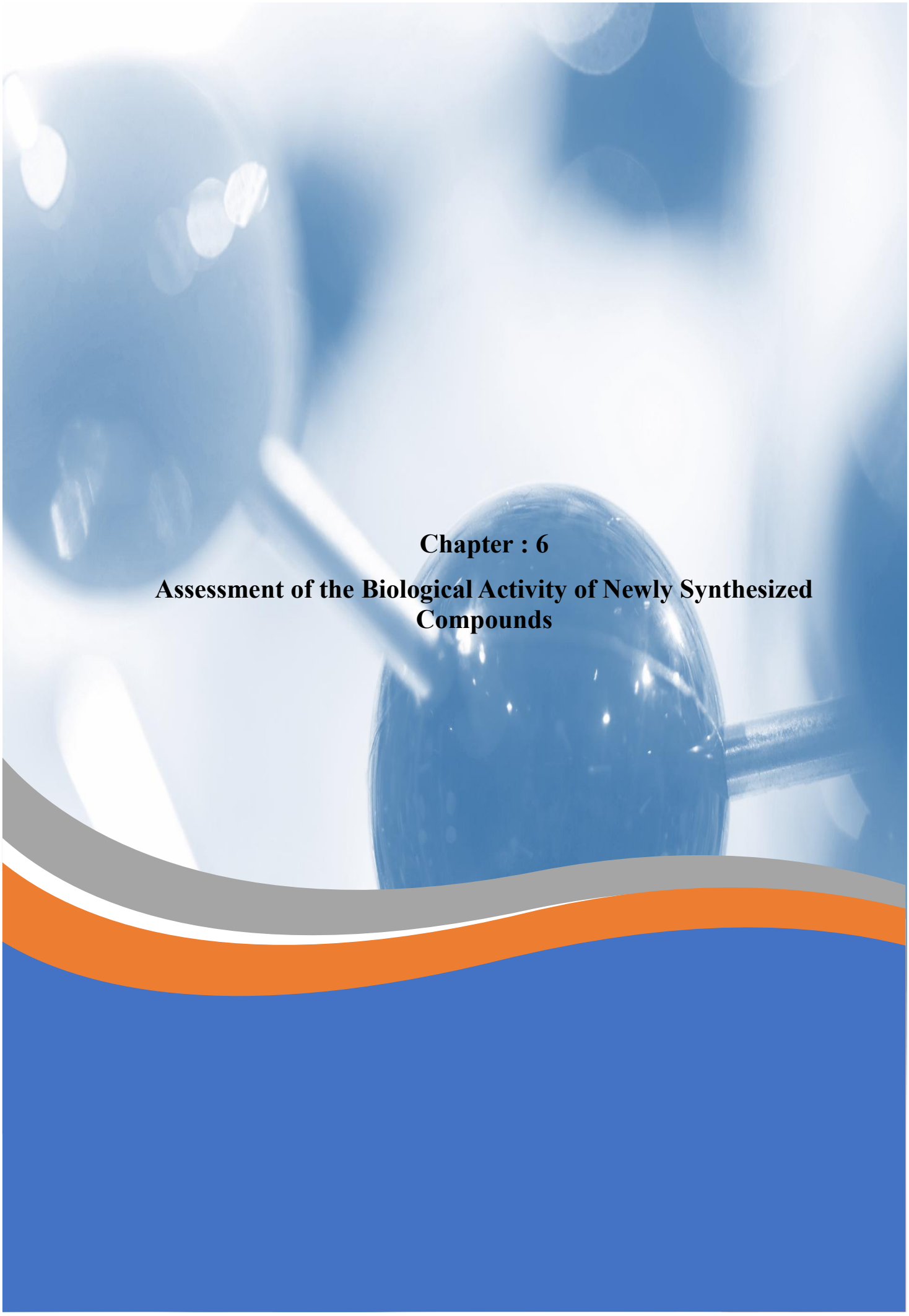


Chapter : 6

Assessment of the Biological Activity of Newly Synthesized Compounds

6.1 Introduction:

The influence of microorganisms on human life is profound, impacting various aspects of our existence significantly. These microscopic entities play a crucial role in shaping our environment and are poised to shape our future. It's crucial to recognize that microorganisms are not separate from humans; rather, they exert a considerable beneficial influence as integral components of our lives. [391] They actively contribute to the production of dairy items, certain foods, the processing of specific medicines and therapeutic agents, and the manufacturing of various chemicals. Their contributions extend to numerous other applications, underscoring the diverse and indispensable role they play in our daily lives.[392]

Despite their established beneficial functions, microorganisms are often associated with food spoilage and various human diseases. Examples of such illnesses include AIDS, herpes, Legionnaires disease, influenza, jaundice, tuberculosis, typhoid, dermatomycoses, dysentery, and malaria, among others. Despite their positive contributions, the notoriety of microorganisms in causing diseases underscores the need for understanding and managing their impact on human health.[393]

Microbial pathogens not only affect humans but also extend their impact to animals and plants. Throughout history, microbial diseases have been prevalent, often leading to disastrous consequences. Microorganisms have not only contributed to diseases but have also played significant roles in areas such as warfare, religion, and population migration. Controlling microbial populations becomes imperative to prevent the transmission of diseases, infections, decomposition, contamination, and spoilage, significantly influencing personal comforts and convenience.[394]

In recent decades, there has been a significant rise in bacterial and fungal infections, partly attributed to the growing number of individuals with compromised immune systems due to factors like AIDS, aging, organ transplantation, or cancer therapy. The heightened prevalence of bacterial and fungal diseases has led to an escalation in morbidity and mortality rates, raising significant concerns. In response, the pharmaceutical industry has developed novel, less toxic anti-infective agents for clinical application.[395]

However, the increased utilization of antibiotics, often over extended durations, has

led to the phenomenon of acquired resistance to existing antibacterial and antifungal agents, posing a significant challenge. Thus, there's a pressing need for continued research and development of antimicrobial agents to effectively combat microbial infections while minimizing the risk of resistance emergence.

6.1.1 Current Investigation - Antimicrobial Assessment:

In this ongoing research, we have conducted an antimicrobial screening of 22 newly synthesized compounds, as outlined in Parts I, II, III and IV of the thesis. The assessment of these derivatives took place in the in-house biology laboratory at Shree M. & N. Virani Science College, Rajkot.

6.2 Study on the Biological Activity of Newly Synthesized Compounds.

6.2.1 Assessment Methods:

The protocol adhered to for the screening of antimicrobial activity is outlined as follows:

1. Close interaction between the test organisms and the substance under evaluation.
2. The essential conditions required for the growth of microorganisms have been provided.
3. Consistency in conditions has been upheld throughout the duration of the study.
4. Aseptic and sterile conditions have been upheld throughout the study to ensure accuracy and reliability.

Different approaches have been employed over time by various researchers to assess antimicrobial activity. The evaluation can be conducted using the following methods:

1) Agar diffusion method
2) Turbid metric method
3) Agar streak dilution method
4) Serial dilution method
5) Broth dilution method

The agar diffusion method is subdivided into the following techniques:

1) Agar Cup method

2) Agar Ditch method

3) Paper Disc method

The "Broth Dilution Method" is employed to assess the antimicrobial activity of the synthesized compounds. This method is among the non-automated in vitro bacterial susceptibility tests. It provides quantitative data regarding the amount of antimicrobial agents required to inhibit the growth of specific microorganisms. The test is conducted in tubes, utilizing both the Macro dilution Method and the Microdilution format, which involves plastic trays.

6.2.2 Materials and Methods:

All microbial cultures are subjected to testing against both known and unknown standard drugs. Mueller Hinton Broth serves as the nutrient medium for cultivating and diluting the drug suspension for the examination.

Testing against standard known and unknown drugs is conducted for all microbial cultures. Mueller Hinton Broth is utilized as the nutrient medium to cultivate and dilute the drug suspension for the examination. DMSO serves as the diluent or vehicle to achieve the desired concentration of drugs for testing against standard bacterial strains.

All essential controls, including Drug, Vehicle, Agar, Organism, and known antibacterial drugs control, have been implemented. The "Broth Dilution Method" is utilized for MIC determination, with serial dilutions prepared in both primary and secondary screenings. Mueller Hinton broth serves as the nutrient medium for cultivating and diluting the drug suspension for the test. Test organisms are cultured in broth for 24 hours for bacteria and 48 hours for fungi at 37°C. Serial dilutions of test compound solutions are prepared, inoculated with test organisms, and then incubated at 37°C for 48 hours. The tubes are subsequently examined for the presence or absence of microbial growth. The lowest concentration displaying no growth is identified as the minimum inhibitory concentration (MIC).

This technique involves preparing serial dilutions of the test compounds in a suitable growth medium, followed by inoculation with standardized bacterial or fungal cultures. The dilutions are then incubated under optimal conditions for microbial growth, allowing the assessment of the compounds' inhibitory effects. The minimum

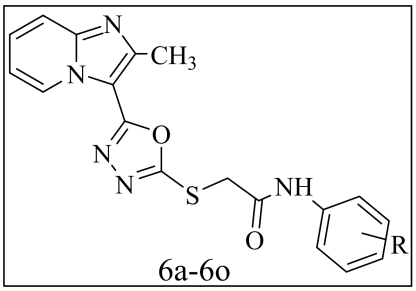
inhibitory concentration (MIC) of each compound is determined, representing the lowest concentration at which microbial growth inhibition is observed. Each synthesized drug and the standard drug were dissolved in a DMSO-water mixture at a concentration of 2 mg/mL. In the primary screening, concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL of the synthesized drugs were tested. Data for the initial solution were not recorded due to the high concentration of DMSO (10%). The active synthesized drugs identified in the primary screening were further diluted to concentrations of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, and 12.5 µg/mL for secondary screening. The highest dilution that showed at least 99% inhibition was determined as the minimum inhibitory concentration (MIC). Each dilution is inoculated with the microbial cultures and incubated for a specified period to allow for microbial growth. Following incubation, the MIC of each compound is determined based on visual inspection of microbial growth or using automated methods, with the lowest concentration exhibiting significant inhibition considered as the MIC. Compounds demonstrating promising activity in the primary screening undergo further evaluation in a secondary screening phase. In this stage, active compounds identified in the primary screening are subjected to additional dilutions to obtain a more refined concentration range. The MIC is again determined for each compound to confirm its inhibitory activity against the target microorganisms.

The evaluation of antimicrobial compounds through comprehensive laboratory testing provides valuable insights into their potential efficacy as antibacterial and antifungal agents. These findings contribute to ongoing research efforts in antimicrobial drug development, aiming to address the growing challenge of antibiotic resistance and improve treatment options for infectious diseases. By identifying novel compounds with potent antimicrobial properties, researchers can advance the development of new therapeutic agents to combat microbial infections and safeguard public health.

6.3 Reading Result:

The MIC is determined as the highest dilution that exhibits at least a 99% inhibition zone. It's important to note that the size of the inoculum significantly impacts this result.

Chapter : 1 Synthesis and Characterization of Oxadiazole Derivatives bearing Imidazo[1,2-*a*]pyridine Scaffold



6a-6o

Where;R=H,4-methyl,4-methoxy,2,4-dimethyl,3-chloro,4-chloro,4-fluoro,4-bromo,2-methoxy,2-methyl,3-methyl,3-methoxy,2-chloro-4-fluro,2,4-difluoro,3,4-dichloro

The antimicrobial activity of the test compounds is evaluated in vitro against 24-hour cultures of various selected bacteria and fungi.

Test Culture:

Gram positive: Staphylococcus aureus, Streptococcus pyogenus

Gram Negative: Escherichia coli, Pseudomonas aeruginosa

Fungi: Aspergillus niger, Candida albicans

Standard Drugs: The standard drugs used in the present study are.;

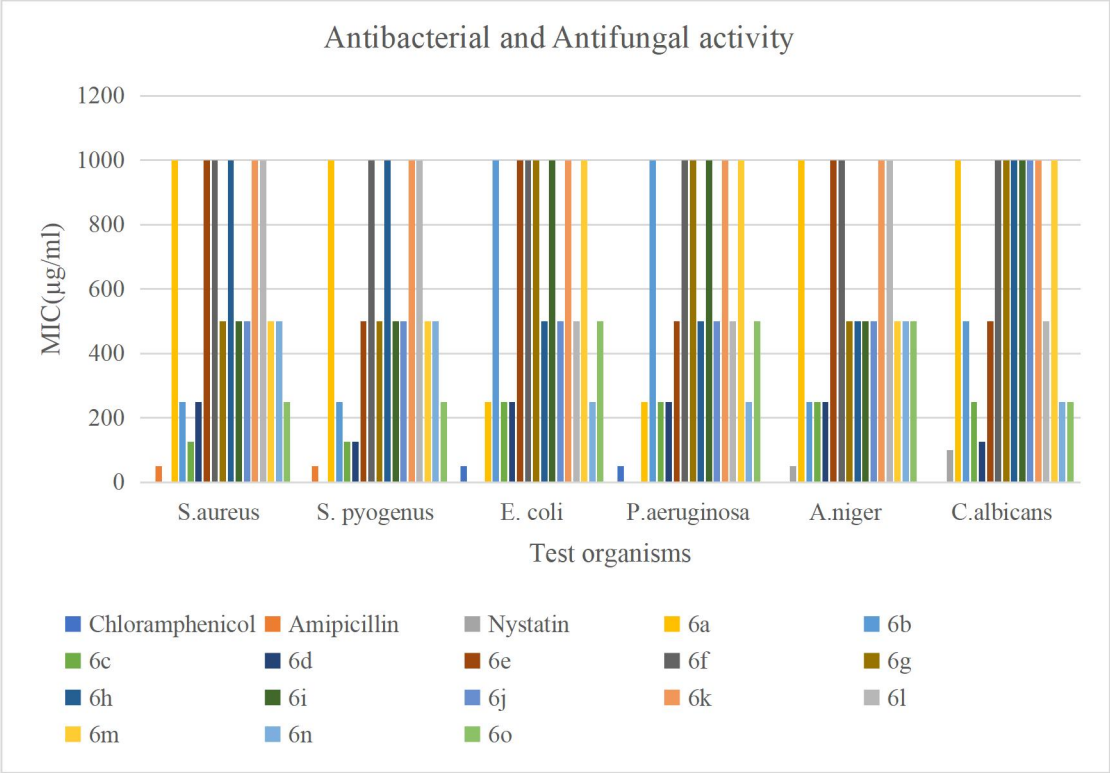
chloramphenicol: Antibacterial activity

Ampicillin: Antibacterial activity

Nystatin: Antifungal activity

Table 6.1: Antibacterial and antifungal activity of 1,3,4-Oxadiazole derivatives (6a-6o):

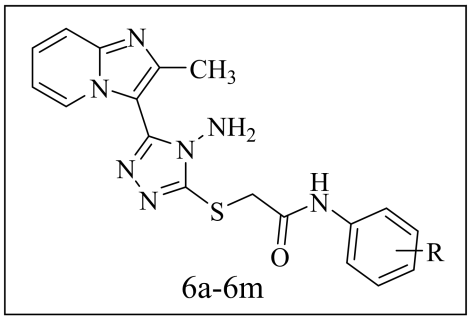
Compound Code	*Antibacterial MIC(µg/mL)*				*Antifungal MIC (µg/mL)*	
	S. aureus MTCC737	S.pyogenus MTCC1925	E. coli MTCC443	P. aeruginosa MTCC5210	A. niger MTCC282	C.albicans MTCC183
chloramphenicol			50	50		
Ampicillin	50	50				
Nystatin					50	100
6a	1000	1000	250	250	1000	1000
6b	250	250	1000	1000	250	500
6c	125	125	250	250	250	250
6d	250	125	250	250	250	125
6e	1000	500	1000	500	1000	500
6f	1000	1000	1000	1000	1000	1000
6g	500	500	1000	1000	500	1000
6h	1000	1000	500	500	500	1000
6i	500	500	1000	1000	500	1000
6j	500	500	500	500	500	1000
6k	1000	1000	1000	1000	1000	1000
6l	1000	1000	500	500	1000	500
6m	500	500	1000	1000	500	1000
6n	500	500	250	250	500	250
6o	250	250	500	500	500	250



The novel derivatives of [2-((5-(2-methylimidazo[1,2-*a*]pyridin-3-yl)-1,3,4-oxadiazol-2-yl)thio)-*N*-phenylacetamide] suggests potential antimicrobial activity based on its structural features. Imidazo[1,2-a]pyridine rings have been associated with antimicrobial properties, indicating potential efficacy against a wide range of microorganisms. Oxadiazole derivatives are known for their antimicrobial activity, often disrupting bacterial cell membranes or interfering with essential cellular processes. Thioether groups, present in the compound, have also demonstrated antimicrobial effects by interacting with bacterial enzymes or disrupting bacterial membranes. Additionally, phenylacetamide groups, found in the compound, have shown antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as some fungi. Considering these structural characteristics, the derivative may possess broad-spectrum antimicrobial activity against various bacteria and fungi. However, experimental testing, such as minimum inhibitory concentration (MIC) assays, would be essential to determine its specific antimicrobial potency and spectrum of activity. Further studies would also be needed to understand its mechanism of action and potential applications as an antimicrobial agent.

The MIC values obtained from the evaluation indicated varying degrees of inhibition for the newly synthesized compounds. Compound 6a demonstrated moderate activity against S. pyogenes and E. coli. Compound 6b exhibited moderate activity against S. aureus, P. aeruginosa, and A. niger strains. Notably, compound 6c displayed good to moderate activity against both Gram-positive and Gram-negative bacteria, including S. aureus, S. pyogenes, E. coli, and P. aeruginosa, as well as against the fungal strains A. niger and Candida albicans. Compound 6d showed good to moderate activity against both Gram-positive and Gram-negative bacterial strains. Compound 6e demonstrated moderate activity overall against S. pyogenes, P. aeruginosa, and Candida albicans bacterial strains. 6i shows moderate towards S. aureus, S. pyogenes and A.niger.6j shows moderate activity towards all bacterial and fungal strain. 6n shows good activity towards gram-negative bacterial strains and also fungal strain Candida albicans. 6o shows good activity towards gram-positive bacterial strains and also fungal strain Candida albicans. Conversely, all other compounds exhibited poor activity against both bacterial and fungal strains. These findings provide valuable insights into the potential effectiveness of the synthesized compounds against specific microbial targets. Chloramphenicol and ampicillin, a standard antibacterial drug, exhibited moderate activity in inhibiting microbial growth, while nystatin, employed for antifungal purposes, demonstrated similar moderate activity in impeding fungal proliferation.

Chapter : 2 Synthesis and Characterization of 2-((4-amino-5-(2-methylimidazo[1,2-*a*]pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)thio)-*N*-phenylacetamide Derivatives



6a-6m

Where;R=H,4-methyl,4-methoxy,2,4-dimethyl,3-chloro,4-chloro,4-fluoro,4-

bromo,2-methoxy,2-methyl,3-methyl,3-methoxy,2-chloro-4-fluro,2,4-difluoro,3,4-dichloro

The antimicrobial activity of the test compounds is evaluated in vitro against 24-hour cultures of various selected bacteria and fungi.

Test Culture:

Gram positive: Staphylococcus aureus, Streptococcus pyogenus

Gram Negative: Escherichia coli, Pseudomonas aeruginosa

Fungi: Aspergillus niger, Candida albicans

Standard Drugs: The standard drugs used in the present study are.;

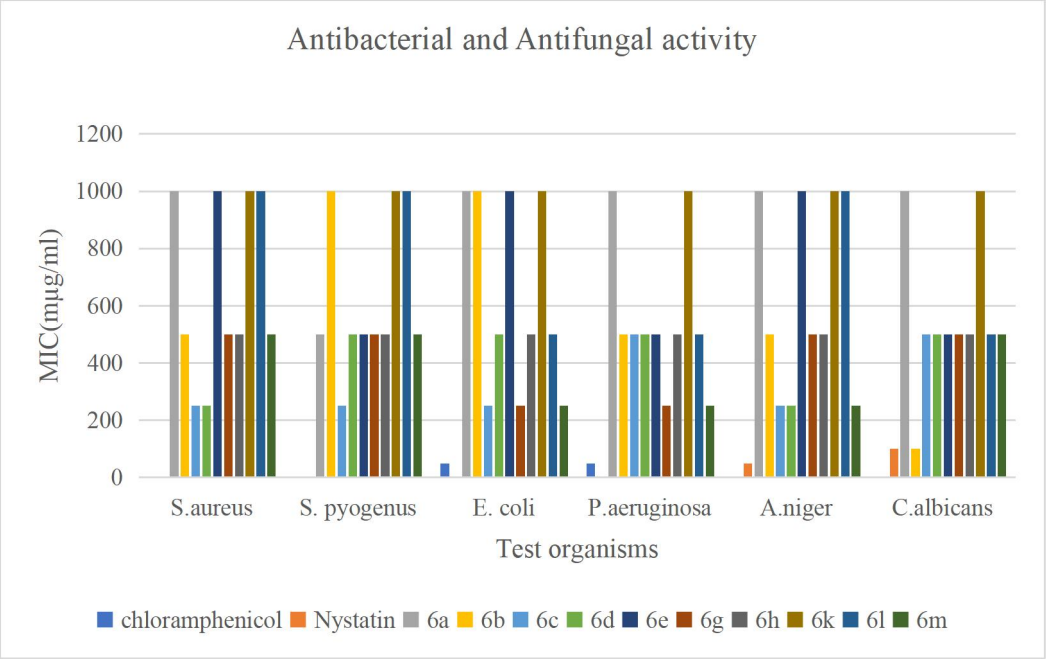
chloramphenicol: Antibacterial activity

Nystatin: Antifungal activity

Table2.1: Antibacterial and antifungal activity of 1,2,4 triazole derivatives (6a-6m):

Compound Code	*Antibacterial MIC(µg/mL)*				*Antifungal MIC (µg/mL)*	
	S. aureus MTCC737	S. pyogenus MTCC1925	E.coli MTCC443	P.aeruginosa MTCC5210	A. niger MTCC282	C. albicans MTCC183
chloramphenicol			50	50		
Nystatin					50	100
6a	1000	500	500	1000	1000	1000
6b	500	1000	1000	500	500	1000
6c	250	250	250	500	250	500
6d	250	500	500	500	250	500
6e	1000	500	1000	500	1000	500
6g	500	500	250	250	500	500
6h	500	500	500	500	500	500
6k	1000	1000	1000	1000	1000	1000
6l	1000	1000	500	500	1000	500

6m	500	500	250	250	250	500

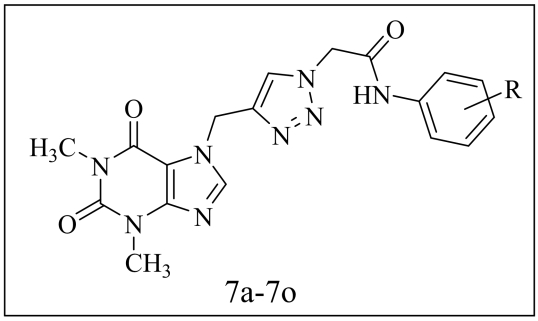


Based on the structural features of the compound [2-((4-amino-5-(2-methylimidazo[1,2-a]pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)thio)-*N*-phenylacetamide], we can anticipate its potential antimicrobial activity. Compounds containing imidazo[1,2-a]pyridine rings are known to possess antimicrobial properties, suggesting activity against a wide range of microorganisms. Triazole derivatives, like the one in this compound, have been extensively studied for their antimicrobial effects, often inhibiting crucial microbial enzymes or interfering with cellular processes. Thioether-containing compounds, such as this derivative, have demonstrated antimicrobial properties by interacting with bacterial enzymes or disrupting bacterial membranes. Additionally, compounds containing phenylacetamide groups, like the one present in this compound, have shown antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as some fungi. Considering these structural features, it's plausible that the derivative may exhibit broad-spectrum antimicrobial activity against various bacteria and fungi. However, experimental testing, such as minimum inhibitory concentration (MIC) assays, would be crucial to determine its specific antimicrobial potency and spectrum of activity. Further research

would also be necessary to elucidate its mechanism of action and explore its potential applications as an antimicrobial agent.

The obtained MIC values revealed varying degrees of inhibition for the newly synthesized compounds. Compound 6a demonstrated moderate activity against Streptococcus pyogenes and Escherichia coli. Compound 6b exhibited moderate activity against Staphylococcus aureus, Pseudomonas aeruginosa, and Aspergillus niger strains. Notably, compound 6c displayed good to moderate activity against both Gram-positive and Gram-negative bacteria, including S. aureus, S. pyogenes, E. coli, and P. aeruginosa, as well as against the fungal strains A. niger and Candida albicans. Compound 6d showed good to moderate activity against both Gram-positive and Gram-negative bacterial strains. Compound 6e demonstrated moderate activity overall against Streptococcus pyogenes, Pseudomonas aeruginosa, and Candida albicans bacterial strains. 6g showed good activity shows towards gram negative bacteria.6m showed good activity towards gram negative bacteria as well as fungal strain. In contrast, all other compounds exhibited poor activity against both bacterial and fungal strains. These findings offer valuable insights into the potential effectiveness of the synthesized compounds against specific microbial targets. Chloramphenicol, a standard antimicrobial drug, exhibited moderate activity in inhibiting microbial growth, while nystatin, used for antifungal purposes, demonstrated similar moderate activity in impeding fungal proliferation.

Chapter:3 Synthesis and Characterization of 1,2,3-triazol Containing Theophylline Moiety



7a-7o

Where;R=H,4-methyl,4-methoxy,2,4-dimethyl,3-chloro,4-chloro,4-fluoro,4-

bromo,2-methoxy,2-methyl,3-methyl,3-methoxy,2-chloro-4-fluro,2,4-difluoro,3,4-dichloro

The antimicrobial activity of the test compounds is evaluated in vitro against 24-hour cultures of various selected bacteria and fungi.

Test Culture:

Gram positive: Bacillus subtilis ,Staphylococcus aureus

Gram Negative: Salmonella spp., Klebsiella pneumonia

Fungi: Candida spp.

Standard Drugs: The standard drugs used in the present study are.;

Ampicillin: Antibacterial activity

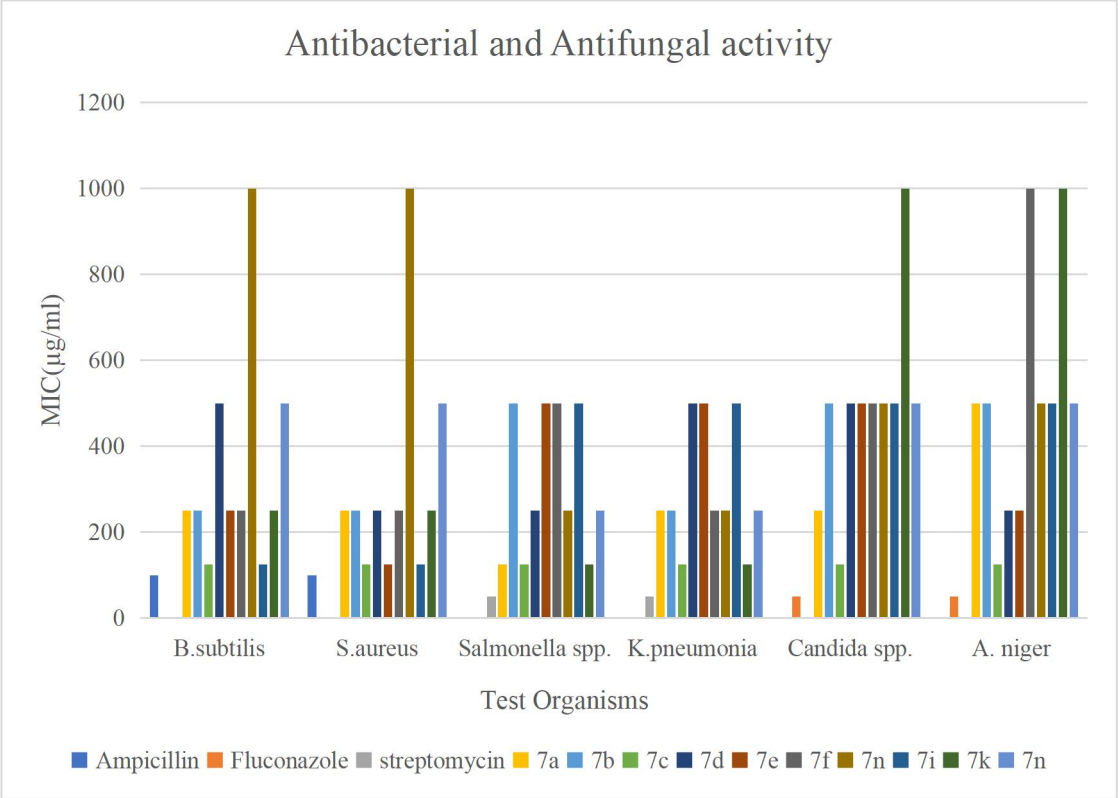
Streptomycin: Antibacterial activity

Fluconazole: Antifungal activity

Table 3.1: Antimicrobial and antifungal activity of novel schiff base derivatives (7a-7o):

Compound	*Antibacterial MIC(µg/ml)*				*Antifungal MIC (µg/ml)*	
Code	B. subtilis MTCC441	S.aureus MTCC737	Salmonella spp. MTCC5690	K.pneumonia MTCC109	Candida spp. MTCC25057	A.niger MTCC282
Ampicillin	100	100				
Fluconazole					50	50
Streptomycin			50	50		
7a	250	250	125	250	250	500
7b	250	250	500	250	500	500
7c	125	125	125	125	125	125
7d	500	250	250	500	500	250
7e	250	125	500	500	500	250
7f	250	250	500	250	500	1000
7h	1000	1000	250	250	500	500
7i	125	125	500	500	500	500

7k	250	250	125	125	1000	1000
7n	500	500	250	250	500	500

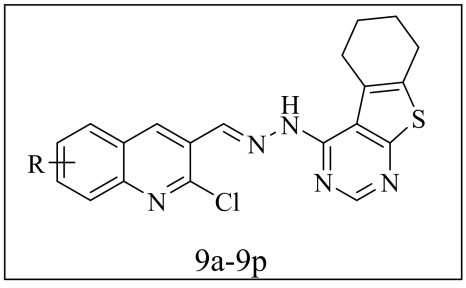


The Antibacterial potential of substituted 2-(4-((1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7*H*-purin-7-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-phenylacetamide primarily relies on the presence of triazoles, purine, and substitution on 2-azido-*N*-phenylacetamide. The electron-withdrawing or electron-releasing nature of these substitutions can significantly influence the compound's interactions with bacterial membranes or proteins. The choice of substituents, whether they withdraw or release electrons, plays a pivotal role in modulating the compound's Antibacterial activity. Electron-withdrawing substituents may enhance the compound's effectiveness by potentially augmenting its affinity towards bacterial targets. Conversely, electron-releasing substituents could alter the compound's interaction profile, potentially affecting its efficacy against various bacterial species.

Therefore, the Antibacterial efficacy of substituted 2-(4-((1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7*H*-purin-7-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-phenylacetamide is intricately linked to the molecular interactions facilitated by its constituent parts and their electronic characteristics, which ultimately dictate its Antibacterial potency.

According to the MIC values, the novel chemical compounds exhibited moderate to excellent inhibition. Compounds 7a, 7b, 7c, 7d, 7e, 7f, 7i, and 7n are efficient antibiotics with a wide spectrum that can suppress the growth of both gram-positive and gram-negative bacteria. 7h exhibits antagonistic effect against primarily gram-positive bacteria. 4a and 4d demonstrate antifungal action. We may deduce that 7a, 7c, and 7k are the most efficient against gram-positive bacteria. 7a, 7d, and 7e also demonstrate antifungal action. As a result, 7a, 7b, 7c, 7d, 7e, 7i and 7n are regarded as strong antibiotics against all gram-positive, negative bacteria, and fungi. the standard drugs ampicillin, fluconazole, and streptomycin exhibit moderate to excellent activity against gram-positive and gram-negative bacteria, as well as fungal strains, respectively.

Chapter :4 Synthesis and Characterization of Schiff Bases Derived from 2-Chloroquinoline-3-Carbaldehyde and Its Derivatives.



9a-9p

Where,R=H,7-methyl,7-methoxy,6,8-dimethyl,6-chloro,7-chloro,7-fluoro,7-bromo,8-methoxy,8-methyl,6-methyl,6-methoxy,7-ethoxy,6-chloro-7-fluro,7,8-difluoro,6,7-dichloro

Evaluation of Antimicrobial Activity:

The antimicrobial activity of the test compounds is evaluated in vitro against 24-hour cultures of various selected bacteria and fungi.

Test Culture:

Gram positive: Staphylococcus aureus, Bacillus subtilis

Gram Negative: Enterobacter aerogenes, Pseudomonas aeruginosa

Fungi: Aspergillus niger, Candida albicans

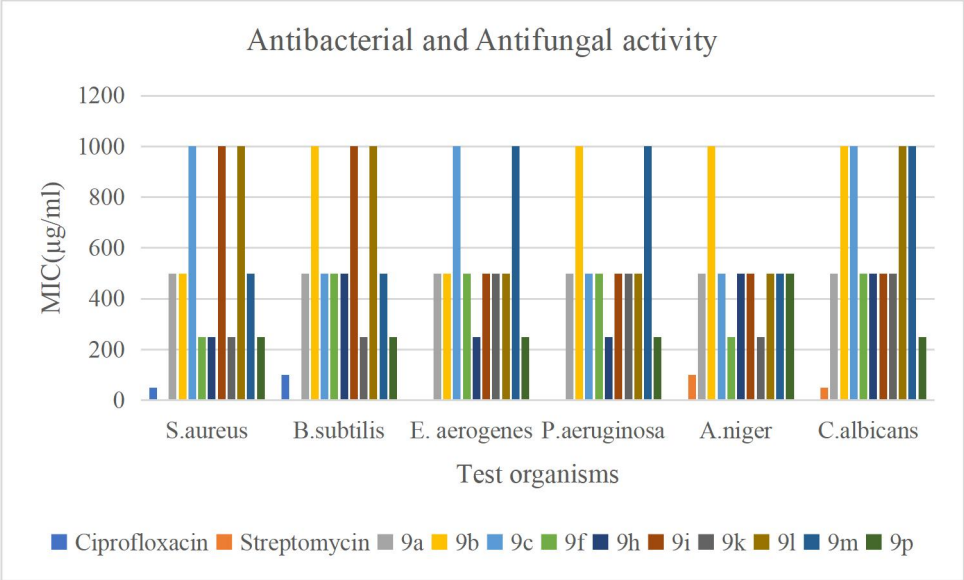
Standard Drugs: The standard drugs used in the present study are.;

Ciprofloxacin: Antibacterial activity

Streptomycin: Antifungal activity

Table 4.1: Antimicrobial and antifungal activity of novel schiff base derivatives (9a-p):

Compound Code	*Antibacterial MIC(µg/mL)*				*Antifungal MIC (µg/mL)*	
	S. aureus MTCC737	B. subtilis MTCC441	E. aerogenes MTCC2823	P. aeruginosa MTCC2453	A. niger MTCC282	C.albicans MTCC183
Ciprofloxacin	50	100				
Streptomycin					100	50
9a	500	500	500	500	500	500
9b	500	1000	500	1000	1000	1000
9c	1000	500	1000	500	500	1000
9f	250	500	500	500	250	500
9h	250	500	250	250	500	500
9i	1000	1000	500	500	500	500
9k	250	250	500	500	250	500
9l	1000	1000	500	500	500	1000
9m	500	500	1000	1000	500	1000
9p	250	250	250	250	500	250



The compound [4-(2-((2,6-dichloroquinolin-3-yl)methylene)hydrazineyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine] contains several functional groups and aromatic rings, which are commonly associated with antimicrobial activity.

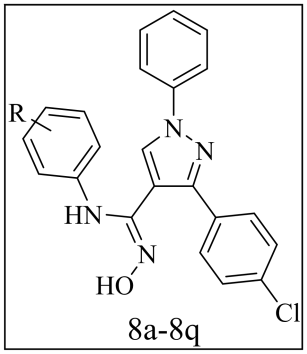
Compounds containing chloroquinoline rings have been reported to exhibit antimicrobial activity against various microorganisms. The chlorine atoms can interact with biological targets, potentially disrupting microbial growth or viability. The presence of a hydrazine group in the compound can also contribute to its antimicrobial activity. Hydrazine derivatives have been studied for their antibacterial and antifungal properties due to their ability to interfere with essential cellular processes. While less commonly explored in the context of antimicrobial activity, the tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine scaffold could potentially contribute to the compound's biological effects through interactions with microbial targets.

To determine the specific antimicrobial activity of this derivative, experimental testing gives results as below. This would involve assessing its effectiveness against a range of bacteria and fungi through methods such as minimum inhibitory concentration (MIC) assays.

The MIC values obtained from the evaluation revealed varying degrees of inhibition for the newly synthesized compounds. Compound 9a demonstrated moderate activity against both Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, Bacillus subtilis, Enterobacter aerogenes, and Pseudomonas aeruginosa, as

well as against the fungal strains A. niger and Candida albicans. Compound 9b exhibited moderate activity against Staphylococcus aureus and Enterobacter aerogenes. Similarly, compound 9c displayed moderate activity against both Gram-positive and Gram-negative bacteria, including Bacillus subtilis, Pseudomonas aeruginosa, and Aspergillus niger strains. Notably, compounds 9f and 9h showed good to moderate activity against both Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, Bacillus subtilis, Enterobacter aerogenes, Pseudomonas aeruginosa, as well as against the fungal strains A. niger and Candida albicans. Compound 9i exhibited moderate activity against Enterobacter aerogenes and Pseudomonas aeruginosa, as well as against the fungal strains A. niger and Candida albicans. 9k and 9p shows good activity towards both bacterial strain and fungal strain. In contrast, all other compounds demonstrated poor activity against both bacterial and fungal strains. These findings offer valuable insights into the potential effectiveness of the synthesized compounds against specific microbial targets. Ciprofloxacin, a standard antimicrobial drug, displayed moderate activity in inhibiting microbial growth, while Streptomycin, used for antifungal purposes, exhibited similar moderate activity in impeding fungal proliferation.

Chapter:5 Synthesis and Characterization of 3-(4-Chlorophenyl)-*N*-hydroxy-*N*,1-diphenyl-1*H*-pyrazole-4-carboximidamide Derivatives.



8a-8q

Where;R=H,4-methyl,4-methoxy,2,4-dimethyl,3-chloro,4-chloro,4-fluoro,4-bromo,2-methoxy,2-methyl,3-methyl,3-methoxy,2-chloro-4-fluro,2,4-difluoro,3,4-dichloro

The antimicrobial activity of the test compounds is evaluated in vitro against 24-hour cultures of various selected bacteria and fungi.

Test Culture:

Gram positive: Bacillus subtilis ,Staphylococcus aureus

Gram Negative: Salmonella spp., Escherichia coli.

Fungi: Candida spp., Aspergillus niger

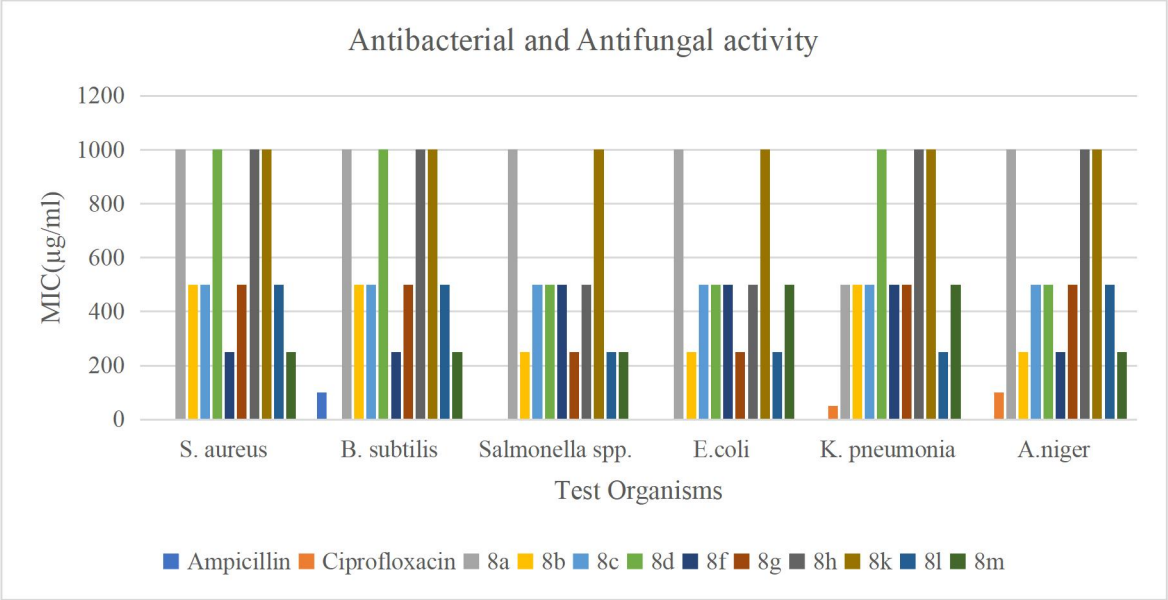
Standard Drugs: The standard drugs used in the present study are.;

Ampicillin: Antibacterial activity

Ciprofloxacin: Antifungal activity

Table 5.1: Antibacterial and antifungal activity of novel3-(4-Chlorophenyl)-*N*-hydroxy-*N*,1-diphenyl-1*H*-pyrazole-4-carboximidamide derivatives (8a-8q):

Compound code	*Antibacterial activity(µg/ml)*				*Antifungal activity(µg/ml)*	
	S.aureus MTCC 737	B. subtilis MTCC 441	Salmonella spp. MTCC5490	E.coli MTCC 443	Candida spp. MTCC25057	A.niger MTCC 282
Ampicillin	100	100				
Ciprofloxacin					50	100
8a	1000	1000	1000	1000	500	1000
8b	500	500	250	250	500	250
8c	500	500	500	500	500	500
8d	1000	1000	500	500	1000	500
8f	250	250	500	500	500	250
8g	500	500	250	250	500	500
8h	1000	1000	500	500	1000	1000
8k	1000	1000	1000	1000	1000	1000
8l	500	500	250	250	250	500
8m	250	250	250	500	500	250



The antimicrobial activity of 3-(4-Chlorophenyl)-*N*-hydroxy-*N*,1-diphenyl-1*H*-pyrazole-4-carboximidamide involves considering several factors, Pyrazole derivatives are known to possess various biological activities, including antimicrobial properties. The presence of a pyrazole ring can enhance the interaction with microbial enzymes or cellular structures, potentially leading to inhibition of microbial growth. The 4-chlorophenyl group introduces a chlorine atom, which is often associated with antimicrobial activity due to its ability to penetrate microbial cell walls and disrupt cellular processes. The *N*-hydroxy group can contribute to the antimicrobial activity by participating in hydrogen bonding with microbial targets, enhancing the binding affinity and inhibitory potential. The presence of two phenyl groups can increase the lipophilicity of the compound, aiding in the penetration of microbial cell membranes and enhancing its overall antimicrobial efficacy. To determine the specific antimicrobial activity of this derivative, experimental testing gives results as below. This would involve assessing its effectiveness against a range of bacteria and fungi through methods such as minimum inhibitory concentration (MIC) assays.

The MIC values obtained from the evaluation revealed varying degrees of inhibition for the newly synthesized compounds. Compound 8b shows good activity towards Salmonella spp. And E.coli bacteria as well as A.niger fungal strain. Compound 8c shows moderate activity towards all bacterial and fungal strains. Compound 8f shows good activity towards S. aureus, B. subtilis as well as A.niger. Compound 8g, 8l, 8m

shows good activity towards Salmonella spp. And E.coli bacteria. 8l shows good activity towards Candida spp. and A.niger both fungal strains. In contrast, all other compounds demonstrated poor activity against both bacterial and fungal strains. These findings offer valuable insights into the potential effectiveness of the synthesized compounds against specific microbial targets. Ampicillin, a standard antimicrobial drug, displayed moderate activity in inhibiting microbial growth, while Ciprofloxacin, used for antifungal purposes, exhibited similar moderate activity in impeding fungal proliferation.

6.4 Application:

In this study, we screened the synthesized derivatives for their antimicrobial activity, revealing promising results as active pharmacophores. Ongoing research endeavors are currently underway to delve into the extent of their diverse biological activities.

6.5 Conclusion:

In conclusion, the compounds discussed in this study show promising potential as antimicrobial agents based on their unique structural features. The novel derivative of [2-((5-(2-methylimidazo[1,2-a]pyridin-3-yl)-1,3,4-oxadiazol-2-yl)thio)-*N*-phenylacetamide] and [2-((4-amino-5-(2-methylimidazo[1,2-a]pyridin-3-yl)-4*H*-1,2,4-triazol-3-yl)thio)-*N*-phenylacetamide] exhibit antimicrobial properties attributed to imidazo[1,2-a]pyridine rings, oxadiazole and triazole derivatives, thioether groups, and phenylacetamide moieties, which are known for their efficacy against a broad spectrum of microorganisms. The antibacterial potential of substituted 2-(4-((1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7*H*-purin-7-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-phenylacetamide is primarily influenced by the electronic characteristics of its substitutions and the presence of triazoles and purine, which significantly impact interactions with bacterial membranes and proteins. Additionally, the compound [4-(2-((2,6-dichloroquinolin-3-yl)methylene)hydrazineyl)-5,6,7,8-

tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine] shows potential antimicrobial activity due to its functional groups, including the chloroquinoline ring and hydrazine group, while the tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine scaffold could contribute to its biological effects. Furthermore, 3-(4-Chlorophenyl)-*N*-hydroxy-*N*,1-diphenyl-1*H*-pyrazole-4-carboximidamide demonstrates antimicrobial efficacy facilitated by pyrazole derivatives and phenylacetamide moieties, which interact with microbial enzymes and cell structures, enhance binding affinity, and facilitate penetration of microbial cell membranes. To fully evaluate their potential, further experimental testing such as minimum inhibitory concentration (MIC) assays is necessary to determine their specific potency and spectrum of activity against various bacteria and fungi, paving the way for future development and potential applications in antimicrobial therapy.

6.6 Acknowledgement:

We express our sincere gratitude to Atmiya In-vitro Testing Laboratory, Rajkot, for conducting the antimicrobial activity studies.