SYNTHESIS, BIOLOGICAL STUDIES OF SOME NEW TRIFLUOROMETHYL AND HALOGEN SUBSTITUTED QUINOLINE DERIVATIVES

Thesis

Submitted in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

by

GARUDACHARI B.

DEPARTMENT OF CHEMISTRY

NATIONAL INSTITUTE OF TECHNOLOGY KARNATAKA,

SURATHKAL, MANGALORE – 575025.

JANUARY-2014

DECLARATION

By the Ph.D. Research Scholar

I hereby **declare** that the Research Thesis entitled "**Synthesis, biological studies of some new trifluoromethyl and halogen substituted quinoline derivatives**" which is being submitted to the **National Institute of Technology Karnataka, Surathkal** in partial fulfillment of the requirements for the award of the Degree of **Doctor of Philosophy** in **Chemistry** is a ***bonafide report of the research work carried out by me***. The material contained in this Research Thesis has not been submitted to any University or Institution for the award of any degree.

Garudachari. B
Reg.No.: 100475CY10P01
Department of Chemistry

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CERTIFICATE

This is to *certify* that the Research Thesis entitled "**Synthesis, biological studies of some new trifluoromethyl and halogen substituted quinoline derivatives**" submitted by **Mr. Garudachari. B** (Register Number: 100475CY10P01) as the record of the research work carried out by him, is *accepted* as the *Research Thesis submission* in partial fulfillment of the requirements for the award of degree of **Doctor of Philosophy**.

Dr. Arun M. Isloor
Research Supervisor
Date:

Dr. M. N. Satyanarayan
Research Co-supervisor
Date:

Chairman- DRPC
Date:


DEDICATED TO
MY BELOVED FAMILY

ACKNOWLEDGEMENT

I whole heartedly express my profound sense of gratitude to Dr. Arun M. Isloor, Associate Professor, Department of Chemistry, NITK, Surathkal for his constant guidance and continuous encouragement throughout the course of the present investigation.

I am thankful to Department of Information Technology (DIT) Delhi, for the financial support throughout the research.

I am extremely grateful to Dr. M.N. Satyanarayan Department of Physics, NITK, Surathkal for their kindness to help me lot and encouragement to pursue my research work.

I am indebted to Dr. A.C. Hegde, Head, Department of Chemistry and Chairman, DRPC and Members of the RPAC Committee Dr. D. Krishna Bhat. Department of Chemistry and Dr. Udaya Bhat K. Department of Metallurgical and material engineering, for their timely assistance and inspiring words.

I thank Dr. A. Nityananda Shetty, Dr. A.V Adhikari, Dr. B. Ramachandra Bhat, Dr. D. Uday Kumar and Dr. Darshak R Trivedi Department of Chemistry, NITK, Surathkal for their good wishes and moral support.

I am extremely grateful to all teaching and non-teaching staffs Department of Physics, NITK, Surathkal their kind co-operation and immense support.

I always do remember and express due respect to all my teachers from childhood, who have been contributed immensely in moulding me.

I would like to express my sincere thanks to my research group at NITK and MIT-Manipal, Dr. Chitrakara Hedge, Dr. Vijesh Nambisan, Dr. Sankappa Rai, Mr. Chandrakanth. B, Dr. Dhanya Sunil, Dr. Mahesh Padaki, Dr. Shridhar Malladi, Mr. Ganesh Shastry, Mr. Rajesh Kumar, Ms. Seema Shenvi, Ms. Rasmi Pereira and Mr. Harikrishna for their help in many ways and co-operation during my course of research work.

I would like to thank Prof. H. K. Fun (School of Physics, Universiti Sains Malaysia, Malaysia), Dr. Rose Haque (School of chemical sciences, Universiti Sains Malaysia, Malaysia), Dr. K. N. Shivananda (Department of Chemistry, Technion Israel Institute of Technology, Haifa, Israel), Dr. Richard Betz (Department of
Chemistry, Nelson Mandela Metropolitan University, South Africa) and Dr. Gurumurthy Hegde (Faculty of Industrial Scainces and Technology, University Malaysia Pahang, Malaysia) for the technical support.

I am also thankful to Head, NMR Research center, IISc, Bangalore, for providing spectral data.

My special thanks are due to Dr. Ananda Kulal, Mr. L. Sathish, Ms. N. Pavithra (Department of Biological Sciences, Poornaprajna Institute of Scientific Research, Devanahalli, Bangalore), Dr. B. Thippeswamy, Mr. C. K. Shivakumar (Department of P.G. Studies and Research in Microbiology, Jnanasahyadri, Kuvempu University) and Dr. S. K. Peethambar (Department of Bio-Chemistry, Kuvempu University) for their help in screening the biological activities.

I thank all my other colleagues in R&D and other Departments of Syngene Intl Ltd. (Biocon) for their help in various activities during my research work.

I remember with pleasure the all-round assistance given by all Research Scholars and the non-teaching staff of the Chemistry Department, NITK, Surathkal.

The help and suggestions received from my friends Mr. Sharath Sydoor, Mr. Jithendra bedur, Mr. Basavanagowda, Mr. Aravind babu, Mr. Sardar Akram and Mr. Vinuthan B Praveen are thankfully acknowledged.

Finally, I express my gratitude to all my well wishers who have helped me in various activities during my research work leading to the submission of Ph. D. thesis.

Above all I humbly thank God Almighty, whose sustaining grace has been sufficient for me to complete this endeavour.

GARUDACHARI. B

ABSTRACT

The heterocyclic chemistry is one of the most important and complex branches of organic chemistry, which provides variety of biologically important compounds. The most recent innovative improvements and developments in health technologies, includes success in the discovery and production of new drugs. In recent years, the mounting threat of bacterial resistance has heightened the urgency to discover and develop anti-ineffective agents with novel mechanism of action and enhanced activity profile.

Quinoline nucleus is an important class of heterocyclic compounds found in many synthetic and natural products with a wide range of pharmacological activities. Quinolone derivatives have significant tissue penetration property and inhibit the DNA synthesis by forming complex with DNA gyrase or topoisomerase II enzyme. Prompted by the biological significance of quinoline and with the aim of finding new trifluoromethylquinoline derivatives having enhanced antimicrobial activity, in the present research work it was planned to synthesize some new quinoline derivatives. Based on the literature survey five series of quinoline derivatives were planned and synthesized. The synthetic and purification methods have been optimized for the new derivatives. Characterizations of newly synthesized compounds were successfully done by means of spectral methods like IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral and elemental analysis. Three dimensional structures of some derivatives were evidenced by X-ray crystallographic study. All the newly synthesized compounds were tested for their *in-vitro* antimicrobial activity. Some of the synthesized compounds were found to exhibit potent activity. A combination of trifluoromethylquinoline with certain substituents has caused an enhanced antimicrobial activity. Hence they are ideally suited for further modifications to obtain more efficient antimicrobial agents.

Key words: Trifluoromethylquinoline, Trifluoromethylquinolone, 1,2,3-Thiazole, Benzimidazole, Oxadiazole, Hydrazones, Click chemistry, Suzuki reaction, Antibacterial studies, Antifungal studies, Antioxidant studies.

CONTENTS

CHAPTER-1 Page No.

GENERAL INTRODUCTION AND OBJECTIVES OF THE WORK

CHAPTER-2

SYNTHESIS AND CHARACTERIZATION OF QUINOLINE INCORPORATED BENZIMIDAZOLE DERIVATIVES

CHAPTER-3

SYNTHESIS AND CHARACTERIZATION OF 7-TRIFLUOROMETHYL QUINOLINE-3-CARBOHYDRAZIDE AND 1,3,4-OXADIAZOLES DERIVATIVES

CHAPTER-4

SYNTHESIS AND CHARACTERIZATION OF SOME NEW 7 AND 8-TRIFLUOROMETHYLQUINOLINE DERIVATIVES

CHAPTER-5

REGIOSELECTIVE SYNTHESIS AND CHARACTERIZATION OF TRIFLUOROMETHYL QUINOLONE DERIVATIVES

CHAPTER-6

SYNTHESIS AND CHARACTERIZATION OF 1,2,3-TRIAZOLE CONTAINING 8-TRIFLUORO METHYLQUINOLINE DERIVATIVES

CHAPTER-7

ANTIMICROBIAL STUDIES

CHAPTER-8

CONCLUSION

LIST OF SCHEMES, TABLES AND FIGURES

SCHEMES

TABLES

FIGURES

NOMENCLATURE

DNA	**:**	Deoxyribonucleic acid
NMR	**:**	Nuclear magnetic resonance
s	**:**	Singlet
d	**:**	Doublet
t	**:**	Triplet
q	**:**	Quartet
m	**:**	Multiplet
DMSO	**:**	Dimethylsulfoxide
$CHCl_3$	**:**	Chloroform
FTIR	**:**	Fourier transform infrared spectroscopy
LCMS	**:**	Liquid chromatography-mass spectrometry
M.P	**:**	Melting point
Mol. Wt	**:**	Molecular weight
TLC	**:**	Thin layer chromatography
MRSA	**:**	Methicilin resistant *Staphylococcus aureus*
DMF	**:**	Dimethyl formamide
Dowtherm	**:**	Biphenyl:biphenyloxide(3:7)
mm	**:**	Millimetre
MIC	**:**	Minimum inhibitory concentration
PPA	**:**	Polyphosphoric acid
COSY	**:**	Correlation spectroscopy
HSQC	**:**	Heteronuclear single-quantum correlation spectroscopy
TFA	**:**	Trifluoroacetic acid
RBF	**:**	Round bottomed flask
MIC	**:**	Minimum inhibition concentration
EDCHCl	**:**	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
HOBT	**:**	1-Hydroxybenzotriazole
DPPH	**:**	2,2-Diphenyl-1-picryhydrazyl
BHT	**:**	Butylated hydroxytoluene
NBS	**:**	N-Bromosuccinimide
$POCl_3$	**:**	Phosphorus oxychloride
THF	**:**	Tetrahydrofuran
QD	**:**	Quinoline Derivatives

CHAPTER-1

GENERAL INTRODUCTION AND OBJECTIVES OF THE WORK

CHAPTER-1

GENERAL INTRODUCTION AND OBJECTIVES OF THE WORK

1.1. INTRODUCTION TO HETEROCYCLIC CHEMISTRY

The heterocyclic chemistry is one of the most important and complex branches of organic chemistry, which deals with the synthesis, properties, and applications of heterocyclic compounds. Majority of heterocycles are biosynthesized by plants and animals. Some of the heterocycles are found in the life such as chlorophyll's, haem. More than half of all known organic compounds, drugs and many natural products are heterocycles and they are used as antibiotics, antituberculosis, anticancer, analgesic and antiviral agents. In the pharmaceutical field most of the drugs are made up of heterocycles of Nitrogen, Oxygen, Sulfur. Some of the common heterocycles are given below.

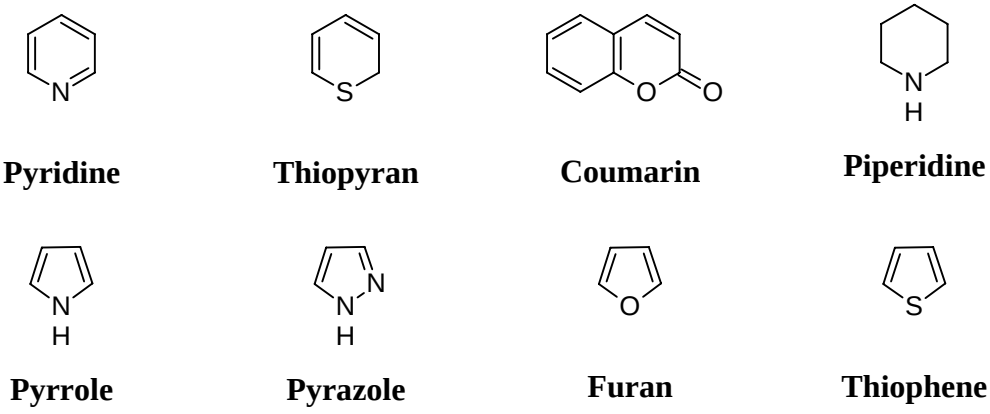


Figure 1.1. Structures of few important heterocycles

The heterocyclic compounds are very important and essential diet ingredients such as amino acids, nicotinamide (Vitamin B_3), thiamine (Vitamin B_1), riboflavin (Vitamin B_2) and many of the enzymes, haemoglobin, nucleic acids and chlorophyll

also contain heterocyclic compounds. Heterocyclic compounds are acquiring more importance in recent years due to their varied pharmacological activities. Nitrogen, Sulphur, Oxygen, containing five/six membered heterocyclic compounds have occupied enormous significance in the field of drug discovery process. These compounds have a wide range of applications in agriculture, photography, biocide formulation and polymer science, predominantly these types of compounds used in pharmaceutical industry.

The scientific understanding of drug action is required to design a compound that will produce a specified therapeutic effect. The contrast of drugs of today and those of older days are dramatically changed. Only a half century ago man relied almost exclusively on nature to produce the organic drugs he needed and the contribution of medicinal chemistry were confined largely to the preparation of extracts and isolation of active pharmaceutical ingredients. Many important biochemical compounds and drugs of natural origin contains heterocyclic ring structure such as pyridine, quinoline, indole, benzimidazoles, imidazoles, pyrazoles, thiazoles, pyrimidine, purines, Schiff and Mannich bases. The various compounds containing the above heterocyclic moieties are possess a wide spectrum of biological properties which include antibacterial, antifungal, anti-inflammatory, anticonvulsant, antiviral, antimalarial, antituberculosis, anticancer, etc. A large number of their new derivatives have been synthesized and extensively studied for various pharmacological properties. The fast growing literature on heterocycles in recent years demonstrates their increasing significance in the pharmaceutical field.

1.2. CHEMISTRY OF QUINOLINE

Quinoline is a heterocyclic aromatic organic compound. It has the formula C_9H_7N (Figure 1.2) and is a colourless hygroscopic liquid. Quinoline is mainly used as a building block to bioactive molecules. Approximately 4 tonnes are produced annually according to a report published in 2005. Its principal use is as a precursor to 8-hydroxyquinoline, which is a versatile chelating agent and precursor to pesticides. Its 2- and 4-methyl derivatives are precursors to cyanine dyes. Oxidation of quinoline affords quinolinic acid (pyridine-2,3-dicarboxylic acid), a precursor to the herbicide sold under the name Assert. Owing to high water solubility, quinoline has significant

potential for mobility in the environment, which may promote water contamination. Fortunately, quinoline is readily degradable by certain microorganisms, such as *Rhodococcus* species Strain Q1, which was isolated from soil and paper mill sludge.

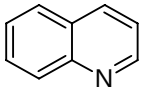
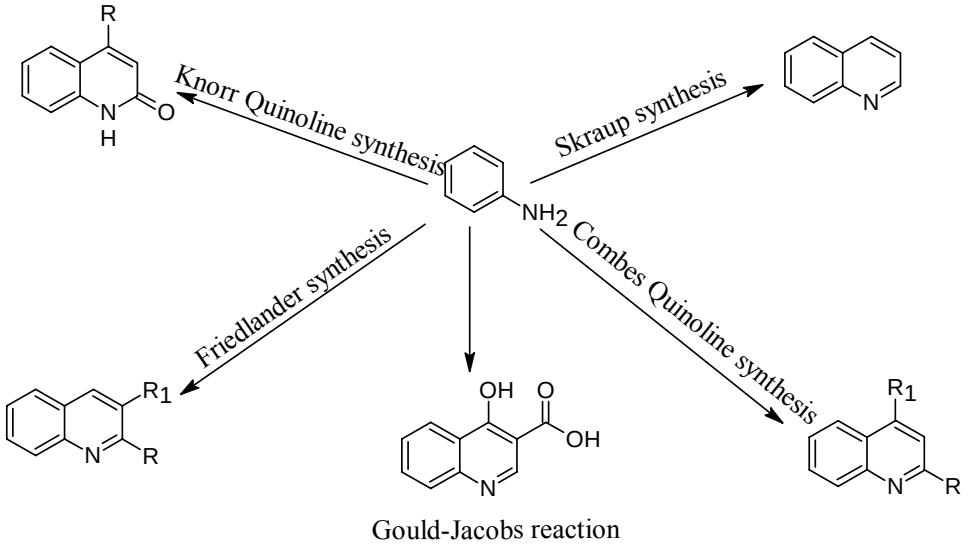


Figure 1.2. General structure of quinoline

Quinolines are known as benzopyridines because they have both a benzene ring and a pyridine ring. Like benzene and pyridine, they are aromatic compounds. Quinoline undergoes electrophilic aromatic substitution on the benzene ring because benzene ring is more reactive than the pyridine ring toward electrophilic substitution. Substitution takes place primarily at C-5 and C-8. Nucleophilic substitution of quinoline undergoes at C-2 and C-4 because pyridine ring is more reactive than benzene.

1.3. GENERAL SYNTHETIC METHODS OF QUINOLINE

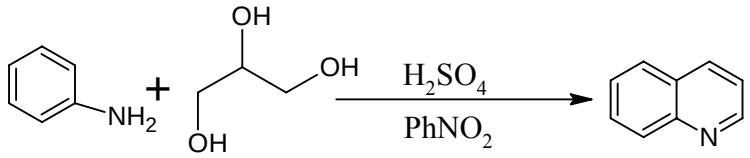
Quinoline was first extracted from coal tar in 1834 by Friedlieb Ferdinand Runge. It can be synthesized from using various methods, such as Skraup synthesis, Knorr Quinoline synthesis, Combes Quinoline synthesis, Friedlander synthesis, Gould-Jacobs reaction (Scheme 1.1).



Scheme 1.1. General routes for the synthesis of quinoline derivatives

(a) Skraup synthesis

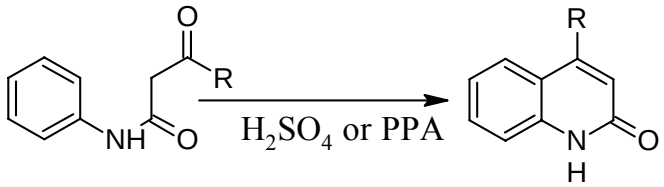
Skraup synthesis is a chemical reaction used to synthesize quinolines using aniline, glycerol and an oxidizing agent nitrobenzene in sulfuric acid media at high temperature. The reaction mechanism is unclear, yet there is a good reason to believe that acrolein (obtained by dehydration of glycerol in presence of concentrated sulfuric acid) is an intermediate, which then undergoes 1,4-addition results in quinoline (Scheme 1.2).



Scheme 1.2. Skraup synthesis

(b) Knorr Quinoline synthesis

The Knorr Quinoline synthesis is an intramolecular organic reaction converting a β-ketoanilide to a 2-hydroxyquinoline using sulfuric acid or polyphosphoric acid as solvent (Scheme1.3). The reaction is a type of electrophilic aromatic substitution followed by elimination of water molecule.

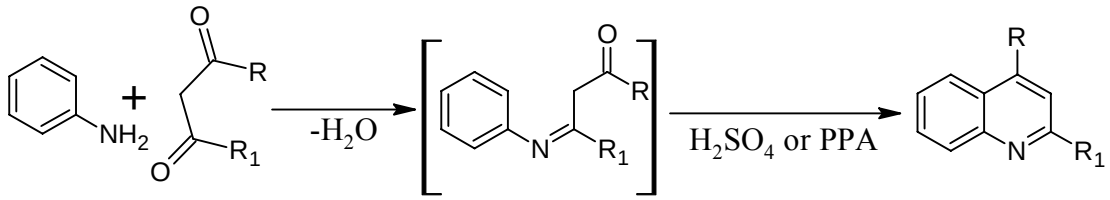


Scheme 1.3. Knorr quinoline synthesis

Where R = Me, Et, OH, Ph.

(c) Combes Quinoline synthesis

The Combes Quinoline synthesis is a condensation reaction between substituted aniline with β-diketones; first formation of Schiff base followed by cyclisation using strong acidic conditions (Scheme1.4).

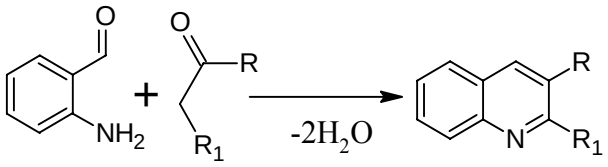


Scheme 1.4. Combes quinoline synthesis

Where R/R_1 = Me, Et, OH, Ph.

(d) Friedlander synthesis

Friedlander Quinoline synthesis is reaction between 2-aminobenzaldehydes with ketones in presence of acid catalysts like trifluoroacetic acid, toluenesulfonic acid and Lewis acids (Scheme 1.5).

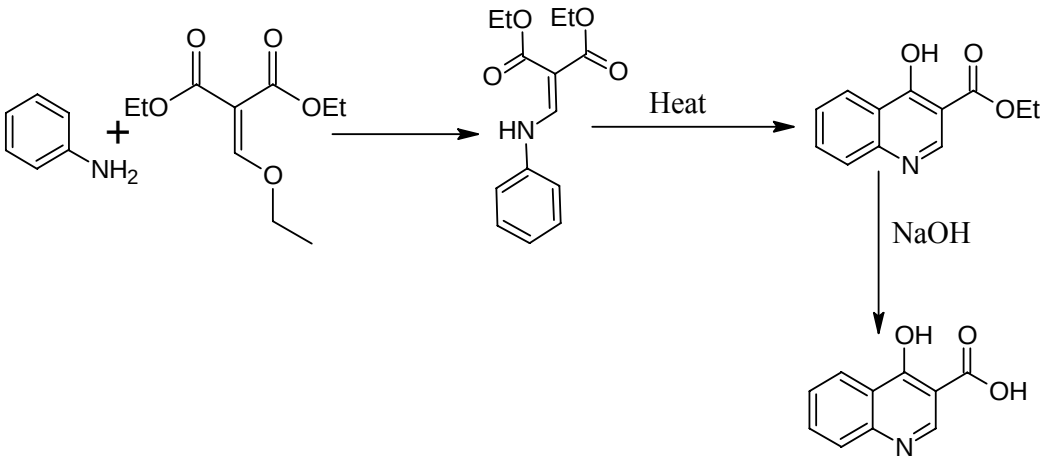


Scheme 1.5. Friedlander synthesis

Where R = Me, CO_2Et, R_1 = Me, Et.

(e) Gould-Jacobs reaction

Gould-Jacobs reaction is an organic reaction for synthesis of 4-hydroxyquinoline-3-carboxylic acid by treating aniline derivative with malonic acid derivatives at high temperature followed by hydrolysis using sodium hydroxide (Scheme 1.6).



Scheme 1.6. Gould-Jacobs reaction

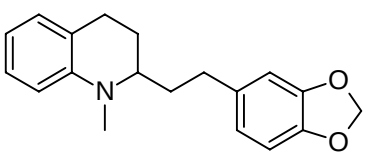
These methods give high yield and good purity of the products. Further, it is possible to synthesize different quinoline derivatives conveniently. New quinoline derivatives containing different pharmacophoric element can be synthesized starting from simple molecules.

1.4. BIOLOGICAL IMPORTANCE OF QUINOLINE

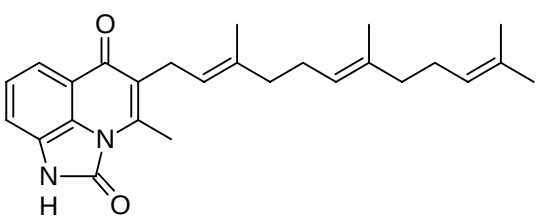
The quinoline nucleus is an important class of heterocyclic compounds found in many synthetic and natural products with a wide range of pharmacological activities. 4-Methanolquinoline derivatives such as Cinchona alkaloids and Mefloquine that are rapidly acting blood schizontocides, these compounds were introduced for routine use in 1985 as an antimalarial. The 4-aminoquinolines such as Chloroquine and Amodiaquine are rapidly acting blood schizontocides with some gametocytocidal activity. The 8-aminoquinolines such as Primaquine are used as tissue schizontocides to prevent relapses of the ovale and vivax malarias. In recent studies it has been established that many quinoline derivatives are being intensively used in medicinal field as anticancer (Nakamura et al. 1999), anti-inflammatory (Bekhit et al. 2004), antibacterial, antifungal (Eswaran et al. 2009) and antiviral (Carta et al. 2011) agents.

The compounds containing novel structure from natural origin represent a major source for the discovery and development of new drugs for several diseases, including plant extracts and compounds isolated from plants, bacteria, fungi and marine organisms. These compounds offer new and novel scaffolds for developing new antimicrobials.

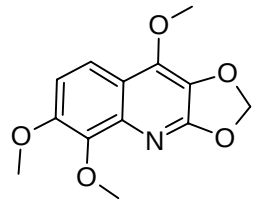
Chavez et al. (1997) reported antimalarial and toxicological activities of the tetrahydroquinoline alkaloids isolated from *Galipea officinalis* bark (**QD-1.1**, **QD-1.2**), showed best antimalarial effect. Leaves of *Acanthosyris paulo-alvinii*, a Brazilian tree belonging to the Santalaceae, yielded the novel compounds **QD-1.3**, **QD-1.4, QD-1.5** and **QD-1.6** an even simpler new alkaloid isolated from the aerial parts of *Ephedra transitoria* (Ephedraceae), inhibited the growth of the common bacteria (Kaur et al. 2010). Some of the naturally occurring quinolines are given below.



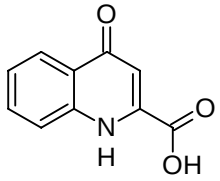
Galipinine
QD-1.1



Aurachin E
QD-1.2

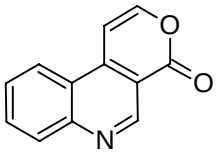


2,3-methylenedioxy-4,7, 8-trimethoxyquinoline
QD-1.3



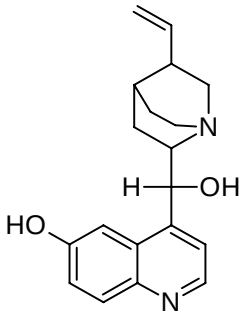
Transtorine

QD-1.4



4H-pyrano[3,4-*c*]quinolin-4-one

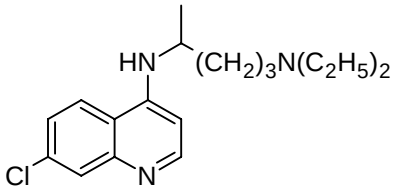
QD-1.5



Cupreine

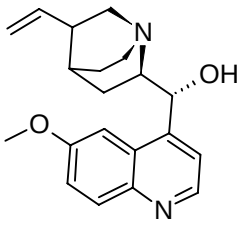
QD-1.6

The quinoline scaffold is prevalent in a variety of pharmacologically active synthetic and natural compounds. The quinolines are historically most important antimalarial drugs ever used. Chloroquine (**QD-1.7**), is the most famous drug of this group provided well-founded hopes for the eradication of malaria. The other known drugs from this family include Quinidine (**QD-1.8**), Quinine (**QD-1.9**), Ciprofloxacin (**QD-1.10**), Grepafloxacin (**QD-1.11**), Antrafenine (**QD-1.12**), Saquinavir (**QD-1.13**), Gemifloxacin (**QD-1.14**), Topotecan (**QD-1.15**), Balofloxacine (**QD-1.16**), Levofloxacin (**QD-1.17**). Drugs which are having quinoline as core molecule are given below.



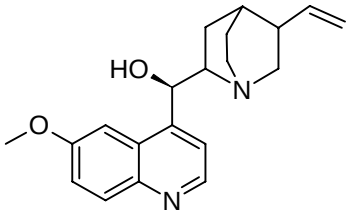
Chloroquine

QD-1.7



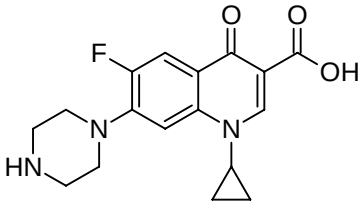
Quinidine

QD-1.8



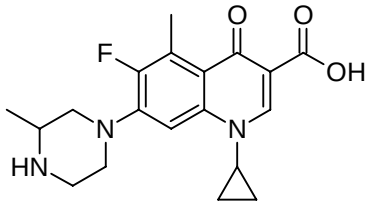
Quinine

QD-1.9



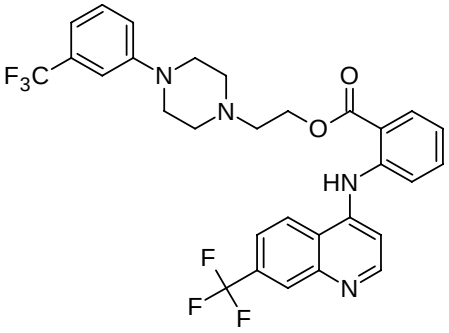
Ciprofloxacin

QD-1.10



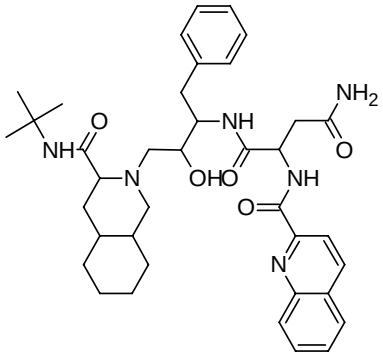
Grepafloxacin

QD-1.11



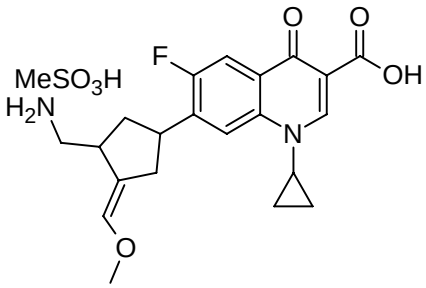
Antrafenine

QD-1.12



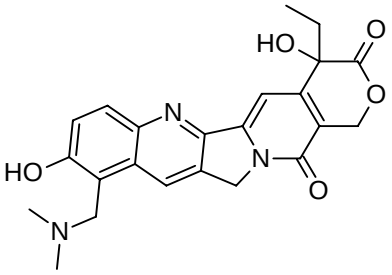
Saquinavir

QD-1.13



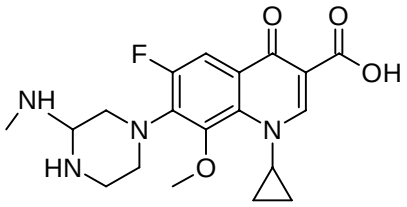
Gemifloxacin

QD-1.14



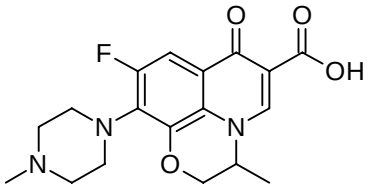
Topotecan

QD-1.15



Balofloxacine

QD-1.16



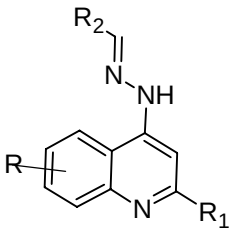
Levofloxacin

QD-1.17

1.4.1. Biological importance of 4-substitutedquinolines

Quinine is a natural white crystalline alkaloid having antipyretic, antimalarial, analgesic, and anti-inflammatory properties and having bitter taste. Chloroquine (**QD-1.7**), a 4-substitutedquinoline, was first chemically synthesized in 1934, as a substitute for quinine (**QD-1.9**). Chloroquine is selectively deposited in the food vacuole of the parasite, exerting its antimalarial effect by preventing the polymerization of the toxic heme. Chloroquine-resistant, antibiotic-resistant started appearing and now drug is virtually ineffective in most parts of the world. As Chloroquine resistance started, several efforts were initiated to develop new antimalarial, antibiotic drugs that target the resistant parasites. The focus of the research was in the direction of synthesis of side-chin modified of 4-substitutedquinolines and hybrid 4-aminoquinolines.

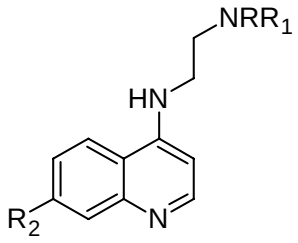
Synthesis of hydrazones of quinoline moiety was reported by Savini et al. (2002). Newly synthesized compounds were screened for their antitubercular activity against *Mycobacterium tuberculosis* H37Rv. All the tested compounds in the series were shown to exhibit excellent inhibitory activity. It was noticed that introduction of 6-cyclohexyl, 7-methoxy, 7-ethoxy and 7-chloro substituents on the quinoline nucleus enhanced the antitubercular activity remarkably**.**



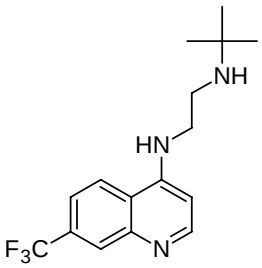
QD-1.18

Where R = H, CH_3, C_6H_5, R_1 = H, F, Cl, 5,7-Cl, OCH_3, OC_2H_5, C_4H_9, C_6H_{11}
R_2 = OCH_3,NO_2, furyl, pyrrolyl, 4-$N(C_2H_5)_2$-C_4H_4, naphthyl, 3,4-(OCH_2)-C_6H_3.

Stocks et al. (2002) showed that the replacement of the diethylamino function with a metabolically stable t-Bu group or heterocyclic ring (piperidyl, pyrrolidinyl, and morpholinyl) in the short chain analogues led to a substantial increase in the antimalarial activity. The most promising analogue (**QD-1.20**) exhibited a 20-fold increase in potency against the chloroquine resistant strain with an IC_{50} value of 9.8 nM.



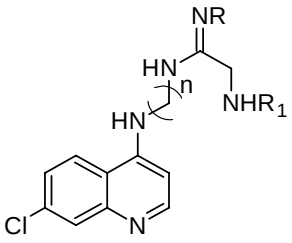
QD-1.19



QD-1.20

Where R = H, Alkyl, R_1 = t-Bu, Alkyl, R_2 = Cl, CF_3.

Solomon et al. (2005) synthesized 4-aminoquinolines by selectively modifying the pendent amino group to facilitate the iraccumulation in the parasite food vacuole to achieve better interaction with the hematin leading to improved antimalarial activity. The compounds (**QD-1.21**) having the Boc group displayed MIC values ranging between 1.02 and 1.08 μM were found to be more active than the corresponding amino compounds.

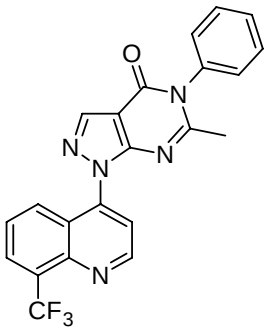


QD-1.21

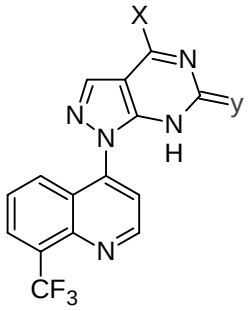
R = H, CH_3, CO_2t-Bu, R_1 = H, CO_2t-Bu, CH_3, n = 2, 3,4.

Holla et al. (2006) reported the synthesis of some new pyrazolo[3,4]pyrimidine derivatives (**QD-1.22 & QD-1.23**) and its antimicrobial

studies. Replacement of 1H of pyrazole of pyrazolo[3,4]pyrimidine ring system by some other bioactive moiety drastically alters its pharmacological properties. Introduction of a fluorine atom as the CF_3 group provides a more lipophilically and pharmacologically interesting compound compared to their non-fluorinated analogues.



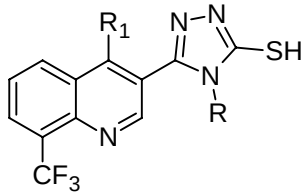
QD-1.22



QD-1.23

X = NH_2, Y = O, S.

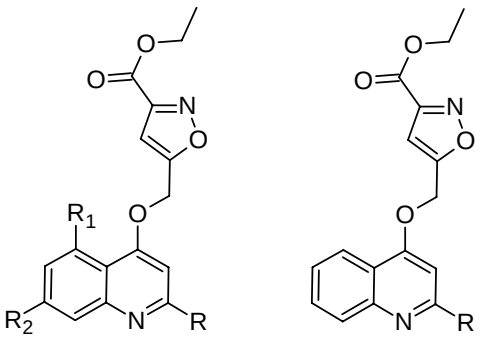
Eswaran et al. (2009) reported synthesis and antimicrobial activity of new 1,2,4-triazole carrying quinoline (**QD-1.24**) derivatives using multistep reactions. The biological results revealed that cyclopropylamine, cychlohexylamine and morpholine at position 4 of quinoline enhanced antibacterial and antifungal activity.



QD-1.24.

R = Ph, -CH_2Ph, -CH_2CH_2OMe, R_1 = Substituted amines.

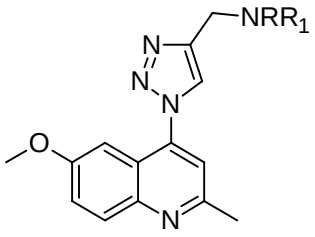
Lilienkampf et al. (2009) identified a class of quinoline-isoxazole hybrid compounds (**QD-1.25**) with good anti-TB (Antitubeculosis) activity. Their investigation report revealed that most of the compound carrying C-2, C-7 trifluoromethyl quinolines and oxazoline at fouth position are better antituberculosis agents. According to authors, the isoxazole moiety played a significant role in the tuberculosis activity.



QD-1.25

Where $R/R_1/R_2$ = H, CF_3.

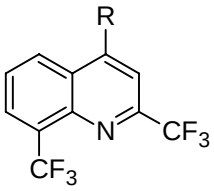
In the recent year Thomas et al. (2010) designed, synthesized and evaluated antimicrobial activity of some new 1,2,3-triazole containing quinoline derivatives (**QD-1.26**). The *in-vitro* preliminary antimicrobial screening revealed that, the presence of active groups like cyclopropyl, substituted piperazines, methoxy and fluoro has contributed significantly in enhancing the activity.



QD-1.26

Where R, R_1 = Alkyl amine.

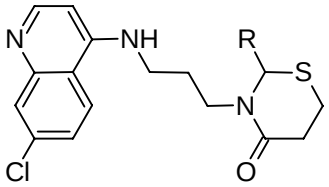
Meshram et al. (2012) discribed synthesis of 2,8-bis(trifluoromethyl)-4-substituted quinoline derivatives (**QD-1.27**) and its anticancer activity. All the synthesized compounds were evaluated for their *in-vitro* cytotoxic activity. The results of their work suggest that hetirocylcic derivatives at fourth position are potent molecules.



QD-1.27

Where R = Substituted aryl, heteroaryl, 3-hydroxypiperidine, morpholine, benzimidazole.

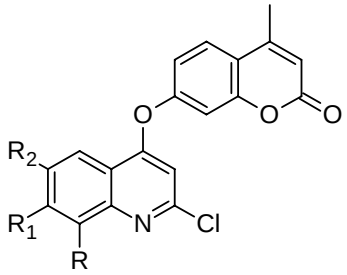
Rudrapal et al. (2012) synthesized and studied antimicrobial activity of some 3-(3-(7-chloroquinolin-4-ylamino)propyl)-1,3-thiazinan-4-one derivatives (**QD-1.28**). They found that most of the tested compounds were useful microbial inhibitors. Results of antibacterial study indicate that aromatic bulky substituents have greater contributing effect than the aliphatic non-bulky group toward the antibacterial activity of the prepared 4-aminoquinoline derivatives.



QD-1.28

Where R = 2-Fluorophenyl, 4-methoxyphenyl, 3-hydroxyphenyl, furan-2-yl-, ethyl, 4-(dimethylamino)phenyl, 5-methyl-thiophen-2yl.

Chromenone fused quinoline derivatives (**QD-29**) were synthesized by Balaji et al. (2013). The targeted final products were prepared using one pot dehydrochlorination of 2,4-dichloroquinoline. The *in-vitro* antibacterial investigation report revealed that some of the compounds were good to moderate antibacterial agents.

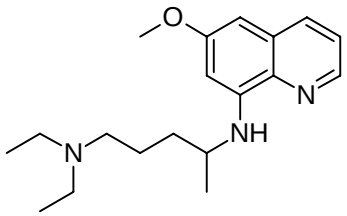


QD-1.29

Where R = H, CH_3, R_1 = H, CH_3, R_2 = H, CH_3, OCH_3.

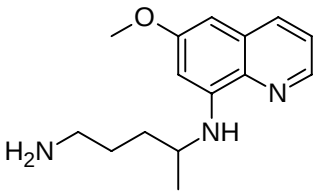
1.4.2. Biological importance of 8-aminoquinolines

Pamaquine (**QD-1.30**) was synthesized in 1952, this 8-aminoquinoline was the first drug capable of preventing the relapses in *Plasmodium vivax* malaria. Toxicological concerns led to restrictions in the use of Pamaquine. Primaquine (**QD-1.31**) another 8-aminoquinoline derivative has been used since 1950's for the eradication of liver stages in course of *Plasmodium vivax* infections.



Pamaquine

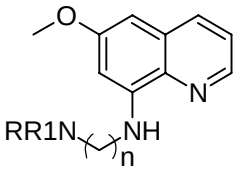
QD-1.30



Primaquine

QD-1.31

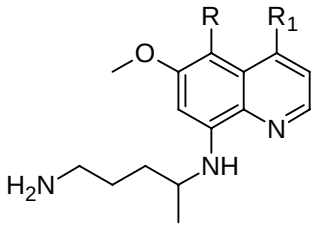
Syntheses of some 8-aminoquinoline derivatives (**QD-1.32**) were reported by Armer et al. (1999). Novel analogues were screened for both *in-vitro* and *in-vivo* anticoccidial activity. Pentyl group at 8^{th} position of quinoline ring showed better anticoccidial activity. Other analogues were less potent *in-vitro* or *in-vivo* or both. Also, an exploration of the terminal nitrogen substitution has revealed the tetrahydropyran group to be optimal.



QD-1.32

Where R, R_1 = H, Alkyl, n = 1,2,3,4,5.

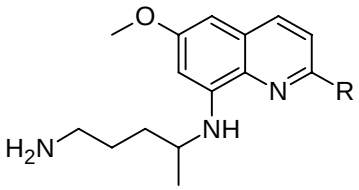
Several ring-substituted 8-aminoquinolines (**QD-1.33**) possessing remarkable antimalarial activities have been reported by Vangapandu et al. (2003). The substitution, R = C_5H_{11} and C_8H_{17} exhibited *in-vitro* and *in-vivo* biological efficacy higher than Chloroquine against both Chloroquine and Chloroquine resistant strains (IC_{50} of 9.4 and 9.7 ng/mL, respectively).



QD-1.33

Where R = C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, R_1 = C_2H_5.

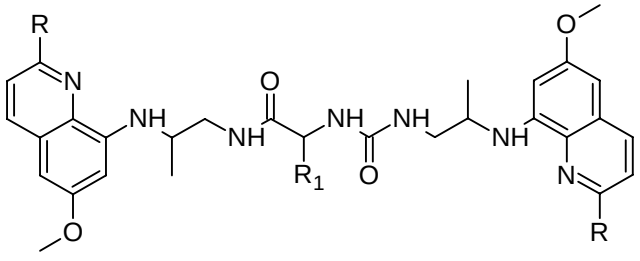
Jain et al. (2004) synthesized a series containing metabolically stable bulky alkyl groups at the C-2 position of the quinoline ring in Primaquine (**QD-1.34**). The most promising analogue, *2-tert*-butyl primaquine displayed potent *in-vitro* antimalarial activity (IC_{50} = 39 ng/mL), superior to that of Chloroquine (IC_{50} = 113ng/ mL).



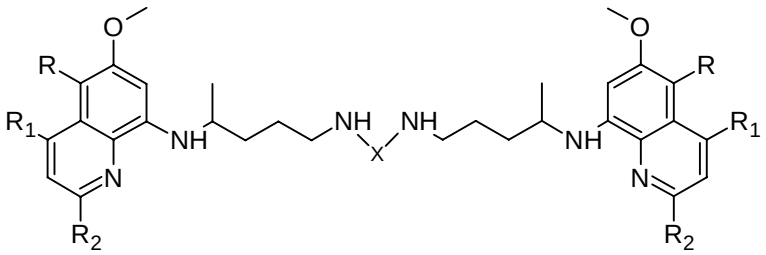
QD-1.34

Where R = H, $C(CH_3)_3$, 1-adamantyl.

Kaur et al. (2011) reported synthesis and antimicrobial, antiprotozoal, antimalarial activity of bisquinoline analogues (**QD-1.35 & QD-1.36**). The bisquinoline analogues exhibited promising *in-vitro* antimicrobial activities against a panel of pathogenic bacteria and fungi. The results of this study provide evidence that bis(8-aminoquinolines) are a promising class of antimalarial agents.



QD-1.35

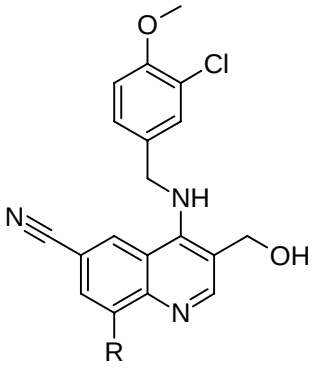


QD-1.36

Where R = H, OC_5H_{11}, R_1 = H, C_2H_5, R_2 = H, $C(CH_3)_3$

X = CO, CS, $COCH_2$, CONHCO, $COOCH_2$, COS, COCO, $CH_2CH_2NHCH_2CH_2$, Alkylaromatic.

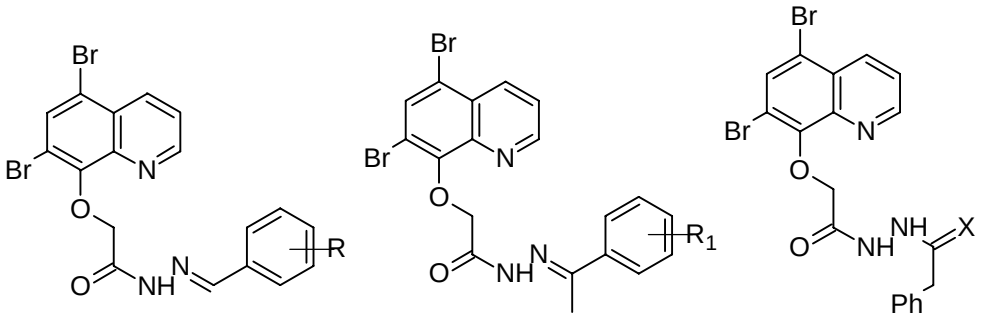
Fiorito et al. (2013) synthesized potent quinoline derivatives (**QD-1.37**) for Alzheimer's disease. Among the synthesized compounds, cyclopropyl amine group at eighth position of the quinoline ring showed good pharmacokinetics profile.



QD-1.37

Where R = Cyclopropylamine, dimethylamine, ethylamine, morpholine, cyclopropyl.

Several derivatives of quinoline scaffold with a flexible, semi-flexible or rigid side chains at position 8 of the quinoline ring were synthesized (**QD-1.38**) by Arafa et al. (2013). Studied *in-vitro* activity versus the human colon cancer cell line HT29 and the human breast cancer cell line MDA-MB231. The derivatives with Schiff's base linkers showed excellent activity.

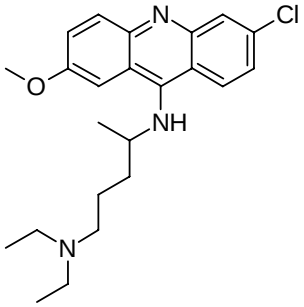


QD-1.38

Where R = H, 4-Cl, 4-Br, 4-NO_2, 3-OMe, 4-OH, 4-OMe, R_1 = H, 4-Br, 4-NO2, X = O, S.

1.4.3. Biological importance of ring modified quinolines

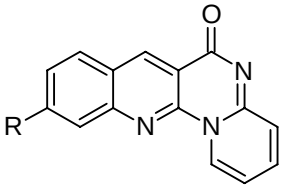
Quinacrine (**QD-1.39**) was initially approved in the 1930s as an antimalarial drug. In addition it has been used for treating tapeworm infections, giardiasis (an intestinal parasite) treatment.



Quinacrine

QD-1.39

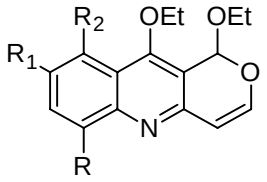
El-Sayed et al. (2002) reported synthesis and antimicrobial evaluation of several quinoline and pyrimidoquinoline derivatives (**QD-1.40**). The pyridine containing compounds were exerted strong antibacterial and antifungal activities, especially when a methoxyl group was located in the 7-position of quinoline nucleus.



QD-1.40

Where R = H, Me, -OCH_3.

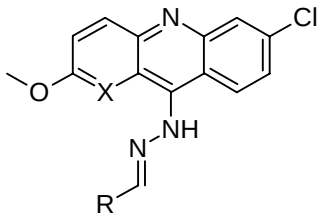
Synthesis of new 1,10-diethoxy-1*H*-pyrano[3, 3]quinolines and their antimicrobial studies were reported by Dhanabal et al. (2006). All the compounds exhibited moderate antibacterial activity. Interestingly the compound 6-methoxy substituted pyranoquinoline showed better activity than the standard Streptomycin in case of *Escherichia coli*.



QD-1.41

Where R = H, Me, R_1 = H, -OMe, Cl, R_2 = H, Me.

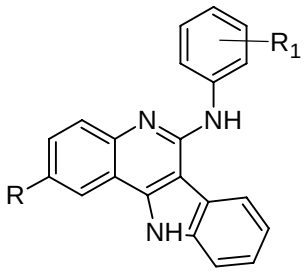
Fattorusso et al. (2008) evaluated N_2-acrydinylhydrazones (**QD-1.42**) for antiplasmodial activity. The imidazoles containing hydrazone derivatives showed better activity against the W2 strain and D10 strain (IC_{50} = 30.8 and 26.9 nM respectively).



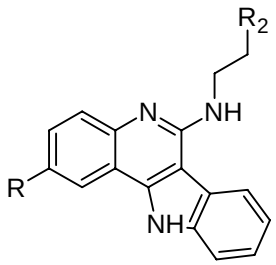
QD-1.42

Where R = Imidazoles and Substituted phenyl, X = CH, N.

Antiproliferative evaluation of certain indolo[3,2]quinoline derivatives were reported by Lu et al. (2010). The introduction of a hydroxyl group at the anilino-moiety resulted in the enhancement of antiproliferative activity in which the activity decreased in an order of *para*-OH > *meta*-OH > *ortho*-OH (**QD-1.43**). The C_6 alkylamino-substituted indolo[3,2]quinoline derivatives (**QD-1.44**), exhibited comparable antiproliferative activities against all tested cancer cells.



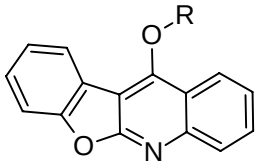
QD-1.43



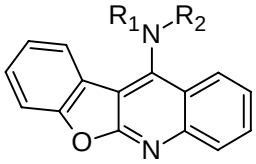
QD-1.44

Where R = H, F, R_1 = H, 4-OH, 3-OH, 2-OH, 4-Me, R_2 = alkyl amine, aromatic alkyl amine.

Series of 4-alkoxylated and 4-aminated benzofuro[2,3]quinoline derivatives (**QD-1.45 & QD-1.46**) was synthesized, evaluated for their anti-TB and cytotoxic activities (Yang et al. 2010). Among the tested compounds, methoxybenzofuro[2,3]quinoline, methylamino-benzofuro[2,3]quinoline, dimethylaminobenzofuro[2,3]quinoline, exhibited significant activities against the growth of *Mycobacterium tuberculosis* (MIC values of <0.20 mg/mL).



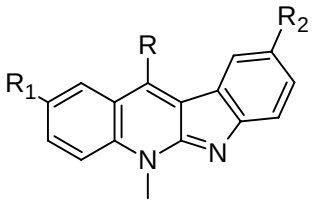
QD-1.45



QD-1.46

Where R = Me, Et, Ph, Ph-4-COMe, R_1 = H, Me, R_2 = H, Me, Ph, Ph-4-COMe, Ph-4-OMe, Ph-3-OMe.

Lu et al. (2013) described the synthesis, and *in-vitro* and *in-vivo* antimalarial evaluations of certain ester modified Neocryptolepine (5-methyl-5H-indolo[2,3-b]quinoline) derivatives (**QD-1.47**). Modification was carried out by introducing ester group at C-2 and C-9 position of Neocryptolepine core. All the tested compounds showed higher activity than the well-known antimalarial drug Chloroquine.

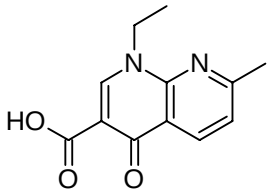


QD-1.47

Where R = Alkylamine, N-substituted alkylamine, alcohol, morpholine, R_1 = H,CO_2Me, Cl, Br, R_2 = H, CO_2Me, Br.

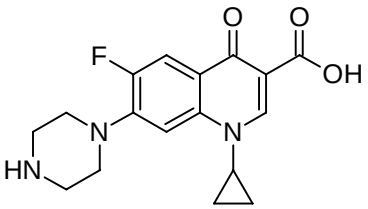
1.4.4. Biological importance of quinolones

The quinolones are a family of synthetic broad-spectrum antibiotics. The first generation of the quinolones begins with the introduction of Nalidixic acid (**QD-1.48**) in 1962 for treatment of urinary tract infections in humans. Nalidixic acid was discovered by George Lesher and co-workers.



Nalidixic acid

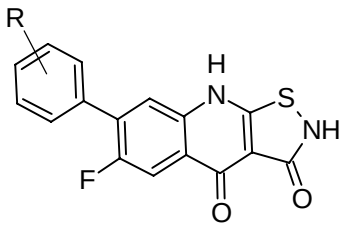
QD-1.48



Ciprofloxacin

QD-1.49

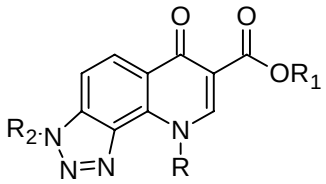
Wiles et al. (2006) reported synthesis of isothiazoloquinolones containing functionalized aromatic hydrocarbons at the 7-position (**QD-1.50**) and its antibacterial activity. The activity of 3-substituted analogues against MRSA was greater than that of the corresponding 4-substituted analogues.



QD-1.50

Where R = H, $NH_2(CO)$, AcNH, Ac, NC, $NCCH_2$, F, OH, OMe, NH_2, $OHCH_2$, 4-OH-2,5-Me, NH_2CH_2, $NH_2(CH_2)_2$, NMe, 3-NH_2-4-Me, 3-NH_2-4-F, Piperidinyl.

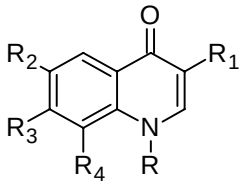
Synthesis of triazole containing quinolones (**QD-1.51**) was reported by Carta et al. (2007). The newly synthesized compounds were screened for their antitubercular activity. N-methyl quinoline ethylester are showed MIC_{90} values in the range 0.5-3.2 μg/mL, while other compounds were inactive at MIC_{90} = 32 μg/mL.



QD-1.51

Where R = H, N-CH_3, R_1 = H, C_2H_5, R_2 = H, C_2H_5.

Ma et al. (2009) reported synthesis, *in-vitro* antitrypanosomal and antibacterial activity of phenoxy, phenylthio or benzyloxy substituted quinolones (**QD-1.52**). The compound having 7-monosustitution showed significant antibacterial activities (MIC < 25 μg/mL). Most 7,8-disubstituted quinolones exhibited significant inhibitory activity against antitrypanosomal agents.

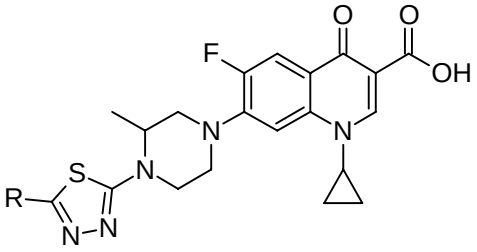


QD-1.52

Where R = C_2H_5, Pr, CH=CH_2, R_1 = COOH, COOMe, COOEt, $CONH_2$, CN, R_2 = F, 4-CH_3-PhO, PhS, $PhCH_2O$, R_3 = 4-CH_3-PhO, 2,4-Dichloro-PhO, PhO, 4-F-PhS, 4-Cl-PhS, 4-CH_3-PhS, $PhCH_2O$, R_4 = 4-CH_3-PhO, 2,4-Dichloro-PhO, 4-OH-PhO, PhO, PhS, 4-F-PhS, 4-Cl-PhS, $PhCH_2O$, F.

Antibacterial evaluation of certain nitroaryl thiadiazole-gatifloxacin hybrids (**QD-1.53**) were reported by Jazayeri et al. (2009). Among synthesized compounds, nitrofuran-1,3,4-thiadiazole moiety attached to the piperazine ring at C-7 position exhibited more potent inhibitory activity against Gram-positive bacteria including *Staphylococcus epidermidis* (MIC = 0.0078 mg/mL), *Bacillus subtilis* (MIC = 0.0039

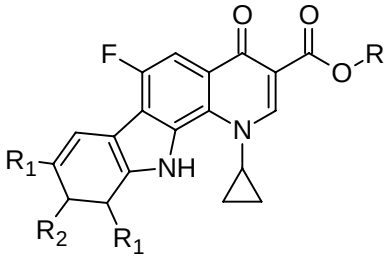
mg/mL), *Enterococcus faecalis* (MIC = 0.125 mg/mL) and *Micrococcus luteus* (MIC = 0.125 mg/mL).



QD-1.53

Where R = Nitrofuran, nitrothiophene, nitrophenyl, N-methyl-nitroimidazole.

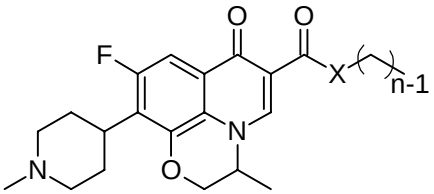
A series of tetracyclic fluoroquinolones (**QD-1.54**) were synthesized by Al-Trawneh et al. (2010). All synthesized derivatives were tested for their *in-vitro* antimicrobial and antiproliferative activity. The fluoroindole fused quinolone compound emerged as the most active antibacterial compound against multidrug-resistant *staphylococci* and the most potent antiproliferative compound against MCF-7 cells.



QD-1.54

Where R = H, Et, R_1 = H, Me, OMe, F, R_2 = H, Me.

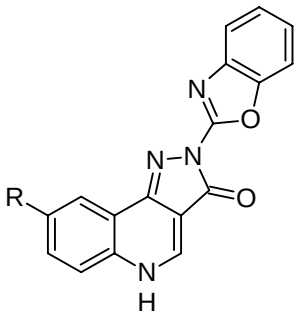
A convenient route for the synthesis of some acyloxymethyl esters and carboxamides (**QD-1.55**) of Levofloxacin (LV) with modulated lipophilicity was described by Korolyov et al. (2010). All newly synthesized compounds were evaluated *in-vitro* antitumor activity against five human cancer cell lines. The most efficient LV derivatives (ester and amide) displayed IC_{50} values in 0.2-2.2 μM range.



QD-1.55

Where X = O, NH, n = 4-15.

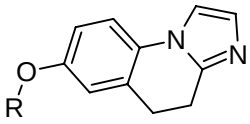
In the recent years, Reis et al. (2011) reported synthesis and anticancer activity of 2-(benzothiazol-2-yl)-8-substituted-2H-pyrazolo[4,3]quinolin-3(5H)-ones (**QD-1.56**). The *in-vitro* anticancer activity of the synthesized compounds revealed, methyl and bromo substitution at C-6 position of quinoline showed considerable effect against three cancer cell lines. IC_{50} value of 2.3 mg/mL against breast cancer (MDA-MB-435), colon (HCT-8) and central nervous system (SF-295) cell lines (IC_{50} values of 4.1 and 4.5 mg/mL, respectively).



QD-1.56

Where R = H, CH_3, Br, OCH_3, Cl, NO_2.

Sun et al. (2013) synthesized 7-alkyloxy-4, 5-dihydro-imidazo[1,2-a]quinoline derivatives (**QD-1.57**) and studied their antimicrobial activity. Most of the compounds exhibited potential antibacterial activity against gram-negative and gram-positive bacteria. The compound (7-heptyloxy-4,5-dihydro-imidazo[1,2-a]quinoline) showed excellent activity than that of reference agent Ciprofloxacin.



QD-1.57

Where R = Aryl, alkyl group.

1.4.5. Important structural features of the quinoline derivatives

From the structure activity relation, the Mono or disubstitutions at the 6, 7, or 8-position of the quinoline nucleus enhances activity relative to the unsubstituted compound, while substitution at the 2-position appears necessary to retard metabolic oxidation of the quinoline skeleton (Novotny et al. 1974).

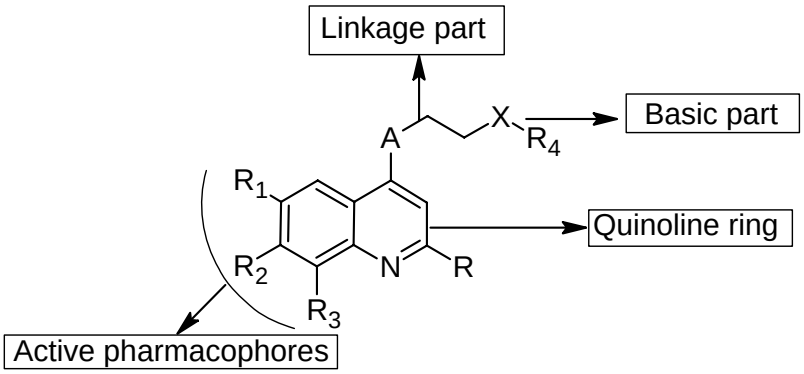


Figure 1.3. Important structural features of the quinoline derivatives.

On the basis of literature review, the structure-activity relationship of compounds with the structure represented in Figure (Figure 1.3) indicated that the basic nitrogen atom is responsible for the accumulation of the compounds in the food vacuoles. The structure-activity relationship study was based on (i) a quinoline ring, (ii) a linkage moiety (iii) hydrophobic moiety. Replacement of the quinoline ring by either a naphthyl or a phenyl ring resulted in the loss of activity. The activity of the compounds is also affected by the distance between the hydrophobic moiety and the basic nitrogen.

From the literature review it was clear that the molecules containing heterocyclic system are having versatile application in the field of medicinal chemistry. Still there is a serious call for innovation of new drug for many diseases.

Keeping this thing in mind it was planned to synthesize new heterocyclic derivatives containing quinoline active site, their characterization by various spectral techniques and to screen them for various biological activities.

1.5. SCOPE OF THE PRESENT WORK

Heterocyclic compounds have a wide range of applications in the modern era. They have been used in pharmaceuticals, agrochemicals and even in veterinary products. Heterocyclic systems viz., quinolines, pyrazoles, triazoles and oxdiazoles etc., have occupied an important place in the field of medicinal chemistry, agriculture and polymer industries. Molecular modification of the promising lead molecule is still a major line of approach for the discovery of new drug. Molecular modification involves substituting, eliminating or adding new moieties to a parent lead compound there by making gradual change in the physicochemical properties of the parent compound and thus biological activity of the compound.

The general observation is that certain structural units of medicinal compounds are also found in those other compounds having similar properties. It is a guiding thought in mapping out structures of further compounds with analogous activities, hopefully more potent, efficient, more specific and less toxic in nature. With that approach it was planned for studying certain heterocyclic systems wherein biologically active moieties are interlinked through active functional groups.

Synthetic design with a combination of heterocycles through active pharmacophor leading to interesting therapeutical drugs, will in turn is the main objective of this research. Quinoline and its derivatives, a class of well-known nitrogen containing heterocyclic compounds, occupy an important position in medicinal and pesticide chemistry with having a wide range of activities such as antibacterial, antifungal, antibiotic, anticancer, anticonvulsant, anti-tuberculosis and anti-inflammatory. Henceforth there is lot of scope for exploring the quinoline chemistry so as to synthesize a molecule of biological importance. The chemistry of quinoline compounds has evoked keen interest and considerable attention in pharmaceuticals. It has been reported that pyrazoles, oxadiazoles, imidazoles and triazole derivatives are some of active constituents of currently used drugs.

Development of resistance to existing drugs is a constant growing phenomenon that has concerned researchers throughout the world and now has reached alarming levels for certain infectious diseases. This combined with the recent decline in the development of new drugs to combat them, can be anticipated to lead to infectious diseases lacking ready treatment regimens. The aim of the present work is to synthesize and characterize new heterocyclic systems having quinoline moiety. These new compounds were screened for their biological activity such as antibacterial and antifungal activity. At present, development of a specific drug with two or more pharmacological properties and minimum toxicity is the dream of researchers in the field of medicinal chemistry.

1.6. OBJECTIVES OF THE WORK

The main objectives of the proposed research plan are as follows

- Synthesis of some new derivatives of quinoline carrying interesting pharmacophores like benzimidazole, carbohydrazide 1,3,4-oxadiazoles, 1,2,3-triazole and N-alkylated quinolones.
- Development of synthetic routes and purification methods for unknown compounds.
- Characterization of new compounds by IR, ^{1}H NMR, ^{13}C-NMR, Mass spectra, followed by elemental analysis. Single crystal x-ray diffraction studies of selected compounds for elucidation of final three-dimensional structure.
- Evaluation of biological studies of new compounds - This includes antibacterial and antifungal activity.

1.7. DESIGN OF NEW TARGETS

Quinolines are broad-spectrum antibiotic agents (effective for both gram negative and gram positive bacteria). The majority of quinoline antibiotics in clinical use belong to the second generation class of fluoroquinolones which are core quinoline framework and which have a fluorine atom attached to the central ring system, typically at the 6-position or C-7 position. During the past couple of decades, enough quinoline based antibiotic drugs have been launched. At present, many drugs carrying quinoline are being used as effective antibiotics in the market. In spite, lots of research activities are still continuing on the synthesis of new quinoline derivatives as potential antimicrobials.

According to the recent literature, fluorinated or trifluoromethyl containing quinoline derivatives are more effective for antimicrobial activity. Incorporation of a trifluoromethyl group instead of hydrogen atom can alter the biological activities. Introduction of trifluoromethyl group provides better electronic effect at neighboring carbon centers, as well as having a substantial effect on the molecule's dipole moment, acidity and basicity of neighboring groups. Fluorine is much more lipophilic than hydrogen, so incorporating of fluorine atoms in a molecule will make it more fat soluble and as a hydrogen bond accepter.

Recent observations suggested that, trifluoromethyl quinoline and its derivatives have wide range of applications in the field of pharmaceuticals as antimalarial, antibacterial, antifungal, antituberculosis, anticancer agents. Ciprofloxacin, Norfloxacin and Trovafloxacin are some of the drugs which contain fluorine group and quinoline core moiety. A detailed literature search reveals that the compounds carrying pharmacophores such as quinoline, quinolone, carbazone, oxadiazole and triazole moieties showed positive response against microbial infection induced by pathogenic microbes. Keeping this in view, it was planned to introduce the active pharmacophores at different positions of quinoline core ring. In view of this, new quinoline centered core ring and active pharmacophores at different position of quinoline was designed. The molecular modifications of new target are given below.

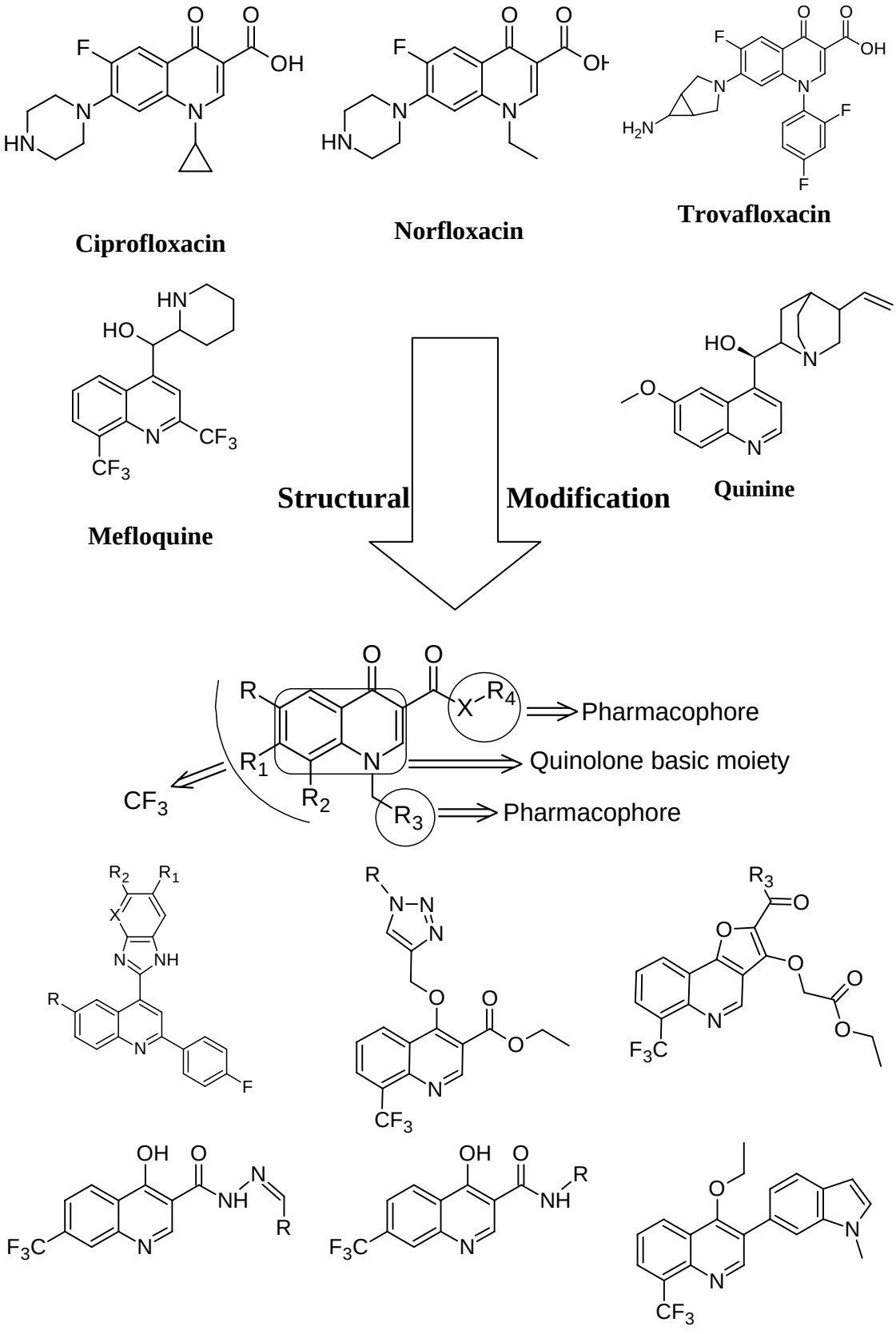


Figure 1.4. Quinoline-based commercial drugs and the modified compounds.

CHAPTER-2

SYNTHESIS AND CHARACTERIZATION OF QUINOLINE INCORPORATED BENZIMIDAZOLE DERIVATIVES

CHAPTER-2

SYNTHESIS AND CHARACTERIZATION OF QUINOLINE INCORPORATED BENZIMIDAZOLE DERIVATIVES

2.1. INTRODUCTION

Benzimidazole is a heterocyclic aromatic organic compound with molecular formula $C_7H_6N_2$. This bicyclic compound consists of the fusion of benzene and imidazole. The most prominent benzimidazole compound in nature is N-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B_{12}. The synthesis of novel benzimidazole derivatives and investigations of their chemical and biological behaviour have gained more importance in recent decades as they possess an extensive spectrum of pharmacological activities.

Azole class of drugs particularly fused imidazole's occupy prominent place in medicinal chemistry because of their broad spectrum of pharmacological activities such as anti-inflammatory, analgesic, anticancer, antiulcer, antimicrobial (Kus et al. 1996; Oren et al. 1999), antiviral, pesticidal, cytotoxicity and anti-arrhythmic (Mann et al. 2001; Sondhi et al. 2002; Horton et al. 2003; Gellis et al. 2008) activities. Omeprazole, Mebendazole, Pimobendan and Albendazole are well-known drugs in the market which contain fused imidazoles as active core moiety. Benzimidazole and its derivatives act on the cell membrane by targeting ergosterol, either by binding to the sterol, forming pores and causing the membrane to become leaky or inhibiting ergosterol biosynthesis.

The transition metal derivatives of heterocyclic imidazole (NHC ligands) are known in the field of organometallic chemistry for more than five decades, due to their pharmacological and catalytic activity. Imidazole carbene complexes have a rich history, starting with Wanzlick's and co-workers in 1970 to Hahn and co-workers at

the end of twentieth century. The carbene complexes of imidazole are well known for their antimicrobial (Ozdemir et al. 2010), anticancer (Iqbal et al. 2013), antimalarial (Hemmert et al. 2013) activity due their tuneable geometry around the central metal.

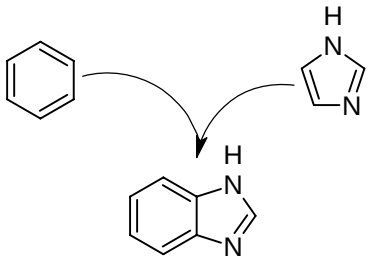
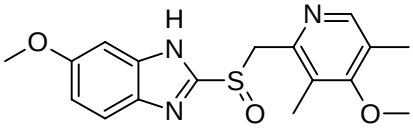
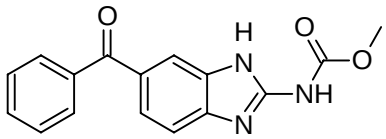


Figure 2.1. Structure of benzimidazole



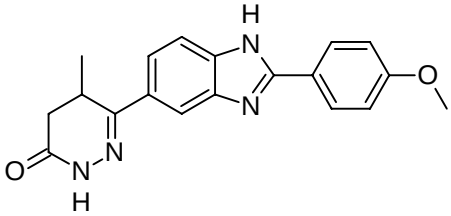
Omeprazole (ulcer disease)

QD-2.1



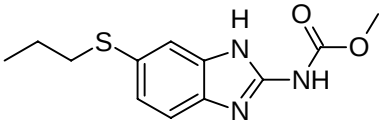
Mebendazole (worm infestations)

QD-2.2



Pimobendan (veterinary medication)

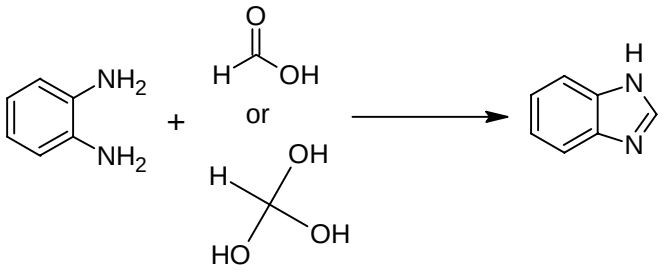
QD-2.3



Albendazole (worm infestations)

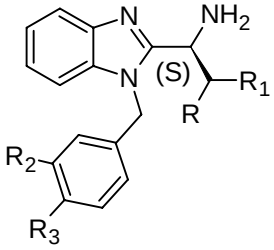
QD-2.4

Benzimidazole usual synthesis involves condensation of o-phenylenediamine with formic acid or Orthoformic acid.



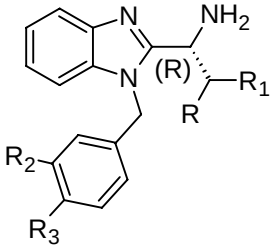
QD-2.5

Bauer et al. (2011) reported a simple and efficient approach for the preparation of biologically active benzimidazole derivatives (**QD-2.6 & QD-2.7**) by ecofriendly (polymer bounded) method. The newly synthesized compounds were screened for their *in-vitro* antifungal activity. It was noticed that (S)-2-aminoalkylbenzimidazoles (**QD-2.6**) are good antifungal agents as that of fluconazole.



Where R = R_1 = CH_3, R_2 = Cl, R_3 = H.

QD-2.6

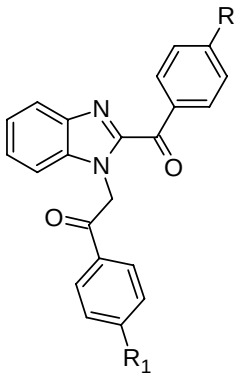


Where R = R_1 = CH_3, R_2 = Cl, R_3 = H.

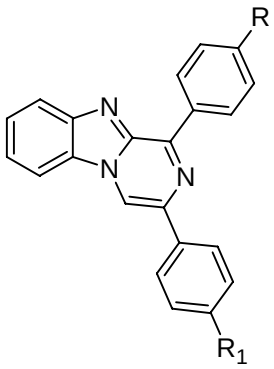
QD-2.7

R = H, CH_3, CH_2CH_3. R_1 = H, CH_3, CH_2CH_3. R_2 = H, F, Cl, CH_3, OCH_3. R_3 = H, Cl.

Synthesis of 1,3-diarylpyrazino[1,2-a]benzimidazole (**QD-2.8 & QD-2.9**) was reported by Demirayak et al. (2011). Novel analogues were screened for anticancer activity. Among the tested compound methoxy substitutions are more active than halogen and methyl substituted derivatives.



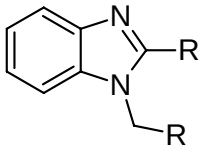
QD-2.8



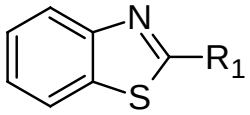
QD-2.9

R = H, OCH_3, Cl. R_1 = H, CH_3, OCH_3, F, Cl.

Bandyopadhyay et al. (2011) studied antibacterial activity of benzimidazole and benzothiazoles (**QD-2.10 & QD-2.11**). The antibacterial activity results revealed that Phenyl substituted benzimidazoles and ethyl, ethoxyphenyl substituted benzothiazoles are more potent antibacterial compounds.



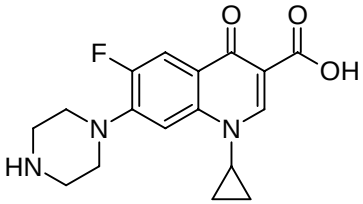
QD-2.10



QD-2.11

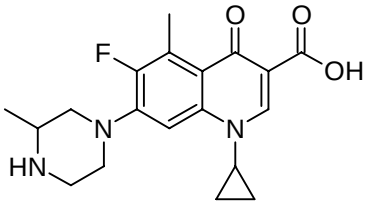
R = C_6H_5, 4-MeC_6H_4, 4-$(Me)_2NC_6H_4$, 4-$(Me)_3CC_6H_4$, 3-EtO-4-HOC_6H_3, 5-Br-2-HOC_6H_3, 4-FC_6H_4, 4-$F_3COC_6H_4$, 3,4-$Cl_2C_6H_3$, Furyl, C_6H_5CH=CH, CH_3-(CH_2))4-CH_2, 3-$NO_2C_6H_4$, 4-$F_3CC_6H_4$, 4-BrC_6H_4, 4-HOC_6H_4. R = 2-EtC_6H_4, 4-HOC_6H_4, 3,4,5-$(MeO)_3C_6H_2$, 4-$EtOC_6H_4$, 4-EtO-3-$MeOC_6H_3$, 5-Br-2-$MeOC_6H_3$, 4-$(Me)_2HCC_6H_4$, 4-$NO_2C_6H_4$.

The development of potent and effective antimicrobial agent is most important to overcome the emerging multi-drug resistance strains of bacteria and fungi such as Methicilin resistant *Staphylococcus aureus* (MRSA) (Chu et al. 1996; Chua et al. 2008). Heterocyclic compounds play an important role in developing new antimicrobial, anticancer, antimalarial, anticonvulsant agents. Recent observations suggested that, quinolines and substituted benzimidazoles have wide range of applications in the field of pharmaceuticals as anticancer (Chen et al. 2004), antimalarial (Nasveld and Kitchener. 2005; Kaur et al. 2010), anti-tuberculosis (Eswaran et al. 2010), antitumor (Yamato et al. 1989; Chou et al. 2010), analgesic, anti-inflammatory (Gaba et al. 2010; Achar et al. 2010), antimicrobial and antiviral (Eswaran et al. 2009; Shingalapur et al. 2009; Vachharajani et al. 2011) agents. The known antibacterial drugs from this family include Ciprofloxacin (**QD-2.12**), Grepafloxacin (**QD-2.13**) which contain quinoline as a core moiety are given below.



Ciprofloxacin (antibacterial)

QD-2.12



Grepafloxacin (antibacterial)

QD-2.13

Literature review revealed that, insertion of pharmacophore at position 4 of quinoline with substituted amines enhances its anti-tuberculosis, antimicrobial activity

(Eswaran et al. 2010). Substituted quinolines at 2 and 3 positions with various benzimidazole derivatives showed excellent pharmacological properties like antimicrobial, antifungal and antitumor activities (Mungra et al. 2011; Perin et al. 2011). Prompted by these observations it was planned to some new benzimidazole derivatives containing substituted quinoline nucleus with the hope of improving its biological properties. These newly synthesized compounds were characterized by spectral and elemental analysis and also were evaluated for their antimicrobial properties.

2.2. MATERIALS AND METHODS

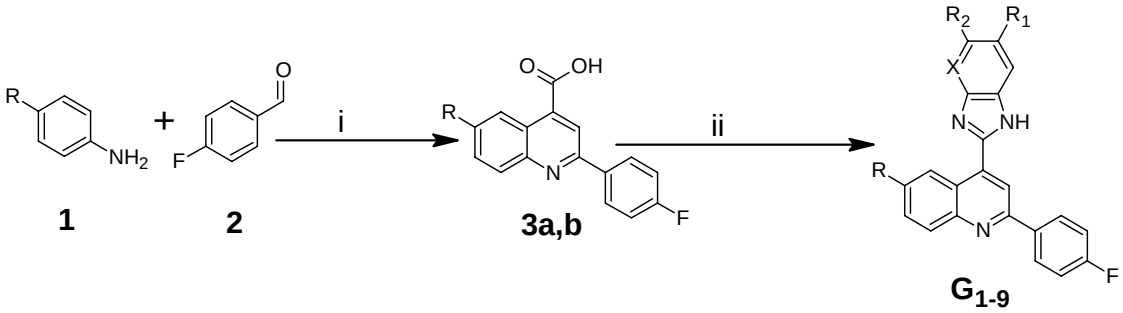
All the chemicals were purchased from Sigma Aldrich, Merck and S. D. Fine chemicals-India. Commercial grade solvents were used and were distilled before use. Melting points were determined by open capillary method and were uncorrected. Newly synthesized compounds were characterized by IR, NMR, mass spectral and C, H, N elemental analyses. The IR spectra (in KBr pellets) were recorded on a JASCO FT/IR-4100 spectrophotometer and Nicolet Avatar 5700 FTIR spectrophotometer (Neat). Bruker, Varian (400 MHz) spectrometer was used to record ^{1}H NMR and ^{13}C NMR spectra (DMSO-d_6, $CDCl_3$) using TMS as internal standard. Chemical shift values were given in δ (ppm) scales. The mass spectra were recorded on LC-MS-Agilent 1100 series and elemental analysis was performed on a Flash EA 1112 series CHNS-O Analyzer. X-Ray diffraction studies were carried on a Bruker SMART APEXII DUO CCD diffractometer. The completion of the reactions was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254). The names of the structures were given as per chemdraw and chemsketch.

2.3. RESULTS AND DISCUSSION

2.3.1. Synthesis of quinoline incorporated benzimidazole derivatives

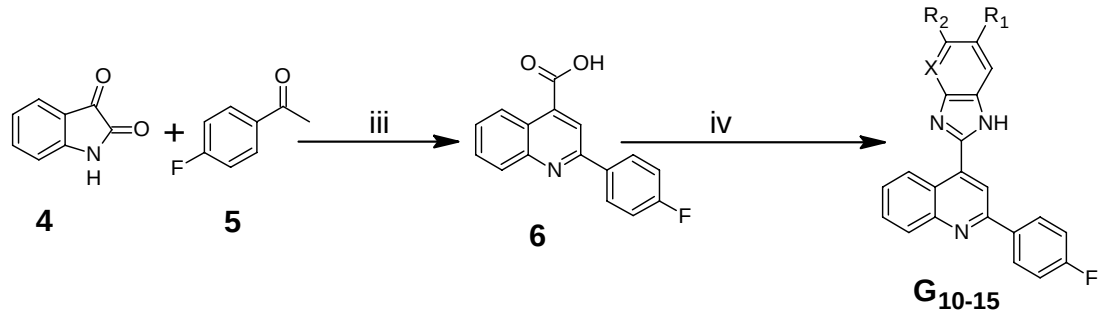
6-Substituted-4-carboxyquinolines (**3a, b**) was synthesized by Doebner reaction using substituted aniline (**1**), 4-fluorobenzaldehyde (**2**), pyruvic acid and a catalytic amount of trifluoroacetic acid in ethanol media. The versatile Pfitzinger reaction was utilized to synthesize 4-carboxyquinoline (**6**) in satisfactory yields by reacting isatin (**4**) with α-methylketone (**5**) in aqueous ethanol (Ivachtchenko et al. 2004); Metwally

et al. 2006; Zarghi et al. 2009). The targeted quinoline incorporated benzimidazole derivatives (**G_{1-9}** and **G_{10-15}**) were synthesized by reacting 6-substituted-4-carboxyquinolines (**3a, b** and **6**) with various aromatic-1,2-diamines in polyphosphoric acid (PPA) media. The crude products were purified by column chromatography. The reaction pathway has been summarized in **Scheme-2.1** and **Scheme-2.2**.



Where R= H, Cl, F; R_1= Cl, F; R_2= H, Cl; X= CH, N

Scheme 2.1. Synthetic route for the compounds G_{1-9}. (i) Pyruvic acid, EtOH, TFA, 13 h; (ii) Aromatic-1,2-diamine, PPA, 250 °C, 2 h.



Where R_1= H, Cl, F; R_2=,H Cl; X= CH, N

Scheme 2.2. Synthetic route for the compounds G_{10-15}. (iii) EtOH (33% KOH), 48%; (iv) Aromatic-1,2-diamine, PPA, 250 °C, 2 h.

Formation of 6-substituted-4-carboxyquinolines (**3a, b** and **6**) was confirmed by recording their IR, ^{1}H NMR, ^{13}C NMR and mass spectra. The formation of quinoline acid (**3a**) was confirmed by the peak at 3384 cm^{-1} in IR spectrum which is due to the -OH stretching of carboxylic acid. Band at 1718 cm^{-1} is due to C=O stretch of acid group. The ^{1}H NMR spectrum of **3a** showed a singlet at δ 14.77 corresponds

to acid -OH proton of quinoline. A singlet at δ 8.53 is due to quinoline-3H proton, 4-fluorophenyl aromatic protons appeared as multiplet at δ 6.98-7.25 further confirmed the structure of the compound. The mass spectrum of **3a** showed a molecular ion peak at m/z = 302 (M+1), which is in agreement with the molecular formula $C_{16}H_9ClFNO_2$.

Table-2.1. Characterization data of the compounds $G_{1\text{-}9}$ and $G_{10\text{-}15}$

Comp. No	R	R_1	R_2	X	Mol. Wt	Mol. Formula	M.p. (°C)	Yield (%)
G_1	Cl	H	H	H	373.82	$C_{22}H_{13}ClN_3$	241-241	60
G_2	Cl	Cl	H	H	408.26	$C_{22}H_{12}Cl_2FN_3$	197-198	50
G_3	Cl	H	H	N	374.80	$C_{21}H_{12}ClFN_4$	266-267	58
G_4	Cl	Cl	Cl	H	442.70	$C_{22}H_{11}Cl_3FN_3$	165-166	40
G_5	F	H	H	H	357.36	$C_{22}H_{13}F_2N_3$	218-219	54
G_6	F	Cl	H	H	391.80	$C_{22}H_{12}ClF_3N_3$	139-139	52
G_7	F	H	H	N	358.34	$C_{21}H_{12}F_2N_4$	132-133	51
G_8	F	Cl	Cl	H	426.25	$C_{22}H_{11}Cl_2F_2N_3$	108-109	42
G_9	F	F	H	H	375.35	$C_{22}H_{12}F_3N_3$	97-98	40
G_{10}	H	H	H	H	339.38	$C_{22}H_{14}FN_3$	164-165	78
G_{11}	H	Cl	H	H	373.81	$C_{22}H_{13}ClFN_3$	234-235	65
G_{12}	H	H	H	N	340.35	$C_{21}H_{13}FN_4$	128-129	68
G_{13}	H	Cl	Cl	H	408.26	$C_{22}H_{12}Cl_2FN_3$	205-206	57
G_{14}	H	F	H	H	357.36	$C_{22}H_{13}ClF_2N_3$	77-78	55
G_{15}	H	OMe	H	H	369.39	$C_{23}H_{16}FN_3O$	74-75	25

Formation of 4-(1H-benzimidazol-2-yl)-2-(4-fluorophenyl)Quinoline (**G_{10}**) was confirmed by the presence of absorption peak at 3307 cm^{-1} in IR spectrum which is due to -NH stretching of benzimidazole. Band at 1690 and 1613 is due to C=N and C=C of benzimidazole, respectively. The ^1H NMR spectrum of compound **G_{10}** showed a broad singlet at δ 13.33 (D_2O exchangeble) which is due to benzimidazole -NH. Singlet at δ 8.66 is due to quinoline -3H, multiplet at δ 7.69-7.91 is due to aromatic protons of benzimidazole ring and multiplet at δ 7.31- 7.50 is due to protons

of 4-fluorophenyl moiety confirmed the structure. The mass spectrum of **G_{10}** showed a molecular ion peak at m/z = 340 (M+1), which is in agreement with the molecular formula $C_{22}H_{14}FN_3$. Similarly the spectral values for all the compounds and C, H, N analyses are presented in the experimental part and the characterization data are provided in **Table-2.1**.

2.4. EXPERIMENTAL

2.4.1. General procedure for the synthesis of 6-substituted-4-carboxyquinoline (3a-b)

An equimolar mixture of 4-substituted aniline (**1**) (10.0 g, 0.078 mol), 4-fluorobenzaldehyde (**2**) (9.72 g, 0.078 mol) in ethanol (100 mL) were refluxed for 1 h, pyruvic acid (10.30 g, 0.117 mol) and trifluoroacetic acid (TFA) (1 mL) were then added to the reaction mass and further refluxed for 12 h. After completion of the reaction, the reaction mixture was poured into ice-cold water. The solid product obtained was filtered, washed with water and recrystallized using ethanol.

2.4.2. Preparation of 2-(4-fluorophenyl)quinoline-4-carboxylic acid (6)

A mixture of isatin (**4**) (10.0 g, 0.067 mol), 100 mL 33% alcoholic potassium hydroxide solution and 4-fluoroacetophenone (**5**) (10.32 g, 0.074 mol) were refluxed for 48 h. After completion of the reaction, the reaction mass was concentrated by distillation and the residue obtained was acidified with 15 % acetic acid. The solid precipitated was filtered, washed with 5 % acetic acid, ethanol, hexane and crystallized in methanol.

2.4.3. General method for the preparation of 4-substituted benzimidoquinoline (G_{1-9} and G_{10-15})

A mixture of 6-substituted-4-carboxyquinoline (**3a, b** and **6**) (0.01 mol), aromatic-1,2-diamine (0.01 mol) and fresh polyphosphoric acid (PPA) (2 mL) were heated to 250 °C for 2 h. The reaction mixture was then poured into 20 mL of 30% aqueous sodium carbonate solution. The solid product obtained was filtered and dried. The crude products were purified by column chromatography using pet ether and ethyl acetate (9:1) as the eluent.

2.5. CHARACTERIZATION OF SYNTHESIZED COMPOUNDS

2.5.1. (6-Chloro-2-(4-fluorophenyl)quinoline-4-carboxylic acid (3a)

Yield: 15.20 g, 64.4%; M.p: 210-211 °C; IR (KBr ν_{max} cm^{-1}): 3384 (O-H-str), 3062, 2983 (C-H-str), 1718 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.98-7.25 (m, 4H, 4-fluorophenyl), 8.49 (d, 2H, quinolone, J = 8.2 Hz), 8.53 (s, 1H, quinoline), 8.74 (m, 1H, quinoline), 14.77 (s, 1H, COOH, D_2O-exchangeble); MS: m/z = 302 (M+1); Anal. calcd. For $C_{16}H_9ClFNO_2$: C, 67.70; H, 3.01; N, 4.64. Found: C, 67.68; H, 3.00; N, 4.67%.

2.5.2. 6-Fluoro-2-(4-fluorophenyl)quinoline-4-carboxylic acid (3b)

Yield: 9.5 g, 36.8%; M.p: 187-188 °C; IR (KBr ν_{max} cm^{-1}): 3400 (O-H-str), 3068, 2981 (C-H-str), 1681 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.90-8.18 (m, 4H, 4-fluorophenyl), 8.49-8.53 (m, 2H, quinoline), 8.54 (s, 1H, quinoline), 8.54 (m, 1H, quinoline), 14.10 (s, 1H, COOH, D_2O-exchangeble); MS: m/z =385; Anal. calcd. For $C_{16}H_9F_2NO_2$: C, 67.37; H, 3.18; N, 4.91. Found: C, 67.70; H, 3.16; N, 4.68%.

2.5.3. 2-(4-Fluorophenyl)quinoline-4-carboxylic acid (6)

Yield: 11.30 g, 62.2%; M.p: 155-156 °C; IR (KBr ν_{max} cm^{-1}): 3400 (O-H-str), 3051, 2989 (C-H-str), 1703 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.09-7.64 (m, 2H, 4-fluorophenyl), 7.68-7.88 (m, 2H, quinoline), 8.76 (d, 2H, 4-fluorophenyl, J = 8.3 Hz), 8.34 (m, 1H, quinoline), 8.45 (s, 1H, quinoline), 8.59-8.61 (m, 1H, quinoline), 14.11 (s, 1H, COOH, D_2O-exchangeble); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.4, 167.2, 162.2, 160.8, 147.9, 134.0, 131.3, 131.0, 130.5, 129.8, 129.6, 129.5, 129.3, 125.6, 123.1, 114.2; MS: m/z = 268 (M+1); Anal. calcd. For $C_{16}H_9ClFNO_2$: C, 71.91; H, 3.77; N, 5.24. Found: C, 72.10; H, 2.98; N, 5.23%.

2.5.4. 4-(1H-Benzimidazol-2-yl)-6-chloro-2-(4-fluorophenyl)Quinoline (G_1)

IR (KBr ν_{max} cm^{-1}): 3356 (-NH stretching for benzimidazole ring), 3087, 2927 (C-H-str), 1621 (C=N), 1597 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28-7.46 (m, 2H, 4-fluorophenyl), 7.67-7.73 (m, 2H, benzimidazole), 7.75-7.88 (m, 2H, 4-fluorophenyl), 8.18 (d, 2H, quinolone, J = 8.2 Hz), 8.44-8.48 (m, 2H, benzimidazole), 8.63 (s, 1H, quinoline), 9.46 (s, 1H, quinoline), 13.45 (s, 1H, -NH, D_2O-exchangeble);

MS: m/z = 374 (M+1). Anal. calcd. For $C_{22}H_{13}FN_3$; Calc: C, 70.69; H, 3.51; N, 11.24; found: C, 70.70; H, 3.50; N, 11.26%.

2.5.5. 6-Chloro-4-(5-chloro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl)quinoline (G_2)

IR (KBr ν_{max} cm^{-1}): 3342 (-NH stretching for benzimidazole ring), 3082, 2935 (C-H-str), 1597 (C=N), 1556 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45-7.87 (m, 4H, 4-fluorophenyl), 7.90 (d, 2H, benzimidazole, J = 8.2 Hz), 8.45 (s, 1H, quinoline), 8.72 (s, 1H, benzimidazole), 8.72 (s, 1H, quinoline), 8.69 (s, d = 8.2 Hz, 2H, quinoline), 13.44 ((s, 1H, -NH, D_2O-exchangeble); MS: m/z = 409 (M+1). Anal. calcd. For $C_{22}H_{12}Cl_2FN_3$; Calc: C, 64.72; H, 2.96; N, 10.29; found: C, 64.75; H, 2.99; N, 10.20%.

2.5.6. 6-Chloro-2-(4-fluorophenyl)-4-(1H-imidazo[4,5]pyridin-2-yl)quinoline (G_3)

IR (KBr ν_{max} cm^{-1}): 3400 (-NH stretching for benzimidazole ring), 3057, 2929 (C-H-str), 1592 (C=N), 1538 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45-7.90 (m, 4H, 4-fluorophenyl), 8.48-8.55 (m, 2H, benzimidazole), 8.73 (s, 1H, quinoline), 8.82 (s, 1H, quinoline), 9.62-9.66 (m, 1H, benzimidazole), 9.84 (d, 2H, quinoline, J = 8.2 Hz), 13.59 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 375 (M+1). Anal. calcd. For $C_{21}H_{12}ClFN_4$; Calc: C, 67.30; H, 3.23; N, 14.95; found: C, 67.34; H, 3.26; N, 14.93%.

2.5.7. 6-Chloro-4-(5,6-dichloro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl) quinoline (G_4)

IR (KBr ν_{max} cm^{-1}): 3362 (-NH stretching for benzimidazole ring), 3054, 2911 (C-H-str), 1627 (C=N), 1550 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.47-7.90 (m, 4H, 4-fluorophenyl), 8.22 (s, 1H, quinoline), 8.45 (s, 1H, quinoline), 8.48 (d, 2H, quinolone, J = 8.3 Hz), 9.58 (s, 1H, benzimidazole), 9.61 (s, 1H, benzimidazole), 13.46 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 443 (M+1). Anal. calcd. For $C_{22}H_{11}Cl_3FN_3$; Calc: C, 59.69; H, 2.50; N, 9.49; found: C, 59.71; H, 2.51; N, 9.55%.

2.5.8. 4-(1H-Benzimidazol-2-yl)-6-fluoro-2-(4-fluorophenyl)quinoline (G_5)

IR (KBr ν_{max} cm^{-1}): 3409, (-NH stretching for benzimidazole ring), 3066, 2925 (C-H-str), 1605 (C=N), 1552 (C=C); ^{1}H NMR (DMSO-d_6) δ: 7.28-7.47 (m, 4H, 4-fluorophenyl), 7.66-7.75-7.81 (m, 2H, benzimidazole), 7.82-7.86 (m, 2H, benzimidazole), 8.45-8.47 (m, 2H, quinoline), 8.76 (s, 1H, quinoline), 9.33-9.40 (m, 1H, quinoline), 13.53 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 358 (M+1). Anal. calcd. For $C_{22}H_{13}F_2N_3$; Calc: C, 73.94; H, 3.67; N, 11.76; found: C, 73.97; H, 3.68; N, 11.77%.

2.5.9. 4-(5-Chloro-1H-benzimidazol-2-yl)-6-fluoro-2-(4-fluorophenyl)quinoline (G_6)

IR (KBr ν_{max} cm^{-1}): 3354 (-NH stretching for benzimidazole ring), 3020, 2979 (C-H-str), 1603 (C=N), 1507 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.34-7.70 (m, 4H, 4-fluorophenyl), 8.26-8.28 (m, 1H, quinoline), 8.47 (s, 1H, quinoline), 8.49 (d, 2H, benzimidazole, J = 8.5 Hz), 8.76 (s, 1H, benzimidazole), 9.42-9.45 (m, 2H, quinoline), 13.45 ((s, 1H, -NH, D_2O-exchangeble); MS: m/z = 392 (M+1). Anal. calcd. For $C_{22}H_{12}ClF_2N_3$; Calc: C, 67.44; H, 3.09; N, 10.72; found: C, 67.42; H, 3.08; N, 10.75%.

2.5.10. 6-Fluoro-2-(4-fluorophenyl)-4-(1H-imidazo[4,5]pyridin-2-yl)quinoline (G_7)

IR (KBr ν_{max} cm^{-1}): 3403 (-NH stretching for benzimidazole ring), 3028, 2921 (C-H-str), 1623 (C=N), 1540 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.39-7.48 (m, 4H, 4-fluorophenyl), 7.50-7.53 (m, 2H, quinoline), 7.83-7.85 (m, 1H, quinoline), 8.28-8.48 (m, 2H, benzimidazole), 8.51 (s, 1H, quinoline), 9.38-9.43 (m, 1H, benzimidazole), 13.73 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 359 (M+1). Anal. calcd. For $C_{21}H_{12}F_2N_4$; Calc: C, 70.39; H, 3.38; N, 15.65; found: C, 70.41; H, 3.35; N, 15.66%.

2.5.11. 4-(5,6-Dichloro-1H-benzimidazol-2-yl)-6-fluoro-2-(4-fluorophenyl) quinoline (G_8)

IR (KBr ν_{max} cm^{-1}): 3355 (-NH stretching for benzimidazole ring), 3057, 2932 (C-H-str), 1660 (C=N), 1539 (C=C); ^1H NMR (400 MHz, DMSO-d_6): δ 7.38-7.83 (m, 4H, 4-fluorophenyl), 8.26 (s, 1H, quinoline), 8.46-8.48 (m, 2H, quinoline), 8.75-8.76 (m, 1H, quinoline), 9.42 (s, 1H, benzimidazole), 9.46 (s, 1H, benzimidazole), 13.42 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 427 (M+1). Anal. calcd. For $C_{22}H_{11}Cl_2F_2N_3$; Calc: C, 61.99; H, 2.60; N, 9.86; found: C, 61.98; H, 2.55; N, 9.89%.

2.5.12. 6-Fluoro-4-(5-fluoro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl)quinoline (G_9)

IR (KBr ν_{max} cm^{-1}): 3400 (-NH stretching for benzimidazole ring), 3057, 2928 (C-H-str), 1584 (C=N), 1540 (C=C); ^1H NMR (400 MHz, DMSO-d_6): δ 7.17-7.73 (m, 4H, 4-fluorophenyl), 7.98-8.28 (m, 2H quinoline), 8.45-8.48 (m, 2H, benzimidazole), 8.74 (s, 1H, quinoline), 9.33-9.40 (m, 2H, benzimidazole), 13.53 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 376 (M+1). Anal. calcd. For $C_{22}H_{12}F_3N_3$; Calc: C, 70.40; H, 3.22; N, 11.20; found: C, 70.45; H, 3.20; N, 11.23%.

2.5.13. 4-(1H-Benzimidazol-2-yl)-2-(4-fluorophenyl)Quinoline (G_{10})

IR (KBr ν_{max} cm^{-1}): 3307 (-NH stretching for benzimidazole ring), 3035, 2972 (C-H-str), 1690 (C=N), 1613 (C=C); ^1H NMR (400 MHz, DMSO-d_6): δ 7.31-7.50 (m, 4H, 4-fluorophenyl), 7.69-7.77 (m, 2H, quinoline), 7.89-8.90 (m, 2H, benzimidazole), 8.18 (d, 1H, benzimidazole, J = 8.3 Hz), 8.46-8.50 (m, 2H, quinoline), 8.65 (s, 1H, quinoline), 9.46 (d, 1H, benzimidazole, J = 8.4 Hz) 13.33 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 340 (M+1). Anal. calcd. For $C_{22}H_{14}FN_3$; Calc: C, 77.86; H, 4.16; N, 12.38; found: C, 77.89; H, 4.14; N, 12.40%.

2.5.14. 4-(5-Chloro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl)quinoline (G_{11})

IR (KBr ν_{max} cm^{-1}): 3325 (-NH stretching for benzimidazole ring), 3066, 2928 (C-H-str), 1601 (C=N), 1508 (C=C); ^1H NMR (400 MHz, DMSO-d_6): δ 7.30-7.73 (m, 4H, 4-fluorophenyl), 7.76-8.44 (m, 4H, quinoline), 8.45-8.48 (m, 2H, benzimidazole), 8.63 (s, 1H, quinoline), 9.46 (s, 1H, benzimidazole), 13.35 (s, 1H, -NH, D_2O-

exchangeble); MS: m/z = 374 (M+1). Anal. calcd. For $C_{22}H_{13}ClFN_3$; Calc: C, 70.69; H, 3.51; N, 11.24; found: C, 70.67; H, 3.53; N, 11.25%.

2.5.15. 2-(4-Fluorophenyl)-4-(1H-imidazo[4,5]pyridin-2-yl)quinoline (G_{12})

IR (KBr ν_{max} cm^{-1}): 3400 (-NH stretching for benzimidazole ring), 3021, 2925 (C-H-str), 1660 (C=N), 1539 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.39-7.77 (m, 4H, 4-fluorophenyl), 7.91-8.31 (m, 2H, benzimidazole), 8.49-8.52 (m, 4H, quinoline), 8.74 (s, 1H, quinoline), 9.48 (m, 1H, benzimidazole), 13.63 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 341 (M+1). Anal. calcd. For $C_{21}H_{13}FN_4$; Calc: C, 74.11; H, 3.85; N, 16.46; found: C, 74.15; H, 3.82; N, 16.47%.

2.5.16. 4-(5,6-Dichloro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl)quinoline (G_{13})

IR (KBr ν_{max} cm^{-1}): 3453 (-NH stretching for benzimidazole ring), 3102, 3015 (C-H-str), 1622 (C=N), 1539 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45-7.76 (m, 4H, 4-fluorophenyl), 7.88-8.47 (m, 4H, quinoline), 8.65 (s, 1H, quinoline), 9.36 (s, 1H, benzimidazole), 9.38 (s, 1H, benzimidazole), 13.66 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 409 (M+1). Anal. calcd. For $C_{22}H_{12}Cl_2FN_3$; Calc: C, 64.72; H, 2.96; N, 10.29; found: C, 64.70; H, 2.92; N, 10 35%.

2.5.17. 4-(5-Fluoro-1H-benzimidazol-2-yl)-2-(4-fluorophenyl)quinoline (G_{14})

IR (KBr ν_{max} cm^{-1}): 3444 (-NH stretching for benzimidazole ring), 3057, 2919 (C-H-str), 1568 (C=N), 1539 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.46-7.77 (m, 4H, 4-fluorophenyl), 7.87-8.20 (m, 4H, quinoline), 8.46-8.49 (m, 1H, benzimidazole), 8.64 (s, 1H, quinoline), 9.39-9.44 (m, 2H, benzimidazole), 13.53 (s, 1H, -NH, D_2O-exchangeble); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.53, 162.67, 155.20, 149.05, 130.74, 130.15, 130.08, 130.00, 127.91, 127.19, 126.68, 124.16, 119.84, 118.74, 116.45, 116.23. MS: m/z = 358 (M+1). Anal. calcd. For $C_{22}H_{13}F_2N_3$; Calc: C, 73.94; H, 3.67; N, 11.76; found: C, 73.97; H, 3.65; N, 11.77%.

2.5.18. 2-(4-Fluorophenyl)-4-(6-methoxy-1H-benzimidazol-2-yl)quinoline (G_{15})

IR (KBr ν_{max} cm^{-1}): 3359 (-NH stretching for benzimidazole ring), 3015, 2924 (C-H-str), 1660 (C=N), 1540 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.01 (s, 3H, -OCH_3), 7.41-7.77 (m, 4H, 4-fluorophenyl), 7.89-8.18 (m, 2H, benzimidazole), 8.20-

8.41 (m, 3H, quinoline), 8.51 (s, 1H, benzimidazole), 9.06 (s, 1H, quinoline), 9.46-9.49 (m, 1H, quinoline), 13.36 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 370 (M+1). Anal. calcd. For $C_{23}H_{16}FN_3O$; Calc: C, 74.78; H, 4.37; N, 11.38; found: C, 74.75; H4.39; N, 11.39%.

2.6. ANALYTICAL DATA

Crystal data	
Empirical formula	$C_{22}H_{13}ClFN_3$
Formula weight	373.81
Crystal system	Triclinic
Crystal dimension	0.20 mm x 0.18 mm x 0.10 mm
Space group	$P\bar{1}$
a(Å)	12.768(5)
b(Å)	17.285(6)
c(Å)	22.636(8)
Angle α, β, γ	97.101, 98.548, 93.455
Temperature (T)	296K
Radiation wavelength (Å)	0.71073
Radiation type	Mo Kα
Radiation source	fine-focus sealed tube
Radiation monochromator	graphite

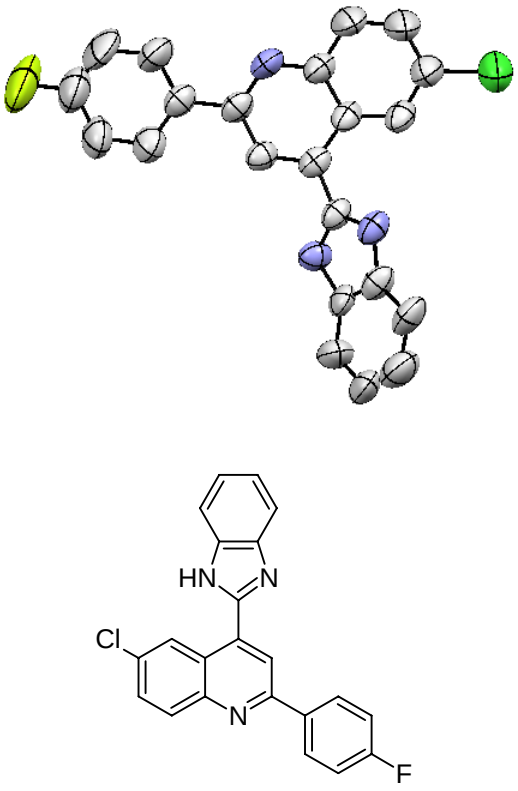


Figure 2.2. ORTEP diagram showing the single crystal structure of compound G_1 (drawn at 50% probability level).

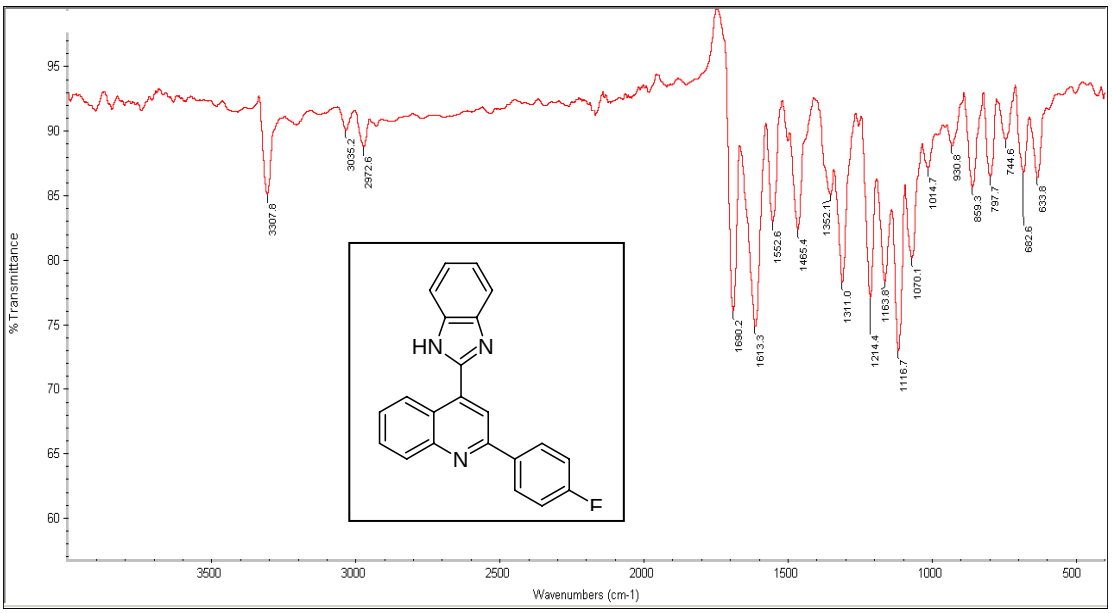


Figure 2.3. IR spectrum of G_{10}

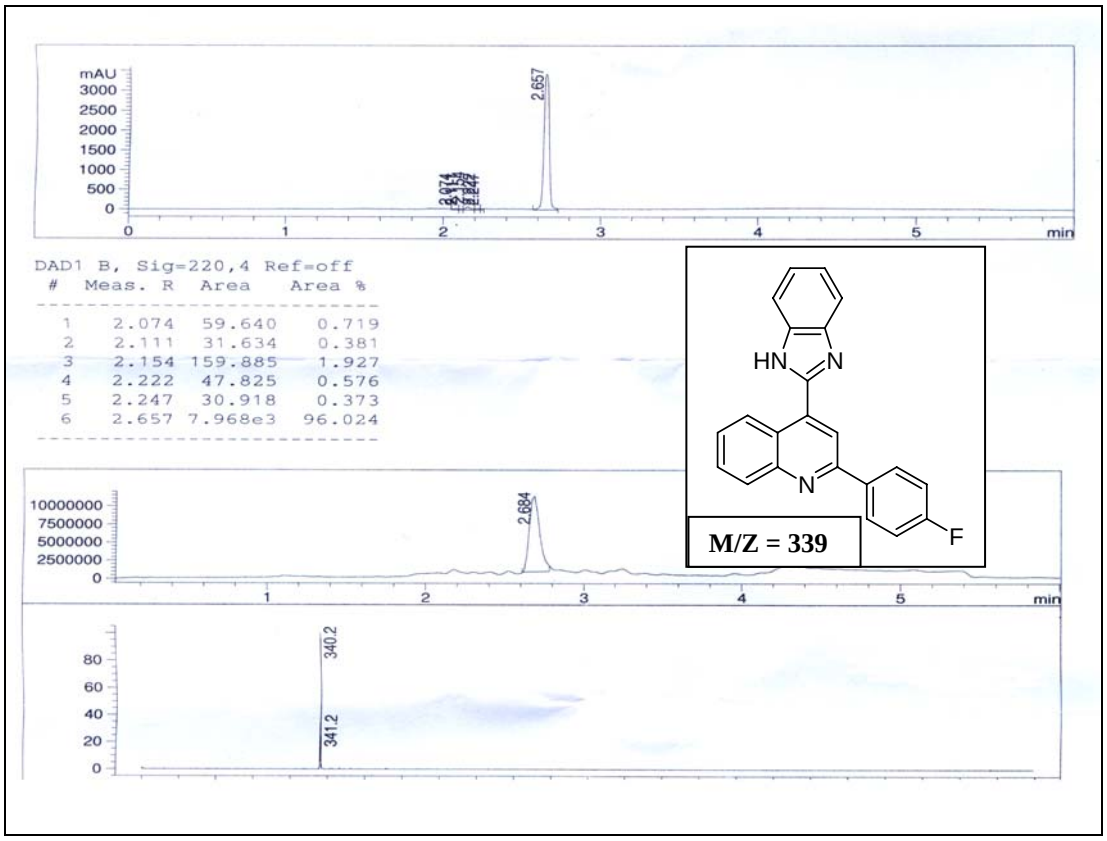


Figure 2.4. LCMS spectrum of G_{10}

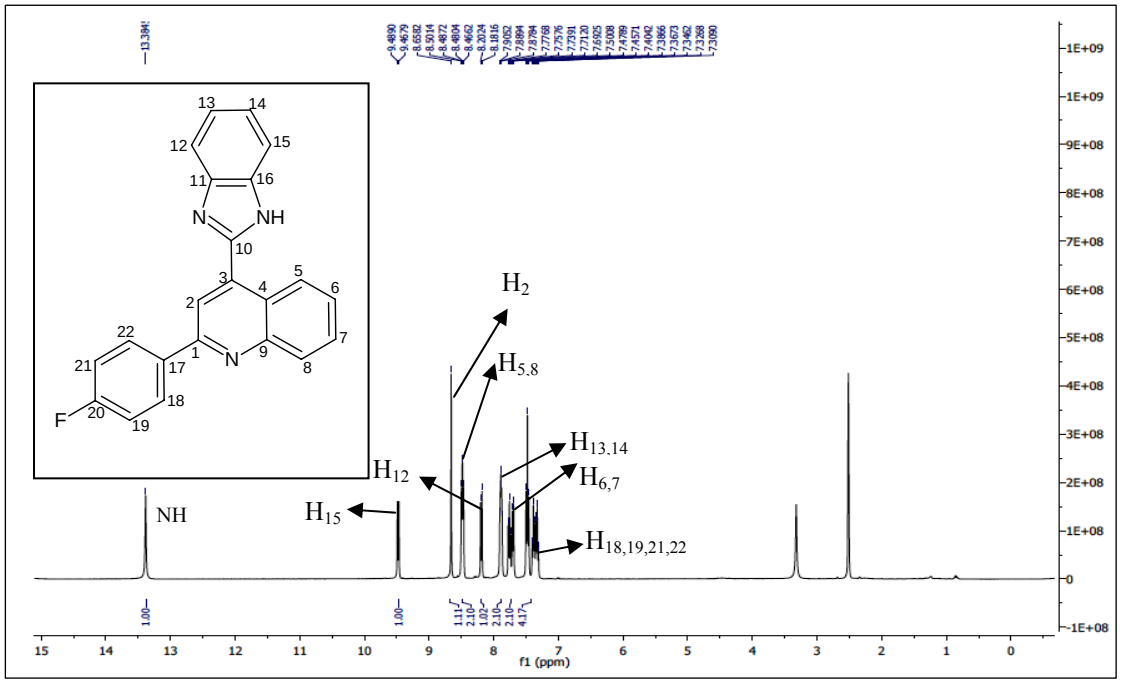


Figure 2.5. ^{1}H NMR spectrum of G_{10}

2.7. CONCLUSION

Two new series of quinoline incorporated benzimidazole derivatives (**G_{1-9}** and **G_{10-15}**) were synthesized from substituted aniline and isatin through multi-step reaction. 6-Substituted-4-carboxyquinolines (**3a, b** and **7**) were synthesized by multi component one pot reactions (via Doebner reaction and Pfitzinger reaction respectively) and the targeted benzimidazole derivatives were obtained by the reaction of 6-substituted-4-carboxyquinolines (**3a, b** and **7**) with substituted aromatic diamines in acidic media. Compounds which have substitution on benzimidazoquinoline were showed lesser yield compared with the unsubstituted derivatives. All the newly synthesized compounds were characterized by IR, NMR, mass spectral study and also by C, H, N analyses. Three-dimensional structure of compound **G_1** was confirmed by single crystal X-ray study. The final compounds were screened for their *in-vitro* antibacterial and antifungal activities by well plate method (zone of inhibition) and the results were discussed in CHAPTER-7.

CHAPTER-3

SYNTHESIS AND CHARACTERIZATION OF 7-TRIFLUOROMETHYL QUINOLINE-3-CARBOHYDRAZIDE AND 1,3,4-OXADIAZOLES DERIVATIVES

CHAPTER-3

SYNTHESIS AND CHARACTERIZATION OF 7-TRIFLUOROMETHYL QUINOLINE-3-CARBOHYDRAZIDE AND 1,3,4-OXADIAZOLES DERIVATIVES

3.1. INTRODUCTION

Pyrazole is a class of simple aromatic compound of the heterocyclic series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions. Pyrazole was first synthesized by German chemist Hans von Pechmann in 1898 using acetylene and diazomethane. It can be synthesized using various reagents, they are cyclisation of 1,3-diketone and hydrazine hydrate (Heller et al. 2006), condensation of substituted aromatic aldehydes and tosylhydrazine followed by cycloaddition with terminal alkynes (Wu et al. 2012), [3+2] annulation approach from arynes and hydrazones (Li et al. 2012).

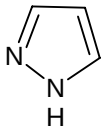
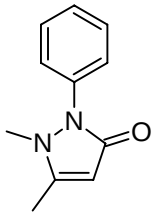
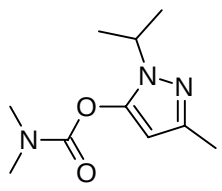


Figure 3.1. Structure of pyrazole

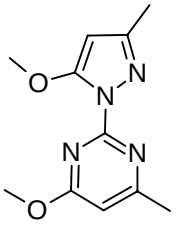
It is having a diverse biological activity when compared to other compounds. They are known for their anti-inflammatory**,** analgesic, antipyretic, anti-arthritic, fungicidal, antibacterial, antitubercular, anticancer and anti-HIV agent. The known drugs from this family include Phenazone, Isolan, Epirizole, Celecoxib.



Phenazone (Analgesic drug)
QD-3.1

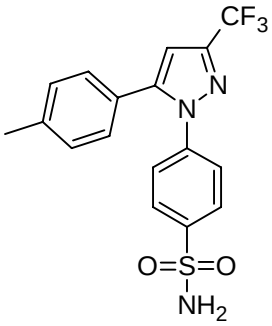


Isolan (Insecticide)
QD-3.2



Epirizole (Analgesic drug)

QD-3.3



Celecoxib (Anti-inflammatory drug)

QD-3.4

Oxadiazole is a heterocyclic aromatic compound with the molecular formula $C_2H_2N_2O$. There are four isomers of oxadiazole, 1,2,4-Oxadiazole, 1,2,5-oxadiazole, and 1,3,4-oxadiazole are known, but the 1,2,3-isomer is unstable. The stable oxadiazoles appear in a variety of pharmaceutical drugs including Raltegravir, Butalamine, Fasiplon, Oxolamine, and Pleconaril.

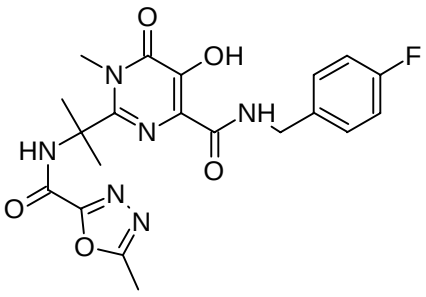
1,2,4-oxadiazole

1,2,5-oxadiazole

1,3,4-oxadiazole

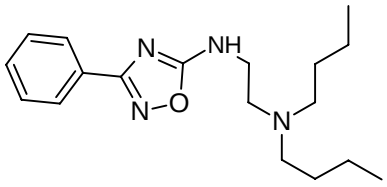
1,2,3-oxadiazole

Figure 3.2. Structure of various oxadiazoles.



Raltegravir (To treat HIV infection)

QD-3.5



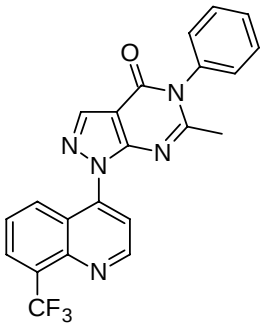
Butalamine (Vasodilation or widening of blood vessels)

QD-3.6

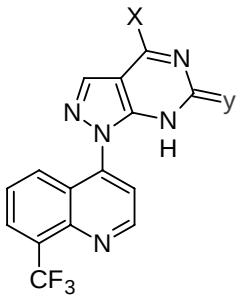
The synthesis of novel oxadiazole derivatives and investigations of their chemical and biological behaviour have gained more importance in recent decades as they possess an extensive spectrum of pharmacological activities. In recent years

[1,3,4]-oxadiazole derivatives were used as antimicrobial , anti-inflammatory, hypoglycemic, anticonvulsant and other activities.

Holla et al. (2006) reported the synthesis of some novel pyrazolo[3,4]pyrimidine derivatives (**QD-3.7 & QD-3.8**) and its antimicrobial studies. Replacement of 1H of pyrazole of pyrazolo[3,4]pyrimidine ring system by some other bioactive moiety drastically alters its pharmacological properties. Introduction of a fluorine atom as the CF_3 group provides a more lipophilically and pharmacologically interesting compound as compared to their non-fluorinated analogues.



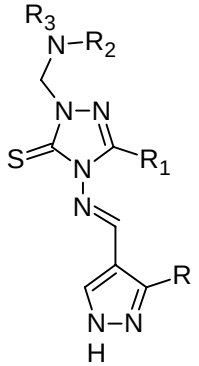
QD-3.7



QD-3.8

$X = NH_2$, Y = O, S.

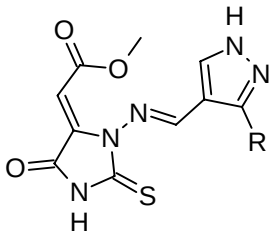
Isloor et al. (2009) reported the synthesis of Schiff and Mannich bases containing pyrazole moiety (**QD-3.9**). The newly synthesized compounds were screened for their antibacterial and antifungal activities. Some of the compounds were found to exhibit significant antimicrobial activity.



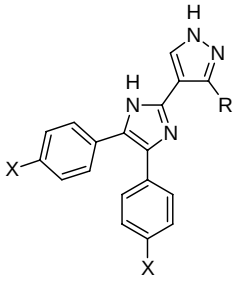
QD-3.9

Where R = CH_3, C_6H_5, P-ClC_6H_4, P-$OMeC_6H_4$, P-$CH_3C_6H_4$, R_1 = CH_3, C_3H_7, C_6H_5
NR_2R_3 = N-methylpiperizine.

Recently, Vijesh et al. (2011) reported the synthesis of two series of novel imidazole derivatives (**QD-3.10 & QD-3.11**) containing substituted pyrazole moiety. Among the synthesized compounds, compound containing 4-$SCH_3C_6H_4$ was found to be potent antimicrobial agent. The acute oral toxicity study for the compound revealed that was safe up to 3000 mg/kg.



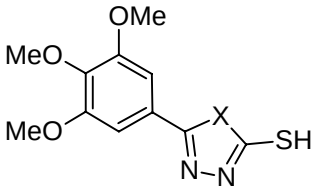
QD-3.10



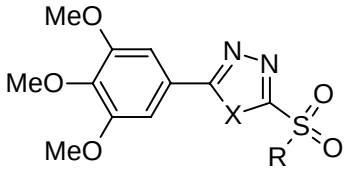
QD-3.11

Where R = 2,4-dichlorophenyl, biphenyl, 2,5-dichlorothiophene, 4-$CH_3C_6H_4$, 4-$SCH_3C_6H_4$, X = H, Br.

Antifungal activitiy of 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives (**QD-3.12 & QD-3.13**) were reported by Chen et al. (2007). The compounds 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole inhibited *mycelia* growth by approximately 50% (EC_{50}) at 2.9–93.3 μg/mL *in-vitro* against 10 kinds of fungi.



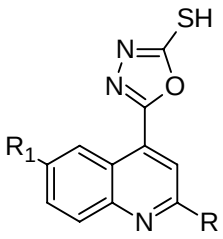
QD-3.12



QD-3.13

Where R = 2-FCH_2Ph, 3-FCH_2Ph, 4-FCH_2Ph, 2-$MeOCH_2Ph$, 3-$MeOCH_2Ph$, 4-$MeOCH_2Ph$, 3-NO_2CH_2Ph, 4-NO_2CH_2Ph, 2-$ClCH_2Ph$, 4-$ClCH_2PhN$, CH_2Ph, CH_3, CH_2CH_3, C_3H_7, CH_2CHCH_2, $CH_3CO_2CH_2$, X = O, S.

1, 3, 4-oxadiazole (**QD-3.14**), its anti-bacterial and anti-fungal activity were reported by Vachharajani et al. (2011). All the compounds showed good antimicrobial activity.



QD-3.14

Where R = 4-FC_6H_4, 4-MeC_6H_4, 4-ClC_6H_4, R_1 = F, Cl, NO_2.

Heterocyclic compounds play an important role in medicinal chemistry. They are well known to possess diverse pharmacological properties, viz. antibacterial, antifungal, anti-inflammatory, anticonvulsant, antiviral, antimalarial, antituberculosis and anticancer (Isloor et al. 2009; Isloor et al. 2010; Rai et al. 2010; Sunil et al. 2011). Resistance to antimicrobial drugs is a serious problem in many countries. Different structural modification has been done to enhance the antimicrobial activity by introducing the different functional groups around the quinoline nucleus. Among the heterocyclic compounds, substituted quinolines are more important because of their wide spectrum of biological activities. A large variety of quinoline derivatives have been used as anticancer (Chen et al. 2004), anti-inflammatory (Bekhit et al. 2004), antimalarial (Nasveld et al. 2005), antibacterial, antifungal (Patel and Patel. 2010; Eswaran et al. 2009), antioxidant (Jayashree et al. 2010), antiviral (Carta et al. 2011), anti-tuberculosis (Thomas et al. 2011) agents.

It has been well established that fluorinated quinolines, in particular, CF_3 substituted quinolines have got a significant place in modern medicinal chemistry. Introduction of trifluoromethyl group provides better electronic effect at neighboring carbon centers, as well as having a substantial effect on the molecule's dipole moment, acidity and basicity of neighboring groups (Hawley et al. 1996). Their biological studies clearly indicated that, the presence of trifluoromethyl group at position 7 and 8 of the quinoline ring are responsible for the biological activity (Holla

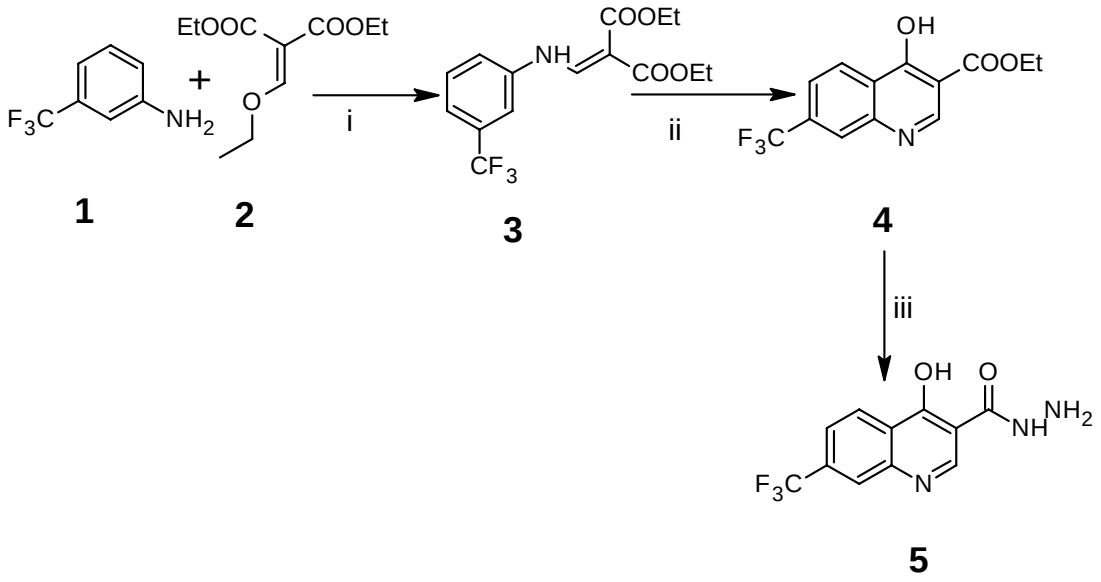
et al. 2006; He et al. 2005; Bi et al. 2004) and are the subject of considerable growing interest. Further, various types of hydrazones have attracted continued interest in the medicinal field owing to their varied biological activities as antimicrobial (Metwally et al. 2006), antimalarial (Fattorusso et al. 2008) and antitubercular properties (Vavrikova et al. 2011). On the other hand, compounds containing 1,3,4-oxadizole rings are very well known to exhibit powerful antimicrobial (Chandrakantha et al. 2010; Chen et al. 2007), analgesic (Ramaprasad et al. 2010), cannabinoid receptor 2 (CB2) agonist (Cheng et al. 2008), VEGFR-2 and Tubulin Inhibitor (Eugene et al. 2010) properties. Therefore there is great importance for the synthesis of oxadiazoles as target structures and evaluation of their biological activities.

In this regard we hereby report the synthesis of new quinoline derivatives and their characterization by spectral studies. Single crystal X-ray analysis was also carried out for one of the synthesized compounds to obtain its three dimensional structure. Further, synthesized compounds were screened for their antimicrobial and *Mycobacterium smegmatis* activities.

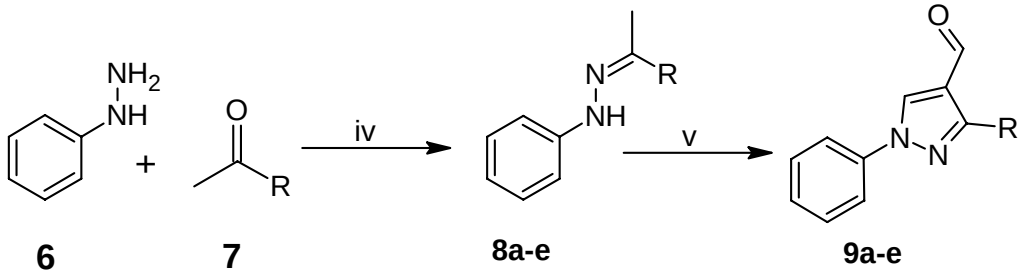
3.2. RESULTS AND DISCUSSION

3.2.1. Synthesis of 7-trifluoromethyl quinoline-3-carbohydrazide and 1,3,4-oxadiazoles derivatives

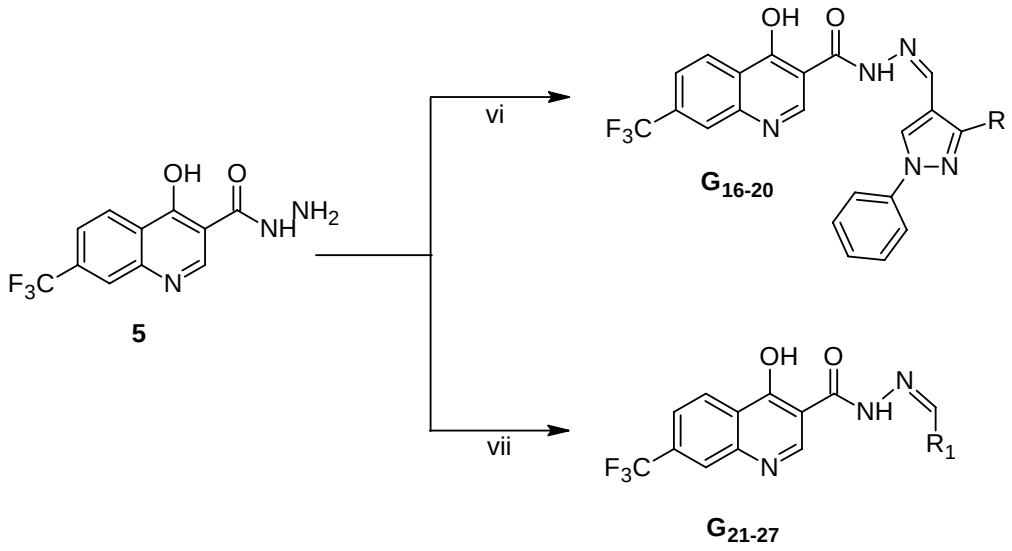
The targeted compounds (**G_{16-20}**, **G_{21-27}** and **G_{28-33}**) were synthesized by employing sequential reactions, which are presented in **Scheme-3.1**, **Scheme-3.2**, **Scheme-3.3** and **Scheme-3.4**. The quinolone skeletons were built up by the Gould-Jacobs procedure starting from 3-(trifluoromethyl)aniline (**1**). Condensation of **1** with diethyl ethoxymethylene malonate and subsequent thermal cyclization in dowtherm (biphenyl:biphenyloxide(3:7)) yielded the 4-hydroxy-quinoline-3-carboxylic ester **4** (Snyder et al. 1947; Bi et al. 2004), which on condensation with hydrazine hydrate in alcoholic medium resulted in 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid hydrazide **5** (Kumar et al. 1988) **(Scheme-3.1)**. Further, the key intermediates, pyrazole-4-carbaldehydes **9a-e** were prepared by the Vilsemeier-Haack reaction of the corresponding hydrazones **8a-e** (Kira et al. 1969) **(Scheme-3.2)**.



Scheme 3.1. Synthetic route for 4-hydroxy-7-(trifluoromethyl)quinoline-3-c carbazide (5): (i) 110 °C, 6 h; (ii) Dowtherm, 250 °C, 5 h; (iii) NH_2NH_2, EtOH, 4 h.

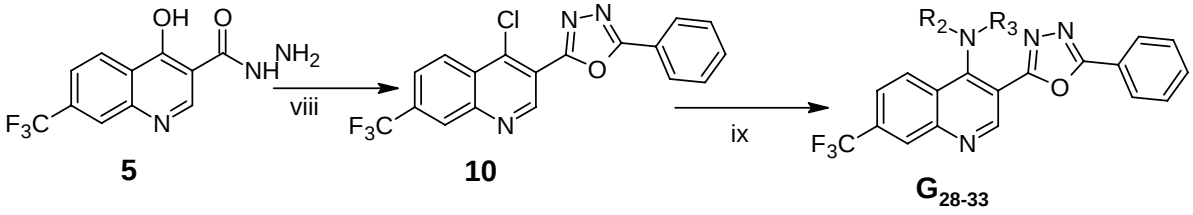


Scheme 3.2. Synthetic route for pyrazole-4-carbaldehyde derivatives (9a-e): (iv) EtOH, AcOH, 25-30 °C, 2 h; (v) $POCl_3$ / DMF, 70 °C, 6 h.



Where R= Phenyl, 4-Methoxyphenyl, 4-Chlorophenyl, 4-Nitrophenyl, 4-Methylphenyl; R_1=4-Methoxybenzaldehyde, 3,4-Dimethoxybenzaldehyde, Thiophene-2-carbaldehyde, 4-N-Methylbenzaldehyde, 3-Ethoxy-2-hydroxyBenzaldehyde, 4-N-Diethyl-2-hyd roxybenzaldehyde, 6-Bromopyridine-3-carbaldehyde.

Scheme 3.3. **Synthetic route for 4-hydroxy-7-(trifluoromethyl)quinoline-3-aryl carbohydrazides (G_{16-20} and G_{21-27}):** (vi) Pyrazolealdehyde, EtOH, 25-30 °C, 1 h; (vii) Aromatic aldehyde, EtOH, 25-30 °C, 1 h.



R_2/R_3= N-Methylpiperidine, Morpholine, Ethanolamine, O-Acetylethanolamine, 3,4,5-Trimethoxyaniline.

Scheme 3.4. Synthetic route for N-alkyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolin-4-amine (G_{28-33}): (viii) Benzoic acid, $POCl_3$, 100 °C, 10 h; (ix) Alkylamine, K_2CO_3, DMF, 110 °C, 8 h.

The targeted 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide derivatives (**G_{16-20}** and **G_{21-27})** were obtained by the reacting quinoline hydrazide **5** with various substituted aldehydes in ethanolic media (**Scheme-3.3**). Further, reaction of **5** with benzoic acid in phosphorus oxychloride ($POCl_3$) yielded 4-chloro-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolines **10**. Finally chlorine in **10** were replaced with various aliphatic and aromatic amines to obtain N-alkyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolin-4-amine derivatives **G_{28-33} (Scheme-3.4)**. The crude products were purified by column chromatography using pet ether and ethyl acetate (7:3) as the eluent.

Formation of 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide derivatives (**G_{16-20}** and **G_{21-27})** were confirmed by recording their IR, ^{1}H NMR, ^{13}C NMR and mass spectra. The IR spectrum of compound **G_{20}** showed two bands at 3385 cm^{-1} and 3073 cm^{-1}, which are due to the hydroxyl and amide groups respectively. Band at 1663 cm^{-1} is due to C=O stretch of carbonyl group. The ^{1}H NMR spectrum of **G_{20}** showed a singlet at δ 8.13 ppm which is due to the imine proton (N=CH). Hydroxyl and amide protons appeared as singlets at δ 12.96 and 13.12 ppm respectively further confirmed the structure of the compound. The mass spectrum of **G_{20}** showed a molecular ion peak at m/z = 516 (M+1), which is in agreement with the molecular formula $C_{27}H_{17}F_3N_6O_4$. Similarly the spectral values for all the compounds and C, H, N analyses are presented in the experimental part and the characterization data are provided in **Table-3.1**.

Table-3.1. Characterization data of the compounds $G_{16\text{-}20}$, $G_{21\text{-}27}$ and $G_{28\text{-}33}$

Compds	$R/R_1/R_2/R_3$	Mol. Formula & Mol. Wt.	Yield (%)	M.p.(°C)	Color & nature
G_{16}	Phenyl	$C_{27}H_{18}F_3N_5O_2$ (501.4)	80	> 300	White solid
G_{17}	4-Methoxy phenyl	$C_{28}H_{20}F_3N_5O_3$ (531.4)	78	> 300	White solid
G_{18}	4-Chloro phenyl	$C_{27}H_{17}ClF_3N_5O_2$ (535.5)	81	> 300	White solid
G_{19}	4-Nitro phenyl	$C_{27}H_{17}F_3N_6O_4$ (546.45)	94	> 300	Pale yellow solid
G_{20}	4-Methyl phenyl	$C_{28}H_{20}F_3N_5O_2$ (515.4)	85	> 300	White solid
G_{21}	4-methoxy benzaldehyde	$C_{19}H_{14}F_3N_3O_3$ (389.3)	90	> 300	White solid
G_{22}	3,4-Dimethoxy benzaldehyde	$C_{20}H_{16}F_3N_3O_4$ (419.35)	87	> 300	White solid
G_{23}	thiophene-2-carbaldehyde	$C_{16}H_{10}F_3N_3O_2S$ (365.3)	63	> 300	Brown solid
G_{24}	4-N-Dimethyl benzaldehyde	$C_{20}H_{17}F_3N_4O_2$ (402.3)	77	> 300	Pale yellow solid
G_{25}	3-ethoxy-2-hydroxy benzaldehyde	$C_{20}H_{16}F_3N_3O_4$ (419.3)	86	> 300	White solid
G_{26}	4-N-Diethyl-2-hydroxybenzaldehyde	$C_{22}H_{21}F_3N_4O_3$ (446.4)	86	> 300	Yellow solid
G_{27}	6-bromopyridine-3-carbaldehyde	$C_{17}H_{10}BrF_3N_4O_2$ (439.18)	90	> 300	Yellow solid
G_{28}	N-Methyl piperidine	$C_{23}H_{20}F_3N_5O$ (439.4)	58	152-154	White crystal
G_{29}	Morpholine	$C_{22}H_{17}F_3N_4O_2$ (426.3)	54	> 300	Pale yellow solid
G_{30}	Ethanolamine	$C_{20}H_{15}F_3N_4O_2$ (400.3)	65	219-221	White solid
G_{31}	O-Acetyl ethanolamine	$C_{22}H_{17}F_3N_4O_3$ (442.3)	63	106-108	Pale yellow solid
G_{32}	3,4,5-Trimethoxy aniline	$C_{27}H_{21}F_3N_4O_4$ (522.5)	82	215-217	Yellow solid
G_{33}	2-Methyl-4amino quinoline	$C_{28}H_{18}F_3N_5O$ (497.4)	80	130-132	Yellow solid

3.3. EXPERIMENTAL

3.3.1. Synthesis of diethyl ({[3-(trifluoromethyl)phenyl]amino}methylidene) propanedioate (3)

3-(Trifluoromethyl) aniline **1** (10.0 g, 0.062 mol) and diethyl ethoxymethylene malonate **2** (18.61 mL, 0.093 mol) were heated to 110 °C for 6 h. The reaction mixture was cooled to room temperature, the solid thus formed was taken in pet ether and stirred for 20 min and filtered to get compound **3** a white crystalline solid.

3.3.2. Synthesis of 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (4)

Diethyl ({[3-(trifluoromethyl)phenyl]amino}methylidene)propanedioate **3** (10.0 g, 0.030 mol) and dowtherm (100 mL) were heated to 250 °C for 5 h. The reaction mixture was then cooled to 25 °C and stirred in 150 mL hexane for 10 min. The solid product obtained was filtered and dried. The crude product obtained was purified by column chromatography using pet ether and ethyl acetate (5:5) as the eluent.

3.3.3. Synthesis of of 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid hydrazide (5)

A mixture of ethyl 4-hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester **10** (5.0 g, 0.017 mol) and hydrazine hydrate (4.1 mL, 0.085 mol, reagent grade 98%) in ethanol (50 mL) were refluxed for 4 h. After the completion of the reaction, the reaction mixture was concentrated and allowed to cool. The solid product obtained was filtered, washed with water and recrystallized from ethanol to give **5** as a white solid.

3.3.4. General method for the preparation of hydrazone derivatives (8a-e)

An equimolar mixture of phenyl hydrazine **6** (2.0 g, 0.018 mol), 4-substituted acetophenone **7** (0.018 mol) and a catalytic amount of acetic acid (0.5 mL) in dry ethanol (20 mL) were stirred at 25 °C for 2 h. Completion of the reaction was monitored by TLC. The precipitated solid was filtered under suction, washed with ethanol and recrystallized from ethanol-dioxane.

3.3.5. General method for the preparation of pyrazole-4-carbaldehyde derivatives (9a-e)

To the dry dimethylformamide (10 mL) in a three necked flask, phosphorus oxychloride ($POCl_3$) (0.028 mol) was added slowly with intensive stirring at 0 °C for 15 min. The solution of hydrazone derivatives (0.009 mol) in dimethylformamide (10 mL) was then slowly added under stirring. The reaction mass was heated at 70 °C for 6 h. After the completion of reaction, the mixture was kept overnight at room temperature. The crude product was precipitated by the addition of crushed ice (50 g) and was isolated by filtration. Further it was recrystallized from hot ethanol (Kira et al. 1969).

3.3.6. General method for the preparation of 4-Hydroxy-7-trifluoro methyl) quinoline-3-carbohydrazide derivatives ($G_{16\text{-}20}$, and $G_{21\text{-}27}$)

An equimolar mixture of 4-hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid hydrazide **5** (0.5 g, 0.0018 mol), pyrazole-4-carbaldehyde (**9a-e**) or aromatic aldehydes (0.002 mol) and a catalytic amount of acetic acid in dry ethanol (5 mL) were stirred at 25 °C for 1 h. Completion of the reaction was monitored by TLC. The precipitated solid was filtered under suction, washed with ethanol and recrystallized from ethanol.

3.3.7. Synthesis of 4-chloro-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl) quinoline (10)

A mixture of 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide **5** (5.0 g, 0.018 mol), benzoic acid (2.44 g, 0.020 mol) and phosphorous oxychloride (50 mL) were heated at 100 °C for 10 h. The reaction mixture was then cooled to room temperature, the excess of phosphorus oxychloride ($POCl_3$) was removed by distillation under vacuum (bath temperature 60 °C, vacuum -200 mm Hg). The residue obtained was quenched to crushed ice and the solid separated was filtered off and dried through the pump. The crude product was purified by column chromatography using pet ether and ethyl acetate (9:1) as the eluent.

3.3.8. General method for the preparation of N-alkyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolin-4-amine derivatives (G_{28-33})

A suspension of compound **10** (0.5 g, 0.0013 mol) in dry dimethylformamide 5 mL was taken in a 25 mL round bottomed flask (RBF), dry potassium carbonate (0.26 g, 0.0019 mol) and substituted amine (0.0019 mol) were then added to the RBF. The reaction mixture was heated at 110 °C for 8 h. After the completion of reaction, the reaction mixture was poured into ice-cold water. The product was extracted in ethyl acetate (15 mL x 3) and concentrated. The crude product was purified by column chromatography using pet ether and ethyl acetate as the eluent.

3.4. CHARACTERIZATION OF SYNTHESIZED COMPOUNDS

3.4.1. Diethyl ({[3-(trifluoromethyl)phenyl]amino}methylidene)propanedioate (3)

Yield: 19.0 g, 92%; M.p: 44-46 °C; IR (Neat ν_{max} cm^{-1}): 3252 (N-H), 3118, 2979 (C-H-str), 1708 and 1616 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.37 (t, 3H, CH_3, J = 5.3 Hz), 1.39 (t, 3H, CH_3, J = 5.3 Hz), 4.26 (q, 2H, CH_2), 4.34 (q, 2H, CH_2), 7.21-8.40 (m, 5H, ArH), 11.49 (s, 1H, NH); MS: m/z = 333 (M+1); Anal. calcd. For $C_{15}H_{16}F_3NO_4$; Calcd: C, 54.38; H, 4.80; N, 4.23; found: C, 54.35; H, 4.80; N, 4.20% (Bi et al. 2004).

3.4.2. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (4)

Yield: 7.2 g, 83.7%; M.p: 298-300 °C; IR (Neat ν_{max} cm^{-1}): 3322 (-OH), 3029, 2970 (C-H-str), 1706 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.22 (t, 3H, CH_3, J = 8.0 Hz), 4.18 (q, 2H, CH_2, J = 8.0 Hz), 7.53 (d, 1H, ArH, J = 12 Hz), 7.81 (d, 1H, ArH, J = 12 Hz), 8.03 (s, 1H, ArH), 8.44 (s, 1H, ArH), 12.30 (s, 1H, -OH); MS: m/z = 286 (M+1); Anal. calcd. For $C_{13}H_{10}F_3NO_3$; Calcd: C, 54.74; H, 3.53; N, 4.91; found: C, 54.77; H, 3.50; N, 4.95% (Bi et al. 2004).

3.4.3. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid hydrazide (5)

Yield: 4.25 g, 89.4%; M.p: 255-257 °C; IR (Neat ν_{max} cm^{-1}): 3442 (-OH), 3296 and 3244 (N-H), 3088, 2963 (C-H-str), 1649 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.67 (s, 2H, -NH_2, D_2O-exchangeble), 7.76 (dd, 1H, ArH, J = 7.7 Hz, J = 1.4 Hz), 8.10 (s, 1H, ArH), 8.45 (d, 1H, ArH, J = 8.4 Hz), 8.90 (s, 1H, ArH), 10.57 (s, 1H,

-NH, D_2O-exchangeble), 12.88 (s, 1H, -OH, D_2O-exchangeble); MS: m/z = 272 (M+1). Anal. calcd. For $C_{11}H_8F_3N_3O_2$; Calcd: C, 48.72; H, 2.97; N, 15.49; found: C, 48.75; H, 2.97; N, 15.59% (Kumar et al. 1988).

3.4.4. 1,3-Diphenyl-1H-pyrazole-4-carbaldehyde (9a)

Yield: 1.85 g, 78.3%; M.p: 142-144 °C; IR (Neat ν_{max} cm^{-1}): 3115, 3058 (C-H-str), 1669 (C=O), 1517 (C=N); ^1H NMR (400 MHz, DMSO-d6): δ ppm 7.42-7.47 (m, 10H, ArH), 8.46 (s, 1H, ArH), 9.70 (s, 1H, CHO); Anal. calcd. For $C_{16}H_{12}N_2O$; Calcd: C, 77.40; H, 4.87; N, 11.28; found: C, 77.40; H, 4.85; N, 11.30% (Kira et al. 1969).

3.4.5. 3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (9b)

Yield: 1.72 g, 74.3%; M.p: 132-134 °C; IR (Neat ν_{max} cm^{-1}): 3116, 3053 (C-H-str), 1665 (C=O), 1517 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 3.29 (s, 1H, CH_3), 7.40-7.32 (m, 9H, ArH), 8.47 (s, 1H, ArH), 9.68 (s, 1H, CHO); Anal. calcd. For $C_{17}H_{14}N_2O_2$; Calcd: C, 73.37; H, 5.07; N, 10.07; found: C, 73.39; H, 5.11; N, 10.10% (Prasath et al. 2011).

3.4.6. 3-(4-Chlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (9c)

Yield: 1.9 g, 82.2%; M.p: 140-142 °C; IR (Neat ν_{max} cm^{-1}): 3118, 3059 (C-H-str), 1664 (C=O), 1511 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.43-7.50 (m, 9H, ArH), 8.42 (s, 1H, ArH), 9.77 (s, 1H, CHO); Anal. calcd. for $C_{16}H_{11}ClN_2O$; Calcd: C, 67.97; H, 3.92; N, 9.91; found: C, 67.90; H, 3.91; N, 9.99% (Fun et al. 2011).

3.4.7. 3-(4-Nitrophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (9d)

Yield: 1.95 g, 85.1%; M.p: 165-167 °C; IR (Neat ν_{max} cm^{-1}): 3124, 3064 (C-H-str), 1679 (C=O), 1517 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.56-7.82 (m, 9H, ArH), 8.41 (s, 1H, ArH), 9.79 (s, 1H, CHO); Anal. calcd. For $C_{16}H_{11}N_3O_3$; Calcd: C, 65.53; H, 3.78; N, 14.33; found: C, 65.51; H, 3.80; N, 14.30% (Kira et al. 1969).

3.4.8. 1-Phenyl-3-p-tolyl-1H-pyrazole-4-carbaldehyde (9e)

Yield: 1.89 g, 81.1%; M.p: 112-114 °C; IR (Neat ν_{max} cm^{-1}): 3116, 3053 (C-H-str), 1665 (C=O), 1517 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 2.42 (s, 3H, CH_3),

7.40-7.45 (m, 9H, ArH), 8.39 (s, 1H, ArH), 9.71 (s, 1H, CHO); Anal. calcd. For $C_{17}H_{14}N_2O$; Calcd: C, 77.84; H, 5.38; N, 10.68; found: C, 77.82; H, 5.40; N, 10.66% (Shetty et al. 2008).

3.4.9. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (1,3-diphenyl-1H-pyrazol-4-ylmethylene)-hydrazide (G_{16})

IR (Neat ν_{max} cm^{-1}): 3410 (O-H), 3138 (N-H), 3064, 2923 (C-H-str), 1668 (C=O), 1604 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.15-7.50 (m, 6H, ArH), 7.52 (d, 1H, ArH, J = 8.4 Hz), 7.75 (d, 2H, ArH, J = 8.0 Hz), 7.96 (d, 2H, ArH, J = 8.0 Hz), 8.07 (s, 1H, N=CH), 8.42 (d, 1H, ArH, J = 8.4 Hz), 8.43 (s, 1H, ArH), 8.96 (s, 2H, ArH), 12.90 (s, 1H, -OH, D_2O-exchangeble), 13.10 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 502 (M+1). Anal. calcd. For $C_{27}H_{18}F_3N_5O_2$; Calcd: C, 64.67; H, 3.62; N, 13.97; found: C, 64.65; H, 3.63; N, 13.90%.

3.4.10. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid [3-(4-methoxy-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-hydrazide (G_{17})

IR (Neat ν_{max} cm^{-1}): 3403 (O-H), 3114 (N-H), 3067, 3010 (C-H-str), 1672 (C=O), 1607 (C=N), 1176 (O-CH_3); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 3.86 (s, 3H, OCH_3), 7.10 (d, 2H, ArH, J = 8.6 Hz), 7.38 (t, 1H, ArH, J = 7.4 Hz), 7.55 (t, 2H, ArH, J = 8.1 Hz), 7.76 (d, 2H, ArH, J = 8.6 Hz), 7.82 (d, 1H, ArH, J = 9.0 Hz), 8.02 (d, 2H, ArH, J = 7.7 Hz), 8.13 (s, 1H, N=CH), 8.45 (s, 1H, ArH), 8.48 (d, 1H, ArH, J = 8.6 Hz), 8.98 (s, 1H, ArH), 9.03 (s, 1H, ArH), 12.99 (s, 1H, -OH, D_2O-exchangeble), 13.15 (s, 1H, -NH, D_2O-exchangeble); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 55.62, 111.43, 118.72, 119.23, 121.28, 124.67, 124.90, 126.69, 127.32, 127.67, 127.88, 129.74, 130.07, 138.96, 146.26, 152.23, 159.54, 175.85. MS: m/z = 532 (M+1). Anal. calcd. For $C_{28}H_{20}F_3N_5O_3$; Calcd: C, 63.28; H, 3.79; N, 13.18; found: C, 63.34; H, 3.72; N, 13.20%.

3.4.11. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid [3-(4-chloro-phenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]-hydrazide (G_{18})

IR ((Neat ν_{max} cm^{-1}): 3411 (O-H), 3129 (N-H), 3015, 2913 (C-H-str), 1667 (C=O),

1605 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.34-7.55 (m, 4H, ArH), 7.75 (d, 2H, ArH, J =8.0 Hz), 7.83 (d, 2H, ArH, J = 8.0 Hz), 7.96-7.98 (m, 3H, ArH), 8.06 (s, 1H, N=CH), 8.42 (s, 1H, ArH), 8.95 (s, 1H, ArH), 8.97 (s, 1H, ArH), 12.96 (s, 1H, -OH, D_2O-exchangeble), 13.10 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 536 (M+1). Anal. calcd. For $C_{27}H_{17}ClF_3N_5O_2$; Calcd: C, 60.51; H, 3.20; N, 13.07; found: C, 60.58; H, 3.21; N, 13.05%.

3.4.12. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid [3-(4-nitro-phenyl) -1-phenyl-1H-pyrazol-4-ylmethylene]-hydrazide (G_{19})

IR (Neat ν_{max} cm^{-1}): 3464 (O-H), 3233 (N-H), 3073, 2923 (C-H-str), 1650 (C=O), 1596 (C=N), 1534 (N-O), 1495 (N-O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.41-7.45 (m, 1H, ArH), 7.56-7.60 (m, 2H, ArH), 7.83 (dd, 1H, ArH, J = 8.76 Hz, J = 1.56 Hz), 8.04-8.07 (m, 2H, ArH), 8.14 (s, 1H, N=CH), 8.23-8.26 (m, 2H, ArH), 8.36-8.39 (m, 2H, ArH), 8.49 (d, 1H, ArH, J = 8.4 Hz), 8.56 (s, 1H, ArH), 9.0 (s, 2H, ArH), 13.03 (s, 1H, -OH, D_2O-exchangeble), 13.14 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 547 (M+1). Anal. calcd. For $C_{27}H_{17}F_3N_6O_4$; Calcd: C, 59.34; H, 3.14; N, 15.38; found: C, 59.38; H, 3.11; N, 15.40%.

3.4.13. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (1-phenyl-3-p-tolyl-1H-pyrazol-4-ylmethylene)-hydrazide (G_{20})

IR (Neat ν_{max} cm^{-1}): 3385 (O-H), 3072 (N-H), 3014, 2919 (C-H-str), 1663 (C=O), 1605 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.41(s, 3H, CH_3), 7.36-7.40 (m, 3H, ArH), 7.55 (t, 2H, ArH, J = 8.0 Hz), 7.70 (d, 2H, ArH, J = 7.8 Hz), 7.83 (d, 1H, ArH, J = 8.7 Hz), 8.01 (d, 2H, ArH, J = 8.0 Hz), 8.13 (s, 1H, N=CH), 8.46 (s, 1H, ArH), 8.47 (d, 1H, ArH, J = 8.7 Hz), 8.98 (s, 1H, ArH), 9.02 (s, 1H, ArH), 12.96 (s, 1H, -OH, D_2O-exchangeble), 13.13 (s, 1H, -NH, D_2O-exchangeble); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 21.18, 111.68, 117.25, 117.42, 121.32, 127.88, 128.83, 129.80, 130.06, 138.51, 139.12, 139.38, 139.56, 141.27, 146.11, 152.07, 160.96, 175.49; MS: m/z = 516 (M+1). Anal. calcd. For $C_{27}H_{17}F_3N_6O_4$; Calcd: C, 65.24; H, 3.91; N, 13.59; found: C, 65.30; H, 3.89; N, 13.55%.

3.4.14. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (4-methoxy-benzy lidene)-hydrazide (G_{21})

IR (Neat ν_{max} cm^{-1}): 3407 (O-H), 3163 (N-H), 3063, 2978 (C-H-str), 1647 (C=O), 1605 (C=N), 1165 (O-CH_3); ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 2.95 (s, 3H, OCH_3), 6.17 (d, 2H, ArH, J = 8.2 Hz), 6.86 (d, 2H, ArH, J = 8.2 Hz), 6.96 (d, 2H, ArH, J = 8.0 Hz), 7.28 (s, 1H, N=CH), 7.53 (s, 1H, ArH), 7.63 (d, 1H, ArH, J = 8.0 Hz), 8.15 (s, 1H, ArH); MS: m/z = 390 (M+1). Anal. calcd. For $C_{19}H_{14}F_3N_3O_3$; Calcd: C, 58.61; H, 3.62; N, 10.79; found: C, 58.72; H, 3.62; N, 10.78%.

3.4.15. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (3,4-dimethoxy-benzylidene)-hydrazide (G_{22})

IR (Neat ν_{max} cm^{-1}): 3410 (O-H), 3170 (N-H), 3015, 2969 (C-H-str), 1661 (C=O), 1582 (C=N), 1173 (O-CH_3), 1156 (O-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 7.06 (d, 1H, ArH, J = 4.4 Hz), 7.28 (dd, 1H, ArH, J = 8.3 Hz, J = 1.8 Hz), 7.39 (d, 1H, ArH, J = 1.8 Hz), 7.82 (dd, 1H, ArH, J = 8.6 Hz, J = 1.3 Hz), 8.15 (s, 1H, N=CH), 8.38 (s, 1H, ArH), 8.50 (d, 1H, ArH, J = 8.4 Hz), 9.02 (s, 1H, ArH), 13.02 (s, 1H, -OH, D_2O-exchangeble), 13.11 (s, 1H, -NH, D_2O-exchangeble); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 55.97, 108.80, 112.09, 117.39, 121.35, 122.06, 127.33, 127.93, 128.53, 139.33, 146.04, 148.60, 149.51, 151.28, 160.97, 160.97, 175.74. MS: m/z = 420 (M+1). Anal. calcd. For $C_{20}H_{16}F_3N_3O_4$; Calcd: C, 57.28; H, 3.85; N, 10.02; found: C, 57.35; H, 3.85; N, 10.00%.

3.4.16. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid thiophen-2-ylmethylene-hydrazide (G_{23})

IR (Neat ν_{max} cm^{-1}): 3407 (O-H), 3061 (N-H), 2983, 2922 (C-H-str), 1649 (C=O), 1607 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.15-7.18 (m, 1H, ArH), 7.47 (d, 1H, ArH, J = 7.6 Hz), 7.69-7.70 (m, 1H, ArH), 7.83 (d, 1H, ArH, J = 8.5 Hz), 8.15 (s, 1H, N=CH), 8.49 (d, 1H, ArH, J = 8.4 Hz), 8.71 (s, 1H, ArH), 9.01 (s, 1H, ArH), 13.03 (s, 1H, -OH, D_2O-exchangeble), 13.12 (s, 1H, - NH, D_2O-exchangeble); MS: m/z = 366 (M+1). Anal. calcd. For $C_{16}H_{10}F_3N_3O_2S$; Calcd: C, 52.60; H, 2.76; N, 11.50; found: C, 52.66; H, 2.72; N, 11.53%.

3.4.17. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (4-dimethylamino-benzylidene)-hydrazide (G_{24})

IR (Neat ν_{max} cm^{-1}): 3417 (O-H), 3100 (N-H), 3075, 3010 (C-H-str), 1645 (C=O), 1602 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.97 (s, 6H, NCH_3), 6.77 (d, 2H, ArH, J = 8.9 Hz), 7.58 (d, 1H, ArH, J = 8.9 Hz), 7.76 (d, 1H, ArH, J = 8.4 Hz), 8.12 (s, 1H, N=CH), 8.26 (s, 1H, ArH), 8.48 (d, 1H, ArH, J = 8.4 Hz), 9.00 (s, 1H, ArH), 13.11 (s, 1H, -OH, D_2O-exchangeble), 13.14 (s, 1H, - NH, D_2O-exchangeble); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 47.05, 53.04, 111.73, 112.04, 117.72, 121.36, 122.38, 127.02, 129.09, 138.96, 145.97, 148.92, 151.27, 160.14, 175.51. MS: m/z = 403 (M+1). Anal. calcd. For $C_{20}H_{17}F_3N_4O_2$; Calcd: C, 59.70; H, 4.26; N, 13.92; found: C, 59.68; H, 4.25; N, 13.82%.

3.4.18. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (3-ethoxy-2-hydroxy -benzylidene)-hydrazide (G_{25})

IR (Neat ν_{max} cm^{-1}): 3400 (O-H), 3222 (O-H), 3189 (N-H), 3049, 2985 (C-H-str), 1660 (C=O), 1615 (C=N), 1206 (O-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.37 (t, 3H, CH_3, J = 6.8 Hz), 4.07 (q, 2H, OCH_2, J = 4.4 Hz), 6.87 (t, 1H, ArH, J = 8.4 Hz), 7.03 (d, 1H, ArH, J = 7.7 Hz), 7.12 (d, 1H, ArH, J = 7.0 Hz), 7.84 (d, 1H, ArH, J = 7.1 Hz), 8.15 (s, 1H, N=CH), 8.50 (d, 1H, ArH, J = 7.0 Hz), 8.69 (s, 1H, ArH), 9.04 (s, 1H, ArH), 11.14 (s, 1H, -OH, D_2O-exchangeble), 13.13 (s, 1H, -OH, D_2O-exchangeble), 13.18 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 420 (M+1). Anal. calcd. For $C_{20}H_{16}F_3N_3O_4$; Calcd: C, 57.28; H, 3.85; N, 10.02; found: C, 57.30; H, 3.86; N, 10.00%.

3.4.19. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (4-diethylamino-2-hydroxy-benzylidene)-hydrazide (G_{26})

IR (Neat ν_{max} cm^{-1}): 3466 (O-H), 3237 (O-H), 3110 (N-H), 2975, 2927 (C-H-str), 1621 (C=O), 1586 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.12 (t, 6H, CH_3, J = 6.9 Hz), 3.37 (q, 4H, NCH_2, J = 7.0 Hz), 6.13 (s, 1H, ArH), 6.29 (dd, 1H, ArH, J = 8.8 Hz, J = 2.4 Hz), 7.22 (d, 1H, ArH, J = 8.8 Hz), 7 .83 (dd, 1H, ArH, J = 8.6 Hz, J = 1.4 Hz), 8.14 (s, 1H, N=CH), 8.46 (s, 1H, ArH), 8.49 (d, 1H, ArH, J = 8.4 Hz), 9.00 (s, 1H, ArH), 11.37 (s, 1H, -OH, D_2O-exchangeble), 12.91 (s, 1H, -OH, D_2O-

exchangeble), 13.10 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 447 (M+1). Anal. calcd. For $C_{22}H_{21}F_3N_4O_3$; Calcd: C, 59.19; H, 4.74; N, 12.55; found: C, 59.20; H, 4.72; N, 12.15%.

3.4.20. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (6-bromo -pyridin-3-ylmethylene)-hydrazide (G_{27})

IR (Neat ν_{max} cm^{-1}): 3464 (O-H), 3048 (N-H), 2974, 2926 (C-H-str), 1541 (C=O), 1580 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.84 (dd, 1H, ArH, J = 8.6 Hz, J = 1.5 Hz), 8.18 (s, 1H, N=CH), 8.34-8.35 (m, 1H, ArH), 8.52 (s, 1H, ArH), 8.76-8.78 (m, 2H, ArH), 8.89 (d, 1H, ArH, J = 8.2 Hz), 9.06 (s, 1H, ArH), 13.16 (s, 1H, -OH, D_2O-exchangeble), 13.28 (s, 1H, -NH, D_2O-exchangeble); MS: m/z = 440 (M+1). Anal. calcd. For $C_{17}H_{10}BrF_3N_4O_2$; Calcd: C, 46.49; H, 2.30; N, 12.76; found: C, 46.45; H, 2.32; N, 12.71%.

3.4.21. 4-Chloro-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinoline (10)

Yield: 3.1 g, 44.9%; M.p: 131-133 °C; IR (Neat ν_{max} cm^{-1}): 3049, 2921 (C-H-str), 1596 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.58-7.64 (m, 2H, ArH), 8.10 (t, 2H, ArH, J = 8.0 Hz), 8.15 (d, 2H, ArH, J = 8.0 Hz), 8.53 (s, 1H, ArH), 8.62 (d, 1H, ArH, J = 8.0 Hz), 9.62 (s, 1H, ArH); MS: m/z = 376 (M+1). Anal. calcd. For $C_{18}H_9ClF_3N_3O$; Calcd: C, 57.54; H, 2.41; N, 11.18; found: C, 57.55; H, 2.38; N, 11.20%.

3.4.22. 4-(4-Methyl-piperazin-1-yl)-3-(5-phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoro methyl-quinoline (G_{28})

IR (Neat ν_{max} cm^{-1}): 3049, 2921 (C-H-str), 1596 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 2.26 (s, 3H, NCH_3), 2.57 (t, 4H, NCH_2, J = 4.2 Hz), 3.21 (t, 4H, NCH_2, J = 4.6 Hz), 7.67-7.70 (m, 3H, ArH), 7.97 (dd, 1H, ArH, J = 8.8 Hz, J = 1.84 Hz), 8.12-8.15 (m, 2H, ArH), 8.39 (s, 1H, ArH), 8.43 (d, 1H, ArH, J = 8.8 Hz), 9.08 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 46.29, 51.91, 55.22, 111.59, 115.86, 122.72, 123.68, 127.14, 127.23, 127.33, 127.50, 127.66, 127.77, 130.10, 132.82, 133.04. MS: m/z = 440 (M+1). Anal. calcd. For $C_{23}H_{20}F_3N_5O$; Calcd: C, 62.86; H,

4.59; N, 15.94; found: C, 62.96; H, 4.55; N, 15.98%.

3.4.23. 4-Morpholin-4-yl-3-(5-phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinoline (G_{29})

IR ((Neat ν_{max} cm^{-1}): 3062, 2959 (C-H-str), 1576 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 3.15 (t, 4H, NCH_2, J = 4.2 Hz), 4.92 (t, 4H, OCH_2, J = 4.4 Hz), 7.65-7.78 (m, 3H, ArH), 7.97-8.14 (m, 3H, ArH), 8.35 (s, 1H, ArH), 8.42 (d, 1H, ArH, J = 8.8 Hz), 9.06 (s, 1H, ArH); MS: m/z = 427 (M+1). Anal. calcd. For $C_{22}H_{17}F_3N_4O_2$; Calcd: C, 61.97; H, 4.02; N, 13.14; found: C, 62.00; H, 4.03; N, 13.10%.

3.4.24. 2-[3-(5-Phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinolin-4-ylamino]-ethanol (G_{30})

IR (Neat ν_{max} cm^{-1}): 3272 (N-H), 3072, 2921 (C-H-str), 1587 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 3.38 (s, 1H, OH), 3.73 (q, 2H, NCH_2, J = 5.0 Hz), 3.93 (q, 2H, OCH_2, J = 5.0 Hz), 5.08 (s, 1H, NH), 7.65-7.78 (m, 3H, ArH), 7.75 (dd, 1H, ArH, J = 8.9, Hz, J = 1.5 Hz), 8.20-8.22 (m, 2H, ArH), 8.70 (d, 1H, ArH, J = 8.9 Hz), 8.96 (t, 1H, ArH, J = 8.7 Hz), 9.21 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 50.98, 60.32, 118.99, 119.70, 121.44, 123.60, 125.68, 126.99, 127.35, 128.06, 129.07, 130.87, 132.36, 148.92, 150.28, 151.24, 162.92, 163.23. MS: m/z = 401 (M+1). Anal. calcd. For $C_{20}H_{15}F_3N_4O_2$; Calcd: C, 60.00; H, 3.78; N, 13.99; found: C, 60.03; H, 3.88; N, 13.94%.

3.4.25. Acetic acid 2-[3-(5-phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinolin-4-ylamino]-ethyl ester (G_{31})

IR (Neat ν_{max} cm^{-1}): 3260 (N-H), 3052, 2981 (C-H-str), 1741 (C=O), 1590 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 2.45 (s, 3H, CH_3), 4.15 (q, 2H, NCH_2, J = 5.1 Hz), 4.67 (q, 2H, OCH_2, J = 5.0 Hz), 5.21 (s, 1H, NH), 7.64-7.70 (m, 3H, ArH), 8.08-8.13 (m, 3H, ArH), 8.51 (d, 1H, ArH, J = 8.9 Hz), 8.61 (t, 1H, ArH, J = 8.6 Hz), 9.89 (s, 1H, ArH); MS: m/z = 443 (M+1). Anal. calcd. For $C_{22}H_{17}F_3N_4O_3$; Calcd: C, 59.73; H, 3.87; N, 12.66; found: C, 59.70; H, 3.81; N, 12.76%.

3.4.26. [3-(5-Phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinolin-4-yl]-(3,4,5-trimethoxy-phenyl)-amine (G_{32})

IR (Neat ν_{max} cm^{-1}): 3260 (N-H), 3025, 2981 (C-H-str), 1590 (C=N), 1116 (O-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.10 (s, 3H, OCH_3), 3.55 (s, 6H, OCH_3), 6.24 (s, 1H, NH), 7.44-7.52 (m, 4H, ArH), 7.72-7.78 (m, 3H, ArH), 8.13 (s, 1H, ArH), 8.66 (d, 1H, ArH, J = 8.0 Hz), 8.82 (s, 1H, ArH); MS: m/z = 508 (M+1). Anal. calcd. For $C_{27}H_{21}F_3N_4O_4$; Calcd: C, 63.90; H, 3.97; N, 8.28; found: C, 63.90; H, 3.97; N, 8.30%.

3.4.27. (2-Methyl-quinolin-4-yl)-[3-(5-phenyl-[1,3,4]oxadiazol-2-yl)-7-trifluoromethyl-quinolin-4-yl]-amine (G_{33})

IR (Neat ν_{max} cm^{-1}): 3245 (N-H), 3061, 2921 (C-H-str), 1606 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.86 (s, 3H, CH_3), 6.30 (s, 1H, NH), 7.30-7.68 (m, 3H, ArH), 7.78 (t, 2H, ArH, J = 8.2 Hz), 7.96-8.06 (m, 3H, ArH), 8.24 (d, 2H, ArH, J = 8.3 Hz), 8.26 (s,1H, ArH), 8.42 (s, 1H, ArH), 8.61 (d, 1H, ArH, J = 8.0 Hz), 9.35 (s, 1H, ArH); MS: m/z = 498 (M+1). Anal. calcd. For $C_{28}H_{18}F_3N_5O$; Calcd: C, 67.60; H, 3.65; N, 14.08; found: C, 67.65; H, 3.64; N, 14.10%.

3.5. ANALYTICAL DATA

Crystal data	
Empirical formula	$C_{23}H_{20}F_3N_5O$
Formula weight	439.44
Crystal system	Triclinic
Crystal dimension	0.44 mm x 0.20 mm x 0.13 mm
Space group	$P\bar{1}$
a(Å)	8.5065 (15)
b(Å)	10.2176 (17)
c(Å)	13.709 (3)
Volume (Å^3)	1060.0 (4)
Angle α, β, γ	103.840, 98.515, 109.034
Z	2
Crystal density	1.377
F_{000}	456
μ (mm^{-1})	0.11
Temperature (T)	296
Radiation wavelength (Å)	0.71073
Radiation type	Mo Kα
Radiation source	fine-focus sealed tube
Radiation monochromator	graphite
h_{min}	- 11
h_{max}	11
k_{min}	- 13
k_{max}	12
l_{min}	- 17
l_{max}	17

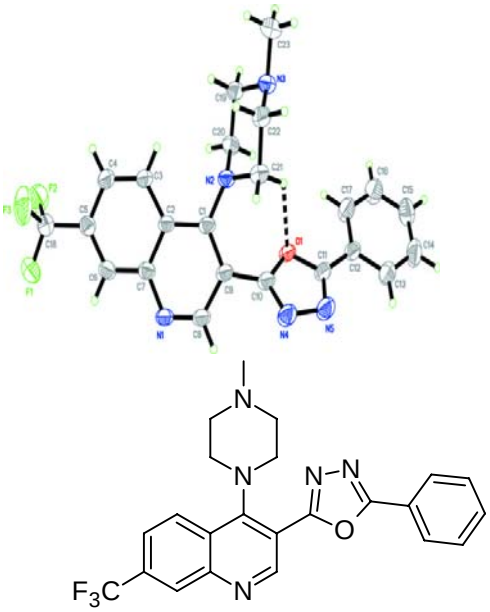


Figure 3.3. ORTEP diagram showing the single crystal structure of compound G_{28} (drawn at 50% probability level).

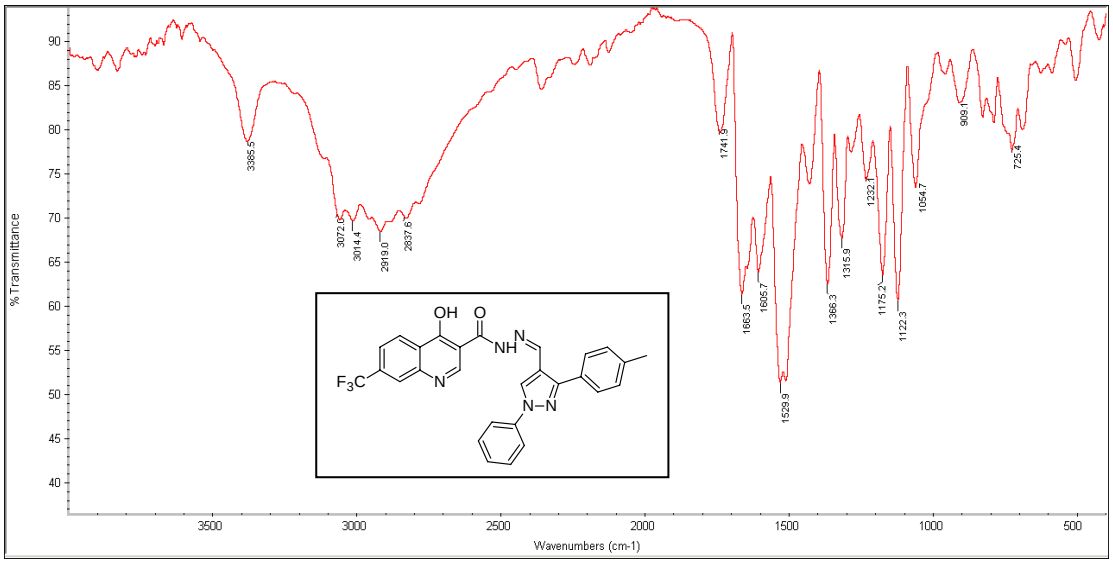


Figure 3.4. IR spectrum of G_{20}

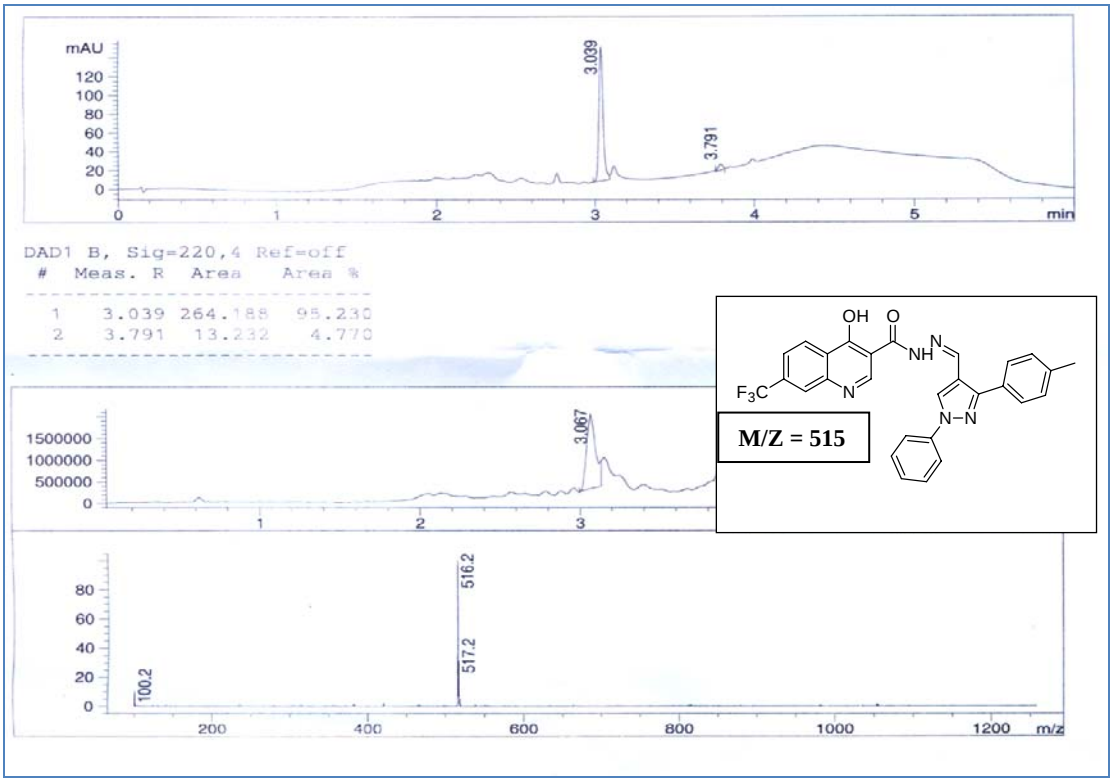


Figure 3.5. LCMS spectrum of G_{20}

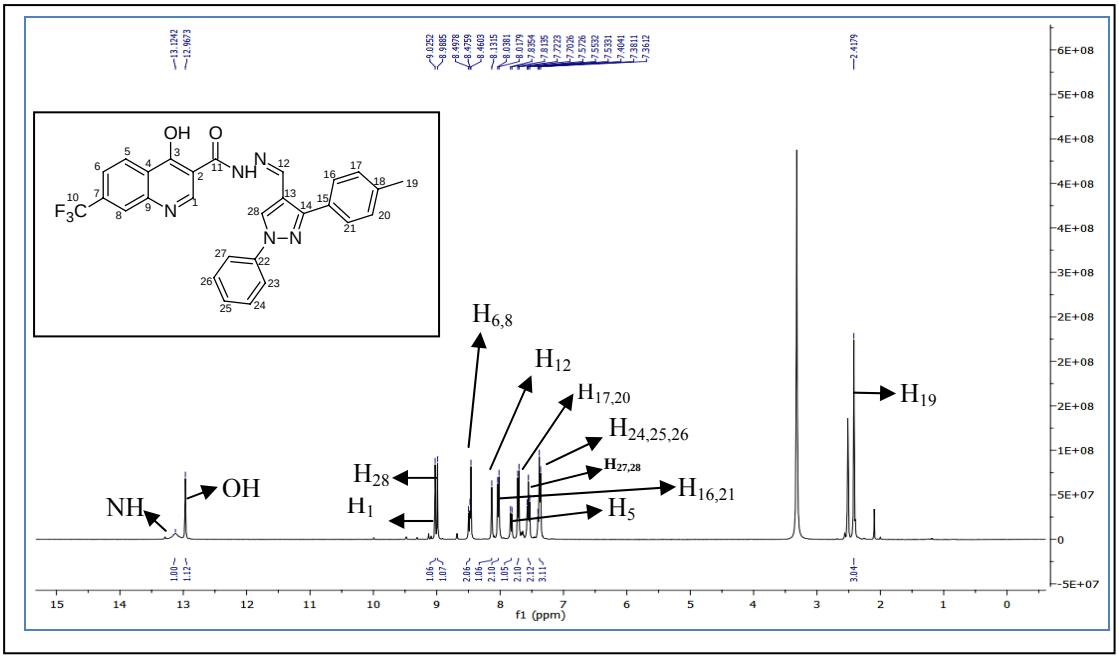


Figure 3.6. ^{1}H NMR spectrum of G_{20}

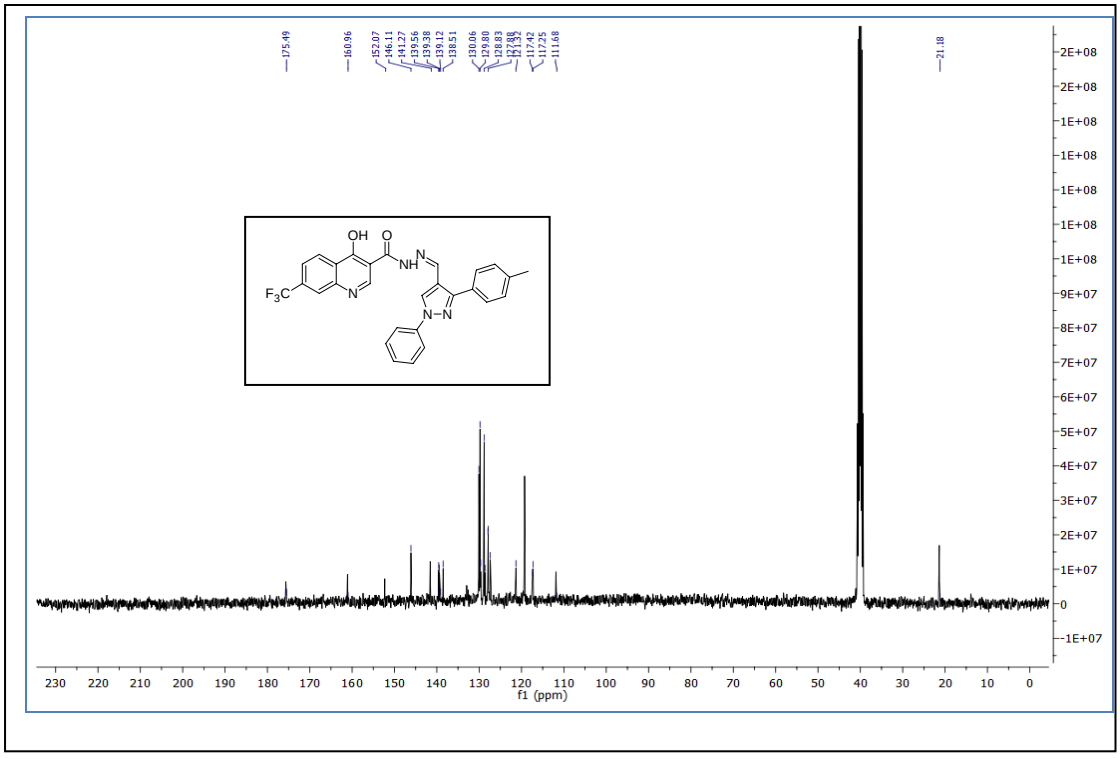


Figure 3.7. ^{13}C NMR spectrum of G_{20}

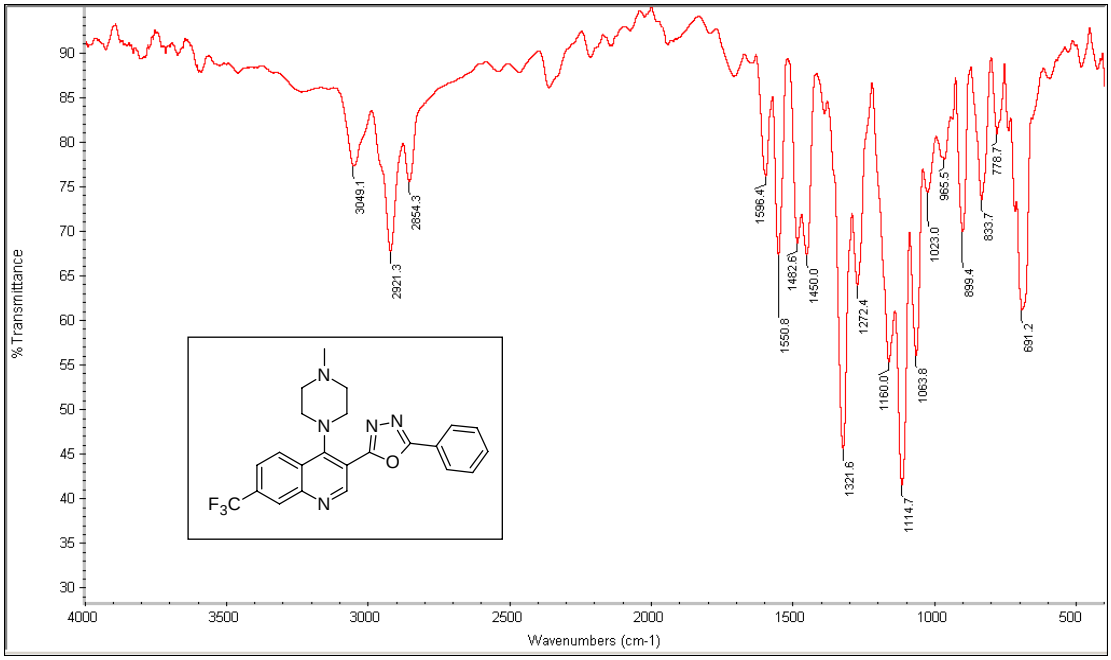


Figure 3.8. IR spectrum of G_{28}

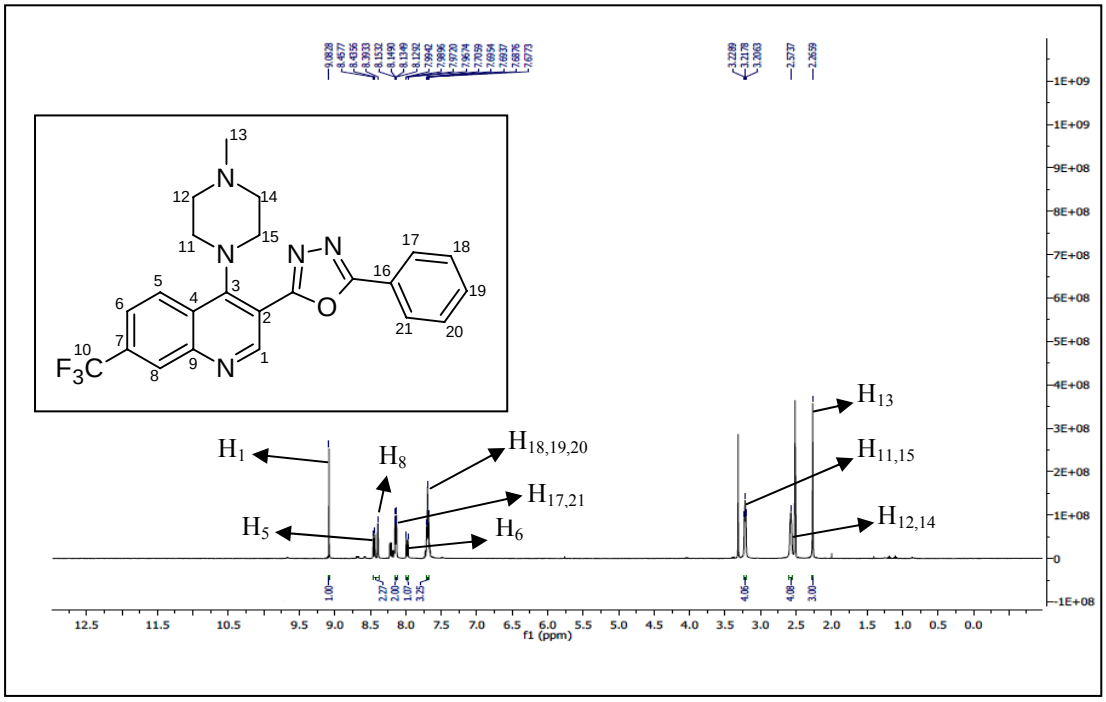


Figure 3.9. ^{1}H NMR spectrum of G_{28}

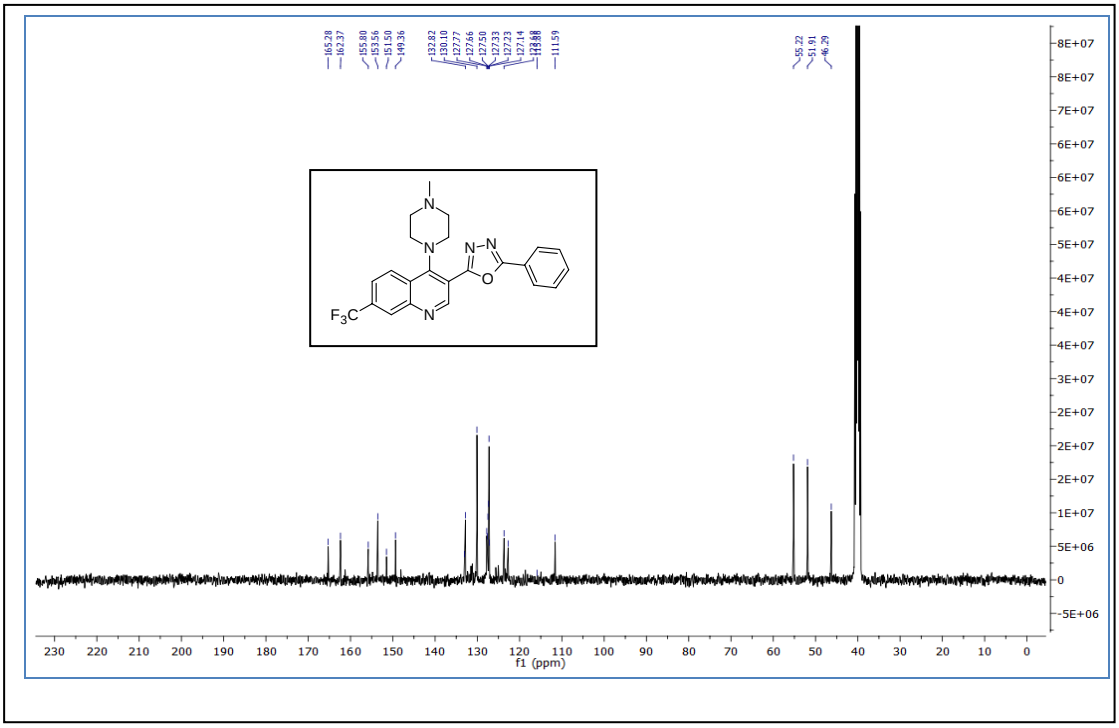


Figure 3.10. ^{13}C NMR spectrum of G_{28}

3.6. CONCLUSION

Two series of new 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide (**G_{16-20}** and **G_{21-27}**) derivatives and N-alkyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolin-4-amine derivatives (**G_{28-33}**) were synthesized in reasonably good yields by multistep reactions. The quinolone skeletons were built up by the Gould-Jacobs procedure starting from 3-(trifluoromethyl)aniline, the key intermediates, pyrazole-4-carbaldehydes (**9a-e)** were prepared by the Vilsemeier-Haack reaction of the corresponding hydrazones. The targeted 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide derivatives (**G_{16-20}** and **G_{21-27})** were obtained by reacting quinoline hydrazide **5** with various substituted aldehydes in ethanolic media and oxadiazoles derivatives were obtained by replacing various aliphatic and aromatic amines with 4-chloro-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolines. Synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry, IR studies and elemental analyses. The structure of **G_{28}** has also been confirmed by X-ray crystallographic study. The *in-vitro* antimicrobial activities were carried out for the final compounds and the results were discussed in CHAPTER-7.

CHAPTER-4

SYNTHESIS AND CHARACTERIZATION OF SOME NEW 7 AND 8-TRIFLUOROMETHYLQUINOLINE DERIVATIVES

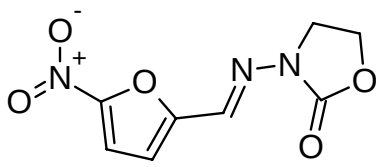
CHAPTER-4

SYNTHESIS AND CHARACTERIZATION OF SOME NEW 7 AND 8-TRIFLUOROMETHYLQUINOLINE DERIVATIVES

4.1. INTRODUCTION

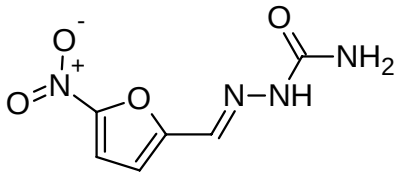
Hydrazones are a class of organic compounds with the structure $R_1R_2C=NNR_3R_4$. They are related to ketones and aldehydes by the replacement of the oxygen with the NNR_3R_4. The formations of hydrazones are used in the identification of organic aldehydes and ketones by making their respective hydrazone derivatives. The compound having N, N-alkylated hydrazones (C=N) are occupy prominent place in pharmaceutical industries. The hydrazone-based molecules are used as antibodies against a certain type of cancer cells, because of their stability at neutral pH (in the blood), but is rapidly destroyed in the acidic environment of lysosomes of the cell.

On the other hand, hydrazide-hydrazones, amides and ester compounds are not only intermediates but they are also very effective organic compounds in their own right. Hydrazones possessing an azometine -NHN=CH- proton constitute an important class of compounds for new drug development. These heterocyclic hydrazones are well known to possess diverse pharmacological properties, viz. antimicrobial (Ozkay et al. 2010), anticancer (Fan et al. 2010), antiamoebic (Siddiqui et al. 2012) activity. Some widely used antibacterial drugs such as Furazolidone (**QD-4.1**), Nitrofural (**QD-4.2**) and Nifuroxazide (**QD-4.3**) are known to contain this pharmacophore group.



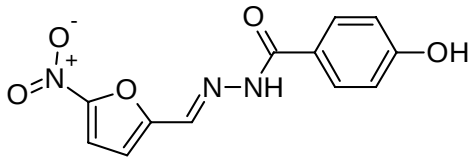
Furazolidone

QD-4.1



Nitrofural

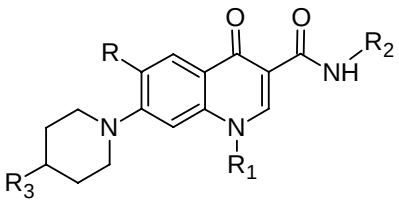
QD-4.2



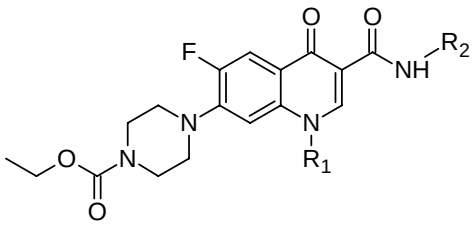
Nifuroxazide

QD-4.3

Amides and ester of fluoroquinolone derivatives (**QD-4.4 & QD-4.5**) were synthesized by Niedermeier et al. (2009). 1-(3-Carbamoyl-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinolin-7-yl)-piperidine-4-carboxylic acid ethyl ester, 1-[3-(2,4-Dichloro-benzylcarbamoyl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinolin-7-yl]-piperidine-4-carboxylic acid ethyl ester, 1-Ethyl-4-oxo-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carboxylic acid 2,4-dichloro-benzylamide, 1-Cyclopropyl-4-oxo-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carboxylic acid 2,4-dichloro-benzylamide derivatives are shown a good antiviral activity.



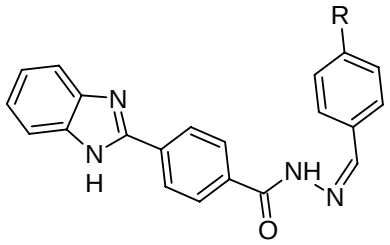
QD-4.4



QD-4.5

Where R = F, Cl, CF_3. R_1 = C_2H_5, C_3H_5. R_2 = 4-Clorobenzyl, 4-methoxybenzyl, 4-aminobenzyl, 2-pyridyl, 2,4-dichlorobenzyl, 4-fluoro-5-chlorobenzylCyclopropyl. R_3 = CF_3, $CONH_2$, $COOC_2H_5$.

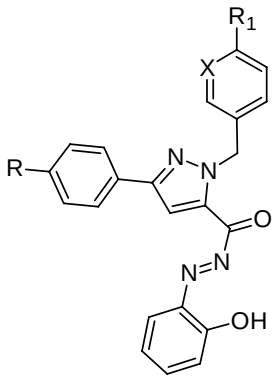
Antimicrobial activity of benzimidazole hydrazone derivatives (**QD-4.6**) was studied by Ozkay et al. (2010). The result revealed that, the presence of chloro, bromo and methyl substituents on the aromatic ring has increased the activity of the compounds compared to those with other substituents.



QD-4.6

Where R = H, OH, F, Cl, Br, CH_3, OCH_3, $N(CH_3)_2$, NO_2, CF_3, COOH, CN.

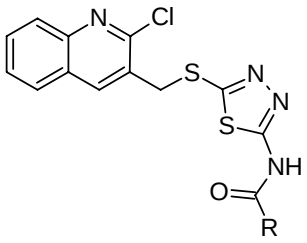
Fan et al. (2010) reported synthesis of some hydrazones of pyrazoles (**QD-4.7**) for anticancer Study. Among the synthesized compounds, the copper complex of (E)-N-(2-hydroxybenzylidene)-1-(4-tert-butylbenzyl)-3-phenyl-1H-pyrazole-5-carbohydrazide showed good activity.



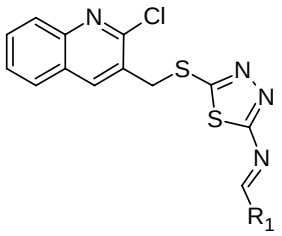
QD-4.7

Where R = H, Cl, OCH_3. R_1 = H, Cl, t-Bu. X = C, N.

Kumar et al. (2012) reported synthesis, anticonvulsant activity of some new amide derivatives (**QD-4.8 & QD-4.9**) of quinoline. The anticonvulsant activity results revealed that Methy, P-chlorophenyl substituted amide derivatives are better anticonvulsant molecules.



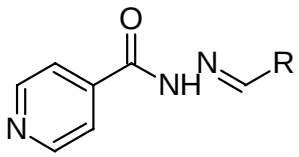
QD-4.8



QD-4.9

Where R = CH_3, CH_2CH_3, C_6H_5, 4-$CH_3C_6H_4$, 4-ClC_6H_4, 4-$OCH_3C_6H_4$, 4-FC_6H_4, 4-$NO_2C_6H_4$. R_1 = CH_3, C_6H_5, 4-$CH_3C_6H_4$, 4-ClC_6H_4, 4-$OCH_3C_6H_4$, 4-FC_6H_4, 4-$NO_2C_6H_4$.

Siddiqui et al. (2012) synthesized some new hydrazone derivatives (**QD-4.10**) and studied their antiamoebic activity. Compounds containing 4-nitrophenyl, 4-methoxyphenyl and 4-methylphenyl substituted pyridylhydrazones are showed better activity.



QD-4.10

Where R = Phenyl, 4-nitrophenyl, 4-methoxyphenyl, 4-methylphenyl, 3-chlorophenyl, 2-hydroxyphenyl, 4-N,N-dimethylphenyl and 4-isopropylphenyl.

Quinoline scaffold is an important pharmaceutically active synthetic and natural compound. Different structural modifications have been done to increase the antimicrobial activity by introducing the different functional groups around the quinoline nucleus (Kategaonkar et al. 2010; Koura et al. 2010). Most of the highly effective quinoline antimicrobials contain the trifluoromethyl group or fluorine atom attached to the quinoline ring (Singh et al. 2006; Kumar et al. 2011). It is capable of altering quite drastically, parameters such as basicity or acidity of the neighboring groups, dipole moment within the molecule, reactivity and stability of neighboring groups (Hawley et al. 1996). It has been shown that incorporation of trifluoromethyl group at 7^{th} and 8^{th} position of the quinoline have a profound effect on biological activity (Bi et al. 2004; He et al. 2005; Holla et al. 2006). A large variety of quinoline

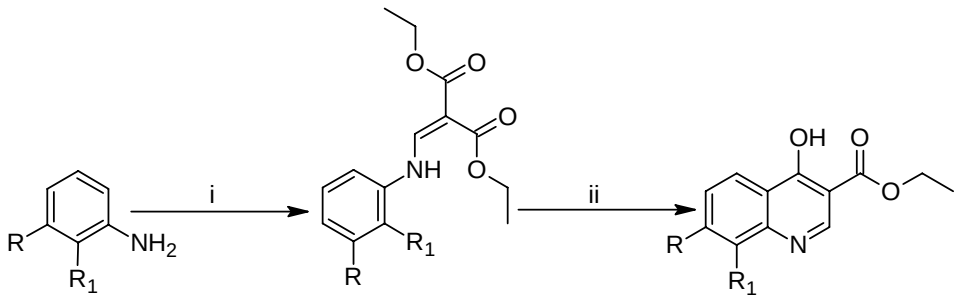
derivatives have been used as anti-inflammatory (Mikhalev et al. 1997), antifungal (Stringacova et al. 2000), antimalarial (Fattorusso et al. 2008; Manohar et al. 2011), anti-tuberculosis (Lilienkampf et al. 2009), antioxidant (Sankaran et al. 2010; Wang et al. 2010), antiviral (Koura et al. 2010), antibacterial (Lingaiah et al. 2012), antitumor (Kumar et al. 2012), anticonvulsant (Kumar et al. 2012; He et al. 2012).

The chemistry of quinoline and their hydrazones has received considerable attention owing to their synthetic and biological importance during the last two decades. Quinoline based compounds are known to exhibit excellent antimicrobial properties (Lingaiah et al. 2012). Several researchers have shown that compounds containing quinoline moiety, such as 8-hydroxyquinoline, 6-fluoro-4-hydroxyquinoline, may act as a free radical scavenger (Zheng et al. 2005), they have potential to protect biological systems against induced oxidative damage (ZQ et al. 2002). On the other hand, compounds containing amide linkages are very well known to exhibit powerful antioxidant (Roopan et al. 2009), antimicrobial, antiviral (Niedermeier et al. 2009; Lingaiah et al. 2012) properties. Therefore there is great importance for the synthesis of hydrazones and amide derivatives of quinoline as target structures and evaluation of their biological activities. By these observations, it was planned for the synthesis of new quinoline derivatives and their characterization. Further, these compounds were evaluated for their antimicrobial and antioxidant properties.

4.2. RESULT AND DISCUSSION

4.2.1. Synthesis of some new 7 and 8-trifluoromethylquinoline derivatives

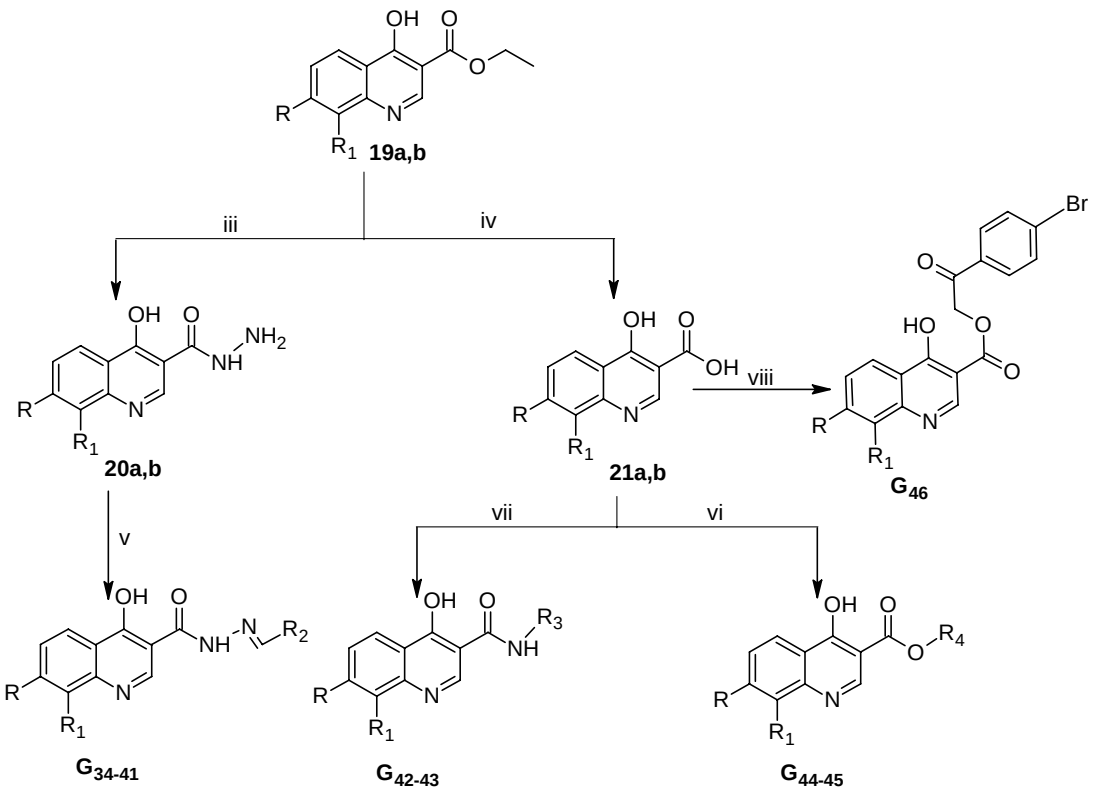
Ethyl 4-hydroxyquinoline-3-carboxylate (**3a**,**b**) were synthesized from Gould-Jacobs procedure as discussed in **chapter-3**. Ethyl 4-hydroxyquinoline-3-carboxylates (**3a,b**) were condensed with hydrazine hydrate in an alcoholic medium resulted trifluoromethyl-4-hydroxyquinoline-3-carbohydrazide (**4a**,**b**) (Kumar et al. 1988; Thomas et al. 2011). The targeted 4-hydroxy-(trifluoromethyl)quinoline-3-carbohydrazide derivatives (**G_{34-41}**) were obtained by the reacting quinoline hydrazide (**4a**,**b**) with various substituted aldehydes in ethanolic media (**Scheme-4.2**). 4-Hydroxy-N-alkylquinoline-3-carboxamide **(G_{42-43})** and alkyl-4-hydroxyquinoline-3-carboxylate (**G_{44-45}**) were obtained by coupling 4-hydroxy-quinoline-3-carboxylic acid (**21a**,**b**), aliphatic amine using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl), 1-hydroxybenzotriazole (HOBT) in chloroform media. Phenacyl ester of the acid (**G_{46}**) was synthesized by reacting quinoline acid (**5a**) with 2-Bromo-1-(4-bromo-phenyl)-ethanone (**scheme-4.2**).



Where R/R_1 = H, CF_3.

Scheme 4.1. Synthetic route for Ethyl 4-hydroxyquinoline-3-carboxylate (3a,b).

(i) 110 °C, 6 h; (ii) Dowtherm, 250 °C, 5 h.



Where R/R_1 =H, CF_3; R_2= Pyridine-4-carbaldehyde, 1H-Indole-3-carbaldehyde, 2-Chloro-quinoline-3-carbaldehyde, 4-Methyl-benzaldehyde, Furan-2-carbaldehyde; R_3= 2-Amino-ethanol; R_4= 2-Amino-ethanol.

Scheme 4.2. Synthetic route for 4-hydroxy quinoline-3-aryl carbohydrazides (G_{34-41}), 4-hydroxy-N-alkylquinoline-3-carboxamide ($G_{42,43}$), Alkyl 4-hydroxyquinoline-3-carboxylate ($G_{44,45}$) and 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid 2-(4-bromo-phenyl)-2-oxo-ethyl ester G_{46}: (iii) NH_2NH_2, EtOH, 4 h; (iv) LiOH, MeOH, 2 h; (v) Aldehyde, EtOH, AcOH, 25-30 °C, 2 h; (vi and vii) Ethanolamine, EDC. HCl, HOBt, DIPEA, 12 h; (viii) 2-bromo-1-(4-bromophenyl) ethanone, K_2CO_3, DMF, 2 h.

Table-4.1. Characterization data of the compounds G_{34-41}, $G_{42,43}$, $G_{44,45}$ and G_{46}

Compd	R	R_1	$R_2/R_3/R_4$	Mol Formula & Mol. Wt.	Yield (%)	M.p. (°C)	Color & nature
G_{34}	CF_3	H	Pyridine-4-carbaldehyde	$C_{17}H_{11}F_3N_4O_2$ 360.29	95	297-299	White solid
G_{35}	CF_3	H	1H-Indole-3-carbaldehyde	$C_{20}H_{13}F_3N_4O_2$ 398.33	92	> 300	White solid
G_{36}	CF_3	H	2-Chloro-quinoline-3-carbaldehyde	$C_{21}H_{12}ClF_3N_4O_2$ 444.79	90	> 300	yellow solid
G_{37}	CF_3	H	4-Methyl-benzaldehyde	$C_{19}H_{14}F_3N_3O_2$ 373.32	87	> 300	White solid
G_{38}	H	CF_3	1H-Indole-3-carbaldehyde	$C_{20}H_{13}F_3N_4O_2$ 398.33	90	248-250	Pale yellow solid
G_{39}	H	CF_3	2-Chloro-quinoline-3-carbaldehyde	$C_{21}H_{12}ClF_3N_4O_2$ 444.79	95	> 300	Pale yellow solid
G_{40}	H	CF_3	Furan-2-carbaldehyde	$C_{16}H_{10}F_3N_3O_3$ 349.26	89	287-289	White solid
G_{41}	CF_3	H	Furan-2-carbaldehyde	$C_{16}H_{10}F_3N_3O_3$ 349.26	84	290-292	Brown solid
G_{42}	CF_3	H	2-Amino-ethanol	$C_{13}H_{11}F_3N_2O_3$ 300.23	39	146-148	Pale yellow solid
G_{43}	H	CF_3	2-Amino-ethanol	$C_{13}H_{11}F_3N_2O_3$ 300.23	33	185-187	White solid
G_{44}	CF_3	H	2-Amino-ethanol	$C_{13}H_{11}F_3N_2O_3$ 300.23	40	156-158	Pale yellow solid
G_{45}	H	CF_3	2-Amino-ethanol	$C_{13}H_{11}F_3N_2O_3$ 300.23	35	125-127	Dark brown solid
G_{46}	H	CF_3	1-(4-Bromo-phenyl)-ethanone	$C_{19}H_{11}BrF_3NO_4$ 454.19	45	87-89	Brown solid

The newly synthesized compounds were characterized by IR, NMR, mass spectral and elemental analyses. The IR spectrum of compound **G_{37}** showed two bands at 3417 cm^{-1} and 3024 cm^{-1}, which are due to the hydroxyl and amide groups respectively. Band at 1645 cm^{-1} is due to C=O stretch of the carbonyl group. The ^{1}H NMR spectrum of **G_{37}** showed singlet's at δ 2.31, 8.08 ppm which is due to the methyl (CH_3) and imine proton (N=CH). Hydroxyl and amide protons appeared as singlets at δ 12.99 and 13.07 ppm respectively further confirmed the structure of the compound. The mass spectrum of **G_{37}** showed a molecular ion peak at m/z = 474 (M+1), which is in agreement with the molecular formula $C_{19}H_{14}F_3N_3O_2$. Similarly the spectral values for all the compounds and C, H, N analyses are presented in the experimental part and the characterization data are provided in **Table-4.1.**

4.3. EXPERIMENTAL

4.3.1. General method for the preparation of [(trifluoromethyl-phenylamino)-methylene]-malonic acid diethyl ester (2a,b)

Trifluoromethylaniline (**1a**,**b**) (10 g, 0.062 mol) and diethyl ethoxymethylene malonate (20.10 g, 0.093 mol) was heated to 110 °C for 6 h. The reaction mixture was cooled to room temperature, the solid thus formed was taken in pet ether and stirred for 20 min and filtered to get compound **2a**,**b** a white crystalline solids.

4.3.2. General method for the preparation of 4-hydroxy-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (3a,b)

2-[(Trifluoromethyl-phenylamino)-methylene]-malonic acid diethyl ester (**2a**,**b**) (15.0 g, 0.045 mol) and dowtherm 100 mL were heated to 250 °C for 5 h. The reaction mixture was then cooled to 25 °C and stirred in 150 mL hexane for 10 min. The solid product obtained was filtered and dried. The crude product obtained was purified by column chromatography using pet ether and ethyl acetate (5:5) as the eluent.

4.3.3. General method for the preparation of 4-hydroxy-trifluoromethyl-quinoline-3-carboxylic acid hydrazide (4a,b)

A mixture of 4-hydroxy-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (**3a**,**b**) (10.0 g, 0.035 mol) and hydrazine hydrate (5.44 mL, 0.175 mol, reagent

grade 98%) in ethanol (100 mL) were refluxed for 4 h. After the completion of the reaction, the reaction mixture was concentrated and allowed to cool. The solid product obtained was filtered, washed with water and recrystallized from ethanol to give **4a**,**b** as white solid.

4.3.4. General method for the preparation of 4-hydroxy-(trifluoromethyl) quinoline-3-carbohydrazide derivatives (G_{34-41})

An equimolar mixture of 4-hydroxy-trifluoromethyl-quinoline-3-carboxylic acid hydrazide (**4a**,**b**) (0.20 g, 0.0007 mol), aromatic aldehydes (0.0008 mol) and catalytic amount of acetic acid in dry ethanol (5 mL) was stirred at 25 °C for 2 h. Completion of the reaction was monitored by TLC. The precipitated solid was filtered under suction, washed with ethanol and recrystallized from ethanol.

4.3.5. General method for the preparation of 4-hydroxy-trifluoromethyl-quinoline-3-carboxylic acid (5a,b)

To a suspension of 4-hydroxy-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester **(3a**,**b)** (5.0 g, 0.017 mol) in methanol (50 mL) at 0 °C was added lithium hydroxide (0.88 g, 0.021 mol) for 10 min. The mixture was allowed to stir for 2 h and was quenched by the slow addition water (50 mL), acidified using dilute HCl. The precipitated solids were collected by filtration and recrystallized by ethanol.

4.3.6. General method for the preparation 4-hydroxy-trifluoromethyl-quinoline-3-carboxylic acid-2-amide (G_{42-43}) and 4-hydroxy-trifluoromethyl-quinoline-3-carboxylic acid ester (G_{44-45})

To a solution of compounds (**5a,b**) (1.0 g, 0.0038 mol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) (0.81 g, 0.0042 mol), 1-hydroxybenzotriazole (HOBt) (0.128 g, 0.0009 mol), N,N-diisopropylethylamine (DIPEA) (0.99 mL, 0.0057 mol) and 2-aminoethanol (034 mL, 0.0057 mol) in chloroform (10 mL) was stirred for overnight and solvents were evaporated under vacuum. The residue obtained was dissolved in water and extracted with ethyl acetate, concentrated under vacuum. The residue obtained was purified by column chromatography using pet ether ethyl acetate as eluents (5:5) to afford solid compounds ($\mathbf{G_{42-43}}$ and $\mathbf{G_{44-45}}$).

4.3.7. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid 2-(4-bromo-phenyl)-2-oxo-ethyl ester (G_{46})

The mixture of 4-hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid (0.5 g, 0.0019 mol) potassium carbonate (0.29 g, 0.0021 mol) and 2-bromo-1-(4-bromophenyl)ethanone (0.58 g, 0.0020 mol) in dimethylformamide (10 mL) was stirred at room temperature for 2 h and was quenched by the slow addition ice-water (10 mL), acidified using dilute HCl. The precipitated solids were collected by filtration and purified by column chromatography using pet ether ethyl acetate (7:3) as eluents.

4.4. CHARACTERIZATION OF SYNTHESIZED COMPOUNDS

4.4.1. 2-[(2-Trifluoromethyl-phenylamino)-methylene]-malonic acid diethyl ester (2a)

Yield: 19.1 g, 92.8%. M.p: 84-86 °C. IR (Neat ν_{max} cm^{-1}): 3278 (N-H), 3176, 2986 (C-H-str), 1706 and 1654 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.33 (t, 3H, CH_3, J = 5.3 Hz), 1.37 (t, 3H, CH_3, J = 5.3 Hz), 4.22 (q, 2H, CH_2), 4.30 (q, 2H, CH_2), 7.21-8.41 (m, 5H, ArH), 11.50 (s, 1H, NH); Anal. calcd. For $C_{15}H_{16}F_3NO_4$; Calcd: C, 54.38; H, 4.80; N, 4.23; found: C, 54.38; H, 4.79; N, 4.21 (Thomas et al. 2011).

4.4.2. 2-[(3-Trifluoromethyl-phenylamino)-methylene]-malonic acid diethyl ester (2b)

Yield: 19.0 g, 92.4%. M.p: 44-46 °C; IR (Neat ν_{max} cm^{-1}): 3252 (N-H), 3118, 2979 (C-H-str), 1708 and 1616 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.37 (t, 3H, CH_3, J = 5.3 Hz), 1.39 (t, 3H, CH_3, J = 5.3 Hz), 4.26 (q, 2H, CH_2), 4.34 (q, 2H, CH_2), 7.21-8.40 (m, 5H, ArH), 11.49 (s, 1H, NH); Anal. calcd. For $C_{15}H_{16}F_3NO_4$; Calcd: C, 54.38; H, 4.80; N, 4.23; found: C, 54.35; H, 4.80; N, 4.20 (Bi et al. 2004).

4.4.3. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (3a)

Yield: 11.9 g, 92.1%. M.p: 295-297 °C. IR (Neat ν_{max} cm^{-1}): 3352 (-OH), 3118, 2979 (C-H-str), 1708 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.23 (t. 3H, J = 8.0 Hz, -CH_3), 4.16 (q, 2H, -CH_2), 7.53 (t, 1H, ArH, J = 8.0 Hz), 8.07 (d, 1H, ArH, 7.8 Hz), 8.41 (d, 2H, ArH, J = 8.0 Hz), 11.62 (s, 1H, -OH, D_2O-exchangeble). ^{13}C NMR: δ ppm 14.70, 60.49, 111.39, 119.07, 124.61, 125.35, 130.99, 131.55, 146.43. MS: m/z

= 286 (M+1). Anal. calcd. For $C_{13}H_{10}F_3NO_3$; Calcd: C, 54.74; H, 3.53; N, 4.91; found: C, 54.75; H, 3.54; N, 4.95 (Thomas et al. 2011).

4.4.4. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (3b)

Yield: 11.0 g, 85.2%. M.p: 298-300 °C. IR (Neat ν_{max} cm^{-1}): 3322 (-OH), 3029, 2970 (C-H-str), 1706 (C=O), ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.22 (t, 3H, CH_3, J = 8.0 Hz), 4.18 (q, 2H, CH_2, J = 8.0 Hz), 7.53 (d, 1H, ArH, J = 12 Hz), 7.81 (d, 1H, ArH, J = 12 Hz), 8.03 (s, 1H, ArH), 8.44 (s, 1H, ArH), 12.30 (s, 1H, -OH); MS: m/z = 286 (M+1). Anal. calcd. For $C_{13}H_{10}F_3NO_3$; Calcd: C, 54.74; H, 3.53; N, 4.91; found: C, 54.77; H, 3.50; N, 4.95 (Bi et al. 2004).

4.4.5. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid hydrazide (4a)

Yield: 8.5 g, 89.4%. M.p: >300 °C. IR (Neat ν_{max} cm^{-1}): 3323 (-OH), 3236 and 33173 (-NH), 3037, 2967 (C-H-str), 1656 (C=O), ^1H NMR (400 MHz, DMSO-d_6): δ ppm 4.65 (s, 2H, $-NH_2$, exchangeble), 7.77 (dd, 1H, ArH, J = 7.8 Hz, J = 1.4 Hz), 8.11 (s, 1H, ArH), 8.45 (d, 1H, ArH, J = 8.4 Hz), 8.92 (s, 1H, ArH), 10.51 (s, 1H, -NH, D_2O-exchangeble), 12.87 (s, 1H, -OH, D_2O-exchangeble), Anal. calcd. For $C_{11}H_8F_3N_3O_2$; Calcd: C, 48.72; H, 2.97; N, 15.49; found: C, 48.74; H, 2.97; N, 15.51 (Thomas et al. 2011).

4.4.6. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid hydrazide (4b)

Yield: 8.3 g, 87.3%. M.p: 255-257 °C. IR (Neat ν_{max} cm^{-1}): 3442 (-OH), 3296 and 3244 (-NH), 3088, 2963 (C-H-str), 1649 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 4.67 (s, 2H, $-NH_2$, exchangeble), 7.76 (dd, 1H, ArH, J = 7.7 Hz, J = 1.4 Hz), 8.10 (s, 1H, ArH), 8.45 (d, 1H, ArH, J = 8.4 Hz), 8.90 (s, 1H, ArH), 10.57 (s, 1H, -NH, D_2O-exchangeble), 12.88 (s, 1H, -OH, D_2O-exchangeble). MS: m/z = 272 (M+1). Anal. calcd. For $C_{11}H_8F_3N_3O_2$; Calcd: C, 48.72; H, 2.97; N, 15.49; found: C, 48.75; H, 2.97; N, 15.59 (kumar et al. 1988).

4.4.7. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid pyridin-4-ylmethylene-hydrazide (G_{34})

IR (Neat ν_{max} cm^{-1}): 3499 (-OH), 3061, 2894 (C-H-str), 1671 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.60 (d, 2H, ArH, J = 8.0 Hz), 7.70 (d, 1H, ArH, J = 8.0 Hz),

8.02 (s, 1H, N=CH), 8.39-8.41 (m, 2H, ArH), 8.59 (m, 2H, ArH), 8.96 (s, 1H, ArH), 13.15 (s, 1H, -OH, D_2O-exchangeble), 13.38 (s, 1H, -NH, D_2O-exchangeble). ^{13}C NMR: δ ppm 111.11, 118.39, 121.02, 121.48, 122.72, 125.43, 127.76, 128.84, 132.36, 132.68, 140.71, 142.09, 145.81, 147.43, 150.69, 162.15, 175.54. MS: m/z = 361 (M+1). Anal. calcd. For $C_{17}H_{11}F_3N_4O_2$; Calcd: C, 56.67; H, 3.08; N, 15.55; found: C, 56.65; H, 3.06; N, 15.58.

4.4.8. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (1H-indol-3-ylmethylene)-hydrazide (G_{35})

IR (Neat ν_{max} cm^{-1}): 3520 (-OH), 3132 (-NH), 3031, 2973 (C-H-str), 1653 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.09-7.18 (m, 2H, ArH), 7.39 (d, 1H, ArH, J = 8.0 Hz), 7.76 (d, 2H, ArH, J = 7.8 Hz), 8.08 (s, 1H, N=CH), 8.21 (d, 1H, ArH, J = 8.0 Hz), 8.44 (d, 1H, ArH, J = 8.0 Hz), 8.51 (s, 1H, ArH), 8.95 (s, 1H, ArH), 11.54 (s, 1H, -NH, D_2O-exchangeble), 12.81 (s, 1H, -OH, D_2O-exchangeble), 13.01 (s, 1H, -NH, D_2O-exchangeble). ^{13}C NMR: δ ppm 112.13, 112.26, 112.35, 120.89, 122.37, 123.11, 124.85, 127.94, 128.60, 131.02, 137.49, 139.29, 145.41, 145.67, 160.54, 175.78. MS: m/z = 399 (M+1). Anal. calcd. For $C_{20}H_{13}F_3N_4O_2$; Calcd: C, 60.30; H, 3.29; N, 14.07; found: C, 60.32; H, 3.26; N, 14.08.

4.4.9. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (2-chloro-quinolin-3-ylmethylene)-hydrazide (G_{36})

IR (Neat ν_{max} cm^{-1}): 3471 (-OH), 3148 (-NH), 3065, 3011 (C-H-str), 1663 (C=O). ^1H NMR (400 MHz, $CDCl_3$): δ ppm 7.19-7.31 (m, 2H, ArH), 7.43-7.49 (m, 2H, ArH), 7.79-7.86 (m, 2H, ArH), 8.15 (s, 1H, N=CH), 8.36-8.40 (m, 2H, ArH), 8.91 (s, 1H, ArH). MS: m/z = 445 (M+1). Anal. calcd. For $C_{21}H_{12}ClF_3N_4O_2$; Calcd: C, 56.71; H, 2.72; N, 12.60; found: C, 56.70; H, 2.70; N, 12.58.

4.4.10. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (4-methyl-benzylidene)-hydrazide (G_{37})

IR (Neat ν_{max} cm^{-1}): 3417 (-OH), 3024 (-NH), 2970, 2908 (C-H-str), 1645 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 2.31 (s, 3H, CH_3), 7.23 (d, 2H, ArH, J = 7.9 Hz), 7.63 (d, 2H, ArH, J = 8.0 Hz), 7.78 (dd, 1H, ArH, J = 8.6 Hz, J = 1.4 Hz), 8.08 (s, 1H, N=CH), 8.36 (s, 1H, ArH), 8.44 (d, 1H, ArH, J = 8.4 Hz), 8.97 (s, 1H, ArH), 12.99 (s,

1H, -OH, D_2O-exchangeble), 13.07 (s, 1H, -NH, D_2O-exchangeble). ^{13}C NMR: δ ppm 25.96, 117.05, 117.27, 120.27, 120.50, 129.87, 129.96, 163.82, 165.92; MS: m/z = 374 (M+1). Anal. calcd. For $C_{19}H_{14}F_3N_3O_2$; Calcd: C, 61.13; H, 3.78; N, 11.26; found: C, 61.15; H, 3.76; N, 11.28.

4.4.11. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid (1H-indol-3-yl methylene)-hydrazide (G_{38})

IR (Neat ν_{max} cm^{-1}): 3547 (-OH), 3171 (-NH), 3104 (-NH), 3048, 2937 (C-H-str), 1653 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.18-7.21 (m, 2H, ArH), 7.39 (d, 1H, ArH, J = 8.0 Hz), 7.44 (d, 1H, ArH, J = 8.0 Hz), 7.64 (t, 1H, ArH, J = 8.0 Hz), 8.02 (d, 1H, ArH, J = 8.0 Hz), 8.19 (s, 1H, N=CH), 8.21-8.23 (m, 1H, ArH), 8.73 (s, 1H, ArH), 9.88 (s, 1H, ArH), 11.53 (s, 1H, -NH, D_2O-exchangeble), 12.66 (s, 1H, -OH, D_2O-exchangeble), 12.67 (s, 1H, -NH). ^{13}C NMR: δ ppm 112.10, 119.49, 122.58, 123.93, 124.20, 130.52, 137.90, 142.81, 146.20, 149.91, 153.50. MS: m/z = 399 (M+1). Anal. calcd. For $C_{20}H_{13}F_3N_4O_2$; Calcd: C, 60.30; H, 3.29; N, 14.07; found: C, 60.33; H, 3.28; N, 14.07.

4.4.12. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid (2-chloro-quinolin-3-ylmethylene)-hydrazide (G_{39})

IR (Neat ν_{max} cm^{-1}): 3468 (-OH), 3062 (-NH), 3002, 2990 (C-H-str), 1649 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.29-7.31 (m, 1H, ArH), 7.50-7.52 (m, 1H, ArH), 7.58-7.66 (m, 2H, ArH), 7.81-7.88 (m, 1H, ArH), 8.19 (s, 1H, N=CH), 8.49-8.50 (m, 2H, ArH), 8.58 (d, 1H, ArH, J = 8.0 Hz), 8.70 (s, 1H, ArH), 13.03 (s, 1H, -OH, D_2O-exchangeble), 13.16 (s, 1H, -NH, D_2O-exchangeble). MS: m/z = 445 (M+1). Anal. calcd. For $C_{21}H_{12}ClF_3N_4O_2$; Calcd: C, 56.71; H, 2.72; N, 12.60; found: C, 56.74; H, 2.70; N, 12.61.

4.4.13. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid furan-2-ylmethyl ene-hydrazide (G_{40})

IR (Neat ν_{max} cm^{-1}): 3517 (-OH), 3132 (-NH), 3045, 2942 (C-H-str), 1667 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 6.60-6.86 (m,2H, ArH), 7.63 (t, 1H, ArH, J = 8.0 Hz), 7.81-8.00 (m, 1H, ArH), 8.18 (s, 1H, N=CH), 8.33 (s, 1H, ArH), 8.56-8.68 (m, 2H, ArH), 12.21 (s, 1H, -OH, D_2O-exchangeble), 12.80 (s, 1H, -NH, D_2O-

exchangeble). ^{13}C NMR: δ ppm 112.69, 114.51, 125.08, 126.00, 128.80, 131.91, 131.98, 138.91, 145.78, 149.88, 156.90, 160.10. MS: m/z = 350 (M+1). Anal. calcd. For $C_{16}H_{10}F_3N_3O_3$; Calcd: C, 55.02; H, 2.89; N, 12.03; found: C, 55.05; H, 2.87; N, 12.01.

4.4.14. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid furan-2-ylmethyl ene-hydrazide (G_{41})

IR (Neat ν_{max} cm^{-1}): 3433 (-OH), 3072 (-NH), 2999, 2948 (C-H-str), 1652 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 6.60-6.61 (m,1H, ArH), 6.86-6.87 (m, 1H, ArH), 7.77-7.82 (m, 2H, ArH), 8.09 (s, 1H, N=CH), 8.33 (s, 1H, ArH), 8.44 (d, 1H, ArH, J = 8.5 Hz), 8.96 (s, 1H, ArH), 12.94 (s, 1H, -OH, D_2O-exchangeble), 13.09 (s, 1H, -NH, D_2O-exchangeble). MS: m/z = 350 (M+1). Anal. calcd. For $C_{16}H_{10}F_3N_3O_3$; Calcd: C, 55.02; H, 2.89; N, 12.03; found: C, 55.04; H, 2.87; N, 12.04.

4.4.15. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid (5a)

Yield: 4.3 g, 95.5%. M.p: 258-260 °C. IR (Neat ν_{max} cm^{-1}): 3440 (-OH), 3113, 3061 (C-H-str), 1720 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.54 (t, 1H, ArH, J = 8.0 Hz), 8.05 (d, 1H, ArH, 7.8 Hz), 8.44 (d, 2H, ArH, J = 8.0 Hz), 11.61 (s, 1H, -OH, D_2O-exchangeble), 14. 43 (s, 1H, OH). Anal. calcd. For $C_{11}H_6F_3NO_3$; Calcd: C, 51.37; H, 2.35; N, 5.45; found: C, 51.39; H, 2.33; N, 5.46 (Snyder et al. 1947).

4.4.16. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (5b)

Yield: 4.4 g, 97.7%. M.p: 249-251 °C. IR (Neat ν_{max} cm^{-1}): 3380 (-OH), 2909, 2955 (C-H-str), 1690 (C=O), ^1H NMR (400 MHz, DMSO-d_6): δ ppm 7.52 (d, 1H, ArH, J = 12 Hz), 7.81 (d, 1H, ArH, J = 12 Hz), 8.03 (s, 1H, ArH), 8.44 (s, 1H, ArH), 12.29 (s, 1H, -OH, D_2O-exchangeble), 14.48 (s, 1H, OH, D_2O-exchangeble). Anal. calcd. For $C_{11}H_6F_3NO_3$; Calcd: C, 51.37; H, 2.35; N, 5.45; found: C, 51.38; H, 2.34; N, 5.45 (Allais et al. 1973).

4.4.17. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid (2-hydroxy-ethyl)-amide (G_{42})

IR (Neat ν_{max} cm^{-1}): 3410 (-OH), 3145 (-NH), 3049, 2933 (C-H-str), 1680 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 3.32-3.52 (m, 4H, -CH_2), 4.77 (s, 1H, -OH, D_2O-

exchangeble), 8.04 (d, 1H, ArH, J = 8.0 Hz), 8.45-8.48 (m, 2H, ArH), 8.81 (s, 1H, -NH, D_2O-exchangeble), 8.98 (s, 1H, ArH). ^{13}C NMR: δ ppm 42.63, 60.04, 124.47, 127.05, 127.57, 127.63, 132.30, 132.69, 138.69, 147.46, 151.04, 164.48. MS: m/z = 301 (M+1). Anal. calcd. For $C_{13}H_{11}F_3N_2O_3$; Calcd: C, 52.01; H, 3.69; N, 9.33; found: C, 52.05; H, 3.66; N, 9.34.

4.4.18. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid (2-hydroxy-ethyl)-amide (G_{43})

IR Neat ν_{max} cm^{-1}): 3420 (-OH), 3286 (-NH), 3100, 3069 (C-H-str), 1650 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 3.37-3.47 (m, 4H, -CH_2), 8.02-8.19 (m, 1H, ArH), 8.56-8.66 (m, 2H, ArH), 8.91 (s, 1H, -NH, D_2O-exchangeble), 8.92 (s, 1H, ArH). MS: m/z = 301 (M+1). Anal. calcd. For $C_{13}H_{11}F_3N_2O_3$; Calcd: C, 52.01; H, 3.69; N, 9.33; found: C, 52.03; H, 3.66; N, 9.38.

4.4.19. 4-Hydroxy-7-trifluoromethyl-quinoline-3-carboxylic acid 2-amino-ethyl ester (G_{44})

IR (Neat ν_{max} cm^{-1}): 3357 (-OH), 3269 (-NH), 3037, 2940 (C-H-str), 1641 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 2.45 (s, 2H, -NH_2, D_2O-exchangeble), 3.62-3.78 (m, 4H, -CH_2), 7.63 (d, 1H, ArH, J = 8.0 Hz), 8.05 (s, 1H, ArH), 8.54 (d, 1H, ArH, J = 8.0 Hz), 8.89 (s, 1H, ArH). ^{13}C NMR: δ ppm 52.48, 60.48, 119.81, 121.72, 126.56, 128.66, 131.22, 150.06, 152.51, 155.45, 168.30. MS: m/z = 301 (M+1). Anal. calcd. For $C_{13}H_{11}F_3N_2O_3$; Calcd: C, 52.01; H, 3.69; N, 9.33; found: C, 52.02; H, 3.61; N, 9.33.

4.4.20. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid 2-amino-ethyl ester (G_{45})

IR (Neat ν_{max} cm^{-1}): 3395 (O-H), 3283 (-NH), 3075, 2929 (C-H-str), 1647 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 2.49 (s, 1H, -NH_2, D_2O-exchangeble), 3.34-3.48 (m, 4H, -CH_2), 8.08-8.14 (m, 1H, ArH), 8.44-8.54 (m, 2H, ArH), 8.87 (s, 1H, ArH), 11.98 (s, 1H, -OH, D_2O-exchangeble). ^{13}C NMR: δ ppm 42.22, 60.06, 123.38, 124.79, 126.13, 130.85, 146.08, 151.42, 154.86, 155.37, 167.98. MS: m/z = 301 (M+1). Anal. calcd. For $C_{13}H_{11}F_3N_2O_3$; Calcd: C, 52.01; H, 3.69; N, 9.33; found: C, 52.01; H, 3.68; N, 9.35.

4.4.21. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid 2-(4-bromo-phenyl)-2-oxo-ethyl ester (G_{46})

IR (Neat ν_{max} cm^{-1}): 3255 (-OH), 3071, 2922 (C-H-str), 1689 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 5.56 (s, 2H, -CH_2), 7.58 (m, 1H, ArH), 7.74-8.09 (m, 5H, ArH), 8.44 (d, 1H, ArH, J = 7.8 Hz), 8.54 (s, 1H, ArH), 11.72 (s, 1H, -OH, D_2O-exchangeble); ^{13}C NMR: δ ppm 60.49, 111.39, 119.07, 124.61, 125.35, 130.99, 131.04, 131.55, 146.43; m/z = 454. Anal. calcd. For $C_{19}H_{11}BrF_3NO_4$; Calcd: C, 50.24; H, 2.44; N, 3.08; found: C, 50.20; H, 2.48; N, 3.10.

4.5. ANALYTICAL DATA

Crystal data of 2a	
Emp rical formula	$C_{15}H_{16}F_3NO_4$
Formula weight	331.29
Crystal system	Triclinic
Crystal dimension	0.55X0.39X0.09mm
Space group	$P\bar{1}$
a(Å)	7.8080 (2)
b(Å)	10.1485 (3)
c(Å)	10.5265 (3)
Volume ($Å^3$)	767.84 (4)
Angle α, β, γ	95.19, 109.18, 99.40
Z	2
μ (mm^{-1})	0.13
Temperature (T)	200 K
Radiation wavelength	0.71073(Å)

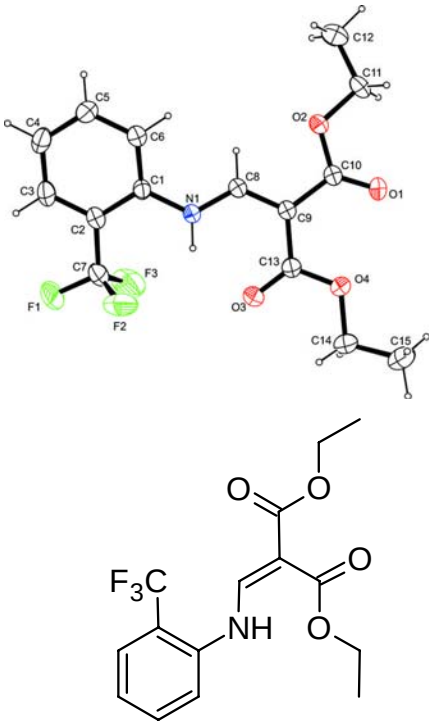


Figure 4.1. ORTEP diagram showing the single crystal structure of compound 2a (drawn at 50% probability level).

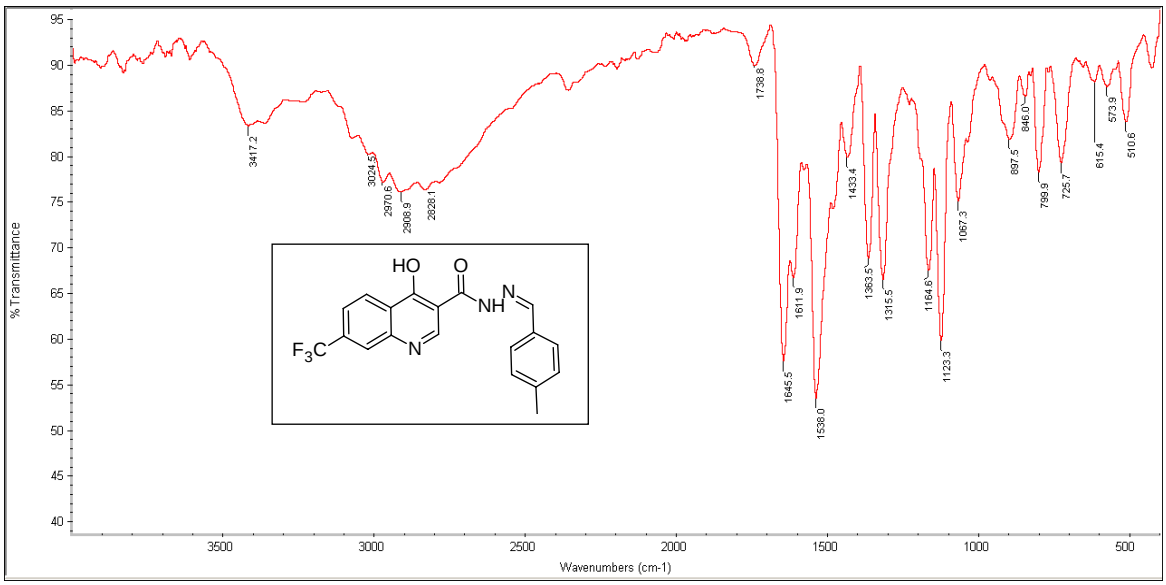


Figure 4.2. IR spectrum of G_{37}.

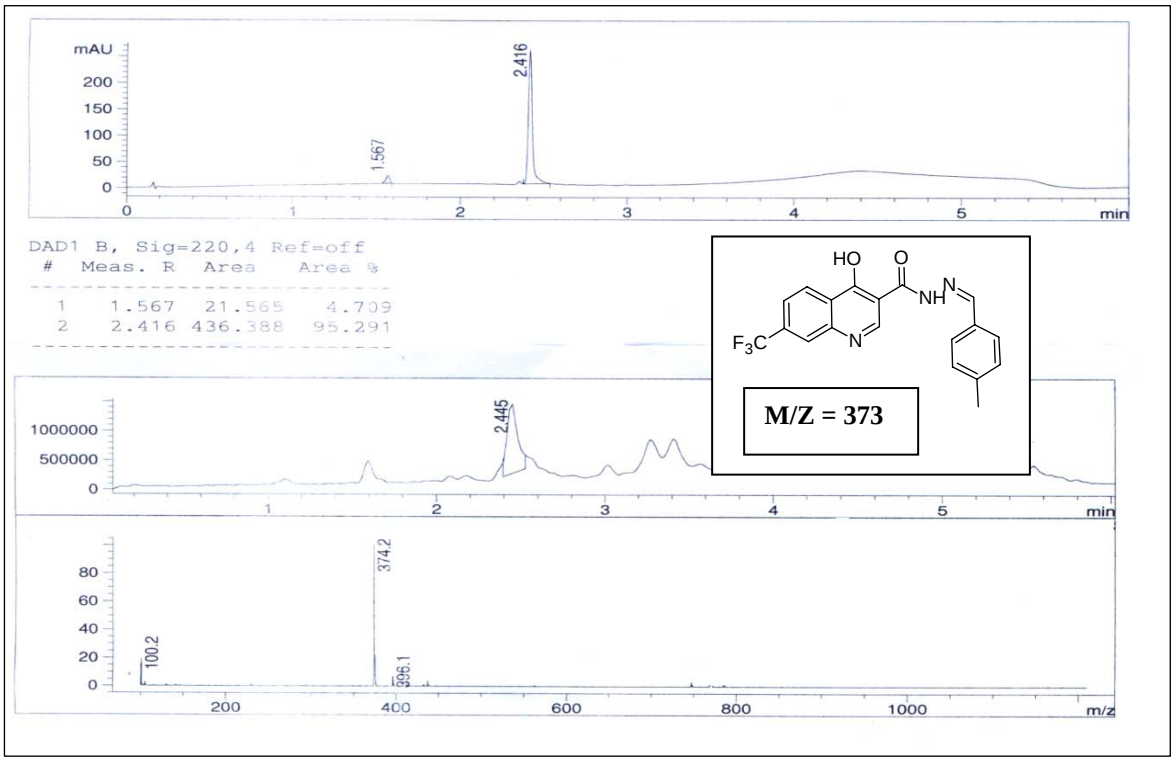


Figure 4.3. LCMS spectrum of G_{37}.

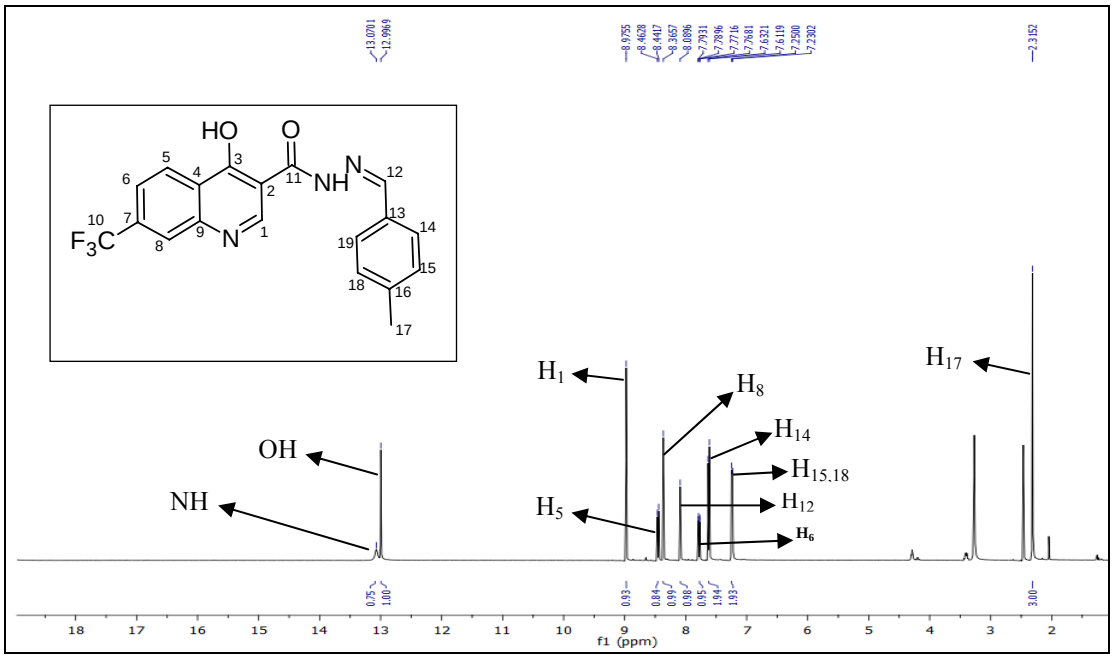


Figure 4.4. ^{1}H NMR spectrum of G_{37}.

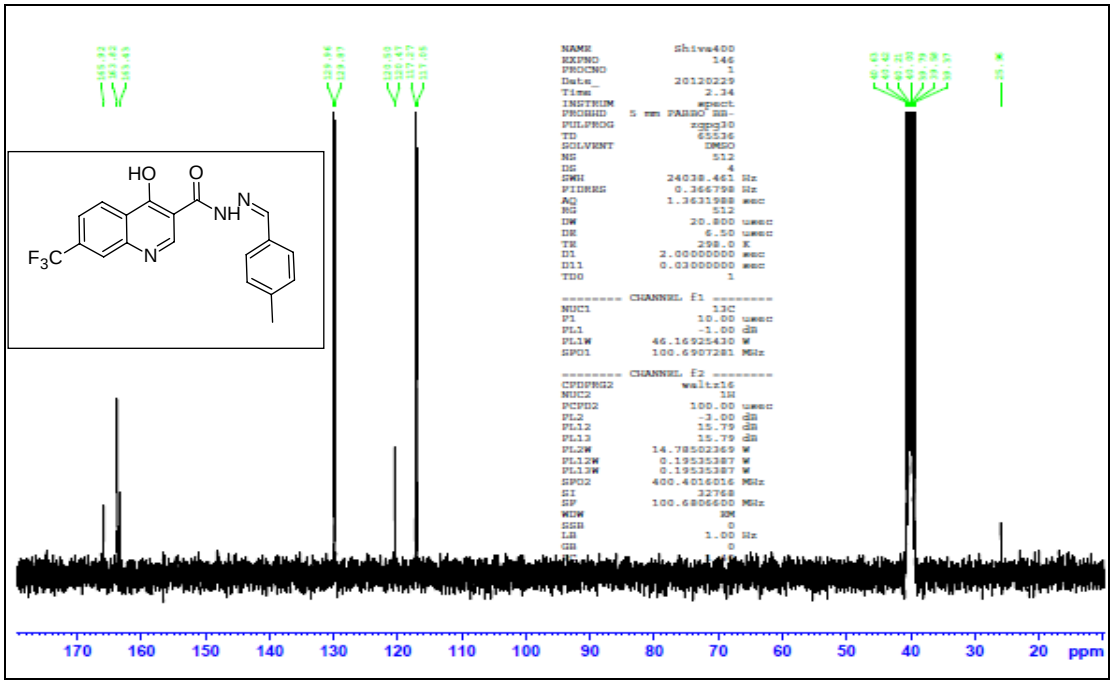


Figure 4.5. ^{13}C NMR spectrum of G_{37}.

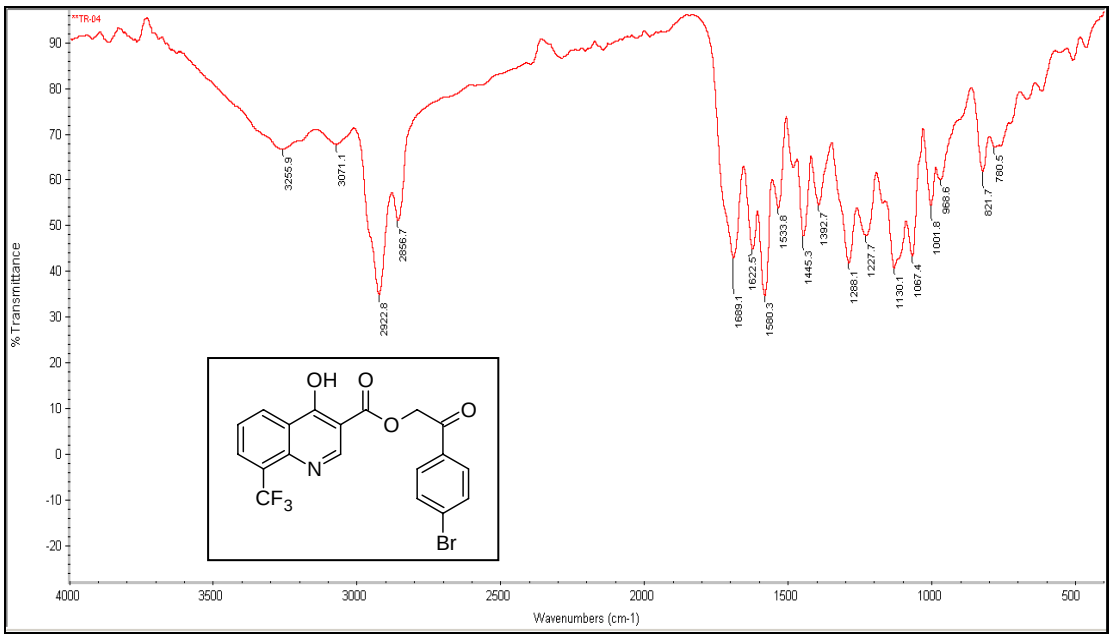


Figure 4.6. IR spectrum of G_{46}.

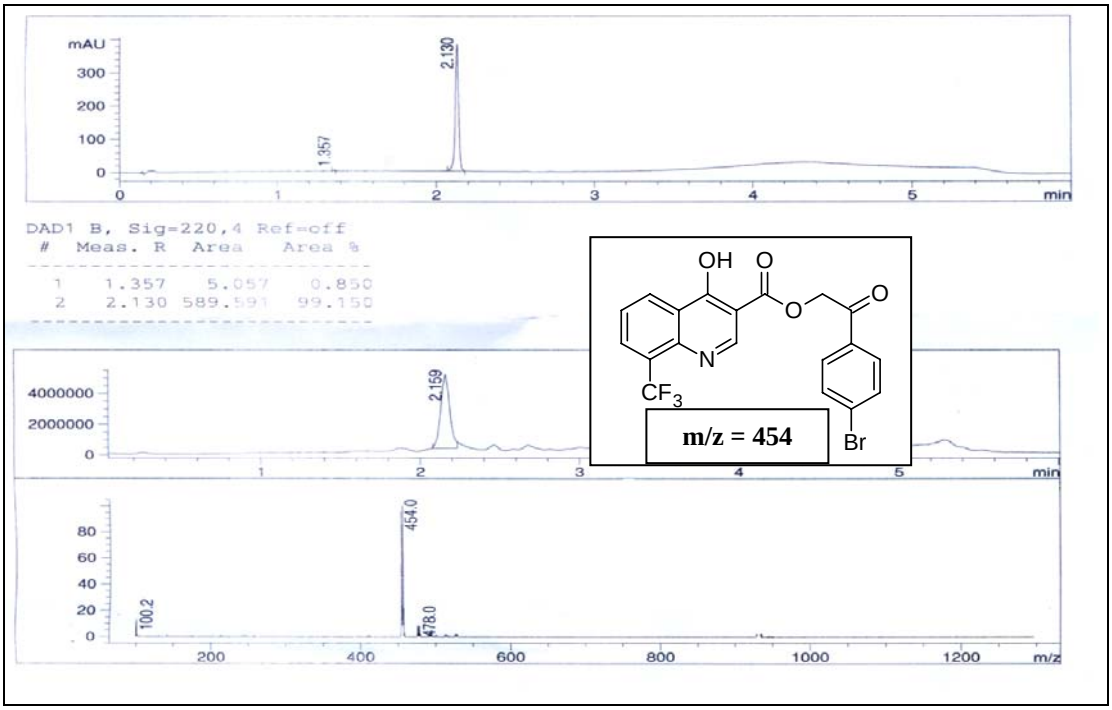


Figure 4.7. LCMS spectrum of G_{46}.

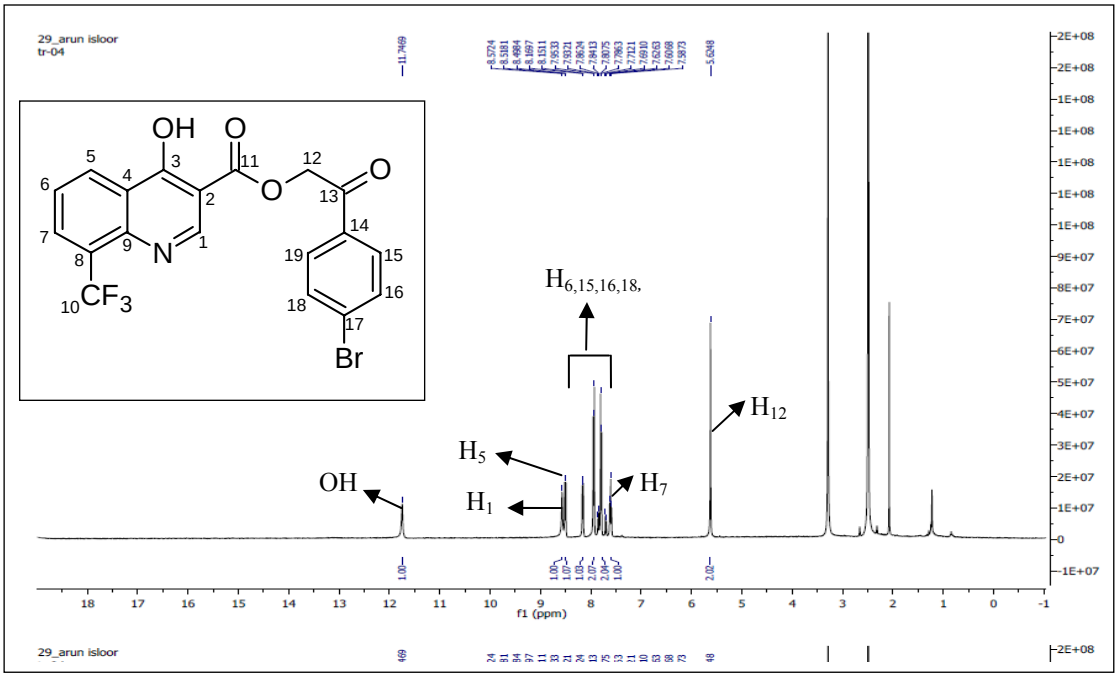


Figure 4.8. ^{1}H NMR spectrum of G_{46}.

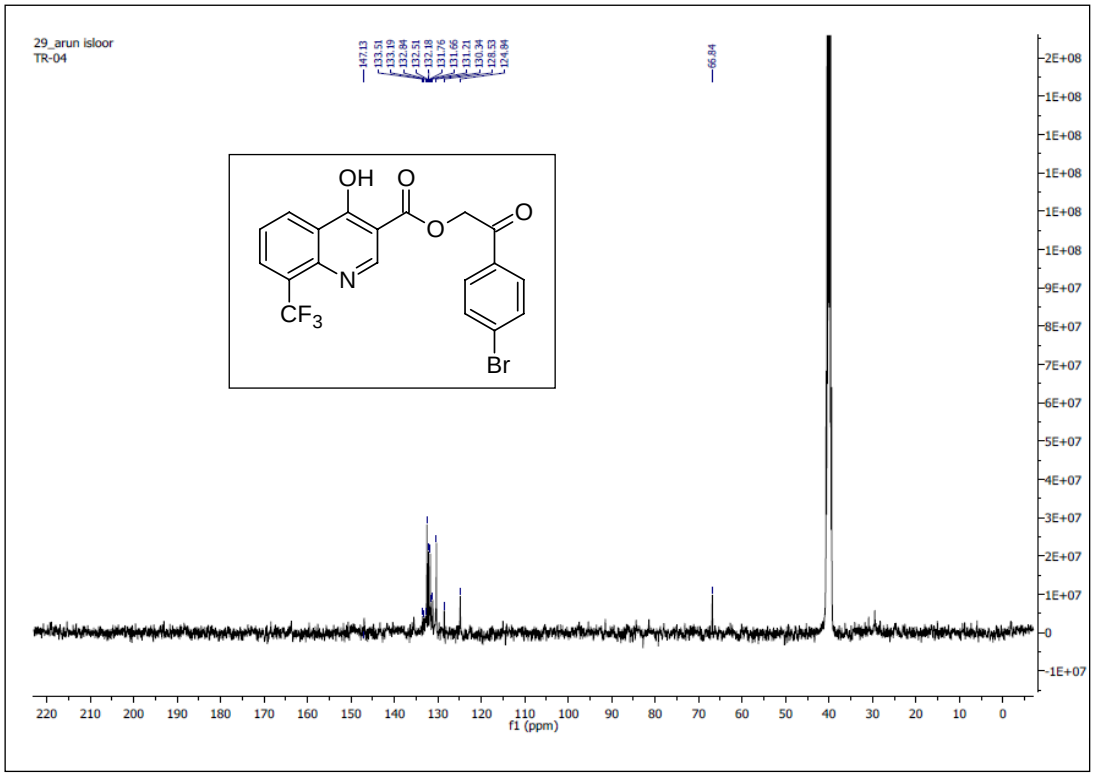


Figure 4.9. ^{13}C NMR spectrum of G_{46}.

4.6. CONCLUSION

A new series of trifluoromethylquinoline derivatives (**G_{34-41}**, **$G_{42,43}$**, **$G_{44,45}$** and **G_{46}**) were synthesized, characterized by IR, NMR, mass spectra and C, H, N analyses. The targeted 4-hydroxy-(trifluoromethyl)quinoline-3-carbohydrazide derivatives (**G_{34-41}**) were obtained by the reacting quinoline hydrazide (**4a**,**b**) with various substituted aldehydes in ethanolic media. The coupling reagents EDC, HOBT were used to synthesize 4-hydroxy-N-alkylquinoline-3-carboxamide derivatives with reasonably good yield. The *in-vitro* antimicrobial and antioxidant activities of the synthesized compounds (**G_{34-41}**, **$G_{42,43}$**, **$G_{44,45}$** and **G_{46}**) were discussed in CHAPTER-7.

CHAPTER-5

REGIOSELECTIVE SYNTHESIS AND CHARACTERIZATION OF TRIFLUOROMETHYLQUINOLONE DERIVATIVES

CHAPTER-5

REGIOSELECTIVE SYNTHESIS AND CHARACTERIZATION OF TRIFLUOROMETHYLQUINOLONE DERIVATIVES

5.1. INTRODUCTION

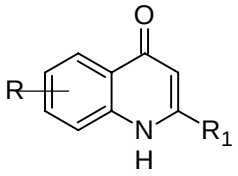
Quinolones are class of bicyclic molecule with chemical structures that are related to the heteroaromatic coal tar isolate quinoline. The most common synthetic methodology to prepare quinolone derivatives is Gould-Jacobs method. In this method quinolones were synthesized by treating substituted aniline with diethyl ethoxymethylenemalonate followed by thermal cyclisation using Dowtherm solvent.

The quinolone and its derivatives are an important class of heterocycles, antibacterial chemotherapeutic agents, which have a broad spectrum of antimicrobial activity. The history of quinolones began in the late 1950s, the breakthrough in the drug design for the scaffold and the basic side chains have allowed improvements to be made to the first new quinolone, Norfloxacin (NFLX). The success of first generation quinolones encouraged the research in this area. Koga and his collaborators introduced Norfloxacin into clinical use in 1980. They enhanced antimicrobial activity of quinolones by introducing piperidine at C-7 and fluorine atom at C-6 of basic quinolone structure, Norfloxacin (Koga et al. 1980). The basic piperazine ring, which can form the zwitterion nature with the carboxylic acid at the C-3 position, has subsequently been shown to increase the ability of the drugs to penetrate the bacterial cells resulting in enhanced activity.

The antibacterial activity of quinolones is the result of the combination of bacterial cell penetration and DNA gyrase inhibitory activity. The antibacterial activity of quinolones depends not only on the quinolone core scaffold but also on the active pharmaphoric substituents around the ring. These substituents exert their influence on bacterial activity by providing additional affinity for bacterial enzymes,

enhancing cell penetration or altering the pharmacokinetics. The antibacterial activity generated by fluoroquinolones is caused by the inhibition of two bacterial enzymes: DNA gyrase (atopoisomerase enzyme in bacteria) and topoisomerase IV enzyme. The general function of topoisomerases is to facilitate the uncoiling of DNA during DNA replication.

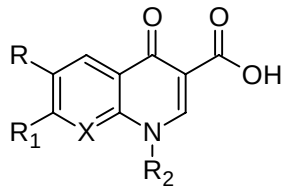
Ding et al. (2006) synthesized a series of 2-aryl-4-quinolones from acylated 2-aminoacetophenones (**QD-5.1**) by irradiating microwave in basic condition. This involves rapid and straightforward strategy for 2-aryl-4-quinolones synthesis.



QD-5.1

Where R = H, 4-Cl, 4,5-dimethoxy, 3,4,5-trimethoxy, R_1 = Phenyl, 4-chlorophenyl, 4-methoxyphenyl, 2-methoxy, 3,4,5-trimethoxy, 2-chloro-3-pyridyl, 2-hiophene.

A novel series of 7-(1,2,3,4-tetrahydropyrrolo[1,2-a]-pyrazin-7-yl) quinolones derivatives (**QD-5.2**) was synthesized by Zhu et al. (2009). The heterocyclic substitutions at C-7 of quinolone were achieved by palladium-mediated coupling reaction. The *in-vitro* antimicrobial activity of 8-methoxy group as well as unsubstituted and (3S)-methyl substituted 1,2,3,4-tetrahydropyrrolo[1,2-a]pyrazin-7-yl side chains showed notable activity against Ciprofloxacin-resistant clinical isolates of *Streptococcus pneumoniae*.

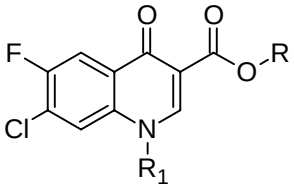


QD-5.2

Where R = H, F, R_1 = Piperidine, substituted piperidine, R_2 = Cyclopropyl, 2,4-difluorophenyl, X = CH, C-OCH_3, C-$OCHF_2$, C-CHF_2.

Dixit et al. (2012) studied *In-vitro* antiplasmodial activities of fluoroquinolone analogs (**QD-5.3**). They found that majority of the compounds showed very good

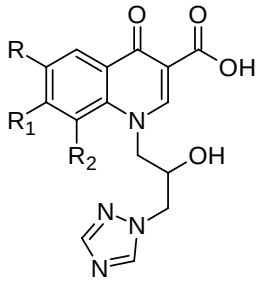
activities with IC_{50} ranging from 1.33 mg/mL to 6.96 mg/mL as compared to Ciprofloxacin (IC_{50} 8.82 mg/mL).



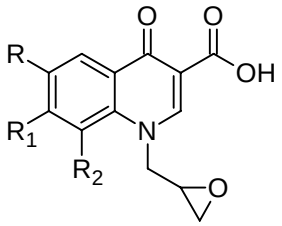
QD-5.3

Where R = H, CH_2CH_3, R_1 = H, CH_3, CH_2CH_3, $-(CH_2)_2CH_3$, $-(CH_2)_3CH_3$, CH_2CN, CH_2Ph, $-(CH_2)_2OH$, $CH(CH_3)_2$, CH_2CCH.

Cui et al. (2013) synthesized triazole, epoxy containing quinolone derivatives (**QD-5.4 & QD-5.5**) and studied their antimicrobial activity. All newly synthesized derivatives exhibited good or even stronger antibacterial and antifungal activities against the tested strains including multi-drug resistant Methicillin-resistant *Staphylococcus aureus* (MRSA).



QD-5.4

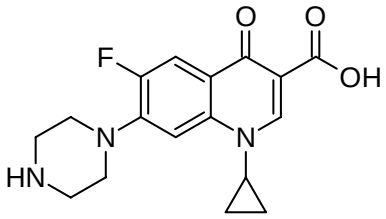


QD-5.5

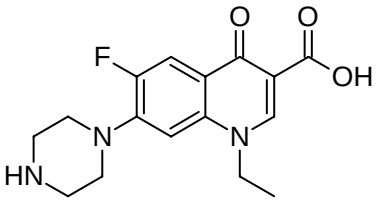
Where R = H, F, Cl, R_1 = H, Cl, CF_3, R_2 = H, F, Cl, Me.

Increase in the microbial infections and pathogenic resistance to the present day drugs (Chu et al. 1996; Chua et al. 2008) leads to urgency to develop novel antimicrobial agents with a new mechanism of action (Sunduru et al. 2011). Quinoline derivatives were well-known heterocyclic compounds with varied biological activity. Quinolone derivatives have significant tissue penetration property and inhibit the DNA synthesis by forming complex with DNA gyrase or topoisomerase II enzyme. Most of the highly effective quinolone antimicrobials contain the trifluoromethyl group or fluorine atom attached to the quinoline ring (Singh et al. 2006; Kumar et al. 2011). Trifluoromethyl or fluorine substituents on quinolone were capable of altering

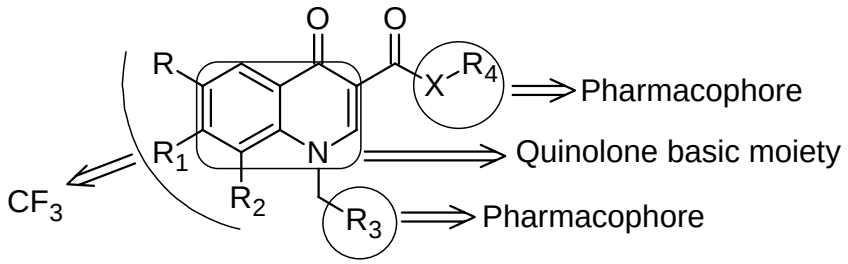
quite drastically, parameters such as basicity or the acidity of neighboringring groups, dipole moment within the molecule, reactivity and stability of neighboring groups (Hawley et al. 1996). Activity and kinetic profiles can be controlled by changing the pharmacophore at C-6, C-7, C-8, C-3 and N-alkylation of the quinoline ring (e.g., Ciprofloxacin, Norfloxacin and Trovafloxacin).



Ciprofloxacin



Norfloxacin



Where X = O, NH. $R/R_1/R_2$ = H, CF_3. R_3 = Acetylene, 4-cyanohenyl, 2,4-dichlorophenyl.

R_4 = H, C_2H_5, NH_2, Acetylene, 4-cyanohenyl, 2,4-dichlorophenyl.

Figure 5.1. Quinoline-based drugs and structural modification

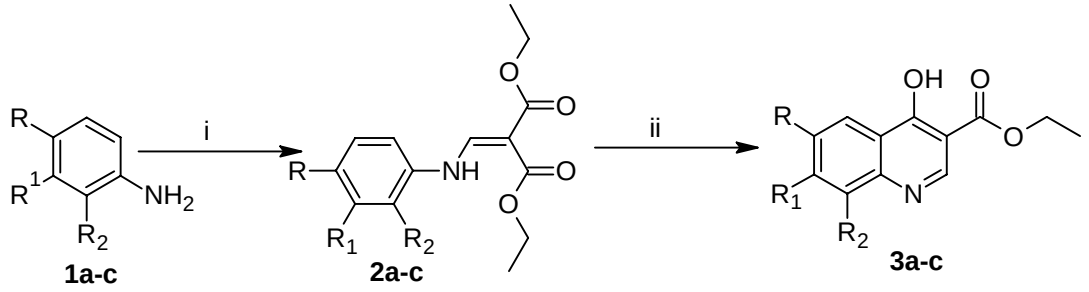
Fluoro and trifluoromethyl substituted quinoline derivatives were received considerable attention owing to their varied biological importance (Asahina et al. 2005; Abadi et al. 2005; Ma et al. 2009). A large variety of quinoline derivatives have been used as antimalarial, antiviral (Mahmoudi et al. 2003), antitubercular (Carta et al. 2007), antibacterial, antifungal (Shafiee et al. 2008), antioxidant (Jayashree et al. 2010), anticancer (Reis et al. 2011) agents. The substitution of trifluoromethyl group at C-6, C-7, C-8 position of the quinoline have a profound effect on biological activity (He et al. 2005; Holla et al. 2006). On the other hand, compounds containing acid, ester, hydrazide at C-3 and N-alkyl substituted quinolones are well-known to exhibit powerful antimicrobial, antioxidant activity (Asahina et al. 2005; Jayashree et al. 2010). Therefore there is great importance in the synthesis of C-3, N-alkyl substituted

trifluoromethylquinolones as target structures and evaluation of their biological activities.

5.2. RESULTS AND DISCUSSION

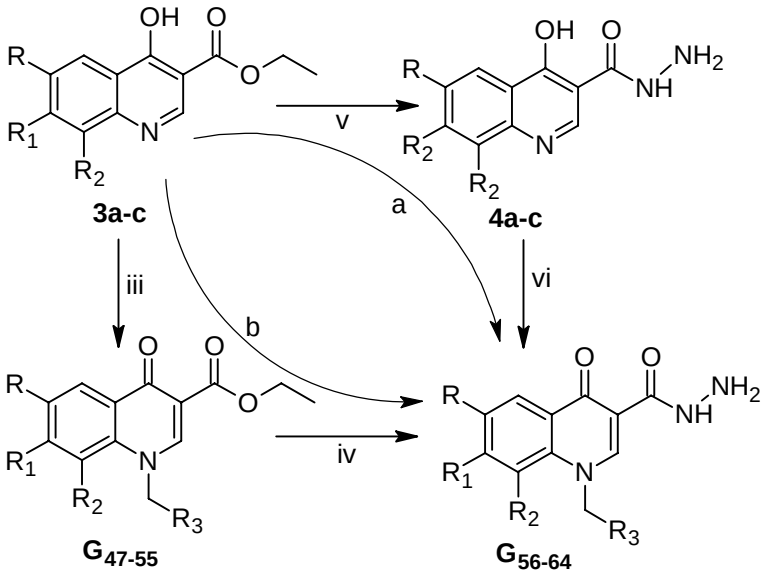
5.2.1. Regioselective synthesis of trifluoromethylquinolone derivatives

Ethyl 4-hydroxy-(trifluoromethyl)quinoline-3-carboxylate (**3a-c**) were synthesized as per Gould-Jacobs as discussed in **chapter-3**. The targeted compounds (**G_{47-55}, G_{56-64}, G_{65-73}** and **G_{74-76}**) were synthesized by employing sequential reactions, which are presented in **Scheme-5.1, Scheme-5.2**, **Scheme-5.3** and **Scheme-5.4**. The alkylation of **3a-c** in dimethylformamide (DMF) resulted in **G_{47-55}**. Hydrazide derivatives (**G_{56-64}**) of quinolones were obtained by condensation of hydrazine hydrate with **G_{47-55}**. The compounds **3a-c** on reaction with hydrazine hydrate produces **4a-c** which on alkylation gives **G_{56-64}** (**Scheme-5.2**). In **Scheme-5.2**, route-b showed a good yield compared with route-a. The quinoline ester **3a-c** on hydrolysis with LiOH gives the corresponding acids **5a-c** which on alkylation yielded major N-alkylated products **G_{65-73}** and minor **G_{74-76}**. In the same way, hydrolysis of N-alkylated products **G_{47-55}** produced exclusively (route-c) N-alkylated acid derivatives **G_{65-73}** (**Scheme-5.3**). The alkylation of trifluoromethyl-4-hydroxyquinoline followed by hydrolysis with LiOH (route-b) and condensation with hydrazine hydrate (route-d) showed a good yield and regioselectivity compared with route-a**,** route-c. The reactions were monitored by thin layer chromatography and crude products were purified by column chromatography using pet ether and ethyl acetate (5:5) as the eluent and recrystallization using ethyl acetate. All the synthesized compounds were characterized by IR, NMR, mass spectral and elemental analyses.



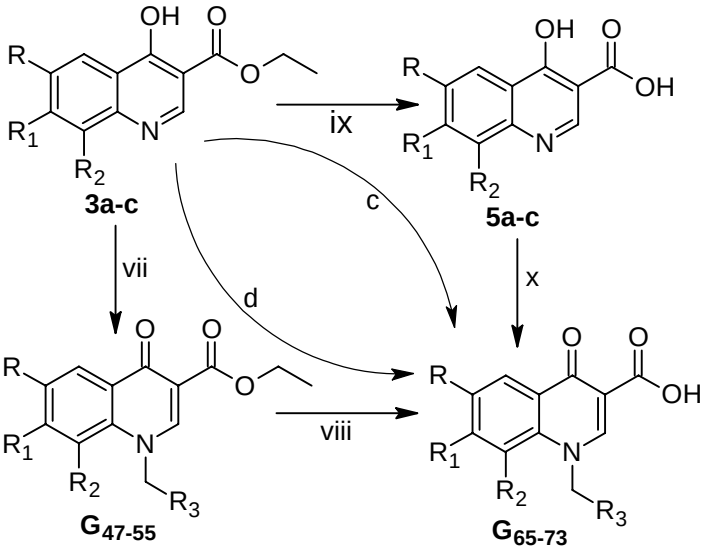
Where $R/R_1/R_2$ =H, CF_3.

Scheme 5.1. Synthetic route for ethyl 4-hydroxy-(trifluoromethyl)quinoline-3-carboxylate (3a-c): (i) 110 °C, 6 h; (ii) Dowtherm, 250 °C, 5 h.



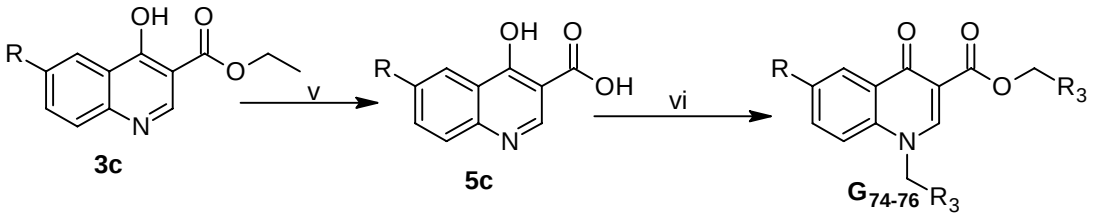
Where $R/R_1/R_2$ = H, CF_3; R_3= Acetylene, 2,4-dichlorophenyl, 4-cyanophenyl.

Scheme 5.2. Synthetic route for ethyl 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{47-55}) and 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{56-64}): (iii) Alkylbromide, K_2CO_3, DMF, 80 °C, 2 h; (iv) NH_2NH_2, EtOH, 80 °C, 4 h; (v) NH_2NH_2, EtOH, 80 °C, 4 h; (vi) Alkylbromide, K_2CO_3, DMF, 80 °C, 2 h.



Where $R/R_1/R_2$ = H, CF_3; R_3= Acetylene, 2,4-dichlorophenyl, 4-cyanophenyl.

Scheme 5.3. Synthetic route for 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydro quinoline-3-carboxylic acid ($G_{65\text{-}73}$): (vii) Alkylbromide, K_2CO_3, DMF, 80 °C, 2 h; (viii) LiOH, MeOH, 2 h; (ix) LiOH, MeOH, 2 h; (x) Alkylbromide, K_2CO_3, DMF, 80 °C, 2 h.



Where R = H, CF_3; R_3= Acetylene, 2,4-dichlorophenyl, 4-cyanophenyl.

Scheme 5.4. Synthetic route for alkyl 1-alkyl-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate derivatives ($G_{74\text{-}76}$): (v) LiOH, MeOH, 2 h; (vi) Alkylbromide, K_2CO_3, DMF, 80 °C, 2 h.

The IR spectrum of compound **G_{51}** showed two bands at 1682 cm^{-1}, 1647 cm^{-1} which are due to the C=O group of ethyl 1-(2,4-dichlorobenzyl)-4-oxo-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate. The ^1H NMR spectrum of **G_{51}** showed a singlet at δ 5.82 ppm which is due to the benzyl proton ($N\text{-}CH_2$). A doublet at δ 7.01 (J = 8.4 Hz) is due to aromatic proton of 2,4-dichlorobenzyl moiety. A singlet at δ 8.94 is due to quinolone-3H proton. The mass spectrum of **G_{51}** showed a

molecular ion peak at m/z = 444 (M^+) which is in agreement with the molecular formula $C_{20}H_{14}Cl_2F_3NO_3$. Three dimensional structure of **G_{51}** was evidenced by X-ray crystallographic study. Similarly the spectral values for all the compounds and C, H, N analyses are presented in the experimental part and the characterization data are provided in **Table 5.1.**

Table 5.1. Characterization data of the compounds (G_{47-55}, G_{56-64}, G_{65-73} and G_{74-76})

Compounds	R	R_1	R_2	R_3	Molecular Formula & Mol. Wt.	M.p.(°C)
G_{47}	H	H	CF_3	Acetylene	$C_{16}H_{12}F_3NO_3$ 323	58-60
G_{48}	H	H	CF_3	2,4-dichlorophenyl	$C_{20}H_{14}Cl_2F_3NO_3$ 444	67-69
G_{49}	H	H	CF_3	4-cyanophenyl	$C_{21}H_{15}F_3N_2O_3$ 400	148-150
G_{50}	H	CF_3	H	Acetylene	$C_{16}H_{12}F_3NO_3$ 323	171-173
G_{51}	H	CF_3	H	2,4-dichlorophenyl	$C_{20}H_{14}Cl_2F_3NO_3$ 444	155-157
G_{52}	H	CF_3	H	4-cyanophenyl	$C_{21}H_{15}F_3N_2O_3$ 400	200-202
G_{53}	CF_3	H	H	Acetylene	$C_{16}H_{12}F_3NO_3$ 323.26	202-204
G_{54}	CF_3	H	H	2,4-dichlorophenyl	$C_{20}H_{14}Cl_2F_3NO_3$ 444.23	183-185
G_{55}	CF_3	H	H	4-cyanohenyl	$C_{21}H_{15}F_3N_2O_3$ 400.35	220-222
G_{56}	H	H	CF_3	Acetylene	$C_{14}H_{10}F_3N_3O_2$ 309	165-167
G_{57}	H	H	CF_3	2,4-dichlorophenyl	$C_{18}H_{12}Cl_2F_3N_3O_2$ 430	208-210
G_{58}	H	H	CF_3	4-cyanophenyl	$C_{19}H_{13}F_3N_4O_2$ 386	136-138
G_{59}	H	CF_3	H	Acetylene	$C_{14}H_{10}F_3N_3O_2$ 309	176-178
G_{60}	H	CF_3	H	2,4-dichlorophenyl	$C_{18}H_{12}Cl_2F_3N_3O_2$ 430	234-236
G_{61}	H	CF_3	H	4-cyanophenyl	$C_{19}H_{13}F_3N_4O_2$ 386	100-102

continued

G_{62}	CF_3	H	H	Acetylene	$C_{14}H_{10}F_3N_3O_2$ 309.24	175-177
G_{63}	CF_3	H	H	2,4-dichlorophenyl	$C_{18}H_{12}Cl_2F_3N_3O_2$ 430.20	236-238
G_{64}	CF_3	H	H	4-cyanophenyl	$C_{19}H_{13}F_3N_4O_2$ 386.32	293-295
G_{65}	H	H	CF_3	Acetylene	$C_{14}H_8F_3NO_3$ 295	206-208
G_{66}	H	H	CF_3	2,4-dichlorophenyl	$C_{18}H_{10}Cl_2F_3NO_3$ 416	182-184
G_{67}	H	H	CF_3	4-cyanophenyl	$C_{19}H_{11}F_3N_2O_3$ 372	210-212
G_{68}	H	CF_3	H	Acetylene	$C_{14}H_8F_3NO_3$ 295	170-172
G_{69}	H	CF_3	H	2,4-dichlorophenyl	$C_{18}H_{10}Cl_2F_3NO_3$ 416	190-192
G_{70}	H	CF_3	H	4-cyanophenyl	$C_{19}H_{11}F_3N_2O_3$ 372	174-176
G_{71}	CF_3	H	H	Acetylene	$C_{14}H_8F_3NO_3$ 295.21	282-284
G_{72}	CF_3	H	H	2,4-dichlorophenyl	$C_{18}H_{10}Cl_2F_3NO_3$ 416.17	248-250
G_{73}	CF_3	H	H	4-cyanophenyl	$C_{19}H_{11}F_3N_2O_3$ 372.29	96-98
G_{74}	CF_3	H	H	Acetylene	$C_{17}H_{10}F_3NO_3$ 333.26	240-242
G_{75}	CF_3	H	H	2,4-dichlorophenyl	$C_{25}H_{14}Cl_4F_3NO_3$ 575.19	85-87
G_{76}	CF_3	H	H	4-cyanophenyl	$C_{27}H_{16}F_3N_3O_3$ 487.42	192-194

5.3. EXPERIMENTAL

5.3.1. General procedure for the synthesis of diethyl ({[(trifluoromethyl)phenyl] amino}methylidene)propanedioate (2a-c)

Trifluoromethylaniline **1a-c** (10.0 g, 0.062 mol) and diethyl ethoxymethylene malonate (20.10 g, 0.093 mol) were heated to 110 °C for 6 h. The reaction mixture was cooled to room temperature, the solid thus formed was taken in pet ether and stirred for 20 minutes. Further it was filtered to get compounds **2a-c** as white

crystalline solid.

5.3.2. General procedure for the synthesis of ethyl 4-hydroxy-(trifluoromethyl) quinoline-3-carboxylate (3a-c)

Diethyl ({[(trifluoromethyl)phenyl]amino}methylidene)propanedioate **2a-c** (10.0 g, 0.030 mol) and dowtherm (100 mL) were heated to 250 °C for 5 h. The reaction mixture was then cooled to 25 °C and stirred in 150 mL hexane for 10 min. The solid product obtained was filtered and dried. The crude product obtained was purified by column chromatography using pet ether and ethyl acetate (5:5) as the eluent to get white solids.

5.3.3. General procedure for the synthesis of ethyl 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{47-55})

A mixture of ethyl 4-hydroxy-(trifluoromethyl)quinoline-3-carboxylate (0.250 g, 0.00087 mol), potassium carbonate (0.132 g, 0.00096 mol) and alkyl bromide (0.00096 mol) in dimethylformamide (5 mL) was stirred at 80 °C for 2 h. After completion of the reaction, the reaction mixture was poured into ice-cold water. The solid product obtained was filtered, washed with water and recrystallized using ethanol.

5.3.4. General procedure for the synthesis of 4-hydroxy-(trifluoromethyl)quinoline-3-carbohydrazide (4a-c)

A mixture of ethyl 4-hydroxy-(trifluoromethyl)quinoline-3-carboxylate (**3a-c**) (5.0 g, 0.017 mol) and hydrazine hydrate (4.13 mL, 0.085 mol, reagent grade 98%) in ethanol (50 mL) were refluxed for 4 h. After the completion of the reaction, the reaction mixture was concentrated and allowed to cool. The solid product obtained was filtered, washed with water and recrystallized from ethanol to give (**4a-c**) as white solids.

5.3.5. General procedure for the synthesis of 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{56-64})

Route-a: A slurry of 4-hydroxy-(trifluoromethyl)quinoline-3-carbohydrazide (**4a-c**) (0.25 g, 0.00092 mol) corresponding alkyl bromide (0.0010 mol) in

dimethylformamide (5 mL) was stirred at 80 °C for 2 h. After completion of the reaction, the reaction mixture was poured into ice-cold water. The solid product obtained was filtered, washed with water and purified by column chromatography using pet ether and ethyl acetate (5:5) as the eluent to get white solids.

Route-b: To a solution of 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (**G_{47-55}**) (0.010 mol) in 5 mL ethanol, hydrazine hydrate (0.015 mol, reagent grade 98%) was added. The mixture was stirred at 80 °C for 4 h. After completion of the reaction, the reaction mixture was poured into ice-cold water. The crude products was recrystallized from ethanol to give (**G_{56-64}**) as white solids.

5.3.6. General procedure for the synthesis of 4-hydroxy-(trifluoromethyl)quinoline-3-carboxylic acid (5a-c)

To a suspension of ethyl 4-hydroxy-(trifluoromethyl)quinoline-3-carboxylate **(3a-c)** (5.0 g, 0.017 mol) in methanol (50 mL) at 0 °C was added lithium hydroxide (0.88 g, 0.021 mol) for 10 min. The mixture was allowed to stir for 2 h and was quenched by the slow addition water (50 mL), acidified using dilute HCl. The precipitated solids were collected by filtration and recrystallized from ethanol.

5.3.7. General procedure for the synthesis of 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{65-73}) and 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{74-76})

Route-a: A mixture of ethyl 4-hydroxy-(trifluoromethyl)quinoline-3-carboxylic acid (**5a-c**) (0.250 g, 0.00097 mol), potassium carbonate (0.147 g, 0.0010 mol) and alkyl bromide (0.00096 mol) in dimethylformamide (5 mL) was stirred at 80 °C for 2 h. The reaction mixture was poured into ice-cold water. The solid product obtained was filtered, washed with water and purified by column chromatography using pet ether and ethyl acetate (5:5) as the eluent to get white solids.

Route-b: To a suspension of 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (**G_{47-55}**) (0.017 mol) in methanol (5 mL) at 0 °C was added lithium hydroxide (0.021 mol) for 10 min. The mixture was allowed to stir for

2 h and was quenched by the slow addition of water (25 mL), acidified by using dilute HCl. The precipitated solids were collected by filtration and recrystallized by ethanol.

5.4. CHARACTERIZATION OF SYNTHESIZED COMPOUNDS

5.4.1. Diethyl ({[2-(trifluoromethyl)phenyl]amino}methylidene)propanedioate (2a)

Yield: 19.1 g, 92.8%. M.p: 84-86 °C. IR (Neat ν_{max} cm^{-1}): 3278 (N-H), 3176, 2986 (C-H-str), 1706 and 1654 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.33 (t, 3H, CH_3, J = 5.3 Hz), 1.37 (t, 3H, CH_3, J = 5.3 Hz), 4.22 (q, 2H, CH_2), 4.30 (q, 2H, CH_2), 7.21-8.41 (m, 5H, ArH), 11.50 (s, 1H, NH). Anal. calcd. For $C_{15}H_{16}F_3NO_4$; Calcd: C, 54.38; H, 4.80; N, 4.23; found: C, 54.38; H, 4.79; N, 4.21 (Thomas et al. 2011).

5.4.2. Diethyl ({[3-(trifluoromethyl)phenyl]amino}methylidene)propanedioate (2b)

Yield: 19.0 g, 92%; M.p: 44-46 °C; IR (Neat ν_{max} cm^{-1}): 3252 (N-H), 3118, 2979 (C-H-str), 1708 and 1616 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.37 (t, 3H, CH_3, J = 5.3 Hz), 1.39 (t, 3H, CH_3, J = 5.3 Hz), 4.26 (q, 2H, CH_2), 4.34 (q, 2H, CH_2), 7.21-8.40 (m, 5H, ArH), 11.49 (s, 1H, NH); Anal. calcd. For $C_{15}H_{16}F_3NO_4$; Calc: C, 54.38; H, 4.80; N, 4.23; found: C, 54.35; H, 4.80; N, 4.20% (Bi et al. 2004).

5.4.3. Diethyl ({[4-(trifluoromethyl)phenyl]amino}methylidene)propanedioate (2c)

Yield: 20.0 g, 97%; M.p: 90-92 °C; IR (Neat ν_{max} cm^{-1}): 3256 (N-H), 2987, 2941 (C-H-str), 1707 and 1653 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.35 (t, 3H, CH_3, J = 5.4 Hz), 1.36 (t, 3H, CH_3, J = 5.3 Hz), 4.26 (q, 2H, CH_2), 4.35 (q, 2H, CH_2), 7.21-8.43 (m, 5H, ArH), 11.42 (s, 1H, NH); Anal. calcd. For $C_{15}H_{16}F_3NO_4$; Calc: C, 54.38; H, 4.80; N, 4.23; found: C, 54.38; H, 4.79; N, 4.22% (Niedermeier et al. 2009).

5.4.4. Ethyl 4-hydroxy-8-(trifluoromethyl)quinoline-3-carboxylate (3a)

Yield: 8.1 g, 94.1%. M.p: 295-297 °C. IR (Neat ν_{max} cm^{-1}): 3352 (-OH), 3118, 2979 (C-H-str), 1708 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.23 (t. 3H, J = 8.0 Hz, -CH_3), 4.16 (q, 2H, -CH_2), 7.53 (t, 1H, ArH, J = 8.0 Hz), 8.07 (d, 1H, ArH, 7.8 Hz), 8.41 (d, 2H, ArH, J = 8.0 Hz), 11.62 (s, 1H, -OH, D_2O-exchangeble). ^{13}C NMR:

δ ppm 14.70, 60.49, 111.39, 119.07, 122.60, 124.00, 124.61, 125.35, 130.99, 131.04, 131.55, 146.43, 164.50. MS: m/z = 286 (M+1). Anal. calcd. For $C_{13}H_{10}F_3NO_3$; Calcd: C, 54.74; H, 3.53; N, 4.91; found: C, 54.75; H, 3.54; N, 4.95.

5.4.5. Ethyl 4-hydroxy-7-(trifluoromethyl)quinoline-3-carboxylate (3b)

Yield: 7.2 g, 83.7%; M.p: 298-300 °C; IR (Neat ν_{max} cm^{-1}): 3322 (-OH), 3029, 2970 (C-H-str), 1706 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.22 (t, 3H, CH_3, J = 8.0 Hz), 4.18 (q, 2H, CH_2, J = 8.0 Hz), 7.53 (d, 1H, ArH, J = 12 Hz), 7.81 (d, 1H, ArH, J = 12 Hz), 8.03 (s, 1H, ArH), 8.44 (s, 1H, ArH), 12.30 (s, 1H, -OH); Anal. calcd. For $C_{13}H_{10}F_3NO_3$; Calc: C, 54.74; H, 3.53; N, 4.91; found: C, 54.77; H, 3.50; N, 4.95% (Bi et al. 2004).

5.4.6. Ethyl 4-hydroxy-6-(trifluoromethyl)quinoline-3-carboxylate(3c)

Yield: 7.9 g, 91.8%; M.p: 327-329 °C; IR (Neat ν_{max} cm^{-1}): 3350 (-OH), 3090, 2996 (C-H-str), 1655 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.24 (t, 3H, CH_3, J = 8.0 Hz), 4.18 (q, 2H, CH_2, J = 8.0 Hz), 7.73 (d, 1H, ArH, J = 12 Hz), 7.82 (d, 1H, ArH, J = 12 Hz), 8.03 (s, 1H, ArH), 8.41 (s, 1H, ArH), 12.30 (s, 1H, -OH, D_2O-exchangeble). MS: m/z = 286 (M+1). Anal. calcd. For $C_{13}H_{10}F_3NO_3$; Calc: C, 54.74; H, 3.53; N, 4.91; found: C, 54.75; H, 3.52; N, 4.90%.

5.4.7. Ethyl 4-oxo-1-(prop-2-yn-1-yl)-8-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{47})

IR (KBr ν_{max} cm^{-1}): 3256 (C≡C-H-str), 3094, 2991 (C-H-str), 1701, 1581 (C=O); ^1H NMR (400 MHz, $CDCl_3$): δ ppm 1.45 (t, 3H, CH_3, J = 7.1 Hz), 2.54 (s, 1H, CH), 4.47 (q, 2H, CH_2, J = 7.1 Hz), 5.08 (s, 2H, CH_2), 7.66 (t, 1H, ArH, J = 7.8 Hz), 8.16 (d, 1H, ArH, J = 7.2 Hz), 8.63 (d, 1H, ArH, J = 8.4 Hz), 9.43 (s, 1H, ArH); MS: m/z = 324 (M+1). Anal. calcd. For $C_{16}H_{12}F_3NO_3$; Calc: C, 59.45; H, 3.74; N, 4.33; found: C, 59.47; H, 3.75; N, 4.33%.

5.4.8. Ethyl 1-(2,4-dichlorobenzyl)-4-oxo-8-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{48})

IR (KBr ν_{max} cm^{-1}): 3066, 2982 (C-H-str), 1715, 1676 (C=O); ^1H NMR (400 MHz, $CDCl_3$): δ ppm 1.41 (t, 3H, CH_3, J = 7.1 Hz), 4.46 (q, 2H, CH_2, J = 7.1 Hz), 5.38 (s, 2H, CH_2), 7.33 (t, 1H, ArH, J = 8.2 Hz), 7.45 (s, 1H, ArH), 7.56-7.65 (m, 2H, ArH),

8.14 (d, 1H, ArH, J = 7.0 Hz), 8.39 (d, 1H, ArH, J = 8.5 Hz), 9.41 (s,1H, ArH); ^{13}C NMR: δ ppm: 54.07, 62.96, 111.46, 119.01, 128.01, 128.45, 129.22, 129.59, 129.71, 129.84, 131.44, 132.31, 141.98, 152.07, 164.26, 172.80; MS: m/z = 445 (M+1). Anal. calcd. For $C_{20}H_{14}Cl_2F_3NO_3$; Calc: C, 54.07; 3.18, H, 2.98; N, 3.15; found: C, 54.07; H, 3.20; N, 3.16%.

5.4.9. Ethyl 1-(4-cyanobenzyl)-4-oxo-8-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{49})

IR (KBr ν_{max} cm^{-1}): 3056, 2930 (C-H-str), 2227 (C≡N), 1660, 1612 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.34 (t, 3H, CH_3, J = 7.0 Hz), 4.19 (q, 2H, CH_2, J = 6.4 Hz), 5.80 (s, 2H, CH_2), 7.49 (d, 2H, ArH, J = 7.9 Hz), 7.57 (d, 2H, ArH, J = 8.4 Hz), 7.81-7.87 (m, 3H, ArH), 8.72 (s, 1H, ArH); MS: m/z = 401 (M+1). Anal. calcd. For $C_{21}H_{15}F_3N_2O_3$; Calc: C, 63.00; H, 3.78; N, 7.00; found: C, 62.08; H, 3.80; N, 7.05%.

5.4.10. Ethyl 4-oxo-1-(prop-2-yn-1-yl)-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{50})

IR (KBr ν_{max} cm^{-1}): 3307 (C≡C-H-str), 3035, 2972 (C-H-str), 1690, 1613 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.31 (t, 3H, CH_3, J = 7.0 Hz), 3.69 (s, 1H, CH), 4.25 (q, 2H, CH_2, J = 7.0 Hz), 5.44 (s, 2H, CH_2), 7.84 (dd, 1H, ArH, J = 8.3 Hz, J = 1.1 Hz), 8.18 (s, 1H, ArH), 8.44 (s, 1H, ArH), 8.92 (s, 1H, ArH); MS: m/z = 324 (M+1). Anal. calcd. For $C_{16}H_{12}F_3NO_3$; Calc: C, 59.45; H, 3.74; N, 4.33; found: C, 59.48; H, 3.72; N, 4.34%.

5.4.11. Ethyl 1-(2,4-dichlorobenzyl)-4-oxo-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{51})

IR (KBr ν_{max} cm^{-1}): 3043, 2989 (C-H-str), 1682, 1647 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.13 (t, 3H, CH_3, J = 7.1 Hz), 4.25 (q, 2H, CH_2, J = 7.1 Hz), 5.82 (s, 2H, CH_2), 7.01 (d, 1H, ArH, J = 8.4 Hz), 7.36 (dd, 1H, ArH, J = 8.4 Hz, J = 2.1 Hz), 7.78-7.79 (m, 2H, ArH), 7.81 (s, 1H, ArH), 8.46 (d, 1H, ArH, J = 7.4 Hz), 8.94 (s, 1H, ArH); MS: m/z = 445 (M+1). Anal. calcd. For $C_{20}H_{14}Cl_2F_3NO_3$; Calc: C, 54.07; H, 3.18; N, 3.15; found: C, 54.07; H, 3.20; N, 3.14%.

5.4.12. Ethyl 1-(4-cyanobenzyl)-4-oxo-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{52})

IR (KBr ν_{max} cm^{-1}): 3065, 2981 (C-H-str), 2225 (C≡N), 1725, 1613 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.31 (t, 3H, CH_3, J = 7.1 Hz), 4.27 (q, 2H, CH_2, J = 7.1 Hz), 5.91 (s, 2H, CH_2), 7.46 (d, 2H, ArH, J = 8.4 Hz), 7.76 (dd, 1H, ArH, J = 8.4 Hz, J = 1.1 Hz), 7.86 (d, 2H, ArH, J = 8.4 Hz), 7.89 (s, 1H, ArH), 8.44 (d, 1H, ArH, J = 8.2 Hz), 9.02 (s, 1H, ArH); MS: m/z = 401 (M+1). Anal. calcd. For $C_{21}H_{15}F_3N_2O_3$; Calc: C, 63.00; H, 3.78; N, 7.00; found: C, 63.01; H, 3.78; N, 7.03%.

5.4.13. Ethyl 4-oxo-1-(prop-2-yn-1-yl)-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{53})

IR (KBr ν_{max} cm^{-1}): 3317 (C≡C-H-str), 3082, 3056 (C-H-str), 1726, 1639 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.30 (t, 3H, CH_3, J = 8.0 Hz), 2.50 (s, 1H, CH), 4.21 (q, 2H, CH_2, J = 8.0 Hz), 5.37 (s, 2H, CH_2), 8.03 (d, 1H, ArH, J = 8.0 Hz), 8.18 (d, 1H, ArH, J = 8.0 Hz), 8.49 (s, 1H, ArH), 8.92 (s, 1H, ArH). MS: m/z = 324 (M+1). Anal. calcd. For $C_{16}H_{12}F_3NO_3$; Calc: C, 59.45; H, 3.74; N, 4.33; found: C, 59.45; H, 3.75; N, 4.30%.

5.4.14. Ethyl 1-(2,4-dichlorobenzyl)-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{54})

IR (KBr ν_{max} cm^{-1}): 3053, 3021 (C-H-str), 1735, 1701 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.26 (t, 3H, CH_3, J = 8.0 Hz), 4.24 (q, 2H, CH_2, J = 8.0 Hz), 5.75 (s, 2H, CH_2), 6.87 (d, 1H, ArH, J = 8.6 Hz), 7.30 (dd, 1H, ArH, J = 8.0 Hz, J = 4.0 Hz), 7.60 (d, 1H, ArH, J = 8.6 Hz), 7.79 (s, 1H, ArH), 8.01 (dd, 1H, ArH, J = 8 Hz, j = 4.0 Hz), 8.50 (s, 1H, ArH), 8.96 (s, 1H, ArH). MS: m/z = 445 (M+1). Anal. calcd. For $C_{20}H_{14}Cl_2F_3NO_3$; Calc: C, 54.07; H, 3.18; N, 3.15; found: C, 54.09; H, 3.17; N, 3.16%.

5.4.15. Ethyl 1-(4-cyanobenzyl)-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{55})

IR (KBr ν_{max} cm^{-1}): 3081, 3008 (C-H-str),2226 (C≡N),1731, 1606 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.30 (t, 3H, CH_3, J = 8.0 Hz), 4.25 (q, 2H, CH_2, J = 8.0 Hz), 5.85 (s, 2H, CH_2), 7.43 (d, 2H, ArH, J = 8.0 Hz), 7.72 (d, 1H, ArH, J = 8.6 Hz), 7.82 (d, 2H, ArH, J = 8.6 Hz), 7.99-8.03 (m, 1H, ArH), 8.49 (s, 1H, ArH), 9.02 (s, 1H,

ArH). MS: m/z = 401 (M+1). Anal. calcd. For $C_{21}H_{15}F_3N_2O_3$; Calc: C, 63.00; H, 3.78; N, 7.00; found: C, 63.02; H, 3.77; N, 7.03%.

5.4.16. 4-Hydroxy-8-(trifluoromethyl)quinoline-3-carbohydrazide (4a)

Yield: 4.4 g, 92.6%. M.p: >300 °C. IR (KBr ν_{max} cm^{-1}): 3323 (-OH), 3236 and 3173 (NH-NH_2), 3037, 2967 (C-H-str), 1656 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 4.65 (s, 2H, -NH_2, exchangeble), 7.77 (dd, 1H, ArH, J = 7.8 Hz, J = 1.4 Hz), 8.11 (s, 1H, ArH), 8.45 (d, 1H, ArH, J = 8.4 Hz), 8.92 (s, 1H, ArH), 10.51 (s, 1H, -NH, D_2O-exchangeble), 12.87 (s, 1H, -OH, D_2O-exchangeble), Anal. calcd. For $C_{11}H_8F_3N_3O_2$; Calcd: C, 48.72; H, 2.97; N, 15.49; found: C, 48.74; H, 2.97; N, 15.51 (Kumar et al. 1988).

5.4.17. 4-Hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide (4b)

Yield: 4.1 g, 86.3%. M.p: 255-257 °C. IR (KBr ν_{max} cm^{-1}): 3442 (-OH), 3296 and 3244 (NH-NH_2), 3088, 2963 (C-H-str), 1649 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 4.67 (s, 2H, -NH_2, D_2O-exchangeble), 7.76 (dd, 1H, ArH, J = 7.7 Hz, J = 1.4 Hz), 8.10 (s, 1H, ArH), 8.45 (d, 1H, ArH, J = 8.4 Hz), 8.90 (s, 1H, ArH), 10.57 (s, 1H, -NH, D_2O-exchangeble), 12.88 (s, 1H, -OH, D_2O-exchangeble). MS: m/z = 272 (M+1). Anal. calcd. For $C_{11}H_8F_3N_3O_2$; Calcd: C, 48.72; H, 2.97; N, 15.49; found: C, 48.75; H, 2.97; N, 15.53 (Niedermeier et al. 2009).

5.4.18. 4-Hydroxy-6-(trifluoromethyl)quinoline-3-carbohydrazide (4c)

Yield: 4.0 g, 85.1%. M.p: >300 °C. IR (KBr ν_{max} cm^{-1}): 3440 (-OH), 3224 and 3249 (NH-NH_2), 3091, 2041 (C-H-str), 1658 (C=O). ^1H NMR (400 MHz, DMSO-d_6): δ ppm 4.62 (s, 2H, -NH_2, exchangeble), 7.77 (dd, 1H, ArH, J = 7.8 Hz, J = 1.5 Hz), 8.10 (s, 1H, ArH), 8.46 (d, 1H, ArH, J = 8.4 Hz), 8.93 (s, 1H, ArH), 10.50 (s, 1H, -NH, D_2O-exchangeble), 12.89 (s, 1H, -OH, D_2O-exchangeble), Anal. calcd. For $C_{11}H_8F_3N_3O_2$; Calcd: C, 48.72; H, 2.97; N, 15.49; found: C, 48.72; H, 2.95; N, 15.50.

5.4.19. 4-Oxo-1-(prop-2-yn-1-yl)-8-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{56})

IR (KBr ν_{max} cm^{-1}): 3278 (C≡C-H-str), 3205, 3066 (NH-NH_2), 3015, 2964 (C-H-str), 1745, 1678 (C=O); ^1H NMR (400 MHz, $CDCl_3$): δ ppm 1.57 (s, 2H, NH_2, D_2O-exchangeble) 2.51 (s, 1H, CH), 4.80 (s, 2H, CH_2), 7.27 (s, 1H, NH, D_2O-exchangeble

)7.71 (t, 1H, ArH, J = 8.0 Hz), 8.08 (d, 1H, ArH, J = 7.1 Hz), 8.26 (d, 1H, ArH, J = 8.4 Hz), 9.13 (s, 1H, ArH). MS: m/z = 310 (M+1). Anal. calcd. For $C_{14}H_{10}F_3N_3O_2$; Calc: C, 54.37; H, 3.26; N, 13.59; found: C, 54.38; H, 3.25; N, 13.55%.

5.4.20. 1-(2,4-Dichlorobenzyl)-4-oxo-8-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{57})

IR (KBr ν_{max} cm^{-1}): 3166, 3091 (NH-NH_2), 3021, 2959 (C-H-str), 1627, 1580 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.27 (s, 2H, NH_2, D_2O-exchangeble), 4.30 (s, 2H, CH_2), 7.32 (dd, 2H, ArH, J = 8.3 Hz, J = 2.0 Hz), 7.50 (s, 1H, ArH), 7.54-7.61 (m, 1H, ArH), 8.12 (d, 1H, ArH, J = 7.3 Hz), 8.43 (d, 1H, ArH, J = 7.8 Hz), 8.56 (s, 1H, ArH), 10.50 (s, 1H, NH, D_2O-exchangeble). MS: m/z = 431 (M+1). Anal. calcd. For $C_{18}H_{12}Cl_2F_3N_3O_2$; Calc: C, 50.25; H, 2.81; N, 9.77; found: C, 50.27; H, 2.80; N, 9.77%.

5.4.21. 1-(4-Cyanobenzyl)-4-oxo-8-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{58})

IR (KBr ν_{max} cm^{-1}): 3148, 3100 (NH-NH_2),, 3069, 2993 (C-H-str), 2223 (C≡N), 1714, 1685 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.37 (s, 2H, NH_2, D_2O-exchangeble) 5.38 (s, 2H, CH_2), 7.64 (d, 1H, ArH, J = 8.2 Hz), 7.67 (d, 1H, ArH, J = 8.2 Hz), 7.73-7.86 (m, 3H, ArH), 8.30 (d, 1H, ArH, J = 7.2 Hz), 8.47 (d, 1H, ArH, J = 8.4 Hz), 8.69 (s, 1H, ArH). MS: m/z = 387 (M+1). Anal. calcd. For $C_{19}H_{13}F_3N_4O_2$; Calc: C, 59.07; H, 3.39; N, 14.50; found: C, 59.09; H, 3.37; N, 14.49%.

5.4.22. 4-Oxo-1-(prop-2-yn-1-yl)-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{59})

IR (KBr ν_{max} cm^{-1}): 3295 (C≡C-H-str), 3242, 3148 (NH-NH_2), 3042, 2963 (C-H-str), 1662, 1599 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 3.29 (s, 1H, CH), 3.68 (s, 2H, NH_2, D_2O-exchangeble), 5.51 (s, 2H, CH_2), 7.85 (d, 1H, ArH, J = 8.4 Hz), 8.22 (s, 1H, ArH), 8.50 (d, 1H, ArH, J = 8.3 Hz), 9.09 (s, 1H, ArH), 10.79 (s, 1H, NH, D_2O-exchangeble); ^{13}C NMR: 46.16, 77.58, 78.51, 79.38, 112.37,121.68, 121.80, 125.68, 129.83, 138.90, 149.73, 162.32, 162.32, 175.12. MS: m/z = 310 (M+1). Anal. calcd. For $C_{14}H_{10}F_3N_3O_2$; Calc: C, 54.37; H, 3.26; N, 13.59; found: C, 54.39; H, 3.25; N, 13.60%.

5.4.23. 1-(2,4-Dichlorobenzyl)-4-oxo-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{60})

IR (KBr ν_{max} cm^{-1}): 3247, 3192 (NH-NH_2), 3032, 2927 (C-H-str), 1666, 1635 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.67 (s, 2H, NH_2, D_2O-exchangeble), 5.94 (s, 2H, CH_2), 7.01 (d, 1H, ArH, J = 8.4 Hz), 7.36 (dd, 1H, ArH, J = 8.4 Hz, J = 2.1 Hz), 7.79 (s, 1H, ArH), 7.83 (d, 1H, ArH, J = 8.4 Hz), 7.95 (s, 1H, ArH), 8.56 (d, 1H, ArH, J = 8.3 Hz), 9.09 (s, 1H, ArH), 10.48 (s, 1H, NH, D_2O-exchangeble). MS: m/z = 431 (M+1). Anal. calcd. For $C_{18}H_{12}Cl_2F_3N_3O_2$; Calc: C, 50.25; H, 2.81; N, 9.77; found: C, 50.23; H, 2.80; N, 9.79%.

5.4.24. 1-(4-Cyanobenzyl)-4-oxo-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{61})

IR (KBr ν_{max} cm^{-1}): 3265, 3215 (NH-NH_2), 3048, 2922 (C-H-str), 2226 (C≡N), 1659, 1601 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.29 (s, 2H, NH_2, D_2O-exchangeble), 6.03 (s, 2H, CH_2), 7.43 (d, 2H, ArH, J = 8.4 Hz), 7.62 (d, 2H, ArH, J = 8.3 Hz), 7.82-7.84 (m, 3H, ArH), 9.17 (s, 1H, ArH), 10.62 (s, 1H, NH, D_2O-exchangeble). MS: m/z = 387 (M+1). Anal. calcd. For $C_{19}H_{13}F_3N_4O_2$; Calc: C, 59.07; H, 3.39; N, 14.50; found: C, 59.09; H, 3.39; N, 14.51%.

5.4.25. 4-Oxo-1-(prop-2-yn-1-yl)-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{62})

IR (KBr ν_{max} cm^{-1}): 3382 (C≡C-H-str), 3325, 3253 (NH-NH_2), 3097, 3034 (C-H-str), 1739, 1647 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.0 (s, 1H, CH), 4.20 (s, 2H, -NH_2, D_2O-exchangeble), 4.29 (s, 2H, CH_2), 7.63 (d, 1H, ArH, J = 8.8 Hz), 7.93 (d, 1H, ArH, J = 10.0 Hz), 7.78 (s, 1H, ArH), 8.84 (s, 1H, ArH), 10.70 (s, 1H, -NH, D_2O-exchangeble). MS: m/z = 310 (M+1). Anal. calcd. For $C_{14}H_{10}F_3N_3O_2$; Calc: C, 54.37; H, 3.26; N, 13.59; found: C, 54.37; H, 3.27; N, 13.59%.

5.4.26. 1-(2,4-Dichlorobenzyl)-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{63})

IR (KBr ν_{max} cm^{-1}): 3287, 3237 (NH-NH_2), 3034, 2960 (C-H-str), 1738, 1642 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.67 (s, 2H, -NH_2, D_2O-exchangeble), 5.87 (s, 2H, CH_2), 6.85 (d, 1H, ArH, J = 8.0 Hz), 7.30 (dd, 1H, ArH, J = 8.4 Hz, J = 2.0 Hz), 7.72-7.78 (m, 2H, ArH), 8.06 (dd, 1H, ArH, J = 9.2 Hz, J = 1.6 Hz), 8.61 (s, 1H,

ArH), 9.10 (s, 1H, ArH), 10.45 (s, 1H, -NH, D_2O-exchangeble). MS: m/z = 431 (M+1). Anal. calcd. For $C_{18}H_{12}Cl_2F_3N_3O_2$; Calc: C, 50.25; H, 2.81; N, 9.79; found: C, 50.25; H, 2.81; N, 9.76%.

5.4.27. 1-(4-Cyanobenzyl)-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carbohydrazide (G_{64})

IR (KBr ν_{max} cm^{-1}): 3312, 3265 (NH-NH_2), 3028, 2965 (C-H-str), 2223 (C≡N),1738, 1651 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.67 (s, 2H, -NH_2, D_2O-exchangeble), 5.87 (s, 2H, CH_2), 6.85 (d, 1H, ArH, J = 8.0 Hz), 7.30 (dd, 1H, ArH, J = 8.4 Hz, J = 2.0 Hz), 7.72-7.78 (m, 2H, ArH), 8.06 (dd, 1H, ArH, J = 9.2 Hz, J = 1.6 Hz), 8.61 (s, 1H, ArH), 9.10 (s, 1H, ArH), 10.45 (s, 1H, -NH, D_2O-exchangeble). MS: m/z = 387 (M+1). Anal. calcd. For $C_{19}H_{13}F_3N_4O_2$; Calc: C, 59.07; H, 3.39; N, 14.50; found: C, 59.07; H, 3.40; N, 14.48%.

5.4.28. 4-Hydroxy-8-(trifluoromethyl)quinoline-3-carboxylic acid (5a)

Yield: 4.3 g, 95.5%. M.p: 258-260 °C. IR (KBr ν_{max} cm^{-1}): 3440 (-OH), 3113, 3061 (C-H-str), 1720 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.54 (t, 1H, ArH, J = 8.0 Hz), 8.05 (d, 1H, ArH, 7.8 Hz), 8.44 (d, 2H, ArH, J = 8.0 Hz), 11.61 (s, 1H, -OH, D_2O-exchangeble), 14. 43 (s, 1H, OH). Anal. calcd. For $C_{11}H_6F_3NO_3$; Calcd: C, 51.37; H, 2.35; N, 5.45; found: C, 51.39; H, 2.33; N, 5.46 (Snyder et al. 1947).

5.4.29. 4-Hydroxy-7-(trifluoromethyl)quinoline-3-carboxylic acid (5b)

Yield: 4.4 g, 97.7%. M.p: 249-251 °C. IR (KBr ν_{max} cm^{-1}): 3380 (-OH), 2909, 2955 (C-H-str), 1690 (C=O). ^{1} H NMR (400 MHz, DMSO-d_6): δ ppm 7.52 (d, 1H, ArH, J = 12 Hz), 7.81 (d, 1H, ArH, J = 12 Hz), 8.03 (s, 1H, ArH), 8.44 (s, 1H, ArH), 12.29 (s, 1H, -OH, D_2O-exchangeble), 14.48 (s, 1H, OH, D_2O-exchangeble). Anal. calcd. For $C_{11}H_6F_3NO_3$; Calcd: C, 51.37; H, 2.35; N, 5.45; found: C, 51.38; H, 2.34; N, 5.45 (Allais et al. 1973).

5.4.30. 4-Hydroxy-6-(trifluoromethyl)quinoline-3-carboxylic acid (5c)

Yield: 4.3 g, 95.5%; M.p: 327-329 °C; IR (KBr ν_{max} cm^{-1}): 3381 (-OH), 3063, 3021 (C-H-str), 1688 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.96 (d, 1H, ArH, J = 12 Hz), 8.10 (d, 1H, ArH, J = 12 Hz), 8.43 (s, 1H, ArH), 8.91 (s, 1H, ArH), 13.80 (s,

1H, -OH, D_2O-exchangeble). MS: m/z = 258 (M+1). Anal. calcd. For $C_{11}H_6F_3NO3$; Calc: C, 51.37; H, 2.35; N, 5.45; found: C, 51.39; H, 2.35; N, 5.45%.

5.4.31. 4-Oxo-1-(prop-2-yn-1-yl)-8-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{65})

IR (KBr ν_{max} cm^{-1}): 3288 (-OH), 3195 (C≡C-H-str), 3078, 3031 (C-H-str), 1718, 1618 (C=O); ^1H NMR (400 MHz, $CDCl_3$): δ ppm 2.61 (s, 1H, CH), 5.07 (s, 2H, CH_2), 7.64 (t, 1H, ArH, J = 7.9 Hz), 8.17 (d, 1H, ArH, J = 7.6 Hz), 8.56 (d, 1H, ArH, J = 8.5 Hz), 9.31 (s, 1H, ArH), 12.03 (s, 1H, OH, D_2O-exchangeble). MS: m/z = 296 (M+1). Anal. calcd. For $C_{14}H_8F_3NO_3$; Calc: C, 56.96; H, 2.73; N, 4.74; found: C, 56.97; H, 2.70; N, 4.78%.

5.4.32. 1-(2,4-Dichlorobenzyl)-4-oxo-8-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{66})

IR (KBr ν_{max} cm^{-1}): 3415 (-OH), 3015, 2974 (C-H-str), 1690, 1617 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 5.28 (s, 2H, CH_2), 7.46 (d, 1H, ArH, J = 8.0 Hz),7.55 (t, 1H, ArH, J = 7.8 Hz), 7.65 (s, 1H, ArH), 7.71 (d, 1H, ArH, J = 8.0 Hz), 8.09 (d, 1H, ArH, J = 8.0 Hz), 8.45 (s, 1H, ArH), 8.47 (s, 1H, ArH), 12.36 (s, 1H, OH, D_2O-exchangeble); ^{13}C NMR: 62.90, 110.40, 112.25, 124.83, 124.98, 128.06, 129.30, 131.57, 131.59, 133.60, 133.92, 146.90, 147.50, 151.70. MS: m/z = 417 (M+1). Anal. calcd. For $C_{18}H_{10}Cl_2F_3NO_3$; Calc: C, 51.95; H, 2.42; N, 3.37; found: C, 51.98; H, 2.43; N, 3.35%.

5.4.33. 1-(4-Cyanobenzyl)-4-oxo-8-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{67})

IR (KBr ν_{max} cm^{-1}): 3419 (-OH), 3005, 2944 (C-H-str), 2222 (C≡N), 1735, 1673 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 5.39 (s, 2H, CH_2), 7.59 (t, 1H, ArH, J = 7.7 Hz), 7.69 (d, 2H, ArH, J = 8.4 Hz), 7.86 (d, 2H, ArH, J = 8.3 Hz), 8.13 (d, 1H, ArH, J = 7.4 Hz), 8.50-8.52 (m, 2H, ArH), 11.73 (s, 1H, OH, D_2O-exchangeble); ^{13}C NMR: 65.05, 110.92, 119.24, 122.62, 124.79, 125.17, 125.23, 125.33, 126.60, 128.38, 131.52, 131.17, 131.69, 132.02, 132.85, 135.50, 142.79, 146.99, 164.57. MS: m/z = 373 (M+1). Anal. calcd. For $C_{19}H_{11}F_3N_2O_3$; Calc: C, 61.30; H, 2.98; N, 7.52; found: C, 61.33; H, 2.97; N, 7.52%.

5.4.34. 4-Oxo-1-(prop-2-yn-1-yl)-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{68})

IR (KBr ν_{max} cm^{-1}): 3427 (-OH), 3306 (C≡C-H-str), 3041, 2962 (C-H-str), 1692, 1609 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.83 (s,1H, CH), 5.39 (s, 2H, CH_2), 7.77 (d, 1H, ArH, J = 8.0 Hz), 8.12 (s, 1H, ArH), 8.38 (d, 1H, ArH, J = 8.0 Hz), 8.89 (s, 1H, ArH), 14.48 (s, 1H, OH, D_2O-exchangeble); ^{13}C NMR: 43.08, 51.99, 79.15, 110.81, 111.59, 115.85, 121.64, 128.68, 128.68, 130.96, 138.96, 150.63, 150.94, 165.03, 172.54. MS: m/z = 296 (M+1). Anal. calcd. For $C_{14}H_8F_3NO_3$; Calc: C, 56.96; H, 2.73; N, 4.74; found: C, 56.96; H, 2.73; N, 4.72%.

5.4.35. 1-(2,4-Dichlorobenzyl)-4-oxo-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{69})

IR (KBr ν_{max} cm^{-1}): 3414 (-OH), 3052, 2955 (C-H-str), 1678, 1632 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 5.83 (s, 2H, CH_2), 7.02 (d, 1H, ArH, J = 8.4 Hz), 7.36 (dd, 1H, ArH, J = 8.4 Hz, J = 2.1 Hz), 7.78-7.82 (m, 3H, ArH), 8.46 (d, 1H, ArH, J = 8.2), 8.99 (s, 1H, ArH) 12.97 (s, 1H, OH, D_2O-exchangeble). MS: m/z = 417 (M+1). Anal. calcd. For $C_{18}H_{10}Cl_2F_3NO_3$; Calc: C, 51.95; H, 2.42; N, 3.37; found: C, 51.96; H, 2.45; N, 3.35%.

5.4.36. 1-(4-Cyanobenzyl)-4-oxo-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{70})

IR (KBr ν_{max} cm^{-1}): 3353 (-OH), 3067, 2956 (C-H-str), 2230 (C≡N), 1728, 1621 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 5.85 (s, 2H, CH_2), 7.40 (d, 2H, ArH, J = 8.0 Hz), 7.70 (d, 2H, ArH, J = 8.0 Hz), 7.77 (d, 1H, ArH, J = 8.0 Hz), 7.84 (s, 1H, ArH), 8.37 (d, 1H, ArH, J = 8.0 Hz), 9.0 (s, 1H, ArH), 12.36 (s, 1H, OH, D_2O-exchangeble); ^{13}C NMR: 51.98, 55.55, 110.55, 11130, 111.33, 116.00, 116.25, 118.94, 127.94, 128.85, 133.35, 139.60, 142.20, 141.89, 151.91, 166.50, 173.25, 187.80, 195.54. MS: m/z = 373 (M+1). Anal. calcd. For $C_{19}H_{11}F_3N_2O_3$; Calc: C, 61.30; H, 2.98; N, 7.52; found: C, 61.34; H, 2.95; N, 7.51%.

5.4.37. 4-Oxo-1-(prop-2-yn-1-yl)-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{71})

IR (KBr ν_{max} cm^{-1}): 3401 (-OH), 3056, 2990 (C-H-str), 1701, 1613 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.18 (s, 1H, CH), 5.16 (s, 2H, CH_2), 7.78 (d, 1H, ArH,

J = 8.8 Hz), 7.95 (d, 1H, ArH, J = 8.8 Hz), 8.78 (s, 1H, ArH), 8.83 (s, 1H, ArH), 14.58 (s, 1H, -OH, D_2O-exchangeble). MS: m/z = 296 (M+1). Anal. calcd. For $C_{14}H_8F_3NO_3$; Calc: C, 56.96; H, 2.73; N, 4.74; found: C, 56.98; H, 2.74; N, 4.73%.

5.4.38. 1-(2,4-Dichlorobenzyl)-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{72})

IR (KBr ν_{max} cm^{-1}): 3455 (-OH), 3048, 3021 (C-H-str), 1728, 1615 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ ppm1.30 (t, 3H, CH_3, J = 8.0 Hz), 2.50 (s, 1H, CH), 4.21 (q, 2H, CH_2, J = 8.0 Hz), 5.37 (s, 2H, CH_2), 8.03 (d, 1H, ArH, J = 8.0 Hz), 8.18 (d, 1H, ArH, J = 8.0 Hz), 8.49 (s, 1H, ArH), 8.92 (s, 1H, ArH). m/z = 417 (M+1). Anal. calcd. For $C_{18}H_{10}Cl_2F_3NO_3$; Calc: C, 51.95; H, 2.42; N, 3.37; found: C, 51.98; H, 2.40; N, 3.35%.

5.4.39. 1-(4-Cyanobenzyl)-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (G_{73})

IR (KBr ν_{max} cm^{-1}): 3445 (-OH), 2230 (C≡N), 3053, 3009 (C-H-str), 1716, 1669 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 5.99 (s, 2H, CH_2), 7.35-7.40 (m, 2H, ArH), 7.47 (d, 1H, ArH, J = 8.0 Hz), 7.83-8.00 (m, 2H, ArH), 8.17 (d, 1H, ArH, J = 7.6 Hz), 8.62 (s, 1H, ArH), 9.40 (s, 1H, ArH), 14.30 (s, 1H, -OH, D_2O-exchangeble). MS: m/z = 373 (M+1). Anal. calcd. For $C_{19}H_{11}F_3N_2O_3$; Calc: C, 61.30; H, 2.98; N, 7.52; found: C, 61.28; H, 2.98; N, 7.52%.

5.4.40. Prop-2-yn-1-yl 4-oxo-1-(prop-2-yn-1-yl)-6-(trifluoromethyl)-1,4-dihydro quinoline-3-carboxylate (G_{74})

IR (KBr ν_{max} cm^{-1}): 3262, 3231 (C≡C-H-str), 3075, 2942 (C-H-str), 1731, 1601 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 3.53 (s, 1H, CH), 3.64 (s, 1H, CH), 4.84 (s, 2H, CH_2), 5.35 (s, 2H, CH_2), 8.01 (d, 1H, ArH, J = 8.9 Hz), 8.15 (dd, 1H, ArH, J = 8.9 Hz, J = 2.0 Hz), 8.45 (s, 1H, ArH), 8.92 (s, 1H, ArH). MS: m/z = 334 (M+1). Anal. calcd. For $C_{17}H_{10}F_3NO_3$; Calc: C, 61.27; H, 3.02; N, 4.20; found: C, 61.29; H, 3.02; N, 4.21%.

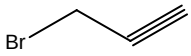
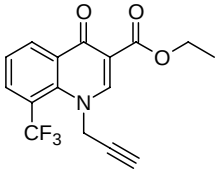
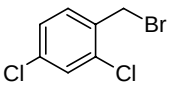
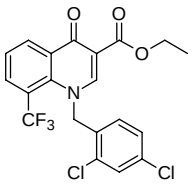
5.4.41. 2,4-Dichlorobenzyl 1-(2,4-dichlorobenzyl)-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate (G_{75})

IR (KBr ν_{max} cm^{-1}): 3072, 2961 (C-H-str), 1727, 1641 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 5.30 (s, 2H, CH_2), 5.72 (s, 2H, CH_2), 6.91 (d, 1H, ArH, J = 8.4 Hz), 7.27 (d, 1H, ArH, J = 8.4 Hz), 7.46 (d, 1H, ArH, J = 8.3 Hz), 7.60 (d, 2H, ArH, J = 9.8 Hz), 7.72 (s, 1H, ArH), 7.75 (s, 1H, ArH), 8.49 (s, 1H, ArH), 8.93 (s, 1H, ArH); ^{13}C NMR: 54.07, 60.27, 62.96, 111.46, 119.74, 128.01, 128.45, 129.22, 129.59, 129.71, 129.84, 131.44, 132.31, 141.98, 152.07, 164.26, 172.80. MS: m/z = 576 (M+1). Anal. calcd. For $C_{25}H_{14}Cl_4F_3NO_3$; Calc: C, 52.20; H, 2.45; N, 2.44; found: C, 52.25; H, 2.45; N, 2.43%.

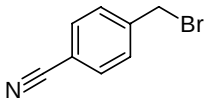
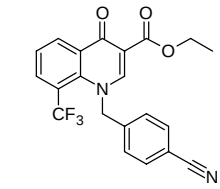
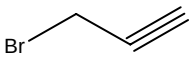
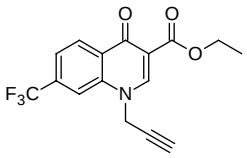
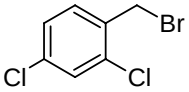
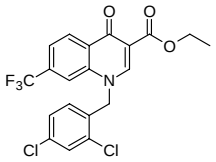
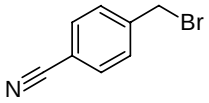
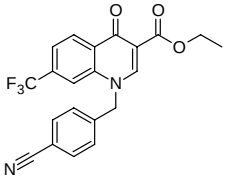
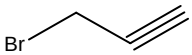
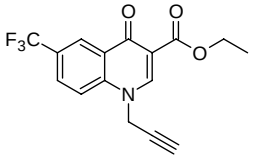
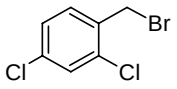
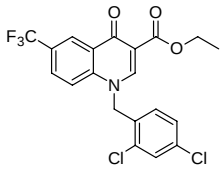
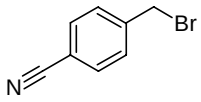
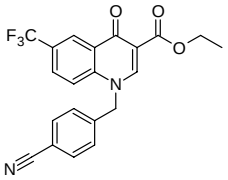
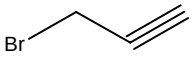
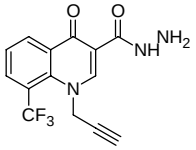
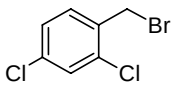
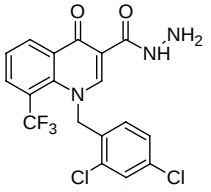
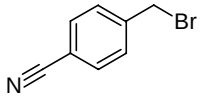
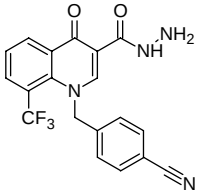
5.4.42. 1-(4-Cyano-benzyl)-4-oxo-6-trifluoromethyl-1,4-dihydro-quinoline-3-carboxylic acid 4-cyano-benzyl ester (G_{76})

IR (KBr ν_{max} cm^{-1}): 3050, 2967 (C-H-str), 2224 (C≡N), 1707, 1649 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 5.38 (s, 2H, CH_2), 5.82 (s, 2H, CH_2), 7.40 (d, 2H, ArH, J = 8.4 Hz), 7.69 (d, 3H, ArH, J = 8.4 Hz), 7.77 (d, 2H, ArH, J = 8.4 Hz), 7.83 (d, 2H, ArH, J = 8.3 Hz), 7.96 (dd, 1H, ArH, J = 8.9 Hz, J = 2.2 Hz), 8.48 (s, 1H, ArH), 9.05 (s, 1H, ArH). MS: m/z = 488 (M+1). Anal. calcd. For $C_{27}H_{16}F_3N_3O_3$; Calc: C, 66.53; H, 3.31; N, 8.62; found: C, 66.55; H, 3.30; N, 8.62%.

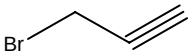
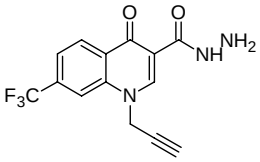
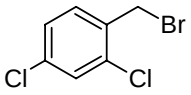
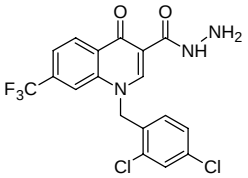
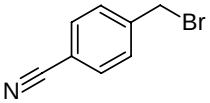
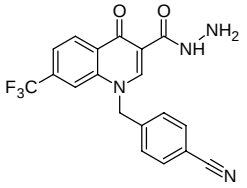
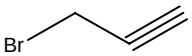
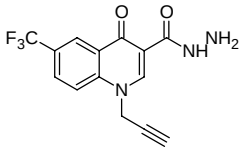
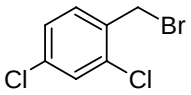
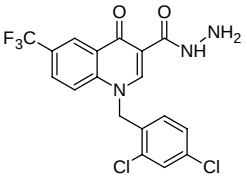
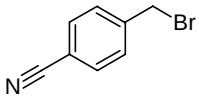
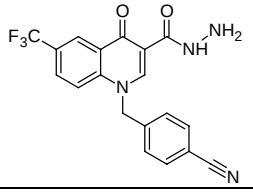
Table 5.2. Synthesis of trifluoromethyl quinolone ester (G_{47-55}) and hydrazide derivatives (G_{56-64}).

Entry	Compounds	R_2	Product	Isolated yield [a,b]
1	**G_{47}**			95
2	**G_{48}**			98

continued

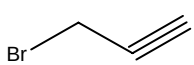
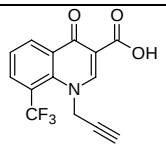
3	**G_{49}**			97
4	**G_{50}**			94
5	**G_{51}**			98
6	**G_{52}**			96
7	**G_{53}**			90
8	**G_{54}**			93
9	**G_{55}**			91
10	**G_{56}**			64, 96
11	**G_{57}**			75, 96
12	**G_{58}**			71, 94

continued

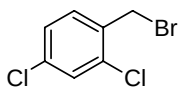
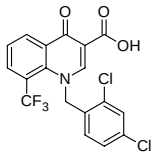
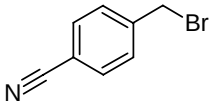
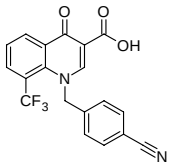
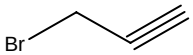
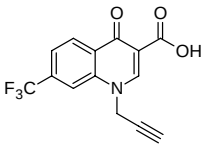
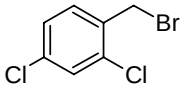
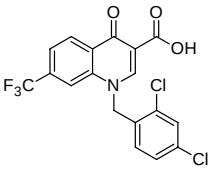
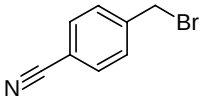
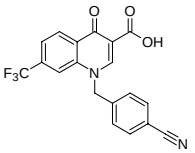
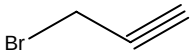
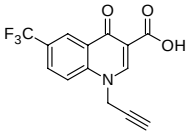
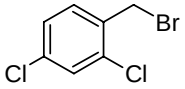
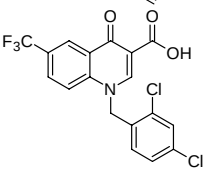
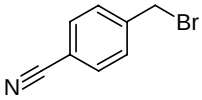
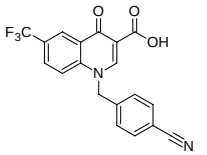
13	**G_{59}**			65, 95
14	**G_{60}**			71, 96
15	**G_{61}**			69, 91
16	**G_{62}**			55, 89
17	**G_{63}**			61, 90
18	**G_{64}**			50, 85

a = Route a, b = Route b

Table 5.3. Synthesis of trifluoromethyl-3-carboxyquinolone derivatives ($G_{65\text{-}73}$) and 1-alkyl-4-oxo-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylate derivatives ($G_{74\text{-}76}$)

Entry	Compounds	R_2	Product	Isolated yield [a,b]
1	**G_{65}**			52, 90

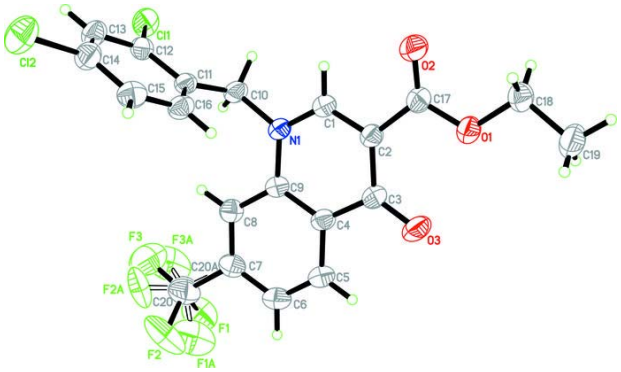
continued

2	**G_{66}**			60, 95
3	**G_{67}**			59, 90
4	**G_{68}**			50, 91
5	**G_{69}**			57, 92
6	**G_{70}**			57, 89
7	**G_{71}**			53, 85
8	**G_{72}**			57, 89
9	**G_{73}**			51, 85

a = Route c. b = Route d

5.5. ANALYTICAL DATA

Crystal data	
Empirical formula	$C_{20}H_{14}Cl_2F_3NO_3$
Formula weight	444.22
Crystal system	Triclinic
Crystal dimension	0.43 mm x 0.18 mm x 0.07 mm
Space group	$P\bar{1}$
a(Å)	8.090 (2)
b(Å)	9.547 (3)
c(Å)	14.047 (4)
Volume (Å^3)	963.3 (5)
Angle α, β, γ	77.299, 76.198, 67.488
Z	2
F_{000}	452
μ (mm^{-1})	0.39
Temperature (T)	296K
Radiation wavelength (Å)	0.71073
Radiation type	Mo Kα
Radiation source	fine-focus sealed tube
Radiation monochromator	graphite



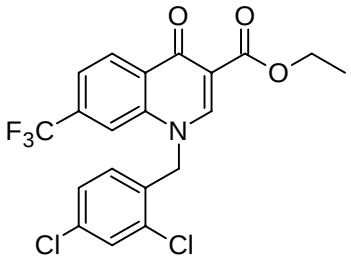


Figure 5.2. ORTEP diagram showing the single crystal structure of compound G_{51} (drawn at 50% probability level).

.

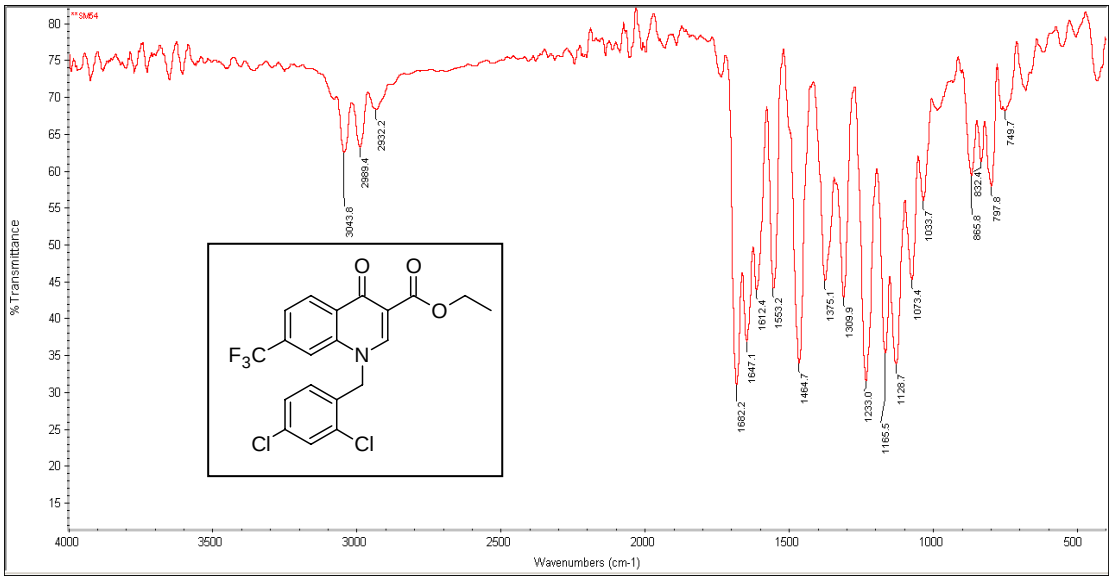


Figure 5.3. IR spectrum of G_{51}.

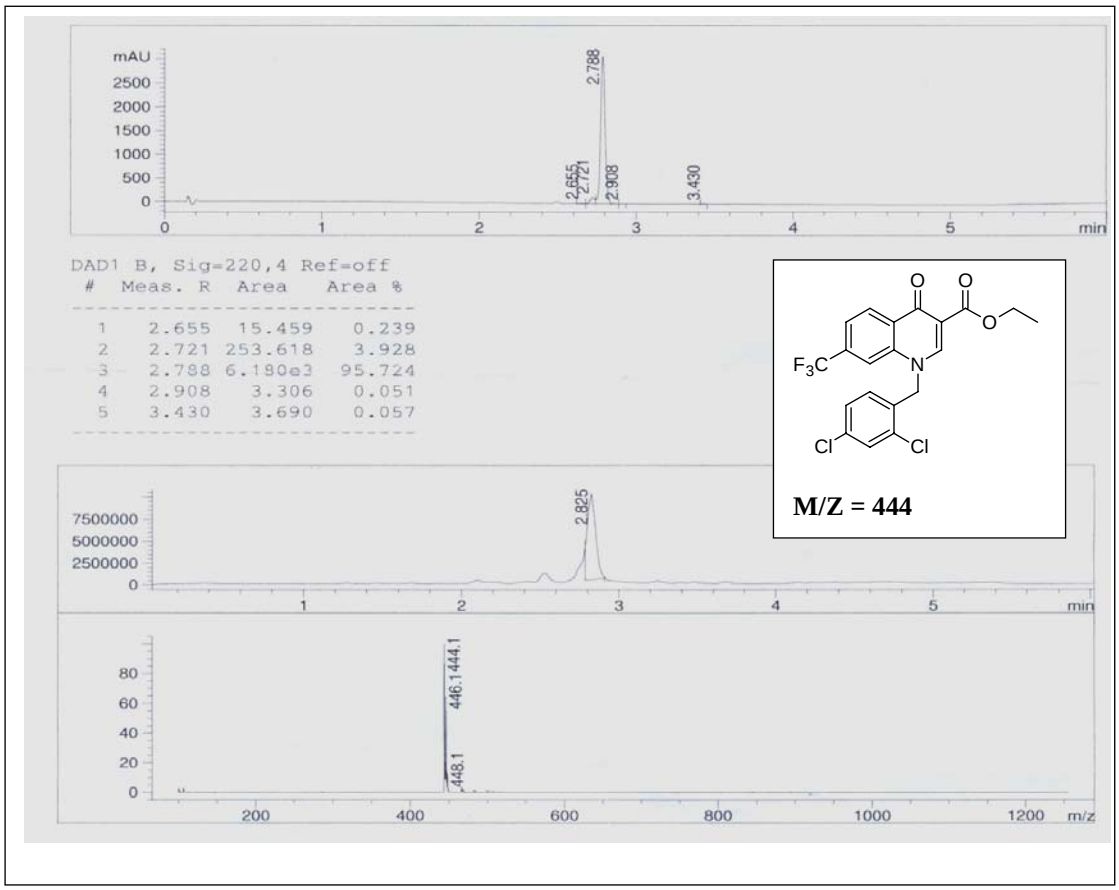


Figure 5.4. LCMS spectrum of G_{51}.

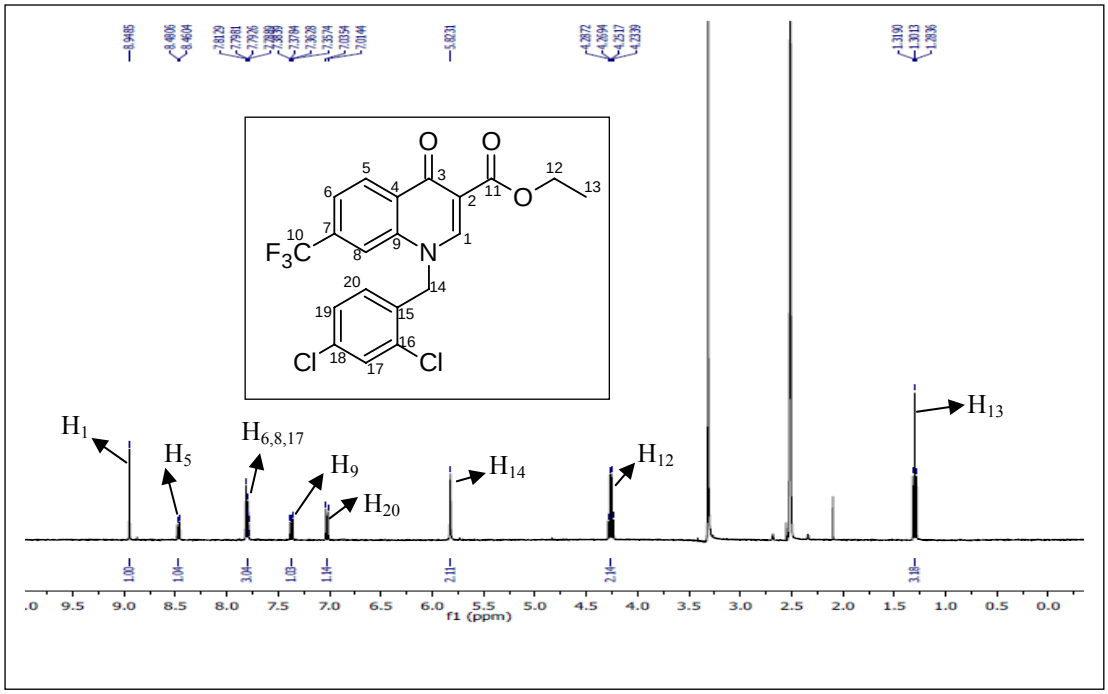


Figure 5.5. ^{1}H NMR spectrum of G_{51}.

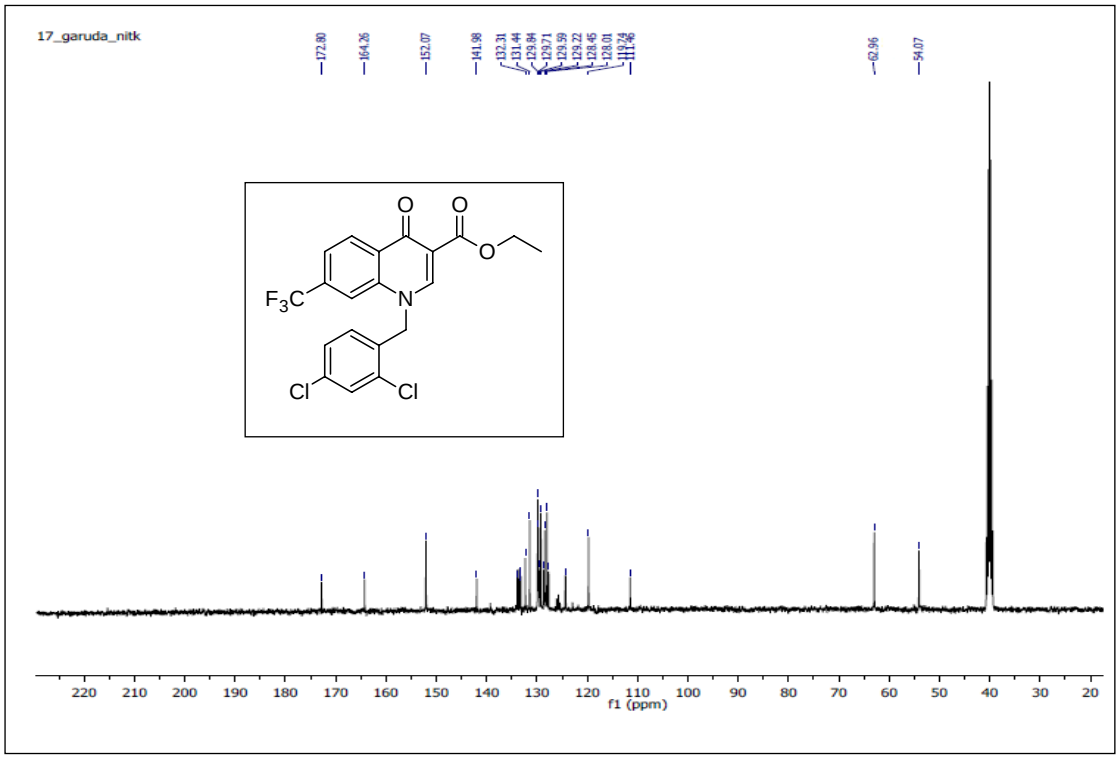


Figure 5.6. ^{13}C NMR spectrum of G_{51}.

5.6. CONCLUSION

Three series of new trifluoromethyl substituted quinolone derivatives were synthesized (**G_{47-55}, G_{56-64}, G_{65-73}** and **G_{74-76}**) by multi-step reactions. The synthetic routs and purification methods were stabilized for regioselective synthesis by approaching two different routes. The alkylation of trifluoromethyl-4-hydroxyquinoline followed by hydrolysis with LiOH (route-b) and condensation with hydrazine hydrate (route-d) showed a good yield and regioselectivity compared with route-a**,** route-c. They were characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry, IR studies and elemental analyses. The structure of **G_{51}** has also been confirmed by single crystal X-ray crystallographic study. All the newly synthesized compounds were screened for *in-vitro* antimicrobial activity by well plate method and the results were discussed in CHAPTER-7.

CHAPTER-6

SYNTHESIS AND CHARACTERIZATION OF

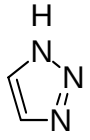
1,2,3-TRIAZOLE CONTAINING
8-TRIFLUOROMETHYLQUINOLINE DERIVATIVES

CHAPTER-6

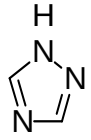
SYNTHESIS AND CHARACTERIZATION OF 1,2,3-TRIAZOLE CONTAINING 8-TRIFLUOROMETHYLQUINOLINE DERIVATIVES

6.1. INTRODUCTION

Triazoles refers to either one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$, having a five-membered ring of two carbon atoms and three nitrogen atoms. In the recent years, azole class of drugs (imidazole and triazole) occupy prominent place in medicinal chemistry because of their broad spectrum of pharmacological activities such as antimicrobial, anti-inflammatory, analgesic, antitumorial, antihypertensive, anticonvulsant and antiviral activities. The known drug in this family includes Fluconazole, Isavuconazole, Voriconazole, Pramiconazole, and Posaconazole.

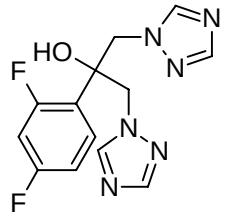


1,2,3-Triazole

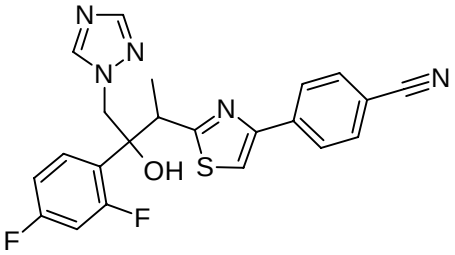


1,2,4-Triazole

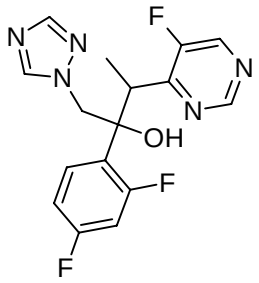
Figure 6.1. Structure of triazoles.



Fluconazole (Antifungal)
QD-6.1

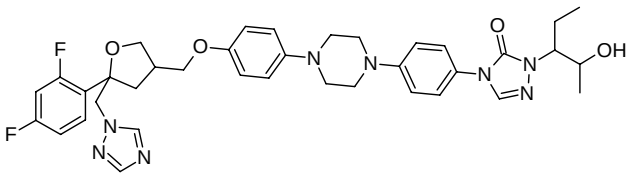


Isavuconazole (Antifungal)
QD-6.2



Voriconazole (Antifungal)

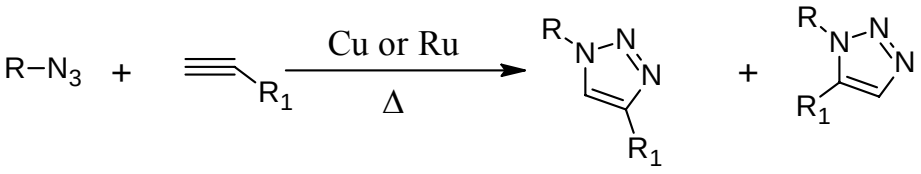
QD-6.3



Posaconazole (Antifungal)

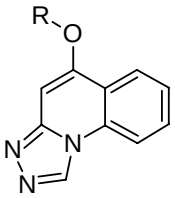
QD-6.4

Triazoles are useful building blocks in chemistry, and are relatively stable to moisture, oxygen, light, and also metabolism in the body. Moreover, these moieties can be tuned to form powerful pharmacophores and also play an important role in bio-conjugation (for example, replacing the phosphate backbone of DNA). Recently, the concept of click chemistry has been explored as a new approach for regioseletivitive synthesis of triazoles for mimicking the nature way of synthesis. Click chemistry concept is the azide alkyne Huisgen cycloaddition using a Copper (Cu) catalyst at room temperature. It was discovered simultaneously and independently by the groups of Valery V. Fokin and K. Barry Sharpless. Copper and ruthenium are the commonly used catalysts in the reaction. The use of copper as a catalyst results in the formation of 1,4-regioisomer, whereas ruthenium results in formation of the 1,5- regioisomer.



1,4-regioisomer 1,5-regioisomer

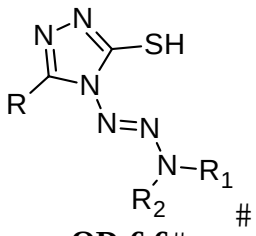
Anticonvulsant activity of 5-alkoxy-[1,2,4]triazolo[4,3]quinoline derivatives (**QD-6.5**) were reported by Guo et al. (2009). The results of these tests showed 5-hexyloxy-[1,2,4]triazolo[4,3]quinoline was the most potent anticonvulsant.



QD-6.5

Where R = CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{12}H_{22}$, CH_2Ph, CH_2Ph(o-F), CH_2Ph(m-F), CH_2Ph(p-F), CH_2Ph(o-Cl), CH_2Ph(m-Cl), CH_2Ph(p-Cl), CH_2Ph(o-Br), CH_2Ph(m-Br), CH_2Ph(p-Br), CH_2Ph(p-Me), CH_2Ph(2,4-Cl_2), CH_2Ph(2,6-Cl_2).

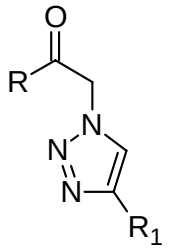
Some new derivatives of 3-substituted -4*H*-1, 2, 4- triazoles (**QD-6.6**) were reported by Goyal et al. (2010). Anti-inflammatory evaluation highlights that compounds containing orthomethoxy group and methyl groups are potent derivatives.



QD-6.6#

Where R = CH_3, C_6H_5, R_1 = H, C_6H_5, R_2 = C_6H_5, p-$NO_2C_6H_4$, o-MeC_6H_4, p-$OMeC_6H_5$.

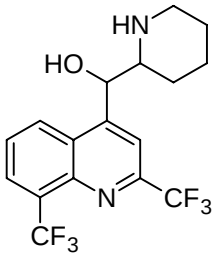
Kumar et al. (2011) reported synthesis of 1,4-disubstituted 1,2,3-triazoles (**QD-6.7**) and their *Src kinase* inhibitory activity. Structure–activity relationship analysis demonstrated that insertion of C_6H_5 and 4-$CH_3C_6H_4$ at position 4 contributed more for the *Src kinase* activity.



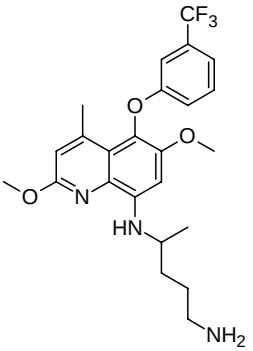
QD-6.7

Where R = C_6H_5, 4-$CH_3C_6H_4$, 4-$OCH_3C_6H_4$, 4-ClC_6H_4, 4-BrC_6H_4, CH_3, OCH_3, $N(C_2H_5)$,. R_1 = C_6H_5, 4-$CH_3C_6H_4$, 4-F-3-$CH_3C_6H_4$, 4-$OCH_3C_6H_4$,

The heterocyclic compounds play an important role in developing new antimicrobial agents. Recent observations suggested that, substitution of trifluoromethyl group at C-8 position of the quinoline have a profound effect on biological activity (Holla et al. 2006; Niedermeier et al. 2009). Trifluoromethyl quinolines and its derivatives have wide range of applications in the field of pharmaceuticals as antimalarial (Lutz et al. 1971), antibacterial, antifungal (Holla et al. 2006), antituberculosis (Mital et al. 2006), anticancer (Wang et al. 2011) agents. Mefloquine and Tafenoquine are some of the drugs which contain trifluoromethyl group and quinoline as a core moiety.



Mefloquine



Tafenoquine

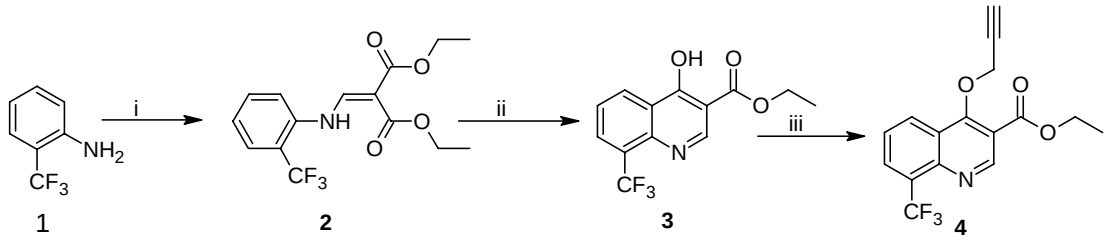
Figure 6.2. Quinoline drugs containing trifluoromethyl group

On the other hand regioisomers, 1,2,3-triazoles and their derivatives form an important class of heterocycles with various pharmacologically interesting activities. Various 1,2,3-triazoles have been reported to possess antibacterial, antifungal, antitubercular, anticancer, antiviral, antihypertensive, anticholinergic and anti-inflammatory properties (Wang et al. 2010; Tripathi et al. 2010; Li et al. 2011; Montagu et al. 2011). Literature review revealed that, insertion of pharmacophore at position-4 of quinoline with heterocyclic derivatives enhances its anti-tuberculosis activity (Mital et al. 2006). The Substitution of trifluoromethyl functional group at 8th position showed excellent pharmacological properties like antimicrobial, antifungal and antitumor activities (Holla et al. 2006; Meshram et al. 2012). Prompted by these observations, A project was carried out for the synthesis of some new 1,2,3-triazole derivatives containing 8-trifluoromethyquinoline nucleus. The final compounds were screened for their antimicrobial properties.

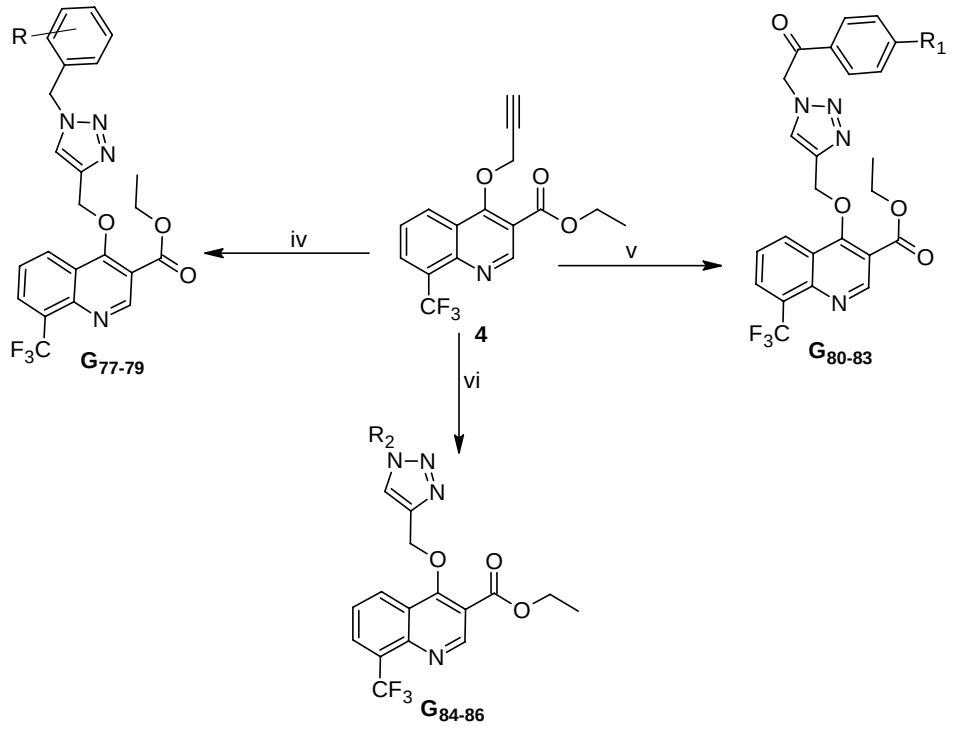
6.2. RESULTS AND DISCUSSION

6.2.1. Synthesis of 1,2,3-triazole containing 8-trifluoromethyl quinoline derivatives

4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (**3**) was synthesized by Gould-Jacobs procedure as discussed in **chapter-3**. The selective O-alkylation of **3** was carried out by treating it with propargylbromide in acetone meadia (**Scheme-6.1**) (Lilienkampf et al. 2009). The targeted 1,2,3-triazole derivatives (**G_{77-79}, G_{80-83}** and **G_{84-86}**) were synthesized by click chemistry approach, reacting 4-prop-2-ynyloxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (**4**) with various benzyl bromides, phenacyl bromides and alkyl bromides (**Scheme-6.2**). 6-Trifluoromethyl-furo[3,2-c]quinoline derivatives (**G_{87-88}**) were synthesized by treating ethyl chloroacetate and 4-hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (**3**) in dimethylformamide using potassium carbonate base followed by the hydrolysis of ester using LiOH (**Scheme-6.3**). The versatile Suzuki reaction was utilized to synthesize 4-ethoxy-3-(1-methyl-1*H*-indol-5-yl)-8-trifluoromethyl-quinoline (**G_{90}**) in satisfactory good yields by reacting N-methylindole-5-boronic acid with 3-bromo-4-ethoxy-8-(trifluoromethyl)quinoline (**G_{89}**) in ethanol-toluene (1:1) mixture (**Scheme-6.4**). The crude products were purified by column chromatography. The reaction pathway has been summarized in **Scheme-6.1**, **Scheme-6.2**, **Scheme-6.3** and **Scheme-6.4**. Newly synthesized compounds were characterized by IR, NMR, mass spectral and C, H, N elemental analyses.

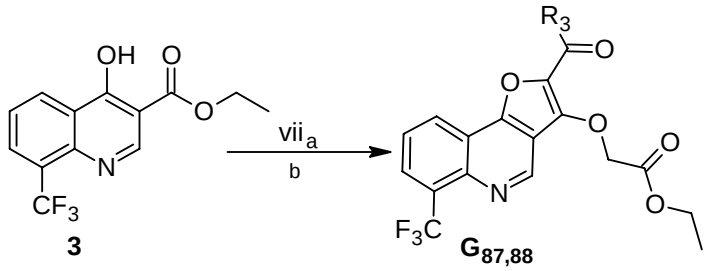


Scheme 6.1. **Synthetic route for 4-prop-2-ynyloxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (4):** (i) 110 °C, 6 h; (ii) Dowtherm, 250 °C, 5 h; (iii) Propargyl bromide, K_2CO_3, Acetone, 50 °C, 12 h.



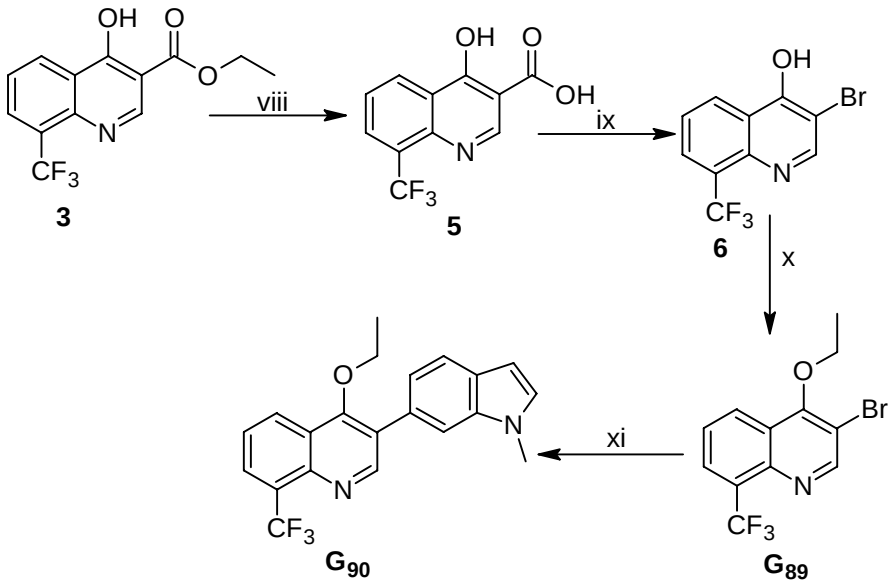
Where R = H, 4-NO_2, 2,4-Cl_2; R_1= F, Cl, Br, OCH_3; R_2= CH_2CH_3, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$.

Scheme 6.2. **Synthetic route for 1,2,3-triazol-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester derivatives (G_{77-79}, G_{80-83} and G_{84-86}):** (iv, v and vi) Alkyl bromide, NaN_3, aqueous PEG 400 (5 mL, 1:1, v/v), sodium ascorbate, 10 mol % of copper sulphate.



Where R_3= OCH_2CH_3, OH.

Scheme 6.3. **Synthetic route for 3-Ethoxycarbonylmethoxy-6-trifluoromethyl-furo[3,2-c]quinoline-2-carboxylic acid ethyl ester (G_{87}) and 3-Ethoxycarbonylmethoxy-6-trifluoromethyl-furo[3,2-c]quinoline-2-carboxylic acid (G_{88}):** (vii) a) Ethyl chloroacetate, K_2CO_3, DMF, 80 °C, 6 h; (vii) b) LiOH, MeOH, 2 h.



Scheme 6.4. **Synthetic route for 4-Ethoxy-3-(1-methyl-1H-indol-5-yl)-8-trifluoromethyl-quinoline (G_{90}):** (vii) LiOH, MeOH, 2 h; (ix) NBS, THF, RT, 12 h; (x) EtI, K_2CO_3, DMF, RT, 2 h; (xi) N-Methylindole-6-boronic acid, Toluene, EtOH (1:1), K_2CO_3, Palladium acetate, 80 °C, 2 h.

The formation of 4-prop-2-ynyloxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (**3**) was conformed by the peaks at 3261 cm^{-1}, 2120 cm^{-1} in IR spectrum which is due to the (C≡C-H-str) stretching of propargyl chain. Bands at 1700 cm^{-1} and 1103 cm^{-1} are due to C=O stretch of carboxylic ester and phenolic ether respectively. The ^1H NMR spectrum of **4** showed triplet and a quartet at δ 1.35, δ 4.38 corresponding to carboxylic ethyl ester. The acetylene CH proton appeared as singlet at δ 3.66. The CH_2 proton appeared as a singlet at δ 5.08. The triplet at δ 7.82 is due to 6^{th} proton of quinoline ring. Doublets at δ 8.28 and δ 8.55 are quinoline 5^{th} and 7^{th} protons respectively. The quinoline third proton appeared as a singlet at δ 9.24.

Table 6.1. Characterization data of the compounds (G_{77-79}, G_{80-83}, G_{84-86}, $G_{87,88}$, G_{89} and G_{90})

Compounds	$R/R_1/R_2/R_3$	Mol. Formula & Mol. Wt.	M.p.(°C)	Yield
G_{77}	H	$C_{23}H_{19}F_3N_4O_3$ 456.4	93-95	67
G_{78}	4-NO_2	$C_{23}H_{18}F_3N_5O_5$ 501.4	152-154	85
G_{79}	2,4-Cl_2	$C_{23}H_{17}Cl_2F_3N_4O_3$ 525.3	150-152	80
G_{80}	F	$C_{24}H_{18}F_4N_4O_4$ 502.4	125-127	79
G_{81}	Cl	$C_{24}H_{18}ClF_3N_4O_4$ 518.8	120-122	75
G_{82}	Br	$C_{24}H_{18}BrF_3N_4O_4$ 563.3	105-107	75
G_{83}	OCH_3	$C_{25}H_{21}F_3N_4O_5$ 514.4	134-136	62
G_{84}	CH_2CH_3	$C_{18}H_{17}F_3N_4O_3$ 394.3	100-102	68
G_{85}	$CH_2CH_2CH_3$	$C_{19}H_{19}F_3N_4O_3$ 408.3	71-73	65
G_{86}	$CH_2CH_2CH_2CH_3$	$C_{20}H_{21}F_3N_4O_3$ 422.4	63-65	60
G_{87}	OCH_2CH_3	$C_{19}H_{16}F_3NO_6$ 411.3	125-127	89
G_{88}	OH	$C_{17}H_{12}F_3NO_6$ 383.2	133-135	95
G_{89}	-	$C_{12}H_9BrF_3NO$ 320.1	106-108	96
G_{90}	-	$C_{21}H_{17}F_3N_2O$ 370.3	60-62	72

Formation of 4-[1-(2,4-dichloro-benzyl)-1H-[1,2,3]triazol-4-ylmethoxy]-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (**G_{79}**) was confirmed by the presence of absorption peak at 1690 cm^{-1} in IR spectrum which is due to (C=O) stretching of carboxylic ester. Band at 1218 cm^{-1} is due to N=N of the 1,2,3-triazole. The ^1H NMR spectrum of compound **G_{79}** showed triplet at δ 1.37 and quartet at δ 4.41 are due to carboxylic ethyl ester. CH_2 protons appeared as singlet at δ 5.45 and δ 5.66. A singlet at δ 8.23 is due to proton of 1,2,3-triazole moiety. All other aromatic protons appeared in δ 7.11 to δ 9.24 regions. The mass spectrum of **G_{79}** showed molecular ion peak at m/z = 626 (M+1), which is in agreement with the molecular formula $C_{23}H_{17}Cl_2F_3N_4O_3$. Three dimensional structures of **G_{79}, G_{82}** and **G_{87}** were evidenced by single crystal X-ray crystallographic study. Similarly the spectral values for all the compounds and C, H, N analyses are presented in the experimental part and the characterization data are provided in **Table 6.1.**

6.3. EXPERIMENTAL

6.3.1. Syntheses of diethyl 2-[(2-trifluoromethyl-phenylamino)-methylene]-malonic acid diethyl ester (2)

2-Trifluoromethyl-phenylamine **1** (10.0 g, 0.062 mol) and diethyl ethoxymethylene malonate (20.10 g, 0.093 mol) were heated to 110 °C for 6 h. The reaction mixture was cooled to room temperature, the solid thus formed was taken in pet ether and stirred for 20 minutes. Further it was filtered to get white solids.

6.3.2. Syntheses of 4-hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (3)

Diethyl 2-[(2-trifluoromethyl-phenylamino)-methylene]-malonic acid diethyl ester **2** (10.0 g, 0.030 mol) and dowtherm (100 mL) were heated to 250 °C for 5 h. The reaction mixture was then cooled to 25 °C and stirred in 150 mL hexane for 10 min. The solid product obtained was filtered and dried. The crude product obtained was purified by column chromatography using pet ether and ethyl acetate (5:5) as the eluent to get white solids.

6.3.3. Syntheses of 4-prop-2-ynyloxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (4)

A mixture of 4-hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (**3**) (5.0 g, 0.017 mol), potassium carbonate (2.66 g, 0.019 mol) and propargylbromide (2.02 g, 0.17 mol) in dry acetone (25 mL) were stirred at 50 °C for 12 h. The completion of reaction was monitored by TLC. After completion of reaction, reaction mixture was concentrated under vacuum and poured into ice-cold water. The solid product obtained was purified by column chromatography using pet ether and ethyl acetate as eluent to get white solids.

6.3.4. General procedure for the syntheses of 1,2,3-triazol-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester derivatives ($G_{77\text{-}79}$, $G_{80\text{-}83}$ and $G_{84\text{-}86}$)

To a stirred solution of alkylbromide (aromatic, aliphatic and phenacyl) (0.50 g, 0.0017 mol), sodium azide (0.117 g, 0.0018 mol) in aqueous PEG 400 (5 mL, 1:1, v/v), 4-Prop-2-ynyloxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (0.58 g, 0.0018 mol), sodium ascorbate (0.356 g ,0.0018 mol), 10 mol % of copper sulphate were added. The heterogeneous mixture was stirred vigorously overnight. Completion of the reaction was monitored by the TLC. The product was extracted in ethyl acetate and concentrated. The crude product was purified by column chromatography using pet ether and ethyl acetate as the eluent.

6.3.5. Synthesis of 3-ethoxycarbonylmethoxy-6-trifluoromethyl-furo[3,2-c]quinoline-2-carboxylic acid ethyl ester (G_{87})

To a suspension of 4-hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (2.0 g, 0.0070 mol), potassium carbonate (1.06 g, 0.0077 mol) in dimethylformamide (20 mL) was added ethyl 4-chloroacetoacetate (2.53 g, 0.0154 mol). The mixture was allowed to stir for 6 h at 80 °C and was quenched by the slow addition water (25 mL), The precipitated solids were collected by filtration and recrystallized from ethanol.

6.3.6. Synthesis of 3-Ethoxycarbonylmethoxy-6-trifluoromethyl-furo[3,2-c]quinoline-2-carboxylic acid (G_{88})

To a suspension of 3-Ethoxycarbonylmethoxy-6-trifluoromethyl-furo[3,2-c]quinoline-2-carboxylic acid ethyl ester (1.0 g, 0.0024 mol) in methanol (10 mL) at 0 °C was added lithium hydroxide (0.06 g, 0.0026 mol) for 5 min. The mixture was allowed to stir for 2 h and was quenched by the slow addition water (25 mL), acidified using dilute HCl. The precipitated solids were collected from filtration and recrystallized by ethanol.

6.3.7. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid (5)

To a suspension of 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (**3**) (5 g, 0.017 mol) in methanol (50 mL) at 0 °C was added lithium hydroxide (0.45 g, 0.019 mol) for 10 min. The mixture was allowed to stir for 2 h and was quenched by the slow addition water (100 mL), acidified using dilute HCl. The precipitated solids were collected by filtration and recrystallized by ethanol.

6.3.8. 3-Bromo-8-trifluoromethyl-quinolin-4-ol (6)

The 4-hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid (5.0 g, 0.019 mol) was taken up in THF (50 mL). NBS (3.73 g, 0.021 mol) was added, and the reaction mixture was allowed to stir at room temperature overnight. After completion of the reaction, the reaction mixture was concentrated under vacuum, poured into ice-cold water. The solid product obtained was filtered, washed with water and recrystallized with ethanol to get white solids.

6.3.9. 3-Bromo-4-ethoxy-8-trifluoromethyl-quinoline (G_{89})

The mixture of 3-bromo-8-trifluoromethyl-quinolin-4-ol (3.0 g, 0.010 mol) potassium carbonate (1.52 g, 0.011 mol) and ethyliodide (2.40 g, 0.015 mol) in dimethylformamide (30 mL) was stirred at room temperature for 2 h. After completion of reaction, the reaction mixture was poured into ice-cold water. The solid product obtained was filtered. The crude product obtained was purified by column chromatography using pet ether and ethyl acetate (9:1) as the eluent to get white solids.

6.3.10. General procedure for the syntheses of 4-ethoxy-3-(1-methyl-1H-indol-5-yl)-8-trifluoromethyl-quinoline (G_{90})

3-Bromo-4-ethoxy-8-(trifluoromethyl)quinoline (0.5 g, 0.0015 mol), N-methylindole-5-boronic acid (0.29 g, 0.0017 mol) were dissolved in toluene, ethanol (5:5, 10 mL). The solution was subsequently stirred for 10 min under nitrogen atmosphere. Potassium carbonate (0.234 g, 0.0017 mol) and palladium acetate (0.050 g, 0.00022 mol) were then added to the reaction mass and refluxed for 2 h under nitrogen atmosphere. After completion of the reaction, the reaction mixture was filtered through celite bed. The crude product obtained was purified by column chromatography using pet ether and ethyl acetate (9:1) as the eluent to get white solids.

6.4. CHARACTERIZATION OF SYNTHESIZED COMPOUNDS

6.4.1. Diethyl 2-[(2-Trifluoromethyl-phenylamino)-methylene]-malonic acid diethyl ester (2)

Yield: 19.1 g, 92.8%. M.p: 84-86 °C. IR (Neat ν_{max} cm^{-1}): 3278 (N-H), 3176, 2986 (C-H-str), 1706 and 1654 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.33 (t, 3H, CH_3, J = 5.3 Hz), 1.37 (t, 3H, CH_3, J = 5.3 Hz), 4.22 (q, 2H, CH_2), 4.30 (q, 2H, CH_2), 7.21-8.41 (m, 5H, ArH), 11.50 (s, 1H, NH); Anal. calcd. For $C_{15}H_{16}F_3NO_4$; Calcd: C, 54.38; H, 4.80; N, 4.23; found: C, 54.38; H, 4.80; N, 4.20 (Thomas et al. 2011).

6.4.2. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (3)

Yield: 8.1 g, 94.1%. M.p: 295-297 °C. IR (Neat ν_{max} cm^{-1}): 3352 (-OH), 3118, 2979 (C-H-str), 1708 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.23 (t. 3H, J = 8.0 Hz, -CH_3), 4.16 (q, 2H, -CH_2), 7.53 (t, 1H, ArH, J = 8.0 Hz), 8.07 (d, 1H, ArH, 7.8 Hz), 8.41 (d, 2H, ArH, J = 8.0 Hz), 11.62 (s, 1H, -OH, D_2O-exchangeble). ^{13}C NMR: δ ppm 14.70, 60.49, 111.39, 119.07, 124.61, 125.35, 130.99, 131.55, 146.43. MS: m/z = 286 (M+1). Anal. calcd. For $C_{13}H_{10}F_3NO_3$; Calcd: C, 54.74; H, 3.53; N, 4.91; found: C, 54.75; H, 3.54; N, 4.95.

6.4.3. 4-Prop-2-ynyloxy-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (4)

Yield: 5.25 g, 57.4%. M.p: 50-52 °C. IR (Neat ν_{max} cm^{-1}): 3261 (C≡C-H-str), 2990, 2948 (C-H-str), 2120 (C≡C), 1700 (C=O), 1103 (C-O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.35 (t, 3H, CH_3, J = 7.10 Hz), 3.66 (s, 1H, CH), 4.38 (q, 2H, CH_2, J = 14.22 Hz, J = 7.10 Hz), 5.08 (s, 2H, CH_2), 7.82 (t, 1H, ArH, J = 7.88 Hz), 8.28 (d, 1H, ArH, J = 7.20 Hz), 8.55 (d, 1H, ArH, J = 7.80), 9.24 (s, 1H, ArH). MS: m/z = 457 (M+1). Anal. calcd. For $C_{23}H_{19}F_3N_4O_3$; Calcd: C, 60.52; H, 4.20; N, 12.28; found: C, 60.53; H, 4.21; N, 12.27.

5.4.4. 4-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethoxy)-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (G_{77})

IR (Neat ν_{max} cm^{-1}): 3031, 2974 (C-H-str), 1724 (C=O), 1206 (N=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.36 (t, 3H, CH_3, J = 7.10 Hz), 4.40 (q, 2H, CH_2, J = 14.2 Hz, J = 7.12 Hz), 5.44 (s, 2H, CH_2), 5.58 (s, 2H, CH_2), 7.16-7.18 (m, 2H, ArH), 7.31-7.32 (m, 3H, ArH), 7.70 (t, 1H, ArH, J = 7.88 Hz), 8.25 (s, 1H, ArH), 8.27 (s, 1H, ArH), 8.35 (d, 1H, ArH, J = 8.24 Hz), 9.24 (s, 1H, ArH). MS: m/z = 457 (M+1). Anal. calcd. For $C_{23}H_{19}F_3N_4O_3$; Calcd: C, 60.52; H, 4.20; N, 12.28; found: C, 60.55; H, 4.21; N, 12.24.

6.4.5. 4-[1-(4-Nitro-benzyl)-1H-[1,2,3]triazol-4-ylmethoxy]-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (G_{78})

IR (Neat ν_{max} cm^{-1}): 3082, 2976 (C-H-str), 1705 (C=O), 1525 (NO_2), 1220 (N=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.37 (t, 3H, CH_3, J = 7.10 Hz), 4.41 (q, 2H, CH_2, J = 14.2 Hz, J = 7.10 Hz), 5.46 (s, 2H, CH_2), 5.76 (s, 2H, CH_2), 7.38 (d, 2H, ArH, J = 8.76 Hz), 7.72 (t, 1H, ArH, J = 7.88 Hz), 8.17 (d, 2H, ArH, J = 8.80 Hz), 8.24 (d, 1H, ArH, J = 7.48 Hz), 8.33 (s, 1H, ArH), 8.36 (d, 1H, ArH, J = 7.64 Hz), 9.24 (s, 1H, ArH); ^{13}C NMR: 14.44, 52.35, 62.17, 69.08, 116.17, 124.30, 124.71, 126.25, 126.76, 129.02, 129.30, 130.86, 142.66, 143.72, 146.85, 147.69, 153.13, 162.78, 164.44. MS: m/z = 502 (M+1). Anal. calcd. For $C_{23}H_{18}F_3N_5O_5$; Calcd: C, 55.09; H, 3.62; N, 13.97; found: C, 55.10; H, 3.60; N, 13.98.

6.4.6. 4-[1-(2,4-Dichloro-benzyl)-1H-[1,2,3]triazol-4-ylmethoxy]-8-trifluoro methyl-quinoline-3-carboxylic acid ethyl ester (G_{79})

IR (Neat ν_{max} cm^{-1}): 3078, 2977 (C-H-str), 1690 (C=O), 1218 (N=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.37 (t, 3H, CH_3, J = 7.10 Hz), 4.41 (q, 2H, CH_2, J = 14.2 Hz, J = 7.12 Hz), 5.45 (s, 2H, CH_2), 5.66 (s, 2H, CH_2), 7.11 (d, 1H, ArH, J = 8.32 Hz), 7.40 (dd, 1H, ArH, J = 8.32 Hz, J = 2.12 Hz), 7.65-7.72 (m, 2H, ArH), 8.23 (s, 1H, ArH), 8.25 (d, 1H, ArH, J = 7.16 Hz), 8.35 (d, 1H, ArH, J = 7.80 Hz), 9.24 (s, 1H, ArH); ^{13}C NMR: 14.43, 50.55, 62.16, 69.05, 116.28, 124.72, 126.22, 126.70, 128.27, 129.03, 129.61, 130.85, 132.19, 132.76, 134.16, 134.46, 142.39, 146.86, 153.13, 162.86, 164.54. MS: m/z = 526 (M+1). Anal. calcd. For $C_{23}H_{17}Cl_2F_3N_4O_3$; Calcd: C, 52.59; H, 3.26; N, 10.67; found: C, 52.58; H, 3.26; N, 10.66.

6.4.7. 4-{1-[2-(4-Fluoro-phenyl)-2-oxo-ethyl]-1H-[1,2,3]triazol-4-ylmethoxy}-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (G_{80})

IR (Neat ν_{max} cm^{-1}): 3069, 2976 (C-H-str), 1710, 1590 (C=O), 1222 (N=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.40 (t, 3H, CH_3, J = 7.10 Hz), 4.45 (q, 2H, CH_2, J = 14.22 Hz, J = 7.10 Hz), 5.49 (s, 2H, CH_2), 6.19 (s, 2H, CH_2), 7.44 (t, 2H, ArH, J = 8.84 Hz), 7.78 (t, 1H, ArH, J = 7.88 Hz), 8.13-8.16 (m,2H, ArH), 8.24 (s, 1H, ArH), 8.28 (d, 1H, ArH, J = 7.60 Hz), 8.43 (d, 1H, ArH, J = 7.76 Hz), 9.26 (s, 1H, ArH). MS: m/z = 503 (M+1). Anal. calcd. For $C_{24}H_{18}F_4N_4O_4$; Calcd: C, 57.37; H, 3.61; N, 11.15; found: C, 57.39; H, 3.62; N, 11.14.

6.4.8. 4-{1-[2-(4-Chloro-phenyl)-2-oxo-ethyl]-1H-[1,2,3]triazol-4-ylmethoxy}-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (G_{81})

IR (Neat ν_{max} cm^{-1}): 3040, 2990 (C-H-str), 1710, 1582 (C=O), 1212 (N=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.36 (t, 3H, CH_3, J = 8.00 Hz), 4.40 (q, 2H, CH_2, J = 12.00 Hz, J = 4.00 Hz), 5.44 (s, 2H, CH_2), 6.15 (s, 2H, CH_2), 7.63 (d, 2H, ArH, J = 8.00 Hz), 7.74 (t, 1H, ArH, J = 8.00 Hz), 8.02 (d, 2H, ArH, J = 8.00Hz), 8.21 (s, 1H, ArH), 8.24 (d, 1H, ArH, J = 8.00 Hz), 8.39 (d, 1H, ArH, J = 8.00 Hz), 9.22 (s, 1H, ArH); ^{13}C NMR: 14.48, 56.40, 62.22, 69.30, 115.98, 124.65, 126.85, 127.34, 129.06, 129.59, 130.58, 133.30, 139.62, 142.35, 146.92, 153.14, 163.03, 164.65, 191.66. MS:

m/z = 519 (M+1). Anal. calcd. For $C_{24}H_{18}ClF_3N_4O_4$; Calcd: C, 55.55; H, 3.50; N, 10.80; found: C, 55.58; H, 3.51; N, 10.75.

6.4.9. 4-{1-[2-(4-Bromo-phenyl)-2-oxo-ethyl]-1H-[1,2,3]triazol-4-ylmethoxy}-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (G_{82})

IR (Neat ν_{max} cm^{-1}): 3085, 2974 (C-H-str), 1706, 1582 (C=O), 1132 (N=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.36 (t, 3H, CH_3, J = 8.00 Hz), 4.40 (q, 2H, CH_2, J = 8.00 Hz), 5.44 (s, 2H, CH_2), 6.15 (s, 2H, CH_2), 7.74-7.79 (m, 3H, ArH), 7.93 (d, 2H, ArH, J = 8.00 Hz), 8.21 (s, 1H, ArH), 8.24 (d, 1H, ArH, J = 8.00 Hz), 8.39 (d, 1H, ArH, J = 8.00 Hz), 9.22 (s, 1H, ArH); ^{13}C NMR: 14.48, 56.38, 62.22, 69.30, 115.97, 124.65, 126.85, 127.33, 128.87, 129.06, 130.63, 130.95, 132.54, 133.62, 142.34, 146.93, 153.15, 163.04, 164.65. MS: m/z = 564 (M+1). Anal. calcd. For $C_{24}H_{18}BrF_3N_4O_4$; Calcd: C, 51.17; H, 3.22; N, 9.95; found: C, 51.17; H, 3.21; N, 9.94.

6.5.10. 4-{1-[2-(4-Methoxy-phenyl)-2-oxo-ethyl]-1H-[1,2,3]triazol-4-ylmethoxy}-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (G_{83})

IR (Neat ν_{max} cm^{-1}): 2979, 2937 (C-H-str), 1707, 1588 (C=O), 1231 (N=N), 1130 (C-O); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.36 (t, 3H, CH_3, J = 8.00 Hz), 4.00 (s, 3H, CH_3), 4.49 (q, 2H, CH_2, J = 12.00, J = 4.00 Hz), 65.44 (s, 2H, CH_2), 6.09 (s, 2H, CH_2), 7.06 (d, 2H, ArH, J = 8.00 Hz), 7.74 (t, 1H, ArH, J = 8.00 Hz), 7.99 (d, 2H, ArH, J = 8.00 Hz), 8.22 (s, 1H, ArH), 8.24 (d, 1H, ArH, J = 8.00 Hz), 8.39 (d, 1H, ArH, J = 8.00 Hz), 9.22 (s, 1H, ArH); ^{13}C NMR: 14.49, 56.04, 56.18, 62.23, 69.32, 114.71, 126.86, 127.39, 129.08, 131.06, 142.24, 153.15, 163.04, 164.41. MS: m/z = 515 (M+1). Anal. calcd. For $C_{25}H_{21}F_3N_4O_5$; Calcd: C, 58.37; H, 4.11; N, 10.89; found: C, 58.39; H, 4.21; N, 10.88.

6.4.11. 4-(1-Ethyl-1H-[1,2,3]triazol-4-ylmethoxy)-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (G_{84})

IR (Neat ν_{max} cm^{-1}): 2984, 2940 (C-H-str), 1699 (C=O), 1251 (N=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.33-1.41 (m, 6H, $2CH_3$), 4.31-4.45 (m, 4H, $2CH_2$), 5.39 (s, 2H, CH_2), 7.74 (t, 1H, ArH, J = 7.86 Hz), 8.23 (s, 1H, ArH), 8.24 (d, 1H, ArH, J = 7.44 Hz), 8.38 (d, 1H, ArH, J = 8.32), 9.21 (s, 1H, ArH); ^{13}C NMR: 14.44, 15.86, 45.08, 62.18, 69.30, 116.11, 124.62, 125.01, 126.78, 129.03, 130.86, 142.25, 146.89,

153.09, 162.89, 164.63. MS: m/z = 395 (M+1). Anal. calcd. For $C_{18}H_{17}F_3N_4O_3$; Calcd: C, 54.82; H, 4.35; N, 14.21; found: C, 54.82; H, 4.36; N, 14.20.

6.4.12. 4-(1-Propyl-1H-[1,2,3]triazol-4-ylmethoxy)-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (G_{85})

IR (Neat ν_{max} cm^{-1}): 2975, 2939 (C-H-str), 1704 (C=O), 1252 (N=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 0.71 (t, 3H, CH_3, J = 7.38 Hz), 1.38 (t, 3H, CH_3, J = 7.10 Hz), 1.70-1.79, (m, 2H, CH_2), 2.48-2.49 (m, 2H, CH_2), 4.42 (q, 2H, CH_2, J = 14.22 Hz, J = 7.10 Hz), 5.43 (s, 2H, CH_2), 7.75 (t, 1H, ArH, J = 7.86 Hz), 8.21 (s, 1H, ArH), 8.26 (d, 1H, ArH, J = 7.16 Hz), 8.38 (d, 1H, ArH, J = 7.76), 9.24 (s, 1H, ArH); $_{13}C$ NMR: 11.04,14.57, 23.57, 51.38, 62.11, 69.24, 116.14, 124.65, 125.52,126.75, 129.06, 130.91, 142.10, 146.82,153.11, 162.85, 164.54. MS: m/z = 409 (M+1). Anal. calcd. For $C_{19}H_{19}F_3N_4O_3$; Calcd: C, 55.88; H, 4.69; N, 13.72; found: C55.87; H, 4.69; N, 13.73.

6.4.13. 4-(1-Butyl-1H-[1,2,3]triazol-4-ylmethoxy)-8-trifluoromethyl-quinoline-3-carboxylic acid ethyl ester (G_{86})

IR (Neat ν_{max} cm^{-1}): 2964, 2930 (C-H-str), 1702 (C=O), 1254 (N=N); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 0.80 (t, 3H, CH_3, J = 7.36 Hz), 1.02-1.12 (m, 2H, CH_2), 1.38 (t, 3H, CH_3, J = 7.10 Hz), 1.65-1.72 (m, 2H, CH_2), 4.31 (t, 2H, CH_2, J = 6.94 Hz), 4.42 (q, 2H, CH_2, J = 14.22 Hz, J = 7.10 Hz), 5.43 (s, 2H, CH_2), 7.73 (t, 1H, ArH, J = 7.88 Hz), 8.19 (s, 1H, ArH), 8.25 (d, 1H, ArH, J = 7.08 Hz), 8.37 (d, 1H, ArH, J = 8.44 Hz), 9.24 (s, 1H, ArH). ^{13}C NMR: 13.65,14.43, 19.32, 31.99,49.50, 62.16, 69.21,116.14,123.00, 124.75, 125.49, 126.69, 129.06,130.88, 142.06, 146.87,153.10, 162.87, 164.57. MS: m/z = 423 (M+1). Anal. calcd. For $C_{20}H_{21}F_3N_4O_3$; Calcd: C, 56.87; H, 5.01; N, 13.26; found: C 56.88; H, 5.00; N, 13.24.

6.4.14. 3-Ethoxycarbonylmethoxy-6-trifluoromethyl-furo[3,2-c]quinoline-2-carboxylic acid ethyl ester (G_{87})

IR (Neat ν_{max} cm^{-1}): 2989, 2958 (C-H-str), 1748, 1711 (C=O), 1134 (C-O); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.14 (t, 3H, CH_3, J = 8.0 Hz), 1.32 (t, 3H, CH_3, J = 8.0), 4.12 (q, 2H, CH_2, J = 8.0 Hz), 4.30 (q, 2H, CH_2, J = 8.0 Hz), 4.98 (s, 2H, CH_2), 7.80 (t, 1H, ArH, J = 8.0 Hz), 8.26 (d, 1H, ArH, J = 8.0 Hz), 8.72 (d, 1H, ArH, J = 8.0

Hz), 9.19 (s, 1H, ArH); ^{13}C NMR: 14.42, 61.46, 62.23, 70.68, 71.95, 114.71, 116.11, 124.23, 126.76, 127.80, 129.43, 131.06, 132.08, 146.79, 153.14, 162.94, 164.37, 168.47, 168.86. MS: m/z = 412 (M+1). Anal. calcd. For $C_{19}H_{16}F_3NO_6$; Calcd: C, 55.48; H, 3.92; N, 3.41; found: C 55.51; H, 3.92; N, 3.40.

6.4.15. 3-Ethoxycarbonylmethoxy-6-trifluoromethyl-furo[3,2-c]quinoline-2-carboxylic acid (G_{88})

IR (Neat ν_{max} cm^{-1}): 3236 (-OH), 3095, 2981 (C-H-str), 1736 (C=O), 1130 (C-O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.17 (t, 3H, CH_3, J = 8.0 Hz), 4.10 (q, 2H, CH_2, J = 8.0 Hz), 5.02 (s, 2H, CH_2), 7.54 (t, 1H, ArH, J = 8.0 Hz), 8.09 (d, 1H, ArH, J = 8.0 Hz), 8.43 (d, 1H, ArH, J = 8.0 Hz), 8.49 (s, 1H, ArH), 11.72 (s, 1H, OH, D_2O-exchangeble). MS: m/z = 384 (M+1). Anal. calcd. For $C_{17}H_{12}F_3NO_6$; Calcd: C, 53.27; H, 3.16; N, 3.65; found: C 53.26; H, 3.19; N, 3.65.

6.4.16. 4-Hydroxy-8-trifluoromethyl-quinoline-3-carboxylic acid (5)

Yield: 4.3 g, 95.5%. M.p: 258-260 °C. IR (Neat ν_{max} cm^{-1}): 3440 (-OH), 3113, 3061 (C-H-str), 1720 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.54 (t, 1H, ArH, J = 8.0 Hz), 8.05 (d, 1H, ArH, 7.8 Hz), 8.44 (d, 2H, ArH, J = 8.0 Hz), 11.61 (s, 1H, -OH, D_2O-exchangeble), 14. 43 (s, 1H, OH). Anal. calcd. For $C_{11}H_6F_3NO_3$; Calcd: C, 51.37; H, 2.35; N, 5.45; found: C, 51.39; H, 2.33; N, 5.46 (Snyder et al. 1947).

6.4.17. 3-Bromo-8-trifluoromethyl-quinolin-4-ol (6)

IR (Neat ν_{max} cm^{-1}): 3165, 3015 (C-H-str), 1607 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.28 (t, 1H, ArH, J = 8.00 Hz), 7.86 (d, 1H, ArH, J = 8.00 Hz), 8.19 (s, 1H, ArH), 8.61 (d, 1H, ArH, J = 8.00 Hz), 11.73 (s, 1H, OH, D_2O-exchangeble); ^{13}C NMR: 105.83, 122.67, 125.38, 130.94, 131.54, 135.49, 141.38, 171.46, 194.17. MS: m/z = 293 (M+1). Anal. calcd. For $C_{10}H_5BrF_3NO$; Calcd: C, 41.13; H, 1.73; N, 4.80; found: C 41.13; H, 1.71; N, 4.82.

6.4.18. 3-Bromo-4-ethoxy-8-trifluoromethyl-quinoline (G_{89})

IR (Neat ν_{max} cm^{-1}): 3015, 2954 (C-H-str), 1573 (C=C), 1128 (C-O); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.50 (t, 3H, CH_3, J = 7.0 Hz), 4.33 (q, 2H, CH_2, J = 7.0 Hz, J = 13.8 Hz), 7.82 (t, 1H, ArH, J = 7.8 Hz), 8.25 (d, 1H, ArH, J = 7.2 Hz), 8.45 (d, 1H, J

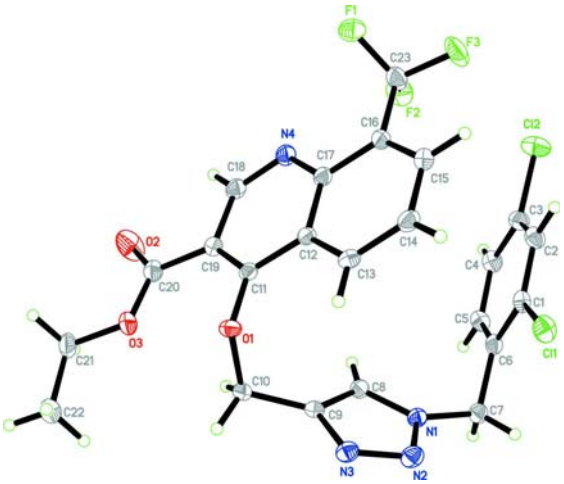
= 8.8 Hz), 9.14 (s, 1H, ArH). MS: m/z = 321 (M+1). Anal. calcd. For $C_{12}H_9BrF_3NO$; Calcd: C, 45.03; H, 2.83; N, 4.38; found: C 45.05; H, 2.83; N, 4.37.3.5.19.

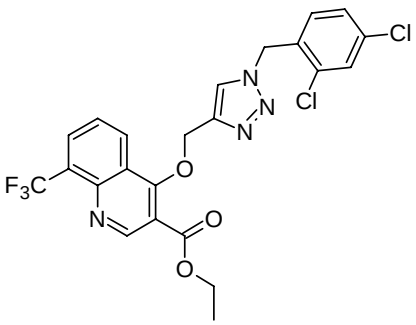
6.4.19. 4-Ethoxy-3-(1-methyl-1H-indol-5-yl)-8-trifluoromethyl-quinoline (G_{90})

IR (Neat ν_{max} cm^{-1}): 2975, 2942 (C-H-str); ^1H NMR (400 MHz, DMSO-d_6): δ ppm 1.13 (t, 3H, CH_3, J = 7.00 Hz), 3.73 (q, 2H, CH_2, J = 13.74 Hz, J = 7.00 Hz), 3.85 (s, 3H, CH_3), 6.52 (d, 1H, ArH, J = 2.96 Hz), 7.40 (d, 1H, ArH, J = 3.04 Hz), 7.46 (dd, 1H, ArH, J = 8.48 Hz, J = 1.44 Hz), 7.58 (d, 1H, ArH, J = 8.52 Hz), 7.76 (t, 1H, ArH, J = 7.84 Hz), 7.86 (s, 1H ArH), 8.16 (d, 1H, ArH, J = 7.2 Hz), 8.55 (d, 1H, ArH, J = 8.24 Hz), 9.02 (s, 1H, ArH). MS: m/z = 371 (M+1). Anal. calcd. For $C_{21}H_{17}F_3N_2O$; Calcd: C, 68.10; H, 4.63; N, 7.56; found: C 68.10; H, 4.61; N, 7.57.

6.5. ANALYTICAL DATA

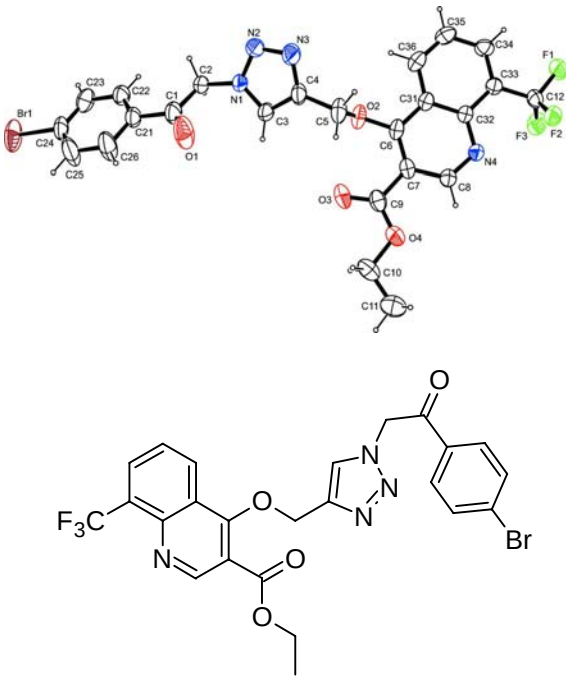
Crystal data of G_{79}	
Empirical formula	$C_{23}H_{17}Cl_2F_3N_4O_3$
Formula weight	525.31
Crystal system	Monoclinic
Crystal dimension	0.32 mm x 0.31 mm x 0.17 mm
Space group	P21/c
a(Å)	10.0414 (6)
b(Å)	18.3997 (11)
c(Å)	15.5456 (7)
Volume ($Å^3$)	2246.0 (2)
Angle α, β, γ	90, 128.559, 90
Z	4
F_{000}	1072
μ (mm^{-1})	0.35
Temperature (T)	100 K
Radiation wavelength (Å)	0.71073
Radiation type	Mo Kα
Radiation source	fine-focus sealed tube
Radiation monochomator	graphite





continued

Crystal data of G_{82}	
Empirical formula	$C_{24}H_{18}BrF_3N_4O_4$
Formu weight	563.33
Crystal system	Monoclinic
Crystal dimension	0.58 mm x 0.16 mm x 0.07 mm
Space group	P21/c
a(Å)	5.2809 (2)
b(Å)	24.5131 (10)
c(Å)	18.3517 (7)
Volume (Å^3)	2342.08 (16)
Angle α, β, γ	90, 99.643, 90
Z	4
μ (mm^{-1})	1.82
Temp rature (T)	200 K
Radiation wavelength (Å)	0.71073



Crystal data of G_{87}	
Empirical formula	$C_{19}H_{16}F_3NO_6$
Formula weight	411.33
Crystal system	Triclinic
Crystal d mension	0.56 mm x 0.38 mm x 0.15 mm
Space group	P$\bar{1}$
a(Å)	8.9167 (3)
b(Å)	8.9223 (3)
c(Å)	13.4125 (5)
Volume (Å^3)	904.22 (5)
Angle α, β, γ	102.895, 97.098, 16.035
Z	2
μ (mm^{-1})	0.13
Temperature (T)	200 K
Radiation wavelength (Å)	0.71073

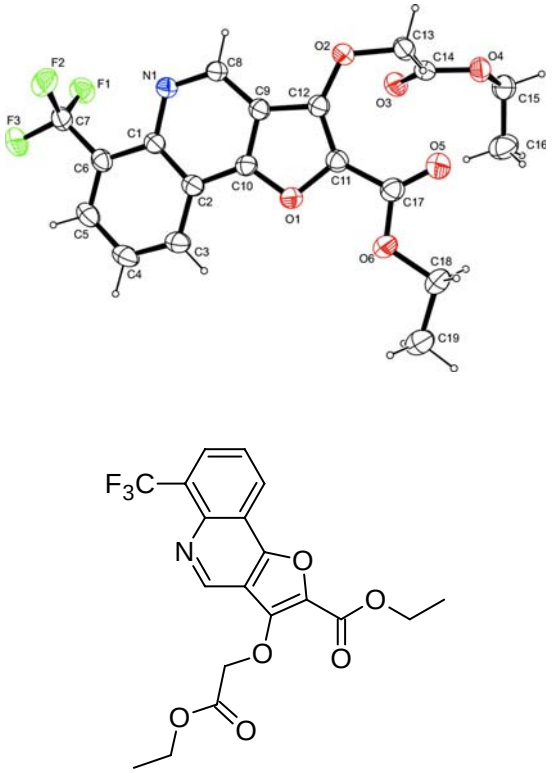


Figure 6.3. ORTEP diagrams showing the single crystal structures of compounds G_{79}, G_{82} and G_{87} (drawn at 50% probability level).

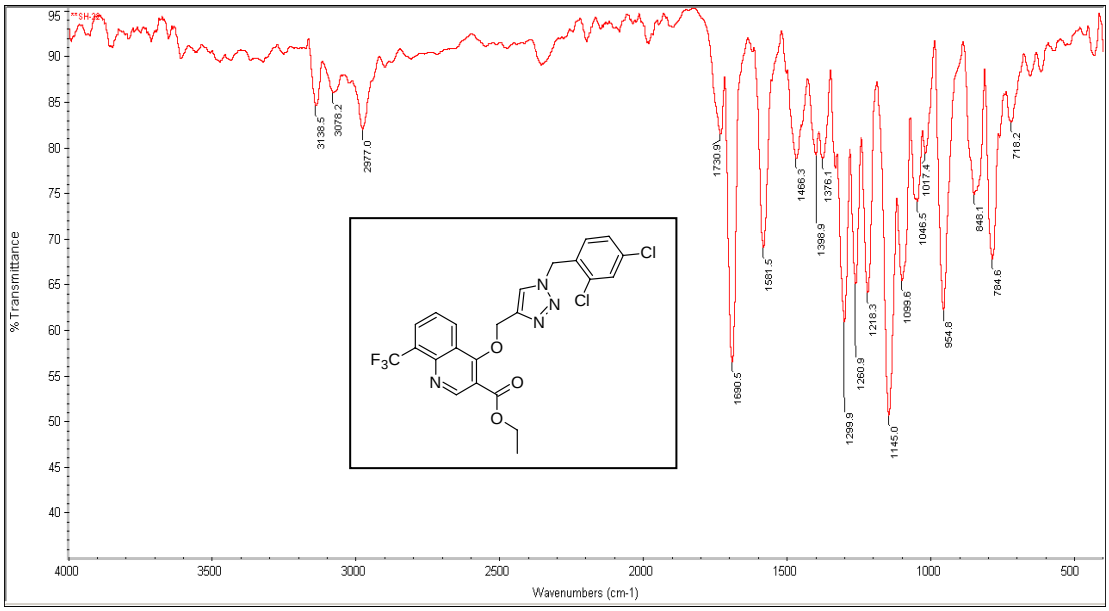


Figure 6.4. IR spectrum of G_{79}.

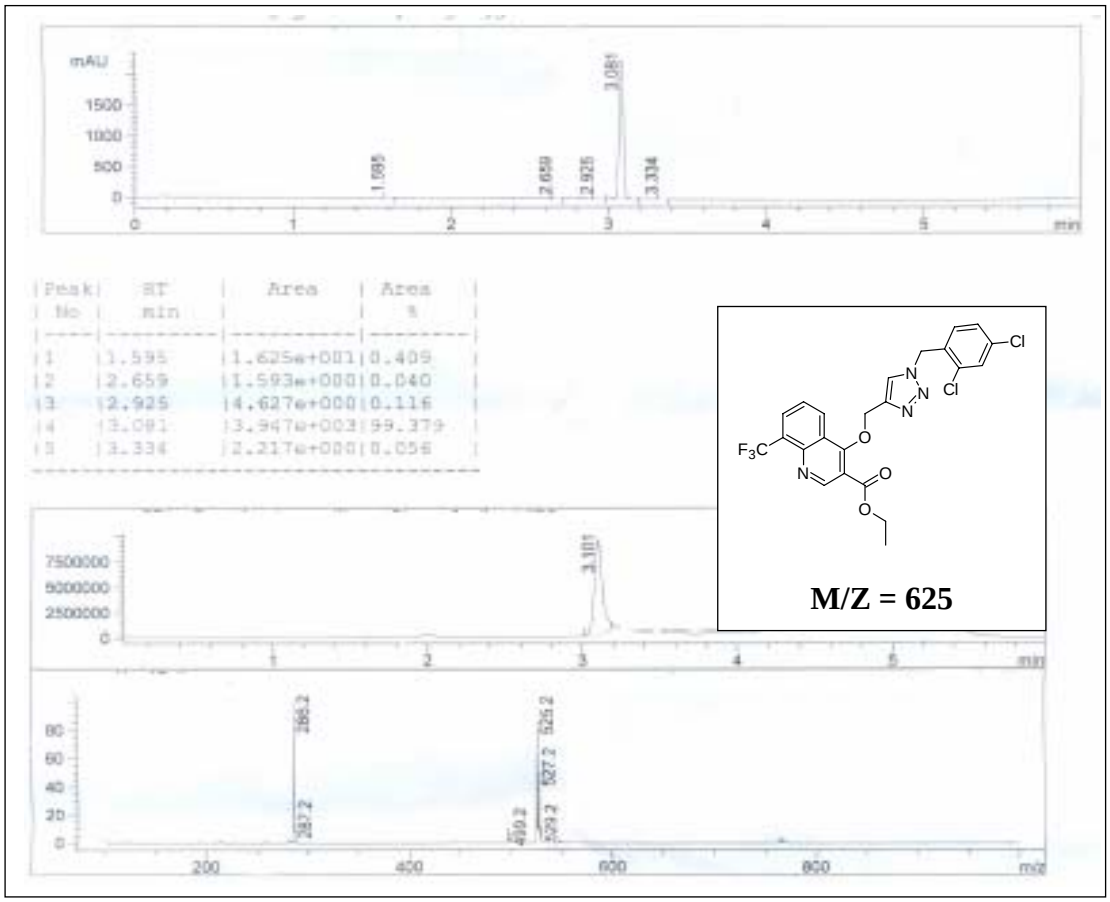


Figure 6.5. LCMS spectrum of G_{79}.

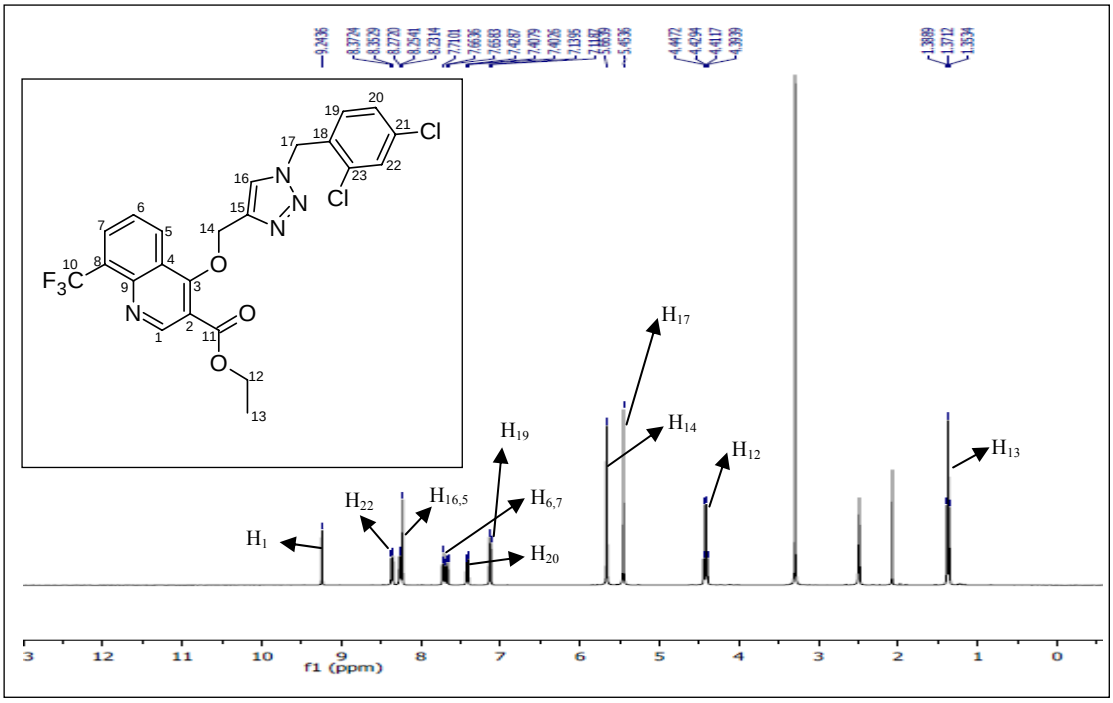


Figure 6.6. ^{1}H NMR spectrum of G_{79}.

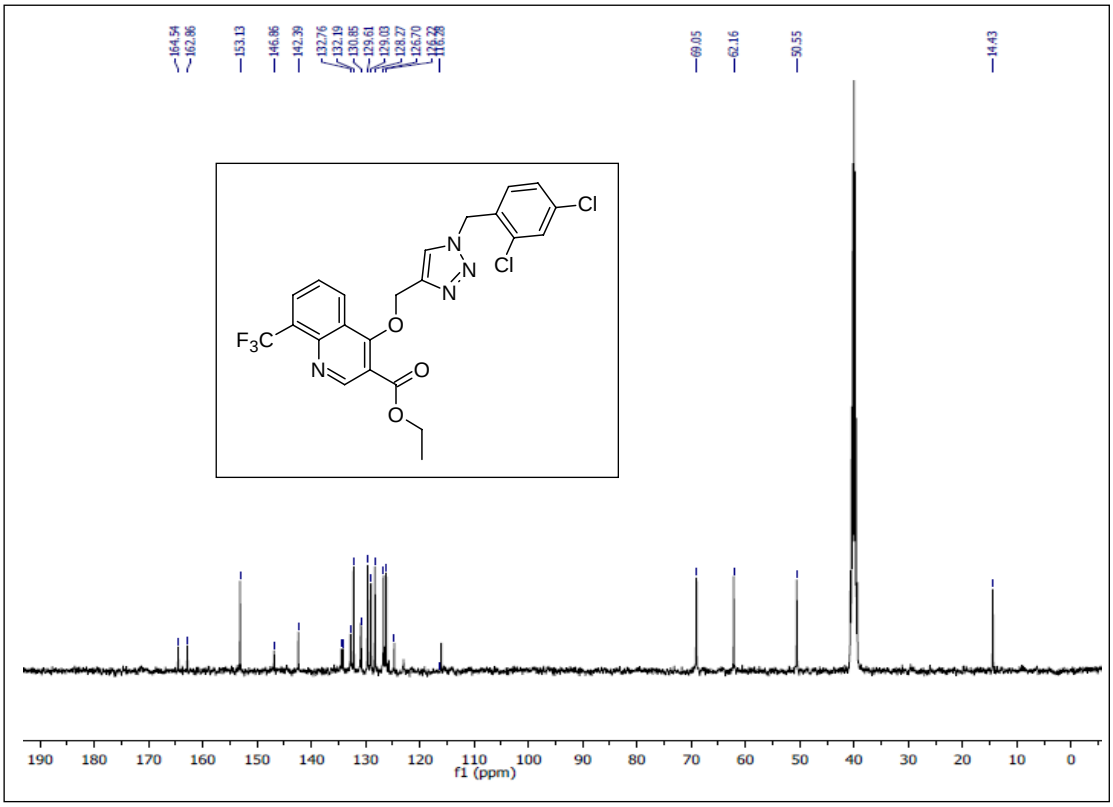


Figure 6.7. ^{13}C NMR spectrum of G_{79}.

6.6. CONCLUSION

Three series of 8-trifluoromethylquinoline based 1,2,3-triazoles derivatives **(G_{77-79}, G_{80-83}, G_{84-86}, $G_{87,88}$, G_{89}** and **G_{90})** were synthesized by multi-step reactions. Compounds with electron withdrawing group in 1,2,3-triazole ring are showed better yield compared with electron donating group. The synthesized compounds were characterized by spectral studies, X-ray analysis. The *in-vitro* antimicrobial activities of the final compounds were performed and the results were discussed in CHAPTER-7.

CHAPTER-7

ANTIMICROBIAL STUDIES

CHAPTER-7

ANTIMICROBIAL STUDIES

7.1. INTRODUCTION

Antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body. The discovery of antimicrobials like Penicillin and Tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short dosage of antimicrobials.

There are mainly two classes of antimicrobial

1. Those obtained from natural sources:
    a) Beta-lactam antibiotic (such as Penicillins, Cephalosporins)
    b) Protein synthesis inhibitors (such as Aminoglycosides, Macrolides, Tetracyclines, Chloramphenicol, Polypeptides)
2. Synthetic agents:
    a) Anti-virals
    b) Anti-fungals
    c) Anti-cancer
    d) Anti-malarials
    e) Anti-tuberculosis
    f) Anti-protozoals

7.1.1. Antimicrobial mechanism of action

Resistance to antibacterial can evolve rapidly, even during a course of treatment. Numerous pathogens, including *Staphylococcus aureus*, *Enterococci,* and *Streptococcus pyogenes* now exhibit resistance worldwide. There are three known mechanisms of resistance. Some types of efflux pumps can act to decrease intracellular quinolone concentration. In Gram-negative bacteria, plasmid-mediated resistance genes produce proteins that can bind to DNA gyrase, protecting it from the action of quinolones. Finally, mutations at key sites in DNA gyrase or topoisomerase IV can decrease their binding affinity to quinolones, thereby decreasing the drug's effectiveness.

The antimicrobial agents function by attacking various cellular targets which include cell wall, plasma membrane, nucleic acids and proteins synthesis of the microbe. The precise mechanisms of the action of antimicrobial drugs are still not clear, but the following possible views were proposed for their mode of action.

- **Inhibition of cell wall synthesis:** β-Lactam antibiotics are bacteriocidal and act by inhibiting the synthesis of the peptidoglycon layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity, especially in gram-positive organisms, being the outermost and primary component of the wall. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidase known as Penicillin-binding proteins (PBPs). PBPs vary in their affinity for binding Penicillin or other β-lactam antibiotics. The amount of PBPs varies among bacterial species.
- **Injury to the plasma membrane:** This is a mode of action for certain antibacterials and antifungals. Antifungals are able to work mostly against fungus cell membranes because they contain ergosterol instead of cholesterol. However, these antimicrobials are potentially toxic to the host. The examples include Polymixins (antibacterial), and Amphotericin B, Miconazole, and Ketoconazole (antifungals).

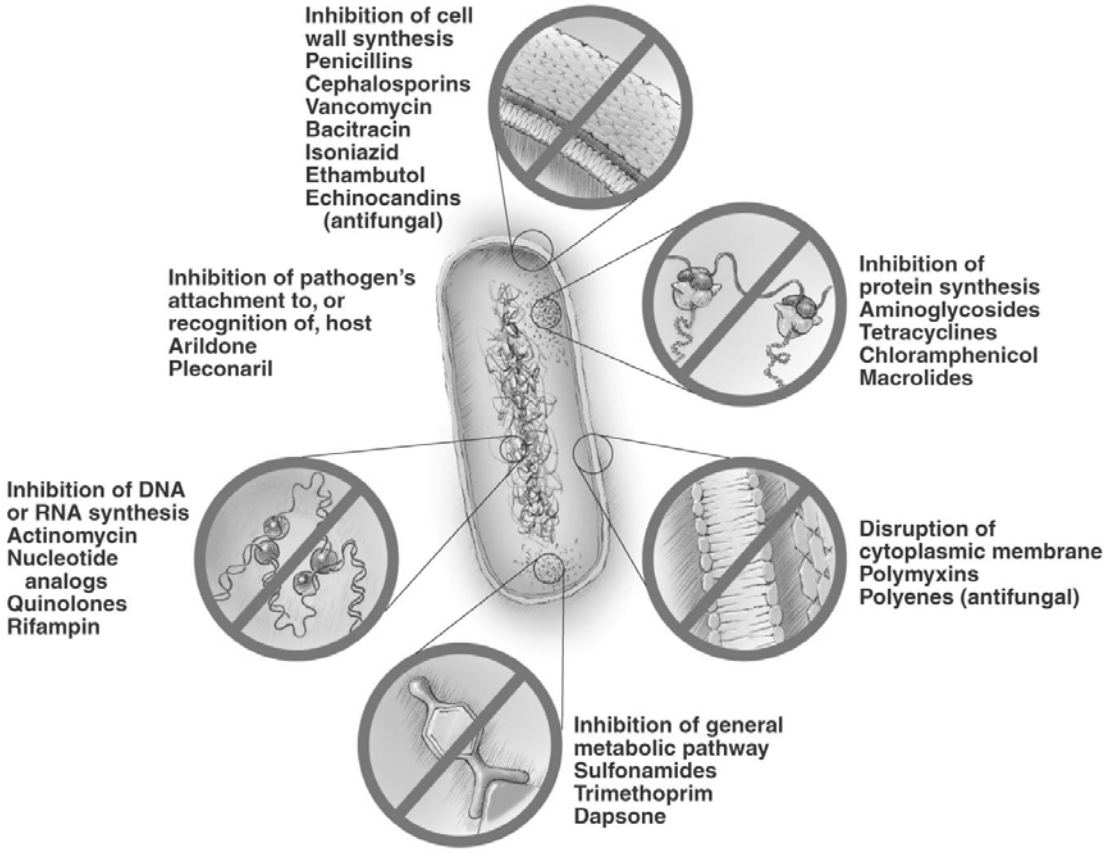


Figure 7.1. Schematic representation of mechanism of action on bacterial cell

(Copied from: thttp://www2.bakersfieldcollege.edu/bio16/10_Antimicrobials.htm)

- **Inhibition of the synthesis of essential metabolites:** Generally sulpha drugs and trimethoprim functions by this mode of action. They interfere with the pathway on which bacteria synthesize folic acid. Since humans produce folic acid by a different pathway, these drugs have less effect on human cells.
- **Inhibition of protein synthesis:** A protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins. Protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins, like initiation, elongation and termination.
- **Inhibition of nucleic acid synthesis:** Antibacterial that acts by inhibiting the production of nucleic acids. There are two major classes, DNA inhibitors and RNA inhibitors. DNA inhibitors such as the quinolines, acting upon DNA gyrase,

RNA inhibitors such as rifampin, acting up on RNA-dependent RNA polymerase. Schematic representation of the mechanism of action of antimicrobial agents is given below (Figure 7.1).

7.1.2. Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

Diffusion	Dilution	Diffusion & Dilution
Stokes method Kirby-Bauer method	Minimum inhibitory concentration a) Broth dilution b) Agar Dilution	E-Test method

7.1.2.1. Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures.

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers. It promotes accurate Antimicrobial susceptibility Testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective.

Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with minimum inhibitory concentration (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.

7.1.2.2. Dilution Methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in $\log_2$ serial dilutions (two fold).

Minimum Inhibitory Concentration (MIC)

Diffusion tests widely used to determine the susceptibility of organisms isolated from clinical specimens have their limitations, when equivocal results are obtained or in prolonged serious infection e.g. bacterial endocarditis, the quantitation of antibiotic action of the pathogen needs to be more precise. Also the terms 'Susceptible' and 'Resistant' can have a realistic interpretation. Thus when in doubt, the way to a precise assessment is to determine the MIC of the antibiotic to the organisms concerned.

There are two methods of testing for MIC:

a) Broth dilution method

b) Agar dilution method.

a) Broth Dilution Method

The broth dilution method is a simple procedure for testing a small number of isolates, even single isolate. It has the added advantage that the same tubes can be taken for Minimum Bactericidal Concentrations (MBC) tests also.

b) Agar dilution Method

Agar dilutions are most often prepared in petri dishes and have advantage that it is possible to test several organisms on single plate. If only one organism is to be tested e.g *Mycobacterium tuberculosis*, the dilutions can be prepared in agar slopes but it will then be necessary to prepare a second identical set to be inoculated with the control organism. The dilutions are made in a small volume of water and added to agar which has been melted and cooled to not more than 60 ^{o}C. Blood may be added and if 'chocolate agar' is required, the medium must be heated before the antibiotic is added.

7.1.2.3. Dilution and Diffusion

'E' test also known as the epsilometer test, is an exponential gradient testing methodology where 'E' in E test refers to the Greek symbol epsilon (ε). The 'E' test which is a quantitative method for antimicrobial susceptibility testing applies both the dilution of antibiotic and diffusion of antibiotic into the medium. A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this 'E' test strip is applied onto an inoculated agar plate, there is an immediate release of the drug. Following incubation, a symmetrical inhibition ellipse is produced. The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy.

'E' test can be used to determine MIC for fastidious organisms like *Streptococcus pneumoniae*, *ß-hemolytic streptococci*, *Neisseria gonorrhoeae*, *Haemophilus* sp. and anaerobes. It can also be used for Nonfermenting Gram Negative bacilli (NFGNB) for eg-*Pseudomonas* sp. and *Burkholderia pseudomallei*.

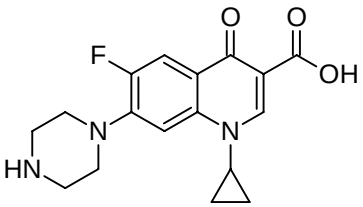
Resistance of major consequence may be detected for e.g., the test is very useful in detecting glycopeptide resistant Enterococci (GRE) and glycopeptide intermediate *Staphylococcus aureus* (GISA) and slow growing pathogens such as *Mycobacterium tuberculosis*. Further it can be used for detection of extended spectrum beta lactamases (ESBL). In conclusion ´E´ test is a simple, accurate and reliable method to determine the MIC for a wide spectrum of infectious agents.

7.2. MATERIAL AND METHODS

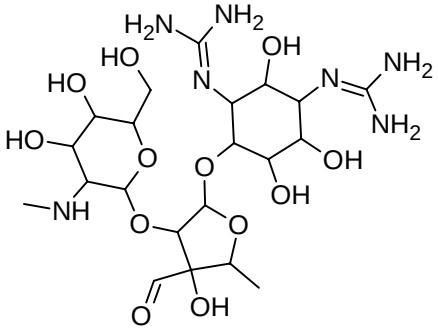
7.2.1. Antibacterial activity

The antibacterial activities of newly synthesized compounds were determined by well plate method in nutrient agar media (Rocha et al. 1995; Arthington-Skaggs et al. 2000). *In-vitro* antibacterial activity of compounds against 24 h old bacterial culture was performed. 15-20 milliliters of nutrient agar media were poured into each petri dish and agar was allowed to solidify by placing inside the laminar air flow for 15 min. 100 µL of 0.5 McFarland standard of bacterial suspension was inoculated by swabbing aseptically on the whole surface of the agar medium with a sterile cotton bud. Using a sterile cork borer, five mm wells were made on the seeded agar plates

and 50 µL of test compound was transferred into the wells. The plates were prepared in triplicate and incubated at 30 °C to 37 °C for 12 h and observed in the zone of inhibition. Standard and working solution of test compounds were prepared by dissolving in dimethylsulfoxide (DMSO). A minimum inhibition concentration (MIC) required for the inhibition was determined using the test compound concentration ranging from 5-100 µg/mL and found out that, 50-100 µg/mL concentration of compound is required. All further antimicrobial activity was determined using 50 µg/mL and 100 µg/mL concentration of test compounds. Antimicrobial activities were determined by measuring the diameter of inhibition zone in millimeter (mm). Ciprofloxacin and Streptomycin were used as antibacterial standards.



Ciprofloxacin

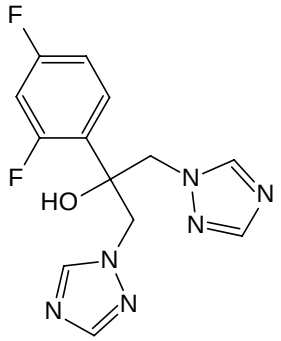


Streptomycin

7.2.2. Antifungal activity

Antifungal studies of synthesized compounds were carried out against different fungal strains. Sabourands agar media was prepared by dissolving peptone (10 g), D-glucose (40 g) and agar (20 g) in distilled water (1000 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37 °C for 1 h. Using a puncher, wells were made on the seeded agar plates. The different concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The petri dishes were prepared in triplicates and maintained at

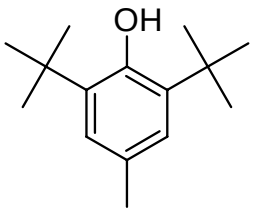
25 °C for 72 h. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with Flucanazole as standard. Zones of inhibition (mm) were determined for all final compounds.



Fluconazole

7.2.3. Antioxidant Activity

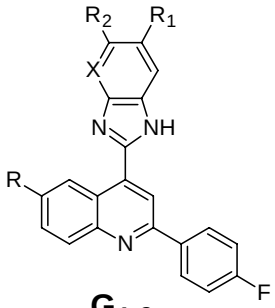
The synthesized compounds were tested for 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity with Butylated hydroxytoluene (BHT) as standard according to the previously reported procedure (Brand-Williamsgive et al. 1995). The following method is based on the reduction of the free radical DPPH by free radical scavengers. The procedure involves the measurement of the decrease in absorbance of DPPH at 517 nm, which is proportional to the activity of free radical scavengers added to the DPPH reagent solution. A stock solution of test compounds (1 mg/mL) and DPPH (0.004 %) was prepared in 95:5 methanol: water. To 3 mL of freshly prepared DPPH solution in a test tube, was added to the stock solution of test compound (100 µg) and reacted for 10 minutes and was measured at 517 nm using UV-visible spectrophotometer. BHT was used as the reference standard and dissolved in distilled water to make the stock solution with the same concentration (1 mg/mL).



Butylated hydroxytoluene (BHT)

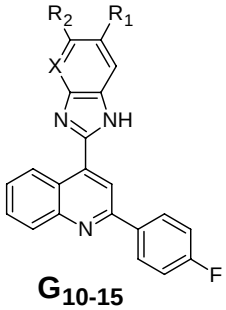
7.3. RESULT AND DISCUSSION

7.3.1. Antimicrobial activity of quinoline incorporated benzimidazole derivatives



G_{1-9}

Where R= H, Cl, F; R_1= Cl, F; R_2= H, Cl; X= CH, N.



G_{10-15}

Where R_1= H, Cl, F; R_2=,H Cl; X= CH, N.

In this series 15 compounds were synthesized. The newly synthesized compounds (**G_{1-9}** and **G_{10-15}**) were screened for their *in-vitro* antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Xanthomonas* and *Salmonella* using Ciprofloxacin as standard by well plate method (zone of inhibition) ((Arthington-Skaggs et al. 2000; Rocha et al. 1995). The test compounds were dissolved in dimethylsulfoxide (DMSO) at concentrations of 6.25 and 12.5 µg/mL.

The antibacterial screening revealed that, few of the tested compounds showed good inhibition against various tested microbial strains compared to the standard drug. Among the synthesized compounds, **G_3** and **G_4** showed significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Compound **G_3** is found to be more potent against *Staphylococcus aureus*. Compounds **G_4** and **G_{13}** exhibited excellent antibacterial activity against *Salmonella* and *Xanthomonas* respectively. Remaining compounds showed moderately better antibacterial activity against all the tested bacterial strains than compared to standard drug Ciprofloxacin. Compounds **G_3** and **G_{12}** contain fused pyridine ring in benzimidazole moiety and a 4-fluorophenyl group on the second position of quinoline ring which has accounted for the enhanced activity. Compounds **G_4** and **G_{13}** contain two chlorine atoms on benzimidazole ring and 4-fluorophenyl group on the second position of quinoline ring which are responsible for the moderately good inhibition. Results of antibacterial studies have been presented in **Table-7.1**.

Table-7.1. Antibacterial activity of the compounds G_{1-9} and G_{10-15}

Compound No.	Zone of inhibition in mm (mean ± S.D.) n=3							
	Staphylococcus aureus		***Escherichia coli***		***Xanthomonas***		***Salmonella***	
Concn. (µg/mL)	6.25	12.5	6.25	12.5	6.25	12.5	6.25	12.5
Standard Ciprofloxacin	18±0.87	20±0.50	19±1.00	21±0.50	18±0.50	22±0.50	19±0.50	31±0.50
Control	-	-	-	-	-	-	-	-
G_1	12±0.50	13±1.00	01±0.50	13±0.50	01±0.50	10±0.50	01±0.50	9±0.50
G_2	16±1.00	16±0.50	12±0.50	12±0.50	01±0.50	10±0.50	10±0.50	13±0.50
G_3	14±0.50	18±1.00	16±0.50	16±0.50	15±1.00	16±0.50	9±0.50	20±0.50
G_4	10±0.50	12±0.50	12±0.50	17±0.50	14±0.50	16±0.50	20±0.50	28±0.50
G_5	14±1.00	15±0.50	10±0.50	15±0.50	14±0.50	14±0.50	15±0.50	15±0.50
G_6	12±0.50	12±0.50	10±0.50	11±0.50	11±0.50	12±0.50	01±0.50	11±0.50
G_7	12±0.50	12±1.00	01±0.50	13±0.50	15±0.50	16±0.50	13±0.50	13±0.50
G_8	14±0.50	15±0.50	01±0.50	12±0.50	12±0.50	16±0.50	9±0.50	12±0.50
G_9	12±0.50	12±0.50	16±0.50	16±0.50	01±0.50	14±0.50	9±0.50	13±0.50
G_{10}	12±1.00	13±1.00	11±1.00	16±0.50	11±0.50	12±0.50	9±0.50	15±0.50
G_{11}	12±1.00	13±0.50	01±0.50	16±0.87	15±0.50	15±0.50	9±0.50	17±0.50
G_{12}	16±1.00	16±1.00	14±0.50	14±0.50	10±0.50	14±0.50	9±0.50	12±0.50
G_{13}	12±0.50	16±0.50	12±0.50	16±0.50	20±1.00	22±0.50	11±0.50	12±0.50
G_{14}	10±1.00	16±0.50	10±0.50	11±0.50	01±0.50	13±0.50	10±0.50	11±0.50
G_{15}	12±0.50	12±0.50	12±0.50	14±0.87	12±0.50	14±0.50	10±1.00	11±0.50

-; Not detected inhibition.

Table-7.2. Antifungal activity of the compounds G_{1-9} and G_{10-15}

	Zone of inhibition in mm (mean ± S.D.) n=3							
Compound No.	***Aspergillus niger***		***Aspergillus flavus***		***Penicillium***		***Aspergillus terrus***	
Concn. (µg/mL)	6.25	12.5	6.25	12.5	6.25	12.5	6.25	12.5
Standard Fluconazole	14±0.50	18±1.00	15±0.50	20±0.50	19±0.50	21±0.50	16±0.50	18±0.50
Control	-	-	-	-	-	-	-	-
G_1	10±1.00	12±0.50	01±0.50	01±0.50	16±0.50	18±0.50	13±0.50	14±0.50
G_2	01±1.00	10±0.50	01±0.50	17±1.00	12±1.00	17±0.50	9±0.50	9±1.00
G_3	12±0.50	14±0.50	01±0.50	10±0.50	01±0.50	13±1.00	9±0.50	15±0.50
G_4	12±1.00	12±1.00	13±0.50	13±0.50	12±0.50	19±0.50	9±0.50	10±0.50
G_5	10±0.50	12±1.00	01±0.50	10±0.50	12±0.50	12±1.00	9±0.50	9±1.00
G_6	01±0.50	10±0.50	01±0.50	10±0.50	15±1.00	17±0.50	10±0.87	14±0.50
G_7	11±1.00	13±0.50	11±0.50	14±0.50	14±0.87	15±0.50	10±0.50	15±0.50
G_8	10±1.00	13±0.50	12±1.00	15±0.50	01±1.00	12±0.50	01±0.50	09±0.50
G_9	10±0.50	17±0.50	01±0.50	01±0.50	13±0.50	13±1.00	10±0.50	9±0.50
G_{10}	11±1.00	12±1.00	11±1.00	12±0.50	15±0.87	17±0.50	12±0.50	14±0.50
G_{11}	15±1.00	18±1.00	11±0.50	16±0.50	12±0.50	12±0.50	10±0.87	16±1.00
G_{12}	14±1.00	16±1.00	01±0.50	11±0.50	16±0.50	17±0.50	9±0.50	10±0.50
G_{13}	10±0.50	12±0.50	10±1.00	10±0.50	12±0.50	12±1.00	10±0.50	11±0.50
G_{14}	01±0.50	14±1.00	01±0.50	14±0.50	12±0.50	15±0.50	12±0.50	12±0.50
G_{15}	10±1.00	12±0.50	01±0.50	10±1.00	16±0.50	18±1.00	12±0.50	13±0.50

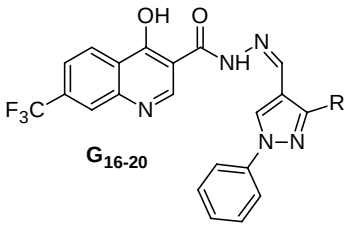
-, Not detected inhibition.

The *in-vitro* antifungal activities of newly synthesized compounds (**G_{1-9}** and **G_{10-15}**) were determined by well plate method (MacLowry et al. 1970; Portillo et al. 2001). In this work *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terrus* and

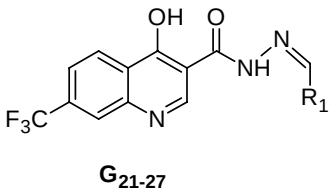
Penicillium were used to investigate the activity. The test compounds were dissolved in dimethylsulfoxide (DMSO) at concentrations of 6.25 and 12.5 µg/mL.

The result indicated that, among the tested compounds, **G_{11}** showed significant antifungal activity against *Aspergillus niger* and *Aspergillus flavus* compared to standard drug Fluconazole. Compounds **G_1**, **G_{10}** and **G_{15}** were active against *Aspergillus terrus* and *Penicillium* fungal strains respectively. The enhanced activity of the compound **G_{11}** may be due to the presence of a chlorine atom on benzimidazole ring and 4-fluorophenyl group on second position of quinoline ring. All other compounds showed less inhibition against all the tested micro organisms as compared to the standard drug. **Table-7.2** depicts the antifungal screening results of the final compounds.

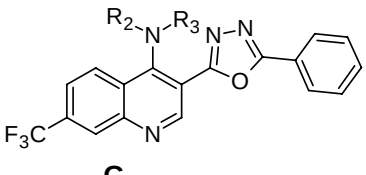
7.3.2. Antimicrobial activity of trifluoromethylquinoline-3-carbohydrazide and 1,3,4-oxadiazole derivatives



$G_{16\text{-}20}$



$G_{21\text{-}27}$



$G_{28\text{-}33}$

Where R= Phenyl, 4-MethoxyPhenyl, 4-Chlorophenyl, 4-Nitrophenyl, 4-Methylphenyl; R_1=4-methoxy benzaldehyde, 3,4-Dimethoxybenzaldehyde, thiophene-2-carbaldehyde, 4-N-imethylbenzaldehyde, 3-ethoxy-2-hydroxyBenzaldehyde, 4-N-Diethyl-2-hyd roxybenzaldehyde, 6-bromopyridine-3-carbaldehyde; R_2/R_3= N-Methylpiperidine, Morpholine, Ethanolamine, O-Acetylethanolamine, 3,4,5-Trimethoxyaniline.

Antibacterial studies of newly synthesized compounds were carried out against two different pathogenic microorganisms. They are Gram-positive *Mycobacterium smegmatis (MTCC 943)* and Gram-negative *Pseudomonas aeroginosa (MTCC4676)*, by well plate method in nutrient agar media (Rocha et al. 1995; Arthington-Skaggs et al. 2000). Antifungal activity was carried out on the fungus *Candida albicans (MTCC 183)* and *Penicillium chrysogenum (MTCC 6795)*

(Mac-Lowry et al. 1970; Portillo et al. 2001). Standard and working solution of test compounds were prepared by dissolving in dimethylsulfoxide (DMSO). A minimum inhibition concentration (MIC) required for the inhibition of pathogenic organisms were determined using the test compound concentration ranging from 5-50 μg/mL. All further antimicrobial activity was determined using 25 μg/mL and 50 μg/mL concentration of test compounds. Antimicrobial activities were determined by measuring the diameter of inhibition zone in millimeter. Ciprofloxacin was the standard drug for antibacterial studies, while Fluconazole was used as a standard for the antifungal studies.

The compounds, **G_{21}** and **G_{30}** are showing significant antibacterial activity against all the tested microorganisms such as Gram-positive and Gram-negative bacteria, non-filamentous and filamentous fungi. The compounds **G_{18}, G_{19}, G_{20}, G_{22}, G_{23}, G_{26}, G_{28}, G_{29}** and **G_{31}** are exhibited excellent antimicrobial activity but these are not able to inhibit the filamentous fungi *Penicillium chrysogenum*. Among all the compounds, **G_{19}** is inhibiting *Mycobacterium smegmatis* to the maximum of 16 mm diameter. The Gram-negative bacteria *Pseudomonas aeroginosa* growth was inhibited to the maximum extent of 19 mm by the compound **G_{20}**. Seven of these compounds inhibited the filamentous fungi *Penicillium chrysogenum* even at 25 μg/mL concentration. All target compounds are inhibiting *Mycobacterium smegmatis* at least at concentration of 50 μg/mL. Except **G_{17}, G_{25}, G_{32}** and **G_{33}** all compounds inhibited Gram-negative bacteria *P. aeroginosa*. Results of antimicrobial studies have been presented in **Table-7.3** and **Table-7.4**.

Table-7.3. Antibacterial activity of the compounds G_{16-20}, G_{21-27} and G_{28-33}

Zone of inhibition in mm (mean ± S.D.) n=3				
Compound No.	***Mycobacterium smegmatis***		***Pseudomonas aeroginosa***	
Concn. (µg/mL)	25	50	25	50
Standard AB	26.33±0.58	28.67±1.15	25.67±0.58	26.67±0.58
Control	-	-	-	-
G_{16}	7.33±0.58	13.67±0.58	6.67±0.58	7.00±0.00
G_{17}	8.33±0.58	13.00±1.00	-	-
G_{18}	8.33±0.58	8.33±0.58	-	7.00±0.00
G_{19}	-	15.33±0.58	9.33±0.58	9.67±1.15
G_{20}	7.17±0.29	9.33±0.58	17.00±0.00	18.33±0.58
G_{21}	-	15.00±0.00	8.00±0.00	8.33±0.58
G_{22}	8.33±0.58	11.33±0.58	11.00±0.00	11.33±0.58
G_{23}	10.67±0.58	10.67±0.58	6.33±0.58	7.00±0.00
G_{24}	7.00±0.00	7.83±0.29	-	6.83±0.29
G_{25}	7.00±0.00	10.67±0.58	-	-
G_{26}	7.17±0.29	14.67±0.58	-	8.33±0.58
G_{27}	7.83±0.29	9.67±0.58	13.67±0.58	13.67±0.58
G_{28}	8.00±0.00	9.33±0.58	9.33±0.58	11.33±0.58
G_{29}	10.67±0.58	10.67±0.58	7.00±0.00	8.67±0.58
G_{30}	12.67±0.58	12.67±0.58	10.00±0.00	10.33±0.58
G_{31}	7.00±0.00	10.33±0.58	10.67±0.58	11.00±0.00
G_{32}	8.67±0.58	8.67±0.58	-	-
G_{33}	8.67±0.58	8.67±0.29	-	-

AB; antibacterial standard Ciprofloxacin, -; not detected inhibition

Control; dimethylsulfoxide

Table-7.4. Antifungal activity of the compounds G_{16-20}, G_{21-27} and G_{28-33}

Zone of inhibition in mm (mean ± S.D.) n=3				
Compound No.	***Candida albicans***		***Penicillium chrysogenum***	
Concn. (µg/mL)	25	50	25	50
Standard AF	20.00±0.00	26.33±0.58	17.00±1.00	20.00±0.00
Control	-	-	-	-
G_{16}	-	-	-	-
G_{17}	-	-	7.00±0.00	8.00±0.00
G_{18}	8.00±0.00	10.00±0.00	-	-
G_{19}	-	7.83±0.29	-	-
G_{20}	8.50±0.50	10.00±0.00	-	-
G_{21}	8.33±0.58	10.00±0.00	7.17±0.29	8.67±0.58
G_{22}	8.00±0.00	10.00±0.00	-	-
G_{23}	7.83±0.29	9.33±0.58	-	-
G_{24}	-	-	7.17±0.29	8.00±0.00
G_{25}	-	-	12.67±0.58	12.67±0.58
G_{26}	7.17±0.29	8.00±0.00	-	-
G_{27}	7.83±0.29	9.67±0.58	13.67±0.58	13.67±0.58
G_{28}	8.00±0.00	9.33±0.58	9.33±0.58	11.33±0.58
G_{29}	10.67±0.58	10.67±0.58	7.00±0.00	8.67±0.58
G_{30}	12.67±0.58	12.67±0.58	10.00±0.00	10.33±0.58
G_{31}	7.00±0.00	10.33±0.58	10.67±0.58	11.00±0.00
G_{32}	8.67±0.58	8.67±0.58	-	-
G_{33}	8.67±0.58	8.67±0.29	-	-

AF; antifungal standard Fluconazole, -; not detected inhibition
Control; dimethylsulfoxide

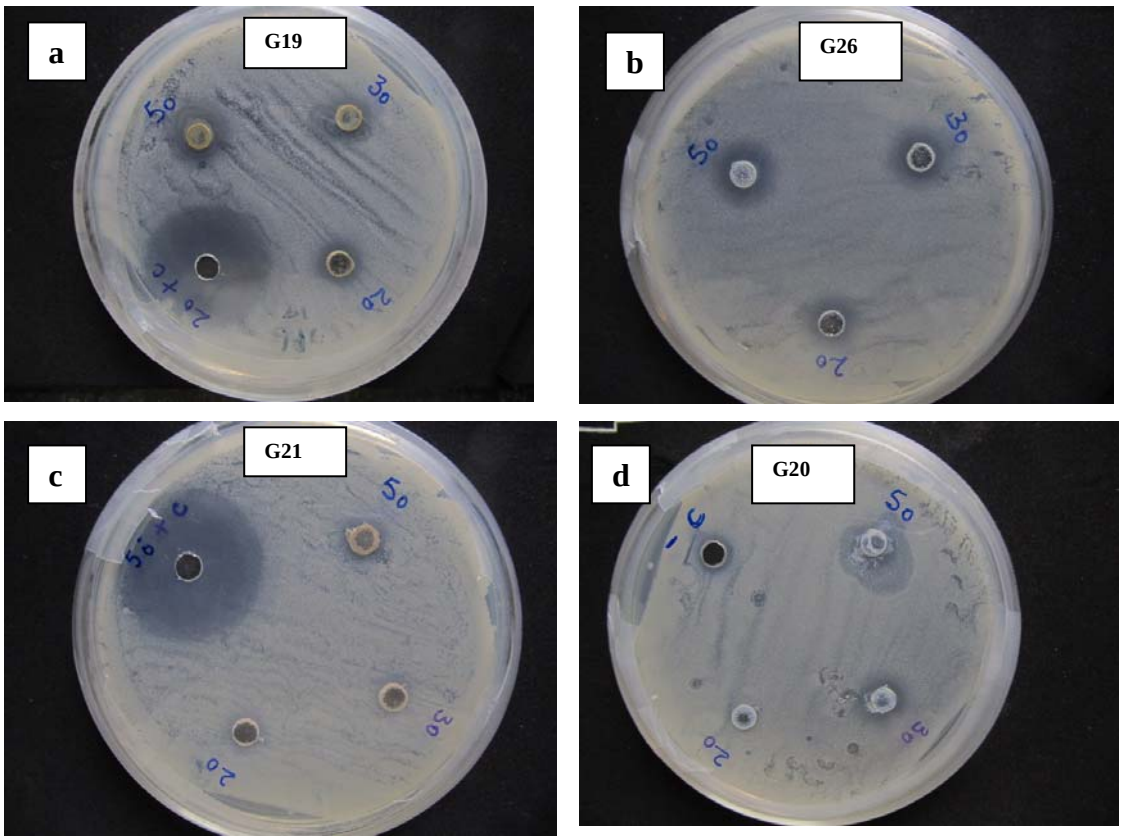
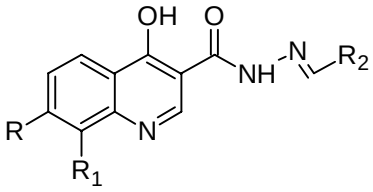


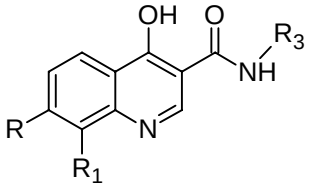
Figure 7.2. Antimicrobial activity image of compounds.

a, b, c = *Mycobacterium smegmatis*. d = *Pseudomonas aeroginosa*.

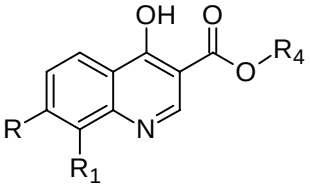
7.3.3. Antimicrobial, antioxidant activity of 7 and 8-trifluoromethylquinoline derivatives



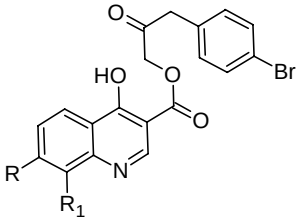
$G_{34\text{-}41}$



$G_{42\text{-}43}$



$G_{44\text{-}45}$



G_{46}

Where R/R_1 =H, CF_3; R_2= Pyridine-4-carbaldehyde, 1H-Indole-3-carbaldehyde, 2-Chloro-quinoline-3-carbaldehyde, 4-Methyl-benzaldehyde, Furan-2-carbaldehyde; R_3= 2-Amino-ethanol; R_4= 2-Amino-ethanol.

The antibacterial activity of the synthesized compounds was done using *Bacillus subtilis* MTCC 441, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. The antifungal activity of the synthesized compounds was done using *Aspergillus flavus* MTCC 3306, *Candida albicans* MTCC 3017 and *Microsporum gypseum* MTCC 3752.

The antimicrobial screening revealed that, among the synthesized compounds, **G_{34}** and **G_{36}** are showed excellent antimicrobial activity. The enhanced activity is due to presence of heterocyclichydrazone (pyridine and quinoline) moieties at third position, hydroxyl at fourth and trifluoromethyl group at seventh position of quinoline. The compounds **G_{38}, G_{39}, G_{40}, G_{42}, G_{45}, G_{46}** and **G_{38}, G_{39}, G_{40}, G_{42}, G_{45}** showed good antibacterial and antifungal activity respectively. The compounds **G_{38}, G_{39}, G_{40}** contains indole, quinoline, furan hydrazones of 4-hydroxy-8-trifluoromethylquinoline at third position which has accounted for the enhanced activity. The compounds containing ethanolamide, aminoester and phenacylester derivatives, at position third of 4-hydroxy(-trifluoromethy)quinoline accounted for the enhanced activity of **G_{42}, G_{45}** and **G_{46}**. Compound **G_{36}** showed minimum inhibitory

concentration (MIC) of 25 µg/mL against *Bacillus subtilis* and 50 µg/mL against *Escherichia coli.* The compound **G_{34}** exhibited MIC of 50 µg/mL against *Bacillus subtilis*, 100 µg/mL *Escherichia coli* and *Pseudomonas aeroginosa*. The compound **G_{46}** showed significant antioxidant activity compared to standard. This is due to 4-bromophenacylester at third position of 4-hydroxy(-8-trifluoromethy)quinoline. Except **G_{39}**, **G_{41}** all other targeted molecules exhibited good antioxidant activity. Results of antimicrobial studies and antioxidant activity have been presented in **Table-7.5, Table-7.6, Table-7.7** and **Table-7.8**.

Table-7.5. Minimum Inhibitory concentration(MIC µg/mL)

Compound	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
G_{34}	100	50	100
G_{36}	50	25	100
G_{38}	250	500	500
G_{39}	250	250	100
G_{40}	500	500	750
G_{41}	500	250	250
G_{43}	500	750	750
G_{45}	750	500	750
G_{46}	500	500	750

Table-7.6. Antibacterial activity

Compound	*Escherichia coli*		*Bacillus subtilis*		*Pseudomonas aeroginosa*	
Concentration In mg/mL	0.5	1	0.5	1	0.5	1
Streptomycin	14±0.2	16±0.3	18±0.1	21±0.2	12±0.3	15±0.2
Control	00	00	00	00	00	00
G_{34}	07±0.2	10±0.2	11±0.2	13±0.3	11±0.3	11±0.3
G_{35}	-	-	-	-	-	-
G_{36}	09±0.2	11±0.2	14±0.2	16±0.3	09±0.1	12±0.3
G_{37}	-	-	-	-	02±0.1	04±0.1
G_{38}	05±0.2	07±0.1	03±0.2	05±0.3	04±0.1	06±0.2
G_{39}	06±0.2	07±0.1	04±0.1	07±0.3	05±0.1	08±0.2
G_{40}	03±0.2	05±0.1	03±0.1	07±0.2	01±0.1	05±0.2
G_{41}	-	-	-	-	-	-
G_{42}	04±0.2	06±0.3	05±0.2	07±0.2	06±0.1	08±0.2
G_{43}	05±0.3	06±0.2	03±0.3	05±0.2	03±0.1	05±0.2
G_{44}	-	-	-	-	-	-
G_{45}	02±0.2	05±0.3	05±0.1	07±0.2	02±0.3	04±0.2
G_{46}	04±0.2	06±0.3	06±0.2	06±0.3	02±0.2	04±0.3

-; not detected inhibition

Table-7.7. Antifungal Activity

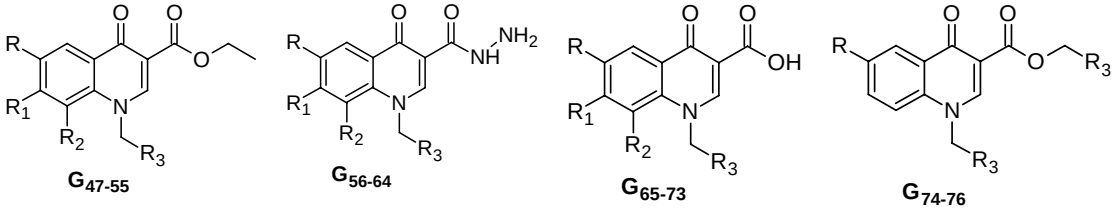
Compound	*Aspergillus flavus*		*Candida albicans*		*Microsporum gypseum*	
Concentration In mg/mL	0.5	1	0.5	1	0.5	1
Flucanazole	10±0.3	13±0.1	13±0.2	16±0.1	15±0.1	17±0.2
Control	00	00	00	00	00	00
G_{34}	04±0.2	06±0.3	06±0.2	08±0.2	02±0.2	04±0.3
G_{35}	-	-	-	-	-	-
G_{36}	05±0.2	07±0.1	03±0.2	05±0.3	04±0.1	06±0.2
G_{37}	-	-	-	-	-	-
G_{38}	03±0.1	05±0.2	03±0.3	05±0.2	03±0.1	07±0.2
G_{39}	02±0.1	04±0.2	02±0.2	05±0.1	05±0.3	06±0.2
G_{40}	03±0.2	05±0.1	04±0.1	07±0.3	01±0.1	05±0.2
G_{41}	-	-	-	-	-	-
G_{42}	04±0.2	06±0.3	05±0.2	07±0.2	06±0.1	08±0.2
G_{43}	-	-	-	-	-	-
G_{44}	-	-	-	-	-	-
G_{45}	02±0.2	04±0.3	05±0.1	07±0.2	06±0.2	07±0.1
G_{46}	-	-	-	-	-	-

-; not detected inhibition

Table-7.8. Antioxidant activity

Compound	% of Inhibition
G_{34}	24.6
G_{35}	22.3
G_{36}	21.1
G_{37}	35.4
G_{38}	37.6
G_{39}	00
G_{40}	23.1
G_{41}	00
G_{42}	29.5
G_{43}	25.3
G_{44}	30.1
G_{45}	36.4
G_{46}	60.5
BHT	90.3

7.3.4. Antimicrobial activity of trifluoromethylquinolone derivatives



Where $R/R_1/R_2$ = H, CF_3; R_3= Acetylene, 2,4-dichlorophenyl, 4-cyanophenyl.

Antibacterial studies of newly synthesized compounds were carried out against two different pathogenic microorganisms by well plate method in nutrient agar media. They are Gram-positive *Mycobacterium smegmatis (MTCC 943), Bacillus subtilis* and Gram-negative *Pseudomonas aeroginosa (MTCC 4673), Salmonella typhimurium (MTCC 3732), Escherichia coli, Pseudomonas aeruginosa.* Antifungal activity was carried out on the fungus *Candida albicans (MTCC 183)* and *Penicillium chrysogenum (MTCC 6795)*.

The antimicrobial screening revealed that, few of the tested compounds showed good inhibition against various tested microbial strains compared to the standard drugs. Among the synthesized compounds, **G_{50}, G_{59}, G_{68}, G_{74}, G_{75}** and **G_{76}** are showed excellent antibacterial activity. The compounds **G_{48}, G_{50}, G_{58}, G_{59}, G_{60}, G_{61}** and **G_{66}** exhibited good activity against gram-positive bacteria *Mycobacterium smegmatis.* Compounds **G_{50}, G_{52}, G_{57}** and **G_{59}** are exhibited excellent activity against gram-negative bacteria *Pseudomonas aeroginosa and Salmonella typhimurium.* The compounds **G_{62}, G_{64}, G_{71}** and **G_{73}** showed moderate antibacterial activity. The compounds **G_{50}, G_{60}, G_{62}, G_{64}, G_{66}, G_{67}** and **G_{68}** showed activity against *Candida albicans* fungal strain but all the compounds failed to show activity against *Penicillium chrysogenum*. The Compounds containing trifluoromethyl group at sixth, seventh and eighth position, ethylester, hydrazide pharmacophore at third position and N-alkylatedquinolone core moiety may be the region for the enhanced antibacterial activity. Antifungal activity of the compounds (**G_{60}, G_{62}, G_{64}, G_{66}, G_{67}** and **G_{68}**) may be due to acid, hydrazide active part at third position and trifluoroquinolone. Results of antimicrobial studies have been presented in **Table 7.9** and **Table 7.10**.

Table 7.9. Antimicrobial activity of the compounds (G_{47-52}, G_{56-61} and G_{65-70})

Compd No.	Zone of inhibition in mm (mean ± S.D.) n=3							
	M.smegmatis		***P.aeruginosa***		***S.typhimurium***		***C.albicans***	
Concn. (µg/mL)	50	100	50	100	50	100	50	100
Stanrd AB/AF	29.0±0	30.0±0.00	26.6±0.57	30.0±0.00	37.0±0.00	37.0±0.00	26.33±0.47	29.0±0.00
Control	-	-	-	-	-	-	-	-
G_{47}	-	-	-	-	-	-	-	-
G_{48}	7.0±0.00	8.0±0.00	7.0±0.00	7.0±0.00	-	-	-	-
G_{49}	-	-	-	-	-	-	-	-
G_{50}	-	9.0±0.00	7.0±0.00	8.0±0.58	10.33±0.58	11.0±0.00	-	8.33±0.00
G_{51}	-	-	-	-	-	-	-	-
G_{52}	7.0±0.00	-	8.0±0.00	8±0.00	7.3±0.57	8.0±0.00	-	-
G_{56}	-	-	-	-	-	-	-	-
G_{57}	-	-	8.0±0.00	8.0±0.00	11.3±0.57	12.0±0.00	-	-
G_{58}	7.3±0.57	8.0±0.00	8.0±0.00	8.0±0.00	-	-	-	-
G_{59}	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00	7.6±0.57	8.0±0.00	-	-
G_{60}	9.0±0.00	9.0±0.00	8.0±0.00	8.0±0.58	-	-	-	7.0±0.00
G_{61}	8.0±0.00	8.0±0.00	8.0±0.00	8±0.00	-	-	-	-
G_{65}	-	-	-	-	-	-	-	-
G_{66}	11.0±0.00	12.0±0.00	13.6±0.57	14.0±0.58	-	-	7.0±0.00	9.0±0.00
G_{67}	-	-	8.0±0.00	8.0±0.00	-	-	-	8.0±0.00
G_{68}	-	7.0±0.00	-	8.0±0.00	8.0±0.00	8.0±0.00	12.0±0.00	12.0±0.00
G_{69}	-	-	-	-	-	-	7.0±0.00	7.0±0.00
G_{70}	-	-	8.0±0.00	8±0.00	-	-	7.6±0.47	8.33±0.00

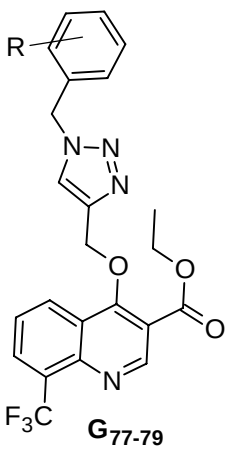
AB/AF; anti-bacterial standard Ciprofloxacin / Antifungal standard Fluconazole.
-; not detected inhibition

Table 7.10. Antibacterial activity of the compounds (G_{53-55}, G_{62-64}, G_{71-73} and G_{74-76}).

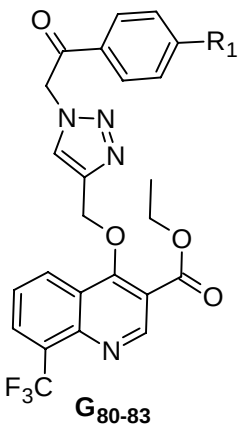
Compound No.	Zone of inhibition in mm (mean ± S.D.) n=3					
	Escherichia coli		*Bacillus subtilis*		*Pseudomonas aeruginosa*	
Concn. (mg/mL)	0.5	1.0	0.5	1.0	0.5	1.0
Standard Streptomycin	17±0.6	19±0.3	19±0.2	22±0.4	15±0.3	19±0.4
Control	-	-	-	-	-	-
G_{53}	-	-	-	-	-	-
G_{54}	-	-	-	-	-	-
G_{55}	-	-	-	-	-	-
G_{62}	-	03±0.5	02±0.4	04±0.5	-	-
G_{63}	-	-	-	-	-	-
G_{64}	03±0.8	06±0.4	-	03±0.3	01±0.1	03±0.3
G_{71}	-	03±0.5	02±0.4	04±0.5	-	-
G_{72}	-	-	-	-	-	-
G_{73}	02±0.4	05±0.3	04±0.7	05±0.3	02±0.6	04±0.3
G_{74}	05±0.5	08±0.1	05±0.7	07±0.6	04±0.3	06±0.3
G_{75}	09±0.5	12±0.3	09±0.7	11±0.3	11±0.5	13±0.4
G_{76}	04±0.5	06±0.3	03±0.5	05±0.7	04±0.5	06±0.7

-; not detected inhibition

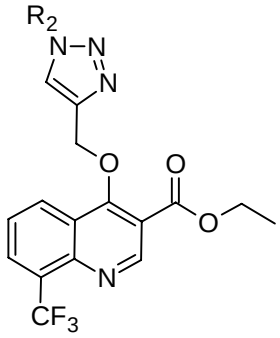
7.3.5. Antimicrobial activity of 1,2,3-triazole containing 8-trifluoromethyl quinoline derivatives



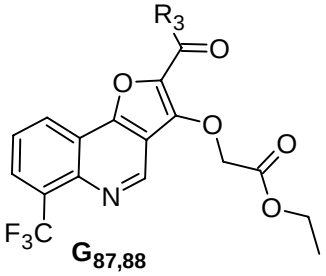
$G_{77\text{-}79}$



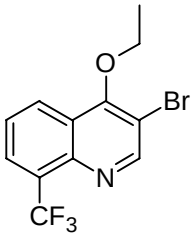
$G_{80\text{-}83}$



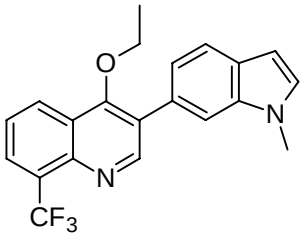
$G_{84\text{-}86}$



$G_{87,88}$



G_{89}



G_{90}

Where R = H, 4-NO_2, 2,4-Cl_2; R_1= F, Cl, Br, OCH_3; R_2= CH_2CH_3, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$; R_3= OCH_2CH_3, OH.

The newly synthesized compounds (**$G_{77\text{-}79}$, $G_{80\text{-}83}$, $G_{84\text{-}86}$, $G_{87,88}$, G_{89}** and **G_{90}**) were screened for their *in-vitro* antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* using Ciprofloxacin as standard by well plate method (zone of inhibition) ((Arthington-Skaggs et al. 2000; Rocha et al. 1995). The test compounds were dissolved in dimethylsulfoxide (DMSO) at concentrations of 0.5 mg/mL and 1.0 mg/mL.

Table 7.11. Antibacterial activity of the compounds (G_{77-79}, G_{80-83}, G_{84-86}, $G_{87,88}$, G_{89} and G_{90})

Zone of inhibition in mm (mean ± S.D.) n = 3						
Compound No.	***Escherichia coli***		***Bacillus subtilis***		***Pseudomonas aeruginosa***	
Concn mg/mL	1	0.5	1	0.5	1	0.5
Standard Ciprofloxacin	24±0.9	22±0.5	25±0.7	22±0.5	28±0.5	24±0.4
Control	00	00	00	00	00	00
G_{77}	04±0.2	02±0.1	05±0.6	03±0.4	06±0.3	04±0.2
G_{78}	10±0.3	08±0.9	10±0.4	07±0.3	09±0.5	06±0.7
G_{79}	15±0.7	13±0.6	13±0.1	11±0.4	16±0.8	13±0.9
G_{80}	04±0.2	02±0.1	06±0.4	03±0.2	05±0.8	04±0.4
G_{81}	15±0.4	14±0.6	11±0.3	08±0.7	13±0.6	10±0.3
G_{82}	10±0.8	07±0.6	06±0.8	04±0.3	08±0.7	06±0.3
G_{83}	06±0.3	04±0.2	05±0.7	03±0.4	06±0.3	05±0.7
G_{84}	12±0.7	08±0.4	08±0.4	06±0.7	08±0.3	05±0.3
G_{85}	08±0.6	05±0.8	08±0.6	07±0.7	06±0.2	04±0.3
G_{86}	07±0.6	04±0.4	09±0.3	06±0.4	07±0.7	06±0.5
G_{87}	10±0.8	08±0.7	06±0.3	04±0.2	11±0.9	08±0.3
G_{88}	12±0.9	10±0.8	10±0.7	07±0.5	12±0.9	09±0.4
G_{89}	16±0.4	04±0.8	15±0.4	13±0.5	17±0.7	15±0.6
G_{90}	18±0.2	17±0.4	16±0.5	15±0.8	17±0.5	15±0.9

Table 7.12. Antifungal activity of the compounds (G_{77-79}, G_{80-83}, G_{84-86}, $G_{87,88}$, G_{89} and G_{90})

Compound No.	Zone of inhibition in mm (mean ± S.D.) n = 3					
	Aspergillus Flavus		*Chrysosporium Keratinophilum*		*Candida Albicans*	
Concn mg/mL	1	0.5	1	0.5	1	0.5
Standard Fluconazole	13±0.2	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2
Control	00	00	00	00	00	00
G_{77}	-	-	-	-	-	-
G_{78}	04±0.2	02±0.1	04±0.6	03±0.5	06±0.3	04±0.1
G_{79}	07±0.4	05±0.3	06±0.5	04±0.5	06±0.4	04±0.6
G_{80}	-	-	-	-	-	-
G_{81}	06±0.7	04±0.2	05±0.2	04±0.4	06±0.3	04±0.3
G_{82}	-	-	-	-	-	-
G_{83}	-	-	-	-	-	-
G_{84}	-	-	-	-	-	-
G_{85}	03±0.3	-	04±0.2	02±0.3	05±0.3	02±0.4
G_{86}	04±0.2	02±0.3	05±0.3	03±0.5	05±0.2	04±0.4
G_{87}	08±0.5	06±0.3	09±0.6	06±0.7	07±0.3	05±0.7
G_{88}	10±0.4	07±0.5	10±0.6	08±0.6	10±0.4	07±0.8
G_{89}	06±0.6	04±0.7	04±0.3	02±0.3	05±0.2	03±0.3
G_{90}	12±0.6	09±0.7	11±0.5	08±0.5	11±0.7	09±0.5

-; not detected inhibition

The *in-vitro* antibacterial activity data revealed that, compounds **G_{79}, G_{81}, G_{88}, G_{89}** and **G_{90}** showed excellent antibacterial activity. The enhanced antibacterial activity of **G_{79}** and **G_{81}** due to presence of chlorine in the 4-benzyl-1,2,3-triazole at the

fourth position of 8-trifluromethylquinoline-3-carboxylic ester. The compounds **G_{88}, G_{89}** and **G_{90}** contains furan ring (**G_{88}**), bromo (**G_{89}**) and N-methylindole (**G_{90}**) groups at third position of 8-trifluromethylquinoline ring which accounts for the enhanced antibacterial activity. Compounds **G_{78}, G_{82}** and **G_{87}** exhibited excellent antibacterial activity against all tested bacterial strains. The enhanced activity may be due to 1,2,3-triazole ring present at 8-trifluromethylquinoline ring.

The *in-vitro* antifungal activities of newly synthesized compounds (**G_{77-79}, G_{80-83}, G_{84-86}, $G_{87,88}$, G_{89}** and **G_{90}**) were determined by well plate method (MacLowry et al. 1970; Portillo et al. 2001). The results indicate that, among the tested compounds **G_{88}** and **G_{90}** were active against all tested fungal strains. The enhanced activities of compounds are due to heterocyclic moieties (furan, N-methyl indole) attached to the 8-trifluoromethylquinoline ring. All other compounds showed less inhibition against all the tested microorganisms as compared to the slandered drug. The **table 7.11** and **Table 7.12** depict the antimicrobial screening results of the final compounds.

7.4. CONCLUSION

Various trifluoromethyl and halogen substituted quinoline derivatives were synthesized from substituted aniline with the objective of developing better antimicrobial agents. Among the sample screened for *in-vitro* antimicrobial activity, nearly 70-80% of all the targeted compounds have been found to be active against at least one of the microbial strains, used in the present investigation. Among ninety compounds of new halogen and trifluoromethyl quinolines, imidazole incorporated quinoline derivatives (**G_{1-15}**), trifluoromethylquinoline-3-carbohydrazide (**G_{16-20}**) and 1,2,3-triazole containing 8-trifluoromethylquinoline derivatives (**G_{87-90}**) showed good *in-vitro* antibacterial activity. The compound **G_4** showed excellent antibacterial activity against *Salmonella* and **G_{11}** showed excellent antifungal activity against *Aspergillus niger* among all the five series. The enhanced activity of compound **G_4** may be due to presence of two chlorine atom on the benzimidazole ring and 4-fluorophenyl group on second position of quinoline ring. The enhanced antifungal activity is due to presence of chlorine atom on benzimidazole ring and 4-fluorophenyl group on second position of quinoline ring.

CHAPTER-8

SUMMARY AND CONCLUSION

CHAPTER-8

SUMMARY AND CONCLUSIONS

8.1. SUMMARY

Antimicrobial drugs have caused a dramatic change not only of the treatment of infectious diseases but of a fate of mankind. Antimicrobial chemotherapy made remarkable advances, resulting in the overly optimistic view that infectious diseases would be conquered in the near future. Antimicrobial resistance is a global public health concern that is impacted by both human and non-human antimicrobial use. The consequences of antimicrobial resistance are particularly important when pathogens are resistant to antimicrobials that are critically important in the treatment of human disease. However, in reality, emerging and re-emerging infectious diseases have left us facing a counter charge from infections. Infections with drug resistant organisms remain an important problem in clinical practice that is difficult to solve.

In view of this, in the present work, it was planned to couple biologically active trifluoromethylquinoline with a series of active heterocyclic moieties through active functional systems to form a new molecular framework and investigate their biological activities. Accordingly, different series, viz. benzimidazole, oxadiazol and triazole derivatives carrying quinoline ring as core structure have been designed and synthesized. Structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectral studies and also by C, H, N elemental analyses. Selected compounds have been subjected to X-Ray crystallographic studies for further confirmation of their structures. The application of newly synthesized compounds was confirmed by biological assays.

8.2. CONCLUSIONS

The conclusions of present research work are summarized as follows:

- Five new series of trifluoromethyl and halogen substituted quinoline derivatives were synthesized by multistep reactions.
- Biologically active heterocyclic pharmacophore were linked by condensation reaction, substitution reaction, click chemistry and Suzuki reactions.
- Synthetic and purification methods for new quinoline compounds have been developed.
- Structures of the new compounds have been confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectral studies and single crystal X- ray study of selected compounds.
- Newly synthesized final molecules have been evaluated for their *in-vitro* antibacterial and antifungal activities.
- Among the Quinoline incorporated benzimidazole derivatives **G_3** and **G_4** showed significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Compound **G_3** is found to be more potent against *Staphylococcus aureus*. Compounds **G_4** and **G_{13}** exhibited excellent antibacterial activity against *Salmonella* and *Xanthomonas* respectively.
- In the trifluoromethylquinoline-3-carbohydrazide and 1,3,4-oxadiazole derivatives, **G_{21}** and **G_{30}** are showing significant antibacterial activity against all the tested microorganisms such as Gram-positive and Gram-negative bacteria, non-filamentous and filamentous fungi. Among all the compounds, **G_{19}** is inhibiting *Mycobacterium smegmatis* to the maximum of 16 mm diameter at 50 µg/mL concentration.
- In the 3-substituted 7 and 8-trifluoromethylquinoline derivatives. Compounds having heterocyclic hydrazones at 3rd position of the quinoline (**G_{34}** and **G_{36}**) showed excellent antimicrobial activity and compound **G_{46}** showed good antioxidant activity compared to the standard drugs.

- In the trifluoromethylquinolone series, compounds **G_{50}, G_{59}** and **G_{68}** showed significant antibacterial activity against all the tested bacterial strains and **G_{68}** have been found to be more potent antifungal agent.
- In the 8-trifluoromethylquinoline based 1,2,3-triazoles derivatives, the compounds **G_{79}, G_{81}, G_{88}, G_{89}, G_{90}** showed excellent antibacterial activity and compounds **G_{88}, G_{90}** were active against all tested fungal strains.
- As regards the relationships between the structure of the heterocyclic scaffold and detected antimicrobial properties of the five newly designed quinoline series, it showed that heterocyclic substitution at third and fourth position of the trifluoromethylquinoline are ideally suited for obtaining more efficient antimicrobial compounds.

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RESEARCH PUBLICATIONS

List of Papers published and communicated in journals

1. **B. Garudachari**, M.N. Satyanarayana, B. Thippeswamy, C.K. Shivakumar, K.N. Shivananda, A.M. Isloor. "Synthesis, characterization and antimicrobial studies of some new quinoline incorporated benzimidazole derivatives." ***European Journal of Medicinal Chemistry***, (2012). 54, 900-906.
2. **B. Garudachari**, A.M. Isloor, M.N. Satyanarayana, H.K. Fun, L. Sathish, A. Kulal. "Design and regioselective synthesis of trifluoromethylquinolone derivatives as potent antimicrobial agents." ***European Journal of Medicinal Chemistry*** (2013). 68, 422-432.
3. **B. Garudachari**, A.M. Isloor, M.N. Satyanarayana, H.K. Fun, Gurumurthy Hegde, "Click chemistry approach: Regioselective one-pot synthesis of some new 8-trifluoromethylquinoline based 1,2,3-triazoles as potent antimicrobial agents." Accepted 3rd January 2014. ***European Journal of Medicinal Chemistry.***
4. **B. Garudachari**, A.M. Isloor, M.N. Satyanarayana, H.K. Fun, L. Sathish, A. Kulal. "*In-vitro Mycobacterium smegmatis* and antimicrobial screening of some new quinoline 3-carbohydrazide and 1,3,4-oxadiazoles." Second Revised Manuscript Submitted, ***European Journal of Medicinal Chemistry***.
5. **B. Garudachari**, A.M. Isloor, M.N. Satyanarayana, S.K. Peethambar. "Synthesis and biological study of some new 7 and 8 trifluoromethylquinoline derivatives." Communicated, ***RSC Advances.***

Acta Crystallographica Section E

1. **B. Garudachari**, A.M. Isloor, M. N. Satyanarayan, T. Gerber, E. Hosten and R. Betz. "Diethyl 2-{[2-(trifluoromethyl)anilino]-methylidene}propanedioate." ***Acta Crystallographica Section E***, (2012). E68, o514-o515.
2. **B. Garudachari**, A.M. Islor, M.N. Satyanarayan, T. Gerber, E. Hosten and R. Betz. "Ethyl 4-oxo-8-trifluoromethyl-1,4-dihydroquinoline-3-carboxylate." ***Acta Crystallographica Section E***, (2012). E68, o3304-o3305.

3. **Garudachari**, A.M. Islor, A.M. Vijesh, T. Gerber, E. Hosten and R. Betz. "Ethyl 3-(2-ethoxy-2-oxoethoxy)-6-(trifluoromethyl)furo[3,2-c]quinoline-2-carboxylate." ***Acta Crystallographica Section E***, (2012). E68, o3389-o3390.
4. A.M. Islor, **B. Garudachari**, K.N. Shivananda, T. Gerber, E. Hosten and R. Betz. " Ethyl 4-({1-[2-(4-bromophenyl)-2-oxoethyl]-1H-1,2,3-triazol-4-yl}methoxy)-8-(trifluoromethyl)quinoli ne-3-carboxylate." ***Acta Crystallographica Section E***, (2012). E68, o3387-o3388.
5. A.M. Islor, **B. Garudachari**, T. Gerber, E. Hosten and R. Betz. "Ethyl (Z)-3-(4-methylanilino)-2-[(4-methylphenyl)carbamoyl]prop-2-enoate." ***Acta Crystallographica Section E***, (2012). E68, o3218.
6. H.K. Fun, C.W. Ooi, **B. Garudachari**, A.M. Isloor and S.A. Rashid. "Ethyl 4-{[1-(2,4-dichlorobenzyl)-1H-1,2,3-triazol-4-yl]methoxy}-8-(trifluoromethyl)quinoline-3-carboxylate." ***Acta Crystallographica Section E***, (2012). E68, o3017-o3018.
7. H.K. Fun, S. Arshad, **B. Garudachari**, A.M. Isloor and M.N. Satyanarayan. "4-(4-Methylpiperazin-1-yl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl) quinoline." ***Acta Crystallographica Section E***, (2011). E67, o3117-o3118.
8. H.K. Fun, C.W. Ooi, **B. Garudachari**, A.M. Isloor and G. Hegde. "Ethyl 1-(2,4-dichlorobenzyl)-4-oxo-7-trifluoromethyl-1,4-dihydroquinoline-3-carboxylate." ***Acta Crystallographica Section E***, (2012). E68, o435-o436.

List of Papers presented in conferences

1. **B. Garudachari**, Rashmi Pereira, Arun M Isloor, S.K. Peethambar, "Design and regioselective synthesis of trifluoromethylquinolone derivatives as potent antimicrobial agents." National Symposium, Chemical Research Society of India (CRSI) Mid Year Symposium 2013 National institute of technology Karnataka during July 12-13, 2013.
2. **B. Garudachari**, A.M. Isloor, M.N. Satyanarayana, H.K. Fun, L. Sathish A. Kulal, "Design, synthesis and biological evaluation of quinoline derivatives." One Day National Conference on "Green & Sustainable Chemistry"(NCGSC-2012) Thumkur university on 25th February 2012.

3. **B. Garudachari**, A.M Isloor and C.K. Shivakumar, "Synthesis of Quinoline Incorporated Benzimidazole Derivatives as Potential Antimicrobial Agents." International Conference on Synthetic and Structural Chemistry (ICSSC-2011) Mangalore university on December 8-10th, 2011.

Department of chemistry

National Institute of Technology

Karnataka

Srinivasanagara, Karnataka-575025

India

Email id : garuda.achar@gmail.com
Mobile No : +91-8095883087

Education

Ph.D., Medicinal Chemistry, National Institute of Technology Karnataka India.

Thesis: Synthesis, biological studies of some new trifluoromethyl and halogen substituted quinoline derivatives,

Supervisor: Dr. Arun M Isloor.

M. Phil., Chemistry, 2009, Periar university, Thamilnadu, India.

M.Sc. General chemistry, Kuvempu university, 2004-2006, Karnataka, India.

Research Experience

Project Assistant, Department of Physics, National Institute of Technology Karnataka, India. June, 2010 to March 2013. Fabrication and Characterization of Blue Organic Light Emitting Diodes (OLEDs).

Project Assistant, Department of Chemistry, Birla Institute of Technology Pilani Rajasthan, India, September, 2009 to February, 2010. Synthesis, Characterization and catalytic application of Ionic liquid tagged Schiff base metal complexes.

Scientist, Syngene intl. Ltd (BIOCON). Bangalore, Karnataka, India, August, 2006 to August, 2009.

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6. Hidayath Ulla, **B. Garudachari**, M. N. Satyanarayan, G. Umesh, A. M. Isloor "Blue Organic Light Emitting Materials: Synthesis and Characterization of Novel 1,8-Naphthalimide Derivatives." Accepted. 2013. ***Optical Materials*.**

7. Hidayath Ulla, **B. Garudachari**, MN Satyanarayan, G Umesh, AM Isloo, "Blue light emitting materials for organic light emitting diodes: Experimental and simulation study." ***Optical Engineering (ICOE)***, 2012, 1-4.

8. Hidayath Ulla, **B. Garudachari**, MN Satyanarayan, G Umesh, AM Isloo, "Tailoring of 1,8-Naphthalimides: Multifunctional Materials for Blue Organic Light Emitting Diodes." Communicated. ***Journal of Photochemistry and Photobiology A***.

Acta Crystallographica Section E

1. **B. Garudachari**, A. M. Isloor, T. Gerber, E. Hosten and R. Betz, "2-(4-Bromophenyl)-2-oxoethyl naphthalene-1-carboxylate." ***Acta Crystallographica Section E,*** (2013). E69, o649.

2. A. M. Isloor, **B. Garudachari**, T. Gerber, E. Hosten and R. Betz, "2,4-Dichlorobenzyl 2-methoxybenzoate." ***Acta Crystallographica Section E,*** (2013). E69, o509.

3. **B. Garudachari**, A. M. Isloor, T. Gerber, E. Hosten and R. Betz, "2-(4-Chlorophenyl)-2-oxoethyl naphthalene-1-carboxylate." ***Acta Crystallographica Section E,*** (2013). E69, o551.

4. A. M. Islor, R. Yaradoni**, B. Garudachari**, T. Gerber, E. Hosten and R. Betz, "3-(4-Chlorophenyl)-5-phenyl-4,5-dihydro-1,3-oxazole." ***Acta Crystallographica Section E,*** (2012). E68, o3215.

5. **B. Garudachari**, Arun M. Isloor, M. N. Satyanarayan, Thomas Gerber, Eric Hostenc and Richard Betz. "Diethyl 2-{[2-(trifluoromethyl)anilino]-methylidene}propanedioate." ***Acta Crystallographica Section E***, (2012). E68, o514-o515.

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9. A. M. Islor, **B. Garudachari**, T. Gerber, E. Hosten and R. Betz. "Ethyl (Z)-3-(4-methylanilino)-2-[(4-methylphenyl)carbamoyl]prop-2-enoate." ***Acta Crystallographica Section E***, (2012). E68, o3218.

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4. **B. Garudachari**, Arun M Isloor and C. K. Shivakumar, "Synthesis of Quinoline Incorporated Benzimidazole Derivatives as Potential Antimicrobial Agents." International Conference on Synthetic and Structural Chemistry (ICSSC-2011) Mangalore university on December 8-10th, 2011.

5. **B. Garudachari** and Bharti Khungar. "Synthesis, Characterization and Catalytic Application of Transition Metal Complexes with Ionic Liquid Tagged Schiff Bases." International conference on Chemical biology for discovery: Perspectives and challenges (ISCBC-2010). Central drug research institute, Lucknow, U.P. India. On January 15 to 18th , 2010.

Personal profile

Age : 29

Date of birth : 10/06/1983

Sex : male

Nationality : Indian

References

Dr. Arun M Isloor

Department of Chemistry,

National Institute of Technology

Karnataka,

Surathkal, Mangalore, Karnataka -575

025, India.

Ph : +91 9448523990.

Email id : isloor@yahoo.com

Dr. M.N. Satyanarayan

Department of Physics,

National Institute of Technology

Karnataka,

Surathkal, Mangalore, Karnataka -575

025, India.

Ph :+ 9686146138

Email id : mn.satyanarayan@gmail.com

Dr.Y.Arthoba Naik

Department of Studies in Chemistry,
Kuvempu University, Shimoga- 577451,
Karnataka, India.
Ph : +9448855078
Email id: yanaik@kuvempu.ac.in,
drarthoba@yahoo.co.in

Dr. Mallem H.V.Ramana RaoV(Group leader)
Syngene intl ltd (Biocon)
Bangalore . Karnataka.India.

Ph : +91-9980515213

Email id: ramana.rao@syngeneintl.com

rraomhv@gmail.com