1.1 Introduction

The occurrence of microbial and fungal infections has increased notoriously in current years[1]. Resistance to antimicrobial agents has increased treatment cost and resulted in mortality and morbidity[2]. The search for new antimicrobial drugs involves active investigation with the goal of overcoming the phenomenon of multiple drug resistance strains of bacteria and fungi[3, 4]. The development of novel structure leads remains a key challenge for medicinal chemists to design new, effective and broad spectrum antimicrobial agents.

Tuberculosis (TB) has become one of the most common lethal infectious disease caused by the transmittable air borne pathogen, *Mycobacterium tuberculosis* [5]. The statistics shows that around three million people die annually from TB throughout the world [6]. Recent frontier therapy for TB recommends combination of different drugs usually isoniazid, rifampin, pyrazinamide and ethambutol over an extended period up to six months[7]. The development of new potent forms like multidrug resistant (MDR) and extensively drug resistant (XDR) TB has become the major threats. These facts demonstrate the urgency for development of novel, more efficient and fast acting anti-TB drugs with low toxicity profiles and performing activity against both drug-sensitive and drug resistant MTB[8].

There is an imperative need to discover and develop novel antibacterial and antifungal agent with novel mechanism of action and enhanced activity profile, high potency without or with at least reduced systemic adverse effects. Nearly one million people die every year mainly of children below the age of five due to malaria caused by genus *Plasmodium*[9, 10]. The disease is predictable to result in nearly 250 million new annual infections worldwide. Owing to this danger, there is an urgent need for the development of novel drugs with fewer side effects and improved efficacy to cure malaria, tuberculosis (TB) and microbial infections.

Heterocycles form the largest of the classical divisions of organic chemistry enjoying enormous importance biologically and industrially. These compounds play a major part in biochemical processes and are part of the most typical and essential constituents of living cells[11]. Most of the significant advances in the treatment of diseases have been often made by designing and testing new heteroaromatic

structures. The majority of pharmaceutical products that mimic natural products with biological activity are heterocycles. In addition, a number of pesticides, antibiotics, alkaloids, and cardiac glycosides are heterocyclic natural products of immense importance to human and animal health. Therefore, numerous efforts are continuously being focused to design and produce better pharmaceuticals, pesticides, insecticides, rodenti-cides, and weed killers following natural models.

Pyrazoles and their derivatives possess several medicinal applications because of their versatile biological activities. They have occupied a distinct place due to a range of bioactivities such as antiproliferative[12] , antimicrobial [13], antidepressant[14], antipyretic [15], anti-inflammatory [16] and anticonvulsant [17]. Pyrazoline is also an important nitrogenous heterocyclic moiety in many drugs[18]. 1,3,4-Oxadiazoles form important class of heterocyclic bioactive compounds which have extensively attracted attention, owing to their remarkable biological and pharmacological properties[19].

Quinoline is the key building core for many naturally occurring (cinchona alkaloids) compounds and pharmacologically active substances. It demonstrates a broad range of biological activity such as antimalarial[20] , antituberculosis[21] , anti-HIV activities[22], antifungal, antibacterial, antiprotozoic and antibiotic[23]. N-functionalized morpholine motifs have been recognized to possess diversified biological activities. Moreover, 1,2,4-oxadiazole derivatives possess significant biological potency in medicinal field.

The substitution of fluorine in to a potential drug molecule can improve effectiveness of drugs by extending pharmacokinetic and pharmacodynamics properties[24]. Trifluoromethyl group is a well known substituent of unique qualities. Its high lipophilicity enables to improve pharmacological activity of the molecule[25, 26].

The present thesis describethe synthesis characterization of some novel fluoro substituted pyrazoles containing polyhydroquinoline, pyrazoline and,1,3,4-oxadiazole scaffolds as well as 2-morpholinoquinoline based pyrazoline and 1,2,4-oxadiazole scaffolds to develop new structural motifs with diverse biological

activities. The improvement of hybrid molecules through the combination of diverse pharmacophores in one frame has led to compounds with interesting biological profiles, which is being reflected in our present work

1.2 Pyrazole

Pyrazole is the class of five membered heterocycles, containing two double bonds, two nitrogen atoms at adjacent positions (Figure 1)that have attracted much more attention in recent time due to their utility in the field of drug discovery and agricultural research.

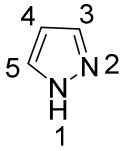
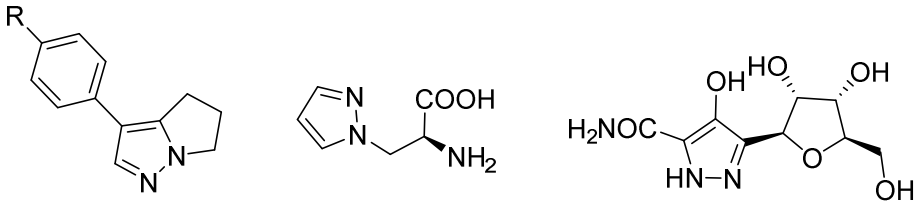


Figure 1.1 Structure of 1H-pyrazole.

1.2.1Pyrazole containing natural products

Akira. Morimoto *et al*.[27] investigatedpyrazole alkaloid, withasomnine was isolated from the *Withaniasomnifera*in 1968.It has diverse biological activities such as CNS-depressant, fungicide, gram(+)icide, gram(-)icide, narcotic and sedative. Moreover, 3-pyrazol-1-ylalanine[28], a nonproteinogenic amino acid with antidiabetic activity and pyrazomycin[29]**,** an antiviral metabolite of *Streptomyces candidus*, are also pyrazole containing natural products (Figure 1.2).



Withasomnine (*s*)-3-pyrazolylalanine Pyrazomycin

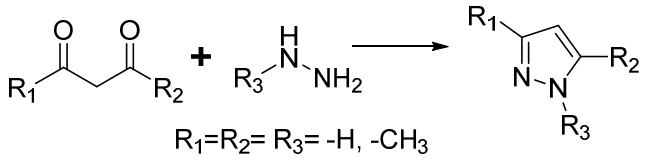
R= -H; Withasomnine
R = -OH; 4'-Hydroxy Withasomnine
R = -OMe; 4'-Methoxy Withasomnine

Figure 1.2 Natural occurrence of pyrazole moiety.

1.2.2Synthetic methods of pyrazole

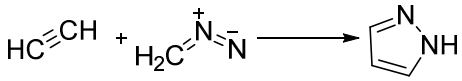
In 1883, Pyrazole derived compound were firstly synthesized by Knorr[30]. Condensation of hydrazines with 1,3-dicarbonyl compounds is the classical method

for the synthesis of pyrazole,which led to discovery of antipyrine and its derivatives(Scheme 1.1).



Scheme1.1 Classical methods for the synthesis pyrazole.

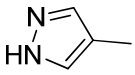
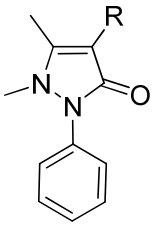
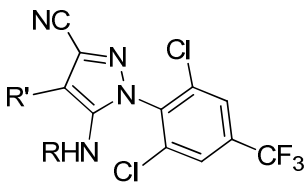
In 1898, Pechmann[31] reported formation of pyrazoles from acetylenes and diazomethane. The analogous addition of diazoacetic esters to the triple bond also yielded pyrazolecarboxylic acid derivatives (Scheme 1.2).



Scheme 1.2 Synthetic pathway for 1H-pyrazole.

1.2.3Pyrazole bearing pharmacological agents

Pyrazoles are an important class of heterocyclespresent in several drugs.Some of the pyrazole containing drugs are mention below:

Name	Structure	Pharmacology
Antizol or Fomepizole		Competitive inhibitor of alcohol dehydrogenate
Phenazone or Antipyrine (R=H)		Non-Steroidal Anti-Inflammatory Drugs (NSAID), powerful analgesic and antipyretic properties
Ampyrone ($R=NH_2$)		
Propyphenazone ($R=CH(CH_3)_2$)		
Aminophenazone ($R=N(CH_3)_2$)		
Novalgin or Metamizole ($R=N(CH_3)CH_2SO_3Na$)		
Aminopropylon ($R=NHCOCH(CH_3)NMe_2$)		
Nifenazone (R=Nicotinamide)		
Fipronil (R=H, R'=$SOCF_3$)		Insecticide
Pyrafluprole ($R=CH_2$-2-pyrazine, R'=SCH_2F)		

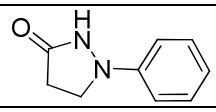
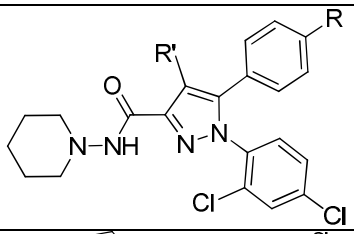
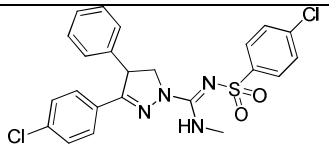
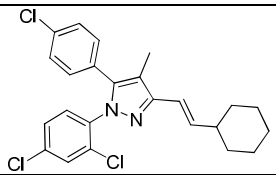
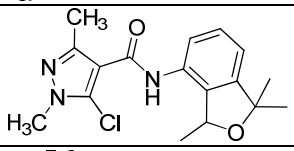
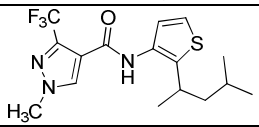
Ethiprol (R=H, R'=SOEt)		
Phenidone or 1-Phenyl-3-pyrazolidinone		Photographic developer
AM251 (R=I, R'=CH_3) Rimonabant (R=Cl, R'= CH_3) Surinabant (R=Br, R'=C_2H_5)		Potent and highly selective Cannabinoid receptor CB_1 antagonists
Ibipinabant		
VCHSR		
Furametpyrad		Fungicides
Penthiopyrad		

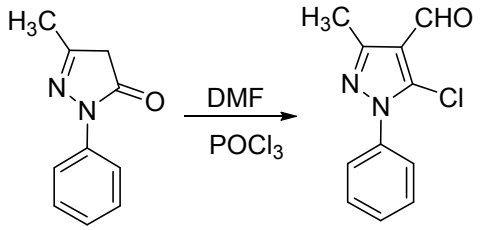
Figure 1.3 Drugs and pesticides containing pyrozole moiety.

1.2.4 1*H*-Pyrazole-4-Carbaldehyde

In the broad field of pyrazole, 1*H*-pyrazole-4-carbaldehyde possesses a prominent position in the intermediate category as it can be utilized for the synthesis of many biologically active heterocyclic derivatives. As the pyrazole derivatives reported in the thesis are derived from 1-aryl-5-chloro-3-methyl-1*H*-pyrazole-4-carbaldehyde**.** The synthetic and biological aspects of 1*H*-pyrazole-4-carbaldehydes are reviewed here.

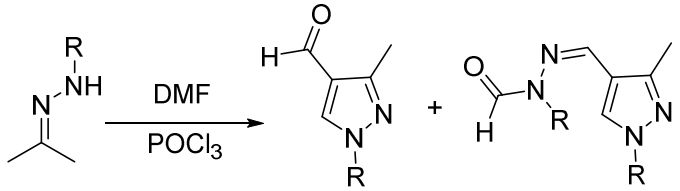
1.2.4.1 Synthesis of 1-Aryl-5-Chloro-3-Methyl-1*H*-Pyrazole-4-Carbaldehyde

The synthesis of 1-aryl-5-chloro-3-methyl-1*H*-pyrazole-4-carbaldehyde was performed by Y. Kvitko and B. A. P. Koshits[32]using Vilsmeier-Haack reaction of 3-methyl-1-phenyl-pyrazol-5(4*H*)-one (Scheme 1.3).



Scheme 1.3 Vilsmeier-Haack reaction of 3-methyl-1-phenyl-pyrazol-5(4*H*)-one.

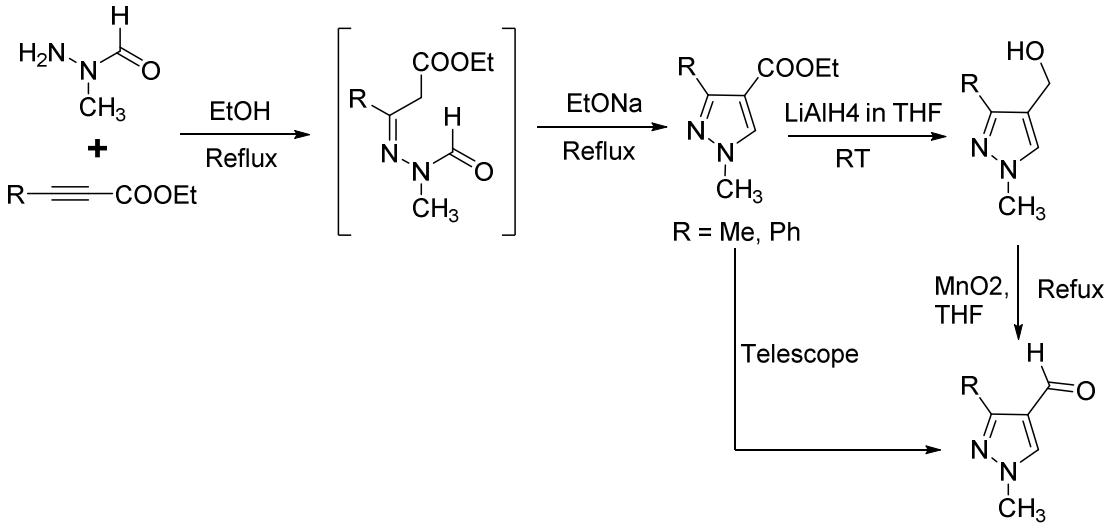
Sergey P. Ivonin*et al*.[33] reported the reactions of N-alkylhydrazones of aliphatic ketoneswith the Vilsmeier–Haack reagent resulting in the formationof 1,3,4-trisubsituted non-symmetric pyrazoles depending on thesubstitution pattern in the startingcompounds (Scheme 1.4).



R = Me, iPr, tBu

Scheme 1.4 Reaction of acetone N-alkylhydrazones with the Vilsmeier–Haack reagent.

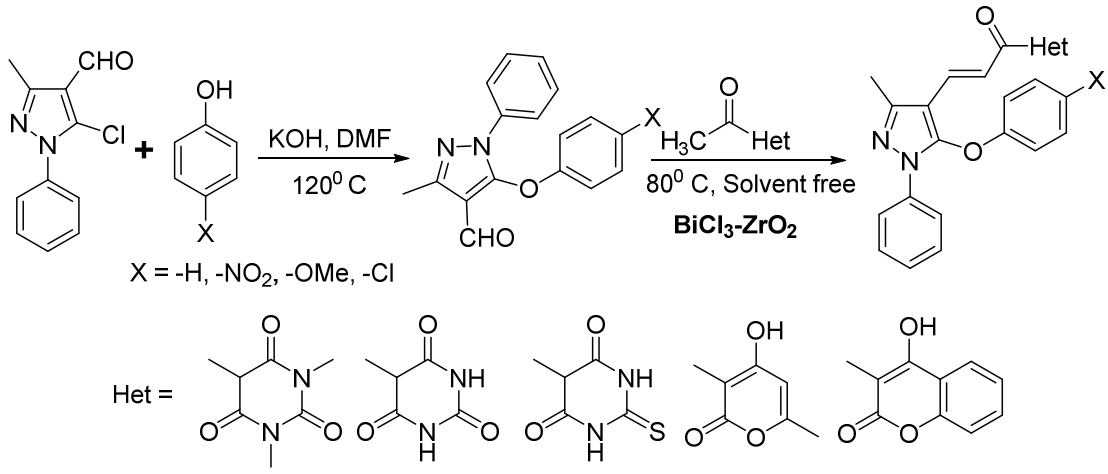
Steven A. Raw and Andrew T. Turner[34]reporteda facile and regioselective one-pot synthesis of 1,3,4-trisubstituted-1H-pyrazoles. Itcomprised a three-step telescoped sequence, which has been utilised in the production of a variety of differentiallysubstituted pyrazoles (Scheme 1.5).



Scheme 1.5 Regioselective one-pot synthesis of 1,3,4-trisubstituted-1H-pyrazoles.

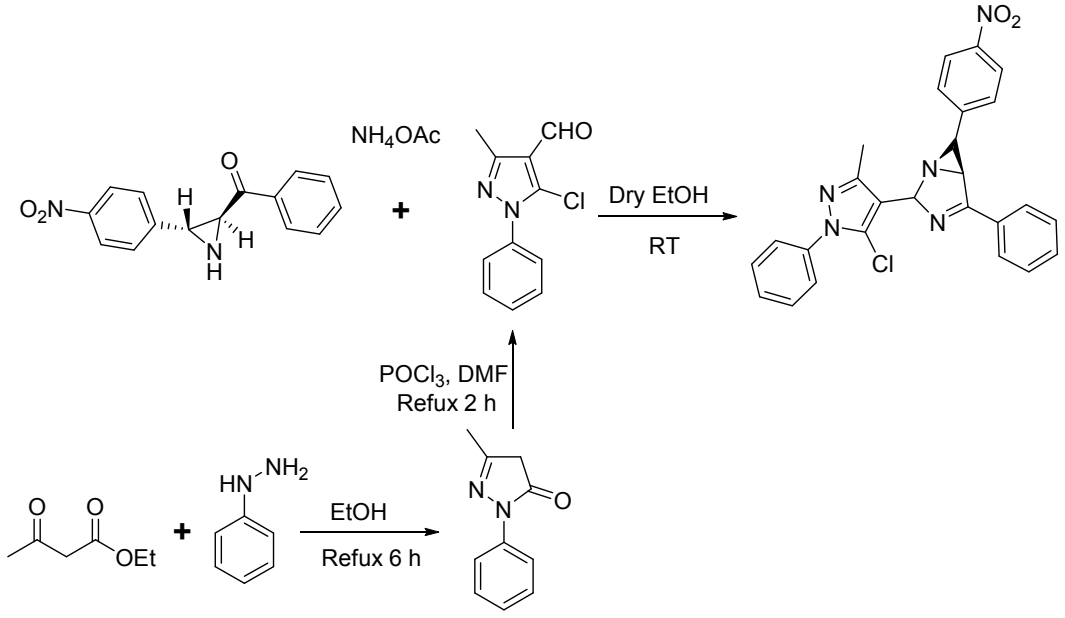
1.2.4.2 Reactions of 1-Aryl-5-Chloro-3-Methyl-1*H*-Pyrazole-4-Carbaldehyde

Zeba N. Siddiqui and Saima Tarannum[35] synthesized and characterized heterogeneousversion of $BiCl_3$ ($BiCl_3$-ZrO_2). The catalytic activity of the catalyst was explored bysynthesizing a library of novel pyrazolyl chalcones in excellent yield (Scheme 1.6).



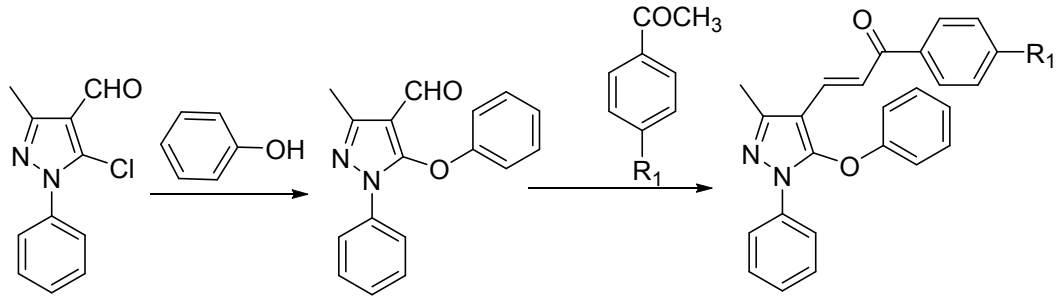
Scheme 1.6 Synthesis of novel pyrazolyl chalcones.

Fereshteh Albooye and Hamzeh Kiyani[36]claimedone-pot, three-component reaction of *trans*-2-benzoyl-3-(4-nitrophenyl)aziridine with 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde andammonium acetate, 2-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-6-(4-nitrophenyl)-4-phenyl-1,3-diazabicyclo[3.1.0]hex-3-enewas achieved in good yield (Scheme 1.7).



Scheme 1.7 Synthesis of pyrazole scaffolds.

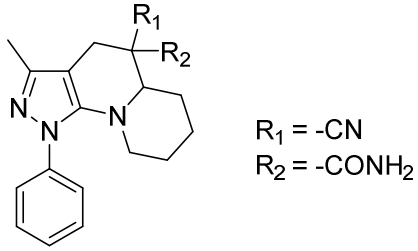
Y.-L. Zhou*et al*. [37]reported to synthesis and crystal structure of 1,3-disubstituted-2-propyleno-1-one containing pyrazole moiety (Scheme 1.8).



R_1 = -H, -Cl, -OMe, $-NO_2$

Scheme 1.8 Synthesis of 1,3-disubstituted-2-propyleno- 1-one containing pyrazole moiety.

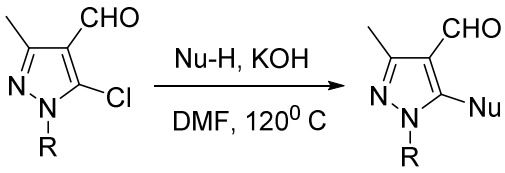
Dipak Prajapati and Kalyan Jyoti Borah[38] synthesized novel fused heterocycles based on reactions proceeding by the mechanism of the tert-amino effect, which generalizedcyclization of certain derivatives of 3-methyl-1-phenyl-2-pyrazolin-5-ones. Using this strategy,diversity of fused heterocycleswere obtained by the Knoevenagel condensation of 5-tert-amino-3-methyl-1-phenylpyrazolone-4-carboxaldehyde with activemethylene compounds such as malononitrile and cyanoacetamide followed by cyclization using anhydrous zinc chloride (Scheme 1.9).



R_1 = -CN
R_2 = $-CONH_2$

Scheme 1.9 Fused pyrazole derivatives*via* tert-amino effect.

Kee-In Leeand his co-workers[39] reported the synthesis of N-Containing heterocycles into pyrazole derivatives (Scheme 1.10).



R = Me, Ph, 2-Py, H

Nu = pyrrole, Imidazole, Pyrazole, Indole, Morpholine, Diethylamine, DMF, Phenol, Thiophenol, Pyrrolidine, piperidine, 1-Methylpiperazine

Scheme 1.10 Synthesis of N-containing heterocycles in to pyrazole scaffolds.

1.2.4.3 Biological screening of 1-Aryl-5-Chloro-3-Methyl-1H-Pyrazole-4-Carbaldehyde Derivatives:

Hai-Liang Zhuand his co-workers[40] reported new series of pyrazole-quinoline-pyridine hybrids based on molecular hybridization technique.The compounds were synthesized by base-catalyzed cyclocondensation reaction through one-pot multicomponent reaction. All compounds were investigated for *in vitro* antibacterial and anticancer activities. Enzyme inhibitory potency of all compounds were carried out against FabH and EGFR. of the compounds studied (Figure1.4).

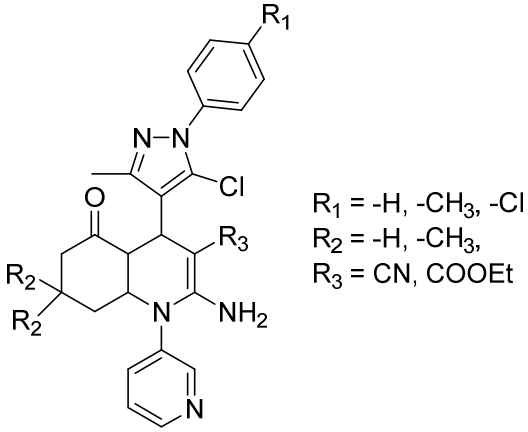


Figure 1.4 Biologically active pyrazole-quinoline-pyridine hybrids.

Ming-Xia Song *et al*. [41]synthesizedrhodanine-based 5-aryloxypyrazolesand evaluated for their antibacterialactivityagainst selected methicillin resistant and quinoline resistantStaphylococcus aureus (MRSA and QRSA) (Figure 1.5).

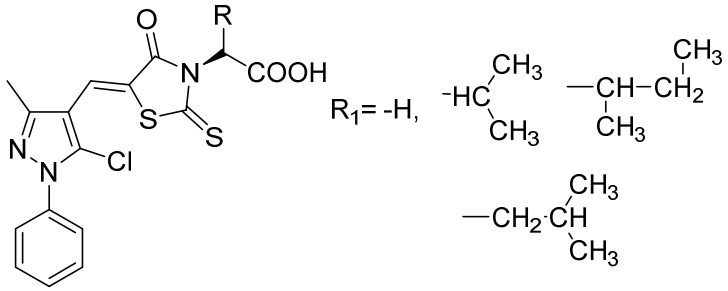


Figure1.5 Antibacterial agents from rhodanine-based 5-aryloxy pyrazole scaffolds.

Amit Trivedi *et al*. [42]described a facile one-pot synthesis of a series of eight pyrazolo[3,4-d]pyrimidinesand evaluatedfor their *in-vitro* antibacterial activity (Figure 1.6).

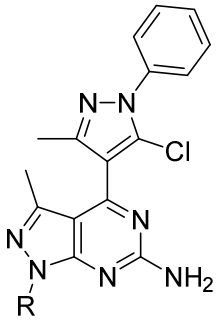


Figure 1.6 Antibacterialsfrom pyrazolo[3,4-d]pyrimidines derivatives.

New substituted5-imidazolinones, sulfonamides, azomethines and formazans derivatives of 1*H*-pyrazole-4-carbaldehydewere synthesized and evaluated for their antimicrobial activity were reported by J. M. Desai and V. M. Shah[43](Figure 1.7).

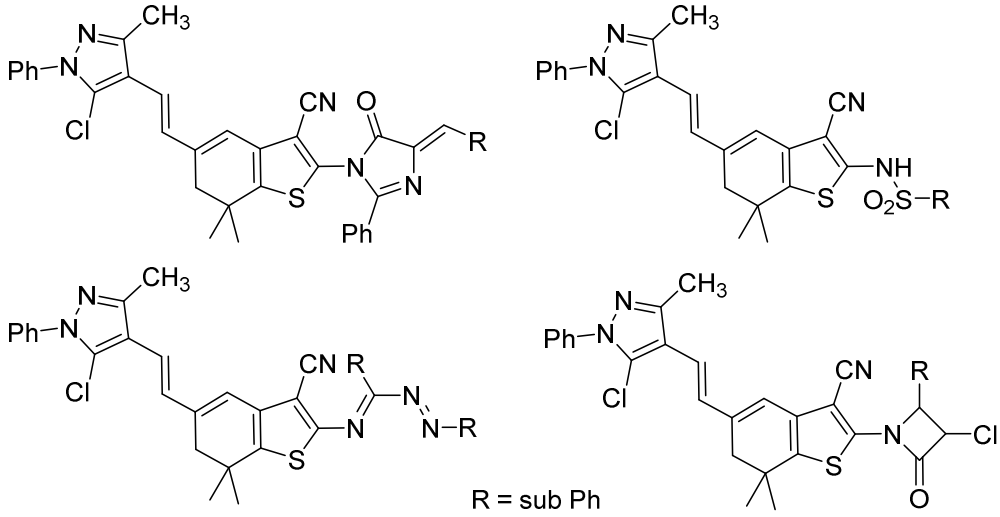


Figure 1.7 Biologically active Pyrazole derivatives.

N. K. Shah *et al*.[44]synthesized a new series of quinoline bearing pyrazole nucleus by condensing various arylidenemalanonitrile and 3-aminocyclohex-2-en-1-onesin alcohol and in the presence of catalytic amount of piperidine in one pot. All compounds were tested for in vitro antibacterial activities (Figure 1.8).

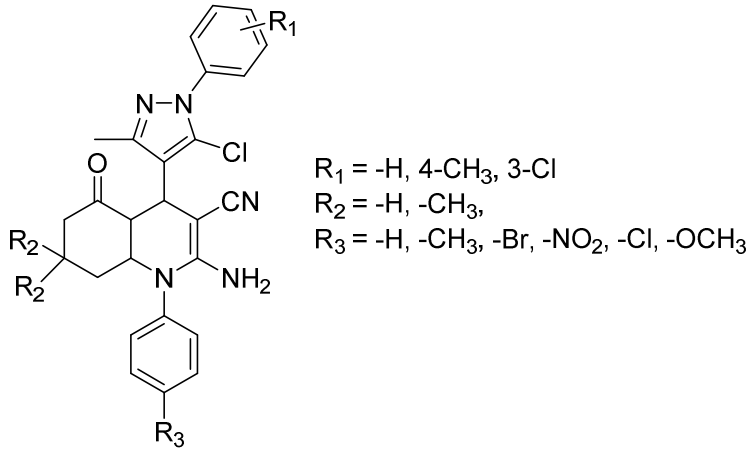


Figure 1.8 Antibacterialscontaining quinoline bearing pyrazole nucleus.

Rakesh kumar *et al*.[45]synthesized new dihydyopyridine and dihydropyrimidone derivatives and evaluated their antimicrobial activity (Figure 1.9).

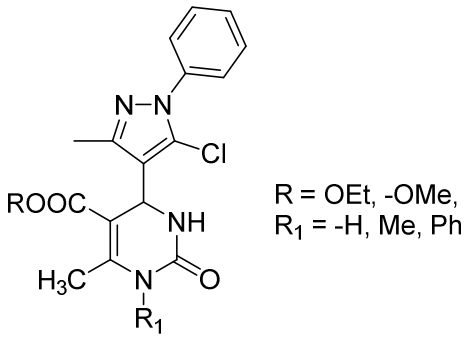


Figure 1.9 Biologically active pyrazole based dihydropyrimidine and dihydro pyrimidinone scaffolds.

1.3 Quinoline:

Quinoline (1-azanaphthalene or benzo[*b*]pyridine) 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 (Figure 1.10). Quinoline, a stable base, was first isolated in impure state from coal-tar distillate. Shortly after the isolation of quinoline from coal tar it was also recognized as a pyrolytic degradation product of cinchonamine, an alkaloid closely associated to quinine, from which the name quinoline is derived.Quinolinewas, probably contaminated by lepidine when obtained by distillation of cinchonine and quinine with caustic alkali and was named as quinoleine. This name was later changed to quinoline by Berzelius.

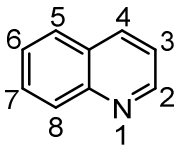


Figure 1.10 Structure of quinoline.

Quinoline can be synthesized from aniline with acrolein under heated sulfuric acid (Skraup synthesis). Numerous quinoline compounds can be prepared by Skraup synthesis using different oxidizing agents. The compounds of Quinoline family are broadly used as a parent compound to make drugs (especially anti-malarial medicines), fungicides, biocides, alkaloids, dyes, rubber chemicals, flavoring agents. They also have antiseptic, antipyretic and antiperiodic properties. They are also employed as catalyst, corrosion inhibitor, preservative and as solvent for resins and terpenes as well as in the production of paints. They are used in transition-metal complex catalyst chemistry for uniform polymerization and luminescence chemistry. They are recognized as good antifoaming agent in refinery field.

1.3.1 Quinoline containing natural products

Quinine[46] is a natural white crystalline alkaloid having antipyretic, antimalarial, analgesic and anti-inflammatory properties (Figure 1.11). In the 17th century, quinine was employed in the first effective treatment for malaria caused by *plasmodium falciparum.*

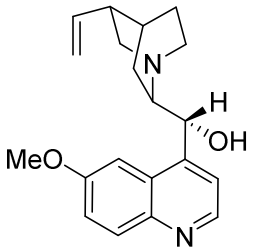
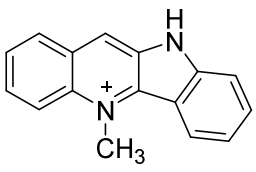
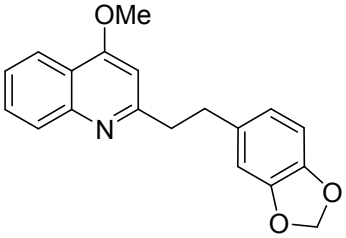


Figure 1.11 Structure of quinine.

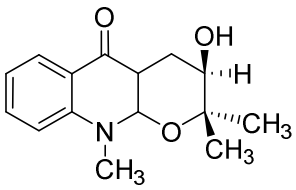
Some of the therapeutically active quinoline alkaloids are presented below.



Cryptolepine
Antimalarial[47]



Cusparine
Antileishmanial[48]



Ribalinine
Calcium channel blocker[49]

1.3.2Synthetic methods of quinoline

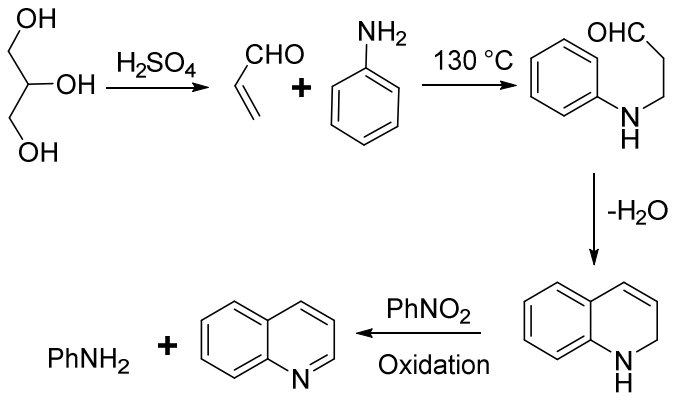
There are several methods reportedfor the synthesis of quinoline.

1. Skraup synthesis
2. Doebner-von miller synthesis
3. Beyer's modification of the dobner-von miller synthesis
4. Conrad-limpach knorr synthesis
5. Combes method
6. Friedlaender synthesis
7. Pfitzinger reaction
8. Gould-jacobs reaction
9. Doebner synthesis

First two methods are described below.

1.3.2.1. Skraup synthesis

The Skraup synthesis[50]is 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 which finally gets dehydrogenated by oxidizing agent to give the quinoline. The Skraup synthesis is the best for the ring synthesis of quinoline unsubstituted on the hetero-ring.

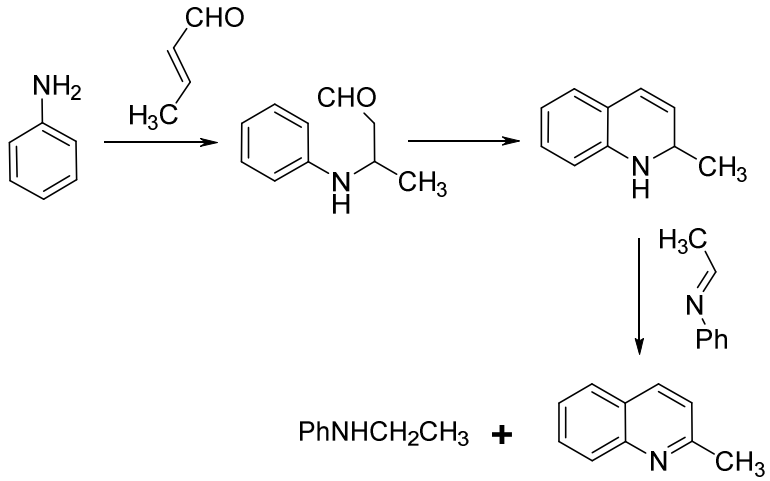


Scheme 1.11 Synthesis of quinoline by Skraup method.

1.3.2.2. Doebner-von miller synthesis for quinoline

This method is the 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[51].

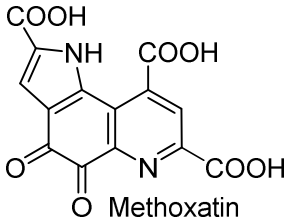
The α,β-unsaturated aldehyde, initially formed from two molecules of aldehydes by acid-catalyzed aldol condensation, reacts with aniline to give secondary amine. Its cyclization in presence of strong acid and dehydrogenation produces quinoline homologue. It is believed that the oxidative step is brought about by the action of schiff base produced *in situ* (from aniline and aldehyde).



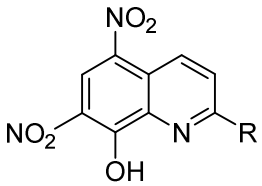
Scheme 1.12 Synthesis of quinoline by Doebner-von miller method.

1.3.3 Quinolinebearing Pharmacological Agents

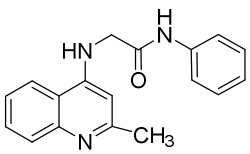
The quinoline scaffold is prevalent in a variety of pharmacologically agents. Some of the therapeutically active quinoline derivatives are listedbelow.



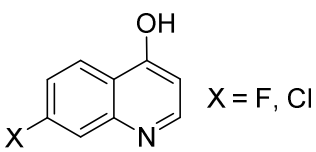
Antimicrobial[52]



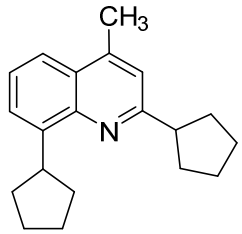
Antifungal[53]



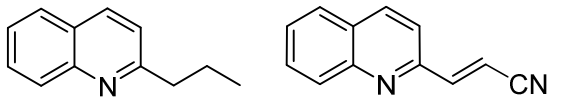
Antileishmanial[54]



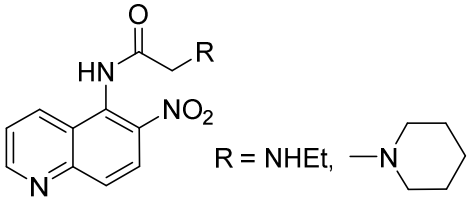
Antioxidative[55]



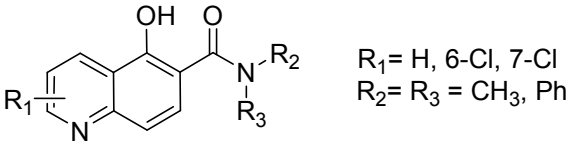
Antitubercular[56]



Antileishmanial[57]



Local anesthetic and Antiarrhythmic[58]

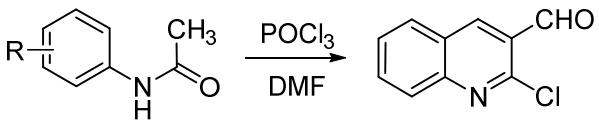


Immuno-modulator[59]

1.3.4 Synthesis of 2-chloro-3-formyl quinoline:

In the broad family of quinoline, 2-chloro-3-formyl quinoline has a prominent position in the intermediate as it can be utilized for the synthesis of many heterocyclic compounds. There has been relentless interest in the direction of 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 $POCl_3$ has involved the attention of synthetic organic chemists since its discovery in 1927[60]. It is one of the most commonly used reagents for the introduction of an aldehydic (-CHO) group into aromatic and heteroaromatic compounds. It is proved to be a mild and efficient method for the formylation[61].The utility of this reagent also explores the powerful route for the synthesis of substituted 2-chloro-3-formyl quinolines (Scheme 1.13).

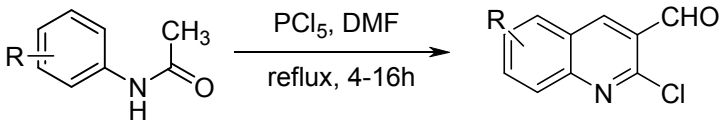
Meth-Cohn Quinoline Synthesis



Scheme 1.13 Synthesis of quinoline.

Angel H. Romero [62] reported synthesis the of 2-chloroquinoline-3-carbaldehyde was carried out by the action of Vilsmeier's reagent on acetanilides

using phosphorus pentachloride as chlorinating agent in place of phosphoryl chloride to obtain good yields for activated acetanilides (Scheme 1.14).

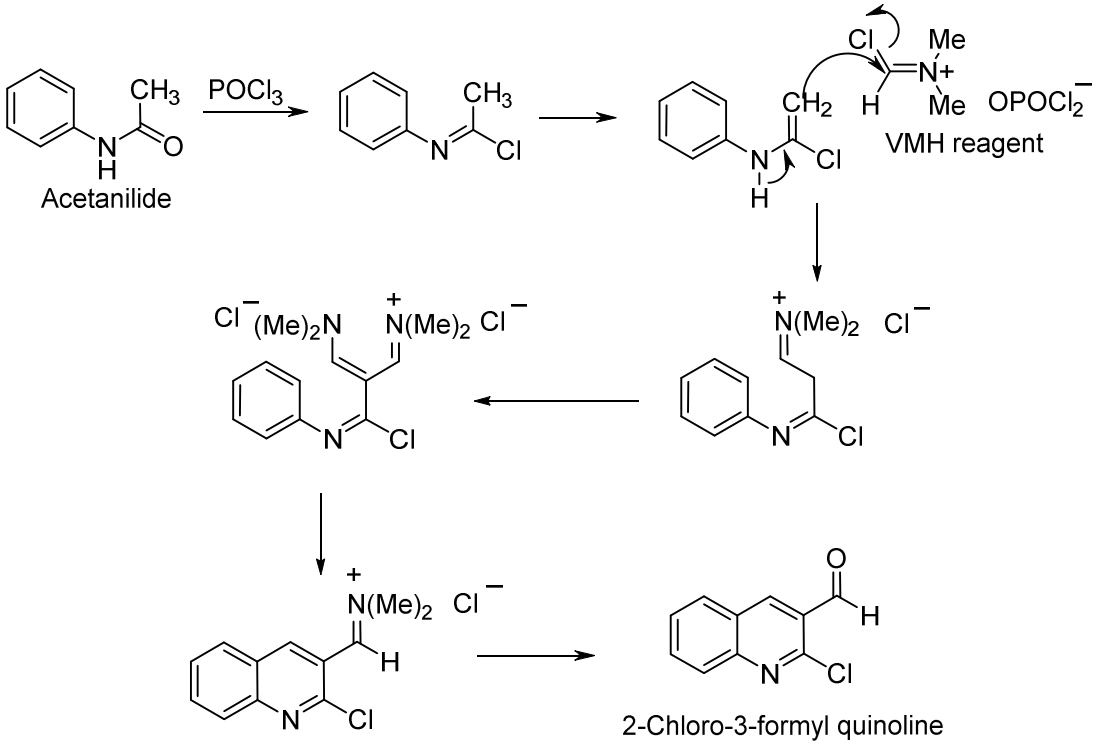


R = -H, 6-CH_3, 7-CH_3, 8-CH_3, 6-OCH_3, 7-OCH_3, 6-Br, 7-Cl, 6-NO_2

Scheme 1.14 Synthesis of 2-chloroquinoline-3-carbaldehyde derivates.

Mechanism

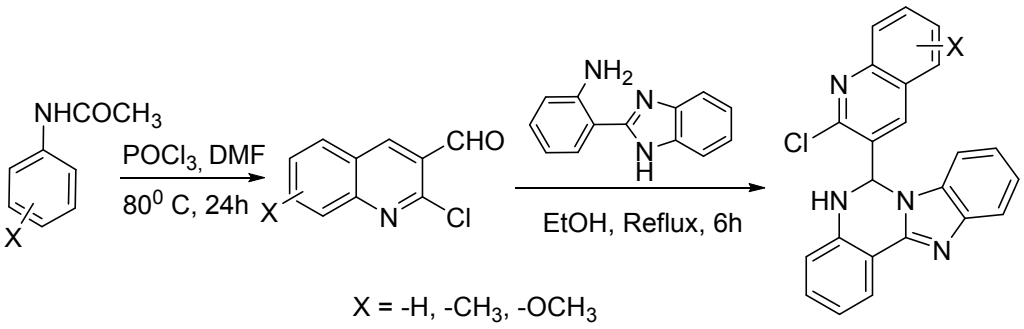
In the Meth-Cohn quinoline synthesis, acetanilide becomes a nucleophile and provides the framework of the quinoline (nitrogen and the 2,3-carbons) and the 4-carbon is derived from the Vilsmeier reagent. The reaction mechanism involves the initial conversion of an acetanilide into an α-iminochloride by the action of $POCl_3$. The *α*-chloroenamine tautomer is subsequently C-formylated by the Vilsmeier reagent derived from $POCl_3$ and DMF. In examples, where acetanilides are employed, a second C-formylation, subsequent cyclisation and aromatization by loss of dimethylamine finally affords 2-chloro-3-formyl quinoline (Scheme 1.15)[63].



Scheme 1.15 Mechanism of 2-cholro-3-formyl-quinoline.

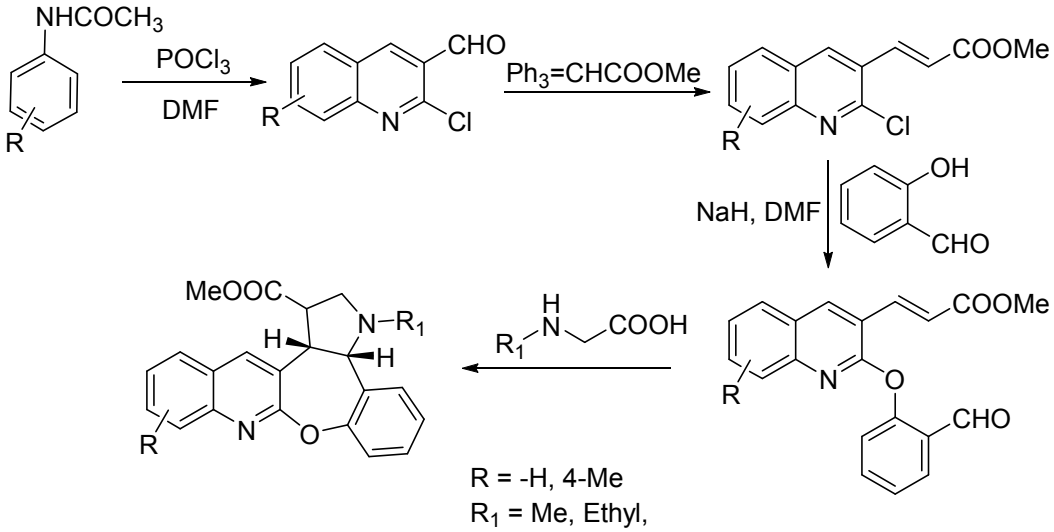
1.3.4.1Reactions of 2-chloro-3-formyl quinoline

Sujay Mukhopadhyay *et al*. [64]investigated three new probes that have the ability to detect Fe^{3+} and Hg^{2+} at ppb level in aqueous acetonitrile medium. These worked efficiently in the pH range of 4–10.5, with insignificant interference from other metal ions. The probes behave as chemosensors for Fe^{3+} and chemodosimeters for Hg^{2+}, with greater sensitivity for Hg^{2+} (Scheme1.16).



Scheme 1.16 Quinoline based prabes to detect Fe^{3+} and Hg^{2+}.

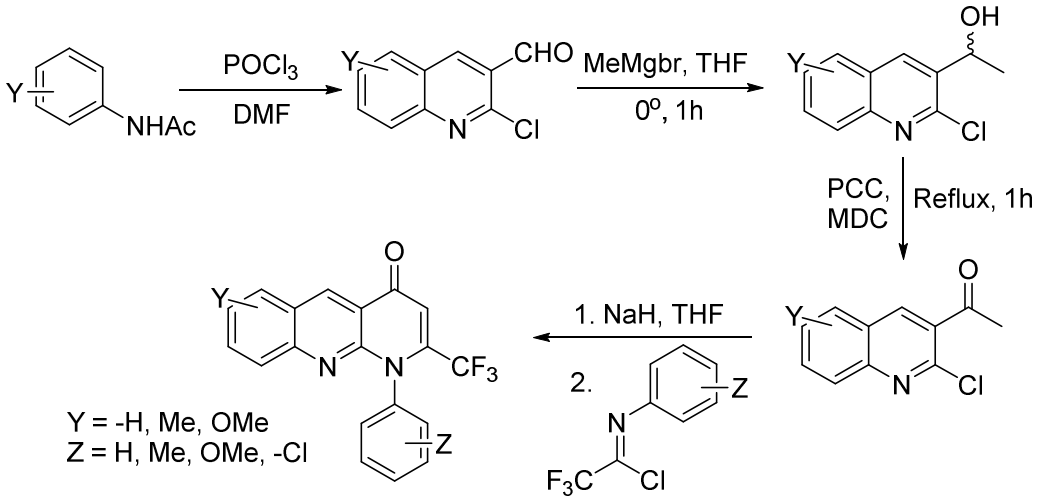
Nagarajan saravanan and his co-workers[65] reported new functionalized quinolo-oxepane achieved by intramolecular 1,3-dipolar cycloaddition reaction of α,β-unsaturated ester with unstabilized azomethine ylides derived from various α-amino acids with high stereo selectivity and good yields (Scheme 1.17).



Scheme 1.17 Synthesis of quinolo-oxepane scaffolds.

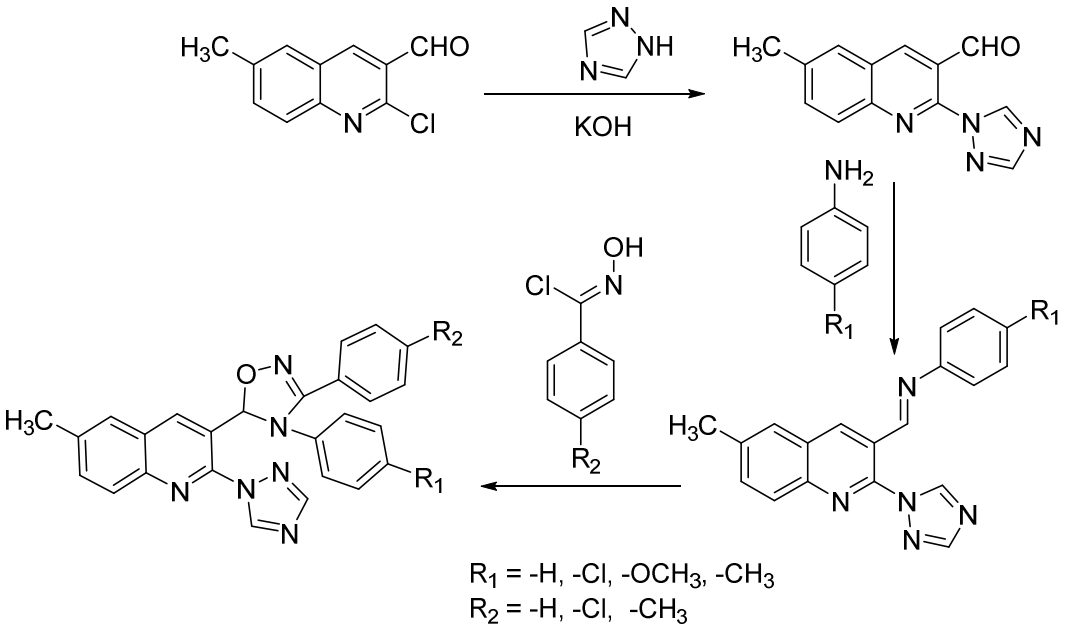
Angel H. Romero *et al*. [66]reported a versatile single step procedure for the preparation of diverse 2-trifluoromethyl-benzo[b][1,8]naphthyridin-4(1H)-ones in

moderate to good yields from readily accessible 2-chloro-3-acetylquinolines (Scheme 1.18).



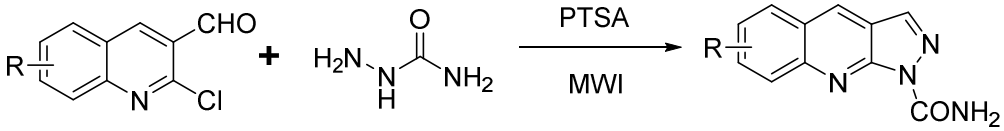
Scheme 1.18 Synthesis of 2-(trifluoromethyl)benzo[b][1,8]naphthyridin-4(1H)-one derivatives.

Fang-Ming Liu and his co-workers[67] prepared a series of novel 1,2,4-oxadiazoline derivatives containing 2-(1,2,4-triazol-1-yl)quinoline were synthesized by the reaction of imines with benzohydroximinoyl chlorides in the presence of Et_3N via 1,3- diplolar cycloaddition reaction (Scheme 1.19).



Scheme 1.19 Synthesis of novel 1,2,4-oxadiazoline derivatives containing 2-(1,2,4-triazol-1-yl)quinoline.

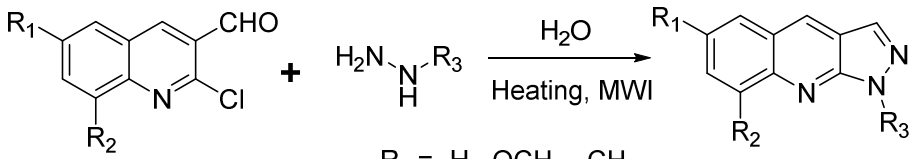
Vetrivel Nadaraj and Senniappan Thamarai Selvi [68]developed a series of quinolines bearing pyrazole nucleus in one pot by condensing various quinolines and semicarbazide in presence of catalytic amount of PTSA (Scheme 1.20).



R_1 = -H, -Cl, $-OCH_3$, $-CH_3$, -Br

Scheme 1.20 Synthesis of quinolines bearing pyrazole nucleus.

Ramrao A. Mane and his co-workers[69]reported one-pot water-mediated synthetic route to prepare pyrazolo[3,4-b]quinolinesby carrying condensation of 2-chloro-3-formyl quinolines and hydrazine hydrate/phenyl hydrazine using thermal/microwave energy sources (Scheme 1.21)

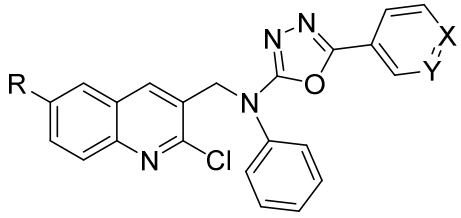


R_1 = -H, $-OCH_3$, $-CH_3$,
R_2 = -H, Et
R_3 = H, Ph

Scheme 1.21 Synthesis of pyrazolo[3,4-b]quinolines.

1.3.4.2 Biological screening of 2-chloro-3-formyl quinoline Derivatives:

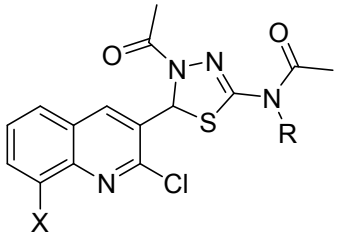
Gaurav G. Ladani and Manish P. Patel[70]reported aseries of quinoline based 1,3,4-oxadiazole derivatives and evaluated their antimicrobial, antitubercular,antimalarial and cytotoxic activities (Figure 1.12)



R = -H, $-CH_3$, $-OCH_3$, -Cl
X = N, CH
Y = CH, N

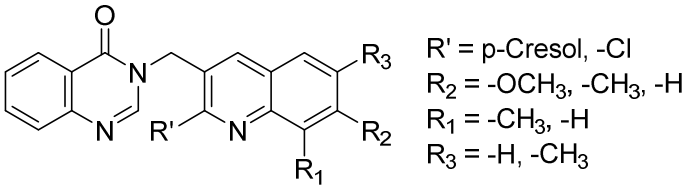
Figure 1.12 Quinoline nucleus incorporated 1,3,4-oxadiazole derivatives.

Abdul R. Bhat*et al*. [71]reporteda new series of thiadiazoles, with an aim to explore their effect on *in vitro* growth of microorganisms causingmicrobial infection (Figure 1.13).



X = -H, -Cl, $-CH_3$
R = Differents aromatic and non aromatic substituents
Figure 1.13 Quinoline basedthiadiazole derivatives

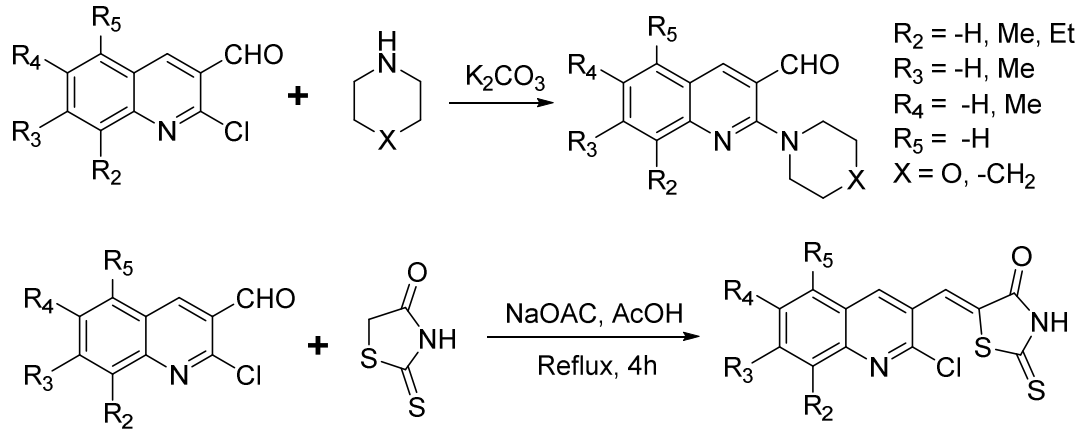
Charansingh H. Gill and his co-workers[72]synthesized new quinazolinone derivatives and evaluated *in vitro* antimicrobial activities of the synthesized compounds (Figure 1.15)



R' = p-Cresol, -Cl
R_2 = $-OCH_3$, $-CH_3$, -H
R_1 = $-CH_3$, -H
R_3 = -H, $-CH_3$

Figure3-((2-chloroquinolin-3-yl)methyl)quinazolin-4(3H)-ones.

Vadla Ramesh*et al*.[73]synthesized rhodanine analoguesbearing 2-chloroquinoline and benzo[h]quinoline scaffolds and evaluated their potential as anticanceragents (Figure1.14).



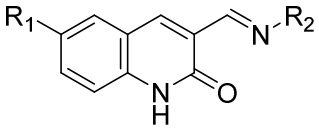
R_2 = -H, Me, Et
R_3 = -H, Me
R_4 = -H, Me
R_5 = -H
X = O, $-CH_2$

R_2 = -H, Me, OMe, Ph, Et
R_3 = -H, Me, OMe, $-O-CH_2-O-$, Ph
R_4 = -H, Me,OMe,$-O-CH_2-O-$
R_5 = -H, Me,OMe,

Figure 1.14 Synthesis of rhodanine analogues bearing 2-chloroquinoline scaffolds.

Ye Zhang*et al*. [74]reported a series of 2-oxo-quinoline-3-carbaldehyde Schiff-base derivatives based on 2-oxo-quinoline structure.*In vitro* antioxidant activitiesof

these compounds were assessed and compared with commercial antioxidants ascorbic acid, BHTand BHA, employing $DPPH^{\cdot}$ assay, $ABTS^{+\cdot}$ assay, $O2^{-\cdot}$ assay and $OH^{\cdot}$assay (Figure 1.16).



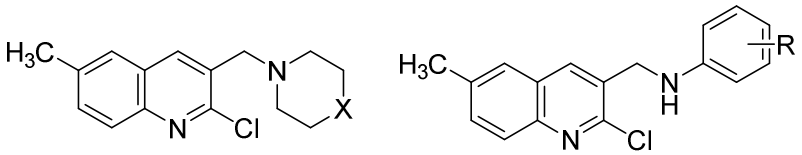
R_1 = -H, $-CH_3$,

R' = p-Cresol, -Cl
R_2 = $-OCH_3$, $-CH_3$, -H
R_1 = $-CH_3$, -H
R_3 = -H, $-CH_3$

R_2 = -OH, C_4H_9, CH_2Ph, CH_2CH_2OH, o-OH-C_6H_4, p-OH-C_6H_4, C_6H_5NH, p-NO_2-C_6H_4NH, NH_2, p-COOH-C_6H_4, 4-Pyridine-scetimido, Amino-sulfoacetimido

Figure 1.162-oxo-quinoline-3-carbaldehyde Schiff-base derivatives.

Suresh Kumar*et al*. [75]prepared a number of secondary and tertiary amines bearing 2-chloro-6-methylquinoline bynucleophilic substitution reaction of 3-(chloromethyl)-2-chloro-6-methylquinoline with substitutedaromatic primary and secondary amines in presence of catalytic amount of triethylamine (TEA) andK_2CO_3.The *invitro*antimicrobial activity of theseries was also studied(Figure 1.17).



X = -H, $-CH_2$, O, N-CH_3 R = -H, $-CH_3$, $-OCH_3$, -F, -Cl, -Be, $-NO_2$

Figure 1.17 Secondary and tertiary amines containing 2-chloro-6-methylquinoline moiety.

1.4 Antimicrobial study

The illnesses originated 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 amplified by at least 10 years since the discovery of antimicrobial agents for the treatment of microbial infections. Considerable attention has been focused on developing more potent and effective anti-microbial agents.

1.4.1.Pathogens

An infectious agent causing disease in a host is a pathogen. There are several pathways whereby pathogens can attack a host.Soil contamination is having the most constant potential for docking a pathogen.An infection is a mini battle between pathogen and host. The resulting infection has three possible outcomes: the host wins and the pathogens are removed; the pathogen wins and kills thehost; or host and pathogen live together whereequilibrium is reached with minimum damage.

1.4.1.1 Bacterial Pathogens

Pathogenic bacteria can cause diseases in humans, animals and in plants. Some bacteria can only make one particular host ill.Depending on the host specificity of bacteria, some can cause trouble in a number of hosts. Bacteria can cause infectious diseases such as pneumonia, food borne illnesses, tetanus, typhoid, diphtheria, syphilis, leprosy and certain forms of cancer. Bacterial replication occurs rapidly by cells growth and division. Many types of biomolecules are to be synthesized or taken up by organismsto grow and divide.

Small microscopic organism with relatively simple and primitive form of the cellular organization known as “prokaryotic” was described as “bacterium” by C. E.Chrenberg. Christian Gram discovered a stain known as Gram stain dividing all bacteria into two classes “Gram positive” and “Gram negative”. The Gram-positive bacteria resist decolouration with acetone, alcohol and remain stained as dark blue with methyl violet, while Gram-negative bacteria are decolorized. We have used following listed bacterial pathogens for antibacterial study of synthesized title derivatives.

Gram positive bacterial pathogens

(i) *Bacillus subtilis*(MTCC 441)[76]

Christian Gottfried Ehrenberg in 1835 named this organism as *Vibrio subtilis*which was renamed as*Bacillus subtilis* in 1872 by Ferdinand Cohn. Other names for these bacteria include *Bacillus uniflagellatus*, *Bacillus globigii*, *Bacillus natto, Hay bacillus andGrass bacillus*. These bacteria are a good model for cellular development and differentiation.

They are strictly aerobic,rod-shaped and Gram-positive bacteria. They are naturally found in soil and vegetation andgrow in the mesospheric temperature range. *Bacillus subtilis* can survive under harsh conditions. One of the survival strategies is the formation of stress resistant endospores. Another strategy is the uptake of external DNA, which allows the bacteria to adapt by recombination. However, these strategies are time-consuming. *Bacillus subtilis* can also gain protection more quickly against many stress situations such as acidic, alkaline, osmotic, oxidative conditions, heat or ethanol. Recent studies show that they can grow in anaerobic conditions making them facultative aerobes.

(ii) *Clostridium tetani*(MTCC 449)[77]

Arthur Nicolaier isolated the strychnine-like toxin of tetanus from free-living, anaerobic soil bacteria *Clostridium tetani*. It is the causative agent of tetanus, a disease categorized by painful muscular spasms that can lead to respiratory failure and 40% of death.The organism is rod-shaped bacterium; found in soil, in the intestinal tracts and feces of various animals. In its vegetative state, *C. tetani* is heat sensitive and cannot survive in the presence of oxygen. However its spores are resistant to heat and some antiseptics, but oxygen rich areas are also toxic to them. The spores can germinate through the dead cells of the body, thus spreading toxins.When in soil, they can last for even years in the proper conditions. *C. tetani* usually enters a host through a wound to the skin, then it replicates. Once an infection is established, *C. tetani* produces potent biological toxins, tetanolysin and tetanospasmin. These toxins are the cause of tetanus.

(iii) *Streptococcus pneumoniae*(MTCC 1936)[78]

In 1881 Louis Pasteur isolated *Streptococcus pneumonia*. The organism was termed *Diplococcus pneumoniae* from 1920 because of its characteristic appearance in Gram-stained sputum. It was renamed *Streptococcus pneumoniae* in 1974 because it was very similar to streptococci. It is the causative agent of pneumonia. *Streptococcus pneumoniae* cells are Gram-positive, lancet-shaped extended cocci with a slightly pointed outer curvature. Usually, they are seen as pairs of cocci, but they may also occur singly and in short chains. When cultured on blood agar, they are alpha hemolytic. They do not form spores, and they are non motile. Like other

streptococci, they lack catalase and ferment glucose to lactic acid.They are normally found in the upper respiratory tract, including the throat and nasal passages. It also causes osteomyelitis, septic arthritis, endocarditic, peritonitis, cellulites and brain abscesses. *Streptococcus pneumoniae* is currently the leading cause of invasive bacterial disease.

Gram negative bacterial pathogens

(i) *Escherichia coli*(MTCC 443)[79]

E. coli was first discovered in 1885 by Theodor Escherich.*E. coli* has been commonly used for biological lab experiment and research. *E. coli* is a facultative gram-negative rod shaped bacteria.It can be commonly found in animal feces, lower intestines of mammalsand on the edge of hot springs. They grow best at 37°C. It is easy to eradicate them by simple boiling or basic sterilization as they cannot sporulate. They are normally present in the intestine without causing problems.A few types cause illness after consuming contaminated food or water.Illness is caused after ingestion of a sufficient number of *E.coli.*It gets attached to the inner surface of the large intestine to cause inflammation of the intestinal wall.It causes infantile diarrhoea, gastroenteritis, traveler's diarrhoea, bacillary dysentery, hemorrhagic colitis, hemolytic uremic syndrome and thrombocytopenic purpura. *E. coli* has long laboratory history and plays an important role in current biological engineering. Most results from *E. coli* research can be applied to humans. It has been widely used to synthesize DNA and proteins. Since *E. coli* can produce human enzymes through recombinant DNA techniques, it is widely used as a very good tool to produce useful compounds or enzymes for medication.The most useful contribution of recombinant DNA from *E. coli* is to use the manipulation of *E. coli* to produce human insulin for diabetes patients.

(ii) *Vibrio cholerae*(MTCC 3906)[80]

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. *Vibrio cholerae* is a "comma" shaped Gram-negative bacteria with a single, polar flagellum for movement. There are numerous strains of *V. cholerae,* some of

which are pathogenic. It enters the human body through ingestion of contaminated food. The bacteria enter the intestine; imbed in the vile of absorptive intestinal cells and releases cholera toxin causing characteristic diarrhoea of the disease cholera. 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. The nontoxic B-units of cholera toxin are used in cellular and molecular biology. The use of nontoxic *V. cholerae* products can be useful in the operating room when small nerves need to be visualized.They can be used to detect neuronswhen attached to a fluorescent protein.

(iii) ***Salmonella typhi***(MTCC 98)[81]

In 1880s, the typhoid bacillus was first observed by C. J. Eberth; Robert Koch confirmed a related finding by Gaffky and succeeded in cultivating the bacterium in 1881. *Salmonella typhi* is a gram negative bacterium that causes systemic infections and typhoid fever in humans. Infection of *S. typhi* leads to the development of enteric fever. This disease is characterized by the sudden onset of a sustained and systemic fever, severe headache, nausea, and loss of appetite. Other symptoms include constipation or diarrhoea, enlargement of the spleen, possible development of meningitis and general malaise. This rod-shaped food born pathogen can grow under aerobic as well as anaerobic conditions. The temperature between 35 - 37°C and *p*H range of 3.8 to 9.5 suit best for its growth.It is killed by heating at 70°C for 1 min or less.*S. typhi* has caused many deaths in developing countries where sanitation is poor and is spread through pollution of water and undercooked food. Eradication seems highly unlikely due to recent emergence of multi drug resistance strains.

1.4.1.2Fungal Pathogens

Fungi differ from both plants and animals.The genetic makeup of fungi is similar to animal cells owing major distinction being a rigid cell wall. The incidence fungal infections are increasing due to increase in the cases of immune suppressed individuals. The most common species of human fungal pathogens are *Candida albicans* and *Aspergillus fumigatus*. They have the mortality rate of 20-40% and 50-90% respectively.

Fungi grow as pathogen on the skin of animals. It causes ringworm, athlete's foot and other more serious diseases in humans. Fungal diseases are very difficult to treat because fungi is more chemically and genetically similar to animals.Fungi and their hosts have eukaryotic cells hence antibiotics cannot be used to treat fungal infections. We have used following listed fungal pathogens to study the synthesized moietiesfor antifungal activity.

(i) *Candida albicans*(MTCC 227)[82]

Candida albicans is a diploidfungus. It grows as yeast and filamentous cells. It causesopportunisticoral and genital infections in humans. This fungus is found among mouth, digestive tract and vagina of completely healthy woman.It may cause severe infections of the skin, nails, mouth, bronchial tubes and lungsunder unknown circumstances. Predisposition is also responsible tocause infection. *C. albicans* lives in 80% of the human population without causing harmful effects, however overgrowth of the fungus results in candidiasis.

(ii) *Aspergillus fumigatus* (MTCC 3008)[83]

It is actually composed of ten species. These species are generally found in decaying vegetationadapted to grow at high temperatures 50-55ºC. *Aspergillus fumigatus* sometimes parasitizes animals. The fungus can attack the embryos of eggs. It also invades the uterus of pregnant cattle and grows through the placenta into the fetus. The fetus is then aborted. It has been observed that 64% of bovine abortions were due to infection of *A. fumigatus*. In people, the fungus can lead to a contagious chronic lung infection.

1.4.2 Antimicrobial agents

Penicillin, the first bacteriocidal substance,wasdiscovered in 1929 by Fleming. In 1935 Domagk discovered the sulfonamides with broad antimicrobial activity. He was also awarded the Nobel Prize for the first synthetic antibacterial compound “prontosil” in 1939.

Antimicrobial agents are either bactericidalor bacteriostatic. They kill thetarget bacterium or inhibitits growth. Bacteriostatic agents allow the normal defense of the host to destroy microorganisms. Antimicrobial agents are also classified as antibacterial, antiviral, antifungal, antiprotozoal and anthelmintic drugs

on the basis of type of organism against which they are active. Various antimicrobial agents can be combinedfor widening the activity spectrums. World Health Organization (WHO) has also approved this type of combinations. This is termed as "synergism". Combination therapy is considered as latest therapy for antimicrobials. Some bacteriostatic agents on novel combination may exhibit bactericidal activity. The combination of Sulphamethoxazole and Trimethoprime is used as a bactericidal combination. Refampin and Dapsone are used in leprosy. Refampin and Isoniazide are used to treat tuberculosis.

Characteristics of antimicrobial agent

- It should have a broad spectrum of potency with the ability to destroy or inhibit pathogenic organisms.
- It should not damage the normal flora of the host.
- It must have solubility in body fluids.
- It should be cheap and easy to produce.
- It should not produce undesirable side effects.
- It should notbe allergenic and toxic to the host.
- It should be able to target the human body where the infection has occurred.
- It should have a long shelf-life.

1.4.3 Antimicrobial Susceptibility Testing

National Committee for Clinical Laboratory Standards (NCCLS) is an international, interdisciplinary, non-profit, non-governmental organization.It is composed of medical professionals, government, industry, healthcare providers, educators etc. It is approved by FDA-USA and recommended by WHO.It deals with following matters in maintaining clinical laboratory standards.

- accurate antimicrobial susceptibility testing (AST)
- appropriate reporting by developing standard reference methods
- quality control parameters for standard test methods
- testing and reporting clinically relevant and cost-effective strategies

Based on study results NCCLS interpretative criteria are revised frequently.

The antimicrobial susceptibility testing is aimed to predict the *in vivo* success of antibiotic therapy. *in vitro*tests are performed to measure the growth response of an isolated organism to various drugs. These tests are performed under standardized

conditions to make them reproducible. The raw data is presented either in the form of zone size or Minimum Inhibitory Concentration (MIC). The evaluation is done by following methods:

Diffusion	Dilution	Diffusion & Dilution
Stokes method Kirby-Bauer method Or Disk diffusion method	Minimum Inhibitory Concentration i) Broth Dilution Method ii)Agar Dilution Method	E-Test method

We have used the **Broth Dilution Method** in the current studyfor antimicrobial study as recommended by NCCLS.[84]

1.4.3.1 Broth Dilution Method

The minimal concentration of antimicrobial to inhibit or kill the microorganism is determined by dilution susceptibility testing methods.The lowest concentration of the assayed antimicrobial agent which can inhibit the visible growth of the pathogen being investigated under defined test conditions is called as minimal inhibitory concentration (MIC). MIC values are used to evaluate the activity of new antimicrobial agents.

Broth Dilution Method yields 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 which is followed in the present work to carry out activity evaluation.

Procedure for 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 $CFUmL^{-1}$ (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
- Sabouraud Dextrose Broth was used for fungal nutrition.
- Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin were used as standard antibacterial drugs.
- Griseofulvin and Nystatin was used as standard antifungal drugs.
- DMSO was used as diluents / vehicle to get desired concentration of preparedmotifs 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 was 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 then put for incubation at 37°C for 24 h for bacteria and 48 h for fungi. The highest dilution (lowest concentration) showing at least 99 % inhibition or preventing appearance of turbidity was considered as Minimal Inhibitory Concentration (μgmL^{-1}) i.e. the amount of growth from the control tube before incubation (which represents the original inoculum) 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}).

1.4.3.2 Factors Influencing Antimicrobial Susceptibility Testing

Theantimicrobial susceptibility testing is greatly influenced by following parameters. Choice of media, size of inoculums, effects of variation in divalent cations, moisture, pH and type of strain are important in performing the tests.

- **Choice of media:** Satisfactory media will provide clear and distinct zones of inhibition. Consistent and reproducible results are obtained in media prepared especially for sensitivity testing.
- **Size of inoculums:** The ideal inoculum 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.
- **Effects of Variation in Divalent Cations:** Excessive cation concentration will reduce zone size, whereas low cation content may result in unacceptably large zones of inhibition.Variations in divalent cations also affect results.
- **Moisture:**when plates are inoculated, droplets of moisture should not be apparent on surface of medium or on petri dish covers.The surface should be moist.
- ***p*H:** The medium should have a *p*H between 7.2 and 7.4 at room temperature after gelling.Aminoglycosides, quinolones and macrolides lose potency at low pH while tetracyclines have excessive activity. If the *p*H is too high, the opposite effects can be expected.
- **Type of strains:** Only aerobic or facultative bacteria can grow well on unsupplemented media. Certain fastidious bacteria do not grow sufficiently on unsupplemented media. These organisms require supplements to grow and they should be tested on suitable media.

1.4.3.3 Conditions required for the Antimicrobial Susceptibility Testing

- There should be intimate contact between the test organisms and the substance to be evaluated.
- For the growth of microorganisms,required conditions should be provided.
- Conditions should be maintainedsame throughout the study.
- Sterile environment should be maintained.

1.4.4 Antimycobacterial Study

Tuberculosis is the global epidemic caused by *Mycobacterium tuberculosis*. It is infectious disease which has affected about one-third of the world population. The global burden of TB remains enormous. In 2011, almost 60,000cases were identified with MDR-TB. India and China together account for almost 40% of the world's TB cases. The current therapy involving first-line and second-line drugs requires 6 months of treatment for TB and 20 months in the case of MDR-TB. HIV positive patients are more susceptible to MTB with a 50-fold risk. TB is currently causing 13% of the deaths due to HIV infection. Hence, new drugs are needed to shorten and simplify the treatment, to improve the efficacy and tolerability of treatment for MDR-TB.

1.4.4.1 Mycobacterium Tuberculosis

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. MTB is the etiologic agent of tuberculosis in humans. Humans are the only reservoir for the bacterium. The bacterium is a facultative intracellular parasite, usually of macrophages. Its virulence is due to slow generation timeof 15-20 hours.

MTB requires oxygen to grow.It is neither Gram positive nor Gram negative; Ziehl-Neelsen staining is used as it does not retain any bacteriological stain due to high lipid content in its wall. When this method is used, the MTB, acid-fast bacilli appear pink in a contrasting background.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.

1.4.4.2 Antimycobacterial Susceptibility Testing

EVALUTION TECHNIQUES:-

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 byRattan[85] to evaluate the anti-tuberculosis activity. It is one of the non automated *in vitro* bacterial susceptibility tests. It is carried out in bottle. This method offers quantitative result for the amount of antimicrobial agents required to inhibit growth of specific microorganisms.

(A)Theminimal inhibition concentration by Lowenstein-Jensen 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 can be carried out with a single drug containing slope although it is preferable to use more than one slope.

Procedure for antimycobacterial study

Primary and secondary screenings were performed as follows. Stock solution was prepared for each synthesized moietyto obtain2000 μgmL^{-1} concentration.

- **Primary screening:** The stock solution was diluted toobtain 6.25 μgmL^{-1}concentrations of the synthesized drugs. The synthesized drugs found active in this primary screening were further evaluated in a second set of dilution against all microorganisms.
- **Secondary screening:** The drugs found active in primary screening were similarly diluted to obtain 500 μgmL^{-1}, 250 μgmL^{-1}, 200μgmL^{-1}, 125μgmL^{-1}, 100 μgmL^{-1}, 50 μgmL^{-1}, 25 μgmL^{-1}, 12.5 μgmL^{-1}, 6.25 μgmL^{-1}, 3.125 μgmL^{-1} and 1.5625 μgmL^{-1} 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 inspissations 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 (5×10^4 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 testedwith known drug Isoniazid and Rifampicin. The screening results are summarized as % inhibition relative to standard drug Isoniazid 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.

1.4.5 Antimalarial Study

Malaria is caused by an infection with protozoa of the genus *Plasmodium*. According to WHO, Each year about 500 million new cases are diagnosed and approximately 1.5 million people die of the disease. The re-emerging of malaria is due to the rapid increase of resistance to most of the available anti-malarial drugs as well as resistance of vectors to insecticides. The difficulty of creating efficient vaccines and also adverse side-effects of the existing anti-malarial drugs encompasses the urgent need for novel, well-tolerated anti-malarial drugs for the treatment of malaria.

1.4.5.1 Antimalarial Susceptibility Testing

The assay development is particularly complicated for *Plasmodium* species because of complex life cycle of the parasite, the requirement for human blood and plasma for media. Some widely used assays are listed below:

(A) Minimum inhibitory concentration (MIC) method

(B) Enzyme-linked immunosorbent assays

(C) Fluorescence-based assays

(D) Molecular methods

We have used the **minimum inhibitory concentration (MIC) method** described by Rieckmann *et al*[86]to evaluate the antimalarial activity in current study. It is one of the non-automated *in vitro* parasite susceptibility tests. Quantitative results are obtained by this method for the amount of antimalarial agents that is required to inhibit growth of *P. falciparum*. It is carried out in 96 well micro titer plates.

- **Microassay protocol of Rieckmann**

P. falciparum strain was acquired from Shree R. B Shah Mahavir Super-speciality hospital, Surat, Gujarat, India, and was used in the *in vitro* tests. Parasites were maintained in continuous culture in A+ human erythrocytes, using RPMI medium supplemented with 10% human serum, as described by Trager and Jensen[87]. The anti-parasitic effect of the compounds was measured by growth inhibition percentage as described by Carvalho and Krettli[88]. Protozoa introphozoite stages in sorbitol synchronized blood[89] were cultured at 1-2% parasitaemia and 2.5% haematocrit and then incubated with the isolated compounds (maximum 1 mg/mL in serial dilutions), diluted with 0.02% final concentration of DMSO in culture medium (RPMI 1640) for a total of 48 h at 37°C. A positive control with reference antimalarial drugs (chloroquine and quinine) in standard concentrations was used in each experiment. The stock solutions were further diluted in complete medium (RPMI 1640 plus 10% human serum) to each of the used concentrations (0.0001 up to 100 mg/mL in seven dilutions). The half-maximal inhibitory (IC_{50}) responses as compared to the drug-free controls were

estimated by interpolation using Microcal Origin software. Each duplicate experiment was repeated three times and blood smears were read blind.

1.5Cytotoxicity

MATERIALS AND METHODS

Schizosaccharomyces pombe

S. pombe Var. Paul Linder 3360 was obtained from IMTECH, Chandigarh. It was maintained on yeast extract:glucose medium having composition 30:5 gml^{-1}.[90]

Bioassay

In vitro bioassay was performed using *S. pombe* cells. *S. pombe* has become an important tool to study cell biology due to its eukaryotic and fairly big size characteristics. *S. pombe* cells were grown in 50 ml liquid yeast extract media in 150 ml Erlenmeyer flask. Flask was incubated at 30°C on shaker at 150 rpm till the exponential growth of *S. pombe* was obtained (24 to 30 hrs). Then the cell culture was treated with the different concentrations (2.5, 5, 7.5, 10, 12.5 µg/ml) and with Dimethylsulphoxide (DMSO) as the control. It was further allowed to grow for 16-18 hrs. Next day, by centrifugation at 10,000 rpm for 10 min; treated cells were collected and dissolved in 500 µl of buffer solution (PBS). 80 µl of yeast culture dissolved in PBS and 20 µl of 0.4 % trypan blue prepared in PBS were mixed and cells were observed in a compound microscope (40X). Live cells resisted the entry of dye whereas dead cells appeared blue. The number of dead cells and number of live cells were counted in one field. Cell counting was repeated in two more of the microscopic fields and percentage of cells died due to synthesized compounds was averaged out.[91]

1.6 Outline of the present study

The present work involves synthesis of fluoro substituted pyrazoles containing polyhydroquinoline, pyrazoline and, 1,3,4-oxadiazole scaffolds as well as 2-morpholinoquinoline based pyrazoline and 1,2,4-oxadiazole scaffolds. 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 analysis as well as some selected compounds were also characterized by mass spectra. All the synthesized compounds enclosed in Chapter 2 to 6 were subjected to *in vitro*

antimicrobial activity against representative panel of human pathogens: Gram positive bacteria, Gram negative bacteria and fungi.

The antitubercular activity was evaluated for five series of compounds (Chapter2to 5). The cytotoxicity was also tested using a bioassay of *Schizosaccharomyces pombe* cells at the cellular level of compounds (Chapter 3, Chapter 5 and Chapter 6). The antimalarial activity was carried out for three series of compounds (Chapter 2, Chapter 3 and Chapter 5).The brief introduction of present work is systematically described below.

Chapter 1

General introduction to heterocyclic compounds, pathogens and antimicrobial study are presented in this chapter. The methods adopted for determination of biological activity are also discussed. The relevant information on the heterocyclic moieties covered under the study is individually presented in their respective chapters.

Chapter 2

This chapter introduces a novel series of fluoro substituted pyrazolylpyrazolines. The targeted compounds were synthesized in good to excellent yield from pyrazole chalcones and substituted phenyl hydrazine hydrochlorides under microwave irradiation. All the synthesized compounds were screened for their *in vitro* antimicrobial, antituberculosis and antimalarial activities (Figure 1.18).[92]

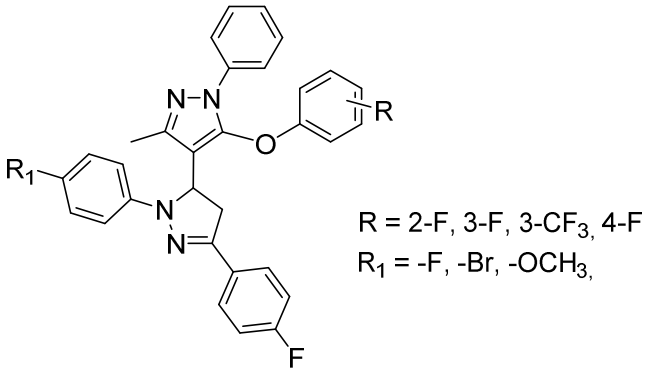


Figure 1.18 Pyrazolylpyrazoline scaffold.

Chapter 3

This chapter describes synthesis of a novel series of polyhydroquinoline scaffolds under ultrasonic irradiation by one-pot three-component cyclocondensation reaction in the presence of basic catalyst. All the synthesized compounds were screened for their *in vitro* antimicrobial, antituberculosis and antimalarial activities. The cytotoxicity of the synthesized compounds was also tested using a bioassay of *Schizosaccharomyces pombe* cells at the cellular level (Figure 1.19).[93]

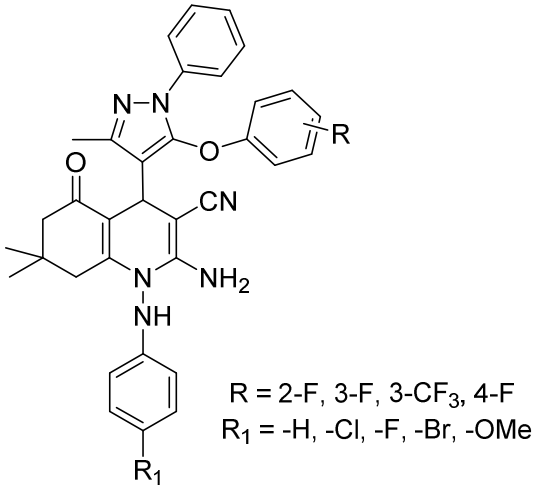


Figure 1.19 Pyrazole based polyhydroquinoline derivatives.

Chapter 4

This chapter deals with synthesis of a novel series of fluoro substituted pyrazole nucleus clubbed with 1,3,4-oxadiazole scaffolds in good yield**.** All the synthesized compounds were screened for their *in vitro* antimicrobial, antituberculosis and antimalarial activities (Figure 1.20).[94]

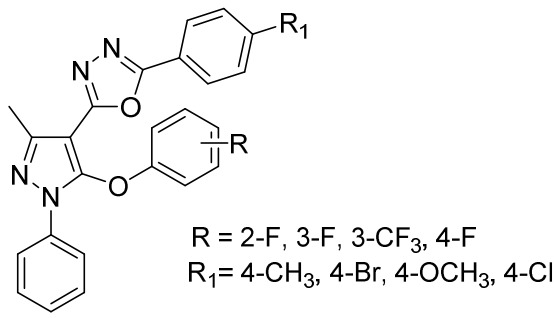


Figure 1.20 Fluorosubstituted pyrazole based 1,3,4-oxadiazole scaffolds.

Chapter 5

The present chapter describes microwave assisted synthesis of series of novel morpholinoquinoline based conjugates with pyrazoline moiety. All the synthesized compounds were screened for their *in vitro* antimicrobial and antituberculosis activities. The cytotoxicity of the synthesized compounds was also tested using a bioassay of *Schizosaccharomyces pombe* cells at the cellular level (Figure 1.21).[95]

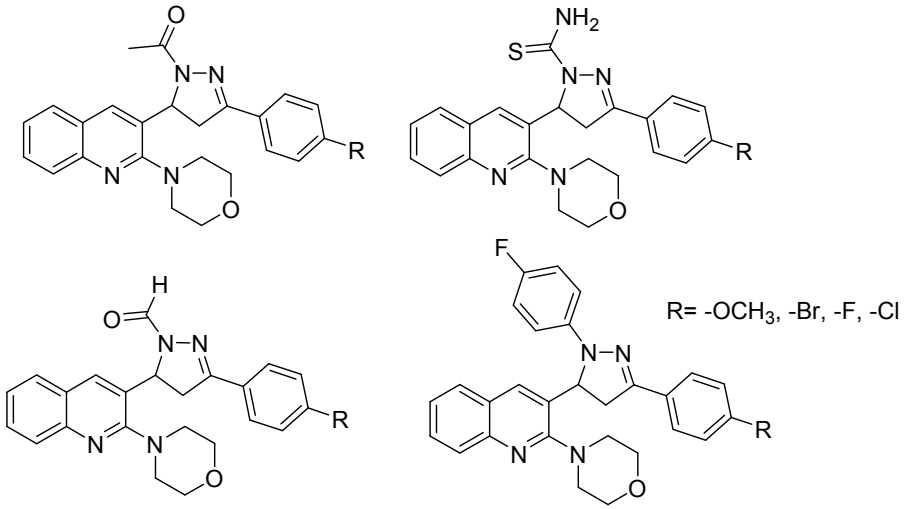
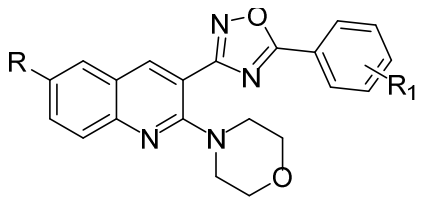


Figure 1.212-morpholinoquinolinebased pyrazoline derivatives.

Chapter 6

This chapter introduces novel series of 2-morpholinoquinoline scaffolds containing 1,2,4-oxadiazole moiety. All the synthesized compounds were screened for their *in vitro* antimicrobial activities. The cytotoxicity of the synthesized compounds was also tested using a bioassay of *Schizosaccharomyces pombe* cells at the cellular level (Figure 1.22).[96]



R = -H, -CH_3
R_1 = -H, 4-Cl, 4-NO_2, 4-CF_3, 4-Br, 3-CF_3, 4-OCH_3

Figure 1.222-morpholinoquinoline scaffolds containing 1,2,4-oxadiazole scaffold.

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