A
Thesis Submitted
In Partial Fulfilment of the Requirement

For the Degree of
Doctor of Philosophy

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME FLUOROQUINOLONES

Submitted by
Salunke Ramkrushna Ashokrao
(951009002)

THAPAR INSTITUTE
OF ENGINEERING & TECHNOLOGY
(Deemed to be University)

Under the Supervision
of

Dr. Manmohan Chhibber
Professor
School of Chemistry and Biochemistry

Dr. BaldevRaj Bansal
Head- Research and Development
M/s Saurav Chemicals Limited
Derabassi – 147507, Mohali, Punjab, India

Thapar Institute of Engineering and Technology
(Deemed to be University)
Patiala -147004, India
August, 2019

Candidate's Declaration

I hereby declare that work presented in the thesis entitled "*SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME FLUOROQUINOLONES*" in partial fulfilment of the requirements for the award of the Doctor of Philosophy, is an authentic record of my own work carried out under the joint supervision of Dr. Manmohan Chhibber, Associate Professor, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala, Punjab, India and Dr. BaldevRaj Bansal, Head – Research and Development, Saurav Chemicals Limited, Derabassi, Mohali, Punjab, India. The matter embodied in the thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or abroad.

30/08/2019

Place: Patiala, India

Date: 30th Aug. 2019

Salunke Ramkrushna Ashokrao

(Reg. No. 951009002)

Certificate

Certified that the thesis entitled "*SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME FLUOROQUINOLONES*" that is being submitted by Mr. Salunke Ramkrushna Ashokrao in partial fulfilment of the requirements for the award of the Doctor of Philosophy, at School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala, Punjab, India is a record of candidate's own independent and original research work carried out by him under our supervision and guidance. The matter embodied in the thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or abroad.

Supervisors

30/8/2019.

Dr. Manmohan Chhibber
Associate Professor
School of Chemistry and Biochemistry,
Thapar Institute of Engineering and Technology,
Patiala, Punjab, India

30-08-2019

Dr. BaldevRaj Bansal
Head- Research and Development
M/s Saurav Chemicals Limited
Derabassi – 147507, Mohali,
Punjab, India

Head

Prof. Amjad Ali
Head
School of Chemistry and Biochemistry,
Thapar Institute of Engineering and Technology,
Patiala, Punjab, India

Place: Patiala, India
Date: 30th Aug. 2019

Dedicated to

MY BELOVED FAMILY

Acknowledgment

Completion of this doctoral thesis was not possible without the support of several people. I would like to express my sincere gratitude to all of them.

As I write this acknowledgement I recall my very first day at the then Thapar University where I met **Dr. Manmohan Chhibber** and he encouragingly guiding me through the process and enrollment of Ph. D degree. I thank my guide for the same. I also thank very calm and supportive personality of **Dr. BaldevRaj Bansal** for his valuable guidance, scholarly inputs, training me to use various industrial techniques, consistent encouragement and discussions throughout the research work.

I would like to thanks my research committee members, **Dr. Kamaldeep Paul**, **Prof. Sanjai Saxena**, **Dr. S.K. Pandey** and **Dr. Bhupesh Goyal** for constructive criticism and useful suggestions during each of the six monthly progress evaluation meetings. Also, I would like to thanks **Prof. Susheel Mittal**, **Prof. Satnam Singh**, **Prof. Amjad Ali**, **Prof. Bonamali Pal**, **Prof. Ranjana Prakash** and **Dr Vijaya Luxami** for their kind support and motivation.

I would like to express my sincere gratitude to my collaborator **Dr. Anil Kumar Pinnaka**, Sr. Scientist IMTECH Chandigarh , **Dr. Chandni Sidhu**, **Dr. Kailash Bhamre** IMTECH Chandigarh and **Dr. Sidharth Chopra**, **Ms. Manjulika Sharma**, **Ms. Grace Kaul** CDRI, CSIR Lucknow for accomplishment of research work and support related to biological activities.

I am also thankful to **SAIF** Chandigarh, **SAI** Patiala, **IMTECH** Chandigarh, **CDRI-CSIR** Lucknow, **M/s Smruti organics** Solapur, **M/s Saurav Chemicals Limited** Derabassi Chandigarh for their valuable help for the analysis, gift in terms of chemical samples and providing infrastructure.

I am thankful to all my friends for their help and kind support. I would like to acknowledge my dear ones, **Dr. Ramandeep Kaur**, **Dr. Giriraj Acharya**, **Dr. Akil Akhatar, Dr. Rampal Saini**, **Dr. Sukhvinder Saini**, **Mr. Ashokkumar Rana** for always standing by my side wherever I was stuck in my research work.

I would like to thanks to **Mr. Pritmohinder Singh** (COO), and **Mr. Rajesh Kaushik** Analytical/Quality experts in M/s Saurav Chemicals limited **Mr. Narender**, **Mr. Amit Sharma** for moral support and constant encouragement. My humble thanks to everyone in **Research and Development Department** of M/s Saurav Chemicals limited, who played an important role for the completion of my research work. I specially thank to **Ms. Nidhi Sharma, Ms. Neha Vaid** and **Mr. K. Mohan Reddy** for their priceless contribution to my research work in terms of resources and ever encouraging and supportive nature. Also I would like to express my gratitude to my industrial mentor **Mr.Nitin Tiwari, Dr. Sanjeev Sharma** and **Mr. Kamesh Yeditha** for their kind support.

A special thanks to my beloved family members, especially my father, **Shree. Ashok Bhagwan Salunke (Tatyya)**, brother, **Mr. Shahaji Salunke (Bhau),** their patience and always standing behind me during my research journey. Thanks to my mother **'Aai'** for her care all the time. I would like to express my gratitude to my better half **'Deepali'** who always supported to me for completion of the research work.

Once again thanks to everyone who played an important role directly or indirectly for the completion of my research work.

30/08/2019

Salunke Ramkrushna Ashokrao

Table of Content

List of Figures

List of Figures

List of Figures

List of Figures

List of Tables

List of Tables

List of Reaction Scheme

Introduction and Literature survey

1.1 Introduction

Infectious diseases remain the main cause of human premature deaths, especially in developing countries. In recent years, there has been a persistent increase in the occurrence of antibiotic resistance to many common bacterial pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis*. Methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *pneumococcus* and vancomycin-resistant *Enterococcus faecalis* (VRE) are now commonplace pathogens that are proving difficult to be treated effectively[1, 2]. Probably more alarming is the emergence of multi-drug resistance[3]. Also, malaria[4] and tuberculosis[5] constitute major causes of mortality in humans. Thus, there is a need for the discovery and development of antibiotics that add to our current arsenal to combat resistant strains.

Fluoroquinolones are a family of compounds that inhibit DNA gyrase represented by general structure as shown on **Figure -1.1**. They have several advantages like convenient synthetic route, low cost, excellent bioavailability and solubility, prolonged serum half-life, both oral and parenteral routes of administration and fewer adverse side effects[6]. In the last six decades, this class of compounds has progressed from Nalidixic acid (**1.2)**, an impurity that was found during the initial synthesis of a chloroquine derivative[7], to well-known antibacterial molecules. Chemically fluoroquinolones are *1,7-disubstituted-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acids* as represented by compound **(1.1)**. Norfloxacin **(1.3)** was the first active fluoroquinolone molecule to be used commercially.

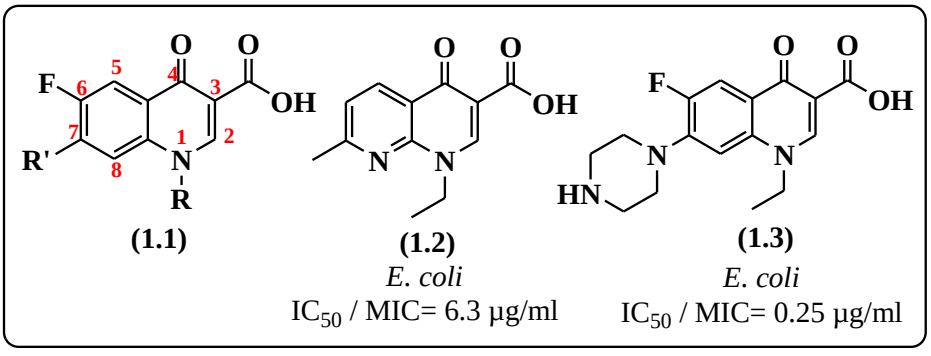


Figure-1.1: Structures of important fluoroquinolone molecules: **1.1-** General Structure of fluoroquinolones, **1.2-** Nalidixic acid, the serendipity molecule discovered while working on chloroquine derivatives and **1.3**-Norfloxacin, the first active fluoroquinolone.

Today, besides several fluoroquinolone molecules that are commercially available for human use, a broad range of such molecules are widely used in food-producing animals, aquaculture and pets to treat or prevent bacterial infections.

Due to the above-mentioned requirements and the advantages, present work was taken up to synthesize and characterize novel C-7 substituted fluoroquinolones for their antibacterial activity. The novelty lies in substitution of bicyclic nortropine ring at the C-7 position of most commercially available fluoroquinolone skeletons. Further, the derivatives of these compounds have also been synthesized and evaluated against different strains of bacteria.

1.2 Fluoroquinolones: Importance of Substituents

Ever since the discovery of nalidixic acid **(1.2, Figure -1.1)** in 1962, a vast number of molecules have been synthesized and tested for their antibacterial activity. The following paragraphs describe the structure-activity relationships of quinolones position by position. The numbering in the quinolone's skeleton has been shown above. **(Compound 1.1** in **Figure-1.1)**

Position N-1: This position represents one of the sensitive sites on the fluoroquinolone skeleton, where the activity is greatly altered by the change of the substituent. Initial results reported a great potential in the presence of aliphatic N-ethyl substituent.[8] As stated above, Norfloxacin **(1.3, Figure-1.1)** was the breakthrough molecule as its potency and spectrum approximated those of the fermentation-derived antibiotics[9]. Later, exploration of the other positions displayed enhancement in the anti-gram-negative potency and breadth of the antibacterial spectrum.

Figure- 1.2 below compares eminent N-1 substituted fluoroquinolone molecules that formed antibacterial arsenal during initial years of their development. The introduction of cyclopropyl moiety at N-1 position created Ciprofloxacin **(1.4)** with enhanced the activity as compared to previously known Norfloxacin **(1.3)**[10]. The former has largely withstood these challenges and remains a market leader to this day.

The bridging between N-1 and C-8 positions of fluoroquinolones by a six-member ring resulted in the tricyclic family of quinolones. The advantage of such a molecule was restricted rotation of N1 substituent and the possibility to introduce the chirality. This feature gave the first fluoroquinolone based anti-infective agent flumequine **(1.5)** that is used primarily for the agricultural purposes today.[11] Ofloxacin, a racemic tricyclic quinolone, and its resolved S-analogue, levofloxacin **(1.6)** were also the result of rigidification of N-1 substituent [12.] This significantly enhanced the anti-Gram-positive activity although some anti-Gram-negative

activity was concomitantly lost. The nature of the atom attached to C-8 seemed comparatively unimportant as C, O, and S bio isosteres possessed a similarly significant activity and the products showed the same chiral dependence.

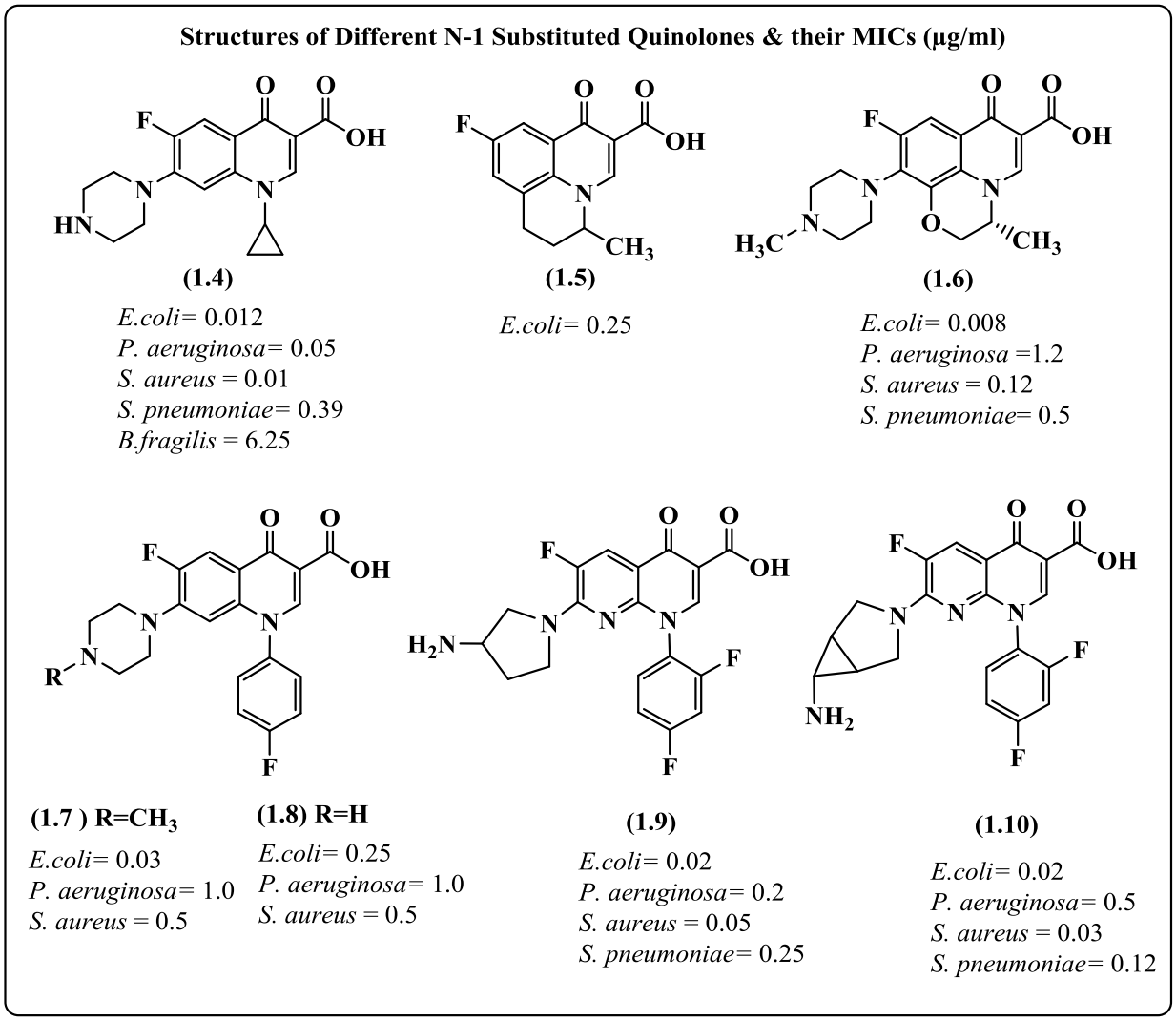


Figure -1.2: Structures of different N-1 substituted quinolones and their antibacterial activity. **1.4-** Ciprofloxacin with cyclopropyl group at N-1 position, **1.5** and **1.6**- Bridged N-1 and C-8 positions of Flumequine and Levofloxacin respectively form tricyclic family of quinolones, **1.7, 1.8, 1.9** and **1.10-** Difloxacin, Sarafloxacin, Tosufloxacin and Trovafloxacin respectively are N-1 fluoro aryl substituents.

N-aryl substituent has proven to be quite successful in specific instances as far as in vitro antibacterial activities were concerned. Most of the molecules in this category contained a second fluorine atom in the aromatic ring. Difloxacin **(1.7)** [13], Sarafloxacin **(1.8)**[14], Tosufloxacin **(1.9)**[15] and Trovafloxacin **(1.10)**[16] were some of the molecules containing fluoroaryl substituent at N-1 position. Compounds **1.9** and **1.10**, however, further enhanced both potencies against Gram-positives and pharmacokinetics and were marketed. Unfortunately, the addition of the difluoro benzene substituent was accompanied by uncommon but severe toxicities that were difficult to detect in clinical trials and were later withdrawn. Different substituted groups at N-1

aryl position gave unsatisfactory results against both Gram-positive and Gram-negative bacterial strains.[17]

Positions C-2, C-3, and C-4: Compared to the other positions not many analogous of fluoroquinolones with substituents at positions 2, 3, and 4 positions have been reported. Among the reported ones, the analogue modified at C-2 position could find a place in market. The co-crystalized structure with protein has revealed that fluoroquinolones form a hydrogen bond with open stranded DNA bases via carboxyl group at C-3 and its co-planer carbonyl group at C-4. The open stranded DNA is made available by strand separation catalysed by DNA gyrase or topoisomerase IV. Substitution at C-2 confronts the steric requirement of the effective hydrogen bond with open stranded DNA there by making this position unfavourable for substitution and biological activity.

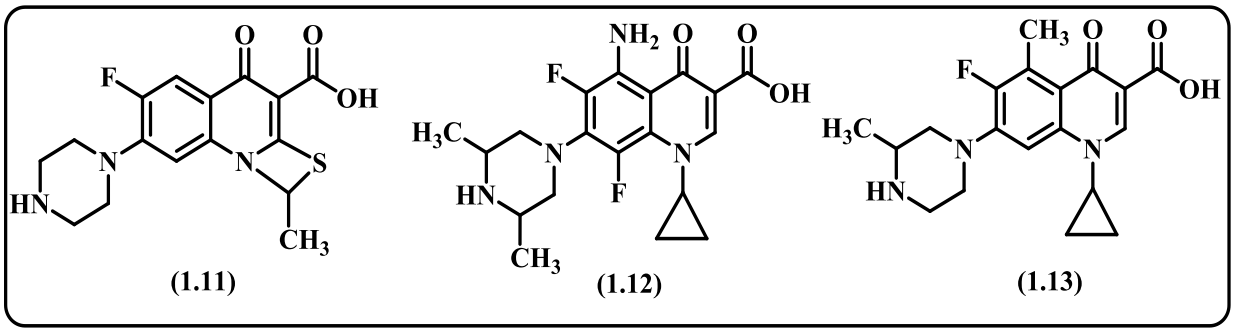


Figure -1.3: Structure of important C-2 and C-5 substituted quinolones: **1.11-**Prulifloxacin, the four membered thiazolone ring compound fused between positions C-2 and N-1, **1.12** and **1.13 -** Sparfloxacin and Grepafloxacin were the most successful C-5 substituted compounds.

However, a fluoroquinolone compound **(1.11)** with four membered thiazolone ring fused between C-2 and an N-1 position was synthesized with an objective to partly overcome the steric crowding and rigidifying the quinolone structure **(Figure -1.3).**[18] This feature led to striking enhancement of antimicrobial potency but also the molecule displayed its toxicity with an ability to kill mammalian cells by damaging DNA through intercalation. This suggested their possible use for the investigation of antitumor agents. Phenyl thiourea derivatives are also important compounds in medicinal chemistry as anti-diabatic, antiviral and antimicrobial agents. Patel *et. al.* synthesized C-3 position related 3-carboxylic ester and thioureidoamide derivatives of ciprofloxacin **(1.4)**. However, not very encouraging results were obtained for the antibacterial activity.[19]

Positions C-5: Substituents at C-5 position are tolerated and help increase potency especially if they are small and polar as well. Groups that have been studied with C-5 substitution include CH_3, C_2H_5, OH, NH_2, NHMe, NMe_2, NHAc, Cl and F. **Figure -1.3** shows sparfloxacin **(1.12)** [20]

and grepafloxacin **(1.13)**[21] fluoroquinolones that were most successful among the discussed. 5-Halo substituents significantly contributed to the phototoxicity to be used as antibacterial. Other substituents did not give very interesting results to preceded further.

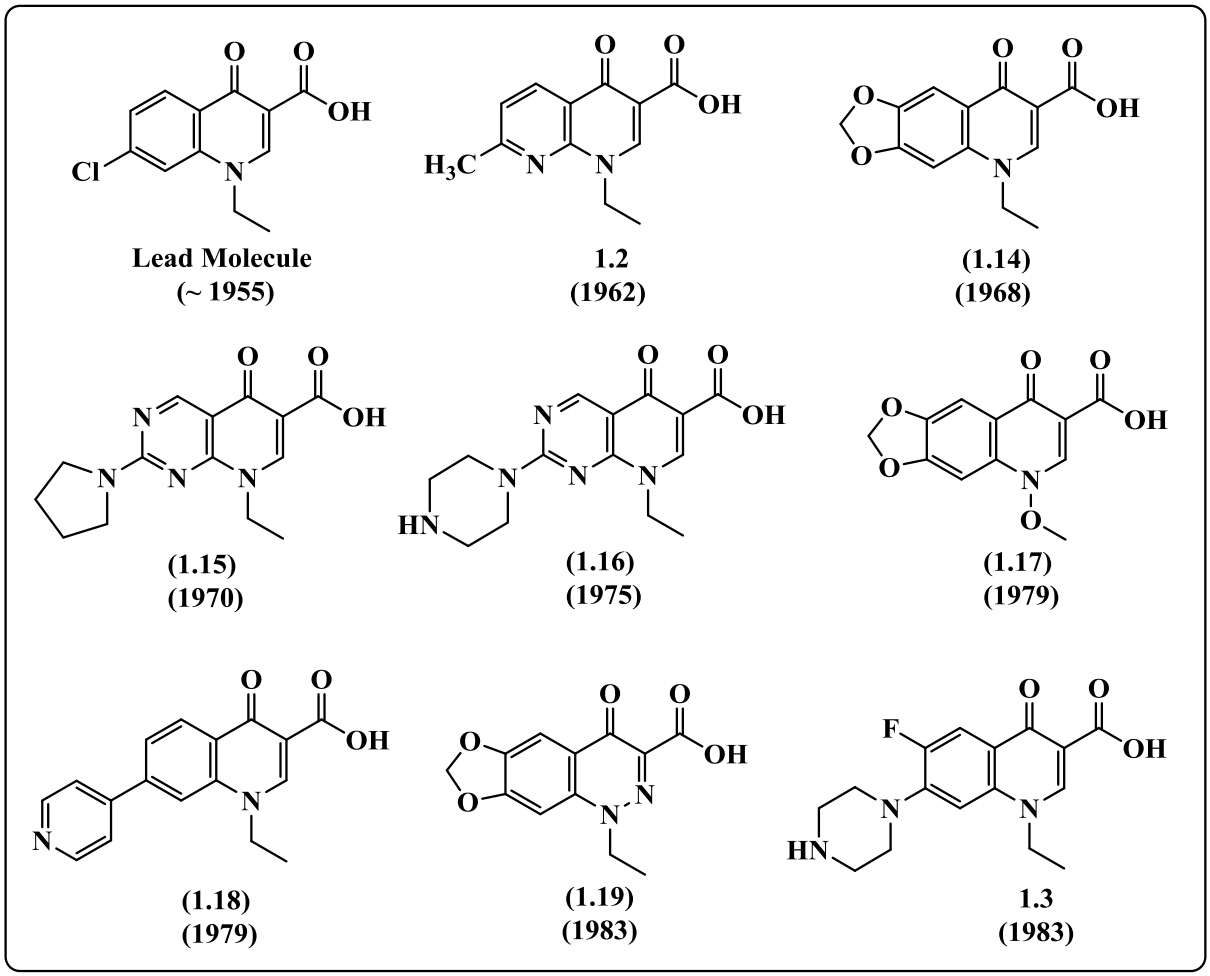


Figure-1.4: Structure of different C-6 substituted quinolone molecules in chronological order. Starting from the lead molecule in 1955 various C-6 substituted derivatives have been reported. Nalidixic acid[7] **(1.2)**, oxolinic acid[25] **(1.14)**, piromidic acid[24]**(1.15)**, pipemidic acid[23]**(1.16)**, miloxacin[26] **(1.17)**, rosoxacin[22] **(1.18)** and cinoxacin[27]**(1.19)** were substituted at C-6 position with CH, N or methylenedioxy bridge to C-7 position before the discovery of fluoroquinolone norfloxacin **(1.3)**.

Position C-6: The advent of norfloxacin **(1.3)** in 1983 led to the sub-classification of quinolones into fluoroquinolones that contained fluorine at the C-6 position. The C-6 fluorine substituted molecule conveyed enhanced DNA gyrase potency and cell penetration. Hence after, the fluorine atom at C-6 position became a regular feature for the quinolone skeleton and further exploration ceased to exist. **Figure- 1.4** above shows the molecules with different C-6 substituents in chronological order before fluoroquinolones. Various groups used as C-6 substituents were CH (Compounds **1.2**[7] and **1.18**[22]) N (Compounds **1.16**[23] and **1.15**[24]) and methylene dioxy bridge to C-7 position (Compounds **1.14**[25], **1.17**[26] and **1.19**[27]).

It has now been shown that if a substituent at other positions is helpful then there is no need for C-6 fluorine. Present synthetic efforts are drifting for non-fluorinated analogues of these molecules as it has been suggested that C-6 fluorine plays a role in the potential mammalian genotoxicity and central nervous system side effects. [28, 29] **Figure-1.5** reveals no difference in the potency against *E. coli*, *E. coli* derived gyrase and *S. aureus* when C-6 substituent is fluorine **(1.20)** and non-fluorine (**1.21)** atom.

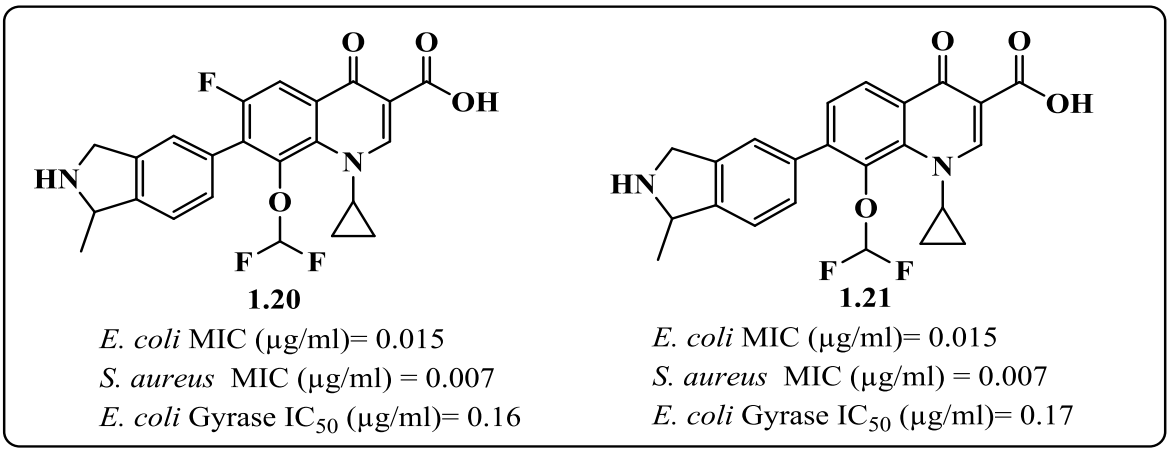


Figure-1.5: Comparison of the potencies of fluorine C-6 substituent and non-fluorine (**1.21)** substituted quinolone molecules.

Position C-7: This is the most important and versatile position for substitution of quinolones and its analogous. A large number of molecules with various substituents at this position have been synthesized. A good substituent results in enhanced potency and favourable pharmacokinetics. The nature of the C-7 substituent (along with C-8) also strongly affects the target preferences of the quinolones. The lead molecule possessed a chlorine molecule **(Figure-1.4)** followed by methyl group in nalidixic acid **(1.2, Figure-1.4)** at the C-7 positions. As discussed above, this was pursued by a methylenedioxy bridge between C-6 and C-7 carbons as represented by molecules **1.14**, **1.17** and **1.19.** Pyrrolidinyl **(1.15)** and piperazinyl **(1.16)** and 4-Pyridyl **(1.18)** moieties also substituted at C-7 position formed important quinolone molecules. Finally, it became known that cyclised amino moieties were superior in biological activity with a caution that such molecules can be photoactive nature .[30, 31] Therefore electron-releasing group at C-7 position is required to be avoided due to its ability to stabilize a radical generated at C-8 position. This ultimately leads to the phototoxicity and genotoxicity of the molecule.

Norfloxacin **(1.3)** established that piperazinyl moiety or its N-methyl analogue were standard feature among biologically active fluoroquinolones. Later it was also found that a pyrrolidinyl substituent with a pendant amine was suitable. [32] However, both the substituents had different

objectives. Whereas piperazinyl analogues usually have enhanced potency against Gram negatives, pyrrolidinyl analogues have enhanced activity against Gram-positives. Subsequently, bicyclic moieties of a variety of types were also introduced.

These however, retained the potency but also diminishing metabolic liability. The position of pendent amino group however, plays an important part. The one that is proximal to the aromatic ring is much less basic than the distal nitrogen atom and is less preferred than later ones[11]. Similarly, chirality that was introduced with Levofloxacin **(1.6)** was also optimized. The stereochemistry of carbon near distal nitrogen atom provided less potent molecules than the ones which were closer to aromatic ring.[15] Broad classification can be done for different substituents that have been attached to C-7 position of fluoroquinolones.

Piperazinyl and Related Moieties: Compounds **1.3**, **1.4**, **1.6**, **1.7** and **1.8** are some of the examples that have piperazinyl or their analogues attached to C-7 position. **Figure-1.6** depicts selection of many simple and fused piperazines and piperidines rings that have been attached to C-7 of quinolones[33, 34].

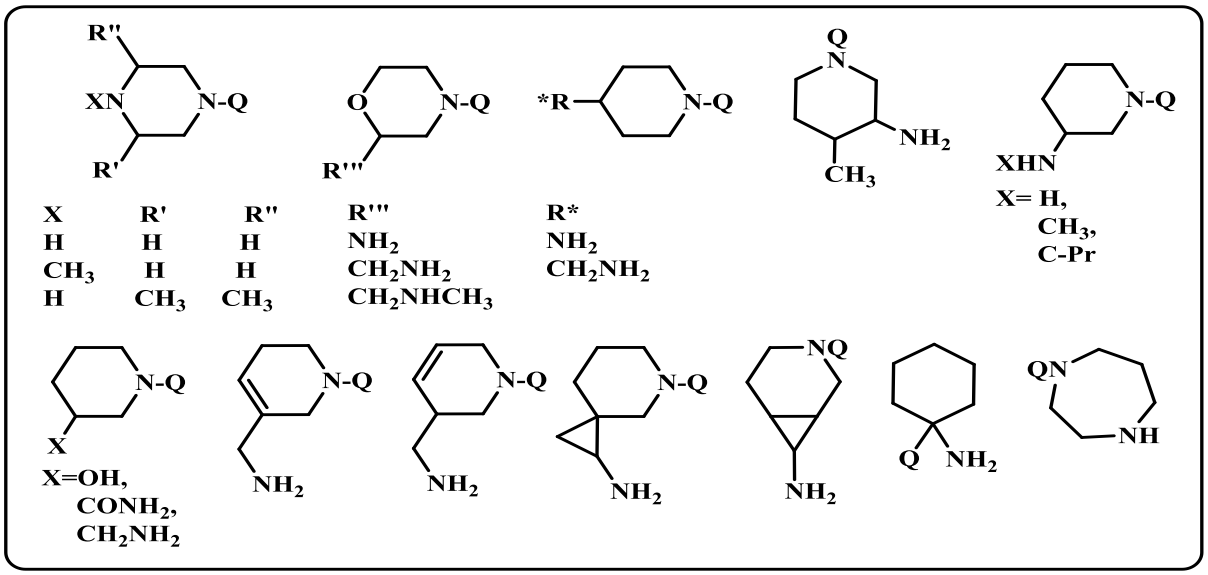


Figure-1.6: A list of cyclic amines substituted at C-7 position for various quinolones and related skeletons as reported by G. Lesher[7]. "Q" represents any one of the quinolones, naphthyridine, or pyridone ring systems.

Bicycloaminyl Moieties: Prominent quinolones with this structural feature included compounds **1.10, 1.20** and **1.21** discussed above. **Figure-1.7(a)** shows additional examples of such molecules.

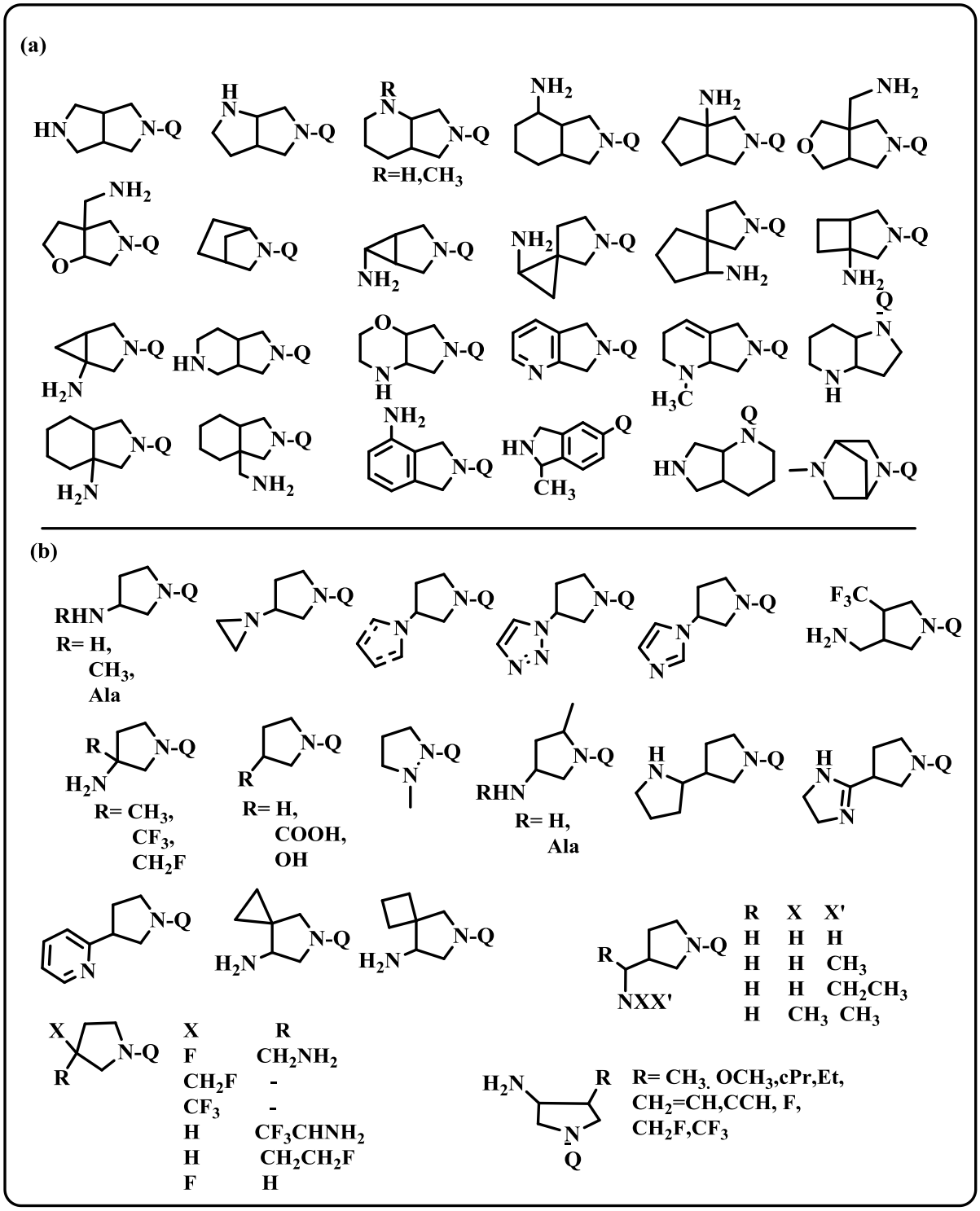


Figure-1.7: A list of (a) Bicyclicamines cyclic amines and (b) pyrrolidines and related moieties substituted at C-7 position for various quinolones [7]. "Q" represents any one of the quinolones, naphthyridine, or pyridone ring systems.

Pyrrolidinyl and Related Moieties: A large number of five-membered heterocyclic rings attached to C-7 position of various quinolones skelton have been reported. Compounds **1.9, 1.10** and **1.15** above are some of the examples discussed above and large number of other examples have been shown in **Figure-1.7(b)**.

Cyclobutylaminyl and Carbon-linked C-7 Substituents: Among the four membered rings substituted at C-7 position very limited number of molecules have been reported. A C-7 substituted four membered rings with 3-amino and 3-methyl amino substituents are less popular fluoroquinolones due to poor biological activity. No prominent quinolones available in market incorporate these moieties as yet.

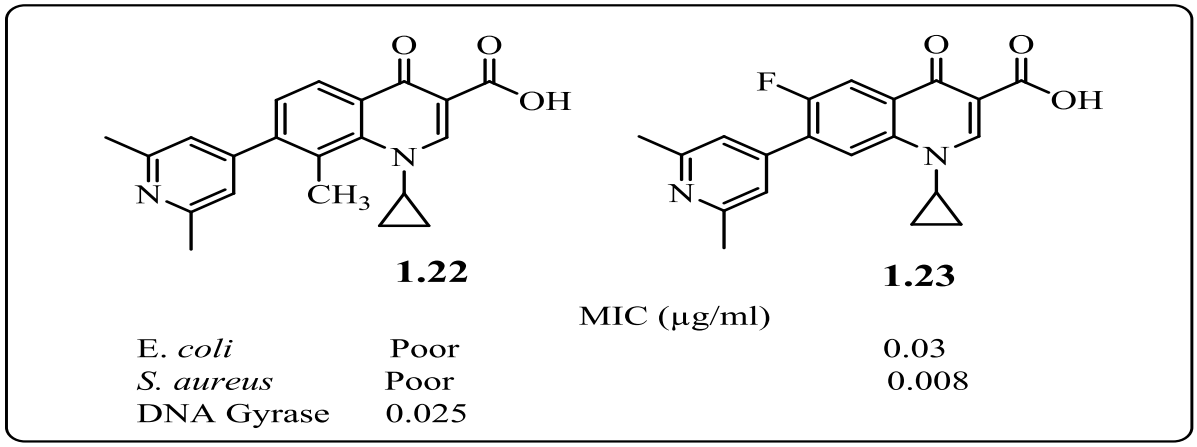


Figure-1.8: Structure of carbon substituted C-7 fluoroquinolones and their activity against bacteria and human gyrase and topoismerase due which they can be toxic to humans

The C-7 carbon linked substituent are also very limited due to their cumbersome synthesis. Most of these substituents are aromatic rings, and a significant number possess enhanced activity against Gram-positive microorganisms. Despite their potential activity against microorganisms, they cannot be used due to their toxic nature. This is due to the inhibition against human topoisomerase. **Figure- 1.5** and **Figure- 1.8** above show structure and biological activity of some of these molecules.

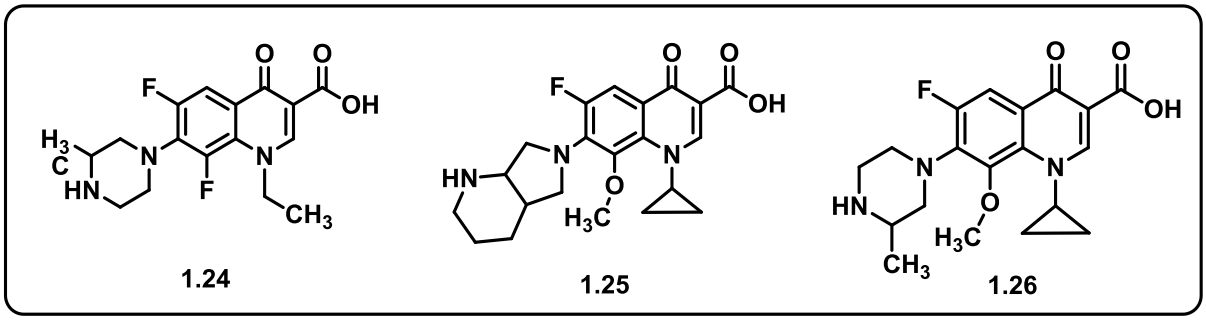


Figure-1.9: Structure of some C-8 substituted fluoroquinolones. With appropriate N-1 substituent the methoxy group increases the potency as compared to fluorine which is generally phototoxic.

Position C-8: The C-8 substituents supported by C-5 substituents play a significant role in determining the affinity of quinolones for DNA gyrase or topoisomerase IV. Fluorine at C-8 position enhances the antibacterial activity but this is also accompanied by phototoxicity and

mammalian toxicity. Compounds **1.12** above and **1.24 (Figure 1.9)** are examples of C-8-F-bearing quinolones.

An O-methyl group at C-8 position or a heteroatom in the aromatic ring like O or S in association with appropriate N-1 substituent increases the potency without compromising on photoxic effect as in case of compound **1.25** and **1.26 (Figure-1.9)**. In fact, compound **1.2** was the first effective C-8 bio isosteric replacement of CH by N. Compounds **1.9**, **1.10 (Figure- 1.2)**, **1.15** and **1.16 (Figure 1.4)** also replace CH at C-8 position by N. It has been reported that the replacement of C or H at C-8 is regularly reported with enhanced pharmacokinetic characteristics. Besides above, other groups tolerated at C-8 include O-ethyl, OH, OCH_2F, $OCHF_2$, OCF_3, and SMe [33].

Thus, it can be concluded that among antibacterial agents, this class of compounds are a saviour for the humanity as in the last five decades approximately twenty quinolone anti-infectives have been launched including ciprofloxacin **(1.4)** and levofloxacin **(1.6)**, that are present market leaders.

1.3 Action of Mechanism of Fluoroquinolones [33, 35, 36]

Fluoroquinolones are known to inhibit enzymes of bacteria that maintain the topology of their DNA. DNA gyrase and topoisomerase IV are both essential enzymes of type II topoisomerases family that operate in similar fashion. As shown in **Figure- 1.10,** both enzymes initially nick the strands of DNA and after the uncut part of the chain has passed through the nick, reseal it back. In the presence of fluoroquinolones, the DNA machinery of bacteria is unable to perform its essential tasks like transcription, translation, repair and storage processes due unorganised topology and this results in organism's death.

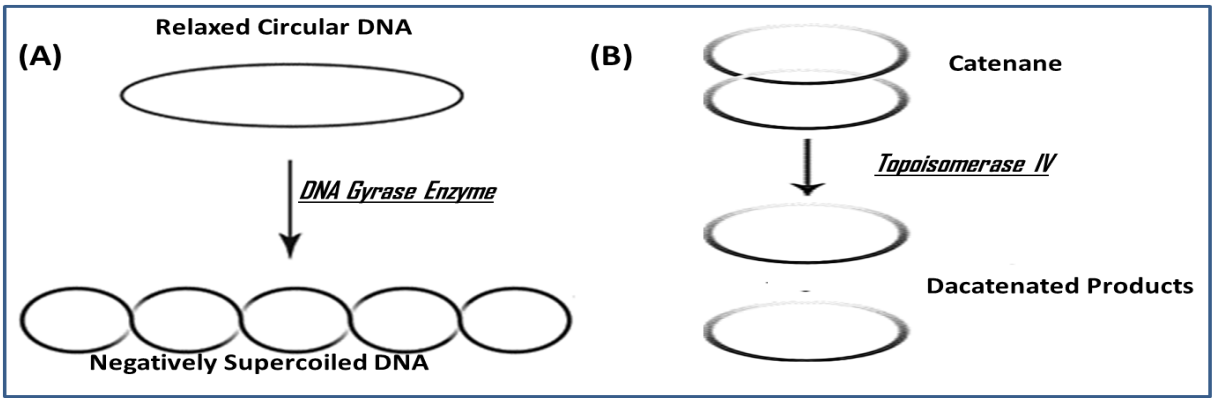


Figure- 1.10: ***(A)*** DNA gyrase converts relaxed circular DNA to negatively supercoiled DNA. **(B)** Topoisomerase IV separates catenated DNA that is produced during DNA replication[33].

Human topoisomerase II also binds to fluoroquinolones, but its affinity is 100 to 1000 times less as compared to that with homologous prokaryotic enzymes. Thus, these molecules do not cause any achievable poisoning in humans. For example, if bacterial DNA gyrase require 0.3 µM of ciprofloxacin **(1.4)**, the requirement for the same molecule for analogous enzyme in human system will be 300 µM.

It is worth mentioning that some molecules like anthracyclines and etoposides inhibit human topoisomerase II enzymes and are used in antitumor chemotherapy against cancer. However, for bacterial system higher dose of these drugs will be required. Thus, selective binding of the molecules for two different enzymes explains the difference in their functioning.

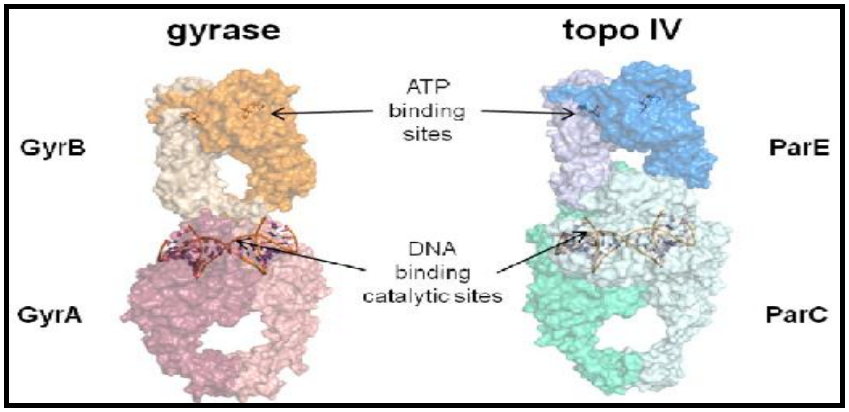


Figure- 1.11: ATP and DNA binding sites of DNA gyrase and Topoisomerase IV[33].

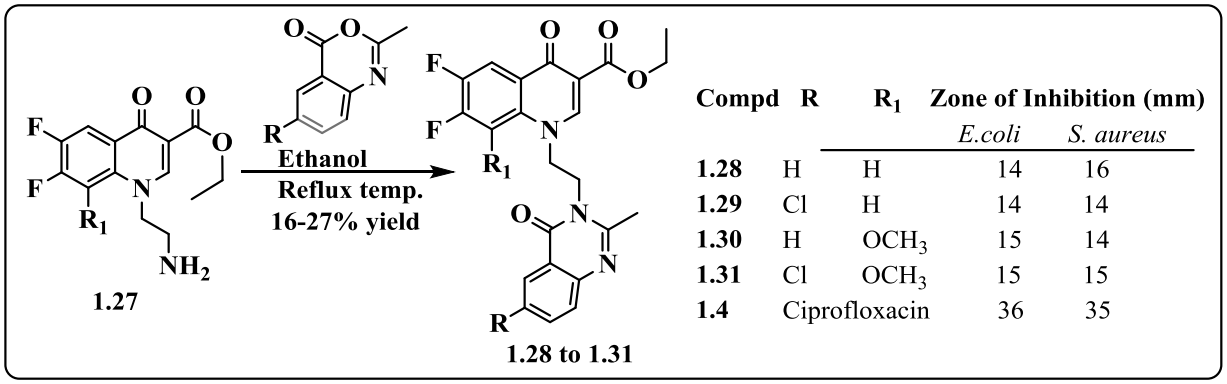
Figure 1.11 shows structure of two enzymes DNA gyrase and topoisomerase IV. Former is primary target of fluoroquinolones in Gram negative bacteria while topoisomerase IV in Gram positive bacteria. Both enzymes have two subunits which in case of gyrase are named as GyrA and Gyr B while in later are named as Par E and Par C. Bottom subunits in DNA gyrase and topoisomerase IV (GyrA and ParC) are binding sites of DNA for nicking and sealing while upper subunits (GyrB and ParE) bind with ATP.

1.4 Examples of Fluoroquinolones from Recent Literature

A brief review of some existing methodologies discovered in the past three decades is presented in the following paragraphs.

Reddy *et. al.* synthesized novel fluoroquinolones with 3-quinazolinones at N-1 position of the compound **1.27.** The 3-quinazolinones moiety was connected via two carbon linker as shown in **Figure-1.12**. The substituent at the C-7 position, in this case, was fluorine. *In vitro* evaluation of

the antibacterial activity against *E. coli* and *S. aureus* strains gave impressive results for all the synthesized compounds **1.28** to **1.31** as compared to standard compound ciprofloxacin **(1.4)** [37].



Compd	R	R_1	Zone of Inhibition (mm)	
			E.coli	*S. aureus*
1.28	H	H	14	16
1.29	Cl	H	14	14
1.30	H	OCH_3	15	14
1.31	Cl	OCH_3	15	15
1.4	Ciprofloxacin		36	35

Figure - 1.12: Novel fluoroquinolones with 3-quinazolinones at N-1 position synthesized by Reddy *et. al.*

Özdemir *et. al.* substituted derivatives of the 1,2,4-triazole**(1.32)** at the C-7 position of fluoro nalidixic acid **(1.33)** skeleton and evaluated antibacterial activity against s *Enterococcus faecalis, Candida albicans, Saccharomyces cerevisiae* and *Mycobacterium smegmatis* microorganisms **(Figure -1.13)**. The compounds **1.34** to **1.36** displayed moderate activity having MIC value in the range of 62- 125 μg/ml[38].

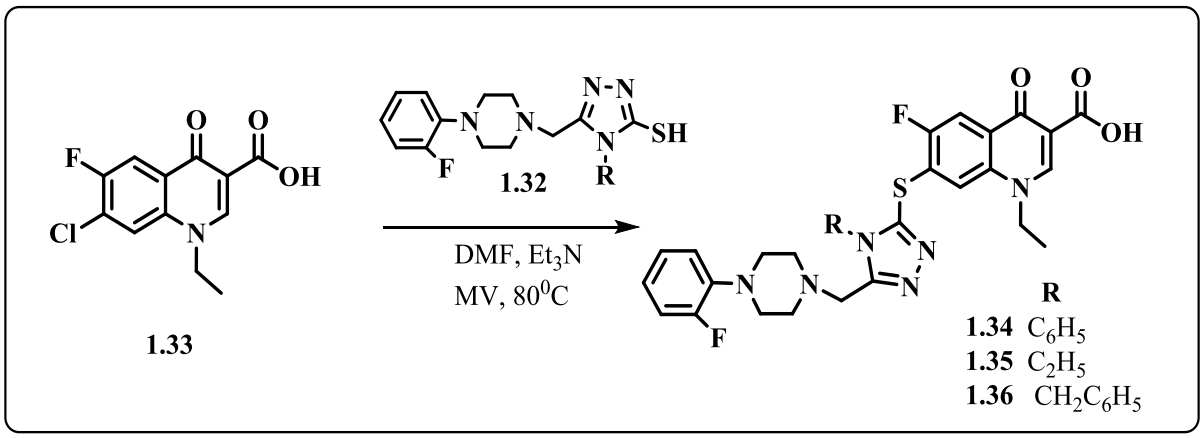


Figure-1.13: 1,2,4-triazole substituted fluoroquinolones derivatives by Özdemir et. al. gave moderate activity MIC values.

Masato Matsuoka *et. al.* synthesized a series of novel fluoroquinolones that contained four-membered cyclic ring bridging N1 and C-2 positions with sulfur planted in the skeleton **(Compound 1.37)** as shown in **Figure 1.14**. Racemic Q-acid obtained in this case was resolved into D and L isomers using HPLC and the C-7 position subsequently tagged with different heterocyclic rings. Hydrolysis was the last step before proceeding for the antibacterial activity against both gram-negative and gram-positive bacteria. The structure-activity relationship of the

compounds revealed order of antibacterial activity concerning C-7 substituents as piperazine > substituted Piperazine > hydroxyl azitizeno > Morpholino > thiomorpholie (not shown in Figure). MIC values for the N-methyl piperazine substituted D isomer **(1.38)**, L isomer **(1.39)** were determined against *Staphylococcus aureus* (0.2 µg/ml, 0.005 µg/ml, 0.1 µg/ml), *Enterococcus faecalis* (3.13 µg/ml, 0.2 µg/ml 1.56 µg/ml), *Escherichia coli* (0.1µg/ml, 0.0125 µg/ml, 0.025 µg/ml) and *Pseudomonas aeruginosa* (0.78 µg/ml,0.2 µg/ml,0.78 µg/ml) with ofloxacin as a reference drug[39].

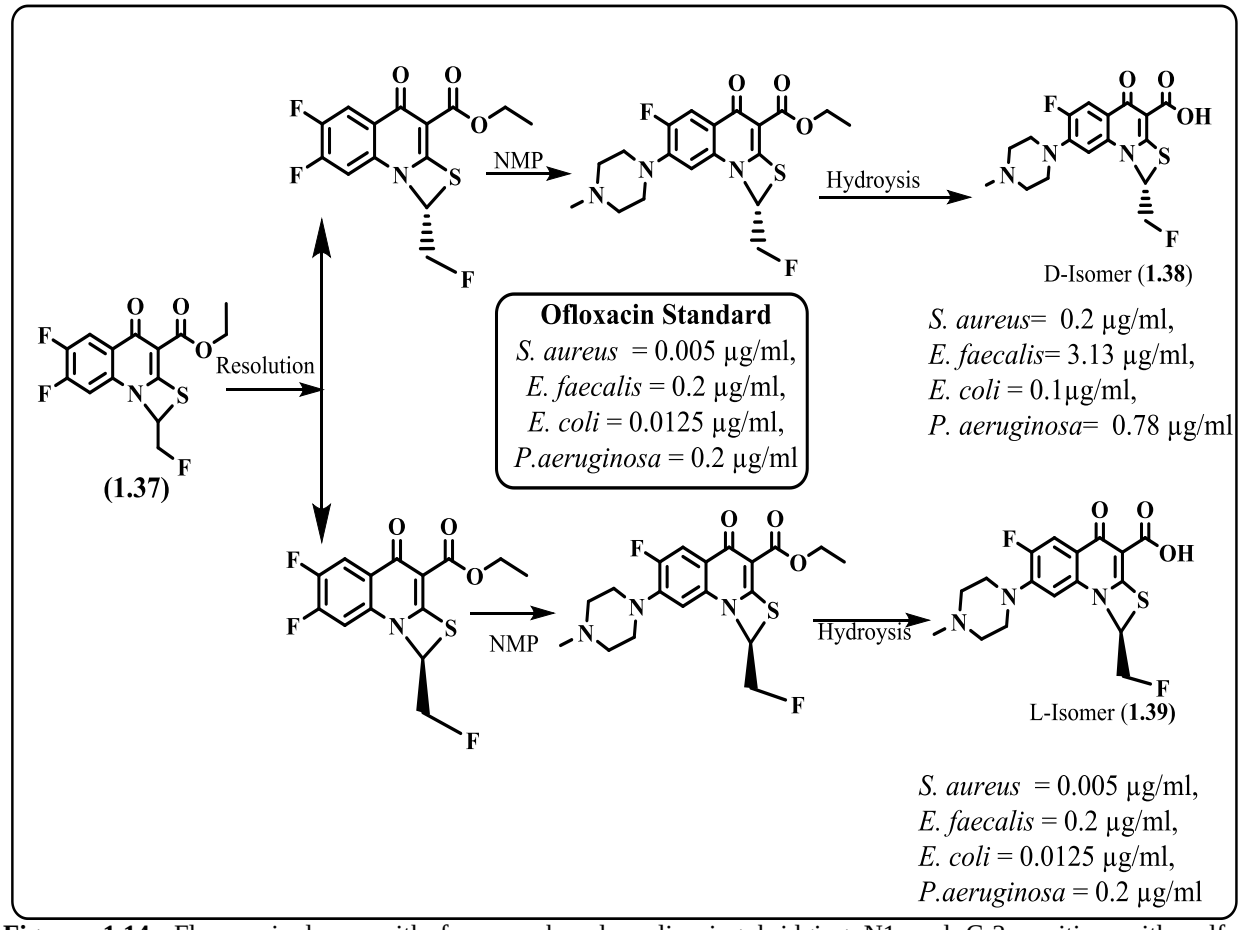


Figure -1.14: Fluoroquinolones with four-membered cyclic ring bridging N1 and C-2 position with sulfur synthesized Masato Matsuoka et. al.

Kiely *et. al.* synthesized and systematically studied four ciprofloxacin derivatives that differed in substitution at the C-5 (R_2) and C-8 (X & R_1) positions as shown in **Figure-1.15.** Thus, the structure of compounds **1.40** to **1.71** was varied by substituting a series of bicyclic compounds at the C-7 position of the parent molecule. The compounds were tested against the bacterial DNA gyrase, the target enzyme, and *S .pneumonia.* All gave nearly equal potencies as compared to parent 7-piperazinyl analogue. Only *endo*-7-(3-amino-8-azabicyclo [3.2.l] oct-8-yl) -l-cyclopropyl -6,8-difluoro -1,4-dihydro-4-oxo-3-quinoline carboxylic acid **(Grey, Figure 1.15)**,

possessed exceptional MICs and in-vivo potency for both Gram-negative (0.05 µg/ml against *E.coli*) and Gram-positive (0.03 µg/ml against *S .pneumonia*) organisms[40].

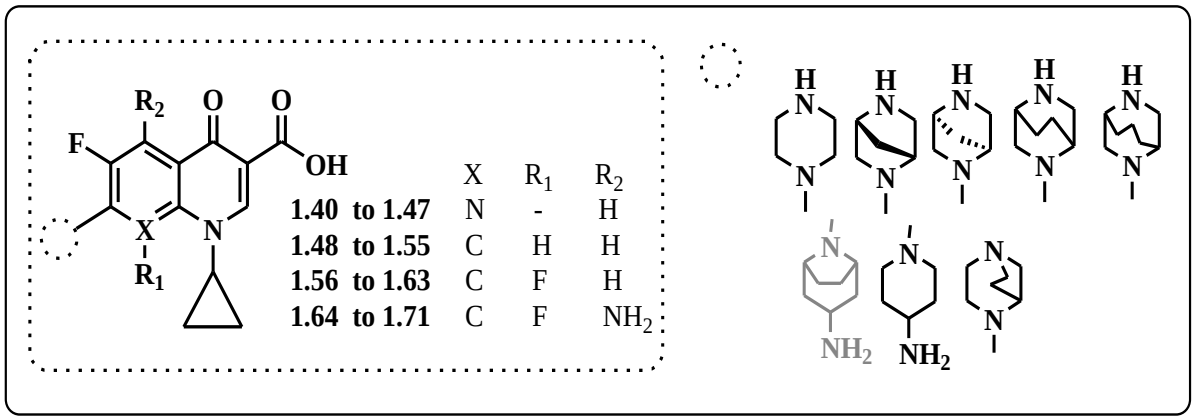


Figure-1.15: Systematic study carried out by Kiely *et. al.* on bicyclic C-7 substituted derivatives of ciprofloxacin.

Yusuf *et. al.* synthesized 8-nitrofluoroquinolone analogs of ciprofloxacin and norfloxacin by Chu-Mitscher protocol and substituted C-7 position with aliphatic and aromatic amines (**1.72** to **1.95)** as shown in **Figure-1.16**. Antibacterial activity of the synthesized compounds was found to be promising against core quinolones named 8-nitroCip and 8-nitroNor having MIC values of 0.97 and 1.2 µg/ml against *S. aureus* (ATCC 6538) and 4.7 and 8.8 µg/ml against *E. coli* (ATCC 8739)[41].

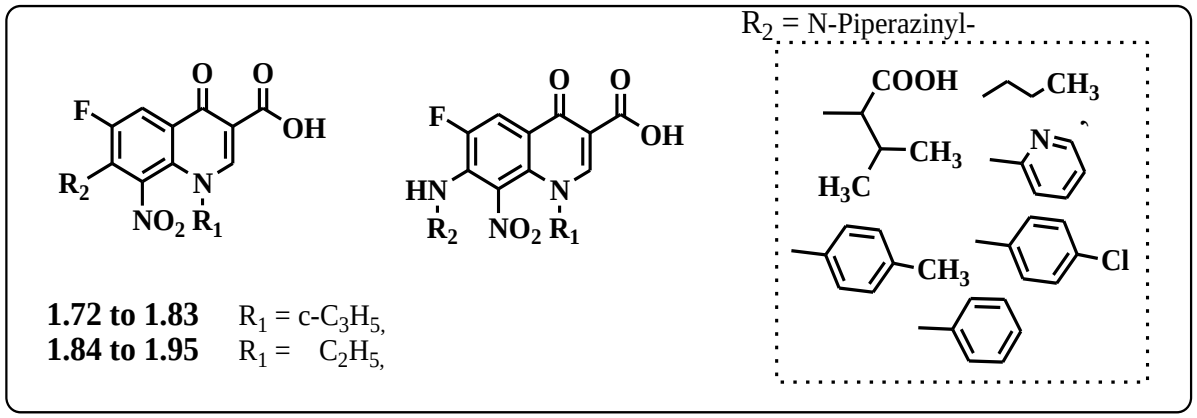


Figure -1.16: 8-Nitrofluoroquinolone derivatives synthesized by Yusuf *et. al. displayed antibacterial activity* against *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8739).

Lingaiah *et.al* synthesized a series of C-3 based amides **(1.96 to 1.99)** using ethyl ester of norfloxacin **(1.3)** with various α- amino acids as shown in **Figure 1.17.** Evaluation against *S. aureus, S. epidermis, B. subtilis, E. coli, P. aeruginosa* and *Klebsiella* showed that phenylalanine based amino acid derivative was found to be most active although overall MIC value was higher as compared to norfloxacin **(1.3)** and ciprofloxacin **(1.4)**[42].

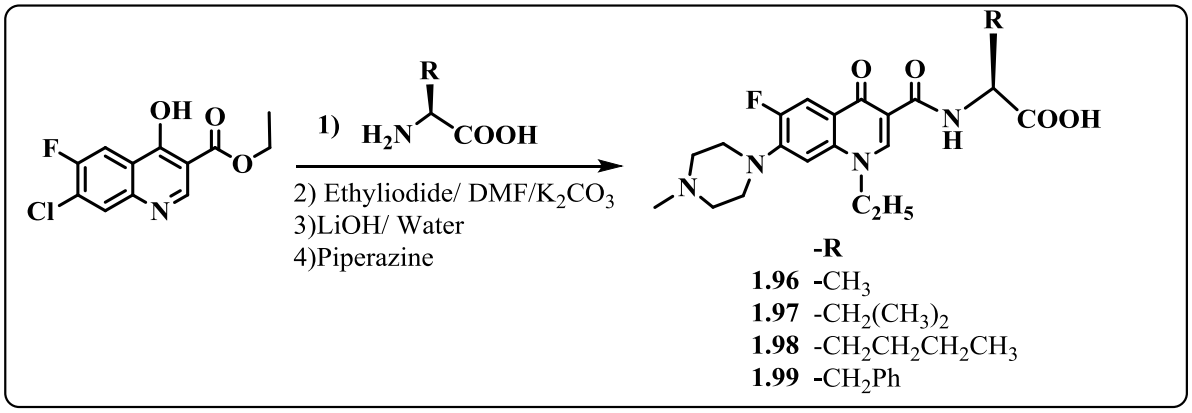
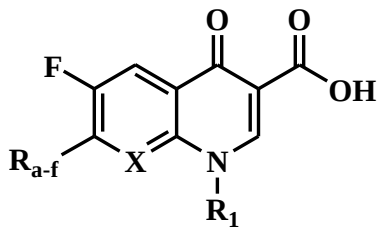


Figure -1.17: C-3 Based amides of norfloxacin ethyl ester using α-amino acids synthesized by Lingaiah

Renau *et. al.* compared the activity of seventy-two fluoroquinolones **(Figure-1.18, Compounds 1.100a-f to 1.111a-f series)** against *Mycobacterium fortuitum* and *Mycobacterium smegmatis* bacterial strains. The work demonstrated that the contribution of the C-8 position against the antibacterial activity of the above-mentioned strains is dependent upon N-1 substituents. With cyclopropyl group at the N1 position, C-8 groups fair as COMe'CBr > CCl > CH'CF'COEt > N > CCF_3 while replacement of cyclopropyl with 2,4-difluorophenyl gives activity in order of N'CH > CF > COMe. However, if N1 has t-butyl or ethyl groups, the C-8 position fairs as N > CH. Overall, the presence of the piperazine substituents at the C-7 was less potent than pyrrolidine substitutions against the said organisms[43].



$R_{a\ to\ f}$

a= Cyclopropyl, b= Ethyl,
c= tert Butyl, d= 2,4-difluorophenyl,
e= Piperazine, f= Pyrrolidine

Series	R_1	X
1.100a-f	C-C_3H_5	CF
1.101a-f	C-C_3H_5	CCl
1.102a-f	C-C_3H_5	CBr
1.103a-f	C-C_3H_5	CCF_3
1.104a-f	C-C_3H_5	COME
1.105a-f	C-C_3H_5	COEt
1.106a-f	C-C_3H_5	N
1.107a-f	CH_2-CH_3	N
1.108a-f	$(CH_3)_3C$	N
1.109a-f	2,4-F_2Ph	CF
1.110a-f	2,4-F_2Ph	COMe
1.111a-f	2,4-F_2Ph	N

Figure-1.18: Structure of fluoroquinolones viz. a viz N-1 to C-8 positions for activity against *Mycobacterium fortuitum* and *Mycobacterium smegmatis*.

J. Huang *et. al*. synthesized a novel series of naphthyridone derivatives having chiral fluoro cyclopropyl ring at N-1 position **(1.112 to 1.116, Figure 1.19)** . The series containing oxime-functionalized piperidines was evaluated against nineteen strains including methicillin-resistance *S.aureus* (MRSA), *S. epidermidis* (MRSE four strains). The synthesized compounds

displayed notable antibacterial activity and were found to be 2 to 250 times more potent (<0.008 to 32 µg/ml) than ciprofloxacin **(1.4)** or levofloxacin **(1.6)** (0.125 to 128 µg/ml)[44]

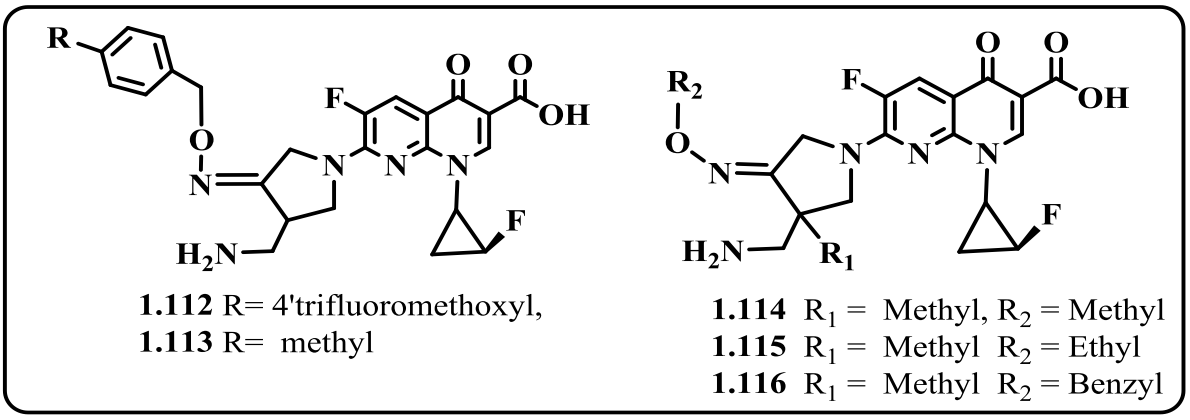


Figure- 1.19: A series of novel naphthyridone derivatives with fluoro substituted chiral cyclopropyl ring evalaued against MRSA strains

Patel and Patel synthesized norfloxacin derivatives with high molecular weight scaffolds piperazine derivatives and evaluated them against *S. aureus, S. pneumonia , E. coli and P. aeruginosa.* **Compound 1.117** in **Figure 1.20** shows one of the compounds in the series. It was observed that 4-nitro and 4-methoxy substituted phenyl rings at C-7 piperazin-yl side chain were the most potent compounds having MIC in the range of 12.5– 25μg/mL against Gram negative and 6.25–25μg/mL against Gram positive bacteria respectively[45].

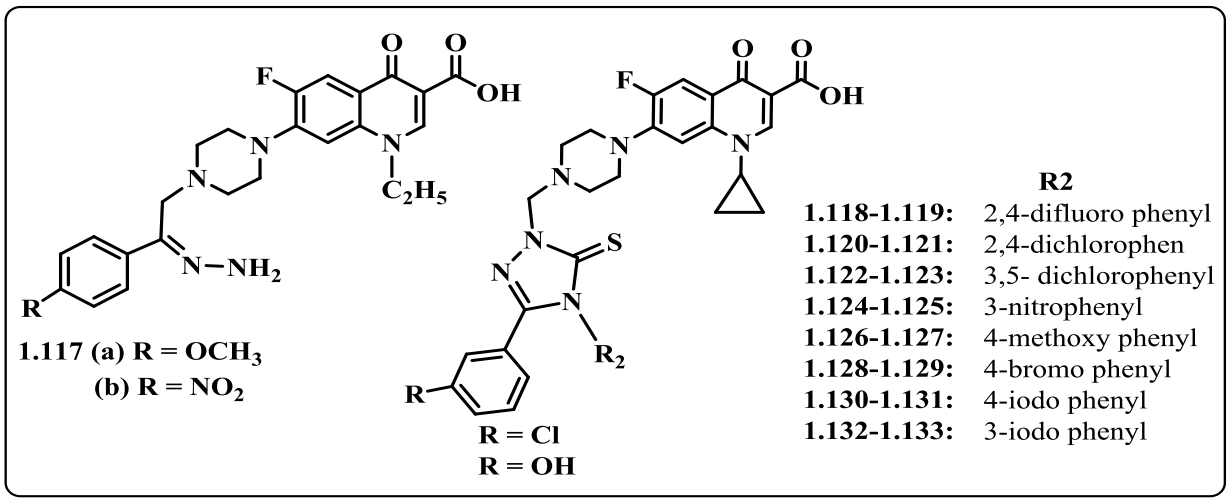


Figure-1.20: Norfloxacin **(1.117a &b)** and ciprofloxacin derivatives **(1.118-1.133)** with oxime and 1, 2, 4-triazole substituents synthesized by Patel et. al. and Patel and Plech et. al.

Plech *et. al.* designed and, synthesized 1, 2, 4-triazole substituted ciprofloxacin derivatives **(1.118-1.133)** and evaluated their antibacterial activity against *E.coli, P. Mirabilis, P. Aeruginosa, S.aureus, S. epidermis, B. Subtilis, B. Cereus, M. Luteus.* Among the 18 synthesized compounds, 11 displayed activity greater than the control, ciprofloxacin **(1.4)**. Most of the derivatives shown in **Figure 1.20** were potent[46].

K. Itoh *et. al.* reported C-7 substitution of the four-membered cyclic amines to the fluoroquinolone that had a difluoro amino pyridine group at N1 position **(Figure 1.21)**. The 7-(3-alkylamino azetidine-1-yl) fluoroquinolone is under clinical trials due to its ability to inhibit *E. coli* DNA gyrase with IC_{50} values ranging from 0.078 to 0.33 mg/L. MIC value of the compound containing t-butyl group was 0.038 mg/L[47].

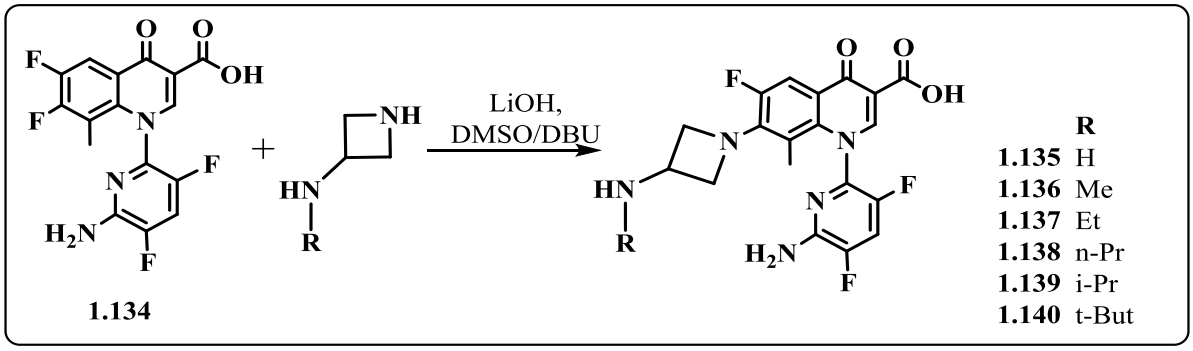


Figure -1.21: Fluoroquinolone molecule with C-7 four-membered ring having t-butyl group as R is in the clinical trial.

Baker *et. al.* synthesized a water-soluble fluoroquinolone with a phosphate group linked **(1.142)** to an N-1 substituted phenol **(1.141)**. This acted as a prodrug to highly active N-1-(4- hydroxyl benzene) substituted fluoroquinolone as shown in **Figure- 1.22**. The studies showed that the phosphate group increases the solubility of fluoroquinolones in water and confirmed that prodrug gets converted into the corresponding hydroxyl group[48].

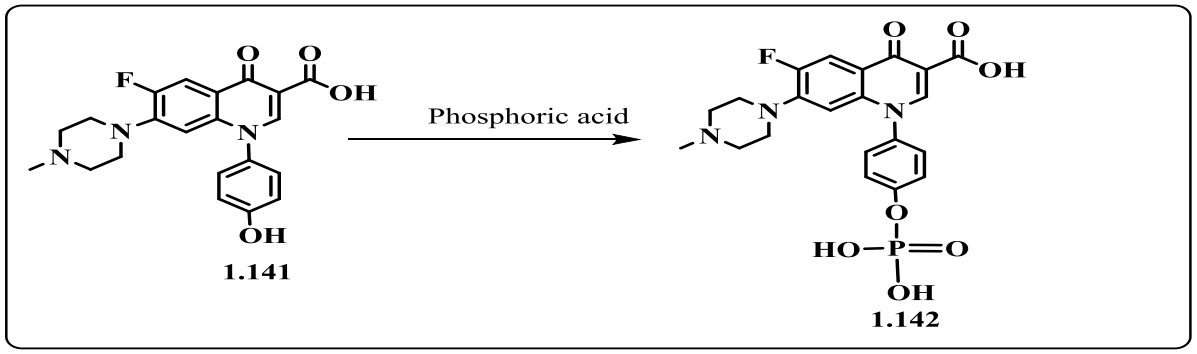


Figure- 1.22: Water-soluble fluoroquinolone with phosphate group acts as a prodrug releasing back the active molecule **1.141** was synthesized by Baker et. al.

Inspired by 3-(2-aminothiazol-4-yl) substituted cephalosporins **(1.143)**, Sheng- Feng Cui *et. al.* synthesized a quinolone to replace the C-3 carboxylic acid group with the 3-(2-aminothiazol-4-yl) motif. Compound **1.144** as shown in **Figure -1.23** exhibited potent antibacterial activity, low cytotoxicity to hepatocyte cells. It also displayed strong inhibitory potency to DNA gyrase and a broad antimicrobial spectrum that included multidrug-resistant strains also. Analysis of structure−activity relationships (SARs) disclosed that the 2-aminothiazole fragment at the 3-position of quinolone plays an important role in exerting antibacterial activity.[49]

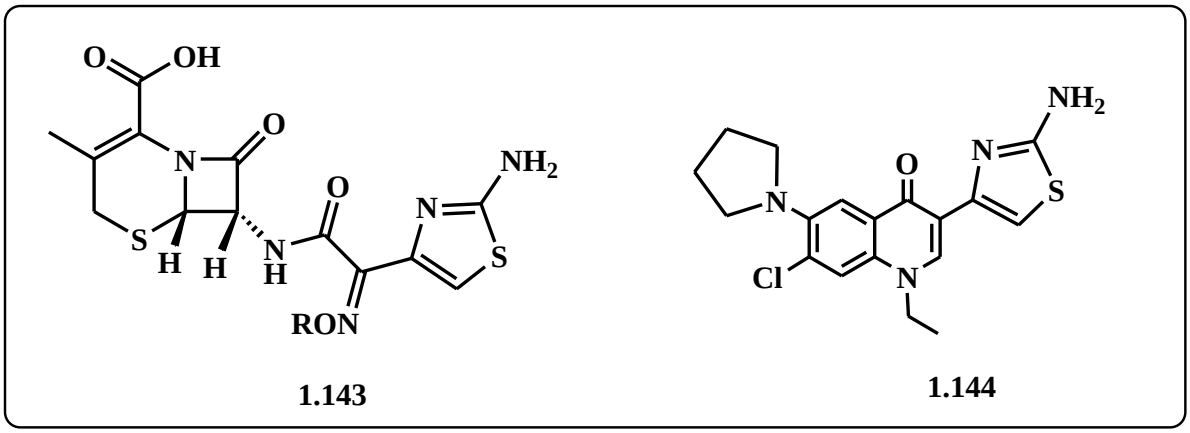


Figure 1.23: Inspired by 3-(2-aminothiazol-4-yl) substituted cephalosporins **(1.143)**, Sheng- Feng Cui *et. al.* synthesized a quinolone to replace the C-3 carboxylic acid group.

The above literature demonstrates the ongoing research in the vast field of fluoroquinolone molecules for their antibacterial activity. The application of such molecules for their anticancer activity has also been demonstrated. For example, 4-piperazineyl ciprofloxacin-chalcones and N-1 decyl chain substituted fluoroquinolones[50,51] reported by M. Abdel-Aziz *et. al.* and Kumar *et. al.* respectively demonstrated their use in anticancer activity. While the former demonstrated their use against nine tumor subpanels including leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancer cell lines the latter exhibited their activity against four human cancer cell lines. (HeLa, MDA-MB-231, MIA PaCa, IMR32) besides against *Mycobacterium tuberculosis* ($H_{37}Rv$).

1.5 Present Work and it's Objective

Despite vast research on fluoroquinolones, there is scope to further explore these molecules. **Figure 1.7** above, illustrates a large number of substituents at the C-7 position in different fluoroquinolones. It can be noted that the number of bridged substituents are very limited. As discussed above (**Figure-1.15)** Kiely *et. al.* systematically studied ciprofloxacin derivatives with different bridged molecules having variation in the substitution at the C-5 (R_2) and C-8 (X & R_1) positions. *Endo*-nortropine with an amino group among all the bridged bicyclic compounds gave exceptional MICs and *in-vivo* potency for both Gram-negative and Gram-positive organisms[40].

The present work utilized e*ndo*-nortropine with an alcohol group at C-7 position for different Q-acids to synthesize fluoroquinolones and evaluate their antibacterial activity. The work was initiated with the following objectives.

1. To synthesize nortropine substituted core fluoroquinolones derivatives and their analogs.
2. To carry the structural characterization of all synthesized compounds.

3. To assess in vitro antibacterial activity of the synthesized compounds against Gram positive and Gram negative bacteria.

The objectives were achieved starting with the basic quinolone skeletons (Q-acids) procured from the market. Endo-nortropine required for the purpose was synthesized by modification of a known procedure and linked to the C-7 position of the Q-acids. More than eighty fluoroquinolones with nortrpoine or its derivatives were synthesized. Purification of the synthesized compounds was done by column chromatography, solubility or crystallization as applicable before its characterization using ^{1}H and ^{13}C NMR, mass and elemental analysis. All the compounds reported in this work were evaluated for their antibacterial activity against Gram positive and Gram negative bacterial strains using ciprofloxacin and Levofloxacin as the standard.

The whole thesis has been divided into five parts. The present chapter (Chapter-1) describes the background of the work in terms of literature and mechanism of action. Chapter-2 describes the synthesis and antibacterial activity of endo-nortropine substituted at the C-7 position of various Q-acid. This is followed by Chapter-3 and Chapter-4 that describe the synthesis of non-polar and polar heterocyclic groups respectively on the hydroxyl of nortropine and their antibacterial activity. Finally in Chapter-5, *in-silico*, docking studies have been done for the biologically active compounds from each chapter to discuss their structure-activity relationship by docking with the target enzyme (DNA gyrase) of the *S.aureus*.

References

1. Pfaller, M.A.; Jones, R.N.; Doem, G.V. and Kugler, K. *Antimicrob. Agents Chemother.* **1998**, 42, 1762–1770.
2. Jones, R.N.; Low, D.E.; and Pfaller, M.A. *Diagn. Microbiol. Infect. Dis*. **1999**, 33, 101–112.
3. Swartz, M.N. Proceedings of the National Academy of Science USA, **1994**, 91, 2420–2427.
4. World Malaria Report **2015.** Geneva, World Health Organization, **2015** (ISBN: 978 9241565158).
5. World Health Organization **(2015)** Fact sheet on tuberculosis. http://www.who.int/mediacentre/factsheets/fs360/en/
6. Joseph, F.J.; Loretta, T.A.; Maple, P.A. H.; Momka, B. *Antimicrob. Agents Chemother.* **1992**, 36, 10, 2346-2348

7. Lesher, G.Y.; Froelich, E.J.; Gruett, M.D.; Bailey, J.H.; Brundage, R.P. *J Med. Pharm. Chem.***1962**, 91, 1063.
8. Albrecht, R. *Prog. Drug Res.* **1977**, 21, 9-104.
9. Koga, H.; Ito, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, 23, 1358-1363.
10. Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* **1983**, 23, 559-564.
11. Gerster, J.F.; Rohlfing; S.R.; Pecore, S.E.; Winandy, R.M.; Stern, R.M.; Landmesser, J.E.; Olsen, R.A.; Gleason, W. B. *J. Med. Chem.* **1987**, 30, 839-843.
12. Hayakawa, I.; Atarashi, S.; Yokohama, S.; Imamura, M.; Sakano, K.; Furukawa, M. *Antimicrob. Agents Chemother.* **1986**, 29, 163-164.
13. Chu, D.T.; Fernandes, P.B.; Claiborne, A.K.; Pihuleac, E.; Nordeen, C.W.; Maleczka, R. E., Jr.; Pernet, A. G. *J. Med. Chem.* **1985**, 28, 1558-1564.
14. Fernandes, P.B.; Chu, D.T.; Bower, R.R.; Jarvis, K.P.; Ramer, N.R.; Shipkowitz, N. *Antimicrob. Agents Chemother.* **1986**, 29, 201-208.
15. Rosen, T.; Chu, D.T.; Lico, I. M.; Fernandes, P.B.; Shen, L.; Borodkin, S.; Pernet, A. G. *J. Med. Chem.* **1988**, 31, 1586-1590.
16. Alghasham, A. A.; Nahata, M. C. *Ann. Pharmacother.* **1999**, 33, 48-60.
17. Roy, A.; Shah, S.; Shah, D.; Lodha, S.; Kalyankar, G. *Int. J. Curr. Pharm. Res.* **2009,** 1, 48-55.
18. Segawa, J.; Kitano, M.; Kazuno, K.; Matsuoka, M.; Shirahase,I.; Ozaki, M.; Matsuda, M.; Tomii, Y.; Kise, M. *J. Med. Chem*. **1992**, 35, 4727-4738.
19. Patel, N.B.; Patel, S.D.; Patel, J.N.; Patel, J.C.; and Gorgamw, Y.S.; *Int. J. Biolo. Chem.* **2011,** 5, 37-45.
20. Miyamoto, T.; Matsumoto, J.; Chiba, K.; Egawa, H.; Shibamori,K.; Minamida, A.; Nishimura, Y.; Okada, H.; Kataoka, M.; Fujita, M . *J. Med. Chem.* **1990**, 33, 1645-1656.
21. Imada, T.; Miyazaki, S.; Nishida, M.; Yamaguchi, K.; Goto, S. *Antimicrob. Agents Chemother.* **1992**, 36, 573-579.
22. Braveny, I.; Machka, K. *Arzneimittel-Forschung* **1980**, 30, 1476 -1478.
23. Corelli, F.; Massa, S.; Stefancich, G.; Artico, M.; Panico, S.;Simonetti, N. *Farmaco Sci*. **1984**, 39, 910-924.
24. Matsumoto, J.; Minami, S. *J. Med. Chem.* **1975**, 18, 74-79.
25. Kaminsky, D.; Meltzer, R. I. *J. Med. Chem.* **1968**, 11, 160-163.

26. Nagate, T.; Komatsu, T.; Izawa, A.; Ohmura, S.; Namiki, S.;Mitsuhashi, S. Antimicrob. *Agents Chemother.* **1980**, 17, 763-769.
27. Lumish, R. M.; Norden, C. W. *Antimicrob. Agents Chemother.***1975**, 7, 159-163.
28. Hayashi, K.; Takahata, M.; Kawamura, Y.; Todo, Y. *Arzneimittelforschung* **2002**, 52, 903-913.
29. Ledoussal, B.; Bouzard, D.; Coroneos, E. *J. Med. Chem.* **1992,** 35, 198 -200.
30. Hayashi, N.; Nakata, Y.; Yazaki, A. *Antimicrob. Agents Chemother.* **2004**, 48, 799-803.
31. Albini, A.; Monti, S. *Chem. Soc. Rev.* **2003**, 32, 238-250.
32. Domagala, J. M. J. *Antimicrob. Chemother.* **1994**, 33, 685-706.
33. Mitscher, L.A; *Chem. Rev.,* **2005**, 105, 559 -592.
34. Huang, X.; Zhang, A.; Chen, D.; Jia, Z.; Xingshu, *Bio. Med. Che. Lett.* **2010,** 20, 2859-2863.
35. Hooper, D. C.; Rubinstein, E. *Quinolone antimicrobial agents,* 3rd ed.; ASM Press: Washington, DC, **2003**.
36. Drlica, K.; Hiasa, H.; Kerns, R.; Malik, M.; Mustaev, A,; Zhao, X. *Curr Top Med Chem*, **2009**, 9, 981-998.
37. Reddy, N.; Rama Mohan H.; Rao, D. M. *Der Pharma Chemica, **2011***, 3, 440-448.
38. Özdemir ,S.B.; *J. Turk. Chem. Soc.* **2016**, 3, 515-534.
39. Matsuoka ,M.; Segawa, J.; Amimoto, I.; Masui, Y.; Tomii ,Y.; Kitano, M.; Kise, M. *Chem Pharm Bull (Tokyo),***1999**, 47, 1765-73.
40. Kiely, J.S.; Hutt, M.P.; Culbertson, T.P.; Bucsh, R.A.; Worth, D.F.; Lesheski, L.E.; Gogliotti, R.D.; Sesnie, J.C.; Solomon, M.; Mich, T.F. *J. Med. Chem.* **1991**, 34, 656-663.
41. Al-Hiari , Y.M.; Al-Mazari, I.S.; Shakya ,A.K.; Darwish R.M.; Abu-Dahab R.; *Molecules*, **2007**, 12, 1240-1258.
42. Lingaiah, B.P.V.; Yakaiah, T.; Chandra Shekhar, A.; Ravikumar, A.; Sathaiah, G.; Raju, K.; Rao, P.S.; Narsaiah B.; PranayKumar, K.; Murthy U.S.N.; Purushotham, U.; Sastry, G.N. *Indian J. Chem.* **2012**, 51B, 969-980.
43. Renau, T.E.; Gage, J.W.; Dever, J.A.; Roland, G.E.; Joannides,E.T.; Shapiro, M.A.; Sanchez, J.P.; Gracheck, S.J.; Domagala, J.M.; Jacobs,M.R.; Reynold, R.C. *Antimicrob Agents and Chemother*. **1996**, 40, 2363–2368.
44. Huang, J.; Liu, H.; Liu, M.; Zhang, R.; Li, L.; Wang, B.; Wang, M.; Wang, C.; Lu, Y. *Bioorg. Med. Chem. Lett*. **2015**, 25, 5058-5063.
45. Patel, M.M.; Patel, L. J. *Sci. World J.* **2014,** 897187. doi:10.1155/2014/897187

46. Plech, T.; Wujec, M.; Kosikowska, U.; Malm,A.; Rajtar, B.; Polz-Dacewicz, B. *Eur. J. Med. Chem.* **2013**, 60, 128-134,
47. Itoh, K.; kuramoto,Y.; Hirotaka,A.; Kazamori, D.; Yazaki , A. *Eur. J. Med. Chem.* **2015**, 103, 354- 360.
48. Baker, W.R.; Cai, S.; Dimitroff, M.; Fang, L.; Huh, K.K.; Ryckman, D.R.; Shang, X.; Shawar, R.M.; Therrien, J.H. *J. Med. Chem.* **2004**, 47, 4693-4709.
49. Cui,S.F.; Addla, D.; Zhou C.H. *J. Med. Chem.* **2016**, 59, 4488−4510.
50. Aziz, M.A; Park, S.E.; Gamal, E.D.; Rahama, A. A.; Sayed, M.A.; Kwon, Y. *Eur. J. Med. Chem.* ***2013***, 69, 427-438.
51. Ravi Kumar, A.; Lingaiah, B.P.V.; Rao, P.S; Narsaiah,B.; Sriram, D.; Sowjanya, P. *Indian J. Chem.* **2015**, 54B, 1495- 1501.

Synthesis of Nortropine Substituted Fluoroquinolones at C-7 Carbon and their Antibacterial Activity

2.1 Introduction

The reviews by Mitscher and Domagala have conferred that substituents at C-7 carbon in fluoroquinolone skeleton play an important part in increasing serum half-life and potency against Gram-positive bacteria[1,2]. This is also evident from the fact that most of the new fluoroquinolone molecules like norfloxacin **(1.3) (Figure 1.1)**, ciprofloxacin **(1.4),** sparfloxacin **(1.18) (Figure 1.2)**, gatifloxacin **(1.26) (Figure 1.9)** and many more have nitrogen-containing heterocyclic compounds at this position[3-7]. However, the literature shows very limited number of reports with bridged molecules at C-7 carbon[2]. Also, very limited exploration on cyclic amino alcohols attached at the position is visible in literature with Nadifloxacin[8] and Delafloxacin[9] perhaps the only two cyclic amino alcohol in the market **(Figure 2.1)**.

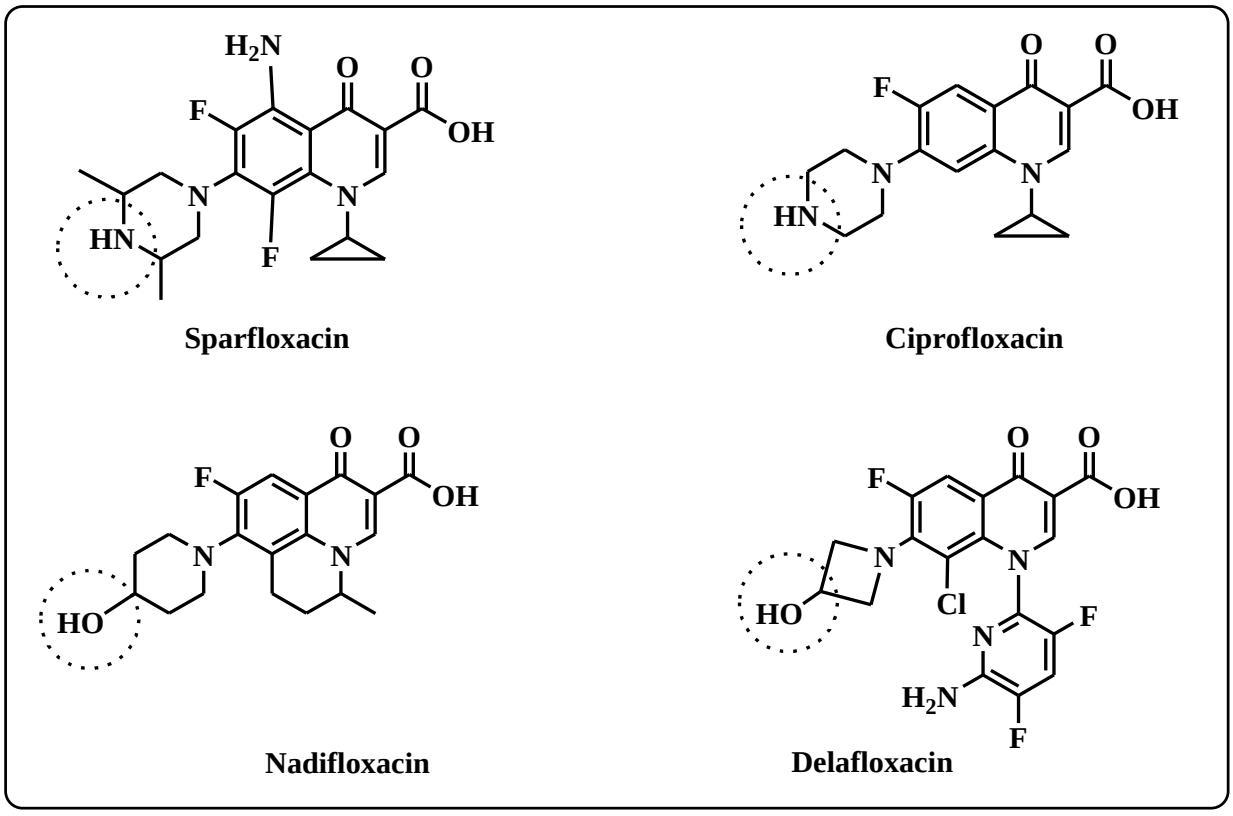


Figure 2.1: Although many nitrogen-containing heterocyclic fluoroquinolones (sparfloxacin and ciprofloxacin) at C-7 position are available in the market, a very limited number of molecules with bridged structure and cyclic amino alcohol (nadifloxacin and delafloxacin) at the same position are available.

Therefore, it was envisaged to incorporate fluoroquinolone skeleton with bridged amino alcohol at C-7 carbon. Many molecules containing derivatives of this alkaloid are used as mydriatics, antiemetics, antispasmodics, anesthetics and bronchodilators drugs[10-12]. **Figure 2.2** shows the structure of molecules used as anticholinergic drugs like homatropine and scopolamine. Tropine and nortropine are the fragments of naturally occurring alkaloids that have bridged structure. Among the two, later is an amino alcohol that was synthesized to attach at the C-7 position of the fluoroquinolones.

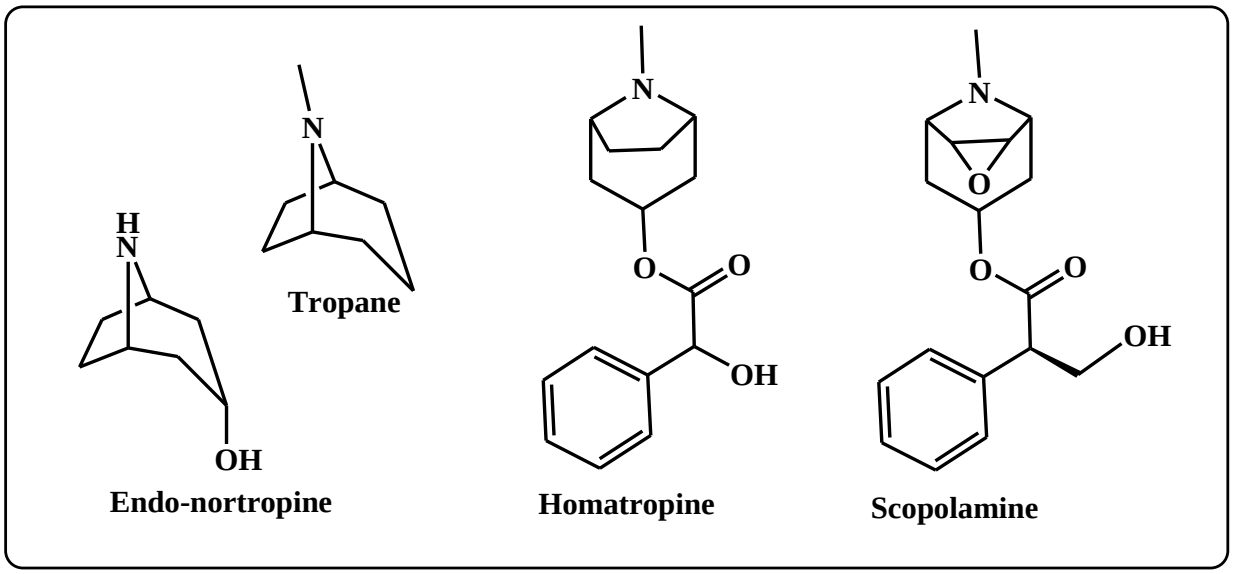


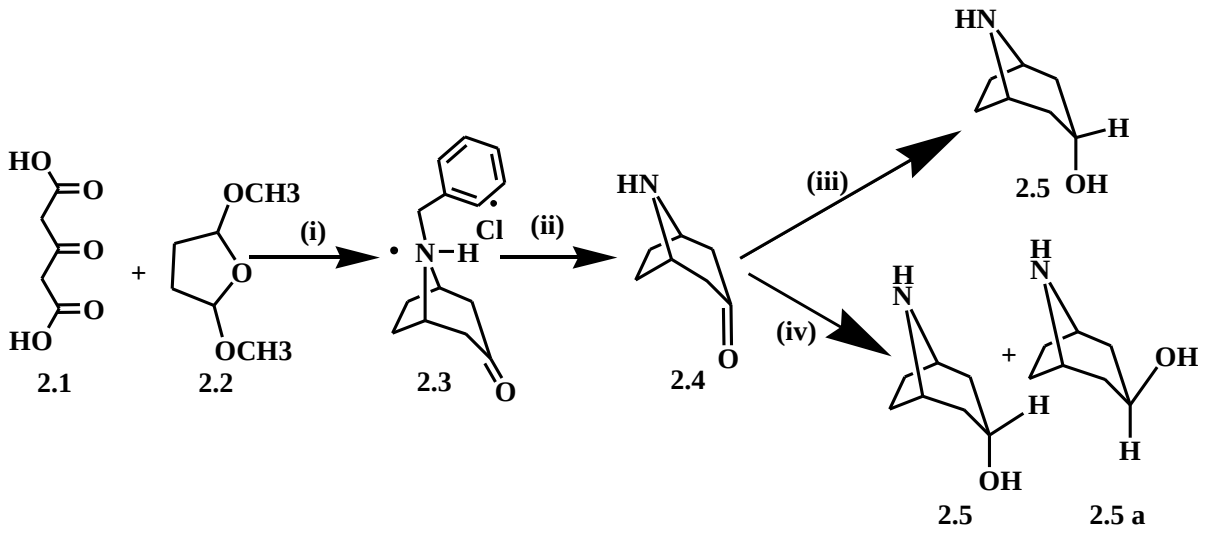
Figure 2.2: Tropine and nortropine are naturally occurring alkaloids having bridged structure. Endo-nortropine is a bridged amino alcohol. Structures of other drugs used as anticholinergic drugs.

2.2 Synthesis

This section describes the synthesis of starting materials that were used for the work described in subsequent chapters. Antibacterial activity of the synthesized fluoroquinolones has been described in the later part **(Section 2.3)** of this chapter. To facilitate the organisation of the work, this section has been divided into three subsections. **Section-A** describes the synthesis and characterization of nortropine molecules that were to be substituted at C-7 position of different fluoroquinolone. **Section-B** gives details of the synthesis and characterization of *endo*-nortropine substituted fluoroquinolones. **Section-C** describes the in-situ synthesis of two fluoroquinolone Q-acids that could not be procured from commercial sources.

SECTION – A: Synthesis of *Endo*-Nortropine and *Exo*-nortropine - Nortropine required for the purpose was synthesized by a known chemical route with slight modification **(Scheme-2.1)**. The natural source of the nortropine is the extract of Australian *Duboisia* and *Solanaceae*

plants.[11-12]. The reaction of 1, 3-acetone dicarboxylic acid **(2.1)** with 2,5-dimethoxy tetrahydrofuran **(2.2)** in the presence of benzyl amine hydrochloride and water at room temperature gave N-benzyl nortropinone hydrochloride **(2.3)** an 80 % yield. The debenzylation of compound **2.3** over Pd/C and hydrogen gave nortropinone **(2.4)** as a semisolid mass under basic pH conditions**.** Selective reduction of the ketone **(2.4)** to give *endo*-nortropine **(2.5)** required reduction over Raney Ni and hydrogen in methanol. Non-selective reduction of **2.4** using potassium borohydride in a mixture of water: methanol (70: 30) gave a mixture of *endo* and *exo* nortropine that was extracted in chloroform after aqueous workup at pH 6.5 to 7.5. The brown colored viscous mass was used as such without any separation. The required *exo* derivatives were separated at the next stage using column chromatography.



Reagents : (i) Benzylamine hydrochloride, Na_2HPO_4, HCl, 25^0C, 24 hrs, (ii) Pd/C, H_2, Liq. ammonia, RT, 24 hrs, (iii) Raney nickel, H_2,25^0C, 24 hrs, (iv) $NaBH_4$, Water, Methanol,Acetic acid, Liq. NH_3, $CHCl_3$, 3 Hrs, 25^0C

Scheme- 2.1 Synthesis of *Endo* and *Exo*-Nortropine

Characterization of the final compounds and the intermediate products was done by ^{1}H, ^{13}C NMR spectroscopy and mass characterization techniques except for the mixture of *endo* and *exo*-nortropine **(2.5 + 2.5 a)** that was used as such for the next step. For compound **2.3**, ^{1}H NMR indicated five protons at δ 7.49 ppm and a singlet due to two protons at δ 4.27 ppm corresponding to benzyl group and methylene (-CH_2-) respectively. Further, the presence of ten protons in the aliphatic region indicated the bicyclic ring. The presence of carbonyl carbon for the ketone in the nortropinone was confirmed by ^{13}C NMR with the characteristic peak at δ 210 ppm along with nine carbons, four of the bicyclic ring and five of aromatic benzyl group. The methylene carbon (-CH_2-) in this case appeared at δ 55.16 ppm. The mass spectrometry gave m/z

value of 216 for a peak corresponding to [M+1] for the compound **2.3**. Formation of compound **2.4** was corroborate by disappearance of aromatic protons due to absence of benzyl group and peaks at δ 4.27 ppm and δ 55.16 ppm corresponding to methylene (-CH_2-) both in ^{1}H and ^{13}C NMR spectra. Compound **2.5**, obtained via Raney nickel reduction, was confirmed by the absence of carbonyl carbon in ^{13}C NMR at δ 207 ppm and simultaneous appearance of broad signals for 2 protons in ^{1}H NMR at δ δ 2.7 ppm due to –NH- and –OH groups respectively. This was further confirmed by mass spectrometry giving a peak at m/z value of 128 corresponding to [M+1] value.

SECTION – B: Synthesis of Nortropine Substituted Fluoroquinolones: The work in the thesis deals with the synthesis of seven new fluoroquinolones starting from corresponding difluoroquinolone acids (Q-Acid) as shown in **Figure-2.3**. Five of the seven Q-acids (**2.6** – **2.10**) were procured from the commercial sources while two (**2.11 - 2.12**) were synthesized by modification of known procedures[13, 14].

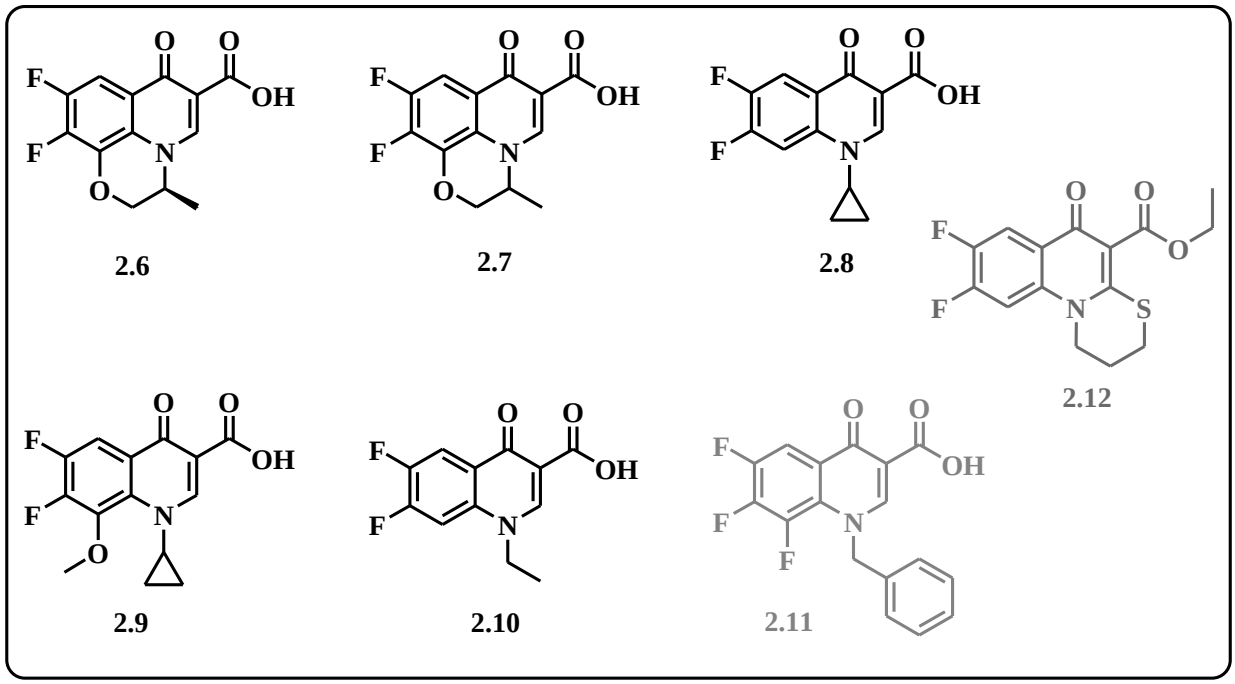
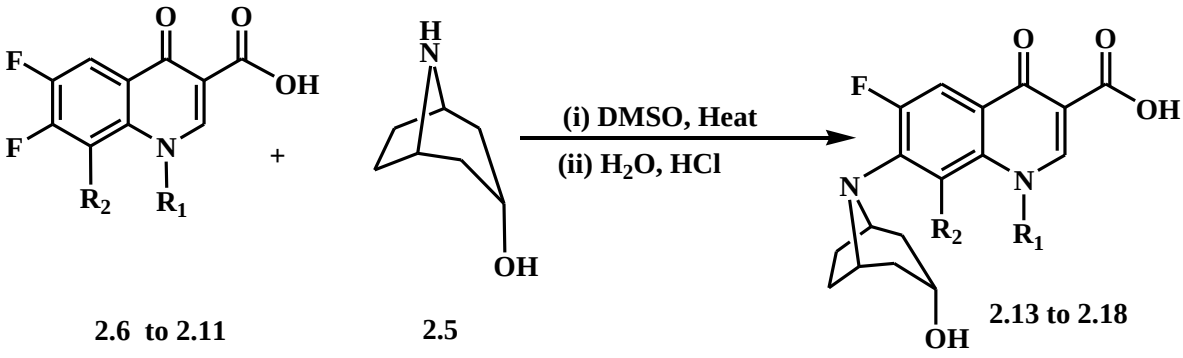


Figure 2.3: Structure of seven difluoroquinolone acids (Q-Acid) used in the study. Compounds 2.6 to 2.10 were procured from commercial sources while compounds 2.11 and 2.12 were synthesized by known procedure.

The desired compounds **(2.13 to 2.19, Scheme 2.2)** were synthesized by a common procedure that involved heating and stirring of the corresponding Q-acid in the presence of *endo*-nortropine **(2.5)** at the desired temperature using DMSO as solvent (**Table 2.1)**. Progress of each reaction was monitored by TLC using a mobile phase of chloroform and methanol in the ratio of 9:1. Workup, after cooling, involved the addition of water to the reaction mixture followed by maintaining the pH to get solid mass that was filtered and then crystallized from methanol in

moderate to good yields. **Table 2.1** details the condition required for an individual reaction while **Scheme 2.2** describes the general reaction conditions.



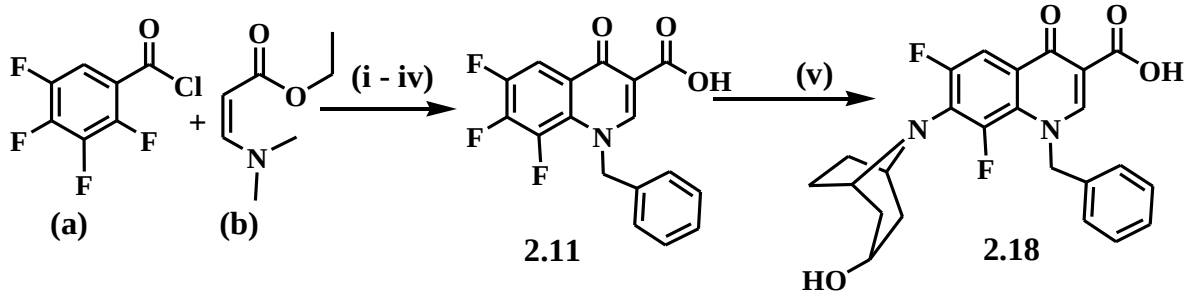
Reactant		Product
2.6	R_1 = N1-*CH(CH_3)CH_2O-C8 =R_2	**2.13**
2.7	R_1 = N1-*CH(CH_3)CH_2O-C8 =R_2	**2.14**
2.8	R_1 = C_3H_5 R_2 = H	**2.15**
2.9	R_1 = C_3H_5 R_2 = OCH_3	**2.16**
2.10	R_1 = C_2H_5 R_2 = H	**2.17**
2.11	R_1 = $CH_2C_6H_5$ R_2 = F	**2.18**

Scheme - 2.2: Reaction conditions for the synthesis of *endo*-nortropine substituted fluoroquinolone

Table-2.1: Conditions for the Synthesis of *Endo*-Nortropine Substituted Fluoroquinolones.

Substrate	R_1	R_2	Temp (°C)	pH	Product	Yield (%)	Melting Pt. (°C)	Mass (M+1)
2.6	-*CH(CH_3)CH_2O-	-	160	2.0-2.5	**2.13**	82.4	270 (dec.)	389
2.7	-CH(CH_3)CH_2O-	-	160	2.0-2.5	**2.14**	79.1	245 (dec)	389
2.8	-C_3H_5	H	160	4.0-4.5	**2.15**	67.36	282-285	373.4
2.9	-C_3H_5	OCH_3	110	4.0-4.5	**2.16**	47.3	258-261	403.3
2.1	-C_2H_5	H	160	4.0-4.5	**2.17**	64.1	301-305	361.2
2.11	-$CH_2C_6H_5$	F	160	4.0-4.5	**2.18**	47.3	245-248	441.3
2.12	–$CH_2CH_2CH_2$S-C2	H	110	6.0-6.5	**2.19**	60	276-280	405.2

Section – C: Synthesis of compounds 2.18 and 2.19: Synthesis of tri fluoroquinolone acid **2.11** was done by modification of Chu- Mitscher synthesis[16] as reported in literature[17]. 2,3,4,5-Tetra fluorobenzoyl chloride and ethyl -3-N-(dimethyl amino) acrylate were heated in the presence of a base in toluene at 50-55° C followed by addition of benzylamine and stirring at room temperature and finally heating at 90°C in DMSO to get N-benzyl substituted quinolone ethyl ester (**Scheme 2.3)**. The ester after workup was subjected to de-ethylation to get trifluoro-Q-acid **2.11**. Finally, as described above heating in the presence of *endo*-nortropine in DMSO gave compound **2.18** as solid when neutralized with hydrochloric acid **(Scheme-2.3)**. Due to the established protocol of the synthetic route only the final product was analyzed in this case using spectroscopic techniques as described in **Tables 2.2** and **2.3**.



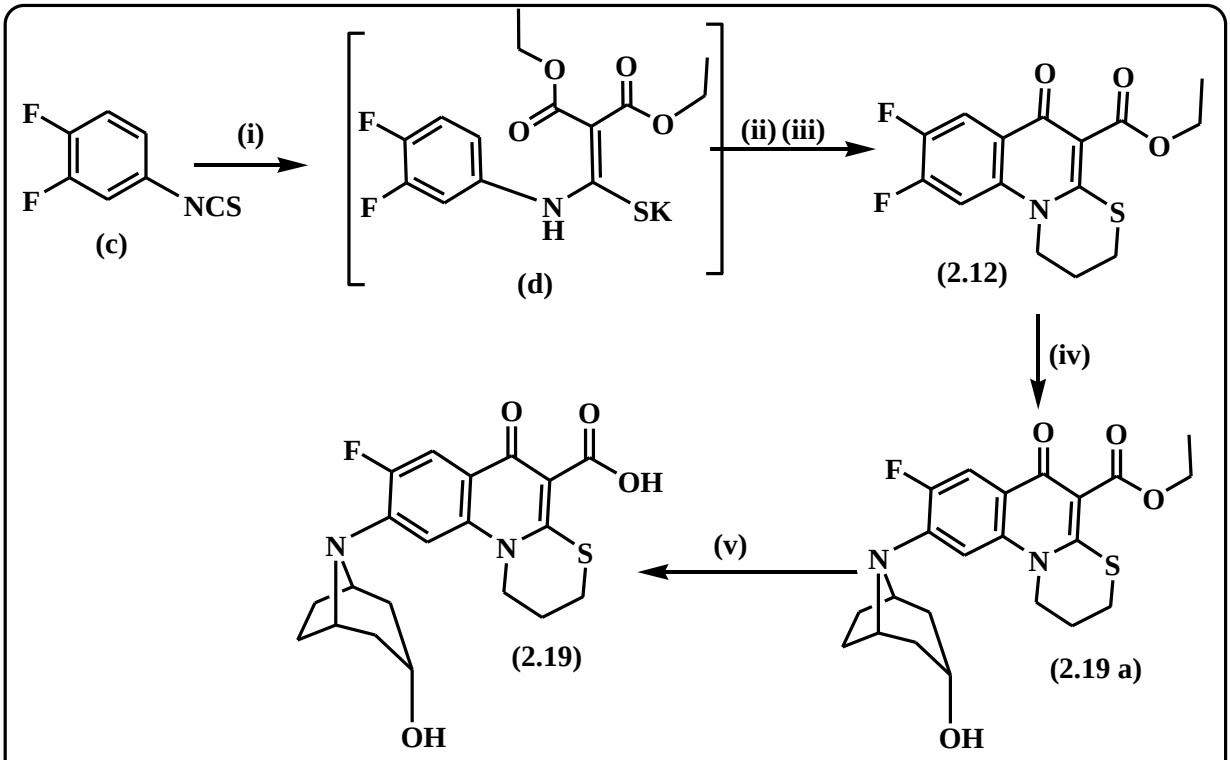
(i) Toulene, Triethyl amine, 55^{o}C; (ii) Benzyl amine, Toulene, 25^{o}C (iii) DMF, K_2CO_3, 90^{o}C (iv) H_2SO_4 Water, Acetic Acid, 50 -60 ^{o}C (v) *Endo*- nortropine, DMSO, 160^{o}C ; HCl and Methanol

Scheme-2.3 : Methodology to synthesize compound - 2.18 by Chu-Mitscher Synthesis

Synthesis of compound **2.19** was done starting from of 3,4-difluorophenyl isothiocyanate **C** that was made to react with diethyl malonate in toluene using potassium hydroxide as a base to get thiolate **D (Scheme 2.4)**. Compound **D** was proceeded further, based on change in TLC from the starting reactants, for the formation of six member cyclic ring containing nitrogen and sulphur using 1,3-dibromopropane in the presence of potassium carbonate and solvent ethyl acetate. The obtained product was subjected to quinoline ring formation, after usual workup and evaporation of the solvent, by refluxing at 100°C in the presence of polyphosphoric acid. The addition of water gave solid residue **2.12** that was characterized using NMR and mass spectroscopic techniques.

Unlike in other cases, where nortropine derivative was synthesized from Q-acids, in this case, ethyl ester of Q-acid **2.12** was substituted at C-7 position with *endo*-nortropine **(2.5)** using

DMSO at 110°C. *Endo*-nortropine substituted cyclic thio compound **(2.19a)** was then de-ethylated by refluxing in the presence of sodium hydroxide to get corresponding final product **2.19** as shown in **Scheme-2.4**.



Reagents : (i) Diethyl malonate, KOH, 1,4-dioxane (ii) 1,3-Dibromopropane, K_2CO_3, Ethyl Acetate (iii) Polyphosphoric Acid, 100^oC (iv) *Endo*-nortropine, DMSO, 110^oC (v) NaOH, Methanol.

Scheme-2.4 : Synthesis of sulphur bridged fluoroquinolone derivative

Characterization: All the synthesized compounds, **2.13-2.18**, were characterized by ^1H and ^{13}C NMR, Mass spectroscopic analysis. The only optically active compound in the series was compound **2.13** having an optical rotation of - 89° in dichloromethane at a w/v concentration of 1%. **Table 2.2** shows peaks due to similar protons present in all the molecules like carboxylic acid and C-2, C-5 and C-8 carbons in ^1H NMR. All the δ values and coupling constants were in the same range as expected from their structural. Similarly, **Table 2.3** shows all regular carbons like carboxylic acid, C-2, C-4 and C-6 carbons having δ values and the coupling constants as expected of their structures. In the case of fluoroquinolones, the splitting of carbon signal due to coupling with C-6 flourine has been well known in literature[15].

Table-2.2: Prominent δ (ppm) value for compounds **2.13** to **2.19** as seen in ^{1}H NMR

Compound Number							
Position	**2.13**	**2.14**	**2.15**	**2.16**	**2.17**	**2.18**	**2.19**
-COOH	15.36	15.32	15.38	15.50	15.57	14.99	17.11
C-2 (H)	8.81	8.80	8.61	8.68	8.85	9.03	-
C-5 (H) (doublet)	7.53-7.50 J=13.8 Hz	7.49- 7.45 J=13.8 Hz	7.83-7.79 J = 14.5 Hz	7.69-7.66 J =14.1 Hz	7.85-7.81 J=14.7Hz	7.79-7.75 J=13.7Hz	7.81-7.78 J=14.4Hz
C-8 (H) (doublet)	- -	- -	7.33-7.32 J=7.6 Hz	- -	6.92-6.91 J=7.5Hz	- -	6.98-6.97 J=7.0Hz

Besides these, chiral and racemic methyl groups in case of compounds **2.13** and **2.14** were observed at δ 1.52 and 1.48 ppm respectively. Single proton due to cyclopropyl ring in case of compounds **2.15** and **2.16** was observed at δ 3.73 and 4.12 ppm respectively. Downfield shift in case of later was due to effect of C-8 methoxy carbon that itself appeared at δ 3.64 ppm. Similarly methylene protons in case of compounds **2.17** and **2.18** appeared at δ 4.52 and 5.79 ppm due to their attachment with N-1 nitrogen of the skelton. Terminal methyl group appeared at δ 1.43 ppm in case of compound **2.17**. Carbons due to methyl group in case of compounds **2.13** and **2.14** appeared at approximately same position of δ 17.98 and 17.97 ppm respectively.

Table 2.3: Prominent δ (ppm) value for compounds **2.13** to **2.19** as seen in ^{13}C NMR

Compound Number							
Position	**2.13**	**2.14**	**2.15**	**2.17**	**2.16**	**2.18**	**2.19**
-COOH	166.0	166.41	166.23	166.41	166.90	165.77	165.54
C-2	145.22	145.44	147.01	147.80,	149.89	145.78	148.25
C-4 (C=O) Doublet	176.18, 176.15 J = 3Hz	176.14 176.10 J = 4Hz	175.99 176.10 J = 4 Hz	175.83, 175.80 J = 3.4 Hz	176.10, 176.07 J = 3.2 Hz	176.29, 176.24 J = 5 Hz	169.75, 169.72 J = 3 Hz
C-6 (C-F) Doublet	154.02 151.58 J =244 Hz	154.01 151.57 J = 244Hz	154.01 149.52 J =246 Hz	151.85 149.38 J = 247 Hz	156.30 153.83 J = 248 Hz	154.13 151.54 J= 259Hz	150.73 148.74 J = 200Hz

^{1}H and ^{13}C NMR and mass spectra of some of the representative compounds have been shown at the end of this chapter.

Characterization of Section C synthesised compounds:

^{1}H NMR of ethyl ester Q-acid 2.12 was in line with its proposed structure showing two aromatic signals at δ 7.8 and 6.6 ppm and a triplet and quartet at δ 1.4 and 4.4 ppm for methyl and methylene protons of the ethyl ester respectively. Triplets at δ 4.1 and 3.0 ppm represented methylene protons adjacent to bridged sulphur and nitrogen respectively. Multiplet at δ 2.4 ppm for methylene protons confirmed presence of two above described adjacent protons. ^{13}C NMR displayed two carbonyls at δ 169.7 and 165.5 ppm, the former showing coupling due to C-6 fluorine. All five aliphatic carbons were observed at δ 60.51, 45.21, 26.80, 23.31, 13.20 ppm. Compound **2.19a**, obtained after substituting *endo*-nortropine at C-7 position of the compound **2.12** gave signals for the bicyclic ring in both ^{1}H and ^{13}C spectra's along with peaks observed in previous compound. A triplet corresponding to *exo*-proton, geminal to hydroxyl group, of nortropine ring was observed at δ 4.1 ppm. Both *exo* and *endo*- protons of two methylenes adjacent to hydroxyl bearing carbon of bicyclic rings were observed separately at δ 2.2 and 2.1 ppm. Similarly, nine aliphatic carbon signals due to bicyclic ring, N1- S2 bridged six membered rings and ethyl group were also observed in ^{13}C NMR at δ 63.93, 60.50, 54.6, 45.20, 35.75, 26.79, 24.82, 23.33, 13.22 ppm. Formation of compound **2.19** was confirmed by absence of peaks due to ethyl group in both ^{1}H and ^{13}C NMR along with mass value at [M+1] m/z value of 405.

2.3 Antibacterial activity

Agar diffusion method: All the synthesized compounds **2.13 to 2.19** shown in **Table 2.1** above were screened for their antibacterail acitvity against Gram positive and Gram Negative bacteria by agar diffusion method. Gram negative bacteria selected for the study were *Pseudomonas aeruginosa* (MTCC 3904) , *Escherichia coli* (MTCC 1687) and *Vibrio cholerae* (MTCC 1934) while Gram positive bacteria were *Staphylococcus aureus* (MTCC 1430) and *Bacillus subtilis* (MTCC 441). Levofloxacin and ciprofloxacin, well known fluoroquinolones, were taken as control.

None of the compounds gave impressive antibacterial activity against *Pseudomonas aeruginosa* (MTCC 3904) and therefore has been eliminated from the date here. **Table 2.4** shows results obtained from the agar diffusion method with zone of inhibition (ZOI in mm) when bacteria is allowed to grow on agar medium having specific dosage (µg/ml) of the synthesized molecules. It can be seen that levofloxacin gave a zone of 10-11 mm for dose ranging from 0.25 µg/ml to 4 µg/ml. While *E.coli* and V. *cholera* required 0.25 µg/ml, *B.subtilis* and *S.aureus* gave almost

similar zone at 0.5 µg/ml and 4 µg/ml respectively. Ciprofloxacin, on the other hand, gave a zone ranging from 10-16 mm for a dose of 2 µg/ml. The largest zone with ciprofloxacin was against *E.coli* (16mm) while the smallest one was against *B. subtilis* (10 mm).

Among the synthesized molecules, benzyl derivative and sulphur bridged tricyclic compounds (**Compound 2.18 and 2.19**) did not show any inhibition zone suggesting no antibacterial activity against all the pathogens except compound **2.18** having 7 mm zone of inhibition against a massive dose of 16µg/ml that was eight to thirty two times higher than that of the control.

Table 2.4: MIC and Zone of inhibition values for the synthesized compounds by agar diffusion method taking Levofloxacin, (Levo), Ciprofloxacin (Cipro) as control.

Compound Name	***E.coli*** **(MTCC 1687)**		***V. cholera*** **(MTCC 1934)**		***B.subtilis*** **(MTCC 441)**		***S.aureus*** **(MTCC 1430)**	
	Zone of inhibition	**MIC value at ZOI**	**Zone of inhibition**	**MIC value at ZOI**	**Zone of inhibition**	**MIC value at ZOI**	**Zone of inhibition**	**MIC value at ZOI**
	(mm) (Mean ±SD)	µg/ml	(mm) (Mean ±SD)	µg/ml	(mm) (Mean ±SD)	µg/ml	(mm) (Mean ±SD)	µg/ml
2.13	11 ± 1	32	9± 1	0.5	14± 1	2	11± 2	0.5
2.14	Nil	128	11± 1	2	12± 1	2	9± 1	2
2.15	10± 2	4	10± 2	4	12± 2	4	10± 1	4
2.16	10± 1	4	10± 1	4	15± 1	4	18± 1	4
2.17	9± 1	16	9 ± 1	4	9± 1	4	10± 2	32
2.18	Nil	128	Nil	128	7± 1	16	Nil	128
2.19	Nil	128	Nil	128	Nil	128	Nil	128
Levo	11± 1	0.25	10± 1	0.25	10± 1	0.5	11± 1	4
Cipro	16± 1	2	14± 1	2	10± 1	2	12± 1	2

It was decided to evaluate further only those compounds and microorganisms by micro-dilution broth assay that gave comparable or higher ZOI (< 9mm) with up to double the dosage as that of controls. Therefore, *E.coli* and above mentioned two compounds were eliminated for further studies. Although compounds **2.15** and **2.16**, both the cyclopropane analogs, fulfilled the criteria for *E.coli* but were dropped due to non availability of sufficient number of compounds against the organism. **Table 2.4** shows that ZOI for five compounds (**2.13** to **2.17)** ranged between 9-15 mm at a dosage range of 0.5 µg/ml to 4 µg/ml. Thus, although all the compounds mentioned above gave good antibacterial activity compound **2.13** was identified as the potential ones with impressive ZOI as compared to controls.

Micro-dilution broth assay: **Table 2.5** shows the minimum inhibitory concentration (MIC) in nM range for all the shortlisted compounds. The assay was carried out using serial dilutions from the stock of each drug taking levofloxacin as a control.

Table 2.5 : MIC value for the compounds screened in Table 2.4 by micro-dilution broth assay

Compound	***S. aureus*** **(MTCC 1430)**	***V. cholerae*** **(MTCC 1934)**	***B. subtilis*** **(MTCC 441)**
	MIC (nM)		
Levofloxacin	125.0	7.8	7.8
2.13	**15.0**	**3.7**	**7.8**
2.14	62.5	31.2	13.2
2.15	25.0	60.0	125.0
2.16	**15.0**	30.0	30.0
2.17	500.0	125.0	250.0

S. aureus: It can be seen (**Table 2.5**) that all compounds except **2.17** gave excellent results against the organism as compared to the control. However, chiral levofloxacin **(2.13)** and ciprofloxacin **(2.16)** derivative with C-8 methoxy group were the potent ones. Both displayed MIC values of 15 nM each that was eight times lower than that of the control. Compounds **2.15** and **2.14** gave MIC of 25nM and 62.5 nM that was five times and half the MIC value of control. N1-ethyl derivative with *endo* nortropine substituent at C-7 position **(2.17)** was the only compound in the series that gave four times higher MIC value (500nM) than that of the control. Thus, it can be concluded that four of the seven synthesized compounds in this chapter were found to be active against *S. aureus* with C-7 *endo*-nortropine and derivative of levofloxacin **(2.13)** being the most potent one.

V. cholera and *B. subtilis*: **Table 2.5** shows that against these two organisms only compound **2.13** was active. While *endo*-nortroine C-7 substituted levofloxacin derivative **(2.13)** gave MIC value half (3.7 nM) to that of control (7.8 nM) in case of *V. cholera,* it was same (7.8 nM) in case of *B. subtilis.*

2.4 Conclusion:

Thus to conclude, the chapter describes synthesis of *endo*-nortropine by selective reduction and its linkage to different fluoroquinolone Q-acid skeletons at C-7 position. Levofloxacin, Norfloxacin and ciprofloxacin along with its C-8 methoxy derivative have been procured from commercial sources. Two Q-acids, having N1-benzyl and C-8 fluoro group **(2.11)** and N1 to C2 carbon bridged via sulphur linkage **(2.12)** were synthesized and characterized.

All the compounds were subjected to antibacterial activity against Gram negative, [*Escherichia coli* (MTCC 1687) and *Vibrio cholerae* (MTCC 1934)] and Gram positive [*Staphylococcus aureus* (MTCC 1430) and *Bacillus subtilis* (MTCC 441)] bacteria. For all the organisms *Agar diffusion* and *Micro-dilution broth* assays gave impressive results against *S. aureus* and *V. Cholera.* Among the synthesized compounds, levofloxacin derivative **2.13** was most impressive followed by C-8 methoxy substituted ciprofloxacin derivative **(2.16)**. Considering that *S. aureus* is one of main organisms for a number and type of infections, including most serious hospital acquired infections and the reports of the re-emergence of *V. cholera* in certain parts of the word results obtained in this chapter are very significant.

2.5 Materials and Methods

All the solvents used in this study were brought from Sigma Aldrich (India) and SD Fine Chemicals (Mumbai, India). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz (SAIF, Panjab University, Chandigarh, India) and Jeol 400 MHz (SAI Labs, Patiala, India) spectrometers and chemical shifts expressed in δ (ppm) with tetramethylsilane (TMS) as an internal reference standard. Autopol V (Rudalph Research) polarimeter was used for measuring the optical rotation. Silica Gel (Merck 60 F_{254}) thin layer chromatography (TLC) plates were used for reaction monitoring along with UV chamber having dual wavelength of 254 nm and 340 nm. Purification of the crude products, wherever required by column chromatography, was done using silica gel (60-120 mesh size).

HPLC Method: Fluoroquinolones pure derivatives were analysed for their purity by analytical HPLC (Shimadzu) using Kromasil C18 column, (150 mm, X 4.6 mm X 5 μm), acetonitrile: buffer (pH=2.2) = 60:40 solvent system, (Buffer preparation: 7.3 gm sodium perchlorate monohydrate and 4.4 gm ammonium acetate dissolved in 1.0 lit of water and pH adjusted 2.2 with orthophosphoric acid) having flow rate 1.5 ml/min (Method A) and 2.0 ml/min (Method B) using UV detector (λ= 294 nm) with 10 μl injection volume.

2.5.1 Synthesis of *Endo* and *Exo*-Nortropine (Scheme-2.1)

8-Benzyl-3-oxo-8-azabicyclo [3.2.1] octane hydrochloride (2.3): To a solution 2,5-dimethoxy tetrahydrofuran (25 g, 180 mmol) in water (200 mL) was added concentrated hydrochloric acid (2 mL). 1,3-Acetonedicarboxylic acid (29 g, 190 mmol), benzyl amine hydrochloride (27 g, 180 mmol) and sodium hydrogen phosphate (6 g, 40 mol) were added after five minutes and the mixture allowed to stir over night. Workup was done after completion of the reaction (TLC

monitoring) by adding sodium hydroxide solution (pH=8-9) followed by extraction with Methylene chloride (150 mL). Combined Methylene chloride washed with water (3 X 35 ml) to remove water soluble impurities. Evaporation of the solvent gave crude product that was crystallized in isopropyl alcohol with isopropyl alcohol hydrochloric acid (25% w/v, 50 mL) as cream solid (38 g) in 80% yield. Melting point: 190-193°C, ^{1}H NMR (D_2O ,400 MHz,); δ 7.49 (m, 5H, Ar), 4.27 (bs, 2H, ArC**H$_2$**-)), 4.13 (s, 2H, bridged **H**), 2.54-2.40 (m, 4H, -CH**(H)**-CH**(H)** &-CH**(H)**-CO-CH**(H)**-), 2.44(bs,2H, -CH**(H)**-CO-CH**(H)**-), 1.96-2.00 (m, 2H, -CH**(H)**-CH**(H)**-); ^{13}C NMR (D_2O, 100 MHz,): δ 210.0 (C, C=O), 130.70 (C-1, -Ar), 130.30 (C-2, -Ar), 130.14 (C-3, -Ar) , 129.49 (C-4 -Ar), 129.36 (C-5, -Ar), 129.20 (C-6, -Ar), 60.90 (CH_2)$_2$-**C**-N), 60.51(CH_2)$_2$-**C**-N), 55.16(-N**C**H$_2$-Ar) , 46.06(CH-**C**H$_2$-CO), 42.02 (CH-**C**H$_2$-CO), 25.08 (-CH-**C**H$_2$-CH$_2$-CH), 23.01(-CH-CH$_2$-**C**H$_2$-CH); EIMS; m/z 216 [M+1] calculated for $C_{14}H_{17}NO$.

3-Oxo-8-azabicyclo [3.2.1] octane (2.4): Compound **2.3** (35 g, 130 mmol) synthesized above was dissolved in water methanol mixture (200 mL, Methanol: Water = 70:30) and stirred under positive pressure of hydrogen gas in the presence of 5% palladium over charcoal (3.5 g, 10% of the reactant). After 16 hours, the solution was filtered to remove palladium and charcoal and methanol evaporated. The pH of left over aqueous solution was adjusted between 9 and 10 with ammonium hydroxide solution and organic compound extracted with methylene chloride (3 X 70 mL). The solvent was evaporated to afford brown coloured semisolid material (14 g) in 81% yield. ^{1}H NMR (D_2O, 400 MHz,): δ 4.33 (bs, 2H, bridged **H**), 2.95-2.90 (dd, 2H, J=18.0 Hz, 4.7 Hz, -CH**(H)**-CO-CH**(H)**-), 2.56-2.52 (d, 2H, J=18.0 Hz -CH**(H)**-CO-CH**(H)**-), 2.20-2.14 (m, 2H, -CH**(H)**-CH**(H)**-), 1.93 -1.87 (m, 2H, -C**H**(H)-C**H**(H)-); ^{13}C NMR (D_2O, 100 MHz,): δ 207.96 (-**C**=O), 54.69 (CH_2-**C**H-N-**C**H-CH_2), 45.53 (-**C**H(H)-CO-**C**H(H)-), 28.59 (CH-**C**H$_2$-**C**H$_2$-CH).

***Endo*-8-azabicyclo [3.2.1] octan-3-ol (2.5):** Compound **(2.4)** (13 g, 100 mmol) synthesized above was taken in methanol (130 mL) and stirred under high pressure of hydrogen gas (10 bar) in the presence of raney nickel (1.3 g, 10% of the reactant). After 24 hours, the solution was filtered to remove catalyst and the solvent evaporated to afford crude product. Crystallization of the obtained product from heptane (70.0 mL) gave light brown coloured solid (11.0 g) in 83% yield). Melting point: 178-182°C, ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 4.05-4.03 (t, 1H, J= 4.9 Hz – C**H**(OH)-), 3.47 (bs, 2H, bridged **H**), 2.7 (bs, 2H, -O**H**& N**H**), 2.22-2.17 (m, 2H, -C**H**(H)-C**H**(H)-), 1.98-1.96 (t, 1H, J=4.2 Hz -C**H**(H)-C(OH)-CH(H)-), 1.94-1.93 (t, 1H, J=4.2 Hz -CH(H)-C(OH)-C**H**(H)-) 1.79-1.71 (m, 4H, -CH**(H)**-CH**(H)**- and -CH**(H)**-C(OH)-CH**(H)**-); ^{13}C NMR ($CDCl_3$,100 MHz,): δ 64.48 (-**C**-OH), 53.46 (CH_2-**C**H-N-**C**H-CH_2), 40.30 (-**C**H(H)-

C(OH)-**C**H(H)-), 29.31(CH-**C**H_2-**C**H_2-CH); EIMS; m/z 128 [M+1] calculated for $C_7H_{13}NO$.

8-Azabicyclo [3.2.1] octan-3-ol (2.5 + 2.5a – Endo + Exo): To the compound **(2.4)** (13 g, 0.10 mol), synthesized above, taken in water: methanol (90: 40 mL) solution was added slowly potassium borohydride (5.14 g , 104 mmol) with stirring and the mass kept at temperature 25-35°C for 2.0 hrs. After completion of the reaction, as monitored by TLC, 90 mL of water was added in reaction mass and the pH adjusted between 6.5 to 7.5 by acetic acid. The crude product was extracted using chloroform (5.0 X 130.0 mL), dried over anhydrous sodium sulphate and distilled off under vacuum completely to get 8.0 g (61% yield) brown coloured viscous mass (Compound - 2.5 & 2.5a) that was further used without any separation or analysis.

2.5.2 Synthesis of Fluoroquinolones with C-7 substituted *Endo*-Nortropine (Scheme-2.2 and Table 2.1)

General Procedure: Difluoroquinolone acid (**Compounds 2.6 to 2.12**, 3.56 mmol) and e*ndo*-nortropine (**Compound 2.4**, 7.88 mmol) were dissolved in DMSO (5 to 25 mL) and heated to the desired temperature **(Table 2.1)** for 5 hours. Reaction monitoring was done by TLC and after completion of the reaction, mixture was cooled to room temperature and quenched in water (150 mL). pH of the reaction mixture was adjusted to the desired value **(Table 2.1)** by drop wise addition of concentrated hydrochloric acid to get solid suspension. Filtration followed by washing with water (2 X 20 mL) to remove excess of acid gave crude product that was re-crystallize from methanol to get pure product (**Compounds 2.13 to 2.18**) in high yields.

(S)-(-)-9-Fluoro-3,7-dihydro-10-(3-hydroxy-8-aza-bicyclo [3.2.1]octan-8-yl)-3-methyl-7-oxo-2H-[1,4]oxazinon [2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (2.13) : Light yellow coloured powder, Yield-82.4%, Purity (HPLC) 98.73% ; Melting point 270°C (decomposes); $[\alpha]^{25}_D$ - 89°(*c* 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$ & DMSO-d_6, 400 MHz,): δ 15.36 (s, 1H, -COO**H**), 8.81 (s, 1H, -NC**H**CCOOH), 7.53-7.50 (d, 1H, J=13.8 Hz, -C(F)C**H**C), 4.82-4.80 (bs, 1H, -NC**H**(CH_3)CH_2-), 4.48-4.42 (m, 2H, -NCH(CH_3)C**H_2**-), 4.32-4.26 (m, 2H, bridged H), 4.00 (bs, 1H, -OH), 2.55-2.54 (m, 1H, -CH_2-C**H**(OH)-CH_2-), 2.33-2.26 (m, 2H, -CH**(H)**-CH**(H)**-), 2.22-2.16 (dt, 1H, J=4.0 Hz, -C**H**(H)-C(OH)-CH(H)-), 2.14-2.09 (dt, 1H, J=4.0 Hz, -CH(H)-C(OH)-C**H**(H)-), 1.97-1.85 (m, 2H, -CH(**H**)-C(OH)-CH(**H**)-), 1.81-1.78 (m, 2H, -C**H**(H)-C**H**(H)-), 1.52-1.53 (d, 3H, J=6.6 Hz,-NCH(C**H_3**)CH_2-); ^{13}C NMR ($CDCl_3$ & DMSO-d_6,100 MHz,):δ (176.18, 176.15, J=3 Hz, -**C**=O), 166.52(-**C**OO), (154.02 ,151.58, J=244 Hz, -**C**F) 145.22 (-N-**C**-C-COO), (135.47, 135.40, J=7 Hz, -**C**-O-CH), (130.82 ,130.69, J=13 Hz, -

CF**C**(N)C), 124.72(-C-O-**C**-N), (116.07, 115.97, J=10 Hz, -**C**-C=O), 106.28 (-C=O**C**-COO), (104.26, 104.01, J=26 Hz, -CF**C**-C), 67.74 (O**C**CH_3), 63.06 (-**C**-OH), 58.14, 58.09, 57.72, 57.66 (CH_2-**C**H-N-**C**H-CH_2), 54.72 (OC**C**CH_3), 39.19 (-**C**H_2-C(OH)-CH_2-), 38.98(-CH_2-C(OH)-**C**H_2-), 28.24 (CH-**C**H_2-CH_2-CH), 28.15 (CH-CH_2-**C**H_2-CH), 17.98 (C-**C**H_3); CHN Anal. Calcd. For $C_{20}H_{21}FN_2O_5$: C, 61.85; H, 5.45; N, 7.21. Found: C, 61.53; H, 5.43; N, 6.93. EIMS m/z 389 [M+1] calculated for $C_{20}H_{21}FN_2O_5$.

(RS)-(±)-9-Fluoro-3,7-dihydro-10-(3-hydroxy-8-aza-bicyclo[3.2.1] octan-8-yl)-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*] quinoline-6-carboxylic acid hydrochloride (2.14): Light yellow colour powder, Yield-79.1%; Purity (HPLC)-96.47%; Melting point 245°C decomposes ; ^{1}H NMR ($CDCl_3$ & DMSO-d_6, 400 MHz,): δ 15.32 (s, 1H, -COOH), 8.80 (s, 1H, -NC**H**CCOOH), 7.49-7.45 (d, 1H, J=13.8 Hz, -C(F)C**H**C), 4.83-4.81 (m, 1H, -NC**H**(CH_3)CH_2-), 4.47-4.24 (m, 5H, -NCH(CH_3)C**H$_2$**- , bridged **H** and -O**H**), 3.96 (m, 1H, -CH_2-C**H**(OH)-CH_2-), 2.54-2.50 (m, 2H, -CH**(H)**-CH**(H)**-), 2.30-2.06 (4 ts, 2H, J=4.0 Hz, -C**H**(H)-C(OH)-C**H**(H)-), 1.93-1.87 (m, 2H, -CH(**H**)-C(OH)-CH(**H**)-), 1.78-1.74 (m, 2H, -C**H**(H)-C**H**(H)-), 1.49-1.47 (d, 3H, J= 6.7 Hz -NCH(C**H**$_3$)CH_2-); ^{13}C NMR ($CDCl_3$ & DMSO-d_6, 100 MHz,): δ (176.14, 176.10, J=4 Hz, -**C**=O), 166.41(-**C**OO), (154.01, 151.57, J=244 Hz, -**C**F), 145.44(-N-**C**-C-COO), (135.64, 135.57, J=7 Hz, -**C**-O-CH), (130.76, 130.63, J=13 Hz, -CF**C**(N)C), 124.75(-C-O-**C**-N), (116.10, 116.0, J =10 Hz, -**C**-C=O), 106.22 (-C=O**C**-COO), (104.12, 103.87, J =25 Hz, -CF**C**-C), 67.70 (O**C**CH_3), 62.96 (-**C**-OH), 58.16, 58.11, 57.73, 57.68 (CH_2-**C**H-N-**C**H-CH_2), 54.71 (OC**C**CH_3), 39.14 (-**C**H_2-C(OH)-CH_2-), 38.93 (-CH_2-C(OH)-**C**H_2-), 28.27 (CH-**C**H_2-CH_2-CH), 28.18 (CH-CH_2-**C**H_2-CH), 17.97 (C-**C**H_3); CHN Anal. Calcd. For $C_{20}H_{21}FN_2O_5$: C, 61.85; H, 5.45; N, 7.21. Found: C, 61.78; H, 5.13; N, 6.89. EIMS m/z 389 [M] + (1) calculated for $C_{20}H_{21}FN_2O_5$.

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(3-hydroxy-8-aza-bicyclo [3.2.1] octan-8-yl)-4-oxo quinoline -3-carboxylic acid(2.15): Yield- 67.4%, Purity (HPLC)-95.18%; Melting point 282-285°C; ^{1}H NMR (400 MHz DMSO-d_6) δ 15.38 (s, 1H, -COO**H**), 8.61 (s, 1H, -NC**H**CCOOH), 7.83-7.79 (d, 1H, J= 14.52 Hz, -C(F)C**H**C), 7.33-7.32 (d, 1H, J= 7.6 Hz, -C(N)C**H**C), 4.65-4.64 (t,1H,-CH_2-C**H**(OH)-CH_2-), 4.53 (bs, 2H, NC**H**$(CH_2)_2$, 3.91 (s, 1H –O**H**), 3.74-3.72 (m, 1H, -NC**H**$(CH_2)_2$, 2.42-2.40 (q, 2H, CH**(H)**-CH**(H)**-), 2.12-2.09 (m, 2H, -CH(**H**)-C(OH)-CH(**H**)-), 2.02-2.00 (m, 2H, -C**H**(H)-C(OH)-C**H**(H)-), 1.80-1.76 (d,2H, C**H(**H)-C**H(**H)-), 1.38-1.33 (q, 2H, -NCHCH(**H**)CH(**H**)-), 1.24-1.17 (q, 2H,-NCHC**H**(H)C**H**(H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (**175.99, 176.10,** J= 4 Hz), 166.23, (**154.01, 149.52,** J= 246 Hz), 147.01, (**140.30,140.19,** J=

18 Hz)**,** 139.63**,** (**115.52, 115.44** J= 8 Hz**),** (**111.48,111.24**, J= 24 Hz)**, 106.38,** (**103.18, 103.0 ,** J= 18 Hz)**,** 62.91, 55.60, 55.53, 36.16, 35.27, 27.30, 7.55., EIMS m/z 373.4 [M+1] calculated for $C_{20}H_{21}FN_2O_4$.

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(3-hydroxy-8-aza-bicyclo[3.2.1]octan-8-yl)-8-methoxy-4-oxoquinoline-3-carboxylic acid (2.16): Yield- 47.3%, Purity (HPLC)- 98.66%; Melting point 258-261°C; ^{1}H NMR (400 MHz $CDCl_3$/ DMSO-d_6) δ 8.68 (s, 1H, -NC**H**CCOOH), 7.69-7.66 (d, 1H, J= 14.08 Hz, -C(F)C**H**C), 4.48 (bs, 2H, -NC**H**$(CH_2)_2$), 4.15-4.12 (m, 1H, -NC**H(**$CH_2)_2$, Cyclopropyl), 4.0-3.98 (t, 1H,CH_2-C**H**(OH)-) , 3.64, (s, 3H, -OC**H**$_3$), 3.45 (s, 1H, –O**H**), 2.36-2.33 (q, 2H, CH**(H)**-CH**(H)**-), 2.15-2.11 (dt, 2H, , -CH(**H**)-C(OH)-CH(**H**)-), 1.97-1.95 (t, 2H , -C**H**(H)-C(OH)-C**H**(H)-), 1.84-1.80 (d,2H, C**H(**H**)**-C**H(**H**)**-), 1.18-1.17 (q, 2H, -NCHCH(**H**)CH(**H**)-), 1.00-0.98 (q, 2H,-NCHC**H**(H)C**H**(H)); ^{13}C NMR (100 MHz, $CDCl_3$/DMSO-d_6) δ (**176.10, 176.07,** J= 3.24 Hz)**,** 165.90, (**153.83, 149.89,** J= 393 Hz**),** (**141.11, 141.03** J= 7.76 Hz)**,** (**136.43,136.33 ,** J = 9.82 Hz)**,** 134.14, (**117.34, 117.25 ,** J= 8.79 Hz **),** **(107.46, 107.21** J= 25.41 Hz **),** 106.43**,** 62.77, 60.10, 57.76, 39.13, 38.98, 38.93, 28.07, 8.97. EIMS m/z 403.3 [M+1] calculated for $C_{21}H_{23}FN_2O_5$.

1-Ethyl-6-fluoro-1,4-dihydro-7-(-3-hydroxy-8-aza-bicyclo[3.2.1]octan-8-yl)-4-oxoquinoline-3-carboxylic acid (2.17): Yield- 64.10%, Purity (HPLC)- 97.28% , Melting point 301-305°C decomposes; ^{1}H NMR (400 MHz DMSO-d_6) δ 15.57 (s, 1H, -COO**H**), 8.85 (s, 1H, -NC**H**CCOOH), 7.85-7.81(d, 1H, J= 14.68 Hz, C(F)C**H**C), 6.92-6.91 (d, 1H, J=7.48 Hz, -C(N)C**H**C), 4.61-4.58 (bs,2H, -NC**H**(CH_2)), 4.55-4.50 (q, 2H, -NC**H$_2$**CH_3), 3.90-3.88 (t,1H, CH_2-C**H**(OH)-CH_2-), 2.71(s, 1H, –O**H**), 2.40-2.46 (q,2H, CH**(H)**-CH**(H)**-), 2.10-1.98 (2 dt, 4H, -CH(**H**)-C(OH)-CH(**H**)-)&-C**H**(H)-C(OH)-C**H**(H)-), 1.77-1.74 (d,2H, C**H(**H**)**-C**H(**H**)**-), 1.45-1.42 (t,3H, -CH_2C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ **(175.83, 175.80 ,** J = 3.39 Hz), 166.41**, (151.85, 149.38 ,** J = 247 Hz**)**, 147.80, (**140.53, 140.42,** J= 10.64 Hz), 137.74, 116.12, (**111.71, 111.47,** J=23.8 Hz)**,** 106.56, (**102.47, 102.42,** J= 5.4 Hz)**,** 62.86, 55.51, 55.44, 48.78, 38.93, 36.30, 27.26, 14.33 EIMS m/z 361.29[M+1] calculated for $C_{19}H_{21}FN_2O_4$.

5.2.3 Synthesis of quinolone acids and then C-7 *Endo*-Nortropine substituted fluoroquinolone (Scheme 2.3 and 2.4 , compound 2.18 and 2.19)

1-Benzyl-6,7,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (2.11, Scheme-2.3)

2,3,4,5-Tetra fluorobenzoyl chloride (5.0 g, 23.5 mmol), ethyl -3-N-(dimethyl amino) acrylate (3.43 g, 23.9 mmol) and triethyl amine (2.42 g, 23.9 mmol) were heated in under nitrogen

atmosphere at 50-55°C in toluene for 12 hours. After completion of the reaction (TLC monitoring) the reaction mass was brought to the room temperature and the hydrochloride salt of triethyl amine separated by filtration. The filtrate containing acrylated derivative in toluene was washed with 5% sodium bicarbonate solution (1x 20 mL) and water (2.0 x 20 mL), followed by addition of sodium sulphate (5 g) to make the reaction mixture anhydrous. Subsequently the dried organic layer (after filtration, to remove sodium sulphate), was stirred in the presence of benzyl amine (2.45 g, 22.8 mmol) at room temperature for 3 hrs and the toluene removed under reduced pressure using rota evaporator. The solid mass obtained was further dissolved in DMF (20.0 mL) and heated at 90°C for 6 hours in the presence of potassium carbonate (4.90 g, 35.5 mmol) to obtain the cyclised ester that was added in water (100 mL) to get solid precipitates to carry out the filtration and washing with water (2 x 20 mL) to remove any residual DMF. Further hydrolysis of the ester obtained was done by heating in the presence of sulphuric acid (25 % V/V, 50 mL) and acetic acid (3.0 mL) at 60°C for 6 hrs. After completion of the reaction (TLC monitoring) the solid obtained was washed with water (2 x 50 mL), purified from methanol to get compound **2.11** (5.0 g) in 67 % yield.

1-Benzyl-6,8-difluoro-1,4-dihydro-7-(3-hydroxy-8-aza-bicyclo[3.2.1]octan-8-yl)-4-oxo quinoline-3-carboxylic acid (2.18): The product was obtained using general procedure as described in **section 2.5.2.** Yield- 47.30%, Melting point 245-248°C decomposes.; ^{1}H NMR (400 MHz $CDCl_3$/ DMSO-d_6) 14.99 (s, 1H, -COO**H**), 9.03 (s, 1H, -NC**H**CCOOH), 7.79-7.75 (d, 1H, J=13.7 Hz, -C(F)C**H**C),7.39-7.12 (m, 5H, -Ar ring), 5.79 (bs, 2H, –NC**H$_2$**Ar), 4.19 (bs, 2H, -N-bridged **H**), 3.88 (t, 1H,CH_2-C**H**(OH)-) , 3.39, (s, 1H, -OH),2.29-2.27(dd, 2H, CH**(H)**-CH**(H)**-), 1.95-1.92 (dt, 2H, , -CH(**H**)-C(OH)-CH(**H**)-), 1.83 (bs, 2H , -C**H**(H)-C(OH)-C**H**(H)-), 1.74-1.70 (d,2H, C**H(**H**)**-C**H(**H**)**-), ^{13}C NMR (100 MHz, $CDCl_3$/DMSO-d_6) δ **(176.29, 176.24,** J= 5 Hz**),** 165.77, (**154.13, 151.54,** J= 2.5 Hz), 145.78, (**135.88, 135.83 ,** J=5.0 Hz), 128.52, 127.70, 125.81, (**116.53, 117.25,** J= 8.79 Hz), (**107.16, 106.86,** J=30.0 Hz **),**106.86**,** 62.59, 60.75, 58.03, 38.96, 38.90, 27.77. EIMS m/z 441.3 [M+1] calculated for $C_{24}H_{22}F_2N_2O_4$.

Ethyl 8,9-difluoro-1,2,3,6-tetrahydro-6-oxo- [1,3] thiazino[3,2-a] quinoline-5-carboxylate (2.12, Scheme 2.4)

Potassium hydroxide powder (4.5 g, 80.1 mmol) was taken in toluene (100 mL) and diethyl malonate (12.6 g, 78.7 mmol) added at 10°C to stir the mixture for 30 min. This was followed by addition of 3,4-difluorophenyl isothiocyanate (10.0 g 58.4 mmol) within 10 to 15 min to continue stirring for another 2-3 hrs. After the completion of the reaction (TLC monitoring) the

mass was filtered, washed with toluene to get potassium salt of compound (2.12a). The obtained potassium salt (19.0 g, 51.2 mmol) was preceded further without any characterisation by dissolving in ethyl acetate (80.0 mL) and further adding potassium carbonate (14.2 g 10.2 mmol), 1,3-dibromopropane (10.5 g, 10.2 mmol) to stir for 16 hrs at room temperature. On completion of the reaction (tracked by TLC monitoring) water (200.0 mL) was added and the material extracted in Ethyl acetate (2 X 20 mL). The combined ethyl acetate layers were washed by water (2 X 50.0 mL) and made anhydrous by addition of sodium sulphate. Evaporation of the solvent using vacuum and temperature below 50°C gave oily residue (15.0 g) that was further cyclised by heating at 100°C for 6.0 hrs in the presence of 60-70 g of polyphosphoric acid. TLC monitoring further conformed the completion of the reaction and the product (2.12) was obtained as precipitates by adding the reaction mixture in water (200.0 mL) that were filtered, washed (water, 2 X 100.0 mL) and purified by crystallizing from methanol (30 mL) to get pure compound 2.12 (12.0 g) in 71% yield. Yield: 1.0 g, (71 %), ^{1}H NMR (400 MHz $CDCl_3$/ DMSO-d_6): 7.88-7.84 (d, 1H, J=14.4 Hz, -C(F)C**H**C), 6.59-6.58 (d, 1H, J=7.08 Hz, C(F)CC**H**), 4.41-4.36 (q,2H,-COOC**H$_2$**CH_3), 4.15-4.12 (t,2H,-SC**H$_2$**CH_2), 3.09-3.05(t,2H, -NC**H$_2$**CH_2CH_2S-), 2.44-2.39 (m, 2H, -NCH_2C**H$_2$**CH_2S-), 1.40-1.36 (t,3H, -$COOCH_2$C**H$_3$**), ^{13}C NMR (100 MHz, $CDCl_3$/DMSO-d_6) δ **(169.76, 169.71 ,** J= 3.0 Hz**),** 165.53, **(151.22, 148.74,** J= 248 Hz), 148.25, 138.98, (138.71, 138.61, J=10.0Hz**), (117.01, 116.94 ,** J=7.0 Hz*)* **,** 114.45**,** (**112.15, 111.92,** J= 20.0 Hz), 99.53, 99.50 , 60.51, 45.21, 26.80, 23.31, 13.20.

Ethyl 8-fluoro-1,2,3,6-tetrahydro-9-(3-hydroxy-8-aza-bicyclo [3.2.1] octan-8-yl)-6-oxo- [1,3] thiazino[3,2-a] quinoline-5-carboxylate (2.19a): Ethyl ester derivative (**2.12**, 1.0 g 3.07 mmol) was heated with *endo*-nortropine (**2.5**, 0.87 g 6.79 mmol) in DMSO (5 ml) at 110°C for 6.0 hrs. After completion of the reaction, as per TLC, the mass was cooled down to room temperature and added to water (25 mL) to get precipitates. It was filtered and washed with water to get nortropine substituted ester derivative. Yield: 1.0 g, (75.75 %), Melting point 268-270°C decomposes. , ^{1}H NMR (400 MHz $CDCl_3$/ DMSO-d_6): 7.88-7.84 (d, 1H, J=14.4 Hz, -C(F)C**H**C), 6.59-6.58 (d, 1H, J=7.08 Hz, C(F)CC**H**), 4.57-4.55 (bs, 2H, -NC**H**(CH_2bridged)), 4.41-4.36 (q, 2H, -COOC**H$_2$**CH_3), 4.15-4.12 (t, 2H, -SC**H$_2$**CH_2), 4.11-4.08 (t, 1H, CH_2-C**H**(OH)-) , 3.09-3.05 (t, 2H, -NC**H$_2$**CH_2CH_2S-), 2.44-2.39 (m, 2H, -NCH_2C**H$_2$**CH_2S-), 2.37-2.33(q, 2H, CH**(H)**-CH**(H)**-), 2.23-2.17 (dt, 2H, , -CH(**H**)-CH(OH)-CH(**H**)-), 2.07-2.05 (t, 2H , -C**H**(H)-C(OH)-C**H**(H)-), 1.90 (s, 1H, –O**H**),1.79-1.76 (d,2H, C**H(**H**)**-C**H(**H**)**-),1.40-1.36 (t, 3H, -$COOCH_2$C**H$_3$**), ^{13}C NMR (100 MHz, $CDCl_3$/DMSO-d_6) δ **(169.75, 169.72 ,** J= 3.0 Hz**),** 165.54, **(150.73, 148.74,** J= 2.0 Hz), 148.25, 138.98, (138.71, 138.61, J=10.0Hz**), (117.02,**

116.95 , J=7.0 Hz*)* **,** 114.45**,** (**112.15, 111.92,** J= 20.0 Hz), (99.53, 99.49), 63.93, 60.50, (54.66, 54.59), 45.20, 35.75, 26.79, 24.82, 23.33, 13.22.

8-Fluoro-1,2,3,6-tetrahydro-9-(3-hydroxy-8-aza-bicyclo [3.2.1] octan-8-yl)-6-oxo-[1,3] thiazino[3,2-a]quinoline-5-carboxylic acid (2.19) :1.0 g (2.31 mmol) of **c**ompound 2.19a further hydrolysed in (20.0 mL) 10% aqueous sodium hydroxide solution at 45˚C for 8.0 hrs, after complies of TLC reaction mass cool down to room temperature and 30.0 mL extra water was added. pH of reaction mass adjusted 6.0 to 6.5 by dil. acetic acid and filtered the material. Solid cake washed with water and purified in methanol 10.0 mL to get 0.75 g of **2.19** *endo* derivative. Yield: 60%, Melting point 276-280°C decomposes. , ^{1}H NMR (400 MHz DMSO d_6) 17.11, (s, 1H, -COO**H**), 7.81-7.78 (d, 1H, J= 14.4 Hz, -C(F)C**H**C), 6.98-6.97 (d, 1H, J =7.02 Hz, C(F)CC**H**), 4.61 (s, 1H, –O**H**),4-4.56 (bs, 2H, -NC**H**(CH_2 bridged)), 4.36 (t, 2H, -SC**H$_2$**CH_2), 3.90 (t, 1H, CH_2-C**H**(OH)-) 3.07-3.04 (t, 2H, -NC**H$_2$**CH_2CH_2S-), 2.42-2.36 (m, 4H , -C**H**(H)-C(OH)-C**H**(H)-) and -NCH_2C**H$_2$**CH_2S-), 2.11-1.98 (dt, 2H,-CH(**H**)-C(OH)-CH(**H**)-), 1.92 (q, 2H, CH**(H)**-CH**(H)**-), 1.78-1.74 (d,2H, C**H(**H**)**-C**H(**H**)**-); ^{13}C NMR (100 MHz, DMSOd6) δ (**169.75, 169.72,** J= 3.0 Hz)**,** 165.54, (**150.73, 148.74,** J= 2.0 Hz), 148.25, 138.98, (**138.71, 138.61,** J=10.0Hz)**,** (**117.02, 116.95 ,** J= 7.0Hz) **,** 114.45**,** (**112.15, 111.92,** J= 20.0 Hz), (99.53,99.49), 63.93, (54.66, 54.59), 45.20, 35.75, 26.79, 24.82, 23.33. EIMS m/z 405.2[M+1] calculated for $C_{20}H_{21}FN_2O_4S$.

2.5.4 Antibacterial activity:

All the synthesised compounds listed in **Table- 2.2** were screened for their *in vitro* antibacterial activity against *Vibrio cholerae* (MTCC 3904), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 1430), *Escherichia coli* (MTCC 1687) and *Pseudomonas aeruginosa* (MTCC 3904) by agar diffusion method taking levofloxacin and ciprofloxacin as a control drug.

Agar diffusion method: Stock solutions (10 mg / mL) of all the compounds were prepared in 100% DMSO and stored in small aliquots at -20 °C. Serial dilutions of the stock were prepared with autoclaved distilled water at 4 °C and assay performed immediately. Final concentration of DMSO in all the dilutions was maintained at less than 2% which is non-inhibitory to the cells. A blank solution containing only 2% DMSO was also used as control. Agar diffusion assay was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Plates were incubated at 37 °C and zone of inhibitions were measured after 36 hours.

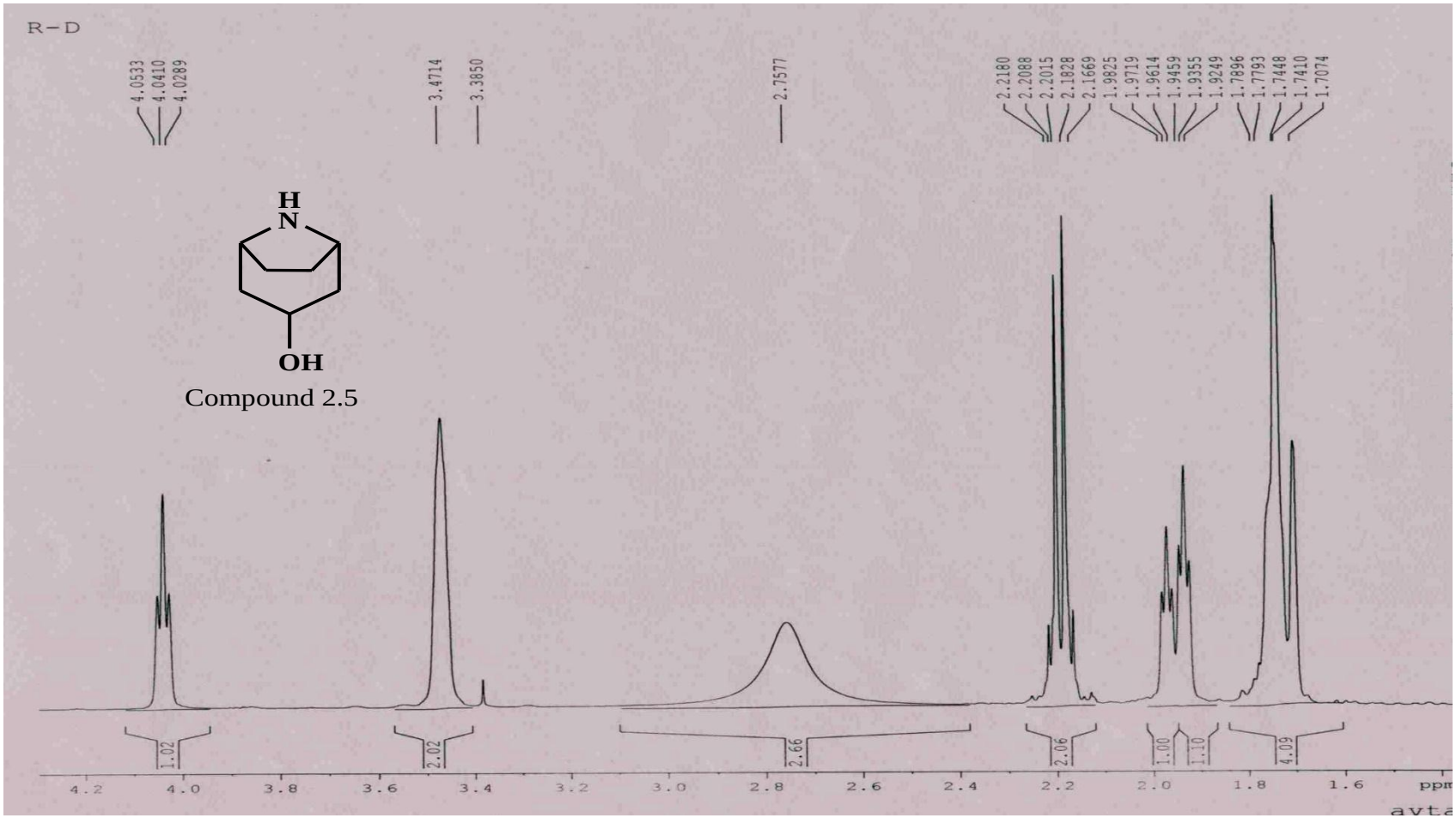
Micro-dilution broth assay: The assay was performed in triplicates according to CLSI guidelines in 96-well microliter plate using Mueller−Hinton broth. Briefly, 100 µL of room

temperature Mueller−Hinton broth was added to each well of 96-well microliter plate. Drug concentration of 128 µg/mL (100 µL) was added to first well and serially diluted each row two-fold across the plate. Bacterial inoculum suspension equivalent to ~4-8 X 10^5 CFU/mL (100 µL) was added to each well resulting in the drug concentration ranges from 32 µg/mL in well 1 to 0.06 µg/mL in well 10. Well 11 was no-drug growth control containing only medium and inoculum whereas well 12 was without inoculum having medium only to check the sterility of the medium used in the plate. Plates were sealed and incubated without shaking at 37°C for 16 hours, absorbance was taken at 600 nm and MIC was calculated according to the absorbance values.

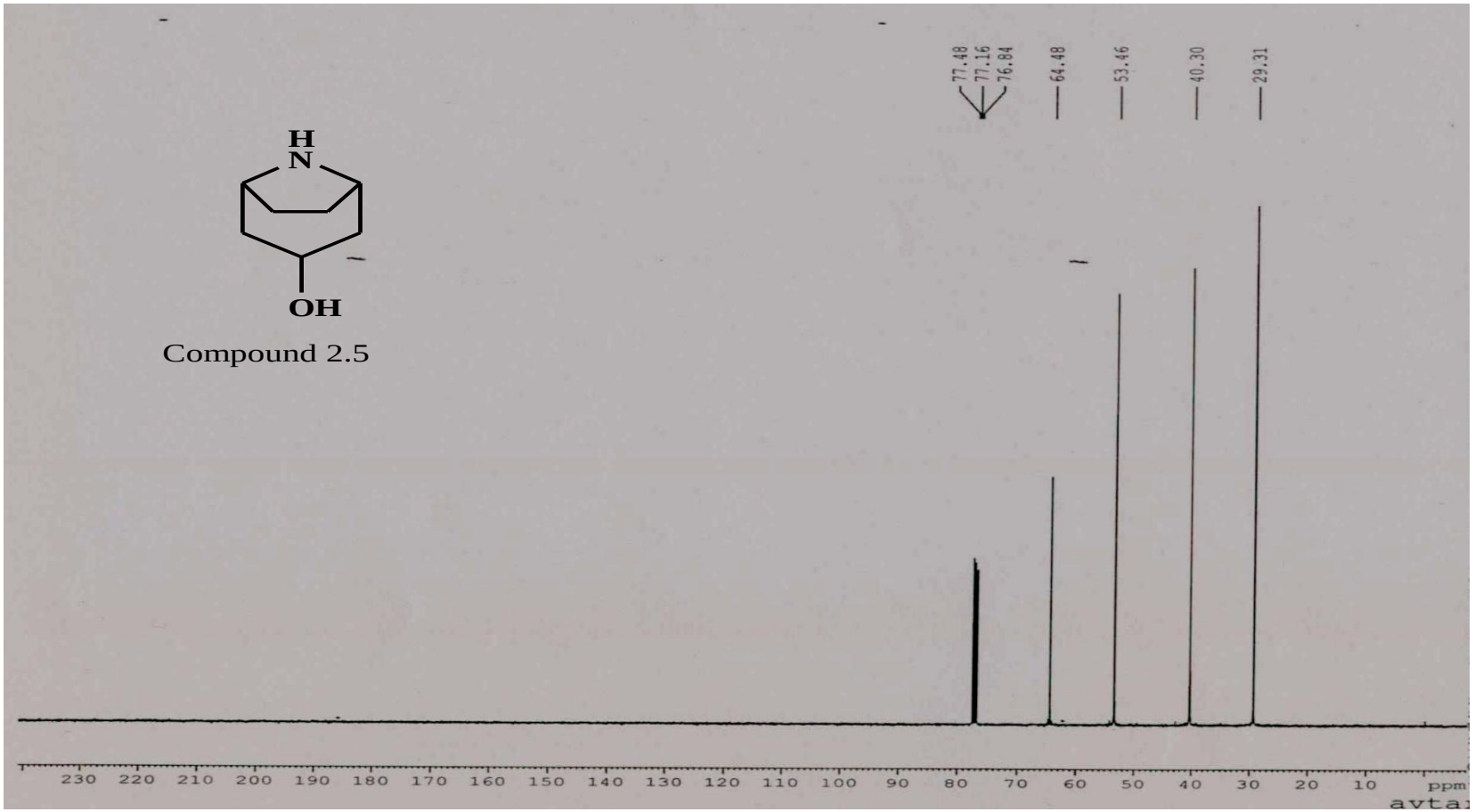
References:

1. Lester, A. M. *Chem. Rev.* **2005**, 105, 559-592.
2. John, M. D. *J. Antimicrob.Chemother.***1994**, 33, 685-706.
3. Maurício, F. S.; Marcus, V. N.; DeSouza, M,E.; Tran, H.D.; Débora, P. A.; Gustavo, S. G.; DeCarvalho, M.V.; Almeida, D. *Carbohydr. Res.* **2010**, 345, 761–767.
4. Qing, R.Q.; Jia, P.; Xiao, Q.G.; Ling, L.W.; Yu, F.L. *Bioorg. Med. Chem. Lett*, **2012**, 22, 7688–7692.
5. Xu-Dong, W.; Wei, W.; Peng, F.W.; Yun, T. T.; Rui-Cheng, D.; Biao, L.; Sha-Sha, Z.; Jing-Wen, Z.; Lei, Z.; Zhu-Ping, X.; Hui, O.; Hai-Liang, Z. *Bioorg. Med. Chem.* **2014**, 22, 3620–3628.
6. Chang, Y. H.; Young, K.K.; Jay, H.C.; Se, H.K.; Hoon, C.; DoHyun, N.; Yong, Z. K.; Jin, H.K. *J. Med. Chem.* **1997**, 40, 3584-3593.
7. Ross, A.G.; Benton, B.M.; Chin, D.; De Pascale, G.; Fuller, J.; Leeds, J.A.; Reck, F.; Richie, D.L.; Vo, J.; LaMarche, M.J. *Bioorg Med. Chem. Lett.* **2015**, 25, 3468–3475.
8. Appelbaum, P.C.; Michael, J. R. *Expert Opin Pharmacother*, **2006**, 7, 1957-1966.
9. Bassetti, M.; Pecori, D.; Cojutti, P.; Righi, E.; Pea, F. *Expert Opin Drug Metab Toxicol*, **2017**, 13, 1193-1200.
10. Amirkia, V.; Heinrich, M.; *Phytochemistry Letters*, **2014**, 10, 48-53.
11. Grynkiewicz G, Gadzikowska M. *Pharmacol Rep*, *Pharmacol Rep*, **2008** , 60, 439-463.
12. R. Sachse, A. Schaupp. US patent 6,191,272 B1, Feb. 20, **2001**.
13. Matsumura, S.; Kise, M.; Tsuda, M. United States Patent 4,426,381, **1984**
14. Thatipally, S.; Dammalapati, V.L.N.R.; Gorantla, S.R.; Chava,S. WO/2014/125506 A2, **2014**

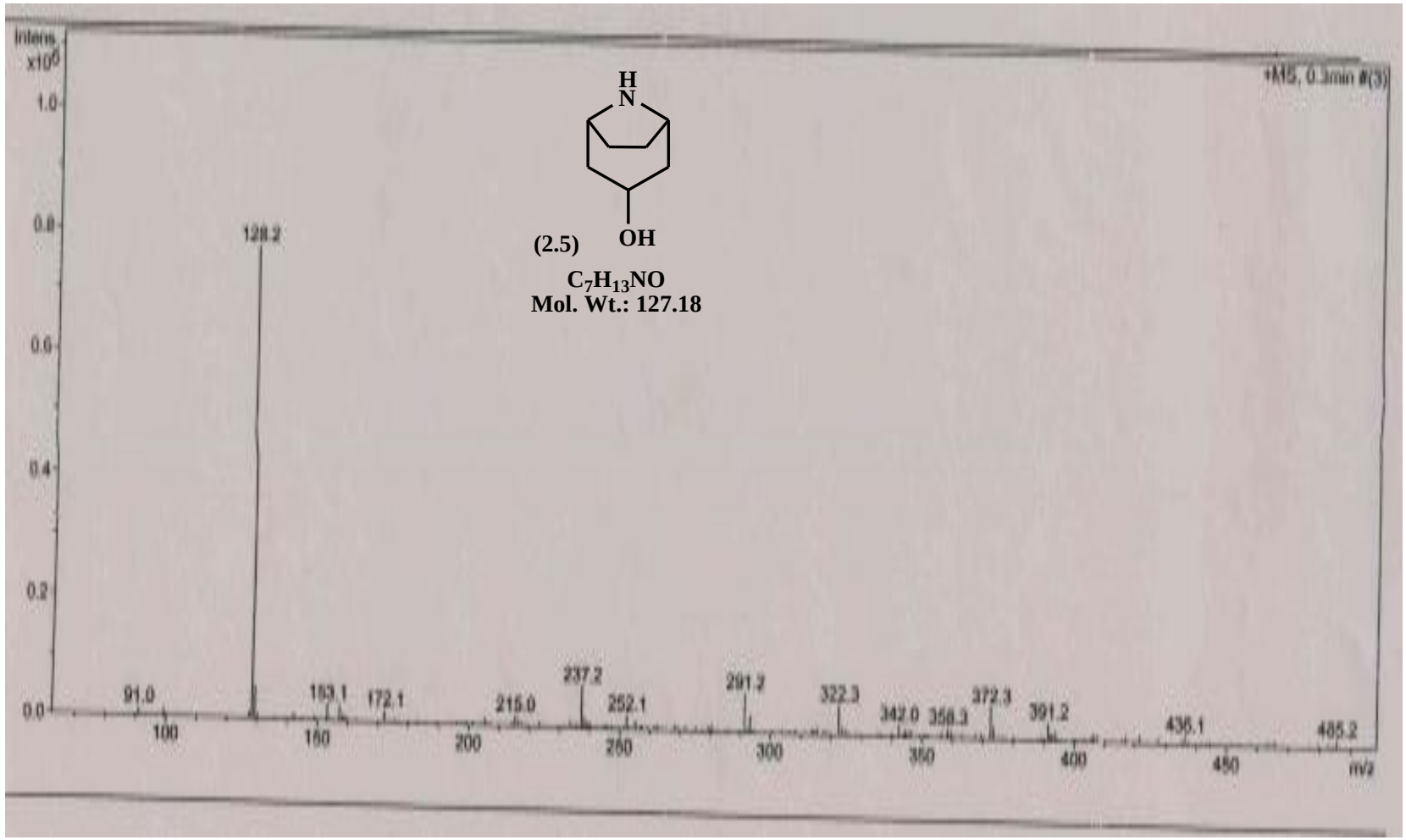
15. Zieba, A.; Maslankiewicz, A.; Sitkowski, J. *Magn. Reson. Chem.***2004**, 42, 903-904
16. Mitscher, L. A.; Sharma, P. N.; Chu, D. T. W.; Shen, L. L.; Pernet, A. G. *J. Med. Chem.* **1987**, 30, 2283-2286.
17. Al-Qawasmeh, R.A.; Zahra, J.A.; Zani, F.; Vicini, P.; Boese, R.;El-Abadelah, M.M. *ARKIVOC*, **2009**, 12, 322-336.



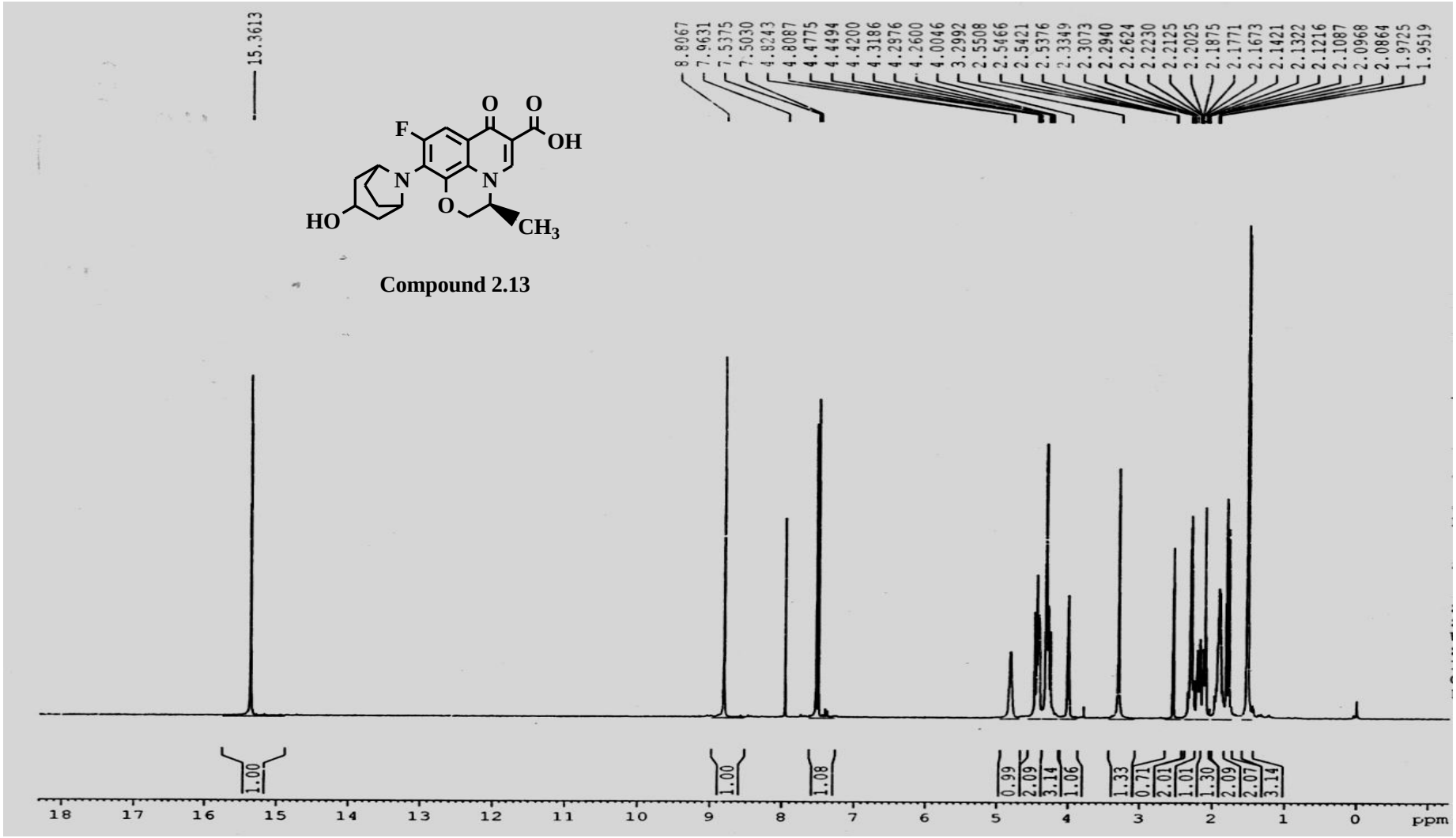
[1]H NMR of compound **(2.5)** (***Endo*-8-azabicyclo[3.2.1]octan-3-ol)**



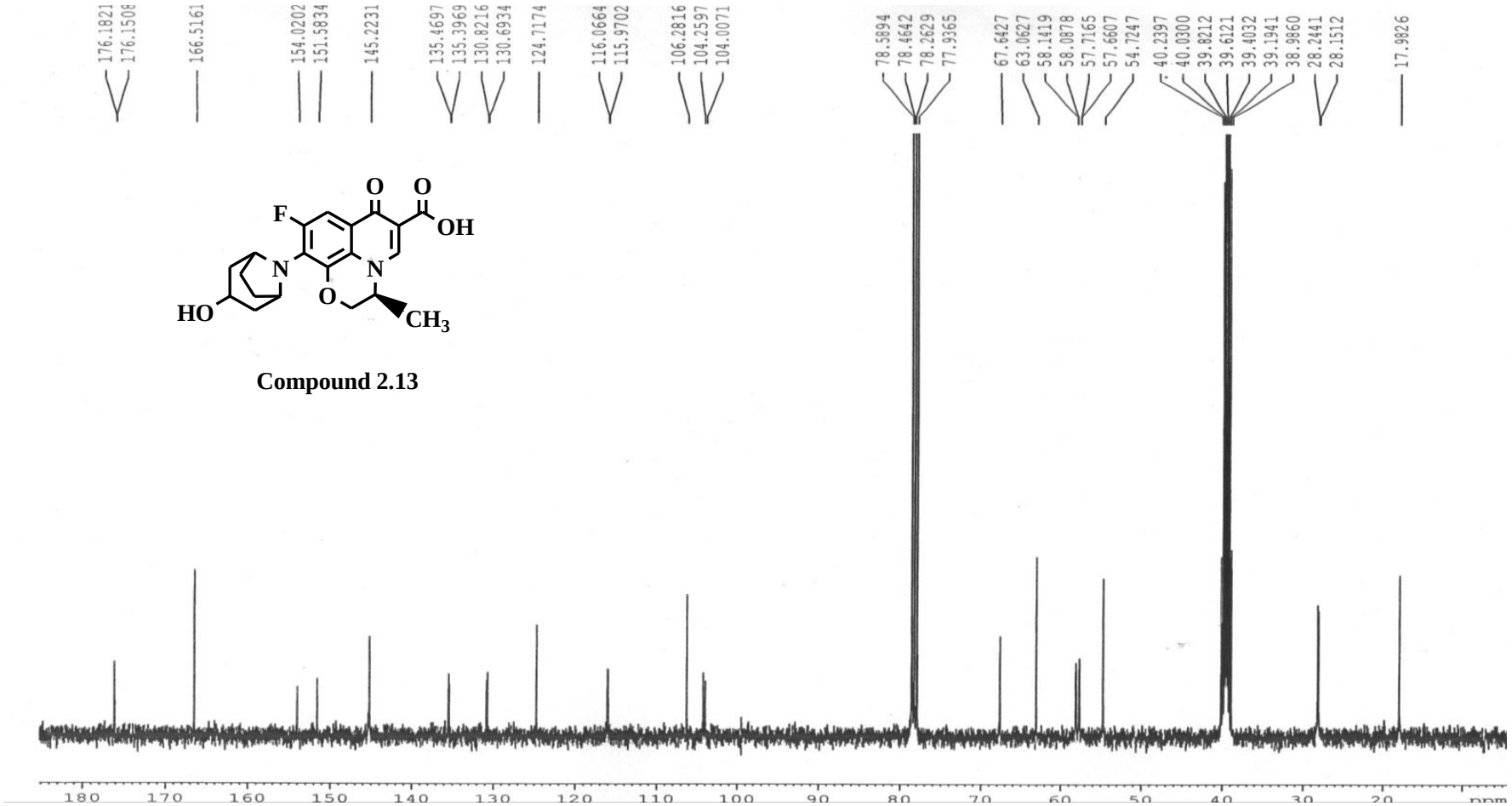
^{13}C NMR of compound **(2.5)** (***Endo*-8-azabicyclo[3.2.1]octan-3-ol)**



Mass spectra of compound **(2.5)** (***Endo*-8-azabicyclo[3.2.1]octan-3-ol)**

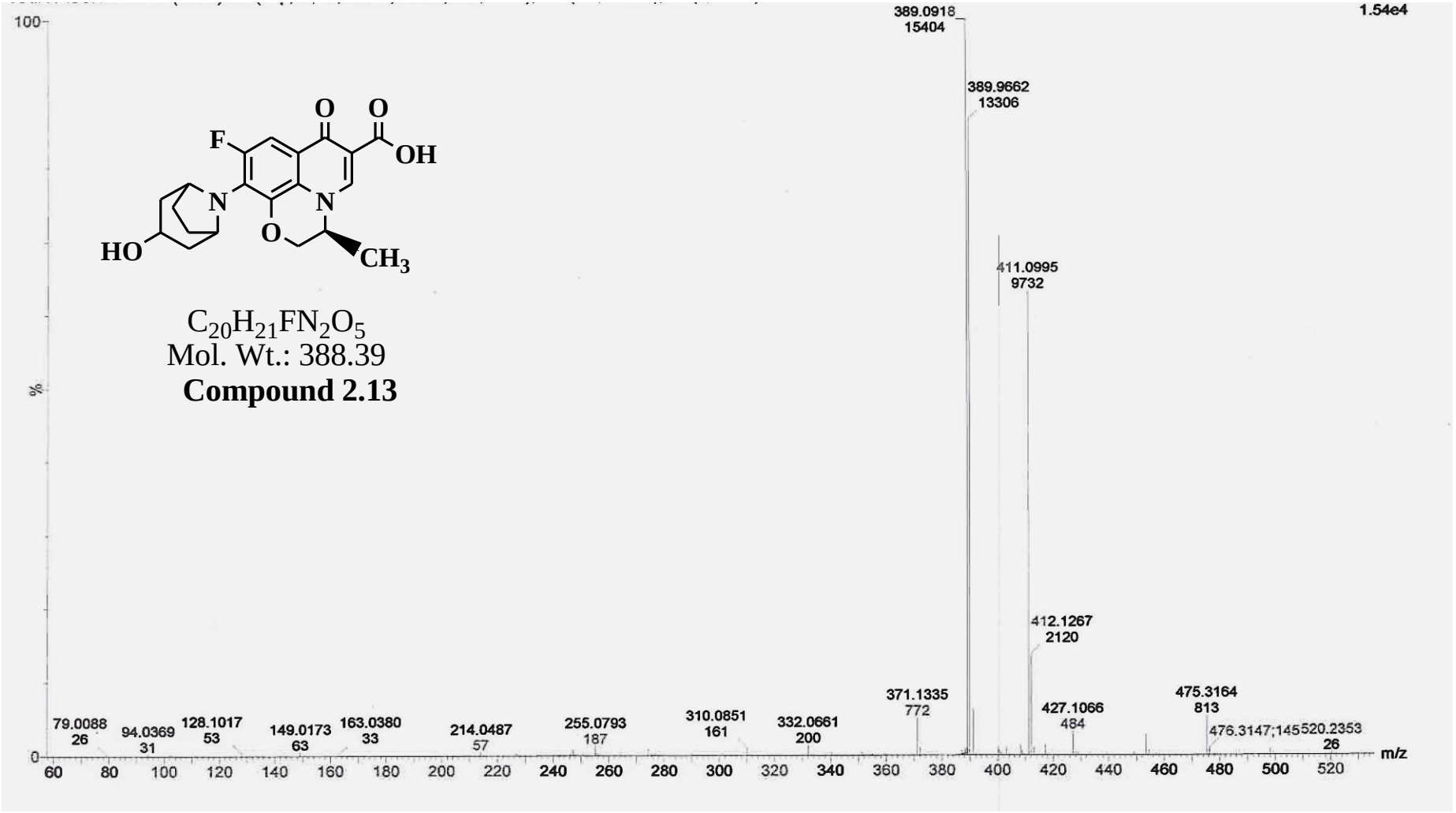


[1]H NMR of compound **2.13**

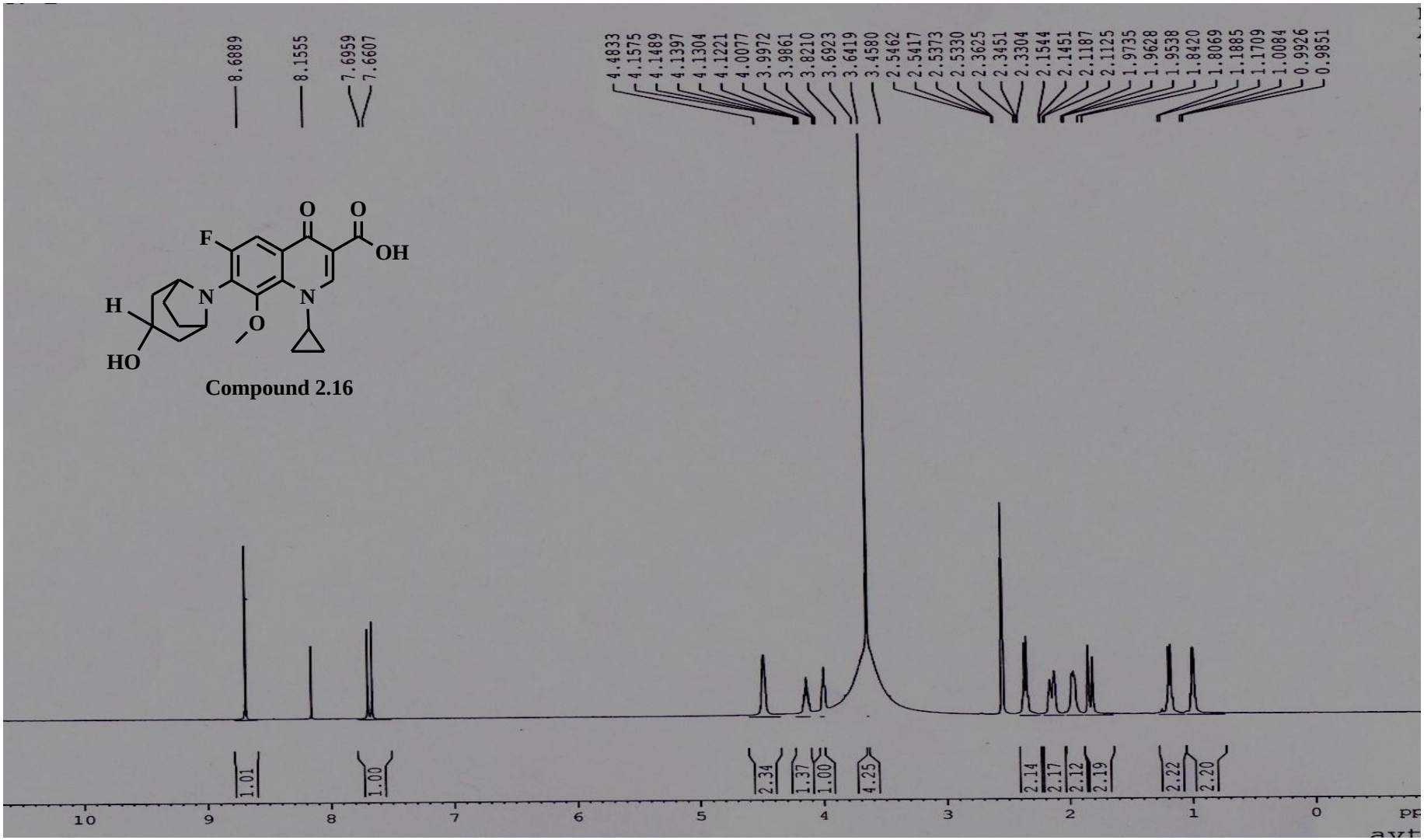


Compound 2.13

^{13}C NMR of compound **2.13**



Mass Spectra of compound 2.13



Compound 2.16

Figure : ^{1}H NMR data of compound 2.16

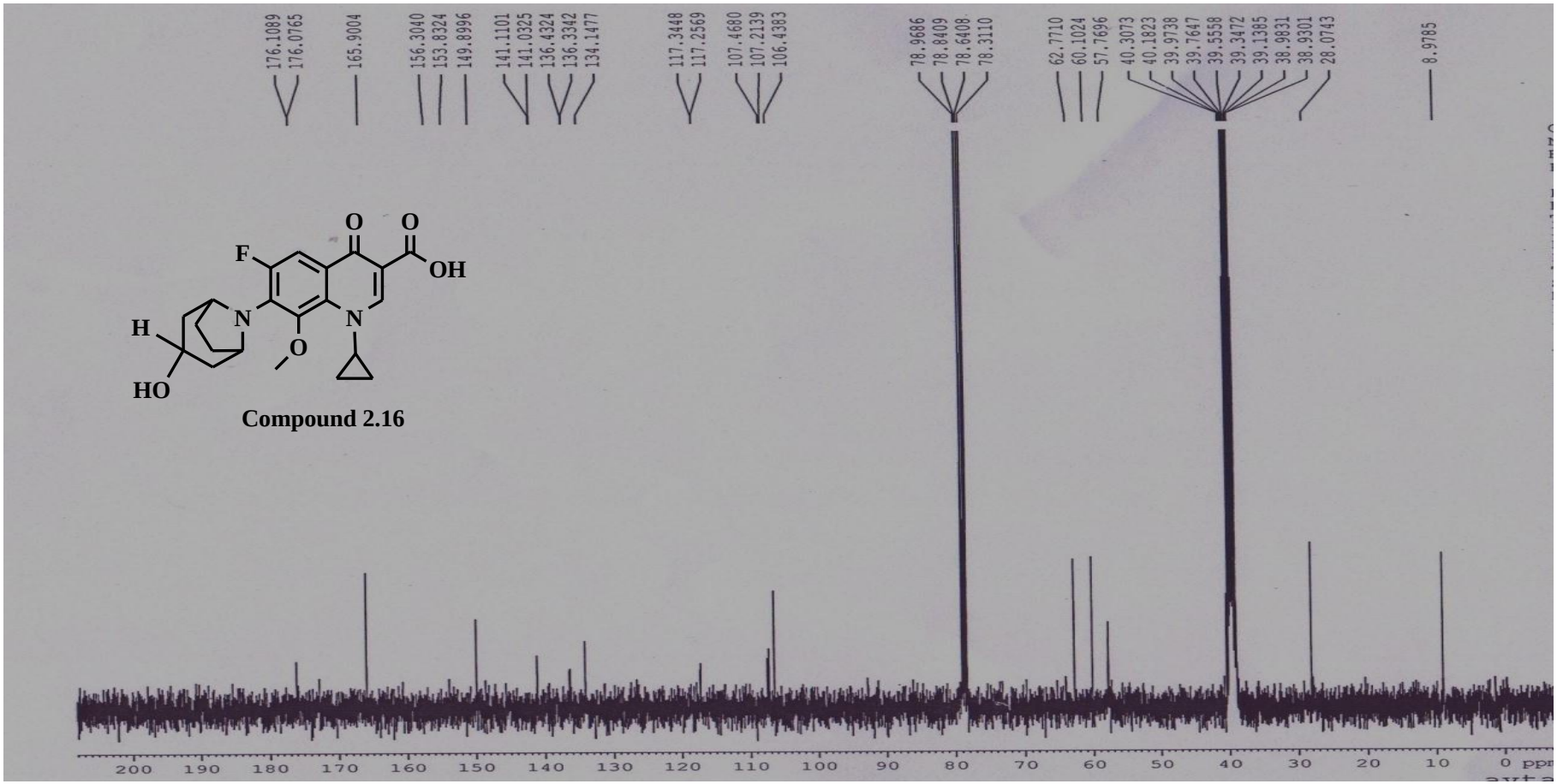


Figure : ^{13}C NMR data of compound 2.16

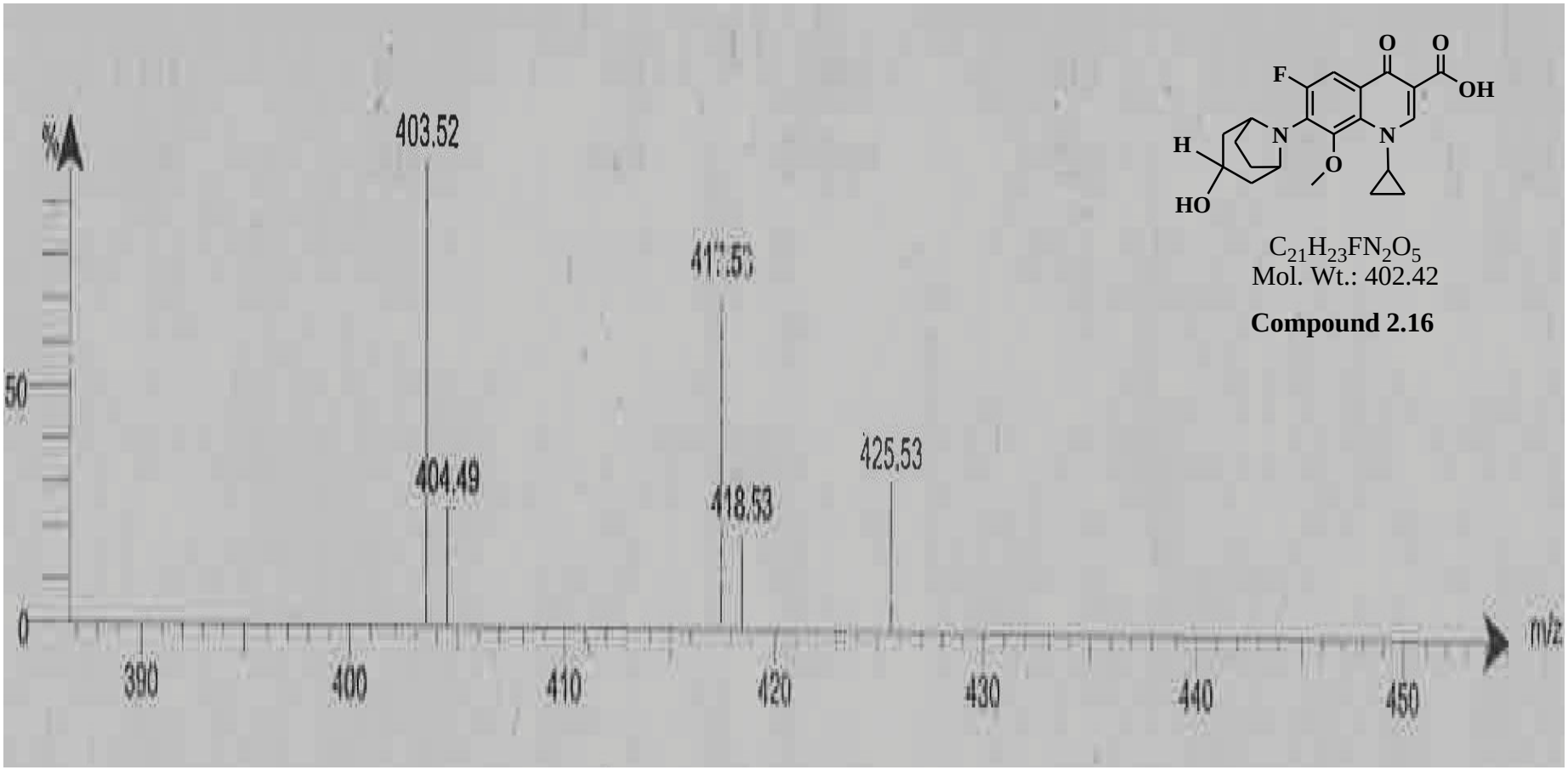


Figure : Mass Spectra of compound 2.16

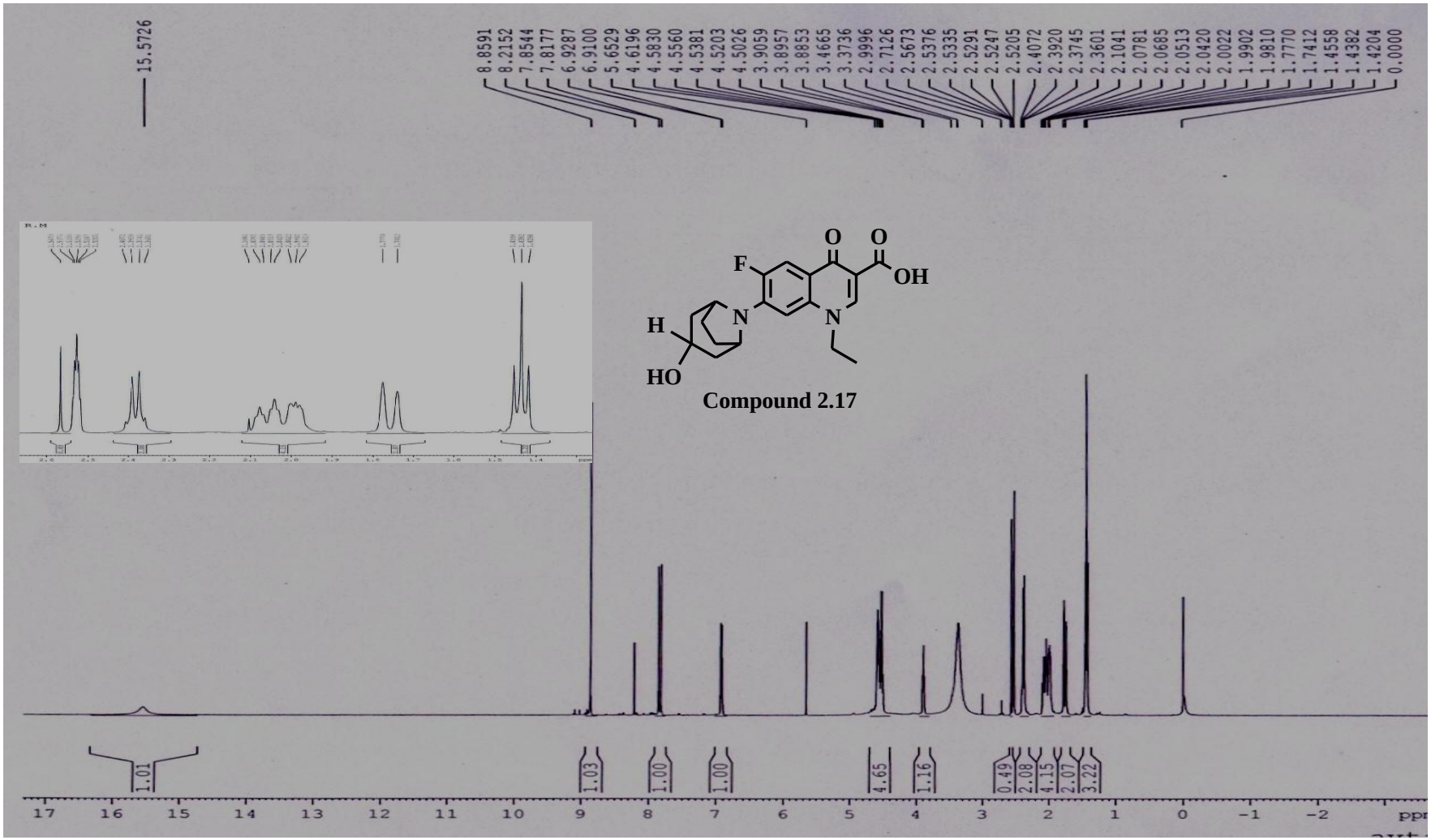


Figure : ^{1}H NMR data of compound 2.17

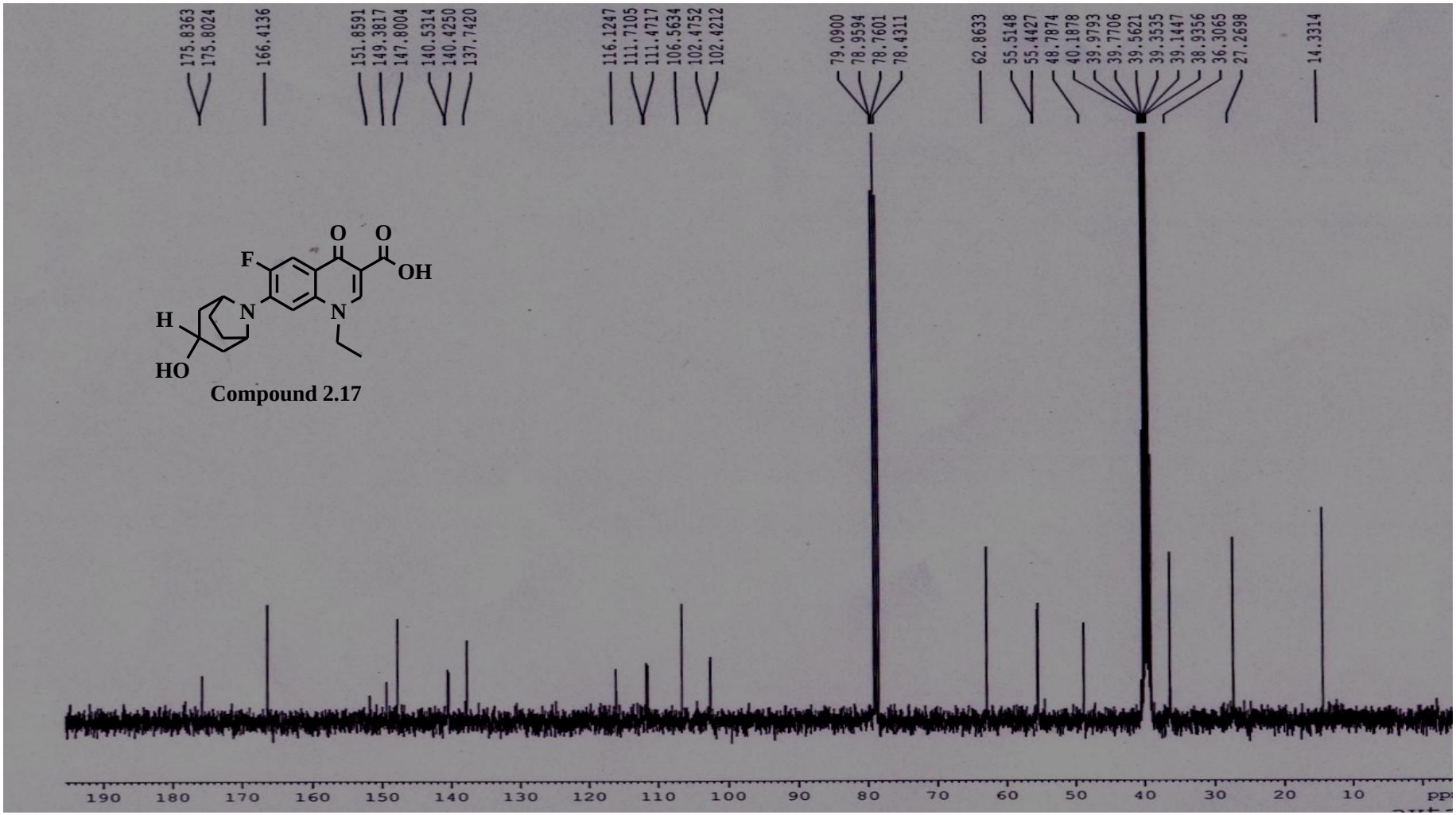


Figure : ^{13}CNMR data of compound 2.17

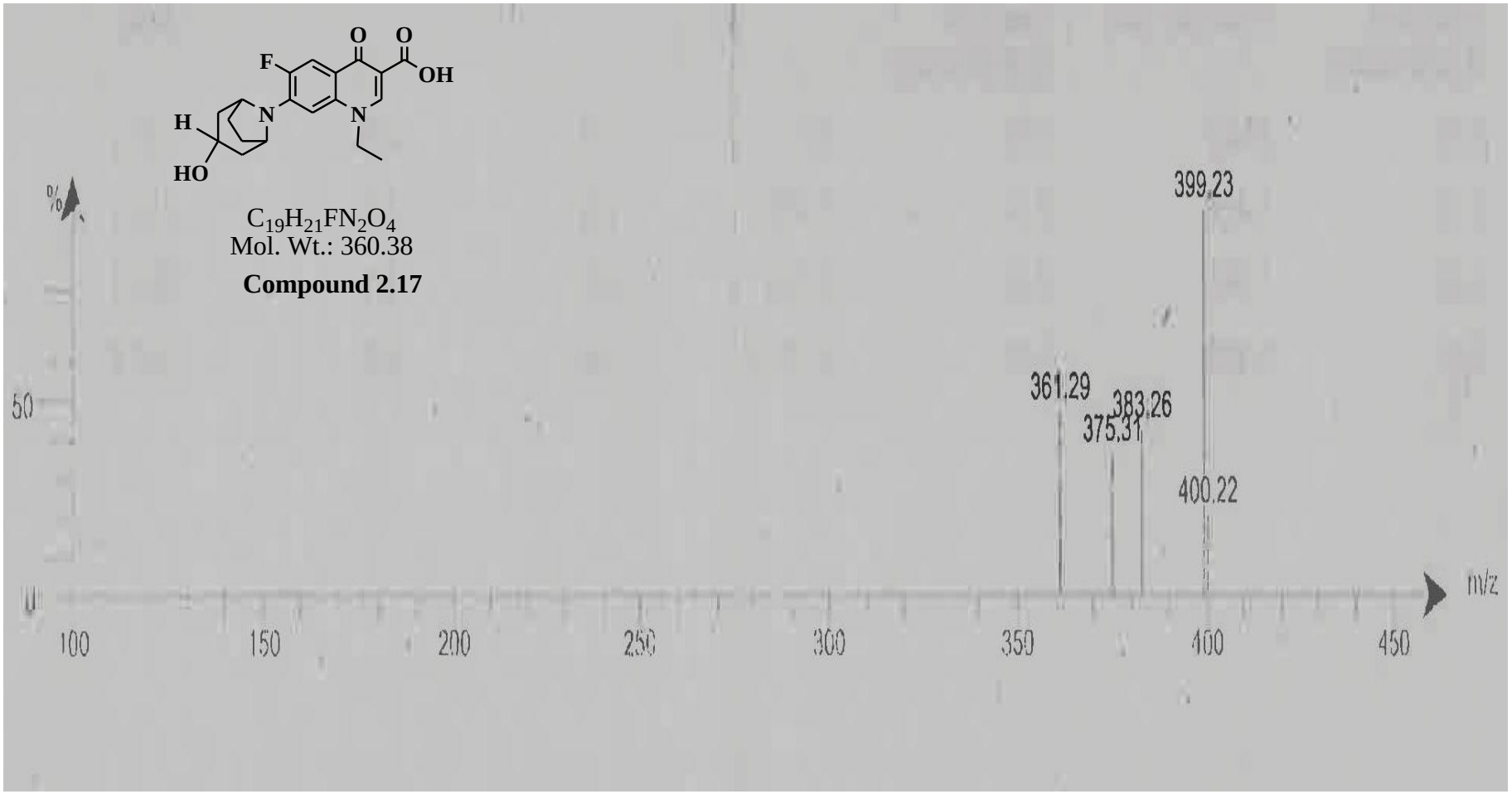
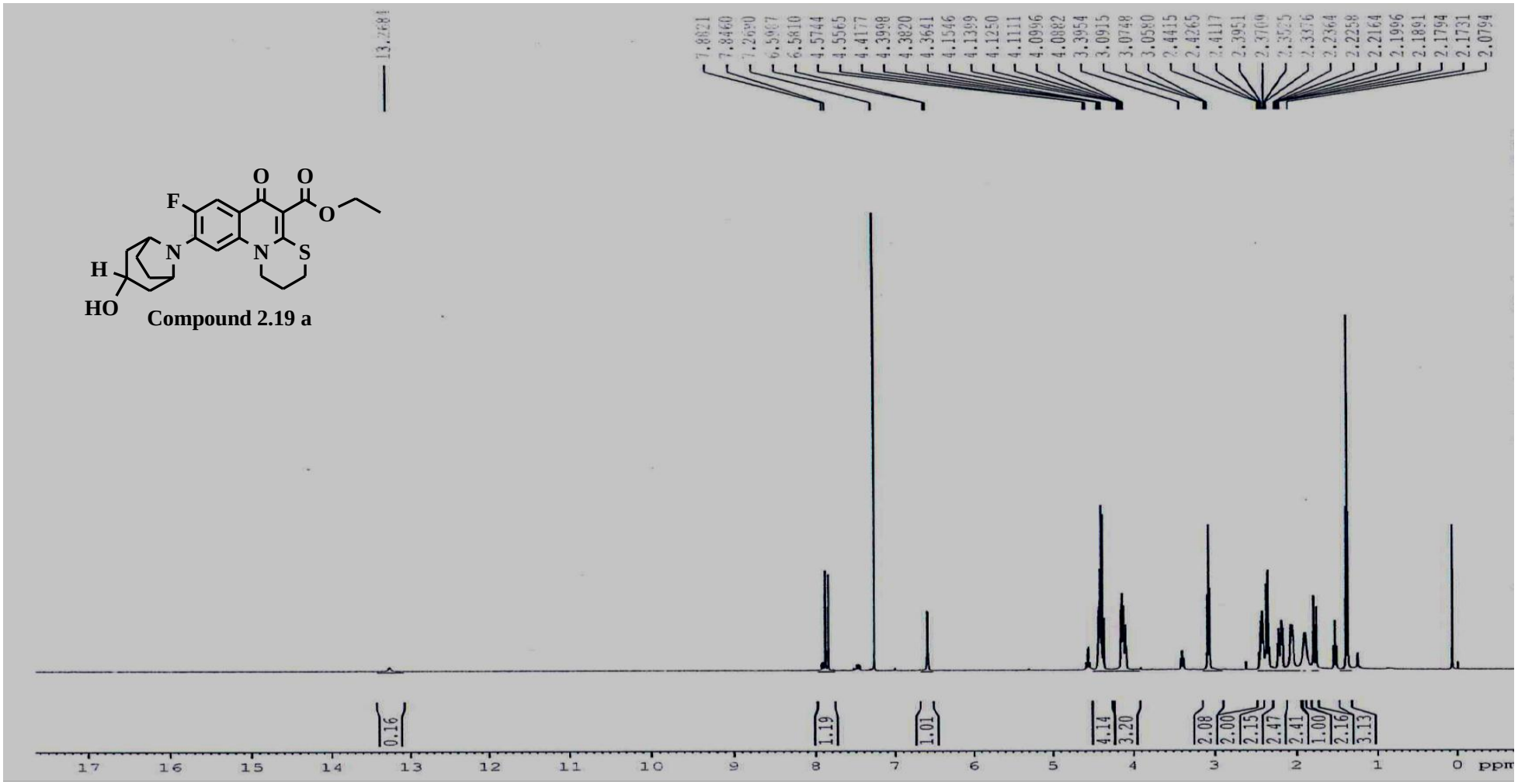
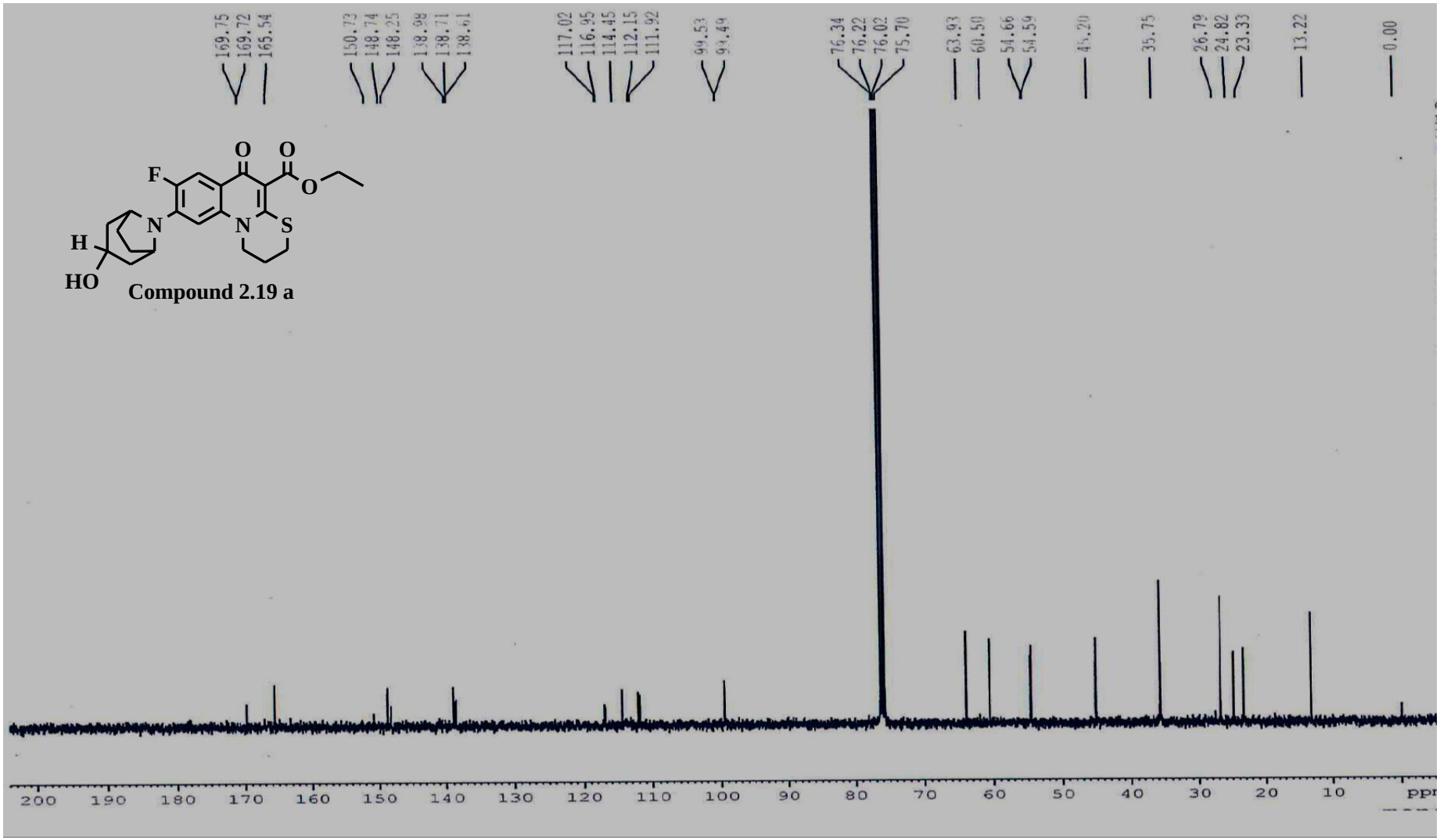


Figure : Mass Spectra of compound 2.17



[1]H NMR data of compound **2.19 a**



^{13}CNMR data of compound **2.19 a**

Aliphatic and Aromatic Esters of Nortropine Substituted Fluoroquinolones

3.1 Introduction

It is well known that C-3 carboxylic and C-4 keto groups are absolutely essential for hydrogen bonding interactions with the bases of the target DNA[1]. Fluoro group at C-6 position is responsible for enhanced potency against DNA gyrase and cell penetration[2]. Thus, C-7 substituent remains the preferred choice to further explore the antibacterial potential of these molecules. Moreover, it has been reported that antibacterial spectrum, bioavailability and safety of fluoroquinolones[3] are associated with this position. Position N-4 of the piperazine has been substituted with different bulky groups in many fluoroquinolones and the structure activity relationship (SAR) of these compounds revealed enhancement of antibacterial potency against Gram-positive bacteria[4-7].

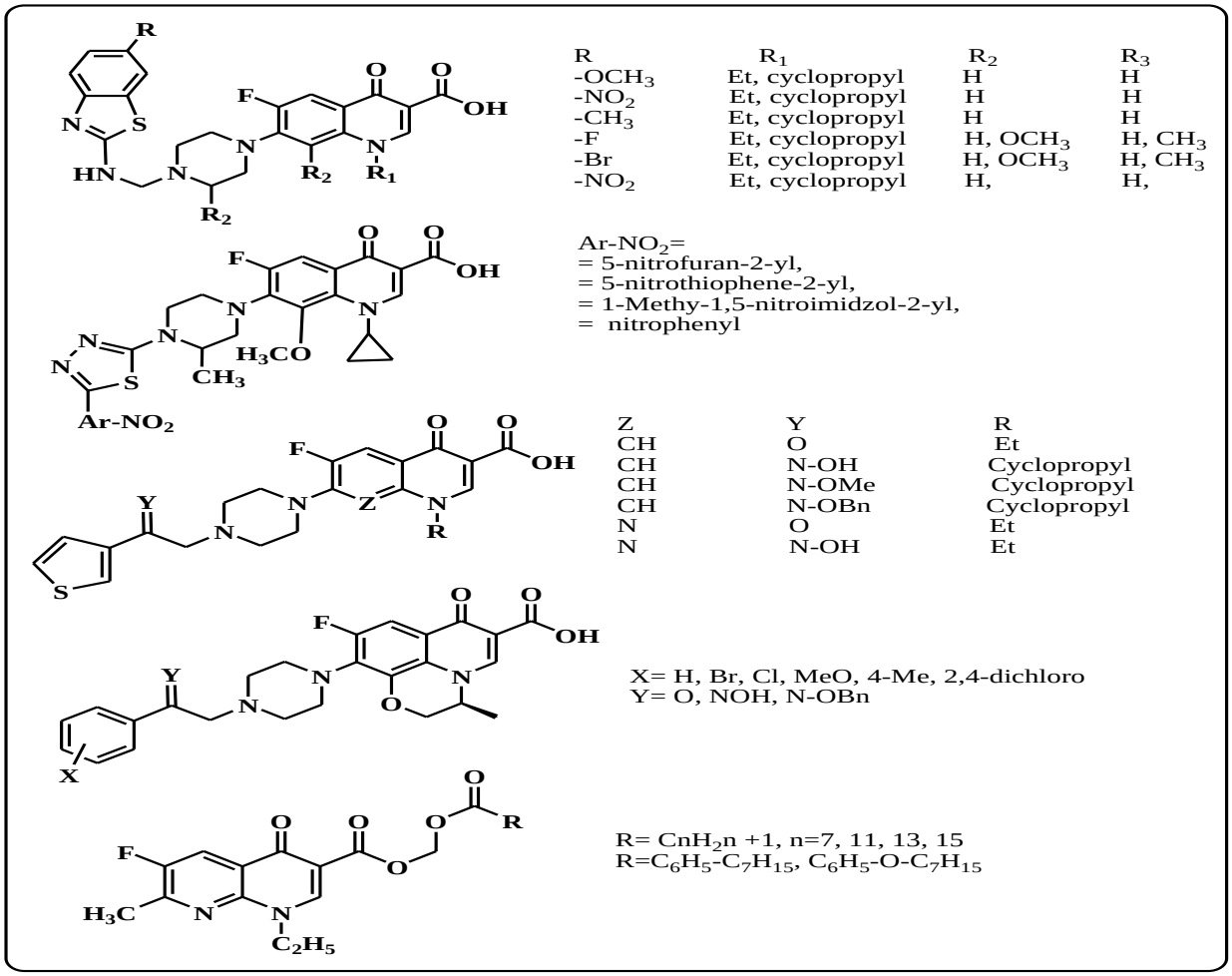


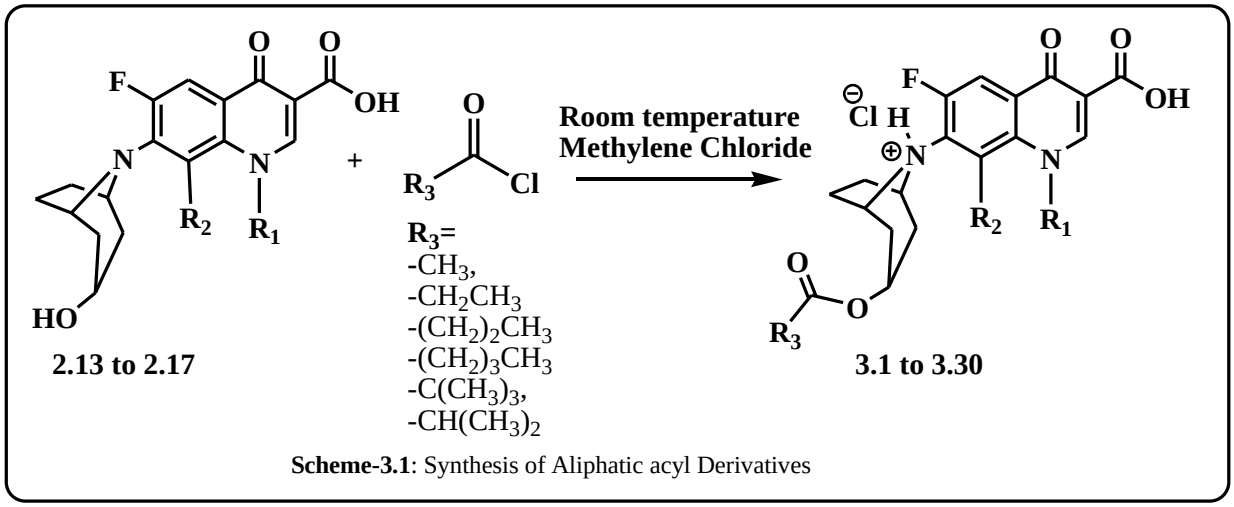
Figure 3.1: Different substituent of N-4 position of piperazine C-7 piperazynyl substituted molecules.[4-7]

Joelle Azema et. al. synthesized long alkyl chain derivatives at C-3 carboxylic terminals of nalixdic acid to evaluate their antibacterial potential and found these molecules to be intra-ocular pro drugs[3]. Thus, it was envisaged to derivatize the fluoroquinolones synthesized in the previous with aliphatic and aromatic acyl chlorides at the hydroxyl group of the C-7 substituted nortropine and observe its effect on the antibacterial activity.

3.2 Synthesis

Compounds **2.13** to **2.17** synthesized in the previous section were reacted with two different substrates namely **(a)** Aliphatic (linear chain length of length up to 4 carbons and branched) and **(b)** aromatic acyl chlorides.

Section-A: Synthesis of Aliphatic Acyl Derivatives: Precursors **2.13** to **2.17** were reacted with two fold excess of acyl chlorides using methylene chloride as solvent at room temperature for 24 hours. Reaction monitoring using TLC indicated formation of the ester that was isolated by distilling of the excess of acid chloride and solvent to get crude product. Solid precipitates were obtained on addition of ethyl acetate that was purified as corresponding hydrochloride salt by crystallization from methanol. It is worth mentioning here that no base was required for initiating the reaction in these cases.



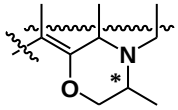
Scheme-3.1: Synthesis of Aliphatic acyl Derivatives

All the synthesized compounds **3.1** to **3.30** were characterized using ^{1}H, ^{13}C NMR and mass spectroscopic techniques. **Scheme-3.1** and **Table 3.1** describes the synthesis and structures and yields of the aliphatic ester derivatives starting from compounds **2.13** to **2.17**.

3.2.1 Characterization: Formation of compounds **3.1** to **3.30** was confirmed by the disappearance of hydroxyl group of the bicyclic ring in the region of δ 1.61- 4.0 ppm. All the terminal methyl protons for the hydrocarbon chain for compounds **3.2 - 3.4**, **3.8 - 3.10**, **3.14 –**

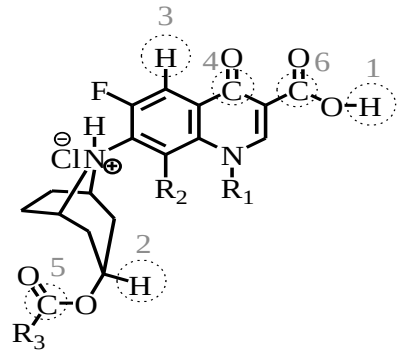
3.16, **3.20 - 3.22** and **3.26 - 3.28** appeared in the range of δ 0.8-0.9 ppm as triplet except for tert-butyl **(3.5**, **3.11**, **3.17**, **3.23** and **3.29)** and vinylic **(3.6**, **3.12**, **3.18**, **3.24** and **3.30)** methyl groups that appeared around δ 1.25 ppm (9 protons) and δ 1.15 ppm (6 protons) as singlet and doublet respectively in ^{1}H NMR. The single methyl group for the acetyl derivatives **(3.1**, **3.7**, **3.13**, **3.19** and **3.25)** appeared downfield around δ 2.00 ppm due to deshielding effect of adjacent carbonyl carbon. The methylene protons also appeared at their usual positions as per distances from the carbonyl carbons in the range of δ 2.41 –1.30 ppm.

Table 3.1: describes the synthesis and structures and yields of the aliphatic ester derivatives starting from compounds **2.13** to **2.17**



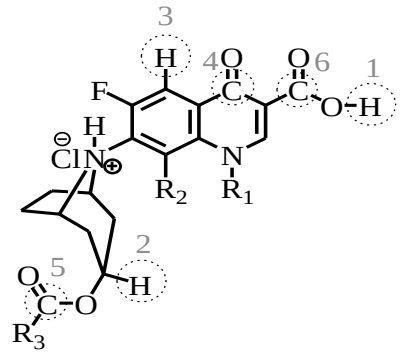
Compound No.	R_1	R_2	R_3	Yield in %
3.1	-*$CH(CH_3)CH_2O$-	-	CH_3	70
3.2	-*$CH(CH_3)CH_2O$-	-	CH_2CH_3	73
3.3	-*$CH(CH_3)CH_2O$-	-	$(CH_2)_2CH_3$	65
3.4	-*$CH(CH_3)CH_2O$-	-	$(CH_2)_3CH_3$	60
3.5	-*$CH(CH_3)CH_2O$-	-	$C(CH_3)_3$	67
3.6	-*$CH(CH_3)CH_2O$-	-	$CH(CH_3)_2$	65
3.7	-$CH(CH_3)CH_2O$-	-	CH_3	76
3.8	-$CH(CH_3)CH_2O$-	-	CH_2CH_3	73
3.9	-$CH(CH_3)CH_2O$-	-	$(CH_2)_2CH_3$	60
3.10	-$CH(CH_3)CH_2O$-	-	$(CH_2)_3CH_3$	63
3.11	-$CH(CH_3)CH_2O$-	-	$C(CH_3)_3$	60
3.12	-$CH(CH_3)CH_2O$-	-	$CH(CH_3)_2$	60
3.13	-C_3H_5	H	CH_3	91
3.14	-C_3H_5	H	CH_2CH_3	89
3.15	-C_3H_5	H	$(CH_2)_2CH_3$	80
3.16	-C_3H_5	H	$(CH_2)_3CH_3$	88
3.17	-C_3H_5	H	$C(CH_3)_3$	88
3.18	-C_3H_5	H	$CH(CH_3)_2$	80
3.19	-C_3H_5	-OCH_3	CH_3	69
3.20	-C_3H_5	-OCH_3	CH_2CH_3	76
3.21	-C_3H_5	-OCH_3	$(CH_2)_2CH_3$	69
3.22	-C_3H_5	-OCH_3	$(CH_2)_3CH_3$	50
3.23	-C_3H_5	-OCH_3	$C(CH_3)_3$	50
3.24	-C_3H_5	-OCH_3	$CH(CH_3)_2$	69
3.25	-C_2H_5	H	CH_3	91
3.26	-C_2H_5	H	CH_2CH_3	84
3.27	-C_2H_5	H	$(CH_2)_2CH_3$	68
3.28	-C_2H_5	H	$(CH_2)_3CH_3$	77
3.29	-C_2H_5	H	$C(CH_3)_3$	77
3.30	-C_2H_5	H	$CH(CH_3)_2$	68

Table 3.2: Summary of prominent ^{1}H, ^{13}C NMR peaks along with the mass spectra of compound **3.1 to 3.30.**



Compd No.	^{1}H NMR in δ ppm			^{13}C NMR in δ ppm			Mass m/z ratio (M+1)*
	1 - COO**H**, **1H, s**	**2** Endo H CH_2C**H**(R)CH_2 1H, t	**3** C-5H -CFC**H**C= 1H, d, J = 14 Hz	**4** C-4 **C**=O J = 4Hz	**5** Ester -O-(**C**=O) R	**6** C-3, **C**OOH	
3.1	15.2	5.1	7.6	176.9	167.4	170.4	432*
3.2	15.2	5.2	7.7	176.9	173.6	167.4	445.46
3.3	15.2	5.2	7.6	176.9	172.9	167.4	460*
3.4	15.2	5.2	7.7	176.4	173.0	167.5	473
3.5	15.2	5.2	7.7	176.9	177.5	167.4	473
3.6	15.2	5.1	7.7	176.9	177.6	167.4	460*
3.7	15.2	5.1	7.5	176.2	166.2	169.7	431
3.8	15.2	5.0	7.5	176.2	172.2	166.2	445.46
3.9	15.2	5.0	7.5	176.9	172.9	167.4	459
3.10	14.7	5.1	7.6	176.4	173.0	167.5	473
3.11	15.1	5.1	7.6	176.9	177.6	167.4	473
3.12	15.1	5.1	7.6	176.9	176.1	167.4	459
3.13	15.3	4.9	7.8	176.8	169.2	166.2	415.36
3.14	15.3	4.9	7.8	176.8	169.8	167.2	429.43
3.15	15.3	5.0	7.8	176.7	172.7	167.2	449.38
3.16	15.3	5.0	7.8	176.8	172.9	167.3	457.49
3.17	15.3	5.0	8.0	177.7	172.9	167.4	457.49
3.18	15.3	5.0	8.0	177.7	172.9	167.4	449.38
3.19	15.3	5.0	8.0	176.5	170.2	167.4	403.52
3.20	15.3	5.1	8.0	176.6	173.6	167.4	417.28
3.21	15.3	5.1	8.0	176.6	172.7	167.4	431.54
3.22	15.3	5.1	7.9	1766	172.8	167.4	445.58
3.23	15.3	5.0	7.9	177.4	176.0	167.4	445.5
3.24	15.3	5.0	7.9	177.4	176.0	167.4	431.58

Table 3.2: Summary of prominent ^{1}H, ^{13}C NMR peaks along with the mass spectra of compound **3.1 to 3.30.**



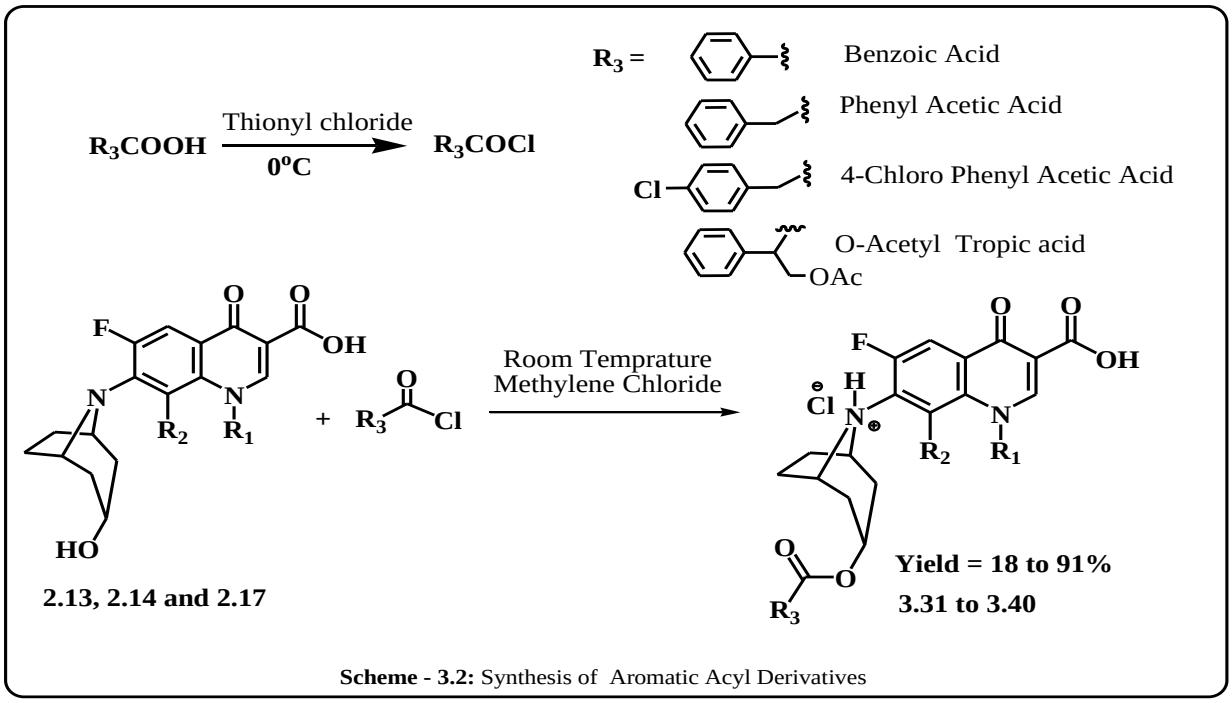
Compd No.	^{1}H NMR in δ ppm			^{13}C NMR in δ ppm			Mass m/z ratio (M+1)*
	1 - COO**H**, **1H, s**	**2** Endo H CH_2C**H**(R)C H_2 1H, t	**3** C-5H -CFC**H**C= 1H, d, J = 14 Hz	**4** C-4 **C**=O J = 4Hz	**5** Ester -O-(**C**=O) R	**6** C-3, **C**OOH	
3.25	14.9	5.1	7.8	175.7	169.2	165.8	445.43
3.26	14.9	5.1	7.8	176.9	169.2	165.8	459.43
3.27	14.9	5.1	7.8	176.9	172.8	167.0	473.45
3.28	14.9	5.2	7.8	176.9	173.0	167.0	487.45
3.29	14.9	5.2	7.8	177.3	173.0	167.0	487.45
3.30	14.9	5.1	7.7	176.1	173.0	167.0	473.48

Appearance of additional carbonyl carbon for all the thirty compounds **(3.1** to **3.30)** around δ 172 ppm in ^{13}C NMR due to ester bond confirmed the substitution of aliphatic acid chlorides to the hydroxyl of the bicyclic ring. This third carbonyl carbon was in addition to the two carbonyls already present in the fluoroquinolone skeleton. Carbonyl at C-4 position coupled with C-6 fluorine to appear as doublet at 176 δ ppm with J =4 Hz while the carboxylic acid ketone appeared around 167 δ ppm. Mass spectra also matched with the molecular mass of synthesized compounds. **Table - 3.2** displays the major ^{1}H, ^{13}C NMR peaks along with the mass spectra of all the compounds.

All levorotatory derivatives **(3.1 to 3.6)** displayed optical rotation when measured by polarimeter indicating that no loss of chirality during acylation reaction.

Section-B: Synthesis of Aromatic Acyl Derivatives: Acid chlorides required for the purpose were freshly prepared, unlike aliphatic acid chloride counterparts discussed above, from aromatic acids namely benzoic acid, phenyl acetic acid, 4-chloro phenyl acetic acid and racemic

o-Acetyl tropic acid using thionyl chloride at low temperature (0°C). Volatile compounds from the reaction mixture viz. excess thionyl chloride and by products hydrochloric acids were removed under vacuum to get corresponding acyl chlorides that were made to react with precursors **2.13** to **2.17**. Unfortunately fluoroquinolones **2.15** and **2.16** did not give the desired product. Conditions for the esterification of fluoroquinolones **2.13**, **2.14** and **2.17** were similar to that used for aliphatic ester derivatives **(3.1 to 3.30)** that did not require use of any base for the reaction completion. However, attempts to afford esterification product for fluoroquinolones **2.15** and **2.16** by use of different bases like potassium carbonate, sodium carbonate, triethyl amine and Biphasic aq. solution of sodium hydroxide both at room temperature and conditions and different solvents were not successful. Thus, in this series only ten compounds **(3.31 to 3.40)** were obtained as shown in **Scheme -3.2** and **Table 3.3.**



Scheme - 3.2: Synthesis of Aromatic Acyl Derivatives

3.2.2 Characterization: Formation of compounds **3.31** to **3.42** was again confirmed by the disappearance of hydroxyl group of the bicyclic ring in the region of δ 1.61- 4.0 ppm. Important peaks of the fluoroquinolone skeleton have been listed in **Table- 3.4**. For compounds **3.32**, **3.33**, **3.36**, **3.37**, **3.39** and **3.40** methylene protons of the benzylic group appeared in the range of δ 3.62 -3.66 and δ 40.7 – 42.3 ppm in ^{1}H and ^{13}C NMR respectively. Aromatic region for all the above six compounds showed 5 **(3.32, 3.36, 3.39)** and 4 **(3.33, 3.37, 3.40)** protons together in the range δ 7.67 -7.22 ppm. Aromatic region of benzoic acid derivatives **(3.31** and **3.35)** appeared as doublet in the range δ 8.04 -8.10 ppm for two protons and two triplets in the range δ 7.62 – 7.55 ppm and δ 7.52 -7.45 ppm for one and two protons respectively. Four tropic acid acetate

derivatives of levofloxacin quinolone acid **(3.34)**, Ofloxacin quinolone acid **(3.38)**, 1N-ethyl Norfloxacin quinolone acid **(3. 41)** and ciprofloxacin quinolone acid **(3.42)** based fluoroquinolone derivatives gave four carbonyl carbons. A distinguishable carbonyl carbon due to tropic acid acetate motif appeared in the range of δ 169 -170 ppm in ^{13}C NMR besides other three carbonyl carbons present the molecule. Terminal carbon for the methyl of the acetate appeared in all the four compounds around δ 20.8 ppm. Protons due to terminal methyl methylene and –CH(R)- of tropic acid acetate appeared around δ 2.0, 3.9 and 4.5 ppm respectively for all the four compounds. Two methylene protons for compounds **3.41** and **3.42** were distinguishable due to adjacent chiral group by approximately δ 0.4 ppm.

Table 3.3: Shows the substituted different aromatic ester derivatives with yield.

Compound No.	R_1	R_2	R_3	Yield in %
3.31	-*CH(CH_3)CH_2O-	-	-C_6H_5	32
3.32	-*CH(CH_3)CH_2O-	-	-$CH_2C_6H_5$	62
3.33	-*CH(CH_3)CH_2O-	-	-$CH_2C_6H_4Cl$	62
3.34	-*CH(CH_3)CH_2O-	-	-$CHC_6H_5CH_2O(CO)CH_3$	36
3.35	-*CH(CH_3)CH_2O-	-	-C_6H_5	30
3.36	-CH(CH_3)CH_2O-	-	-$CH_2C_6H_5$	50
3.37	-CH(CH_3)CH_2O-	-	-$CH_2C_6H_4Cl$	62
3.38	-CH(CH_3)CH_2O-	-	-$CHC_6H_5CH_2O(CO)CH_3$	18
3.39	-C_2H_5	H	-$CH_2C_6H_5$	76
3.40	-C_2H_5	H	-$CH_2C_6H_4Cl$	46
3.41	-C_2H_5	H	-$CHC_6H_5CH_2O(CO)CH_3$	40
3.42	-C_3H_5	H	-$CHC_6H_5CH_2O(CO)CH_3$	47

Levorotatory derivatives **(3.31 to 3.34)** displayed optical rotation when measured by polarimeter indicating that no loss of chirality during acylation reaction.

3.3 Antibacterial Activity

All the synthesized compounds were evaluated for their *in vitro* antibacterial activity against Gram negative and Gram positive strains. The Gram negative included Escherichia *coli* (MTCC: 1687), *Pseudomonas aeruginosa* (MTCC: 3904), *Vibrio cholera* (MTCC 1934), while Gram positive included *Staphylococcus aureus* (MTCC: 1430) and *Bacillus subtilis* (MTCC 441). The screening was done by agar diffusion method taking levofloxacin (LFX) and ciprofloxacin (CFX) as control drugs. **Table -3.5** shows the results measured in terms of zone of inhibition (ZOI) with diameter in millimetres' against the used dosage of the molecule in µg/ml. of the five organisms shortlisted for the study only have been listed in table because *Pseudomonas aeruginosa* (MTCC: 3904) was inactive against all the synthesized molecules and thus has been omitted.

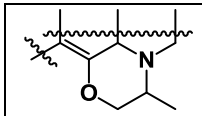
The molecules were shortlisted as active if their ZOI was up to 4 times to that of any of the controls for the organism taken for the study. Thus, for *V. cholera*, *B. subtilis*, and *E. coli* any molecule having dosage up to 8 µg/ml giving comparable ZOI (±2mm) to that of standard was termed as active. The dosage for defining active molecule in case of S. *aureus* was 16 µg/ml with comparable ZOI (±2mm).

Table -3.5 shows that most of the molecules were inactive against *V. cholera* and *E.coli.* The only exception in case of *V. cholera* was molecule **3.25**, N-1 ethyl substituted fluoroquinolone having acetyl substituted at *endo*-nortropine hydroxyl group. In fact, all fluoroquinolones synthesized in this section having acetyl substituted at *endo*-nortropine hydroxyl group were found to be active against rest of the two organisms' B. *subtilis* and *S. aureus.* Thus, compounds **3.1** and **3.7**, acetyl substituted levofloxacin Q-acid and ofloxacin Q-acid derivatives were active against *B. subtilis* and *S. aureus.* Besides these two, acetyl and

Table 3.4: Summary of prominent ^{1}H, ^{13}C NMR peaks along with the mass spectra of all the aromatic acyl chloride compounds.

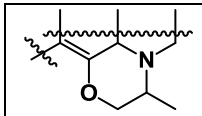
Compound No.	**^{1}H NMR in δ ppm**			**^{13}C NMR in δ ppm**			**Mass m/z ratio (M+1)**
	1 - COO**H**, **1H, s**	**2** Endo H CH_2C**H**(R)CH_2 1H, t	**3** C-5H -CFC**H**C= 1H, d, J = 14 Hz	**4** C-4 **C**=O J = 4Hz	**5** Ester -O-(**C**=O) R	**6** C-3, **C**OOH	
3.31	15.4	5.4	7.7	176.0	170.6	167.4	493.4
3.32	15.3	5.1	7.5	176.3	169.5	166.4	542.1
3.33	15.3	5.1	7.6	176.0	170.7	166.4	507.1
3.34	15.2	5.1	7.7	175.9	170.7	167.3	579.2
3.35	15.1	5.4	7.7	176.2	170.7	167.4	493.4
3.36	15.1	5.1	7.6	176.8	170.2	167.4	542.1
3.37	15.2	5.1	7.5	176.8	170.7	167.4	507.1
3.38	15.1	5.2	7.7	175.9	170.7	167.3	579.2
3.39	15.2	5.1	7.9	176.6	170.5	167.4	479.3
3.40	15.2	5.1	8.0	176.6	170.6	167.4	513.5
3.41	15.2	5.1	8.0	177.2	170.6	167.4	551.3
3.42	15.3	4.7	7.9	177.7	170.6	167.2	563.5

Table 3.5: Preliminary *in-vitro* biological activity of aliphatic substituted derivatives against some Gram positive and Gram negative strains. (Results measured in terms of zone of inhibition in mm (ZOI) with concentration in µg/ml.)



Cmpd. No	R_1	R_2	R_3	*V. cholera* MTCC 1934		*B. subtilis* MTCC 441		*S. aureus* MTCC 1430		*E. coli* MTCC 1687	
				Zone of inhibition in mm	MIC value at ZOI in µg/ml	Zone of inhibition in mm	MIC value at ZOI in µg/ml	Zone of inhibition in mm	MIC value at ZOI in µg/ml	Zone of inhibition in mm	MIC value at ZOI in µg/ml
	LFX	-	-	10	0.25	10	0.5	11	4	11	0.25
	CFX	-	-	14	2	10	2	12	2	16	2
3.1	-*$CH(CH_3)CH_2O$-	-	**CH_3**	12	32	**10**	**2**	**11**	**2**	0	128
3.2	-*$CH(CH_3)CH_2O$-	-	CH_2CH_3	12	32	10	32	11	32	0	128
3.3	-*$CH(CH_3)CH_2O$-	-	**$(CH_2)_2CH_3$**	11	32	**8**	**8**	11	32	0	128
3.4	-*$CH(CH_3)CH_2O$-	-	$(CH_2)_3CH_3$	12	32	11	32	11	32	0	128
3.5	-*$CH(CH_3)CH_2O$-	-	$C(CH_3)_3$	10	32	10	32	9	32	0	128
3.6	-*$CH(CH_3)CH_2O$-	-	$CH(CH_3)_2$	10	32	8	16	10	32	0	128
3.7	-$CH(CH_3)CH_2O$-	-	**CH_3**	10	16	**13**	**4**	**9**	**2**	0	128
3.8	-$CH(CH_3)CH_2O$-	-	CH_2CH_3	10	32	8	32	10	32	0	128
3.9	-$CH(CH_3)CH_2O$-	-	**$(CH_2)_2CH_3$**	10	128	10	16	**10**	**16**	0	128
3.10	-$CH(CH_3)CH_2O$-	-	$(CH_2)_3CH_3$	10	128	7	32	9	128	0	128
3.11	-$CH(CH_3)CH_2O$-	-	$C(CH_3)_3$	8	128	8	32	9	128	0	128
3.12	-$CH(CH_3)CH_2O$-	-	$CH(CH_3)_2$	8	128	8	32	9	128	0	128
3.13	-C_3H_5	H	**CH_3**	11	32	10	16	**11**	**16**	10	128
3.14	-C_3H_5	H	CH_2CH_3	11	32	10	16	**11**	**16**	10	128
3.15	-C_3H_5	H	$(CH_2)_2CH_3$	9	64	8	128	8	64	0	128

Table 3.5: Preliminary *in-vitro* biological activity of aliphatic substituted derivatives against some Gram positive and Gram negative strains. (Results measured in terms of zone of inhibition in mm (ZOI) with concentration in µg/ml.)



Cmpd. No	**R_1**	**R_2**	**R_3**	***V. cholera* MTCC 1934**		***B. subtilis* MTCC 441**		***S. aureus* MTCC 1430**		***E. coli* MTCC 1687**	
				Zone of inhibition in mm	MIC value at ZOI in µg/ml	Zone of inhibition in mm	MIC value at ZOI in µg/ml	Zone of inhibition in mm	MIC value at ZOI in µg/ml	Zone of inhibition in mm	MIC value at ZOI in µg/ml
3.16	$-C_3H_5$	H	$(CH_2)_3CH_3$	4	128	0	128	4	128	0	128
3.17	$-C_3H_5$	H	$C(CH_3)_3$	6	128	6	128	10	64	0	128
3.18	$-C_3H_5$	H	$CH(CH_3)_2$	4	128	8	128	8	64	0	128
3.19	$-C_3H_5$	$-OCH_3$	**CH_3**	12	**32**	**10**	**4**	14	32	8	128
3.20	$-C_3H_5$	$-OCH_3$	CH_2CH_3	10	64	8	64	11	64	6	128
3.21	$-C_3H_5$	$-OCH_3$	$(CH_2)_2CH_3$	0	128	7	64	10	32	0	128
3.22	$-C_3H_5$	$-OCH_3$	$(CH_2)_3CH_3$	4	128	0	128	4	128	0	128
3.23	$-C_3H_5$	$-OCH_3$	$C(CH_3)_3$	8	128	8	128	8	65	0	128
3.24	$-C_3H_5$	$-OCH_3$	$CH(CH_3)_2$	4	128	8	128	8	64	0	128
3.25	$-C_2H_5$	H	**CH_3**	**10**	**4**	**13**	**4**	**12**	**32**	10	64
3.26	$-C_2H_5$	H	CH_2CH_3	10	64	13	128	10	128	8	128
3.27	$-C_2H_5$	H	$(CH_2)_2CH_3$	8	128	6	128	4	128	0	128
3.28	$-C_2H_5$	H	$(CH_2)_3CH_3$	6	128	4	128	0	128	0	128
3.29	$-C_2H_5$	H	$C(CH_3)_3$	4	128	0	128	0	128	0	128
3.30	$-C_2H_5$	H	$CH(CH_3)_2$	4	128	0	128	4	128	0	128

and propionyl substituted ciprofloxacin Q-acid derivative, (**3.13**, **3.14** respectively) and n-butyryl ofloxacin Q-acid derivative **(3.9)** were also found to be active against S. *aureus.* Other two acetyl substituted compounds **3.19** and **3.25** were not active against S. *aureus.* However, both these compounds were active against *B. subtilis.*

Thus, with an exception of ciprofloxacin skelton derivatives, all four of the five acetyl substituted fluoroquinolones synthesized in this section were active against B. *subtilis.* Inactivity of the rest molecules, with an exception of one **(3.3)**, suggest that increasing chain length at the C-7 *endo*- nortropine substituent renders the molecules inactive against the organism.

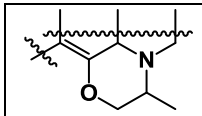
S. aureus on the other hand could be seized with broad molecular range of synthesized compounds having linear substituents up to four carbon atoms at C-7 substituent position. However, acetyl substituted fluoroquinolones having methoxy group at C-8 position and ethyl group at N-1 position, **3.19** and **3.25** respectively, were well endured by the organism.

Table 3.6 shows the results for aromatic derivatives against the above four organisms talking same molecules as control drugs. In this case also, *Pseudomonas aeruginosa* (MTCC: 3904) was inactive and therefore has been omitted.

It can be seen that only p-chlorobenzyl substituted ofloxacin Q-acid and N1- ethyl sub. fluoroquinolones (**3.37** and **3.40** respectively) were active against the two organisms B. *subtilis* and *S. aureus*. While ofloxacin derivative, **3.37**, was active against both organisms compound 3.40 showed activity against B. *subtilis* only. Besides this *S. aureus* could be clutched with ofloxcin's benzyl derivative, **3.36** also with comparable dosage and zone of inhibition as that of compound **3.37**.

It can be seen that only p-chlorobenzyl substituted ofloxacin Q-acid and N1- ethyl sub. fluoroquinolones (**3.37** and **3.40** respectively) were active against the two organisms B. *subtilis* and *S. aureus*. While ofloxacin derivative, **3.37**, was active against both organisms compound 3.40 showed activity against B. *subtilis* only. Besides this *S. aureus* could be clutched with ofloxcin's benzyl derivative, **3.36** also with comparable dosage and zone of inhibition as that of compound **3.37**.

Table 3.6: Preliminary *in-vitro* biological activity of aliphatic substituted derivatives against some Gram positive and Gram negative strains. (Results measured in terms of zone of inhibition in mm (ZOI) with concentration in µg/ml.)



Cmpd. No.	R_1	R_2	R_3	*V. cholera* MTCC 1934		*B. subtilis* MTCC 441		*S. aureus* MTCC 1430		*E. coli* MTCC 1687	
				Zone of inhibition in mm	MIC value at ZOI in µg/ml	Zone of inhibition in mm	MIC value at ZOI in µg/ml	Zone of inhibition in mm	MIC value at ZOI in µg/ml	Zone of inhibition in mm	MIC value at ZOI in µg/ml
	LFX	-	-	10	0.25	10	0.5	11	4	11	0.25
	CFX	-	-	14	2	10	2	12	2	16	2
3.31	-*CH(CH_3)CH_2O-	-	-C_6H_5	ND	ND	ND	ND	ND	ND	ND	ND
3.32	-*CH(CH_3)CH_2O-	-	-$CH_2C_6H_5$	10	128	9	128	10	128	0	128
3.33	-*CH(CH_3)CH_2O-	-	-$CH_2C_6H_4Cl$	8	64	9	64	11	64	0	128
3.34	-*CH(CH_3)CH_2O-	-	Tropic acid acetate	9	32	8	64	8	32	0	128
3.35	-CH(CH_3)CH_2O-	-	-C_6H_5	ND	ND	ND	ND	ND	ND	ND	ND
3.36	-CH(CH_3)CH_2O-	-	**-$CH_2C_6H_5$**	10	16	11	16	**13**	**16**	0	128
3.37	-CH(CH_3)CH_2O-	-	**-$CH_2C_6H_4Cl$**	10	16	**12**	**8**	**12**	**16**	0	128
3.38	-CH(CH_3)CH_2O-	-	Tropic acid acetate	10	32	9	32	10	64	0	128
3.39	-C_2H_5	H	-$CH_2C_6H_5$	14	128	10	64	0	128	0	128
3.40	-C_2H_5	H	**-$CH_2C_6H_4Cl$**	11	64	**12**	**8**	0	128	0	128
3.41	-C_2H_5	H	Tropic acid acetate	0	128	11	16	0	128	0	128
3.42	-C_3H_5	H	Tropic acid acetate	0	128	11	16	0	128	0	128

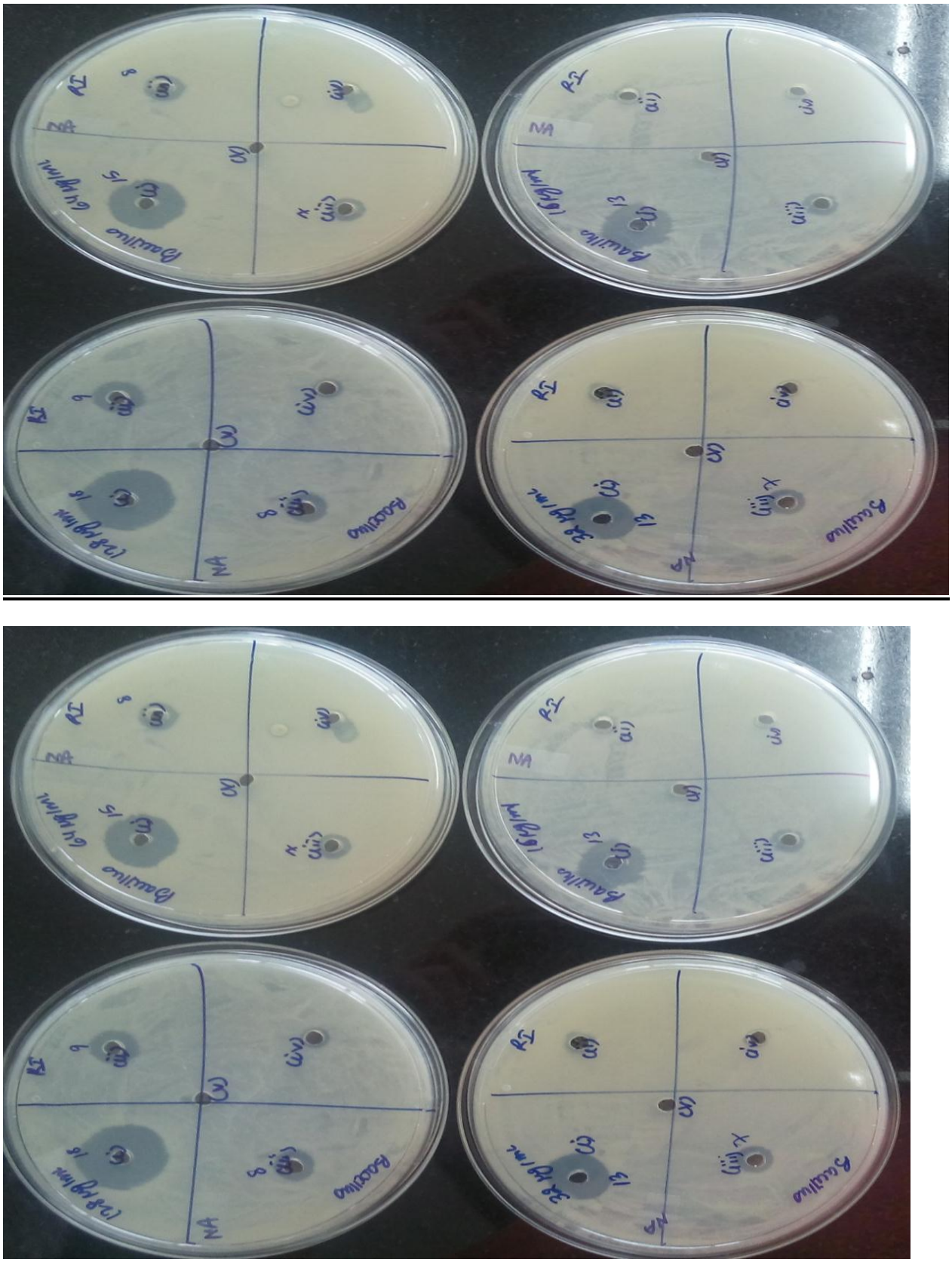


Figure 3.2 shows that the antibacterial activity of some compounds against *Vibrio cholera*, *Bacillus subtilis* and *Staphylococcus aureus* by agar diffusion method.

3.4 Conclusion:

In conclusion, of the five categories of fluoroquinolone skeletons substituted with different aliphatic and aromatic acyl groups three were active against both B. *subtilis* and *S. aureus*. Common feature among the three classes was methyl **(3.1, 3.7** and **3.25)** and p-chloro benzyl substitution **(3.37)** at the hydroxyl group of the C-7 *endo*-nortropine substituent. Besides this while B. *subtilis* could be seized with only other methyl substituted fluoroquinolones **(3.19** and **3.25)** *S. aureus* was distressed by **(3.9** and **3.14)** longer chains also.

3.5 Materials and methods

3.5.1 General procedure for the synthesis of compound 3.1 to 3.41: Compound **2.13** to **2.17** (1.0 mole eq) dissolved in Methylene dichloride (20 ml) and was added drop wise corresponding acid chlorides (2.0 mole eq) and the stirring continued for 24 hrs. After completion of the reaction, (TLC monitoring), excess of acid chloride was distilled off under vacuum. To the remaining solid mass was added ethyl acetate (2 X 10 ml) to dissolve impurities and filtered the product. Further purification, if required, was done by column chromatography using ethyl acetate and hexane solvent system or by re-crystallization in methanol to get 18-91% yield of compound **3.1** to **3.42**.

3.5.2 <u>Aliphatic Acid chloride substituted derivatives:</u>

(S)-10-(3-Acetoxy-8-aza-bicyclo [3.2.1] octan-8-yl)-9-Fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*] quinoline-6-carboxylic acid hydrochloride(3.1) : Light yellow colour powder, Yield- 70% (0.35 g), HPLC Purity- 99.88% , Melting point 261°C decomposes, $[\alpha]^{25}_{D}$ - 77°(*c* 0.04, CH_2Cl_2), ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 15.22 (s, 1H, -COO**H**), 8.59 (s, 1H, -NC**H**CCOOH), 7.68-7.65(d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.14 -5.12 (t, 1H, J= 4.8 Hz, -CH_2-C**H**(OR)-CH_2-), 4.54-4.52 (m, 1H, -NC**H**(CH_3)CH_2-), 4.44-4.42 (m, 2H, -NCH(CH_3)C**H_2**-), 4.35-4.33 (2bs, 2H, bridged **H**), 2.39-2.28(4ts, 2H, J=4.0 Hz , -C**H**(H)-C(OR)-C**H**(H)-), 2.18-2.14 (m, 2H, -CH(**H**)-C(OR)-CH(**H**)-), 2.10-2.03 (m, 5H, C**H**$_3$CO- and -CH**(H)**-CH**(H)**-), 1.90 -1.86 (2bs, 2H, -C**H(**H**)**-C**H(**H**)**-), 1.62 -1.61 (d, 3H, J= 6.7 Hz, -NCH(C**H**$_3$)CH_2-); ^{13}C NMR ($CDCl_3$, 100 MHz,): δ 176.94, 170.43, 167.45, (**154.77,152.32**, J= 245 Hz), 144.63, (**135.87, 135.80**, J= 7 Hz), (**131.25,131.11**, J= 14 Hz), 125.02, (**117.36, 117.26**, J=10 Hz),107.40**,** (**105.69, 105.44** , J=25 Hz), 68.04, 67.86, 58.31, 58.25, 57.86, 57.80, 55.54, 37.38, 37.29, 28.61, 28.56, 21.65, 18.40 ; CHN Anal. Calcd. For $C_{22}H_{23}FN_2O_6$: C, 61.39; H, 5.39; N, 6.51. Found: C, 61.14; H, 5.33; N, 6.23. EIMS m/z 432[M] +(2) calculated for $C_{22}H_{23}FN_2O_6$.

(S)-10-(3-Propionyloxy -8-aza-bicyclo [3.2.1] octan-8-yl)-9-Fluoro-3,7-dihydro-3-methyl-7-

oxo-2H-[1,4]oxazino [2,3,4-*ij*] quinoline-6-carboxylic acid hydrochloride (3.2) : Light yellow colour powder, Yield- 73% **(**0.38 g), HPLC Purity- 99.68% , Melting point 241°C decomposes, $[\alpha]^{25}_{D}$ - 83°(*c* 0.05, CH_2Cl_2), ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 15.18 (s, 1H, -COO**H**), 8.58 (s, 1H, -NC**H**CCOOH), 7.71-7.67 (d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.16 -5.15 (t, 1H, J=4.5 Hz, -CH_2-C**H**(OR)-CH_2-), 4.19 (m, 1H, -NC**H**(CH_3)CH_2-), 4.44-4.41 (m, 2H, -NCH(CH_3)C**H_2**-), 4.34-4.33 (2bs, 2H, bridged **H**), 2.40-2.28 (q and dt, 4H, -C**H**(H)-C(OR)-C**H**(H)-) and (CH_3C**H_2**CO-), 2.20-2.13 (m, 2H, -CH(**H**)-C(OR)-CH(**H**)-), 2.06-2.04 (m, 2H, -CH**(H)**-CH**(H)**-), 1.89 -1.85 (2bs, 2H, -C**H(**H**)**-C**H(**H**)**-), 1.62 -1.61 (d, 3H, J=6.7 Hz -NCH(C**H_3**)CH_2-), 1.20-1.17 (t, 3H, J=7.52, C**H_3**CH_2CO-); ^{13}C NMR ($CDCl_3$, 100 MHz,): δ 176.93, 173.66, 167.45, (**154.77, 152.32**, J= 245 Hz), 144.63, (**135.87, 135.80**, J= 7 Hz, (**131.25, 131.11**, J= 14 Hz, 125.02, (**117.36, 117.26**, J= 10 Hz) ,107.40**,** (**105.69, 105.44** , J= 25 Hz), 68.09, 67.70, 58.33, 58.28, 57.88, 57.83, 55.59, 37.44, 37.35, 28.62, 28.27, 18.40, 9.14. EIMS m/z 445.45[M] + (1) calculated for $C_{23}H_{25}FN_2O_6$.

(S)-10-(3-Butyryloxy-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*] quinoline-6-carboxylic acid hydrochloride (3.3): Off white colour powder, Yield- 65% **(**0.32 g); HPLC Purity 98.61% , Melting point 230-232°C, $[\alpha]^{25}_{D}$ -90°(*c* 0.05, CH_2Cl_2), ^{1}H NMR ($CDCl_3$,400 MHz): δ 15.25 (s, 1H, -COO**H**), 8.61 (s, 1H, -NC**H**CCOOH), 7.63-7.60(d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.18 -5.15 (t, 1H, J=4.7 Hz, -CH_2-C**H**(OR)-CH_2-), 4.60 (m, 1H, -NC**H**(CH_3)CH_2-), 4.44-4.35 (m, 4H, -NCH(CH_3)C**H_2**- and bridged **H**), 2.40-2.30 (m, 4H, -C**H**(H)-C(OR)-C**H**(H)- and –COC**H_2**CH_2CH_3), 2.20-2.06 (m, 4H, -CH(**H**)-C(OR)-CH(**H**)- and -CH**(H)**-CH**(H)**-), 1.88 -1.85 (2bs, 2H, -C**H(**H**)**-C**H(**H**)**-), 1.75 -1.62 (m, 5H, –$COCH_2$C**H_2**CH_3 and -NCH(C**H_3**)CH_2-), 1.01-0.98 (t, 3H, J= 7.4 Hz, –$COCH_2CH_2$C**H_3**); ^{13}C NMR ($CDCl_3$, 100 MHz,):δ (**176.88, 176.85**, J=3 Hz), 172.92, 167.45, (**154.76, 152.31**, J=245 Hz), 144.78, (**135.92, 135.85**, J=7 Hz), (**131.24, 131.11**, J=13 Hz), 125.02, (**117.25, 117.16**, J= 9 Hz), 107.26, (**105.51, 105.26**, J= 25 Hz), 68.13, 67.52, (58.30, 58.25, 57.86, 57.80, 55.58, 37.48 , 37.38 , 36.88 , 28.61, 28.57, 18.46, 13.78; CHN Anal. Calcd. For $C_{24}H_{27}FN_2O_6$: C, 62.87; H, 5.94; N, 6.11. Found: C, 62.85; H, 5.93; N, 5.93. EIMS m/z 460 [M]+(2) calculated for $C_{24}H_{27}FN_2O_6$.

(S)-10-(3-Pentanoyloxy-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*] quinoline-6-carboxylic acid hydrochloride (3.4): Off white colour powder, Yield- 60% (0.36 g), HPLC Purity- 98.19% , Melting point 196°C , $[\alpha]^{25}_{D}$ -109° (*c* 0.04, CH_2Cl_2), ^{1}H NMR ($CDCl_3$,400 MHz,): δ 15.19 (s, 1H, -COO**H**), 8.58 (s, 1H, -NC**H**CCOOH), 7.71-7.68 (d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.16 -5.14 (t, 1H, J=5.0 Hz, -CH_2-

C**H**(OR)-CH_2-), 4.50-4.34 (m, 5H, -NC**H**(CH_3)CH_2-, -NCH(CH_3)C**H**$_2$- and bridged **H**), 2.4-2.27(m, 4H, -C**H**(H)-C(OR)-C**H**(H)- and –COC**H**$_2$$CH_2$$CH_2$$CH_3$), 2.17-2.05 (m, 4H, -CH(**H**)-C(OR)-CH(**H**)- and -CH(**H**)-CH(**H**)-), 1.88 -1.85 (2bs, 2H, -C**H**(H)-C**H**(H)-), 1.69 -1.61 (m, 5H, – $COCH_2$C**H**$_2$$CH_2$$CH_3$ and -NCH(C**H**$_3$)CH_2-), 1.41-1.39 (m, 2H, –$COCH_2CH_2$C**H**$_2$$CH_3$), 0.96-0.93 (t, 3H, J=7.4 Hz, –$COCH_2CH_2CH_2$C**H**$_3$); ^{13}C NMR ($CDCl_3$,100 MHz,); δ (**176.42, 176.39**, J= 3 Hz),173.05, 167.59, (**154.83, 152.37**, J= 246 Hz), 144.78, (**136.0, 135.93**, J= 7 Hz), (**131.18, 131.05**, J= 13 Hz), 125.12, (**117.01, 116.91**, J= 10 Hz),107.02, (**105.46, 105.21**, J= 25 Hz), 68.17, 67.44, 58.45, 58.40, 57.99, 57.94 , 55.70 , 37.44 , 37.34 , 34.68 , 28.55, 28.50 , 27.03 , 22.32, 18.46, 13.77 ; CHN Anal. Calcd. For $C_{25}H_{29}FN_2O_6$: C, 63.55; H, 6.19; N, 5.93. Found: C, 63.35; H, 6.20; N, 5.81. EIMS m/z 473[M]+(1) calculated for $C_{25}H_{29}FN_2O_6$.

(S)-10-(3-(Pivaloyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride(3.5): Off white colour powder, Yield- 67% (0.40 g), Melting point 262°C, $[\alpha]^{25}_D$ -123° (*c* 0.018, CH_2Cl_2), ^{1}H NMR ($CDCl_3$, 400 MHz); δ 15.19 (s, 1H, -COO**H**), 8.58 (s, 1H, -NC**H**CCOOH), 7.70-7.67(d, 1H, J=13.5 Hz, -C(F)C**H**C), 5.19 -5.12 (t, 1H, J=5.1 Hz, -CH_2-C**H**(OR)-CH_2-), 4.51-4.49 (m, 2H, -NC**H**(CH_3)CH_2-),4.44-4.41(m, 2H, -NCH(CH_3)C**H**$_2$-) 4.35-4.32(2bs, 2H bridged **H**), 2.42-2.29 (4ts, 2H, J=4.0 Hz -C**H**(H)-C(OR)-C**H**(H)-), 2.20-2.03 (m, 4H, -CH(**H**)-C(OR)-CH(**H**)- and-CH(**H**)-CH(**H**)-), 1.86-1.82 (2bs, 2H, -C**H**(H)-C**H**(H), 1.62-1.61 (d, 3H, J=6.6 Hz, –NCH(CH_3)CH_2), 1.24 (s, 9H, -C(C**H**$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.68, (**176.91, 176.89**, J=2 Hz) , 167.44 , (**154.76, 152.32**, J=244 Hz), 144.61 , (**135.88**, **135.79**, J=9 Hz) , (**131.26, 131.12**, J = 14 Hz) , 125.02, (**117.39, 117.30,** J = 9 Hz), 107.45, (**105.77, 105.52**, J= 25 Hz), 68.13, 67.52, 58.30 ,58.25,57.86, 57.80, 55.58 , 37.48 , 37.38 , 36.88 , 28.61 , 28.57 , 18.46, 13.78 ; CHN Anal. Calcd. For $C_{25}H_{29}FN_2O_6$: C, 63.55; H, 6.19; N, 5.93. Found: C, 63.29; H, 5.89; N, 5.54. EIMS m/z 473 [M] + (1) calculated for $C_{25}H_{29}FN_2O_6$.

(S)-10-(3-(Isobutyryloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.6): Off white colour powder, Yield- 65% (0.32 g), HPLC Purity- 98.20%, Melting point 206°C , $[\alpha]^{25}_D$ -129° (*c* 0.04, CH_2Cl_2), ^{1}H NMR ($CDCl_3$,400 MHz); δ 8.60 (s, 1H, -NC**H**CCOOH), 7.72-7.66 (d, 1H, J=13.5 Hz, -C(F)C**H**C), 5.15-5.14 (t, 1H, J=5.1 Hz, -CH_2-C**H**(OR)-CH_2-), 4.53-4.32 (m, 5H, -NC**H**(CH_3)CH_2-, (-NCH(CH_3)C**H**$_2$-) and (2bs, 2H bridged **H**), 2.61-2.53 (m, 1H, (CH_3)$_2$C**H**), 2.40-2.29 (4ts, 2H, -C**H**(H)-C(OR)-C**H**(H)-) 2.18-2.16 (m, 2H, -CH(**H**)-C(OR)-CH(**H**)-), 2.09-2.05 (m, 2H, -CH(**H**)-CH(**H**)-), 1.87-1.83 (2bs, 2H, -C**H**(H)-C**H**(H), 1.62-1.61 (d, 3H, J=6.8 Hz, –NCH(CH_3)CH_2), 1.21 (d, 6H, -CH(C**H**$_3$)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ

177.68, 176.91, 167.41, (**154.76, 152.32**, J= 244 Hz), 144.57, (**135.78, 135.69**, J= 9 Hz) ,(**131.15, 131.01**, J= 14 Hz), 125.02, (**117.39, 117.30** J= 9 Hz), 107.31, (**105.11, 104.86** J= 25 Hz), 67.98, 67.39, 58.27, 58.21, 57.80, 57.74, 55.49, 37.42, 37.32, 34.36, 28.55, 18.46, 18.36 , CHN Anal. Calcd. For $C_{24}H_{27}FN_2O_6$: C, 62.87; H, 5.94; N, 6.11. Found: C, 62.85; H, 5.93; N, 5.93. EIMS m/z 460[M]+(2) calculated for $C_{24}H_{27}FN_2O_6$.

10-(3-Acetoxy-8-aza-bicyclo[3.2.1]octan-8-yl)-9-Fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.7) :light yellow coloured powder, Yield- 76 % (0.42 g), HPLC Purity- 99.90%, Melting point 264°C decomposes, ^{1}H NMR ($CDCl_3$ &DMSO-d_6, 400 MHz) : δ 15.27 (s, 1H, -COO**H**), 8.85 (s, 1H, -NC**H**CCOOH), 7.53-7.50 (d, 1H, J=13.5 Hz, -C(F)C**H**C), 5.00 (bs, 1H, -CH_2-C**H**(OR)-CH_2-), 4.86-4.85 (m, 1H, -NC**H**(CH_3)CH_2-), 4.50-4.39 (m, 2H, -NCH(CH_3)C**H**$_2$-), 4.29-4.27 (2bs, 2H, bridged **H**), 2.31-2.20 (m, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.12-2.10 (m, 2H, -CH(**H**)-C(OR)-CH(**H**)-), 2.02-1.89 (m, 5H, C**H**$_3$CO- and -CH(**H**)-CH(**H**)-), 1.81 -1.77 (2bs, 2H, -C**H**(H)-C**H**(H)-), 1.49 -1.47 (d, 3H, J=6.4 Hz -NCH(C**H**$_3$)CH_2-); ^{13}C NMR ($CDCl_3$ & DMSO-d_6, 100 MHz,): δ (**176.21, 176.17**, J= 5 Hz),169.37, 166.29, (**154.16, 151.73**, J= 243 Hz), 145.68, (**136.21, 136.15** , J= 5 Hz), (**130.29, 130.16**, J = 13 Hz), 124.75, (**116.77, 116.67** J= 10 Hz), 106.36 , (**104.07,103.83** J= 24 Hz), 67.85, 67.09, 57.66, 57.27, 57.21, 54.70, 36.82, 36.71, 28.15, 21.19, 17.98 ; CHN Anal. Calcd. For $C_{22}H_{23}FN_2O_6$: C, 61.39; H, 5.39; N, 6.51. Found: C, 61.15; H, 5.11; N, 6.14. EIMS m/z 431[M] + (1) calculated for $C_{22}H_{23}FN_2O_6$.

10-(3-Propionyloxy -8-aza-bicyclo [3.2.1] octan-8-yl)-9-Fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino [2,3,4-*ij*] quinoline-6-carboxylic acid hydrochloride (3.8) : Light yellow colour powder, Yield- 73% ,(0.38 g), HPLC Purity- 99.68% , Melting point 243°C decomposes, ^{1}H NMR (DMSO d_6, 400 MHz,): *δ* 15.24 (s, 1H, -COO**H**), 8.88 (s, 1H, -NC**H**CCOOH), 7.55-7.52 (d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.04 -5.03 (t, 1H, J=4.5 Hz, -CH_2-C**H**(OR)-CH_2-), 4.89 -4.84 (m, 1H, -NC**H**(CH_3)CH_2-), 4.52-4.39 (m, 2H, -NCH(CH_3)C**H$_2$**-), 4.30-4.26 (2bs, 2H, bridged **H**), 2.51-2.50 (q ,2H, (CH_3C**H$_2$**CO-) , 2.34-2.28 (dt, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.25-2.20 (m, 2H, -CH(**H**)-C(OR)-CH(**H**)-), 2.14-2.08 (m, 2H, -CH**(H)**-CH**(H)**-), 1.81 -1.77 (2bs, 2H, -C**H(**H**)**-C**H(**H**)**-), 1.48 -1.47 (d, 3H, J=6.7 Hz, -NCH(C**H**$_3$)CH_2-), 1.10-1.07 (t, 3H, J=7.52, C**H$_3$**CH_2CO-); ^{13}C NMR (DMSO D_6, 100 MHz,): *δ* (**176.23, 176.18**, J= 5 Hz), 172.52, 166.26, (**154.47, 152.96,** J= 245 Hz), 145.77, 137.43, 136.32, (**131.36, 131.13**, J= 13 Hz) , 124.83, (**116.79, 116.69,** J= 10 Hz), 106.34**,** (**105.61, 105.44** , J= 18 Hz), 67.836, 66.94, 57.67, 54.70 , 36.86, 36.75, 28.13, 27.51, 17.97, 8.84. EIMS m/z 445.45[M] + (1) calculated for $C_{23}H_{25}FN_2O_6$.

10-(3-Butyryloxy-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.9): Off white colour powder ,Yield- 60% (0.35 g), HPLC Purity- 98.36%, Melting point 238-239°C, ^{1}H NMR ($CDCl_3$,400 MHz,): δ 15.24 (s, 1H, -COO**H**), 8.88 (s, 1H, -NC**H**CCOOH), 7.55-7.52 (d, 1H, J=13.6 Hz, -C(F)C**H**C), 5.04 -5.01 (t, 1H, J=4.9 Hz, -CH_2-C**H**(OR)-CH_2-), 4.86-4.85 (m, 1H, -NC**H**(CH_3)CH_2-), 4.52-4.26 (m, 4H, -NCH(CH_3)C**H**$_2$- and bridged **H**), 2.43-2.30(m, 4H, -C**H**(H)-C(OR)-C**H**(H)- and –COC**H**$_2$$CH_2CH_3$), 2.20-2.17 (m, 2H, -CH(**H**)-C(OR)-CH(**H**)), 2.15-2.03 (m, 2H, -C**H**(H)-C**H**(H)-), 1.90 -1.86 (2bs, 2H, -CH(**H**)-CH(**H**)-), 1.76 -1.66 (m, 2H, –$COCH_2$C**H**$_2$$CH_3$), 1.64-1.62 (d, 3H -NCH(C**H**$_3$)CH_2-), 1.02-0.98 (t, 3H, J=7.4 Hz, –$COCH_2CH_2$C**H**$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz,): δ (**176.87, 176.83**, J= 4 Hz), 172.90, 167.44, (**154.78, 152.33**, J= 245 Hz), 144.66, (**135.90, 135.82**, J= 8 Hz), (**131.22, 131.09**, J= 13 Hz,) 125.04, (**117.35, 117.25**, J= 10 Hz), 107.39, (**105.70, 105.45**, J= 25 Hz), 68.07, 67.48, 58.37, 58.32, 57.91, 57.85, 55.57 , 37.47, 37.38, 36.88, 28.60, 28.55, 18.46, 13.40, 13.78 ; CHN Anal. Calcd. For $C_{24}H_{27}FN_2O_6$: C, 62.87; H, 5.94; N, 6.11. Found: C, 62.49; H, 6.11; N, 5.981. EIMS m/z 459 [M] + (1) calculated for $C_{24}H_{27}FN_2O_6$.

10-(3-Pentanoyloxy-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.10): Off white colour powder , Yield- 63% (0.38g), HPLC Purity- 98.21%, Melting Point 190°C , ^{1}H NMR ($CDCl_3$,400 MHz,): δ 14.74 (s, 1H, -COO**H**), 8.68 (s, 1H, -NC**H**CCOOH), 7.65-7.61 (d, 1H, J=13.5 Hz, -C(F)C**H**C), 5.16 -5.14 (t, 1H, J=4.9 Hz, -CH_2-C**H**(OR)-CH_2-), 4.69 (bs, 1H, -NC**H**(CH_3)CH_2-), 4.47-4.37 (m, 4H, -NCH(CH_3)C**H**$_2$- and bridged **H**), 2.42-2.31 (m, 4H, -C**H**(H)-C(OR)-C**H**(H)- and –COC**H**$_2$$CH_2CH_2CH_3$), 2.21-2.03 (m, 4H, -CH(**H**)-C(OR)-CH(**H**)- and -C**H**(H)-C**H**(H)-), 1.89-1.85 (2bs, 2H, -CH(**H**)-CH(**H**)-),1.69-1.62 (m,5H,– $COCH_2$C**H**$_2$ CH_2CH_3 and -NCH(C**H**$_3$)CH_2-), 1.43-1.33 (m, 2H, –$COCH_2CH_2$C**H**$_2$$CH_3$), 0.96-0.93 (t, 3H, J=7.4 Hz, –$COCH_2CH_2CH_2$C**H**$_3$); ^{13}C NMR ($CDCl_3$,100 MHz,): δ (**176.42, 176.39**, J=3 Hz), 173.05, 167.59, (**154.83, 152.37**, J= 246 Hz), 144.78, (**136.0, 135.93**, J=7 Hz), (**131.18, 131.05**, J=13 Hz), 125.12, (**117.01, 116.91**, J=10 Hz), 107.02 , (**105.46, 105.21**, J=25 Hz) , 68.17, 67.44, 58.45, 58.40, 57.99, 57.94, 55.70, 37.44, 37.34, 34.68, 28.55, 28.50,27.03 , 22.32 , 18.46, 13.77 ; CHN Anal. Calcd. For $C_{25}H_{29}FN_2O_6$: C, 63.55; H, 6.19; N, 5.93. Found: C, 63.44; H, 5.82; N, 5.79. EIMS m/z 473[M] + (1) calculated for $C_{25}H_{29}FN_2O_6$.

10-(3-(Pivaloyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.11): Off white colour powder, Yield- 60% **(**0.36 g), HPLC Purity- 98.38%, Melting Point 250°C , ^{1}H NMR

($CDCl_3$,400 MHz,): δ 15.13 (s, 1H, -COO**H**), 8.53 (s, 1H, -NC**H**CCOOH), 7.64-7.61 (d, 1H, J=13.5 Hz, -C(F)C**H**C), 5.07 -5.05 (t, 1H, J=5.0 Hz, -CH_2-C**H**(OR)-CH_2-), 4.48-4.28 (m, 5H, -NC**H**(CH_3)CH_2-, -NCH(CH_3)C**H**$_2$- bridged **H**), 2.36-2.22 (4ts, 2H, J=4.1 Hz -C**H**(H)-C(OR)-C**H**(H)-) 2.13-1.98 (m, 4H, -CH(**H**)-C(OR)-CH(**H**)- and-C**H**(H)-C**H**(H)-), 1.79-1.75 (2bs, 2H, -CH(**H**)-CH(**H**), 1.56-1.55 (bs, 3H, –NCH(C**H**$_3$)CH_2), 1.17 (s, 9H, -C(C**H**$_3$)$_3$); ^{13}C NMR ($CDCl_3$,100 MHz,): δ 177.68, (**176.91, 176.89,** J= 2.32 Hz), 167.43, (**156.19, 153.99**, J= 219 Hz), 144.63, (**135.88, 135.81**, J=7 Hz), (**131.25, 131.11**, J=14 Hz,),125.04, (**117.42, 117.31**, J=11 Hz), 107.48, (**105.79, 105.54**, J= 25 Hz), 68.09, 67.38, 58.34, 58.29, 57.89, 57.83, 55.58, 38.63, 37.48, 37.39, 28.56, 28.52, 27.10 , 18.40; CHN Anal. Calcd. For $C_{25}H_{29}FN_2O_6$: C, 63.55; H, 6.19; N, 5.93. Found: C, 63.26; H, 6.11; N, 5.98. EIMS m/z 473 [M] + (1) calculated for $C_{25}H_{29}FN_2O_6$.

10-(3-(Isobutyryloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.12): Off white colour powder, Yield- 60% (0.35 g), Melting Point 216°C , ^{1}H NMR ($CDCl_3$,400 MHz); δ 15.06 (s, 1H, -COO**H**), 8.53 (s, 1H, -NC**H**CCOOH), 7.63-7.60 (d, 1H, J=13.5 Hz, -C(F)C**H**C), 5.08-5.07 (t, 1H, J=5.1 Hz, -CH_2-C**H**(OR)-CH_2-), 4.46-4.28 (m, 5H, -NC**H**(CH_3)CH_2-, (-NCH(CH_3)C**H**$_2$-) and (2bs, 2H bridged **H**), 2.53-2.46 (m, 1H, (CH_3)$_2$C**H**), 2.35-2.22 (4ts, 2H, -C**H**(H)-C(OR)-C**H**(H)-) 2.11-2.07(m, 2H, -CH(**H**)-C(OR)-CH(**H**)-), 2.03-1.99 (m,2H, -CH(**H**)-CH(**H**)-), 1.80-1.77 (2bs, 2H, -C**H**(H)-C**H**(H)), 1.56-1.54 (d, 3H, J=6.8 Hz, – NCH(CH**3**)CH_2), 1.15 (d, 6H, -CH(C**H$_3$**)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (**176.88, 176.84,** J= 4 Hz) , 176.18, 167.43, (**154.79, 152.34**, J= 244 Hz), 144.64, (**135.90, 135.83**, J=7 Hz), (**131.22, 131.08**, J=14 Hz), 125.04, (**117.39, 117.30** J=9 Hz), 107.43 , (**105.76, 104.50** J=26 Hz), 68.07, 67.41, 58.38 ,58.33,57.92, 57.86, 55.57, 37.46, 37.36, 34.40, 28.58, 28.54, 18.88, 18.40, CHN Anal. Calcd. For $C_{24}H_{27}FN_2O_6$: C, 62.87; H, 5.94; N, 6.11. Found: C, 62.85; H, 5.93; N, 5.93. EIMS m/z 460[M]+(2) calculated for $C_{24}H_{27}FN_2O_6$.

7-(3-Acetoxy-8-aza-bicyclo[3.2.1]octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo quinoline-3-carboxylic acid (3.13) Off white colour powder , Yield- 91% (0.3 g), HPLC Purity- 99.20% , Melting point 306°C decomposes, ^{1}H NMR (400 MHz DMSO-d_6) *δ* 15.35 (s, 1H, -COO**H**), 8.65 (s, 1H, -NC**H**CCOOH), 7.88-7.74 (d, 1H, J= 14.4 Hz, C(F)C**H**C), 7.38-7.36 (d, 1H, J= 7.5 Hz, -C(N)C**H**C), 4.97-4.94 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.58 (bs, 2H, -N-bridged **H**), 3.75 (m, -N-Cyclopropyl C**H**) , 2.26-2.23 (m, 4H, -CH(**H**)-C(OCOR)-CH(**H**)- and -C**H**(H)-C(OCOR)-C**H**(H)-), 2.18-2.13 (q, 2H, CH**(H)**-CH**(H)**-), 2.11-2.07 (s, 3H, -OCOC**H$_3$**), 1.84, 1.81 (d, 2H, C**H(**H)-C**H(**H)-), 1.39-1.34 (q, 2H, -NCHCH(**H**)CH(**H**)-), 1.24-1.18 (q,

2H,-NCHC**H**(H)C**H**(H)); ^{13}C NMR (100 MHz, DMSO-d_6) δ (**176.10, 176.07** J= 3.0 Hz), 169.29 , 166.23, (**152.34, 149.67** J= 267 Hz), 147.07, (**142.06, 142.04** J= 2.0 Hz), 139.55, (**115.52, 115.45,** J= 7.0 Hz), (**111.70, 111.46** J= 24.0 Hz)**,** 106.55, (**103.50, 103.32** J= 18.0 Hz)**,** 66.76, 55.04,54.97, 35.27, 33.30,27.08,21.14,7.59 MS (EI): m/z calculated for $C_{22}H_{23}FN_2O_5$ $[M]^+$: 414.43,found: 415.36.

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-(propionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl) quinoline-3-carboxylic acid (3.14) Off white colour powder, Yield- 88.5% (0.3 g), HPLC Purity- 98.95% , Melting point 313°C decomposes, ^{1}H NMR (400 MHz DMSO-d_6) δ 15.35 (s, 1H, -COO**H**), 8.62 (s, 1H, -NC**H**CCOOH), 7.88-7.75 (d, 1H, J= 13.6 Hz, C(F)C**H**C), 7.42-7.40 (d, 1H, J= 7.9 Hz, -C(N)C**H**C), 4.95 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.58,(bs, 2H, -N-bridged **H**) , 3.79 (m, -N-Cyclopropyl C**H**) , 2.42-2.37 (q, 2H, -OCOC**H$_2$**CH_3), 2.35-2.09 (m, 6H, -CH(**H**)-C(OCOR)-CH(**H**)- , -C**H**(H)-C(OCOR)-C**H**(H)- and CH**(H)**-CH**(H)**-), 1.80, 1.77 (d, 2H, C**H(**H**)**-C**H(**H**)**-), 1.31-1.24 (q, 2H, -NCHCH(**H**)CH(**H**)-Cyclopropyl), 1.17 (bs, 2H, -NCHC**H**(H)C**H**(H) cyclopropyl), 1.01 (t, 3H, -OCOCH_2C**H$_3$**); ^{13}C NMR (100 MHz, DMSO-d_6) δ (**176.10, 176.07** J= 3.0 Hz), 169.78, 167.23, (**15.35, 149.56** J= 255 Hz), 147.07, (**142.06, 142.04,** J= 2.0 Hz), 139.57, (**115.52, 115.45,** J= 7.0 Hz), (**111.68, 111.44,** J= 24.0 Hz)**,** 106.55, (**103.50, 103.32,** J= 18.0 Hz)**,** 66.76, 55.04, 54.97, 35.27, 33.30, 27.08, 21.14, 17.97, 7.59 MS (EI): m/z calculated for $C_{23}H_{25}FN_2O_5$ $[M]^+$: 428.45,found: 429.43

7-(3-(Butyryloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (3.15) Off white colour powder, Yield- 80% (0.28 g), HPLC Purity- 98.93% , Melting point 303°C decomposes, ^{1}H NMR (400 MHz DMSO-d_6) δ 15.33 (s, 1H, -COO**H**), 8.63 (s, 1H, -NC**H**CCOOH), 7.88-7.85 (d, 1H, J= 14.3 Hz, C(F)C**H**C), 7.41- 7.39 (d, 1H, J= 7.7 Hz, -C(N)C**H**C), 4.96 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.59,(bs, 2H, -N-bridged **H**) , 3.78 (m, -N-Cyclopropyl C**H**) , 2.32-2.30 (t, 2H, -OCOC**H$_2$**CH_2CH_3), 2.26-2.09 (m, 6H, -CH(**H**)-C(OCOR)-CH(**H**)- , -C**H**(H)-C(OCOR)-C**H**(H)- and CH**(H)**-CH**(H)**-), 1.80, 1.77 (d, 2H,C**H(**H**)**-C**H(**H**)**-),1.67-1.58 (m,2H,-OCOCH_2C**H$_2$**CH_3), 1.34-1.33 (q, 2H, -NCHCH(**H**)CH (**H**)-Cyclopropyl), 1.24-1.17 (bs, 2H, -NCHC**H**(H)C**H**(H) cyclopropyl), 0.96-0.86 (t, 3H, -OCOCH_2CH_2C**H$_3$**); ^{13}C NMR (100 MHz$CDCl_3$ and DMSO-d_6) δ (**176.71, 176.0,** J= 3.39 Hz), 172.73 ,167.29, (**152.64, 150.16,** J= 248 Hz), 147.12, (**140.52, 140.41,** J= 11.0 Hz), 139.68, (**116.93, 116.85,** J= 8 Hz), (**112.90, 112.66 ,** J= 24.0Hz), 107.59, (**102.33, 102.28** J= 5.0 Hz), 67.00, 55.51, 55.44, 36.73, 35.05, 33.91, 27.53, 18.38, 13.72, 8. 15., MS (EI): m/z calculated for $C_{24}H_{27}FN_2O_5$ $[M]^+$: 442.48, found: 449.38

7-(3-(Pentanoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (3.16) light yellow colour powder, Yield- 88%, (0.31 g) , HPLC Purity- 95.15% , Melting Point 276-278°C decomposes, ^{1}H NMR (400 MHz DMSO-d_6) δ 15.33 (s, 1H, -COO**H**), 8.64 (s, 1H, -NC**H**CCOOH), 7.89-7.85 (d, 1H, J= 14.3 Hz, - C(F)C**H**C), 7.40- 7.38 (d, 1H, J= 7.7 Hz, -C(N)C**H**C), 4.97 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.59,(bs, 2H, -N-bridged **H**), 3.77 (m, -N-Cyclopropyl C**H**), 2.35-2.32 (t, 2H, -OCOC**H$_2$**CH_2 CH_2CH_3), 2.28-2.17 (m, 4 H, -CH(**H**)-C(OCOR)-CH(**H**)- and CH**(H)**-CH**(H)**-), 2.12-2.10 (t, 2H, -C**H**(H)-C(OCOR)-C**H**(H)-), 1.81,1.77 (d, 2H, C**H(**H)-C**H(**H)-), 1.61-1.56 (m, 2H, -$OCOCH_2$C**H$_2$**CH_2CH_3), 1.38-1.30 (m, 4H, -$OCOCH_2CH_2$C**H$_2$**CH_3 and -NCHCH(**H**)CH(**H**)-Cyclopropyl), 1.24-1.17 (bs, 2H, -NCHC**H**(H)C**H**(H) cyclopropyl), 0.94-0.87 (t, 3H, -$OCOCH_2CH_2CH_2$C**H$_3$**); ^{13}C NMR (100 MHz DOSO-d_6) δ (**176.77, 176.0,** J= 3.39 Hz), 172.92 ,167.33, (**152.64, 150.16** J= 248 Hz**)**, 147.16, (**140.53, 140.43** J= 10.0 Hz), 139.69, (**116.97, 116.85,** J= 8 Hz), **(112.97, 112.72,** J= 25.0 Hz **),** 107.64, (**102.28, 102.28,** J= 5.0 Hz)**,** 67.02, 55.52, 55.45 , 35.05, 34.58, 33.90, 27.54, 26.95, 22.29, 13.73, 8.16, MS (EI): m/z calculated for $C_{25}H_{29}FN_2O_5$ $[M]^+$: 456.51,found: 457.49

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-(pivaloyloxy)-8-aza-bicyclo[3.2.1] octan-8-yl) quinoline-3-carboxylic acid (3.17) light yellow colour powder**,** Yield- 88%, (0.31 g), HPLC Purity- 96.59% , Melting point 309°C decomposes, ^{1}H NMR (400 MHz $CDCl_3$) δ , 15.36 (s, 1H, -COO**H**), 8.72 (s, 1H, -NC**H**CCOOH), 8.01-7.97 (d, 1H, J= 14.2 Hz, C(F)C**H**C), 7.21-7.19 (d, 1H, J= 7.3 Hz, -C(N)C**H**C), 5.05-5.02 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.54 (bs, 2H, -N-bridged **H**) , 3.49 (m, -N-Cyclopropyl C**H**) , 2.31-2.17 (m, 6H, -CH(**H**)-C(OCOR)-CH(**H**)- , -C**H**(H)-C(OCOR)-C**H**(H)- and CH**(H)**-CH**(H)**-), 1.89, 1.81 (d, 2H, C**H(**H)-C**H(**H)-), 1.36-1.34 (q, 2H, -NCHCH(**H**)CH(**H**)-Cyclopropyl), 1.25 (s, 9H, -OCOC(C**H$_3$**)$_3$) 1.21-1.18 (bs, 2H, -NCHC**H**(H)C**H**(H) cyclopropyl), ^{13}C NMR (100 MHz, $CDCl_3$) δ (**177.64, 176.0** J= 3.39 Hz), 172.92, 167.42, (**152.64, 150.16,** J= 248 Hz), 147.23, (**140.53, 140.43** J= 10.0 Hz),139.68, (**116.97, 116.85 ,** J= 8 Hz), (**112.97, 112.72,** J= 25.0 Hz)**,**107.80, (**102.20, 102.28,** J= 5.0 Hz)**,** 66.74, 55.24, 55.45 ,38.69, 34.79, 33.91, 33.82, 27.53, 27.04,8.18, MS (EI): m/z calculated for $C_{25}H_{29}FN_2O_5$ $[M]^+$: 456.51,found: 457.49

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(3-(isobutyryloxy)-8-aza-bicyclo [3.2.1] octane -8-yl) -4-oxoquinoline-3-carboxylic acid (3.18) light yellow colour powder, Yield- 80%(0.28 g), HPLC Purity- 96.35% , Melting point 318-320°C decomposes, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.36 (s, 1H, -COO**H**), 8.73 (s, 1H, -NC**H**CCOOH), 8.02-7.99 (d, 1H, J=14.2 Hz, C(F)C**H**C), 7.21- 7.19 (d, 1H, J= 7.3 Hz, -C(N)C**H**C), 5.06-5.03 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.54 (bs,

2H, -N-bridged **H**) , 3.84 (m, -N-Cyclopropyl C**H**) ,2.59 (m, 1H, -OCOC**H**$(CH_3)_2$), 2.30-2.17 (m, 6H, -CH(**H**)-C(OCOR)-CH(**H**)- , -C**H**(H)-C(OCOR)-C**H**(H)- and CH**(H)**-CH**(H)**-), 1.86, 1.82 (d, 2H, C**H(**H**)**-C**H(**H**)**-), 1.57 (d, 6H,-OCOCH(C**H$_3$**$)_2$), 1.38-1.33 (q, 2H, -NCHCH (**H**)CH(**H**)-Cyclopropyl), 1.20-1.18 (m, 2H, -NCHC**H**(H)C**H**(H) Cyclopropyl), ^{13}C NMR (100 MHzCDCl$_3$ and DMSO-d$_6$) δ (**176.71, 176.0,** J= 3.39 Hz), 172.73 ,167.29, (**152.64, 150.16,** J= 248 Hz), 147.12, (**140.52, 140.41,** J= 11.0 Hz), 139.68, (**116.93, 116.85,** J= 8 Hz), (**112.90, 112.66 ,** J= 24.0Hz), 107.59, (**102.33, 102.28** J= 5.0 Hz), 67.00, 55.51, 55.44, 36.73, 35.05, 33.84, 27.44, 18.87, 8. 15. MS (EI): m/z calculated for $C_{24}H_{27}FN_2O_5$ $[M]^+$: 442.48, found: 449.38

7-(3-Acetoxy-8-aza-bicyclo[3.2.1]octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy -4-oxoquinoline-3-carboxylic acid (3.19) light yellow colour powder, Yield- 69% (0.38 g) , HPLC Purity- 98.66% , Melting point 288-290°C, ^{1}H NMR (400 MHz CDCl$_3$) 14.93 (s, 1H, -COO**H**), 8.78 (s, 1H, -NC**H**CCOOH), 7.85-7.81 (d, 1H, J =13.84 Hz, -C(F)C**H**C), 5.14-5.11 (t, 1H, J= 4.8 Hz, -CH$_2$-C**H**(OCOR)-CH$_2$-), 4.49 (bs, 2H, bridged **H**), 4.03-3.98 (m, 1H, m, 1H, -N cyclopyopyl C**H**), 3.66, (s, 3H, -OC**H**$_3$), 2.33-2.27 (dt, 2H, , -CH(**H**)-C(OH)-CH(**H**)-) , 2.23-2.15 (q, 2H, CH**(H)**-CH**(H)**-),2.10-2.08 (t, 2H , -C**H**(H)-C(OH)-C**H**(H)-), 1.93-1.89 (d,2H, C**H(**H**)**-C**H(**H**)**-), 1.23-1.17 (q, 2H, -NCHCH(**H**)CH(**H**) cyclopropyl-), 0.99 -0.95 (q, 2H, -NCHC**H**(H)C**H**(H) cyclopropyl); ^{13}C NMR (100 MHz, CDCl$_3$) δ (**175.78, 175.75,** J= 3 Hz) **,** 169.26, 165.89, (**153.41, 150.94,** J= 246 Hz), 148.81,(**140.63, 140.55,** J= 8 Hz)**,** (**135.71, 135.60,** J= 11 Hz)**,** 133.17, (**117.87, 117.78 ,** J= 9 Hz)**,** (**107.79, 107.53,** J =26 Hz), 106.50, 66.42, 59.33, 56.89, 39.23, 35.65, 27.31, 20.50, 8.40; MS (EI): m/z calculated for $C_{23}H_{25}FN_2O_6$ $[M]^+$: 444.45,found: 445.43

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(3-(propionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl) quinoline-3-carboxylic acid (3.20) light yellow colour powder **,** Yield- 76% (0.43 g), HPLC Purity- 98.45%, Melting point 305-307°C , ^{1}H NMR (400 MHz CDCl$_3$) 14.93 (s, 1H, -COO**H**) , 8.78 (s, 1H, -NC**H**CCOOH), 7.84-7.80 (d, 1H, J= 13.84 Hz,-C(F)C**H**C), 5.16-5.13 (t, 1H, J=4.8 Hz, -CH$_2$-C**H**(OCOR)-CH$_2$-), 4.50 (bs, 2H, bridged **H**), 4.03-4.0 (m, 1H, -N cyclopyopyl C**H**), 3.66, (s, 3H, -OC**H**$_3$), 2.41-2.35 (q , 2H, -OCOC**H$_2$**CH$_3$), 2.32-2.29 (dt, 2H, , -CH(**H**)-C(OH)-CH(**H**)-), 2.29 (q, 2H, CH**(H)**-CH**(H)**-), 2.21-2.18 (t, 2H, -C**H**(H)-C(OH)-C**H**(H)-),1.92-1.88 (d,2H,C**H(**H**)**-C**H(**H**)**-), 1.21-1.15 (m,5H, -OCOCH$_2$C**H$_3$**and-NCHCH(**H**)CH (**H**) cyclopropyl-), 0.99 -0.95 (q, 2H, -NCHC**H**(H)C**H**(H) cyclopropyl); ^{13}C NMR (100 MHz, CDCl$_3$)δ (**175.78, 175.75,** J= 3 Hz)**,** 169.26, 165.89, (**153.41, 150.94,** J= 246 Hz), 148.81, (**140.63, 140.55,** J= 8 Hz)**,** (**135.71, 135.60,** J= 11 Hz)**,** 133.17, (**117.87, 117.78 ,** J= 9 Hz)**,** (

107.79, 107.53, J =26 Hz), 106.50, 66.28, 59.28, 56.90, 39.21, 35.65, 27.31, 20.41, 8.40 MS (EI): m/z calculated for $C_{24}H_{27}FN_2O_6$ $[M]^+$: 458.48,found: 459.43

7-(3-(Butyryloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (3.21); light yellow colour powder, Yield- 69 % (0.4 g), HPLC Purity- 98.65%, Melting point 256-258°C, ^{1}H NMR (400 MHz $CDCl_3$) 14.98 (s, 1H, -COO**H**) 8.79 (s, 1H, -NC**H**CCOOH), 7.84-7.81 (d, 1H, J= 13.84 Hz, -C(F)C**H**C), 5.15 (t, 1H, J=4.8 Hz, -CH_2-C**H**(OCOR)-CH_2-), 4.50 (bs, 2H, bridged **H**), 4.02- (m, 1H, -N cyclo propyl C**H**), 3.67, (s, 3H, -OC**H**$_3$), 2.35-2.29 (m , 4H , -OCOC**H$_2$**CH_2CH_3 and-CH(**H**)-C(OH)-CH(**H**)-), 2.21-2.15 (q, 2H, CH**(H)**-CH**(H)**-) , 2.14-2.09 (t, 2H , -C**H**(H)-C(OH)-C**H**(H)-), 1.91-1.88 (d,2H, C**H(**H**)**-C**H(**H**)**-), 1.75-1.66 (m, 2H, -OCOCH_2C**H$_2$**CH_3 **),**1.20 (m, 2H **,** -NCHCH(**H**)CH(**H**) cyclopropyl-), 1.01-0.97 (m, 5H, -NCHC**H**(H)C**H**(H) cyclopropyl and -OCOCH_2CH_2C**H$_3$**); ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.89, 176.85,** J= 4 Hz), 172.88, 167.01, (**154.56, 152.08,** J= 248 Hz),150.04 , (**141.76, 141.68 ,** J= 8 Hz)**, (136.86, 136.77 ,** J= 9 Hz)**,** 134.33, (**118.98, 118.89**, J = 9 Hz)**,** (**108.91, 108.66** J= 25 Hz), 107.64, 67.19, 60.64, 58.10, 40.49, 36.90, 36.86, 28.46, 18.46, 13.78, 9.61 ; MS (EI): m/z calculated for $C_{25}H_{29}FN_2O_6$ $[M]^+$: 472.51,found: 473.45

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(3-(pentanoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl) quinoline-3-carboxylic acid (3.22) light yellow colour powder, Yield- 50% (0.3 g), HPLC Purity- 99.65%, Melting point 214-216°C. , ^{1}H NMR (400 MHz $CDCl_3$) 14.86 (s, 1H, -COO**H**), 8.80 (s, 1H, -NC**H**CCOOH), 7.86-7.83 (d, 1H, J =13.88 Hz, -C(F)C**H**C), 5.18-5.16 (t, 1H, J= 4.9 Hz, -CH_2-C**H**(OCOR)-CH_2-), 4.51 (bs, 2H, bridged **H**), 4.03- (m, 1H, -N cyclopropyl C**H**), 3.68, (s, 3H, -OC**H**$_3$), 2.38-2.30 (m , 4H , -OCOC**H$_2$**CH_2CH_3 and -CH(**H**)-C(OH)-CH(**H**)-), 2.24-2.15 (q, 2H, CH**(H)**-CH**(H)**-), 2.12-2.10 (t, 2H , -C**H**(H)-C(OH)-C**H**(H)-), 1.93-1.89 (d, 2H, C**H(**H**)**-C**H(**H**)**-), 1.71-1.63 (m, 2H, -OCOCH_2C**H$_2$**CH_2CH_3 **),** 1.45-1.35 (m, 2H**,** -OCOCH_2CH_2C**H$_2$**CH_3 **),** 1.22-1.21 (m, 2H **,** -NCHCH(**H**)CH(**H**) cyclopropyl-), 0.98-0.95 (m, 5H, -NCHC**H**(H)C**H**(H) cyclopropyl and –OCO$(CH_2)_3$C**H$_3$**); ^{13}C NMR (100 MHz, $CDCl_3$) δ (**176.89, 176.85,** J= 4 Hz)**,** 173.05, 167.00, (**154.55, 152.07,** J= 248 Hz), 149.96 , (**141.75, 141.69** J= 6 Hz)**,** (**136.87, 136.75,** J= 12 Hz), 134.31, (**118.99, 118.90 ,** J= 9 Hz)**,** (**108.91, 108.66** J= 25 Hz**),** 107.64 ,67.19, 60.49, 58.04, 40.38, 36.88, 34.68, 28.45, 27.04, 22.34, 13.78, 9.55 .MS (EI): m/z calculated for $C_{26}H_{31}FN_2O_6$ $[M]^+$: 486.53,found: 487.45

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(3-(pivaloyloxy)-8-aza-bicyclo [3.2.1]octan-8-yl) quinoline-3-carboxylic acid (3.23): light yellow colour powder, Yield- 50%, (0.3 g), HPLC Purity- 99.07% , Melting point 285°C decomposes , ^{1}H NMR (400 MHz $CDCl_3$)

14.90 (s, 1H, -COO**H**), 8.81 (s, 1H, -NC**H**CCOOH),7.87-7.84 (d, 1H, J= 13.84 Hz , -C(F)C**H**C) , 5.16-5.15 (t, 1H, J= 4.8 Hz, -CH_2-C**H**(OCOR)-CH_2-), 4.52 (bs, 2H, bridged **H**),4.03- (m, 1H, -N cyclopropyl C**H**), 3.68, (s, 3H, -OC**H**$_3$), 2.35-2.32 (dt , 2H , -CH(**H**)-C(OH)-CH(**H**)-), 2.25-2.19 (q , 2H, CH**(H)**-CH**(H)**-), 2.13- 2.12 (t, 2H,-C**H**(H)-C(OH)-C**H**(H)-), 1.90-1.87 (d, 2H, C**H(**H)-C**H(**H)-), 1.27 (s, 9H, -OCO(C**H$_3$)$_3$)**,1.26-1.21 (m, 2H **,** -NCHCH(**H**)CH(**H**) cyclo propyl-), 1.11-0.95 (m, 2H, -NCHC**H**(H)C**H**(H) cyclopropyl); ^{13}C NMR (100 MHz, $CDCl_3$)δ (**177.64, 176.90,** J= 26 Hz), 173.07, 167.01, (**154.62, 152.14 ,** J= 248 Hz)**,**149.98, (**141, 79, 141.72,** J =7 Hz)**,** (**136.89 , 136.81,** J =9 Hz), 134.29, (**118.92 , 118. 83,** J= *9 H*z)**,** (**108.93, 108.67,** J= 26 Hz)**,** 107.66, 67.09, 60.53, 58.09, 40.40, 38.63, 36.91, 28.41, 27.10, 9.56. MS (EI): m/z calculated for $C_{26}H_{31}FN_2O_6$ $[M]^+$: 486.53, found: 487.45

1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(3-(isobutyryloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-8-methoxy-4-oxoquinoline-3-carboxylic acid (3.24): light yellow colour powder, Yield-69% (0.4 g), Melting Point 265-267°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 14.86 (s, 1H, -COO**H**), 8.72 (s, 1H, -NC**H**CCOOH), 7.79-7.75 (d, 1H, J =13.88 Hz ,-C(F)C**H**C), 5.08-5.07-5.15 (t, 1H, J= 4.9 Hz, -CH_2-C**H**(OCOR)-CH_2-), 4.43 (bs, 2H, bridged **H**), 3.95-3.92 -(m, 1H, -N cyclopropyl C**H**), 3.61 (s, 3H, -OC**H**$_3$), 2.54-2.47 (m, 1H, OCOC**H**$(CH_3)_2$), 2.26-2.22 (dt, 2H, -CH(**H**)-C(OH)-CH(**H**)-), 2.14-2.09 (q , 2H, CH**(H)**-CH**(H)**-), 2.02-2.02 (t, 2H,-C**H**(H)-C(OH)-C**H**(H)-),1.83-1.79(d, 2H, C**H(**H)-C**H(**H)-), 1.16-1.12 (m, 8H, -OCOCH(C**H$_3$)$_2$** and-NCHCH(**H**)CH(**H**) cyclopropyl-), 0.92-0.90 (m, 2H, -NCHC**H**(H)C**H**(H) cyclopropyl)., ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.11 , 176.06,** J= 5 Hz)**,** 173.05, 166.99, (**156.30, 153.83,** J= 247 Hz), 149.95, (**141.11, 141.03,** J= 8 Hz), (**136.43, 136.33,** J= 10 Hz),134.29**,** (**117.34, 117.25** J= 9 Hz)**,** (**107.46, 107.20,** J= 26 Hz)**,** 106.43, 67.12, 60.47,58.07, 40.34, 36.87, 34.39, 28.44, 18.87, 9.53 MS (EI): m/z calculated for $C_{25}H_{29}FN_2O_6$ $[M]^+$: 472.51,found: 473.48

7-(3-Acetoxy-8-aza-bicyclo [3.2.1] octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (3.25): Off white colour powder, Yield- 91% (0.5 g), Melting point 267°C , ^{1}H NMR (400 MHz $CDCl_3$) δ 14.91 (s, 1H, -COO**H**), 8.60 (s, 1H, -NC**H**CCOOH), 8.0-7.97 (d, 1H, J= 14.68 Hz, C(F)C**H**C), 6.62-6.64 (d, 1H, J= 7.36 Hz, -C(N)C**H**C), 5.04 (t, 1H, CH_2-C**H**(OCOR)-CH_2-), 4.53 (bs,2H, -N Bridged **H**), 4.28-4.27 (q, 2H, -NC**H$_2$**CH_3-), 2.26-2.23 (m, 4H, CH**(H)**-CH**(H)**- and -CH(**H**)-C(OCOR)-CH(**H**)-) , 2.17-2.15 (dt, 2H, -C**H**(H)-C(OCOR)-C**H**(H)-), 1.95 (s, 3H, -OCOC**H$_3$**)**,** 1.89-1.85 (d,2H, C**H(**H)-C**H(**H)-), 1.59-1.55 (t,3H, -CH_2C**H$_3$**), ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.49, 176.0,** J= 3.39 Hz), 170.20, 167.49, (**152.46, 149.98,** J= 248 Hz), 146.85, (**140.72, 140.62,** J= 10.0 Hz), 137.73, 116.12, (**117.66, 117.58** J= 8 Hz), (**113.27, 113.04,** J= 23.0)**,** 107.82, (**101.12, 101.0,** J= 5.4 Hz), 67.28, 55.51, 55.45, 49.59,

33.93, 29.67, 27.51 21.54 , 14.42; MS (EI): m/z calculated for $C_{21}H_{23}FN_2O_5$ $[M]^+$: 402.42,found: 403.52

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-(propionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl) quinoline-3-carboxylic acid (3.26): Off white colour powder, Yield- 84% (0.48 g), HPLC Purity- 98.76% , Melting point 271-272°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 14.91 (s, 1H, -COO**H**), 8.60 (s, 1H, -NC**H**CCOOH), 8.01-7.97 (d, 1H, J= 14.68 Hz, C(F)C**H**C), 6.66-6.64 (d, 1H, J= 6.88 Hz, -C(N)C**H**C), 5.07 (t, 1H, CH_2-C**H**(OCOR)-CH_2-), 4.53 (bs,2H, -N Bridged **H**), 4.30-4.25 (q, 2H, -NC**H$_2$**CH_3-), 2.41-2.36 (q, 2H, -OCOC**H$_2$**CH_3), 2.27-2.23(m, 4H ,CH**(H)**-CH**(H)**- and -CH(**H**)-C(OCOR)-CH(**H**)-), 2.17-2.15 (t, 2H, -C**H**(H)-C(OCOR)-C**H**(H)-), 1.88-1.84 (d,2H, C**H(**H)-C**H(**H)-), 1.59-1.55 (t, 3H, -OCOCH_2C**H$_3$**), 1.21-1.17 (t, 3H -NCH_2C**H$_3$**); ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.64, 176.0,** J= 3.39 Hz), 173.64, 167.48, (**152.45, 149.97,** J =248 Hz), 146.84, (**140.73, 140.62,** J= 11.0 Hz), 137.73,(**117.57, 117.58,** J= 8 Hz), (**113.27, 113.03,** J=24.0 Hz), 107.82, **(101.13, 101.0,** J= 5.4 Hz),67.06, 55.51, 55.44 ,49.58, 33.97, 28.13, 27.51, 14.41, 9.05.MS (EI): m/z calculated for $C_{22}H_{25}FN_2O_5[M]^+$: 416.44,found: 417.28

7-(3-(Butyryloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxo quinoline-3-carboxylic acid (3.27): Off white colour powder, Yield- 68% (0.24 g) , HPLC Purity- 98.72%, Melting point 286-288°C., ^{1}H NMR (400 MHz $CDCl_3$) δ 14.91 (s, 1H, -COO**H**), 8.61 (s, 1H, -NC**H**CCOOH), 8.02-7.98 (d, 1H, J= 14.68 Hz, C(F)C**H**C), 6.66-6.64 (d, 1H, J= 6.88 Hz, -C(N)C**H**C), 5.07 (t, 1H, CH_2-C**H**(OCOR)-CH_2-), 4.53 (bs,2H, -N Bridged **H**), 4.28-4.24 (q, 2H, -NC**H$_2$**CH_3-), 2.36-2.32 (t, 2H, -OCOC**H$_2$**CH_2CH_3), 2.27-2.20 (m, 4H , CH**(H)**-CH**(H)**- and -CH(**H**)-C(OCOR)-CH(**H**)-),2.19-2.14 (dt, 2H -C**H**(H)-C(OCOR)-C**H**(H)-), 1.87-1.84 (d, 2H,C**H(**H)-C**H(**H)-),1.73-1.66 (m, 2H, -OCOCH_2C**H$_2$**CH_3), 1.59-1.55 (t, 3H, -OCOCH_2CH_2C**H$_3$**), 1.01-1.0.97 (t,3H, -NCH_2C**H$_3$**); ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.64, 176.0,** J= 3.39 Hz), 172.71, 167.49, (**152.45, 149.97,** J= 248 Hz),146.85, (**140.62, 140.62,** J= 11.0 Hz), 137.73, (**117.68, 117.60,** J=8 Hz), **(113.31, 113.06,** J= 25.0 Hz), 107.85, **(101.06, 101.0** J= 5.4 Hz)**,** 66.94, 55.51, 55.41, 49.57, 36.72, 34.01, 37.52, 18.38, 14.41, 13.72.MS (EI): m/z calculated for $C_{23}H_{27}FN_2O_5[M]^+$: 430.47, found: 431.54

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-(pentanoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl) quinoline-3-carboxylic acid (3.28): Off white colour powder, Yield- 77%, (0.28 g), HPLC Purity- 98.37% , Melting point 239°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 14.92 (s, 1H, -COO**H**), 8.59 (s, 1H, -NC**H**CCOOH), 7.97-7.93 (d, 1H, J= 16 Hz, C(F)C**H**C), 6.66-6.64 (d, 1H, J= 6.88 Hz, -C(N)C**H**C), 5.07 (t, 1H, CH_2-C**H**(OCOR)-CH_2-), 4.53 (bs,2H, -N Bridged **H**), 4.30-4.25 (q, 2H, -NC**H$_2$**CH_3-), 2.36-2.32 (t, 2H, -OCOC**H$_2$**$CH_2CH_2CH_3$), 2.27-2.22 (m, 4H , CH**(H)**-

CH**(H)**- and -CH(**H**)-C(OCOR)-CH(**H**)-) , 2.17-2.15 (t, 2H -C**H**(H)-C(OCOR)-C**H**(H)-), 1.87-1.84 (d, 2H, C**H(**H**)**-C**H(**H**)**-), 1.69-1.61 (m, 2H,-OCOCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.58-1.55 (t, 3H -NCH$_2$C**H$_3$**), 1.41-1.35(m,2H,-OCOCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.97-0.93(t, 3H, OCOCH$_2$CH$_2$CH$_2$C**H$_3$**); ^{13}C NMR (100 MHz, CDCl$_3$) δ (**176.64, 176.0**, J= 3.39 Hz), 172.87, 167.45, (**152.45, 149.97,** J= 248 Hz), 146.79, (**140.62, 140.62** J= 11.0 Hz), 137.71, (**117.68, 117.60** J= 8 Hz), (**113.15, 112.90,** J= 25.0 Hz), 107.72, (**101.05, 101.10,** J= 5.0 Hz)**,** 66.95, 55.50, 55.43, 49.57, 34.54, 33.99, 27.49, 26.92, 22.26, 14.38, 13.70.MS (EI): m/z calculated for $C_{24}H_{29}FN_2O_5[M]^+$: 444.54, found: 445.58.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-(pivaloyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl) quinoline-3-carboxylic acid (3.29): Off white colour powder, Yield- 77 %, (0.28 g), 77.0 %, HPLC Purity- 98.91% , Melting point 305-307°C, ^{1}H NMR (400 MHz CDCl$_3$) δ 14.92 (s, 1H, -COO**H**), 8.60 (s, 1H, -NC**H**CCOOH), 7.99-7.95 (d, 1H, J=16 Hz, C(F)C**H**C), 6.66-6.64 (d, 1H, J =6.88 Hz,-C(N)C**H**C), 5.07 (t, 1H, CH$_2$-C**H**(OCOR)-CH$_2$-), 4.53 (bs,2H, -N Bridged **H**), 4.30-4.25 (q, 2H, -NC**H$_2$**CH$_3$-), 2.32-2.15 (m, 6H, CH**(H)**-CH**(H)**- , -CH(**H**)-C(OCOR)-CH(**H**)-) and --C**H**(H)-C(OCOR)-C**H**(H)-), 1.85-1.82 (C**H(**H**)**-C**H(**H**)**-), 1.57 (t , 3H, -NCH$_2$C**H$_3$**), 1.25 (s, 9H, -OCOC(C**H$_3$**)$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ (**177.48, 176.0** J= 3.39 Hz), 176.07, 167.45, (**152.45, 149.97,** J= 248 Hz),146.82, **(140.62, 140.62,** J= 11.0 Hz),137.71, (**117.68, 117.60,** J= 8 Hz), (**113.21, 112.96,** J= 25.0 Hz), 107.76, (**101.17, 101.12,** J= 5.0 Hz), 66.86, 55.47, 55.39 , 49.58, 38.55, 34.00, 27.47, 27.01, 14.37. MS (EI): m/z calculated for $C_{24}H_{29}FN_2O_5$ $[M]^+$: 444.54, found: 445.5

1-Ethyl-6-fluoro-1,4-dihydro-7-(3-(isobutyryloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-4-oxoquinoline-3-carboxylic acid (3.30): Off white colour powder, Yield: 0.24 gm, 68.0 %, HPLC Purity 99.55% , Melting point 280-280°C , ^{1}H NMR (400 MHz CDCl$_3$) δ 14.92 (s, 1H, -COO**H**), 8.60 (s, 1H, -NC**H**CCOOH), 7.99-7.96 (d, 1H, J= 14.2 Hz, C(F)C**H**C), 6.66-6.64 (d, 1H, J= 6.88 Hz, -C(N)C**H**C), 5.07 (t, 1H, CH$_2$-C**H**(OCOR)-CH$_2$-),4.53 (bs,2H, -N Bridged **H**), 4.28-4.27 (q, 2H, -NC**H$_2$**CH$_3$-), 2.61 (m, 1H, -OCOC**H**(CH$_3$)$_2$) , 2.26-2.10 (m, 6H, CH**(H)**-CH**(H)**- , -CH(**H**)-C(OCOR)-CH(**H**)-) and -C**H**(H)-C(OCOR)-C**H**(H)-), 1.87-1.83 (C**H(**H**)**-C**H(**H**)**-), 1.59 (t , 3H, -NCH$_2$C**H$_3$**), 1.23 (d, 6H, -OCOCH(C**H$_3$**)$_2$) , ^{13}C NMR (100 MHz, CDCl$_3$)δ (**177.48, 176.0,** J= 3.39 Hz), 175.99, 167.45, (**152.45, 149.97,** J= 248 Hz), 146.82, (**140.62, 140.62,** J= 11.0 Hz),137.71, (**117.68, 117.60** J= 8 Hz), (**113.21, 112.96,** J= 25.0 Hz)**,** 107.77, (**101.15, 101.10,** J= 5.0 Hz), 66.90, 55.49, 55.41 , 49.58, 34.27, 33.98, 27.49, 18.79, 14.39. MS (EI): m/z calculated for $C_{23}H_{27}FN_2O_5$ $[M+H]^+$: 430.47, found: 431.54

3.5.3 Aromatic Acid chloride substituted derivatives:

(S)-10-(3-(Benzoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.31): light yellow colour powder, Yield- 32%, (0.20 g), Melting point 245°C decomposes, $[\alpha]^{25}_{D}$ -73° (*c* 0.30, CH_2Cl_2), ^{1}H NMR ($CDCl_3$,400 MHz,) : δ 15.19 (s, 1H, -COO**H**), 8.58 (s, 1H, -NC**H**CCOOH), 8.07-8.05 (d, 2H, -Ar ring), 7.74-7.71 (d, 1H, J=13.3 Hz, -C(F)C**H**C), 7.62-7.59 (t, 1H, - Ar ring), 7.51-7.48 (t, 2H, -Ar ring), 5.44-5.43 (t, 1H, J= 5.0 Hz, -CH_2-C**H**(OR)-CH_2-), 4.50-4.4.30 (m, 5H, -NC**H**(CH_3)CH_2-, -NCH(CH_3)C**H**$_2$- and bridged **H**), 2.51-2.39 (4ts, 2H, -C**H**(H)-C(OR)-C**H**(H)), 2.32-2.31 (m, 2H, CH(**H**)-C(OR)-CH(**H**)),2.16- 2.14-(q, 2H, -C**H**(H)-C**H**(H))., 2.05-2.01 (bs, 2H, -CH(**H**)-CH(**H**)), 1.64-1.62 (d, 3H, J= 6.5 Hz, – NCH(C**H**$_3$)CH_2); ^{13}C NMR ($CDCl_3$,100 MHz,): δ (**176.04, 176.0,** J= 4.0 Hz) , 170.70, 167.40 ,(**154.00, 152.00,** J= 200 Hz), 144.52, (**135.10, 135.00**, J= 10 Hz) , 133.93, 129.23, 128.63, 127.61 , 124.93, (**117.50, 117.40**, J= 10 Hz), 107.35,(**105.67, 105.42**, J= 25 Hz), 68.38, 67.93, 58.08, 57.67, 55.49, 37.22, 37.12, 28.73, 18.24. EIMS m/z 493.4 [M] + (1) calculated for $C_{27}H_{25}FN_2O_6$.

(S)-10-(3-(2-(4-Chlorophenyl)acetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.32) : light yellow colour powder, Yield- 62%, (0.42 g), Melting point 235°C; $[\alpha]^{25}_{D}$ -96° (*c* 0.30, CH_2Cl_2); ^{1}H NMR (DMSO d_6 ,400 MHz,) : δ 15.34 ((s, 1H, -COO**H**), 8.82 (s, 1H, -NC**H**CCOOH), 7.58-7.55(d, 1H, J=13.5 Hz, -C(F)C**H**C), 7.33-7.26 (q, 4H, -CH_2-**ArCl**), 5.10-5.09 (t, 1H, J= 4.5 Hz, -CH_2-C**H**(OR)-CH_2-), 4.83-4.81 (m, 1H, -NC**H**(CH_3)CH_2-), 4.48-4.28 (m, 4H, -NCH(CH_3)C**H$_2$**- and bridged **H**), 3.65 (s, 2H, -C**H**$_2$-Ar), 2.35-2.23(4ts, 2H, J=4.0 Hz -C**H**(H)-C(OR)-C**H**(H)), 2.00-1.86 (m, 4H, CH(**H**)-C(OR)-CH(**H**)), and C**H**(H)-C**H**(H))., 1.80-1.76 (bs, 2H, -CH(**H**)-CH(**H**)), 1.55-1.53 (d, 3H, J=6.5 Hz, –NCH(C**H**$_3$)CH_2); ^{13}C NMR (DMSO d_6,100 MHz,): δ (**176.31, 176.28,** J= 4.0 Hz), 169.48, 166.42 ,(**151.81, 149.36**, J= 245 Hz), 145.26, (**135.97, 135.87**, J= 10 Hz), 132.56, 132.08 ,(**130.43, 130.30,** J= 13 Hz), 128.63,124.66, (**116.82 ,116.71**, J= 11 Hz) , 106.50,(**104.29, 104.04**, J= 25 Hz), 67.85, 67.76 ,57.51 , 57.12, 54.71, 40.67, 36.74, 36.64, 27.94, 27.89, 17.94 EIMS m/z 542.1[M]+(1) calculated for $C_{28}H_{26}ClFN_2O_6$.

(S)-10-(3-(2-Phenylacetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.33): light yellow colour powder, Yield-62%, (0.40 g), HPLC Purity- 99.65% ; Melting point 235°C; $[\alpha]^{25}_{D}$ -93° (*c* 0.33, CH_2Cl_2); ^{1}H NMR ($CDCl_3$,400 MHz,) : δ 15.34 ((s, 1H, -COO**H**), 8.52 (s, 1H, -NC**H**CCOOH), 7.67-7.64(d, 1H, J=13.3 Hz, -C(F)C**H**C), 7.67-7.28 (m, 5H, -CH_2-**ArH**),

5.15-5.12 (t, 1H, J=5.3 Hz, -CH_2-C**H**(OR)-CH_2-), 4.38-4.25 (m, 5H, -NC**H**(CH_3)CH_2-, -NCH(CH_3)C**H**$_2$- and bridged **H**), 3.65 (s, 2H, -C**H**$_2$-Ar),), 2.35-2.23(4ts, 2H, J= 4.0 Hz -C**H**(H)-C(OR)-C**H**(H)), 1..91-1.86 (m, 2H, CH(**H**)-C(OR)-CH(**H**)),1.82-1.80 (2bs, 2H, -C**H**(H)-C**H**(H))., 1.68 (bs, 2H, -CH(**H**)-CH(**H**)) , 1.60-1.58 (d, 3H, J= 5.5 Hz, – NCH(C**H**$_3$)CH_2); ^{13}C NMR ($CDCl_3$,100 MHz,): δ (**176.04, 176.0,** J= 4.0Hz), 170.70, 167.40,(**154.54, 152.00** , J= 246 Hz), 144.52 , (**135.10, 135.00**, J= 10 Hz) , 133.93,129.23,128.63 127.61 , 124.9 , (**117.50 ,117.40**, J= 10 Hz), 107.35,(**105.67, 105.42,** J= 25 Hz), 68.25, 67.93 ,58.08 , 57.67, 55.49, 42.24 ,37.22, 37.12, 28.30, 18.33 ; CHN Anal. Calcd. For $C_{28}H_{27}FN_2O_6$: C, 66.39; H, 5.37; N, 5.53. Found: C, 66.12; H, 5.12; N, 5.31. EIMS m/z 507.1[M]+(1) calculated for $C_{28}H_{27}FN_2O_6$.

(S)-10-(3-(3-Acetoxy-2-phenylpropanoyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid hydrochloride (3.34): light yellow colour powder, Yield- 36%, (0.24 g), Melting point 204°C; ^{1}H NMR ($CDCl_3$,400 MHz,): δ 15.18 (s, 1H, -COO**H**), 8.54 (s, 1H, -NC**H**CCOOH), 7.72-7.69 (d, 1H, J= 13.44 Hz -C(F)C**H**C), 7.38-7.32 (m, 5H, -CH_2-Ar**H**), 5.18 -5.16 (t, 1H, -CH2-C**H**(OR)-CH_2-), 4.67-4.65 (m, 1H, -C**H**-Ar), 4.45-4.36 (m, 2H, -NCH(CH_3)C**H**$_2$-), 4.29, 4.26 (bs, 2H, bridged **H**), 4.19 (m, 1H, -NC**H**(CH_3)CH_2-), 3.97-3.93 (m , 2H, (-ArCH)C**H$_2$**($COOCH_3$), 2.35-2.05 (4ts, 2H, J= 4.0 Hz -C**H**(H)-C(OR)-C**H**(H)), 2.14 (s, 3H, -CH_3COO), 2.02-1.54 (m, 6H, CH(**H**)-C(OR)-CH(**H**),-C**H**(H)-C**H**(H)),-CH(**H**)-CH(**H**)),1.54-1.52 (d, 3H,–CH(C**H**$_3$)CH_2); ^{13}C NMR ($CDCl_3$,100 MHz,): δ (**175.95, 175.91**, J=4 Hz,),172.05, 170.69, 167.31 ,(**154.22, 151.77**, J=245 Hz ,144.46, (**135.89, 135.81**, J=8 Hz), 134.86, 132.52, 129.03, 128.03, 128.08, 125.80, 118.52, 107.61, (**104.46, 104.21**, J= 25 Hz) 68.69, 68.05, 61.46, 61.38, 55.40, 51.14, 37.03, 37.02, 28.21, 22.4,18.35. EIMS m/z 579.2[M]+(1) calculated for $C_{31}H_{31}FN_2O_8$.

10-(3-(Benzoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.35): light yellow colour powder, Yield- 30% (0.18 g), Melting point 252°C decomposes, ^{1}H NMR ($CDCl_3$,400 MHz,) : 15.08, (s, 1H, -COO**H**), δ 8.55 (s, 1H, -NC**H**CCOOH), 8.10-8.04 (d, 2H, -Ar ring), 7.74-7.76(d, 1H, J=13.3 Hz, -C(F)C**H**C), 7.62-7.55 (t, 1H, - Ar ring), 7.52-7.45 (t, 2H, -Ar ring), 5.45-5.41 (t, 1H, J=5.0 Hz, -CH_2-C**H**(OR)-CH_2-), 4.50-4.4.35 (m, 5H, -NC**H**(CH_3)CH_2-, -NCH(CH_3)C**H**$_2$- and bridged **H**), 2.59-2.41 (4ts, 2H, -C**H**(H)-C(OR)-C**H**(H)), 2.30-2.29 (m, 2H, CH(**H**)-C(OR)-CH(**H**)), 2.15- 2.13-(q, 2H, -C**H**(H)-C**H**(H))., 2.04-2.01 (bs, 2H, -CH(**H**)-CH(**H**)) ,1.65-1.63 (d, 3H, J=6.5 Hz, –NCH(C**H**$_3$)CH_2); ^{13}C NMR ($CDCl_3$,100 MHz,): δ (**176.25, 176.21**, J= 4 Hz), 170.79, 167.46, (**154.54, 152.00**, J= 246 Hz), 144.26 , 135.21, 135.22, 133.99, 129.26, 128.52, 127.32, 124.90, (**117.70, 117.60** J= 10 Hz), 107.31, (**105.69,**

105.44, J= 25 Hz), 68.30, 67.90, 58.04, 57.62, 55.50, 37.20, 37.10, 28.72, 18.20; EIMS m/z 542.1[M]+(1) calculated for $C_{28}H_{26}ClFN_2O_6$.

10-(3-(2-(4-Chlorophenyl)acetoyloxy)-8-aza-bicyclo[3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H- [1,4] oxazino[2,3,4-*ij*] quinoline-6-carboxylic acid hydrochloride (3.36): light yellow colour powder, Yield- 62%, (0.42 g), Melting point 247°C, ^{1}H NMR ($CDCl_3$,400 MHz,) : δ 15.15 (s, 1H, -COO**H**), 8.59 (s, 1H, -NC**H**CCOOH), 7.62-7.59(d, 1H, J=13.5 Hz, -C(F)C**H**C), 7.32-7.22 (q, 4H, -CH_2-**ArCl**), 5.15-5.14 (t, 1H, J= 4.5 Hz, -CH_2-C**H**(OR)-CH_2-), 4.55-4.27 (m, 5H, -NC**H**(CH_3)CH_2-), -NCH(CH_3)C**H$_2$**- and bridged **H**), 3.62 (s, 2H, -C**H**$_2$-Ar), 2.38-2.28(4ts, 2H, J=4.0 Hz -C**H**(H)-C(OR)-C**H**(H)), 2.27-2.25 (m, 2H, CH(**H**)-C(OR)-CH(**H**)),1.98-1.77(m, 4H, C**H**(H)-C**H**(H)) and -CH(**H**)-CH(**H**)) , 1.59-1.58 (d, 3H, J=6.5 Hz, –NCH(C**H**$_3$)CH_2); ^{13}C NMR ($CDCl_3$,100 MHz,): δ (**176.85, 176.82,** J= 3 Hz), 170.22, 167.37, (**154.77, 152.32** , J= 245 Hz) , 144.73, 135.92, 135.85, 133.15 , 132.48 ,(**131.13, 131.00,** J= 13 Hz), 130.64, 128.80, 125.03, (**117.35, 117.26**, J= 9 Hz) , 107.34, (**105.53, 105.28**, J= 25 Hz), 68.80, 68.10, 58.15 , 58.10, 57.73, 57.67, 55.52, 41.52, 37.29, , 37.20 28.42, 28.38, 18.34 EIMS m/z 542.1[M]+(1) calculated for $C_{28}H_{26}ClFN_2O_6$.

10-(3-(2-Phenylacetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic acid hydrochloride (3.37): light yellow colour powder, Yield- 50%,(0.32 g), HPLC Purity- 96.63%; Melting point 204°C, ^{1}H NMR ($CDCl_3$,400 MHz,): δ 15.22 (s, 1H, -COO**H**), 8.59 (s, 1H, -NC**H**CCOOH), 7.58-7.54 (d, 1H, J= 13.44 Hz, -C(F)C**H**C), 7.36-7.30 (m, 5H, -CH_2-Ar**H**), 5.13 (t, 1H, -CH2-C**H**(OR)-CH_2-), 4.57-4.26 (m, 5H, -NC**H**(CH_3)CH_2-, -NCH(CH_3)C**H**$_2$- and bridged **H**), 3.65 (s, 2H, -C**H**$_2$-Ar),), 2.35-2.24(4ts, 2H, J=4.0Hz -C**H**(H)-C(OR)-C**H**(H)), 1.91-1.77 (m, 6H, CH(**H**)-C(OR)-CH(**H**),-C**H**(H)-C**H**(H)),-CH(**H**)-CH(**H**)), 1.57 (Bs,3H,–CH(C**H**$_3$)CH_2); ^{13}C NMR ($CDCl_3$,100 MHz,): δ (**176.81, 176.78,** J=3 Hz,), 170.70, 167.39 ,(**154.71, 152.26,** J= 245 Hz), 144.75 , (**135.89, 135.81**, J=8 Hz, ,134.00, (**131.16, 131.02**, J=14 Hz,), 129.26, 128.65, 127.16, 124.98, (**117.17, 117.08** J=9 Hz), 107.17, **(105.37, 105.11**, J=26 Hz), 68.33, 68.02, 58.14, 58.09, 57.71, 57.66, 55.50, 42.26, 37.26, 37.16, 29.68, 28.35, 28.30, 18.37; CHN Anal. Calcd. For $C_{28}H_{27}FN_2O_6$: C, 66.39; H, 5.37; N, 5.53. Found: C, 66.84; H, 5.28; N, 5.39. EIMS m/z 507[M]+(1) calculated for $C_{28}H_{27}FN_2O_6$.

10-(3-(3-Acetoxy-2-phenylpropanoyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid hydrochloride (3.38): light yellow colour powder, Yield- 18% (0.12 g), Melting point 224°C, ^{1}H NMR ($CDCl_3$,400 MHz): δ 15.08 (s, 1H, -COO**H**), 8.54 (s, 1H, -NC**H**CCOOH), 7.70-7.67 (d, 1H, J=

13.44 Hz -C(F)C**H**C), 7.38-7.31 (m, 5H, -CH_2-Ar**H**), 5.18-5.16 (t, 1H, -CH2-C**H**(OR)-CH_2-), 4.67-4.62 (m, 1H, -C**H**-Ar), 4.45-4.36 (m, 2H, -NCH(CH_3)C**H**$_2$-), 4.29, 4.26 (bs, 2H, bridged **H**), 4.19 (m, 1H, -NC**H**(CH_3)CH_2-), 3.97-3.93 (m , 2H, (-ArCH)C**H$_2$**($COOCH_3$), 2.39-2.09(4ts, 2H, J=4.0Hz -C**H**(H)-C(OR)-C**H**(H)),2.04 (s, 3H, -CH_3COO), 2.02-1.54 (m, 6H, CH(**H**)-C(OR)-CH(**H**),-C**H**(H)-C**H**(H)),-CH(**H**)-CH(**H**)),1.53-1.52 (d,3H,–CH(C**H**$_3$)CH_2); ^{13}C NMR ($CDCl_3$,100 MHz,): δ (**175.95, 175.93**, J=3 Hz), 172.0, 170.65, 167.28 ,(**154.18, 152.12**, J=245 Hz) 144.46, (**135.89, 135.81**, J=8 Hz), 134.85, 132.52, 129.02, 128.13, 128.04, 125.82, 118.62, 107.65, 104.01**,** 68.67, 68.00, 61.36, 61.28, 55.47, 51.04, 37.83, 37.02, 28.41, 22.0,18.30 ; EIMS m/z 579.2[M]+(1) calculated for $C_{31}H_{31}FN_2O_8$.

7-(3-(2-Phenylacetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (3.39): Yellow coloured powder, Yield- 76%, (0.3 g), Melting point 218-220°C, ^{1}H NMR (400 MHz $CDCl_3$) *δ* 15.24 (s, 1H, -COO**H**), 8.58 (s, 1H, -NC**H**CCOOH), 7.98-7.94 (d, 1H, J= 14.36 Hz, C(F)C**H**C), 7.37-7.26 (m, 5H, -Ar), 6.60-6.58 (d, 1H, J= 7.48 Hz -C(N)C**H**C), 5.05-5.03 (t, 1H,CH_2-C**H**(OCOR)-CH_2), 4.28 (bs,2H, -NC**H**(CH_2)), 4.24-4.21 (q, 2H, -NC**H$_2$**CH_3-), 3.66 (s, 2H, -OCOC**H$_2$**Ar), 2.22-2.16 (dt, 2H, -CH(**H**)-C(OCOR)-CH(**H**)-) 2.04-1.95 (t , 2H , C**H**(H)-C(OOCOR)-C**H**(H)-), 1.88-1.85 (q, 2H, CH**(H)**-CH**(H)**-) , 1.79-1.76 (d,2H, C**H(**H**)**-C**H(**H**)**-), 1.55-1.52 (t,3H, -CH_2C**H$_3$**), ^{13}C NMR (100 MHz, $CDCl_3$)*δ* (**176.70, 176.66** J= 4.0 Hz), 170.49, 167.42 , 166.41**,** (**152.49, 150.01,** J= 248 Hz)**,** 146.85, (**142.02, 141.98** J= 4.0 Hz)**,** 137.77, 133.83, 129.22, 128.74, 127.30, (**117.75, 117.68,** J= 7 Hz **),** (**113.32, 113.08,** J= 24 Hz)**,** 107.96**,** (**101.09, 101.05,** J= 4.0 Hz)**,** 67.74, 55.47,55.40 ,49.54, 42.23, 33.92, 27.28, 14.40.MS (EI): m/z calculated for $C_{27}H_{27}FN_2O_5[M]^+$: 478.51, found: 479.32

7-(3-(2-(4-Chlorophenyl)acetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride (3.40): Yellow coloured powder, Yield- 46% (0.32 g), Melting point 227-228°C, ^{1}H NMR (400 MHz $CDCl_3$) *δ* 15.22 (s, 1H, -COO**H**), 8.60 (s, 1H, -NC**H**CCOOH), 8.02-7.98 (d, 1H, J= 14.28 Hz, C(F)C**H**C), 7.34-7.22 (m, 4H, -Ar), 6.62-6.60 (d, 1H, J= 7.04 Hz, -C(N)C**H**C), 5.05 (t ,1H, CH_2-C**H**(OCOR)-CH_2), 4.44 (bs,2H, -NC**H**(CH_2)), 4.26-4.23 (q, 2H, -NC**H$_2$**CH_3-), 3.63 (s, 2H, -OCOC**H$_2$**Ar), 2.23-2.16 (dt, 2H, -CH(**H**)-C(OCOR)-CH(**H**)-), 2.01-2.0 (t , 2H , C**H**(H)-C(OOCOR)-C**H**(H)-), 1.95-1.87 (q, 2H,CH**(H)**-CH**(H)**-), 180-1.76 (d,2H, C**H(**H**)**-C**H(**H**)**-),1.55-1.53 (t,3H, -CH_2C**H$_3$**), ^{13}C NMR (100 MHz, $CDCl_3$)*δ* (**176.70, 176.66** J= 4.0 Hz), 170.49, 167.42 , 166.41**,** (**152.49, 150.01,** J= 248 Hz)**,** 146.85, (**142.02,141.98** J= 4.0 Hz)**,** 137.77, 133.83, 129.22, 128.74, (**117.75, 117.68,** J= 7 Hz **),** (**113.32, 113.08,** J= 24 Hz)**,** 107.96**,** (**101.09, 101.05** J= 4.0 Hz) **,** 67.74, 55.47,55.40

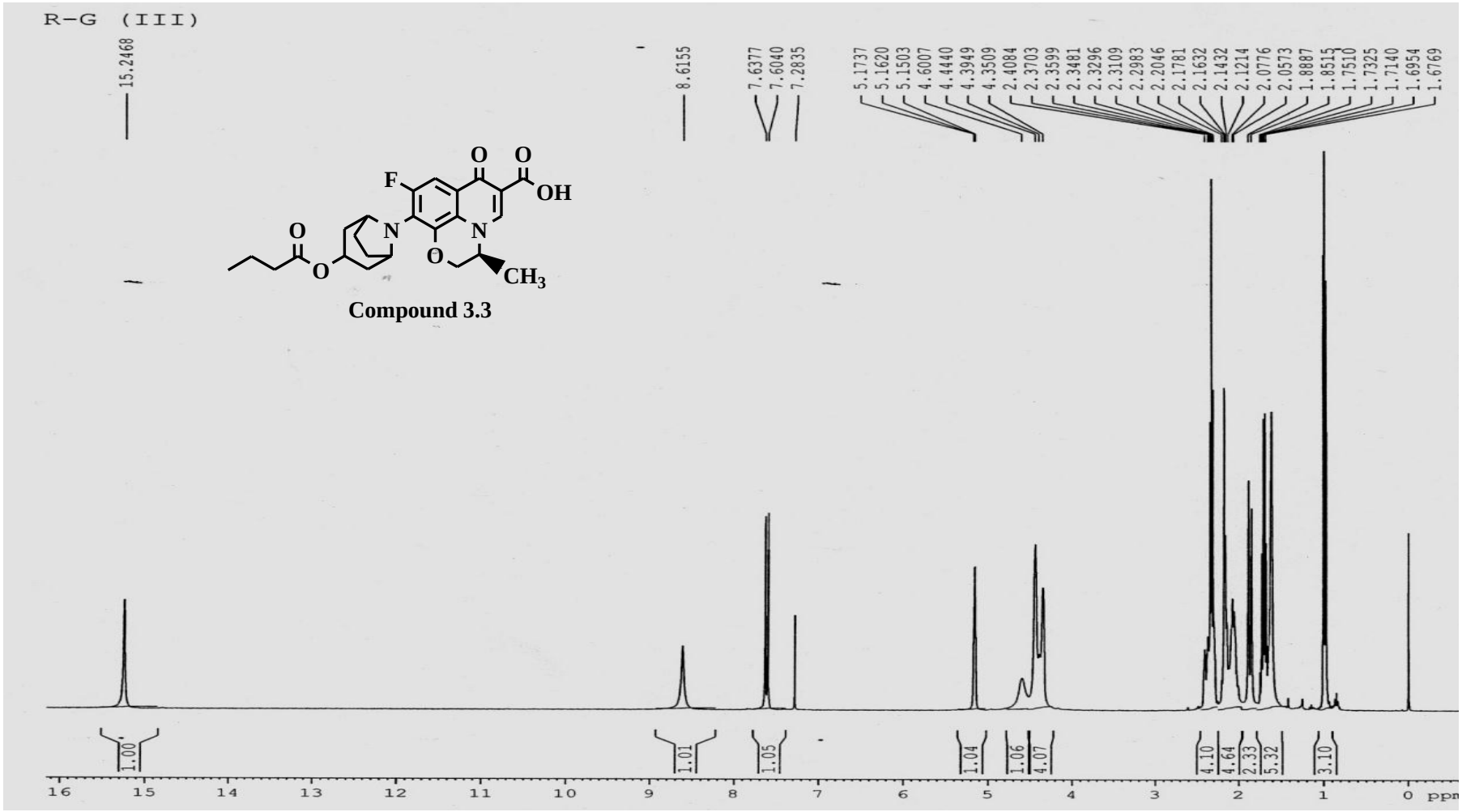
,49.54, 42.23, 33.92, 27.28, 14.40 ; MS (EI): m/z calculated for $C_{27}H_{26}ClFN_2O_5[M]^+$: 512.96, found: 513.58,

7-(3-(3-Acetoxy-2-phenylpropanoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride (3.41): Yellow coloured powder, Yield-40%, (0.3 g), Melting point 190-193°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.21 (s, 1H, -COO**H**), 8.61 (s, 1H, -NC**H**CCOOH), 8.01-7.97 (d, 1H, J= 14.32 Hz, C(F)C**H**C), 7.40-7.26 (m, 5H, -Ar), 6.60-6.58 (d, 1H, J= 7.48 Hz, -C(N)C**H**C), 5.09-5.06 (t , 1H, CH_2-C**H**(OCOR)-CH_2) ,4.68-4.65 (t, 1H, -OCO(C**H)**Ar) ,4.49 &4.34 (two bs 2 H , -NC**H**(CH_2)), 4.42 and 3.98 (m, 2H, OCOCH(C**H$_2$**)OCO-) , 4.25-4.20 (q, 2H, -NC**H$_2$**CH_3-), 2.26-2.15 (2 dt, 2H , -CH(**H**)-C(OCOR)-CH(**H**)-), 2.04 (s, 5H, -OCOC**H$_3$** and C**H**(H)-C(OOCOR)-C**H**(H)-),), 1.89-1.81 (q, 2H, CH**(H)**-CH**(H)**-), 1.68-1.64 (d,2H, C**H(**H)-C**H(**H)-), 1.58-1.50 (t, 3H, -CH_2C**H$_3$**); ^{13}C NMR (100 MHz, $CDCl_3$)δ (**177.18, 177.13,** J= 5.0 Hz), 170.62,169.94, 167.47, (**151.94, 149.51** J= 243 Hz),146.89, (**142.45, 142.42** J= 3.0 Hz**),** 136.95, 134.71, 129.11, 128.26, 128.0,**(117.89, 117.80,** J= 9.0 Hz), (**113.20, 112.95 ,** J= 25 Hz)**,** 108.05**, (101.07, 101.02** J= 5.0 Hz), 68.10, 64.65, 55.40,55.20, 50.98, 49.52, 34.06, 33.51, 27.38, 26.91, 20.83, 14.42, MS (EI): m/z calculated for $C_{30}H_{31}FN_2O_7[M]^+$: 550.57, found: 551.3

7-(3-(3-Acetoxy-2-phenylpropanoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-cyclopropyl -6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (3.42): Light yellow coloured powder, Yield- 47% (0.20 g), HPLC Purity- 95.62% , Melting point 225-227°C , ^{1}H NMR (400 MHz $CDCl_3$) δ 15.33 (s, 1H, -COO**H**), 8.65 (s, 1H, -NC**H**CCOOH), 7.92-7.89 (d, 1H, J=14.2 Hz, C(F)C**H**C), 7.40-7.31 (m. 5H, -OCOCH-**Ar**), 7.14- 7.12 (d, 1H, J=7.3 Hz, -C(N)C**H**C), 5.08 (t, 1H, -CH_2-C**H**(OCOR)-CH_2-),4.68-4.63 (t. 1H, -OCOC**H**-(CH_2)-(Ar**)**) , 4.49 &4.35 (two bs 2 H, -N-bridged **H**), 4.42-4.38 and 3.98-3.94 (m, 2H, OCOCH(C**H$_2$**)OCO-) 3.46 (m, -N-Cyclopropyl C**H**) ,2.29-2.19 (m, 2H, -CH(**H**)-C(OCOR)-CH(**H**)-), 2.04 (s, 3H, -OCOC**H$_3$**), 1.87-1.83 (q, 2H, -CH**(H)**-CH**(H)**-),1.67-1.65 (d, C**H(**H)-C**H(**H)-), 1.57-1.52 (m 2H -C**H**(H)-C(OCOR)-C**H**(H)-), 1.33-1.32 (q, 2H, -NCHCH(**H**)CH(**H**)-Cyclopropyl), 1.17 (bs, 2H, -NCHC**H**(H)C**H**(H) cyclopropyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ (**177.76, 176.73** J= 3 Hz), 170.62, 170.56, 167.19, (**152.66, 150.18** J= 248 Hz), 147.13, (**140.52, 140.41** J= 11.0 Hz), 139.70, 134.72, 129.10, 128.25, 128.0, (**117.06, 117.00,** J= 8 Hz), (**112.96, 112.72,** J= 25.0 Hz)**,** 107.73, (**102.31, 102.26,** J= 5.0 Hz)**,** 66.20, 64.67, 55.46, 55.38 , 55.28, 55.21, 50.98, 35.04, 33.96, 33.40, 27.39, 26.93, 20.83, 8.15, MS (EI): m/z calculated for $C_{31}H_{31}FN_2O_7$ $[M]^+$: 562.59,found: 563.51

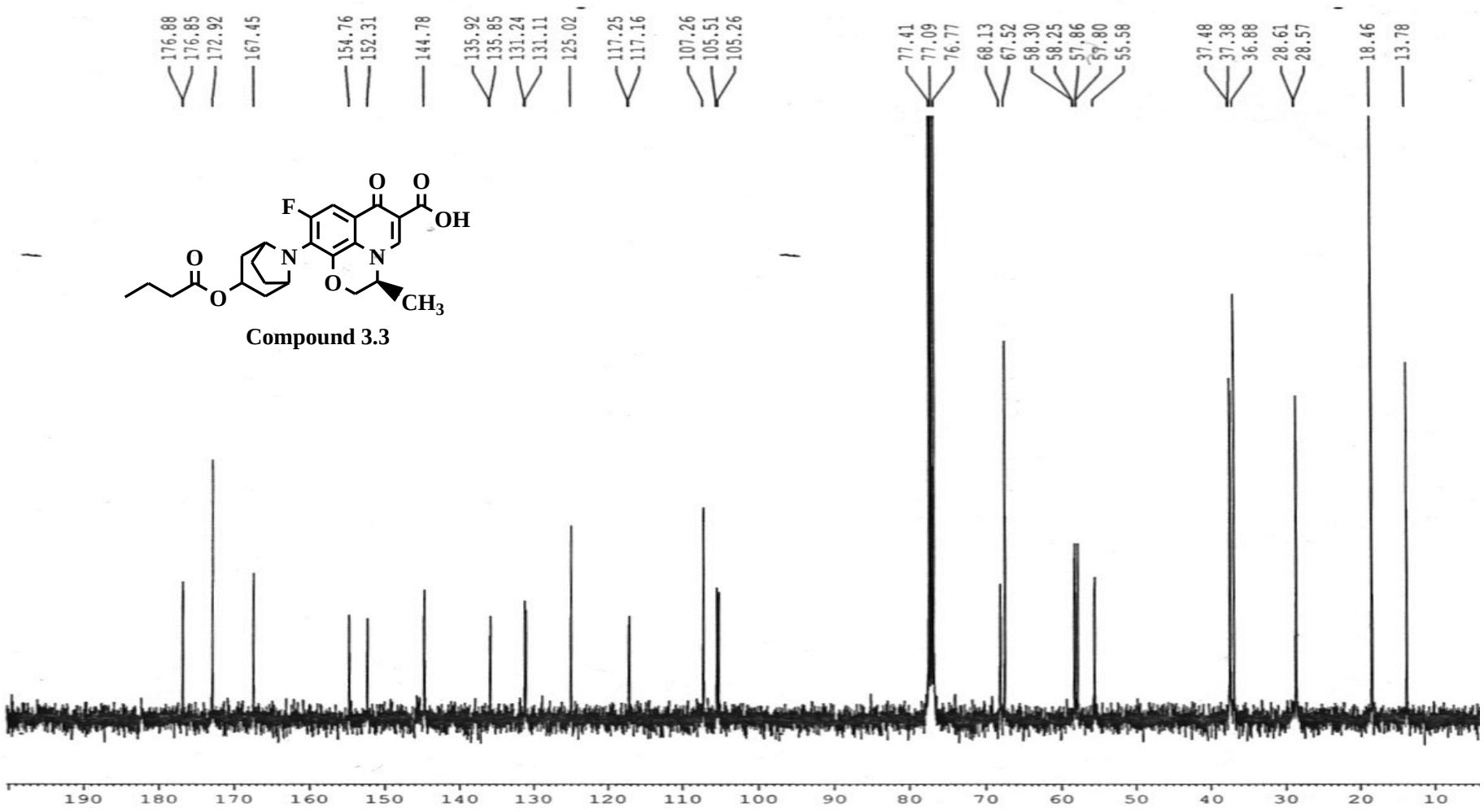
References

1. Joelle, A.; Brigitte, G.; Alexander, K.; Robert, K.; Christine, R.; Patricia, C.; Mamadou, D.; Myriam M.M. *Eur. J. Med. Chem*, **2011**, 46, 6025-6038.
2. Lester A. M. *Chem. Rev.*, **2005**, 105, 559-592.
3. Azéma, J.; Guidetti, B.; Malet-Martino, M.; Martino, R.; Roques, C. *Bioorg. Med. Chem.* **2006**, 14, 2569-2580.
4. Sharma,P.C.; Jain, A.; Yar, M.S.; Pahwa, R.; Singh, J.; Goel, S. *Arab. J. Chem.* **2015**, 8, 671–677.
5. Jazayeri, S.; Moshafi, M.H.; Firoozpour,L.; Emami, S.; Rajabalian, S.; Haddad, M.; Pahlavanzadeh, F.; Esnaashari, M.; Shafiee, A.; Foroumadi, A. *Eur. J. Med. Chem.* **2009,** 44, 1205-1209.
6. Letafat, B.; Emami, S.; Mohammadhosseini, N.; Faramarzi, M.; Samadi, N,; Shafiee, A.; Foroumadi, A. *Chem. Pharm. Bull.* **2007**, 55, 894-898.
7. Foroumadi,A.; Emami, S.; Mansouri, S.; Javidnia, A.; Saeid-Adeli,N.; Shirazi, F.H.; Shafiee, A. *Eur. J. Med. Chem.* **2007**, 42, 985-992.



Compound 3.3

Fig.-: ^{1}H NMR of Compound 3.3



Compound 3.3

Fig.-: ^{13}C NMR of Compound 3.3

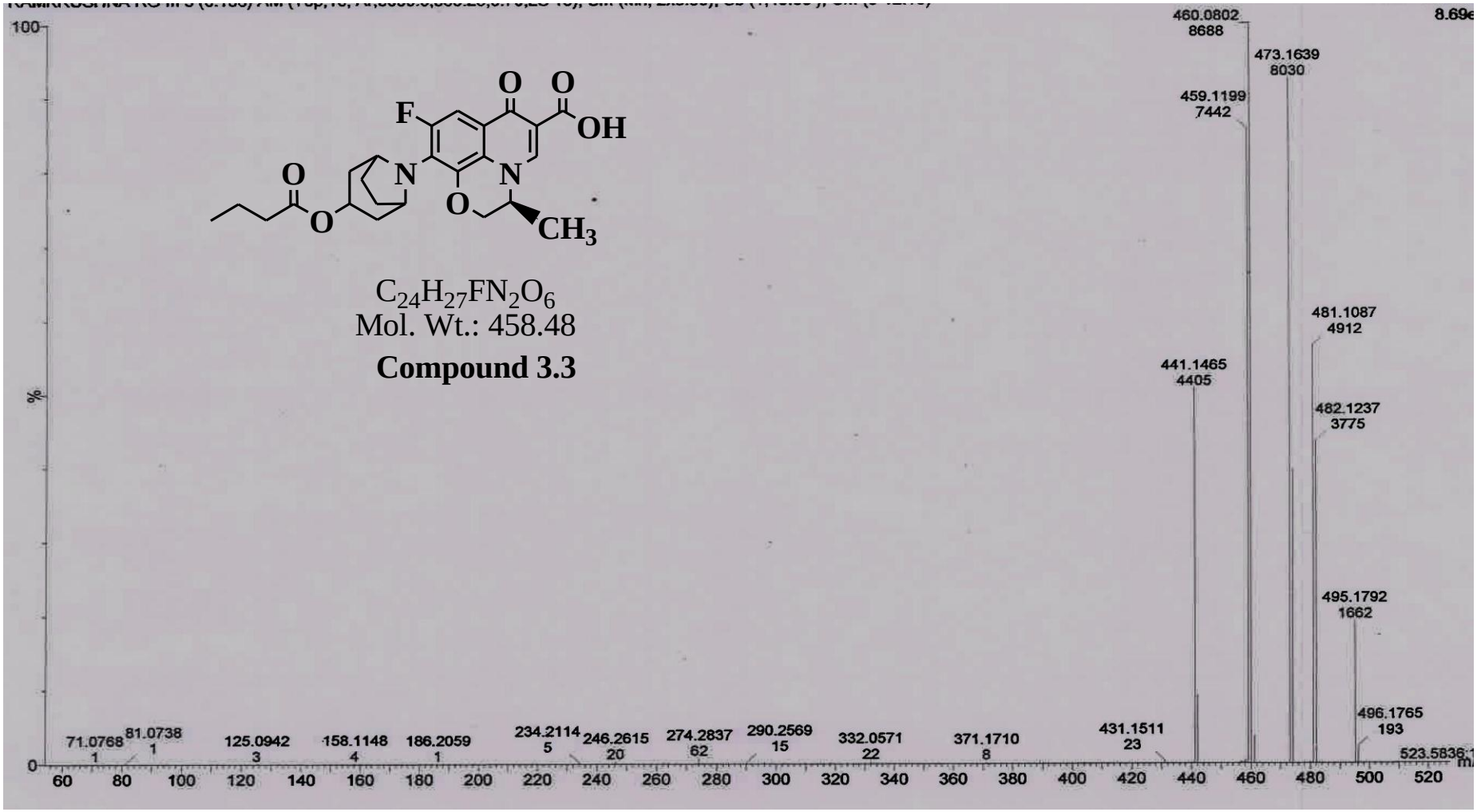
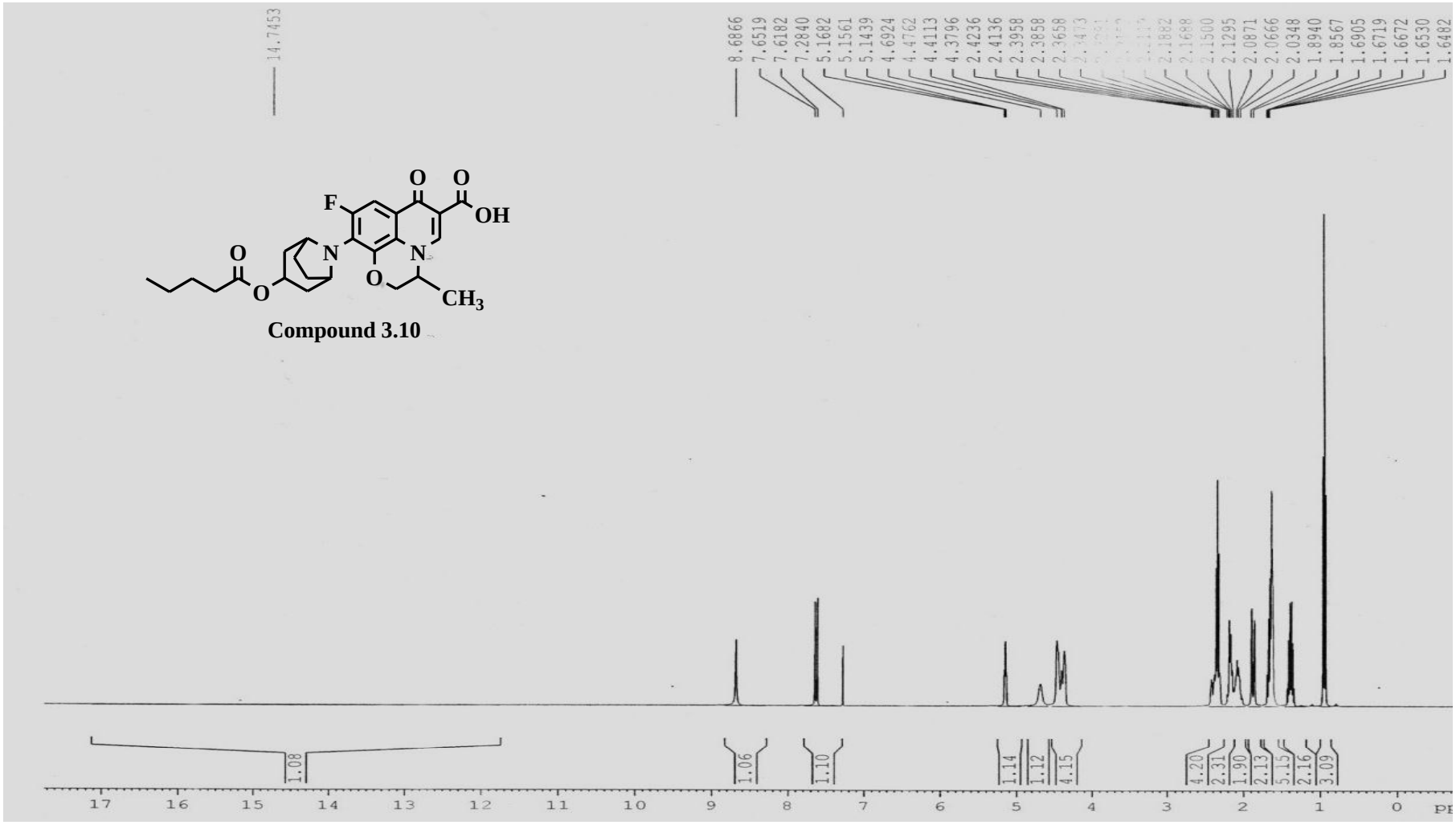
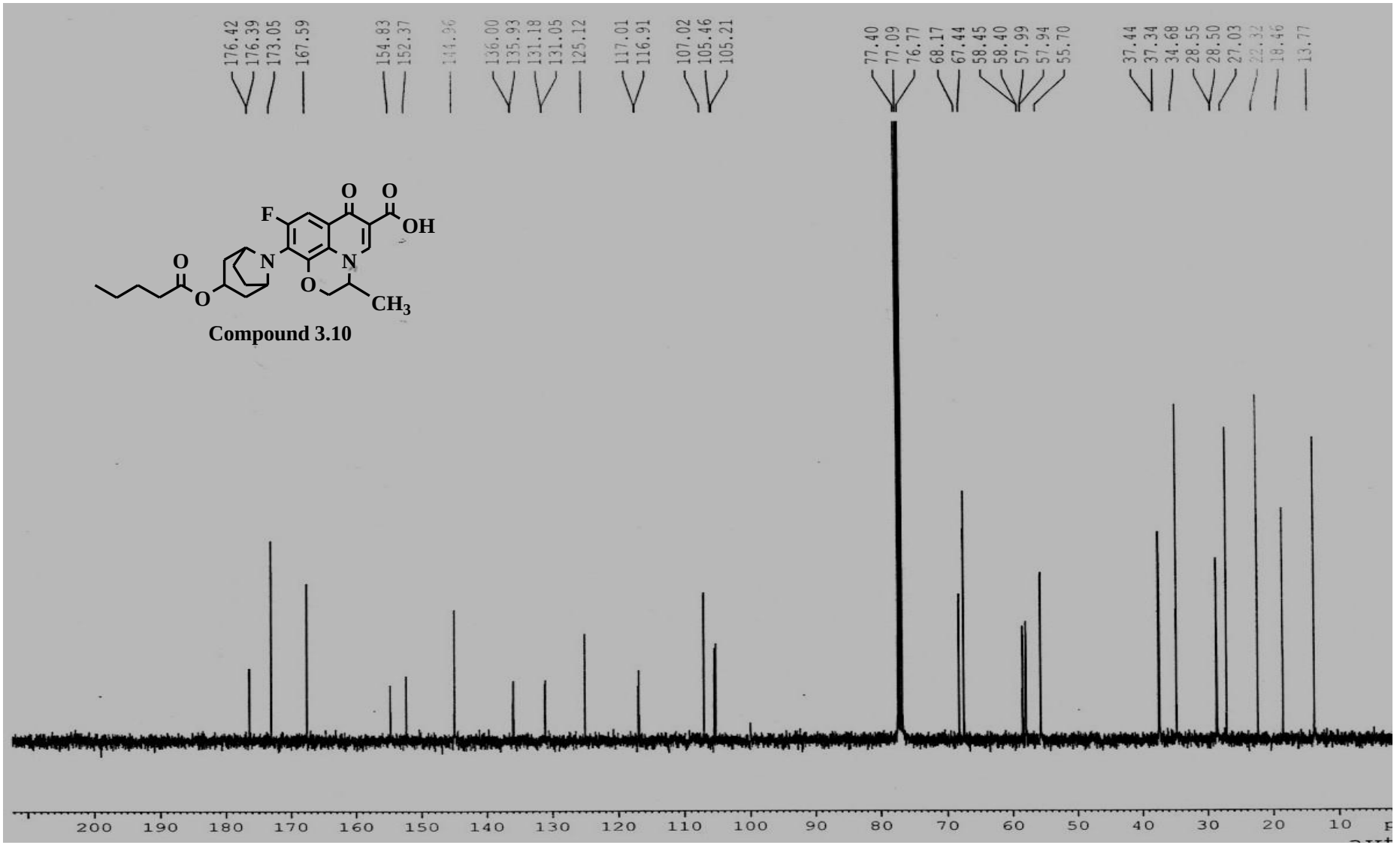


Figure: Mass Spectra of compound 3.3



Compound 3.10

Fig.-: ^{1}H NMR of Compound 3.10



Compound 3.10

Fig.-: ^{13}C NMR of Compound 3.10

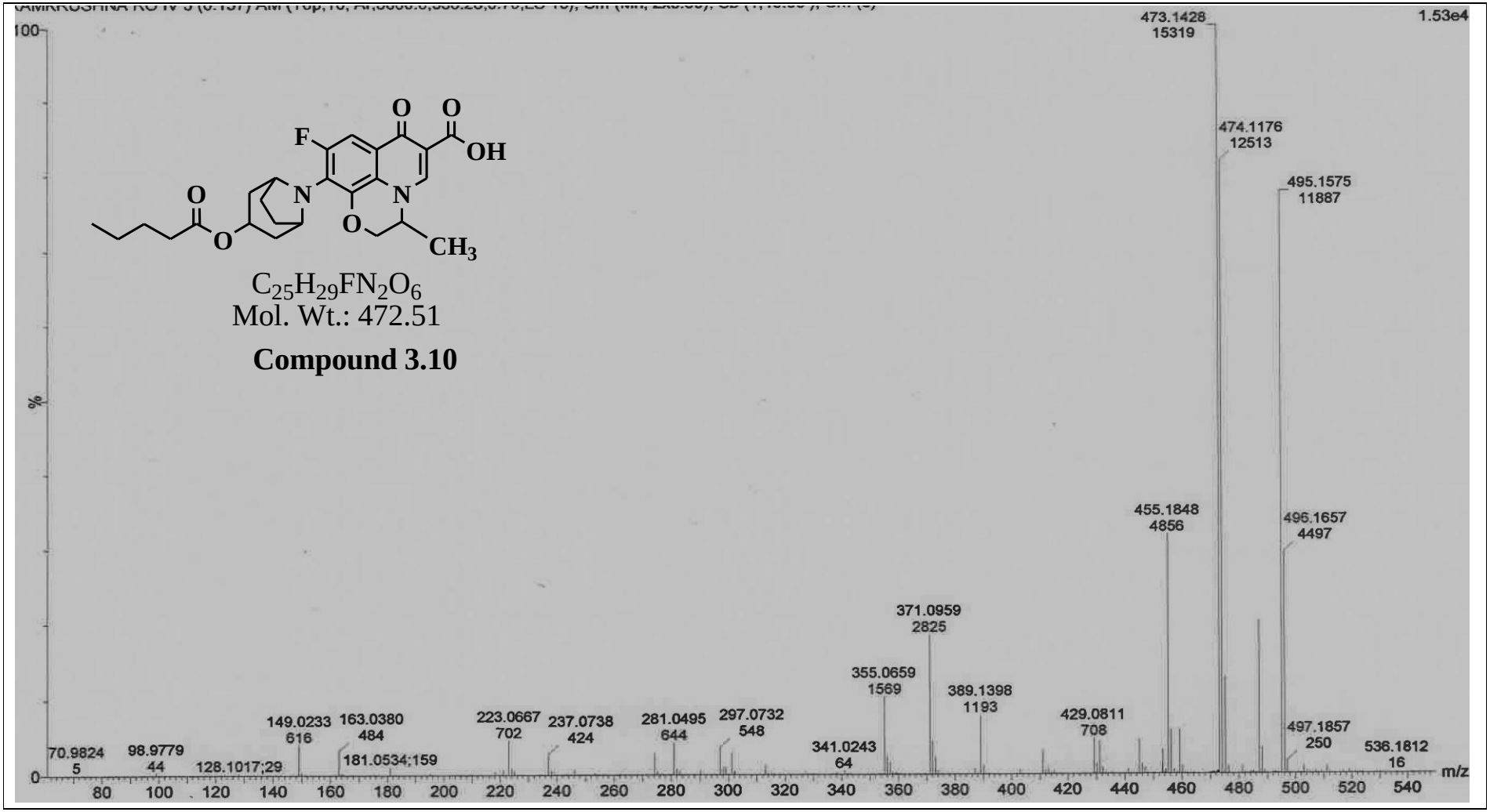


Figure: Mass Spectra of compound 3.10

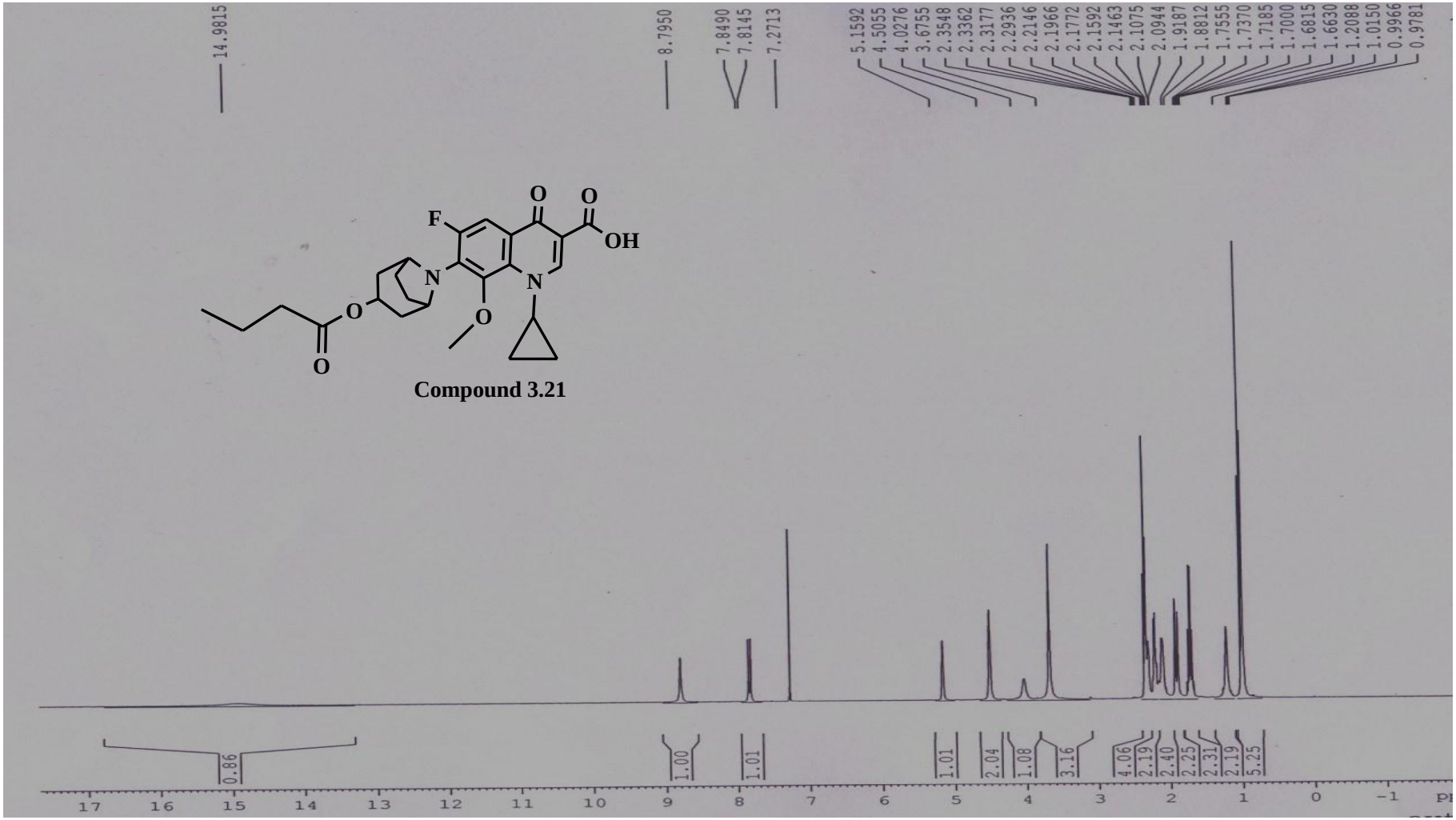


Fig.-: ^{1}H NMR of Compound 3.21

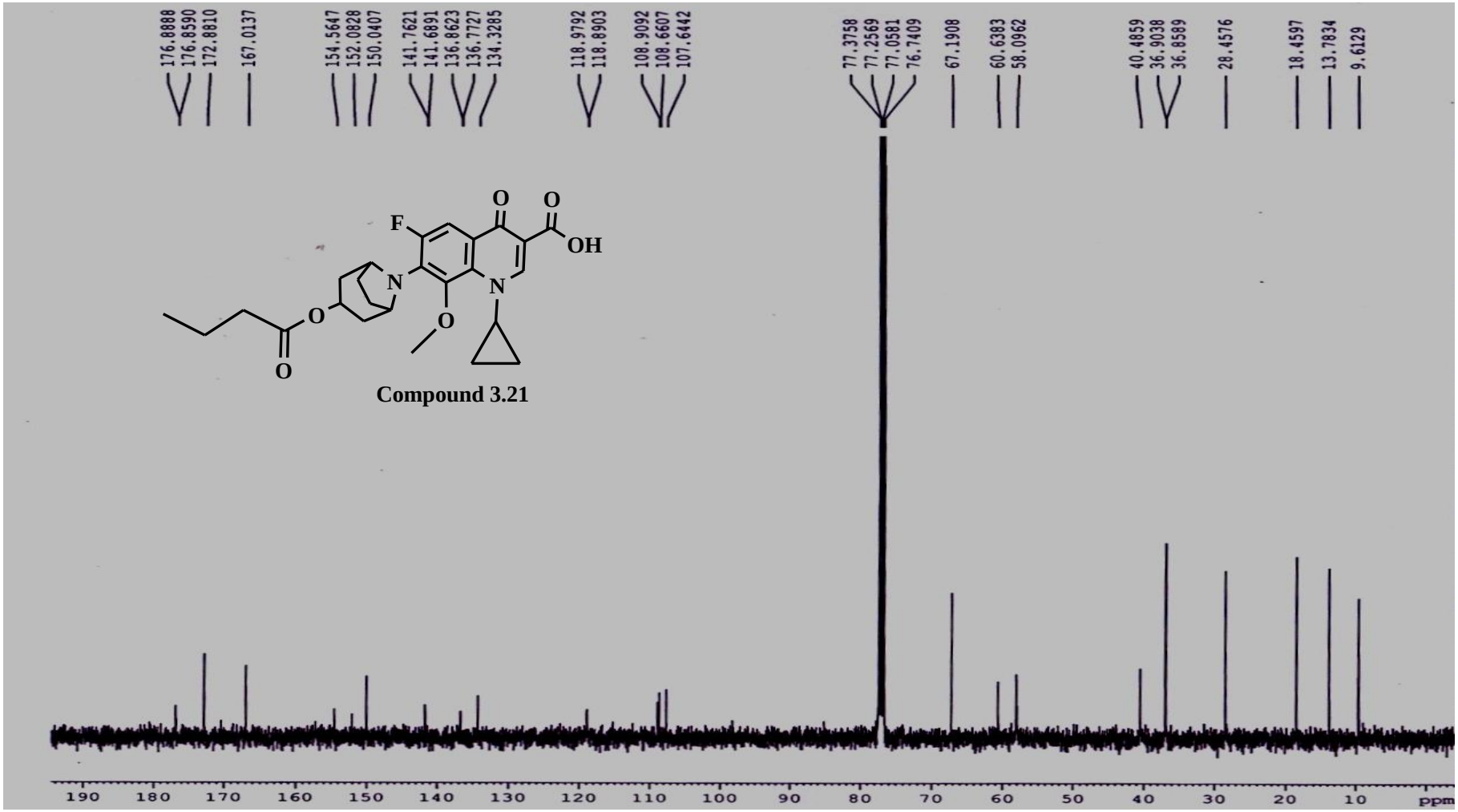


Fig.-: ^{13}C NMR of Compound 3.21

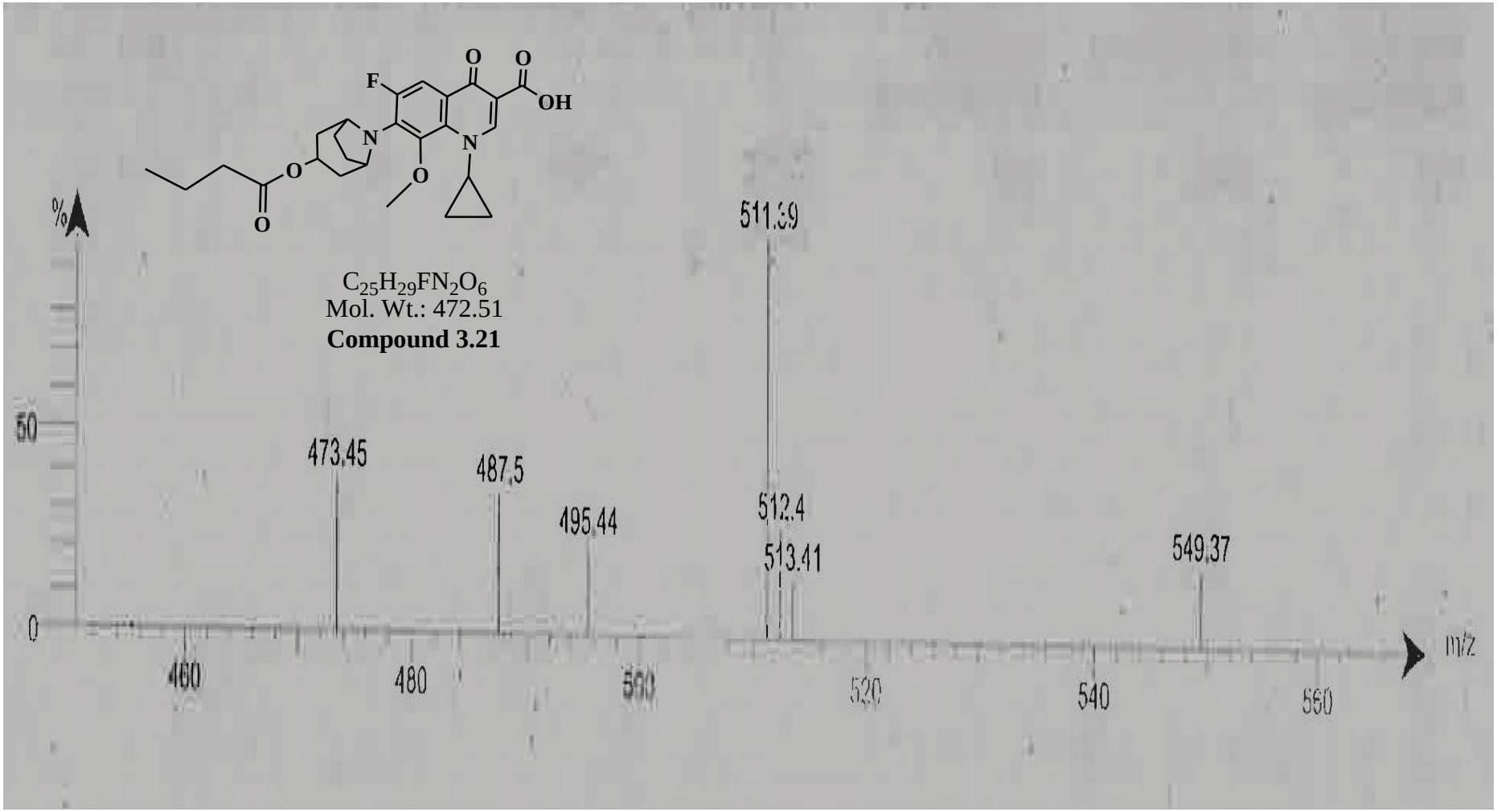


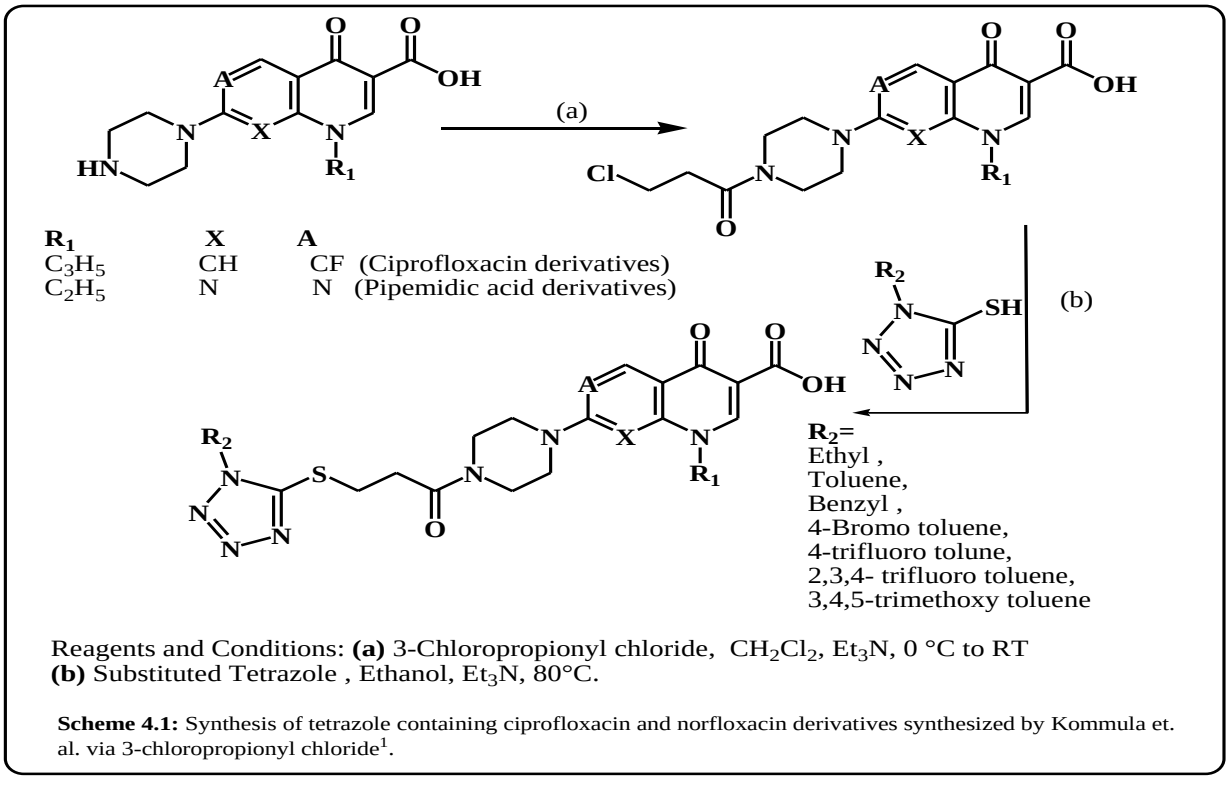
Figure: Mass Spectra of compound 3.21

Heterocyclic Amine Linked Esters of Nortropine Substituted Fluoroquinolones

4.1 Introduction

Compounds with aliphatic and aromatic esters of the nortropine substituted fluoroquinolones, synthesized in the previous section, could not be evaluated beyond initial screening due to the lack of encouraging results for their antibacterial activity.

Literature reports suggested tagging of similar organic motifs at the C-7 substituent for improved antibacterial activity. Kommula et.al synthesized tetrazole linked derivatives of ciprofloxacin and Pipemidic acid **(Scheme-4.1)** and evaluated their antibacterial activity against *Escherichia coli, Bacillus subtilis, Bacillus megaterium, Micrococcus luteus, Salmonella typhi, Pseudomonas aeruginosa*[1].

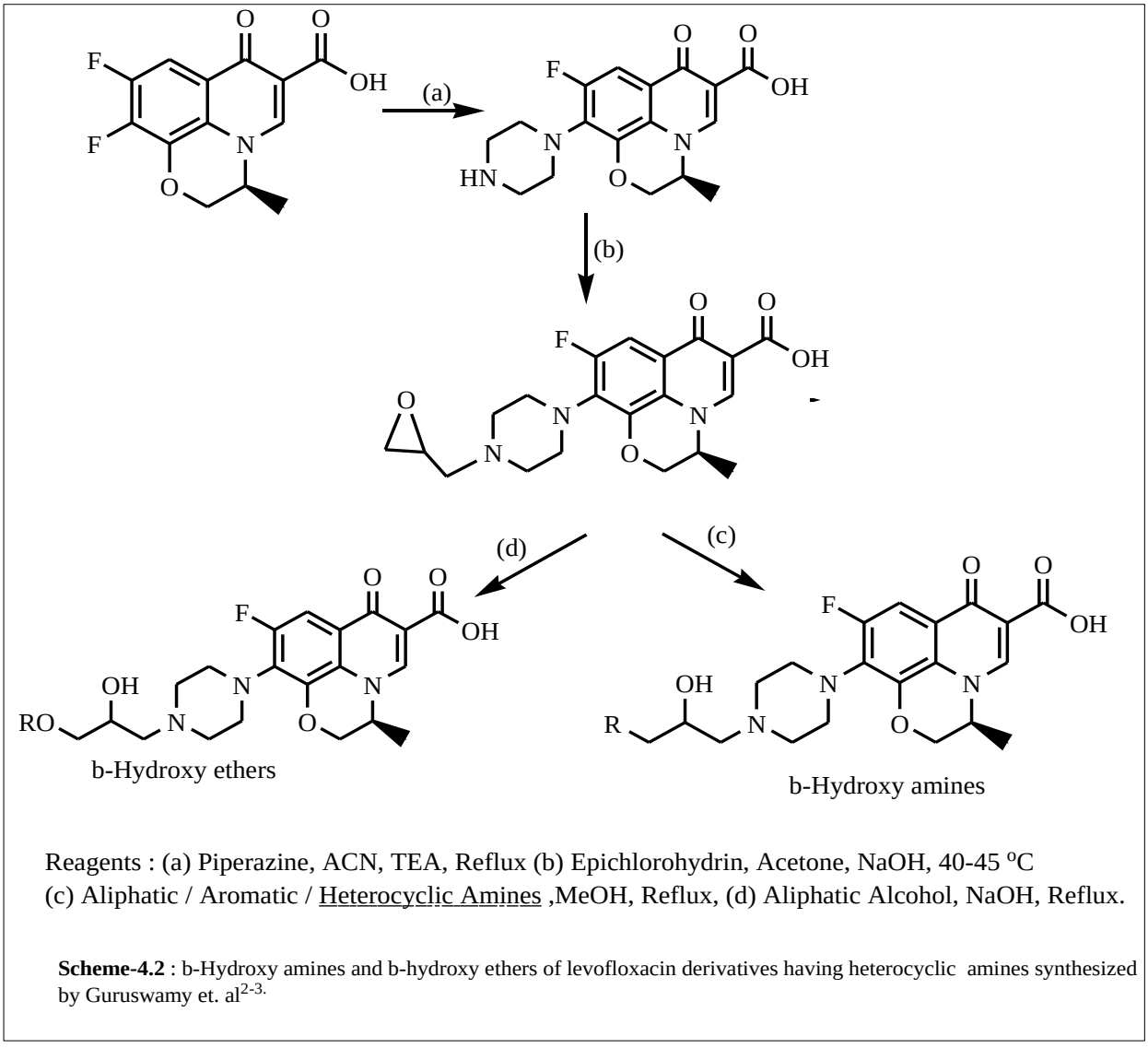


Reagents and Conditions: **(a)** 3-Chloropropionyl chloride, CH_2Cl_2, Et_3N, 0 °C to RT
(b) Substituted Tetrazole , Ethanol, Et_3N, 80°C.

Scheme 4.1: Synthesis of tetrazole containing ciprofloxacin and norfloxacin derivatives synthesized by Kommula et. al. via 3-chloropropionyl chloride[1].

The research group observed that ciprofloxacin derivatives gave better antibacterial activity as compared to pipemidic acid derivatives and one of the tetrazole linked derivatives of ciprofloxacin gave one fold higher MIC value as compared to its parent compound. Similarly,

Guruswamy et. al. synthesized β-hydroxy amines and β-hydroxy ethers on levofloxacin derivatives substituted with piperazine at C-7 position **(Scheme 3.2)**. Among all the synthesized compounds, the antibacterial activity of β-hydroxy amines having heterocyclic rings was impressive as compared to others[2-3].



Reagents : (a) Piperazine, ACN, TEA, Reflux (b) Epichlorohydrin, Acetone, NaOH, 40-45 ^{o}C
(c) Aliphatic / Aromatic / Heterocyclic Amines ,MeOH, Reflux, (d) Aliphatic Alcohol, NaOH, Reflux.

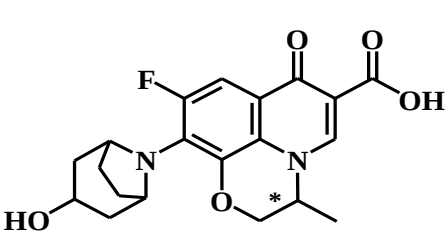
Scheme-4.2 : b-Hydroxy amines and b-hydroxy ethers of levofloxacin derivatives having heterocyclic amines synthesized by Guruswamy et. al[2-3.]

Therefore, it was contemplated to substitute esters containing heterocyclic amines at the hydroxyl group of nortropine.

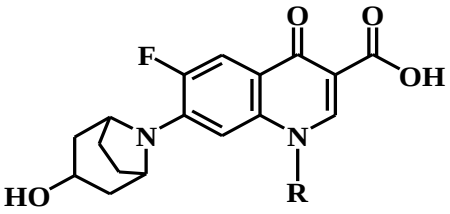
4.2 Synthesis

Four of the synthesized fluoroquinolones described in Chapter -2 were taken for further synthesis of esters containing heterocyclic amines in this section. The selection was based upon the promising biological activity of the compounds. **Figure – 4.1** shows the nortropine derivatives of levofloxacin (**2.13**), ofloxacin (**2.14**), ciprofloxacin (**2.15**) and norfloxacin (**2.17**) that were converted to their corresponding chloro esters using corresponding acid chlorides in

anhydrous chloroform. Chloroacetyl chloride and 2-chloropropionyl chloride were used as short, two carbon linkers containing chloro group at the β- position.



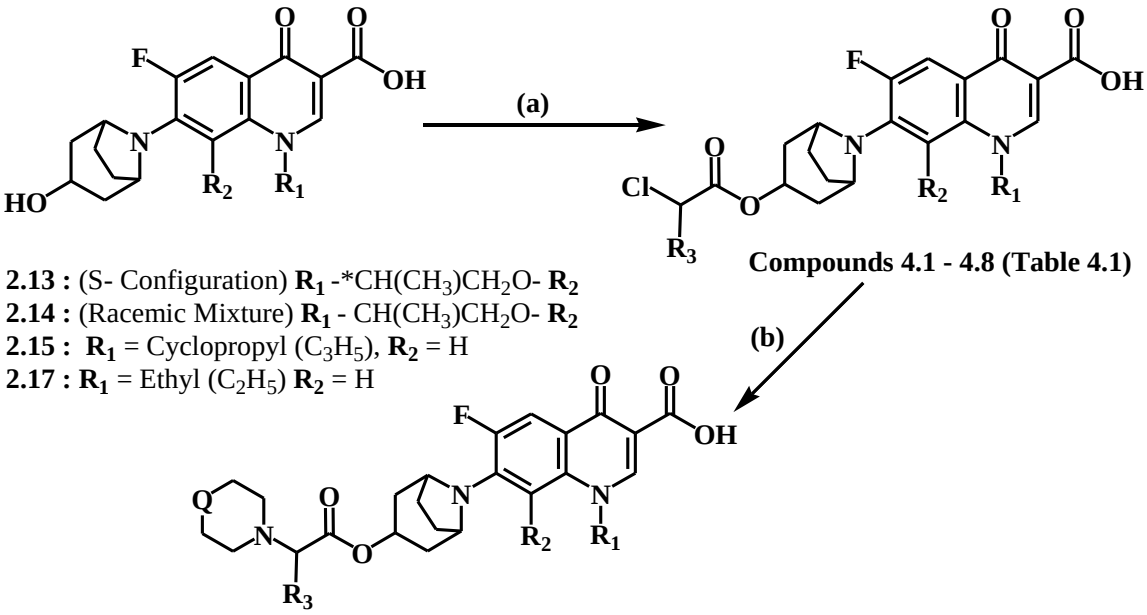
Compound 2.13 * = S - configuration
Compound 2.14 * = R + S (Racemic)



Compound 2.15 R = Cyclopropyl
Compound 2.17 R = Ethyl

Figure - 4.1 : Fuoroquinolones synthesized in chapter -2 taken for further synthesis of esters containing heterocyclic amines in this section.

Three heterocyclic amines namely piperidine, N-methylpiperazine and morpholine were linked to the β-position in the presence of chloroform at room temperature using simple stirring and ethyl acetate used for crystallization. The basic conditions required for this reaction were compensated by taking three fold excess of cyclic amines.



2.13 : (S- Configuration) $\mathbf{R_1}$ -*$CH(CH_3)CH_2O$- $\mathbf{R_2}$
2.14 : (Racemic Mixture) $\mathbf{R_1}$ - $CH(CH_3)CH_2O$- $\mathbf{R_2}$
2.15 : $\mathbf{R_1}$ = Cyclopropyl (C_3H_5), $\mathbf{R_2}$ = H
2.17 : $\mathbf{R_1}$ = Ethyl (C_2H_5) $\mathbf{R_2}$ = H

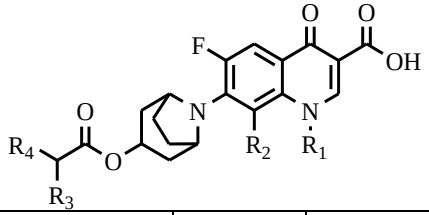
Compounds 4.1 - 4.8 (Table 4.1)

Compounds 4.9 - 4.32 (Table 4.1)
Q = CH_2 (Piperidine), N-CH_3 (N-methylpiperazine) and O (morpholine)

Reagents : **(a)** Chloroacetyl chloride or 2-Chloropropionyl chloride, $CHCl_3$, RT, 24 h **(b)** Cyclic amine (Piperidine / N-Methylpiperazine / Morpholine), $CHCl_3$ / $C_2H_5OCOCH_3$, RT, 24 h

Scheme 4.3: Route of synthesized heterocyclic amine linked ester dericatives of nortropine substituted fluoroquinolones

Table - 4.1: Structure and yield of all the compounds synthesized in Scheme 4.3



Compd. No.	R_1	R_2	R_3	R_4	Yield in %
4.1(Chiral)	-*$CH(CH_3)CH_2O$-	-	H	Cl	82.3
4.2 (Chiral)	-*$CH(CH_3)CH_2O$-	-	CH_3	Cl	86.0
4.3 (Racemic)	-$CH(CH_3)CH_2O$-	-	H	Cl	84.0
4.4 (Racemic)	-$CH(CH_3)CH_2O$-	-	CH_3	Cl	83.0
4.5		H	H	Cl	93.0
4.6		H	CH_3	Cl	93.0
4.7	-CH_2CH_3	H	H	Cl	90.0
4.8	-CH_2CH_3	H	CH_3	Cl	91.0
4.9	-*$CH(CH_3)CH_2O$-	-	H		90.0
4.10	-*$CH(CH_3)CH_2O$-		H		79.0
4.11	-*$CH(CH_3)CH_2O$-		H		85.0
4.12	-*$CH(CH_3)CH_2O$-	-	CH_3		92.0
4.13	-*$CH(CH_3)CH_2O$-	-	CH_3		70.0
4.14	-*$CH(CH_3)CH_2O$-	-	CH_3		93.0
4.15	-$CH(CH_3)CH_2O$-	-	H		88.0
4.16	-$CH(CH_3)CH_2O$-	-	H		72.0
4.17	-$CH(CH_3)CH_2O$-	-	H		86.0
4.18	-$CH(CH_3)CH_2O$-	-	CH_3		90.0
4.19	-$CH(CH_3)CH_2O$-	-	CH_3		72.0
4.20	-$CH(CH_3)CH_2O$-	-	CH_3		93.0
4.21		H	H		93.0
4.22		H	H		80.0
4.23		H	H		70.0
4.24		H	CH_3		93.0
4.25		H	CH_3		80.0
4.26		H	CH_3		70.0
4.27	-CH_2CH_3	H	H		91.0
4.28	-CH_2CH_3	H	H		80.0
4.29	-CH_2CH_3	H	H		80.0
4.30	-CH_2CH_3	H	CH_3		91.0
4.31	-CH_2CH_3	H	CH_3		91.0
4.32	-CH_2CH_3	H	CH_3		80.0

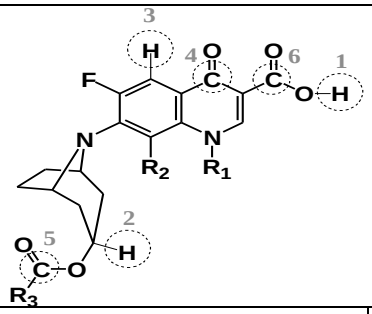
Scheme 4.3 and **Table 4.1** shows the route for the synthesis for compounds **4.1** to **4.32**. All the synthesized compounds were characterised using ^{1}H, ^{13}C and CHN analysis.

4.2.1 Characterization

^{1}H NMR of the compounds synthesized in step-1 **(Scheme-4.3)** was in agreement with the proposed structures. For compounds **4.1**, **4.3**, **4.5** and **4.7**, singlet for the two methylene ($-CH_2-$) protons in the region δ 4.15 to 4.45 ppm was observed. Similarly, two signals due to methine (-CH-) and methyl ($-CH_3$) protons for compounds **4.2**, **4.4**, **4.6** and **4.8** appeared as quartet and doublet in the region of δ 4.34 to 4.76 ppm and δ 1.59-1.74 ppm respectively. Additional carbonyl carbon due to ester, formed by nortopine's hydroxyl and chloroacetyl chloride / chloropropionyl chloride, was observed in ^{13}C NMR around δ 169 ppm along with C-3 and C-4 carbonyl carbons. This was also complimented by absence of hydroxyl group in ^{1}H NMR due to C-7 substituted nortropine.

Substitution of cyclic amines with the chloro group at α position in the 2nd step was confirmed by downfield shifting of methylene singlet (in case of compounds **4.9-4.11**, **4.15-4.17**, **4.21-4.23** and **4.27-4.29**) and methine quartet (in case of compounds **4.12-4.14**, **4.18-4.20, 4.24-4.26** and **4.30-4.32**) from a region of δ 4.34- 4.76 ppm to δ 3.40 -3.25 ppm. This was due to the replacement of electronegative chloro group by nitrogen of the cyclic amine. Three broad signals in the ratio of 2: 2: 1 corresponding to piperidine ring were observed in the ^{1}H NMR spectra of eight compounds (**4.9**, **4.12**, **4.15**, **4.18**, **4.21**, **4.24**, **4.27** and **4.30**). N-methyl piperazine ring present in the respective compounds (**4.10**, **4.13**, **4.16**, **4.19**, **4.22**, **4.25**, **4.28** and **4.31**) was identified by singlet at δ 2.31-2.43 ppm and broad signals at δ 2.47-2.80 ppm corresponding to three N-methyl and eight ring protons in ^{1}H NMR. Later eight protons of the N-methyl piperazine ring appeared at different δ values in the ratio of 1:1 due to change in their respective environments. Compounds with morpholine ring (**4.11**, **4.14**, **4.17**, **4.20**, **4.23**, **4.26**, **4.29** and **4.32**) displayed wider separation for the eight ring protons due to deshielding by electronegative oxygen. Four protons in the vicinity of oxygen appeared at δ 3.69-3.81 ppm while other four in the vicinity of nitrogen appeared at 2.61-2.80 δ ppm in equal ratios. Carbons for the cyclic amines appeared at their respective positions in ^{13}C NMR spectra along with other prominent peaks as shown in **Table 4.2.**

Table 4.2: summary of prominent ^{1}H, ^{13}C NMR peaks of compound 4.1 to 4.32.

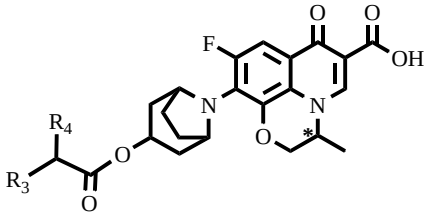


Compd No.	^{1}H NMR in δ ppm			^{13}C NMR in δ ppm		
	1 -COO**H**, **1H, s**	**2** Endo H CH_2C**H**(R)CH_2 1H, t	**3** C-5H -CFC**H**C= 1H, d, J = 14 Hz	**4** C-4 **C**=O J = 4Hz	**5** Ester -O-(**C**=O) R	**6** C-3, **C**OOH
4.1	15.2	5.1	7.5	176.2	166.3	166.1
4.2	15.1	5.2	7.6	176.8	169.1	167.4
4.3	15.3	5.1	7.5	176.2	166.3	166.1
4.4	15.2	5.2	7.6	176.8	169.1	167.4
4.5	15.3	5.0	7.8	176.6	169.3	167.2
4.6	15.3	4.9	7.8	176.6	169.8	167.2
4.7	15.4	5.1	7.9	176.6	169.8	167.4
4.8	15.4	5.1	8.0	176.6	168.9	167.5
4.9	15.6	5.2	7.7	176.9	169.3	167.3
4.10	15.1	5.2	7.7	176.0	169.5	167.3
4.11	15.2	5.2	7.6	176.9	169.1	167.4
4.12	15.1	5.2	7.6	176.9	169.4	167.3
4.13	15.1	5.2	7.6	176.9	171.9	167.3
4.14	15.1	5.2	7.6	176.9	169.8	167.4
4.15	15.1	5.2	7.6	176.9	169.5	167.4
4.16	15.1	5.2	7.7	176.9	169.4	167.3
4.17	15.1	5.2	7.6	176.9	168.9	167.3
4.18	15.1	5.2	7.7	176.9	172.3	167.3
4.19	15.1	5.2	7.6	176.9	172.2	167.3
4.20	15.1	5.2	7.7	176.9	169.3	167.3
4.21	14.8	5.1	7.9	176.6	169.9	167.2
4.22	15.1	5.1	7.9	176.7	169.5	167.2
4.23	15.1	5.1	7.9	176.7	169.3	167.2
4.24	15.1	5.1	7.9	176.7	172.4	167.2
4.25	15.1	5.1	7.9	176.7	171.9	167.2
4.26	15.1	5.1	7.9	176.9	171.9	167.4
4.27	15.2	5.1	7.9	176.6	167.9	167.4
4.28	15.1	5.1	7.9	176.7	169.5	167.5
4.29	15.1	5.1	7.9	176.6	169.3	167.4
4.30	15.2	5.1	7.9	176.6	172.3	167.4
4.31	15.1	5.1	7.9	176.7	171.9	167.5
4.32	15.3	5.1	7.9	176.6	171.9	167.4

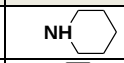
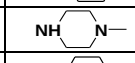
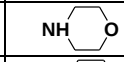
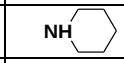
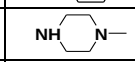
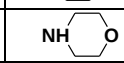
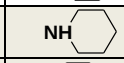
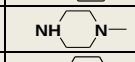
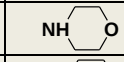
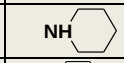
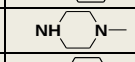
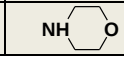
4.3 Antibacterial Activity

All compounds synthesized in this section were screened for their antibacterial activity against a set of five bacterial strains namely *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (BAA 1705), *A. baumannii* (BAA 1605) and *S. aureus* (ATCC 29213).

Table 4.3 - Results of *in vitro* screening of levofloxacin and ofloxacin derivative against five set of bacterial strains.



MIC (µg/ml)

Comd. No.	R_3	R_4	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 29212)	*K. pneumoniae BBA 1705*	*A. baumannii BBA 1605*	*P. aeruginosa* (ATCC 27853)
4.1*	H	Cl	2	0.125	>64	>64	>64
4.2*	$-CH_3$	Cl	4	0.5	>64	>64	>64
4.3	H	Cl	16	0.5	>64	>64	>64
4.4	$-CH_3$	Cl	32	1	>64	>64	>64
4.9*	H		8	1	>64	>64	>64
4.10*	H		16	1	>64	>64	>64
4.11*	H		32	1	>64	>64	>64
4.12*	$-CH_3$		16	1	>64	>64	>64
4.13*	$-CH_3$		8	2	>64	>64	>64
4.14*	$-CH_3$		32	2	>64	>64	>64
4.15	H		8	2	>64	>64	>64
4.16	H		16	2	>64	>64	>64
4.17	H		16	2	>64	>64	>64
4.18	$-CH_3$		64	2	>64	>64	>64
4.19	$-CH_3$		4	2	>64	>64	>64
4.20	$-CH_3$		32	2	>64	>64	>64
2.13			1	0.125	>64	>64	32
2.14			2	0.25	>64	>64	64
Levofloxacin			0.015	0.25	64	8	1
Moxifloxacin			0.03	0.06	64	8	2

Note: All compounds with * represent chiral levofloxacin derivatives while without star represent racemic ofloxacin derivatives.

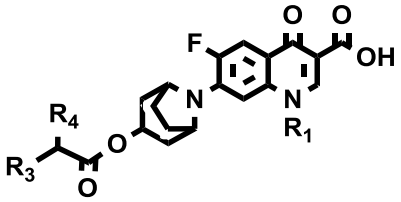
E. coli (ATCC 25922), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (BAA 1705) and *A. baumannii* (BAA 1605) among these were gram negative while *S. aureus* (ATCC 29213) is gram positive. Based on the initial results of antibacterial activity, the discussion in the following section has been divided into two parts.

Antibacterial activity of Levofloxacin and Ofloxacin derivatives: **Table 4.3** shows the MIC values in µg/ml for all the bacterial strains taken for this study. Levofloxacin, Moxifloxacin and the parent compounds of the series, compounds **2.13** and **2.14,** were taken as standards. It can be observed that, of the five selected strains considered, only *E. coli* and *S. aureus* gave encouraging results having MIC values ranging between 2-32 µg/ml (*E. coli)* and an impressive 0.125-2 µg/ml (*S. aureus*). Rest of the three strains namely, *P. aeruginosa*, K. *pneumonia* and *A. baumannii,* gave MIC values higher than 64 µg/ml and therefore were not considered further.

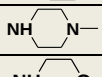
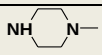
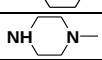
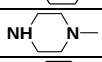
A comparison of MIC values for *E. coli* between standard drugs levofloxacin (0.015 µg/ml), moxifloxacin (0.03 µg/ml) and the synthesized compounds (2-32 µg/ml) indicated that standards were in excess of hundred (levofloxacin) to fifty (moxifloxacin) times more potent than the most active of the synthesized compounds (**Table 4.3**). However, MIC values of the parent compounds in the series were comparable to that of synthesized compounds. Thus it was concluded not to take up further either of the synthesized or parent compounds for study against *E. coli*.

In case of *S. aureus*, one of the standard drugs, levofloxacin (MIC = 0.25 µg/ml), was only eight times more potent than the least potent compounds of the series (MIC = 2 µg/ml). In fact, compound **4.1, 4.2** and **4.3** were having MIC values comparable to that of standard levofloxacin. From the medicinal chemistry point of view, MIC values with a multiplication factor of ±2 are considered comparable. It can be distinguished that compounds having heterocyclic amine groups at R_4 position were less impressive than the ones having chloro group at the same position. From absolute MIC values (**Table 4.3**), it can also be seen that chloroacetyl derivatives, compounds **4.1** and **4.3**, were more potent than chloropropionyl derivatives **4.2** and **4.4**. Similarly, chiral compounds **4.1** and **4.2** were more impressive than racemic compounds **4.3** and **4.4**. Thus, it can be conclude that compound **4.1** was the most impressive of the series having MIC value comparable to the other standard drug moxifloxacin (MIC = 0.6 µg/ml). Parent compounds **2.13** and **2.15**, synthesized in chapter-2 gave reproducible results that were comparable to compounds synthesized in this series. Never the less, it was decided to evaluate further all compounds of this series against fluoroqunolone resistant strains of *S. aureus*.

Table 4.4 - Results of *in vitro* screening of ciprofloxacin and norfloxacin derivative against five set of bacterial strains.



MIC (µg/ml)

Comd. No.	R_1	R_3	R_4	*E. coli* ATCC 25922	*S. aureus* ATCC 29212	*K. pneumoniae BBA 1705*	*A. baumannii BBA 1605*	*P. aeruginosa* (ATCC 27853)
4.5	C_3H_5	H	Cl	2	1	>64	>64	32
4.6	C_3H_5	CH_3	Cl	>64	2	>64	>64	>64
4.7	C_2H_5	H	Cl	16	2	>64	>64	>64
4.8	C_2H_5	CH_3	Cl	16	4	>64	>64	>64
4.21	C_3H_5	H		8	4	>64	>64	>64
4.22	C_3H_5	H		16	8	>64	>64	>64
4.23	C_3H_5	H		>64	8	>64	>64	>64
4.24	C_3H_5	CH_3		>64	8	>64	>64	>64
4.25	C_3H_5	CH_3		16	16	>64	>64	>64
4.26	C_3H_5	CH_3		32	8	.64	.64	.64
4.27	C_2H_5	H		32	16	>64	>64	>64
4.28	C_2H_5	H		64	64	>64	>64	>64
4.29	C_2H_5	H		>64	16	>64	>64	>64
4.30	C_2H_5	CH_3		64	8	>64	>64	>64
4.31	C_2H_5	CH_3		64	32	>64	>64	>64
4.32	C_2H_5	CH_3		>64	16	>64	>64	>64
2.15				0.25	0.5	>64	>64	32
2.17				4	1	>64	>64	>64
Levofloxacin				0.015	0.25	64	8	1
Moxifloxacin				0.03	0.06	64	8	2

Antibacterial activity of Ciprofloxacin and Norfloxacin derivatives: **Table 4.4** shows MIC values for ciprofloxacin and norfloxacin derivatives taking levofloxacin and moxifloxacin as control drugs along with their parent compounds **2.15** and **2.17** synthesized in chapter-2. The results of antibacterial activity against five strains taken for this study were similar to levofloxacin derivatives as discussed above. All compounds of the series were fairly impressive

against *S. aureus* only. Levofloxacin (MIC = 0.25 µg/ml) was four to two fifty times more potent than most of the compounds that gave MIC in the range of 1-64 µg/ml.

Table 4.5 : Results of antibacterial activity of shortlisted compounds against fluoroquinolone resistant strains of *S. aureus.*

S. No	Compd. No.	MIC (µg/ml)		
		FQ Resistant Strain		FQ sensitive strain
		S. aureus **NARSA 10198**	***S. aureus*** **NARSA 10193**	***S. aureus*** **ATCC 29213**
1.	**2.13**	2	4	≤0.5
2.	**2.14**	4	8	≤0.5
3.	**2.15**	64	64	≤0.5
4.	**4.1***	8	4	≤0.5
5.	**4.2 ***	8	8	≤0.5
6.	**4.3**	8	8	≤0.5
7.	**4.4**	16	16	1
8.	**4.5**	16	16	≤0.5
9.	**4.6**	8	>64	1
10.	**4.7**	16	16	2
11.	**4.8**	8	8	4
12.	**4.9***	32	32	1
13.	**4.10***	64	64	1
14.	**4.11***	32	32	0.5
15.	**4.12***	32	32	1
16.	**4.13***	64	64	1
17.	**4.14***	64	64	1
18.	**4.15**	64	64	2
19.	**4.16**	>64	>64	2
20.	**4.17**	64	>64	2
21.	**4.18**	32	32	2
22.	**4.19**	>64	>64	2
23.	**4.20**	>64	>64	4
24.	**4.21**	16	16	1
25.	**4.22**	64	64	4
26.	Vancomycin	2	2	1
27.	Linezolid	2	2	2
28.	Levofloxacin	32	32	0.5

It was again observed that compounds having heterocyclic amine groups at R_4 position were less impressive, having MIC in the range of 4-64 µg/ml, than the ones having chloro group at the same position. MIC value for these compounds ranged between 1 - 4 µg/ml having four to sixteen times less potency as compared to levofloxacin (**Table 4.3**). However, as compared to moxifloxacin standards (MIC = 0.06 µg/ml) potency of chloro compounds was sixteen to sixty six times reduced. Nevertheless, all chloro compounds **4.5-4.8** were shortlisted for advance study against fluoroquinolone resistant strains of *S. aureus*. In fact parent compounds of the series **2.13**

and 2.14, synthesized in chapter -2 gave reproducible results of MIC = 0.5 and 1 μg/ml respectively and were also considered for the resistant strains.

Antibacterial Activity against Fluoroquinolone Resistant strains of *S. aureus:* All compounds that gave MIC value up to 4 μg/ml against *S. aureus* were evaluated further against two fluoroquinolone MRSA strains (NARSA 10193 and NARSA 10198) using linezolid, vancomycin and levofloxacin as standards. *S. aureus* strain (FQ-sensitive, ATCC 29213) used for initial screening was also taken as control.

Table 4.5 shows that against FQ-resistant strains, MIC value of six compounds namely **2.13**, **2.14**, **4.1 - 4.3** and **4.8** ranged between 2 - 8 μg/ml making them two to four times less potent than standards linezolid and vancomycin (MIC = 2 μg/ml) but four to sixteen times more effective than standard levofloxacin (MIC = 32 μg/ml). Five among these compounds were chloro esters of chiral levofloxacin (**4.1** and **4.2**) and ofloxacin derivatives (**4.3**) and their parent compounds (**2.13** and **2.14**). Sixth compound (**4.8**) was chloropropionyl esters of norfloxacin. Other chloro esters that gave MIC comparable with standard levofloxacin (32 μg/ml), were chloropropionyl esters of ofloxacin **(4.4)**, chloroacetyl ester of ciprofloxacin **(4.5)** and norfloxacin **(4.7)** having MIC = 16 μg/ml. Only exception among chloro esters was chloropropionyl esters of ciprofloxacin (**4.6)** that gave MIC greater than 64 μg/ml.

All six heterocyclic amine esters of levofloxacin (**4.9 - 4.14**), two of the four ofloxacin (**4.15 and 4.18**) and one of the two ciprofloxacin (**4.22**) derivatives considered for MIC against FQ resistant *S. aureus* gave values (32- 64 μg/ml) on the higher side as compared to that of their chloro esters precursors. Only exception in this series was compound **4.21**, that gave MIC of 16 μg/ml comparable to chloro esters.

4.4 Conclusion:

Over all, it can be concluded that parent compounds of levofloxacin and ofloxacin derivatives (**2.13** and **2.14**) described in chapter-2, their corresponding chloro esters (**4.1-4.5** and **4.7- 4.8**) and one heterocyclic amine ester of ciprofloxacin (**4.21**), described in this section, gave impressive results against fluoroquinolone resistant and sensitive strains of *S. aureus.* Further studies can be carried out on these molecules as they have the potential to be developed further for treatment against fluoroquinolone-resistant strains of *S. aureus*.

4.5 Material and Methods:

4.5.1 Experimental: All the solvents used in this study were brought from Sigma Aldrich and SD Fine Chemicals, India. ^{1}H and ^{13}C NMR spectrum ware recorded on Bruker 400 MHz and Jeol 400 MHz spectrometers. Chemical shifts are expressed in δ (ppm) with tetramethylsilane as internal reference standard. Merck silica gel 60 F254 plate were used for analytical TLC and silica gel 60-120 mesh size were used for column chromatography.

General procedure for the synthesis of compound 4.1 to 4.8: Compound **2.13**, **2.14**, **2.15** and **2.16** (1.0 mol eq) were taken in chloroform (20 mL) and acid chlorides (chloroacetyl chloride/ 2-chloropropionyl chloride) (3.0 mol eq) added. The reaction mixture was stirred overnight. After completion of the reaction, (TLC monitoring), chloroform along with excess of acid chloride was distilled off under vacuum. To the remaining solid mass ethyl acetate (2 X 10 mL) was added to dissolve impurities, traces of acid chlorides and the solid product filtered. Re-crystallization from methanol gave 82-93 % yield. Further purification, if required, was done by column chromatography using ethyl acetate and hexane solvent system.

General procedure for synthesis of compounds 4.9 – 4.32: (Method A): To the chloro compound (**4.1** to **4.8**) (1.0 mol eq) was added corresponding cyclic amines (3.0 mol eq) in the chloroform (20) mL and the stirring continued for 24 hrs. After completion of the reaction, (TLC monitoring), solvent and excess of amines were distilled off under vacuum. To the sticky mass was added ethyl acetate (20.0 mL) to get solid mass that was re-crystallized from methanol (10.0 mL) to get 70-93% yield of compound **4.9** to **4.32**.

General procedure for synthesis of compounds 4.9 – 4.32: (Method B): To the chloro compound (**4.1** to **4.8**) (1.0 mol eq) was added corresponding cyclic amines (3.0 mol eq) in the chloroform (20) mL and the stirring continued for 24 hrs. After completion of the reaction, (TLC monitoring), water (20.0 mL) was added and the aqueous and organic layers separated out. The chloroform layer was washed with water (2 X 20.0 mL) to remove excess of amines. Chloroform after drying with calcium chloride was evaporated and to the remaining sticky mass was added ethyl acetate to get solid material that was filtered and re-crystallized from methanol to get 70-79 % yield of compound **4.9** to **4.32**

4.5.2 Bacterial strains:

The synthesized compounds were screened against a bacterial panel consisting of ESKAPE pathogens namely *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (BAA-1705), *Acinetobacter baumannii* (BAA-1605), *Pseudomonas aeruginosa* (ATCC 27853) and

Staphylococcus aureus (ATCC 29213). The strains were obtained from the ATCC, USA and were routinely cultivated in MH (Difco) Agar medium. The panel was further expanded to include drug-resistant clinical *S. aureus* strains including Vancomycin and other glycopeptide resistant strains. These strains were procured from BEI/NARSA/ATCC, USA and routinely cultivated in MHB (Difco) medium. Before starting the experiment, single colony was picked from MHA plate and was inoculated in liquid medium and incubated at 37 °C for overnight to get the starter culture.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out by broth micro dilution assay following the standard CLSI guidelines. Test compounds were prepared in DMSO as stock solutions (10 mg/mL). Bacterial cultures were inoculated in Muller-Hinton broth. Optical density (OD) of the cultures was measured at the wavelength of 600 nm followed by dilution to achieve ~10^6 CFU/mL. The concentrations of test compounds used in the study ranged from 64-0.5 mg/L in serially diluted fashion in DMSO from stock solutions and 2.5 µL of each concentration was added to each well of a 96-well microliter plate (Polypropylene, Corning Inc., Corning, USA). Later, 97.5 µL of bacterial suspension in MH medium was added to each well containing the test compound. Two controls were also included i.e., cells alone and media alone (without compound + cells) and plates were incubated at 37˚C for 16-18 h. MIC values were observed by the absence or presence of visible growth. For each compound, MIC determinations were carried independently 3 times using duplicate samples.

Experimental data:

(S)-10-(3-Chloroacetoxy-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4.1) : Light yellow colour powder, Yield- 82.0% (0.49 g), Melting point 256.7°C decomposes., $[\alpha]^{D}_{25}$ - 94˚ (c 1.00, $CHCl_3$) ,^{1}H NMR (400 MHz $CDCl_3$ and $DMSOd_6$) δ 15.22 (s, 1H, -COO**H**), 8.89 (s, 1H, -NC**H**CCOOH), 7.59-7.56(d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.17 -5.15 (t, 1H, J = 4.8 Hz, -CH_2-C**H**(OR)- CH_2-), 4.90-4.88 (m, 1H, -NC**H** (CH_3) CH_2-), 4.43-4.43 (m, 2H, -NCH (CH_3)C**H_2**-O), 4.33-4.27 (2bs, 2H, bridged **H**),4.15 (s, 2H, ClC**H_2**COO-), 2.40-2.35 (4ts, 2H, -C**H**(H)-C(OR)-C**H**(H)-),2.19-2.17 (q, 2H,-C**H(**H**)**-C**H(**H**)**-), 2.04-1.99 (dt, 2H, -CH(**H**)-C(OR)-CH(**H**)-), 1.89-1.86 (2 bs,2H,-CH**(H)**-CH**(H)**-),1.53 -1.51 (d, 3H, J= 6.7 Hz -NCH(C**H_3**)CH_2-); ^{13}C NMR (100 MHz, $CDCl_3$ and DMSO d_6) δ (**176.25, 176.21**, J= 4 .0 Hz), 166.30, 166.13, (**154.24, 151.80**, J= 244 Hz),

145.68, (**136.26, 136.18**, J = 8 Hz),(**132.37, 132.23**, J= 14 Hz) , 124.99, (**118.23, 118.13**, J= 10 Hz) , 106.41, (**103.88, 103.77**, J = 11 Hz) , 69.39 , 67.86, 57.13, 57.09, 54.69, 41.12, 36.67, 36.56**,** 28.07, 28.05, 17.98. CHN Anal. Calcd. for $C_{22}H_{22}ClFN_2O_6$: C,56.84; H, 4.77; N, 6.03. Found C, 56.68; H, 4.52; N, 5.98.

(S)-10-(3-(2-Chloropropionyloxy)-8-aza-bicyclo [3.2.1]octan-8-yl)-9-fluoro-3,7-Dihydro -3-methyl-7-oxo-2H- [1,4] oxazino[2, 3,4-ij] quinoline-6-carboxylic acid (4.2) : Light yellow colour powder , Yield: 86 % (0.53 g) , Melting point 249.6°C decomposes, $[\alpha]^{D}_{25}$ - 103˚ (c 1.00, $CHCl_3$) ^{1}H NMR (400 MHz $CDCl_3$) δ 15.12 (s, 1H, -COO**H**), 8.61 (s, 1H, -NC**H**CCOOH), 7.69-7.65 (d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.22 -5.21 (t, 1H, J= 4.8 Hz, -CH2-C**H**(OR)-CH_2-), 4.56-4.52 (m, 1H, -NC**H**(CH_3)CH_2-), 4.45-4.34 (m, 6H, -NCH(CH_3)C**H_2**-O), bridged **H** and Cl(C**H)**CH_3COO-),2.44-2.33 (4ts, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.23-2.17 (q, 2H, -C**H(**H)-C**H(**H)-), 2.12-2.05 (dt, 2H, -CH(**H**)-C(OR)-CH(**H**)-),1.93-1.89 (2 bs,2H, -CH **(H)**-CH**(H)**-),1.74-1.71(d, 3H, J=5 Hz, Cl(CH)C**H_3**COO-), 1.63 -1.61 (d, 3H, J= 6.7 Hz, -NCH(C**H_3**)CH_2-); ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.87, 176.84**, J= 3 Hz), 169.13, 167.45, (**154.80, 152.35**, J=245 Hz), 144.69, (**135.96, 135.89**, J =7 Hz), (**131.13, 131.00** , J= 13 Hz) , 125.02, (**117.47, 117.36**, J= 10 Hz), 107.39, (**105.69, 105.44**, J= 25 Hz) , 69.85, 68.08, 58.20, 58.15, 57.75, 57.70**,** 55.55, 52.85, 37.17, 37.10, 28.53, 28.49, 21.31, 18.39. CHN Anal. Calcd. for $C_{23}H_{24}ClFN_2O_6$: C, 57.68; H, 5.05; N, 5.85. Found C, 57.58; H, 4.98; N, 5.65.

10-(3-Chloroacetoxy-8-aza-bicyclo[3.2.1]octan-8-yl)-9-Fluoro-3,7-dihydro-3-methyl-7-oxo -2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4.3) : Light yellow colour powder**,** Yield**:** 84%, **(**0.50 g), Melting point 253°C decomposes, ^{1}H NMR (400 MHz DMSO d_6) δ 15.37 (s, 1H, -COO**H**), 8.90 (s, 1H, -NC**H**CCOOH), 7.59-7.56 (d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.17 -5.15 (t, 1H, J=4.8 Hz,-CH_2-C**H**(OR)-CH2-), 4.90-4.88 (m, 1H, -NC**H**(CH_3)CH_2-), 4.53-4.43 (m, 2H, -NCH(CH_3)C**H_2**-O), 4.33-4.29 (2bs, 2H, bridged **H**),4.27 (s, 2H, ClC**H_2**COO-), 2.40-2.17 (4ts, 2H, -C**H**(H)-C(OR)-C**H**(H)-),2.19-2.17 (q, 2H,-C**H(**H)-C**H(**H)-), 2.03-2.00 (dt, 2H, -CH(**H**)-C(OR)-CH(**H**)-), 1.89-1.86 (2 bs,2H,-CH**(H)**-CH**(H)**-),1.53 -1.51 (d, 3H, J= 6.7 Hz -NCH(C**H_3**)CH_2-); ^{13}C NMR (100 MHz, $CDCl_3$ and DMSO d6) δ (**176.25,176.21**, J= 4 Hz),166.30, 166.13, (**154.24, 151.80**, J= 244 Hz), 145.68 , (**136.26, 136.18**, J = 8 Hz), (**132.37, 132.23**, J= 14 Hz), 124.99,(**118.23, 118.13,** J= 10 Hz), 106.41, (**103.88, 103.77**, J= 11 Hz), 69.39, 67.86, 57.13, 57.09, 54.69, 41.12, 36.67, 36.56, 28.07,28.05,17.98. CHN Anal. Calcd. for $C_{22}H_{22}ClFN_2O_6$: C, 56.84; H,4.77; N, 6.03. Found C, 56.68; H, 4.52; N, 5.98.

10-(3-(2-Chloropropionyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H- [1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.4): Light yellow colour powder, Yield**:** 83% , **(**0.51 g) **,** Melting point 232°C decomposes., ^{1}H NMR (400 MHz $CDCl_3$) 15.25 (s, 1H, -COO**H**), 8.61 (s, 1H, -NC**H**CCOOH), 7.67-7.64(d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.22 -5.21 (t, 1H, J=4.8 Hz, -CH_2-C**H**(OR)-CH_2-), 4.57-4.55 (m, 1H, -NC**H**(CH_3)CH_2-), 4.45-4.40 (m, 4H, -NCH(CH_3)C**H$_2$**-O), and Cl(C**H)**CH_3COO-),4.37-4.35 (bs, 2H, bridged **H**), 2.44-2.33 (4ts, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.25-2.04 (m, 4H, -C**H(**H)-C**H(**H**)**- and -CH(**H**)-C(OR)-CH(**H**)-), 1.93-1.89 (2 bs,2H,- CH**(H)**-CH**(H)**-),1.74-1.72(d, 3H, J= 7 Hz, Cl(CH)C**H$_3$**COO-), 1.63-1.61(d, 3H, J= 6.7 Hz -NCH(C**H**$_3$)CH_2-); ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.91,176.88**, J= 3 Hz**),** 169.14, 167.41, (**154.79,152.34**, J= 245 Hz), 144.69, (**135.98, 135.90,** J= 8 Hz),(**131.13, 131.00**, J= 13 Hz) , 125.01, (**117.45, 117.36,** J= 9 Hz), 107.37, (**105.62, 105.37,** J= 25 Hz), 69.87, 68.08, 58.17, 58.12, 57.72, 57.66, 55.53, 52.86, 37.19, 37.16, 28.53,28.50, 21.31, 18.38. CHN Anal. Calcd. for $C_{23}H_{24}ClFN_2O_6$: C, 57.68; H, 5.05; N, 5.85. Found C,57.58; H, 4.98; N, 5.65.

7-(3-(2-Chloroacetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.5): Light yellow colour powder, Yield: 93%, (0.3 g), Melting point 299.8°C , ^{1}H NMR (400 MHz DMSO d_6) δ 15.31 (s, 1H, -COO**H**), 8.64 (s, 1H, -NC**H**CCOOH), 7.89-7.85(d, 1H, J= 14.4 Hz, C(F)C**H**C), 7.40-7.39 (d, 1H, J= 7.48 Hz, -C(N)C**H**C), 5.06-4.94 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.60,(bs, 2H, -N-bridged **H**) , 4.32, (s, 2H, -OCOC**H$_2$**Cl), 3.77 (m, 1H, -N-Cyclopropyl C**H**) , 2.29-2.26 (m, 4H, -CH(**H**)-C(OCOR)-CH(**H**)- and -C**H**(H)-C(OCOR)-C**H**(H)-), 2.12-2.10 (q, 2H, CH**(H)**-CH**(H)**-), 1.87, 1.84 (d, 2H, C**H(**H)-C**H(H)**-), 1.38-1.35 (q, 2H, -NCHCH(**H**)CH(**H**)-), 1.24-1.18 (q, 2H, -NCHC**H**(H)C**H**(H), ^{13}C NMR (100 MHz, $CDCl_3$)*δ* (**176.74, 176.70,** J=4.0 Hz), 169.37, 167.20, **(152.69, 150.21,** J= 245 Hz**)**, 147.14, **(140.55, 140.44,** J= 11.0 Hz), 139.70, (**117.05, 116.98,** J= 7.0 Hz), **(112.92, 112.68** J= 24.1 Hz**),**107.66, **(102.39, 102.34,** J =5.0 Hz**),** 67.87, 55.50, 55.42, 39.98, 35.09, 33.95, 27.58, 8.18. CHN Anal. Calcd. For $C_{22}H_{22}FN_2O_5$: C, 58.87; H, 4.94; N, 6.24 Found C, 58.58, ; H, 4.59; N, 6.06.

7-(3-(2-Chloropropionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.6): Light yellow colour powder, Yield:93% (0.3 g), Melting point 297°C , ^{1}H NMR (400 MHz DMSO d_6) δ 15.31 (s, 1H, -COO**H**), 8.58 (s, 1H, -NC**H**CCOOH), 7.86-7.83(d, 1H, J= 12.0 Hz, - C(F)C**H**C), 7.41-7.39 (d, 1H, J= 8.0 Hz, -C(N)C**H**C), 4.96 (Bs,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.77-4.72 (q, 1H, Cl(C**H)**CH_3COO-),

4.55,(bs, 2H, -N-bridged **H**) , 3.77 (m, 1H, -N-Cyclopropyl C**H**) , 2.28-2.17 (m, 4H, -CH(**H**)-C(OCOR)-CH(**H**)- and -C**H**(H)-C(OCOR)-C**H**(H)-), 2.05-2.03 (q, 2H, CH**(H)**-CH**(H)**-), 1.77, 1.73 (d, 2H, C**H(**H)-C**H(**H)-), 1.61-1.59 (d, 3H, Cl(CH**)**C**H$_3$**COO-), 1.27-1.13 (m , 4H, -NCHCH(**H**)CH(**H**)-), and -NCHC**H**(H)C**H**(H), (100 MHz, $CDCl_3$) δ **(176.79, 176.76,** J=4.0 Hz), 169.8, 167.24, **(152.69, 150.21,** J =248 Hz**,** 147.19, **(140.54, 140.43,** J= 11.0 Hz), 139.72, (**117.04, 116.96,** J=8.0 Hz), **(113.00, 112.76,** J= 24.0 Hz**),** 107.72, (**102.35, 102.30,** J=5.0 Hz**),** 66.76, 55.04, 55.02, 54.97, 35.27, 33.30, 27.08, 21.14, 7.59 CHN Anal. Calcd. for $C_{23}H_{24}FN_2O_5$: C, 59.58; H, 5.23; N, 6.05. Found C, 59.02; H, 4.98 ;N, 5.92 .

7-(3-(2-Chloroacetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.7) Off-white coloured powder, Yield: 91% (0.5 g), Melting point 249°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.41, (s, 1H, -COO**H**), 8.61 (s, 1H, -NC**H**CCOOH), 7.99-7.96 (d, 1H, J=14.28 Hz, -C(F)C**H**C), 6.66-6.64 (d, 1H, J=7.08, -C(N)C**H**C), 5.12-5.09 (t, 1H, CH_2-C**H**(OCOR)-CH_2-), 4.52 (bs,2H, -N Bridged **H**), 4.45 (s, 2H, ClC**H2**COO-) 4.30-4.25 (q, 2H, -NC**H$_2$**CH_3-), 2.29-2.22 (m, 4H, CH**(H)**-CH**(H)**- and -CH(**H**)-C(OCOR)-CH(**H**)-) ,2.19-2.15 (m, 2H, -C**H**(H)-C(OCOR)-C**H**(H)-), 1.90-1.86 (d,2H, C**H(**H)-C**H(**H**)**-), 1.62-1.57 (t,3H, -CH_2C**H$_3$**), ^{13}C NMR (100 MHz, $CDCl_3$) δ **(176.70, 176.66,** J= 4.0 Hz), 169.87, 167.49 , (**152.51, 150.04,** J= 247 Hz), 146.89, (**140.78, 140.67,** J= 11.0 Hz), 137.78,, (**117.69, 117.57,** J= 8 Hz), (**113.34, 113.10,** J= 24.0 Hz**),** 107.91, **(101.16, 101.12,** J=5.0 Hz), 67.50, 55.54, 55.47,55.42, 49.68, 34.01,34.05, 27.56, 14.45. CHN Anal. Calcd. for $C_{21}H_{22}FN_2O_5$: C, 57.74; H, 5.08; N, 6.41. Found C, 57.12; H, 4.91;N, 6.02.

7-(3-(2-Chloropropionyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.8): Light Yellow colour powder, Yield:93% (0.3 g), Melting point 274°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.04 (s, 1H, -COO**H**), 8.62 (s, 1H, -NC**H**CCOOH), 8.02-7.99 (d, 1H, J= 14.3 Hz, C(F)C**H**C), 6.68-6.66 (d, 1H, J= 7.12 Hz, -C(N)C**H**C) , 5.13-5.11 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.54,(bs, 2H, -N-bridged **H**) , 4.46-4.40 (q, 1H,-OCO(C**H)**$ClCH_3$), 4.31-4.26 (q, 2H,-NC**H$_2$**CH_3), 2.32-2.27 (m, 4H, -CH(**H**)-C(OCOR)-CH(**H**)- and -C**H**(H)-C(OCOR)-C**H**(H)-), 2.19-2.16 (t, 2H, CH**(H)**-CH**(H)**-), 1.93, 1.89 (d, 2H, C**H(**H)-C**H(**H**)**-), 1.74-1.72 (d, 3H,-OCO(CH**)**ClC**H$_3$**) 1.59-1.16 (t, 3H, -NCH_2C**H$_3$**) ^{13}C NMR (100 MHz, $CDCl_3$) δ (**176.63, 176.61** J =3.0 Hz), 168.99 , 167.53, (152.54, 150.07 J= 247 Hz, **)**, 146.96, **(140.83, 140.72,** J= 11.0 Hz), 137.80, (**117.82, 117.74,** J =8.0 Hz), **(113.40, 113.16,** J= 24.0 Hz**),** 107.89, (**101 .20, 101.15** J= 5.0 Hz), 69.26, 55.50, 55.47, 55.42, 55.40, 52.67, 49.66, (33.90, 33.79), 27.45, 21.24, 14.45.

(S)-10-(3-(2-(Piperidin-1-yl)acetoyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4.9) off-white colour powder, Yield: 0.2 g , (90%), Melting point 220.7°C dec,^{1}H NMR (400 MHz $CDCl_3$) 15.61, (s, 1H, -COO**H**), 8.59 (s, 1H, -NC**H**CCOOH), 7.71-7.68 (d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.21 -5.19 (t, 1H, J= 4.72 Hz, -CH2-C**H**(OR)-CH2-), 4.51 (Bs,1H,-NC**H**(CH_3) CH_2-), 4.43-4.33 (2 bs, 4H, -NCH (CH_3)C**H_2**- and bridged H), 3.23 (s, 2H, Piperidine C**H_2** COO-), 2.58(bs, 4H,-N(C**H_2**)**$_2$**),2.45-2.29 (dt, 2H,-C**H**(H)-C(OR)-C**H**(H)-), 2.17-2.14 (m, 2H, -C**H**(H)-C**H**(H)), 2.07-2.05 (m ,2H,CH(**H**)-C(OR)-CH(**H**)-),1.91-1.87 (2bs, 2H,-CH**(H)**-CH**(H)**-), 1.67 (m, 4H, -N(CH_2)$_2$(C**H_2**)**$_2$**CH_2 and 1.62 (bs, 2H, -NCH(C**H_3**)), 1.48-1.47 (m, 2H, -N(CH_2)$_2$(CH_2)$_2$C**H_2**),^{13}C NMR (100 MHz, $CDCl_3$) δ (**176.98 ,176.94**, J= 4 Hz), 169.35, 167.39, (**154.77 ,152.32**, J= 245 Hz), 144.57, (**135.89,135.79**, J= 10 Hz) , (**131.20 , 131.07**, J= 13 Hz), 125.01, (**117.47, 117.37**, J= 10 Hz), 107.51, (**105.78, 105.53**, J= 25 Hz), 68.16, 68.04, 60.41,58.26, 58.21, 57.81, 57.74, 55.54, 54.39, 37.43, 37.34, 28.61, 28.57, 25.69, 23.76, 18.38. CHN Anal. Calcd. for $C_{27}H_{32}FN_3O_6$: C, 63.15; H, 6.28; N, 8.18. Found C, 62.92; H, 6.02; N, 8.02.

(S)-10-(3-(2-(4-Methylpiperazin-1-yl) acetoyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6- carboxylic acid (4.10) : Off-white colour powder, Yield: 79% (0.18 g), Melting point 218.4°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.14 (s, 1H, -COO**H**)**,** 8.58 (s, 1H, -NC**H**CCOOH), 7.72-7.68 (d, 1H, J 16 Hz, -C(F)C**H**C), 5.20 (t, 1H, J= 4.9 Hz, -CH_2-C**H**(OR)-CH_2-), 4.50 (Bs 1H, -NC**H**(CH_3)CH_2-), 4.41 (Bs, 2H,-NCH(CH_3)C**H_2**)**,** 4.34 (Bs 2H, - bridged H), 3.25 (s, 2H , Piperazine C**H_2**COO-) , 2.72-2.67 (Bs, 8H, piperazine ring) , 2.41(s, 3H, -NC**H_3**), 2.34-2.30 (dt, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.14-2.08 (m ,4H, -C**H**(H)-C**H**(H)-), and (-CH(**H**)-C(OR)-CH(**H**)-), 1.90-1.87 (Bs, 2H, -CH**(H)**-CH**(H)**-), 1.63- 1.61(d,3H, J= 8 Hz -NCH(C**H_3**)),^{13}C NMR (100 MHz, $CDCl_3$) δ (**176.04 , 175.99**, J= 5 Hz), 169.52, 167.37, (**153.17, 150.70**, J= 247 Hz), 144.56, (**134.43, 134.36**, J= 7 Hz), (**131.16, 131.09**, J= 7 Hz), 125.01, (**117.54, 117.44**, J= 10 Hz),107.55,(**104.68,104.42,** J= 26 Hz), 68.38, 68.04, 59.51, 58.23, 58.18,57.76,57.71, 55.52,54.62 52.44, 45.52 37.41, 37.32, 28.60, 18.36. CHN Anal. Calcd.for $C_{27}H_{33}FN_4O_6$: C, 61.35; H, 6.29; N, 10.60. Found C, 61.03; H, 6.12; N, 10.26.

(S)-10-(3-(2-Morpholinoacetoyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H- [1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.11): Light yellow colour powder, Yield**:** 85% **(**0.17 g), Melting point 230.3°C , ^{1}H NMR (400 MHz $CDCl_3$) δ

15.21 (s, 1H, -COO**H**), 8.61 (s, 1H, -NC**H**CCOOH), 7.67-7.63 (d, 1H, J =13.5Hz, -C(F)C**H**C), 5.23-5.21 (t, 1H, J =4.9 Hz, -CH_2-C**H**(OR)-CH_2-), 4.57-4.56 (bs 1H, -NC**H**(CH_3)CH_2-), 4.44-4.42 (bs, 2H, -NCH(CH_3)C**H_2**-), 4.36-4.34 (bs, 2H , bridged H), 3.81-3.78 (t, 4H , N-C**H_2** of morpholine ring), 3.27 (s, 2H ,Morpholine C**H_2**COO-) , 2.67 (bs, 4H, O-C**H_2**of morpholine ring) , 2.43-2.30 (dt, 2H, -C**H**(H)- C(OR)-C**H**(H)-), 2.14-2.04 (m , 4H, -C**H**(H)-C**H**(H)-), and (-CH(**H**)-C(OR)-CH(**H**)-), 1.91-1.87 (bs, 2H, -CH**(H)**-CH**(H)**-), 1.62-1.60 (d,3H, J = 6.7 Hz -NCH(C**H_3**)),^{13}C NMR (100 MHz,$CDCl_3$) δ (**176.93,176.89**, J= 4 Hz),169.13, 167.44, (**154.78,152.32**, J=246 Hz), 144.68, (**135.96, 135.88**, J= 8 Hz), (**131.14,131.00**, J= 14 Hz),125.00, (**117.45, 117.35,** J= 10 Hz), 107.38, (**105.64, 105.39**, J= 25 Hz), 68.54, 68.07, 66.62, 59.75, 58.20, 58.15, 57.75, 57.69, 55.51, 53.34, 37.41, 37.31, 28.64, 28.59,18.39. CHN Anal. Calcd. For $C_{26}H_{30}FN_3O_7$: C, 60.57; H, 5.87; N, 8.15. Found C, 60.18; H, 5.61; N, 7.96.

(S)-10-(3-(2-(Piperidin-1-yl) propionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H- [1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.12); light yellow colour powder, Yield: 92% (0.20 g), Melting point 238.4°C, ^{1}H NMR (400 MHz $CDCl_3$), 15.15 (s, 1H, -COO**H**), 8.58 (s, 1H, -NC**H**CCOOH), 7.70-7.67(d, 1H, J=12 Hz, -C(F)C**H**C), 5.22 -5.21 (t, 1H, J= 5.0 Hz, CH_2-C**H**(OR)-CH_2-), 4.53-4.49 (m, 1H,-NC**H**(CH_3)CH_2-), 4.44-4.41 (m, 2H, -NCH(CH_3)C**H_2**), 4.35-4.33 (Bs, 2H, bridged **H**), 3.40 (Bs, 1H , Piperidine(C**H)**CH_3COO-), 2.71(Bs, 4H, -N(C**H_2**)**$_2$**), 2.43-2.32 (dt, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.17-2.06 (m , 4H, -C**H**(H)-C**H**(H)-and -CH(**H**)-C(OR)-CH(**H**)-), 1.91-1.84 (t, 2H, -CH**(H)**-CH**(H)**-), 1.68 (Bs, 4H, -N(CH_2)$_2$(C**H_2**)**$_2$**CH_2, 1.63-(d,3H,-NCH(C**H_3**)), 1.49-1.40 (m, 2H, -N(CH_2)$_2$(CH_2)$_2$C**H_2**),1.39-1.25 (d, 3H, Piperidine(CH)C**H_3**COO) , ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.96 , 176.93**, J= 3 Hz) , 169.42, 167.35, (**154.78, 152.33**, J= 245 Hz), 144.58, (**135.89, 135.81**, J= 8 Hz), (**131.17 ,131.04**, J= 13 Hz), 125.01, (**117.50, 117.41**, J= 9 Hz),107.51,(**105.75, 105.50**, J= 25 Hz), 68.05, 65.61, 63.23, 58.23, 58.19, 57.74, 55.52, 50.61, 37.66, 37.57, 37.47, 28.61, 28.57, 25.85, 18.37, 14.35.CHN Anal. Calcd. for $C_{28}H_{34}FN_3O_6$: C, 63.74; H, 6.50; N, 7.96. Found C, 63.18; H, 6.23; N, 7.50.

(S)-10-(3-(2-(4-Methylpiperazin-1-yl)propionyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H- [1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.13): Light yellow colour powder, Yield**:** 70% **(**0.16 g) **,** Melting point 196°C decomposes., ^{1}H NMR (400 MHz $CDCl_3$) δ 15.17 (s, 1H, -COO**H**), 8.59 (s, 1H, -NC**H**CCOOH), 7.71-7.68

(d, 1H, J = 12 Hz, -C(F)C**H**C), 5.21-5.20 (t, 1H, J= 4.6 Hz, -CH_2-C**H**(OR)-CH_2-), 4.49-4.34(m,5H, -NC**H**(CH_3)CH_2-),-NCH(CH_3)C**H_2**- and bridged H), 3.33-3.30 (m,1H , Piperazine (C**H)**CH_3COO -) , 2.80 (Bs, 4H,piperazine ring) , 2.68 (Bs, 4H, Piperazine ring), 2.43 (s, 3H, -NC**H_3**), 2.35-2.32 (dt, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.17-2.06 (m , 4H, -C**H**(H)-C**H**(H)-), and (-CH(**H**)-C(OR)-CH(**H**)-), 1.92-1.85(t, 2H, -CH**(H)**-CH**(H)**-), 1.63-1.61 (d,3H, J= 8 Hz -NCH(C**H_3**)), 1.35-1.34 (d, 3H, Piperazine(CH)C**H_3**COO-); ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.98 ,176.95**, J= 3 Hz) , 171.93, 167.37, (**156.08 ,153.67**, J= 241 Hz), 144.58, (**135.94, 135.87**, J= 7 Hz), (**131.18 ,131.05,** J= 13 Hz),124.99, (**117.52, 117.43**, J = 9 Hz), 107.52, (**105.78, 105.52**, J= 26 Hz), 68.09, 68.03, 62.69, 58.24, 58.19, 57.78, 57.72, 55.52, 54.99, 53.75, 45.37, 37.56, 37.46, 28.63, 28.59, 18.37,14.30. CHN Anal. Calcd. for $C_{28}H_{35}FN_4O_6$: C, 61.98; H, 6.50; N, 10.33. Found C, 61.40; H, 6.23; N, 10.02

(S)-10-(3-(2-Morpholinopropionyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro -3-methyl-7-oxo-2H- [1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.14) : Light yellow colour powder, Yield**:** 93% **(**0.2 g), Melting point 232.6°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.17 (s, 1H, -COO**H**), 8.60 (s, 1H, -NC**H**CCOOH), 7.71-7.68 (d, 1H, J=12 Hz, -C(F)C**H**C), 5.24-5.23(t, 1H, J= 4 Hz, -CH_2-C**H**(OR)-CH_2-), 4.45-4.36 (m, 5H, -NC**H**(CH_3) CH_2-), -NCH(CH_3)C**H_2**- and bridged **H),** 3.81 (Bs,4H , N-C**H_2** of morpholine ring), 3.28-3.25 (m, 1H , morpholine(C**H)**CH_3COO -), 2.80 (Bs, 4H,O-C**H_2**of morpholine ring) , 2.41-2.34 (dt, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.17-2.08 (m , 4H, -C**H**(H)-C**H**(H)-), and (-CH(**H**)-C(OR)-CH(**H**)-), 1.92-1.84 (t, 2H, -CH**(H)**-CH**(H)**-), 1.63-1.61(d,3H, J= 8 Hz -NCH(C**H_3**)),1.44 (Bs, 3H, Piperidine(CH)C**H_3**COO-), ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.97, 176.94**, J= 3 Hz) , 169.87, 167.41, (**154.80, 152.32**, J= 248 Hz), 144.62, (**135.95, 135.87**, J = 8 Hz), (**131.12, 130.99**, J= 13 Hz), 125.00, (**117.58, 117.48**, J = 10 Hz), 107.49, (**105.77, 105.51**, J= 26 Hz), 68.55, 68.05, 58.19, 58.13, 57.74,57.67, 55.51, 49.74, 37.63, 37.53, 37.43, 28.58, 18.38, 14.04. CHN Anal. Calcd. for $C_{27}H_{32}FN_3O_7$:C, 61.24; H, 6.09; N, 7.93. Found C, 61.05; H, 5.92; N, 7.48.

10-(3-(2-(Piperidin-1-yl) acetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro -3-methyl-7-oxo-2H-[1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.15): Light yellow colour powder, Yield: 88%, (0.19 g), Melting point 247°C decomposes., ^{1}H NMR (400 MHz $CDCl_3$) δ 15.18 (s, 1H, -COO**H**), 8.59 (s, 1H, -NC**H**CCOOH), 7.69-7.66 (d, 1H, J= 13.5Hz, -C(F)C**H**C), 5.21 -5.20 (t, 1H, J= 4.9 Hz, -CH_2-C**H**(OR)-CH_2-), 4.53-4.52 (m, 1H, -NC**H**(CH_3)CH_2-), 4.49-4.41 (dd, 2H, -NCH(CH_3)C**H_2**-), 4.35-4.32 (m,2H, bridged H),

3.25 (s, 2H , PiperidineC**H**$_2$COO-), 2.61 (bs, 4H,-N(C**H**$_2$)$_2$) , 2.41-2.30 (dt, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.15-2.10 (q, 2H, -C**H**(H)-C**H**(H)-), 2.07-2.04 (m, 2H, -CH(**H**)-C(OR)-CH(**H**)-), 1.91-1.87 (m, 2H, -CH**(H)**-CH**(H)**-), 1.71-1.65 (q, 2H, -N(CH$_2$)$_2$(C**H**$_2$)$_2$CH$_2$), 1.62-1.60 (d,3H, J= 6.7 Hz -NCH(C**H**$_3$)), 1.48-1.47 (m, 2H,- N(CH$_2$)$_2$(CH$_2$)$_2$C**H**$_2$),^{13}C NMR (100 MHz, CDCl$_3$) δ (**176.95 ,176.92**, J= 3 Hz),169.56, 167.40, (**154.76, 152.31**, J= 245 Hz), 144.61, (**135.89, 135.81**, J= 8 Hz), (**131.18 ,131.05**, J= 13 Hz), 125.00, (**117.43, 117.34**, J= 9 Hz),107.44, (**105.71,105.46**, J= 25 Hz), 68.27, 68.04, 60.20, 58.24, 58.18,57.78, 57.72 ,55.52, 54.30, 37.41, 37.31, 28.61, 28.56, 25.57, 23.67, 18.38. CHN Anal. Calcd. for $C_{27}H_{32}FN_3O_6$: C, 63.15; H, 6.28; N, 8.18.Found C, 62.97; H, 6.08; N, 7.98.

10-(3-(2-(4-Methylpiperazin-1-yl) acetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.16) : Light yellow colour powder, Yield**:** 72% **(**0.16 g), Melting point 212°C dec., ^{1}H NMR (400 MHz CDCl$_3$) δ 15.14 (s,1H, -COO**H**), 8.58 (s, 1H, -NC**H**CCOOH), 7.73-7.69(d, 1H, J=16 Hz, -C(F)C**H**C), 5.21-5.20 (t,1H, J= 4 Hz, -CH$_2$-C**H**(OR)-CH$_2$-), 4.50-4.48 (m, 1H, -NC**H**(CH$_3$)CH$_2$-), 4.44-4.42 (d, 2H, -NCH(CH$_3$)C**H**$_2$-), 4.34-4.31 (Bs, 2H, bridged H), 3.26 (s, 2H , PiperazineC**H**$_2$COO-) , 2.76 (Bs,8H, piperazine ring) , 2.43 (s, 3H, -NC**H**$_3$), 2.38-2.30 (dt, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.13-2.08 (m , 4H, -C**H**(H)-C**H**(H)-), and (-CH(**H**)-C(OR)-CH(**H**)-), 1.90-1.87 (Bs, 2H, -CH**(H)**- CH**(H)**-), 1.63-1.61 (d,3H, J= 8 Hz -NCH(C**H**$_3$)),^{13}C NMR (100 MHz, CDCl$_3$)δ (**176.98 , 176.95**, J= 3 Hz),169.48, 167.35, (**154.81 ,152.32**, J= 249 Hz), 144.53, (**135.20, 135.14**, J= 6 Hz), (**130.73 ,130.60**, J= 13 Hz), 124.99, (**115.37, 115.27**, J= 10 Hz), 107.56, (**105.81, 105.56**, J= 25 Hz), 68.42, 68.03,59.44, 57.75, 57.70, 55.51, 54.56, 52.29, 52.25,45.41 , 37.41, 37.31, 28.64, 28.59, 18.36. CHN Anal. Calcd. for $C_{27}H_{33}FN_4O_6$: C, 61.35; H,6.29; N, 10.60 Found C, 61.08; H, 6.02; N, 10.06.

10-(3-(2-Morpholinoacetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H- [1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.17); Light yellow colour powder, **:**Yield**:** 86% **(** 0.19 g), Melting point 225°C decomposes., ^{1}H NMR (400 MHz CDCl$_3$) δ 15.16 (s, 1H, -COO**H**),8.59 (s, 1H, -NC**H**CCOOH), 7.70-7.67 (d, 1H, J= 13.5 Hz, -C(F)C**H**C), 5.23-5.22 (t, 1H, J= 4.9 Hz,-CH$_2$-C**H**(OR)-CH$_2$-), 4.54-4.49 (Bs 1H, -NC**H**(CH$_3$)CH$_2$-), 4.44-4.43 (Bs, 2H, -NCH(CH$_3$)C**H**$_2$-), 4.35-4.32 (Bs, 2H , bridged H), 3.82-3.80 (t, 4H , N-C**H**$_2$ of Piperidine ring), 3.30 (s, 2H ,Piperidine C**H**$_2$COO-) , 2.71 (Bs, 4H, O-C**H**$_2$ of Piperidine ring) , 2.43-2.30 (4dt, 2H, -C**H**(H)- C(OR)-C**H**(H)-), 2.17-2.02 (m , 4H, -C**H**(H)-C**H**(H)-), and (-CH(**H**)-C(OR)-CH(**H**)-), 1.91-1.87 (Bs, 2H, -CH**(H)**-CH**(H)**-),

1.62-1.61 (d,3H, J= 6.7 Hz -NCH(C**H$_3$**)),^{13}C NMR (100 MHz, $CDCl_3$) δ(**176.97,176.94,** J= 3 Hz), 168.91,167.37, (**154.77, 152.33**, J= 244 Hz), 144.60,(**135.93, 135.85**, J = 8 Hz), (**131.13, 131.00**, J= 13 Hz), 125.00, (**117.54, 117.44**, J= 10 Hz) 107.50, (**105.75, 105.50**, J= 25 Hz), 68.66, 68.05, 66.50, 59.56, 58.20, 58.15, 57.74, 57.68, 55.51, 53.25, 37.40, 37.30, 28.63, 28.59,18.37. CHN Anal. Calcd. for $C_{26}H_{30}FN_3O_7$: C,0.57; H, 5.87; N, 8.15. Found C, 60.18; H, 5.61; N, 7.96.

10-(3-(2-(Piperidin-1-yl)propionyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.18): Light yellow colour powder, Yield**:** 90% **(**0.19 g) **,** Melting point 236°C dec., ^{1}H NMR (400 MHz $CDCl_3$) 15.15 (s, 1H, -COO**H**), 8.58 (s, 1H, -NC**H**CCOOH), 7.72-7.69 (d, 1H, J= 12 Hz, -C(F)C**H**C), 5.23 -5.21 (t, 1H, J=8 Hz, -CH_2-C**H**(OR)-CH_2-), 4.51-4.32 (m. , 5 H, -NC**H**(CH_3)CH_2-), -NCH(CH_3)C**H$_2$**- and bridged **H**), 3.34 (Bs, 1H , Piperidine(C**H)**CH_3COO-), 2.75 (Bs, 4H, -N(C**H$_2$**)$_2$), 2.33-2.09 (dt, 2H, -C**H**(H)-C(OR)- C**H**(H)-), 2.18-2.07 (m , 4H, -C**H**(H)-C**H**(H)-and -CH(**H**)-C(OR)-CH(**H**)-), 1.91-1.84 (t, 2H, -CH**(H)**-CH**(H)**-), 1.63 (Bs, 4H, -N(CH2)2(C**H$_2$**)**$_2$**CH_2), 1.61-1.58 (d, 3H, -NCH(C**H$_3$**)) , 1.50-1.43 (Bs, 2H, -N(CH_2)$_2$(CH_2)$_2$C**H$_2$**),1.25 (d, 3H, Piperidine(CH)C**H**$_3$COO) , ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.98 ,176.94**, J= 4 Hz) , 172.36, 167.36, (**154.78 ,152.33**, J= 245 Hz), 144.56, (**135.88, 135.81**, J= 7 Hz), (**131.16, 131.03**, J= 13 Hz), 125.01, (**117.44, 117.34**, J= 10 Hz), 107.54, (**105.79, 105.53**, J= 25 Hz) , 68.04, 68.25, 63.16, 58.22, 58.17, 57.78, 57.72, 55.52, 53.07, 37.64, 37.55, 37.44, 28.60, 28.56, 25.85, 18.37, 14.33. CHN Anal.Calcd. for $C_{28}H_{34}FN_3O_6$: C, 63.74; H, 6.50; N, 7.96. Found C,63.25; H, 6.03; N, 7.42.

10-(3-(2-(4-Methylpiperazin-1-yl) propionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro -3,7-dihydro-3-methyl-7-oxo-2H- [1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.19) : Light yellow colour powder, Yield**:**72% (0.16 g), Melting point 228°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.16 (s, 1H, -COO**H**), 8.59 (s, 1H, -NC**H**CCOOH), 7.72-7.68 (d, 1H, J= 16 Hz, -C(F)C**H**C), 5.21-5.20 (t, 1H, J= 4Hz, -CH_2-C**H**(OR)-CH_2-), 4.49-4.35 (m, 5H, -NC**H**(CH_3)CH_2-), -NCH(CH_3)C**H$_2$**-and bridged H), 3.34-3.28 (m, 1H , Piperazine (C**H)**CH_3 COO -) , 2.73 (Bs, 4H, piperazine ring), 2.57 (Bs, 4H, Piperazine ring), 2.42-2.38 (dt , 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.35 (s, 3H, -NC**H$_3$**), 2.18-2.06 (m , 4H, -C**H**(H)-C**H**(H)-), and (-CH(**H**)-C(OR)-CH(**H**)-), 1.91-1.85 (t, 2H, -CH**(H)**-CH**(H)**-), 1.63-1.61(d,3H, J= 8 Hz -NCH(C**H$_3$**), 1.35-1.33 (d, 3H, Piperazine(CH)C**H**$_3$COO-) . ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.97 ,176.94**, J= 3 Hz) , 172.02, 167.39, (**154.77 ,152.31**, J= 246 Hz), 144.55, (**135.90,**

135.82, J= 8 Hz), (**131.17, 131.06**, J= 11 Hz) ,125.0, (**117.50, 117.40**, J= 10 Hz), 107.51,(**105.78, 105.53**, J= 25 Hz), 68.03, 67.93, 62.78, 58.22, 58.20, 57.79, 57.73, 55.52, 55.16, 52.03, 45.74, 37.56, 37.45, 28.57, 18.36 ,14.33.CHN Anal. Calcd. for $C_{28}H_{35}FN_4O_6$: C, 61.98; H, 6.50; N, 10.33. Found C, 61.58; H, 6.16; N, 9.96.

10-(3-(2-Morpholinopropionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H- [1,4] oxazino[2,3,4-ij] quinoline-6-carboxylic acid (4.20) : Light yellow colour powder, Yield**:** 93% **(**0.20 g **),** Melting point 237°C., ^{1}H NMR (400 MHz $CDCl_3$) δ 15.13 (s, 1H, -COO**H**),8.59 (s, 1H, -NC**H**CCOOH), 7.73-7.69 (d, 1H, J=14 Hz, -C(F)C**H**C), 5.24-5.23 (t, 1H, J= 4 Hz, -CH_2-C**H**(OR)-CH_2-), 4.44-4.34 (m, 5H, -NC**H**(CH_3)CH_2-), -NCH(CH_3)C**H$_2$**- and bridged **H),** 3.81 (Bs,4H , N-C**H$_2$** of morpholine ring), 3.40 (m, 1H , morpholine(C**H)**CH_3COO -), 2.78 (Bs, 4H, O-C**H**$_2$ of morpholine ring) , 2.45-2.34 (dt, 2H, -C**H**(H)-C(OR)-C**H**(H)-), 2.18-2.06 (m, 4H,-C**H**(H)-C**H**(H)-), and (-CH(**H**)-C(OR)-CH(**H**)-), 1.92-1.84 (t,2H, -CH**(H)**-CH**(H)**-), 1.63-1.61(d,3H, J= 8 Hz -NCH(C**H$_3$**)),1.43 (Bs, 3H, Piperidine(CH)C**H$_3$**COO-), ^{13}C NMR (100 MHz,$CDCl_3$)δ (**176.99, 176.96**, J= 3 Hz), 169.39,167.34, (**154.79, 152.32**, J= 247 Hz),144.55, (**136.78, 136.72**, J= 6 Hz), (**130.95, 130.82**, J= 13 Hz),125.00, (**117.71, 117.61,** J= 10 Hz),107.58, (**105.82, 105.57**, J= 25 Hz), 68.34, 68.05, 62.90, 58.19, 57.77, 57.67, 55.51, 49.71, 37.54, 37.43, 28.58, 28.60 , 18.36, 14.04. CHN Anal. Calcd. For $C_{27}H_{32}FN_3O_7$: C, 61.24; H, 6.09; N, 7.93. Found C, 61. 12; H, 5.98; N,7.48.

7-(3-(2-(Piperidin-1-yl)acetoyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.21); Light yellow colour powder, Yield: 93% (0.32 g), Melting point 235°C , ^{1}H NMR (400 MHz $CDCl_3$) δ 14.80 (s, 1H, -COO**H**), 8.66 (s, 1H, -NC**H**CCOOH), 7.92-7.88 (d, 1H, J= 14.28 Hz, -C(F)C**H**C), 7.19-7.17 (d, 1H, J= 7.32 Hz, -C(N)C**H**C,), 5.11-5.09 (t, 1H, -CH_2-C**H**(OCOR)-CH_2-), 4.53,(bs, 2H, -N-bridged **H**) , 3.50 (m, 1H, -N-Cyclopropyl C**H**), 3.20 (s, 2H, -OCOC**H$_2$**-Piperidine), 2.54-2.52 (t, 4H, N-(C**H$_2$**)$_2$) 2.31-2.15 (m, 6H, CH(**H**)-C(OCOR)-CH(**H**)- and -C**H**(H)-C(OCOR)-C**H**(H)-), CH**(H)**-CH**(H)**-), 1.89, 1.85 (d, 2H, C**H(**H)-C**H(**H)-), 1.66-1.61 (q, 4H, Piperidine ring), 1.48-1.45 (q , 2H, Piperidine C**H$_2$**), 1.38-1.36 (q, 2H, NCHCH(**H**)CH(**H**)-), 1.19 (q, 2H, -NCHC**H**(H)C**H**(H),^{13}C NMR (100 MHz, $CDCl_3$) δ (**176.68, 176.64 ,** J=4.0 Hz), 169.96, 167.25, **(152.68, 150.20** J= 248 Hz), 147.12, **(140.56, 140.45** J= 11.03 Hz), 139.70, (**116.97, 116.89,** J= 7.5 Hz), (**112.86, 112.67,** J= 24.0 Hz **),** 107.65, (**102.38, 102.33,** J= 4.75 Hz)**,**

67.53, 60.65, 55.54, 55.47**,** 54.50, 35.09, 33.96, 27.56, 25.84, 23.86, 8.17. CHN Anal. Calcd. For $C_{27}H_{32}FN_3O_5$: C, 65.18; H, 6.48; N, 8.45. Found C, 64. 96 ; H, 5.98; N,7.98.

7-(3-(2-(4-Methylpiperazin-1-yl)acetoyloxy)-8-aza-bicyclo[3.2.1] octan-8-yl)-1-cyclopropyl -6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.22): Light yellow coloured powder, Yield: 80.0% (0.28 g), Melting point 237°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.15 (s, 1H,-COO**H)**, 8.68 (s, 1H, -NC**H**CCOOH), 7.96-7.92 (d, 1H, J 14.24 Hz, -C(F)C**H**C), 7.19-7.18 (d, 1H, J 7.36 Hz, -C(N)C**H**C), 5.13-5.09 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.53,(bs, 2H, -N-bridged **H**) , 3.51-3.48 (m, 1H, -N- Cyclopropyl C**H**), 3.24 (s, 2H, -OCOC**H_2-**NMP), 2.65-2.52 (m, 4H, N-(C**H_2**)$_2$) 2.44 (m, 4H, CH_3N-(C**H_2**)$_2$), 2.31 (s, 3H, -NC**H_3**), 2.29-2.15 (m, 6H, CH(**H**)-C(OCOR)-CH(**H**)-, -C**H**(H)-C(OCOR)-C**H**(H)-)and CH**(H)**-CH**(H)**-), 1.89, 1.85 (d, 2H, C**H(**H**)**-C**H(**H**)**-), 1.38-1.33 (q, 2H, NCHCH(**H**)CH(**H**)-), 1.21-1.17 (q, 2H, -NCHC**H**(H)C**H**(H),^{13}C NMR (100 MHz, $CDCl_3$) δ **(176.79, 176.75,** J= 4.0 Hz), 169.53, 167.26, **(152.70, 150.22,** J= 248 Hz**),** 147.18, **(140.56, 140.45,** J= 11.03 Hz), 139.72, (**117.09, 117.01 Hz,** J=8.0 Hz), **(112.99, 112.75,** J= 24.0 Hz**),** 107.72, (**102.37, 102.33** J= 4.75 Hz**),** 67.74, 59.71, 55.51, 55.43 , 54.86 , 53.15, 46.00, 35.08, 33.93, 27.58, 8.19. CHN Anal. Calcd. For $C_{27}H_{33}FN_4O_5$: C, 63.27; H, 6.49; N, 10.93. Found C, 63.24; H, 6.29; N, 10.86.

7-(3-(2-Morpholinoacetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.23): Light yellow colour powder, Yield: 70% (0.26 g), Melting point 281°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.15 (s, 1H,-COO**H),** 8.66 (s, 1H, -NC**H**CCOOH), 7.93-7.89 (d, 1H, J=14.24 Hz, -C(F)C**H**C), 7.19-7.18 (d, 1H, J= 7.36 Hz, -C(N)C**H**C), 5.13-5.11 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.53,(bs, 2H, -N-bridged **H**) , 3.78-3.75 (t, 4H, O(C**H_2**)$_2$), 3.51-3.49 (m, 1H-N-Cyclopropyl C**H**), 3.24 (s, 2H, -OCOC**H_2-**Morpholine), 2.62-2.60 (t, 4H, N-(C**H_2**)$_2$) , 2.34-2.32 (2H dt, CH(**H**)-C(OCOR)-CH(**H**)-),2.30-2.16 (m, 4H, -C**H**(H)-C(OCOR)-C**H**(H)-) and CH**(H)**-CH**(H)**-, 1.89, 1.85 (d, 2H, C**H(**H**)**-C**H(**H**)**-), 1.38-1.34(q, 2H, NCHCH(**H**)CH(**H**)-), 1.21-1.17 (q, 2H, -NCHC**H**(H)C**H**(H),^{13}C NMR (100 MHz, $CDCl_3$) δ **(176.74, 176.71,** J=2.66 Hz **),** 169.37, 167.20, (**152.69, 150.21,** J= 245 Hz**)**, 147.14, (**140.55, 140.44,** J= 10.80 Hz**)**, 139.70, (**117.05, 116.98,** J= 7.31 Hz), (**112.92, 112.68,** J= 24.1 Hz**),**107.66, (**102.39,102.34,** J=4.75 Hz**),** 67.87, 66.79, 59.99, 55.50, 55.42, 53.47, 35.09, 33.95 , 27.58, 8.18. CHN Anal. Calcd. For $C_{26}H_{30}FN_3O_6$: C, 62.51 ; H, 6.05; N, 8.41 Found C, 62.40, ; H, 5.98; N, 8.26.

7-(3-(2-(Piperidin-1-yl) propionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.24): Light yellow colour powder,

Yield: 93% (0.32 g), Melting point 271.0°C ,^{1}H NMR (400 MHz $CDCl_3$) δ 15.11 (s, 1H, -COOH), 8.67 (s, 1H, -NC**H**CCOOH), 7.95-7.91(d, 1H, J= 14.28 Hz, -C(F)C**H**C), 7.20-7.18 (d, 1H, J= 7.36 Hz, -C(N)C**H**C), 5.11-5.09 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.54,(bs, 2H, -N-bridged **H**), 3.52-3.46 (m, 1H, -N-Cyclopropyl C**H**), 3.32-3.27 (q, 1H, -OCO(C**H)**CH_3 Piperidine), 2.63-2.53 (m, 4 H, N-(C**H$_2$**)$_2$of Piperidine ring), 2.33-2.27 (m, 4H, CH(**H**)-C(OCOR)-CH(**H**)- and -C**H**(H)-C(OCOR)-C**H**(H)-), 2.21-2.16 (m, 2H, CH**(H)**-CH**(H)**-), 1.88, 1.82 (dd, 2H, C**H(**H)-C**H(**H**)**-), 1.66-1.58 (q, 4H, Piperidine ring), 1.48-1.42 (q , 2H, Piperidine C**H$_2$**), 1.38-1.31 (m, 5H, NCHCH(**H**)CH(**H**)- and -OCO(CH)C**H$_3$**), 1.19-1.17 (q, 2H, -NCHC**H**(H)C**H**(H), ^{13}C NMR (100 MHz, $CDCl_3$)δ **(176.77,176.73,** J=4.0 Hz), 172.44, 167.27, (**152.70, 150.22,** J= 248 Hz**),** 147.15, (**140.56, 140.45,** J= 11.03 Hz**),**139.72, (**117.04, 116.96,** J= 8.0 Hz), (**112.96, 112.71,** J= 24.0 Hz **),**107.72, (**102.38, 102.33,** J= 4.75 Hz**),** 67.09, 63.44, 55.55, 55.49, 50.71, 35.08, 34.19, 34.15, 27.54, 26.35, 24.52, 14.30, 8.18. CHN Anal. Calcd. For $C_{28}H_{34}FN_3O_5$: C, 65.74; H, 6.70; N, 8.21. Found C, 65.62; H, 6.21 ; N,8 .08.

7-(3-(2-(4-Methylpiperazin-1-yl)propionyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-1-cyclo propyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.25); Light yellow coloured powder, Yield: 80%, (0.28 g), Melting point 251°C , ^{1}H NMR (400 MHz $CDCl_3$) δ 15.11 (s, 1H, -COOH), 8.68 (s, 1H, -NCHCCOOH), 7.96-7.92 (d, 1H, J= 14.24 Hz, -C(F)C**H**C), 7.20-7.18 (d, 1H, J= 7.28 Hz, -C(N)C**H**C), 5.10 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.50,(bs, 2H, -N-bridged **H**) , 3.49- (m, 1H, -N-Cyclopropyl C**H**), 3.34 -3.29 (q, 1H, -OCO(C**H)**CH_3NMP), 2.68 (m, 4H, N-(C**H$_2$**)$_2$) 2.47 (m, 4H, CH_3N-(C**H$_2$**)$_2$), 2.32-2.25 (m , 7 H, -NC**H$_3$**, CH(**H**)-C(OCOR)-CH(**H**)-, -C**H**(H)-C(OCOR)-C**H**(H)-), 2.19-2.18(q, 2H ,CH**(H)**-CH**(H)**-), 1.89, 1.83 (dd, 2H, C**H(**H)-C**H(**H**)**-), 1.36-1.33 (m, 5H, NCHCH(**H**) CH(**H**)- and -OCO(CH)C**H$_3$**), 1.19 (q, 2H, -NCHC**H**(H)C**H**(H),^{13}C NMR (100 MHz, $CDCl_3$) δ**(176.79, 176.75,** J= 4.0 Hz), 171.94, 167.27, **(152.70, 150.23,** J= 247 Hz**),** 147.18, **(140.54, 140.43,** J= 11 Hz), 139.72, (**117.07, 116.99,** J=8.0), **(112.98, 112.75,** J= 24.0 Hz**),** 107.71, (**102.35, 102.30,** J= 5.0 Hz**),** 67.35, 62.80, 55.44, 55.34, 49.41, 46.04, 35.09, 34.11 29.17, 27.58 14.24, 8.19. CHN Anal. Calcd. For $C_{28}H_{35}FN_4O_5$: C, 63.86; H, 6.70; N, 10.70. Found C, 63.52; H, 5.98 ; N,10.20.

7-(3-(2-Morpholinopropionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.26): Light yellow colour powder, Yield:70% (0.26 g), Melting point 244°C, ^{1}H NMR (400 MHz $CDCl_3$) δ 15.16 (s, 1H,-COO**H),** 8.68 (s, 1H, -NC**H**CCOOH), 7.96-7.92 (d, 1H, J=14.2 Hz,-C(F)C**H**C), 7.20-7.19 (d, 1H, J= 7.32 Hz, -C(N)C**H**C), 5.12 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.54,(bs, 2H, -N-bridged

H) , 3.73-3.69 (Bs, 4H, O(C**H₂**)₂), 3.50-3.49 (m, 1H -N-Cyclopropyl C**H**), 3.31-3.26 (q, 1H, -OCO(C**H)**CH_3-Morpholine), 2.66-2.64 (t, 4H, N-(C**H₂**)₂)2.35-2.17 (m, 6H, -CH(**H**)-C(OCOR)-CH(**H**)-), -C**H**(H)-C(OCOR)-C**H**(H)-) and CH**(H)**-CH**(H)**-), 1.89, 1.83 (dd, 2H, C**H(**H)-C**H(**H)-),1.36-1.31 (d,5H, NCHCH(**H**)CH(**H**)- and-OCOCHC**H₃**), 1.20 (q, 2H, -NCHC**H**(H) C**H**(H),^{13}C NMR (100 MHz, $CDCl_3$) δ **(176.79, 176.76,** J=4.0 Hz), 171.97, 167.24, **(152.69, 150.21,** J =248 Hz**,** 147.19, **(140.54, 140.43,** J= 11.0 Hz), 139.72, (**117.04, 116.96,** J=8.0 Hz), **(113.00, 112.76,** J= 24.0 Hz**),** 107.72, (**102.35, 102.30, J=5.0 Hz),** 67.47, 67.21, 63.11, 55.49, 55.43**,** 49.96, 35.08, 34.12, 27.57, 14.11,8.19. CHN Anal. Calcd. For $C_{27}H_{32}FN_3O_6$: C, 63.15; H, 6.28; N, 8.18. Found C, 62.98 H, 5.98 ; N,8.01

7-(3-(2-(Piperidin-1-yl)acetoyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.27): Off-white coloured powder, Yield: 91% (0.5 g), Melting point 249°C, ^{1}H NMR (400 MHz $CDCl_3$) δ15.29, (s, 1H, -COO**H**), 8.61 (s, 1H, -NC**H**CCOOH), 8.01-7.97 (d, 1H, J=14.28 Hz, -C(F)C**H**C), 6.66-6.64 (d, 1H, J=7.08, -C(N)C**H**C), 5.12-5.09 (t, 1H, CH_2-C**H**(OCOR)-CH_2-), 4.53 (bs,2H, -N Bridged **H**), 4.30-4.25 (q, 2H, -NC**H₂**CH_3-), 3.21 (s, 2H,-OCOC**H₂**-Piperidine), 2.56-2.53 (t, 4H, N-(C**H₂**)₂), 2.29-2.22 (m, 4H, CH**(H)**-CH**(H)**- and -CH(**H**)-C(OCOR)-CH(**H**)-) ,2.19-2.15 (m, 2H, -C**H**(H)-C(OCOR)-C**H**(H)-), 1.90-1.86 (d,2H, C**H(**H)-C**H(**H)-), 1.67-1.63 (q, 4H, Piperidine ring), 1.62-1.57 (t, 3H, (t,3H, -CH_2C**H₃**), 1.49-1.46 (q , 2H, Piperidine C**H₂**), ^{13}C NMR (100 MHz, $CDCl_3$) δ **(176.70, 176.66,** J= 4.0 Hz), 169.87, 167.49 , (**152.51, 150.04,** J= 247 Hz), 146.89, (**140.78, 140.67,** J= 11.0 Hz), 137.78,, (**117.69, 117.57,** J= 8 Hz), (**113.34, 113.10,** J= 24.0 Hz**),** 107.91, **(101.16, 101.12,** J=5.0 Hz), 67.50, 60.58,55.54, 55.47, 54.50, 49.60, 34.05, 27.56, 25.80, 23.83 14.45. CHN Anal. Calcd. For $C_{26}H_{32}FN_3O_5$: C, 64.31; H, 6.64; N, 8.65. Found C, 64.03 H, 6.01 ; N,8.11

7-(3-(2-(4-Methylpiperazin-1-yl) acetoyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.28): Off-white coloured powder, Yield: 80% (0.28 g), Melting point 212°C, ^{1}HNMR (400 MHz $CDCl_3$) δ 15.28 (s, 1H, -COO**H**) 8.59 (s, 1H, -NC**H**CCOOH), 7.99-7.96 (d, 1H, J= 14.32 Hz, -C(F)C**H**C), 6.64-6.63 (d, 1H, J= 6.8 Hz, -C(N)C**H**C), 5.10-5.08 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.51,(bs, 2H, -N-bridged **H**) ,4.27-4.25(q, 2H, -NC**H₂**CH_3-), 3.23 (s, 2H, -OCOC**H₂**-NMP), 2.64- (bs, 4H, N-(C**H₂**)₂) 2.54 (bs, 4H, CH_3N-(C**H₂**)₂), 2.31 (s, 3H, -NC**H₃**), 2.27-2.13 (m, 6H, CH(**H**)-C(OCOR)-CH(**H**)-, -C**H**(H)-C(OCOR)-C**H**(H)-) and CH**(H)**-CH**(H)**-), 1.88,1.84 (d, 2H, C**H(**H)-C**H(**H)-), 1.57-1.54 (t,3H, -CH_2C**H₃**),^{13}C NMR (100 MHz, $CDCl_3$)δ **(176.74, 176.71,** J= 3.0 Hz), 169.54, 167.52, (**152.45, 149.97** J= 248 Hz**)**, 146.94 (-N-**C**-C-COO), **(140.81,**

140.70, J=11.0 Hz**),** 137.82, **(117.85, 117.77,** J= 8 Hz), (**113.40, 113.16,** J=24.0 Hz), 107.96, **(101.22, 101.17,** J= 5.0 Hz**),** 67.76, 59.70, 55.55, 55.48**,** 54.85, 53.08, 49.65, 46.09, 34.08, 27.61, 14.49. CHN Anal. Calcd. For $C_{26}H_{33}FN_4O_5$: C, 62.39; H, 6.64; N, 11.19. Found C, 61.88; H, 6.01 ; N,10.96.

7-(3-(2-Morpholinoacetoyloxy)-8-aza-bicyclo[3.2.1]octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.29) Off-white coloured powder,Yield:80.0% (0.28 g), Melting point 246°C , ^{1}HNMR (400 MHz $CDCl_3$) δ 15.11 (s, 1H, -COOH), 8.60 (s, 1H, -NC**H**CCOOH), 8.00-7.97 (d, 1H, J= 14.28 Hz,), 6.66-6.64 (d, 1H, J=7.12 Hz, -C(N)C**H**C), 5.13-5.10 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.53,(bs, 2H, -N-bridged **H**) ,4.30-4.24 (q, 2H, -NC**H$_2$**CH_3-), 3.78-3.76 (t, 4H, O(C**H$_2$**)$_2$), 3.24 (s, 2H, -OCOC**H$_2$**-Morpholine), 2.63-2.61 (t, 4H, N-(C**H$_2$**)$_2$) , 2.30-2.15 (m, 6H, CH(**H**)-C(OCOR)-CH(**H**)-), -C**H**(H)-C(OCOR)-C**H**(H)- and CH**(H)**-CH**(H)**-, 1.90, 1.86 (d, 2H, C**H(**H)-C**H(**H)-), 1.59-1.55 (t, 3H, -CH_2C**H$_3$)** ,^{13}C NMR (100 MHz, $CDCl_3$) δ **(176.69, 176.66,** J=3.0 Hz), 169.31, 167.46, (**152.51, 150.03**, J= 248 Hz**)**, 146.90, (**140.75, 140.64 ,**J=11.0 Hz), 137.76, (**117.82, 117.74,** J=8.0 Hz), (**113.36, 113.11,** J= 24.0 Hz**),**107.91, (**101.18, 101.13** J=5.0 Hz)**,** 67.83, 66.76, 59.93, 55.49, 55.42,53.46, 49.59, 34.04, 27.57, 14.44. CHN Anal. Calcd. For $C_{25}H_{30}FN_3O_6$: C, 61.59; H, 6.20; N, 8.62. Found C, 61.18; H, 5.98 ; N,8.16

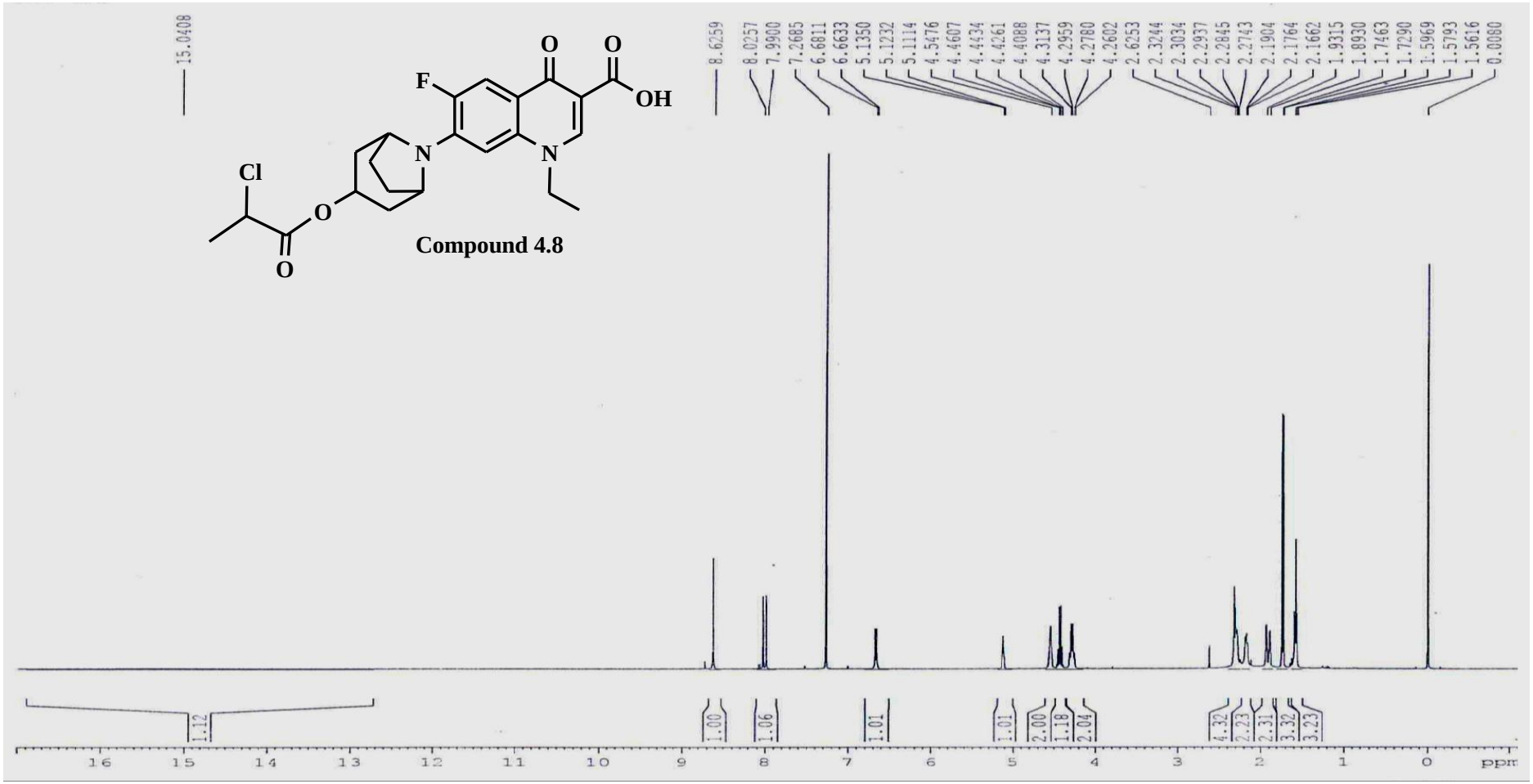
7-(3-(2-(Piperidin-1-yl) propionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.30): Off-white coloured powder,Yield: 91.0%(0.5 g), Melting point 243°C.,^{1}H NMR (400 MHz $CDCl_3$)δ 15.29, (s, 1H, -COO**H**), 8.60 (s, 1H, -NC**H**CCOOH), 8.01-7.98 (d, 1H, J= 14.3 Hz, -C(F)C**H**C), 6.66-6.64 (d, 1H, J= 7.08 Hz -C(N)C**H**C), 5.12-5.09 (t, 1H, CH_2-C**H**(OCOR)-CH_2-), 4.54 (bs,2H, -N Bridged **H**), 4.29-4.24 (q, 2H, -NC**H$_2$**CH_3), 3.32-3.30 (q, 1H, -OCO(C**H)**CH_3), 2.61-2.59 (m , 4H, N-(C**H$_2$**)$_2$), 2.30-2.27(m, 4H, CH**(H)**-CH**(H)**- and -CH(**H**)-C(OCOR)-CH(**H**)-) ,2.18-2.14 (m, 2H, -C **H**(H)-C(OCOR)-C**H**(H)-), 1.89-1.83(dd,2H, C**H(**H)-C**H(**H)-), 1.62-1.55 (m , 7H, Piperidine ring and -NCH_2C**H$_3$**), 1.48-1.44 (q , 2H, Piperidine C**H$_2$**), 1.34-1.32 (d, 3H,-OCO(CH**)**C**H$_3$**), ^{13}C NMR (100 MHz, $CDCl_3$)δ **(176.71, 176.68,** J= 3.0 Hz), 172.36, 167.49, (**152.31, 150.03,** J= 248 Hz**),** 146.90, (**140.77, 140.66,** J= 11.0 Hz), 137.78, (**117.77, 117.69,** J= 8 Hz), (**113.37, 113.12,** J= 25.0 Hz), 107.92, (**101.17, 101.12,** J= 5.0 Hz**),** 67.07, 63.40, 55.57, 55.53, 55.49, 55.46 , 50.70, 49.61, 34.25, 27.52, 26.28, 24.47, 14.45,14.28. CHN Anal. Calcd. For $C_{27}H_{34}FN_3O_5$: C, 64.91; H, 6.86; N, 8.41. Found C, 64.23 H, 6.31 ; N,8.05.

7-(3-(2-(4-Methylpiperazin-1-yl) propionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.31): Off-white coloured powder, Yield:91.0% (0.5 g), Melting point 212°C., ^{1}H NMR (400 MHz $CDCl_3$) δ15.08, (s, 1H, -COO**H**), 8.60 (s, 1H, -NC**H**CCOOH), 8.00-7.96 (d, 1H, J= 14.3 Hz, -C(F)C**H**C), 6.66-6.64 (d, 1H, J= 7.08 -C(N)C**H**C), 5.11-5.09 (t, 1H, CH_2-C**H**(OCOR)-CH_2-), 4.53 (bs,2H, -N Bridged **H**), 4.30-4.24 (q, 2H, -NC**H$_2$**CH_3), 3.34-3.29 (q, 1H, -OCO(C**H)**CH_3), 2.68(bs, 4H, N-(C**H$_2$**)$_2$), 2.49 (bs, 4H, CH_3N-(C**H$_2$**)$_2$), 2.32 (s, 3H, -NC**H$_3$**), 2.27-2.11 (m, 6H, CH(**H**)-C(OCOR)-CH(**H**)-, -C**H**(H)-C(OCOR)-C**H**(H)-) and CH**(H)**-CH**(H)**-), 1.90,1.83 (dd, 2H, C**H(**H**)**-C**H(**H**)**-), 1.58-1.55(t,3H, -NCH$_2$C**H$_3$**),1.35-1.33(d, 3H,-OCO(CH**)**C**H$_3$**), ^{13}C NMR (100 MHz, $CDCl_3$)δ**(176.72, 176.69,** J= 3.0 Hz), 171.98, 167.53, **(152.54, 150.06,** J= 248 Hz), 146. 93, (**140.78, 140.67,** J= 11.0 Hz), 137.81, (**117.79, 117.72,** J= 7.0 Hz), (**113.36, 113.12,** J= 24.0 Hz**),** 107.92, **(101.23, 101.19,** J =4 Hz**),** 67.36, 62.81,55.54, 55.49, 55.74, 55.31, 49.64, 49.34, 45.99, 34.25, 29.17, 27.59, 27.56, 14.46,14.26. CHN Anal. Calcd. For $C_{27}H_{35}FN_4O_5$: C, 64.01; H, 6.86; N, 10.89. Found C, 63.83; H, 6.01 ; N,10.11

7-(3-(2-Morpholinopropionyloxy)-8-aza-bicyclo [3.2.1] octan-8-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4.32): Off-white coloured powder,Yield:80 % (0.28 g), Melting point 228°C, ^{1}HNMR (400 MHz $CDCl_3$) δ 15.34 (s, 1H, -COO**H**), 8.60 (s, 1H, -NC**H**CCOOH), 8.00-7.97 (d, 1H, J= 14.28 Hz, C(F)C**H**C), 6.66-6.64 (d, 1H, J= 7.12 Hz, -C(N)C**H**C), 5.13-5.11 (t,1H,-CH_2-C**H**(OCOR)-CH_2-), 4.54,(bs, 2H, -N-bridged **H**) ,4.30-4.24 (q, 2H, -NC**H$_2$**CH_3-), 3.74-3.72 (t, 4H, O(C**H$_2$**)$_2$), 3.31-3.26 (q, 1H, -OCO(C**H)**CH_3), 2.66-2.64 (t, 4H, N-(C**H$_2$**)$_2$) , 2.32-2.17 (m, 6H, CH(**H**)-C(OCOR)-CH(**H**)-), -C**H**(H)-C(OCOR)-C**H**(H)-) and CH**(H)**-CH**(H)**-, 1.89, 1.83 (dd, 2H, C**H(**H**)**-C**H(**H**)**-), 1.59-1.55 (t, 3H, -CH$_2$C**H$_3$)** , 1.36-1.34(d, 3H,-OCO(CH**)**C**H$_3$**), ^{13}C NMR (100 MHz, $CDCl_3$)δ (**176.69, 176.66,** J =3.0 Hz**),** 171.96, 167.47, (**152.51, 150.03** J= 248 Hz**)**, 146.91, (**140.74, 140.63** J= 11 Hz**),** 137.77, (**117.81, 117.73,** J= 8.0 Hz**),** (**113.35, 113.11,** J=24.0 Hz), 107.91, **(101.21, 101.16,** J= 4 Hz), 67.41, 67.20, 63.10, 55.49, 55.44, 55.42, 49.96, 49.61,34.22, 27.55, 14.44, 14.10. CHN Anal. Calcd. For $C_{26}H_{32}FN_3O_6$: C, 62.26; H, 6.43; N, 8.38. Found C, 62.03 H, 6.01 ; N,8.01.

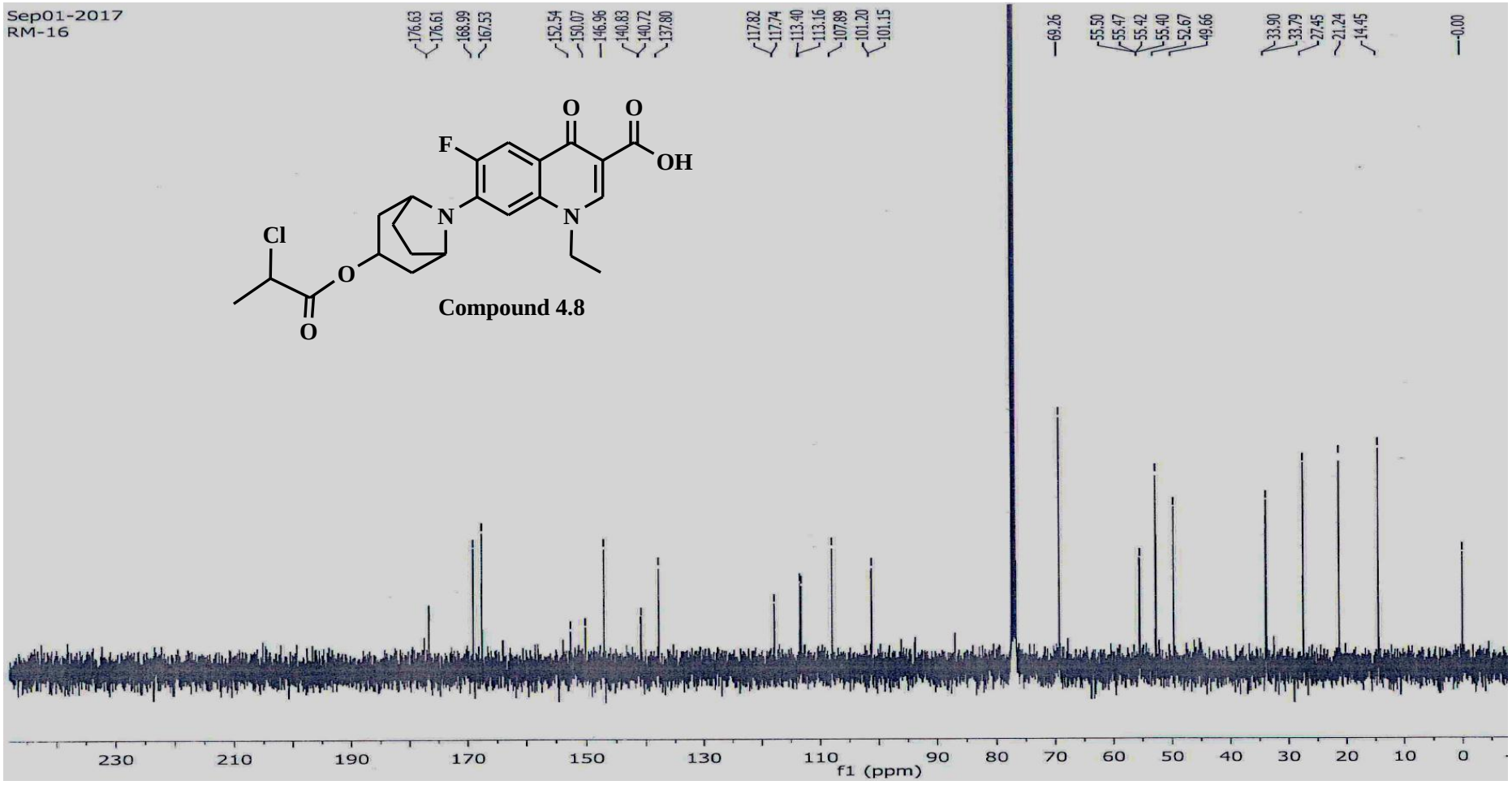
References:

1) Dileep,K.; Polepalli, S.; Jain, N.; Buddana, S, K.; Prakasham R,S.; Murty M,S,R. *Mol. Divers*, **2018** , 22, 83-93.
2) Guruswamy, B.; Arul, R. *Lett Drug Des Discov*, **2013**, 10, 86-93.
3) Guruswamy, B.; Arul, R. *J. Heterocycl. Chem*,. **2015**, 53,284-293.

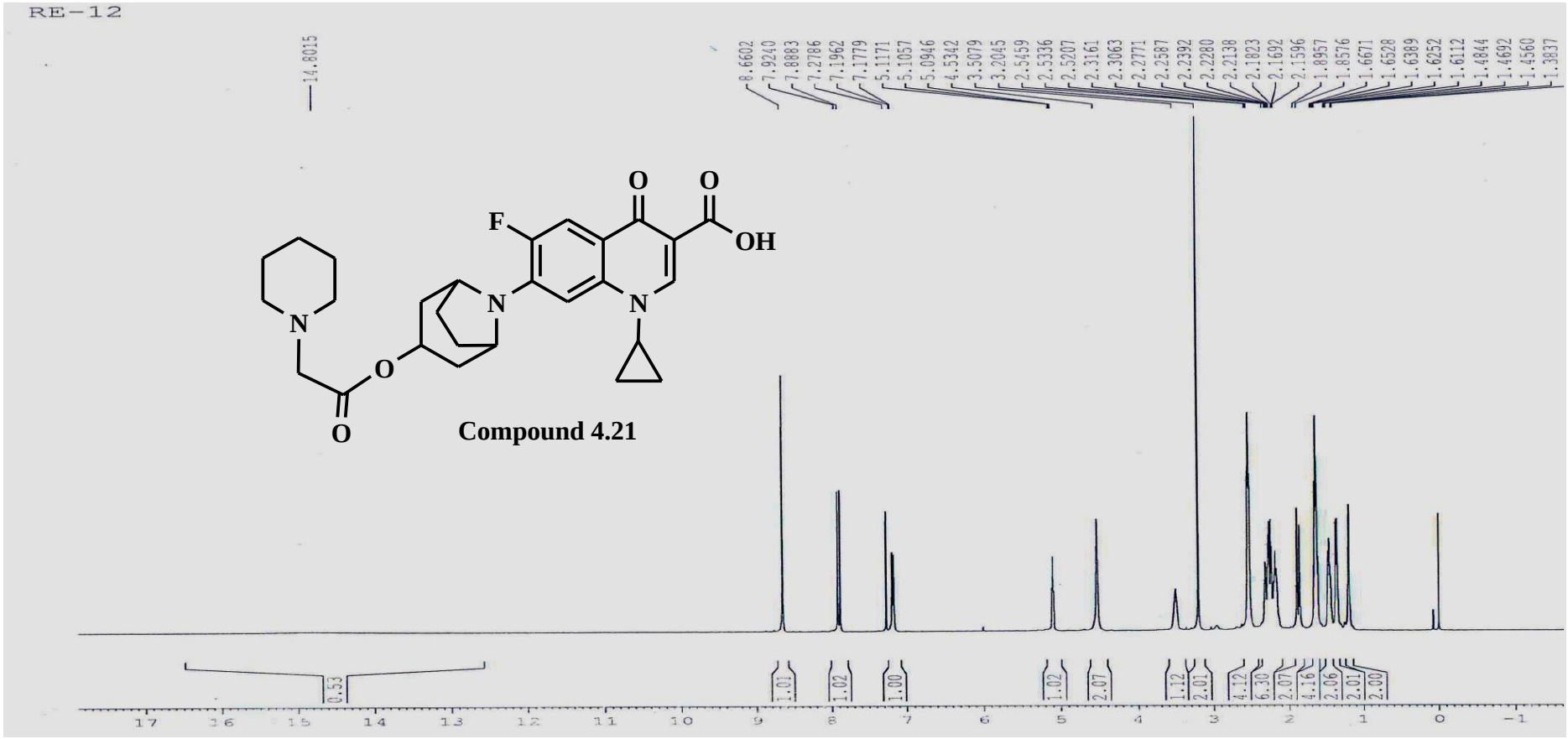


Compound 4.8

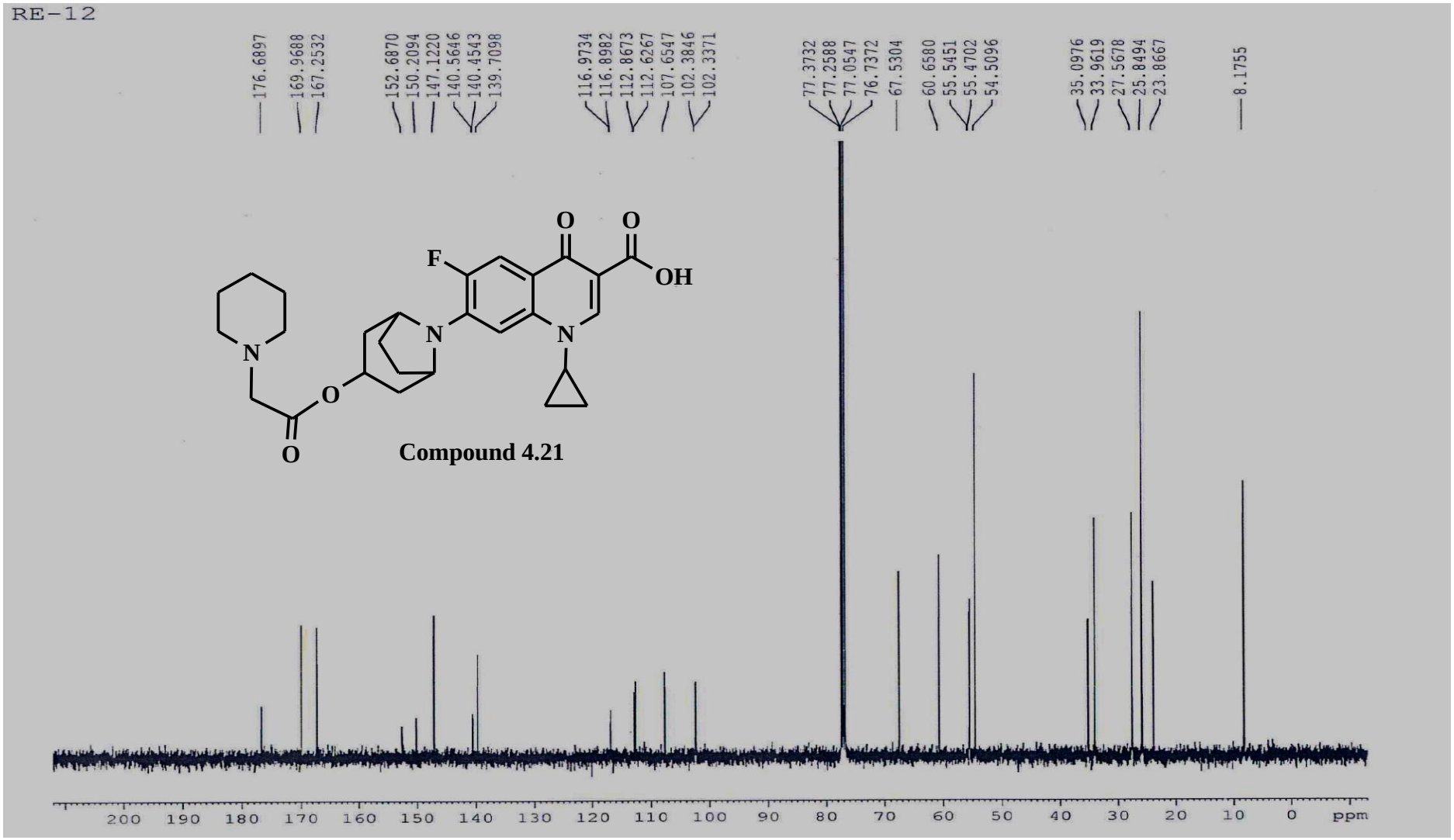
[1]H NMR spectra of compound 4.8



^{13}C NMR spectra of compound 4.8

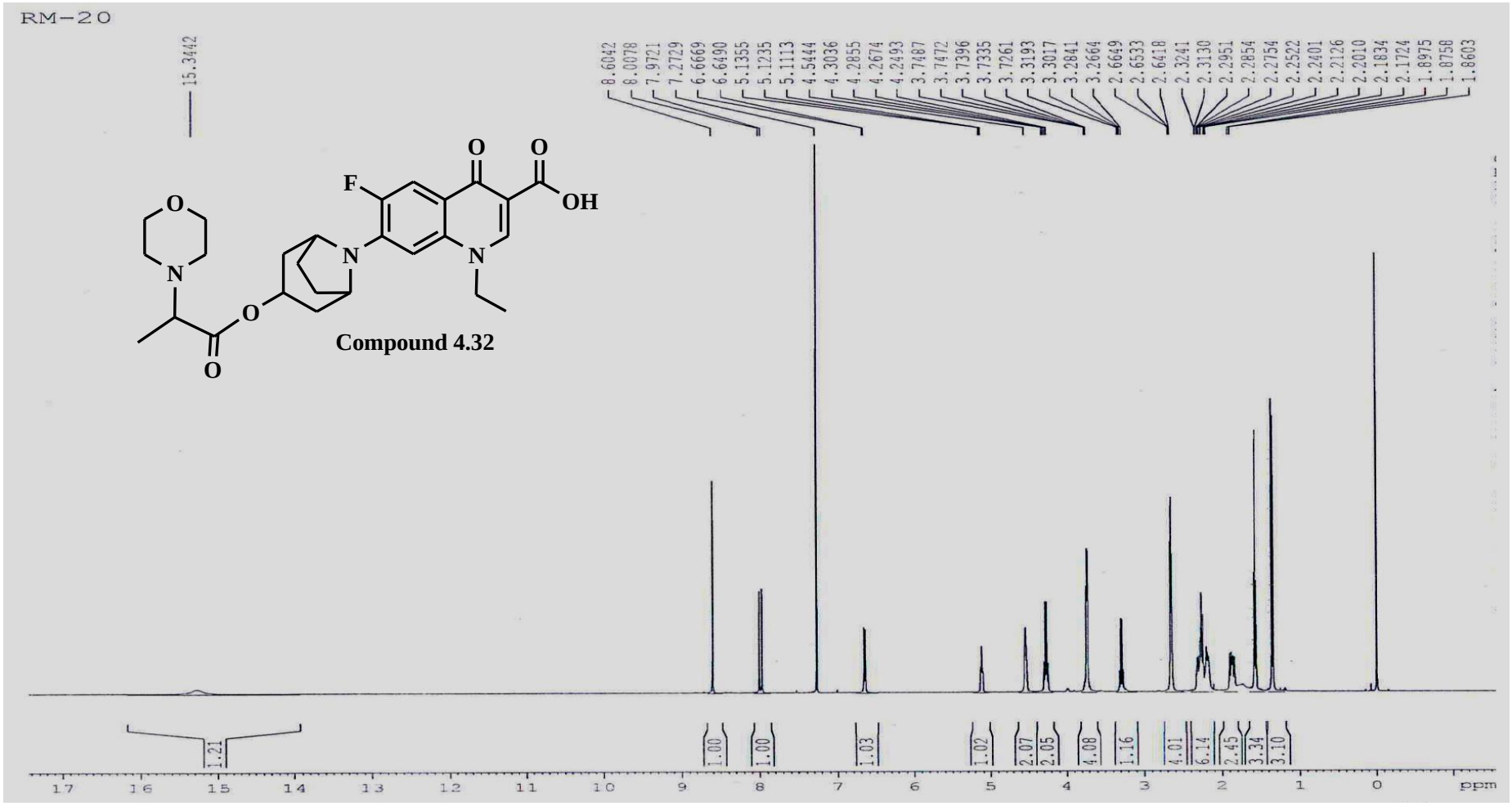


[1]H NMR spectra of compound 4.21

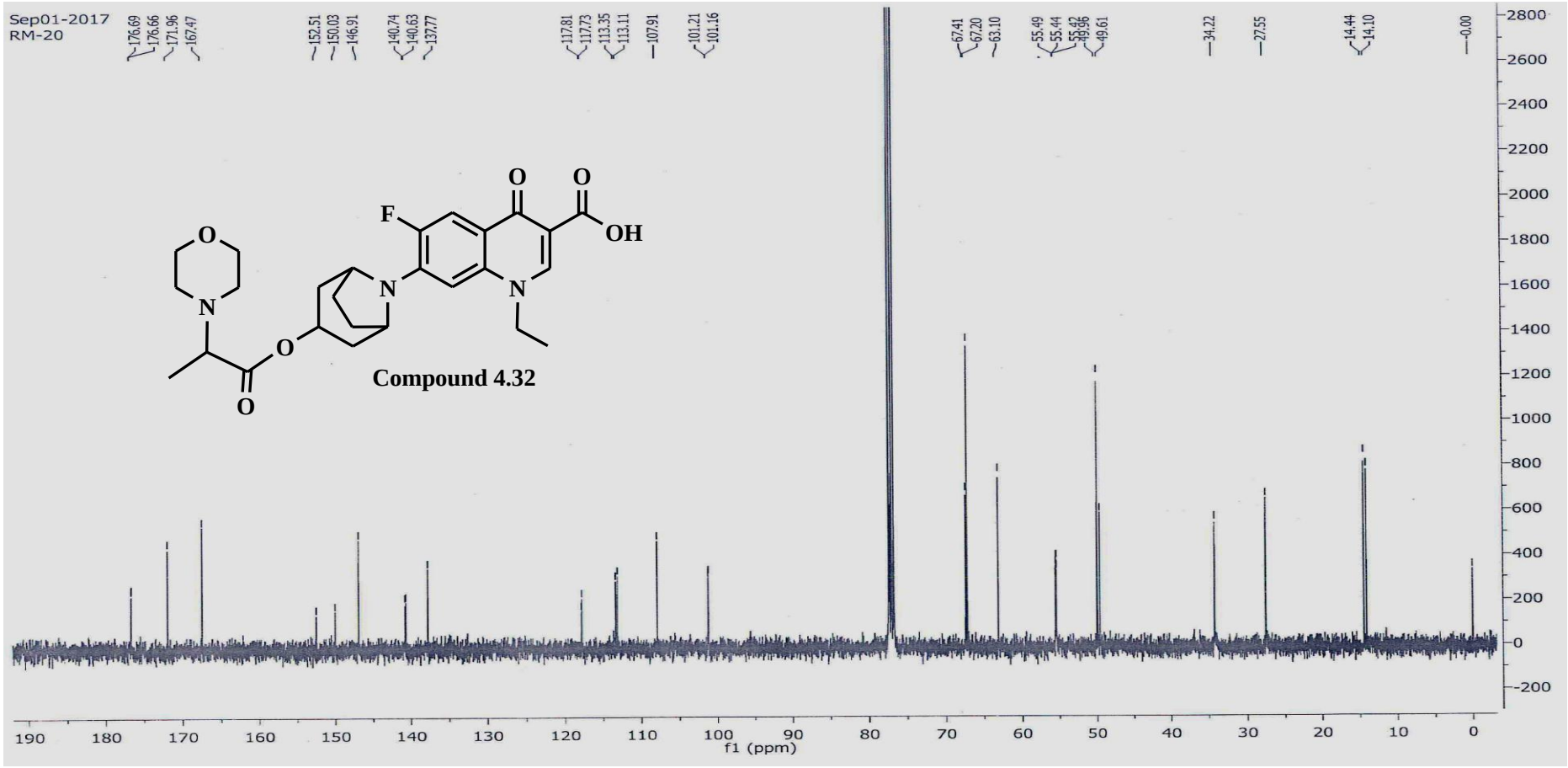


Compound 4.21

^{13}C NMR spectra of compound 4.21



[1]H NMR spectra of compound 4.32



^{13}C NMR spectra of compound 4.32

Chapter-5

Molecular Docking of the Synthesized Molecules with Target Protein

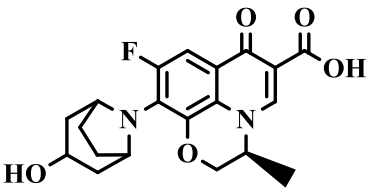
5.1 Introduction

Molecular docking has emerged as an important *in silico* tool for rationalizing biological activity of the new molecules based on interactions with their target protein[1]. Recent reports have predicted the averaged success rates of ten such docking programs between 54.0 and 68 percent[2]. This suggests that the algorithm can be used to explore the conformation of the protein-ligand complexes in its binding pocket to sufficient accuracy[3]. It was therefore envisaged to use docking for the biologically evaluated compounds, in the present work, to understand the molecular level interactions with the target protein.

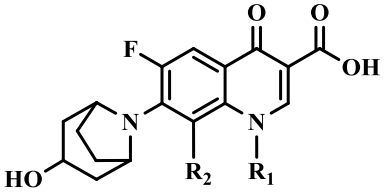
This chapter, therefore, reflects the results of antibacterial activity in the light of molecular interactions between the synthesized compounds and the target protein. The choice of target protein among all seven microorganisms evaluated in this study was *S. aureus*. This was due to the relatively inspiring results of all the synthesized series of compounds against *S. aureus* as compared to other microorganisms. The target chosen for the study was DNA gyrase B subunit of the chosen microorganism from PDB database[27]. The docking studies were done using AutoDock Vina software.

5.2 Docking studies with Nortropine Substituted Fluoroquinolones: All the compounds listed in **Table 2.5 (Chapter-2)** were docked with the target protein after preliminary studies of identifying the active site of the target using blind docking. **Table-5.1** below summarizes results for both the MIC and binding energy values. Compound **2.13** (-8.7 kcal/mol) displayed binding energy reduced by 0.7 kcal/mol with the target as compared to the reference molecule, Levofloxacin (-8.0 kcal/mol). This can be attributed to the increased number of hydrogen-bonding interactions in the case of compound **2.13** as discussed and shown below.

Table-5.1: Binding energies of the synthesized compounds docked against DNA gyrase B subunit of the *S. aureus* calculated using Auto Dock Vina software. MIC values have also been shown for the comparison.



Compound 2.13



2.15 : R_1= C_3H_5, R_2=H
2.16 : R_1=C_3H_5, R_2=OCH_3
2.17 : R_1=C_2H_5 R_2=H

Compound	*MIC* Value (nM)	Binding energy (kcal/mol)
Std. (levofloxacin)	125.0	-8.0
2.13*	**15.0**	**-8.7**
2.15	25	-8.6
2.16	15	-8.3
2.17	500	-8.4

* Compound **2.14** was the racemic mixture of compound **2.13** and therefore was not considered.

Figures 5.1a displays the only hydrogen bonding interaction of the C4 carbonyl in levofloxacin with arginine 1122 whereas in the case of compound **2.13** equivalent C4 carbonyl is involved in three hydrogen bonding interactions. **Figure 5.1b** illustrates two of these interactions with the arginine 1122 and one with the guanine base of the complexed DNA. Besides these, oxygen of the hydroxyl group in COOH at the C3 position can be seen as involved in hydrogen bonding interaction with the arginine 1122. Nortropine substituent at the C7 position, on the other end of the molecule **2.13**, forms two additional hydrogen-bonding interactions with the aspartic acid 1083 that are absent in the case of levofloxacin at the C7 end of the molecule with the piperazine. White arrows marked in **figures 5.1a** and **5.1b** display comparative interactions that have been discussed above.

Enhanced interactions in the case of nortropine derivatives can be attributed to the replaced position of the quinolone skeleton as contrasted to levofloxacin in **Figure 5.1c**. Besides, the larger size of the bicyclic nortropine as compared to piperazine in the case of synthesized compounds brings about additional interactions that account for the lower binding energy. **Figure 5.1d** displays the hydrogen bonding interactions both for compound **2.13** and levofloxacin in an overlay positions while docked in the protein structure.

Compounds **2.15** to **2.17** also display enhanced interaction of the C4 carbonyl group and the C3 hydroxyl group of COOH **(Figure 5.2)**. In the case of compounds **2.15** and **2.17 (Figure 5.2a** and **c** respectively**)**, the same amino acid residue, arginine 1122, as in the case of compound **2.13** can be seen interacting with C4 carbonyl.

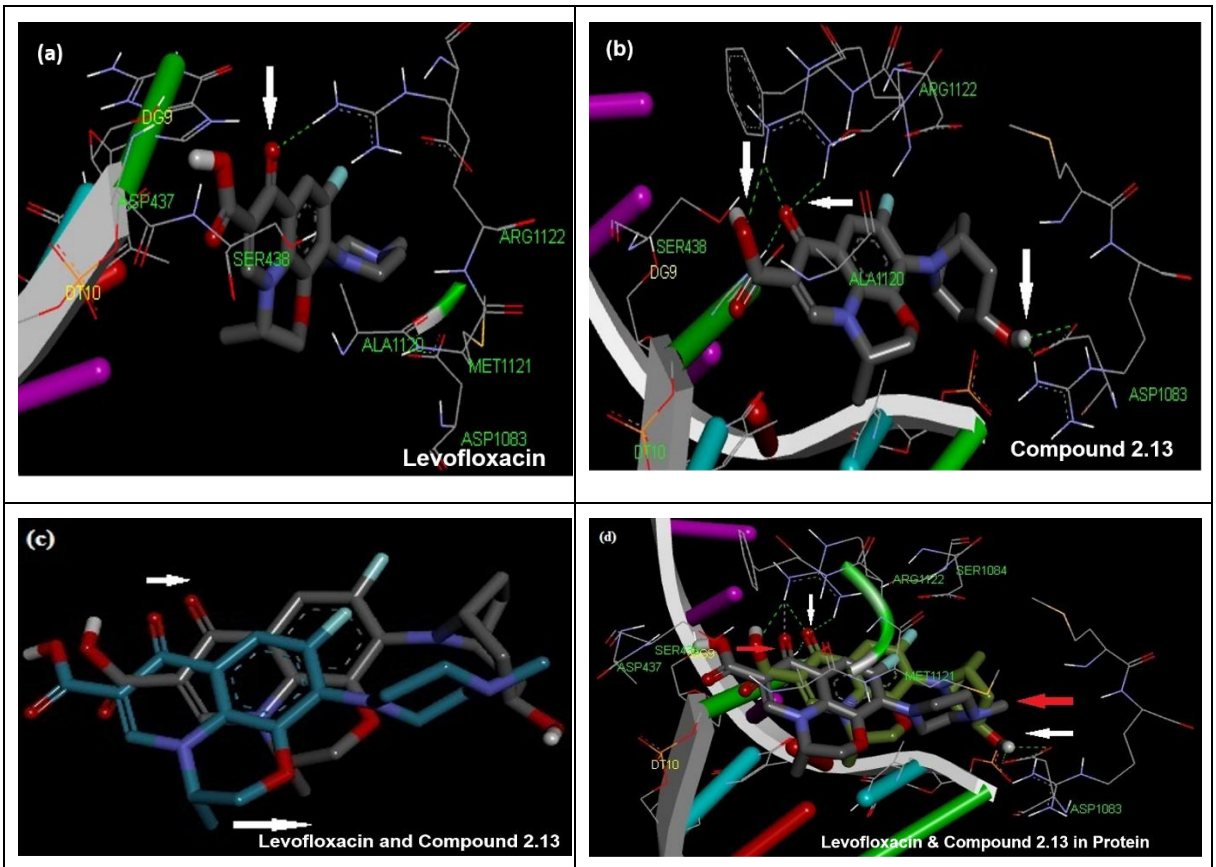


Figure-5.1: Ternary complex of the DNA gyrase B subunit of *S. aureus* from (PDB 2xct), DNA and **(a)** Standard drug Levofloxacin **(b)** Compound **2.13** using AutoDock Vina. (**c)** Replaced position of the quinolonine skelton in case of compound **2.13** as compared to standard levofloxacin in an overlay without pritein **(d)** and with the protein. Red arrow displays interaction with levofloxacin while white arrow is for compound **2.13.**

However, for compound **2.16**, it is the aspartic acid 1083 that interacts with C4 carbonyl **(Figure 5.2b)**. This may be due to the presence of the C8 methoxy group that drift the entire molecule away from arginine 1122 reducing the hydrogen-bonding interactions and thus comparatively reduced binding energy. Also, C4 carbonyl in the case of compounds **2.15** shows hydrogen bonding interactions with the base pair of DNA which is missing in the case of compound **2.17** but supplemented by a second interaction with the same arginine 1122 residue.

Both the compounds get further stabilized by interacting with serine 1084 and aspartic acid 1083 residues at C3 carbonyl of the COOH. The nortropine end of both the molecules remains interaction-free, unlike compound **2.13** justifying later's highest binding energy in the selected lot. Compared with compound **2.16** the interactions at C4, C3 carbonyls and the nortropine end are observable but are less in number as compared to compound **2.15** and compound **2.16** and thus lower binding energy.

The N1 substituted cyclopropyl derivatives, compounds **2.15** and **2.16** when compared for binding energy *vis-a-vis* MIC values, present an interesting case. It can be inferred from **Figure 5.2 (a, b)** and **Table 5.1** that although the displaced position of molecule **2.16** destabilized the binding energy by 0.3 kcal/mol, the MIC value showed, in contrast, shows enhancement of one and a half times. Compound **2.17**, on the other hand, gave an unexpected poor MIC value of 500 nM despite a comparable BE to that of compound **2.16**.

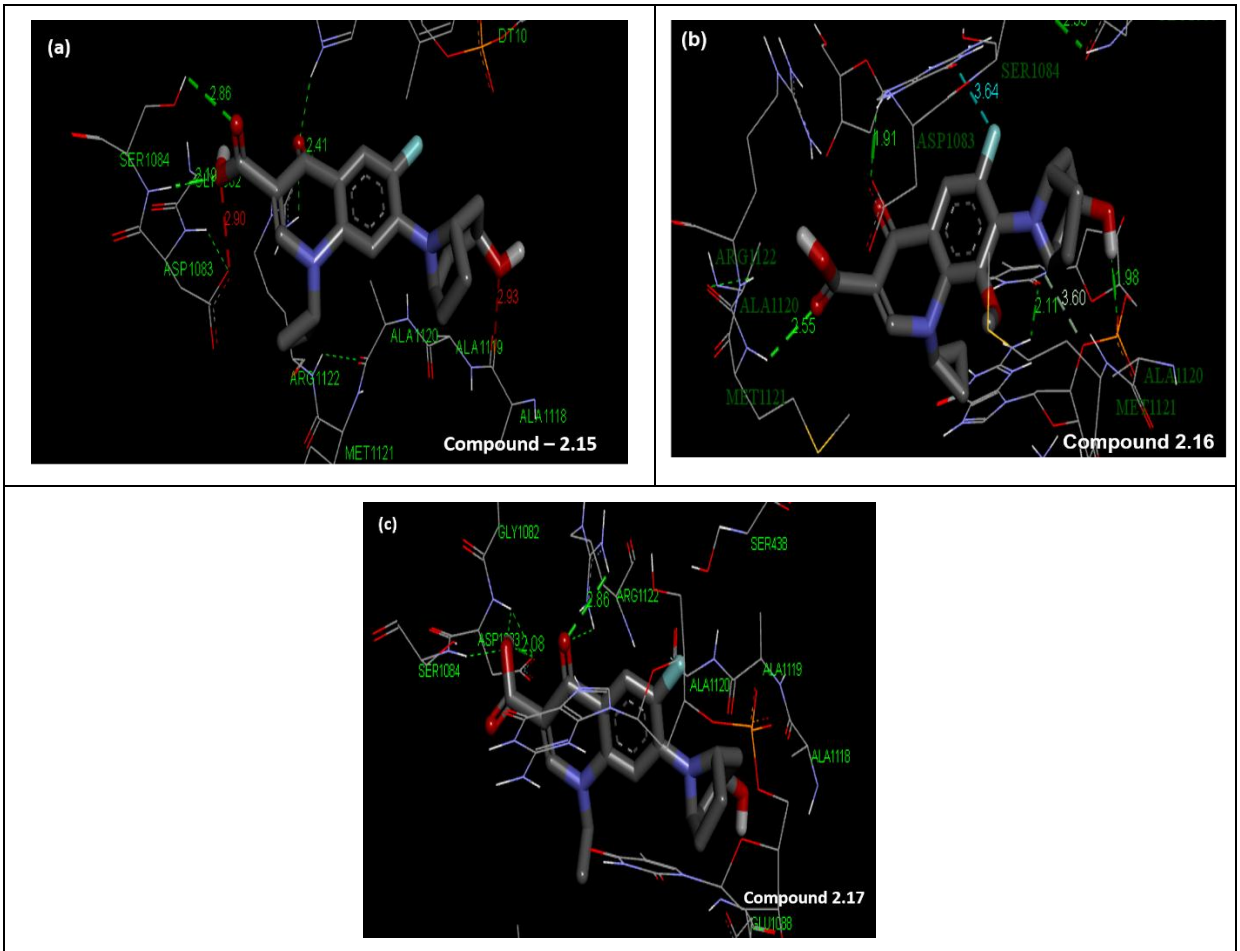


Figure 5.2: (a) Hydrogen bonding interactions with C4 and C3 carbonyls of the compound **2.15** at the quinolone end. **(b) I**nteractions at C4, C3 carbonyls, and the nortropine end in the case of compound **2.16**. **(c)** Hydrogen bonding interactions with C4 and C3 carbonyls of the compound **2.17** at the quinolone end.The nortropine end of compound **2.15** and **2.17** remains interaction-free,

This can be attributed to the low bioavailability of the N1 ethyl substituted class of fluoroquinolones that belong to the norfloxacin category[5]. Thus, docking studies and biologically established results gave a moderate doctrine about the antibacterial activity of the synthesized compounds.

5.3 Docking Studies with Aliphatic Esters of Nortropine Substituted Fluoroquinolones

This section correlates the binding of compounds listed in **Table 3.5 (Chapter-3)** with the

DNA gyrase B subunit of *S. aureus* as described above[27]. A glance at **Table 5.2** reveals that compound **3.1** was the most effective in series when compared with standard drug levofloxacin. As discussed in Chapter -3, the methyl ester derivative of compounds **2.13** displayed an improved MIC with the same zone of inhibition at half the concentration to that of the standard and the binding energy lowered by 0.8 kcal/mol.

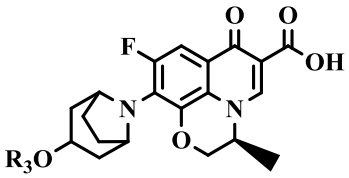
The methyl esters of the other parents namely compounds **3.13**, **3.19** and **3.25** displayed an equivalent zone of inhibition but with a dosage that was four to eight times higher as compared to the standard drug levofloxacin **(Table 5.2)**. Similarly, ethyl esters analogs compounds **3.2**, **3.14**, **3.20** and **3.26** furnished identical zone of inhibition at exceptionally higher concentrations ranging from four to thirty-two times to that of the standard. All higher esters specifically propyl, butyl, tert-butyl and isopropyl derivatives also, did not show any encouraging results to be considered as antibacterials.

This is interesting to note that despite the meager antibacterial activity, the binding energy of all such molecules was considerably improved, as contrasted to standard drug levofloxacin. Theoretical calculations for all the esters displayed, (**Table 5.2**) binding energy within the same range as that of their potentially antibacterial parent compounds. As compared to standard levofloxacin (8.0 kcal/mol), the binding energy was appreciably improved for the derivatives of compounds **2.13** and **2.15 (**up to 0.8 kcal/mol) and moderately improved for the derivatives of compounds **2.16** and **2.17** (up to 0.4 kcal /mol). The only molecule that gave binding energy compatible with its MIC value was compound **3.1**.

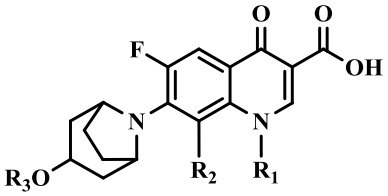
A glimpse of the DNA gyrase B subunit docked with ester derivatives shows the same set of amino acid residues as that their parent compounds suggesting the matching binding pocket for the former **(Figure 5.3)**.

A closer look at the quinolone skeleton of the molecules shown in **Figure 5.3** displays the interaction of either C4 carbonyl or C3 carboxylic acid with amino acid residues. Hydroxyl group of C3 carboxylic acid in compound **3.2** can be observed in hydrogen bonding interactions with aspartic acid 437 (**Figure 5.3a)**. Compound **3.17** is observably interacting with the serine 438 amino acid residue at C4 carbonyl while its adjacent carbonyl peptide bond interacts with the C3 hydroxyl group (**Figure 5.3b)**. Interestingly, the C6 fluorine for the first time shows its affinity with both amino groups of the arginine 1122. Compounds **3.20** and **3.25** show single interaction on the quinolone side with alanine 1120 and with serine 1084 residues at the carbonyl and the hydroxyl group of the C3 carboxylic acid respectively.

Table-5.2: Binding energies of the aliphatic esters of the nortropine substituted fluoroquinolones docked against DNA gyrase B subunit of the *S. aureus* calculated using Auto Dock Vina software. MIC values have also been shown for the comparison.



Compounds 3.1 to 3.6



Compounds 3.13 to 3.30

Parent Compound ***(Binding Energy - kcal/mol)***	**Compound**	**R_1**	**R_2**	**R_3**	***S. aureus* MTCC 1430**		**Binding Energy (kcal/mole)**
					ZOI (mm)	**MIC at ZOI (µg/ml)**	
	Levofloxacin	-	-	-	**11**	**4**	-8.0
2.13 *(-8.7)*	3.1	-*CH(CH_3)CH_2O-	-	**CH_3**	**11**	**2**	-8.8
	3.2	-*CH(CH_3)CH_2O-	-	CH_2CH_3	11	32	-8.7
	3.3	-*CH(CH_3)CH_2O-	-	$(CH_2)_2CH_3$	11	32	-8.6
	3.4	-*CH(CH_3)CH_2O-	-	$(CH_2)_3CH_3$	11	32	-8.6
	3.5	-*CH(CH_3)CH_2O-	-	$C(CH_3)_3$	9	32	-8.5
	3.6	-*CH(CH_3)CH_2O-	-	$CH(CH_3)_2$	10	32	-8.4
2.15 *(-8.6)*	3.13	$-C_3H_5$	H	**CH_3**	**11**	**16**	-8.6
	3.14	$-C_3H_5$	H	**CH_2CH_3**	**11**	**16**	-8.2
	3.15	$-C_3H_5$	H	$(CH_2)_2CH_3$	8	64	-8.0
	3.16	$-C_3H_5$	H	$(CH_2)_3CH_3$	4	128	-8.3
	3.17	$-C_3H_5$	H	$C(CH_3)_3$	10	64	-8.4
	3.18	$-C_3H_5$	H	$CH(CH_3)_2$	8	64	-8.4
2.16 *(-8.3)*	3.19	$-C_3H_5$	-OCH_3	**CH_3**	**14**	**32**	-8.3
	3.20	$-C_3H_5$	-OCH_3	**CH_2CH_3**	**11**	**64**	-8.2
	3.21	$-C_3H_5$	-OCH_3	$(CH_2)_2CH_3$	10	32	-8.1
	3.22	$-C_3H_5$	-OCH_3	$(CH_2)_3CH_3$	4	128	-8.0
	3.23	$-C_3H_5$	-OCH_3	$C(CH_3)_3$	8	65	-8.0
	3.24	$-C_3H_5$	-OCH_3	$CH(CH_3)_2$	8	64	-8.2
2.17 *(-8.4)*	3.25	$-C_2H_5$	H	**CH_3**	**12**	**32**	-8.4
	3.26	$-C_2H_5$	H	CH_2CH_3	10	128	-8.1
	3.27	$-C_2H_5$	H	$(CH_2)_2CH_3$	4	128	-8.4
	3.28	$-C_2H_5$	H	$(CH_2)_3CH_3$	0	128	-8.3
	3.29	$-C_2H_5$	H	$C(CH_3)_3$	0	128	-8.3
	3.30	$-C_2H_5$	H	$CH(CH_3)_2$	4	128	-8.2

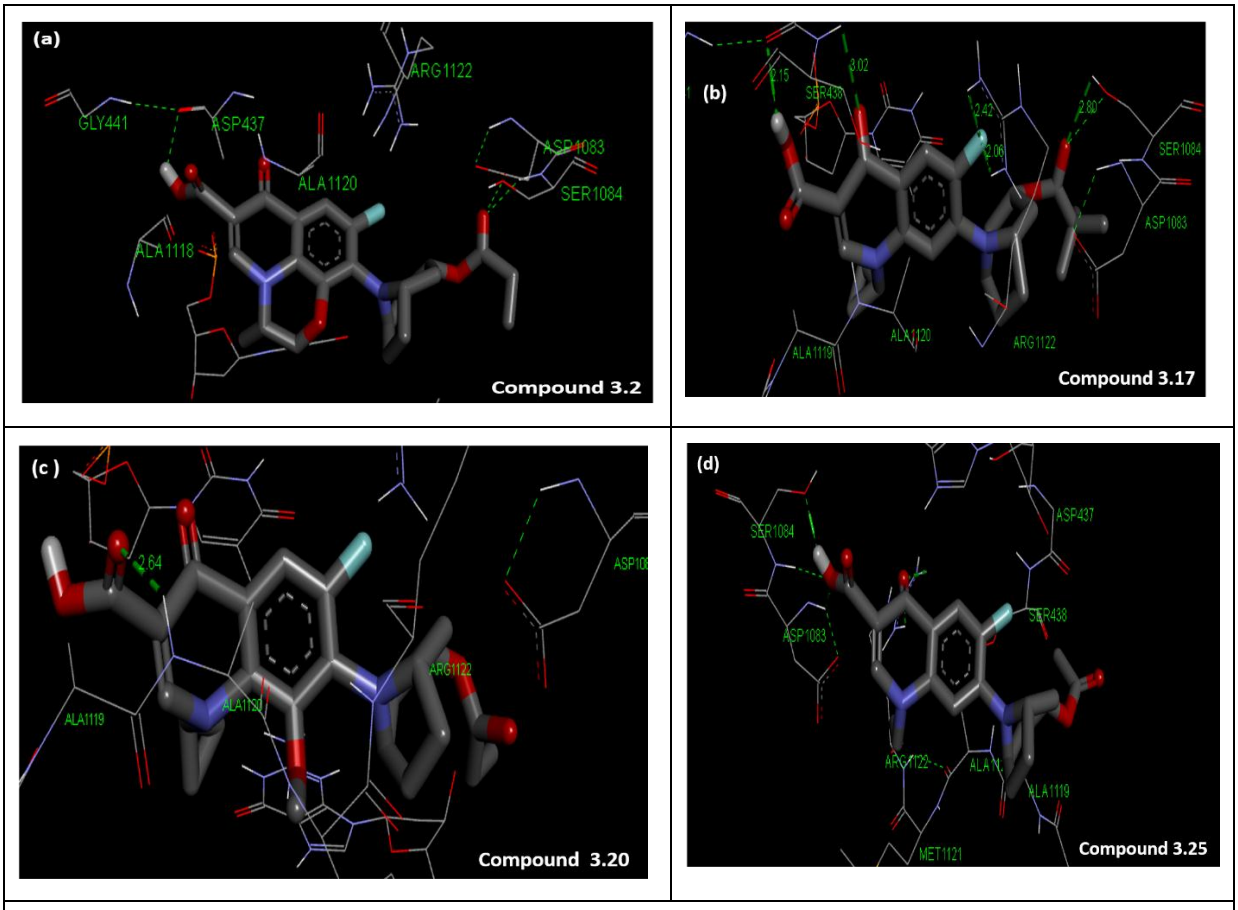


Figure 5.3: (a) Compound **3.2** can be observed in hydrogen bonding interactions with aspartic acid 437, 1083 and serine 1084 **(b)** Compound **3.17** is observably interacting with the serine 438 and 1084 **(c)** Compound **3.20** shows interaction with alanine 1120 **(d)** Compound **3.25** shows interaction with serine 1084 residues. The nortropine end of compound **3.20** and **3.25** remains interaction-free.

The fact that methyl and ethyl ester derivatives (**3.20** and **3.25 Figure 5.3c and d**) are in the vicinity of the same amino acid residues as their parent compounds (2.16 and **2.17 Figure 5.2b and c**) evidence that both categories fit in the same binding pocket. Therefore, methyl esters reporting comparable binding energy and antibacterial activity is not surprising.

At the nortropine end of all the four molecules displayed above, only two compounds namely **3.2** and **3.17** show interaction with the amino acid residues. Serine 1084 is the common amino acid residue that interacts with both the compounds via the carbonyl group of the ester. In the case of compound **3.2** an additional amino acid residue aspartic acid 1083 form an additional hydrogen bond. In compound **3.20**, the same aspartic acid 1083 residue can be observed in compound **3.20 (Figures 5.3 (c))** without any interactions. The smaller methyl group of the ester on the nortrpoine side drifts the molecule thereby moving both these residues towards the quinolone side.

The reduced bioavailability due to increased hydrophobicity as a result of the aliphatic ester chain can be hypothesized reason for the compromised antibacterial activity for this series of

compounds. Comparable binding energy to that of their parent compounds and the marginally improved antibacterial activity of methyl esters as compared to the rest of the respective lot is evidence of that.

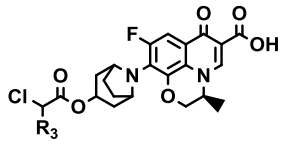
5.4 Docking Studies with Chloroacetyl and 2-Chloropropionyl Linked Esters of Nortropine Substituted Fluoroquinolones: This section correlates the binding of the compounds with the DNA gyrase B subunit of *S. aureus* that gave MIC value in the range of 4 to 16 μg/ml for the fluoroquinolone-resistant strains listed in **Table 4.5 (Chapter-4)**. The selection eliminated all heterocyclic-linked derivatives as their MIC values ranged from 32 to more than 64 μg/ml. Therefore **Table 5.3** below shows the structure, MIC values and the binding energy of the shortlisted compounds. Further, compounds **4.3** and **4.4** were not considered due to their racemic nature in the light of their optically pure analogs **4.1** and **4.2**. Remarkably, the MIC value against fluoroquinolone sensitive strains for the selected compounds ranged from less than or equal to 0.5 and 4 μg/ml **(Table 5.3)**.

A glance at the listed compounds in **Table 5.3** shows that 2-chloropropionyl linked esters (**R_3 = CH_3** Compounds **4.2**, **4.6** and **4.8**) gave comparatively higher binding energy as compared to chloroacetyl esters analogs (**R_3 = H** Compounds **4.1**, **4.5** and **4.7**). Levofloxacin, the reference molecule, that gave MIC values comparative to the synthesized compounds against fluoroquinolone sensitive strains (0.5 μg/ml) however gave binding energy values lower by 0.5 to 1.6 kcal/mol.

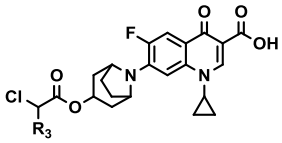
The fact that the same amino acid residues namely arginine 1122, alanine 1120, aspartic acid 1083 and serine 1084 interacted with the synthesized compounds shows that the binding pocket was the same as that of the other compounds discussed above **(Figure 5.4)**. Remarkably, five of the six selected compounds displayed interaction with the C6 fluorine and that accounts for the high stability (due to lower binding energy) of the molecules in the system. The binding energy was ranged between -9.6 kcal/mol to -8.5 kcal/mol for all the molecules.

At the quinoline end, the C4 carbonyl interacted with amino acid arginine 1122 in the case of compounds **4.1**, **4.2** and **4.6 (Figure 5.4a, b** and **d)**. However, in the case of compounds **4.5** and **4.7 (Figure 5.4c** and **e)** same C4 carbonyl interacted with alanine 1120 and serine 1084 respectively. No interaction of the C4 carbonyl was observed in the case of compound 4.8 (**(Figure 5.4f)**.

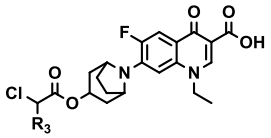
Table-5.3: Binding energies of the chloroacetyl and 2-chloropropionyl linked esters of nortropine substituted fluoroquinolones docked against DNA gyrase B subunit of the *S. aureus* calculated using Auto Dock Vina software. MIC values have also been shown for the comparison.



Compounds 4.1 and 4.2



Compounds 4.5 and 4.6



Compounds 4.7 and 4.8

S. No	Compd. No.	MIC (µg/ml)				Binding energy in Kcal/mol
		R_3	FQ Resistant Strain		FQ sensitive strain	
			S. aureus MRSA 10198	*S. aureus* MRSA 10193	*S. aureus* 29213	
29.	**4.1**	H	8	4	≤0.5	-9.0
30.	**4.2**	CH_3	8	8	≤0.5	-9.6
31.	**4.5**	H	16	16	≤0.5	-8.5
32.	**4.6**	CH_3	8	>64	1	-9.0
33.	**4.7**	H	16	16	2	-8.7
34.	**4.8**	CH_3	8	8	4	-8.9
35.	Levofloxacin		32	32	0.5	-8.0

Unlike most of the molecules discussed in previous sections, where either C3 carboxylic acid or C4 carbonyl interacted with the amino acid residues, in this case, interactions with both C3 carboxylic acid and C4 carbonyl groups were observed here. Carbonyl group of the C3 carboxylic acid in compounds **4.5**, **4.7** and **4.8 (Figure 5.4c, e** and **f)** interacted with arginine 1122, methionine 1121 and alanine 1120 and DNA base guanine respectively. Hydroxy group of the C3 carboxylic acid interacted with serine 1084 in compound **4.6 (Figure 5.4d)**. No interactions at this position were observed in case levofloxacin derivatives, compounds **4.1** and **4.2.**

As mentioned above C6 fluorine interacted with aspartic acid 1083 in the case of compounds **4.1** and **4.2 (Figure 5.4a** and **b)**, with glutamic acid 1088 in the case of compound **4.5** and with DNA base pairs in the case of compound **4.7** and **4.8**. The fluorine in the case of compound **4.6** did not show any interaction.

At the nortropine end, a common amino acid residue, alanine 1120, was involved in the interaction with compounds **4.1**, **4.2**, **4.6** and **4.7** via the carbonyl group of the chloro ester. The same carbonyl group of the compound **4.8** displayed double interactions with serine 1084 and DNA base pair.

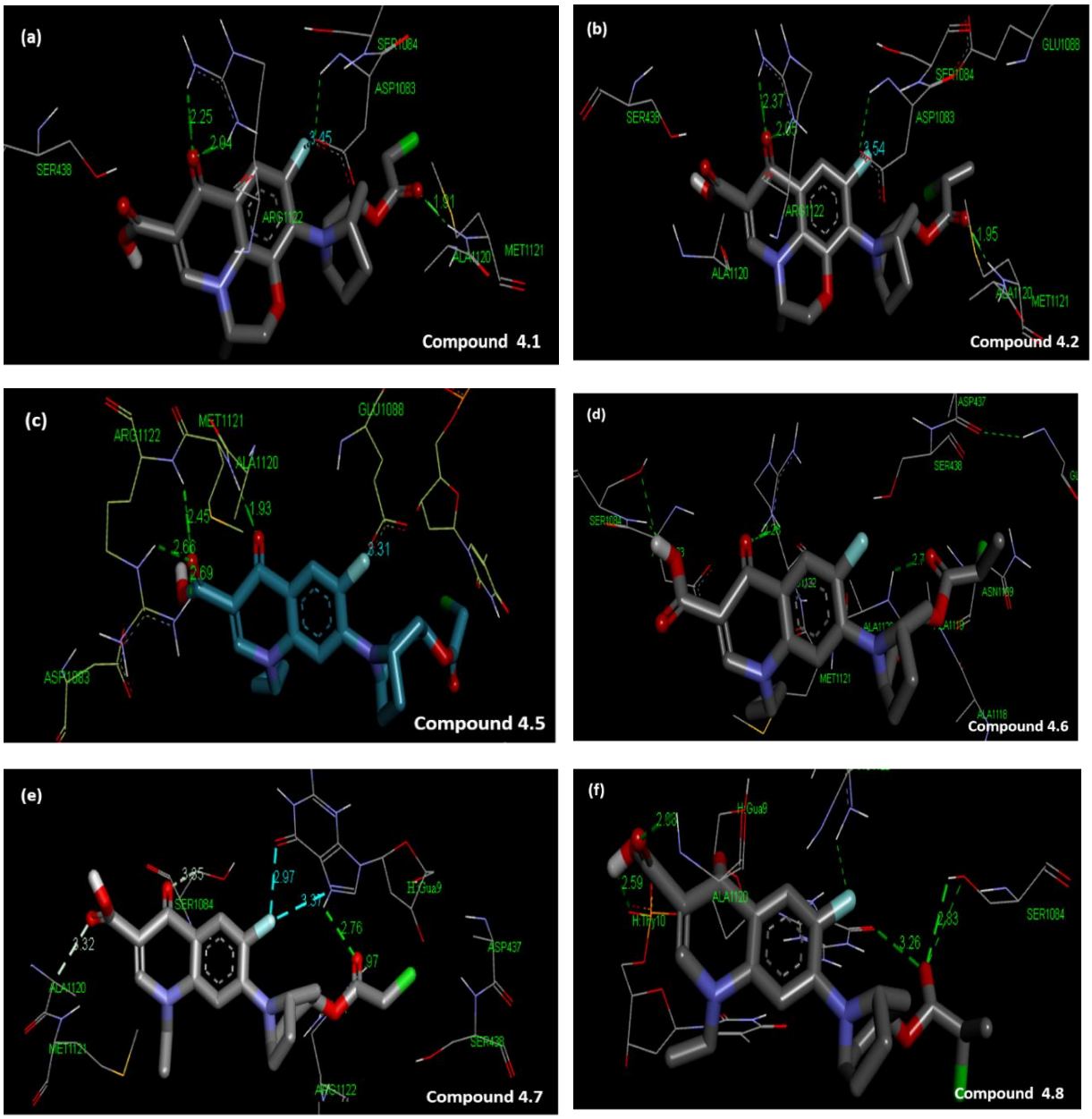


Figure 5.4: (a) Compound **4.1** **(b)** Compound **4.2** interacting with protein at quinolone, nortropine ends and C6 fluorine. **(c)** Compound **4.5** interacting at quinoline end and C6 fluorine. **(d)** Compound **4.6** interacting at quinoline quinolone and nortropine end. **(e)** Compound **4.7** and (f) Compound **4.8** interacting with protein at quinolone, nortropine ends and C6 fluorine

Overall, three sets of compounds were prepared. In the parent compound series compounds **2.13** and **2.16** were the most potent ones (MIC 15 nM) and displayed interactions both at the quinolone and at the nortrpoine ends. Similarly, in the last discussed chloroester series compounds **4.1**, **4.2** and **4.8** (MIC value ranging from 4 to 8 μg/ml) displayed interaction not only at quinolone and nortropine ends but also with C6 fluorine. Aliphatic ester series of the compounds although displayed interactions at both end and gave very high binding energy values but were did not pass the threshold to qualify as antibacterials due to the reduced bioavailability as a result of introduced hydrophobic (aliphatic) ester groups.

In summary, the docking of the synthesized molecules with the target protein of the *S. aureus* could identify and correlate the antibacterial activity of C7 substituted fluoroquinolones. This included carbonyl on the nortropine esters, C6 fluorine and already known C3 carboxylic acid and C4 carbonyl groups. The studies also concluded that polar moieties on the nortropine side help in enhancing the antibacterial activity while hydrophobic moieties reduce the antibacterial activity. The results and conclusions will help design future molecules not only for antibacterial activity but also for other biological activities.

References:

1. Jerome, R.; Guillaume, B.; Ralf, B.; Marc, L. *Adv Appl Bioinform Chem*. **2016**; 9, 1–11.
2. Nataraj, P.; Khajamohiddin, S.; Jack, T. Biophys Rev. 2017; 9 (2), 91–102.
3. Ferreira, G.; Ricardo, S.; Glaucius, O.; Adriano, A. Molecules. 2015 ; 20 (7), 13384–13421**.**
4. Bax , B.D.; Chan, P. F.; Eggleston , D.S.; Fosberry , A; Gentry , D.R.; Gorrec , F.; Giordano , I ; Hann , M.M.; Hennessy, A.; Hibbs , M.; Huang, J.; Jones, E.; Jones , J .; Brown , K.K.; Lewis , C.J.; May , E.W.; Saunders , M.R.; Singh , O.; Spitzfaden, C.E.; Shen , C.; Shillings, A.; Theobald , A.J. ; Wohlkonig , A.; Pearson , N.D.; Gwynn, M.N; *Nature*, **2010**, 466, 935-940.
5. Thu, D.; Zyta. M. Z.; Mark. B. *Medchemcomm.* **2019** ; 10(10), 1719–1739.

Chapter-6

Conclusion and Outlook

The emergence of resistance against antibacterials is a common problem and fluoroquinolones are no exception to this. Thus, there is a need to develop new molecules to hold off the resistance continuously. Prominent molecules ever since the discovery of Nalidixic acid have been highlighted in the introduction and literature survey part of this work as a background to the work entitled "synthesis, characterization and antibacterial activity of some fluoroquinolones". This section discusses the importance of substituents at different positions of fluoroquinolones and their influence on biological activity. From the discussion, it emerges that C-7 substituents of the fluoroquinolone molecules are the most important, explored and commercialized ones, consequently being the successful antibacterials. However, not many bicyclic rings containing nitrogen linkage at the C-7 position have been reported so far. Therefore, the rationale to substitute *endo-nortropine*, a naturally occurring bridged alkaloid, at the position is pretty justified. The section further describes the mechanism of action for this class of molecules that target essential bacterial enzymes DNA gyrase and topoisomerase for antibacterial activity and concludes by informing some new molecules' synthesis and antibacterial activity from the recent literature.

Chapter-2 of the work describes the synthesis of seven new fluoroquinolones **(Scheme -2.2; Compounds 2.13 to 2.19)** having *endo-nortropine* at the C-7 position of different Q-acids. Five of the seven starting Q-acids **(2.6 to 2.11)** were procured from commercial sources while the precursors for the two products (**2.18** and **2.19)** were synthesized starting from 2,3,4,5-tetrafluorobenzoyl chloride and 3-4-difluorophenyl isothiocyanate respectively using the known protocol **(Scheme- 2.3 and 2.4)**. *Endo-nortropine* required for the purpose was also synthesized by an already reported chemical route with a slight modification as depicted in **Scheme 2.1.** The starting Q-acids differed in the substitution at the N-1 position except for compounds **2.6** and **2.7** that were the same but chiral and racemic respectively. The section discusses the results of the spectroscopic (^{1}H, ^{13}C NMR, Mass) and chromatographic (HPLC) investigation of all the synthesized compounds.

Antibacterial activity of all the synthesized compounds (**2.13 to 2.19)** was carried out in two phases. Initial screening against Gram-positive [*Staphylococcus aureus* (MTCC 1430) and

Bacillus subtilis (MTCC 441)] and Gram-Negative [*Escherichia coli* (MTCC 1687) and *Vibrio cholerae* (MTCC 1934)] bacteria by the agar diffusion method identified five compounds (**2.13** to **2.17)** that gave zone of inhibition (ZOI) in the range of 9-15 mm with a dosage ranging between 0.5 µg/ml to 4 µg/ml **(Table 2.4)**. Levofloxacin and ciprofloxacin were used as controls. Compound **2.13** was identified as the most impressive with ZOI comparable to controls.

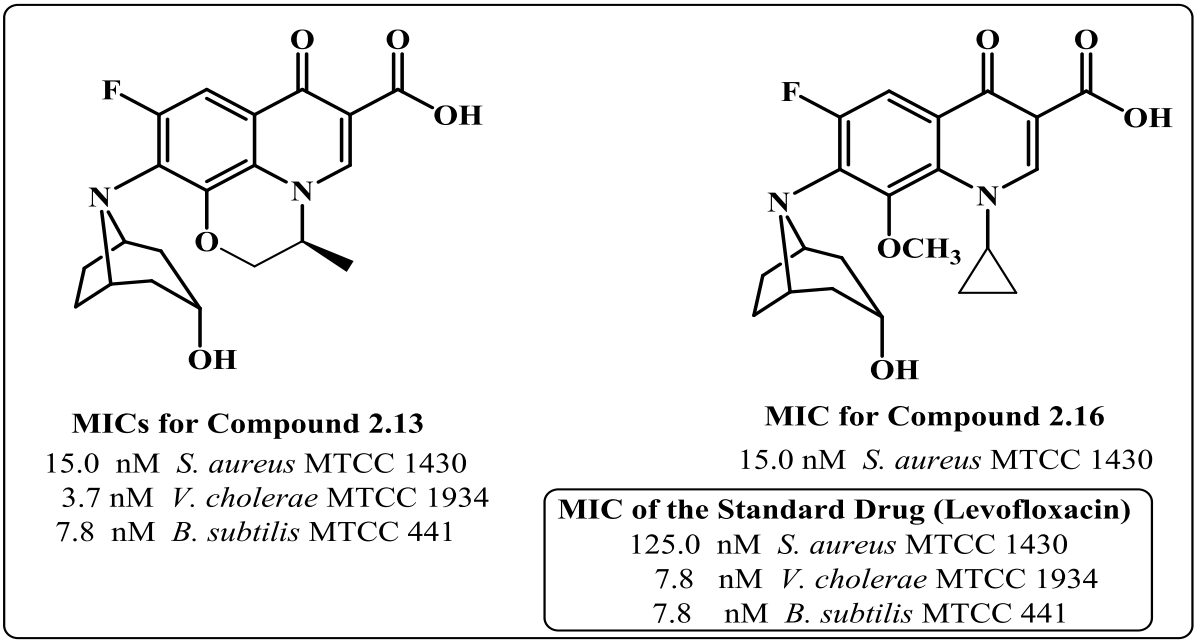


Figure – 6.1: Structure and the MICs of the potential compounds of C7 substituted nortropine derivatives of the fluoroquinolones (Parent Compounds) described in Chapter-1.

Minimum inhibitory concentration (MIC) of the shortlisted compounds (**2.13** to **2.17)** using microdilution broth assay gave results in the nM range **(Table 2.4)** for all the shortlisted compounds. The assay was carried out using serial dilutions from the stock of each drug taking levofloxacin as a control. Compounds **2.13 and 2.16**, the C-7 *endo-nortropine* derivative of the levofloxacin and ciprofloxacin/Moxifloxacin respectively, were eight times more potent than standard drug levofloxacin against S. *aureus.* Compound **2.13** was two times more potent against *V. cholera* equally potent against *B. subtilis* as compared to control levofloxacin. The results in this part of the study hold great significance cconsidering that *S. aureus* is one of the main organisms for several infections types, including most serious hospital-acquired infections and the reports of the re-emergence of *V. cholera* in certain parts of the word.

Further, as part of the next study **(Chapter-3)**, it was envisaged to derive the fluoroquinolones synthesized in the previous section with aliphatic and aromatic acyl chlorides at the hydroxyl group of the C-7 substituted nortropine to evaluate their antibacterial activity. The literature and structure-activity relationship evidenced that the N-4 position of the piperazine substituted with bulky groups in many fluoroquinolones enhanced antibacterial potency against Gram-positive bacteria.

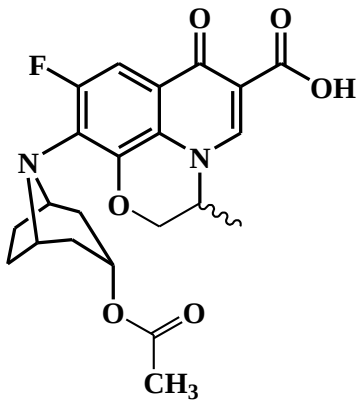
Thus, compounds **2.13** to **2.17,** synthesized previously, were reacted with aliphatic and aromatic acyl chlorides to yield forty-two compounds. Thirty of these compounds **(Table 3.1, compound 3.1 to 3.30)** with Acetyl, propanoyl, butyryl, valeryl, isobutyryl and pivaloyl chlorides **(Section-A)** gave moderate to excellent yields. In contrast, with aromatic acid chlorides, only twelve **(Table 3.3, compound 3.31 to 3.42)** products could be afforded **(Section-B)** in moderate to poor yields. Only three of the five fluoroquinolones **(2.13, 2.14** and **2.17)** afforded reactions with chlorides of the benzoic acid, phenylacetic acid, 4-chloro phenylacetic acid and racemic o-acetyl tropic acid. Attempts to afford esterification product using precursors **2.15** and **2.16** using different bases like potassium carbonate, sodium carbonate, triethylamine and a biphasic aqueous solution of sodium hydroxide were unsuccessful. All the synthesized compounds were characterized using spectroscopic (^{1}H, ^{13}C NMR, Mass) and chromatographic (HPLC) investigations and systematically discussed in the section.

Screening of all the compounds for the antibacterial activity against Gram-negative [Escherichia *coli* (MTCC: 1687), *Pseudomonas aeruginosa* (MTCC: 3904), *Vibrio cholera* (MTCC 1934)] and Gram-positive [*Staphylococcus aureus* (MTCC: 1430) and *Bacillus subtilis* (MTCC 441)] strains was done by agar diffusion method taking levofloxacin (LFX) and ciprofloxacin (CFX) as controls. The results, expressed in terms of the zone of inhibition (ZOI) with diameter in millimeters and the corresponding dosage in μg/ml have been presented in **Table 3.5.** *Pseudomonas aeruginosa* (MTCC: 3904), being inactive against all the synthesized molecules, has been omitted. Molecules with the comparable ZOI (±2mm) and a dosage up to four times that of the control were termed as active for each organism. Thus, for *V. cholera*, *B. subtilis,* and *E. coli* a molecule with a dosage of up to 8 μg/ml and S. *aureus, a dosage up to* 16 μg/ml was considered active **(Table 3.5)**.

Most of the aliphatic esters were inactive against *V. cholera* and *E.coli.* Compound **3.25,** the N-1 ethyl substituted fluoroquinolone with acetyl group at the *endo*-nortropine, was the only exception against *V. cholera* that displayed the same ZOI with twice the dosage as that of

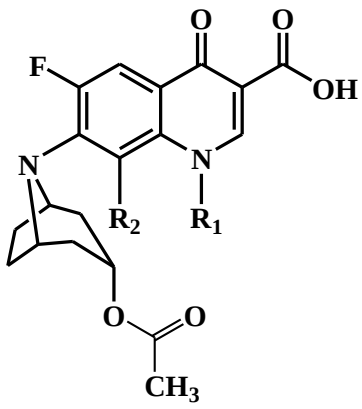
control. Most of the molecules with the acetyl group at the *endo*-nortropine were active against the other two organisms, *B. subtilis* and S. *aureus.* Compounds **3.1**, **3.7** were active against both the organisms at the exact or double dosage as that of control. Among other acetyl derivatives, compound **3.13** inhibited S. *aureus,* and compounds **3.19** and **3.25** inhibited *B. subtilis* at four and two times the dosage as that of control, respectively. Overall except for the ciprofloxacin derivatives, all four of the five acetyl substituted fluoroquinolones were active against B. *subtilis.* However, for S. *aureus*, besides acetyl, propyl **(3.9)** and ethyl **(3.14)** derivatives were four times active as that of the control.

Aromatic acyl chlorides (**Table 3.6)** were inactive against *V. cholera, E. coli, and P. aeruginosa.* However, only three of the twelve synthesized esters **(3.36**, **3.37** and **3.40)** displayed antibacterial activity. While ofloxacin derivative **3.37**, was active against both organisms, compounds **3.36** and **3.40** with phenyl acetyl derivative and 4-clorophenyl acetyl groups respectively were active against S. *aureus* and B. *subtilis,* all at four times the dosage as that of control.



ZOI for the Chiral **Compound 3.1**
11 mm at 2μg/ml for *S. aureus* MTCC 1430
10 mm at 2μg/ml for *B. subtilis* MTCC 441

ZOI for the Racemic **Compound 3.7**
9 mm at 2μg/ml for *S. aureus* MTCC 1430
13 mm at 4μg/ml for *B. subtilis* MTCC 441



Compound 3.19 : R_1= C_3H_5 , R_2 =OCH_3
10 mm at 4μg/ml for *B. subtilis* MTCC 441

Compound 3.25 : R_1= C_2H_5 , R_2 =H
10 mm at 4μg/ml for V.*cholera* MTCC 1430
13 mm at 4μg/ml for *B. subtilis* MTCC 441

ZOI for the Standard Drug Levofloxacin
11 mm at 4μg/ml for *S. aureus* MTCC 1430
10 mm at 0.25 μg/ml for *V. cholerae* MTCC 1934
10 mm at 0.5 μg/ml for *B. subtilis* MTCC 441

Figure – 6.2: Structure and the ZOI for the most impressive aliphatic substituted nortropine derivatives of the fluoroquinolones described in Chapter-3. MIC was not determined due to lack of encouraging results for the antibacterial activity.

Considering the lack of encouraging results for the antibacterial activity of the compounds in this section, further evaluation beyond initial screening was not taken up.

Further, based on the literature reports that heterocyclic amines moieties at the C-7 substituents improved antibacterial activity, it was envisaged to substitute esters containing heterocyclic amines at the hydroxyl group of the nortropine **(Chapter-4)**. Thus, four parent molecules synthesized initially (Figure 4.1) were converted to their corresponding chloro esters using chloroacetyl chloride and 2-chloropropionyl chloride. The chloro group was then replaced with three heterocyclic amines namely piperidine, N-methyl piperazine and morpholine in each case to afford a total of thirty-two **(Scheme 4.3, Compounds 4.1** to **4.32)** molecules in good yields that included chloro ester precursors as well **(Table- 4.1)**. All the synthesized compounds were characterized using spectroscopic (^{1}H, ^{13}C NMR, Mass) and chromatographic (HPLC) investigations and systematically discussed in the section **(Table 4.2)**.

The antibacterial activity of all the thirty-two compounds against Gram-negative [*E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (BAA 1705) *A. baumannii* (BAA 1605)] and Gram-positive [*S. aureus* (ATCC 29213)] bacteria with Levofloxacin and Moxifloxacin and corresponding parent molecules as controls showed that the series was active against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 29213) only. **Table 4.3** shows MIC values for levofloxacin and ofloxacin derivatives while **Table 4.4** shows MIC values for the ciprofloxacin and norfloxacin derivatives.

In the former case **(Table 4.3)** controls were hundred (levofloxacin) to fifty (moxifloxacin) times were more potent than most of the synthesized compounds in the case of *E. coli* while in the case of *S. aureus* they were only eight times more potent than the least potent compounds of the series. Thus further studies against *E. coli* were not taken up. Against, *S. aureus* compounds **4.1, 4.2** and **4.3** gave comparable MIC values to that of standard levofloxacin and the parent compounds **2.13** and **2.14**. Unfortunately, the heterocyclic amines were less impressive than the ones having chloro group at the same position.

The ciprofloxacin and norfloxacin derivatives **(Table 4.4)** were also impressive against *S. aureus* only. Of the sixteen compounds in the series, ten gave MIC value that was four to thirty-two times greater than the control levofloxacin. The MIC values of these compounds when compared to their parent molecules **(2.15** and **2.17)** were only two to eight times higher.

Once again in this case also, the chloro compounds **4.5-4.8** were more impressive than their heterocyclic amines analogs.

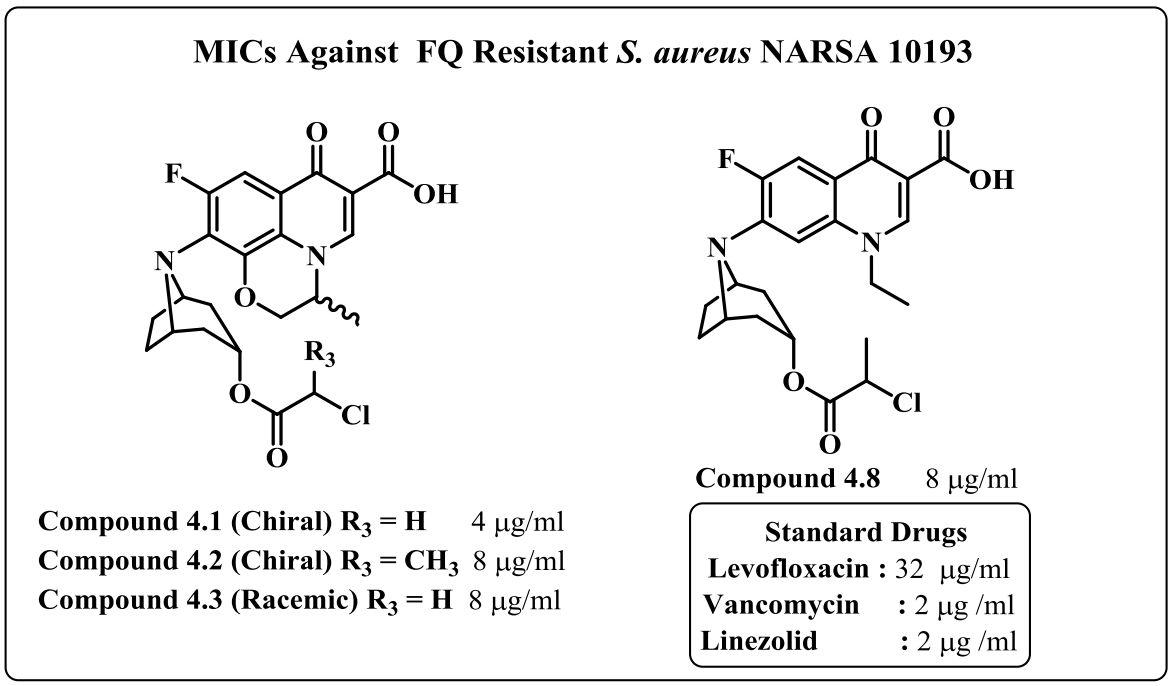


Figure – 6.3: Structure and the MICs of the potential compounds of nortropine substituted chloroesters series described in Chapter-4.

Encouraged by impressive results against *S. aureus,* it was decided to evaluated further all compounds (**Table 4.3 and 4.4)** that gave MIC value up to 4 μg/ml against two fluoroquinolone MRSA strains (NARSA 10193 and NARSA 10198) using linezolid, vancomycin and levofloxacin as standards. *S. aureus* strain (FQ-sensitive, ATCC 29213) used for initial screening was also taken as control. **Table 4.5** shows that besides the levofloxacin and ofloxacin parent precursors (**2.13**, **2.14)** their chloro derivatives, synthesized in this section **(4.1 - 4.3)** along with norfloxacin's chloro derivative (**4.8)** has the potential to be developed further as antibacterial drugs **(Figure – 6.3)**.

Finally, in **Chapter-5**, the *in silico* molecular docking results reflect antibacterial activity in the light of interactions of the synthesized compounds with the target protein. The DNA gyrase B subunit of *S. aureus* from the PDB database was the target protein of choice due to the consistent antibacterial activity against the organism across all the synthesized series of the fluoroquinolones.

Table-5.1 shows that the most the potent compound **(2.13)** in the parent series effectively interacted with the target protein thus displaying the lowest binding energy as compared to the reference molecule, Levofloxacin. Relatively increased hydrogen-bonding interactions

both at C-4 and C-7 terminals for compound **2.13** via arginine 1122, guanine (of DNA) (C-4) and aspartic acid 1083 (C-7) at their respective terminals (**Figure 5.1)** contributed to the lowering of the binding energy. Compounds **2.15** and **2.17**, the cyclopropyl and ethyl substituents at N-1 positions respectively, also interlaced with arginine 1122 via their C-4 terminal **(Figure 5.2 a** and **c)**. However, no interaction was visible at the C-7 terminal in both cases and their MICs were also found higher than that of compound **2.13**. The displacement of compound **2.16** while interacting with the target protein subunit due to the presence of the C-8 methoxy group, made C-4 carbonyl of the molecule interact with aspartic acid 1083 instead of arginine 1122 **(Figure 5.2 b)**. Despite the lower binding energy of compound **2.16,** compared to compound **2.13**, its MIC value was comparable to the latter due to its interaction with the DNA's phosphate group at the C-7 nortropine hydroxyl.

Docking studies with aliphatic esters of nortropine substituted fluoroquinolones gave improved binding energy as contrasted to standard drug levofloxacin despite relatively meager antibacterial activity. The only molecule that gave binding energy compatible with its MIC value was compound **3.1**. Theoretical calculations for all the esters displayed, (**Table 5.2**) binding energy within the same range as that of their potentially antibacterial parent compounds. The methyl and ethyl ester derivatives (**3.20** and **3.25 Figure 5.3c and d**) interact with same amino acid residues as their parent compounds (**2.16** and **2.17 Figure 5.2b and c**) evidence that both category of molecules fit in the same binding pocket. Thus, the methyl esters reporting comparable binding energy and antibacterial activity is understandable. The compromised antibacterial activity for this series of compounds can be attributed to the reduced bioavailability due to increased hydrophobicity as a result of the aliphatic ester chains.

For chloroacetyl and 2-chloropropionyl linked esters of nortropine substituted fluoroquinolones, only six compounds as shown in **Table 5.3** were docked with the target protein subunit due to their promising MIC values (in the range of 4 to 16 μg/ml) as compared to the rest of the molecules of this series. The racemic compounds **4.3** and **4.4** were also not considered in the light of their optically pure analogues **4.1** and **4.2.** Docking results indicated that binding energy for the 2-chloropropionyl linked esters (**R_3 = CH_3** Compounds **4.2**, **4.6** and **4.8**) was higher as compared to chloroacetyl esters analogues (**R_3 = H** Compounds **4.1**, **4.5** and **4.7**). Levofloxacin - the reference molecule, however, despite showing comparable MIC values gave binding energy value lowered by 0.5 to 1.6 kcal/mol.

Also, the fact that the same amino acid residues namely arginine 1122, alanine 1120, aspartic acid 1083 and serine 1084 interacted with this series of the compounds suggested the same binding pocket as that for the other compounds discussed above **(Figure 5.4)**. The highlight of docking studies with the chloroacetyl and 2-chloropropionyl linked esters of nortropine was the revelation that C6 fluorine interacted with the binding pocket (aspartic acid 1083, glutamic acid 1088 and DNA base pairs) of the target in the case of five of the six selected compounds accounting for the high stability. Another unique feature of this study was the interaction of the C3 carboxylic acid group with the binding pocket (arginine 1122, methionine 1121, alanine 1120, serine 1084 and DNA base guanine) besides regular interaction with C4 carbonyl except in the case of compounds **4.1** and **4.2**. At the nortropine end, alanine 1120 was the common residue that engaged compounds **4.1**, **4.2**, **4.6** and **4.7** via the carbonyl group of the chloro ester. In the case of compound **4.8**, however, the group displayed interactions with serine 1084 as well as DNA base pair.

Overall, off three series of the prepared compounds, the parent series compounds **2.13** and **2.16** gave the most potent compounds (MIC 15 nM) and displayed enhanced interactions both at the quinolone and at the nortrpoine ends as compared to standard levofloxacin. Aliphatic ester series of the compounds did not pass the threshold to qualify as antibacterial despite displaying interactions at both the ends that we propose due to the reduced bioavailability as a result of hydrophobic (aliphatic) ester groups. The chloroester series also gave impressive compounds **4.1**, **4.2** and **4.8** having MIC value ranging from 4 to 8 μg/ml displaying interaction not only at quinolone and nortropine ends but also with C6 fluorine also.

FUTURE DIRECTION: The work above, presents only a small fraction of the modifications carried out on the quinolone nucleus ever since the discovery of nalidixic acid. Over the years researchers have carried out with synthesis and modification of the nucleus with different objectives that includes enhancement of the potency, spectrum of the antibacterial activity, prolong half-life, improved pharmacokinetic and pharmacodynamics properties. This work presents studies carried out on a range of Gram-positive and Gram-negative bacteria, the results of which indicate a potential to develop C-7 substituted *endo-nortropine* fluoroquinolnes as antibacterials against *S. aureus* and *V. cholera*. The chloroester linked *endo-nortropine* molecules have also given optimistic results to develop antibacterials against fluoroquinolone-resistant *S. aureus.*

Thus, future direction to the present work requires carrying out elaborate work on the toxicity, half-life, pharmacokinetic and pharmacodynamics parameters on the lead molecules like compounds **2.13** and **2.16**.

Increase in the resistance against fluoroquinolones is on rise in many organisms in certain parts of the world. Therefore, working to explore and understand the mechanism of resistance for the synthesized molecules, especially the ones that have shown activity against fluoroquinolone-resistant *S. aureus* will be useful.

The input in this work in terms of resources, efforts and energy cannot be justified by only analyzing the antibacterial parameter of the molecules. The unwanted side effects of the currently available anticancer agents and the possible ability of fluoroquinolones to bind eukaryotic topoisomerases point to explore the use of all the synthesized compounds in this work for antiproliferative activity.
