1. INTRODUCTION

Heterocyclic chemistry represents a vast and important area of research which is of interest to a wide spectrum of chemists. Heterocyclic compounds are cyclic compounds with two or more different kinds of atoms as a part of the ring structure. The frequent heteroatoms are nitrogen, oxygen and sulphur. The number of atom in the heterocyclic ring can range from three to many. The smallest ring possible is three members e.g. azirine but very large rings are possible in the case of crown ethers. Heterocyclic compounds can be synthesized by cyclization reaction, addition reaction, ring transformation or replacement involving groups. They have contributed to the development of society from a biological and industrial point of view as well as to the understanding of life processes and to the efforts to improve the quality of life.

Heterocyclic compounds are widely distributed in nature and essential to life in various ways, most of the sugars and their derivatives including vitamins and some members of vitamin B group possess heterocyclic rings containing nitrogen as a heteroatom. The numerous plant alkaloids are example of complex nitrogenous ring compounds. They have been used for thousands of years in various religious, cultural and medicinal applications. With the advent of modern organic chemistry more and more of the basic principles for their activities has been elucidated. Heterocyclic compounds are important components of the side chain of the amino acid, histidine. They are found at the active site of many enzymes, where they are involved in proton transfer reactions. The discovery of penicillin and its remarkable bactericidal properties and the urge to accomplish its synthesis, provoked intensive research in the field of heterocycles.

The heterocyclic ring systems play vital role in daily life of animals and plants. Majority of the drugs being introduced in pharmacopeias every year are heterocyclic compounds. Coenzymes originator such as thiamine, riboflavin, nicotinic acid, pyridoxine, folic acid, adenine, biotin, photosynthesizing pigment chlorophyll, the oxygen transporting pigment hemoglobin, purine and pyrimidine components of nucleic acid and their breakdown products uric acid, alloxan, allantion and the amino acids histidine, tryptophan, proline and other metabolically active compounds like
heteroauxin, serotonin and histamine all are heterocyclic compounds. Some heterocycles are fundamental to life such as haem derivatives in blood and the chlorophylls essential for photosynthesis. Likewise, the paired bases found in RNA and DNA are heterocycles. The interesting biological activities of heterocycles have stimulated considerable research work in recent years including synthetic utility. Synthetic heterocyclic compounds are found to use as antimicrobial, antimalarial, antitubercular, hypnotics, anticonvulsants, anticancer, antihistaminics, antiseptics as well as herbicides and pesticides etc.

Over the past few decades, the incidence of fungal and bacterial infections has increased dramatically. The problems posed by multi-drug resistant microorganisms have reached an alarming level in many countries around the world. The use of most antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by the unsatisfactory status of the present treatment of bacterial and fungal infections. The infections have soundly affected humankind as well as animals and it is caused by those microorganisms represent a serious challenge to the medical community; hence, the development of new antimicrobial agents is an important goal. Extensive attention has been focused on the development of more potent and effective antimicrobial agents as the devotion to the antimicrobial medicine research.

Present thesis explores synthesis, characterization and biological evaluation of some new heterocyclic compounds bearing 2-chloroquinoline, 2-morpholinoquinoline and 2-thiophenoxyquinoline as a prime nucleus, where in other biologically active heterocycles such as pyridine, N-arylquinoline, chromene, pyran and benzimidazole derivatives have been introduced with the hope that the assimilation of more than one bioactive nucleus in a single scaffold may produced novel heterocycles with fascinate pharmacological effect along with efficient bioactivity. Hence, it is worthy here to provide brief introduction of quinoline moiety, their synthesis and medicinal application of various quinoline compounds. The introduction of other bioactive heterocycles are presented chapter wise.
1.1 QUINOLINE

Quinoline is an aromatic nitrogen containing compound characterized by a double-ring structure where a benzene ring is fused to pyridine at two adjacent carbon atoms. Quinoline was first isolated in an impure state in 1834 by Runge from coal-tar distillate\(^1\). Shortly after the isolation of quinoline from coal tar it was also recognized as a pyrolytic degradation product of cinchonamine, an alkaloid closely related to quinine, from which name quinoline is derived; the word quinine, in turn, derives from quina, a Spanish version of a local South American name for the bark of quinine-containing Cinchona species. Gerhardt obtained quinoline, probably contaminated by lepidine by distillation of cinchonine and quinine with caustic alkali, and named it quinoleine\(^2\). This name was subsequently changed to quinoline by Berzelius.

Quinoline can be prepared from aniline with acrolein under heated sulfuric acid (Skraup synthesis). Various quinoline compounds can be prepared by Skraup synthesis using different oxidizing agents. Quinoline family compounds are widely used as a parent compound to make drugs possessing activity like anti-malarial, antihypertensive, antimicrobial, analgesic, antitubercular etc. They are useful for the synthesis of alkaloids, dyes, rubber chemicals and flavoring agents. Moreover, they are also used as catalyst, corrosion inhibitor, preservative and as solvent for resins and terpenes and in the production of paints and in transition-metal complex catalyst chemistry for uniform polymerization and luminescence chemistry. They are used as antifoaming agent in refinery field. Quinaldine, 2-methylquinoline, is used as to prepare antimalarial drugs and used in manufacturing oil soluble dyes, food colorants, pharmaceuticals, pH indicators and other organic compounds.
The remarkable capability of quinoline alkaloids as well as synthetic quinoline derivatives with biological activity have received considerable attention from the chemical community, especially from biochemists and synthetic organic chemists who are concerned with human and animal health problems.

1.2 NATURAL OCCURANCE

Quinine\(^3\) is natural white crystalline alkaloids having antipyretic, antimalarial, analgesic and anti-inflammatory properties and a bitter taste. It is a stereoisomer of quinidine.

\[
\begin{align*}
\text{Quinine} & \quad \text{Quinine} \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\end{align*}
\]

Quinine was the first effective treatment for malaria caused by plasmodium falciparum, appearing in therapeutic in the 17\(^{th}\) century.

The stereoisomer of quinine, (+)-Quinidine is originally derived from the bark of the cinchona tree and acts as a class-I anti-arrhythmic agent in the heart.

\[
\begin{align*}
\text{Quinidine} & \quad \text{Quinidine} \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\end{align*}
\]

A considerable number of medicinally important alkaloids have been isolated from the plant of Rutaceae family\(^4\). Representative example of this class of compound includes atanine, the angular alkaloid araliopsine and linear alkaloid isoplatydesmine.
These types of compounds have been shown to exhibit a variety of pharmacological properties including antimicrobial, antiviral, mutagenic and cytotoxic activities.

Some of the therapeutically active quinoline alkaloids are reviewed here.

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cusparine</td>
<td>Antileishmanial</td>
</tr>
<tr>
<td>Ribalinine</td>
<td>Calcium channel blocker</td>
</tr>
<tr>
<td>Buchapine</td>
<td>HIV</td>
</tr>
<tr>
<td>Semecarpifoline</td>
<td>Antiplatelet aggregation</td>
</tr>
<tr>
<td>Galipeine</td>
<td>Antimalarial and cytotoxic activity</td>
</tr>
</tbody>
</table>

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Cusparine

Ribalinine

Buchapine

Semecarpifoline

Galipeine
1.3 SYNTHETIC QUINOLINE DERIVATIVES AS THERAPEUTIC AGENTS

A number of derivatives of quinoline serve as valuable therapeutic agents. Some hundred years ago cinchona bark was introduced for the treatment of malaria, and until very recently quinine has remained the standard remedy for this disease. Several other synthetic antimalarial drugs are based on quinoline nucleus e.g. Chloroquine. Ciprofloxacin is one of the effective antibacterial agent in the market.

Considerable interest has been created in the chemistry of quinoline due to their wide spectrum of therapeutic activities including antibacterial\(^{15}\), antifungal\(^{16}\), antimycobacterial\(^{17}\), antimalarial\(^{18-19}\), anti-inflammatory\(^{20}\), anticancer activity\(^{21}\) etc.

Some of the therapeutically active quinoline derivatives are reviewed here.

<table>
<thead>
<tr>
<th>Quinoline derivatives</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aaptamine</td>
<td>Cardiac(^{14})</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Antimicrobial(^{22})</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
</tr>
</tbody>
</table>
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- Antileishmanial\textsuperscript{23}

- Antioxidative\textsuperscript{24}

- Antituberculosis\textsuperscript{25}

- Antimalarial\textsuperscript{26}

- Anticancer\textsuperscript{27}

- Anti-convulsant\textsuperscript{28}

- Antimalarial\textsuperscript{29}
1.4 SYNTHETIC ROUTES FOR QUINOLINE

Quinoline derivatives can be synthesized by using various routes. Some of these are summarized below.

(A) Skraup synthesis

The Skraup synthesis is probably the most important synthetic route to quinoline derivatives. Quinoline is produced when aniline, concentrated sulphuric acid, glycerol and oxidizing agent are heated together. The reaction has been shown to proceed by dehydration of glycerol to acrolein to which aniline then adds in conjugate fashion. Acid-catalyzed cyclization produces a 1,2-dihydroquinoline finally dehydrogenated by oxidizing agent to give quinoline. Skraup synthesis is the best for the ring synthesis of quinoline unsubstituted on the hetero-ring.

(B) Doebner V. Miller synthesis

This is a modification of Skraup synthesis of quinolines and consists in heating primary aromatic amine and aldehyde with sulfuric acid. In this synthesis glycerol is replaced by two molecules of aldehydes.
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The α,β-unsaturated aldehyde (1), initially formed from two molecules of aldehydes by acid-catalyzed aldol condensation, reacts with aniline to give (2). Its cyclization in presence of strong acid and dehydrogenation yields quinoline homologue. It is believed that the oxidative step is brought about by the action of schiff base (3) produced in situ (from aniline and aldehyde).

(C) Beyer’s modification of the Dobner-V. Miller synthesis

Substitution of a methyl ketone for the second molecule of aldehyde in the Dobner-V. Miller synthesis\textsuperscript{34} results in the formation of a 2,4-disubstituted quinoline.

(D) Conrad-Limpach-Knorr synthesis

β-Keto ester, such as ethyl acetoacetate can react with an aromatic amine in either of two ways. The factors governing the manner in which the condensation takes place have been greatly clarified by Houser and Reynolds\textsuperscript{35}.
(E) Combes method

Combes method resembles the Conrad-Limpach-Knorr synthesis so closely that it must be classed as a variant of this method. Aromatic amines are condensed with 1,3-diketones and the resulting substances are then ring-closed to 2,4-disubstituted quinolines.\(^\text{36}\)

(F) Friedlander synthesis

Friedlander\(^\text{37}\) obtained quinoline by the condensation of o-aminobenzaldehyde with acetaldehyde in the presence of sodium hydroxide. The Friedlander ring closure involves two distinct reactions: (1) Schiff base formation between the amino group of the aniline and the carbonyl group of the acetaldehyde and (2) an internal claisen type of condensation between the aryl aldehyde group and the α-hydrogens of the acetaldehyde. Piperidine is a condensing agent.\(^\text{38}\)
Besthorn and Fischer on the basis of Friedlander’s synthesis of quinoline demonstrated the mode of formation of flavanilne. When acetanilide is heated with zinc chloride, the acetyl group migrates in part to the ortho position and in part to the para position. The resulting o-acetyl aniline and p-acetyl aniline then undergo condensation in sense of Friedlander’s synthesis of quinoline to yield flavanilne.

\[
\begin{align*}
\text{NH}_2 & \quad \text{NH}_2 \\
\text{COCH}_3 & + \text{NH}_2
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 & \quad \text{NH}_2
\end{align*}
\]

\[\text{Flavanilne} \]

(G) **The Pfitzinger reaction**

The reaction is carried out by Pfitzinger in 1886, by boiling the isatin with sodium hydroxide solution, and the resultant isatic acid is condenses directly with the ketone. Isatin is hydrolyzed to an o-amino keto acid which condense with ketones or acids that have a reactive methylene group\(^{39}\).

(H) **Gould-Jacobs reaction**

The Gould-Jacobs reaction is an organic synthesis for the preparation of quinolines\(^{40}\). In this reaction aniline or an aniline derivative first reacts with malonic acid derivative \textit{ethyl ethoxymethylene malonate} with substitution of the ethoxy group by nitrogen. A benzannulation takes place by application of heat to a quinoline. The ester group is hydrolyzed by sodium hydroxide to the carboxylic acid followed by decarboxylation again by application of heat to 4-hydroxyquinoline.
(I) Meth-Cohn synthesis

In the Meth-Cohn quinoline synthesis, the acetanilide becomes a nucleophile and provides the framework of the quinoline (nitrogen and the 2,3-carbons) and the 4-carbon is derived from the Vilsmeier reagent. The reaction mechanism involves the initial conversion of an acetanilide into an \( \alpha \)-iminochloride (a) by the action of POC\(_3\). The \( \alpha \)-chloroenamine tautomer (b) is subsequently C-formylated by the Vilsmeier reagent derived from POC\(_3\) and DMF. In examples where acetanilide is employed, a second C-formylation of (c) occurs to afford (d); subsequent cyclisation and aromatisation by loss of dimethylamine finally affords the 2-chloroquinoline-3-carbaldehyde (I).
Other methods for the synthesis of quinoline and its various derivatives have been reported in literature\textsuperscript{42-47}.

1.5 SYNTHESIS, REACTIONS AND BIOLOGICAL ACTIVITIES OF 2-CHLOROQUINOLINE-3-CARBALDEHYDE

As the quinoline compounds reported in the thesis are derived from 2-chloroquinoline-3-carbaldehyde, more details regarding its synthesis and reactions are also reviewed here.

(A) Synthesis of 2-chloroquinoline-3-carbaldehyde

In the broad field of quinoline, 2-chloroquinoline-3-carbaldehyde possesses a prominent position in the intermediate category as it can be utilized for the synthesis of many heterocyclic compounds. There has been relentless interest towards the use of Vilsmeier-Haack reagent in organic synthesis of several nitrogen and oxygen heterocycles. The Vilsmeier-Haack reagent (VMH) (Halomethyleneiminium salt) formed from the interaction of dialkyl formamides such as DMF with \( \text{POCl}_3 \) has
attracted the attention of synthetic organic chemists since its discovery in 1927\textsuperscript{48}. It is one of the most commonly used reagents for the introduction of an aldehydic (-CHO) group in to aromatic and heteroaromatic compounds. It is proved to be a mild and efficient method for the formylation\textsuperscript{49-53}. The utility of this reagent also explores the powerful route for the synthesis of substituted 2-chloroquinoline-3-carbaldehyde.

Rajana et al\textsuperscript{54} have recently demonstrated that acetanilides undergo rapid cyclisation in micellar media to afford 2-chloroquinoline-3-carbaldehyde. Cyclisation in the presence of cetyl trimethylammonium bromide (CTAB) under Vilsmeier-Haack conditions afforded 2-chloroquinoline-3-carbaldehyde in good yield. As the current trend of green chemistry they have also synthesized the 2-chloroquinoline-3-carbaldehydes using ultrasound in the presence of micelles like CTAB (cetyltrimethylammonium bromide). Under ultrasonic irradiation the reaction time were reduced with dramatic enhancement in the yield of reaction products\textsuperscript{55}.

Gupta et al\textsuperscript{56} reported the Vilsmeier-Haack cyclization of acetanilides under microwave-irradiation using silica as a support in solvent-free condition. This method is rapid and efficient.

P. T. Perumal and R. R. Amaresh have reported synthesis of 4-chloro-3-quinolinecarbaldehyde from o-aminoacetophenone using Vilsmeier reagent\textsuperscript{57}.
Mazahir Kidwai and Shelly Jindal have described the method for the preparation of 4-methyl-2-chloroquinoline-3-carbaldehyde starting from acetoacetanilide\textsuperscript{58}.

P. A. Pawar, P. B. Bajare and coworkers have reported synthesis of 4-methyl-2-chloroquinoline-3-carbaldehyde from acetophenone oxime under the Vilsmeier cyclization conditions\textsuperscript{59}.

(B) Reactions of 2-chloroquinoline-3-carbaldehyde:-

As 2-chloroquinoline-3-carbaldehyde is having reactive functional group at position-2 and -3, various reactions have been carried out and some of the reactions are depicted below.

The formyl group of 2-chloroquinoline-3-carbaldehyde is highly reactive and gives variety of Schiff's bases by reaction with hydrazine hydrate, phenyl hydrazine and hydroxylamine hydrochloride\textsuperscript{60}. 
R. P. Shrivastava et al have described the grignard reaction of 2-chloro-3-formyl quinoline\(^6\) (1), and K. R. Rao et al has reported the oxidation reaction of 2-chloroquinoline-3-carbaldehyde\(^2\) (2).

2-Chloro-3-cyanoquinoline derivatives have been carried out by reaction of 2-chloroquinoline-3-carbaldehyde with aq. Ammonia in presence of iodine as a catalyst\(^3\).

S. Kumae et al\(^4\) have reduced the 2-chloro-6-methylquinoline-3-carbaldehyde to 2-chloro-3-(hydroxymethyl)-6-methylquinoline with solid sodium borohydride in methanol, which on subsequent chlorination with thionyl chloride in dry benzene gave
2-chloro-3-(chloromethyl)-6-methylquinoline and then upon reaction with aniline to give N-[(2-chloro-6-methylquinolin-3-yl)methyl]aniline

\[
\begin{align*}
\text{H}_2\text{C}-\text{N}-\text{Cl} & \xrightarrow{\text{CH}_3\text{CHO}} \text{H}_2\text{C}-\text{N}-\text{Cl} \\
& \xrightarrow{\text{i. NaBH}_4} \text{H}_2\text{C}-\text{N}-\text{Cl} \\
& \xrightarrow{\text{ii. SO}_2} \text{EtOH/TEA} \quad \text{H}_2\text{C}-\text{N}-\text{Cl}
\end{align*}
\]

\(O,O\)-diethyl \(o\)-(2-chloroquinolin-3-yl)-methyl phosphorothioate derivatives in almost quantitative yield have been prepared by the reaction of 2-chloro-3-(hydroxymethyl)-quinoline with \(O,O\)-diethyl phosphorochloridithioate in acetone in the presence of sodium hydroxide\(^65\).

\[
\begin{align*}
\text{R}_1\text{N}-\text{Cl} & \xrightarrow{\text{NaBH}_4} \text{R}_1\text{N}-\text{Cl} \\
& \xrightarrow{\text{MeOH}} \text{R}_1\text{N}-\text{Cl} \\
& \xrightarrow{\text{(EtO)}_2\text{P(S)Cl}} \text{Me}_2\text{CO}, \text{NaOH} \quad \text{R}_1\text{N}-\text{Cl} \\
& \xrightarrow{\text{R}_1=\text{H, CH}_3, \text{OCH}_3, \text{OCH}_2\text{CH}_3} \text{R}_1\text{N}-\text{Cl} \\
& \xrightarrow{\text{R}_2=\text{H, CH}_3, \text{OCH}_3} \text{R}_1\text{N}-\text{Cl} \\
& \xrightarrow{\text{R}_3=\text{H, CH}_3, \text{OCH}_2\text{CH}_3} \text{R}_1\text{N}-\text{Cl}
\end{align*}
\]

B. Bhat and coworkers have reported the reaction of 2-chloroquinoline-3-carbaldehyde with nitromethane or nitroethane in the presence of sodium acetate in methanol\(^66\).

\[
\begin{align*}
\text{EtO}-\text{N}-\text{Cl} & \xrightarrow{\text{CH}_3\text{NO}_2 \text{ or C}_2\text{H}_5\text{NO}_2} \text{EtO}-\text{N}-\text{Cl} \\
& \xrightarrow{\text{MeOH, NaOAc}} \text{EtO}-\text{N}-\text{Cl} \\
& \xrightarrow{\text{R=H, CH}_3} \text{EtO}-\text{N}-\text{Cl}
\end{align*}
\]

Acrylonitrile derivatives have been synthesized by reaction of various 2-chloroquinoline-3-carbaldehyde and 2-cyanomethyl-4-ketoquinazolinone derivatives\(^67\).
N. J. Datta and coworkers have reported synthesis of some new 4-thiazolidinones\(^{68}\) using 2-chloroquinoline-3-carbaldehyde.

The reaction between 2-chloroquinoline-3-carbaldehyde and substituted acetophenones has been carried out in domestic microwave oven and in presence of anhydrous K\(_2\)CO\(_3\) to gives chalcones in good yields\(^{69}\).
A. H. Kategaonkar and coworkers have reported the synthesis of new 2-chloro-3-((4-phenyl-1\(H\)-1,2,3-triazol-1-yl)methyl)quinoline derivatives\(^7\). 

![Chemical structure diagram]

Synthesis of tetrazolo[1,5-\(a\)]quinoline-3-carbaldehyde derivatives have been achieved by the reaction of 2-chloroquinoline-3-carbaldehyde with sodium azide\(^7\). 

![Chemical structure diagram]

M. Yu. Onysko and V. G. Lendel have reported the synthesis of 2-allyl(propargyl)oxyquinoline-3-carbaldehydes\(^7\) from 2-chloroquinoline-3-carbaldehyde.
The reaction of 2-chloroquinoline-3-carbaldehydes with thioglycolic acid in the presence of sodium hydroxide in absolute ethanol afforded two compounds. (i) [(3-formylquinolin-2-yl)thio]acetic acids with a 60–70% yield and (ii) thieno[2,3-b]quinoline-2-carboxylic acids with 30–40% yield.

A number of 2-chloro-quinoline-3-carbaldehyde were reacted with aniline to produce substituted dibenzo[b,g][1,8]naphthyridines.

A synthesis of substituted [1,2,4]-triazolo[1′,2′:1,2]pyrimido[6,5-b]-quinoline has been reported in literature using various catalyst using conventional as well as MWI method.
A convenient, one-pot, copper-free, Pd-catalyzed methodology has been described for the synthesis of 1,3-disubstituted pyrano[4,3-b]quinolines from 2-chloroquinoline-3-carbaldehydes. Formation of annulated products is attributed to the presence of Pd(OAc)$_2$ and PPh$_3$. Further, PPh$_3$ in the reaction mixture promotes the cyclization by reducing the reaction time and increasing the yield of cyclized product$^{76}$.

![Chemical structure of reaction](image)

The fused tetracyclic derivatives, 3-aryl/alkyl-9-substituted quinolino[3,2-f]1,2,4-triazolo[3,4-b]1,3,4-thiadiazepines have been reported by B. Kalluraya and coworkers from reaction of 6-substituted-2-chloroquinoline-3-carbaldehydes and 3-substituted-4-amino-5-mercapto-1,2,4-triazole$^{77}$.

![Chemical structure of reaction](image)

R. Nandha Kumar and co-worker$^{78}$ have prepared 2,3-heteroannelated quinoline derivatives like quino[3,2-f]benzoxazepines from reaction of substituted 2-chloroquinoline-3-carbaldehyde and 2-aminophenol in methanol.
H. R. P. Naik et al\textsuperscript{79} have reported the synthesis of 2-Mercaptopyrano[2,3-\(b\)]quinolin-2-ol and 2-Selenopyrano[2,3-\(b\)]quinolin-2-ol.

Some reactions of 2-chloroquinoline-3-carbaldehydes as per current green chemistry trends:–

Kidwai et al\textsuperscript{80} reported Biginelli reaction in which neat reactants were subjected under microwave irradiations.

M. Kidwai and S. Saxena\textsuperscript{81} have synthesized the 2-amino-3-cyanopyran derivatives of 2-chloroquinoline-3-carbaldehyde using aq. potassium carbonate.
Naik et al. have carried out the nano-titanium dioxide ($\text{TiO}_2$) mediated simple and efficient modification to Biginelli reaction with some 2-substituted-3-carbaldehyde quinoline derivatives.\(^{82}\)

One-pot synthesis of some new quinoline derivatives under microwave irradiation conditions have been reported recently.\(^{83}\)

Adhikari and coworkers\(^{84}\) have reported the synthesis of dihydropyrimidine derivatives from 2-chloroquinoline-3-carbaldehyde using microwave irradiation as well as conventional method.
Pyrazolo[3,4-\(b\)]quinolines have been synthesized from 2-chloroquinoline-3-carbaldehyde and hydrazine hydrate/phenylhydrazine using \(p\)-TsOH under microwave irradiation by Gupta and coworkers\(^{85}\).

\[
\begin{align*}
\text{R}^1 & = \text{H, CH}_3, \text{OCH}_3, \text{R} = \text{C}_6\text{H}_5 \\
\end{align*}
\]

M. Raghavendra\(^ {86}\) and coworkers have reported microwave induced one-pot synthesis of some new thiopyrano[2,3-\(b\)]quinolin-2-ones.

Substituted 2-chloroquinoline-3-carbaldehyde reacts with carbodimide in presence of catalytic amount of \(p\)-TsOH in anhydrous DMF under microwave irradiation method\(^ {87}\).
(C) 2-Chloroquinoline-3-carbaldehydes as therapeutic agents:

Some of the therapeutically active compounds derived from 2-chloroquinoline-3-carbaldehyde derivatives are reviewed here.

S. U. F. Rizvi and coworkers have reported the antileishmanial activity against *Leishmania major* of novel quinolyl-thienyl chalcones and their 2-pyrazoline derivatives.

V. K. Bhovi and coworkers have reported analgesic and antimicrobial activity of some Schiff's base derivatives of 2-chloroquinoline-3-carbaldehyde.

Nirmal et al. have reported antimicrobial activity of some new biquinoline derivatives derived from 2-chloroquinoline-3-carbaldehyde.
New quinolinyl chalcones as potent antiplasmodial agent have been reported in the literature\textsuperscript{91}.

\[
\begin{array}{c}
\text{R} & \text{Cl} & \text{CHO} + \text{H}_2\text{COOC} & \text{R}_3 & \text{R} & \text{Cl} & \text{N} & \text{OH} & \text{R}_1 & \text{N} & \text{OH} & \text{R}_2 & \text{N} & \text{OH} & \text{R}_3 & \text{R} & \text{Cl} & \text{CHO} \\
\text{R} & = & \text{H}, \text{OEt} & \text{R}_1 & = & \text{H}, \text{CH}_3, \text{Cl}, \text{Br}, \text{I}, \text{NO}_2; \text{R}_2 & = & \text{H}, \text{OCH}_3 \\
\text{R}_3 & = & \text{H}, \text{CH}_3, \text{Cl}, \text{Br}, \text{I}, \text{NO}_2, \text{NH}_2 \\
\end{array}
\]

A. Mohmmed and co-workers\textsuperscript{92} have reported synthesis and anti-inflammatory activities of some new hydrazones of aryl alkanoic acid.

\[
\begin{array}{c}
\end{array}
\]

M. Kedwai and N. Negi have reported analgesic activity of following 3,3'-bi-quinoline derivatives\textsuperscript{93} derived by reaction of 2-chloroquinoline-3-carbaldehyde with ethylene diamine and \(\alpha\)-phenylene diamine.

\[
\begin{array}{c}
\end{array}
\]

Rajiv Gupta et al.\textsuperscript{94} have reported anti-inflammatory, antibacterial and antifungal activities of quinoline derivatives, 2-chloro-6/8-substituted-3-(3-alkyl/aryl-5,6-dihydro-5-triazolo-[3,4-b][1,3,4]thiadiazol-6-yl)-quinoline.
Fathy N. M. and Aly A. S. have reported azomethine derivatives of 2-chloro-3-formyl quinoline, which shows bactericidal and fungicidal activity.  

Parikh et al. have reported antimicrobial activities of some Schiff bases, 2-azetidinones, pyrazoline and isoxazole derivatives of 2-chloro quinoline-3-carbaldehyde.

Murlidhar S. Shingare and coworkers have reported synthesis and antibacterial activities of α-hydroxyphosphonates and α-acetyloxyphosphonates derived from 2-chloro quinoline-3-carboxaldehyde.
M. Kedwai and N. Negi have reported analgesic activity of some fused quinoline derivatives.

R. R. Kamble and coworkers have reported the synthesis as well as anticancer and antitubercular activity of some 1,3,4-thiadiazole derivatives bearing quinoline nucleus.
N. V. Kumar et al.\textsuperscript{101} have reported 2-oxo-pyrano[2,3-\textit{b}]quinoline derivatives and these were subjected to ammonia treatment to yield the corresponding 2-oxo-pyrido[2,3-\textit{b}]quinoline derivatives. The prepared compounds were tested for their antimalarial, diuretic, clastogenic and antimicrobial properties.

Senniappan Thamarai Selvi et al.\textsuperscript{102} have reported antimicrobial activity of pyrimido[4,5-\textit{b}] and pyrazolo[3,4-\textit{b}]quinoline derivatives.

S. P. Rajendran and R. Karvembu have prepared Schiff's bases, which displayed an antifungal activity\textsuperscript{103}.
1.6 ANTIMICROBIAL STUDY

Humankind has been subjected to infections by microorganisms since before the dawn of recorded history. Until after the discovery of bacteria 300 years ago and subsequent understanding of their role in infection about 150 years ago, there was no hope for the rational therapy. The past few decades have witnessed a significant increase in microbial diseases. The infections caused by bacteria and fungi has affected human as well as animals. Control of microbial population is necessary to prevent transmission of disease, infection, decomposition, contamination and spoilage caused by them. Humankind’s personal comforts and convenience depend to a large extent on the control of microbial population. It has been estimated that the life expectancy of humans has increased by at least 10 years since the discovery of antimicrobial agents for the treatment of microbial infections. Substantial attention has been focused on developing a more potent and effective anti-microbial agents.

1.6.1 Pathogens:-

The microorganism, or infectious agent or more commonly germ, is a biological agent capable of producing diseases in host are known as pathogen. There are several substrates and pathways whereby pathogens can invade a host; the principal pathways have different episodic time frames, but soil contamination has the longest or most persistent potential for harboring a pathogen.

Pathogens have certain characteristics that they need and use, to cause disease. These so-called virulence factors have specific functions in the successive steps that result in an infection. An infection can be seen as a miniature battle between pathogen and host, the first trying to remain present and to feed and multiply, while the host is trying to prevent this. The resulting infection is a process with three possible outcomes: the host wins and the pathogen are removed (possibly with the help of medication) so that the host can recover; the pathogen win the ultimate battle and kill their host; or an equilibrium is reached in which host and pathogen live involuntarily together and damage is minimized.
Bacterial Pathogens

Bacteria that cause disease are called pathogenic bacteria. Bacteria can cause diseases in humans, in other animals and also in plants. Some bacteria can only make one particular host ill; others cause trouble in a number of hosts, depending on the host specificity of the bacteria. The diseases caused by bacteria are almost as diverse as the bugs themselves and include infectious diseases such as pneumonia, foodborne illnesses, tetanus, typhoid fever, diphtheria, syphilis and leprosy and even certain forms of cancer. Bacterial cells grow and divide, replicating repeatedly to form large numbers present during an infection or on the surfaces of the body. To grow and divide, organisms must synthesize or take up many types of biomolecules.

In 1928, a German scientist C. E. Chrenberg first used the term “bacterium” to denote small microscopic organism with a relatively simple and primitive form of the cellular organization known as “prokaryotic”. The Danish physician Christian Gram in 1884 discovered a stain known as Gram stain, which can divide all bacteria into two classes “Gram positive” and “Gram negative”. The Gram-positive bacteria resist decolouration with acetone, alcohol and remain stained (methyl violet) as dark blue color, while Gram-negative bacteria are decolorized. We have used following listed bacterial pathogens for antibacterial study of synthesized title derivatives.

Gram positive bacterial pathogens

*Streptococcus pneumoniae*: They are lancet-shaped cocci, fermentative aerotolerant anaerobe. Usually, they are seen as pairs of cocci (diplococci), but they may also occur singly and in short chains. When cultured on blood agar, they are alpha hemolytic. Individual cells are between 0.5 and 1.25 micrometers in diameter. They do not form spores and they are nonmotile. Individual bacteria are between 0.5 and 1.25 micrometers in diameter. They do not form spores and are non-motile. They are mesophillic, living optimally at temperatures between 30 and 35 °C. It was isolated in 1881 by Louis Pasteur. The species was then known as pneumococcus due to its role in the disease, pneumonia. It was termed Diplococcus pneumonia in 1926 due to its propensity to exist in pairs of
cells and renamed *Streptococcus pneumoniae* in 1974 because of its formation of chains in liquid. They are found normally in the upper respiratory tract, including the throat and nasal passages. It infects the upper respiratory tract and can cause pneumonia, as well as infections in other parts of the body such as in the bloodstream (bacteremia), lining of the brain and spinal cord (meningitis), bones (osteomyelitis), joints (arthritis), ears (otitis media) and sinuses (sinusitis).

*Clostridium tetani*[^1]:

It is a mobile, spore-forming, obligate anaerobic, cannot survive in high oxygen situations, rod-shaped bacterium, found as spores in soil or as parasites in the gastrointestinal tract of animals. The rods arrange themselves as pairs and chains as well as single-celled and do not contain any membrane-bound organelles, such as a nucleus. Its appearance on gram stain is said to resemble tennis rackets or drumsticks. It is a non-halophilic bacterium with an optimal temperature of 37 °C, making it mesophilic. In 1884, Arthur Nicolaier isolated the strychnine-like toxin of tetanus from free-living, anaerobic soil bacteria. *C. tetani* produces a potent biological toxin, tetanospasmin and is the causative agent of tetanus, a disease characterized by painful muscular spasms that can lead to respiratory failure and, in up to 40% of cases, death.

*Bacillus subtilis*[^2]:

They are rod-shaped with rounded ends, more or less strictly, aerobic, found in soil and vegetation. They are motile and sporulating. They are small in size, occurring single or in short chains. *B. subtilis* produces the proteolytic enzyme subtilisin. *Bacillus subtilis* grow in the mesophilic temperature range. The optimal temperature is 25-35° C and a basic pH of 8. In 1835, the bacterium was originally named Vibrio subtilis by Christian Gottfried Ehrenberg and renamed *Bacillus subtilis* by Ferdinand Cohn in 1872. They can contaminate food; however, they seldom result in food poisoning. *B. subtilis* spores can survive the extreme heating that is often used to cook food and it is responsible for causing ropiness — a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides — in spoiled bread dough.
Gram negative bacterial pathogens

*Salmonella typhi*\textsuperscript{107}:

This rod-shaped food born pathogen has adapted to grow under both an aerobic and anaerobic conditions. It grows best between 35 and 37° C and pH range of 3.8 to 9.5. It was discovered by C. J. Eberth in 1880. Its infections cause systemic infections and typhoid fever in humans. It is killed by heating, 70° C for 1 min or less. Transmission of disease is mainly through food, water or human carriers. *S. typhi* usually invades the surface of the intestine in humans, but have developed and adapted to grow into the deeper tissues of the spleen, liver and the bone marrow. Symptoms most characterized by this disease often include a sudden onset of a high fever, a headache and nausea. Other common symptoms include loss of appetite, diarrhea and enlargement of the spleen (depending on where it is located).

*Vibrio cholerae*\textsuperscript{108}:

It has a "comma" shaped cell body and contains a singular polar flagellum used for motility. It enters the human body through ingestion of contaminated food or water. The bacteria enter the intestine; imbed itself in the villi of absorptive intestinal cells and releases cholera toxin. The bacteria infects the intestine and increases mucous production causing diarrhea and vomiting which result in extreme dehydration and, if not treated, death. It is usually transmitted through the feces of an infected person, often by way of unclean drinking water or contaminated food results in epidemic cholera. Filippo Pacini first discovered *V. cholerae* in Italy in 1854, though it was originally believed to be Robert Koch who discovered it thirty years later in Berlin in 1884.

*Escherichia coli*\textsuperscript{109}:

They are rods, 2 to 4 micro by 0.4 micro in size, commonly seen in coccobacillary form and rarely in filamentous form. Colonies are circular, raised and smooth and emit a faecal odour. It grows best at 37° C, through a pH range of 4.4 to 9.0, in the presence or absence of oxygen. Escherichia is discovered by T. Escherich in 1885.
They are normally present in the intestine without causing problems, but a few types cause illness after consuming contaminated food or water, when the bacteria produces toxin in the intestine causing diarrhoea. It causes infantile diarrhoea, Gastroenteritis, traveller’s diarrhoea, causes bacillary dysentery, causes Haemorrhagic colitis, Haemolytic uraemic syndrome (HUS), or Thrombocytopenic purpura. It does not form toxin in food, this is formed in the intestine of infected people. Illness is caused after ingestion of a sufficient number of E.coli when the bacteria travels through the stomach and small intestine, attaches itself to the inside surface of the large intestine and causes inflammation of the intestinal wall.

**Fungal Pathogens**

Fungi are one of the five kingdoms of life. They are plant-like organisms that lack chlorophyll. Since they do not have chlorophyll, fungi absorb food from others. Since they don't use light to make food, they can live in damp and dark places. Fungi are saprophytic organism, as they grow on dead organic matter such as soil or dead plant material. Fungi are nonphotosynthetic eukaryotes growing either as colonies of single cells (yeasts) or as filamentous multicellular aggregate [molds]. Fungi comprise a eukaryotic kingdom of microbes that are usually saprophytes but can cause diseases in humans, animals and plants.

The incidence of fungal infections has increased dramatically in the past 20 years. Accordingly, the increase in rates of morbidity and mortality because of fungal infections has been now recognized as a major problem. Most fungal infections are due to opportunistic pathogens; these affect people who are already ill or have a suppressed immune system (e.g. in patients who have been given an organ transplant, or in AIDS patients), although fungi are common problems in the immunocompetent population as the causative agents of skin, nail or yeast infections. Most commonly, fungi grow as pathogen on the skin of animals or people. This is sometimes called Ringworm symptom. Fungi also cause a number of plant and animal diseases: in humans, ringworm, athlete's foot and several more serious diseases are caused by fungi. Because fungi are more chemically and genetically similar to animals than other organisms, this makes fungal diseases very difficult to treat. Plant diseases
caused by fungi include rusts, smuts and leaf, root and stem rots and may cause severe damage to crops. Most antibiotics that function on bacterial pathogens cannot be used to treat fungal infections due to the fact that fungi and their hosts both have eukaryotic cells. The typical fungal spore size is 1-40 micrometer in length. We have used following listed fungal pathogens for antifungal study of synthesized title derivatives.

_Candida albicans_\(^{10}\):

It is a dimorphic fungus. That is, it grows as both mycelium and yeasts. This is one reason why there were so many names given to this fungus. This fungus is found among the normal flora of the mouth, digestive tract and vagina of perfectly healthy people, but under some circumstances and for reasons unknown, it may cause severe and even fatal infections, with lesions and eruptions of the skin, nails, mouth, bronchial tubes and lungs. The reason for this outbreak is difficult to pinpoint since the fungus is generally present on and within the body of healthy individuals. There are suggestions that there are special strains of this species that are pathogenic. This is suggested by the fact that this disease can be contagious and epidemics have occurred. Predisposition may also play a role in infection. An oral infection known as thrush is relatively common. There are various infections on the body due to it.

_Aspergillus fumigatus_\(^{11}\):

It is a species complex rather than a single species. It is actually composed of ten species. These species are commonly found in decaying vegetation, especially when the latter is undergoing microbiological heating, because this complex is thermophilic, adapted to growing at high temperatures 50-55 °C. _Aspergillus fumigatus_ sometimes parasitizes animals, especially birds, infecting mainly lungs and causing heavy mortality - up to 50% in young turkeys and up to 90% in young chicks. Heavy losses have also been reported in herring gulls, ostriches and diving ducks in the wild and in penguins in zoos. The fungus can also invade the embryos of eggs in incubators and probably does the same in eggs in nest in the wild. It also invades the uterus of pregnant cattle and grows through the placenta into the fetus, which then dies and is aborted. It has been estimated that 64% of bovine abortion investigated were due to
infection of *A. fumigatus*. In people, the disease can lead to a chronic lung infection which is apparently very contagious. The fungus is thought to cause death, but that is not certain. In patients that have died and *A. fumigatus* has been isolated, many have also had underlying disease that possibly lowered their resistance to the fungus.

### 1.6.2 Antimicrobial agents:

The modern era of antimicrobial chemotherapy began following Fleming's discovery in 1929 of the powerful bactericidal substance penicillin and Domagk's discovery in 1935 of synthetic chemicals (sulfonamides) with broad antimicrobial activity. In 1939 Gerhard Domagk, a German Bacteriologist and Pathologist, awarded the Nobel Prize for discovery of the first synthetic antibacterial compound “prontosil”.

Antimicrobial agents may be either bactericidal, killing the target bacterium or fungus or bacteriostatic, inhibiting its growth. Bactericidal agents are more effective, but bacteriostatic agents can be extremely beneficial since they permit the normal defenses of the host to destroy microorganisms. Antimicrobial agents may be classified according to the type of organism against which they are active i.e. antibacterial, antiviral, antifungal, antiprotozoal and anthelmintic drugs. It can also be useful to combine various antimicrobial agents for broadening the activity spectrums and to minimize the possibility of the development of bacterial resistance. Some antibiotic combinations are more effective together than the combine effectiveness of the single agent. This is termed as Synergism. Combination therapy has proved its value as latest therapy for antimicrobials. Some bacteriostatic agents on novel combination give bactericidal activity. Sulphamethoxazole is bacteriostatic and Trimethoprim is also bacteriostatic but combination of both the drugs is now widely used as a bactericidal combination. Two such bactericidal drugs are also used in combination therapy. Refampin + Dapsone are used in leprosy, Refampin + Isoniazide in Tuberculosis. WHO has also approved this type of combination.

Most microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious disease: antibiotics, which are natural substances produced by certain groups of microorganisms and chemotherapeutic agents, which are chemically
synthesized. A hybrid substance is a semisynthetic antibiotic, wherein a molecular version produced by the microbe is subsequently modified by the chemist to achieve desired properties. Furthermore, some antimicrobial compounds, originally discovered as products of microorganisms, can be synthesized entirely by chemical means. In the medical and pharmaceutical worlds, all these antimicrobial agents used in the treatment of disease are referred to as antibiotics, chemicals that are produced by living organisms which, even in minute amounts, inhibit the growth of or kill another organism.

**Characteristics of antimicrobial agent**

- It should have a wide spectrum of activity with the ability to destroy or inhibit many different species of pathogenic organisms.
- It should be nonallergenic and nontoxic to the host and without undesirable side effects.
- It should not eliminate the normal flora of the host.
- It should be able to reach the part of the human body where the infection is occurring.
- It should be inexpensive and easy to produce.
- It should be chemically-stable (have a long shelf-life).
- Microbial resistance is uncommon and unlikely to develop.
- It must have solubility in body fluids to be active and can rapidly penetrate body tissues.

**1.6.3 Antimicrobial Susceptibility Testing:**

The goal of antimicrobial susceptibility testing is to predict the *in vivo* success or failure of antibiotic therapy. Tests are performed *in vitro* and measure the growth response of an isolated organism to a particular drug or drugs. The tests are performed under standardized conditions so that the results are reproducible. The raw data are either in the form of a zone size or Minimum Inhibitory Concentration (MIC). i.e. antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated
techniques are variations of the above methods. The evaluation can be done by the following methods:

<table>
<thead>
<tr>
<th>Diffusion</th>
<th>Dilution</th>
<th>Diffusion &amp; Dilution</th>
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<tbody>
<tr>
<td>Stokes method</td>
<td>Minimum Inhibitory Concentration:</td>
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<tr>
<td>Kirby-Bauer method</td>
<td>i) Broth Dilution Method</td>
<td>E-Test method</td>
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<tr>
<td>Or Disk diffusion method</td>
<td>ii)Agar Dilution Method</td>
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We have used the **Kirby-Bauer method** and **Broth Dilution method** for antimicrobial study recommended by the National Committee for Clinical Laboratory Standards (NCCLS)\(^{112}\).

**Kirby-Bauer Method:-**

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing. The Kirby-Bauer method is well documented and standard zones of inhibition can be determine for susceptible and resistant values.

The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures i.e. many conditions can affect a disc diffusion susceptibility test. When performing these tests certain things are held constant so only the size of the zone of inhibition is variable. Conditions that must be constant from test to test include the media (agar) used, the amount of organism used, the concentration of chemical used and incubation conditions (time, temperature and atmosphere). The depth of the agar in the plate is a factor to be considered in the disc diffusion method.

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies...
that are clinically relevant and cost-effective. Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with MIC’s and the results have corroborated with clinical data. Based on study results NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

**Broth Dilution Method:**

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism i.e. aim of broth dilution methods is to determine the lowest concentration of the assayed antimicrobial agent (MIC) that, under defined test conditions, inhibits the visible growth of the pathogen being investigated. MIC values are used to determine susceptibilities of pathogen to drugs and also to evaluate the activity of new antimicrobial agents.

This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. The tube dilution test is the standard method for determining levels of resistance to an antibiotic.

**Procedure for Performing the Broth Dilution Method**

- The *in vitro* antimicrobial activity of the synthesized compounds and standard drugs were assessed against three representative of Gram-positive bacteria *viz.* *Streptococcus pneumoniae* (MTCC 1936), *Clostridium tetani* (MTCC 449), *Bacillus subtilis* (MTCC 441), three Gram-negative bacteria *viz.* *Salmonella typhi* (MTCC 98), *Vibrio cholerae* (MTCC 3906), *Escherichia coli* (MTCC 443) and two fungi *viz.* *Aspergillus fumigatus* (MTCC 3008) and *Candida albicans* (MTCC 227) and the strains employed for the activity were procured from (MTCC – Micro Type Culture Collection) Institute of Microbial Technology, Chandigarh.
- Inoculum size for test strain was adjusted to $10^8$ CFU mL$^{-1}$ (Colony Forming Unit per milliliter) by comparing the turbidity (turbidimetric method).
- Mueller Hinton Broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose Broth used for fungal nutrition.
- Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin and Norfloxacin were used as standard antibacterial drugs, whereas griseofulvin and nystatin was used as standard antifungal drugs.
- DMSO was used as diluents / vehicle to get desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains.
- Serial dilutions were prepared in primary and secondary screening. Each synthesized compound and standard drugs were diluted obtaining 2000 $\mu\text{g} \text{mL}^{-1}$ concentration, as a stock solution. In primary screening 1000, 500 and 250 $\mu\text{g} \text{mL}^{-1}$ concentrations of the synthesized drugs were taken. The active synthesized compounds found in this primary screening were further diluted to obtain 200, 100, 62.5, 50, 25, 12.5 and 6.25 $\mu\text{g} \text{mL}^{-1}$ concentrations for secondary screening to test in a second set of dilution against all microorganisms.
- The control tube containing no antibiotic is immediately sub cultured (before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism. The tubes are then put for incubation at 37° C for 24 hr for bacteria and 48 hr for fungi. The highest dilution (lowest concentration) showing at least 99% inhibition or preventing appearance of turbidity is considered as Minimal Inhibitory Concentration ($\mu\text{g} \text{mL}^{-1}$) i.e. the amount of growth from the control tube before incubation (which represents the original inoculum) is compared. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. The result of this is much affected by size of the inoculum. The test mixture should contain $10^8 \text{CFU} \text{mL}^{-1}$ organisms. The protocols were summarized and compared with standard drugs as the Minimal Inhibitory Concentration ($\mu\text{g} \text{mL}^{-1}$).

Factors Influencing Antimicrobial Susceptibility Testing:
- **Choice of media**: Consistent and reproducible results are obtained in media prepared especially for sensitivity testing. Satisfactory media will provide essentially clear, distinct zones of inhibition 20 mm or greater in diameter.
Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

- **Size of inoculums:** Although large numbers of organisms do not markedly affect many antibiotics, the ideal inoculum is one, which gives an even dense growth without being confluent. Overnight broth cultures of organisms and suitable suspensions from solid media can be diluted appropriately to give optimum inoculum for sensitivity testing.

- **pH:** The medium used should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected.

- **Moisture:** The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

- **Effects of Variation in Divalent Cations:** Variations in divalent cations affect results. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptably large zones of inhibition.

- **Testing strains that fail to grow satisfactorily:** Only aerobic or facultative bacteria that grow well on unsupplemented media should be tested on that medium. Certain fastidious bacteria do not grow sufficiently on unsupplemented media. These organisms require supplements or different media to grow and they should be tested on the media.

The Following Conditions must be met for the Antimicrobial Susceptibility Testing:

- There should be intimate contact between the test organisms and substance to be evaluated.
- Required conditions should be provided for the growth of microorganisms.
- Conditions should be same through the study.
  Aseptic / sterile environment should be maintained.
1.7 PRESENT WORK

The present work involves synthesis of new heterocyclic compounds bearing 2-chloroquinoline, 2-morpholinoquinoline and 2-thiophenoxy quinoline as a parent moiety. These new heterocyclic compounds have been characterized by using various spectroscopic and analytical methods such as $^1$H-NMR, $^{13}$C-NMR, FT-IR and elemental analyses as well as some selected compounds were characterized by mass spectra. All the synthesized compounds were subjected to in vitro antimicrobial activity against eight human pathogens viz. three Gram positive bacteria (Bacillus subtilis, Clostridium tetani, Streptococcus pneumoniae), three Gram negative bacteria (Escherichia coli, Vibrio cholerae, Salmonella typhi) and two fungi (Aspergillus fumigatus, Candida albicans). The entire work of thesis is distributed in to following five chapters.

Chapter-2:- Synthesis and in vitro antimicrobial evaluation of penta-substituted pyridine derivatives bearing the quinoline nucleus

![Chemical structure image]

Where, $R_1 = H, CH_3, OCH_3, Cl$
$R_2 = H, CH_3$
$R_3 = H, Cl, CH_3$

Chapter-3:- Synthesis and in vitro antimicrobial activity of N-arylquinoline derivatives bearing 2-morpholinoquinoline moiety

![Chemical structure image]

Where, $R_1 = H, CH_3, OCH_3$
$R_2 = R_3 = H, CH_3$
$R_4 = H, CH_3, OCH_3, Cl$
Chapter-4:-

PART-I Microwave assisted synthesis and antimicrobial evaluation of new fused pyran derivatives bearing 2-morpholinoquinoline nucleus

\[
\begin{align*}
R &= H, \text{CH}_3, \text{OCH}_3 \\
&
\end{align*}
\]

PART-II Diversity-synthesis and antimicrobial evaluation of pyrano[4,3-\textbf{b}]pyran and pyrano[3,2-\textbf{c}]chromene bearing 2-thiophenoxyquinoline nucleus

\[
\begin{align*}
R_1 &= H, \text{CH}_3, \text{OCH}_3 \\
R_2 &= H, \text{Cl}, \text{CH}_3 \\
R_3 &= H, \text{CH}_3 \\
&
\end{align*}
\]
Chapter-5: Synthesis and *in vitro* antimicrobial activity of new 3-(2-morpholinoquinolin-3-yl) substituted acrylonitrile and propanenitrile derivatives.

![Chemical Structure](image1)

Where, $R_1 = \text{H, CH}_3, \text{OCH}_3$

$R_2 = \text{H, CH}_3$

Chapter-6: Synthesis and *in vitro* antimicrobial evaluation of 2-thiophenoxyquinoline based new hydrazone derivatives

![Chemical Structure](image2)

$R_1 = \text{H, CH}_3, \text{OCH}_3$

$R_2 = R_3 = \text{H, Cl, CH}_3$
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Chapter-1

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