Design, Synthesis and In-vitro Evaluation of thiazeto [2, 3-*a*] quinolones as Potential Bioactive Molecules

by
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A

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Abstract

The varied nature of biological profiles of the quinolone scaffold has attracted researcher's interests in exploring novel quinolone-based compounds. Novel quinolone derivatives are designed to overcome unwanted effects such as bacterial resistance, toxicities and side effects of existing molecules. Based on understanding of mechanism of action of quinolones, while their side effects, profile, bacterial resistance and efficacy may be altered.

Among the newly synthesized biologically active polycyclic quinolones, the sulfur-containing polycyclic quinolone nuclei such as thiazolo-, thiazeto- and thieno-derivatives are of biological interest. While there are numerous research reports available on various polycyclic quinolones, for instance 4-oxo-thiazolo [3,2-*a*] quinoline-3-carboxylic acid derivatives, 4-oxo-thieno[3,2-*a*]quinoline-3-carboxylic acid derivatives etc., there are very few studies reported for the 4-oxo-thiazeto [3,2-*a*] quinolines. The work presented in this thesis was performed in order to discover novel 4-oxo-thiazeto [3, 2-*a*] quinolone derivatives with potential antibacterial activity, focussing on activity against resistant bacterial strains. This work traverses from design and synthesis of a series of 4-oxo-thiazeto [3, 2-a] quinoline derivatives to their *in vitro* evaluation for microbiological activity, and finally ends with docking studies. Unfortunately, the antibacterial activity of the synthesized compounds was insignificant. Thus, in order to comprehend the mechanism behind the insignificant antibacterial activity, docking studies were performed on the DNA gyrase enzyme for a certain compounds.

The results obtained from docking studies showed that the synthesized 4-oxo-thiazeto [3, 2-*a*] quinolone derivatives could interact with the DNA gyrase enzyme, forming

reversible binding interaction with the various domains of this enzyme, while other reported active antibacterial compounds from this class bind irreversibly with the ATP binding site.

Acknowledgement

Research is an eternal process by an individual or a group inquisitive into never endings and newer avenues of science. I am grateful to almighty for providing me the opportunity to complete such a wonderful project. Additionally, this project would not be possible without sincere support and cooperation of my veteran supervisor, respected parents, supporting friends, kind seniors and well wishers. With deep sense of gratitude, I acknowledge my indebtness to one and all.

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ATP	Adenosine triphosphate
CNS	Central nervous system
DMF	Dimethyl formamide
DNA	Deoxyribonucleic acid
EC_{50}	Half Maximal Effective Concentration
GABA	γ amino butyric acid
HRMS	High resolution mass spectroscopy
HCMV	Human cytomegalovirus
HSV	Herpes simplex virus
HIV	Human immunodeficiency virus
HCl	Hydrochloric acid
IN	HIV- integrase
IUPAC	International union of pure and applied chemistry
LGA	Lamarckian genetic algorithm
MIC	Minimum inhibitory concentration
MP	Melting point
MRSA	Methicillin-resistant Staphylococcus aureus
NMR	Nuclear magnetic resonance
PDB	Protein data bank
RNA	Ribonucleic acid
STAT	Signal transducers and activators of transcription
SAR	Structure activity relationship
THF	Tetrahydrofuran
TLC	Thin layer chromatography

Chapter 1

Introduction

1.1 Antibiotics

1.1.1 Introduction

Antibiotics are simply the chemical compounds which can inhibit the growth of and/or kill bacteria and other micro-organisms [1]. Antibiotics may be of natural origin obtained directly from micro-organisms. This definition covers traditional antibiotic agents such as aminoglycosides, tetracyclines, macrolides and β-lactams. The up-to-date definition of the antibiotic has been modified with the inclusion of chemically modified antibiotics and those of fully synthetic chemical origins, such as quinolones [4(1*H*)-oxoquinoline-3-carboxylic acid derivatives], sulfonamides, and oxazolidinones, etc. [2]. Antibiotics may act by inhibition of various biological processes in bacterial cells, such as inhibition of protein biosynthesis and RNA/DNA replication/repair in bacterial cell wall synthesis. Ideal antibiotics are compounds which can selectively affect bacteria with minimum or no effect on the host cells. This characteristic of antibiotics contributes to reduction of undesired effects on host body cell.. However, among the above-mentioned mechanisms, inhibition of bacterial cell wall synthesis and folate coenzyme synthesis are unique to bacterial cells, whereas, the two processes of inhibition of protein biosynthesis and RNA/DNA replication/repair synthesis are not unique to bacterial cells and are also present in mammalian cells as well [3].

The story of naturally occurring antibiotics originated in 1928, when Alexander Fleming discovered penicillin while working on *Staphylococcus* bacteria. When culturing a sample of *Staphylococcus aureus*, he observed the growth of a mold (via contamination) in some areas of the agar plate. Interestingly, no bacteria were found to grow in the region surrounding the mold. This suggested that the mold, which was later identified as *Penicillium notatum*, inhibited the

growth of *Staphylococcus aureus* [4]. Later on, the first antibiotic penicillin was isolated from cultures of the above mold. Penicillin was first used clinically during World War II and became widely available to the civilian market shortly after the war through the increase in bulk manufacturing capability [5]. Over the next couple of decades, thriving antibiotic research produced numerous natural product derived classes of antibiotics including tetracyclines [6], glycopeptides [7], macrolides, and phenylpropanoids [8]. Daptomycin, a lipopeptide representative of the naturally derived antibiotic class, was introduced into the market in 2003 [9].

The first sulfonamide as well as the first synthetic commercially available antibacterial agent was Prontosil, which was marketed by Bayer in 1935 [10]. It took almost 30 years before the next fully synthetic antibacterial class of compounds, the quinolones (Nalidixic acid), hit the market. It was discovered by Lesher *et al.* in 1962 as an impurity while manufacturing chloroquine, an antimalarial agent at that time. Nalidixic acid was found to be effective in the treatment of urinary tract infections [11]. Nearly four more decades passed before the next synthetic antibiotic class, the oxazolidinones, would enter clinical use in 2000 [12]. Figure 1.1 illustrates the classification of antibiotics.

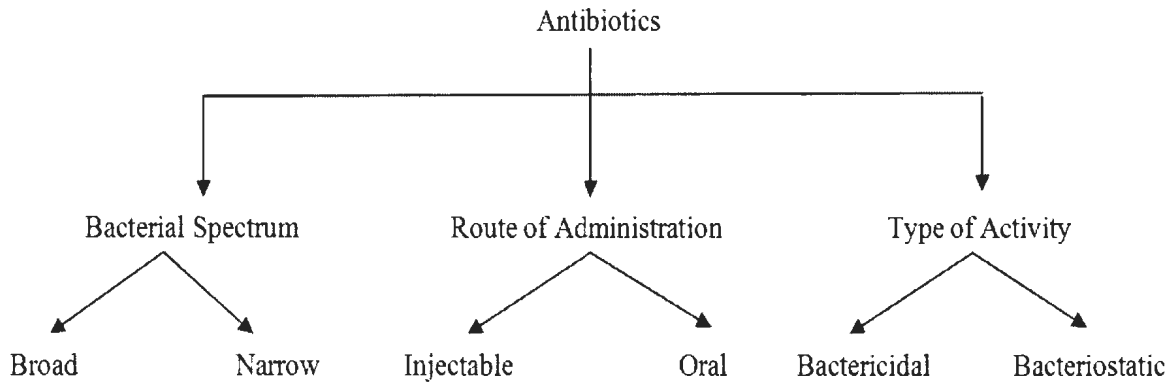


Figure 1.1. Common plan of classifications for antibiotics [13]

The long periods of relative idleness in research on antibiotics can be attributed to numerous causes such as the belief that bacterial infections would shortly be no longer a threat in most countries, along with changes in research priorities at major pharmaceutical companies. Blossoming genomics research and ignorance for discovery of new chemical entities/ drugs, as well as a major shift in the source of new drug molecules from natural products to small synthetic molecules were also other reasons for the idleness in natural product antibiotic research.

1.1.2 Mechanism of action of Antibiotics and Resistance

While antibiotics may act by targeting various biological processes in bacterial cells (Figure 1.2 [14] and Table 1.1), inhibition of bacterial cell wall formation is known to be the most common mechanism of action of antibacterial agents [13, 14].

Antibacterial agents can also inhibit protein synthesis (translation). Some other mechanisms

include: alteration of cell membranes, inhibition of nucleic acid synthesis and antimetabolite activity. Bacteria can become resistant to a drug by a specific internal mechanism. There are few proposed mechanisms for the development of bacterial resistance including enzymes which can deactivate a drug *via* a chemical modification. Another path is the structural modification through which bacteria can modify target structures which render the drugs ineffective. Bacteria can also stimulate efflux proteins which can pump the drug out of the cell. Albeit, researchers were cognizant of bacterial resistance in the early 1960s [15, 16], and even with the vigilant and responsible use of clinical antibiotics, resistance has become increasingly challenging [17, 18]. In this context, even third line antibiotics, such as vancomycine, have become ineffective against resistant pathogens [19, 20]. The contemporary increase in the risk of community-acquired- and healthcare-acquired-methicillin resistant *Staphylococcus aureus* (MRSA) has brought the threat of antibiotic resistant into the public perception [19, 20].

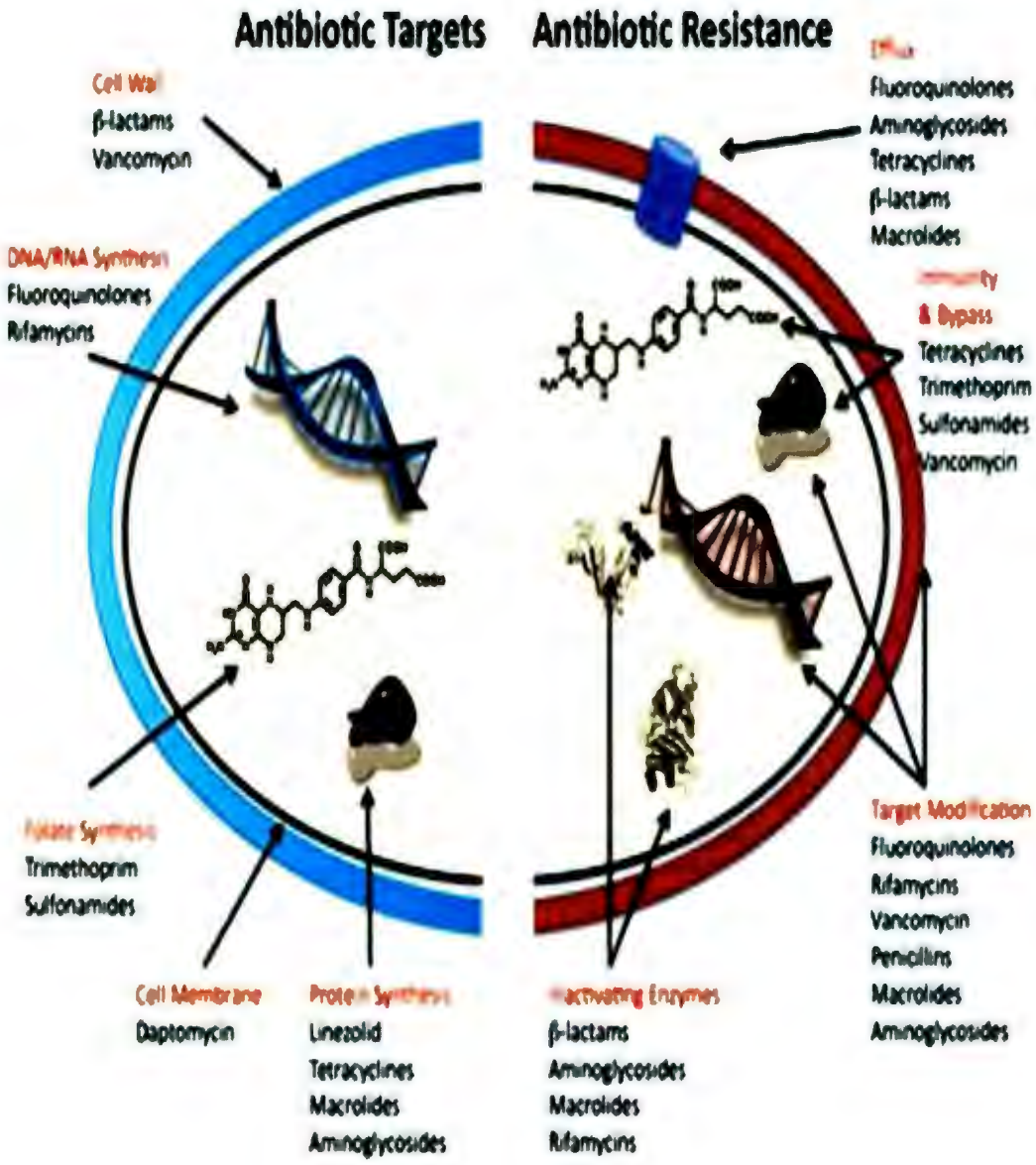


Figure 1.2. Antibiotic targets and mechanisms of resistance (Figure adopted from web source: http://www.gustrength.com/health:antibiotic-resistance-questions-and-answers)[14]

Table 1.1. Examples and mechanism of action of antibiotic class by origin

Origin	**Class**	**Mechanism**	**Example**
Natural Product	Penicillins	Inhibition of cell wall biosynthesis	Penicillin G
	Tetracyclines	Protein synthesis inhibition by inhibiting 30S ribosomal subunit	Doxycycline
	Glycopeptides	Inhibition of cell wall biosynthesis	Vancomycin
	Macrolides	Protein synthesis inhibition by blocking 50S rRNA complex	Erythromycin
	Phenylpropanoids	Protein synthesis inhibition	Chloramphenicol
	Lipopeptides	Cell membrane disruption/depolarization	Daptomycin
Synthetic	Sulfonamides	Folate synthesis inhibition (by direct inhibition of dihydropterate	Prontosil
	Fluoroquinolones	Inhibition of DNA replication	Ciprofloxacin
	Oxazolidinones	Protein synthesis inhibition.	Linezolid

1.1.3 Natural vs. Synthetic antibiotics

Natural antibiotics are metabolites produced by living organisms (obtained directly from the source, which is typically a plant, animal or a microorganism), whereas synthetic antibiotics are the product of laboratory-based chemical synthesis which may be prepared by complicated or simple processes.

Moreover, these antibiotics differ from each other in terms of the onset and the duration of action. As observed to date, with few exceptions, synthetic antibiotics work faster and longer than the natural antibiotics. This phenomenon gives a good rational for the use of synthetic antibiotics in medical emergencies [21].

Additionally, safety profile is another characteristic in which these substances differ from each other. Even though synthetic antibiotics are very target specific compared to natural antibiotics, natural antibiotics are considered safer. Noteworthy, this comparison is not on the transient effect of a particular agent but is all about adverse effect upon long term basis. As we know, most of the synthetic antibiotics cause potentially dangerous adverse effects on long term use. However, with reference to the above discussion, natural antibiotics should be the preferred agents for the treatment of bacterial infections. On the contrary, most bacteria or microorganisms are capable of developing resistance to natural antibiotics if used for a long period of time. Emerging bacterial resistance generates the need of relatively novel antibacterial agents and natural antibiotics are not sufficient to satisfy this need.

In consideration of the severity of widespread bacterial resistance in addition to multidrug resistance, there is an incessant need for novel natural or synthetic antimicrobial agents [21].

1.2 Quinolones: An Overview

Quinolones are of prominent therapeutic importance because of a privileged basic ring skeleton. The concept of ''privileged basic ring skeleton'' was first proposed by Evans [22]. Privileged structure refers to a molecular scaffold whose structure contains two or three ring systems that are arranged in such a manner so as to allow a very high degree of versatility in binding with the receptors. In this perspective, a quinolone scaffold can be considered as a privileged basic ring skeleton [23].

Since the discovery of nalidixic acid by Lesher *et al.* in 1962, more than 10,000 quinolone derivatives have been patented or published [24]. Among these are clinically important antibacterial agents such as norfloxacin, ciprofloxacin, temafloxacin, difloxacin and ofloxacin which possess the 1-substituted 4-oxo-1, 4-dihydroquinoline-3-carboxylic acid moiety. Based on their main clinical use and decade of discovery, they can be categorized into four different groups (A, B, C, and D) as depicted in Figure 1.3.

Quinolones have an important place in the field of antibacterial drugs. Their therapeutic values as antibacterial agents have been known for over 50 years. They are still a hot topic for concurrent research because of continuous development of bacterial resistance against currently used antibiotics. This has directed the current trend of antibiotic research to focus mainly on the design and development of novel quinolone antibacterials that are active against resistant strains of bacteria [24, 25].

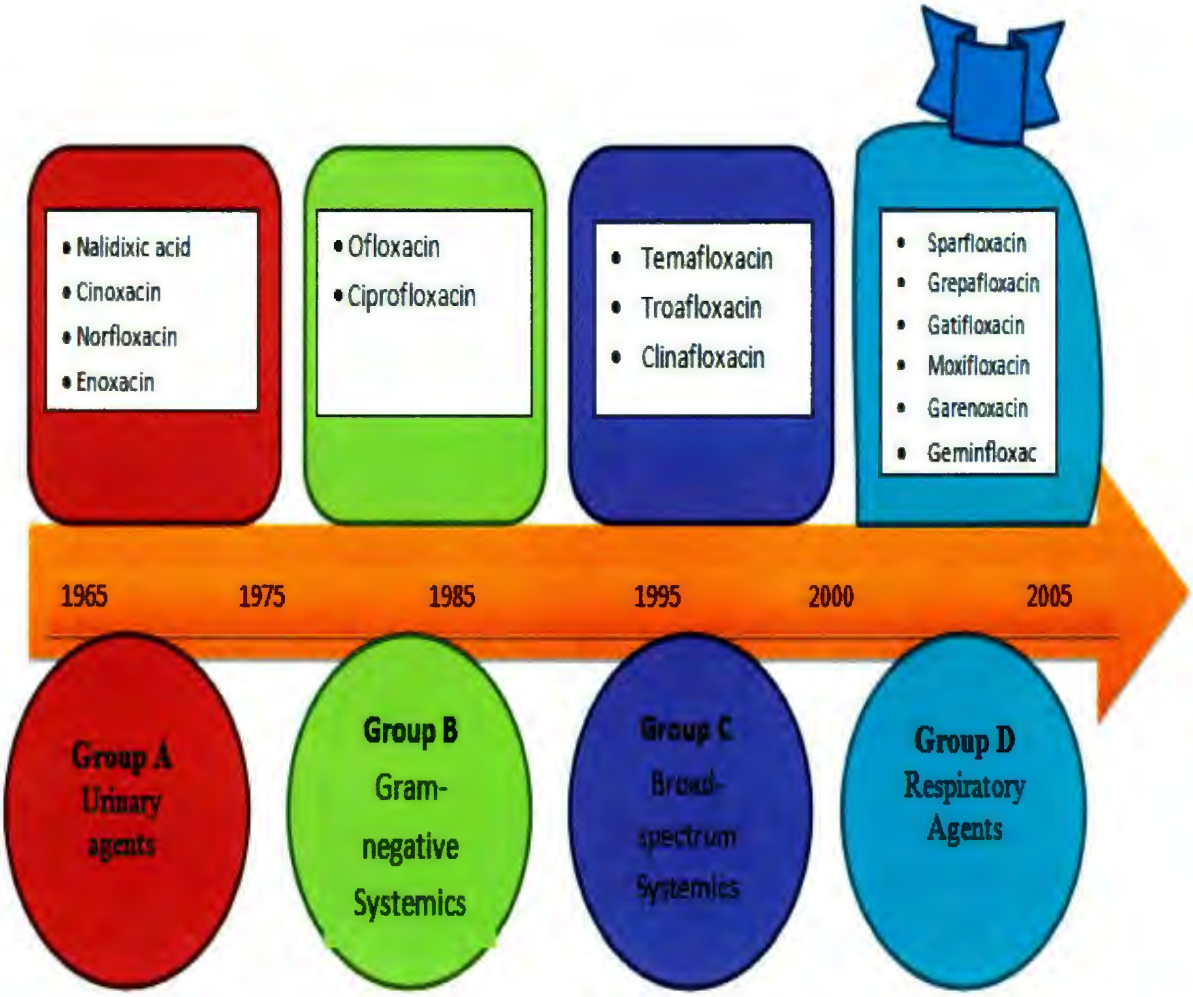


Figure 1.3. Chronological development of quinolones (suggested by the author of this thesis)

1.2.1 Classification of Quinolones:

Originally, the antibacterial quinolone framework was derived from the naphthyridone-based compound, nalidixic acid (Table 1.2). Quinolones can be classified into four generations based

on antimicrobial activity, structural modifications and chronological order of discovery [26, 27]. The only characterization that is universally accepted is that the non-fluorinated quinolone derivatives are classified as the first generation and the fluorinated counterparts are grouped into the later generations. Conversely, some lately discovered broad spectrum antibacterial quinolones, such as garenoxacin, do not have fluorine in their structure, despite being derived from the later generations of the fluoroquinolone class [26].

First generation: This generation of quinolones has been used for the treatment of uncomplicated urinary tract infections since their advent to the market. Nalidixic acid, first reported in 1962, was biologically active and is the parent compound of this generation [26, 27]. The first generation agents display activity against Gram-negative bacteria, with the exception of *Pseudomonas* species. They are used less often today because of their side effects and emergence of bacterial resistance. Interestingly, few first generation quinolones are still in use even after their genotoxic and carcinogenic effects [25].

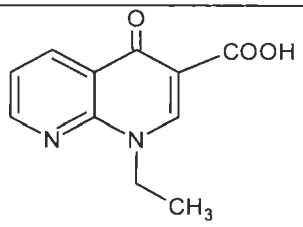
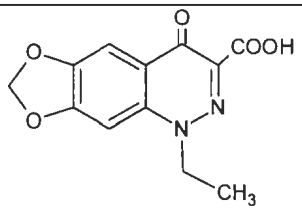
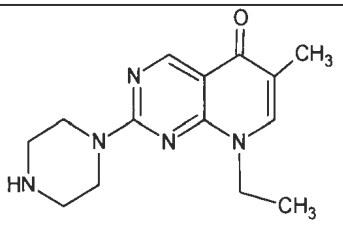
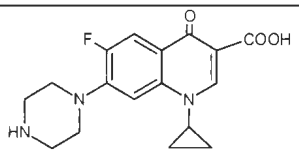
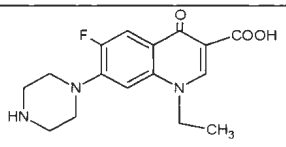
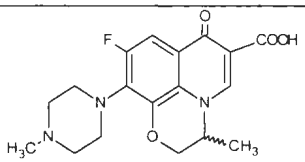
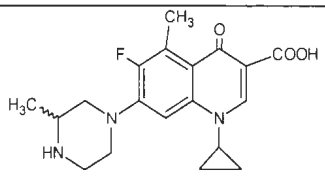
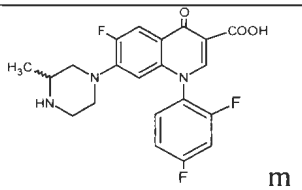
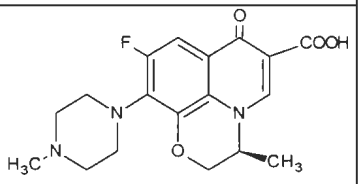
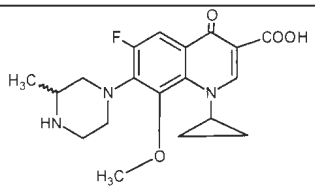
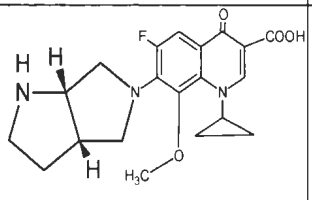
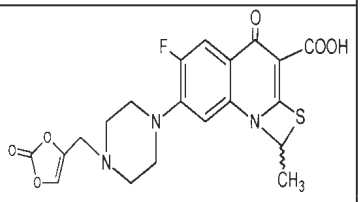
Second generation: This generation of quinolones differs structurally from the first generation by containing a fluorine atom at the C-6 position. This generation of quinolones is also called "fluoroquinolones" (Table 1.2). Activities of the second generation agents are circumscribed to Gram-negative pathogens and recently included *Pseudomonas* species, but they also display activity against some Gram-positive species, such as *Staphylococcus aureus* [26]. Diseases that have been treated using second generation fluoroquinolones include urinary tract infections (both complicated and uncomplicated), pyelonephritis, sexually transmitted diseases, prostatitis, localized skin and soft tissue infections [27]. The second generation quinolones are further separated into subclasses IIa and IIb. Subclass IIb compounds tend to have broad-spectrum uses,

particularly improved activity against Gram-positive bacteria and respiratory infections, and their long duration of action permit once daily dosing [27]. Perhaps the greatest well publicized use of a second generation fluoroquinolone, ciprofloxacin (Cipro), was when several government workers took it for prophylaxis during an anthrax scare in the fall of 2001 [26].

Third generation: Fluoroquinolones have applications against respiratory infections [27]. They exhibit broader-spectrum activity as compared to the first and second generation agents in the treatment of expanded Gram-positive and a few penicillin resistant bacteria. Third generation quinolones are also divided further in two subgroups of IIIa and IIIb. The latter group is now considered as the fourth generation [27].

The difference between third and fourth generation fluoroquinolones is not well defined. Some agents such as moxifloxacin can be considered as either third or fourth generation, depending on the source by which they are obtained. Usually, fourth generation fluoroquinolones have actions against similar infections as third generation drugs; additionally they demonastrate activity against anaerobic microbes as well as infections of the pelvis, abdomen, and nosocomial pneumonia. As compared to the first generation, activity profile of the second and third generation quinolones is not only retained, but is increased. Another characteristic of this class is that the fourth generation fluoroquinolones have activity against ciprofloxacin-resistant *Pneumonococci* [27].

Table 1.2. Some examples of each of quinolone generation

First Generation	 Nalidixic acid	 Cinoxacin	 Pimemidic Acid
Second Generation	 Ciprofloxacin	 Norfloxacin	 Ofloxacin
Third Generation	 Grepafloxacin	 Temafloxacin	 Levofloxacin
Fourth Generation	 Gatifloxacin	 Moxifloxacin	 Prulifloxacin

1.2.2 Chemistry of Quinolones

Replacement of a single carbon atom on a benzene ring with nitrogen results in pyridine skeleton, while the same replacement on a naphthalene nucleus (with fused pair of benzene rings) results in a quinoline or isoquinoline nuclei depending on the position of the carbon atom being replaced by nitrogen (Figure 1.4). Quinoline is a liquid at room temperature with a high boiling point, first extracted in 1834 from coal tar. Isoquinoline is a solid substance with a low melting point, first extracted in 1885 from the same source [28].

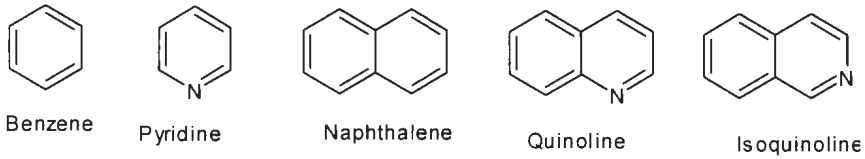


Figure 1.4. Structures of pyridine and quinoline nuclei.

The Schematic numbering of a quinoline nucleus is shown in Figure 1.5.

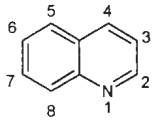


Figure 1.5. Schematic numbering of quinoline nucleus.

When a hydroxyl group is added to the C-2 or C-4 positions of a quinoline ring the corresponding tautomeric forms of hydroxquinolines and quinolones are formed as depicted in Figures1.6 and 1.7.

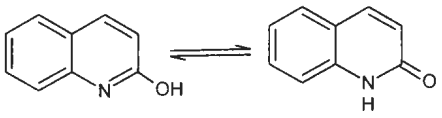


Figure 1.6. Tautomeric forms of 2-hydroxyquinoline and 2(1*H*)--quinolone

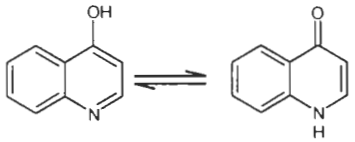
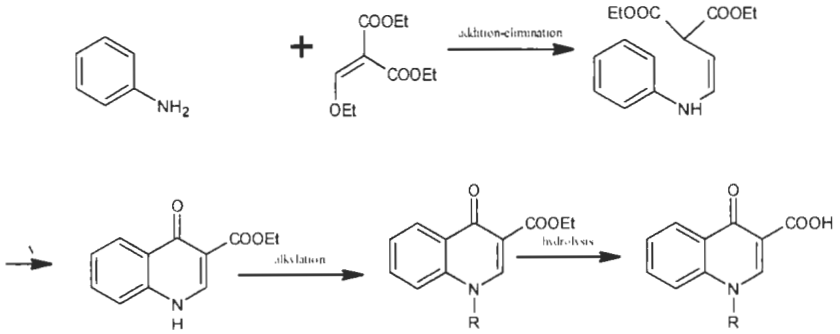


Figure 1.7. Tautomeric forms of 4-hydroxyquinoline and 4 (1*H*)-quinolone

Various synthetic methods have been reported so far for the preparation of quinolones, few of which are described in the following section.

1.2.2.1 Gould-Jacob's Quinolone Synthesis

Until the end of the 1970s, the Gould Jacob's quinolone synthesis was the most commonly used procedure for synthesizing 1,4-dihydro-4-oxo-quinoline-3-carboxylic acid derivatives. This method of preparation uses an *addition-elimination* reaction of various substituted anilines and dialkyl alkoxymethylenemalonate as shown in Scheme 1.1 [28]. The product of this reaction undergoes thermal cyclization when heated and produces quinoline-4(1*H*)-one system. *N*-Alkylation of this product, followed by hydrolysis of the ester group, affords *N*-substituted-4-(1*H*)-quinolone-3-carboxylic acid.

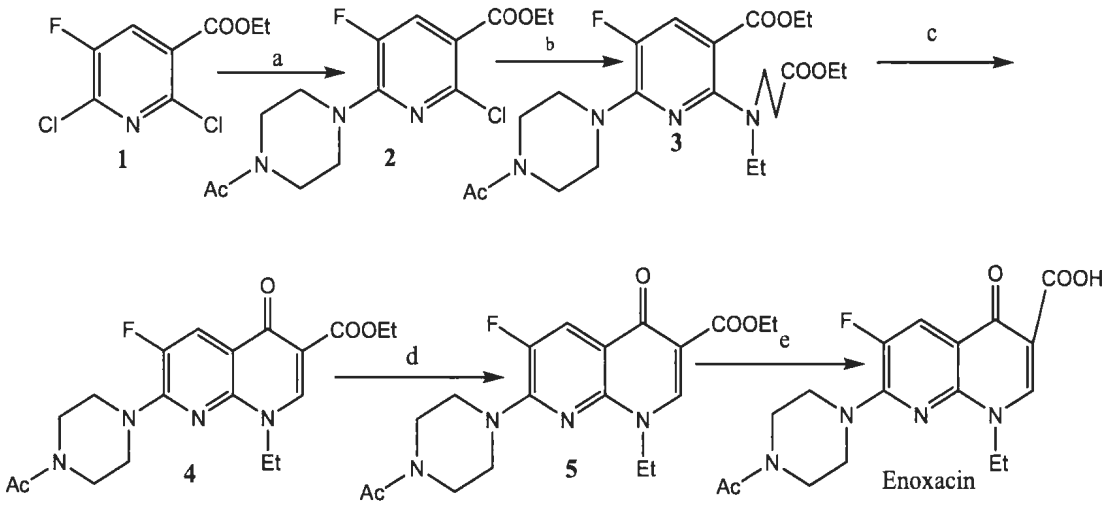


Scheme 1.1. Gould-Jacobs reaction.

1.2.2.2 Dieckmann Cyclization of Diesters

Pesson *et al.* reported a simple and convenient, 5-step procedure for the synthesis of aza- and diazaquinolone carboxylic acids [29]. This process involves an intramolecular cyclization of diesters in the presence of a base as depicted in Scheme 1.2.

The first step is a regioselective reaction between ethyl ester of 2,6-dichloro-5-fluoronicotonic acid (**1**) with *N*-acetylpiperazine which leads to the formation of 6-piperazinyl derivative **2**. Further reaction of Compound **2** with the ethyl ester of 3-ethylaminopropionic acid affords Compound **3,** which is a diester. Compound 3 would further undergo intramolecular ring formation with potassium *tert*-butoxide to yield ethyl ester of tetrahydroquinoline carboxylic acid **4**. The dehydrogenation of Compound **4** by means of chloranil gives rise to the ethyl ester of naphthyridone carboxylic acid **5** which yields the corresponding fluoroquinolone (enoxacin) after hydrolysis.



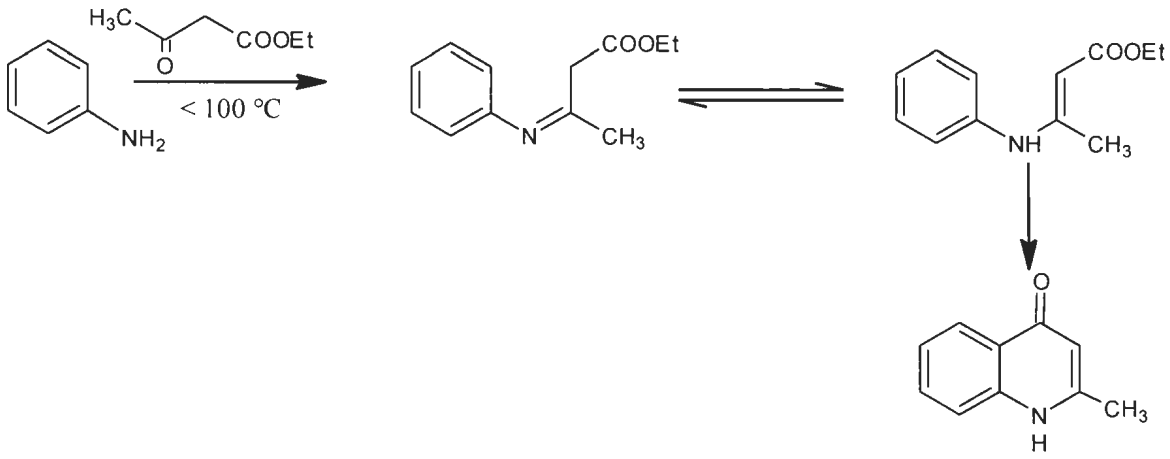
[a) N-acetylpiperazine, CH_3CN; b) $EtNH(CH_2)_2CO_2Et$, DMF, $NaHCO_3$, 120 °C; c) $KOC(CH_3)_3$, toluene; d) chloranil; e) OH^-]

Scheme 1.2. Synthesis of enoxacin by Dieckmann cyclization method

1.2.2.3 Conrad-Limpach 4-quinolone synthesis

This reaction is a modification of Knorr quinoline synthesis in which condensation of β-keto-esters and arylamines occurs at 110 ^{0}C, then the resulting anilides further cyclizes when reacted with concentrated sulphuric acid to yield 4-hydroxyquinolines. Mechanism of this reaction is a nucleophilic attack by aniline at the carbonyl group of the ester, followed by cyclization of the intermediate anilide.

Modified reactions employed for the synthesis of 4-quinolones involves the reaction between aniline and 3-oxobutanoate at a temperature below 100 ^{0}C. The second step in this reaction involves cyclization in an inert solvent at a temperature around 250 ^{0}C to yield 4-quinolone derivative as shown in Scheme 1.3 [30].

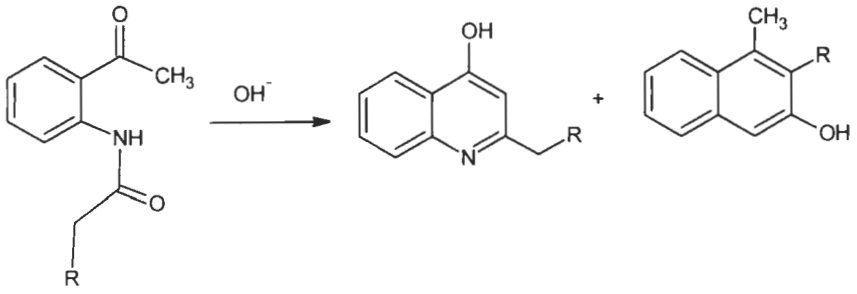


Scheme 1.3. Conrad-Limpach 4-quinolone synthesis

1.2.2.4 Camps quinoline synthesis

In this reaction the intramolecular cyclization and condensation occur under basic conditions. Fundamentally, it is the alkaline cyclization of an *N*-acylated-*o*-

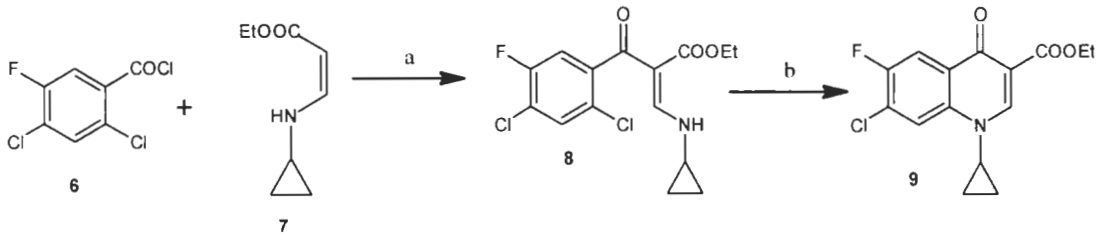
aminoacetophenone which affords the corresponding 2- or 4-hydroxyquinolines as shown in Scheme 1.4 [31-37].

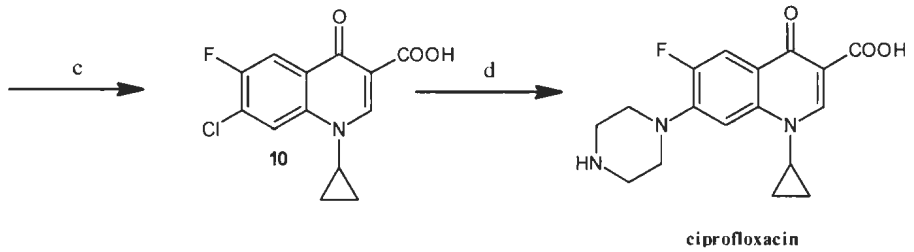


Scheme 1.4. Camps quinoline synthesis

1.2.2.5 Synthesis of quinolones by cycloaracylation reaction

This cycloaracylation procedure has been developed by Grohe *et al.* [38] in 1987. It starts with the formation of acylated product **8** *via* the reaction between 2,4-dichloro-5-fluorobenzoyl chloride (**6**) and ethyl 3-cyclopropylaminoacrylate (**7**) in the presence of triethylamine. Cyclization of **8** under the catalytic influence of potassium carbonate yields the ethyl ester of quinolone carboxylic acid **9**, which is subsequently hydrolysed to its carboxylic acid derivative **10.** The reaction of compound **10** with piperazine leads to the generation of a quinolone with antimicrobial properties (in this case, ciprofloxacin). Detailed procedure is depicted in Scheme 1.5 [37].



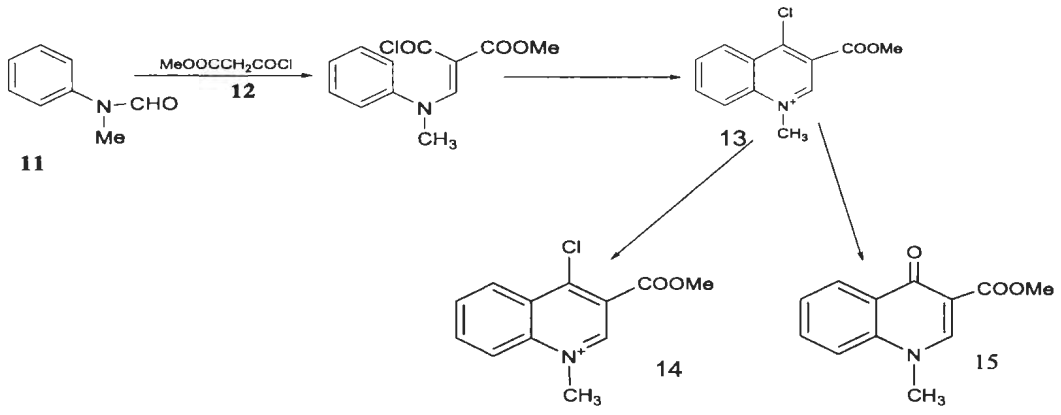


[a) NEt_3, toluene; b) K_2CO_3, DMF; c) KOH; d) piperazine, DMSO]

Scheme 1.5. Synthesis of ciprofloxacin by cycloaracylation reaction

1.2.2.6 Meth-Cohn Quinolone Synthesis

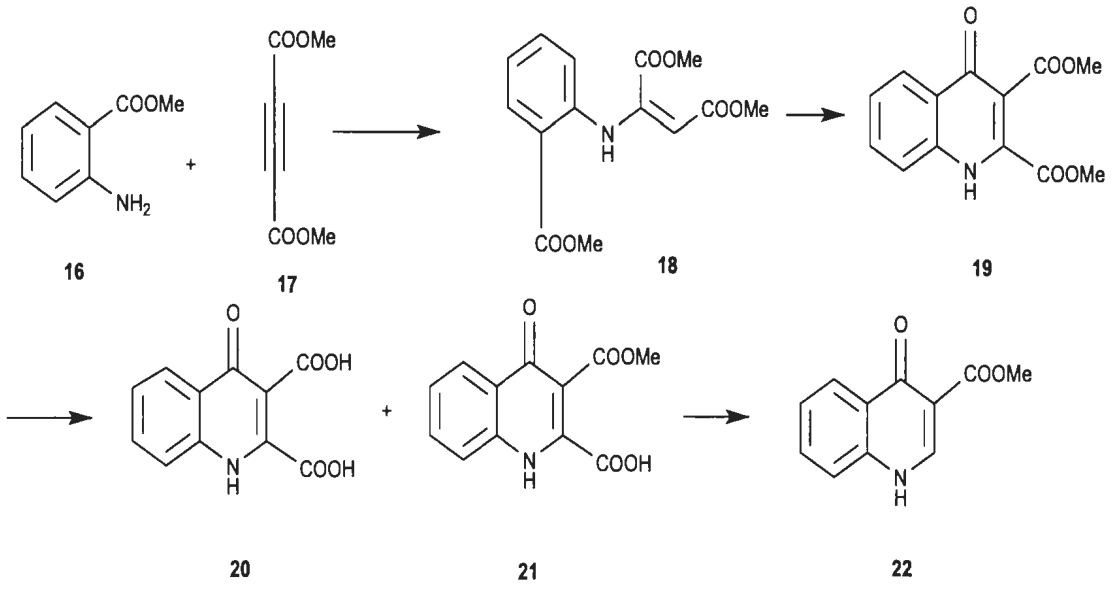
This approach of quinolone synthesis was developed by Meth-Cohn in 1986 [39]. In this reaction a Vilsmeier complex, which consist of *N*-methylformanilide (**11**) and phosphoryl chloride, is reacted with methylmalonyl chloride (**12**) to produce its quinolinium salt **13**. This quinolinium salt further reacts with ammonium hexafluorophosphate to afford 4-chloro-3-methoxycarbonyl-1-methylquinolinium hexafluorophosphate (**14**). Compound **13** is also converted to methyl 1-methyl-4(1*H*)-quinolone-3-carboxylate (**15**) by treatment with excess alkali followed by acidification as depicted in Scheme 1.6.



Scheme 1.6. Meth- Cohn quinolone synthesis

1.2.2.7 Biere and Seelen Approach for quinolone synthesis

This method for quinolone synthesis has been developed in 1976 by Biere and Seelen as shown in Scheme 1.7 [40]. The reaction progresses with the Michael addition of methyl anthranilate (**16**) to dimethylacetylene dicarboxylate (**17**) to yield the enamino esters (**18**). This enamino ester undergoes further cyclization to the quinolone dicarboxylic acid ester (**19**) in the presence of strong base such as sodium hydride or potassium *tert*-butoxide. The dicarboxylic acid (**20**) or the ester-carboxylic acid (**21**) are obtained after regioselective or complete alkaline hydrolysis of **19**. The quinolone carboxylic acid ester (**22**) is then obtained by thermal decarboxylation reaction at very high temperature from compound **21**.



Scheme 1.7. Biere and Seelen approach for quinolone synthesis

1.2.3 Mechanism of action of fluoroquinolones as antibacterial agents

The fluoroquinolone class of compounds exert their effect by inhibition of bacterial type II topoisomerases (DNA gyrase) or topoisomerase IV. Topoisomerase IV and DNA gyrase are the enzymes which pave the way for DNA replication by relaxing supercoiled DNA and once replication is done they induce supercoiling of DNA. Generally, topo IV and gyrase enzymes work to relax negatively supercoiled DNA by forming a break in one strand of double stranded DNA, passing the complementary strand through the break, then combining the separated ends again [41]. (Figure 1.8)

A fluoroquinolone usually binds with the DNA topoisomerase units and form a ternary complex that inhibits DNA replication, thereby preventing bacterial cell division [42, 43]. Most fluoroquinolones predominantly bind with either topo IV or DNA gyrase. There are some fluoroquinolones that can bind with both enzymes and have nearly equal potency against Gram-positive and Gram-negative bacteria, therefore being called as dual target agents [44]. The obvious fact is that the dual target agents are likely to be more active against resistant mutants.

Quinolones are generally bactricidal in nature meaning that the ternary complexes formed by the first and second generation quinolones exclusively result in inhibition of bacterial growth [45, 46].

By a unique mechanism which is novel but partially explained, few third and fourth generation fluoroquinolones are known to damage the ternary complex, resulting in fragmented DNA. This process is termed as a "rapid lethality" [47]. Additionally, there can be two diverse paths in the new rapid lethality mechanism; first route requires protein synthesis while the second path does not depend on protein synthesis. The type of pathway is determined by particular the

fluoroquinolone in the ternary complex, but to date it is not known what properties of the fluoroquinolone lead to one mechanism over the other.

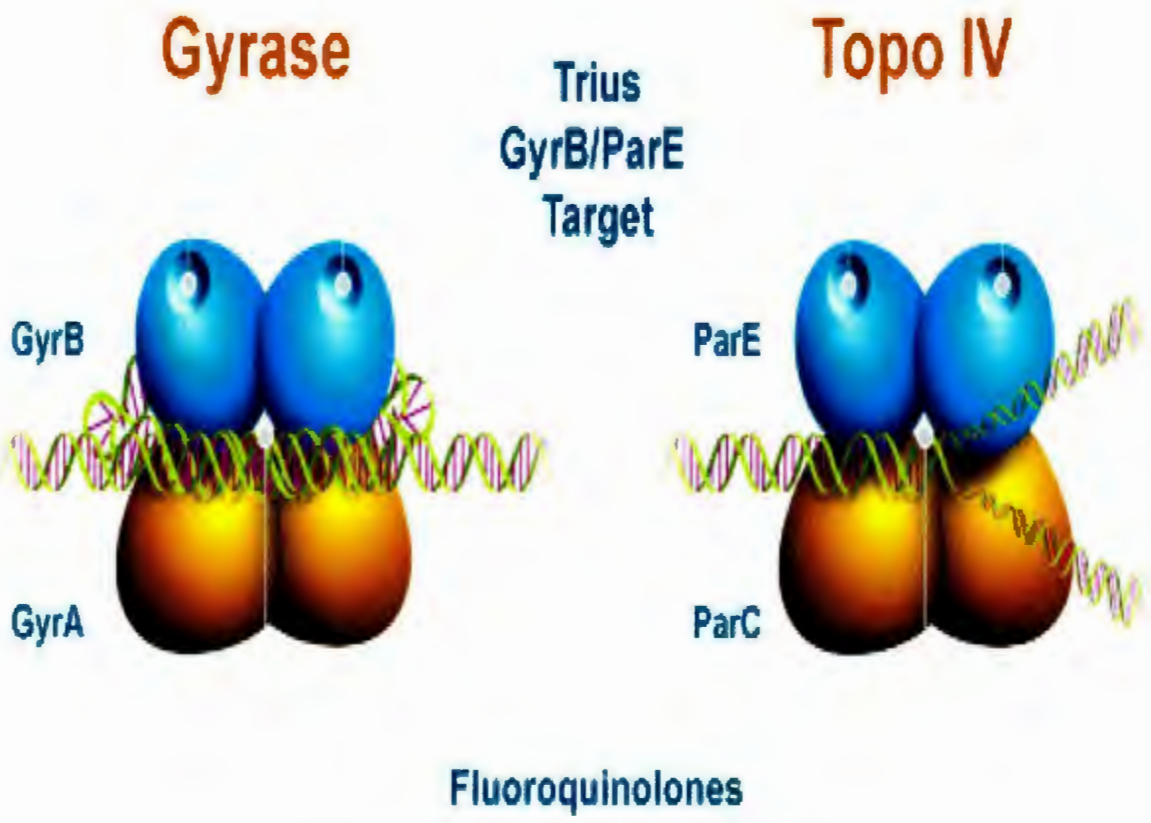


Figure 1.8. Quinolone-enzyme Complex
(Figure adapted from web sources http://www.triusrx.com/trius-therapeutics-gyrbpare.php) [48]

1.2.4 Bacterial Resistance

Emerging bacterial resistance to most of the known antibiotics is a serious problem which needs immediate attention. To date, bacteria and fungi have been known to develop drug resistance by the following mechanisms: **(a)** producing enzymes that inactivate the drug or **(b)** modifying their

targets to render the drugs useless, or simply 'pump' the drugs out with the help of efflux proteins in order to lower their concentrations. [49]

As stated, most common Gram-positive and Gram-negative bacteria have already developed resistance against various quinolone antibiotics. Bacteria usually opt for changing their target structures to acquire resistance to fluoroquinolones. They are reported to change or modify their target structure and/or conformation as a result of mutations leading to amino acid changes. More specifically, they get the mutation in the area of the active site of GyrA (DNA gyrase) or ParC (Topo IV), from positions 67-106 [50]. Mutation of amino acid positions 83 and 87 (of GyrA) is often associated with clinical resistance [51] and is known as the quinolone resistance determining region (QRDR) [52] and is located within the DNA binding interface of gyrase. Fluoroquinolone antibacterials known to bind to both DNA and the topoisomerase IV with amino acids in the QRDR form a binding site. Obviously, mutations in this region results in decreased affinity for fluoroquinolone binding followed by decreased activity leading to resistance [53].

1.3 DNA, DNA super coiling, replication and topoisomerase

DNA is a prominent macromolecule among four macromolecules (nucleic acids, proteins, carbohydrates and lipids) which are crucial for all forms of life. DNA carries genetic information in a series of nucleobases (guanine, adenine, thymine, and cytosine) denoted by letters G, A, T, and C. In fact, DNA is a polymer of deoxyribose nucleotides, which fundamentally composed of a nitrogenous base, a five-carbon sugar (deoxyribose) and phosphate groups. It functions as genetic carrier of all known living organisms and many viruses. It also allows mutation and recombination and serves as a template for semi-conservative replication [54]. The polymer is

linked by phosphate groups and hydrogen bonds between nucleotides of opposing chains (strands) form a double helical structure. Usually, DNA is found as double-stranded helices, composed of two long polymers of simple units called nucleotides. DNA is well-equipped for storing biological information, since the double-stranded structure provides the molecule with an replica of the encoded data. Followings here are some basic characteristics of DNA molecule and its orientation in the nucleus.

1) In *the E. coli* bacterium, the DNA is approximately 1000 μm long [55], as compared to the cell which is 1-2 μm. In order to accommodate such elongated strand of DNA in a relatively small cell, it must be highly condensed. Thus, the chromosomal DNA is coiled around itself in purported supercoiling. The supercoiling facilitates this 1000 μm long chromosome to accommodate within the cell with an actual girth of about 1 μm. Despite its super condensation state, DNA is always able to replicate, and allows transcription of the individual strands as required without becoming fatally entangled.
2) Usually DNA possesses double- helical structure. With each turn of a helix, two single strands are winding with each other. On an average around 10.4 base pairs separates two consecutive turns of DNA. Likewise, an *E. coli* chromosome composed of 4 million base pairs. This suggests that essentially the *E. coli* chromosome must be intertwined about 400,000 times. This double helical structure must unwind by the same number of times whenever DNA undergoes semi-conservative replication.
3) Prokaryotic DNA differs from eukaryotic DNA in many instances. Prokaryotic DNA undergoes less than one turn for each 10.4 base pairs [56] leading to negative supercoiling of DNA. Negative supercoiling is a fundamental characteristic of prokaryotic DNA to ease DNA replication and transcription and the whole process is

done with the help of the energy of the cell in the form of ATP. In summary, the creation and maintenance of negative supercoiled state of DNA uses a tremendous amount of energy.

4) Replication and transcription of DNA requires its helical structure to be unwound into two separated parallel strands facilitated by several enzymes to make the unwinding possible. RNA polymerase is one amongst those enzymes. RNA polymerase binds to the DNA and, with other cofactors, unwinds the DNA to create an initiation bubble so that the RNA polymerase can access to the single-stranded DNA template. However, unwinding during DNA replication means generation of positive supercoils ahead of the replication fork [57-60].Generation of positive supercoils is also a highly energy consuming process. Uninterrupted generation of positive supercoils causes scarcity of energy with in the cell and eventually it becomes difficult for RNA polymerase to unwind the DNA and carry out transcription, in energy deficient environment. This generation of positive supercoils, if not resolved, will lead to inefficiency in the transcription and replication processes.

   The process of generation of positive and negative supercoils is well regulated by a group of enzymes known as Topoisomerases [56, 61-63]. This group of enzymes fix the number of times one single strand of DNA duplex winds around its complementary strand. Fundamentally, we can assume that these enzymes are crucial for cell growth and replication.

   Three members in this group of topoisomerase enzymes have been discovered so far are categorized as:

1) Type I Topoisomerase - represented by topoisomerase I (in both eukaryotes and prokaryotes) and III in prokaryotes.

2) Type II Topoisomerase - represented by DNA gyrase (bacterial topoisomerase II and mammalian topoisomerase II) and Bacterial topoisomerase IV.

3) Special Topoisomerase - represented by those catalyzing transposition.

1.3.1 Bacterial Topoisomerase I

Topoisomerase I was discovered by James Wang in 1969 from *E. coli* [64]. Type I topoisomerase is a 110-kDa protein and is encoded by the *topA* gene [65].

Type I topoisomerase breaks one strand of DNA and passes it through the other. Topoisomerase I (bacterial as well eukaryotic), were able to release negative supercoils from DNA, even in the absence of ATP [64]. On the other hand, eukaryotic topoisomerase I was also able to remove positive supercoils. In a prokaryotic cell, DNA gyrase and topoisomerase I, both regulate the negative super- coiling of DNA [66, 67] and hence these enzymes are essential for transcription to take place [68]. Pruss *et al.* conducted a study on *topA* mutants of *E coli* [69]. These mutants were not able to produce a functional topoisomerase enzyme. This incident defers by excessive accumulation of negative supercoils in plasmid pBR322 DNA of *topA* mutant bacteria. This excessive accumulation of negative supercoils was dependant on transcription of *tet* gene. Hence, this study proved that topoisomerase I is required for removal of negative supercoil behind the progressive *transcriptional bubble.*

In general, quinolones inhibit the DNA relaxation activity of topoisomerase I at very high concentrations only [70,71].

1.3.2 Bacterial topoisomerase II (DNA gyrase)

DNA gyrase was first isolated by Martin Gellert and associates in 1976 from *E. coli* bacterium [56, 61-63, 72]. Based on high resolution microscopy, it was confirmed that gyrase had a heart shaped structure [73]. DNA gyrase is made up of four different subunits comprising of two A subunits (GyrA) and two B subunits (GyrB) [74, 75]. The GyrA subunit is encoded by the *gyrA* gene and GyrB subunit is encoded by *gyrB* gene. Both GyrA and GyrB subunits have specific functions. GyrA subunit is mainly required for DNA cleavage and re-ligation using a specific tyrosine residue (Tyr-122), which forms a temporary phosphotyrosine bond with the broken strand of DNA. GyrB subunit controls ATPase activity of the enzyme [75]. Several research studies have been conducted using the fragments of GyrA and GyrB subunits to scrutinize various functional domains of these subunits.

Reece *et al.* found out that the addition of a C-terminal 33-kDa GyrA fragment improved the efficiency of the enzyme and was able to stabilize the complex [76]. A 47-kDa C-terminal fragment of GyrB, when complexed with the GyrA subunit, was able to cause DNA relaxation but not ATP hydrolysis. Further work was subsequently carried out to identify the ATP binding site in the gyrase enzyme. The *N*-terminal region (amino acid 2 through 220) of the GyrB subunit is now considered as ATP binding region [77]. This finding is based on the study of the x-ray crystallographic structure of the gyrase enzyme. The structure of the gyrase enzyme complexing with the DNA has been studied extensively [73, 78]. It was found in those studies that a single turn of DNA is wrapped around the enzyme. ATP, when bound to the GyrB subunit, was able to induce structural change to the enzyme which eventually results in increased supercoiling [78]. In other

words, ATP is required by the gyrase to function [79]. DNA gyrase also requires a divalent cation (mainly magnesium) to function. Replacement of magnesium with calcium has been shown to result in an abortive DNA breakage reaction [76].

1.3.3 Bacterial Topoisomerase III

Srivenugopal *et al.* discovered topoisomerase III in 1984 [80]. This enzyme can remove the negative supercoils in DNA without energy consumption, but it needs energy in the form of ATP to remove positive supercoils [80]. On the other hand, it is less efficient as compared to other types of topoisomerase [81]. Topoisomerase III has affinity for both DNA, as well as RNA [82]. It is encoded by the *topB* gene located at 38.7 min on the *E. coli* genetic map [83]. Studies were conducted on mutants where genetic information was removed from 38.4 min to 39 min. Survival of these mutant bacteria have proved that topoisomerase III enzyme is a non-essential enzyme [83].

1.3.4 DNA Gyrase: primary target for quinolone action

In 1977, GyrA subunit was reported as a primary target of nalidixic and oxolinic acids by Gellert [84] and Cozzarelli [85]. Research has been done on purified gyrase enzyme in which GyrA subunit was collected from nalidixic acid resistant mutants and GyrB subunit was derived from the wild type population. This modified enzyme was active in the presence of normal concentrations of nalidixic acid that inhibited the wild type enzymes. Same experiments were repeated for *B. subtilis* [86], *Enterococcus faecalis* [87], *S. aureus* [88], *Pseudomonas aeruginosa* [89-92], and *Haemophilus influenzae* [93]. These experiments endorsed the same assertion that the GyrA subunit is also a target of quinolone action.

Two different mutations identified on the *gyrB* gene that are responsible for nalidixic acid resistance [94-96]. The first one, was responsible for increased resistance to newer quinolones. The second one caused increased susceptibility to quinolones containing piperazine at the C-7 position [97]. These studies confirm that both GyrA and GyrB subunits are targets for quinolone action.

The quinolone binds to the DNA - DNA Gyrase complex and blocks the religation of the cut DNA strands [98]. The ternary complex forms a "roadblock" for the replication machinery. Quinolones exert their inhibitory effect by binding to the Gyrase-DNA or Topoisomerase IV- DNA complexes. Upon binding with the complex, quinolones induce a conformational change in the enzyme. As a result of this conformational change, gyrase can still break the strands of DNA but loses its ability to re-ligate it (Figure 1.9). The overall result is the formation of a quinolone–enzyme–DNA complex. This complex formation reversibly inhibits DNA and cell growth and is responsible for the bacteriostatic action of the quinolones [97, 98].

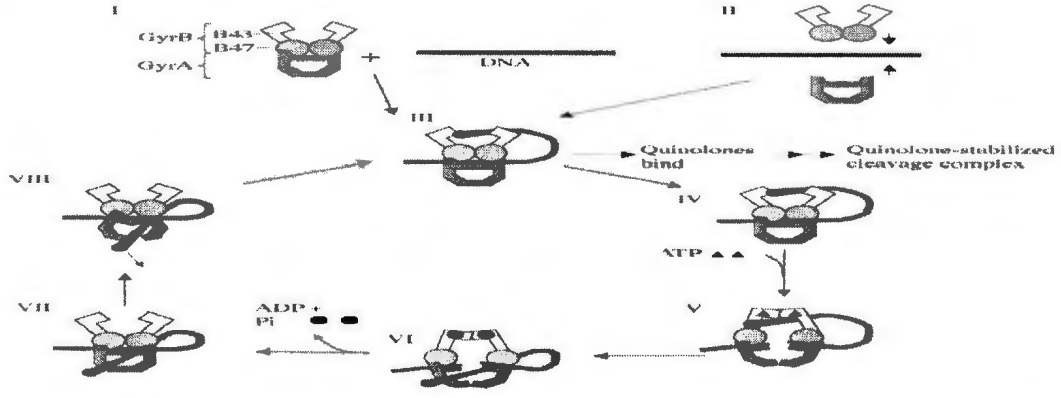


Figure 1.9. DNA gyrase super coiling cycle showing the point of action of quinolones [98]

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Conclusion

Antibiotics are Chemicals which kill and/or stop the growth of bacteria and other microorganisms. These drugs may be of natural origin or may be synthetic depending on their source. In synthetic antibiotics, the quinolone molecule is one of the most promising scaffold which can be customized in several ways to achieve a wide range of pharmacological activities. These activities can range from being antibacterial, anti-ischemic, antitumor or antiviral. Due to emerging bacterial resistance to existing antibacterial agents, the discussion on quinolones in antibacterial research is still an extremely important topic.

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Chapter -2

SAR and Non-Classical Use of Quinolones

2.1 Structure-Activity Relationships of Quinolones

Biological activity of a compound depends upon its structural feature which is responsible for interation with a specific receptor. Structure-Activity Relationships (SAR) is a comprehensive review on structural modifications of a specific class of biologically active compounds in order to improve or optimize some critical factors such factors include potency, efficacy, pharmacokinetics/pharmacodynamics, and safety aspects of that class and, as a result, identification of a lead compound for further preclinical studies. Fluoroquinolone skeleton is well studied by various researchers, in order to find the role of a specific substitution and effect on receptor. Figure 2.1 comprehensively explains the role of various substitutions on the fluoroquinolone pharmacophore and its impact on microbiological activity of the respective compound.

Position-1

Anti bacterial activity against Gram-negatives decreases when substitution at N-1 position is an alkyl or arylmethyl moiety, while the same structural pattern causes an increase in the activity against Gram-positive bacteria [1,2]. On the contrary, activity is retained with the addition of fluorine atom to the substitution at the N-1 position [3]. These studies also assert 2, 4-difluorophenyl as the second most important moiety at this position to improve the antimicrobial activity of quinolones [4].

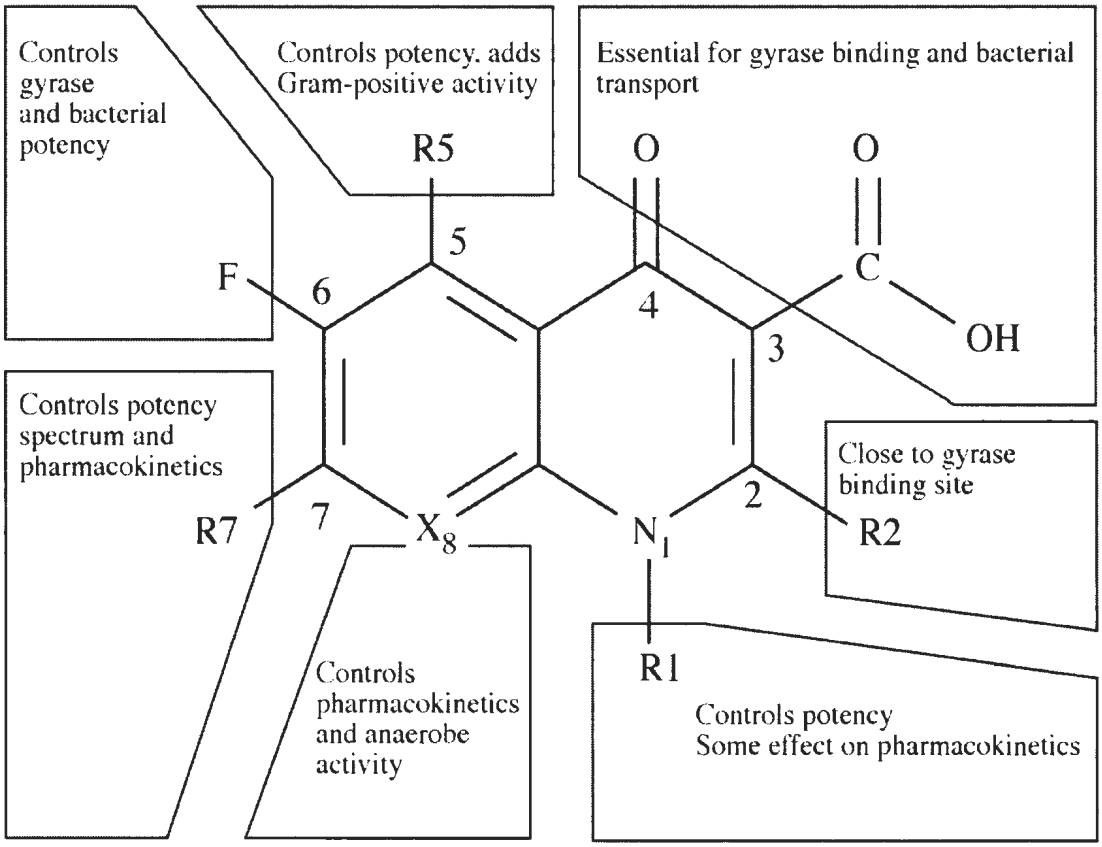


Figure 2.1. General structure of most commonly used quinolone molecules.

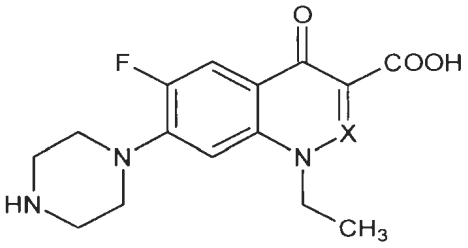
(Reproduced with permission from copyright holder; see appendix **A-2** for details)

Position-2

Before the discovery of fluoroquinolones, substitution at this position of a quinolone nucleus was reported to cause diminishing or loss of antibacterial activity [5]. As this position is in proximity to the gyrase binding site, various large substitutions at this position were considered to hamper the transfer of quinolones across the bacterial cells which ultimately results in reduction of antimicrobial activity [1]. Even addition of a small substitution such as methyl group at this

position was reported to result in complete loss of antibacterial activity. These results give us an inkling of another phenomenon that a large substitution at this position impedes the interaction between DNA gyrase and drug molecule by interfering with the co planarity of the C-3 carboxyl and C-4 keto groups of quinolone ring. However, when sulfur is incorporated in a small ring to replace the hydrogen, the antibacterial activity is reported to be retained [1]. During the synthesis and antibacterial evaluation of norfloxacin analogues, it was observed that the antibacterial activity was retained when nitrogen replaced carbon at the position 2while potency was significantly decreased. This can be inferred from comparative MIC values in Table 2.1 [6, 7].

Table 2.1. MIC of C-2 variants

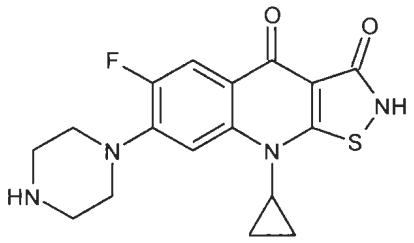


Drug	X	MIC (μg/ml) against *E. Coli*
Norfloxacin	CH	0.1
--	N	3.13

Positions-3 and -4

Positions 3 and 4 handle the gyrase binding and bacterial transport processes. A carboxyl substitution is found to be superior compared to the esters, phosphonates and sulphonates [8]. In the same fashion, a fused thiazolidone ring at the C-2 and C-3 positions of quinolones demonstrates analogy to a carboxylic acid group because of its aromatic resonance, and results in

compounds with outstanding *in vitro* potencies. Comparative MIC values against various bacterial strains are shown in Table 2.2 [9-11].



A

Table 2.2. MIC of C-3 variants

Structure	MIC (μg/ml)			DNA gyrase IC_{50} (μg/ml)
	E. coli	*P. aeruginosa*	*S. aureus*	
Ciprofloxacin	0.004	0.125	0.25	0.3
Compound A	0.005	0.02	0.02	0.09

Position-5

Substituents at this position form hydrogen bond with DNA. So this substituent must not be colossal or sterically hindered, and should be able to form hydrogen bond with the DNA for increased potency [12]. Antimicrobial activity against *E. coli* was found to increase after the addition of a primary amine substitution at C-5 [12]. Some electron-donating groups, for example hydroxyl, methyl etc., were also noticed to increase *in vitro* activity against Gram-positive bacteria. See Table 2.3 for MIC data [13].

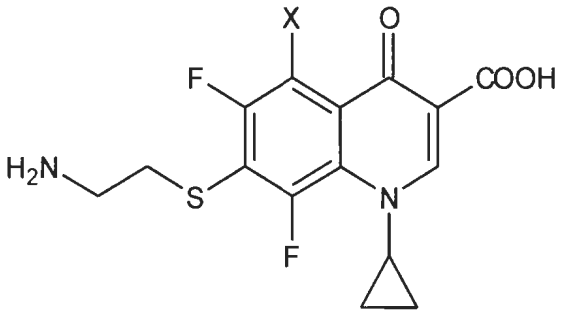


Table 2.3. MIC of C-5 variants

X	MIC (μg/ml) for:		
	E. coli	*P. aeruginosa*	*S. aureus*
NH_2	0.025	0.1	0.05
OH	1.56	6.25	1.56
F	0.78	3.13	3.13

Position- 6

After the discovery of fluoroquinolones, the fluorine substitution at this position was found to be essential for the antibacterial activity as shown in Table 2.4 [14]. In this context, Ledoussal *et al.* [15] were able to confirm the above phenomenon by making several C-6 fluorinated quinolones and compare their antibacterial profile with those molecules lacking the C-6 fluorine substituent.

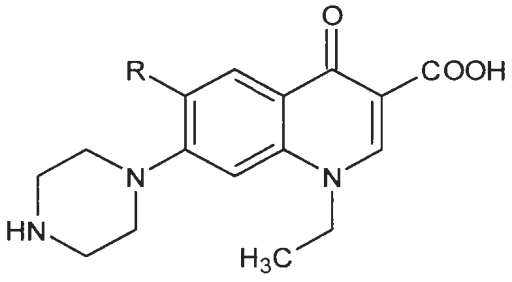


Table 2.4. MIC of C-6 variants

R	MIC (μg/ml) for:	
	E. coli	*P. aeruginosa*
H	0.78	3.13
F	0.05	0.39

Position-7

This position controls potency and pharmacokinetics of the compounds. Also, it is involved in direct interaction with the DNA gyrase [16]. Five- or six-membered nitrogen heterocyclic moieties (piperazinyl, *N*-methylpiperazinyl, pyrollidinyl, etc.) are the most common substituents at this position.

Position- 8

Substituents at this position contribute not only to pharmacokinetics properties of drug molecule and spectrum of activity but also play a role in reducing the development of bacterial resistance

[17]. Alkylation at this position is favourable since it not only improves the antibacterial activity against Gram-positive bacteria; it also improves the overall pharmacokinetic parameters.

2.2 Uses of Quinolones

2.2.1 Quinolones as Antitumor Agents

Cancer, the second largest cause of mortality in the world, is continuing to be a major health problem in the developed as well as developing countries. Tumor cells divide in an uncontrolled manner as compared to the other normal body cells. Human topoisomerases are of key importance in DNA replication and play important roles in cleaving the DNA chain at a particular point to reduce the stress of the growing replication fork and also simplify the annealing of the separated strains once replication has been done. Inhibition of these enzymes affects the vital functions of cell and more specifically arrests the cell cycle. Many topoisomerase directed cytotoxic agents are developed based on the idea of targeting human topoisomerases [18].

As described in Chapter-1 of this thesis, human topoisomerase II is analogous to DNA gyrase of prokaryotic organisms. Traditional targeting agents of this enzyme like the natural pentacyclic alkaloid and camptothecin, usually binds to topoisomerase II and forms a stable binary DNA-topoisomerase complex. This complex is composed of a single-strand nick thereby inhibiting DNA replication and ultimately arrests cell growth causing cell death during the S phase of the cell cycle. Many compounds like teniposide, etoposide, adriamycin etc., target topoisomerase II to exert their cytotoxic profile [18]. DNA intercalating agents, which are well established in the treatment of cancer, are also known to cause DNA damage as a mode of their cytotoxic action

[18 19]. Various studies have revealed that topoisomerases are involved in mediating the effect of various cytotoxic drugs, including DNA intercalating agents [18, 19].

Since the past two decades, quinolones have earned a special place in the treatment of infectious diseases due to their antibacterial activity as they cause potential chromosomal DNA damage within the bacterial cells [20, 21]. Further research found that formation of gyrase-DNA complexes give rise to the development of extra quinolone binding pockets which further bolster the mechanism of DNA damage [22]. Most quinolone derivatives have very high specificity for the enzyme such as bacterial or human topoisomerase. However, these enzymes are homologues so there is a great possibility of interaction with both enzymes by some compounds. Indeed, several antibacterial quinolones that also inhibit P4 DNA- unknotting activity of various eukaryotic type II topoisomerase have been identified [23-25].

Another study done by Kohlbrenner *et al.* [26] has reported two novel antibacterial isothiazoloquinolones, A-65281 and A-65282 (Figure 2.3) which target both bacterial and eukaryotic topoisomerases. These compounds were shown to induce DNA strand breakage as well as to inhibit the P4- unknotting reaction catalyzed by calf thymus topoisomerase II. This study, again, introduces a new class of topoisomerase II directed compounds, which were able to interact with both bacterial and eukaryotic enzymes.

Robinson *et al.* [27] reported **CP-115,953** which shows twice as much potency as that of etoposide at enhancing topoisomerase II mediated DNA cleavage. When the cyclopropyl group was replaced with an ethyl group at N-1 position, potency was found to be reduced by 20%. Compound **WIN 57294** [28], **A-62176** [29], and **A-85226** [30] were also found to be effective against cancer cell growth. Compound **A-65282**, which is an isothiazoloquinolone derivative,

was reported to demonstrate comparable potency to that of teniposide in enhancing topoisomerase II-facilitated DNA breakage. Compound **WIN 57294,** which is a 7-(2, 6-dimethyl-4-pyridyl) derivative, was found to have an EC_{50} value of 7.6 μM in a DNA cleavage assay using HeLa topoisomerase II. Compound **A-62176** and **A-85226** were found to have broad activity against human and murine tumour cell lines. Compound **AG-7352** [31] was also found to be more cytotoxic against human cell lines as compared to etoposide.

Signal transducers and activators of transcription (STATS) are proteins which are responsible for modulation of signals and transcription in the cell. Vinkemeier *et al.*have defined them as "A family of transcription factors that are specifically activated to regulate gene transcription when cells encounter cytokine and other growth factors" [32]. By this encounter, STATS regulate several processes that drive tumorigenesis, including proliferation, cell survival and angiogenesis, and also play an important role in immune surveillance. There are seven members in the STATS family discovered so far. Out of those seven different subtypes of mammalian STAT protein, STAT3 and STAT5 are oncogenic STATS which are constitutively activated in a variety of tumors. STAT1, whose function is assumed to antagonize the activities of STAT3, possesses tumor suppressor properties. Therefore, this protein has a discrete place in anticancer therapy as its expression has been shown to be reduced in several types of tumors. Also, STAT3 has achieved a pivotal position as a target in anticancer therapy [33].

A recent high throughput screening [34] carried out on large number of compounds found that a highly fluorinated quinolone derivatives, inhibits STAT3 pathway and causing apoptosis (EC_{50} = 4.6μM). Around 30 percent increase in STAT3 pathway inhibitory potency has been noticed by further optimisation of the structure of this fluorinated compound (Figure 2.2) [34].

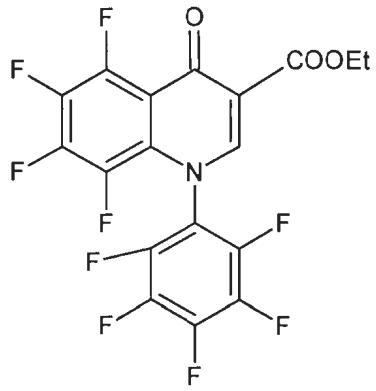
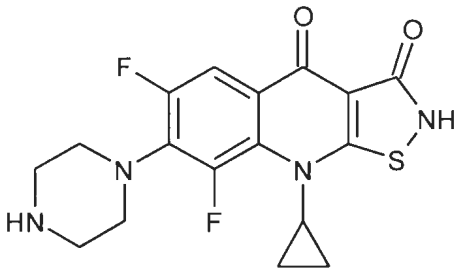
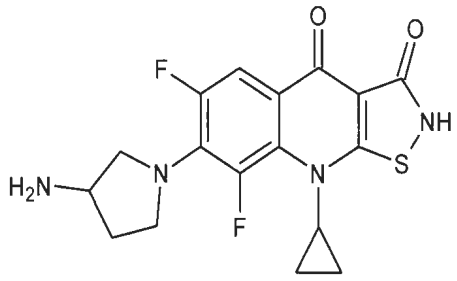


Figure 2.2. Structure of highly fluorinated quinolone derivative

Fertile results encourage researchers to perform a receptor-based virtual screening on a large array of compounds (around 70,000 different compounds). The results have revealed that some of the 3-carboxy-4(1*H*)-quinolones have very powerful human protein kinase CK2 inhibitory activity [35]. Protein kinase CK2 plays a significant role in the progress of various types of cancer and has been also demonstrated to play a pivotal role in viral infection and inflammatory processes. The chemical structures of the above mentioned compounds are depicted in Figure 2.3 [35].



A-65281



A-65282

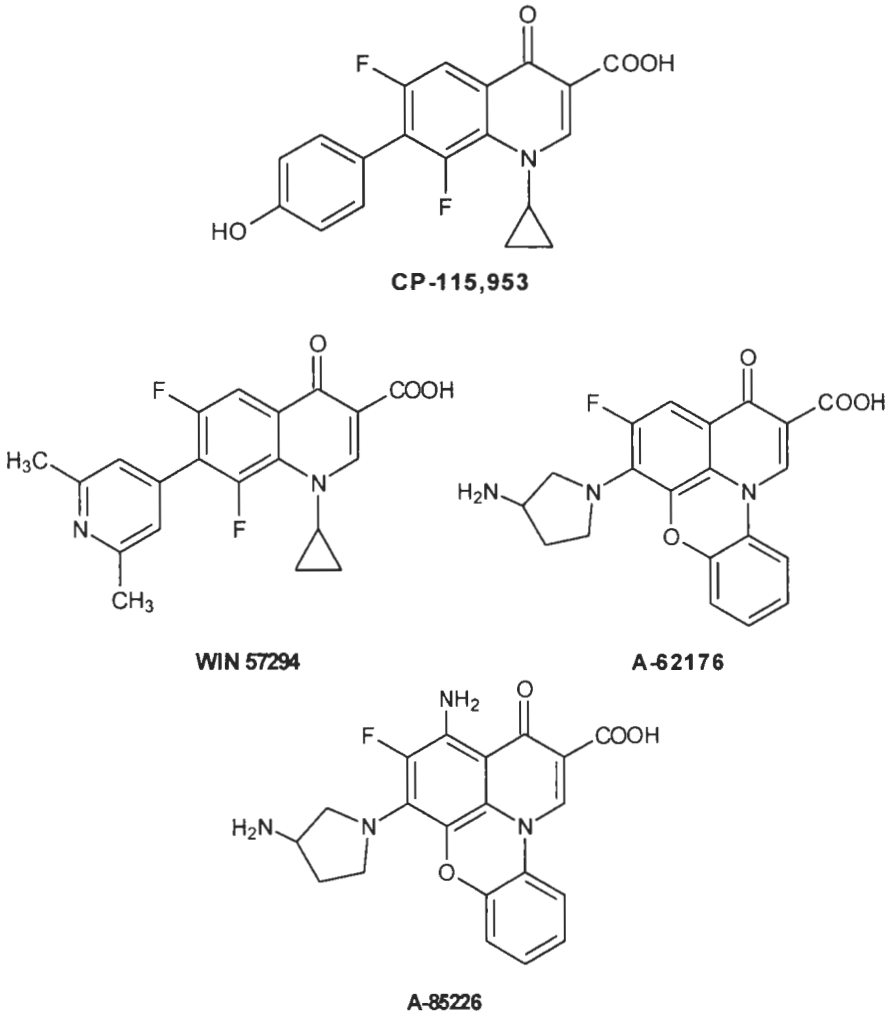


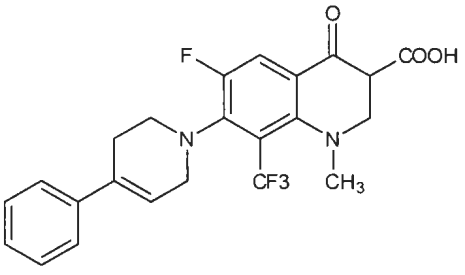
Figure 2.3. Structures of novel quinolone derivatives having anticancer activity

2.2.2 Quinolones as Antiviral Agents

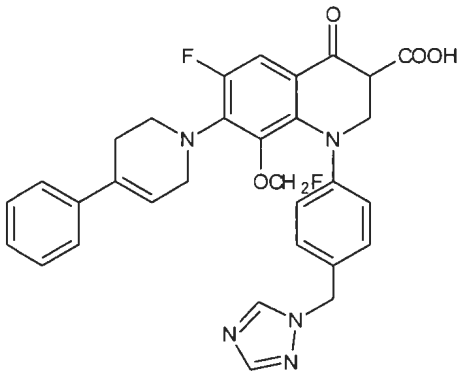
2.2.2.1 Quinolones as HIV-1 Inhibitors

Despite the availability of several classes of anti-HIV agents, there are limitation of options on therapeutic agents with sustained activity and reasonable efficacy against this viral infection. One of the most important limitations is the rapid emergence of resistant strains and inability of current treatment regimens to completely eradicate viral infection. To circumvent these problems

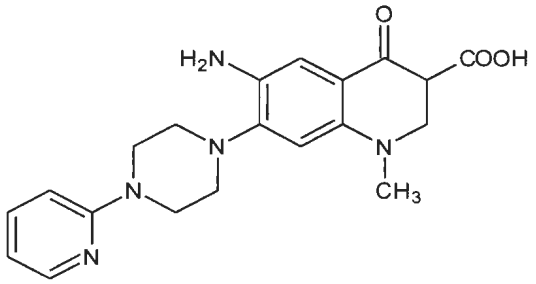
researchers are working towards devising novel molecules possessing new mechanisms of action, which are assumed to be a solution for these short- comings. Viral replication is again a set of reactions, one and most important of which is the transcription of its genome into mRNA. Most of the antiviral agents act by inhibiting this step which eventually leads to suppress HIV replication in both acutely as well as chronically infected cells [36]. Baba and coworkers suggested for the first time that some antibacterial fluoroquinolones can also exhibit antiviral activity. Two analogues, **K-37** and **K-38** (Figure 2.4), having a 3, 4-dehydro-4-phenyl-1-piperidinyl moiety at the C-7 position, exhibited $EC_{50} < 50$ nM in chronically infected cells. Cecchetti and coworkers [37] began a study on various quinolone derivatives including 6-fluoroquinolones and 6-desfluoroquinolones, and discovered that 6-amino-1-*tert*-butyl-7-[4-(pyridin-2-yl)]-1-piperazinyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid exhibited good antiviral activity and was taken as the lead molecule. To establish the structure-activity relationships of this lead, a series of variously substituted molecules were synthesized. Among those molecules, Compound **24** (Figure 2.4), which has a methyl substitution at the N-1 position and a 4-(2-pyridinyl)-1-piperazine moiety at position 7, was found to be the most potent molecule with $EC_{50} = 0.1\mu M$.



K-37



K-38



Compound 24

Figure 2.4. Structure of **K-37**, **K-38** and compound **24**

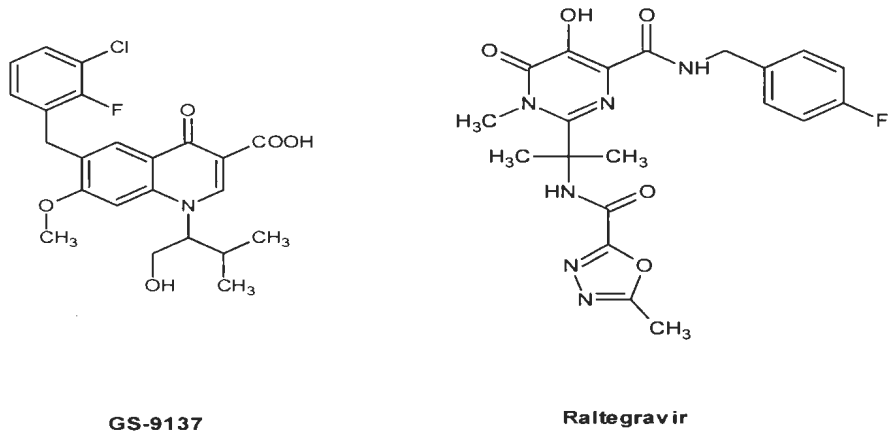
2.2.2.2 Quinolones as HIV-1 Integrase (IN) Inhibitors

Three most important enzyme encoded by the HIV-1 viral genome are: 1) HIV-1 reverse transcriptase; 2) HIV-1 protease; and 3) HIV-1 Integrase.

HIV-1 Integrase (IN), integrates viral cDNA into the genome of the host cell [38]. The insertion reaction catalyzed by IN involves two different steps. First step, usually takes place in the host cell cytoplasm and in this step IN cleaves GT nucleotides from the conserved 3'-CAGT motif. This reaction is known as 3' processing and produces unprotected hydroxyl groups at the both 3' ends of viral cDNA. The processed viral cDNA along with IN then moves to the nucleus of the host cell, where the second reaction takes place. In the second step of the integration, IN facilitate the nucleophilic attack of both 3'-end hydroxyls of the viral cDNA onto the host DNA. This process is usually termed as the strand transfer reaction.

The diketo acid (DKA) class of IN inhibitors were the first milestones for the inhibition of IN. For example, Raltegravir has wide spectrum of activity as an IN inhibitor. Recently discovered

GS-9137 (Figure 2.5) [39], which is a derivative of 4-quinolone-3-carboxylic acid, is found to be effective as an IN inhibitor, and is currently under clinical evaluation [40].



GS-9137 Raltegravir

Figure 2.5. Structures of **GS-9137** and **Raltegravir**

2.2.2.3 Quinolones as Anti-HSV-1 Agents

Herpes simplex virus (HSV) is globally a very common pathogen for various infections. Predominantly, HSV-1 causes infections on the face and lips, which includes cold sores and fever. Conventionally, acyclovir is widely used as a chemotherapeutic agent for the treatment of these infections or lesions. Unfortunately, development of resistance against the effect of acyclovir in immuno-compromised patients is the most encountered problem[41]. Recent research for quinolone-based derivatives that can be used in treating HSV-1 infections discovered Compound **23** (Figure 2.6). It is a quinolone acyclonucleoside derivative which has shown reasonable activity against HSV-1 [41]. At the concentration of 50 μM, this compound has demonstrated efficacy in reducing the viral load in the range of 70-99%. In the same research, it was found out that the carboxylic acid derivatives of quinolones were more effective than their corresponding esters. Various other structure analogues of compounds (**23**) were also

tested for cytotoxicity in Vero cells. Recently discovered quinolone derivatives were found effective in preventing the cytopathogenic effect of HSV-1 at μM concentrations. Among all, Compound **23** was found to be the most effective anti-HSV-1 derivative with almost 1.5-fold potency as that of acyclovir.

Further screening of latter compounds and a naphthalene carboxamide derivative have revealed the inhibitory activity of these non-nucleoside molecules against human cytomegalovirus (HCMV) polymerase. **PNU-183792** (Figure 2.7) acts as a competitive inhibitor of herpes virus polymerase which was effective against both human and animal herpes viruses. However, when toxicity assays were performed on **PNU-183792** using four different mammalian cell lines, the results were not encouraging enough for cytotoxic activity at relevant drug concentrations (CC_{50} value > 100μM).

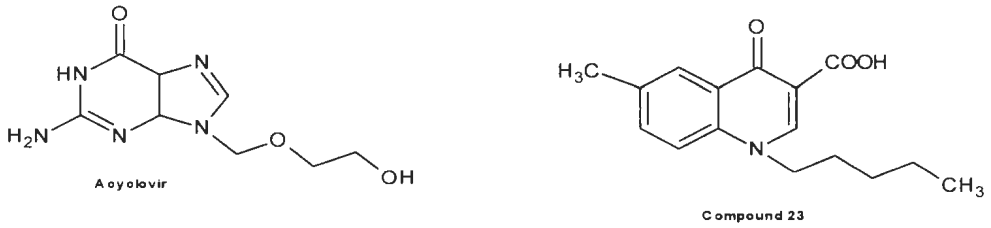


Figure 2.6. Structures of Acyclovir and compound **23**

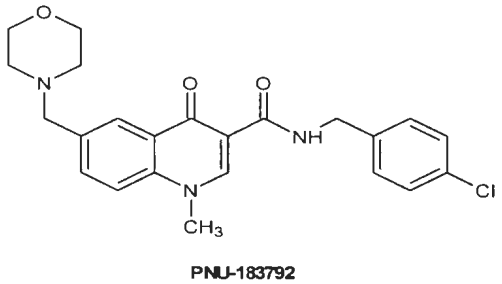
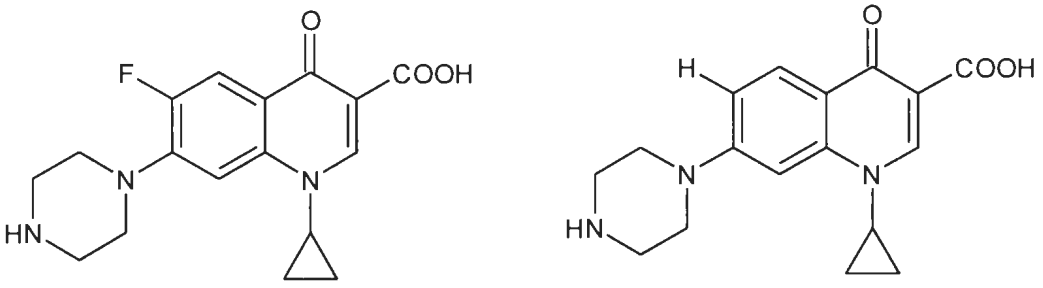


Figure 2.7. Structure of **PNU-183792**

2.2.3 Quinolones as Anti-Ischemic Agents

Ischemia occurs when partial or complete blockages of coronary arteries hamper the blood flow to the heart muscle. This type of ischemic cell injury further contributes to fatality or life-long disability. Thrombolytic therapy is the most common treatment for ischemic cell injury to overcome this obstruction. Thrombolytic agents help to restore blood flow to the ischemic region. However, thrombolytic therapy is also endowed with various limitations. Lately, on-going research on a few antibiotics has found them to be potential anti-ischemic agents. Antibiotics are used to treat inflammation and/or infection linked to ischemia; hence they can be viewed as prospective anti-ischemic agents [42]. Park *et al.*, has proposed in his research that ciprofloxacin exhibited *in vivo* and *in vitro* anti-ischemic activity. Lead optimization study on various known and novel molecules, with an objective to differentiate anti-ischemic activity from the anti-bacterial activity, resulted in the discovery of **SQ-4004**. This compound exhibited potent cell viability in addition to neuroprotective as well as cardioprotective effects. On the other hand, the antibacterial activity of this compound was reduced.(Figure 2.8) [42].



Ciprofloxacin **SQ-4004**

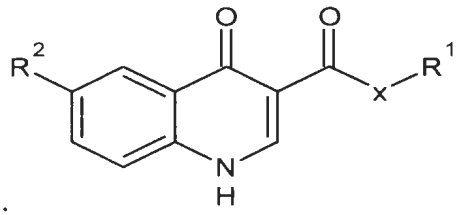
Figure 2.8. Structures of Ciprofloxacin and SQ-4004

2.2.4 Quinolones as Anxiolytic Agents

Anxiety can be defined as disturbances in psychological and physiological state which are characterized by somatic, emotional, cognitive, and behavioural components [43]. In order to maintain normal well-coordinated activity, there are two types of neurotransmitters found in the CNS, namely excitatory and inhibitory. γ-aminobutyric acid (GABA) is one of the most important amongst all inhibitory neurotransmitters in the central nervous system. It exhibits its effects by acting primarily on GABA receptors. Structurally, $GABA_A$ receptor composes of a pentameric macromolecular complex which regulates the opening and closing operations of ligand-gated chloride ion channels. In humans $GABA_A$ receptors are made up of six types of α subunits, three different types of β and γ subunits as well as a δ and θ subunits. Each of these subunits has different functions which are implemented via different physiological effects (for example α_1-containing receptors are involved in sedation, α_2 and α_3 in anxiolytic activity) [44]. The benzodiazepine class of drugs has been widely used as anti-anxiety agents for a long time. Benzodiazepines are non-selective against the receptors on which they act and allosterically control the GABA-mediated chloride ion influx through the chloride ion channel. On the contrary, benzodiazepines cause considerable amount of adverse effects. This problem incited the need of better anxiolytic agents.

A study carried out by Kahnberg *et al.* [45] found that 3-ethoxycarbonyl-6-trifluoromethyl-4 (1*H*)-quinolone (**15**) was a very fruitful lead compound for developing novel molecules that can effectively bind to the binding site of benzodiazepines on $GABA_A$ receptors. Further optimisation of this compound led to a large number of novel molecules that were able to bind to $GABA_A$ receptors with very vibrant affinity. However, in this study only compounds **20-22** were

tested on the $\alpha_1\beta_2\gamma_{2s}$ and $\alpha_3\beta_2\gamma_{2s}$ $GABA_A$ receptor subtypes. Compounds **21** and **22** were found to be selective for α_1- vs. α_3-containing receptors. The K_i ratio for compounds **21** and **22** was found to be 22 and 27, respectively. These values for receptor selectively were found comparable to that of the well known α_1 subunit selective drug, **Zolpidem**. Structures of compounds **15-22** are shown in Figure 2.9.



Compound	R^1	R^2	X
15	CH_2CH_3	CF_3	O
16	$CH_2CH_2CH_3$	$CH_2C_6H_5$	O
17	$CH_2CH_2CH_2CH_3$	CH_2CH_3	NH
18	$CH_2CH_2CH_3$	CH_2CH_3	NH
19	$CH_2CH_2CH_3$	$CH_2C_6H_5$	NH
20	CH_2CH_3	Br	O
21	$CH_2CH_2CH(CH_3)_2$	CH_2CH_3	O
22	CH_2CH_3	$CH_2C_6H_5$	O

Figure 2.9. Novel quinolone derivatives with anti-anxiety effect

2.3 Thiazetoquinolones

2.3.1 Introduction

Like most of the currently utilized antibacterial agents, emerging bacterial resistance, especially methicilline-resistant *Staphylococcus aureus* (MRSA), has become a major barrier in chemotherapeutic application of different generations of quinolones. Thus the need for more effective agents to treat infectious diseases caused by drug-resistant bacteria, such as quinolone-resistant MRSA, is essential [46, 47].

While searching for new quinolone antibacterial agents scientists came across a tricyclic sulfur-containing quinolone derivative in which a 4-(1*H*) quinolone system is fused to a thietane ring at N-1 and C-2 junctions (Figure 2.10). This building block was later named thieazetoquinolone [46, 48].

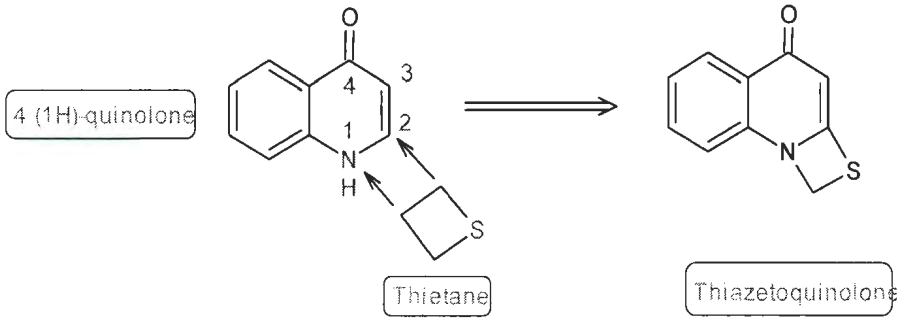


Figure 2.10. Thiazetoquinolone nucleus

2.3.2 Some structural features of thiazetoquinolones

As discussed in SAR of quinolones, any polycyclic structure with a bridge connecting the N-1 and C-2 positions of the quinolone would prevent the free rotation of the substituent attached to the C-2 position, thereby affecting the interaction of these compounds with topoisomearase II. Researchers expect that such structure should retain its activity against DNA gyrase [48]. In this context, M. Matsuoka *et al.* were able to synthesize a series of compounds and noted the excellent antibacterial activity in 6-fluoro-1-methyl-4-oxo-7-(1-piperazinyl)-4*H*-[1,3] thiazeto[3,2-*a*]quinoline carboxylic acid (Figure 2.11) [49].

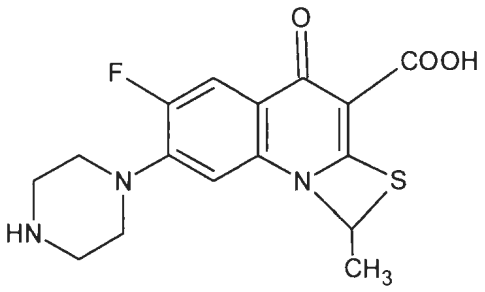


Figure 2.11. Structure of 6-fluoro-1-methyl-4-oxo-7-(1-piperazinyl)-4*H*-[1, 3] thiazeto[3,2-*a*]quinoline carboxylic acid.

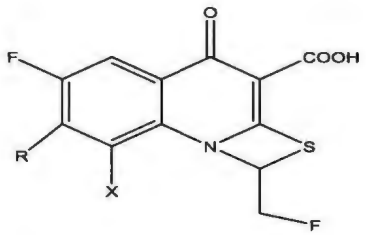
Generally, substitution of a cyclic amino moiety at the C-7 position of a quinolone scaffold plays an important role in antibacterial potency of these molecules. This phenomenon is also applicable in thiazetoquinolines when the C-7 position of this scaffold is substituted by a 3-hydroxyazetidinyl moiety. In fact, the 3-hydroxyazetidinyl-substituted derivative displays the best activity against Gram-positive bacteria amongst all other counterparts including morpholinyl , piperazinyl, and thiomorpholinyl analogues. An analogue with piperidine substitution has shown least activity amongst all [49, 50]. The same authors also synthesized some 8-

unsubstituted-1-fluoromethylthiazetoquinolone derivatives, among which, 7-(3-hydroxyazetidinyl) analogue was showing the most significant antibacterial activity against Gram-positive bacteria including MRSA. The 7-(4-methyl-1-piperazinyl) analogue and 7-morpholino analog showed considerable antibacterial activity against Gram-positive resistant bacteria, whereas other analogues like 7-(3-hydroxyazetidinyl) analogue showed no significant activity [49].

On the other hand, the order of *in vitro* activity against Gram-negative bacteria was completely different. The analogue with 4- methyl piperazinyl and piperazinyl substituents were showing very good activity amongst all. Whereas, the analogue with thiomorpholinyl or 4-hydroxypiperidinyl and also 4-aminomethylpiperidinyl moieties showed the lowest activity [49, 50]. Among all the derivatives prepared by M. Matsuoka *et al.* 7-piperazinyl analogues were found to exhibit the most balanced *in vitro* activity against both Gram-positive and Gram-negative bacteria including quinolone-resistant MRSA [49].

Insertion of a fluoromethyl moiety at the C-1 position of thiazetoquinolone ring also influences profile and antibacterial potency of this class of compounds. In the 8-unsubstituted-7-(4-methyl-1-piperazinyl) derivatives, 1-fluoromethyl analogue was showing more potent antibacterialactivity against quinolone-resistance MRSA as compared to 1- methyl analogue, but exhibiting the same or less activity against other bacteria. Whereas, in the 7-(1-piperazinyl) derivatives, the substitution of fluoromethyl at C-1 position did not affect its potency against Gram-positive bacteria, it even decreased activity against Gram-negative bacteria considerably[49]. Methoxy group at C-8 position of the 1-fluoromethyl analogue resulted in moderate increases in antibacterial activity against Gram-positive bacteria including quinolones-

resistant MRSA.The addition of fluorine at C-8 position led to decreased antibacterial activity (Figure 2.12) [49].



R	X	Gram-positive MIC(μg/ml) (*Staphylococcous aureus*)	Gram-negative MIC (μg/ml) (*E. Coli*)
4-Methylpiperazinyl	H	0.1	0.05
4-Methylpiperazinyl	MeO	0.05	0.025
4-Methylpiperazinyl	F	0.2	0.2
Piperazinyl	H	0.05	0.025
Cis-3,5-dimethylpiperazinyl	H	0.1	0.05
Morpholino	H	0.05	0.2
Thiomorpholino	H	0.1	0.78
4-Hydroxypiperidino	H	0.05	0.2
4-Aminopiperidino	H	0.1	0.2
4-Hydroxymethylpiperidino	H	0.1	3.13
4-Aminomethylpiperidino	H	0.2	1.56
3-Hydroxyazetidino	H	≤0.00625	0.05
4-Methylpiperazinyl	H	0.2	0.1
4-Methylpiperazinyl	H	0.025	0.0125

Figure 2.12. *In vitro* antibacterial activity of Thiazetoquinolone derivatives

2.4 Conclusion

The quinolone nucleus is one of the most promising scaffolds which can be modified in a number of ways to achieve diverse pharmacological activity. Huge array of activities including antibacterial, anti-ischemic, antitumor, antiprotozoal, antihelminthic and antiviral have been displayed by this class of compounds. Despite decades of research on their activities, it is still a hot topic for researchers. Even after discovery of involvement in various activities, there are still

a lot of unsolved problems to resolve due to development of resistant bacterial strains or unexpected adverse effect profiles of the existing regimens.

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Chapter-3

Design, Synthesis and In-vitro Evaluation of thiazeto[2,3-*a*]quinolones as Potential Bioactive Molecules

3.1 Introduction

As discussed in Chapter 2 of this thesis, quinolone derivatives can act as anticancer agents. Anticancer quinolones cause cytotoxicity through DNA intercalation or by causing interference in the replication process. As mentioned in Chapter-1 of this thesis, human topoisomerase II is analogous to DNA gyrase enzyme of prokaryotic organisms. Some antibacterial or anticancer quinolones such as actinomycin D, doxorubicin, mitoxantrone and streptonigrin display their biological activities through DNA intercalation [1]. Despite the vast number of quinolone analogues reported with reasonable biological activity, researchers are still delving into this field for synthesis of new compounds with a novel structure and different mechanisms of action. Due to bacterial antibiotic resistance and toxicity profiles of compounds, research is moving towards the extension of quinolone genera with formation of novel polycyclic quinolone analogues with a good biological activity and less toxicity.

Our research group has long been involved in developing a novel series of polycyclic quinolones with diverse biological activities [2a-c]. In this context our previous group member, Dr. Abeer Ahmed, was able to isolate and identify novel 4-oxo-benzo[*h*]thiazetoquinoline derivatives [3] (Figure 3.1). The discovery of this compound was considered as serendipity due to unexpected structural feature of the product while attempting to synthesize a thieno [2,3-*b*]benzo[*h*]quinoline derivative. While there are numerous research reports available on various polycyclic quinolones, for example 4-oxo-thiazolo [3-2*a*]quinoline-3-carboxylic acid derivatives, 4-oxo-thieno[3,2-*a*] quinoline 3- carboxylic acid derivatives etc., there are very limited reports on the 4-oxo-thiazeto [3, 2-*a*] quinolines [4-7]. The research reported by Dr. Ahmed prompted us to continue our research on 4-oxo-thiazeto[3, 2-*a*]quinolines.

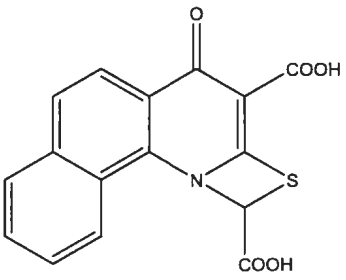


Figure 3.1. 4-oxo-benzo[*h*] Thiazeto[3,2-*a*]quinoline derivatives [2a]

An extensive literature search on the chemistry of thiazeto[3,2-*a*] quinolines led us to a series of publications related to the synthesis and antibacterial activity of a 4-oxo-thiazeto[3,2-*a*] quinoline-3-carboxylic acid, Prulifloxacin [8] (Figure 3.2). Actually, it is a prodrug which metabolizes in the body to the active compound ulifloxacin [9]. The first synthesis of this compound was reported almost two decades ago by Nippon Shinyaku Co. and patented in Japan in 1987 and in the United States in 1989. [10-13]

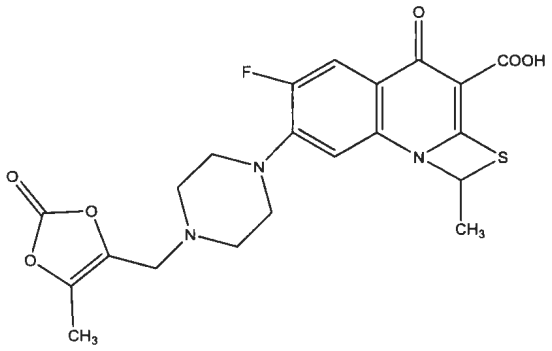


Figure 3.2. Structure of prulifloxacin

As discussed in Chapter 2 of this thesis the rationale for prulifloxacin synthesis was to decrease the steric obstruction of the C-2 substituent toward the C-3 carboxyl group *via* formation of a 4-membered ring that connects the N-1 and C-2 atoms. This would possibly improve the binding potential of quinolone system to the bacterial enzyme [14].

On the same path, Matsuoka *et al.*, reported the synthesis of 6-fluoro-1-methyl-4-oxo-7-(1-piperazinyl)-4H-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid (NM394). This compound was also showing excellent *in vitro* antibacterial activity [15] (Figure 3.3).

Ito *et al.*, have reported another compound from this series , a 4-oxo-1,4-dihydro-[1,3] thiazeto[3,2-*a*]quinoline-3-carboxylic acid derivative, as a potent antibacterial, anticancer, and anti-HIV agent [16] (Figure 3.4].

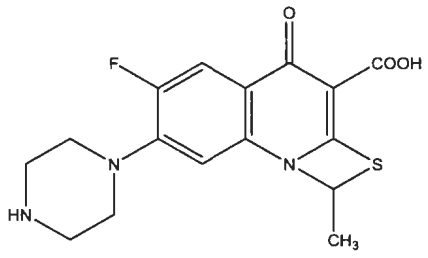


Figure 3.3. Compound reported by Matsuoka *et al.*[15]

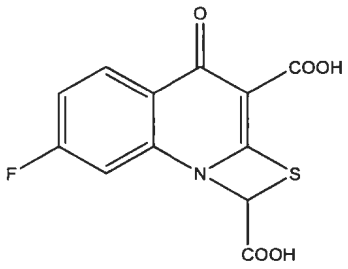


Figure 3.4. Compound reported by Ito *et al.*[16]

The novel synthetic procedure reported by A. Ahmed *et al.*, for the synthesis of the 4-oxo-1,4-dihydrobenzo[*h*][1,3]thiazeto[3,2-*a*]quinoline-1,3-dicarboxylic acid is shown in Figure 3-5. In this procedure, sodium salt of diethyl malonate was allowed to react with naphthylisothiocyanate and the resulting product was further reacted with ethyl bromoacetate. The open chain derivative obtained was then cyclised by heating under vacuum at 180-185 ^{0}C to afford the corresponding

benzo[h]quinolone carboxylic acid ester. This scaffold was subjected to several oxidative cyclization reactions to obtain the targeted thiazeto[3,2-*a*]quinoline derivatives [2c,3].

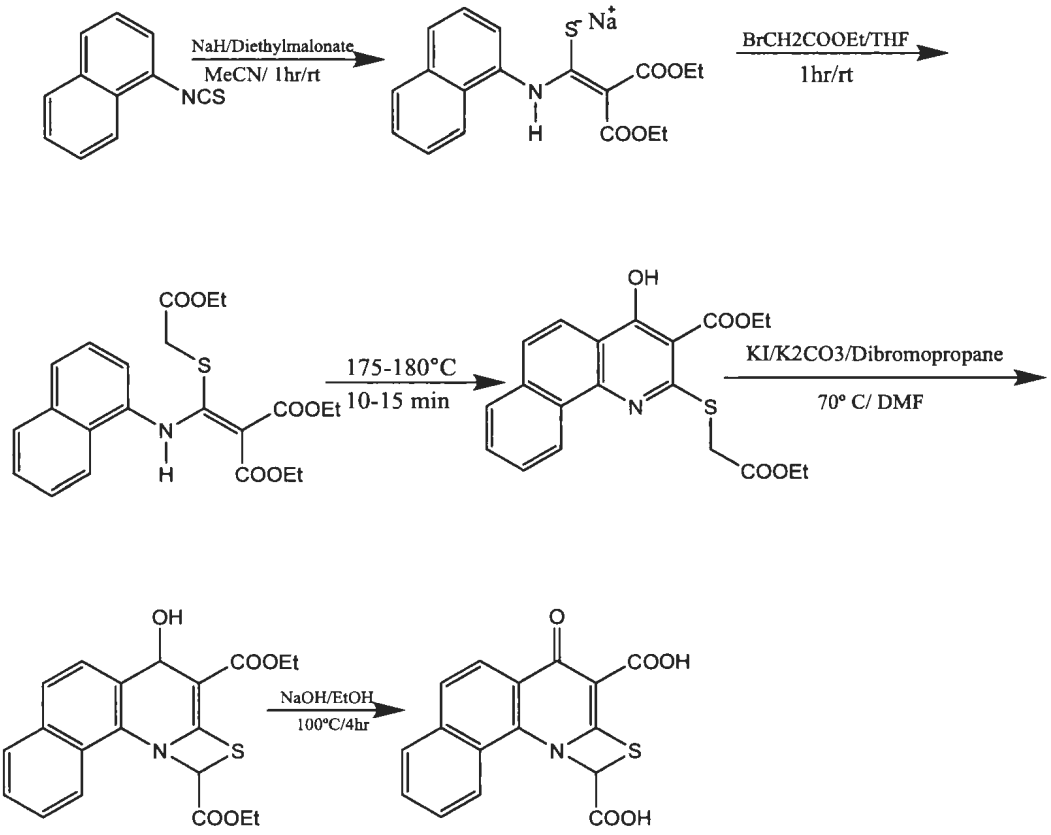
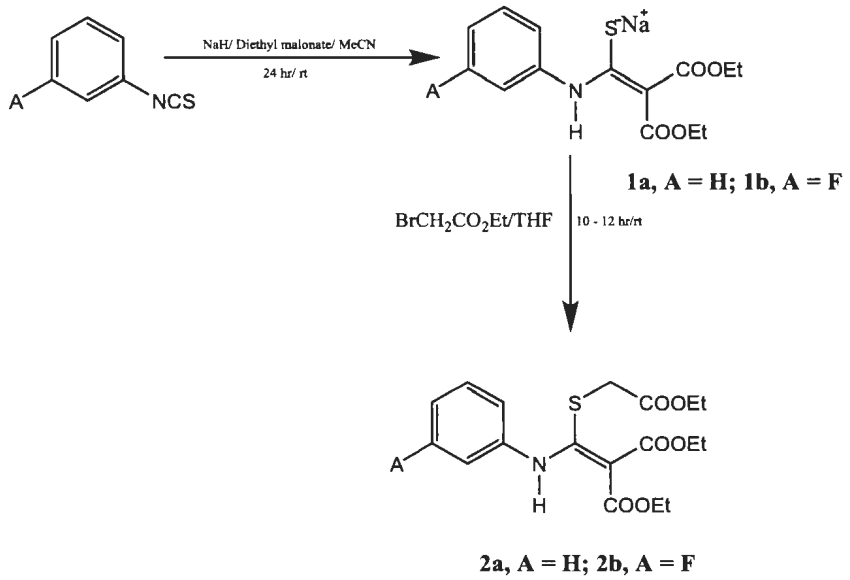


Figure 3.5. Synthesis of 4-oxo-thiazeto [3, 2-*a*]quinoline nucleus.

In continuation of our current research on thiazeto [3, 2-*a*]quinolones and in order to expand our knowledge on the chemistry and biological profiles of this nucleus, we attempted the synthesis and biological evaluation of modified thiazeto[3,2-*a*]quinolone analogues of compounds reported by Ahmed *et. Al.* [2a].

3.2 Materials and Methods

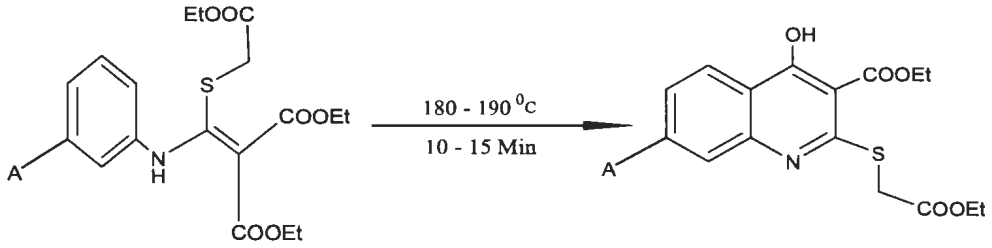
Our approach to the synthesis of the target compounds starts with 3-fluorophenylisothiocyanate or simply phenylisothiocynate. In the first step, sodium hydride is reacted with diethylmalonate in acetonitrile to afford sodium salt of diethyl malonate. To this a solution of phenylisothiocyanate or 3-fluorophenylisothiocyanate is added drop-wise and the mixture is stirred at room temperature for 4 hour to yield the salts **1a-b.** These salts are further reacted with ethylbromoacetate in anhydrous THF under ice cooling to obtain compound **2a-b** as shown in Scheme 3.1a.



2a, A = H; 2b, A = F

Scheme 3.1a

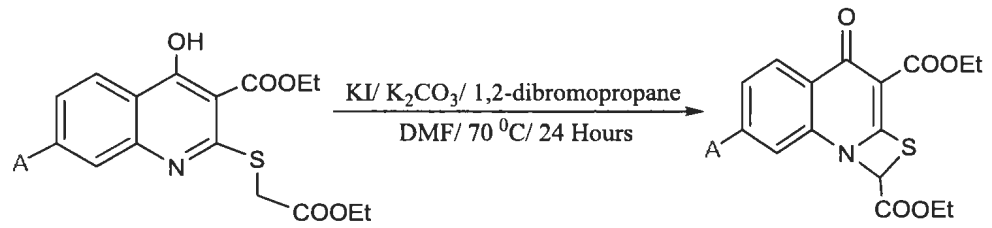
Compounds **2a-b,** which are open-chain unstable intermediates, are immediately subjected to thermal cyclization by heating for 10-15 min under vacuum at 180-190 ^{0}C to afford Compounds**3a-b**, as shown in Scheme 3.1b.



2a, A = H; 2b, A = F **3a, A = H; 3b, A = F**

Scheme: 3.1b

Following the oxidative cyclization procedures reported by Ahmed, A. *et al.* [2b] with minor modifications, compounds **3a-b** are later reacted with 1,2-dibromopropane in the presence of KI and K_2CO_3 to obtain compounds **4a** and **4b**, as depicted in Scheme 3.2.

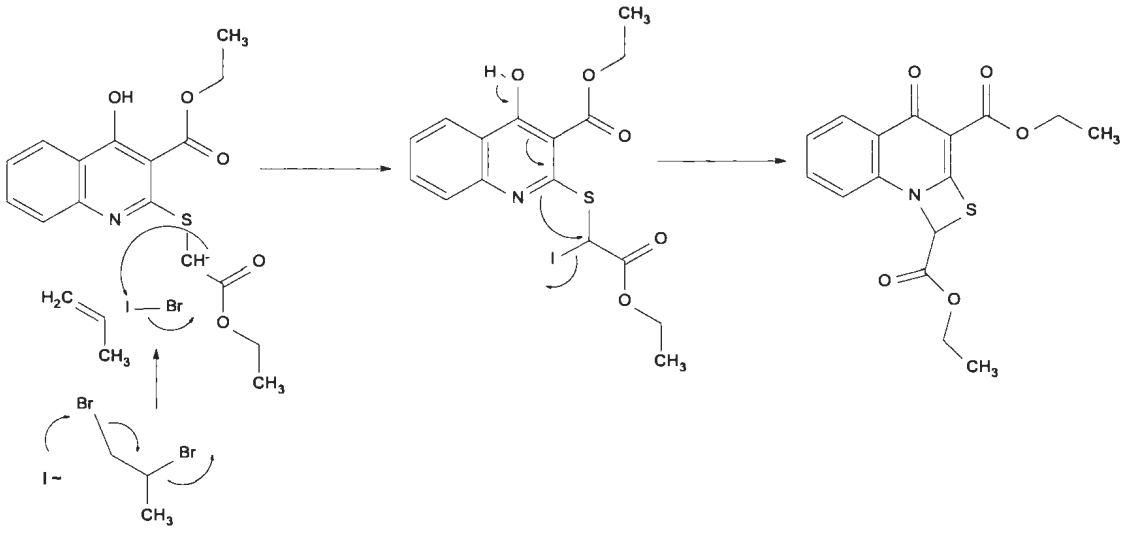


3a, A = H; 3b, A = F **4a, A = H; 4b, A = F**

Scheme 3.2. Formation of compounds **4a-b**

According to all previously reported procedures this cyclization reaction can only be possible in presence of vicinal dihaloalkane and but not in presence germinal analogues. [9-13]. It is interesting to know that no part of the vicinal dihaloalkane participates in the final structure of the thiazetoquinolne structural features. This phenomenon suggests the catalytic role of the vicinal dihaloalkane in this oxidative cyclization procedure, rather than a contributor to the structural feature of the target compounds. [2c]

Based on our previously reported mechanism of formation of vicinal dihaloalkane catalyzed thiazeto-quinolines [2c], a likely pathway for formation of our tricyclic derivatives is suggested as following. Namely, the 4-oxo-thiazetoquinoline nucleus is formed *via* reaction of the carbanion of the ethoxycarbonylmethylsulfide group at the C-2 position of the quinoline ring with a pseudo halogen (IBr), formed *via* reaction of iodide anion with the vicinal dihaloalkane, or a halogen (I_2), followed by nucleophilic attack of the N-1 on the halogenated carbon and the departure of halogen. The role of the vicinal dihaloalkane in this process is the provision of a pseudo halogen such a IBr without direct interaction with quinoline system as depicted in the following scheme [2c].

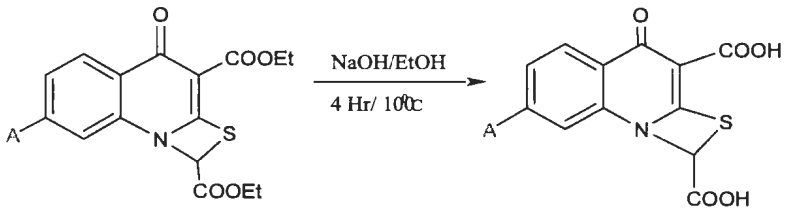


Mechnism 3.1 Proposed Mechanism for scheme 3.2

In order to reassure this mechanism and participation of the pseudohalide (iodobromide), formed *via* the reaction of 1,2-dibromopropane and KI, in the oxidative cyclization of Compound **3** to Compound **4**, we attempted the reaction of **3a** with iodobromide or iodine in presence of K_2CO_3. In both attempts we were able to obtain Compound **4** in reasonable yields.

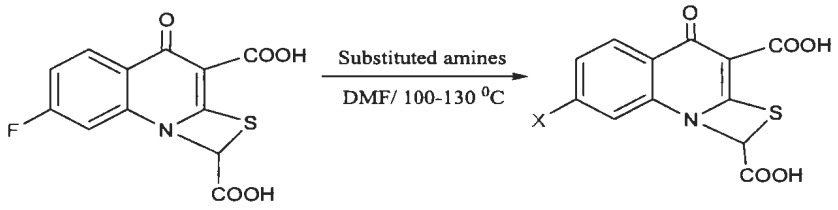
Compounds **4a-b** were further saponified by heating in alkaline alcoholic solution at 100^0C for 4 hours, as shown in Scheme 3.3.

Compound **5b** was further gone under nucleophilic substitution reaction with several amines using the procedures reported by Tabart, M. *et al.* [17], Antoine, M. *et al.* [18], and Massari, S. *et al.*[19] with some alterations to obtain the corresponding C-7 substituted derivatives **6a-h** as depicted in Scheme 3.4.



4a, A = H; 4b, A = F **5a, A = H; 4b, A = F**

Scheme 3.3. Formation of compounds **5a-b**



5b **6a-e**

Scheme 3.4. Synthesis of compounds **6a-h**

6a; X= Piperazine, 30%
6b; X= methylpiperazine 27%
6c; X= Pyrrolidine 17%
6d; X= Morpholine 21%
6e; X= Pyrrol 37%

3.3 *In vitro* microbiological testing

In order to evaluate the biological activities of newly synthesized compounds **5a-b** and **6a-c**, antimicrobial screening was carried out, using broth dilution method to determine their MICs. The 96-well microtiter plate-based serial dilution method was adopted [20]. In accordance with

the protocols followed by Tahlan bacteriology Laboratory at the Dept. of Biology, Memorial University, nalidixic acid and ampicillin were used as controls. *E. coli*, *B. subtilis*, *S. epidermidis*, *P. fluorescens*, and *M. luteus* were used as the test organisms. Stock solution of concentration 10000 µM was prepared by appropriate weight of test compounds. 100 µl of sterile media was added to the wells 1A-1H (Figure 3.6). To the wells 2A-2H to 11A-11H, 50 µl of sterile media was added. To each well 1A-1H, 2 µl of the stock solution of test compound was added and mixed properly. Using a multichannel pipette, 50 µl of the media from wells 1A-1H was transferred to the wells 2A-2H and mixed properly. This procedure was repeated till the wells 11A-11H. To the wells 12A-12H, only 50 µl of the sterile media was added without any test compound. To each well, 50 µl of various microbial suspensions was then added. This resulted in the effective concentrations of 100, 50, 25, 12.5, 36.25, 3.125, 1.5625, 0.78125, 0.390625 and 0.1953125 µM in the wells 1-11, respectively. Wells were then allowed to incubate at the respective temperatures. Plates containing *M. luteus* and *B. subtilis* were incubated at 30^0C for 48 hours. Plates containing *S.epidermidis, P. fluorescens*, and *E.coli* were incubated at 37^0C for 48 hours. Nalidixic acid and ampicillin were used as the reference standards. After incubation, plates were observed visually to detect the presence of bacterial growth.

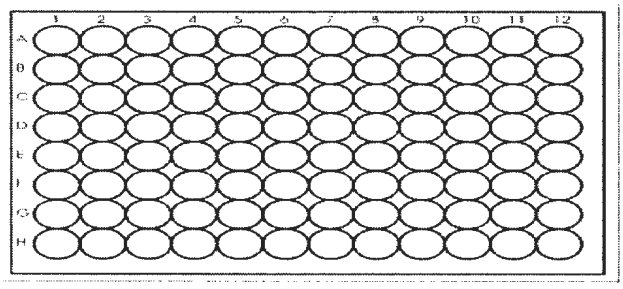


Figure 3.6. A 96 well plate

3.4 Molecular Docking

3.4.1 Methods

Docking calculations were carried out according to the Docking Server methodology [21]. The MMFF94 force field [22] was used for energy minimization of ligand molecule using Docking Server. PM6 semi empirical charges calculated by MOPAC2009 (J. P. Stewart, Computer code MOPAC2009, Stewart Computational Chemistry, 2009) were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined.

Docking calculations were carried out on human topoisomerase II and IV structures with the pdb codes 4FGH, 1S16 and 3FOE. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [23]. Affinity (grid) maps of 25×25×25 Å grid points and 0.375 Å spacing were generated using the Autogrid program [23]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the vander Waals and the electrostatic terms, respectively.

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [23]. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 100 different runs that were set to terminate after a maximum of 2500000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

3.4.2 Background

Topoisomerases are ATPases belonging to the GHKL (Gyrase, Hsp90, histidine Kinase, mutL) family. Their mechanism of function is to cut both strands of the DNA helix using the energy

derived from ATP hydrolysis. As a consequence, there are more ways to inhibit the enzyme's action, such as inhibiting ATP binding or blocking DNA cleveage. Thus, molecular docking studies on topoisomerase inhibition are focused on the ATP binding site and on a possible ligand binding site at the DNA-protein interface.

Quinolones are known to inhibit type IIA topoisomerases, the topoisomerase II (bacterial DNA gyrase) and the bacterial topoisomerase IV. Therefore, the goal of this study was to evaluate the inhibition potential of these compounds on topoisomerase II and IV.

There are a number of topoisomerase structures available in Protein Data Bank; however, architecture of the full-length enzyme has remained undefined until recently. In 2012, Schmidt, B.H. *et al.*, has published the first full length structure of topoisomerase II of *Saccharomyces cerevisiae* S288c (PDB code **4GFH**) complexed with DNA and a non-hydrolysable ATP analog enabling molecular docking studies on an enzyme in a biologically relevant conformation. Although this structure (**4GFH**) does not contain bound ligand at the DNA-protein interface, alignment with topoisomerase II structure (3QX3) with a bound ligand has revealed the potential binding site of an inhibitor exerting its effect at the DNA binding site of the protein, as shown in Figure 3.7 [24].

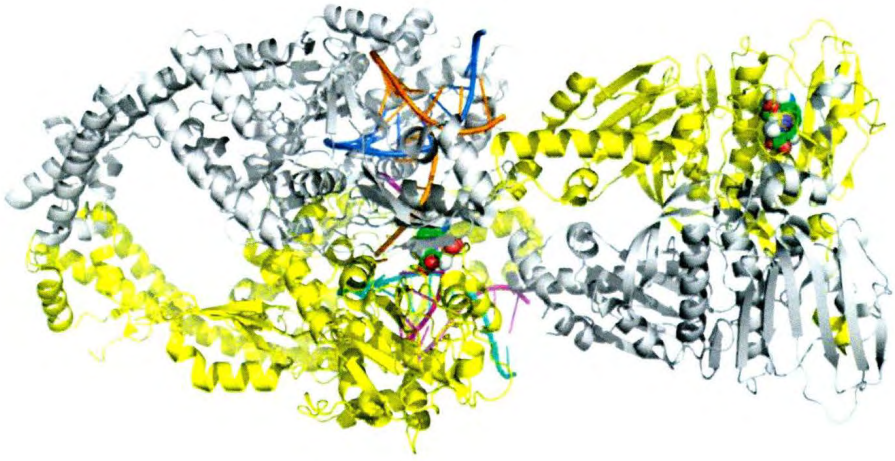


Figure 3.7. ATP and DNA binding sites in PDB accession code 4GFH.(Ligands are indicated with spheres)[24]

In contrast to topoisomerase II, no full length structure of bacterial topoisomerase IV exists in Protein Data Bank. Thus, DNA bound and ATP bound structures of bacterial topoisomerase IV were selected separately. PDB entry **1S16** was used for the ATP binding site, whereas PDB entry **3FOE** was used for docking at the DNA-protein interface.

3.4.3 Results

The resulting docking energies are shown on Table 1. All compounds had more favorable binding on the DNA binding site as compared to the ATP binding site. It can be seen that binding of larger derivates is more favorable at the DNA binding site; while the smaller ATP binding site accommodates smaller ligands due to possible steric reasons.

Table 3.1. Lowest docking energies of the investigated compounds at topoisomerase II and IV binding sites. (Most favorable energies are indicated with bold letters)

Ligand/Protein	4GFH ATP binding site	4GFH DNA binding site	1S16 ATP binding site	3FOE DNA binding site
6a	-8.2 kcal/mol	**-13.2 kcal/mol**	-4.5 kcal/mol	-8.6 kcal/mol
6c	-8.2 kcal/mol	-11.4 kcal/mol	-6.2 kcal/mol	-8.4 kcal/mol
6b	-8.6 kcal/mol	**-13.0 kcal/mol**	-5.8 kcal/mol	-8.2 kcal/mol
5b	-7.9 kcal/mol	-11.6 kcal/mol	-7.0 kcal/mol	-7.0 kcal/mol
5a	-7.6 kcal/mol	-11.5 kcal/mol	-7.2 kcal/mol	-7.2 kcal/mol

Figure 3.8 shows the interactions of Compound **6a** with the ATP binding site of 4GFH. A strong coulomb interaction can be observed between the terminal amino group of LYS147 and carboxylate group of the ligand. This carboxylate accepts a hydrogen bond from ASN70 at the same time. ILE120, PHE121 and ILE104 form hydrophobic interactions with the aromatic rings of the ligand.

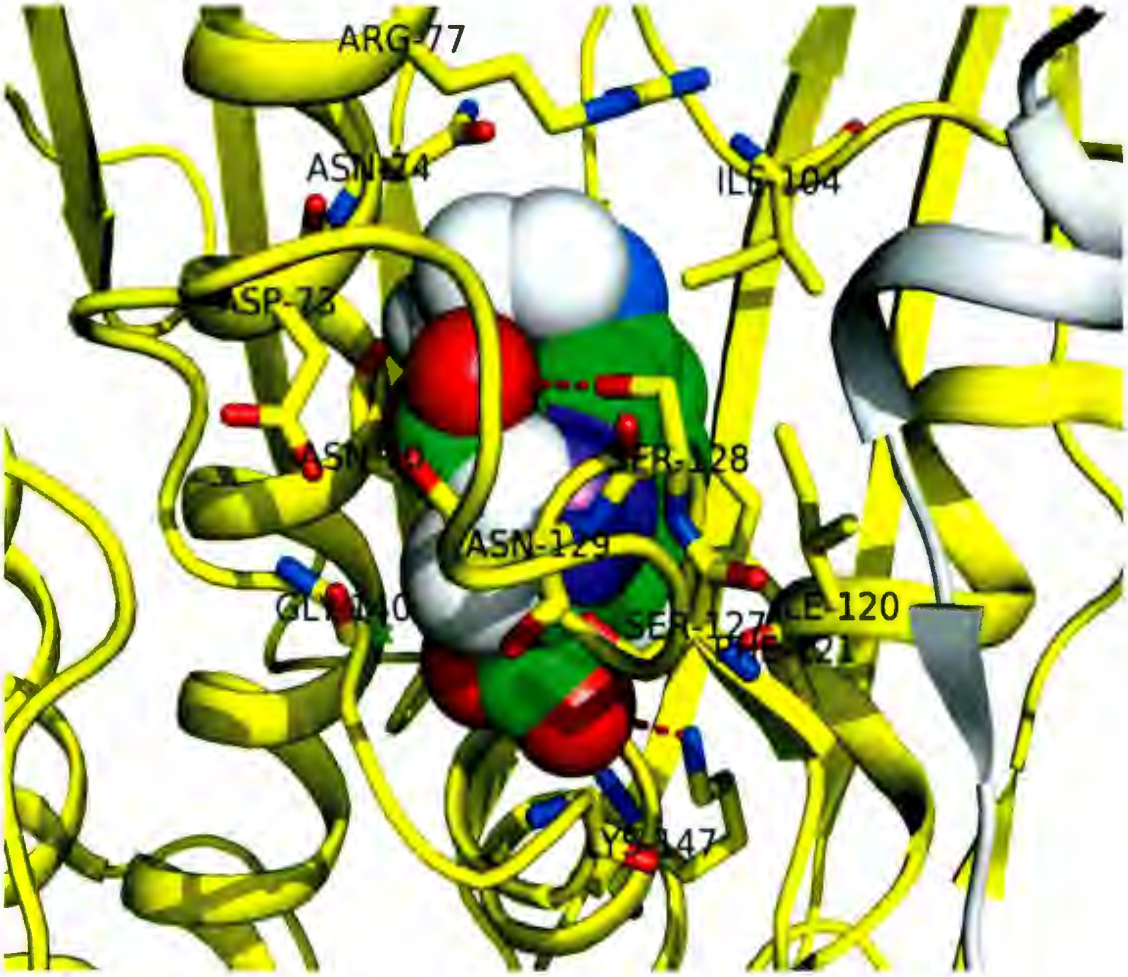


Figure 3.8 Lowest energy docking result of compound **1** at the 4GFH ATP binding site

Figure 3-9 shows the interaction of compound **6a** at the DNA binding site of 4FGH. As can be seen, DNA bases form strong hydrogen bonding interactions with the ligand forming a very stable complex. This complex is further stabilized with a coulomb interaction formed with ARG475.

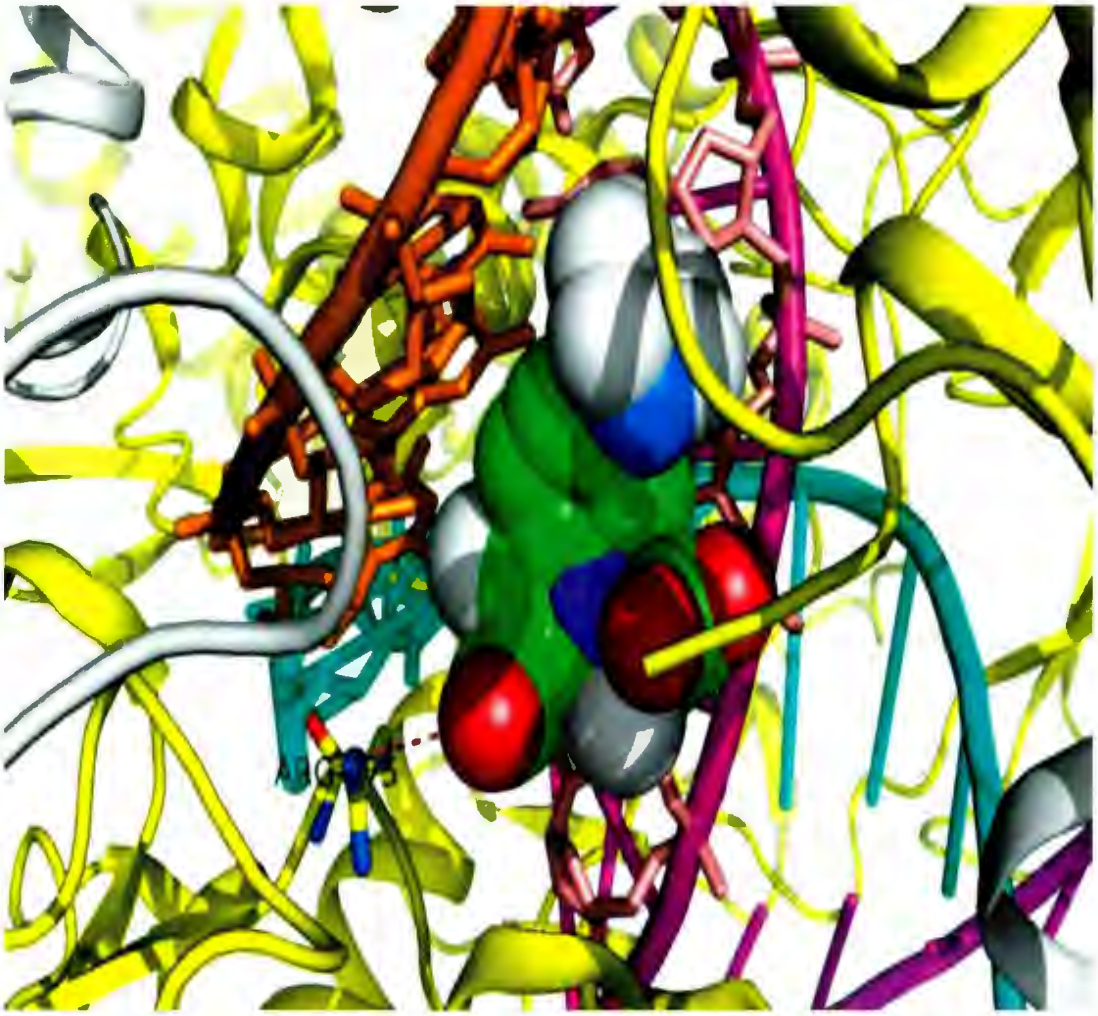


Figure 3.9. Lowest energy docking result of compound **1** at the 4GFH DNA binding site

Figure 3.10 shows the highest energy binding of compound **6a** at 1S16 ATP binding site. The high docking energy indicates that the binding site of 1S16 is not able to accommodate compound **6a** due to steric hindrance. Therefore, compound **6a** can only bind in a nonfavourable conformation. The intermolecular interactions are favorably stabilizing the complex.

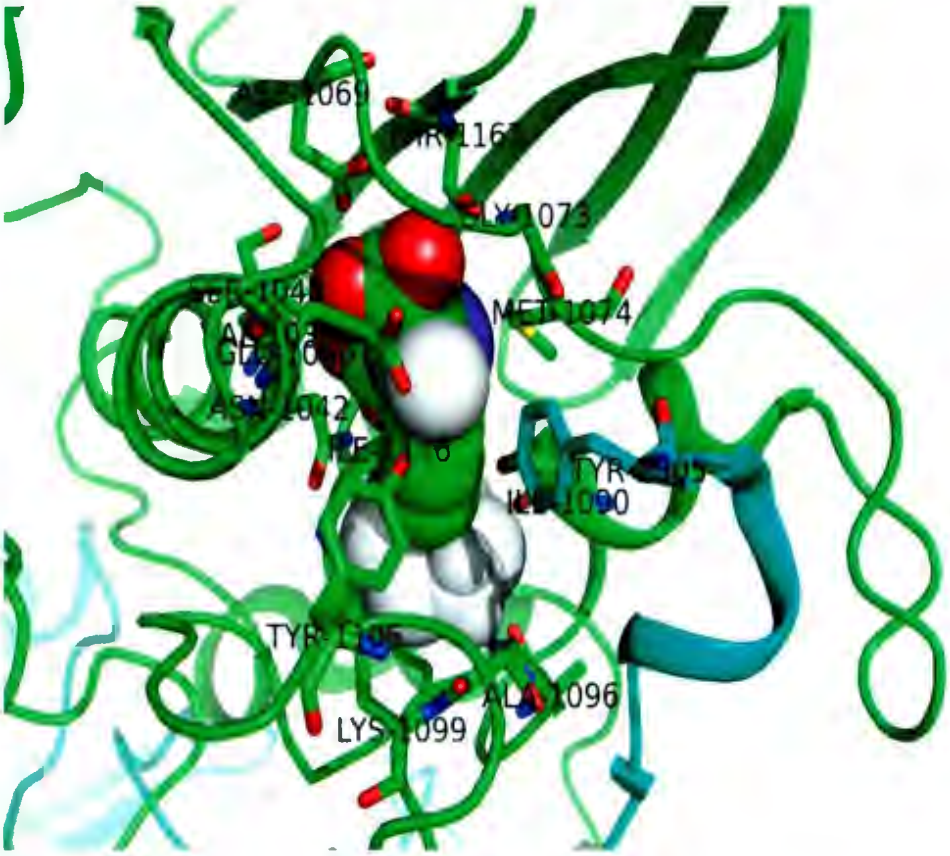


Figure 3.10. Lowest energy docking result at the 1S16 ATP binding site

Figure 3-11 shows the binding of compound **6a** at the 3FOE DNA binding site. The interactions are very similar to that of observed at the DNA binding site of 4GFH. Namely, strong hydrogen bonding interactions are observed with DNA bases, and ARG456 also contributes to complex stabilization by forming a strong coulomb interaction with the carboxylate group of the ligand. As there are fewer bases forming interactions with the ligand, higher docking energy is calculated as compared to 4GFH binding at the DNA binding site.

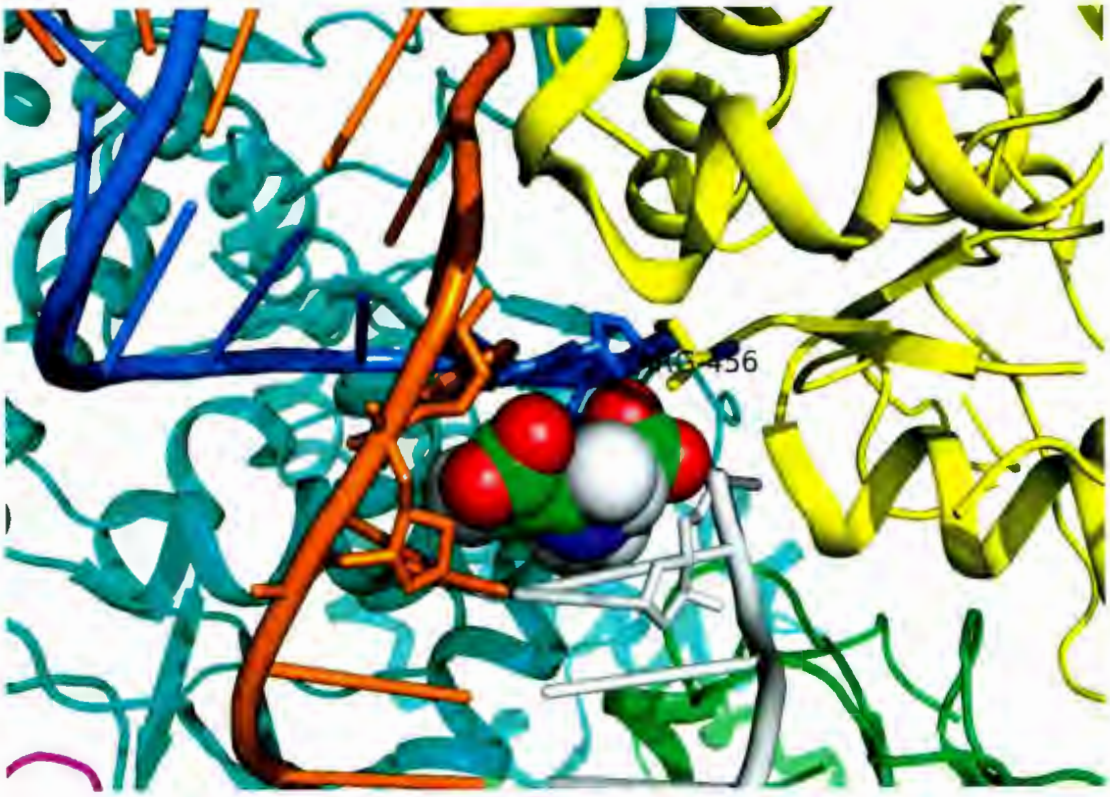


Figure 3.11. Lowest energy docking result at the 3FOE DNA binding site

3.4.4 Summary

Interactions of thiazetoquinolone derivates were investigated with topoisomerase II and IV proteins using molecular docking methods. Analyzing the available X-Ray structures with bound ligand, two potential binding sites were defined; namely, the ATP binding site and a site at the protein-DNA interface in a DNA bound conformation. The ligands were docked into both binding sites of topoisomerase II and IV. Our results indicate that the DNA binding sites are more favorable for binding the investigated compounds. Moreover, it was shown that binding of larger derivates is more favorable at the DNA binding site; while the smaller ATP binding site accommodates smaller ligands due to steric reasons.

3.5 Results and discussions

3.5.1 Antimicrobial assay results

Compounds were tested against *E. coli, B. subtilis, S. epidermidis, P. fluorescens, M. luteus* using nalidixic acid and ampicillin as standard. Biological Assay results against *Testes* microorganisms are recorded in Table 3.2.

Table 3.2. Microbiological assay results for tested compounds

Compound	MIC(μM)				
	M. *luteus*	E. *coli*	S. *epidermidis*	*B. subtilis*	*P. fluorescens*
6a	>100	>100	>100	>100	>100
6b	>100	>100	>100	>100	>100
6c	>100	>100	>100	>100	>100
5a	>100	>100	>100	>100	>100
5b	>100	>100	>100	>100	>100
Nalidixic acid	>100	1.6	>100	>100	>100
Ampicillin	>125	>62.5	>62.5	>125	>62.5

3.6 Conclusion

The quinolone-based system is an excellent scaffold for design of various classes of bioactive molecules. Its several distinctive structural features have led researchers to design and synthesize different kinds of molecules. In this study, a series of novel C_7-substitutedthiazeto[3,2-*a*]quinolones derivative were synthesized and tested for their antibacterial activity against *E. coli, B. subtilis, S. epidermidis, P. fluorescens,* and *M. luteus.* The test results revealed the lack of

antimicrobial activity of these compounds. In order to explore the cause behind the lack of activity, docking experiments were carried out against DNA gyrase and topoisomerase IV. Based on our docking results, we concluded that in this type of polycyclic quinolone systems, deletion of fluorine can consequently eliminate the antimicrobial activity. This can be due to formation of a reversible and weak complex with the ATP binding region of the DNA gyrase as well as with DNA-GyrA complex. Further structural optimisation and bioassays of these compounds to find additional possible bioactivities are ongoing in the Daneshtalab group.

3.7 Experimental

Solvents and reagents were obtained from commercial suppliers and were used without further purification. Melting points are not corrected. ^{1}H NMR spectra were recorded on a Bruker AVANCE-500 MHz NMR spectrometer and on Bruker AVANCE-300 MHz NMR spectrometer using tetramethylsilane as an internal standard. Mass spectra were obtained on an Agilent 1100 series LC/MSD chromatographic system. High-resolution mass spectra (EI or ESI) were obtained on Waters GCT Premier Micromass Spectrometer. Silicycle Ultrapure silica gel (0-20 μm) G and F-254 were used for thin-layer chromatography (TLC), and Silicycle silia-P Ultrapure Flash silica gel (40-63 μm) was used for flash column chromatography. TLC was conducted on Polygram SIL G/UV254 precoated plastic sheets. The reaction yields are included in corresponding schemes.

Sodium 3-ethoxy-2-(ethoxycarbonyl)-3-oxo-1-(phenylamino)prop-1-ene-1-thiolate (1a)

To a suspension of sodium hydride (0.6 g, 25 mmol) in acetonitrile (50 mL) at 5-10 ^{0}C, diethylmalonate (4 mL, 26.34 mmol) was added gradually with constant stirring over a period of 15 min. This mixture was then stirred at 5-10 ^{0}C for additional 30 min, then brought to room temperature, allowed to stir for another 30-40 min. Phenyl isothiocyanate (19.41 g, 143.6 mmol) was added to the reaction mixture portion wise over a period of 30 minutes at the same temperature and the mixture was stirred overnight. After around 24 hours of stirring, evaporation of MeCN yielded a yellowish a yellow solid which was washed with Et_2O. ^{1}H NMR (300 MHz, Chloroform-d) δ 7.10 – 6.96 (m, 2H), 6.76 – 6.58 (m, 3H), 4.19 (q, J = 8.0 Hz, 4H), 3.60 (s, 1H), 1.26 (t, J = 16.0 Hz, 6H). APCI-MS: 318.068 (M^+ +1,100).

Sodium 3-ethoxy-2-(ethoxycarbonyl)-1-[(3-fluorophenyl)amino]-3-oxoprop-1-ene-1-thiolate (1b)

This compound was prepared according to the same procedure as that applied for **1a** using 3-fluorophenylisothiocyanate (22 g, 143.6 mmol) instead of phenyl isothiocyanate; yellow crystals; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.10 (td, J = 7.5, 5.7 Hz, 1H), 6.79 (dt, J = 8.9, 2.0 Hz, 1H), 6.65 – 6.51 (m, 1H), 6.41 (dt, J = 7.5, 2.0 Hz, 1H), 4.19 (q, J = 8.0 Hz, 4H), 3.60 (s, 1H), 1.26 (t, J = 16.0 Hz, 6H). APCI-MS: 335.30 (M^+ +1,100).

Ethyl 2-[(ethoxycarbonylmethyl)sulfanyl]-4-hydroxyquinoline-3-carboxylate (3a)

To the above yellow solid **1a** (1.75 g, 5.44 mmol) in THF (50 mL) was added $BrCH_2CO_2Et$ (0.6 mL, 5.44 mmol) dropwise at 0 ^{0}C and the mixture was stirred for 10-12 hour at room temperature. The solvent was then evaporated, extracted with $CHCl_3$ and dried over Na_2SO_4.

The organic layer was evaporated by rotary evaporator to give yellow oil (**2**). The obtained oil was heated at 180-190 ^{0}C in an oil bath under vacuum for 10-15 min. The resulting oil was solidified, then washed with ether to afford compound **3a** as dark yellow amorphous solid. Mp 145-150 ^{o}C, ^{1}H NMR (300 MHz, Chloroform-d) δ 7.88 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.79 – 7.61 (m, 2H), 7.43 (td, *J* = 7.4, 1.6 Hz, 1H), 4.35 – 4.12 (m, 6H), 1.27 (dt, *J* = 21.1, 5.9 Hz, 6H). HR-MS (TOFEI) calcd for $C_{16}H_{17}NO_5S$: (335.374); Found (335.330), Calculated LogP: 4.78 +/- 0.69.

Ethyl 2-[(ethoxycarbonylmethyl)sulfanylyl]-7-fluoro-4-hydroxyquinoline-3-carboxylate (3b)

This compound was prepared according to the same procedure as that applied for **3a** using **1b** (1.85 g, 5.44 mmol) instead of **1a** ; brick red crystals; Mp 148-152 ^{o}C. ^{1}H NMR (300 MHz, Chloroform-d) δ 7.97 (dd, *J* = 7.5, 5.0 Hz, 1H), 7.46 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.16 (td, *J* = 7.7, 1.4 Hz, 1H), 4.35 – 4.12 (m, 6H), 1.27 (dt, *J* = 21.1, 5.9 Hz, 6H). HR-MS (TOFEI) calcd for $C_{16}H_{16}NO_5SF$: (353.364); Found (353.301). Calculated LogP: 4.95+/- 0.72

Diethyl 4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-1,3-dicarboxylate (4a)

1) Oxidative Cyclization with KI and 1,2-dibromopropane

To a mixture of **3a** (0.335 g, 1 mmol) and K_2CO_3 (0.386 g , 2.8 mmol) in anhydrous DMF (25 mL) under nitrogen atmosphere was added 1,2-dibromopropane (0.56 g, 2.8 mmol) along with KI (0.464 g, 2.8 mmol). The reaction mixture was heated at 70 ^{0}C for 24 h, then brought it at room temperature and with help of conc HCl acidify it till *p*H 1-2 and then poured into ice-

H_2O. The resulting thiazetoquinoline derivative was collected by filtration and recrystrallized with hexane: $CHCl_3$ (1:4) to afford brick red crystals; yield 68%.

2) Oxidative cyclization with iodobromide and/or iodine

To a mixture of **3a** (0.335 g, 1 mmol) and K_2CO_3 (0.386 g, 2.8 mmol) in anhydrous DMF (25 mL) under nitrogen atmosphere was added idobromide and /or iodine (2.8 mmol). The reaction mixture in case of iodobromide was stirred at room temperature for 24 h (in case of Iodine the reaction mixture was heated at 70 °C), then brought it at room temperature and with help of conc. HCl acidify it till *p*H 1-2 and then poured into ice-H_2O. The resulting thiazetoquinoline derivative was collected by filtration and recrystallized from hexane: $CHCl_3$ (1:4) to afford Brick red crystals. Yield: 44 % , Mp 225-229 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 8.14 (dd, J = 7.5, 2.0 Hz, 1H), 7.65 (td, J = 7.5, 2.0 Hz, 1H), 7.10 (dd, J = 7.5, 2.0 Hz, 1H), 6.90 (td, J = 7.4, 2.0 Hz, 1H), 5.73 (s, 1H), 4.21 (dq, J = 14.3, 5.9 Hz, 4H), 1.24 (dt, J = 9.0, 5.9 Hz, 6H). HR-MS (TOFEI) calcd for $C_{16}H_{15}NO_5S$: (333.358); Found (333.315), Calculated LogP: 4.38+/-1.10.

Diethyl 7-fluoro-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-1,3-dicarboxylate(4b)

1) ***Oxidative Cyclization with KI and 1,2-dibromopropane***

This compound was prepared according to the same procedure as that applied for **4a** using **3b** instead of **3a.**

2) ***Oxidative cyclization with iodobromide and/or iodine***

This compound was prepared according to the same procedure as that applied for **4a** using **3b** instead of **3a.** Mp 223-228 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 8.09 – 7.98 (m, 1H), 6.92 – 6.79 (m, 2H), 5.73 (s, 1H), 4.22 (dq, *J* = 17.1, 5.9 Hz, 4H), 1.24 (dt, *J* = 9.0, 5.9 Hz, 6H). HR-MS (TOFEI) calcd for $C_{16}H_{14}NO_5SF$: (351.348); Found (351.302)), Calculated LogP: 4.56+/- 1.14.

4-Oxo-4*H*-[1,3]thiazeto[3,2-a]quinoline-1,3-dicarboxylic acid (5a) As the procedure reported, we have stirred and heated the mixture of ester **4a** (.333 g, 1 mmol) and sodium hydroxide in water (20 mL) at 100 °C for 3-4 h. After cooling, the reaction mixture was neutralized with hydrochloric acid (1M), extracted with CH_2Cl_2, dried over $MgSO_4$, then the solvent was evaporated. The solid obtained was purified by recrystallization from EtOH to afford compound **5**a as red amorphous powder. Mp 220-225 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 9.67 (s, 2H), 8.15 (dd, *J* = 7.5, 2.0 Hz, 1H), 7.65 (td, *J* = 7.5, 2.0 Hz, 1H), 7.09 (dd, *J* = 7.5, 2.0 Hz, 1H), 6.90 (td, *J* = 7.4, 2.0 Hz, 1H), 5.77 (s, 1H). HR-MS (TOFEI) calcd for $C_{12}H_7NO_5S$: (277.250); Found (277.004). Calculated Log P: 2.34+/- 1.10.

7-Fluoro-4-oxo-4*H*-[1,3]thiazeto[3,2-a]quinoline-1,3-dicarboxylic acid(5b) This compound was prepared according to the same procedure as that applied for **5a** using **4b** instead of **4a.** Brick red solid was extracted. Mp 226-230 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 9.67 (s, 2H), 8.10 – 7.98 (m, 1H), 6.93 – 6.80 (m, 2H), 5.78 (s, 1H). HR-MS (TOFEI) calcd for $C_{12}H_6NO_5SF$: (295.240); Found (294.990), Calculated LogP : 2.52+/- 1.14.

4-Oxo-4 *H*-7-(piperazin-1-yl)[1,3]thiazeto[3,2-*a*]quinoline-1,3-dicarboxylic acid (6a)

To a mixture of compound **5b** (0.79 g, 2.00 mmol) in DMF (10 mL), 0.86 g (10 mmol) of piperazine was added and the mixture was stirred at 110 ^{0}C for 15-18 h. It was then brought to room temperature. The title compound was precipitated by the addition of 100 ml saturated solution of sodium chloride. The precipitate was washed with water and purified by recrystallization from EtOH; yellow solid; Mp 154-159 ^{o}C. ^{1}H NMR (300 MHz, Chloroform-d) δ 9.67 (s, 2H), 7.83 (d, *J* = 7.5 Hz, 1H), 6.57 (dd, *J* = 7.4, 2.0 Hz, 1H), 6.37 (d, *J* = 2.0 Hz, 1H), 5.71 (s, 1H), 3.50 (t, *J* = 5.0 Hz, 2H), 3.22 (t, *J* = 5.1 Hz, 2H), 2.89 (t, *J* = 5.1 Hz, 4H), 1.18 (s, 1H). HR-MS (TOFEI) calcd for $C_{16}H_{14}NO_5SF$: (361.231); Found (361.302), Calculated LogP: 1.23+/- 1.45.

7-(4-Methylpiperazin-1-yl)-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-1,3-dicarboxylic acid (6b)

This compound was prepared utilising the procedure employed for the preparation of compound **6a**, using (0.59 g, 2.00 mmol) of compound **5b** and 1.0 g (10 mmol) of methylpiperazine; Black powder; Mp 130-135 ^{o}C. ^{1}H NMR (300 MHz, Chloroform-d) δ 9.67 (s, 2H), 7.83 (d, *J* = 7.5 Hz, 1H), 6.58 (dd, *J* = 7.4, 2.0 Hz, 1H), 6.42 (d, *J* = 1.9 Hz, 1H), 5.77 (s, 1H), 3.54 (t, *J* = 5.1 Hz, 2H), 3.35 (dd, *J* = 5.4, 4.8 Hz, 2H), 3.20 (t, *J* = 5.1 Hz, 4H). HR-MS (TOFEI) calcd for $C_{17}H_{17}N_3O_5S$: (375.242); Found (375.302), Calculated LogP: 1.93+/- 1.44.

7-(Morpholin-4-yl)-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-1,3-dicarboxylic acid (6c)

Prepared according to the procedure employed for the preparation of compound **7a**, using (0.59 g, 2.00 mmol) of compound **5b** and morpholine (0.87 g, 10 mmol); dark yellow precipitates;

Mp 136-140 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 9.67 (s, 2H), 7.85 (d, *J* = 7.5 Hz, 1H), 6.56 (dd, *J* = 7.5, 2.0 Hz, 1H), 6.35 (d, *J* = 2.1 Hz, 1H), 5.76 (s, 1H), 3.73 (t, *J* = 4.5 Hz, 4H), 3.37 (t, *J* = 4.5 Hz, 4H). HR-MS (TOFEI) calcd for $C_{16}H_{14}N_2O_6S$: (362.238); Found (362.302), Calculated LogP: 1.48+/- 1.44.

4-Oxo-4*H*-7-(pyrrolidin-1-yl)-[1,3]thiazeto[3,2-*a*]quinoline-1,3-dicarboxylic acid (6d)

Prepared utilising the procedure employed for the preparation of compound **5b**, using (0.59 g, 2.00 mmol) of compound **5b** and pyrrolidine (0.71 g,10 mmol); yellow powder; Mp 136-140 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 9.67 (s, 2H), 7.80 (d, *J* = 7.5 Hz, 1H), 6.43 (dd, *J* = 7.6, 2.0 Hz, 1H), 6.25 (d, *J* = 2.0 Hz, 1H), 5.77 (s, 1H), 3.49 – 3.33 (m, 4H), 2.46 – 2.28 (m, 4H). HR-MS (TOFEI) calcd for $C_{16}H_{14}N_2O_5S$: (348.231); Found (348.860), Calculated LogP: 2.54+/- 1.44.

4-Oxo-4*H*-7-(1*H*-pyrrol-1-yl)-[1,3]thiazeto[3,2-*a*]quinoline-1,3-dicarboxylic acid (6e)

To a mixture of compound **5b** (0.59 g, 2.00 mmol) in DMF (10 mL), pyrrol (0.68 g, 10 mmol) was added and the mixture was stirred at 110 ^{0}C for 15-18 h. It was then cooled down to room temperature and the title compound was precipitated by the addition of 100 ml saturated solution of sodium chloride. The precipitate was washed with water and purified by recrystallization from EtOH. Yellow powder, Mp 145-150 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 9.67 (s, 2H), 8.14 (d, J = 7.5 Hz, 1H), 7.79 (d, *J* = 2.0 Hz, 1H), 7.70 (dd, *J* = 7.5, 2.0 Hz, 1H), 7.43 (dd, *J* = 5.6, 3.4 Hz, 2H), 6.35 (dd, *J* = 5.6, 3.5 Hz, 2H), 5.78 (s, 1H). HR-

MS (TOFEI) calcd for $C_{16}H_{10}N_2O_5S$: (342.231); Found (341.892), Calculated LogP: 3.20+/-1.44.

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Chapter-4

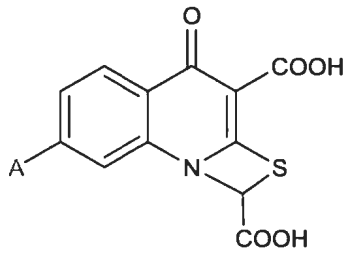
Conclusion and Future research

4.1 Conclusion and future research

Antibacterial drugs are those which kill or prevent the growth of various strains of bacteria. Chemical compounds which have quinolones in their basic ring skeleton cover a large part of whole antibacterial group. Nalidixic acid the aborigine of quinolone antibacterial class and other very first discovered members of this group were eventually fazed out of therapeutic system, either because of development of bacterial resistance or their toxic side effects. As discussed in chapter 2 of this thesis, the emerging bacterial resistance against quinolone antibacterials is the biggest challenge for researchers. Few recent studies on the synthesis and structure-activity relationships of quinolone antibacterials have shown that non-fluorinated analogues display additional antibacterial activity against resistant antibacterial species [1-5].

Daneshtalab group has long been involved in quinolone-based research. Numerous quinolone based compounds have been synthesized and reported by our group. A research done by our previous group member Abeer Ahmed has reported the synthesis of a novel 4-oxo-benzo[h]thiazetoquinoline derivative [6, 7].

As detailed in previous chapters of this thesis, while she was attemting the synthesis and biological evaluation of polycyclic quinolones using conventional synthetic procedures, she unexpectedly discovered a novel polycyclic 4-oxo-benzo[*h*]thiazetoquinoline derivative. Unfortunately the biological data of this compound were not encouraging. In continuation of her research and to generate more data for structure-activity relationships of thiazetoquinolone derivatives, we further studied other structural analogues of compound shown in Figure 4.1.



Where, A is piperazine/methyl piperazine/Morpholine etc.

Figure 4.1. Structure of synthesized compounds

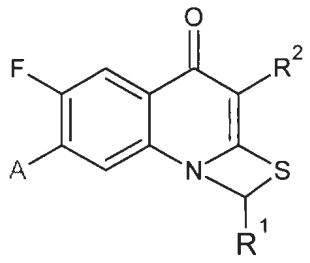
In this project we have synthesized a series of non-fluorinated derivatives of thiazetoquinolones using the same synthetic procedure as that of Abeer Ahmed with minor modifications. In fact, there are numerous studies available on the synthesis and bioactivity of angular 4-oxo-thiazolo[3, 2-*a*]quinoline-3-carboxylic acids and other sulfur containing polycyclic quinolone derivatives, while there are very few studies on the synthesis of 4-oxo-thiazeto [3,2-*a*]quinolines. In the synthesis described in this theses, the 4-oxo-thiazetoquinoline nucleus was formed by the reaction of the carbanion at the alkyl sulfide group at the C-2 position of the quinoline ring with the pseudohalogen (IBr), formed when iodide ion reacted with the *vic*-dihaloalkane, or a halogen (I_2) followed by the nucleophilic attack of the *N*-1 on halogenated carbon followed departure of halogen. The role of *vic*-dihaloalkane in this reaction is to generate the pseudohalogen without direct involvement in the quinoline nucleus. Following a series of five reactions, we were able to collect various 4-oxo-thiazetoquinoline-3-carboxylic acid derivatives, in reasonable yields. After completing synthesis of considerable number of novel compounds, we proceeded for evaluation of their biological activity against different bacterial strains. Unluckily, none of the

synthesized compounds have shown any encouraging results in biological activity. To justify the reason behind the loss of antibacterial activity in this series of compounds, we attempted molecular docking on DNA gyrase enzyme. This is a bacterial enzyme which is inhibited by quinolones, in order to show their antimicrobial activity. The findings of docking studies were quiet interesting. Our synthesized compounds were able to interact with the ATP binding domain of DNA gyrase as well as the DNA-DNA gyrase complex. Based on docking energies, it was found that, although the test compounds were interacting with the enzyme, they were forming a reversible binding with various domains of the gyrase enzyme. In general, it has been reported that the clinically used quinolone antibacterials form a irreversible binding with the ATP binding site of the gyrase molecule. The reversible interaction of the test compounds with the ATP binding site of the gyrase may be the main reason for the low activity of these compounds.

Quinolones must possess two distinct features in order to act on DNA topoisomerases. First feature comes with their ability to chelate the Mg^{2+} at the active site and second to complex with the ATP binding region. Compounds synthesized by Dr. Abeer Ahmed were able to bind with the ATP binding region but were not able to make appropriate chelation with the Mg^{+2}. This was the cause behind the absence of cytotoxicity [8a-b]. This may also be the reason for insignificant activity of our compounds.

As a part of my future research plan, I will try to work on further structural optimisation of my test compounds, to explore various other biological activities. One of them will include development of some structural analogue of my compound which includes the substitution of carboxylic acid group with $COOC_2H_5$ or $CONH_2$ (Figure 4.2). These

substituents will definitely help to chelate the Mg^{+2} at the active site of topoisomerase, to a larger extent, as compared with the compound which I already synthesized (Figure 4.1).



Where, A is piperazine/methyl piperazine/Morpholine etc.

R^1 and R^2 are COOH/ $COOC_2H_5$/$CONH_2$

Figure 4.2: Structure of proposed compound for future research

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