IN SILICO SCREENING, SYNTHESIS AND CHARACTERIZATION OF IMIDAZOLE DERIVATES WITH POTENTIAL ANTI-HYPERTENSIVE ACTIVITY

BY

OLOWO JUDE OLUWATOBI

PHA1908573

SUPERVISED BY:

DR. UNUIGBE ISONAH

A PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work was carried out by **OLOWO JUDE OLUWATOBI** in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Edo State.

_____ _____

DR. UNUIGBE ISONAH **DATE**

PROJECT SUPERVISOR

_____ _____

DR. VINCENT IMIEJE **DATE**

HEAD OF DEPARTMENT

DEDICATION

This project work is dedicated to the Almighty God for His grace and faithfulness in my life

ACKNOWLEDGEMENT

I am profoundly grateful to God Almighty for his abundant grace, strength, and guidance throughout this academic journey.

My sincere appreciation goes to my project supervisor, Dr. Unuigbe Isonah, whose support, guidance, and consistent encouragement were invaluable to the successful completion of this work. I am also thankful to all the academic and non-academic staffs of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin; for their support, and for creating a conducive learning environment that fostered the success of this project.

My deepest gratitude also goes to my Dad, Pastor (Dr.) Olowolaiyemo Raphael and my Mum, Mrs Olowo Janet, and my siblings for their sacrifices, prayers encouragement and unwavering support throughout this journey.

Special recognition to my project partners — Ebere, Osas and Neina, for their commitment, assistance, and valuable contributions. Working alongside them was truly a privilege.

My sincere appreciation extends to Pharm. Dr. Emmanuel Etudaye, Eimoga Nathan, Idowu Emmanuel, Amedu Blessing, Engineer Ugboke Precious, Mr. Charles, Mr. Mayowa, Excellent, Esther, and Nosakhare, all of whom have supported me in various ways throughout my academic journey. This project stands as a testament to the collective support, prayers and goodwill of all these remarkable individuals. I remain sincerely grateful for their contributions.

Lastly, I want to thank me. I want to thank me for believing in me. I want to thank me for doing all this hard work. I want to thank me for having no days off. I want to thank me for never quitting. And finally, I want to thank me for becoming the person who could stand here today.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Hypertension affects approximately 1.3 billion people globally and remains a leading cause of cardiovascular morbidity and mortality. Despite available treatments, challenges including resistant hypertension, poor adherence, and adverse effects persist. This study employed integrated computational and experimental approaches to design and synthesize novel imidazole-based antihypertensive compounds. Molecular docking using AutoDock Vina evaluated four imidazole derivatives against key hypertension-related targets: 6L88, 7BVQ, 5XPR, and 1O86. The compound 2-hexyl-4-phenyl-1H-imidazole exhibited superior binding with affinities of -6.3 to -7.9 (kcal/mol) to all the targets used.

SwissADME analysis predicted favorable pharmacokinetics: high gastrointestinal absorption, optimal lipophilicity, and full Lipinski compliance. ProTox-3.0 toxicity profiling also showed acceptable safety with no predicted mutagenicity, carcinogenicity, immunotoxicity, or cytotoxicity. Based on these results, 2-hexyl-4-phenyl-1H-imidazole was synthesized via Debus-Radziszewski condensation with 77.3% yield and melting point of 184-186°C. Structure-activity analysis confirmed both hexyl and phenyl substituents are essential for optimal binding. This work demonstrates rational drug design principles in identifying promising antihypertensive leads and establishes a foundation for developing imidazole-based cardiovascular agents

While complete spectroscopic characterization (FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry) is pending, this work successfully demonstrates the application of rational drug design principles in identifying promising lead compounds for antihypertensive therapy. The findings establish a foundation for further development of imidazole-based cardiovascular agents.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Hypertension: A Global Health Challenge

Hypertension, commonly known as high blood pressure, is one of the most pressing public health issues worldwide. The World Health Organization reported in 2023 that roughly 1.3 billion people live with hypertension, yet only one in five achieves adequate blood pressure control below 140/90 mmHg (Richardson et al., 2025). This condition is a major driver of cardiovascular disease, stroke, kidney failure, and early death. Between 2000 and 2021, ischemic heart disease remained the leading cause of death from non-communicable diseases, with hypertension identified as one of the top three contributing risk factors.

The impact of hypertension goes beyond direct cardiovascular effects. Elevated blood pressure also significantly increases the risk of heart disease, stroke, chronic kidney disease, and dementia, making it a leading preventable cause of death globally (Zhou et al., 2021). The economic burden is staggering, with global costs estimated at $820 billion annually, covering both medical expenses and productivity losses. If hypertension were effectively managed worldwide, an estimated 120 million strokes, 79 million heart attacks, 17 million cases of heart failure, and 76 million deaths could be prevented between 2023 and 2050 (Wandile, 2024).

While the global prevalence of hypertension has remained relatively stable at around 32-33% from 1990 to 2019, the absolute number of affected individuals doubled during this period (Longkumer et al., 2023). Developed and high-income countries have seen declining rates, but lower-middle-income countries now account for over 82% of people living with

hypertension accounting for more than 1 billion individuals. These patterns reveal growing health disparities and unequal access to quality healthcare.

Despite numerous effective medications, blood pressure control remains poor in many patients. Clinical studies show that only 30-35% of treated hypertensive patients reach their target blood pressure levels (Petramala et al., 2024). This gap reflects challenges like poor medication adherence, side effects, and the complexity of hypertension itself.

1.1.1 Definition and Classification

Hypertension is defined as persistently elevated arterial blood pressure above levels associated with increased cardiovascular risk. Classification systems have evolved over time, with different international guidelines taking varied approaches to make diagnosis practical and easy to identify without unnecessary labeling.

The 2024 European Society of Cardiology guidelines introduced a simplified three-tier system: non-elevated (below 120/70 mmHg, no drug treatment needed), elevated (120-139/70-89 mmHg, treatment for select high-risk individuals), and hypertension (140/90 mmHg or higher, treatment recommended for most) (McCarthy et al., 2025). This approach aims to make clinical decisions clearer while still capturing meaningful risk differences.

In contrast, the 2023 European Society of Hypertension guidelines maintain a more granular classification with six categories: optimal, normal, high-normal, and grades 1-3 hypertension. Grade 1 begins at 140/90 mmHg, grade 2 at 160/100 mmHg, and grade 3 at 180/110 mmHg or higher (Meyer & Redford, 2024).

Category	Systolic BP (mmHg)	Diastolic BP (mmHg)
Optimal	< 120	< 80
Normal	120–129	80–84
High Normal	130–139	85–89
Grade 1 Hypertension (Mild)	140–159	90–99
Grade 2 Hypertension (Moderate)	160–179	100–109
Grade 3 Hypertension (Severe)	≥ 180	≥ 110

Figure 1.1 showing the 2023 European Society of Hypertension guidelines

The American approach differs notably. The 2017 ACC/AHA guidelines lowered the diagnostic threshold to 130/80 mmHg for stage 1 hypertension, based on clinical trial evidence showing cardiovascular benefits at these levels. Blood pressure is categorized as normal (below 120/80 mmHg), elevated (120-129/below 80 mmHg), stage 1 (130-139/80-89 mmHg), or stage 2 (140/90 mmHg or higher) (Meyer & Redford, 2024).

An important subtype is isolated systolic hypertension, defined as systolic pressure above 140 mmHg with diastolic below 90 mmHg. This affects approximately 15% of people over 60 and is the most common and high-risk form of hypertension in older adults, primarily resulting from reduced arterial elasticity and increased stiffness with aging (Wang et al., 2024).

Proper diagnosis requires standardized blood pressure measurement. Both European and American guidelines emphasize using validated cuff devices and increasingly recommend out-of-office monitoring to avoid misdiagnosis from white-coat effect or masked hypertension (Meyer & Redford, 2024).

1.1.2 Pathophysiology of Hypertension

Hypertension develops through complex interactions among multiple biological systems. Despite differences in origin between primary and secondary hypertension, overlapping physiological pathways contribute to impaired blood pressure regulation. Understanding these mechanisms is crucial for identifying therapeutic targets.

(i) The Renin-Angiotensin-Aldosterone System (RAAS)

The RAAS is one of the most extensively studied pathways in hypertension. It regulates blood volume, electrolyte balance, and vascular resistance by responding to decreased renal blood pressure, reduced salt delivery to the kidney, and beta-receptor stimulation (Fountain et al., 2023). The system operates through enzymatic reactions that produce angiotensin II (a powerful vasoconstrictor) and aldosterone (which promotes sodium retention).

RAAS activation is central to hypertension pathogenesis. All components exist in the kidneys at higher concentrations than in plasma, and the kidneys can produce angiotensin II independently of systemic RAAS (Moon JY, 2013). Kidney angiotensin II concentrations are 50-100 times higher than systemic levels, highlighting the importance of local tissue-based activity. This suggests therapeutic interventions targeting RAAS may work through both locally and systemically.

Recent research has revealed alternative RAAS pathways with protective effects. The Mas receptor pathway, involving angiotensin-converting enzyme type 2 (ACE2), angiotensin 1-7, and Mas receptors, demonstrates antihypertensive properties including vasodilation, nitric oxide release, and anti-inflammatory effects (Martyniak & Tomasik, 2023). These alternative pathways may offer new therapeutic targets to counteract classical RAAS hypertensive effects.

(ii) The Sympathetic Nervous System

The sympathetic nervous system plays a central role in blood pressure control through effects on the heart, blood vessels, and kidneys. Growing evidence shows it contributes to hypertension development from early stages through mechanisms involving genetic factors and immune system interactions with sympathetic activation (Seravalle & Grassi, 2022). In essential hypertension, the brain, heart, and kidneys all show heightened sympathetic activity.

Measurements in younger essential hypertension patients reveal increased sympathetic outflow to the heart, kidneys, and skeletal muscle blood vessels, which both initiates and sustains elevated blood pressure (Esler, 2000). Centrally acting drugs like moxonidine work by targeting imidazoline receptors in the brain, reducing sympathetic outflow. Studies show moxonidine significantly decreases muscle sympathetic nerve activity and plasma norepinephrine, lowering blood pressure through reduced central sympathetic drive (Wenzel et al., 1998).

(iii) RAAS - Sympathetic System Interaction

These two systems don't only work in isolation, they also interact closely. RAAS activation enhances sympathetic transmission and blocks norepinephrine reuptake at nerve terminals, creating a positive feedback loop that amplifies hypertensive effects (Sowers et al., 2001). This interaction explains why achieving adequate blood pressure control is difficult and has led to therapeutic strategies targeting both systems simultaneously.

(iv) Endothelial Dysfunction and Oxidative Stress

The endothelium lining blood vessels acts as a barrier between blood and tissues, producing substances with anti-clotting and anti-inflammatory properties. When damaged by oxidative stress and inflammation, endothelial function shifts from protective to harmful, promoting

vasoconstriction and clot formation (Drożdż et al., 2023). Reduced nitric oxide availability plays a central role in hypertension-related endothelial dysfunction.

Oxidative stress involves reactive oxygen species that cause endothelial damage, blood vessel dysfunction, cardiovascular remodeling, kidney problems, sympathetic nervous system overactivity, and systemic inflammation (Griendling et al., 2021). Factors like impaired blood flow, inflammation, and RAAS activation all contribute to endothelial dysfunction in hypertension (Gallo et al., 2021).

Angiotensin II promotes blood vessel inflammation by activating enzymes that produce oxidative stress molecules and increase production of endothelin-1, a vasoconstrictor (Zhang et al., 2023). This creates a harmful loop where RAAS activation, oxidative stress, and endothelial dysfunction cause and amplify hypertensive complications.

1.1.3 Cardiovascular and Organ Complications

Hypertension drives a wide range of complications that contribute substantially to disease burden and death worldwide. Sustained blood pressure elevation damages the heart, brain, kidneys, and blood vessels through different mechanisms including mechanical stress, vascular remodeling, and accelerated atherosclerosis. Some of these complications includes:

(i) Heart Disease

The heart is one of the primary targets of chronic hypertension. Prolonged pressure overload causes structural and functional changes known collectively as hypertensive heart disease. In the United States, approximately 75 million adults have hypertension, with studies showing an avarage time of 14.1 years between hypertension onset and progression to heart failure (Shams et al., 2025).

Hypertensive heart disease leads to dangerous heart rhythm disturbances, including ventricular tachycardia and fibrillation, particularly in advanced cases. It can also cause slow heart rhythms and conduction problems. Between 1990 and 2019, hypertensive heart disease caused over 1.1 million deaths in the United States, with higher mortality in men and younger adults (Shams et al., 2025).

Heart failure is a major complication. Patients with preserved ejection fraction and poorly controlled hypertension face 50% higher hospitalization risk and 30% higher mortality compared to those with well-controlled blood pressure (Burlacu et al., 2025). Atrial fibrillation, another common complication, increases stroke risk by 70% in hypertensive patients.

(ii) Stroke and Brain Complications

Hypertension is the single most important modifiable stroke risk factor. Large studies demonstrate it also increases risk for heart failure, atrial fibrillation, chronic kidney disease, and dementia (Fuchs & Whelton, 2020). Both ischemic and hemorrhagic strokes are closely linked to hypertension, even without obvious strokes, hypertension can cause silent brain infarcts, white matter damage, and cognitive decline that may lead to vascular dementia.

(iii) Kidney Disease

Hypertension and chronic kidney disease (CKD) create a cycle where each worsens the other. Hypertension is the leading modifiable cause of premature death and affects most CKD patients. Both conditions are intrinsically linked. Hypertension worsens kidney and cardiovascular outcomes, while declining kidney function further elevates blood pressure. (Burnier & Damianaki, 2023).

CKD is both a cause and complication of uncontrolled hypertension. The interaction is complex and increases adverse cardiovascular and cerebrovascular outcomes, particularly in resistant hypertension commonly seen with kidney disease (Hamrahian & Falkner, 2017). Multiple mechanisms contribute to elevated blood pressure in CKD, including neural and hormonal changes that disrupt normal blood pressure regulation (Ameer, 2022).

The progression from hypertension to end-stage renal disease follows a predictable pattern: glomerular hyperfiltration, protein in urine, progressive decline in kidney filtration, and eventually the need for dialysis or transplant. Uncontrolled hypertension accelerates kidney function decline. The pathophysiology is complex but largely relates to reduced functional kidney tissue, sympathetic overactivity, RAAS involvement, and widespread endothelial dysfunction (Hebert & Ibrahim, 2022).

(iv) Blood Vessel Complications

Chronic hypertension promotes widespread vascular damage through endothelial dysfunction, accelerated atherosclerosis, arterial stiffening, and blood vessel remodeling. These changes affect both large arteries and small resistance vessels, contributing to problems in multiple organ systems. Other serious complications associated with hypertension include peripheral arterial disease, aortic aneurysm, and aortic dissection. Persistent high pressure on weakened arterial walls can lead to vessel rupture or dissection.

1.1.4 Current Treatment Approaches

Hypertension management has evolved substantially over recent decades, with five major drug classes now available: ACE inhibitors, angiotensin receptor blockers (ARBs), calcium channel blockers, diuretics, and beta-blockers. Achieving good blood pressure control leads

to significant improvements in outcomes, with reductions in heart attack, heart failure, stroke, and chronic kidney disease risk (Mahfoud et al., 2024).

(i) RAAS Inhibitors

ACE inhibitors and ARBs are cornerstones of hypertension treatment, both carrying the strongest recommendation in major guidelines as first-line agents (Chen et al., 2021; Sobhy et al., 2024). They're the preferred choice for patients with heart failure and chronic kidney disease, especially when protein appears in the urine (Khalil & Zeltser, 2023).

Recent evidence shows ARBs are as effective as ACE inhibitors for lowering blood pressure but with better tolerability (Cutrell et al., 2023). When considering all available evidence, these drug classes have similar effectiveness, but ARBs consistently show fewer side effects (Turner & Kodali, 2020). The most notable difference is dry cough with ACE inhibitors, which occurs due to bradykinin accumulation and affects 5-20% of patients, often leading to treatment discontinuation.

(ii) Calcium Channel Blockers

Calcium channel blockers prevent calcium entry into blood vessel smooth muscle and heart cells, promoting vasodilation and reducing vascular resistance. They're among the most widely studied drugs and are recommended as first-line therapy alone or in combination based on extensive knowledge of their mechanisms and minimal side effects (Jones et al., 2024).

These drugs are divided into two classes: dihydropyridines (like amlodipine and nifedipine) and non-dihydropyridines (verapamil and diltiazem). Dihydropyridines are more selective for blood vessels with minimal heart effects, making them particularly effective for blood pressure reduction. Non-dihydropyridines also affect heart conduction and contraction.

Compared to beta-blockers, calcium channel blockers reduce major cardiovascular events, stroke, and cardiovascular death. Compared to ACE inhibitors, they reduce stroke but slightly increase heart failure risk (Zhu et al., 2022).

(iii) Diuretics

Thiazide and thiazide-like diuretics have been used for decades and remain important first-line options. They work primarily by promoting sodium excretion and reducing blood volume, with additional direct vasodilatory effects. Thiazide-like diuretics, particularly chlorthalidone and indapamide, have longer durations of action than hydrochlorothiazide and may provide superior cardiovascular protection, though with higher rates of metabolic side effects like low potassium.

Studies show no significant mortality difference between calcium channel blockers and diuretics. In chronic kidney disease patients already taking RAAS inhibitors, those who added diuretics had lower risk of kidney disease progression and similar cardiovascular event risk compared to those adding calcium channel blockers (Faucon et al., 2023), suggesting potential kidney-protective effects beyond blood pressure reduction.

(iv) Beta-Blockers

Beta-blockers reduce blood pressure through multiple mechanisms including decreased cardiac output, reduced renin secretion, and modulation of central sympathetic outflow. While the 2023 European guidelines state that all five major drug classes improve cardiovascular outcomes similarly and can be used alone or combined, some experts still debate using beta-blockers as first-line agents without specific indications (Mahfoud et al., 2024).

Beta-blockers are particularly beneficial for patients with coronary artery disease, heart failure with reduced ejection fraction, or certain arrhythmias. Their relative position in hypertension management has been debated because evidence suggests they may be less effective than other classes in preventing stroke and may increase new-onset diabetes risk, particularly when combined with thiazide diuretics.

(v) Combination Therapy

Most hypertensive patients need multiple drugs to achieve adequate control. RAAS blockers combined with either diuretics or calcium channel blockers are preferred combinations in most guidelines and are the most frequently used in clinical practice (Rimoldi et al., 2015). Current guidelines recommend starting with a thiazide diuretic, calcium channel blocker, ACE inhibitor, or ARB, then increasing doses or adding other classes if blood pressure targets aren't met (Oh et al., 2017).

Combination therapy allows simultaneous targeting of multiple pathways, achieves greater blood pressure reduction than single drugs, and potentially minimizes side effects by using lower individual drug doses. Fixed-dose combination pills containing two or more drugs improve adherence and simplify treatment. Contemporary guidelines increasingly recommend starting with combination therapy rather than single drugs in patients with substantially elevated blood pressure or high cardiovascular risk.

1.1.5 Treatment Challenges and Limitations

Despite numerous effective medications, significant challenges persist that limit optimal blood pressure control at the population level. These include resistant hypertension, poor medication adherence, side effects affecting quality of life, therapeutic inertia among healthcare providers, and socioeconomic barriers to accessing treatment.

(i) Resistant Hypertension

Resistant hypertension is defined as blood pressure above goal despite confirmed adherence to three first-line drugs at maximum doses, or when controlled with four or more medications. These patients face higher cardiovascular risk and are more likely to have treatable secondary causes (Cluett & William, 2024). While true resistant hypertension affects less than 10% of treated patients, the absolute number is large and increasing (Lauder et al., 2024).

Evaluating apparent resistant hypertension requires distinguishing true resistance from other factors, including poor medication adherence and white-coat effect(a temporary rise in blood pressure that occurs when a patient is in a medical setting, usually in the presence of a doctor or nurse but is normal out of office) (Carey et al., 2018). Accurate diagnosis is critical because true resistant hypertension carries substantially higher cardiovascular risk and may warrant evaluation for secondary causes or interventional approaches.

(ii) Medication Adherence Problems

Non-adherence to hypertension medications is a major but often underrecognized barrier to blood pressure control. Globally, non-adherence rates range from 27-40%, with adherence tending to decline over time (Altoum et al., 2023). Studies show only about 42% of patients adhere to their medications (Algabbani & Algabbani, 2020). Analysis of Medicare data demonstrates significant associations between non-adherence and cardiovascular deaths (Gavrilova et al., 2021).

Multiple factors contribute across different domains. The WHO identifies five obstacle categories: patient demographics, healthcare system issues, therapy concerns like side effects, disease-related factors, and patient-related challenges including fear of adverse effects

(Altoum et al., 2023). Undertreatment (therapeutic inertia) and non-adherence are well-recognized but underestimated barriers (Hamrahian et al., 2022). Adherence is particularly challenging for long-term treatment versus short-term symptom relief (Nikolic et al., 2023).

(iii) Side Effects and Tolerability

Drug side effects significantly impact adherence and long-term continuation. In one study, 85% of participants experienced side effects, and 34.5% became non-adherent, with adherence being 6 percentage points lower among those with more side effects (Tedla et al., 2015). Increased number of prescribed drugs and choice of drug class affect adherence risk, with diuretics or diuretic-containing combinations associated with higher non-adherence rates (Hamrahian et al., 2022).

Diuretic non-adherence is often explained by side effects directly affecting quality of life (Altoum et al., 2023). Adherence was 6.5 percentage points lower with excessive urination and 7.6 percentage points lower with decreased sexual drive (Tedla et al., 2015). These findings suggest certain side effects disproportionately impact medication-taking behavior, highlighting the importance of considering tolerability when selecting treatment regimens.

(iv) Treatment Complexity

Complex regimens, particularly requiring multiple medications, pose additional adherence challenges. Adherence was better among patients taking fewer than four medications (47.1%) compared to those taking four or more (31.3%) (Algabbani & Algabbani, 2020). Multiple daily doses and managing medications for other conditions contribute to pill burden and increase non-adherence likelihood.

Fixed-dose combination medications address these challenges by reducing pill burden and simplifying regimens. However, access to these products and their clinical use varies across

healthcare settings and regions. Finding the right balance between effective blood pressure control and keeping medication regimens tolerable and manageable still remains a constant challenge.

1.1.6 Novel Therapeutic Approaches and Their Limitations

Recent years have seen development of novel approaches for resistant hypertension, including new drugs and device-based interventions, but these have encountered significant challenges. For example, firibastat failed to meet its primary endpoint in a phase 3 trial for treatment-resistant hypertension, showing similar blood pressure reduction to placebo (Flack et al., 2024).

Renal denervation initially showed promise for resistant hypertension but faced setbacks. In the SPYRAL HTN-ON MED trial, it did not significantly lower blood pressure measured over 24 hours, though office blood pressure measurements showed significant decreases (Flack et al., 2024). This discordance highlights the complexity of blood pressure assessment and importance of rigorous study design.

One novel agent, aprocitentan, received FDA approval in March 2024 for use with other antihypertensive medications in adults with inadequately controlled hypertension. However, limitations include fluid retention concerns at higher doses, potentially higher cost than traditional medications, and smaller blood pressure reductions than some existing therapies (Pena et al., 2025).

Broader Barriers

Beyond clinical challenges, socioeconomic and healthcare system factors significantly impact control rates. Medication access, treatment affordability, insurance coverage, healthcare infrastructure, and health literacy all influence patients' ability to receive and adhere to

effective therapy. These barriers are particularly pronounced in low- and middle-income countries but also affect vulnerable populations in high-income countries.

The complexity of hypertension combined with current treatment limitations highlights the ongoing need for innovation in drug development. Novel compounds with improved effectiveness, tolerability, and adherence profiles could address many unmet needs. Developing agents targeting alternative mechanisms, represents one promising avenue for expanding treatment options and improving outcomes for patients with difficult-to-control hypertension.

1.2 Heterocyclic Compounds and Imidazoles in Medicinal Chemistry

1.2.1 Overview of Heterocyclic Compounds in Drug Discovery

Heterocyclic compounds are organic molecules containing at least one heteroatom (nitrogen, oxygen, or sulfur) within a ring structure, distinguishing them from all-carbon rings. These compounds include five-membered rings like pyrrole, furan, and imidazole, or six-membered rings like pyridine. They're critical in medicinal chemistry due to their structural diversity and ability to interact with biological targets such as enzymes and receptors.

Figure 1.2 Different common heterocyclic compounds

Heteroatoms enable hydrogen bonding, dipole interactions, and hydrophobic effects, enhancing how drugs bind to their targets and optimizing properties like solubility and bioavailability. Heterocycles are found in over 85% of biologically active compounds. Nitrogen-containing heterocycles, such as imidazoles and pyrimidines, appear in over 75% of FDA-approved small-molecule drugs. These "privileged structures" bind multiple targets with high affinity, making them ideal for drug discovery.

Their ability to mimic natural biomolecules like peptides or nucleotides, combined with improved metabolic stability, explains their prevalence in modern medicine. In drug discovery, heterocycles are integral to structure-activity relationship studies, allowing precise optimization of potency and selectivity. For example, imidazole derivatives like losartan target the RAAS for antihypertensive therapy, while pyrimidine-based drugs like imatinib inhibit tyrosine kinases in cancer treatment.

Synthetic advancements have simplified the production of diverse heterocyclic scaffolds, accelerating drug development. These compounds are also prominent in natural products, guiding synthetic analogs with enhanced therapeutic profiles. Heterocycles are essential across different therapeutic fields, including antimicrobial, anticancer, and cardiovascular applications, ensuring continued innovation in addressing unmet medical needs.

1.2.2 Chemistry of Imidazoles

Structure and Properties

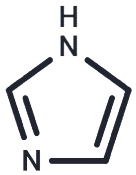

Figure 1.3 The structure of imidazole

Imidazole is a five-membered aromatic heterocycle with the molecular formula $C_3H_4N_2$, containing two nitrogen atoms at positions 1 and 3. Its aromaticity arises from a delocalized system of six π-electrons, where the "pyrrole-like" nitrogen (N-1) donates two electrons from its lone pair to the π-system, while the "pyridine-like" nitrogen (N-3) retains its lone pair for protonation or coordination, giving basic properties (Tolomeu et al., 2023).

This dual nitrogen character results in amphoteric behavior making imidazoles acts as both a weak acid (pKa ≈ 14.5) and a base (pKa of conjugate acid ≈ 7.0), with it being approximately 60 times more basic than pyridine (Rasajna et al., 2023). The planar ring has a resonance energy of about 14.2 kcal/mol and a dipole moment of 3.61 D, contributing to high water solubility (633 g/L) and compatibility with polar solvents (Ali et al., 2013).

Physically, imidazole is a white or pale yellow solid with a melting point of 89–91°C and boiling point of 256°C, driven by intermolecular hydrogen bonding. It exhibits tautomerism, where the hydrogen atom shuttles between N-1 and N-3, rendering the 4- and 5-positions equivalent in unsubstituted imidazole. The electron-rich nature favors electrophilic substitution at C-4/C-5 and nucleophilic attack at C-2, while its hydrogen-bonding capability (N-1 as donor, N-3 as acceptor) and metal coordination properties are critical for biological interactions (Rasajna et al., 2023).

Different Methods for Synthesis of Imidazoles

Imidazole synthesis has advanced from classical condensation reactions to modern catalytic and multicomponent strategies.

(i) The Debus-Radziszewski reaction, pioneered by Heinrich Debus in 1858, is a cornerstone method involving condensation of glyoxal (or α-dicarbonyls), an aldehyde, and ammonia to form 2,4,5-trisubstituted imidazoles (Tolomeu et al., 2023). Traditional conditions required

harsh parameters like high temperatures and strong acids, but modern modifications employ magnetic nanoparticles, metal-organic frameworks, and ionic liquids, achieving yields exceeding 90% with milder conditions and shorter reaction times.

Green chemistry innovations including ultrasonic irradiation, microwave assistance, and solvent-free conditions have revolutionized this reaction, enabling yields exceeding 95% with reaction times as short as 5-30 minutes. Recent developments include bio-based green solvents like ethyl lactate, which eliminate traditional catalysts while providing good yields.

(ii) The Van Leusen synthesis, developed by A. M. van Leusen in 1977, uses tosylmethyl isocyanide (TosMIC) with imines under basic conditions to generate 1,4,5-trisubstituted imidazoles. This method proceeds under mild conditions, typically at room temperature, and exhibits excellent functional group tolerance, making it suitable for complex molecular architectures.

(iii) The Phillips-Ladenburg reaction provides direct access to benzimidazole derivatives through condensation of o-phenylenediamine with carboxylic acids or aldehydes. Traditional conditions required temperatures exceeding 170°C, but modern modifications using green catalysts reduce reaction times to 10-30 minutes while achieving yields of 80-99%.

Copper-catalyzed multi-component reactions have been optimized to provide tri-substituted imidazoles in excellent yields under relatively mild conditions, demonstrating broad reaction scope.

1.2.3 Substituted Imidazoles and Their Derivatives

Substituted imidazoles, featuring alkyl, aryl, or other functional groups at positions 1, 2, 4, or 5, significantly enhance pharmacological utility. Mono-substituted derivatives, such as N-arylimidazoles, are synthesized via copper-catalyzed N-arylation, offering quantitative yields.

Di-substituted imidazoles are prepared from α-ketoaldehydes with ammonium acetate, demonstrating antiproliferative activity.

Trisubstituted imidazoles, such as 2,4,5-triaryl derivatives, are accessed through condensations or multicomponent reactions. Notable derivatives include nitroimidazoles, fused systems like imidazo[1,2-a]pyridines, and benzimidazoles. Substituents profoundly influence activity: electron-withdrawing groups at C-2 enhance antimicrobial effects, while aryl groups at C-4/C-5 improve kinase inhibition (Ali et al., 2013).

Pharmacologically relevant examples include antifungal azoles like ketoconazole, anticancer EGFR inhibitors with nanomolar IC50 values, and antiparasitic compounds.

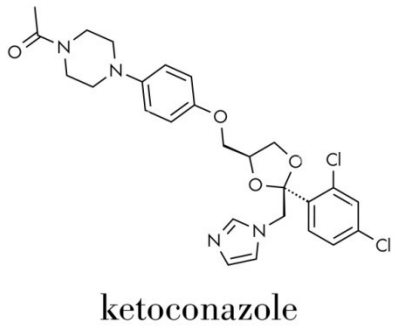

Figure 1.4 The structure of Ketoconazole

1.2.4 Pharmacological Significance of Imidazole Derivatives

Imidazoles represent a privileged scaffold in pharmacology, exhibiting diverse therapeutic activities due to their ability to interact with enzymes, receptors, and ion channels. This versatility arises from the imidazole ring's capacity for hydrogen bonding, π-π stacking, and metal coordination. Recent advances include ionic liquid formulations to enhance bioavailability and target specificity (Sahu & Satapathy, 2023).

In antimicrobial therapy, imidazoles exhibit potent antibacterial and antifungal effects by disrupting cellular membranes and inhibiting key enzymes like 14α-demethylase in fungal ergosterol biosynthesis. Azole antifungals such as ketoconazole and fluconazole are widely used against Candida and Aspergillus species. Recent advancements include imidazole-based ionic liquids and silver complexes showing broad-spectrum activity with minimum inhibitory concentrations in the μg/mL range (Sahu & Satapathy, 2023).

In oncology, imidazoles target pathways like tubulin polymerization, kinase inhibition, and DNA intercalation. Compounds have shown efficacy against colorectal, breast, and prostate cancers. Dacarbazine, an imidazole-containing alkylating agent, is used in melanoma treatment, while newer hybrids demonstrate potent cytotoxicity via apoptosis induction (Sharma et al., 2021).

Beyond these, imidazoles possess anti-inflammatory and analgesic properties by inhibiting cyclooxygenase enzymes and cytokine release. Antiviral applications include inhibition of viral replication against HIV and hepatitis. In metabolic disorders, they modulate PPARγ receptors for antidiabetic effects. Their role in central nervous system disorders encompasses sedative and anticonvulsant activities, exemplified by midazolam.

1.3 Imidazole-Based Antihypertensive Agents

1.3.1 Imidazole Derivatives in Antihypertensive Therapy

Imidazole derivatives play a crucial role in antihypertensive therapy by targeting key cardiovascular systems, including the RAAS and sympathetic nervous system. Their mechanisms include angiotensin II receptor antagonism, vasorelaxation, and central sympathetic inhibition, which collectively reduce vascular resistance and blood pressure. Biphenyl-imidazole scaffolds selectively block AT1 receptors, preventing vasoconstriction

and aldosterone release, offering advantages over ACE inhibitors by avoiding bradykinin-related side effects like cough (Rasajna et al., 2025).

Imidazoline derivatives, a subclass, act centrally to inhibit sympathetic outflow from the medulla oblongata. Initially thought to activate α2-adrenoceptors, recent evidence supports involvement of I1-imidazoline receptors, leading to reduced norepinephrine release and peripheral vasodilation (Szabo, 2002). Benzo[d]imidazole analogues synthesized with electron-withdrawing groups like nitro or trifluoromethyl exhibit endothelium-dependent vasorelaxation in rat models, with potency correlated to substituent electronics (Navarrete-Vázquez et al., 2010).

Hybrid imidazoles combining with other heterocycles enhance multitarget profiles, addressing hypertension comorbidities like inflammation or oxidative stress. Patents for substituted imidazoles highlight their diuretic and antithrombotic properties, broadening therapeutic scope (Rasajna et al., 2025).

1.3.2 Examples of Clinically Approved Imidazole-Based Antihypertensive Drugs

Several imidazole-based drugs are clinically approved for hypertension, exemplifying the scaffold's therapeutic potential. **Losartan**, a biphenyl-tetrazole-imidazole derivative, is a prototypical angiotensin II receptor blocker that selectively antagonizes AT1 receptors, reducing blood pressure by inhibiting vasoconstriction. It is effective in mild-to-moderate hypertension and offers kidney-protective benefits in diabetic nephropathy (Rasajna et al., 2025). **Eprosartan**, another ARB with a similar imidazole core, demonstrates high affinity for AT1 receptors and is used for essential hypertension.

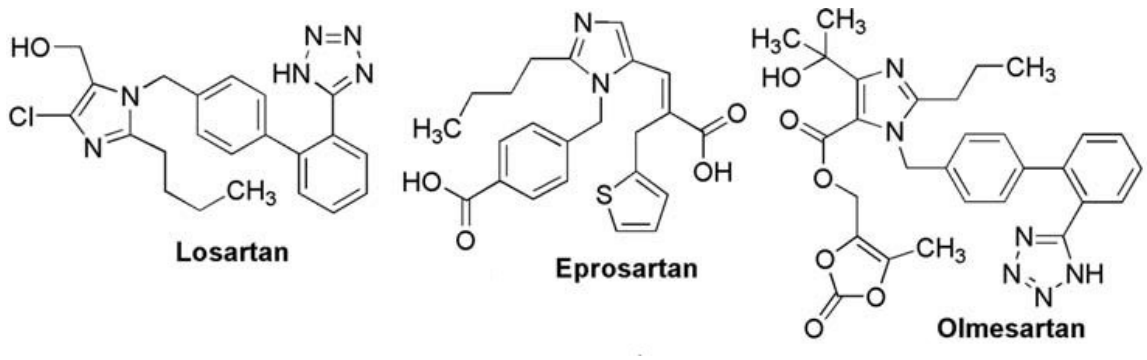

Figure 1.5 The structures of some clinically approved imidazole-based antihypertensive drugs

Imidazoline-based agents include **clonidine**, which lowers blood pressure via central α2-adrenoceptor activation and I1-imidazoline receptor stimulation, inhibiting sympathetic tone. It is employed in hypertensive emergencies but has limitations like sedation (Szabo, 2002). Second-generation imidazolines like **moxonidine** and **rilmenidine** offer improved selectivity for I1 receptors, minimizing side effects. Moxonidine reduces peripheral vascular resistance and benefits metabolic syndrome-associated hypertension, while rilmenidine provides similar effects with better tolerability.

Experimental derivatives, such as nitro-substituted benzo[d]imidazoles, exhibit potent vasorelaxation and dose-dependent antihypertensive activity in spontaneously hypertensive rat models, surpassing reference compounds (Navarrete-Vázquez et al., 2010).

1.3.3 Structure–Activity Relationship (SAR) of Antihypertensive Imidazoles

Understanding the SAR of imidazole derivatives is critical for designing optimized drug candidates. SAR studies systematically analyze how structural modifications influence pharmacological activity, selectivity, and therapeutic potential. For imidazole-based compounds, key structural features, such as substituent nature and position, electronic

properties, steric effects, and hydrogen-bonding capabilities, profoundly impact interactions with biological targets.

Structural Features Influencing Activity

The imidazole ring's amphoteric nature, high solubility, and ability to engage in hydrogen bonding, π-π stacking, and metal coordination make it versatile (Verma et al., 2013). Substitutions at the 1-, 2-, 4-, and 5-positions, as well as fusion with other heterocycles, allow fine-tuning of properties like lipophilicity, basicity, and steric bulk, directly influencing biological activity (Sharma et al., 2021).

For antihypertensive therapy, SAR is driven by:

1. **Substituent Effects**: Electron-withdrawing or electron-donating groups alter the ring's electronic environment, affecting receptor binding
2. **Steric Considerations**: Bulky substituents can enhance or hinder active site access
3. **Hydrogen Bonding**: N-1 and N-3 nitrogens facilitate hydrogen bond formation, critical for receptor interactions
4. **Lipophilicity**: Alkyl or aryl substituents modulate membrane permeability and bioavailability

General SAR Trends

Across therapeutic applications, SAR studies reveal consistent patterns:

- **Electron-Withdrawing Groups**: Nitro, chloro, or cyano groups at C-2 or C-5 enhance activity by increasing electron density and stabilizing receptor interactions (Sharma et al., 2021)

- **Aryl Substituents**: Phenyl or biphenyl groups at C-4 or C-5 improve hydrophobic interactions, critical for receptor binding (Timmermans et al., 1993)
- **Hydrogen-Bonding Groups**: Hydroxyl, amino, or carboxyl groups enhance solubility and receptor affinity
- **Steric Constraints**: Excessive bulk reduces activity due to steric hindrance

SAR studies guide rational drug design by identifying features that enhance potency, selectivity, and pharmacokinetic profiles. Computational tools like molecular docking and QSAR models further refine these efforts by predicting binding affinities and properties, accelerating therapeutic development (Sharma et al., 2021).

1.4 *In silico* Drug Discovery Approaches

In silico screening has become essential in modern drug discovery, allowing researchers to test thousands of compounds computationally before laboratory work begins. This approach saves time, reduces costs, and minimizes animal testing by predicting how molecules bind to targets or behave in the body. For antihypertensive imidazoles, *In silico* methods focus on RAAS and sympathetic pathways, using tools like molecular docking and virtual libraries to find promising leads (Uniyal et al., 2020).

Computer-aided drug design strategies have become vital, with several drugs being discovered and optimized using these technologies. They include Aliskiren, boceprevir, captopril, and saquinavir (Ece, 2023). Structure-based virtual screening is a key computational method that can identify potentially highly active compounds from large ligand databases by determining binding affinities between receptors and ligands (Zhang et al., 2022).

1.4.1 Fundamentals of Computer-Aided Drug Design (CADD)

Computer-aided drug design has emerged as an indispensable component of modern drug discovery, integrating computational techniques with biological knowledge to identify and optimize potential drug candidates. This interdisciplinary field combines computational methods with biological understanding, contributing to pharmaceutical industry versatility and effectiveness (Niazi & Mariam, 2024).

CADD is valuable because it expedites and lowers the cost of drug development. Traditional drug discovery is expensive and time-consuming, frequently taking 10-15 years for a drug to become commercially available (Vemula et al., 2023). Within CADD, adherence to Lipinski's rule is important for achieving optimal oral drug characteristics, where compounds ideally minimize violations of criteria such as molecular weight, lipophilicity, hydrogen bond donors, and acceptors (Niazi & Mariam, 2024).

Structure-Based and Ligand-Based Approaches

CADD encompasses two fundamental complementary approaches. Structure-based drug design leverages knowledge of the three-dimensional structure of biological targets to understand how potential drugs can fit and interact with them. Ligand-based drug design doesn't require target structure knowledge but instead focuses on known drug molecules and their pharmacological profiles to design new candidates (Niazi & Mariam, 2024).

Structure-based methods analyze macromolecular target 3D structural information, typically of proteins or RNA, to identify key sites and interactions important for biological functions. Ligand-based methods focus on known antibiotic ligands for a target to establish relationships between their physiochemical properties and activities, referred to as structure-activity relationships (Negron & MacKerell, 2017).

Structure-based design relies on detailed knowledge of target architecture, typically obtained through X-ray crystallography, nuclear magnetic resonance spectroscopy, or cryogenic electron microscopy. When experimental structures are unavailable, homology modeling can construct 3D models based on sequence similarity. With artificial intelligence advances, deep learning-driven methods like AlphaFold can now predict most protein 3D structures approaching experimental accuracy (Yu et al., 2023).

Ligand-based design becomes valuable when structural information about biological targets is unavailable or limited. This approach leverages knowledge from compounds with known biological activities to identify structural and physicochemical features correlating with desired properties. When no protein structure is available, if a 3D structure can't be constructed, then ligand-based tools such as quantitative structure-activity relationship analysis are employed (Liu et al., 2023).

Integration in Drug Discovery Workflows

The practical application of CADD involves integration with experimental techniques in iterative cycles that progressively refine drug candidates. The workflow starts with biological identification of a target to which ligand binding should lead to therapeutic activity, followed by CADD screening using computational methods. Information from CADD is used to design compounds subjected to chemical synthesis and biological assay, with information from those experiments used to further develop the structure-activity relationship (Negron & MacKerell, 2017).

This iterative workflow enables continuous learning throughout drug discovery. Initial computational screening identifies promising chemical scaffolds and prioritizes compounds for synthesis based on predicted binding affinity and drug-like properties. Experimental

testing provides validation of computational predictions and generates SAR data informing subsequent design cycles.

For imidazole-based antihypertensive drug discovery, CADD provides essential capabilities for designing compounds that selectively interact with key molecular targets involved in blood pressure regulation while possessing favorable pharmacokinetic properties. Computational prediction of receptor-ligand interactions enables rational modification of the imidazole scaffold to enhance affinity, selectivity, and drug-likeness.

1.4.2 Molecular Docking and Virtual Screening

Molecular docking is a cornerstone technique in structure-based drug design, enabling prediction of how small molecules bind to protein targets and estimation of binding affinities. Structure-based virtual screening is a key routine computational method that can identify potentially highly active compounds, speeding up novel drug design progress. Molecular docking-based virtual screening helps find active compounds from large ligand databases by identifying binding affinities between receptors and ligands (Zhang et al., 2022).

The docking process involves two critical steps: predicting the binding pose of the ligand within the target protein's active site and estimating binding affinity through scoring functions that evaluate protein-ligand interaction favorability. In semi-flexible docking, the ligand is allowed to be flexible while the receptor structure remains rigid, and docking methods are commonly used for virtual screening calculations as ligand molecules are relatively small (Zhang et al., 2022).

Modern docking algorithms employ various search strategies including systematic searches, random or stochastic methods, and genetic algorithms to efficiently explore conformational space of ligand-receptor complexes. Machine learning integration with molecular docking

has revolutionized virtual screening capabilities, enabling screening of ultra-large chemical libraries containing billions of compounds.

A strategy combining machine learning and molecular docking enables rapid virtual screening of databases containing billions of compounds, where a classification algorithm is trained to identify top-scoring compounds based on molecular docking of one million compounds to the target protein (Carlsson & Luttens, 2024). A highly accurate structure-based virtual screening method outperforms other state-of-the-art methods on various benchmarks, partially due to modeling receptor flexibility, and incorporation into an open-source AI-accelerated virtual screening platform enables screening of multi-billion compound libraries (Zhou et al., 2024).

For antihypertensive drug discovery targeting AT1 receptors and I1-imidazoline receptors, molecular docking provides insights into binding modes of imidazole derivatives and helps identify structural features enhancing receptor affinity and selectivity. Docking studies against these targets enable rational design of imidazole compounds with optimized binding properties and predicted pharmacological activity.

1.4.3 Pharmacophore Modeling and Ligand-Based Design

Pharmacophore modeling is a ligand-based drug design approach that identifies essential structural features required for biological activity and uses this information to guide new compound design. Pharmacophore modeling systematically analyzes essential features contributing to a molecule's pharmacological activity, enhancing understanding of ligand-receptor interactions and empowering researchers to rationally design compounds with enhanced efficacy and reduced side effects (Niazi & Mariam, 2024).

A pharmacophore is defined as the spatial arrangement of molecular features necessary for a molecule to bind to its biological target and elicit a pharmacological response, typically including hydrogen bond donors and acceptors, hydrophobic regions, aromatic rings, and charged groups. The modeling process involves selection of a training set of compounds with known biological activities, conformational analysis to identify bioactive conformations, alignment of active compounds based on structural features, and identification of common pharmacophoric features correlating with activity.

Features required in binding ligands with critical amino acid residues are constructed from the target biological macromolecule's binding site. A pharmacophore can be used as a 3D query to find drug candidates for further optimization from databases consisting of millions or billions of molecules through virtual screening, while only thousands of compounds are generally screened using molecular docking (Ece, 2023).

For imidazole-based antihypertensive agents, pharmacophore models can capture key structural elements responsible for I1-receptor binding and selectivity over α2-adrenergic receptors. These models incorporate features such as the imidazole ring system, aromatic substituents, and flexible linker groups determining receptor affinity and pharmacological profile. By using pharmacophore models as search queries, researchers can rapidly screen large chemical databases to identify novel imidazole scaffolds with predicted antihypertensive activity.

1.4.4 ADMET Prediction and Drug-Likeness Evaluation

Prediction of absorption, distribution, metabolism, excretion, and toxicity properties is a critical component of i*n silico* drug discovery, enabling early identification and elimination of compounds with unfavorable pharmacokinetic or safety profiles. For a new molecular entity to become a drug, it must not only have the right biological activity and be safe and efficient,

but also have a favorable pharmacokinetic profile including toxicity. ADMET prediction during early development stages increases the success rate of compounds reaching lead optimization (Dulsat et al., 2023).

Poor pharmacokinetic properties and toxicity issues account for significant proportions of drug failures in clinical development, making early ADMET assessment essential for efficient drug discovery. The concept of drug-likeness provides useful guidelines for designing molecules with favorable pharmacokinetic properties.

Lipinski and coworkers proposed the Rule of Five in 1997, the original and most well-known rule-based filter of drug-likeness distinguishing whether a molecule is orally absorbed well or not: molecular weight ≤500, octanol-water partition coefficient ≤5, hydrogen bond donors ≤5, and hydrogen bond acceptors ≤10. A molecule is predicted not to be orally active if it violates two or more of these four rules (Yang et al., 2019). Oral bioavailability of possible active compounds can be calculated through Lipinski's Rule of Five and Veber's rule, while Muegge's rule determines the possibility of a compound becoming a successful drug molecule by pharmacophore point calculation (Uniyal et al., 2020).

Modern ADMET prediction employs computational tools that estimate various pharmacokinetic parameters based on molecular structure. *In silico* tools such as SwissADME and admetSAR webserver can lead to early predictions of physicochemical properties, ADMET, and drug-likeness properties (Dulsat et al., 2023). These tools predict parameters including aqueous solubility, intestinal absorption, blood-brain barrier permeability, cytochrome P450 enzyme interactions, plasma protein binding, renal clearance, and various toxicity endpoints such as hepatotoxicity, cardiotoxicity, and mutagenicity.

For imidazole derivatives intended as antihypertensive agents, ADMET prediction helps identify compounds with optimal oral bioavailability, appropriate brain penetration for

centrally acting agents, minimal drug-drug interaction potential, and acceptable safety profiles.

1.5 Synthesis and Characterization of Imidazole Derivatives

The synthesis of imidazole derivatives is fundamental to medicinal chemistry and pharmaceutical research, with these versatile heterocyclic compounds serving as essential building blocks in therapeutic agent development. Imidazole was first synthesized by Heinrich Debus in 1858 through reaction of glyoxal and formaldehyde in ammonia, initially called glyoxaline (de Siqueira et al., 2023).

The significance of developing efficient synthetic methodologies stems from their widespread pharmaceutical applications and biological activities. Recent advancements focus on diverse multicomponent reactions conducted under different conditions, highlighting the role of catalysts and conditions that optimize synthetic efficiency (Devi et al., 2024). The evolution of imidazole synthesis has progressed from classical methodologies to contemporary approaches incorporating green chemistry principles, atom economy, and sustainable practices. Modern techniques include microwave-assisted reactions, nanoparticle-catalyzed reactions, light-mediated synthesis, electrochemical reactions, and transition metal-free synthesis (Saha & Mukhopadhyay, 2024).

For developing imidazole-based antihypertensive agents, synthetic methodology selection must consider regioselectivity of substitution, compatibility with target functional groups, scalability for biological testing, and environmental impact.

1.5.1 The Debus-Radziszewski Synthesis

The Debus-Radziszewski reaction is the most historically significant and extensively utilized method for imidazole synthesis. Originally discovered by Heinrich Debus in 1858 and refined

by Bronisław Radziszewski in 1882, this multicomponent reaction enables efficient assembly of molecular complexity from simple readily available precursors.

The reaction proceeds through condensation of a 1,2-dicarbonyl compound (typically glyoxal) with an aldehyde and ammonia or primary amine. The mechanism involves initial double condensation forming a diimine intermediate, followed by cyclization with the aldehyde component, tautomerization, and dehydration to afford the aromatic imidazole ring. When ammonia is employed, the reaction yields 2,4,5-trisubstituted imidazoles, whereas substituting ammonia with a primary amine affords 1,2,4,5-tetrasubstituted derivatives.

The dicarbonyl component can be glyoxal, benzil, or other 1,2-diketones, while the aldehyde component tolerates electron-donating and electron-withdrawing substituents on aromatic rings, as well as aliphatic and heteroaromatic aldehydes. This methods makes the reaction ideal for producing a variety of compounds for biological evaluation.

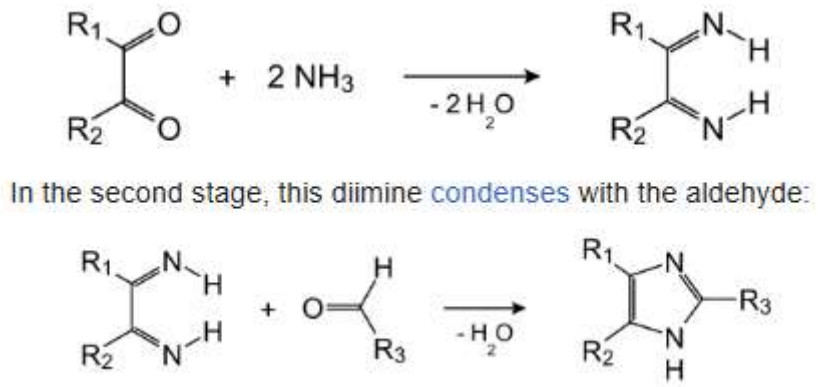

Figure 1.6 The Debus-Radziszewski reaction scheme for the synthesis of Imidazoles

Traditional conditions required elevated temperatures often exceeding 100°C, extended reaction times, strong acid catalysts, and complex purifications. Contemporary modifications have addressed these shortcomings through diverse catalytic systems. Modern adaptations employ magnetic nanoparticles, metal-organic frameworks, heterogeneous solid acid

catalysts, ionic liquids, and transition metal complexes. These catalytic systems offer enhanced reaction rates, improved yields often exceeding 90%, milder conditions with temperatures reduced to 60-100°C, shorter reaction times measured in minutes, easy catalyst recovery, and reduced waste.

1.5.2 Characterization Techniques

Following successful synthesis, comprehensive structural characterization using spectroscopic and analytical techniques provides essential confirmation of molecular identity and purity.

Thin-Layer Chromatography and Column Chromatography

Prior to comprehensive spectroscopic characterization, crude products require preliminary assessment to evaluate reaction progress and guide purification strategies. Thin-layer chromatography serves as an essential preliminary technique for monitoring reaction completion and assessing product purity through rapid qualitative analysis requiring minimal sample quantities.

The technique involves differential migration of compounds on silica gel stationary phase coated on glass or aluminum supports, with separation driven by polarity differences and interactions with the mobile phase solvent system. For imidazole derivatives, mobile phase compositions typically consist of different solvent systems like hexane-dichloromethane or ethyl acetate mixtures adjusted to achieve optimal separation. Visualization employs multiple detection methods including ultraviolet illumination at 254 nm for compounds containing chromophores, or chemical staining with iodine vapor and phosphomolybdic acid for universal detection.

Column chromatography represents the primary preparative purification technique for isolating imidazole derivatives in quantities sufficient for biological evaluation and characterization. The technique involves packing a glass column with silica gel, loading the crude product mixture, and eluting with gradually increasing polarity solvent systems that selectively separate compounds based on retention characteristics.

Fourier-Transform Infrared Spectroscopy (FT-IR)

Fourier-transform infrared spectroscopy provides rapid, non-destructive analysis of functional groups present in imidazole derivatives through measurement of molecular vibrations induced by absorption of infrared radiation at characteristic frequencies. Molecules absorb infrared radiation at frequencies matching natural vibrational frequencies of bonds within the structure, with absorbed energy causing bond stretching, bending, and deformation.

Sample preparation commonly employs the potassium bromide pellet technique or attenuated total reflectance methods. The potassium bromide pellet method involves mixing 1-2 milligrams of sample with approximately 100 milligrams of dried potassium bromide powder, followed by compression under high pressure to form a transparent disk. Attenuated total reflectance techniques have gained popularity due to simplicity and ability to analyze samples directly in solid or liquid form without extensive preparation.

The infrared spectrum of imidazole derivatives exhibits several characteristic absorption bands that confirm presence of the heterocyclic core. The nitrogen-hydrogen stretching vibration of unsubstituted imidazole nitrogen appears as a broad band in the region of 3100-3300 cm^{-1}, with breadth resulting from hydrogen bonding interactions. For N-substituted imidazoles, this N-H stretching absorption is absent, providing immediate information about

substitution pattern. Aromatic carbon-hydrogen stretching vibrations produce bands around 3050-3150 cm^{-1}, distinguished from aliphatic C-H stretches appearing below 3000 cm^{-1}.

The fingerprint region between 1600-1400 cm^{-1} contains absorption bands from carbon-nitrogen stretching and aromatic ring deformations diagnostic for imidazole systems. Absorption bands around 1600-1550 cm^{-1} correspond to aromatic ring stretching vibrations, while the region around 1400-1500 cm^{-1} shows bands from ring deformations characteristic of the imidazole structure.

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR)

Proton nuclear magnetic resonance spectroscopy is the most informative technique for elucidating detailed structure of imidazole derivatives, providing comprehensive information about hydrogen atoms within the molecular framework. The technique involves alignment of nuclear magnetic moments in a strong magnetic field, with absorption of radiofrequency energy at specific resonance frequencies depending on the chemical environment experienced by each nucleus. Chemical shifts, expressed in parts per million relative to a reference standard, quantify resonance frequency differences. Modern high-field spectrometers operating at 400, 500, or 600 MHz deliver exceptional spectral resolution and sensitivity.

The proton NMR spectrum of imidazole derivatives exhibits characteristic chemical shift ranges enabling structural identification. The nitrogen-bound proton in unsubstituted imidazoles appears as a broad singlet in the region of 10-13 ppm due to its highly deshielded position between two electronegative nitrogen atoms. This exchangeable proton can be confirmed through deuterium oxide exchange experiments, where addition of D_2O causes disappearance of the N-H signal. Aromatic protons attached to imidazole ring carbons produce signals between 7-8.5 ppm, with exact positions influenced by electronic effects of substituents.

Carbon-13 Nuclear Magnetic Resonance Spectroscopy (^{13}C-NMR)

Carbon-13 nuclear magnetic resonance spectroscopy provides complementary structural information by detecting signals from carbon atoms throughout the molecular framework, enabling direct observation of both protonated and quaternary carbon centers. The technique exploits the magnetic properties of the carbon-13 isotope, which represents approximately 1.1% of naturally occurring carbon. Modern carbon-13 experiments typically employ proton decoupling techniques that simplify spectra by collapsing multiplets into single lines for each chemically distinct carbon atom.

The carbon-13 spectrum of imidazole derivatives exhibits characteristic signals for the heterocyclic ring carbons and substituent groups. The carbon at position 2 (between the two nitrogen atoms) typically appears furthest downfield in the region of 135-145 ppm due to its location between electronegative nitrogen atoms. The carbons at positions 4 and 5 produce signals in the region of 115-130 ppm, with relative positions influenced by substituents. Aromatic substituents contribute signals in the aromatic region between 120-160 ppm, while aliphatic carbons produce signals in the upfield region of 10-60 ppm.

Mass Spectrometry (MS)

Mass spectrometry provides definitive confirmation of molecular weight and molecular formula through measurement of mass-to-charge ratios for intact molecular ions and their fragments. Modern mass spectrometers employ electrospray ionization and atmospheric pressure chemical ionization that enable analysis of polar pharmaceutical compounds without extensive derivatization.

The mass spectrum of imidazole derivatives typically displays a molecular ion peak corresponding to the intact molecule, with the mass-to-charge value providing direct

confirmation of molecular weight. For electrospray ionization, the spectrum often shows protonated molecular ions $[M+H]^+$ or sodium adducts $[M+Na]^+$ with mass-to-charge values one or twenty-three mass units higher than the nominal molecular weight.

Fragment ion peaks appearing at lower mass-to-charge values provide structural information about molecular connectivity and fragmentation pathways. Common fragmentation patterns for imidazole derivatives include loss of alkyl substituents, cleavage of benzylic bonds, and elimination of small neutral molecules such as water or carbon dioxide. High-resolution mass spectrometry enables determination of accurate masses with errors less than five parts per million, sufficient to calculate unambiguous elemental compositions.

1.6 Justification for the Study

Hypertension remains one of the leading causes of cardiovascular diseases and premature deaths globally. Despite the wide range of available antihypertensive drugs, effective long-term management remains challenging due to drug resistance, side effects, and variability in patient response. Many currently available drugs act on similar molecular targets such as the RAAS, which limits therapeutic diversity and contributes to treatment failure in some patients. This situation calls for the discovery of novel agents with improved selectivity, fewer adverse effects, and possibly new mechanisms of action.

Imidazole compounds have proven to be among the most promising scaffolds in medicinal chemistry because of their chemical versatility and strong biological relevance. Several clinically approved antihypertensive agents, such as losartan and moxonidine, contain the imidazole moiety, indicating its significance in modulating cardiovascular targets. However, the potential of imidazole-based structures remains underexplored, especially in the design of newer derivatives that can overcome limitations associated with existing therapies.

This research seeks to contribute to the development of safer and more effective antihypertensive agents by designing and developing novel imidazole derivatives through an integrated approach that combines *in silico* screening, chemical synthesis, and characterization. The application of computational methods enables efficient identification of promising lead compounds prior to synthesis, while subsequent experimental validation provides a solid foundation for future biological evaluation. By systematically exploring the imidazole derivatives through rational drug design principles, this study aims to identify novel compounds with enhanced antihypertensive potential and improved pharmacological profiles.

1.7 Aim of the Study

The aim of this study is to design, synthesize, and characterize novel imidazole derivatives with potential antihypertensive activity using a combination of *in silico* screening, chemical synthesis, and analytical characterization techniques.

1.8 Specific Objectives

The specific objectives of this study are to:

1. Perform *in silico* screening of imidazole-based compounds to identify potential antihypertensive candidates with strong affinity toward hypertension-related biological targets and favorable ADMET properties.
2. Synthesize the most promising imidazole derivative using efficient and reproducible chemical methods.
3. Characterize the synthesized compounds using spectroscopic and chromatographic techniques such as FT-IR, NMR, and mass spectrometry to confirm their structures and purity.

CHAPTER TWO

MATERIALS AND METHOD

2.1 MATERIALS

This chapter describes the computational tools, databases, and laboratory reagents used in this study. The research was conducted in two phases: computational screening (*In silico* analysis) and chemical synthesis (wet laboratory work).

2.1.1 Computational Tools

1. Protein Data Bank (PDB)

The Protein Data Bank (PDB) website (https://www.rcsb.org/) is an essential resource for structural biology research, providing access to a vast repository of three-dimensional structural data for biological macromolecules. Its user-friendly interface and advanced search functionalities enable scientists to explore and retrieve molecular structures based on various criteria such as PDB ID, keywords, and experimental methods. The website also offers interactive visualization tools for in-depth analysis and facilitates data deposition, fostering collaboration and knowledge dissemination within the scientific community. In this study, PDB was used to obtain structures of target proteins implicated in hypertension.

2. PubChem Database

PubChem is a comprehensive chemical database maintained by the National Center for Biotechnology Information (NCBI), a division of the National Institutes of Health (NIH). It serves as a repository of chemical information, including chemical structures, biological activities, molecular weight, and formula. The database features tools for chemical similarity

searching, structure-activity relationship analysis, and provides information on compound properties essential for drug discovery research.

3. PyRx Software

PyRx is a powerful open-source software tool designed for virtual screening and molecular docking studies in computational drug discovery. Its intuitive graphical interface facilitates docking experiments without requiring extensive programming skills. It supports popular docking algorithms like AutoDock Vina and AutoDock 4, enabling accurate predictions of ligand-protein interactions. The software streamlines ligand and protein preparation steps, conducts virtual screening, and provides robust analysis tools for interpreting docking results.

4. Discovery Studio 2025 Client

Discovery Studio 2025 Client is a comprehensive software suite for molecular modeling and simulation, focusing on drug discovery and structural biology. It offers tools for molecular visualization, protein-ligand interaction analysis, and pharmacophore modeling. The software enables researchers to visualize complex molecular structures and derive valuable insights for drug design and optimization.

5. Marvin Sketch

Marvin Sketch, developed by ChemAxon, is a molecular drawing and visualization software that enables users to sketch chemical structures, manipulate molecular properties, and perform structural analysis. It provides customizable visualization options and supports various chemical file formats, making it invaluable for designing and modifying chemical structures.

6. PyMOL

PyMOL is a molecular visualization software that aids in visualizing, manipulating, and analyzing molecular structures. It offers real-time interaction, precise measurement and evaluation of molecular interactions, customizable visualization options, and the production of high-quality graphical outputs. In this study, PyMOL was used to identify active amino acid residues for target-directed docking.

7. SwissADME

SwissADME is a web-based tool designed specifically for drug discovery endeavors (http://www.swissadme.ch/). It provides a suite of instruments for evaluating the pharmacokinetic and physicochemical properties of small molecules, essential for selecting promising drug candidates. Through predictive models, SwissADME offers insights into absorption, distribution, metabolism, and excretion (ADME) properties, enabling researchers to assess compound viability early in drug development. The platform calculates key physicochemical parameters including lipophilicity, water solubility, and molecular weight, and drug-likeness assessment.

8. ProTox-3.0

ProTox-3.0 is an advanced computational platform designed for toxicological analysis (https://tox.charite.de/protox3/). It offers a comprehensive suite of predictive models for assessing the potential toxicity of chemical compounds, leveraging machine learning techniques and extensive toxicological databases to forecast various toxicity endpoints including acute toxicity, mutagenicity, and organ-specific toxicities. This tool was used in early-stage safety screening of these compounds.

2.1.2 Instruments and Equipments

The following instruments were used for the chemical synthesis:

1. Magnetic stirrer and heating apparatus
2. Three-necked round-bottom flask
3. Weighing balance
4. Reflux condenser
5. Melting Point Apparatus
6. Beakers
7. Test Tubes
8. TLC plates
9. Ultra-violet lamp
10. Thermometer
11. Forceps

2.1.3 Chemicals and Reagents

1. 2-oxo-2-phenylacetaldehyde
2. Ammonium acetate
3. Glacial acetic acid
4. Heptanal
5. Dimethyl sulfoxide (DMSO)
6. Silica Gel
7. Dichloromethane

8. Ethyl Acetate
9. Acetone
10. Ethanol
11. N – Hexane

2.2 METHODS

2.2.1 *In silico* Screening and Molecular Docking

1. Protein Target Retrieval and Preparation

The three-dimensional structures of the target proteins were retrieved from the RCSB Protein Data Bank (PDB) using their respective identification codes and downloaded in 3d formats. The proteins include 6L88, 7BVQ, 5XPR, and 1O86 respectively. The binding pocket of each protein was then identified using PyMOL by visualizing the co-crystallized ligand within the downloaded protein structure. The amino acid residues surrounding this region were recorded as the active site residues, and their coordinates were later used to define the docking grid box in PyRx.

The proteins were prepared using Discovery Studio Visualizer to ensure they were suitable for molecular docking. All non-essential components such as water molecules, cofactors, and unrelated heteroatoms were removed, retaining only the functional receptor chain required for ligand binding. Hydrogen atoms were added to stabilize the geometry, and the cleaned protein structure was saved in PDB format.

2. Ligand Preparation

The structures of the imidazole-based compounds used for the docking studies were drawn using Marvin Sketch and were imported as SDF files into PyRx. The compounds were

minimized using the built-in energy minimization tool in PyRx to obtain stable conformations. The minimized structures were then converted into the AutoDock-compatible PDBQT format for docking. This process ensured that each ligand was geometrically optimized and properly formatted for compatibility with the docking software.

All ligands were verified to ensure they contained no missing atoms or improper valences before proceeding to the docking stage. Each compound was assigned a unique identifier corresponding to its structure for easy recognition during result analysis.

3. Molecular Docking

Molecular docking studies were performed using AutoDock Vina within the PyRx platform to evaluate the binding affinity of the ligands toward the selected protein targets. The prepared receptor and ligands were imported into the workspace, and the docking grid was defined to cover the active site region corresponding to the co-crystallized ligand in the retrieved protein. The grid dimensions were adjusted to ensure the inclusion of all the key amino acid residues around the binding pocket.

Docking parameters were set to default conditions, with the exhaustiveness value set at 8. Each ligand was docked individually against the receptor, and AutoDock Vina generated several possible binding poses ranked by their predicted binding affinities (expressed in kcal/mol). The conformation with the lowest binding energy was considered the most stable and was selected for further evaluation.

The best docking poses were visualized and analyzed using Discovery Studio Visualizer to study the nature of interactions between the ligand and receptor. Non-covalent interactions such as hydrogen bonding, hydrophobic contacts, and π–π stacking were identified to gain insight into the potential binding mechanism of the imidazole derivatives.

4. ADMET and Drug-Likeness Analysis

To evaluate the pharmacokinetic suitability of the most promising docked compounds, **ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity)** analyses were carried out using SwissADME and ProTox online platforms. The canonical SMILES of each ligand were obtained and entered into both websites for prediction.

Key parameters analyzed included gastrointestinal absorption, water solubility, bioavailability, blood–brain barrier permeability, and potential interaction with cytochrome P450 enzymes. Toxicological parameters such as hepatotoxicity, mutagenicity, nephrotoxicity and other relevant parameters were also assessed. Drug-likeness was determined based on Lipinski's Rule of Five, which assesses molecular weight, partition coefficient (LogP), hydrogen bond donors, and acceptors.

All ADMET results were saved alongside docking scores in a spreadsheet for transparent selection of lead compounds.

2.2.2 SYNTHESIS OF 2-HEXYL-4-PHENYL-1H-IMIDAZOLE

A mixture of 2-oxo-2-phenylacetaldehyde (3.35g, 25mmol) and ammonium acetate (10 g) was dissolved in glacial acetic acid and heated under reflux at 80–100 °C for 1 hour with continuous magnetic stirring to ensure uniform mixing.

A solution of heptanal (3.5ml) in 12.5 mL of glacial acetic acid was prepared and added dropwise to the reaction mixture over a period of 15–20 minutes while maintaining the same temperature and constant stirring. The mixture was then allowed to continue refluxing for an additional 5 hours, and the progress of the reaction was monitored using thin-layer chromatography (TLC).

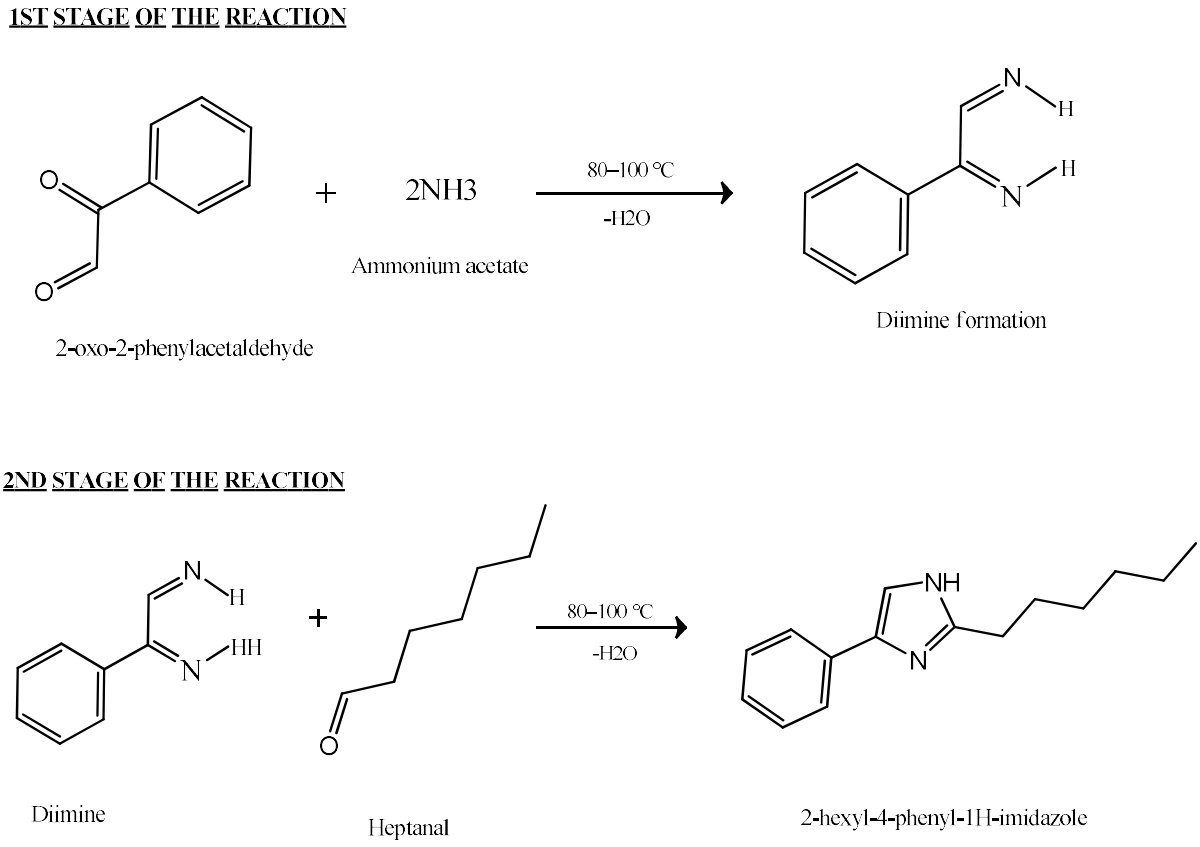

Figure 2.1 showing reaction scheme for the synthesis of 2-hexyl-4-phenyl-1H imidazole

Upon completion, the reaction mixture was allowed to stand overnight at room temperature. The reaction mixture was filtered off to remove any precipitate present and 300 mL of cold deionized water was added to the filtrate to induce further precipitation of the product. The solid formed was collected by filtration, washed thoroughly with water to remove residual acetic acid and ammonium salts, and then air-dried to obtain the crude imidazole derivative.

2.2.3 Purification

Prior to purification, the crude product was analyzed using thin-layer chromatography (TLC) to determine a suitable solvent system for column chromatography. Small portions of the sample were spotted on silica gel TLC plates and developed using different solvent mixtures, including ethyl acetate–hexane and hexane–dichloromethane systems in different ratios.

Among the mixtures tested the 2:1 (hexane:dichloromethane) system showed good separation with distinct and well-resolved spots.

Based on this observation, the crude sample was subjected to column chromatography using silica gel as the stationary phase and the 2:1 hexane–dichloromethane mixture as the mobile phase. Fractions collected from the column were analyzed by thin-layer chromatography (TLC) to confirm the presence and purity of the desired compound. Fractions showing identical TLC profiles were combined, and the solvent was allowed to evaporate at room temperature, to yield the purified imidazole derivative as a solid product.

CHAPTER 3

RESULTS

3.1 Molecular Docking Results

Molecular docking studies were conducted to evaluate the binding interactions and affinities of selected imidazole derivatives with key protein targets associated with hypertension. The results gotten asre as follows.

3.1.1 Binding Affinities

The results of the binding sites for protein with PDB ID 6L88, 7BVQ, 5XPR, 1O86 identified within 5A with PyMOL as shown in table 3.1, 3.2, 3.3 and 3.4

Table 3.1: Amino acid binding sites of protein 6L88 identified with PyMOL

S/N	**Amino acid binding sites**
1	LEU 766, 769, 772, 809, 814
2	ASN 770
3	ALA 773, 813
4	GLN 776
5	VAL 780
6	TRP 806
7	SER 810
8	MET 807, 845, 852

9	CYS 942
10	LYS 873
11	ARG 817
12	TYR 869

Table 3.2: Amino acid binding sites of protein 7BVQ identified with PyMOL

S/N	**Amino acid binding sites**
1	PHE 1218, 1340, 1341
2	SER 1228, 1229, 1232
3	ASN 1344, 1363
4	THR 1220, 1135, 1143
5	VAL 1139, 1142
6	ALA 1225
7	TYR 1224, 1367
8	ASP 1138
9	TRP 1134

Table 3.3: Amino acid binding sites of protein 5XPR identified with PyMOL

S/N	Amino acid binding sites
1	ARG 343
2	HIS 150, 340
3	LEU 277, 339
4	TRP 167, 336
5	ILE 372
6	ALA 375
7	SER 376
8	LYS 161, 183, 273
9	TYR 281
10	VAL 177, 185
11	GLU 236
12	PHE 240
13	GLN 181
14	PRO 178
15	CYS 174, 255
16	ASN 158

17	ASP 154

Table 3.4: Amino acid binding sites of protein 1O86 identified with PyMOL

S/N	**Amino acid binding site**
1	HIS 353, 383, 387, 513
2	ALA 356, 354
3	GLU 162, 384, 411
4	SER 355
5	VAL 380, 518
6	ASP 377
7	TYR 520, 523
8	PHE 457, 512, 527
9	LYS 511
10	GLN 281

KEY:

PHE: Phenylalanine **LEU**: Leucine **GLU**: Glutamic acid **ALA**: Alanine

MET: Methionine **ILU**: Isoleucine **TYR**: Tyrosine **ARG**: Arginine

TRP: Tryptophan **CYS**: Cysteine **GLN:** Glutamine. **VAL**: Valine

ASN: Asparagine **ASP:** Asparagine **GLY:** GLycine **HIS:** Histidine

LYS: Lysine **PRO:** Proline **SER:** Serine **THR:** Threonine

The results of the binding affinities of the selected imidazole compounds and the control ligand with the proteins 6L88, 7BVQ, 5XPR and 1O86 are shown in the table 3.5, 3.6, 3.7 and 3.8 respectively.

The chemical structures of the selected imidazole ligands with used in this study are shown in figures 3.1, 3.2, 3.3, 3.4.

Table 3.5: Binding affinities for the selected imidazole compounds for 6L88 protein

S/N	Compound	CID	ΔG Energy (Kcal/mol)	RSMD
1	2-hexyl-4-phenyl-1H-imidazole	69620	-7.3	0
2	4-Phenylimidazole	69590	-6.3	0
3	1H-benzimidazole	5798	-5.3	0
4	2,4-Dimethylimidazole	70259	-4.3	
5	Esaxerenone	25052023	-8.2	0

Table 3.6: Binding affinities for the selected imidazole compounds for 7BVQ protein

S/N	Compound	CID	ΔG Energy (Kcal/mol)	RSMD
1	2-hexyl-4-phenyl-1H-imidazole	69620	-7.9	0
2	4-Phenylimidazole	69590	-6.7	0

3	1H-benzimidazole	5798	-5.9	0
4	2,4-Dimethylimidazole	70259	-4.4	
5	Carazolol	71739	-9.7	0

Table 3.7: Binding affinities for the selected imidazole compounds for 5XPR protein

S/N	Compound	CID	ΔG Energy (Kcal/mol)	RSMD
1	2-hexyl-4-phenyl-1H-imidazole	69620	-6.3	0
2	4-Phenylimidazole	69590	-5.4	0
3	1H-benzimidazole	5798	-5.0	0
4	2,4-Dimethylimidazole	70259	-4.1	0
5	Bosentan	104865	-8.6	0

Table 3.8: Binding affinities for the selected imidazole compounds for 1O86 protein

S/N	Compound	CID	ΔG Energy (Kcal/mol)	RSMD
1	2-hexyl-4-phenyl-1H-imidazole	69620	-7.0	0
2	4-Phenylimidazole	69590	-6.2	0
3	1H-benzimidazole	5798	-5.9	0
4	2,4-Dimethylimidazole	70259	-4.5	0

5	Lisinopril	5362119	-7.4	0

3.1.2 Chemical structures of the selected imidazole compounds

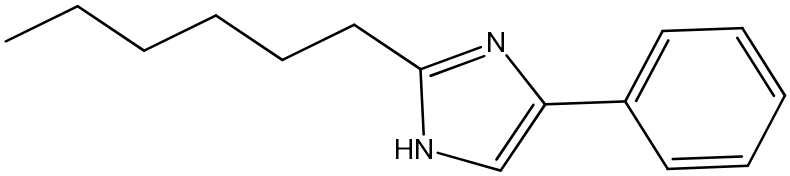

Figure 3.1 Chemical structure of 2-hexyl-4-phenyl-1H-imidazole

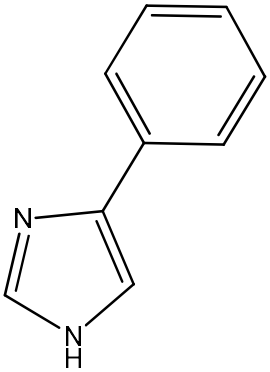

Figure 3.2 Chemical structure of 4-Phenylimidazole

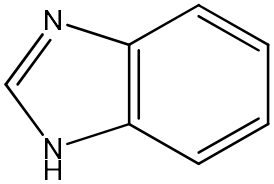

Figure 3.3 Chemical structure of 1H-1,3-benzodiazole

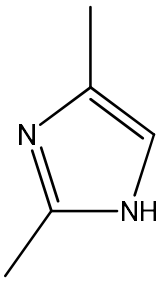

Figure 3.4 Chemical structure of 2,4-Dimethylimidazole

3.1.3 Binding interactions between proteins

The images of 2D and 3D H-bond interactions between protein 6L88 and the selected imidazole ligands figures 3.5, 3.6 and 3.7 and 3.8 respectively.

The images of 2D and 3D H-bond interactions between protein 7BVQ and the selected imidazole ligands figures 3.9, 3.10 and 3.11 and 3.12 respectively.

The images of 2D and 3D H-bond interactions between protein 5XPR and the selected imidazole ligands figures 3.13, 3.14 and 3.15 and 3.16 respectively.

The images of 2D and 3D H-bond interactions between protein 1O86 and the selected imidazole ligands figures 3.17, 3.18 and 3.19 and 3.20 respectively.

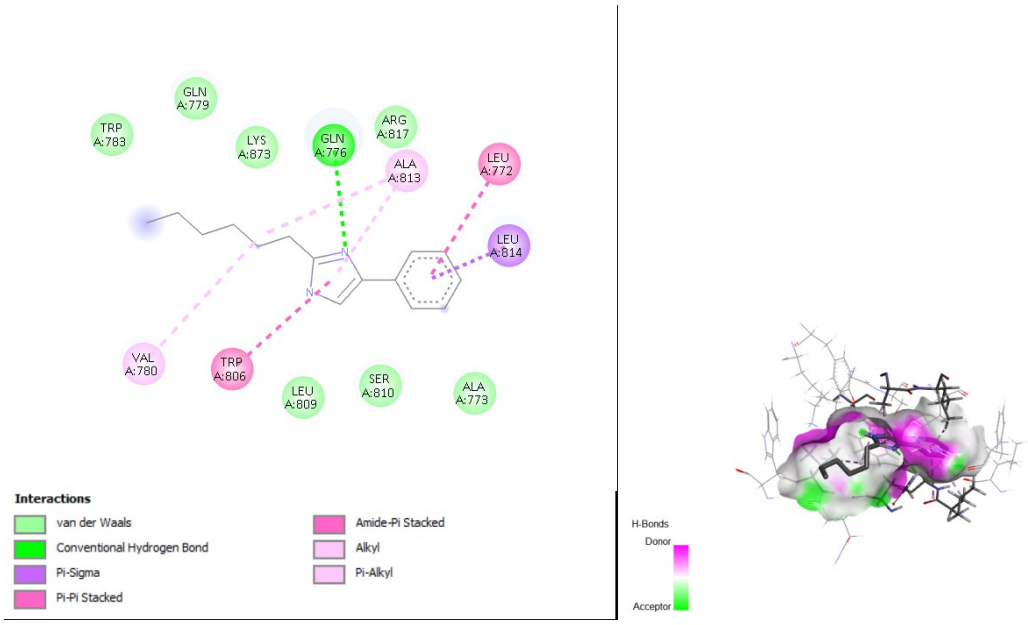

Figure 3.5: showing the 2D (A) and 3D H-bond (B) interactions between protein 6L88 and ligand 69620 with binding affinity of -7.5

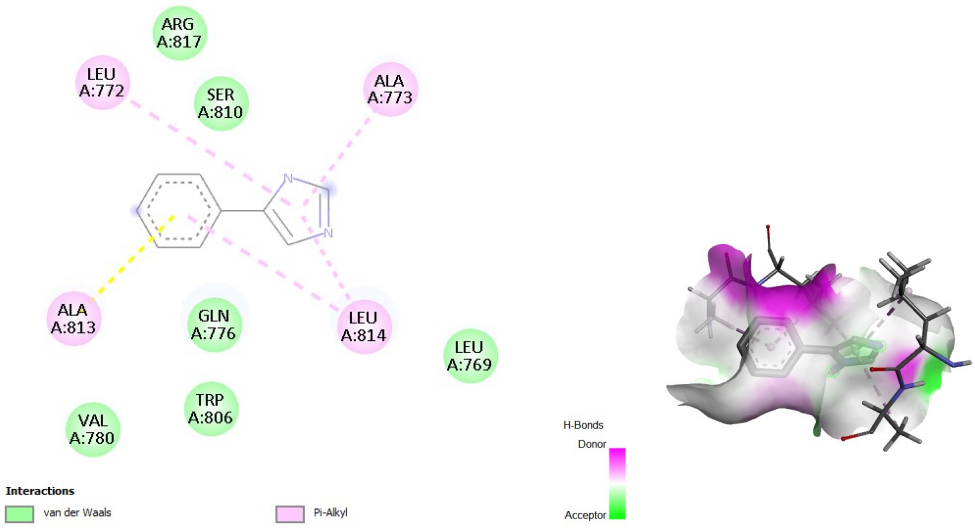

Figure 3.6: showing the 2D (A) and 3D H-bond (B) interactions between protein 6L88 and ligand 69590 with binding affinity of -6.3

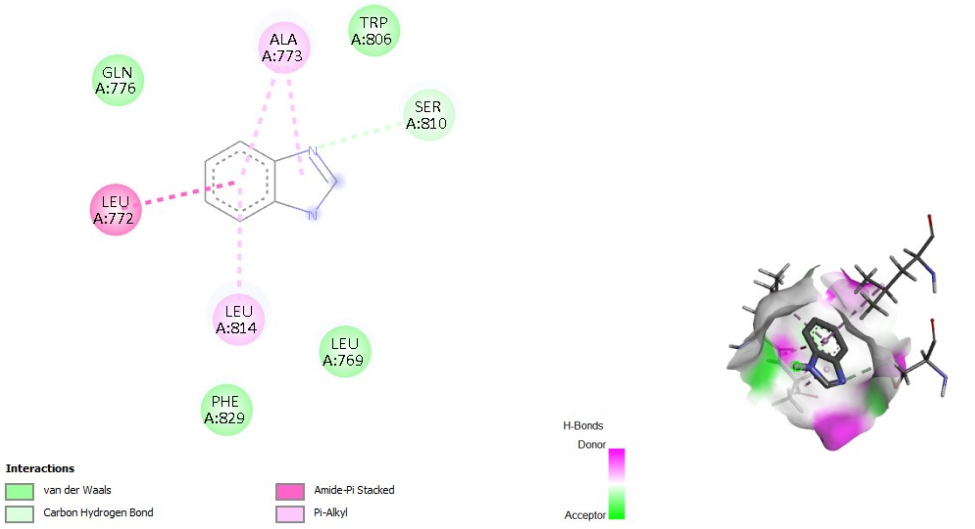

Figure 3.7: showing the 2D (A) and 3D H-bond (B) interactions between protein 6L88 and ligand 5798 with binding affinity of -5.3

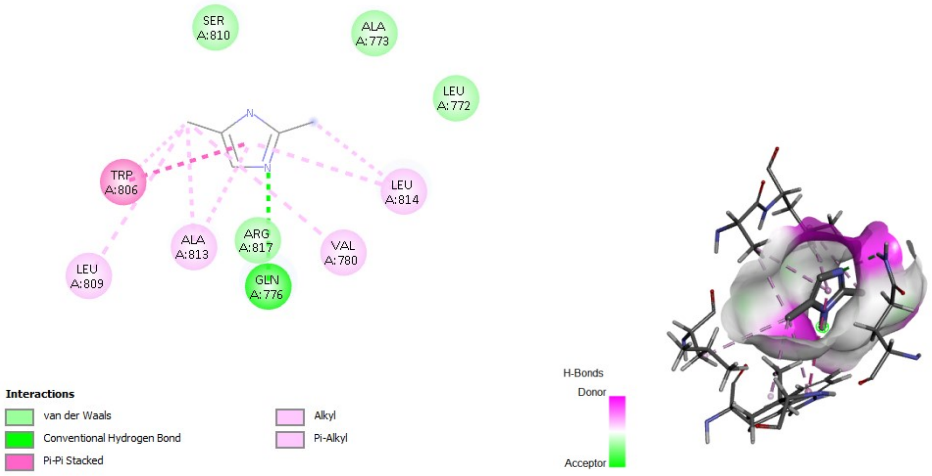

Figure 3.8: showing the 2D (A) and 3D H-bond (B) interactions between protein 6L88 and ligand 70259 with binding affinity of -4.3

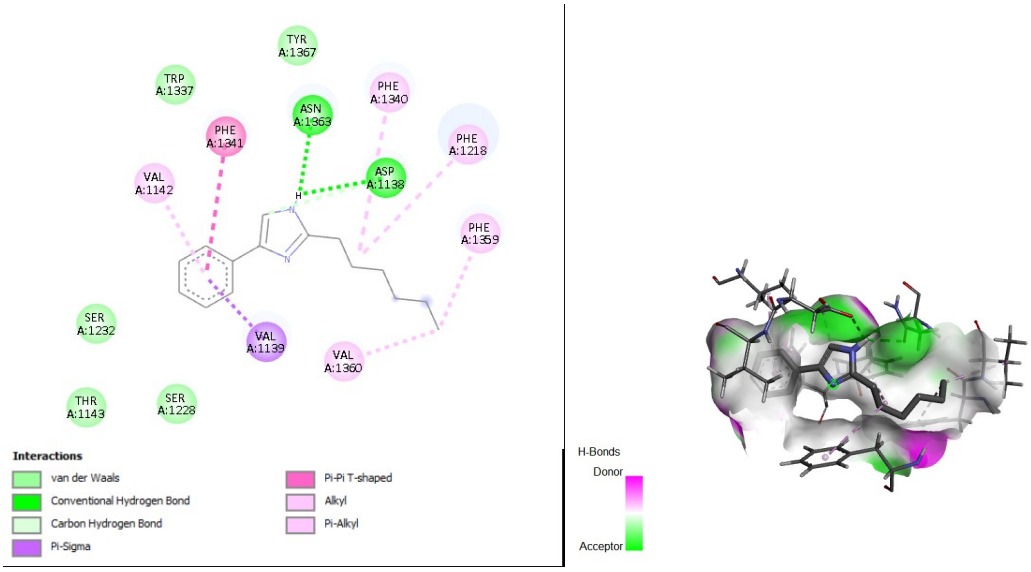

Figure 3.9: showing the 2D (A) and 3D H-bond (B) interactions between protein 7BVQ and ligand 69620 with binding affinity of -7.9

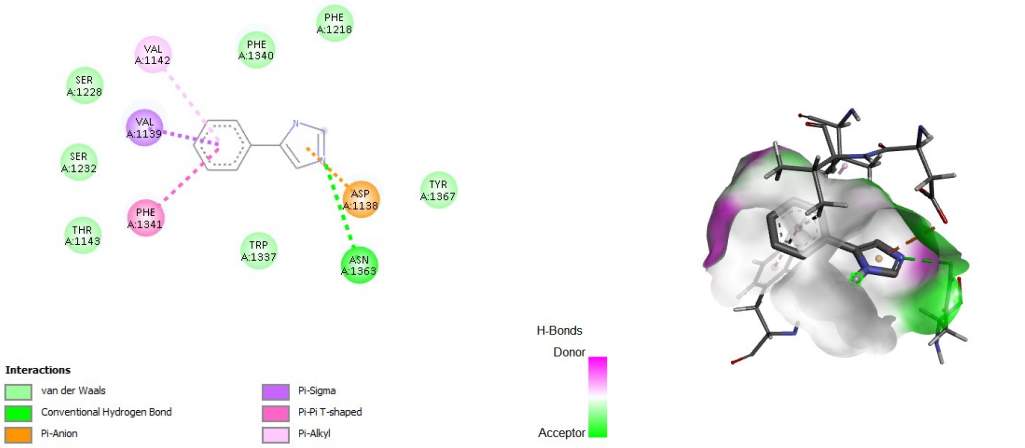

Figure 3.10: showing the 2D (A) and 3D H-bond (B) interactions between protein 7BVQ and ligand 69590 with binding affinity of -6.7

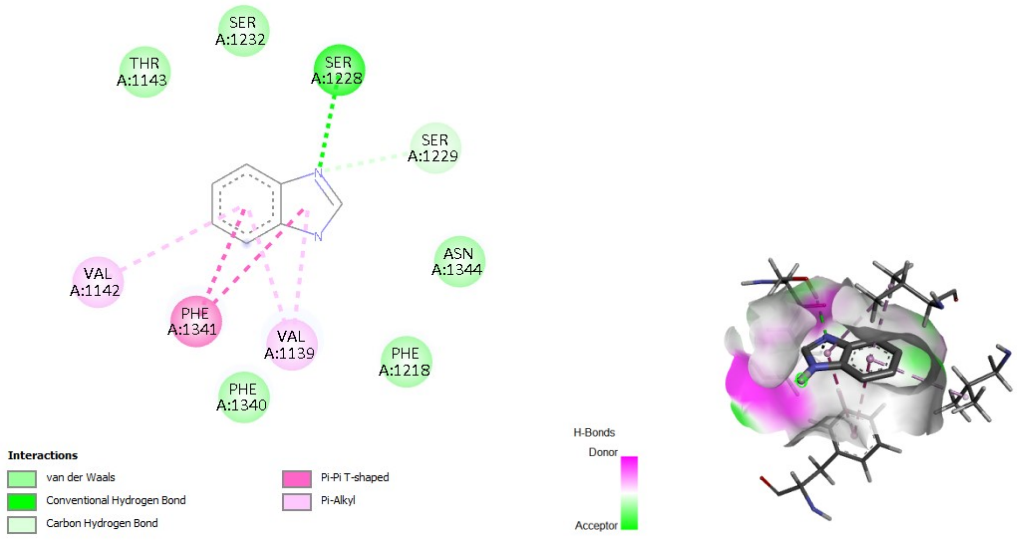

Figure 3.11: showing the 2D (A) and 3D H-bond (B) interactions between protein 7BVQ and ligand 5798 with binding affinity of -5.9

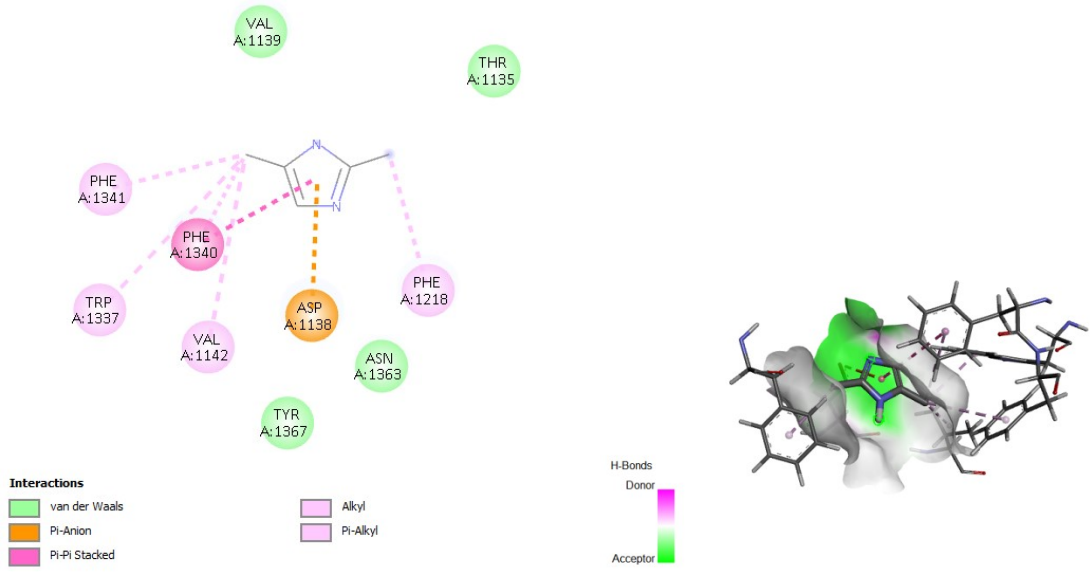

Figure 3.12: showing the 2D (A) and 3D H-bond (B) interactions between protein 7BVQ and ligand 70259 with binding affinity of -4.4

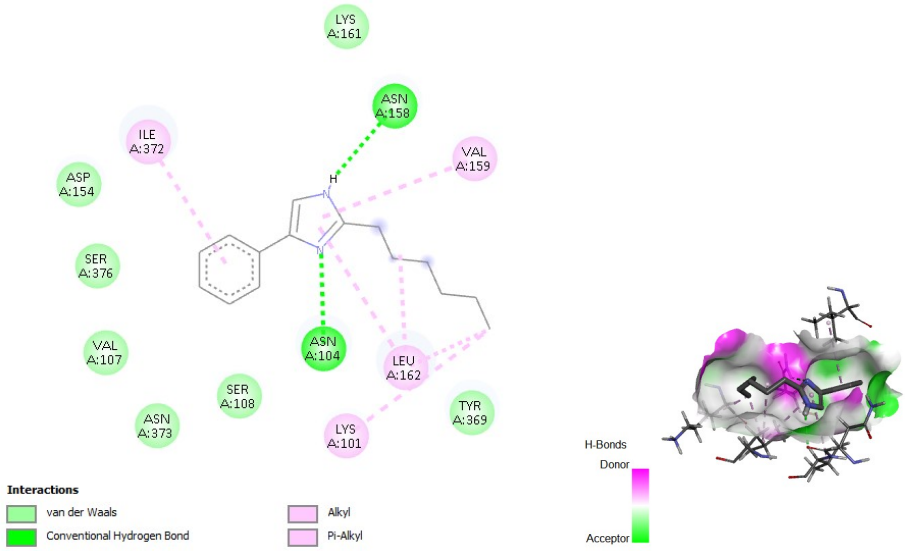

Figure 3.13: showing the 2D (A) and 3D H-bond (B) interactions between protein 5XPR And ligand 69620 with binding affinity of -6.3

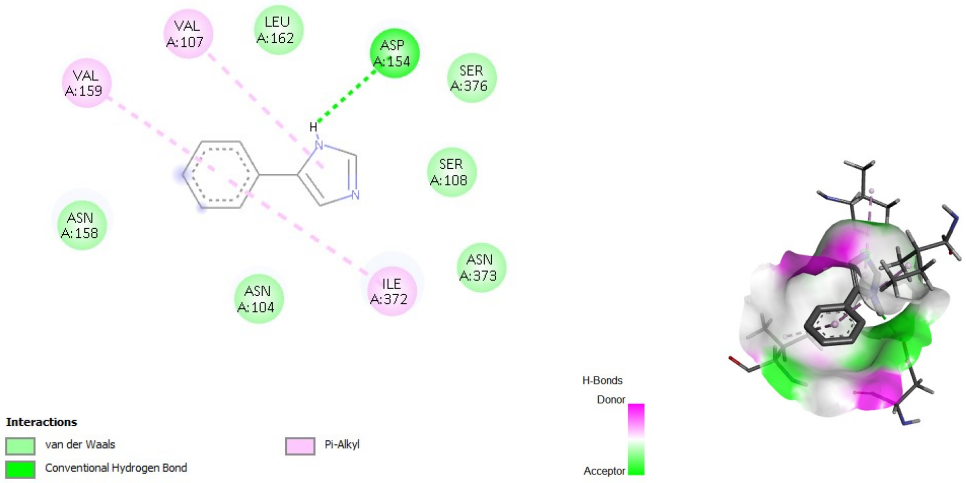

Figure 3.14: showing the 2D (A) and 3D H-bond (B) interactions between protein 5XPR and ligand 69590 with binding affinity of -5.4

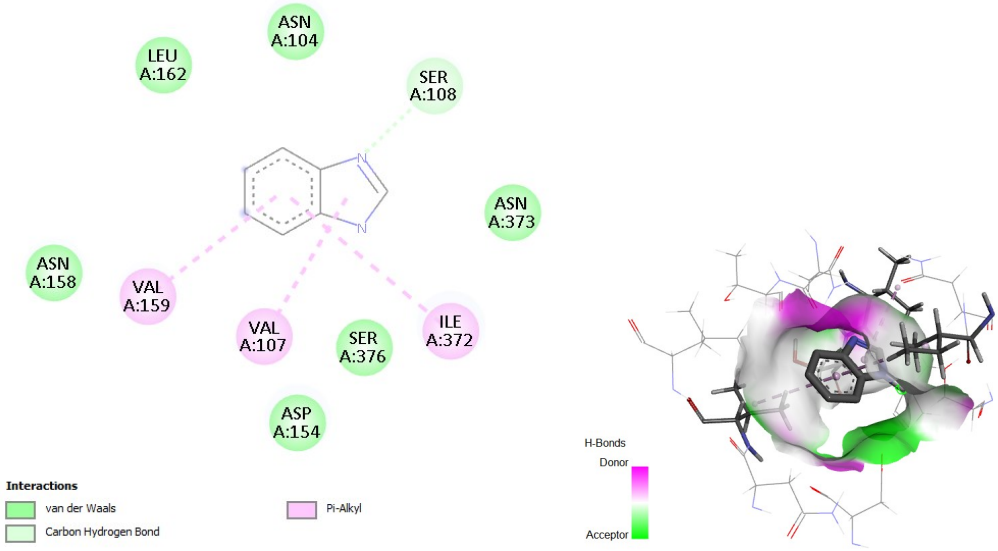

Figure 3.15: showing the 2D (A) and 3D H-bond (B) interactions between protein 5XPR and ligand 5798 with binding affinity of -5.0

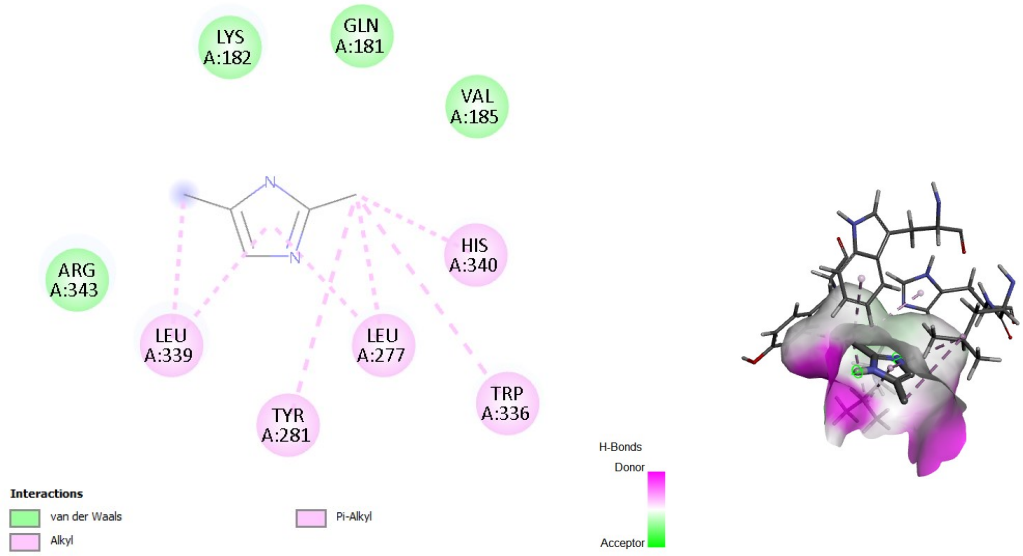

Figure 3.16: showing the 2D (A) and 3D H-bond (B) interactions between protein 5XPR and ligand 70259 with binding affinity of -4.1

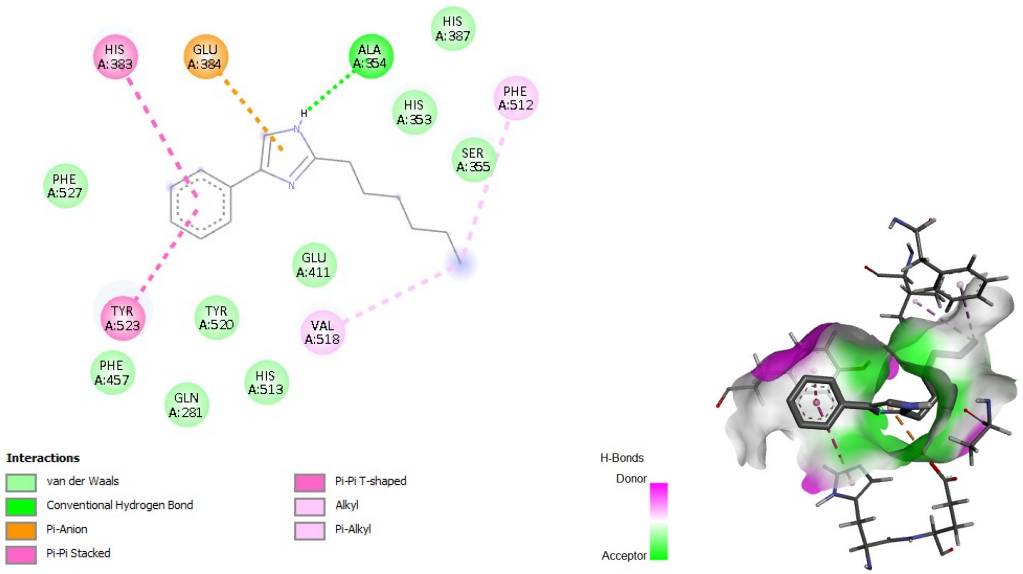

Figure 3.17: showing the 2D (A) and 3D H-bond (B) interactions between protein 1O86 and ligand 69620 with binding affinity of -7.0

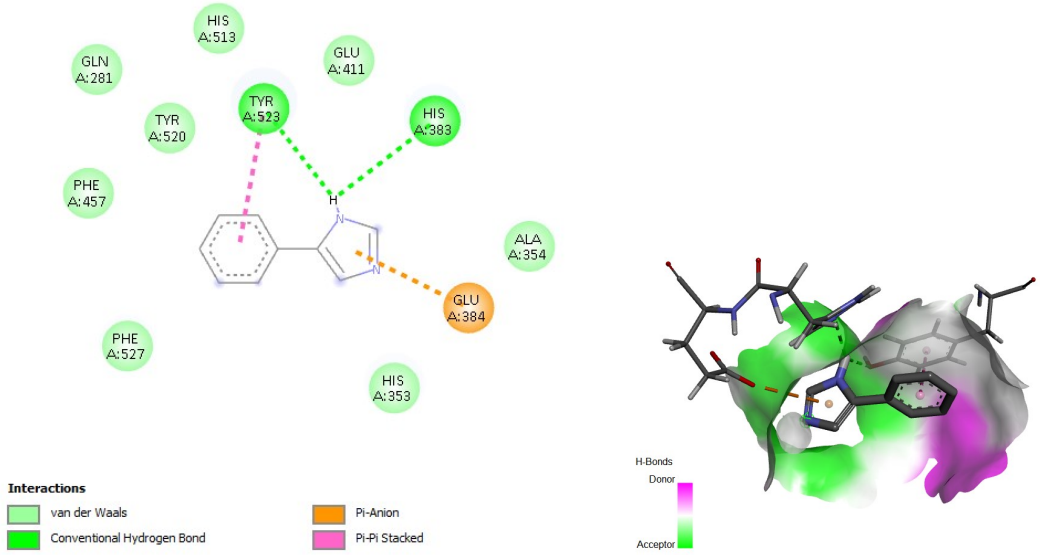

Figure 3.18: showing the 2D (A) and 3D H-bond (B) interactions between protein 1O86 and ligand 69590 with binding affinity of -6.3

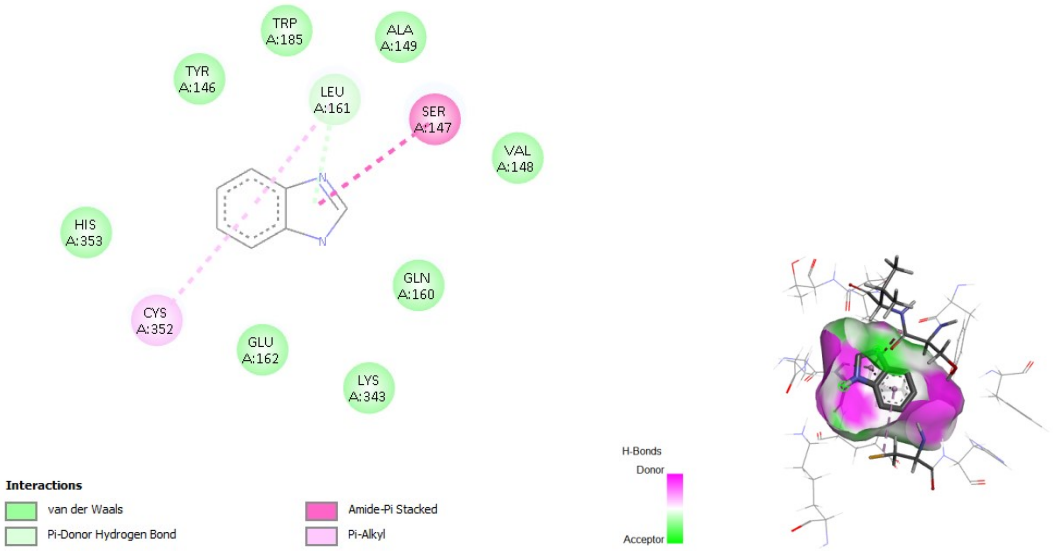

Figure 3.19: showing the 2D (A) and 3D H-bond (B) interactions between protein 1O86 and ligand 5798 with binding affinity of -5.9

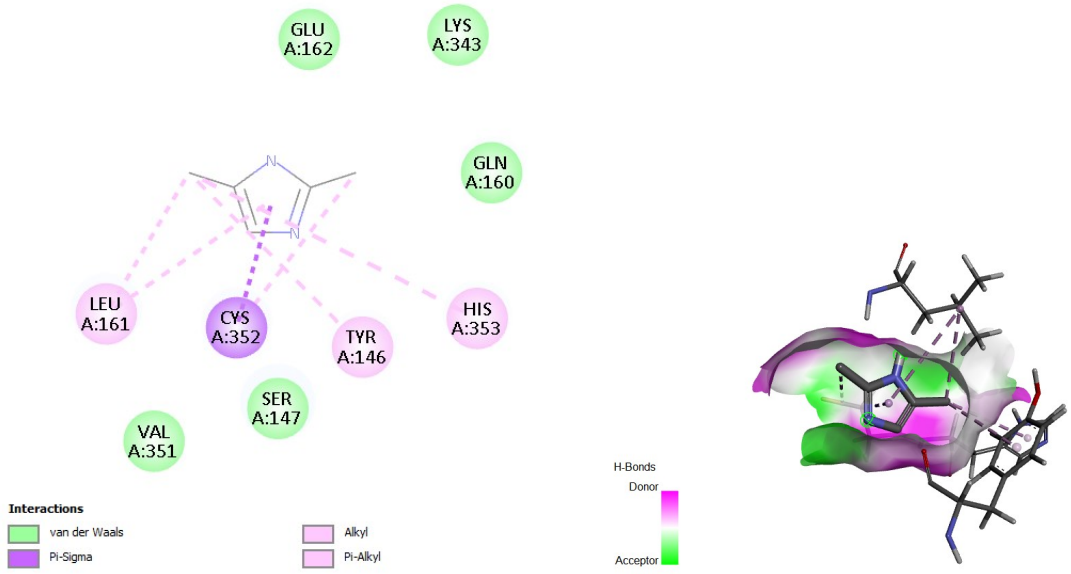

Figure 3.20: showing the 2D (A) and 3D H-bond (B) interactions between protein 1O86 and ligand 70259 with binding affinity of -4.5

3.2 Post Docking Analysis

The physicochemical properties, pharmacokinetic and lipophilicity parameters of the selected imidazole ligands obtained using SwissADME are shown in table 3.9 and 3.10 and 3.11 respectively.

Table 3.9: Physicochemical properties of the selected imidazole compounds

S/N	Pubchem CID	Molecular Formular	Molecular weight	No of Heavy atoms	Fraction CSP3	No of Rotatable bonds	No of H bond donors	Molar Refractivity	TPSA
1	69620	C15H20N2	228.33	17	0.4	6	1	73.02	28.68
2	69590	C9H8N2	144.17	11	0.0	1	1	44.02	28.68
3	5798	C7H6N2	118.14	9	0.0	0	1	36.09	28.68
4	70259	C5H8N2	96.13	7	0.4	0	1	28.52	28.68

Table 3.10: Pharmacokinetic properties of the selected imidazole compounds

S/N	PubChem CID	GI absorption	BBB Permeability	P-gp Substrate	CPY1A2 Inhibitor	CP2C9 Inhibitor	CP2D6 Inhibitor	CP3A4 Inhibitor	Log Kp (Skin permeation (cm/s)
1	69620	High	Yes	No	Yes	Yes	Yes	No	-4.47
2	69590	High	Yes	No	Yes	No	No	No	-5.96
3	5798	High	Yes	No	Yes	No	Yes	No	-5.74
4	70259	High	Yes	No	No	No	No	No	-6.28

Table 3.11: Lipophilicity Characteristics and Drug likeness of the selected imidazole compounds

S/N	PubChem CID	Lipophilicity (Log Po/w (!LOGP))	Lipophilicity (Log Po/w (XLOGP3))	Lipophilicity (Log Po/w (MLOGP))	Lipophilicity (Log Po/w (SILICOS IT))	Drug likeness (Lipinski)
1	69620	2.69	4.54	2.72	4.78	Yes, 0 violation
2	69590	1.26	1.72	1.06	2.65	Yes, 0 violation
3	5798	0.90	1.81	0.98	2.15	Yes, 0 violation
4	70259	1.00	0.86	-0.06	1.87	Yes, 0 voliation

Table 3.12: Toxicity Profile of the selected imidazole compounds

S/N	PubChem CID	Hepato Toxicity	Neuro Toxicity	Nephro Toxicity	Respiratory Toxicity	Cardio Toxicity	Carcino Genicity	Immuno Toxicity	Mutagenicity (cm/s)	Cyto Toxicity
1	69620	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive
2	69590	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
3	5798	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
4	70259	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive

3.3 Synthesis of 2-Hexyl-4-Phenyl-1H-Imidazole

3.2.1 Reaction Overview

The synthesis of 2-hexyl-4-phenyl-1H-imidazole was successfully carried out using a Debus-Radziszewski-type condensation reaction under reflux conditions. The reaction involved 2-oxo-2-phenylacetaldehyde, heptanal, and ammonium acetate as starting materials. Upon completion of the reaction and workup procedures, the crude product was isolated as a brownish solid with a mass of 4.45 g.

Based on the stoichiometry of the reaction and considering the limiting reagent, the theoretical yield was calculated to be 5.76 g, resulting in an experimental percentage yield of 77.3%.

The melting point of the synthesized compound was determined using a standard melting point apparatus and recorded as 184–186°C. This narrow range indicates a consistent thermal property for the obtained solid.

Purity assessment by thin-layer chromatography (TLC) revealed a single spot when visualized under UV light at 254 nm, with a retention factor (Rf) value of 0.48, using a mobile phase of hexane and dichloromethane in a 2:1 ratio.

Solubility tests indicated that the compound was poorly soluble in ethanol, acetone, n-hexane, water, ethyl acetate, and dichloromethane at room temperature. However, it showed good solubility in dimethyl sulfoxide (DMSO).

The molecular properties of the synthesized compound were confirmed as follows: molecular formula $C_{15}H_{20}N_2$ and molecular weight 228.34 g/mol. The physical appearance, yield, melting point, TLC behavior, and solubility profile are summarized in Table 3.13.

Table 3.13: Physical and Chemical Properties of Synthesized 2-Hexyl-4-Phenyl-1H-Imidazole

Parameter	**Observation/Value**
Molecular Formula	$C_{15}H_{20}N_2$
Molecular Weight	228.34 g/mol
Physical Appearance	Brownish solid
Weight of Crude Product	4.45 g
Theoretical Yield	5.76 g
Percentage Yield	77.3%
Melting Point	184-186°C
Rf Value	0.48 (Hexane:Dichloromethane = 2:1, UV 254 nm)

CHAPTER FOUR

DISCUSSION

4.1 Identification of Protein Active Sites

Identifying active binding sites before molecular docking ensures that computational efforts focus on biologically relevant regions where ligands naturally interact with their targets. This approach improves both prediction accuracy and computational efficiency.

Using PyMOL software, amino acid residues within 5Å of the binding sites were identified for four hypertension-related proteins. The mineralocorticoid receptor (6L88) showed binding sites comprising LEU 766, 769, 772, 809, 814; ASN 770; ALA 773, 813; GLN 776; VAL 780; TRP 806; SER 810; MET 807, 845, 852; CYS 942; LYS 873; ARG 817; and TYR 869

The β-adrenergic receptor (7BVQ) displayed PHE 1218, 1340, 1341; SER 1228, 1229, 1232; ASN 1344, 1363; THR 1220, 1135, 1143; VAL 1139, 1142; ALA 1225; TYR 1224, 1367; ASP 1138; and TRP 1134

The endothelin receptor (5XPR) exhibited ARG 343; HIS 150, 340; LEU 277, 339; TRP 167, 336; ILE 372; ALA 375; SER 376; LYS 161, 183, 273; TYR 281; VAL 177, 185; GLU 236; PHE 240; GLN 181; PRO 178; CYS 174, 255; ASN 158; and ASP 154.

Finally, angiotensin-converting enzyme (1O86) revealed HIS 353, 383, 387, 513; ALA 356, 354; GLU 162, 384, 411; SER 355; VAL 380, 518; ASP 377; TYR 520, 523; PHE 457, 512, 527; LYS 511; and GLN 281.

The presence of both hydrophobic residues (PHE, LEU, VAL, ALA, TRP) and polar/charged residues (HIS, GLU, ASP, LYS, ARG, SER, THR) in these binding pockets indicates that

successful ligands must engage in diverse molecular interactions including hydrogen bonding, hydrophobic contacts, π-π stacking, and electrostatic interactions.

4.2 Molecular Docking and Binding Affinity Analysis

Molecular docking simulations evaluated the binding strength of four imidazole compounds against the proteins involved. Binding affinity values (ΔG in Kcal/mol) indicate interaction strength, with more negative values signifying tighter binding and potentially greater biological activity.

Against the mineralocorticoid receptor (6L88 protein), 2-hexyl-4-phenyl-1H-imidazole achieved -7.3 Kcal/mol, approaching esaxerenone's -8.2 Kcal/mol with only 0.9 Kcal/mol difference. The 2D and 3D analysis revealed hydrogen bonds and hydrophobic interactions with key pocket residues. Other compounds showed weaker binding: 4-phenylimidazole (-6.3 Kcal/mol), 1H-benzimidazole (-5.3 Kcal/mol), and 2,4-dimethylimidazole (-4.3 Kcal/mol). The hexyl chain and phenyl ring appear crucial for optimal binding through enhanced hydrophobic interactions and π-π stacking.

For the β-adrenergic receptor (7BVQ), 2-hexyl-4-phenyl-1H-imidazole demonstrated the highest affinity of all protein-ligand combinations at -7.9 Kcal/mol, compared to carazolol's -9.7 Kcal/mol. The interaction diagrams showed multiple hydrogen bonds with SER 1228, 1229, and THR 1220, plus hydrophobic contacts with PHE 1218, 1340, and TYR 1224. This strong binding suggests potential β-blocking activity that could reduce cardiac output and heart rate. Other compounds displayed moderate to weak binding: 4-phenylimidazole (-6.7 Kcal/mol), 1H-benzimidazole (-5.9 Kcal/mol), and 2,4-dimethylimidazole (-4.4 Kcal/mol).

Against the endothelin receptor (5XPR protein), 2-hexyl-4-phenyl-1H-imidazole showed -6.3 Kcal/mol versus bosentan's -8.6 Kcal/mol. The 2.3 Kcal/mol gap suggests endothelin receptor

antagonism may not be the primary mechanism, though even modest interaction could contribute to overall antihypertensive effects.

The most clinically significant finding emerged from ACE docking, where 2-hexyl-4-phenyl-1H-imidazole achieved -7.0 Kcal/mol, remarkably close to lisinopril's -7.4 Kcal/mol—only 0.4 Kcal/mol difference. Structural analysis revealed critical interactions with zinc-binding histidine residues at positions 383 and 513, plus glutamate 384, which comprise ACE's catalytic machinery. This near-equivalent binding affinity strongly suggests genuine ACE inhibitory potential comparable to lisinopril, a widely used clinical drug.

The structure-activity relationship is clear: 2-hexyl-4-phenyl-1H-imidazole consistently outperformed all other compounds across targets, with affinities between -6.3 and -7.9 Kcal/mol. The hexyl chain provides crucial hydrophobic interactions while the phenyl ring enables aromatic stacking. Removing either substituent consistently weakened binding by approximately 1.0 Kcal/mol or more, demonstrating that both structural elements work synergistically. These results indicate multi-target activity with potential to modulate several blood pressure regulatory pathways simultaneously.

4.3 ADMET Properties and Drug-likeness

The physicochemical properties showed that 2-hexyl-4-phenyl-1H-imidazole has a molecular weight of 228.33 g/mol, 17 heavy atoms, 6 rotatable bonds, and fraction Csp3 of 0.4, indicating balanced flexibility. The topological polar surface area of 28.68Ų predicts excellent membrane permeability. All compounds shared this favorable TPSA value.

Drug-likeness assessment using Lipinski's Rule of Five revealed zero violations for all compounds, indicating excellent drug-like properties. For 2-hexyl-4-phenyl-1H-imidazole,

lipophilicity values ranged from iLOGP = 2.69 to SILICOS-IT = 4.78, with consensus around 3.4, an optimal range balancing membrane permeability with adequate aqueous solubility.

Pharmacokinetic predictions showed all compounds have high gastrointestinal absorption, critical for oral bioavailability. All demonstrated blood-brain barrier permeability and none were P-glycoprotein substrates, suggesting they won't be actively pumped out of cells. However, 2-hexyl-4-phenyl-1H-imidazole was predicted to inhibit CYP1A2, CYP2C9, and CYP2D6, raising drug-drug interaction concerns, though sparing CYP3A4 is favorable. The compound 2,4-dimethylimidazole showed no CYP inhibition, suggesting lowest interaction potential. Skin permeability values (-4.47 to -6.28 cm/s) indicated limited transdermal penetration, typical for oral drugs.

Overall, 2-hexyl-4-phenyl-1H-imidazole exhibits favorable pharmacokinetic properties with high GI absorption, appropriate lipophilicity, and compliance to Lipinski compliance rules.

4.4 Toxicity Assessment

Computational toxicity profiling using ProTox-3.0 revealed varied safety profiles. Importantly, none of the compounds showed immunotoxicity, mutagenicity, or cytotoxicity safety parameters. The absence of mutagenicity is particularly significant for drugs requiring chronic administration.

The compound 2-hexyl-4-phenyl-1H-imidazole was inactive for hepatotoxicity, nephrotoxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity, but showed predicted neurotoxicity and respiratory toxicity. The overall profile appears relatively favorable with most major organ toxicities inactive. The compound 4-phenylimidazole displayed hepatotoxicity and neurotoxicity, while 1H-benzimidazole

showed only neurotoxicity. The compound 2,4-dimethylimidazole exhibited neurotoxicity and carcinogenicity, with the latter representing a significant concern.

Notably, all compounds triggered neurotoxicity predictions, suggesting a class effect possibly related to the imidazole core or blood-brain barrier permeability. Interestingly, some central nervous system activity might be therapeutically relevant, as drugs like clonidine and moxonidine work by reducing sympathetic outflow from the brain. The key question is whether predicted neurotoxicity represents actual harm or beneficial CNS activity that contributes to efficacy.

Comparing safety profiles, 2-hexyl-4-phenyl-1H-imidazole and 1H-benzimidazole appear most favorable with limited organ toxicity predictions. These computational predictions require experimental validation through in vitro and in vivo toxicity studies, as many successful drugs contain structural alerts that don't manifest as actual toxicity at therapeutic doses.

4.5 Chemical Synthesis

Based on its superior binding affinity, favorable pharmacokinetic parameters, and acceptable predicted toxicity profile, 2-hexyl-4-phenyl-1H-imidazole was selected for laboratory synthesis. The compound was prepared through a Debus–Radziszewski-type condensation reaction, involving the reflux of 2-oxo-2-phenylacetaldehyde, heptanal, and ammonium acetate.

The reaction proceeded successfully, yielding a brownish crystalline solid. The crude product weighed 4.45 g, corresponding to a percentage yield of 77.3%, based on a theoretical yield of 5.76 g. The relatively high yield indicates that the synthetic pathway was efficient under the applied conditions.

The melting point of the compound was determined to be 184-186°C. The molecular formula ($C_{15}H_{20}N_2$) and molecular weight (228.34 g/mol) were consistent with the expected values for the target compound, though structural confirmation is yet to be established through spectroscopic techniques.

Thin-layer chromatography (TLC) using a hexane:dichloromethane (2:1, v/v) solvent system produced a single spot with an Rf value of 0.48 when visualized under UV light at 254 nm, indicating the presence of a predominant product with minimal detectable impurities.

Solubility testing showed that the compound was insoluble or sparingly soluble in ethanol, methanol, ethyl acetate, dichloromethane, acetone and water, but exhibited good solubility in dimethyl sulfoxide (DMSO). This solubility behavior reflects its lipophilic nature and low topological polar surface area (TPSA). While the limited aqueous solubility may present formulation challenges, DMSO solubility makes it suitable for initial in vitro screening and pharmacological evaluation.

4.6 Conclusions and Future Directions

This study successfully identified 2-hexyl-4-phenyl-1H-imidazole as a promising lead compound through integrated computational and experimental approaches. The compound demonstrated:

- **Multi-target binding activity** with affinities ranging from -6.3 to -7.9 Kcal/mol across four hypertension-related proteins
- **Particularly strong ACE inhibition potential**, coming within 0.4 Kcal/mol of lisinopril with coordination to zinc-binding residues
- **Favorable pharmacokinetic profile** including high GI absorption, balanced lipophilicity (~3.4), zero Lipinski violations, and no P-glycoprotein liability

- **Acceptable safety profile** with no predicted mutagenicity, carcinogenicity, immunotoxicity, or cytotoxicity

Structure-activity analysis confirmed that combining the hexyl chain with the phenyl ring on the imidazole scaffold is essential for optimal binding, as simpler derivatives showed substantially reduced potency.

The compound may function through multiple complementary mechanisms: ACE inhibition reducing angiotensin II formation, β-adrenergic receptor blockade decreasing cardiac output, mineralocorticoid receptor antagonism promoting natriuresis, and modest endothelin receptor modulation. This multi-target profile could offer advantages over single-mechanism drugs.

However, several limitations require attention. Computational predictions need experimental validation through enzyme inhibition assays, receptor binding studies, and functional cellular assays. Pharmacokinetic models require confirmation via permeability studies, metabolic stability assays, and animal pharmacokinetic experiments. Toxicity predictions must be verified through cytotoxicity testing, genotoxicity assays, and animal studies. The poor aqueous solubility requires pharmaceutical development efforts.

Future research should prioritize biochemical validation of ACE inhibition and receptor binding, blood pressure measurements in hypertensive animal models, medicinal chemistry optimization to improve solubility while maintaining potency, comprehensive safety pharmacology studies, and formulation development for enhanced bioavailability. While substantial additional research is needed, this work provides a solid foundation for developing novel antihypertensive agents based on the imidazole scaffold.

CHAPTER FIVE

CONCLUSION

This study successfully employed computational and experimental approaches to identify and characterize imidazole derivatives with potential antihypertensive activity. Through systematic molecular docking simulations against four key hypertension-related protein targets - mineralocorticoid receptor (6L88 protein), β-adrenergic receptor (7BVQ protein), endothelin receptor (5XPR protein), and angiotensin-converting enzyme (1O86 protein). The compound 2-hexyl-4-phenyl-1H-imidazole emerged as the most promising lead candidate.

This compound demonstrated consistently strong binding affinities across all four targets, ranging from -6.3 to -7.9 Kcal/mol, with values comparable to clinically established antihypertensive drugs. Most notably, its binding affinity to ACE (-7.0 Kcal/mol) was remarkably close to that of lisinopril (-7.4 Kcal/mol), differing by only 0.4 Kcal/mol. The interaction pattern with ACE's zinc-binding histidine residues strongly suggests genuine enzyme inhibitory potential, positioning this compound as a possible ACE inhibitor with multi-target activity.

ADMET analysis revealed favorable drug-like properties, with the compound exhibiting high gastrointestinal absorption, appropriate lipophilicity, complete compliance with Lipinski's Rule of Five (zero violations), and no P-glycoprotein substrate liability. These characteristics predict good oral bioavailability and tissue distribution, essential requirements for oral antihypertensive therapy.

Toxicity profiling indicated an acceptable safety profile, with no predicted mutagenicity, carcinogenicity, immunotoxicity, or cytotoxicity. While neurotoxicity and respiratory toxicity

were flagged computationally, these predictions require experimental validation and may be manageable through appropriate dosing strategies.

The successful chemical synthesis of 2-hexyl-4-phenyl-1H-imidazole via Debus-Radziszewski condensation yielded a stable brownish crystalline solid (melting point 182-184°C) with properties matching computational predictions. Structure-activity relationship analysis confirmed that both the hexyl chain and phenyl ring are essential for optimal binding, as simpler imidazole derivatives showed substantially reduced affinities across all targets.

The multi-target binding profile of 2-hexyl-4-phenyl-1H-imidazole suggests potential for addressing hypertension through complementary mechanisms, ACE inhibition, β-adrenergic receptor blockade, mineralocorticoid receptor antagonism, and endothelin receptor modulation. This polypharmacology could offer therapeutic advantages over single-target drugs by addressing multiple pathways simultaneously.

However, several challenges remain. The poor aqueous solubility observed experimentally necessitates formulation development through strategies such as salt formation, solid dispersion, or nanoparticle technology. More importantly, all computational predictions require rigorous experimental validation through enzymatic assays, receptor binding studies, in vitro cellular assays, and ultimately in vivo pharmacological studies in hypertensive animal models.

In conclusion, this study has identified 2-hexyl-4-phenyl-1H-imidazole as a promising lead compound for antihypertensive drug development, with particularly strong potential as an ACE inhibitor. The compound exhibits favorable pharmacokinetic predictions, acceptable safety profiles, and successful laboratory synthesis. The structure-activity relationships established provide valuable guidance for future medicinal chemistry optimization aimed at

improving solubility while maintaining or enhancing potency. While substantial additional research, including functional validation, comprehensive toxicity testing, and in vivo efficacy studie, is essential before clinical consideration, this work establishes a solid foundation for developing novel imidazole-based antihypertensive agents. The findings contribute to the ongoing search for innovative therapeutic options for hypertension, a condition affecting millions worldwide and requiring continuous advancement in treatment strategies.

REFERENCES

Algabbani F.M., Algabbani A.M. (2020). Treatment adherence among patients with hypertension: findings from a cross-sectional study. Clinical Hypertension, 26, Article 18.

Altoum, S. M., Al-Mahayri, Z. N., & Ali, B. R. (2023). Antihypertensives associated adverse events: a review of mechanisms and pharmacogenomic biomarkers available evidence in multi-ethnic populations. Frontiers in Pharmacology, 14, 1286494.

Ameer, O. Z. (2022). Hypertension in chronic kidney disease: What lies behind the scene. Frontiers in Pharmacology, 13, 949260.

Burlacu A, Kuwabara M, Brinza C, Kanbay M. Key Updates to the 2024 ESC Hypertension Guidelines and Future Perspectives. Medicina. 2025; 61(2):193.

Burnier M, Damianaki A. (2023). Hypertension as cardiovascular risk factor in chronic kidney disease. Circulation Research, 132(8), 1050-1063.

Carlsson, J., & Luttens, A. (2024). Structure-based virtual screening of vast chemical space as a starting point for drug discovery. Current Opinion in Structural Biology, 87, 102829.

Chen, R., Suchard, M. A., Krumholz, H. M., Schuemie, M. J., Shea, S., Duke, J., Pratt, N., Nguyen, H., Viernes, B., Matcho, A., Schuemie, M., Hripcsak, G., Madigan, D., & Ryan, P. B. (2021). Comparative first-line effectiveness and safety of ACE (angiotensin-converting enzyme) inhibitors and angiotensin receptor blockers: A multinational cohort study. Hypertension, 78(3), 591–603.

Chunxue Y, Jianming Z, Jianbing W. (2022). Effect of calcium channel blockers versus diuretics for hypertension. Computational and Mathematical Methods in Medicine, 2022:3975130.

Cluett JL & William JH. (2024). Evaluation and management of resistant hypertension: Core Curriculum 2024. American Journal of Kidney Diseases, 84(3), 395–407.

Cutrell, S., Alhomoud, I. S., Mehta, A., Talasaz, A. H., Van Tassell, B., & Dixon, D. L. (2023). ACE-Inhibitors in hypertension: A historical perspective and current insights. Current Hypertension Reports, 25(9), 243–250.

Devi, M. M., Singh, O. M., & Singh, T. P. (2022). Synthesis of N-containing heterocycles in water. Physical Sciences Reviews, 9(2), 463-497.

Devi, M., Devi, K., Singh, O., & Singh, T. (2024). Synthesis of imidazole derivatives in the last 5 years: An update. Heterocyclic Communications, 30(1), 20220173.

Drożdż, D., Drożdż, M., & Wójcik, M. (2023). Endothelial dysfunction as a factor leading to arterial hypertension. Pediatric Nephrology, 38(9), 2973–2985.

Dulsat, J., López-Nieto, B., Estrada-Tejedor, R., & Borrell, J. I. (2023). Evaluation of free online ADMET tools for academic or small biotech environments. Molecules, 28(2), 776.

Ece, A. (2023). Computer-aided drug design. BMC Chemistry, 17(1), 26.

Ernsberger, P., Giuliano, R., Willette, R. N., & Reis, D. J. (1997). The I1-imidazoline receptor: From binding site to therapeutic target in cardiovascular disease. Journal of Hypertension, 15(Suppl 1), S9–S23.

Esler M. The sympathetic system and hypertension. Am J Hypertens. 2000 Jun;13(6 Pt 2):99S-105S. doi: 10.1016/s0895-7061(00)00225-9. PMID: 10921528.

Faucon A-L, Lambert O, Massy Z, et al. (2023). A nationwide cohort study comparing the effectiveness of diuretics and calcium channel blockers on top of renin-angiotensin system inhibitors on chronic kidney disease progression and mortality. Kidney International. [Epub ahead of print].

Flack, J. M., Buhnerkempe, M. G., & Moore, K. T. (2024). Resistant hypertension: Disease burden and emerging treatment options. Current Hypertension Reports, 26(5), 183–199.

Fountain JH, Kaur J, Lappin SL. (2023). Physiology, renin angiotensin system. In StatPearls. StatPearls Publishing.

Fountain, J. H., Kaur, J., & Lappin, S. L. (2023). Physiology, renin angiotensin system. In StatPearls. StatPearls Publishing.

Fuchs, F. D., & Whelton, P. K. (2020). High blood pressure and cardiovascular disease. Hypertension, 75(2), 285–292.

Gallo G., Volpe M., & Savoia C. (2021). Endothelial dysfunction in hypertension: Current concepts and clinical implications. Frontiers in Medicine (Lausanne), 8, 798958.

Gavrilova A., Šūmane J., Repek I., Mezale D., & Erglis A. (2021). Adherence Level to Arterial Hypertension Treatment: A Cross-Sectional Patient Survey and Retrospective Analysis of the NHS Prescription Database. Healthcare (Basel), 9(8), 1085.

Giacomo Buso, Claudia Agabiti-Rosei, Matteo Lemoli, Federica Corvini, Maria Lorenza Muiesan, The Global Burden of Resistant Hypertension and Potential Treatment Options, European Cardiology Review 2024;19:e07.

Grassi, G, (2021). The sympathetic nervous system in hypertension: Roadmap update of a long journey. American Journal of Hypertension, 34(12), 1247–1254.

Griendling KK, Camargo LL, Rios FJ, Alves-Lopes R, Montezano AC, Touyz RM. (2021). Oxidative stress and hypertension. Circulation Research, 128(7):993–1020.

Hamrahian S.M., Maarouf O.H., Fülöp T. (2022). A critical review of medication adherence in hypertension: Barriers and facilitators clinicians should consider. Patient Preference and Adherence, 16, 2749-2757.

Hamrahian SM, Falkner B. (2017). Hypertension in chronic kidney disease. Advances in Experimental Medicine and Biology, 956, 307-325.

Hebert SA, Ibrahim HN. (2022). Hypertension Management in Patients With Chronic Kidney Disease. Methodist Debakey Cardiovascular Journal, 18(3), 229-237.

Jampilek, J. (2019). Heterocycles in Medicinal Chemistry. Molecules, 24(21), 3839. https://pmc.ncbi.nlm.nih.gov/articles/PMC6864827/

Jones, K. E., Hayden, S. L., Meyer, H. R., Sandoz, J. L., Arata, W. H., Dufrene, K., Ballaera, C., Lopez Torres, Y., Griffin, P., Kaye, A. M., Shekoohi, S., & Kaye, A. D. (2024). The evolving role of calcium channel blockers in hypertension management: Pharmacological and clinical considerations. Current Issues in Molecular Biology, 46(7), 6315–6327.

Katke SP. Imidazole: Chemistry, Synthesis, Properties, Industrial Applications and Biological and Medicinal Applications. Environ Sci Ind J. 19(1): 257

Kerru, N., Gummidi, L., Maddila, S., Gummidi, S., Jonnalagadda, S. B., & Basireddy, M. (2020). A review on recent advances in nitrogen-containing heterocycles as anticancer

agents. Molecules, 25(17), 3931. https://doi.org/10.3390/molecules25173931 (PMC PMCID: PMC7221918)

Khalil H, Zeltser R. (2023). Antihypertensive Medications. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from:

Lauder L., Mahfoud F., & Böhm M. (2024). Management of resistant hypertension. Annual Review of Medicine, 75, 443–457.Carey RM, Calhoun DA, Bakris GL, et al. (2018). Resistant hypertension: Detection, evaluation, and management: A scientific statement from the American Heart Association. Hypertension, 72(5), e53-e90.

Lee, H., Kwon, S. H., Jeon, J. S., Noh, H., Han, D. C., & Kim, H. (2022). Association between blood pressure and the risk of chronic kidney disease in treatment-naïve hypertensive patients. Kidney Research and Clinical Practice, 41(1), 31–42.

Longkumer, I., Yadav, S., Rajkumari, S., & Saraswathy, K. N. (2023). Trends in hypertension prevalence, awareness, treatment, and control: an 8-year follow-up study from rural North India. Scientific Reports, 13, 9910.

Mahfoud F., Wang J., Ray S. (2024). The current position of β-blockers in hypertension: guidelines and clinical practice. Curr Med Res Opin, 40(suppl 1):25-32.

Martyniak A, Tomasik PJ. A New Perspective on the Renin-Angiotensin System. Diagnostics. 2023; 13(1):16.

McCarthy C.P., Bruno R.M., Rahimi K., Touyz R.M., McEvoy J.W. (2025). What is new and different in the 2024 European Society of Cardiology guidelines for the management of elevated blood pressure and hypertension. Hypertension, 82(3), 432-444. Meyer R, Redford D. (2024). 2023 ESH Hypertension Guideline Update – Bringing Us Closer to the U.S. Approach. J Am Coll Cardiol (Acc latest-in-cardiology article). Available at:

Moon JY. Recent Update of Renin-angiotensin-aldosterone System in the Pathogenesis of Hypertension. Electrolyte Blood Press. 2013 Dec;11(2):41-5. doi: 10.5049/EBP.2013.11.2.41. Epub 2013 Dec 31. PMID: 24627703; PMCID: PMC3950224.

NavarreteVázquez, G., Castillo, R., ContrerasPliego, J., Uribe, M., MedinaFranco, J. L., & EstradaSoto, S. (2010). Synthesis, vasorelaxant activity and antihypertensive effect of benzo[d]imidazole derivatives. Biomedical Reports, 3(4), 113–119. https://doi.org/10.1016/S09680896(10)003317

Nematpour, M. N., Chermahini, Z. R., & Teymourlouy, A. A. (2024). Sustainable synthesis of imidazoles using a catalyst-free approach and ethyl lactate as a bio-based green solvent in the Debus-Japp-Radziszewski reaction. Synthetic Communications, 54(7), 592-599.

Niazi, S. K., & Mariam, Z. (2024). Computer-aided drug design and drug discovery: A prospective analysis. Pharmaceuticals, 17(1), 22.

Nikolić A., Mišić V., Mihailović M., et al. (2023). Predictors of Non-Adherence to Medications in Hypertensive Patients: A Single-Center Observational Study in Serbia. Risk Management and Healthcare Policy, 16, 2253–2265. https://doi.org/10.2147/RMHP.S392566 (PMCID: PMC10362828)

Oh, G.C., Lee, HY., Chung, W.J. et al. Comparison of effects between calcium channel blocker and diuretics in combination with angiotensin II receptor blocker on 24-h central blood pressure and vascular hemodynamic parameters in hypertensive patients: study design for a multicenter, double-blinded, active-controlled, phase 4, randomized trial. Clin Hypertens 23, 18 (2017).

Pena D-L., Aurelian J., Grigore M., Hodorogea A-S., Weiss E., Bădilă E., Ilieșiu A-M., & Balahura A-M. (2025). Emerging therapeutic frontiers in hypertension management. Frontiers in Cardiovascular Medicine, 12, 1550181.

Petramala, L., Gigante, A., Sarlo, F., Servello, A., Circosta, F., Marino, L., Ciccarelli, A., Cavallaro, G., & Letizia, C. (2024). Systematic review article: new drug strategies for treating resistant hypertension—the importance of a mechanistic, personalized approach. Frontiers in Cardiovascular Medicine, 11, 1369090.

Rasajna, G. (2025). A review of synthesis, structure-activity relationship and therapeutic applications of imidazole and its derivatives: Review article. Journal of Pharma Insights and Research, 3(1), 121–129. https://doi.org/10.69613/z6yaks23

Rasajna, G., Hema Latha, N., Sunitha, P., Krishna Veni, V., Shanmitha Jahnavi, Likitha, G., Anantha, E., Lakshmi Aswini, K., Bhargavi, V., & Vijaya, V. (2025). A Review of Synthesis, Structure Activity Relationship and Therapeutic Applications of Imidazole and Its Derivatives. Journal of Pharma Insights and Research.

Rasajna, G., Hema Latha, N., Sunitha, P., Krishna Veni, V., Shanmitha Jahnavi, Likitha, G., Anantha, E., Lakshmi Aswini, K., Bhargavi, V., & Vijaya, V. (2025). A Review of Synthesis, Structure Activity Relationship and Therapeutic Applications of Imidazole and Its Derivatives. Journal of Pharma Insights and Research.

Richardson, C., Battle, S.J. & DiPette, D.J. Population-based approaches to increase hypertension control: an urgent need. Hypertens Res 48, 1195–1197 (2025).

Rimoldi SF, Scherrer U, Messerli F. (2015). Efficacy and safety of calcium channel blocker/thiazide (-like) diuretic combination therapy in hypertensive patients: A meta-

analysis. Journal of Hypertension, 33(5), 924–932. https://doi.org/10.1186/s40885-017-0074-0 (PMC9461694)

Sahu, M., & Satapathy, P. (2023). Recent development and pharmaceutical significance of imidazolebased ionic liquids [Review Article – Open Access]. International Journal of Green & Natural Sciences, 5(1), 45–59.

Seravalle, G., & Grassi, G. (2022). Sympathetic nervous system and hypertension: New evidences. Autonomic Neuroscience, 238, 102954.

Shaker, R. M. (2012). Recent Advances in the Chemistry of Imidazoles. Heterocycles, 85(7), 1529-1605.

Shams P, Tackling G, Borhade MB. Hypertensive Heart Disease. [Updated 2025 Apr 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539800/

Sharma, P., LaRosa, C., Antwi, J., Govindarajan, R., & Werbovetz, K. A. (2021). Imidazoles as Potential Anticancer Agents: An Update on Recent Studies. Archiv der Pharmazie, 354(9), e2100024.

Singh B, Cusick AS, Goyal A, et al. (2025). ACE Inhibitors. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from:

Sobhy M., Eletriby A., Ragy H., Kandil H., Saleh M.A., Farag N., et al. (2024). ACE inhibitors and angiotensin receptor blockers for the primary and secondary prevention of cardiovascular outcomes: Recommendations from the 2024 Egyptian cardiology expert consensus in collaboration with the CVREP Foundation. Cardiology and Therapy, 13(4):707-736.

Sowers, J. R., Epstein, M., & Frohlich, E. D. (2001). Diabetes, hypertension, and cardiovascular disease: An update. Hypertension, 37(4), 1053–1059.

Szabo, B. (2002). Imidazoline Antihypertensive Drugs: A Critical Review on Their Mechanism of Action. Pharmacology & Therapeutics, 93(1), 1-35.

Tan, J. L., & Thakur, K. (2023). Systolic hypertension. In StatPearls. StatPearls Publishing.

Taylor, A. P., Robinson, R. P., Fobian, Y. M., Blakemore, D. C., Jones, L. H., & Fadeyi, O. (2016). Modern advances in heterocyclic chemistry in drug discovery. Organic & Biomolecular Chemistry, 14, 66116637. https://doi.org/10.1039/C6OB00936K

Tedla YG, Saboo-Borg M, Birungi J, Ali MK, Kaanoumani W, Munjanja SP, et al. (2015). Drug side-effect symptoms and adherence to antihypertensive drug therapy in south western Uganda. Patient Preference and Adherence, 9, 1281–1291. https://doi.org/10.2147/PPA.S90647 (PMCID: PMC5863783)

Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JA, Smith RD. Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol Rev. 1993 Jun;45(2):205-51. PMID: 8372104.

Tolomeu, H. V., & Fraga, C. A. M. (2023). Imidazole: Synthesis, functionalization and physicochemical properties of a privileged structure in medicinal chemistry. Molecules, 28(2), 507. https://doi.org/10.3390/molecules28020507

Turner, J. M., & Kodali, R. (2020). Should angiotensin-converting enzyme inhibitors ever be used for the management of hypertension? Current Cardiology Reports, 22(10), 95.

Uniyal, A., Mahapatra, M. K., Tiwari, V., Sandhir, R., & Kumar, R. (2020). Targeting SARS-CoV-2 main protease: Structure based virtual screening, in silico ADMET studies and molecular dynamics simulation for identification of potential inhibitors. Journal of Biomolecular Structure & Dynamics.

Vemula, D., Jayasurya, P., Sushmitha, V., Kumar, Y. N., & Bhandari, V. (2023). CADD, AI and ML in drug discovery: A comprehensive review. European Journal of Pharmaceutical Sciences, 181, 106324.

Verma, A., Joshi, S., & Singh, D. (2013). Imidazole: Having Versatile Biological Activities. Journal of Chemistry, 2013, 329412.

Wandile, P. M. (2024). Hypertension and comorbidities: A silent threat to global health. World Journal of Biology Pharmacy and Health Sciences, 17(1), 182–189.

Wang C., Liu S., Miao W., Ye N., Xie Z., Qiao L., et al. (2024). Intensive blood pressure control in isolated systolic hypertension: a post hoc analysis of a cluster randomized trial. Lancet Reg Health West Pac, 48, 101111.

Wenzel RR, Spieker L, Qui S, Shaw S, Lüscher TF, Noll G. (1998). I1-imidazoline agonist moxonidine decreases sympathetic nerve activity and blood pressure in hypertensives. Hypertension, 32(6):1022–1027.

Yang, H., Lou, C., Sun, L., Li, J., Cai, Y., Wang, Z., Li, W., Liu, G., & Tang, Y. (2019). admetSAR 2.0: web-service for prediction and optimization of chemical ADMET properties. Bioinformatics, 35(6), 1067–1069.

Yang, X.-L., Feng, Y.-H., Zheng, X.-N., Gao, J.-F., Zhang, L.-G., Yu, Y.-L., Xu, J.-H., & Zhang, D.-W. (2023). Synthesis of imidazole-based molecules under ultrasonic irradiation approaches. Molecules, 28(12), 4845.

Yu, W., Weber, D. J., & MacKerell, A. D., Jr. (2023). Computer-Aided Drug Design: An Update. In P. Sass (Ed.), Antibiotics (Methods in Molecular Biology, Vol. 2601, pp. 123–152). Humana.

Zhang Z., Zhao L., Zhou X., Meng X., Zhou X. (2023). Role of inflammation, immunity, and oxidative stress in hypertension: New insights and potential therapeutic targets. Frontiers in Immunology, 13, 1098725.

Zhang, B., Li, H., Yu, K., & Jin, Z. (2022). Molecular docking-based computational platform for high-throughput virtual screening. CCF Transactions on High Performance Computing, 4(1), 63–74.

Zhang, L., Peng, X. M., Damu, G. L. V., Geng, R. X., & Zhou, C. H. (2014). Comprehensive Review in Current Developments of Imidazole-Based Medicinal Chemistry. Medicinal Research Reviews, 34(2), 340-437. =

Zhou, B., Perel, P., Mensah, G. A., & Ezzati, M. (2021). Global epidemiology, health burden and effective interventions for elevated blood pressure and hypertension. Nature Reviews Cardiology, 18(11), 785–802.

Zhou, G., Rusnac, D.-V., Park, H., Canzani, D., Nguyen, H. M., Stewart, L., Bush, M. F., Tran Nguyen, P., Wulff, H., ... & DiMaio, F. (2024). An artificial intelligence accelerated virtual screening platform for drug discovery. Nature Communications, 15, 7761. https://doi.org/10.1038/s41467-024-52061-7

Zhu, J., Chen, N., Zhou, M., Guo, J., Zhu, C., Zhou, J., Ma, M., & He, L. (2022). Calcium channel blockers versus other classes of drugs for hypertension. Cochrane Database of Systematic Reviews, 1(1), CD003654.

APPENDICES