CHAPTER 4

Biological Evaluation of the Synthesized Compounds in Chapter 2 and Chapter 3
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➢ Antimalarial activity
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INTRODUCTION

ANTIMICROBIAL DRUGS

Term “antimicrobials” include all agents that perform against all types of microorganisms – bacteria (antibacterial), viruses (antiviral), fungi (antifungal) and protozoa (antiprotozoal).

The science dealing with the study of the avoidance and treatment of diseases caused by micro-organisms is known as medical microbiology. Its sub-disciplines are bacteriology (study of bacteria), mycology (study of fungi), protozoology (study of protozoa), virology (study of viruses) and phycology (study of algae). For the treatment of diseases, inhibitory chemicals used to kill micro-organisms or avert their growth, are called antimicrobial agents.

Antimicrobial drugs are the utmost contribution of the 20th century to therapeutics. Their introduction changed the outlook of the physician about the power drugs can have on diseases. They are one of the few drugs which can cure, and not just palliate disease. Their significance is magnified in the developing countries, where infective diseases predominate.

Drugs in this class vary from all others in that they are considered to inhibit/kill the infecting organism and to have no/minimal effect on the recipient. This type of therapy is generally called chemotherapy which means ‘treatment of systemic infections with specific drugs that selectively restrain the infecting microorganism without significantly affecting the host.’ The basis of selective microbial toxicity is the action of the drug on a component of the microbe (e.g. bacterial cell wall) or metabolic processes (e.g. folate synthesis) that is not found in the host, or high affinity for certain microbial biomolecules (e.g. trimethoprim for bacterial dihydrofolate reductase).\(^1\)

Antibiotics are substances produced by microorganisms, which selectively restrain the growth of or kill other microorganisms at very low concentrations. These excludes other natural substances which also inhibit microorganisms but are produced by higher forms (e.g. antibodies) or even those produced by microbes but are needed in high concentrations.
CLASSIFICATION

Antimicrobial drugs can be classified in many ways:

A. Chemical structure

- Sulfonamides and related drugs: Sulfadiazine and others, Sulfones—
  Dapsone (DDS), Paraaminosalicylic acid (PAS).
- \( \beta \)-Lactam antibiotics: Penicillins, Cephalosporins, Monobactams,
  Carbapenems.
- Quinolones: Nalidixic acid, Norfloxacin, Ciprofloxacin, Prulifloxacin, etc.
- Diaminopyrimidines: Trimethoprim, Pyrimethamine
- Tetracyclines: Oxytetracycline, Doxycycline, etc.
- Nitrobenzene derivative: Chloramphenicol.
- Macrolide antibiotics: Erythromycin, Clarithromycin, Azithromycin, etc.
- Aminoglycosides: Streptomycin, Gentamicin, Amikacin, Neomycin, etc.
- Oxazolidinone: Linezolid.
- Glycopeptide antibiotics: Vancomycin, Teicoplanin.
- Polypeptide antibiotics: Polymyxin-B, Colistin, Bacitracin, Tyrothricin.
- Nitroimidazoles: Metronidazole, Tinidazole, etc.
- Nitrofuran derivatives: Nitrofurantoin, Furazolidone.
- Nicotinic acid derivatives: Isoniazid, Pyrazinamide, Ethionamide.
- Azole derivatives: Miconazole, Clotrimazole, Ketoconazole, Fluconazole.
- Polyene antibiotics: Nystatin, Amphotericin-B, Hamycin.
- Others: Rifampin, Spectinomycin, Sod. fusidate, Cycloserine, Viomycin,
  Ethambutol, Thiacetzone, Clofazimine, Griseofulvin.
B. Mechanism of action (Fig. 4.1²)

- Inhibit cell wall synthesis: Penicillins, Cycloserine, Cephalosporins, Vancomycin and Bacitracin.
- Cause misreading of m-RNA code and affect permeability: Aminoglycosides—Streptomycin, Gentamicin, etc.
- Inhibit DNA gyrase: Fluoroquinolones—Ciprofloxacin and others.
- Interfere with DNA synthesis: Acyclovir, Zidovudine.
- Inhibit protein synthesis: Tetracyclines, Chloramphenicol, Erythromycin, Clindamycin and Linezolid.
- Interfere with DNA function: Rifampin.
- Interfere with intermediary metabolism: Sulfonamides, Sulfones, Trimethoprim, PAS, Pyrimethamine and Metronidazole.

C. Type of organisms against which primarily active

- Antibacterial: Penicillins, Aminoglycosides, Erythromycin, Fluoroquinolones, etc.
- Antifungal: Griseofulvin, Amphotericin B, Ketoconazole, etc.
- Antiprotozoal: Chloroquine, Pyrimethamine, Metronidazole, Diloxanide, etc.
- Antiviral: Acyclovir, Amantadine, Zidovudine, etc.
- Anthelmintic: Mebendazole, Pyrantel, Niclosamide, Diethyl carbamazine, etc.

D. Spectrum of activity

Narrow-spectrum

Broad-spectrum.
E. Type of action

Primarily bacteriostatic- Sulfonamides, Erythromycin, Tetracyclines, Clindamycin, Chloramphenicol, Linezolid and Ethambutol.

Primarily bactericidal- Penicillins, Cephalosporins, Aminoglycosides and Vancomycin.

Some primarily static drugs may become cidal at higher concentrations (as attained in the urinary tract), e.g. erythromycin, nitrofurantoin. On the other hand, some cidal drugs, e.g. cotrimoxazole, streptomycin may only be static under certain circumstances.

F. Antibiotics are obtained from

Fungi- Penicillin, Griseofulvin, Cephalosporin.

Bacteria- Polymyxin B, Tyrothricin, Colistin, Aztreonam, Bacitracin.

Actinomycetes- Aminoglycosides, Macrolides, Tetracyclines, Polyenes, Chloramphenicol.

![Diagram of bacterial cell processes](fig.4.1)
MICROBIALS

BACTERIA

In 1928, a German scientist C.E. Chrenberg first used the term “bacterium” to designate small microscopic organism with a relatively simple and primal form of the cellular organization known as “Prokaryotic”.

Bacteria are generally unicellular e.g. cocci, bacilli, etc. filamentous, eg. actinomycetes, some being sheathed having certain cells specialized for reproduction. The microorganisms are capable of producing diseases in host are known as ‘pathogenic’. Most of the microorganisms present on the skin and mucous membrane are non pathogenic and are often referred to as “commensals” or if they live on food residues as in intestine, they may be called “saprophytes”. Generally, the pathogenic cocci and bacilli are gram positive and the pathogenic coco bacilli are gram negative.

GRAM POSITIVE AND GRAM NEGATIVE BACTERIA (wikipedia)

Gram-positive bacteria show colour in the gram stain test. These bacteria adopt the crystal violet stain used in the test, and when seen through a microscope, appear to be purple-coloured. The thick peptidoglycan layer of the cell wall retains the stain when it is washed away from the rest of the sample, in the decolorization stage of the test so it appeared purple in colour. Despite their thicker peptidoglycan layer, gram-positive bacteria are more accessible to antibiotics than gram-negative, by reason of the absence of the outer membrane.

Gram Negative bacteria cannot retain the violet stain after the decolorization process. In this stage alcohol is used which degrades the outer membrane of gram-negative cells making the cell wall more porous and unable of retaining the crystal violet stain. Their peptidoglycan layer is much thinner.

Cell wall structure of Gram positive and gram negative bacteria is shown in (Fig. 4.4). (http://wikieducator.org/File:Gramstain.jpg)
A fungus is any member of the group of eukaryotic organism that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. In 1969, an American biologist, Whittaker, accepted that fungi are dissimilar from other eukaryotes in many essential aspects, so he designated them to a new kingdom. Most fungi live as saprobes in soil or dead plant material and are important in mineralization of organic matter. Some of the fungi are beneficial to humans. A small proportion of these fungi are pathogenic to animals and plants. 

https://upload.wikimedia.org/wikipedia/commons/f/fc/Fungi_collage.jpg

(Fig. 4.4)
EXPERIMENT

ABSTRACT

A brief account of activity profile of some newly synthesized compounds has been discussed in this chapter. All the newly synthesized compounds in Chapter 2 were screened for their in vitro antimicrobial activities. Antibacterial activity was tested against clinically isolated bacterial strains such as Gram positive (Staphylococcus aureus, Streptococcus pyogenes) and Gram negative (Escherichia coli) using Ampicillin as standard drug. Antifungal activity was tested for three fungal strains (Candida albicans, Aspergillus niger and Aspergillus clavatus) using Griseofulvin as standard drugs. Broth Dilution Method was used to evaluate antimicrobial activity. Results are given in MIC (µg/mL).

EVALUATION TECHNIQUES

- Many methods have been used by numerous workers to evaluate the antimicrobial activity such as Turbidometric, Agar streak dilution, Agar diffusion and Serial dilution methods.

- For Agar Diffusion method Paper Disc, Agar Cup and Agar Ditch techniques are used

- We have used the **Broth Dilution Method** to evaluate the antimicrobial activity. It is one of the non automated in vitro microbial susceptibility tests. This standard method yields a quantitative result for the amount of antimicrobial agents that is needed to restrain growth of specific microorganisms. It is carried out in tubes.
  - Macrodilution Method in Tubes
  - Microdilution format using plastic trays

- The advantage of the ‘Broth Dilution Method’ for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

- The following conditions must be met for the evaluation of antimicrobial activity:
Necessary conditions should be provided for the growth of microorganisms.

There should be close contact between the test organisms and substance to be evaluated.

Conditions should be same throughout the study.

Sterile environment should be maintained.

**MINIMAL INHIBITION CONCENTRATION [MIC]** (wikipedia)

In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of a chemical that prevents visible growth of bacteria (in other words, at which it has bacteriostatic activity), whereas the Minimum bactericidal concentration (MBC) is the concentration that results in microbial death (in other words, the concentration at which it is bacteriocidal)\(^1\).

The MIC of a chemical is determined by preparing solutions of the chemical at increasing concentrations, incubating the solutions with the separate batches of cultured bacteria, and measuring the results using agar dilution and broth microdilution, usually following the guidelines of a references such as the BSAC, CLSI and EUCAST (European Committee on Antimicrobial Susceptibility Testing)\(^4\).

**MATERIAL AND METHODS**\(^5\text{-}^9\)

- All the synthesized drugs were used for Antimicrobial test procedures.
- All necessary controls are:
  - Drug Control
  - Agar Control
  - Vehicle Control
  - Organism Control
  - Known Antibacterial Drugs Control
- All MTCC cultures were tested against mentioned standard drugs and synthesized drugs.
Inoculum size for test strain was adjusted to $10^8$ Cfu [Colony Forming Unit] per milliliter by comparing the turbidity.

Mueller Hinton Broth was used as nutrient medium to grow and dilute the drug suspension for the test strains.

DMSO was used as diluents to get desired concentration of drugs to test upon strains.

Following common standard strains were used for screening of antibacterial and antifungal activities: The strains were procured from Institute of Microbial Technology, Chandigarh.

- *Staphylococcus aureus* MTCC 96 (Gram positive)
- *Streptococcus pyogenes* MTCC 442 (Gram positive)
- *Escherichia coli* MTCC 443 (Gram negative)
- *Candida albicans* MTCC 227
- *Aspergillus niger* MTCC 282
- *Aspergillus clavatus* MTCC 1323

1. Serial dilutions were prepared in primary and secondary screening.

2. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at $37^0C$ overnight.

3. The MIC of the control organism is read to check the accuracy of the drug concentrations.

4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.

5. The amount of growth from the control tube before incubation [which represents the original inoculum] is compared.
PRIMARY AND SECONDARY SCREENING

Each synthesized drug was diluted obtaining 2000 µg/mL concentration as a stock solution.

**Primary screen-** In primary screening 1000 µg/mL, 500 µg/mL and 250 µg/mL concentrations of the synthesized drugs were taken. The synthesized drugs which found active in this primary screening were further tested in a second set of dilution against all microorganisms.

**Secondary screen-** The drugs found active in primary screening were similarly diluted to get 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.250 µg/mL concentrations.

OBSERVATIONS

The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculum. The test mixture should contain $10^8$ organism/mL.

*Bold numbers indicate more or equivalent potent compounds compared to standard drugs; SA, *S. aureus*; SP, *S. pyogenes*; EC, *E. coli*; CA, *C. albicans*; AC, *A. clavatus*; AN, *A. niger*; MTCC, microbial type culture collection*

<table>
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<th>Compounds</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
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<tbody>
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<td></td>
<td>Gram +ve</td>
<td>Gram –ve</td>
</tr>
<tr>
<td></td>
<td>SA</td>
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</tr>
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<td>2.I.IVc</td>
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<td>125</td>
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<tr>
<td>2.I.IVd</td>
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<tr>
<td>Griseofulvin</td>
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Graph 4.I: Graphical presentation of MICs (µg/mL) against bacterial strains

Graph 4.II: Graphical presentation of MICs (µg/mL) against fungal strains
Table 4.II: *In vitro* Antimicrobial activity of Synthesized Compounds 2.II.Ia-c, 2.II.IIa-d and 2.II.IIIa-c MICs (µg/mL)

<table>
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<tr>
<th>Comp.</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>Griseofulvin</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Bold numbers indicate more or equivalent potent compounds compared to standard drugs; SA, *S. aureus*; SP, *S. pyogenes*; EC, *E. coli*; CA, *C. albicans*; AC, *A. clavatus*; AN, *A. niger*; MTCC, microbial type culture collection*
Graph 4.III: Graphical presentation of MICs (µg/mL) against bacterial strains

Graph 4.IV: Graphical presentation of MICs (µg/mL) against fungal strains
Table 4.III: *In vitro* Antimicrobial activity of Synthesized Compounds

2.III.III.a-d and 2.III.Va-d MICs (µg/mL)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Gram +ve</td>
<td>Gram –ve</td>
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<td>2.III.IIIa</td>
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<td>2.III.IIIb</td>
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</tr>
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<td>2.III.Vb</td>
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</tr>
<tr>
<td>2.III.Vc</td>
<td>250</td>
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<tr>
<td>2.III.Vd</td>
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</tr>
<tr>
<td>Ampicillin</td>
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<td>100</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Bold numbers indicate more or equivalent potent compounds compared to standard drugs; SA, *S. aureus*; SP, *S. pyogenes*; EC, *E. coli*; CA, *C. albicans*; AC, *A. clavatus*; AN, *A. niger*; MTCC, microbial type culture collection*
Graph 4.V: Graphical presentation of MICs (µg/mL) against bacterial strains

Graph 4.VI: Graphical presentation of MICs (µg/mL) against fungal strains
RESULTS AND DISCUSSION

The observed minimum inhibitory concentrations (MICs \(\mu g/mL\)) for compounds synthesized in Part I of chapter 2 are given in Table 4.I. The results revealed that some of the synthesized compounds were showing good to moderate activity against bacterial and fungal strains. For gram positive bacteria \(S.aureus\) compounds 2.I.IVc and 2.I.VId showed excellent activity, while compounds 2.I.IVb and 2.I.VIc were equipotent as compared to standard drug ampicillin (MIC = 250 \(\mu g/ ml\)). Compound 2.I.IVb was found equipotent to ampicillin (MIC = 100 \(\mu g/ ml\)) against \(S. pyogenes\). For gram negative bacteria \(E. coli\) compound 2.I.IVc exhibited the highest activity whereas compounds 2.I.VIb and 2.I.VIc exhibited equally good activity as compared to standard drug. The investigation of antifungal activity revealed that compounds 2.I.IVc and 2.I.IVd showed equipotent activity as compared to griseofulvin (MIC = 100 \(\mu g/ ml\)) against \(A. clavatus\). For \(C. albicans\) compounds 2.I.IVc, 2.I.VIb and 2.I.VIc were found to be more active as compared to griseofulvin (MIC = 500 \(\mu g/ ml\)), whereas compounds 2.I.IVd and 2.I.VIc were equally active.

From Table 4.II results, following inferences can be drawn for Part II compounds of chapter 2. In study, it is found that Mannich bases and tetrazole derivatives exhibited good activity. Compound 2.II.Ia showed the highest inhibitory effect against \(E. coli\) while compounds 2.II.Ic, 2.II.IIb and 2.II.IIIc are equally active. Compounds 2.II.Ia, 2.II.Ic, 2.II.IIb, 2.II.IIIb and 2.II.IIIc showed equipotent activity against \(S. aureus\) to standard ampicillin (MIC = 250 \(\mu g/ ml\)). Against \(S. pyogenes\) only compounds 2.II.Ia and 2.II.IIIb showed equal potency. The results of antifungal studies revealed that tetrazole derivatives were showing significant activity against all the tested strains especially compound 2.II.IIIa demonstrated excellent activity than standard drug for \(A. clavatus\). Amongst the other derivatives compounds 2.II.Ia, 2.II.Ic, 2.II.IIIa and 2.II.IIIb exhibited stronger activity against \(C. albicans\) as compared to griseofulvin (MIC = 500 \(\mu g/ ml\)) while compounds 2.II.Ib, 2.II.IIIc and 2.II.IIIc are equally potent. Compounds 2.II.IId, 2.II.IIIb and 2.II.IIIc exhibited potent activity against \(A. niger\).
Table 4.III describes minimum inhibition concentration for antibacterial and antifungal activities for Part III of chapter 2. Compound 2.III.IIIc was significantly superior to other compounds in exhibiting antimicrobial activity. The compounds 2.III.IIIb and 2.III.IIId showed equal activity as standard against S. aureus whereas 2.III.IIIc demonstrated an excellent activity against both S. aureus, S. pyogenes as standard drug ampicillin (MIC = 250 µg/ ml, 100 µg/ ml respectively). For E. coli bacteria compounds 2.III.IIIa, 2.III.IIIc and 2.III.Vc were equally active as compared to ampicillin (MIC = 100 µg/ ml). In antifungal study compounds 2.III.IIIb exhibited highest activity while 2.III.IIIc and 2.III.Vc found equally good as griseofulvin (MIC = 100 µg/ ml) against A. clavatus. For A. niger only compound 2.III.IIIa exhibited moderate activity. Compounds 2.III.IIIb, 2.III.IIIc and 2.III.Ve showed strongest activity for C. albicans.

ANTIMALARIAL ACTIVITY

INTRODUCTION

(Fig. 4.6)
Malaria is endemic in most parts of India and other tropical countries. It is one of the major health problems. It is caused by a protozoan parasite of the Plasmodium genus, with 107 countries and territories having areas at risk of transmission. The World Health Organization (WHO) reported the occurrence of 214 million cases worldwide in 2015, and the death of 438,000 people, mostly children in the African region. This disease is mainly attributable to five species of plasmodium:

1. *P. vivax*
2. *P. falciparum*
3. *P. ovale*
4. *P. malariae*
5. *P. knowlesi*

*Plasmodium falciparum* (*P. falciparum*) is the most dangerous of the malaria parasites and its resistance to currently available drugs continues to grow, and presents an impediment to attempts to successfully deal with the disease. There is, therefore, great need and challenge to continuously develop new inhibitors, with the goal to overcome parasite resistance. However, the mechanism by which these compounds apply their anti-malarial properties is still not fully clear. One suggested mechanism is the formation of a complex with heme within the food vacuole that inhibits hematin polymerization.

A *Plasmodium* from the saliva of a female mosquito moving across a mosquito cell is shown in (Fig. 4.6). (Image by Ute Frevert; false color by Margaret Shear - http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.0030192, CC BY 2.5, https://commons.wikimedia.org/w/index.php?curid=219588)

Life cycle of the malaria parasite illustrating the various stages (Fig. 4.7)

About four centuries ago, a treatment for malaria was discovered in the bark of the Cinchona tree. Quinine was the first effective treatment for malaria caused by *P. falciparum*. It was used as anti-malarial medication from the seventeenth century until the 1920s, when Chloroquine, a more effective synthetic anti-malarial, became available.
Though a number of antimalarial drugs such as quinine, chloroquine, mefloquine, pyrimethamine, primaquine, piperaquine, amodiaquine (Fig. 4.8) are available, these drugs are rapidly losing their therapeutic potential due to emerging drug resistance in Plasmodium\textsuperscript{16, 17}.

Different approaches adopted till now by the scientific communities for antimalarial drug discovery are mainly based upon\textsuperscript{18} (a) chemical modification of known drugs and compound classes (mefloquine, primaquine, chloroquine, artesunate) (b) combination of existing drugs (artemether-lumefantrine, amodiaquine-artesunate, dihydroartemisinin-piperaquine) (c) use of natural products (quinine, artemisinin) (e) use of drug resistance reversers (verapamil, desipramine, trifluoperazine)\textsuperscript{19} (d) in vitro whole cell parasitic assay (KAE609 (formerly known as NITD609) and KAF156 under phase II clinical trials)\textsuperscript{20}. (f) “piggy back approach” for the compounds active against other diseases (antifolates, tetracyclines, atovaquone).
Several novel drug candidates based on the chloroquine structure, with modifications of both the side chain and the quinoline ring, have been reported\textsuperscript{21–24}.

\textbf{Fig. 4.8}

**CLASSIFICATION\textsuperscript{1}**

Antimalarial drugs are classified in following ways:

- 4-Aminoquinolines- Chloroquine (CQ), Amodiaquine (AQ), Piperaquine
- Quinoline methanol- Mefloquine
- Cinchona alkaloid- Quinine, Quinidine
- Biguanide- Proguanil (Chloroguanide)
- Diaminopyrimidine- Pyrimethamine
- 8-Aminoquinoline- Primaquine, Tafenoquine
- Sulfonamides and sulfone – Sulfadoxine, Sulfamethopyrazine, Dapsone
- Antibiotics- Tetracycline, Doxycycline, Clindamycin
- Sesquiterpine lactones – Artesunate, Artemether, Arteether, Arterolane
- Amino alcohols- Halofantrine, Lumefantrine
- Naphthyridine- Pyronaridine
- Naphthoquinone- Atovaquone

EXPERIMENT

ABSTRACT

All the final products of Part 2.III (Chapter 2) and Chapter 3 were screened for antimalarial activity. For antimalarial activity mean IC₅₀ values are given. Chloroquine and Quinine were used as the reference drug.

MATERIAL AND METHODS

IN VITRO ANTIMALARIAL SCREENING²⁵⁻³⁰

All the synthesized compounds were evaluated for antimalarial activity in the Microcare laboratory & TRC, Surat, Gujarat. The in vitro antimalarial assay was carried out in 96 well microlitre plates by following the microassay protocol of Rieckmann and co-workers with minor modifications. The cultures (P. falciparum strain) were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 0.23% sodium bicarbonate, 1% D-glucose and 10% heat inactivated human serum. The asynchronous parasites of P. falciparum were synchronized after 5% D-sorbitol treatment to attain only the ring stage parasitized cells. For execution of the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µL of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O⁺). A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture
medium. The diluted samples in 20 μL volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4 µg/mL to 100 µg/mL in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37°C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were observed microscopically to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Chloroquine and quinine were used as the reference drug.

OBSERVATIONS

The mean number of rings, trophozoites and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 hours, and percent maturation inhibition with respect to control group. Results are given in the Table 4.IV and 4.V.

Table 4.IV: Antimalarial activity (MIC) of Compounds Synthesized in Scheme 2.III

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Compounds</th>
<th>Molecular Formula</th>
<th>Mean IC&lt;sub&gt;50&lt;/sub&gt; values (µg/mL)</th>
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Graph 4.VII: Graphical presentation of mean IC$_{50}$ value (μg/mL) against *Plasmodium falciparum*

Graph 4.VIII: Graphical presentation of Mean IC$_{50}$ value (μg/mL) against *Plasmodium falciparum*
RESULTS AND DISCUSSION

Compounds synthesized in Scheme 2.III (Chapter 2) and Scheme 3 (Chapter 3) evaluated for antimalarial activity. All the synthesized compounds were screened against Plasmodium falciparum. Table 4.IV depicted that few of the compounds were good active and most showed moderate activity. From the results it is evident that compounds 2.III.IIIb, 2.III.IIIc and 2.III.Vc exhibited potent activity as compared to Quinine. Compounds 2.III.IIIa, 2.III.IIId and 2.III.Vb are moderately active.

The investigation of antimalarial activity results in Table 4.V revealed that all tested compounds showed moderate antimalarial activity. Among the series 3.IIa-f and 3.IIIa-f compounds 3.IIa and 3.IIb showed potent activity whereas compounds 3.IIc, 3.IIIc and 3.IIId are moderately active as compared to Quinine.

CONCLUSION

We have described simple and efficient synthesis of pyrimidine and acridine derivatives with good yields. 26 compounds in chapter 2 and 12 compounds in chapter 3 were synthesized. Structures of the synthesized compounds were established by IR, 1H NMR, MASS spectral data and elemental analysis. All the newly synthesized compounds of Chapter 2 have been evaluated for their in vitro antimicrobial activity. Final Products of Chapter 3 and part III of Chapter 2 were screened for antimalarial activity. The results of antimicrobial and antimalarial analysis reveals that the synthesizd compounds are promisingly significant and most of them possess excellent antimicrobial activity than antimalarial activity. Conclusion can be summarized as following-

- Heterocycles accommodating pyrimidine with morpholine and tetrazole are good antibacterial and antifungal agents respectively.

- The structure-activity relationship studies revealed that –Cl and –F functional groups substituted benzo[b]thiophene derivatives have shown better activity than others. These results indicate that electron
withdrawing group substituted derivatives may be useful leads for antimicrobial agent in the future.

- These new pyrimidine and acridine derivatives have proved to be promising candidates for further efficacy evaluation.
REFERENCES


