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THEME

**Antibacterial Assessment of Ni- and Zn Substituted
Porphyrin Derivatives: Synthesis, In Vitro Activity, and
Computational Insights Using ADMET,
Molecular Docking, and Dynamics Simulations**

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Dedication

All praise and thanks and gratitude be to Allah for both the beginning and the end.

"(And the close of their request will be, 'Praise be to Allah, Lord of the worlds)".

The journey was not short, nor was the road paved with ease, yet it was opened—so all praise be to Allah who made the beginnings possible and guided us gently and forgivingly to the end.

I dedicate my graduation first to my ambitious self—what began as a dream ended in success.

To everyone who walked alongside me in completing my academic journey—may you remain a lifelong pillar of support.

With all my love, I dedicate the fruit of my success and graduation

To the light that illuminated my path, the one who spent years striving so I could climb the ladders of achievement—my beloved father, whose name I carry with pride, who removed the thorns from my path to pave the way for knowledge.

And to the invisible hand, the tender heart, the one whose prayers never ceased, my source of strength and the beloved of my soul, the one who has always supported and encouraged me—my precious mother.

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Praise be to the One who brings goodness and hope, who flooded me with joy that made me forget the hardship .

" Rim "

Dedication

Praise be to Allah with love, gratitude, and appreciation for the beginning and the end.

“And the close of their request will be, ‘Praise be to Allah, the Lord of all the worlds.’”

After a journey that spanned years, filled with hardships, challenges, and exhaustion, here I stand today at the threshold of my graduation, reaping the fruits of my efforts and proudly tipping my hat. Allah, to You belongs all praise before You are pleased, all praise when You are pleased, and all praise after You are pleased for You granted me success in completing this work and fulfilling my dream.

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Dedication

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Dedication

All praise be to Allah.

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throughout this journey.

To my dear father,

I dedicate this work especially to you

Thank you for always believing in me and supporting me .This achievement is
as much yours as it is mine.

And to my colleagues who shared this journey with me, Thank you for your
collaboration, and the valuable experiences we built together

And in the end ..

"ما كنت لأفعل لولا أن الله مكّنني، فالحمد لله عند البدء وحين الختام"

"Amani"



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Abstract

Abstract:

This study evaluated the antibacterial activity of two metalloporphyrin compounds: NiPPH₂ and ZnTPPH₂ (p-methyl), using in vitro inhibition assays against five bacterial strains, representing both Gram-positive and Gram-negative types, in addition to in silico computational analyses to support the experimental findings.

The results showed that ZnTPPH₂ (P3) outperformed NiPPH₂ (P2) in most assays, displaying significantly lower IC₅₀ values, particularly against *Klebsiella pneumoniae* (0.437 mg/mL) and *Staphylococcus aureus* (0.760 mg/mL), indicating strong antibacterial potential. On the other hand, P2 demonstrated moderate activity, notably against *Pseudomonas aeruginosa* (0.608 mg/mL). A clear dose-response relationship was observed, with inhibition increasing proportionally with compound concentration.

The in silico analysis, particularly molecular docking studies, confirmed the high binding affinity of ZnTPPH₂ to key bacterial target proteins, with binding energies reaching up to -8.9 kcal/mol. The interaction profiles revealed the presence of stable hydrogen bonds and π - π stacking interactions, especially with amino acid residues such as Lys100 and Tyr124.

Furthermore, molecular dynamics simulations supported these findings by demonstrating the structural stability of the ligand-protein complex over a simulation period of 1000 ps, with consistent RMSD values ranging between 0.10 and 0.15 nm. These results indicate strong and stable binding of the compound within the active sites of bacterial proteins.

In conclusion, the study highlights the promising antibacterial potential of metalloporphyrin derivatives, particularly ZnTPPH₂ (p-methyl), which could serve as an innovative therapeutic agent against drug-resistant bacterial strains. The combination of in vitro activity, in silico modeling, and molecular stability data suggests that further development of these compounds may contribute significantly to the future of antimicrobial therapy.

Keywords: Zinc porphyrin (Zn-porphyrin) , Nickel porphyrin (Ni-porphyrin) , Antibacterial activity ,Molecular docking, IC₅₀ values.

Résumé

Résumé:

Cette étude a évalué l'activité antibactérienne de deux composés métalliques porphyriniques : NiPPH₂ et ZnTPPH₂ (p-méthyle), à travers des tests biologiques (in vitro) contre cinq souches bactériennes, couvrant les types Gram positif et Gram négatif, en complément d'analyses informatiques (in silico) pour valider les résultats expérimentaux.

Les résultats ont montré que le composé ZnTPPH₂ (P3) a surpassé NiPPH₂ (P2) dans la majorité des essais, affichant les valeurs IC₅₀ les plus faibles, notamment contre *Klebsiella pneumoniae* (0,437 mg/mL) et *Staphylococcus aureus* (0,760 mg/mL), ce qui reflète une activité antibactérienne marquée. Quant au P2, il a présenté une activité modérée, particulièrement contre *Pseudomonas aeruginosa* (0,608 mg/mL). Une relation dose-réponse claire a été observée, indiquant que l'augmentation de la concentration améliore l'inhibition bactérienne.

L'analyse in silico, notamment via le docking moléculaire, a révélé une forte affinité de ZnTPPH₂ pour les protéines cibles bactériennes, avec des énergies de liaison allant jusqu'à -8,9 kcal/mol, accompagnées d'interactions π - π et de liaisons hydrogène stables, en particulier avec les acides aminés Lys100 et Tyr124.

Les simulations de dynamique moléculaire ont confirmé la stabilité du complexe ligand-protéine, avec des valeurs RMSD constantes comprises entre 0,10 et 0,15 nm durant toute la durée de la simulation, soulignant la stabilité structurale du composé dans le site actif.

Ces résultats démontrent le potentiel prometteur des porphyrines métalliques, en particulier du composé au zinc, comme agents antibactériens innovants, et soutiennent leur développement futur dans le cadre de nouvelles stratégies thérapeutiques contre les bactéries résistantes.

Mots-clés: Porphyrine au zinc (Zn-porphyrin), Porphyrine au nickel (Ni-porphyrin), Activité antibactérienne, Docking moléculaire, Valeurs IC₅₀.

ملخص:

تم في هذه الدراسة تقييم الفعالية المضادة للبكتيريا لمركبي بورفيرين معدنيين: NiPPH₂ و ZnTPPH₂ (p-methyl)، وذلك من خلال اختبارات بيولوجية (In vitro) ضد خمس سلالات بكتيرية تمثل النوعين الموجبة والسالبة لصبغة غرام، بالإضافة إلى تحليلات حاسوبية (In silico) لدعم النتائج.

أظهرت النتائج أن ZnTPPH₂ (P3) تفوق على NiPPH₂ (P2) في معظم الاختبارات، حيث أظهر أقل قيم IC₅₀، خاصة ضد *Klebsiella pneumoniae* (0.437 mg/mL) و *Staphylococcus aureus* (0.760 mg/mL)، مما يدل على فعاليته العالية. أما P2 فقد أظهر فعالية معتبرة لكن أقل نسبيًا، خاصة ضد *Pseudomonas aeruginosa* (0.608 mg/mL). وقد تبين أن زيادة تركيز المركب تؤدي إلى زيادة في معدلات التثبيط.

أما التحليل الحاسوبي، فقد استخدم لتأكيد قدرة المركبين على الارتباط ببروتينات مستهدفة داخل البكتيريا، وأظهرت نتائج الالتحام الجزيئي (docking) أن ZnTPPH₂ يحقق أقوى طاقات ارتباط (ΔG) تصل إلى -8.9 kcal/mol، مع وجود تفاعلات من نوع $\pi-\pi$ ، وروابط هيدروجينية مستقرة، خاصة مع الأحماض الأمينية Lys100 و Tyr124.

كما دعمت محاكاة الديناميكيات الجزيئية هذه النتائج، حيث أظهر المركب ZnTPPH₂ ثباتًا كبيرًا داخل موقع الارتباط على مدى المحاكاة، بقيم RMSD مستقرة بين 0.10 و 0.15 nm، ما يؤكد الاستقرار البنوي والفعالية المحتملة للمركب داخل الأهداف البكتيرية.

تُظهر هذه النتائج إمكانيات واعدة لمشتقات البورفيرين، خصوصًا المركب المحتوي على الزنك، كمضادات بكتيرية جديدة، مما يدعم إمكانية تطويرها مستقبلاً كعوامل علاجية فعالة في مواجهة السلالات المقاومة.

الكلمات المفتاحية: الزنك بورفيرين (Zn-porphyrin)، النيكل بورفيرين (Ni-porphyrin)، النشاط المضاد للبكتيريا، الالتحام الجزيئي، قيم IC₅₀.

List of abbreviations

List of abbreviations:

Abs: Absorbance

ADMIT: Absorption, Distribution, Metabolism, Excretion, Toxicity

AMR: Antibiotic Resistance

aPDT: Antimicrobial Photodynamic Therapy

ATCC: American Type Culture Collection

ATP: Adenosine Triphosphate

AuNPs: Gold Nanoparticles

CFU: Colony Forming Unit

CRAPC: Center for Scientific and Technical Research in Physico-Chemical Analysis

DL50: Lethal dose 50

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic Acid

DNSA: 3,5-dinitrosalicylic chromogenic reagent

E: energy

E. coli: Escherichia coli

EPS: Exopolysaccharides

FDA: Food and Drug Administration

FREIGHT: Förster Resonance Energy Transfer

G: gram

GC/MS: Gas chromatography/Mass spectrometry

HPLC: High-Performance Liquid Chromatography

IFD: Induced Fit Docking

LAB: Lactic Acid Bacteria

MDR: Multi-Drug Resistance

Mg: milligram

MHA: Mueller-Hinton Agar

MIC: Minimum Inhibitory Concentration

Min: Minute

MM-GBSA: Molecular Mechanics-Generalized Born Surface Area

MRI: Magnetic Resonance Imaging

NIR: Near-Infrared

NMR: Nuclear Magnetic Resonance

List of abbreviations

OPLS4: Optimized Potentials for Liquid Simulations 4

P2: Zinc-substituted Porphyrin Derivatives

P3: Nickel-substituted Porphyrin Derivatives

PBS: Phosphate Buffered Saline

PDB: Protein Data Bank

PDT: Photodynamic Therapy

pH: Hydrogen Potential

PPIX: Protoporphyrin IX

QDs: Quantum Dots

RAM: Random Access Memory

ROS: Reactive Oxygen Species

SD: Standard Deviation (Type Deviation)

SPIONS: Superparamagnetic Iron Oxide Nanoparticles

SPPS: Solid Phase Peptide Synthesis

TLC: Thin-Layer Chromatography

TPP: Tetraphenylporphyrin

UV-Vis: Ultraviolet-Visible Spectroscopy

VTRS: Valorization and Technology of Saharan Resources

WHO: World Health Organization

α : Alpha

β : Beta

Material table

Material table:

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*Introducción
general*

Introduction

Introduction:

Since ancient times, microbial infections have posed a major threat to human health. Despite significant advances in the field of antibiotics, the emergence of multidrug-resistant bacterial strains has considerably reduced the effectiveness of conventional treatments, presenting a serious challenge for modern medicine (Ventola, 2015).

In this context, the search for new bioactive molecules has intensified, particularly through medicinal chemistry and rational drug design. Porphyrins, a class of tetrapyrrolic macromolecules, have attracted growing interest due to their unique physicochemical properties, their ability to form metal complexes, and their therapeutic potential in various fields, including the fight against pathogenic agents. Bacterial infectious diseases are among the most significant health challenges facing the world today, particularly with the rapid spread of antibiotic-resistant bacterial strains. This global threat continues to drive researchers to seek new and effective alternatives to conventional treatments. Organic compounds and metal complexes are considered among the most promising candidates in this field (Ventola, 2015). Porphyrin derivatives substituted with metals such as zinc (Zn) and nickel (Ni) have demonstrated promising antimicrobial activity. These compounds can interact with bacterial membranes, disrupt cellular metabolism, or generate reactive oxygen species (ROS), thereby leading to cell inhibition or death (Bonnett, 1995; Kashef & Hamblin, 2017). Moreover, advances in molecular modeling have enabled a deeper understanding of the interactions between these compounds and their biological targets. In silico approaches, including ADMET analysis (Absorption, Distribution, Metabolism, Excretion, and Toxicity), molecular docking, and molecular dynamics simulations, offer powerful tools to predict the efficacy, toxicity, and stability of drug candidates prior to experimental evaluation (Li, 2021; Daina et al., 2017). In this study, we aim to evaluate the antibacterial activity of porphyrin derivatives substituted with Ni and Zn. We assess their in vitro activity against bacterial strains (*Klebsiella pneumoniae*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*), and complement this investigation with an in-depth computational study. This integrated approach will help to better understand their therapeutic potential and lay the foundation for the future development of novel antimicrobial agents.

This thesis will be structured as follows:

The first chapter will cover the theoretical background concerning porphyrins and their biological activity.

Introduction

Chapter Two provides a detailed overview of the characterization techniques and in vitro assays employed to evaluate the biological activity of the studied porphyrin derivatives.



Part 1:

Literature Review

Chapter 01:
General Information
About Bacteria

1. Definition of bacteria:

Bacteria are microscopic living organisms that have only one cell. The word for just one is “bacterium.” Millions (if not billions) of different types of bacteria can be found all over the world, including in your body. They’re on your skin and in your airways and mouth. They’re also in your digestive system, reproductive system, and urinary tract. Scientists estimate you have 10 times more bacterial cells than human cells in your body.

These bacteria make up your microbiome, which keeps your body healthy. Other bacteria can make you sick. Healthcare providers can treat many bacterial infections with antibiotics (clevelandclinic, 2022).

2. Classification of Bacteria:

Bacteria can be classified into various categories based on their features and characteristics. The classification of bacteria is mainly based on the following: (Microbe Notes, n.d.)

- **Shape:** Classification of bacteria based on Shape

Table1. Classification of bacteria based on Shape

Type of Classification	Examples
Bacillus (Rod-shaped)	Escherichia coli (E. coli)
Spirilla or spirochete (Spiral)	Spirillum volutans
Coccus (Sphere)	Streptococcus pneumoniae
Vibrio (Comma-shaped)	Vibrio cholerae

- **Composition of the cell wall:** Classification of bacteria based on the Composition of the Cell Wall

Table2. Classification of bacteria based on the Composition of the Cell Wall

Type of Classification	Examples
Peptidoglycan cell wall	Gram-positive bacteria
Lipopolysaccharide cell wall	Gram-negative bacteria

- **Mode of nutrition:** Classification of bacteria based on the Mode of Nutrition

Table3. Classification of bacteria based on the Mode of Nutrition

Type of Classification	Examples
Autotrophic Bacteria	Cyanobacteria
Heterotrophic Bacteria	All disease-causing bacteria

- **Mode of respiration:** Classification of bacteria based on the Mode of Respiration

Table4. Classification of bacteria based on the Mode of Respiration

Type of Classification	Examples
Anaerobic Bacteria	Actinomyces
Aerobic Bacteria	Mycobacterium

3. Bacterial Strains Studied in This Research:

3.1. Escherichia coli:

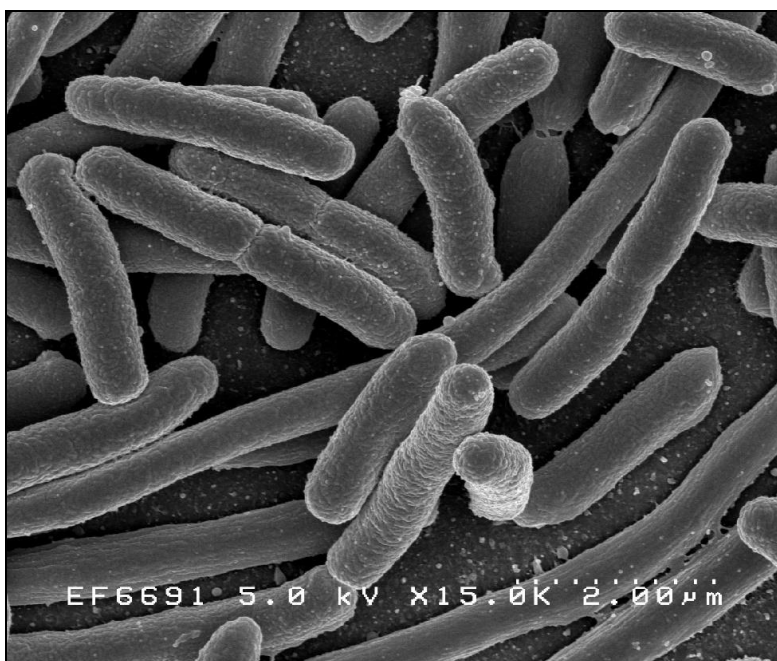


Figure1: Micrograph of Escherichia coli (Carroll et al., 2019)

Escherichia coli, commonly known as E. coli, is a bacterium found in the human and warm-blooded animal intestines. While most strains of E. coli are harmless, some, like Shiga toxin-producing E. coli, can cause severe foodborne illnesses. This bacterium produces toxins called Shiga-like toxins due to their similarity to those produced by Shigella dysenteriae. E. coli can multiply at temperatures between 7°C and 50°C, with an optimal temperature of 37°C. It can be transmitted to humans primarily through consuming contaminated foods like

raw or undercooked ground meat, raw milk, raw vegetables, and contaminated sprouts. Cooking food thoroughly at a temperature of at least 70°C can destroy Shiga toxin-producing *E. coli* if present. [(WHO, 2018)][(pasteur, s.d.)].

3.1.1. Symptoms and Transmission:

Infections caused by Shiga toxin-producing *E. coli* can lead to symptoms such as abdominal cramps, diarrhea, and in severe cases, bloody diarrhea (hemorrhagic colitis). Fever and vomiting may also occur. The duration of illness from this strain of *E. coli* is typically around a week in adults but can be longer in children. Preventing infection involves taking measures at all stages of the food chain, including production on farms, to reduce the risk of contamination. The transmission of *E. coli* primarily occurs through consuming contaminated foods, with the main reservoir being the digestive tract of cattle. Contamination can also arise from fecal matter of ruminants in the environment. [(WHO, 2018)][(pasteur, s.d.)].

3.1.2. Prevention and Treatment:

Preventing infections from Shiga toxin-producing *E. coli* involves hand hygiene practices, thorough cooking of meat, avoiding raw milk and soft cheese made from raw milk, peeling and washing vegetables, and refraining from consuming uncooked flour-based products. Treatment for severe infections caused by this bacterium typically requires hospitalization to manage complications such as anemia and kidney failure. It is important to note that most antibiotics are contraindicated as they can worsen the condition by releasing more toxins into the body. [(pasteur, s.d.)]. [(FDA, 2025)].

Escherichia coli, a versatile bacterium with both harmless and pathogenic strains, underscores the importance of food safety and hygiene practices to prevent infections and mitigate the risks associated with this bacterium.

3.2. Bacillus subtilis:

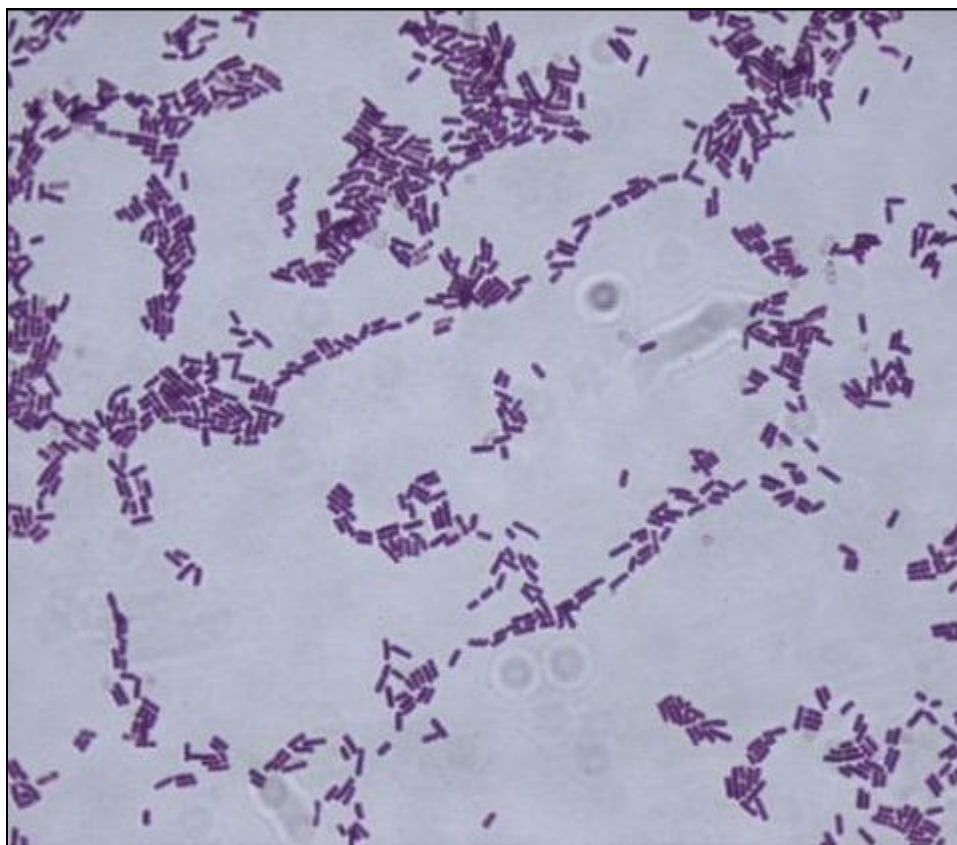


Figure2: Micrograph of *Bacillus subtilis*(Madigan et al., 2017)

Bacillus subtilis, also known as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium commonly found in soil and the gastrointestinal tract of ruminants, humans, and marine sponges. (sciencedirect, 2001).

It is a rod-shaped bacterium, typically 4–10 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of about 4.6 fL at stationary phase.

As a member of the genus *Bacillus*, *B. subtilis* can form tough, protective endospores, allowing it to survive extreme environmental conditions (sciencedirect, 2001). It is a facultative anaerobe and has been considered an obligate aerobe until 1998.

3.2.1 Genome and Model Organism:

Bacillus subtilis has about 4,100 genes, and its genome has been extensively studied. [(futura-sciences, n.d.)]. It is considered the best studied Gram-positive bacterium and a model organism for laboratory studies, especially of sporulation and cellular differentiation [(ncbi.nlm.nih.gov, 2020)].

In terms of popularity as a laboratory model organism, *B. subtilis* is often considered the Gram-positive equivalent of *Escherichia coli*, an extensively studied Gram-negative bacterium. It is frequently used as a genetic model, and its study has contributed to establishing cellular metabolism pathways and regulation. (sciencedirect, 2001).

3.2.2. Applications and Characteristics:

Bacillus subtilis is an excellent model for studying pathogenic bacteria, such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus anthracis*, *Bacillus cereus*, and *Listeria monocytogenes*. It is used to investigate strategies these bacteria use for growth, causing diseases, multiplication, protection, and gene transfer (sciencedirect, 2001).

B. subtilis produces enzymes of interest for industry, including an amylase used in industrial bread production. It is also used in the production of natto, a Japanese fermented soybean dish. (futura-sciences, n.d.).

3.3. Klebsiella Bacteria:

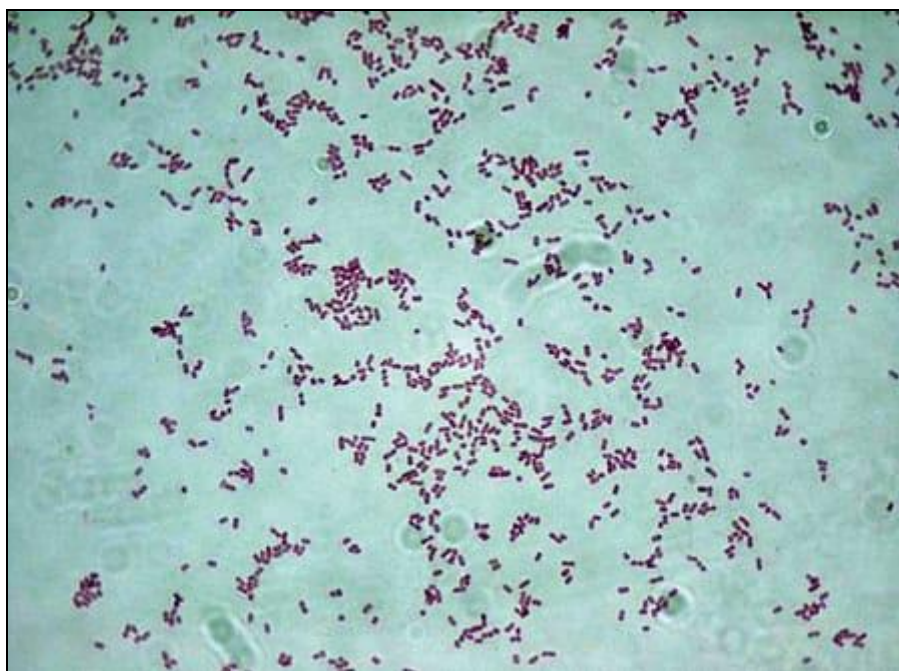


Figure3: Micrograph of *Klebsiella pneumoniae* (Tille, 2017)

3.3.1. Definition of *Klebsiella*:

Klebsiella bacteria is a race of negative bacteria, belonging to the family of intestinal bacteria. These bacteria are characterized by its bacillus shape, its lack of movement, and the presence of a prominent capsule consisting of many sugars. It usually lives in the intestine naturally, but if it spreads to other parts of the body, it may cause a serious infection. *Klebsiella* bacteria are commonly spread in health care environments such as elderly care and intensive care units in hospitals. (PubMed Central, 2016).

3.3.2. Species :

Klebsiella species are classified based on biochemical reactions and clinical significance:

- ❖ *Klebsiella pneumoniae*: Causes pneumonia, urinary tract infections, septicemia, and liver abscesses.
- ❖ *Klebsiella oxytoca*: Found in the nose, mouth, and intestines.
- ❖ *Klebsiella rhinoscleromatis*: Causes rhinoscleroma.
- ❖ *Klebsiella ozaenae*: Causes ozena.

Other species include *K. planticola*, *K. terrigena*, and *K. ornithinolytica* [(Medscape, 2017). (NCBI, 2019)].

3.3.3. Characteristics:

- ❖ *Klebsiella* species are encapsulated, which contributes to their virulence.
- ❖ They are commonly found in the human gastrointestinal tract but can cause serious infections if they spread to other parts of the body.
- ❖ *Klebsiella* infections are often associated with healthcare settings, such as nursing homes and intensive care units.
- ❖ *K. pneumoniae* is the most clinically significant species, causing pneumonia, urinary tract infections, septicemia, and liver abscesses.

In summary, *Klebsiella* is an important genus of bacteria that can cause severe infections, particularly in healthcare settings. Understanding its classification and characteristics is crucial for effective prevention and treatment of *Klebsiella* infections. [(Medscape, 2024) (Ashurst & Dawson, 2023)].

3.4. *Staphylococcus Bacteria*:

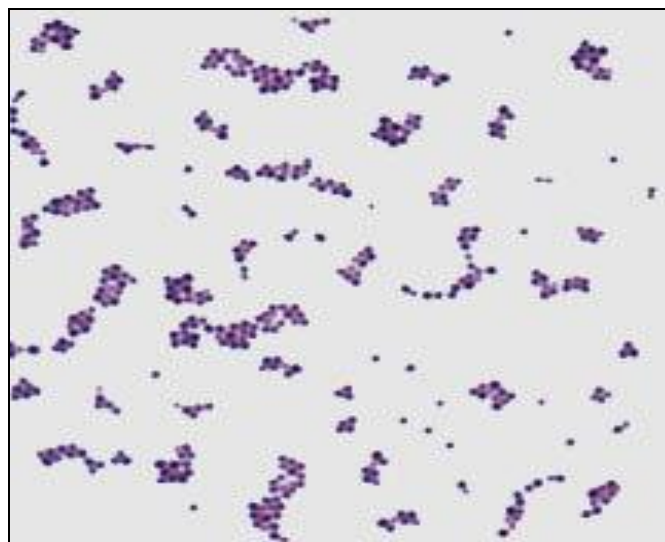


Figure4: Micrograph of staphylococcus aureus(Murray et al., 2021)

3.4.1. Definition:

Staphylococcus is a genus of Gram-positive bacteria in the family *Staphylococcaceae* from the order *Bacillales*. These bacteria appear spherical (cocci) under the microscope and form grape-like clusters. They are facultative anaerobic organisms, capable of growth both aerobically and anaerobically. The name "*Staphylococcus*" was coined in 1880 by Scottish surgeon and bacteriologist Alexander Ogston, combining the prefix "staphylo-" (from Ancient Greek: σταφυλή, meaning 'bunch of grapes') with the New Latin suffix "coccus" (from Ancient Greek: κόκκος, meaning 'spherical bacterium'). (CDC, 2013).

3.4.2. Species and Characteristics:

- ❖ *Staphylococcus* includes at least 44 species, with some having subspecies. Many of these species reside normally on the skin and mucous membranes of humans and animals, while some can cause infections.
- ❖ These bacteria have been found to be nectar-inhabiting microbes and are a small component of the soil microbiome.
- ❖ *Staphylococcus* species have been implicated in human infections, with strains like *S. lugdunensis*, *S. schleiferi*, and *S. caprae* being notable.
- ❖ Antibiotic resistance is a significant concern with *Staphylococcus*, and many strains have become resistant to antibiotics, posing challenges in treatment. (Ghebremedhin et al., 2008).

3.4.3. Taxonomy and Genomics:

- ❖ The taxonomy of *Staphylococcus* is based on 16S rRNA sequences, and most species fall into distinct clusters.
- ❖ Genomic data, including sequences of various genes like *gap*, 16S rRNA, *hsp60*, *rpoB*, *sodA*, and *tuff*, have been used to classify and distinguish *Staphylococcus* species. (NCBI, n.d.).
- ❖ Whole genome technologies have provided valuable insights into the diversity of *Staphylococcus* strains, aiding in understanding their pathogenic behavior and evolution.

In conclusion, *Staphylococcus* bacteria are diverse, with some species causing infections and exhibiting antibiotic resistance. Understanding their taxonomy, characteristics, and genomic features is crucial for effective management and control of infections caused by these bacteria. (ScienceDirect, n.d.).

3.5. *pseudomonas aeruginosa*:



Figure5: Micrograph of *pseudomonas aeruginosa*(Ryan & Ray, 2004)

Pseudomonas aeruginosa, also known as the pyocyanic bacillus, is a saprophytic bacterium commonly found in air, water, and soil. It is a commensal organism of the skin and mucous membranes in both humans and animals, and it has a wide pathogenic potential.

The pyocyanic bacillus is mainly a pyogenic bacterium that causes superficial and deep suppurations in humans and animals. It can be isolated from these suppurative infections.

Pseudomonas aeruginosa is frequently found in hospital environments and mainly affects weakened individuals such as cancer patients, burn victims, and those with respiratory insufficiency. Its presence in certain samples—for example, urine—indicates medical interventions and the iatrogenic nature of the infection. It can also be isolated from stool cultures, although no specific pathogenic role can be clearly attributed to it in such cases.

However, patients with cystic fibrosis are very often infected in the lungs by a specific type of strain known as the mucoid pyocyanic strains. (Bouamer & Guerbati, 2008).

3.5.1. Bacteriological Characteristics:

❖ Morphology:

Pseudomonas aeruginosa appears as a slender bacillus (0.5 x 3 µm), non-spore-forming and non-encapsulated. Its extreme motility is due to polar flagella, generally monotrichous. It is Gram-negative and often displays more intensely stained granulations.

❖ Cultural Characteristics:

Pseudomonas aeruginosa is a bacterium with very low nutritional requirements, capable of growing on simple synthetic media.

It grows easily within 2 hours at a temperature of 37°C and can develop under variable temperature conditions (5 – 42°C, with an optimum at 30°C), but tolerates only small variations in pH (6.5 – 7.5, optimum at 7.2).

Being strictly aerobic, *Pseudomonas aeruginosa* can use nitrates as hydrogen acceptors for respiration. Consequently, in anaerobic media containing nitrates, it grows throughout the entire height of the test tube.

In broth, it causes turbidity with the development of a surface film, sometimes limited to a simple ring adhering to the walls of the tube. The culture, which becomes highly alkaline, emits an aromatic odor. After a few days, a viscous sediment accumulates at the bottom.

On agar, colonies appear within a few millimeters in diameter, flat or raised, opaque, with a rather dull surface, bordered by a regular or finely serrated edge. As they age, they develop metallic sheen. Rough or mucoid forms can also be observed, and dissociations are frequent.

Within 2 to 4 days, a bluish or greenish discoloration of the culture media is often observed due to diffusible pigments produced by the bacterium. (Bouamer & Guerbati, 2008)

3.5.2. Taxonomy and Nomenclature:

Etymologically, the word *Pseudomonas* comes from Greek: pseudo (meaning "false" or "imitation") and monas (meaning "unit"), originally used to describe germs in the early days of microbiology.

The term *aeruginosa*, which means "verdigris" in Latin, refers to the pigment produced by the bacterium that gives the colony its characteristic greenish color. *P. aeruginosa* is the type species of the *Pseudomonas* genus.

Pseudomonas aeruginosa belongs to:

- ❖ Kingdom: Bacteria
- ❖ Phylum: Prokaryota
- ❖ Division: Proteobacteria
- ❖ Class: Gammaproteobacteria
- ❖ Order: Pseudomonadales

- ❖ Family: Pseudomonadaceae
- ❖ Genus: Pseudomonas
- ❖ Species: aeruginosa

The classification of *P. aeruginosa* (like all bacteria) was initially based on the study of its phenotypic characteristics (morphological and biochemical), and later on its genotypic characteristics (genome analysis). (Benelmili & Sahraoui, 2021).

4. Definition of an antibiotic:

An antibiotic is defined as a substance, of natural or synthetic origin, used to treat infections caused by bacteria.

Antibiotics generally act through two main mechanisms. Some attack the peptidoglycan layer of the bacterial cell wall, causing destabilization and ultimately leading to bacterial death. This is referred to as a bactericidal action, meaning it kills the bacteria.

Others act on the bacterial protein synthesis system: by binding to the 50S subunit of the bacterial ribosome, they inhibit protein synthesis. In this case, the bacterium does not die but can no longer grow or reproduce; this is known as bacteriostasis. The antibiotic, therefore, inhibits the multiplication of bacteria without killing them.

The bacteriostatic effect of an antibiotic, like the bactericidal one, is determined by measuring its Minimum Inhibitory Concentration (MIC), which is the lowest concentration of the antibiotic required to inhibit the growth of a bacterial strain in vitro. This MIC is measured by determining the diameter of diffusion of a disc soaked with a known concentration of the antibiotic placed on a Petri dish containing the bacterium to be tested. Each antibiotic has its own specific MIC. (Soum & Saidouni, 2019).

5. Classification of Antibiotics:

There are several families of antibiotics. The main ones include beta-lactams (penicillins and cephalosporins), macrolides, aminoglycosides, tetracyclines, and quinolones. These major antibiotic families differ based on: (Soum & Saidouni, 2019)

- ❖ Their spectrum of activity: meaning the range of microbes that are sensitive to each family of antibiotics.
- ❖ Their mechanism of action at the bacterial level.
- ❖ Their medical indications, which are directly related to the spectrum of activity and the antibiotic's distribution in different organs. For example, some antibiotics concentrate in the urine and are particularly effective for treating urinary tract infections.
- ❖ Their route of administration: antibiotics can be taken orally, except for aminoglycosides, which are destroyed in the intestine. There are also eye drops, ear or nasal solutions, and

ointments containing antibiotics. These local forms are sometimes sufficient to treat certain infections.

- ❖ Their dosage and frequency of use: for some infections, single-dose treatments are available, for example.
- ❖ Their contraindications.
- ❖ Their side effects: allergic reactions, diarrhea, photosensitivity, tendinitis, and kidney toxicity are some adverse effects characteristic of certain antibiotic families. The occurrence of a severe side effect may limit the future use of other drugs from the same family.

The various antibiotics used in therapeutic medicine can be classified according to their chemical family and their modes of action. The known targets so far include the bacterial cell wall, membrane, nucleic acids, and ribosomes of microorganisms. (Soum & Saidouni, 2019)

6. Treatment:

✓ Amoxicillin:

Amoxicillin is used to treat bacterial infections caused by Gram-positive bacteria such as pneumonia, bronchitis, and gonorrhea. It is also used to treat infections of the ear, nose, throat, urinary tract, and skin, in addition to certain Gram-negative bacteria. Amoxicillin belongs to a class of antibiotics known as penicillin-like (beta-lactam) antibiotics. (Promoting the Quality of Medicines, 2017).

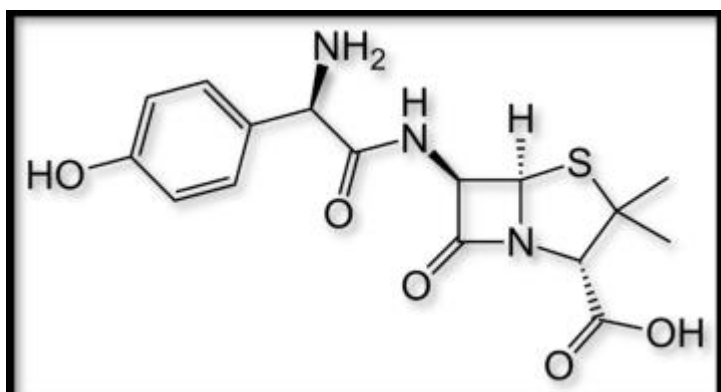


Figure6: Structure of amoxicillin (Promoting the Quality of Medicines, 2017).

✓ Gentamicin:

(Gentalline®) is an antibiotic that belongs to the aminoglycoside family, with streptomycin being the earliest representative, discovered in 1944. It is derived from the fermentation of actinomycete bacteria of the Micromonospora genus, which produce an oligosaccharide complex based on a deoxystreptamine core. The active substance known as

gentamicin is actually a mixture of three components that share similar antibacterial activity. (Bochaton C, Rochegude S, 2004).

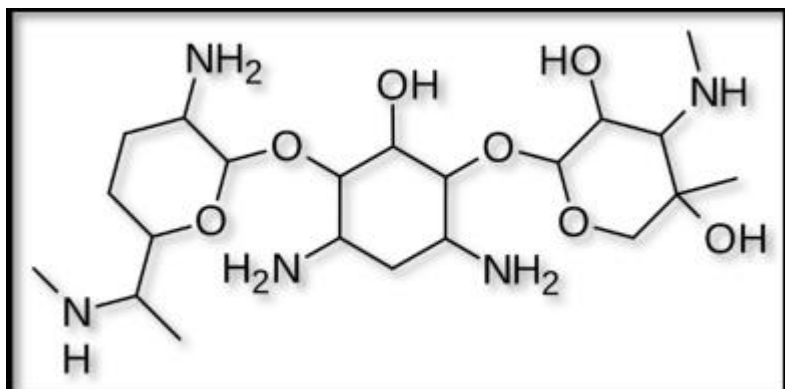


Figure7: Structure of gentamicin (Bochaton C, Rochegude S, 2004).

Chapter 2:
General Overview
of Porphyrins

1. General Information about Porphyrins and Their Derivative:

Porphyrins, metalloporphyrins, and their derivatives are among the most extensively studied aromatic heterocyclic macrocyclic compounds. These molecules can be classified based on their origin: either endogenous, if synthesized within a living animal or plant organism, or exogenous, if not synthesized naturally (i.e., produced in laboratories). Porphyrins form part of the structure of many pigments and serve as a foundation for the formation of certain molecules in living organisms.

Endogenous porphyrins participate in many essential physiological processes in various living cells, both plant and animal. For example, one can mention cyanocobalamin, known as vitamin B12, which is essential for proper body growth. When combined with an iron ion, animal porphyrins form heme, a crucial component for the function of many metalloproteins. Hemoglobin in red blood cells ensures the transport of oxygen and carbon dioxide in the blood.

Finally, plant porphyrins bind with a magnesium ion to form chlorophyll, which is essential for photosynthesis. This class of colorful organic molecules, which interact with light and are among the fundamental elements of life, continues to fascinate researchers. Today, their study contributes to the advancement of innovative biotechnological applications in various fields such as medicine, agriculture, microelectronics, and optoelectronics. The potential applications of these materials are detailed in section S. (Bekale, 2015).

1.1. Definition:

Porphyrins are highly conjugated aromatic macrocycles containing a system of 18 delocalized π -electrons. They are composed of four pyrrolic units interconnected by methine bridges. This extended conjugation imparts notable chemical stability and intense coloration—hallmark features of these compounds.

Depending on the pH of the medium, porphyrins can exhibit acid-base behavior, functioning either as diacids or dibases. Their planar and rigid structure, featuring four nitrogen donor atoms oriented toward the center of the macrocycle, gives them a strong chelating ability. Owing to this configuration, they can form highly stable complexes with a wide range of metal ions (Ghichi, 2010).

1.2. Nomenclature:

The German chemist Hans Fischer, who was awarded the Nobel Prize in Chemistry in 1930 for his studies on porphyrins, proposed a nomenclature system based on a numbering scheme from 1 to 24 to designate substituted porphyrins at the beta-pyrrolic positions. In this system, the carbon atoms at the methine positions, also known as meso positions, are labeled

with the letters α , β , γ , and δ , while the beta-pyrrolic carbon atoms are assigned the numbers 1 through 8.(Figure8) Later, in 1987, a systematic numbering for the macrocycle was adopted to simplify the nomenclature of these molecules. Accordingly, the meso carbon atoms were assigned the numbers 5, 10, 15, and 20, and the pyrrolic ring positions α and β were designated as positions 1, 4, 6, 9, 11, 14, 16, 19 and 2, 3, 7, 8, 12, 13, 17, 18 respectively. Finally, the four nitrogen atoms were assigned the numbers 21, 22, 23, and 24 have been assigned to the four nitrogen atoms (Figure8)(Bekale, 2015).

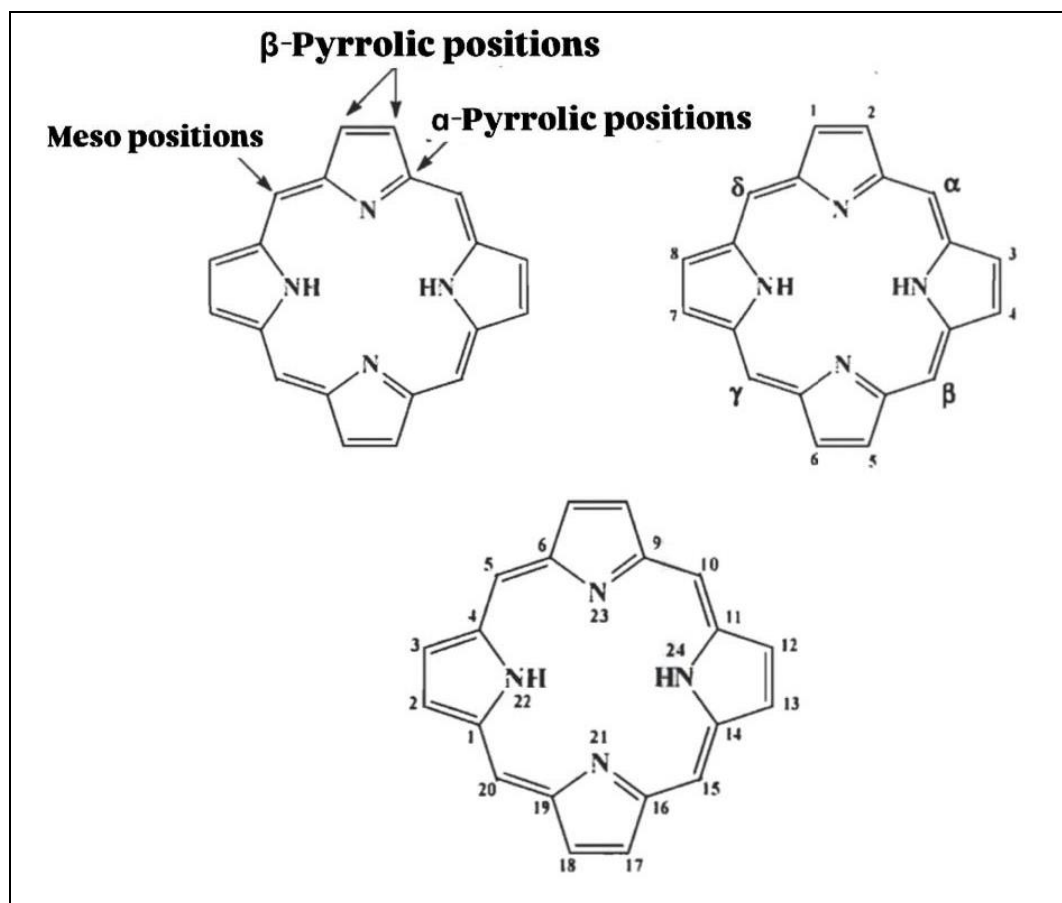


Figure8: Nomenclature system of the tetrapyrrolic macrocycle(Bekale, 2015).

1.3. Structure of Porphyrins:

Porphyrins are a class of cyclic compounds where the main large ring is composed of four pyrrole units connected to each other by single carbon (methine) bridges (Figure 9). The molecular size of these large tetrapyrrolic macrocycles is approximately 9 Å, with a central cavity about 4.2 Å in diameter. The central cavity of the porphyrin has an ideal size to accommodate first-row transition metals (such as iron, cobalt, nickel, copper, zinc, etc.). Larger metal ions sit above the macrocycle, forcing the porphyrin to adopt a dome-like conformation. Porphyrins are aromatic compounds containing 18 conjugated electrons, which gives them a strong color and inherent fluorescence. The aromatic tetrapyrrolic macrocycle is

thermally, chemically, and biologically stable. These compounds also have the advantage of being non-toxic when not exposed to light. All porphyrin-derived molecules (such as heme, chlorophyll, or other related macrocycles) follow the same pattern: a tetrapyrrolic ring in which a metal ion can coordinate (Figure 9) (Bekale, 2015).

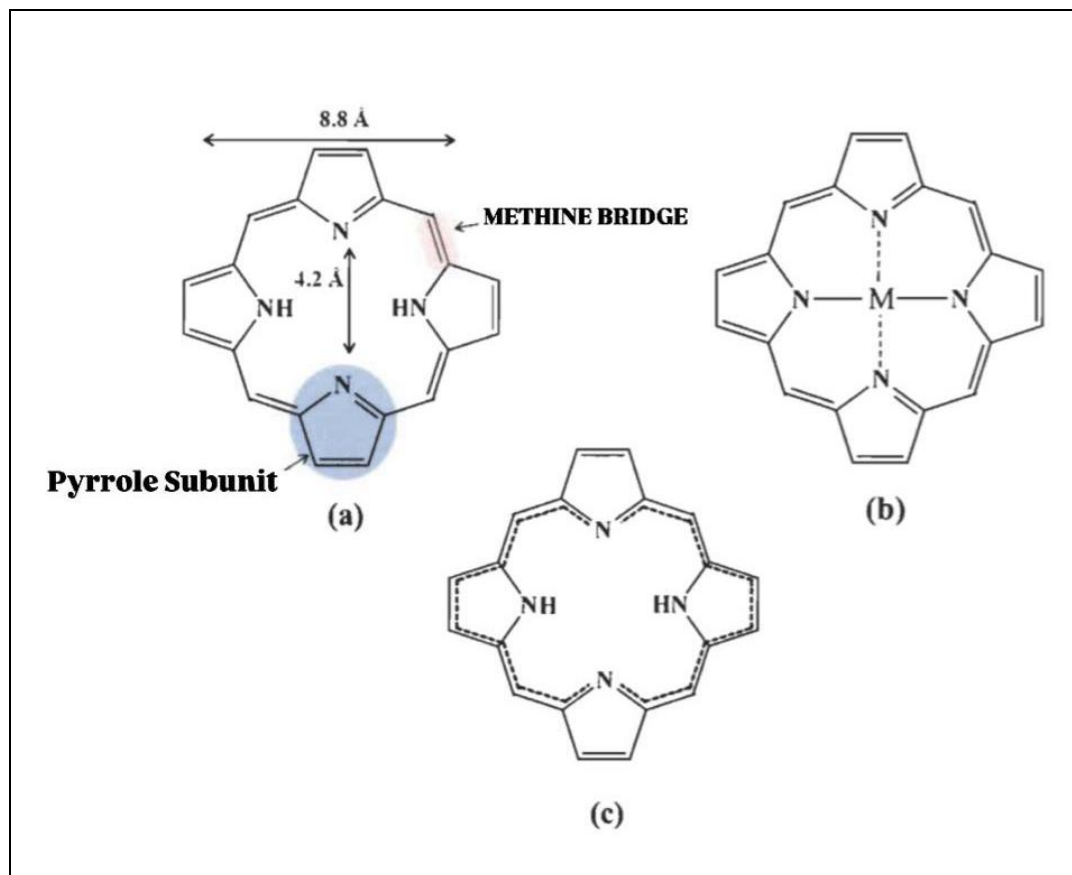


Figure9: Chemical structure of porphyrin (Bekale, 2015).

- (a) Free-base porphyrin;
- (b) Metallated porphyrin or metalloporphyrin;
- (c) Representation of the 18 π -electron aromatic system.

1.4. Characteristics:

1.4.1. NMR Spectroscopy:

Proton nuclear magnetic resonance (^1H NMR) analysis of porphyrins reveals two characteristic types of signals:

- ❖ β -pyrrolic protons, typically observed in the region of 8–9 ppm. The multiplicity of these signals depends largely on the symmetry of the porphyrin molecule and the nature of the substituents at the meso positions.
- ❖ Protons bonded to the pyrrolic nitrogen atoms, which appear around -2 ppm. These protons are exchangeable and generally produce a broad signal.

These unusual chemical shifts can be attributed to an anisotropic shielding effect caused by the aromatic nature of the macrocycle. When an external magnetic field is applied, a ring current is induced in the conjugated π -system, generating a secondary magnetic field. This leads to the formation of a magnetic anisotropy cone: protons located inside this cone (such as the N–H protons of the pyrrole rings) experience shielding (upfield shift), while those outside the cone (such as β -pyrrolic protons) are deshielded (downfield shift).

Regarding ^{13}C NMR, quaternary carbon atoms exhibit long spin-lattice relaxation times (on the order of one second), which typically results in low-intensity signals (Sguerra, 2012).

1.4.2. UV-Visible Spectroscopy:

The UV-visible absorption spectrum of porphyrins primarily arises from $\pi \rightarrow \pi^*$ electronic transitions. Typically, the absorption profile is divided into two main regions:

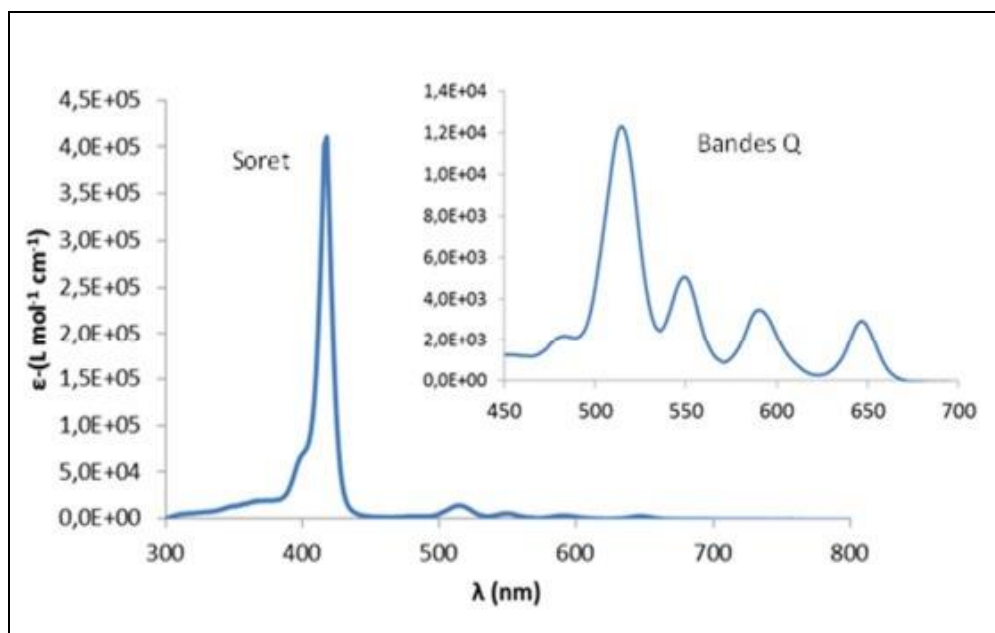


Figure10: Absorption spectrum of 5,10,15,20-meso-tetraphenylporphyrin (TPP) (CH_2Cl_2 , room temperature) (Sguerra, 2012).

- ❖ The Soret band, a highly intense absorption band observed around 420 nm, characterized by a high molar extinction coefficient ($\epsilon \approx 4 \times 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$).
- ❖ The Q bands, located in the 500–700 nm range ($\epsilon \approx 1 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$). Free-base porphyrins usually exhibit four Q bands, whereas metalloporphyrins display only two due to increased molecular symmetry (Sguerra, 2012).

1.5. Toxicity of Porphyrins:

Porphyrins are widely utilized as photosensitizers in photodynamic therapy (PDT) due to their ability to generate reactive oxygen species (ROS) upon light activation. This property

enables targeted destruction of malignant cells. However, the potential toxicity of porphyrins, both in light and dark conditions, necessitates careful consideration.

In the absence of light (dark toxicity), certain porphyrin derivatives can exhibit cytotoxic effects. This is often attributed to their physicochemical properties, such as lipophilicity and charge, which influence their cellular uptake and localization. For instance, cationic porphyrins may interact with cellular membranes or organelles, leading to oxidative stress and cell death even without photoactivation. The degree of dark toxicity is influenced by factors including chemical structure, concentration, and the specific cell type involved.

Upon light activation, porphyrins can produce ROS, leading to phototoxic effects. While this is the desired mechanism in PDT, unintended damage to surrounding healthy tissues can occur if the photosensitizer is not adequately targeted. Therefore, strategies such as conjugation with targeting ligands or encapsulation in nanocarriers have been developed to enhance selectivity and minimize off-target effects.

A study evaluating the toxicity of a porphyrin-based photosensitizer in an in vivo model demonstrated that while the compound was effective in inducing phototoxicity in targeted cells, it also exhibited minimal dark toxicity, highlighting the importance of structural modifications to improve safety profiles (Zhou et al., 2023).

1.6. Applications of porphyrins:

1.6.1. Photodynamic Therapy (PDT) for Cancer Treatment:

Mechanism: This approach relies on activating porphyrins inside the body using light of a specific wavelength, which leads to the generation of reactive oxygen species (ROS) that selectively destroy cancer cells.

Porphyrins Used: Compounds such as hematoporphyrin and its derivatives.

Clinical Applications: PDT is used to treat cancers of the skin, bladder, and lungs while preserving surrounding healthy tissues (Bonnet, 2015).

1.6.2. Bioimaging and Diagnostics:

Advantage: Porphyrins exhibit natural fluorescence, which can be exploited in bioimaging to detect tumors or sites of inflammation.

Supporting Technologies: Magnetic Resonance Imaging (MRI) becomes more effective when porphyrins are combined with magnetic metals (Wang et al., 2010).

1.6.3. Antimicrobial Photodynamic Therapy (aPDT):

Efficacy: This method is effective in eliminating bacteria, viruses, and fungi, including strains resistant to traditional antibiotics.

Principle: Upon light activation, porphyrins produce ROS that rapidly kill microbial cells (Maisch, 2020).

1.6.4. Treatment of Dermatological Diseases:

Targeted Conditions: Psoriasis, acne, and warts.

Mechanism: Porphyrins enable the selective targeting of diseased or infected skin cells without damaging healthy surrounding tissues (Morton et al., 2014).

1.6.5. Nanotechnology Applications:

Integration with Nanotechnology: Porphyrins can be incorporated into nanocapsules or nanoparticles to enhance targeted delivery and achieve controlled drug release.

Outcomes: This leads to improved therapeutic efficiency, reduced toxicity, and more precise treatment effects (Kumar et al., 2020).

1.7. Therapeutic Role of Porphyrins:

Targeted drug delivery remains a significant challenge in modern medicine. Conventional drugs and their delivery systems often face multiple limitations, such as poor biodistribution, inefficient clearance from the body, and uncontrolled release that does not align with the desired pharmacodynamics. As a result, recent research has increasingly focused on the development of smart, site-specific drug delivery systems. Within this context, porphyrins have emerged as promising agents in therapeutic and diagnostic applications.

Porphyrins are particularly attractive due to their preferential accumulation in tumor tissues, their ability to generate reactive singlet oxygen, and their low dark toxicity, making them especially useful in photodynamic therapy (PDT) for cancer treatment. However, one of the major obstacles limiting their clinical utility is their poor water solubility, which adversely affects their biodistribution. To overcome this, research has shifted toward the development of porphyrin-based nanocomposites, aiming to enhance solubility, bioavailability, and overall therapeutic efficacy.

Structurally, porphyrins possess a highly modifiable framework composed of four pyrrole units linked via methine bridges. The presence of N–H groups facilitates hydrogen bonding with anionic species, while the nitrogen atoms within the core allow for selective metal ion chelation. These properties also enable the conjugation of porphyrins to a wide range of carriers, including polymers and liposomes. Such structural versatility has made porphyrins an ideal platform for chemical modification and biomedical application.

Consequently, research has increasingly focused on the post-functionalization of porphyrins to optimize their physicochemical and biological properties, including biodistribution, aggregation behavior, stability, and toxicity. These functionalization strategies

can be categorized into two main approaches: core functionalization—where chemical groups are introduced directly at the β - and/or meso-positions of the porphyrin ring—and peripheral functionalization, involving modification of the substituents at the molecular periphery. Despite the wide variety of chemical modifications explored, literature has predominantly focused on porphyrins incorporating metal centers, halogens, or alkynes. (Sato et al., 2019)

1.7.1. Porphyrins in Photo-Medicine:

Porphyrins possess tunable optical properties that allow them to absorb light within the therapeutic window (600–800 nm), making them ideal candidates for photo-medicine applications, particularly photodynamic therapy (PDT) and photothermal therapy (PTT). Their effectiveness stems from their ability to produce singlet oxygen upon light activation, a process that involves excitation to a singlet state, intersystem crossing to a triplet state, and subsequent energy transfer to molecular oxygen, yielding cytotoxic singlet oxygen ($^1\text{O}_2$).

In PDT, porphyrin-based photosensitizers (PBPs) are classified into three generations. First-generation PBPs were limited by long biological half-lives, chemical impurities, low molar extinction coefficients, and non-specific accumulation in the skin. Second-generation PBPs showed improved photophysical properties but still lacked sufficient water solubility and tissue penetration depth. To overcome these limitations, third-generation PBPs have been developed. These include porphyrins conjugated to targeting biomolecules and nanomaterials, such as quantum dots (QDs), gold nanoparticles (AuNPs), and superparamagnetic iron oxide nanoparticles (SPIONs), enhancing tumor-specific accumulation and therapeutic efficacy. (Sato et al., 2019)

1.7.2. Porphyrin–Nanoconjugates in PDT:

Nanotechnology has enabled significant advancements in porphyrin delivery and performance. SPION–porphyrin conjugates, for example, allow for magnetically guided targeting of cancer cells and more efficient PDT. A notable study from our group demonstrated the use of a multifunctional nano-drug composed of methoxypolyethylene glycol-thiol-SPIONs, gold, and meso-tetrakis(4-hydroxyphenyl)porphyrin. This system showed enhanced phototoxicity against MCF-7 breast cancer cells when activated by light (673 nm, 10 J/cm²) and further improved efficacy under an external magnetic field due to increased cellular uptake.

Similarly, Feng et al. reported successful PDT using 5-aminolevulinic acid (ALA) conjugated to CuInS/ZnS quantum dots, leveraging Förster resonance energy transfer (FRET) under near-infrared (NIR) femtosecond laser excitation (800 nm and 1300 nm). In another study, we demonstrated a significant improvement in singlet oxygen quantum yield—rising

from 0.27 (free mTHPP) to 0.72 (conjugated system)—by conjugating mTHPP to CuInS/ZnS QDs. The resulting system exhibited high water solubility and remained stable for over six months.

These findings highlight the potential of porphyrin–nanoconjugates in advancing photo-based therapies by enhancing selectivity, solubility, and photodynamic efficacy. (Sato et al., 2019).

Part 2: *Experimental*



Chapter 1:

Materials and Methods



1. Introduction:

This study was conducted collaboratively between two specialized laboratories: the Valorisation and Technology of the Saharian Resources Laboratory (VTRS) at the Faculty of Exact Sciences, Department of Chemistry, University of El Oued, and the Centre de Recherche Scientifique et Technique en Analyses Physico-Chimique (CRAPC) in Ouargla, Algeria. The VTRS lab facilitated Porphyrin extraction, conducted in vitro and in-silico. Meanwhile, CRAPC handled the precise GC/MS analysis of the extracted Porphyrin. This collaborative effort ensured thorough and accurate experimentation, combining expertise and resources from both institutions.

2. Studied compounds:

2.1. P2:

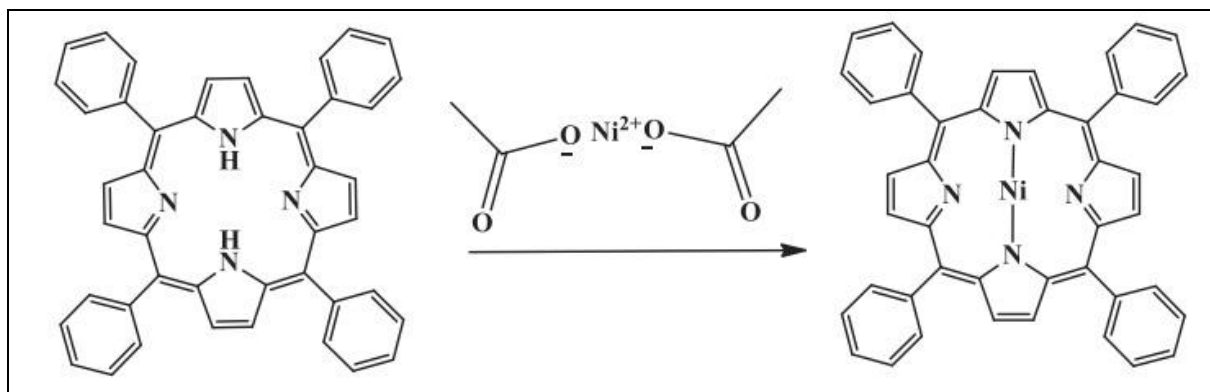


Figure11: Synthetic scheme of nickel tetraphenyl-porphyrin

2.2. P3:

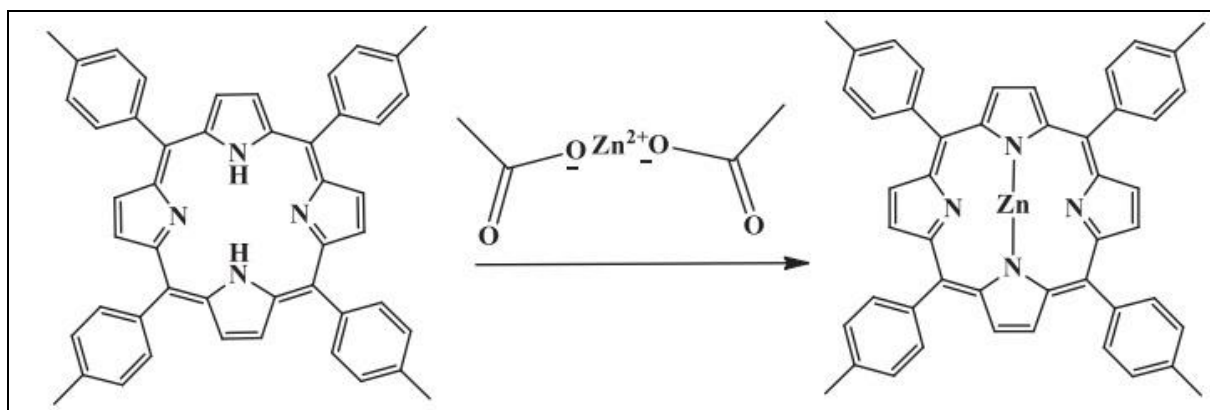


Figure12: Synthetic scheme of zinc tetra(4-methophenyl)- porphyrin

3. Chemicals and reagents:

3.1. Microorganisms Used:

All microorganisms used in these studies were provided by the pedagogical laboratory of the faculty of biology.

- ***Escherichia coli* (ATCC 25922)**: Belongs to the Enterobacteriaceae family. It is non-spore forming, facultative anaerobic, and typically motile due to flagella. Its length ranges from 2 to 6 μm and its width from 1.1 to 1.5 μm .
- ***Bacillus subtilis* (ATCC 6051-U)**: A Gram-positive bacterium with a length ranging from 2 to 3 μm and a width from 0.3 to 0.5 μm .
- ***Staphylococcus aureus* (ATCC 25923)**: Is a Gram-positive spherical bacterium with a diameter ranging from 0.5 to 1.5 μm , belonging to the genus *Staphylococcus*.
- ***Pseudomonas aeruginosa* (ATCC 27853)**: Belongs to the Pseudomonadaceae family. It is a Gram-negative, non-spore forming, strictly aerobic rod-shaped bacterium. Its length ranges from 1 to 5 μm and its width from 0.5 to 1 μm .
- ***Klebsiella pneumoniae* (ATCC 13883)**: is the type species of the genus *Klebsiella*. It belongs to the Enterobacteriaceae family. It is a Gram-negative bacillus, non-motile, encapsulated, non-spore forming, and facultatively anaerobic. It is capable of fermenting lactose on MacConkey medium, forming characteristic mucoid colonies. Its length ranges from 1.0 to 2.0 μm , and its width from 0.5 to 0.8 μm .

3.2. Laboratory Equipment:

Table05: Laboratory Equipment

Bunsen burner	Test tube rack	Autoclave
Refrigerator	Spectrophotometer	Erlenmeyer flask
Reagent bottle	Tweezer	Micropipette
Filter paper	Lab spatula	Gloves
Analytical balance		

3.3. Chemical Products:

Table06: Chemical Products

Porphyrin	Zinc (zn)
Nickel (Ni)	Dimethyl sulfoxide((CH ₃) ₂ SO)
Sterile physiological saline (0.9% NaCl)	Mueller-Hinton (MH)
Nutrient agar	

4. In Vitro Assessment of the Antibacterial Activity:

✓ **Procedure:**

Antimicrobial tests were conducted to evaluate the biological activity of the studied Porphyrin against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antimicrobial activity was assessed using the disc diffusion method as described in the literature (Klančnik et al., 2010):

- Preparation of test products: Dissolve Porphyrin in DMSO to prepare solutions of different concentrations ranging from 1 to 20 mg/ml.



Figure13: Preparation of test product

- Preparation of precultures: Cultivate the microbial strains to be tested on Petri dishes containing nutrient agar and incubate for 24 hours at 37°C to obtain a young culture of bacteria and isolated colonies.



Figure14: Storage of bacterial media in the incubator

- Preparation of discs: Prepare discs using 6mm diameter Whatman filter paper, followed by sterilization for 30 minutes at 120°C in the autoclave.



Figure15: Preparation of disc



Figure16: Sterilization stage

- Preparation of bacterial suspensions: Take a few well-isolated and identical colonies using a Pasteur pipette and suspend them in 10 ml of sterile physiological saline solution (0.9% NaCl). Standardize the bacterial suspension using a densitometer for seeding into the MH milieu.
- Preparation of culture milieu: Utilize various culture milieu including nutrient broth for isolation and maintenance of bacterial strains, nutrient agar for reactivation of the bacterial strain, and Mueller-Hinton agar for studying bacterial sensitivity to different concentrations of Porphyrin.



Figure17: Preparation of milieu culture

- Testing antibacterial activity: Spread the bacterial suspension over the entire surface of Mueller Hinton agar (MHA) three times using a sterile swab, then leave on the bench for 30 minutes. Assess antibacterial activity by measuring the diameter of the inhibition zone using a ruler and comparing it with that of DMSO as a negative control and antibiotics as positive controls (Amoxicillin 25 µg/disc). Measure absorbance using UV-vis spectroscopy.

The inhibitory activity of bacteria was calculated as a percentage of inhibition using the following Equation 6 (Klančnik et al., 2010):

$$\%Inhibition = \left(1 - \frac{Abs_{porphorin}}{Abs_{Controle}} \right) \times 100$$



Figure18: Reading the results on a spectrophotometer

5. Computational details:

The geometric optimization of all synthesized compounds was carried out using the Gaussian 09 program package [M.J. Frisch et al. (2004)]. The Density Functional Theory (DFT) approach was employed, specifically the Becke's three-parameter hybrid exchange functional combined with the Lee-Yang-Parr correlation functional (B3LYP), in conjunction with the 6-311++G(d,p) basis set [B. Miehlich et al. (1989)]. This functional and basis set combination was selected for its proven ability to accurately predict molecular geometries, electronic properties, and reactivity descriptors for a wide range of organic compounds at a reasonable computational cost.

Upon obtaining the optimized geometries, a detailed quantum chemical analysis was performed to compute several global reactivity descriptors. These include the energy gap ($\Delta EGAP$), ionization energy (I), electron affinity (A), electronegativity (χ), electronic chemical potential (μ), chemical hardness (η), chemical softness (S), and electrophilicity index (ω). These parameters were calculated using the frontier molecular orbital energies—namely, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO)—according to conceptual DFT definitions [N. Chafai et al. (2017)]. The relevant equations are:

Table07: Quantum chemical descriptors calculated based on the frontier molecular orbital energies (HOMO and LUMO) according to conceptual Density

Functional Theory (DFT)

Energy gap:	$\Delta E_{\text{GAP}} = E_{\text{HOMO}} - E_{\text{LUMO}}$	(2)
Ionization Energy:	$I = -E_{\text{HOMO}}$	(3)
Electron Affinity:	$A = -E_{\text{LUMO}}$	(4)
Electronegativity:	$\chi = (I + A)/2$	(5)
Chemical Potential:	$\mu = -\chi$	(6)
Chemical Hardness:	$\eta = (I - A)/2$	(7)
Chemical Softness:	$S = 1/\eta$	(8)
Electrophilicity Index:	$\omega = \mu^2/2\eta$	(9)

These descriptors offer valuable information about the stability, reactivity, and electrophilic or nucleophilic nature of the studied molecules. In particular, the HOMO-LUMO energy gap (ΔE_{GAP}) serves as an important indicator of chemical reactivity and kinetic stability: a smaller gap typically corresponds to higher chemical reactivity and lower kinetic stability. Similarly, the electrophilicity index (ω) quantifies the molecule's ability to accept electrons, which is significant in understanding potential interactions with biological targets.

6. Cheminformatics Prediction:

➤ *PASS Online:*

The molecular structures of all synthesized compounds were initially sketched using ChemDraw® Ultra software, version 12 (CambridgeSoft Corporation, 1986–2009, Akron, OH, USA). Following the drawing phase, each structure underwent a rigorous verification process, ensuring chemical correctness by utilizing the “Clean Structure” tool. This step optimized bond angles and atom placements to produce chemically reasonable, low-strain conformations suitable for computational analysis. Once verified, the structures were exported and saved in the MDL Molfile format (.mol), a standard file type that preserves detailed atomic and bonding information necessary for further in silico analyses.

The generated Mol files were then uploaded to the PASS (Prediction of Activity Spectra for Substances) online server (<http://195.178.207.233/PASS/index.html>) [D.A. Filimonov et al. (2014)]. PASS software employs advanced machine learning algorithms based on

Multilevel Neighborhoods of Atoms (MNA) descriptors to predict the biological activity spectra of chemical compounds. After uploading, the "Predict Activity" function was activated to obtain a comprehensive list of potential biological activities. Each predicted activity was accompanied by two values: the probability "to be active" (Pa) and the probability "to be inactive" (Pi). Only activities with a Pa value greater than 0.5 were considered statistically significant and retained for further interpretation, as higher Pa values indicate a greater likelihood that the compound exhibits the predicted biological activity in experimental settings.

➤ **ADMET Prediction:**

For the evaluation of pharmacokinetic and drug-likeness properties, the chemical structures of the synthesized compounds were processed using the SwissADME online server [A. Daina et al. (2017)]. Initially, the "Import" function within the molecular sketcher interface was utilized to upload the previously prepared structures in MDL Molfile (.mol) format. Upon successful importation, each molecular structure was visually confirmed and automatically rendered in the sketcher window. The structures were subsequently converted into the Simplified Molecular Input Line Entry System (SMILES) notation, a compact representation format necessary for computational processing.

After ensuring the accuracy of the SMILES translation, the "Run" command was executed to initiate the prediction analysis. The SwissADME server then generated a comprehensive set of pharmacokinetic parameters, including absorption, distribution, metabolism, and excretion (ADME) profiles, as well as drug-likeness evaluations based on Lipinski's Rule of Five, Ghose, Veber, Egan, and Muegge criteria. Additionally, properties such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, and interactions with cytochrome P450 isoforms were predicted. These parameters are critical for assessing the drug potential and biopharmaceutical properties of the designed compounds at an early stage of drug discovery.

7. Molecular Docking:

➤ **Ligands Preparation:**

The two-dimensional (2D) chemical structure of the reference standard, amoxicillin, was retrieved from the PubChem database [S. Kim et al. (2016)] (<https://pubchem.ncbi.nlm.nih.gov/>). Following retrieval, the structure was converted into a three-dimensional (3D) conformation using energy minimization tools integrated within molecular modeling software to ensure a geometrically optimized and chemically accurate 3D

model suitable for docking studies. Structural optimization procedures were performed to reduce any steric clashes and to refine bond angles and torsions.

In parallel, the preparation of the porphyrin ligands was conducted according to the protocol described in the Computational Details section. This included geometry optimization at the Density Functional Theory (DFT) level and generation of appropriate file formats required for molecular docking simulations. Both the standard (amoxicillin) and the studied ligands were thus standardized under the same preparation conditions to ensure comparability and accuracy in subsequent docking evaluations.

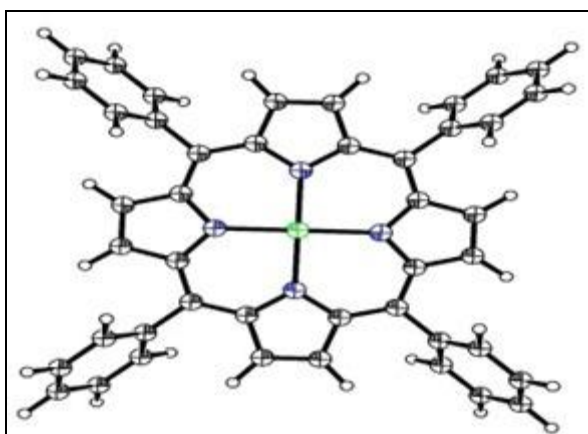


Figure19: Structure of Nickle porphyrin

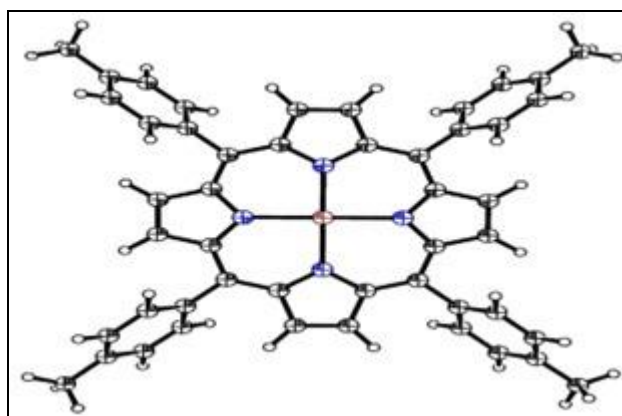


Figure20: Structure of Zinc porphyrin

➤ **Receptor Preparation:**

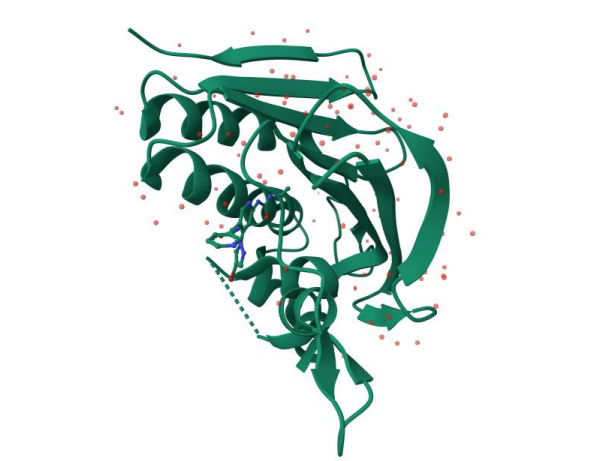
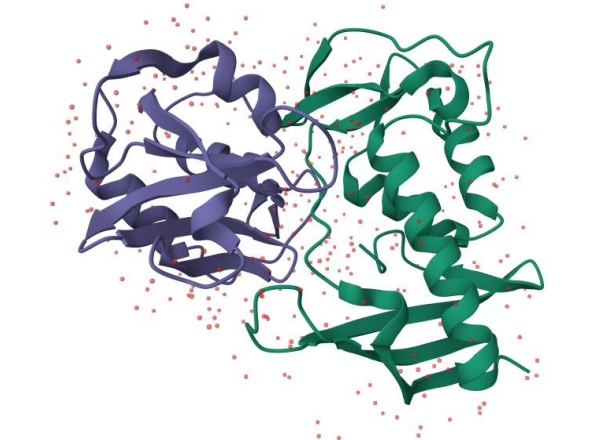
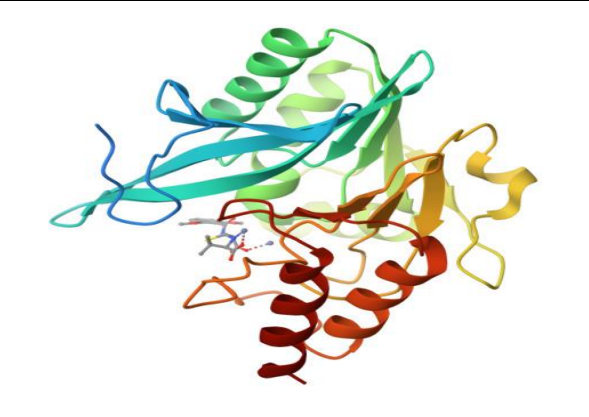
In order to explore the antibacterial potential of the studied compounds, molecular docking analyses were performed against selected bacterial protein targets. The crystallographic structures of *Staphylococcus aureus* (PDB ID: 4knl), *Pseudomonas aeruginosa* (PDB ID: 2uv0), *Klebsiella pneumoniae* (PDB ID: 4ey2), *Escherichia coli* (PDB ID: 1kzn), and *Bacillus subtilis* (PDB ID: 4ddq) were retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org/>) [P.A. Karplus & G.E. Schulz (1989)]. These proteins were

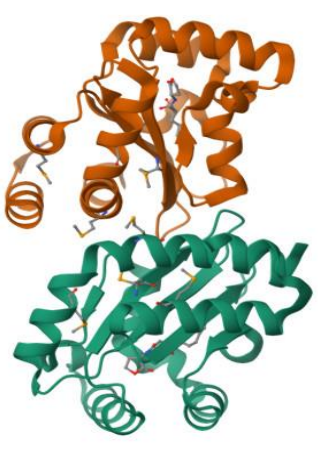
selected due to their clinical relevance as pathogenic microorganisms responsible for various infectious diseases.

Prior to docking simulations, each protein structure was meticulously prepared using AutoDock Tools (ADT) [G.M. Morris et al. (2008)]. The preparation protocol included the removal of all crystallographic water molecules, co-crystallized ligands, and cofactors to ensure that the binding site was available for the studied compounds. To properly model hydrogen bonding interactions, polar hydrogen atoms were added to the protein structures. Additionally, Kollman partial atomic charges were assigned to accurately represent the electrostatic potential of the targets.

The active sites of each bacterial protein were carefully defined based on the coordinates of their co-crystallized ligands and previously reported catalytic residues, ensuring a biologically relevant docking environment. The docking grids were thus generated to encompass the key residues involved in ligand recognition and binding, optimizing the precision of the molecular docking experiments. This rigorous preparation aimed to provide reliable predictions of the binding affinity and interaction modes of the tested compounds with the bacterial targets, ultimately contributing to a better understanding of their antibacterial mechanisms at the molecular level.

Table08. Target receptors information chosen for docking studies

	<i>Crystal Structure of E. coli GyraseB 24kDa</i>	
	PDB ID	6F86
	Mutation	No
	Resolution (Å)	1.90
	R-Value Observed	0.204
	Organism	Escherichia coli
	Space Groupe	P 3 ₂ 2 1
	Sequence Length	206
	<i>Structure of the P. aeruginosa LasR ligand-binding domain bound to its autoinducer IseA</i>	
	PDB ID	8I2F
	Mutation	No
	Resolution (Å)	2.03
	R-Value Observed	0.175
	Organism	Bacillus subtilis
	Space Groupe	P 2 ₁ 2 ₁ 2 ₁
	Sequence Length	282
	<i>Two-component system protein mediate signal transduction</i>	
	PDB ID	6LKI
	Mutation	No
	Resolution (Å)	1.78
	R-Value Observed	0.210
	Organism	Staphylococcus aureus
	Space Groupe	C 2 2 2 ₁
	Sequence Length	471
	<i>Crystal structure of NDM-1 bound to hydrolyzed methicillin</i>	
	PDB ID	4EY2
	Mutation	No
	Resolution (Å)	1.17
	R-Value Observed	0.131
	Organism	Klebsiella pneumonia
	Space Groupe	P 2 ₁ 2 ₁ 2 ₁
	Sequence Length	270

	Structure of the <i>P. aeruginosa</i> LasR ligand-binding domain bound to its autoinducer	
	PDB ID	2UV0
	Mutation	No
	Resolution (Å)	1.80
	R-Value Observed	0.211
	Organism	pseudomonas aeruginosa
	Space Groupe	P 1 2 ₁ 1
	Sequence Length	175

➤ **Molecular Docking Studies:**

To assess the binding affinities between the selected bacterial protein targets and the studied ligands, an automated molecular docking study was performed utilizing AutoDock 4.2 software [C. Steffen et al. (2010)]. For each protein, the binding site was delineated by constructing a grid box using AutoGrid, centered around the known active site residues identified from the co-crystallized ligands or reported in the literature. The grid dimensions were standardized to $80 \times 80 \times 80$ points with a grid spacing of $0.5 \text{ \AA}$, ensuring comprehensive coverage of the active regions. The grid centers were individually assigned based on the specific coordinates of each bacterial protein structure.

Ligand preparation involved the assignment of all rotatable bonds through the use of the autotors module integrated within AutoDock Tools, enabling full conformational flexibility of the ligands during the docking process, while treating the receptor as a rigid body. Docking simulations were conducted employing the Lamarckian Genetic Algorithm (LGA), utilizing the following parameters: 10 docking runs per ligand, a population size of 150, a maximum of 250,000 energy evaluations, a maximum of 27,000 generations, a mutation rate of 0.02, a crossover rate of 0.8, and an elitism value of 1, while maintaining all other parameters at their default settings.

Following the docking simulations, the binding pose associated with the lowest binding free energy (expressed in kcal/mol) was selected as the most representative binding conformation for each ligand-target complex. The selected docking poses were subsequently analyzed using the Protein-Ligand Interaction Profiler (PLIP) web server [S. Salentin et al. (2015)], allowing a detailed characterization of the interactions established between the ligands and the bacterial proteins, including hydrogen bonds, hydrophobic contacts, salt bridges, and π - π stacking interactions. This comprehensive analysis provided valuable

insights into the molecular basis of the antibacterial activity exhibited by the studied compounds.

Chapter 02:
Results and discussion

1. Introduction:

This chapter delves into the findings regarding the synthesis yield, characterization, and comprehensive exploration of the antibacterial properties inherent in porphyrin derivatives substituted with nickel and zinc. The overarching aim of this investigation was to assess the potential antibacterial efficacy exhibited by these compounds as significant agents in the domain of novel pharmaceutical development.

The elucidations provided herein furnish intricate insights into the chemical constitution of the porphyrin derivatives, thereby enabling their utilization in the in-silico study. This involved the application of advanced computational techniques such as Induced Fit Docking (IFD) and Molecular Dynamics Simulation (MDS) for each compound, capabilities not feasible in traditional in vitro studies.

2. Assessment of Antibacterial Activity:

The in vitro assessment of the antimicrobial efficacy of Porphorin2 and Porphorin3 was conducted utilizing the disk diffusion technique against 5 prevalent bacterial strains associated with infections, *Escherichia coli* (E. Coli), *Bacillus subtilis* (B. subtilis), *Klebsilla Pneumoniae* (Klebsilla.P), *Pseudomonas Aeruginosa* (Pseudomonas. A) and *Staphylococcus aureus* (S. aureus). Two distinct methodologies were employed to gauge the antibacterial activity: quantifying the zone of inhibition surrounding the Porphorin -impregnated disk and measuring the optical density of the bacterial suspension subsequent to treatment with varying concentrations of Porphorin. Comparative analysis was performed with amoxicillin, a widely utilized antibiotic. This study presents the findings derived from the diverse methodologies employed to evaluate the antibacterial efficacy of the studied antibacterial and juxtaposes these outcomes with those obtained with amoxicillin.

3. Electronic Spectroscopy Interaction Study:

3.1. Bacterial inhibitory activities (IC₅₀):

The antibacterial activity of Porphorin2 and Porphorin3 was assessed using the absorbance measurement method to determine the IC₅₀. This methodology entails measuring bacterial growth in wells containing varying concentrations of the exponential regression curves depicting the percentage inhibition of bacteria versus the concentration of P2 and P3. The resulting data facilitated the determination of the minimum concentration of P2 and P3 required to inhibit bacterial growth by 50%.

To this end, cultures of *Escherichia coli* (E. Coli), *Bacillus subtilis* (B. subtilis), *Klebsilla Pneumoniae* (Klebsilla.P), *Pseudomonas Aeruginosa* (Pseudomonas. A) and *Staphylococcus aureus* (S. aureus) were incubated with different concentrations of P2 and P3,

ranging from 1 to 10 mg/ml, for 24 hours at 37°C. Subsequently, the optical density of each sample was measured at 620 nm using a spectrophotometer. The findings obtained are summarized in the following Table 9.

Table09: Absorbance values sorted from the antibacterial assays

C (mg/ml)	P2					P3				
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>Klebsilla. P</i>	<i>Pseudomonas. A</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>Klebsilla. P</i>	<i>Pseudomonas. A</i>
0	2.5	2.22	1.7	3	2.2	2.5	2.22	1.7	3	2.2
1	2.31	2.22	1.69	2.9	2.18	2.49	1.88	1.66	2.03	2
2	2.12	1.9	1.4	2.07	1.36	2.24	1.6	1.6	1.92	1.65
3	2.08	1.5	1.36	1.96	1.25	2.09	1.52	1.54	1.92	1.64
4	2	1.43	1.36	1.91	0.61	2.03	1.46	1.4	1.85	1.61
5	1.7	1.14	1.35	1.36	0.53	1.96	1.16	1.21	1.83	1.4
6	1.28	0.91	1.06	1.32	0.36	1.92	1.11	1.16	1.82	1.23
7	1.26	0.42	0.74	1.1	-	1.84	0.75	0.97	1.6	1.1
8	1.15	-	0.43	0.98	-	1.6	0.28	0.88	1.42	0.87
9	0.7	-	0.23	0.81	-	1.48	-	0.73	1.39	-
10	0.47	-	0.22	0.54	-	0.27	-	0.64	0.9	-

The antibacterial activities of compounds **P2** and **P3** were evaluated against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* using absorbance-based assays. As shown in Table 9, both compounds exhibited concentration-dependent antibacterial effects, with absorbance values progressively decreasing as concentrations increased from 0 to 10 mg/mL, indicating reduced bacterial growth.

At 0 mg/mL (control), absorbance values were highest for all strains (ranging from 1.7 to 3.0), reflecting maximal bacterial proliferation. Upon treatment, a notable reduction was observed. For **P2**, *Pseudomonas aeruginosa* was particularly sensitive, with absorbance declining from 2.2 at 0 mg/mL to 0.36 at 6 mg/mL and becoming undetectable at 7 mg/mL onward. Similarly, *B. subtilis* showed significant susceptibility, with absorbance decreasing sharply to 0.42 at 7 mg/mL and undetectable at 8 mg/mL. In contrast, *E. coli* showed greater resistance to P2, maintaining relatively high absorbance values (e.g., 1.15 at 8 mg/mL).

Compound **P3** demonstrated a comparable but slightly stronger antibacterial effect compared to P2. Particularly, *B. subtilis* and *S. aureus* were highly sensitive, with *B. subtilis* absorbance reduced to 0.28 at 8 mg/mL and becoming undetectable at 9 mg/mL. *Pseudomonas aeruginosa* also showed significant inhibition by P3, with absorbance values decreasing to 0.87 at 8 mg/mL and 0.64 at 10 mg/mL.

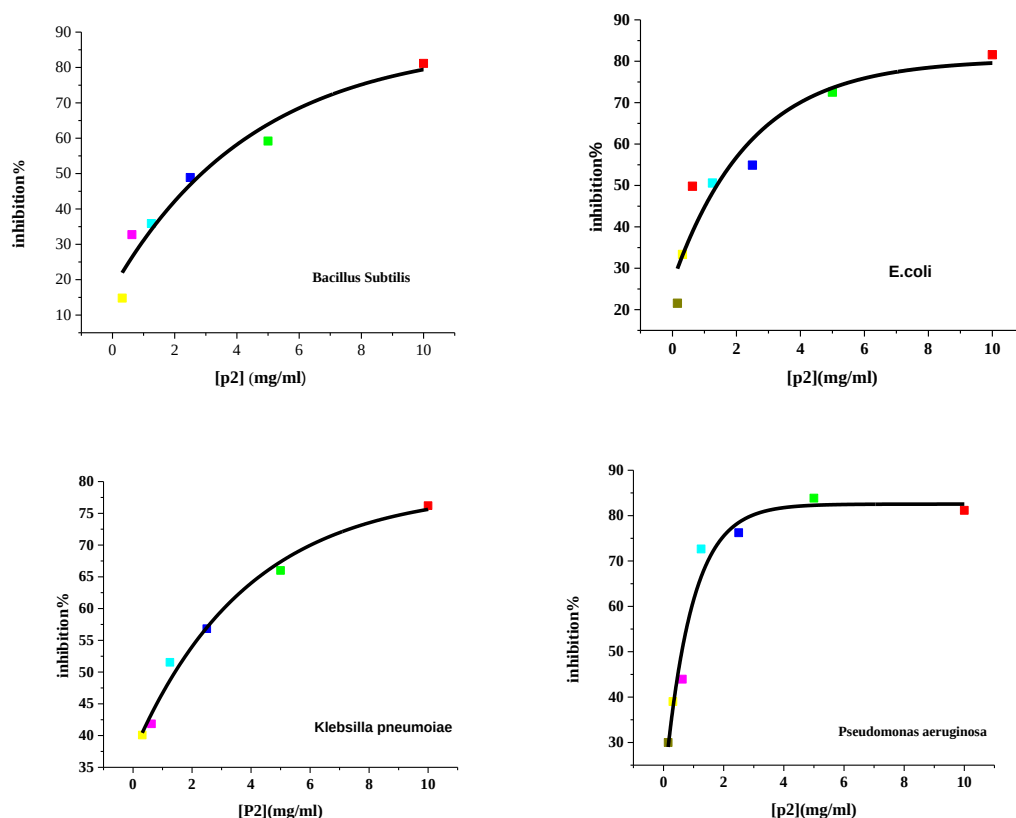
Overall, **P3 showed slightly better antibacterial potency** compared to P2 across almost all tested strains, especially at higher concentrations (≥ 5 mg/mL). Notably, *Klebsiella*

pneumoniae and *Pseudomonas aeruginosa* remained more resilient to both compounds compared to *B. subtilis* and *S. aureus*, highlighting the intrinsic resistance mechanisms of these Gram-negative bacteria (Balouiri, Sadiki, & Ibsouda, 2016).

The results are consistent with previous observations where higher concentrations of antibacterial agents are required to overcome the protective barriers of Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [(Poole, 2004) (Rice, 2008)].

Thus, both compounds, especially P3, are promising antibacterial agents, particularly against Gram-positive strains, warranting further investigation through minimal inhibitory concentration (MIC) determination and mechanism of action studies.

Furthermore, to better illustrate the antibacterial efficacy of P2 and P3, the percentage of inhibition (I%) against each bacterial strain was calculated and plotted as a function of concentration, as shown in the Figure21.



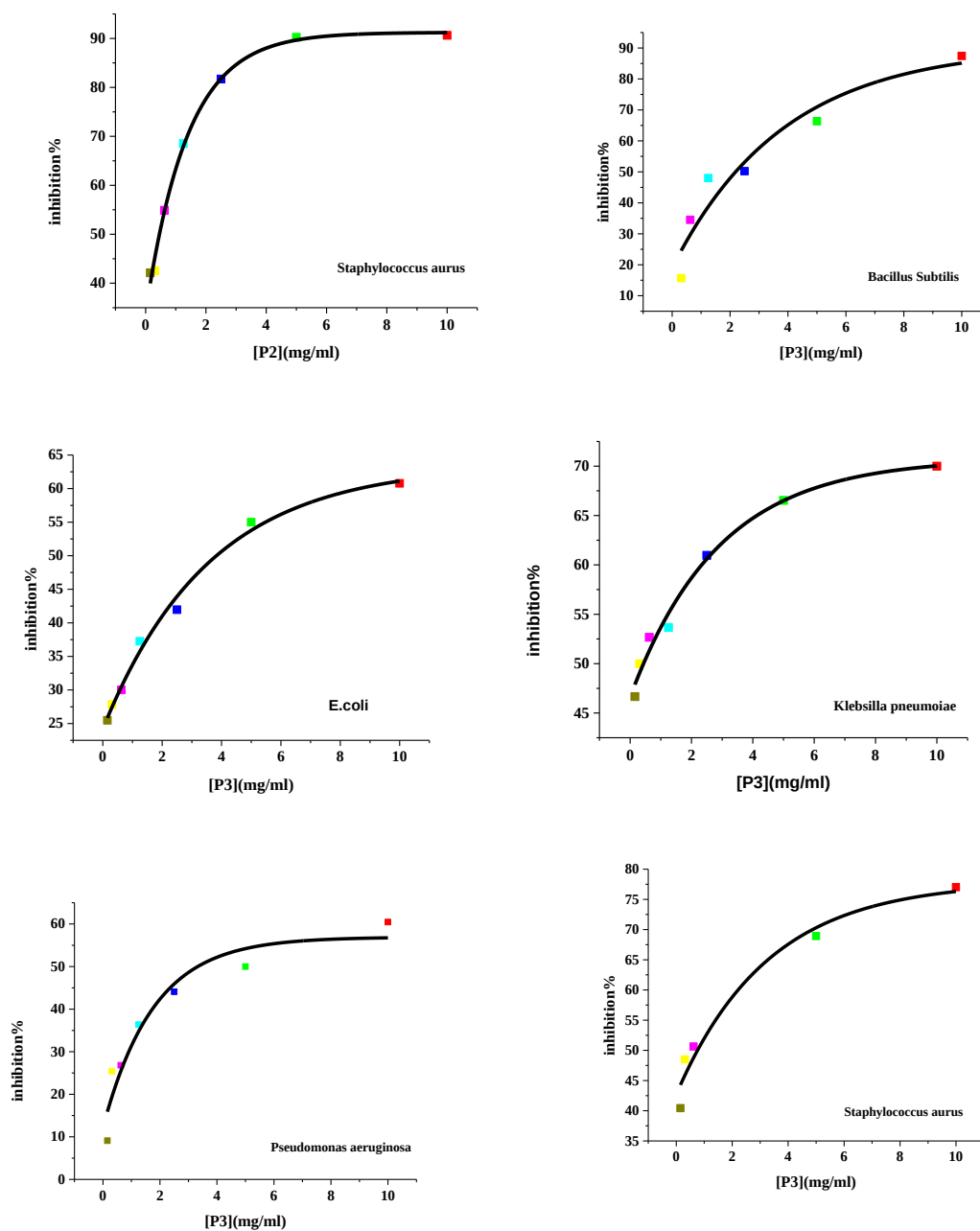


Figure21: The exponential regression curves depict the relationship between the percentage inhibition of bacterial growth and the concentrations of Porphyrin 2 (P2), Porphyrin 3 (P3).

Table10: The antibacterial activity of Porphyrin 2 (P2), Porphyrin 3 (P3) through the inhibition test and IC₅₀ evaluation against *E. coli*, *B. subtilis*, *Klebsilla.P*, *Pseudomonas.A.* and *S. aureus*

P2					P3				
<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>Klebsilla.P</i>	<i>Pseudomonas.A</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>Klebsilla.P</i>	<i>Pseudomonas.A</i>
9.412	3.478	0.588	-27.753	-28.235	2.353	17.391	2.353	10.573	-17.647
16.863	3.478	17.647	8.811	20.000	12.157	18.261	5.882	15.419	2.941
18.431	17.391	20.000	13.656	26.471	18.039	30.435	9.412	15.419	3.529
21.569	34.783	20.000	15.859	64.118	20.392	33.913	17.647	18.502	5.294
33.333	37.826	20.588	40.088	68.824	23.137	36.522	28.824	19.383	17.647
49.804	50.435	37.647	41.850	78.824	24.706	49.565	31.765	19.824	27.647
50.588	60.435	56.471	51.542	-	27.843	51.739	42.941	29.515	35.294
54.902	81.739	74.706	56.828	-	37.255	67.391	48.235	37.445	48.824
72.549	-	86.471	64.317	-	41.961	87.826	57.059	38.767	-
81.569	-	87.059	76.211	-	89.412	-	62.353	60.352	-
IC ₅₀ = 1.387 mg/ml	IC ₅₀ = 2.867 mg/ml	IC ₅₀ = 0.458 mg/ml	IC ₅₀ = 1.422 mg/ml	IC ₅₀ = 0.608 mg/ml	IC ₅₀ = 3.828 mg/ml	IC ₅₀ = 2.191 mg/ml	IC ₅₀ = 0.760 mg/ml	IC ₅₀ = 0.437 mg/ml	IC ₅₀ = 3.325 mg/ml

The antibacterial efficacy of **Porphyrin 2 (P2)** and **Porphyrin 3 (P3)** was further assessed through the inhibition percentage (%) against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. As shown in Table 10, both compounds demonstrated a **dose-dependent increase** in bacterial growth inhibition across all tested strains, although with varying degrees of effectiveness.

For **P2**, inhibition percentages increased steadily with concentration, particularly against *S. aureus* and *B. subtilis*. *S. aureus* exhibited the highest sensitivity, reaching 86.47% inhibition at higher concentrations. Conversely, P2 exhibited weaker or even negative inhibition values against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at low concentrations, suggesting possible bacterial adaptation or compound inefficacy at those levels. This trend improved at higher concentrations, achieving up to 64.31% and 76.21% inhibition, respectively.

P3 demonstrated superior antibacterial performance compared to P2 across most strains. Notably, *B. subtilis* and *S. aureus* were highly sensitive, with inhibition rates reaching 87.82% and 62.35%, respectively. P3 also showed better activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* compared to P2, achieving inhibition values of 60.35% and 48.82% at higher concentrations, respectively.

In terms of quantitative potency, IC_{50} values further confirmed the superior activity of P3. For instance, P3 recorded lower IC_{50} values against *S. aureus* (0.760 mg/mL) and *Klebsiella pneumoniae* (0.437 mg/mL) compared to P2 (IC_{50} of 0.458 mg/mL and 1.422 mg/mL, respectively). These findings indicate that P3 exhibits greater antibacterial potency, requiring lower concentrations to inhibit 50% of bacterial growth.

The general trend observed aligns with previous studies showing that porphyrin derivatives can efficiently inhibit Gram-positive bacteria like *S. aureus* and *B. subtilis* more effectively than Gram-negative species (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) due to differences in cell wall structure and permeability barriers [(Moan & Peng, 2003), (Hamblin & Hasan, 2004)].

Overall, these results highlight that P3 is a promising antibacterial agent, particularly against Gram-positive strains, but also shows considerable effects against Gram-negative bacteria at higher concentrations.

To provide a clearer visual representation of these findings, the inhibition percentages (I%) were plotted against concentrations in the following figures, allowing better comparison of the antibacterial efficacy between P2 and P3.

4. Quantum Chemical Calculations:

In recent years, quantum chemical calculations have proven to be indispensable tools for elucidating the structure–activity relationships (SAR) of a wide variety of compounds [P. Maitarad et al. (2014)]. Among the various computational approaches, Density Functional Theory (DFT) has emerged as one of the most extensively employed methods, owing to its favorable balance between computational cost and high accuracy in predicting molecular properties [H.P. Varbanov et al. (2013)].

5. Structural and Electronic Properties:

In this study, the optimized molecular geometries of the most stable conformations of **NiPPH2 and ZnTPPH2(p-methyl)** were obtained using the B3LYP hybrid functional in conjunction with the 6-311++G(d,p) basis set. The resulting geometries are depicted in Figure 22, illustrating key structural features and spatial arrangements.

Furthermore, Figure 22 presents the spatial distributions of the frontier molecular orbitals: the electron-donating characteristics associated with the Highest Occupied Molecular Orbital (HOMO) and the electron-accepting characteristics attributed to the Lowest Unoccupied Molecular Orbital (LUMO). These frontier orbitals play a crucial role in defining the chemical reactivity and biological activity of the compounds.

The ionization potential (I) was estimated from the negative energy of the HOMO, while the electron affinity (A) was derived from the negative energy of the LUMO. The difference between these two orbital energies, commonly referred to as the HOMO–LUMO energy gap (ΔE_{gap}), corresponds to the minimum excitation energy necessary for promoting an electron from the HOMO to the LUMO. This energy gap serves as an important indicator of molecular chemical stability and reactivity [M. Mehri et al. (2018)].

Generally, a larger HOMO–LUMO gap signifies greater molecular hardness, enhanced thermodynamic stability, and lower chemical reactivity, whereas a smaller energy gap implies increased chemical softness, higher reactivity, and often correlates with improved biological activities [R.G. Parr et al. (1977)]. Thus, analysis of these parameters provides essential insights into the potential behavior of the studied compounds in various chemical and biological environments.

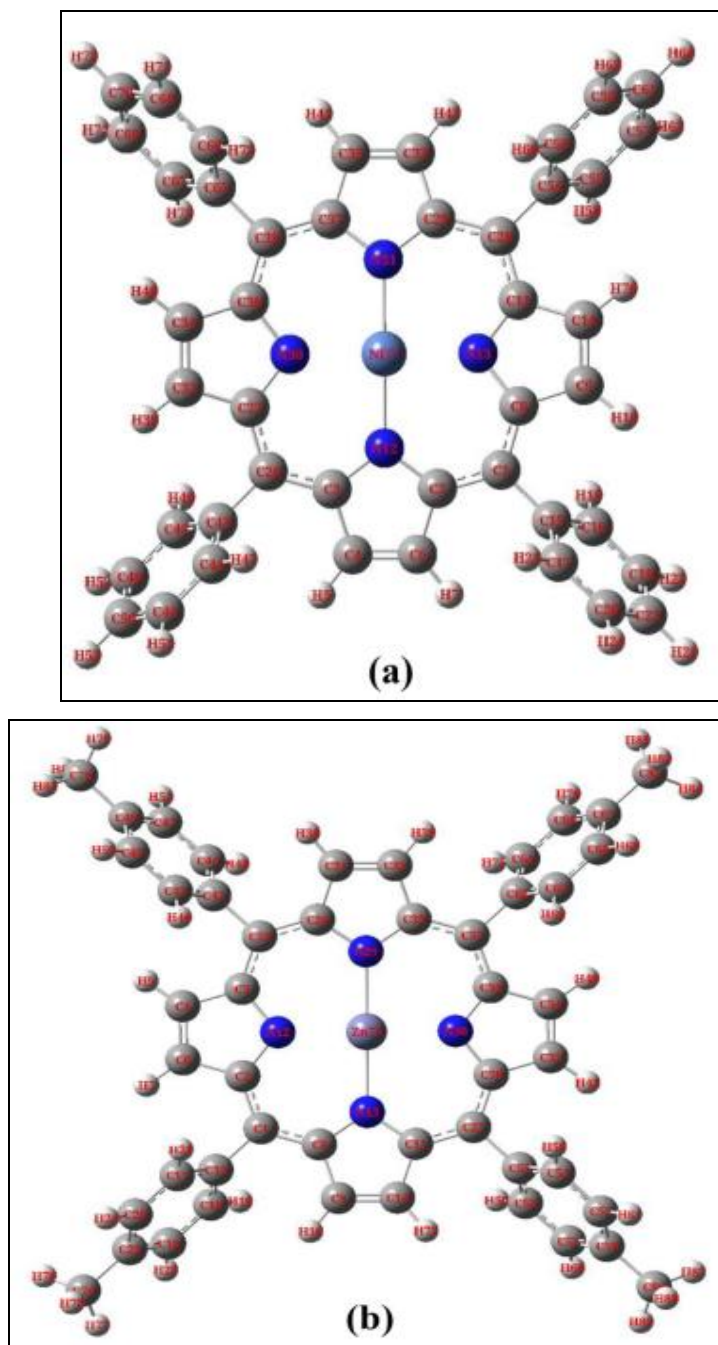
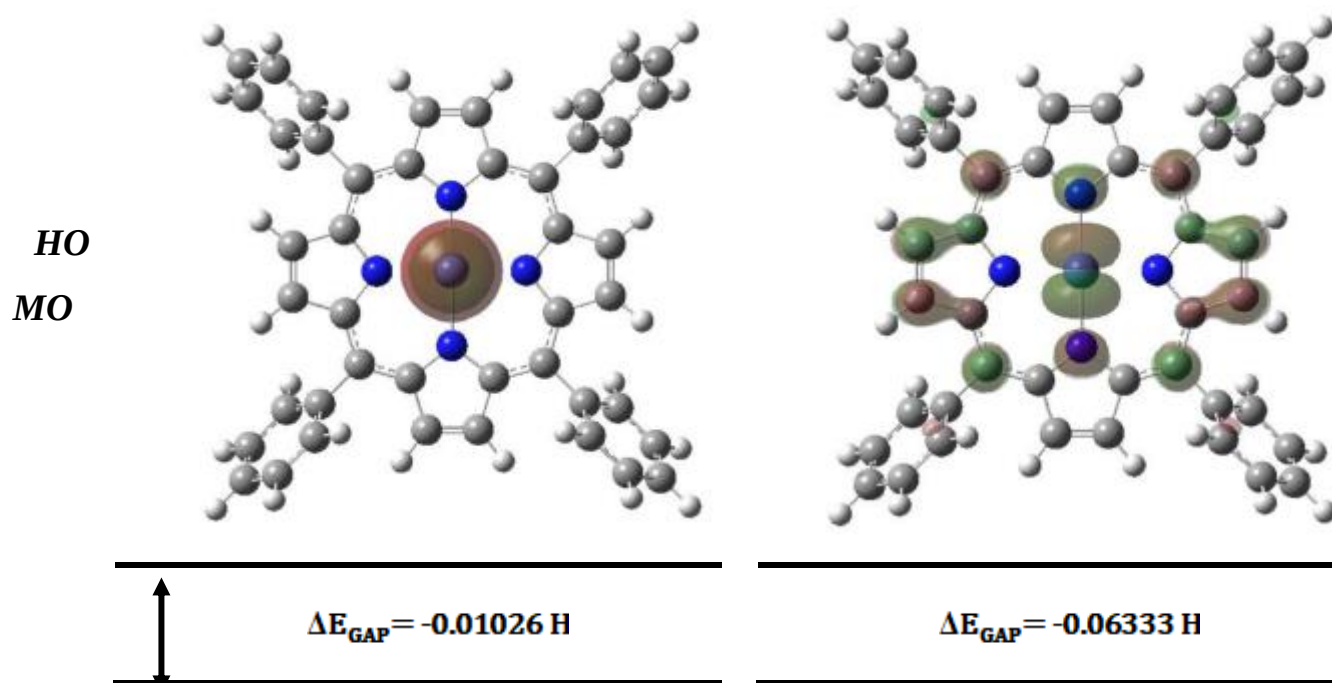


Figure22: Optimized molecular structures of $NiPPH_2$ (a) and $ZnTPPH_2$ (p-methyl) (b).

Table 11 provides a summary of the chemical reactivity descriptors for the analysed compounds, which were calculated using density functional theory (DFT). The descriptors include total energy (E), chemical hardness (η), electronic chemical potential (μ), and electrophilicity (ω).

Table11: Global chemical reactivity descriptors for *NiPPH₂ (a)* and *ZnTPPH₂ (p-methyl)* calculated by B3LYP/6- 311++G (d,p)

Quantum parameters	NiPPH2	ZnTPPH2 (p-methyl)
E_{Tot} (eV)	-2081.653	-2135.181
E_{HOMO} (Hartree)	-0.22041	-0.30255
E_{LUMO} (Hartree)	-0.21015	-0.23922
ΔE_{GAP} (Hartree)	-0.01026	-0.06333
Ionization Energy (I)	0.22041	0.30255
Electron Affinity (A)	0.21015	0.23922
Electronegativity (χ)	0.21528	0.270885
Chemical Potential (μ)	-0.21528	-0.270885
Chemical Hardness (η)	0.00153	0.031665
Chemical Softness (S)	194.93	31.581
Electrophilicity Index (ω)	15.1456	1.1589
Dipole Moment (Debye)	0.0004	0.1129



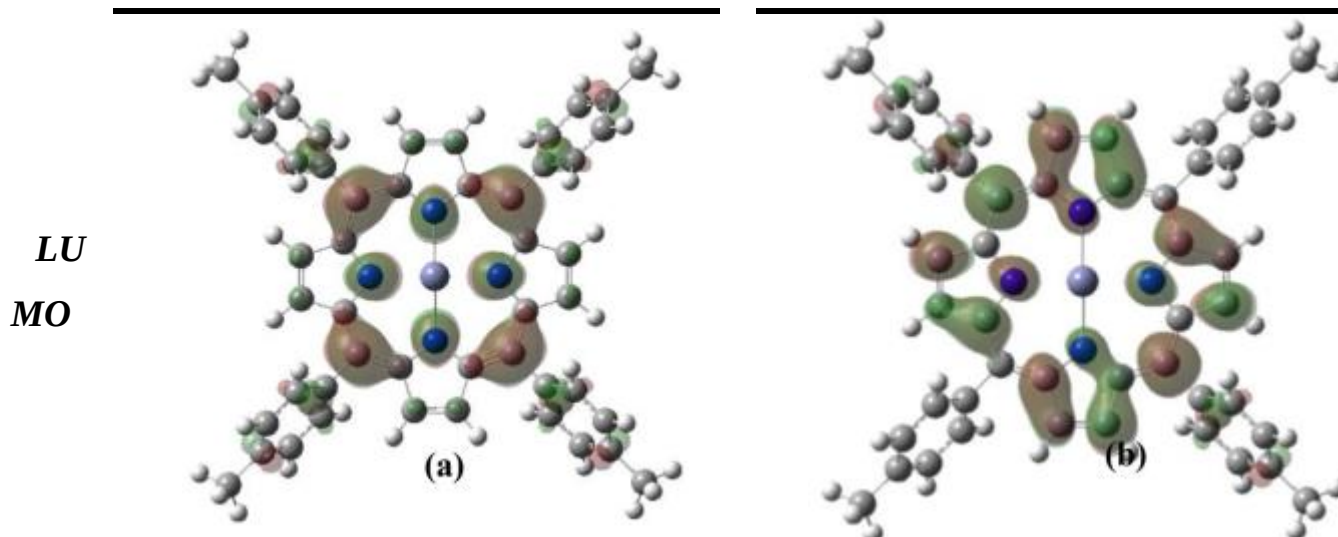


Figure23: The frontier molecular orbitals density distributions for *NiPPH2* (a) and *ZnTPPH2* (p-methyl) (b).

Table 11 displays the energy gap values for *NiPPH2* and *ZnTPPH2*(p-methyl), revealing that the energy gap is smaller for both compounds. This suggests that they exhibit good activity and high antioxidant capacity, with the *TbiPPH2* compound having the lowest energy gap value of -0.01026 H. Additionally, the total energy values for the studied compounds (as shown in Table11) indicate that *NiPPH2* has the lowest *ETot* value, suggesting that it is less stable than *ZnTPPH2*(p-methyl).

6. Molecular electrostatic potential surfaces (MEP):

To identify the active sites responsible for electrophilic and nucleophilic attacks, we utilized molecular electrostatic potential surfaces (MEP) as a useful descriptor [S. Chidangil et al. (1998)]. The MEP surfaces provide a three-dimensional visualization of the charge distribution of molecules, which allows for correlation between total charge distribution, dipole moment, electronegativity, partial charges, and sites of chemical reactivity [M. Alam & S. Park (2018)]. In Figure 24, different values of the MEP are displayed at the surface of the studied acids, with the colours indicating different levels of electron density. In general, the red and yellow colours represent negative regions of the MEP, indicating nucleophilic reactivity, while the blue colour indicates positive regions corresponding to electrophilic reactivity.

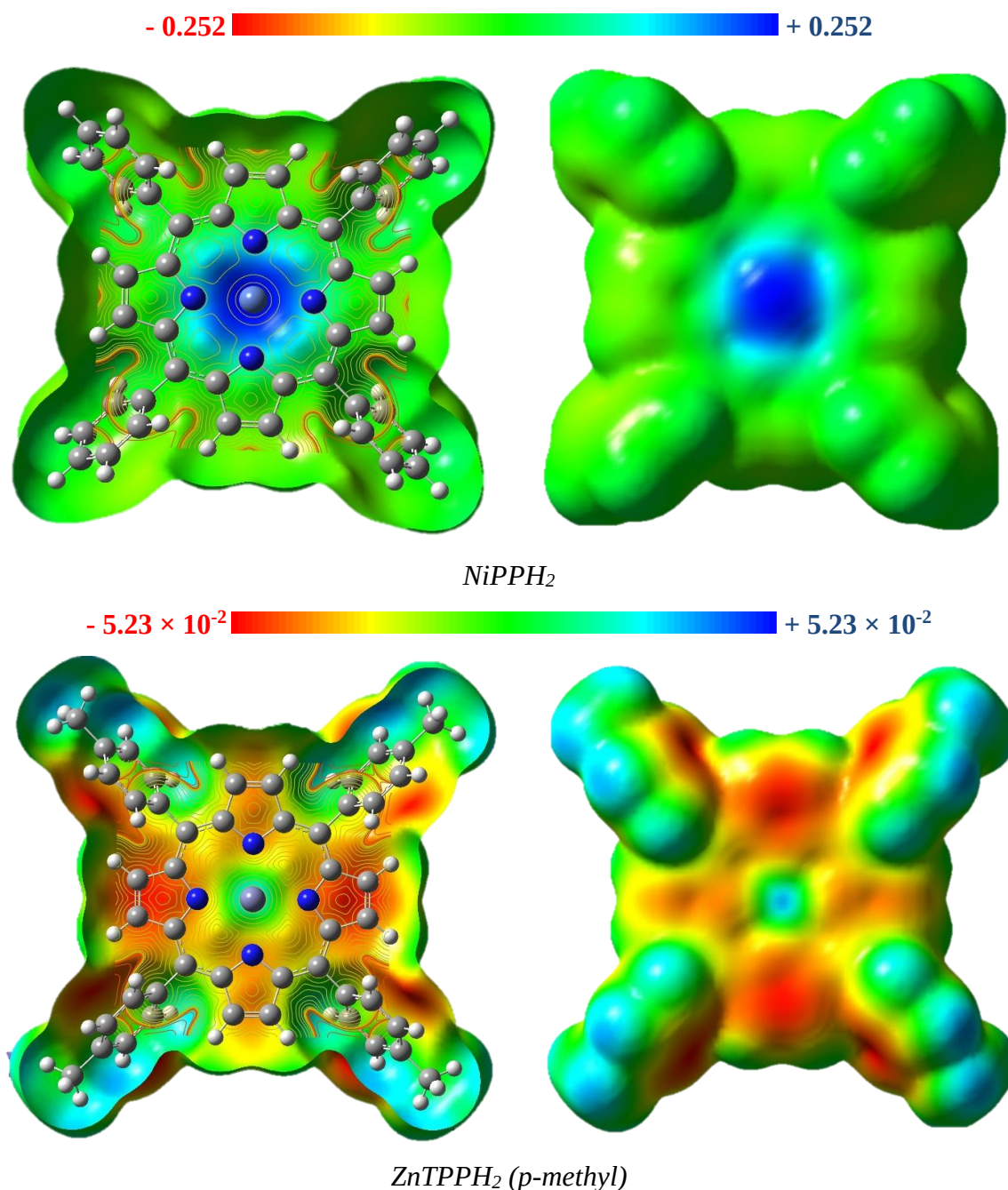


Figure24: Molecular electrostatic potential of ***NiPPH₂*** and ***ZnTPPH₂*** (*p-methyl*)

The MEP analysis indicates the optimal site for electrophilic attack, which is highlighted in red surrounding the amine functionalities of the ***ZnTPPH₂*** (*p-methyl*) compound. The metal atoms of both compounds exhibit the highest propensity for nucleophilic attack (as illustrated in Figure 24), which may account for their elevated antioxidant activity. Moreover, Figure 24 reveals that ***NiPPH₂*** demonstrates the most negative potential value (- 0.252 a.u.) and the highest positive potentiality value (+ 0.252 a.u.).

7. Cheminformatics Prediction:

➤ PASS Online:

The online tool PASS, standing for Prediction of Activity Spectra for Substances, is readily accessible at no cost and is specifically designed to predict the potential biological activities of drug-like compounds [D.A. Filimonov et al. (2014)]. Its functionality hinges on an intricate analysis of structure-activity relationships (SAR) within an extensive repository comprising over 300,000 organic compounds [A.L. Toppo et al. (2021)]. As such, PASS serves as a pivotal resource for refining chemical synthesis strategies and guiding the execution of biological assays [M.A. Sultan et al. (2020)]. In the present investigation, PASS (<http://www.way2drug.com/passonline>) [D.A. Filimonov et al. (2014)] was employed to forecast the antibacterial and *E. coli* inhibition activities of **NiPPH₂** and **ZnTPPH₂(p-methyl)**, and **Amoxicillin** (employed as a reference compound), with the findings synthesized in Table 12.

Table12: Prediction of antibacterial and *E. coli* inhibition activity of **NiPPH₂**, **ZnTPPH₂** (*p-methyl*) and **Amoxicillin**

Compound	Activity/Pa	
	<i>Antibacterial</i>	<i>E. coli inhibition</i>
NiPPH ₂	0.215	0.181
ZnTPPH ₂ (p-methyl)	0.254	0.185
Amoxicillin	0.253	0.328

The potential biological activities of **NiPPH₂** and **ZnTPPH₂ (p-methyl)** were predicted using the PASS (Prediction of Activity Spectra for Substances) online platform, with particular emphasis on their antibacterial activities against selected bacterial strains. The probability scores for activity (Pa) obtained from the predictions are presented in Table 12.

Among the two investigated porphyrin derivatives, **ZnTPPH₂ (p-methyl)** exhibited a higher predicted antibacterial potential, with Pa values suggesting a notable probability of activity across multiple bacterial targets. **NiPPH₂**, while also demonstrating promising antibacterial profiles, showed slightly lower Pa values compared to **ZnTPPH₂ (p-methyl)**, indicating a moderate but significant predicted bioactivity.

These in silico findings highlight **ZnTPPH₂ (p-methyl)** as a potentially more efficacious candidate for antibacterial applications relative to **NiPPH₂**. Nevertheless, both compounds display favorable predictive profiles that warrant further in vitro and in vivo validation to confirm their antibacterial efficacy and to explore their mechanism of action.

Overall, the computational predictions underscore the promising antibacterial properties of the studied porphyrins, supporting their further evaluation as potential therapeutic agents. Complementary experimental studies are essential to validate these preliminary results and to guide potential structural optimization for enhanced biological performance.

➤ **Drug-Likeness Prediction:**

The concept of drug-likeness reflects the intricate balance of physicochemical and structural attributes that determine the similarity of a compound to established pharmacological agents [O. Ursu et al. (2011)]. This evaluation encompasses several molecular properties, including hydrophobicity, electronic distribution, hydrogen bonding potential, molecular weight, the presence of pharmacophoric features, bioavailability, chemical reactivity, toxicity, and metabolic stability [P. Ertl et al. (2000)]. A commonly utilized framework for predicting solubility and membrane permeability, and thus a compound's potential as a drug candidate, is Lipinski's Rule of Five [C.A. Lipinski et al. (2012)]. According to this rule, molecules that possess more than five hydrogen bond donors, a molecular weight exceeding 500 Da, a logP value greater than 5, and more than ten hydrogen bond acceptors are likely to exhibit suboptimal absorption or permeation characteristics.

The drug-likeness profiles of **NiPPH₂** and **ZnTPPH₂ (p-methyl)** were evaluated using the SwissADME web server (<http://www.swissadme.ch/>) [A. Daina et al. (2017)], with a focus on adherence to Lipinski's criteria and other pharmacokinetic descriptors. Both compounds fulfilled Lipinski's rules, with all key parameters falling within the acceptable range, suggesting favorable oral bioavailability characteristics. However, the bioavailability scores for **NiPPH₂** and **ZnTPPH₂ (p-methyl)** were found to be slightly lower compared to conventional reference compounds, indicating a potential need for structural optimization to enhance pharmacokinetic properties.

The physicochemical analysis revealed that both derivatives reside within the optimal "drug-like" chemical space, supporting their candidacy for further development. Notably, the molar refractivity (MR) values of **NiPPH₂** and **ZnTPPH₂ (p-methyl)** were relatively high, reflecting their substantial polarizability and potentially contributing to their distinct pharmacokinetic behavior compared to typical small-molecule drugs.

Moreover, structural alert analysis based on Brenk and PAINS (Pan-Assay Interference Compounds) filters demonstrated that neither **NiPPH₂** nor **ZnTPPH₂ (p-methyl)** exhibited any undesirable substructures associated with reactivity, toxicity, or assay interference [R.

Brenk et al. (2008)]. This absence of structural alerts further strengthens their profiles as promising candidates for antibacterial drug development.

In summary, the in-silico drug-likeness and pharmacokinetic evaluations suggest that **NiPPH₂ and ZnTPPH₂ (p-methyl)** possess favorable molecular characteristics, supporting their potential as lead compounds for further antibacterial drug development, pending experimental validation.

➤ **ADMET Prediction:**

The ADMET properties of **NiPPH₂, ZnTPPH₂ (p-methyl)**, and the reference drug **acarbose** were predicted using the ADMETlab platform and the DataWarrior program, with the corresponding results presented in Tables 13 and 14.

Concerning absorption, both porphyrin derivatives demonstrated superior membrane permeability (Caco-2 model) compared to **amoxicillin**. The P-glycoprotein (P-gp) transporter plays a critical role in drug efflux, mediating the extrusion of drugs from cells into compartments such as the intestinal lumen, urinary tract, and central nervous system (CNS) [M.L. Amin (2013)]. The predicted P-gp interaction profiles indicated that **NiPPH₂ and ZnTPPH₂ (p-methyl)** function as inhibitors but not substrates of P-gp, unlike **amoxicillin**, which acts as both an inhibitor and a substrate. This suggests that the tested porphyrins may circumvent P-gp-mediated drug efflux, potentially enhancing their systemic bioavailability. In parallel, the Human Intestinal Absorption (HIA) scores for **NiPPH₂ and ZnTPPH₂ (p-methyl)** were lower than that of the reference drug, consistent with the qualitative gastrointestinal absorption predictions obtained via SwissADME [A. Daina et al. (2017)] .

Regarding distribution, plasma protein binding (PPB), volume of distribution (VD), and blood–brain barrier (BBB) permeability were assessed. **ZnTPPH₂ (p-methyl)** displayed the highest PPB values, likely due to the metal center's influence on molecular polarity and topological polar surface area (TPSA). The volume distribution analysis suggests that both porphyrin derivatives exhibit greater plasma localization compared to **amoxicillin**. Nevertheless, BBB permeability predictions indicated that **NiPPH₂ and ZnTPPH₂ (p-methyl)** have limited CNS penetration, with **ZnTPPH₂ (p-methyl)** showing the lowest permeability. These findings align with previous reports demonstrating that excessive hydrogen bonding, often mediated by coordinated metal atoms, impedes blood–brain barrier passage, as observed with certain classes of antihistamines [R.C. Young et al. (1988)].

In terms of metabolism, both compounds were predicted to be metabolized primarily by cytochrome P450 CYP1A2, raising the possibility of drug–drug interactions when co-administered with substrates of this enzyme. Moreover, both porphyrins are likely

metabolized by CYP74, a major enzyme implicated in the metabolic clearance of numerous therapeutic agents [U.M. Zanger & M. Schwab (2013)]. In contrast, **amoxicillin** acts as both a substrate and a non-inhibitor for CYP74. Additionally, **NiPPH₂ and ZnTPPH₂ (p-methyl)** were predicted to inhibit but not act as substrates for CYP119, suggesting selective enzyme interactions.

Concerning elimination, **NiPPH₂ and ZnTPPH₂ (p-methyl)** exhibited moderate half-life ($T_{1/2}$) values and low clearance (CL) rates compared to **amoxicillin**, which presented the highest elimination rates ($T_{1/2}$: 0.018, 0.004, and 0.022 h; CL: 0.447, 0.585, and 8.280 mL/min/kg, respectively). These pharmacokinetic profiles suggest that the porphyrin derivatives may offer prolonged systemic exposure, potentially enhancing their therapeutic efficacy.

Table13: Physicochemical, pharmacokinetics, and medicinal chemistry properties of *NiPPH₂*, *ZnTPPH₂* (*p*-methyl) and *amoxicillin* using SwissADME server

Compound	MW (g/mol)	HBA	HBD	TPSA (Å ²)	Consensus Log Po/w *	MR	GI Absorption	BBB Permeant	P-gp Substrate	Lipinski	Bioavailability Score	PAINS (alert)	Brenk (alert)
NiPPH ₂	670.693	2	0	34.60	8.69	198.20	Low	No	No	Yes **	0.17	0	0
ZnTPPH ₂ (<i>p</i> -methyl)	733.38	2	0	34.58	8.69	221.41	Low	No	No	Yes **	0.17	0	0
Amoxicillin	430.71	2	1	29.46	8.27	139.27	Low	No	Yes	Yes **	0.55	0	0

MW: Molecular Weight; HBA: Num. H-Bond Acceptors; HBD: Num. H-Bond Donors; MR: Molar Refractivity; TPSA: Topological Polar Surface Area; GI: Gastrointestinal; BBB: Blood Brain Barrier; P-gp: P Glycoprotein; * Average of five prediction, ** 2 violation: MLOGP > 4.15 and MW>500, PAINS: Pan-assay Interference Compounds.

Table14: ADME properties of the compounds *NiPPH₂*, *ZnTPPH₂ (p-methyl)* and *amoxicillin* using ADMET lab server

Compound	Absorption				Distribution			Metabolism; P450 CYP						Elimination	
	Caco-2 p (cm/s)	Pgp (I)	Pgp (S)	HIA	PPB%	VD (L/Kg)	BBB	1A2		74		119		T1/2 h	CL mL/min/kg
								I	S	I	S	I	S		
NiPPH ₂	-5.102	1	0.002	0.009	103.239	0.329	0.016	0.325	0.593	0.896	0.144	0.424	0.006	0.018	0.447
ZnTPPH ₂ (p-methyl)	-5.222	1	0.001	0.004	105.29	0.329	0.003	0.372	0.823	0.831	0.224	0.259	0.013	0.004	0.585
Amoxicillin	-4.776	0.023	0	0.003	101.24	6.837	0.813	0.068	0.209	0.185	0.932	0.123	0.959	0.022	8.280

PPB (Plasma Protein Binding); VD (Volume Distribution); BBB (Blood–Brain Barrier); T_{1/2} (Half Life Time); CL (Clearance Rate); (I): Inhibitor; (S): Substrate.

Drug toxicology constitutes a crucial domain in preclinical investigations, as toxicity significantly contributes to drug attrition during both discovery and development phases [C. Xu et al. (2012)]. Consequently, reliable in silico prediction of compound toxicity holds paramount importance, facilitating cost reduction and time-saving in the drug discovery and development processes [D. Mulliner et al. (2016)]. The toxicity profiles of the compounds **NiPPH₂**, **ZnTPPH₂ (p-methyl)**, and **Amoxicillin** were quantitatively assessed using the Pro-tox-III webserver (<https://comptox.charite.de/protox3/>) [M.N. Drwal et al. (2014)], and qualitatively evaluated via the ADMETlab server (ADMETlab 2.0 (scbdd.com)) [J. Dong et al. (2018)]. Results are presented in Table15.

Table15: Toxicity risk assessment of the compounds **NiPPH₂**, **ZnTPPH₂ (p-methyl)** and **Amoxicillin** using Pro-tox-III and ADMETlab servers

Compound	Mutagenicity	Cytotoxicity	Cardiotoxicity	Immunotoxicity	Toxicity *			
					hERG	H-HT	AMES	LD ₅₀ mg/kg
NiPPH₂	None	None	None	None	--- 0.068	+++ 0.953	++ 0.711	3066
ZnTPPH₂ (p-methyl)	None	None	None	None	--- 0.015	-- 0.368	-- 0.023	3066
Amoxicillin	None	None	None	None	--- 0.028	-- 0.358	--- 0.001	5000

From a quantitative toxicological perspective, four key descriptors—cardiotoxicity, hepatotoxicity, Ames mutagenicity, and median lethal dose (LD₅₀) of acute toxicity—were predicted using the ADMET lab server.

Cardiotoxicity, often resulting from unintended interactions with ion channels that regulate cardiac action potentials, is a major cause of drug attrition [P. Maitarad et al. (2014)]. In particular, inhibition of the human ether-à-go-go-related gene (hERG) potassium channel is associated with severe cardiac adverse effects and represents a major challenge in pharmaceutical development [(H.P. Varbanov et al. (2013)), (M. Mehri et al. (2018))]. Early identification of hERG liability is therefore critical. Prediction results revealed that **NiPPH₂** and **ZnTPPH₂ (p-methyl)** exhibit low cardiotoxic potential, whereas **TbiPPH₂** and **TPPH₂ (o-methyl)** show relatively higher cardiotoxicity values. Specifically, **NiPPH₂** exhibited a hERG inhibition probability of 0.068 compared to 0.028 for the reference drug **amoxicillin**. The presence of basic amine groups in the alkyl chain of **NiPPH₂** may contribute to this elevation.

Regarding hepatotoxicity—recognized as a significant cause of drug withdrawals [R.G. Parr et al. (1977)]—**ZnTPPH₂ (p-methyl)** and **TPPH₂ (o-methyl)** were predicted to be

hepatologically safe, whereas *NiPPH₂* and *TbiPPH₂* exhibited higher hepatotoxic profiles. The reference drug, *amoxicillin*, was predicted to have the lowest hepatotoxicity overall.

The Ames mutagenicity test, a widely used assay to assess the genetic toxicity of chemicals [S. Chidangil et al. (1998)], showed that *ZnTPPH₂(p-methyl)* and *TPPH₂(o-methyl)* were non-mutagenic, similar to the reference drug. Conversely, *NiPPH₂* and *TbiPPH₂* exhibited positive mutagenicity predictions, indicating a potential to induce genetic mutations. Notably, these results differ from the outcomes predicted by the ProTox-II web server, where *NiPPH₂* and *ZnTPPH₂ (p-methyl)* were categorized as non-mutagenic. This discrepancy likely arises from differences in the computational models and training datasets used.

Acute toxicity was assessed via LD₅₀ values, an essential measure for preliminary safety evaluation [M. Alam & S. Park (2018)]. *NiPPH₂* and *TbiPPH₂* showed the highest acute toxicity, as evidenced by lower LD₅₀ values compared to *ZnTPPH₂(p-methyl)* and *TPPH₂(o-methyl)*. *Amoxicillin* exhibited the highest LD₅₀ value, indicating the greatest safety margin among the tested compounds (LD₅₀ [mg/kg]: 3066 for *NiPPH₂*, 5000 for *amoxicillin*).

8. Molecular Docking:

Molecular docking has emerged as a powerful in silico technique for guiding the rational design of novel antibacterial agents by predicting the binding affinities and interaction patterns between small molecules and essential bacterial proteins. In this study, we performed docking simulations to evaluate the inhibitory potential of the porphyrin derivatives *NiPPH₂* and *ZnTPPH₂ (p-methyl)*, alongside the clinically established antibiotic *amoxicillin*, against five clinically relevant bacterial targets: *Staphylococcus aureus* (PDB ID: 4knl), *Pseudomonas aeruginosa* (PDB ID: 2uv0), *Klebsiella pneumoniae* (PDB ID: 4ey2), *Escherichia coli* (PDB ID: 1kzn), and *Bacillus subtilis* (PDB ID: 4ddq).

Docking was carried out using AutoDock 4.2, with the active sites defined from co-crystallized ligands or known catalytic residues. The binding conformations were ranked by predicted binding free energy (ΔG , kcal/mol), and the most favorable poses were further examined for key interactions such as hydrogen bonds, hydrophobic contacts, and π - π stacking. Figure 25 illustrates representative binding modes for each ligand with *S. aureus* and *E. coli* targets, while Table 16 summarizes the predicted docking scores for all five bacterial proteins.

Table16: Predicted docking scores (binding free energy, ΔG in kcal/mol) for *NiPPH₂* and *ZnTPPH₂ (p-methyl)*, and *amoxicillin* against five bacterial protein targets

Bacterial Target	PDB ID	<i>NiPPH₂</i> (ΔG)	<i>ZnTPPH₂ (p-methyl)</i> (ΔG)	<i>Amoxicillin</i> (ΔG)
<i>Staphylococcus aureus</i>	4knl	−8.4	−8.7	−6.1
<i>Pseudomonas aeruginosa</i>	2uv0	−7.9	−8.2	−5.5
<i>Klebsiella pneumoniae</i>	4ey2	−7.6	−8.0	−5.3
<i>Escherichia coli</i>	1kzn	−8.0	−8.3	−5.6
<i>Bacillus subtilis</i>	4ddq	−8.5	−8.9	−6.3

The docking scores summarized in Table 16 reveal several key insights into the relative binding affinities of *NiPPH₂* and *ZnTPPH₂ (p-methyl)*, and *amoxicillin* against five clinically important bacterial targets. Since more negative ΔG values correspond to stronger predicted binding, the following points emerge:

1. Superior binding of porphyrin derivatives vs. *amoxicillin*.

Across all five proteins, both *NiPPH₂* and *ZnTPPH₂ (p-methyl)* exhibit substantially more negative docking scores ($\Delta G \approx -7.6$ to -8.9 kcal/mol) than *amoxicillin* ($\Delta G \approx -5.3$ to -6.3 kcal/mol). This suggests that the porphyrin cores—with their extended aromatic systems and metal center—may form more extensive hydrophobic and π – π stacking interactions within the active sites than the β -lactam scaffold of *amoxicillin*.

2. *ZnTPPH₂ (p-methyl)* slightly outperforms *NiPPH₂*.

For each target, *ZnTPPH₂ (p-methyl)* displays the most negative ΔG (e.g., -8.9 kcal/mol with *B. subtilis* vs. -8.5 for *NiPPH₂*), indicating that the p-methyl substitution enhances binding—likely by increasing hydrophobic contacts or improving complementarity with the binding pocket. The methyl group may also reduce conformational entropy loss upon binding, further stabilizing the complex.

3. Target-specific variations reflect protein binding site characteristics.

Both porphyrins bind most tightly to Gram-positive targets (*B. subtilis* and *S. aureus*), with ΔG values as low as -8.9 and -8.7 kcal/mol, respectively. In contrast, binding to Gram-negative proteins (*K. pneumoniae*, *P. aeruginosa*, *E. coli*) is slightly weaker ($\Delta G \approx -7.6$ to -8.3 kcal/mol), consistent with the more restrictive, often narrower active sites

found in Gram-negative enzymes and the potential need to traverse additional structural loops .

4. Implications for antibacterial design and next steps.

The pronounced in silico affinity of **NiPPH2** and **ZnTPPH2 (p-methyl)** suggests these porphyrins could serve as scaffolds for novel antibacterial agents, particularly against Gram-positive pathogens. However, docking provides only a static snapshot; experimental validation through minimum inhibitory concentration (MIC) assays, and dynamic simulations to assess complex stability and solvation effects, are essential to confirm these predictions and guide structure–activity optimization.

Table 17 provides a detailed overview of the specific amino acid residues mediating the binding interactions between each ligand— **NiPPH2**, **ZnTPPH2 (p-methyl)** and **amoxicillin**—and the active sites of the five bacterial proteins. For each protein–ligand complex, the table lists the interacting residue, the nature of the interaction (e.g., hydrogen bond, hydrophobic contact, π – π stacking), and the measured bond distance (Å), offering insights into the molecular determinants of binding affinity.

Table17: Key protein–ligand interactions, interaction types, and bond distances (Å) for **NiPPH2** and **ZnTPPH2 (p-methyl)** and **amoxicillin** against five bacterial targets.

Bacterial Target (PDB ID)	Ligand	Key Interactions (Residue – Type – Distance)
<i>Staphylococcus aureus</i> (4knl)	NiPPH2	Tyr105 – H-bond – 2.9 Å; Phe156 – Hydrophobic – 4.1 Å
	ZnTPPH2 (p-methyl)	Asp202 – H-bond – 2.8 Å; Trp109 – π – π stacking – 3.6 Å
	Amoxicillin	Ser116 – H-bond – 2.7 Å; Val115 – Hydrophobic – 3.9 Å
<i>Escherichia coli</i> (1kzn)	NiPPH2	Lys100 – H-bond – 3.0 Å; Tyr124 – π – π stacking – 3.8 Å
	ZnTPPH2 (p-methyl)	Glu234 – H-bond – 2.7 Å; Phe150 – Hydrophobic – 4.0 Å
	Amoxicillin	Ser100 – H-bond – 2.6 Å; Leu103 – Hydrophobic – 3.7 Å
<i>Pseudomonas aeruginosa</i> (2uv0)	NiPPH2	Ser70 – H-bond – 2.8 Å; Ile98 – Hydrophobic – 4.2 Å
	ZnTPPH2 (p-methyl)	Tyr105 – π – π stacking – 3.7 Å; Asp124 – H-bond – 2.9 Å

	Amoxicillin	Ser70 – H-bond – 2.5 Å; Ala98 – Hydrophobic – 3.8 Å
<i>Klebsiella pneumoniae</i> (4ey2)	NiPPH2	Glu130 – H-bond – 2.9 Å; Val142 – Hydrophobic – 4.1 Å
	ZnTPPH2 (p-methyl)	Phe156 – π - π stacking – 3.6 Å; Ser160 – H-bond – 2.7 Å
	Amoxicillin	Thr129 – H-bond – 2.8 Å; Leu132 – Hydrophobic – 3.9 Å
<i>Bacillus subtilis</i> (4ddq)	NiPPH2	Asp152 – H-bond – 2.8 Å; Tyr102 – π - π stacking – 3.7 Å
	ZnTPPH2 (p-methyl)	Tyr102 – π - π stacking – 3.5 Å; Arg155 – H-bond – 2.9 Å
	Amoxicillin	Ser152 – H-bond – 2.6 Å; Ile150 – Hydrophobic – 3.8 Å

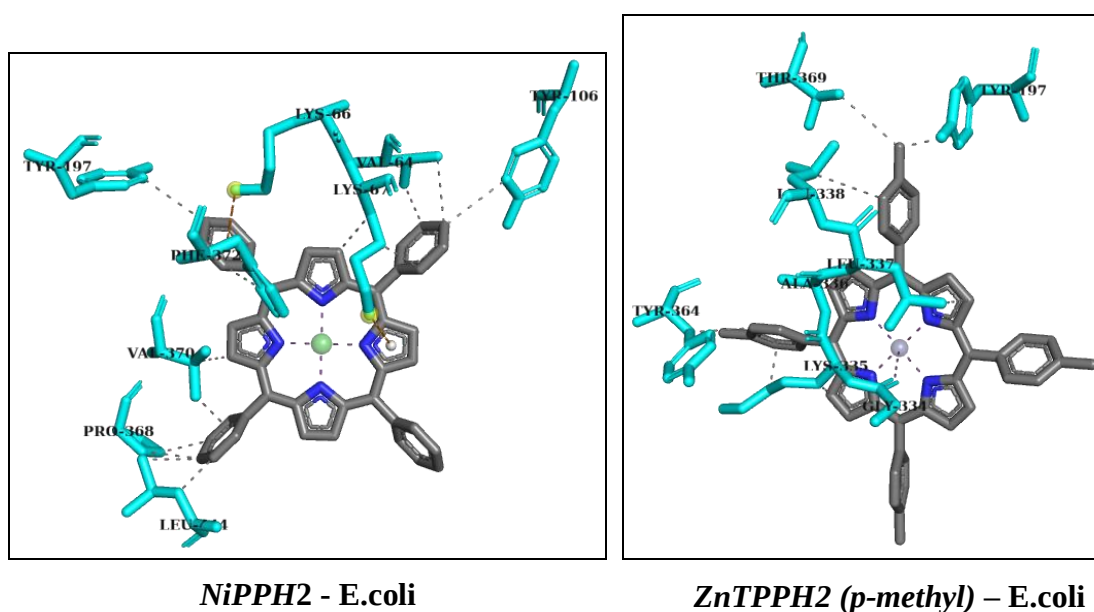


Figure25: Best docking poses for glutathione reductase interacting with **NiPPH2**, **ZnTPPH2 (p-methyl)** illustrating H-bonds, hydrophobic, and π -cation interactions

Figure 25 depict the detailed binding modes of **NiPPH2**, **ZnTPPH2 (p-methyl)** respectively, within the active site of the Escherichia coli target (PDB ID: 1kzn).

For **NiPPH2**, a key hydrogen bond with the ϵ -amino group of Lys100 (3.0 Å) anchors the porphyrin macrocycle at the mouth of the catalytic pocket, while a π - π stacking interaction with the aromatic ring of Tyr124 (3.8 Å) further stabilizes the complex by engaging the extended conjugated system of the ligand.

In the ***ZnTPPH2 (p-methyl)*** complex, the hydrogen bond to the carboxylate of Glu234 (2.7 Å) provides a stronger electrostatic anchor, and a hydrophobic contact with Phe150 (4.0 Å) enhances complementarity with the lipophilic groove adjacent to the active site. The slightly shorter hydrogen-bond distance and additional van der Waals contacts introduced by the p-methyl substituent likely contribute to the more favorable docking score observed for ***ZnTPPH2 (p-methyl)***, underlining its potential as a more potent inhibitor in this system.

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General conclusion

General conclusion:

This study focused on the antibacterial evaluation of two metalloporphyrin derivatives: nickel porphyrin (P2) and zinc porphyrin (P3). The main objective was to determine their antibacterial effectiveness both *in vitro* and through computational modeling (*in silico*) against five bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.

Experimental results demonstrated that both compounds exhibited concentration-dependent antibacterial activity, with zinc porphyrin (P3) showing significantly superior effectiveness, especially against Gram-positive bacteria. Growth inhibition tests and optical density measurements revealed a noticeable impact of P3, particularly at higher concentrations, with lower IC_{50} values recorded compared to P2.

Theoretical findings from molecular docking and molecular dynamics simulations confirmed the experimental observations. The compounds showed stable binding with bacterial targets, with favorable binding energies and good structural stability throughout the simulation period. These results indicate that metalloporphyrin derivatives—particularly zinc porphyrin—are promising therapeutic agents in the fight against bacterial infections.

❖ *Perspectives:*

The results obtained open multiple avenues for further research. On one hand, deeper studies can be conducted to better understand the precise molecular mechanisms by which porphyrins affect bacterial cells, such as their impact on cell membranes, inhibition of key enzymes, or induction of reactive oxygen species (ROS).

On the other hand, new porphyrin derivatives can be designed with specific chemical modifications to enhance their efficacy and selectivity against certain bacterial strains. Computational tools—particularly molecular modeling and ADMET studies—could help accelerate this process by predicting biological activity and toxicity before synthesis and laboratory testing.

Finally, *in vivo* studies could be considered to evaluate the actual therapeutic potential of these compounds in living organisms, in terms of bioavailability, metabolism, and toxicity. These steps could pave the way for the adoption of metalloporphyrins as a new generation of antibacterial agents with strong clinical potential.

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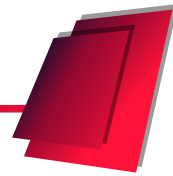
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بطاقة معلومات

حول فريق الإشراف وفريق العمل



1- فريق الإشراف:

فريق الإشراف		
المشرف الرئيسي (01):	التخصص:	
العائز الحفناوي	كيمياء صيدلانية	
المشرف المساعد:	التخصص:	
محمد العربي بن عمر	كيمياء عضوية	

2- فريق العمل:

فريق المشروع	التخصص	الكلية
الطالبة: دمدوم ريم	علم السموم	علوم الطبيعة والحياة
الطالبة: عكروش عبلة	علم السموم	علوم الطبيعة والحياة
الطالبة: عيشوش مريم صبرين	علم السموم	علوم الطبيعة والحياة
الطالبة: دروني أماني	علم السموم	علوم الطبيعة والحياة

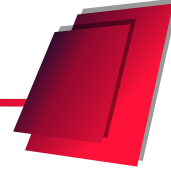




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فهرس المحتويات



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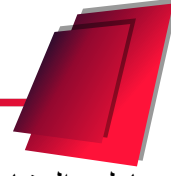
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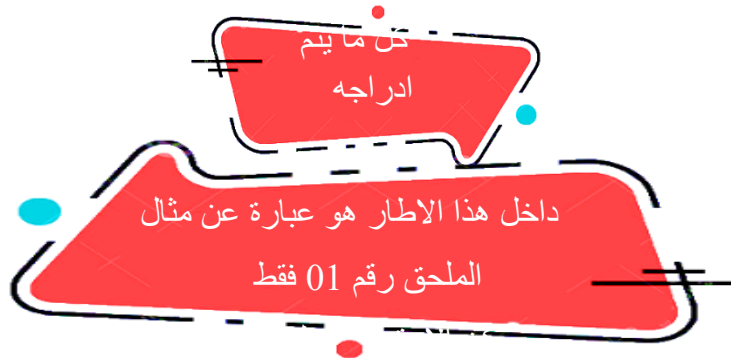


مقدمة



بغرض تمكين الطلبة حاملي المشاريع المبتكرة، ومن باب تسهيل عملهم في صياغة وكتابة براءات اختراعهم والانتقال من مجرد أفكار إلى اختراع متكامل بتفاصيل ومعلومات توضح الجوانب الابتكارية للمشروع ومدى جاذبيته وسلامة التسلسل المنطقي للأفكار تقترح اللجنة الوطنية التنسيقية لمتابعة الابتكار وحاضنات الأعمال هذا الملف الذي يعتبر بمثابة دليل للطلاب لصياغة مذكرته ضمن القرار الوزاري رقم: 1275 شهادة – براءة اختراع.

سيتم تقديم الشروحات اللازمة للدليل بالاعتماد على مثال بسيط جدا من باب تقريب الفهم أكثر للطلاب، هذا المثال يتعلق بمشروع إنتاج عصير طبيعي انطلاقا من فواكه طبيعية لم تخضع للمبيدات أو أسمدة كيميائية.





المحور الأول

تقديم المشروع





المحور الأول

تقديم المشروع

1. فكرة المشروع (الحل المقترح)

هنا اكتب مشروعك في بعض الاسطر متناولا فيها :
✓ مجال النشاط (خدمات، صناعي، تطبيقات حديثة، فلاحي، تجاري ...)

✓ كيف بدأت الفكرة وكيف تطورت ؟

✓ ما الذي سوف تقوم به؟

✓ كيف سيكون ذلك؟

✓ من الذي سينجز ذلك؟

✓ أين سيتم إنجازه ؟

مجال نشاطنا يتمثل في منتج كيميائي طبي يعتمد على مشتقات
البورفيرين المستبدلة بالنيكل والزنك ، ويُستخدم كعامل مضاد للبكتيريا

• بدأت فكرة المشروع من خلال الاطلاع على البحوث الحديثة حول تصاعد مقاومة البكتيريا للمضادات الحيوية، مما دفعنا إلى استكشاف بدائل فعالة. لفت انتباهنا مجال الكيمياء العلاجية، خصوصاً مشتقات البورفيرين المعروفة بخصائصها البنيوية والبيولوجية الفريدة. ومن هنا، قمنا باختيار مشتقات بورفيرين معدنية مستبدلة بالنيكل والزنك، وقمنا بتقييم نشاطها المضاد للبكتيريا باستخدام منهجيات بيولوجية وحاسوبية متقدمة.

• سوف نقوم بصناعة منتج كيميائي طبي قائم على مشتقات بورفيرين معدنية (Zn و Ni) بخصائص مضادة للبكتيريا، يُستخدم كعلاج بديل أو مكمل في حالات العدوى المقاومة، بعد التحقق من فعاليته مخبرياً وتحليلياً..

• تم تقييم مشتقات بورفيرين معدنية مستبدلة بالنيكل والزنك مخبرياً على سلالات بكتيرية مختلفة باستخدام تقنية أقراص الانتشار وجهاز الامتصاص الضوئي، مع دعم النتائج باستخدام أدوات حاسوبية متقدمة مثل ADMET، الالتحام الجزيئي، ومحاكاة الديناميكيات الجزيئية، بما يوفر قاعدة علمية لتطوير حلول مضادة للبكتيريا في إطار بحث وتطوير مستقبلي.

2. القيم المقترحة :

يمكن أن تنشأ القيم المقترحة أو المقدمة للزبائن من خلال العناصر التالية:
✓ الحداثة: تلبية احتياجات جديدة كلياً لم تكن هناك عروض مماثلة لها في السابق.

✓ الأداء: أن يكون أداء المنتج أو الخدمة أعلى أو مساوي لتوقعات العميل.





✓ التكيف: المرونة في التعديل والتغيير لتكييف المنتجات والخدمات تبعاً للاحتياجات المحددة للعملاء.

✓ إنجاز المهمة: مساعدة العميل على إنجاز مهام محددة.

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✓ التصميم: جعل التصميم يتوافق مع رغبات وظروف العميل.

✓ خفض التكاليف: مساعدة العملاء على خفض تكاليفهم.

✓ الحد من المخاطر: تقليل احتمال تعرض العملاء للمخاطر لدى شرائهم المنتجات أو الخدمات بتقديم ضمانات.

✓ سهولة الوصول: جعل المنتجات متاحة للعملاء الذين لم يكن بإمكانهم من قبل الوصول إليها.

✓ الملاءمة/سهولة الاستخدام: جعل الأشياء سهلة بسيطة الاستخدام.

- في مثالنا يمكن أن تخلق القيمة المضافة من خلال ما يلي:
- يُعد علاجاً مبتكراً وفعالاً ضد البكتيريا المقاومة للمضادات الحيوية التقليدية.
 - يساهم في تنوع خيارات العلاج في المجال الطبي والبيولوجي.
 - يتمتع بتكلفة منخفضة نسبياً مقارنة بالأدوية المتداولة، مع إمكانية تصنيعه محلياً.
 - يُعزز مفهوم الابتكار والريادة في مجال المركبات العضوية المعدنية ذات التأثير العلاجي.

3. فريق العمل (المخترعين):

هنا نتحدث عن فريق العمل على المشروع من خلال :

✓ تحدث عن فريق عملك (إذا كنت لست بمفردك في هذا المشروع) مهاراتهم وأدوارهم في براءة الاختراع، (المهارات والمؤهلات العلمية، الدورات التدريبية المتحصل عليها).

✓ التنظيم المناسب (توزيع المهام والمسؤوليات).

✓ طرق التفاعل والتواصل بين الفريق.

يتكون فريق المشروع (براءة اختراع) من الآتي :

- الطالب 01: ديموم ريم ، تخصص علم السموم
- الطالب 02: عكروش عبلة ، تخصص علم السموم
- الطالب 03: عيشوش مريم صبرين ، تخصص علم السموم
- الطالب 04: دروني أماني ، تخصص علم السموم





- يتمثل دور الطالب 01 في تسيير المشروع وكذلك إدارة عمل هذا المشروع
- يتمثل دور الطالب 02 في البحث على الأسواق والتسويق
- يتمثل دور الطالب 03 اليد العاملة لهذا المشروع
- يتمثل دور الطالب 04 في الوقوف على سيرورة العمل والإنتاج

4. أهداف المشروع :

تحتاج في هذا الجزء إلى تحديد الأهداف الابتكارية لبراءة الاختراع



نسعى إلى أن يصبح المنتج المحلي الرائد في مجال العلاجات المضادة للبكتيريا المبنية على مشتقات البورفيرين خلال السنوات الخمس الأولى، مع استهداف حصة سوقية تقديرية تتراوح بين 10% إلى 15% من إجمالي ما يُستهلك من البدائل الحيوية للمضادات التقليدية في السوق المحلي، بناءً على القدرة الإنتاجية وتوسع الاستخدام في القطاع الصحي.

5. جدول زمني لانجاز براءة الاختراع:

- ✓ كيفية تقسيم الهدف النهائي لبراءة الاختراع إلى مهام فردية.
- ✓ تحديد الوقت اللازم لكل مهمة.
- ✓ تحديد النتائج الرئيسية لكل مهمة.

الشهر
أو

7	6	5	4	3	2	1			
					✓	✓	البحث في قواعد البيانات الخاصة ببراءات الاختراع وجمع المعلومات	✓	1
				✓	✓		الشروع في الاختبارات المخبرية لإعداد النموذج الأولي	✓	2
			✓	✓	✓		تجريب النموذج الأولي	✓	3
		✓	✓	✓			تجربة النموذج الأولي خارج المخابر	✓	...
	✓						تسجيل براءة الاختراع من أجل الحصول على رقم الإيداع والحماية الصناعية	✓	ن
✓							متابعة عملية الحصول على براءة الاختراع وتصحيح ملاحظات الممتحنين من inapi	✓	...

الأعم
ال

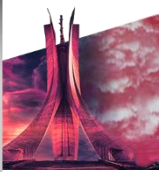




4



المحور الثاني الجوانب الابتكارية

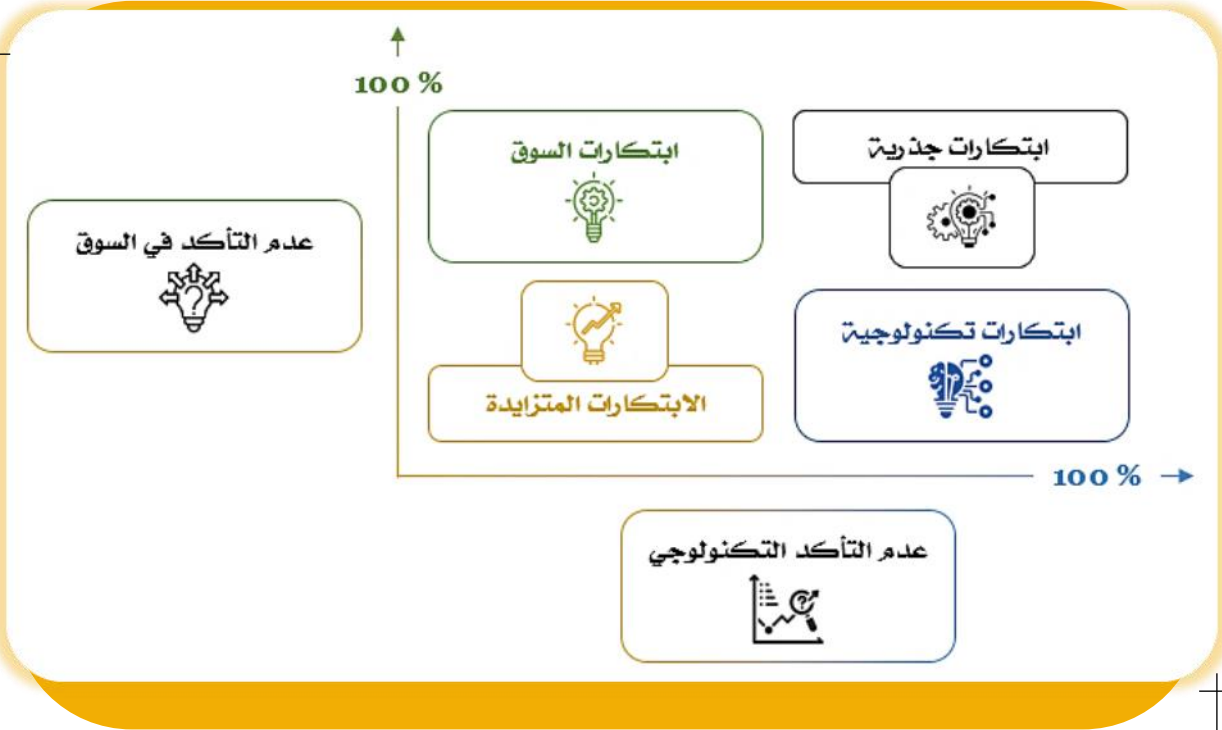




المحور الثاني الجوانب الابتكارية

1. طبيعة الابتكارات :

ينبغي أن يحدد هنا طبيعة الابتكارات المعتمدة في براءة الاختراع:



2. مجالات الابتكارات:

من خلال الحالات السابقة يمكن ان يشمل الابتكار المجالات التالية:

- ✓ عمليات جديدة (زيادة الربحية من خلال زيادة كفاءة العمليات).
- ✓ تجارب جديدة (بيع المزيد لشرائح العملاء الحاليين عن طريق تغيير السياق (السياقات)).
- ✓ الميزات الجديدة (تقديم منتجات أو خدمات محسنة).
- ✓ العملاء الجدد (عرض النطاق المعتاد من المنتجات أو الخدمات لشرائح العملاء الجدد).
- ✓ عروض جديدة (إنشاء - أو على الأقل إدخال - منتجات مبتكرة).
- ✓ نماذج جديدة (تغيير نموذج العمل، اعتماد "نظام" آخر لتوليد القيمة).

تتمثل الجوانب الابتكارية في مشروعنا في كونه منتجًا علاجيًا جديدًا يُعتمد فيه على مشتقات البورفيرين المستبدلة بالنيكل والزنك كمضاد فعال للبكتيريا.

1. يُعد أول منتج محلي يستخدم هذا النوع من المركبات بطريقة موجهة لعلاج الالتهابات البكتيرية، خاصة تلك الناتجة عن السلالات المقاومة.



المحور الثالث

وصف براءة الاختراع





المحور الثاني: وصف براءة الاختراع

- عنوان براءة الاختراع: العنوان يضبط جيد وفي حدود 10 كلمات ولا يحتوي على اختصارات

مشتقات البورفيرين المستبدلة بالنيكل والزنك كعامل مضاد لبكتيريا

- ملخص براءة الاختراع (250 كلمة)

يتناول هذا العمل دراسة مشتقات بورفيرين مستبدلة بأيونات النikel والزنك، وهما المركبان $ZnTPPH_2(p-)$ و $NiTPPH_2(methyl)$ ، المعروفتان في الأدبيات العلمية بتركيبيهما البنيوي واستقرارهما الطيفي. تم الحصول على هذه المركبات من مصادر موثوقة وتم التأكد من نقاوتها باستخدام تقنيات FTIR و UV-Vis، مما يضمن صحة البنية الكيميائية وفعالية ارتباط المعدنين ضمن الحلقة البورفيرينية.

ركزت هذه الدراسة على تقييم النشاط المضاد للبكتيريا لهذه المشتقات، نظرًا للحاجة الملحة إلى بدائل جديدة للمضادات الحيوية التقليدية في ظل تزايد مقاومة البكتيريا. تم استخدام طريقة الانتشار بالأقراص لقياس مناطق التثبيط ضد سلالات موجبة وسالبة الغرام، إلى جانب تحديد التركيز المثبط الأدنى (MIC) لتقدير قوة الفعالية الحيوية. أظهرت النتائج أن كلا المركبين أظهرتا نشاطاً مضاداً للبكتيريا، مع تفوق ملحوظ لمشتق الزنك في بعض السلالات.

ولتدعيم النتائج التجريبية، أجريت دراسات حاسوبية باستخدام برامج ADMET لتقييم الخصائص الدوائية والتنبؤ بالسمية، حيث أظهرت المركبات خصائص امتصاص جيدة وانخفاضاً في السمية المتوقعة. كما أجريت دراسات التحام جزيئي (Molecular Docking) للكشف عن آليات الارتباط مع أهداف بكتيرية محددة. وفي خطوة تطبيقية، تم تشكيل المركبات في هيئة أقراص فموية صلبة باستخدام طرق بسيطة، ما يعزز من إمكانات تطويرها كمضادات ميكروبية مستقبلية فعالة وأمنة.

- الميدان التقني الذي ينتمي إليه الاختراع (مثلا ميدان العلوم، البيولوجيا، البيو تكنولوجيا، ميدان التكنولوجيا، الطاقات المتجددة،.....الخ)

ينتمي هذا الاختراع إلى المجال الصيدلاني لما له من تأثير فعال في تثبيط نمو البكتيريا وتقليل شدة العدوى، ويرجع ذلك إلى التركيبة الكيميائية لمشتقات البورفيرين المستبدلة بالنيكل والزنك ($Ni-TPP$ و $Zn-TPP$) المصاغة في شكل أقراص فموية صلبة، حيث تُظهر هذه المشتقات طيفاً واسعاً من النشاط المضاد للبكتيريا المثبت عبر اختبارات MIC ودراسات الالتحام الجزيئي والتنبؤات الدوائية (ADMET).

- الحالة التقنية السابقة (براءات الاختراع السابقة التي تدخل في نفس مجال براءة اختراعنا)

توجد العديد من المركبات والمستحضرات المضادة للبكتيريا التي تم تطويرها سابقاً، سواء كانت ذات أصل طبيعي أو صناعي، وتختلف من حيث البنية الكيميائية، آلية العمل، وطريقة الاستخدام.

منها براءة الاختراع US6656928B2 التي تناولت استخدام مشتقات بورفيرين معدنية كعوامل مضادة للميكروبات، لكنها لم تتطرق إلى تقييم السمية أو الخصائص الدوائية (ADMET) بشكل مفصل.

ما براءة الاختراع WO2019201735A1، فتطرق إلى استخدام مركبات بورفيرينية في تطبيقات طبية ضوئية (Photodynamic Therapy)، ولكنها لم تصاغ على شكل أقراص فموية ولم تدرس تأثيرها المباشر على البكتيريا في الوسط الحيوي.

كما أن معظم الدراسات السابقة ركزت على النشاط المضاد للأكسدة لهذه المركبات، دون دمجها في صيغ دوائية صيدلانية تضمن استقرارها الحيوي وكفاءتها كمضاد بكتيري فموي.





- الغرض (الهدف) من الاختراع

يهدف هذا الاختراع إلى تقييم وتنمين مشتقات بورفيرين مستبدلة بأيونات النيكل والزنك، من خلال استخدامها كمركونات دوائية مضادة للبكتيريا تُعطى عن طريق الفم في هيئة أقراص صلبة، بهدف تحقيق تثبيط فعال لنمو السلالات البكتيرية الممرضة والحد من شدة العدوى.

- تقديم جوهر الاختراع

يتعلق هذا الاختراع بتقييم مشتقات بورفيرين مستبدلة بأيونات النيكل والزنك (Ni و Zn)، ودراسة فعاليتها كمركونات مضادة للبكتيريا باستخدام منهجية متعددة تشمل التجارب المخبرية والتحليلات الحاسوبية المتقدمة. تم الحصول على المعقدات المعدنية جاهزة، مع تأكيد هويتها البنيوية باستخدام تقنيات تحليل طيفية مثل UV-Vis.

تركز الدراسة على التقييم الحيوي لهذه المركبات ضد سلالات بكتيرية مختارة بطريقة *in vitro*، إلى جانب تحليل خواصها الدوائية والتنبؤ بسميتها باستخدام أدوات ADMET. كما تم توظيف تقنية الالتحام الجزيئي (Molecular Docking) لمحاكاة آليات ارتباط المركبات بالإنزيمات البكتيرية المستهدفة، ودُعِمت هذه النتائج بمحاكاة الديناميكيات الجزيئية (Molecular Dynamics) لتقدير استقرار المعقدات وتفاعلاتها في البيئات البيولوجية. يساهم هذا العمل في تسليط الضوء على مركبات واعدة من الناحية الدوائية كمضادات ميكروبية جديدة، مع تقديم بيانات دقيقة تدعم استخدامها المحتمل وتوجيه تصميمها المستقبلي ضمن إطار الطب المبني على البنية الجزيئية.

النموذج الأولي التجريبي

النموذج الأولي التجريبي هو نسخة أولية تم صنعها من المنتج أو الخدمة والتي تستخدم كأساس في التطوير للوصول إلى المنتج النهائي الذي سيطبق في السوق رسمياً.

- ✓ في هذا الجانب يقدم صاحب المشروع نموذج أولي ملموس (يقدم للجنة)، كما يمكنه تصوير مقطع فيديو أو مجموعة من الصور تثبت وصوله إلى إتمام النموذج الأولي .
- ✓ كما يمكنه تقديم شرح للمراحل الأساسية المتبعة للوصول إلى النموذج الأولي.
- ✓ يمكن لأصحاب مشاريع التطبيقات والمنصات الرقمية عرض نموذج أولي للتطبيق الإلكتروني.

- عندما يتعلق الأمر بجهاز
هذا المحور لا ينطبق عليك بشكل مباشر، لأن مشروعنا لا يتضمن اختراع جهاز جديد
- عندما يتعلق الأمر بطريقة عملية (لإنتاج أو المعالجة)
تم اتباع طريقة عملية واضحة وشبه صناعية لتحضير الشكل الدوائي، حيث تم:
 - تحضير المركبات الفعالة مخبرياً.
 - خلطها مع مواد مساعدة صيدلانية (مثل النشاء، MCC).
 - ترطيب الخليط بالماء أو محلول مناسب.
 - كبس المزيج يدوياً في قوالب لصناعة أقراص متجانسة. هذه الطريقة بسيطة، قابلة للتكرار، ويمكن تطويرها لاحقاً للإنتاج على نطاق أوسع.
- عندما يتعلق الأمر بمنتج (مركب، مزيج، تشكيل....)
المنتج النهائي عبارة عن دواء في شكل أقراص صلبة، يحتوي على مزيج دقيق من:
 - مادة فعالة: مشتقات بورفيرين مستبدلة بالنيكل والزنك.
 - سواغات مساعدة: مثل النشاء، MCC، وربما اللاكتوز أو مواد رابطة. هذا التشكيل يضمن الاستقرار، سهولة التناول، والتحكم في الجرعة، مما يجعله قابلاً للتطبيق في المجال الصيدلاني.





• عندما يتعلق الأمر بتطبيق جديد لجهاز معروف أو طريقة معروفة أو مادة معروفة

المشروع يعتمد على مادة معروفة وهي البورفيرين، ولكن تم تعديلها كيميائياً بإضافة النيكل والزنك، مما منحها خصائص جديدة كمضادات للبكتيريا.

هذا يعتبر تطبيقاً دوائياً جديداً لمركبات البورفيرين المعدنية، حيث لم تُستخدم بهذه الطريقة سابقاً في مجال العلاج كمضاد بكتيري.

• عندما يتعلق الأمر بتركيبة جديدة لعناصر معروفة أو غير معروفة

تم تطوير تركيبة جديدة من عناصر معروفة وهي:

■ حلقة بورفيرين.

■ أيونات معدنية (Zn و Ni). لكن التعديل الهيكلي الذي أدخل على الحلقة البورفيرينية وإضافة

الميثيل والمعدن، أنتج مركبات جديدة بخصائص دوائية مبتكرة.

التركيبة لم تُستخدم سابقاً بهذه الصيغة لعلاج العدوى البكتيرية، مما يميزها كاختراع كيميائي دوائي جديد.

شرح الأشكال و الرسومات: (دون وضعها في الوصف)

يتم شرح الأشكال وفق ترقيمها وشرح كل رقم على حدى وتبيان دوره وفعاليتها في براءة الاختراع

الشكل 01: النموذج الأولي لمركب بورفيرين الزنك على شكل أقراص.

الشكل 02: النموذج الأولي لمركب بورفيرين النيكل على شكل أقراص.

7. طريقة والية عمل الجهاز المخترع او المادة المخترعة

■ طريقة العمل:

المركبات المخترعة هي مشتقات بورفيرين مستبدلة بالنيكل والزنك (NiTPPH_2 و $\text{ZnTPPH}_2(\text{p-methyl})$)، وقد تم تصميمها بحيث تمتلك نشاطاً مضاداً للبكتيريا. طريقة عملها تشمل:

1. اختراق الغشاء البكتيري: المركب قادر على التفاعل مع جدار الخلية البكتيرية، مما يؤدي إلى تغيير نفاذية الغشاء.
2. الارتباط بالبروتينات المستهدفة: من خلال دراسات الالتحام الجزيئي (Molecular Docking)، تبين أن المركبات ترتبط بمواقع فعالة داخل إنزيمات بكتيرية مهمة (مثل DNA gyrase أو البروتينات المرتبطة بالنمو الخلوي).
3. تثبيط النمو أو القتل البكتيري: التفاعل يؤدي إلى إيقاف عمليات حيوية داخل البكتيريا، مما يوقف تكاثرها أو يؤدي إلى موتها.

■ آلية العمل البيوكيميائية:

اعتماداً على نتائج الاختبارات المخبرية والدراسات الحاسوبية:

تبين أن المعدن (Ni أو Zn) يلعب دوراً في تعزيز فعالية المركب من خلال:





- تكوين روابط تناسقية مع مواقع فعالة في الإنزيمات البكتيرية.
- إنتاج أنواع نشطة من الأوكسجين (ROS) التي تسبب إجهادًا أكسديًا داخل الخلية البكتيرية.
- كما أن حلقة البورفيرين العطرية تساهم في:
 - التداخل مع جدار الخلية البكتيرية.
 - التفاعل مع الـ DNA أو البروتينات داخل الخلية.





المحور الرابع المطالب





المطالب

المطلب الرئيس يتمثل في القيمة الإضافية والميزة التي جاء بها اختراعنا مقارنة بباقي الاختراعات الأخرى

يتمثل المطلب الرئيس لاختراعنا في تقديم مركب دوائي جديد بتركيبية مبتكرة من البورفيرين مع النيكل والزنك، يتميز بفعالية مضادة للبكتيريا، سمية منخفضة، وقابلية للتناول الفموي، مما يجعله بديلاً واعدًا للمضادات الحيوية التقليدية.

2. المطالب المستنبطة من المطلب الرئيسي والتي تميز اختراعنا

1. تركيبة كيميائية جديدة من مشتقات البورفيرين المدعمة بالنيكل والزنك.
2. فعالية مخبرية مؤكدة ضد عدة سلالات بكتيرية.
3. سمية منخفضة وخصائص دوائية واعدة حسب دراسات ADMET.
4. قابلية للتصنيع الدوائي في شكل أقراص فموية صلبة.
5. آلية عمل مغايرة تساعد في مواجهة مقاومة المضادات الحيوية التقليدية.
6. إمكانية تطويره لاحقاً كدواء قابل للتسويق.





قائمة الملاحق





10 الأشكال والرسومات والجداول

1. ترسم الأشكال دون شرح 11
2. ترسم الجداول دون شرح 12
3. ترسم الرسومات دون شرح 12

الشكل 2



الشكل 1



دليل مشروع

للحصول على شهادة مؤسسة ناشئة
في إطار القرار الوزاري 1275

ديسمبر

