerinde orta derecede etki göstermiştir (MIC_{50} değerleri 18,9 µM). Yeni güçlü antimikrobiyal ajanların keşfinde değerli olabilecek çalışmamız ülkemizdeki insan sağlığı ve ilaç endüstrisine katkı sağlayacaktır. Bu sonuçlar, florlu 5-tert-butil salisilaldehitlerin bakterilerin kontrolünde yeni antibiyotik ajan adayları olarak kullanılması olasılığının önünü açmaktadır. Gelecekteki çalışmalar, potansiyel toksik reaksiyonları ve gerçek etkinliği belirlemek için *in vivo* ve klinik testleri içerecektir.

ANAHTAR KELİMELER: Antibakteriyel aktivite, Schiff bazları, Florlu salisilaldehitler, MIK_{50}, MTT yöntemi.

ABSTRACT

MSc Thesis

INVESTIGATION OF THE ANTIBACTERIAL EFFECTS OF ON BACTERIA THAT HAVE THE RISK OF DEVELOPING DRUG RESISTANCE

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Year: 2021, Page:71

Antibiotic resistance has been documented in a variety of microorganisms and those with multi-resistance phenotypes over the last few decades. According to the World Health Organization, this is a concerning situation, and several researchers are working to develop new therapeutic options. In our study, we aimed to discover new antimicrobial drugs against microbes to solve the resistance and evaluate the potentiality and effectiveness of Fluorinated 5-tert-butyl salicylaldehydes from Schiff bases antibacterial agents. In this study, *Escherichia coli* (ATCC, 25922), *Pseudomonas aeruginosa* (ATCC, 27853), as well as *Staphylococcus aureus* (ATCC, 29213), bacterial strains incubated in 50 ml in Nutrient Broth (NB) at 37 °C for 24 h. For serial cultures, 1 ml of the initiating culture sample was incubated in 100 ml NB at 37 °C for 24 h. Schiff base derivatives of fluorınated 5-tert-butyl salicylaldehydes (Compound 1-5) added to 96-well culture plates in triplicate order at 1 to 1000 µM doses. Bacteria, set to 0.5 McFarland standards in NB, were added in 100 µl to each culture plate wells. The plate was incubated at 37 °C for 18 hours and then placed in a 37 °C incubator. After the incubation time, a total of 10 µl of a 300-µM MTT solution was added to each well, and the plates were left to incubate for 4 hours. Diluted with 100 microliters of dimethyl sulfoxide, the MTT-formazan crystals were dissolved. The culture plate was read on the ELIZA microplate reader at 570 nm, and the optical density (OD) values for each well were recorded. MIC_{50} value of each compound was calculated by linear regression analysis using OD values. MIC50 values for gentamicin were found on *P. aeruginosa*, *E. coli* and *S. aureus* 1.43, 1.93, 1.98 µM, respectively. The results showed that the antibiotics have a significant inhibitory effect on the growth of tested strains. The most potent inhibitory effects against *P. aeruginosa* were obtained by Compounds 2, 3, and 5 (MIC_{50} values were 9.56, 9.9, 3.46 µM, respectively). The most potent effect on *S. aureus* was obtained by C5 and moderate impact by Compound 1, 2 and 4 (MIC_{50} values were 5.12, 15.8, 15.1, 12.7 µM, respectively). Compound 1 was showed a moderate effect on *E. coli* (MIC_{50} values was 18.9 µM). Our research, which may be beneficial in discovering new potent antimicrobial agents, will benefit the human health care system and the pharmaceutical industry in our country. These results pave the way toward the possibility of using the fluorınated 5-tert-butyl salicylaldehydes as new antibiotic agent candidates in control of bacteria. Future studies will be containing the *in vivo* and clinical tests for determining the potential toxic reactions and actual effectiveness.

KEY WORDS: Antibacterial activity, Schiff bases, Fluorınated salicylaldehydes, MIC_{50}, MTT assay.

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Karzan Hassan HAJI

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SYMBOLS and ABBREVIATIONS

ATCC	American Type Culture Collection
AMPs	Antimicrobial Peptides
AgNP	Silver nanoparticles
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
EC_{50}	Half maximal effective concentration
ELIZA	Enzyme-Linked Immunosorbent Assay
g	gram
h	Hour
HIV	Human Immunodeficiency Virus
MDR	Multidrug Resistant
mg	milligram
MIC	Minimum Inhibitory Concentration
min	Minute
mL	Mililitre
mM	Milimolar
MRSA	Methicillin-Resistant *S. aureus*
MTT	Thiazolyl Blue Tetrazolium Bromide
MW	Molecular weight
NB	Nutrient Broth
nm	Nanometer
OD	Optical Density
OMKO1	A lytic bacteriophage
PDR	Pan-drug-resistant
USA	United States
UV	Ultraviolet
WHO	World Health Organization
wksl3	A virulent bacteriophage
XDR	Extensively drug-resistant
µm	Micrometer
µM	Micromoller
µL	Microlitre
^{o}C	Degrees centegrade
rpm	Rotation per minute

1. INTRODUCTION

One major disadvantage of this method is that pathogenic bacteria have developed resistance to different antibiotic treatments, causing a significant public health concern since there are few potent antimicrobial medicines obtainable for diseases due to bacterial (Magiorakos et al., 2012).

On the other hand, multidrug resistance is linked to the widespread use and abuse of antibiotics in individuals, agriculture, and animal production (O'neill, 2014; Harbarth et al., 2015; Christaki et al., 2020).

Antibiotic-resistant pathogen infections are more difficult to medicate, and they can recur, causing significant morbidity and mortality. Therefore, the very efficient treatment of bacterial infections with antibiotics, which is limited to the treatment of microbial disease, is one of the most significant medical achievements of our period (Marchese et al., 2016; Barbieri et al., 2017; Vivas et al., 2019; Mühlberg et al., 2020).

Pathogenic bacterial strains commonly encountered in health care settings such as *S. aureus, P. aeruginosa, Enterobacteriaceae, Acinetobacter* spp. and *Enterococcus* spp. Clinical, reference and public health microbiology laboratories will use these definitions to grade various antibiotic resistance profiles using a consistent nomenclature (Jones and Masterton, 2001; Carmeli et al. 2010; Magiorakos et al., 2012).

S. aureus detected increased resistance to erythromycin, methicillin, and trimethoprim-sulfamethoxazole, for *E. coli* ciprofloxacin, ceftriaxone, ampicillin, amoxicillin/clavulanic acid and trimethoprim. This increase in two of the most often diagnosed bacterial illnesses is alarming since it may compromise empirical therapy in a world where laboratory testing is restricted (Monteıro et al., 2020).

Gram-negative microbial, like *P. aeruginosa, E. coli,* and *Klebsiella* sp., are the most frequent, accounting for up to 80% of polymicrobial bacteremias, while additional *Enterobacteriaceae, Stenotrophomonas maltophilia*, and *Acinetobacter* sp. Can also be identified (Rolston et al., 2007; Bassetti and Righi, 2013).

Antimicrobial resistance is responsible for 700,000 deaths each year worldwide, with the figure predicted to rise to 10 million by 2050. Antimicrobial-resistant bacteria, such as multidrug-resistant methicillin-resistant *S. aureus* (MRSA), cause infection, *Acinetobacter baumannii* and *Klebsiella pneumonia* (*K. pneumonia*), prolong hospitalization and place a significant financial strain on national healthcare systems (Ventola et al., 2015)

Depend on a 2014 (WHO) analysis, Antimicrobial resistance is developing across the world, putting the effective prevention and treatment of common diseases caused by bacteria, parasites, viruses, and fungi at threat (Serpi et al., 2016).

Furthermore, the process of discovering a novel antibiotic and bringing it to market takes about ten years. As a result, new antibacterial drugs are urgently needed to combat harmful microbes (Wang et al., 2018).

Schiff bases are medically intriguing molecules with an expanded range of pharmacological effects, involving antipyretic, anti-inflammatory, and antibacterial capabilities. In addition, Schiff bases (–C=N–) are frequently utilized for biological actions such as antibacterial, antifungal, anticancer, antimalarial, anti-HIV, trypanocidal, anti-urease and anti-inflammatory, (Da Silva et al., 2011; De Fátima et al., 2018; Abdel-Rahman[1] et al., 2016; Abdel-Rahman[2] et al.; 2017).

The Schiff bases were tested for antibacterial activity with some bacterial resistance may be *Staphylococcus Sp., E. coil, Pseudomonas sp., Streptococcus* sp., *Klebsiella* sp. and other organisms with different prevalence rates. In recent years, it has been discovered that Schiff bases possess different properties, such as enzyme

inhibition, cleavage activity, DNA binding, as well as cytotoxicity (Joseyphus and Nair, 2008).

Fluorinated molecules have recently gained popularity due to their therapeutic potential in a variety of medical fields. Because of their well-defined biological actions, fluoro substituted derivatives have shown to be attractive "druggable" heterocycles. For example, 5-fluorouracil, as well as another fluoro derivative, has gotten a lot of interest. Fluorine compounds have received significant attention due to their numerous biological properties, including antibacterial and anesthetic properties (Shanmugam et al., 2013, Gurol et al., 2017).

Fluorinated organic compounds are a unique class of xenobiotics with a significant possibility for developing new physiologically valuable chemicals. One of the essential methods in the successful creation of novel medicines is the insertion of fluorine atoms into a core pharmacophore. As a result, it's not strange that at least one fluorine atom is present in about 25% of pharmaceutical line medications. Moreover, fluorination has proven to be a very effective technology in recent decades, enabling new marketable medicines for various diseases. For example, Tavaborole (antifungal), finafloxacin (antibiotic), fluoxetine (antidepressant), and chidamide (antitumor) and are just a few of the medicines included (Avila-Sorrosa et al., 2020).

Our study tested the antimicrobial effects of Fluorinated 5-tert-butyl Salicylaldehydes from Schiff bases on P. aeruginosa, E. coli and S. aureus with the MTT method to develop alternative antimicrobial agents to drug resistance. In addition, we aimed to create new antimicrobial drugs or improve existing ones to combat microbe mutation, solve resistance, and evaluate the potentiality and effectiveness of newer Schiff base as antibacterial agents, which will be tested to specify new antimicrobial agents.

2. PREVIOUS STUDIES

2.1. Drug Resistance

2.1.1. Definition of drug resistance

Antibiotic resistance develops when bacteria acquire or develop the ability to bypass antibiotics' processes to combat them. As a result, antibiotic-resistant pathogen infections are harder to treat and can repeat, causing considerable morbidity and death (Christaki et al., 2020).

There are multiple definitions of In the medical literature, the terms extensively drug-resistant (XDR), multidrug-resistant (MDR) & pan-drug-resistant (PDR) Pathogens are being used to analyze the different shapes of resistance shown in universal health care antibiotic-resistant microorganisms.

The European Committee on Antimicrobial Susceptibility Testing (ECAST) and the United States (US) Food and Drug Administration (FDA) are both involved in antimicrobial susceptibility testing. MDR has been identified as acquired non-susceptibility to at least each antimicrobial agent in 3 or even more antimicrobial classifications, XDR when non-susceptibility to the at least one antimicrobial agent in any antimicrobial varieties except two or fewer as PDR as non-susceptibility to everyone agents throughout all antimicrobial types. To guarantee that these definitions are applied correctly, bacterial separate would be screened toward whole or virtually whole antimicrobial agents within an antimicrobial category, and findings should not be selectively reported or suppressed (Magiorakos et al., 2012).

Few people outside of the medical field are aware that antimicrobials are used in life-saving treatments, including chemotherapy, organ transplants, major surgery, and the treatment of autoimmune diseases and infections in newborns. Nonetheless,

always we use antibiotics, we put bacteria under selection pressure to mutation or exchange DNA, potentially leading to drug resistance (Teillant et al., 2015).

Worryingly, worldwide usage of antibiotics increased approximately 70% between 2000 and 2010. Before the age of 18, each individual in industrialized nations is prescribed between 10 and 20 courses of treatment. Thus, almost two tons of antibiotics are used every 10 minutes worldwide, all too frequently without a prescription or control (Blaser 2011; Mattar et al., 2020).

Antimicrobial resistance affects both the Gram-negative as well as Gram-positive bacteria when it emerges also spreads. To combat this increasingly global issue, new antimicrobial methods that are non-incursive, non-toxic, and work more effectively and quickly than existing antibiotics are needed (Ibrahim et al., 2000; Anderson et al., 2006; Amos-Tautua et al., 2019).

Antimicrobial resistance is linked to widespread use and misuse of antibiotics in people, agriculture, animal farming, and industry, necessitating a One-Health strategy since human health is inextricably linked to animal health and the sustainability of agriculture, animal farming, and industry (Harbarth et al., 2015)

PDR, XDR and MDR strains of bacteria commonly encountered in hospital settings are defined as an example (*S. aureus*, *P. aeruginosa*, *Enterococcus* sp., *Enterobacteriaceae* and *Acinetobacter* sp.). Therefore, clinical, reference and public healthcare microbiology labs would utilize these criteria to grade diverse antibiotic resistance profiles uniformly. This will lead to a uniform report of similar data that could track antimicrobial resistance trends both locally and globally. Furthermore, the usage of the standard terminology would strengthen the epidemiological monitoring system by enabling information sharing between medical professionals, public health officials, and policymakers due to encouraging the wise utilization of the antimicrobials as well as other public healthcare initiatives (Jones and Masterton, 2001; Carmeli et al., 2010), Table 2.1.

Table 2.1. General Characteristics of Multidrug-Resistant Organisms (Alekshun and Levy, 2007).

Organism	Common Infections	Key Antibiotic Resistances	Drugs Considered for Treatment of MDR
P. aeruginosa	Lung, wound	β-lactams, fluoroquinolones, aminoglycosides	Colistin
Acinetobacter spp.	Lung, wound, bone, blood	β-lactams, fluoroquinolones, aminoglycosides	Colistin, tigecycline
E. coli and K. pneumonia bearing extended-spectrum β-lactamas	Urinary, biliary, gastrointestinal tracts, lung, blood.	β-lactams, fluoroquinolones, aminoglycosides	Colistin (for K. pneumoniae), tigecycline
Vancomycin-resistant *Enterococci*	Blood, heart, intra-abdominal	Vancomycin	Quinupristin-dalfopristin, linezolid, daptomycin
MRSA	Skin and soft tissue, respiratory tract, blood	β-lactams, fluoroquinolones, macrolides	Quinupristin-dalfopristin, daptomycin, linezolid, tigecycline, vancomycin

MDR is an abbreviation for "resistant to even more than one antimicrobial agent." however, the medical community has yet to agree on a uniform meaning for the acronym. Some definitions are used to describe MDR patterns in Gram-positive and Gram-negative organisms. Clinical trial methods with no defined criteria for MDR result in results that are difficult to compare (Falagas et al., 2006; MacGowan 2008; Cohen et al., 2008).

Various authors and agencies utilize in vitro antimicrobial susceptibility test data to categorize organisms as MDR while they test "resistance to numerous antimicrobial agent, classes, or subclasses of the antimicrobial agent." 'Resistants to 3 or more of the antimicrobial classes' is the most common Definition for the Gram-negative & Gram-positive bacteria. An understanding of the MDR in the *P.*

aeruginosa, as well as *Acinitobacter baumannii*, provides an overview of the diversity of these classifications, noting that while a large numerous of publications don't offer any particular explanations for MDR, the several describe MDR as "resistance to 3 or more antimicrobial classes (Magiorakos et al., 2012).

When bacteria were resistant's to at least one antimicrobial agent, they are classified as MDR. These bacterial isolates are potentially relevant for public health since they are resistant to a single antimicrobial agent. However, they commonly show cross- or co-resistances to the multiple antimicrobial classes, indicating MDR. The benefit of employing these techniques for the surveillance aims was that they could be quickly implemented. Create the acronym for the bacteria rely on its resistance to significant antimicrobial agents such as MRSA instantly show its epidemiological importance (Hidron et al., 2008; Becher et al., 2011).

2.1.2. Drug resistance mechanisms

Antibiotic resistance is a developing threat today as a result of the unregulated use of antibiotics. As a result, selected pathogenic microorganisms that are resistant to several medicines have proliferated. In addition, Antimicrobial-drug resistance's economic effect is growing alarming, owing to rising medical and human costs (Singh et al., 2014).

Antimicrobial resistance develops in microbial populations in a variety of ways. The velocity with which a gene mutates to cause the antibiotic resistance phenotype is a complex process involving the environment, bacterial genetics, cell physiology, and population trends (Baquero et al., 2005).

Furthermore, due to the antimicrobial targets' genetic redundancy, mutations must occur in many genes for complete resistance to emerge. For example, the fluoroquinolone targets genes gyrB, gyrA, parC & parA, were the whole target for a fluoroquinolone antibiotic and must full had mutations for complete resistances

phenotypes to emerge Table 2.2. Bacteria can also acquire antimicrobial resistance genes through horizontal or lateral gene transfer (Roe and Pillai, 2003).

Table 2.2. Representing the mechanism of drug resistance of common antibiotics (Supriya et al., 2019).

P-Lactums	Penicillins ,Cephalosporins, Penems, Monobactams	Hydrolysis, efflux, altered target
Aminoglycosides	Gentamicin, Streptomycin, Spectinomycin	Phosphorylation, acetylation, nucleotidylation, efflux, altered target
Glycopeptides	Vancomycin, Teicoplanin	Reprogramming peptidoglycan biosynthesis
Tetracyclines	Minocycline, Tigecycline	Monooxygenation, efflux, altered target
Macrolides	Erythromycin azithromycin	Hydrolysis, glycosylation, phosphorylation, efflux, altered target
Lincosamides	Clindamycin	Nucleotidylation, efflux, altered target
Streptogramins	Synercid	Carbon-Oxygen lyase, acetylation, efflux, altered target
Oxazolidinones	Linezolid	Efflux, altered target
Phenicols	Chloramphenicol	Acetylation, efflux, altered target
Quinolones	Ciprofloxacin	Acetylation, efflux, altered target
Pyrimidines	Trimethoprim	Efflux, altered target
Sulfonamides	Sulfamethoxazole	Efflux, altered target
Rifamycins	Rifampin	ADP-ribosylation, efflux, altered target
Lipopeptides	Daptomycin	Altered target
Cationic	Colistin	peptides Altered target, efflux

The bacterial cell could get genetic sequences from another species along with various mechanisms. The first is the uptake of naked DNA from their immediate environment, referred to as transformation. The frequency at which bacteria acquire DNA from the environment is determined by several parameters, including cell wall construction and bacterial type, and can be as low as 10^{-7}. To accept foreign DNA

through transformation, bacterial cells have to be "competent (Alekshun and Levy, 2007).

Bacteria could transfer also acquire genetic material via plasmid / self-replicating extra-chromosomal DNA. However, this requires physical contact among cells for plasmids to be transferred among donor and recipient cells. The floR gene, which encodes florofenicol resistance in the *E. coli* discovered in cattle, was the example above; see figure 2.1. (Cloeckaert et al., 2000).

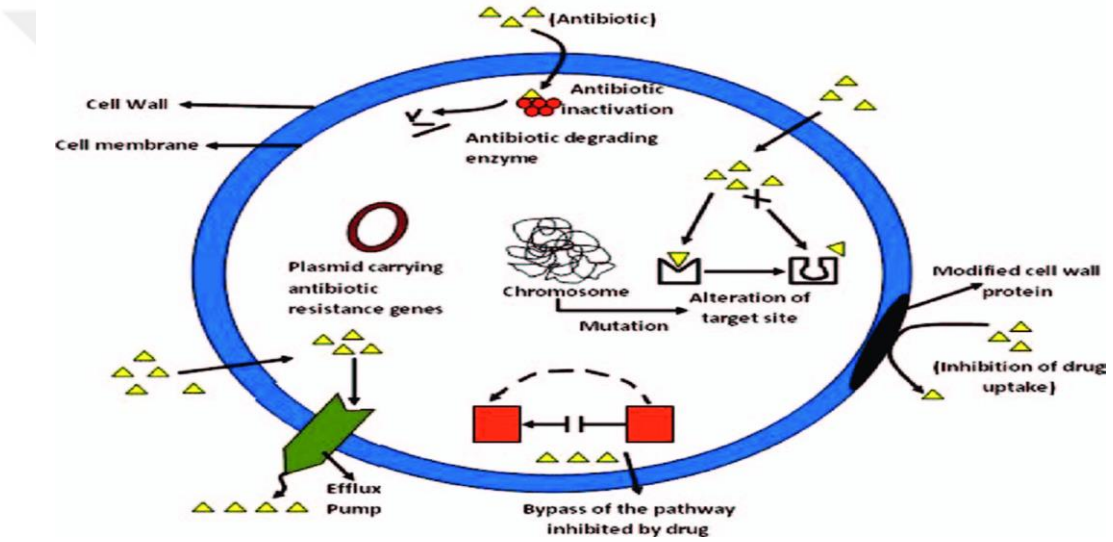


Figure 2.1. Mechanisms of antibiotic resistance.

2.1.3. Strategies to Combat MDR

The battle against infectious illnesses is recognized as a central theme in human history. However, antibiotic resistance has arisen as a significant worldwide health problem, rather than monitoring the decline of bacterial infections. Antibiotic resistance strategies in bacteria include targets preservation, target substitutions, antibiotic detoxification, and suppression of the accumulation of the intracellular antibiotic (Sultan et al., 2018). Observing bacteria progressively grow resistant to various antibiotics as resistances genes to the same also other bacteria genera are

acquired and added to the existing situation. Bacteria with higher adaptability, distribution of antibiotic resistances genes to reduce antibiotic potents by numerous methods, In addition to horizontal gene transfer (conjugation, transformation, as well as transduction), the rapid expansion of antibiotic resistance between many bacteria was aided by mobile elements (transposons, insertion sequences, plasmids, integrons, as well as integrative-conjugative elements) and also the bacterial toxin-antitoxin framework. The ability of the bacteria to receive resistances gene resulted in an uncomfortable scenario; this is a severe yet often unnoticed characteristic of resistance gene transfer (Sultan et al., 2018).

Antibiotic resistance is a global issue that must be addressed. As a result of increased antibiotic use, the creation and spread of antibiotic-resistant bacteria have damaged antibiotics' therapeutic value. Techniques that materialize perfectly among the various tactics employed include;

- Antimicrobial peptides (AMPs; Microcins, Cathelicidins, Bacteriocins, so on.) via a larger spectrum of the target are being developed
- To destroy antibiotic-resistant bacteria, phage treatment uses a lytic bacteriophage such as OMKO1 and (a virulent bacteriophage) wksl3
- Mixture treatment (utilizing a combination of the antibiotics, such as colistin in conjunction via tigecycline, meropenem, aminoglycoside, so on .) or a combination of antibiotic & inhibitor, like Augmention (amoxicillin and clavulanate)
- AMPs, Drugs, also essential oil are delivered the nanoparticles (NPs) to prolonged regulated reduction (amoxicillin, erythromycin, penicillin G, as well as vancomycin silver nanoparticles (AgNP)
- Liposomes medication delivery systems
- Flavonoids, alkaloids, coumarins, and other natural chemicals are used. and
- Plazomicin (ACHN-490) is a derivative of the sisomicin created by adding the hydroxy-aminobutyric acid substituents at position one and the hydroxyethyl substituent at position one one-six, is an example of antibacterial modification (Chan et al., 2016; Gordya et al., 2017; Soudeihaa

et al., 2017; Wang et al., 2017;, Poerio et al., 2017; Chandra et al., 2017; Lopez-Diaz et al., 2017).

Other strategies include using genomics to discover novel bacterial targets and optimizing emerging methods that target bacterial pathogens to decrease pathogenicity if bacteria develop resistance to therapeutic intervention. In addition, designing compounds that could prevent bacterial adhesion to the surface also targets bacterial virulence factors and contributes to the creation of inactivating antibodies, which appear to be viable alternative methods for combating the threat of drug resistance, see figure 2.2. ((Brooks and Brooks, 2014; Sultan et al., 2018).

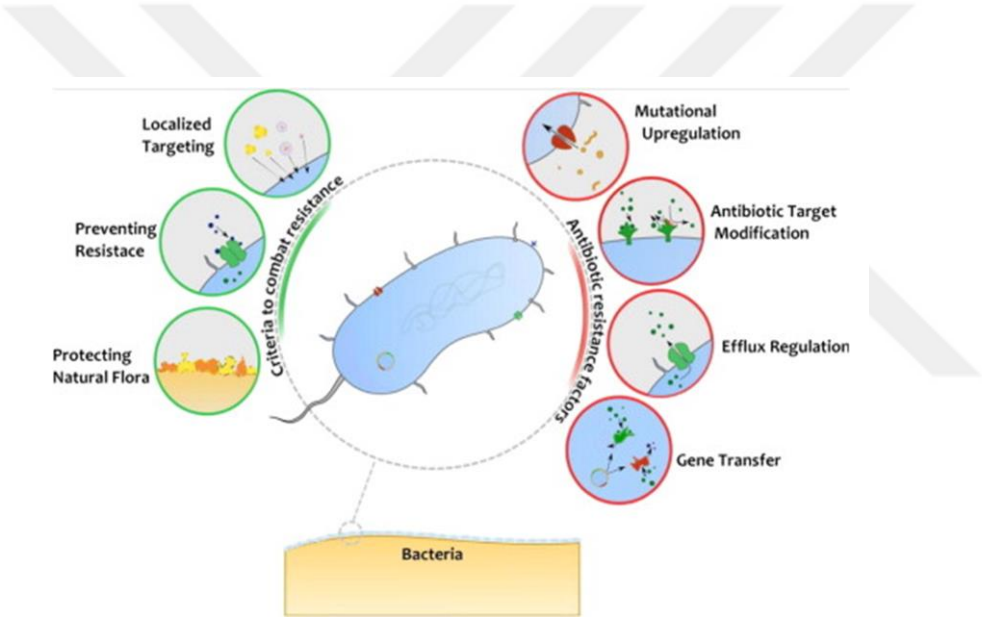


Figure 2.2. Diagram illustrating the three main criteria (on the left) required for just about any approach created to prevent the four primary modes of antibiotic resistance (on the right)

Antibiotic combination therapy has been widely utilized in the treatment of infections with high death rates. In addition, this method is frequently used when monotherapy is demonstrated to have disadvantages like toxicity, potency, or inadequate penetrations, which would likely result in lower efficacies. Combination therapy is used for a variety of reasons, including increasing antibiotic activity through the synergistic effects, removing resistance, obtained antibacterial activities towered microorganisms that form a biofilm, improving antibacterial agent

penetrations into cells and tissues, toxins product inhibition, also enzyme productions reduction (Khameneh et al., 2016).

2.2. Schiff Base

2.2.1. Definition of Schiff bases

In Schiff bases, which are multifunctional C=N (Imine) comprising molecules with a broad spectrum of biological activity, the integration of metals in the form of complexes exhibited some degree of antibacterial, antifungal, anticancer, and anti-inflammatory action (Xavier and Srividhya 2014).

One of the most widely used chemical compounds is Schiff base, which would be the nitrogen equivalent of a ketone or aldehyde for which the carbonyl group (C=O) was substituted via an imine or azomethine group. Hugo Schiff first created it in 1864. Schiff base legends were a famous class of compounds with the general formula R1 R2 C=NR3 (via R3 H), (Figure 2.1) (Da Silva et al. 2011; De Fátima et al., 2018).

Researchers are interested in the bioactivity of the Schiff base molecules and their metal complexes. Antifungal, antibacterial, anticancer, and anti-inflammatory effects of Schiff base molecules also their metals complexes have been widely reviewed (Kumar et al., 2009; Abdel-Rahman[3] et al., 2016; Wang et al., 2018).

Schiff base has several biological effects, involving anti-HIV, anti-malaria, and anti-urease properties. Since the imine group in all of these compounds is essential for their biological action, that moiety has received a lot of attention in searching for recent bioactive chemicals (Bringmann et al., 2004; Kajal et al., 2013; de Fátima et al., 2018).

R-NH2+R-CHOR-N=CH-R + H_2O

Primary amine + Ketone / Aldehyde = Schiff bases + water

Figure 2.3. The general structure of the Schiff Bases

Since Werner's time, the wide variety of physicochemical applications of azomethine imine functionalities has long been known. For example, it could be -CH=N- / >C=N- Focusing on whether condensation happened among primary amine and ketonic or aldehydic groups. As a result, the importance of inorganic and organic chemists designing compounds with novel functionalities has grown exponentially (Majid et al., 2019).

The imine nitrogen is a basic pharmacophore that is responsible for several activities. Imine or azomethine groups can be found in several natural, natural-derived, non-natural compounds, Figure 2.4 for many instances (Da Silva et al., 2011). In these substances, the existence of an imine group was shown to be critical to their pharmacological processes (Bringmann et al., 2004; Guo et al., 2007; Majid et al., 2019).

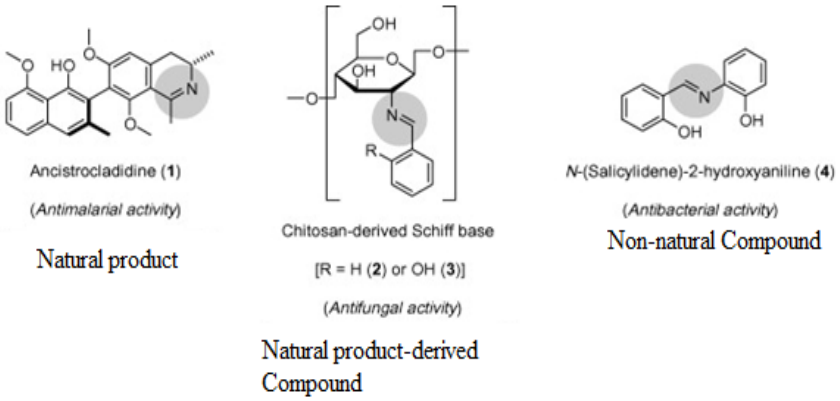


Figure 2.4. As an example, consider Schiff bases that have bioactive properties. In each molecule, the imine or azomethine group is shaded.

2.2.2. Synthesis and mechanism of schiff bases

Xavier and Srividhya (2014) studied that the Synthesis of Schiff base is usually catalyzed by acids or bases or by heat. Most major Schiff bases were crystalline solid that was mildly basic yet, under certain conditions, create insoluble salt via strong acid. Schiff bases were utilized as ligands to manufacture different

compounds with a range of structures or as intermediates in the Synthesis of amino acids.

In a Schiff base, a deprotonated phenolic group's O atoms, as well as a azomethine group's N atoms, typically coordinate as a Flexi-dentate ligand. The chemistry of Schiff base azomethane nitrogen and other donor atoms, such as oxygen, must be coordinated. As a result, an attempt is made to investigate the coordination chemistry of reduced Schiff base interactions with biologically active transition metals (Worku et al., 2002; Xavier and Srividhya, 2014).

Aromatic aldehydes, particularly those with a sound conjugation system, produce a persistent Schiff base, but aliphatic aldehyde was unstable and polymerized quickly. Schiff bases ligands were generated more easily with aldehydes than via ketone (carbonyl carbon) (Gaikwad et al., 2018).

Schiff bases are chelate ligands generated from very stable metal ion complexes bi, tri, or tetra-dentate. Preparative functions, qualifications, protections, as well as quantization of ketones and aldehydes, isolations of the carbonyl and amino substance, and Synthesis of this substance in the complicated or sensitive processes, had all been explored using their chemical and physical capabilities (Paquette 1968; Yoshizawa et al., 2005).

The production of the Schiff bases from the ketones/aldehydes was the reversible reaction that happens most frequently when aldehydes or ketones are heated or under acid or base catalysis. Isolation of a product, prevent the water, or both, is usually used to complete the formation. Aqueous acid or base may hydrolyze several Schiff bases back to their aldehydes, ketones, and amines (Solankee and Thakor, 2006) figure 2.5

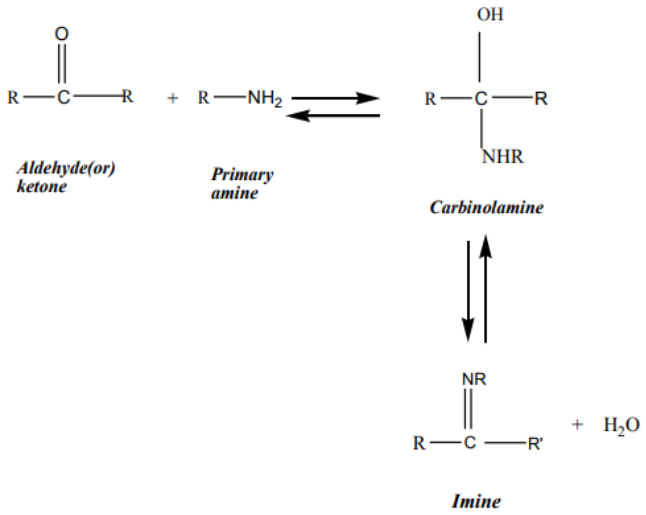


Figure 2.5. The development of such a Schiff base with an aldehyde

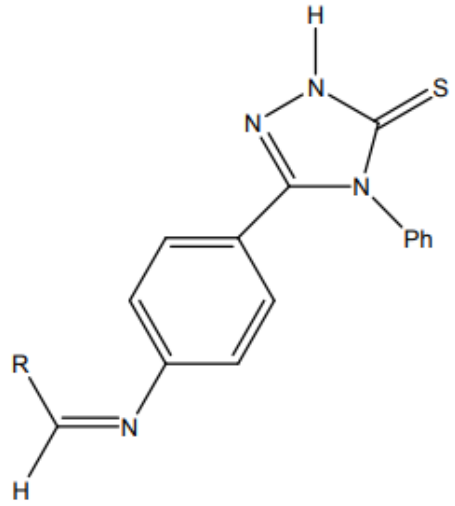
2.2.3. Biological significance of the schiff base

Schiff base has a varied range of structural features and is quite helpful in the science establishment. Because of their various uses, the imine characteristics of Schiff bases have piqued the interest of many scientists across the world. They're utilized as colours, chelating, antibacterial, and just as the model for the manufacture of antibiotics. Antibacterial, antifungal, antiviral, anti-inflammatory, antimalarial, and antioxidant capabilities of imines Schiff bases have been reported. Numerous ketones /aldehydes and amines utilized in the condensation processes, mostly not exclusively aromatic but heterocyclic rings, are responsible for their multifunctional features (El-Sayed et al. 2010; Tobriya, 2014).

The antibacterial and anticancer effects of Schiff bases' nitro and halo derivatives have been demonstrated. Several Schiff base has been shown to had antimicrobial and antifungal properties. Some Schiff base derivatives and Beta-Lactam are effective antibacterial agents (Meenachi and Chitra, 2015). The peak ethers of Schiff bases are also investigated and shown to have a significant probable for antibacterial activity (Rani et al., 2015)

Schiff's base in thiazoles, so their transition metals complexes have been demonstrated to have a broad spectrum of biological functions. Similarly, there are several Schiff bases via transitions metal compounds which indicate the extensive range of antibacterial action (Chandraleka et al., 2011; Ashraf et al., 2011)

In methanol, condensation 5-(4-aminophenyl) (4-aminophenyl) -4-phenyl-1,2,4-triazole To make a novel Schiff base, combine 3-thione with 4-methoxy benzaldehyde. The antibacterial activity of the chemicals generated was tested in vitro against bacterial strains, Gram-positive/Gram-negative. Against the bacterial that are examined, the synthesized chemical displayed distinct inhibitory zones (Rani et al., 2015).



R= 4 MeOC6H4

Figure 2.6. Schiff bases ligands prepare from the 5-(4-aminophenyl)-4-phenyl-1,2,4-triazole-3- thione via 4-methoxy benzaldehyde.

Schiff base of the Salicaldehyde and sulfonamides were created by gently adding sulphonamide to Salicaldehyde in an ethanol solution containing rare droplets of glacial acetic acid. The antibacterial screening of suitably described Schiff bases was carried out utilizing the paper disc method against various *Salmonella enteritidis* and *S. aureus* pathogenic strains (Figure 2.7). Schiff bases were shown to be effective against Salmonella enteritidis. In addition, the new Schiff bases provided a good response against *S. aureus* at all concentrations studied while examining the belongings of attention on the zone of inhibition of both components (Rani, 2015).

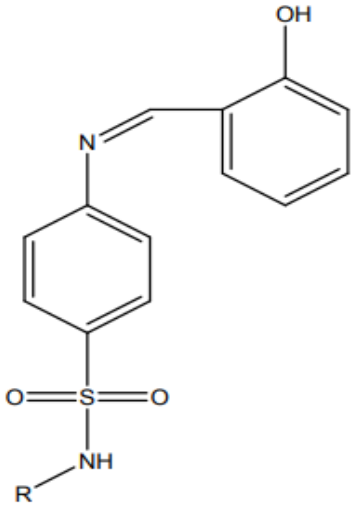


Figure 2.7. Schiff bases are derived from Salicaldehyde and sulfonamides.

A different Schiff bases from the three ethoxy salicy aldehyde and sulpha pyridine. A Schiff base was described and discovered to have significant antibacterial and antifungal action against various microbes (Figure 2.8). (Rani et al., 2015).

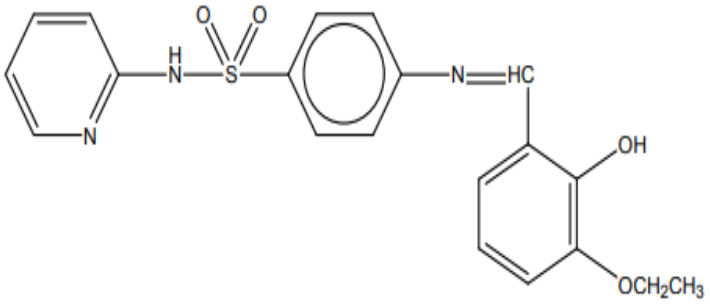


Figure 2.8. Schiff bases ligands, 4-(3-ethoxy-2-hydroxybenzylideneamino)-N-(pyridin-2- yl) benzenesulfonamide prepared from sulfapyridine & 3-ethoxysalicyl aldehyde

2.2.4. Antimicrobial studies with Schiff bases

2.2.4.1. Antibacterial activity

Both Gram-positive and Gram-negative bacteria are harmful to people's health and influence it. The consumption of germs such as *Salmonella typhi* and *E. coli* from contaminated water and food. Bacteria, such as *Clostridium*, *Pseudomonas* and

Bacillus sp., can spread diseases including typhoid, fever, and diarrhea in humans and animals (Myer et al., 2005; Rajabally et al., 2013).

Due to microbial resistance, therapeutic measures to treat these infections have several disadvantages. Although Schiff bases were potent antibacterial agents, metal complexations had been demonstrated in the literature to improve their biological activity, with bacteria responding differentially (Crowther-Gibson et al., 2011).

Shanty studied that, Seven distinct heterocyclic Schiff bases are also synthesized their antibacterial activity was evaluated. To make Schiff base ligands, carbonyls (pyrrole-2-carboxaldehyde & thiophene-2-carboxaldehyde) were mixed via aminophenol derivative (2-amino 4-methyl phenol, 2-amino 4-nitrophenol, 2-aminophenol, or 2-amino benzimidazole,) in the warm methanol. A antibacterial activity of a generated ligands is found utilizing the agar diffusion technique toward *Salmonella typhi, Bacillus pumills, K. pneumonia* bacteria, *Clostridium, Bacillus circulans, Bacillus coagulans, Pseudomonas,* and *E. coli* (Shanty et al., 2017).

A compound in (figure 2.9.) was the most effective ligand against *Salmonella typhi,* with 17 mm diameter zones to inhibited growth thing also a MIC of 25 µg/mL. Other strains investigated showed low to moderate activity, and test chemicals had a little cytotoxic impact on *E. coli. Bacillus subtilis* and *E. coli* were used to investigate the antibacterial activities of a metal-free ligand and complex. Six 2-Chloro substitute ligand and their metals analogues have a higher MIC (325-425 µg/mL) against these bacteria than another substituted ligand (MIC 330-455 µg/mL). According to the structure-activities connection of ligands, enlarged heterocyclic rings (indoline-2,3-dione) significantly boosted the pharmacological activity, making it more active against all bacteria tested (Fonkui et al., 2018).

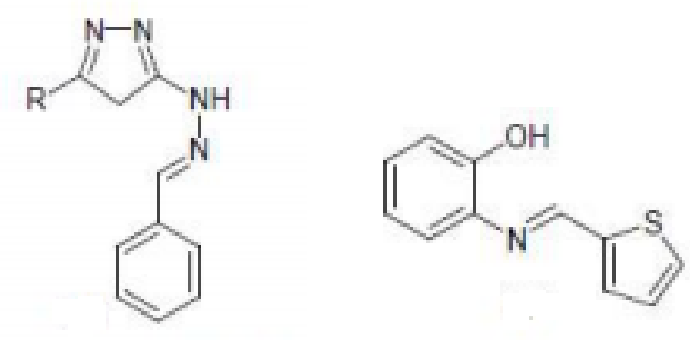


Figure 2.9. Molecular structure of certain Schiff base substances with antibacterial activity.

2.2.4.2. Antifungal activity

Fungi were tiny organisms that cause a broad range of illnesses also were significant producers of secondary metabolites like mycotoxins (Njobeh et al., 2009).

It has been recorded that inorganic compounds like silica-based material, like. Binders, as well as their chemicals alterations, are used to reduce fungal infection. Their health consequences and presence in commodities, on the other hand, frequently harm food quality. For these motives, the need to discover new chemicals to combat microbial resistance to commercially available medicines will never go away (Diaz et al., 2004; García-Pérez et al., 2013).

Recently, Maddila et al. (2016), reported on the novel Schiff bases built from benzothiazole pyrimidine derivatives: production and antifungal activity. A broth dilutions technique is utilized to evaluate a ligands' MIC on the *Candida albicans(C. albicans), Aspergillus flavus, Penicillium marneffei, Aspergillus fumigates* and *Penicillium mucor.* The whole of the organism tested is sensitive to a ligand with moderate to excellent antifungal activities at 100-200 µg/mL doses. It is discovered which the para-substitution is superior to the ortho-substitution. It has a stronger antifungal activities (MIC 12.5-25 µg/mL) than ciprofloxacin (MIC 12.5-50 µg/mL).

The metabolic activity of all analyzed fungal strains was affected by the produced seven Schiff base ligands. The chemicals 3,5-bis(trifluoromethyl)aniline and salicylaldehyde exhibit more action against the strains tested (MIC 12.5 µg/mL) clotrimazole (MIC 25 µg/mL). The ligand is two times more potent than clotrimazole toward *A. flavus* and *A. fumigatus,* also four times more effective toward *P. mu*cor *and C. Albicans,* according to these data. Lipophilicity scores ranging from 5.4 to 6.17 may be directly related to the efficacy of a chemical (Yıldız et al., 2015).

Using the disc diffusion technique, *A. niger*, *Rhizopus oryzae,* and *C. albicans* were used to investigate antifungal activities of the Schiff bases produced from the variety of the Schiff bases 1,3-benzothiazole-2-yl-hydrazones. All of the compounds were made by combining 6-chloro2-benzothiazol-2-yl-hydrazine with various aromatic carbonyls in hot ethanol. Compounds A and B, for example, were equally efficient toward *A. niger*, while Compounds A is more powerful toward *Rhizopus oryzae* and *C.a albicans.* Furthermore, the activities of this compound were improved by substituting electrons losing groups (-CH3) in the para positions. Also, electrons withdraw groups (-NO_2 and -Br) in the exact position in figure 2.10 (Asati et al., 2015).

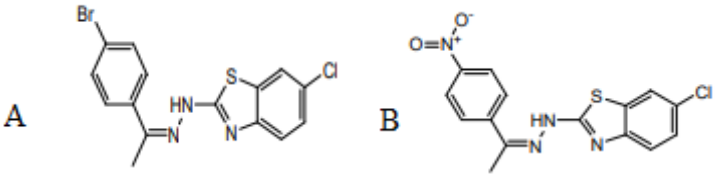


Figure 2.10. Molecular structure of certain Schiff base substances or rather their metal complexes via antifungal activity

2.2.4.3. Antimalarial activity

Malaria parasites have infected over 40% of the world's population as a result of an increase in pesticide-resistant vectors, the proliferation of drug-resistant malaria parasites, as well as the health effects of existing antimalarial drugs, necessitate the development of more effective unique and far less toxic antimalarial drugs

via different mechanisms of action. Compounds containing azomethine (C=N) have the potentials to help combat drug resistance. Sulphonamide and its derivatives and Schiff bases containing thiosemicarbazone moieties have been proven to be efficient antimalarial parasites (Supuran and Scozzafava, 2007; Katwal et al., 2013).

Katwal et al. (2013), studied that, A set of 50 Schiff bases generated from the aromatics sulfonamide also analogues were tested therefore inhibitor of the *Plasmodium falciparum* carbonic anhydrases enzymes, which is required for the pyrimidine biosynthetic pathway and whose metabolic activity in malaria parasites is explained below. The effectiveness of these Schiff bases on the *Plasmodium* enzyme was compared to that of the therapeutic medication acetazolamide. The antimalarial activity of the compounds, according to the authors, was primarily determined by the type of the substituted aromatic aldehydes employed to make a ligand. Sulphonamide-derive Schiff base includes modified (2-methoxyphenyl; 2- or 4-chlorophenyl; 2- or 4-hydroxyphenyl; 3-methoxy-4-hydroxy-5-bromophenyl) aldehydes, have been discovered to be potent inhibitors of carbonic anhydrase and other enzymes. Via the affinity constant (KI) ranging to the 0.54-1.23 µg/mL, substances 2.26-2.29 (Figure 2.11) reduced parasite activity, while substances 2.30 and 2.32 (Figure 2.11) are more effective derivatives against carbonic anhydrase enzyme, with KI ranging from 80 to 0.5 ng/mL. The unsubstituted compound 2.31 has antimalarial action as well. Compared to acetazolamide (KI, 315 ng/mL), the substances are four times more productive at KI (80 ng/mL).

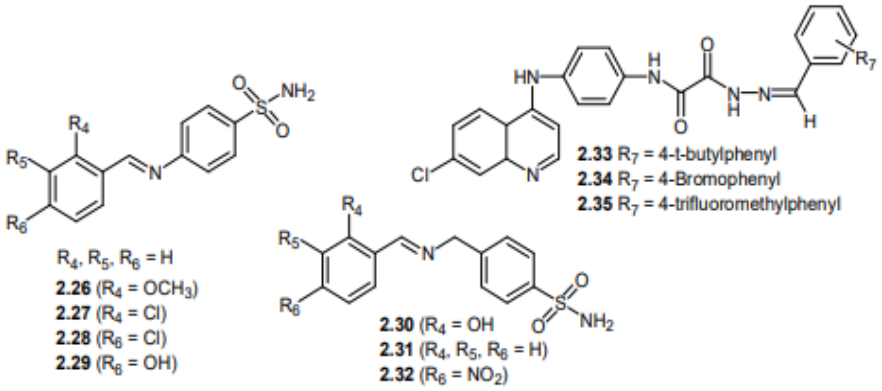


Figure 2.11. The molecular structures of a few antimalarial Schiff base substances

2.2.4.4. Antiviral activity

Medicine resistance caused by viruses is a significant community health concern, and treating associated diseases necessitates the development of new bioactive chemicals.

The antiviral effects of metal complexes of Schiff bases ligands produced to 5-amino-4-phenyl-4H-1,2,4-triazole-3-thiol have been studied. Al-Masoudi et al. (2009) performed the MT-4/MTT test to investigate a synthesis also antiviral activities of various Schiff bases in the human T-lymphocyte (MT-4) cells toward HIV-1 (strain IIIB) & HIV-2 (strain ROD). An EC_{50} (effective concentration of the chemical (µM) providing 50% protection against the cytopathic impact of HIV in MT-4 cell lines) was determined. The studied strains were shown to be sensitive to ligand, so their complexes, via metal-based ligands showing the most activity. The most potent anti-HIV drugs tested from this class were compounds 2.41 and 2.42 (Figure 2.12) that also inhibited HIV-2 replication through cultured cells at EC50 10.2 g/mL via an index specificity of 9.Compound 2.42, a gold complex, had more muscular antiviral activity than the other compounds, possibly due to gold's antibacterial properties and Tweedy's hypothesis may have also affected membrane permeability.

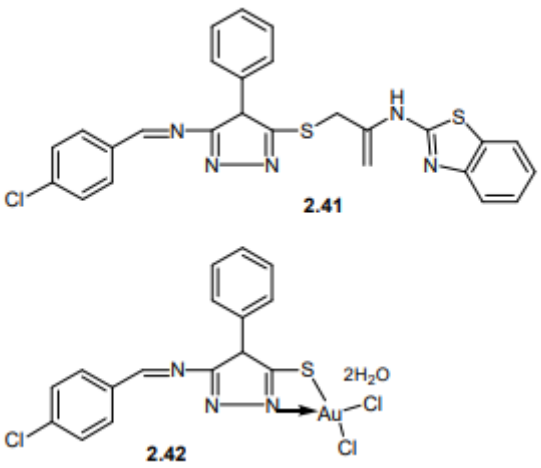


Figure 2.12. The molecular structure of certain antiviral Schiff bases as well as complexes

2.3. Methods Used in Antimicrobial Agent Research

Antimicrobial susceptibility testing uses mainly two methods. These methods are categorized as "diffusion" and "dilution."

2.3.1. Diffusion tests

Bacterial antimicrobial susceptibility testing is a typical problem in standard laboratories. Drug research, epidemiology, and treatment outcome prediction can benefit from antimicrobial susceptibility testing (Nagy et al., 2018).

2.3.1.1. Agar disk-diffusion approach

The disks diffusions test is frequently employed. However, anaerobes take advantage of the vast variation in diameter to the individual MIC and the weak relationship found among a MIC's value so diameter zones, which leads to significant and severe errors. Agar disk-diffusion test, established in 1940, was a widely used method for regular antimicrobial susceptibility in several clinical microbiology labs (Heatley, 1944). The Laboratory / Clinical Standard Institutes now provide recognized and certified standards for bacteria and yeast testing (Clsi and Wayne, 2004). While this method could be utilized to evaluate only those fastidious bacteria, it's been used to standardize the evaluation of specific particular bacterial pathogens, including *streptococci, Haemophilus parainfluenzae, meningitidis, Neisseria gonorrhoeae, Haemophilus influenza* and *Neisseria*, along with the use of specific culture media, different incubation conditions, or rather appropriate help for inhibition areas figure 2.13, (Balouiri et al., 2016).

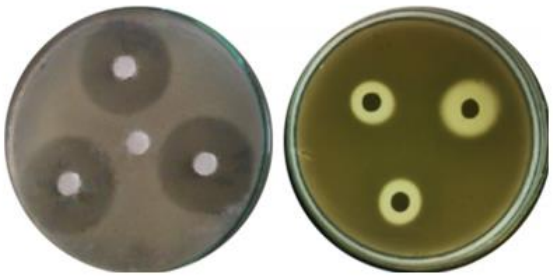


Figure 2.13. Disk-diffusion technique, using agar diffusion methods.

2.3.1.2. E-test (Antimicrobial Gradient technique)

An antimicrobial gradients technique with the concepts of the dilution & diffusion processes to get the MIC values. It is concerned with an antimicrobial agent's ability to produce a concentration gradient in agar media. An E-tests are the for-profit variation of this method. In this approach, agar surfaces previously infected via a microorganism under investigation was covered with the strip impregnated via the enhancing concentrations gradient of an antimicrobial agent to one end to another, figure 2.14. Antibiotics, antifungals, and anti-mycobacterials' MICs are determined using this method (Hausdorfer et al., 1998). MIC values measured by E-test also these acquired by the broth dilutions or the agar dilutions method have demonstrated a high agreement in earlier investigationsfigure 2.14. This method may also look at the antibacterial interactions of two medicines (Gupta et al., 2015; Bailey et al., 2018).

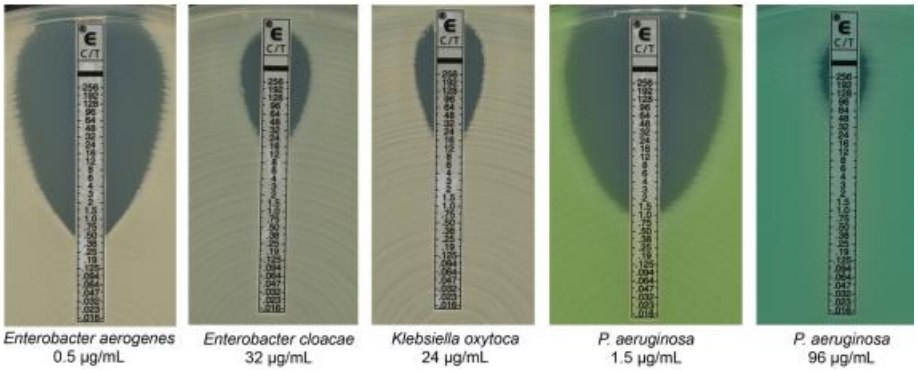


Figure 2.14. Interpretation of E-test results.

2.3.2. Dilution tests

The diluent methods which aid in determining the density of the tested antimicrobial agents in agar (agar dilutions) or broth intermediate are best suited for deciding MIC value (microdilution/microdilution). A broth or the agar dilute technique could be utilized to measure in vitro antimicrobial activities toward fungi & bacteria. A MIC value, usually stated in mg/mL or mg/L, was the lowest dosage to a tested antimicrobial agent, suppressing an obvious growth thing of a microorganism assayed. A few widely recognized concepts for dilutions antimicrobial susceptibility test of fastidious and non-fastidious bacteria, yeast, or even filamentous fungi (Pfaller et al., 2004).

2.3.2.1. Broth dilution technique

Among the most fundamental antimicrobial susceptibility, clinical trials are the broth micro- or macro-dilution method. Making two-fold dilute of antimicrobial agents (like., 1, 2, 4, 8, 16, & 32 mg/mL) in the liquids growth media provided in the tubes with the minimum capacity of the 2 mL (microdilution) or utilizing a 96-good microtitration plate with lower quantities are part of the method (microdilution). Evey tube or good has been injected with a microbial inoculum processed in the same medium after a standardized microbial suspension was diluted to 0.5 McFarland scale. After thoroughly mixed, inoculation tubes or 96-well microtitration plates were incubated under optimal conditions, relying on the testing microorganism (Balouiri et al., 2016).

2.3.2.2. Agar dilution technique

Agar dilutions were the golden standard for measuring MICs since it offers the most precise and repeatable results. However, it is time-consuming and challenging to utilize in everyday operations, figure 2.15. Antibiotics and other compounds with antibacterial action (bactericidal activity) or antibacterial activity (bacteriostatic

activity) are added to agar dilution. These techniques evaluate antibiotic susceptibility, not other antimicrobial biocides such as preservatives and disinfectants (Wiegand et al., 2008; Rocha-Santos and Duarte, 2014).

After integrating required amounts of antimicrobial agents into the agar media (molten agar media) via consecutive two-fold dilution, a agar dilution technique involves inoculating a predefined microbial inoculum on an agar plate's surfaces. Under optimum culture conditions, the MIC endpoint was well-defined; therefore, a lowermost dose of the antimicrobial agents fully suppresses growths. Antibacterial and antifungal susceptibility may be determined using this approach. When several separations were being tasted toward the single component, or when the chemical (or extract) being tested has a colour that hides Bacterial growth in a liquid media, the agar dilutions technique was usually preferred over broth dilutions for the MIC determinations. For fastidious organisms like anaerobes and *Helicobacter* spp., agar dilutions were frequently suggested, therefore the standardized approach. It's also been used to cure *Candida* spp., *A. niger*, *Fusarium niger*, and dermatophytes using antifungal agent-drug combinations (Balouiri et al., 2016).

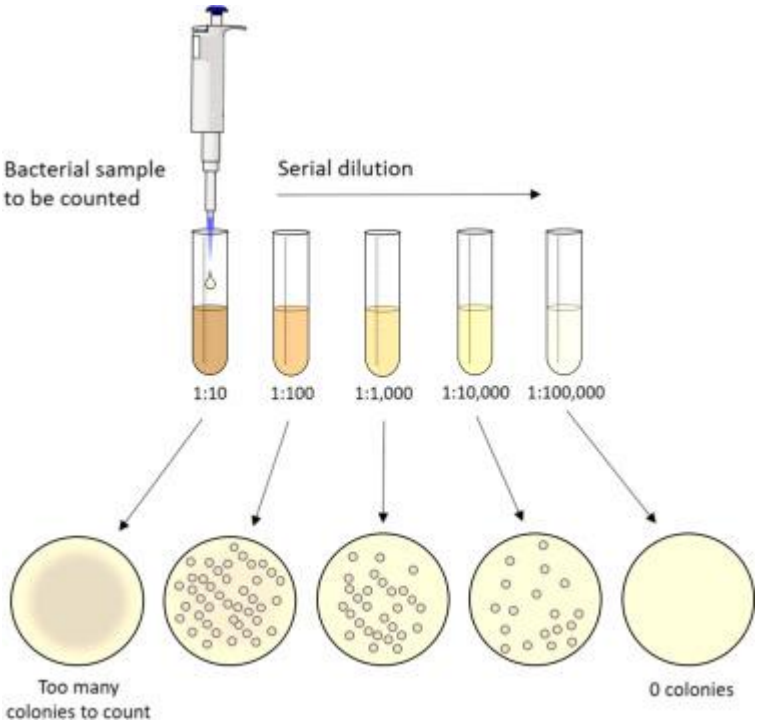


Figure 2.15. Schematic representation of the colony-forming unit (CFU) growth on agar plates.

2.4. Types of Bacteria used in our study

2.4.1. *P. aeruginosa*

P. aeruginosa was the Gram-negative, aerobic rod bacterium of the *Pseudomonadaceae* family (a *Gammaproteobacteria* member) and a harmful pathogenic bacterium that sources mild to long-lasting, life-threatening infections in patients with weakened immune systems, including chronic and acute infections. It has a pearlescent look and smells like grapes or tortillas. *P. aeruginosa* thrives at temperatures ranging from 25 to 37 ° C, and its ability to develop at 42 ° C sets it apart from many other *Pseudomonas* sp. *P. aeruginosa* is a common bacteria that may thrive in a range of environments. Increased mortality is due mainly to the development of drug-resistant diseases (El Zowalaty et al.,2015, Fujii et al., 2014).

Intrinsically chromosomal encode or genetically sent resistances to determine to impact the main types of the antibiotics like quinolones, aminoglycosides, β-lactams and polymyxins in *P. aeruginosa*. Aminoglycosides carbapenems (meropenem, imipenem,), (netilmicin, tobramycin, gentamicin, amikacin), cephalosporins (ceftazidime, cefepime,), fluoroquinolones (levofloxacin, ciprofloxacin,), and penicillin via β–lactamase are among the antibiotics often used to treat *P. aeruginosa* Alterations in regulatory systems that regulate the expression of the resistances mutations, determinant, membranes permeability alters, also horizontal acquisitions to the antibiotic-inactivating enzyme or enzyme that promote targets alters all contribute to an emergence of MDR, XDR, and PDR strains. The simultaneous development of these mechanisms confers multi-resistance to multiple songs, which is noteworthy (El Zowalaty et al., 2015; Musthafa et al., 2017; Bassetti et al., 2018).

2.4.2. *E. coli*

E. coli was a gram-negative, facultative anaerobic bacterium that looks like a rod with a short tail under the microscope. Catalase is produced but not oxidase. It thrives in temperatures ranging from 37 to 44 ° C. According to taxonomy classification, it belongs to the class *Gammaproteobacteria,* the order *Enterobacteriales,* and the family *Enterobacteriaceae.* Pathogenic strains can cause gastroenteritis, urinary tract infections, meningitis, and wound infections. Some *E. coli* serotypes can release toxins that cause bloody diarrhoea or hemolytic-uremic syndrome. Humans and other animals' digestive tracts are host to bacteria from this species, which are a significant part of their microbiota (Leimbach et al., 2013; Adeolu et al., 2016; Vijayakumar et al., 2018; de Mello Santos et al., 2020).

E. coli MDR had become the major problem which was enhancing being encountered in the humans and veterinary across the world. Even though E. coli was naturally resistant to almost whole therapeutically related antimicrobial treatments; it has a significant capacity for resistance gene accumulation, primarily by gene transfering. The acquisition of genes encoding extended-spectrum -lactamases is amongst the most complicated processes (which confer resistances to the broad-spectrum cephalosporins), carbapenemases (which confer resistance to the carbapenems), 16S rRNA methylase (which confer pan-resistances to the aminoglycosides), and plasmid-mediated quinolone resistances gene (which confer resistance to [fluoro]quinolones) (Poirel et al., 2018; Croxen and Finlay, 2020).

2.4.3. *S. aureus*

S. aureus is a Gram-positive, non-motile, coagulase-positive coccoid bacteria that often infect humans and belongs to the *Firmicutes* phylum. *S. aureus* is a leading cause of bloodstream infection in people of all ages, both in the community and in hospitals (Asgeirsson et al., 2018; Chang et al., 2020).

S. aureus was discovered in surgical wound pus for the first time by Alexander Ogston, who named the bacteria micrococci at the time. Ogston named the bacterium *staphylococci* for their distinctive appearance of grape-such clusters (staphyle in the Greek) of the sphere-shaped bacterial, which isolates them to chain-forming *streptococci*, which are involved via surgical wounds infection (Thomer et al., 2016; Said et al., 2020).

Antimicrobial resistance and evasion of the human immune system make *S. aureus* a therapeutically important pathogen. *S. aureus* with *β*-lactam resistance and resistance to additional antibiotic classes such as Vancomycin, Daptomycin, and Clindamycin in most clones. MRSA's clinical symptoms range from asymptomatic nasal mucosa colonization to moderate skin and soft tissue infections to fulminant invasive diseases with a high death rate (Archer et al., 2011; Liu et al., 2011; Lee et al., 2018)

Approximately 20–25 % of humanity have been colonizing on a long-term basis, while another 75–80 per cent had been colonized sporadically or not at all (Kluytmans et al., 1997; Dall'Antonia, 2005). *S. aureus* colonizes the nares, the skin, in a stable manner. However, *S. aureus* is also a pathogen that causes skin and soft tissue infections through bloodstream infections. Once S. aureus enters circulation, it multiplies and disseminates to several sites, cause severing disorder manifestations like sepsis, infectives endocarditis, and deep-seated abscesses in nearly every organ tissue (David et al., 2010).

Our study tested the antimicrobial effects of Fluorinated 5-tert-butyl Salicylaldehydes from Schiff bases on *P. aeruginosa*, *E. coli* and *S. aureus* with the MTT method to develop alternative antimicrobial agents to drug resistance. In addition, we wanted to create new antimicrobial medications or improve existing ones to combat bacterial mutation, overcome resistance, and evaluate the potentiality and effectiveness of newer Schiff base as antibacterial agents, which will be tested to specify new antimicrobial agents.

3. MATERIAL and METHOD

3.1. Materials

3.1.1. Chemical compounds

Fluorınated 5-tert-butyl Salicylaldehyde from Schiff bases Compound 1-5, obtained from Harran University, Faculty of Arts and Sciences, Chemistry Department, Inorganic Chemistry Department. After the chemicals are dissolved in 10 mM alcohol, the syringe type is sterilized by passing through 20 µm pore diameter filters.

Compound 1: 2,3, fluoro-5-tert-butyl Salicylaldehyde
M.W: 289 g/mol

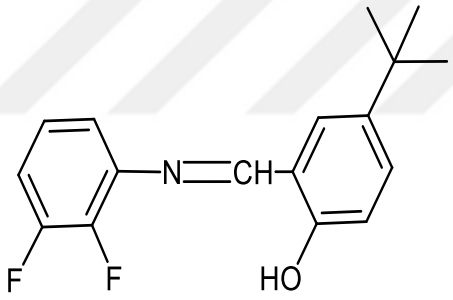


Figure 3.1. The open formula of compound 1.

Compound 2: 2,4, fluoro-5-tert-butyl Salicylaldehyde
M.W: 289 g/mol

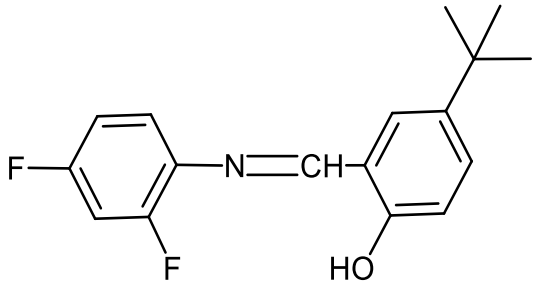


Figure 3.2. The open formula of compound 2.

Compound 3: 2,5, fluoro-5-tert-butyl Salicylaldehyde

M.W: 289 g/mol

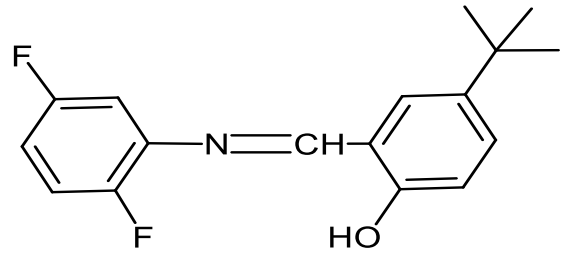


Figure 3.3. The open formula of compound 3.

Compound 4: 3,4, fluoro-5-tert-butyl Salicylaldehyde

M.W: 289 g/mol

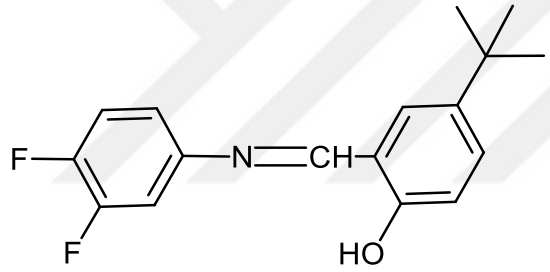


Figure 3.4. The open formula of compound 4.

Compound 5: 3,5, fluoro-5-tert-butyl Salicylaldehyde

M.W: 289 g/mol

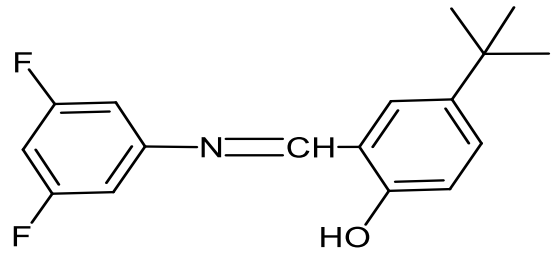


Figure 3.5. The open formula of compound 5.

3.1.2. MTT Stain

MTT (3- (4,5-Dimethylthiazol-2-yl) -2,5-Diphenyltetrazolium Bromide) colour utilized to determines a cytotoxicity and antimicrobial effect of newly synthesized Fluorınated 5-tert-butyl salicylaldehydes from Schiff bases on *S. aureus*, *P. aeruginosa* and *E. coli*. MTT stain prepared as 300 µM.

3.1.3. Bacteria Strains

E. coli (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 29213), lyophilized bacterial strains purchased in the study. These strains are among the bacteria that develop the most antibiotic resistance and are on the WHO's list of bacteria for which urgent drug development is desired.

3.1.4. Media

Resuscitation medium utilized for ta revitalization of lyophilized bacteria, nutrient agar to reproduce bacteria, and nutrient Broth medium for antibacterial effect tests.

3.1.5. McFarland standard

McFarland standard prepared to measure the number of bacteria ($0.5x10^6$ CFU / mL) in the medium of 0.5 McFarland equivalent.

3.2. Method

3.2.1. Preparation of compounds for analysis

Fluorınated 5-tert-butyl salicylaldehydes from Schiff bases and molar solutions of compounds, It was weighed on a precision balance (Sartorius) to a concentration of 10 mM relative to their molecular weight.

3.2.1.1. Sterilization of compounds

Compounds dissolved in alcohol were sterilized using a 20 µm pore diameter injector type filter (Minisart®, Biotech, USA) to be 10 mM. Figure 3.6.

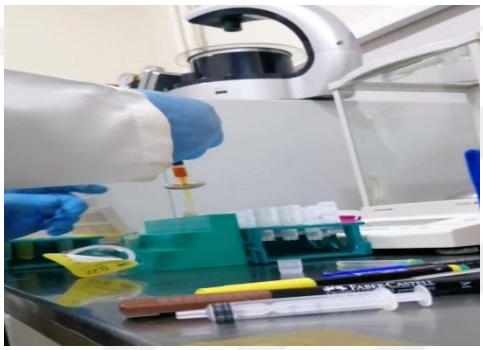


Figure 3.6. sterilization of compounds

3.2.1.2. Dose adjustment of compounds

Fluorınated 5-tert-butyl salicylaldehydes from Schiff bases, ten mM Stock solutions were prepared after dissolving in alcohol as one µM, ten µM, 100 µM and 1000 µM.

3.2.1.3. Preparation of culture plates

Compounds; 1 µM, ten µM, 100 µM, & 1000 µM are added to 96-well culture plates in 10 µl volumes, in a triple pattern. Alcohol utilized just as solvent was added to control wells in books of 10 µL. Figure 3.7.

To compare the antimicrobial effects of the chemicals, gentamicin was utilized just as positive control; also, pure DMSO was being used as solvent blank.

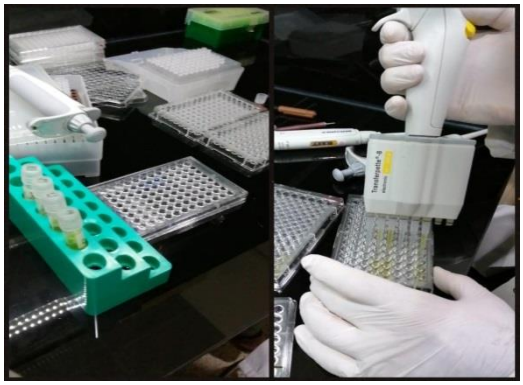


Figure 3.7. Preparation of culture plates

3.2.2. Bacterial culture

First, cultures of Lyophilized bacterial strains started. Resuscitation medium prepared for the bacteria in the lyophilized form provided. The resuscitation medium was prepared as 50 mL in a 100 mL flask sterilized in an autoclave at 121 °C for 15 minutes to stimulate the bacteria. Next, the bacteria culture in lyophilized form was transferred under aseptic conditions to the sterilized resuscitation medium (initial volume 1 mL, after dissolution transfer to 50 mL). These revitalization environments are kept in the incubator that shakes in a circular motion at 37 °C and 120 rpm for 24 hours to revitalize the bacteria culture. After 24 h, the bacteria culture taken from the resuscitation medium was transferred to the solid medium prepared in petri dishes (Nutrient Agar 28 g/L). It was kept in the incubator at 37 ° C for 24 h allowing the bacteria to grow on the solid media surface. Petri dishes taken from the incubator at the end of 24 hours, kept at +4 ° C for later applications.

For micro-dilution, after adding 100 µl to each of the wells containing liquid medium, the compounds were added at varying doses (1, 10, 100 and 1000 µM) in triple order. Finally, bacterial strains were added in an amount of $0.5x10^6$ CFU / ml

to each well where the compounds were planted, so incubated at thirty-seven ° C for 18 hours.

3.2.3. Cytotoxicity (MTT Method)

After the 18 h incubation of bacteria in 96-well culture plates to which Fluorınated 5-tert-butyl salicylaldehydes ligands were added, MTT test was applied to determine the ratio of live and dead bacteria in the culture medium. For this purpose, 10 µl of MTT solution at a dose of 300 µM add to every culture good also incubated for 4 hours in the same culture medium. In the meantime, MTT converted into MTT-formazan by living bacteria in culture wells and yellow colour resulting from MTT in culture wells converted into purple tetrazolium salts. Dimethylsulfoxide (DMSO) was added to each well at a dose of 100 µl to dissolve MTT-formazan formed after incubation. With the effect of DMSO, an increase in colour intensity can be observed with the amount of MTT-formazan dissolved in the culture wells.

Results were analyzed calorimetrically in the spectrophotometer. For this purpose, culture plates loaded into ELIZA plate reader (Thermo Scientific, Country) read at 570 nm wave-length where optimum absorbance obtained. Optical density (OD) values obtained from culture wells were used to determine each compound's MIC values, and gentamycin was used as the positive control.

3.2.4. Statistical analysis

The data obtained are expressed, therefore mean and (SD). The reliability of data obtained from the test wells was tested by performing the Homogeneity test from the OD values obtained from the MTT test. Linear regression analysis was used to calculate MIC values. OD values of solvent blank wells were derived from the OD values obtained with test wells and control.

4. RESEARCH FINDINGS and DISCUSSION

4.1. Research outcomes

4.1.1. Antibacterial effects of gentamicin on *P. aeroginosa*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of gentamicin are presented in Table 4.1.

Table 4.1. OD values were obtained from the incubation of *p. aeroginosa* with gentamicin and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Genta	OD Values	3.90±0.53	2.097±0.84	1.847±0.42	1.181±0.79	1.094±0.12	P=0.264
				MIC_{50}: 1.43 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation to ***P. aeroginosa*** via gentamicin during the culture process is presented in Figure 4.1.

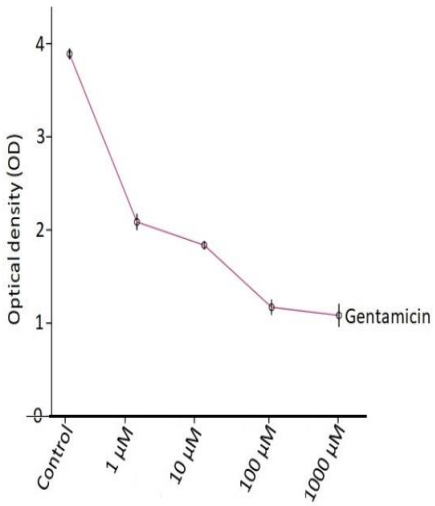


Figure 4.1. Antibacterial effects of gentamicin on *P. aeroginosa.*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of gentamicin on *P. aeroginosa* was found to be 1.43 µM.

4.1.2. Antibacterial effects of compound 1 on *P. aeroginosa*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 1 are presented in Table 4.2.

Table 4.2. OD values were obtained from the incubation of *p. aeroginosa* with Compound 1 and MIC_{50} values calculated using OD values.

		Doses (µM)					
		Control	1	10	100	1000	Homogeneity of variance
Compound 1	OD values	3.90±0.53	2.146±0.53	1.971±0.01	1.814±0.1	1.710±0.92	P=0.038
				MIC_{50}: 11.5 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of the *P.aeroginosa* via Compound 1 during the culture process is presented in Figure 4.2.

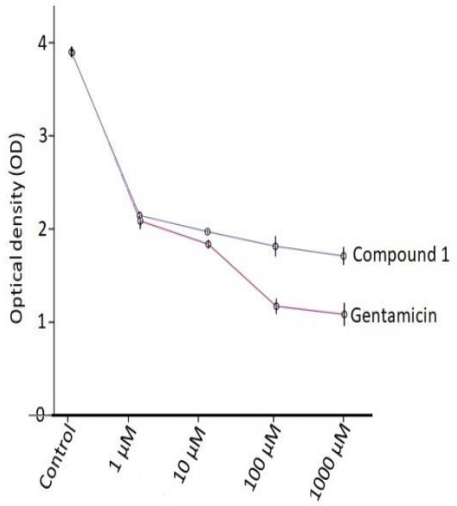


Figure 4.2. Antibacterial effects of Compound 1on *P. aeroginosa*.

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 1 on *P. aeroginosa* was found to be 11.5 µM.

4.1.3. Antibacterial effects of compound 2 on *P. aeroginosa*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 2 are presented in Table 4.3.

Table 4.3. OD values were obtained from the incubation of *p. aeroginosa* with Compound 2 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 2	OD values	3.90±0.53	2.768±0.03	2.012±0.07	1.840±0.03	1.735±0.00	P=0.06
				MIC_{50}: 9.56 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of the *P. aeroginosa* via Compound two during the culture process is presented in Figure 4.3.

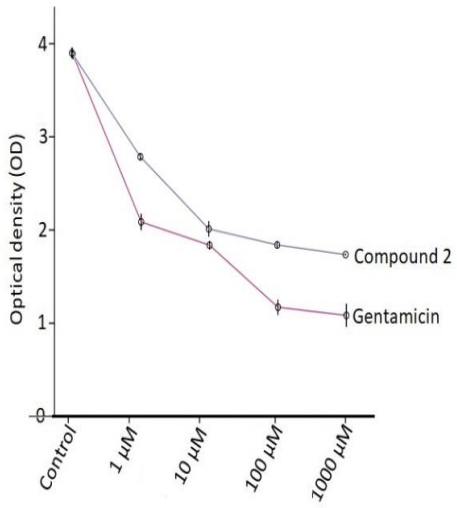


Figure 4.3. Antibacterial effects of Compound 2 on *P. aeroginosa*.

In the regression analysis using OD values obtained from the MTT test,

the MIC_{50} value of Compound 2 on *P. aeroginosa* was found to be 9.56 µM.

4.1.4. Antibacterial effects of compound 3 on *P. aeroginosa*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 3 are presented in Table 4.4.

Table 4.4. OD values were obtained from the incubation of *p. aeroginosa* with Compound 3 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 3	OD values	3.90±0.53	2.819±0.1	2.428±0.08	1.852±0.05	1.712±0.07	P=0.84
				MIC_{50}: 9.9 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of the *P. aeroginosa* via Compound three during the culture process is presented in Figure 4.4.

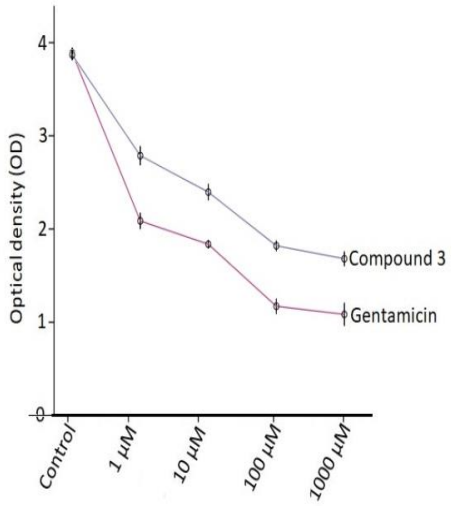


Figure 4.4. Antibacterial effects of Compound 3 on *P. aeroginosa.*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 3 on *P. aeroginosa* was found to be 9.9 µM.

4.1.5. Antibacterial effects of compound 4 on *P. aeroginosa*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 4 are presented in Table 4.5.

Table 4.5. OD values were obtained from the incubation of *p. aeroginosa* with Compound 4 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 4	OD values	3.90±0.53	3.073±0.09	2.351±0.03	2.048±0.11	1.868±0.00	P=0.07
				MIC_{50}: 12.6 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of the *P. aeroginosa* via Compound four during the culture process is presented in Figure 4.5.

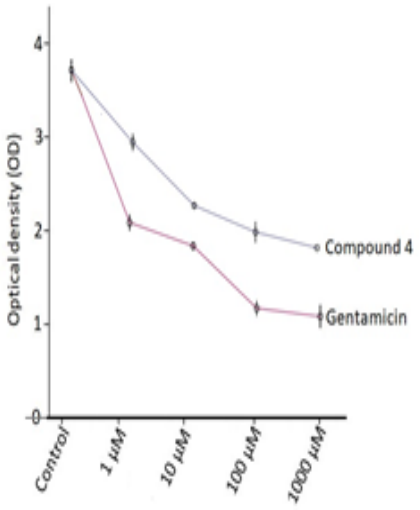


Figure 4.5. Antibacterial effects of Compound 4 on *P. aeroginosa.*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 4 on *P. aeroginosa* was found to be 12.6 µM.

4.1.6. Antibacterial effects of compound 5 on *P. aeroginosa*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 5 are presented in Table 4.6.

Table 4.6. OD values were obtained from the incubation of *p. aeroginosa* with Compound 5 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 5	OD values	3.900±0.05	3.150±0.03	2.675±0.5	2.238±0.02	1.111±0.03	P=0.00
				MIC_{50}: 3.46 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of the *P. aeroginosa* via Compound 5 during the culture process is presented in Figure 4.6.

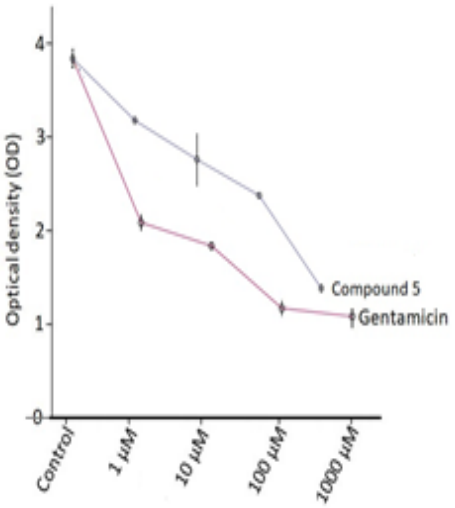


Figure 4.6. Antibacterial effects of Compound 5 on *P. aeroginosa.*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 5 on *P. aeroginosa* was found to be 3.46 µM.

4.2.1. Antibacterial effects of gentamicin on *E. coli*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of gentamicin are presented in Table 4.7.

Table 4.7. OD values were obtained from the incubation of *E. Coli* with gentamicin and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Genta	OD values	3.887±0.08	1.341±0.06	1.270±0.02	1.196±0.05	0.982±0.05	P=0.36
				MIC_{50}: 1.93 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of ***E. Coli*** via gentamicin during the culture process is presented in Figure 4.7.

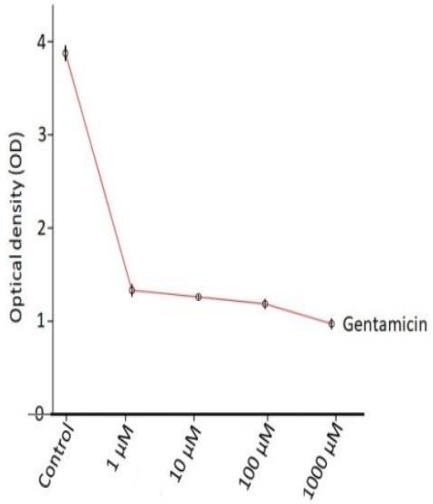


Figure 4.7. Antibacterial effects of gentamicin on *E. coli*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of gentamicin on *E. coli* was 1.93 µM.

4.2.2. Antibacterial effects of compound 1 on *E. coli*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 1 are presented in Table 4.8.

Table 4.8. OD values were obtained from the incubation of *E. coli* with Compound 1 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 1	OD Values	3.887±0.08	3.697±0.05	3.079±0.03	2.546±0.04	2.004±0.06	P=0.64
				MIC_{50}: 18.9 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of *E. coli* via Compound 1 during the culture process is presented in Figure 4.8.

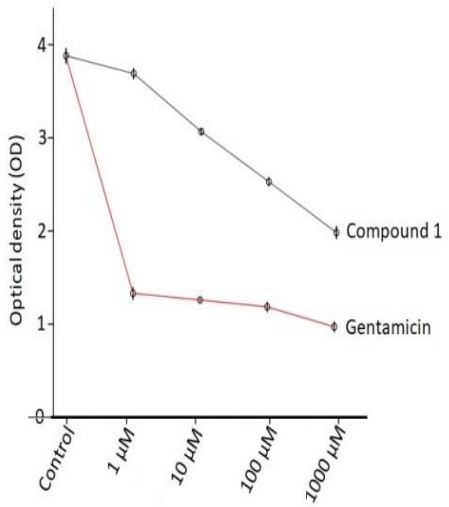


Figure 4.8. Antibacterial effects of Compound 1on *E. coli*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 1 on *E. coli* was 18.9 µM.

4.2.3. Antibacterial effects of compound 2 on *E. coli*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 2 are presented in Table 4.9.

Table 4.9. OD values were obtained from the incubation of *E. coli* with Compound 2 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 2	OD values	3.887±0.08	3.787±0.13	3.618±0.05	3.306±0.05	3.031±0.12	P=0.26
				MIC_{50}: 40.4 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of *E. coli* via Compound two during the culture process is presented in Figure 4.9.

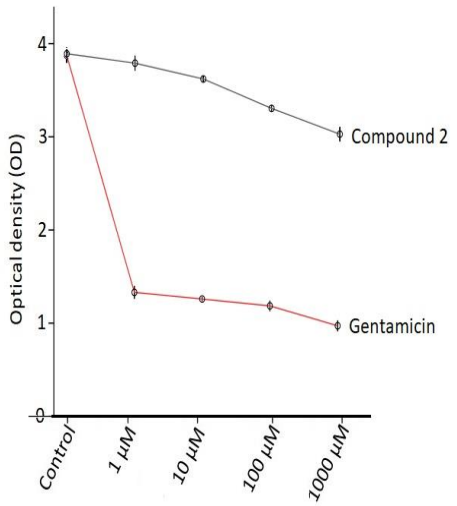


Figure 4.9. Antibacterial effects of Compound 2 on *E. coli*

In the regression analysis using OD values obtained from the MTT test,

the MIC_{50} value of Compound 2 on *E. coli* was 40.4 µM.

4.2.4. Antibacterial effects of compound 3 on *E. coli*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 3 are presented in Table 4.10.

Table 4.10. OD values obtained from the incubation of *E. coli* with Compound 3 and MIC_{50} values were calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 3	OD values	3.887±0.08	3.558±0.12	3.445±0.04	3.307±0.08	2.926±0.08	P=0.68
				MIC_{50}: 39.4 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of *E. coli* via Compound three during the culture process is presented in Figure 4.10.

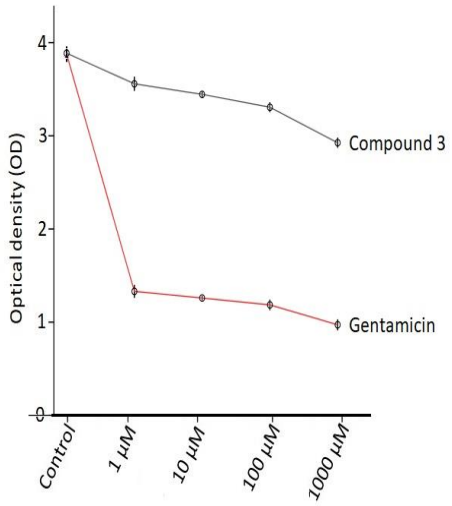


Figure 4.10. Antibacterial effects of Compound 3 on *E. coli*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 3 on *E. coli* was 39.4 µM.

4.2.5. Antibacterial effects of compound 4 on *E. coli*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 4 are presented in Table 4.11.

Table 4.11. OD values were obtained from the incubation of *E. coli* with Compound 4 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 4	OD values	3.887±0.08	3.811±0.14	3.545±0.14	3.285±0.04	3.145±0.03	P=0.05
				MIC_{50}: 41.6 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of the 18 h incubation of *E. coli* via Compound 4 during the culture process is presented in Figure 4.11.

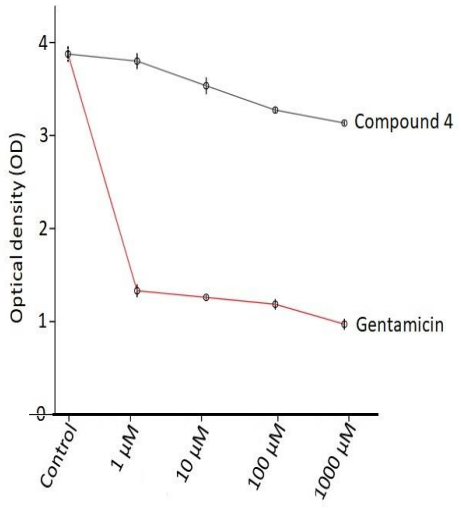


Figure 4.11. Antibacterial effects of Compound 4 on *E. coli*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 4 on *E. coli* was 41.6 µM.

4.2.6. Antibacterial effects of compound 5 on *E. coli*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 5 are presented in Table 4.12.

Table 4.12. OD values were obtained from the incubation of *E. coli* with Compound 5 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 5	OD values	3.887±0.08	3.536±0.05	3.149±0.08	2.635±0.14	2.337±0.1	P=0.36
				MIC_{50}: 25.1 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of the 18h incubation of the *E. coli* via Compounds five during the culture process is presented in Figure 4.12.

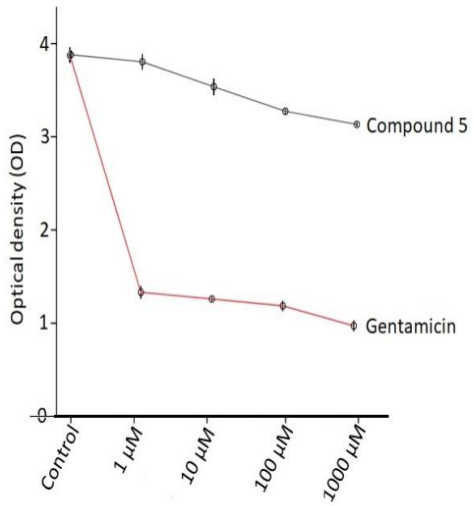


Figure 4.12. Antibacterial effect of the Compounds five on the *E. coli*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 5 on *E. coli* was 25.1 µM.

4.3.1. Antibacterial effects of gentamicin on *S. aureus*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of gentamicin are presented in Table 4.13.

Table 4.13. OD values were obtained from the incubation of *S. aureus* with gentamicin, and MIC_{50} values were calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Genta	OD values	3.857±0.12	2.165±0.03	1.852±0.11	1.009±0.03	0.757±0.01	P=0.1
				MIC_{50}: 1.98 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of the 18 h incubation of ***S. aureus*** with gentamicin during the culture process is presented in Figure 4.13.

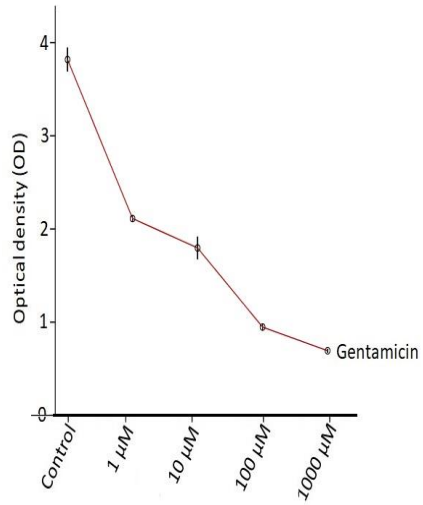


Figure 4.13. Antibacterial effects of gentamicin on *S. aureus*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of gentamicin on *S. aureus* was found to be 1.98 µM.

4.3.2. Antibacterial effects of compound 1 on *S. aureus*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 1 are presented in Table 4.14.

Table 4.14. OD values were obtained from the incubation of *S. aureus* with Compound 1 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 1	OD values	3.857±0.12	3.768±0.23	3.369±0.04	2.361±0.11	1.869±0.37	P=0.15
				MIC_{50}: 15.8 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of the *S. aureus* via Compounds 1 during the culture process is presented in Figure 4.14.

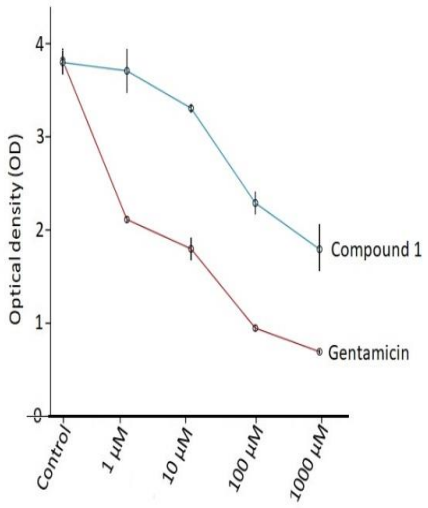


Figure 4.14. Antibacterial effects of Compound 1on *S. aureus*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 1 on *S. aureas* was 15.8 µM.

4.3.3. Antibacterial effects of compound 2 on *S. aureas*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 2 are presented in Table 4.15.

Table 4.15. OD values were obtained from the incubation of *S. aureas* with Compound 2 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 2	OD values	3.857±0.12	3.446±0.04	2.686±0.18	2.369±0.14	1.815±0.14	P=0.27
				MIC_{50}: 15.1 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of *S. aureas* via Compounds 2 during the culture process is presented in Figure 4.15.

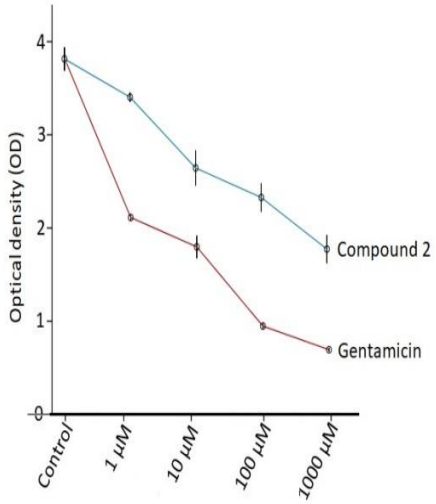


Figure 4.15. Antibacterial effects of Compound 2 on *S. aureas.*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 2 on *S. aureas* was found to be 15.1 µM.

4.3.4. Antibacterial effects of compound 3 on *S. aureas*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 3 are presented in Table 4.16.

Table 4.16. OD values were obtained from the incubation of *S. aureas* with Compound 3 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 3	OD values	3.857±0.12	3.091±0.06	2.534±0.06	2.426±0.04	2.215±0.06	P=0.28
				MIC_{50}: 22.4 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of *S. aureas* via Compound three during the culture process is presented in Figure 4.16.

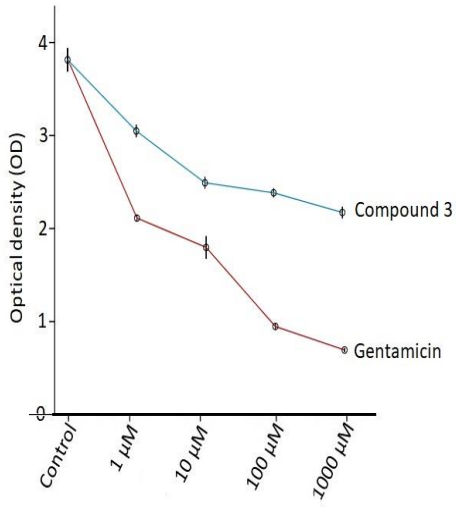


Figure 4.16. Antibacterial effects of Compound 3 on *S. aureas.*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 3 on *S. aureas* was found to be 22.4 µM.

4.3.5. Antibacterial effects of compound 4 on *S. aureas*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 4 are presented in Table 4.17.

Table 4.17. OD values were obtained from the incubation of *S. aureas* with Compound 4 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 4	OD values	3.857±0.12	3.694±0.03	2.948±0.12	2.167±0.04	1.796±0.07	P=0.24
				MIC_{50}: 12.7 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of an 18 h incubation of *S. aureas* via Compounds 4 during the culture process is presented in Figure 4.17.

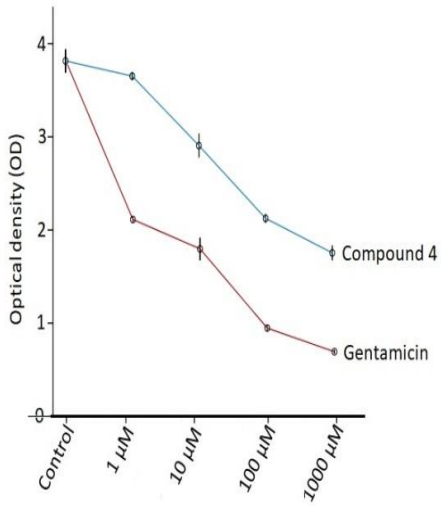


Figure 4.17. Antibacterial effects of Compound 4 on *S. Aureas*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 4 on *S. aureas* was 12.7 µM.

4.3.6. Antibacterial effects of compound 5 on *S. aureas*

The mean±SD values of OD data obtained from the MTT test, homogeneity of variance analysis results (P values) and MIC_{50} value of Compound 5 are presented in Table 4.18.

Table 4.18. OD values were obtained from the incubation of *S. aureas* with Compound 5 and MIC_{50} values calculated using OD values.

		Doses (µM)					Homogeneity of variance
		Control	1	10	100	1000	
Compound 5	OD values	3.857±0.12	3.646±0.04	2.697±0.07	2.369±0.04	1.171±0.09	P=0.3
				MIC_{50}: 5.12 µM			

The line graphic of the OD values obtained by the MTT test applied at the end of the 18 h incubation of *S. aureas* via Compounds 5 during the culture process is presented in Figure 4.18.

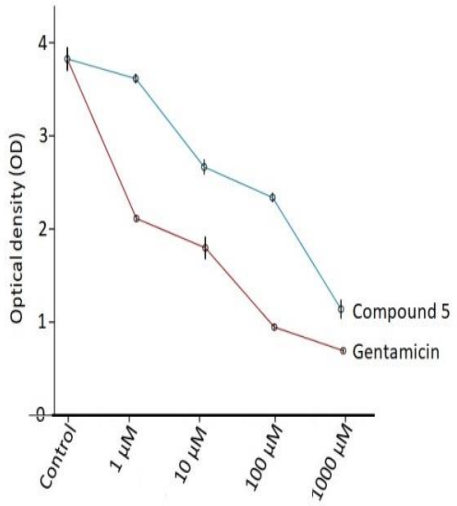


Figure 4.18. Antibacterial effects of Compound 5 on *S. aureas*

In the regression analysis using OD values obtained from the MTT test, the MIC_{50} value of Compound 5 on *S. aureas* was 5.12 µM.

4.2. Discussion

Antibiotic resistance has been identified in various microbes in recent decades, including those with multi-resistance phenotypes. As the WHO has highlighted, this is a concerning condition, and many researchers are working to discover new treatment options.

Another important finding was that the Schiff bases via such the 2,4-dichloro-5-fluorophenyl moiety similarly suppress bacterial growth. *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. All of the *K. pneumoniae* strains were inhibited. The MIC values of these Schiff base molecules range from 6.3 to 12.5 µg/mL, which were similar in many aspects for the reference antibiotic ciprofloxacin (Karthikeyan et al., 2006). Madurahydroxylactone Imines derive from natural sources were known as Schiff base. For example, *Actinomadura Rubra* produces madurahydroxylactones, secondary metabolites (Paulus et al., 1994). The imines are Schiff bases that fall into this category. *S. aureus*, *B. subtilis*, *Micrococcus flavus*, and *Sarcina lutea* growth were all inhibited in vitro by all madurahydroxylactone-derived compounds, with MICs ranging from 0.2 to 3.1 µg/mL (Heinisch et al., 1999).

For cancer therapy, a growing variety of fluorinated antimitotic/antitumour medicines are present worldwide. 5-fluoropyrimidines, like5-fluorouracil (5-FU) and 5-fluoro-20-deoxyuridine, were 5-fluoropyrimidines (FdUrd) 2 their pro-drug counterparts are the most commonly utilized. 3–8 In vivo, these pro-drugs are transformed to bioavailable (Danenberg et al., 2004).

The most exciting finding was that Sak et al. (2021), Compound 6, which includes five fluorine atoms and has an IC_{50} of 0.64 µM, had the most lethal effect on A549 lung cancer cells. Compound 5 (PI: 4.95) with two fluorine atoms had the highest antiproliferative impact on A549 cells. Induction of apoptosis resulted in cell death.

Panneerselvam et al. (2005), described that 20 morpholine-derived Schiff bases (compounds 18–20) were made and confirmed for antibacterial activity in vitro. The microorganisms most susceptible to a morpholine-derived Schiff bases 18 are *S. aureus* & *Micrococcus luteus* (MIC = 20 and 32 µg/mL, respectively). A morpholine-derived Schiff base 19 (MIC = 17 µg/mL) was more sensitive to *Streptococcus epidermidis*, whereas Compound 20 was more sensitive to *Bacillus cereus* and *E. coli (*MIC = 21 and 16 l µg/mL, respectively).

These results reflect those of Shanmugam[1] et al. (2013), who also found that Nucleophiles were combined with cyanuric chloride in a stoichiometric ratio in the presence of the triethylamine/alkaline media to produce new s-triazine nucleobase derivatives (1b–5b, 1a–5a and1c–5c). Antimicrobial activity was tested on all of the compounds against a variety of fungal & bacterial species. Compounds 1a and 1b displayed great moderates antibacterial activities toward *S. aureus, P. aeruginosa, K. pneumoniae,* and *S. typhi* bacterial strains, whereas compounds 2a, 5b, 1c, and 2c had better antibacterial activity against *S. typhi*. Substances 1b–2b, 1a–5a, & 1c–5c were discovered to have effective antifungal activities toward the fungal types *Candida albicans, Aspergillus niger* and *Fusarium oxysporum*. Also, the overall antifungal investigations revealed the following pattern. 1a–5a > 1b–5b > 1c–5. 1a–5a > 1b–5b > 1c–5.

Wang et al. (2018), another important finding was that the Cinnamaldehyde-amino acid Schiff bases substances were antibacterial molecules that work against both gram-positive & gram-negative bacteria. A two best Quantitative structure-activity relationship models were R2 = 0.9354, F = 57.96, and S2 = 0.0020 *against E. coli* and R2 = 0.8946, F = 33.94 and S2 = 0.0043 toward S. *aureus*. The antibacterial activity value of the novel compounds was equivalent to that of ciprofloxacin, indicating that they had high antibacterial activity.

The most exciting finding was that Shanmugam[2] et al. (2013), A variety of physiologically relevant substituted 3-fluorosalicylaldehyde derivative (1a–h) via several primary amines are synthesize utilized an ionic liquids (1-Butyl-3-

methylimidazolium bis(trifluoromethylsulfonyl)imide) just as the efficient catalysts. The antibacterial activity of the produced compounds is evaluated toward Gram-positive bacteria S. *aureus, B. subtilis*, & Gram-negative bacteria *P. aeruginosa* and *E. coli K. pneumoniae, S. typhi*, by differencing their MIC value to the standards antibiotic streptomycin. When all of the MIC values for substances (1a–h) were plotted together, it can be seen which substances (1d) and (1f) have three times the activities toward *K. pneumoniae* at the MIC 12.5 µg/mL compared to the streptomycin standards (50 µg / mL). Substance 1d is also shown to be twice therefore efficient toward *S. Typhi, S. aureus* (MIC 25 µg /mL), *B. subtilis* (MIC 6.25 µg /mL) and *P. aeruginosa* (MIC 12.5 µg/mL) than the standard. The compounds 1g, 1b, and 1c, on the other hand, were more effective toward *K. pneumoniae* (25 µg/mL). Furthermore, compounds 1a and 1g and 1a exhibited enhanced efficacy toward *S. Typhi* (25 µg/mL). Moreover, even at the highest concentration, compound 1b showed more significant action against *K. pneumoniae* at a MIC of 25 µg/mL, but no activity against *B. subtilis* or *P. aeruginosa.* Furthermore, against *E. coli*, compounds 1c and 1g outperformed the reference streptomycin drug, whereas compound 1c outperformed the reference streptomycin drug against *K. pneumoniae, B. subtilis* and *S. aureus.*

Shi et al. (2007), one interesting finding that Schiff bases made to the condensations of the antibacterial activity were found in 5-chloro-salicylaldehyde and primary amines. At least one of the investigated bacterial species was extremely aggressive against a 5-chloro-salicylaldehyde-Shiff base derivative 6–15. Compounds 6–11 and 13–15 are particularly sensitive to *Pseudomonas fluorescence*, with MIC values ranging from 2.5 to 5.2 µg/mL for each drug. Kanamycin, a reference medicine, has a MIC of 3.9 µg/mL against another bacterial strain, which is similar to the findings of this investigation. When the MIC value for kanamycin is 3.9 µg/mL, the MIC value for Schiff bases 6, 7, 9–11, 14, and 15 against *E. coli* is 1.6–5.7 µg/mL. B. subtilis is only sensitive to Schiff bases 14 (MIC = 1.8 µg/mL).

Duan et al. (2017), The outcome of this studies show For the 1st time, A novel class of Schiff base-bridged tetrahydroprotoberberine (THPB) triazoles have been

developed. The in vitro biological assessment revealed that some of the synthesized compounds showed good to better antibacterial activities when compared to the reference medications. MRSA was successfully inhibited by THPB triazole 7a, which had MIC values of 2 µg/mL. Further study revealed that compound 7a might kill MRSA quickly while causing bacterial resistance to develop more slowly than norfloxacin.

Salem et al. (2019) studied that the target compounds were made by reacting alginate dialdehyde with o-phenylenediamine analogues as a starting material. The antimicrobial activity test indicated that items 1,2,5,6 and 8 have antifungal activity against the pathogenic fungi tested. In addition, the condensation products (1,2,5 and 6) shown antibacterial activities toward gram negative bacteria and Gram-positive.

5. CONCLUSION and SUGGESTIONS

In our study, we aimed to discover new antimicrobial drugs against microbes to solve the resistance and evaluate the potentiality and effectiveness of Fluorinated 5-tert-butyl salicylaldehydes from Schiff bases antibacterial agents.

In this study, *P. aeruginosa* (ATCC, 27853), *E. coli* (ATCC, 25922), & *S. aureus* (ATCC, 29213), bacterial strains incubated in 50 ml in Nutrient Broth (NB) at 37 °C for 24 hours. For serial cultures, 1 ml of the initiating culture sample was incubated in 100 ml NB at 37 °C for 24 h. Schiff base derivatives of fluorınated 5-tert-butyl salicylaldehydes (Compound 1-5) added to 96-well culture plates in triplicate order at 1 to 1000 µM doses. Bacteria, set to 0.5 McFarland standards in NB, were added in 100 µl to each culture plate wells. Plates are incubated at 37 °C for 18 hours. After a culture period, 10 µl of MTT solutions (300 µM concentration) is added to every well and incubated for 4 hours. In addition, 100 µl of dimethyl sulfoxide is added to every good to dissolve MTT-formazan crystals. Culture plates are read on an ELIZA microplate reader at 570 nm, and the optical density (OD) values for each well were recorded. MIC_{50} value of each compound was calculated by linear regression analysis using OD values.

MIC_{50} values for gentamicin were found on *P. aeruginosa*, *E. coli* and *S. aureus* 1.43, 1.93, 1.98 µM, respectively. The results showed that the antibiotics have a great inhibitory effect on the growth of the tested strains. The most potent inhibitory effects against *P. aeruginosa* were obtained by Compounds 2, 3, and 5 (MIC_{50} values were 9.56, 9.9, 3.46 µM, respectively). The most potent effect on *S. aureus* was obtained by C5 and moderate impact by Compound 1, 2 and 4 (MIC_{50} values were 5.12, 15.8, 15.1, 12.7 µM, respectively). Compound 1 was showed a moderate effect on *E. coli* (MIC_{50} values was 18.9 µM).

Our study, which can be valuable in discoveries of recent potent antimicrobial agents, will contribute to our country's human health care and drug industry. These

results pave the way toward the possibility of using the fluorınated 5-tert-butyl salicylaldehydes as new antibiotic agent candidates in control of bacteria. Future studies will be containing the *in vivo* and clinical tests for determining the potential toxic reactions and actual effectiveness.

Overall, this study supports the notion that, in the context of patient support, political commitment from national governments, the business sector, technical partners, civil society, and financial agencies is required to adopt new and current recommendations, as well as their safe and timely delivery. More high-quality research is still needed to improve treatment outcomes and quality of life for MDR patients worldwide, including operational research and randomized controlled trials. This will most likely be a long-term "war" against living things that have a high capacity to adapt and survive.

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