CHAPTER 1:
Introduction
The carbocyclic compounds are the cyclic organic compound containing all carbon atoms in the ring. If at least one atom other than carbon forms a part of the ring system, then it is designated as a heterocyclic compound\(^1\). Nitrogen, oxygen and sulfur are the most common heteroatoms of the heterocyclic compounds. Heterocyclic compounds are widely distributed in nature and are essential to life in many ways, most of the sugars and their derivatives, including vitamins possess heterocyclic rings commonly containing nitrogen as a heteroatom.

In pharmaceutics, heterocyclic compounds have great applicability in because they have specific chemical reactivity and provide false synths in biosynthetic process or block the normal functioning of biological receptors. From the huge library of heterocyclic compounds, the heterocycles containing nitrogen are more abundant than those containing oxygen or sulfur owing to their wide distribution in nucleic acid instance and involvement in almost every physiological process of plant and animals. Naturally occurring heterocycles such as alkaloids and glycosides have been used for old age, as remedial agents. Febrical alkaloid from ancient Chinese drug chang shan, reserpine from Indian rouwolfia, curen alkaloid from arrow poison codeiene, tropine and strychnine are all examples of heterocyclic compounds.

In the case of medicinal chemistry, antibiotics like penicillin, cephalosporin, norfloxacin etc. Veterinary products like atrazine and simazine are well known heterocyclic compounds. The role of heterocyclic compounds in medicinal chemistry is more significant due to their synthetic utility as synthetic intermediates, protecting groups, chiral auxiliaries, organ catalysts, and metal ligands in asymmetric catalysts inorganic synthesis. Therefore, substantial attention has been paid to develop efficient new methods to synthesize heterocycles. Synthetic heterocyclic chemistry has influenced almost every place of human life and the synthesized compounds have found their application in diverse field as medicine, agriculture and various industries. Synthetic heterocyclic drugs are used in various fields such as hypnotics, anticonvulsants, antineoplastics, antihistamines, antithyroid, antiseptics, antimicrobials etc.

In the pharmaceutical field, the need for new and novel chemical inhibitors of biological function these have always been and will continue. Our efforts are focused on the introduction of chemical diversity in the molecular frame work in order to synthesizing pharmacologically interesting compounds of different composition.
1.1 PYRAZOLE

The pyrazole ring consists of a doubly unsaturated five membered ring containing two adjacent nitrogen atoms. In 1883, L. Knorr\textsuperscript{2} first synthesized 3-methyl-1-phenyl-1\(H\)-pyrazol-5(4\(H\))-one by the reaction of ethyl acetoacetate with phenyl hydrazine. The name pyrazole was given to such compounds because the nucleus was derived from pyrrole by replacement of carbon by nitrogen.

After the discovery of pyrazole, till 1930s very little research work had been done for the synthesis of pyrazole derivatives. In 1938 L. Ruzicka et al.\textsuperscript{3} have reported the first steroidal pyrazole and only a single derivative; cholest-4-eno[3,2-\(c\)]pyrazole-5-carboxylic acid.

Celecoxib is the first to market of a number of selective cycloxygenase-2 (COX-2) inhibitors\textsuperscript{4} which show great promise as anti-inflammatory and analgetic agents, without the undesirable side effects associated with other non-steroidal anti-inflammatories drugs.

Antipyrine is an analgesic\textsuperscript{5}, a non-steroidal anti-inflammatory and an antipyretic drug. There are many pyrazole dyestuffs, the food colourant tatrazine is one such substance\textsuperscript{6}. Tatrazine is known as “Acid Yellow 23”.

The pyrazole ring system has been studied in detail due to its important properties in photography, dyes and as pharmaceutical agents. Phenyl butazone has been utilised for some time in the treatment of severe arthritis\textsuperscript{7}.

During the past years, considerable evidence has accumulated to demonstrate the efficacy of pyrazole derivatives including antitumor\textsuperscript{8}, antibacterial and antifungal\textsuperscript{9}, antiviral\textsuperscript{10}, analgesic\textsuperscript{11}, anti-proliferative\textsuperscript{12}, antileukemic\textsuperscript{13,14}. 

1.1.1 NATURAL OCCURRENCE OF PYRAZOLE

Very few pyrazole derivatives are naturally occurring may be due to the difficulty of living organisms to construct the \( N-N \) bond. Withasomnine which is a pyrazole alkaloid, isolated from the Withania somnifera by H. Schröter et al.\(^\text{15}\) in 1968, has found to have various biological activities such as anti-inflammatory and anti-arthritic activity, anti-oxidant activity\(^\text{16}\) skin lesions, ulcers, boils, swelling, to reduce pus formation, and inflammation\(^\text{17}\). Moreover, \((s)-3\)-pyrazolylalanine\(^\text{18}\), a nonproteinogenic amino acid with antidiabetic activity\(^\text{19,20}\), and pyrazomycin\(^\text{21}\), an antiviral metabolite of \textit{Streptomyces candidus}, are also pyrazole containing natural products. In 1960, pyrazolyl-alanine, was isolated from seeds of watermelons\(^\text{22}\).

<table>
<thead>
<tr>
<th>ALKALOID</th>
<th>PHARMACOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Withasomnine" /></td>
<td>Anti-inflammatory and Anti-arthritic activity, Anti-oxidant activity(^\text{16}) skin lesions, ulcers, boils, swelling, and to reduce pus formation(^\text{17})</td>
</tr>
<tr>
<td><img src="image" alt="Pyrazomycin" /></td>
<td>Antidiabetic(^\text{19,20})</td>
</tr>
<tr>
<td><img src="image" alt="Pyrazolylalanine" /></td>
<td>Antiviral metabolite(^\text{21})</td>
</tr>
</tbody>
</table>
1.1.2 SYNTHETIC APPROACH FOR PYRAZOLE

Although there are many ways to prepare the pyrazole ring, the condensation of the 1,3-dicarbonyl and its variation remains the most common and facile way to assemble this ring system. The Knorr pyrazole synthesis\(^2\) is an organic reaction used to convert a hydrazine or its derivatives and a 1,3-dicarbonyl compound to a pyrazole using an acid catalyst. The mechanism begins with an acid catalyzed imine formation, where in the case of hydrazine derivatives the attack can happen on either carbonyl carbon and result in two possible products. The other nitrogen of the hydrazine derivative then attacks the other carbonyl group which has also been protonated by the acid and forms a second imine group. This diimine compound gets deprotonated to regenerate the acid catalyst and provide the final pyrazole product.

\[
\begin{align*}
\text{Hydrazine or} & \quad \text{1,3-dicarbonyl} \\
R’ \quad \text{its derivatives} & \quad \text{compound}
\end{align*}
\]

where \(R, R_1, R_2, R_3 = H\) or various alkyl or aryl or hetaryl group

L. Wu *et al.*\(^{23}\) have reported an efficient, general, one-pot, three-component procedure for the preparation of 3,5-disubstituted-1H-pyrazoles includes condensation of substituted aromatic aldehydes and tosylhydrazine followed by cycloaddition with terminal alkynes.

\[
\begin{align*}
\text{Ar} & = \text{Ph, alkyl, vinyl}
\end{align*}
\]

C. Reddy *et al.*\(^{24}\) have developed a new approach for synthesis of 3,5-disubstituted 1H-pyrazoles from propargylic alcohols in good overall yields proceeds via an acid-catalyzed propargylation of \(N,N\)-diprotected hydrazines followed by base-mediated 5-endo-dig cyclization.

\[
\begin{align*}
\text{Ar} & = \text{Ph, Pr, CH}_2\text{OBn} \\
\text{Ar} & = \text{Various aromatic compounds}
\end{align*}
\]
The reaction of terminal alkynes with n-BuLi, and then with aldehydes, followed by the treatment with molecular iodine, and subsequently hydrazines or hydroxylamine provided the corresponding 3,5-disubstituted pyrazoles in good yields and with high regioselectivity.\textsuperscript{25}

\[
\begin{align*}
\text{Ar} & \overset{\text{1.1 eq. n-BuLi (1.6 M in hex), THF, 0°C, 1.5 h}}{\rightleftharpoons} \overset{\text{2 eq. NH}_3\text{NH}_2\text{H}_2\text{O, 1h}}{\text{Ar}^{'}} \\
& \text{Ar} = \text{Ar}' = \text{Ph}, 4-\text{MeO-Ph}, 4-\text{Me-Ph}, 2-\text{Me-Ph}, 3-\text{Me-Ph}, 4-\text{Cl-Ph}, 4-\text{CF}_2\text{-Ph}
\end{align*}
\]

A simple and straightforward multicomponent reaction of vinyl azide, aldehyde, and tosylhydrazine affords 3,4,5-trisubstituted 1\textit{H}-pyrazoles regioselectively in good yields in the presence of a base.\textsuperscript{26}

\[
\begin{align*}
\text{R}_1 & \overset{\text{NHTs NH}_2}{\overset{\text{5 eq. NaOH or Cs}_2\text{CO}_3, \text{DMF, 2-12 h}}{\rightleftharpoons}} \overset{\text{2 eq. NMM, r.t., 3-6 h}}{\text{R}_2} \\
& \text{R}_1 = \text{Ph}, 4-\text{BrPh}, 4-\text{OMePh}, 4-\text{NO}_2\text{Ph}; \quad \text{R}_2 = \text{PhCO}, 4-\text{MePhCO}, 4-\text{ClPhCO}, \text{COOEt}, \text{Ph} \\
& \text{R}_2 = \text{Ph}, 4-\text{MePh}, 4-\text{BrPh}, 3-\text{BrPh}, 4-\text{NO}_2\text{Ph}
\end{align*}
\]

A tandem catalytic cross-coupling/electrocyclization allows the conversion of differentially substituted acyclic and cyclic enol triflates and an elaborated set of diazoacetates to provide the corresponding 3,4,5-trisubstituted pyrazoles with a high degree of structural complexity.\textsuperscript{27}

G. Mariappan et al.\textsuperscript{28} have reported that the synthesis of pyrazolone by reaction of ethyl acetoacetate with hydrazine hydrate in absolute alcohol. In presence of alcoholic sodium hydroxide, reaction of pyrazolone with different substituted aromatic aldehydes gives corresponding substituted pyrazolones.
G. Murineddu, et al.\textsuperscript{29} have synthesized a series of dihydroindeno substituted pyrazole carboxamide derivatives and evaluated for its cannabinoid receptor affinity.

A. Bernardino and co-workers\textsuperscript{30} have synthesized different 1-(4-X-phenyl)-N'-[(4-Y-phenyl)methylene]-1H-pyrazole-4-carboxhydrazides and investigated their leishmanicidal \textit{in vitro} activities and cytotoxic effects were investigated. Among all the 1H-pyrazole-4-carboxhydrazides derivatives examined, the most active compounds were those with X = Br, Cl and Y = NO\textsubscript{2} derivatives.

P. Chovatia et al.\textsuperscript{31} have synthesized a series of 1-acetyl-3,5-diphenyl-4,5-dihydro-(1H)-pyrazole derivatives and these compounds were tested \textit{in vitro} for their antitubercular and antimicrobial activities.
M. Martins et al.\textsuperscript{32} have synthesized ethoxycarbonylpyrazoles, by the cyclocondensation of $\beta$-alkoxyvinyl trichloromethyl ketones with hydrazine hydrochloride under mild conditions.

\[
\begin{align*}
\text{CH}_2\text{O} & \xrightarrow{\text{reflux, 4h}} \text{EtOOC-} \\
\text{N}_2\text{H}_4\cdot\text{HCl / EtOH} & \xrightarrow{\text{EtO}_2\cdot\text{CCHN}_2} \text{N} \\
R = \text{H, Me, Et} & , \quad R_1 = \text{H, Me, Ph} \quad \text{R}_2 = \text{H, Me, Ph}
\end{align*}
\]

$\beta$-keto-ester is reacted with phenyl hydrazine to give pyrazolines\textsuperscript{33}.

The cycloaddition of stanny alkynes\textsuperscript{34} produces tin derivatives of the pyrazole heterocycle.

\[
\begin{align*}
\text{HC=Sn n-Bu}_3 & \xrightarrow{\text{EtO}_2\cdot\text{CCHN}_2} \text{EtO}_2\cdot\text{C} \\
\text{Sn n-Bu}_3 & \xrightarrow{\text{EtO, rt}} \text{N} \\
\text{Sn n-Bu}_3 & \xrightarrow{\text{EtO}_2\cdot\text{CCHN}_2} \text{N} \\
\text{Sn n-Bu}_3 & \xrightarrow{\text{EtO, rt}} \text{N}
\end{align*}
\]

1.1.3 SYNTHETIC PYRAZOLE DERIVATIVES AS THERAPEUTIC AGENTS

In pharmaceutical chemistry large number of derivatives of pyrazole is found as important therapeutic agents. Some of the listed below.

<table>
<thead>
<tr>
<th>STRUCTURE</th>
<th>NAME</th>
<th>PHARMACOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Antizol" /></td>
<td><strong>Antizol</strong> or <strong>Fomepizole</strong>\textsuperscript{35}</td>
<td>Competitive inhibitor of alcohol dehydrogenase</td>
</tr>
<tr>
<td><img src="image" alt="Phenazo" /></td>
<td>Phenazo or Antipyrine\textsuperscript{5}</td>
<td>Non-Steroidal Anti-Inflammatory Drug (NSAID) and powerful analgesic and antipyretic properties</td>
</tr>
<tr>
<td><img src="image" alt="Aminophenazo" /></td>
<td>Aminophenazo\textsuperscript{38} (R = N(CH(_2))(_2))</td>
<td>Non-Steroidal Anti-Inflammatory Drug (NSAID) and powerful analgesic and antipyretic properties</td>
</tr>
<tr>
<td><img src="image" alt="Novalgin" /></td>
<td>Novalgin or Metamizole\textsuperscript{39} (R = N(CH(_3))CH(_3)SO(_3))</td>
<td>Non-Steroidal Anti-Inflammatory Drug (NSAID) and powerful analgesic and antipyretic properties</td>
</tr>
<tr>
<td>Chemical Structure</td>
<td>Name</td>
<td>Properties</td>
</tr>
<tr>
<td>-------------------</td>
<td>------</td>
<td>------------</td>
</tr>
</tbody>
</table>
| ![Aminopropylon](image) | Aminopropylon<sup>40</sup>  
(R = NHCOCH(CH₃)NMe₂) | Non-Steroidal Anti-Inflammatory Drug (NSAID) and powerful analgesic and antipyretic properties |
| ![Nifenazone](image) | Nifenazone<sup>41</sup>  
(R = Nicotinamide) | |
| ![AM251](image) | AM251<sup>42</sup>  
(R = I, R’ = CH₃) | Acts as a potent and highly selective Cannabinoid receptor CB₁ antagonist |
| ![Rimonabant](image) | Rimonabant<sup>43</sup>  
(R = Cl, R’ = CH₃) | |
| ![Surinabant](image) | Surinabant<sup>44</sup>  
(R = Br, R’ = C₂H₅) | |
| ![Ibipinabant](image) | Ibipinabant<sup>45</sup> | CB₁ antagonist |
| ![VCHSR](image) | VCHSR<sup>46</sup> | |
| ![Sulfamazone](image) | Sulfamazone<sup>47</sup> | Antibiotic with Antipyretic properties |
| ![Celecoxib](image) | Celecoxib<sup>48</sup>  
(R = F & R’ = CH₃ & R” = H) | |
| ![Deracoxib](image) | Deracoxib<sup>49</sup>  
(R = H & R’ = OCH₃ & R” = F) | |
| ![Kebuzone or Ketophenylbutazone](image) | Kebuzone or Ketophenylbutazone<sup>50</sup>  
(R = -(CH₂)₂COCH₃ & R’ = H & R” = H) | Non-Steroidal Anti-Inflammatory Drug (NSAID) |
| ![Phenyl butazone](image) | Phenyl butazone<sup>51</sup>  
(R = -(CH₂)₂CH₃ & R’ = H & R” = H) | |
Suxibuzone\textsuperscript{52}

Oxyphenbutazone\textsuperscript{53}

Non-Steroidal Anti-Inflammatory Drug (NSAID)

Sulfinpyrazone\textsuperscript{5}

Lonazolac\textsuperscript{55}

Tepoxalin\textsuperscript{56}

Tartrazine or E102\textsuperscript{57} or C.I. 19140

Food coloring

Phenidone or 1-Phenyl-3-pyrazolidinone\textsuperscript{58}

Photographic developer

Furametpyr\textsuperscript{59}

Fungicidal activity
Penthiopyrad$^{60}$ Fungicidal activity

Pyraclostrobin$^{61}$

Zaleplon$^{62}$ Sedative, Hypnotic

Sulfaphenazole$^{63}$ Antibacterial

Quinpirole$^{64}$ Psychoactive drug, D receptor agonist

Betazole$^{65}$ H$_2$ receptor

1.1.4 SYNTHESIS, REACTIONS AND BIOLOGICAL ASPECTS OF 3-SUBSTITUTED-$1H$-PYRAZOLE-4-CARBALDEHYDES

1.1.4A SYNTHESIS OF 3-SUBSTITUTED-$1H$-PYRAZOLE-4-CARBALDEHYDES

As the pyrazole compounds reported in the thesis have been derived from 3-substituted-$1H$-pyrazole-4-carbaldehydes, details regarding its synthesis and reaction are also reviewed here.

A. Lebedev et al.$^{66}$ have reported the synthesis of 3-substituted-$1H$-pyrazole-4-carbaldehydes by Vilsmeier-Haack reaction of semicarbazone.
A. Kira et al.\textsuperscript{67} have reported that 1,3-diarylpentazole-4-carboxaldehyde, which is synthesized by reaction of acetophenone phenylhydrazone with 2 moles of DMF-POCl\textsubscript{3} in DMF at 70-80°C for 6 h which gave immonium perchlorate. Alkaline hydrolysis of immonium perchlorate gives product as 1,3-diarylpentazole-4-carboxaldehyde.

A. Kira et al.\textsuperscript{68} have synthesized 3-substituted pyrazole-4-carboxaldehyde by the reaction of semicarbazones with 2 moles of POCl\textsubscript{3} in DMF.

M. Bavatenko et al.\textsuperscript{69} have developed synthesis of substituted pyrazoles by cyclizing aryl hydrazones under Vilsmeier conditions.
1.1.4B REACTIONS AND BIOLOGICAL SIGNIFICANCE OF 3-SUBSTITUTED-1H-PYRAZOLE-4-CARBADEHYDE

A. Isloor et al.\textsuperscript{70} have been synthesized a series of new 4[(3-substituted-1H-pyrazol-4-yl)methyleneamino]-5-substituted-2-[(4-methylpiperazine-1-yl)methyl]-2H-1,2,4-triazole-3(4H)-thiones by the aminomethylation of 4-(3-substituted-1H-pyrazol-3-yl)methyleneamino-5-substituted-4H-1,2,4-triazole-3-thiols with formaldehyde and \(N\)-methylpiperazine. These synthesized schiff and manich bases were found as potent antibacterial and antifungal agents.

\[ \text{CHO} \overset{\text{POCl}_3/\text{DMF}}{\longrightarrow} \text{CHO} \]

where \( Ar = \text{C}_6\text{H}_5, 4-F, \text{C}_6\text{H}_4, 4-\text{CH}_3\text{C}_6\text{H}_4, 4-\text{CH}_3\text{O-C}_6\text{H}_4 \)

R. Murugan et al.\textsuperscript{71} have used magnesium oxide nanotubes for synthesis of pyrazolyl 1,4-dihydropyridine derivatives, this protocol having advantages like simple workup, short reaction times, high yields and reusability of the MgO nanotube catalyst.

\[ \text{R} = \text{Et, H} \]
\[ R_1 = \text{H, 4-OCH}_3, 4-F, 4-\text{Cl, 2,4-Cl}_2 \]
S. Malladi et al.\textsuperscript{72} have synthesized a series of pyrazole based Schiff bases and all synthesized compounds were screened for their antibacterial activity.

\begin{equation}
\text{HS}\text{N}=\text{N}\text{R} + \text{C}=\text{N}R_1 \xrightarrow{\text{EtOH/H}^+ \Delta, 7h} \text{N} \equiv \text{N} \text{R} \quad R = \text{CH}_3, \text{C}_2\text{H}_5, \text{C}_6\text{H}_5, R_1 = \text{CH}_3, \text{C}_2\text{H}_5, 4\text{-Cl}, \text{C}_6\text{H}_4, 4\text{-OCH}_3, \text{C}_6\text{H}_4, 4\text{-CH}_2\text{C}_6\text{H}_4
\end{equation}

A. Vijesh et al.\textsuperscript{73} have reported 2,4-disubstituted thiazole derivatives containing substituted pyrazole moiety, and antibacterial studies against \textit{Staphylococcus aureus, Bacillus subtilis, Escherichia coli} and \textit{Pseudomonas aeruginosa} that representing good results.

A. Vijesh et al.\textsuperscript{74,75} have prepared novel imidazole derivatives containing substituted pyrazole moiety by two different pathway. (1) Reaction of 3-aryl-1\textit{H}-pyrazole-4-carbaldehyde thiosemicarbazones with DMAD and (2) Reaction of 3-aryl-1\textit{H}-pyrazole-4-carbaldehydes with 1,2-diketones in the presence of ammonium acetate. All compounds were showing excellent antifungal and antibacterial activities.
A. Vijesh et al.\textsuperscript{76} have synthesized three series of new 1,2,4-triazole and benzoxazole derivatives containing substituted pyrazole moiety, all the synthesized compounds were screened for their analgesic and antimicrobial activity.

S. Dhanya et al.\textsuperscript{77} have synthesized two triazolo-thiadiazoles derivatives and investigated the \textit{in vitro} antioxidant property by spectrophotometric DPPH and ABTS radical scavenging methods as well as by lipid peroxide assay.
1.2 QUINOLONE

The carbon framework of 2-quinolone is isomeric to 4-quinolones. Ciprofloxacin, ofloxacin, lomefloxacin, enoxacin etc are well known antibacterial agents which bears 4-quinolones skeleton. While 2-quinolones contains benzopyranone ring so its carbon framework is isosteric to compounds of coumarins (i.e. chlorobiocin, novobiocin) and flavones (has shown DNA gyrase inhibition). 2-quinolones, also called carbostyrils or 1-aza coumarins.

1.2.1 NATURAL OCCURRENCE

The 2-quinolone core is found widely in various alkaloids, especially in the Rutaceae family, many of which possess interesting biological activity. Some of them are listed below with its pharmacology.

<table>
<thead>
<tr>
<th>ALKALOID</th>
<th>PHARMACOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Image of Buchapine]</td>
<td>Anti-HIV\textsuperscript{79}</td>
</tr>
<tr>
<td>[Image of Semecarpifoline]</td>
<td>Antiplatelet aggregation\textsuperscript{80}</td>
</tr>
</tbody>
</table>
Chapter 1

Antifungal and strong cytotoxic agent\textsuperscript{81}

Cytotoxic agent against human tumor cell lines\textsuperscript{82}

Antioxidative activity\textsuperscript{83}

Nitric oxide production inhibitory activity\textsuperscript{84}

Antimicrobial agent\textsuperscript{85}
1.2.2 SYNTHETIC APPROACH FOR QUINOLONE

For the synthesis of 2-quinolones the classic methods used as acid-catalyzed cyclization of acylacetoanilides (Knorr synthesis), cyclization/rearrangement and Heck reaction. The most general method is the Knorr synthesis. The evolving importance of these compounds has led to the development of new methods for their synthesis, including solid-phase synthesis, microwave-enhanced synthesis, acylation/cyclodehydration of anilines and o-aminobenzophenones, palladium-catalyzed carboxylative annulation of alkynes, palladium-catalyzed amidation of o-carbonyl-substituted aryl halides, palladium-catalyzed Ullmann cross-coupling of 1-bromo-2-nitroarenes with α-halo-esters, electrocyclic reaction of o-isocyanatostyrenes, cyclization of Baylis-Hillman adducts and cyclization of o-amino-functionalized benzoylecetates. The synthesis of 2-quinolones from o-aminoarylketone and β-ketoester or ethyl cyanoacetate or ethyl malonyl chloride / diethyl malonate / malonic acid is also well known.

V. Nadaraj and S. Selvi have prepared 2-oxo-3-formylquinoline from 2-chloro-3-formyl quinoline via microwave assisted synthesis in presence of sodium acetate and acetic acid.

\[
\text{Flindersine (R = H) N-Methylflindersine (R = Me)}
\]

\[
\text{R = H: Zanthobungeanine R = OMe: Vepristine}
\]

SRS-A antagonists
A general and efficient reaction of readily available $N$-methyl-$N$-phenylcinnamamides with phenyliodine bis(trifluoroacetate) (PIFA) in the presence of Lewis acids provides various 3-arylquinolin-2-one compounds in good yields. This novel approach features not only metal-free oxidative $C(\text{sp}^2)$$-C(\text{sp}^2)$ bond formation but also an exclusive 1,2-aryl migration.\(^{106}\)

\[
\begin{array}{c}
R_1\quad \mathrm{N}^+\quad \text{O}
\end{array}
\]

\[
\begin{array}{c}
R_2
\end{array}
\]

\[
\begin{array}{c}
R_3
\end{array}
\]

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

\[
\begin{array}{c}
1.5 \text{ eq. PIFA, 10 eq. TFA}
\end{array}
\]

\[
\begin{array}{c}
1 \text{ eq. BF}_3 \text{Et}_2\text{O}
\end{array}
\]

\[
\begin{array}{c}
\text{DCE, rt, 6h}
\end{array}
\]

\[
\begin{array}{c}
R_1 = \text{H, Me, OMe, F, Cl, Br, CF}_3, \text{COOMe}
\end{array}
\]

\[
\begin{array}{c}
R_2 = \text{Me, Bn, } \text{tPr, cyclopropymethyl}
\end{array}
\]

\[
\begin{array}{c}
R_3 = \text{H, Ar}
\end{array}
\]

$\text{Ar} = \text{Various phenyl/heteryl substrate}$

A Ru-catalyzed cyclization of anilides with propiolates or acrylates affords 2-quinolones having diverse functional groups in good to excellent yields. 2-quinolinones can be converted into 3-halo-2-quinolines and 2-chloroquinolines.\(^{107}\)

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

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

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

\[
\begin{array}{c}
1.5 \text{ eq.}
\end{array}
\]

\[
\begin{array}{c}
\left[\text{RuCl}_2(\text{p-cymene})\right]_2
\end{array}
\]

\[
\begin{array}{c}
0.2 \text{ eq. AgSbF}_6
\end{array}
\]

\[
\begin{array}{c}
10 \text{ eq. PivOH}
\end{array}
\]

\[
\begin{array}{c}
\text{1PrOH, 130°C, 24h}
\end{array}
\]

$R = \text{OMe, Me, OH, H, F, Cl, Br, COOMe, COOMe, NO}_2$

A silver-catalyzed method provides a practical, highly efficient, and straightforward route to substituted quinolin-2-ones or 3,4-dihydroquinolin-2-ones in one step through an intermolecular radical addition/cyclization in aqueous solution. A mechanism for the formation of quinolin-2-ones is proposed.\(^{108}\)

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

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

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

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

\[
\begin{array}{c}
0.2 \text{ eq. AgNO}_3
\end{array}
\]

\[
\begin{array}{c}
4 \text{ eq. K}_2\text{S}_2\text{O}_8
\end{array}
\]

\[
\begin{array}{c}
\text{MeCN} / \text{H}_2\text{O} (1:1)
\end{array}
\]

\[
\begin{array}{c}
100°C, 12h
\end{array}
\]

$\text{Ar} = \text{Ph, 2-Cl-Ph, 4-Br-Ph, 4-OMe-Ph, 3,4-OMe-Ph, R = Ph, 3-Br-Ph, } \text{N}(\text{Me})\text{-Ph}$

4-hydroxyquinolin-2(1$H$)-one derivatives have attracted much attention due to their biological benefits, but up to now harsh reactions conditions must be employed to provide these key compounds. Various $o$-alkynylanilines can be converted under
mild reaction conditions to 4-hydroxyquinolin-2(1H)-one derivatives in high yield in the presence of a catalytic amount of a silver salt, carbon dioxide and a base.\textsuperscript{109}

\[
\begin{array}{c}
\text{R} \\
\text{NHNH}_2
\end{array} & \xrightarrow[0.1 \text{ eq. AgNO}_3 \text{ 1 eq. DBU}]{} & \xrightarrow{} & \begin{array}{c}
\text{O} \\
\text{NCO}
\end{array} & \xrightarrow{} & \begin{array}{c}
\text{OH} \\
\text{N}
\end{array}
\]

\[\text{R} = \text{Ar}, \text{Bu}, \text{H}, \text{COPh}\]

1.2.3 SYNTHETIC QUINOLONE DERIVATIVES AS THERAPEUTIC AGENTS

As per the literature survey it is found that N-substituted-2-quinolone possesses significant differences in pharmacological activities compared with parent nucleus\textsuperscript{110}, for example substituted \(N\)-aryl-2-quinolones represent the structural basis of many biologically active compounds, such as antiviral agents, hypoglycemics, immunomodulators, protein kinase inhibitors, anti-ulcer agent and farnesyl transferase inhibitors. Some of the therapeutically active quinolone derivatives are reviewed here with their pharmacology.

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Possesses anti-hepatitis B activity\textsuperscript{115}

Anti-HIV activity\textsuperscript{116}

Photoantiproliferative activity\textsuperscript{117}

Rho-kinase inhibitors\textsuperscript{118}

Farnesyltransferase inhibitors\textsuperscript{119}

Androgen receptor\textsuperscript{120}

FMS kinase inhibitors\textsuperscript{121}

Antiproliferative activity and inhibition of topoisomerase II activity\textsuperscript{122}
1.2.4 SYNTHESIS, REACTIONS AND BIOLOGICAL ASPECTS OF N-ALLYL QUINOLONE

1.2.4A SYNTHETIC ROUTE FOR N-ALLYL QUINOLONE

In recent years quinolone derivatives represent a new class of potent medicinal active compounds, which prompted our continued attempt to demarcate a pharmacophoric pattern, from which a recognized target might be deduced. On the other hand, from the synthetic point of view, the presence of electrophile favored (nucleophilic) ‘nitrogen’ on 2-oxo-3-formylquinoline encouraged us to synthesize N-allyl-3-formylquinolone from an easily available allyl bromide.

Herein, we developed hybrid molecules via incorporation of pharmacophoric allyl group at the N-atom of 2-oxo-3-formylquinoline which lead to compounds with amplified pharmacological activity.

Quinolone is the inner core structure for the synthesis of several bioactive heterocyclic compounds in the large field of quinoline. The substituted 2-chloro-3-formyl quinolines are the unique intermediates as they can be utilized for various functional group interconversions (FGIs). 2-oxo-3-formylquinoline can be obtained by O-nucleophilic substitution reaction of 2-chloro-3-formylquinoline in presence of aqueous acetic acid or 50% dilute hydrochloric acid.
K. Park and J. Lee,\textsuperscript{127} have reported the reaction quinolone with alkyl halide in DMF/K$_2$CO$_3$ which gave mixtures of the corresponding $N$-alkylated and $O$-alkylated products. The reaction with methyl iodide gave mostly $N$-alkylated product.

Substituted $N$-allyl quinolone was prepared from carbostyril derivative by using potassium hexamethyldisilazane (KHMDS) in tetrahydrofuran under microwave irradiation in excellent yield.\textsuperscript{128}

$N$-allyl quinolone was synthesized by allylation of tert-butylidiphenylechlorosilane (TBDPSCl) substituted quinolone derivative in the presence of potassium carbonate in acetone and evaluated for \textit{in vitro} anti-hepatitis B virus (anti-HBV) activity.\textsuperscript{129}
1.2.4B REACTIONS AND BIOLOGICAL SIGNIFICANCE OF N-ALLYL QUINOLONE

The amidation of N-allyl quinolone ester by alkylaminoalkylamines was carried out by heating equimolar amounts of the reagents in ethanol at reflux over several hours to develop new highly efficient opioid receptor antagonists.\(^{130}\)

Reaction of the 1-R-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acids hydrazides with acetone or methyl ethyl ketone gave the corresponding isopropylidene and sec-butylidene derivatives in high yields. These derivatives were screened for their antitubercular activity\(^{131}\).

I. Ukrainets and co-workers\(^{132}\) have synthesized 2-bromomethyl-5-oxo-1,2-dihydro-5H-oxazolo[3,2-a]quinoline-4-carboxylic acid pyrimidin-2-ylamide derived from bromination of 1-allyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid pyrimidin-2-ylamide and evaluated for their and antitubercular activity.
I. Ukrainets and co-workers\textsuperscript{133} have published three-component condensation reaction of \(N\)-allyl-4-hydroxyquinolone, isatin and malononitrile which gave a 4,3'–spiro[(6-allyl-2-amino-5-oxo-5,6-dihydro-4H-pyrano-[3,2-c]quinoline-3-carbonitrile)-2'-oxindole].

![Chemical structure]

Y. Lee \textit{et al.}\textsuperscript{134} have developed the synthesis of dihydrofuroquinolinones and from \(N\)-allyl-4-hydroxyquinolone and 2-methoxyprop-1-ene in the presence of \(\text{Ag}_2\text{CO}_3/\text{Celite}\) in moderate yields.

![Chemical structure]

N. Parmar \textit{et al.}\textsuperscript{135} have reported one-pot solvent-free procedure for the synthesis of diazepinone derivatives of \(N\)-allyl quinolone and evaluated their \textit{in vitro} antimicrobial, antitubercular and antioxidant activities.
Bromination of 1-allyl-substituted 3-(arylamimethylene)quinoline-2,4-(1H,3H)-diones with one equivalent of molecular bromine in glacial acetic acid and subsequent dilution of the reaction mixture with water is accompanied by halocyclization and hydrolysis to form 2-bromomethyl-5-oxo-1,2-dihydro-5H-oxazolo[3,2-a]quinoline-4-carbaldehyde\textsuperscript{136}.

![Chemical Reaction Diagram]

1.3 ANTIMICROBIAL STUDY

An anti-microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects. 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.

1.3.1 PATHOGENS

The microorganisms, or infectious agents or more commonly germs, are biological agents capable of producing diseases in host are known as pathogens. 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 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 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, food borne 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. 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.
Bacillus subtilis, as with many in the Bacillus genus, is an extremely common bacterium. It is found in soil, water, air, and decomposing plant matter. Bacteria in the Bacillus genus are spore-forming, which means that they create a thick wall which surrounds their DNA and other internal cell structures. In this way, they are very hardy and impervious to extreme temperatures, chemicals, environmental factors, even some types of radiation. This makes them excellent for use in industrial processes.

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

GRAM POSITIVE BACTERIAL PATHOGENS

(i) Bacillus subtilis (MTCC 441)\textsuperscript{137}
single or in short chains. *B. subtilis* produces the proteolytic enzyme subtilisin. *B. 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.

(ii) *Clostridium tetani* (MTCC 449)\(^{138}\)

*Clostridium tetani* is a rod-shaped, anaerobic bacterium of the genus species Clostridium. Like other Clostridium genus species, it is Gram-positive, and its appearance on a gram stain resembles tennis rackets or drumsticks. *C. tetani* is found as spores in soil or in the gastrointestinal tract of animals.

It is a mobile, spore-forming, obligate anaerobic, cannot survive in high oxygen situations, rod-shaped bacterium. 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. 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.
(iii) *Staphylococcus aureus* (MTCC 96)\(^{139}\)

*Staphylococcus aureus* is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the respiratory tract and on the skin. It is often positive for catalase and nitrate reduction. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of *S. aureus* such as MRSA is a worldwide problem in clinical medicine.

The individual cells of *S. aureus* are 0.8 to 0.9 micrometres in diameter. They are ovoid or spherical, non motile, non capsulated, non sporing stain with ordinary aniline dyes and gram positive, typically arranged in groups of irregular clusters like branches of groups found in pus, singly or in pairs. The optimum condition for the growth of *S. aureus* is 37 °C, pH 7.4 to 7.6. They produce golden yellow pigment, which develops best at room temperature. They cause pyoregenic of pus forming conditions, mastitis of women and cows, boils, carbuncles infantile impetigo, internal abscess and food poisoning.

(iv) *Streptococcus pneumoniae*\(^{140}\)

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 mesophilic, 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 pneumoniae 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).

-GRAM NEGATIVE BACTERIAL PATHOGENS

(i) *Escherichia coli* (MTCC 443) 141

They are rods, 2 to 4 micro by 0.4 micrometres in size, commonly be seen in cocccobacillary 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.

![Image](image_url)

It causes infantile diarrhoea, gastroenteritis, traveller’s diarrhoea, bacillary dysentery, haemorrhagic colitis, haemolytic uraemic syndrome (HUS) and thrombocytopaenic 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.

(ii) *Vibrio cholerae* (MTCC 3906)\(^{142}\)

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 results in an 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 epidermic 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.

(iii) *Salmonella typhi* (MTCC 98)\(^{143}\)

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.
\textit{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).

(iv) \textit{Pseudomonas aeruginosa} (MTCC 1688)

\textit{P. aeruginosa} occurs as a commensal in the intestine of human and animals but the defensive mechanism of the body is poor. It acts as a minor pathogen producing suppurative wound, otitis media, peritonitis, cystitis, bronchopneumonia and empyema. In children it causes diarrhea and septicemia. The pus produced by \textit{P. aeruginosa} is greenish blue. These are gram negative, actively motile, non sporing organisms having 1.5 to 3.0 micrometres size and 0.5 micrometres with rounded ends of short chains. They grow well in ordinary media under aerobic conditions, producing diffusible pigment.

(B) \textbf{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 immune competent 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. Fungi make fungal diseases very difficult to treat because they are more chemically and genetically similar to animals compare to other organisms. 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.

(i) *Candida albicans* (MTCC 227)

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. 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.

(ii) *Aspergillus niger* (MTCC 282)\textsuperscript{146}

*A. niger* is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. *A. niger* is included in *Aspergillus* subgenus *Circumdati*, section *Nigri*. *A. niger* is less likely to cause human disease than some other *Aspergillus* species, if large amounts of spores are inhaled, a serious lung disease, aspergillosis can occur. *A. niger* is one of the most common causes of otomycosis, which can cause pain, temporary hearing loss, and, in severe cases, damage to the ear canal and tympanic membrane.

(iii) *Aspergillus fumigates* (MTCC 3008)\textsuperscript{147}

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.

(iv) *Trichophyton rubrum* (MTCC 296) 148

*T. rubrum* is a fungus that is the most common cause of athlete's foot, jock itch and ringworm. This fungus was first described by Malmsten in 1845. The growth rate of *Trichophyton* colonies in the lab can be slow to rather quick. Their texture is waxy, smooth and even to cottony. From the top, the color is white to bright yellowish beige or red violet. Reverse is pale, yellowish, brown, or reddish-brown. Although *Trichophyton rubrum* is the most common of the dermatophytes causing fingernail fungus infections, there are others. Positive, selective diagnosis of *T. rubrum* is difficult as many members of the genus react similarly with test reagents.

1.3.2 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. World Health Organization (WHO) has also approved this type of combinations.

Most microbiologists explain that the antimicrobial agents are used in the treatment of infectious disease: antibiotics, which are natural substances produced by certain groups of microorganisms. 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.

1.3.3 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:

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<tr>
<td>Or</td>
<td>ii) Agar Dilution Method</td>
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<tr>
<td>Disk diffusion method</td>
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We have used the **Broth Dilution Method** for antimicrobial study recommended by the National Committee for Clinical Laboratory Standards (NCCLS). \(^{149}\)

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
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(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.

1.3.3 (A) 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.

Following is the typical procedure to carry out Broth Dilution Method.

- **Procedure for performing the Broth Dilution Method**

  - The *in vitro* antimicrobial activity of the synthesized compounds and standard drugs were assessed against representative panel of Gram-positive bacteria, Gram-negative bacteria and fungi. 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 $10^8 \text{ CFU mL}^{-1}$ (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, 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 µgmL\(^{-1}\) concentration, as a stock solution. In primary screening 1000, 500 and 250 µgmL\(^{-1}\) 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 6.250 µgmL\(^{-1}\) 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 (µgmL\(^{-1}\)) 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\(^8\) CFUmL\(^{-1}\) organisms. The protocols were summarized and compared with standard drugs as the Minimal Inhibitory Concentration (µgmL\(^{-1}\)).

1.3.3 (B) 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 surface of medium or on petri dish covers when 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.

1.3.3 (C) **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.

1.4 **ANTIMYCOBACTERIAL STUDY**

1.4.1 **MYCOBACTERIUM TUBERCULOSIS (MTB)**

**General Characteristics**

MTB is the etiologic agent of tuberculosis in humans. Humans are the only reservoir for the bacterium. First discovered in 1882 by Robert Koch, *Mycobacterium tuberculosis* is a fairly large nonmotile rod-shaped bacterium distantly related to the actinomycetes. Many non pathogenic mycobacteria are components of the normal flora of humans, found most often in dry and oily locales. The rods are 2-4 micrometers in length and 0.2-0.5 micrometers in width.
MTB is an obligate aerobe. For this reason, in the classic case of tuberculosis, MTB complexes are always found in the well-aerated upper lobes of the lungs. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15-20 hours, a physiological characteristic that may contribute to its virulence.

MTB requires oxygen to grow. It does not retain any bacteriological stain due to high lipid content in its wall, and thus is neither Gram positive nor Gram negative; hence Ziehl-Neelsen staining or acid-fast staining, is used. *Mycobacterium* species, along with members of a related genus *Nocardia*, are classified as acid-fast bacteria due to their impermeability by certain dyes and stains. Despite this, once stained, acid-fast bacteria will retain dyes when heated and treated with acidified organic compounds. One acid-fast staining method for *Mycobacterium tuberculosis* is the Ziehl-Neelsen stain. When this method is used, the MTB, smear is fixed, stained with carbol-fuchsin (a pink dye), and decolorized with acid-alcohol. The smear is counterstained with methylene-blue or certain other dyes. Acid-fast bacilli appear pink in a contrasting background.

*M. tuberculosis* divides every 15–20 hours, which is extremely slow compared to other bacteria, which tend to have division times measured in minutes (*Escherichia coli* can divide roughly every 20 minutes). Tubercle bacilli are aerobes, grow slowly (generation time 14-15 hours), optimum temperature 37 °C, pH 6.4-7.0. They grow only in specially enriched media containing egg, asparagines, potatoes, serum and meat extracts. Colonies appear in 2-6 weeks. Two media are used to grow MTB Middlebrook's medium which is an agar based medium and Lowenstein-Jensen medium which is an egg based medium. MTB colonies are small and buff colored when grown on either medium. Both types of media contain inhibitors to keep
contaminants from out-growing MT. It takes 4-6 weeks to get visual colonies on either type of media.

The drug susceptibility test may be performed by either the direct or the indirect method.

The direct drug susceptibility test is performed by using a subculture from a primary culture as the inoculum.

1.4.2 ANTIMYCOBACTERIAL SUSCEPTIBILITY TESTING

Three well-known measures of sensitivity test are available:

(A) The minimal inhibition concentration or the MIC
(B) The resistance ratio or the RR
(C) The proportion method

These tests are set up on solid media.

We have used the Minimal Inhibition Concentration reported by A. Rattan\textsuperscript{150} to evaluate the anti-tuberculosis activity. It is one of the non automated \textit{in vitro} bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in bottle.

1.4.3 MINIMAL INHIBITION CONCENTRATION BY L.J SLOPE METHOD

MIC is defined as the minimal concentration of the drug required to inhibit the growth of the organisms, where growth is defined as 20 colonies or more. This definition of growth is chosen so that only a small proportion (e.g. 1%) of wild strains would be classified as resistant by its use. This method is simple and be carried out with a single drug containing slope although it is preferable to use more than one slope.

\textbf{Procedure for antimycobacterial study}

- **Methods used for primary and secondary screening:** Each synthesized drug was diluted obtaining 2000 \( \mu \text{g}\text{mL}^{-1} \) concentration, as a stock solution.
- **Primary screening:** In primary screening 6.25 \( \mu \text{g}\text{mL}^{-1} \) concentration of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.
Secondary screening: The drugs found active in primary screening were similarly diluted to obtain 500 µg/mL, 250 µg/mL, 200 µg/mL, 125 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL and 1.5625 µg/mL concentrations.

A primary screen was conducted at 6.25 µg/ml against *M. tuberculosis* H37Rv by Lowenstein-Jensen (LJ) MIC method where primary 6.25 µg/ml dilution of each test compound were added to liquid Lowenstein-Jensen Medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein-Jensen Medium was harvested in 0.85% saline in bijou bottles. DMSO was used as vehicle to get desired concentration. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 × 10⁴ bacilli per tube). These tubes were then incubated at 37°C. Growth of bacilli was seen after 12, 22, and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound. The standard strain *M. tuberculosis* H37Rv was tested with known drug Isoniazide and Rifampicin. The screening results are summarized as % inhibition relative to standard drug Isoniazide and Rifampicin. Compounds effecting < 90% inhibition in the primary screen were not evaluated further. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentration (MIC) in a Lowenstein-Jensen medium.

### 1.5 ANTIOXIDANT STUDY

Generation of reactive oxygen species (ROS) and free radicals *in vivo* is involved in a wide range of human diseases. ROS, including superoxide anion, hydrogen peroxide and hydroxyl radical, are by-products of a variety of pathways of aerobic metabolism. They are unstable and react readily with a wide range of biological substrates, such as lipids, DNA, and proteins, resulting in cell damage. The human body possesses innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The unbalance between formation and detoxification of free radical species results in the progression of oxidative stress and leads to the development of a wide spectrum of serious diseases, such as, cancer, atherosclerosis, aging, immunosuppression, inflammation, ischemic heart disease, diabetes, hair loss, and neurodegenerative
disorders such as Alzheimer’s and Parkinson’s diseases. Many studies have suggested that agents (antioxidants) with the ability to protect against ROS may be therapeutically useful in these diseases.

**General Characteristics**

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases like chronic diseases including heart disease and some cancers.

### 1.5.1 ANTIOXIDANTS

Various antioxidant activity assays are used to monitor the antioxidant activity. Some widely used assays are mentioned hereafter:

(A) Ferric Reducing Antioxidant Power (FRAP) assay
(B) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay
(C) Oxygen Radical Absorbance Capacity (ORAC) assay
(D) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay

We have used **Ferric Reducing Antioxidant Power (FRAP) assay** reported by I. Benzie and J. Strain\textsuperscript{151} to measure antioxidant capacity of the synthesized compounds. This assay has the ability of quantitative determination of the amounts of antioxidants in samples. Also, it is rapid, simple and inexpensive among all above mentioned assays.

### 1.5.2 ANTIOXIDANT ACTIVITY TESTING

**FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY**

Ferric reducing antioxidant power (FRAP) of newly synthesized compounds was determined chemically using modified FRAP method.

- **Principle**

  FRAP method depends upon the reduction of ferric tripyridyltriazine complex (Fe$^{3+}$-TPTZ) to the ferrous tripyridyltriazine (Fe$^{2+}$-TPTZ) by a reductant (antioxidant)
at the low pH. This ferrous tripyridyltriazine complex has an intensive blue colour and can be monitored at 593 nm.

The antioxidant potentials of the compounds were estimated as their power to reduce the TPTZ-Fe(III) complex to TPTZ-Fe(II) complex. The ascorbic acid was used as a standard antioxidant compound and the results were expressed as ascorbic equivalent (mmol/100 gm compound).

- **Reagents and Samples**
  (i) Buffer solution: 0.187 gm sodium acetate and 1.6 mL acetic acid was dissolved in double distilled water to make 100 mL.
  (ii) TPTZ: 0.155 gm TPTZ was dissolved in 100 mL 40 mM HCl.
  (iii) FeCl₃ solution: 0.324 gm FeCl₃ was dissolved in 100 mL distilled water.
  (iv) Standard Ascorbic acid: 0.176 gm of standard ascorbic acid was dissolved in 100 mL distilled water.
  (v) Sample solution: 5.0 mg of sample was dissolved in DMF to make 25 mL.

- **Procedure**
  Fe(II)-TPTZ(2,4,6-tripyridyl-s-triazine) reagent was prepared by mixing a 10.0 mL TPTZ solution, 10 mL FeCl₃ solution and 100 mL acetate buffer of pH 3.6. A mixture of 200.0 µL sample solution and 3 mL of Fe(II)TPTZ reagent was incubated at 37 °C for 15 min. The absorbance of colour complex Fe(II)TPTZ was measured at 593 nm using ascorbic acid as standard. The results were expressed as ascorbic equivalent (mmol/100 gm compound).

  **Ascorbic acid taken = 1.99 x 10⁻⁴ mm; Sample taken = 0.04 mg**

  The Ferric Reducing Antioxidant Power (FRAP) can be calculated using the following equation:

  \[
  \text{FRAP value} \ (\text{mm A.A./100 g sample}) = \frac{\Delta OD_{593 \ nm \ of \ sample}}{\Delta OD_{593 \ nm \ of \ standard}} \times \frac{\text{mm of standard}}{\text{sample weight (mg)}} \times 10^5
  \]
1.6 PRESENT STUDY

Literature survey reveals that 1H-pyrazole and N-allyl-quinolone nucleus possesses tremendous therapeutic activity but very few references have been found of these compounds. The aim of the present investigation is to synthesize some new heterocyclic compounds bearing 1H-pyrazole and N-allyl-quinolone biologically active moieties, with hope to find potent compounds. With this aim and objective various new heterocyclic compounds containing these moieties have been synthesized. The various synthesized intermediates, the newly synthesized compounds, their characterization and biological studies have described systematically in different chapters.

CHAPTER 2:


\[
\text{Where, } R_1 = \text{Ph, 4-F-Ph, 4-Cl-Ph and 4-Br-Ph}
\]

CHAPTER 3:

Part 1: Microwave assisted synthesis, characterization of 3-substituted-pyrazol-1H-pyrazolo[1,2-b]phthalazine-5,10-dione derivatives and their in vitro antimicrobial activities.

\[
\text{Where } R_1 = \text{Ph, 4-F-Ph, 4-Cl-Ph and 4-Br-Ph}
\]
\[
R_2 = \text{CN, COOCH}_3, \text{COOC}_2\text{H}_5, \text{COOCH(CH}_3)\text{)}_2
\]
Part 2: Microwave-induced CAN promoted synthesis of 2,4,6-tri substituted pyridine derivatives and \textit{in vitro} study of the effect of halogen substitution on antimicrobial activities.

\[
\begin{align*}
\text{HN} - \text{N} & \\
\text{R}_1 & \\
\text{R}_2 & \\
\text{R}_2 & \\
\text{R}_2 & \\
\end{align*}
\]

Where, 
\(\text{R}_1\) and/or \(\text{R}_2\) = H, F, Cl and Br

CHAPTER 4:


\[
\begin{align*}
\text{HN} - \text{N} & \\
\text{R}_1 & \\
\text{R}_2 & \\
\text{R}_3 & \\
\text{R}_3 & \\
\end{align*}
\]

Where, 
\(\text{R}_1\) = Ph, 4-F-Ph, 4-Cl-Ph and 4-Br-Ph 
\(\text{R}_2\) = CN, COOCH\(_2\)CH\(_3\), COOCH(CH\(_3\))\(_2\) 
\(\text{R}_3\) = H or CH\(_3\)


\[
\begin{align*}
\text{HN} - \text{N} & \\
\text{R}_1 & \\
\text{R}_2 & \\
\text{R}_3 & \\
\text{R}_3 & \\
\text{R}_3 & \\
\end{align*}
\]

Where, 
\(\text{R}_1\) = Ph, 4-F-Ph, 4-Cl-Ph and 4-Br-Ph 
\(\text{R}_2\) = CN, COOCH\(_2\)CH\(_3\), COOCH(CH\(_3\))\(_2\) 
\(\text{R}_3\) = H or CH\(_3\)

CHAPTER 5:
Zinc triflate promoted a general synthetic protocol for the facile construction of chromenes and pyrimidines bearing N-allyl quinolone nucleus and their in vitro antimicrobial activity.

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