



**HAWASSA UNIVERSITY**

**SCHOOL OF GRADUATE STUDIES**

**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES**

**DEPARTMENT OF CHEMISTRY**

**DESIGN AND SYNTHESIS OF SOME NOVEL HYBRID MOLECULES  
CONTAINING INDOLE AND OXADIAZOLE RING FOR EVALUATION  
OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES**

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**MAY, 2024**

**HAWASSA, ETHIOPIA**

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THE REQUIREMENTS FOR DEGREE OF MASTERS OF SCIENCE IN  
ORGANIC CHEMISTRY

MAY, 2024

HAWASSA, ETHIOPIA

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## Table of Contents

<b>Contents</b>	<b>Page</b>
DECLARATIONs.....	ii
ADVISORS' APPROVAL SHEET.....	iii
EXAMINER 'S APPROVAL SHEET .....	iv
ACKNOWLEDGMENTs.....	v
List of Abbreviations .....	x
List of Figures.....	xi
List of Tables .....	xii
List of Schemes.....	xiii
List of Appendices .....	xiv
Abstract.....	xv
CHAPTER ONE.....	1
1. INTRODUCTION .....	1
1.1. Background of the study .....	1
1.2. Problem statement.....	4
1.3. General Objective.....	5
1.3.1. Specific Objectives .....	5
1.4. Significance of the study.....	5
CHAPTER TWO .....	7
2. LITERATURE REVIEW .....	7
2.1. Chemistry of indoles .....	7
2.2. Methods for synthesizing the indole ring and its derivatives.....	8
2.2.1. Conventional Methods .....	8
2.2.2. Novel Methods.....	9
2.2.3. Green Methodologies.....	11

2.2.3.1.	Synthesis of Indole Derivatives Using Water as the Solvent .....	12
2.2.3.2.	Synthesis of Bioactive Indole Derivatives using Indole as a Precursor .....	13
2.2.3.3.	Preparation of Indole Derivatives (Catalyst-free Conditions).....	14
2.2.3.4.	Synthesis of Indole Derivates - Solid Acid Catalyst .....	15
2.2.3.5.	Synthesis of Indole Derivatives using Nanoparticles .....	16
2.2.3.6.	Microwave-assisted Synthesis .....	17
2.3.	Importance of indole and its derivatives .....	19
2.4.	Indole in natural products.....	20
2.5.	Indole in synthetic products .....	21
2.6.	Pharmacological activity of indole derivatives .....	22
2.6.1.	Antioxidant activities .....	23
2.6.2.	Antifungal activities.....	25
2.6.3.	Antimalarial activities .....	27
2.6.4.	Antiviral activities.....	28
2.6.5.	Anticancer activities.....	30
2.6.6.	Anti-inflammatory activities.....	32
2.6.7.	Antimicrobial activity .....	34
2.6.8.	Antidepressant activity.....	37
2.6.9.	Anticonvulsant activity .....	39
2.6.10.	Antidiabetic activity .....	40
2.7.	Chemistry of Oxadiazoles .....	41
2.7.1.	Synthesis Techniques for 1,3,4-Oxadiazoles rings and its derivatives.....	42
CHAPTER THREE .....		44
3.	MATERIALS AND METHODS.....	44
3.1.	Materials.....	44

3.1.1.	Instruments.....	44
3.1.2.	Chemicals and Apparatus .....	44
3.2.	Methods.....	45
3.2.1.	General procedures for the synthesis of target compounds .....	45
3.2.2.	Synthesis of the Intermediates .....	45
3.2.2.1.	Synthesis of Methyl 2-(1 <i>H</i> -indol-3-yl)acetate (106a') .....	45
3.2.2.2.	Synthesis of methyl 4-(1 <i>H</i> -indole-3-yl)butanoate (106b') .....	46
3.2.2.3.	Synthesis of 2-(1 <i>H</i> -indol-3-yl)acetohydrazide (107a').....	47
3.2.2.4.	Synthesis of 4-(1 <i>H</i> -indole-3-yl)butaneydrazide (107b').....	47
3.2.3.	Synthesis of the Target Compounds .....	48
3.2.3.1.	Synthesis of 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1 <i>H</i> -indole (110a')... 48	
3.2.3.2.	Synthesis of 3-(3-(5-phenyl-1,3,4-oxadiazol-2-yl)propyl)-1 <i>H</i> -indole (110b') 48	
3.2.3.3.	Synthesis of 2-(5-((1 <i>H</i> -indol-3-yl)methyl)-1,3,4-oxadiazol-2-yl)phenol (111a') 49	
3.2.3.4.	Synthesis of 3-(3-(5-((1 <i>H</i> -indol-3-yl)methyl)-1,3,4-oxadiazol-2-yl)propyl)-1 <i>H</i> - indole (111b').....	50
3.3.	<i>In vitro</i> antibacterial activity tests .....	50
3.4.	<i>In vitro</i> antioxidant activity Assay .....	51
3.4.1.	DPPH Assay.....	51
3.4.2.	Procedure for the DPPH assay .....	52
3.4.3.	Calculation of IC <sub>50</sub> .....	52
CHAPTER FOUR.....		53
4.	RESULTS AND DESCUSSION .....	53
4.1.	Methods.....	53
4.2.	Partial Characterization of the Synthesize Compounds .....	54
4.2.1.	Interpretation of Methyl 2-(1 <i>H</i> -indol-3-yl)acetate (106a') .....	54

4.2.2.	Interpretation of methyl 4-(1 <i>H</i> -indole-3-yl)butanoate (106b') .....	55
4.2.3.	Interpretation of 2-(1 <i>H</i> -indol-3-yl)acetohydrazide (107a').....	55
4.2.4.	Interpretation of 4-(1 <i>H</i> -indol-3-yl)butanehydrazide (107b') .....	56
4.2.5.	Interpretation of 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1 <i>H</i> -indole (110a')....	56
4.2.6.	Interpretation of 3-(3-(5-phenyl-1,3,4-oxadiazol-2-yl)propyl)-1 <i>H</i> -indole (110b').	57
4.2.7.	Interpretation of 2-(5-((1 <i>H</i> -indol-3-yl)methyl)-1,3,4-oxadiazol-2-yl)phenol (111a')	57
4.2.8.	Interpretation of 3-(3-(5-((1 <i>H</i> -indol-3-yl)methyl)-1,3,4-oxadiazol-2-yl)propyl)-1 <i>H</i> - indole (111b') .....	58
4.3.	Antibacterial activity test results .....	59
4.3.1.	Structure Activity Relationship for Antibacterial activity test results .....	60
4.4.	Antioxidant activity assay results of the synthesized compounds .....	61
4.4.1.	DPPH Radical Scavenging Activities and Structure–Activity Relationships .....	64
CHAPTER FIVE .....		65
5.	CONCLUSION AND RECOMMENDATION.....	65
5.1.	Conclusion.....	65
5.2.	Recommendation.....	66
6.	REFERENCES .....	67
7.	APPENDICES .....	85

## List of Abbreviations

DMSO	Dimethyl sulphoxide
POCl <sub>3</sub>	Phosphorus oxychloride
DPPH	1,1-diphenyl-2-picrylhydrazyl
FT-IR	Fourier transform infrared
IC <sub>50</sub>	Half maximal inhibitory concentration
<b>NPs</b>	Nanoparticles
R <sub>f</sub>	Retention Factor
RT	Room Temperature
SAR	Structure activity relationships
SD	Standard Deviation
TLC	Thin Layer chromatography
UV-VIS	Ultraviolet visible
RSA	Radical Scavenging activity

## List of Figures

Figure	Page
Figure 1: Chemical structure of indole .....	7
Figure 2: Indole scaffolds in biologically important compounds .....	19
Figure 3: Indole alkaloids found in natural products .....	21
Figure 4: The Chemical structure of marketed indole derivatives.....	22
Figure 5: Pharmacological profile of the Indole scaffold .....	23
Figure 6: Chemical structure of indole derivative compounds that show antioxidant activity ....	25
Figure 7: Chemical structure of indole derivative compounds that show antifungal activity .....	26
Figure 8: Chemical structure of indole derivative compounds that show antimalarial activity ...	28
Figure 9: Chemical structure of indole derivative compounds that show antiviral activity .....	30
Figure 10: Chemical structure of indole derivative compounds that show anticancer activity ....	32
Figure 11: Chemical structure of indole derivative compounds that show anti-inflammatory activity.....	34
Figure 12: Chemical structure of indole derivative compounds that show antimicrobial activity	37
Figure 13: Chemical structure of indole derivative compounds that show antidepressant activity .....	38
Figure 14: Chemical structure of indole derivative compounds that show anticonvulsant activity .....	40
Figure 15: Chemical structure of indole derivative compounds that show antidiabetic activity..	41
Figure 16: Reaction between DPPH• and antioxidant to form reduced DPPH .....	51
Figure 17: Chemical structure of the compound 106a' .....	55
Figure 18: Chemical structure of compound 106b'.....	55
Figure 19: Chemical structure of compound 107a'.....	56
Figure 20: Chemical structure of compound 107b'.....	56
Figure 21: Chemical structure of compound 110a'.....	57
Figure 22: Chemical structure of compound 110b'.....	57
Figure 23: Chemical structure of compound 111a'.....	58
Figure 24: Chemical structure of compound 111b'.....	59
Figure 26: DPPH radical scavenging activity of synthesized compounds and Ascorbic Acid .	63
Figure 25: Mean inhibition zone of synthesized compounds in mm (mean ± SD) at 150 mg/mL. ....	98

## List of Tables

<b>Table</b>	<b>Page</b>
Table 1: Physical properties of the synthesized compounds .....	54
Table 3: The average diameter (mm) of the Zone of Inhibition for synthesized compounds was measured against selected bacterial strains.....	59
Table 4: % radical scavenging activities of the synthesized compounds and ascorbic acid.....	62
Table 2: The diameter (mm) of the Zone of inhibition of synthesized compounds was measured against selected bacterial strains. ....	97

## List of Schemes

Scheme	Page
Scheme 1: Discovery of indole by Adolf von Baeyer .....	7
Scheme 2: Synthesis of indole and its derivatives by conventional methods.....	9
Scheme 3: Synthesis of indoles and their derivatives by novel methods .....	11
Scheme 4: Synthesis of an indole derivative using Cu(PPh <sub>3</sub> )Cl in aqueous media.....	12
Scheme 5: Synthesis of hemiaminal indole in aqueous medium.....	12
Scheme 6: Synthesis of 3-substituted indole using [Sc(DS) <sub>3</sub> ] as a catalyst in water.....	13
Scheme 7: Preparation of bis(indolyl)methane derivatives under visible light .....	13
Scheme 8: Preparation of bis(indolyl)methane derivatives from solid cellulose sulfuric acid.....	13
Scheme 9: Indion Ina 225H resin-based preparation of bis (indolyl) methane .....	14
Scheme 10: Catalyst-free preparation of an indole derivative by the use of polyethylene glycol	14
Scheme 11: Synthesis of an indole derivative by the use of PEG-600.....	15
Scheme 12: Catalyst-free preparation of 3-sulfenylindoles.....	15
Scheme 13: Synthesis of an indole derivative using a solid acid catalyst .....	16
Scheme 14: Heteropoly acid catalyst-based preparation of 3-substituted indole derivatives.....	16
Scheme 15: Preparation of indole derivatives using MgO nanoparticles .....	17
Scheme 16: Preparation of indole derivatives using Fe <sub>3</sub> O <sub>4</sub> nanoparticles .....	17
Scheme 17: Synthesis of thienindoles using microwave irradiation .....	17
Scheme 18: Preparation of indole derivatives by microwave irradiation .....	18
Scheme 19: Preparation of substituted benzindoles using microwave irradiation .....	18
Scheme 20: Synthesis of indole derivatives by cycloisomerization using MWI.....	18
Scheme 21: The initial preparation of 1,3,4-oxadiazole through thermolysis.....	42
Scheme 22: Possible methods for preparations of 1,3,4-oxadiazole derivatives.....	43
Scheme 23: Reagents and conditions: (a) Methanol, H <sub>2</sub> SO <sub>4</sub> (99.8%), reflux at 80 °C, 8h; (b) NH <sub>2</sub> NH <sub>2</sub> .H <sub>2</sub> O (80%), methanol, stir at RT, 14h; (c) POCl <sub>3</sub> , reflux at 60-80 °C, 9-10h. ....	45
Scheme 24: Chemical reactions for Synthesis of compound 106a' .....	46
Scheme 25: Chemical reactions for Synthesis of compound 106b' .....	46
Scheme 26: Chemical reactions for Synthesis of compound 107a' .....	47
Scheme 27: Chemical reactions for Synthesis of compound 107b' .....	48
Scheme 28: Chemical reactions for Synthesis of compound 110a' .....	48
Scheme 29: Chemical reactions for Synthesis of compound 110b' .....	49
Scheme 30: Chemical reactions for Synthesis of compound 111a' .....	50
Scheme 31: Chemical reactions for Synthesis of compound 111b' .....	50

## List of Appendices

Appendix	Page
Appendix 1: Interpolated regression graphs for synthesized compounds (107a', 107b', 110a', 110b', 111a' and 111b') and standard ascorbic acid for calculating its IC <sub>50</sub> value .....	85
Appendix 2: UV-Vis Absorption Spectrum of compound 106a' .....	87
Appendix 3: FT-IR Absorption Spectrum of compound 106a' .....	87
Appendix 4: UV-Vis Absorption Spectrum of compound 106b' .....	88
Appendix 5: FT-IR Absorption Spectrum of compound 106b' .....	88
Appendix 6: UV-Vis Absorption Spectrum of compound 107a' .....	89
Appendix 7: FT-IR Absorption Spectrum of compound 107a' .....	89
Appendix 8: UV-VIS Absorption Spectrum of compound 107b' .....	90
Appendix 9: FT-IR Absorption Spectrum of compound 107b' .....	90
Appendix 10: UV-Vis Absorption Spectrum of compound 110a' .....	91
Appendix 11: FT-IR Absorption Spectrum of compound 110a' .....	91
Appendix 12: UV-Vis Absorption Spectrum of Compound 110b' .....	92
Appendix 13: FT-IR Absorption Spectrum of Compound 110b' .....	92
Appendix 14: UV-Vis Absorption Spectrum of Compound 111a' .....	93
Appendix 15: FT-IR Absorption Spectrum of Compound 111a' .....	93
Appendix 16: UV-Vis Absorption Spectrum of Compound 111b' .....	94
Appendix 17: FT-IR Absorption Spectrum of Compound 111b' .....	94
Appendix 18: Zone of inhibition on the grown bacteria on the prepared culture media on petri-dish by picture .....	95
Appendix 19: The diameter (mm) of the Zone of inhibition of synthesized compounds was measured against selected bacterial strains .....	97
Appendix 20: Mean inhibition zone of synthesized compounds in mm (mean ± SD) at 150 mg/mL .....	98

## Abstract

Indole-containing oxadiazole compounds have emerged as a versatile class of nitrogen-containing heterocyclic compounds with a wide range of biological activities. These compounds and their derivatives exhibit a diverse array of properties such as antiviral, anticancer, anti-HIV, antioxidant, antimicrobial, antidiabetic, and antimalarial activities. The aim of the study was to design and synthesize some novel hybrid molecules containing an indole and oxadiazole rings, followed by partial characterization of the synthesized compounds using UV-vis and FT-IR spectroscopic techniques, and assessment of their antibacterial and antioxidant activities. The synthetic methodology was initiated by esterification of 2-(1*H*-indol-3-yl) acetic acid (105a') and 4-(1*H*-indol-3-yl) butanoic acid (105b') using a catalytic amount of sulfuric acid and excess methanol. This process resulted in the formation of methyl 2-(1*H*-indol-3-yl)acetate (106a') and methyl 4-(1*H*-indole-3-yl)butanoate (106b') respectively. These esters were further converted to 2-(1*H*-indol-3-yl)acetohydrazide (107a') and 4-(1*H*-indole-3-yl)butaneydrazide (107b') respectively. Finally, the compounds (107a') and (107b') underwent a reaction with substituted carboxylic acids in the presence of POCl<sub>3</sub> to produce the desired targeted compounds: 110a', 111a', 110b', and 111b'. The synthesized compound yields were as follows: 110a' (62%), 111a' (55%), 110b' (57%), and 111b' (53%). The chemical structures of these synthesized compounds were partially characterized by using spectroscopic techniques like UV-Vis and FT-IR. The synthesized compounds were evaluated for their *in vitro* antibacterial activity test against four bacterial strains: *E. coli*, *P. aeruginosa*, *S. pyogenes* and *S. aureus* by the disk diffusion method. Among synthesized compounds, compound 111b' showed potent inhibitory activity against Gram-negative, *E. coli* with  $11.57 \pm 0.15$  mm zone of inhibition compared to the standard drug tetracycline ( $13 \pm 0.5$  mm) at 150 mg/mL. The RSA of synthesized compounds were assessed through DPPH radical assay, revealing that compounds 111a' and 110a' demonstrated higher %RSA (91.83% and 89.9%, respectively) with IC<sub>50</sub> values of 4.95 and 5.03, respectively, compared to the standard ascorbic acid of %RSA (87.5%) with an IC<sub>50</sub> of 31.44 and than other synthesized compounds. Hence, the studies have indicated that all the synthesized compounds could be considered as potential candidates for further investigation in antibacterial and antioxidant research.

**Key words:** Antibacterial, Anticancer, Antioxidant, Antiviral, Design, Indole, Synthesis

# CHAPTER ONE

## 1. INTRODUCTION

### 1.1. Background of the study

Microbial infections are a growing concern worldwide, particularly in developing countries, where they are a leading cause of morbidity and mortality. This resistance is attributed to the emergence and spread of antimicrobial and antioxidant drug resistance, which has made it increasingly difficult to treat these infections effectively [1]. Microbial resistance is a significant concern in healthcare and public health due to its potential to cause treatment failure, prolonged illness, and increased healthcare costs. Moreover, these antimicrobial agents can also pose a threat to the effectiveness of existing antimicrobial drugs and their ability to control infectious diseases [2]. According to a study published in *The Lancet Infectious Diseases*, the prevalence of antimicrobial resistance is on the rise, and 10 million deaths are projected to occur annually by 2050 if not addressed [3]. Another study published in the *Journal of Global Antimicrobial Resistance* showed that the misuse and overuse of antibiotics in both humans and animals contribute to the development of antimicrobial resistance [4]. Additionally, the World Health Organization has highlighted the urgent need for global action to combat antimicrobial resistance, as this action poses a significant threat to public health and can undermine the progress made in modern medicine [5]. Therefore, it is crucial to implement effective strategies to prevent and control microbial infections and reduce the development of antimicrobial resistance [6].

Heterocyclic compounds are an important class of organic compounds that contain at least one heteroatom, such as nitrogen, oxygen, or sulfur, in their ring structure. These compounds have a wide range of applications in the fields of pharmaceuticals, agrochemicals, materials science, and organic electronics [7]. Heterocyclic compounds containing nitrogen are known to play a very important role in the field of medicinal chemistry. The key field of medicinal chemistry is the investigation of heterocycles as favored structures in drug discovery [8, 9].

Indole is an aromatic heterocyclic compound with unique reactivity. It consists of a benzopyrrole fused at the 2- and 3-positions of the pyrrole ring. Indole can be deprotonated at the nitrogen end, forming salts that are good nucleophiles. It is found in many natural products, such as fungal metabolites, indole alkaloids, and marine natural products, and highly ionic salts (e.g.,  $\text{Li}^+$  and  $\text{K}^+$ ) favor N-substitutions, while softer counterions favor C-3 substitution [10]. The indole ring

system represents one of the most abundant and important heterocycles in nature [11]. Indoles are found in various natural products and have been identified as compounds of chemical and biological importance. Indole is a highly conserved heterocyclic molecule that acts as a free radical scavenger and has a broad spectrum of antioxidant activity [12].

The presence of indole motifs in proteins, amino acids, bioactive alkaloids, and drugs highlights the importance of heterocyclic compounds in biological systems. This finding suggested that heterocyclic compounds may have a natural advantage in interacting with biological targets, making them promising candidates for drug discovery and development. Furthermore, the versatility of indole and other heterocyclic compounds containing an indole moiety in the synthesis of bioactive drugs underscores their potential as valuable tools in medicinal chemistry [13]. Several studies have reported the potential of indole-containing compounds as anticancer agents. For instance, 5-fluoroindole, a synthetic indole derivative, has shown promising results in inhibiting the growth of cancer cells [14]. Similarly, indole-3-carbinol, a natural compound found in cruciferous vegetables, has been found to possess anticancer effects by modulating estrogen metabolism and inducing apoptosis in cancer cells [15]. In addition to their anticancer potential, indole-containing compounds have also been investigated for their antimicrobial activity. For example, indole derivatives have been reported to exhibit antibacterial activity against a range of gram-positive and gram-negative bacteria [16].

Indole derivatives are a class of organic compounds with diverse therapeutic potential that are found in natural sources such as plants, fungi, and bacteria. They have gained attention in medicinal chemistry due to their broad range of pharmacological activities, including anticancer, anti-inflammatory, and antifungal properties [17]. 3-Substituted indole derivatives exhibit various pharmacological effects, such as antimicrobial [18], anti-inflammatory [19], antitumor [20], anticancer [21], antiviral [22], and anti-HIV [23] activities.

Heterocyclic molecules called oxadiazoles have two nitrogen atoms and one oxygen atom arranged in a five-membered ring, and they have a variety of beneficial biological benefits [24]. Because it can form hydrogen bonds, is lipophilic, and inhibits a variety of enzymes, 1,3,4-oxadiazole is an essential scaffold. Compounds containing 1,3,4-oxadiazole have a wide range of biological activities, including anticancer effects, because of these special characteristics [25]. Additionally, oxadiazole derivatives have shown potent antimicrobial activity against various bacterial and fungal strains. Literature report showed that, oxadiazole derivatives have shown

promising antimicrobial activity against both gram-positive and gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Pseudomonas aeruginosa* [26]. Another study, reported that oxadiazole derivatives demonstrated potent antifungal activity against *Candida albicans* and *Aspergillus fumigatus* [27]. These findings suggest that oxadiazole derivatives could be developed as effective antimicrobial agents for combating antibiotic-resistant bacterial and fungal infections.

Oxadiazole and indole-containing substances exhibit diverse biological roles [28]. Moreover, the presence of oxadiazole and indole moieties in several natural products has led to their investigation as potential sources of new drugs. Overall, the diverse biological activities exhibited by oxadiazole- and indole-containing compounds highlight their potential as valuable scaffolds for drug discovery and development. Several alkaloids containing oxadiazole and indole rings have been isolated from plants and marine organisms and have shown promising pharmacological activities [29]. In addition to their potential as drug scaffolds, oxadiazole- and indole-containing compounds have also shown promise in other areas of research. For example, a study revealed that indole compounds derived from cruciferous vegetables may have anti-inflammatory effects [30]. Another study, reported the development of indole-based inhibitors for the treatment of cancer [31]. Therefore, the linked molecules of 3-substituted indole and oxadiazole frame structures, indole-based oxadiazoles, are useful physiologically active agents. Indole-substituted 1,3,4-oxadiazoles exhibit a broad spectrum of biological activities, including anticancer activity [32]. The combination of indole and oxadiazole moieties in a single molecular entity could lead to the development of new drugs with enhanced biological activity against multidrug-resistant microbial infections [33]. This is because the incorporation of multiple pharmacophores can lead to synergistic effects, resulting in improved efficacy and potency. Additionally, the use of indole derivatives in combination with other pharmacophores has been shown to improve pharmacokinetic properties, such as bioavailability and metabolic stability, which can further enhance their therapeutic potential. Therefore, this approach represents a promising avenue for the discovery of novel antimicrobial agents [34]. The purpose of this work was to examine the antibacterial and antioxidant activities of synthesized hybrid molecules containing indole and oxadiazole rings.

## 1.2. Problem statement

Microbial infections are causing increased illness and death in developing countries due to increasing resistance to antibiotics. This is a significant global health issue, as many clinically important microorganisms are becoming resistant to multiple antibiotics. An increase in antibiotic resistance in developing countries is leading to increased illness and death, emphasizing the need for new antibacterial agents. Effective antibacterial and antioxidant agents are crucial for treating infectious diseases as pathogens continue to mutate. The burden of antibiotic-resistant bacteria on the human population highlights the importance of developing new treatments. The development of new antibacterial agents is essential for combating the increasing threat of antibiotic resistance in developing countries. With pathogens constantly mutating and evolving, effective antibacterial and antioxidant compounds are crucial for treating infectious diseases. The burden of antibiotic resistance on the human population cannot be ignored, and it is imperative that new treatments are developed to prevent the transmission of infections. As current antibacterial drugs lose their potential and effectiveness due to overuse or misuse, the urgency to create new products has never increased. Failure to address this issue could have devastating consequences for global public health.

To maintain the constantly evolving resistance of pathogens, there is a growing need for the development of new broad-spectrum antimicrobial agents. This study has focused on synthesizing nitrogen-containing heterocycles, which serve as pharmacophoric fragments or synthetically active organic compounds. However, investigations into the specific mechanism of action and mode of binding of the synthesized hybrid molecules of indole containing oxadiazole ring with microbial and oxidant targets are lacking. While studies aim to evaluate the antibacterial and antioxidant activities of the synthesized novel compounds, further research is needed to understand how these compounds interact with microbial cells and how they can be optimized for improved efficacy. Currently, the increasing resistance of pathogens to antibacterial compounds is a common and serious problem. Therefore, it is necessary to carry out research on new compounds that possess good activity against pathogens from the compounds that bear indole derivatives.

### 1.3. General Objective

The overall objective of this study was to design and synthesize some novel hybrid molecules containing an indole and oxadiazole rings for the evaluation of their antibacterial and antioxidant activities.

#### 1.3.1. Specific Objectives

- To design some novel hybrid molecules containing an indole and oxadiazole rings.
- To synthesize the designed hybrid molecules using appropriate synthetic methodologies.
- To elucidate the structure of the synthesized compounds using some spectroscopic techniques, such as UV-Vis, and FT-IR.
- To evaluate the *in vitro* antibacterial activity of the synthesized compounds against four bacterial strains: *E. coli*, *P. aeruginosa*, *S. pyogenes* and *S. aureus*
- To assess *in vitro* antioxidant activity of the synthesized compounds using DPPH free radical assay.

### 1.4. Significance of the study

- ✚ The significance of this study lies in the potential development of some novel antibacterial and antioxidant agents.
- ✚ The design and synthesis of hybrid molecules containing an oxadiazole rings could lead to the discovery of new compounds with enhanced antibacterial and antioxidant activity.
- ✚ This approach is particularly important in the face of increasing antibiotic resistance, where there is a critical need for the development of new antibacterial agents.
- ✚ The evaluation of the antibacterial and antioxidant activity of these novel hybrid molecules could provide valuable insights into their potential as effective antibacterial and antioxidant agents, which could ultimately contribute to the development of new treatments for infectious diseases.
- ✚ This study could also contribute to the advancement of the field of medicinal chemistry by expanding the knowledge of chemical structures that exhibit antibacterial and antioxidant activities.
- ✚ The results of this study provide information about synthetic strategies for accessing biologically active indole derivatives.
- ✚ The study would be an initiative for researchers who conduct their studies in the field of synthetic and medicinal chemistry.

- ✚ Recently, synthesizing and characterizing compounds from privileged structures have been new approaches and have become very important strategies for finding new drug molecules.
- ✚ This study involved design and synthesize some novel hybrid molecules containing an oxadiazole rings for the evaluation of their antibacterial and antioxidant activities, which could be crucial for obtaining new and useful compounds.

## CHAPTER TWO

### 2. LITERATURE REVIEW

#### 2.1. Chemistry of indoles

Indole is an aromatic heterocyclic organic compound with a bicyclic structure consisting of a six-membered benzene ring fused to the 2,3 position of the five-membered nitrogen-containing pyrrole ring, as indicated in Figure 1 [35].

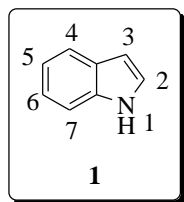
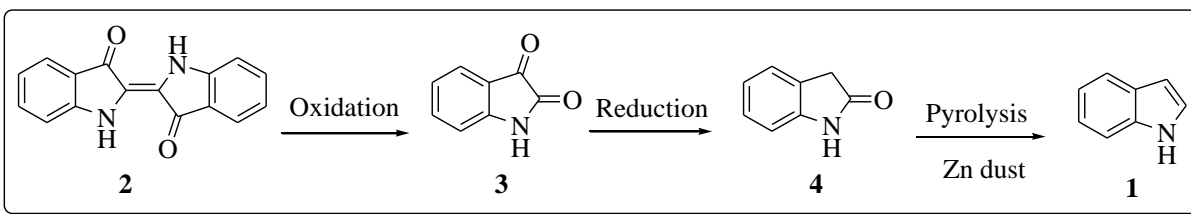


Figure 1: Chemical structure of indole

The word “indole” is derived from the word “indigo”, as the heterocycle was first isolated from the blue dye “indigo”, which was produced in India during the sixteenth century. In 1866, Adolf von Baeyer isolated Indole (**1**) by the pyrolysis of oxindole (**4**) with Zn dust. Oxindole (**4**) was originally obtained by the reduction of isatin (**3**), which in turn was isolated by the oxidation of Indigo (**2**) in Scheme 1 [36].

Scheme 1: Discovery of indole by Adolf von Baeyer



Compound **1** is also known as benzo[*b*]pyrrole and contains a benzenoid nucleus and has 10  $\pi$ -electrons (two lone pairs on nitrogen and two bonds providing eight electrons), which make them aromatic in nature [37]. Chemically, compound **1** is very weakly basic. This is due to the delocalization of the nitrogen lone pair into the  $\pi$ -electronic system, which is free to circulate throughout the indole ring. Therefore, the lone pair of electrons on nitrogen is not available for protonation and becomes protonated at C-3 because this position, due to the retention of aromaticity, is thermodynamically more stable [38]. Therefore, it is involved in various chemical reactions, e.g., cycloaddition [39], carbon lithiation [40], oxidation [41], electrophilic substitution [42], and organometallic indole anion complexes [43]; additionally, it occurs

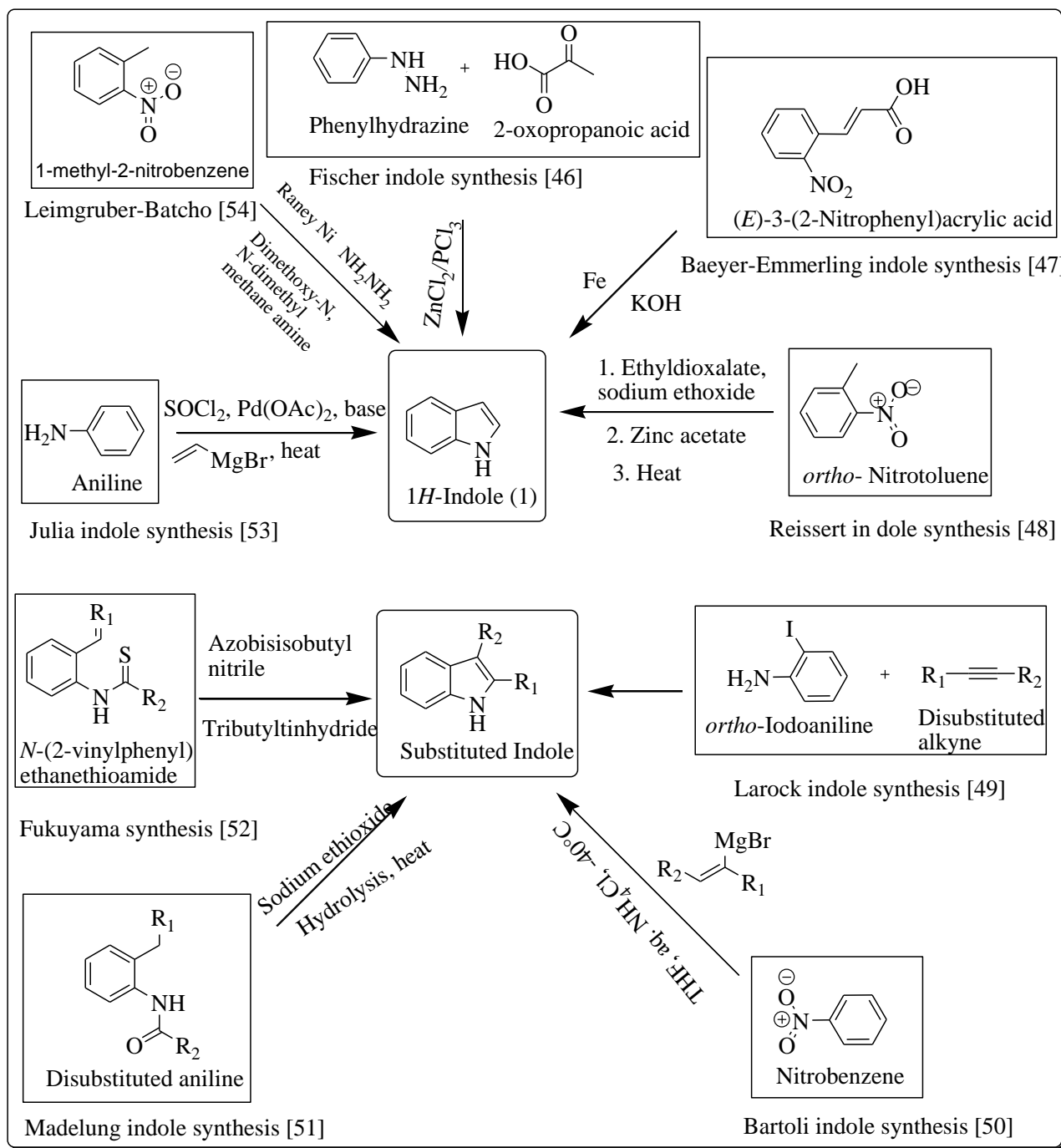
particularly at the C-3 position. Compound **1** is a white crystalline solid at room temperature and has a melting point of 52-54°C. It is slightly soluble in water but highly soluble in many organic solvents, such as ethanol and acetone [44]. Compound **1** exists naturally in the feces of human beings and has a peculiar smell. However, at lower concentrations, it has a flowery smell and is the main component of flower scents, coal tar, and perfumes [38]. In addition, compound **1** is associated with numerous biological reactions in the human body. It regulates various aspects of bacterial physiology, including spore formation, virulence, and resistance to drugs, biofilm formation and plasmid stability [45].

## **2.2. Methods for synthesizing the indole ring and its derivatives**

### **2.2.1. Conventional Methods**

A number of methods for the conventional synthesis of the indole nucleus and its derivatives have been described in the literature. The method involves a number of starting materials and different strategies, as mentioned in Scheme 2, which include Fischer indole synthesis [46], Baeyer-Emmerling indole synthesis [47], Reissert indole synthesis [48], Larock indole synthesis [49], Bartoli indole synthesis [50], Madelung indole synthesis [51], Fukuyama synthesis [52], Julia indole synthesis [53], and Leimgruber-Batcho synthesis [54].

Scheme 2: Synthesis of indole and its derivatives by conventional methods



### 2.2.2. Novel Methods

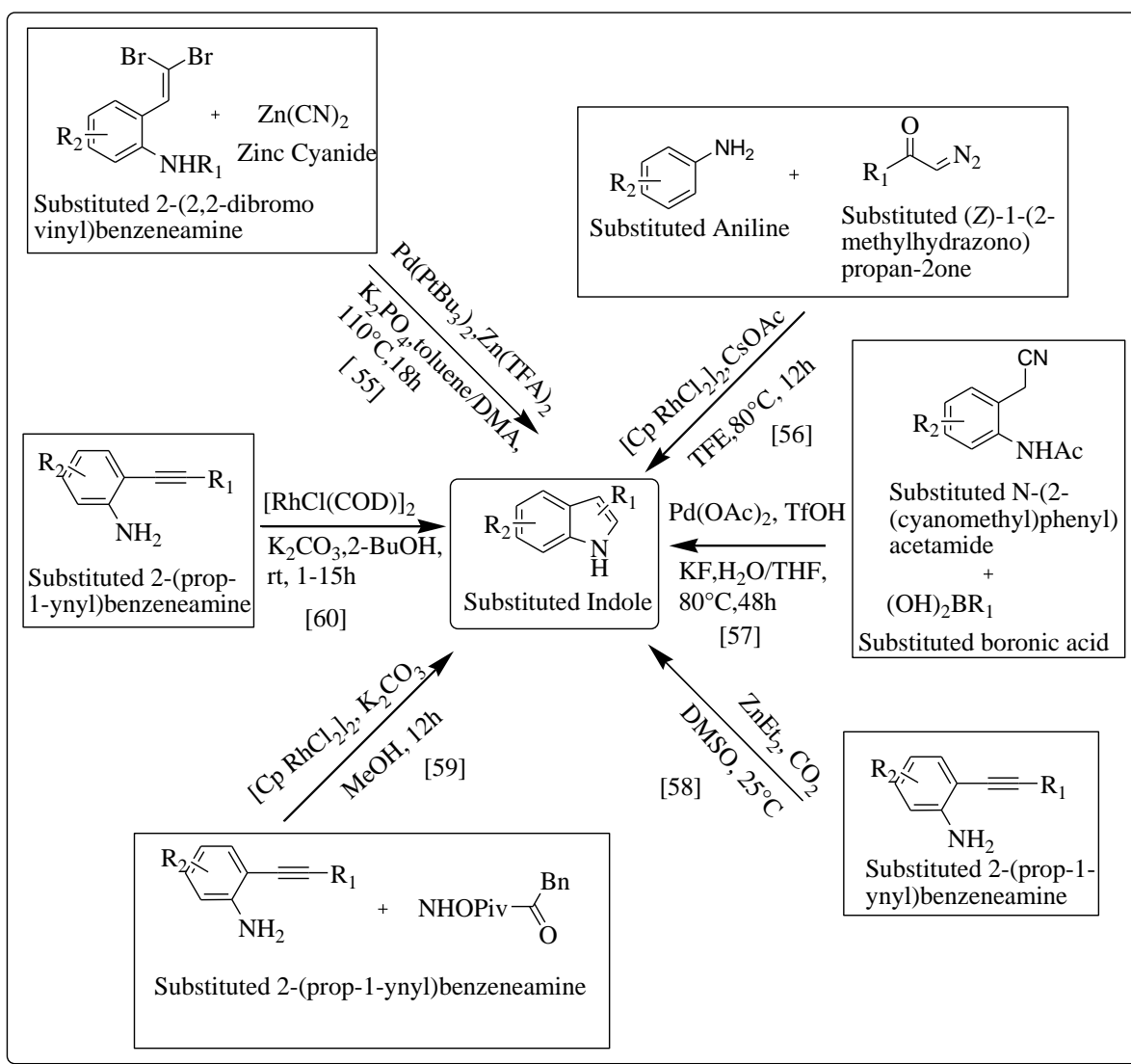
Recently, several novel methods using various effective catalysts have been explored, and the results are discussed and presented in Schemes 3. Zeidan *et al.* recently published a study on the conversion of 2-gem-dihalovinylanilines into substituted indoles using palladium catalysis. To

prolong the catalytic activity,  $\text{Zn}(\text{TFA})_2$  was added, and  $\text{Zn}(\text{CN})_2$  was used as a source of cyanide. The concentration of cyanide was maintained throughout the reaction by the solubility of  $\text{Zn}(\text{CN})_2$  in two solvent mixtures. This reaction can lead to the production of cyanoindole derivatives, which are found in various plant sources and pharmaceutical drugs [55]. Shi *et al.* utilized CeH activation in a rhodium-catalyzed coupling reaction of butyl oxycarbonyl hydrazones with diazodiester. This was achieved under mild temperature conditions using TFA as a reaction medium, resulting in a yield of approximately 45% [56]. In 2017, Yu *et al.* presented a novel approach for the synthesis of organic compounds involving the use of palladium as a catalyst in the reaction between aminoarylacetonitriles and arylboronic acid. The reaction proceeded via nucleophilic addition and intramolecular cyclization, resulting in good compatibility with halogens and potential for derivatization. This method expands the possibilities for the synthesis of diverse organic compounds and offers a promising avenue for future research in the field [57].

In 2016, Miao *et al.* introduced a novel approach for the direct synthesis of bioactive indole derivatives using alkynylanilines and diethyl zinc under 1 atm of  $\text{CO}_2$ . This method offers several advantages, including the availability of starting materials and the potential for creating a library of diverse compounds. Additionally, this technique has been successfully applied in the synthesis of the drug "Lotronex" for the treatment of irritable bowel syndrome, highlighting its potential for developing new therapeutics. Overall, this approach represents a promising avenue for future research in the field of organic synthesis [58]. In 2016, Hu *et al.* introduced an innovative approach for the synthesis of heterocyclic fused indole nuclei using a rhodium-catalyzed cascade cyclization with N-pivaloyloxylamides as the nitrogen source. This method offers several advantages, including mild temperature conditions and the potential for creating a diverse range of compounds. Furthermore, this technique has been successfully applied in the synthesis of various bioactive molecules, highlighting its potential for developing new therapeutics. Overall, this approach represents a significant breakthrough in the field of organic synthesis and holds great promise for future research [59]. Similarly, in 2016, Mizukami *et al.* reported the use of rhodium as a catalyst in the synthesis of heterocyclic fused indole nuclei and the use of a cyclization addition mechanism for the synthesis of compounds from ethylanilines and isocyanates, which are both innovative approaches that have significant potential for advancing the field of organic synthesis. These methods not only involve mild temperature

conditions and the ability to create diverse compounds but also have been successfully applied in the synthesis of bioactive molecules, highlighting their potential for developing new therapeutics. With their high yields and ability to work with substrates containing different functional groups, these techniques represent exciting breakthroughs in the field of organic chemistry and offer promising avenues for future research [60].

Scheme 3: Synthesis of indoles and their derivatives by novel methods



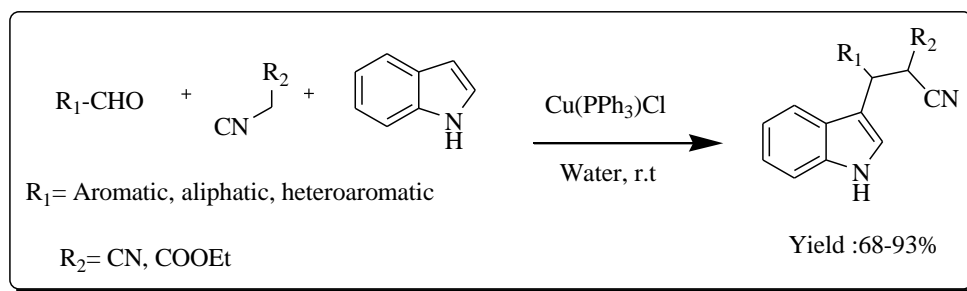
### 2.2.3. Green Methodologies

Green chemistry protocols offer various techniques for the preparation of indole derivatives. Some of these methods are outlined below.

### 2.2.3.1. Synthesis of Indole Derivatives Using Water as the Solvent

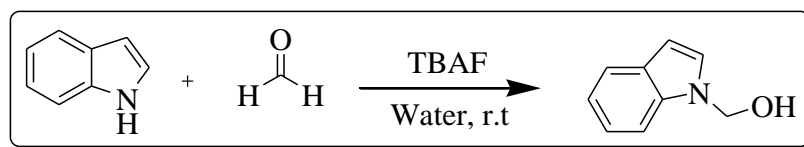
Indole derivatives can be synthesized using green chemistry protocols, such as water as the solvent. This approach minimizes the use of organic solvents and reduces the environmental impact. Several successful reactions have been carried out using this method. For example, A.N. Prasad *et al.* utilized a multicomponent reaction to prepare 3-substituted indoles. The reaction was conducted in water as a medium, with indole, aldehydes, and active methylene compounds in the presence of the catalyst  $\text{Cu}(\text{PPh}_3)\text{Cl}$ , as indicated in Scheme 4. This process resulted in the production of potentially important indole derivatives with high yields ranging from 68-93% [61]. The use of water as a solvent and the high efficiency of the catalyst make the process more cost-effective and environmentally friendly.

Scheme 4: Synthesis of an indole derivative using  $\text{Cu}(\text{PPh}_3)\text{Cl}$  in aqueous media



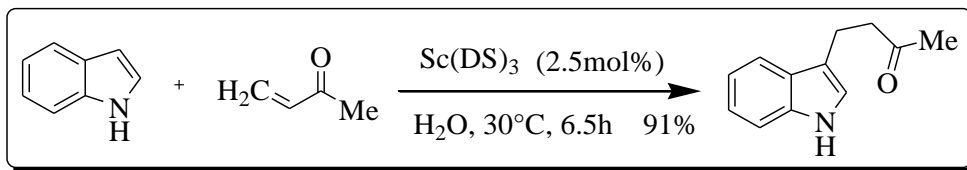
Similarly, H.M. Meshram *et al.* utilized a phase-transfer catalyst in water to prepare hemiaminal indole moieties with yields ranging from 84-96% [62]. The mild reaction conditions and reusability of the reaction media make these processes environmentally friendly and cost-effective. Scheme 5 provides a straightforward synthesis pathway for hemiaminal.

Scheme 5: Synthesis of hemiaminal indole in aqueous medium



Additionally, Kobayashi *et al.* used scandium tris(dodecylsulfate) [ $\text{Sc}(\text{DS})_3$ ] as a new catalyst for the reaction of indoles with electron-deficient enones for the preparation of indole derivatives in water, as indicated in Scheme 6. The reaction was successful with both solid and liquid olefins such as  $\beta$ -nitrostyrene and 3-butenone [63].

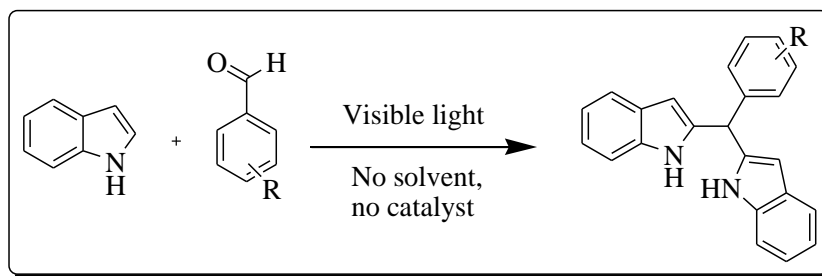
Scheme 6: Synthesis of 3-substituted indole using [Sc(DS)<sub>3</sub>] as a catalyst in water



### 2.2.3.2. Synthesis of Bioactive Indole Derivatives using Indole as a Precursor

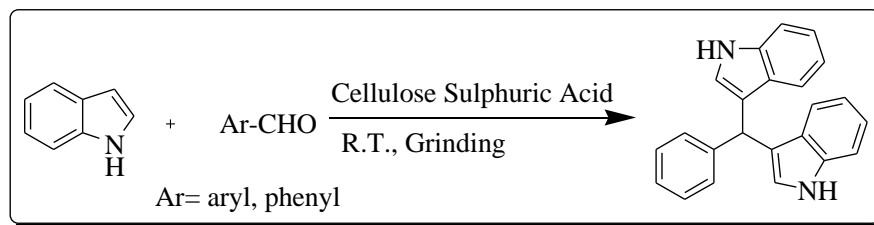
Green chemistry techniques that use water as a solvent have proven effective in synthesizing indole derivatives in high yields. B.S. Hote *et al.* successfully synthesized indole derivatives without the use of solvents or catalysts, achieving high yields (80-90%) in short time intervals (30-90 minutes) under visible light irradiation, as indicated in Scheme 7 [64].

Scheme 7: Preparation of bis(indolyl)methane derivatives under visible light



Additionally, S.A. Sadaphal *et al.* utilized cellulose sulfuric acid (CSA) as a catalyst to produce indole derivatives under the solvent-free conditions shown in Scheme 8 [65]. The nonhygroscopic solid nature of CSA allows for its reuse at least twice without any significant loss of catalytic activity, making this method environmentally friendly and cost-effective.

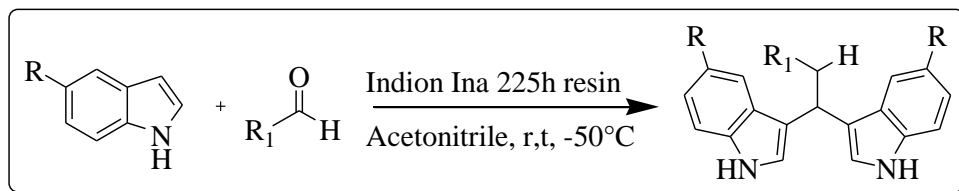
Scheme 8: Preparation of bis(indolyl)methane derivatives from solid cellulose sulfuric acid



In addition to the above mentioned green chemistry techniques for synthesizing indole derivatives using water as a solvent, R. Surasani *et al.* successfully synthesized certain indole derivatives using the Indion Ina 225 h resin catalyst indicated in Scheme 9 [66]. This method not only yields high product yields in less time but is also more selective for aldehydes than for ketones. Moreover, the catalyst can be reused up to five times, making the process economically

and environmentally friendly compared to other catalysts reported in the literature. Thus, the use of green chemistry techniques and catalysts is an effective approach for synthesizing indole derivatives with minimal environmental impact and cost.

Scheme 9: Indion Ina 225H resin-based preparation of bis (indolyl) methane

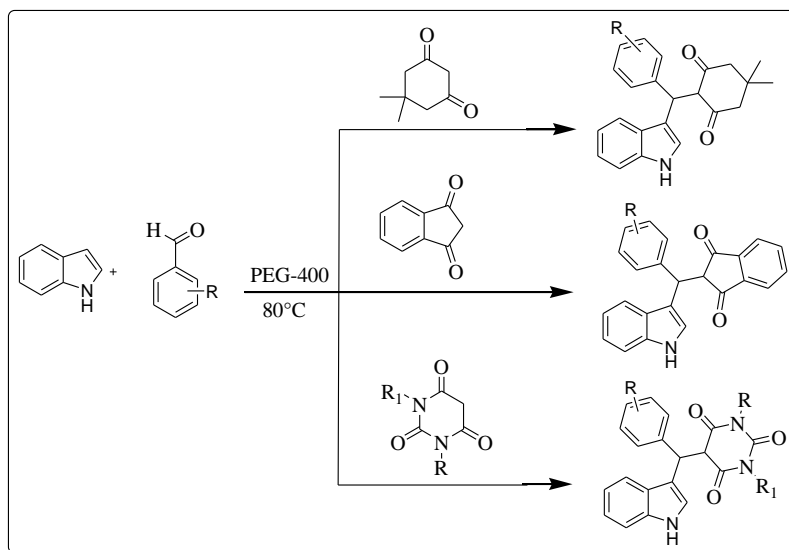


### 2.2.3.3. Preparation of Indole Derivatives (Catalyst-free Conditions)

Catalyst-free conditions for the preparation of indole derivatives have garnered significant attention due to their potential advantages, such as simplicity, cost-effectiveness, and environmental friendliness. This approach eliminates the need for expensive and potentially toxic catalysts, making the synthesis process more sustainable and accessible.

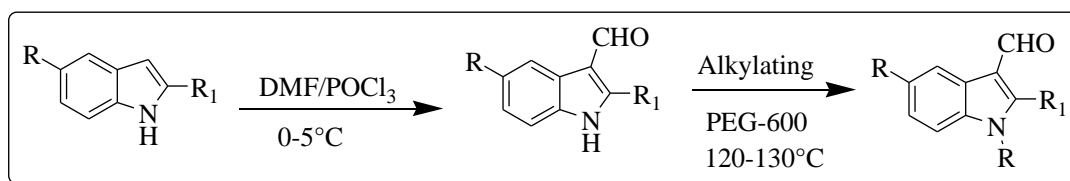
Indole derivatives can also be synthesized using a multicomponent reaction (MCR) process, as demonstrated by R. Kardooni *et al.* The MCR process involves the use of polyethylene glycol 400 as a reaction promoter and medium and C-H activated acids to prepare the desired indole derivatives. This method is catalyst free and selective for accessing heterodimeric products rather than for accessing homodimeric adducts such as bisindole/xanthine. The yield of 3-substituted indoles using this method is between 86 and 96%, and these reactions can be achieved in 35-80 minutes explained in Scheme 10 [67].

Scheme 10: Catalyst-free preparation of an indole derivative by the use of polyethylene glycol



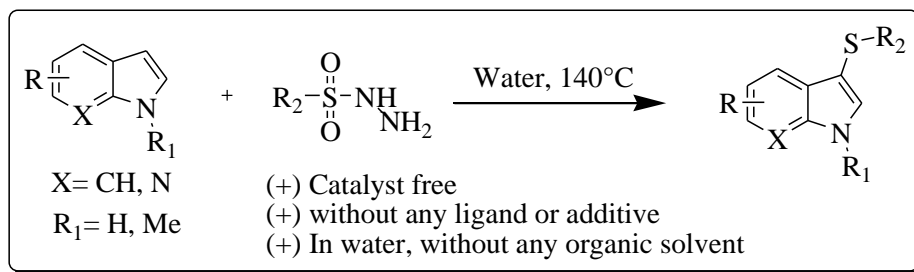
P.K. Dubey *et al.* devised an effective and eco-friendly approach for synthesizing indole derivatives, specifically N-alkyl/aralkyl and indole-3-carboxaldehydes, using polyethylene glycol (PEG-600). This low-cost and rapid methodology offers a sustainable alternative to traditional synthesis methods, making it an ideal choice for industries seeking to reduce their environmental impact explained in Scheme 11 [68].

Scheme 11: Synthesis of an indole derivative by the use of PEG-600



An alternative approach for synthesizing 3-sulfenylindoles was developed by Y. Yang *et al.*, which does not require the use of catalysts, ligands, or additives. This method involves thiolation of indoles using sulfonyl hydrazides to produce the desired products and is water-based. The simplicity and efficiency of this technique make it a promising alternative to traditional synthesis methods, particularly for industries seeking to reduce their dependence on expensive and environmentally damaging catalysts and solvents. A diagram outlining the steps involved in this innovative technique can be found in Scheme 12 [69].

Scheme 12: Catalyst-free preparation of 3-sulfenylindoles

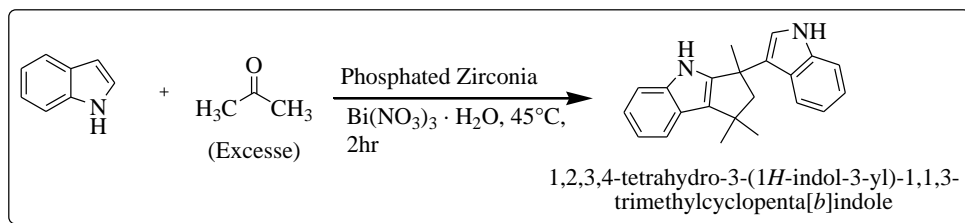


#### 2.2.3.4. Synthesis of Indole Derivates - Solid Acid Catalyst

Solid acid catalysts, such as zeolites and sulfated zirconia, are widely used in the synthesis of indole derivatives due to their high catalytic activity and selectivity. These compounds promote the cyclization of N-acyl or N-alkylated tryptamines or tryptophans to form the indole ring. Solid acid catalysts offer advantages over traditional methods, including improved stability and reusability; easy separation from the reaction mixture; and milder reaction conditions that improve yield and reduce side reactions. Various substituted cyclo[b]indoles were successfully

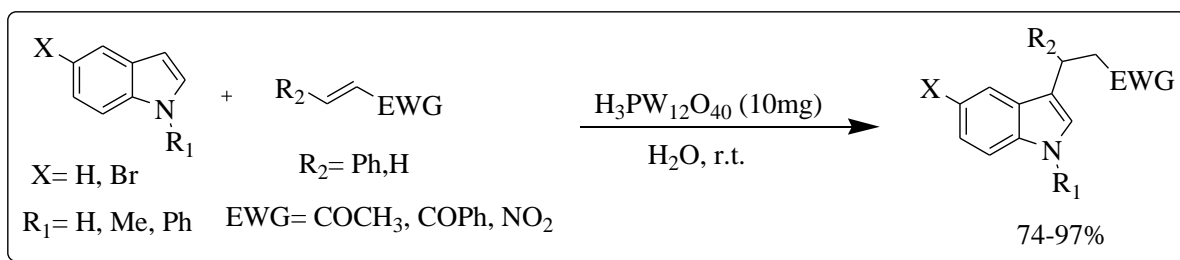
synthesized by S.V. Nadkarni *et al.* using a solid acid catalyst, namely, phosphate zirconia (P-Zr) and  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ , as demonstrated in Scheme 13 [70].

Scheme 13: Synthesis of an indole derivative using a solid acid catalyst



M.M. Heravi *et al.* was reported an alternative approach to the synthesis of 3-substituted indoles with improved properties. Their method involves the reaction between indole (or its derivative) and a Michael acceptor in the presence of a heteropoly acid (HPA) catalyst such as  $\text{H}_3\text{PW}_{12}\text{O}_{40}$ . The products are obtained via Friedel–Crafts alkylation at C3 of the indole or its derivative explained in Scheme 14 [71].

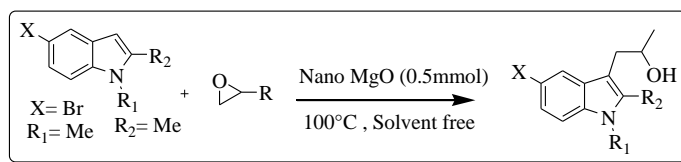
Scheme 14: Heteropoly acid catalyst-based preparation of 3-substituted indole derivatives



### 2.2.3.5. Synthesis of Indole Derivatives using Nanoparticles

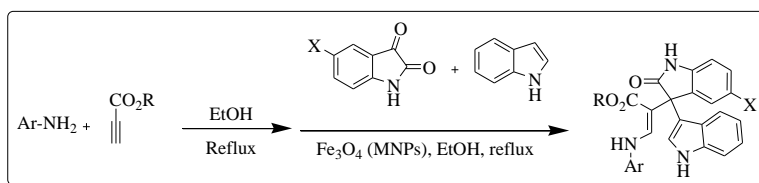
NPs are increasingly being used as catalysts in the synthesis of indole derivatives due to their unique properties, such as high surface area, reactivity, and tunable catalytic activity. Gold and palladium nanoparticles have been found to be particularly effective at catalyzing reactions with high selectivity and yield. NPs offer advantages over traditional catalysts, such as easy synthesis and functionalization, better control of reaction conditions, and easy separation for reuse in subsequent reactions. In a recent study, Hosseini-Sarvari *et al.* utilized nano-MgO as a catalyst for producing indole derivatives at the C-3 position through Friedel-Craft alkylation. The researchers found that under solvent-free conditions, treating indole with epoxides using magnesium oxide nanoparticles as a catalyst resulted in the production of bioactive indole derivatives. Magnesium oxide was found to be an eco-friendly catalyst in this process, catalyzing the ring opening of epoxide by indole (Scheme 15) [72].

Scheme 15: Preparation of indole derivatives using MgO nanoparticles



Another study conducted by H. Hajighasemi *et al.* utilized magnetic oxide (III) nanoparticles (Fe<sub>3</sub>O<sub>4</sub> MNPs) as a catalyst for synthesizing the 3-indol-3-yl-oxoindolin-3-yl-3-acrylates. This approach eliminates the need for hazardous solvents and instead employs highly efficient and recyclable catalysts, which can be easily separated from the reaction mixture. The use of Fe<sub>3</sub>O<sub>4</sub> MNPs allowed the production of bioactive indole derivatives with improved sustainability and reduced environmental impact indicated in Scheme 16 [73].

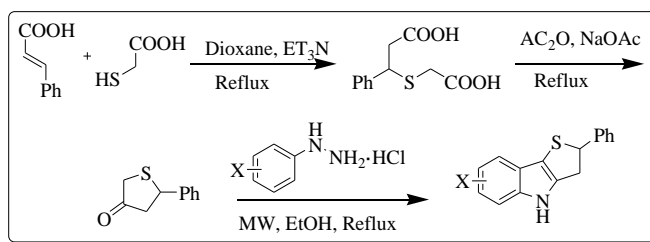
Scheme 16: Preparation of indole derivatives using Fe<sub>3</sub>O<sub>4</sub> nanoparticles



### 2.2.3.6. Microwave-assisted Synthesis

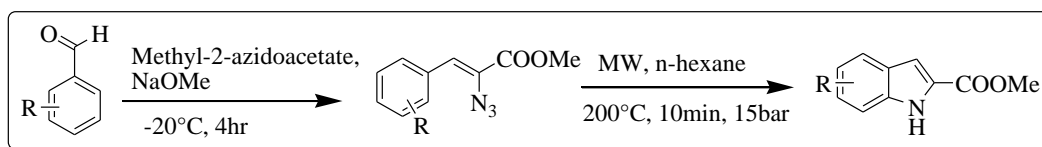
Microwave-assisted synthesis is an efficient and rapid technique for synthesizing indole derivatives. This approach allows for faster reaction rates and shorter reaction times, resulting in higher yields and improved efficiency. The use of microwave-assisted synthesis has been shown to be a highly efficient and rapid method for synthesizing indole derivatives. Researchers have successfully synthesized 2-aryl-3,4-dihydro-2H-thieno[3,2-b]indoles in excellent yields through this method. This approach involves the reaction of arylhydrazine hydrochloride with 5-aryldihydro-3(2H)-thiophenones, affording the desired products in yields ranging from 85-98%. This method is considered to be an effective and fast route for the regioselective synthesis of indole derivatives via the Fischer process (Scheme 17) [74].

Scheme 17: Synthesis of thienoindoles using microwave irradiation



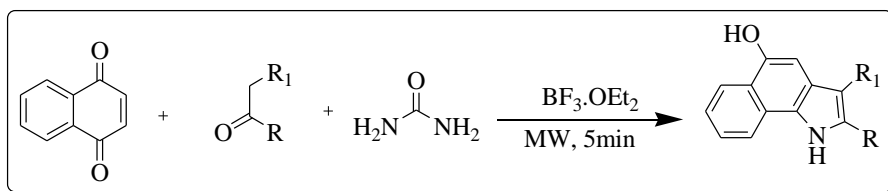
In a study by F. Lehmann *et al.*, Hemetsberger–Knittel indole synthesis was performed using microwave irradiation. The process involved the conversion of benzaldehyde or its derivatives to alpha azidocinnamates, which subsequently underwent ring closure to form various indole derivatives. This method proved to be a quick and simple approach for synthesizing different types of indole derivatives (Scheme 18) [75].

Scheme 18: Preparation of indole derivatives by microwave irradiation



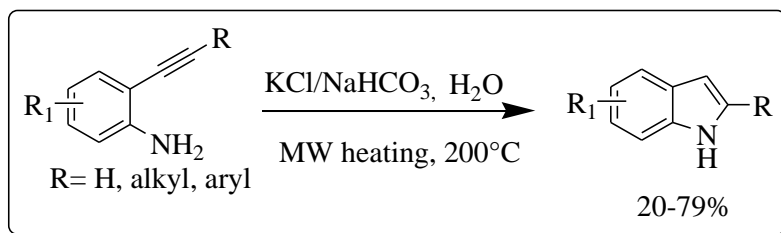
M. Borthakur *et al.* successfully achieved the one-pot synthesis of benzoindoles under microwave irradiation. They prepared benzoindoles by reacting omega-substituted acetophenones with naphthoquinone and urea (Nenitzescu reaction) under solvent-free conditions, eliminating the need for hazardous substances. The use of  $\text{BF}_3 \cdot \text{OEt}_2$  as the catalyst resulted in a high yield and enhanced reaction rates (Scheme 19) [76].

Scheme 19: Preparation of substituted benzoindoles using microwave irradiation



In a study conducted by A. Carpita *et al.*, a straightforward and efficient method for synthesizing indole derivatives was demonstrated using microwave irradiation. The desired product was obtained through the cycloisomerization of 2-alkynylanilines in the presence of inorganic salts, such as  $\text{KCl}/\text{NaHCO}_3$ , as catalysts and water as the reaction medium. This method offers a convenient approach for the preparation of indole derivatives (Scheme 20) [77].

Scheme 20: Synthesis of indole derivatives by cycloisomerization using MWI



### 2.3. Importance of indole and its derivatives

Indole is widely used in the synthesis of pharmaceuticals, agrochemicals, and other organic compounds due to its unique chemical and biological properties. It is also widely distributed in nature and is found in a variety of plants, including jasmine, orange blossoms, and tuberose, as well as in coal tar and certain animal feces [78, 79]. The chemical structure of indole gives it unique properties and makes it an important building block in the synthesis of various compounds. It is also a key component in the structure of many important biological molecules, including the amino acid tryptophan, the neurotransmitter serotonin, and various alkaloids and hormones [80]. Tryptophan (**5**), which contains indole, is an essential amino acid found in many natural resources. It is also a biosynthetic precursor of tryptamine, 5-hydroxy-tryptophan, and an immediate precursor of serotonin (Figure 2) [81]. Serotonin (**6**) is an important neurotransmitter associated with the transmission of nerve impulses. Furthermore, melatonin serves as a precursor to melatonin, a neuro-hormone secreted by the pineal gland. In addition, melatonin (**8**) is involved in several physiological processes beyond the circadian rhythm [82]. Indole-3-acetic acid (**7**) is the most common and widely studied plant hormone in the auxin class. It is a plant hormone produced by the degradation of tryptophan in higher plants (Figure 2) [83].

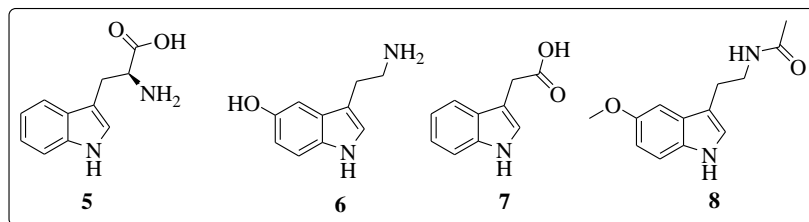


Figure 2: Indole scaffolds in biologically important compounds

In terms of chemical reactivity, indole is a versatile compound that can undergo a variety of reactions. It can be easily functionalized at nitrogen and carbon atoms, allowing for the synthesis of a wide range of derivatives with different properties and applications. For example, indole derivatives are used in pharmaceuticals, agrochemicals, dyes and they also have potential applications in materials science and organic electronics [84]. Indole and its derivatives are also of interest in the field of medicinal chemistry because they have been found to exhibit various biological activities, including anti-inflammatory, antioxidant, antimicrobial, and anticancer effects, making them potential candidates for the development of new drugs [85]. They have been extensively studied for their ability to interact with various cellular targets, such as enzymes, receptors, and DNA [86]. Several natural and synthetic indole derivatives have been

developed as drugs, such as the anticancer drugs vinblastine and vincristine, the antipsychotic drug clozapine, and the antidepressant drug fluoxetine [87].

#### **2.4. Indole in natural products**

Indole is a heterocyclic aromatic organic compound that is commonly found in natural products such as plants, bacteria, and marine organisms. It is known for its diverse biological activities and is a key structural component of many biologically active molecules. This lead compound is a universal constituent of pharmacologically active natural products. Indole alkaloids are common in various plant families, e.g., *Apocyanaceae*, *Loganiaceae*, *Rubiaceae* and *Nyssaceae* [88]. The indole core, which is a fundamental structure, found in many alkaloids, including auxins and tryptophan, is widely distributed in nature. Auxin, a plant growth hormone that is crucial for both cellular division and expansion, is derived from indole-3-acetic acid [89]. Tryptophan is an amino acid that is necessary for the synthesis of proteins, is not only a building block for biological compounds but also serves as a precursor for various important molecules. For instance, it is involved in the production of serotonin, a neurotransmitter that regulates mood and sleep, and melanin, a neurohormone that is responsible for skin pigmentation. Additionally, indole alkaloids, which are derived from tryptophan, have been found to have effects on both the central and peripheral nervous systems (Figure 3) [88].

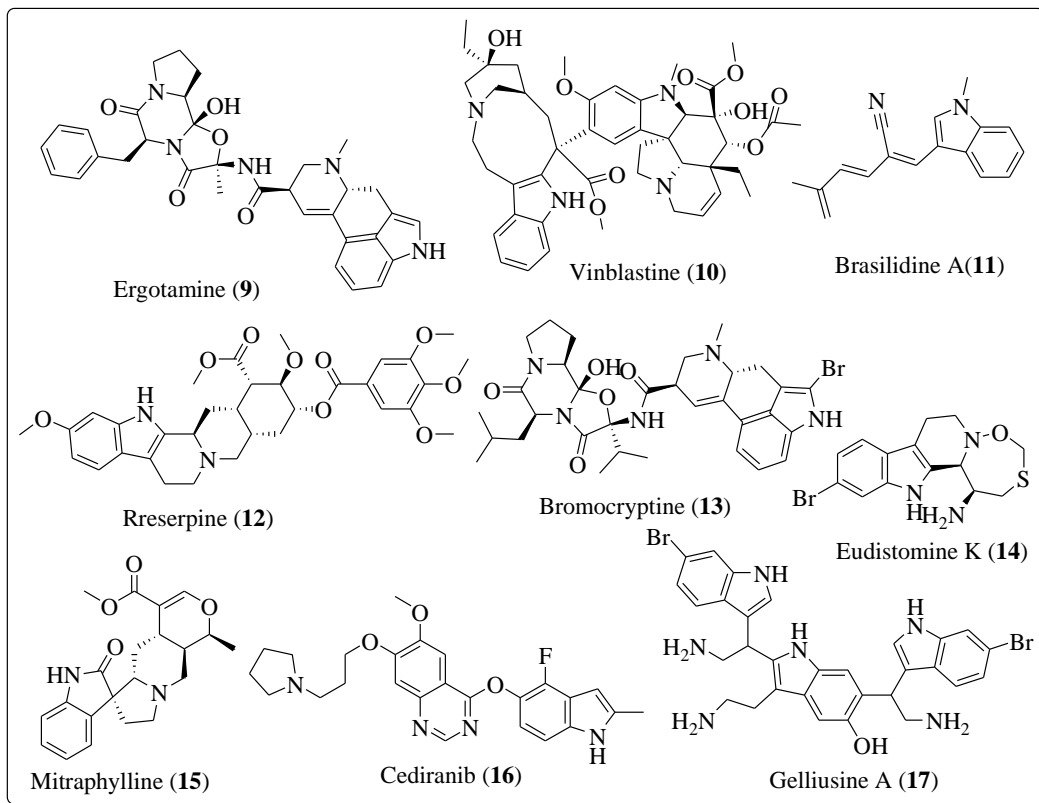


Figure 3: Indole alkaloids found in natural products

## 2.5. Indole in synthetic products

Indole is also widely used in the synthesis of various synthetic products due to its unique chemical properties. For example, indole derivatives are utilized in the production of dyes, pharmaceuticals, and agrochemicals. Indole-based dyes are used in the textile industry for coloring fabrics, while indole-containing pharmaceuticals are used for the treatment of various diseases, including cancer, depression, and inflammation. Indole derivatives are also used as plant growth regulators and insecticides in agriculture [90]. Moreover, indole has been extensively studied for its potential use in organic synthesis. It serves as a versatile building block for the construction of complex molecules due to its ability to undergo a variety of chemical reactions, such as electrophilic substitution, nucleophilic addition, and oxidation. Indole derivatives have been utilized in the synthesis of natural products, such as alkaloids and terpenoids, as well as in the development of new drugs and materials [91].

Numerous scientists have been inspired to create synthetic compounds containing indoles due to the pharmacological activity of various natural indole derivatives. Medicinal chemists are currently utilizing different heterocyclic moieties with indoles to develop compounds that can

combat a variety of illnesses. For instance, Desai *et al.* synthesized pyridine and oxadiazole derivatives of indole as antitubercular agents, while Shakuja *et al.* created bis spiroindole derivatives as antibacterial agents.

A wide range of indole derivatives are now accessible in commercial preparations [92, 93]. These efforts have resulted in the availability of a wide range of indole derivatives on the market, including pindolol **18** for hypertension [94], indapamide **19** for heart failure and hypertension [95], and delavirdine **20** for HIV-1 [96]. Other notable indole derivatives include indomethacin **21** [97], which is considered a promising anti-inflammatory and analgesic drug, and yohimbine **22**, which is effective against sexual dysfunction and reduces the risk of diabetes (Figure 4) [98].

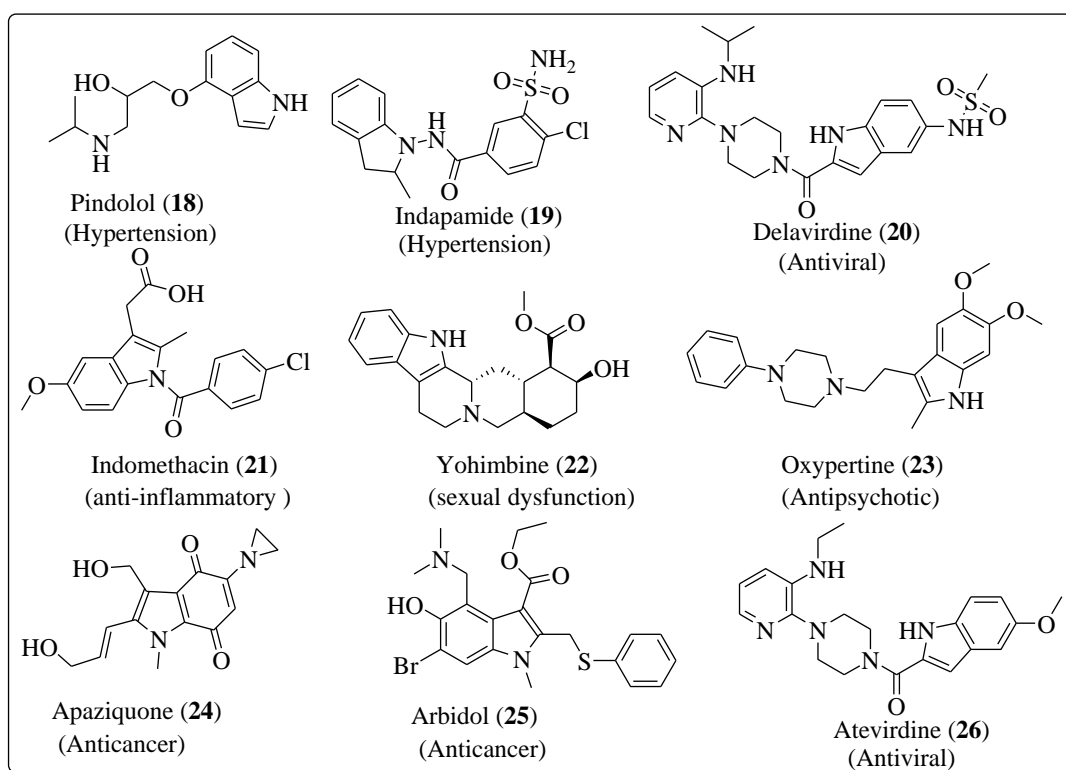


Figure 4: The Chemical structure of marketed indole derivatives

## 2.6. Pharmacological activity of indole derivatives

Due to the versatile nature of indole, it has gained increased popularity among organic and medicinal chemists. A number of drug molecules containing indole nuclei are involved in the treatment of various disease conditions, such as antimalarial, antitubercular, antifungal, anti-inflammatory, antibacterial, antioxidant, anticonvulsant, anti-allergic, enzyme inhibitor, herbicidal, anti-HIV, antidiabetic, anticancer, and insecticidal agents (Figure 5).

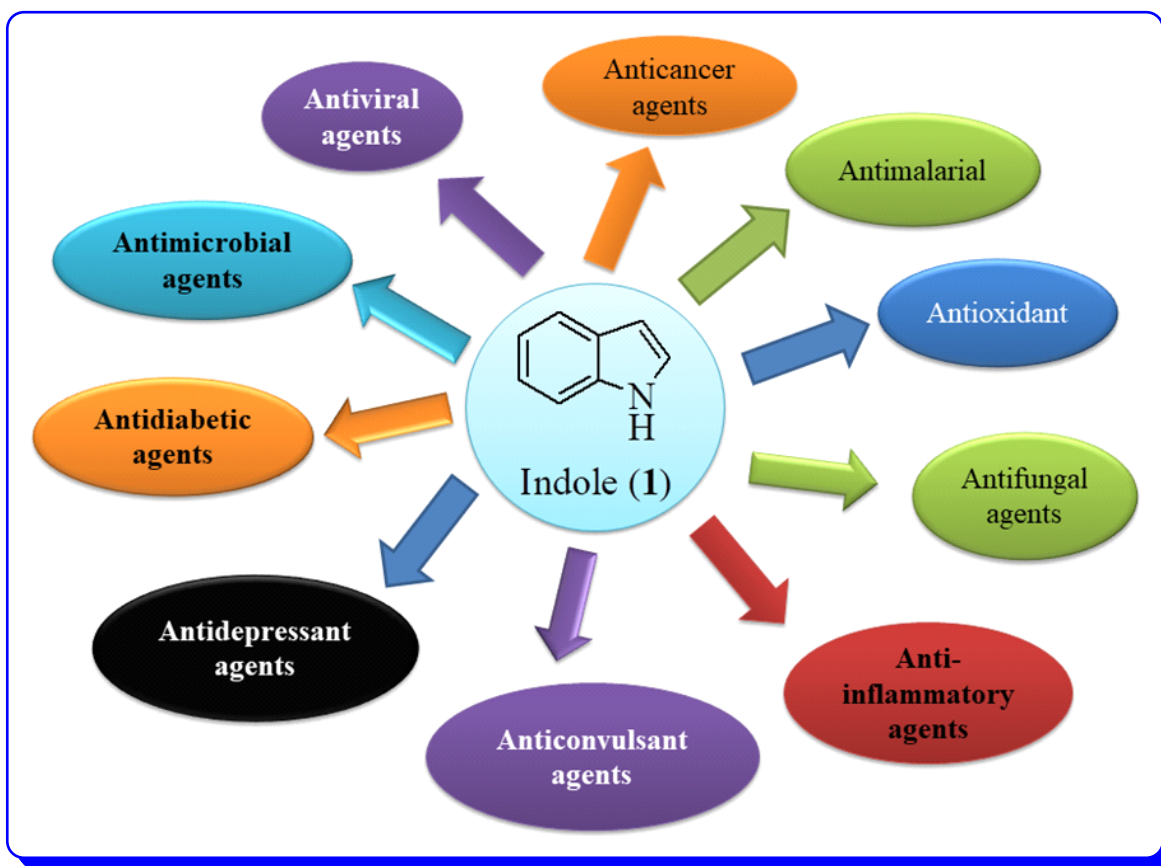


Figure 5: Pharmacological profile of the Indole scaffold

### 2.6.1. Antioxidant activities

Indole derivatives are utilized as pharmacological agents due to their diverse biological activities. Antioxidants play a crucial role in preventing cell damage caused by oxidation reactions and can be found in both natural chemicals in foods and body tissue, as well as in added products. These agents have been shown to have a positive impact on neurodegenerative diseases, e.g., Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [99]. Oxidation is a chemical reaction that generates free radicals, leading to cell damage through chain reactions. Antioxidants are molecules that inhibit the oxidation of other molecules, such as ascorbic acid, and can terminate these chain reactions and prevent cell damage. Antioxidants work by inhibiting these chain reactions, preventing cell damage. The term "antioxidant" refers to two distinct groups of substances: those added to products to prevent oxidation and natural chemicals found in foods and body tissue that have beneficial health effects [100].

In 2016, Orhan *et al.* synthesized and evaluated indole-based melatonin derivatives and reported that hydrazone derivatives of indole with *o*-halogenphenyl and 3,5-difluorophenyl substituents

were favorable for antioxidant activity. The synthesized derivatives were screened for ROS-induced DCFH-DA oxidation and were investigated for their ability to protect against  $\beta$ -amyloid-induced damage. Compounds **27a** ( $IC_{50} = 38.3 \pm 8.9 \mu M$ ) and **27b** ( $IC_{50} = 37.0 \pm 2.0 \mu M$ ) were found to be highly potent antioxidants and cytoprotective agents in neuronal and nonneuronal cells compared with the standard drug melatonin [101]. In 2013, Silveira *et al.* conducted a study on the antioxidant activity of C-3 sulfenyl indoles. The study concluded that the presence of a bis-indole system connected through a sulfide group at the C-3 position leads to increased potency. Substitution at the C-3 position leads to better stabilization of the indole ring and delocalization of electrons. The compounds (**28**) bis(indol-3-yl)sulfide and (**29**) bis(indol-3-yl)sulfone exhibited potent antioxidant activity. Compound **28** showed greater potency than the other compounds, with an activity of less than 96.8% [102].

Baytas *et al.* synthesized various triazole-substituted indole derivatives and tested them for DPPH and superoxide radical scavenging activities. Structure–activity relationship studies revealed that the unsubstituted 1,2,4-triazole-5(4H)-thione ring attached at the 2<sup>nd</sup> position of the indole is favorable for activity. Compounds **30a** and **30b** were found to be highly potent, with % inhibition of  $87 \pm 3$  and  $88 \pm 4$ , respectively, compared to the standard drug butylated hydroxytoluene (BHT) [103]. In the same year, Suzen *et al.* investigated the antioxidant activity of indole-based melatonin derivatives via LP inhibition and DPPH radical scavenging activities. Structure–activity relationship studies revealed that benzoylpyrrolidine is favorable for activity. Compound **31** showed better results [104]. Additionally, researchers have synthesized and assessed a range of aminomethyl-indole derivatives for their ability to scavenge superoxide radicals and inhibit lipid peroxidation activities. The results indicated that electron-withdrawing groups, as well as phenyl and pyrrole substituents, were advantageous for activity. Of all the synthesized derivatives, **32a** and **32b** exhibited high potency, with a percentage inhibition of 36–42%, compared to the standard drug butylated hydroxyl toluene [105].

Shirinzadeh *et al.* synthesized melatonin-based indole derivatives and tested them for their ability to reduce the oxidation of a redox-sensitive fluorescent probe, protect against  $H_2O_2$ , and inhibit AAPH. Their studies revealed that the presence of halogen substituents, particularly *o*- and *m*-halogens in the aromatic side chain, significantly enhanced the antioxidant activity of the compounds. Additionally, the substitution of a methyl group at the indole nitrogen and a halogenated side chain resulted in highly potent compounds. Among all the synthesized

derivatives, **33a** and **33b** were identified as the most promising compounds [106]. In the same year, Estevao *et al.* conducted a study in which they synthesized and assessed tryptophan and tryptamine derivatives of indole via a prenylation system. The scavenging activity of all the synthesized compounds was tested against superoxide, hydrogen peroxide, and hypochlorous acid radicals. Their research revealed that the presence of a carbomethoxy group, a free amine group in the side chain, and a free indolic nitrogen were beneficial for enhancing the activity of the compounds. Among all the synthesized derivatives, compounds **34** ( $IC_{50} = 4.13 \text{ } 0.17 \text{ mM}$ ) and **35** ( $IC_{50} = 4.56 \text{ } 0.48 \text{ mM}$ ) were identified as highly potent (Figure 6) [107].

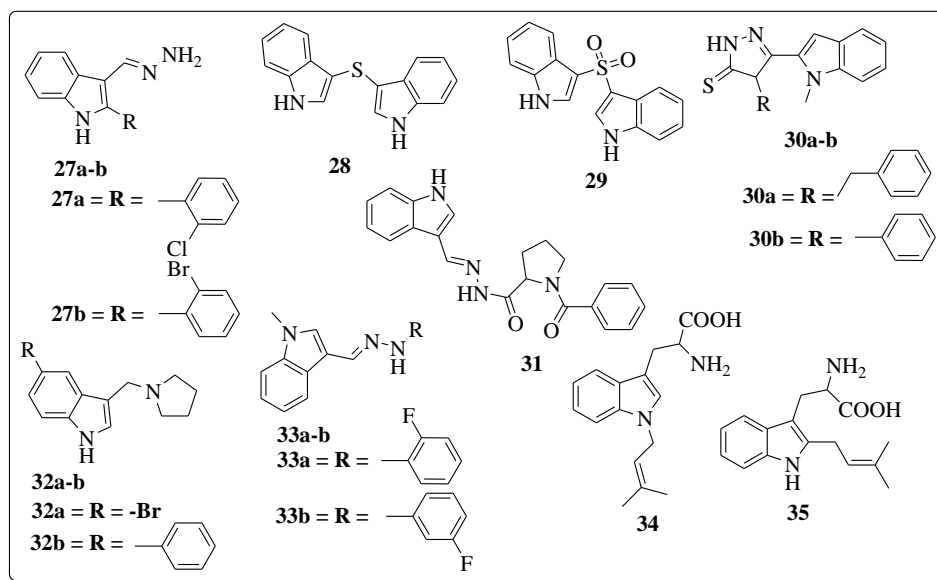


Figure 6: Chemical structure of indole derivative compounds that show antioxidant activity

### 2.6.2. Antifungal activities

Antifungal activity is the ability of a substance to inhibit the growth of fungal pathogens. Antifungal agents are substances or medications used to treat fungal infections and are most commonly located on the skin, hair, or nails. They work by either killing fungal cells by affecting the substances of the cell membrane, leading to leakage of cell components and cell death, or by preventing the growth and reproduction of fungal cells [108].

Indole derivatives have gained recognition for their effectiveness as antifungal agents in recent years. In 2020, Kumar *et al.* synthesized and tested 1H-Indole derivatives against *Aspergillus niger* and *Candida albicans* fungi. Compound **36**, with a zone of inhibition of  $16 \pm 2 \text{ mM}$ , demonstrated superior potency compared to the reference drug ampicillin, which had a zone of inhibition of  $25 \pm 2 \text{ mM}$  [109]. In a separate study, Song *et al.* synthesized and evaluated the

antifungal activity of 2-(indole-3-yl)-thiochroman-4-ones in vitro. The derivatives showed better activity than fluconazole. Compound **37**, which is a 6-chloro-2-(5-chloro-1H-indol-3-yl)thiochroman-4-one, exhibited potent antifungal activity [110]. In 2013, Zhang *et al.* synthesized three series of novel indole-based 1,3,4-oxadiazoles and reported that several of the synthesized compounds exhibit greater antifungal activity than does pimprinine. Among all the synthesized derivatives, compound **38**, which is 2-(1H-indol-3-yl)-5-(trifluoromethyl)-2,5-dihydro-1,3,4-oxadiazole, was found to be the most active in biological assays [111]. In another study, Zhang *et al.* prepared and investigated the antifungal activity of streptochlorin analogs. Compound **39** has shown remarkable potency (81–100%) in controlling this disease [112]. In 2018, Mishra *et al.* conducted a study to determine how to enhance the antifungal activity of indole triazole-amino acid conjugates. Compound **40**, which was prepared, exhibited better activity than the reference drugs [113]. In 2018, Jia *et al.* conducted a study on streptochlorin and its analogs, testing their effectiveness against various fungi, including *Pythium dissimile*, *Alternaria solani*, *Gibberella zea*, *Botrytis cinerea*, *Rhizoctonia solani*, *Alternaria blotch*, and *Colletotrichum chumcapsica*. Among the compounds tested, **41** were found to be highly potent [114]. In 2014, Pooja *et al.* synthesized an indole moiety appended with amino acids and evaluated its antifungal activity against *Candida albicans*. Compound **42** was found to demonstrate effective antifungal properties [115]. The structures of the indole derivative-based antifungal agents [**36-42**] are shown in Figure 7.

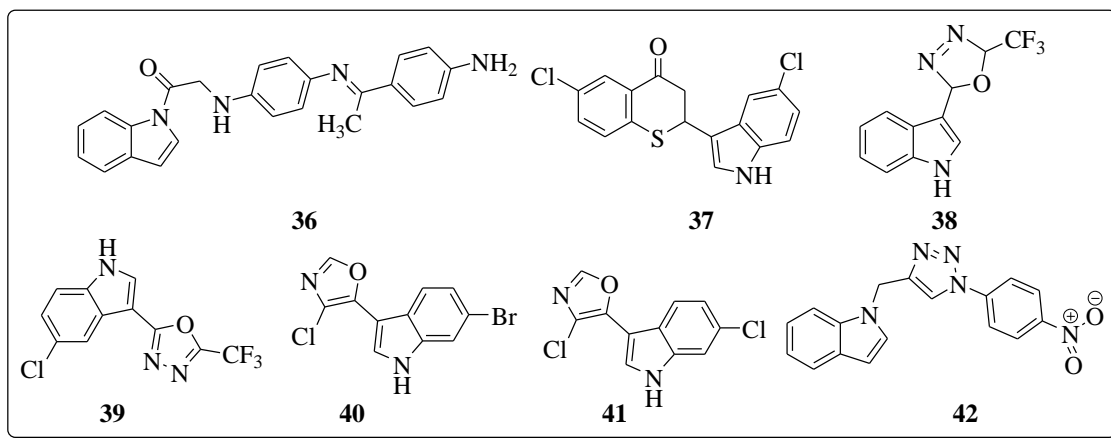


Figure 7: Chemical structure of indole derivative compounds that show antifungal activity

### 2.6.3. Antimalarial activities

Malaria is a chronic infectious disease caused by Plasmodium parasites that leads to thousands of deaths annually. Literature data showed that, among the 216 million reported cases of malaria, 731,000 died worldwide, with most cases occurring in Africa. The development of antimalarial drugs is necessary to combat this problem [116]. Indole derivatives have been investigated as potential antimalarial agents, and some of the promising derivatives are discussed below.

In 2016, Yadav *et al.* investigated the use of numerous indole derivatives for their antimalarial activity against *Plasmodium falciparum*. Compounds **43a** and **43b** showed high potency, with MIC values not > 0.70 µg/mL compared to the standard drugs quinine (MIC = 0.270 µg/mL) and chloroquine (MIC = 0.02 µg/mL) [117]. In 2015, Santos *et al.* investigated various indole-based piperidine derivatives, and in vitro studies were conducted in *P. falciparum* culture. The activity was measured in terms of the EC<sub>50</sub>. Lipophilicity was also calculated in terms of the partition coefficient (clogP) to further assess the activity of the synthesized derivatives. The piperidinyl moiety was found to be critical for the activity. Compound **44**, which was synthesized in their study, demonstrated maximum potency (EC<sub>50</sub> ~ 3 µM, partition coefficient (clogP) = 2.42 and MW = 305) against malaria parasites without showing any resistance when compared to reference drugs such as chloroquine, artesunate, atovaquone, and amodiaquine. The EC<sub>50</sub> values for these reference drugs were 285±58 µM, 1.97±0.43 µM, 0.35±0.14 µM, and 12.30±4.21 µM, respectively [118]. In 2014, Singh *et al.* synthesized and tested various melatonin-based indole derivatives and evaluated their inhibitory effect on the cell cycle of *P. falciparum*. Among these compounds, compound **45** exhibited superior antimalarial activity, with an IC<sub>50</sub> value of 2.93 µM [119].

Meridianin G is an indole alkaloid that is extracted from the marine invertebrate *Aplidium meridianum*. It has been found to be an inhibitor of cyclin-dependent protein kinase, which is involved in the progression of malaria. Bharate *et al.* conducted a study to evaluate the effectiveness of various meridianin G-based indole derivatives against chloroquine-sensitive and chloroquine-resistant clones of *P. falciparum* through plasmodial LDH activity. Compound **46** was found to be the most effective, with an IC<sub>50</sub> value of less than 4.01 µM when compared to the standard drugs artemisinin and chloroquine, which had IC<sub>50</sub> values of less than 0.09 µM and 0.72 µM, respectively [120]. In 2013, Teguh *et al.* synthesized several quinoline-indole conjugates and tested their effectiveness against the K1 strain of *P. falciparum*. Compound **47**

demonstrated the highest antimalarial activity, with an  $IC_{50}$  value of less than  $0.4 \pm 0.2 \mu\text{g/ml}$  [121]. Moreover, in 2014, Schuck *et al.* developed a novel series of melatonin analogs and tested them against *P. falciparum* cultures. Derivative compound **48** was effective against *P. falciparum* and hindered its growth [122]. The structures of the indole derivative-based active antimalarial compounds [**43a, b-48**] are shown in Figure 8.

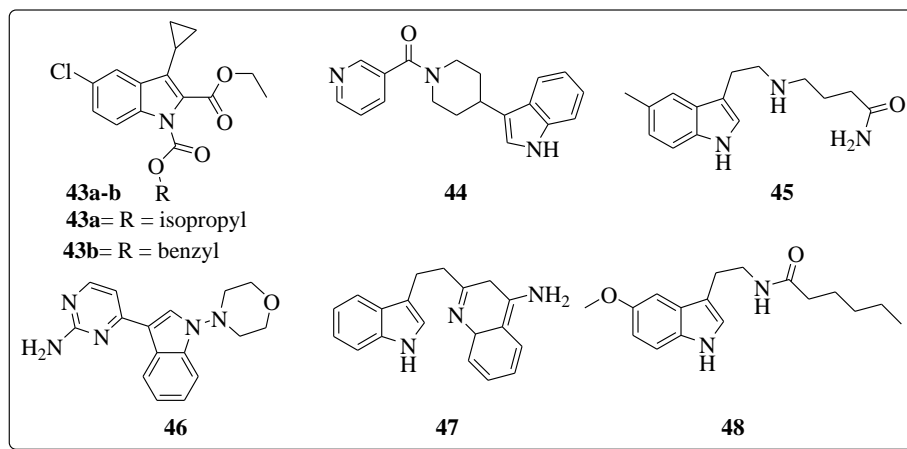


Figure 8: Chemical structure of indole derivative compounds that show antimalarial activity

#### 2.6.4. Antiviral activities

Viruses are highly infectious and can spread rapidly throughout the body, causing a range of illnesses. Antiviral drugs have been developed to combat viruses such as HIV, herpes, and hepatitis B and C. These drugs work by inhibiting viral replication and preventing the virus from entering host cells. In addition, some antiviral drugs can stimulate the immune system to fight infections. With viral infections being responsible for approximately 60% of illnesses in developed countries, it is crucial to develop safe and effective antiviral drugs. Researchers are currently working on developing new antiviral drugs with a broad range of activities to combat these fast-replicating viruses [123].

In 2017, Scutto *et al.* designed a series of novel multitarget indole-3- carboxylate derivatives as antiviral agents. All the synthesized compounds were evaluated against *Chikungunya* virus in Vero cell culture by a CPE reduction assay. Compound **49** was found to be most active ( $EC_{50} = 6.5 \pm 1$ ), which is 10-fold greater than that of the standard drug arbidol [124]. In 2017, Chen *et al.* studied the synthesis and investigation of integrated indoles and spiroindolines. The authors evaluated the efficacy of the prepared compounds against TMV both in vitro and in vivo. Compound **50** exhibited greater potency, with an inhibition rate of  $56 \pm 2\%$  at a concentration of  $500 \mu\text{g/ml}$ , than did the reference drugs ribavirin (% inhibition =  $36 \pm 1\%$ ) and harmine (%

inhibition = 45±1%) [125]. In a separate study by Musella *et al.*, in 2016, amide-substituted indole derivatives were designed and synthesized for the treatment of human *Varicella zoster* virus (VZV). Compound **51** (CC50 = 39 µM) was found to be more potent than the reference drugs briuvudine (CC50 = 160 µM) and acyclovir (CC50 = 191 µM) [126]. In 2018, Sanna *et al.* designed and synthesized new indole thiourea hybrid derivatives for the treatment of HIV-1. Compound **52** (EC<sub>50</sub> = 8.7 ± 0.4 µM) exhibited greater potency than the standard drug efavirenz (EC<sub>50</sub> = 0.002 ± 0.0002 µM) [127].

Dussan *et al.* carried out a study in 2016 in which they developed and evaluated several indole derivatives for their efficacy against HIV. Among these, compound **53** (EC<sub>50</sub> < 0.011 µM) was found to possess exceptionally strong anti-HIV activity [128]. Numerous indole-pyrido derivatives have been synthesized and investigated for anti-HIV activity. Ashok *et al.* (2015) studied the molecular properties of indole-pyrido derivatives to monitor HIV-infected cells. The synthesized compound **54** (EC<sub>50</sub>=0.53 µM) exhibited greater potency than the reference drug zidovudine, with an EC<sub>50</sub>=0.002 µM [129]. In 2014, Jiang *et al.* synthesized trifluoromethylindole analogs that have shown improved resistance to anti-HIV-1 NNRTIs. Compound **55** (EC<sub>50</sub> < 133.33 µM) has shown better potency than the reference drug nevirapine, with an EC<sub>50</sub>=0.4 µM, and efavirenz, with an EC<sub>50</sub>=0.08 µM [130].

In 2014, Ferro *et al.* conducted a study in which they created and assessed various indole derivatives by docking analysis with HIV-1 integrase. The results of the docking analysis indicated that a larger substituent on the benzyl group, such as tert-butyl or trifluoromethyl, was advantageous for interaction with the HIV-1 integrase protein. Among the compounds evaluated, compound **56** (IC<sub>50</sub> = 0.4 mM) demonstrated significant potency against HIV-1[131]. In a separate study, Regina *et al.* synthesized and assessed nitrogen-containing indole 2-carboxamide derivatives against mutant strains of HIV-1, including Y181C, Y188L, K103N, K101Q, IRL98, and G190A. The findings of the SAR studies indicated that a pyridine 4-yl methyl substituent was advantageous for activity. Compound **57** (EC<sub>50</sub> = 2.0 ± 0.2 nM) exhibited greater potency than did standard drugs, such as zidovudine (EC<sub>50</sub> = 2.0 ± 0.2 nM) and efavirenz (EC<sub>50</sub> = 6.3 ± 3.2 nM) [132]. In 2014, Xue *et al.* conducted a study on the antiviral potency of indole-2-carboxylate derivatives. Among the compounds synthesized, compound **58** demonstrated notable effectiveness against influenza A virus [133]. The structures of the indole derivative-based active antiviral compounds [49-58] are shown in Figure 9.

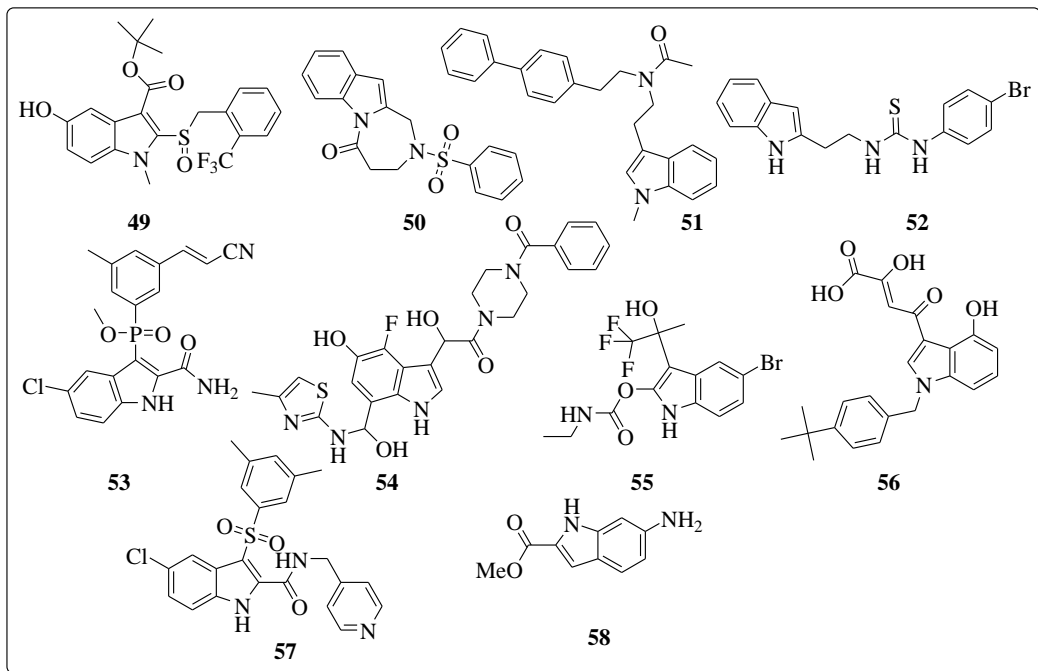


Figure 9: Chemical structure of indole derivative compounds that show antiviral activity

### 2.6.5. Anticancer activities

Cancer is a life-threatening disease characterized by the uncontrolled growth and spread of abnormal cells in the body. These abnormal cells can cause tumors to form or invade nearby tissues and organs, potentially spreading to other parts of the body through the bloodstream or lymphatic system. There are many different types of cancer, each with its own unique characteristics and treatment options. Common types of cancer include breast cancer, lung cancer, prostate cancer, and skin cancer [134]. While cancer can be a serious and life-threatening condition, early detection and treatment can greatly improve the outcomes and quality of life for those affected. Hence, novel indole derivatives with different mechanisms of action must be developed. In recent years, various indole derivatives have been designed as anticancer agents that act *via* various targets, such as histone deacetylases (HDACs), sirtuins, and DNA topoisomerase [135].

In 2015, Panathur *et al.* developed a series of indole-isoxazolone hybrids through a simple synthetic method. The compounds were then tested against three human cancer cell lines to assess their cytotoxicity *in vitro*. Compounds **59** and **60** exhibited the highest inhibitory activity against SIRT1, with  $IC_{50}$  values of 35.25 and 37.36  $\mu M$ , respectively [136]. Another study conducted by Parkash *et al.* in 2018 focused on the preparation and investigation of reciprocated

heteroannulated indole derivatives for their cervical anticancer activity. A docking study revealed that the electron-withdrawing group located at the 8<sup>th</sup> position of the carbon displayed favorable interactions at the target site through hydrogen bonding and van der Waals interactions. Compounds **61a** and **61b** demonstrated excellent potency, with IC<sub>50</sub> values of 13.41 μM and 14.67 μM, respectively, which were comparable to that of the reference drug cisplatin (IC<sub>50</sub> = 13.20 μM) [137]. Furthermore, in 2017, Tocco *et al.* synthesized and evaluated bis-indole derivatives for their cytotoxicity against hepatocarcinoma cells. Compound **62** demonstrated superior activity with an IC<sub>50</sub> range of 20-100 μM, outperforming the standard drug indole 3-carbinol [138].

In 2018, Coriglino *et al.* focused on the synthesis of 2,4-thiazolidinedione indole analogs as potential anticancer agents. The synthesized compounds were tested on human breast cancer cells (MCF-7) and PC3 human prostate cancer cells. Compound **63** demonstrated high potency with an IC<sub>50</sub> value of 5 μM [139]. In 2017, Romagnoli *et al.* synthesized and screened a range of 3-substituted 2-oxindole hybrid derivatives. Compound **64** exhibited high levels of activity (IC<sub>50</sub> < 5500 μM) on HL-60 cells, potentially due to its increased interactions with cellular nucleophiles compared to those of the standard drug [140]. In a study conducted by Bakherad *et al.* in 2019, thiosemicarbazone indole derivatives were synthesized and evaluated in MCF-7 (breast cancer), A-549 (lung cancer), and Hep-G2 (liver cancer) cell lines. Compound **65** had potent effects on the A-549 (IC<sub>50</sub> = 12.5 μM) and Hep-G2 (IC<sub>50</sub> = 56 ± 6.30 μM) cell lines and was comparable to the reference drugs etoposide on the A-549 (IC<sub>50</sub> = 38.23 ± 1.89 μM) and Hep-G2 (IC<sub>50</sub> = 33.17 ± 3.19 μM) cell lines, as was colchicine on the A-549 (IC<sub>50</sub> = 1.9 ± 0.23 μM) and Hep-G2 (IC<sub>50</sub> = 6 ± 0.49 μM) cell lines [141]. Moreover, El-Sharief *et al.* investigated the effects of isoindole derivatives on three cancer cell lines and determined that phenylacetamide and malononitrile substitution were favorable for activity. Compounds **66** and **67** were highly potent, with IC<sub>50</sub> values of <6.67 ± 0.36 μM and 6.34 ± 0.21 μM, respectively, compared to the standard drugs isatin and doxorubicin, with IC<sub>50</sub> values of < 41.83 ± 0.67 μM and < 7.03 ± 0.21 μM, respectively [142].

In 2016, Chang *et al.* synthesized and evaluated novel series of bis-(hydroxymethyl)indolizino [8,7-b] indole hybrids as anti-small cell lung cancer agents. Hybridization of two nuclei led to the inhibition of tumor cell growth via two mechanisms: topoisomerase II inhibition and induction of DNA cross-linking. Compound **68** demonstrated greater potency (IC<sub>50</sub> = 0.49 μM)

against the growth of small cell lung cancer (SCLC) H526 cells in a xenograft model than did cisplatin ( $IC_{50} = 0.63 \mu\text{M}$ ) [143]. Hu *et al.* conducted a study on the anticancer properties of various new series of 2,5-disubstituted indole derivatives. Among the compounds tested, compound **69** exhibited greater potency ( $IC_{50} < 8.70 \pm 0.11 \mu\text{g/mL}$ ) than did the reference drug cisplatin ( $IC_{50} < 6.10 \pm 0.09 \mu\text{g/mL}$ ) [144]. The structures of the indole derivative-based anticancer agents [**59-69**] are shown in Figure 10.

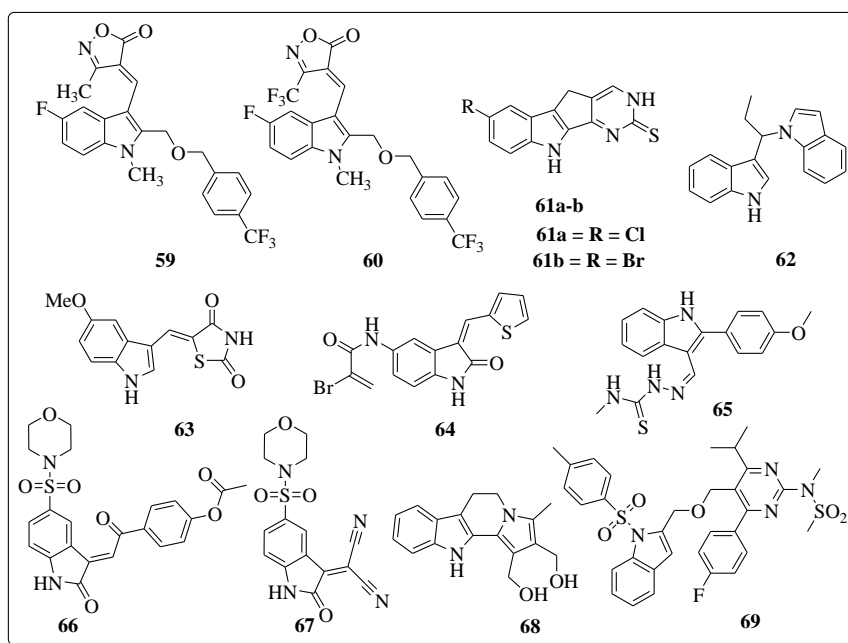


Figure 10: Chemical structure of indole derivative compounds that show anticancer activity

### 2.6.6. Anti-inflammatory activities

Inflammation is a complex biological response of vascular tissue to harmful stimuli such as pathogens, damaged cells, or irritants. Self-healing is a protective process by which an organism removes injurious stimuli, initiates the healing process and restores both structure and function. Inflammation can be acute or chronic and can have negative effects on the body in its chronic form. Anti-inflammatory agents are used to treat inflammation by reducing swelling and pain [145]. Indole is a natural compound found in cruciferous vegetables such as broccoli, kale, and cabbage. It has been shown to have anti-inflammatory properties and is a major inhibitor of cyclooxygenase [146].

In 2018, Chancharunee *et al.* developed various capsaicin-based indole and nitroindole derivatives and evaluated their effectiveness against the proinflammatory kinase  $\text{TNF-}\alpha$ . The SAR study highlighted the favorable activity of capsaicin alkyl chain systems and nitro

substituents. Compounds **70a** (relative % inhibition = 47.65%) and **70b** (relative % inhibition = 51.95%) were found to be highly potent in terms of relative % inhibition compared to the standard drug capsaicin (relative % inhibition = 65.55%) [147]. In the same year, Bhat *et al.*, 2018, prepared and investigated acetohydrazide-indole hybrid derivatives for their COX-2 inhibitory activity. A docking study of compound **71** (potency = 0.79%) revealed potent selective inhibition compared to the reference drug indomethacin (potency = 1.0%) [148]. Similarly, Shaker *et al.* conducted a study on indole derivatives containing methyl sulfonyl and aryl-substituted derivatives for COX-2 inhibition. Compounds **72a** (IC<sub>50</sub> = 0.11 μM, SI = 107.63) and **72b** (IC<sub>50</sub> = 0.15 μM, SI = 76.6) demonstrated greater potency than did the reference drug indomethacin (IC<sub>50</sub> = 0.49 μM, SI = 0.079) [149].

In 2017, Fatahala *et al.* synthesized a series of indole derivatives and evaluated their anti-inflammatory activity using the rat paw edema method and docking analysis. Compound **73** showed the highest activity, with a %inhibition of 92%, compared to the standard drugs ibuprofen (inhibition = 69.84%) and indomethacin (inhibition = 78.58%) [150]. In 2017, Shroff *et al.* synthesized novel indolyl-pyrazoline derivatives and evaluated their anti-inflammatory activity using the carrageenan-induced paw edema method. Compounds **74a** and **74b** showed greater potency, with 63.90% and 57.46% inhibition, respectively, than the reference drug indomethacin (61.36%) [151]. In 2016, Liu *et al.* conducted a study on indole-2-carboxamide derivatives and identified potent compounds through in vivo screening of their biological activity. The study revealed that the substitution of an oxazole or amine at C-5 with a carboxamide had a significant impact on activity, and an increase in methoxy substituents led to increased activity. Moreover, the substitution of certain benzyl groups at N1 resulted in compounds with good anti-inflammatory activity. Compounds **75a** and **75b** were found to be highly potent, with % inhibition  $< 2.90 \pm 0.73\%$  and  $< 2.67 \pm 0.76\%$ , respectively [152].

In 2014, Shaveta *et al.* synthesized numerous chromone-substituted oxindole compounds and investigated their effects on COX-1, COX-2, and 5-LOX. Compounds **76a** (IC<sub>50</sub> = 9.5 ± 0.8 μg/mL) and **76b** (IC<sub>50</sub> = 10.0 ± 4.2 μg/mL) exhibited greater potency than did the reference drug indomethacin (IC<sub>50</sub> = 0.7 ± 0.2 μg/mL) [153]. In 2015, Ozdemir *et al.* reported on indole-chalcone derivatives. All the synthesized compounds were evaluated against COX-1 and COX-2, and SAR studies revealed that methoxy, bromo, and sulphonyl-substituted chalcone derivatives were favorable for activity. Among all the synthesized compounds, **77a** and **77b** were found to

be highly potent [154]. The structures of the indole derivative-based compounds with anti-inflammatory activity [**89a-95b**] are shown in Figure 11.

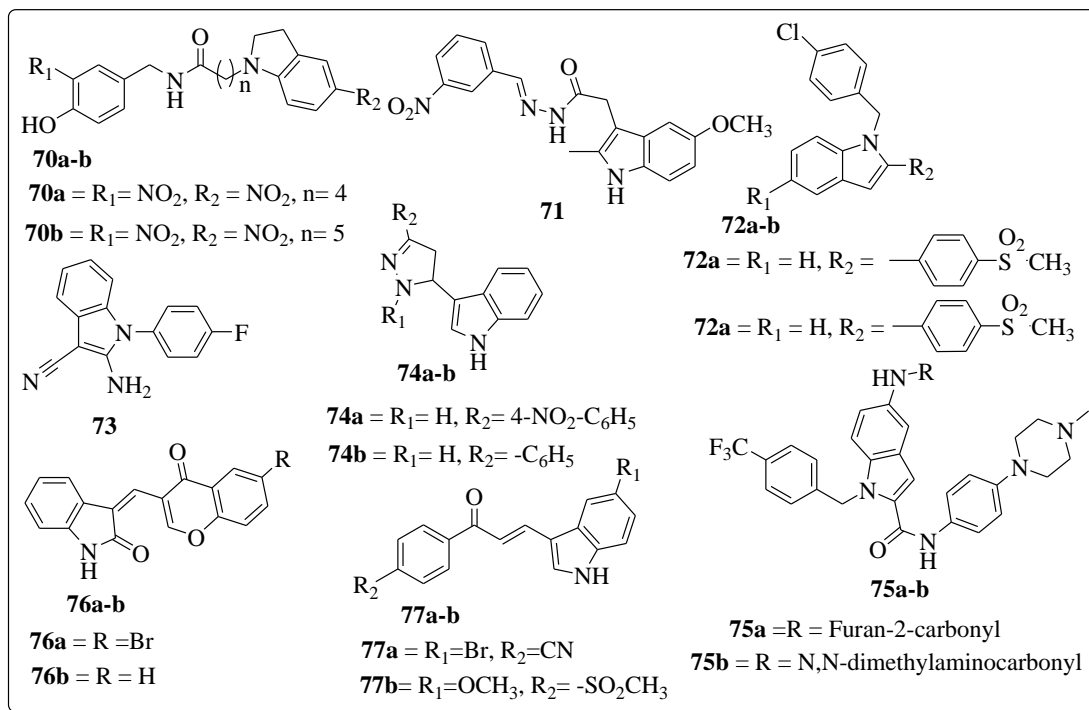


Figure 11: Chemical structure of indole derivative compounds that show anti-inflammatory activity

### 2.6.7. Antimicrobial activity

The emergence of drug resistance has become a critical challenge since the introduction of the first antimicrobial agent into clinical practice in the 1940s. To combat microbial resistance, it is crucial to preserve existing antimicrobial agents through responsible use while simultaneously developing new lead molecules. According to the latest survey report from the World Health Organization, more than 0.5 million individuals in 22 countries have developed antibiotic resistance, underscoring the urgent need for continued efforts to address this global health issue [155]. To address the problem of antibiotic resistance, it is necessary to develop new indole derivatives with different mechanisms of action. Numerous indole derivatives have been identified and studied as potential antimicrobial agents. To combat the issue of antimicrobial resistance, new indole derivatives that target microorganisms through different mechanisms must be developed. Various indole derivatives have been identified and evaluated as potential antimicrobial agents, which are discussed below.

In 2018, Sayed *et al.* synthesized and evaluated indole derivatives with heterocyclic nuclei as potential antimicrobial agents. The presence of thiophene and imidazole rings in these compounds increased their antimicrobial activity. Compound **78** exhibited high antibacterial activity with a minimum inhibitory concentration (MIC) of less than 8 µg/mL, while compound 79 exhibited high antifungal activity with an MIC of less than 6 µg/mL [156]. In 2018, Sanna *et al.* synthesized hybrids of indole-thiourea and tested them on a group of gram-positive and gram-negative microbes. The synthesized Compound **80**, which has a minimum inhibitory concentration of less than 12.5 µg/ml, was found to have greater potency than the reference drug ciprofloxacin, which has an MIC of less than 1.0 µg/ml [157].

In 2016, Mane *et al.* synthesized and assessed various indole-2-carboxamide derivatives for their antioxidant and antimicrobial properties. The compounds were tested against several microbes, including *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. typhi*, *C. albicans*, *C. neoformans*, *A. fumigates*, and *C. parapsilosis*. Compound **81** demonstrated maximum antimicrobial activity with an MIC of less than 6.25 µg/mL, outperforming the standard drug gentamicin (MIC of less than 3.0 µg/mL) [158]. Pyrazole and imidazole exhibit maximum antimicrobial properties because of the presence of nitrogen atoms in their nucleus, which functions by restraining DNA synthesis [159]. The antimicrobial activity of these heterocyclics has led various scientists to attach pyrazole and imidazole rings to the indole nucleus to prevent microbial resistance. In 2017, Quazi *et al.* focused on the synthesis and evaluation of different indole-pyrazole derivatives. Among all the compounds, compound **82a** exhibited favorable activity against gram-positive bacteria with a zone of inhibition less than 0.5 cm, while compound **82b** demonstrated good activity against the fungal strains *Macrophomina phaseolina* and *Sclerotium rolfsii* with a zone of inhibition less than 0.1 cm [160].

In 2017, Rajaraman *et al.* conducted a study on the synthesis and molecular docking of several indole derivatives for antimicrobial activity. Compound **83** exhibited favorable stable bond parameter reactivity due to the presence of negative charges on nitrogen and oxygen atoms and had a minimum inhibitory concentration of less than 12.5 µg/ml. This concentration was comparable to that of the standard drug methicillin, which has a minimum inhibitory concentration of less than 6.25 µg/ml [161]. In 2015, El-Sayed *et al.* synthesized and tested a new series of indole derivatives, specifically bisindolyl-substituted cycloalkane indoles, for their antibacterial activity against methicillin-resistant *S. aureus* (MRSA) and *S. aureus*. The

researchers discovered that compound **84**, which contain a cyclohexane indole moiety, exhibited significant activity against both strains of bacteria [162].

In 2015, Gali *et al.* synthesized and investigated thiazolyl coumarin-substituted indole derivatives and treated *E. coli* and *B. subtilis*. Compound **85** (with a zone of inhibition < 18 mm) exhibited greater potency than did the reference drug streptomycin (with a zone of inhibition < 30 mm) [163]. Hydrazone derivatives are abundant in various biological components and exhibit numerous pharmacological effects, including but not limited to anticonvulsant, antiviral, antibacterial, antitubercular, and anticancer activities [164]. In 2011, Shirizadeh *et al.* synthesized and investigated several indole-hydrazone derivatives to address the issue of multidrug-resistant bacteria. Compound **86** exhibited greater potency (MIC < 25 µg/ml) than did the reference drugs fluconazole (MIC < 0.78 µg/ml) and ciprofloxacin (MIC < 0.19 µg/ml) [165]. In a separate study by Shi *et al.*, in 2015, numerous indole derivatives were prepared and evaluated for antibacterial activity. Thirteen novel indole derivatives were synthesized using ultrasound irradiation with 2-mercapto-5-substituted 1,3,4-oxadiazoles and 4-amino-5-(1H-indol-3-yl)-4H-[1,2,4] TRIzole-3thiol. Of these, compounds **87** and **88** exhibited exceptional antimicrobial activity against *E. coli* and *S. aureus* [166].

In a study conducted by Nassar *et al.*, various pyrazoline, pyridine, and pyrimidine-substituted indole derivatives were synthesized as potential antimicrobial agents. The compounds were evaluated against a range of microorganisms, including *S. aureus*, *E. coli*, *P. aeruginosa*, *Fusarium*, *A. niger*, and *C. albicans*. The results of the study indicated that the methoxyphenyl substitution played a crucial role in the activity of the compounds. Compound **89** exhibited promising antibacterial activity (zone of inhibition < 34 mm) when compared to the standard drugs ciprofloxacin (zone of inhibition < 44 mm) and nystin (zone of inhibition < 44 mm) [167]. The structures of indole nucleus-based antimicrobial compounds [78-89] are shown in Figure 12.

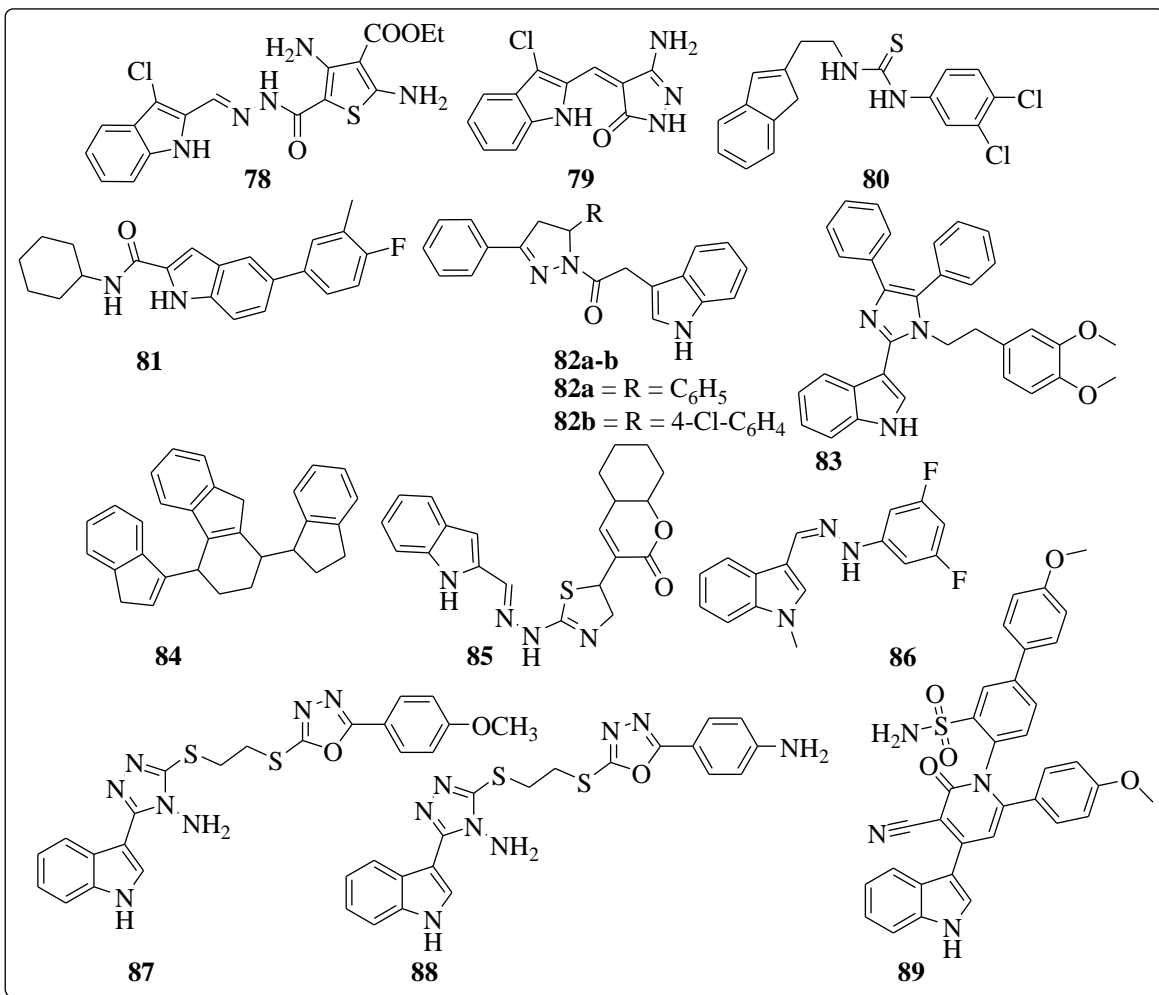


Figure 12: Chemical structure of indole derivative compounds that show antimicrobial activity

### 2.6.8. Antidepressant activity

Depression affects people's thoughts, behaviors, tendencies, and potential feelings characterized by low mood. Depression is the leading cause of ill health and disability worldwide [168]. Approximately 300 million people were suffering from depression, representing an increase of 18% between 2005 and 2015. Recent data show that 8.1% of American adults experienced depression within the 2-week period. Women suffer more than men [169]. Researchers are involved in the discovery of drugs with advanced structural modifications that have potent effects on the brain. Various indole derivatives have been reported to function as antidepressant agents.

In 2018, Kerazare *et al.* synthesized oxindole derivatives containing an azetidinone moiety and conducted an animal study using a forced swim test. The results showed that compound **90a**

reduced immobility to 66.82%, and compound **90b** reduced immobility to 65.61%, indicating their high potency compared to the reference drug fluoxetine, which only reduced immobility to 70.93% [170]. Similarly, Patil and Bari (2016) synthesized a variety of indole derivatives containing a dihydropyrazole moiety and evaluated their antidepressant activity using a forced swim test. Compounds **91a** and **91b** demonstrated greater potency than the reference drug fluoxetine, reducing immobility to 77.4% and 75.5%, respectively, compared to the 77.4% reduction in fluoxetine and 75.5% reduction in imipramine [171]. The same group previously synthesized isoxazoline-fused indole derivatives as antidepressant agents and conducted a detailed SAR analysis, which confirmed that the activity of these compounds was due to the presence of heterocyclic rings such as pyridine and pyrrole. Furthermore, the substitution of electron-withdrawing systems (F, Br, Cl) at the *para*-position resulted in good activity, while electron-releasing groups (CH<sub>3</sub>, OH, OCH<sub>3</sub>) decreased activity. Compounds **92a** and **92b** were found to be highly potent [172].

More recently, Zhen *et al.*, in 2015, introduced a series of 2-(5-methyl-2,3-dioxindolin-1-yl) acetamide derivatives and reported that compounds with 3-Br phenyl, 4-Br-phenyl, 3-CF<sub>3</sub>-phenyl, and benzyl group attachments exhibited good potency in a forced swim test. Compounds **93a** (65.77 ± 17.8) and **93b** (75.77 ± 18.7) showed promising results compared to the standard drug fluoxetine (58.5 ± 9.3) [173]. These studies demonstrate the potential of indole derivatives as effective antidepressant agents and highlight the importance of structural modifications in enhancing their activity. The structures of indole derivative-based compounds with antidepressant activity [**91a-93b**] are shown in Figure 13.

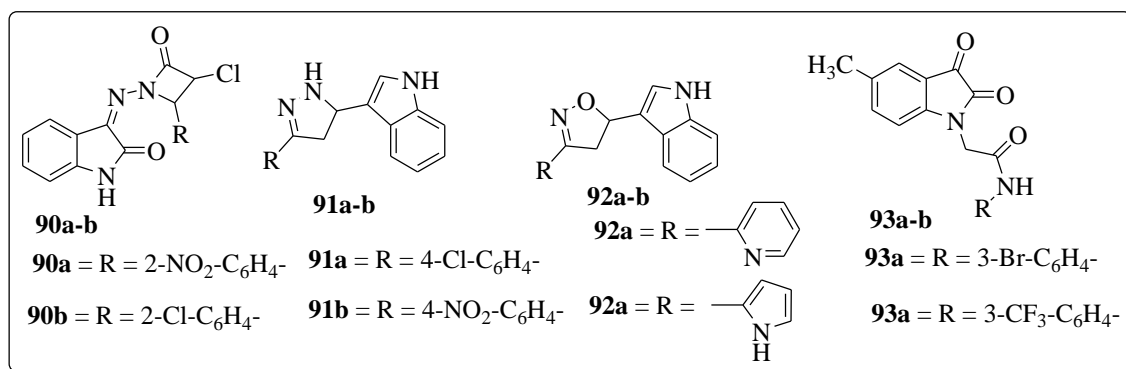


Figure 13: Chemical structure of indole derivative compounds that show antidepressant activity

### 2.6.9. Anticonvulsant activity

Convulsion is a neurological disorder that causes sudden and involuntary muscle contractions, often leading to jerking movements of the body. It can be caused by various factors and may indicate a serious underlying condition requiring immediate medical attention. Epilepsy is a prevalent neurological disease characterized by brief episodes of seizures and/or loss of consciousness. Researchers have synthesized and evaluated various indole derivatives as anticonvulsant agents [174].

Swathi *et al.*, in 2015, designed, synthesized and evaluated dialkylaminoalkoxy-oxindole derivatives. The anticonvulsant activities of the synthesized compounds were investigated by the pentylenetetrazole (PTZ)-induced convulsion method and maximal electroshock seizure (MES) test. Compound **94** has shown ( $IC_{50} < 67.18 \pm 0.23 \mu\text{g/mL}$ ) better anticonvulsant activity than the standard drug phenytoin, with an  $IC_{50}$  of  $100 \mu\text{g/mL}$  [175]. In 2017, Madhira *et al.* conducted a study to assess the anticonvulsant activity of benzohydrazide-oxindole derivatives. Compound **95** (protection=83.19%) demonstrated greater potency than did the reference drug phenytoin (protection=100%) [176]. In 2016, Raju *et al.* prepared and investigated novel indole carboxylate derivatives for anticonvulsant activity by the MES method. Compound **96** ( $108.3 \pm 0.7$ ) was more potent than the reference drug phenytoin (100%) [177].

In 2014, Yar *et al.* investigated the synthesis of indole-hydrazide derivatives as dual binding site cholinesterase inhibitors both in vitro and through docking studies. The majority of the tested compounds demonstrated significant inhibition of acetylcholinesterase and butyrylcholinesterase, with the presence of aryl and aryl halide substituents on the indole enhancing their activity. Notably, compound **97** ( $IC_{50} = 91.21 \pm 0.06 \mu\text{M}$ ) and compound **98** ( $IC_{50} = 68.52 \pm 0.04 \mu\text{M}$ ) displayed promising acetylcholinesterase inhibitory activity compared to the standard drug eserine ( $IC_{50} < 0.85 \pm 0.0001$ ), with compound **98** also exhibiting two-point attachment to acetylcholinesterase. Furthermore, compound **97** exhibited high potency against butyrylcholinesterase due to the presence of an amide linkage [178]. These findings suggest that indole-hydrazide derivatives may have potential as cholinesterase inhibitors for therapeutic applications. In 2014, Ahuja and Siddiqui investigated the synthesis of indole-1,2,4-triazine analogs and their potential as anticonvulsant agents against maximal electric shock (MES) and subcutaneous pentylenetetrazole (scPTZ). Compound **99**, which contains nitro groups that bind to the receptor, displayed greater potency, with a % protection of 100%. These findings suggest

that indole-1,2,4-triazine analogs may have potential as anticonvulsant agents for therapeutic applications [179]. The structures of the indole derivative-based anticonvulsant activity compounds [94-99] are shown in Figure 14.

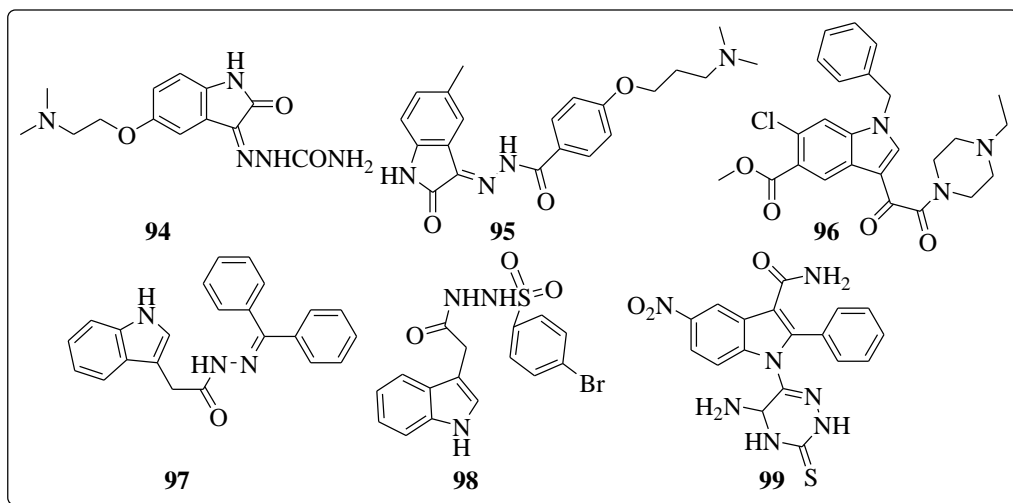


Figure 14: Chemical structure of indole derivative compounds that show anticonvulsant activity

### 2.6.10. Antidiabetic activity

Diabetes mellitus (DM) is a chronic metabolic disorder that affects millions of people worldwide. The high blood sugar levels associated with diabetes can lead to a range of complications, including renal and eye problems. In fact, diabetes is the leading cause of kidney failure and blindness in adults. It is important for individuals with diabetes to maintain their blood sugar levels through proper diet, exercise, and medication to prevent or delay the onset of these complications. Regular check-ups with healthcare professionals can also help individuals detect and treat any issues early on, improving overall outcomes and quality of life for those living with diabetes [180].

In addition to the traditional methods of managing diabetes, such as diet, exercise, and medication, the development of novel antidiabetic drugs could provide a valuable tool for combating this disease. Indole derivatives have been identified as potential candidates for these new drugs due to their demonstrated antidiabetic activity. Furthermore, nitrogen-containing heterocyclic nuclei, such as oxadiazole derivatives, have been shown to manage type II diabetes mellitus through their  $\alpha$ -glucosidase inhibitory activity [181].

In 2018, Rajan *et al.* prepared and investigated indole-triazole derivatives for their antidiabetic properties using a Syrian golden hamster model. Among the synthesized compounds, compound

**100** showed the maximum potency [182]. Moreover, Nazir *et al.* investigated indole oxadiazole hybrid analogs for inhibiting  $\alpha$ -glucosidase activity, with compounds **101** ( $IC_{50} = 9.46 \pm 0.03 \mu M$ ) and **102** ( $IC_{50} = 9.37 \pm 0.03 \mu M$ ) showing greater potency than acarbose ( $IC_{50} = 37.38 \pm 0.12 \mu M$ ) [183]. In vivo studies conducted by Srividya and Reddy in 2017 investigated the efficacy of synthesized indole derivatives using a diabetes-induced chick model, with compound **103** demonstrating particularly strong antidiabetic activity (29.6-38.6%) compared to the standard drug glibenclamide (57.10%) [184].

In 2017, Taha *et al.* combined indole and oxadiazole to create a new chain of tris-indole-oxadiazole analogs. Compound **104** ( $IC_{50}=2.00 \pm 0.001 \text{ mM}$ ) showed high potency compared to the reference drug acarbose ( $IC_{50}=895.09 \pm 2.04 \text{ mM}$ ), demonstrating the potential of these compounds as effective antidiabetic agents [185]. The structures of indole derivative-based compounds with antidiabetic activity [**100-104**] are shown in Figure 15.

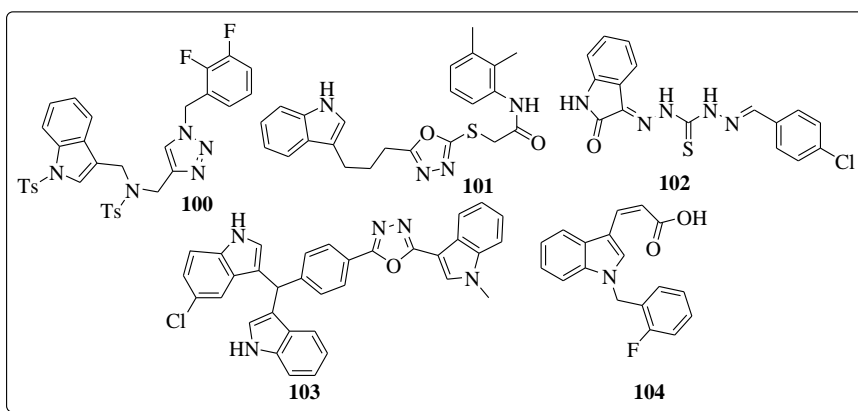


Figure 15: Chemical structure of indole derivative compounds that show antidiabetic activity

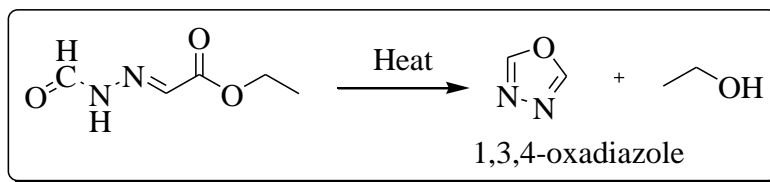
## 2.7. Chemistry of Oxadiazoles

These compounds consist of a five-membered heterocyclic ring that includes two nitrogen atoms and one oxygen atom. Oxadiazoles exhibit different isomeric forms based on the arrangement of the hetero-toms, such as 1,3,4-oxadiazole, 1,2,3-oxadiazole, 1,2,4-oxadiazole, and 1,2,5-oxadiazole. Those with aromatic systems are referred to as azoxins, while the five-membered cyclic molecules containing an equal number of nitrogen and oxygen atoms and have undergone partial reduction are termed furoxanes [186].

### 2.7.1. Synthesis Techniques for 1,3,4-Oxadiazoles rings and its derivatives

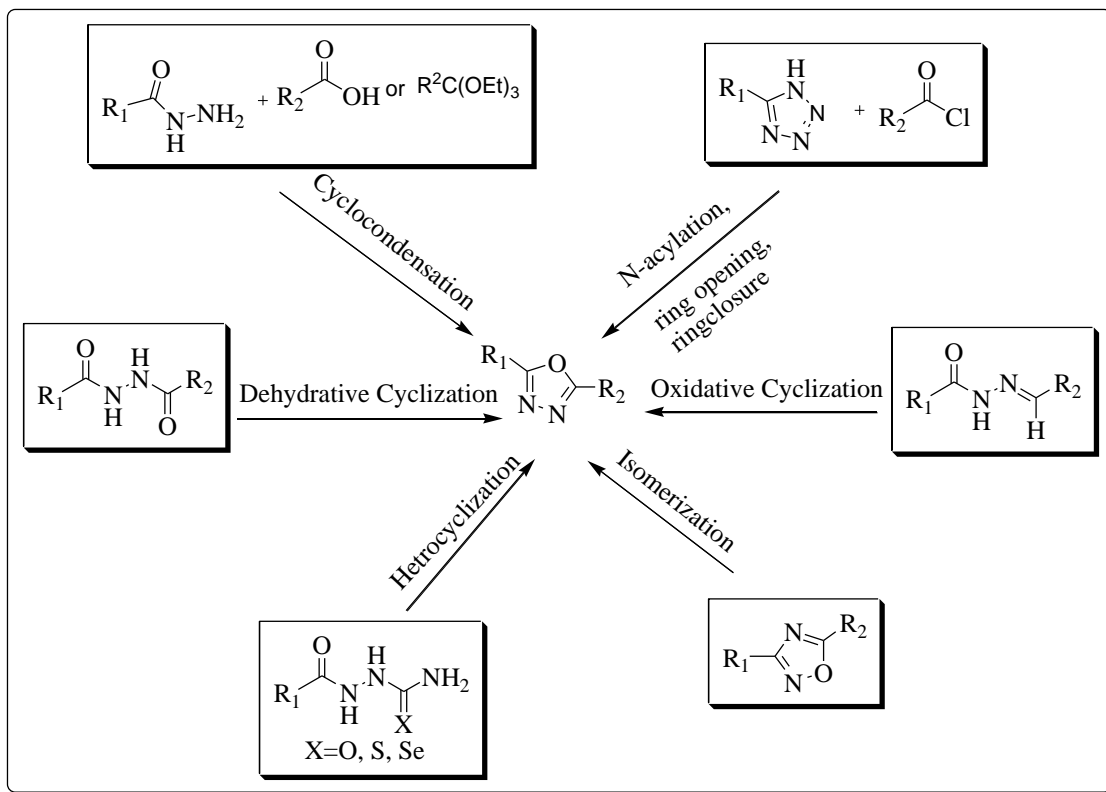
In 1965, Ainsworth initially detailed the synthesis of unsubstituted 1,3,4-oxadiazole. This method involved the application of thermolysis under atmospheric pressure using formylhydrazone ethylformate [187].

Scheme 21: The initial preparation of 1,3,4-oxadiazole through thermolysis.



The synthesis of basic unsubstituted 1,3,4-oxadiazole involves a straightforward process wherein the simplest *N,N'*-diformylhydrazine reacts with phosphorus pentoxide in the presence of polyphosphoric acid. The method proposed by Schwarzer et al. entails preheating the polyphosphoric acid to approximately 100 °C initially, followed by the addition of P<sub>2</sub>O<sub>5</sub>. Subsequently, hydrazine is introduced to the mixture, and the reaction is allowed to proceed at an elevated temperature for several hours. The resulting unsubstituted 1,3,4-oxadiazole can then be neutralized with sodium bicarbonate. The predominant methods for synthesizing 1,3,4-oxadiazole derivatives involve the utilization of *N,N'*-diacylhydrazines or *N*-acylhydrazones. Cyclodehydration of *N,N'*-diacylhydrazines is commonly achieved using various agents such as polyphosphoric acid (PPA), H<sub>2</sub>SO<sub>4</sub>, POCl<sub>3</sub>, SOCl<sub>2</sub>, (CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>O, P<sub>2</sub>O<sub>5</sub>, BF<sub>3</sub>·OEt<sub>2</sub>, or Burgess reagent [188]. Furthermore, 1,3,4-oxadiazole derivatives can also be synthesized through the oxidative cyclization of *N*-acylhydrazones using oxidizing agents like ceric ammonium nitrate (CAN), Br<sub>2</sub>, KMnO<sub>4</sub>, PbO<sub>2</sub>, chloramine T, or hypervalent iodine reagents. The literature also documents one-pot methods for synthesizing oxadiazole groups by utilizing acid hydrazides along with carboxylic acids and ortho-esters in the presence of an acid catalyst. Alternative synthesis approaches involve acylation, followed by the opening and closing of the tetrazole ring, as well as the conversion of 1,2,4-oxadiazole derivatives through UV radiation-induced reactions. Additionally, heterocyclization methods utilizing semicarbazides, thiosemicarbazides, or selenosemicarbazides are also explored for generating oxadiazole compounds [189].

Scheme 22: Possible methods for preparations of 1,3,4-oxadiazole derivatives



## CHAPTER THREE

### 3. MATERIALS AND METHODS

#### 3.1. Materials

##### 3.1.1. Instruments

A Rotary Evaporator (Laborota 4000, Heidolph, Germany) was employed to remove solvents from the samples. The spots of the synthesized compounds were visualized on TLC using UV light at 254 and 365 nm with an HP-UV/visible lamp. The melting points of all synthesized compounds were determined in open capillary tubes utilizing a Gallenkamp melting point apparatus (Gallenkamp, CAT. No. 66600-003). Electronic spectra of the synthesized compounds were measured using a T80 UV/VIS Spectrometer (PG Instruments Ltd) within the range of 200-800 nm at room temperature. An oven (OV 150 SS, England) was used to dry and sterilize all glassware utilized in the study. A precision Electronic Balance (SPU2001, China) was utilized to weigh solid samples. A magnetic stirrer hotplate (H3770-HS, Chain) was employed to reflux the reaction mixture. IR spectra (in terms of wave numbers,  $\text{cm}^{-1}$ ) of all synthesized compounds were recorded using the KBr pellet method in a PerkinElmer FT-IR (Frontier) spectrophotometer. The UV-Vis and FT-IR analyses were conducted at the Department of Chemistry, Hawassa University, Ethiopia.

##### 3.1.2. Chemicals and Apparatus

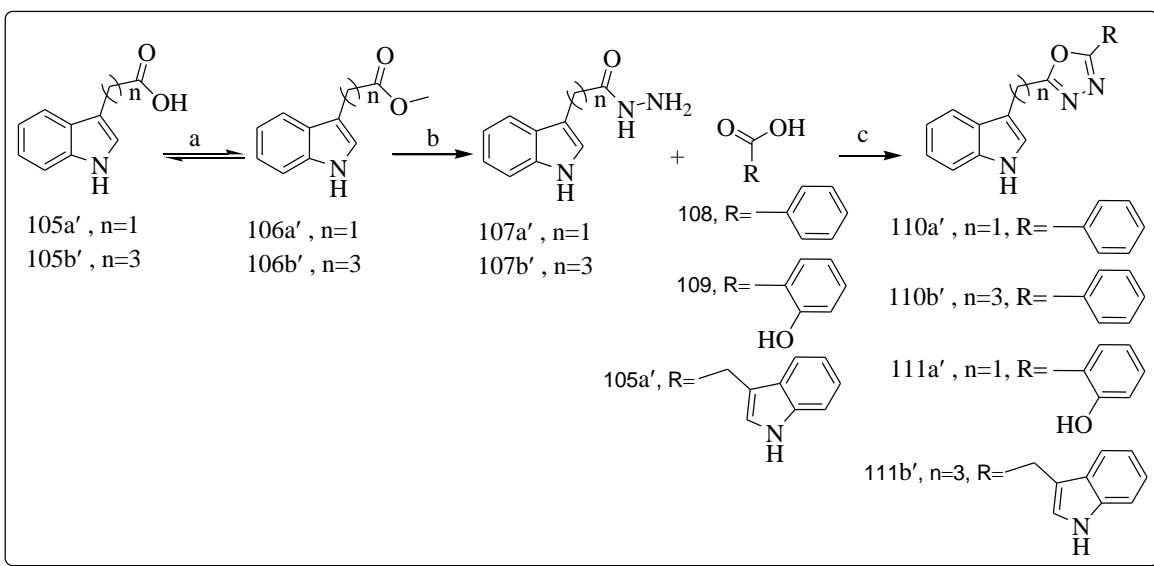
All reagents and solvents utilized in the experiment were sourced from commercially available suppliers and were used without any additional purification steps. The chemicals and reagents employed during the study included: 2-(1*H*-indol-3-yl) acetic acid (98%, Loba Chemie, India), 4-(1*H*-indol-3-yl) butanoic acid (98%, SRL, Mumbai), Hydrazine monohydrate (80%, Loba Chemie, India), Phosphorous oxychloride (98%, Loba Chemie, India), Hexane (99%, Loba Chemie, India), Methanol (99.8%, Loba Chemie, Mumbai), silica gel (60-120 mesh, Loba Chemie, India), ethyl acetate (AR/ACS, CDH, Delhi-110002), anhydrous sodium sulfate (99%, Loba Chemie, India), sodium bicarbonate (99.5%, Fisher Scientific), Chloroform (AR, CDH, Delhi), Sulfuric acid (98% AR, Loba Chemie, Mumbai-400005), potassium carbonate (99.5%, SRL, India), and acetone (AR, Loba Chemie, India). The apparatus utilized in the experiment included precoated aluminum sheets (Silica gel 60 F254, Merck Germany) for thin-layer chromatography (TLC). Standard laboratory equipment was employed throughout the study.

## 3.2. Methods

### 3.2.1. General procedures for the synthesis of target compounds

Starting with 2-(1*H*-indol-3-yl) acetic acid (**105a'**) and 4-(1*H*-indol-3-yl) butanoic acid (**105b'**), the synthesis of the target compounds involved their conversion to intermediate compounds **106a'** and **106b'**, respectively, through a reaction in absolute methanol with concentrated sulfuric acid. Subsequently, the intermediate compounds **106a'** and **106b'** were further transformed into intermediate compounds **107a'** and **107b'**, respectively, by reaction with hydrazine monohydrate in methanol. Finally, the intermediate compounds **107a'** and **107b'** underwent a reaction with substituted carboxylic acids in the presence of POCl<sub>3</sub> to yield the desired target compounds, as illustrated in Scheme 23 [190].

Scheme 23: Reagents and conditions: (a) Methanol, H<sub>2</sub>SO<sub>4</sub> (99.8%), reflux at 80 °C, 8h; (b) NH<sub>2</sub>NH<sub>2</sub>.H<sub>2</sub>O (80%), methanol, stir at RT, 14h; (c) POCl<sub>3</sub>, reflux at 60-80 °C, 9-10h.



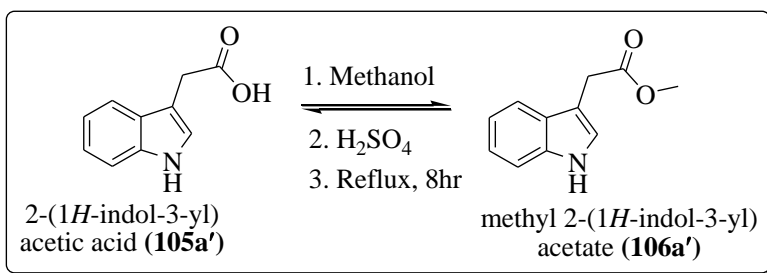
### 3.2.2. Synthesis of the Intermediates

#### 3.2.2.1. Synthesis of Methyl 2-(1*H*-indol-3-yl)acetate (**106a'**)

In a 100 mL round-bottom flask, a solution of 2-(1*H*-indol-3-yl)acetic acid (**105a'**) (0.8 g; 4 mmol) in 25 mL of absolute methanol was prepared. To this solution, 2.5 mL of concentrated sulfuric acid (99.8%) was added, and the mixture was refluxed at 90°C for 8 hours until the reaction reached completion. The progress of the reaction was monitored by thin-layer chromatography using a suitable solvent system (n-hexane/ethyl acetate, 7:3 v/v). Upon completion of the reaction, the mixture was neutralized with 10% aqueous sodium carbonate (10

mL), followed by the addition of water (40 mL). The product was extracted with chloroform (60 mL  $\times$  3), and the organic phase was collected and dried over anhydrous sodium sulfate and concentrated under reduced pressure using a rotary evaporator. The resulting product was purified by column chromatography using a solvent mixture of n-hexane/ethyl acetate (8:2 v/v) to yield the pure product **106a'** as a reddish-brown liquid. The chemical synthesis of compound **106a'** is outlined in Scheme 24.

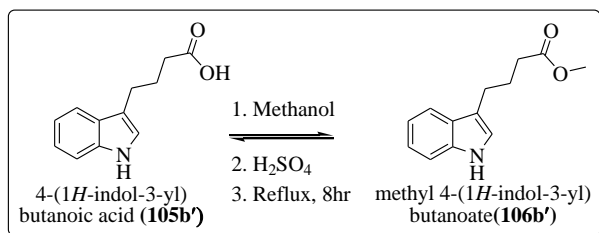
Scheme 24: Chemical reactions for Synthesis of compound 106a'



### 3.2.2.2. Synthesis of methyl 4-(1*H*-indole-3-yl)butanoate (**106b'**)

In a 100 mL round-bottom flask, a solution of 4-(1*H*-indol-3-yl)butanoic acid (**105b'**) (0.8 g; 3.9 mmol) in 25 mL of absolute methanol was prepared. To this solution, 2.5 mL of concentrated sulfuric acid (99.8%) was added, and the mixture was refluxed at 90°C for 8 hours until the reaction reached completion. The progress of the reaction was monitored by thin-layer chromatography using a suitable solvent system (n-hexane/ethyl acetate, 7:3 v/v). Upon completion of the reaction, the mixture was neutralized with 10% aqueous sodium carbonate (10 mL), followed by the addition of water (40 mL). The product was extracted with chloroform (60 mL  $\times$  3), and the organic phase was collected and dried over anhydrous sodium sulfate and concentrated under reduced pressure using a rotary evaporator. The resulting product was purified by column chromatography using a solvent mixture of n-hexane/ethyl acetate (8:2 v/v) to yield the pure product **106b'** as a white crystalline solid. The chemical synthesis of compound **106b'** is outlined in Scheme 25.

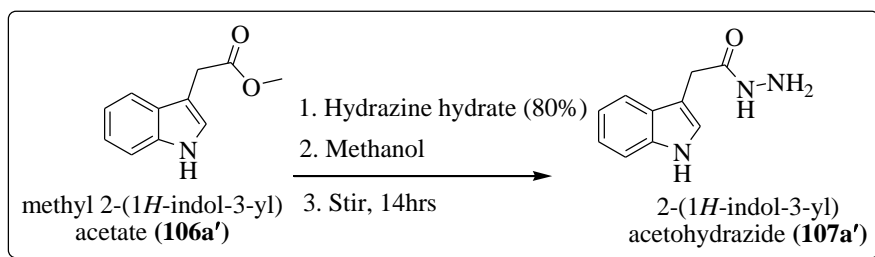
Scheme 25: Chemical reactions for Synthesis of compound 106b'



### 3.2.2.3. Synthesis of 2-(1*H*-indol-3-yl)acetohydrazide (**107a'**)

In a 100 mL round-bottom flask, a solution of methyl 2-(1*H*-indol-3-yl)acetate (**106a'**) (0.6 g; 3.2 mmol) in 20 mL of methanol was combined with hydrazine monohydrate (80%; 5 mL). The reaction mixture was stirred at room temperature for 14 hours. The progress of the reaction was monitored by thin-layer chromatography using a suitable solvent system (Chloroform/methanol, 8:2 v/v). Upon completion, the methanol was removed from the reaction mixture using a rotary evaporator to obtain the acid hydrazide. The resulting precipitate was filtered, washed with cold n-hexane, and air-dried to yield compound **107a'** as product. The product was further purified by column chromatography using a solvent mixture of chloroform/methanol (9:1 v/v) to afford the pure product **107a'** as a brownish crystalline solid. The synthesis of compound **107a'** is described in Scheme 26.

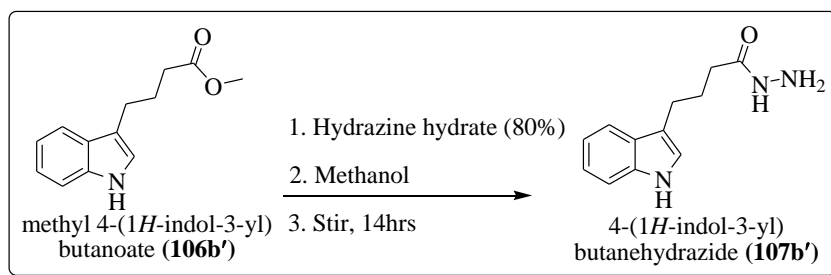
Scheme 26: Chemical reactions for Synthesis of compound 107a'



### 3.2.2.4. Synthesis of 4-(1*H*-indole-3-yl)butaneydrazide (**107b'**)

In a 100 mL round-bottom flask, a solution of methyl 4-(1*H*-indol-3-yl)butanoate (**106b'**) (0.6 g; 2.7 mmol) in 20 mL of methanol was combined with hydrazine monohydrate (80%; 5 mL). The reaction mixture was stirred at room temperature for 14 hours. The progress of the reaction was monitored by thin-layer chromatography using a suitable solvent system (Chloroform/methanol, 8:2 v/v). Upon completion, the methanol was removed from the reaction mixture using a rotary evaporator to obtain the acid hydrazide. The resulting precipitate was filtered, washed with cold n-hexane, and air-dried to yield compound **107b'** as product. The product was further purified by column chromatography using a solvent mixture of chloroform/methanol (9:1 v/v) to afford the pure product **107b'** as a white crystalline solid. The synthesis of compound **107b'** is described in Scheme 27.

Scheme 27: Chemical reactions for Synthesis of compound 107b'

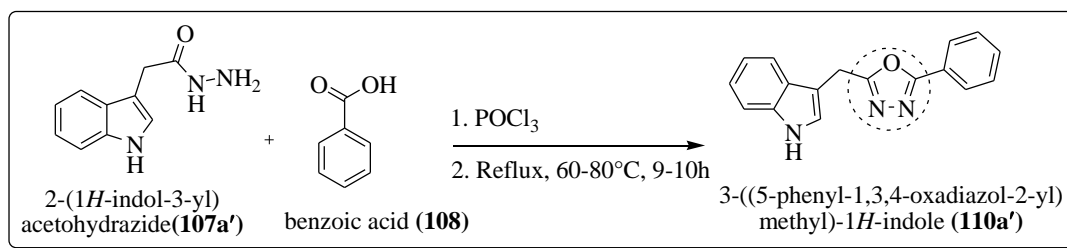


### 3.2.3. Synthesis of the Target Compounds

#### 3.2.3.1. Synthesis of 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-indole (**110a'**)

In a clean and dry 100 mL round-bottom flask, 2-(1H-indol-3-yl)acetohydrazide (**107a'**) (0.4 g; 2.1 mmol) was dissolved by the slow addition of phosphorus oxychloride (2.5 mL) at room temperature. Benzoic acid (**108**) (0.28 g; 2.3 mmol) was then introduced to the solution, and the resulting mixture was refluxed with stirring for 9-10 hours at 60-80°C. The progress of the reaction was monitored by thin-layer chromatography using a suitable solvent system (n-hexane/ethyl acetate, 8:2 v/v). Upon completion of the reaction, the mixture was cooled to room temperature and poured over crushed ice while stirring. Neutralization of the mixture was achieved by adding a 10% aqueous sodium bicarbonate solution (20 mL). The resulting precipitate was filtered, washed with distilled water several times, and then dried. The product obtained was subsequently purified by column chromatography using a solvent mixture of n-hexane/ethyl acetate (9:1 v/v) to yield the pure product **110a'** as a white crystalline solid. The chemical synthesis of compound **110a'** is outlined in Scheme 28.

Scheme 28: Chemical reactions for Synthesis of compound 110a'

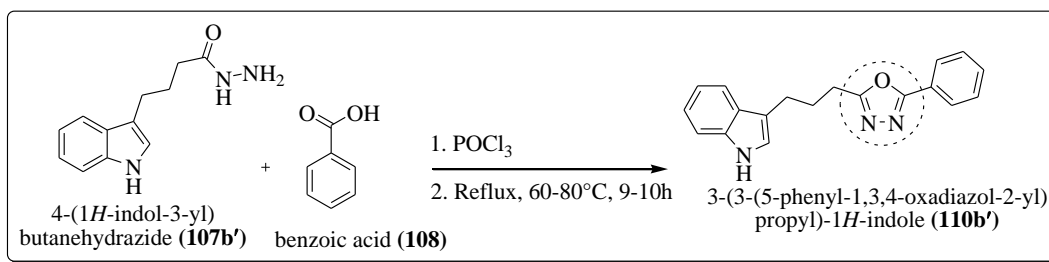


#### 3.2.3.2. Synthesis of 3-(3-(5-phenyl-1,3,4-oxadiazol-2-yl)propyl)-1H-indole (**110b'**)

In a clean and dry 100 mL round-bottom flask, 4-(1H-indole-3-yl)butanehydrazide (**107b'**) (0.4 g; 1.8 mmol) was dissolved by the slow addition of phosphorus oxychloride (2.5 mL) at room temperature. Benzoic acid (**108**) (0.28 g; 2.3 mmol) was then introduced to the solution, and the

resulting mixture was refluxed with stirring for 9-10 hours at 60-80°C. The progress of the reaction was monitored by thin-layer chromatography using a suitable solvent system (n-hexane/ethyl acetate, 8:2 v/v). Upon completion of the reaction, the mixture was cooled to room temperature and poured over crushed ice while stirring. Neutralization of the mixture was achieved by adding a 10% aqueous sodium bicarbonate solution (20 mL). The resulting precipitate was filtered, washed with distilled water multiple times, and then dried. The product obtained was subsequently purified by column chromatography using a solvent mixture of n-hexane/ethyl acetate (9:1 v/v) to yield the pure product **110b'** as a white crystalline solid. The synthesis of compound **110b'** is described in Scheme 29.

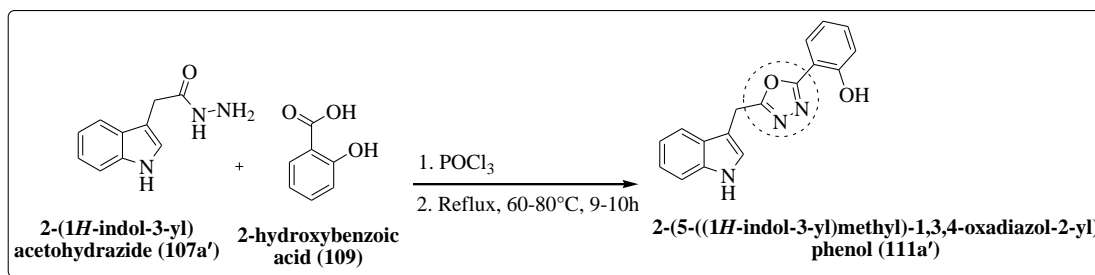
Scheme 29: Chemical reactions for Synthesis of compound 110b'



### 3.2.3.3. Synthesis of 2-(5-((1*H*-indol-3-yl)methyl)-1,3,4-oxadiazol-2-yl)phenol (**111a'**)

In a clean, dry 100 mL round bottom flask, 2-(1*H*-indol-3-yl)acetohydrazide (**107a'**) (0.4 g; 2.1 mmol) was dissolved by adding phosphorus oxychloride (2.5 mL) dropwise at room temperature. Salicylic acid (**109**) (0.36 g; 2.6 mmol) was added to the solution, and the mixture was refluxed with stirring for 9-10 hours at 60-80°C. The progress of the reaction was monitored by TLC using a suitable solvent system (n-hexane/ethyl acetate, 8:2 v/v). Upon completion of the reaction, the mixture was cooled to room temperature and poured over crushed ice with stirring. The resulting mixture was neutralized with a 10% aqueous sodium bicarbonate solution (20 mL). The precipitate formed was filtered, washed with distilled water several times, and then dried. The product obtained was purified by column chromatography using a solvent mixture of n-hexane/ethyl acetate (9:1 v/v) to yield the pure product **111a'** as a white crystalline solid. The synthesis of compound **111a'** is outlined in Scheme 30.

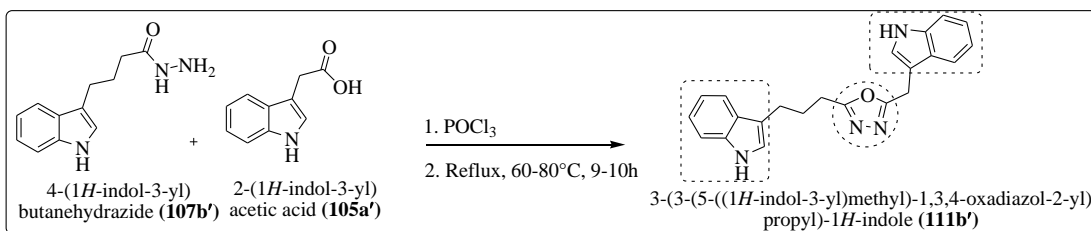
Scheme 30: Chemical reactions for Synthesis of compound 111a'



### 3.2.3.4. Synthesis of 3-(3-(5-((1*H*-indol-3-yl)methyl)-1,3,4-oxadiazol-2-yl)propyl)-1*H*-indole (**111b'**)

In a clean, dry 100 mL round bottom flask, 4-(1*H*-indole-3-yl)butaneydrazide (**107b'**) (0.4 g; 1.8 mmol) was dissolved by adding phosphorus oxychloride (2.5 mL) dropwise at room temperature. Subsequently, 2-(1*H*-indol-3-yl)acetic acid (**105a'**) (0.38 g; 2.2 mmol) was added to the solution, and the mixture was refluxed with stirring for 9-10 hours at 60-80°C. The progress of the reaction was monitored by TLC using an appropriate solvent (n-hexane/ethyl acetate, 8:2 v/v). Once the reaction was complete, the mixture was cooled to room temperature and poured over crushed ice with stirring. The resulting mixture was neutralized with a 10% aqueous sodium bicarbonate solution (20 mL). The precipitate formed was filtered, washed several times with distilled water, and then dried. The product obtained was purified by column chromatography using a solvent mixture of n-hexane/ethyl acetate (9:1 v/v) to yield the pure product **111b'** as a reddish solid. The synthesis of compound **111b'** is outlined in Scheme 31.

Scheme 31: Chemical reactions for Synthesis of compound 111b'



### 3.3. *In vitro* antibacterial activity tests

The synthesized compounds **107a'**, **107b'**, **110a'**, **111a'**, **110b'**, and **111b'** were tested for their antibacterial activity against four bacterial strains: *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), and *Streptococcus pyogenes* (*S. pyogenes*). The *in vitro* antibacterial susceptibility testing was conducted using the disc diffusion method as described in reference [191]. Mueller Hinton agar medium was prepared by dissolving

38 g of the medium in 1000 mL of distilled water, followed by autoclaving at 121 °C for 15 minutes. The autoclaved medium was then poured into sterile plates (20-25 mL per plate) and allowed to solidify under sterile conditions at room temperature. Subsequently, the plates were inoculated with an overnight culture of approximately  $1.5 \times 10^8$  CFU/mL by swabbing evenly onto the surface of the medium using a sterile cotton swab.

The synthesized compounds were dissolved in DMSO at a concentration of 150 mg/mL. Discs approximately 6 mm in diameter were prepared using Whatman filter paper no. 1. The sterile discs were impregnated with the synthesized compounds and placed on the surface of Mueller Hinton agar using sterile forceps, ensuring contact with the medium. Tetracycline was used as a positive control, while DMSO served as the negative control. The plates were then inverted and incubated at 37 °C for 24 hours. Following incubation, the inhibition zones produced by the synthesized compounds were assessed by measuring the diameter (in mm) of the clear zone around the discs against the test organisms using a ruler. The inhibition zone of each synthesized compound was measured three times. The antibacterial analysis data generated by triplicate measurements were reported as mean  $\pm$  standard deviation. The antibacterial activity was tested at Adama Science and Technology University, Department of Applied Biology by Yeshane Adimasu Lemenh (Microbiologist).

### 3.4. *In vitro* antioxidant activity Assay

#### 3.4.1. DPPH Assay

The DPPH free radical is an organic nitrogen radical known for its vibrant purple color. When this radical is exposed to an antioxidant, its color changes from purple to yellow, forming the corresponding hydrazine (Figure 16). The effectiveness of antioxidants in reducing DPPH can be determined by observing the decrease in absorbance at 515-528 nm. The outcomes are typically presented as  $IC_{50}$  values or as a percentage of DPPH• scavenging at a specific antioxidant concentration for all samples [192].

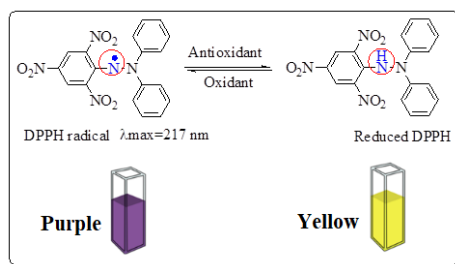


Figure 16: Reaction between DPPH• and antioxidant to form reduced DPPH

### 3.4.2. Procedure for the DPPH assay

The DPPH solution was prepared by accurately measuring 100 mg of DPPH using a chemical balance, dissolving it in 1000 mL of 99.5% ethanol, and then allowing it to sit in darkness for 2 hours. The synthesized compounds **107a'**, **107b'**, **110a'**, **111a'**, **110b'**, and **111b'** were dissolved in methanol at a concentration of 20 mg/L, 40 mg/L, 60 mg/L, and 80 mg/L respectively. In a separate test tube, 1,000  $\mu$ L of DPPH solution was combined with 800  $\mu$ L of Tris-HCl buffer (pH 7.4), followed by the rapid addition of 200  $\mu$ L of each test sample solution. The mixture was then kept at room temperature for 30 minutes, and the absorbance of each solution at 517 nm was measured. A blank solution consisting of 1,200  $\mu$ L of ethanol and 800  $\mu$ L of Tris-HCl buffer (pH 7.4) was used for reference. Ascorbic acid was used as a reference standard. The control was carried without addition of DPPH and the synthesized compounds.

The radical scavenging activity (%) was obtained from the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{A1 - A2}{A1} \times 100$$

Where A1 is the absorbance of the addition of ethanol instead of testing sample and A2 is the absorbance of testing sample solution.

### 3.4.3. Calculation of IC<sub>50</sub>

The IC<sub>50</sub> for each analytical sample was determined as follows: Inhibition ratios (y) were plotted against sample concentrations (x) at four data points, and a regression line ( $y = ax + b$ ) was fitted to the data. To calculate the IC<sub>50</sub> value, an interpolation method was used by connecting two points around the 50% inhibition with a straight line on the slightly curved inhibition curve. The two points, corresponding to a 50% inhibition ratio, were identified for the interpolation. The resulting regression line ( $Y = AX + B$ ) did not need to pass through the origin. The sample concentration (X) was calculated by substituting the value of Y as 50 in the regression equation  $Y = AX + B$ . The interpolated regression graph of the synthesized compounds **107a'**, **107b'**, **110a'**, **110b'**, **111a'** and **111b'** and ascorbic acid is presented in Appendix 1.

## CHAPTER FOUR

### 4. RESULTS AND DESCUSSION

#### 4.1. Methods

The general methods used to synthesize the compounds are described in Scheme 21. In the first step, the synthesis began with the esterification of 2-(1*H*-indol-3-yl)acetic acid (**105a'**) and 4-(1*H*-indol-3-yl)butanoic acid (**105b'**) using a small amount of H<sub>2</sub>SO<sub>4</sub> as a catalyst in excess methanol. The excess methanol was used to favor the formation of the products, namely methyl 2-(1*H*-indol-3-yl)acetate (**106a'**) and methyl 4-(1*H*-Indol-3-yl)butanoate (**106b'**), due to the reversible nature of the reaction. After refluxing the reaction mixture for 8 hours, the product was isolated through solvent extraction following the addition of a 10% aqueous Na<sub>2</sub>CO<sub>3</sub> to neutralize any remaining catalyst and unreacted starting acid. The acids' salts moved into the aqueous layer, while the resulting ester remained in the organic phase after solvent extraction. Thus, the compounds methyl 2-(1*H*-indol-3-yl)acetate (**106a'**) and methyl 4-(1*H*-indol-3-yl)butanoate (**106b'**) were obtained as a reddish-brown liquid and white crystalline solid, respectively. In the second step of the reaction, esters **106a'** and **106b'** were treated with nucleophilic hydrazine monohydrate in methanol as the solvent. The mixture was stirred at room temperature for 14 hours to allow the reaction to proceed. Once the conversion was complete, the methanol was evaporated, and the resulting product was purified by washing with cold n-hexane. This purification process led to the successful isolation of the corresponding 2-(1*H*-indol-3-yl)acetohydrazide (**107a'**) and 4-(1*H*-indole-3-yl)butaneydrazide (**107b'**) were obtained as a brownish crystal and white crystalline solid, respectively in a favorable yield. In the third step, cyclization reactions were conducted to form hetrocyclic rings (1,3,4-oxadiazole ring) by refluxing acid hydrazide **107a'** with compounds **108** and **109** in the presence of phosphorus oxychloride for 9-10 hours, resulting in the formation of compounds **110a'** and **111a'**, respectively. Additionally, a similar cyclization reaction was carried out to form hetrocyclic rings (1,3,4-oxadiazole ring) by refluxing acid hydrazide **107b'** with compounds **108** and **105a'** in the presence of phosphorus oxychloride for 9-10 hours, leading to the synthesis of compounds **110b'** and **111b'**, respectively in a good yield.

The confirmation of the compound structures were achieved through the analysis of melting point determination and interpretation of UV-Vis and FT-IR spectra, providing crucial insights into the chemical composition of the synthesized compounds **106a'**, **106b'**, **107a'**, **107b'**, **110a'**,

**111a'**, **110b'**, and **111b'**. The physical properties of the synthesized compounds are described in Table 1.

Table 1: Physical properties of the synthesized compounds

Compounds	Molecular formula	Colour	M.P (°C)	Yield (%)	R <sub>f</sub> value
<b>106a'</b>	C <sub>11</sub> H <sub>11</sub> NO <sub>2</sub>	Reddish-brown liquid	125	65	0.6
<b>106b'</b>	C <sub>13</sub> H <sub>15</sub> NO <sub>2</sub>	White crystalline solid	74-76	70	0.52
<b>107a'</b>	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O	Brownish crystals	144	63	0.57
<b>107b'</b>	C <sub>12</sub> H <sub>15</sub> N <sub>3</sub> O	White crystalline solid	132-134	72	0.54
<b>110a'</b>	C <sub>17</sub> H <sub>13</sub> N <sub>3</sub> O	White crystalline solid	148	62	0.46
<b>110b'</b>	C <sub>19</sub> H <sub>17</sub> N <sub>3</sub> O	White crystalline solid	136-140	57	0.48
<b>111a'</b>	C <sub>17</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>	White crystalline solid	158-160	55	0.44
<b>111b'</b>	C <sub>22</sub> H <sub>20</sub> N <sub>4</sub> O	Reddish-brown solid	164-166	53	0.38

Mobile phase for TLC: For **106a'** & **106b'**: n-hexane: ethyl acetate (7:3), for **107a'** & **107b'**: chloroform: methanol (8:2) and for **110a'**, **111a'**, **110b'**, and **111b'**: n-hexane: ethyl acetate (8:2)

## 4.2. Partial Characterization of the Synthesize Compounds

The products' structures were partially characterized through the analysis of IR and UV-visible spectra. The spectroscopic data from both IR and UV-Visible analyses are in good agreement with the structure of the synthesized compound.

### 4.2.1. Interpretation of Methyl 2-(1*H*-indol-3-yl)acetate (**106a'**)

In the UV-Vis absorption spectrum (Appendix 2) recorded in methanol, two distinct bands were observed for compound **106a'** in Figure 17. The band at  $\lambda_{\text{max}} = 280$  nm is attributed to the  $\pi \rightarrow \pi^*$  transitions of the C=C group, while the band at  $\lambda_{\text{max}} = 224$  nm is attributed to both  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions of the carbonyl C=O group.

The FT-IR absorption spectrum (KBr pellet,  $\nu$ ,  $\text{cm}^{-1}$ , Appendix 3) of the compound reveals significant absorption features (Figure 17). A high-intensity single band at  $3416 \text{ cm}^{-1}$  is attributed to the N-H stretch of the amine group. Absorption bands at  $3056$  and  $2952 \text{ cm}^{-1}$  correspond to the presence of aromatic and aliphatic C-H stretching, respectively. An intense absorption band at  $1732 \text{ cm}^{-1}$  indicates the presence of the carbonyl C=O group stretching. Additionally, medium absorption bands at  $1622 \text{ cm}^{-1}$  are associated with the aromatic C=C double bond stretch, while the medium absorption band at  $1096$  and  $1010 \text{ cm}^{-1}$  are related to C-O-C stretching.

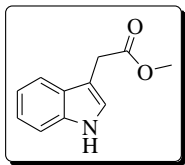


Figure 17: Chemical structure of the compound 106a'

#### 4.2.2. Interpretation of methyl 4-(1*H*-indole-3-yl)butanoate (106b')

In the UV-Vis absorption spectrum (Appendix 4) of the compound **106b'** (Figure 18) recorded in methanol, a single band at  $\lambda_{\text{max}} = 278$  nm was observed, attributed to the  $\pi \rightarrow \pi^*$  transitions of the C=C group.

The FT-IR absorption spectrum (KBr pellet,  $\nu$ ,  $\text{cm}^{-1}$ , Appendix 5) of the compound reveals important absorption bands (Figure 18). A high-intensity single band at  $3338 \text{ cm}^{-1}$  is associated with the N-H stretch of the amine group. Absorption bands at  $3048$  and  $2928 \text{ cm}^{-1}$  are indicative of aromatic and methyl C-H stretching, respectively. An intense absorption band at  $1718 \text{ cm}^{-1}$  indicates the presence of the carbonyl C=O group stretching. Additionally, medium absorption bands at  $1556 \text{ cm}^{-1}$  are attributed to the presence of the aromatic C=C double bond stretch, while the medium absorption band at  $1184 \text{ cm}^{-1}$  is related to C-O-C stretching.

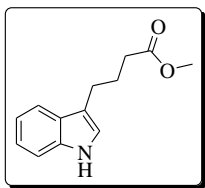


Figure 18: Chemical structure of compound 106b'

#### 4.2.3. Interpretation of 2-(1*H*-indol-3-yl)acetohydrazide (107a')

The UV-Vis absorption spectrum (Appendix 6) of compound **107a'** (Figure 19) in methanol displayed two distinct electronic absorption bands. The band at  $\lambda_{\text{max}} = 280$  nm is attributed to the  $\pi \rightarrow \pi^*$  transitions of the C=C group, while the band at  $\lambda_{\text{max}} = 228$  nm is attributed to both  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions of the carbonyl C=O group.

The FT-IR absorption spectrum (KBr pellet,  $\nu$ ,  $\text{cm}^{-1}$ , Appendix 7) of the compound reveals several important absorption features (Figure 19). A medium-intensity band at  $3304$  and  $3222 \text{ cm}^{-1}$  are associated with the asymmetric and symmetric N-H stretch of the amine group respectively. An absorption band at  $3054 \text{ cm}^{-1}$  indicates aromatic C-H stretching. The intense absorption band at  $1668 \text{ cm}^{-1}$  corresponds to the presence of the carbonyl C=O group of an

amide. Additionally, medium absorption bands at  $1552\text{ cm}^{-1}$  are attributed to the aromatic C=C double bond stretch, while medium band at  $1451\text{ cm}^{-1}$  is related to C-N stretching.

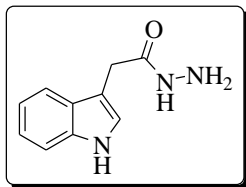


Figure 19: Chemical structure of compound 107a'

#### 4.2.4. Interpretation of 4-(1H-indol-3-yl)butanehydrazide (107b')

The UV-Vis absorption spectrum (Appendix 8) of compound **107b'** (Figure 20) recorded in methanol displayed two distinct electronic absorption bands. The band at  $\lambda_{\text{max}} = 282\text{ nm}$  is attributed to the  $\pi \rightarrow \pi^*$  transitions of the C=C group, while the band at  $\lambda_{\text{max}} = 228\text{ nm}$  is attributed to both  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions of the carbonyl C=O group.

The FT-IR absorption spectrum (KBr pellet,  $\nu$ ,  $\text{cm}^{-1}$ , Appendix 9) shows several significant features (Figure 20). There is a medium intensity band at  $3324$  and  $3268\text{ cm}^{-1}$  attributed to the asymmetric and symmetric N-H stretch of the amine group respectively. An absorption band at  $3054\text{ cm}^{-1}$  is indicative of aromatic C-H stretching. The medium absorption band at  $1626\text{ cm}^{-1}$  corresponds to the presence of the carbonyl C=O group of an amide. Additionally, medium absorption bands at  $1528\text{ cm}^{-1}$  are associated with the aromatic C=C double bond stretch, while medium band at  $1458\text{ cm}^{-1}$  is related to C-N stretching.

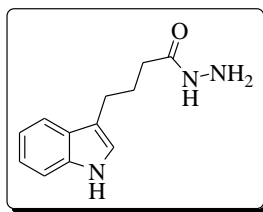


Figure 20: Chemical structure of compound 107b'

#### 4.2.5. Interpretation of 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-indole (110a')

The UV-Vis absorption spectrum (Appendix 10) of compound **110a'** (Figure 21) in methanol displayed two distinct electronic absorption bands. The absorption band at  $\lambda_{\text{max}} = 280\text{ nm}$  suggests the presence of an aromatic ring, while the absorption band at  $\lambda_{\text{max}} = 212\text{ nm}$  indicates conjugation, corresponding to the  $\pi\text{-}\pi^*$  transition of C=C double bond and C=N group.

The FT-IR absorption spectrum (KBr pellet,  $\nu$ ,  $\text{cm}^{-1}$ , Appendix 11) shows several important features (Figure 21). There is a medium intensity band at  $3452\text{ cm}^{-1}$  attributed to the N-H stretch

of the amine group. An absorption band at  $3054\text{ cm}^{-1}$  is indicative of aromatic C-H stretching. The medium absorption band at  $1702\text{ cm}^{-1}$  corresponds to the C=N stretching of the oxadiazole ring. Additionally, medium absorption bands at  $1606\text{ cm}^{-1}$  are associated with the aromatic C=C double bond stretch. The bands at  $1548$ ,  $1482$ , and  $1446\text{ cm}^{-1}$  are attributed to oxadiazole ring stretching, while the absorption band at  $1266$  and  $1072\text{ cm}^{-1}$  are related to C-O-C stretching.

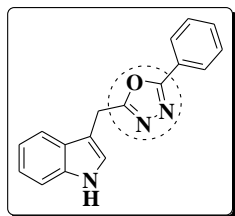


Figure 21: Chemical structure of compound 110a'

#### 4.2.6. Interpretation of 3-(3-(5-phenyl-1,3,4-oxadiazol-2-yl)propyl)-1H-indole (110b')

The UV-Vis absorption spectrum (Appendix 12) of compound **110b'** (Figure 22) in methanol revealed two distinct electronic absorption bands. The absorption band at  $\lambda_{max} = 278\text{ nm}$  suggests the presence of an aromatic ring, while the absorption band at  $\lambda_{max} = 212\text{ nm}$  indicates characteristics of conjugation, corresponding to the  $\pi\text{-}\pi^*$  transition of C=C double bond of aromatic ring and C=N group.

The FT-IR absorption spectrum (KBr pellet,  $\nu$ ,  $\text{cm}^{-1}$ , Appendix 13) indicates several key features (Figure 22). There is a medium intensity band at  $3450\text{ cm}^{-1}$  attributed to the N-H stretch of the amine group. A medium absorption band at  $3056\text{ cm}^{-1}$  is indicative of aromatic C-H stretching. The absorption band at  $1698\text{ cm}^{-1}$  corresponds to the C=N stretching of the oxadiazole ring. Additionally, medium absorption bands at  $1606\text{ cm}^{-1}$  are associated with the aromatic C=C double bond stretch. The bands at  $1548$ ,  $1482$ , and  $1448\text{ cm}^{-1}$  are attributed to oxadiazole stretching, while the absorption band at  $1264$  and  $1072\text{ cm}^{-1}$  are related to C-O-C stretching.

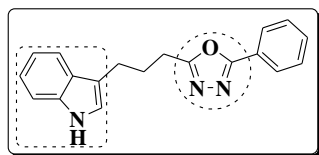


Figure 22: Chemical structure of compound 110b'

#### 4.2.7. Interpretation of 2-(5-((1H-indol-3-yl)methyl)-1,3,4-oxadiazol-2-yl)phenol (111a')

The UV-Vis absorption spectrum (Appendix 14) of compound **111a'** (Figure 23) in methanol displayed two distinct electronic absorption bands. The absorption band at  $\lambda_{max} = 251\text{ nm}$

suggests the presence of an aromatic ring, while the absorption band at  $\lambda_{max} = 221 \text{ nm}$  indicates conjugation, corresponding to the  $\pi\text{-}\pi^*$  transition of C=C double bond of aromatic ring and C=N group.

The FT-IR absorption spectrum (KBr pellet,  $\nu$ ,  $\text{cm}^{-1}$ , Appendix 15) shows several important features (Figure 23). There is a broad intensity band at  $3268 \text{ cm}^{-1}$  attributed to the O-H stretch of the 2-hydroxy group. An absorption band at  $3059 \text{ cm}^{-1}$  is indicative of aromatic C-H stretching. The medium absorption band at  $1623 \text{ cm}^{-1}$  corresponds to the C=N stretching of the oxadiazole ring. Additionally, medium absorption bands at  $1593 \text{ cm}^{-1}$  are associated with the aromatic C=C double bond stretch. The bands at  $1593$ ,  $1547$ ,  $1483$ , and  $1409 \text{ cm}^{-1}$  are attributed to oxadiazole ring stretching, while the absorption band at  $1259 \text{ cm}^{-1}$  is related to C-O-C stretching.

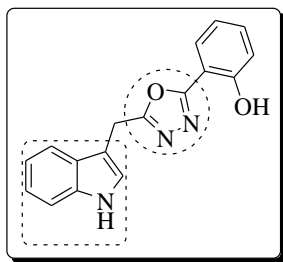


Figure 23: Chemical structure of compound 111a'

#### 4.2.8. Interpretation of 3-(3-(5-((1H-indol-3-yl)methyl)-1,3,4-oxadiazol-2-yl)propyl)-1H-indole (111b')

The UV-Vis absorption spectrum (Appendix 16) of compound **111b'** (Figure 24) in methanol revealed two distinct electronic absorption bands. The absorption band at  $\lambda_{max} = 252 \text{ nm}$  suggests the presence of an aromatic ring, while the absorption band at  $\lambda_{max} = 222 \text{ nm}$  indicates characteristics of conjugation, corresponding to the  $\pi\text{-}\pi^*$  transition of C=C double bond and C=N group.

The FT-IR absorption spectrum (KBr pellet,  $\nu$ ,  $\text{cm}^{-1}$ , Appendix 17) indicates several key features (Figure 24). There is a weak intensity band at  $3446 \text{ cm}^{-1}$  attributed to the N-H stretch of the amine group of indole ring. A medium absorption band at  $3063$  and  $3036 \text{ cm}^{-1}$  are indicative of aromatic C-H stretching. The absorption band at  $1681 \text{ cm}^{-1}$  corresponds to the C=N stretching of the oxadiazole ring. Additionally, medium absorption bands at  $1603 \text{ cm}^{-1}$  are associated with the aromatic C=C double bond stretch. The bands at  $1547$ ,  $1480$ ,  $1264$ , and  $1025 \text{ cm}^{-1}$  are attributed to oxadiazole ring stretching, while the absorption band at  $1264$ ,  $1072$  and  $1025 \text{ cm}^{-1}$  are related to C-O-C stretching.

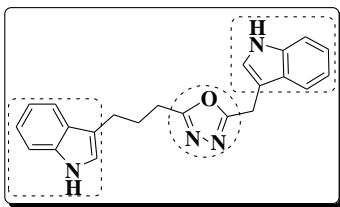


Figure 24: Chemical structure of compound 111b'

### 4.3. Antibacterial activity test results

The antibacterial activity test of the synthesized compounds were evaluated using the disc diffusion method to measure the zone of inhibition on cultured bacteria on petri dishes (Appendix 18). The diameter and average diameter of the zone of inhibition (mm) were recorded and compared with standard drug tetracycline. The results are summarized in Table 2 (Appendix 19) and Table 3.

Table 2: The average diameter (mm) of the Zone of Inhibition for synthesized compounds was measured against selected bacterial strains.

Compound	Concentration (mg/mL)	Bacteria Species and Zone of Inhibitions in mm			
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. pyogene</i>
<b>107a'</b>	150	7.46 ± 0.50	7.36 ± 0.32	6.93 ± 0.40	8.30 ± 0.26
<b>107b'</b>	150	8.33 ± 0.28	8.50 ± 0.10	7.70 ± 0.26	9.43 ± 0.37
<b>110a'</b>	150	7.96 ± 0.45	8.36 ± 0.32	7.50 ± 0.50	8.36 ± 0.15
<b>110b'</b>	150	8.63 ± 0.35	9.10 ± 0.36	8.33 ± 0.30	10.56 ± 0.20
<b>111a'</b>	150	7.70 ± 0.30	8.43 ± 0.40	7.40 ± 0.40	9.40 ± 0.17
<b>111b'</b>	150	11.57 ± 0.15	10.73 ± 0.2	9.20 ± 0.26	11.3 ± 0.28
<b>Tetracycline (+ve control)</b>	150	13 ± 0.5	13.3 ± 0.25	12.36 ± 0.32	14.53 ± 0.06

The values are presented as the mean ± standard deviation of three replicates. The size of 6 mm means that no activity (there was no zone of inhibition)

The results showed that all tested compounds demonstrated varying levels of antibacterial activity, with inhibition zones ranging from 6.93±0.40 to 11.57±0.15 mm as detailed in Table 3 and Figure 25 (see Appendix 20). Compound **111b'** and **110b'** demonstrated potent inhibitory activity against all bacterial strains used in the study, particularly compound **111b'**, which exhibited very good inhibitory potential with inhibition zones of 11.57±0.15, 11.3±0.28 and 10.73±0.2 mm against *E. coli*, *S. pyogenes* and *S. aureus*, respectively, comparable to the inhibition zones of tetracycline (13±0.5, 14.53±0.06 and 13.3±0.25 mm) against the same strains.

Compound **107b'** also displayed very good antibacterial activity with an inhibition zone of  $9.43\pm 0.37$  mm against *S. pyogenes*.

Compounds **110a'** and **111a'** exhibited better activity against *S. pyogenes*, with inhibition zones of  $8.36\pm 0.15$  and  $9.40\pm 0.17$  mm, respectively, and moderate activity against *S. aureus*, with inhibition zones of  $8.36\pm 0.15$  and  $8.43\pm 0.40$  mm, respectively, comparable to the standard drug tetracycline with an inhibition zone of  $13.3\pm 0.25$  mm. Additionally, compounds **110a'** and **111a'** also demonstrated moderate activity against *E. coli*, with inhibition zones of  $7.96\pm 0.45$  and  $7.70\pm 0.30$  mm, respectively, compared to tetracycline with an inhibition zone of  $13\pm 0.5$  mm. The remaining compounds also demonstrated moderate antibacterial potential overall. To explore the full potential of indole-containing oxadiazole moieties, further tests on a larger variety of bacterial strains are recommended for the discovery of new antibacterial agents.

#### **4.3.1. Structure Activity Relationship for Antibacterial activity test results**

The structural activity relationship has been conducted for all the target compounds in this study. The compound **111b'**, having indole-methyl groups located on the 5-position of the 1,3,4-oxadiazole rings, was identified as the most potent analog within the series. The heightened inhibitory efficacy of this compound is likely attributed to the proximity of the indole-methyl groups.

When comparing compound **111b'**, which exhibited an inhibition zone of  $11.57\pm 0.15$ , with other compounds such as compound **111a'** with an inhibition zone of  $9.40\pm 0.17$  containing an ortho-hydroxyphenyl group on the 5-position of the 1,3,4-oxadiazole ring, compound **110a'** with an inhibition zone of  $8.36\pm 0.15$  containing a phenyl group on the 5-position of the 1,3,4-oxadiazole ring, and compound **110b'** with an inhibition zone of  $10.56\pm 0.20$  containing phenyl-oxadiazole-propyl groups on the 3-position of indole, it was observed that compound **111b'** is significantly more potent. This heightened potency indicates that the indole-methyl groups located on the 5-position of the 1,3,4-oxadiazole rings play a crucial role in the inhibitory potential.

When comparing compound **110a'** and **110b'**, it was found that compound **110b'** exhibited the highest inhibitory activity against all the tested bacterial strains. Interestingly, the only structural difference between the two compounds is the presence of an ethyl group attached between the 3-position of the indole and the 2-position of the oxadiazole ring. These results suggest that the antibacterial activities of the synthesized compounds increase as the number of carbons attached between the 3-position of the indole and the 2-position of the oxadiazole ring increases.

When comparing compound 110a' and 111a', it was observed that compound 110a' exhibited the highest inhibitory activity against Gram-negative bacteria, specifically *E. coli* and *P. aeruginosa*, with inhibition zones of  $7.96\pm 0.45$  mm and  $7.50\pm 0.50$  mm, respectively. Conversely, compound 111a' demonstrated the highest inhibitory activity against Gram-positive bacteria, namely *S. pyogenes* and *S. aureus*, with inhibition zones of  $9.40\pm 0.17$  mm and  $8.43\pm 0.40$  mm, respectively. Interestingly, the only structural difference between the two compounds is the presence of a hydroxyl group attached to the phenyl ring. These results indicate that an unsubstituted phenyl ring at the 5-position of the 1,3,4-oxadiazole ring enhances antibacterial activity against Gram-negative bacteria, while a substituted phenyl ring with an electron-donating group (such as hydroxyl) at the 5-position of the 1,3,4-oxadiazole ring decreases antibacterial activity against Gram-negative bacteria.

#### **4.4. Antioxidant activity assay results of the synthesized compounds**

The DPPH method is a straightforward technique used to assess the antioxidant activity of a compound by measuring the reduction in absorbance of DPPH at 517 nm. This assay involves testing synthesized compounds for their ability to scavenge free radicals by reacting with stable DPPH radicals. The decrease in DPPH absorbance at 517 nm is monitored to track the reduction of DPPH and determine the antioxidant capacity of the compounds. The DPPH radical scavenging activity was expressed as % of radical scavenging activity and  $IC_{50}$  values are tabulated in Table 4.

Table 3: % radical scavenging activities of the synthesized compounds and ascorbic acid

Compounds	Coc. (mg/L)	Absorbance	% R.S.A
<b>107a'</b>	20	0.82	60.57
	40	0.74	64.42
	60	0.64	69.23
	80	0.58	72.11
	<b>IC<sub>50</sub></b>		<b>6.1</b>
<b>107b'</b>	20	0.9	56.73
	40	0.8	61.54
	60	0.68	67.31
	80	0.57	72.59
	<b>IC<sub>50</sub></b>		<b>12.04</b>
<b>110a'</b>	20	0.75	63.94
	40	0.48	76.92
	60	0.37	82.21
	80	0.21	89.90
	<b>IC<sub>50</sub></b>		<b>5.03</b>
<b>110b'</b>	20	0.72	65.38
	40	0.51	75.48
	60	0.45	78.36
	80	0.28	86.54
	<b>IC<sub>50</sub></b>		<b>6.92</b>
<b>111a'</b>	20	0.6	71.15
	40	0.44	78.84
	60	0.31	85.09
	80	0.17	91.82
	<b>IC<sub>50</sub></b>		<b>4.95</b>
<b>111b'</b>	20	1.12	46.15
	40	0.84	59.61
	60	0.56	73.07
	80	0.32	84.61
	<b>IC<sub>50</sub></b>		<b>24.74</b>
<b>Ascorbic Acid</b>	20	1.3	37.5
	40	0.93	55.29
	60	0.72	65.38
	80	0.26	87.5
	<b>IC<sub>50</sub></b>		<b>31.44</b>

% R.S.A is % radical scavenging activities. Absorbance (A1) of control is 2.08

The DPPH radical scavenging activity was carried out at concentration of 20, 40, 60 and 80 mg/L of the synthesized compounds and standard ascorbic acid, the results were reported in a Table 4 and Figure 26. When, DPPH interacts with antioxidant compounds, the reduction in absorbance seen as a result of the reaction between antioxidant molecules and radicals progresses, leading to the elimination of the radical through hydrogen donation. The hydrogen

donation activity measured at 517 nm demonstrated a strong correlation between the concentration of compounds and the percentage of inhibition.

The DPPH radical scavenging activities of synthesized compounds **107a'**, **107b'**, **110a'**, **110b'**, **111a'**, and **111b'** were found to be **72.11%**, **72.59%**, **89.90%**, **86.54%**, **91.82%**, and **84.61%**, respectively at a concentration of 80 mg/L (Figure 26) and standard ascorbic acid was found to be **87.5%** at the same concentration. It was observed that the DPPH scavenging activity increased with increasing concentration of the synthesized compounds in the assay.

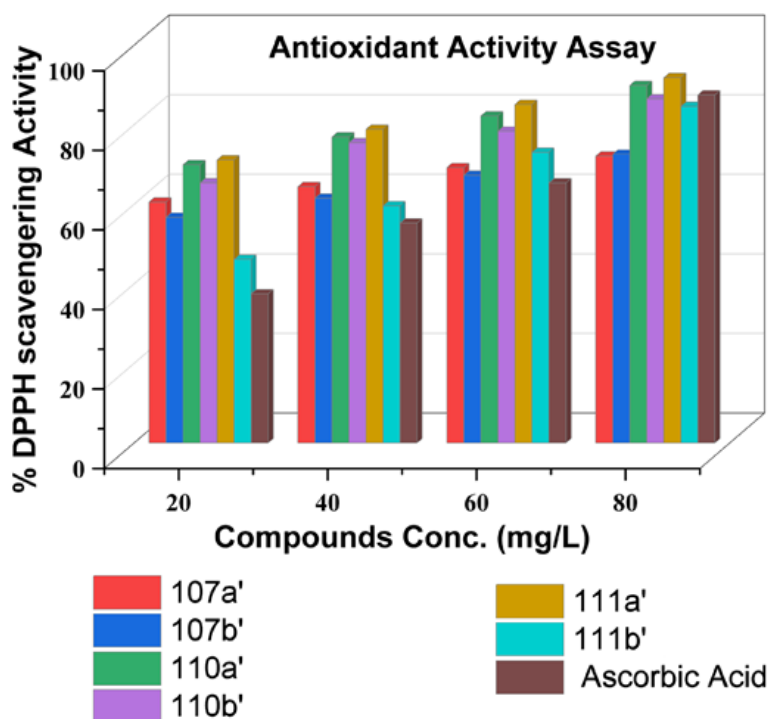


Figure 25: DPPH radical scavenging activity of synthesized compounds and Ascorbic Acid. Among the synthesized compounds **107a'**, **107b'**, **110a'**, **110b'**, **111a'**, and **111b'**, the compounds **111a'** (91.83%) and **110a'** (89.9%) demonstrated the highest DPPH free radical scavenging activity. Compounds **111a'** and **110a'** also exhibited significant DPPH free radical scavenging activity at concentrations of 20, 40, and 60 mg/L. Additionally, the compounds **110b'** (86.54%) and **111b'** (84.61%) displayed good radical scavenging activity. Conversely, the Compounds **107a'** (72.11%), **107b'** (72.59%) showed lower DPPH free radical scavenging activity compared to the other compounds.

The DPPH reducing capabilities of all synthetic compounds were assessed by determining their  $IC_{50}$  values. The antioxidant activity analysis of the six synthetic compounds revealed a significant capacity for reducing activity. The  $IC_{50}$  values for all compounds in the DPPH assays

can be found in Table 4. The  $IC_{50}$  value represents the concentration of a sample needed to scavenge 50% of the free radicals in the reaction mixture. Lower  $IC_{50}$  values indicate a more potent antioxidant activity of the compound.

As depicted in Table 4, all compounds exhibited free radical scavenging activity in the DPPH assay, with  $IC_{50}$  values ranging from 4.95 to 31.44 mg/L. Among the different concentrations (20, 40, 60, 80 mg/L) of synthesized compounds tested, compounds **111a'** and **110a'** demonstrated higher percent inhibition of DPPH with  $IC_{50}$  values of **4.95** and **5.03**, respectively, compared to the standard ascorbic acid with an  $IC_{50}$  of **31.44** and the other synthesized compounds.

#### **4.4.1. DPPH Radical Scavenging Activities and Structure–Activity Relationships**

The structural activity relationship has been conducted for all the target compounds in this study. Among the synthesized target compounds (**110a'**, **110b'**, **111a'** and **11b'**), compound **111a'** having *ortho* hydroxyl phenyl groups located on the 5-position of the 1,3,4-oxadiazole rings, was found to be highest DPPH radical scavenging activities. The heightened radical scavenging activities of this compound is likely attributed to the proximity of the *ortho* hydroxyl phenyl groups which donates hydroxyl proton to DPPH free radical shows highest antioxidant activity. When comparing compound **110a'** and **111a'**, it was found that compound **111a'** exhibited the highest percent DPPH Radical Scavenging activities (91.82%) with  $IC_{50}$  value of 4.95 as compared to compound **110a'** moderate percent DPPH Radical Scavenging activities (89.90%) with  $IC_{50}$  value of 5.03. Interestingly, the only structural difference between the two compounds is the presence of a hydroxyl group attached on the phenyl ring at the 2-position of the oxadiazole ring. The general activity pattern revealed that compounds with hydroxyl substitutions at phenyl rings were better antioxidizing agents compared to unsubstituted ones.

## CHAPTER FIVE

### 5. CONCLUSION AND RECOMMENDATION

#### 5.1. Conclusion

In the current investigation, some novel indole-containing oxadiazole derivatives were successfully synthesized via cyclization reaction, yielding between 53-72%. Partial characterizations of the synthesized compounds were performed using spectroscopic techniques including UV-Vis and FT-IR in conjunction with melting point analysis. Evaluation of the synthesized compounds for *in vitro* antibacterial activity test against four bacterial strains (*E. coli*, *P. aeruginosa*, *S. pyogenes*, and *S. aureus*) revealed that Compound **111b'** and **110b'** exhibited significant inhibitory effects against all bacterial strains tested. Compound **111b'** demonstrated notable inhibitory potential with inhibition zones measuring  $11.57\pm 0.15$ ,  $11.3\pm 0.28$ , and  $10.73\pm 0.2$  mm against *E. coli*, *S. pyogenes*, and *S. aureus*, respectively, comparable to the inhibition zones of tetracycline ( $13\pm 0.5$ ,  $14.53\pm 0.06$  and  $13.3\pm 0.25$  mm, respectively) against the same strains (Table 3). Compound **110b'** displayed moderate inhibitory activity with inhibition zones of  $10.56\pm 0.20$ ,  $9.10\pm 0.36$ , and  $8.63\pm 0.35$  mm against *S. pyogenes*, *S. aureus*, and *E. coli*, respectively, also comparable to the inhibition zones of tetracycline ( $14.53\pm 0.06$ ,  $13.3\pm 0.25$ , and  $13\pm 0.5$  mm, respectively) against the same strains (Table 3). Furthermore, the antioxidant activity of the synthesized compounds was evaluated by % radical scavenging activities and  $IC_{50}$  values. Among the synthesized compounds, compounds **111a'** and **110a'** demonstrated higher percent inhibition of DPPH (91.82% and 89.90%, respectively) with  $IC_{50}$  values of 4.95 and 5.03, respectively, compared to the standard ascorbic acid (87.5%) with an  $IC_{50}$  of 31.44 and the other synthesized compounds. Overall, the synthesized compounds show promise as potential candidates for future drug development studies, particularly in the areas of antibacterial and antioxidant research.

## 5.2. Recommendation

Building upon the findings of this study, the following recommendations are put forth:

- In this study the synthesized compounds were partially characterized using UV-Vis and FT-IR spectroscopic techniques, additional characterization utilizing  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, MS analysis and X-ray Crystallography are essential to elucidate the chemical structures of the synthesized compounds comprehensively.
- This study focused on the synthesis of a limited number of indole derivatives due to the lack of finance to purchase chemicals. Subsequent research is warranted to expand the synthesis of additional indole derivatives for evaluation of their antibacterial and antioxidant activity to substantiate their potential medicinal applications. Furthermore, exploration of other activities such as anticancer properties is also recommended.
- It is recommended to explore the potential of indole-containing oxadiazole moieties in  $\alpha$ -glucosidase inhibitory, hemolytic, and molecular docking studies.

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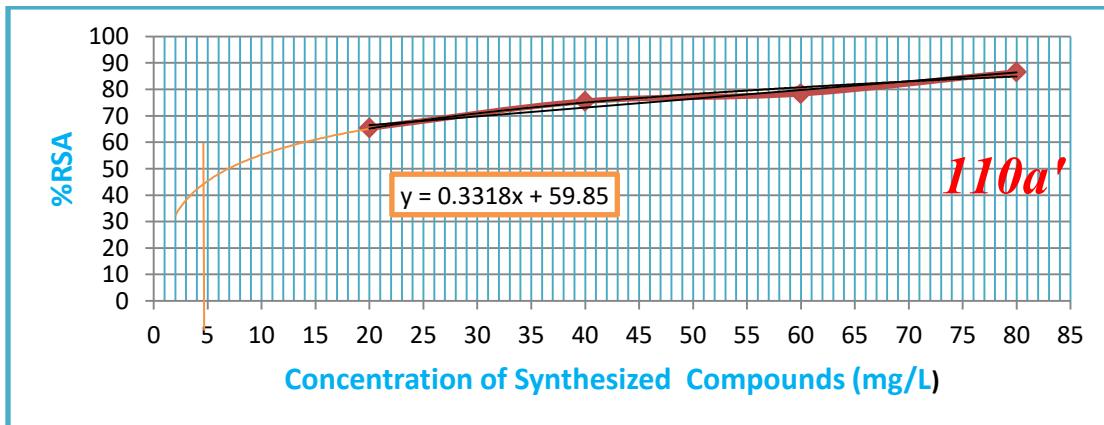
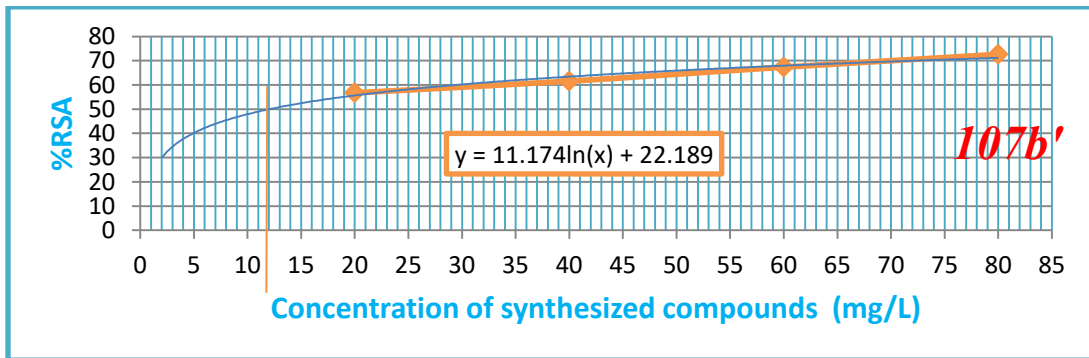
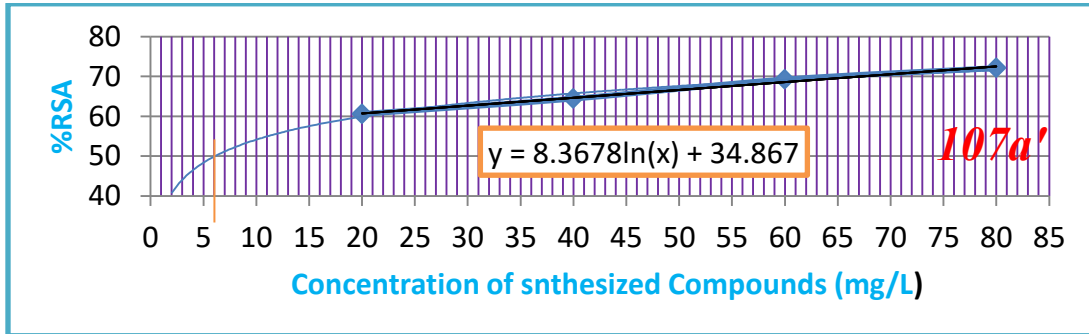
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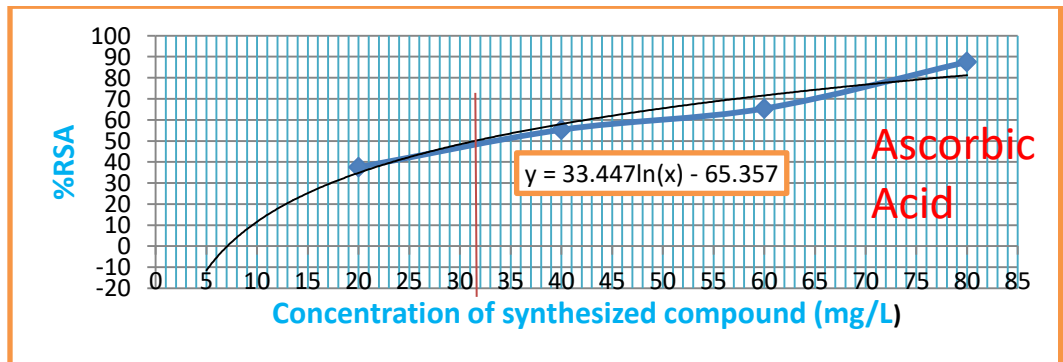
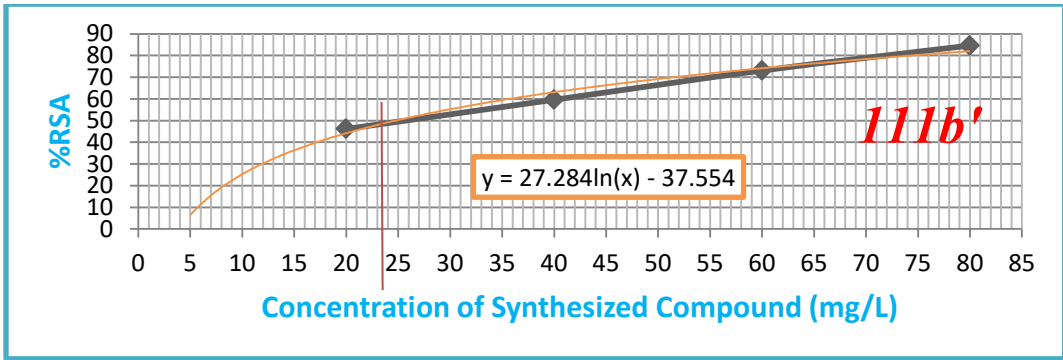
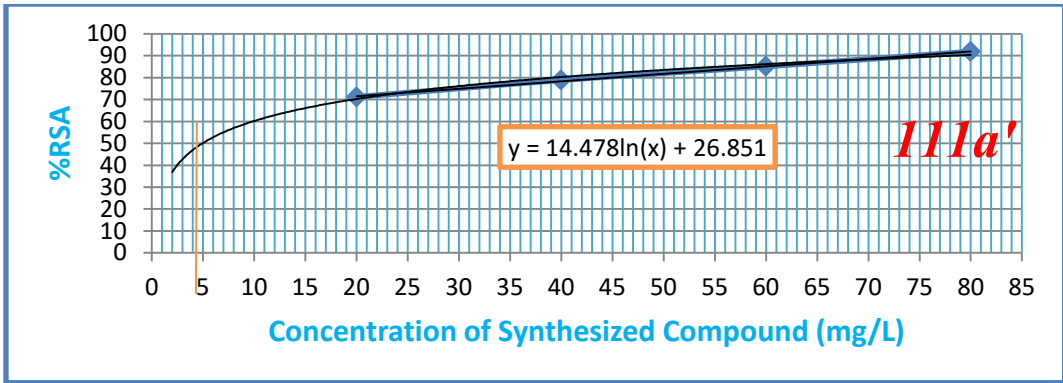
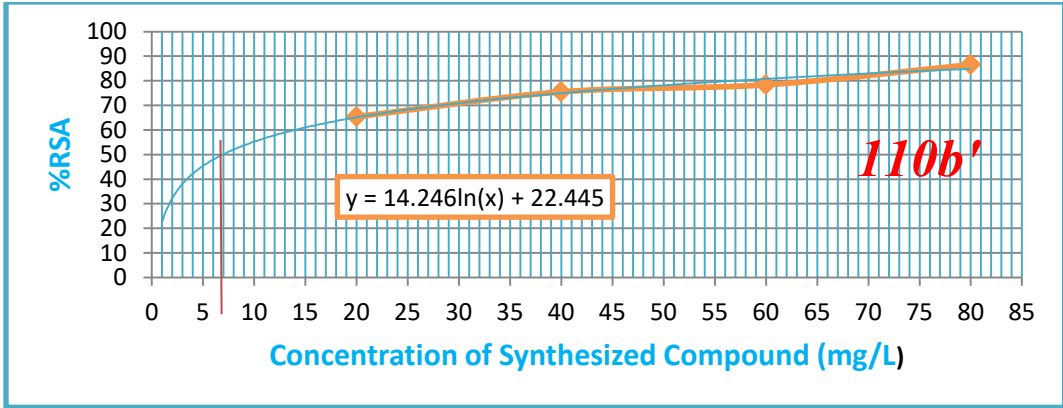
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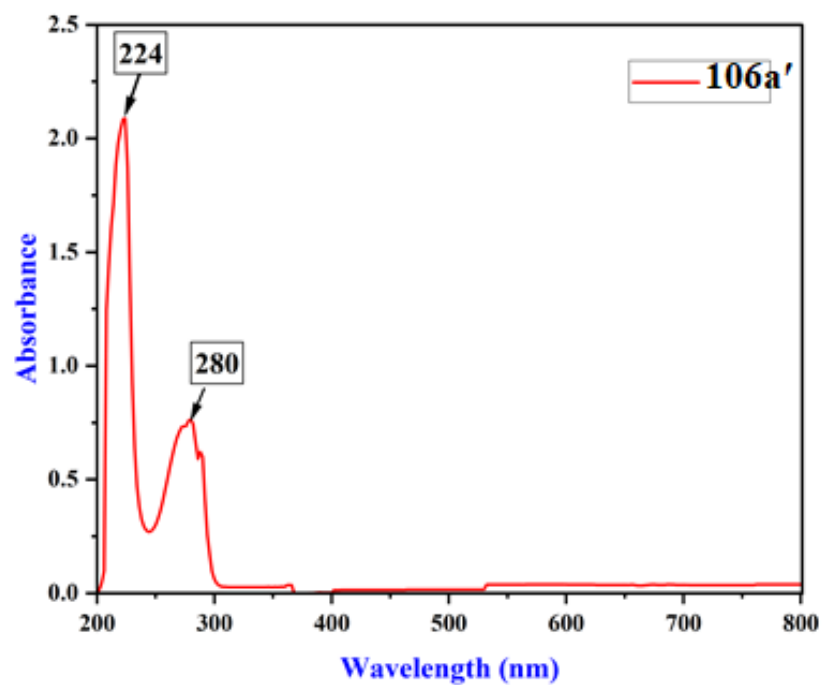
## 7. APPENDICES

Appendix 1: Interpolated regression graphs for synthesized compounds (107a', 107b', 110a', 110b', 111a' and 111b') and standard ascorbic acid for calculating its IC<sub>50</sub> value

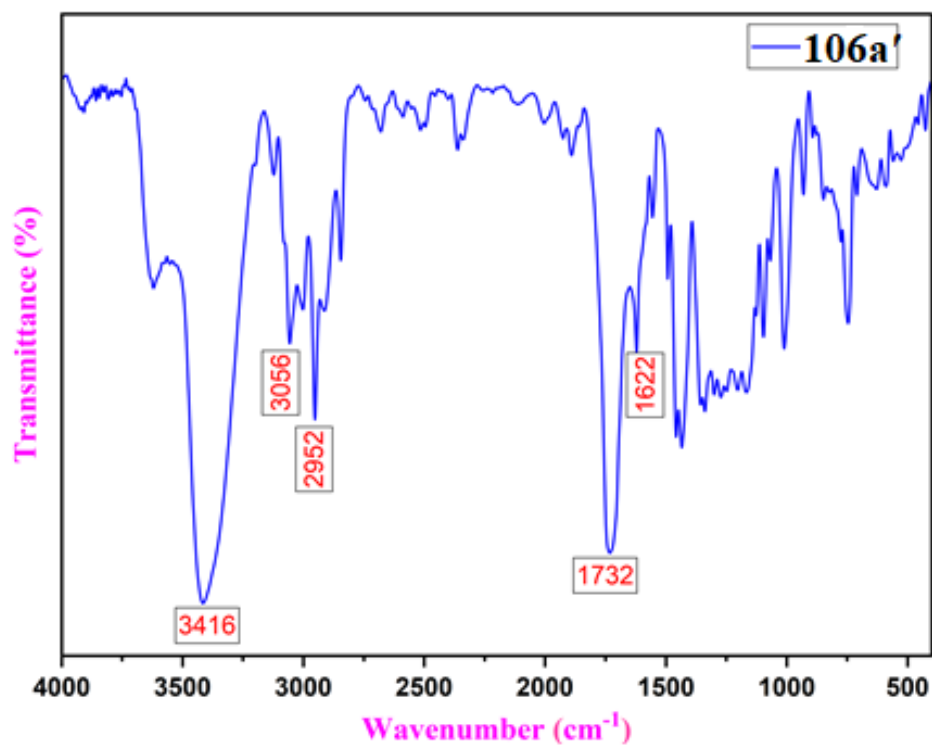




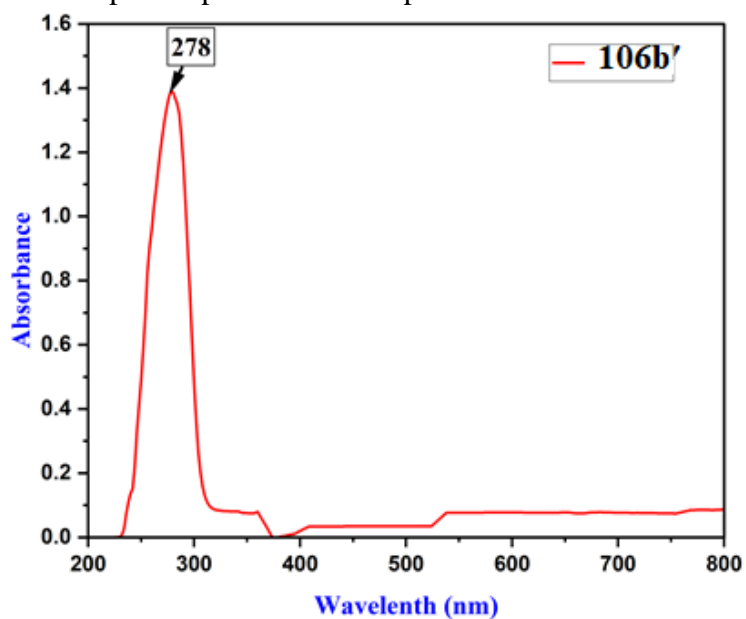
Appendix 2: UV-Vis Absorption Spectrum of compound 106a'



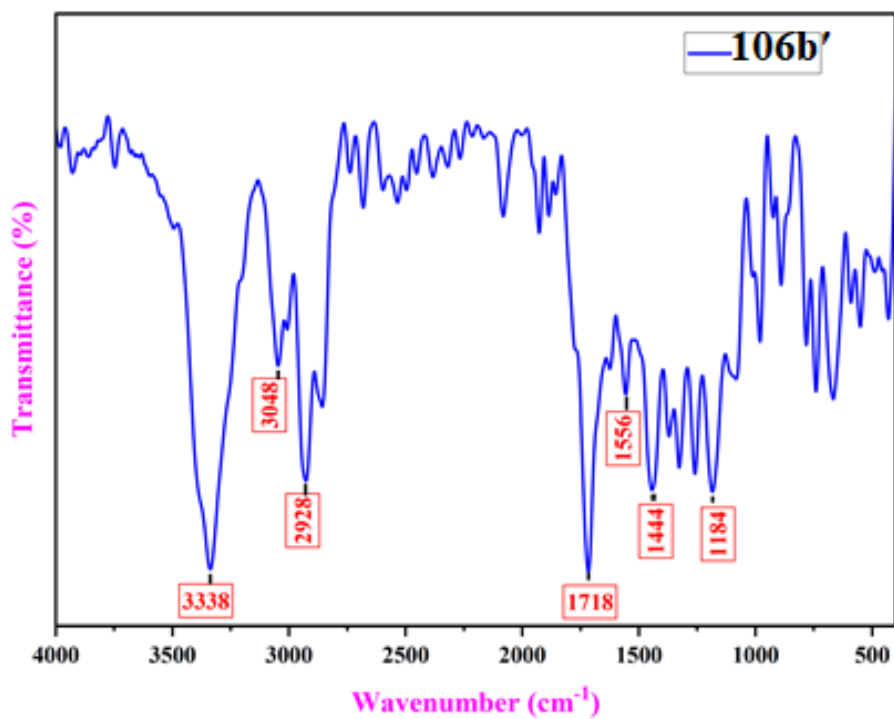
Appendix 3: FT-IR Absorption Spectrum of compound 106a'



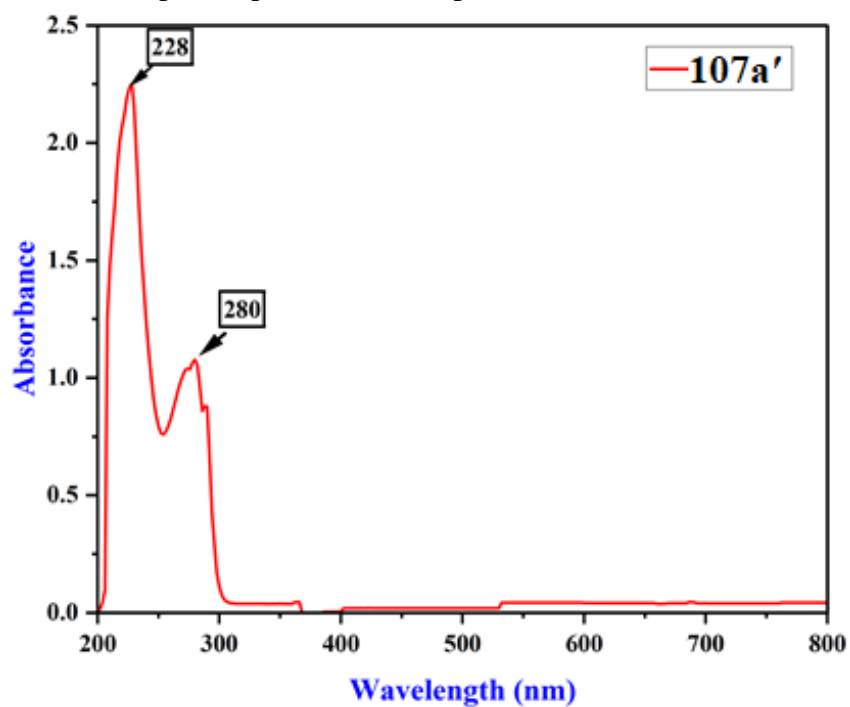
Appendix 4: UV-Vis Absorption Spectrum of compound 106b'



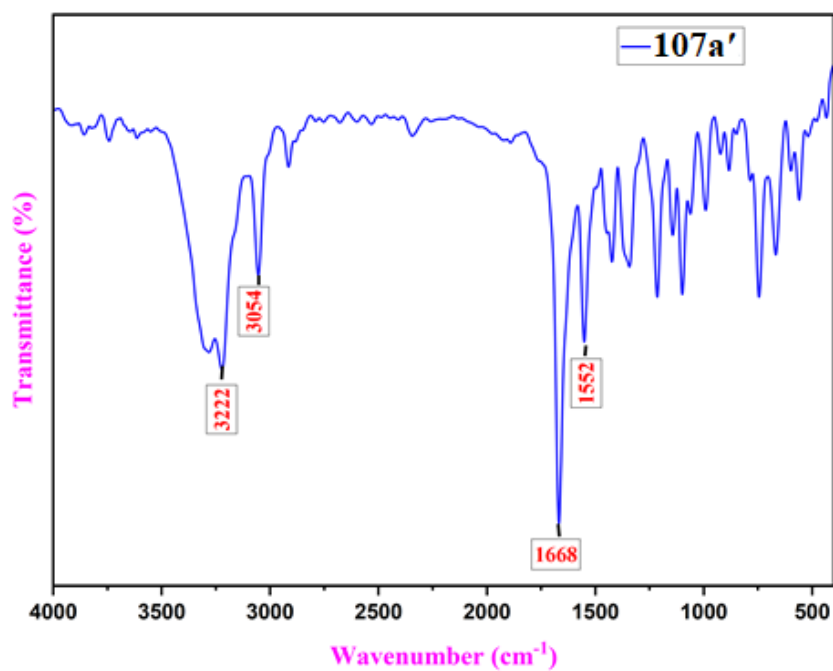
Appendix 5: FT-IR Absorption Spectrum of compound 106b'



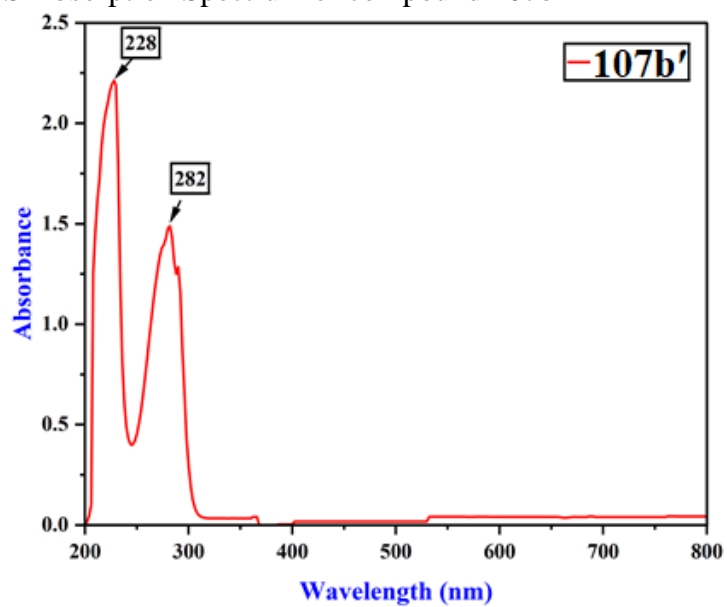
Appendix 6: UV-Vis Absorption Spectrum of compound 107a'



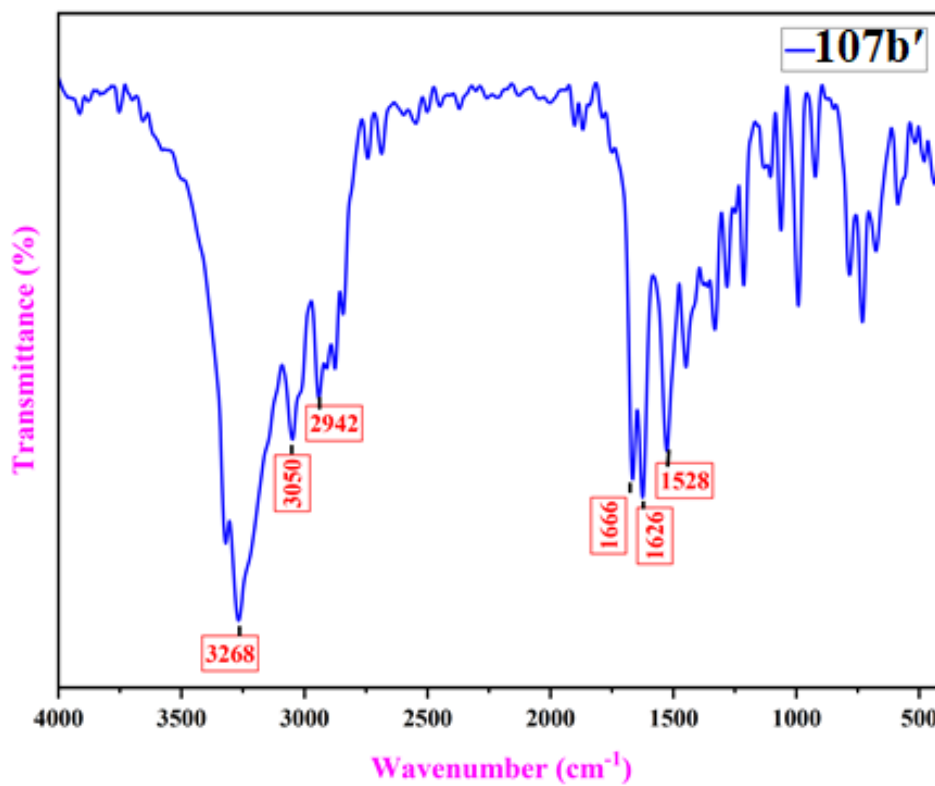
Appendix 7: FT-IR Absorption Spectrum of compound 107a'



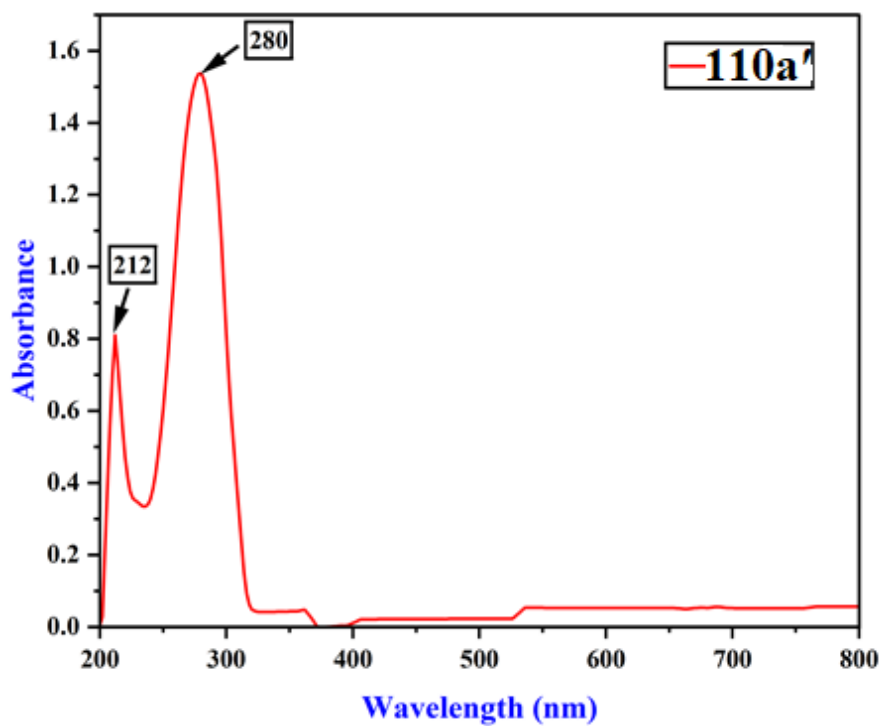
Appendix 8: UV-VIS Absorption Spectrum of compound 107b'



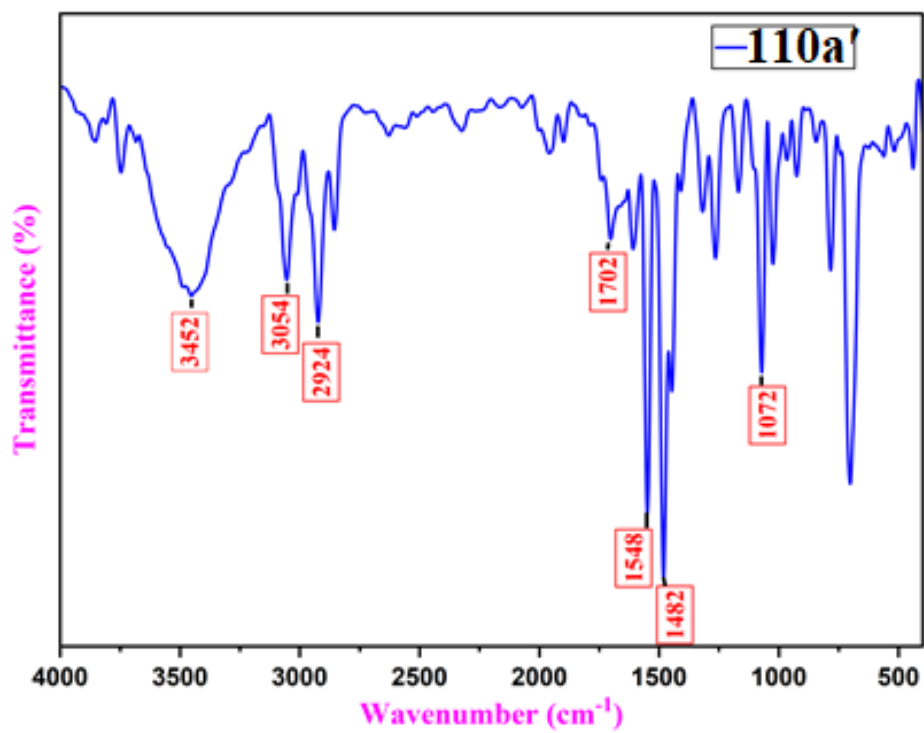
Appendix 9: FT-IR Absorption Spectrum of compound 107b'



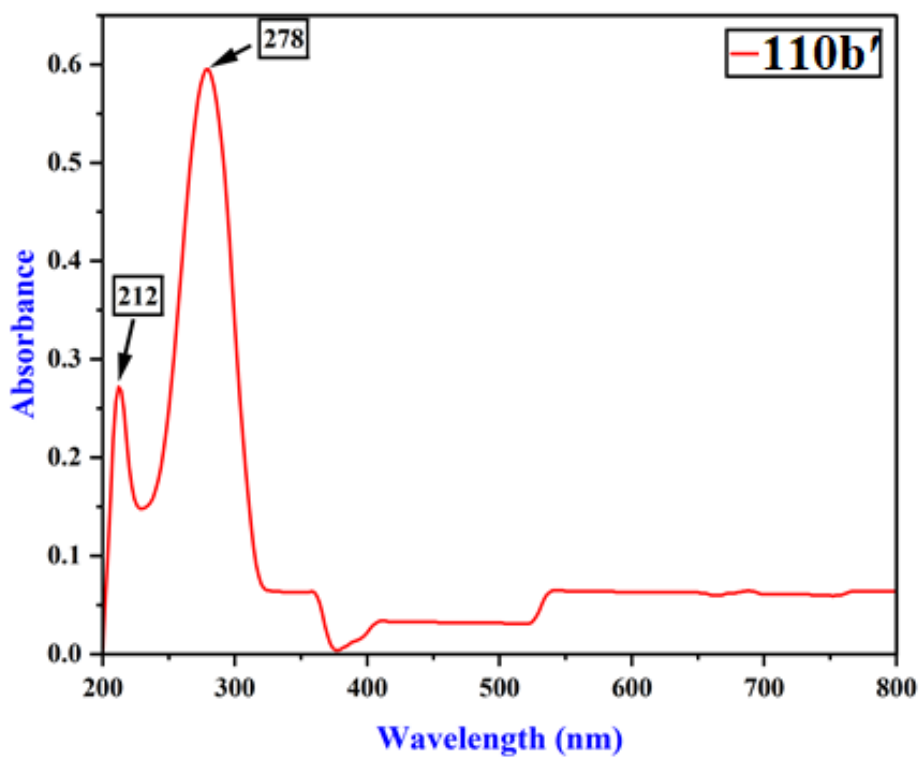
Appendix 10: UV-Vis Absorption Spectrum of compound 110a'



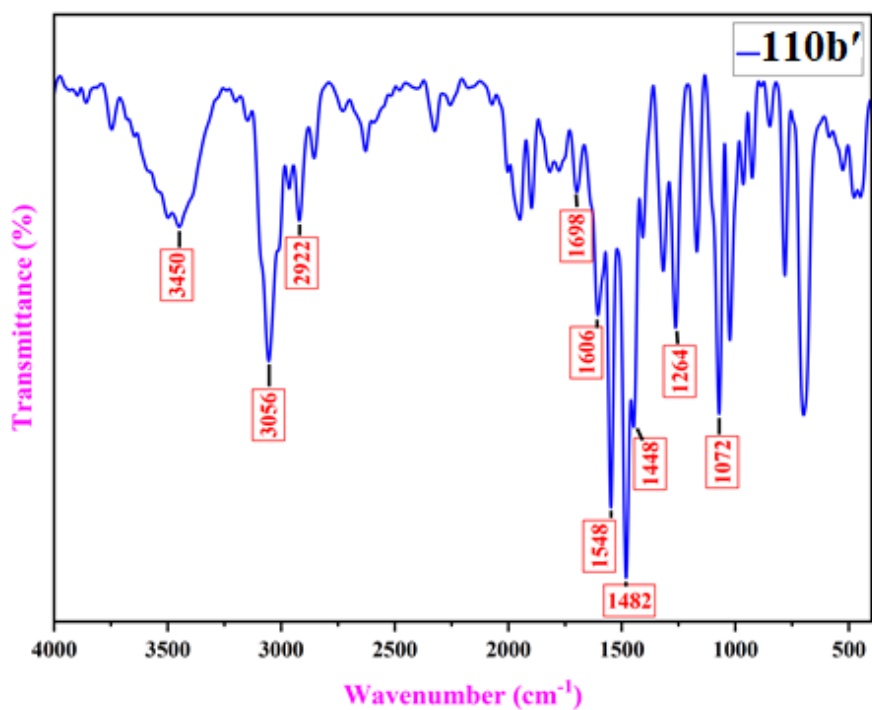
Appendix 11: FT-IR Absorption Spectrum of compound 110a'



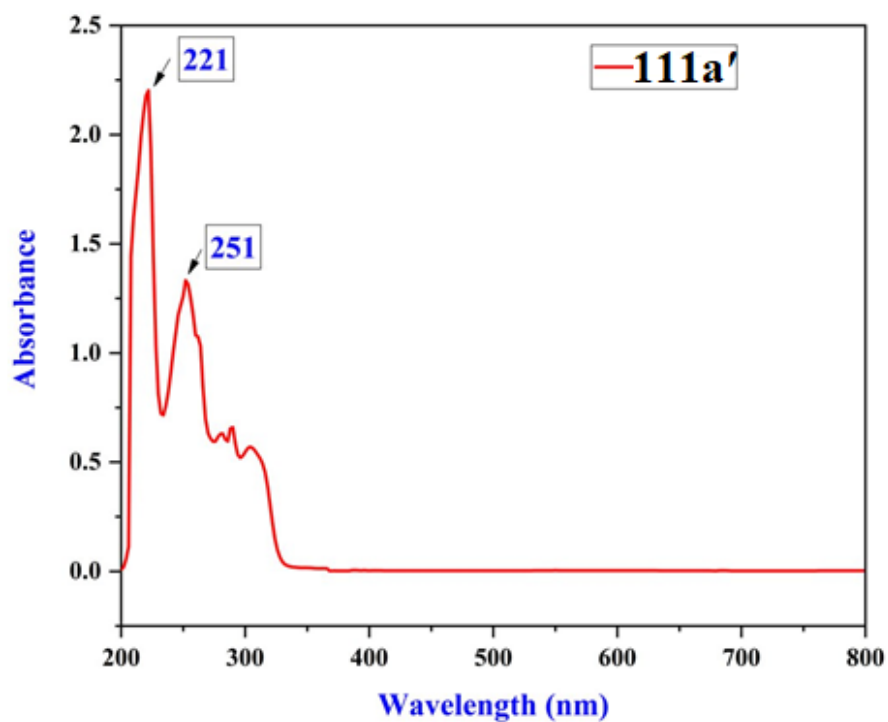
Appendix 12: UV-Vis Absorption Spectrum of Compound 110b'



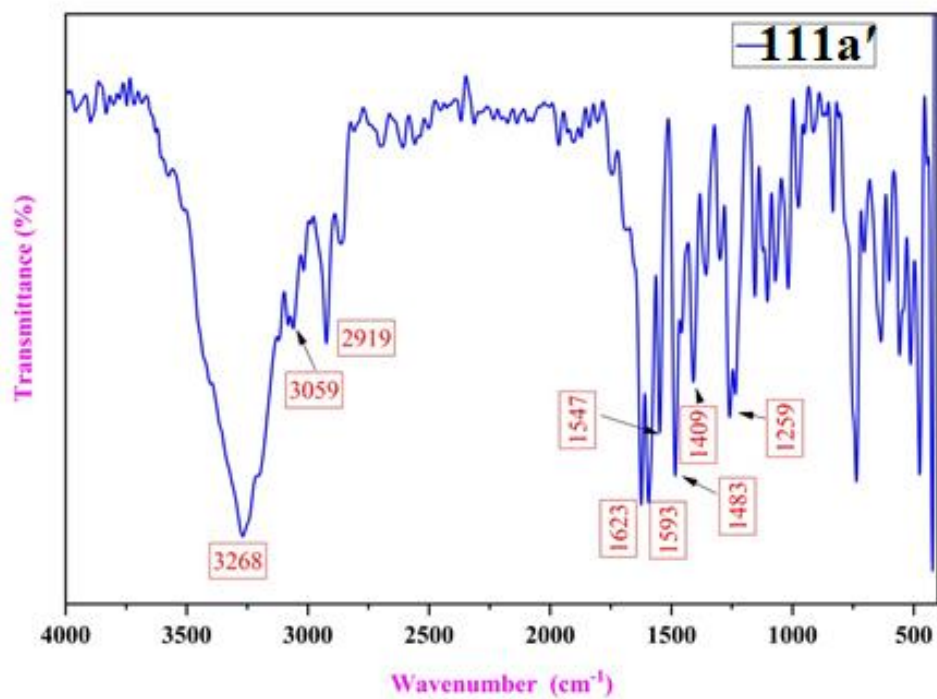
Appendix 13: FT-IR Absorption Spectrum of Compound 110b'



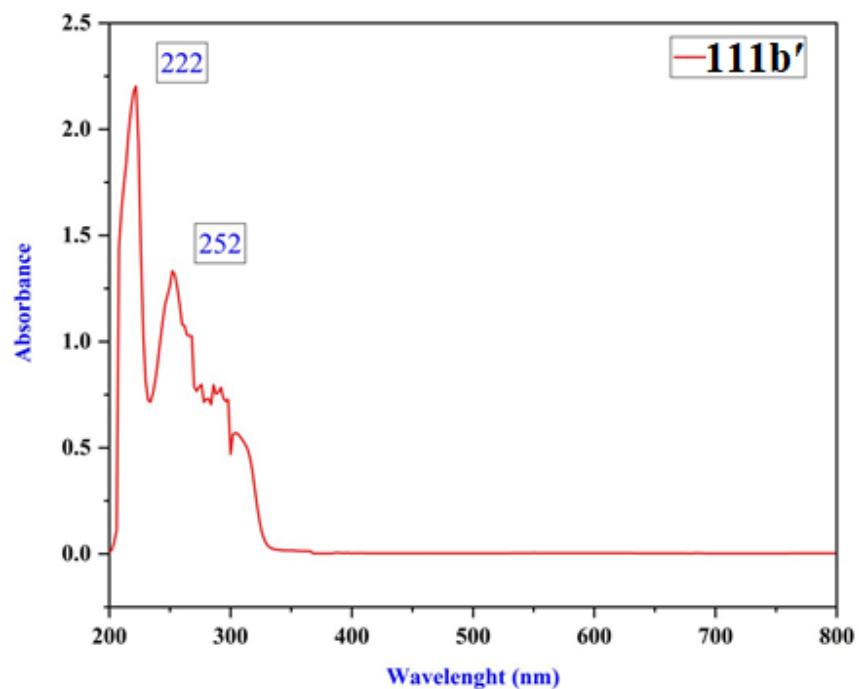
Appendix 14: UV-Vis Absorption Spectrum of Compound 111a'



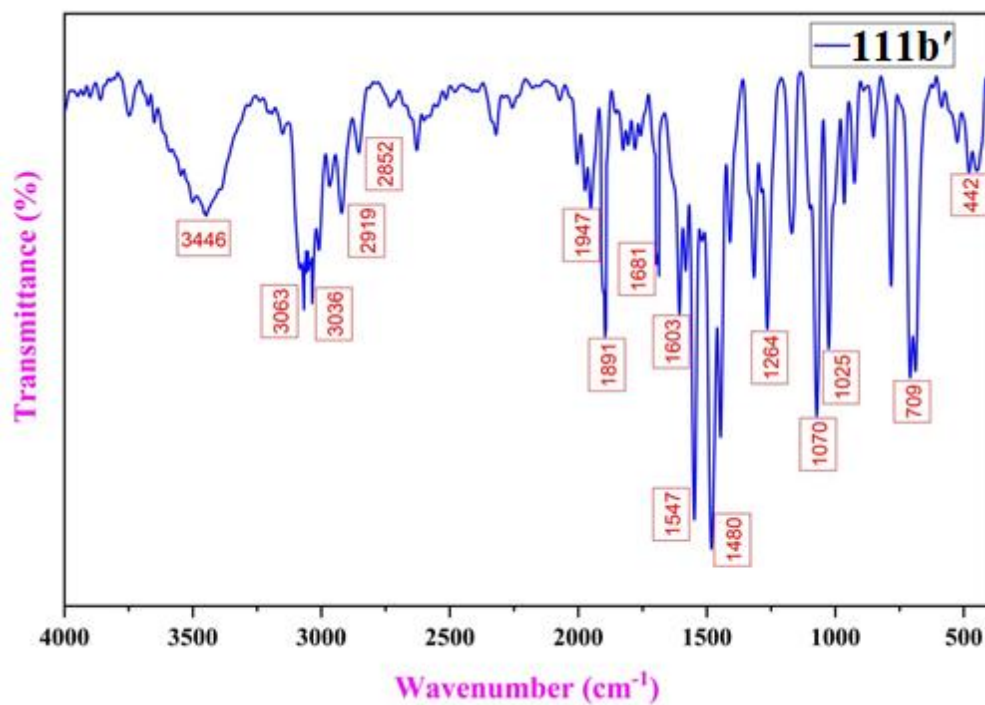
Appendix 15: FT-IR Absorption Spectrum of Compound 111a'



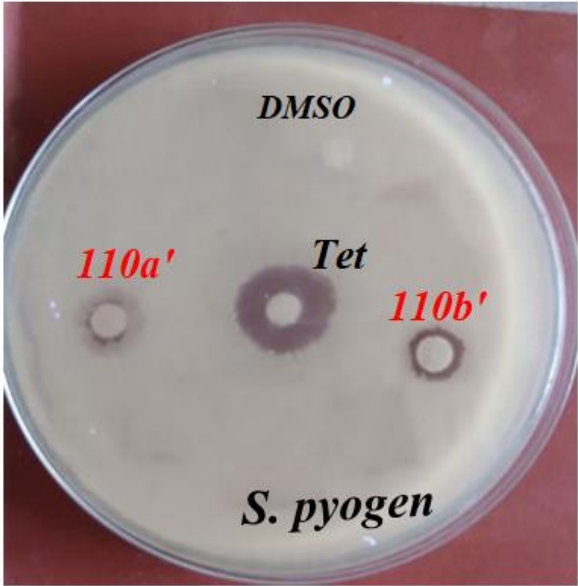
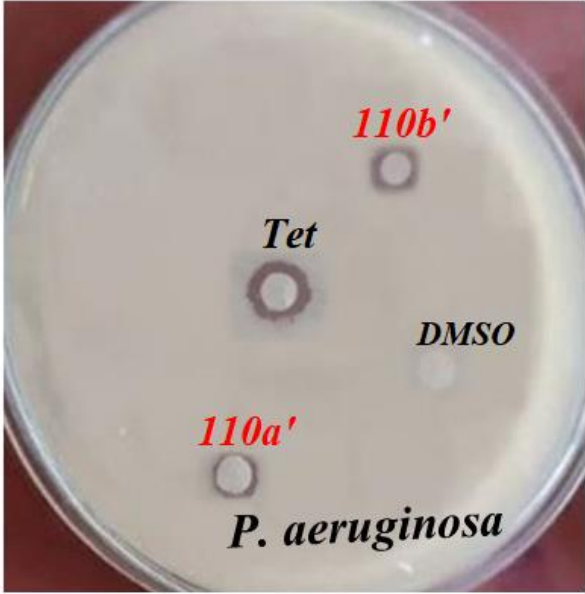
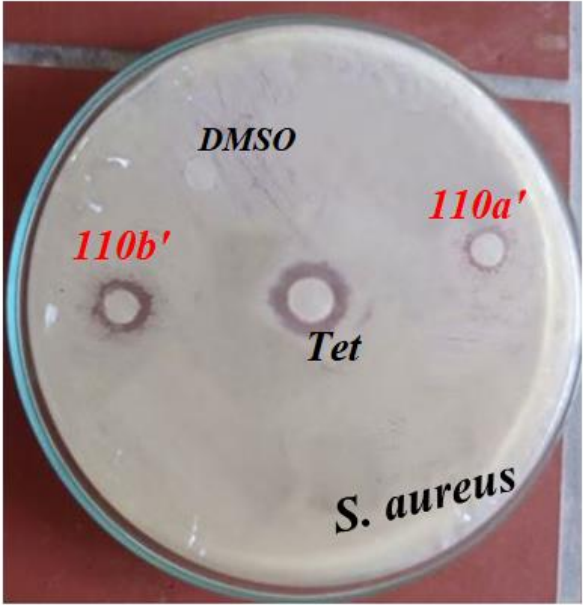
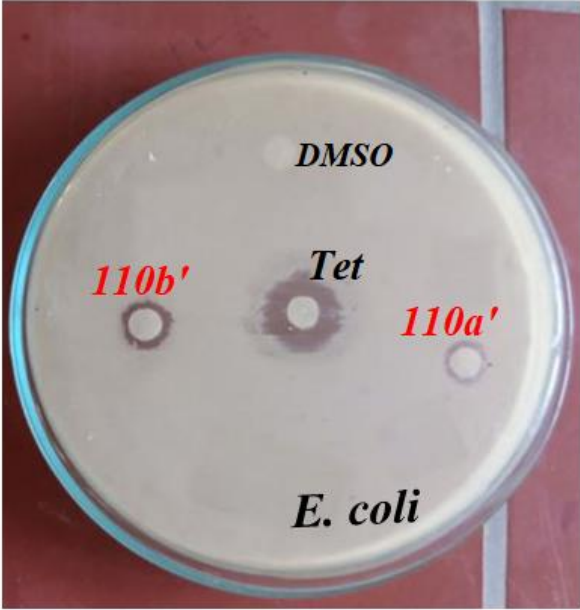
Appendix 16: UV-Vis Absorption Spectrum of Compound 111b'

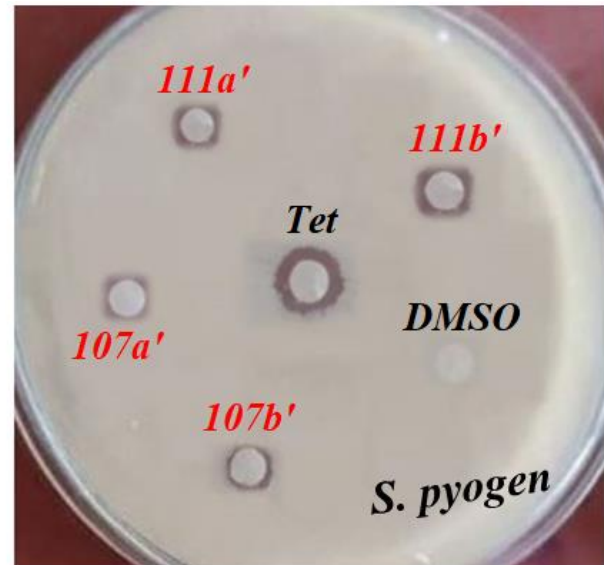
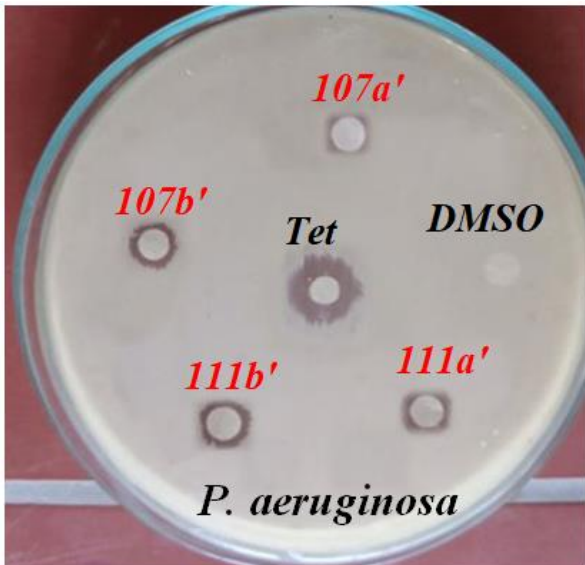
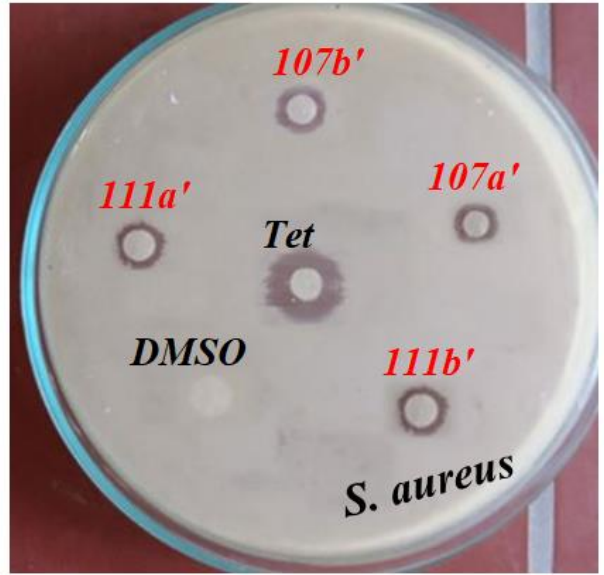
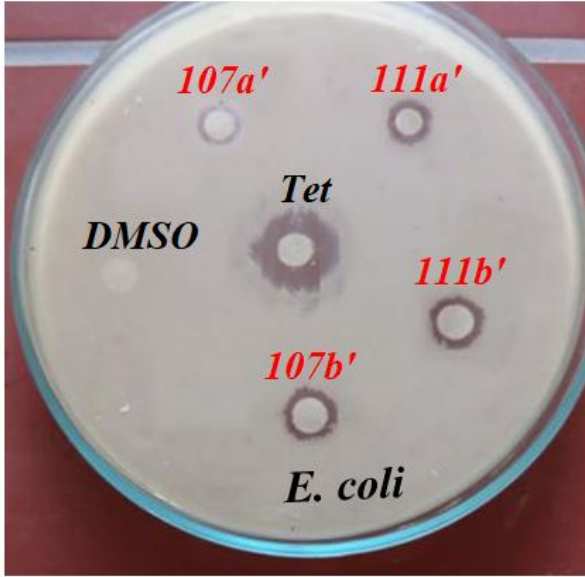


Appendix 17: FT-IR Absorption Spectrum of Compound 111b'



Appendix 18: Zone of inhibition on the grown bacteria on the prepared culture media on petri-dish by picture





Appendix 19: The diameter (mm) of the Zone of inhibition of synthesized compounds was measured against selected bacterial strains.

Table 4: The diameter (mm) of the Zone of inhibition of synthesized compounds was measured against selected bacterial strains.

Compound	Concentration (mg/mL)	Entry	Bacteria Species and Zone of Inhibitions in mm			
			<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. pyogene</i>
<b>107a'</b>	150	1	7	7.6	6.5	8
		2	7.4	7	7.3	8.5
		3	8	7.5	7	8.4
<b>107b'</b>	150	1	8.5	8.6	7.5	9
		2	8	8.5	7.6	9.6
		3	8.5	8.4	8	9.7
<b>110a'</b>	150	1	7.5	8	8	8.5
		2	8.4	8.5	7.5	8.2
		3	8	8.6	7	8.4
<b>110b'</b>	150	1	8.6	9	8	10.4
		2	9	9.5	8.4	10.5
		3	8.3	8.8	8.6	10.8
<b>111a'</b>	150	1	7.7	8	7	9.5
		2	7.4	8.5	7.4	9.2
		3	8	8.8	7.8	9.5
<b>111b'</b>	150	1	11.6	10.5	9	11.5
		2	11.4	10.5	9.5	11
		3	11.7	10.9	9.1	11.5
<b>Tetracycline (+ve control)</b>	150	1	13	13.5	12.6	14.5
		2	13.5	13.3	12	14.6
		3	12.5	13	12.5	14.5

The values are presented as three replicates.

Appendix 20: Mean inhibition zone of synthesized compounds in mm (mean  $\pm$  SD) at 150 mg/mL.

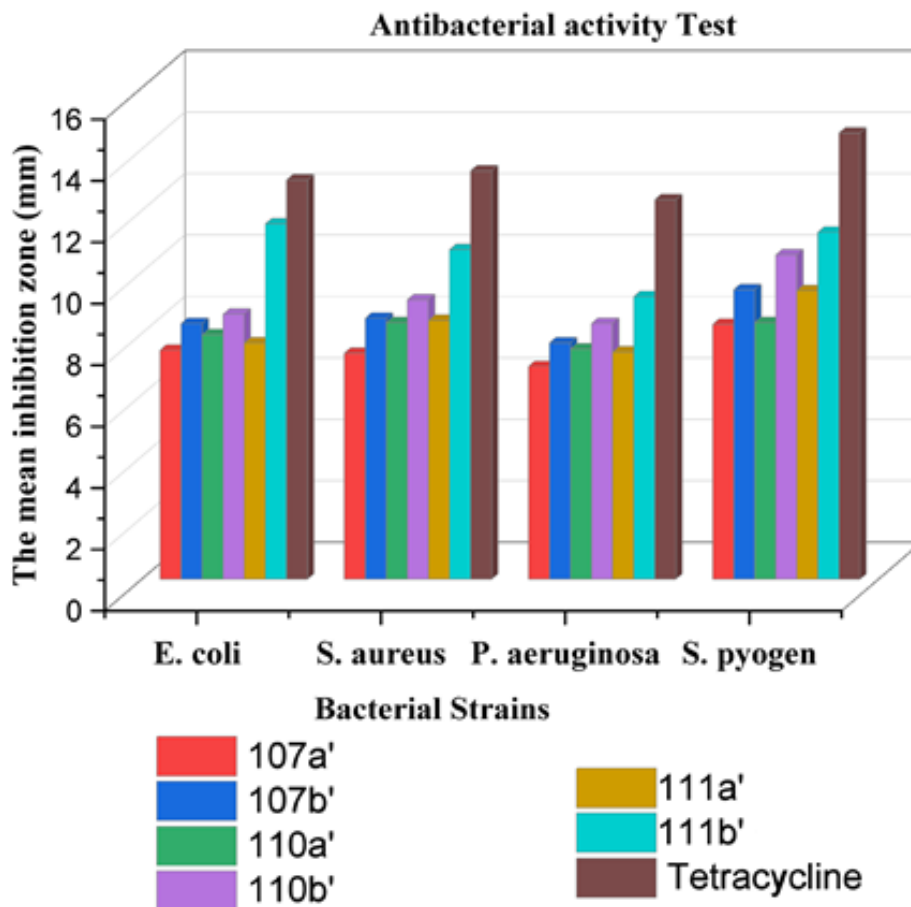


Figure 26: Mean inhibition zone of synthesized compounds in mm (mean  $\pm$  SD) at 150 mg/mL.