

**NATURAL PRODUCT BASED GREEN SYNTHESIS OF
HETEROCYCLIC ANALOGS AS ANTIDIABETIC,
ANTIBACTERIAL AND ANTIOXIDANT AGENTS:
A COMBINED EXPERIMENTAL AND COMPUTATIONAL STUDY**



Demis Zelelew Habtegiyorgies

**A dissertation submitted to the Department of Applied Chemistry
School of Applied Natural Science**

**Presented in Partial Fulfillment for the Requirements of the Degree of Doctor
of Philosophy in Applied Chemistry (Organic Synthesis)**

**Office of Graduate Studies
Adama Science and Technology University**

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**Natural product based green synthesis of heterocyclic analogs as
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APPROVAL PAGE

We hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the dissertation entitled “**Natural product based green synthesis of heterocyclic analogs as antidiabetic, antibacterial and antioxidant agents: A combined experimental and computational study**” by Demis Zelelew.

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DECLARATION AND RECOMMENDATION

Declaration

I hereby declare that this Dissertation entitled “**Natural product based green synthesis of heterocyclic analogs as antidiabetic, antibacterial and antioxidant agents: A combined experimental and computational study**” is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials used for this thesis have been duly acknowledged through appropriate citations.

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We, the supervisors of this dissertation, hereby certify that we have read and revised the dissertation entitled “**Natural product based green synthesis of heterocyclic analogs as antidiabetic, antibacterial and antioxidant agents: A combined experimental and computational study**” prepared under our guidance by Demis Zelelew submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Chemistry (Organic Synthesis). Therefore, we recommend the submission of the dissertation to the department for further review and defense.

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DEDICATION

I dedicate this work to my family, for their inspiration, loving support, moral guidance, and timely help in various ways throughout my life.

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LIST OF ACRONYMS AND ABBREVIATIONS

AA	Ascorbic acid
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
AGIs	α -glucosidase inhibitors
AMR	Antimicrobial resistance
ANOVA	Analysis of variance
ASTU	Adama Science and Technology University
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute's standard protocols
d	Doublet
dd	Doublet of doublets
DCM	Dichloromethane
DM	<i>Diabetes mellitus</i>
DNS	Dinitrosalicylic acid color reagent
DIZ	Diameter of inhibition zone
DMSO- d_6	Deuterated dimethyl sulfoxide-deuterated
DOS	Diversity-oriented synthesis
DPPH	1,1-diphenyl-2-picrylhydrazyl
brs	Broad singlet
EDG	Electron donating group
EPHI	Ethiopian public health institute
EPHARM	Ethiopian Pharmaceutical Manufacturing Factory
EWG	Electron withdrawing group
FDA	Food and Drug Administration
FT-IR	Fourier transmission infrared spectroscopy
IC ₅₀	Half-maximal inhibitory concentration
IDDM	Insulin-dependent Diabetes Mellitus
IDF	International Diabetes Federation
IZD	Inhibition zone diameter
LPO	Lipid peroxidation

m	Multiplet
MDR	Multidrug resistance
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
MHz	Megahertz
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 <i>H</i> -tetrazoliumbromide
NMR	Nuclear Magnetic Resonance
NIDDM	Non-insulin-dependent Diabetes Mellitus
PDB ID	Protein data bank identity
PE	Petroleum ether
q	Quartet
R _f	Retention factor
ROS	Reactive oxygen species
RSA	Radical scavenging activity
s	Singlet
SAR	Structure-activity relationship
SEM	Standard Error of Mean
SMILES	Simplified molecular-input line-entry system
t	Triplet
T2DM	Type 2 <i>Diabetes Mellitus</i>
TLC	Thin layer chromatography
TMS	Tetramethylsilane
US	Ultrasonic irradiation
UV-Vis	Ultraviolet -Visible
WHO	World Health Organization
ZnO-NPs	Zinc Oxide nanoparticles

ABSTRACT

Heterocyclic compounds and their analogs possess a broad spectrum of antidiabetic and antibacterial properties, which block postprandial hyperglycemia, and bacterial growth owing to their structural features and the generation of antioxidation products. They have a broad range of therapeutic potential with the ability to regulate the activity of numerous metabolic enzymes. In this study, a greener and more efficient approach has been developed to synthesize a novel series of thiazole-pyrazoline Schiff base hybrids, thiazolo[3,2-*a*] pyrimidine and heterocyclic C₅-curcuminoid analogs using ZnO nanoparticle and ultrasonic irradiation assisted protocol affords good to excellent yields (78.3-96.9%) with improved reaction time. All the synthesized compounds were characterized using a combination of physicochemical parameters and spectroscopic techniques (UV-visible, ¹H-NMR, and ¹³C-NMR). Among the synthesized compounds, **81**, **83**, **93**, **94**, **96**, **98**, **100**, **108**, **111**, and **116** showed good activities against *P. aeruginosa* with mean inhibition zone of 13.33±0.58, 17.33±0.58, 11.00±1.00, 12.00±1.00, 14.67±0.58, 12.33±0.58, 10.67±1.15, 17.67±0.57, 14.33±0.57 and 23.33±0.00 mm diameter, respectively, at 500 µg/mL compared to amoxicillin (15.00±1.00, 12.33±0.58, and 13.75±1.83 mm, respectively) at same concentration. While, compounds **78**, **81**, **83**, **94**, **96**, **100**, **108**, and **111** showed good activities against *E. coli* with mean inhibition zone of 12.00±1.00, 13.33±0.58, 17.00±1.00, 12.33±1.15, 12.67±0.58, 12.00±1.00, 13.00±0.57, and 19.66±0.00 mm diameter, respectively, at 500 µg/mL compared to amoxicillin (14.67±0.58, 13.33±1.15, and 12.87±1.41 mm, respectively) at same dose. While, compounds, **80**, and **99** showed good activities against *S. aureus* with mean inhibition zone of 11±1.00, 12.00±1.73 at 250 µg/mL where as compound, **112** exhibited 11.33±0.57 mm diameter at 500 µg/mL, compared to amoxicillin (14±0.01, 16.33±0.58, and 10.75±1.83 mm, respectively). Compounds **77**, **78** and **80** showed good activities against *S. pyogenes* with mean inhibition zone of 18.33±0.58, 17.00±1.00 and 22.33±1.53 mm diameter, respectively, at 250 µg/mL compared to amoxicillin (29.67±2.52 mm). Compounds **80**, **94**, **111** displayed a higher binding affinities of -8.3, -7.9, -7.5 against Penicillin binding proteins (PBPs) and compounds **83**, **96**, **111** displayed a higher binding affinities of -7.6, -8.3, -8.3 Kcal/mol with β-lactamases producing bacterial strains, compared to amoxicillin (-7.2 and -7.9 Kcal/mol, respectively), these results are in good agreement with the in vitro antibacterial activities. In vitro testing for antidiabetic potential on α-amylase enzyme revealed that compounds **80**, **81**, **82**, **83**, **94**, **98**, **99**, **111**, and **116** with IC₅₀ values of 6.90, 6.74, 6.67, 6.80, 6.56, 6.80, 6.75, 7.59 and 4.08 µg/mL, respectively have comparable to potent inhibitory activities with reference drug, acarbose (IC₅₀= 4.42, 4.42 and 8.0 µg/mL, respectively). The obtained results revealed that, both the biological activities and in silico computational results revealed that compounds **81**, **83**, **94**, **96**, **98**, **100**, **111**, and **116** are good antidiabetic agents against α-amylase enzymes and antibacterial agents against *P. aeruginosa* and *E. coli*. On the other hand, compounds **80**, **81**, **95**, and **112** were found to be promising antibacterial, against *S. aureus*, and antioxidant agents. Compounds **80**, **99**, and **108** displayed significant inhibition of DPPH (IC₅₀= 4.63, 9.48, and 2.44 µg/mL) compared to ascorbic acid (IC₅₀ = 3.21 µg/mL), and compounds **80**, **99**, and **116** displayed 80.01±0.07, 76.28 ± 0.12, and 89.92±0.42% inhibition of peroxide formation suggesting its potential in preventing the formation of lipid peroxides, respectively. The results of the ADMET study showed that all synthesized compounds obeyed Lipinski's rule of five, good oral drug-likeness and drug-score properties without toxic effects to become orally active molecules. Moreover, the molecular docking studies of the most active

compounds showed stronger binding interactions and they are in good agreement with their in vitro biological activities as well as with in silico ADMET profiles, demonstrating promising anti-diabetic properties as well as moderate to strong antibacterial and free radical scavenging activities that may also assist decrease lipid profiles. According to our findings, an efficient synthesis of natural product base heterocyclic compounds via greener protocols of ZnO nanoparticles and ultrasonic-aided approaches are preferable due to increased yields, time savings, and achievement of environmentally friendly reactions, which makes it a useful and eco-friendly strategy compared to the conventional method. Hence, the present study revealed that the synthesized compounds appear to become valuable lead molecules for rational drug design.

Keywords: α -amylase, antibacterial, antioxidant, thiazole-pyrazoline Schiff base, thiazolo [3,2- α] pyrimidine, heterocyclic curcumin, Molecular docking, ADMET, ZnO nanoparticles, Ultrasonic-irradiation

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

Globally, the ever-growing population along with the lifestyle of people has put the life of humankind on an edge of being targeted by various diseases among which *diabetes mellitus* and bacterial infection that are resistant to existing antibiotic agents are the most common (Akash, Rehman, Fiayyaz, Sabir, & Khurshid, 2020; Carrillo-Larco et al., 2022). Over the past decades, the global incidence rate of diseases has risen at an alarming rate hence making it a major health problem of the 21st century (M. B. Gomes et al., 2019). *Diabetes mellitus* (DM) is a chronic metabolic and epidemic disease, which is characterized by prolonged hyperglycemia, and impairment of carbohydrate, fat, and protein metabolism resulting from a deficiency in insulin production or impaired insulin function. Insulin resistance leads to an increased risk of cancer, stroke, cardiovascular disease, retinopathy, nephropathy, and metabolic syndrome (Ibrar et al., 2017).

In 2018, the World health organization (WHO), estimated that over 422 million population around the globe have been affected by DM and this number is expected to increase to about 629 million by 2040 (Organization, 2018). By 2030, the frequency of DM is projected to be double with a 69% increase in developing countries and a 20% increase between the grownups in developed countries (Shaw, Sicree, & Zimmet, 2010). According to the estimates of the International Diabetes Federation (IDF) in 2020, about 4.2 million people aged 20–79 years died as a consequence of Type 2 DM (T2DM) and its complications (F. Hussain et al., 2019). Several strategies being are used for DM management and one of them is controlling postprandial hyperglycemia, by retarding, regulating, and/or inhibiting carbohydrate hydrolytic enzymes. One solution to mitigate DM, especially T2DM, is the regulation of postprandial blood glucose by inhibiting the main carbohydrate hydrolyzing enzymes of α -glucosidase and α -amylase in the intestine (Nyenwe, Jerkins, Umpierrez, & Kitabchi, 2011). α -amylase (α -1,4-glucan-4-glucanohydrolases, E.C.3.2.1.1) is accountable for hydrolyzes α -1,4-glycosidic bonds of starch to simple sugar which can be absorbed by cells (M. Saeedi et al., 2019). Inhibition of the activity of α -glucosidase and α -amylase could reduce postprandial plasma glucose levels and

suppress postprandial hyperglycemia, and thus α -glucosidase and α -amylase has been considered an important target for the treatment T2DM (non-insulin-dependent type 2 DM). Furthermore, they have been recognized as a therapeutic target for other carbohydrate-mediated diseases including cancer, viral infections, HIV, hepatitis, and Alzheimer's disease (M. Saeedi et al., 2019). Currently, prescribed acarbose as both sugar mimics oral α -glucosidase and α -amylase inhibitors remain in parallel with voglibose, and miglitol the most common drugs of T2DM. However, their application is hindered by the side effects of hepatotoxicity and gastrointestinal symptoms. Moreover, the associated disadvantages that require tedious multi-steps reactions for preparation, and low natural abundance prompted to explore new molecules as inhibitors for the targeted enzyme (Rahim et al., 2015). Among present approaches, the use of non-sugar molecules as α -glucosidase/amylase inhibitors has attracted much attention due to their increased availability *via* easy chemical synthesis (Iqbal et al., 2017). Indeed, there is great structural diversity among α -glucosidase/amylase inhibitors, which are not based on a sugar scaffold (Asgari et al., 2020; Gupta, Dutta, Gajbhiye, Jaisankar, & Sen, 2017). Various α -glucosidase/amylase inhibitors have been isolated from nature and synthetic mimics are reported. However, selective and potent α -glucosidase/amylase inhibitors are still in search (Alqahtani et al., 2019; Kazeem, Adamson, & Ogunwande, 2013; Santoso et al., 2022).

It's interesting to note that people with uncontrolled DM (regardless of type) have changes in healing latency and a higher risk of contracting several newly emerging infectious (mostly bacterial) infections. Due to the immune system's inability to successfully defend against invasive microorganisms, infections are a major problem in diabetics (Huang et al., 2018). The increase in bacterial resistance and the rapid emergence of new infections have drastically decreased the efficacy of the drugs employed in the treatment of pathologies caused by certain microorganisms (Dhingra et al., 2020). Antimicrobial resistance (AMR) was once largely confined to hospitals and long-term care facilities, it has now emerged in community settings and represents one of the most pressing global public health concerns (Dhingra et al., 2020). Bacteria can develop resistance by changing target sites, making specialized efflux pumps, mutating their DNA to create new metabolic pathways, or creating β -lactamases, an enzyme that breaks down β -lactams. In extreme cases, drug-resistant bacteria are no longer susceptible to any of the licensed or currently available antibacterial agents (Bernal, Hammann, & Kloss, 2022). Drug-

resistant diseases caused by AMR kill roughly 700,000 people worldwide each year, and without effective intervention, 10 million individuals are expected to die and the global economy will lose around a hundred trillion dollars around the year 2050 (O'Neill, 2016). The lack of novel antibiotic classes since the beginning of the new millennium, the so-called 'antibiotic innovation gap', is a contributing factor to repeated calls from various organizations for the development of new antibiotics (Martelli & Giacomini, 2018). Thus, the discovery and development of new α -glucosidase/amylase inhibitors, antibacterial and antioxidants possessing high efficacy and low side effects are still in high demand as an attractive target (Luepke & Mohr III, 2017; Qadri et al., 2022).

In hyperglycemia and bacterial infectious diseases, the Reactive oxygen species (ROS) reduces insulin and *vice versa* for resistant bacterial strains level which controls the gene transcription and ultimately causes apoptosis. ROS are thought to damage cells by forming free radicals and lipid peroxides in cell membranes (Rao, Kalva, Yerramilli, & Mamidi, 2011; Su et al., 2019). Antioxidants that scavenge ROS are of great value in preventing the development and spread of oxidative diseases (Mady, Awad, & Jørgensen, 2014). They play an important role in protecting against deadly diseases like diabetics and bacterial infectious diseases by turning free radicals and lipid peroxides into ineffective states (Shah et al., 2022). Lipid peroxidation proceeds in the form of a self-sustaining chain reaction and is very harmful and it can be initiated by ROS such as hydroxyl radicals by receiving a hydrogen atom from lipids and forming a conjugated lipid radical. This reacts rapidly with oxygen to form a lipid radical until the chain reaction is terminated. The lipid peroxidation adducts may induce the oxidation of biomolecules, such as DNA, proteins, and other lipids, resulting in cellular damage (Rao et al., 2011; Su et al., 2019). In such events, it would be favorable to find compounds that can have both antidiabetic, and antibacterial agents in combination with antioxidant activities in a single molecule with no toxic effects on health is therefore highly anticipated (Rao et al., 2011). Therefore, there is an imperative need to search for the use of antioxidants in combination with an anti-diabetic and antibiotic drugs greatly help the immune system to combat insulin and drug resistance microbes, respectively (F. Hussain et al., 2019; Volpe, Villar-Delfino, Dos Anjos, & Nogueira-Machado, 2018).

Natural product scaffolds play a key role in drug discovery and in optimizing chemical diversity for human use. The quality of leads arising from natural product discovery is better and often more biologically friendly, due to their co-evolution with the target sites in biological systems (Gerry & Schreiber, 2020). The large number of natural products-derived compounds in various stages of clinical development indicates that the use of natural product templates is still a viable source of new drug candidates. In the drug discovery process, the incorporation of an active pharmacophoric moiety into core bioactive natural products is a key step to obtain a wide range of New Chemical Entities (NCEs) with enhanced biological activities (Katiyar, Gupta, Kanjilal, & Katiyar, 2012). Modifications in the biologically privileged scaffolds have been recognized as an important strategy to search new bioactive compounds for the treatment of various diseases. Of particular interest are the heterocyclic scaffolds, which are prevalent in drugs and natural products. The natural products-based heterocyclic compounds increase metabolic stability, water solubility, and infuse capability to readily interact with biological targets with low toxicity and high efficacy (Nepali, Sharma, Sharma, Bedi, & Dhar, 2014).

Natural and synthetic compounds such as flavonoids, particularly chalcones, and curcumins derivatives have received a lot of attention in medicinal chemistry since they possess incredible synthetic utility (G. Rajendran et al., 2022; Urošević et al., 2022). This has been attributed due to the aryl moieties or the enone linker in the chalcones and curcumins primary structure that can undergo several structural alterations (Prabakaran, Manivarman, & Bharanidharan, 2022). Furthermore, heterocyclic compounds containing nitrogen and sulfur, especially those in the thiazole family, have generated special interest in terms of their synthetic chemistry, which is attributable to their ubiquitous existence in pharmacologically dynamic natural products (H. Kumar et al., 2022; Obaid et al., 2022). They are as overwhelmingly powerful pharmaceuticals due to they contain electron-donating (-S-) and electron-accepting (C=N-) groups, as well as the chirality aspects of these molecules, which have critical influences on activity (Kerru, Gummidi, Maddila, Gangu, & Jonnalagadda, 2020). Owing to this, they exhibited a broad range of bioactivity, and free radical scavenging capacity by delocalization to produce stable DPPH fragments (Youssef, Fouda, & Faty, 2018). Recent clinical trials have increased the significance of thiazole derivatives due to their application as antimicrobial (Khidre & Radini, 2021), antiviral (Dawood, Eldebss, El-Zahabi, & Yousef, 2015), anti-diabetic

(Gollapalli et al., 2019), anticonvulsant (Siddiqui, Partap, Khisal, Yar, & Mishra, 2020), antioxidant (Jaishree, Ramdas, Sachin, & Ramesh, 2012), anti-HIV (Kasralikar, Jadhavar, Goswami, Kaminwar, & Bhusare, 2019), anti-inflammatory (Modrić, Božičević, Faraho, Bosnar, & Škorić, 2021), Alzheimer's disease (Ghotbi et al., 2020), and antitumor activities (Kesari et al., 2021). Therefore, this study is focused on the synthesis of nitrogen-containing heterocycles with a sulfur nucleus, like thiazoles derivatives of thiazole-pyrazoline Schiff base, thiazolo[3,2- α] pyrimidine, and heterocyclics curcumin analogs, are an important class of compounds (Nehra et al., 2020; Petrou, Fesatidou, & Geronikaki, 2021; Rodrigues, Kumar, & Thakur, 2021). In addition, this work focused on the preparations of nitrogen-containing compounds of *N*-pyrazoline, pyrimidine-2-ol, thiazolo[3,2- α] pyrimidine, and β -substituted imidazole analogs through heterocyclization of suitably functionalized substrates of curcuminoid derivatives. On the other hand, in recent years, the use of ZnO nanoparticle and ultrasonic radiation are gaining popularity for a variety of organic transformations due to their ability to reduced pollution, low cost, offer high yields in shorter reaction time, experimental simplicity, greater selectivity properties (B. Banerjee, 2017).

On account of the aforementioned facts, this study was aimed to synthesize natural products, namely; isoflavonoid (chalcone) and curcumin-based heterocyclic analogs (i.e. thiazolyl-pyrazoline Schiff base, thiazolo[3,2- α] pyrimidine, and heterocyclics curcumin) by following a greener route of ZnO nanoparticle and ultrasonic radiation protocols, respectively. The synthesized compounds were evaluated for α -amylase enzyme inhibitory activity via α -amylase inhibition assay to examine the anti-diabetic potential of the compounds as well as their antibacterial against a panel of Gram-positive and Gram-negative bacteria including drug-resistant pathogens along with antioxidant potentials, with the ultimate attempt of obtaining new, safer, potent, and relatively low-cost antidiabetic, antibacterial and antioxidant agents. A preliminary structure-activity relationship (SAR) study was also carried out to investigate the structural features responsible for the antidiabetic, antibacterial, and antioxidant activities of the newly synthesized compounds. Moreover, the *in silico* computational prediction of ADMET and molecular docking studies were performed to confirm drug-like properties and predict the binding interaction of the synthesized compounds in the binding pocket of the target enzymes, respectively.

1.2. Statement of the problem

The treatment of non-communicable diseases like DM and AMR remains a vital and challenging problem due to the rapid rise in insulin resistance, and the limited number of their drugs (Miethke et al., 2021; Toniolo et al., 2019). Recently, WHO has identified insulin and antimicrobial resistance as one of the greatest threats to human health and has adopted a global action plan that outlines particular attention being given to increase investments in the development of new antidiabetic and antimicrobial agents. Nowadays, DM and bacterial infections are the major health challenges of the present era, affecting millions of people worldwide (large number of deaths and increased economic losses), especially in developing countries and newly industrialized countries (Tesfaye et al., 2019). The development of DM is projected to reach pandemic proportions over the next 10-20 years (C.-Y. Wang, Neil, & Home, 2018). The annual health costs caused by DM and its complications account for around 6-12% of all health-care expenditures and are likely to occur predominantly in low- to middle-income countries, where approximately three-quarters of people live with T2DM (Organization, 2016). Nowadays, α -glucosidase/amylase inhibitors (acarbose, miglitol, and voglibose) are a unique class of oral hypoglycaemic agents approved for the prevention and management of insulin-resistant T2DM clinically. However, current approaches including α -amylase have some shortcomings such as safety concerns, limited efficiency, failure in metabolism adjustment, and the prevention of diabetic complications, which necessitates the development of new anti-diabetic agents with improved efficacy and reduced side effects for T2DM therapy (Nazir et al., 2018). In addition, the problem of microbial resistance is growing and the future prospect of the use of antimicrobial drugs is uncertain (Aslam et al., 2018). As a result, currently available antibiotics have undesirable toxicity, and the widespread use of these drugs has led to the rapid development of drug-resistant strains. This resistant strains cause failure in clinical applications. Hence, the design and discovery of effective small organic molecules with convenient synthesis with high inhibitory activity against target enzymes, and low side effects is an attractive target for synthetic chemists. In addition, the synthesis of novel bioactive compounds using green methods that minimize the use and generation of hazardous substances is a major aim for many researchers. Therefore, searching for new antidiabetic, antibacterial and antioxidant drugs based on structural modification of natural products towards their heterocycles through green synthetic

routes of ZnO nanoparticle and ultrasonic irradiation -assisted conditions for new therapies to minimize patients suffering and reduce costs with today's expensive treatments are needed urgently.

1.3. Justification of the study

Natural products are structurally fine-tuned by nature for optimum bioactivity, often only minor structural modifications are necessary to optimize drugability (W. Liu et al., 2019). Among these, chalconoid and curcuminoids are major classes of natural products with widespread distribution in nature. They have attracted great interest for the synthesis of five, and six-membered heterocyclic compounds, in addition to their pharmacological activities (Jangir, Dhadda, & Jangid, 2022). It is therefore, not surprising that many synthetic methods have been developed for the preparation of chalcones/curcumins and their heterocycles starting from their precursors that have been tested for their potential therapeutic applications. Keeping in view the potential biological activities of chalcone and/or curcumin scaffolds and their heterocyclic derivatives, it was perceived that the synergistic effect of the two moieties in a single nucleus might result in the formation of some worthwhile molecules from the biological point of view (Elkanzi et al., 2022; D. Kumar, Kumar, Kumar, & Kumar Singh, 2013). On the other hand, green chemistry methods have gained much attention over conventional techniques for rapid organic synthesis (Rubab et al., 2022). Hence, both ZnO nanoparticles and ultrasonic irradiation conditions are nowadays well-established eco-friendly approaches for the synthesis of various heterocycles due to their spectacular results such as reduced pollution, low cost, offer high yields in shorter reaction time, experimental simplicity, greater selectivity together with simplicity in processing, and handling (Kaur, 2018; Mohammadi Ziarani, Kheilkordi, & Gholamzadeh, 2020). Thus, a greener and more efficient synthesis, and development of new antidiabetic, and antibacterial in combination with antioxidant agents possessing high efficacy and low side effects from heterocyclization of natural products precursors are still in high demand as an attractive target.

1.4. Significance of the study

Treating hyperglycemia, drug resistance, and controlling subsequent complications are the main outcomes of diabetics and infectious diseases therapy. The limitation of currently available oral α -glucosidase/amylase inhibitors and antibiotics either in terms of efficacy/safety coupled with the emergence of insulin resistance and drug-resistant bacteria into the global epidemic has encouraged alternative therapy that can manage T2DM, and antibiotic resistant infectious diseases more efficiently and safely (Hegazy, Rajab, Lila, & Abbas, 2021). In this study, the synthesis and identification of a novel class of non-glycosidic natural product-inspired heterocyclic analogs, which was expected to overcome insulin and drug resistance, side effects and increase antidiabetic, antibacterial, and antioxidant bioactivities thereby helping in addressing drug resistance-related issues. Moreover, the design and synthesis strategy used in this study would help in reducing the cost and providing affordable health care to all. Furthermore, limited study has been reported on natural products derived heterocyclic conjugates as antidiabetic, antibacterial, and antioxidant agents evidenced by literature search to date. Hence, this research addresses investigation for new heterocyclic compounds derived from natural product scaffolds, since numerous natural product hybrids have not yet been explored. This study could also serve as a road map for further structural-based drug design and development.

CHAPTER TWO

2. OBJECTIVES OF THE STUDY

2.1. General Objective

The main objective of this study was to synthesize and evaluate *in vitro* and *in silico* biological activities of natural product-based heterocyclic analogs as antidiabetic, antibacterial and antioxidant agents

2.2. Specific Objectives

- To synthesize thiazolyl-pyrazoline Schiff base hybrids, and thiazolo[3,2- α] pyrimidine analogs derived from chalcone derivatives using ZnO nanoparticle assisted reaction condition compared to conventional approach.
- To synthesize heterocyclic C₅-curcumin analogs derived from curcumin derivatives using ultrasonic irradiation-assisted reaction condition compared to conventional approach.
- To characterize the structures of the synthesized compounds using physicochemical parameters (m.pt, R_f, and color) and spectroscopic techniques (UV-Vis, ¹H-NMR, and ¹³C-NMR).
- To evaluate the antibacterial activities of synthesized compounds against pathogenic bacterial strains of Gram-positive bacteria, including methicillin-resistant *S. aureus* (ATCC 29213), and *S. pyogenes* (ATCC 27853), and Gram-negative bacteria, *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853), respectively using disc diffusion method and compared with the reference drug.
- To investigate the *in vitro* antioxidant activity of all synthesized compounds using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and lipid peroxidation inhibition assays
- To evaluate α -amylase enzyme inhibitory activity *via* α -amylase inhibition assay to examine the anti-diabetic potential of the compounds.
- To study *in silico* ADMET prediction and molecular docking studies of synthesized compounds against selected protein targets' inhibitory potential using SwissADME/PreADMET and Autodoc vina 4.2 version software programs, respectively.

CHAPTER THREE

3. LITERATURE REVIEW

3.1. *Diabetes mellitus and oral antidiabetic agents*

DM is a metabolic disorder characterized by chronic hyperglycemia that can cause further serious health problems such as chronic microvascular, macrovascular, neurological, and cardiovascular complications (F. Hussain et al., 2019). In 2018, approximately half a billion people (463 million) worldwide were affected by DM, and this number is predicted to reach 578 million and 700 million by 2030 and 2045, respectively (P. Saeedi et al., 2019). The WHO has estimated that 439 million people will be diabetic in 2030 (Organization, 2016). Report to IDF 2017, there are 422 million people experiencing diabetes in the world and this figure is expected to rise to 642 million in 2045 (Cho et al., 2018). Globally, the number of people living with diabetes mellitus quadrupled between 1980 and 2016 (Krug, 2016). Despite a paucity of updated regional data in Africa, IDF 2015 estimated a regional prevalence of 2.1-6.7% in sub-Saharan Africa as depicted in [Figure 1](#) (Zheng, Ley, & Hu, 2018).

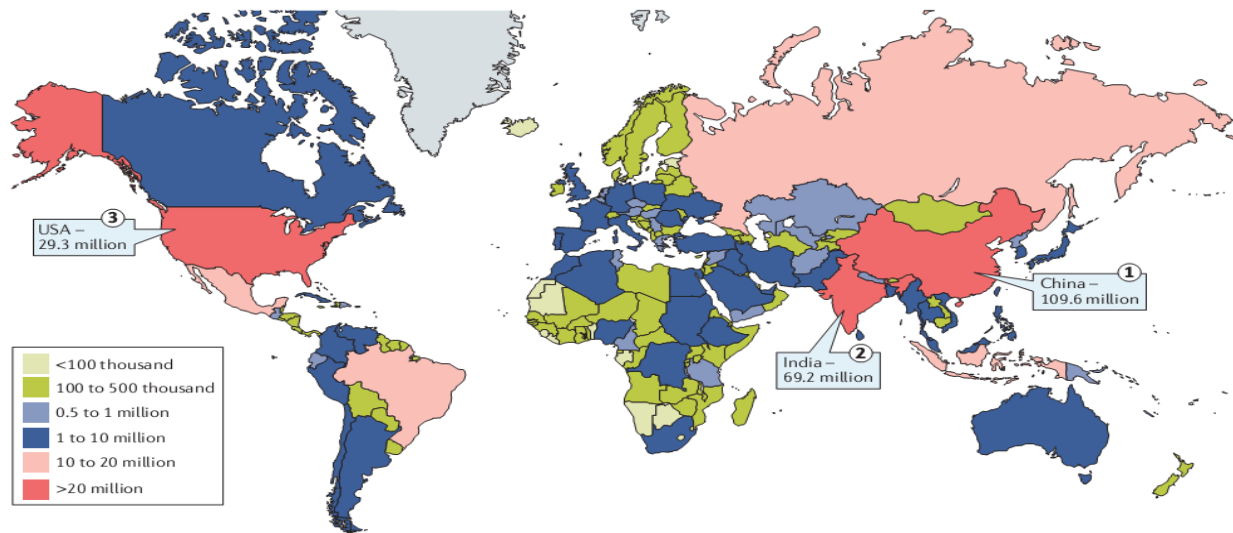


Figure 1. Estimated total number of adults living with DM for the number of adults with diabetes mellitus in 2015 (Zheng et al., 2018).

The global prevalence of DM is increasing across the world, with over 70% of morbidity and 88% of mortality because of diabetes occurring in low and middle-income countries (LMICs). This is due partly to rapid lifestyle modification, urbanization, as well as an increase in life expectancy (Bekele, 2019). The burden of DM is high in Sub-Saharan Africa countries following Asian countries. According to IDF 2017 report, about 15.5 million adults aged 20-79 years were living with DM in the Africa region, representing a continental prevalence of 3.3%. The highest prevalence of diabetes in the region is found in adults aged 55-64 years (Motala, Mbanya, Ramaiya, Pirie, & Ekoru, 2022; Sivieri, de Oliveira, de Souza Marquez, Pérez-Jiménez, & Diniz, 2022). Developing countries will share the burden of DM to the magnitude of 77% of the global burden in the 21st century. This possibility is due to population growth, aging, unhealthy diets, obesity, and sedentary lifestyles (Zeru, Tesfa, Mitiku, Seyoum, & Bokoro, 2021). Oral hypoglycemics and insulin were the most common treatments. These classes of drugs are either administered as monotherapy or given in combination with other hypoglycaemics. Severe hypoglycemia, weight gain, lower therapeutic efficacy owing to improper or ineffective dosage regimen, low potency and altered side effects due to drug metabolism and lack of target specificity, solubility, and permeability problems are the major draw-backs associated with the use of the above mentioned conventional drugs. Despite the advent of promising anti-hyperglycemic agents, the major challenges in efficient diabetes treatment include optimizing the existing therapies to guarantee optimum and balanced glucose concentrations, as well as reducing long-term diabetes-related complications (Padhi, Nayak, & Behera, 2020).

3.1.1. Types of *Diabetes mellitus*

Depending on the mechanism by which it occurs, diabetes can be of three types: Type 1 diabetes (T1DM), Type 2 diabetes (T2DM), and Gestational diabetes. T1DM affects about 5–10% of the total diabetic patients and is caused due to autoimmune-mediated selective destruction of β cells of the pancreas that produce insulin, leading to absolute insulin deficiency, hyperglycemia, oxidative stress, inflammation, and other metabolic complications (Rashid, Chowdhury, Ghosh, & Sil, 2017). Whereas T2DM or non-insulin dependent DM is described by insulin deficiency related to dysfunction of these β - cells and insulin resistance in target cells (Zimmet, Alberti, & Shaw, 2001). Insulin resistance due to insulin receptor (IR) insensitivity, chronic hyperglycemia,

low-grade inflammation, and dyslipidemia are the features of T2DM (Esser, Paquot, & Scheen, 2015). Gestational diabetes is diagnosed in pregnant women, featuring adverse clinical conditions in the mother and offspring (Jiao, Hua, Huang, Zhang, & Yan, 2018) (Figure 2). So, controlling the blood glucose level in diabetic patients is vital (Hossain, Das, Ghosh, & Sil, 2020).

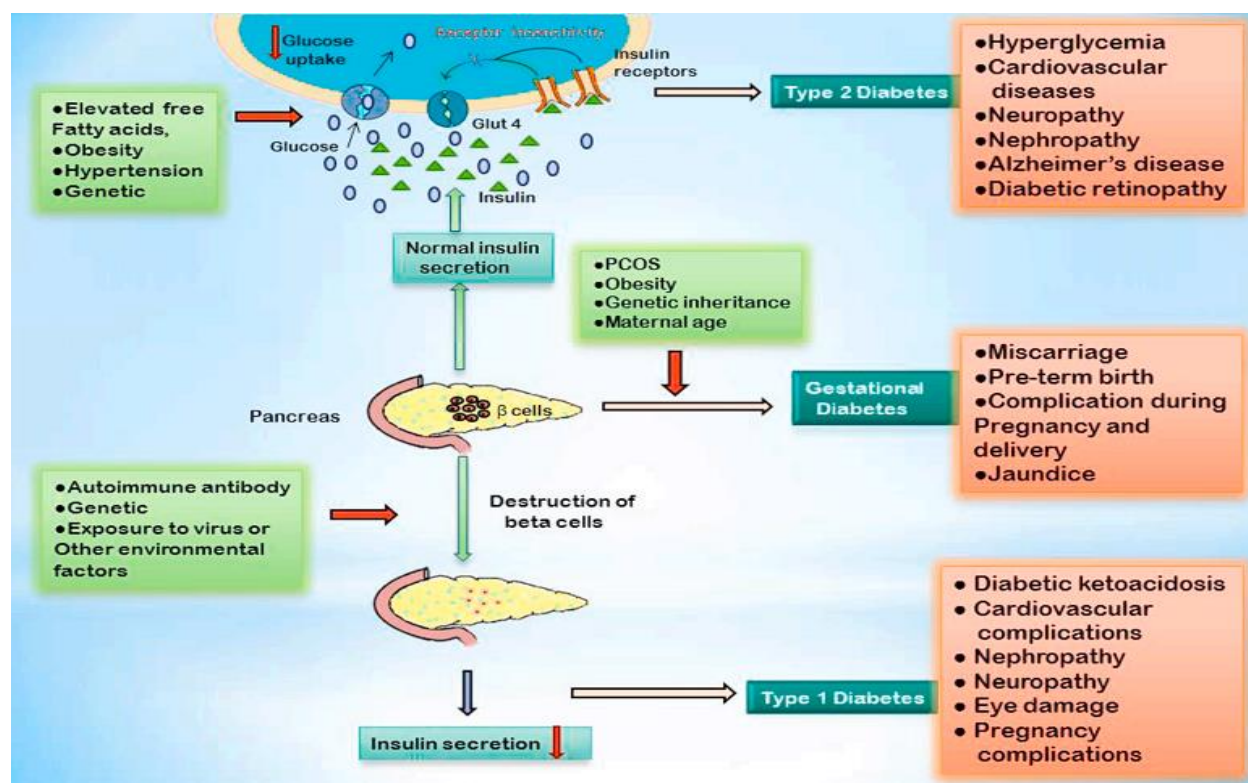


Figure 2. Schematic diagram of different types of diabetes, their cause, and consequences (Hossain et al., 2020).

3.1.2. Role of α -glucosidase/amylase inhibitors in controlling postprandial hyperglycemia

Hyperglycemia is a critical condition in both T1DM and T2DM patients and is the main contributing factor behind oxidative stress and its deleterious consequences. Hyperglycemia can induce ROS generation and accumulation via various metabolic pathways (Vanessa Fiorentino, Priolella, Zuo, & Folli, 2013). In diabetic conditions, as glucose uptake in insulin-dependent tissues (fat and muscle) is minimized, uptake of glucose is elevated in insulin-independent

tissues (King & Loeken, 2004). This excessive intracellular glucose is converted to polyalcohol sorbitol, resulting in a decrease in NADPH/NADP⁺ ratio and glutathione (GSH) concentration. In addition, hyperglycemia leads to the activation of PKC (protein kinase C) isoforms, induction of hexokinase pathway, and overproduction of advanced glycation end products (AGEs). All these effects of hyperglycemia are responsible for removing antioxidant agents and overproduction and accumulation of reactive oxygen species, which ultimately leads to oxidative stress. The detrimental consequences of this oxidative stress condition are long-term damage and pathophysiological conditions of various organs like kidney, heart, liver, testis, spleen etc. (Sumit Ghosh, Chowdhury, Sarkar, & Sil, 2018). For this reason, researchers are trying to control hyperglycemic conditions in order to reduce various diabetic complications. The most eminent hyperglycemia-controlling agents are the AGIs, which slow down α -glucosidase activity and efficiently diminish postprandial hyperglycemia. According to the guidelines of IDF, AGIs in combination with insulin, metformin, and sulfonylureas are the best treatment option for uncontrolled hyperglycemia in diabetic patients (Lozano-Ortega et al., 2016).

Almost all AGIs structurally resemble disaccharides or oligosaccharides and can bind to the active site of α -glucosidase/amylase, forming complexes with stronger affinity than that of the carbohydrate- α -glucosidase complex. This results in the competitive inhibition of α -glucosidase/amylase activity and diminishes carbohydrate hydrolysis and glucose absorption in the brush boarder site of the small intestine (Figure 3). Most of the carbohydrates that are not hydrolyzed are subsequently broken down in the lower parts of the small intestine and result in delayed glucose absorption after meal. This mechanism of action of AGIs reduces postprandial hyperglycemia, which is an efficient remedy against various diabetic complications. Another striking characteristic of AGIs is that they can assist in the stimulation of glucagon-like peptide (GLP1) (an incretin hormone) secretion, which helps lower postprandial hyperglycemia by triggering insulin secretion and inhibiting glucagon secretion (Drucker & Nauck, 2006). GLP1 is secreted from intestinal L cells, on sensing food intake. AGIs delay polysaccharide digestion that results in increased local carbohydrate concentration in the lower gut. Since the lower gut has a sufficient amount of GLP1-secreting cells, belated carbohydrate absorption helps to stimulate GLP1 secretion properly. Thus, AGI helps in GLP1 secretion, which in turn stimulates insulin secretion (Hossain et al., 2020).

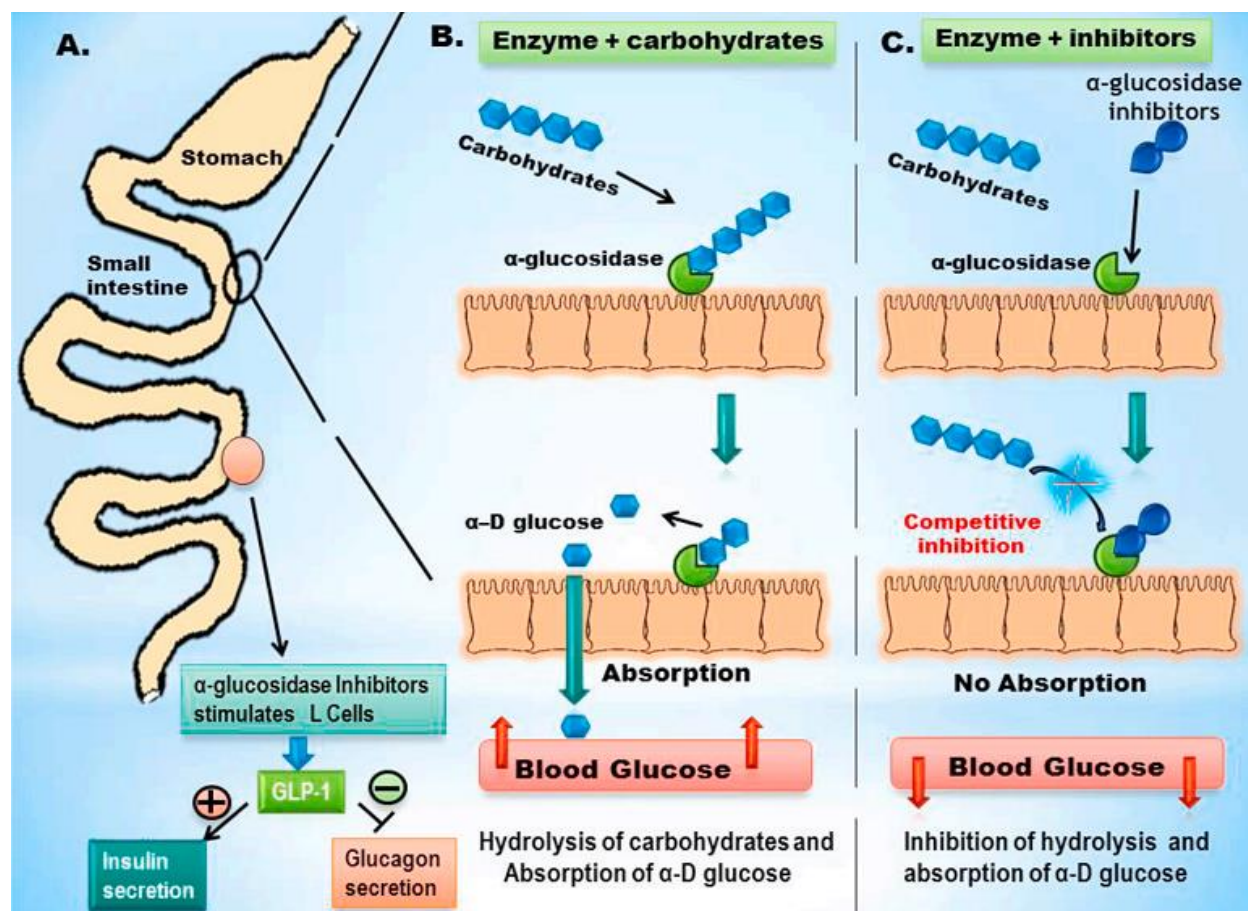


Figure 3. Role of α -glucosidase/amylase inhibitors in controlling postprandial hyperglycemia (Hossain et al., 2020).

Where, (A) schematic diagram of the small intestine; stimulation of L-cells by α -glucosidase/amylase inhibitors and secretion of glucagon-like peptide-1 (GLP-1) from the lower part of small intestine; (B) hydrolysis of carbohydrates by α -glucosidase/amylase along the brush border of small intestine, the release of α -D glucose and its absorption leading to elevation of blood glucose level; (C) Competitive inhibition of α -glucosidase/amylase by its inhibitors and restrict the hydrolysis of carbohydrates and absorption of α -D glucose, leading to decrease in postprandial elevation of blood glucose level.

3.1.3. Anti-diabetic potential of natural products targeting α -glucosidase/ amylase enzyme

Natural products have played a critical role as lead molecules in drug discovery (Atanasov, Zotchev, Dirsch, & Supuran, 2021). Reports also showed that synthetic and semi-synthetic derivatives of natural products exhibit various biological activities (B. Chopra & Dhingra, 2021). Fortunately, naturally occurring anti-diabetic drugs have never been obsoleted and still play a

principal role in the management of T2DM, many of which are known to be effective against DM (Xu, Li, Dai, & Peng, 2018). Metformin, derived from *Galega officinalis*, is typical for treating diabetes, which has been used as a first-line drug for T2DM for 60 years (Bailey, 2017). In spite of the availability of numerous drugs for the regulation of hyperglycemia, there has been a growing demand for the further design and synthesis of potential agents for the treatment of DM and its associated pathologies from natural products derived scaffolds (Marín-Peñalver, Martín-Timón, Sevillano-Collantes, & del Cañizo-Gómez, 2016).

Retarding glucose absorption by inhibiting carbohydrate digestive enzymes activities is considered effective management of T2DM (Perez Gutierrez, 2016). Different therapeutic approaches used for the cure of diabetes are motivating endogenous secretion of insulin, reduction of insulin demands, increasing the action of insulin at the target tissue, and inhibiting the degradation of di- and oligosaccharides. Pancreatic alpha-amylase is a metallo-enzyme containing Ca^{2+} ions in its active site and speeds up hydrolysis of starch into maltose and glucose. This enzyme fascinated extensive attention due to its capability of hydrolyzing α -1,4-glycosidic connection of starch and activities that can be carried out due to hydrolysis (Bashary et al., 2020). There are many alpha-amylases present in human digestive system including pancreatic-amylase (EC3.2.1.1), which help in the hydrolysis of α -1,4-glycosidic linkage of glycogen, amylopectin, amylose, and play role in the starch digestion (Mahnashi et al., 2022). Competitive inhibitors of these enzymes effectively delay and slow down the absorption of glucose and control the increase of blood sugar level (Wani, John-Kalarickal, & Fonseca, 2008).

The insulin plays its role, prevents the blood sugar level from rising, and stimulates the cells to convert them into an energy unit, glycogen. Several studies reveal that with excessive enzymatic activity, there is resistance or deficiency of insulin which leads to hyperglycemia. Several oral hypoglycemic drugs for the treatment of hyperglycemia include sulfonylureas (enhance the release of insulin from pancreatic islets); biguanides, thiazolidinediones (reduces hepatic glucose production); peroxisome proliferator-activated receptor- γ (PPAR γ) agonists (boosts the action of insulin); α -glucosidase/amylase inhibitors (interferes with absorption of glucose in the gut) are common medical recommendations for controlling diabetes. Acarbose, miglitol, and voglibose are important inhibitors used in clinical treatment (Chaudhury et al., 2017; Z. Liu & Ma, 2017).

These drugs are used along with other diabetic drugs for better efficacy. New therapy with a low risk of side effects for diabetes needs to be explored.

The most common and commercially available synthetic anti-diabetic drugs are Acarbose (Glucobay **1**, BAY g 5421) (inhibits the activity of maltase α -amylase and sucrase), Miglitol (Glyset **2**, inhibits glucoamylase and the activity of isomaltose and starch) and Voglibose (Volix **3**, Basen, bacterial origin and it inhibits the activities of sucrose and maltase) (Huneif et al., 2022) (Figure 4). Of these drugs, the first to be approved was acarbose, which is the most widely used α -glucosidase inhibitor. However, these drugs are expensive and commonly associated with side-effects and draw-backs like insulin resistance, anorexia nervosa, brain atrophy, hepatotoxicity, abdominal pain, and flatulence, which limit their applications (Abbas, Al Harrasi, Hussain, Hamaed, & Supuran, 2019). Moreover, such inhibitors, which are of great structural diversity, require tedious multi-steps for preparation, and their clinical use is associated with gastrointestinal side effects. Besides, α -glucosidase/ amylase inhibitors are a unique class of antidiabetic drugs, described as an attractive therapeutic target for type-2 diabetes, and also be used for other diseases in which carbohydrates or their biosynthesis are potential targets including cancer, viral infections, HIV, hepatitis and Alzheimer's disease. Indeed, there is great structural diversity among glucosidase inhibitors, which are not based on a sugar scaffold. Therefore, more effective and safer therapeutic drugs remain to be urgently developed. Therefore, the development of potent α -glucosidase/amylase inhibitors with small structures and low side effects has attracted great attention in recent years by mimicking nature.

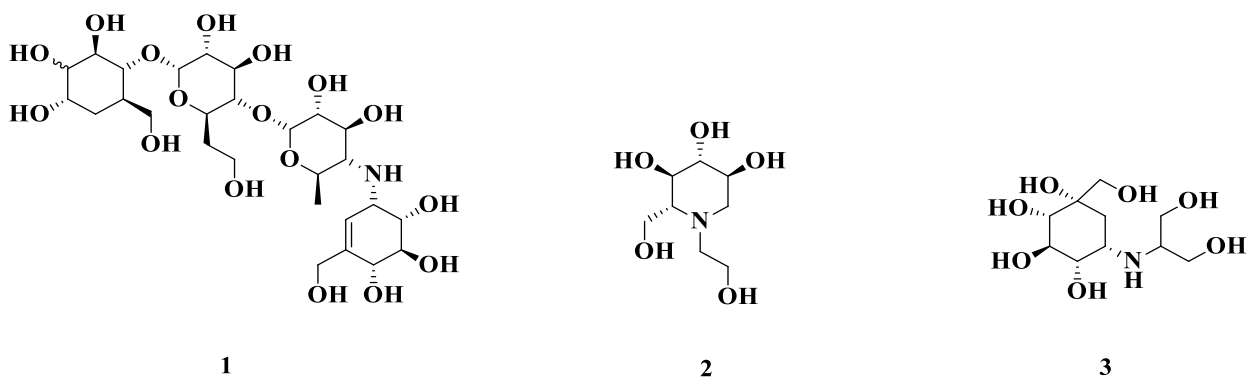


Figure 4. Structures of commercially available α -glucosidase/amylase inhibitors (Dowarah & Singh, 2020; Z. Liu & Ma, 2017).

3.2. Bacterial resistance and factors contributing to resistance

Bacteria can evade the antimicrobial activity of antibiotics *via* three different but related mechanisms: resistance, tolerance, and persistence. Resistance is the ability of bacteria to grow in the presence of high concentrations of an antibiotic and is caused by inherited mutations, which affect the efflux pumps, the drug target, or the antibiotic molecule itself (Tanwar, Das, Fatima, & Hameed, 2014). Pathogenic bacterial organisms adopt multiple antimicrobial resistance mechanisms to overcome the effect of different antibacterial agents (Kapoor, Saigal, & Elongavan, 2017). In general, AMR mechanisms are divided into four major classes: A) drug uptake limitation, B) drug target modification, C) drug inactivation, and D) active drug efflux. Restricted drug uptake, drug inactivation, and drug efflux are some examples of intrinsic resistance mechanisms adopted by different bacterial organisms; while drug target modification, inactivation of the drug, and drug efflux are examples of acquired resistance mechanisms. The different kinds of resistant processes used by gram-negative bacteria and gram-positive bacteria differ due to changes in their structure etc (Reygaert, 2018). Overuse/misuse of antibiotics, unsuitable and wrongly given antibiotics, large agricultural utilization of antibiotics, and the accessibility of only a few innovative antibiotics are the factors contributing to the establishment of antibiotic tolerance issues and crises (Ventola, 2015). On the other hand, due to scientific constraints, clinical impediments, and poor economic yields, development in discovering novel antimicrobial agents has slowed. Since the quality of pharmaceutical drugs is still in question, developing countries and regions with low resources, a defective pharmaceutical supply chain and poor health service management systems may contribute most to the development of the AMR phenomenon (Chokshi, Sifri, Cennimo, & Horng, 2019). AMR propagation has been accelerated during and after the COVID-19 outbreak due to overutilization and misuse of current antimicrobial drugs, in addition to other well-known variables that drive the AMR phenomenon (Lobie et al., 2021). Faced with this reality, the need for action to avert a developing global crisis in health care is imperative. Although the acquisition of resistance is a natural process, it is exacerbated by the misuse of antibiotics, inadequate surveillance, and the poorly controlled regulation of antibiotics in clinical medicine and in the livestock industry, which led to the appearance and spread of drug resistance bacteria all over the world (Levy, 2002).

3.2.1. Mechanism of action of membrane targeting antibiotics

One of the potential targets of novel antibiotics that could be effective against resistant bacteria is the bacterial cell membrane. Many antibiotics target the bacterial membrane, but new, more potent ones are needed. One of the reasons why the bacterial membrane is an attractive target for new antibiotics is that the bacterial membrane is arranged differently from a mammalian membrane (Belete, 2019). An important difference is that ionic lipids are exposed on the surface of bacterial membranes, while in eukaryotic membranes, anionic lipids are sequestered to the monolayer facing the interior of the cell or organelle. For this reason, many of the antimicrobial agents are cationic so they have greater selectivity for bacterial membranes. The molecular components and morphology of membranes from Gram-positive bacteria are fundamentally different from those of Gram-negative bacteria (Bharadwaj, Rastogi, Pandey, Gupta, & Sohal, 2022) (Figure 5).

Gram-positive bacteria keep their complex stain and look purple, but Gram -negative bacteria lose their complex stain and appear pink owing to counter stain. The change in cell wall composition between the two species of bacteria is the cause of this discrepancy. The outer membrane, which serves as a protective barrier, has a unique characteristic that distinguishes Gram-positive bacteria from Gram-negative bacteria (Epand, Walker, Epand, & Magarvey, 2016). The outer monolayer of the membrane contains lipopolysaccharide (LPS) as the major lipid component, a lipid species unique to Gram-negative bacteria. Although Gram-positive bacteria do not have the additional outer membrane layer which Gram-negative bacteria do, they share the commonality of having a cell wall surrounding the cytoplasmic membrane of the cell (Epand et al., 2016).

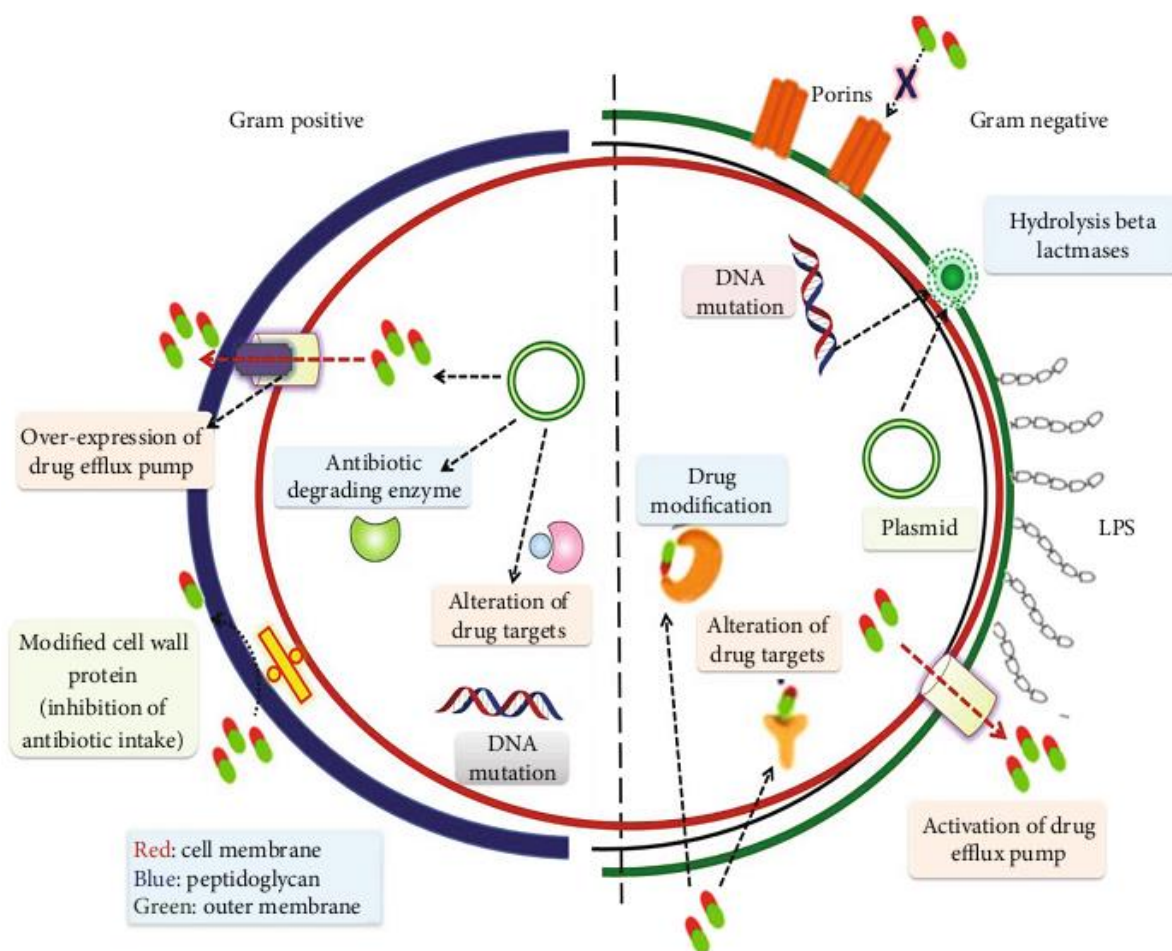


Figure 5. Resistance mechanisms and comparing Gram-positive (left) and Gram-negative (right) bacterial cell membranes (Bharadwaj et al., 2022; Epand et al., 2016).

The cell wall is comprised of peptidoglycan. In addition, the cell wall of Gram-positive bacteria contains teichoic acids. The major lipid components of the inner monolayer of the outer membrane of Gram-negative bacteria, as well as both monolayers of the cell membrane of both types of bacteria, include phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and cardiolipin (CL). Different species of bacteria have very different proportions of these three lipids (I. Chopra, 1988). Some bacterial species also have significant amounts of glycosyl diglycerides in addition to a number of other minor lipid components.

3.3. Natural products and their semi-synthetic derivatives as a source of antidiabetics, antibiotics and antioxidants

Natural molecules are natural arsenal against diseases for mankind and living beings. Natural products have been playing an important role in drug discovery, even in the area of diabetics and infectious diseases (Atanasov et al., 2021). They are versatile building block for lead generation, and easily accessible to diverse derivatives for subsequent lead optimization, due to they playing a pivotal role in antidiabetic, antioxidant, and antibiotic drug discovery (Wright, Seiple, & Myers, 2014). Increasingly these natural molecules have inspired synthetic chemists to modify the natural product molecules in the desired way to incorporate therapeutic potential in them. This fashion of tailoring the natural molecules in desired way arises the area of natural product-inspired synthesis (Tripathi, 2021).

Over the last two decades, much effort had been devoted by synthetic chemists toward the synthesis and bio-evaluation of nature-inspired molecules (Guo, 2017). Hence, natural products-based drug discovery is characterized by a starting point of an active compound that necessitates an ideal or individualized manipulation of the structure to reach a drug criterion (W. Liu et al., 2019). Natural and synthetic compounds containing α,β -unsaturated ketones, especially (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one, **4** and 1,3-diarylprop-2-en-1-ones, **11** or **12**, are commonly known as curcumin, and chalcones, have received much attention in medicinal chemistry as they possess a strong and broad variety of biological activities including antibacterial, antifungal, antidiabetic and antioxidant (Detsi, Majdalani, Kontogiorgis, Hadjipavlou-Litina, & Kefalas, 2009; Mishra, Patel, & Halpani, 2019; Tiwari, Bendi, & Bhathiwal, 2021). Recently, research has been focused on natural products containing heterocycles, which are powerful pharmacophores for potent and selective antidiabetic, antibiotic, and antioxidant drugs in pharmaceutical evaluations (Kerru et al., 2020).

Thus, the search for heterocyclic compounds from natural origin for the development of new antidiabetic and antibacterial drugs remains an important strategy in the fight against insulin and bacterial resistance, respectively.

3.3.1. Naturally occurring biologically active curcumin derivatives

Curcumin (diferuloylmethane or (1*E*, 6*E*)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, (**4**) Figure 6) is a natural phenolic compound isolated as a yellow pigment from the dried rhizome of *Curcuma longa* (turmeric), “Golden spice,” a plant that is commonly used as a spice, as a food colorant, and even as a food preservative. Importantly, curcumin has potent antioxidant properties due to the presence of phenolic and enolic hydroxyl groups. Curcumin has various biological applications including antibacterial, antiviral, anti-angiogenesis, anti-diabetes, anti-Alzheimer, and anti-inflammatory activities. Curcumin is a bis- α , β -unsaturated β -diketone which exists both in the keto as well as the enol form in equilibrium. It is observed that in neutral and acidic conditions, the keto form predominates whereas, in alkaline conditions, the enolic form predominates (Abbas et al., 2019).

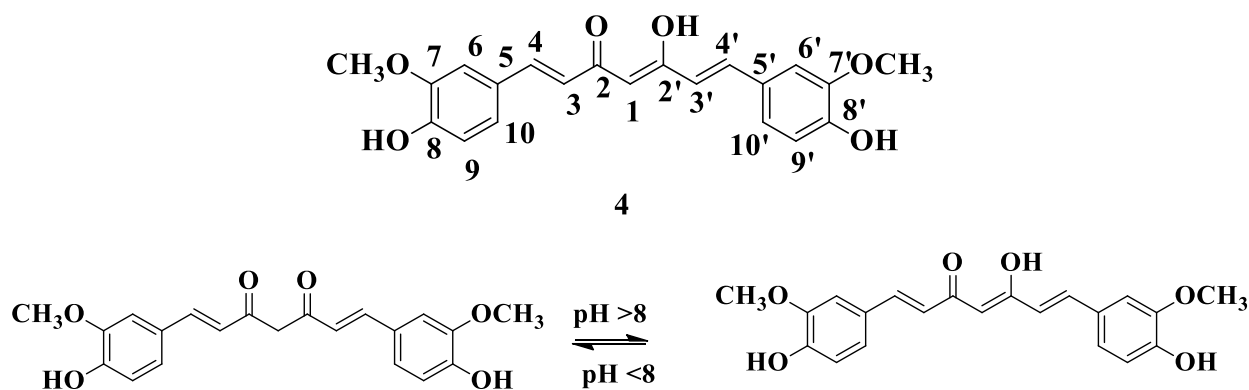


Figure 6. Chemical structure of curcumin and its keto-enol tautomerism (Salem, Rohani, & Gillies, 2014; Urošević et al., 2022).

Moreover, the poor solubility, absorption, bioavailability, and rapid metabolism of curcumin in a human probably limit its clinical efficacy. The variation on curcumin has been done with active methylene groups/replacing the β -diketone bridge to enhance the biological activity than the natural curcumin. To overcome many of such limitations, mono-carbonyl analogs of curcumin have been synthesized by deleting the reactive β -diketone moiety (which was considered to be responsible for the pharmacokinetic limitation of curcumin) through several chemical modifications in the basic structure of curcumin to increase its potential, stability, and

bioavailability. Curcumin is one of the key components of the curcuminoids group. The non-toxic food origin and wide range of pharmaceutical drug properties of curcuminoids ensure they are promising lead molecules for medicinal chemistry. The natural curcuminoids of significance are curcumin (4), demethoxycurcumin (5), bisdemethoxycurcumin (6), and cyclocurcumin which are present in approximately 77%, 18% and 5% of the composition of turmeric, respectively. In addition, tetrahydroxycurcumin (7), hexahydroxy curcumin (8), cyclocurcumin (9) and monocarbonyl curcumin (10) (Figure 7) (Amalraj, Pius, Gopi, & Gopi, 2017; Priyadarsini, 2014). Thus, the syntheses of new curcuminoids are very interesting and important research. Thus, the researchers have been focused on developing new curcumin derivatives with enhanced biological activity and pharmacological properties.

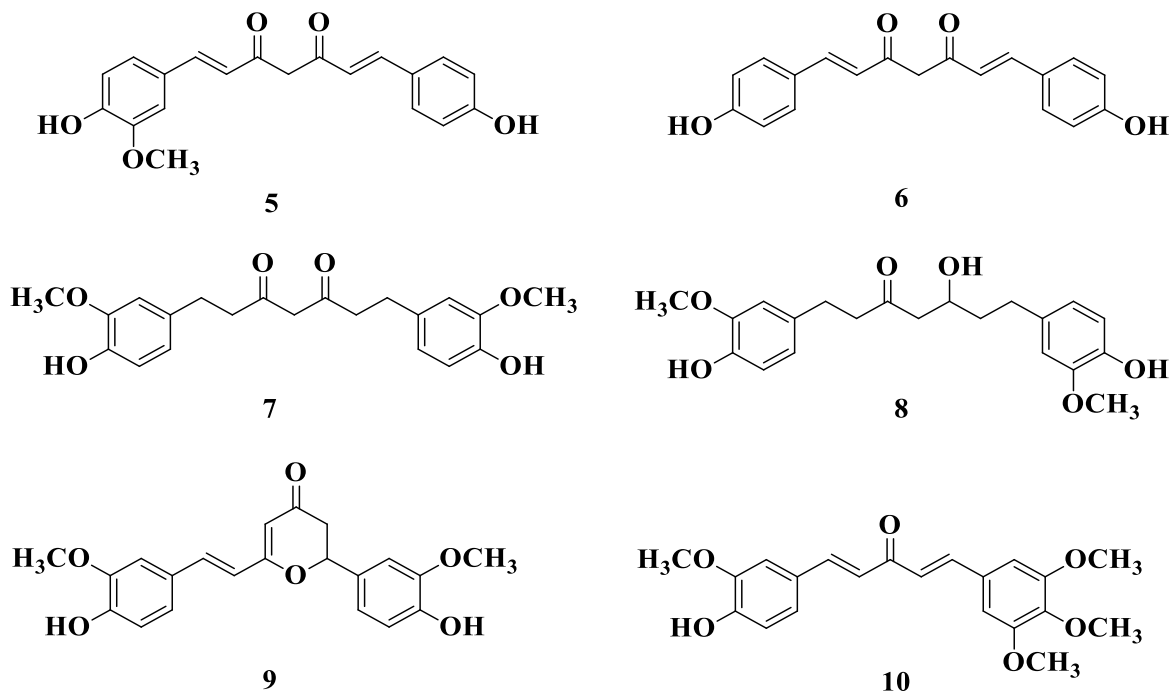


Figure 7. Structure of most potent curcuminoids and C₅-curcuminoid derivatives (Amalraj et al., 2017; Anand et al., 2008).

3.3.2. Naturally occurring biologically active chalcone derivatives

Chalcones (1,3-diaryl-2-propenones, Figure 8) which belong to the flavonoid family, are open-chained molecules bearing an α, β -unsaturated carbonyl system between two aromatic rings and

represent a crucial class of molecules that are abundant in edible plants (Pérez-González, Castañeda-Arriaga, Guzmán-López, Hernández-Ayala, & Galano, 2022). Chalcones are an important group of natural compounds that are particularly abundant in many plant species, some of which have been used for centuries in traditional herbal medicine (Rammohan, Bhaskar, Venkateswarlu, Gu, & Zyryanov, 2020). Chalcones reportedly possess beneficial biological activities, which remain a fascination among researchers in the 21st century due to their simple chemistry, ease of synthesis, and a large number of replaceable hydrogens to yield a variety of derivatives. Many studies on chalcone derivatives have shown that they demonstrate antioxidant, antibacterial, antimalarial, antifungal, antidiabetic, antimalarial, and anticancer activities (Chinthala et al., 2015; Dhaliwal et al., 2022).

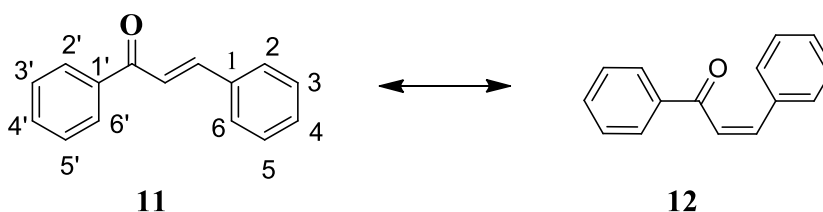


Figure 8. Chemical structure of *trans* (E) (**11**) and *cis* (Z) (**12**) –chalcones (M. N. Gomes et al., 2017).

In this regard, natural and synthetic flavonoids, especially 1,3-diarylprop-2-en-1-ones, commonly known as chalcones, have received much attention in medicinal chemistry due to their possessing a strong and broad variety of biological activities (Detsi et al., 2009). During the last decade, several chalcone analogs and derivatives have been designed with the enone moiety replaced by a heterocyclic ring in order to get rigid analogs. They are ubiquitous, and their derivatives possess diverse biological properties the most potent agents are those possessing a trimethoxy unit on one aryl ring and a 3-hydroxy-4-methoxy phenyl ring as the other aryl ring and belong to the family of flavonoids example licochalcone A (**13**), licochalcone D (**14**), morachalcone A (**15**), butein (**16**), xanthohumol (**17**) and dorsmannin (**18**) are most potent chalcones exhibited antibacterial activities (Figure 9) (Okolo et al., 2021; Salehi et al., 2021).

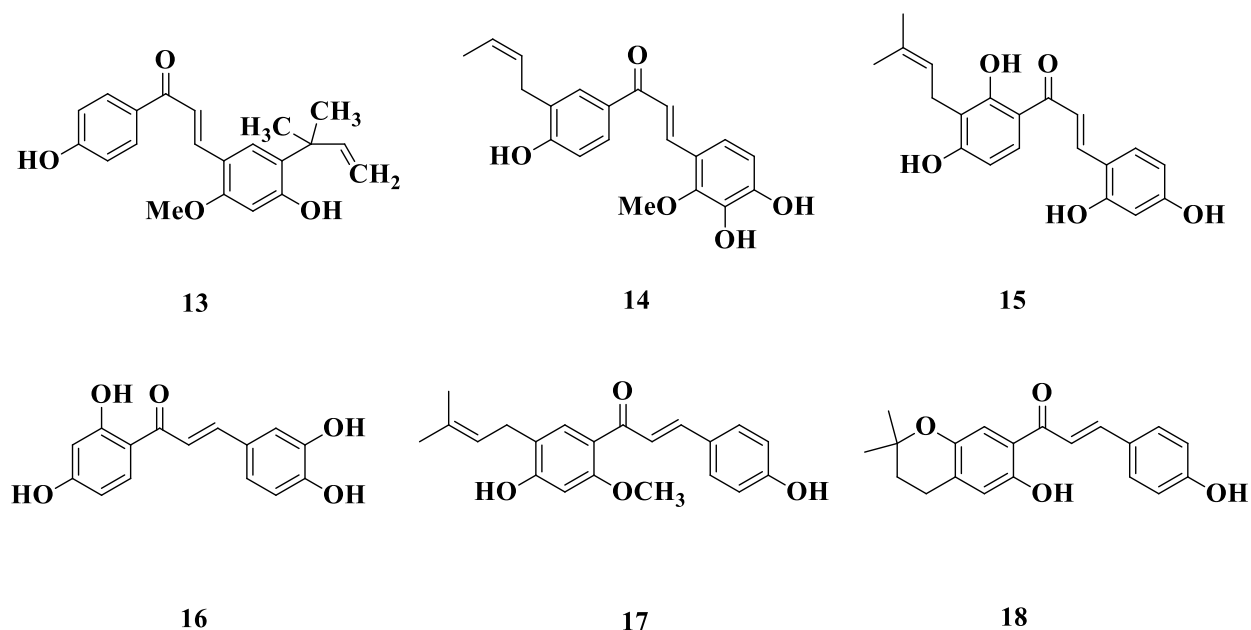


Figure 9. Chemical structure of the some of potent chalcone derivatives (Dhaliwal et al., 2022).

3.4. Natural products as versatile scaffolds for synthesis of heterocyclic compounds

Nitrogen-containing heterocycles with sulfur nucleus compounds play an important role in the drug discovery process, as approximately 75% of FDA (Food and Drug Administration)-approved small-molecule drugs contain one or more nitrogen-based heterocycles (Kerru et al., 2020). The chemistry of chalcones and curcumin has attracted the attention of scientists not only for their synthesis but also for their transformations to other biologically important compounds of 5- and 6-membered nitrogen heterocyclic compounds (Nayak, Gaonkar, & Sabu, 2022; Rodrigues et al., 2021). The α , β -unsaturated carbonyl system of chalcones/ curcumins possesses two electrophilic reactive centers allowing them to participate in addition reactions *via* attack to the carbonyl group (1,2-addition) or involving the β -carbon (1,4-conjugate addition), leading to the synthesis of promising bioactive heterocyclic compounds (MT Albuquerque, MM Santos, AS Cavaleiro, & MS Silva, 2014). The most recent advances in the synthesis of pyrroles, indoles, isoxazoles, imidazoles, pyrazoles, indazoles, triazoles, tetrazoles, pyridines, pyrimidines,

pyrimidine-2-ol, and thiazolo[3,2- α] pyrimidine, etc analogs through heterocyclization of suitably functionalized substrates of chalconoid/curcuminoid derivatives (Mallia & Sloop, 2023).

3.4.1. Pharmacological activities of Thiazole-based compounds

Many natural and synthetic compounds containing thiazole moiety in their structures have biological applications as antibacterial, antioxidant, antifungal, and anti-cancer agents (Ayati, Emami, Asadipour, Shafiee, & Foroumadi, 2015). Its diverse biological activity is reflected in a large number of clinically approved thiazole-containing compounds with an extensive range of pharmacological activities significance in medicinal chemistry, being a valuable scaffold in the design and synthesis of new drugs (H. Kumar et al., 2022; Singh, Malhotra, Singh, Chadha, & Bedi, 2022). The five-membered heterocyclic rings incorporating heteroatoms of nitrogen, sulfur, and oxygen are a fascinating strategy in the eyes of chemists to improve the activity of potential therapeutic molecules (Ismail, Abdulwahab, Nossier, El Menofy, & Abdelkhalek, 2020). Among these, thiazoles represent a class of heterocyclic compounds of great importance in biological chemistry and they are a basic scaffold found in many natural compounds such as vitamin B₁-thiamine, alkaloids, anabolic steroids, and flavones (Ayati et al., 2015; Jadhav et al., 2021). Nowadays, thiazole and its derivatives exist as part of the structure of molecules of great importance such as penicillin, cephalosporines, thiamine, and various natural bioactive compounds (Modrić et al., 2021; T Chhabria, Patel, Modi, & S Brahmksatriya, 2016). There are many drugs with this scaffold such as an antidepressant drug (pramipexole) [13], antiulcer agent (nizatidine) (Mohareb, Zaki, & Abbas, 2015), anti-inflammatory drug (fanetizole, meloxicam, and fentiazac) (Mohareb et al., 2015; Zia ur Rehman, Choudary, & Ahmad, 2005), HIV/AIDS drug (ritonavir) (Souza & Almeida, 2003), anticancer drug (tiazofurin) (Popsavin et al., 2006), antitumor (dasatinib, tiazofurin,), antiviral agents (brecanavir, and ritonavir) (MH Abbas, M Gomha, A Farghaly, & M Abdalla, 2016), antibacterial agents (sulfathiazole; aztreonam; and numerous cepheems) (Bikobo et al., 2017), antifungal agents (ravuconazole, myxothiazol, abafungin, and ethaboxam) (Pasqualotto, Thiele, & Goldani, 2010), antineoplastic agents (tiazofurin and dasatinib) (Das et al., 2006), antiparasitic agent (nitazoxanide), and insecticide agent (thiamethoxam) (Ayati et al., 2015; Shah et al., 2022) are some examples of thiazole

bearing products. In the initial stages of the COVID-19 pandemic outbreak, ritonavir was used to fight against the novel coronavirus COVID-19 (Zhu et al., 2020).

3.4.1.1. Antidiabetic agents thiazole-based derivatives

Type II diabetes is one of the most important metabolic disorders with a prevalence of 6.4% of people over the world which is accompanied by insulin resistance and dyslipidemia (Tangvarasittichai, 2015). In an attempt to find a new drug for the treatment of diabetes, diverse compounds with thiazole heterocycle were designed and some of them showed very potent activity. Stearoyl-CoA desaturase-1 (SCD1) is an important enzyme for controlling the high level of triglyceride and plasma insulin in humans. Therefore, compounds with SCD1 inhibitory activity are useful for treatment of obesity and type II diabetes (Attie et al., 2002). Various thiazole carboxamide analogs were designed, synthesized and examined as SCD1 inhibitors. The unsubstituted amide compound **19** (Figure 10) exhibited the highest potency in the whole cell assay ($IC_{50}=0.3$ mM) against the hSCD1 (delta-9 desaturase) (Li et al., 2009). With considering another target for treatments of type II diabetes, Kang *et al.*, (Kang, Song, Lee, Lee, & Lee, 2010) described benzylthiazolyl analogues **20** (Figure 10) as sodium glucose co-transporter inhibitors (SGLT2). Based on their results, almost all the compounds had moderate inhibitory activity. However, 4-ethylbenzyl-5-chlorothiazole derivative (R=Et), exhibited the most potent inhibition ($IC_{50} = 121$ nM) Peroxisome proliferator-activated receptors (PPARs) which consist of three subtypes (α , γ , and β/δ) are a member of transcription factors family. The potency of PPAR β/δ in the oxidation of lipids could lead to find novel treatment for obesity and type II diabetes (Oliver Jr et al., 2001). A number of tri-substituted thiazoles (Figure 10) were synthesized as new analogs of GW501516, and their transcriptional activities were evaluated by using a PPAR β/δ ‘reporter’ cell line. Almost all tested compounds had similar potencies at high concentrations and showed PPAR β/δ selectivity. Compound with 2-furyl substituent (**21-22**) exhibited the most potent transcriptional activity comparable with GW501516 ($EC_{50}= 4$ nM) (Pereira et al., 2006). Sodelglitazar (GW 677954, Figure 10) is a thiazole PPAR δ receptor agonist developed by Glaxo Smith Kline. Although it is primary considered as PPAR δ receptor agonist, it is a pan-agonist active against all PPARs. Sodelglitazar is a phase II clinical candidate for treatment of type II diabetes (X.-Y. Liu et al., 2011). Uzma Salar *et al.*, 2000 investigated compounds **23-28** along

with their intervening intermediates, which were screened for *in vitro* α -amylase inhibitory, DPPH, and ABTS radical scavenging activities. All compounds showed good inhibition potential in the range of IC_{50} = 2.186–3.405 μ M as compared to standard acarbose having IC_{50} value of 1.9 ± 0.07 μ M. It is worth mentioning that compounds also demonstrated good DPPH (IC_{50} = 0.09–2.233 μ M) and ABTS (IC_{50} = 0.584–3.738 μ M) radical scavenging activities as compared to standard ascorbic acid having IC_{50} = 0.33 ± 0.18 μ M for DPPH and IC_{50} = 0.5 ± 0.3 μ M for ABTS radical scavenging activities. In addition to that cytotoxicity of the compounds was checked on NIH-3T3 mouse fibroblast cell line and found to be non-toxic, **29-33** (Figure 10).

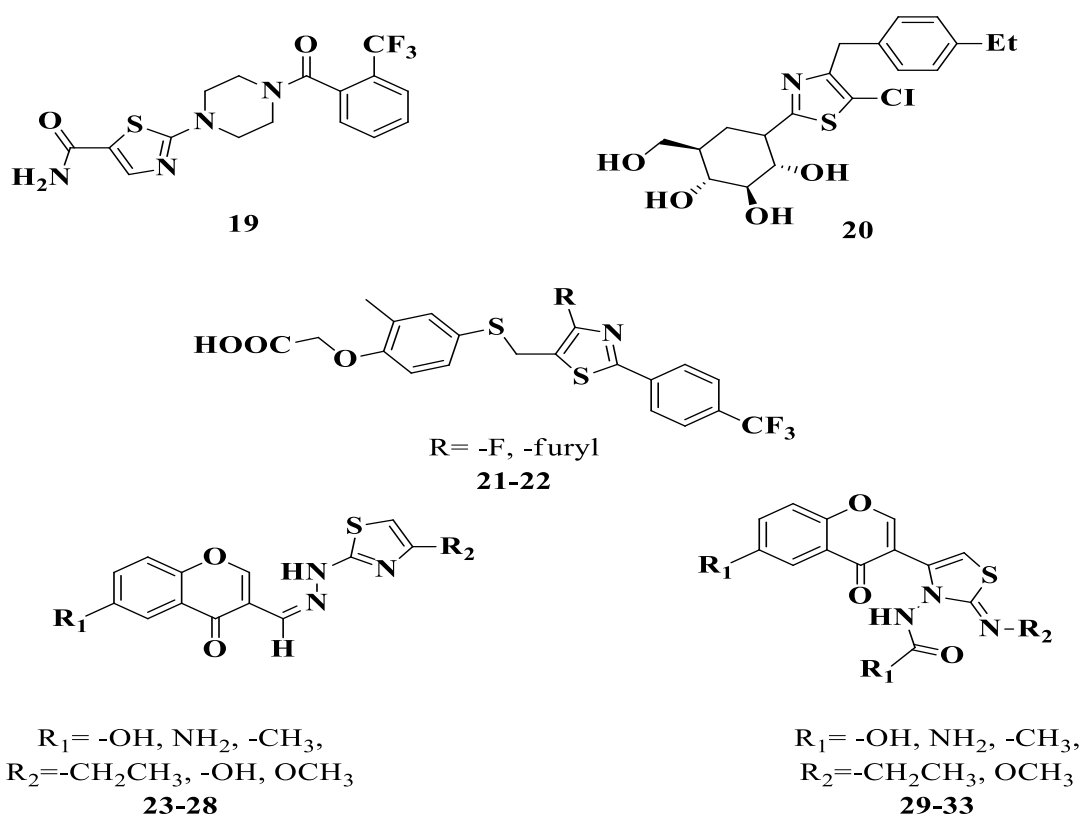


Figure 10. Antidiabetic thiazole-based compounds reported.

3.4.1.2. Antimicrobial agents thiazole-based derivatives

The resistance of pathogenic bacteria toward available drugs has been reported worldwide. Therefore, the research has been focused on the development of new antimicrobial agents with

novel targets. Currently, research in the area of antimicrobial drug design and development has been stimulated due to the expansion of the bacterial resistance phenomenon, with the emergence of multidrug-resistant strains such as *Staphylococcus aureus*, *Enterococcus sp.*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae*, and *Candida sp.* with intrinsic and acquired resistance to fluconazole (Bezerra et al., 2017). Nowadays, particular attention is given to hybrid molecules containing a thiazole nucleus combined with different heterocycles were designed, synthesized, and evaluated as antimicrobial agents (Qadri et al., 2022). In this regard, different thiazole-bearing compounds showed promising antimicrobial activities.

Recently, (Cheng, Xue, & Zhu, 2013) reported 2-(phenylacetamido)thiazole derivatives as ecKAS III inhibitors. Based on their results, N-(4-(4-bromophenyl thiazol-2-yl)-2-(3-chlorophenyl) acetamide **34** (Figure 11) had the highest antibacterial activity with MIC of 1.56-6.25 mg/mL against all bacterial stains (*E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*) and showed the most potent ecKAS III inhibitory activity (IC_{50} = 5.3 mM). A series of novel 2-(3-pyridyl)-4,5-disubstituted thiazoles **35** (Figure 12) were synthesized and evaluated for their antimicrobial activity against ten bacterial and five fungal human pathogenic strains using the disc diffusion assay. Among them, 5-acetyl-4-methyl-2-(3-pyridyl) thiazole exhibited the most potent antibacterial activity against *Staphylococcus epidermidis* with a MIC value of 0.24 mg/mL (2 fold more potent than ampicillin) and also anti-fungal activity similar to amphotricin B against *Geotricum candidum* (MIC = 0.48 mg/mL) (Bondock, Naser, & Ammar, 2013). Based on the structure-activity relationship study (SAR), the replacement of bulky substitutions (in both positions 4 or 5) on the thiazole nucleus decreased the antimicrobial activity. In a study by (Ghasemi, Beyzaei, Hashemi, & Askari) reported 3-(2-thiazolyl)-2-styryl-4(3H)-quinazolinone derivatives **36** (Figure 11) were synthesized and screened for their *in vitro* antibacterial and antifungal activities against *E. coli*, *P. aeruginosa*, *B. megaterium*, *B. subtilis*, and *A. niger*. The results showed that compounds with R = Br and Ar = Ph, 2-NO₂-Ph or 4-Cl-Ph had comparable inhibitory effects with respect to norfloxacin and clotrimazole as standard drugs. Some thiazole-based 1,3,4-oxadiazoles **37** (Figure 11) were synthesized and assayed for their *in vitro* antibacterial activities by (Desai, Bhatt, Somani, & Trivedi, 2013). Compounds possessing fluoro or nitro-substitution in the para-position of phenyl ring showed 2-4-fold higher antibacterial activities than the chloramphenicol. Thiazole-based chalcone derivatives **38** (Figure 11) have

been synthesized by the condensation reactions between 1-(4-methyl-2-(methylamino) thiazol-5-yl)ethanone with different aromatic aldehydes. Compounds were tested as novel antimicrobial agents against eight Gram-positive and Gram-negative bacteria, and fungal species. Based on the results, compounds with electron-withdrawing nitro and chloro groups had better antibacterial activity. It could be noticed that these compounds showed comparable antibacterial activity with respect to ampicillin (MIC range of 7.6 4-45.87 mmol/mL), while their antifungal activity was less than ketoconazole or bifonazole (Abd El-Wahab, Abd El-Fattah, Abd El-Khalik, Nassar, & Abdelall, 2014; Liaras, Geronikaki, Glamočlija, Ćirić, & Soković, 2011). In the search for new antimicrobial agent with potential application in polyurethane coating, (Abd El-Wahab et al., 2014) have synthesized coumarin thiazole derivative 2-(2-amino-1,3-thiazol-4-yl)-3H-benzo[f]chromen-3-one **39** (Figure 11). This compound was evaluated against eight different microorganisms when physically incorporated into a polyurethane varnish formula. The results indicated that the polyurethane varnishes containing compound **39** show a very good antimicrobial effect.

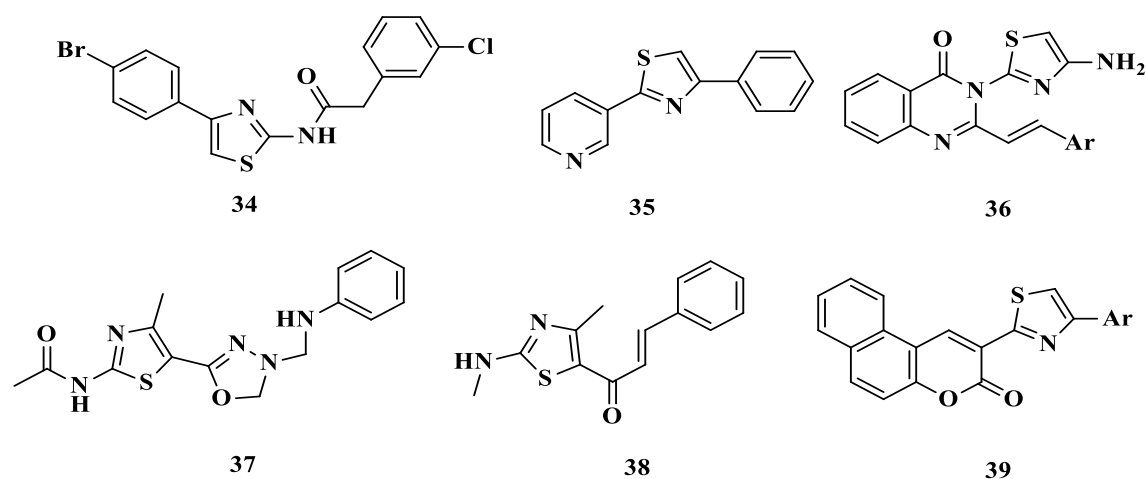


Figure 11. Antimicrobial Thiazole-based compounds reported.

3.4.1.3. Antioxidant agents thiazole-based derivatives

Increased level of reactive oxygen species (ROS) under free radical oxidative stress condition causes some damage to cell growth, gene expression, and host defense. Therefore controlling the

level of free radicals in cells seems to be essential to decrease damage (P. Rajendran et al., 2014). The antioxidant activity of 2,4-dioxo-1,3-thiazolidine thiazoles has been studied by Yadla team (Koppireddi et al., 2013) using the DPPH method. Based on their report, *para*-bromo derivative **40** (Figure 12) showed the highest antioxidant potency (EC_{50} = 49.71 μ g/mL), comparable to ascorbic acid and luteolin as reference compounds. In an attempt to find new antioxidant agents, 1-thiazolyl-2-pyrazoline derivatives were synthesized and evaluated for xanthine oxidase (XO) inhibitory and free radical scavenging activities. Among the synthesized derivatives, compound **41-44** (Figure 12) bearing 2-(benzylthio)-5-chloro-thiophen-3-yl moiety had the best XO inhibition (IC_{50} = 6.2 mM) and free radical scavenging activity (IC_{50} = 15.6 mM), which were comparable to allopurinol and ascorbic acid as reference drugs (Mandawad, Dawane, Beedkar, Khobragade, & Yemul, 2013). To find new therapeutic agents for the treatment of neurodegenerative disorders, Kim *et al.*, (K.-M. Kim et al., 2003) designed novel antioxidants **45**, and **46** (Figure 12) containing *N*-*t*-butyl-*N*-hydroxyl aminophenyl moiety. The *N*-*t*-butyl-*N*-hydroxyl aminophenyl scaffold could have potential antioxidant activity because of the high stability of radical species generated from an abstraction of hydrogen on N-OH by reactive oxygen species

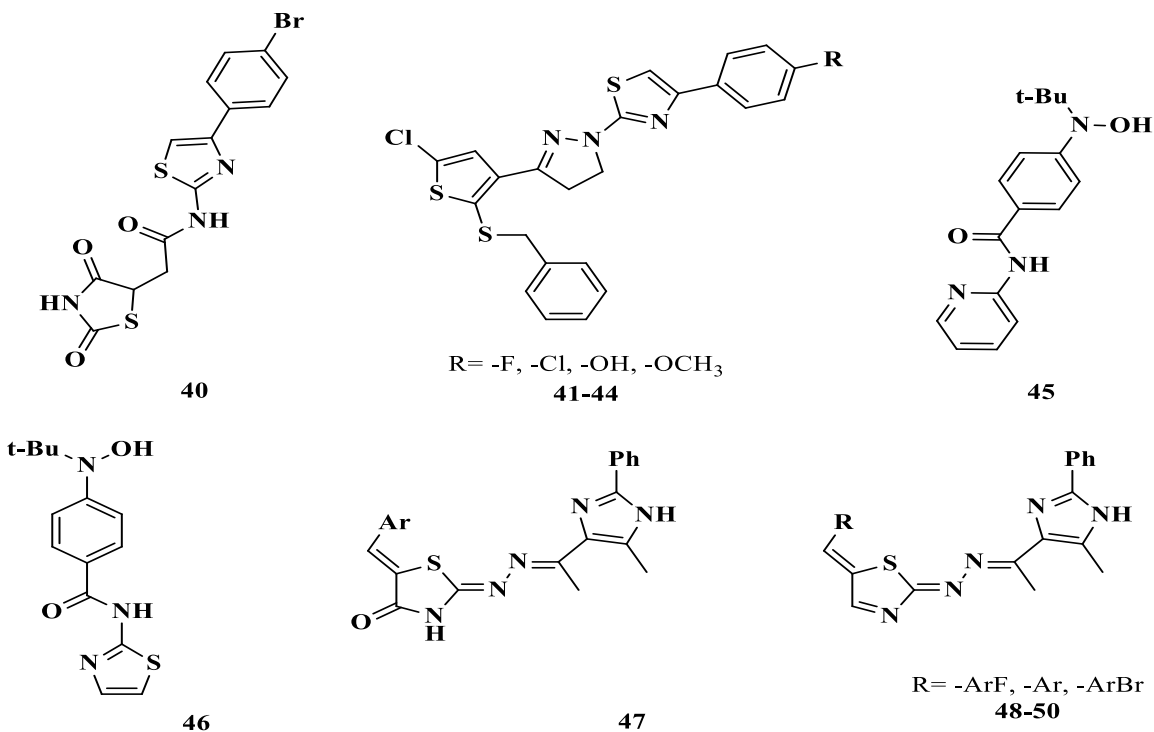


Figure 12. Antioxidant thiazole derivatives and related compounds reported.

This study has indicated that thiazole-possessing compounds remarkably decreased the neurotoxicity induced by ROS at 10 mM concentrations. Also, the mentioned compound showed the best lipid peroxidation (LPO) inhibition and *in vivo* neuroprotective activities using gerbils as animal models. Several series of thiazole-modified compounds **47** (Figure 12) containing hydrazine-imidazolyl moiety were evaluated for their antioxidant ability using DPPH method. Among the synthesized compounds, 4-fluorobenzylidene-thiazolidin-4-one derivative, **48-50** (Ar= 4-F-Ph) was the most potent compound (Abdel-Wahab, Awad, & Badria, 2011). Therefore, thiazole compounds constitutes the skeleton of various commercially marketed drug candidates and the heart-core in a diverse range of entities of biological and medicinal interest (Jadhav et al., 2021). Many natural and synthetic compounds containing thiazole and another heterocyclic moiety in their structures have biological applications as antidiabetic, antibacterial, antioxidant, antifungal, and anti-cancer agents, **51-59** (Figure 13) (Arshad et al., 2022).

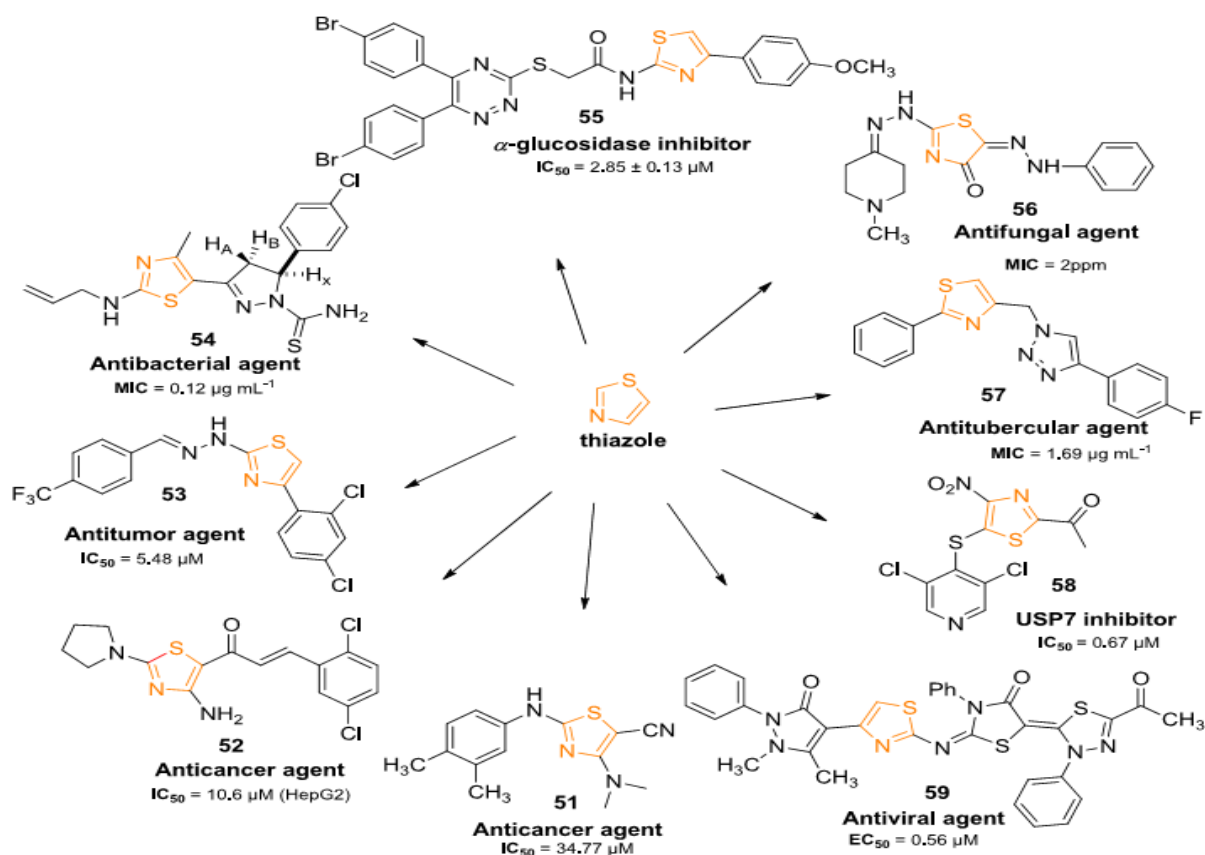


Figure 13. Thiazole-based most active compounds reported for different molecular targets (Singh et al., 2022).

In 2015, there are 23 drugs based on thiazole ring approved by the Food and Drug Administration (FDA) agency, which is much more than for any other related 5-membered heterocycle (Mak, Xu, & Fairlie, 2015). Due to their synthetic and competitive biological significance, the chemistry of thiazole, and their fused heterocyclic derivatives received substantial attention. Recent reports have declared the applications of thiazole core structure in drug design and the development of novel therapeutic agents. Hence, thiazole ring has exerted various roles in lead identification and optimization, including as pharmacophoric, bio-isosteric elements, and as a spacer. Also, the presence of thiazole ring as a part of the drug structure can be a determinant of its physicochemical and pharmacokinetic properties (Arshad et al., 2022; T Chhabria et al., 2016).

3.5. Diversity -oriented synthetic (DOS) strategy

In recent times, diversity-oriented synthesis of small chemical entities has emerged as a possible tool for exploring the intersections between chemistry and biology (Tan, 2005). Achieving a large coverage of chemical space simply by a random synthesis of compounds is not practicable or desirable; it is widely accepted that it is not synthetically feasible to produce all theoretically stable, small carbon-based molecules, and making and screening molecules cost, both in terms of time and money (Galloway, Isidro-Llobet, & Spring, 2010b). The overall aim of a DOS is to efficiently generate a library of small molecules with a high degree of structural, and thus functional, diversity that interrogates large areas of chemical space. These small molecules can be used as probes for biological targets or as modulators of disease states. Indeed, molecules capable of modulating protein-protein interactions, transcription factor activity, and multidrug resistance in pathogens have all been discovered using DOS libraries, whereas the typical compound libraries employed by pharmaceutical companies or commercial vendors have been largely unsuccessful in providing hits against such challenging and underexploited targets (Galloway, Isidro-Llobet, & Spring, 2010a).

In an attempt to increase the biological diversity and relevance of libraries prepared by DOS, several variations of this approach, such as incorporating natural product scaffolds, have been developed. Similarly, another DOS-based approach involves the inclusion of so-called privileged

scaffolds or substructures (*pDOS*) beyond those found in natural products. A substrate-based *pDOS* by Kim et al., recently reported (J. Kim, Kim, & Park, 2014). More recently, attention has been focused on the ability to produce relevant biological active molecules *via* the incorporation of essential privileged structures (privileged-substructure-based *DOS* (*pDOS*)). A privileged scaffold is a structural framework that is common to many different molecules that serve as ligands for a variety of biological targets. Indeed, because derivatives of privileged structures frequently display biological activity, drug discovery campaigns often involve molecules incorporating such motifs.

3.6. Greener synthetic approaches

During the last decade, the topic of ‘green chemistry’ has received increasing attention as alternative synthesis protocol. The green chemistry approach refers to the utilization of a set of principles that reduces the generation of chemical hazardous during the design, manufacture, and use of chemical substances (Bouherrou et al., 2017). This protocol plays a major role in controlling environmental pollution by using safer solvents, catalysts, suitable reaction conditions and thereby increasing the atom economy and energy efficiency of the synthetic process (Banik et al., 2021). The development of simple and eco-friendly procedures for the synthesis of compounds with biological interest is the driving force for the discovery and design of new bioactive compounds. Recently, the development of an efficient and green procedure in the synthesis of drugs has received great interest in modern heterocyclic chemistry (Rubab et al., 2022). The fundamental challenge for today’s methodologists is to make their protocols more environmentally benign and sustainable by avoiding the extensive use of hazardous reagents and solvents, harsh reaction conditions, and toxic metal catalysts. Among these procedures, ZnO nanoparticles and ultrasound irradiation-assisted synthesis fulfilled all the mentioned challenges to various organic transformations.

3.6.1. ZnO nanoparticles assisted synthesis

Nanotechnology is a fast-evolving field that deals with manufacturing new materials on the nanoscale level. In other words, nanotechnology aims to synthesize, characterize, and manipulate

matter in size of 1-100 nm (Roco, Mirkin, & Hersam, 2011). The utilization of metal oxide nanoparticles is a unique material that has prompted a high interest in the past decade namely because of their applications in catalysis (Bouherrou et al., 2017). Metal oxides have been used as efficient heterogeneous catalysts in various organic reactions. Their application depends on the structure (pore size, surface area, etc.) and on the morphology (that depends, e.g., on the metal and its oxidation state). Although metal oxide surfaces exhibit both Lewis acid and base properties, the nature of metal cation and the surface area of metal oxides have extensively manipulated their catalytic properties. Hence, nanotechnology has led to a new research interest in using nanometer-sized particles as an alternative matrix for catalytic reactions. Several reports showed a surprising level of the performance of nanoparticles as a catalyst in terms of reactivity, selectivity, and improved yields of products.

Among various metal oxides, zinc oxide nanoparticles (NPs) is the most appealing multifunction materials, which is used in agriculture, biomedical, biosensors, gas sensors, cosmetics, and drug-delivery systems. Because of biocompatibility, ZnO is considered a safe drug carrier system when the particles ranged bigger than 100 nm, as stated by the FDA (Arvanag, Bayrami, Habibi-Yangjeh, & Pouran, 2019). Moreover, ZnO nano particles can produce ROSs, which result in its antidiabetic, antibacterial, and antioxidant properties. Currently, nano zinc oxide has received considerable attention as a solid catalyst for various organic transformations due to favorable physical and chemical properties like inexpensive, non-toxic, low corrosion, waste minimization, recycling nature (reusability), large surface area, high pores volume, environmental sustainability, and commercial availability make this Lewis acidic heterogeneous nanocatalyst superior to others. Although a considerable amount of work has been devoted to such a system, the use of nano-catalysts is still under studied, mainly due to the cost involved in their fabrication both industrial and nano type has been used as a professional catalyst in various organic transformations (Mashrai, Khanam, & Aljawfi, 2017; Tamaddon, Sabeti, Jafari, Tirgir, & Keshavarz, 2011).

3.6.2. Ultrasonic (sonochemical) accelerated synthesis

In recent years, ultrasound irradiation organic synthesis, as a synthetic approach, has gained recognition as a clean and advantageous approach in organic synthesis. The sonochemical phenomenon is the result of the interaction of a suitable field of acoustic waves with a potentially reacting chemical system. Sonochemical methods have recently proved a powerful technique for facilitating various chemical reactions (Hussein & Khairou, 2014). Ultrasound has been recognized as an important technique for green and sustainable synthetic processes (Mohammadi Ziarani et al., 2020). This phenomenon occurs through the adiabatic collapse of transient cavitation bubbles of gaseous substances. Owing to this cavitation effect, solubility, diffusivity, penetration, mass transportation, and turbulent flow of chemical species are enhanced in certain reactions (Shabir, Shafique, & Saeed, 2022). The phenomenon of cavitation in an irradiated solution may be expressed as a sequential process involving the bubble formation, its growth, and breakdown. The cavitation phenomenon develops high temperature and pressure in the micro environment which creates turbulence and facilitates the mass transfer in the neighborhood. This cavitationally induced phenomenon is known to activate reactant molecules entering into it and thereby converting them into reactive intermediates. In addition, such a phenomenon occurs more easily in solvents with low vapor pressure (Mardjan et al., 2022).

Compared to conventional heating which provides thermal energy in the macro system, ultrasonification reduces reaction times, improves yields, and minimizes side product formation by providing the activation energy in micro environment. Studies have shown that this technique can be applied to a variety of organic syntheses accomplishing better yields, under mild reaction conditions and shorter reaction times. It is also observed that reactions under ultrasound irradiation are commonly easier to work-up than those in conventional stirring methods (Safaei-Ghomi & Masoomi, 2015). Additionally, in many cases, reactions under ultrasound irradiation represent environmentally friendly processes, using small amounts of solvents and consuming less energy. Furthermore, ultrasonic irradiation provides minimal side reactions.

CHAPTER FOUR

4. MATERIAL AND METHODS

All starting chemicals and materials used were analytical grades. Merck (Germany), Sigma Aldrich Sigma Chemical, Co. (USA), and Loba Chemie, Pvt Ltd. (India) provided chemicals and reagents, which were purchased from commercial vendors and used immediately without additional purification. All the chemicals of acetophenone, *para*-nitro acetophenone, *ortho*-hydroxy acetophenone (99%), benzaldehyde, *para*-bromo benzaldehyde, *ortho*-hydroxy benzaldehyde (99%), Vanillin (4-hydroxy-3-methoxybenzaldehyde) (99%), Iodine beads, urea, thiourea (99%), Sodium thiosulfate (99.55%), hydrazine hydrate (80%), formic acid (85%), Glacial acetic acid (99.55%), KOH (85%), NaOH (99%), Na metal in ethanol (99%), Zinc Oxide nanoparticles, Sodium bicarbonate (99.5%), ethanol (99%) and methanol (99%). Column chromatography was performed using silica gel (particle size 60-120 mm) (Merck) with *n*-hexane, ethyl acetate, and dichloromethane as mobile phase. ZnO nanoparticles were obtained from the Department of Applied Chemistry, Material Chemistry research team of ASTU. Ultrasonic-assisted reactions were performed in a “Spectralab model UMC 20 Ultrasonic cleaner” (horn type, made in ACE, USA). The instrument had an operating frequency of 40 kHz, output (nominal) power of 250 W, 1.3×10^{-2} m diameter of stainless-steel tip with the intensity of 3.4×10^5 W/m² and 1.32×10^{-4} m² surface area of the ultrasound irradiating face at Department of Materials Science and Engineering Laboratory, School of Mechanical, Chemical and Materials Engineering, Adama Science and Technology University. Amoxicillin, Acarbose and *L*-ascorbic acid (99 %) were employed as standards, are obtained from Ethiopian Pharmaceutical Manufacturing Factory (EPHARM).

Purification of synthesized compounds (**108-116**) was carried out by using silica gel column chromatography (60-120 mesh size) and reaction progress was monitored by analytical thin-layer chromatography (TLC) carried out on silica gel pre-coated aluminum plate 60 F₂₅₄ (Merck, Darmstadt, Germany) plates by using visualizing spots in UV light of long and short wavelength (254 and 365 nm). Melting points were determined on a Gallen-Kamp MFB-595 apparatus, uncorrected. UV spectra were obtained using a double-beam UV-Visible spectrophotometer (Model 2201, India). The ¹H-NMR and ¹³C-NMR spectra were recorded at 400 and 100 MHz,

respectively, on a Bruker Avance III 400 nano spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift (δ) values are given in parts per million (ppm) relative to MeOD- d_4 , CDCl₃, or DMSO- d_6 and coupling constants (J values) in Hertz. The chemical shifts are expressed in δ (ppm) and reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), and brs (broad singlet), respectively. SwissADME, PreADMET, ProTOX II and Autodoc vina 4.2.6 version software packages were used to undertake *in-silico* ADMET and molecular docking simulation investigations, respectively.

4.1. Synthesis procedure of thiazolyl-pyrazoline Schiff base hybrids

4.1.1. Synthesis of chalcones (1, 3-diaryl-2-propen-1-ones), **62-64**

The synthesis of chalcone derivatives by a base catalyzed Claisen-Schmidt condensation reaction reported (Yadav et al., 2017). To an equimolar amount of *p*-nitro acetophenone (**60**, 0.06 mol) and the corresponding aldehyde derivatives (**61**, 0.06 mol) in 5-10 mL of ethanol, then NaOH 60% (w/v) was added dropwise (3 mL) and stirred in an ice bath for 30 min and then kept at rt until the precipitate was formed within 3 h (Scheme 1). The reaction progress was monitored by TLC with an eluent of *n*-hexane: ethyl acetate (9:1). The precipitate was filtered off and washed thoroughly with cold distilled water and cold methanol (2×20 mL). The resulting solid products (**62-64**) were recrystallized from absolute ethanol to yield a yellow precipitate of chalcone derivatives. The ¹H and ¹³C-NMR spectra of all the synthesized compounds are presented in Appendix 1-6.

(*E*)-1-(4-nitrophenyl)-3-phenylprop-2-en-1-one (**62**). Orange solid powder; yield: 90.1 %; m.p.: 153-154°C; R_f (1 % MeOH in DCM) =0.74; ¹H-NMR (400 MHz, DMSO- d_6): δ 7.93 (d, J = 15.4 Hz, 2H), 7.83 (d, J = 7.5 Hz, 2H), 7.75 (dd, J = 15.5, 8.2 Hz, 1H), 7.73 (d, J = 16.2, 7.3 Hz, 1H), 7.50 (dd, J = 7.6, 7.2 Hz, 2H), 7.45 (t, J = 7.7 Hz, 1H), 7.23 (d, J = 8.4 Hz, 2H); ¹³C-NMR (101 MHz, DMSO- d_6): δ 188.6, 162.8, 150.9, 146.0, 145.8, 134.6, 130.3, 129.1, 128.5, 124.3, 122.1.

(*E*)-3-(4-bromophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**63**). Yellowish solid powder; yield: 93.6 %; m.p.: 167-169°C; R_f (1 % MeOH in DCM) =0.63; ¹H-NMR (400 MHz, DMSO- d_6): δ 8.04 (d, J = 8.5 Hz, 2H), 8.00 (dd, J = 8.5 Hz, 2H), 7.93 (d, J = 8.3 Hz, 2H), 7.91 (dd, J =

16.2, 8.5 Hz, 1H), 7.85 (dd, $J = 15.7, 7.7$ Hz, 1H), 7.72, 7.52 (d, $J = 7.7$ Hz, 2H); ^{13}C -NMR (101 MHz, DMSO- d_6): δ 196.3, 144.5, 143.5, 141.6, 150.3, 129.9, 128.0, 127.6, 124.2, 124.2, 122.8.

(*E*)-3-(2-hydroxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**64**). Yellowish solid powder; yield: 87.8 %; m.p.: 160-162 °C; R_f (1 % MeOH in DCM) =0.60; ^1H -NMR (400 MHz, DMSO- d_6): δ 7.98 (s, OH), 7.81 (d, $J = 8.4$ Hz, 1H), 7.75 (d, $J = 8.5$ Hz, 1H), 7.66 (d, $J = 8.4$ Hz, 1H), 7.57 (d, $J = 15.7$ Hz, 1H), 7.29 (d, $J = 15.6$ Hz, 1H), 6.82 (t, $J = 7.7$ Hz, 1H), 6.47 (d, $J = 8.2$ Hz, 1H), 6.40 (t, $J = 7.5$ Hz, 1H); ^{13}C -NMR (101 MHz, DMSO- d_6): δ 188.5, 167.2, 151.2, 148.2, 141.6, 130.8, 129.9, 124.1, 121.4, 111.9.

4.1.2. Synthesis of 2-amino-4-(substituted-phenyl)-1, 3-thiazole derivatives, **69-71**

The thiazole moiety was synthesized using Hantzsch's approach, which involved a solvent-free reaction of substituted acetophenones (**65-67**) derivatives with thiourea (**68**) and iodine under thermal conditions resulting in 2-amino-4-aryl thiazoles derivatives (**69-71**), as illustrated in [Scheme 2](#) (Zitko et al., 2018). Thiourea (40 mmol, 2 molar equiv.) was mixed with iodine beads (20 mmol, 1 molar equiv.) in a mortar and the mixture was homogenized with a pestle. The solids were scratched off into a 250 mL round-bottom flask with a magnetic stirring bar and charged with the corresponding acetophenone (20 mmol, 1 molar equiv.). The flask was heated in an oil bath under an air-cooled condenser at 100 °C with stirring. The reaction progress was monitored using TLC in *n*-hexane and ethyl acetate (4:1). After 3 h, the mixture was cooled and it solidified again. A saturated aqueous solution of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 5%) was added with concomitant mechanical disruption of the solid matrix in a sufficient amount to reduce the remaining iodine. The product as a suspension was filtered off and carefully washed with 20 mL of diethyl ether to remove the residues of unreacted acetophenone. The crude product was dissolved in hot water, filtered, and the hot filtrate was adjusted to pH=9 by aqueous NaHCO_3 . After cooling, the precipitated product was collected and recrystallized using ethanol. The ^1H and ^{13}C -NMR spectra of all the synthesized compounds are presented in [Appendix 7-12](#).

4-phenylthiazol-2-amine (**69**). White solid powder; yield: 96.6%; m.p.: 144-146 °C; R_f (20 % EtOAc in *n*-hexane) =0.73; ^1H -NMR (400 MHz, DMSO- d_6): δ 7.35 (d, $J = 7.7$ Hz, 2H), 6.94 (t, $J = 7.6$ Hz, 2H), 6.84 (d, $J = 7.3$ Hz, 1H), 6.55 (s, 1H, CH of thiazole), 2.1 (d, 2H, $-\text{NH}_2$);

^{13}C -NMR (100 MHz, DMSO- d_6): δ 168.7 (C-2), 150.2 (C-4), 135.1 (C-5'), 128.9 (C-2'), 127.7 (C-1',3'), 125.9 (C-4',6'), 102.0 (C-5).

4- (4-Nitrophenyl) thiazol-2-amine (**70**). Orange solid powder; yield: 86.6%; m.p.: 269.-270 °C; R_f (20 % EtOAc in *n*-hexane) =0.44; ^1H -NMR (400 MHz, DMSO- d_6): δ 7.82 (d, J = 8.7 Hz, 2H), 7.62 (d, J = 8.7 Hz, 2H), 6.96 (s, 1H, CH of thiazole), 6.77 (d, J = 8.1 Hz, 2H), 2.11 (d, J = 2.0 Hz, 2H, -NH $_2$); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 169.1 (C-2), 148.2 (C-4), 146.3 (C-2'), 141.1 (C-5'), 126.7 (C-1',3'), 124.4 (C-2',4'), 107.1 (C-5).

2-(2-aminothiazol-4-yl) phenol (**71**). Pale yellow solid powder; yield: 74.2%; m.p.: 138-140 °C; R_f (20 % EtOAc in *n*-hexane) =0.61; ^1H -NMR (400 MHz, DMSO- d_6): δ 11.56 (s, 1H, -OH), 7.24 (d, J = 7.7 Hz, 1H), 7.00 (s, 1H, CH of thiazole), 6.70 (t, J = 7.7 Hz, 1H), 6.61 (d, J = 8.2 Hz, 1H), 6.38 (t, J = 8.1 Hz, 1H), 2.09 (d, J = 2.7 Hz, 2H, -NH $_2$); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 168.6 (C-2), 155.5 (C-4'), 147.3 (C-4), 129.3 (C-2'), 126.4 (C-6'), 119.3 (C-1'), 118.4 (C-5'), 117.1 (C-3'), 100.8 (C-5).

4.1.3. Synthesis of 3, 5-diphenyl-4,5-dihydro-1H-pyrazol-1-carbaldehydes, **72-74**

Chalcones **62-64** (0.04 mol), 9.72 mL hydrazine hydrate (80 %, 0.2 mol), and ethanol (15 mL) mixture were refluxed for 10 min (Illicachi et al., 2017). After cooling, a mixture of the NH-pyrazoline (0.04 mol) and formic acid (85%, 20 mL) was added and the solution was stirred at room temperature for 30 min until complete consumption of the NH-pyrazoline. The progress of the reaction was monitored using TLC in *n*-hexane and ethyl acetate (9:1) as the mobile phase. When the reaction was completed, the warm reaction mixture was poured into ice-cold water (50 mL), and the precipitate was filtered and rinsed with water and ethanol using suction filtration. The solid product was recrystallized with ethanol to yield *N*-formylpyrazoline derivatives with good yields (Scheme 3). The ^1H and ^{13}C -NMR spectra of all the synthesized compounds are presented in Appendix 13-18.

4,5-dihydro-3-(4-nitrophenyl)-5-phenylpyrazole-1-carbaldehyde (**72**). Reddish solid powder; yield: 87.3%; m.p.: 130-132 °C; R_f (30 % EtOAc in *n*-hexane)=0.79; ^1H -NMR (400 MHz, DMSO- d_6): δ 8.48 (s, 1H, CHO), 7.82 (d, J = 8.5 Hz, 2H), 7.50 (d, 2H), 7.26 (dd, J = 16.9, 9.1 Hz, 2H), 6.85 (d, J = 8.2 Hz, 2H), 6.16 (d, J = 8.4 Hz, 1H), 5.11 (dd, 3J = 11.72 Hz, 3J = 4.92

Hz, 1H, C₅-H_X), 3.54 (dd, ²J=17.47 Hz, ³J= 11.60 Hz, 1H , C₄-H_M), 3.01 (dd, ²J= 17.74 Hz, ³J=4.91 Hz, 1H, C₄-H_A); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 165.9 (C-13), 161.1 (C-3), 155.4 (C-10), 148.9 (C-1'), 141.7 (C-7), 137.5 (C-9,11), 128.6 (C-2',6'), 126.5 (C-8,12), 124.8 (C-3',5'), 114.3 (C-1'), 59.9 (C-5), 42.9 (C-4).

5-(4-bromophenyl)-4,5-dihydro-3-(4-nitrophenyl)pyrazole-1-carbaldehyde (73). Yellow solid powder; yield: 78.9 %; m.p.: 202-204 °C; R_f (30 % EtOAc in *n*-hexane) =0.61; ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.50 (s, 1H, CHO), 7.87 (dd, *J* = 8.8, 3.5 Hz, 4H), 7.38 (d, *J* = 8.6 Hz, 3H), 7.11 (d, *J* = 8.0 Hz, 5H), 6.89 (d, *J* = 8.1 Hz, 2H), 4.55 (dd, ³J= 11.81 Hz, ³J= 5.29 Hz, 1H, C₅-H_X), 3.58 (dd, ²J=17.27 Hz, ³J= 11.71 Hz, 1H , C₄-H_M), 3.10 (dd, ²J= 17.24 Hz, ³J=4.81 Hz, 1H, C₄-H_A); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 166.5 (C-13), 160.3 (C-3), 155.9 (C-10), 146.2(C-1'), 141.9 (C-7), 131.3(C-3',5'), 128.8 (C-9,11), 125.9 (C-8,12), 120.3 (C-2',6'), 63.3 (C-5), 48.6 (C-4).

4,5-dihydro-5-(2-hydroxyphenyl)-3-(4-nitrophenyl)pyrazole-1-carbaldehyde (74). Pale yellow solid powder; yield: 92.5 %; m.p.: 175-177 °C; R_f (30 % EtOAc in *n*-hexane) =0.54; ¹H-NMR (400 MHz, DMSO-*d*₆): δ 10.65 (s, 1H), 8.55 (s, 1H, CHO), 7.58 (d, *J* = 8.2 Hz, 2H), 7.25 (d, *J* = 7.7 Hz, 2H), 7.00 (d, *J* = 7.6 Hz, 2H), 5.81 (dd, ³J= 11.71 Hz, ³J= 4.94 Hz, 1H, C₅-H_X), 2.76 (dd, ²J=17.27 Hz, ³J= 11.71 Hz, 1H , C₄-H_M), 2.10 (dd, ²J= 17.14 Hz, ³J=5.29 Hz, 1H, C₄-H_A); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 167.3 (C-13), 163.7 (C-3), 159.4 (C-6'), 148.5(C-10), 134.2 (C-9,11), 131.8 (C-4'), 128.0 (C-2'), 124.5 (C-8,12), 120.5 (C-5'), 118.8 (C-3'), 79.8 (C-5), 49.4 (C-4).

4.1.4. Synthesis of thiazolyl-pyrazoline Schiff base hybrids in the presence of ZnO nanoparticles as a catalyst derived from chalcone, **75-83**

The target Schiff base hybrids were synthesized by condensation of an equimolar ethanolic solution (20 mL) of *N*-formylpyrazoline derivatives, **72-74** (3 mmol), with substituted 2-amino-4-(substituted phenyl) thiazole derivatives, **69-71** (3 mmol equiv.) in ZnO nanoparticles (43 mg, 0.2 mmol, 20 mol %) as a catalyst in methanol (20 mL) under reflux for 3 h. The progress of the reaction was monitored using TLC using *n*-hexane: ethyl acetate (7:3). At the end of the reaction, the solution was slowly evaporated, and the precipitate was filtered, washed with ethanol, and dried under a vacuum. Finally, the resulting products were recrystallized from ethanol to provide

solid products (Scheme 4). The structures of the synthesized compounds, **75-83** are characterized using physicochemical (M.pt, color, and R_f) and spectroscopic (UV-Vis, ^1H -NMR, and ^{13}C -NMR) techniques. The ^1H and ^{13}C -NMR spectra of all the synthesized compounds are presented in Appendix 19-45.

(18E)-N-((4,5-dihydro-3-(4-nitrophenyl)-5-phenylpyrazol-1-yl)methylene)-4-phenylthiazol-2-amine (**75**). Reddish yellow solid powder; yield: 94.7%; m.p.: 258-260 °C; R_f (20 % EtOAc in *n*-hexane) =0.39; UV-Visible (MeOH) λ_{max} =285 and 385 nm; ^1H -NMR (400 MHz, DMSO- d_6): δ 8.49 (s, 1H, CH =N), 7.94-7.81 (m, 1H), 7.78 (s, 1H, sp^2 methine of thiazole-H), 7.56 (d, J = 8.5 Hz, 2H), 7.37 (d, J = 8.5 Hz, 2H), 7.10 (d, J = 8.3 Hz, 2H), 6.89 (d, J = 8.0 Hz, 2H), 6.78 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 8.4 Hz, 1H), 5.13 (d, J = 6.8 Hz, 2H), 4.55 (dd, 3J = 11.82, 3J = 6.24 Hz, 1H, C₅-H_X), 3.12 (dd, 2J =17.77, 3J = 11.82 Hz, 1H, C₄-H_M), 2.48 (dd, 2J = 17.84, 3J =4.62 Hz, 1H, C₄-H_A); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 160.7 (C-2'), 156.4 (C-6), 155.1 (C-4'), 153.1 (C-3), 148.4 (C-10'), 146.6 (C-1'), 142.3 (C-7'), 139.8 (C-2',6'), 137.4 (C-4'), 131.9 (C-8',12'), 129.2 (C-3',5'), 128.6 (C-8), 128.2 (C-10,12), 126.4 (C-8), 124.4 (C-11), 121.1 (C-9,13), 120.7 (C-5'), 63.7 (C-5), 49.0 (C-4).

(18E)-N-((4,5-dihydro-3-(4-nitrophenyl)-5-phenylpyrazol-1-yl)methylene)-4-(4-nitrophenyl)thiazol-2-amine (**76**). Orange solid powder; yield: 90.7 %; m.p.: 255-257 °C; R_f (20 % EtOAc in *n*-hexane) =0.27; UV-Visible (MeOH) λ_{max} =285 and 340 nm; ^1H -NMR (400 MHz, DMSO- d_6): δ 8.50 (s, 1H, CH =N), 7.85 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 8.5 Hz, 1H), 7.38 (s, 1H, sp^2 methine of thiazole-H), 6.92 (d, J = 8.2 Hz, 2H), 6.85-6.74 (m, 1H), 6.74 (d, J = 8.1 Hz, 2H), 6.18 (d, J = 8.3 Hz, 1H), 5.15 (dd, 3J = 11.12, 3J = 5.74 Hz, 1H, C₅-H_X), 4.50 (dd, 2J =18.21, 3J = 12.21 Hz, 1H, C₄-H_M), 3.57 (dd, 2J = 17.44, 3J =5.19 Hz, 1H, C₄-H_A); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 169.1 (C-2'), 160.7 (C-6), 159.5 (C-4'), 157.2 (C-3), 155.0 (C-10'), 151.6 (C-11), 148.5 (C-7'), 146.3 (C-9',11'), 137.1 (C-10', 12'), 129.2 (C-8',12'), 126.7 (C-9,13), 124.4 (C-4'), 124.3 (C-2',6'), 118.0 (C-8), 113.9 (C-3',5'), 107.1 (C-5'), 59.3 (C-5), 42.7 (C-4).

2-((2E)-2-((4,5-dihydro-3-(4-nitrophenyl)-5-phenylpyrazol-1-yl)methyleneamino)thiazol-4-yl)phenol (**77**). Orange solid powder; yield: 90.2 %; m.p.: 198-200 °C; R_f (20 % EtOAc in *n*-hexane) =0.17; UV-Visible (MeOH) λ_{max} =285 and 390 nm; ^1H -NMR (400 MHz, DMSO- d_6): δ 8.47 (s, 1H, CH =N), 7.81 (d, J = 8.3 Hz, 2H), 7.75 (s, 1H, sp^2 methine of thiazole-H), 7.52 (d, J

= 8.4 Hz, 2H), 7.21 (d, J = 7.9 Hz, 2H), 6.98 (d, J = 8.2 Hz, 2H), 6.89 (t, J = 7.6 Hz, 1H), 6.77 (d, J = 7.6 Hz, 2H), 6.57 (d, J = 8.0 Hz, 1H), 6.36 (d, J = 8.2 Hz, 2H), 6.17 (t, J = 8.8 Hz, 1H), 5.11 (dd, 3J = 11.62, 3J = 4.95 Hz, 1H, C₅-H_X), 4.53 (dd, 2J = 17.91, 3J = 11.23 Hz, 1H, C₄-H_M), 3.53 (dd, 2J = 18.43, 3J = 6.23 Hz, 1H, C₄'-H_A); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 168.8 (C-2'), 160.6 (C-6), 155.7 (C-4'), 155.0 (C-13), 151.6 (C-3), 148.5 (C-7'), 147.5 (C-9', 11'), 142.8 (C-10'), 141.2 (C-9), 137.1 (C-11), 129.5 (C-3', 5'), 128.1 (C-8', 12'), 126.62 (C-2', 6'), 126.1 (C-10), 124.2 (C-12), 117.3 (C-4'), 113.9 (C-5'), 59.5 (C-5), 42.5 (C-4).

(18E)-N-((5-(4-bromophenyl)-4,5-dihydro-3-(4-nitrophenyl)pyrazol-1-yl)methylene)-4-phenylthiazol-2-amine (**78**). Yellow solid powder; yield: 92.6 %; m.p.: 251-253 °C; R_f (20 % EtOAc in *n*-hexane) = 0.70; UV-Visible (MeOH) λ_{max} = 285 and 390 nm; ^1H -NMR (400 MHz, DMSO- d_6): δ 8.44 (s, 1H, CH = N), 7.78 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 11.8 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.25 (d, J = 8.7 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 6.96 – 6.54 (m, 5H), 6.51 (s, 1H, sp^2 methine of thiazole-H), 6.42 (d, J = 6.5 Hz, 1H), 6.14 (d, J = 8.2 Hz, 1H), 5.10 (dd, 3J = 12.82, 3J = 6.85 Hz, 1H, C₅-H_X), 4.47 (dd, 2J = 18.22, 3J = 12.13 Hz, 1H, C₄-H_M), 3.50 (dd, 2J = 18.43, 3J = 6.23 Hz, 1H, C₄'-H_A); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 169.1 (C-2'), 160.6 (C-6), 155.0 (C-4'), 148.5 (C-3), 141.6 (C-10'), 137.1 (C-1'), 134.1 (C-7'), 129.5 (C-9', 11'), 128.4 (C-4'), 125.9 (C-3', 5'), 124.3 (C-8', 12'), 118.2 (C-4'), 114.0 (C-8), 102.2 (C-5'), 59.5 (C-5), 42.5 (C-4).

(18E)-N-((5-(4-bromophenyl)-4,5-dihydro-3-(4-nitrophenyl)pyrazol-1-yl)methylene)-4-(4-nitrophenyl)thiazol-2-amine (**79**). Yellow solid powder; yield: 89.8 %; m.p.: 247-249 °C; R_f (20 % EtOAc in *n*-hexane) = 0.71; UV-Visible (MeOH) λ_{max} = 285 and 380 nm; ^1H -NMR (400 MHz, DMSO- d_6): δ 8.49 (s, 1H, CH = N), 7.92 (dd, J = 11.4, 7.0 Hz, 1H), 7.89- 7.62 (m, 1H), 7.57 (dd, J = 11.4, 7.0 Hz, 2H), 7.36 (d, J = 9.4 Hz, 1H), 7.10 (d, J = 9.6 Hz, 1H), 6.92 (s, 1H, sp^2 methine of thiazole-H), 6.78 (d, J = 8.3 Hz, 1H), 5.54 (dd, 3J = 11.13, 3J = 5.95 Hz, 1H, C₅-H_X), 3.52 (dd, 2J = 17.49, 3J = 4.99 Hz, 1H, C₄-H_M), 2.89 (dd, 2J = 18.73, 3J = 6.44 Hz, 1H, C₄'-H_A); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 169.1 (C-2'), 160.7 (C-6), 156.4 (C-8), 155.0 (C-3), 153.0 (C-10'), 148.2 (C-7'), 146.3 (C-9', 11'), 143.7 (C-11), 141.1 (C-4'), 132.4 (C-2', 6'), 129.9 (C-3', 5'), 128.6 (C-8', 12'), 127.6 (C-8), 126.7 (C-9, 13), 121.1 (C-10, 12), 107.1 (C-4'), 59.9 (C-5), 48.5 (C-4).

2-((2E)-2-((5-(4-bromophenyl)-4,5-dihydro-3-(4-nitrophenyl)pyrazol-1-yl)methylene amino) thiazol-4-yl)phenol (**80**). Pale yellow solid powder; yield: 78.3 %; m.p.: 258-260 °C; R_f

(20 % EtOAc in *n*-hexane) =0.26; UV-Visible (MeOH) λ_{max} =285 and 395 nm; $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 8.49 (s, 1H, CH =N), 7.92-7.80 (m, 2H), 7.76 (d, J = 8.2 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.56 (dd, J = 11.9, 8.3 Hz, 2H), 7.23 (d, J = 7.8 Hz, 1H), 7.09 (d, J = 7.8 Hz, 3H), 6.75 (dd, J = 10.9, 8.1 Hz, 2H), 6.60 (s, 1H, sp^2 methine of thiazole-H), 6.38 (d, J = 8.5 Hz, 2H), 5.12 (dd, J = 12.0, 5.5 Hz, 1H), 4.51 (dd, 3J = 11.79, 3J = 4.94 Hz, 1H, C₅-H_X), 3.33 (dd, 2J = 17.18, 3J = 5.22 Hz, 1H, C₄-H_M), 3.06 (dd, 2J = 17.73, 3J = 5.47 Hz, 1H, C₄'-H_A); $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6): δ 169.1 (C-2'), 160.7 (C-6), 156.4 (C-4'), 146.7 (C-3), 142.3 (C-13), 140.6 (C-10'), 139.8 (C-1'), 139.3 (C-8), 137.0 (C-7'), 131.8 (C-9', 11'), 129.2 (C-4''), 126.6 (C-3', 5'), 126.4 (C-2', 6'), 124.4 (C-8', 12'), 119.4 (C-9, 12), 117.3 (C-10, 11), 101.0 (C-5'), 63.7 (C-5), 43.8 (C-4).

2-(1-((E)-(4-phenylthiazol-2-ylimino)methyl)-4,5-dihydro-3-(4-nitrophenyl)-1H-pyrazol-5-yl)phenol (81). Yellow solid powder; yield: 86.3 %; m.p.: 248-250 °C; R_f (20 % EtOAc in *n*-hexane) =0.76; UV-Visible (MeOH) λ_{max} =285 and 395 nm; $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 8.56 (s, 1H, CH =N), 8.41 (d, J = 14.3 Hz, 1H), 7.90 (d, J = 8.5 Hz, 2H), 7.81 (s, 1H, sp^2 methine of thiazole-H), 7.86-7.69 (m, 2H), 7.62 (dd, J = 16.8, 8.7 Hz, 1H), 7.36-7.23 (m, 2H), 7.05-6.95 (m, 2H), 5.01 (dd, 3J = 12.33, 3J = 6.12 Hz, 1H, C₅-H_X), 3.96 (dd, 2J = 17.41, 3J = 5.79 Hz, 1H, C₄-H_M), 3.55 (dd, 2J = 17.72, 3J = 4.92 Hz, 1H, C₄'-H_A); $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6): δ 175.1 (C-2'), 166.3 (C-6), 163.4 (C-4'), 162.7 (C-3), 158.4 (C-10'), 155.8 (C-3), 148.1 (C-1'), 147.8 (C-9', 11'), 143.1 (C-6''), 133.1 (C-8', 12'), 130.8 (C-2'', 5''), 128.0 (C-3'', 4''), 127.6 (C-8), 127.0 (C-9, 13), 123.4 (C-11), 119.5 (C-10, 12), 116.3 (C-5'), 75.6 (C-5), 48.4 (C-4).

2-(1-((E)-(4-(4-nitrophenyl)thiazol-2-ylimino)methyl)-4,5-dihydro-3-(4-nitrophenyl)-1H-pyrazol-5-yl)phenol (82). Yellow solid powder; yield: 91.6 %; m.p.: 200-220 °C; R_f (20 % EtOAc in *n*-hexane) =0.56; UV-Visible (MeOH) λ_{max} =285 and 385 nm; $^1\text{H-NMR}$ (400 MHz, DMSO- d_6): δ 8.54 (s, 1H, CH =N), 7.88 (d, J = 8.7 Hz, 1H), 7.80 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.33-7.22 (m, 1H), 6.99 (d, J = 7.0 Hz, 1H), 6.75 (s, 1H, sp^2 methine of thiazole-H), 6.56 (d, J = 4.7 Hz, 1H), 5.17 (dd, 3J = 11.35, 3J = 4.59 Hz, 1H, C₅-H_X), 3.94 (dd, 2J = 17.49, 3J = 4.59 Hz, 1H, C₄-H_M), 3.25 (dd, 2J = 17.92, 3J = 4.92 Hz, 1H, C₄'-H_A); $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6): δ 169.1 (C-2'), 163.3 (C-6), 161.8 (C-4'), 158.9 (C-6''), 156.4 (C-3), 148.7 (C-10'), 146.3 (C-7'), 143.7 (C-8), 141.1 (C-9', 11'), 133.8 (C-8', 12'), 131.5 (C-2''), 128.6 (C-11), 126.7 (C-4''), 124.4 (C-9, 13), 124.0 (C-10, 12), 120.1 (C-3''), 116.9 (C-5''), 107.1 (C-5'), 66.3 (C-5), 49.0 (C-4).

2-(1-((E)-(2-thiazol-4-yl)phenol)methyl)-4,5-dihydro-3-(4-nitrophenyl)-1H-pyrazol-5-yl)phenol (83). Bright yellow solid powder; yield; 92.9 %; m.p.: 220-222 °C; R_f (20 % EtOAc in *n*-hexane) = 0.51; UV-Visible (MeOH) λ_{max} = 285 and 395 nm; 1H -NMR (400 MHz, DMSO- d_6): δ 10.77 (s, OH), 8.52 (s, 1H, CH = N), 8.38 (s, 1H, sp^2 methine of thiazole-H), 7.85 (d, J = 9.0 Hz, 4H), 7.83-7.76 (m, 1H), 7.80-7.63 (m, 5H), 7.33-7.18 (m, 5H), 7.02-6.93 (m, 5H), 6.84-6.61 (m, 1H), 6.59 (d, J = 17.0 Hz, 2H), 6.55-6.45 (m, 2H), 6.38 (d, J = 7.9 Hz, 2H), 6.20 (s, 1H, sp^2 methine of thiazole-H), 5.57 (dd, 3J = 11.95, 3J = 5.22 Hz, 1H, C₅-H_X), 3.94 (dd, 2J = 17.51, 3J = 5.29 Hz, 1H, C₄-H_M), 3.88 (dd, 2J = 17.52, 3J = 5.22 Hz, 1H, C₄-H_A); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 168.8 (C-2'), 163.9 (C-6), 163.3 (C-3), 159.1 (C-13), 156.4 (C-6''), 155.7 (C-7'), 148.7 (C-9', 11'), 148.4 (C-10), 147.5 (C-8', 12'), 143.6 (C-4''), 133.7 (C-9), 131.4 (C-10'), 128.2 (C-10), 127.7 (C-3''), 126.6 (C-11), 124.0 (C-2''), 118.4 (C-12), 116.8 (C-5''), 101.1 (C-5'), 49.0 (C-5), 40.1 (C-4).

4.2. Synthesis procedures of 5H-thiazolo [3,2- α] pyrimidines analogs

4.2.1. Synthesis of chalcones (1,3-diaryl-2-propen-1-ones), 88-90

An equimolar amount of substituted acetophenone derivatives (**84-86**) (0.06 mol) and the 2-hydroxy benzaldehyde (**87**) (0.06 mol), were dissolved in ethanol (5-10 mL), and aqueous NaOH (3 mL, 60 %) was added dropwise (Yadav et al., 2017). The obtained solid products (**88-90**) were recrystallized from absolute ethanol to yield a yellow precipitate of chalcone derivatives (Scheme 6). The 1H and ^{13}C -NMR spectra of all the synthesized compounds are presented in Appendix 46-51.

(E)-3-(2-hydroxyphenyl)-1-phenylprop-2-en-1-one (88). Orange solid powder; yield: 86.7 %; m.p.: 167-169°C; R_f (1 % EtOAc in *n*-hexane) = 0.64; 1H -NMR (400 MHz, DMSO- d_6): δ 7.88 (s, OH), 7.50 (d, J = 15.4 Hz, 1H), 7.44 (d, J = 7.5 Hz, 2H), 7.33 (d, J = 15.5 Hz, 1H), 7.03 (t, J = 7.3 Hz, 1H), 6.96 (dd, J = 7.6, 7.2 Hz, 2H), 6.51 (t, J = 7.7 Hz, 1H), 6.18 (d, J = 8.4 Hz, 1H), 5.93 (t, J = 7.4 Hz, 1H); ^{13}C -NMR (101 MHz, DMSO- d_6): δ 190.6, 166.2, 143.9, 139.0, 132.8, 132.6, 130.3, 129.1, 128.5, 122.3, 119.7, 118.1, 115.1.

(E)-1,3-bis(2-hydroxyphenyl)prop-2-en-1-one (89). Yellowish solid powder; yield: 92.6 %; m.p.: 153-154°C; R_f (1 % EtOAc in *n*-hexane) = 0.44; 1H -NMR (400 MHz, DMSO- d_6): δ

10.80 (s, OH), 8.36 (s, OH), 7.90 (d, $J = 8.5$ Hz, 1H), 7.85 – 7.77 (m, 1H), 7.74 (d, $J = 8.3$ Hz, 1H), 7.59 (dd, $J = 19.2, 8.5$ Hz, 1H), 7.24 (d, $J = 7.7$ Hz, 1H), 6.98 (t, $J = 7.7$ Hz, 1H), 6.56 (dd, $J = 8.3, 4.9$ Hz, 1H); ^{13}C -NMR (101 MHz, DMSO- d_6): δ 197.9, 166.9, 158.5, 150.8, 150.3, 148.0, 147.8, 144.3, 141.6, 129.9, 128.0, 127.6, 124.2, 123.9, 123.9.

(*E*)-3-(2-hydroxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**90**). Reddish solid powder; yield: 94.1 %; m.p.: 160-162 °C; R_f (1 % EtOAc in *n*-hexane) = 0.44; ^1H -NMR (400 MHz, DMSO- d_6): δ 7.98 (s, OH), 7.81 (d, $J = 8.4$ Hz, 1H), 7.75 (d, $J = 8.5$ Hz, 1H), 7.66 (d, $J = 8.4$ Hz, 1H), 7.57 (d, $J = 15.7$ Hz, 1H), 7.29 (d, $J = 15.6$ Hz, 1H), 6.82 (t, $J = 7.7$ Hz, 1H), 6.47 (d, $J = 8.2$ Hz, 1H), 6.40 (t, $J = 7.5$ Hz, 1H); ^{13}C -NMR (101 MHz, DMSO- d_6): δ 197.8, 167.2, 158.2, 150.2, 149.9, 141.6, 133.1, 129.9, 124.2, 124.1, 121.4, 120.8, 119.8.

4.2.2. Synthesis of 5H-thiazolo [3,2- α] pyrimidines analogs in the presence of ZnO nanoparticles as a catalyst derived from chalcone, **92-100**

A solution of substituted 2-amino-4-substituted phenylthiazole, **69-71** (1.5 mmol) in ethanol (20 mL) was mixed with the appropriate α , β -unsaturated ketones (chalcone), **88-90** (1.5 mmol) in presence of Na metal (0.5 g) under use of ZnO nanoparticles as a catalyst (0.43 mg, 0.2 mmol, 20 mol %). The mixture was heated under reflux for 3 h and then the solvent was evaporated under a vacuum. The residue was dissolved in chloroform and washed with water. The organic layer was separated, dried. The obtained solid was recrystallized from ethanol to yield thiazolo[3,2- α] pyrimidine analogs, **92-100** (Scheme 8) and recyclability study of the ZnO particles was conducted by taking a model reaction. The structures of the synthesized compounds, **92-100** are characterized using physicochemical (M.pt, color, and R_f) and spectroscopic (UV-Vis, ^1H -NMR, and ^{13}C -NMR) techniques. The ^1H and ^{13}C -NMR spectra of all the synthesized compounds are presented in Appendix 52-78.

2-(3, 7-diphenyl-5H-thiazolo[3,2- α]pyrimidin-5-yl)phenol (**92**). Brown cream solid powder; yield: 87.9 %; m.p.: 166-167 °C; R_f (1 % MeOH in DCM) = 0.74; UV-Visible (MeOH) λ_{max} = 290 and 420 nm; ^1H -NMR (400 MHz, DMSO- d_6): δ 7.68 (s, 1H, sp^2 methine of thiazole-H), 7.47 (s, 1H, CH=C), 7.15-6.93 (m, 1H), 6.92 (d, $J = 7.7$ Hz, 1H), 6.81 (d, $J = 7.9$ Hz, 1H), 6.65 (d, $J = 7.8$ Hz, 1H), 6.63- 6.52 (m, 1H), 6.52-6.45 (m, 1H), 6.38 (dd, $J = 17.2, 8.4$ Hz, 2H),

6.31 (dd, $J = 13.9, 7.7$ Hz, 3H), 6.24 (dd, $J = 13.0, 6.9$ Hz, 2H), 6.01 (d, $J = 7.8$ Hz, 1H), 5.99-5.60 (m, 1H), 4.66 (s, 1H, benzylic-H); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 200.6 (C-2a), 168.9 (C-6), 164.8 (C-14'), 161.0 (C-3'), 150.3 (C-8), 144.4 (C-9,13), 135.1 (C-11), 133.0 (C-10,12), 132.3 (C-4'), 129.5 (C-5',9'), 129.1 (C-6',8'), 129.0 (C-7'), 128.5 (C-12'), 127.8 (C-10'), 126.7 (C-10), 125.9 (C-11), 119.2 (C-11), 116.9 (C-5), 102.2 (C-2'), 49.1 (C-4).

2-(5-(2-hydroxyphenyl)-7-phenyl-5H-thiazolo[3,2- α]pyrimidin-3-yl)phenol (93). Brown solid powder; yield: 92.1 %; m.p.: 168-169 °C; R_f (1 % MeOH in DCM) = 0.46; UV-Visible (MeOH) λ_{max} = 290 and 320 nm; ^1H -NMR (400 MHz, DMSO- d_6): δ 8.96 (s, 1H, sp^2 methine of thiazole-H), 7.42 (s, 1H, CH=C), 7.20 (d, $J = 10.1$ Hz, 1H), 6.89 (d, $J = 7.4$ Hz, 1H), 6.74 (d, $J = 7.7$ Hz, 1H), 6.33 (m, $J = 8.8, 5.0$ Hz, 2H), 6.24 (d, $J = 7.7$ Hz, 2H), 5.73 (d, $J = 7.6$ Hz, 2H), 4.71 (s, 1H, benzylic-H); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 192.5 (C-2a), 177.5 (C-6), 173.3 (C-3'), 171.7 (C-14'), 168.5 (C-5'), 162.3 (C-8), 161.3 (C-10,12), 139.4 (C-9'), 136.7 (C-10'), 132.8 (C-5'), 130.85 (C-11'), 130.1 (C-8'), 129.6 (C-12'), 129.2 (C-7'), 128.5 (C-9,13), 128.1 (C-11), 124.8 (C-13'), 122.3 (C-4'), 120.1 (C-6'), 117.7 (C-5), 116.5 (C-2'), 49.2 (C-4).

2-(3-(4-nitrophenyl)-7-phenyl-5H-thiazolo[3,2- α]pyrimidin-5-yl)phenol (94). Brown cream solid powder; yield: 96.3 %; m.p.: 173-174 °C; R_f (1 % MeOH in DCM) = 0.44; UV-Visible (MeOH) λ_{max} = 290 and 390 nm; ^1H -NMR (400 MHz, DMSO- d_6): δ 14.78 (s, OH), 7.83 (s, 1H, sp^2 methine of thiazole-H), 7.66 (d, $J = 8.5$ Hz, 1H), 7.45 (d, $J = 8.6$ Hz, 1H), 7.29 (d, $J = 7.3$ Hz, 2H), 7.11 (dd, $J = 14.7, 8.0$ Hz, 1H), 7.02 (d, $J = 8.2$ Hz, 1H), 6.84-6.56 (m, 4H), 6.47 (d, $J = 7.6, 6.9$ Hz, 1H), 6.24-6.11 (m, 1H), 6.13-5.97 (m, 1H), 5.91 (s, 1H), 5.06 (s, 1H, CH=C), 4.57 (s, 1H, benzylic-H); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 176.8 (C-2a), 171.2 (C-6), 169.1 (C-3'), 167.9 (C-7'), 162.1 (C-14'), 160.7 (C-8), 150.1 (C-6), 148.0 (C-5',9'), 146.3 (C-4'), 141.0 (C-9, 13), 139.4 (C-10,12), 130.9 (C-11), 129.6 (C-9), 129.4 (C-11), 127.7 (C-12'), 126.6 (C-10), 124.4 (C-13'), 116.2 (C-6',8'), 112.8 (C-5), 107.1 (C-2'), 48.9 (C-4).

2-(7-(4-nitrophenyl)-3-phenyl-5H-thiazolo[3,2- α]pyrimidin-5-yl)phenol (95). Grayish solid powder; yield: 94.1 %; m.p.: 171-172 °C; R_f (1 % MeOH in DCM) = 0.66; UV-Visible (MeOH) λ_{max} = 290 and 350 nm; ^1H -NMR (400 MHz, DMSO- d_6): δ 7.45 (s, 1H, sp^2 methine of thiazole-H), 7.30 (d, $J = 8.2$ Hz, 1H), 6.86 (dd, $J = 43.0, 7.6$ Hz, 2H), 6.66 (dd, $J = 25.9, 8.3$ Hz, 2H), 6.61 – 6.47 (m, 1H), 6.39 (dt, $J = 13.4, 6.5$ Hz, 4H), 6.37-6.16 (m, 3H), 6.02 (d, $J = 7.4$ Hz, 3H), 5.62 (d, $J = 8.5$ Hz, 3H), 5.50 (d, $J = 8.1$ Hz, 1H), 5.08 (s, 1H, CH=C), 4.67 (s, 1H, benzylic-H); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 196.3 (C-2a), 171.2 (C-6), 168.8 (C-3'), 167.9

(C-11), 154.1 (C-14'), 150.1 (C-10,12), 135.1 (C-9), 131.1 (C-12'), 131.0 (C-11), 129.7 (C-8), 129.5 (C-10'), 128.9 (C-4'), 128.1 (C-9'), 127.8 (C-6',8'), 127.7 (C-7'), 126.7 (C-5'.9'), 125.9 (C-9,13), 113.0 (C-5), 102.1 (C-2'), 49.0 (C-4).

2-(5-(2-hydroxyphenyl)-7-(4-nitrophenyl)-5H-thiazolo[3,2- α]pyrimidin-3-yl)phenol (96). Grayish solid powder; yield: 91.6 %; m.p.: 183-184 °C; R_f (1 % MeOH in DCM) = 0.41; UV–Visible (MeOH) $\lambda_{\max}$ = 290 and 385 nm; ^1H –NMR (400 MHz, DMSO- d_6): δ 7.99 (s, 1H sp^2 methine of thiazole-H), 7.80 (s, 1H, CH=C), 7.39 (dt, J = 18.2, 9.1 Hz, 1H), 7.24 (t, J = 8.9 Hz, 3H), 7.16 (d, 1H), 7.14 (s, 1H), 6.93-6.71 (m, 1H), 6.62-6.46 (m, 1H), 6.22 (t, J = 7.8 Hz, 2H), 6.14 (d, J = 8.4 Hz, 2H), 6.02 (d, J = 8.2 Hz, 1H), 5.35 (d, J = 181.7 Hz, 3H), 4.70 (s, 1H, benzylic-H); ^{13}C –NMR (100 MHz, DMSO- d_6): δ 196.2 (C-2a), 172.5 (C-6), 167.5 (C-3'), 162.5 (C-11), 154.0 (C-8), 150.1 (C-14'), 132.2 (C-5'), 131.1 (C-10, 12), 131.0 (C-10', 9'), 130.5 (C-7', 12'), 125.2 (C-6', 13'), 117.0 (C-11' 8'), 116.2 (C-10'), 113.0 (C-5), 112.8 (C-2'), 49.0 (C-4).

2-(3,7-bis(4-nitrophenyl)-5H-thiazolo[3,2- α]pyrimidin-5-yl)phenol (97). Brown solid powder; yield: 90.8 %; m.p.: 198-199 °C; R_f (1 % MeOH in DCM) = 0.44; UV–Visible (MeOH) $\lambda_{\max}$ = 290 and 365 nm; ^1H –NMR (400 MHz, DMSO- d_6): δ 9.37 (s, OH), 7.81 (s, 1H, sp^2 methine of thiazole-H), 7.61 (s, 1H, CH=C), 7.07 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.1 Hz, 2H), 6.97 (d, J = 8.3 Hz, 2H), 6.92 (d, 1H), 6.42 (t, J = 7.2 Hz, 4H), 6.00 (d, J = 8.1 Hz, 2H), 5.88 (d, J = 8.2 Hz, 1H), 5.43- 5.02 (m, 1H), 4.55 (s, 1H, benzylic-H); ^{13}C – NMR (100 MHz, DMSO- d_6): δ 196.7 (C-2a), 177.2 (C-6), 172.5 (C-3'), 171.2 (C-11), 168.2 (C-7'), 161.0 (C-10,12), 154.2 (C-12'), 150.3 (C-8), 147.3 (C-5',9'), 132.2 (C-6',8'), 131.3 (C-11), 131.2 (C-9,13), 127.7 (C-4'), 125.3 (C-10'), 122.2 (C-13'), 113.2 (C-5), 113.0 (C-2'), 49.2 (C-4).

2-(5-(2-hydroxyphenyl)-3-phenyl-5H-thiazolo[3,2- α]pyrimidin-7-yl)phenol (98). Reddish brown solid powder; yield: 96.9 %; m.p.: 178-179 °C; R_f (1 % MeOH in DCM) = 0.83; UV–Visible (MeOH) $\lambda_{\max}$ = 290 and 420 nm; ^1H –NMR (400 MHz, DMSO- d_6): δ 8.30 (s, OH), 8.19 (s, OH), 7.97 (s, 1H, sp^2 methine of thiazole-H), 7.78 (s, 1H, CH=C), 7.41 (d, J = 7.2 Hz, 2H), 7.37-7.25 (m, 1H), 7.21 (d, J = 8.5 Hz, 1H), 7.15 (dd, J = 12.0, 8.3 Hz, 1H), 7.01-6.82 (m, 4H), 6.79 (d, J = 7.6 Hz, 1H), 6.72 (d, J = 7.4 Hz, 1H), 6.53 (s, 1H, CH=C), 6.58-6.46 (m, 2H), 6.00 (d, J = 8.1 Hz, 1H), 5.12 (s, 1H, benzylic-H); ^{13}C –NMR (100 MHz, DMSO- d_6): δ 167.6 (C-2a), 131.1 (C-6), 129.4 (C-3'), 128.9 (C-4'), 127.8 (C-8), 127.5 (C-13,10'), 126.7 (C-9,14'), 125.9 (C-11,12'), 125.2 (C-10,13'), 113.0 (C-5), 112.8 (C-2'), 49.0 (C-4).

2-(5-(2-hydroxyphenyl)-3-phenyl-5H-thiazolo[3,2- α]pyrimidin-7-yl)bisphenol (99).

Brown solid powder; yield: 94.5 %; m.p.: 189-190 °C; R_f (1 % MeOH in DCM) = 0.57; UV–Visible (MeOH) λ_{max} = 290 and 420 nm; ^1H –NMR (400 MHz, DMSO- d_6): δ 8.31 (s, OH), 7.99 (s, 1H, sp^2 methine of thiazole-H), 7.45 (dt, J = 25.8, 7.1 Hz, 1H), 7.37 (s, 1H, CH=C), 7.31-7.09 (m, 5H), 6.56-6.44 (m, 1H), 6.25-5.99 (m, 5H), 5.56 (d, 2H), 5.11 (s, 1H, benzylic-H); ^{13}C –NMR (100 MHz, DMSO- d_6): δ 196.2 (C-2a), 167.4 (C-6), 154.0 (C-3'), 146.6 (C-8), 139.1 (C-9,14',5'), 132.2 (C-12,10',8), 131.1 (C-11,12',7'), 130.5 (C-13,10',9'), 128.1 (C-4'), 125.2 (C-10'), 116.2 (C-10,13',6'), 113.7 (C-5), 113.0 (C-2'), 49.0 (C-4).

2-(5-(2-hydroxyphenyl)-3-(4-nitrophenyl)-5H-thiazolo[3,2- α]pyrimidin-7-yl) phenol

(100). Reddish brown solid powder; yield: 90.8 %; m.p.: 192-193 °C; R_f (1 % MeOH in DCM) = 0.64; UV–Visible (MeOH) λ_{max} = 290 and 420 nm; ^1H –NMR (400 MHz, DMSO- d_6): δ 8.32 (s, OH), 7.99 (s, 1H, sp^2 methine of thiazole-H), 7.60 (q, J = 8.0 Hz, 1H), 7.41 (dd, J = 19.1, 8.0 Hz, 1H), 7.33-7.17 (m, 3H), 7.19-7.06 (m, 1H), 6.55 (t, J = 9.1 Hz, 1H), 6.14 (d, J = 8.5 Hz, 3H), 6.02 (d, J = 8.1 Hz, 1H), 5.57 (s, 1H, CH=C), 5.11 (s, 1H, benzylic-H); ^{13}C –NMR (100 MHz, DMSO- d_6): δ 196.2 (C-2a), 167.4 (C-6), 154.0 (C-4'), 137.2 (C-7'), 131.1 (C-8), 125.2 (C-4'), 118.4 (C-6',8'), 117.3 (C-9',5'), 113.6 (C-12,11'), 113.0 (C-5), 79.4 (C-2'), 49.0 (C-4).

4.2.3. Regeneration of Catalyst

To examine the reusability of ZnO nanoparticles, after completion of the model reaction in specified time, the catalyst was recovered by filtration from the reaction mixture, after washed with dichloromethane and methanol, and dried at 100 °C for 5 hr before being used in the next cycle and reused as such for subsequent experiments under similar reaction conditions. The observed fact that yields of the product remained comparable in these experiments established the recyclability and reusability of the catalyst without any significant loss of activity.

4.3. Synthesis procedures of heterocyclic C₅-curcumin analogs

4.3.1. Synthesis of curcumin (1*E*,4*E*)-1,5-Bis(4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one, **103** & dehydrozingerone (*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one, **104**

C₅-curcumin, **103** and dehydrozingerone, **104** were synthesized via the condensation reaction under the basic conditions (Ning et al., 2013). A mixture of vanillin (4-hydroxy-3-methoxybenzaldehyde, 8.84 (1.35 g) and 4.42 mmol (0.68 g) mmol) (**101**), respectively in 5-mL acetone (**102**) was added to a well with a closed round-bottom flask and stirred for 5 min in an ice bath. After that, 1 mL of 20% NaOH in ethanol-water (1:1) was added dropwise, and the solution was then stirred at room temperature for approximately 1 h. During the reaction period, the reactor was covered with aluminum foil. The progress of the reaction was monitored by TLC using methanol/ dichloromethane (1:9) as a mobile phase. The reaction was worked up by adding 10% HCl solution to provide a solid product. The resulting solids were crushed into smaller pieces and filtered through filter paper under a vacuum, washed with cold water, and dried in a vacuum oven. A C₅-curcumin, **103** and dehydrozingerone, **104** was finally purified by recrystallization in ethanol to obtain a light-yellow solid (Scheme 9). Further purifications of the synthesized compounds were done using column chromatography. The ¹H and ¹³C-NMR spectra of all the synthesized compounds are presented in Appendix 79-80 and 81-82.

(1E, 4E)-1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (103). Yellow solid powder; yield: 86.7%; m.p.: 144-146 °C; R_f (30% EtOAc in *n*-hexane) = 0.30; ¹H-NMR (400 MHz, MeOD-*d*₄): 8.28 (s, 1H), 8.26 (s, 1H), 7.92 (d, *J* = 15.6 Hz, 2H), 7.76 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.45 (d, *J* = 15.9 Hz, 2H), 7.13 (d, *J* = 8.2 Hz, 2H), 6.58 (dd, *J* = 15.8 Hz, 2H), 3.83 (s, 3H); ¹³C-NMR (100 MHz, MeOD-*d*₄): δ 190.0 (C-3), 153.1 (C-4',4''), 149.9 (C-3',3''), 143.2 (C-1,5), 139.3 (C-2,4), 130.1 (C-1,1''), 125.0 (C-6,6''), 118.4 (C-2,2''), 110.9 (C-5,5''), 55.7 (C-7,7').

(E)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (104). Light-yellow solid powder; yield: 78.9%; m.p.: 157-159 °C; R_f (30% EtOAc in *n*-hexane) = 0.38; ¹H-NMR (400 MHz, MeOD-*d*₄): 7.42 (d, 1H, *J* = 16.5 Hz), 7.06 (dd, 1H, *J* = 8.5, 1.8 Hz), 7.03 (d, 1H, *J* = 1.9 Hz), 6.91 (d, 1H, *J* = 8.2 Hz), 6.56 (d, 1H, *J* = 15.6 Hz), 6.05 (br, 1H), 3.90 (s, 3H), 2.34 (s, 3H); ¹³C-NMR (100 MHz, MeOD-*d*₄): δ 201.4 (C,3), 150.9 (C,3'), 149.5 (C,4'), 146.5 (C,1'), 127.7 (C,4), 124.9 (C,6'), 124.5 (C,2), 116.5 (C,5'), 111.9 (C,2'), 56.4 (C,7'), 27.1 (C,4).

4.3.2. Synthesis of curcuminoid derivatives from dehydrozingerone, **105-107**

Vanillin (5 g, 0.033 mol) was dissolved in acetone (80 mL) to which, 50 ml NaOH (2.0 g, 0.25 mol) was slowly added with continuous stirring for 2-3 h. Excess acetone was removed under reduced pressure. Upon acidification with 0.1 N HCl, a yellow precipitate was obtained, which was extracted with CHCl₃ and the organic layer was dried over anhydrous sodium sulfate. Excess solvent was removed under reduced pressure and the yellow solid of (E)-4-(4-hydroxy-3-methoxyphenyl) but-3-en-2-one, (**104**) obtained was recrystallized from ethanol. Then, an equimolar amount of dehydrozingerone, (**104**) (0.06 mol), and the appropriate aromatic aldehyde derivatives (0.06 mol) were dissolved in a minimum amount of ethanol (5-10 mL), aqueous KOH (3 mL, 30 %) was added dropwise slight modification method (Yadav et al., 2017). The solid that appeared in the mixture was stirred in an ice bath for 30 min then at rt until the precipitate was formed within 3 h. The reaction mixture was stirred at room temperature until benzaldehyde consumption as monitored by TLC with an eluent of *n*-hexane: ethyl acetate (9:1). The precipitate will be filtered off and washed thoroughly with cold distilled water and cold methanol (2×20 mL). The C₅-curcumin analogs, **105-107** were recrystallized from absolute ethanol to yield a precipitate (Scheme 10). Then, the products were further purified using column chromatography. The ¹H and ¹³C-NMR spectra of all the synthesized compounds are presented in Appendix 83-88.

(1E,4E)-1-(4-hydroxy-3-methoxyphenyl)-5-(4-nitrophenyl)penta-1,4-dien-3-one (**105**). Brown solid powder; yield: 86.7 %; m.p.: 171-173 °C; R_f (30% EtOAc in *n*-hexane) = 0.73; ¹H-NMR (400 MHz, MeOD-*d*₄): 8.54 (s, 1H), 7.70 (d, *J* = 15.4 Hz, 1H), 7.64 (d, *J* = 15.6 Hz, 1H), 7.54 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.10 (d, *J* = 15.7 Hz, 1H), 7.07 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.99 (s, 1H), 6.95 (d, *J* = 11.1 Hz, 1H), 6.80 (d, *J* = 15.4 Hz, 1H), 6.74 (d, *J* = 9.0 Hz, 1H), 6.59 (d, *J* = 8.2 Hz, 1H), 3.83 (s, 3H); ¹³C-NMR (100 MHz, MeOD-*d*₄): δ 191.4 (C-3), 165.0 (C-4'), 153.81 (C-3'), 152.9 (C-10'), 147.7 (C-1), 145.5 (C-5), 144.5 (C-2), 131.5 (C-4), 127.9 (C-1'), 124.2 (C-7'), 121.9 (C-6'), 119.6 (C-5'), 113.1 (C-9',11'), 111.2 (C-8',12'), 110.8 (C-2') 55.8 (C-13').

(1E,4E)-1-(4-(dimethylamino)phenyl)-5-(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (**106**). Reddish solid powder; yield: 86.7 %; m.p.: 200-202 °C; R_f (30% EtOAc in *n*-hexane) = 0.71; ¹H-NMR (400 MHz, MeOD-*d*₄): 8.55 (s, 1H), 7.93 (d, *J* = 6.9 Hz, 1H), 7.78 (d, *J* = 15.4

Hz, 1H), 7.69 (dd, $J = 6.1, 2.1$ Hz, 2H), 7.66 (s, 1H), 7.40 (dd, $J = 10.5, 7.1$ Hz, 1H), 7.34 (dd, $J = 16.2, 1.4$ Hz, 1H), 7.26 (d, $J = 15.9$ Hz, 1H), 7.11 (dd, $J = 15.8, 2.1$ Hz, 1H), 6.81 (d, $J = 15.4$ Hz, 1H), 6.58 (d, $J = 8.2$ Hz, 1H), 3.84 (s, 3H), 3.31 (s, 6H); ^{13}C -NMR (100 MHz, MeOD- d_4): δ 191.1 (C-3), 170.3 (C-4'), 153.3 (C-3'), 149.3 (C-1), 142.7 (C-5), 136.7 (C-2), 131.2 (C-1'), 130.2 (C-7'), 129.9 (C-10'), 129.4 (C-5'), 128.7 (C-6'), 127.2 (C-4), 120.9 (C-8',12'), 118.3 (C-9',11'), 110.9 (C-2), 55.8 (C-13'), 24.2 (C-14',15').

(1E,4E)-1-(4-hydroxy-3-methoxyphenyl)-5-phenylpenta-1,4-dien-3-one (**107**). Dark brown solid powder; yield: 86.7 %; m.p.: 164-166 °C; R_f (30% EtOAc in *n*-hexane) = 0.89; ^1H -NMR (400 MHz, MeOD- d_4): 8.49 (s, 1H), 7.49 (d, $J = 15.8$ Hz, 1H), 6.95 (s, 1H), 6.93 (dd, $J = 8.3, 2.2$ Hz, 2H), 6.51 (d, $J = 7.9$ Hz, 2H), 6.35 (d, $J = 15.8$ Hz, 1H), 3.73 (s, 3H); ^{13}C -NMR (100 MHz, MeOD- d_4): δ 180.4 (C-3), 170.5 (C-4'), 165.1 (C-3'), 161.3 (C-1), 152.9 (C-5), 149.5 (C-7'), 127.7 (C-1'), 120.3 (C-2), 119.9 (C-4), 119.8 (C-10'), 110.8 (C-2'), 55.8 (C-13').

4.3.3. Synthesis procedures of heterocyclic C₅-curcumin analogs, **108-116** via ultrasonic irradiation-assisted approach

*General procedure for the synthesis of title compounds, **108-116** under conventional heating conditions:*

A mixture of C₅-curcumin derivatives, **105-107**, and different nucleophilic reagents, namely, hydrazine hydrate, urea, 2-amino-4-substituted phenylthiazole, and imidazole in catalytic condition under ethanol (30 mL) was refluxed for 4-8 h. After completion of the reaction, as indicated by TLC the reaction was quenched using ice-cold water (10 mL) was added and the resulting mixture was stirred for 30 min, and the precipitate thus formed was filtered under suction. The precipitated product was filtered, washed with water and acetone then dried under a vacuum. Further purifications of the synthesized compounds were done using column chromatography. The solid obtained was recrystallized using ethanol as presented below for synthesis of title compounds, **108-116**.

*General procedure for the synthesis of title compounds, **108-116** under ultrasonic radiation promoted procedure:*

A mixture of C₅-curcumin derivatives, **105-107** and different nucleophilic reagents, namely, hydrazine hydrate, urea, 2-amino-4-substituted phenylthiazole, and imidazole in catalytic condition under ethanol (10 mL) was sealed in the Pyrex tube and sonicated in an ultrasonic bath at 45-60 °C for the appropriate time. Cold/hot water was added and removed manually to maintain the temperature of the ultrasonic bath. After completion of the reaction as indicated by TLC using *n*-hexane: ethyl acetate (9:1 v/v) as eluent, the reaction mixture was cooled to room temperature. The precipitated product further purified by using silica gel column chromatography (60-120 mesh size) and washed with acetone, and then vacuum-dried. The obtained solid (**107-116**) was recrystallized from ethanol. In most cases, no further purification was necessary (Shabalala, Pagadala, & Jonnalagadda, 2015). The structures of the synthesized compounds, **108-116** are characterized using physicochemical (M.p, color, and R_f) and spectroscopic (UV-Vis, ¹H-NMR, and ¹³C-NMR) techniques. The substitution pattern at the aryl rings was carefully selected to confer versatile electronic environments which would certainly affect the anti-diabetic, anti-bacterial, and anti-oxidant activity pattern of the target molecules.

4.3.3.1. Synthesis of *N*-pyrazoline analogs, **108-109**

A mixture of curcumin derivatives, **105-107** (1 mmol), and hydrazine hydrate (10 mmol, 98%) in absolute ethanol (10 mL) then a homogeneous reaction mixture were sealed in the Pyrex tube was sonicated in an ultrasonic bath at 45-60 °C for 30 min. Cold/hot water was added or removed manually to maintain the temperature of the ultrasonic bath. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to room temperature and water. The precipitated product was filtered, washed with water and acetone, and then vacuum-dried. The obtained solid products of C₅-curcumin-based *N*-pyrazoline analogs (**108-109**) were recrystallized from ethanol (Scheme 11). Further purifications of the synthesized compounds were done using column chromatography. The ¹H and ¹³C-NMR spectra of all the synthesized compounds are presented in Appendix 89-92.

4-((1E)-2-(3-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazol-5-yl)vinyl)-2-methoxyphenol (**108**). Pale yellow solid powder; yield: 87.8%; m.p.: 215-217 °C; R_f(30% EtOAc

in *n*-hexane) = 0.67; ¹H-NMR (400 MHz, DMSO-*d*₆): 7.51 (s, 1H), 7.22 (s, 1H, NH), 7.14 (dd, *J* = 7.9, 2.1 Hz, 1H), 6.90 (d, *J* = 15.1 Hz, 1H), 6.72 (d, *J* = 10.6 Hz, 1H), 6.48 (dd, *J* = 6.9, 1.6 Hz, 2H), 6.38 (d, *J* = 8.2 Hz, 1H), 6.29 (d, *J* = 2.4 Hz, 1H, olefinic H), 6.29-6.23 (m, 1H), 5.90 (d, *J* = 15.8 Hz, 1H, olefinic H), 4.11 (dd, *J* = 11.6, 4.0 Hz, 1H, pyrazoline H_X), 3.39 (s, 3H, OCH₃), 2.47 (dd, *J* = 17.6, 11.6 Hz, 1H, pyrazoline H_M), 2.07 (dd, *J* = 17.6, 4.0 Hz, 1H, pyrazoline H_A); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 155.1 (C-4'), 153.2 (C-3'), 150.9 (C-12'), 144.3 (C-3), 136.2 (C-11', 13'), 134.7 (C-7'), 128.6 (C-6'), 128.4 (C-8'), 126.6 (C-2'), 118.5 (C-5'), 114.3 (C-9'), 114.2 (C-10', 14'), 59.4 (C-15'), 55.5 (C-5), 44.4 (C-4), 39.6 (C-16', 17').

4-((1*E*)-2-(4,5-dihydro-3-phenyl-1*H*-pyrazol-5-yl)vinyl)-2-methoxyphenol (**109**). Yellow solid powder; yield: 91.2%; m.p.: 146-148 °C; *R*_f (30% EtOAc in *n*-hexane) = 0.68; ¹H-NMR (400 MHz, DMSO-*d*₆): 8.51 (s, 1H), 7.42 (s, 1H, NH), 7.91 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.74 (dd, *J* = 15.6, 7.7 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.35 (s, 1H), 7.26 (dd, *J* = 8.1, 4.5 Hz, 1H), 7.12 (d, *J* = 2.1 Hz, 2H), 6.92 (d, *J* = 6.3 Hz, 1H, olefinic H), 6.82 – 6.70 (m, 1H), 6.64 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.59 (d, *J* = 16.2 Hz, 1H, olefinic H), 4.78 (dd, *J* = 10.2, 4.0 Hz, 1H, pyrazoline H_X), 3.29 (dd, *J* = 16.0, 10.6 Hz, 1H, pyrazoline H_M), 2.69 (dd, *J* = 10.9, 5.5 Hz, 1H, pyrazoline H_A), 3.78 (s, 3H, OCH₃); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 153.3 (C-4'), 151.9 (C-3'), 150.9 (C-3), 148.2 (C-9'), 147.2 (C-7'), 143.3 (C-6'), 132.6 (C-8'), 128.5 (C-5'), 126.6 (C-11', 13'), 120.6 (C-10', 14'), 119.1 (C-12'), 115.9 (C-9'), 110.0 (C-5'), 63.5 (C-15'), 55.7 (C-5), 43.9 (C-4).

4.3.3.2. Synthesis of 4-phenyl-6-styrylpyrimidine-2-ol analogs, **110-111**

The synthesis of 4-phenyl-6-styrylpyrimidine-2-ol compounds involves the reaction of monocarbonyl curcumin derivatives, **105-107** (1 mmol) with cyclization of nucleophilic reagents, urea (0.6 g, 1 mmol) was dissolved in an ethanolic sodium hydroxide (4 g NaOH and 10 mL ethanol) and then subjected to the ultrasonic irradiation. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to room temperature. The precipitated product was filtered, washed with water and acetone, and then vacuum-dried. The 4-phenyl-6-styrylpyrimidine-2-ol analogs precipitate obtained (**110-111**) was filtered, washed, and recrystallized in ethanol (Scheme 11). Further purifications of the synthesized compounds were

done using column chromatography. The ^1H and ^{13}C -NMR spectra of all the synthesized compounds are presented in [Appendix 93-96](#).

4-(4-hydroxy-3-methoxystyryl)-6-(4-(dimethylamino)phenyl)pyrimidin-2-ol (110). Brown solid powder; yield: 90.3%; m.p.: 184-186 °C; R_f (30% EtOAc in *n*-hexane) = 0.59; ^1H -NMR (400 MHz, DMSO- d_6): 9.40 (s, 1H), 8.23 (s, 1H), 7.43 (d, J = 9.0 Hz, 2H), 7.66 (d, J = 15.6 Hz, 1H), 7.33 (dd, J = 8.8, 1.8 Hz, 1H), 6.75 (d, J = 15.8 Hz, 1H), 6.53 (d, J = 9.1 Hz, 2H), 6.48 (d, J = 9.0 Hz, 1H, olefinic H), 6.32 (d, J = 6.5 Hz, 1H, olefinic H), 5.32 (s, 1H, Ar-CH, pyrimidine H-4), 2.78 (s, 6H, $-\text{N}(\text{CH}_3)_2$); ^{13}C -NMR (100 MHz, DMSO- d_6): δ 166.6 (C-2-OH), 160.2 (C-6), 154.4 (C-4), 151.9 (C-4'), 151.1 (C-12), 142.7 (C-11), 131.8 (C-2',6'), 130.3 (C-1'), 128.1 (C-3',5'), 124.6 (C-13), 122.3 (C-8), 121.1 (C-12), 119.1 (C-9), 112.0 (C-7), 111.2 (C-10), 110.7 (C-5), 55.58 (C-15), 43.3 (C-15,17).

4-(4-hydroxy-3-methoxystyryl)-6-phenylpyrimidin-2-ol (111). Brown solid powder; yield: 86.1%; m.p.: 166-168 °C; R_f (30% EtOAc in *n*-hexane) = 0.68; ^1H -NMR (400 MHz, DMSO- d_6): 8.91 (s, 1H), 8.19 (s, 1H), 7.56 (dd, J = 7.2, 2.2 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H), 7.11-6.88 (m, 1H), 6.63 (d, J = 8.2 Hz, 1H), 6.10 (d, J = 18.6 Hz, 1H, olefinic H), 5.87 (d, J = 8.3 Hz, 1H, olefinic H), 5.29 (s, 1H, Ar-CH, pyrimidine H-4), 4.20 (s, 1H). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 175.14 (C-2-OH), 171.0 (C-6), 169.9 (C-4), 166.7 (C-12), 160.3 (C-11), 140.5 (C-8), 129.2 (C-1'), 128.9 (C-7), 128.8 (C-4'), 128.3 (C-3,5'), 127.3 (C-2',6'), 127.2 (C-14), 126.6 (C-13), 117.7 (C-5), 54.8 (C-15).

4.3.3.3. Synthesis of 5*H*-thiazolo[3,2- α] pyrimidines analogs, **112-114**

A solution of 2-amino-4-substituted phenylthiazole (1.5 mmol) in ethanol (10 mL) was mixed with the appropriate α , β -unsaturated ketones/ C_5 -curcumin derivatives **105-107** (1.5 mmol) in presence of Na metal (0.5 g) and then subjected to the ultrasonic irradiation. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to room temperature. The precipitated product was filtered, washed with water and acetone, and then vacuum-dried. The obtained solid thiazolo[3,2- α] pyrimidine analogs (**112-114**) was recrystallized from ethanol ([Scheme 11](#)). In most cases no further purification was necessary. The ^1H and ^{13}C -NMR spectra of all the synthesized compounds are presented in [Appendix 97-102](#).

2-(7-(4-hydroxy-3-methoxystyryl)-5-(4-nitrophenyl)-5H-thiazolo[3,2-a]pyrimidin-3-yl)phenol (112). Brown solid powder; yield: 93.7%; m.p.: 177-179 °C; R_f (30% EtOAc in *n*-hexane) = 0.71; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 9.12 (s, 1H), 8.35 (s, 1H), 7.94 (s, 1H, sp^2 methine of thiazole-H) 7.54 (d, J = 9.4 Hz, 2H), 7.01 (dd, J = 15.2, 1.9 Hz, 2H), 6.95 (d, J = 8.3 Hz, 1H), 6.81 (d, J = 15.2 Hz, 1H, olefinic H), 6.56- 6.43 (m, 6H), 6.06 (d, J = 8.3 Hz, 1H, olefinic H), 6.29 (s, 1H, Ar-CH, pyrimidine H), 5.41 (s, 1H, benzylic-H), 3.58 (s, 3H, OCH_3); $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ 186.2 (C-2a), 175.5 (C-12), 171.9 (C-6), 170.2 (C-11), 166.9 (C-13'), 162.7 (C-5',9'), 159.9 (C-6'8'), 151.3 (C-8), 131.9 (C-14), 131.5 (C-12',14), 130.1 (C-7), 124.5 (C-3'), 120.4 (C-5',9'), 117.8 (C-7'), 117.2 (C-11'15'), 116.9 (C-5), 116.1 (C-2'), 115.8 (C-4), 54.6 (C-15).

2-(7-(4-hydroxy-3-methoxystyryl)-5-(4-(dimethylamino)phenyl)-5H-thiazolo[3,2-a]pyrimidin-3-yl)phenol (113). Pale yellow solid powder; yield: 91.0 %; m.p.: 196-198 °C; R_f (30% EtOAc in *n*-hexane) = 0.66; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 9.14 (s, 1H), 8.41 (s, 1H), 7.60 (d, J = 9.1 Hz, 1H), 7.58 (d, J = 2.1 Hz, 0H), 7.05 (d, J = 7.3 Hz, 1H), 6.84 (s, 1H, sp^2 methine of thiazole-H), 6.70 (d, J = 9.0 Hz, 1H), 6.55-6.48 (m, 6H), 6.07 (d, J = 8.3 Hz, 1H), 5.40 (s, 1H, Ar-CH, pyrimidine H), 4.26 (s, 1H, benzylic-H), 3.56 (s, 3H), 2.16 (s, 6H, $\text{N}(\text{CH}_3)_2$); $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ 189.9 (C-2a), 185.9 (C-6), 171.8 (C-4), 170.7 (C-13'), 166.7 (C-12), 162.8 (C-11), 159.8 (C-13), 154.3 (C-8), 131.6 (C-9), 131.4 (C-7), 130.0 (C-10'), 124.5 (C-3'), 117.4 (C-12',14'), 116.0 (C-14), 115.8 (C-5), 111.1 (C-2'), 54.5 (C-15), 40.1 (C-4), 27.4 (C-15',16').

2-(7-(4-hydroxy-3-methoxystyryl)-5-phenyl-5H-thiazolo[3,2-a]pyrimidin-3-yl)phenol (114). Brown solid powder; yield: 89.6%; m.p.: 142-144 °C; R_f (30% EtOAc in *n*-hexane) = 0.77; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 9.08 (s, 1H), 8.28 (s, 1H), 8.22 (s, 1H, Ar-CH, pyrimidine H-4), 7.71 (d, J = 7.4 Hz, 1H), 7.50 (dd, J = 7.6, 2.7 Hz, 1H), 7.11 (dd, J = 12.3, 6.9 Hz, 1H), 6.98 (d, J = 2.8 Hz, 1H), 6.96 (s, 1H, sp^2 methine of thiazole-H), 6.91 (d, J = 8.4 Hz, 1H), 6.78 (d, J = 12.7 Hz, 1H, olefinic H), 6.48-6.37 (m, 6H), 6.04 (d, J = 8.3 Hz, 1H, olefinic H), 5.38 (s, 1H, Ar-CH, pyrimidine H), 4.22 (s, 1H, benzylic-H), 3.22 (s, 3H); $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ 186.5 (C-22a), 175.8 (C-6), 172.2 (C-3'), 170.1 (C-12), 167.2 (C-11), 163.2 (C-9), 162.5 (C-10), 160.1 (C-8), 151.3 (C-7), 131.6 (C-14), 130.2 (C-13), 129.1 (C-5',9'), 129.0 (C-4'), 127.3 (C-6'8'), 120.3 (C-7'), 118.1 (C-10',15'), 116.4 (C-10), 121.8 (C-5), 115.9 (C-2'), 54.6 (C-15), 40.0 (C-4).

4.3.3.4. Synthesis of Michael addition products of *N*-Imidazole analogs, **115-116**

A mixture of imidazole (1 mmol) and C₅-curcumin derivatives, **105-107** (1.2 mmol) was added to a 10-mL flask containing anhydrous K₃PO₄ (0.25 mmol) in CH₃CN (10 mL), and the mixture was stirred at room temperature for 30 min and then subjected to the ultrasonic irradiation. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to room temperature. The precipitated product was filtered, washed with water and acetone, and then vacuum-dried. The obtained corresponding Michael adducts (**115-116**) were recrystallized from ethanol ([Scheme 11](#)). In most cases no further purification was necessary. The ¹H and ¹³C-NMR spectra of all the synthesized compounds are presented in [Appendix 103-106](#).

(E)-1-(4-hydroxy-3-methoxyphenyl)-5-(1*H*-imidazol-1-yl)-5-(4-nitrophenyl)pent-1-en-3-one (**115**). Greenish solid powder; yield: 81.5%; m.p.: 177-179 °C; R_f(30% EtOAc in *n*-hexane) = 0.77; ¹H-NMR (400 MHz, DMSO -*d*₆): 8.53 (s, 1H), 8.22 (d, *J* = 13.8 Hz, 1H), 8.09 (d, *J* = 13.0 Hz, 1H), 7.96 (d, *J* = 6.8 Hz, 7H), 7.60 (d, *J* = 16.4 Hz, 4H), 7.56 (s, 1H, imidazole CH, -HC=CH-), 7.51 (s, 1H, imidazole CH, -HC=CH-), 7.49 (s, 1H, -CH), 7.42 (d, *J* = 15.8 Hz, 1H), 6.86 (s, 1H, olefinic H), 6.41 (d, *J* = 14.8 Hz, 1H), 6.03 (m, 1H), 3.76 (dd, *J* = 21.1, 4.6 Hz, 1H), 3.73 (dd, *J* = 16.9, 8.8 Hz, 1H), 3.63 (s, 3H, OCH₃); ¹³C-NMR (100 MHz, DMSO -*d*₆): δ 195.5 (C-8), 183.6 (C-20), 147.6 (C-11), 145.7 (C-10), 140.8 (C-16), 134.3 (C-2), 132.1 (C-4), 126.6 (C-5), 124.4 (C-19,21), 120.5 (C-16), 119.1 (C-15), 115.4 (C-17), 112.3 (C-18,22), 110.9 (C-9), 63.4 (C-23), 54.8 (C-6), 44.9 (C-7).

(E)-5-(4-(dimethylamino)phenyl)-1-(4-hydroxy-3-methoxyphenyl)-5-(1*H*-imidazol-1-yl)pent-1-en-3-one (**116**). Reddish solid powder; yield: 89.7%; m.p.: 184-186 °C; R_f(30% EtOAc in *n*-hexane) = 0.72; ¹H-NMR (400 MHz, DMSO-*d*₆): 8.55 (s, 1H), 8.50 (s, 1H, imidazole CH, -HC=CH-), 7.96 (s, 1H, -CH), 7.69 (d, *J* = 9.0 Hz, 1H), 7.66 -7.62 (m, 2H), 7.60-7.50 (m, 2H), 7.42 (d, *J* = 15.8 Hz, 1H), 7.11 (s, 1H, imidazole CH, -HC=CH-), 7.06-6.95 (m, 4H), 6.76 (dd, *J* = 16.1, 9.0 Hz, 3H), 6.53-6.34 (m, 1H), 3.77 (dd, *J* = 15.8, 3.6 Hz, 1H), 3.75 (dd, *J* = 14.2, 2.9 Hz, 1H), 3.72 (s, 3H), 3.04 (s, 6H); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 186.6 (C-8), 165.8 (C-14), 159.8 (C-20), 154.2 (C-13), 151.5 (C-10), 150.0 (C-4), 145.3 (C-7), 143.8 (C-5), 141.0 (C-16), 135.2 (C-15), 130.1 (C-11), 129.8 (C-17), 124.5 (C-12), 122.5 (C-19,21), 121.7 (C-18,22), 63.2 (C-23), 55.2 (C-6), 40.6 (C-7), 27.5(C-24,25).

4.4. Biological activity evaluations

4.4.1. *In vitro* antibacterial activity using agar disc diffusion assay

The antibacterial susceptibility of each bacterial strain to all synthesized compounds was assessed using the Clinical and Laboratory Standards Institute (CLSI) procedures using agar disc diffusion assay (Balouiri, Sadiki, & Ibnsouda, 2016; Humphries et al., 2018). The inhibition zone technique shows the ability of a compound or drug to prevent microbial growth (Hudzicki, 2009). The Adama Public Health Research & Referral Laboratory Center provided us four strains of pathogenic microorganisms from the American Type Culture Collection of two Gram-positive bacteria, including the MRSA strain of *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 27853), and two gram-negative bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Colony morphology and Gram staining, as well as routine biochemical assays, were used to identify and authenticate the bacterial strains, according to Bergey's Manual of Determinative Bacteriology (Bergey, 1994).

The bacterial strains were brought to the microbiology lab with nutrient agar and kept at 4 °C until they were needed. Microbial cultures were cultured in nutrient broth overnight at 37 °C, adjusted to 0.5 McFarland standards with distilled water, and then inoculated onto Mueller-Hinton agar plates. The tested compounds were freshly dissolved in 1 mL of DMSO and adjusted to a concentration of 250 and 500 µg/mL. After incubation, the test organism was inoculated into the Muller-Hinton agar medium using a sterile cotton swab. Then sterile filter paper (6 mm diameter) was soaked in 1mL DMSO solution of the compounds at 250 and 500 µg/mL concentrations separately loaded with 10 µl of prepared compounds, along with the positive and negative controls, and left till dryness and then positioned on a previously inoculated nutrient agar with the target microbial strain. After 2 h of diffusion at 4 °C, the plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms and comparing it to amoxicillin as a reference drug at 25 and 50 mg/ml, respectively, while the negative control was DMSO. The experiments were repeated three times, and the average clear zone (inhibition zone) around each compound was measured with a caliper in millimeters (mm)(Eloff, 2019).

4.4.2. *In vitro* α -amylase inhibitory assay

The pancreatic porcine α -amylase inhibitory activity was adapted with modifications (Chortani et al., 2021). Briefly, the enzyme, and soluble starch were dissolved in 20 mM sodium phosphate buffer containing 6 mM NaCl (pH 6.9). The synthesized compounds at different concentrations were dissolved in the buffer solution. To a test tube, 250 μ L of pancreatic porcine α -amylase (1 U/mL, dissolved in the buffer (pH 6.9) and 100 μ L of synthesized compounds at a concentration ranging from 1.25 to 10 μ g/mL were added. The mixture was pre-incubated at 37 °C for 15 min, before the addition of 250 μ L of 0.5% starch. The mixture was then vortexed and incubated again at 37 °C for 15 min followed by the reaction termination using 1 mL of dinitrosalicylic acid (DNS) color reagent. The tubes were placed in a boiling water bath for 5 min, cooled to room temperature, and diluted. Two hundred microliters of the reaction mixture were taken into a 96-well clear plate, and the absorbance was read at 540 nm by Thermo Scientific™ GO Microplate Spectrophotometer. The control α -amylase at 1 U/mL without any inhibitor represented 100% enzyme activity. Appropriate synthesized compounds controls containing the reaction mixture except the enzyme were used to correct for the color interference. A known α -amylase inhibitor, acarbose was used for comparison studies. The percentage inhibition of the test sample on α -amylase was calculated as:

$$\% \text{ Alpha-amylase inhibition} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100 \quad (1)$$

Where, A sample and A control are the absorbance of the sample and control, respectively. By graphing inhibition percentages versus sample concentrations, the concentration of the drug that provides 50% inhibition (IC_{50}) was determined. Each assay was performed in triplicate.

4.4.3. *In vitro* DPPH free radical scavenging assay

The ability of the synthesized compounds to transfer hydrogen atoms or electrons was measured by bleaching the purple color of a methanolic solution of DPPH. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) approach was used to examine the *in vitro* radical scavenging activity of

the synthesized compounds with minor adjustments (Ullah et al., 2017). To a solution of 1 ml of 0.004% methanolic solution of diphenyl picrylhydrazyl (DPPH), 4 ml of various sample concentrations were added. After 60 minutes in the dark, the absorbance was measured against a blank at 517 nm using a double-beam UV-Vis spectrophotometer. The blank reading solution was methanol, while the standard solution was ascorbic acid, which was calculated by the following equation:

$$\% \text{ DPPH free radical scavenging} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad (2)$$

Where, A_{control} is the absorbance of the control (DPPH without compound), and A_{sample} is the absorbance of the test sample. To determine the IC_{50} , different concentrations of the test compounds in methanol (12.5 to 200 $\mu\text{g/mL}$) were added to a methanolic solution of DPPH (the concentration that makes 50 % inhibition of the DPPH color). All IC_{50} values were computed from the corresponding sigmoidal dose-response curve according to their best-fit shapes based on at least five reaction points. All experiments were carried out in triplicates to calculate the mean and standard deviation statistically.

4.4.4. *In vitro* lipid peroxidation inhibition assay

Lipid peroxidation was estimated by 2-thiobarbituric acid (TBA) reaction with malondialdehyde (MDA) a product formed due to the peroxidation of membrane lipids (Varma et al., 2020). The lipid peroxidation inhibition assay was conducted according to the procedure described with slight modifications (Yehye et al., 2012). This assay is based on the reaction of a chromogenic reagent, TBA, with MDA at room temperature. One molecule of MDA reacts with two molecules of TBA *via* condensation to yield a chromophore with an absorbance maximum at 532 nm. The synthesized compounds and linoleic acid were homogenized using the homogenizer, and the supernatant was used for the assay. The Thiobarbituric acid-Trichloroacetic acid (TBA-TCA) mixture was prepared by dissolving 0.392 g TBA in 75 mL of 0.25 M HCl, followed by the addition of 5 g TCA, and made up to 100 mL of 0.25 M HCl. In this assay, 500 μL of the

TBA-TCA mixture was added to a mixture containing 250 mL of homogenate and 200 mL PBS buffer, in a clean test tube. These test tubes were covered with aluminum foil and boiled in a water bath at 100 °C for 30 min. The mixture was allowed to cool at room temperature, centrifuged at 2000 rpm for 10 min and the supernatant was transferred into a 96-microwell plate. Absorbance was read on a microplate reader at 532 nm. The percentage of lipid peroxidation inhibition was calculated from the following equation:

$$\% \text{ lipid peroxidation inhibition} = \frac{A_{\text{control}} - A_{\text{compound}}}{A_{\text{compound}}} \times 100 \quad (3)$$

Where A compound and A control are Absorbance of the compound, and control, respectively. The percentage of lipid peroxidation inhibition was plotted against the synthetic compound concentration to determine the concentration required to achieve 50% inhibition of phospholipid oxidation (IC₅₀) in linoleic acid; ascorbic acid was used as a positive control.

4.5. *In silico* computational studies

4.5.1. ADMET, drug-likeness, and toxicity prediction

The prediction of the *in silico* ADMET parameters before the experimental studies is one of the most important aspects of drug discovery and development processes, it helps to understand whether a particular drug has properties suitable for being an orally active drug. ADMET studies play a critical role in helping to optimize the pharmacokinetic and pharmacodynamic properties of new drugs (Rao et al., 2011). This prediction is based on an already established concept by the Lipinski rule of five (Veber et al., 2002) and the Veber rule (Daina, Michielin, & Zoete, 2017). Properties of the compounds were estimated using ADMET descriptors by using SwissADME (<http://www.swissadme.ch/>), PreADMET (<https://preadmet.webservice.bmdrc.org/>), and AdmetSAR (<http://lmmd.ecust.edu.cn/admetSar1>) online toolkit servers. Structures of compounds were submitted to SwissADME tools and converted to their canonical simplified molecular-input line-entry system (SMILES), to estimate *in silico* pharmacokinetic parameters and other molecular properties based on the reported method (Ahmed & Alkali, 2019). The

organ toxicity profiles and toxicological endpoints of the ligands and toxicity class were predicted using Pro Tox II web server explore (<https://tox-new.charite.de/>).

4.5.2. Molecular Docking study

A molecular docking study was performed to investigate the binding modes of the synthesized compounds against target enzymes. ChemDraw program was used to draw chemical structures of compounds with suitable 2D orientation, and ChemDraw Ultra 8.0 was employed to minimize the energy of ligands and standard drugs. The protein preparation and docking simulation were then carried out using the energy-minimized ligand molecules as input to AutoDock Vina 4.2.6 (Seeliger & de Groot, 2010). The crystallographic structures of DNA gyrase B (PDB ID: 6F86), PqsA (PDB ID: 5OE3), Pyruvate Kinase (PDB ID: 3T07), LuxS (PDB ID: P0C0C7), Penicillin binding proteins (PBPs) (PDB ID: 1VQQ), β -lactamases (PDB ID: 1IYS), Human peroxiredoxin 5 (PDB ID: 1HD2) and α -amylase enzyme (PDB ID: 4W93) were retrieved from the Protein Data Bank (<https://www.rcsb.org>) to investigate the interaction of the synthesized compounds with the active sites of protein targets using the following grid box parameters (PDB ID: 6F86) [62x30x64], [15x14x19], (PDB ID:5OE3) [38x-2x17], [19x21x14], (PDB ID:3T07) [-15x-3x2],[18x14x16], (PDB ID: P0C0C7) [46x45x39], [14x12x17],], (PDB ID: 1VQQ) [18x32x38], [16x18x17], (PDB ID: 1IYS) [-5x40x29], [21x22x14], (PDB ID:1HD2) [6x44x33], [15x18x17] and (PDB ID: 4W93) [-11.95x10.64x-17.96], and [26.5x 15.8x16.39] Å (x, y, and z) (Trott & Olson, 2010). The best-docked conformation between ligand and protein was searched using the docking algorithm with AutoDock Vina. During the docking process, a maximum of nine conformers were considered for each ligand. Meanwhile, the Discovery Studio Visualizer 2021 (Biovia) was used to inspect the possible interactions and ligands orientations between the target receptor and ligands by identifying conformations to optimize the docking function (Jones, Willett, Glen, Leach, & Taylor, 1997).

4.6. Statistical data analysis

All experiments were performed in triplicate ($n = 3$). The *in vitro* antidiabetic and antioxidant activities of synthetic compounds were expressed as 50% inhibitory concentration (IC_{50}) values. All data were presented as the mean $\pm$ standard error of the mean (95% confidence intervals) of three independent experiments. Office Excel 2016 software (Microsoft, Redmond, WA, USA) was used for the statistical and graphical analysis. Statistical significance was assessed using a one-way analysis of variance ANOVA followed by a t-test to determine the degree of significance with the single normal and experimental control group. When the P-value < 0.05 , differences between data sets were judged statistically significant.

CHAPTER FIVE

5. RESULTS AND DISCUSSIONS

Considering the pace at which drug resistance is emerging among the pathogens of public health significance, drug design, discovery, and development are the current pressing hot issues (Streicher, 2021). This study was done in the search for new small organic molecules with significant antidiabetic, antioxidant, and antibacterial activities with a huge potential for new medicine development. A series of novel thiazolyl-pyrazoline Schiff base hybrids (**75-83**) and thiazolo[3,2- α] pyrimidines analogs (**92-100**) were synthesized derived from chalcone *via* ZnO nanoparticle as a Lewis acid catalyst to activate the carbonyl groups to afford the target products with good to excellent yields (78.3-96.9%). The intrinsic Lewis acidity of the zinc ion makes ZnO nanoparticles highly stable and capable of taking on various shapes and sizes. These characteristics were the major factors for the synthesis of compounds. Shorter reaction times were achieved for the ZnO nanoparticle-catalyzed approach compared to the conventional glacial acetic acid-catalyzed reactions under reflux conditions. When compared to typical conditions, this approach is experimentally simple, higher yield, and greener approach, with improved reaction times (Chacko & Samanta, 2017; Z. Hussain, Yousif, Ahmed, & Altaie, 2014; Wu, Luo, & Hu, 2018). Thus, the green synthesis approach using ZnO nanoparticles as catalysts was found to be an efficient method to synthesize such biologically active compounds compared to the conventional method. NMR spectral data of the synthesized compounds are presented in [Appendix 19-78](#).

5.1. Recyclability study of catalyst

An important investigation into catalysis is the recyclability study, thus, good catalysts should be stable and have high activity for scale-up applications. As a result, this work study the thiazolyl-pyrazoline Schiff base with compound, **78** and thiazolo[3,2- α] pyrimidine analogs' with compound, **92** recyclability under ideal reaction conditions. After the reaction was completed, the catalyst was filtered, washed with dichloromethane and methanol, and dried at 100 °C for 5 hr before being used in the next cycle with no apparent loss of activity, making this technique cost-effective and environmentally benign ([Table 1](#) and [Figure 14](#)) (Piltan & Ghomi, 2016). The

catalyst ZnO-NPs were observed to have good catalytic activity for six consecutive runs with no significant catalytic activity loss and provided the product with a small decrease in reaction yield as shown in Figure 14. As presented in Table 1, ZnO nanoparticles were successfully recovered and reused after the reaction was completed, with no apparent loss of activity, making this process cost-effective and environmentally friendly. When compared to traditional acid catalysts, the efficient utilization of ZnO nanoparticles allows for a faster overall process time and higher product yields by reducing undesirable by-product generation during the reaction (Aldalbahi et al., 2020).

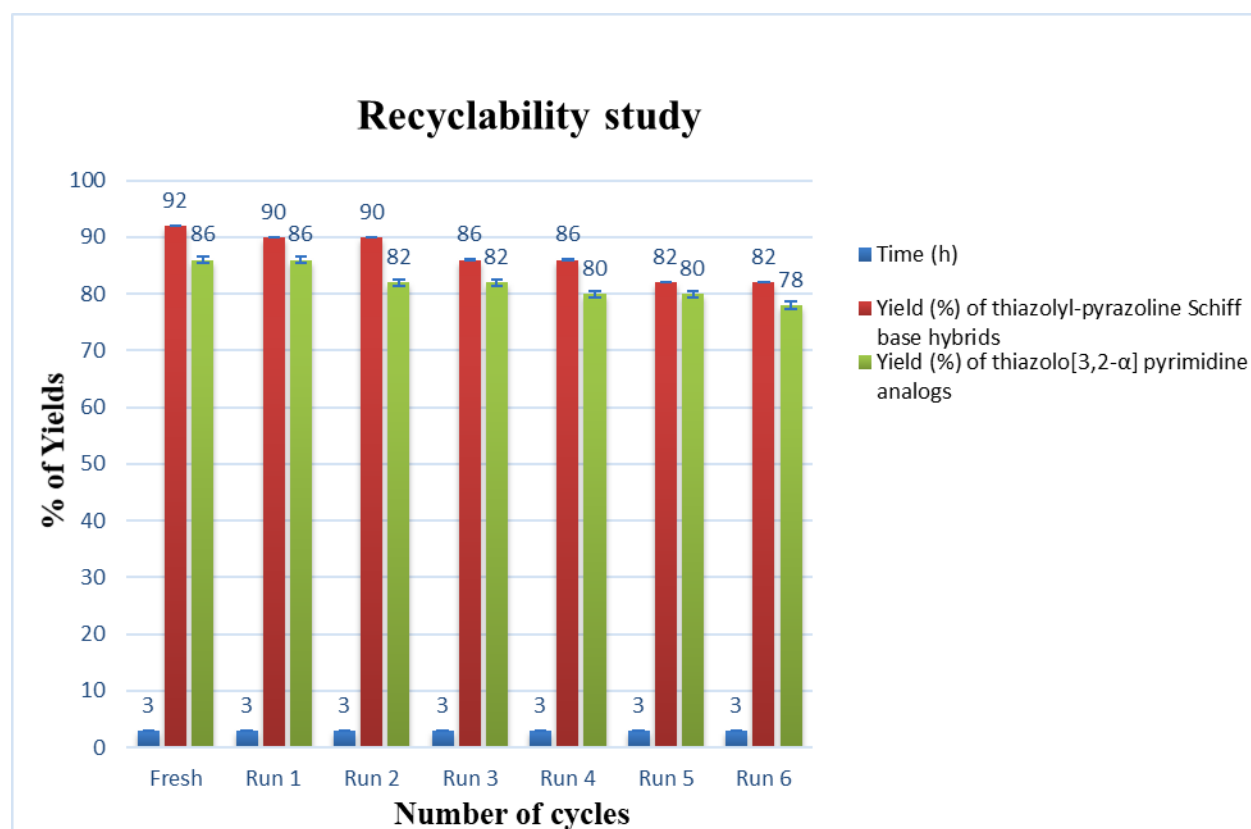


Figure 14. The synthesis of thiazolyl-pyrazoline Schiff base hybrids, **75-83** and thiazolo[3,2- α] pyrimidine analogs, **92-100** from chalcone catalyzed by ZnO-NPs investigated for recyclability.

Table 1. Recycling study of ZnO NPs solid catalyst for the model reaction

Entry	Time (h)	Synthesized compound, 78 isolated Yield (%) ^a	Synthesized compound, 92 isolated Yield (%) ^a
Fresh	3	92	86
Entry 1	3	90	86
Entry 2	3	90	82
Entry 3	3	86	82
Entry 4	3	86	80
Entry 5	3	82	80
Entry 6	3	82	78

^a Yields are related to isolated pure products.

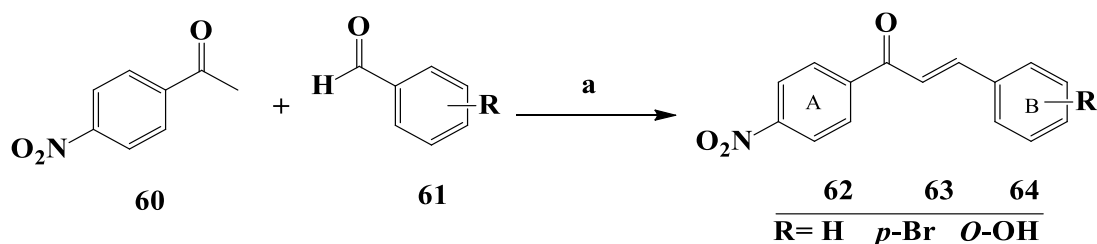
5.2. Chemistry of thiazolyl-pyrazoline Schiff base analogs

The title hybrid compounds were synthesized by a Schiff base reaction, in which a 2-amino thiazole derivative and carbonyl compound (*N*-formyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole) in ethanol was refluxed in the presence of glacial acetic acid and ZnO nanoparticles, undergo nucleophilic addition reaction, to form a hemiaminal derivatives. This reaction was followed by dehydration to generate a stable imine, forming Schiff base products (Cordeiro & Kachroo, 2020). The plausible reaction mechanism for the synthesis of the title compounds, a synthetic approach utilizing zinc oxide nanoparticles act as a Lewis acid to activate the carbonyl groups, resulting in the synthesis of thiazolyl-pyrazoline Schiff base hybrids. Finally, ZnO nanoparticles were effectively recovered and reused for numerous runs without apparent loss of activity, making this approach cost-effective and hence environmentally benign.

5.2.1. Synthesis of chalcone derivatives, 62-64

Chalcone scaffolds were synthesized at room temperature by reacting *p*-nitro acetophenone with substituted aryl aldehyde derivatives *via* a base catalyzed Claisen-Schmidt condensation reaction (Scheme 1). The substitution pattern at the aryl rings was carefully selected to confer versatile electronic environments which would certainly affect the antidiabetic, antibacterial, and

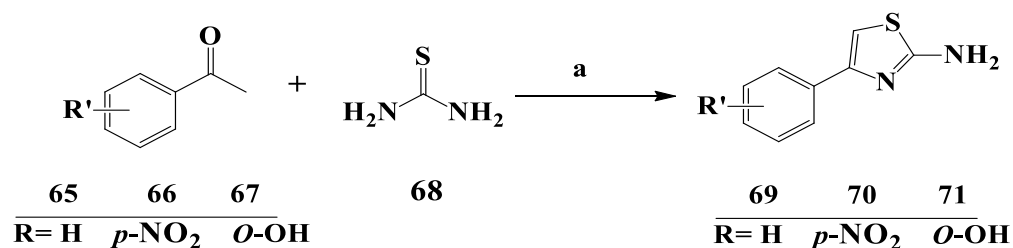
antioxidant activity patterns of the title molecules. In the ^1H -NMR spectrum of compounds (**62-64**), H- β and H- α protons of α , β -conjugated system of chalcone appeared as a broad doublet in between δ 7.73-7.75 and δ 7.32-7.39, respectively, with a coupling constant of $^3J = 15.6$ Hz, which agrees with a trans configuration. For compound **62**; AAXX' spin system aromatic protons for ring A and monosubstituted ring B are evident at δ 7.43-7.95. For compound **63**, two sets of AAXX' spin system aromatic protons are observed between δ 7.23-7.46 and δ 7.52-7.96. For compound **64**, AAXX' spin system aromatic protons for ring A and disubstituted ring B are evident at δ 7.70-7.88 and δ 8.00-8.04, respectively. In the ^{13}C -NMR spectrum of compounds (**62-64**), the C- β and C- α signals appeared at δ 138.4 and 120.1, respectively, while the carbonyl group appeared around δ 167-189.9.



Scheme 1. Synthesis of chalcone derivatives, **62-64**. *Reagent and conditions:* (a) NaOH (60%), ethanol, rt, 3 h.

5.2.2. Synthesis of 2-amino-4-(substituted-phenyl)thiazole derivatives, **69-71**

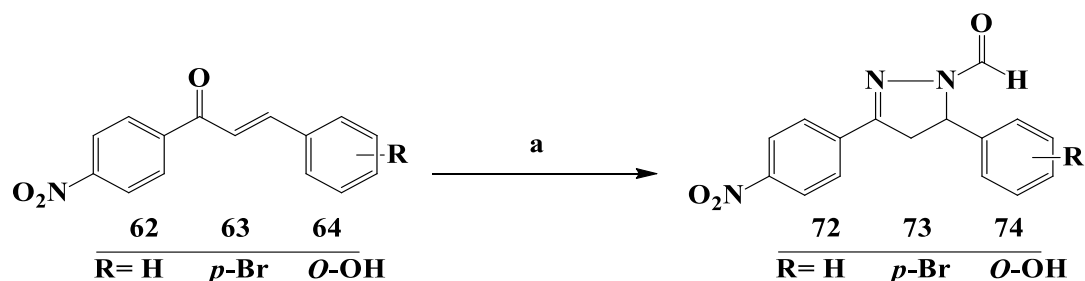
Thiazole amine derivatives, **69-71** were synthesized by reacting substituted acetophenone and thiourea in the presence of a catalyst ([Scheme 2](#)), the intermediate 4-(4-substituted phenyl)thiazol-2-amines were obtained from good to excellent yields (48.2-96.6 %). The aryl ring substitution pattern was carefully chosen to provide a variety of electronic environments that would affect the molecules' bioactivity patterns. The singlet at δ 2.9 in the ^1H -NMR spectrum of **69-71** confirms the existence of the $-\text{NH}_2$ group. The singlet at δ 6.9-7.80 suggests the presence of sp^2 methine in the thiazole ring. Aromatic methine peaks were observed between δ 6.93-7.52. The ^{13}C -NMR spectrum i.e. phenyl ring peaks at δ 119.2, 127.6, 128.4, 131.2, and 143.3, and peaks that belong to the thiazole ring appeared at δ 107.4, 148.2 and 165.1.



Scheme 2. Synthesis of 2-amino-4-(substituted-phenyl)thiazole derivatives, **69-71**. *Reagent and conditions:* (a) I₂, 100 °C, reflux for 3h.

5.2.3. Synthesis of *N*-formylpyrazoline derivatives, **72-74**

Chalcones (**62-64**) were used as the starting material for a nucleophilic cycloaddition reaction with hydrazine hydrate to form intermediates of *N*-formyl pyrazolines' (**72-74**). The *N*-formyl pyrazoline ring is produced by cyclization and dehydration of chalcone derivatives after a 1,4-Michael addition of hydrazine followed by formylation reaction with formic acid. The synthesis of *N*-formyl pyrazoline derivatives (**72-74**) was found in excellent yields (80-96%) *via* in two steps (Scheme 3). In the ¹H-NMR spectrum, the diastereotopic protons H_A, H_M, and H_X that correspond to the pyrazoline nucleus display an AMX spin pattern where H_A and H_M each appear as a doublet of a doublet at H_A appeared at δ 2.91-3.06 (dd, ²J_{AM}= 17.41, ³J_{AX}=4.91 Hz, C₄-H_A of pyrazoline) and H_M δ 3.42-3.90 (dd, ²J_{MA}=17.27 Hz, ³J_{MX}= 11.60 Hz, C_{4'}-H_M of pyrazoline). The H_X methine (C-H) proton of pyrazoline moiety appeared in the range of δ 4.60-5.65 with coupling constants of (dd, ³J_{AX}= 11.71, ³J_{MX}= 4.92 Hz, C₅-H_X of pyrazoline) suggesting vicinal coupling of methine with magnetically non-equivalents methylene protons. The aldehyde proton signal appeared as a singlet at δ 8.93-9.23. The ¹³C-NMR spectrum shows that the methylene carbon, sp³ methine, and sp² quaternary carbons of the pyrazoline ring appeared C-4 at δ 42.2-44.9, C-5 at δ 62.7-65.2, and C-3 at δ 155.8, respectively. Whereas, the aldehyde carbonyl carbon appeared in between at δ 160.1-167.5 range confirming the formation of *N*-formyl pyrazoline derivatives (**72-74**). All the other aromatics were observed in the expected regions.



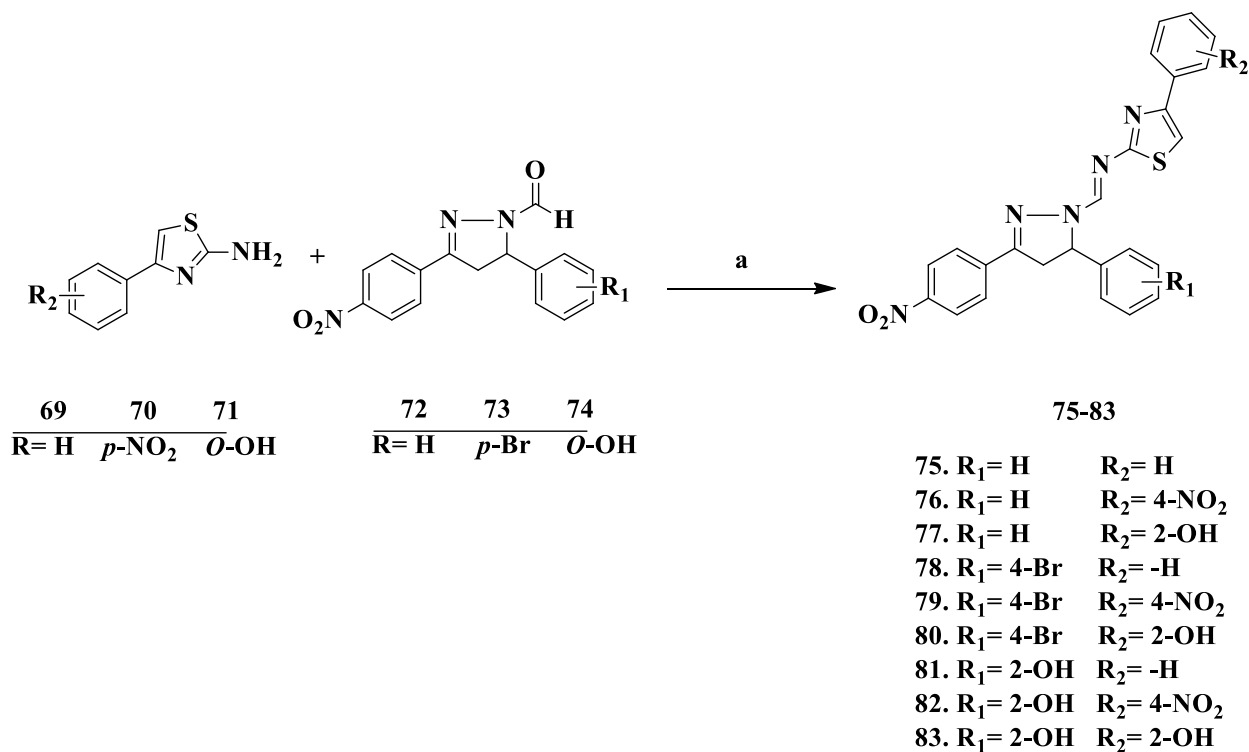
Scheme 3. Synthesis of *N*-formylpyrazoline derivatives, **72-74**. *Reagent and conditions:* (a) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ (80%), HCOOH , reflux at 101 °C, 2–4 h.

5.2.4. Synthesis of thiazolyl-pyrazoline Schiff base hybrids, **75-83**

A series of novel thiazolyl-pyrazoline Schiff base hybrids were synthesized *via* a Schiff base reaction between 2-amino-4-(substituted-phenyl)thiazole (**69-71**) and *N*-formyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives (**72-74**) in the presence of ZnO nanoparticles as a heterogeneous catalyst in ethanol reflux. The scope of the reaction was checked with electron-donating and withdrawing substituents at phenyl rings. ^1H -NMR spectra of target products (**75-83**) revealed three doublets of doublets as an AMX spin pattern with varying coupling constant (J) values, two diastereotopic methylene protons of H_A and H_M , and sp^3 methine proton H_X of pyrazoline moiety. Protons H_A and H_B appeared as two doublets of a doublet in the range of δ 3.09–3.35 (dd, $J_\text{AM} = 17.2$ Hz, $J_\text{AX} = 4.78$ Hz, C-4- H_A) and δ 3.42–3.90 (dd, $J_\text{MA} = 17.60$ Hz, $J_\text{MX} = 11.20$ Hz, C-4'- H_M) due to vicinal and geminal coupling, respectively. Proton H_X exhibited a signal having doublet of doublet in the region δ 4.60–5.64 with $J_\text{XA} = 11.38$ Hz, $J_\text{MX} = 4.92$ Hz, C5- H_X due to vicinal coupling with two nonequivalent methylene protons of *N*-formylpyrazoline nucleus. A signal having a singlet observed at δ 7.00–8.68 attributed to sp^2 methine proton of thiazole ring. The azomethine sp^2 methine proton ($\text{CH}=\text{N}$) was also detected as a singlet at δ 9.19. In addition, all the other aromatic protons were found in the expected regions.

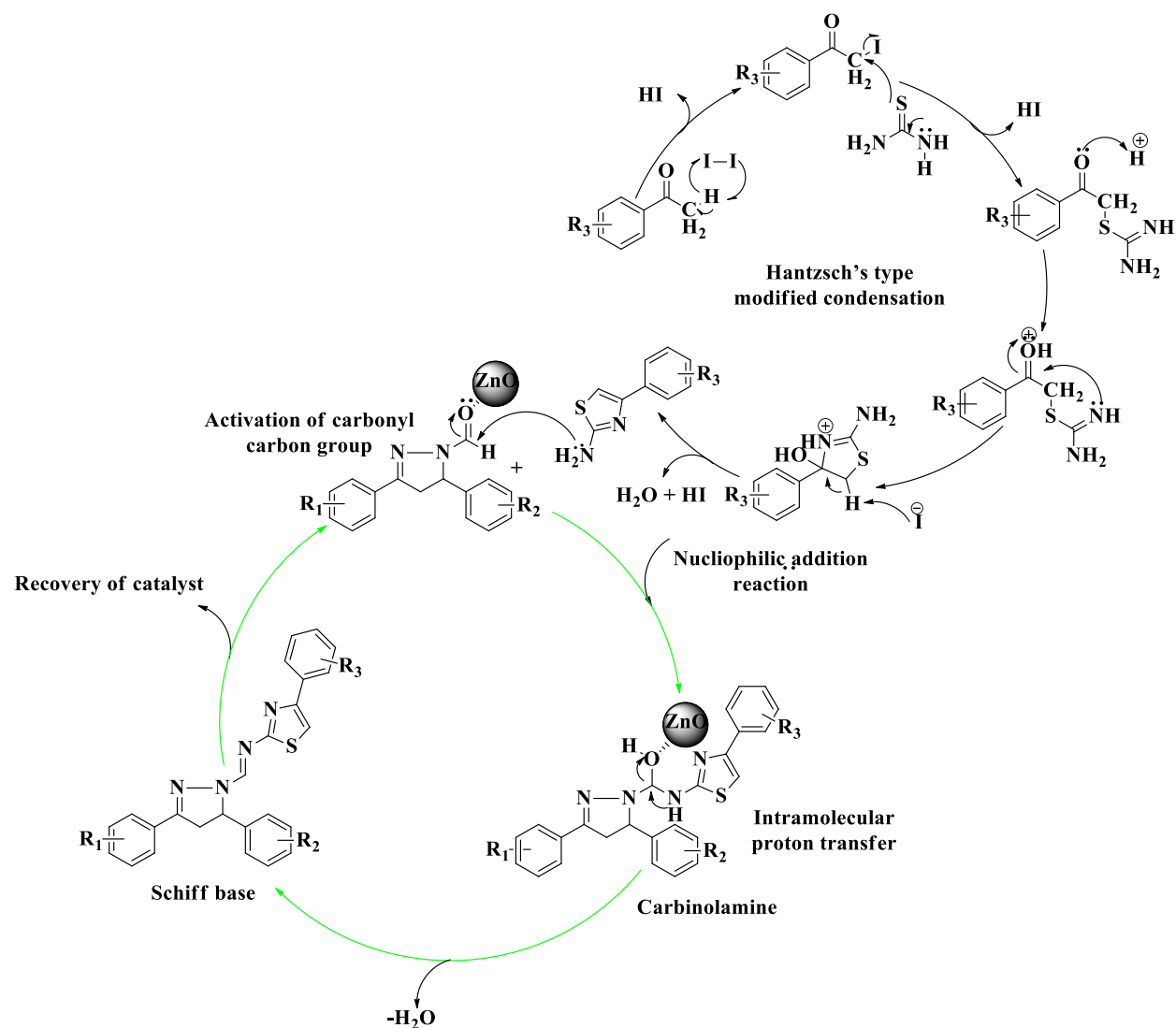
The ^{13}C -NMR spectrum of the compounds (**75-83**) showed peaks at δ 151.1 attributed to sp^2 methine of azomethine group ($-\text{CH}=\text{N}$) ring. The signals at δ 164.8–165.1, 148.6–152.3, and 102.8–109.5 attributed to carbons of thiazole rings. Moreover, sp^2 quaternary carbon, sp^3

methine, and methylene carbons of the pyrazoline moiety were observed around δ 152.4–153.8, 63.2–64.7, and 43.4–43.9, respectively. Furthermore, aromatic ring sp^2 methines and sp^2 quaternary carbons appeared within the expected ranges of δ 117.4–164.1 (Scheme 4). A plausible mechanism for the synthesis of thiazolyl-pyrazoline Schiff base hybrids *via* ZnO-nanoparticles as a catalyst was investigated as shown in Scheme 5.



Scheme 4. Synthesis of thiazolyl-pyrazoline Schiff base hybrids derived from chalcone, 75-83.

Reagent and conditions: (a) ZnO NPs (20 mol %) in methanol reflux, 3h.



Scheme 5. A plausible mechanism for the synthesis of thiazolyl-pyrazoline Schiff base hybrids via ZnO-nanoparticle as a catalyst.

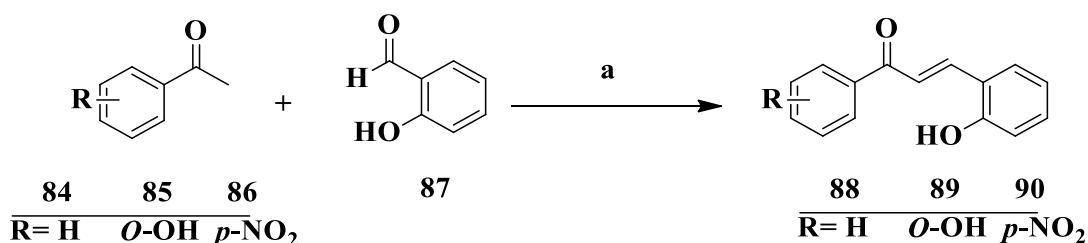
5.3. Chemistry of thiazolo[3,2-*a*] pyrimidine analogs

Thiazolo[3,2-*a*] pyrimidine belongs to the bridgehead nitrogen derivatives, and a number of methods were reported for the synthesis of its derivatives (Wu et al., 2018). However, most of the reported strategies have drawbacks, such as extended reaction times, the use of flammable organic solvents, hazardous catalysts, low yields, and harsh reaction conditions (Wu et al., 2018). In this study, an alkene of α , β -unsaturated ketones (chalcone) with the 2-amino thiazole

undergoes nucleophilic addition, forming a pyrimidine ring, as the amino group of the thiazole, as a Michael donor, attacks the alkene of the, α , β - unsaturated intermediate, making a nucleophilic attack on the β -carbon of the activated chalcone, which is electron deficient due to the electron-withdrawing influence of the carbonyl group, leading to the formation of Michael type addition product, which undergoes dehydration to give the title compounds. The plausible reaction mechanism for the synthesis of the title compounds, a synthetic approach utilizing ZnO nanoparticles could act as a Lewis acid to activate the carbonyl groups, resulting in the synthesis of thiazolo[3,2- α] pyrimidine analogs. Finally, ZnO nanoparticles were effectively recovered and reused for numerous runs without apparent loss of activity, making this approach cost-effective and hence environmentally benign.

5.3.1. General procedure for the synthesis of chalcone derivatives, **88-90**

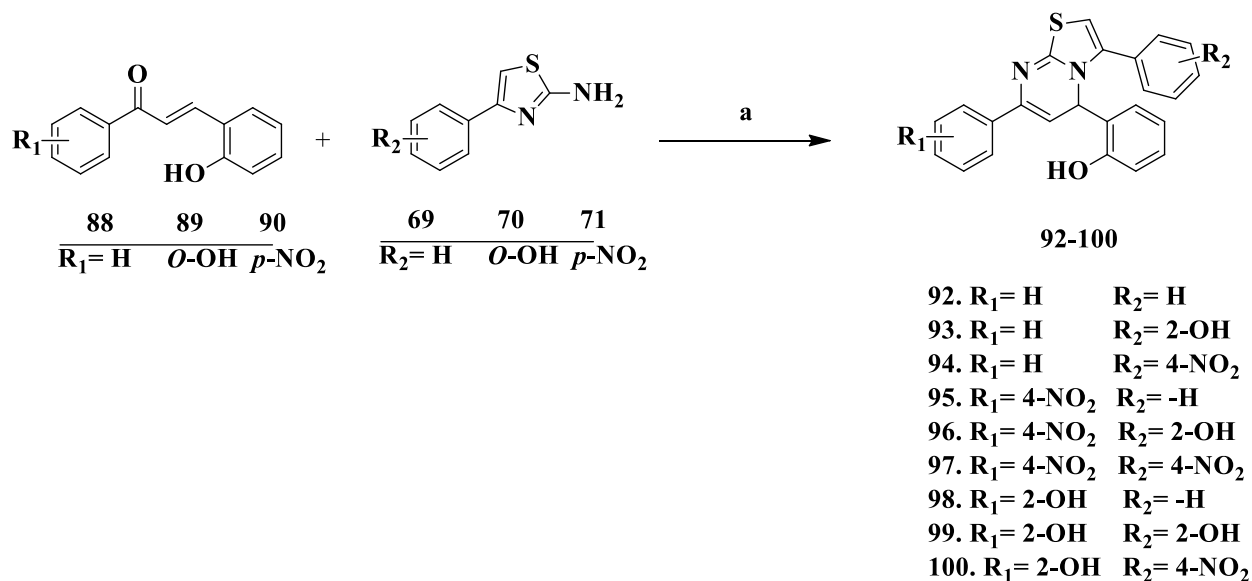
Initially, the synthesis of chalcone derivatives were done at room temperature using substituted acetophenone with 2-hydroxy benzaaldehydes using a basic catalyzed Claisen–Schmidt condensation reaction (Scheme 6) (Elkanzi et al., 2022). In the ^1H -NMR spectra of compounds, **88-90** the H- β and H- α protons appeared each as a doublet at δ 7.7-7.7 and δ 7.3-7.4 respectively, with a coupling constant between them of $^3J = 15.6$ Hz, which agrees with a *trans* configuration. The compounds displayed multiplet at δ 7.9-8.5, indicating the aromatic C-H linkage. In the ^{13}C -NMR spectrum for the compounds the C- β and C- α signals appeared at δ 138.4-148.2 and δ 120.1-123.6 ranges respectively, while the C=O appeared at δ 188.9-190.7.



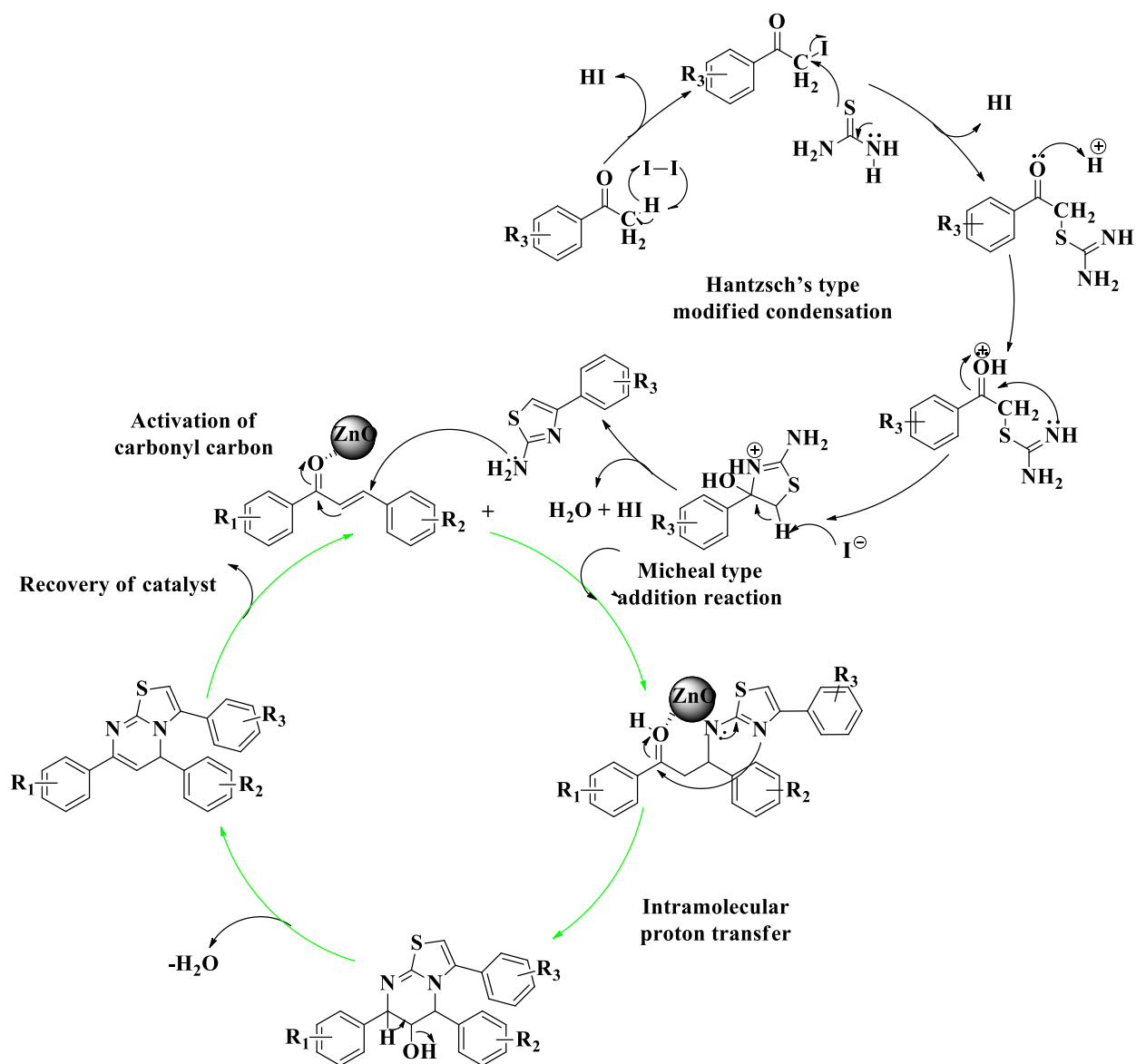
Scheme 6. Synthesis of chalcone derivatives, **88-90**. *Reagent and conditions:* (a) NaOH (60%), ethanol, rt, 3 h.

5.3.2. General procedure for the synthesis of thiazolo[3,2- α] pyrimidine analogs, **92-100**

The synthetic strategy for the preparation of thiazolo[3,2- α] pyrimidine analogs is illustrated in [Scheme 7](#). Substituted acetophenones were combined with different 2-hydroxy benzaldehyde derivatives in an ethanolic solution of sodium hydroxide to produce the reported chalcone derivatives (**88-90**). The title compounds were obtained by reacting either 2-amino-4-substituted phenylthiazole or its substituted derivatives, with chalcone derivatives in sodium ethoxide in the presence of ZnO nanoparticles as a heterogeneous catalyst. In this case, acetophenone was used as the ketone of choice and the reaction was also carried out with sodium ethoxide as solvent. The $^1\text{H-NMR}$ spectra of target compounds, **92-100** revealed the presence singlet peak (1H) at δ 12.0-10.0 and 7.8 of hydroxyl and thiazole protons, and the spectrum shows a singlet signal at δ 5.4-6.9 assigned for the C-H thiazolopyrimidine proton (pyrimidine H-4) and δ 7.4 for methine proton and the aromatic region shows multiplet at δ 6.9-7.5, indicating the aromatic C-H linkage.



Scheme 7. Synthesis of thiazolo[3,2- α] pyrimidine analogs, **92-100**. *Reagent and conditions:* (a) Na metal, ethanol, ZnO-NPs (20 mol %), 3 h.



Scheme 8. Plausible reaction mechanism for the synthesis of thiazolo[3,2-*a*] pyrimidine analogs via ZnO-nanoparticle as a catalyst.

The ¹³C-NMR shows a characteristic peak at δ 167.0-168.0 corresponding to C-2 of the thiazole ring. The peak appeared around δ 168.0-195.7 corresponding to (-S-C(N)=N- of thiazolo-pyrimidine, the appearance of downfield shift around δ 43.9 for the pyrimidine C-4 indicates the effect of the environment around carbon-4 (aromatic environment) and consequently, has assigned target structures for the reaction products. As a result, distinct chemical shift positions

resonated with these protons. This change in the structure of the scaffold is caused by a change in the substituent position of thiazole and chalcone phenyl groups, which causes different chemical shift values. This procedure is efficient for most of the substances used and furnishes the desired product in very good to excellent yields. A 1,4-nucleophilic addition of 2-aminothiazole on, a β -unsaturated intermediate resulting from the reaction between the aromatic aldehyde and the molecule bearing the activated methylene group is one proposed reaction mechanism (Scheme 8). The respective thiazolo[3,2- α] pyrimidine analogs were obtained in a synthetically useful manner, then all the obtained products were purified and characterized by physicochemical and spectroscopy techniques.

5.4. Chemistry of heterocyclic curcumin analogs

During the last decade, synthetic modifications of the curcumin molecular framework, mainly aimed at improving its bioactivities, have been studied intensively (Borik, Fawzy, Abu-Bakr, & Aly, 2018; Rodrigues et al., 2021). Due to an unstable β -diketone moiety in the curcumin structure, its poor stability, low bioavailability, and pharmacokinetic limitations have hindered the therapeutic efficacy and largely contributed to curcumin instability under physiological conditions (Azzi et al., 2019; da Silva et al., 2019). To overcome such limitations, several approaches have been explored and the replacement of central β -diketo functionality with mono carbonyl has resulted in many compounds with improving pharmacokinetic properties and bioavailability of curcumin (Singh et al., 2019). According to the literature, monocarbonyl curcuminoids' bioactive representations exhibit fascinating biological activities that are superior to curcumin itself and are chemically more stable than curcumin *in vitro* (Sharma, Gupta, Saxena, & Bedi, 2015). Studies have identified the diketo moiety as the cause of curcumins' instability and it has been hypothesized that adding a heterocyclic ring structure to the heart of the curcumin scaffold would increase the molecule's stability as well as its activity by a wide margin. Curcuminoids incorporated with a heterocyclic structure at the diketo site tend to have reduced rotational freedom, absence of tautomerism, and minimized metal chelation properties (Rodrigues et al., 2021).

This dissertation focused on the preparations of nitrogen-containing compounds of *N*-pyrazoline, pyrimidine-2-ol, thiazolo[3,2- α] pyrimidine, and β -substituted imidazole analogs through heterocyclization of suitably functionalized curcuminoid derivatives. Thus, nine heterocyclic analogs derived from C₅-curcumin derivatives were synthesized under the ultrasonic-assisted method, and their anti-diabetic, anti-bacterial, and antioxidant activities were evaluated. The synthesis of title compounds of heterocyclic C₅-curcumin analogs achieved through the reaction of monocarbonyl curcumin derivatives, **105-107** (1 mmol), in the presence of different nucleophilic reagents, namely; hydrazine hydrate (10 mmol), urea (1 mmol), 2-amino-4-substituted phenylthiazole (1.5 mmol), and imidazole (1 mmol) were dissolved in ethanolic sodium hydroxide resulted in the formation of the corresponding products of pyrazolines (**108-109**), 4-phenyl-6-styryl pyrimidine-2-ol (**110-111**), thiazolo[3,2- α] pyrimidines (**112-114**) and β -substituted imidazole compounds (**115-116**). The respective heterocyclic C₅-curcumin analogs (**108-116**) were obtained in a synthetically useful manner, then all the obtained products were purified and characterized by physicochemical properties, and spectroscopy techniques.

Table 2. Time and yield data of synthesized compounds (**108-116**) using ultrasonic irradiation techniques.

Synthesized compound ^a	Ultrasonic irradiation method			
	Time (min)	Power (W)	Temperature (°C)	Percentage (%) yield ^b
108	30	250	45	87.8
109	30	250	45	91.2
110	30	250	45	90.3
111	30	250	45	86.1
112	60	250	60	93.7
113	60	250	60	91.0
114	60	250	60	89.6
115	30	250	45	81.5
116	30	250	45	89.7

^a Reactions were conducted in an ultrasonic cleaner bath in a sealed tube (Anton 300™)

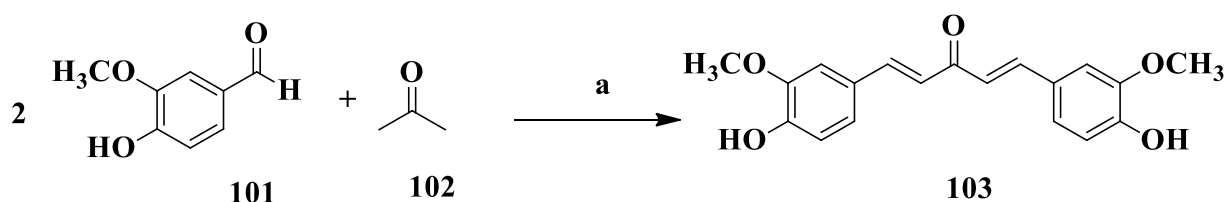
^b Isolated yields.

In terms of the environment, reaction time, high yield, simplicity of workup, and isolation of products, ultrasound-promoted technology is more effective (Nagasundaram et al., 2022). Additionally, ultrasonic irradiation has a number of benefits due to solvents being costly, hazardous, and challenging to remove especially when they are aprotic dipolar solvents with a high boiling point and harmful to the environment (Lahyani & Trabelsi, 2016). The reaction time and yield data of newly synthesized compounds by ultrasonic methods were presented in [Table 2](#). According to these results, the reaction of the ultrasonically-assisted synthesis was completed in a short time with a relatively high yield compared with the conventional method. It was observed that the reactions which required 6-8 h by a conventional method were completed within 30-60 min by the ultrasonic irradiation method at 45-60 °C, and the yields of corresponding products have been improved in excellent yields (81.4 to 93.7%). It has also been observed that reactions under ultrasonic irradiation are generally easier to work up and have less energy consumption than conventional heating method. This finding is due to the cavitation phenomenon, which induces very high local temperature and pressure within the bubble, resulting in turbulence in the liquid and enhanced mass transfer in that region, which typically require long reaction times, high temperatures, and expensive reagents.. This method has riddled the limitations such as high temperature, extended reaction time, expensive reagents, and hazardous reaction conditions. Additionally, reactions under ultrasound irradiation represent environmentally friendly processes, using small amounts of solvents and consuming less energy (Zhang et al., 2017).

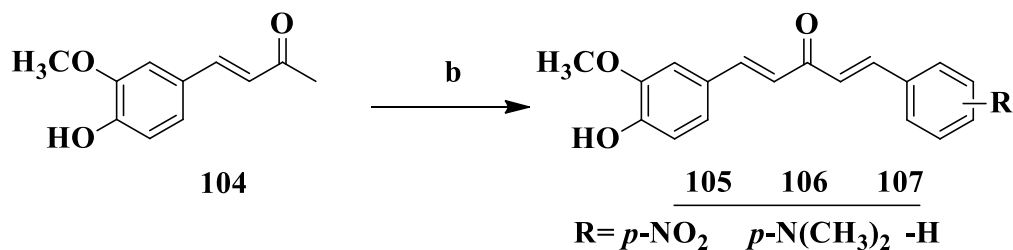
5.4.1. Synthesis of curcumin (1*E*,4*E*)-1,5-bis(4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one, and its curcuminoid derivatives, **103** and **105-107**

Synthesis of C₅-curcumin and its derivatives was done at room temperature using vanillin (**101**) with acetone (**102**) or dehydrozingerone (**104**) with various substituted aryl aldehydes using a basic catalyzed Claisen–Schmidt condensation reaction to form symmetrical and asymmetrical C₅-curcumin derivatives, respectively ([Schemes 9](#) and [10](#)). In the ¹H-NMR spectra of compounds, **103** and **105-107** the H-β and H-α protons appeared each as a doublet at δ 7.7-7.9 and δ 7.3-7.4 respectively, with a coupling constant between them of ²J = 15.6 Hz, which agrees with a *trans* configuration. The synthesized compounds displayed multiplet at δ 7.9-8.5,

indicating the aromatic C-H linkage, and the methoxy and hydroxyl groups appeared at δ 3.97 and 8.28 ranges, respectively. As a result, distinct chemical shift positions resonated with these protons. This change in the structure of the scaffold is caused by a change in the substituent position of C₅-curcumin phenyl groups, which causes different chemical shift values. In the ¹³C-NMR spectrum for the compounds the C- β and C- α signals appeared at δ 148.2 and δ 146.6 ranges respectively, while the C=O appeared at δ 188.8-190.7. However, all the aromatic carbons peaks appeared in the expected regions.



Scheme 9. Synthesis monocarbonyl curcumin compound, **103**. *Reagent and conditions:* (a) NaOH (20%), ethanol, rt, stir for 1 h.



Scheme 10. Synthesis of dehydrozingerone-based monocarbonyl curcumin derivatives, **105-107**. *Reagent and conditions:* (b) substituted benzaldehyde, KOH (30%), ethanol, rt, stir for 2-3 h, left overnight

5.4.2. Synthesis of *N*-pyrazoline analogs derived from curcumin derivatives, **108-109**

Cyclization of C₅-curcumin precursors (**105-107**) using a 1,4-Michael addition of hydrazine derivatives in 10.0 mL of ethanol gives pyrazoline derivatives. The reaction progress was monitored by TLC. The precipitate formed was filtered off, washed with cold water, and dried.

The solid product was recrystallized from ethanol. The synthesis of new monocarbonyl curcumin-based pyrazoline derivatives, **108-109** followed the general pathway outlined in [Scheme 11](#). The ^1H -NMR spectrum of the pyrazoline ring showed three different signals as doublet of doublet attributed to diastereotopic protons (H_A , H_M and H_X) protons due to the AMX spin system in the structures. The H_A , H_M , and H_X protons appeared at 3.07-3.38 ppm (J_AM =17.24 Hz), 3.77-3.98 ppm (J_AX = 5.98 Hz), and 5.41-5.68 ppm (J_MX = 11.90 Hz), suggesting vicinal coupling of methine with magnetically non-equivalents methylene protons, respectively. NH- protons of pyrazoline groups appeared as a singlet peak at 6.09-7.22 (s, NH, 1H). The α , β -unsaturated double bond appear at 6.00-6.89, with a coupling constant between them of J =15.6 Hz, which agrees with a *trans* configuration. The ^{13}C -NMR spectrum shows that the methylene carbon, sp^3 methine, and sp^2 quaternary carbons of the pyrazoline ring appeared at C3 (147.5–148.7), C5 (57.1–63.5) and C4 (41.5–43.5) ppm carbons of the pyrazoline ring, respectively. The C- α and C- β signals appeared around δ 146.2-146.6 and δ 148.6-148.8 ranges, respectively. All the other aromatic protons and peaks were observed in the expected regions.

5.4.3. Synthesis of 4-phenyl-6-styrylpyrimidine-2-ol analogs derived from curcumin derivatives, **110-111**

The preparation of 4-phenyl-6-styrylpyrimidine-2-ol derivatives involves starting from equimolar quantities of C₅-curcumin derivatives (**105-107**) by cyclization with the nucleophilic reagent of urea separately, in NaOH, and 10.0 mL ethanol. The reaction progress was monitored using TLC. The precipitate formed was filtered off, washed with cold water, and dried. The solid product of 4-phenyl-6-styrylpyrimidine-2-ol (**110-111**) were recrystallized from ethanol ([Scheme 11](#)). The ^1H -NMR spectrum displayed singlet at δ 3.79, and 5.04, revealed the presence of methoxy, and hydroxy functionality of pyrimidin-2-ol, respectively. The methine proton of pyrimidine ring appears singlet at δ 5.35. The H- β and H- α protons appeared each as a doublet at δ 7.7-7.7 and δ 7.3-7.4 ranges, respectively, with a coupling constant between them of J =15.6 Hz, which agrees with a *trans* configuration. The ^{13}C -NMR spectrum revealed that δ 88.1 (pyrimidine-CH), 165.2, 157.52, 150.2 (Pyrimidine-C 2/4/6), respectively. The peaks of C- β and C- α signals appeared at δ 148.2 and δ 146.6 ranges, respectively. All the other aromatic protons and peaks were observed in the expected regions.

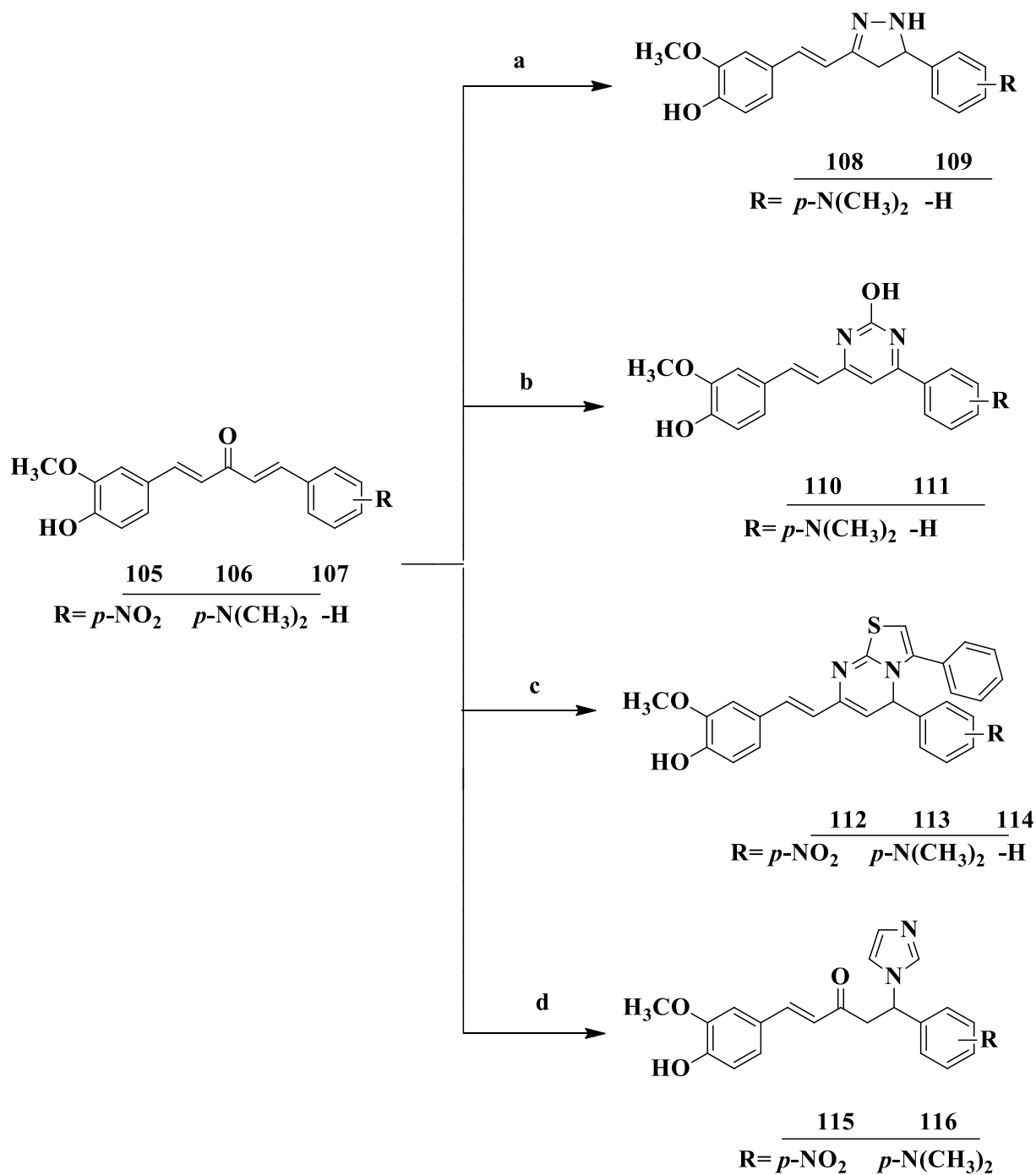
5.4.4. Synthesis of thiazolo [3,2- α] pyrimidine analogs derived from curcumin derivatives, **112-114**

The synthetic strategy for the preparation of C₅-curcumin-based thiazolo[3,2- α] pyrimidine analogs is illustrated in [Scheme 11](#). The target compounds were obtained by a 1,4-nucleophilic addition of 2-amino-4-substituted phenylthiazole (4-(2-aminothiazol-4-yl)phenol) with C₅-curcumin derivatives with sodium ethoxide as solvent. The ¹H-NMR spectra of target compounds, **112-114** revealed the presence of singlet peak (1H, br) at δ 6.05 and at δ 3.90 of hydroxyl and methoxy protons, respectively. The spectrum shows a singlet signal at δ 5.4-6.9 assigned for the C-H thiazolopyrimidine proton (pyrimidine H-4), δ 7.4 for methine proton and the thiazole ring proton shows singlet around δ 6.9-8.5 ranges. The H- β and H- α protons appeared each one as a doublet at δ = 7.75–7.73 (1H, d, J_{ortho} = 8.16 Hz), and δ = 7.32–7.39 (1H, d, J_{trans} = 16.20 Hz) ppm respectively. The ¹³C-NMR shows a characteristic peak at δ 167.0-168.0 corresponding to C-2 of the thiazole ring. The peak appeared around δ 195.7-168.0 corresponding to (-S-C(N)=N- of thiazolo-pyrimidine, the appearance of this downfield shift around δ 43.9 for the pyrimidine CH-4 indicates the effect of the environment around carbon-4 (aromatic environment). The peaks of the C- β and C- α signals appeared at δ 148.2 and δ 146.6 ranges respectively. All the other aromatic protons and peaks were observed in the expected regions, depending on the substitution patterns.

5.4.5. Synthesis of the aza-Michael addition product of β -substituted imidazole analogs derived from curcumin derivatives, **115-116**

The C₅-curcumin derivatives and imidazole were reacted via an aza-Michael addition type reaction in the presence of anhydrous K₃PO₄ in CH₃CN to produce β -substituted imidazole analogs ([Scheme 11](#)). ¹H-NMR spectra of target compounds, (**115-116**) revealed the presence of singlet peak (1H, br) at δ 6.05 and (3H, s) at δ 3.90 of hydroxyl and methoxy protons. The spectrum shows δ 3.2 (dd, J_{AM} = 4.6 Hz, J_{AX} = 16.9 Hz; 1H), 3.6 (dd, J_{AM} = 8.8 Hz, J_{AX} = 16.9 Hz, 1H), 4.98–5.03 (m, 1H), due to the vicinal coupling with the non-equivalent protons of the methylene and methine group. The spectrum H- β and H- α protons appeared each as a doublet at δ = 7.75–7.73 (1H, d, J_{ortho} = 2.16 Hz), and δ = 7.32–7.39 (1H, d, J_{trans} = 16.20 Hz),

respectively, with a coupling constant between them of $J = 15.6$ Hz, which agrees with a trans-configuration. The imidazole C₂ and vinylic protons appear singlet at 7.96 (s, 1H, -CH), and doublet at 7.76-8.50 (1H, d, imidazole CH, $J = 15.6$ Hz, -HC=CH-), respectively. Besides, the ¹³C NMR chemical shift values of carbonyl carbon peak appear at δ 200.4, the methine carbon around δ 69.9, and methylene carbon at δ 40.3-47.3, while C- β and C- α signals appeared around δ 148.2 and δ 146.6 ranges respectively. The peaks of imidazole C₂ signals appeared at δ 138.2-140.6 ranges. However, other chemical shift values of all the other aromatic protons were found in the expected regions.



Scheme 11. Ultrasonic irradiation assisted synthesis of heterocyclic C₅-curcuminoid analogs. **108-116 Reagents and conditions:** (a) hydrazine hydrate, EtOH; (b) NH₂CONH₂, 40% NaOH, EtOH; (c) Na metal, ethanol; (d) Imidazole, 25 mol% K₃PO₄, CH₃CN,), 45-60 °C, 250 W for 30-60 min.

5.5. *In vitro* antibacterial activity

5.5.1. *In vitro* antibacterial activities of 75-83 compounds using agar disk diffusion assay

The antibacterial activity of the synthesized thiazolyl-pyrazoline Schiff base hybrids (75-83) was evaluated by *in vitro* disk diffusion bioassay and measuring inhibition zone diameters (IZDs, expressed as mean $\pm$ SD mm) at 500 and 250 $\mu\text{g/mL}$ against the following strains of bacteria: *Staphylococcus aureus* (ATCC 29213), *Streptococcus pyogenes* (ATCC 27853), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) (Rani & Mohamad, 2014; Shakhathreh et al., 2016). Compounds **81** and **83** displayed potent inhibitory activity against *P. aeruginosa* (13.33 ± 0.58 mm and 17.33 ± 0.58 mm, respectively) at 500 $\mu\text{g/mL}$ (Table 3 and Figure 15) compared to amoxicillin (15.00 ± 1.00 mm). Compounds **78** and **83** displayed promising activity against *E. coli* (12.00 ± 1.00 mm and 17.00 ± 1.00 mm, respectively) at 500 $\mu\text{g/mL}$ compared to amoxicillin (14.67 ± 0.58 mm). Compounds **77**, **78**, **80**, and **83** displayed promising activity against *S. pyogenes* (19.33 ± 0.58 mm, 19.33 ± 0.58 mm, 18.67 ± 2.52 mm, and 15.67 ± 0.58 mm, respectively) at 500 $\mu\text{g/mL}$ whereas compounds **77** and **80** displayed promising activity against *S. aureus* (10.00 ± 1.00 mm, and 11.00 ± 1.00 mm, respectively) at 500 $\mu\text{g/mL}$ compared to amoxicillin (33.33 ± 1.53 mm, and 15.00 ± 1.00 mm, respectively). The rest of the compounds showed moderate to weak activity against the tested bacterial strains. Thus, these potent antibacterial compounds could be considered promising lead compounds to develop new antibacterial drugs (Appendix 107) (U. Bhadraiah et al., 2021).

Design and development of new antibacterial drugs depend heavily on structure-activity-based mechanisms of antibacterial action. Preliminary structure-activity relationship studies (SARs) provide those biological activities can be modulated by the presence of unsubstituted aromatic ring as well as the position of substituents on the phenyl moiety via electron-withdrawing groups (EWGs) or electron-donating groups (EDGs) effects (Hammouda et al., 2022). The results demonstrate that compound **77**, with a *p*-OH substituent on the aromatic ring of thiazole moiety, resulted in an increase of antibacterial activity towards *S. aureus* at 250 $\mu\text{g/mL}$ and *S. pyogenes* at 250 and 500 $\mu\text{g/mL}$ compared to compound **75**.

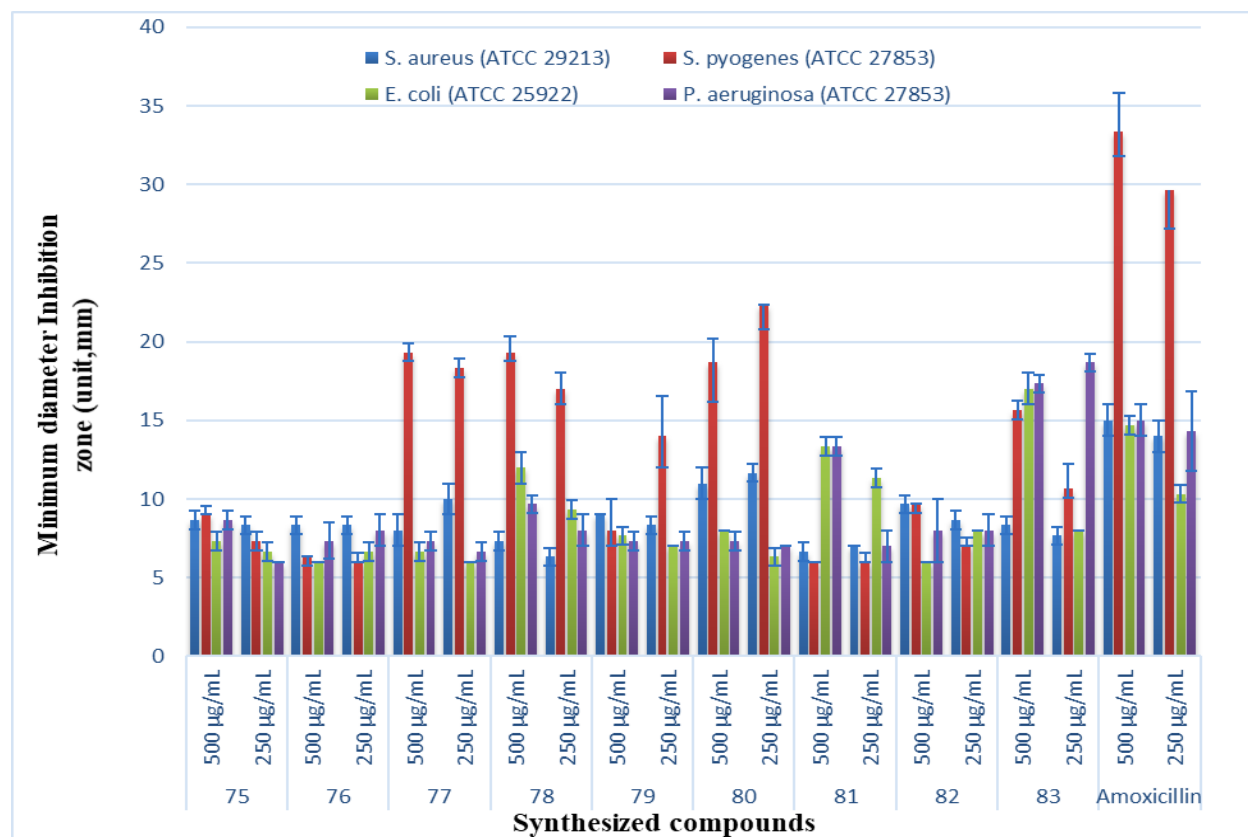


Figure 15. Diameter inhibition zone of the synthetic compounds, **75-83** in mm (mean $\pm$ SD) at 250 and 500 $\mu\text{g/mL}$ concentrations.

Compound **80**, with a *p*-OH substituent on the aromatic ring of thiazole moiety, resulted in an increase of antibacterial activity towards *S. aureus* and *S. pyogenes* at 500 $\mu\text{g/mL}$ compared to compound **78**, which has hydrogen at the *para* position of the aromatic ring of thiazole moiety, whereas compound **82**, with a *p*-NO₂ substituent on the aromatic ring of thiazole moiety, resulted in an increasing antibacterial activity trend towards *S. aureus* and *S. pyogenes* at 500 $\mu\text{g/mL}$ compared to compound **81**. On the other hand, compound **80**, with a *p*-OH substituent on the aromatic ring of thiazole moiety, resulted in an increase of antibacterial activity towards *S. pyogenes*, *E. coli*, and *P. aeruginosa* at 500 $\mu\text{g/mL}$ compared to compound **78**, which has hydrogen at the *para* position of the aromatic ring of thiazole moiety. In addition, the presence of *p*-Br, and *p*-OH groups' pyrazoline moiety bearing with substituted phenyl rings might be the most relevant cause for enhancing the antibacterial activities (Login et al., 2019; Mohanty et al., 2021).

Table 3. Antibacterial activity of thiazolyl-pyrazoline Schiff base hybrids, **75-83** and amoxicillin against selected bacterial strains

Synthesized compounds		Mean Inhibition zone diameter (IZD) in mm			
		Gram-positive bacteria		Gram-negative bacteria	
		<i>Staphylococcus aureus</i> (ATCC 29213)	<i>Streptococcus pyogenes</i> (ATCC 27853)	<i>Escherichia coli</i> (ATCC 25922)	<i>Pseudomonas aeruginosa</i> (ATCC 27853)
75	500 µg/mL	8.67±0.58	9.00±0.00	7.33±0.58	8.67±0.58
	250 µg/mL	8.33±0.58	7.33±0.58	6.67±0.58	6.00±0.00
76	500 µg/mL	8.33±0.58	6.33±0.58	6.67±0.00	8.33±1.15
	250 µg/mL	8.33±0.58	6.00±0.00	6.00±0.58	7.00±1.00
77	500 µg/mL	8.00±1.00	19.33±0.58	6.67±0.58	7.33±0.58
	250 µg/mL	10.00±1.00	18.33±0.58	6.00±0.00	6.67±0.58
78	500 µg/mL	7.33±0.58	19.33±0.58	12.00±1.00	9.67±0.58
	250 µg/mL	6.33±0.58	17.00±1.00	9.33±0.58	8.00±1.00
79	500 µg/mL	9.00±0.00	8.00±1.00	7.67±0.58	7.33±0.58
	250 µg/mL	8.33±0.58	14.00±2.00	7.00±0.00	7.33±0.58
80	500 µg/mL	11.67±1.00	18.67±2.52	8.00±0.00	7.33±0.58
	250 µg/mL	11.00±0.58	22.33±1.53	6.33±0.58	7.00±0.00
81	500 µg/mL	7.00±0.58	6.00±0.00	13.33±0.58	13.33±0.58
	250 µg/mL	6.67±0.00	6.00±0.00	11.33±0.58	7.00±1.00
82	500 µg/mL	9.67±0.58	9.67±0.58	8.00±0.00	8.00±2.00
	250 µg/mL	8.67±0.58	7.00±0.00	6.00±0.00	8.00±1.00
83	500 µg/mL	8.33±0.58	15.67±0.58	17.00±1.00	18.67±0.58
	250 µg/mL	7.67±0.58	10.67±0.58	8.00±0.00	17.33±0.58
Amoxicillin	500 µg/mL	15.00±1.00	33.33±1.53	14.67±0.58	15.00±1.00
	250 µg/mL	14.00±1.00	29.67±2.52	10.33±0.58	14.33±2.52
DMSO		0±0.00	0±0.00	0±0.00	0±0.00

^a The experiment was carried out in triplicate and the average zone of inhibition was calculated. ^bATCC: American Tissue Culture Collection. ^cIZD, Inhibition zone diameter.

5.5.2. *In vitro* antibacterial activities of **92-100** compounds using agar disk diffusion assay

The antibacterial activity of the nine series of target compounds (**92-100**) was evaluated by measuring the size of the zone inhibition against the test organisms as depicted in [Table 4](#) and [Figure 16](#). Results showed that all synthetic compounds had better antibacterial activity *in vitro* with mean inhibition zones displayed in the ranges between 6.33-22.33 mm for tested bacterial strains. Among the synthesized analogs, the *in vitro* antibacterial activity displayed by compound **96** showed higher activity (14.67 ± 0.58 mm at 500 $\mu\text{g/mL}$) against *P. aeruginosa* compared to amoxicillin (12.33 ± 0.58 mm at 500 $\mu\text{g/mL}$) whereas compounds **94** and **98** showed comparable activity (12.00 ± 0.00 mm and 12.33 ± 0.58 mm at 500 $\mu\text{g/mL}$ and 250 $\mu\text{g/mL}$, respectively) against the same strain. Therefore, due to the presence of the electron-donating nature of the *p*-OH substituent on the aromatic ring of the thiazole moiety in compound **96**, it leads to increased antibacterial activity against *S. aureus*, *S. pyogenes*, *E. coli*, and *P. aeruginosa* at 500 $\mu\text{g/mL}$. Compounds **94**, **96**, **98**, and **100** displayed comparable activity (12.33 ± 1.15 mm, 12.65 ± 0.58 mm, 12.33 ± 0.58 mm, 12.00 ± 1.00 mm, respectively, at 500 $\mu\text{g/mL}$) against *E. coli* compared to amoxicillin (13.33 ± 1.15 mm at the same concentration). Compound **99** showed good activity (12.00 ± 1.72 mm at 500 $\mu\text{g/mL}$) against *S. aureus* compared to amoxicillin (16.33 ± 0.58 mm at the same concentration)(U. K. Bhadraiah et al., 2021). However, the rest of the compounds show low to moderate antibacterial activity against all tested microorganisms at the respective doses as shown in [Appendix 108](#).

In terms of SAR, the results suggest that the antibacterial activity of the investigated compounds was influenced by the EDG and EWG properties of the type and position of substituent on the aromatic rings of thiazole and chalcone moieties. The results demonstrate that compound **94**, with a *p*-NO₂ substituent on the aromatic ring of thiazole moiety, resulted in an increase of antibacterial activity towards *S. aureus*, *E. coli*, and *P. aeruginosa* at 500 $\mu\text{g/mL}$ compared to compound **92**, which have hydrogen at the *para* position of the aromatic ring of thiazole moiety. On the other hand, compound **96**, with a *p*-OH substituent on the aromatic ring of thiazole moiety, resulted in an increase of antibacterial activity towards *S. aureus*, *S. pyogenes*, *E. coli*, and *P. aeruginosa* at 500 $\mu\text{g/mL}$ compared to compound **95**, which have hydrogen at the *para* position of the aromatic ring of thiazole moiety. Compound **99**, with a *p*-OH substituent on the

aromatic ring of thiazole moiety, resulted in an increase of antibacterial activity towards *S. aureus* and *S. pyogenes* at 500 µg/mL compared to compound **98**, which has hydrogen at the *para* position of the aromatic ring of thiazole moiety, whereas compound **100**, with a *p*-NO₂ substituent on the aromatic ring of thiazole moiety, resulted in an increasing antibacterial activity trend towards *E. coli* and *P. aeruginosa* at 500 µg/mL compared to compound **98**. The presence of both electron-withdrawing and donating groups in the most active compounds of thiazole moiety have broad-spectrum activity against the tested Gram-positive and Gram-negative bacterial strains (Moty, Hussein, Aziz, & Abou-Salim, 2016). The results of the antibacterial activities of all the synthesized compounds were further presented in Figure 16.

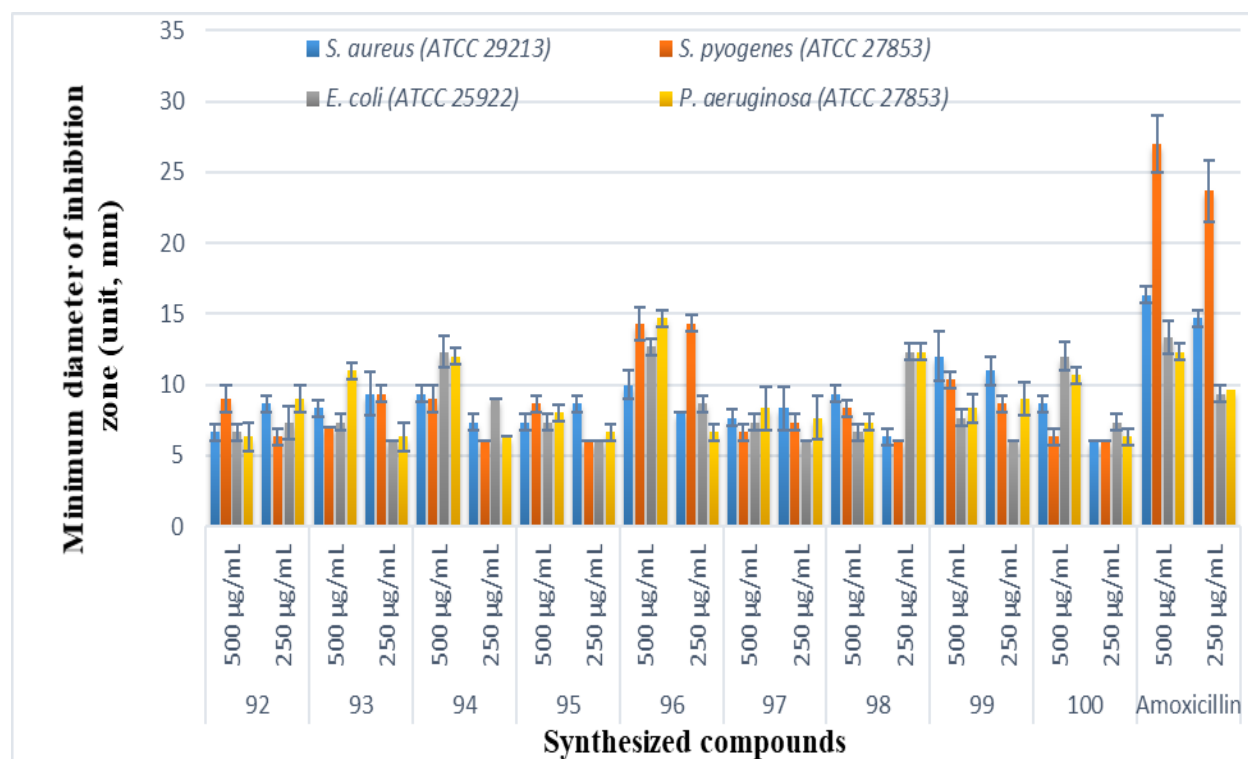


Figure 16. Diameter of inhibition zone of the compounds, **92-100** in mm (mean ± SD) at 250 and 500 µg/mL concentrations.

Table 4. Antibacterial activity of thiazolo[3,2- α] pyrimidine analogs, **92-100** against selected bacterial strains.

Synthesized compounds		Mean Diameter of inhibition zones (DIZ) in mm			
		Gram-positive bacteria		Gram-negative bacteria	
		<i>Staphylococcus aureus</i> (ATCC 29213)	<i>Streptococcus pyogenes</i> (ATCC 27853)	<i>Escherichia coli</i> (ATCC 25922)	<i>Pseudomonas aeruginosa</i> (ATCC 27853)
92	500 μ g/mL	8.67 $\pm$ 0.58	9.00 $\pm$ 1.00	7.67 $\pm$ 0.58	9.33 $\pm$ 0.58
	250 μ g/mL	6.67 $\pm$ 0.58	6.33 $\pm$ 0.58	6.33 $\pm$ 1.15	6.00 $\pm$ 1.00
93	500 μ g/mL	9.33 $\pm$ 0.58	9.00 $\pm$ 0.00	7.33 $\pm$ 0.58	11.00 $\pm$ 1.00
	250 μ g/mL	8.33 $\pm$ 1.53	7.33 $\pm$ 0.58	6.00 $\pm$ 0.00	6.33 $\pm$ 0.58
94	500 μ g/mL	9.33 $\pm$ 0.58	9.00 $\pm$ 1.00	12.33 $\pm$ 1.15	12.00 $\pm$ 1.00
	250 μ g/mL	7.33 $\pm$ 0.58	6.00 $\pm$ 0.00	9.00 $\pm$ 0.00	6.33 $\pm$ 0.58
95	500 μ g/mL	8.33 $\pm$ 0.58	8.67 $\pm$ 0.58	7.33 $\pm$ 0.58	8.00 $\pm$ 0.00
	250 μ g/mL	7.67 $\pm$ 0.58	6.00 $\pm$ 0.00	6.00 $\pm$ 0.00	6.67 $\pm$ 0.58
96	500 μ g/mL	10.00 $\pm$ 1.00	14.33 $\pm$ 1.15	12.67 $\pm$ 0.58	14.67 $\pm$ 0.58
	250 μ g/mL	8.00 $\pm$ 0.00	14.33 $\pm$ 0.58	8.67 $\pm$ 0.58	6.67 $\pm$ 0.58
97	500 μ g/mL	8.67 $\pm$ 0.58	7.67 $\pm$ 0.58	7.33 $\pm$ 0.58	8.33 $\pm$ 0.58
	250 μ g/mL	7.33 $\pm$ 1.53	6.33 $\pm$ 0.58	6.00 $\pm$ 0.00	7.67 $\pm$ 1.53
98	500 μ g/mL	9.33 $\pm$ 0.58	8.33 $\pm$ 0.58	6.67 $\pm$ 0.58	7.33 $\pm$ 1.53
	250 μ g/mL	6.33 $\pm$ 0.58	6.00 $\pm$ 0.00	12.33 $\pm$ 0.58	12.33 $\pm$ 0.58
99	500 μ g/mL	12.00 $\pm$ 1.73	10.33 $\pm$ 0.58	7.67 $\pm$ 0.58	9.33 $\pm$ 0.58
	250 μ g/mL	11.00 $\pm$ 1.00	8.67 $\pm$ 0.58	6.00 $\pm$ 0.00	8.00 $\pm$ 1.00
100	500 μ g/mL	8.67 $\pm$ 0.58	6.33 $\pm$ 0.58	12.00 $\pm$ 1.00	10.67 $\pm$ 1.15
	250 μ g/mL	6.00 $\pm$ 0.00	6.00 $\pm$ 0.00	7.33 $\pm$ 0.58	6.33 $\pm$ 0.58
Amoxicillin	500 μ g/mL	16.33 $\pm$ 0.58	27.00 $\pm$ 2.00	13.33 $\pm$ 1.15	12.33 $\pm$ 0.58
	250 μ g/mL	14.67 $\pm$ 0.58	23.67 $\pm$ 2.21	9.33 $\pm$ 0.58	9.67 $\pm$ 0.58
DMSO		0 $\pm$ 0.00	0 $\pm$ 0.00	0 $\pm$ 0.00	0 $\pm$ 0.00

^a The experiment was carried out in triplicate and the average zone of inhibition was expressed in mean $\pm$ standard deviation (mm). ^b

N.A. (no activity). ^c ATCC: American Tissue Culture Collection. ^d DIZ: Diameter of Inhibition Zone.

5.5.3. *In vitro* antibacterial activities of **108-116** compounds using agar disk diffusion assay

The results of the antibacterial activity of all the synthesized compounds were further presented in [Figure 17](#) and [Table 5](#). The antibacterial activity of the nine series of compounds (**108-116**) was evaluated by measuring the zone of inhibition against the test organisms. Among these, compounds **108**, and **111** exhibited the highest antibacterial activities against *E. coli* with mean inhibition zone of 13.00 ± 0.57 , and 19.66 ± 0.00 mm diameter, respectively, at 500 $\mu\text{g/mL}$ compared to the results of standard drug amoxicillin (12.87 ± 1.41 mm) at the same concentration, while compound **110** (11.00 ± 0.57 mm) demonstrated comparable activities at the same concentration. In addition, compounds **108**, **111**, and **116** showed excellent activities against *P. aeruginosa* with mean inhibition zone of 17.67 ± 0.57 , 14.33 ± 0.57 , and 23.33 ± 0.00 mm diameter, respectively, at 500 $\mu\text{g/mL}$ compared to amoxicillin (13.75 ± 1.83 mm) at the same concentration. Moreover, compound **111** showed comparable activities with mean inhibition zone of 17.00 ± 0.57 at 250 $\mu\text{g/mL}$ compared to amoxicillin (9.37 ± 1.50 mm) at the same concentration. On the other hand, compounds **109** and **111** displayed comparable activities against *S. pyogenes* with mean inhibition zone of 11.33 ± 0.57 mm and 15.67 ± 0.57 mm at 500 $\mu\text{g/mL}$, respectively, compared to reference drug amoxicillin (14.75 ± 2.00 mm) at the same dose ([Appendix 109](#)). The remaining synthesized compounds exhibited moderate to weak antibacterial activities in the tested bacterial strains (Hamed et al., 2013). Thus, these powerful antibacterial compounds might be thought of as viable lead compounds for the development of new antibacterial drugs.

Compounds that contain electron-donating and electron-withdrawing substituents on phenyl rings are more potent than those with none substituted systems. The presence of electron-withdrawing groups ($p\text{-NO}_2$) at the para position of compounds **108**, **111**, and **116** on pyrazoline, 4-phenyl-6-styrylpyrimidine-2-ol and β -substituted imidazoles analogs derived from C_5 -curcuminoid moieties may enhance (increase) the antibacterial activity against *E. coli* and *P. aeruginosa*. Moreover, the presence of electron donating groups ($-\text{N}(\text{CH}_3)_2$) at the para position of compound **111** on 4-phenyl-6-styrylpyrimidine-2-ol analogs derived from C_5 -curcuminoid moieties could increase the antibacterial activity against *S. aureus* (Liang et al., 2008).

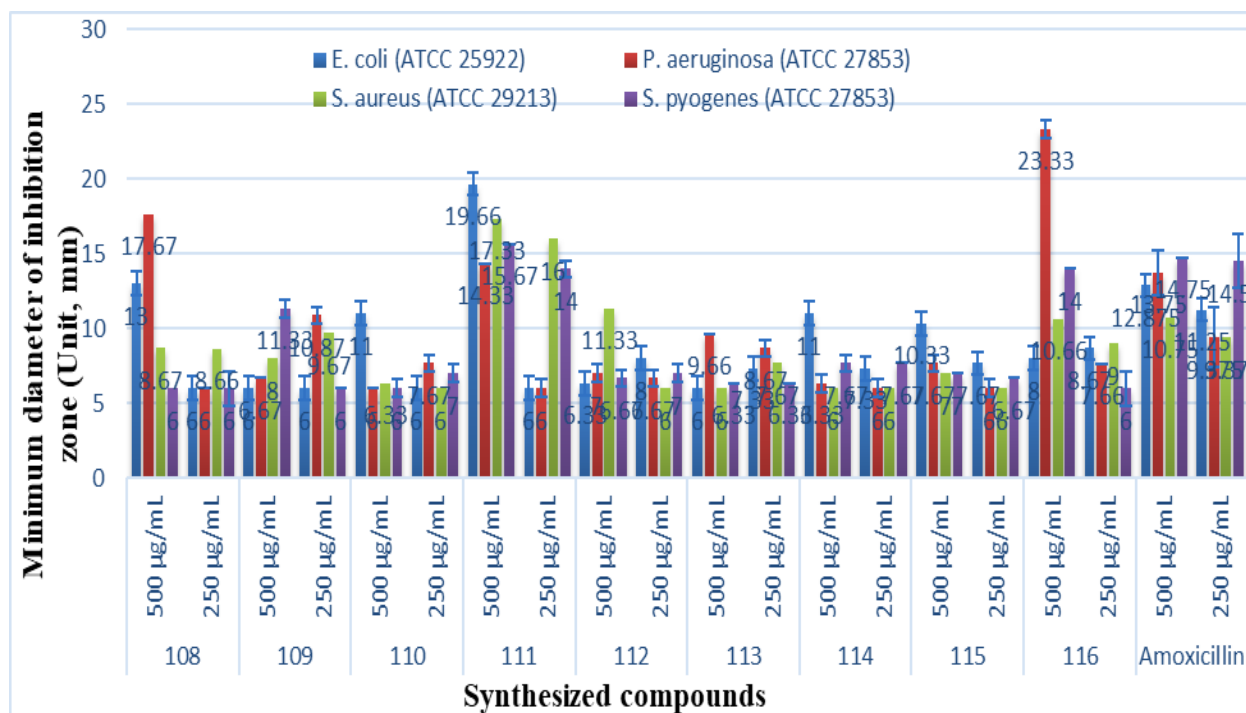


Figure 17. Diameter of inhibition zone of the synthesized compounds, **108-116** in mm (mean $\pm$ SD) at 250 and 500 $\mu\text{g/mL}$ concentration

Table 5. Antibacterial activity of heterocyclics C₅-curcuminoid analogs, **108-116** against selected bacterial strains.

		Mean Diameter of inhibition zones (DIZ) in mm			
Synthesized compounds		Gram-negative bacteria		Gram-positive bacteria	
		<i>Escherichia coli</i> (ATCC 25922)	<i>Pseudomonas aeruginosa</i> (ATCC 27853)	<i>Staphylococcus aureus</i> (ATCC 29213)	<i>Streptococcus pyogenes</i> (ATCC 27853)
108	500 µg/mL	13.00±0.57	17.67±0.57	8.67±1.15	6.00±0.00
	250 µg/mL	6.00±0.00	6.00±0.00	8.66±0.57	6.00±0.00
109	500 µg/mL	6.00±0.00	6.67±0.57	9.00±0.00	11.33±0.57
	250 µg/mL	6.00±0.57	10.87±0.57	8.67±0.57	6.00±0.00
110	500 µg/mL	11.00±0.57	7.67±0.00	6.33±0.57	7.00±0.57
	250 µg/mL	6.00±0.57	7.00±0.57	6.00±0.00	6.00±0.00
111	500 µg/mL	19.66±0.00	14.33±0.57	17.33±0.57	15.67±0.57
	250 µg/mL	6.00±0.57	6.00±0.00	16.00±0.57	14.00±0.57
112	500 µg/mL	6.67±0.00	7.00±0.57	11.33±0.57	7.67±0.57
	250 µg/mL	6.33±0.00	6.67±1.15	6.00±0.00	6.00±0.00
113	500 µg/mL	7.00±0.57	9.66±0.57	7.67±0.00	6.33±0.57
	250 µg/mL	6.33±0.57	8.67±1.15	6.00±0.57	6.00±0.57
114	500 µg/mL	11.00± 0.57	6.33±0.57	6.00±0.00	7.67±0.57
	250 µg/mL	7.33±0.57	6.00±0.00	6.00±0.00	7.33±0.57
115	500 µg/mL	10.33±0.57	7.67±0.57	7.00±0.00	7.00±0.57
	250 µg/mL	7.67±0.57	6.00±0.57	6.00±0.00	6.67±0.57
116	500 µg/mL	8.67±0.00	23.33±0.00	10.66±1.15	14.00±0.00
	250 µg/mL	8.00±0.00	7.66± 0.57	9.00±0.00	6.00± 1.52
Amoxicillin	500 µg/mL	12.875±1.41	13.75±1.83	10.75±1.83	14.75±2.00
	250 µg/mL	11.25±1.33	9.375±1.50	9.37±1.50	14.50±1.25
DMSO		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

^a The experiment was carried out in triplicate and the average zone of inhibition was expressed in mean ± standard deviation (mm). ^b N.A. (no activity). ^cATCC: American Tissue Culture Collection. ^d DIZ: Diameter of Inhibition Zone.

5.6. *In vitro* α -amylase inhibitory activity

5.6.1. *In vitro* α -amylase inhibitory activities of compounds, 75-83

Inhibition of the digestive enzyme (α -amylase) decreases the rate of glucose absorption by extending the digestion and absorption time of carbohydrates and is one of the therapeutic approaches for controlling the level of glucose in T2DM (Chortani et al., 2021; S. Khan et al., 2022). The inhibitory effect of this enzyme results in lowering postprandial hyperglycemia (PPHG), and blood glucose levels. The synthesized compounds were evaluated *in vitro* inhibitory potencies against the α -amylase enzyme. The synthesized compounds were evaluated *in vitro* inhibitory potencies against α -amylase enzyme in a dose-dependent manner (1.25-10 $\mu\text{g/mL}$). Based on the obtained results of % inhibition of alpha-amylase enzyme and IC_{50} values results are presented in Table 6 and Figure 21. Interestingly, the tested thiazolyl-pyrazoline Schiff base hybrids (75-83) showed modest α -amylase inhibitory activity with IC_{50} ranging from 6.67 ± 0.002 to 7.98 ± 0.002 $\mu\text{g/mL}$. Among these, compounds 80, 81, 82, and 83 have shown the highest activity against α -amylase with $\text{IC}_{50} = 6.90, 6.74, 6.67,$ and 6.80 $\mu\text{g/mL}$, respectively as compared to standard drug, acarbose which has ($\text{IC}_{50} = 4.42$ $\mu\text{g/mL}$). It was observed that compound 82 (6.67 $\mu\text{g/mL}$) was found to be the most active inhibitor with an inhibitory effect comparable to acarbose. All other analogs showed good to moderate inhibitory potential is with $\text{IC}_{50} < 500$ μM . As the concentration of tested compounds increases, the percentage of inhibition was also observed to increase. The structural activity relationship was established for all compounds which depend upon the different substituents EWG or EDG on the phenyl ring of thiazolyl-pyrazoline Schiff base part. Compound 80 was found to be the most potent ($\text{IC}_{50} = 6.90 \pm 0.005$ $\mu\text{g/mL}$). This might be due to the presence of the electron donating group of hydroxyl ($-\text{OH}$) moiety on the *para*- position of the phenyl ring of thiazole ring and an electron-withdrawing group of $-\text{Br}$ on the *para*- position of the phenyl ring of pyrazoline moiety, while the rest compounds 81, 82, and 83 exhibited better potentials with electron donating group of $-\text{OH}$ on ortho position and electron withdrawing group $-\text{NO}_2$ on the *para* positions of pyrazoline phenyl moiety with variety of substituted groups at thiazole group, are another useful moiety that might facilitate its binding with the active site of the α -amylase (Stagos, 2019). Hence, the observed results revealed that the introduction of strong electron-withdrawing group *p*- NO_2 and –

Br substitutions into the *N*-pyrazoline moiety can enhance the inhibitory activity among these series.

5.6.2. *In vitro* α -amylase inhibitory activities of compounds, **92-100**

The *in vitro* studies results of % inhibition of alpha-amylase enzyme and IC_{50} values results are presented in Table 7 and Figure 21. Interestingly, the tested thiazolo [3,2- α] pyrimidines analogs (**92-100**) showed potent α -amylase inhibitory activity with IC_{50} ranging from 6.56 ± 0.002 to 8.26 ± 0.003 $\mu\text{g/mL}$. Among these, compounds **94**, **98**, and **99** have shown the moderate activity against α -amylase with $IC_{50} = 6.56 \pm 0.002$, 6.80 ± 0.004 and 6.75 ± 0.002 $\mu\text{g/mL}$, respectively as compared to standard drug, acarbose which has ($IC_{50} = 4.42 \pm 0.001$ $\mu\text{g/mL}$). It was observed that compound **98** was found to be the most active inhibitor with an inhibitory effect comparable with acarbose. All other analogs showed good to moderate inhibitory potential with $IC_{50} < 500$ μM . As the concentration of tested compounds increases, the percentage of inhibition is also increasing. The structural activity relationship was established for all compounds which depend upon the different substituents EWG or EDG on the phenyl ring of phenyl groups of thiazolo[3,2- α] pyrimidines part. Compounds **98** and **99** were found most potent this might be due to the presence of electron donating group of hydroxyl ($-\text{OH}$) moiety on the *ortho*- and *para*-position of the phenyl ring of core chalcone and thiazole analogs, while compound **95** exhibited better potentials with electron-withdrawing group ($-\text{NO}_2$) on the phenyl ring of thiazole phenyl ring, is another useful moiety that might facilitate its binding with the active site of the α -amylase. Based on the results, the introduction of a strong electron-donating group of *ortho*-OH substitution plays an important role in the thiazolo[3,2- α] pyrimidines moiety can have enhanced the inhibitory activity.

5.6.3. *In vitro* α -amylase inhibitory activities of compounds, **108-116**

Based on the obtained results of % inhibition of alpha-amylase enzyme and IC_{50} values are illustrated in Figure 21 and the results are displayed in Table 8, interestingly, the tested heterocyclic C_5 -curcumin analogs, **108-116** showed excellent to potent α -amylase inhibitory activity with IC_{50} ranging from 4.08 ± 0.23 to 102.65 ± 0.23 $\mu\text{g/mL}$. Among these, compounds **116**,

111, and **108** showed the highest activity against α -amylase with IC_{50} = 4.08, 7.59, and 22.03 μ g/mL, respectively as compared to the standard drug, acarbose which has (IC_{50} = 8.0 μ g/mL). These compounds inhibited the α -amylase activity of 85.32 ± 0.36 %, 85.17 ± 0.55 %, and 87.15 ± 0.46 at 10 μ g/mL concentration, respectively. It was observed that compound **116** (IC_{50} = 4.08 μ g/mL), was found to be the most active inhibitor with an inhibitory effect up to 2-fold better than acarbose. This may be due to the presence of imidazole moiety and hydroxyl functionality at aromatic ring that interacts with the target enzyme. This enhanced activity of compounds **108** and **116** was found to be the most potent this might be due to the presence of electron-donating a group of dimethyl amino ($-N(CH_3)_2$) moiety on the *para*-position of the aryl ring of *N*-pyrazolines and β -substituted imidazoles analogs, while compound **111** exhibited better potentials with unsubstituted at phenyl ring of 4-phenyl-6-styrylpyrimidine-2-ol group, is another useful moiety that might facilitate its binding with the active site of the α -amylase (Rossi & Ciofalo, 2020).

However, as the concentrations of tested compounds increases as the percentage of inhibition is also increasing. The structural activity relationship was established for all compounds which depend upon the different substituents EWG or EDG on the phenyl ring of curcumin and its heterocyclic part. Based on the results, the constituent that is responsible for an α -amylase inhibitor is possibly the phenols in curcumin since their antioxidant properties can prevent damage from reactive oxygen species and also bind and inhibit an α -amylase (Stagos, 2019). The introduction of strong electron-withdrawing group *p*-NO₂ substitution (**112**, IC_{50} = 102.65 ± 0.23 μ g/mL) into the thiazolo[3,2-*a*] pyrimidines moiety can reduce the inhibitory activity. Thus, the most active compounds diminished the levels of enzymes, which are in charge of catalyzing the hydrolysis of complex carbohydrates, and improved the utilization rate of glucose by lowering postprandial hyperglycemia (Chortani et al., 2021).

Table 6. % inhibition of α -amylase enzyme and IC₅₀ values of thiazolyl-pyrazoline Schiff base hybrids analogs, **75-83** and standard acarbose at different concentrations.

Synthesized Compounds	% of inhibition (mean $\pm$ S.D.) * (μ g/mL)				IC ₅₀ $\pm$ SEM ^a (μ g/mL)
	1.25	2.5	5	10	
75	8.21 $\pm$ 0.001	17.76 $\pm$ 0.002	31.82 $\pm$ 0.007	69.32 $\pm$ 0.006	7.42 $\pm$ 0.003
76	7.85 $\pm$ 0.001	16.98 $\pm$ 0.001	30.39 $\pm$ 0.002	66.28 $\pm$ 0.007	7.65 $\pm$ 0.003
77	7.52 $\pm$ 0.001	16.26 $\pm$ 0.002	29.15 $\pm$ 0.004	63.44 $\pm$ 0.001	7.98 $\pm$ 0.002
78	7.75 $\pm$ 0.005	16.78 $\pm$ 0.001	30.07 $\pm$ 0.003	65.53 $\pm$ 0.005	7.73 $\pm$ 0.002
79	8.25 $\pm$ 0.000	17.85 $\pm$ 0.001	31.96 $\pm$ 0.001	69.69 $\pm$ 0.004	7.28 $\pm$ 0.002
80	9.77 $\pm$ 0.000	21.15 $\pm$ 0.002	37.89 $\pm$ 0.004	70.64 $\pm$ 0.011	6.90 $\pm$ 0.005
81	9.45 $\pm$ 0.001	20.43 $\pm$ 0.001	36.57 $\pm$ 0.001	73.86 $\pm$ 0.004	6.74 $\pm$ 0.001
82	9.02 $\pm$ 0.001	19.50 $\pm$ 0.001	34.92 $\pm$ 0.000	76.13 $\pm$ 0.005	6.67 $\pm$ 0.002
83	8.84 $\pm$ 0.000	19.12 $\pm$ 0.008	34.24 $\pm$ 0.002	74.62 $\pm$ 0.003	6.80 $\pm$ 0.003
Acarbose ^b	14.73$\pm$0.005	34.85$\pm$0.006	58.37$\pm$ 0.004	102.46$\pm$0.005	4.42$\pm$0.001

* Values are expressed as mean $\pm$ SD (n=3)

^a SEM (standard error mean)

^b Acarbose (Standard for α -amylase inhibitory activities)

Table 7. % inhibition of α -amylase enzyme and IC₅₀ values of thiazolo [3,2- α] pyrimidines analogs, **92-100** and standard acarbose at different concentrations.

Synthesized Compounds	% of inhibition (mean $\pm$ S.D.) * (μ g/mL)				IC ₅₀ $\pm$ SEM ^a (μ g/mL)
	1.25	2.5	5	10	
92	7.26 $\pm$ 0.000	15.72 $\pm$ 0.002	28.15 $\pm$ 0.003	61.36 $\pm$ 0.008	8.26 $\pm$ 0.003
93	8.10 $\pm$ 0.001	17.51 $\pm$ 0.002	31.33 $\pm$ 0.002	68.37 $\pm$ 0.009	7.42 $\pm$ 0.004
94	9.19 $\pm$ 0.001	19.85 $\pm$ 0.002	35.55 $\pm$ 0.003	77.46 $\pm$ 0.005	6.56 $\pm$ 0.002
95	7.96 $\pm$ 0.001	17.22 $\pm$ 0.002	30.82 $\pm$ 0.002	67.23 $\pm$ 0.008	7.55 $\pm$ 0.003
96	7.95 $\pm$ 0.001	17.18 $\pm$ 0.001	30.78 $\pm$ 0.003	67.04 $\pm$ 0.003	7.56 $\pm$ 0.001
97	7.36 $\pm$ 0.001	15.87 $\pm$ 0.002	28.46 $\pm$ 0.005	61.93 $\pm$ 0.007	8.18 $\pm$ 0.003
98	10.14 $\pm$ 0.008	21.93 $\pm$ 0.001	39.26 $\pm$ 0.001	71.02 $\pm$ 0.007	6.80 $\pm$ 0.004
99	9.61 $\pm$ 0.001	20.76 $\pm$ 0.001	37.17 $\pm$ 0.002	73.29 $\pm$ 0.006	6.75 $\pm$ 0.002
100	8.21 $\pm$ 0.002	17.72 $\pm$ 0.002	31.76 $\pm$ 0.005	69.13 $\pm$ 0.007	7.34 $\pm$ 0.002
Acarbose ^b	14.73$\pm$0.005	34.85$\pm$0.006	58.37$\pm$ 0.004	102.46$\pm$0.005	4.42$\pm$0.001

* Values are expressed as mean $\pm$ SD (n=3)

^a SEM (standard error mean)

^b Acarbose (Standard for α -amylase inhibitory activities)

Table 8. % inhibition of α -amylase enzyme and IC₅₀ values of heterocyclics C₅-curcumin analogs, **108-116** and standard acarbose at different concentrations.

Synthesized Compounds	% of inhibition (mean $\pm$ S.D.) * (μ g/mL)				IC ₅₀ $\pm$ SEM ^a (μ g/mL)
	1.25	2.5	5	10	
108	58.19 $\pm$ 0.08	62.19 $\pm$ 0.09	74.12 $\pm$ 0.08	87.15 $\pm$ 0.46	22.03 $\pm$ 0.19
109	45.89 $\pm$ 0.06	57.82 $\pm$ 0.17	66.98 $\pm$ 0.38	78.89 $\pm$ 1.09	95.75 $\pm$ 0.46
110	50.29 $\pm$ 0.01	59.97 $\pm$ 0.08	72.39 $\pm$ 0.14	82.56 $\pm$ 0.47	50.51 $\pm$ 0.20
111	58.12 $\pm$ 0.09	66.31 $\pm$ 0.08	76.33 $\pm$ 0.13	85.32 $\pm$ 0.36	7.59 $\pm$ 0.13
112	39.82 $\pm$ 0.11	50.21 $\pm$ 0.18	58.27 $\pm$ 0.43	69.26 $\pm$ 0.60	102.65 $\pm$ 0.23
113	51.21 $\pm$ 0.09	62.71 $\pm$ 0.14	75.98 $\pm$ 0.17	86.99 $\pm$ 0.65	45.10 $\pm$ 0.26
114	39.11 $\pm$ 0.04	54.21 $\pm$ 0.05	68.89 $\pm$ 0.18	83.94 $\pm$ 0.32	86.44 $\pm$ 0.13
115	52.71 $\pm$ 0.07	62.62 $\pm$ 0.15	75.56 $\pm$ 0.36	87.61 $\pm$ 0.62	41.65 $\pm$ 0.25
116	58.19 $\pm$ 0.04	67.32 $\pm$ 0.12	77.01 $\pm$ 0.28	85.17 $\pm$ 0.55	4.08 $\pm$ 0.23
Acarbose^b	57.38$\pm$ 0.36	67.99$\pm$ 0.47	77.99$\pm$ 0.56	85.78$\pm$ 0.45	8.01$\pm$0.08

* Values are expressed as mean $\pm$ standard deviation (n = 3).

^a SEM (standard error mean).

^b Acarbose (Standard for α -amylase inhibitory activities).

5.7. *In vitro* Antioxidant activities and lipid peroxidation inhibition

5.7.1. DPPH free radical scavenging and lipid peroxidation inhibition activities of **75-83**

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity is a rapid technique for assessing the ability of synthetic compounds or extracts as scavengers of free radicals (Bouherrou et al., 2017). The DPPH free radical is long lived organic nitrogen radical with purple color. When the DPPH solution is mixed with antioxidant, its color turns from purple to yellow of the corresponding hydrazine. The reducing ability of antioxidants towards DPPH was evaluated by measuring the decrease of its absorbance at 517 nm. Oxidative degradation of polyunsaturated fatty acids in membrane phospholipids is known as lipid peroxidation (Halliwell & Chirico, 1993). The increased concentration of lipid peroxidation end products increased free radicals, which enhanced the oxidative stress level in living organisms and resulted in cell death. The antioxidant activity of compounds **75-83** were determined by the DPPH assay. From the results obtained (Table 9, Figure 21 and Appendix 110), compound **80** displayed significant antioxidant activity ($IC_{50} = 4.63 \mu\text{g/mL}$) compared to the positive control, ascorbic acid ($IC_{50} = 3.21 \mu\text{g/mL}$). The rest of the compounds showed moderate to weak activity. Most of the synthesized compounds, except **81** showed greater than 80% of DPPH radical scavenging activity at a concentration of 200 $\mu\text{g/mL}$ (Figure 18). The presence of electron-donating hydroxyl functionality at of thiazole and Br-at the phenyl groups could be the most possible reason which might stabilize the radicals for their strong electron-donating nature (Shah et al., 2022).

Compounds **80** and **83** inhibited peroxide formation by $80.01 \pm 0.07\%$ and $79.44 \pm 0.05\%$, respectively, demonstrating their potential in preventing lipid peroxide formation as presented (Table 9). Thus, the results showed that the studied thiazole-pyrazoline based Schiff base hybrids remove the free radicals, especially the lipid peroxide radicals, by giving them their hydrogen atom and thus, inhibit the lipid peroxidation reaction.

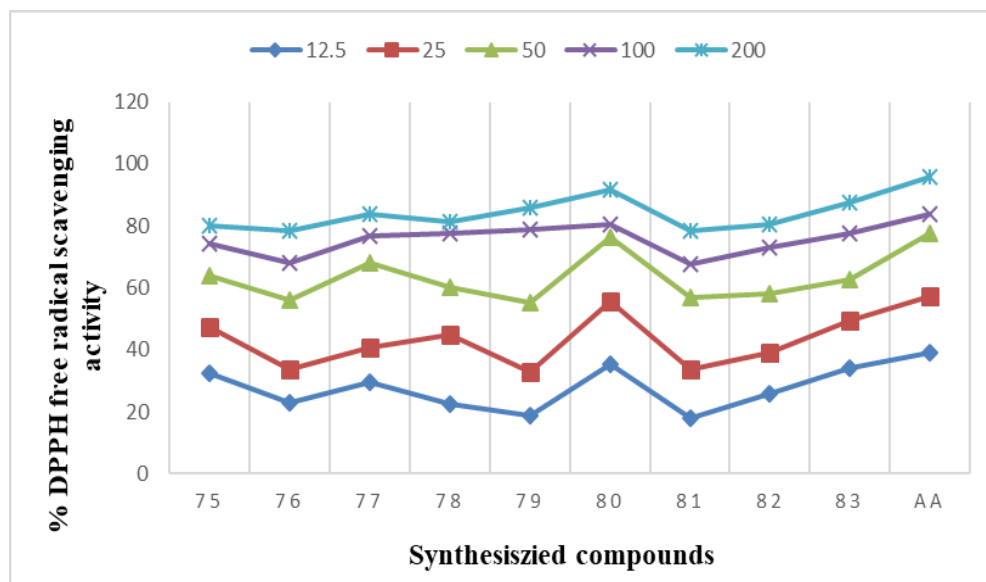


Figure 18. Percentage inhibition of the synthesized compounds, **75-83** at different concentration ranges.

5.7.2. DPPH free radical scavenging and lipid peroxidation inhibition activities of **92-100**

Synthesized compounds (**92-100**) were assessed for their antioxidant activities as presented in [Table 10](#). The compounds were first screened at five different concentration ranges, namely 12.5, 25.0, 50.0, 100.0, and 200.0 µg/mL ([Appendix 111](#)). The antioxidant activities were expressed as % DPPH inhibition. As a result, a lower IC_{50} value suggests that the compound has a stronger ability to operate as a DPPH scavenger and vice versa. As shown in [Figure 19](#) and [Figure 21](#), among the tested compounds, compound **99** showed the highest percentage of DPPH inhibition with an IC_{50} value of 9.48 µg/mL compared with the standard drug ascorbic acid (IC_{50} = 3.21 µg/mL) respectively. This might be due to the presence of hydroxyl groups at the *ortho*-positions of phenyl rings of thiazole and chalcone moieties that stabilize the radicals for their strong electron-donating nature. The synthesized compounds (**92-100**) were also assessed for their lipid peroxidation potentials. As seen in [Table 10](#), compounds **96** and **99** inhibit peroxide formation by 70.11 ± 0.04 and 76.28 ± 0.12 % demonstrating their potential in preventing the formation of lipid peroxides. However, the other synthesized compounds exhibited a moderate ability to inhibit peroxide formation compared with the natural antioxidant, ascorbic acid. The obtained results

showed that the studied thiazolo[3,2- α] pyrimidine analogs remote the free radicals, especially the lipid peroxide radicals, by giving their hydrogen atom and thus, inhibiting the lipid peroxidation reaction. The presence of electron-donating hydroxyl functionality at *ortho*- and *para*-positions of aryl thiazolo[3,2- α]pyrimidine groups, could be the most possible reason which might stabilize the radicals for their strong electron-donating nature (Shah et al., 2022).

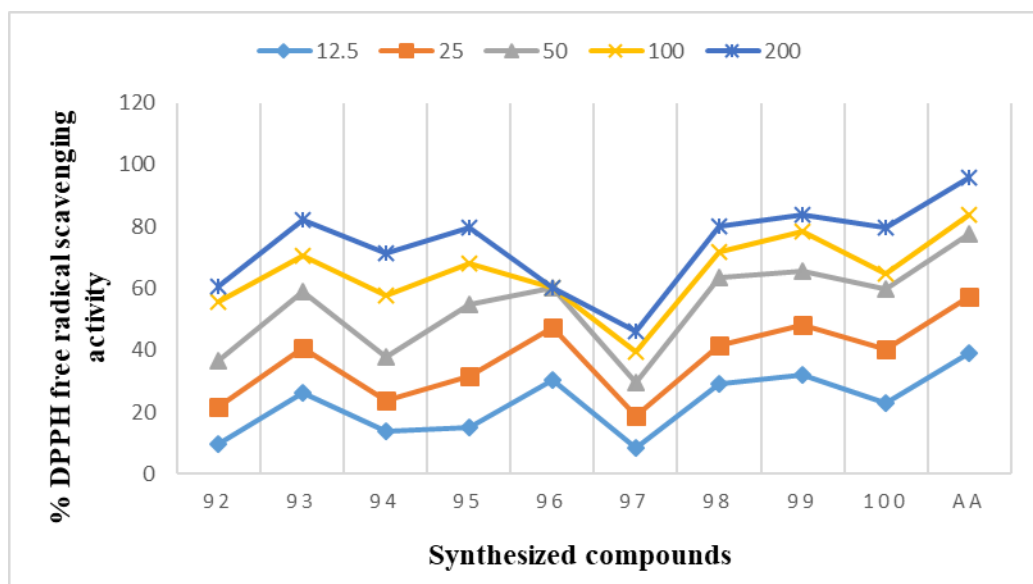


Figure 19. Percentage inhibition of the synthesized compounds, **92-100** at the different concentration ranges.

5.7.3. DPPH free radical scavenging and lipid peroxidation inhibition activities of **108-116**

In the presence of scavengers in the fractions, the lower the absorption, the more effective the antioxidant activity is (Idhayadhulla et al., 2014). Based on the experimental results, the synthesized compounds (**108-116**) series exhibit good *in vitro* DPPH radical scavenging abilities, and the results are presented in [Table 11](#) and [Appendix 112](#). The results of antioxidant by DPPH assay revealed that the percentage of inhibition was increased with the increase in concentration test samples. Most of the compounds demonstrated greater than 80-94% of DPPH free radical scavenging activity at a concentration of 200 $\mu\text{g/mL}$ ([Figure 20](#)). Of the tested compounds, the IC_{50} of antioxidant activity data of compound **108** showed the highest percent inhibition of the

DPPH with an IC_{50} value of 2.44 $\mu\text{g/mL}$ compared with standard ascorbic acid drug ($IC_{50} = 1.24 \mu\text{g/mL}$), indicating that heterocyclics derived C_5 -curcumin, **108** exhibited highest antioxidant activity compared to ascorbic acid. In other words, these compounds have the ability of electron donors to scavenge free radicals. This might be due to the presence of hydroxyl groups at the *para* positions (*p*-OH) of curcumin phenyl rings or pyrazoline moiety itself that stabilize the radicals for their strong electron-donating nature, respectively. The antioxidant capacity of all the tested compounds is explained by the redox potential of phenolic compounds, which allows them to function as reducing agents, hydrogen donors, and singlet oxygen scavengers. Figure 21, illustrated a comparison between the IC_{50} values of % radical scavenging activity, as well as the IC_{50} of the tested compounds against alpha-amylase along with acarbose and ascorbic acid reference drugs.

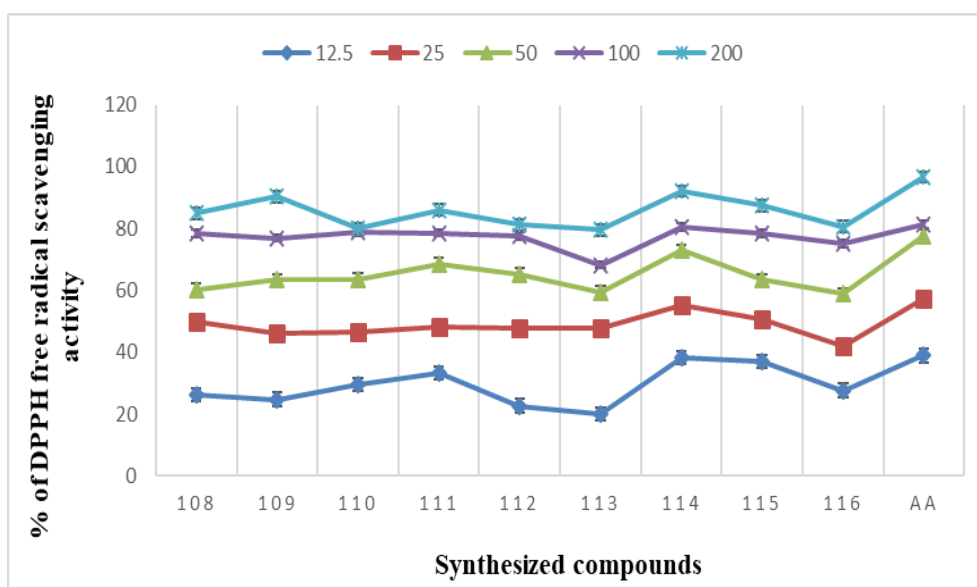


Figure 20. Percentage inhibition of the synthesized compounds, **108-116** at the different concentration range

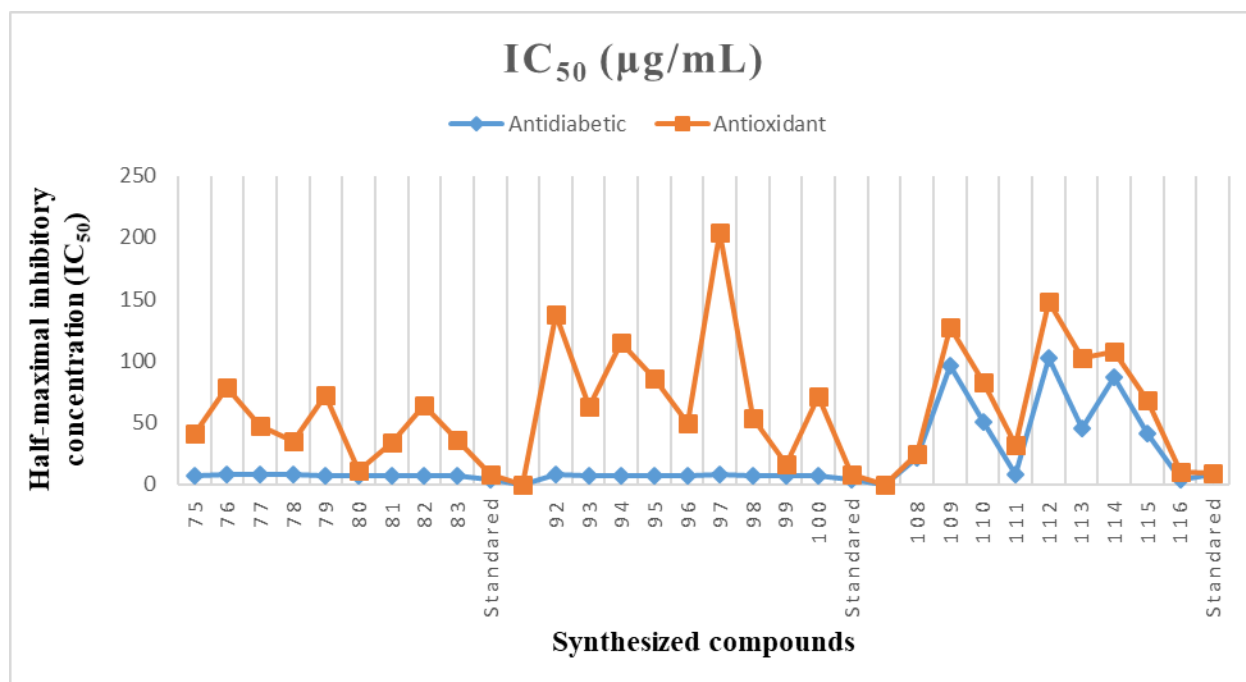


Figure 21. Half-maximal inhibitory concentration (IC₅₀) of synthesized compounds, **75-83**, **90-100** and **108-116** as compared to that of standard drugs, acarbose, and ascorbic acid.

The lipid peroxide removal effects of heterocyclic C₅-curcumin analogs reflect their antioxidant activities of them. The synthesized compounds, **108-116** were also investigated for their anti-lipid peroxidation potential with the result summarized in [Table 11](#). As seen from lipid-peroxidation data, compound **116** inhibits peroxide formation by 89.92±0.42% demonstrating their potential in preventing the formation of lipid peroxides. However, the synthesized compounds exhibited a moderate ability to inhibit peroxide formation compared with the natural antioxidant, ascorbic acid. The obtained results displayed that, the studied heterocyclic C₅-curcumin analogs remove the free radicals, especially the lipid peroxide radicals, by giving their hydrogen atom and thus, inhibit the lipid peroxidation reaction.

Table 9. The *in vitro* antioxidant and anti-lipid peroxidation inhibition test with various concentrations of **75-83**.

Conc. (µg/mL)	% of inhibition of the synthesized compounds*									
	75	76	77	78	79	80	81	82	83	Ascorbic acid ^a
12.5	32.32±0.50	22.75±0.31	29.67±0.24	22.69±0.54	19.00±0.01	35.32±0.03	18.13±0.55	25.96±0.08	34.02±0.02	38.94±0.04
25.0	47.25±0.36	33.59±0.41	40.95±0.12	44.90±0.02	32.73±0.11	55.73±0.04	33.82±0.36	38.99±0.07	49.63±0.25	57.16±0.13
50.0	63.9±0.30	56.0±0.36	67.9±0.16	60.2±0.20	55.4±0.39	76.3±0.11	56.9±0.41	58.1±0.28	62.9±0.16	77.6±0.18
100.0	74.33±0.29	68.21±0.74	76.92±0.03	77.61±0.06	78.92±0.04	80.44±0.11	67.83±0.11	73.19±0.21	77.53±0.34	83.73±0.06
200.0	80.25±0.35	78.44±0.38	83.74±0.17	81.22±0.14	85.74±0.23	91.49±0.05	78.45±0.30	80.53±0.13	87.41±0.02	95.71±0.13
IC₅₀(µg/mL)*	34.25	70.93	39.79	27.34	64.62	4.63	27.32	57.35	29.08	3.21
% Anti-lipid peroxidation inhibition	55.74±0.09	68.05±0.54	65.00±0.14	70.66±0.52	69.07±0.52	80.01±0.07	58.88±0.25	69.66±0.11	79.44±0.05	80.34±0.12

*IC₅₀ is the 50% inhibitory concentration of the samples, and the results were represented as Mean ± SD.

^a Ascorbic acid was used as a positive control.

Table 10. *In vitro* antioxidant and anti-lipid peroxidation inhibition properties of compounds, **92-100**.

Conc.(µg/mL)	% of inhibition of the Synthesized compounds*									
	92	93	94	95	96	97	98	99	100	Ascorbic Acid ^a
12.5	9.85±0.06	26.19±0.99	13.82±0.33	15.12±0.27	30.35±0.30	8.52±0.12	29.23±0.47	32.09±0.03	22.89±0.05	38.94±0.04
25.0	21.81±0.32	40.93±0.37	23.83±0.10	31.60±0.05	47.58±0.52	18.70±0.04	41.58±0.14	48.01±0.03	40.21±0.09	57.16±0.13
50.0	36.8±0.10	58.9±0.03	37.9±0.24	55.0±0.04	60.4±0.06	29.6±0.05	63.6±0.21	65.6±0.20	59.7±0.15	77.6±0.18
100.0	55.64±0.03	70.42±0.12	57.91±0.21	67.89±0.08	72.31±0.03	39.66±0.01	71.74±0.27	78.61±0.06	64.78±0.06	83.73±0.06
200.0	60.69±0.23	82.01±0.01	71.54±0.24	79.56±0.51	81.54±0.28	46.27±0.05	80.19±0.06	83.92±0.13	79.70±0.08	95.71±0.13
IC₅₀ (µg/mL)	128.98	56.02	107.93	78.04	41.72	195.94	46.77	9.48	64.06	3.21
% Anti-lipid peroxidation inhibition	56.91±0.37	61.33±0.06	43.65±0.05	67.12±0.04	70.11±0.04	55.43±0.21	49.29±0.52	76.28±0.12	57.19±0.07	80.34±0.12

*IC₅₀ is the 50% inhibitory concentration of the samples, and the results were represented as Mean ± SD.

^a Ascorbic acid was used as a positive control.

Table 11. *In vitro* antioxidant and anti-lipid peroxidation inhibition test with various concentrations of **108-116**.

Conc.(µg/mL)		12.5	25.0	50.0	100.0	200.0	IC ₅₀ (µg/mL)	%Anti-lipid peroxidation inhibition
% of inhibition of the Synthesized compounds*	108	47.66±0.22	53.89±0.31	68.9±0.13	79.19±0.77	93.53±0.02	2.44	82.47±0.33 %
	109	39.11±0.41	45.95±0.12	59.4±0.16	76.92±0.33	90.24±0.27	31.29	76.88±0.76 %
	110	39.44±0.87	46.58±0.24	58.6±0.23	71.74±0.27	80.1±0.07	32.55	82.49±0.26 %
	111	38.29±0.33	50.01±0.33	61.6±0.33	73.61±0.06	80.02±0.12	24.00	82.18±0.42 %
	112	56.23±0.30	63.86±0.17	70.2±0.26	83.43±0.42	94.01±0.17	45.35	80.67±0.33%
	113	28.91±0.01	43.73±0.47	53.5±0.42	68.21±0.45	79.47±0.77	57.79	78.43±0.33 %
	114	38.32±0.04	51.16±0.04	62.88±0.14	80.44±0.17	92.09±0.21	21.20	81.71±0.27 %
	115	37.12±0.42	50.67±0.25	61.4±0.18	74.53±0.67	87.42±0.21	26.95	79.66±0.05 %
	116	42.69±0.54	52.9±0.22	65.2±0.20	73.51±0.16	81.12±0.22	5.85	89.92±0.42 %
	AA	38.94±0.04	57.16±0.13	77.6±0.18	81.27±0.66	96.61±0.13	1.24	80.34±0.12%
DPPH								

*IC₅₀ is the 50% inhibitory concentration of the samples, and the results were represented as Mean ± SD.

^aAscorbic acid was used as a positive control.

5.8. *In silico* ADMET prediction study

5.8.1. *In silico* prediction of drug-likeness, and pharmacokinetic study of **75-83**

SwissADME/Pre ADMET are a web-based tool that provides important information about drug-likeness, pharmacokinetic, and pharmacodynamics properties of bioactive molecules (Abishad et al., 2021; Ibrahim et al., 2021). In this perspective, the theoretical agreement of synthesized compounds to Lipinski's rule of five and Veber's rule (Daina et al., 2017; Lipinski, Lombardo, Dominy, & Feeney, 1997; Veber et al., 2002), as well as the pharmacokinetic and toxicity parameters were estimated using ADMET descriptors by a SwissADME (Daina et al., 2017), and PreADMET (Lee et al., 2003) online servers. The percent absorption (%Abs) of the synthesized compounds was computed using the formula $\%Abs = 109 - 0.345TPSA$ [46]. The filter that combines Lipinski's "rule of five" refers physicochemical properties of a molecule to become a drug i.e. ($MW \leq 500$ Daltons, $\log P \leq 5$, H-bond donors ≤ 5 , and H-bond acceptors ≤ 10) (Lipinski et al., 1997) and Veber's parameters ($TPSA \leq 140 \text{ \AA}^2$ and rotatable bonds ≤ 10) (Veber et al., 2002). Pro Tox II web server exploration was used to forecast the ligands' organ toxicity profiles and toxicological endpoints, as well as their LD_{50} (P. Banerjee, Eckert, Schrey, & Preissner, 2018; Subhasis Ghosh, Tripathi, Talukdar, & Talapatra, 2019).

The results showed that all of the compounds obeyed Lipinski's rule of five (Table 12), with only minor deviations for **78-80** and **82**, thus they have the potentials to be developed as an orally bioavailable drugs. All synthesized compounds have a higher bioavailability score of 0.55 and were suitable for oral administration, indicating drug-like qualities (Martin, 2005). Their HBD values are <3 , indicating that they are highly soluble in cellular membranes, with HBA values ranging from 5-8. The $\log P$ values for all compounds are <5 , notably for **79** and **81-83** indicating the compounds have good lipophilicity characteristics which may protect them against ROS degradation. TPSA ranged from 86.68-152.73 $\text{\AA}^2$, while the consensus $\log P_{o/w}$ (an indicator of lipophilicity) was observed in an optimal range from 2.06 to 3.64. Compounds that satisfy the parameters of $TPSA \leq 140 \text{ \AA}^2$ are expected to have adequate solubility, oral bioavailability, and cell permeability (Ertl, Rohde, & Selzer, 2000). All compounds (**75-83**) other than compound **82**, exhibited better percent absorption (% Abs) than amoxicillin suggesting good oral bioavailability.

In silico pharmacokinetic characteristics results (Table 13) revealed that compounds **75-83** had superior intestinal absorption to amoxicillin, ranging from 90.80 to 99.82% absorption with HIA values near 100%. *In vitro*, Caco-2 and MDCK cells have moderate to high permeability, notably **77**, **81**, and **83**, and especially **78** and **83**, which are better than amoxicillin (0.39), respectively. Skin permeability (SP, Log_{K_p}) was found to be somewhat below the permitted range of -5.42 to -6.59 cm/sec and a substance with a low negative log _{K_p} value has higher absorption into human skin. Results indicate that the synthesized compounds are showing good absorption properties to cross the gut wall by passive diffusion mechanism to reach the target. All of the compounds were poorly absorbed into the CNS *in vivo*, showing that they have a decreased ability to cross the CNS, with a total clearance value of **83** (0.07), which is comparable to amoxicillin (0.06). All of the substances have a high PPB%, with values of more than 90% suggesting that they are strongly bound. The results revealed that compounds **77** (99.75), and **83** (97.31) had high PPB values, indicating longer half-lives than amoxicillin (23.35)(Alonso et al., 2019). Furthermore, glycoprotein (P-gp) inhibition measurements are utilized to forecast the target drugs' excretion properties. P-gp and cytochrome P450 (CYP) are known to aid in the protective efflux (biotransformation) of biological membranes (GI tract or brain) from xenobiotics to protect tissues (Finch & Pillans, 2014). The compounds' metabolic properties were predicted by the behavior of cytochrome P450 and their isoform enzymes. In this investigation, compounds, **75-83** showed no inhibitory activity against CYP2C19, CYP2C9, CYP2C6, or CYP3A4, except **82** and **83**, which showed inhibitory activity against CYP2D9. In our study, none of the synthesized compounds inhibited P-gp substrates, except **75** and **78**.

Toxicity data must be connected with *in vitro* safety investigations before being examined in relevant laboratory animal models using ProTox II service (Goswami, 2019). In addition, all compounds were predicted for their computational toxicity risks, such as the AMES test (for mutagenicity prediction), tumorigenicity carcino-Mouse/Rat (for carcinogenicity prediction), and skin irritancy hERG-inhibition (for checking cardiac toxicity), and cytotoxicity effect (Table 14). Based on toxicity profiles, all compounds (**75-83**) and amoxicillin showed mutagenic behavior in the AMES test. All compounds, except for **76**, **79**, **82**, **83**, and amoxicillin, exhibited no possible carcinogenic effect in mice and have potential carcinogenic effect in rats. All of the compounds exhibited a low risk of being cardiotoxic or cytotoxic, as well as being safer than standard drugs.

All of the synthesized compounds tested had predicted LD₅₀ (mg/kg) values ranging from 800 to 1,000 putting them in ProTox-II's class-4, indicating that they could be harmful if swallowed ($300 < LD_{50} \leq 2000$) (Drwal, Banerjee, Dunkel, Wettig, & Preissner, 2014). The bioavailability radar plots of the tested compounds (**75-83**) indicated that all of the compounds were completely within the pink area, indicating that they have drug-like qualities with an improved bioavailability profile ([Appendix 113](#)) (Islam & Pillay, 2020). Even though the chemical violates type Fsp3<0.25, degree of unsaturation score (INSATU), it is verifiable that it does not violate any of Lipinski and Veber's standards, satisfying the qualifications within the ideal spectrum that combines drug-likeness criteria. Using wLogP and TPSA, the BOILED-Egg server predicts these pharmacokinetics (Daina & Zoete, 2016). As a result, pharmacokinetics was evaluated using the Boiled-Egg model, which appeared in the white ellipse (HIA) justifying their high probability of being passively absorbed by the gastro-intestinal tract, with red points for **77**, **80**, and **83** (non-substrate of P-gp), and blue points for **75** and **78** (substrate of P-gp), respectively, ([Appendix 113](#)). They were predicted to be no P-gp substrate, despite their promising intestinal absorption and bioavailability, and they can't pass through the blood-brain barrier (BBB), demonstrating they are not able to cross the blood-brain barrier to the brain when it attaches to certain receptors.

Table 12. The parameters of *in silico* bioavailability prediction and physicochemical properties of compounds, **75-83** and reference drugs.

Compound	MW	iLogP	HBD (noHNH)	HBA (non)	nroth	MR	TPSA	%ABS	Lipinski #violations	Bio availability score
Lipinski*	≤500	≤5	≤5	≤10	≤10	-	-	100%		
Veber**	-	-	-	-	-	-	≤ 140			
75	453.52	3.64	0	5	6	140.68	86.68	79.09	0	0.55
76	498.51	3.31	0	7	7	149.51	132.51	63.28	0	0.55
77	469.52	3.09	1	6	6	142.71	106.91	72.11	0	0.55
78	532.41	3.23	0	5	6	148.38	86.68	79.09	1	0.55
79	577.41	2.81	0	7	7	157.21	132.51	63.28	1	0.55
80	548.41	3.54	1	6	6	150.41	106.91	72.11	1	0.55
81	469.52	2.66	1	6	6	142.71	106.91	72.11	0	0.55
82	514.51	2.06	1	8	7	151.53	152.73	56.30	1	0.17
83	485.51	2.57	2	7	6	144.73	127.14	65.13	0	0.55
Amoxicillin	365.4	1.46	4	6	6	94.59	132.96	63.12	0	0.55
Acarbose	657.66	1.16	14	18	10	145.22	311.94	1.38	3	0.17

*Reference values of Lipinski; **Reference values of Veber; (-): Not tested. MW, Molecular weight; iLogP, Lipophilicity (O/W, octanol-water partitioning coefficient); HBD, Number of hydrogen bond donors (OH and NH groups); HBA, Number of hydrogen bond acceptors (O and N atoms); nVs, Number of Lipinski rule violations; n roth, Number of Rotatable Bonds; MR, Molar refractivity; TPSA, Topological polar surface area; (Å²); %ABS, Percentage of absorption (%Abs=109-[0.345×TPSA]).

Table 13. Pharmacokinetic (ADME) properties prediction of compounds, **75-83** and reference drugs using preADMET online server.

Compound	Absorption			Distribution			Metabolism and Excretion				
	HIA (%) ^a	Caco2(n m/sec) ^b	SPLogKp (cm/hour) ^c	MDCK(nm/sec) ^d	PPB % ^e	BBB(c.b rain/c.bl ood) ^f	CYP 2C1 9 ^g	CYP2C9 ^h	CYP2D6 ⁱ	CYP3 A4 ^j	Pgp_ Inhibiti on ^k
75	99.82	15.60	-5.80	0.047	96.53	0.80	Non	Non	Non	Non	Inhibitor
76	96.14	9.39	-5.81	0.047	95.90	0.21	Non	Non	Non	Non	Non
77	98.56	4.35	-5.61	0.044	99.75	0.34	Non	Non	Non	Non	Non
78	98.43	27.34	-6.59	0.021*	93.54	1.59	Non	Non	Non	Non	Inhibitor
79	99.40	17.52	-6.60	0.018*	93.82	0.41	Non	Non	Non	Non	Non
80	98.02	18.88	-6.40	0.030*	93.30	0.63	Non	Non	Non	Non	Non
81	98.56	7.78	-5.61	0.044	94.41	0.24	Non	Non	Non	Non	Non
82	90.80	11.84	-5.62	0.044	94.60	0.12	Non	Inhibitor	Non	Non	Non
83	95.95	5.52	-5.42	0.044	97.31	0.07	Non	Inhibitor	Non	Non	Non
Amoxicillin	62.93	0.34	-0.21	0.398	23.35	0.06	Non	Non	Inhibitor	Non	Non
Acarbose	96.03	0.8882	-15.69	-	30.20	0.82	Non	Non	Non	Non	Non

^a HIA: Human intestinal absorption; ^b Caco2:Caco2 cell permeability; ^c SP, LogKp: Skin permeability; ^d MDCK: Maden-Darby canine kidney; ^e PBB: Plasma protein binding; ^f BBB level: Blood-brain barrier level; ^{g-h} CYP2D6: Cytochrome P.; ^k P-gp: glycoprotein inhibition

Table 14. *In silico* toxicity prediction of compounds, **75-83** and reference drugs using preADMET and Pro Tox-II web service.

Compound	Toxicity						Class
	AMES (Mutagenicity prediction)	Carcino_Mouse (for carcinogenicity prediction of the tested compounds)	Carcino_Rat (for carcinogenicity prediction of the tested compounds)	hERG_ inhibition (for checking the cardiac toxicity)	Cytotoxicity	^a LD ₅₀ (mg/kg)	
75	Mutagen	Negative	Positive	Low_risk	Negative	1000	4
76	Mutagen	Positive	Positive	Low_risk	Negative	1000	4
77	Mutagen	Negative	Positive	Low_risk	Negative	800	4
78	Mutagen	Negative	Positive	Low_risk	Negative	800	4
79	Mutagen	Positive	Positive	Low_risk	Negative	1000	4
80	Mutagen	Negative	Positive	Low_risk	Negative	800	4
81	Mutagen	Negative	Positive	Low_risk	Negative	800	4
82	Mutagen	Positive	Negative	Low_risk	Negative	800	4
83	Mutagen	Negative	Negative	Low_risk	Negative	1000	4
Amoxicillin	Mutagen	Positive	Negative	Low_risk	Negative	15000	4
Acarbose	Mutagen	Negative	Positive	Low_risk	Negative	540	4

^a LD₅₀: lethal dose parameter

5.8.2. *In silico* prediction of drug-likeness, and pharmacokinetic study of **92-100**

The therapeutic activity of small molecules depends on their ability to reach the target of interest at sufficient concentrations as defined by the properties of ADMET (Abishad et al., 2021). The results demonstrated that all compounds, **92-100** showed no violation of Lipinski's rule of five, these zero violations are indicative of their good drug-likeness as illustrated in [Table 15](#). Furthermore, all synthesized compounds exhibited better bioavailability scores of 0.55, suggesting that they have drug-like properties (Martin, 2005). Their HBD values (n_{OHNH}) are <3 , especially in compounds **92**, **94**, **95**, and **97** giving their higher solubility in cellular membranes with also HBA values (n_{ON}) in the range of 2-6, basically for compound **92**. The ilogP values for all compounds below are <5 , especially for compounds **96** and **98** highlighting their good lipophilic properties. The higher lipophilic nature of these compounds may protect against the harmful effects of ROS and is partly due to their smaller TPSA (Daina, Michielin, & Zoete, 2014). TPSA values ranged from 37.53 to 129.17 Å², while the consensus log Po/w was observed to range from 2.37 to 3.25. TPSA is a parameter used to predict the transport properties of drugs in passive molecular transport (Ertl et al., 2000). The tested compounds exhibited better oral absorption than amoxicillin. The compounds have molar refractive index values in the range of 117.77-135.41, suggesting their need in preclinical development and are likely to be a drug.

All compounds showed a better intestinal absorption range of 94.625-99.028 showed good intestinal absorption with HIA values close to 100% absorption, especially (**92**, **95**, and **100**). The median permeability of Caco-2 cells *in vitro* ranged from 12.19 to 55.91, and the permeability of MDCK cells *in vitro* was low to moderate, with **94**, **95**, **96**, **98**, and **100** being better than amoxicillin (0.39). This indicates that the compound exhibits good absorption properties for passing through the intestinal wall and reaching the target by a passive diffusion mechanism. Skin permeability was found to be slightly below the acceptable range of -4.99 to -5.78 cm/s this corresponds to higher absorption into human skin (Zafar et al., 2020). Predicted *in vivo* BBB indicated lower CNS uptake for all compounds, suggesting a lower ability for these compounds to cross the CNS and this indicates these compounds have less capability to cross the CNS with a total clearance value of basically, **96**, **98**, and **100** more than amoxicillin (0.06). All compounds have strong PPB%, a value greater than 90% indicates strong binding, of which

compound **93** (99,07) in particular showed strong PPB values indicative of prolonged half-lives, more than amoxicillin (23,35)(Alonso et al., 2019). In this study, compounds **92-100** exhibited no inhibitory behavior against CYP2C19 and CYP3A6 except for **92**, but they did against CYP2C9 and CYP2D4, except for **92**, **94**, and **95**. In this study, the compounds (**92-100**) exhibited non-P-gp substrate inhibition, except **98** and **99**, thus, their liver dysfunction effects are not expected upon administration (Table 16). Based on toxicity profiles assessed using the ProTox II service, active compounds were screened for carcinogenicity, mutagenicity, and hERG inhibition *in vitro* (cardiotoxicity) as shown in Table 17. All compounds showed a positive carcinogenic effect in mice and a negative carcinogenic effect in rats. All compounds were found to be non-cardiogenic, non-cytotoxic, or safer than amoxicillin, giving it an acceptable profile as a lead for the development of safe and effective drugs in the future. The predicted LD₅₀ (mg/kg) for all tested synthetic compounds is 540; therefore, they have been classified by Protox-II as grade 4 toxicity, indicating that the compounds can be harmful if ingested ($300 < LD_{50} \leq 2000$) (Drwal et al., 2014).

The bioavailability radar plots for the tested compounds, **92-100** (Appendix 114) indicated that all compounds were located within the pink region, confirming their similarity to the drug with a better bioavailability profile (Islam & Pillay, 2020). It exhibits ideal physicochemical properties that make the title compounds good candidates for oral drugs. Despite violating the type Fsp3 < 0.25 degree of unsaturation score (INSATU), the compound does not violate the criteria of Lipinski's and Veber's rules and is within the ideal spectrum combined with drug-likeness criteria. The BOILED-Egg server uses wLogP and TPSA to predict these pharmacokinetics (Daina & Zoete, 2016). Therefore, the pharmacokinetics were assessed by the boiled egg model (Appendix 114) for **92-100**, it appeared in the white ellipse (HIA) justifying their high probability to be passively absorbed by the gastro-intestinal tract, with red points **92**, **93**, **94**, **95**, **96**, **97**, and **100** (non-substrate of P- gp) and blue point for **98** and **99** (substrate of P-gp), respectively. Interestingly, they are predicted not to be P-gp substrates that provide their promising bioabsorption and bioavailability, which are unable to cross the blood-brain barrier permeability, approving their ability cannot cross the blood-brain barrier to the brain when it binds to specific receptors.

Table 15. Calculated parameters of *in silico* bioavailability (drug-likeness) prediction & physicochemical properties of the synthesized compounds, **92-100** and reference drugs.

Compound	MW	iLogP	HBD (noHNH)	HBA (noN)	nrotb	MR	TPSA	%ABS	Lipinski #violations	Synthetic Accessibility	Bio availability score
Lipinski*	≤500	≤5	≤5	≤10				100%			
Veber**	-	-	-	-	≤10		≤ 140 Å ²			-	
92	382.48	3.25	1	2	3	117.77	37.53	96.05	0	4.48	0.55
93	398.48	2.81	2	3	3	119.79	57.75	89.07	0	4.55	0.55
94	427.48	2.58	1	4	4	126.59	83.35	80.24	0	4.51	0.55
95	427.48	2.78	1	4	4	126.59	83.35	80.24	0	4.51	0.55
96	443.47	2.37	2	5	4	128.61	103.58	73.26	0	4.59	0.55
97	472.47	2.54	1	6	5	135.41	129.17	64.43	0	4.58	0.55
98	398.48	3.19	2	3	3	119.79	57.75	89.07	0	4.54	0.55
99	414.48	2.81	3	4	3	121.81	77.98	82.09	0	4.61	0.17
100	443.47	2.62	2	5	4	128.61	103.58	73.26	0	4.58	0.55
Amoxicillin	365.4	1.46	4	6	5	94.59	132.96	63.12	0	4.17	0.55
Acarbose	657.66	1.16	14	18	10	145.22	311.94	1.38	3	7.54	0.17

*Reference values of Lipinski; **Reference values of Veber; MW, Molecular weight; iLogP, Lipophilicity (O/W, octanol-water partitioning coefficient); HBD, Number of hydrogen bond donors (OH and NH groups); HBA, Number of hydrogen bond acceptors (O and N atoms); nVs, Number of Lipinski rule violations; n rotb, Number of Rotatable Bonds; MR, Molar refractivity; TPSA, Topological polar surface area; (Å²); %ABS, Percentage of absorption (%Abs=109-[0.345×TPSA]).

Table 16. Pharmacokinetic (ADME) properties prediction of compounds, **92-100** and reference drugs using preADMET online server.

Compound	Absorption			Distribution			Metabolism and Excretion				
	HIA (%) ^a	Caco2 (nm/sec) ^b	SPLogKp (cm/hour) ^c	MDCK (nm/sec) ^d	PPB % ^e	BBB (c.brain/c .blood) ^f	CYP 2C19 ^g	CYP2C 9 ^h	CYP2D 6 ⁱ	CYP3A 4 ^j	Pgp_ Inhibition ^k
92	97.153	55.911	-4.99	27.995	96.507	1.0887	Non	Non	Non	Non	Non
93	96.158	41.105	-5.34	12.477	99.071	1.9138	Non	Inhibitor	Non	Inhibitor	Non
94	97.136	27.533	-5.39	0.0691	94.092	0.0161	Non	Inhibitor	Non	Non	Non
95	97.136	20.334	-5.39	0.0572	94.100	0.0161	Non	Inhibitor	Non	Non	Non
96	96.028	12.196	-5.73	0.0561	95.193	0.0218	Non	Inhibitor	Non	Inhibitor	Non
97	96.783	16.635	-5.78	0.0436	95.188	0.0290	Non	Inhibitor	Non	Inhibitor	Non
98	96.158	41.105	-5.34	12.477	93.616	1.9138	Non	Inhibitor	Non	Inhibitor	Inhibitor
99	94.625	23.381	-5.69	4.1508	95.564	2.3180	Non	Inhibitor	Non	Inhibitor	Inhibitor
100	96.028	17.575	-5.73	0.0644	94.514	0.0219	Non	Inhibitor	Non	Inhibitor	Non
Amoxicillin	62.936	0.3483	-9.94	0.3985	23.353	0.0606	Non	Non	Inhibitor	Non	Non
Acarbose	96.03	0.8882	-15.69	-	30.20	0.8250	Non	Non	Non	Non	Non

^a HIA: Human intestinal absorption; ^b Caco2: Caco2 cell permeability; ^c SP, LogKp: Skin permeability; ^d MDCK: Maden-Darby canine kidney; ^e PBB: Plasma protein binding; ^f BBB level: Blood-brain barrier level; ^{g-i} CYP2D6: Cytochrome P450 inhibition; ^k P-gp: glycoprotein inhibition

Table 17. *In silico* pharmacodynamics (toxicity) prediction of compounds, **92-100** and reference drugs using preADMET and Pro Tox-II online servers.

Compound	<i>In silico</i> pharmacodynamics (Toxicity)						Class
	AMES (Mutagenicity prediction)	Carcino_Mouse (for carcinogenicity prediction of the tested compounds)	Carcino_Rat (for carcinogenicity prediction of the tested compounds)	hERG_ inhibition (for checking the cardiac toxicity)	Cytotoxicity	LD ₅₀ (mg/kg)	
92	Mutagen	Positive	Negative	Low_risk	Negative	540	4
93	Mutagen	Positive	Negative	Low_risk	Negative	540	4
94	Mutagen	Positive	Negative	Low_risk	Negative	540	4
95	Mutagen	Positive	Negative	Low_risk	Negative	540	4
96	Mutagen	Positive	Negative	Low_risk	Negative	540	4
97	Mutagen	Positive	Negative	Low_risk	Negative	540	4
98	Mutagen	Positive	Negative	Low_risk	Negative	540	4
99	Mutagen	Positive	Negative	Low_risk	Negative	540	4
100	Mutagen	Positive	Negative	Low_risk	Negative	540	4
Amoxicillin	Mutagen	Positive	Negative	Low_risk	Negative	540	4
Acarbose	Mutagen	Negative	Positive	Low_risk	Negative	540	4

^a LD₅₀: lethal dose parameter

5.8.3. *In silico* prediction of drug-likeness, and pharmacokinetic study of **108-116**

Oral bioavailability is accounted as a crucial characteristic for the discovery of efficacious medicinal drugs. The obtained results as illustrated in [Table 18](#), showed all compounds are in agreement with Lipinski's rule of five, confirming their easy binding to receptors. MW of all the synthesized analogs was found to be <500, for most of the derivatives indicate easy absorption, diffusion, and transportation. Molecular aquaphobicity (iLog P) values of all the synthesized compounds were found to be <5, demonstrating good membrane permeability of the compounds. The number of HBD (n_{HBNH}) and the number of HBA (n_{ON}) values in all the synthesized analogs were determined to be <5, which is in the range of 1-2, and <10, which is in the range of 3-7, respectively. Topological polar surface area (TPSA) is a very useful parameter for the prediction of the transport of drug molecules. All the tested compounds had TPSA values <140 Å, in the range of 57.09–121.29 Å, with only one violation of **112**, suggesting promising oral availability and were thus, they are expected to have good solubility, capacity for penetrating cell membranes, and intestinal absorption. It can be noticed that all the titled compounds displayed %Abs ranging from 58.16- 81.43 % indicating significant oral bioavailability and the numbers of rotational bonds are less than 10, confirming their good flexibility. They exhibited good lipophilicity (a major factor in the quality of drug candidate) given by the values of consensus Log $P_{\text{o/w}}$ in the range of 2.04-4.23 with better bioavailability score (0.55) meaning acceptable drug-likeness criteria.

The ilogP values for all compounds are <5, notably indicating the compounds have good lipophilicity characteristics which may protect them against ROS degradation. The lower lipophilicity of these compounds **108**, **109**, **111**, **112**, **114**, and **116** may protect against ROS damage and is due in part to their smaller polar surface area which is another useful descriptor of the oral bioavailability and drug transport properties. The value of mlogP (octanol-water partition coefficient) of all compounds are less than 5.0 (2.04-4.23) showing their high affinity and excellent permeability across the lipid bilayers of biological membranes. The results revealed that compounds, **108**, **111**, and **116** have the least lipophilicity (mlogP) and are biologically more active, but the remaining compounds (greater lipophilicity) are less active against the tested bacteria. Our results exhibited that there is a clear relationship between

lipophilicity and antibacterial activity (Brown et al., 2021). MR values are in the range of 93.38-149.63 for tested compounds suggesting their need in preclinical development and likely to be a drug. In light of the above results, we achieved that all tested compounds satisfy the “rule of five” and meet all criteria for good permeability and bioavailability.

AdmetSAR online database was utilized to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the target compounds as illustrated in [Table 19](#) and [Appendix 115](#). *In silico* pharmacokinetic characteristics, results revealed that most active compounds had superior intestinal absorption to amoxicillin with HIA and PPB values near (1) 100%, indicating that the synthesized compounds are showing good absorption and brain penetration properties to cross the gut wall by passive diffusion mechanism to reach the target but are impermeable to Caco-2. All of the substances have a high PPB%, with values of more than 75%, indicating prolonged half-lives more than amoxicillin (31.05). The results revealed that compounds **108** (0.888), **111** (0.946), and **116** (0.757) had high PPB values, indicating longer half-lives than amoxicillin (0.348)(Alonso et al., 2019). Skin permeability (SP, Log_{Kp}) was found to be somewhat below the permitted range of -5.25 to -6.69 cm/sec and a substance with a low negative log_{Kp} value has higher absorption into human skin. The compounds' metabolic properties were predicted by the behavior of cytochrome P450 and their isoform enzymes. In this investigation, almost all compounds, showed inhibitory activity against CYP3A4, CYP2C9, CYP2C19, but they did not show inhibitory behavior towards CYP2D6. In our study, none of the synthesized compounds inhibited P-gp substrates, except **108**, and **109**. Computationally toxicity risks such as AMES Mutagenicity, hepatotoxicity, carcinogenicity, and skin sensitization were investigated. The *in silico* pharmacodynamics studies indicated that compounds **108**, **111**, and **116** were predicted to be non-mutagenic, non-tumorigenic, non-hepatotoxic, non-irritant, AMES nontoxic, and without any skin-sensitive effects, which gives them the property to be acceptable lead-like potential for future development of safe and efficient drugs. For further investigation of the *in vivo* antibacterial activity, the computed LD_{50} in rat from the safer category III acute oral toxicity model seems to be sufficiently safe (2.29–2.41 mol/kg) as shown in [Table 20](#).

The bioavailability radar plot indicates that **108**, **111**, and **116** fall entirely in the pink area confirming their potent drug-likeness and their better bioavailability profiles (Islam & Pillay, 2020). Even though the chemical violates type Fsp3<0.25, and degree of unsaturation score (INSATU), it is verifiable that it does not violate any of Lipinski and Veber's parameters, satisfying the qualifications within the ideal spectrum that combines drug-likeness criteria. Using wLogP and TPSA, the BOILED-Egg server predicts these pharmacokinetics (Daina & Zoete, 2016). As a result, pharmacokinetics were evaluated using the Boiled-Egg model, compounds **108**, **109**, **111**, and **115** appeared in the white ellipse (HIA) justifying their high probability of being passively absorbed by the gastro-intestinal tract and bioavailability, whereas, compounds **110**, **112-114** and **116** which appeared in the yellow ellipse (BBB) justifying their high probability of being passed through the blood-brain barrier, demonstrating they can cross the blood-brain barrier to the brain when it attaches to certain receptors with red points (non-substrate of P-gp), respectively ([Appendix 115](#)). All the above *in silico* ADME parameters of the most active compounds (**108**, **111**, and **116**) may help in the further development of new drug candidates with good and acceptable oral bioavailability.

Table 18. Bioavailability, and drug-likeness assessment of synthesized compounds, **108-116** using SwissADME online server.

Compound	Bioavailability and Drug-likeness											
	MW	iLogP	HBD (noHNH)	HBA (non)	nroth	MR	MlogP	TPSA	%Abs	Lipinski #violations	Synthetic Accessibil ity	Bio availability score
Lipinski*	≤500	≤5	≤5	≤10					100%			
Veber**	-	-	-	-	≤10			≤ 140 Å ²			-	
108	337.42	2.84	2	3	5	109.78	2.37	57.09	80.31	0	3.77	0.55
109	294.35	2.98	2	3	4	95.57	2.47	53.85	81.43	0	3.61	0.55
110	363.41	3.29	2	5	5	107.58	1.89	78.71	72.85	0	3.18	0.55
111	320.34	2.95	2	5	4	93.38	1.99	75.47	73.97	0	2.97	0.55
112	499.54	3.41	2	6	6	144.24	2.47	141.04	60.35	0	4.88	0.55
113	497.61	4.23	2	4	6	149.63	3.23	98.46	66.04	0	5.09	0.55
114	454.54	4.01	2	4	5	135.42	3.38	95.22	67.15	0	4.84	0.55
115	393.39	2.04	1	6	8	109.55	0.81	110.17	62.00	0	3.35	0.55
116	391.46	2.85	1	4	8	114.93	1.62	67.59	76.68	0	3.44	0.55
Amoxicillin	365.4	1.46	4	6	6	94.59	0.23	132.96	63.12	0	4.17	0.55
Acarbose	657.66	1.16	14	18	10	145.22	-6.22	311.94	1.38	3	7.54	0.17

*Reference values of Lipinski; **Reference values of Veber; MW, Molecular weight; iLogP, Lipophilicity (O/W, octanol-water partitioning coefficient); HBD, Number of hydrogen bond donors(OH and NH groups); HBA, Number of hydrogen bond acceptors(O and N atoms); nVs, Number of Lipinski rule violations; nroth, Number of Rotatable Bonds; MR, Molar refractivity; TPSA, Topological polar surface area; (Å²); %ABS, Percentage of absorption (%Abs=109-[0.345×TPSA]).

Table 19. Pharmacokinetic (ADME) properties prediction of synthesized compounds **108-116** using AdmetSAR online platform.

Compound	<i>In silico</i> pharmacokinetics									
	HIA (%) ^a	Caco2 (nm/sec) ^b	SPLogKp (cm/hour) ^c	PPB % ^e	BBB (c.brain/c.blood) ^f	CYP3A4 inhibition ^g	CYP2C9 inhibition ^h	CYP2C19 inhibition ⁱ	CYP2D6 inhibition ^j	Pgp_Inhibition ^k
108	+ 0.9823	-0.5430	-5.72	0.888	+ 0.7500	+0.8430	+0.5591	+ 0.7526	- 0.6935	-0.5156
109	+0.9955	-0.6153	-5.55	0.747	+0.7750	+0.7003	+0.6373	+0.7714	-0.6785	-0.7846
110	+0.9589	-0.5810	-5.75	1.011	+0.7000	+0.5879	-0.6971	+0.5235	-0.8204	+0.6709
111	+0.9906	-0.7344	-5.58	0.946	+0.6750	-0.6245	-0.5691	+0.5531	-0.8888	-0.6383
112	+0.9032	-0.7649	-5.64	0.994	-0.5000	+0.8888	+0.7218	+0.7388	-0.7795	+0.6958
113	+0.9628	-0.7247	-5.42	0.992	+0.6000	+0.7544	+0.7852	+0.8738	-0.6652	+0.8564
114	+0.9802	-0.7183	-5.25	1.067	+0.5750	+0.7941	+0.8129	+0.9119	-0.5571	+0.7374
115	+0.9349	-0.6547	-6.69	0.75	+0.7000	+0.7868	+0.5325	-0.5487	-0.8399	-0.5705
116	+0.9816	-0.5967	-6.47	0.757	+0.7000	+0.7895	-0.6185	+0.7159	-0.5568	+0.6521
Amoxicillin	-0.8790	-0.9249	-0.21	0.348	-1.0000	-0.8309	-0.9070	-0.9150	-0.9231	-0.9167
Acarbose	-0.9603	-0.8882	-15.69	0.302	-0.8250	-0.9864	-0.8896	-0.8707	-0.9030	-0.5735

^a HIA: Human intestinal absorption; ^b Caco2: Caco2 cell permeability; ^c SP, LogKp: Skin permeability; ^d; ^e PBB: Plasma protein binding; ^f BBB level: Blood-brain barrier level; ^{g,j} CYP: Cytochrome P450 inhibition; ^k P-gp: glycoprotein inhibition.

Table 20. *In silico* toxicity prediction of compounds, **108-116** and amoxicillin using AdmetSAR and Pro Tox-II online servers.

Compound	<i>In silico</i> pharmacodynamics (toxicity)							
	Mutagenic	Tumorigenic	Irritant	Carcinogenicity	Ames toxicity	Hepatotoxicity	Skin sensitization	Rat acute toxicity (LD50; mol/kg)
108	Active	Active	-0.9185	-0.7628	-0.680	-0.5040	-0.8228	III 0.5884
109	Active	Active	-0.8995	-0.7828	-0.770	+0.5072	-0.8100	III 0.5871
110	Inactive	Inactive	-0.6400	-0.7928	-0.570	+0.5264	-0.8903	III 0.6840
111	Active	Inactive	+0.636	-0.8128	-0.600	+0.5251	-0.8883	III 0.5457
112	Active	Active	-0.8660	-0.7012	+0.750	+0.5625	-0.8225	III 0.5803
113	Inactive	Inactive	-0.8495	-0.9000	+0.510	-.6375	-0.8314	III 0.6147
114	Inactive	Inactive	-0.7894	-0.9300	-0.550	-0.5625	-0.8340	III 0.5783
115	Inactive	Inactive	-0.9478	-0.6607	+0.580	+0.6625	-0.8544	III 0.6763
116	Inactive	Inactive	-0.9497	-0.7800	-0.740	-0.6125	-0.8819	III 0.7078
Amoxicillin	Active	Inactive	-0.9872	+0.6977	-0.960	+0.9875	-0.7693	IV 0.5992
Acarbose	Active	Active	-0.9391	-0.9800	+0.580	+0.7573	-0.8755	IV0.5397

^a LD₅₀: Lethal dose parameter

5.9. *In silico* molecular docking simulation

Computational simulation approaches are gaining popularity because the experimental determination of complex structures is rather difficult and expensive (Lin, Li, & Lin, 2020). Over the years, the speed and accuracy of computational docking methods have improved, and these methods now play a significant roles in structure-based drug design (Agnihotry, Pathak, Srivastav, Shukla, & Gautam, 2020). Molecular docking was considered to be a powerful computational technique in structure-based drug development that allowed the prediction of protein-ligand binding interactions of new molecules at the binding sites of suitable targets. Studies of docking simulations were carried out to identify the binding pattern of the synthesized compounds (**75-83**, **92-100**, and **108-116**) to selected protein target active sites (i.e. DNA gyrase B, PqsA, Pyruvate Kinase, LuxS, Penicillin binding proteins (PBPs) and β -lactamases, Human peroxiredoxin 5 and α - amylase) (Hillman, 2022). The binding affinity, hydrogen bond, and amino acid residual interactions (hydrophobic/ π -cation/ π -anion/ π -alkyl, and Van der Waals interactions) of ligands (**75-83**, **92-100**, and **108-116**) and standards are presented ([Table 21-41](#)). The majority of the hybrids investigated showed the best docking scores and good interactions (2D and 3D) with different amino acid residues in the ligand binding sites of protein targets([Appendix 116, a-j](#) to [Appendix 137, a-k](#)). Ribbon model showing the structure of the target protein binding pocket with hydrogen bonds between compounds and amino acids indicated by green dotted lines and hydrophobic interactions indicated by pink lines.

5.9.1. *In silico* molecular docking simulation study of ompounds, **75-83**

5.9.1.1. Molecular docking studies of synthesized compounds, **75-83** against DNA Gyrase.

DNA gyrase B B (PDB ID: 6F86) is a member of bacterial type IIA topoisomerase enzymes that control the topology of DNA during processes of transcription, replication, and recombination by introducing transient breaks (negative supercoils) to both DNA strands, which provide the energy necessary for the catalytic function of the enzyme through ATP hydrolysis (T. Khan et al., 2018). Inhibiting the ATPase activity of DNA Gyrase B blocks the introduction of negative supercoils in DNA and traps the chromosome in a positive impact on cell physiology and

division (Metwally, Abdallah, & Mohsen, 2020). The synthesized compounds (**75-83**) were docked towards the bacterial DNA gyrase B receptor to determine their binding affinity and the results were compared to amoxicillin ([Appendix 116, a-j](#), and [Table 21](#))(Durcik et al., 2020). The minimum binding energies of the synthesized compounds (**75-83**) ranged from -7.4 to -8.5 kcal/mol, showing that thiazolyl-pyrazoline Schiff base hybrids have a thermodynamically favorable inhibitory action on DNA gyrase B. Compounds **78** (-8.0 Kcal/mol), **79** (-7.8 Kcal/mol), **80** (-7.9 Kcal/mol), **81** (-8.5 Kcal/mol), and **83** (-7.9 Kcal/mol) have high binding interactions with DNA Gyrase B, respectively ([Figures 22](#), and [Appendix 139, j](#)) compared to amoxicillin (-7.1 Kcal/mol). The synthesized compounds displayed similar residual interactions with amino acid residues of Ile-78 and Ile-94, as well as hydrogen bonding interaction patterns with Val-120, Asn-46, and Ser-121 when compared to standard amoxicillin. The docking results (high negative binding affinity values) are in good agreement with the *in vitro* antibacterial activity results of compounds **78**, **81**, and **83** against Gram-negative bacteria namely *E. coli*.

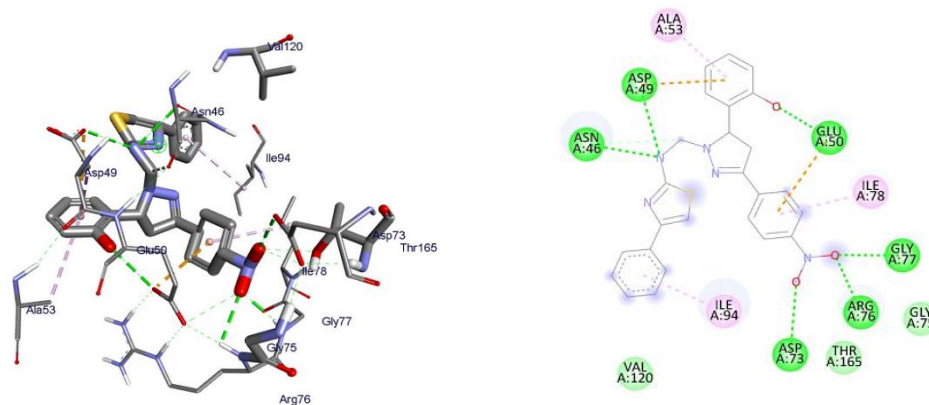


Figure 22. 3D (right) and 2D (left) representations of the binding interactions of **81** against DNA Gyrase B.

5.9.1.2. Molecular docking studies of synthesized compounds, **75-83** against the N-terminal domain of PqsA.

PqsA protein (PDB ID: 5OE3) is responsible for priming anthranilate for entry in to the 2-alkyl-4(1*H*)-quinolones (*Pseudomonas* quinolone signal, PQS) biosynthesis pathway and that this enzyme may serve as a useful *in vitro* indicator for potential agents to disrupt quinolone

signaling in *P. aeruginosa*. PqsA catalyzes the formation of anthraniloyl-CoA from anthranilate, ATP and CoA (Coleman et al., 2008). Inhibition of the PQS quorum sensing system is an attractive target for the development of alternative therapies against multidrug-resistant *P. aeruginosa*. Anthraniloyl-AMP mimics are competitive antagonists of the enzyme PqsA, and block the production (secretion) of virulence factors PQS and biofilm formation ability of *P. aeruginosa* are significantly reduced without affecting bacterial growth (Witzgall, Ewert, & Blankenfeldt, 2017). The binding affinities of synthesized compounds (**75-83**) were evaluated towards the N-terminal domain of PqsA in the 6-fluoroanthranilamidoyl-AMP complex (Ji et al., 2019) and compared with amoxicillin ([Appendix 117, a-j](#) and [Table 22](#)). The synthesized compounds (**75-83**) have minimum binding energy in the binding pocket of the N-terminal domain of PqsA ranging from -7.8 to -10.7 kcal/mol. Compounds **76** (-9.5 Kcal/mol), **78** (-9.6 Kcal/mol), **79** (-9.5 Kcal/mol), **81** (-10.7 Kcal/mol), **82** (-9.6 Kcal/mol) and **83** (-9.9 Kcal/mol) have large binding interactions. Higher binding affinity was observed for compounds **81** and **83**, with the binding affinity of -10.7 and -9.9 Kcal/mol respectively, against PqsA compared to amoxicillin (-7.3 Kcal/mol) suggesting the compounds are promising antibacterial agents against *P. aeruginosa* ([Figures 23, 25](#), and [Appendix 117, a-j](#)) in good agreement with the *in vitro* antibacterial activity against Gram-negative *P. aeruginosa* observed. The synthesized compounds revealed similar amino acid residue interactions with Thr-232, Arg-128, Asp-231, Asp-132, Ala-124, Ala-126, Phe-209, Gly-302, and H-bonding interactions profiles with Asn-61, Ser-65, and Pro-129 as compared to the amoxicillin drug.

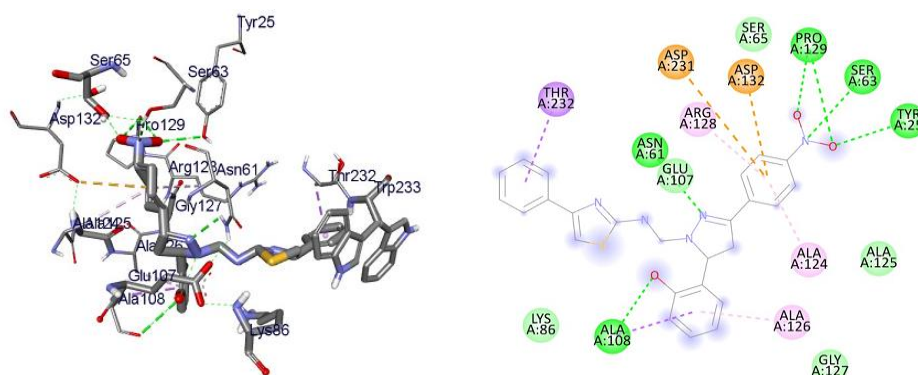


Figure 23. 3D (right) and 2D (left) representations of the binding interactions of **81** against the N terminal domain of PqsA.

5.9.1.3. Molecular docking studies of synthesized compounds, **75-83** against *S. aureus* Pyruvate Kinase.

Pyruvate Kinase (PK, PDB ID: 3T07) enzyme catalyzes the rate-limiting, final step in glycolysis (carbohydrate metabolism) involving the irreversible conversion of phosphoenolpyruvate (PEP) into pyruvate with the subsequent phosphorylation of ADP into ATP. Both the products and substrates of PK are involved in a number of additional biological pathways, therefore providing a critical intervention point to disrupt whole-cell bacterial metabolism (Veale et al., 2015). Its inhibition would lead to reduced ATP levels, causing bacterial metabolism to be severely disrupted, leading to a lack of bacterial growth (N. S. Kumar et al., 2014). The synthesized compounds (**75-83**) were subjected to a molecular docking analysis to determine their binding affinity towards Pyruvate Kinase in a complex containing the natural bis-indole alkaloid and the results were compared to amoxicillin ([Appendix 118, a-j](#), and [Table 23](#))(El Sayed et al., 2020; N. S. Kumar et al., 2014).

It was found that the synthesized compounds to show minimum binding energy values in the range of -4.8 to -6.4 kcal /mol. Compounds **77** (-5.5 Kcal/mol), **78** (-5.0 Kcal/mol), **79** (-5.5 Kcal/mol), **80** (-5.2 Kcal/mol), **81** (-5.9 Kcal/mol), **82** (-6.4 Kcal/mol) and **83** (-5.0 Kcal/mol) have better binding interactions, with the best docking results obtained for compounds **80**, **81**, and **82** with the binding affinities of -5.2, -5.9, and -6.4 Kcal/mol, respectively, against Pyruvate Kinase compared to amoxicillin (-4.7 Kcal/mol) ([Figures 24](#), and [Appendix 118, j](#)). This suggests that these compounds are effective antibacterial agents against *S. aureus*. Amino acid residue interactions with Pro-402, Lys-425, Leu-355, Ile359, and H-bonding interactions patterns with Ala-358, Thr-464, Gly-462, and Ser-383 were similar in the synthesized compounds. The *in vitro* biological results of **80** against Gram-positive bacteria of *S. aureus* is in good agreement with molecular docking results to some extent.

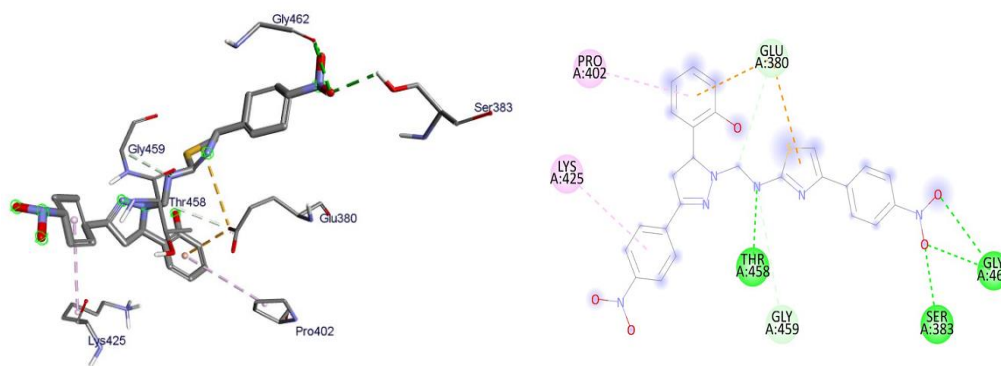


Figure 24. 3D (right) and 2D (left) representations of the binding interactions of **82** against *S. aureus* Pyruvate Kinase.

5.9.1.4. Molecular docking studies of synthesized compounds, **75-83** against LuxS of *S. pyogenes*.

LuxS protein (PDB ID: P0C0C7) is required for the biosynthesis of the type 2 autoinducer, AI-2, which is involved in quorum sensing in a wide range of bacterial species including *S. pyogenes* (Zhou, Zhang, Ge, Zhu, & Pan, 2020). Bacteria sense the density of the surrounding population via AI-2 molecules, and this regulates gene expression and biofilm formation (Y. Wang, Liu, Grenier, & Yi, 2019). The binding affinities of the synthesized compounds (**75-83**) were examined against LuxS and compared to amoxicillin (Appendix 119, a-j, and Table 24). The synthesized compounds (**75-83**) had minimal binding energies ranging from -3.7 to -5.1 kcal/mol. The binding affinity of **80** against LuxS was nearly identical (-5.1 Kcal/mol) to that of amoxicillin (-5.5 Kcal/mol), indicating that these compound is promising antibacterial agents against *S. pyogenes* (Figures 25, and Appendix 119, a-j), in good agreement with the *in vitro* biological results of observed by **80** against Gram-positive bacteria of *S. pyogenes*. The synthesized compounds have similar amino acid residue interactions with Ala-64, His-61, Cys-127, Pro-120, Pro-79, Leu-56, and H-bonding interactions profiles with His-57, Gly-128, and His-61 when compared to a standard antibiotic drug (amoxicillin).

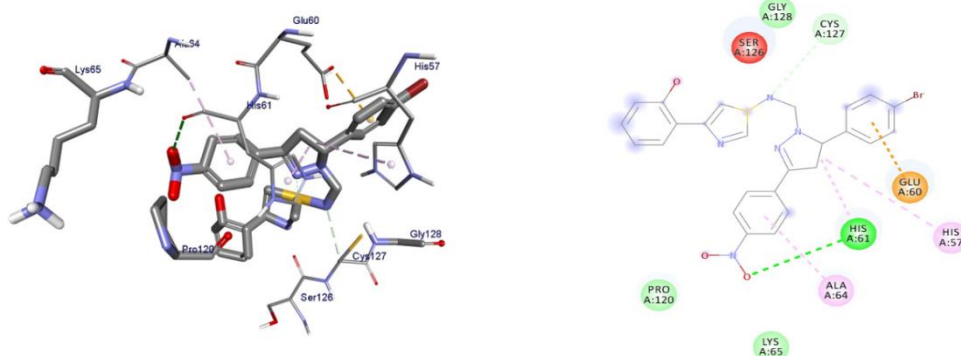


Figure 25. 3D (right) and 2D (left) representations of the binding interactions of **80** against LuxS of *S. pyogenes*.

5.9.1.5. Molecular docking studies of synthesized compounds, **80**, **81**, and **83** against Penicillin binding proteins (PBPs)

Penicillin binding proteins (PBPs) remain attractive targets for developing new antibiotic agents due to, they are involved in the end stages of the synthesis of bacterial cell wall biosynthesis of peptidoglycan (Nikolaidis, Favini-Stabile, & Dessen, 2014). The inhibition of PBPs leads to irregularity in the form of bacterial cell wall structure such as elongation, lesions, the loss of permeability, and cell lysis (Y. Liu & Breukink, 2016). The β -lactam antibiotics inhibit both transpeptidase and DD-carboxypeptidase activities by acylating the active-site serine of PBPs. The synthesized compounds, **80**, **81**, and **83** were found to have minimum binding energies of -7.4 to -7.6 kcal/mol within the binding pocket of the Penicillin binding proteins (PBPs), as shown in [Table 25](#) and [Appendix 120, a-d](#). The highest results was attained with compound **83** (-7.6 kcal/mol) compared to reference drug amoxicillin (-7.2 kcal/mol), suggesting that these compounds are promising antibacterial agents against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* ([Figure 26](#)). The results of *in silico* docking are in good correlation with the results of bioactivity against Penicillin binding proteins (PBPs) producing bacterial strains. Compound **83** was involved in hydrogen bonding of Asn-104 with –OH groups of chalcone and Ser-70, with thiazole. Hydrophobic/ π -cation interactions were observed for Ser-70, Tyr-105, Asn-132, Arg-274, and Pro-167 aromatic rings of chalcone, thiazole –OH and Schiff base moiety are responsible for the activation of the β -lactam ring, this provides an anionic site (hydrophobicity),

which appears to be necessary for interacting with the target enzymes, and encourages the acylation of the PBPs' active site serine.

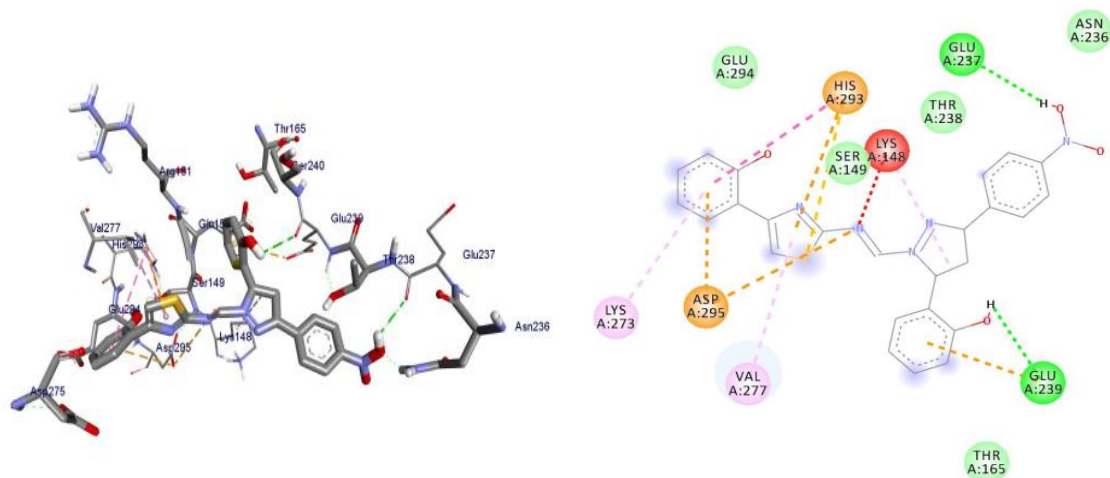


Figure 26. 3D (right) and 2D (left) representations of the binding interactions of **83** against PBPs.

5.9.1.6. Molecular docking studies of synthesized compounds, **80**, **81**, and **83** against β -lactamases.

The production of β -lactamases is the major mechanism of bacterial resistance to β -lactam antibiotics (penicillins, cephalosporins and carbapenems) and have been used effectively over several decades against different types of bacterial infections (Bush & Bradford, 2016). This irreversible inhibition of the PBPs prevents the final cross linking (transpeptidation) of the nascent peptidoglycan layer, disrupting cell wall synthesis (Sauvage, Kerff, Terrak, Ayala, & Charlier, 2008). In the presence of these antibiotics, the PBPs form a lethal covalent penicilloyl-enzyme complex that blocks the normal transpeptidation reaction; this finally results in bacterial death. The synthesized compounds, were found to have minimum binding energies of -7.9 to -8.3 kcal/mol within the binding pocket of the β -lactamases protein, as shown in [Table 25](#) and [Appendix 121, a-d](#). The best highest results was obtained with compound **80** (-8.3 kcal/mol) compared to reference drug amoxicillin (-7.9 kcal/mol), demonstrated that these compound is promising antibacterial agents against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* ([Figure 27](#)). The results of *in silico* docking are in good correlation with the results of bioactivity against β -lactamases producing bacterial strains. Compound **80** was involved in hydrogen bonding with

Glu-237, Asp-295 at thiazole of –OH. Hydrophobic/ π -cation interactions were observed for Lys-148, His-293, Asp-295, Arg-241, Arg-151, and Val-277.

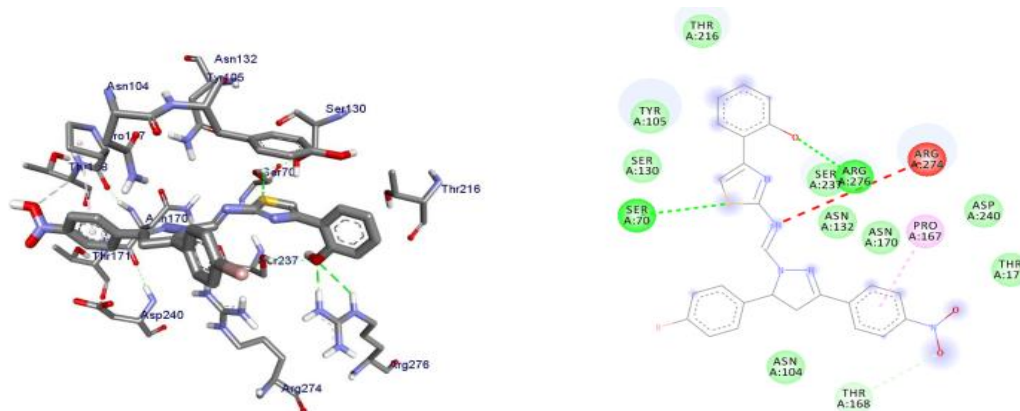


Figure 27. 3D (right) and 2D (left) representations of the binding interactions of **80** against β -lactamases.

5.9.1.7. Molecular docking studies of synthesized compounds, **75-83** against Human peroxiredoxin 5.

Peroxiredoxin 5 (PRDX5, PDB ID: 1HD2) is a novel thioredoxin peroxidase recently identified in mammals, participating directly in eliminating hydrogen peroxide and neutralizing other reactive oxygen species (Declercq et al., 2001; Yuan et al., 2004). Functionally, PRDX5 has been implicated in antioxidant protective mechanisms as well as in signal transduction in cells (Plaisant, Clippe, Vander Stricht, Knoops, & Gressens, 2003). Target synthetic compounds (**75-83**) were docked in to the binding site of the human protein target peroxidase 5 receptor and compared to ascorbic acid ([Appendix 122, a-j](#), and [Table 26](#))(Radyuk et al., 2009). The synthesized compounds were found to show minimum binding energies ranging from (-6.7 to -7.8 kcal/mol) of which compounds **76** (-7.4 Kcal/mol), **77** (-7.4 Kcal/mol), **78** (-7.2 Kcal/mol), **80** (-7.8 Kcal/mol), **81** (-7.5 Kcal/mol) and **83** (-7.3 Kcal/mol) have high binding interactions. A docking score observed for compound **80** (-7.8 Kcal/mol) higher than ascorbic acid (-5.2 Kcal/mol) suggesting that the compound to have a promising antioxidant activities ([Figure 28](#), and [Appendix 122, a-j](#)), in good agreement with the *in vitro* antioxidant result displayed by compound **80**.

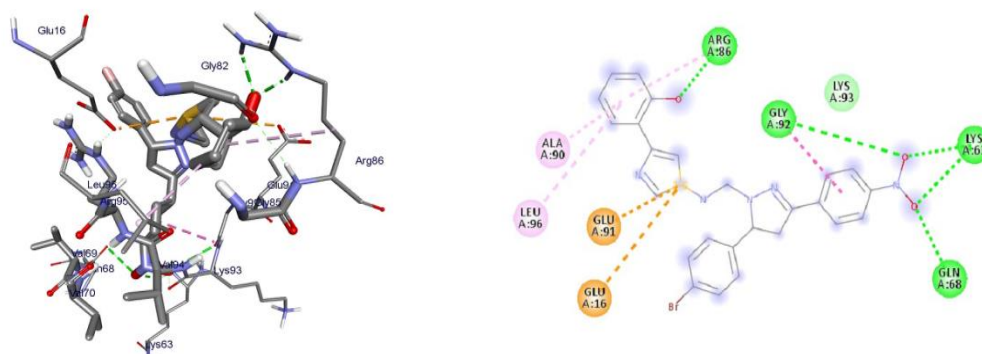


Figure 28. 3D (right) and 2D (left) representations of the binding interactions of **80** against Human peroxiredoxin 5.

5.9.1.8. Molecular docking studies of synthesized compounds **80**, **81**, **82**, and **83** against α -amylase enzyme.

Alpha-amylase (PDB ID: 4W93) is a metalloenzyme containing Ca^{2+} ions in its active site and catalyzed the hydrolysis of starch into simple sugars. This enzyme fascinated extensive attention due to its capability of hydrolyzing the α -1,4-glycosidic connection of starch (Chortani et al., 2021). Inhibition of this enzymes leads to minimization of absorption of carbohydrates from small intestine, can control postprandial hyperglycemic and reduce the risk of developing diabetics (Hossain et al., 2020). It was found that the synthesized compounds have low binding energy values from -8.4 to -9.6 kcal/mol in the binding pocket of the α -amylase enzyme, with the best result achieved for compounds **80**, **81**, **82**, and **83** with -8.6, -9.5, -9.6 and -8.4 kcal/mol, respectively as compared to acarbose (-7.5 kcal/mol), signifying that the compounds are promising antidiabetic agents against the α -amylase enzyme, as depicted in [Appendix 137, a-d](#) and [k](#) and [Table 27](#). Thus, the results of molecular docking are in good correlation with the results of **81**, and **82** *in vitro* α -amylase enzyme inhibition activities ([Figures 29](#) and [30](#) and [Appendix 137, a-d](#)). Ile-235, Asp-197, Lys-200, Glu-233, Arg-195, Gln-63, Ile-235 was involved in hydrogen bonding $-\text{NO}_2$ group of with *N*-pyrazoline moiety, respectively. The oxygen lone pair is strategically positioned within the *N*-pyrazoline conjugated system to delocalize and increase electron density, which may account for the remarkable potency of these molecules. The delocalization is better able to stabilize (+ve)-charges over the benzene, which in turn improves the inhibitory action of the corresponding enzyme (Rahim et al., 2019). Hydrophobic/ π -cation

Figure 29. 3D (right) and 2D (left) representations of the binding interactions of **81** against α -amylase enzyme

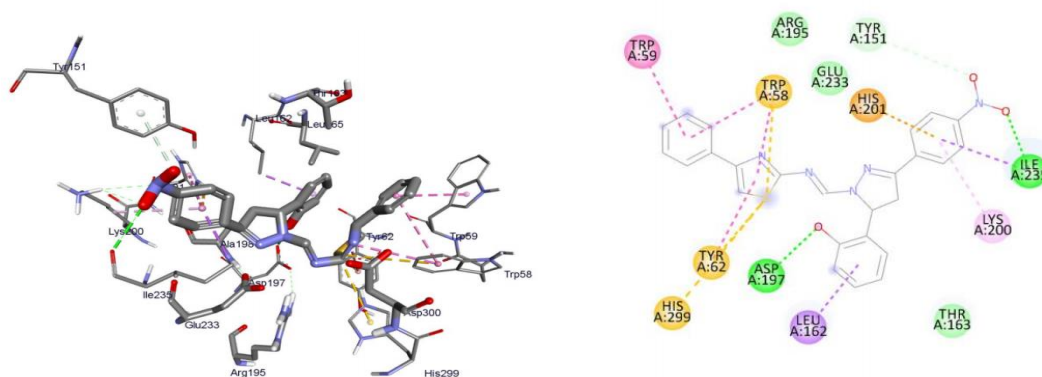


Figure 29. 3D (right) and 2D (left) representations of the binding interactions of **81** against α -amylase enzyme

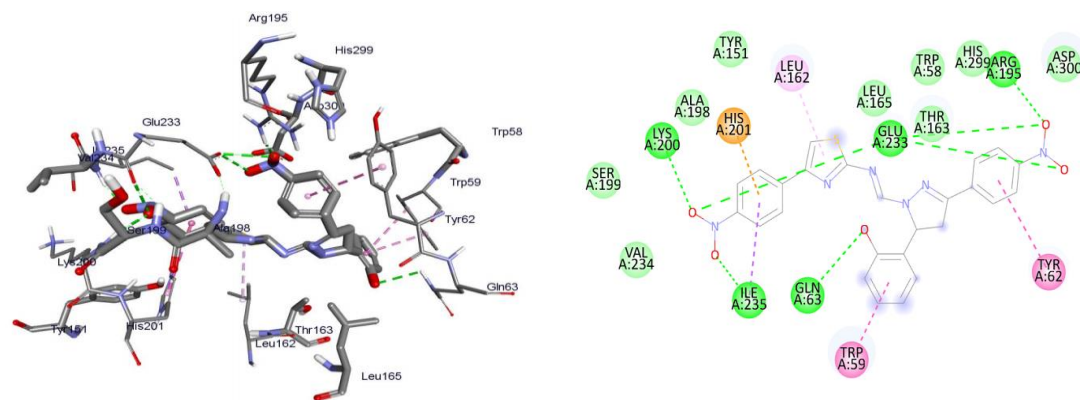


Figure 30. 3D (right) and 2D (left) representations of the binding interactions of **82** against α -amylase enzyme

Table 21. Molecular docking scores and residual amino acid interactions of synthesized compounds, **75-83** against *E. coli* Gyrase B. (PDB ID: 6F86).

Synthesized compounds (Ligands)	Docking scores/Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
75	-7.6	Ile-94, Val-97, Ser-121, Val-120	Pro-79, Ile-78	Thr-165, Asp-73, Gly-119, Leu-98
76	-7.9	Arg-76, Gly-77, Asp-73, Asn-46, Gly-117, Val-118, Leu-98	Glu-50, Asp-49, Ile-94, Ile-78	--
77	-7.8	Glu-50, Arg-76, Asp-73	Ala-53, Ile-94, Asn-46, Ile-78, Asp-49	Thr-165, Gly-75, Gly-77
78	-8.0	Val-120, Ser-121	Ile-78, Pro-79, Ile-94	Val-97, Gly-119, Leu-98, Asp-73, Thr-165, Gly-77
79	-7.8	Val-120, Ser-121	Ile-78, Pro-79, Ile-94	Asp-73, Thr-165, Asn-46, Gly-119, Val-97, Leu-98, Gly-77, Asp-49
80	-7.9	Asp-73, Arg-76, Glu-50	Asn-46, Asp-49, Ala-53, Ile-94, Ile-78	Thr-165, Gly-77, Gly-75
81	-8.5	Asp-49, Asn-46, Glu-50, Gly-77, Arg-76, Asp-73	Ala-53, Ile-78, Ile-94	Gly-75, Thr-165, Val-120
82	-7.4	Glu-50, Arg-76, Gly-77, Asp-73	Ile-94, Ala-90, Val-93, Pro-79, Ile-78	Arg-136, Thr-165
83	-7.9	Glu-50, Arg-76, Asp-73, Asn-46	Ile-94, Asp-49, Ala-53, Ile-78, Gly-77	Thr-165, Gly-75
Amoxicillin	-7.1	Asn-46, Leu-98, Val-120, Ser-121	Ile-78	Asp-73, Ile-94, Val-97, Gly-119

Table 22. Molecular docking scores and residual amino acid interactions of synthesized compounds, **75-83** against the N-terminal domain of PqsA (PDB ID: 5OE3).

Synthesized compounds (Ligands)	Docking scores \Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
75	-7.8	Lys-206, Ile-204	Ile-257, Val-254, Pro-234, Pro-205, Phe-208, Trp-233	Phe-209
76	-9.5	Ser-63, Asn-61, Pro-85, Ser-87	Ala-108, Glu-107, Ala-110, Asp-231, Ala-124, Arg-128, Ala-126	Gly-127, Glu-89, Arg-106, Lys-86, Pro-111, Phe-134, Ser-65, Leu-66, Leu-60, Ala-125
77	-8.0	Gly-169, Arg-88	Trp-233, Ala-91, Pro-171	Phe-208, Phe-209, Tyr-163, Ile-95, Asp-94
78	-9.6	Asn-61, Ser-65	Thr-232, Ala-108, Ala-126, Ala-124, Arg-128, Leu-60	Lys-86, Gly-127, Asp-231, Glu-107, Phe-134, Leu-66, Ser-63
79	-9.5	Asn-61, Ser-65	Thr-232, Ala-108, Ala-126, Ala-124, Arg-128, Leu-60	Lys-86, Gly-127, Asp-231, Glu-107, Phe-134, Leu-66, Ser-63
80	-8.1	Glu-89, Arg-88	Trp-233, Lys-86, Ala-91	Ser-87
81	-10.7	Ala-108, Tyr-25, Ser-63, Pro-129, Asn-61	Thr-232, Arg-128, Asp-231, Asp-132, Ala-124, Ala-126	Glu-107, Ser-65, Ala-125, Gly-127, Lys-86
82	-9.6	Pro-129, Tyr-25, Ser-63, Asp-132, Asn-61, Lys-86, Arg-106, Pro-85, Ser-87	Glu-107, Ala-110, Ala-124, Arg-128, Ala-108, Ala-126	Gly-27, Ala-125, Ser-65, Asp-231, Glu-89
83	-9.9	Asp-299, Thr-323	Ile-301, Pro-281, Ser-280, Val-254, Phe-209, Gly-279, Ala-303	Gly-300, His-394, Arg-397, Thr-380, Tyr-378, Glu-305, Thr-304
Amoxicillin	-7.3	Arg-372, Tyr-378, Thr-380, Arg-397	Phe-209, Gly-302	Gly-279, Ala-303, Glu-305

Table 23. Molecular docking studies of synthesized compounds, **75-83** against *S. aureus* Pyruvate Kinase (PDB ID: 3T07).

Synthesized compounds (Ligands)	Docking scores /Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
75	-5.5	Ala-358, Asn-465	Met-466, Met-467, Ile-359	Ser-362, Thr-427, Val-456
76	-4.8	Asn-369	His-365, Ile-361, Ala-358	Ser-362
77	-5.5	Thr-461, Gly-459, Lys-390, Asn-357	--	Th-463, Ser-381, Thr-458, Gly-462, Ser-383, Val-356
78	-5.0	Asn-465, Ala- 358, Ser-362	Met-466, Met-467, Ile-359	Thr-427, Val-456
79	-5.5	Lys-390, Leu-269, Thr-387	Arg-386, Ser-381	Gly-462, Ser-383, Lys-271, Thr-463
80	-5.2	Asn-465, Val-456, Ile-469	Leu-431, Lys-468	Met-467
81	-5.9	Glu-380, Gly-459, Gly-462, Ser-383	Pro-402, Lys-425	Ser-381, Thr-461, Thr-458, Gly-423
82	-6.4	Gly-462, Ser-383, Thr-458	Glu-380, Pro-402, Lys-425, Gly-459	--
83	-5.0	Ser-362, Ala-358	Ile-361, Met-467, Thr-366	His-365, Asn-369, Ile-359
Amoxicillin	-4.7	Ala-358, Thr-464	Leu-355, Ile359	Asn-465, Ser-362

Table 24. Molecular docking studies of synthesized compounds, **75-83** against LuxS of *S. pyogenes* (PDB ID: P0C0C7).

Synthesized compounds (Ligands)	Docking scores /Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
75	-4.0	His-61, Gly-121	Gly-128, Cys-127, Ala-64, His-57	Ser-126
76	-3.7	His-57	His-61, Cys-127, Ala-64	Pro-120, Arg-68, Asn-129
77	-4.7	Glu-60, Cys-77	His-57, Ser-126, His-61, Ala-64	
78	-4.5	His-57, Cys-127, His-61	Ala-64	Gly-128, Gly-121
79	-4.0	--	His-61, Pro-120, Ala-64, Gly-121	Gly-128, Glu-60, Thr-122, Val-119
80	-5.1	His-61	Ser-126, Glu-60, His-57, Ala-64	Pro-120, Lys-65, Cys-127, Gly-128
81	-4.2	Thr-122	Cys-127, His-61, Ala-64, Pro-120	Gly-128, Thr-123
82	-4.0	Gly-128, His-57, Glu-60	Ala-64, His-61, Cys-127	Asn-129, Gly-121, Pro-120
83	-3.9	Cys-77	Ser-126, His-61, His-57, Cys-127, Ala-64, Asp-76	Pro-120, Gly-121, Gly-128, Asn-129
Amoxicillin	-5.5	Asp-132, Asn-129, Cys-127, His-57, Gly-128, Ser-126, His-61	Pro-79, Leu-56	Glu-60, Phe-80

Table 25. Molecular docking studies of most active synthesized compounds (**80, 81, and 83**) against Penicillin binding proteins (PBPs) (PDB ID: 1VQQ) and β -lactamases (PDB ID: 1IYS).

Synthesized compounds (Ligands)	Docking scores/Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions

80	-7.5	Ser-70, Arg-276	Pro-167	Thr-216, Tyr-105, Ser-130, Ser-23, Asn-132, Asn-170, Asp-240, Thr-171, Asn-104, Thr-108
81	-7.4	Asn-104	Ser-70, Tyr-105, Asn-132, Arg-274, Pro-167	Thr-216, Arg-276, Asp-240, Thr-171, Asn-170, Ser-237, Ser-130, Thr-168
83	-7.6	Ser-70, Asn-104	Ser-70, Tyr-105, Asn-132, Arg-274, Pro-167	Thr-168, Ser-287, Asn-170, Thr-171
80	-8.3	Glu-237	Lys-148, His-293, Asp-295, Arg-241, Arg-151, Val-277	Ser-240, Glu-239, Thr-165, Ser-149, Glu-294, Asp-275, Asn-236
81	-7.9	-	Lys-148, Asp-295, His-293, Val-277, Arg-241, Val-256, Met-372	Ser-240, Tyr-373, Pro-258, Glu-257, Ser-149, Arg-151, Glu-294, Lys-273
83	-8.2	Glu-237, Glu-239	His-293, Lys-148, Asp-295, Lys-273, Val-277	Glu-294, Ser-149, Thr-238, Asn-236, Thr-165
Amoxicillin	-7.2	Lys-148, Asp-275	Val-277, His-293, Arg-241, Arg-151	Ala-276, Ser-149, Asn-164, Gly-166, Thr-165, Ser-240
Amoxicillin	-7.9	Thr-235, Ser-130, Ser-237, Asn-132, Glu-166, Asn-170, Thr-216	Arg-276, Tyr-105	Asn-104, Lys-234

Table 26. Molecular docking studies of synthesized compounds, **75-83** against Human peroxiredoxin 5 (PDB ID: 1HD2).

Synthesized compounds (Ligands)	Docking scores /Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
75	-6.7	Gly-92, Gln-68, Lys-63, Val-70, Lys-93	Arg-86, Glu-91, Glu-16, Arg-95, Val-69	Gly-17, Asn-21, Val-94
76	-7.4	Glu-18, Asn-21, Gly-92, Val-70, Lys-93, Lys-63	Arg-86, Ala-90, Arg-95, Glu-16	Gly-85, Leu-96, Gly-17, Gln-68, Val-69, Val-94, Glu-91
77	-7.4	Arg-86, Val-70, Lys-63, Lys-93	Leu-96, Ala-90, Glu-91, Glu-16	Gly-82, Gly-92
78	-7.2	Lys-93, Lys-63, Gln-68, Gly-92, Val-94	Arg-86, Glu-16, Arg-95, Val-69	Asn-21, Gly-17, Leu-96, Glu-91, Val-70
79	-7.2	Gly-92, Lys-93, Gln-68, Lys-63	Arg-86, Glu-16, Arg-95, Val-69	Asn-21, Leu-96, Val-94, Val-70
80	-7.8	Arg-86, Gly-92, Lys-63, Gln-68	Glu-91, Glu-16, Ala-90, Leu-96	Gly-85, Gly-82, Arg-95, Lys-93, Val-69, Val-70, Val-94
81	-7.5	Glu-16, Arg-95, Lys-63, Val-70, Lys-93	Leu-96, Val-69, Glu-91, Ala-90	Gly-85, Gln-68, Val-94, AsN-21
82	-7.2	Val-70, Lys-93, Gly-92, Lys-22	Val-69, Lys-32, Leu-28, Val-23	Lys-63, Gln-68, Glu-27, Asn-24, Arg-95
83	-7.3	Gln-68, Lys-63, Gly-92, Arg-86	Arg-95, Glu-16, Glu-91, Ala-90, Leu-96	Val-94, Gly-85, Gly-82, Lys-93, Val-69, Val-70
Vitamin C	-5.5	Gly-92, Val-94, Ala-90, Glu-16, Gly-82, Arg-86, Glu-91	--	Arg-95, Lys-93, Leu-96, Gly-85

Table 27. Molecular docking studies of most active synthesized compounds **80**, **81**, **82**, and **83** against α -amylase enzyme (PDB ID: 4W93).

Synthesized compounds (Ligands)	Docking scores/Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
80	-8.6	Glu-233, Asp-197, Arg-195, His-299	Tyr-151, Ile-235, Tyr-62, Trp-59	Asp-300, Asn-298, Gln-63, Thr-163, Leu-162
81	-9.5	Ile-235, Asp-197	Trp-59, Trp-58, His-201, Tyr-151, Lys-200, Leu-162, Tyr-62, His-299	Arg-195, Glu-233, Thr-163
82	-9.6	Lys-200, Glu-233, Arg-195, Gln-63, Ile-235	His-201, Leu-162, Tyr-62, Trp-59	Val-234, Ser-199, Ala-198, Tyr-151, Leu-165, Thr-163, Trp-58, His-299, Asp-300
83	-8.4	Arg-195, His-299, Asp-197, Glu-233, Gln-63	His-305, Asp-300, Tyr-62, Trp-59	Asp-356, Trp-58, Ala-198, Val-107, Ala-106, Gly-104, Thr-163
Acarbose	-7.5	Asp-300, Ile-235, Trp-59, Glu-240	Ala-198, Leu-162	Gly-00, His-305, Trp-58, Ala-307, Leu-237, Leu-165, His-101, Asp-197

5.9.2. *In silico* molecular docking simulation study of **92-100**

5.9.2.1. Molecular docking studies of the synthesized compounds, **92-100** against *E. coli* DNA Gyrase B.

Synthesized compounds (**92-100**) were evaluated by molecular docking to bacterial DNA gyrase B, and the results were compared with a standard antibacterial drug (amoxicillin), as shown in [Appendix 123, a-j](#), and [Table 28](#) (Metwally et al., 2020). The minimum binding energies of the synthesized compounds, **92-100** ranged from -6.9 to -7.6 kcal/mol, suggesting the inhibitory effect of all compounds against *E. coli* DNA gyrase B. Compared to amoxicillin (-7.1 kcal/mol), synthesized compounds (**92, 95, 96, and 98**) showed better results, and (**94, 97, 99, and 100**) showed similar binding affinity and residual interaction profiles with amino acid residues. The higher binding affinity obtained for compounds **92, 93, 95, and 98** is due to the compounds having a similar hydrogen interaction profile with amino acid residues of Asn-46, Ser-121 and residual interactions of Asn-46, Ile-78, and Asp-73, Val-97, Gly-119 (hydrophobic / π -cation/ π -anion/ π -alkyl or van der Waals interactions) ([Figures 31, 32](#), and [Appendix 123, j](#)). According to the results of molecular docking analysis, all the synthesized compounds showed better residual interaction and docking scores than amoxicillin. The docking results (high negative binding affinity) agree well with the *in vitro* biological activity results of **94** and **96** against *E. coli*.

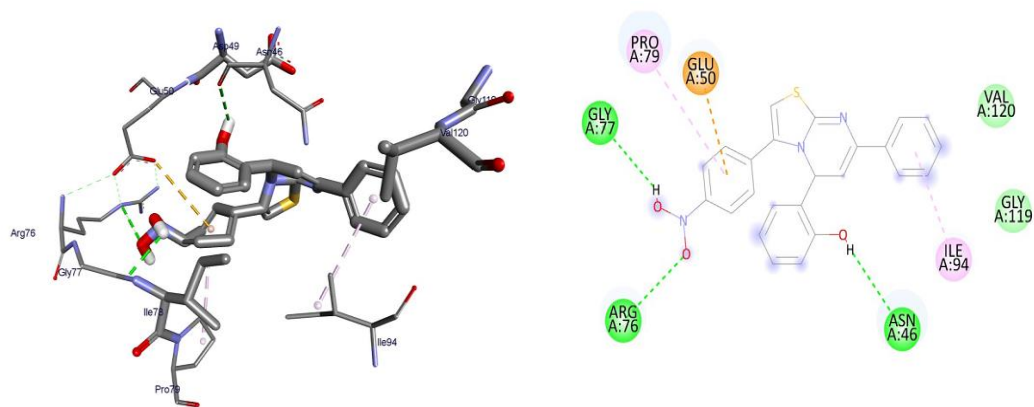


Figure 31. 3D (right) and 2D (left) representations of the binding interactions of **94** against *E. coli* DNA Gyrase B.

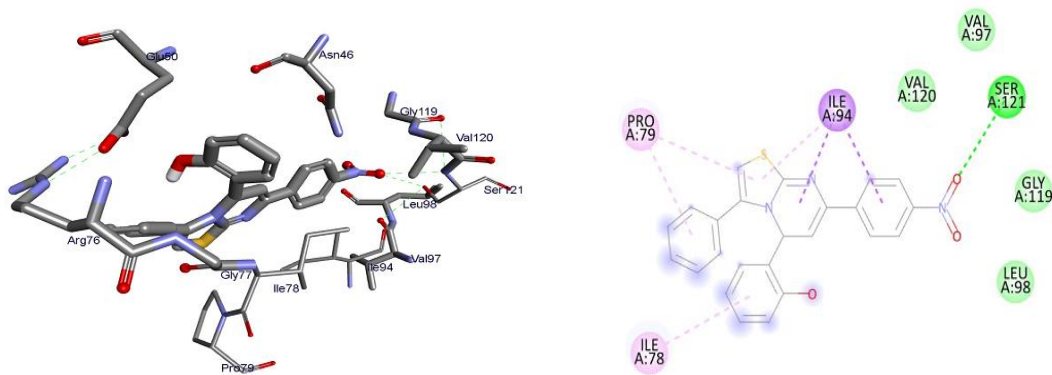


Figure 32. 3D (right) and 2D (left) representations of the binding interactions of **95** against *E. coli* DNA Gyrase B.

5.9.2.2. Molecular docking studies of synthesized compounds, **92-100** against the N-terminal domain of PqsA.

The synthesized compounds were subjected to docking analysis to study their binding mode to the N-terminal domain of PqsA in a complex with 6-fluoroanthraniloyl-AMP target, and the results were compared with those of standard amoxicillin drugs for comparison, as shown in [Appendix 124, a-j](#) and [Table 29](#) (Witzgall et al., 2017). The synthesized compounds were found to have minimum binding energy between -6.3 to -8.4 kcal/mol in the binding pocket of the N-terminal domain of PqsA, with the best result achieved using compounds **95**, **96**, and **98** (-8.4, -8.1 and -8.1 kcal/mol), indicates that the compounds are promising antibacterial agents against *P. aeruginosa*. Compared to amoxicillin (-7.3 kcal/mol), the synthesized compounds (**92**, **93**, **95-97**, **98**, and **99**) have shown better binding affinity and similar amino acid residual interaction profiles. Compounds **95** obtained higher binding affinities due to the interaction of these compounds with amino acid residues of Phe-209, Ala-278, Gly-279, Gly-302, Ala-303, His-308, Arg-397, and Asp-382 (hydrophobic/ π -cation/ π -anion/ π -alkane) interactions and van der Waals forces, respectively ([Figures 33](#), and [Appendix 124, j](#)). Thus, the results of molecular docking are in good correlation with the results of **93**, **96**, and **98** *in vitro* bioactivity against *P. aeruginosa*.

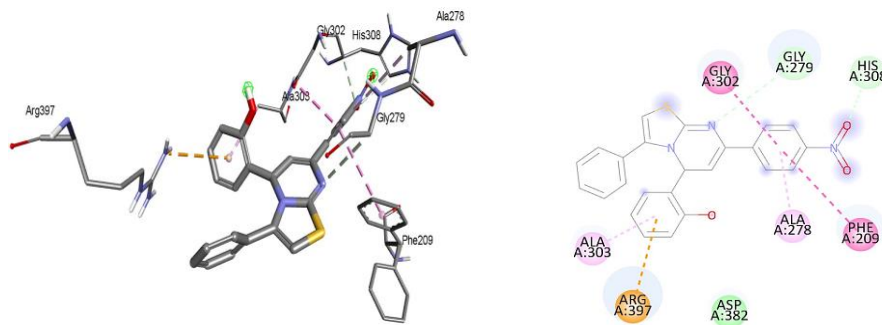


Figure 33. 3D (right) and 2D (left) representations of the binding interactions of **95** against the N-terminal domain of PqsA.

5.9.2.3. Molecular docking studies of synthesized compounds, **92-100** against *S. aureus* Pyruvate Kinase.

Docking analysis of the synthesized compounds was performed to investigate their binding pattern with Pyruvate Kinase in a complex containing the natural bis-indole alkaloid and the results were compared with standard amoxicillin as shown in [Appendix 125, a-j](#) and [Table 30](#) (Veale et al., 2015). The results show that the synthesized compounds have minimum binding energy values in the range from -6.3 to -8.4 kcal/mol in the pyruvate kinase binding pocket, while the best result achieved with compound **95** (-8.4 kcal/mol) indicates that the compounds are promising antibacterial agents against *S. aureus* ([Figures 34](#), and [Appendix 125, j](#)). Compared to amoxicillin (-4.7 kcal/mol), the synthesized compounds (**92**, **93**, **95-97**, **98**, and **99**) have shown better binding affinities and similar residual interaction profiles with amino acid residues. The higher binding affinity obtained for compounds **93**, **95-97**, **98**, and **99** interact with amino acid residues Ala-358 and residues Phe-209, and Gly-302 via hydrophobic/ π -cation/ π -anion/ π -alkyl, and van der Waals interactions, respectively. The docking results are consistent with the results of **96** and **99** *in vitro* bioactivity against *S. aureus*.

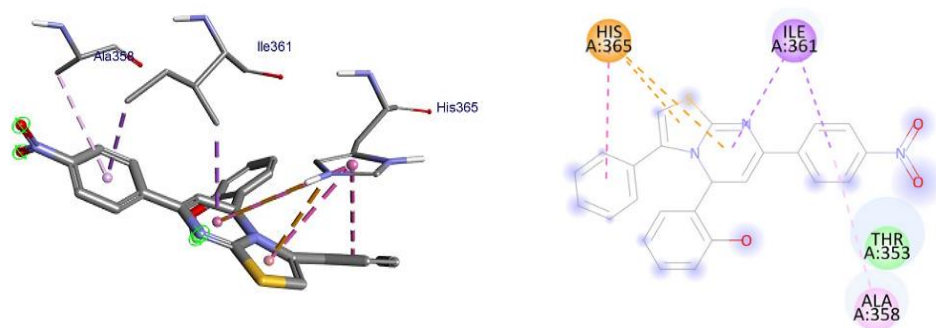


Figure 34. 3D (right) and 2D (left) representations of the binding interactions of **95** against *S. aureus* Pyruvate Kinase.

5.9.2.4. Molecular docking studies of synthesized compounds, **92-100** against LuxS of *S. pyogenes*.

Docking analysis of the synthesized compounds was performed to examine their binding pattern with LuxS and the results were compared to the amoxicillin drug as shown in [Appendix 126, a-j](#) and [Table 31](#) (Zhou et al., 2020). The synthesized compounds were found to have minimum binding energies of -4.5 to -6.5 kcal/mol within the binding pocket of LuxS protein. The best results obtained with compound **99** (-6.5kcal/mol) indicated that these compounds are promising antibacterial agents against *S. pyogenes* ([Figures 35](#), and [Appendix 126, j](#)). Compounds, **92-94**, **95**, **97**, and **98-100** have better binding affinity and similar amino acid residual interactions compared to standard amoxicillin drug (-5.7 kcal/mol). The higher binding affinities obtained with compounds **92-94**, **96**, **97**, and **98-100** are amino acid residues Ala-358 and residual interactions Phe-209, and Gly-302. Compounds **96** (Phe-80), **98** (His-61) and **93** (Asn-129) exhibited additional hydrogen and **95** (Pro-79, Arg-83, Cys-127), **96** (His-57, Glu-60, Pro-79, Arg-83), **98** (Leu-56, His-57, Glu-60, Pro-79) remaining interactions, respectively. The results of *in silico* docking are in good correlation with the results of biological activity against *S. pyogenes*.

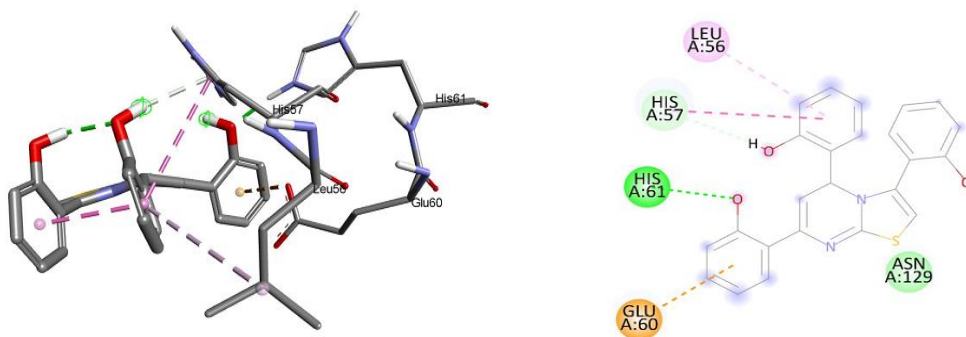


Figure 35. 3D (right) and 2D (left) representations of the binding interactions of **99** against LuxS of *S. pyogenes*.

5.9.2.5. Molecular docking studies of synthesized compounds, **94**, **98**, and **99** against Penicillin binding proteins (PBPs)

The synthesized compounds, were found to have minimum binding energies of -7.5 to -7.9 kcal/mol within the binding pocket of the PBPs, as shown in [Appendix 127, a-c](#) and [Table 32](#). The highest results was attained with compound **94** (-7.9 kcal/mol) compared to reference drug amoxicillin (-7.2 kcal/mol), suggesting that these compounds are promising antibacterial agents against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* ([Figure 36](#)). Compound **94** was involved in hydrogen bonding of Asp-295 with $-\text{NO}_2$ group of thiazole moiety. Hydrophobic/ π -cation interactions were observed for His-293, Val-277, and Glu-294 aromatic rings of chalcone and thiazole moiety.

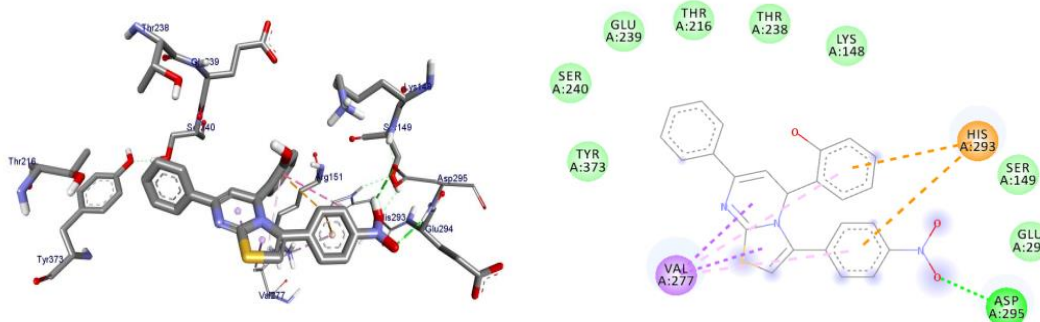


Figure 36. 3D (right) and 2D (left) representations of the binding interactions of **94** against PBPs.

5.9.2.6. Molecular docking studies of synthesized compounds, **94**, **98**, and **99** against β -lactamases.

Compounds, were found to have minimum binding energies of -8.2 to -8.3 kcal/mol within the binding pocket of the β -lactamases protein, as shown in [Appendix 128, a-c](#) and [Table 32](#). The best highest results was obtained with compound **98** (-8.3 kcal/mol) compared to reference drug amoxicillin (-7.9 kcal/mol), demonstrated that these compound is promising antibacterial agents against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* ([Figure 37](#)). Compound **98** was involved in hydrogen bonding with Ser-70, Ser-130 at thiazole of -OH. Hydrophobic/ π -cation interactions were observed for Arg-274, and Tyr-105 with aromatic groups of thiazole and pyrimidine moiety.

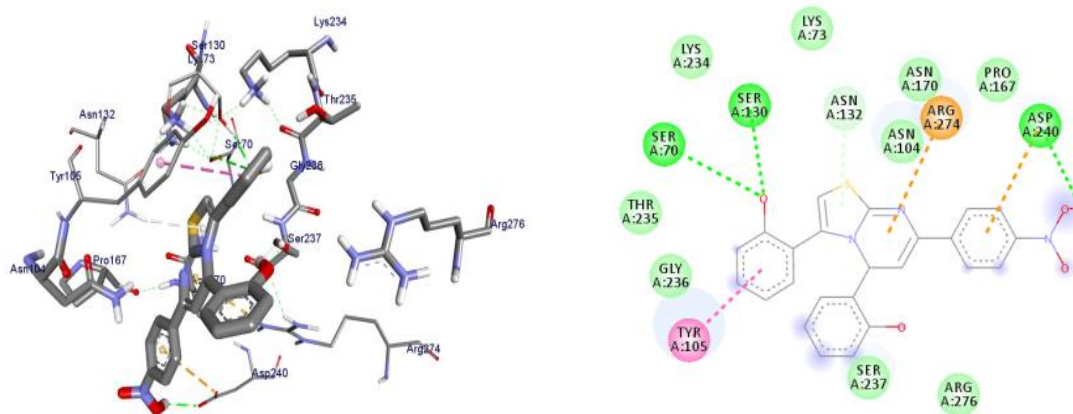


Figure 37. 3D (right) and 2D (left) representations of the binding interactions of **98** against β -lactamases.

5.9.2.7. Molecular docking studies of synthesized compounds, **92-100** against Human peroxiredoxin 5.

The molecular docking of target synthetic compounds (**92-100**) at the binding site of human peroxidase 5 was also analyzed, and the results were compared with the standard antioxidant ascorbic acid, as shown in [Appendix 129, a-j](#), and [Table 33](#). The synthesized compounds were found to have minimum binding energies ranging from (-3.0 to -4.8 kcal/mol) with a moderate result achieved using compound **92** (-4.8 kcal/mol), which revealed that the compounds are

promising antioxidants within the target. Antioxidant analysis of the synthetic compounds showed moderate to good activity. In addition, all compounds showed relevant binding affinities and residual interaction for vitamin C as shown in (Figures 38, and Appendix 129, j).

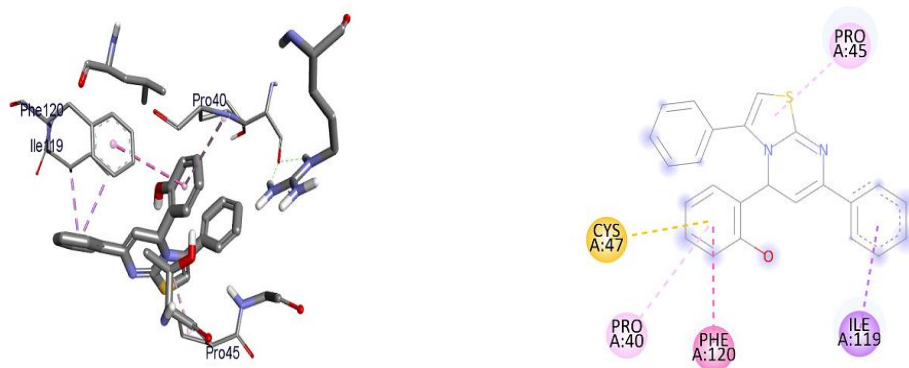


Figure 38. 3D (right) and 2D (left) representations of the binding interactions of **92** against Human peroxiredoxin 5.

5.9.2.8. Molecular docking studies of synthesized compounds (**94**, **98**, and **99**) against α -amylase enzyme.

It was found that the synthesized compounds have minimum binding energy ranging from -8.6 to -8.8 kcal/mol in the binding pocket of the α -amylase enzyme, with the best result achieved for compounds **94**, **98**, and **99** (-8.8, -8.7, and -8.6 kcal/mol, respectively) as compared to acarbose (-7.0 kcal/mol) (Figures 39-41 and Appendix 137, e-g), signifying that the compounds possess promising antidiabetic properties against the α -amylase enzyme, as depicted in Appendix 137, e-g and 11 and Table 34. Thus, the results of molecular docking are in good correlation with the results of **94**, **98**, and **99** *in vitro* α -amylase enzyme inhibition activities. Gln-63, Arg-195, Asp-300, Tyr-151 and was involved in hydrogen bonding with chalcone of -OH, -NO₂, and pyrimidine nitrogen of thiazolo[3,2- α] pyrimidine moiety, respectively. The oxygen lone pair is strategically positioned within the curcumin-conjugated system to delocalize and increase electron density, which may account for the remarkable potency of these molecules. The delocalization is better able to stabilize (+ve)-charges over the benzene, which in turn improves the inhibitory action of the corresponding enzyme (Rahim et al., 2019). Hydrophobic/ π -cation interactions were observed for Tyr-62, His-299, Lys-200, Ile-235, His-201 and Leu-162, His-201,

Ile-235, Lys-200, Tyr-62 and Leu-162, His-201, Lys-200, Ile-235, Ala-198, Tyr-62 with phenyl groups of curcumin. Thus, the docking calculation data were found to be in good correlations with the experimental results. Which show the most potent anti- α -amylase activity of compounds **94**, **98**, and **99** compared to its analogs. These shows that the compounds posses promising α -amylase enzyme inhibitory activities.

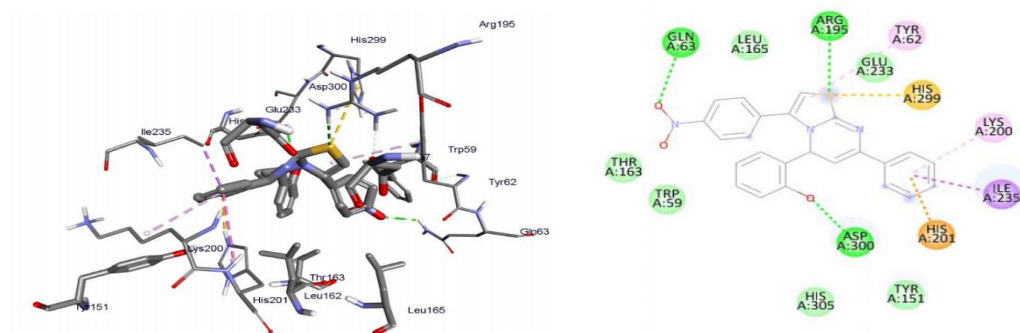


Figure 39. 3D (right) and 2D (left) representations of the binding interactions of **94** against α -amylase enzyme.

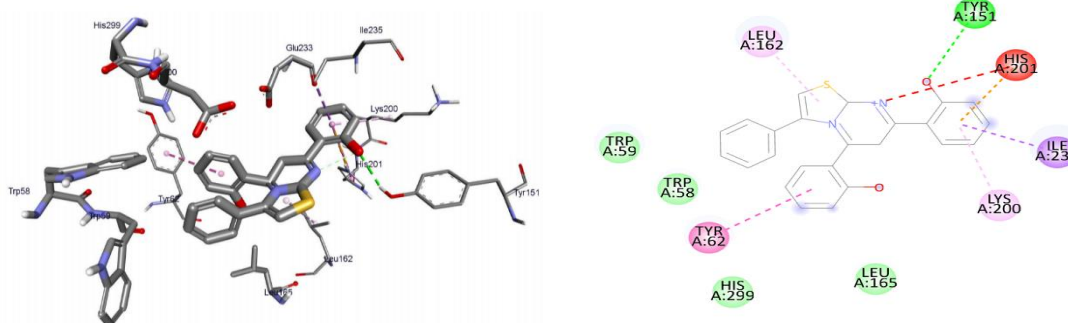


Figure 40. 3D (right) and 2D (left) representations of the binding interactions of **98** against α -amylase enzyme.

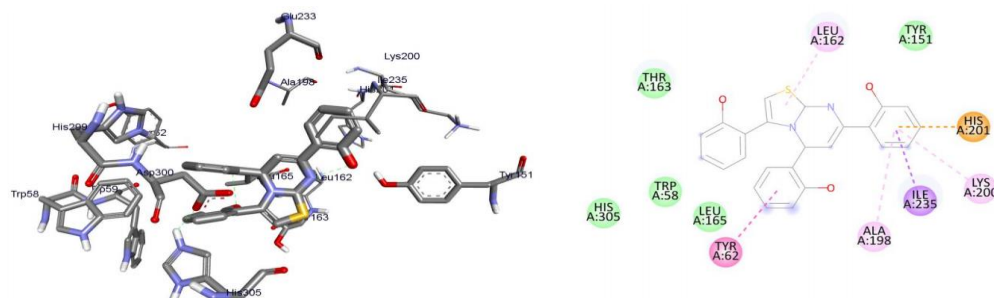


Figure 41. 3D (right) and 2D (left) representations of the binding interactions of **99** against α -amylase enzyme.

Table 28. Molecular docking scores and residual amino acid interactions of synthesized compounds, **92-100** against *E. Coli* Gyrase B. (PDB ID: 6F86).

Synthesized compounds (Ligands)	Docking scores/Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/Pi-cation/ Pi-anion/ Pi-alkyl interactions	Van der Waals interactions
92	-7.4	Asn-46, Thr-165	Val-43, Ala-47, Gly-77, Ile-78, Ile-94, Val-167,	Asp-49, Glu-50, Asp-73, Arg-76, Pro-79
93	-7.6	Gly-77, Thr-165	Val-43, Ala-47, Asp-73, Ile-78,	Pro-79
94	-7.0	Asn-46, Arg-76, Gly-77	Glu-50, Pro-79, Ile-94	Gly-119, Val-120
95	-7.4	Ser-121	Ile-78, Pro-79, Ile-94	Val-97, Leu-98, Gly-119, Val-120
96	-7.3	Glu-50,	Arg-76, Ile-78, Pro-79	Ala-47, Asp-73, Gly-77, Thr-165
97	-6.9	Arg-136	Asn-46, Arg-76, Ile-78, Pro-79	Asp-49, Gly-77
98	-7.6	Asn-46	Val-43, Ala-47, Asp-73, Gly-77, Ile-78, Ile-94, Thr-165, Val-167	Val-71, Val-120
99	-7.1	Thr-165	Val-43, Asn-46, Ala-47, Arg-76, Ile-78, Val-167	Asp-49, Asp-73, Gly-77, Ile-94
100	-6.9	Glu-50, Arg-56, Gly-77,	Asn-46, Pro79	Asp-49, Ile-94, Gly-119, Val-120, Arg-136
Amoxicillin	-7.1	Asn-46, Leu-98, Val-120	Ile-78	Asp-73, Ile-94, Val-97, Gly-119

Table 29. Molecular docking scores and residual amino acid interactions of synthesized compounds, **92-100** against the N-terminal domain of PqsA (PDB ID: 5OE3).

Synthesized compounds (Ligands)	Docking scores \Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/Pi-cation/ Pi-anion/ Pi-alkyl interactions	Van der Waals interactions

92	-7.4	Asp-382	Pro-281, Ile-301, Ala-303, Arg-397	Thr-164, Thr-380, His-394
93	-7.8	-	Phe-209, Gly-279, Ser-280, Pro-281, Ile-301, Gly-302, Asp-382	-
94	-6.6	-	Phe-209, Pro-281, Ile-301, Ala-303	Arg-397
95	-8.4	-	Phe-209, Ala-278, Gly-279, Gly-302, Ala-303, His-308, Arg-397	Asp-382
96	-8.1	Gly-279, Asp-382	Phe-209, Ile-301, Gly-302, His-308, Arg-397	Thr-164, Ala-278, Ser-280, Ala-303, Thr-304
97	-7.4	Thr-164, Thr-323	Pro-281, Ile-301, His-394, Arg-397	Phe-209, Ala-303, Thr-304, Asp-382
98	-8.1	Gly-279, Gly-300, Asp-382, Arg-397	Phe-209, Ser-280, Pro-281, Ile-301, Gly-302	Thr-164
99	-7.2	Gly-302	Phe-209, Gly-210, Gly-279, Ile-301, His-308, Asp-382	Thr-164, Tyr-211, Ser-280, Ala-303, Thr-304, Arg-397
100	-6.3	-	Pro-281, Ala-303, Asp-382, His-394, Arg-397	Gly-279, Gly-302, Thr-304
Amoxicillin	-7.3	Arg-372, Tyr-378, Thr-380, Arg-397	Phe-209, Gly-302	Gly-279, Ala-303, Glu-305

Table 30. Molecular docking studies of synthesized compounds, **92-100** against *S. aureus* Pyruvate Kinase (PDB ID: 3T07).

Synthesized compounds (Ligands)	Docking scores /Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/Pi-cation/ Pi-anion/ Pi-alkyl interactions	Van der Waals interactions
92	-5.3	-	Thr-353, Ala-358, Ile-361, His-365	-
93	-5.0	-	Ile-361, Ser-362	Met-467
94	-4.5	-	Ala-358, Ile-361	Ser-362, His-365
95	-5.9	-	Ala-358, Ile-361, His-365	Thr-353
96	-5.6	Ala-358, Asn-369	Ile-361, His-365	Ser-362,
97	-5.3	Leu-355, Ser-362, Met-467	Ala-358, Ile-359, Met-466	Thr-353, Ile-361
98	-5.3	-	Thr-353, Ala-358, Ile-361, Ser-362	-

99	-5.0	Ser-362	Thr-353, Ala-358, Ile-359, Ile-361	-
100	-4.3	-	Ala-358, Ile-361, His-365	-
Amoxicillin	-4.7	Ala-358, Thr-464	Leu-355, Ile-359	Asn-465, Ser-362

Table 31. Molecular docking studies of synthesized compounds, **92-100** against LuxS of *S. pyogenes* (PDB ID: P0C0C7).

Synthesized compounds (Ligands)	Docking scores /Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
92	-6.0	-	Leu-56, His-57, Glu-60, Pro-79	-
93	-6.1	Asn-129	His-57, Glu-60	Pro-79
94	-6.2	-	Leu-56, His-57, Glu-60, Pro-79	Arg-83
95	-4.5	Glu-60	Pro-79, Arg-83, Cys-127	His-57
96	-5.0	Phe-80	His-57, Glu-60, Pro-79, Arg-83	-
97	-6.1	-	Leu-56, His-57, Glu-60, Pro-79	-
98	-6.4	His-61	Leu-56, His-57, Glu-60, Pro-79	-
99	-6.5	His-61	Leu-56, His-57, Glu-60	Asn-129
100	-5.9	-	Leu-56, His-57, Glu-60, Pro-79	Arg -83
Amoxicillin	-5.7	His-57, Glu-60, Pro-79	-	-

Table 32. Molecular docking studies of most active synthesized compounds **94, 98,** and **99** against Penicillin binding proteins (PBPs) (PDB ID: 1VQQ) and β -lactamases (PDB ID: 1IYS).

Synthesized compounds (Ligands)	Docking scores/Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions

94	-8.2	Asn-170, Tyr-105	Asp-240, Arg-274, Tyr-105	Pro-167, Ser-237, Thr-216, Ser-130, Ser-70, Ser-130, Asn-132, Lys-73
98	-8.3	Ser-70, Ser-130, Asp-240	Arg-274, Trp-105	Lys-234, Thr-235, Glu-236, Asn-132, Asn-170, Pro-167, Ser-237, Arg-276
99	-8.2	-	Tyr-105, Arg-274, Asn-104, Asn-170	Ser-130, Ser-70, Asn-132, Pro-167, Asp-240
94	-7.9	Asp-295	His-293, Val-277, Glu-294	Ser-240, Glu-239, Thr-216, Thr-238, Lys-148, Tyr-373, Ser-149, Glu-294
98	-7.5	Asp-295	His-293, Val-277, Glu-294	Thr-238, Glu-239, Ser-240, Ala-270
99	-7.5	-	Lys-148, Asp-295, His-293, Val-277, Ala-276	Thr-238, Glu-239, Arg-151, Ser-149, Glu-294, Asp-275
Amoxicillin	-7.2	Lys-148, Asp-275	Val-277, His-293, Arg-241, Arg-151	Ala-276, Ser-149, Asn-164, Gly-166, Thr-165, Ser-240
Amoxicillin	-7.9	Thr-235, Ser-130, Ser-237, Asn-132, Glu-166, Asn-170, Thr-216	Arg-276, Tyr-105	Asn-104, Lys-234

Table 33. Molecular docking studies of synthesized compounds, **92-100** against Human peroxiredoxin 5 (PDB ID: 1HD2).

Synthesized compounds (Ligands)	Docking scores /Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/Pi-cation/ Pi-anion/ Pi-alkyl interactions	Van der Waals interactions
92	-4.8	-	Pro-40, Pro-45, Cys-47, Ile-119, Phe-120	-
93	-4.4	-	Pro-40, Pro-45, Cys-47, Ile-119, Phe-120	Leu-116, Arg-127, Thr-147
94	-4.0	-	Leu-116, Ile-119	-
95	-4.3	-	Pro-40, Pro-45, Cys-47, Ile-119	Thr-44, Gly-46, Phe-120, Thr-147, Leu-149
96	-4.4	-	Pro-40, Pro-45, Cys-47, Ile-119	Thr-44, Gly-46, Arg-124, Arg-

				127
97	-3.1	Arg-124	Pro40, Pro-45, Ile-119	Gly-46, Arg-127, Thr-147
98	-4.1	-	Pro-40, Pro-45, Cys-47, Ile-119, Phe-120	Thr-44, Gly-46, Thr-147
99	-3.6	Gly-46	Pro-40, Pro-45, Ile-119, Phe-120	Thr-44, Cys-47, Arg-127
100	-3.0	Thr-147	Pro-45, Leu-116, Ile-119	Thr-44, Cys-47, Phe-120, Asp-145
Vitamin C	-5.2	Gly-46, Cys-47, Thr-147	-	Pro-40, Thr-44, Pro-45, Arg-127, Gly-148, Leu-149

Table 34. Molecular docking studies of most active synthesized compounds, **94**, **98**, and **99** against α -amylase enzyme (PDB ID: 4W93).

Synthesized compounds (Ligands)	Docking scores/Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
94	-8.8	Gln-63, Arg-195, Asp-300	Tyr-62, His-299, Lys-200, Ile-235, His-201	Trp-59, Thr-163, Leu-165, Glu-233, Tyr-151, His-305
98	-8.7	Tyr-151	Leu-162, His-201, Ile-235, Lys-200, Tyr-62	Trp-59, Trp-58, His-299, Leu-165
99	-8.6		Leu-162, His-201, Lys-200, Ile-235, Ala-198, Tyr-62	Thr-163, Tyr-151, Leu-165, Trp-58, His-305
Acarbose	-7	His-305, Asp-300, His-299, Arg-195, Asp-197, Glu-233	Gly-306, Tyr-62	Ala-307, Trp-58, Leu-162, Ala-198, His-101, Leu-165, Thr-163

5.9.3. *In silico* molecular docking simulation study of **108-116**

5.9.3.1. Molecular docking studies of synthesized compounds, **108-116** against *E. coli* DNA Gyrase B.

The minimum binding energies of the synthesized compounds (**108-116**) ranged from -6.4 to -7.9 kcal/mol, suggesting the inhibitory effect of all compounds against *E. coli* DNA gyrase B, as shown in [Appendix 130, a-j](#) and [Table 35](#), respectively. The docking results (high negative binding affinity) agree well with the *in vitro* biological activity results of **111** (-7.9 kcal/mol) against *E. coli* as compared to amoxicillin (-6.1 kcal/mol) as shown in [Figure 42](#) and [Appendix 123, j](#). Gly-77, and Arg-76 were involved in hydrogen bonding with O–H of styrylpyrimidine-2-ol group. Hydrophobic/ π -cation interactions were observed for Thr-165, Ala-47, Ile-94, Pro-79, Ile-78, and Asn-46 with methoxy and pyrimidine rings, respectively. On the other hand, Asp-73, Gly-75, and Glu-50 exhibited Van der Waals residual interactions, respectively.

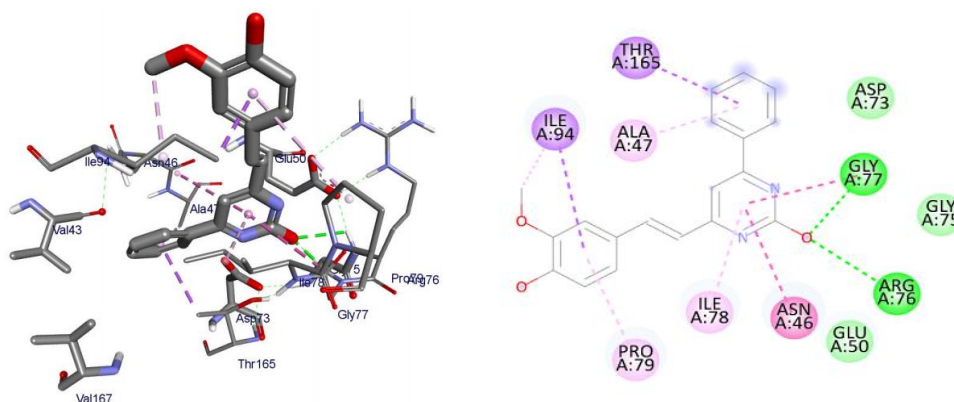


Figure 42. 3D (right) and 2D (left) representations of the binding interactions of **111** against *E. coli* DNA Gyrase B.

5.9.3.2. Molecular docking studies of synthesized compounds, **108-116** against the N-terminal domain of PqsA.

It was found that the synthesized compounds have minimum binding energy ranging from -4.6 to -9.1 kcal/mol in the binding pocket of the N-terminal domain of PqsA, with the best result achieved using compounds **116** (-9.1 kcal/mol) as compared to amoxicillin (-7.9 kcal/mol),

indicating that the compounds are promising antibacterial agents against *P. aeruginosa*., as depicted in [Appendix 131, a-j](#) and [Table 36](#). Thus results of molecular docking are in good correlation with the results of **116** *in vitro* bioactivity against *P. aeruginosa* ([Figure 43](#) and [Appendix 131, j](#)). Val-309 was involved in hydrogen bonding with O–H of curcumin moiety. Hydrophobic/ π -cation interactions were observed for His-308, Gly-302, Asp-382 (at imidazole group), Ala-303, Ile-301, Gly-279, Asp-299, Ala-278, Gly-300, His-394, and Phe-209 with nitrogen atom of $-N(CH_3)_2$ group. While, Thr-304, Thr-164, Arg-397, Thr-380, Ser-280, Asp-299, Ala-278, Gly-300, His-394, and Phe-209 showed Van der Waals residual interactions, respectively.

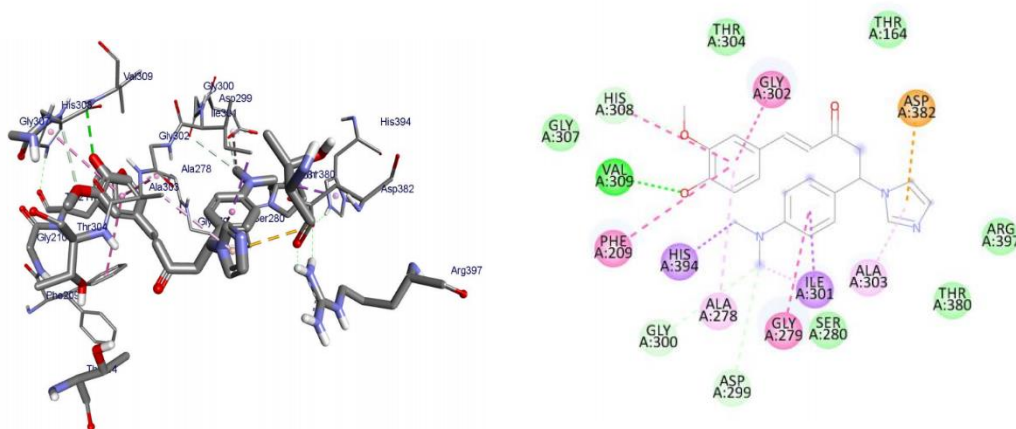


Figure 43. 3D (right) and 2D (left) representations of the binding interactions of **116** against the N-terminal domain of PqsA.

5.9.3.3. Molecular docking studies of synthesized compounds, **108-116** against *S. aureus* Pyruvate Kinase.

It was found that the synthesized compounds have minimum binding energy in the range from -5.1 to -5.8 kcal/mol in the pyruvate kinase binding pocket, while the best result achieved with compound **112** (-5.8 kcal/mol) indicates that the compounds are promising antibacterial agents against *S. aureus*, as presented in [Appendix 132, a-j](#) and [Table 37](#). The docking results are consistent with the results of **112** *in vitro* bioactivity against *S. aureus* ([Figures 44](#) and [Appendix 132, j](#)). Compound **112** (Asn-465, Val-456, Gly-462, Thr-463) was involved in hydrogen bonding with -OH thiazole moiety. Hydrophobic/ π -cation interactions were observed for Ile-359,

Ala-358, Pro-457, Leu-355 with nitrogen atom of $-N(CH_3)_2$ and pyrimidine-2-ol nitrogen atom. Whereas, Thr-461, Asn-465, and Ser-362 displayed Van der Waals residual interactions, respectively.

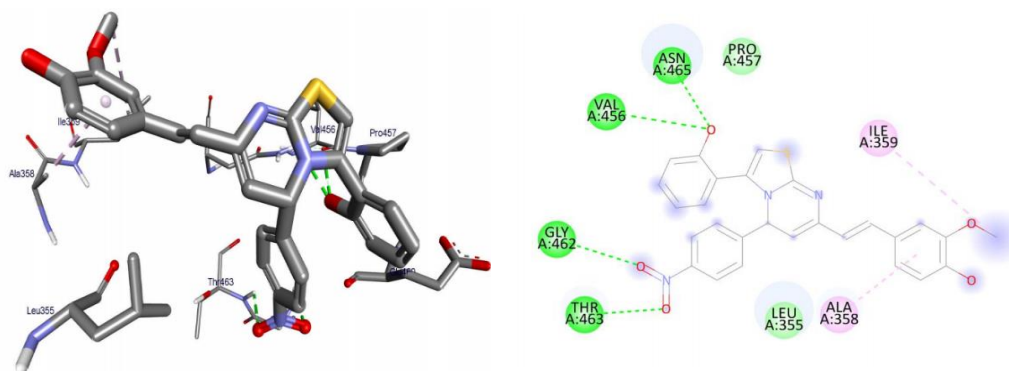


Figure 44. 3D (right) and 2D (left) representations of the binding interactions of **112** against *S. aureus* Pyruvate Kinase.

5.9.3.4. Molecular docking studies of synthesized compounds, **108-116** against LuxS of *S. pyogenes*.

The synthesized compounds (**108-116**) were found to have minimum binding energies of -1.6 to -5.6 kcal/mol within the binding pocket of the LuxS protein, as shown in [Appendix 133, a-j](#), and [Table 38](#). The best results obtained with compound **111** (-5.6 kcal/mol) indicated that these compounds are promising antibacterial agents against *S. pyogenes* ([Figure 45](#) and [Appendix 133, j](#)). The results of *in silico* docking are in good correlation with the results of biological activity against *S. pyogenes*. Hydrophobic/ π -cation interactions were observed for Ser-126, His-61, Pro-79, Leu-56, and His-57 with O-H and methoxy groups of curcumin moiety. While, Gly-128, Asn-129, and Thr-53 showed Van der Waals residual interactions, respectively.

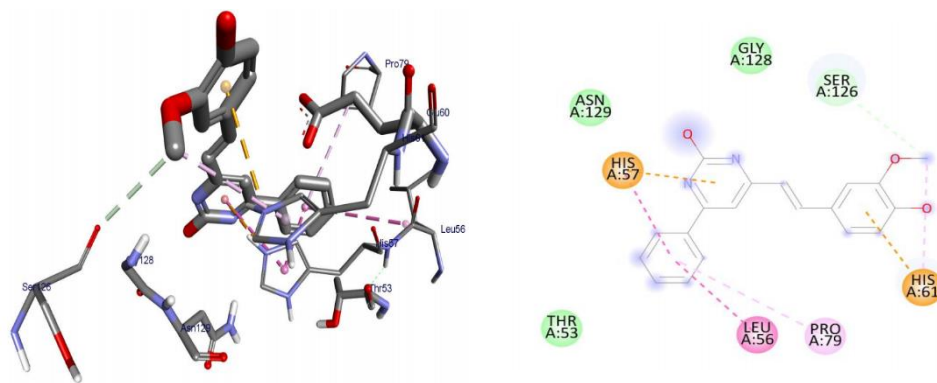


Figure 45. 3D (right) and 2D (left) representations of the binding interactions of **111** against LuxS of *S. pyogenes*.

5.9.3.5. Molecular docking studies of synthesized compounds, **108-116** against Penicillin binding proteins (PBPs)

The synthesized compounds, **108**, **111** and **116** were found to have minimum binding energies of -6.7 to -7.5 kcal/mol within the binding pocket of the Penicillin binding proteins (PBPs), as shown in [Appendix 134, a-c](#), and [Table 39](#). The highest results was attained with compound **111** (-7.5 kcal/mol) compared to reference drug amoxicillin (-7.2 kcal/mol), suggesting that these compounds are promising antibacterial agents against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* ([Figure 46](#)). The results of *in silico* docking are in good correlation with the results of bioactivity against Penicillin binding proteins (PBPs) producing bacterial strains. Compound **111** was involved in hydrogen bonding of Ser-240 with –OH groups of curcumin. Hydrophobic/ π -cation interactions were observed for His-293, Val-277, Asp-295, Ala-276, and Lys-273 aromatic rings of curcumin moiety. While, Met-372, Glu-239, Ser-149, Lys-148, and Glu-294 showed Van der Waals residual interactions, respectively.

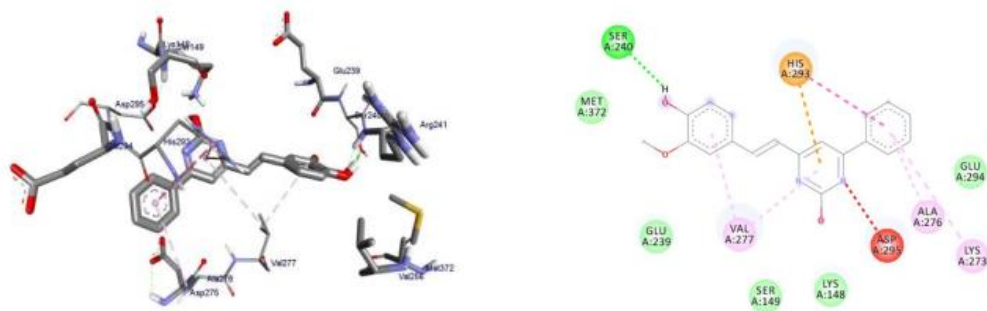


Figure 46. 3D (right) and 2D (left) representations of the binding interactions of **111** against PBPs.

5.9.3.6. Molecular docking studies of synthesized compounds, **108-116** against β -lactamases.

The synthesized compounds, **108**, **111** and **116** were found to have minimum binding energies of -6.9 to -7.7 kcal/mol within the binding pocket of the β -lactamases protein, as shown in [Appendix 135, a-c](#), and [Table 39](#). The best highest results was obtained with compound **111** (-8.3 kcal/mol) compared to reference drug amoxicillin (-7.9 kcal/mol), demonstrated that these compound is promising antibacterial agents against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* ([Figure 47](#)). The results of *in silico* docking are in good correlation with the results of bioactivity against β -lactamases producing bacterial strains. Compound **111** was involved in hydrogen bonding with Ser-70 at pyrimidine-2-ol of -OH. Hydrophobic/ π -cation interactions were observed for Tyr-105, Asp-240, and Pro-167 with aromatic groups of curcumin moiety. While, Asn-132, Ser-237, Thy-168, Thr-171, Asn-104, Asn-170, Glu-166, and Ser-130 showed Van der Waals residual interactions, respectively.

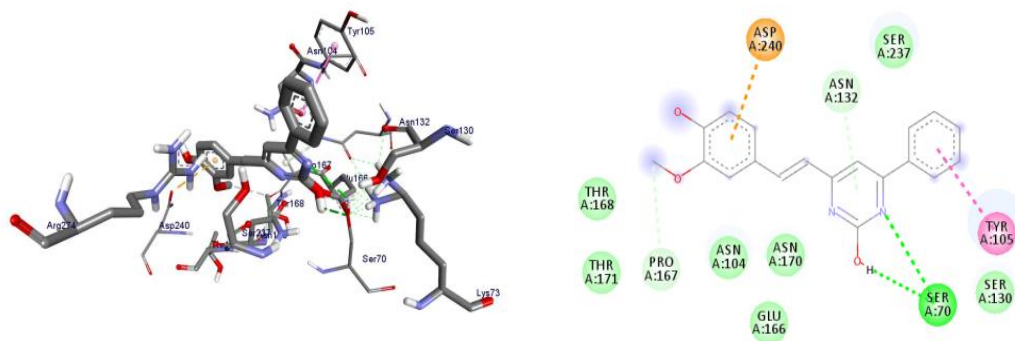


Figure 47. 3D (right) and 2D (left) representations of the binding interactions of **111** against PBPs.

5.9.3.7. Molecular docking studies of synthesized compounds, **108-116** against Human peroxiredoxin 5.

Molecular docking was performed on the active site of Human peroxiredoxin 5. The synthesized compounds were found to have minimum binding energies ranging from (-4.6 to -5.6 kcal/mol) with a moderate result achieved using compound **108** (-5.6 kcal/mol), which revealed that the compounds are promising antioxidants within the target ([Appendix 136, a-j](#) and [Table 40](#)). Antioxidant analysis of the synthetic compounds showed moderate to good activity. In addition, all compounds showed relevant binding affinity and residual interaction for the natural antioxidant of vitamin C. Compound **108** exhibited Thr-147 was involved in hydrogen bonding with O–H and methoxy groups of curcumin moiety. Hydrophobic/ π -cation interactions were observed for Leu-116 with the aromatic group. While, Arg-127, Asn-76, Arg-124, Phe-120, Thr-44, and Ile-119 showed Van der Waals residual interactions, respectively ([Figure 48](#)).

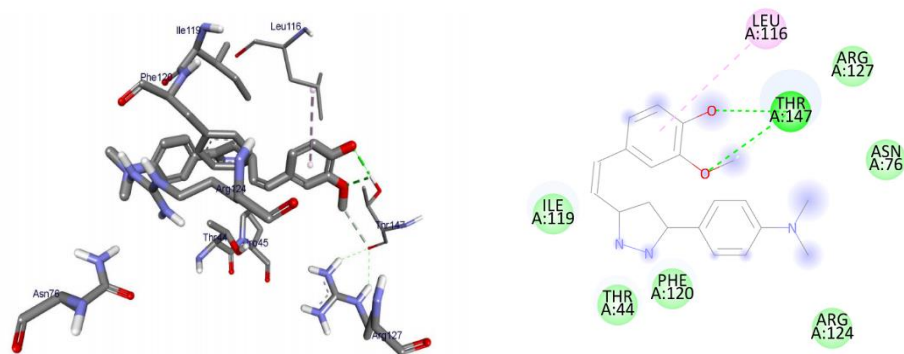


Figure 48. 3D (right) and 2D (left) representations of the binding interactions of **108** against Human peroxiredoxin 5.

5.9.3.8. Molecular docking studies of synthesized compounds (**108**, **111**, and **116**) against α -amylase enzyme.

It was found that the synthesized compounds (**108-116**) have minimum binding energy ranging from -7.4 to -8.4 kcal/mol in the binding pocket of the α -amylase enzyme, with the best result achieved for compounds **108** and **116** (-8.4 and -8.2 kcal/mol, respectively) and while comparable docking score exhibited for compound **111** (-7.4 kcal/mol) as compared to acarbose (-7.5 kcal/mol), signifying that the compounds are promising antidiabetic agents against the α -amylase enzyme, as depicted in [Appendix 137, h-k](#) and [Table 41](#). Thus, the results of molecular docking are in good correlation with the results of **108** and **116** *in vitro* α -amylase enzyme inhibition activities ([Figures 49, 50](#) and [Appendix 137, k](#)). Glu-233 was involved in hydrogen bonding with *N*-pyrazoline and O–H of curcumin moiety, respectively. The oxygen lone pair is strategically positioned within the curcumin-conjugated system to delocalize and increase electron density, which may account for the remarkable potency of these molecules. The delocalization is better able to stabilize (+ve)-charges over the benzene, which in turn improves the inhibitory action of the corresponding enzyme (Rahim et al., 2019). Hydrophobic/ π -cation interactions were observed for His-201, Trp-59, Trp-58, Ile-235 and His-299, Tyr-62, Ala-198, Leu-162 with phenyl groups of curcumin. While, Arg-195, Ala-198, Lys-200, Tyr-151, Tyr-62, Gln-63, and Tyr-58, Asp-300, Asn-298, Leu-165, Thr-163 showed Van der Waals residual interactions, respectively. Thus, the docking calculation was found in good correlations with the

experimental results, which show the most potent anti- α -amylase activity of compounds **108**, **111**, and **116** compared to its analogs.

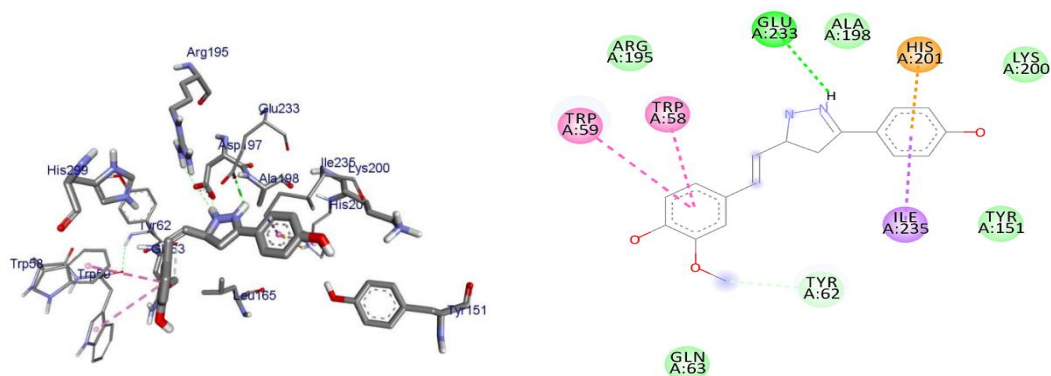


Figure 49. 3D (right) and 2D (left) representations of the binding interactions of **108** against α -amylase enzyme.

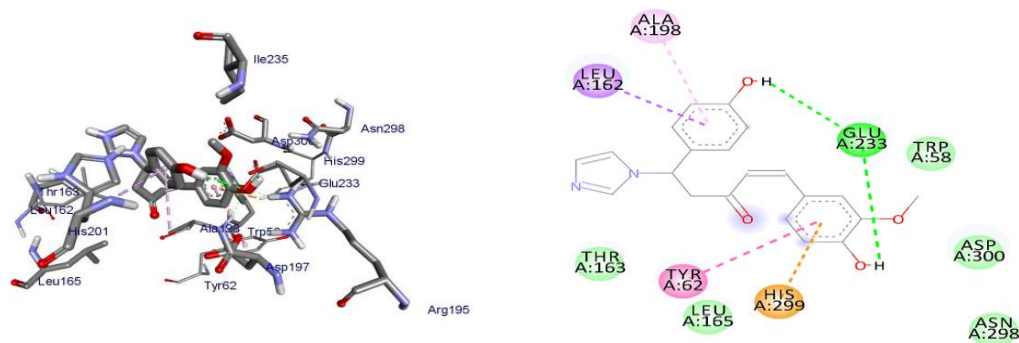


Figure 50. 3D (right) and 2D (left) representations of the binding interactions of **116** against α -amylase enzyme.

Table 35. Molecular docking scores and residual amino acid interactions of synthesized compounds, **108-116** against *E. coli* Gyrase B. (PDB ID: 6F86).

Synthesized compounds (Ligands)	Docking scores /Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
108	-6.5	Pro-79, Arg-136, Arg-76, Ile-78, Gly-77, Asn-46, Ala-47, Asp-73		Glu-50, Thr-165
109	-7.6	Arg-76	Asn-46, Thr-165, Ala-47, Glu-50, Pro-79	Gly-77, Val-167, Val-43
110	-7.5	Arg-76, Arg-136	Glu-50, Pro-79, Ala-47, Asp-73, Asn-46, Ile-78	Asp-49, Val-167, Thr-165
111	-7.9	Gly-77, Arg-76	Thr-165, Ala-47, Ile-94, Pro-79, Ile-78, Asn-46	Asp-73, Gly-75, Glu-50
112	-6.4	Arg-76, Arg-136	Ala-47, Thr-165, Val-167, Ile-78, Val-120, Pro-79, Met-95	Asp-73, Val-43, Asn-46, Glu-50, Gly-77
113	-6.4	Asp-73, Thr-165	Ile-94, Ile-78, Ala-47, Pro-79	Arg-76, Asn-46, Gly-77
114	-7.6	Gly-77, Thr-165	Ile-78, Glu-50, Ile-94	Val-120, Ala-47, Asn-46, Asp-49
115	-7.2	Val-167	Arg-76, Asn-46, Ile-78, Ala-47, Thr-165, Pro-79	Met-166, Val-71, Val-43, Asp-73, Arg-136, Gly-77
116	-6.5		Pro-79, Thr-165, Ala-47, Ile-78, Ile-94	Arg-136, Asp-73
Amoxicillin	-6.1	Asp-73, Arg-76, Gly-77, Asn-46	Pro-79	Ile-94, Arg-136, Thr-165

Table 36. Molecular docking scores and residual amino acid interactions of synthesized compounds, **108-116** against the N-terminal domain of PqsA (PDB ID: 3T07).

Synthesized compounds (Ligands)	Docking scores \Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
108	-5.2	Gly-462, Glu-460	Ile-359, Ala-358, Asn-465, Val-456	Ser-362, Thr-463, Thr-461, Gly-455
109	-5.8	Ser-383	Gly-270, Arg-386, Val-356	Thr-387, Leu-269, Lys-390, Asn-357, Glu-352, Ser-354
110	-5.6	Glu-460, Gly-462, Thr-463	Val-456, Leu-355, Ala-358, Met-467, Ile-359	Thr-461, Asn-465, Ser-362
111	-5.3	Thr-463, Gly-462, Glu-460	Leu-355, Ala-358, Ile-359, Val-456	Asn-465, Thr-461
112	-5.8	Asn-465, Val-456, Gly-462, Thr-463	Ile-359, Ala-358, Pro-457, Leu-355	
113	-5.6	Val-456, Asn-465	Ile-359, Ala-358, Thr-463, Glu-460	Pro-457, Gly-462, Leu-355
114	-5.5	Asn-465, Val-456	Ala-358, Ile-359, Pro-457	Gly-462, Thr-463, Glu-460
115	-5.1	Ser-362, Asn-465	Leu-355, Met-467, Ile-359, Ala-358	Ser-354, Thr-353
116	-5.3	Gly-462, Glu-460	Val-456, Asn-465, Ile-359, Ala-358, Met-467	Ser-362, Leu-355, Thr-461, Thr-463
Amoxicillin	-4.9	Thr-464, Thr-463, Leu-355, Ala-358	Ile-359	Ser-362, Ile-361

Table 37. Molecular docking studies of synthesized compounds, **108-116** against *S. aureus* Pyruvate Kinase (PDB ID: 4XCH).

Synthesized compounds (Ligands)	Docking scores /Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
108	-3.8	Ser-126	Cys-127, His-57	Glu-60, Gly-128, Asn-129, Pro-120
109	-4.5	Cys-127, Glu-60	His-57, His-61	Gly-128, Asn-129, Pro-120, Ser-126, Gly-121
110	-5.3	Glu-60	Ala-64, His-61, His-57	Gly-121, Ser-126, Gly-128, Asn-129
111	-5.6		Ser-126, His-61, Pro-79, Leu-56, His-57	Gly-128, Asn-129, Thr-53
112	-1.6		Ala-64, His-61, His-57, Leu-56, Cys-127, Pro-79, Ser-126, Gly-121	Pro-120, Glu-60, Thr-122, Thr-123
113	-2.1	Ser-126, Asn-129	Ala-64, Cys-127, Gly-121, Pro-79, Leu-56, His-57, His-61, Glu-60, Cys-77	Arg-68, Gly-128, Asp-132, Thr-53
114	-2.3	Asn-129	Ser-126, His-61, His-57, Glu-60, Ala-64, Cys-127	Gly-128, Pro-120, Gly-121
115	-3.1		Cys-127, Ala-64, His-57	Asn-129, Gly-128, Pro-120, Gly-121, Arg-68, Cys-77, Glu-60
116	-5.1		Pro-79, Glu-60, Ala-64, His-61, Pro-120, His-57	Ser-126, Gly-128, Gly-121, Asn-129
Amoxicillin	-2.9	His-61, Glu-60	His-57, Cys-127, Gly-121	Ser-126, Gly-128, Asn-129

Table 38. Molecular docking studies of synthesized compounds, **108-116** against LuxS of *S. pyogenes* (PDB ID: 5OE3).

Synthesized compounds (Ligands)	Docking scores /Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
108	-7.6	Thr-304	Asp-299, Gly-300, Ser-280, Gly-279, Ile-301, Ala-278, Phe-209	Thr-323, Pro-281, His-394, Asp-382, Gly-302, Arg-397
109	-8.1	Thr-304	Glu-305, Ala-303, Ser-280, Pro-281, Gly-279, Ile-301	Thr-164, Tyr-211, Gly-300, Gly-210, Arg-397
110	-7.6		His-308, Gly-307, Ala-303, Asp-382, Arg-397, His-394, Pro-281, Gly-302, Ala-278, Phe-209, Tyr-211	Thr-164, Thr-304, Gly-210, Gly-279
111	-8.6	Thr-304, Tyr-211, Asp-382	Pro-281, His-394, Ser-280, Ile-301, Gly-279, Arg-397	Gly-210, Phe-209, Thr-323, Asp-299, Gly-300, Gly-302, His-308
112	-5.7	Gly-307	Asp-382, Arg-397, Gly-279, Ile-301, Tyr-211, Ala-278, Val-309, His-308, Phe-209, Gly-210, Gly-302	Ala-303, Thr-304, Gly-300
113	-4.6	Thr-380, Arg-372, Arg-397	Glu-305, Ala-303, Gly-302, Phe-209, Tyr-211, Ala-278, His-308, Ile-301	Ser-380, Tyr-378, Thr-164, Val-309
114	-7.0	Tyr-378, Glu-305	Thr-164, Thr-304, Ala-303, His-308, Phe-209, Gly-302, Ala-278, Arg-397	Thr-380, Arg-372, Gly-210, Gly-307, Tyr-211
115	-8.8	Thr-304, Val-309	Ala-303, Arg-397, Ile-301, Gly-210, His-308, Gly-302	Thr-164, Arg-372, Thr-380, Tyr-378, Asp-382, Ser-280, Gly-279, Tyr-211
116	-9.1	Val-309	His-308, Gly-302, Asp-382, Ala-303, Ile-301, Gly-279, Asp-299, Ala-278, Gly-300, His-394, Phe-209	Thr-304, Thr-164, Arg-397, Thr-380, Ser-280, Asp-299, Ala-278, Gly-300, His-394, Phe-209
Amoxicillin	-7.9	Glu-305, Asp-299, Thr-323, Gly-302, Gly-279	Ser-280, Pro-281, Ile-301, Asp-382	Thr-304, Gly-300, Leu-282, Arg-397, Thr-380, Tyr-378

Table 39. Molecular docking studies of most active synthesized compounds (**108**, **111**, and **116**) against Penicillin binding proteins (PBPs) (PDB ID: 1VQQ) and β -lactamases (PDB ID: 1IYS).

Synthesized compounds (Ligands)	Docking scores/Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
108	-6.7	Arg-241, Ser-240	Asp-295, Asp-275, Val-277	Lys-148, Glu-294, Lys-273, Val-256, Met-372, Try-373
111	-7.5	Ser-240	His-293, Val-277, Asp-295, Ala-276, Lys-273	Met-372, Glu-239, Ser-149, Lys-148, Glu-294
116	-6.9	Ser-149	His-293, Glu-150, Val-277, Thr-165, Pro-258, Met-372	Asp-295, Arg-241, Thr-165, Ser-240, Val-256, Gly-257, Tyr-373
108	-6.9	Tyr-105, Asn-104, Asn-170	Asn-132	Trr-129, Thr-215, Ser-218, Thr-126, Ala-219, Ser-130, Asn-132, Ser-132, Glu-166, Ser-70
111	-8.3	Ser-70	Tyr-105, Asp-240, Pro-167	Asn-132, Ser-237, Thy-168, Thr-171, Asn-104, Asn-170, Glu-166, Ser-130
116	-7.7	Arg-276, Thr-216, Ser-70, Lys-73	Gly-236, Tyr-105	Asn-132, Ser-130, Gly-236, Asn-104, Ser-237, Asn-170, Thr-235, Gly-236
Amoxicillin	-7.2	Lys-148, Asp-275	Val-277, His-293, Arg-241, Arg-151	Ala-276, Ser-149, Asn-164, Gly-166, Thr-165, Ser-240
Amoxicillin	-7.9	Thr-235, Ser-130, Ser-237, Asn-132, Glu-166, Asn-170, Thr-216	Arg-276, Tyr-105	Asn-104, Lys-234

Table 40. Molecular docking studies of synthesized compounds, **108-116** against Human peroxiredoxin 5 (PDB ID: 1HD2).

Synthesized compounds (Ligands)	Docking scores/Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
108	-5.6	Thr-147	Leu-116	Arg-127, Asn-76, Arg-124, Phe-120, Thr-44, Ile-119
109	-5.4	Thr-147	Leu-116, Thr-44, Phe-120	Arg-127, Phe-43
110	-5.4	Ile-119, Gly-46	Leu-116, Thr-147, Pro-45	Thr-44, Ala-147
111	-5.5		Phe-120, Ile-119, Ala-42, Pro-45	Phe-43, Thr-44, Leu-149, Gly-46, Arg-127, Thr-147, Leu-116
112	-4.9	Thr-147, Gly-46, Ile-119	Leu-116, Phe-43, Pro-45	Arg-127, Gly-148, Asp-145, Thr-44
113	-4.6	Thr-147, Gly-46	Ile-119, Pro-45, Phe-120, Thr-44	Arg-127, Phe-43
114	-5.6	Thr-147, Arg-127, Ile-119	Ala-42, Pro-45	Gly-148, Gly-46, Thr-44
115	-4.8	Thr-44, Gly-46, Arg-127, Cys-47, Thr-147	Pro-45, Leu-116, Asp-145	
116	-4.9	Ile-119, Thr-44	Thr-147, Leu-116, Pro-45, Ala-42, Phe-120, Pro-40, Cys-47	Asp-145, Arg-124, Gly-46, Arg-127
Amoxicillin	-5.4	Thr-147, Arg-127, Gly-46	Ile-119, Phe-120, Pro-45	Thr-44, Cys-47, Leu-149
Vitamin C	-5.8	Thr-44, Arg-127, Thr-147, Gly-46, Cys-47		Leu-149, Pro-40

Table 41. Molecular docking studies of most active synthesized compounds **108**, **111**, and **116** against α -amylase enzyme (PDB ID: 4W93).

Synthesized compounds (Ligands)	Docking scores/Affinity (kcal/mol)	H-bonds	Amino acid residual interactions	
			Hydrophobic/ π -cation/ π -anion/ π -alkyl interactions	Van der Waals interactions
108	-8.4	Glu-233	His-201, Trp-59, Trp-58, Ile-235	Arg-195, Ala-198, Lys-200, Tyr-151, Tyr-62, Gln-63
111	-7.4	His-299	Arg-195, Asp-197, His-201, Ile-235, Lys-200, Tyr-62, Leu-165	Tyr-59, Gln-63, His-101, Tyr-151, Ala-198, Glu-233
116	-8.2	Glu-233	His-299, Tyr-62, Ala-198, Leu-162	Tyr-58, Asp-300, Asn-298, Leu-165, Thr-163
Acarbose	-7.5	Asp-300, Ile-235, Trp-59, Glu-240	Ala-198, Leu-162	Gly-00, His-305, Trp-58, Ala-307, Leu-237, Leu-165, His-101, Asp-197

6. CONCLUSION AND RECOMMENDATIONS

In the present study, an eco-friendly and efficient ZnO nanoparticle and ultrasonic-irradiation-assisted protocol have been adopted for the synthesis of new series of thiazolyl-pyrazoline Schiff base hybrids, fused rings of thiazolo [3,2- α] pyrimidine and heterocyclic C₅-curcumin analogs as antidiabetic, antibacterial and antioxidant agents along with the prediction of their ADMET profiles, and molecular docking simulation study. The structures of all the synthesized compounds were determined using combined physicochemical properties and spectroscopic techniques (UV-Vis, ¹H-NMR, and ¹³C-NMR). The results of antibacterial screening indicated that compounds **81**, **83**, **93**, **94**, **96**, **98**, **100**, **108**, **111**, and **116** showed good activities against *P. aeruginosa* with mean inhibition zone of 13.33±0.58, 17.33±0.58, 11.00±1.00, 12.00±1.00, 14.67±0.58, 12.33±0.58, 10.67±1.15, 17.67±0.57, 14.33±0.57 and 23.33±0.00 mm diameter, respectively, at 500 µg/mL compared to amoxicillin (15.00±1.00, 12.33±0.58, and 13.75±1.83 mm, respectively) at same concentration. Whereas, compounds **78**, **81**, **83**, **94**, **96**, **100**, **108**, and **111** showed good activities against *E. coli* with mean inhibition zone of 12.00±1.00, 13.33±0.58, 17.00±1.00, 12.33±1.15, 12.67±0.58, 12.00±1.00, 13.00±0.57, 14.33±0.57 and 19.66±0.00 mm diameter, respectively, at 500 µg/mL compared to amoxicillin (14.67±0.58, 13.33±1.15, and 12.87±1.41 mm, respectively) at same dose. Compounds **80**, **99** and **112** showed good activities against *S. aureus* with mean inhibition zone of 11±1.00 mm, 12.00±1.73 and 11.33±0.57 mm diameter at 250 and 500 µg/mL, respectively compared to amoxicillin (14±0.01, 16.33±0.58, 10.75±1.83 mm, respectively) at same concentration. Compounds **77**, **78** and **80** showed good activities against *S. pyogenes* with mean inhibition zone of 18.33±0.58, 17.00±1.00 and 22.33±1.53 mm diameter, respectively, at 250 µg/mL compared to amoxicillin (29.67±2.52 mm). The remaining compounds showed moderate to weak activity against the tested bacterial strains. *In vitro* results for α -amylase enzyme revealed that compounds **80**, **81**, **82**, **83**, **94**, **98**, **99**, **111**, and **116** with IC₅₀ values of 6.90, 6.74, 6.67, 6.80, 6.56, 6.80, 6.75, 7.59 and 4.08 µg/mL, respectively have comparable inhibitory activities with reference drug, acarbose (IC₅₀= 4.42, 4.42 and 8.0 µg/mL, respectively). Additionally, compounds **80**, **99**, and **116** displayed the highest percent inhibition of DPPH with IC₅₀ values of 4.63, 9.48, and 2.44 g/mL compared to ascorbic acid (3.21 g/mL) and promising inhibition of peroxide formation by 80.01±0.07,

76.28±0.12, and 89.92±0.42%, respectively, indicating their promising suppression of peroxide formation.

Molecular docking analysis of compounds **81**, **83**, **94**, **96**, **98**, **100**, **109**, and **116** revealed higher DNA Gyrase B inhibitory effect with the binding affinity of -8.5, -7.9, -7.1, -7.3, -7.6, -6.9, -7.9, and -5.8 Kcal/mol, respectively, and compounds **81**, **83**, **96**, **98**, **99**, **109** and **112** displayed promising binding affinities of -10.7, -9.9, -8.1, -8.1, -7.2 -7.9, and -5.8 Kcal/mol, respectively, against PqsA, and pyruvate kinase compared to amoxicillin (-7.1, -7.3, -6.1, -7.9 and -4.9 Kcal/mol, respectively). Compound **80** displayed a binding affinity of -7.8 Kcal/mol with LuxS, compared to amoxicillin (-5.5 Kcal/mol), these results are in correlation with the *in vitro* antibacterial activities displayed by these compounds against *E. coli*, *P. aeruginosa*, and *S. pyogenes*, respectively. Compound **80**, **94**, **111** and **83**, **98**, **111** displayed a higher binding affinities of -8.3, -7.9, -7.5 and -7.6, -8.3, -8.3 Kcal/mol with Penicillin binding proteins (PBPs) and β -lactamases producing bacterial strains, compared to amoxicillin (-7.2 and -7.9 Kcal/mol, respectively), these results are in good agreement with the *in vitro* antibacterial activities exhibited by these compounds against *E. coli*, *P. aeruginosa*, and *S. aureus*. This, is due to it involved in hydrogen bonding with Serine-residue of -OH group, which is able to acylate the active Serine residue. Compounds **80** and **99** displayed a binding affinity of -5.1 Kcal/mol and -3.6 Kcal/mol against Human peroxiredoxin 5 compared to ascorbic acid (-5.2 Kcal/mol). Compounds **81**, **82**, **94**, **98**, **99**, **108** and **116** displayed a binding score of -9.5, -9.6, -8.8, -8.7, -8.6, -8.4 and -8.2 Kcal/mol, respectively with α -amylase enzyme compared to acarbose (-7.5, -7.0 and -7.5 Kcal/mol, respectively), these results are in strong correlation with *in vitro* α -amylase inhibitory effects. The results of the ADMET study showed that all synthesized compounds followed Lipinski's rule of five and drug-like properties. *In silico* toxicity study showed that all synthesized compounds had LD₅₀ (mg/kg) values ranging from 300 to 1,000 putting them in Protox-II's class-3 and 4. Hence, the *in vitro* antibacterial activity and molecular docking analysis showed that compounds **81**, **83**, **94**, **96**, **98**, **108**, **111**, and **116** is a more potent antibacterial therapeutics agents against *P. aeruginosa* and *E. coli*, whereas compound **80**, **81**, **95**, and **112** were found to be a promising antibacterial, against *S. aureus*, and antioxidant agents.

Moreover, the green synthesis approach using ZnO nanoparticles and ultrasonic-irradiation-promoted conditions was found to be a very efficient and eco-friendly method to synthesize

biologically active natural product based heterocyclic analogs compared to the conventional method. Consequently, *in silico* ADMET predictions and molecular docking studies of most active synthesized compounds showed a strong correlation with the *in vitro* studies of antidiabetic, antibacterial, and antioxidant biological results suggesting the synthesized compounds appear to be lead compounds for rational drug design. Future attempts should be carried out to synthesize a series of related natural product-based heterocyclic compounds for comprehensive structure-activity relationships and *in vivo* studies to develop and optimize lead antidiabetic, antibacterial, and antioxidant drugs. Thus, further derivatization or modification might help to obtain more potent and selective agents.

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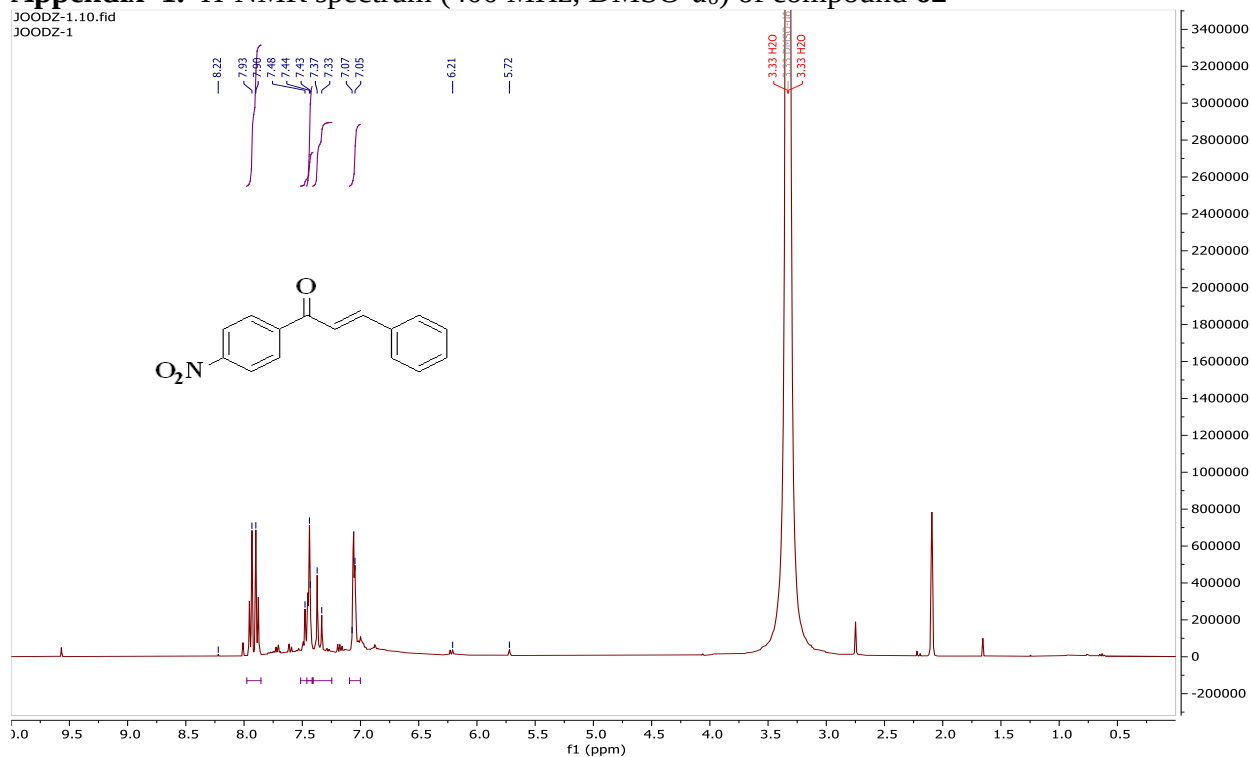
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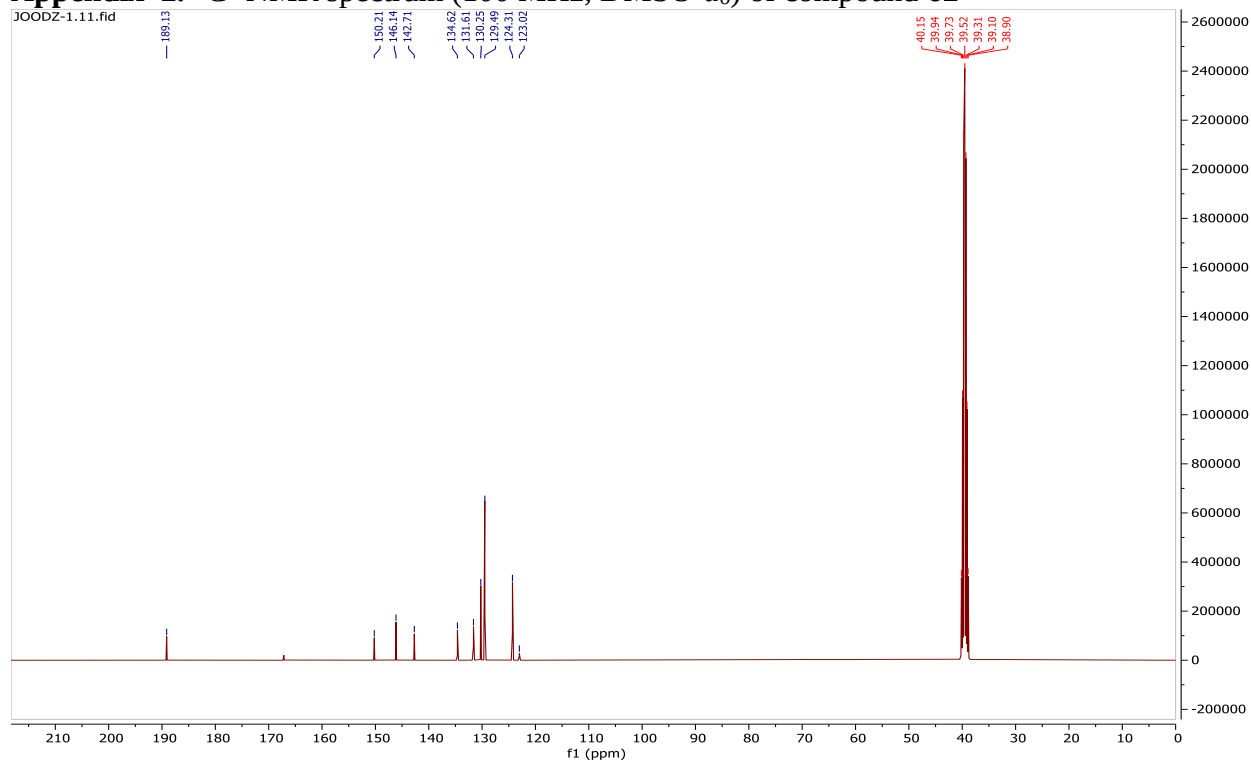
LIST OF APPENDICES

¹H- and ¹³C-NMR spectrum (400 MHz, and 100 MHz, respectively) of compounds **60-116**

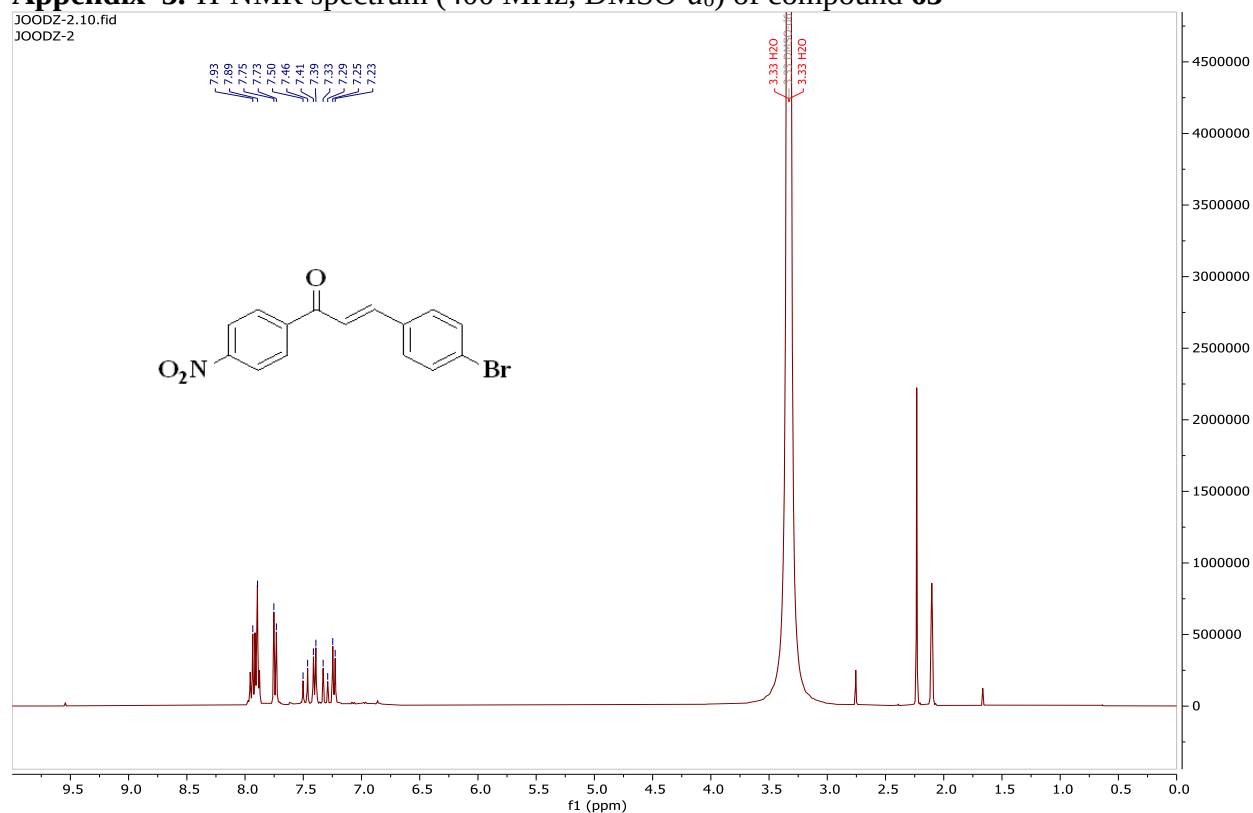
Appendix 1. ^1H -NMR spectrum (400 MHz, DMSO- d_6) of compound **62**



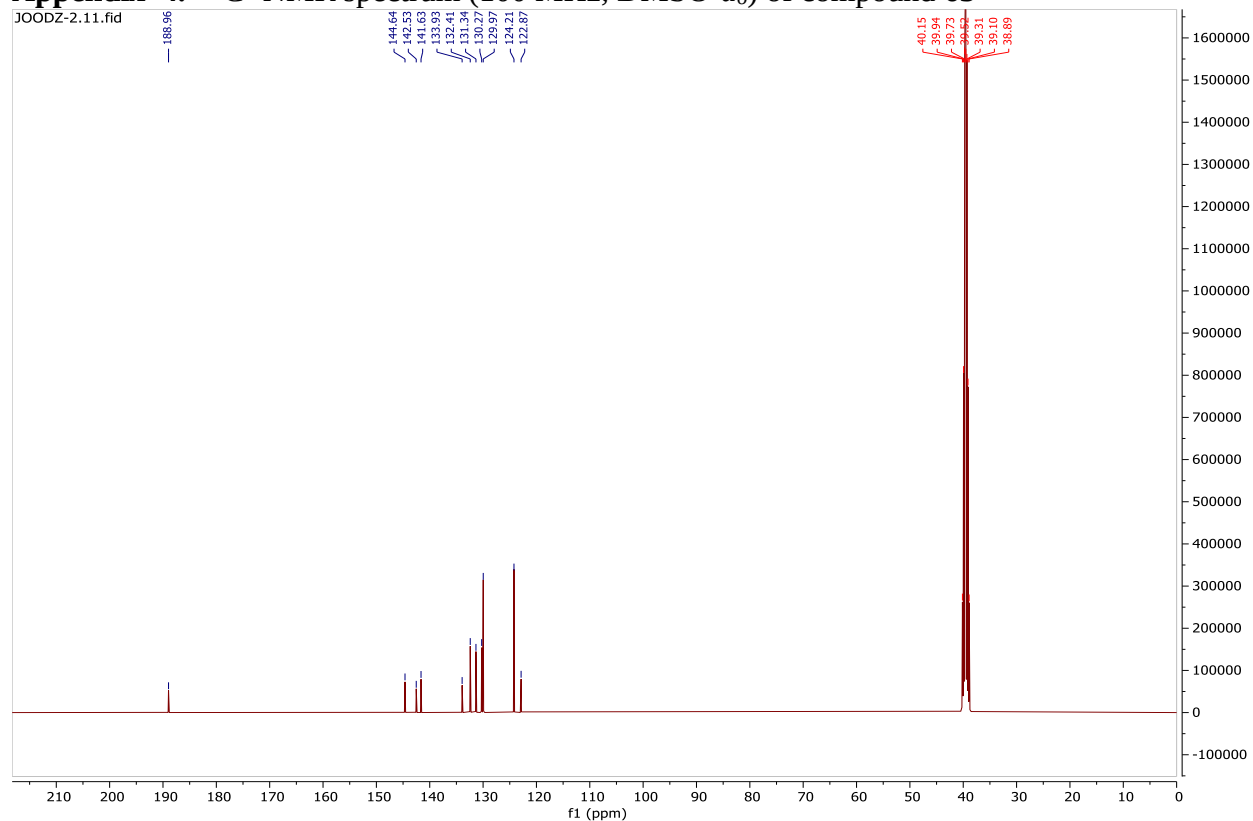
Appendix 2. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **62**



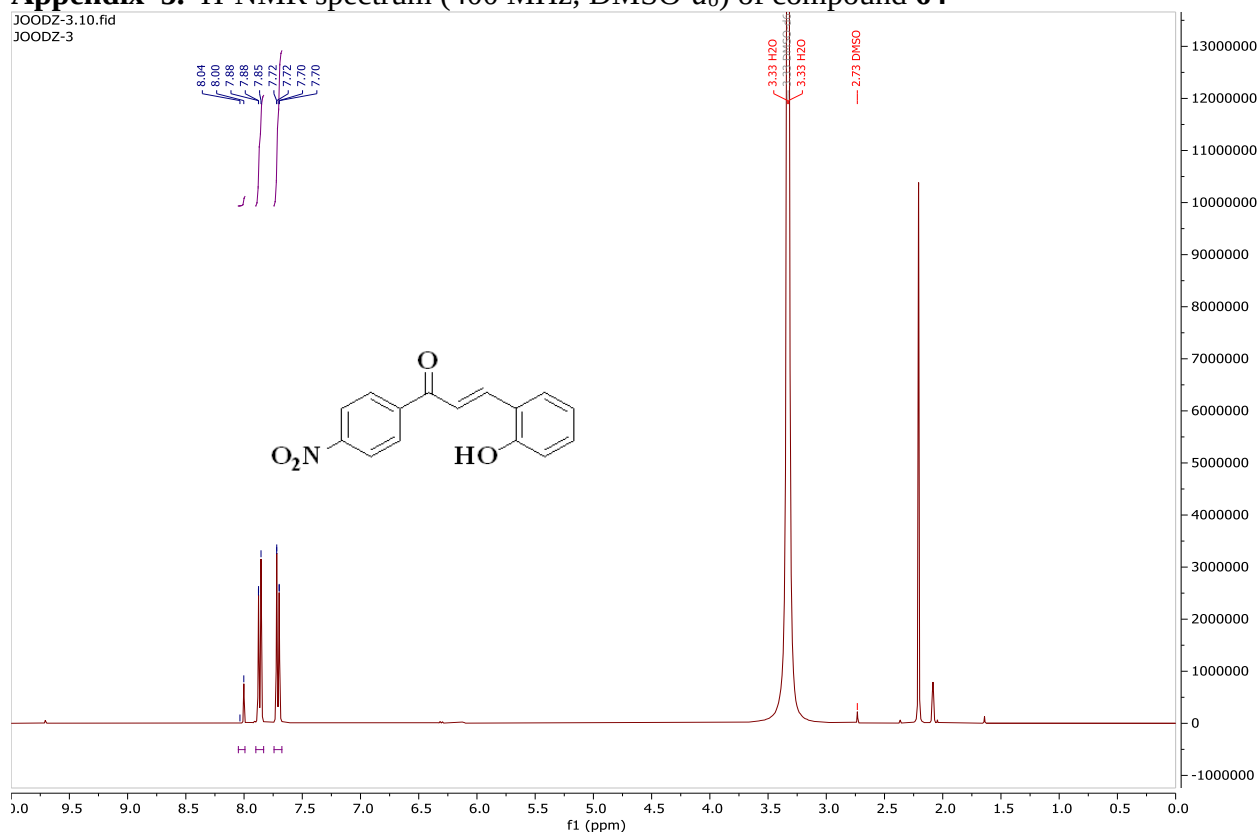
Appendix 3. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **63**



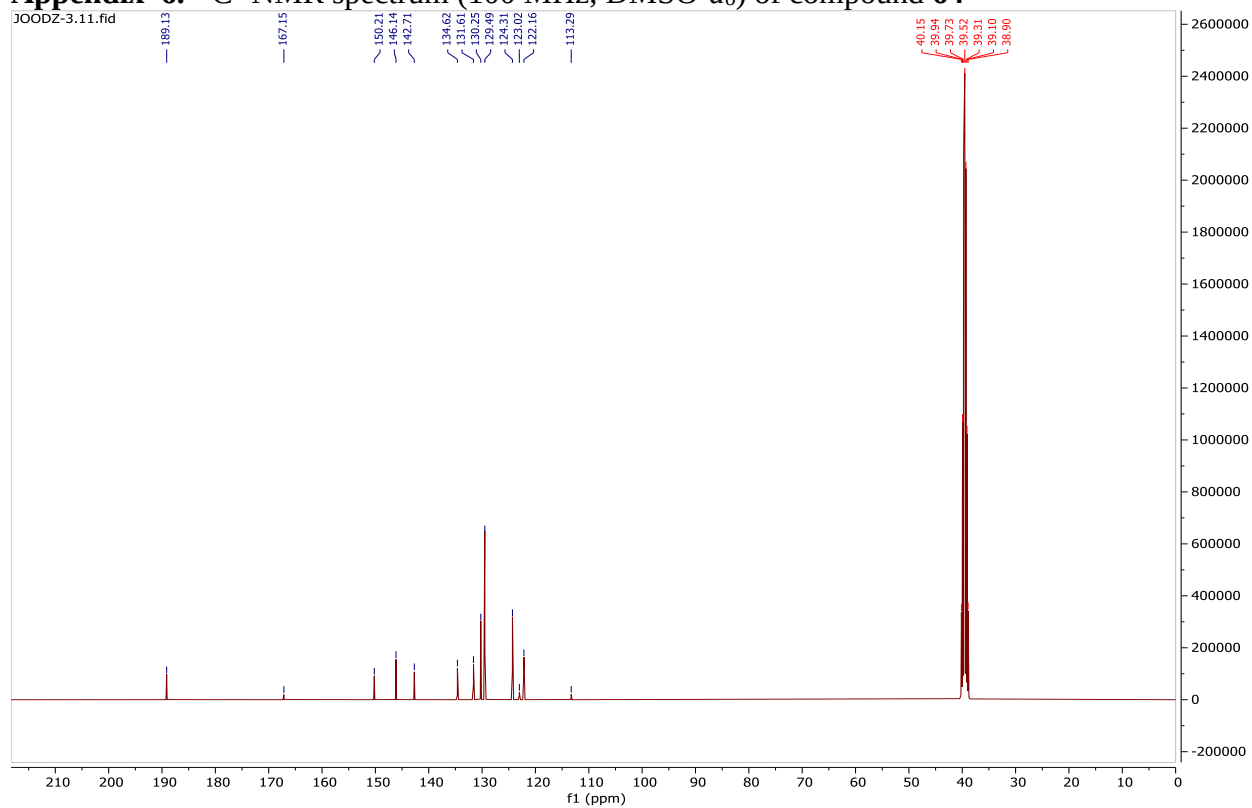
Appendix 4. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **63**



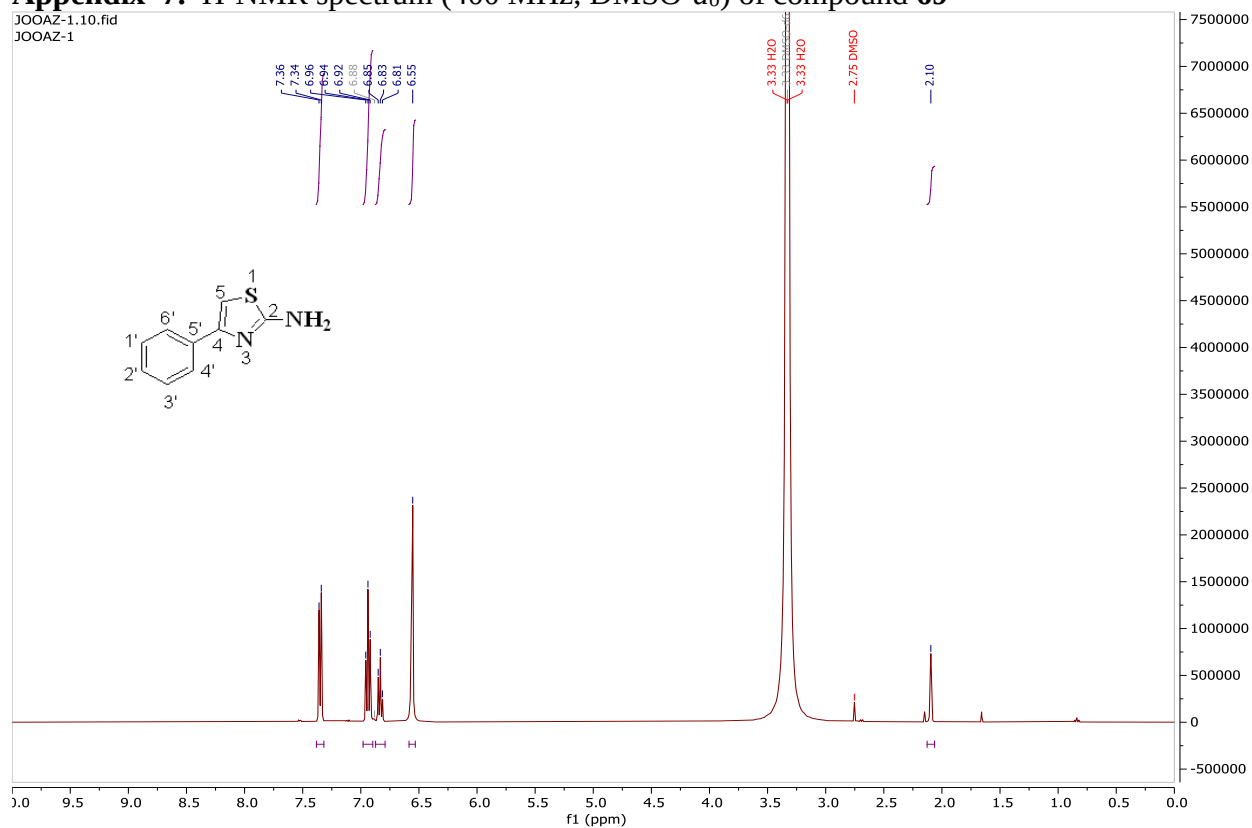
Appendix 5. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **64**



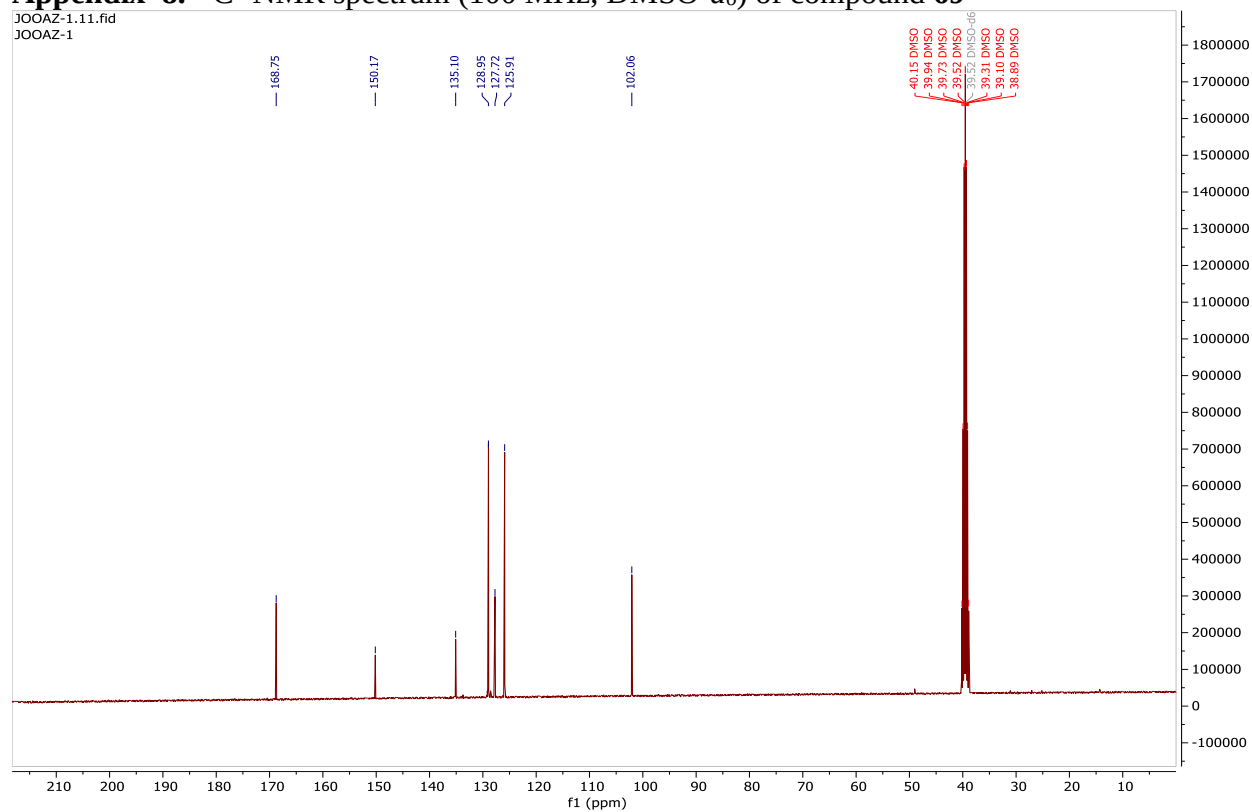
Appendix 6. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **64**



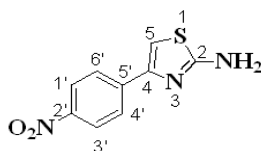
Appendix 7. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **69**



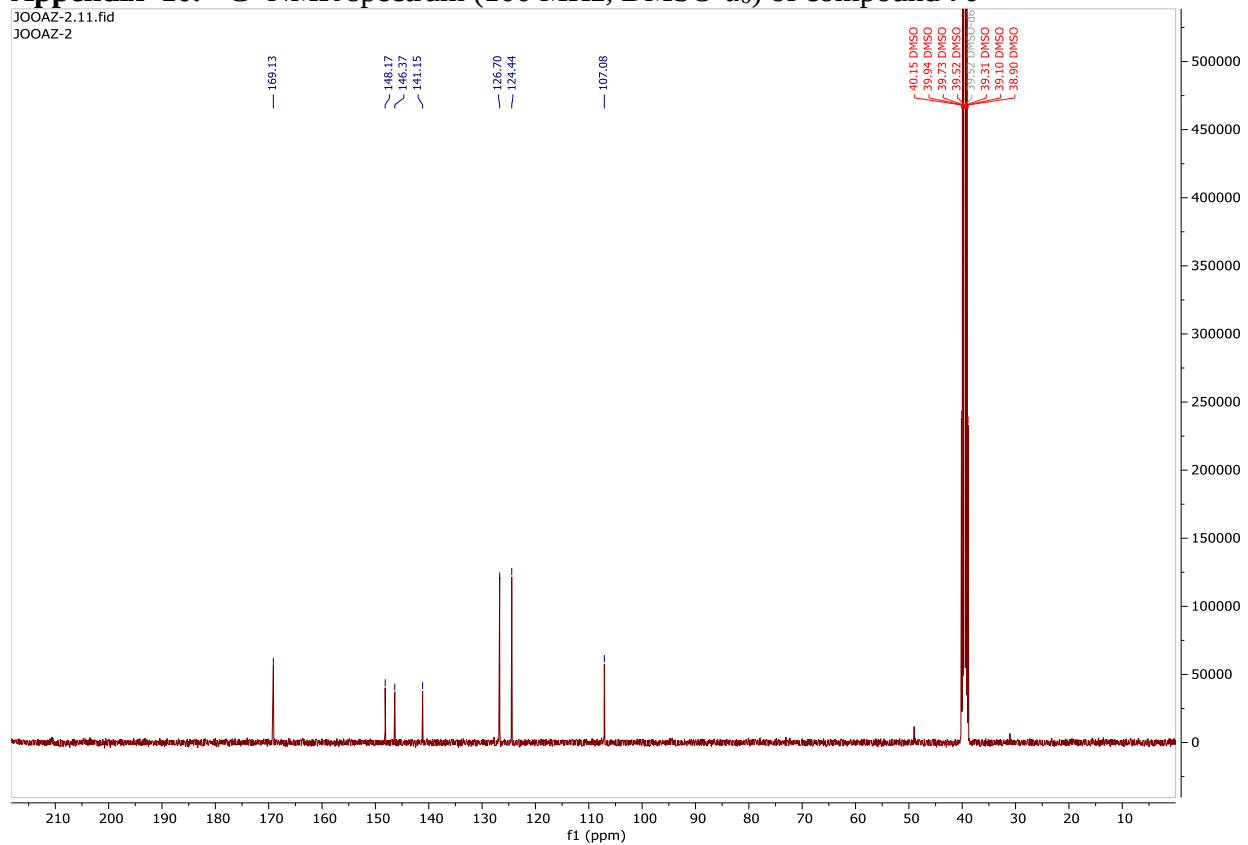
Appendix 8. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **69**



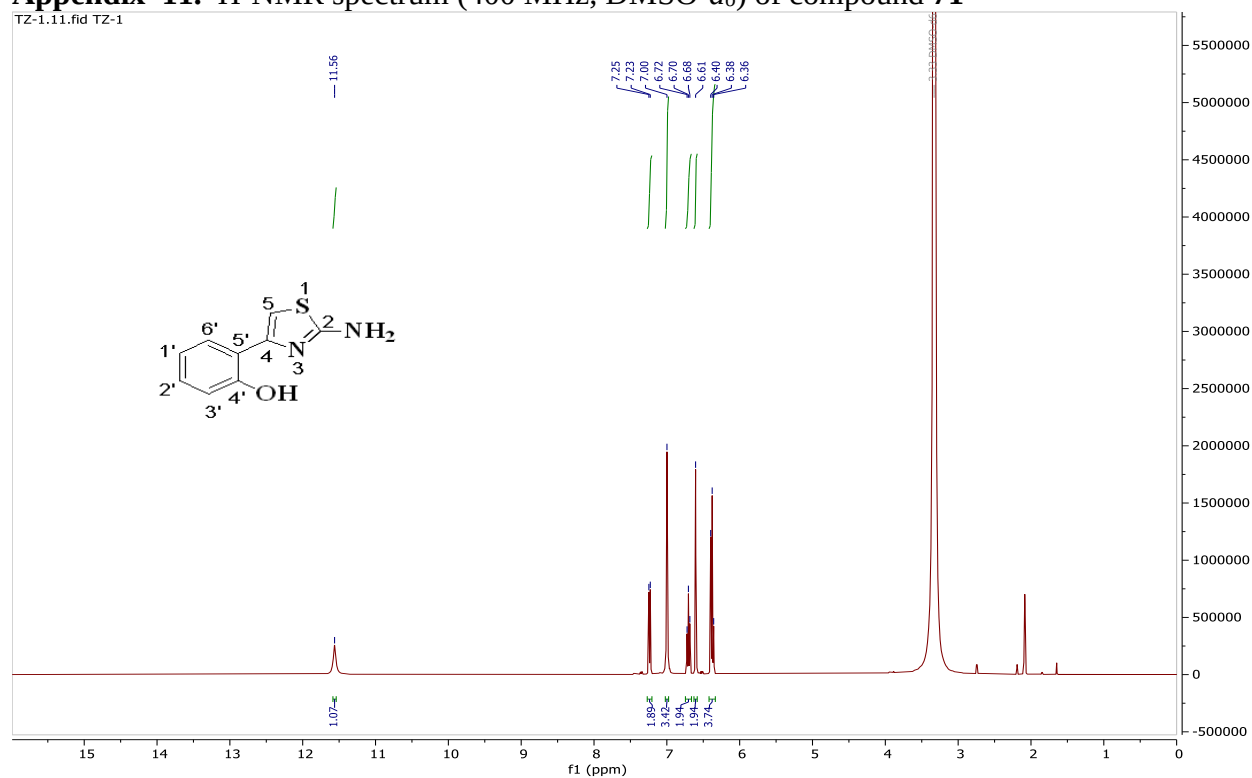
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JOOAZ-2



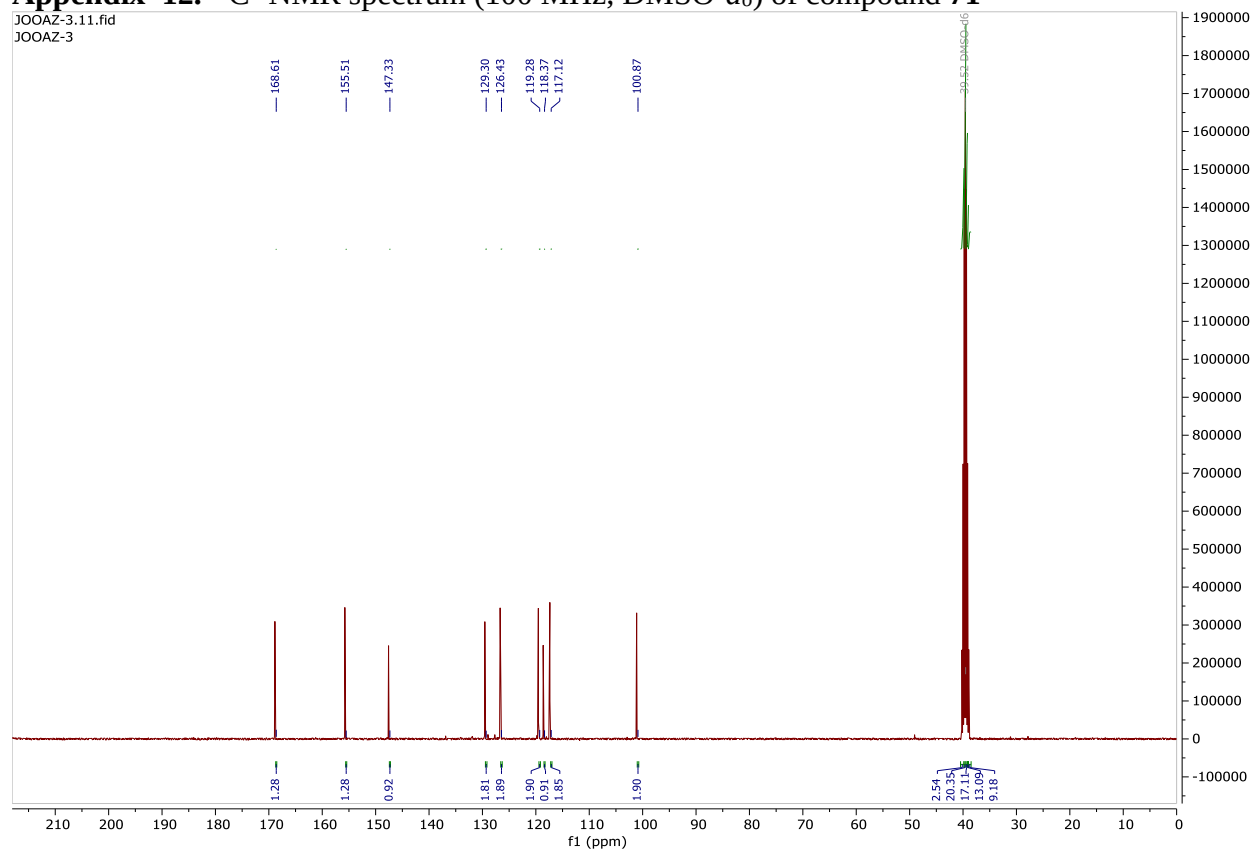
JOOAZ-2.11.fid
JOOAZ-2



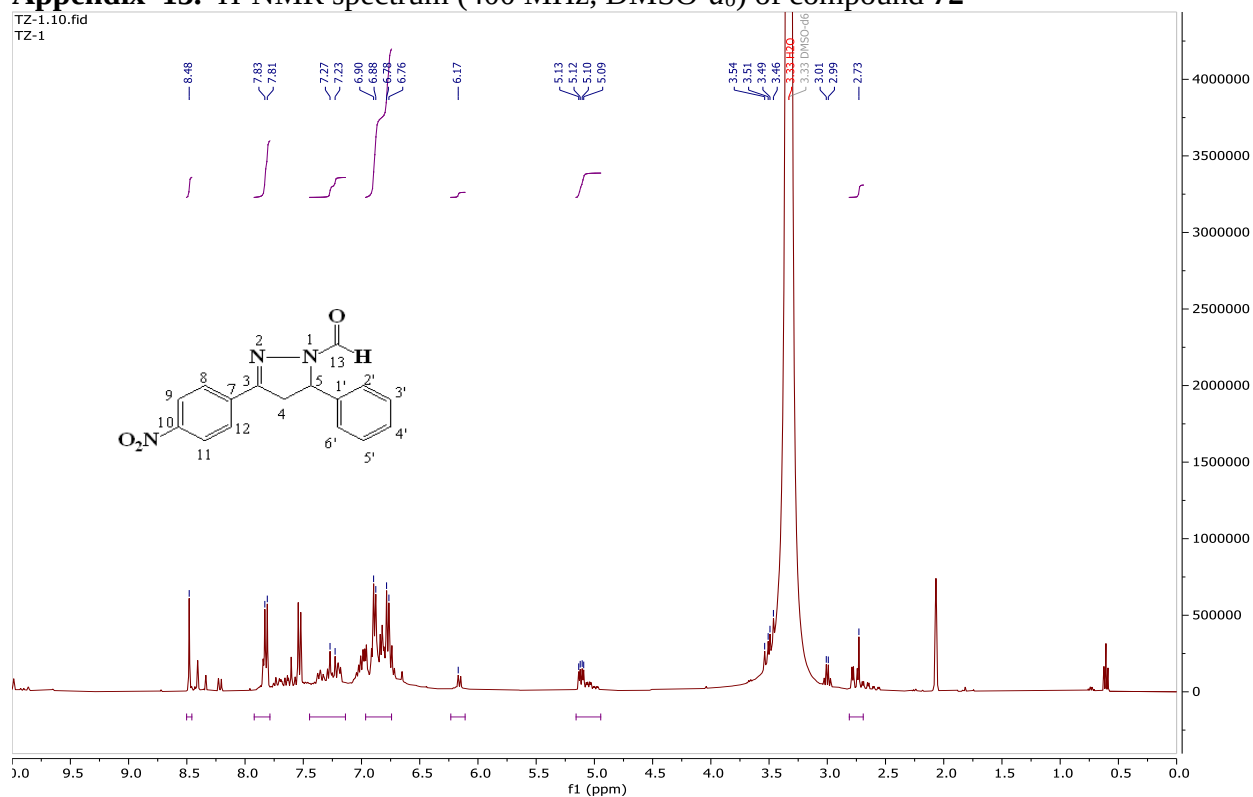
Appendix 11. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound 71



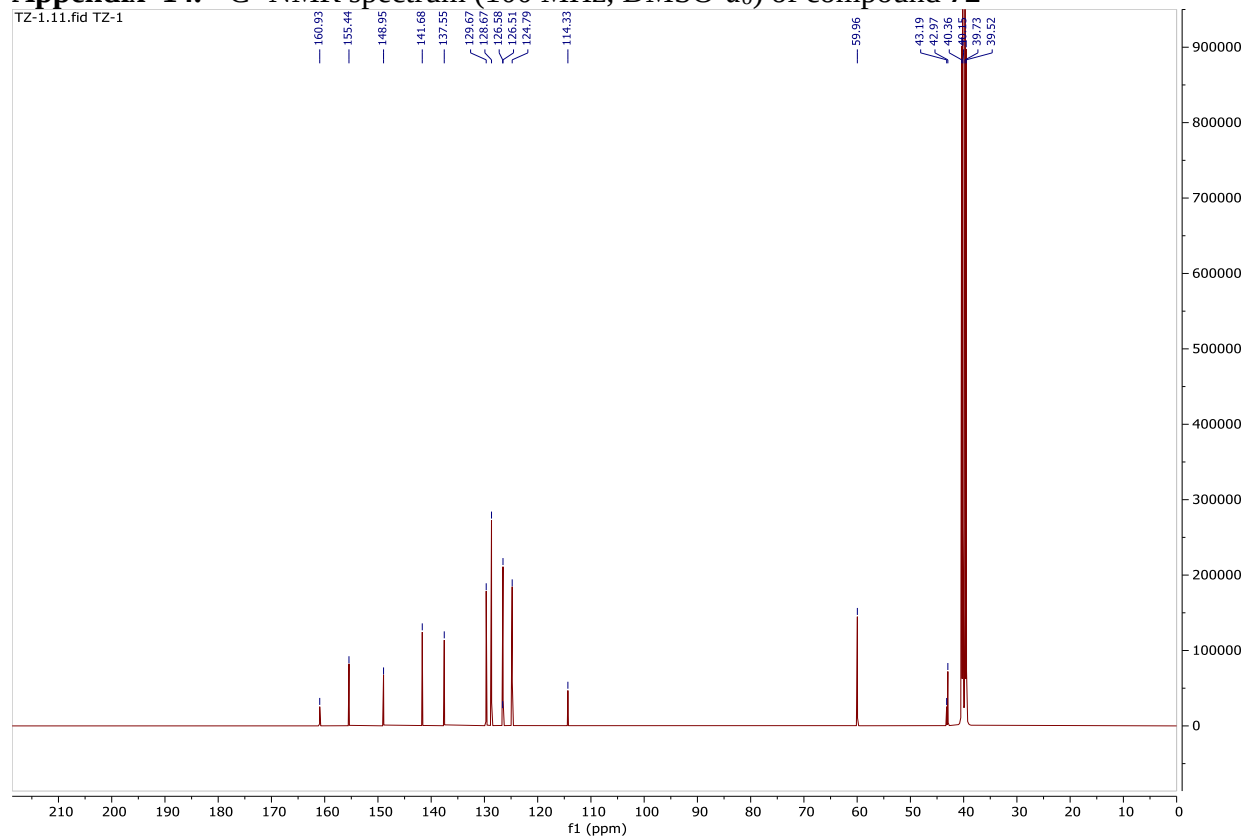
Appendix 12. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound 71



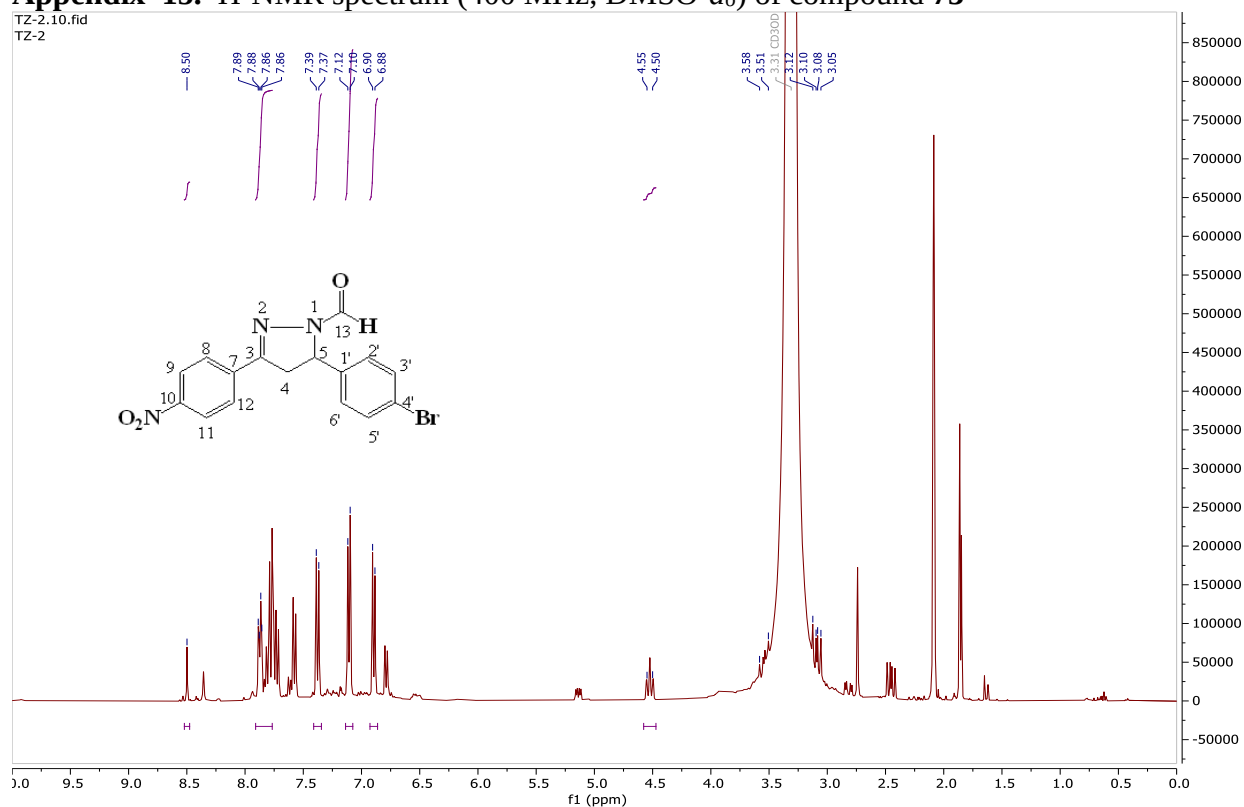
Appendix 13. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound 72



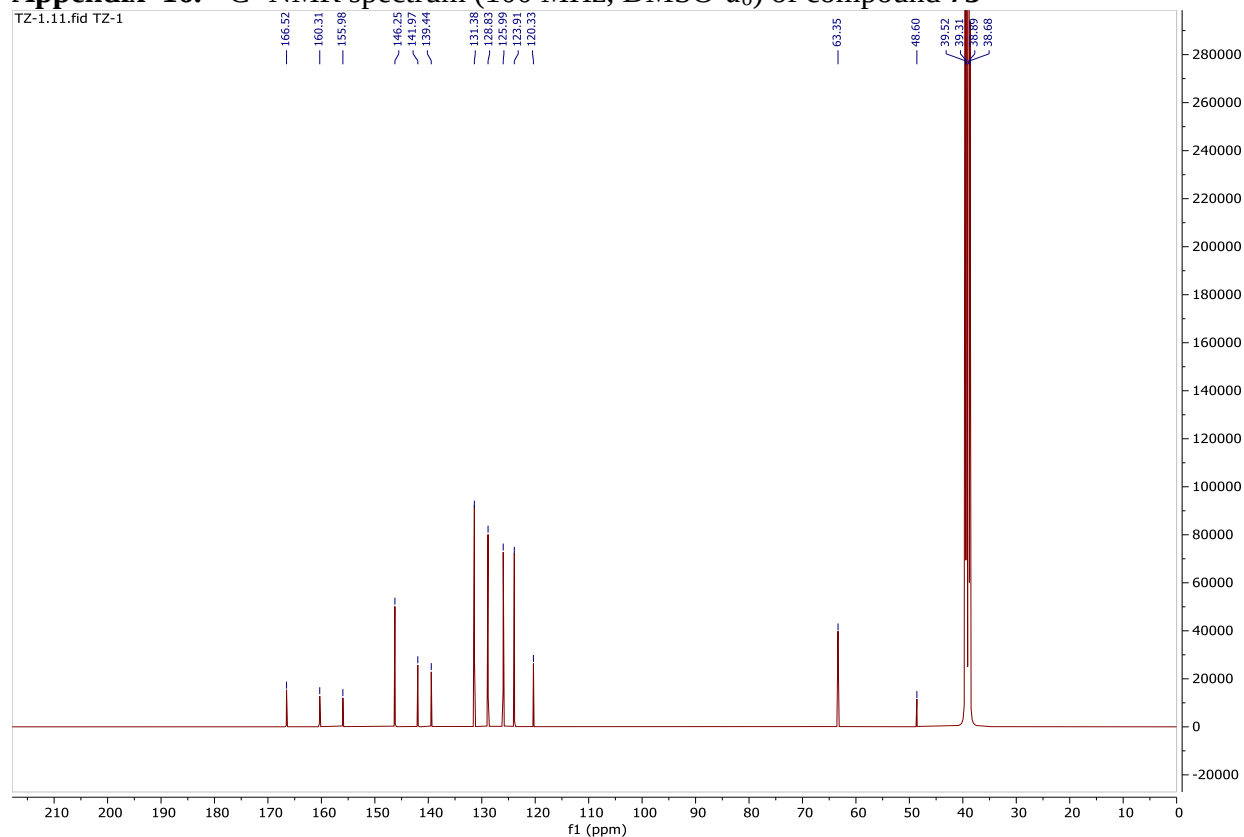
Appendix 14. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound 72



Appendix 15. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound 73



Appendix 16. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound 73



¹H NMR spectrum (400 MHz, DMSO-d₆) of compound 1

Chemical structure of compound 1 is shown, with atoms numbered 1 through 13. The structure is a benzimidazole derivative with a nitro group and a hydroxyl group.

The ¹H NMR spectrum (400 MHz, DMSO-d₆) is displayed, showing peaks in the aromatic region (6.5-8.6 ppm) and aliphatic region (2.1-3.4 ppm). The x-axis represents the chemical shift in ppm (f1), ranging from 0.0 to 9.5. The y-axis represents the intensity, ranging from -100,000 to 1,500,000.

Key peaks and integrations are labeled:

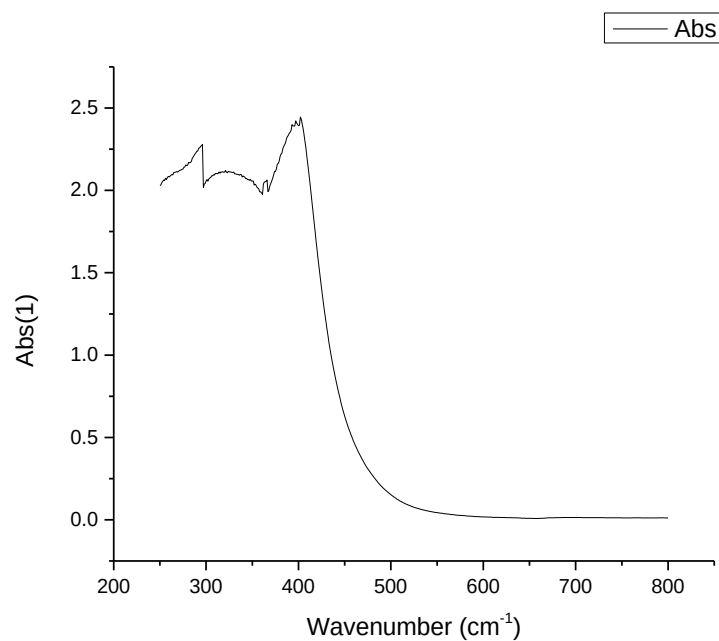
- 8.55, 8.37, 7.89, 7.87, 7.83, 7.81, 7.59, 7.57, 7.26, 7.24, 7.01, 6.99, 6.57, 5.81 (aromatic region, integration values shown above the peaks).
- 3.33 (NH), 3.29 (H₂O), 2.76, 2.10 (aliphatic region, integration values shown above the peaks).

The spectrum shows a complex pattern of peaks in the aromatic region, likely corresponding to the protons of the benzimidazole core and the nitro-substituted benzene ring. The aliphatic region shows a broad peak at 3.33 ppm (NH) and a sharp peak at 2.10 ppm (H₂O).

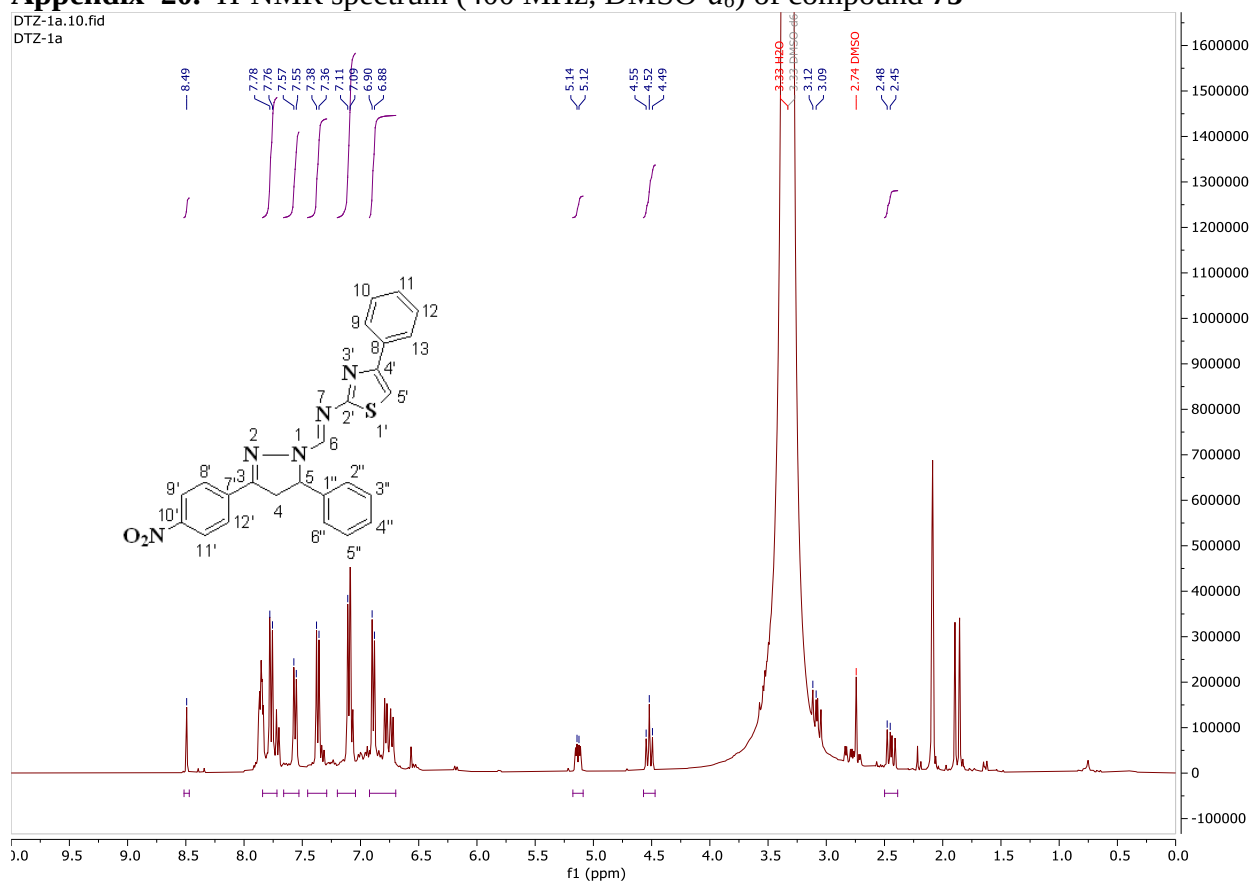
13C NMR Spectrum of 10b

Chemical shift (ppm): 167.36, 163.76, 159.40, 148.48, 134.20, 131.82, 128.46, 124.43, 124.37, 120.56, 118.87, 117.36, 65.70, 49.44, 40.36, 39.73.

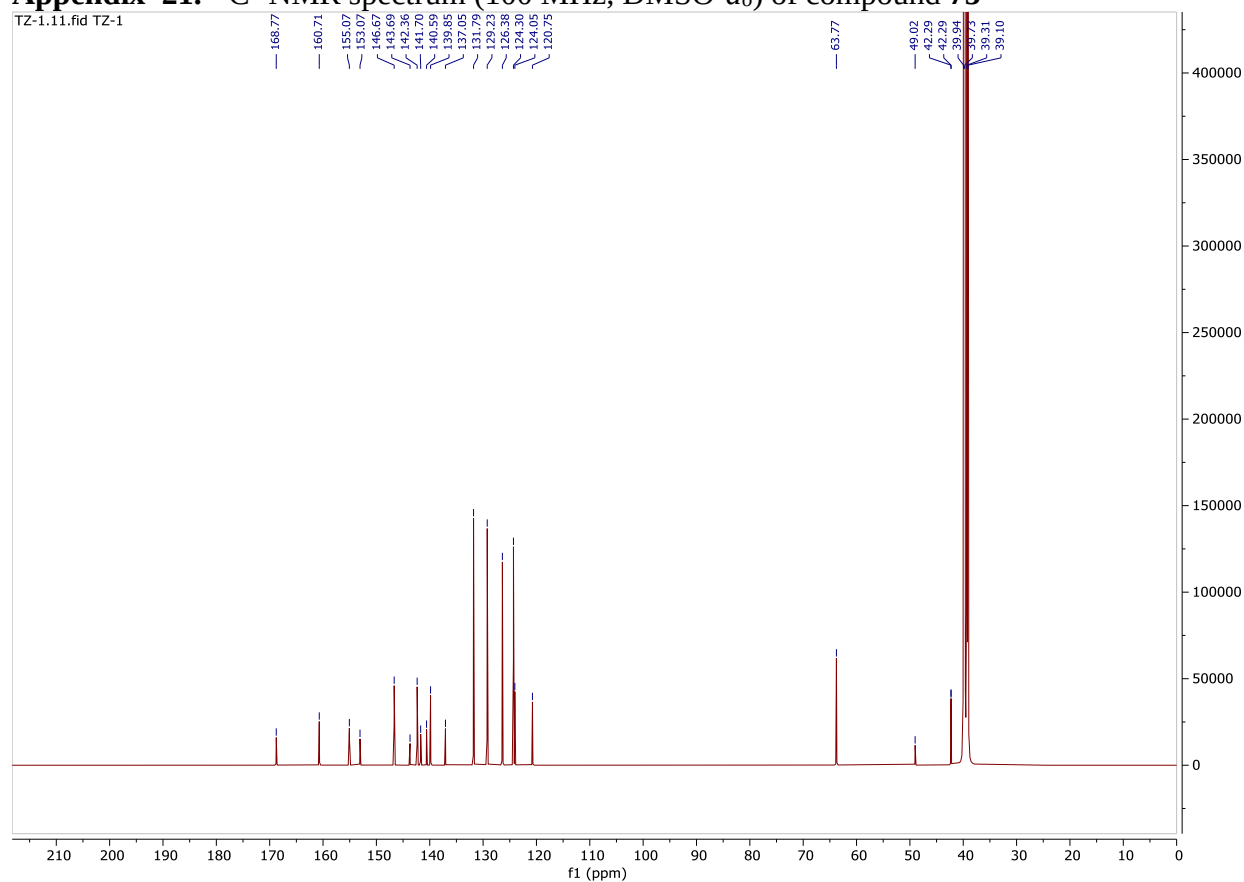
Appendix 19. UV-Visible spectrum of compound 75



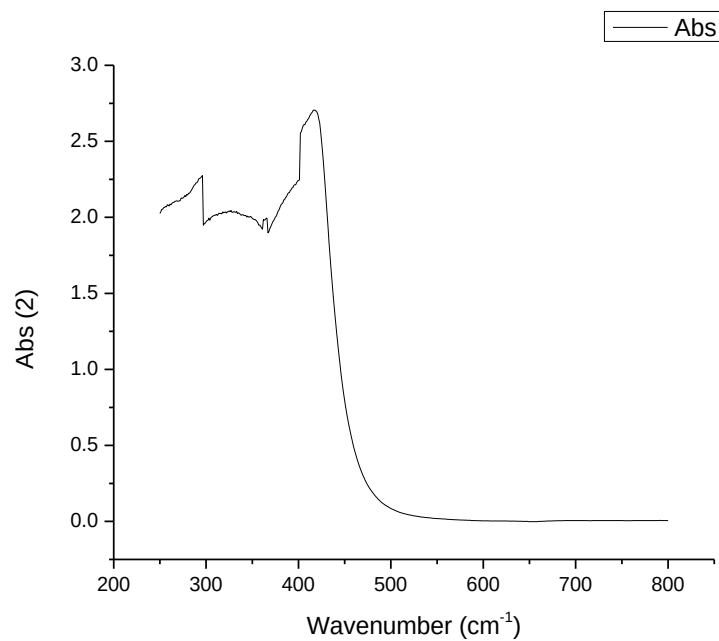
Appendix 20. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **75**



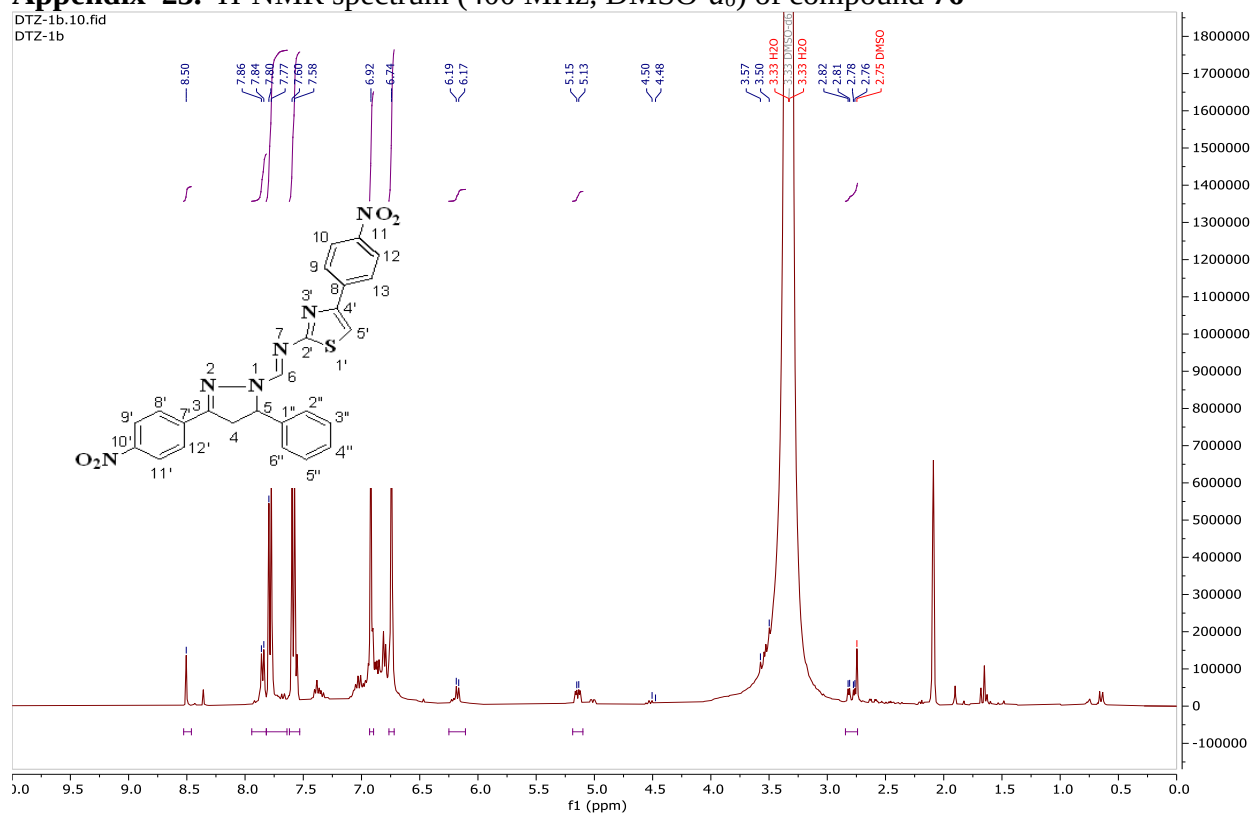
Appendix 21. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound 75



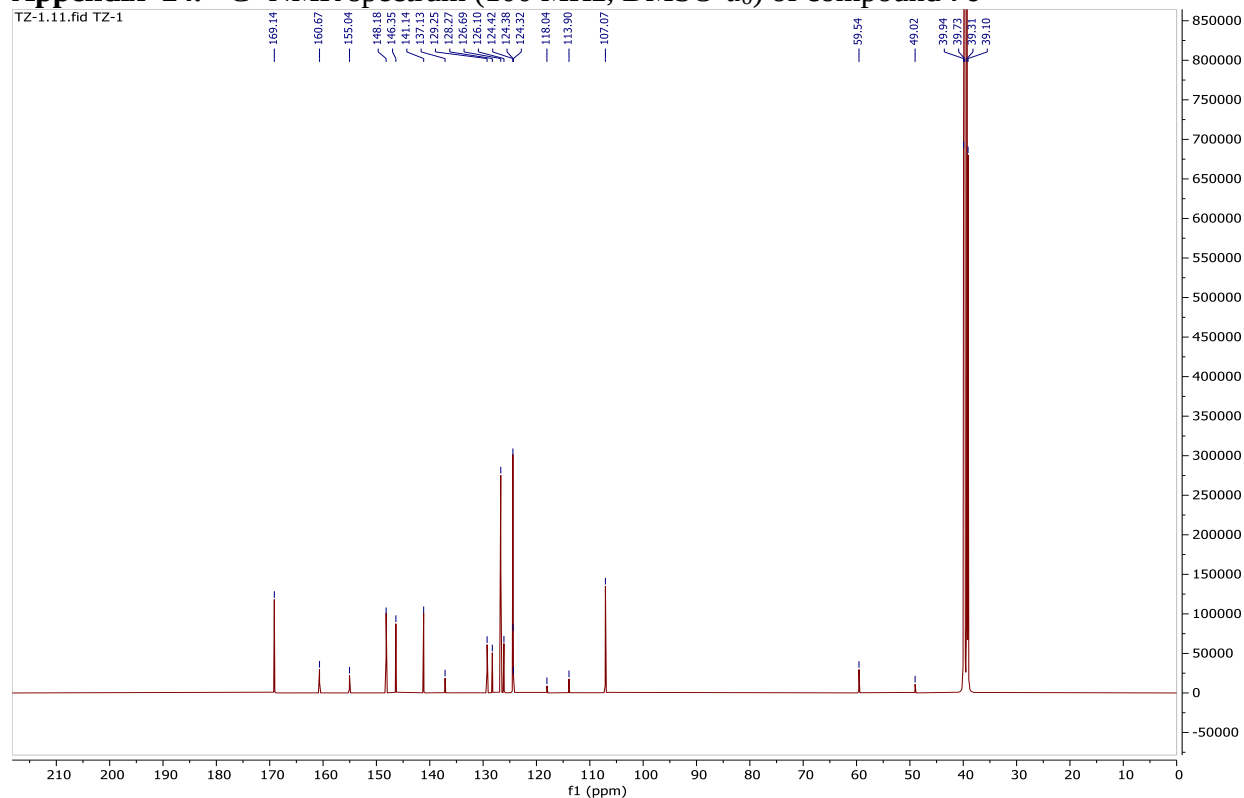
Appendix 22. UV-Visible spectrum of compound 76



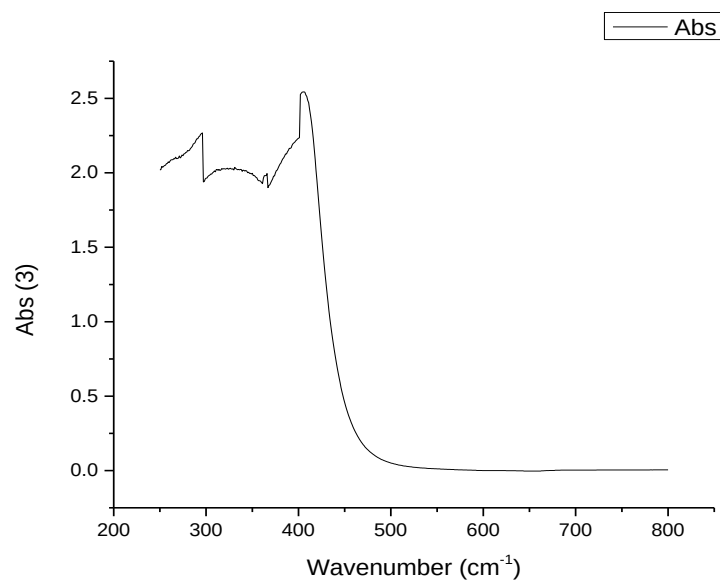
Appendix 23. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **76**



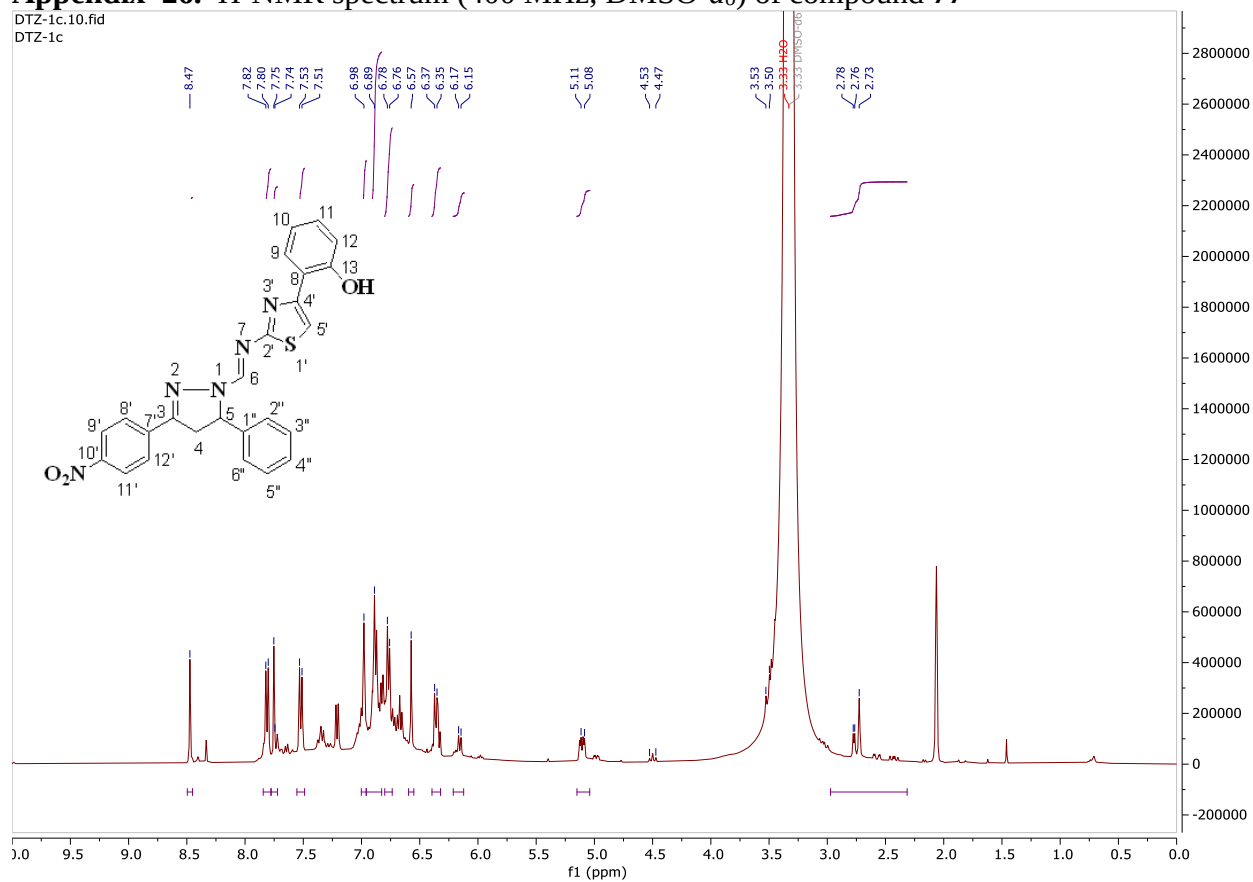
Appendix 24. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **76**



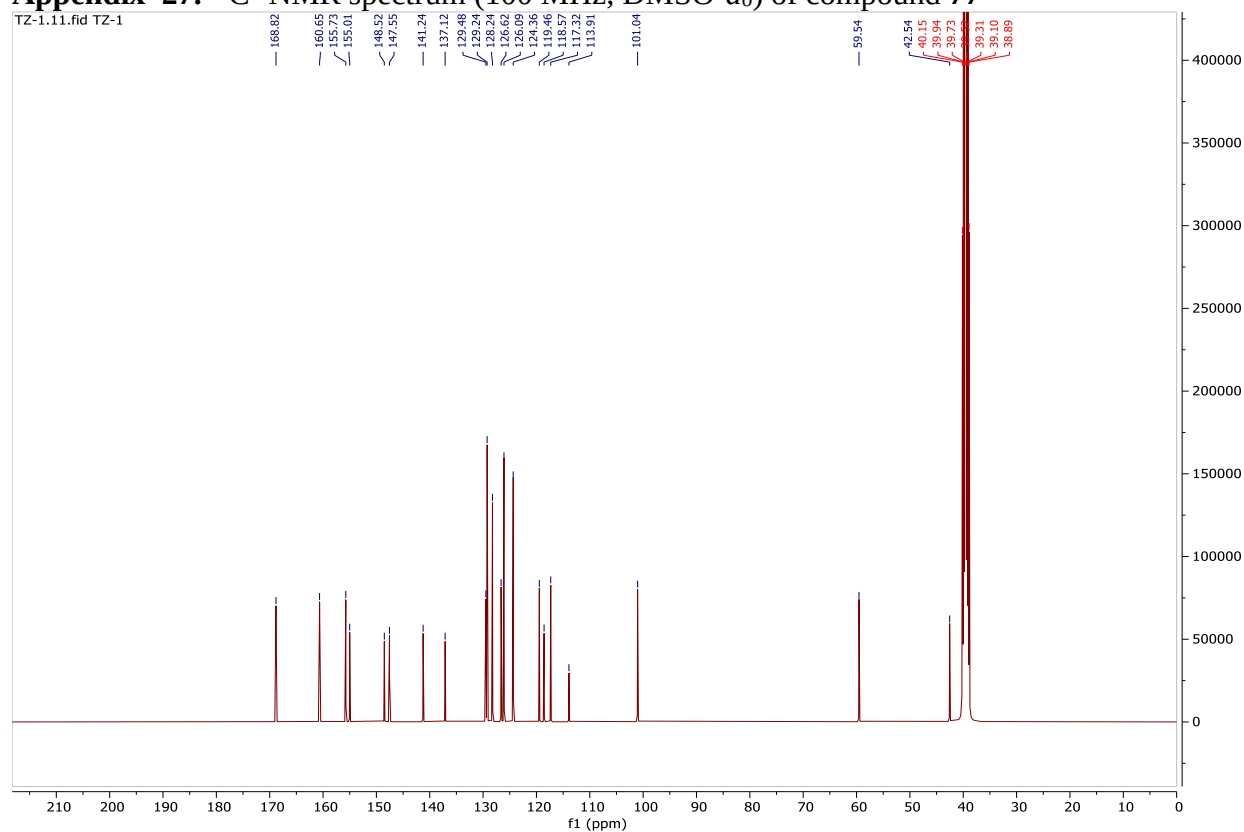
Appendix 25. UV-Visible spectrum of compound 77



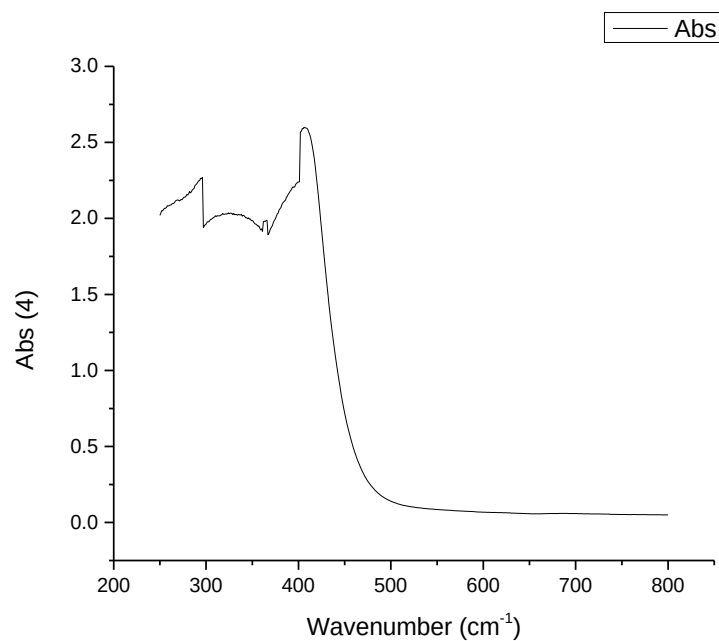
Appendix 26. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound 77



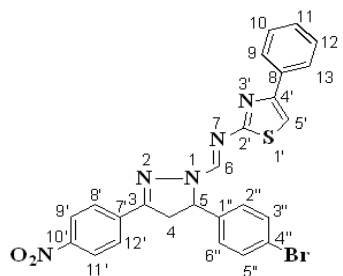
Appendix 27. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **77**



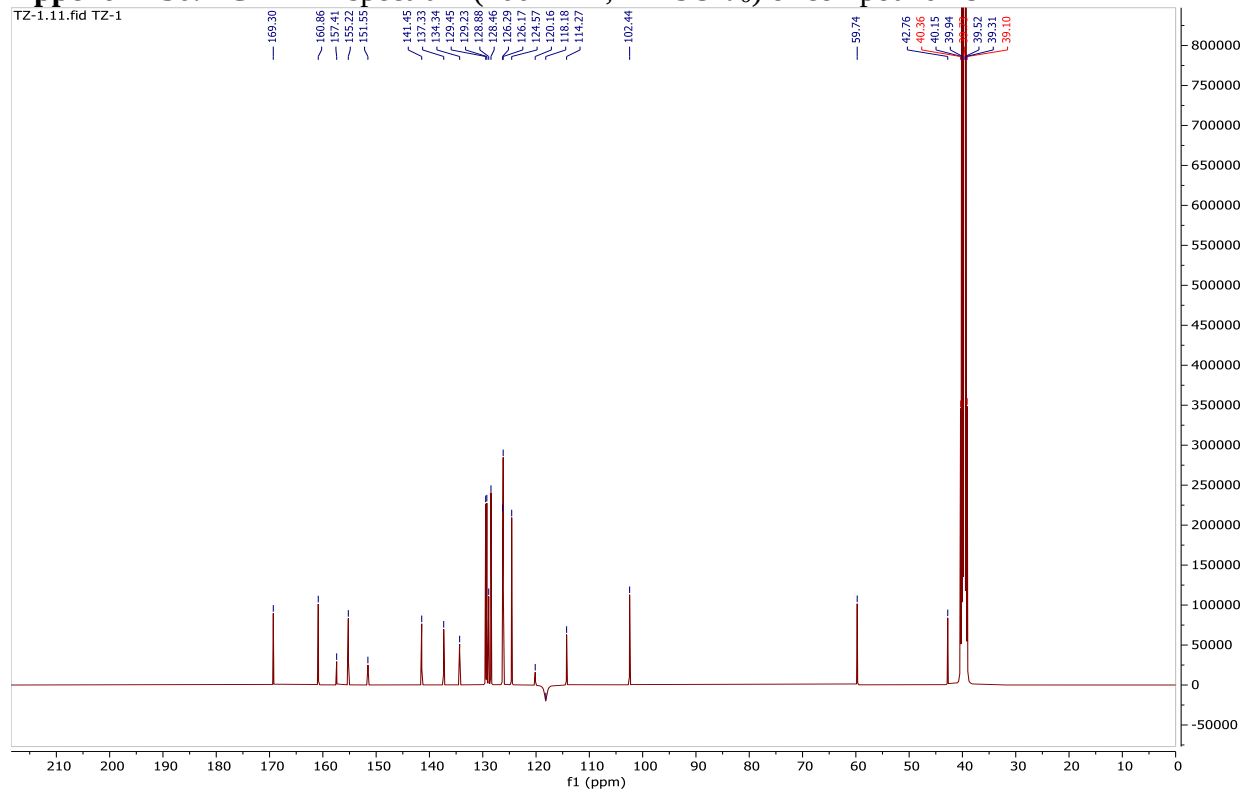
Appendix 28. UV-Visible spectrum of compound **78**



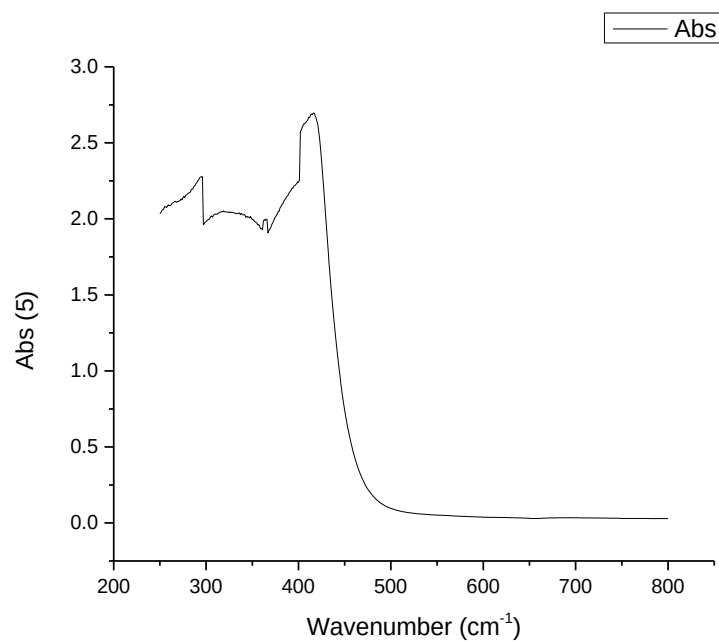
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DTZ-2a



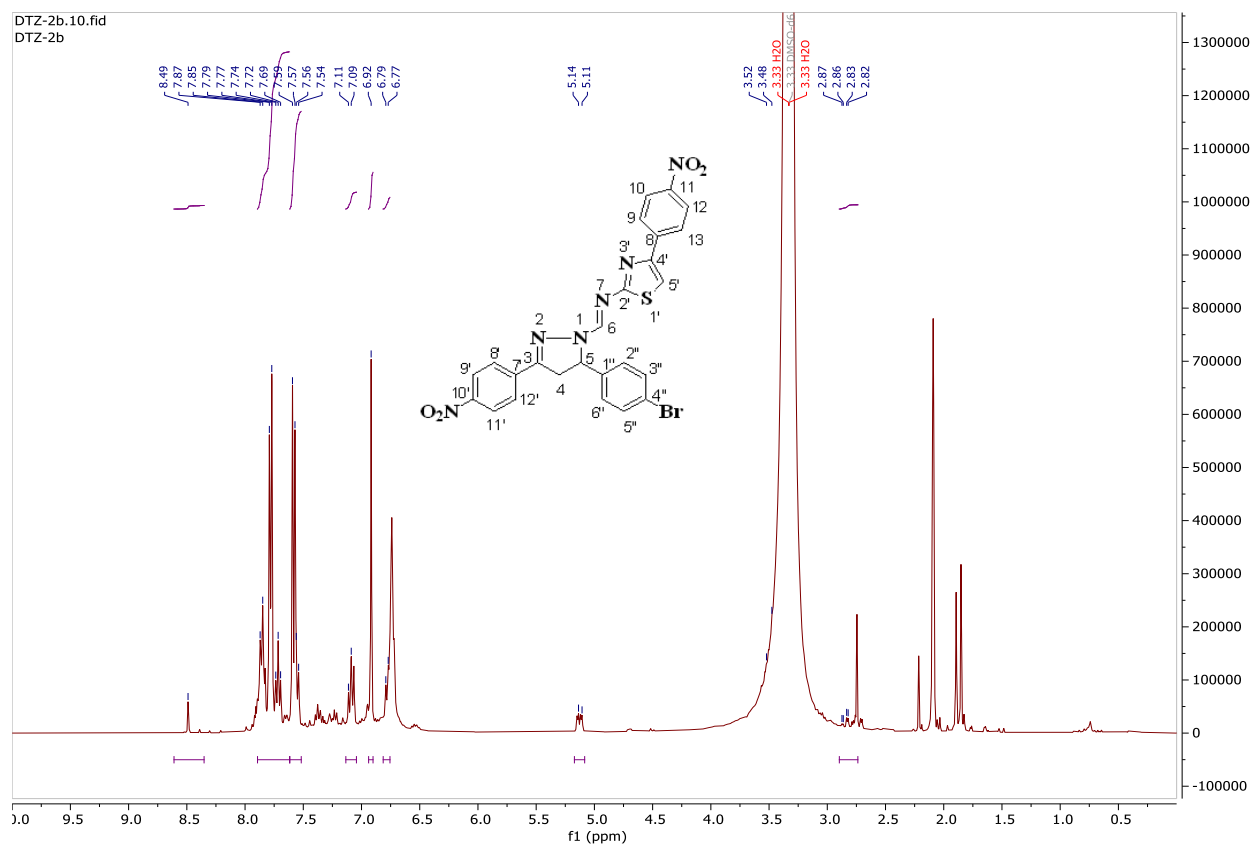
TZ-1.11.fid TZ-1



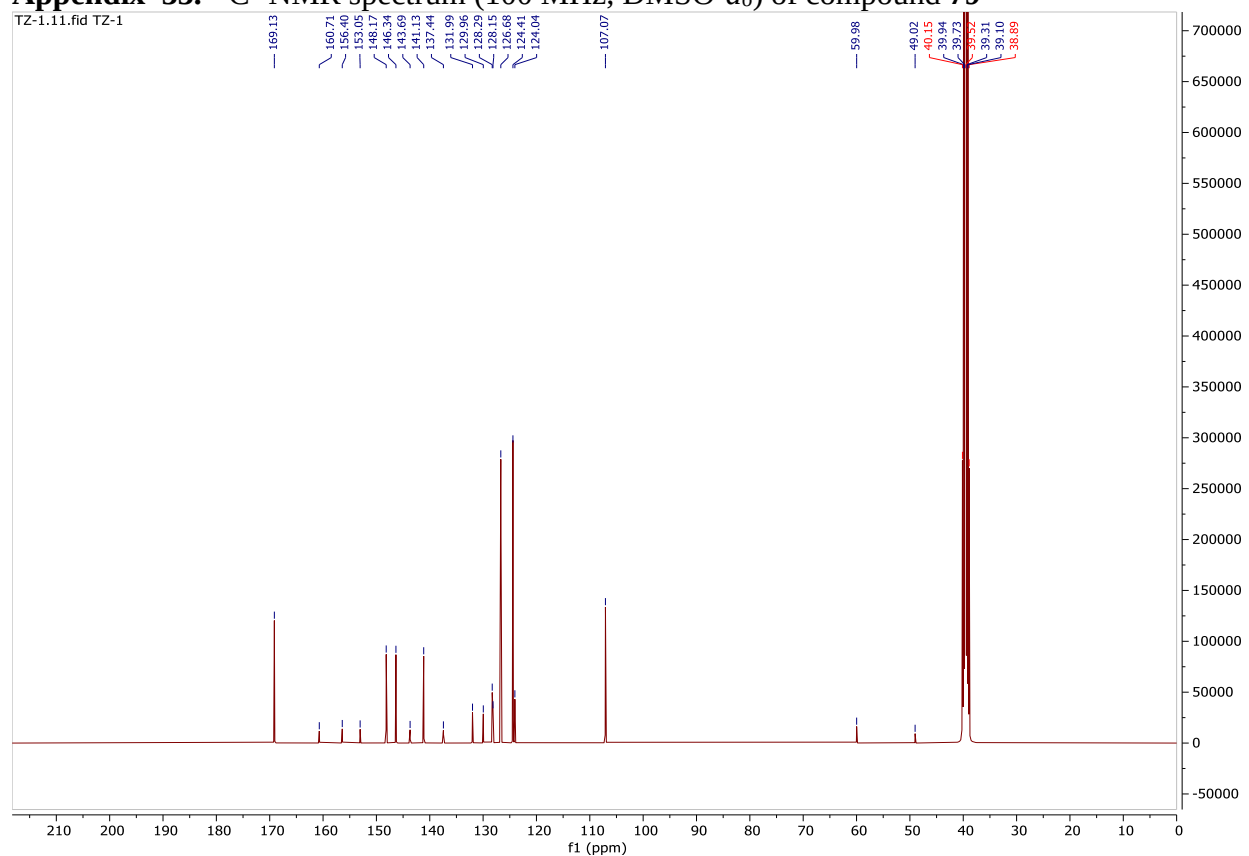
Appendix 31. UV-Visible spectrum of compound 79



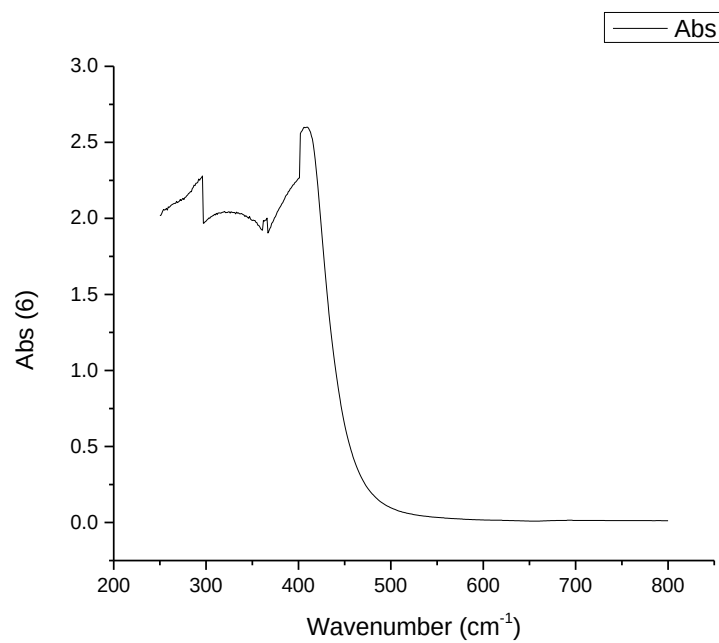
Appendix 32. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound 79



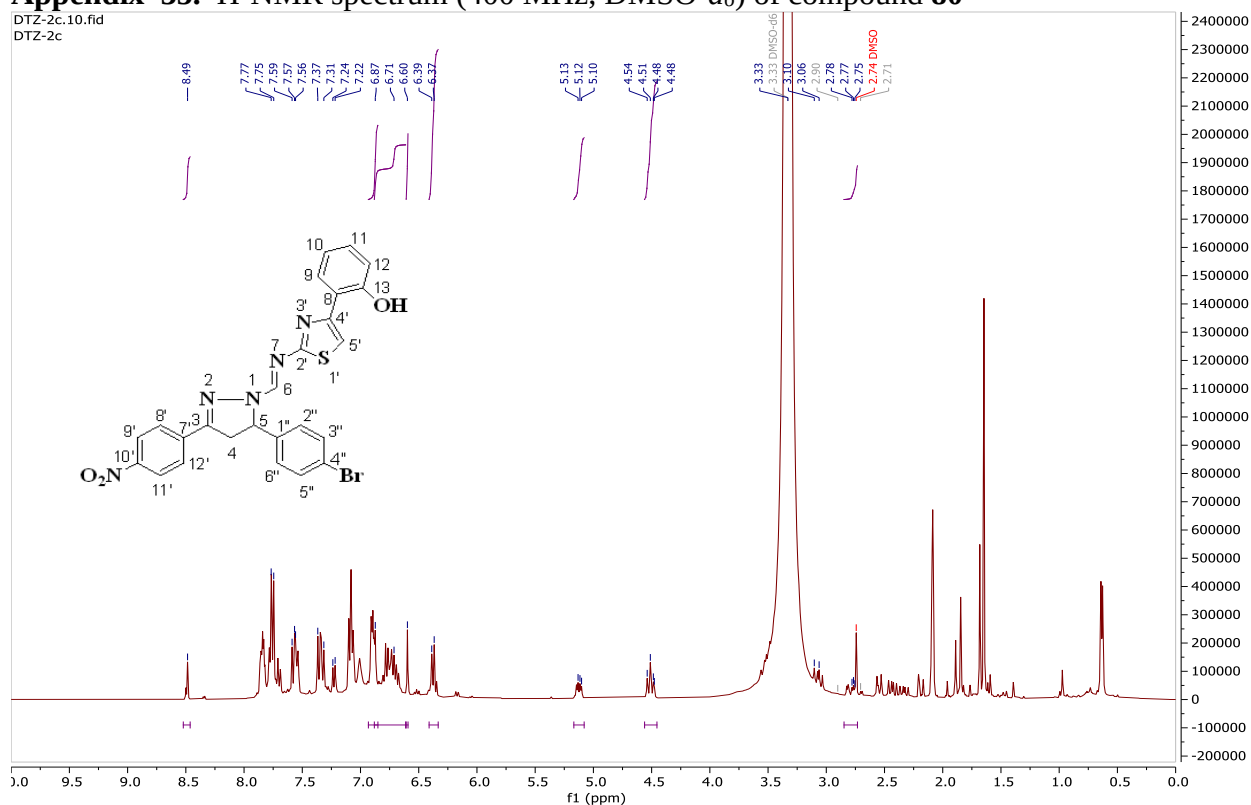
Appendix 33. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **79**



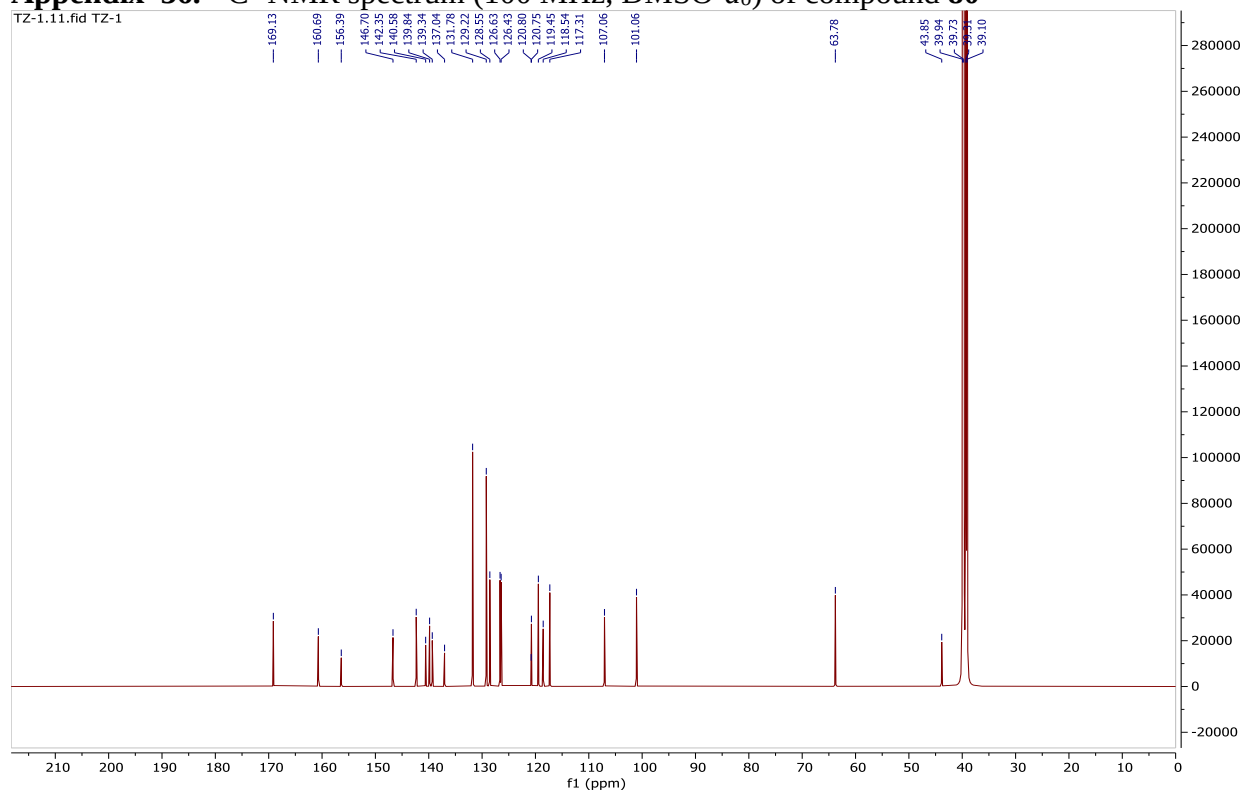
Appendix 34. UV-Visible spectrum of compound **80**



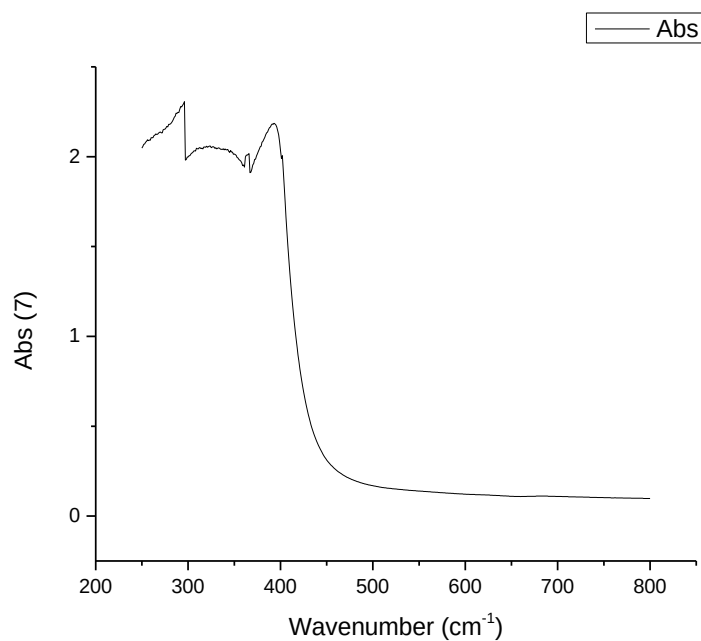
Appendix 35. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **80**



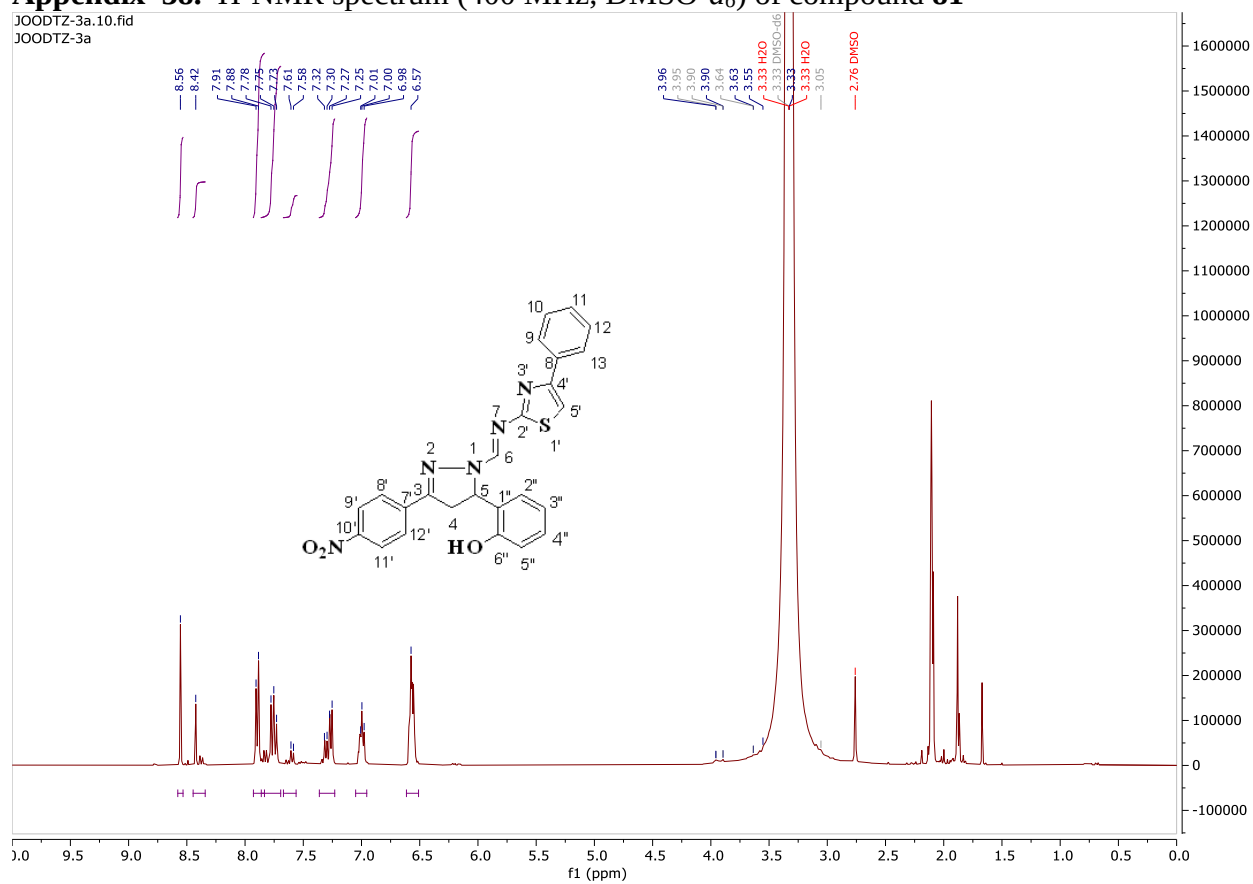
Appendix 36. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **80**



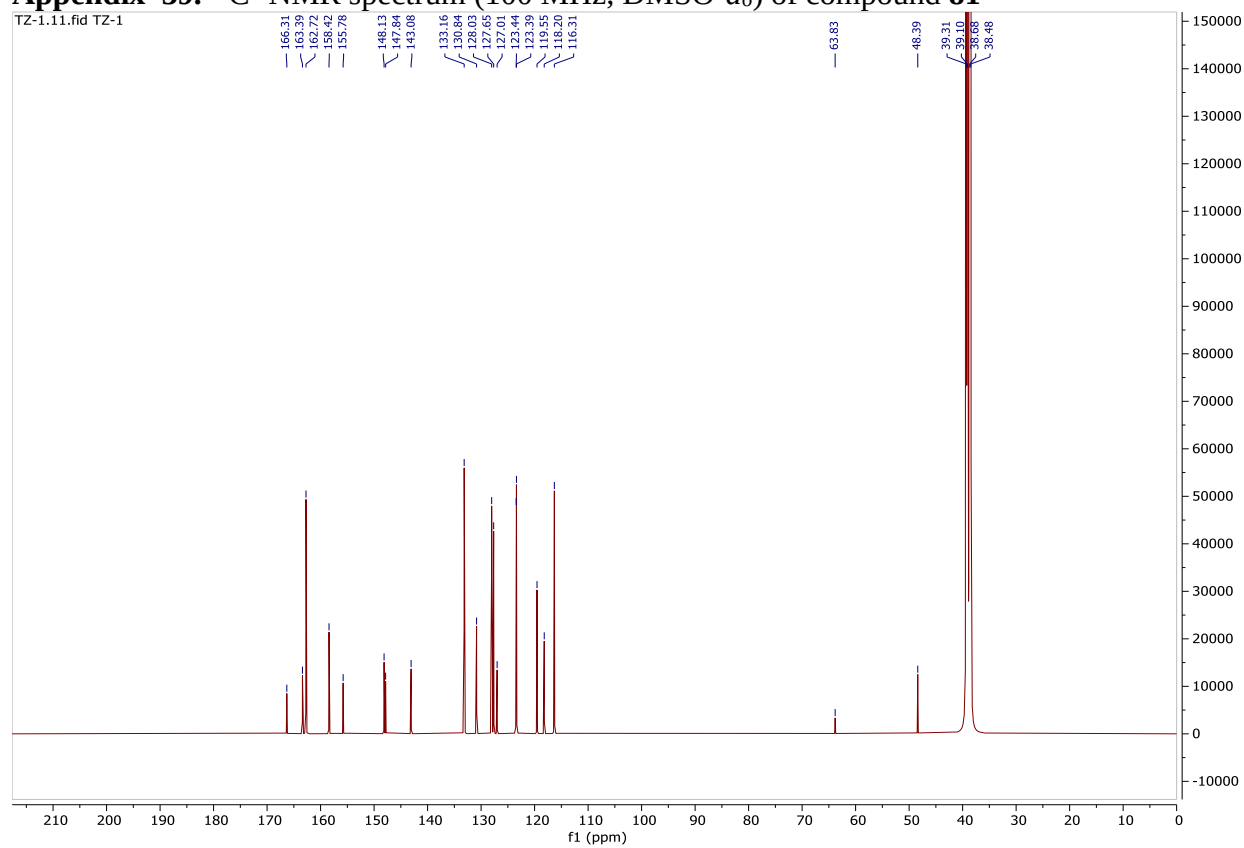
Appendix 37. UV-Visible spectrum of compound **81**



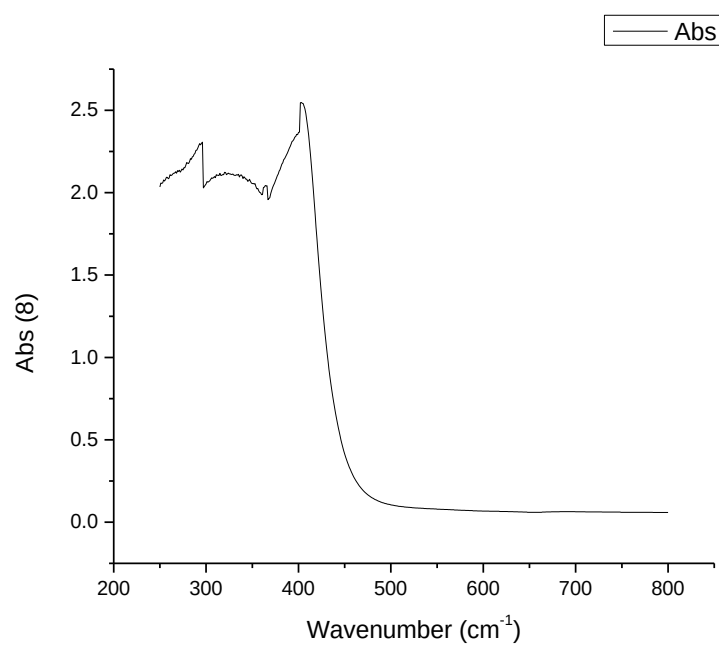
Appendix 38. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound **81**



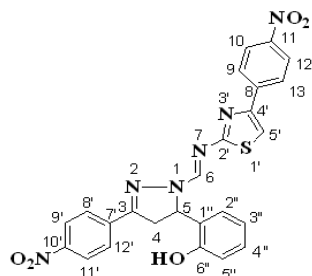
Appendix 39. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **81**



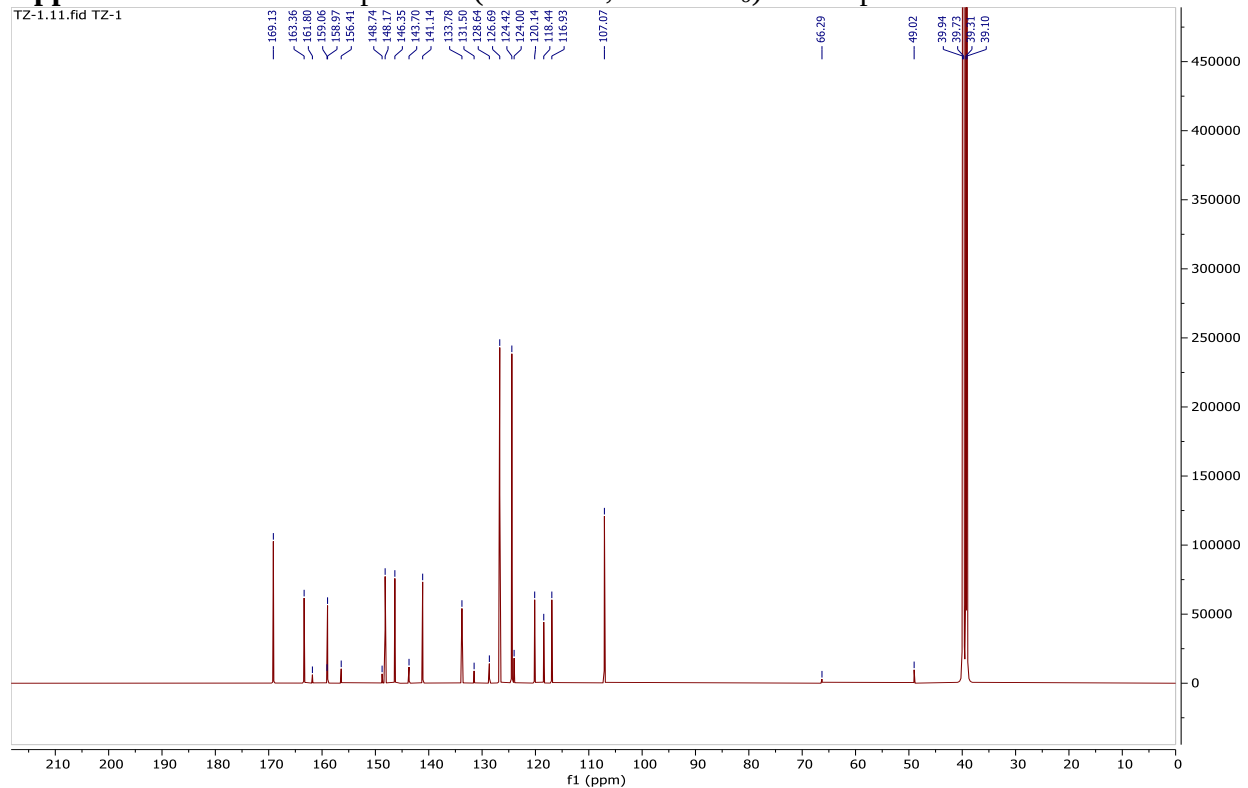
Appendix 40. UV-Visible spectrum of compound **82**



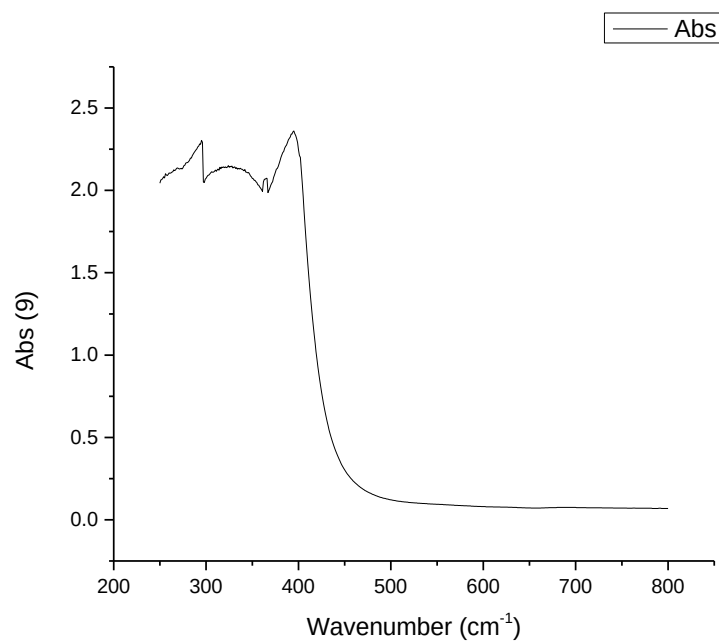
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JOODTZ-3b



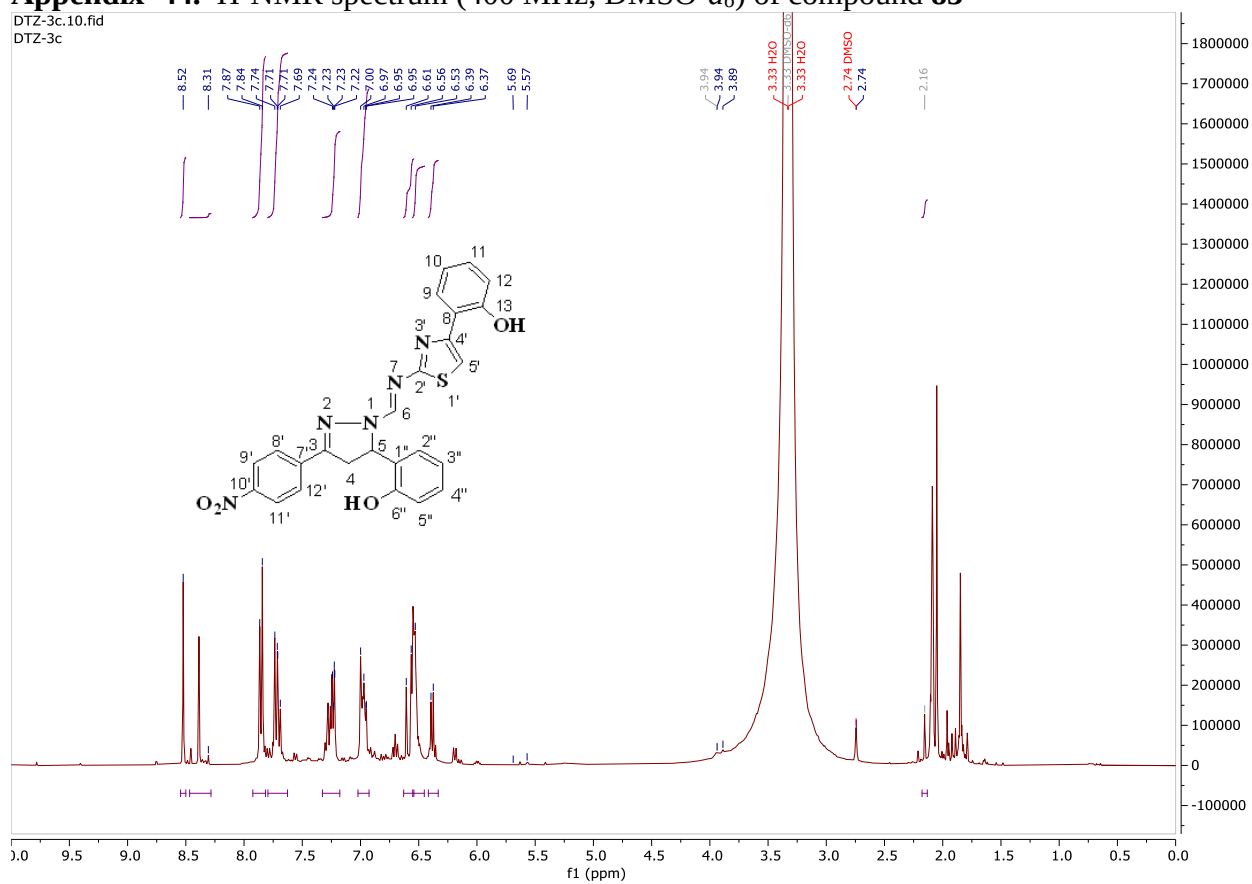
TZ-1.11.fid TZ-1



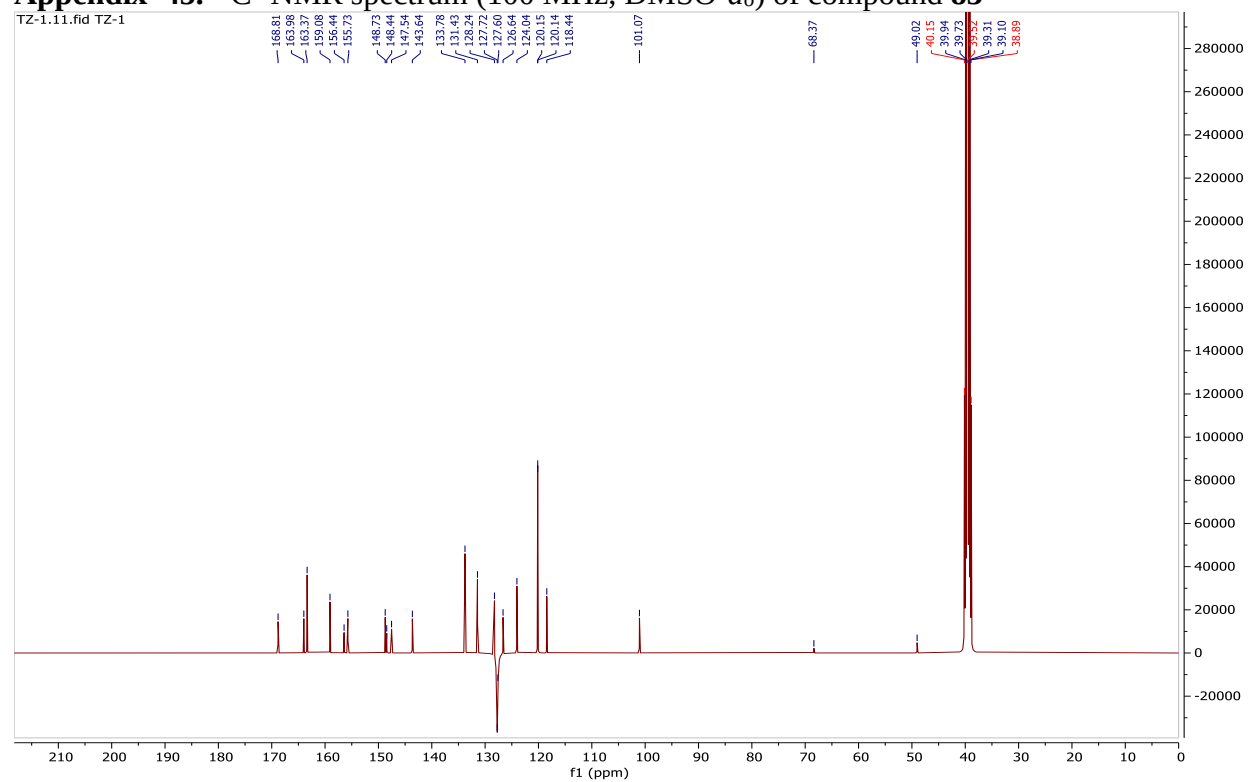
Appendix 43. UV-Visible spectrum of compound 83



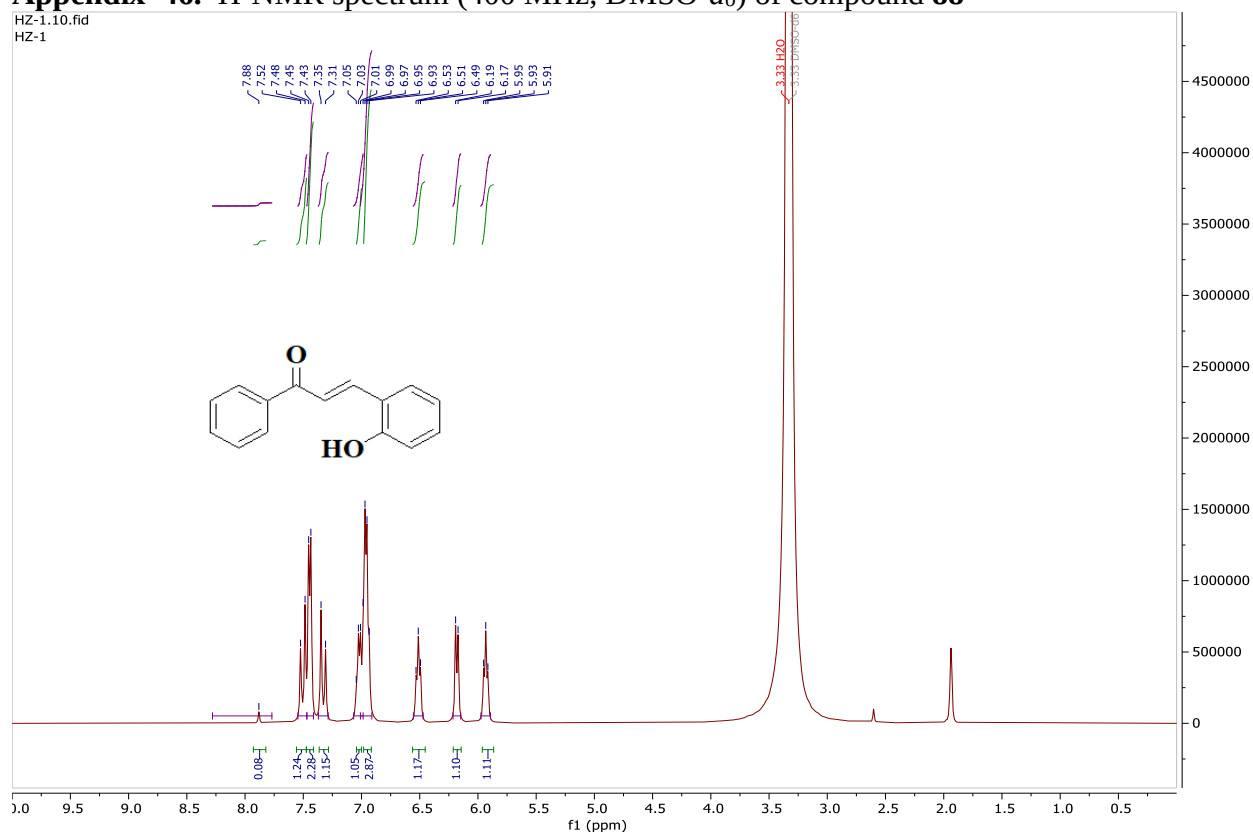
Appendix 44. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound **83**



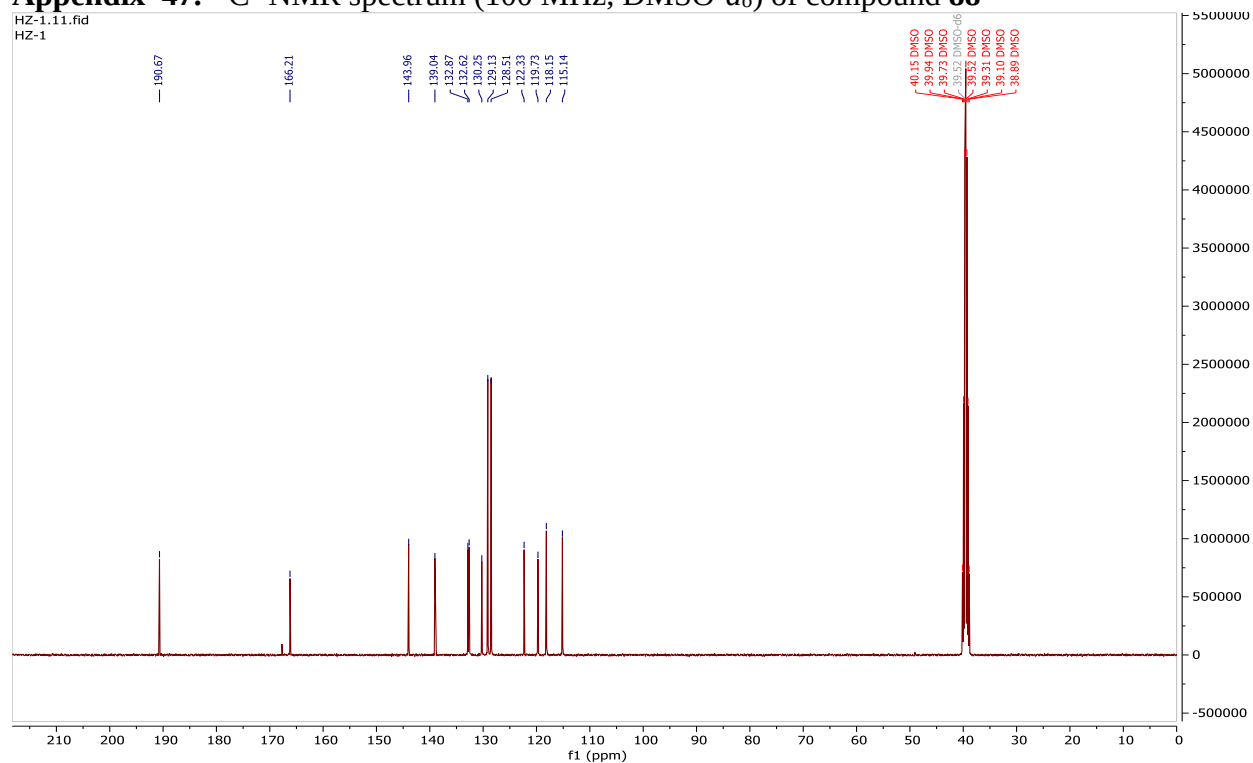
Appendix 45. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **83**



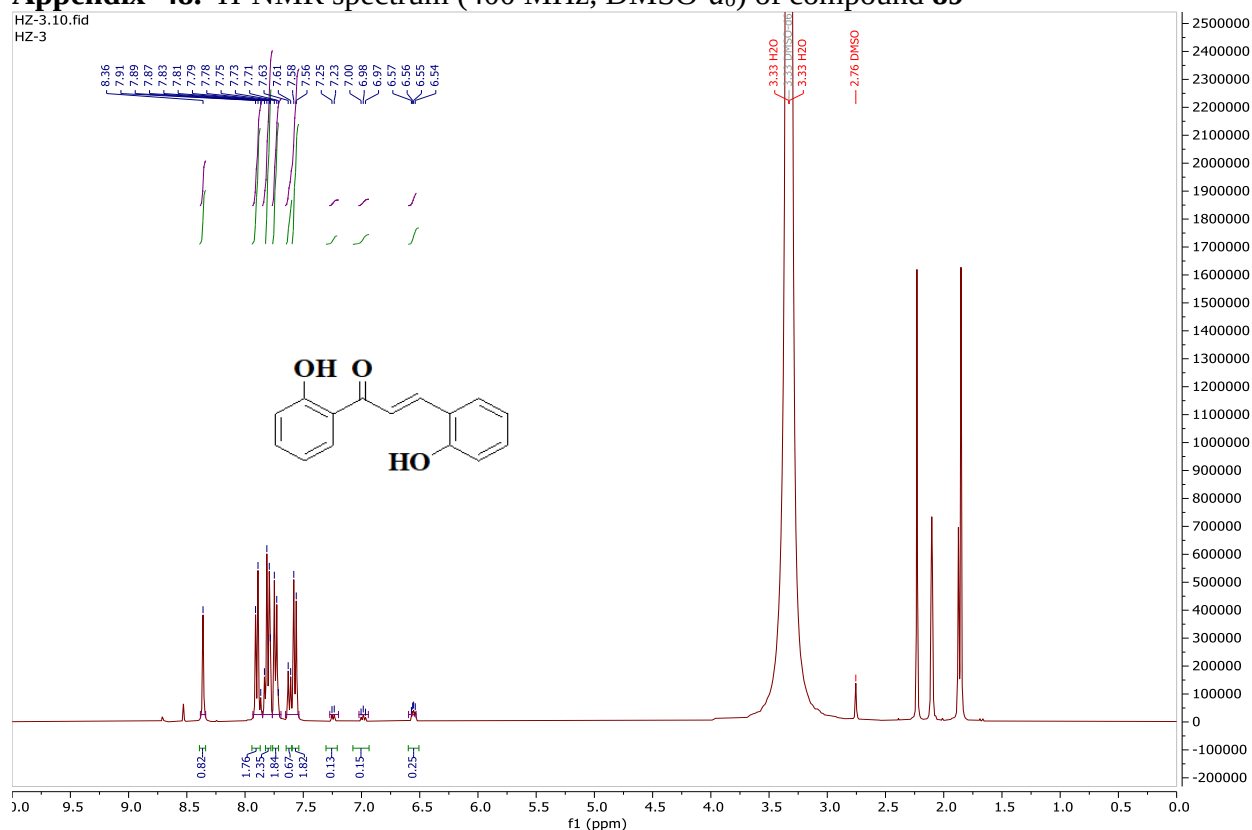
Appendix 46. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **88**



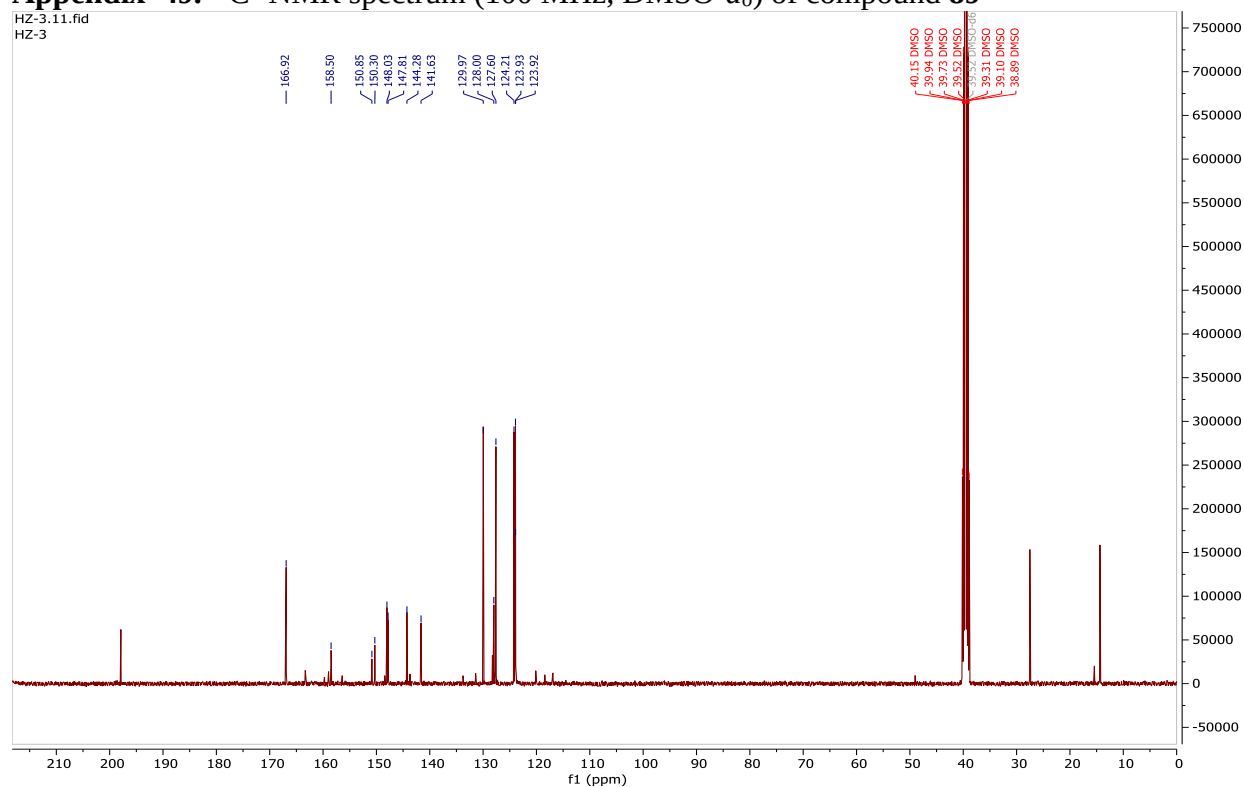
Appendix 47. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **88**



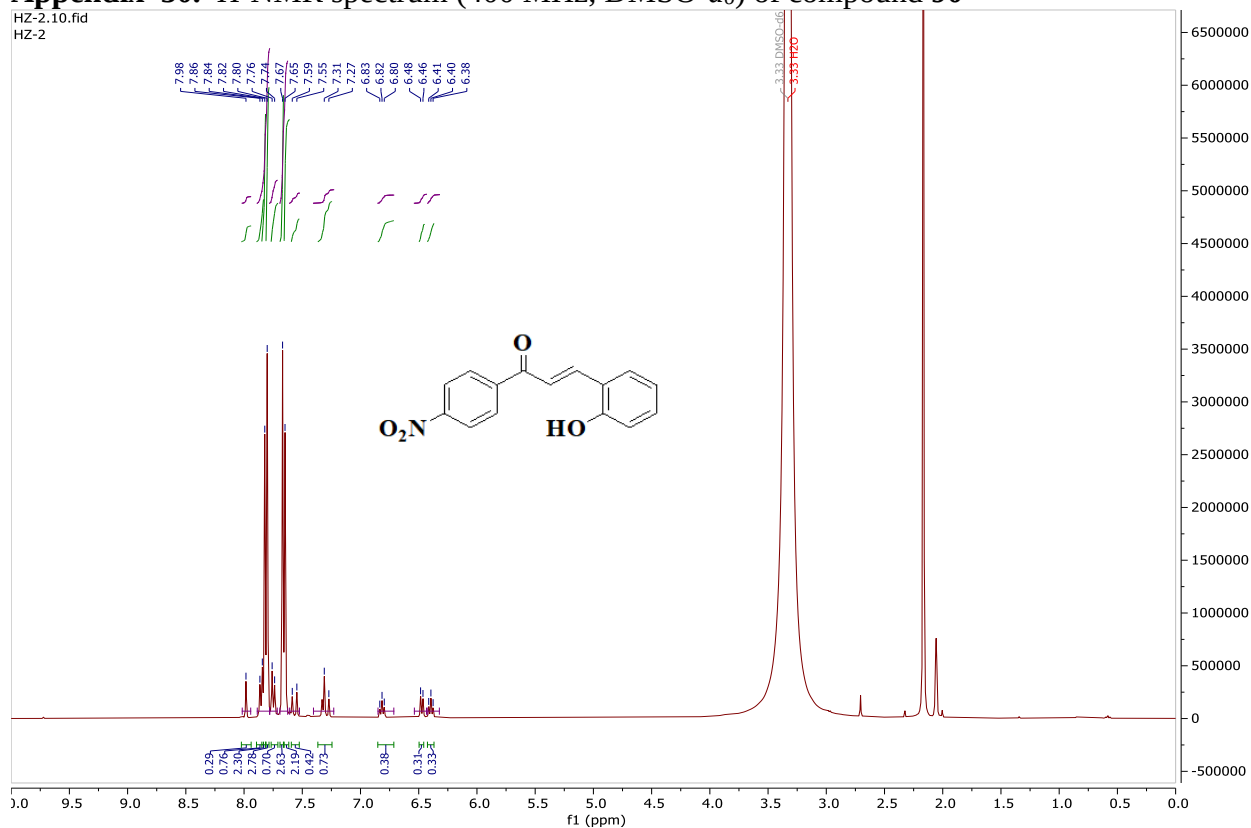
Appendix 48. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **89**



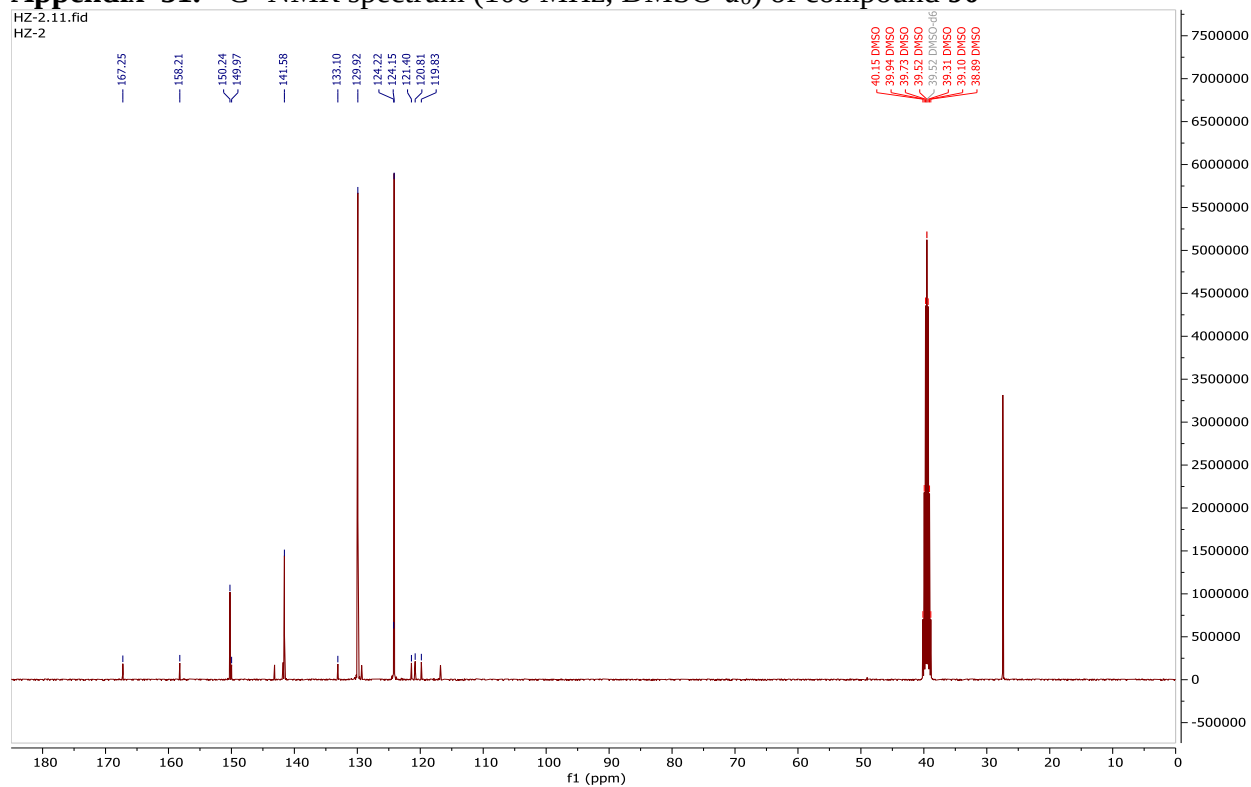
Appendix 49. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **89**



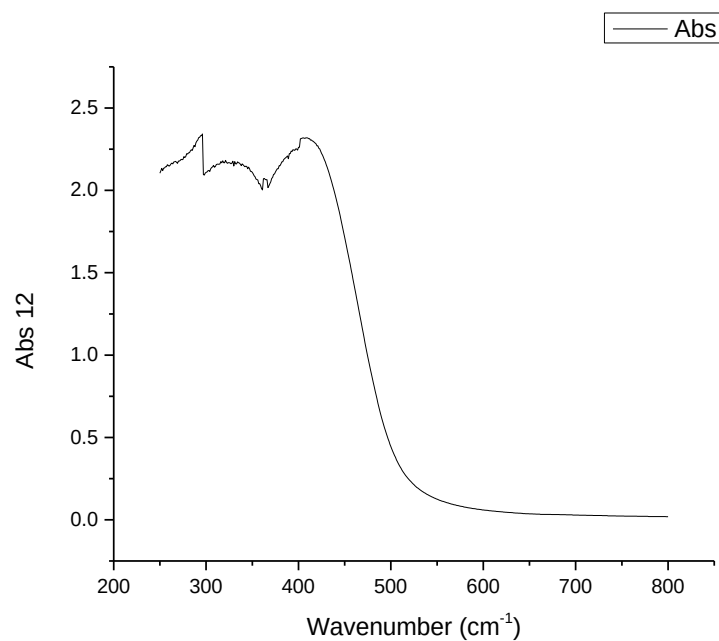
Appendix 50. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound **90**



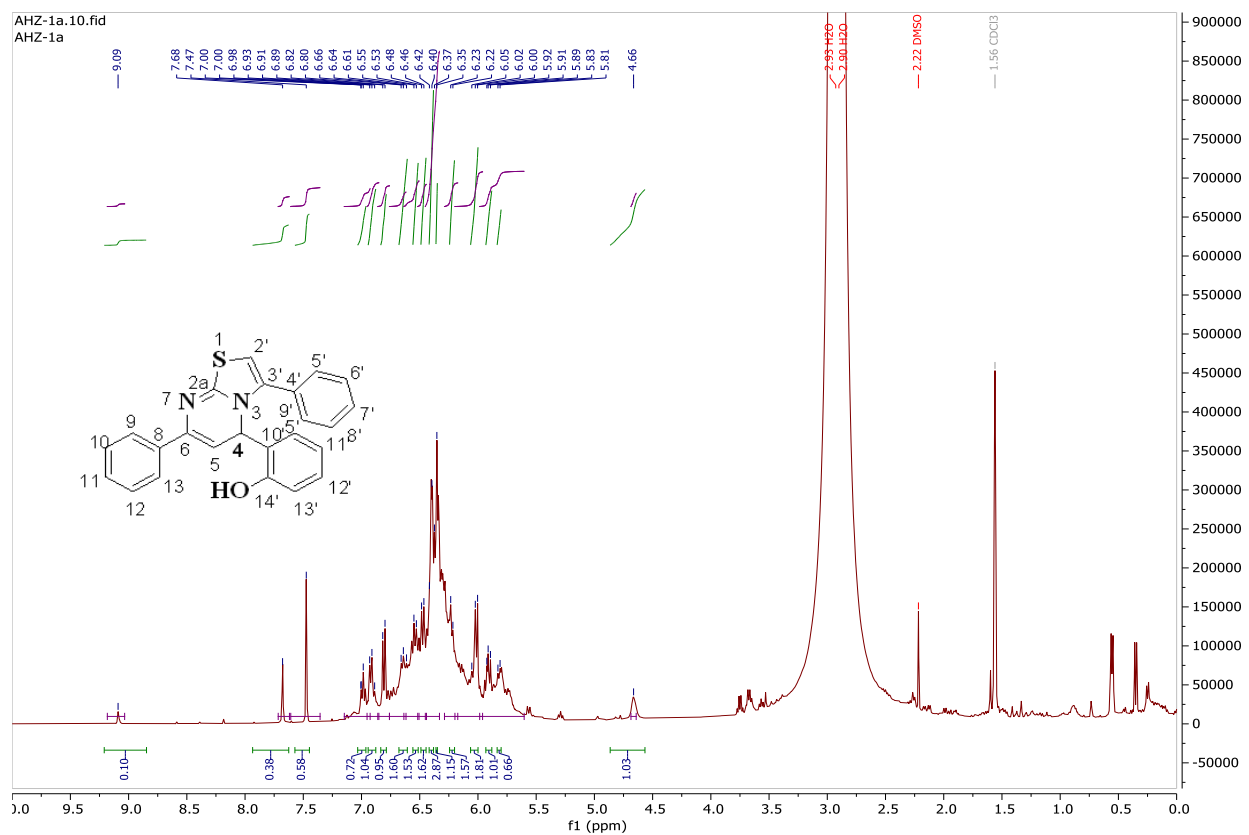
Appendix 51. ¹³C-NMR spectrum (100 MHz, DMSO-*d*₆) of compound **90**



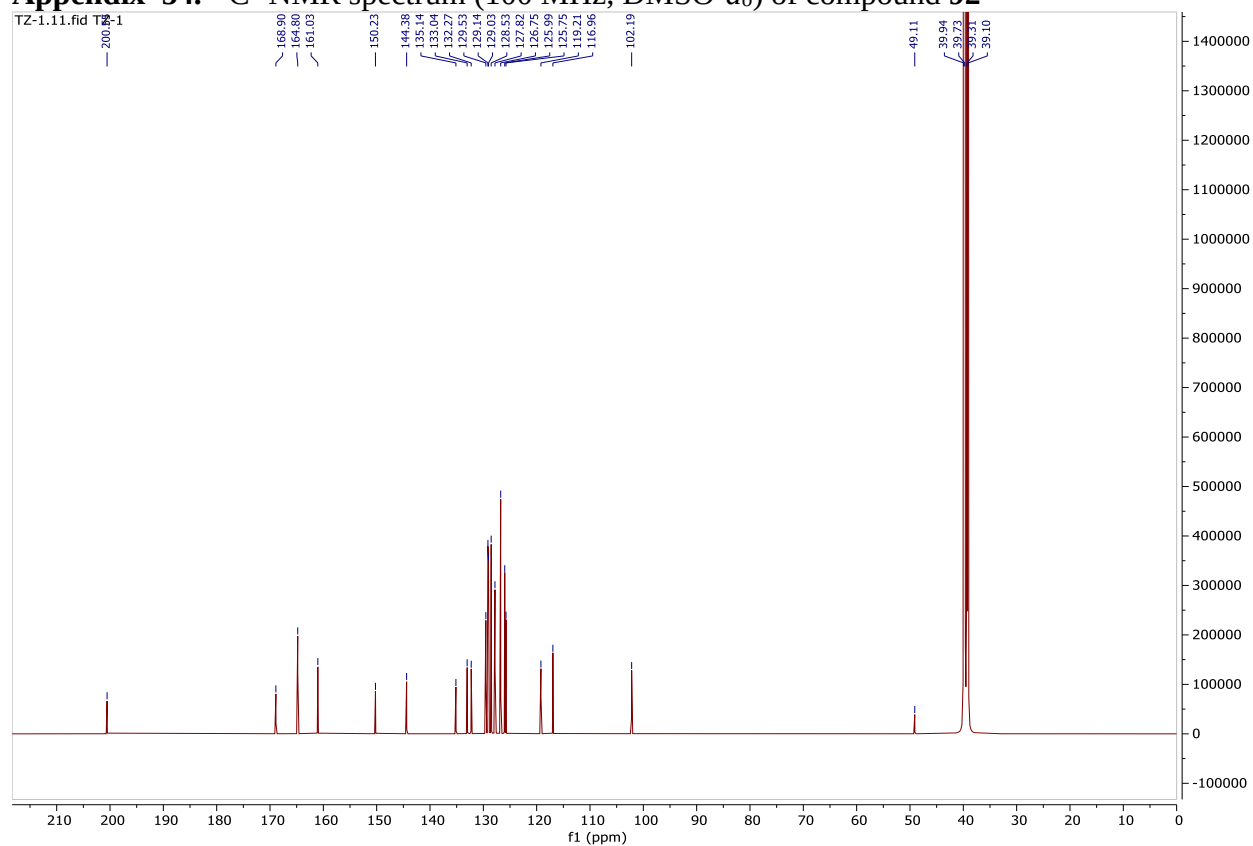
Appendix 52. UV-visible spectrum of synthesized compound **92**



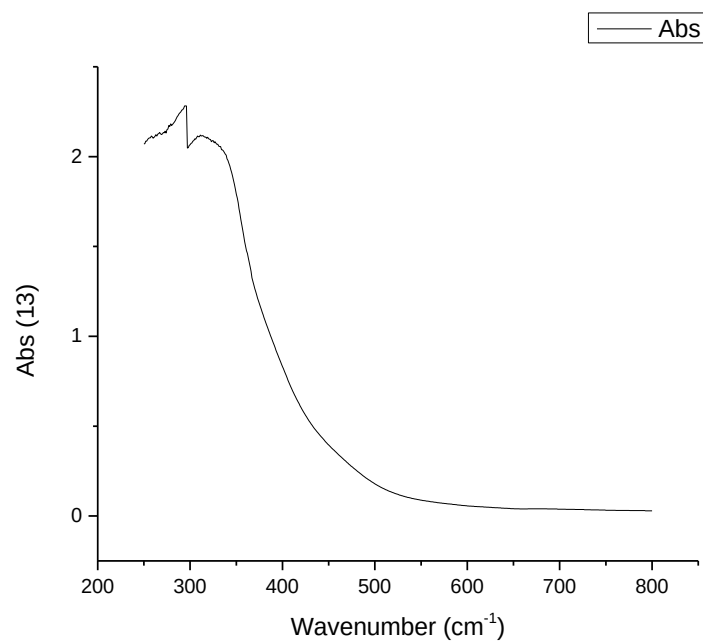
Appendix 53. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound **92**



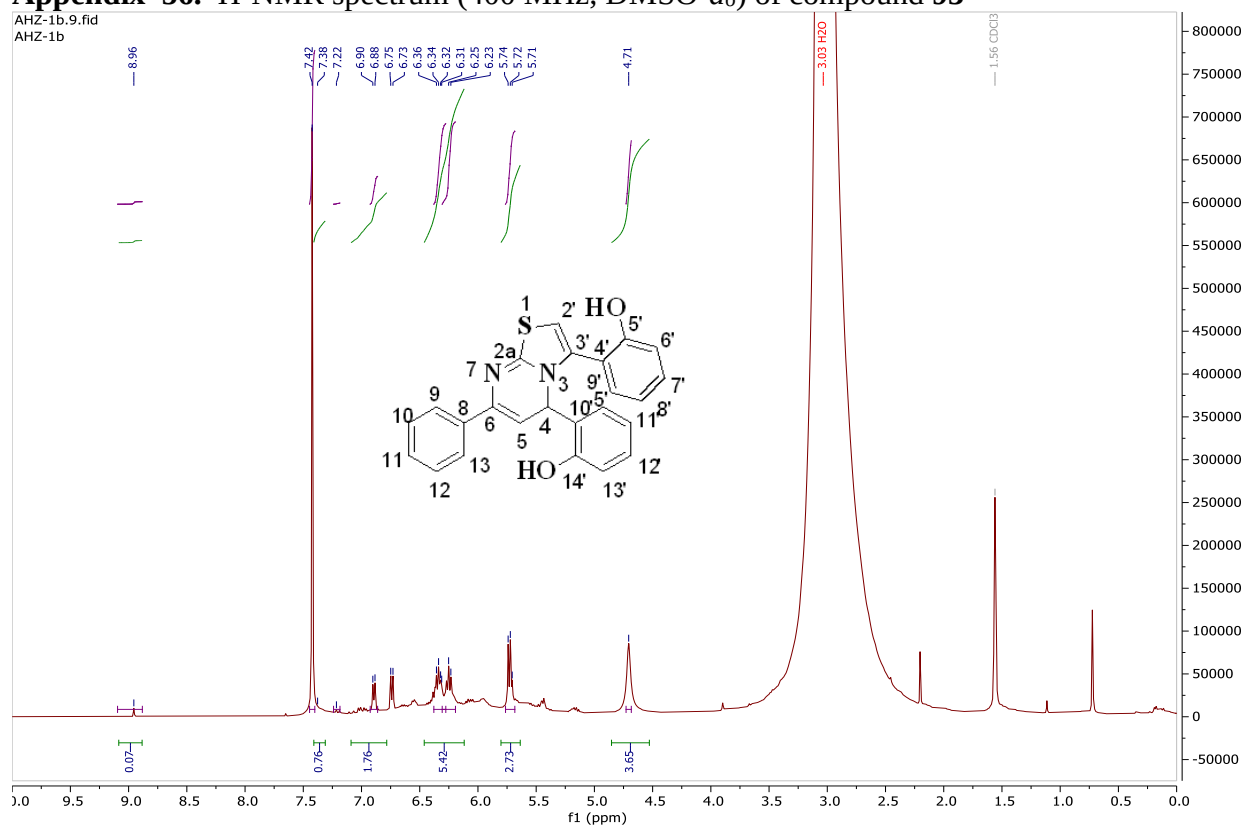
Appendix 54. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **92**



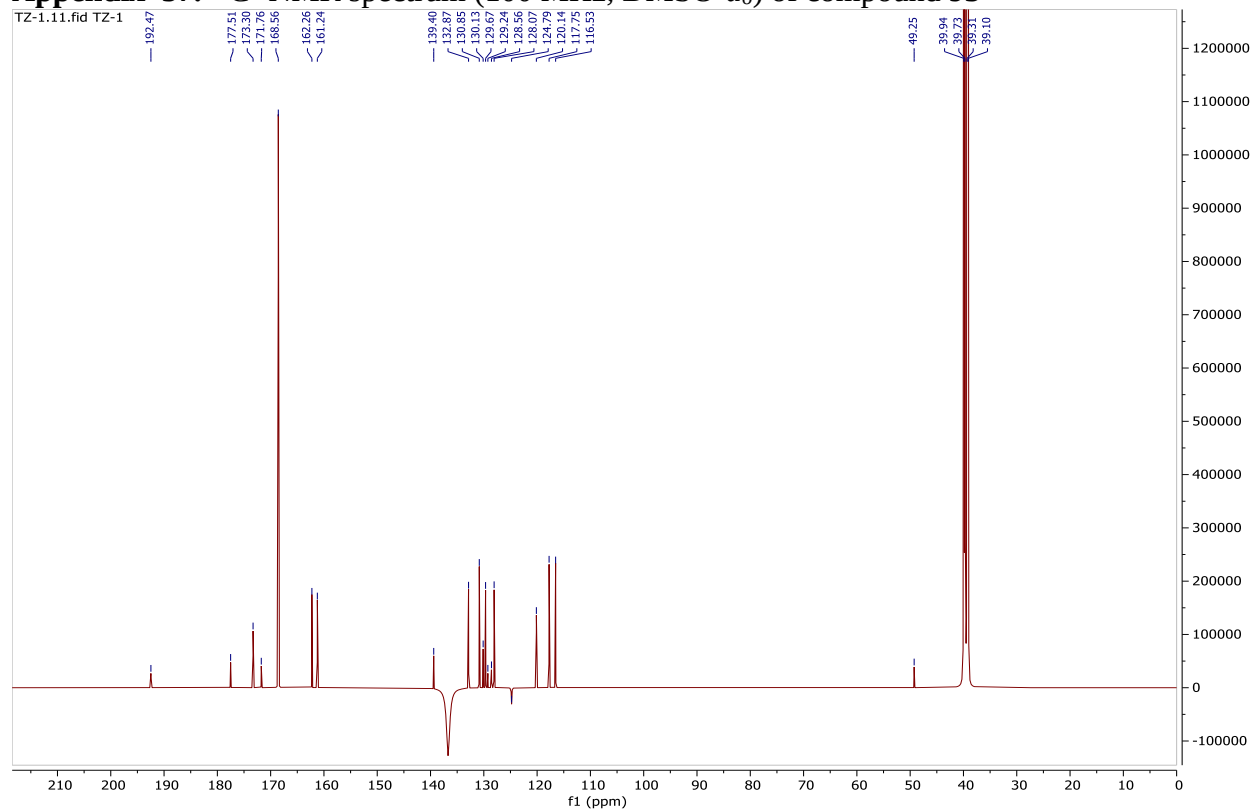
Appendix 55. UV-visible spectrum of synthesized compound **93**



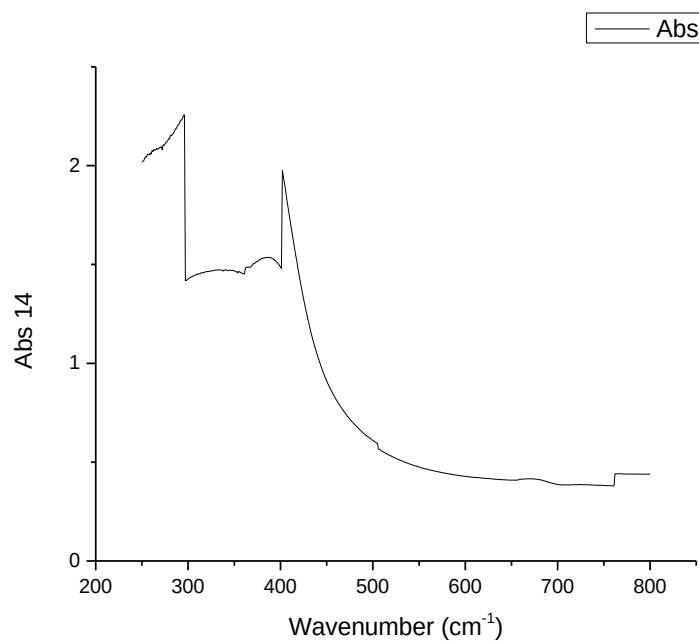
Appendix 56. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **93**



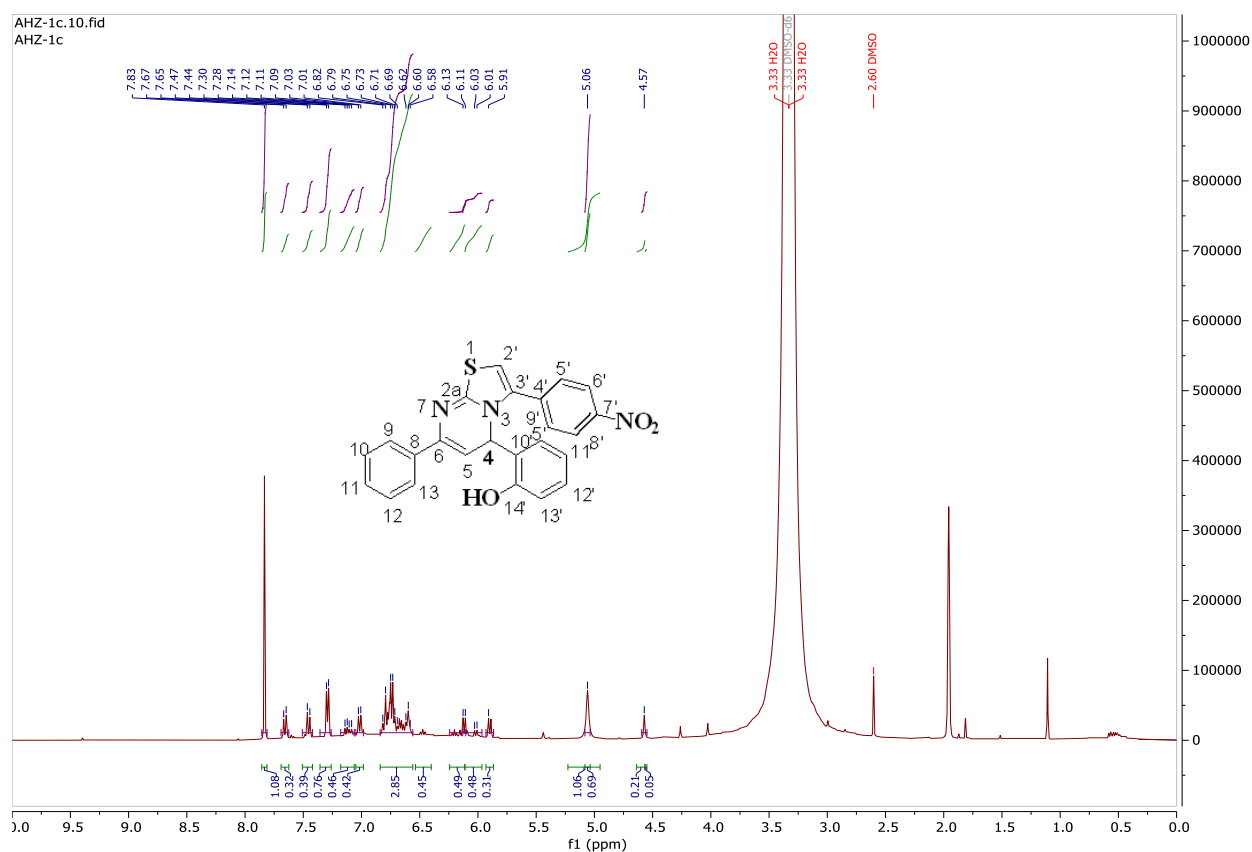
Appendix 57. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **93**



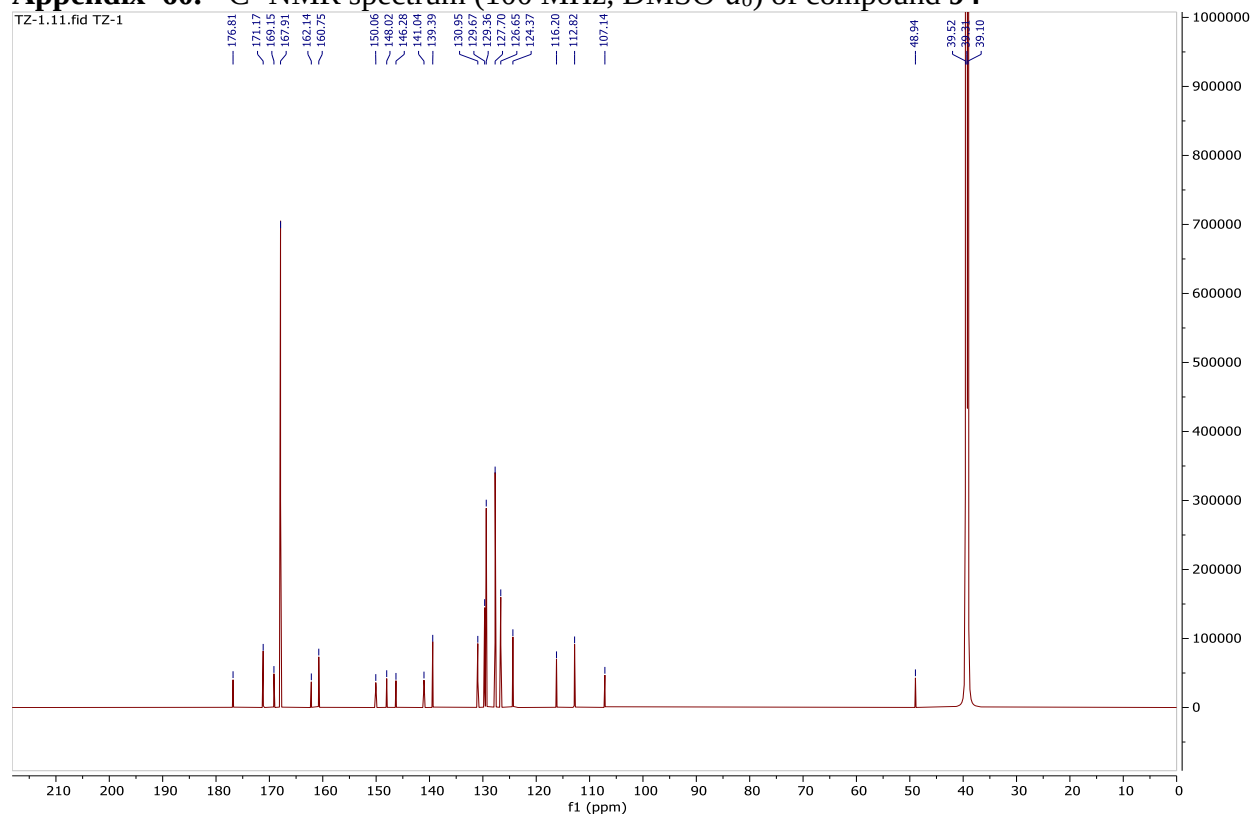
Appendix 58. UV-visible spectrum of synthesized compound **94**



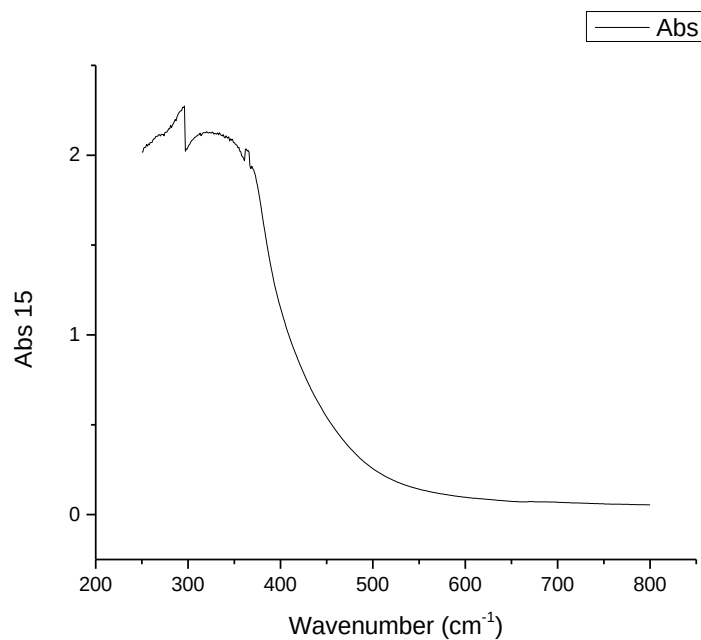
Appendix 59. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound **94**



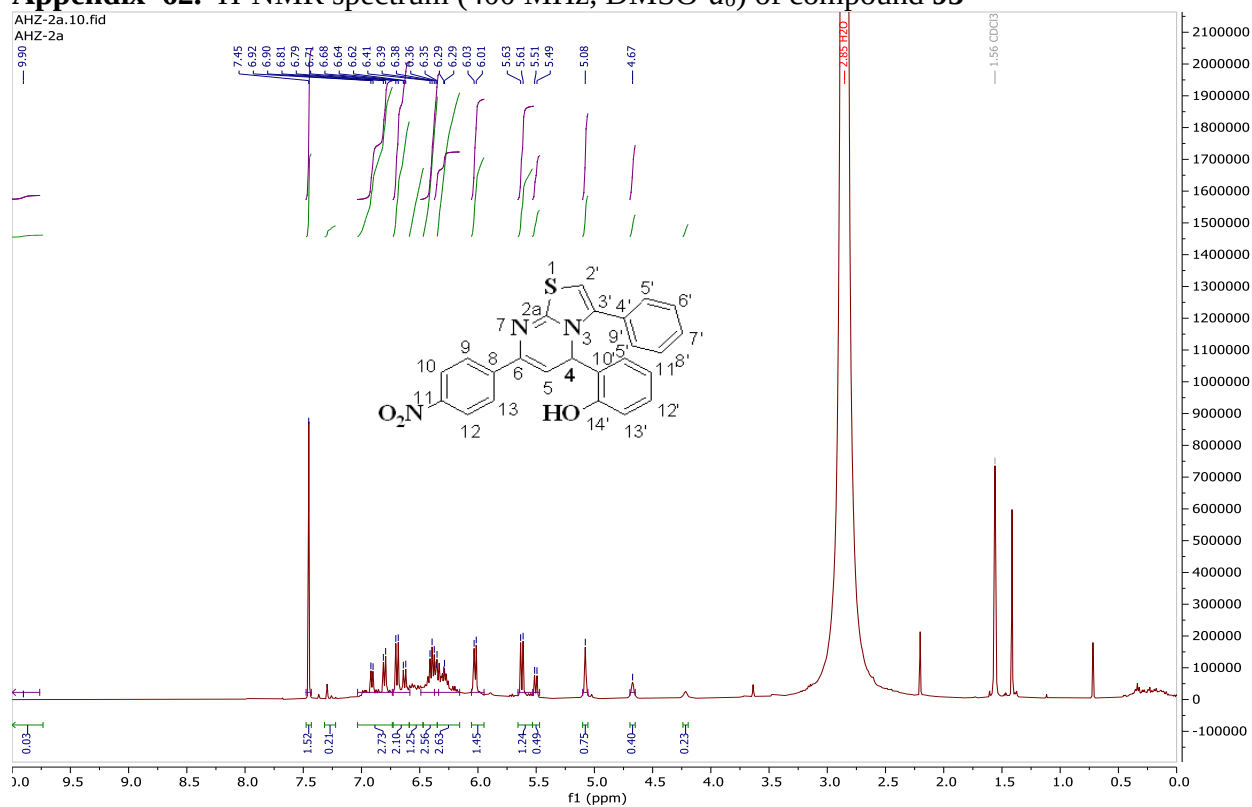
Appendix 60. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **94**



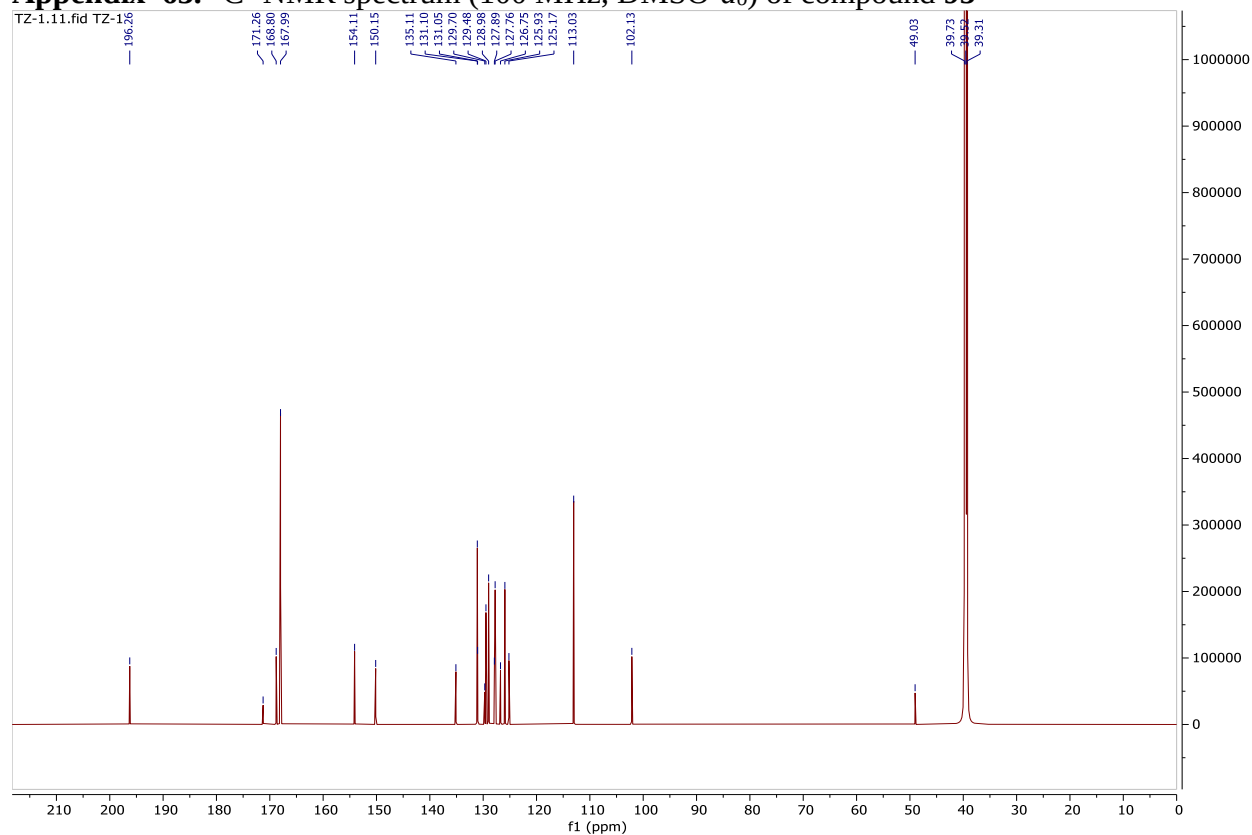
Appendix 61. UV-visible spectrum of synthesized compound **95**



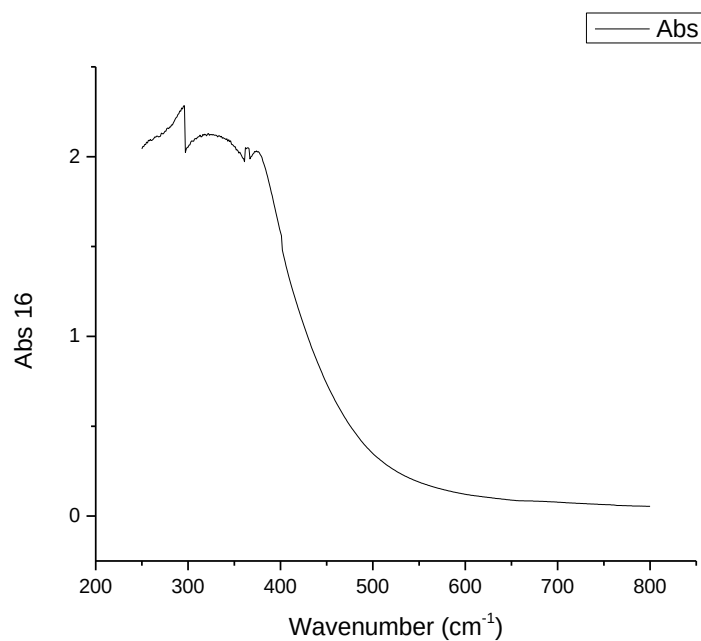
Appendix 62. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound 95



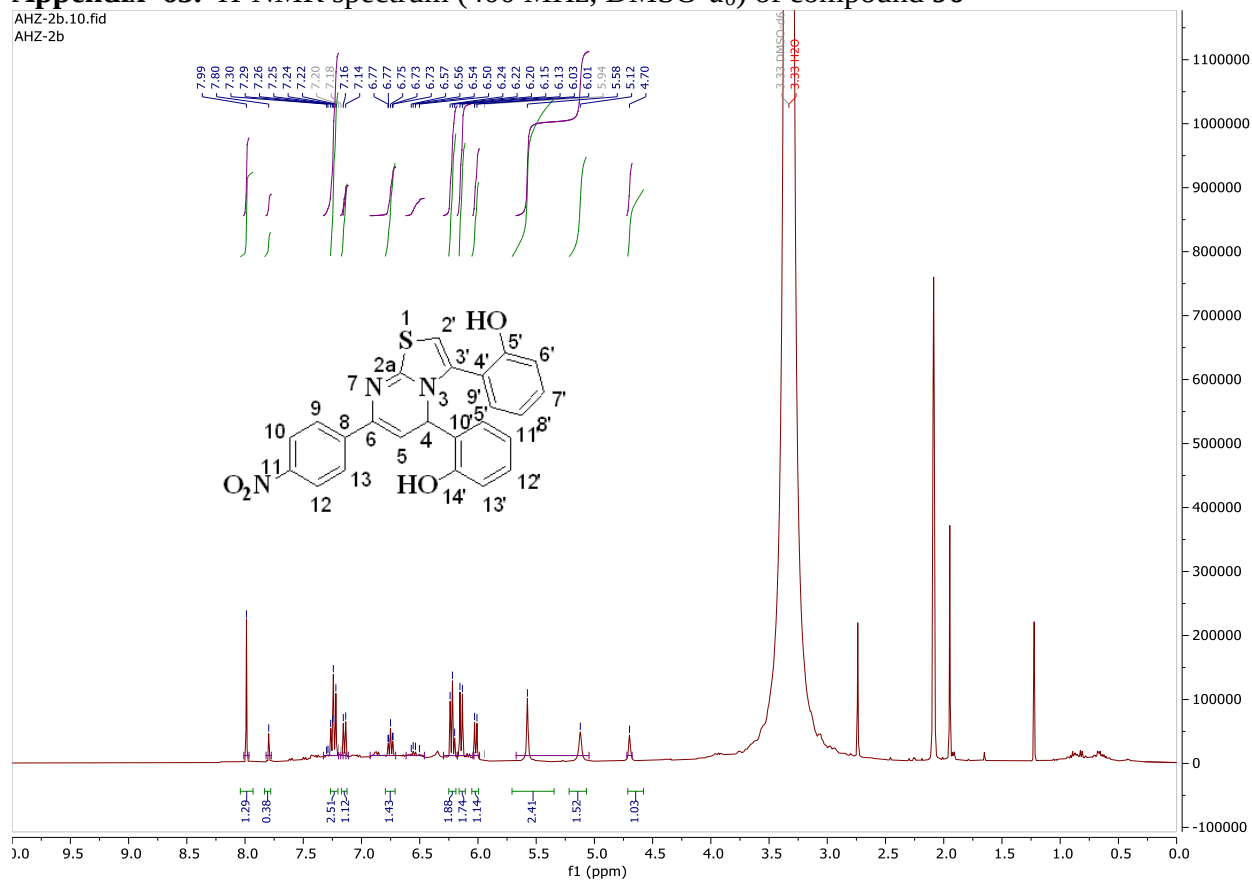
Appendix 63. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound 95



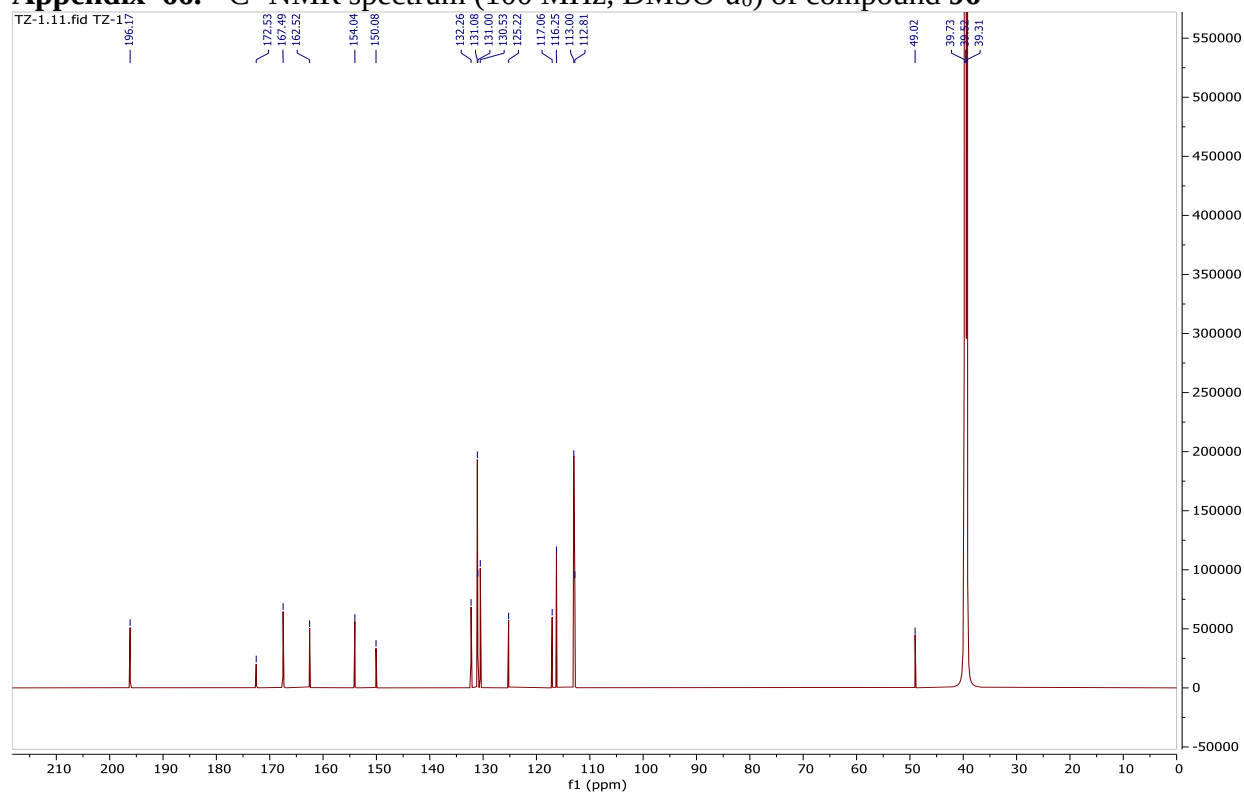
Appendix 64. UV-visible spectrum of synthesized compound **96**



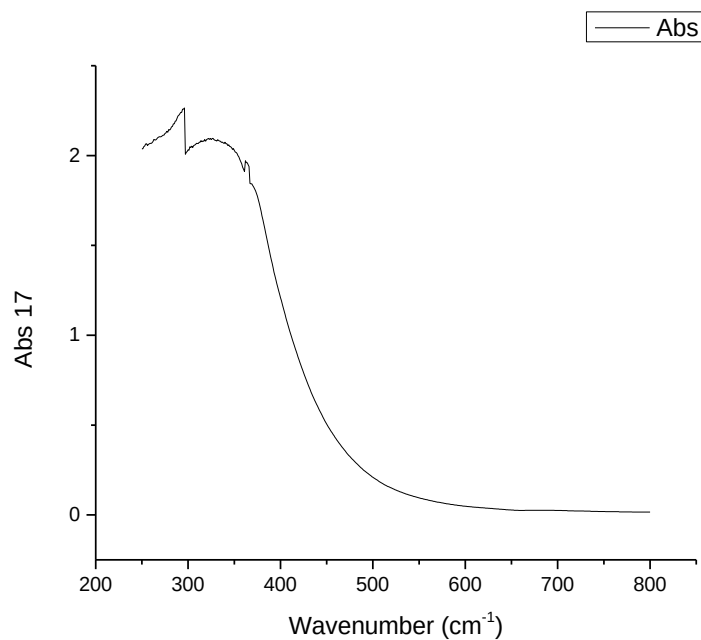
Appendix 65. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound **96**



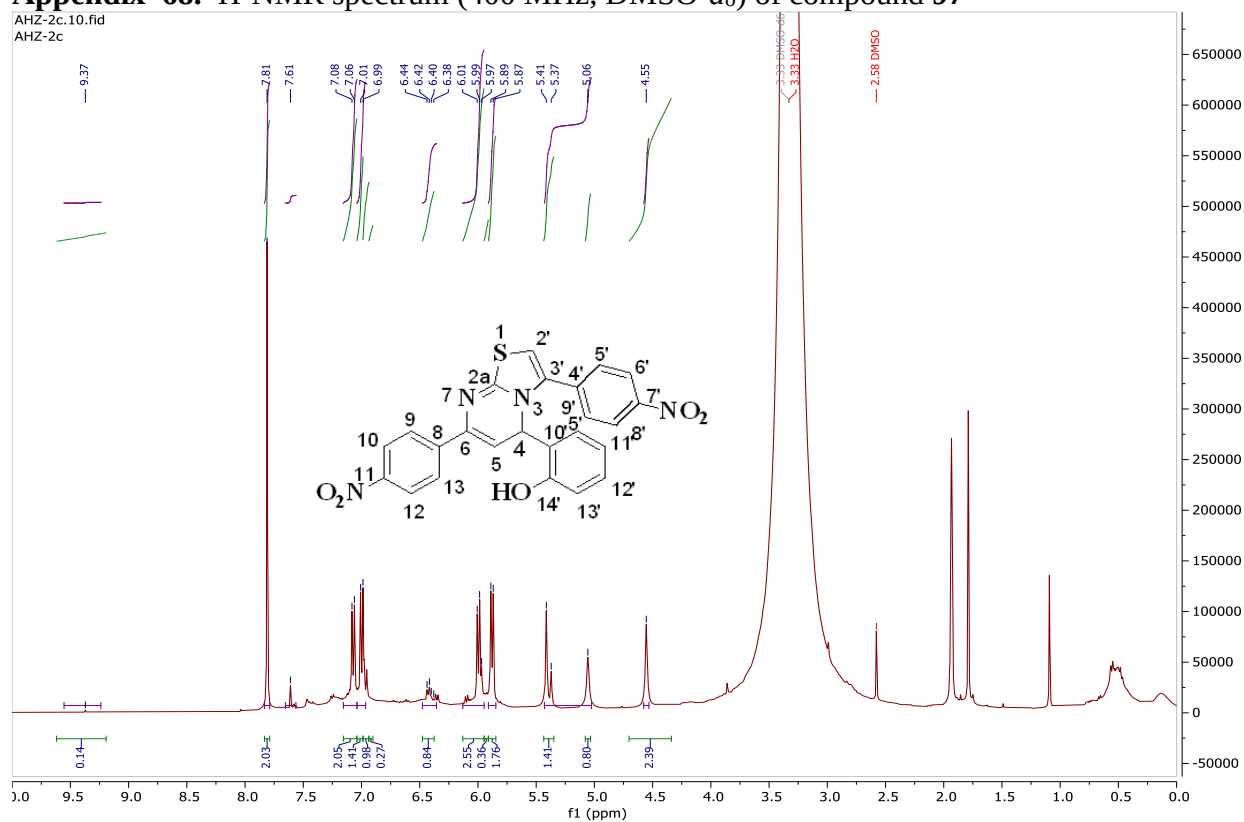
Appendix 66. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **96**



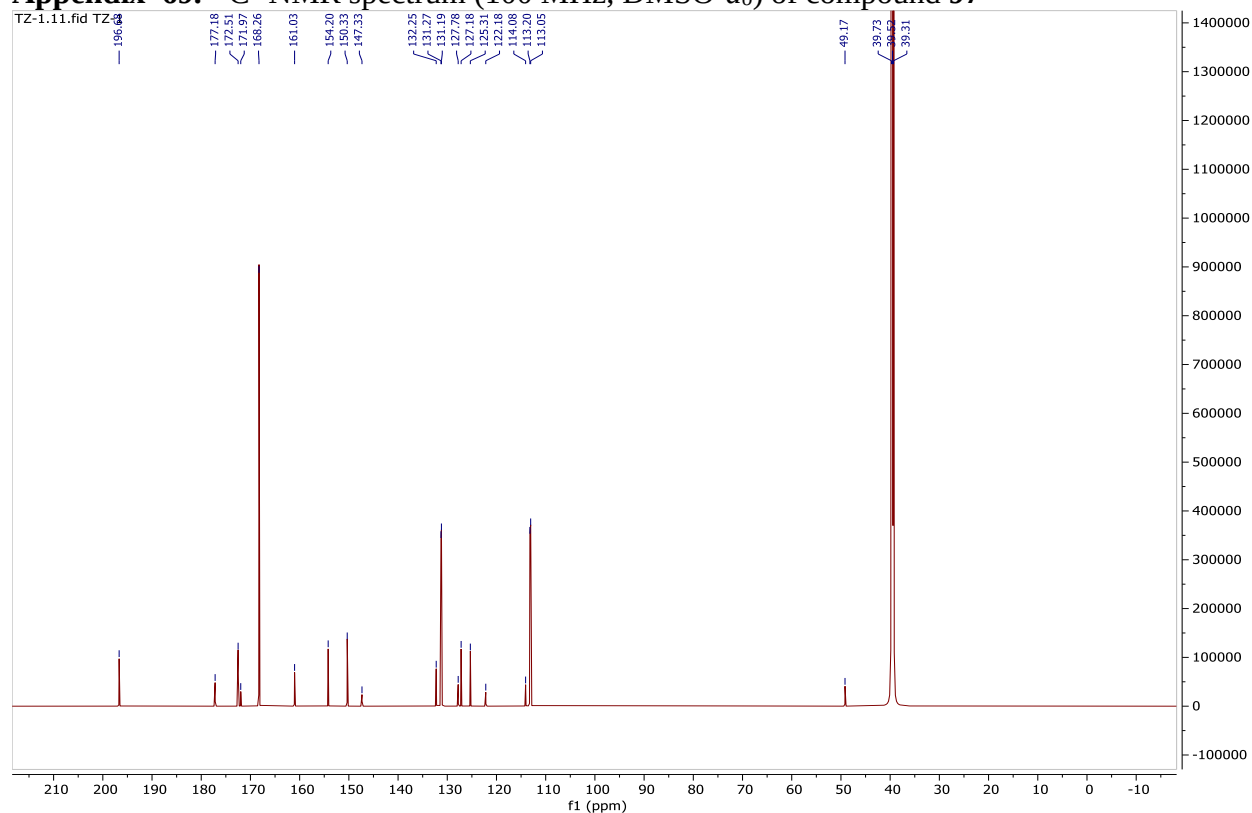
Appendix 67. UV-visible spectrum of synthesized compound **97**



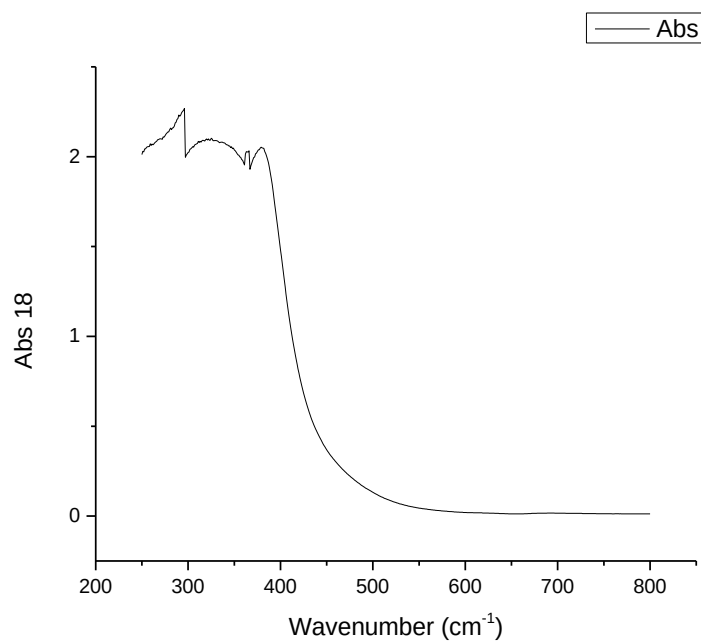
Appendix 68. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **97**



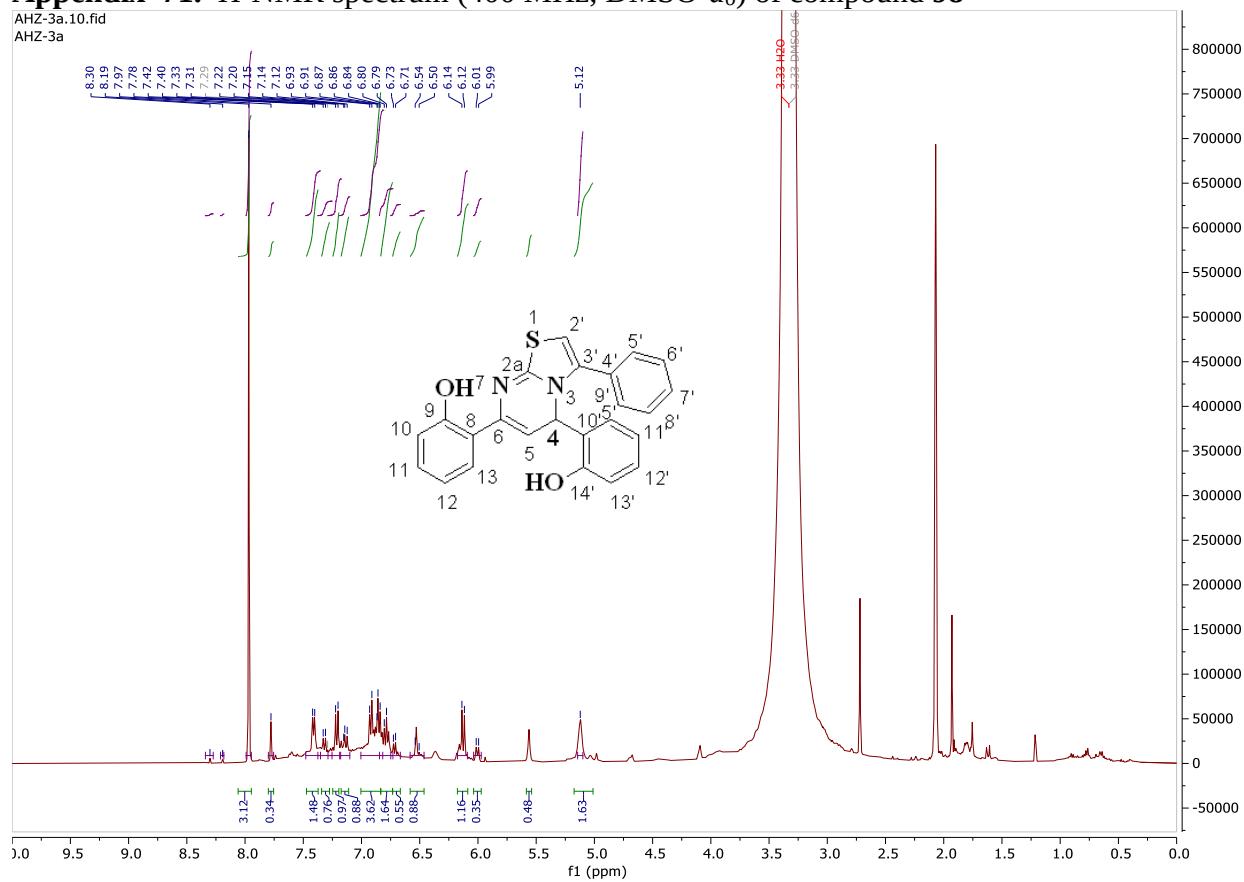
Appendix 69. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **97**



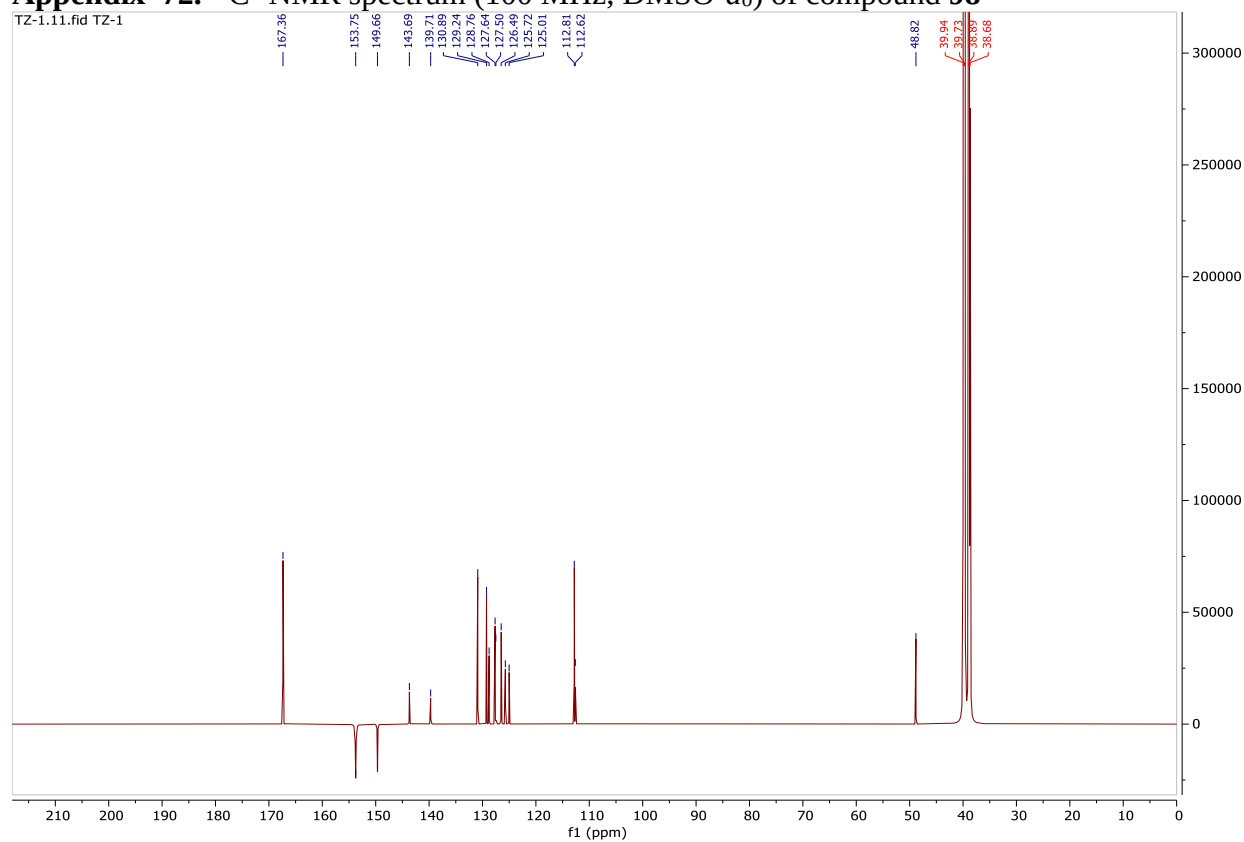
Appendix 70. UV-visible spectrum of synthesized compound **98**



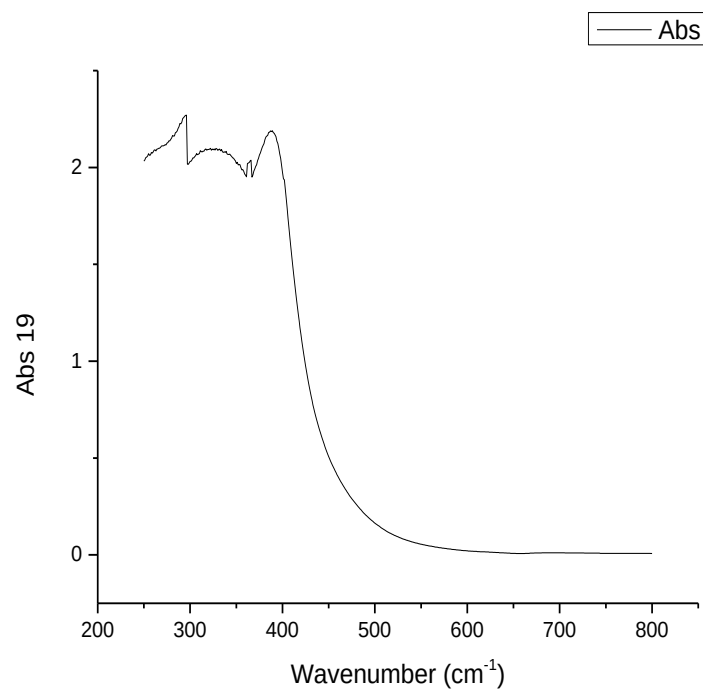
Appendix 71. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound **98**



Appendix 72. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **98**

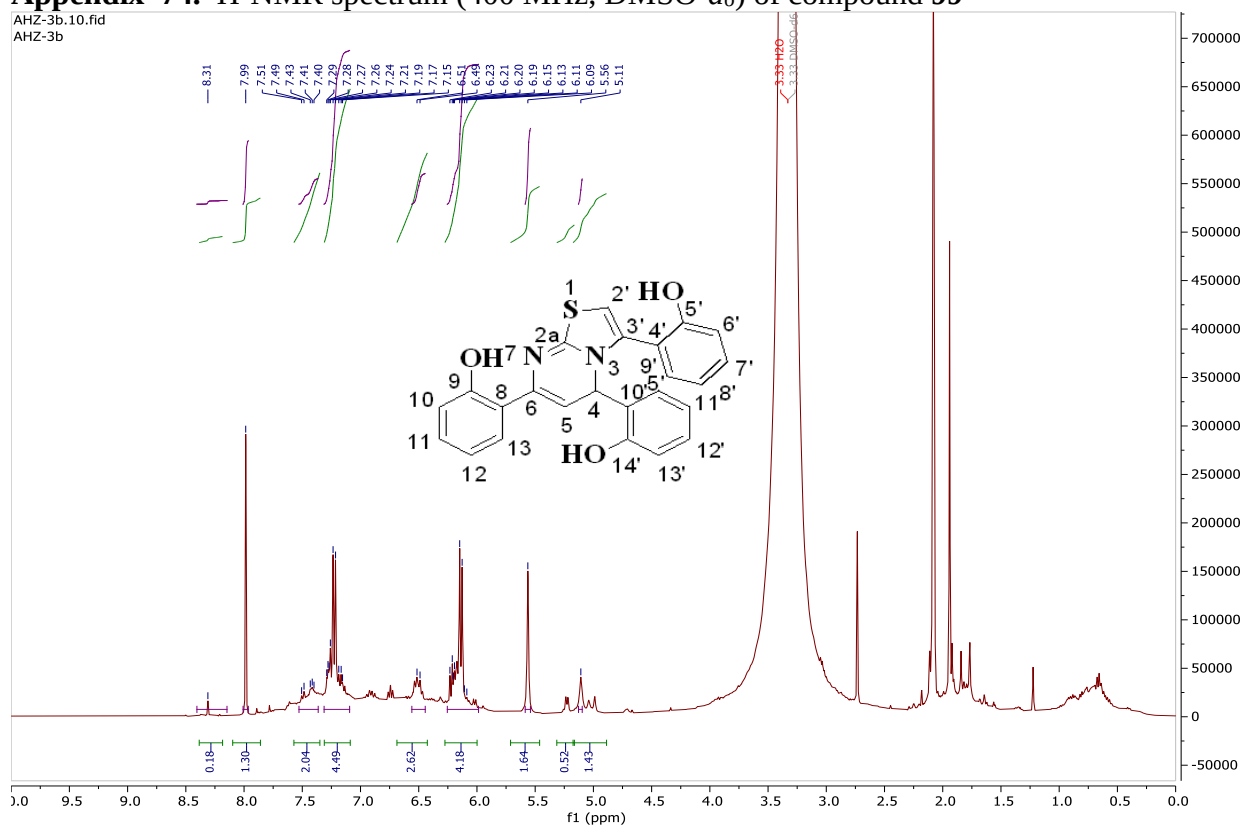


Appendix 73. UV-visible spectrum of synthesized compound **99**



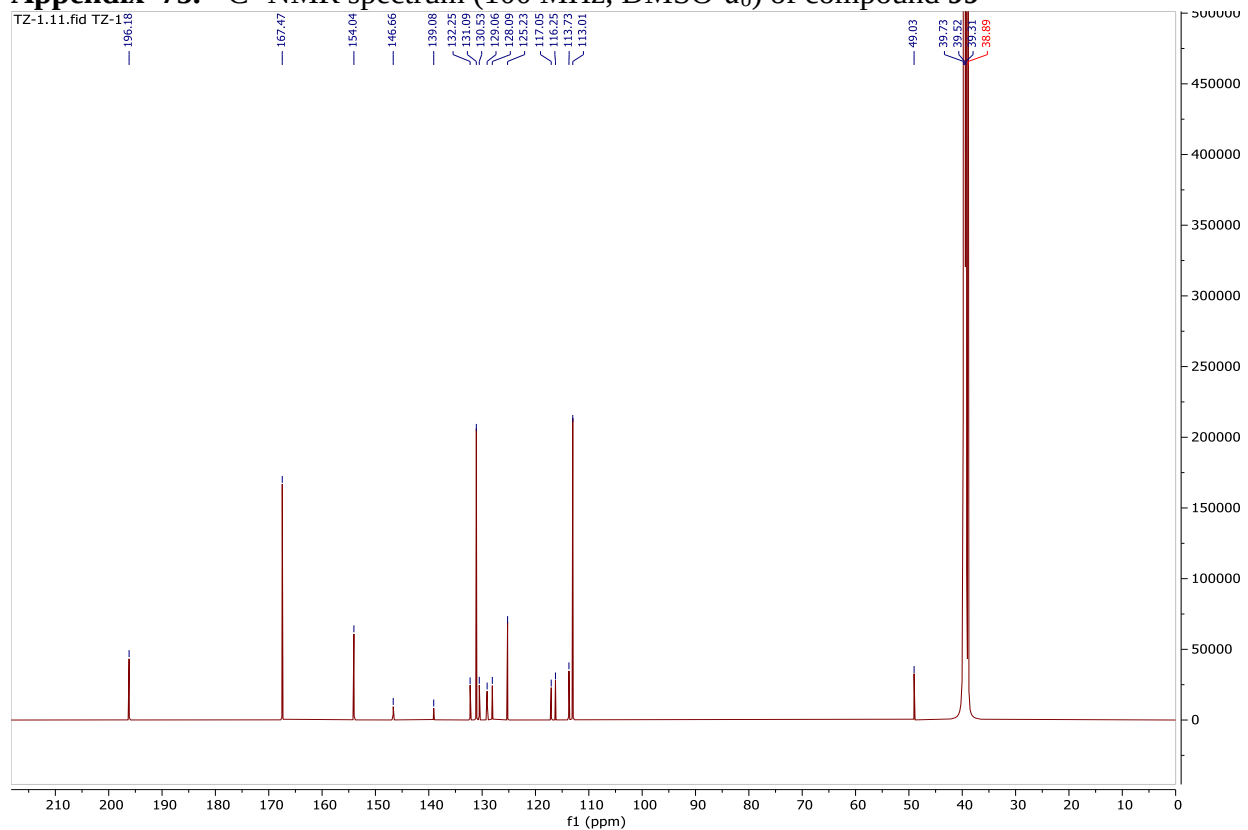
Appendix 74. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **99**

AHZ-3b.10.fid
AHZ-3b

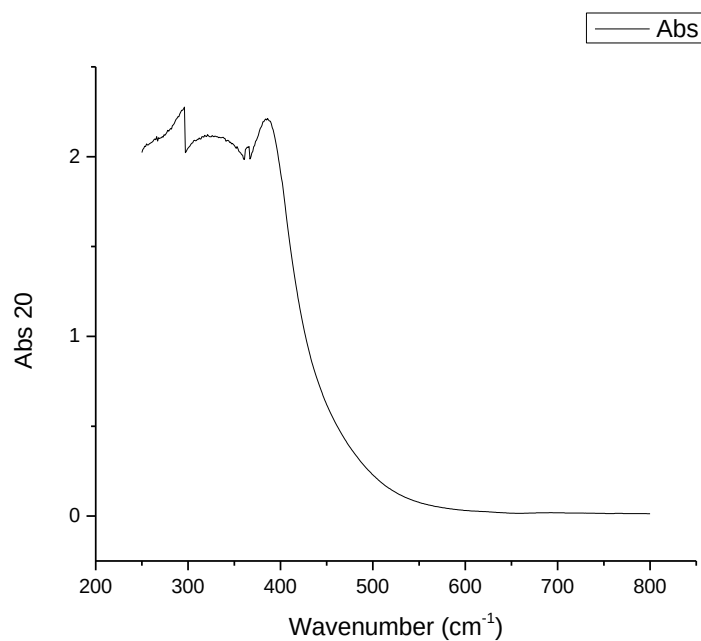


Appendix 75. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **99**

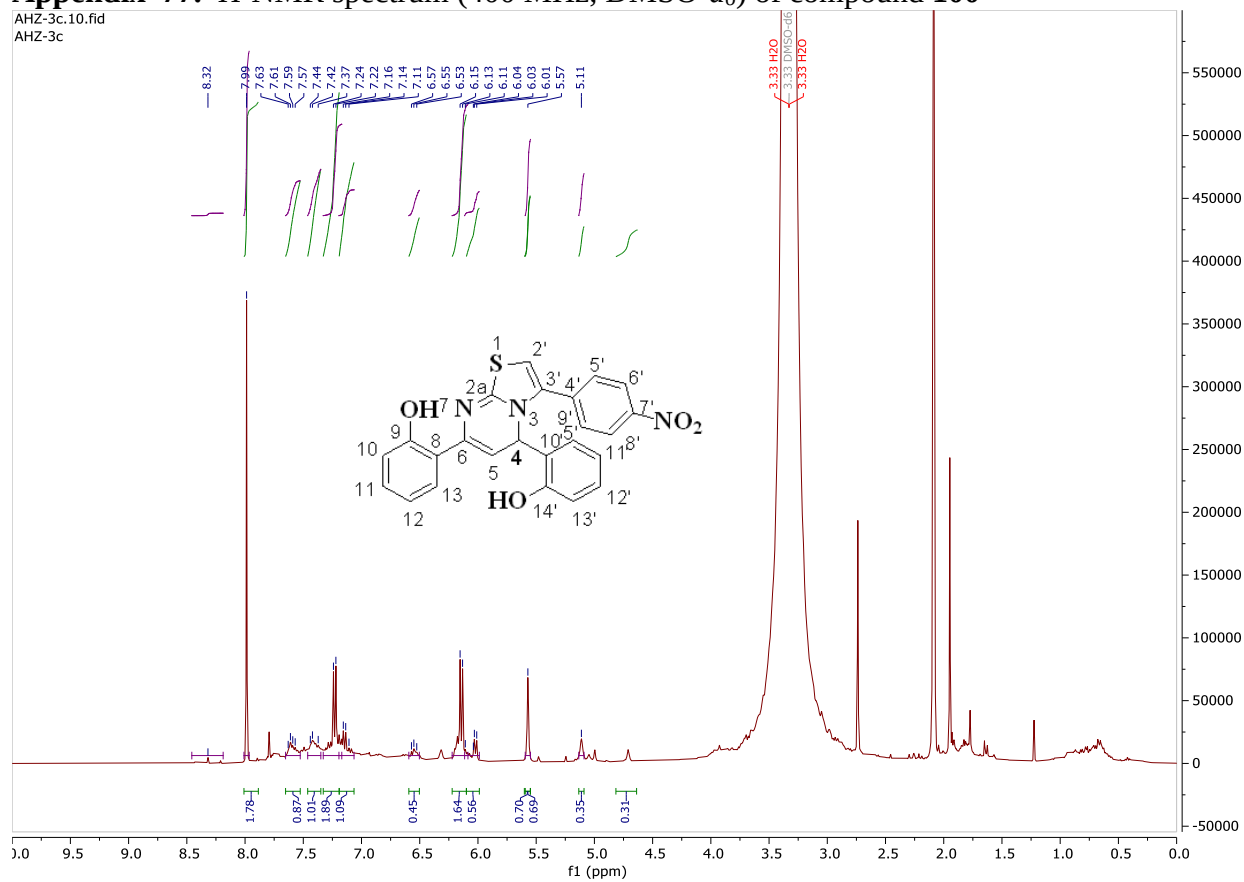
TZ-1.11.fid TZ-18



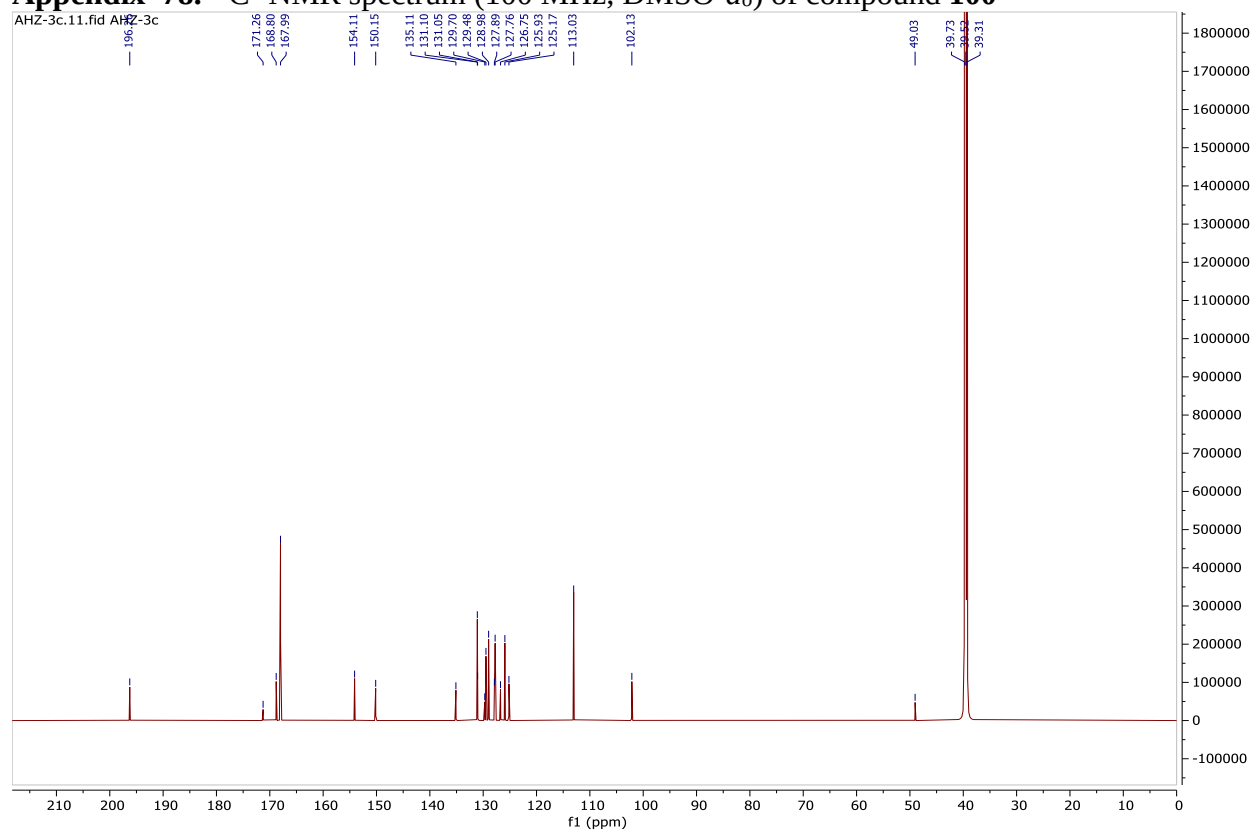
Appendix 76. UV-visible spectrum of synthesized compound **100**



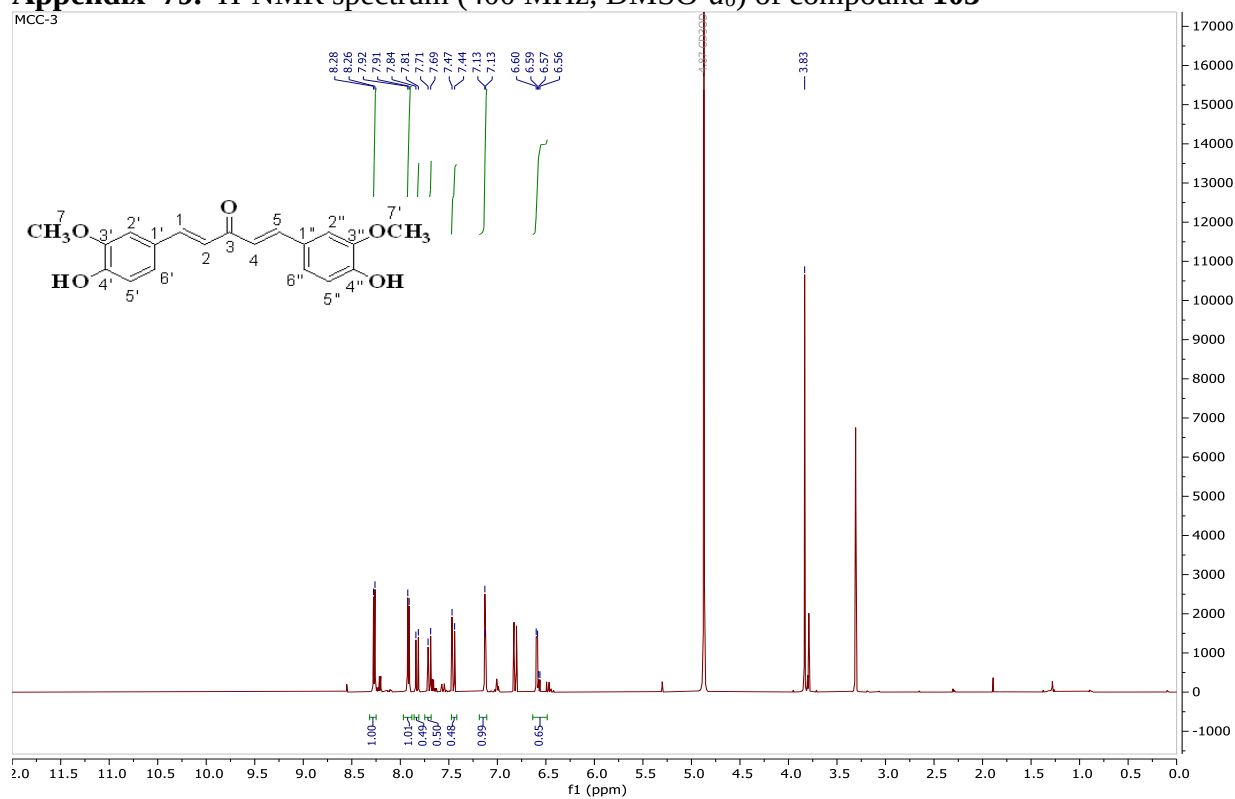
Appendix 77. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound **100**



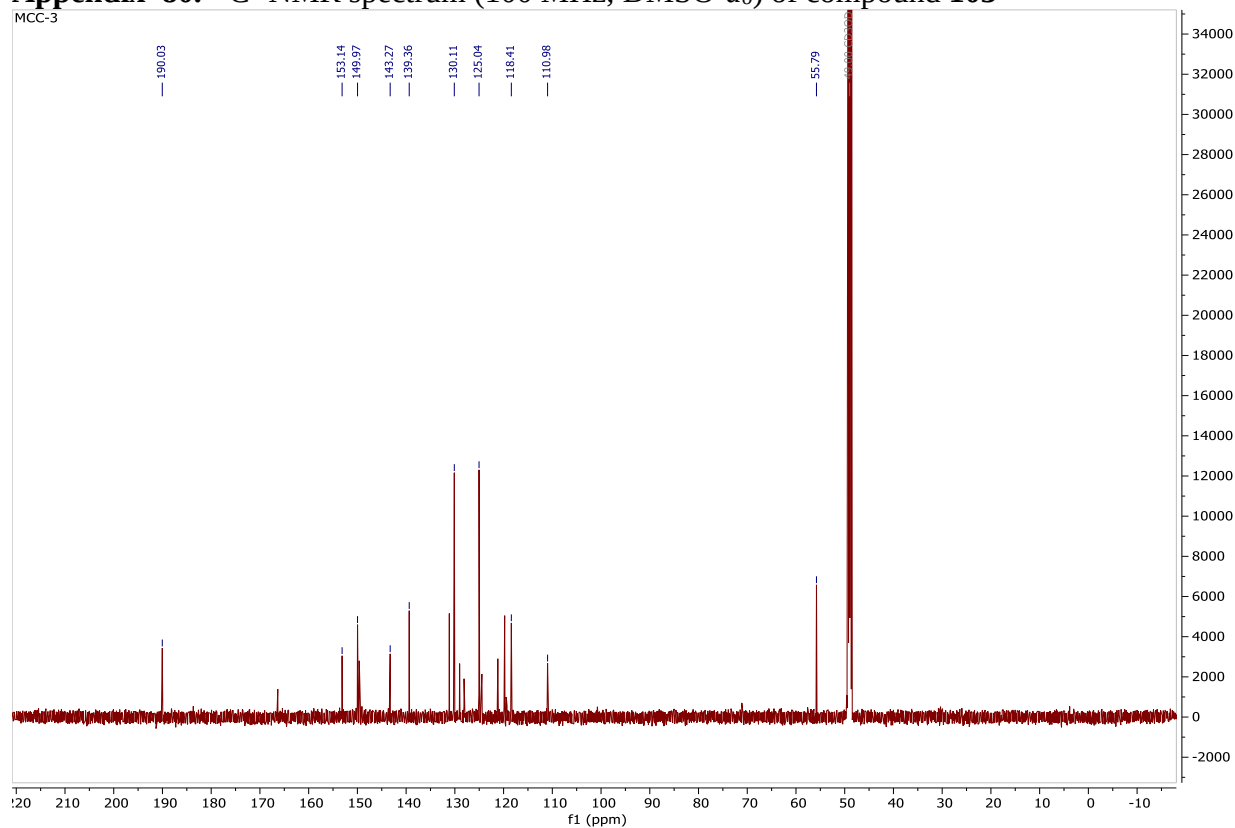
Appendix 78. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **100**



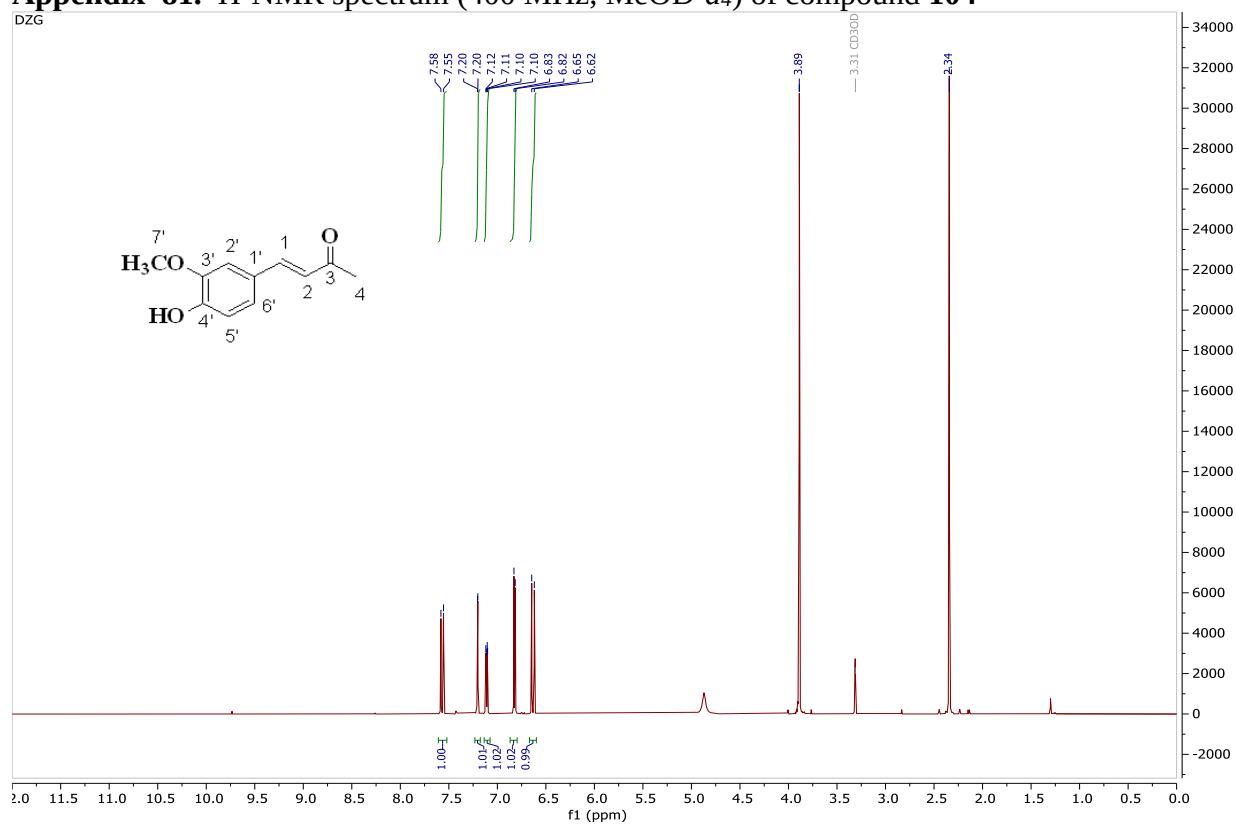
Appendix 79. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **103**



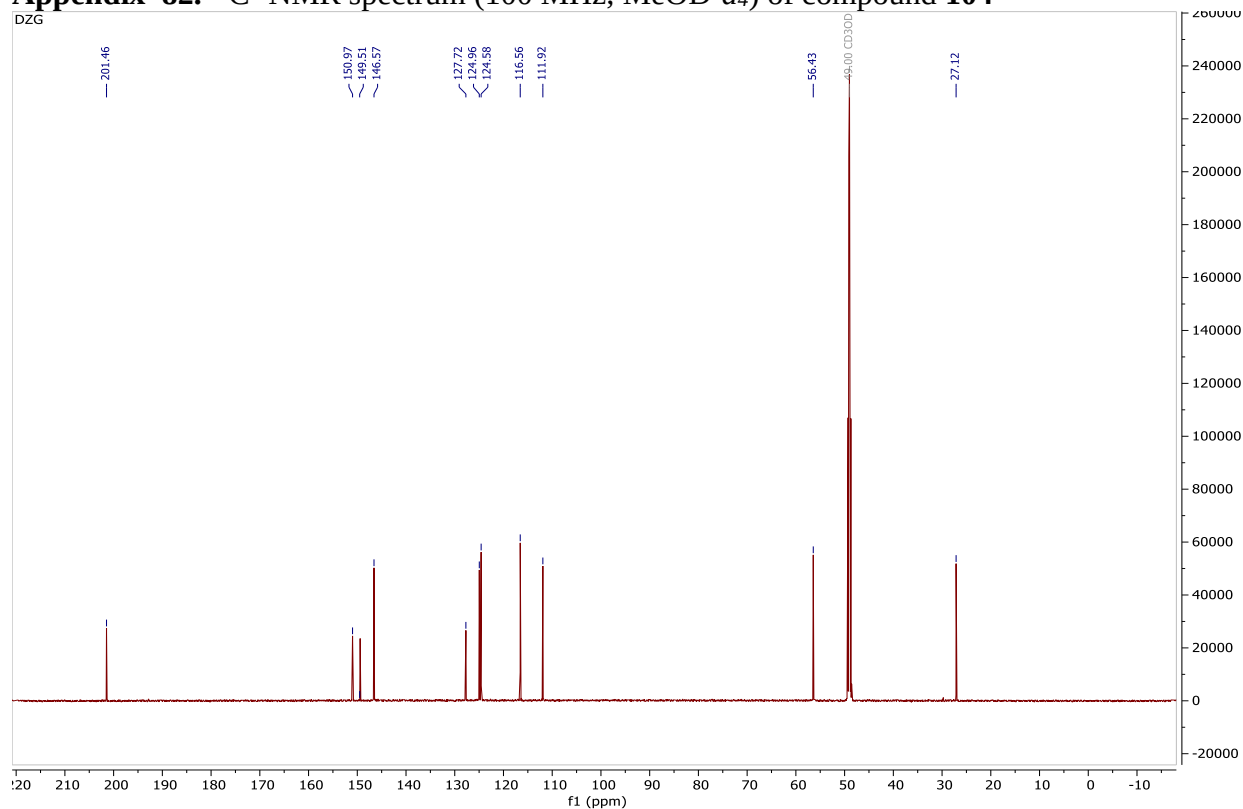
Appendix 80. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **103**



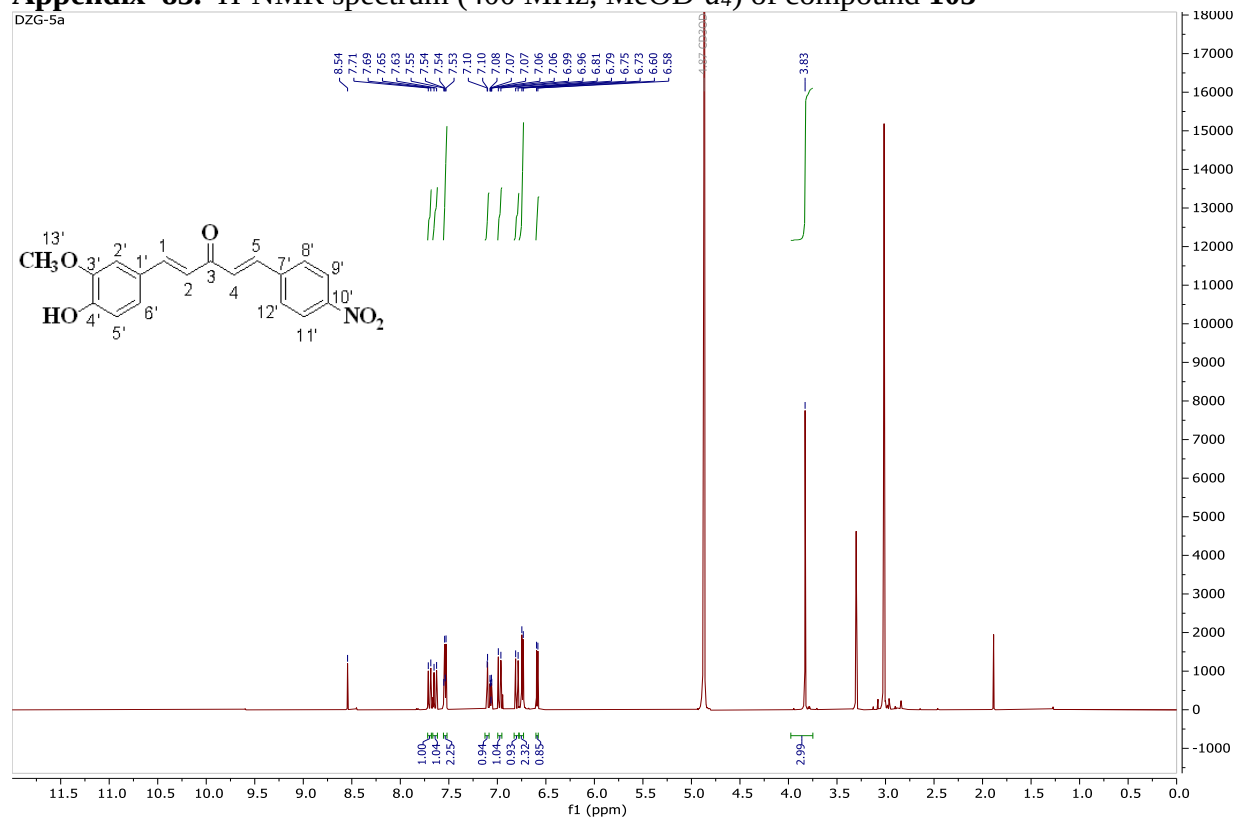
Appendix 81. ^1H -NMR spectrum (400 MHz, $\text{MeOD-}d_4$) of compound **104**



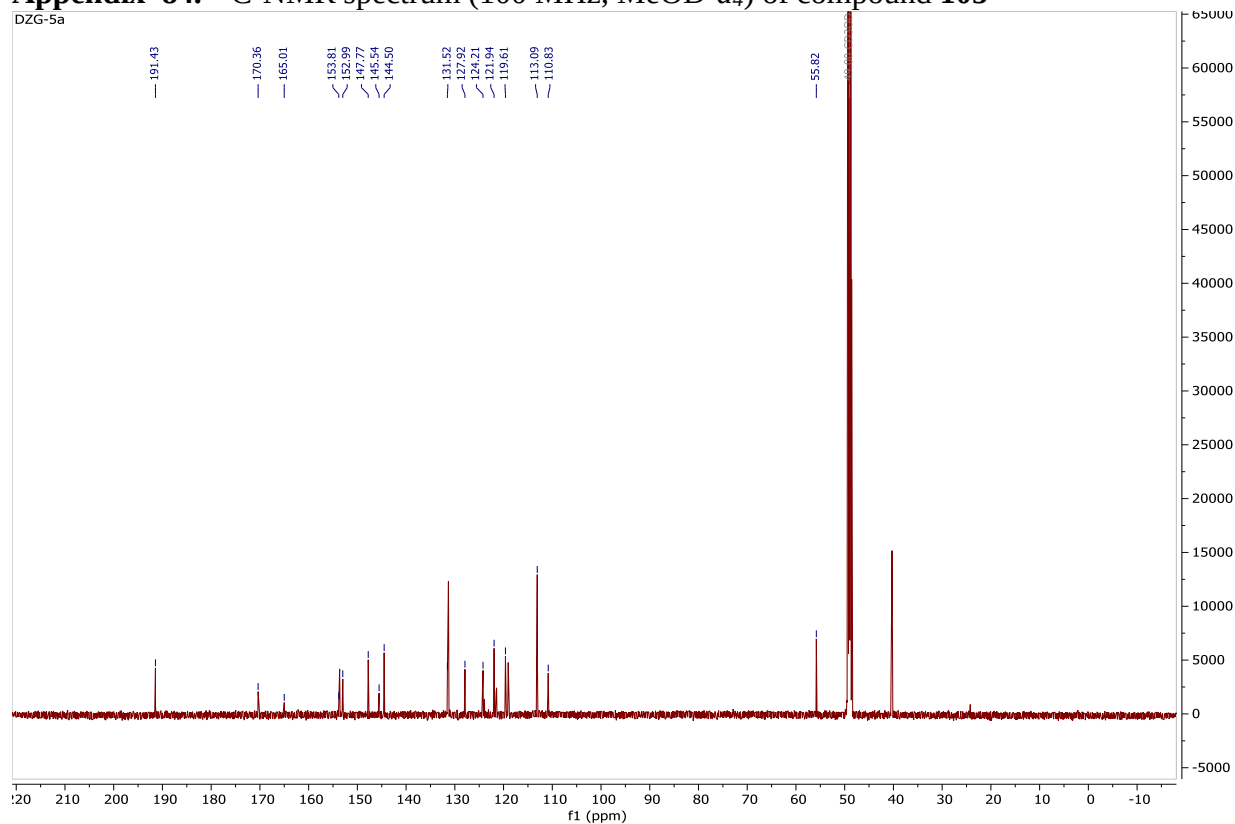
Appendix 82. ^{13}C -NMR spectrum (100 MHz, $\text{MeOD-}d_4$) of compound **104**



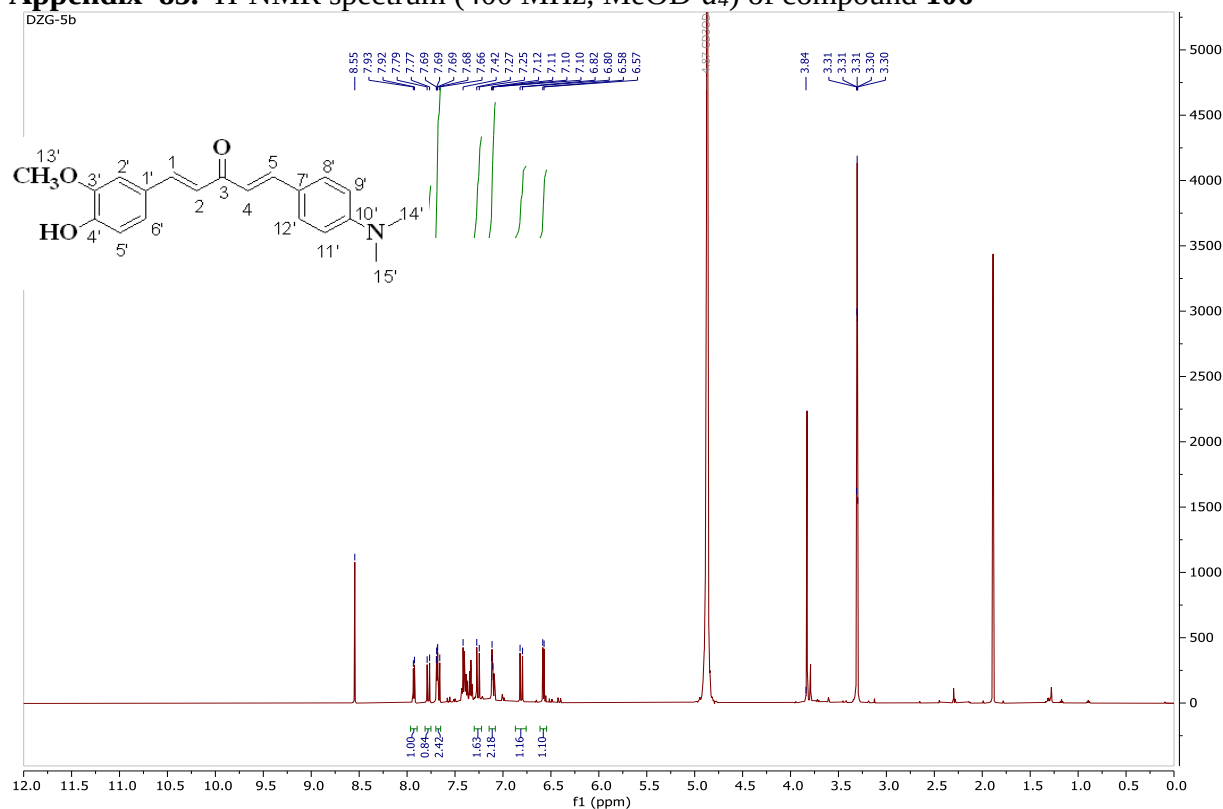
Appendix 83. ^1H -NMR spectrum (400 MHz, $\text{MeOD-}d_4$) of compound **105**



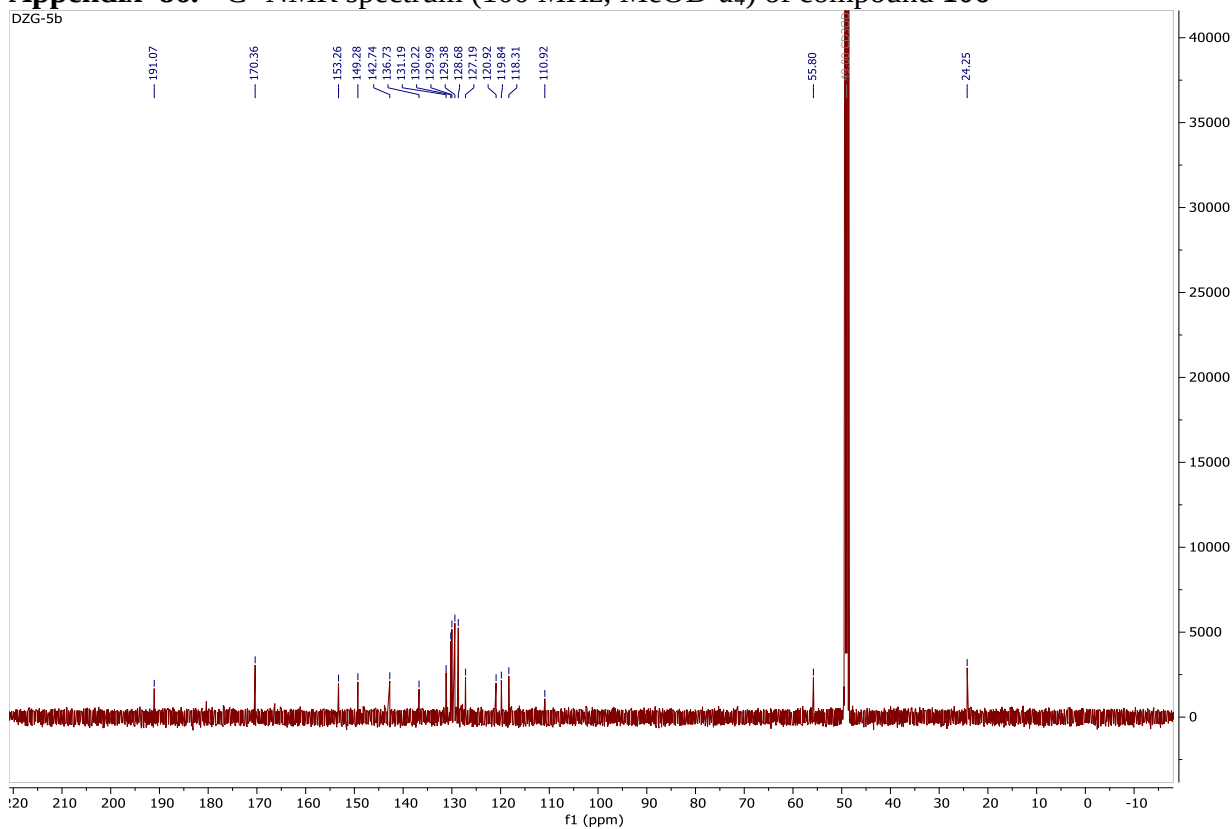
Appendix 84. ^{13}C -NMR spectrum (100 MHz, $\text{MeOD-}d_4$) of compound **105**



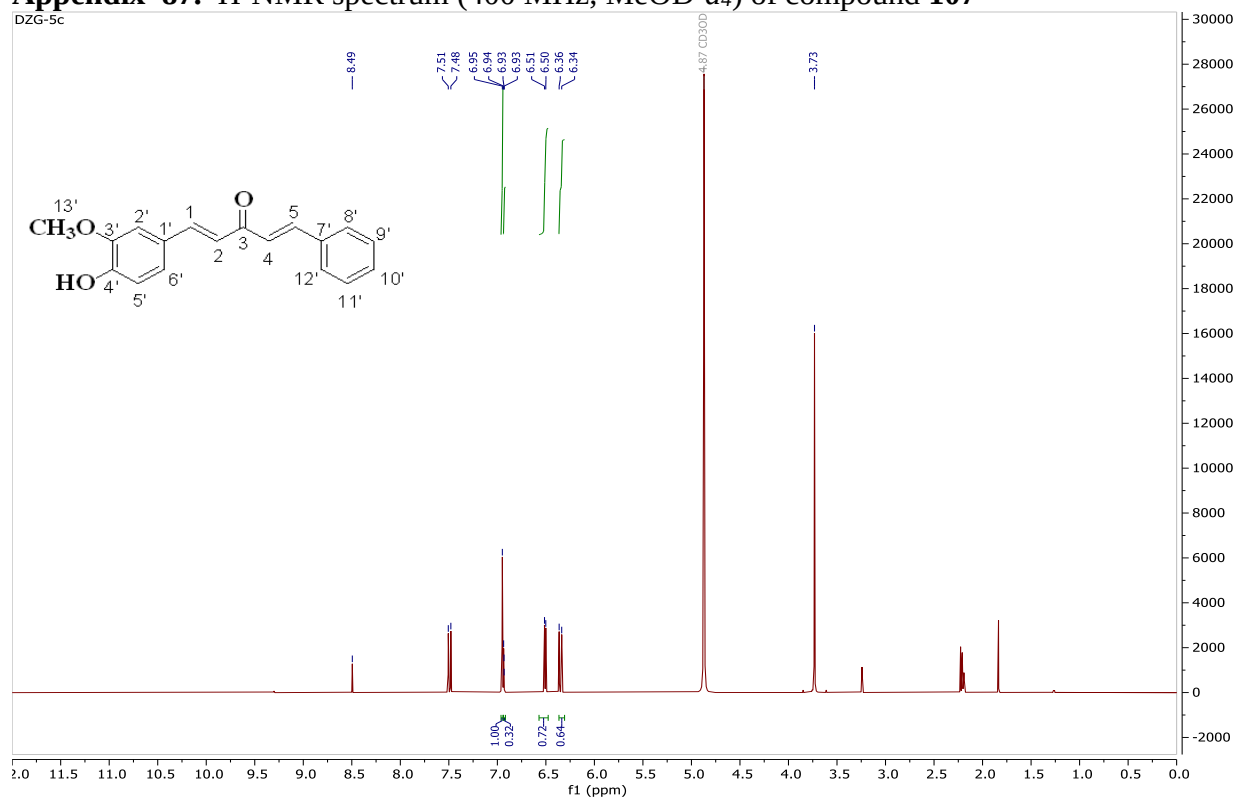
Appendix 85. ^1H -NMR spectrum (400 MHz, $\text{MeOD-}d_4$) of compound **106**



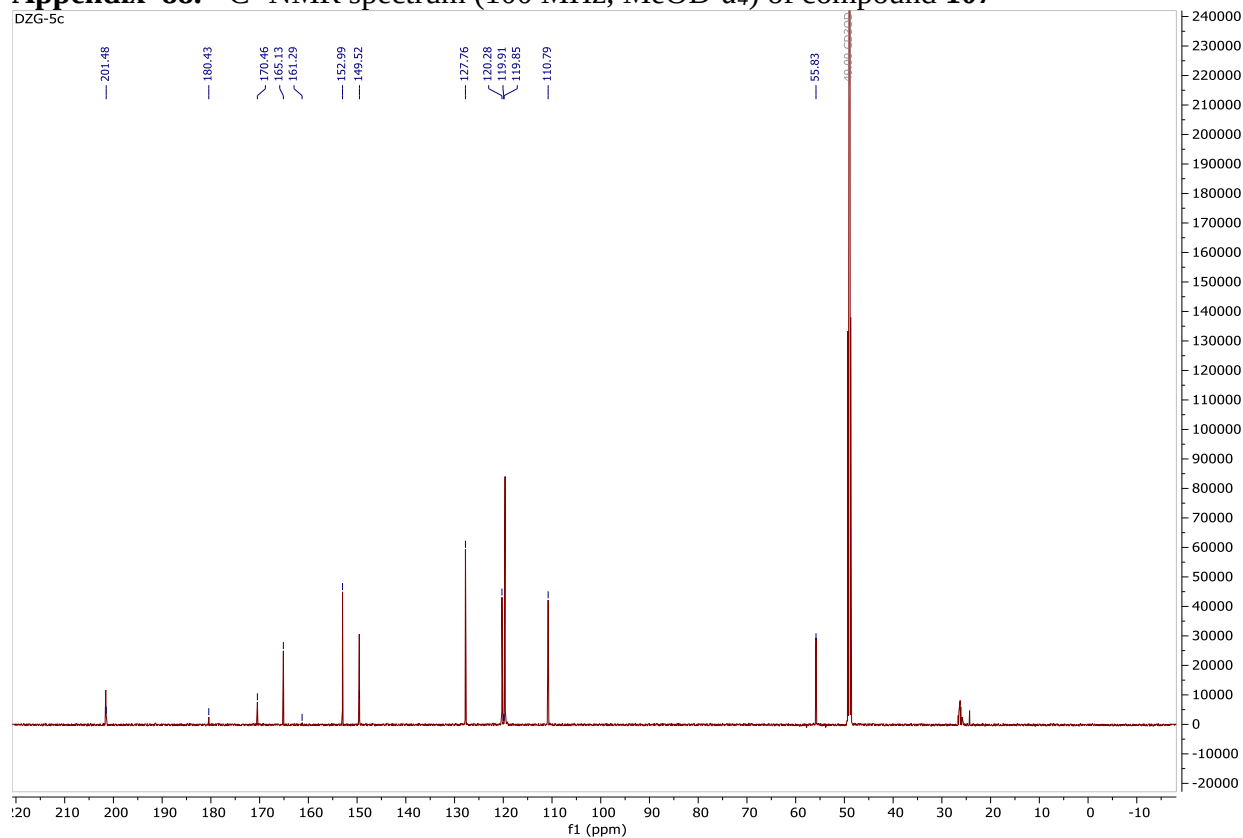
Appendix 86. ^{13}C -NMR spectrum (100 MHz, $\text{MeOD-}d_4$) of compound **106**



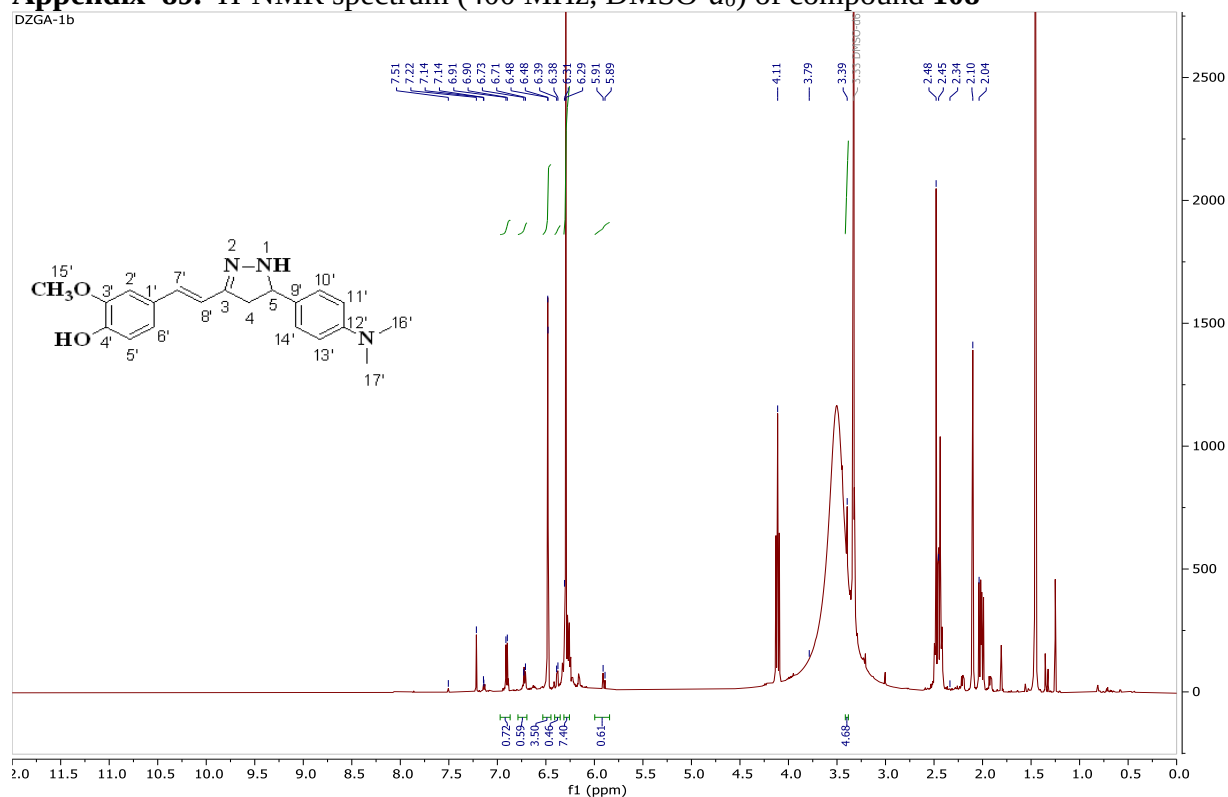
Appendix 87. ^1H -NMR spectrum (400 MHz, $\text{MeOD-}d_4$) of compound **107**



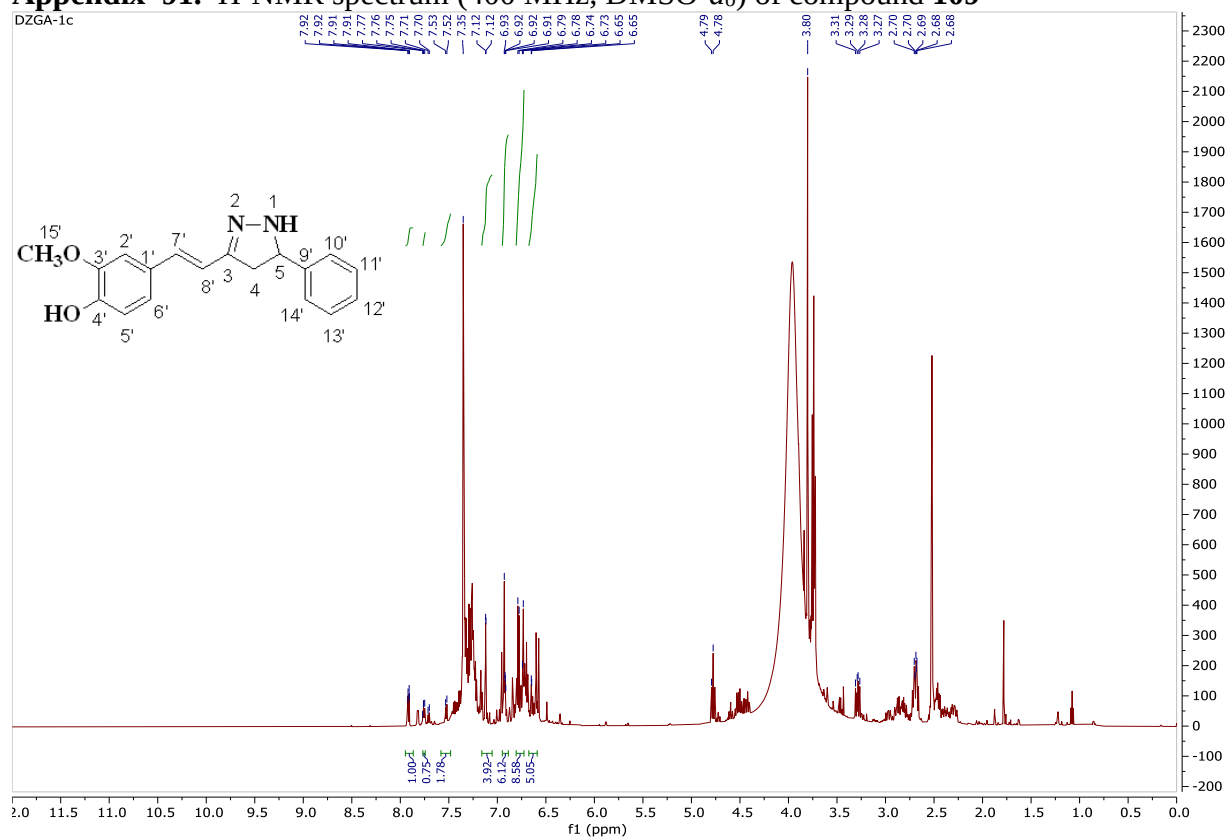
Appendix 88. ^{13}C -NMR spectrum (100 MHz, $\text{MeOD-}d_4$) of compound **107**



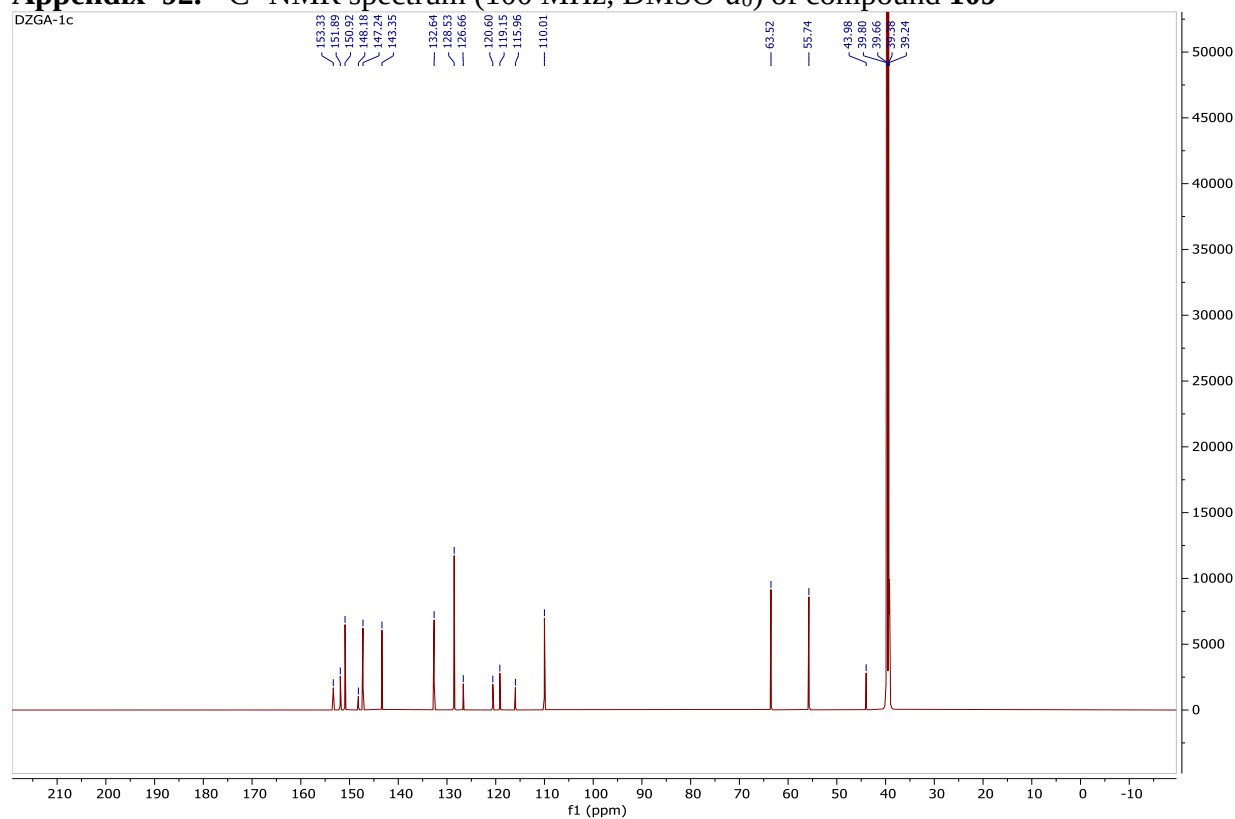
Appendix 89. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **108**



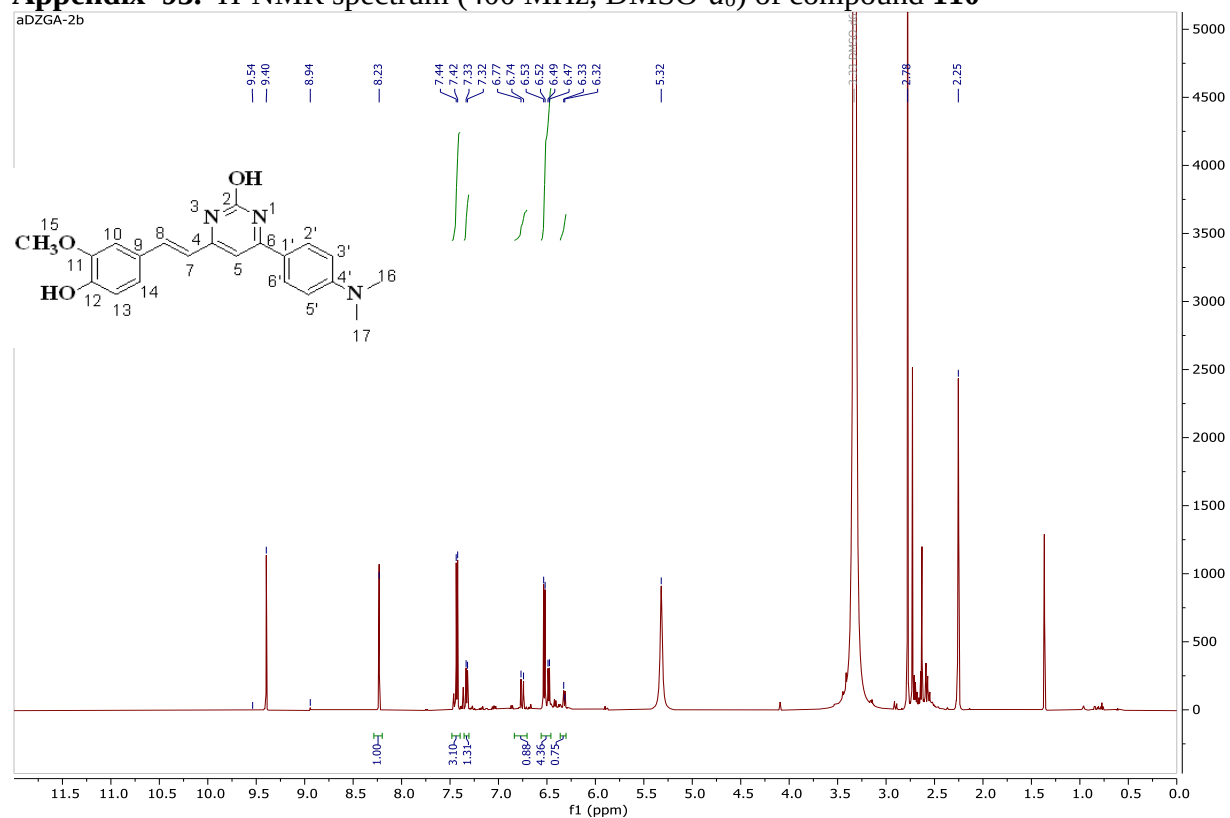
Appendix 91. ¹H-NMR spectrum (400 MHz, DMSO-*d*₆) of compound **109**



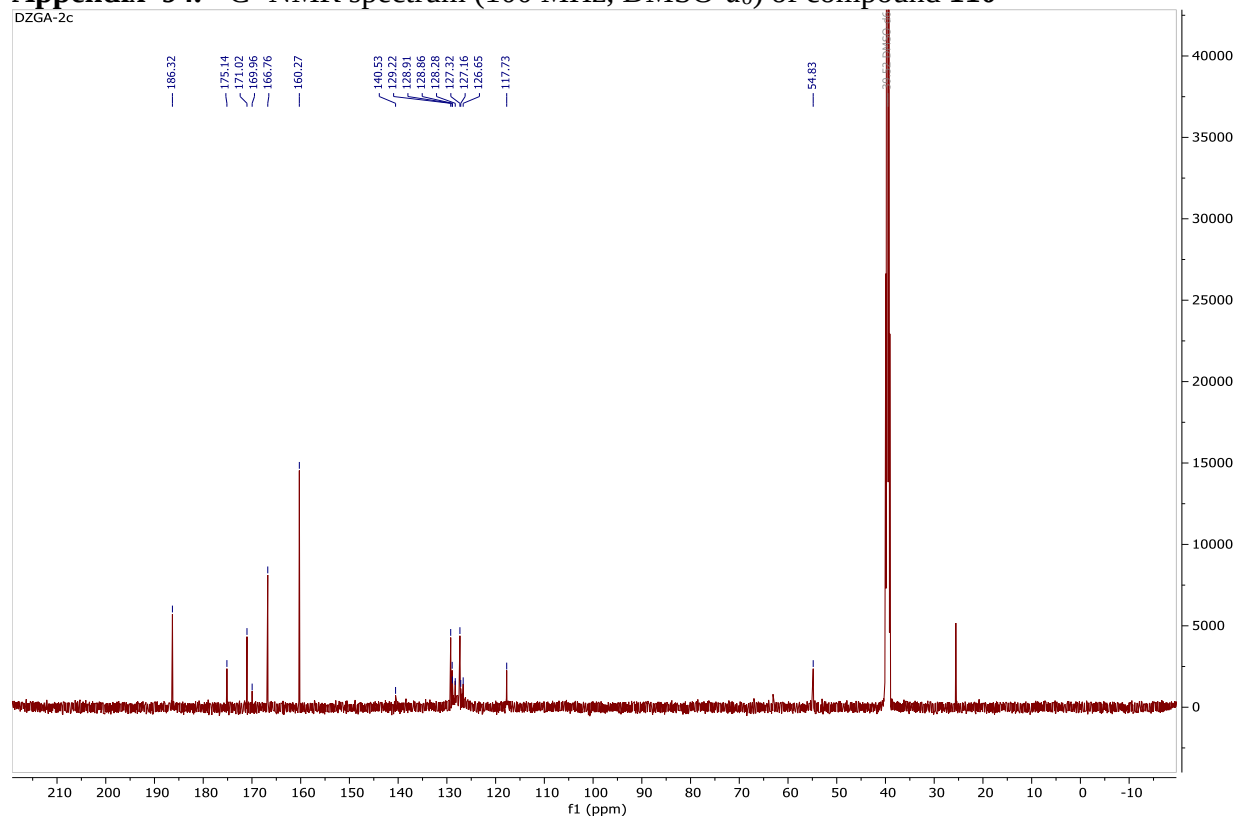
Appendix 92. ¹³C-NMR spectrum (100 MHz, DMSO-*d*₆) of compound **109**



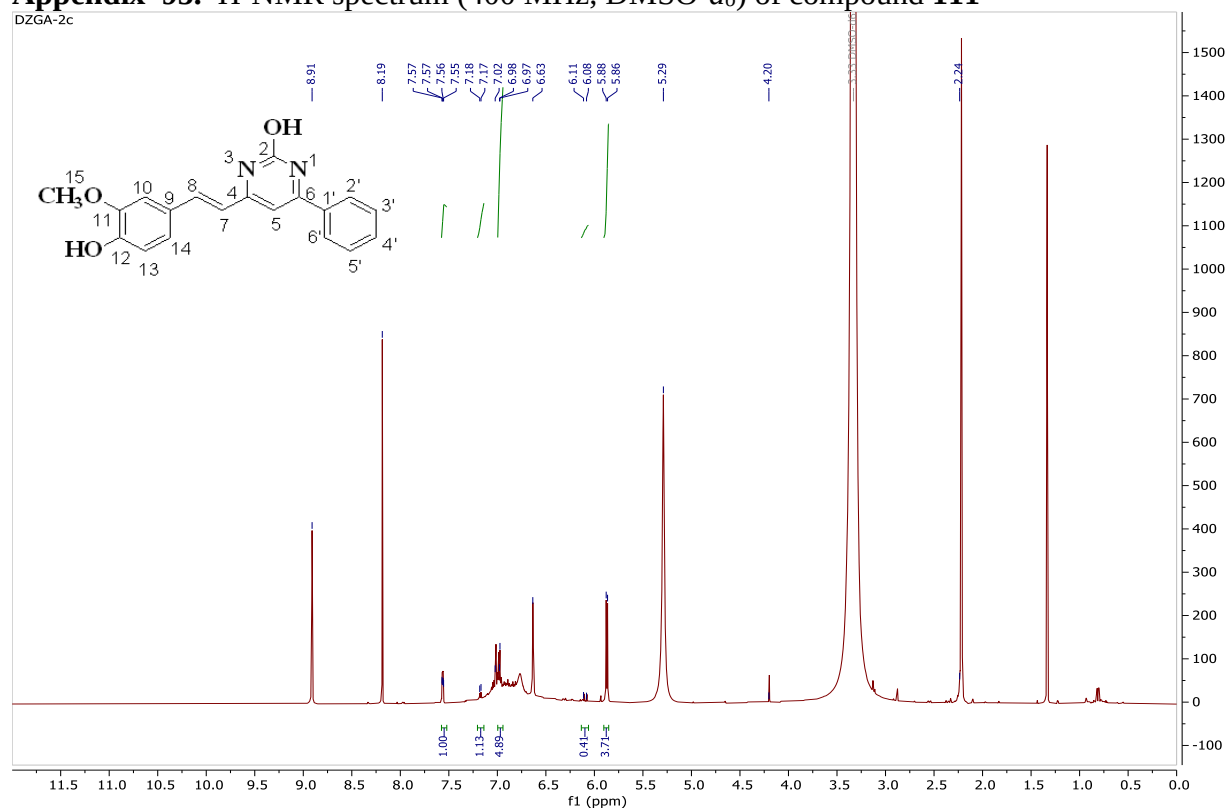
Appendix 93. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **110**



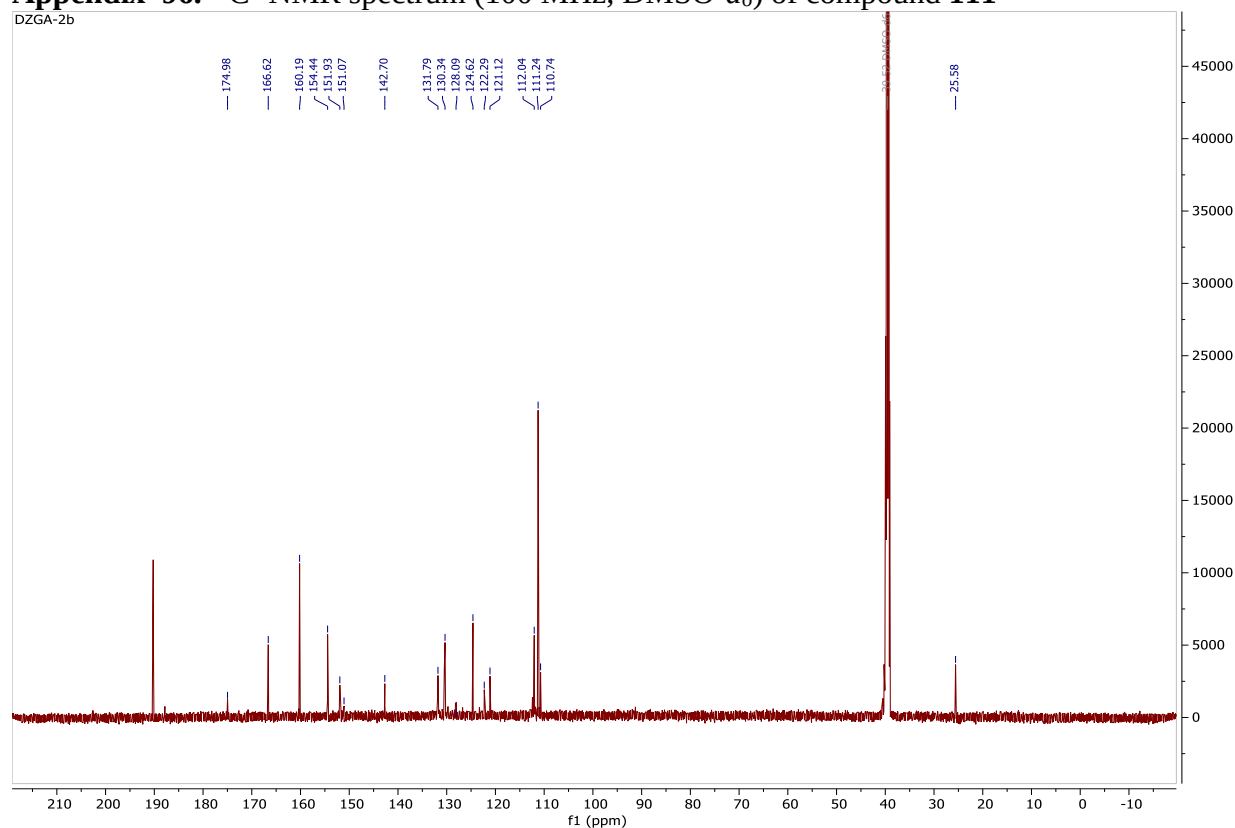
Appendix 94. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **110**



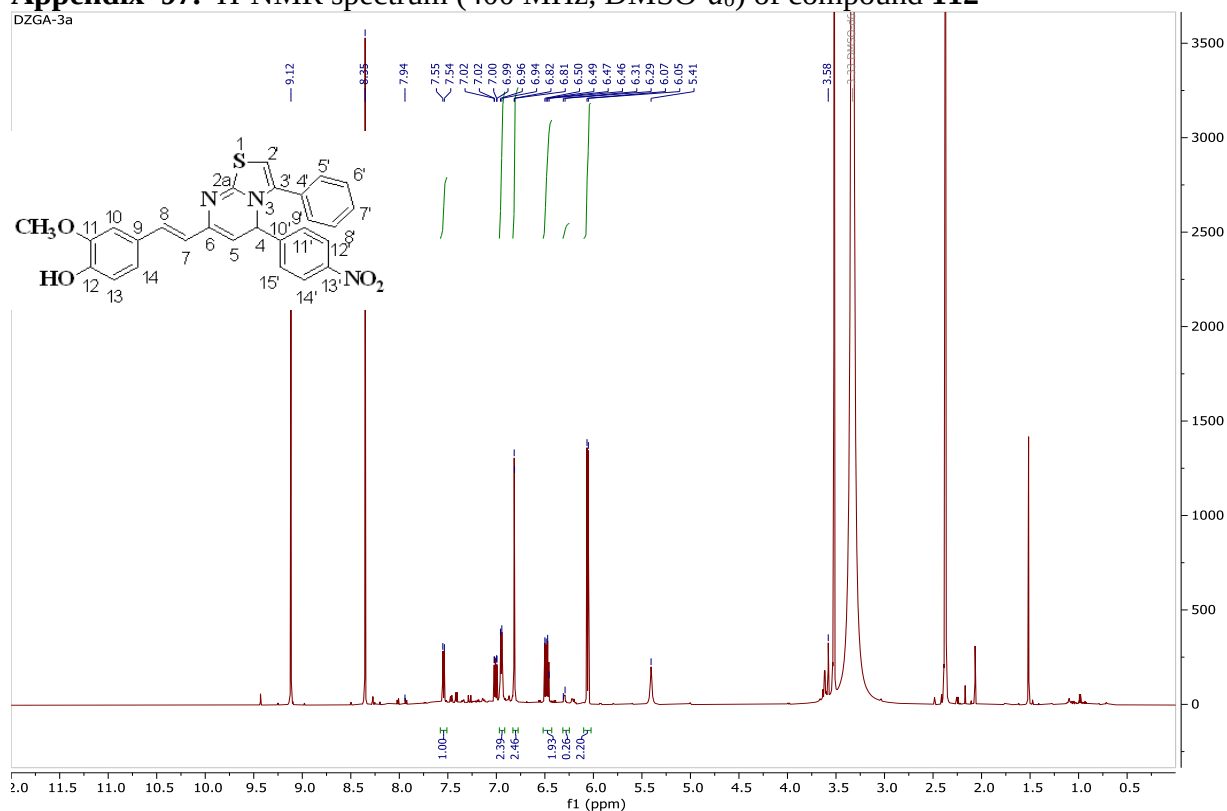
Appendix 95. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **111**



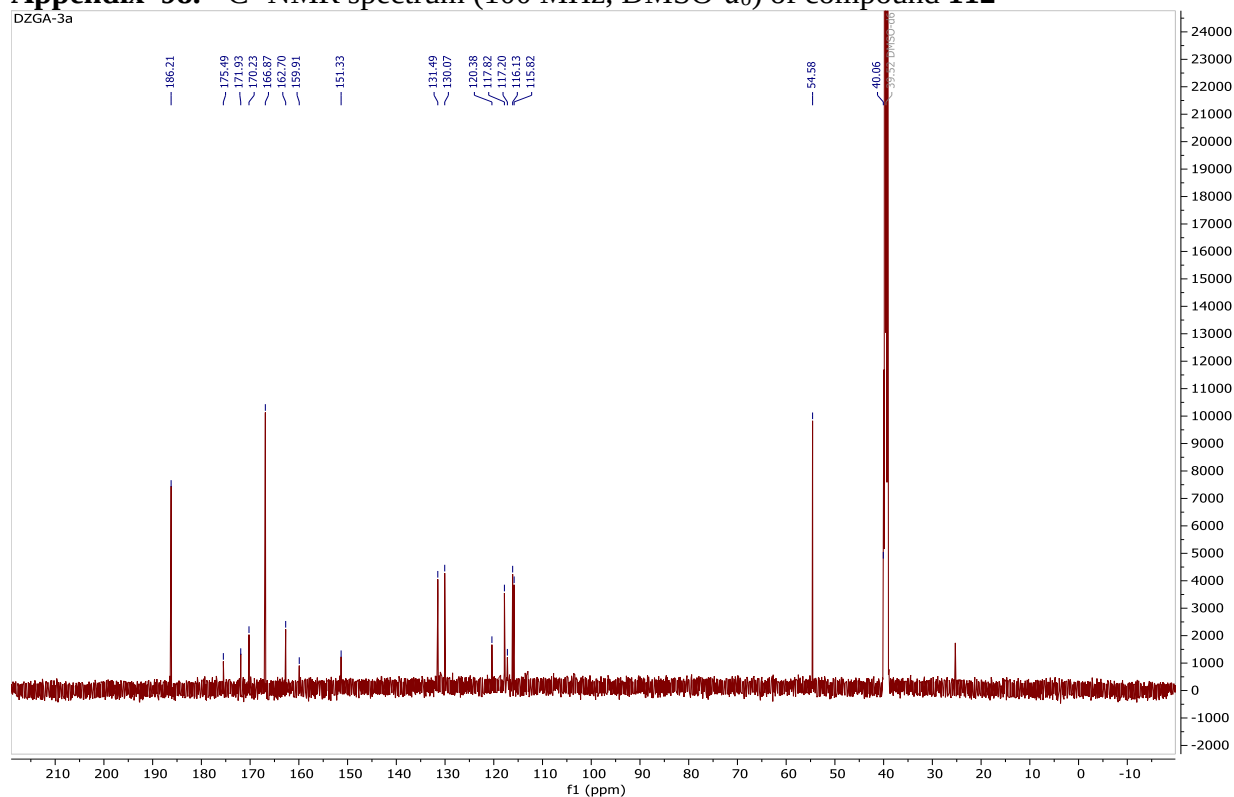
Appendix 96. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **111**



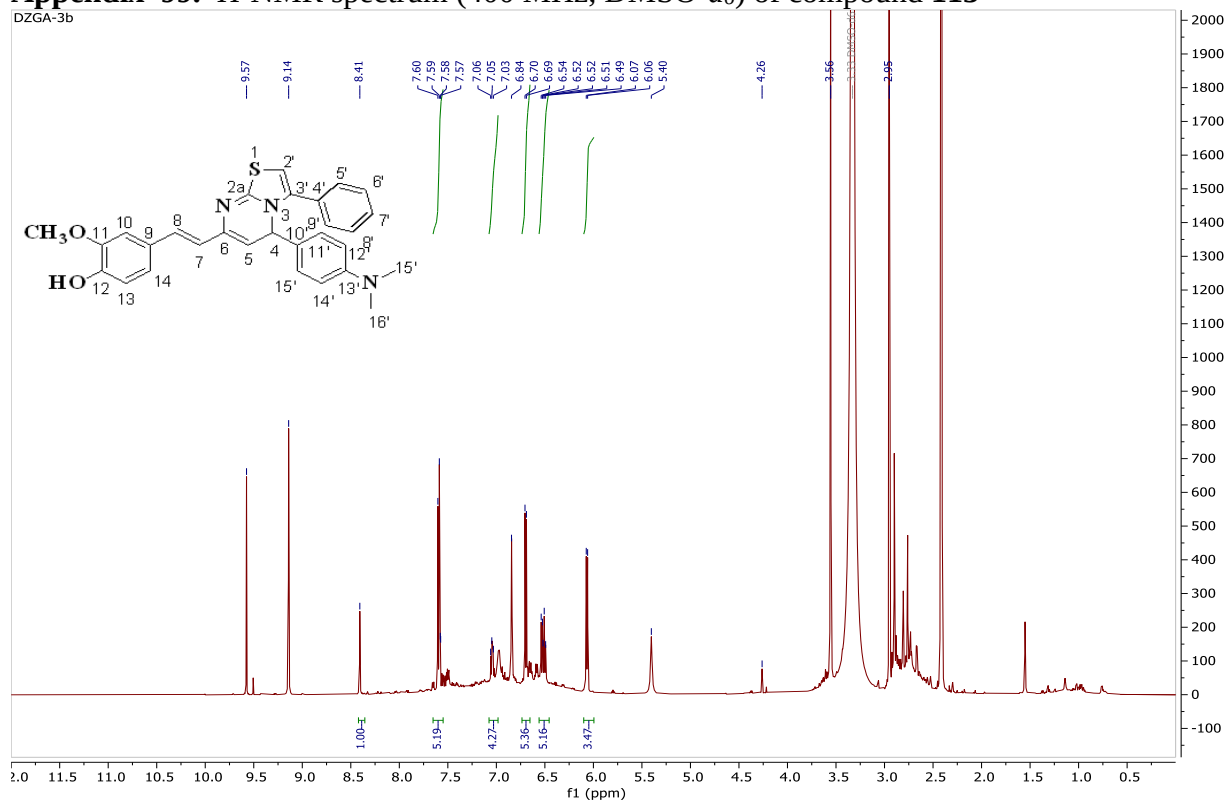
Appendix 97. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound 112



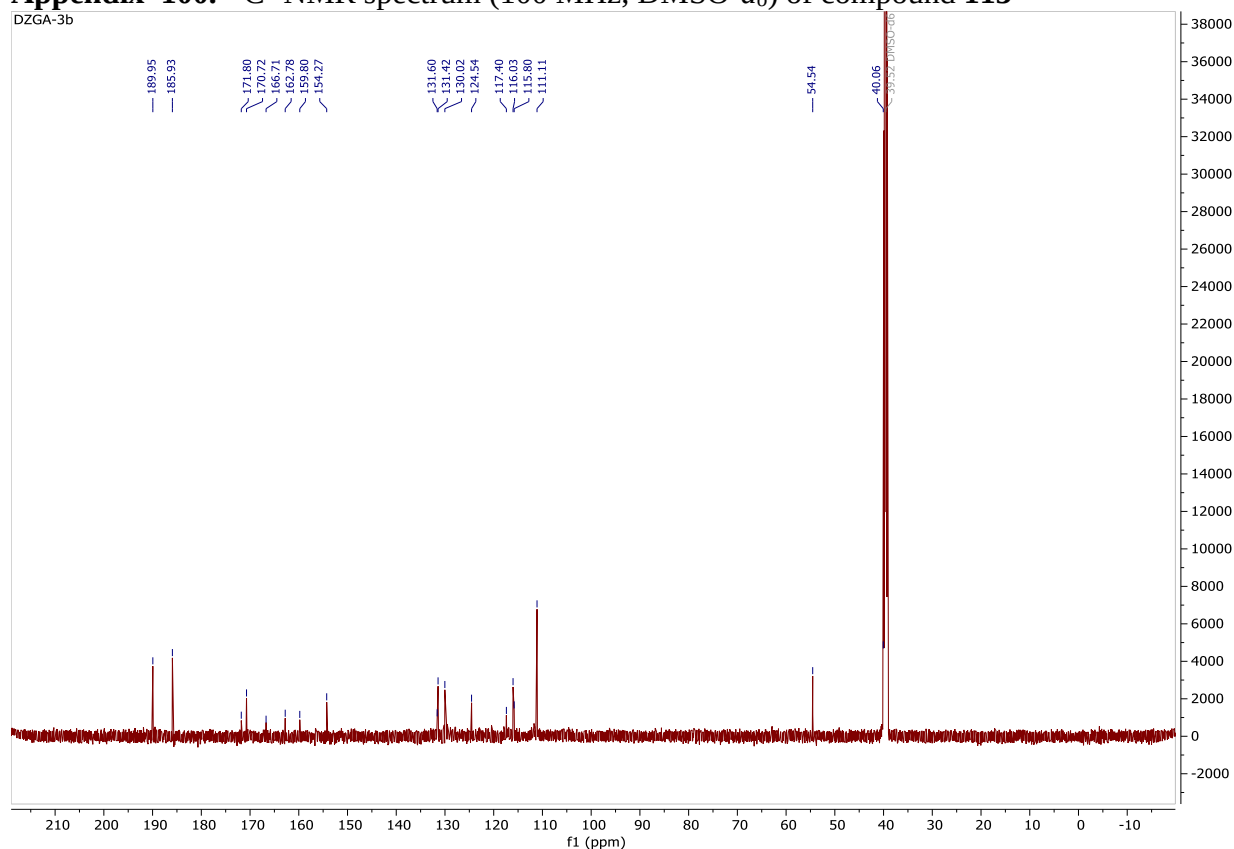
Appendix 98. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound 112



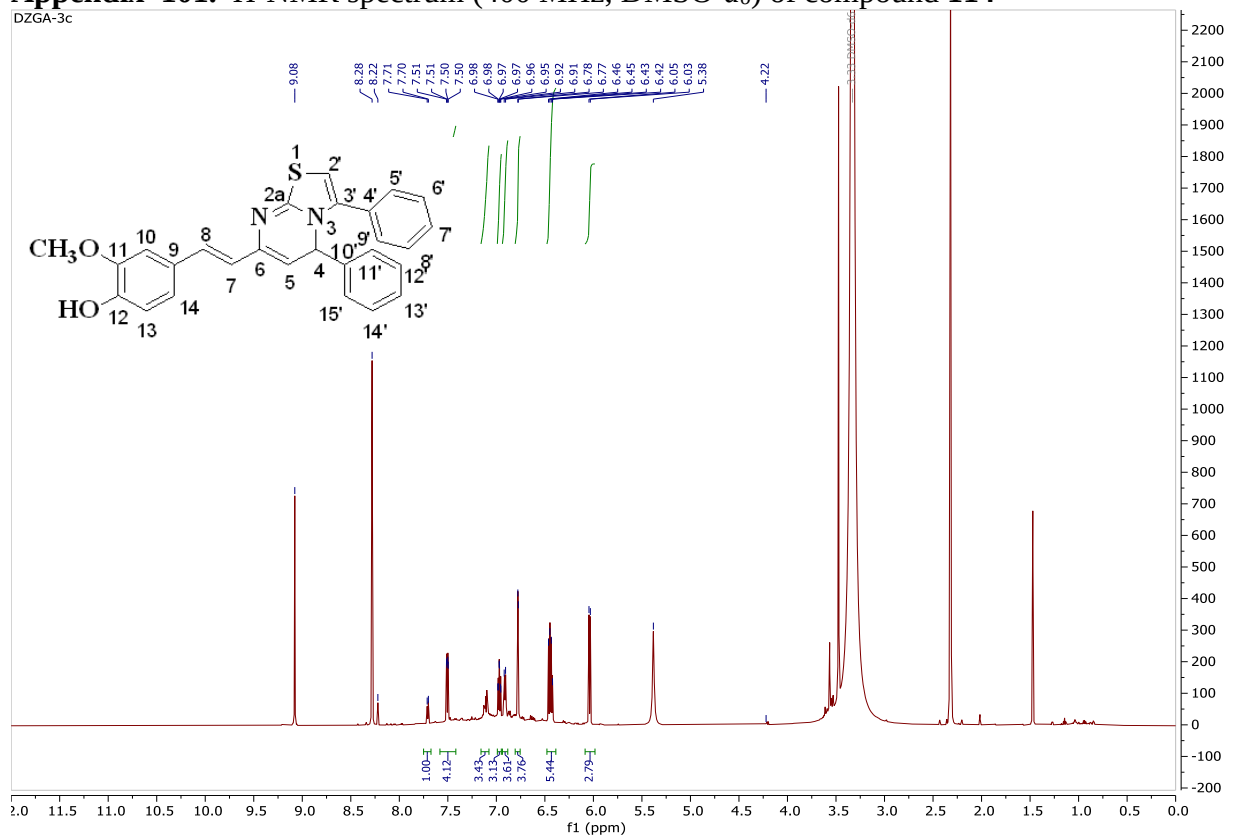
Appendix 99. ^1H -NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound 113



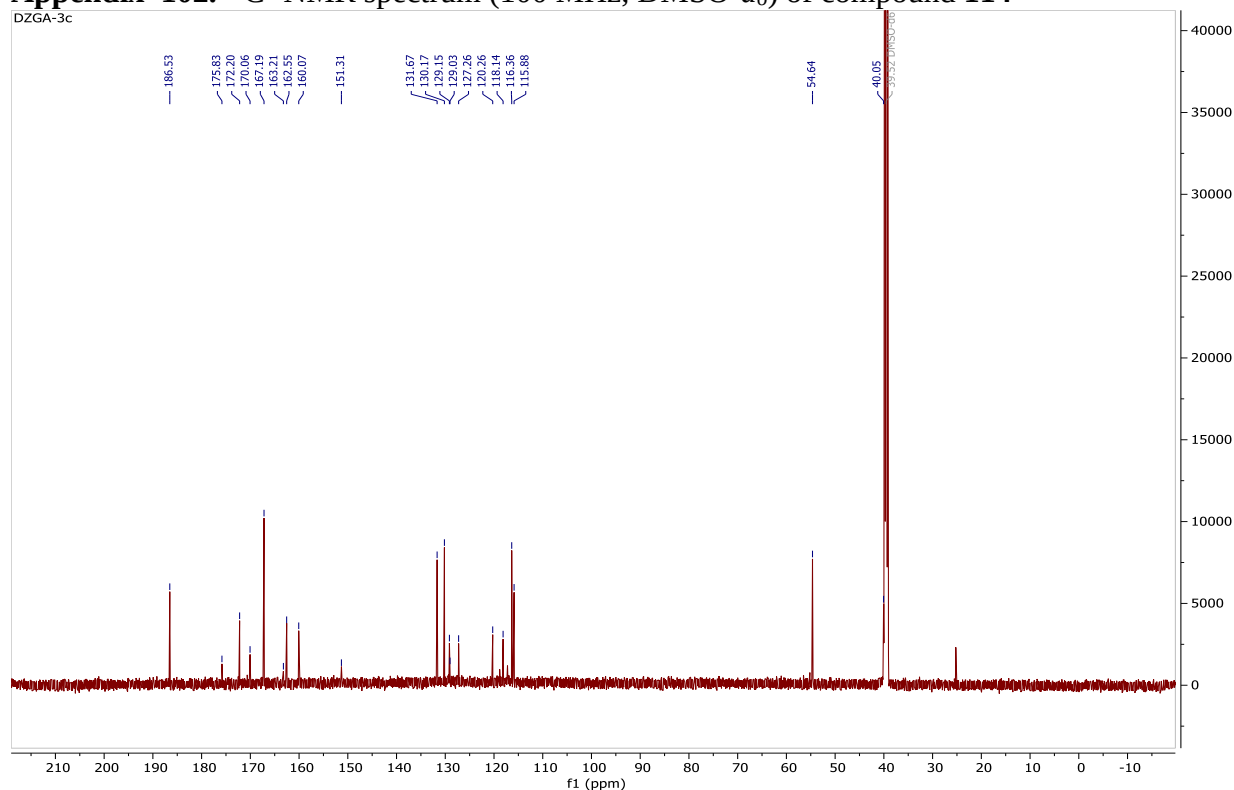
Appendix 100. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound 113



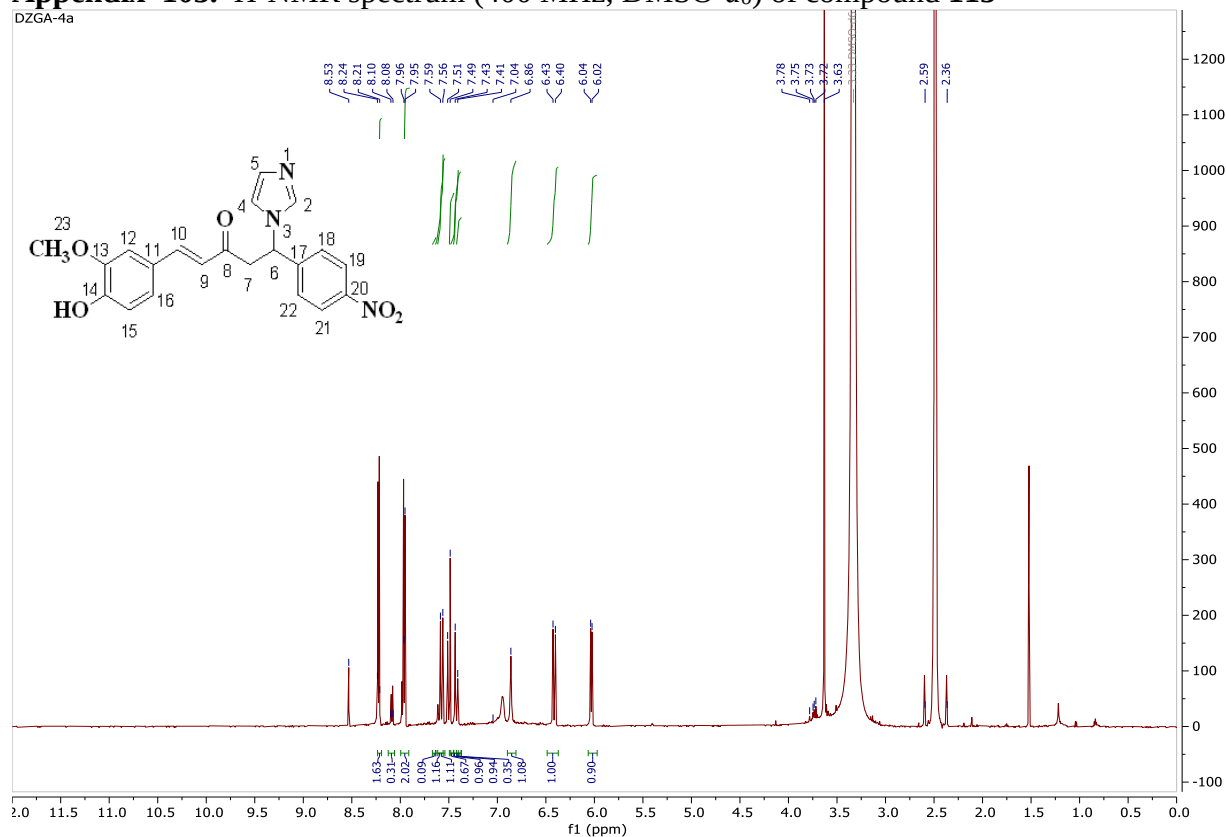
Appendix 101. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **114**



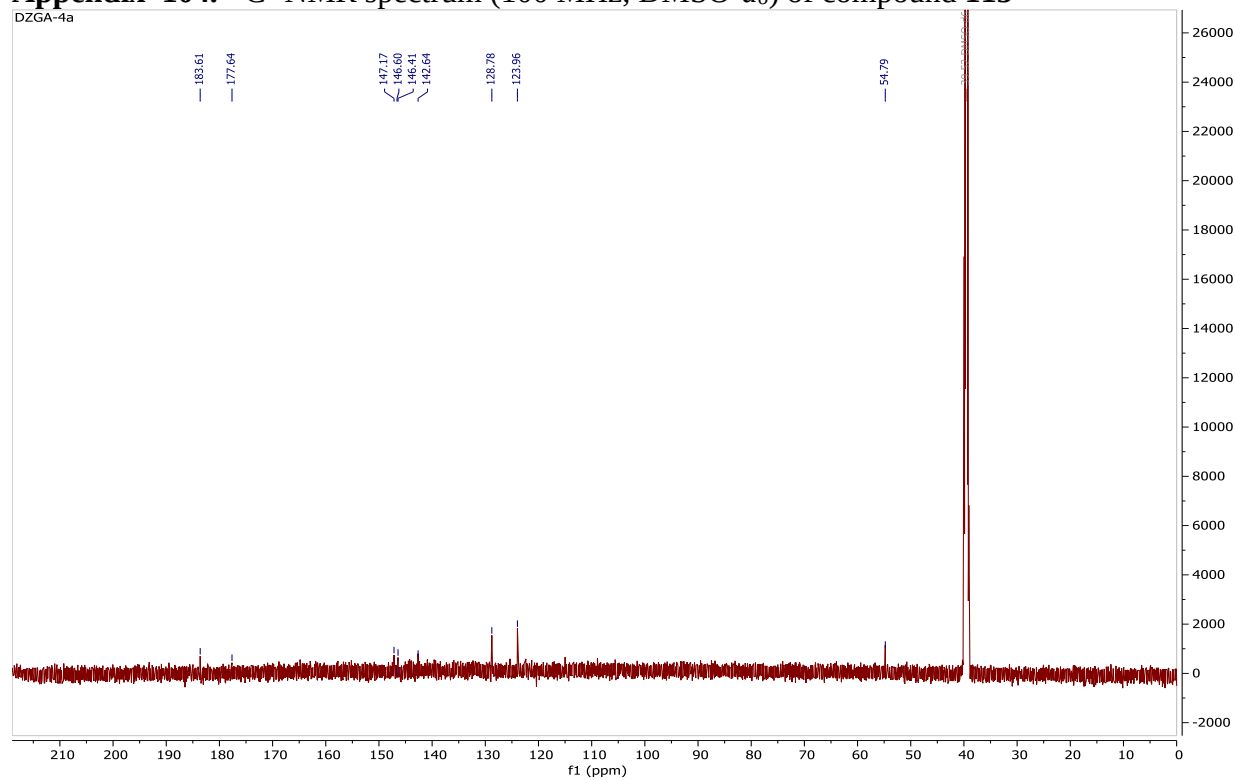
Appendix 102. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **114**



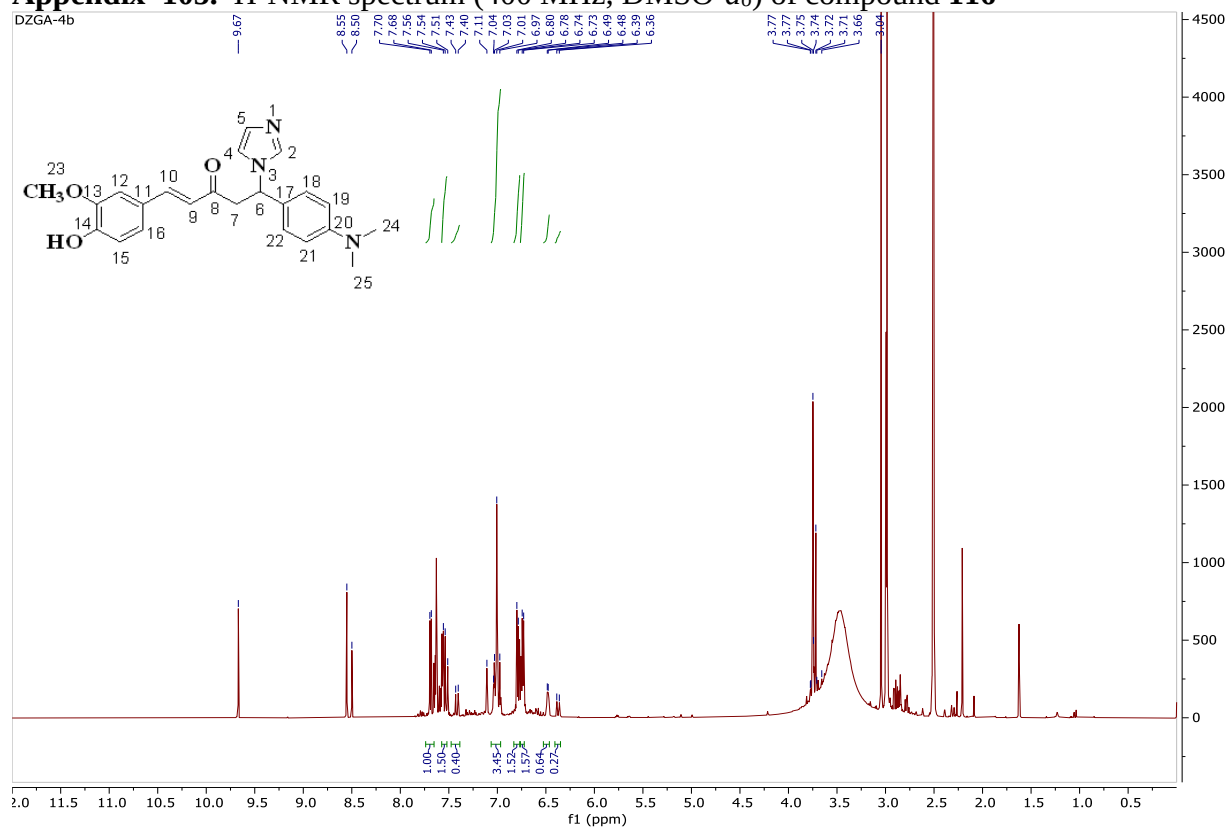
Appendix 103. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **115**



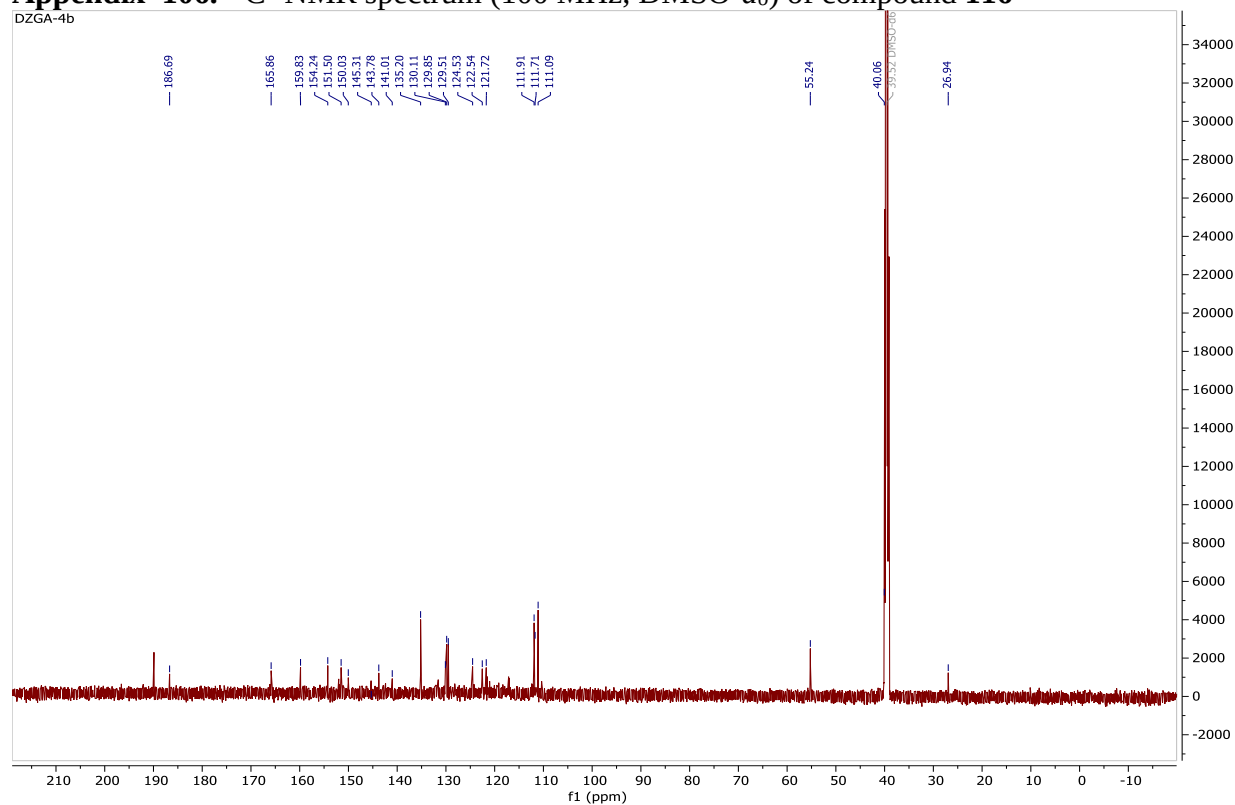
Appendix 104. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **115**



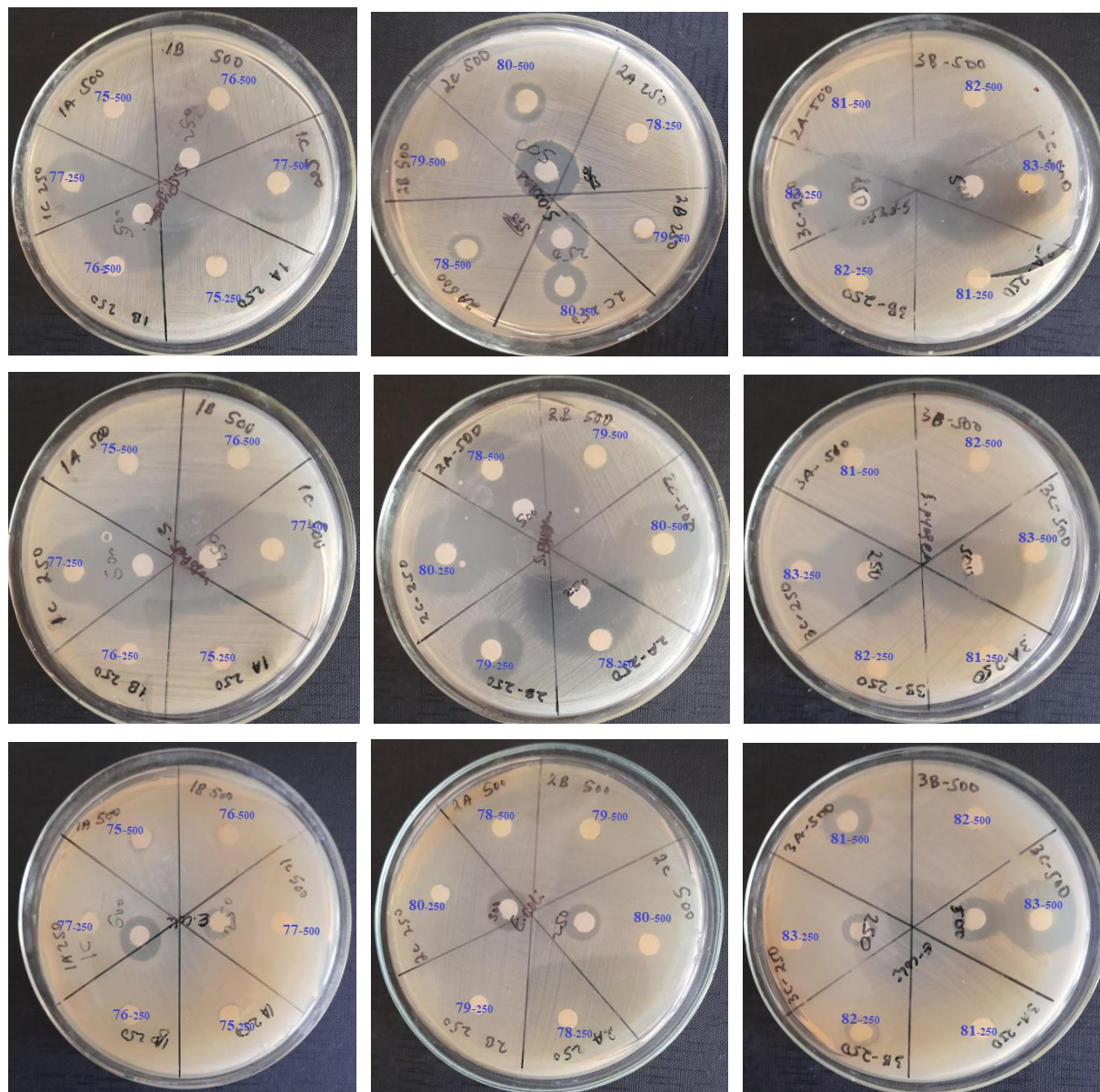
Appendix 105. ^1H -NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **116**

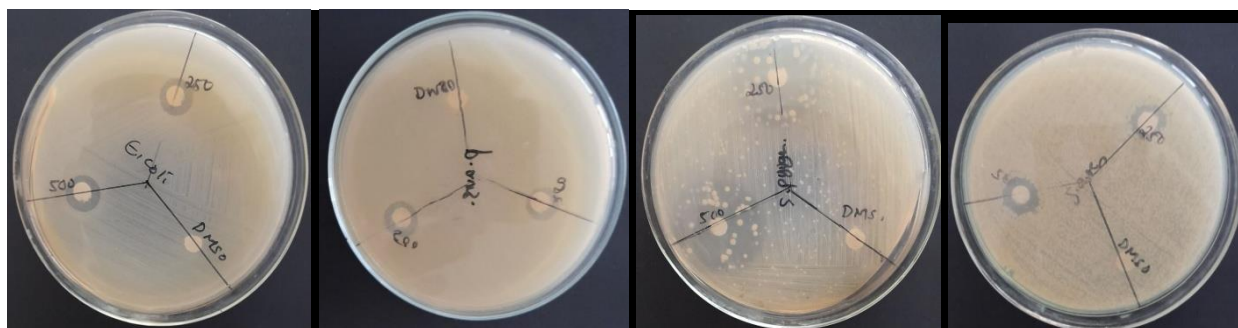
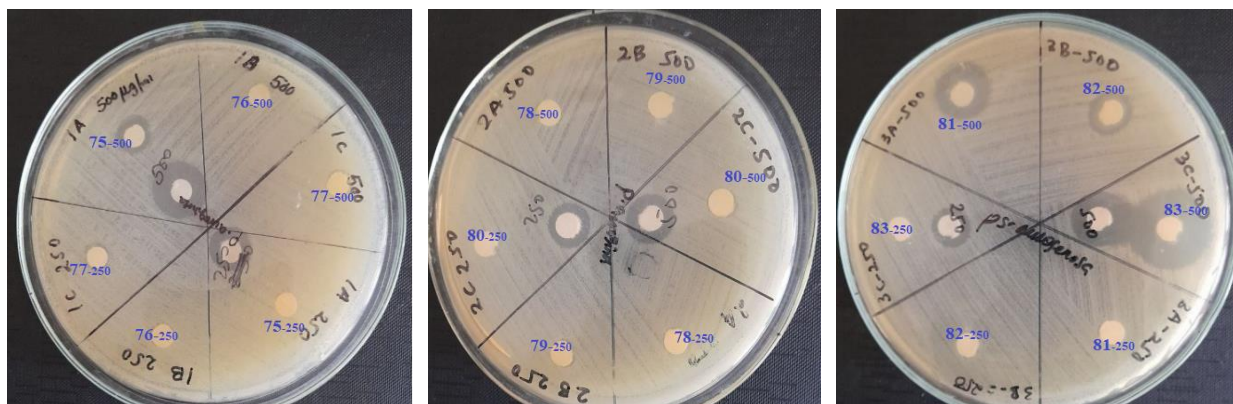


Appendix 106. ^{13}C -NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **116**

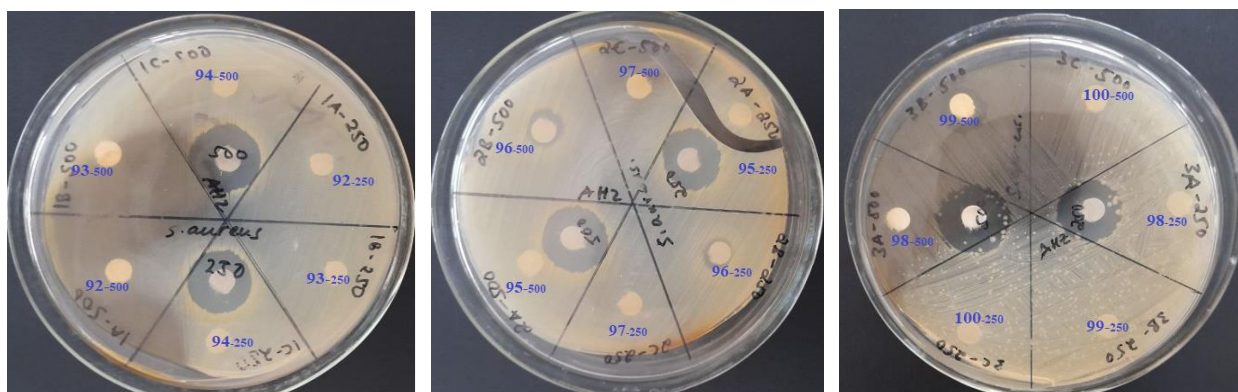


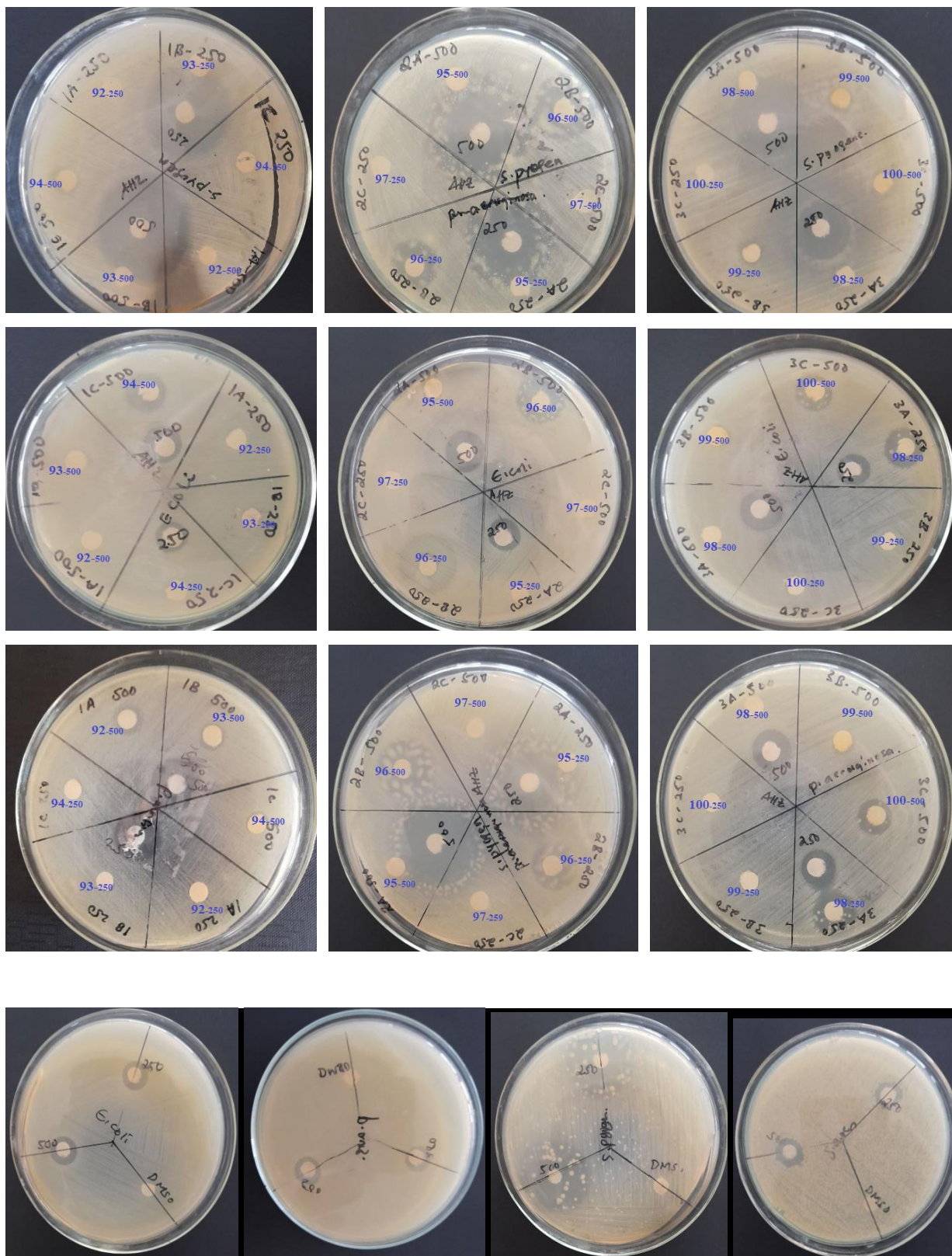
Appendix 107. *In vitro* antibacterial activity of thiazolyl-pyrazoline Schiff base hybrids (75-83) against pathological strains expressed as inhibition diameter zones in millimeters (unit, mm) based on agar well diffusion assay compared to positive control (amoxicillin) and negative control (DMSO), results given in $\mu\text{g/mL}$ sample



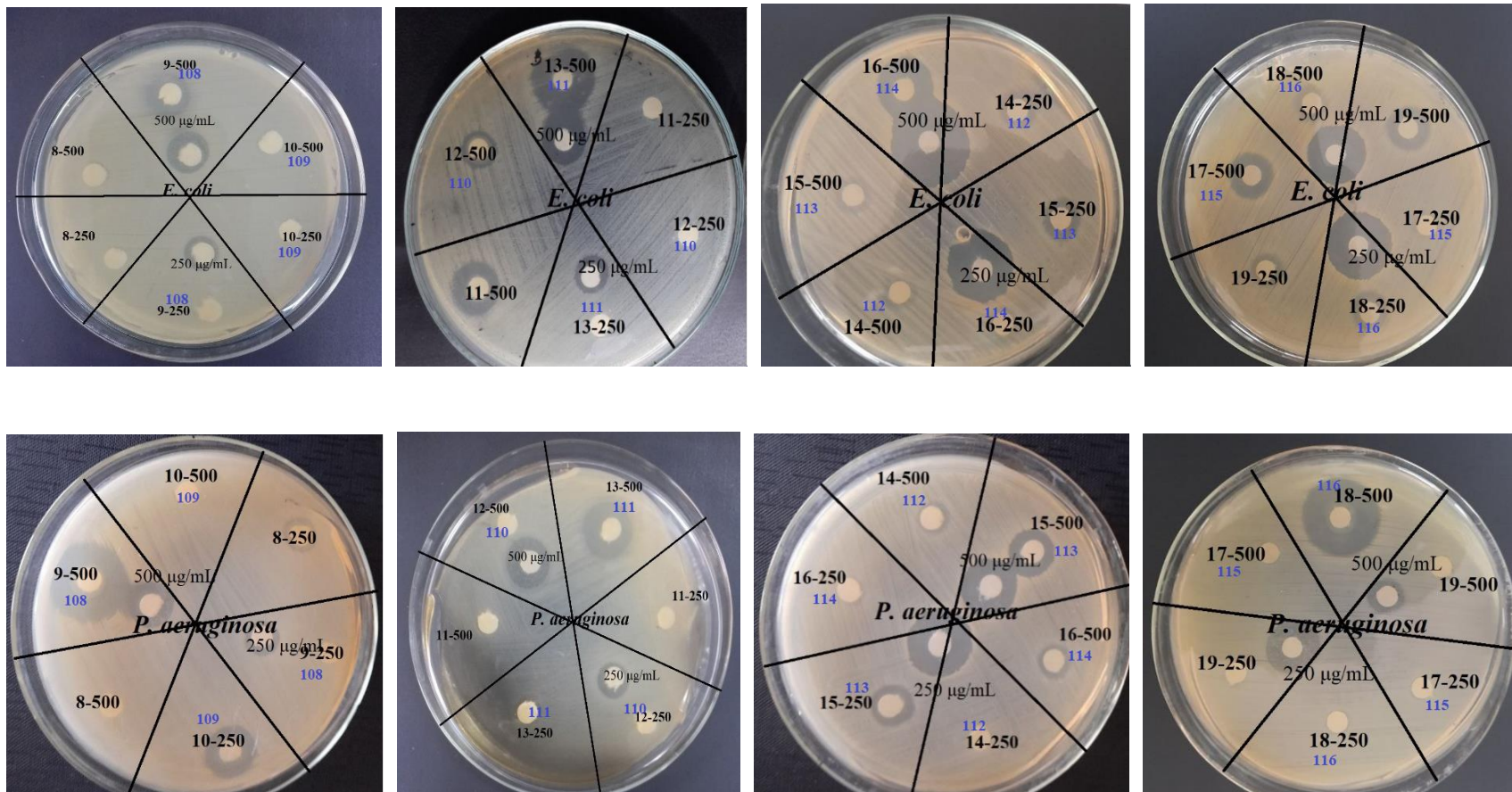


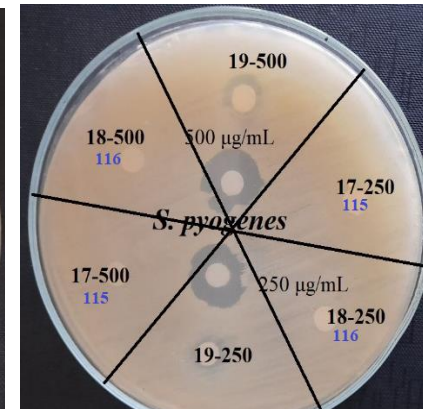
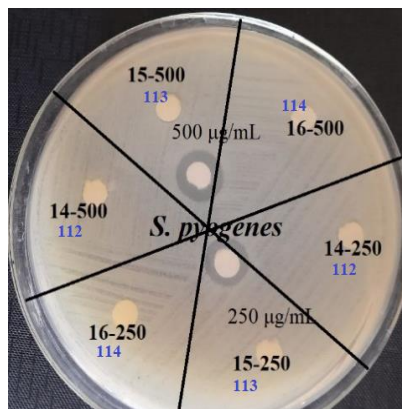
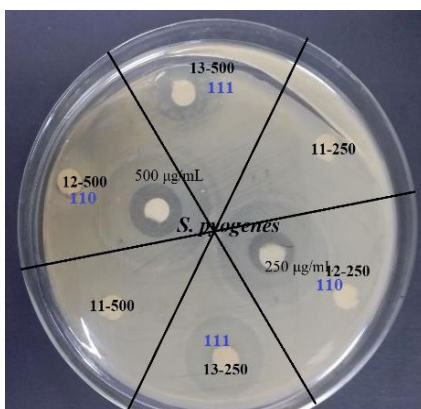
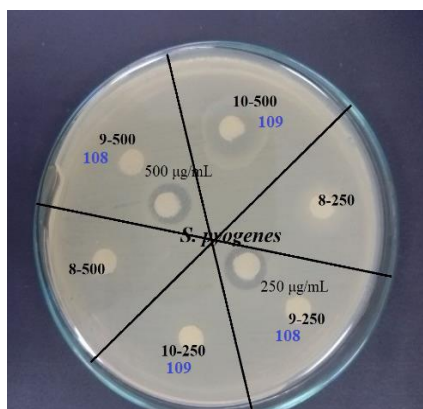
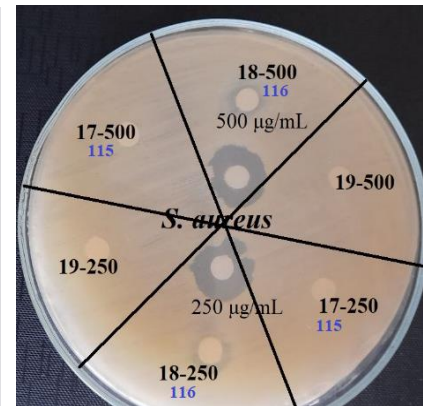
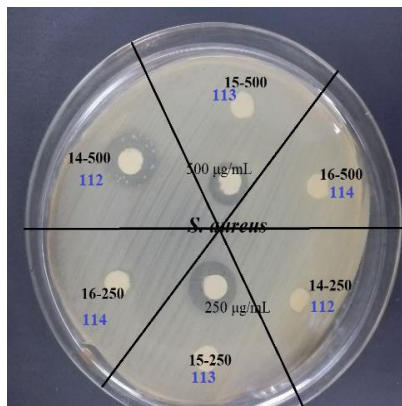
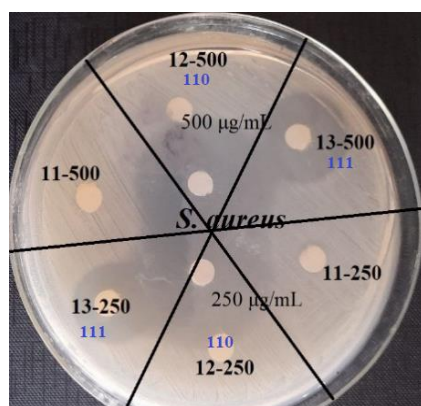
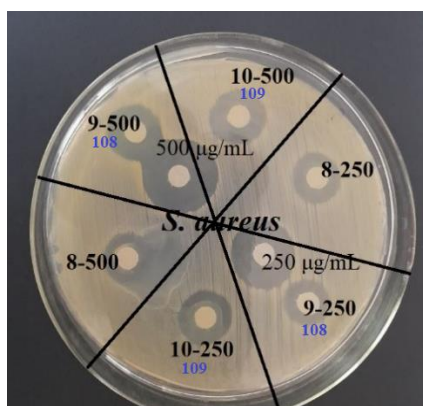
Appendix 108. *In vitro* antibacterial activity of thiazolo[3,2- α] pyrimidine analogs (92-100) against pathological strains expressed as the diameter of inhibition zones in millimeters (unit, mm) based on agar well diffusion assay compared to a positive control (amoxicillin) and negative control (DMSO), results given in $\mu\text{g/mL}$ sample

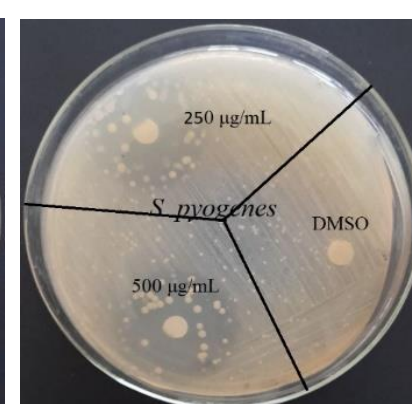
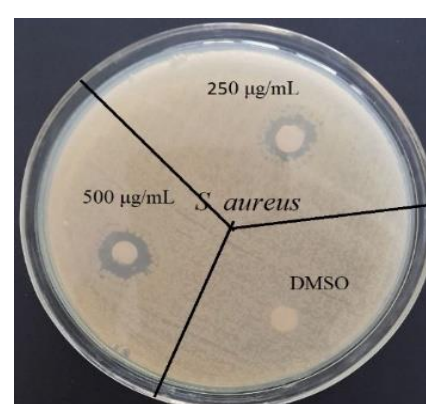
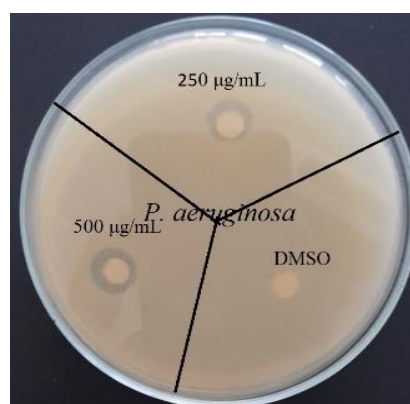
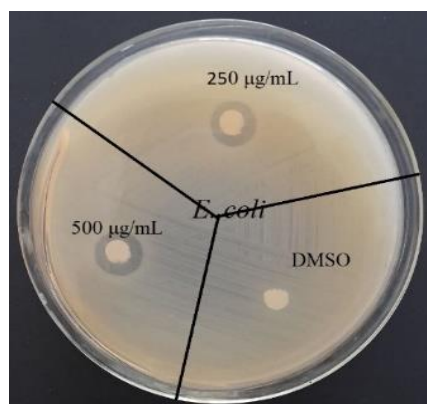




Appendix 109. *In vitro* antibacterial activity of heterocyclics C₅-curcuminoid analogs (**108-116**) against pathological strains expressed as the diameter of inhibition zones in millimeters (unit, mm) based on agar well diffusion assay compared to a positive control (amoxicillin) and negative control (DMSO), results given in $\mu\text{g/mL}$ sample



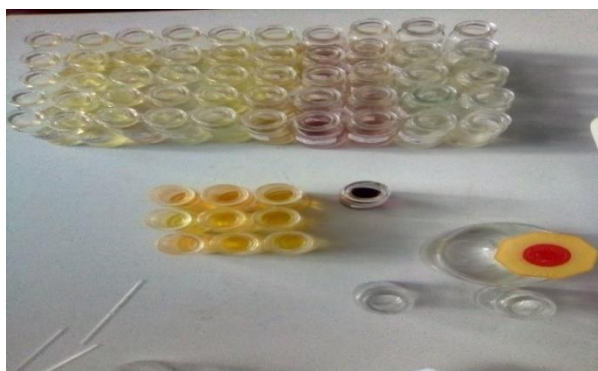




Appendix 110. DPPH free radical scavenging activity of the synthesized compounds (75-83) and the standard drug at the different concentration ranges (namely 12.5, 25.0, 50.0, 100.0, and 200.0 $\mu\text{g/mL}$)



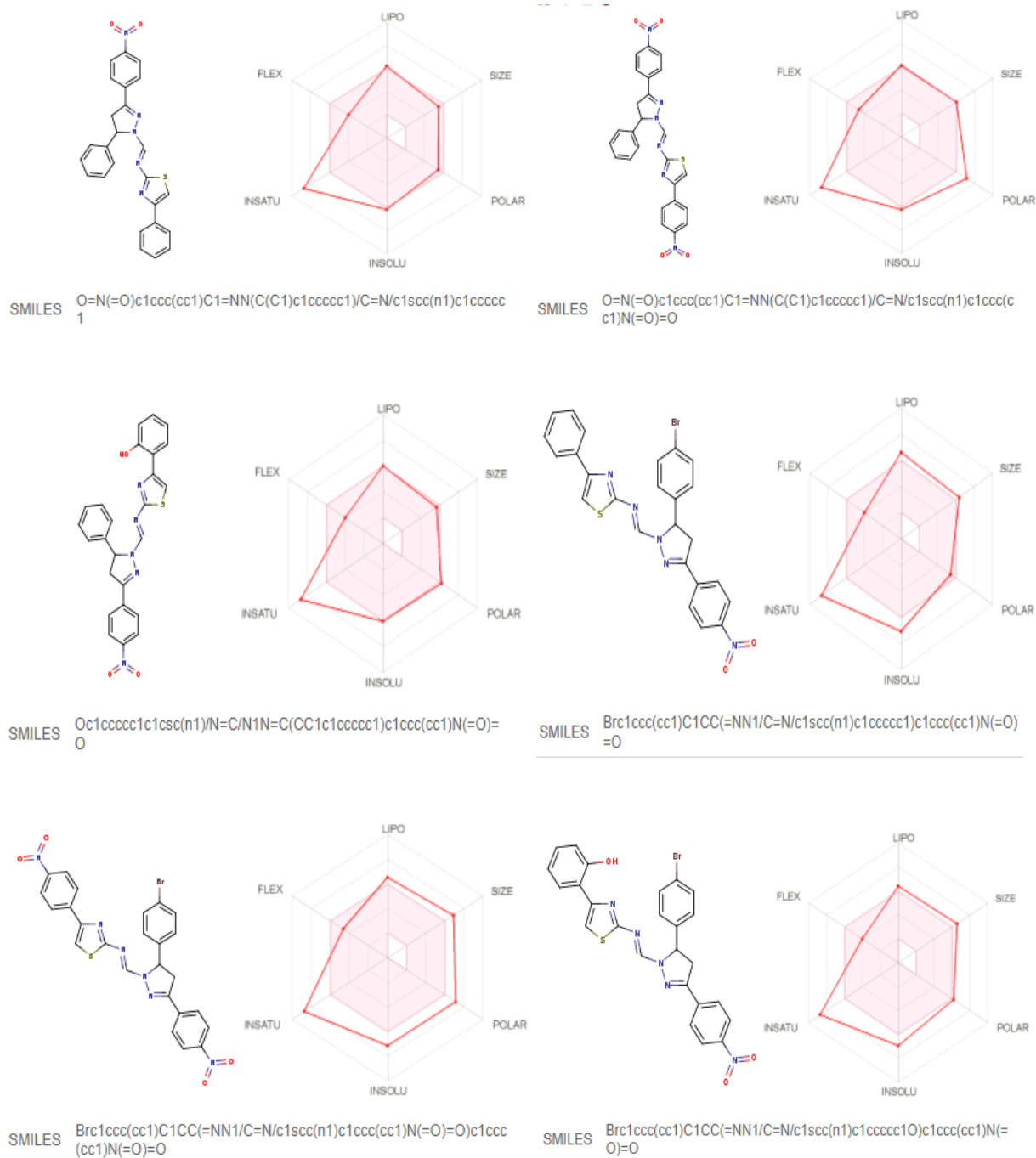
Appendix 111. DPPH free radical scavenging activity of the synthesized compounds (92-100) and the standard drug at the different concentration ranges.

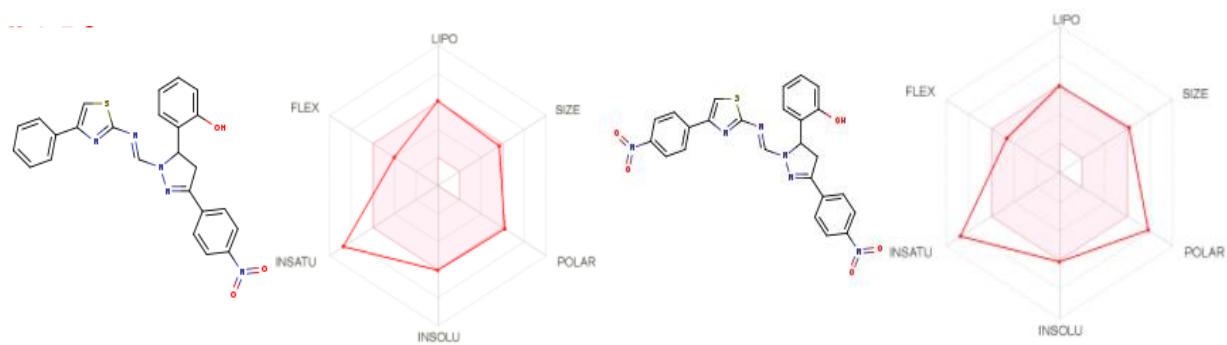


Appendix 112. DPPH free radical scavenging activity of the synthesized compounds (108-116) and the standard drug at the different concentration ranges.



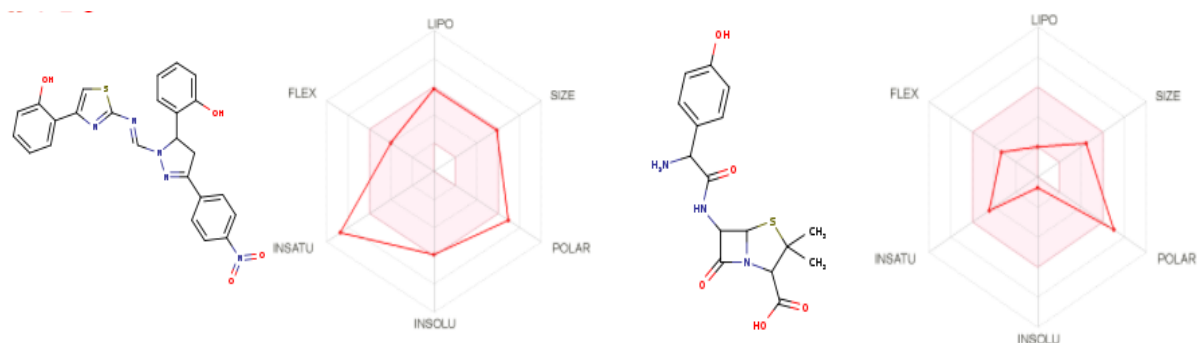
Appendix 113. Bioavailability radar and Boiled-Egg model of the title compounds, **75-83** by the druglikeness criteria





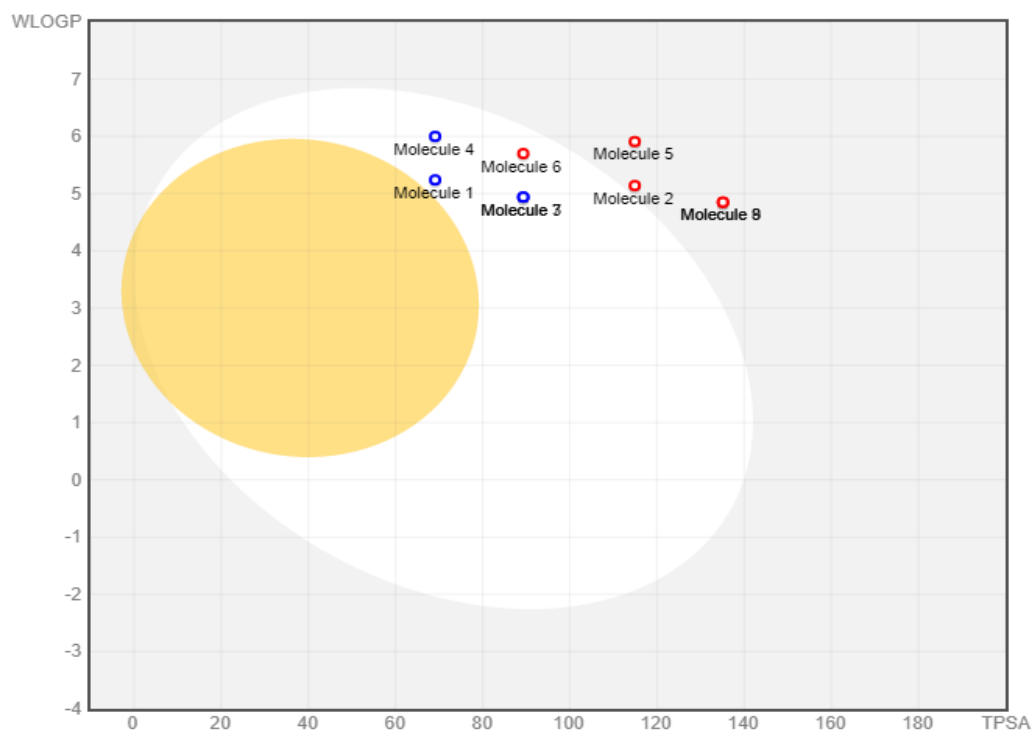
SMILES Oc1ccccc1C1CC(=NN1/C=N/c1sc(n1)c1ccccc1)c1ccc(cc1)N(=O)=O

SMILES Oc1ccccc1C1CC(=NN1/C=N/c1sc(n1)c1ccc(cc1)N(=O)=O)c1ccc(cc1)N(=O)=O

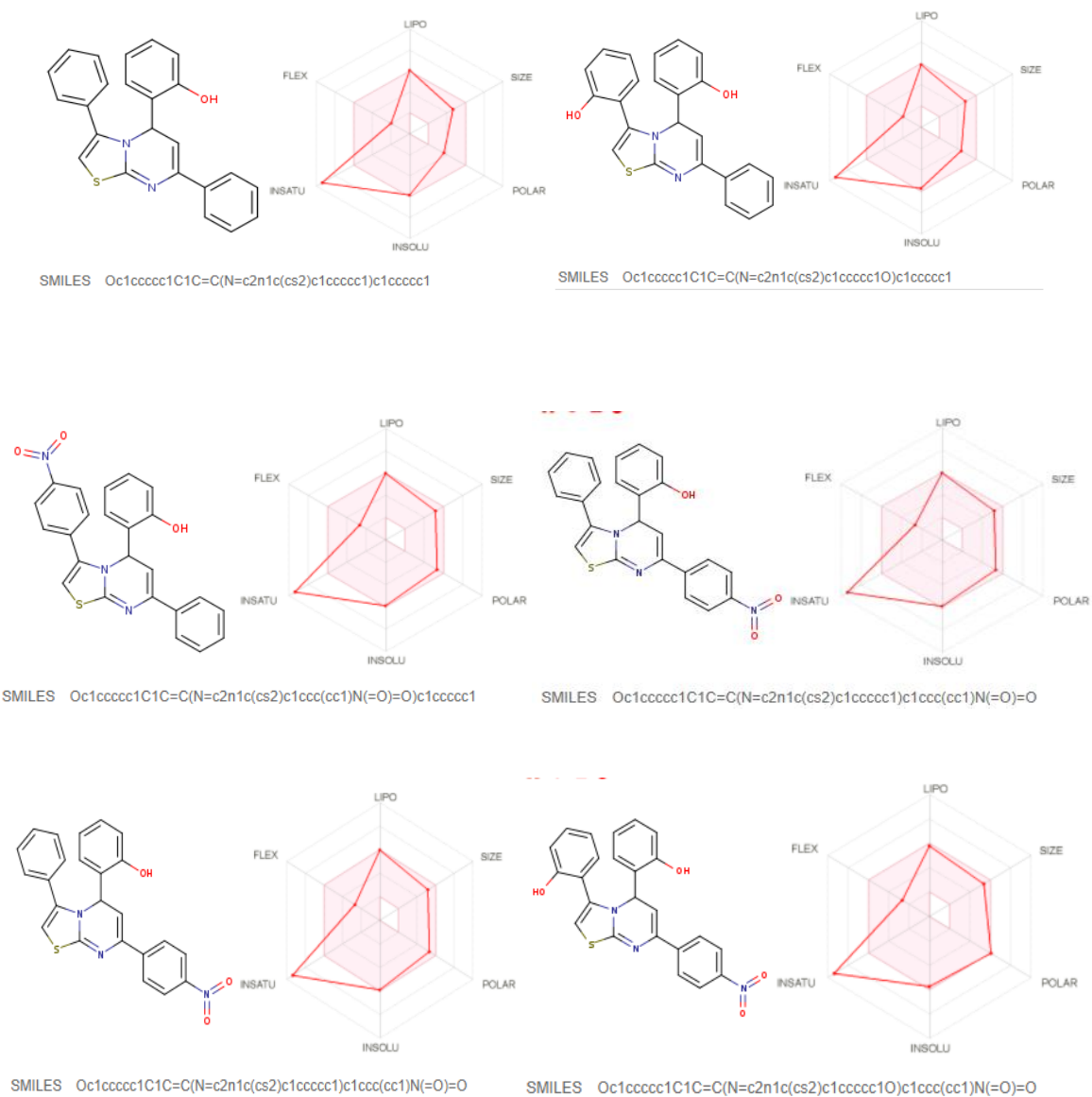


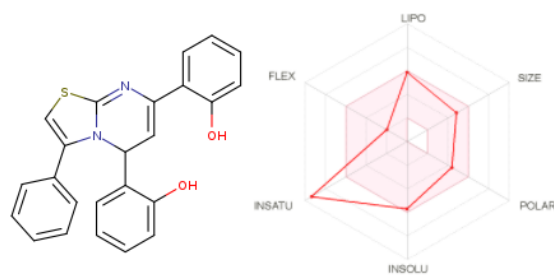
SMILES Oc1ccccc1C1CC(=NN1/C=N/c1sc(n1)c1ccccc1O)c1ccc(cc1)N(=O)=O

SMILES O=C(C(c1ccc(cc1)O)N)NC1C(=O)N2C1SC(C2C(=O)O)(C)C

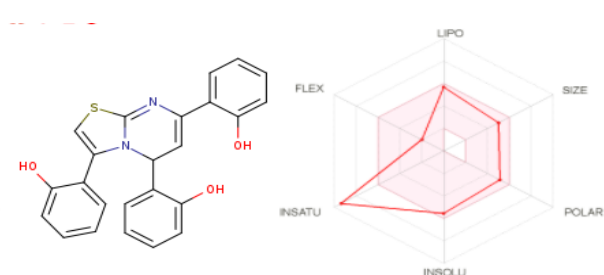


Appendix 114. Bioavailability radar and Boiled- Egg model of the title, **92-100** by the druglikeness criteria

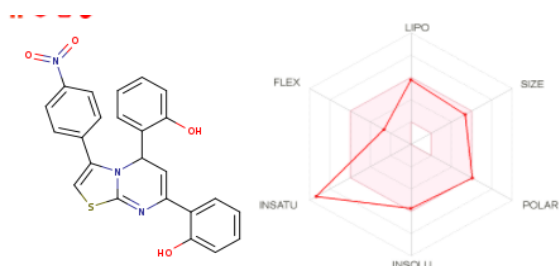




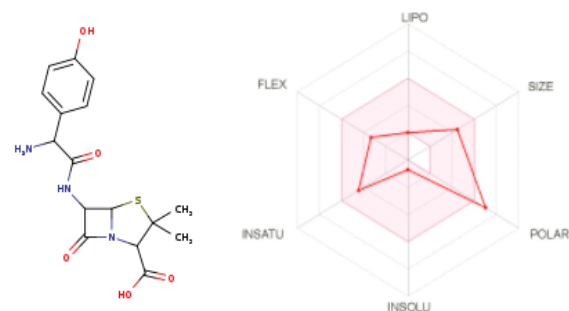
SMILES Oc1cccc1C1=CC(n2c(=N1)sc2c1cccc1)c1cccc1O



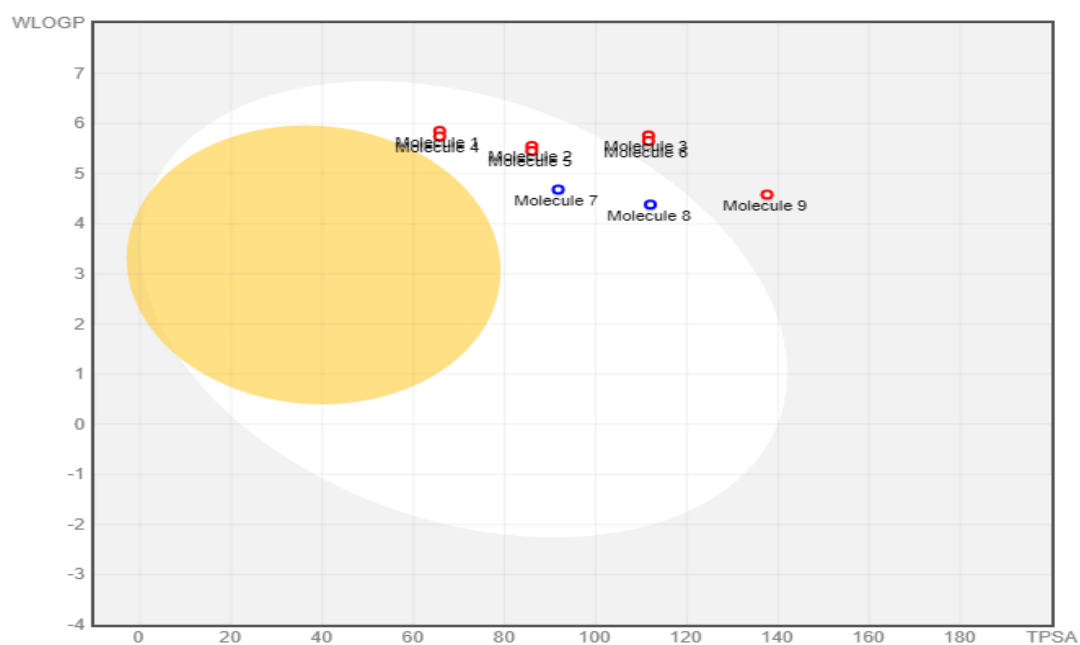
SMILES Oc1cccc1C1=CC(n2c(=N1)sc2c1cccc1O)c1cccc1O



SMILES Oc1cccc1C1C=C(N=c2n1c(cs2)c1ccc(cc1)N(=O)=O)c1cccc1O

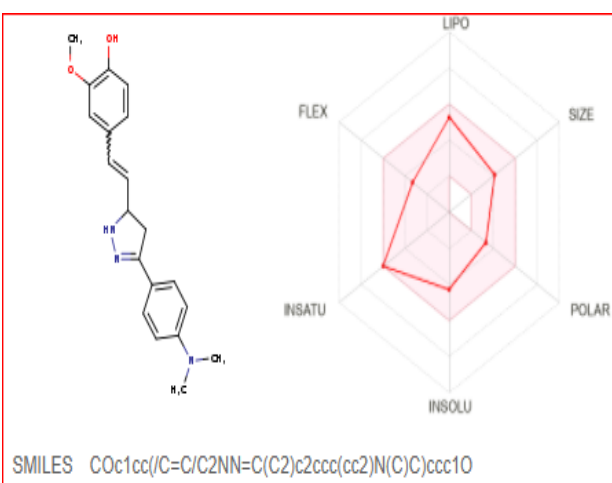
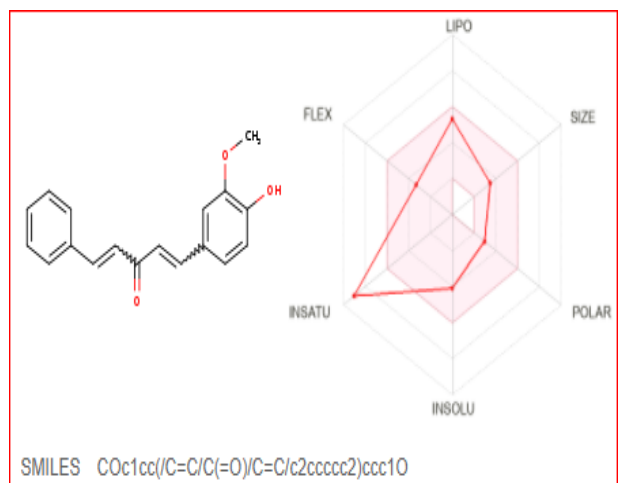
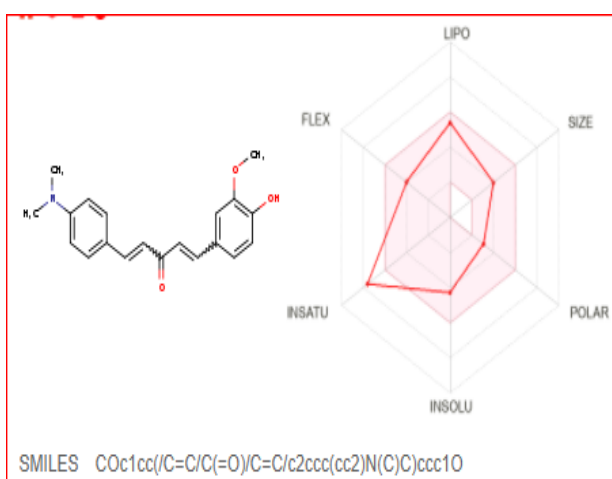
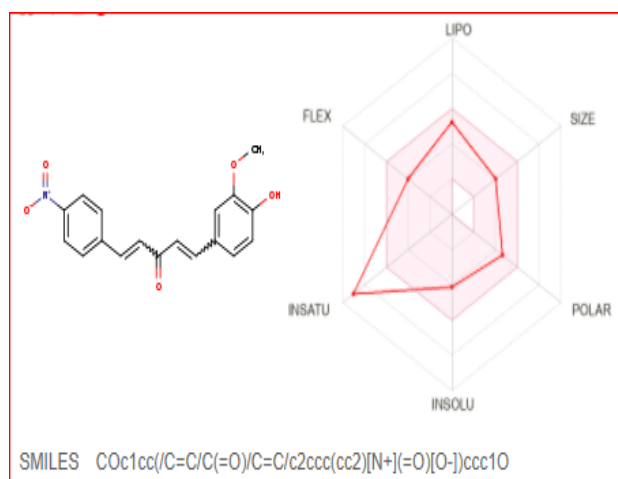
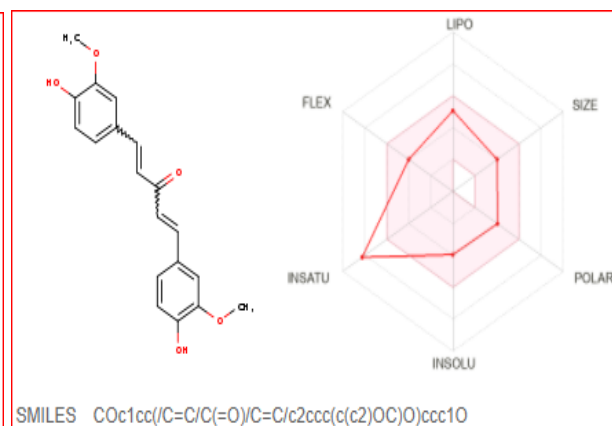
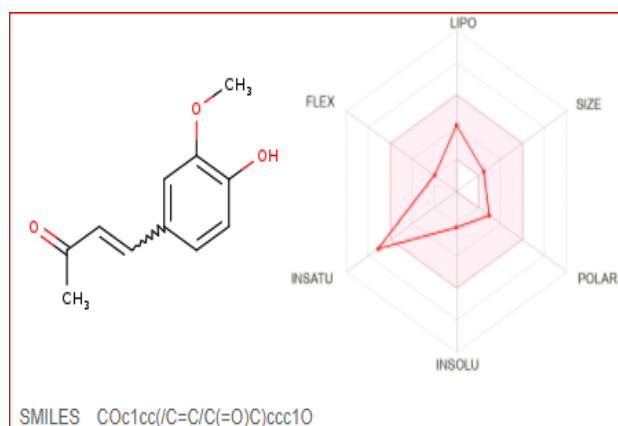


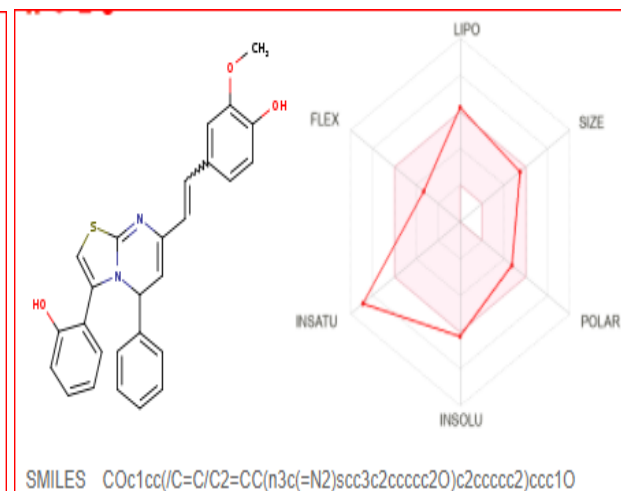
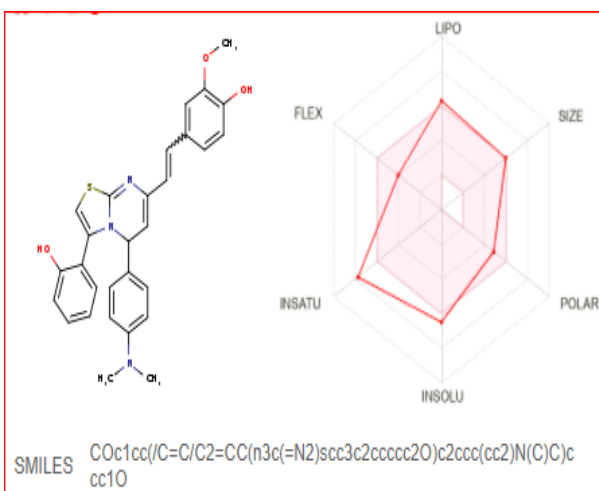
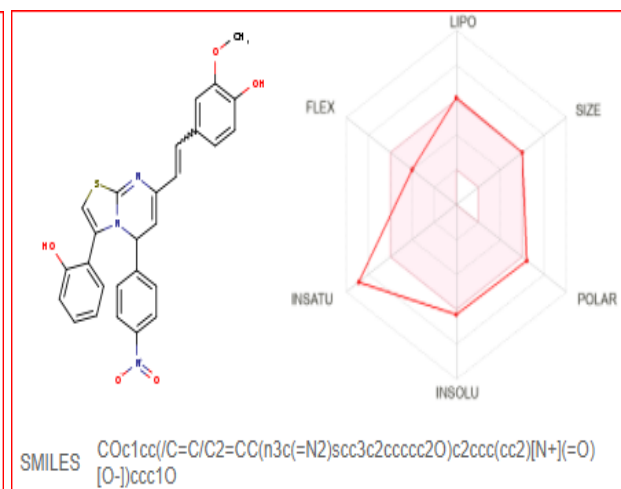
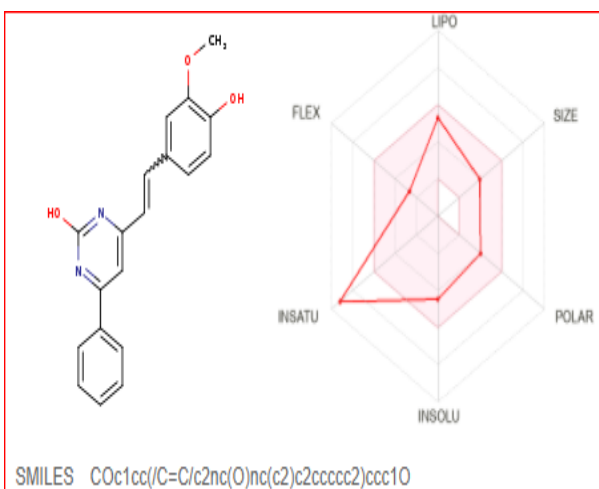
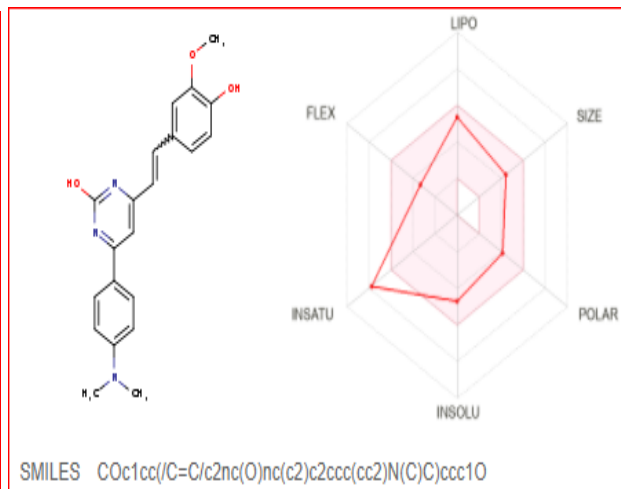
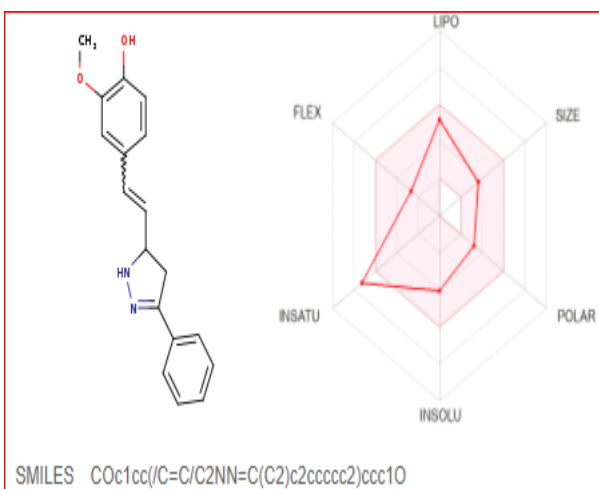
SMILES O=C(C(c1ccc(cc1)O)N)NC1C(=O)N2C1SC(C2C(=O)O)(C)C

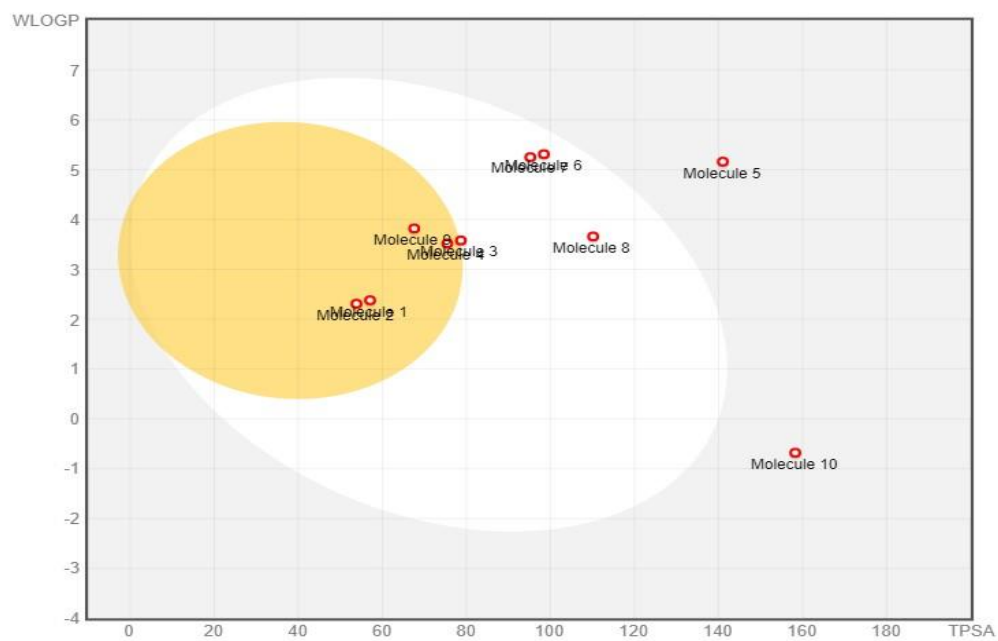
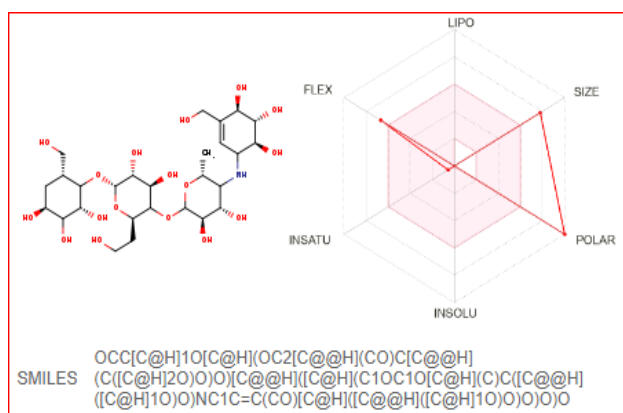
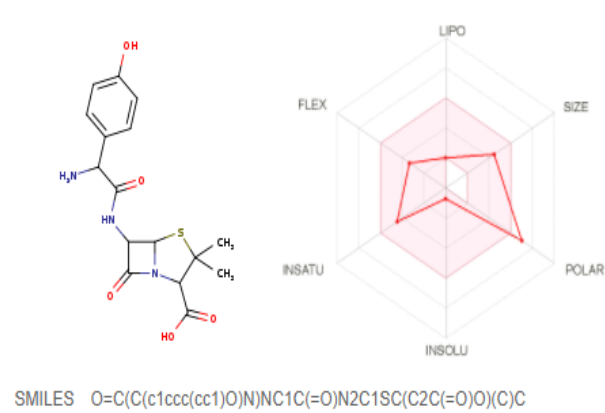
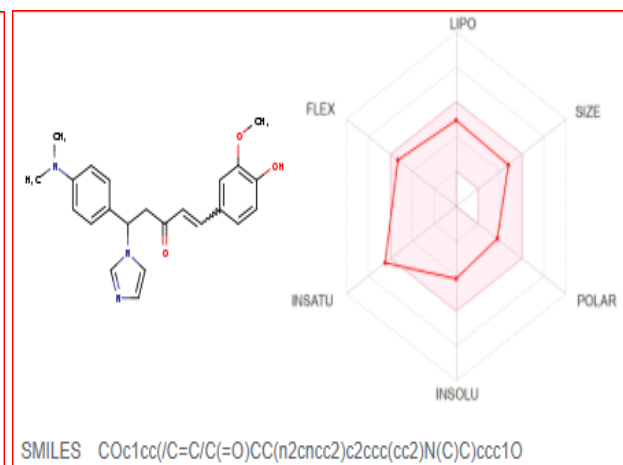
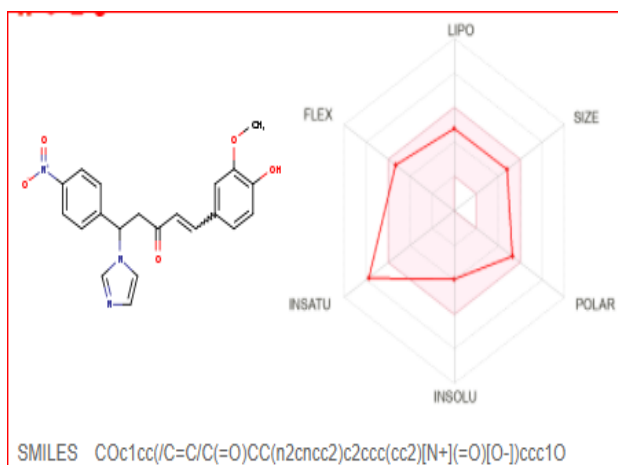


Where: (Lipophilicity ($-0.7 < \log P < 5.0$), size ($150 \text{ g/mol} < \text{MW} < 500 \text{ g/mol}$), polarity ($20^\circ \text{A}^2 < \text{TPSA} < 140^\circ \text{A}^2$), insolubility ($-6 < \log S < 0$), unsaturation ($0.25 < \text{Fraction Csp}^3 < 1$) and flexibility ($0 < \text{Num. rotatable bonds} < 9$)

Appendix 115. Bioavailability radar and Boiled- Egg model of the title, **108-116** by the drug-likeness criteria







Appendix 116. a-j. 3D (right) and 2D (left) representations of the binding interactions of **75-83** and amoxicillin against *E. coli* DNA Gyrase B (PDB ID: 6f861)

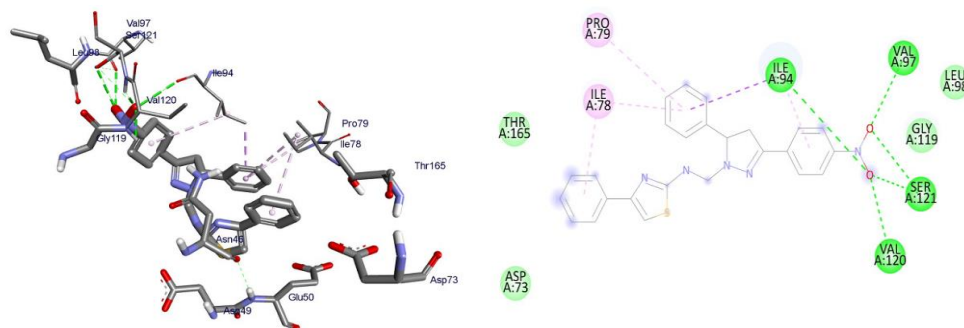


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **75**

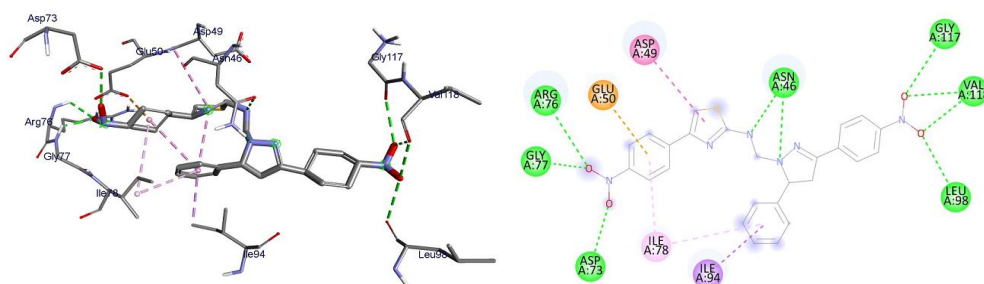


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **76**

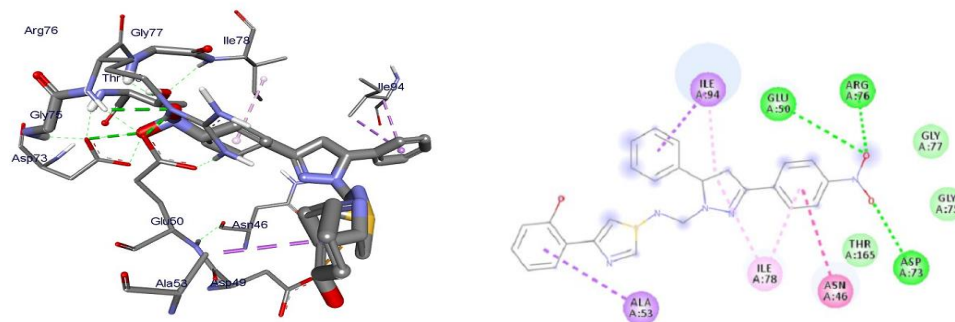


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **77**

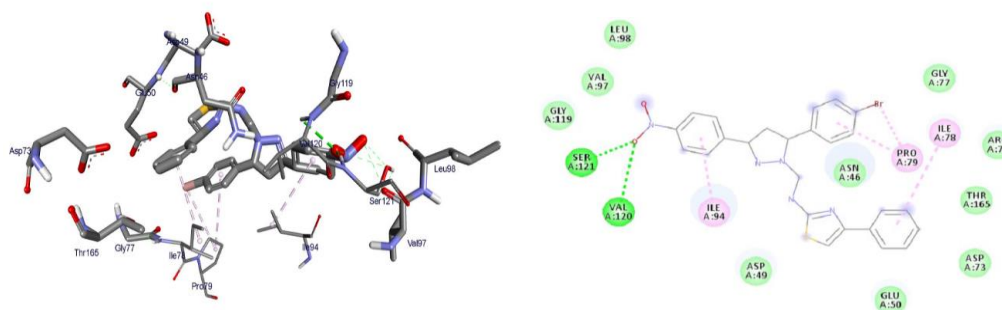


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **78**

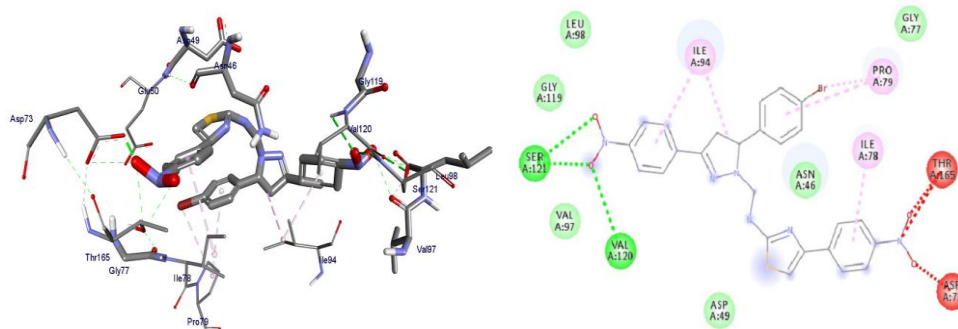


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **79**

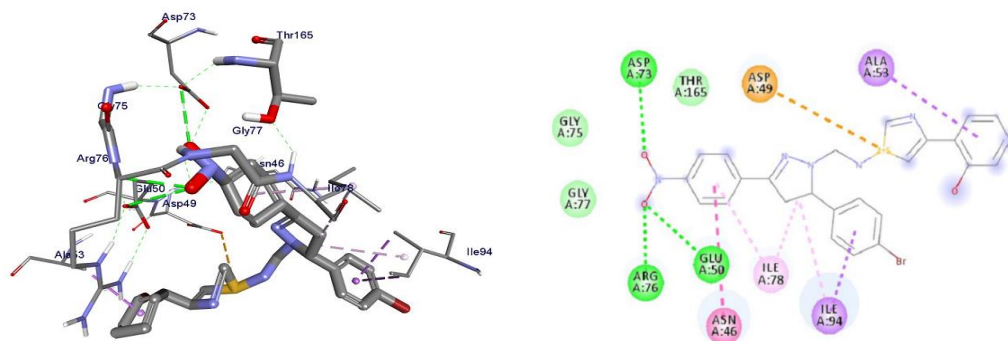


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **80**

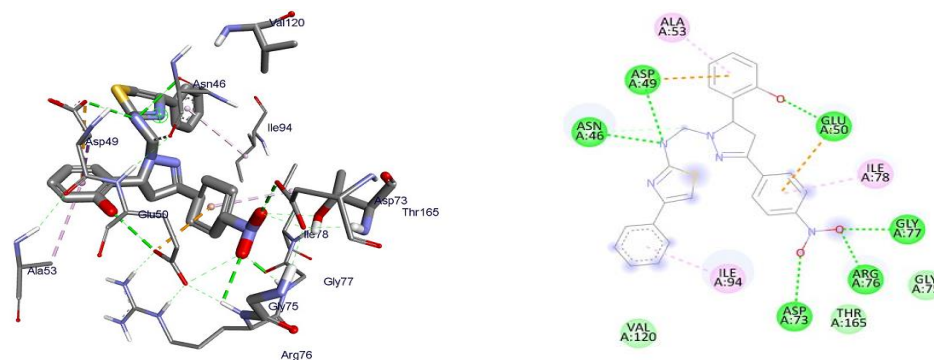


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **81**

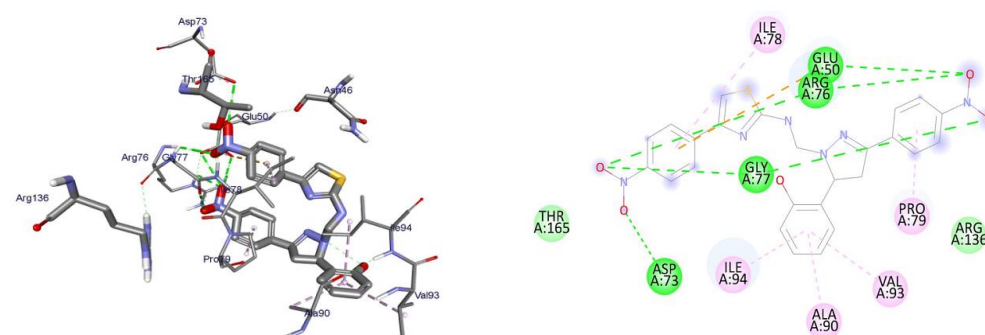


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **82**

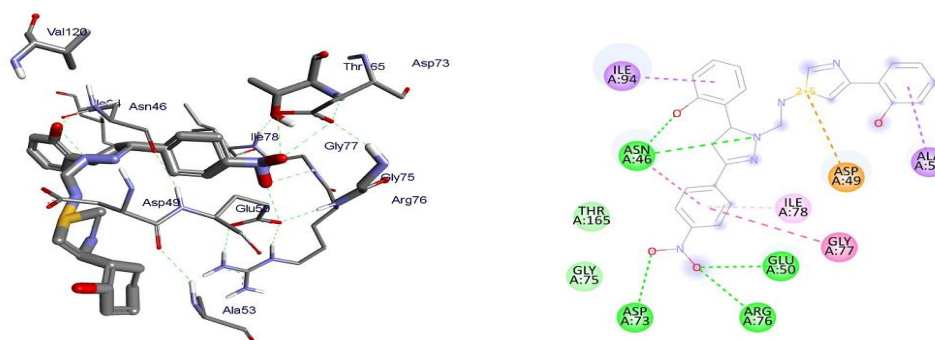


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **83**

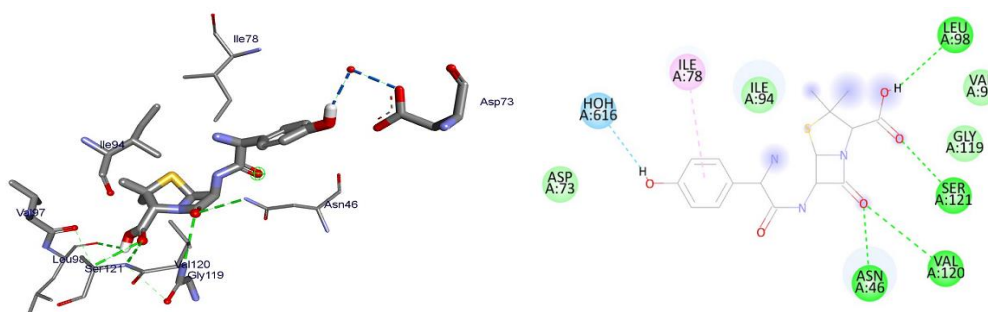


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**

Appendix 117. a-j. 3D (right) and 2D (left) representations of the binding interactions of **75-83** and amoxicillin against N-terminal domain of (PDB ID: 5oe3)

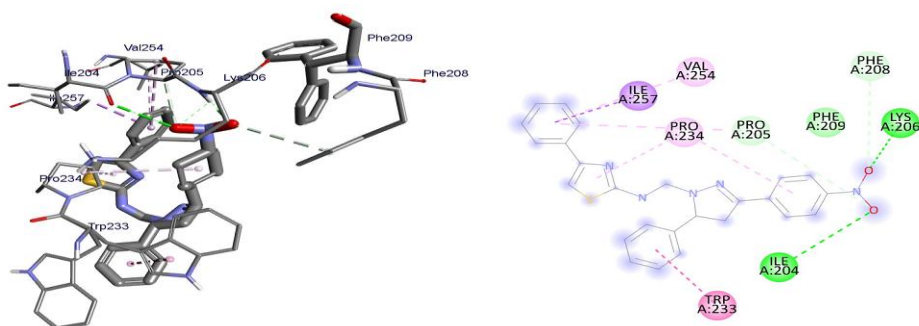


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **75**

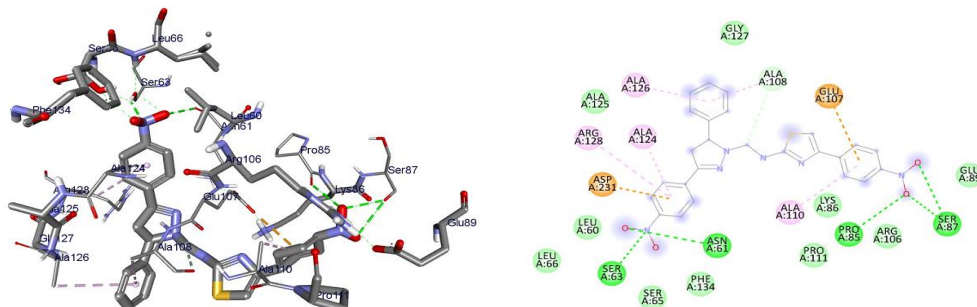


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **76**

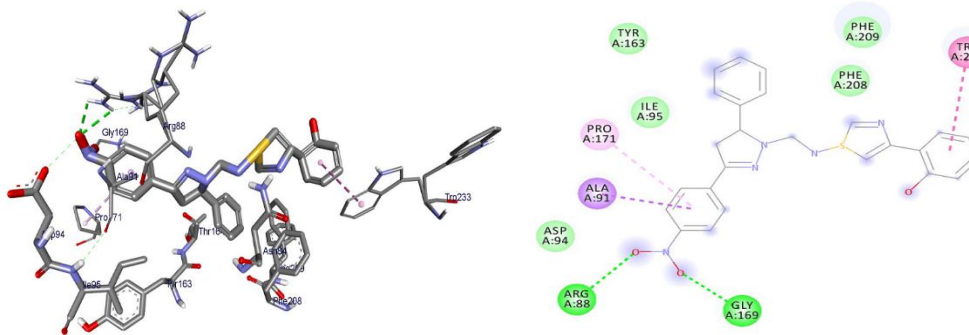


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **77**

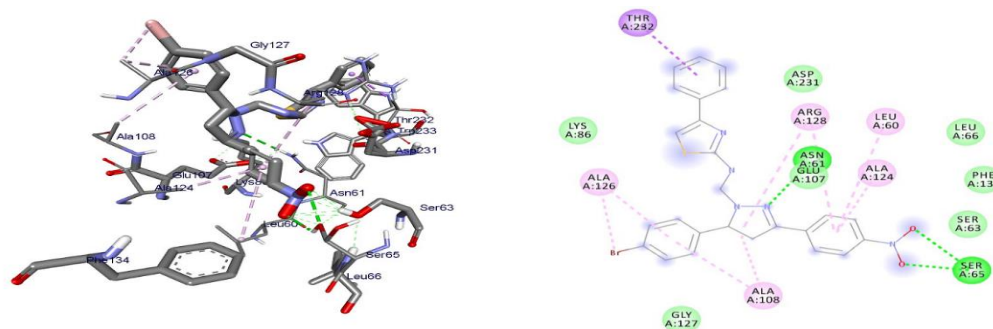


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **78**

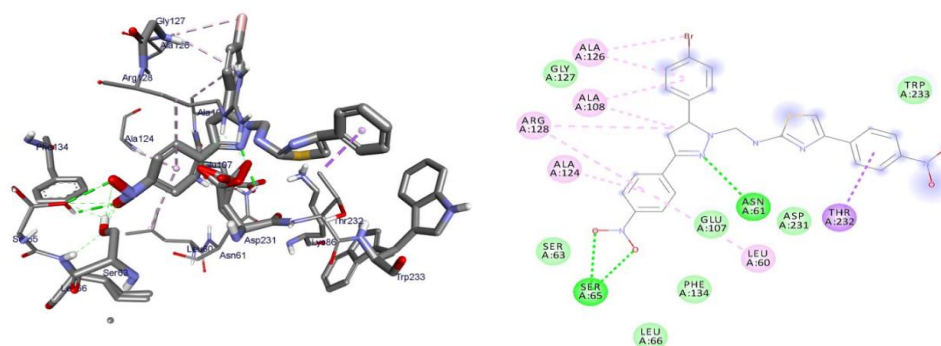


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **79**

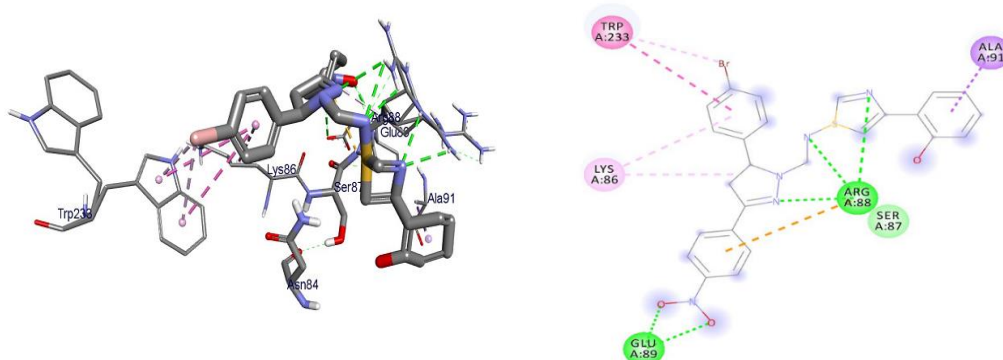


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **80**

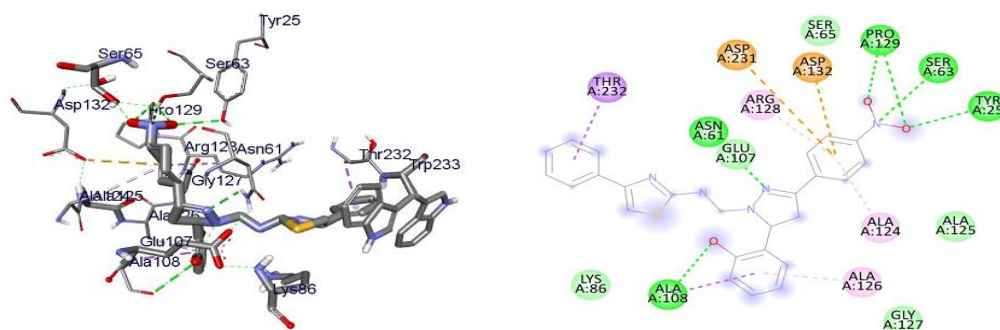


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **81**

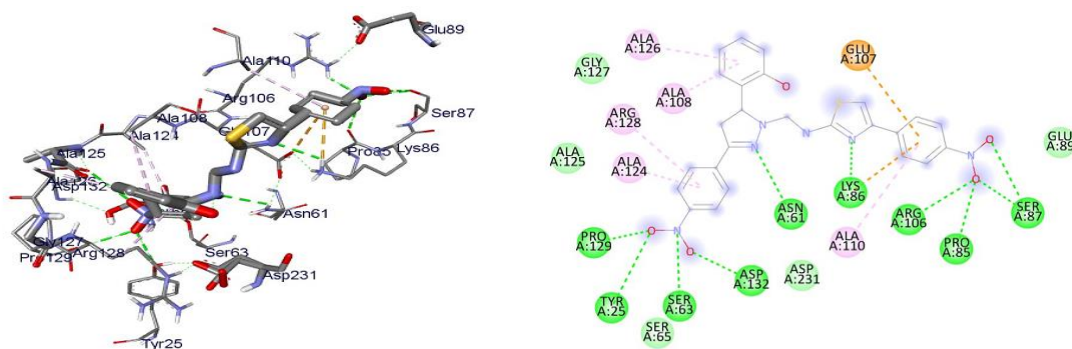


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **82**

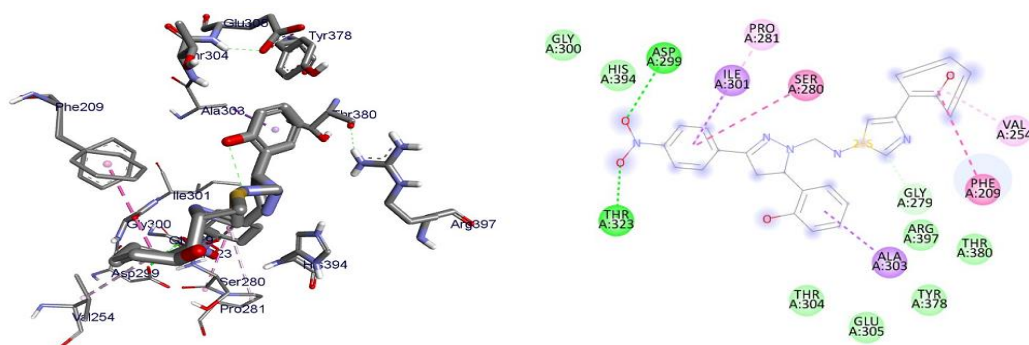


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **83**

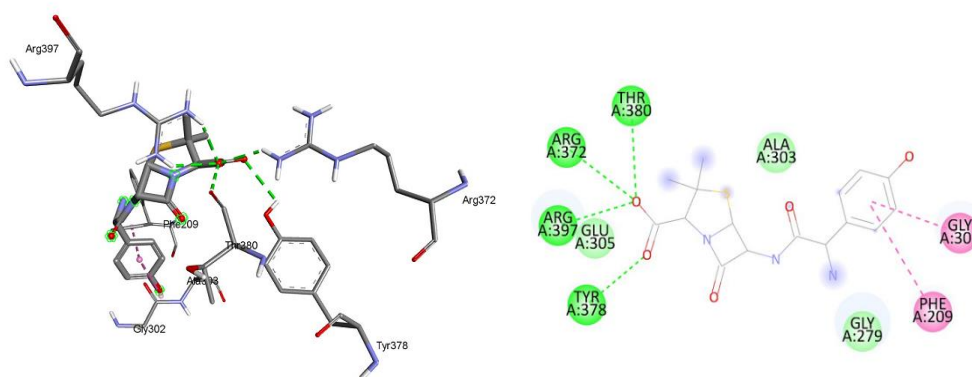


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**

Appendix 118. a-j. 3D (right) and 2D (left) representations of the binding interactions of **75-83** and amoxicillin against *S. aureus* Pyruvate Kinase (PDB ID: 3t07)

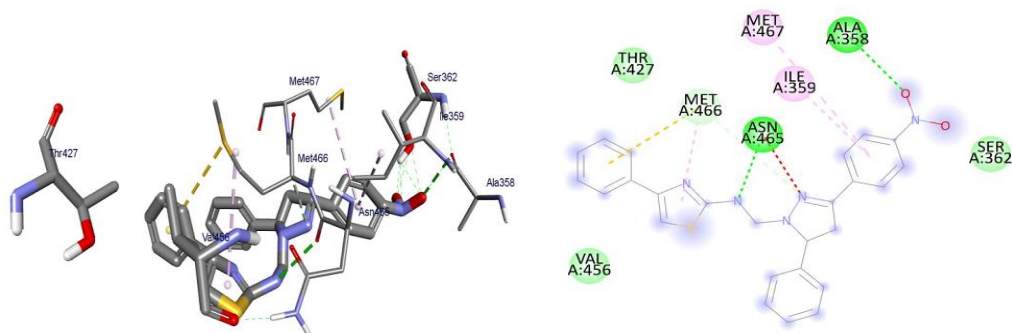


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **75**

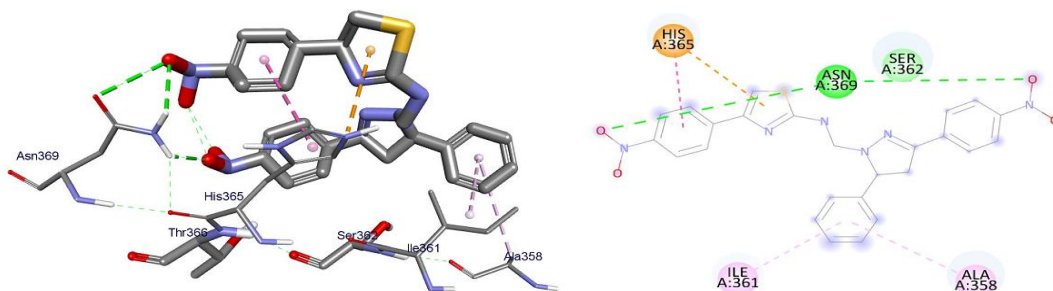


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **76**

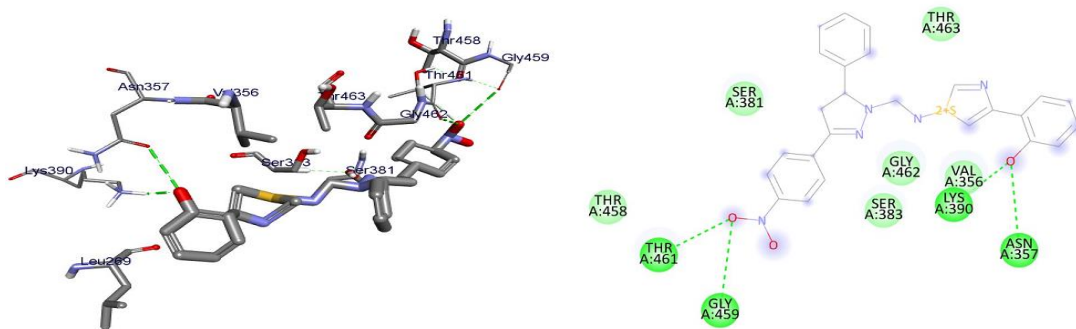


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **77**

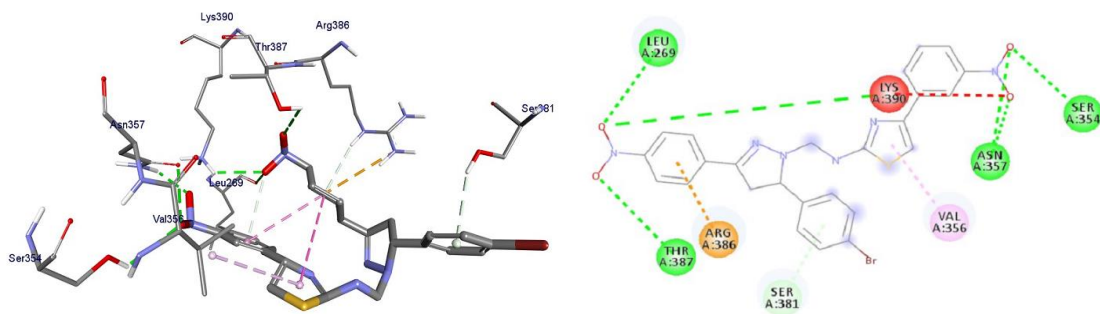


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **78**

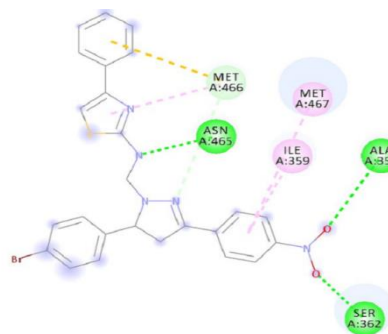
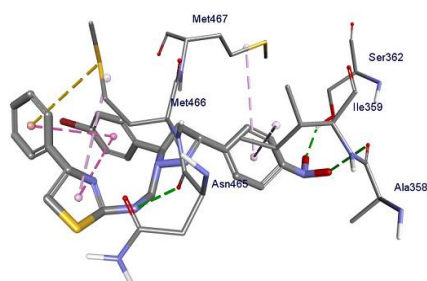


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **79**

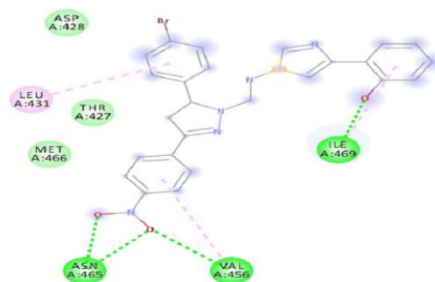
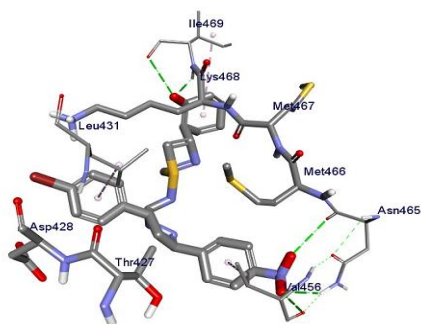


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **80**

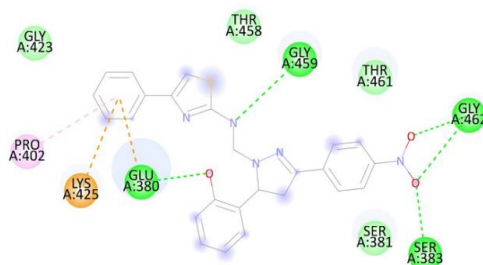
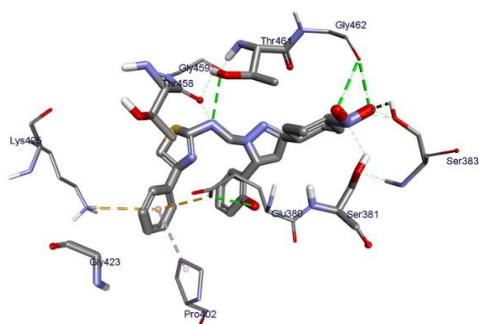


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **81**

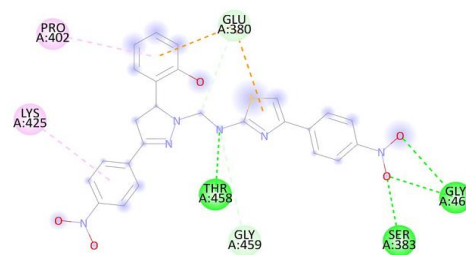
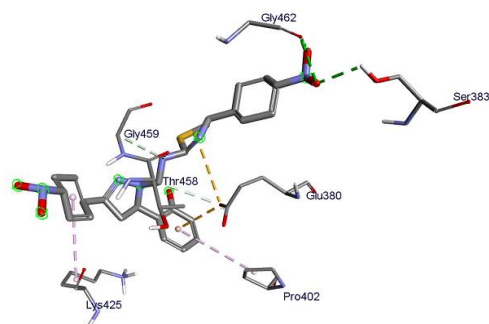


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **82**

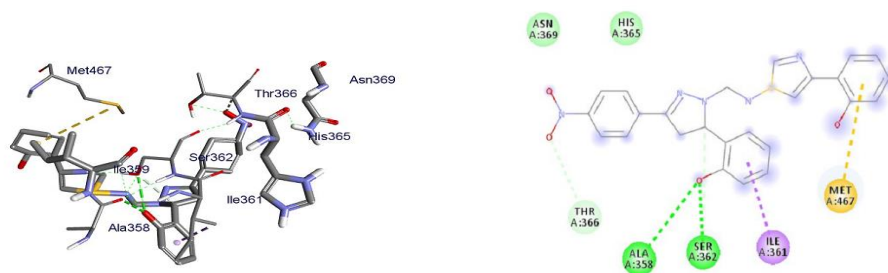


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **83**

Appendix 119. a-j. 3D (right) and 2D (left) representations of the binding interactions of **75-83** and amoxicillin against LuxS of *S. pyogenes* (PDB ID: P0C0C7)

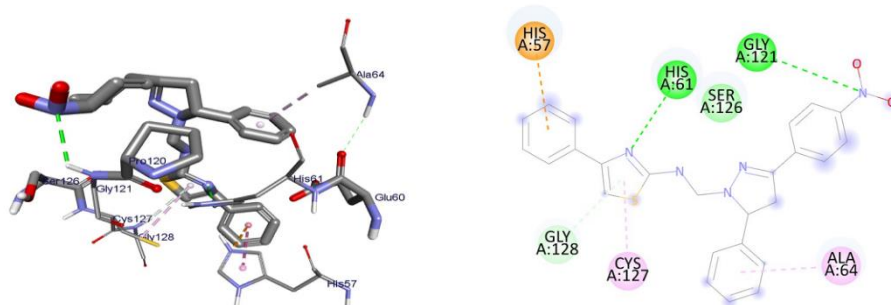


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **75**

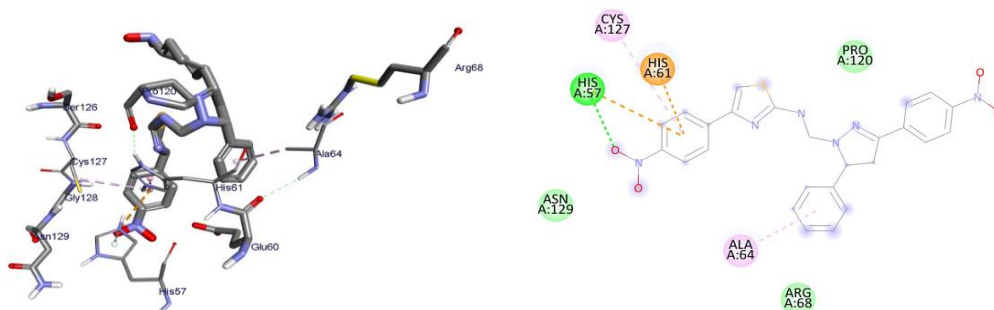


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **76**

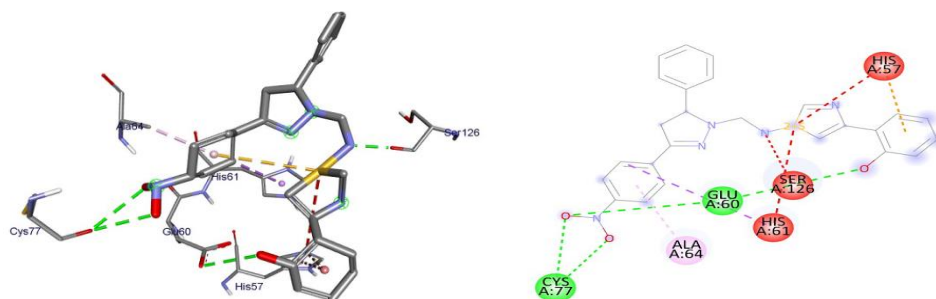


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **77**

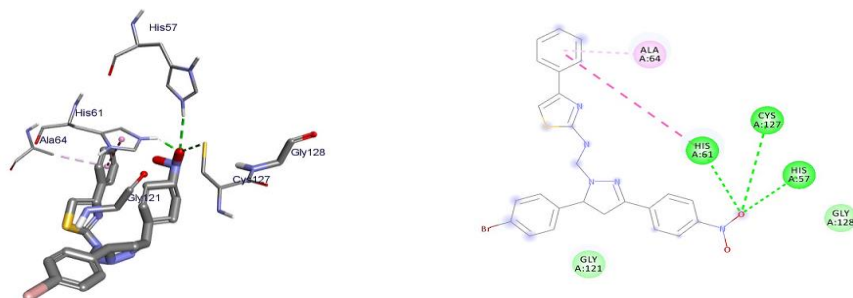


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **78**

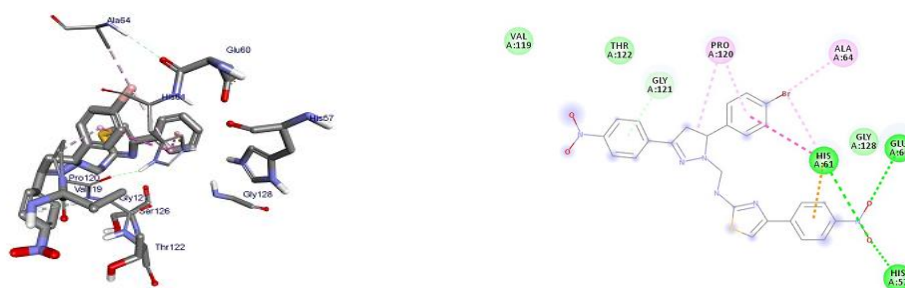


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **79**

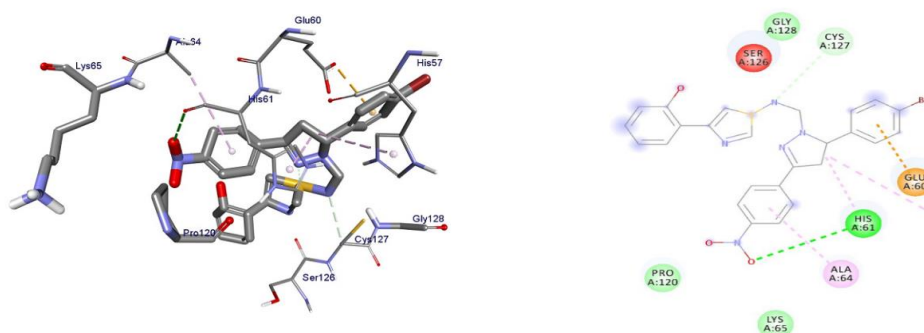


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **80**

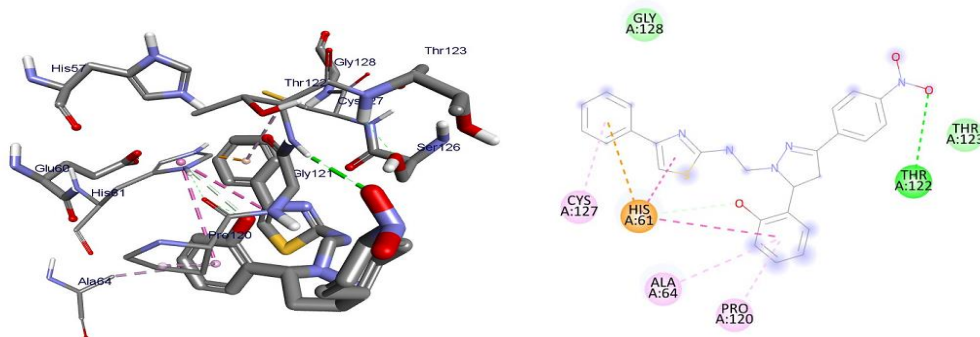


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **81**

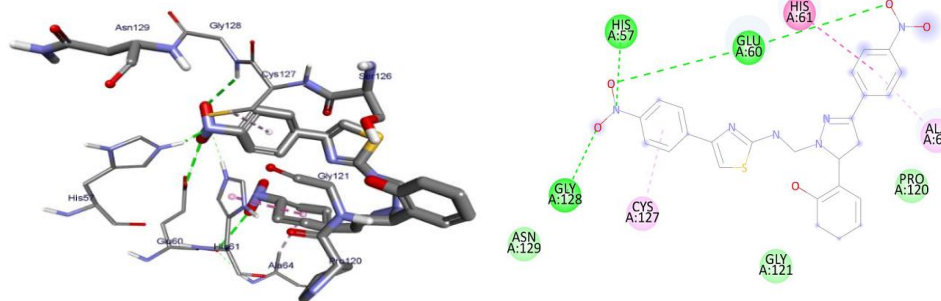


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **82**

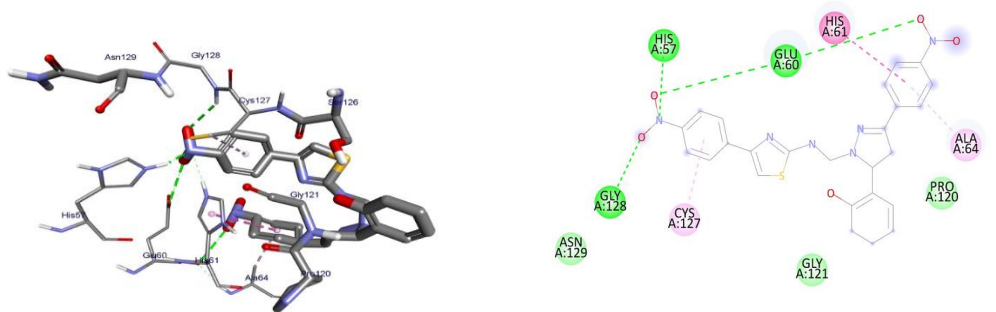


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **83**

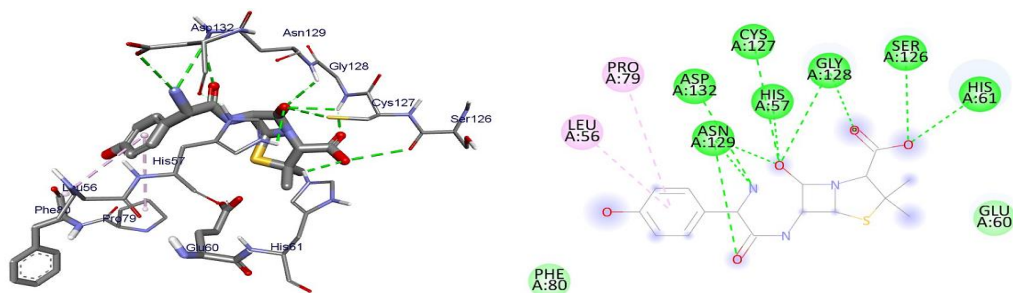


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**

Appendix 120.a-d. 3D (right) and 2D (left) representations of the binding interactions of synthesized compounds, **75-83** against Penicillin binding proteins (PBPs) (PDB ID: 1VQQ).

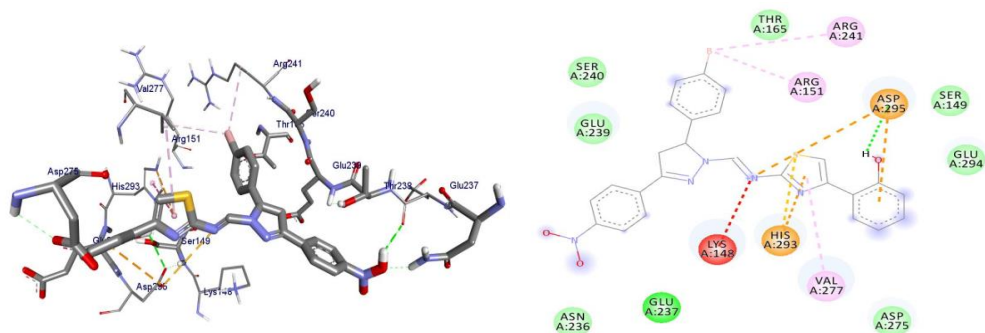


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **66**

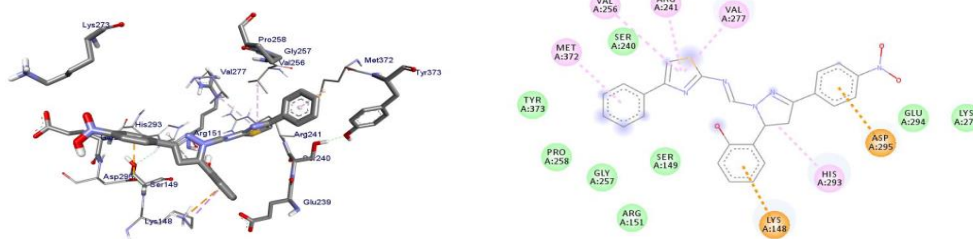


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **67**

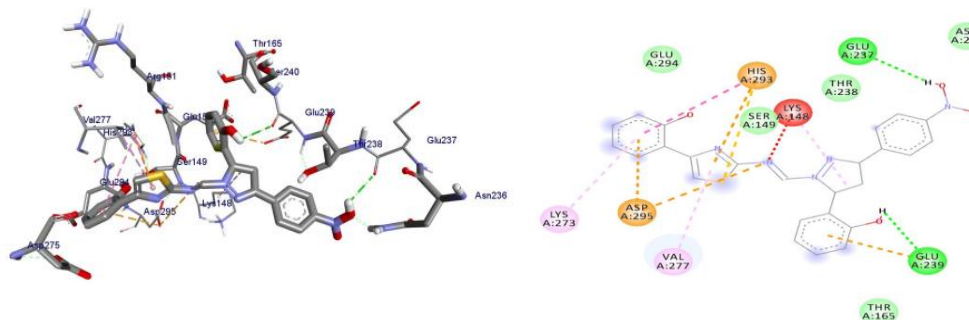


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **69**

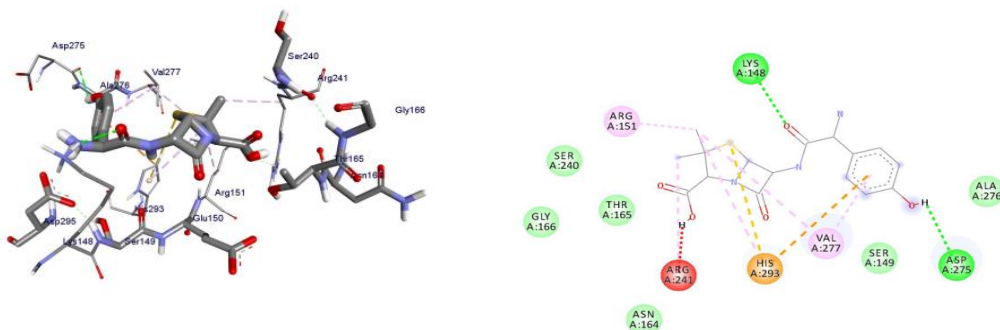


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**

Appendix 121. a-d. 3D (right) and 2D (left) representations of the binding interactions of synthesized compounds, **75-83** against against β -lactamases (PDB ID: 1IYS).

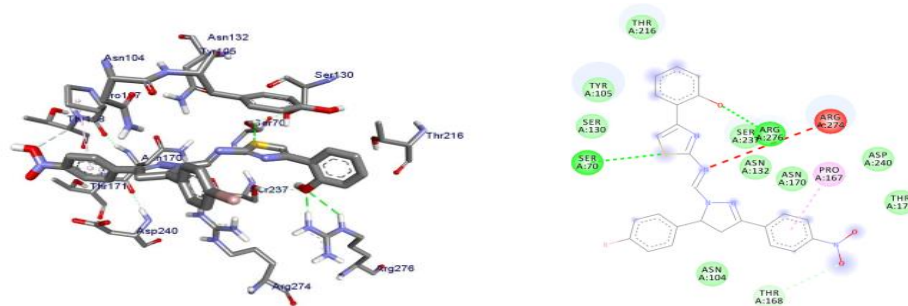


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **66**

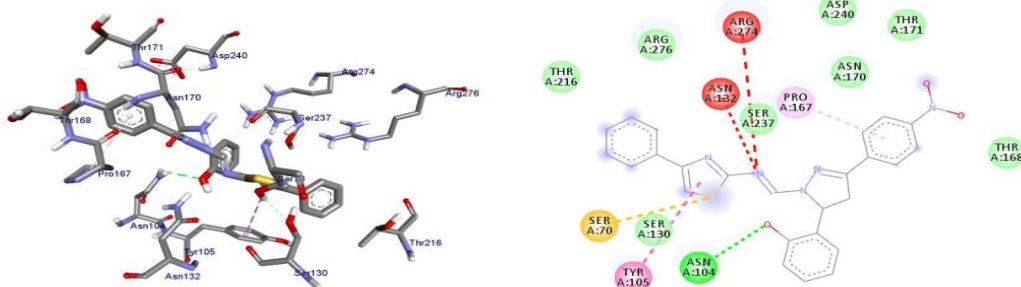


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **67**

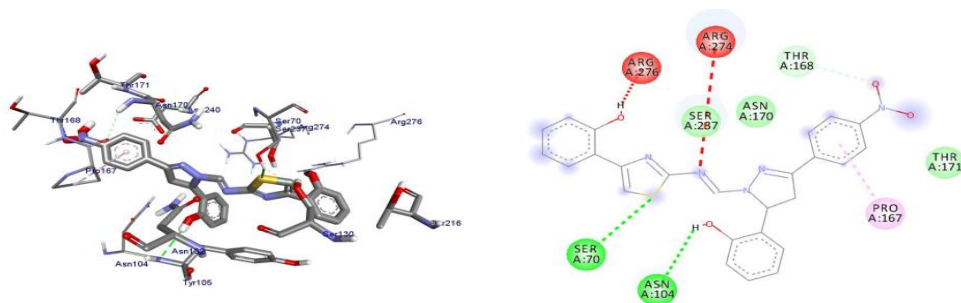


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **69**

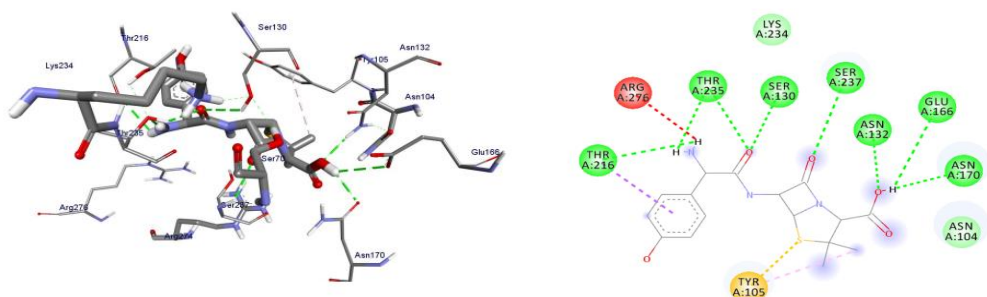


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**

Appendix 122. a-j. 3D (right) and 2D (left) representations of the binding interactions of **75-83** and amoxicillin against Human peroxiredoxin 5 (PDB ID: 1hd2).

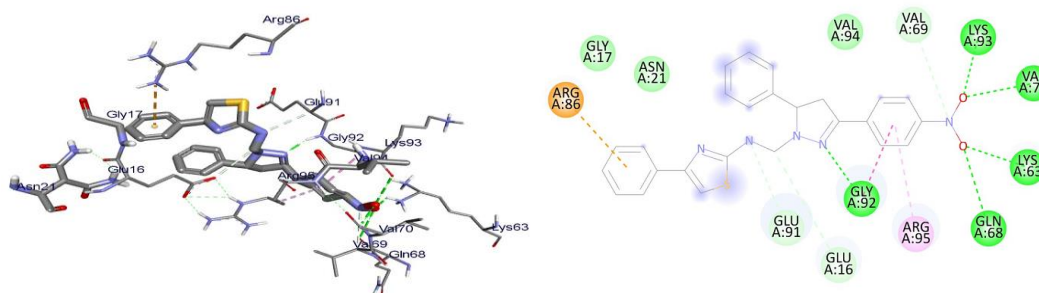


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **75**

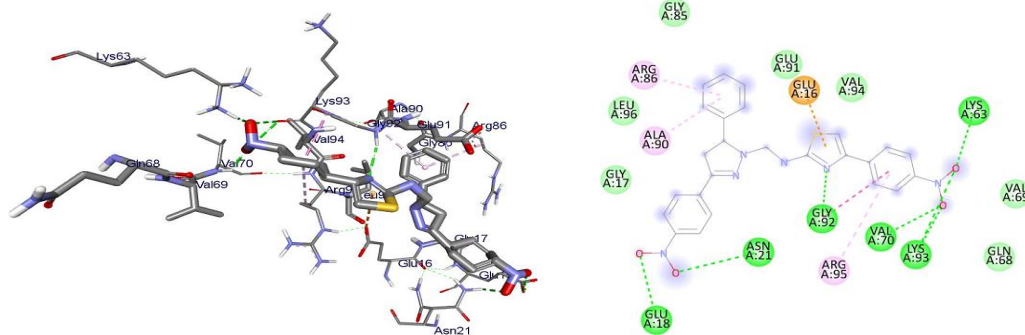


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **76**

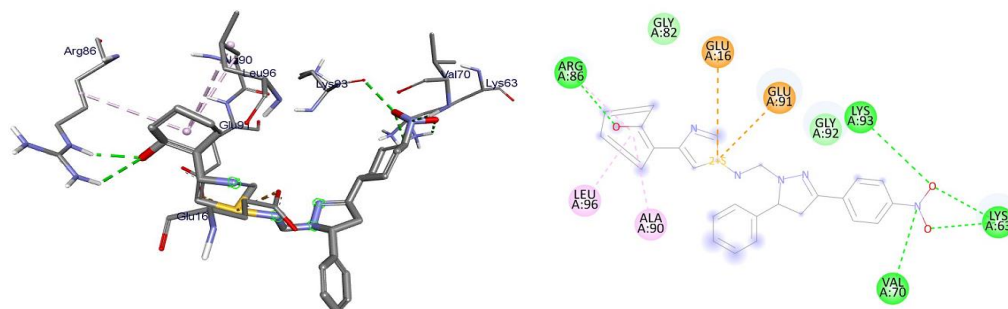


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **77**

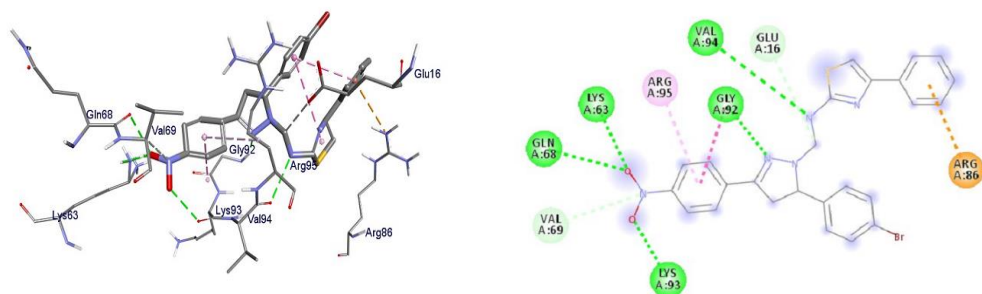


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **78**

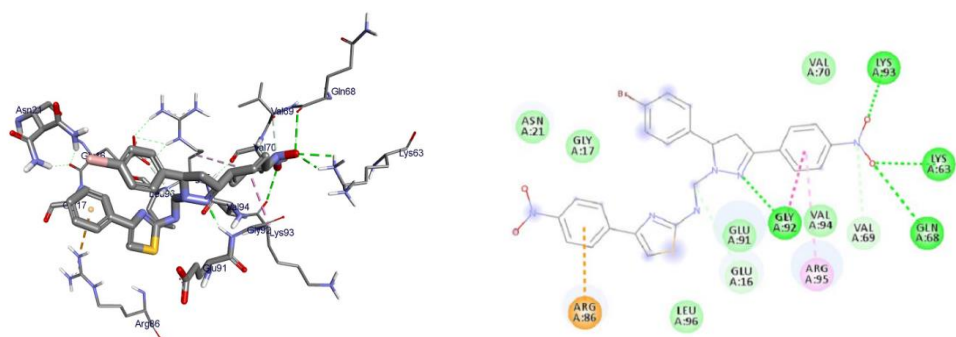


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **79**

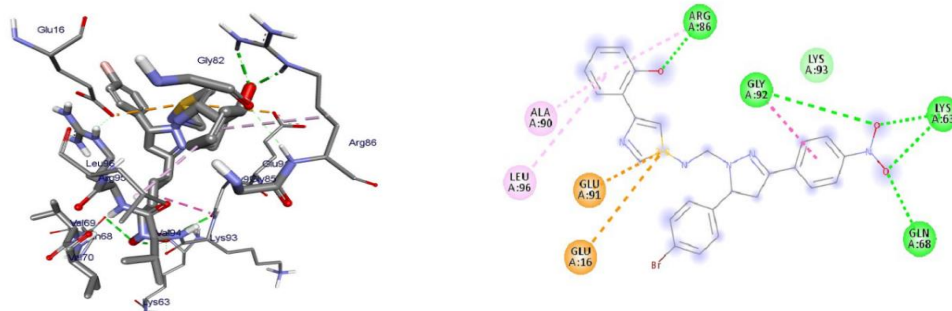


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **80**

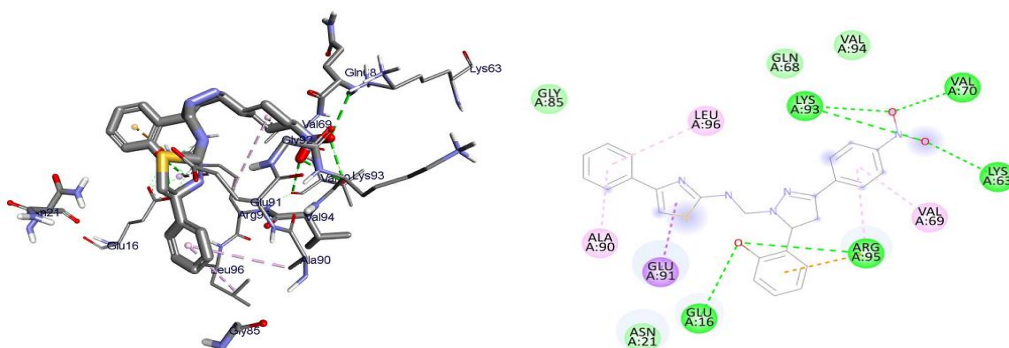


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **81**

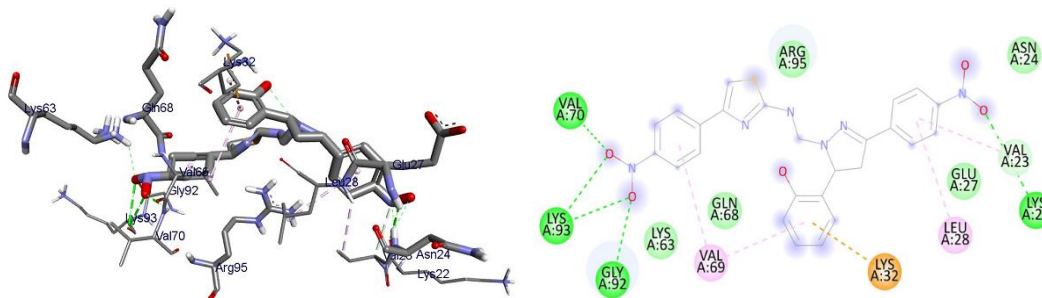


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **82**

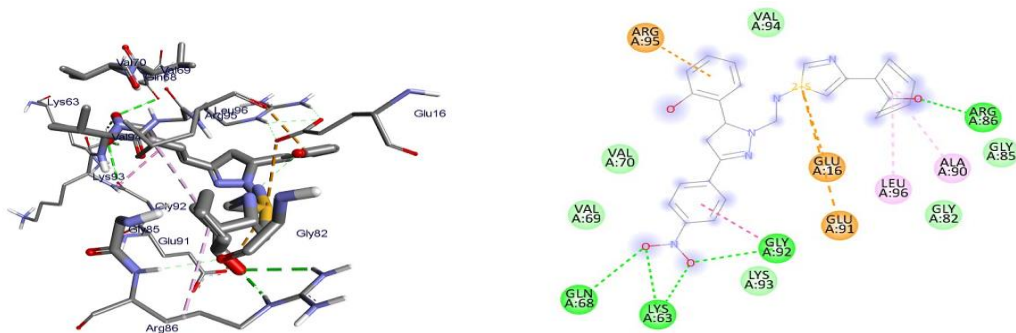


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **83**

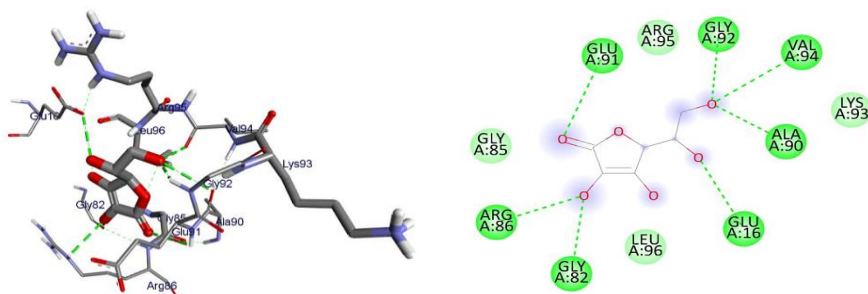


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **vitamin C**
Appendix 123. a-j. 3D (right) and 2D (left) representations of the binding interactions of **92-100** and amoxicillin against *E. coli* DNA Gyrase B (PDB ID: 6f861)

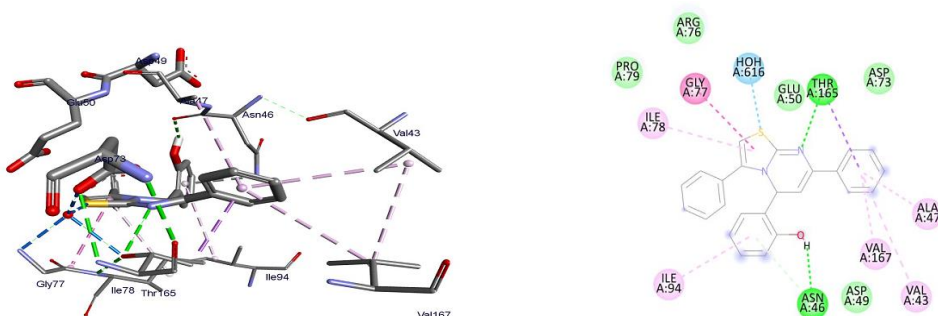


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **92**

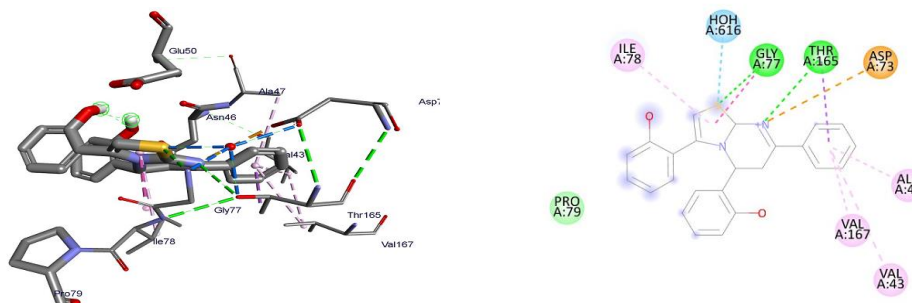


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **93**

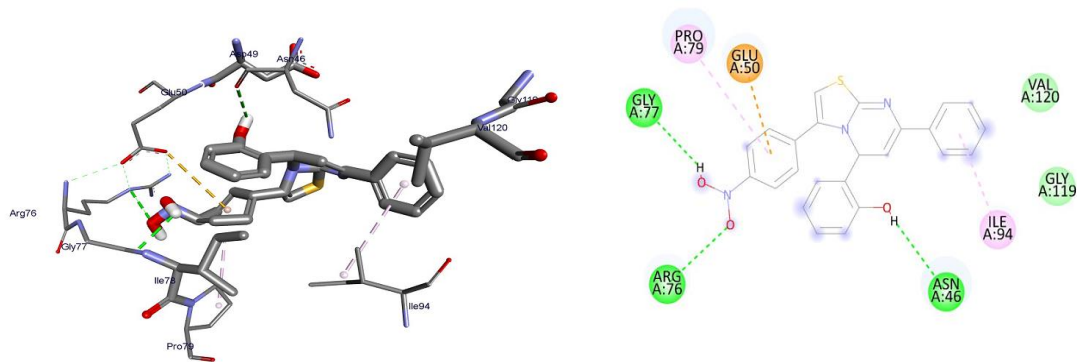


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **94**

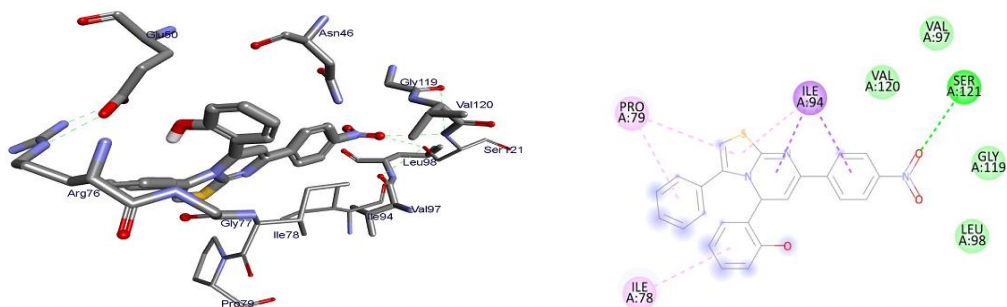


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **95**

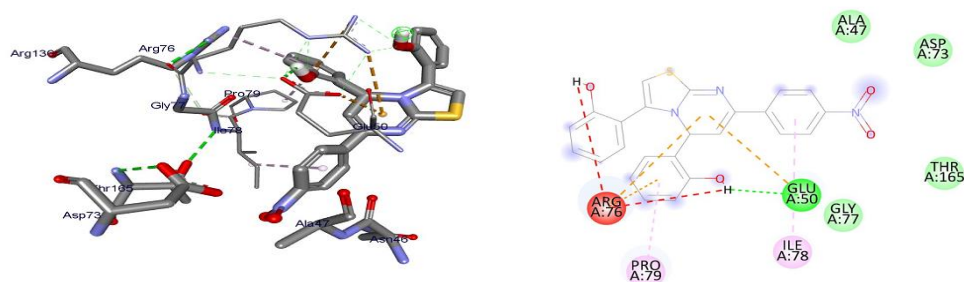


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **96**

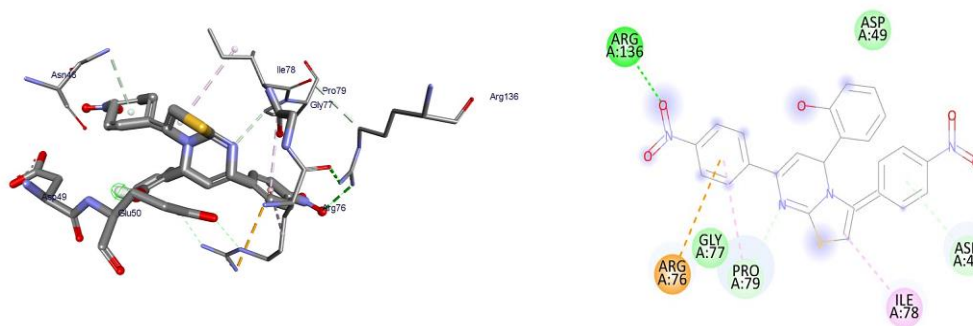


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **97**

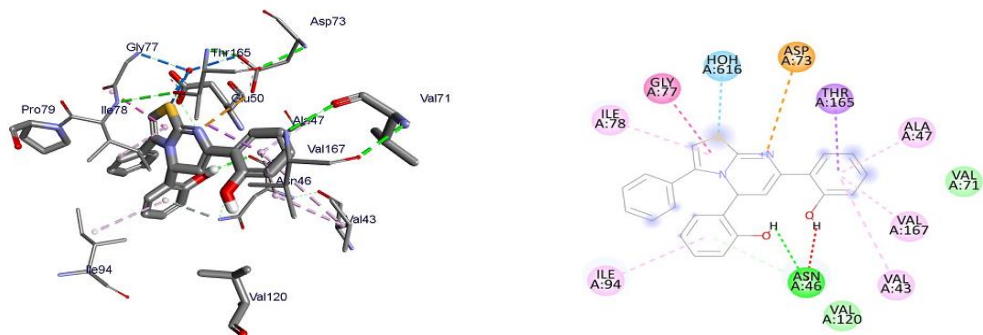


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **98**

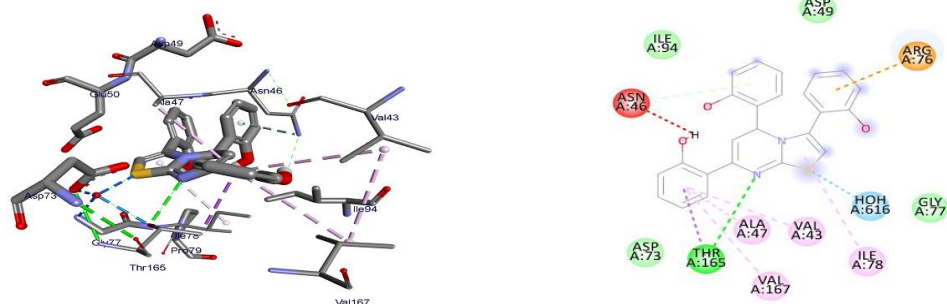


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **99**

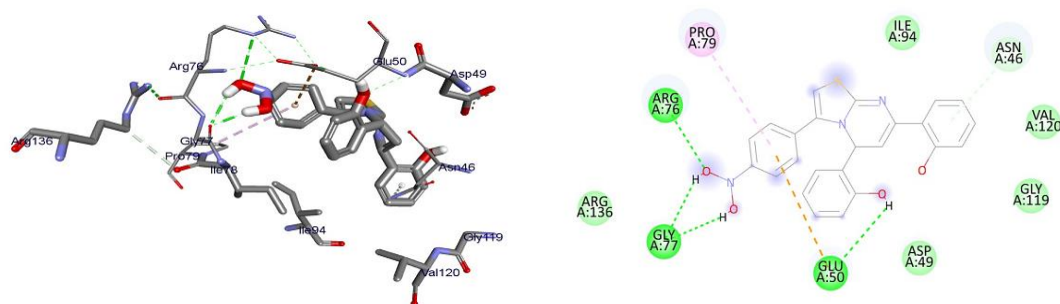


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **100**

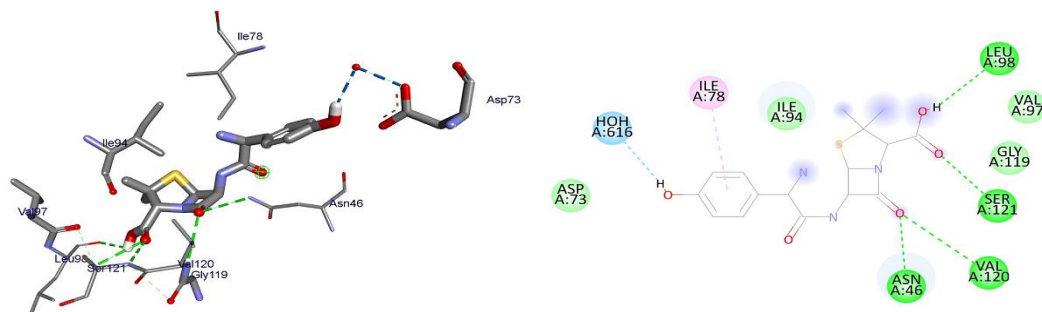


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**

Appendix 124. a-j. 3D (right) and 2D (left) representations of the binding interactions of **92-100** and amoxicillin against N-terminal domain of PqsA (PDB ID:5oe3)

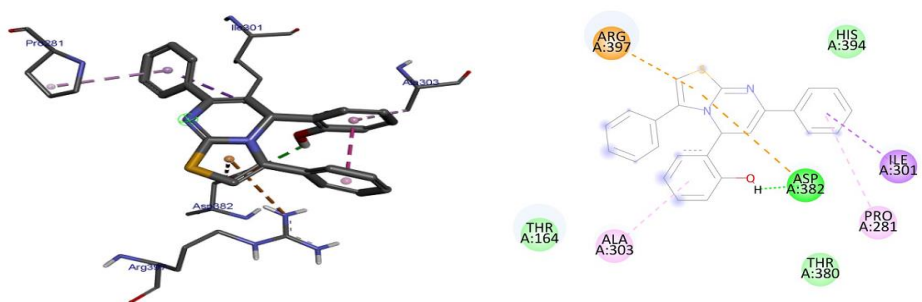


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **92**

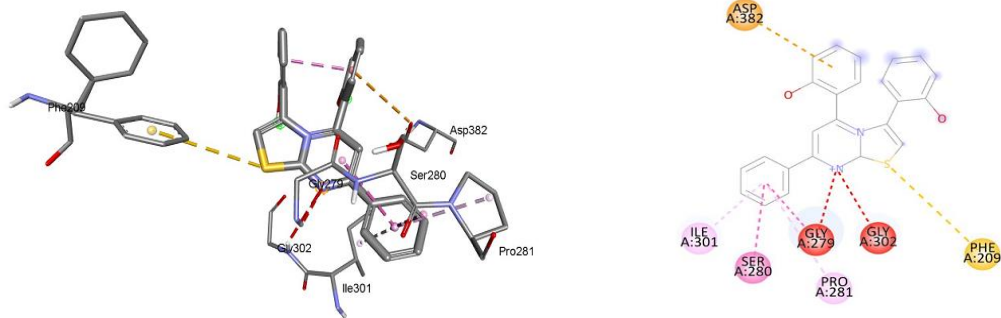


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **93**

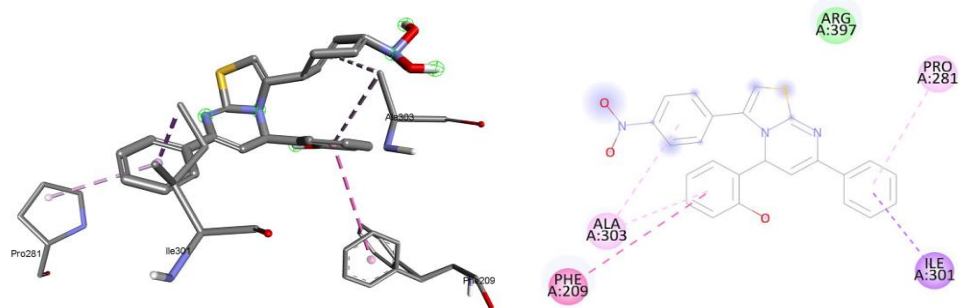


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **94**

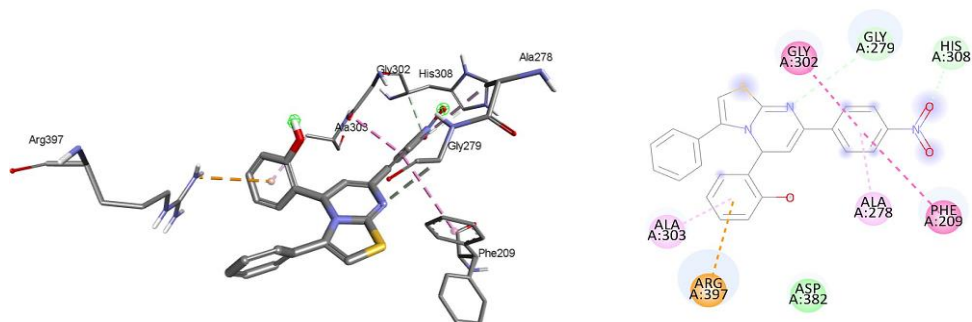


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **95**

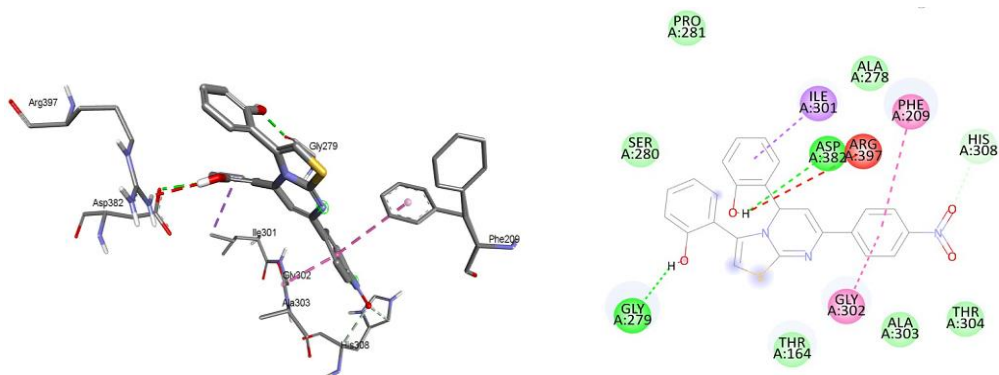


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **96**

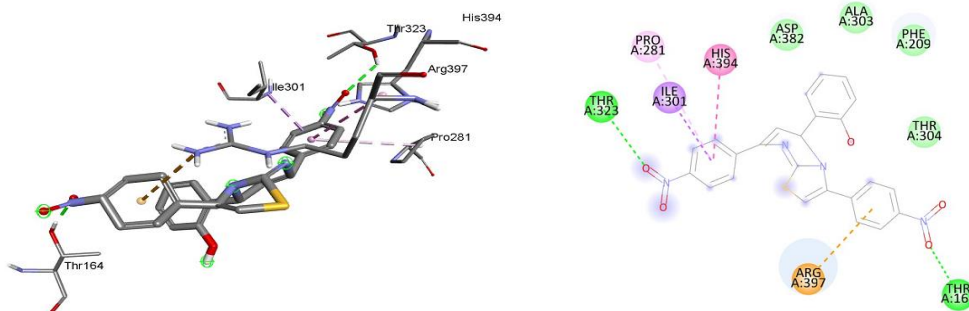


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **97**

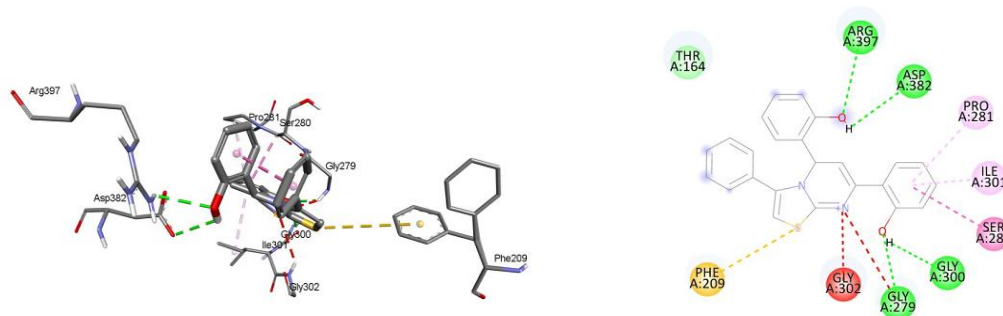


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **98**

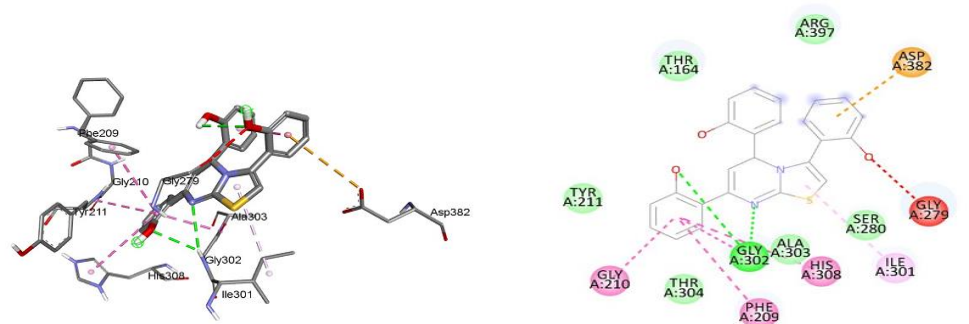


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **99**

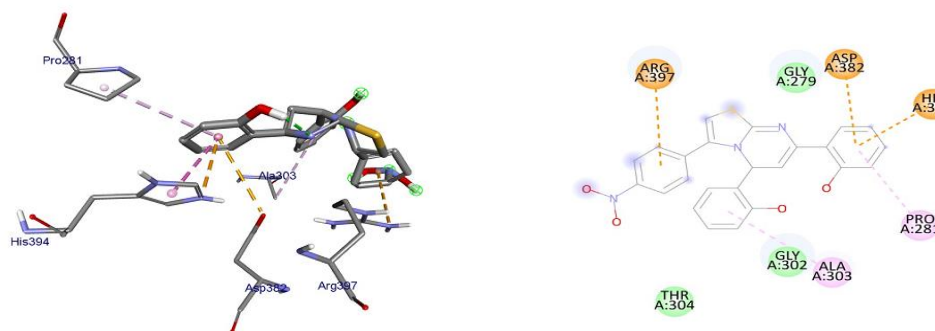


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **100**

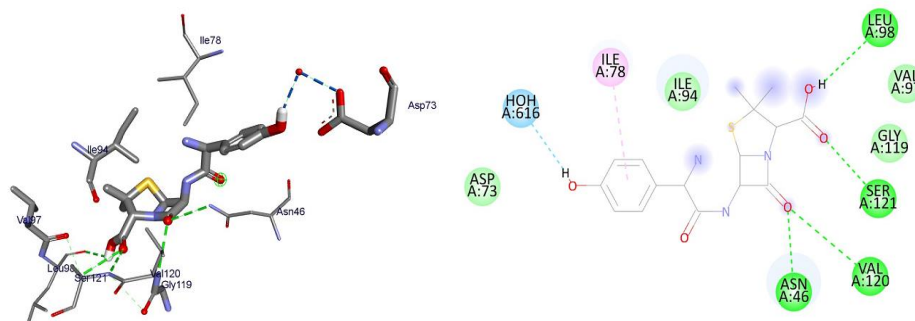


Figure j: 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**
Appendix 125. a-j. 3D (right) and 2D (left) representations of the binding interactions of **92-100** and amoxicillin against *S. aureus* Pyruvate Kinase (PDB ID:3t07)

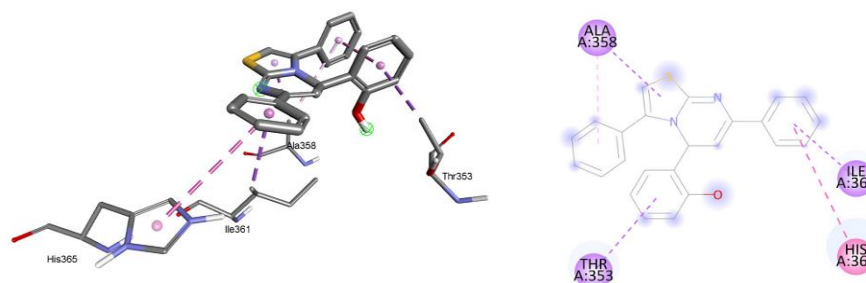


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **92**

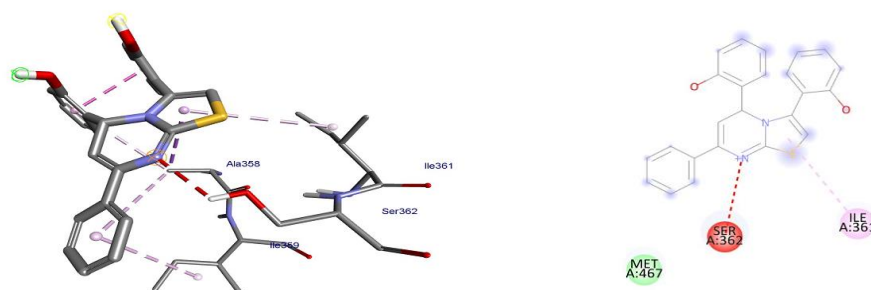


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **93**

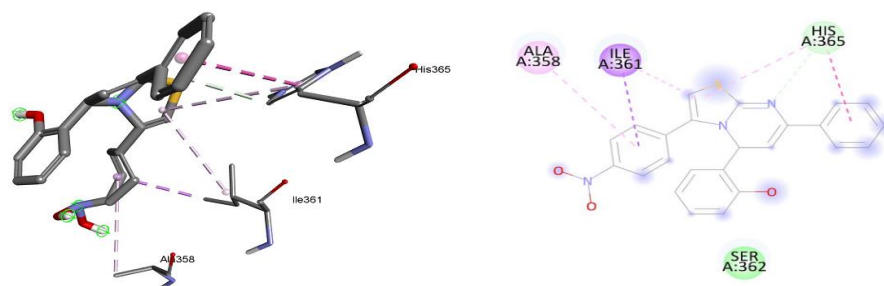


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **94**

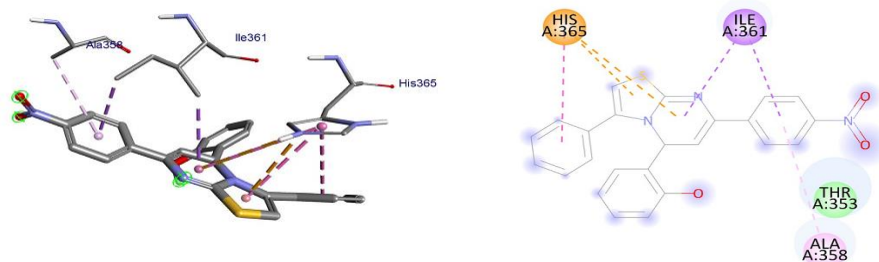


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **95**

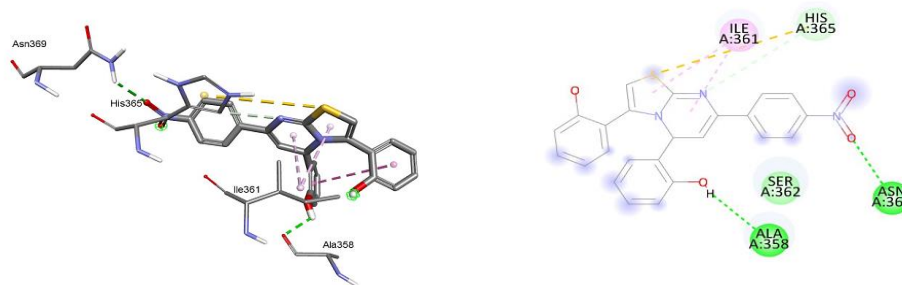


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **96**

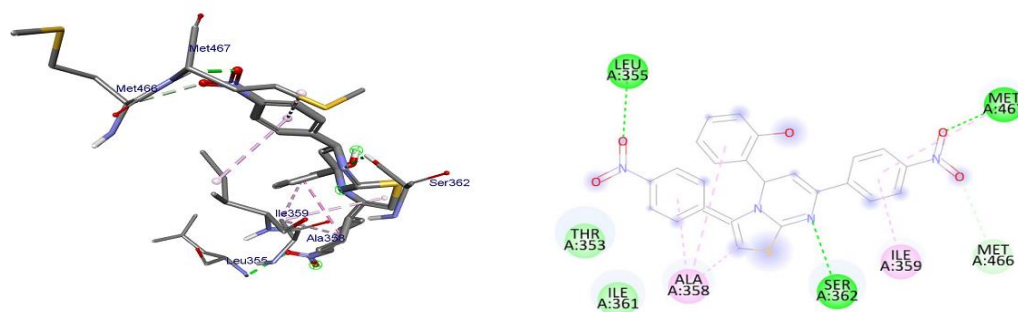


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **97**

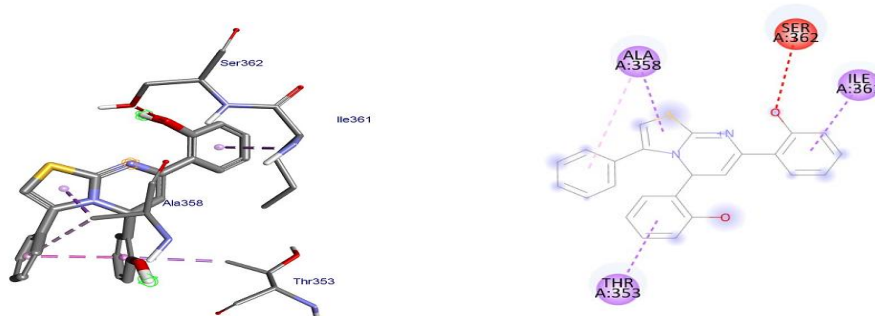


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **98**

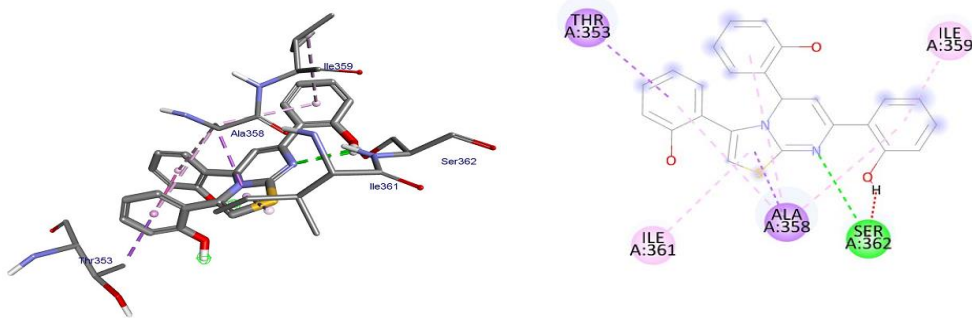


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **99**

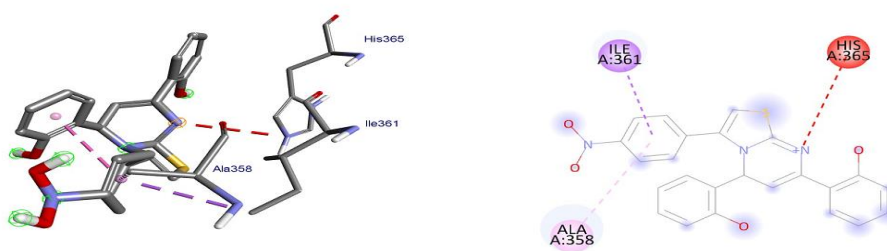


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **100**

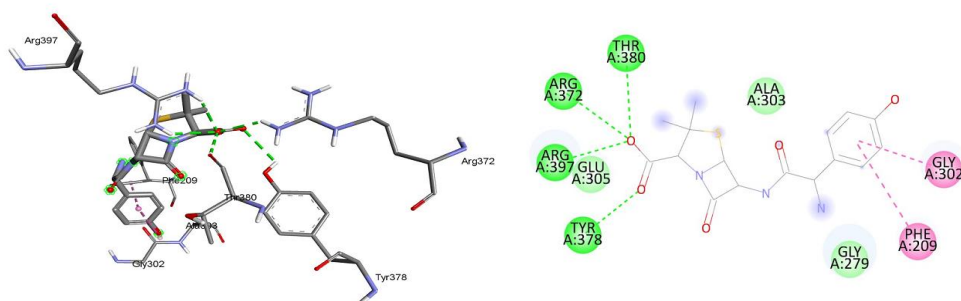


Figure j: 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**

Appendix 126. a-j. 3D (right) and 2D (left) representations of the binding interactions of **92-100** and amoxicillin against LuxS of *S. pyogenes* (PDB ID: P0C0C7)

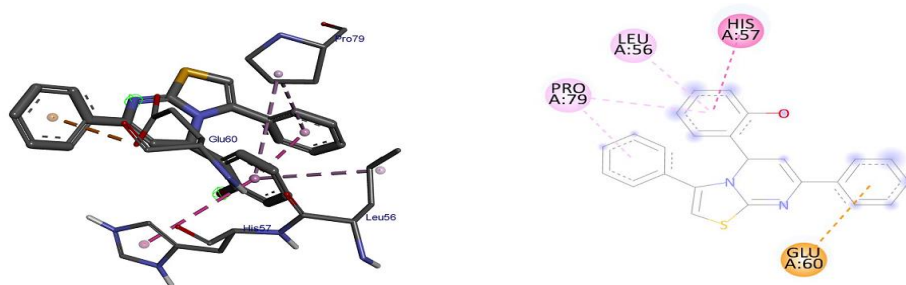


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **92**

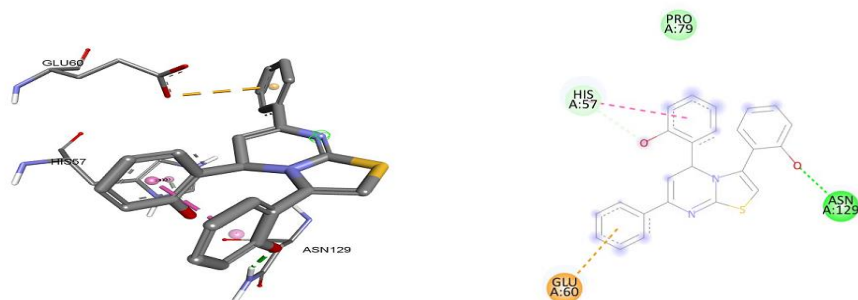


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **93**

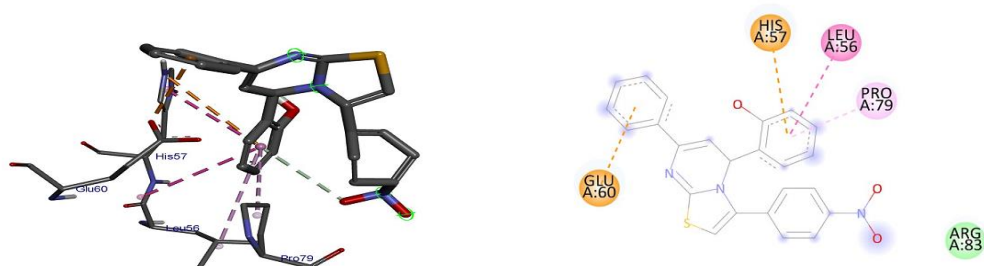


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **94**

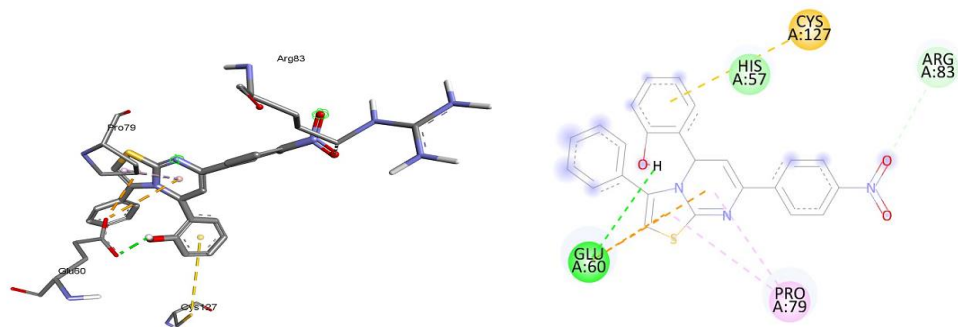


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **95**



Figure e. 3D (right) and 2D (left) representations of the binding interactions of **96**

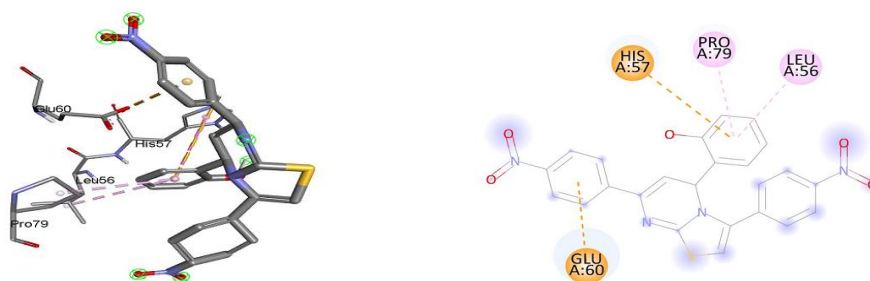


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **97**

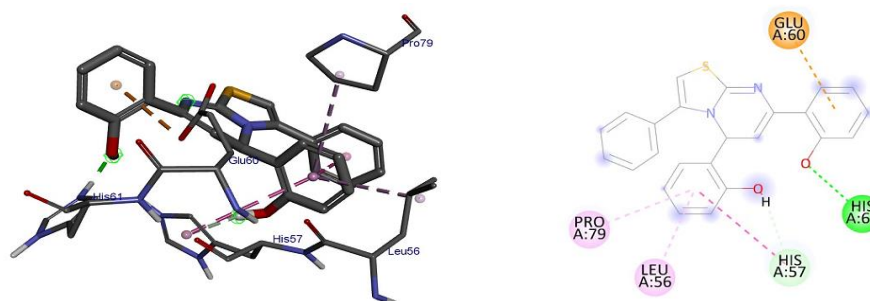


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **98**

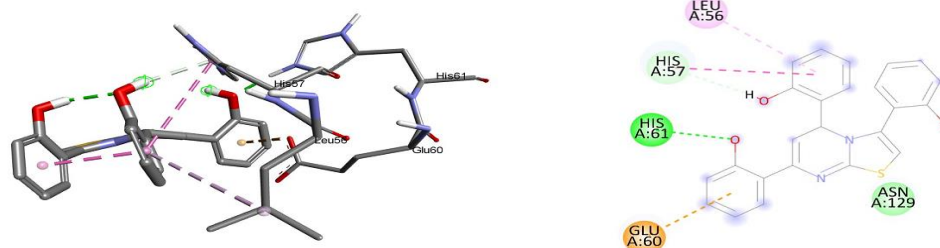


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **99**

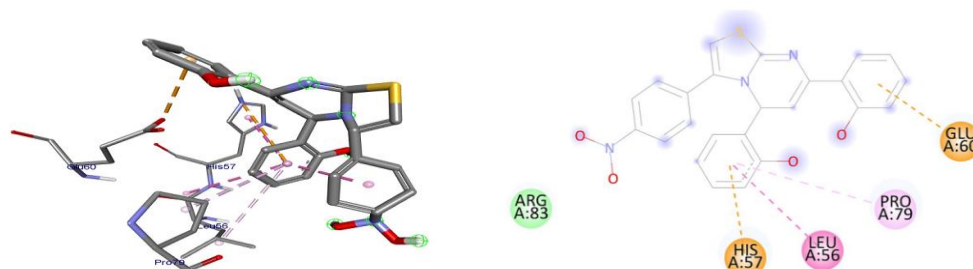


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **100**

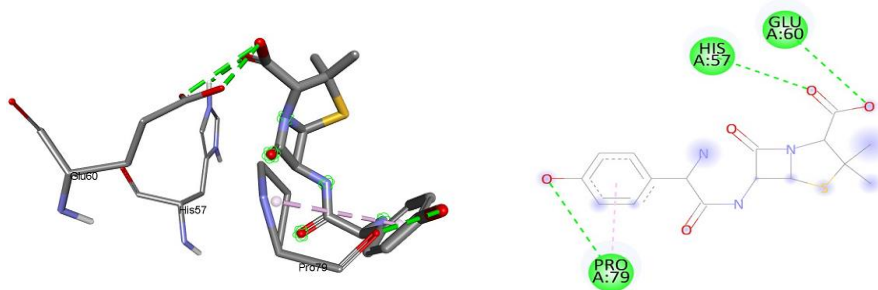


Figure j: 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**

Appendix 127. a-c. 3D (right) and 2D (left) representations of the binding interactions of synthesized compounds, **92-100** against Penicillin binding proteins (PBPs) (PDB ID: 1VQQ).

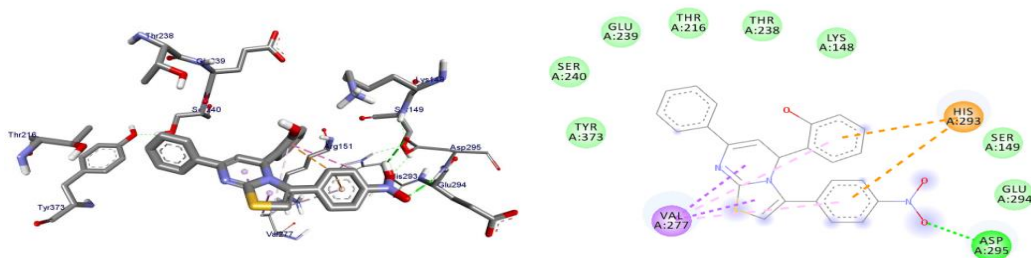


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **94**

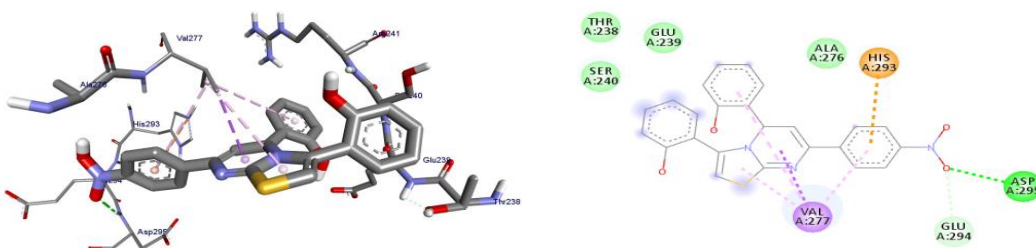


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **98**

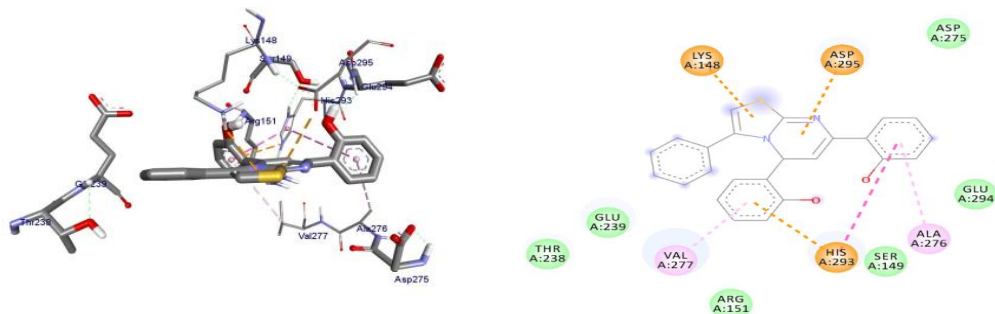


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **99**

Appendix 128. a-c. 3D (right) and 2D (left) representations of the binding interactions of synthesized compounds, **92-100** against against β -lactamases (PDB ID: 1IYS).

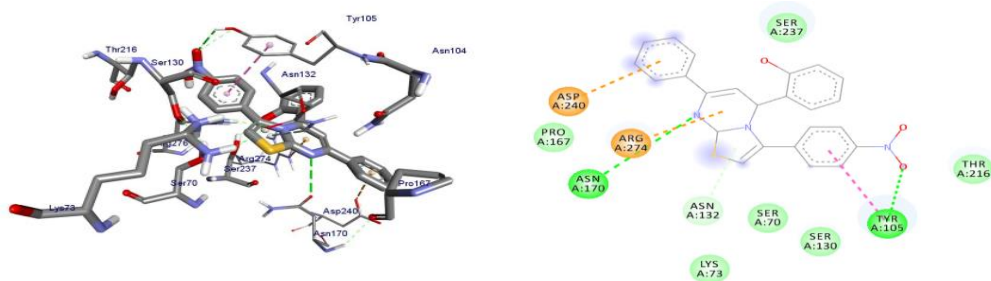


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **94**

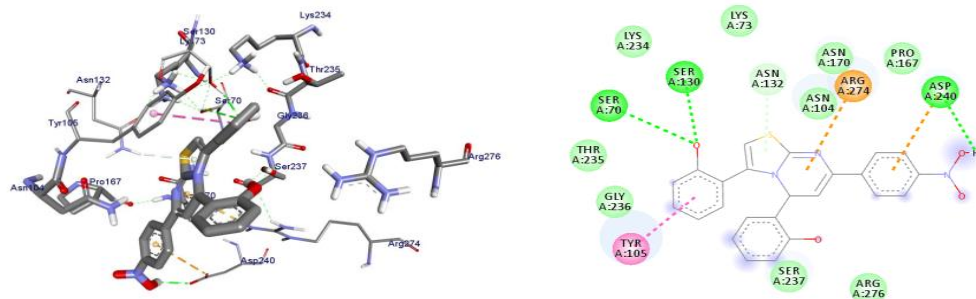


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **98**

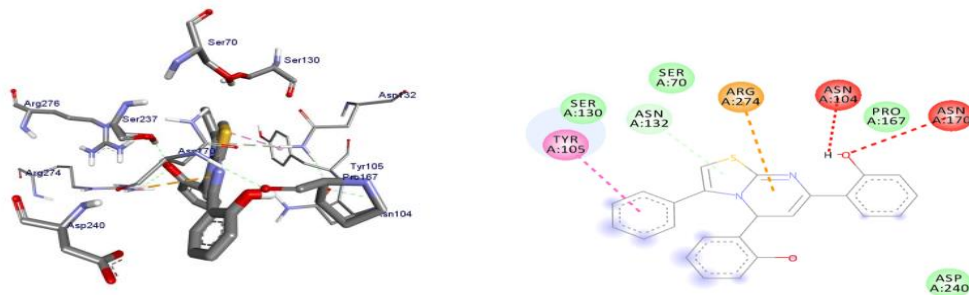


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **99**

Appendix 129. a-j, 3D (right) and 2D (left) representations of the binding interactions of **92-100** and amoxicillin against Human peroxiredoxin 5 (PDB ID: 1hd2).

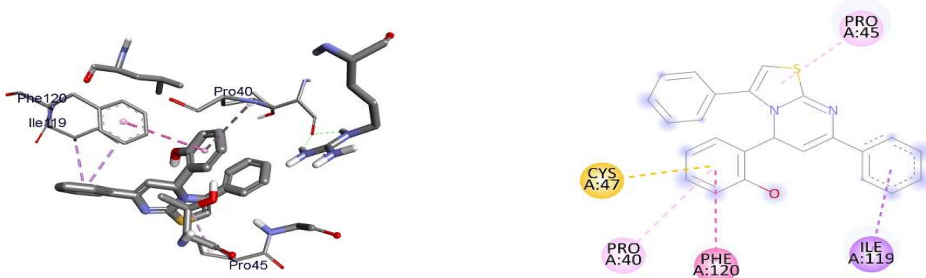


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **92**

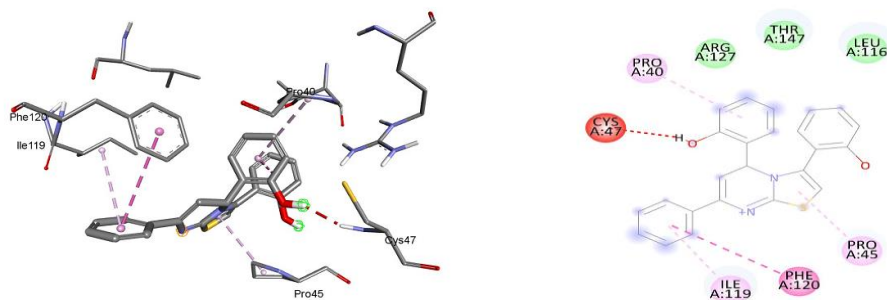


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **93**

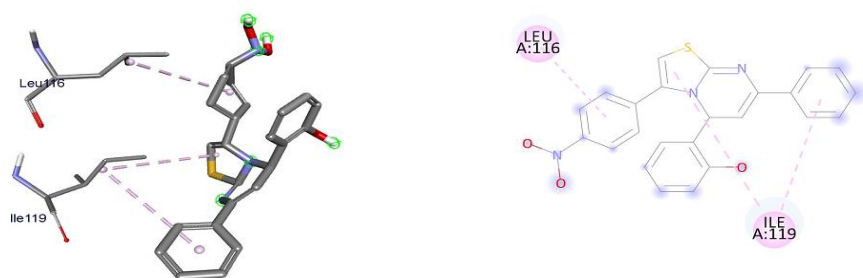


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **94**

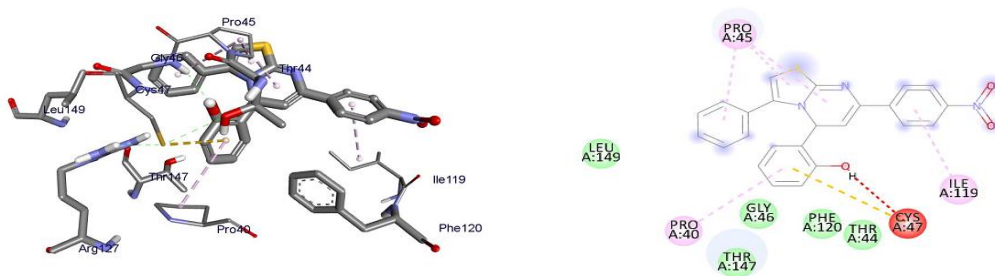


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **95**

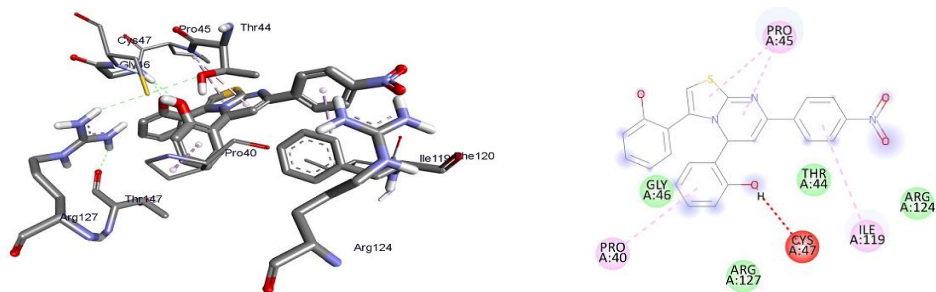


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **96**

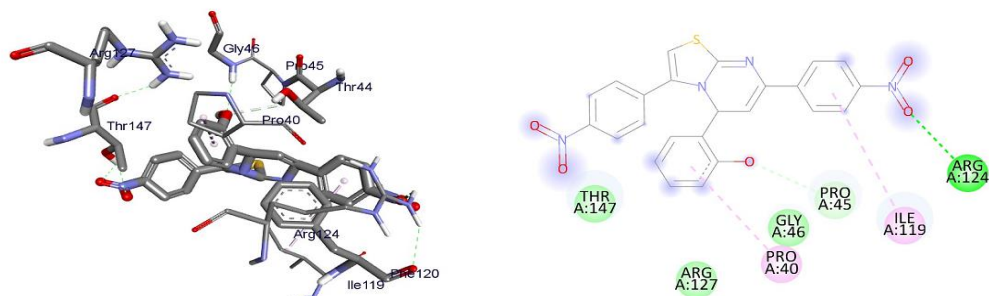


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **97**

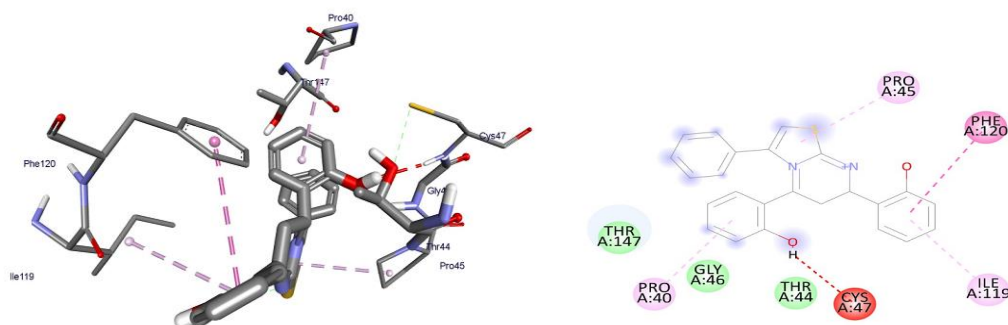


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **98**

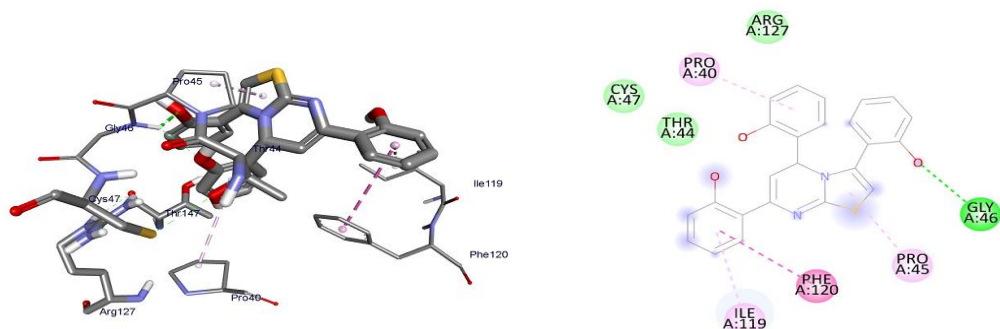


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **99**

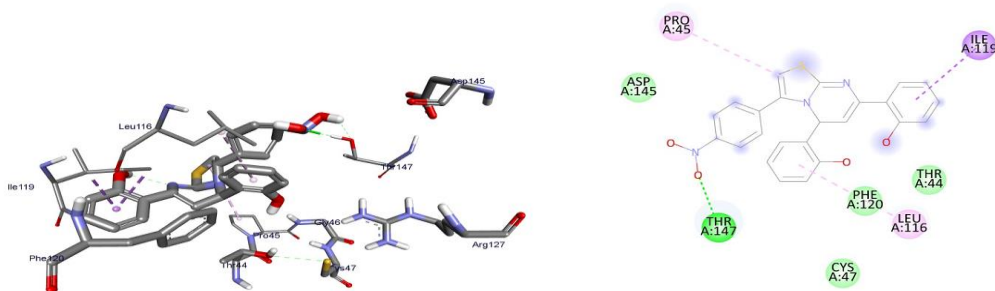


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **100**

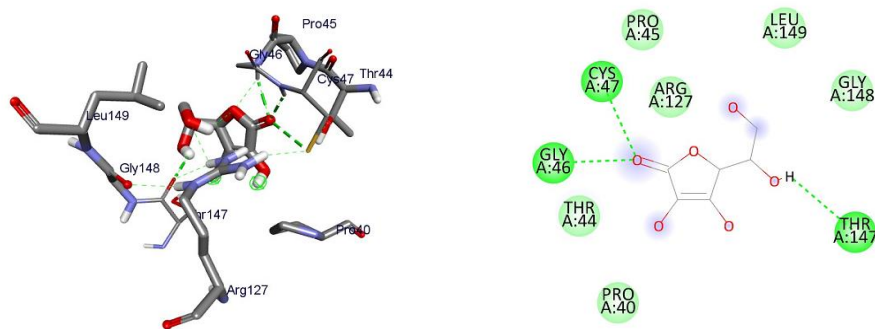


Figure j: 3D (right) and 2D (left) representations of the binding interactions of **vitamin C**
Appendix 130. a-j. 3D (right) and 2D (left) representations of the binding interactions of **(108-116)** and amoxicillin against *E. coli* DNA Gyrase B (PDB ID: 6f86)

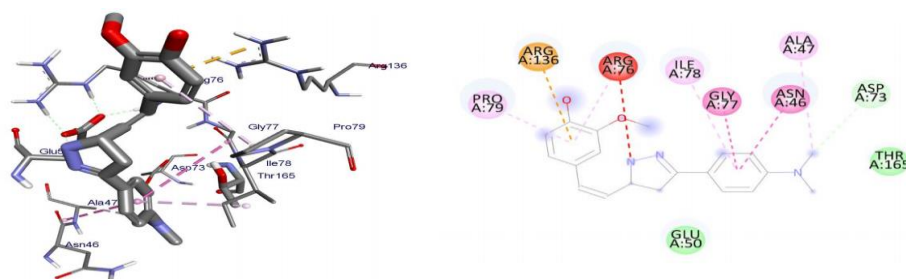


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **108**

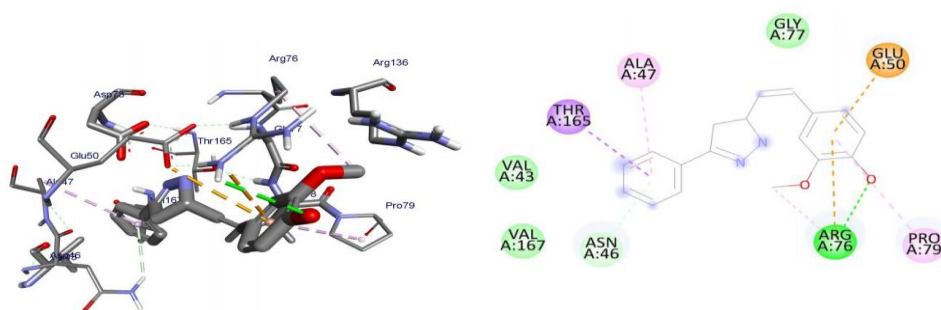


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **109**

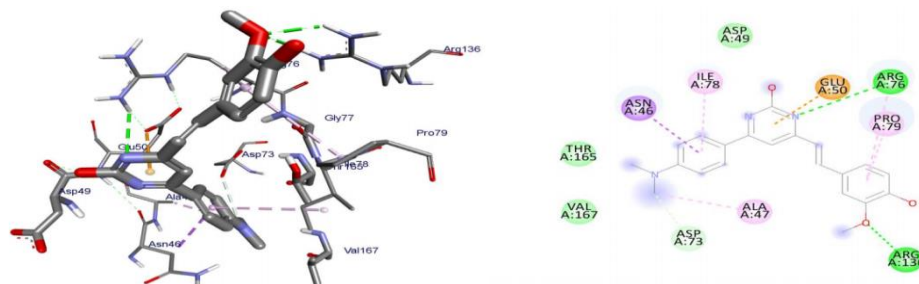


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **110**

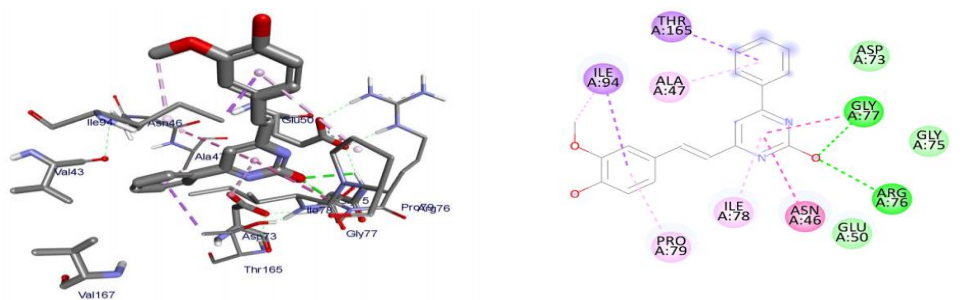


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **111**

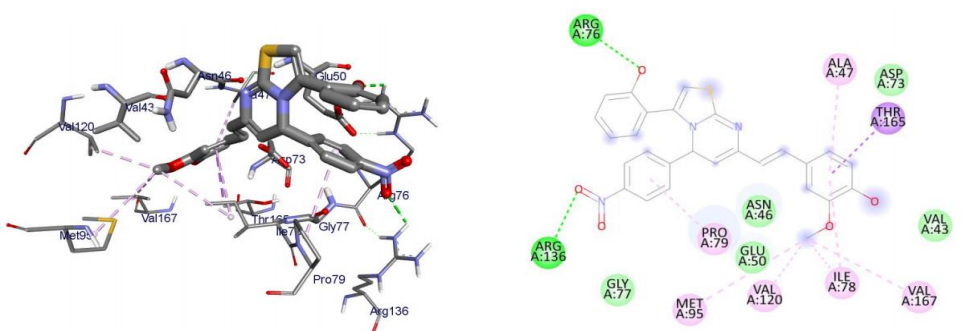


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **112**

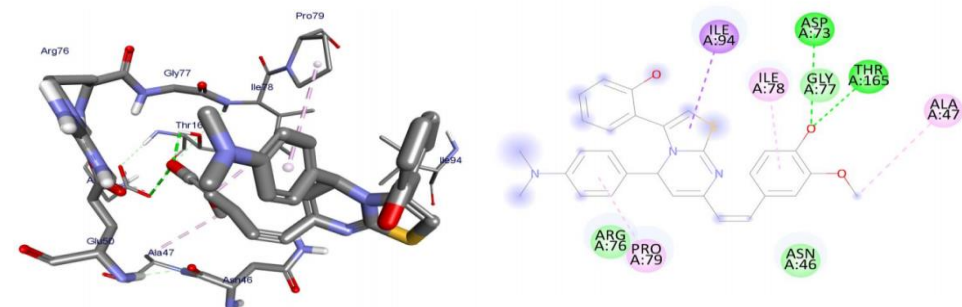


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **113**

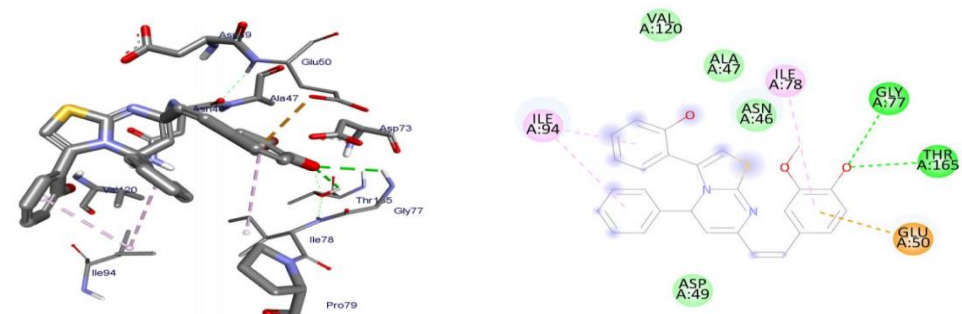


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **114**

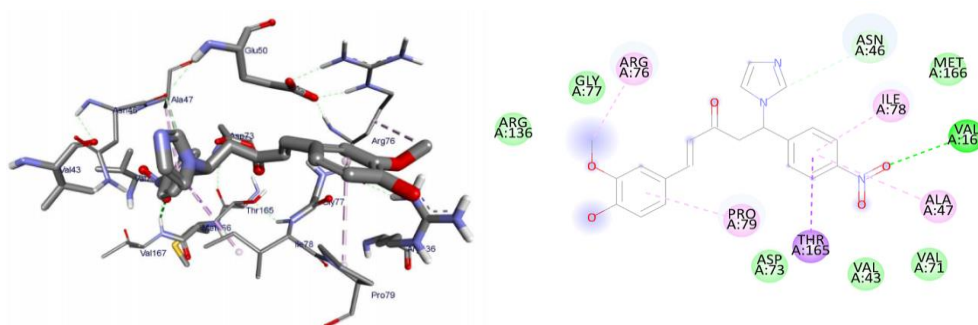


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **115**

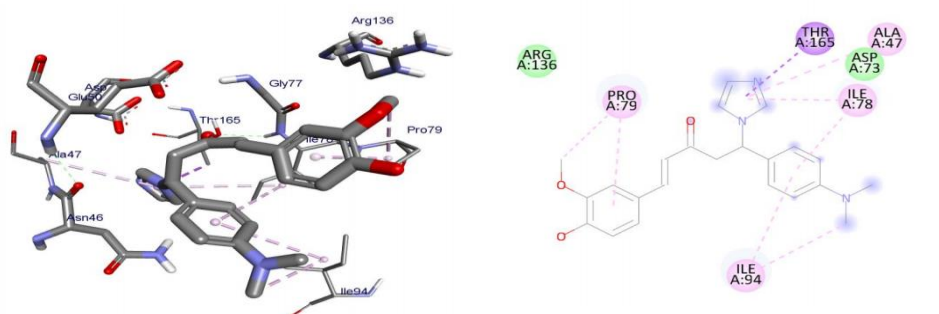


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **116**

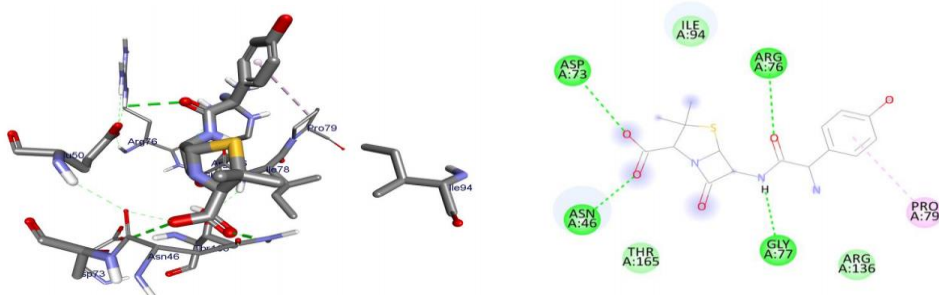


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**.

Appendix 131. a-j. 3D (right) and 2D (left) representations of the binding interactions of (**108-116**) and amoxicillin against N-terminal domain of PqsA (PDB ID: 5oe3).

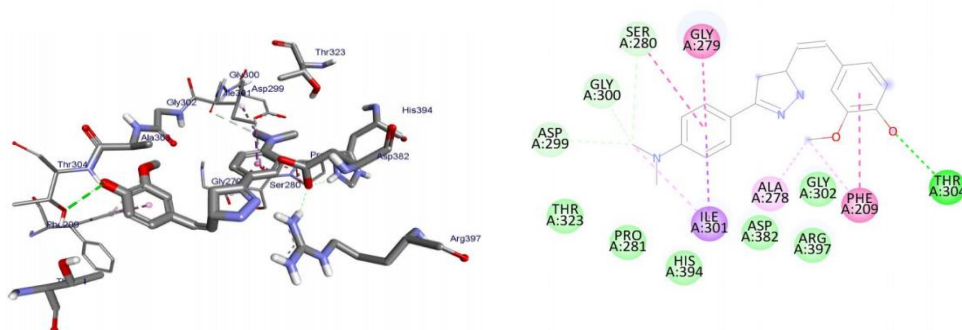


Figure a. 3D (right) and 2D (left) representations of the binding interactions **108**

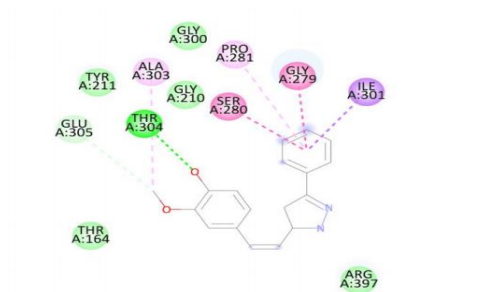
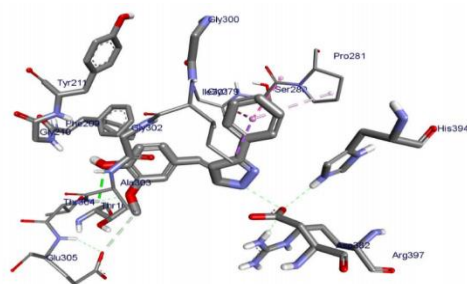


Figure b. 3D (right) and 2D (left) representations of the binding interactions **109**

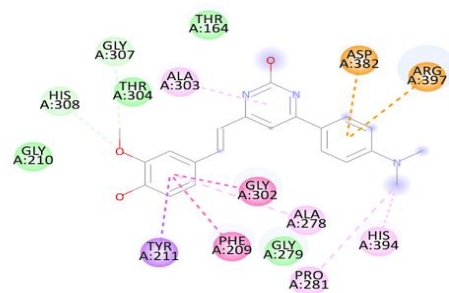
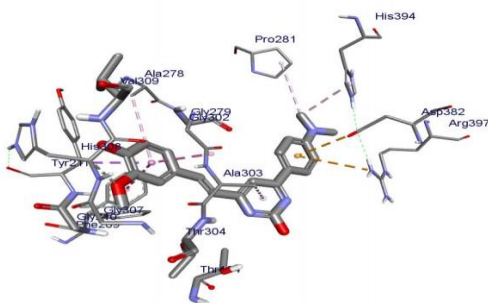


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **110**

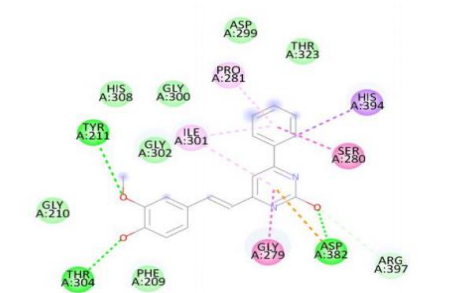
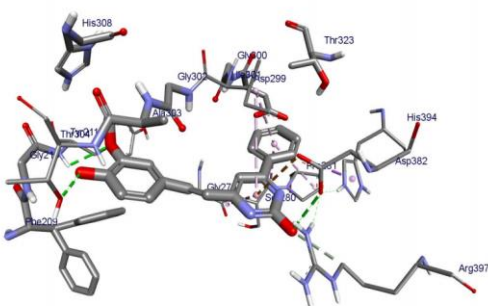


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **111**

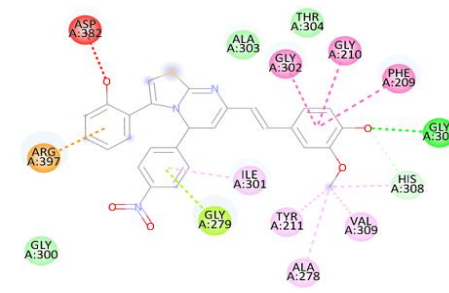
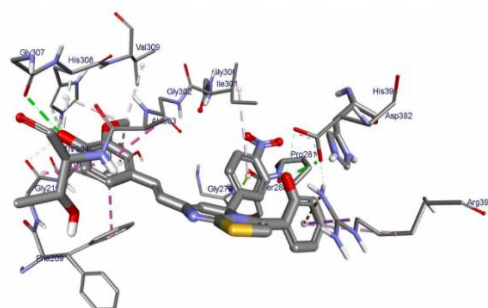


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **112**

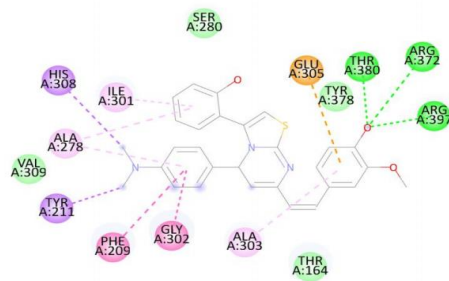
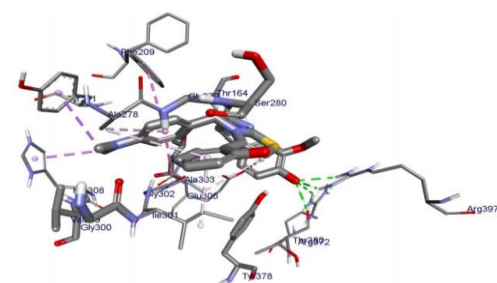


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **113**

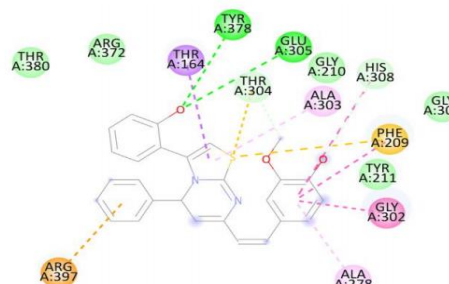
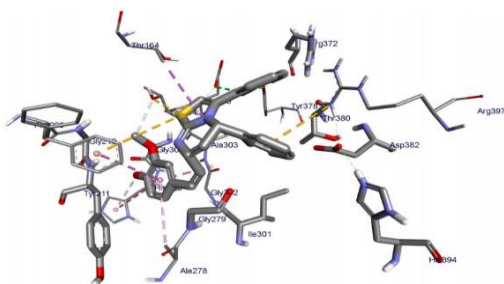


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **114**

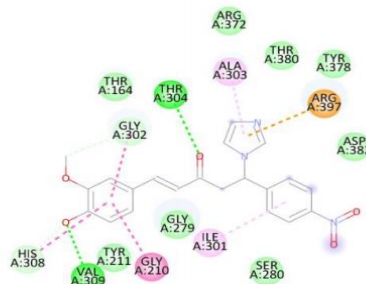
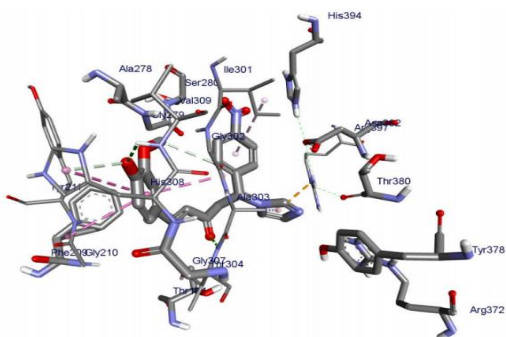
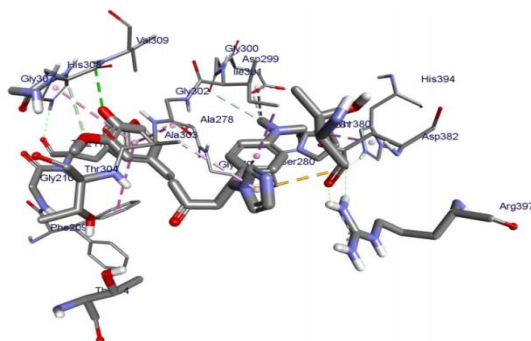


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **115**



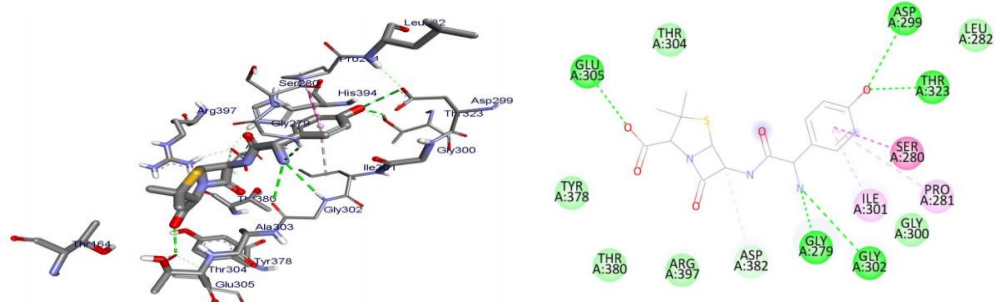


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**.

Appendix 132. a-j. 3D (right) and 2D (left) representations of the binding interactions of (**108-116**) and amoxicillin against *S. aureus* Pyruvate Kinase (PDB ID: 3t07).

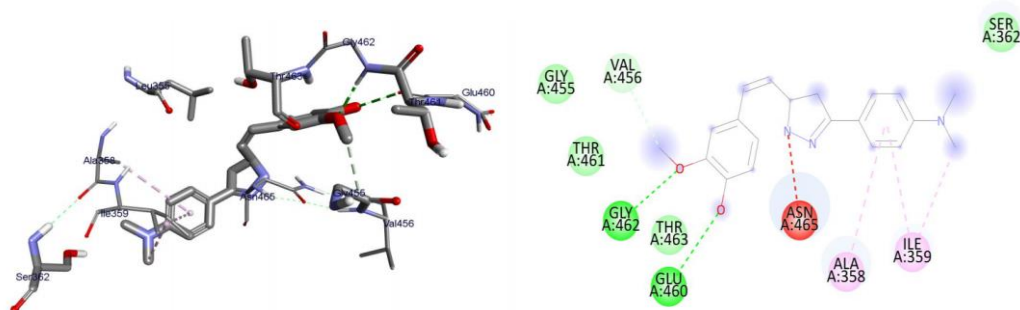


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **108**

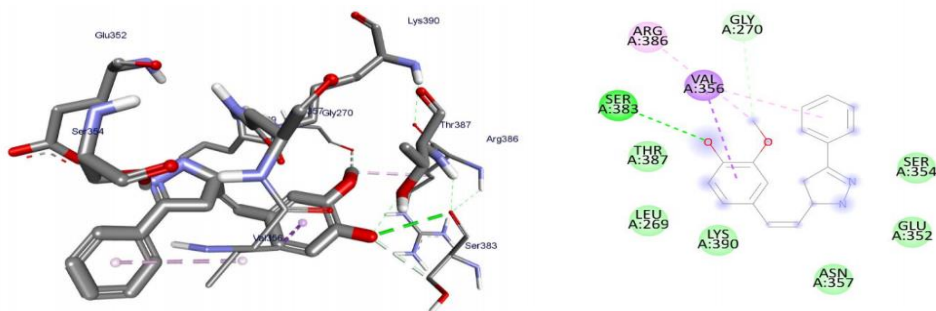


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **109**

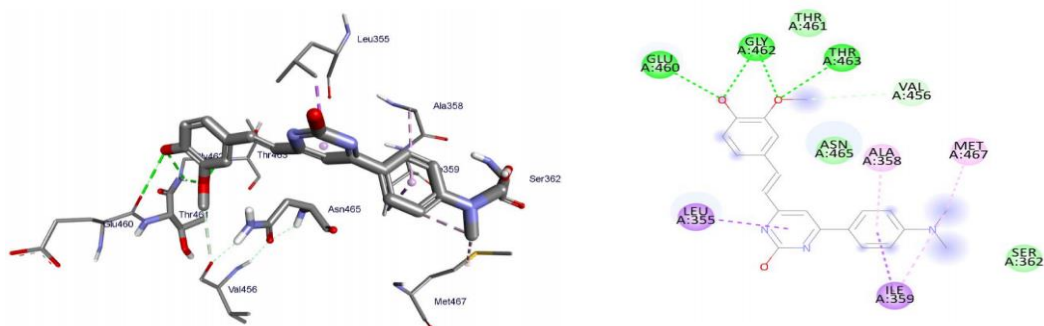


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **110**

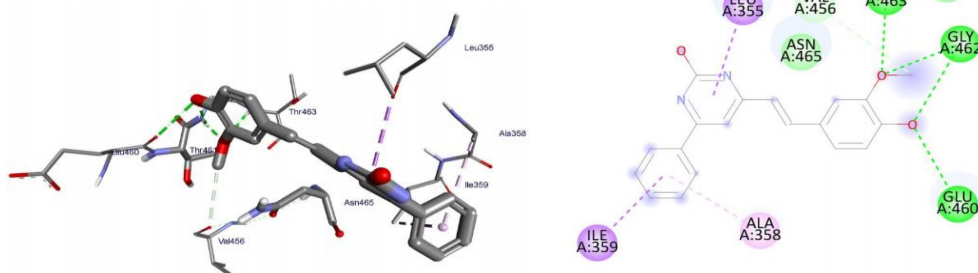


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **111**

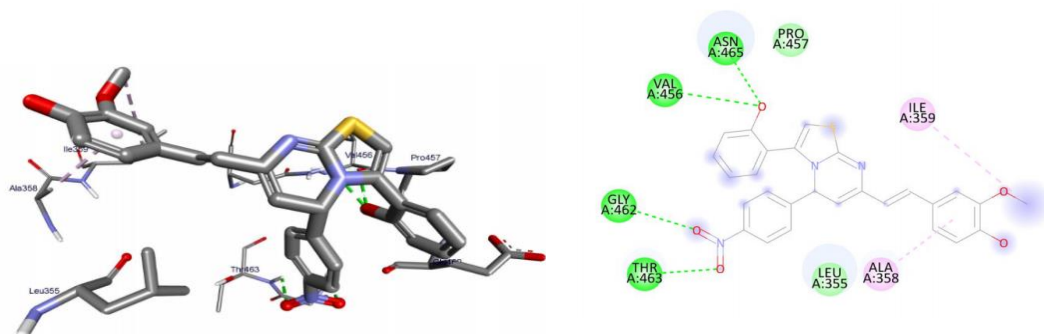


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **112**

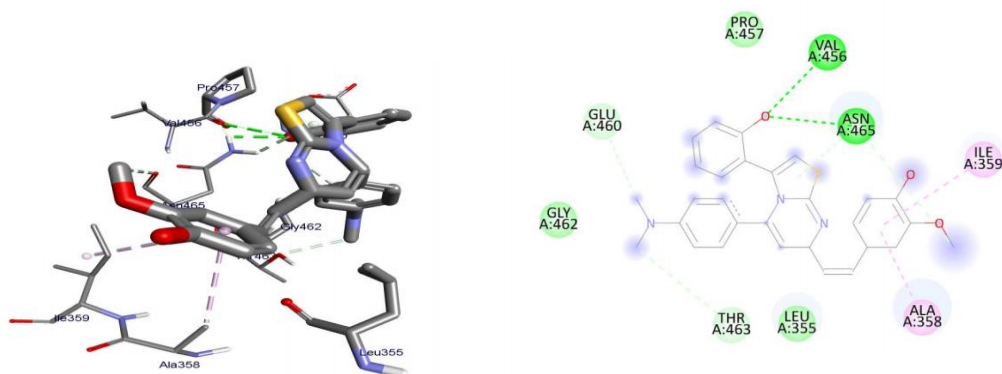


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **113**

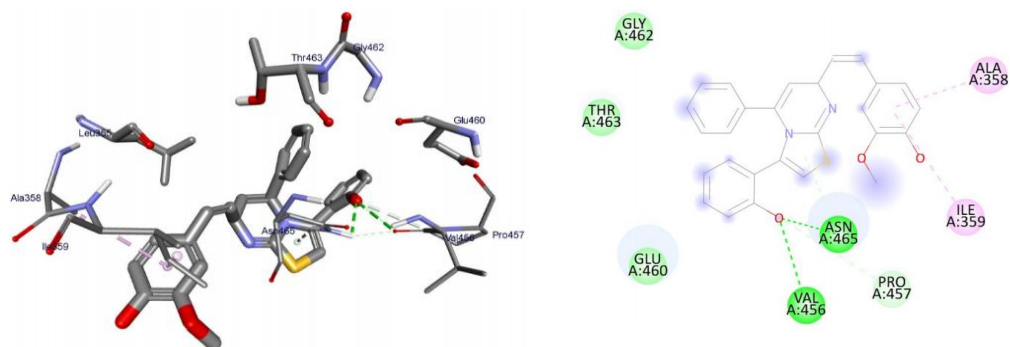


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **114**

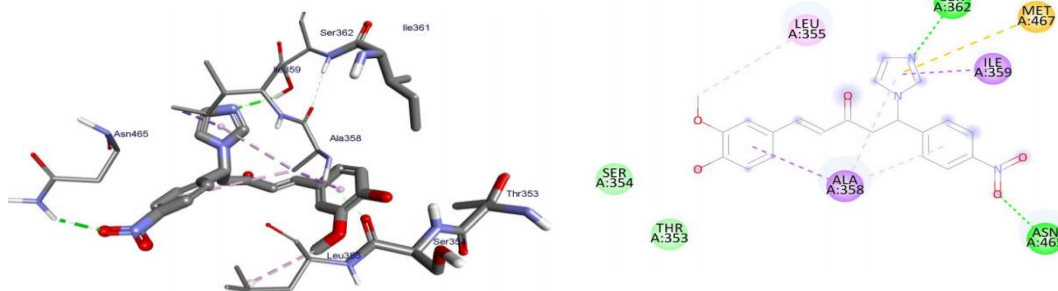


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **115**

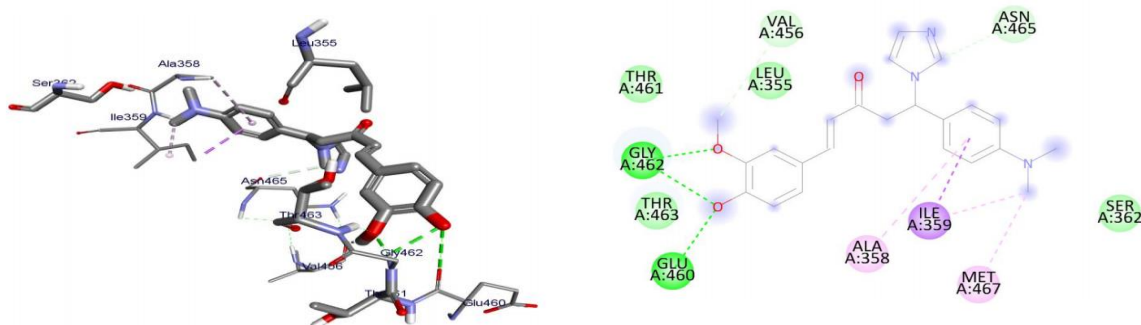


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **116**

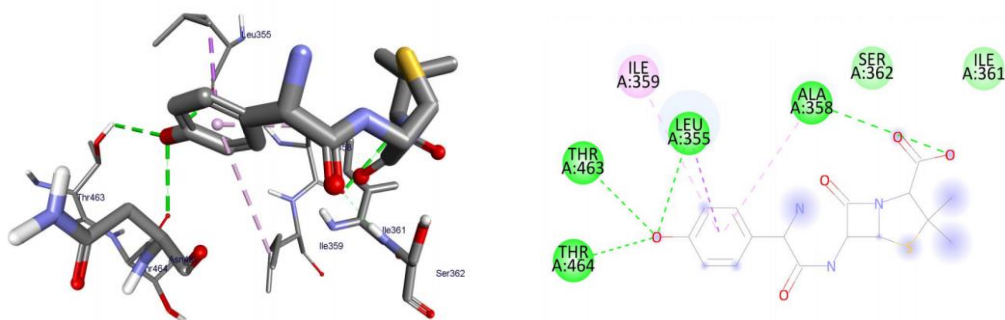


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **amoxicillin**.

Appendix 133. a-j. 3D (right) and 2D (left) representations of the binding interactions of (**108-116**) and amoxicillin against LuxS of *S. pyogenes* (PDB ID: 4xch)

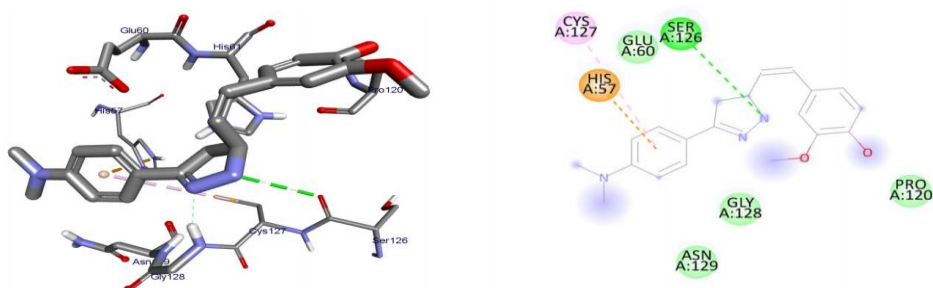


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **108**

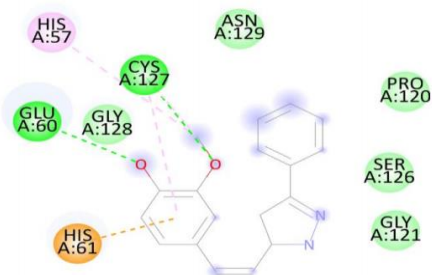
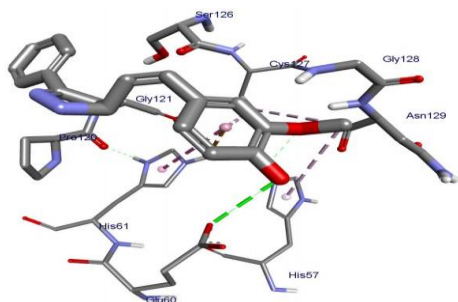


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **109**

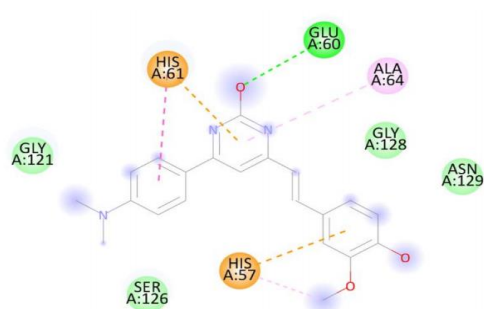
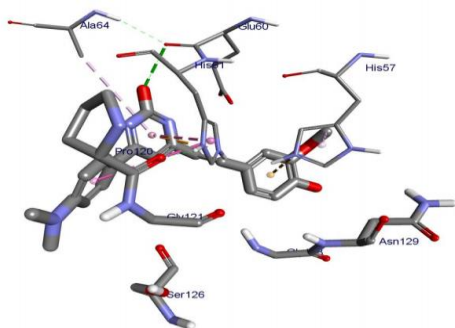


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **110**

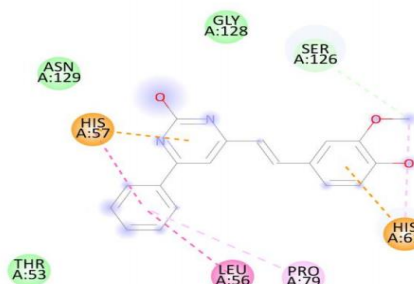
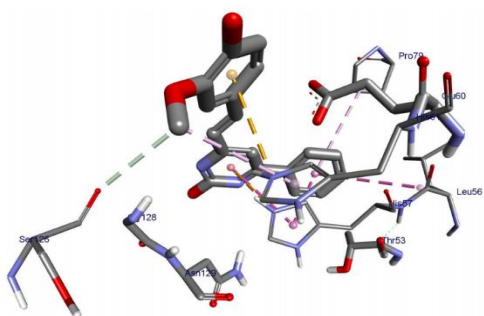


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **111**

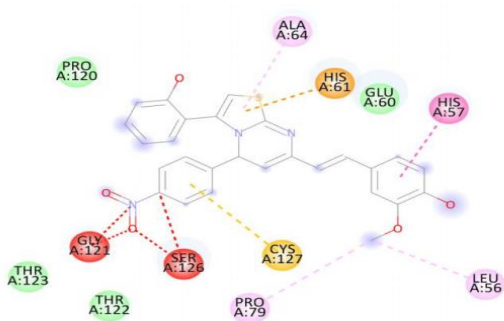
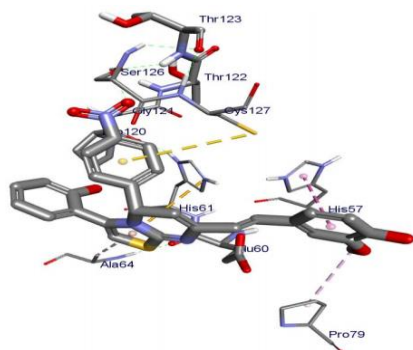


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **112**

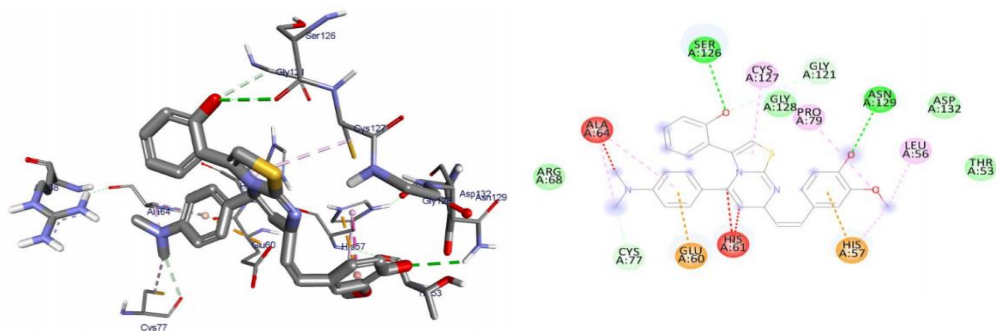


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **113**

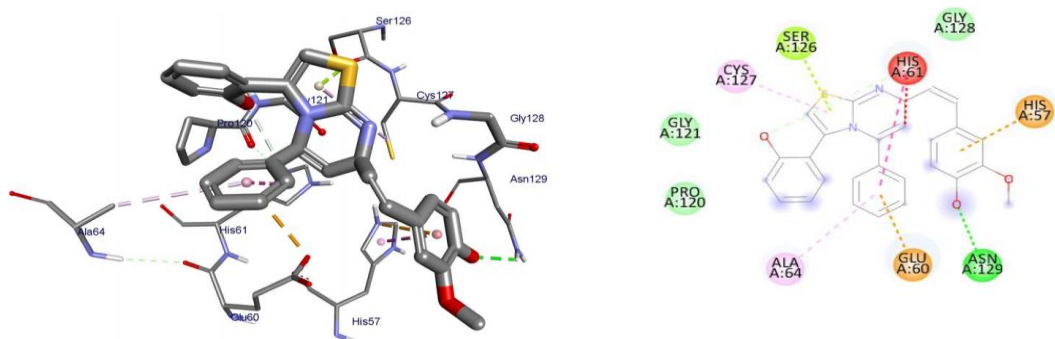


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **114**

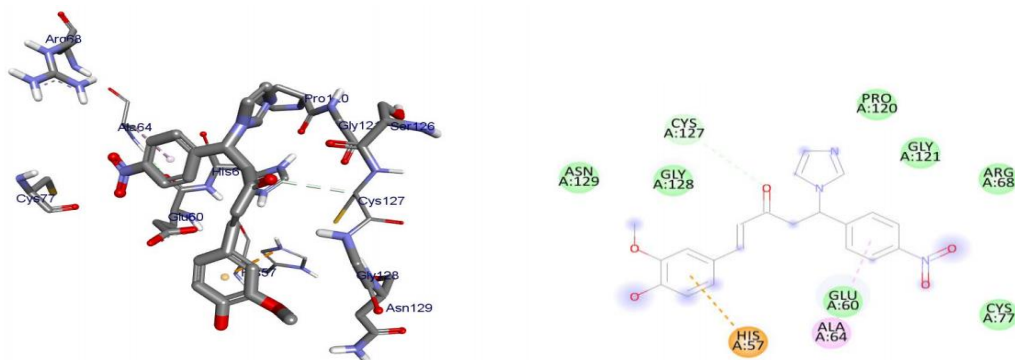


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **115**

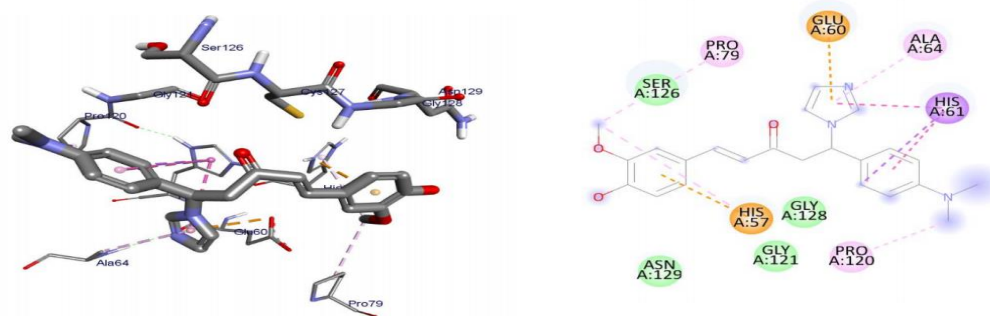


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **116**

Appendix 135. a-c. 3D (right) and 2D (left) representations of the binding interactions of synthesized compounds, **108-116** against against β -lactamases (PDB ID: 1IYS).

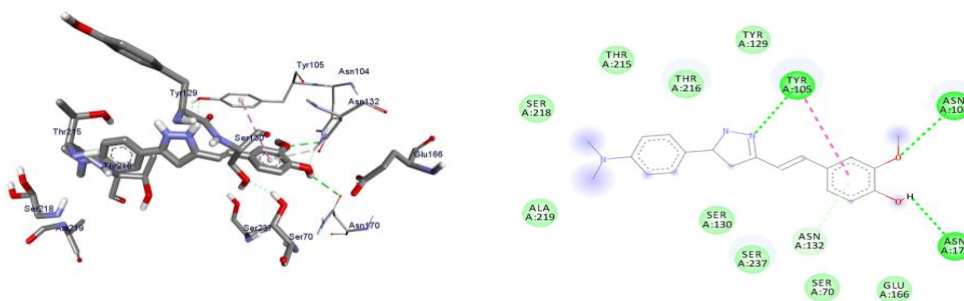


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **108**

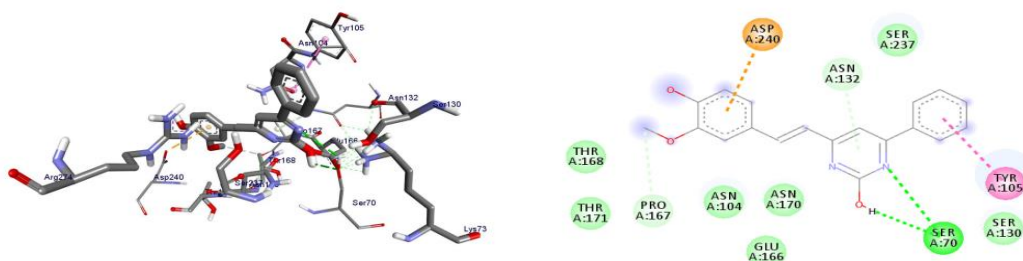


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **111**

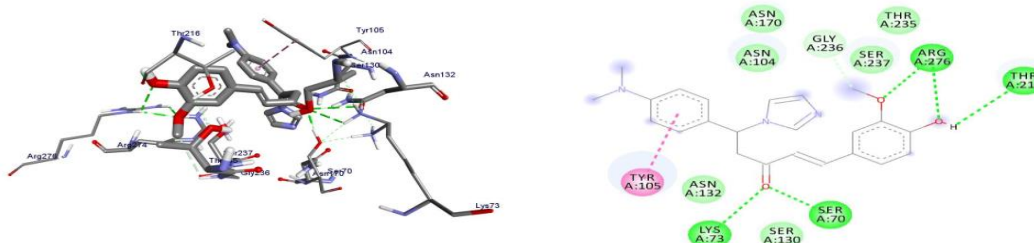


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **116**

Appendix 136. a-j. 3D (right) and 2D (left) representations of the binding interactions of (**108-116**) and amoxicillin and vit C against Human peroxiredoxin 5 (PDB ID: 1hd2).

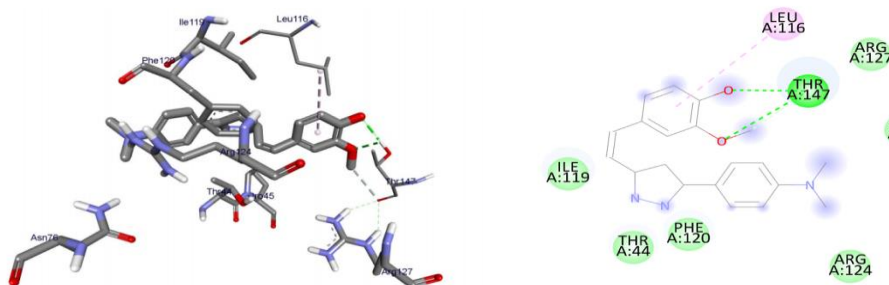


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **108**

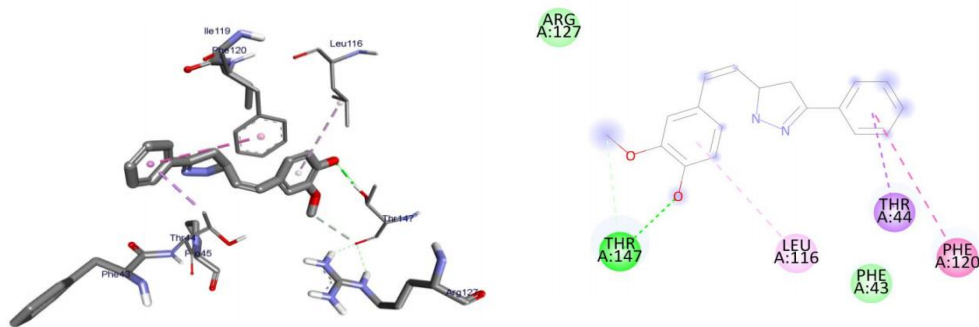


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **109**

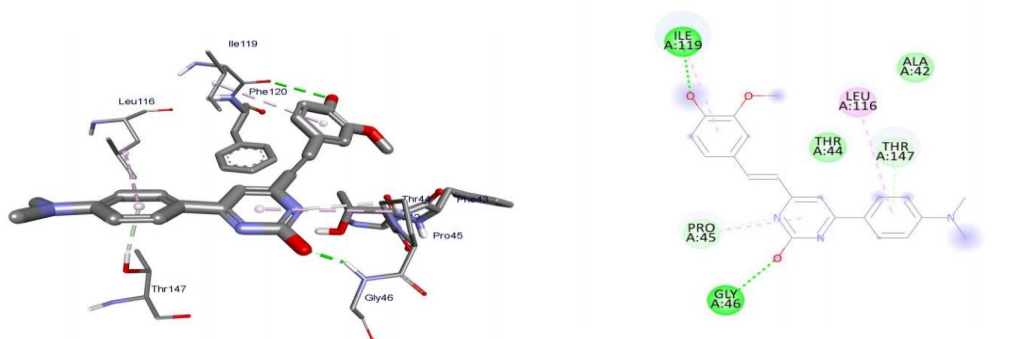


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **110**

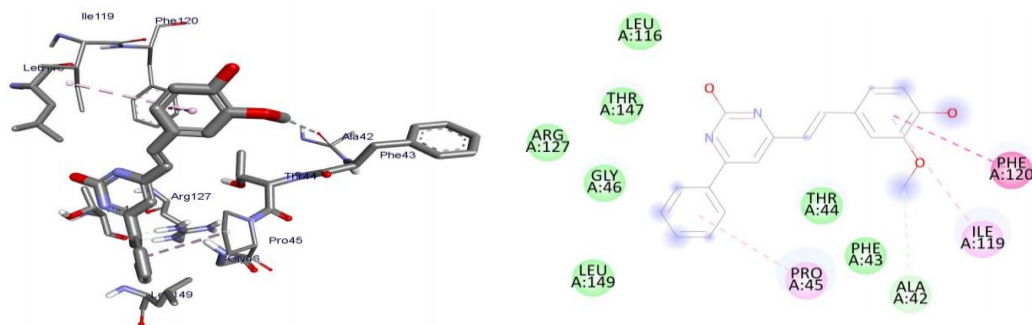


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **111**

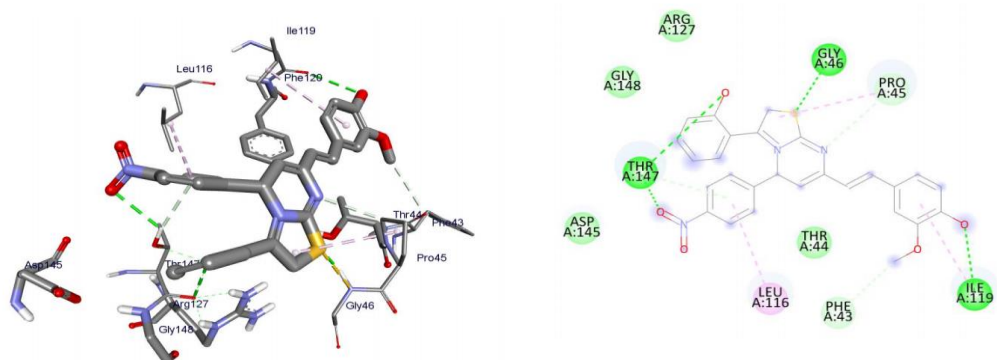


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **112**

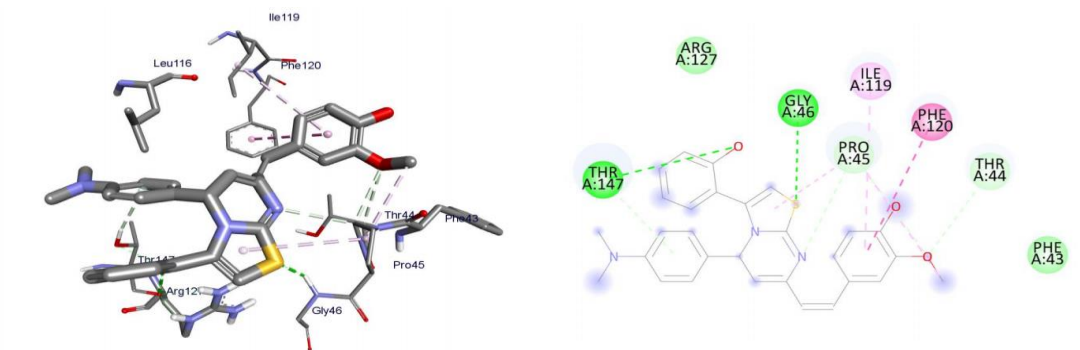


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **113**

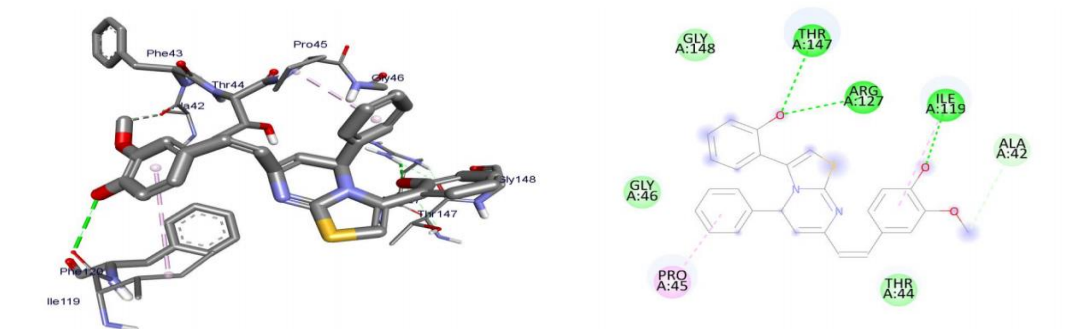


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **114**

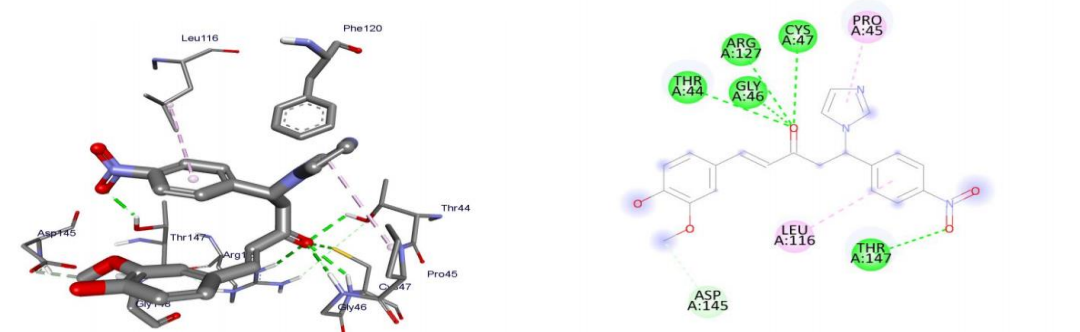


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **115**

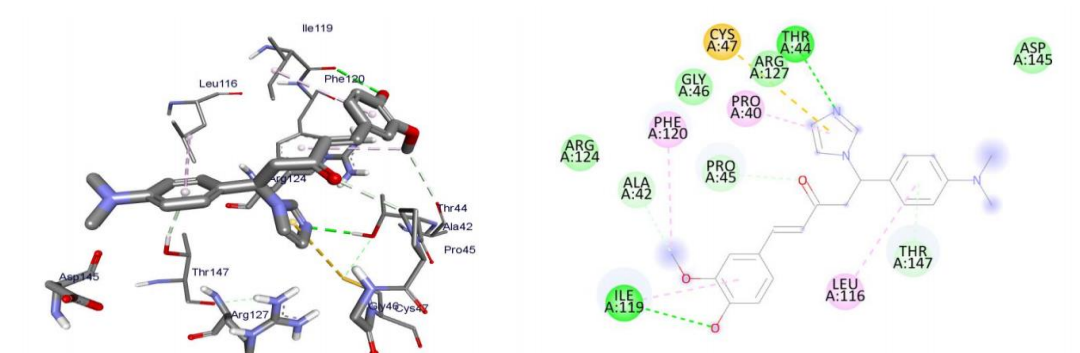


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **116**

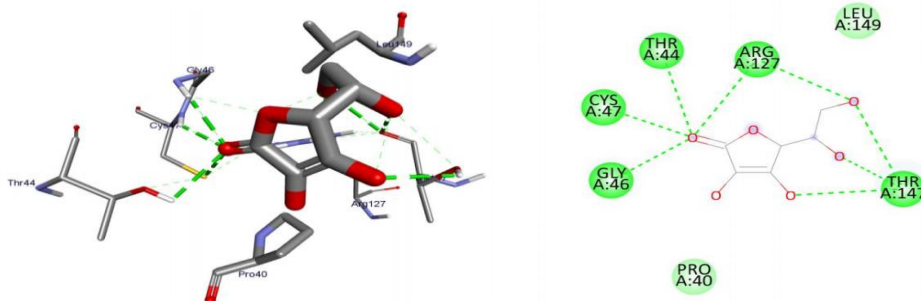


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **vitamin C**
Appendix 137.a-k. 3D (right) and 2D (left) representations of the binding interactions of (**80**,
81, **82**, **83**, **94**, **98**, **99**, **108**, **111**, **116**) and acarbose against α -amylase enzyme (PDB ID: 4w93).

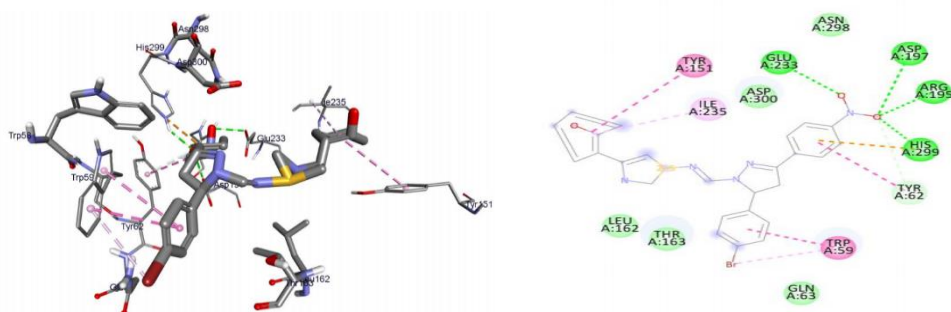


Figure a. 3D (right) and 2D (left) representations of the binding interactions of **80**

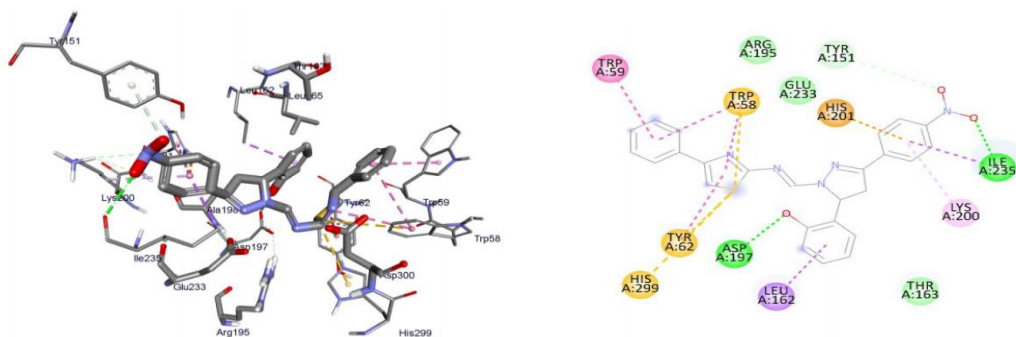


Figure b. 3D (right) and 2D (left) representations of the binding interactions of **81**

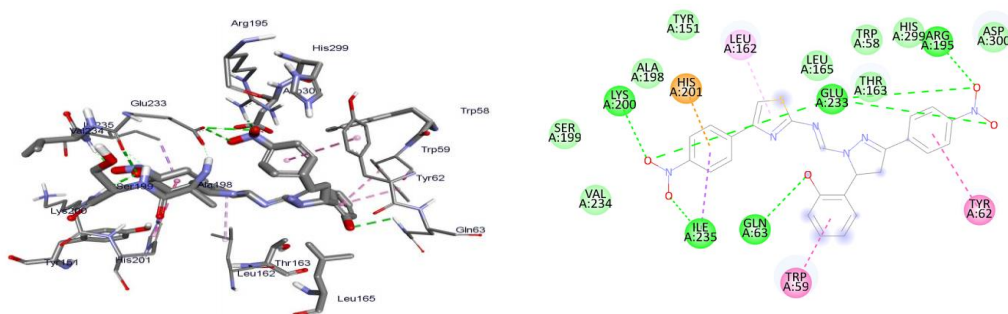


Figure c. 3D (right) and 2D (left) representations of the binding interactions of **82**

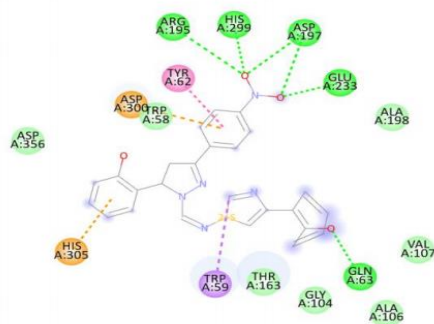
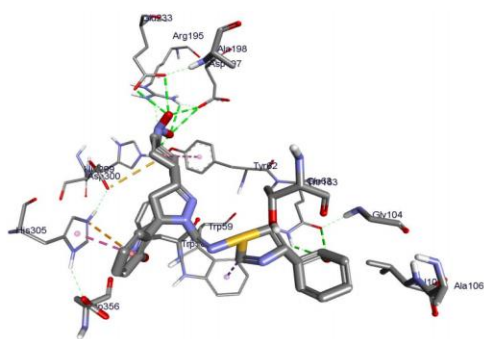


Figure d. 3D (right) and 2D (left) representations of the binding interactions of **83**

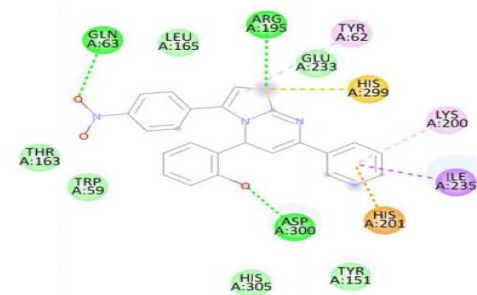
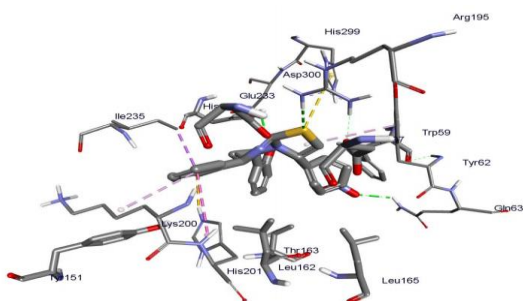


Figure e. 3D (right) and 2D (left) representations of the binding interactions of **94**

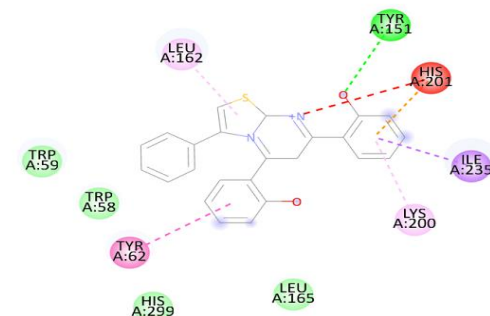
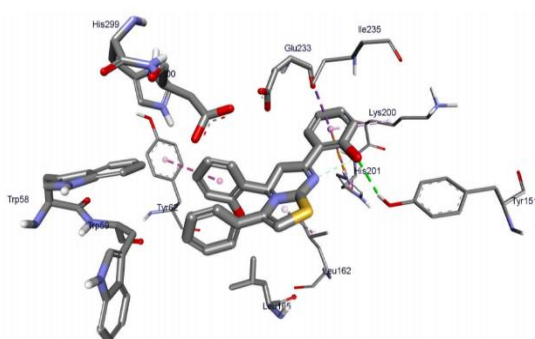


Figure f. 3D (right) and 2D (left) representations of the binding interactions of **98**

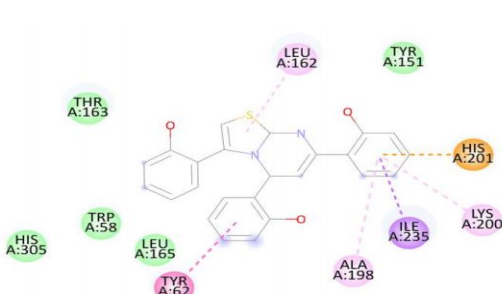
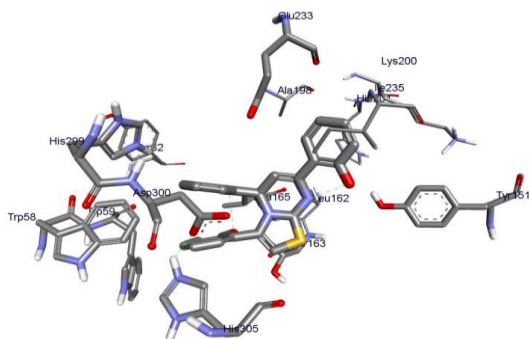


Figure g. 3D (right) and 2D (left) representations of the binding interactions of **99**

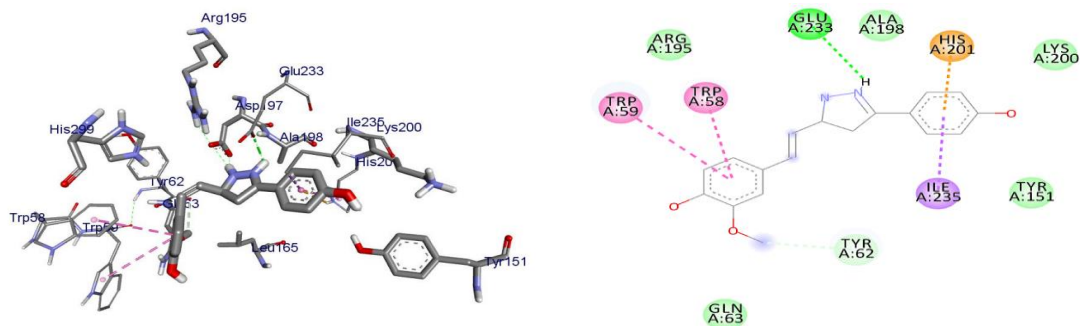


Figure h. 3D (right) and 2D (left) representations of the binding interactions of **108**

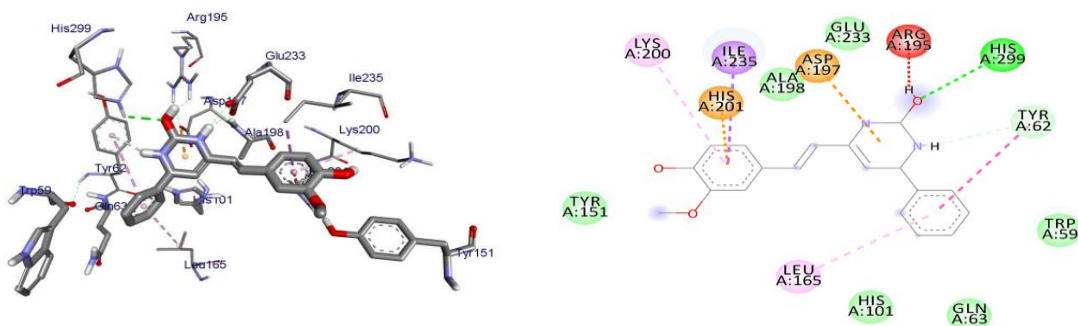


Figure i. 3D (right) and 2D (left) representations of the binding interactions of **111**

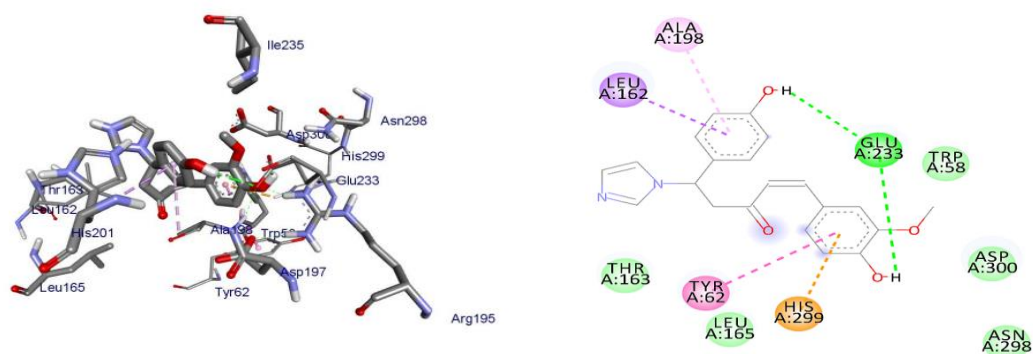


Figure j. 3D (right) and 2D (left) representations of the binding interactions of **116**

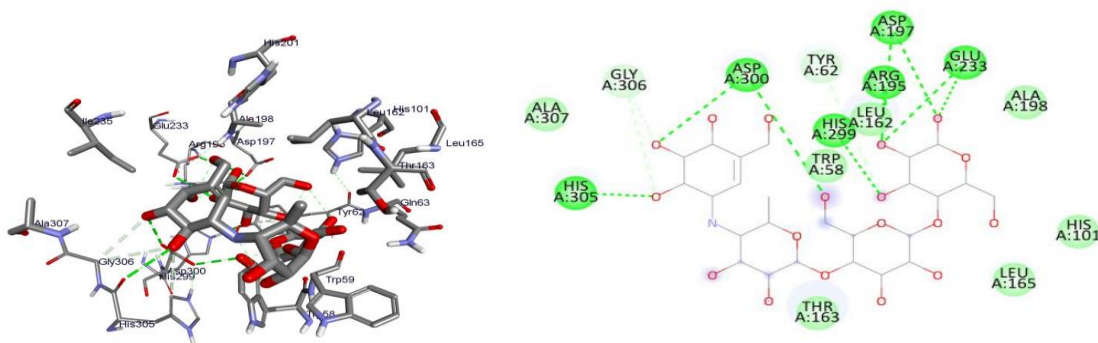


Figure k. 3D (right) and 2D (left) representations of the binding interactions of **acarbose**

LIST OF PUBLISHED ARTICLES

1. Demis Zelelew, Milkyas Endale, Yadessa Melaku, Fedlu Kedir, Taye B. Demissie, Japheth O. Ombito, Rajalakshmanan Eswaramoorthy (2022). Synthesis, antibacterial and antioxidant activities of thiazolyl-pyrazoline Schiff base hybrids: A combined experimental and computational study, *Journal of Chemistry*, Volume 2022, Article ID 3717826, 19 pages, <https://doi.org/10.1155/2022/3717826>
2. Demis Zelelew, Milkyas Endale, Yadessa Melaku, Taye B. Demissie, Japheth O. Ombito, Rajalakshmanan Eswaramoorthy (2022). ZnO nanoparticle assisted synthesis of Thiazolo[3,2- α] pyrimidine analogs: Antibacterial and antioxidant activity, *in silico* molecular docking and ADMET prediction study, *Journal of Chemistry*, Volume 2022, Article ID 1346856, 20 pages, <https://doi.org/10.1155/2022/1346856>
3. Demis Zelelew, Milkyas Endale, Yadessa Melaku, Teshome Geremew, Rajalakshmanan Eswaramoorthy, Lemma Teshome Tufa, Youngeun Choi, Jaebeom Lee (2023). Ultrasonic-assisted synthesis of heterocyclic curcumin analogs as Antidiabetic, Antibacterial, and Antioxidant Agents combined with *in vitro* and *in silico* studies, *Adv Appl Bioinform Chem*. 2023; 16: 61–91, <https://doi.org/10.2147/AABC.S403413>