POLYCYCLIC QUINOLONES:
DESIGN, SYNTHESIS, PRELIMINARY In Vitro AND In Silico studies

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Polycyclic Quinolones: Design, Synthesis, Preliminary *In Vitro* and *In Silico studies*

By

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ABSTRACT

4 (1*H*)-Quinolone is an important pharmacophore found in a variety of biologically active molecules including clinically used antimalarial, antibacterial, anti-inflammatory, anticancer and antiviral agents. The diverse biological profiles of quinolone derivatives have attracted researchers interested in exploring novel quinolone-based compounds that may overcome the unwanted effects of life-saving drugs while presenting new mechanisms of action and high efficacy.

Among the quinolone derivatives, 4 (1*H*)-quinolone-3carboxylic acid derivatives have displayed an excellent antibacterial profile. This includes high efficacy, broad spectrum of activity, high tolerability, and effectiveness against bacterial strains resistant to available antibiotics. The work presented in this thesis was performed in order to discover novel polycyclic quinolones with potential antibacterial activity, focussing on activity against resistant bacterial strains. This work involves the design and synthesis of various fused quinolones and their *in vitro* microbiological activity. In order to understand the mechanism behind the loss of activity, *in silico* molecular docking was carried out on the DNA gyrase enzyme.

The results obtained in this study showed that the synthesized quinolone derivatives have good binding affinities to DNA gyrase. However, these derivatives were not able to form stable bonds with the enzyme, which may account for the lack of bioactivity exhibited by these derivatives.

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List of Abbreviations

ATP	Adenosine triphosphate
DKA	Diketo acid
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
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
LC	Lipid chromatography
MIC	Minimum inhibitory concentration
MP	Melting point
NMR	Nuclear magnetic resonance
PDB	Protein data bank
RNA	Ribonucleic acid
STAT	Signal transducers and activators of transcription
SAR	Structure activity relationship
TLC	Thin layer chromatography

CHAPTER 1

Quinolones: An Overview

1.1 Introduction

A pharmacophore is a part of a molecule which is responsible for interaction with receptors, biomolecules etc. IUPAC defines a pharmacophore as "An ensemble of steric and electronic feature that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger its biological response" [1a]. It is well known that certain types of pharmacophores have influence on improving the drug ability profile of some molecules as compared to others. These specific types of pharmacophores are associated with improvement in the bioavailability of drugs and, as a result, modifying their pharmacokinetic profiles. The concept of ''privileged structures'' was first proposed by Evans [1b]. Privileged structure refers to a molecular scaffold whose structure contains two or three ring systems, arranged in such a manner so as to allow a very high degree of versatility in binding with the receptors [2]. In this context, a quinolone scaffold can be considered as a truly privileged structure.

The very first quinolone was discovered by serendipity. It was discovered by Lesher *et al.* (1962) as an impurity while manufacturing chloroquine, an antimalarial agent [3]. Since that time, numerous derivatives of quinolones have been discovered, and many of them are in clinical use. Based on their main clinical use and decade of discovery, they can be placed in four different groups (A, B, C,D) as depicted in **Figure 1-1.**

Quinolones as antibacterial agents have been known for a very long time. However, they are still a hot topic for current research. This is mainly because of continuous development of bacterial resistance against currently used antibiotics. This has

guided the current trend of antibiotics research to focus mainly on development of novel quinolone antibacterials which will be active against highly resistant strains [1-4].

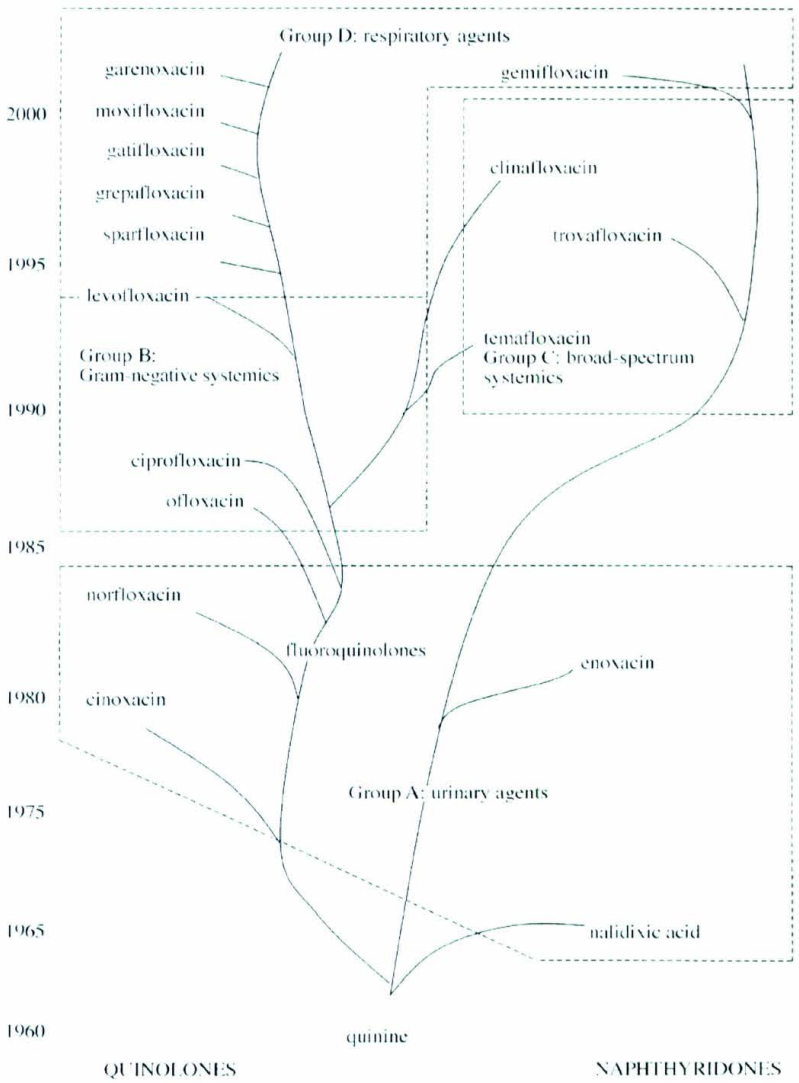

Figure 1-1: Development of quinolones

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1.2 Chemistry of Quinolones

Substitution of a single carbon atom on a benzene ring with nitrogen gives rise to the pyridine skeleton, while the same substitution on a napthalene nucleus results in a quinoline or isoquinoline nucleus (**Figure 1-2**). Quinoline is a liquid with a high boiling point, first extracted in 1834 from coal tar. Isoquinoline is a solid with a low melting point, first extracted in 1885 from the same source [14].

Figure 1-2 : Structures of pyridine and quinoline nuclei.

The Schematic numbering of a quinoline nucleus is shown in **Figure 1-3**.

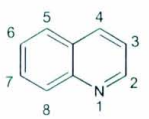


Figure 1-3: Schematic numbering of quinoline nucleus.

A quinolone is the keto tautomer form of a quinoline substituted with a hydroxyl functional group. Quinolones exist in isomeric forms of 4- and 2-quinolones as depicted in **Figures 1-4** and **1-5**.

Figure 1-4: Tautomeric forms of 2-quinolone

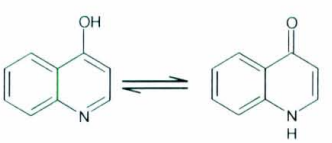
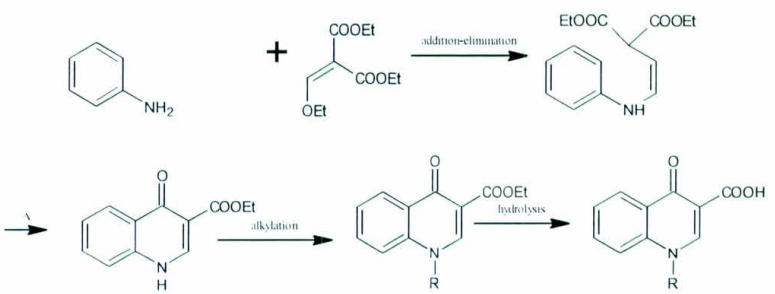


Figure 1-5: Tautomeric forms of 4-quinolone

Among the various known methods for the synthesis of quinolones, a few important procedures are described below.

1.2.1 Gould Jacob's Quinolone Synthesis

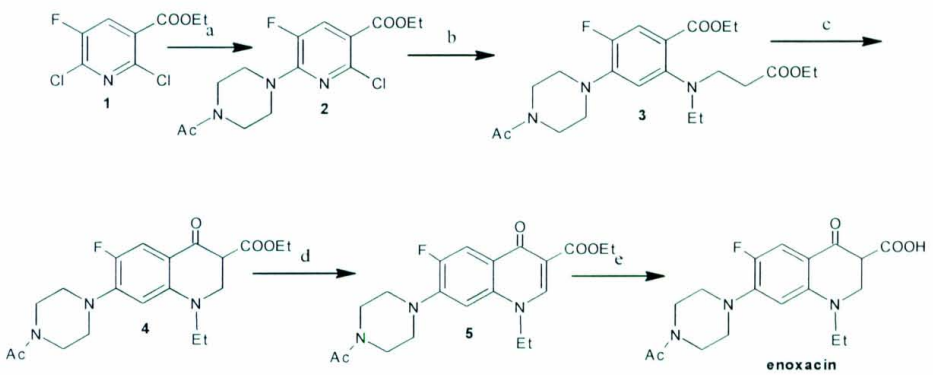
This is one of the oldest and established methods for the syntheses of quinolones. Preparation of quinolones by this method relies on an *addition-elimination* reaction between various substituted anilines and dialkyl alkoxymethylenemalonate as shown in **Scheme 1-1** [5]. The product formed by the addition-elimination reaction between substituted aniline and dialkyl alkoxymethylenemalonate undergoes thermal cyclization at high temperature to give quinoline-4(1*H*)-one system. *N*-Alkylation of this scaffold, 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 Dieckmann Cyclization of Diesters

This is a suitable procedure for the synthesis of aza- and diazaquinolone carboxylic acids that was reported by Pesson *et al.* [6]. This process is based on base catalyzed intramolecular cyclization of diesters as depicted in **Scheme 1-2**. The first step involves the regioselective reaction between ethyl ester of 2,6-dichloro-5-fluoronicotonic acid (**1**) with *N*-acetylpiperazine to form 6-piperazinyl derivative **2**. The treatment of compound **2** with the ethyl ester of 3-ethylaminopropionic acid leads to the formation of diester **3**, which in turn undergoes intramolecular ring closure with potassium *tert*-butoxide to form ethyl ester of tetrahydronaphthyridine carboxylic acid **4**. The dehydrogenation of **4** by the means of chloranil yields the ethyl ester of naphthyridone carboxylic acid **5** which then gets hydrolysed to the fluoroquinolone (enoxacin).

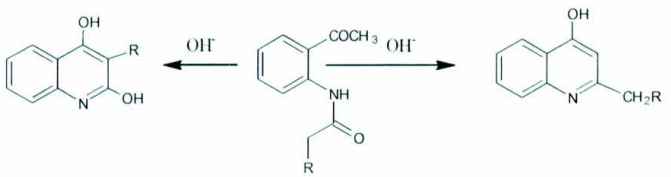


[a) N-acetylpiperazine, CH_3CN; b) $EtNH(CH_2)_2CO_2Et$, DMF, $NaHCO_3$, 120 °C; c) $KOC(CH_3)_3$, toluene; d) chloranil; e) OH^-]

Scheme 1-2: Synthesis of enoxacin by Dieckmann Cyclization method

1.2.3 Camps quinoline synthesis

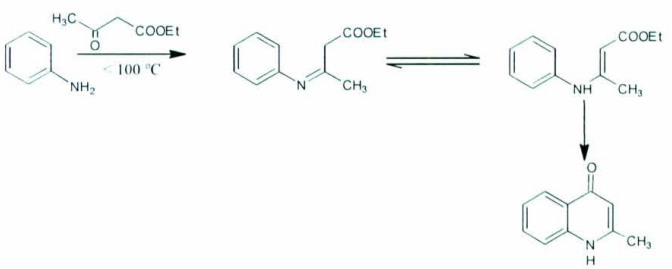
In Camps quinoline synthesis, alkaline cyclization of an *N*-acylated-*o*-aminobenzophenone affords the corresponding 2- or 4-hydroxyquinolines as shown in **Scheme 1-3** [7-13].



Scheme 1-3: Camps quinoline synthesis

1.2.4 Conrad-Limpach 4-quinolone synthesis

This is another name reaction that is employed for the synthesis of 4-quinolones. It 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 1-4.**

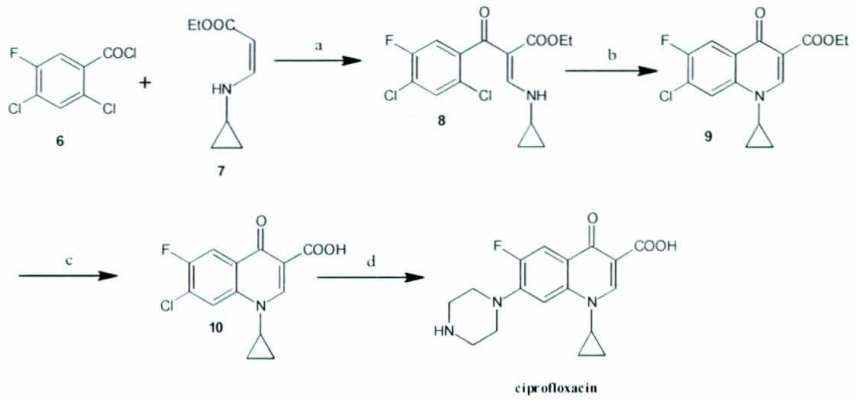


Scheme 1-4: Conrad-Limpach 4-quinolone synthesis

One important modification of this reaction is the use of a temperature above 100^0C in the first step that results in the formation of a 3-oxobutanoic acid amide, which upon further cyclization affords the corresponding 2-quinolone derivative [15].

1.2.5 Synthesis of quinolones by cycloaracylation reaction

The cycloaracylation procedure was developed by Grohe *et al.* [16] in 1987. This procedure is depicted in **Scheme 1-5**. In this procedure, 2,4-Dichloro-5-fluorobenzoyl chloride (**6**) reacts with ethyl 3-cyclopropylaminoacrylate (**7**) in the presence of triethylamine to yield the acylated product **8**. Cyclization of **8** under the catalytic influence of potassium carbonate produces the ethyl ester of quinolone carboxylic acid **9**, which is subsequently hydrolysed to its carboxylic acid derivative **10.** The reaction of **10** with piperazine leads to the generation of quinolone antimicrobial (in this case, ciprofloxacin) [17]

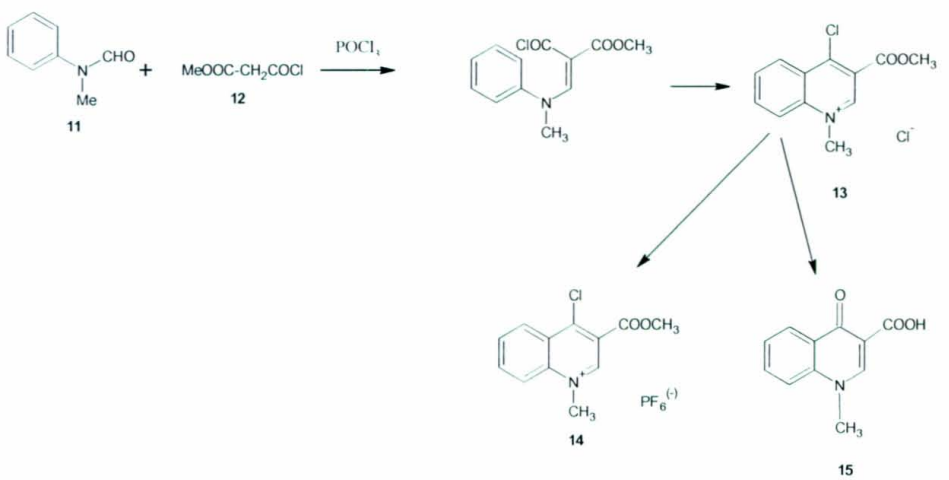


[a) NEt_3, toluene; b) K_2CO_3, DMF; c) KOH; d) piperazine, DMSO]

Scheme 1-5: Synthesis of ciprofloxacin by cycloaracylation reaction

1.2.6 Meth-Cohn Quinolone Synthesis

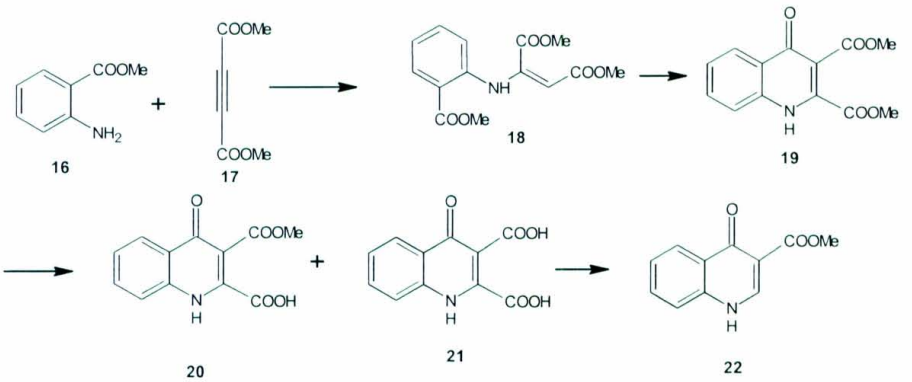
This approach of quinolone synthesis was developed by Meth-Cohn in 1986 [18]. In this reaction a Vilsmeier complex consisting of N-methylformanilide (**11**) and phosphoryl chloride is reacted with methylmalonyl chloride (**12**) to produce its quinolinium salt **13**. Treatment of this quinolinium salt with ammonium hexafluorophosphate affords 4-chloro-3-methoxycarbonyl-1-methylquinolinium hexafluorophosphate (**14**). Compound **13** is converted to 1-methyl-4-quinolone-3-carboxylic acid **15** by treatment with excess alkali followed by acidification as depicted in **Scheme 1-6**.



Scheme 1-6: Meth- Cohn quinolone synthesis

1.2.7 Biere and Seelen Approach for quinolone synthesis

This method for quinolone synthesis was developed by Biere and Seelen in 1976 as shown in **Scheme 1-7** [19]. This procedure involves the Michael addition of methyl anthranilate (**16**) to dimethylacetylene dicarboxylate (**17**) to give rise to the enamino esters **18**. This enamino ester then undergoes cyclization to the quinolone dicarboxylic acid ester **19** in the presence of strong base such as sodium hydride or potassium *tert*-butoxide. The ester-acid **20** or the dicarboxylic 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 from compound **20**. This method of quinolone synthesis has never been used for the synthesis of fluoroquinolones, mainly because of expensive starting material and requirement of very a high temperature for the decarboxylation reaction to take place.



Scheme 1-7: Biere and Seelen approach for quinolone synthesis

1.3 DNA Super coiling, replication and topoisomerase

Deoxyribonucleic acid (DNA) is a linear, double helical structure that carries genetic information from one generation to another, allows mutation and recombination and serves as a template for semi conservative replication [20]. The way and orientation in which DNA is present in the cell is associated with certain problems.

1) DNA is present in a super condensed state inside the nucleus of cell. For example in *E. coli* bacterium, the DNA is approximately 1000 μm long [21], as compared to the cell which is 1-2 μm. To accommodate such a long strand of DNA in a relatively small cell, it must be highly condensed. However despite super condensation, DNA must be able to replicate, and allow transcription of individual genes as required without becoming lethally entangled.
2) DNA has a double- helical structure. With each turn of a helix, two single strands are wrapped around each other. Also, on an average two turns of DNA are separated by 10.4 base pairs. An *E. coli* chromosome has 4 million base pairs. This would mean that essentially the *E. coli* chromosome is intertwined about 400,000 times. In order for DNA to undergo semi conservative replication, it must unwind by the same number of times.
3) In reality, prokaryotic DNA undergoes less than one turn for every 10.4 base pairs [22]. This results in negative super coiling of DNA. Negative super coiling is an inherent feature of prokaryotic DNA to facilitate DNA replication and transcription. However it is an energy consuming process. Hence in order for bacterium to generate and maintain its DNA in a negative super coil state, it must use a tremendous amount of energy.

4) A fourth problem arises when the DNA undergoes replication or transcription. Replication or transcription of DNA requires its helical structure to be unwrapped into two parallel strands. This unwrapping is done by RNA polymerase. However this causes generation of positive super coils ahead of the replication fork [23-26]. With continuous generation of positive super coils, eventually it becomes difficult and a highly energy consuming process for RNA polymerase to unwind the DNA and carry out replication and transcription. This generation of positive super coils, if not resolved, will lead to inefficiency of the transcription and replication processes.

Each of these problems is resolved by a class of enzymes known as Topoisomerases [22, 27-29]. These are the group of enzymes that alter the number of times one single strand of a DNA duplex winds around its complementary strand. Hence, these enzymes are very essential for cell growth and replication.

Three subtypes of topoisomerase enzyme have been discovered so far. They are:

1) Type I Topoisomerase - represented by topoisomerase I 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 first isolated by James Wang [30] in 1969 from *E. coli*. Type I topoisomerase is a 110-kDa protein and it is encoded by the *topA* gene [31]. This *topA* gene is located at 28 min on the bacterial chromosome of *E coli*.

Type I topoisomerase breaks only one strand of DNA and passes it through the other. Wang *et al.* [30] observed that topoisomerase I from both bacterial and eukaryotic sources, were able to remove negative super coils from DNA in the absence of ATP. However eukaryotic topoisomerase I was also able to remove positive super coil. In a prokaryotic cell, DNA gyrase along with topoisomerase I are responsible for regulation of negative super coiling of DNA [32, 33] and hence the enzyme is required for transcription to take place [34]. Pruss *et al.* conducted a study [35] on *topA* mutants of E coli. These mutants were not able to produce a functional topoisomerase enzyme. As a result it was observed that, there was excessive accumulation of negative super coils in Plasmid pBR322 DNA of *topA* mutant bacteria. This excessive accumulation of negative super coils was dependant on transcription of *tet* gene. Hence, this study gave direct evidence that topoisomerase I is required for removal of negative super coil behind the progressive *replication fork*. Quinolones can inhibit the DNA relaxation activity of topoisomerase I at very high concentrations only [36-37].

1.3.2 Bacterial topoisomerase II (DNA Gyrase)

DNA gyrase was first isolated by Martin Gellert and associates in 1976 from *E. Coli* bacterium [22, 27, 28, 29, 38]. Based on studies conducted with the help of High Resolution Microscopy, it was found that the gyrase enzyme has a heart shaped structure [39]. DNA gyrase is made up of four different subunits and is comprised of two A subunits (GyrA) and two B subunits (GyrB) [40, 41]. The GyrA subunit is encoded by the *gyrA* gene and GyrB subunit is encoded by *gyrB*

gene. By using the method of small-angle neutron scattering, the mass of the holoenzyme was found to be 353 kDa [41].

Both GyrA and GyrB subunits are responsible for different functions. GyrA is mainly required for DNA cleavage and re-ligation with the tyrosine residue at position 122 (Tyr-122), hence creating a temporary phosphotyrosine bond with the broken strand of DNA. GyrB subunit, on the other hand, is mainly responsible for the ATPase activity of the enzyme. Various studies have been conducted using the fragments of GyrA and GyrB subunit with the aim of identifying the functional domain of these subunits. Reece *et al.* [42] found that the addition of a C-terminal 33-kDa GyrA fragment improved the efficiency of the enzyme and was able to stabilize the complex. 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 on the gyrase enzyme. The N-terminal region (amino acid 2 through 220) of the GyrB subunit is now known to contain the ATP binding region [43]. 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 [39, 44]. 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 super coiling [44]. In other words, hydrolysable ATP is required by the gyrase to function [45]. 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 [42].

1.3.3 Bacterial Topoisomerase III

Srivenugopal *et al.* discovered topoisomerase III in 1984 [46]. This enzyme was able to remove the negative super coil from DNA without any energy requirement. However it needed energy in the form of ATP to remove positive super coils [46]. However its activity is less efficient as compared to other types of topoisomerase [47]. Topoisomerase III can bind to both DNA as well as RNA [49]. It is encoded by the *topB* gene located at 38.7 min on the *E. coli* genetic map [48]. Studies were conducted on mutants where genetic information was removed from 38.4 min to 39 min. Survival of these mutant bacterium have shown that topoisomerase III enzyme is not an essential enzyme [48].

1.4 DNA Gyrase: primary target for quinolone action

In 1977, GyrA subunit was reported as a primary target of Nalidixic and Oxolinic acid by Gellert [50] and Cozzarelli [51]. Experiments were conducted by these investigators on purified gyrase enzyme in which GyrA subunit was isolated 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. Similar studies conducted on *B. subtilis* [52], *Enterococcus faecalis* [53], *S.*

aureus [54], *Pseudomonas aeruginosa* [55-58], *Haemophilus influenzae* [59] have shown that the GyrA subunit is also a target of quinolone action.

Two different mutations that are responsible for nalidixic acid resistance have also been identified on the *gyrB* gene [60-62]. One of these mutations, nalD, was responsible for increased resistance to newer quinolones. The other mutation, nalC, surprisingly, produced increased susceptibility to quinolones containing piperazine at the 7^{th} position [63]. It was concluded in these studies that both GyrA and GyrB subunits are targets for quinolone action.

Quinolones exert their inhibitory effect by binding to the Gyrase-DNA or Topoisomerase IV- DNA complex. Upon binding with the complex, quinolones induce a conformational change in the enzyme. As a result of this conformational change, gyrase can now break the strands of DNA but loses its ability to re-ligate it (**Figure 1-6**). 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 [64].

1.5 Important structural features of antibacterial quinolones

The essential structural features for a quinolone based molecule to show antibacterial activity is shown in **Figure 1-7**.

Position 1: This position forms part of the enzyme-DNA binding complex [65]. Small, saturated or unsaturated, alkyl moieties enhance the antibacterial action especially against Gram negative microbes [66]. Cyclopropyl substitution is the most potent substitution at this position [67].

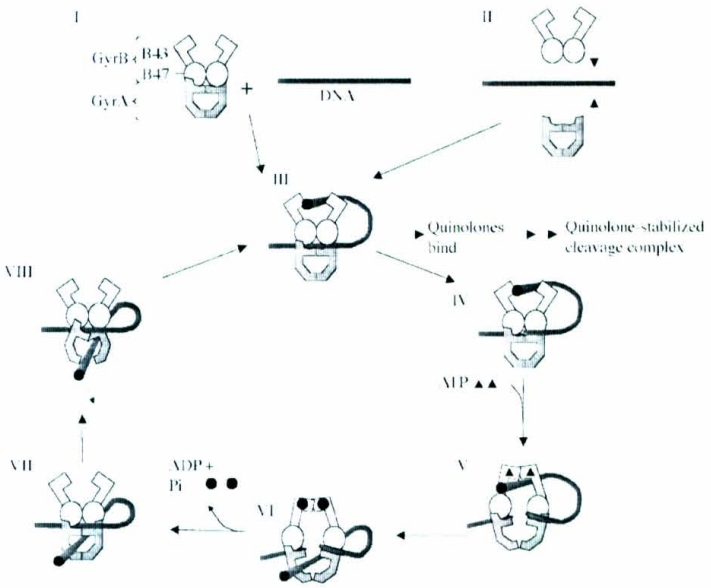


Figure 1-6: DNA gyrase super coiling cycle showing the point of action of quinolones

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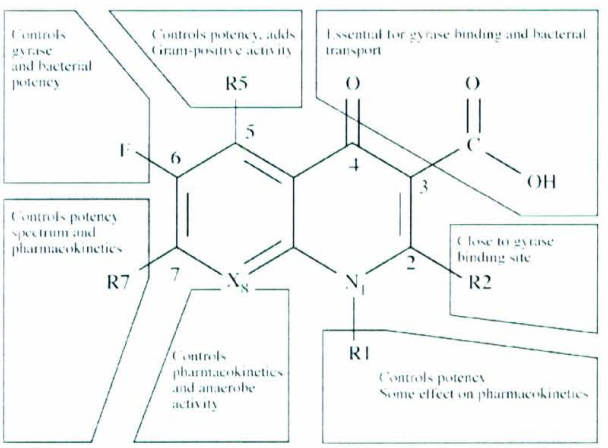

Figure 1-7: General structure of most commonly used quinolone molecules.

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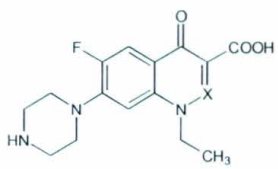
Replacing the alkyl/aryl substitution at *N*-1 position by an alkyl-arylmethyl moiety was found to decrease antibacterial activity against Gram negatives while it increased the activity against Gram positive bacteria [68]. It was also found that replacing the hydrogen atoms on the *N*-1 substituents by fluorine atoms preserved the activity [69]. Based on these studies, the 2,4-difluorophenyl moiety is considered as the second important substitution required for antimicrobial activity of quinolones [67].

Position 2: In the pre-fluoroquinolone era, addition of various substituents at this position has been shown to diminish antimicrobial activity [70]. This position is close to the gyrase binding site. Bulky substitution at this position inhibits the transport of quinolones across the bacterial cells and leads to diminished antimicrobial activity [65]. However, when sulfur is incorporated in a small ring, it can replace the hydrogen without adversely affecting the activity [65]. Norfloxacin analogues prepared by the bioisosteric replacement of C-2-CH by N, ware found to have comparable activity. However, their potency was found to be reduced significantly. Comparative MIC values are shown in **Table 1-1** [71, 72].

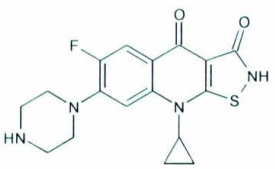


Drug	X	MIC (μg/ml) against *E. Coli*
Norfloxacin	CH	0.1
--	N	3.13

Table 1-1: MIC of C-2 variants

(Data taken from reference 71)

***Position 3 and 4*:** Position 3 and 4 are responsible for gyrase binding and bacterial transport. A carboxyl substitution is found to be superior to the esters, phosphonates and sulphonates [73]. Fusing a thiazolidone ring between C-2 and C-3 of quinolones, because of its aromatic resonance, can mimic the carboxyl group and resulted in compounds with outstanding *in vitro* potencies. Comparative MIC values against various bacterial strains are shown in **Table 1-2** [74-76].



A

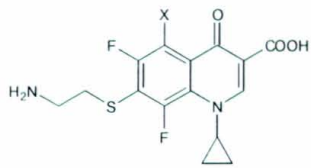
Structure	MIC (μg/ml)			DNA gyrase IC_{50} (μg/ml)
	E. coli	*P. aeruginosa*	*S. aureus*	
Ciprofloxacin	0.004	0.125	0.25	0.3
A	0.005	0.02	0.02	0.09

Table 1-2: MIC of C-3 variants

(Data taken from reference 74-76)

Position 5: Substituents present in this position must not be big and they should be able to form hydrogen bond with the DNA, for increased potency [77]. Introduction of a primary amine substitution at C-5 was found to increase the antimicrobial activity against *E. coli*

[77]. Other electron donating groups such as hydroxyl, methyl etc., were also found to increase *in vitro* activity against gram-positive bacteria as shown in **Table 1-3** [78].

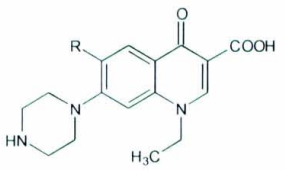


X	MIC (μg/ml) for:		
	E. coli	*P. aeruginosa*	*S. aureus*
NH_2	0.025	0.1	0.05
OH	1.56	6.25	1.56
F	0.78	3.13	3.13

Table 1-3: MIC of C-5 variants

(Data taken from reference 81)

Position 6: Fluorine substitution at this position has been found to markedly increase the anti- bacterial activity as shown in **Table 1-4,** and has resulted in the modern day fluoroquinolones [66]. Ledoussal *et al.* [79] found that replacement of an hydrogen at the 6th position with fluorine has resulted in analogues with better activity.



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

Table 1-4: MIC of C-6 variants

(Data taken from reference 66)

Position 7: Substituents at this position are responsible for controlling potency and pharmacokinetics. This position directly interacts with the DNA gyrase [80]. Five- or six-membered nitrogen heterocycles are generally present at this position.

Position 8: Substituent at position 8 has also been shown to control pharmacokinetics, spectrum of activity and reducing the development of bacterial resistance [82]. Alkylation at this position has not only been shown to increase activity against Gram positive bacteria, but it also improves overall pharmacokinetic parameters.

1.6 Non classical use of quinolones

1.6.1 Quinolones as Antitumor agents

Cancerous cells divide and multiply at a much faster rate than that of normal cells. Topoisomerase is a key enzyme in DNA replication which is responsible for cleaving the

DNA chain at specific locations to relieve the strain of the growing replication fork and resealing the cleaved strains afterwards. Inhibition of this key enzyme causes hindrance with the proper functioning of cell machinery and eventually leads to arrest of the cell cycle. Many topoisomerase directed cytotoxic agents are based on this principle [84]. Topoisomerase II is an eukaryotic analogue of the DNA gyrase enzyme. Many compounds like teniposide, etoposide, adriamycin, m-ASA etc., target Topoisomerase II to exert their cytotoxic profile [84]. 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 [84-85]. Various studies have revealed that topoisomerases are involved in mediating the effect of various cytotoxic drugs, including DNA intercalating agents [84-85].

Quinolones are well known for their antibacterial activity and they cause excessive chromosomal damage within the bacterial cells [86, 87]. In a separate study, it was found that formation of the gyrase-DNA complexes leads to exposure of extra quinolone binding pockets which plays a significant role in DNA damage [88]. Although many well known quinolones show a high degree of specificity towards either bacterial or eukaryotic Topoisomerase enzymes, since both enzymes are homologues and share mechanistic similarity, there is high possibility of interaction with both enzymes by some compounds. Indeed, several antibacterial quinolones that inhibit P4 DNA- unknotting activity of various eukaryotic Type – II Topoisomerase have been identified [89, 90].

Kohlbrenner *et al.* [91] reported two novel antibacterial isothiazoloquinolones, A-65281 and A- 65282 (**Figure 1-8**) as potent inhibitors of both bacterial and eukaryotic Topoisomerase enzymes. These compounds were shown to induce DNA breakage as well

as to inhibit the P4- unknotting reaction catalyzed by calf thymus Topoisomerase II. In this study, a new class of Topoisomerase II directed compounds, which were able to interact with both bacterial and eukaryotic enzymes, were discovered.

Robinson *et al.* [92] reported CP-115,953 which displayed double potency of 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 [93], A-62176 [94], and A-85226 [95] were also found out to be effective against cancer cell growth. Compound A-65282 which is an isothiazoloquinolone derivative was having comparable potency with 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 a broad activity against human and murine tumour cell lines. Compound AG-7352 [96] was also found to be more cytotoxic against human cell lines compared to etoposide.

Signal transducers and activators of transcription (STATs) are a group of proteins which are primarily responsible for modulation of signal transcription in the cell. Vinkemeier *et al.* [97] 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". Out of seven different subtypes of mammalian STAT protein, STAT3 has achieved an important position in anti cancer therapy [98].

A high throughput screening [99] was conducted recently on a large number of compounds and it was found that a compound which was a highly fluorinated quinolone

derivative, inhibited STAT3 pathway and eventually caused cell apoptosis (EC_{50} = 4.6μM). Further optimisation of structure of this fluorinated compound resulted in a 30-fold increase in STAT3 pathway inhibitory potency [99]. Following these encouraging results, a receptor based virtual screening was performed on nearly 70,000 different compounds. The results have identified a few 3-carboxy-4(1H)-quinolones as very potent human protein kinase CK2 inhibitors [100]. Protein kinase CK2 plays an important role in development of some types of cancer. They have also been shown to play an important role in viral infection and inflammatory failures. From this point of view, quinolones can not only be considered as potential anticancer agents but also anti-infectious and anti-inflammatory agents. The chemical structures of the above compounds are depicted in **Figure 1-8**.

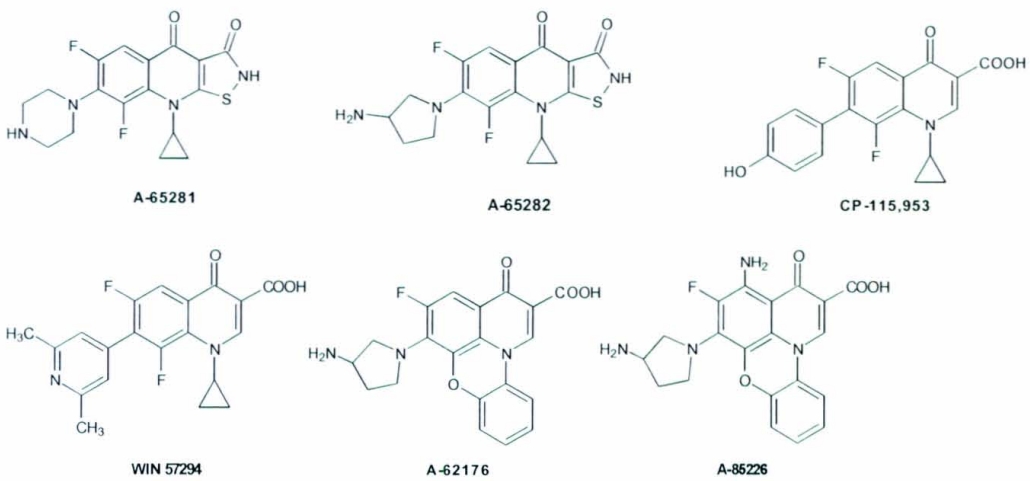


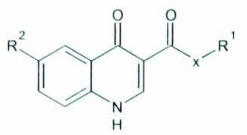
Figure 1-8: Novel quinolone derivatives having anticancer activity

1.6.2 Quinolones as Anxiolytic agents

Seligman *et al.* [101] have defined anxiety as a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioural components. γ-Aminobutyric acid (GABA) is one of the most important inhibitory neurotransmitters in the central nervous system. It exerts its effects by acting primarily on GABA receptors. The $GABA_A$ receptor is a pentameric macromolecular complex that regulates the opening and closing of ligand-gated chloride ion channels. $GABA_A$ receptors in humans 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 are responsible for different physiological effects (α1-containing receptors are involved in sedation, α2 and α3 in anxiolytic activity) [102]. The benzodiazepine class of drugs has been widely used as anti-anxiety agents. They generally show no selectivity against the receptor on which they act and allosterically modulate the GABA-mediated chloride ion influx through the chloride ion channel. However, benzodiazepines are associated with a considerable amount of undesirable side effects. This problem has led the search for finding better anxiolytic agents.

Kahnberg *et al.* [103] found out that 3-ethoxycarbonyl-6-trifluoromethyl-4-quinolone (**15**) is a very interesting lead compound for developing molecules that can effectively bind at the benzodiazepine site of $GABA_A$ receptors. Further lead optimisation of this compound led to a large number of novel molecules that were able to bind to $GABA_A$ receptors with very high affinity. Binding affinities for the compounds **16-19** were found to be in the sub-nanomolar range. 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. Compound **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 ware found comparable to that of the well known α_1 subunit selective drug, Zolpidem. Structures of compounds **15-22** are shown in **Figure 1-9.**



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 1-9: Novel quinolone derivatives with anti-anxiety effect

1.6.3 Quinolones as Antiviral agents

1.6.3.1 Quinolones as Anti-HSV-1 agents

Oral and facial lesions in humans are mainly caused by Herpes Simplex Virus type 1 (HSV-1). Although, Acyclovir is available for use in the chemotherapy for these

lesions, development of resistance against the effect of acyclovir in immuno-compromised patients is often a problem. Recent works on discovering quinolone-based derivatives that can be used in HSV-1 infection led to the identification of compound **23** (**Figure 1-10**). It is a quinolone acyclonucleoside derivative having high activity against HSV-1[104]. Most of the synthesized derivatives were effective in reducing the viral load in the range of 70-99% at the concentration of 50 μM. It was also discovered that acidic derivatives of quinolones were more effective than their corresponding ester counterparts. Compounds **23a-d** were also tested for cytotoxicity in Vero cells. Many novel quinolone derivatives were effective in preventing the cytophatic effect of HSV-1 at micromolar concentrations. Compound **23b** was found to be the most effective anti-HSV-1 derivative with a potency that was 1.5-fold of acyclovir.

Further screening was performed on these compounds and a naphthalene carboxamide was identified as a non-nucleoside inhibitor of human cytomegalovirus (HCMV) polymerase. **PNU-183792** (**Figure 1-11**) was found to be a competitive inhibitor of herpes virus polymerase that was effective against both human and animal herpes viruses. However, when toxicity assays were performed on **PNU-183792** using four different mammalian cell lines, it was not found to be cytotoxic at relevant drug concentrations (CC_{50} value > 100μM).

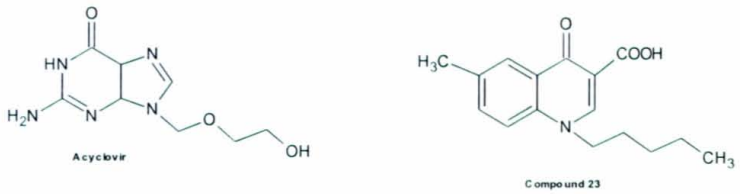


Figure 1-10: Structure of Acyclovir and compound **23**

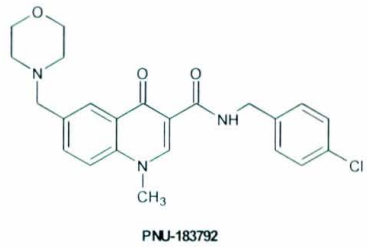


Figure 1-11: Structure of **PNU-183792**

1.6.3.2 Quinolones as HIV-1 Inhibitors

Current chemotherapy for the treatment of HIV infection is endowed with many shortcomings which include emergence of resistant strains and inability of current treatment regimens to completely eradicate viral infection. This has led to the search for novel molecules possessing new mechanisms of action which might be able to overcome the current shortcomings. One of the crucial steps in viral replication is the transcription of its genome into mRNA. Inhibitors of this step will be able to suppress HIV replication in both acutely as well as chronically infected cells [105]. It was discovered by Baba and coworkers that some antibacterial fluoroquinolones can also exhibit antiviral activity. Two analogues, **K-37** and **K-38** (**Figure 1-12**), 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 [106] began 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 1-8**), which has a methyl substitution at the N- position and a 4-(2-pyridinyl)-1-piperazine moiety at position 7, was found to be the most potent compound with EC_{50} = 0.1μM.

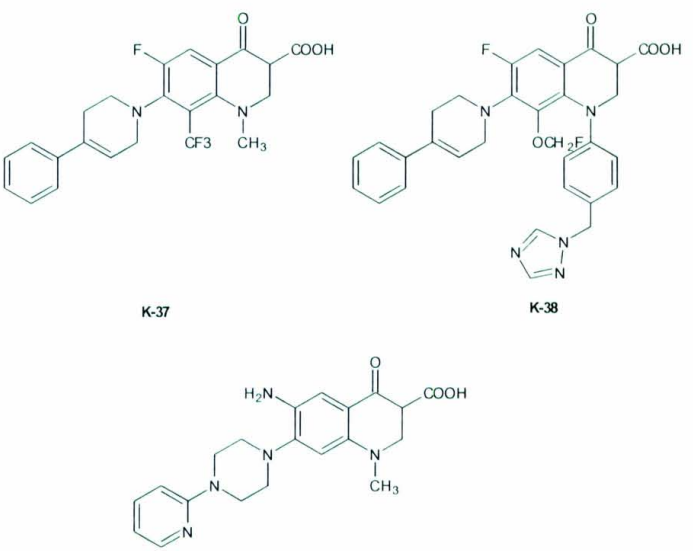
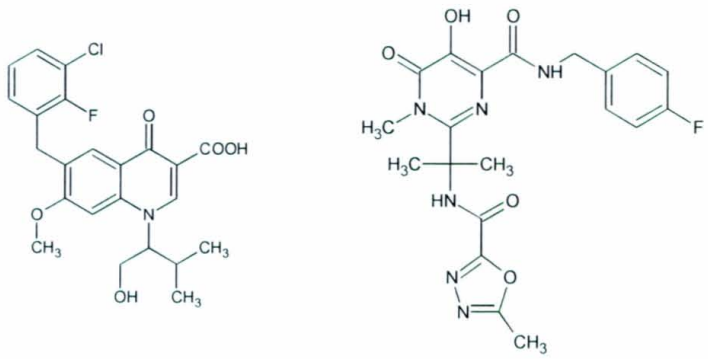


Figure 1-12: Structure of **K-37**, **K-38** and compound **24**

1.6.3.3 Quinolones as HIV-1 Integrase Inhibitor

HIV-1 Integrase (IN) is a viral enzyme that is responsible for the insertion of viral cDNA into the genome of the host cell. It is one of the three most essential enzymes, encoded by the viral genome, and plays an important role in viral survival [107]. There are two different steps involved in the insertion reaction catalyzed by IN. The first step takes place in the host cell cytoplasm where IN cleaves GT nucleotides from the DNA conserved 3'-CAGT end. This reaction is also known as 3' processing and results in unprotected hydroxyl groups at the both 3' ends of viral cDNA. The enzyme along with processed viral cDNA then moves to the nucleus of the host cell where the second reaction takes place. In the second stage of the integration, IN catalyzes the nucleophilic attack of both 3'-end hydroxyls of the viral cDNA onto the host DNA. This process is also known as the strand transfer reaction.

The Diketo acid (DKA) class of IN inhibitors were once milestones for the inhibition of IN. Raltegravir has shown a wide spectrum of activity as an IN inhibitor. GS-9137 (**Figure 1-13**) [108], a derivative of 4-quinolone-3-carboxylic acid, is effective as an IN inhibitor, and is currently under clinical evaluation [109].



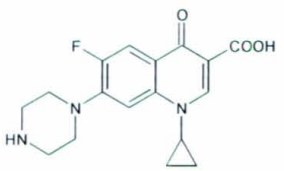
GS-9137 Raltegravir

Figure 1-13: Structure of **GS-9137** and **Raltegravir**

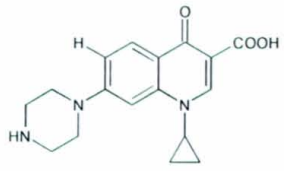
1.6.4 Quinolones as Anti-Ischemic Agents

Ischemic cell injury, in many cases, is responsible for fatality or life-long disability. The most common treatment for ischemic cell injury relies on the use of thrombolytic therapy, which restores blood flow to the ischemic region. However, thrombolytic therapy has its own limitations. Recently, studies conducted on known antibiotics have found them to be potential anti-ischemic agents. Antibiotics can inhibit inflammation and/or infection associated with ischemia; hence they can be regarded to have a potential role as anti-ischemic agents. [110]. In a study published by Park *et al.*, ciprofloxacin exhibited *in vivo* and *in vitro* anti-ischemic activity. Lead optimization study, on various known and novel molecules, with an aim to separate anti-ischemic activity from the anti-bacterial activity, resulted in the discovery of compound SQ-4004. This compound exhibited potent cell viability with reduced anti-bacterial activity.

SQ-4004 has also shown neuroprotective as well as cardioprotective effects (**Figure 1-14**)[110].



Ciprofloxacin



SQ-4004

Figure 1-14: Structure of **Ciprofloxacin** and **SQ-4004**

1.7 Conclusion

The quinolone molecule is one of the most promising scaffold which can be modified in a number of ways to achieve diverse pharmacological activity. These activities can range from being antibacterial, anti-ischemic, antitumour, antiviral. Despite great progress towards improving their antibacterial profile, they are still a hot topic of antibacterial research, mainly because of development of resistant bacterial strains.

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CHAPTER 2

Polycyclic Quinolones: Design, Synthesis, Preliminary *in vitro* and *in silico* study.

2.1 Introduction

Quinoline derivatives form a major class of heterocycles. The quinolone nucleus is present in a diverse range of bioactive molecules, most important being antibacterial agents. Many quinolone-based antibiotics were introduced and later withdrawn from the market, mainly because of development of bacterial resistance towards them.

It was discovered that non-fluorinated quinolones have better activity against resistant species [1-5]. Compound **PGE 9262932** and **PGE 9509924** reported by Jones *et al.* [3] were found to have good activity especially against antibacterial resistant strains (**Figure 2-1**).

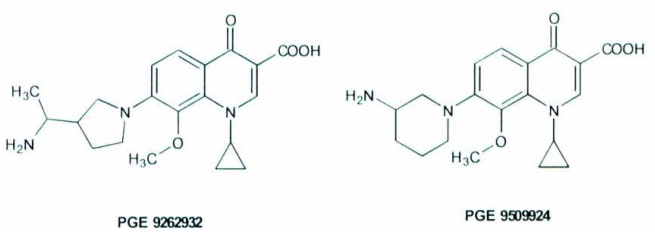


Figure 2-1: Structure of **PGE 9262932** and **PGE 9509924**

Our research group has long been involved in developing a novel series of polycyclic quinolones with diverse biological activities [6a-c]. In the search for novel polycyclic quinolones with potential antibacterial activity, a patent by Katsumi *et al.* [7] was of interest. In their report, **compound A** was mentioned as a molecule with a good antibacterial profile (**Figure 2-2**). This compound was a fluorinated derivative of a polycyclic quinolone. Based on the data, that the non-fluorinated quinolone derivatives are more effective against resistant bacterial species, we attempted the design, synthesize

and evaluation of various non-fluorinated analogues of **compound A**. In this context, our research was focussed on the syntheses of *O*- and *N*-alkylated analogues.

Compound A

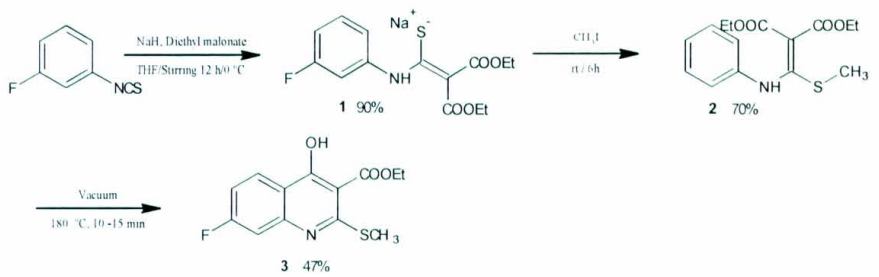
Figure 2-2: Structure of Compound **A** (Patent by Katsumi *et al.* [7])

2.2 Material and Methods

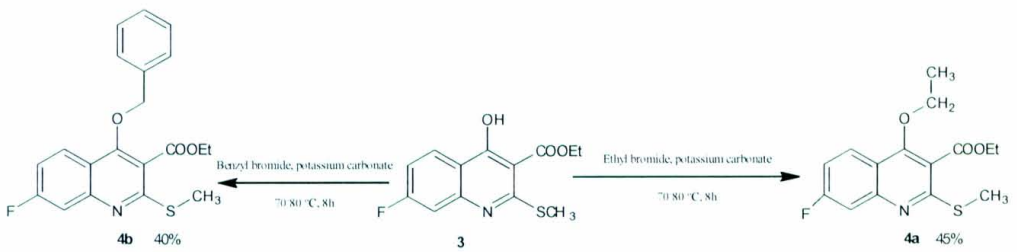
2.2.1 *O*- Alkylated quinolones

In our synthetic approach, 3-fluorophenylisothiocyanate was used as the main starting material which, upon reaction with diethyl malonate and sodium hydride in tetrahydrofuran, afforded the salt **1**. This salt was allowed to react with iodomethane to obtain compound **2**. This compound was immediately subjected to thermal cyclization under vacuum to afford compound **3** in 47% yield [6,8] as shown in **Scheme 2-1**.

Following the alkylation procedures reported by Garlapati, R. *et al.* [6a] and Hassan A *et al.* [9] with minor modifications, compound **3** was allowed to react with ethyl bromide and benzyl bromide to give compounds **4a** and **4b**, as depicted in **Scheme 2-2**.

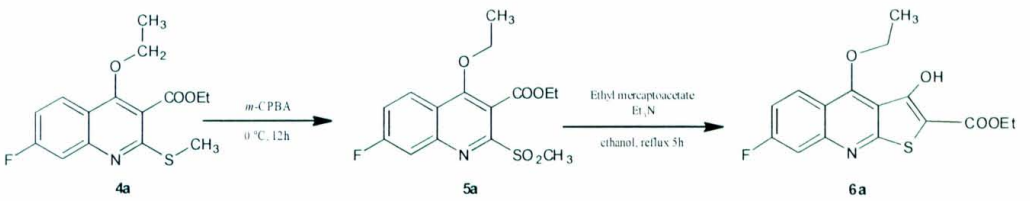


Scheme 2-1: Synthesis of compound **3**



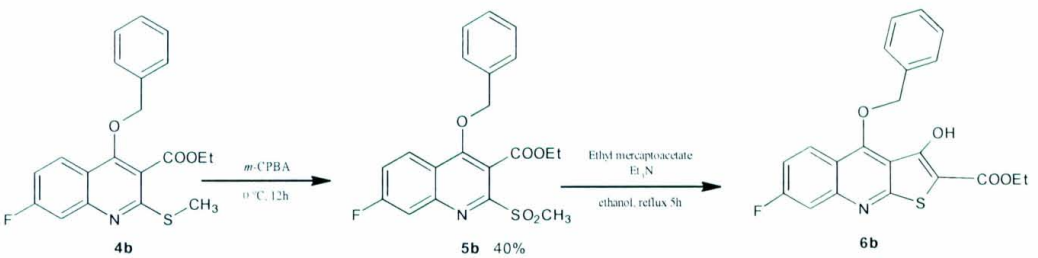
Scheme 2-2: Synthesis of compound **4a** and **4b**

According to the procedures reported by Garlapati, R. *et al.* [6a] and Venkatesh, C. *et al.* [10] with minor modifications, compound **4a** was subjected to oxidation using *m*-chloroperoxybenzoic acid to give **5a**. A five-membered ring containing sulfur as heteroatom, was incorporated in compound **5a** by reacting it with ethyl mercaptoacetate in presence of triethylamine as a catalyst to give compound **6a**, following the conditions reported in references [6a], [11-13] with minor modifications. (**Scheme 2-3**).



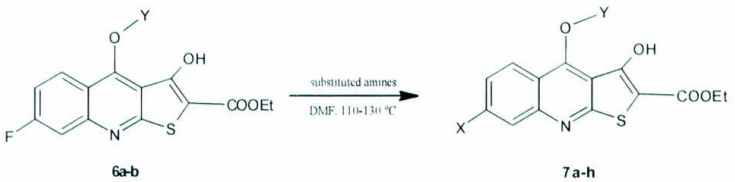
Scheme 2-3: Synthesis of compound **6a**

The same conditions as those used in the conversion of compound **4a** to **6a** were applied to compound **4b** to obtain compound **5b**, as shown in **Scheme 2-4**.



Scheme 2-4: Synthesis of compound **6b**

Following the procedures reported by Tabart, M. *et al.* [14], Antoine, M. *et al.* [15], and Massari, S. *et al.* [16] with minor modifications, nucleophilic substitution was carried out on compounds **6a-b** using various substituted amines to yield the final products **7a-h** as depicted in **Scheme 2-5**. Compounds **3**, **4a**, **4b**, **5a**, **5b**, **6a**, **6b** have also been prepared by our research group, previously as a part of a different project [6a]



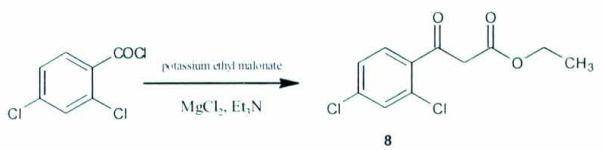
6a-b **7a-h**

7a; Y= -C_2H_5, X= Piperazine, 30%
7b; Y= -C_2H_5, X= methylpiperazine 27%
7c; Y= -C_2H_5, X= Morpholine 21%
7d; Y= -C_2H_5, X= Pyrrolidine 17%
7e; Y= -$CH_2C_6H_5$, X= Piperazine 37%
7f; Y= -$CH_2C_6H_5$, X= Methylpiperazine 40%
7g; Y= - $CH_2C_6H_5$, X= Morpholine 26%
7h; Y= - $CH_2C_6H_5$, X= Pyrrolidine 19%

Scheme 2-5: Synthesis of compound **7a-h**

2.2.2 *N*- Alkylated quinolones

In this approach, 2,4-dichlorobenzoylchloride was used a starting material for the preparation of *N*-alkylated derivatives. This compound was reacted with potassium ethyl malonate, magnesium chloride and triethyl amine to afford compound **8** as a β keto ester, following the conditions reported in references [6a], [17] with minor modifications. (**Scheme 2-6**).



8

Scheme 2-6: Preparation of β keto ester, **8**

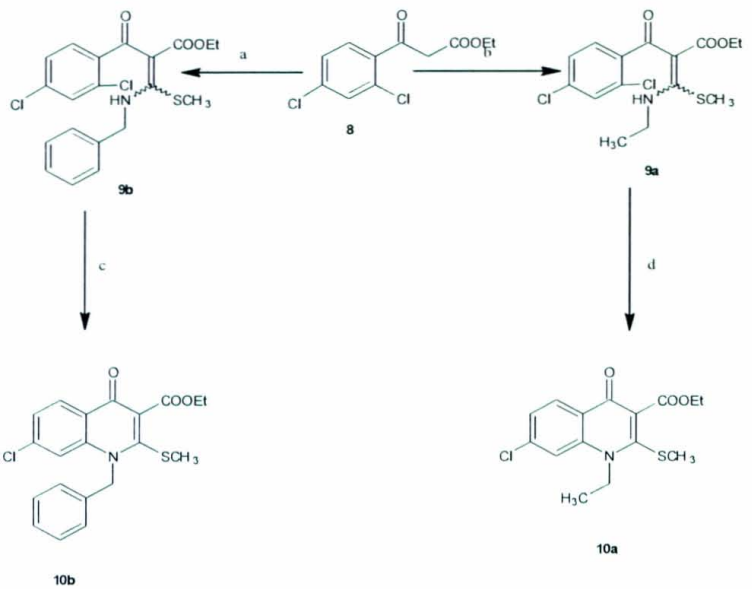
The β keto ester derivative **8** was allowed to react with ethyl or benzyl isothiocyanate in the presence of potassium hydroxide and *n*-tetrabutylammonium bromide as catalyst to afford compounds **9a** and **9b**, respectively. Compound **9a** and **9b,** being unstable open

ring molecules, were immediately subjected to cyclization using *t*-BuOk to afford compounds **10a** and **10b,** respectively, following the conditions reported in references [6a] and [17] with minor modifications. (**Scheme 2-7**).

Compound **10a** was subjected to oxidation using *m*-chloroperoxybenzoic acid to give **11a** following the conditions reported in references [6a] and [10] with minor modifications. A five-membered ring containing sulfur as heteroatom, was incorporated into compound **11a** by reacting it with ethyl mercaptoacetate in presence of triethylamine as catalyst to give compound **12a** following the reaction conditions reported in references [6] and [11-13] with minor modifications. (**Scheme 2-8**).

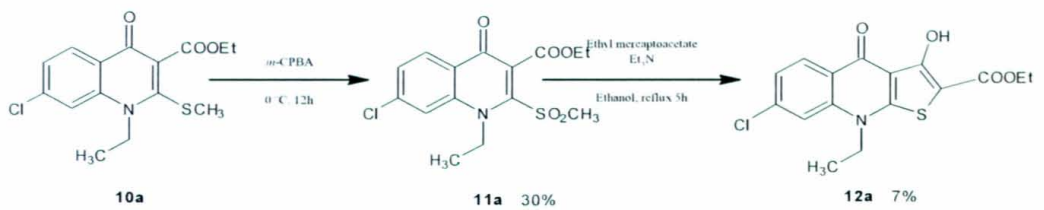
The same conditions as those used in the conversion of compound **10a** to **11a** and **12 a** were applied to compounds **10b** and **11b** to obtain compound **12b**, as shown in **Scheme 2-9**.

Nucleophillic substitution was carried out on compounds **12a-b** using various substituted amines to yield the final products **13a-h** (**Scheme 2-10**) following the conditions reported in references [14-16] with minor modifications. Compounds **10a**, **10b**, **11a**, **11b**, **12a**, **12b** have also been previously prepared by our research group, as a part of different project [6a].

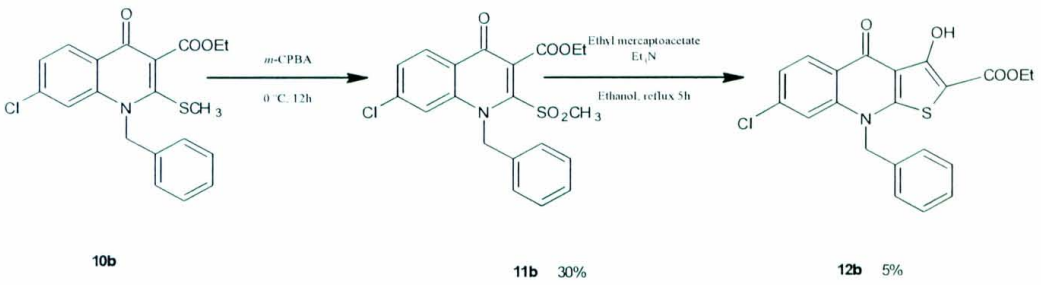


a) KOH, n-tetrabutylammonium bromide, benzyl isothiocyanate, CH_3I, 0°C, stir 16h.
b) KOH, n-tetrabutylammonium bromide, ethyl isothiocyanate, CH_3I, 0°C, stir 16h.
c) and **d)** *t*-BuOk, reflux 20 h

Scheme 2-7: Synthesis of compound **10a-b**

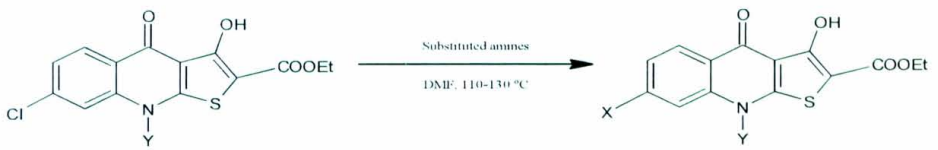


Scheme 2-8: Synthesis of compound **12a**



10b **11b** 30% **12b** 5%

Scheme 2-9: Synthesis of compound **12b**



12a-b **13a-h**

13a; Y= -C_3H_5, X= Piperazine, 32%
13b; Y= -C_3H_5, X= methylpiperazine 24%
13c; Y= -C_3H_5, X= Morpholine 20%
13d; Y= -C_3H_5, X= Pyrrolidine 15%
13e; Y= -$CH_2C_6H_5$, X= Piperazine 40%
13f; Y= -$CH_2C_6H_5$, X= Methylpiperazine 41%
13g; Y= - $CH_2C_6H_5$, X= Morpholine 22%
13h; Y= - $CH_2C_6H_5$, X= Pyrrolidine 18%

Scheme 2-10: Synthesis of compound **13a-h**

2.2.3 *In vitro* microbiological testing

Antimicrobial screening of the synthesized compounds **7a-h** and **13a-h** was carried out using the Broth dilution method to determine their minimum inhibitory concentration (MIC). The 96-well microtitreplate-based serial dilution checkerboard method was adopted [18]. Mueller Hinton broth was used as a liquid medium for sensitivity test. In

accordance with the protocols followed by *Tahlan* Laboratory, Nalidixic acid and Rifampicins were used as controls and *E. coli*, *M. luteues*, *S. epidermidis*, *M. segmentis* were used as the test organisms. Stock solution of concentration 10000 µM was made using the appropriate weight of test compounds. 100 µl of sterile media was added to the well 1A-1H (**Figure 2-3**). 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, 6.25, 3.125, 1.5625, 0.7812, 0.3906, 0.1953 and 0.0976 µM in the wells 1-11, respectively. Wells were then allowed to incubate at the respective temperature. Plates containing *M. luteus* was incubated at 30^0C for 48 hours. Plates containing *Mycobacterium smegmatic* and *E. coli* were incubated at 37^0C. Similarly, *Staphylococcus epidedermis* was incubated at 37^0C for 48 hours. Rifampicin and Nalidixic acid were used as the reference standards. After incubation, plates were observed visually to detect the presence of bacterial growth.

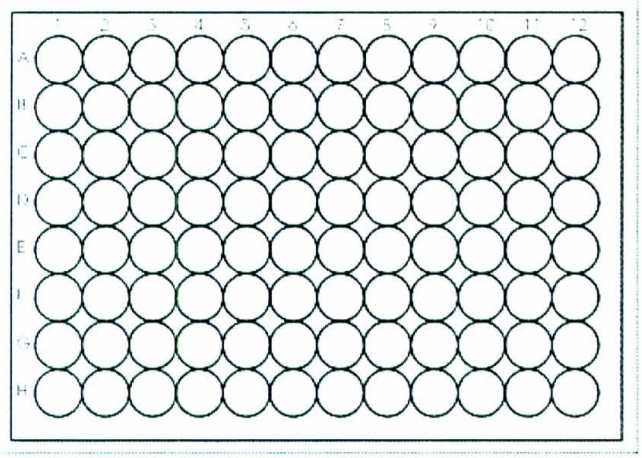


Figure 2-3: A 96 well plate

2.3 Molecular Docking

2.3.1 Methods

DNA gyrase + DNA model was generated by superposition onto the crystal structure of the topoisomerase IV (pdb code **3FOF**). Chains A and B from **3IFZ** were superimposed to the corresponding chains from **3FOF**, respectively, and the DNA structure from topoisomerase IV was exported to the DNA gyrase structure. Docking calculations were carried out according to the Docking Server methodology [19]. The MMFF94 force field [20] was used for energy minimization of ligand molecule using DockingServer. PM6 semi-empirical charges calculated by MOPAC2009 were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on *Mycobacterium tuberculosis* DNA gyrase structure with the pdb code **3IFZ**, the constructed **3IFZ**+DNA model and *Escherichia coli* DNA gyrase subunit B (PDB entry: **1EI1**). Essential hydrogen atoms,

Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [21]. Affinity (grid) maps with 80x80x80 Å and 25×25×25 Å dimension (blind and focused docking, respectively) and 0.375 Å spacing were generated using the Autogrid program [21]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [22]. 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.

2.3.2 Docking calculations to GyrA region of *Mycobacterium tuberculosis* DNA gyrase

2.3.2.1 Background and availability of the models

Mycobacterium tuberculosis DNA gyrase is an important target protein against multidrug-resistant tuberculosis. There are two deposited structures of this DNA gyrase. The GyrA N-terminal domain from *Mycobacterium tuberculosis* X-ray structures available in Protein Data Bank are **3IFZ** and **3ILW** (**Table 2-1**).

Entry	Method	Resolution (Å)	Chains	Positions	Ligand
3IFZ	X-ray	2.70	A/B	1-501	(4*S*)-2-methyl-2,4-pentanediol
3ILW	X-ray	1.60	A/B	34-500	-

Table 2-1: Available X-Ray structures of DNA gyrase GyrA N-terminal domain from Mycobacterium tuberculosis

The structure of PDB entry **3ILW** has been resolved with higher resolution, however, **3IFZ** structure covers the whole N-terminal region and more importantly, it was determined as a complex with a bound ligand ((4*S*)-2-methyl-2,4-pentanediol). Therefore, PDB entry **3IFZ** seems to be a more adequate target than that of **3ILW** to carry out docking calculations. It is important to note that the structure of a homologous protein, DNA topoisomerase IV complexed with DNA and quinolone is also available (PDB entry: **3FOF**). This structure reveals that the quinolone is intercalated at the highly bent DNA gate. Therefore, a binding site composed by DNA gyrase and DNA should be also considered.

.2.3.2.2 Blind Docking Experiment

Three ligands, compound **13a**, compound **7a** and compound **A**, (Page 51, **Figure 2-2**), were docked to DNA gyrase. A blind docking calculation covering the whole protein structure (PDB entry: **3IFZ**) was carried out to identify possible binding sites. In a second step, ligands were docked to **i)** the site identified in the blind docking calculations, **ii)** to the ligand binding site of 3IFZ and **iii)** to a constructed DNA gyrase-DNA complex. The calculated docking energies are summarized in **Table 2-2**.

Compound/docking type	blind	focused	focused2	DNA complex
Compound 13a	-7.74	-8.02	-7.14	-9.85
Compound 7a	-7.72	-8.26	-7.06	-9.69
Compound A	-8.91	-9.1	-7.47	-9.95

Table 2-2: Calculated docking energies in kcal/mol

In order to define possible binding sites in the protein, *Mycobacterium tuberculosis* DNA gyrase, blind docking procedure was applied. According to our calculations, all compounds were docked to Potential Binding Site I. (**Figure 2-4**). Therefore, this site was selected for focused docking calculations. It is important to note that buried binding sites cannot be identified by blind docking. Therefore, the binding site of (4*S*)-2-methyl-2,4-pentanediol (Potential Binding Site II., **Figure 2-4**) was also selected for focused docking calculations.

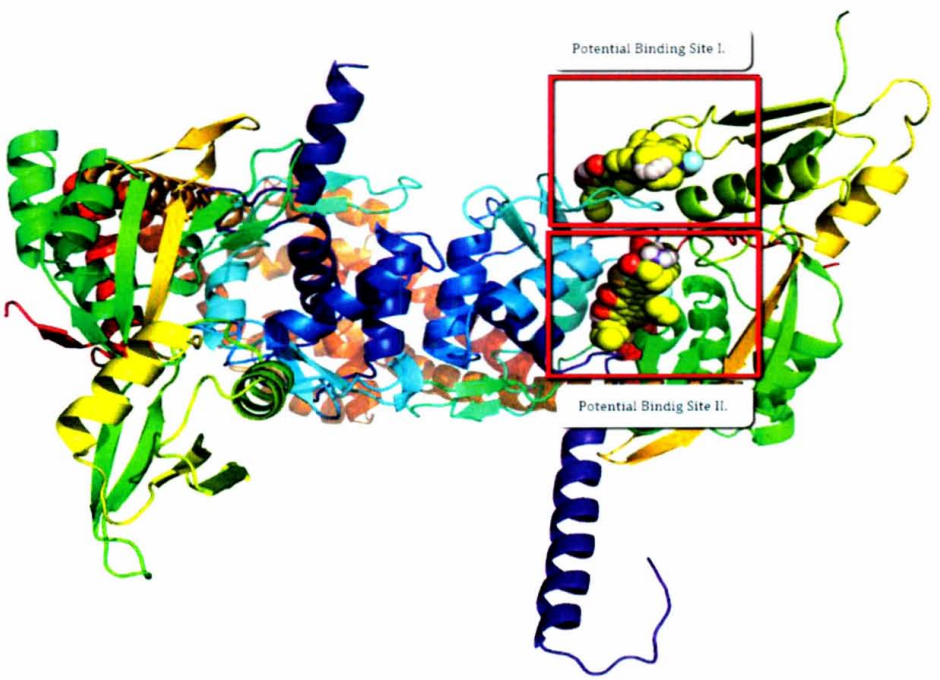
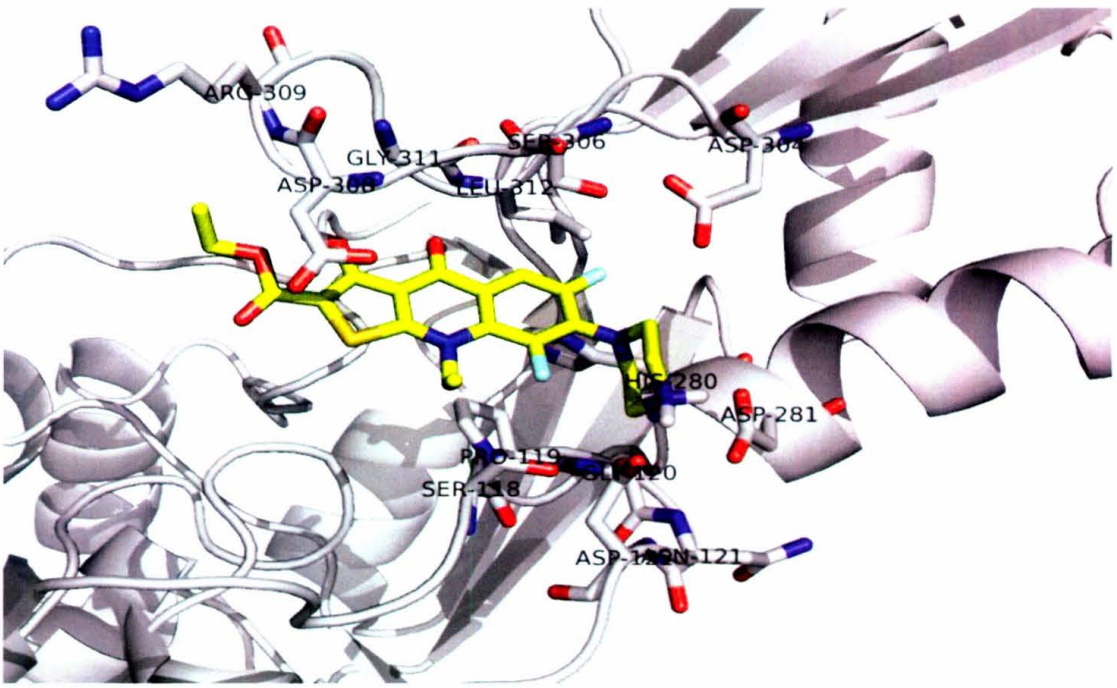


Figure 2-4: Potential binding sites of PDB entry **3IFZ**

2.3.2.3 Focused Docking Experiment

Compound A, as well as compounds **13a** and **7a** were subjected to focused docking calculations at the potential binding site I of the protein. The docking geometries and interactions are shown in **Figure 2-5**.

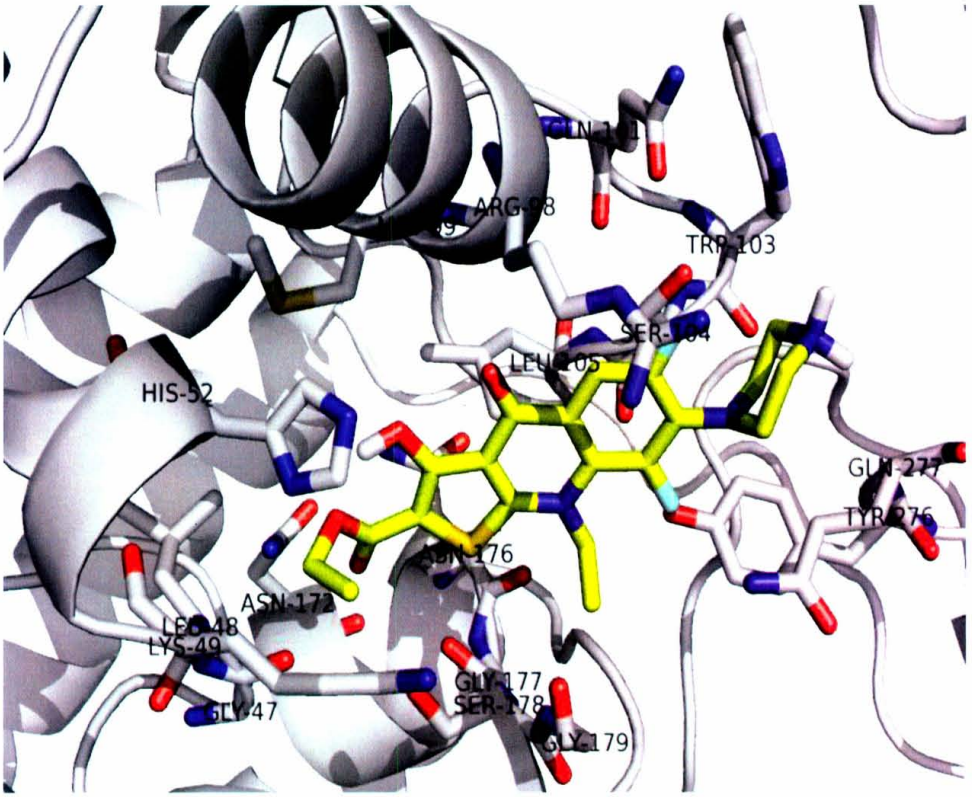


Compound 13a	Compound 7a	Compound A
ASN115 PO OT	ASP122 HB PO OT	ASP122 HB PO OT
ASP122 HB PO OT	ASP281 HB PO OT	ASP281 HB PO OT
ASP281 HB PO OT	ASP304 OT	ASP304 HB OT
ASP308 OT	ASP308 PO OT	ASP308 PO OT
PHE116 HY	HIS280 HY OT	HIS280 HY OT
PRO102 HY	PRO119 HY OT	LEU312 OT
PRO119 HY OT	SER118 PO OT	PRO119 HY OT
SER118 OT	TRP103 HY	SER118 PO HB OT
TRP103 HY OT		SER306 HB OT

Figure 2-5: Docked structure of compound **A** at potential binding site I and calculated interactions of the docked compounds (HB: hydrogen bond, PO: polar, HY: hydrophobic, CA: cation-pi, OT: other)

As the results show, the investigated compounds form similar interactions with the protein. As can be seen in **Figure 2-5** and the corresponding interaction table, the docked compounds are bound with many specific interactions. Compound **A** forms strong hydrogen bonds (or Coulomb interaction) with ASP122 and ASP281, additionally Ser118, Ser306 and ASP304 are involved a halogen bond. While there are many polar interactions involved in ligand binding, the number of hydrophobic residues and consequently hydrophobic interactions is very limited at potential binding site I. Therefore, the planar aromatic ring is not involved in specific interactions. The absence of the specific hydrophobic interactions can hinder ligand binding to this site in vitro.

In the next step, compound **A**, compounds **13a** and **7a** were subjected to focused docking calculations at the potential binding site II of the protein. The docking geometries and interactions are shown in **Figure 2-6.**



Compound 13a	Compound 7a	Compound A
ARG98 PO	ARG98 PO OT	ARG98 PO OT
ASN172 PO	ASN172 PO OT	ASN172 PO
GLN277 PO OT	ASN176 OT	GLN101 HA
HIS52 PO HY CA OT	GLN101 OT	GLN277 OT
LEU105 HY OT	GLN277 PO OT	HIS52 PO HY CA OT
LEU48 HY OT	GLY47 HB	LEU105 HY OT
LYS49 OT	HIS52 HY OT	LEU48 HY OT
MET99 OT	LEU105 HY	LYS49 OT
SER104 PO OT	LEU48 OT	MET99 OT
SER178 OT	SER104 PO OT	SER104 HA OT
TRP103 PO HY CA	SER178 PO OT	SER178 OT
TYR276 PO HY OT	TRP103 HY OT	TRP103 HY
	TYR276 PO OT	TYR276 HA OT

Figure 2-6: Docked structure of compound A at potential binding site II and calculated interactions of the docked compounds (HB: hydrogen bond, PO: polar, HY: hydrophobic, CA: cation-pi, HA: halogene bond, OT: other)

In contrast to potential binding site I, many hydrophobic residues form hydrophobic interactions at potential binding site II (TRP103, TYR276, LEU48, MET99), however, the number of polar interactions is limited at this site. No specific hydrogen bonds can be found for compound **A**, additionally, there is no interaction partner for the quaternary nitrogen. Therefore, the calculated docking energies for this site are higher than that of potential binding site I.

As it was mentioned earlier, the structure of a homologous protein, DNA topoisomerase IV complexed with DNA and quinolone is also available (PDB entry: 3FOF). This structures reveals quinolone intercalated at the DNA gate. Therefore, we constructed a model for DNA gyrase-DNA complex, and focused docking calculations were carried out to the interaction interface. **Figure 2-7** shows compound **A** docked to this potential binding site. The charged quaternary nitrogen forms strong Coulomb interaction with ASP89 and hydrogen bond with HIS87. The planar aromatic ring is intercalated in the DNA structure. This interaction is ideal for both the hydrophilic and hydrophobic parts of the investigated compounds; docking energies were calculated to be the lowest at this potential site.

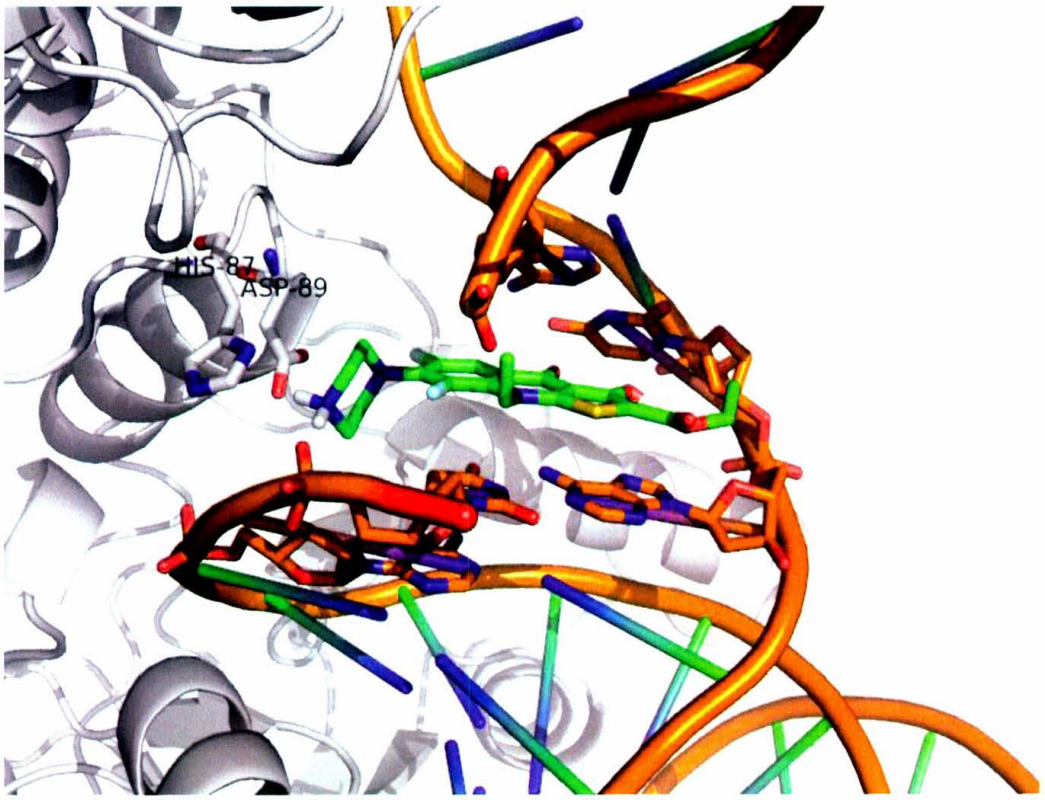


Figure 2-7: Compound A docked to DNA gyrase-DNA complex

2.4 Background and availability of the models

The structure of DNA gyrase subunite B (gyrB) from *Mycobacterium tuberculosis* DNA gyrase subunit B is only known in the 448-675 sequence region (PDB entries: **3M4I**, **3IG0, 2ZJT**). This region does not include the ATP binding region and therefore cannot be used for docking calculations. The ATP binding region of *Escherichia coli* DNA gyrase subunit B is determined (PDB entry: **1EI1**). The sequence alignment of *Mycobacterium tuberculosis* DNA gyrase subunit B and *Escherichia coli* DNA gyrase subunit B is shown in **Figure 2-8**.

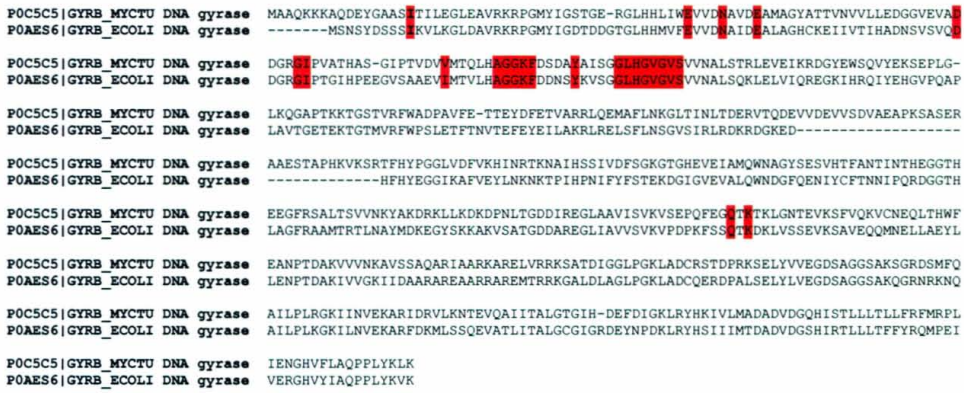


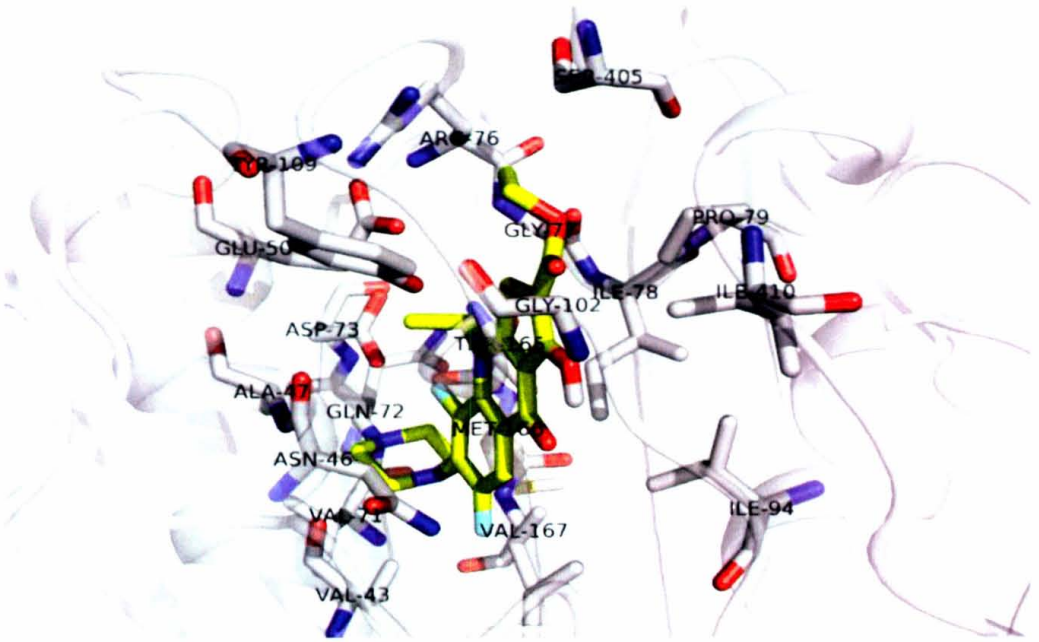
Figure 2-8: Sequence alignment of *Mycobacterium tuberculosis* DNA gyrase subunit B and *Escherichia coli* DNA gyrase subunit B (ATP binding site residues are shaded with red color)

2.5 Docking calculations to GyrB region of *Escherichia coli* DNA gyrase

The ATP binding region (**Figure 2-8**) is identical for the two species expcept the sole valine/isoleucine conservative change. This high sequence identity allows us to carry out docking calculations on *Escherichia coli* DNA gyrase subunit B (PDB entry **1EI1**).

2.5.1 Focused Docking Experiment

Due to the closet structural similarity of compound **13a** and **7a** with compound **A**, they were chosen for docking calculations. Docking calculations were carried out to the known ATP binding site. The calculated docking energies of compound **A**, compounds **13a** and **7a** to gyrB shows strong potential binding (-9.73 kcal/mol, -9.06 kcal/mol, -8.27 kcal/mol, respectively). The compounds were docked in a similar conformation to the ATP binding site of gyrB (**Figure 2-10**). The lowest energy docking result for compound **A** is shown in **Figure 2-9**.



Compund 13A	Compound 7a	Compound A
ALA47 HY	ALA47 HY OT	ALA47 HY OT
ARG76 OT	ARG76 PO	ARG76 OT
ASN46 PO OT	ASN46 OT	ASN46 OT
ASP73 HB PO OT	ASP73 HB PO OT	ASP73 HB PO HA OT
GLU50 OT	GLU50 PO OT	GLU50 OT
ILE410 OT	ILE410 HY OT	ILE410 HY OT
ILE78 HY OT	ILE78 HY OT	ILE78 HY OT
ILE94 HY OT	ILE94 OT	ILE94 OT
PRO79 HY OT	LYS103 OT	PRO79 HY OT
SER405 OT	PRO79 HY OT	SER405 OT
THR165 HB PO OT	SER108 OT	THR165 HB PO HA OT
TYR109 PO OT	SER405 OT	TYR109 OT
VAL120 HY OT	SER409 OT	VAL120 HY OT
VAL167 HY OT	THR165 HB OT	VAL167 HY OT
VAL43 HY OT	TYR109 PO HY OT	VAL43 HY OT
VAL71 HB HY OT	VAL120 HY	VAL71 HB HY OT
	VAL167 HY OT	
	VAL43 HY OT	
	VAL71 HB OT	

Figure 2-9: Docked structure of compound A at ATP binding site and calculated interactions of the docked compounds (HB: hydrogen bond, PO: polar, HY: hydrophobic, CA: cation-pi, HA: halogen bond, OT: other).

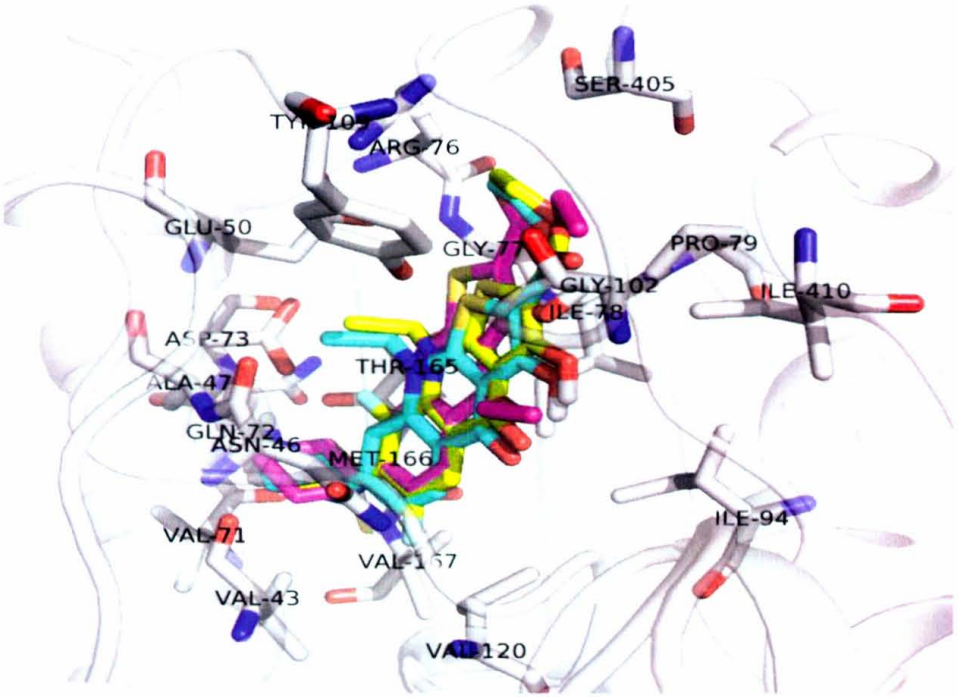


Figure 2-10: Comparison of docking geometries of compound **A** (yellow), compounds **13a** (blue) and **7a** (violet) in the ATP binding site.

The docked compounds are deeply buried in the binding site and form many hydrophilic and hydrophobic interactions. The protonated quaternary nitrogen and ASP73 forms strong Coulomb interaction. ASP73 is also involved in halogen bonding. Similarly, THR165 forms a hydrogen bond with the investigated compounds and can be involved in halogen bonding. The backbone oxygen of VAL71 donates a hydrogen bond from the protonated nitrogen. Additionally, there are many hydrophobic interactions at the binding site interacting with the planar aromatic ring, e.g. ILE78, ILE94, ILE410, VAL43,

VAL71, VAL120, VAL167 and TYR109. These interactions and the calculated docking energies suggest strong potential binding of the investigated compounds to gyrB in vitro.

2.6 Results and discussions

2.6.1 Antimicrobial assay results

Compounds were tested against *Mycobacterium luteues, Mycobacterium smegmatic, E. coli* and *Staphalycoccus epidedermis* using Rifampicin and Nalidixic acid as standard. Assay results against *Mycobacterium luteues* (**Table 2-3**), *Mycobacterium smegmatic* (**Table 2-4**), *E. coli* (**Table 2-5**) and *Staphalycoccus epidedermis* (**Table 2-6**) are shown below.

2.6.2 Docking Result

The GyrA region of bacterial topoisomerase forms a complex with DNA and carries out its function. Quinolones interact with this DNA-GyrA complex and hence cause inhibition of the whole complex. In our docking experiments, we found that our standard compound A, interacts with the DNA-GyrA complex with strongest affinity as compared to our test compounds. This is concluded as a result of lowest docking energy of compound A with DNA-GyrA complex (**Table2-2**). Additionally, when compound **A**, **13a**, **7a** were docked to the ATP binding region of GyrB subunit. It was found that Compound **A** has lowest docking energy (-9.73kcal/mol) as compared to our test compounds. The main difference between the interaction of compound **A** and compounds **13a**, **7a** to our target protein is their ability to form stable complex with target proteins. Based on the calculated docking energy, we hypothesize that these compounds might not

be able to form a stable complex with the target site. This may explain the lack of antibacterial activity with compounds **13a** and **7a**.

Compound	MIC (μM)
7a	>100
7b	>100
7c	>100
7d	>100
7e	>100
7f	>100
7g	>100
7h	>100
13a	>100
13b	>100
13c	>100
13d	>100
13e	>100
13f	>100
13g	>100
13h	>100
Rifampicin	6.25
Nalidixic acid	Not active

Table 2-3: Assay against *M. luteus*

Compound	MIC (μM)
7a	>100
7b	>100
7c	>100
7d	>100
7e	>100
7f	>100
7g	>100
7h	>100
13a	>100
13b	>100
13c	>100
13d	>100
13e	>100
13f	>100
13g	>100
13h	>100
Rifampicin	1.5625
Nalidixic acid	6.25

Table 2-4: Assay against *E. coli*

2.7 Conclusion

The quinolone based system is an excellent scaffold for design of various classes of bioactive molecules. Its various unique structural features have led to design and synthesis of various diverse ranges of molecules. In this study, a series of novel non-fluorinated polycyclic quinolones were designed based on compound A, in order to study the effects of absence of fluorine on its activity. These compounds were tested for their susceptibility against *Mycobacterium luteues, Mycobacterium smegmatic, E. coli* and

Staphalycoccus epidedermis. The test results revealed the lack of antimicrobial activity of these compounds. In order to explore the cause behind the lack of activity, docking-

Compound	MIC (μM)
7a	>100
7b	>100
7c	>100
7d	>100
7e	>100
7f	>100
7g	>100
7h	>100
13a	>100
13b	>100
13c	>100
13d	>100
13e	>100
13f	>100
13g	>100
13h	>100
Rifampicin	0.0976
Nalidixic acid	Not active

Table 2-5: Assay against *S. epidedermis*

Compound	MIC (μM)
7a	>100
7b	>100
7c	>100
7d	>100
7e	>100
7f	>100
7g	>100
7h	>100
13a	>100
13b	>100
13c	>100
13d	>100
13e	>100
13f	>100
13g	>100
13h	>100
Rifampicin	6.25
Nalidixic acid	Not active

Table 2-6: Assay against *M. smegmatic*

-experiments were carried out against DNA gyrase using "**Compound A**", as the standard DNA gyrase inhibitor. Based on our docking results, we concluded that in this type of polycyclic quinolone systems, removal of fluorine can dramatically abolish 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 other potential bioactivities are ongoing in the Daneshtalab group.

2.8 Experimental

Solvents and reagents were obtained from commercial suppliers and were used without further purification. Melting points are not corrected. ^{1}H and ^{13}C 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.

Ethyl 7-Fluoro-4-hydroxy-2-(methylsulfonyl)quinoline-3-carboxylate (3)

To a suspension of sodium hydride (3.655 g, 145.1 mmol) in tetrahydrofuran (300 ml) maintained at 5-10 ^{0}C, diethylmalonate (23.23 g, 145.1 mmol) was added gradually with constant stirring. The mixture was then brought to room temperature, allowed to stir for a further 30-40 minutes and then 3-fluorophenyl isothiocyanate (22.00 g, 143.6 mmol)

was added gradually over a period of 30 minutes under ice cooling. Stirring was then stopped and the reaction mixture was allowed to stand overnight at 4 ^{0}C. The resulting precipitate was collected by filtration and washed with diethyl ether to afford compound **1** as a yellow solid. The yellow solid was then dissolved in DMF (50 ml) and methyl iodide (34.66 g, 244.2 mmol) was added gradually under ice cooling and the mixture was allowed to stir at the 0 ^{0}C for 2 hours. It was then slowly warmed up to room temperature and stirred for another 4 hours. The reaction mixture was then poured into ice-water and extracted with chloroform (25 ml x 3). The chloroform layer was separated and dried over sodium sulfate and concentrated under vacuum to yield compound **2** as a yellow oil. This oil was used in the next step without purification. Compound **2** was heated at 180 ^{0}C under vacuum for 15-20 minutes to afford compound **3**. Purification by recrystallization from a mixture of hexane and ethyl acetate (1:1) afforded compound **3** as a white powder; mp 160-162 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.09 (dd, *J* = 7.4, 5.0 Hz, 1H), 7.61 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.21 (td, *J* = 7.7, 1.5 Hz, 1H), 2.59 – 2.44 (m, 3H), 4.29 (q, *J* = 5.9 Hz, 2H), 4.04 (s, 1H, OH), 1.30 (t, *J* = 5.9 Hz, 3H). ^{13}C NMR (175 MHz, Chloroform-d) δ 176.6, 167.1, 163.1, 161.7, 151.2, 125.6, 118.5, 115.4, 108.8, 105.9, 61.2, 14.6, 13.9; HR-MS (TOFEI) calcd for $C_{13}H_{12}NO_3FS$ (281.0521); found (281.0521).

Ethyl 4-Ethoxy-7-fluoro-2-(methylsulfonyl)quinoline-3-carboxylate (4a)

To a stirred solution of compound **3** (5.00 g, 17.77 mmol) and potassium carbonate (4.893 g, 35.4 mmol) in DMF(20 ml), (3.85 g, 35.4 mmol) of ethyl bromide

was added gradually and the reaction mixture was allowed to stir at 70-80 ^{0}C overnight. The reaction mixture was then warmed up to the room temperature and poured into a large quantity of cold water. The aqueous layer was extracted with chloroform. The organic extract was dried over sodium sulfate and evaporated under reduced pressure to give the crude compound **4a**, which was purified by column chromatography (0.5% ethyl acetate in Hexane) to obtain the pure compound as a yellow liquid; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.24 (dd, *J* = 7.5, 4.9 Hz, 1H), 7.52 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.30 (td, *J* = 7.7, 1.6 Hz, 1H), 4.29 (q, *J* = 5.9 Hz, 2H), 4.07 (q, *J* = 5.9 Hz, 2H), 2.54 (s, 3H), 1.34 (t, *J* = 5.9 Hz, 3H), 1.30 (t, *J* = 5.9 Hz, 3H). ^{13}C NMR (175 MHz, Chloroform-d) δ 169.4, 163.2, 162.0, 158.6, 132.9, 128.8, 126.0, 123.8, 118.7, 114.3, 66.4, 61.2, 14.6, 13.9, 13.8; APCI-MS: 292.1 (M^{+}+1).

Ethyl 4-(Benzyloxy)-7-fluoro-2-(methylsulfonyl)quinoline-3-carboxylate (4b)

Utilizing the same alkylation procedure as applied for the preparation of **4a** using benzyl bromide (6.05 g, 35.4 mmol); yellow liquid; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.18 (d, *J* = 6.3 Hz, 1H), 7.99 (dd, *J* = 9.1, 6.2 Hz, 1H), 7.57-7.50 (m, 1H), 7.47-7.39 (m, 5H), 5.16 (d, *J* = 4.1 Hz 2H), 4.54 (q, *J* = 7.1 Hz, 2H), 2.57 (s, 3H), 1.53 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 169.6, 165.0, 163.2, 159.5, 138.8, 134.1, 130.2, 130.0, 129.4, 129.3, 127.2, 125.0, 119.7, 116.2, 75.5, 62.4, 15.8, 15.1; APCI-MS: 372.1 (M^{+}+1).

Ethyl 4-(Benzyloxy)-7-fluoro-3-hydroxythieno[2,3-*b*]quinoline-2-carboxylate (6b)

To a solution of compound **4b** (1.50 g, 3.72 mmol) in dichloromethane maintained at 0 ^{0}C, m-chloroperoxybenzoic acid(0.962 g, 5.58 mmol) was added and the reaction mixture was allowed to stir at the same temperature for 2-3 hours. It was then warmed up to room temperature and allowed to stir overnight. The reaction mixture was washed with 30% sodium bicarbonate solution (50ml) and the organic layer was separated, dried over sodium sulfate and filtered. The filtrate was evaporated under reduced pressure to yield compound **5b** as a white amorphous solid, which was subjected to further chemical reaction without purification. The white amorphous precipitate obtained was suspended in dry ethanol (30ml) and ethyl mercaptoacetate (0.60 g, 5.00 mmol,) and triethylamine (1.51 g, 15.00 mmol) was added to it. The reaction mixture was refluxed for 5 h under argon atmosphere. The solvent was then evaporated to dryness under reduced pressure and ice cold water was added to the remaining mixture. The resulting mixture was extracted with chloroform, dried over sodium sulfate and evaporated under reduced pressure to yield compound **6b** as a highly viscous yellow liquid; ^{1}H NMR (500 MHz, Chloroform-d) δ 13.32 (s 1H), 8.18 (d, *J* = 6.3 Hz, 1H), 7.99 (dd, *J* = 9.1, 6.2 Hz ,1H), 7.57-7.50 (m, 1H), 7.47-7.39 (m, 5H), 5.16 (d, *J* = 4.1 Hz, 2H), 4.54 (q, *J* = 7.1 Hz, 2H), 1.53 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (175 MHz, Chloroform-d) δ 168.5, 167.0, 166.4, 165.8, 164.6, 159.1, 154.9, 137.5, 129.0, 128.1, 125.7, 118.9, 115.8, 115.6, 110.8, 109.9, 109.8, 95.8, 74.5, 61.4, 14.6; APCI-MS: 398.0 (M^{+}+1).

Ethyl 4-(Ethoxy)-7-fluoro-3-hydroxythieno[2,3-*b*]quinoline-2-carboxylate (6a)

This compound was prepared by utilising the same procedure employed for the preparation of compound **6b** using compound **4a** (1.17 g, 3.8 mmol); highly viscous yellow liquid; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.35 (dd, *J* = 7.5, 4.9 Hz, 1H), 7.69 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.32 (td, *J* = 7.9, 1.4 Hz, 1H), 5.86 (s, 1H), 4.37 (q, *J* = 5.9 Hz, 2H), 4.07 (q, *J* = 5.9 Hz, 2H), 1.34 (t, *J* = 5.9 Hz, 6H). ^{13}C NMR (175 MHz, Chloroform-d) δ 167.4, 166.0, 165.8, 165.4, 162.0, 154.3, 125.7, 118.0, 115.7, 111.2, 109.8, 102.4, 66.4, 61.4, 14.6, 13.8; APCI-MS: 336.1 (M^{+}+1)

Ethyl 4-(Ethoxy)-3-hydroxy-7-(piperazin-1-yl)thieno[2,3-*b*]quinoline-2-carboxylate (7a)

To a mixture of compound **6a** (0.67 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 hours. 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 ethanol; white powder; mp 250-252 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.20 (d, *J* = 7.5 Hz, 1H), 7.90 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.43 (d, *J* = 1.4 Hz, 1H), 6.16 (s, 1H), 4.37 (q, *J* = 5.9 Hz, 2H), 4.07 (q, *J* = 5.9 Hz, 2H), 3.73 (t, *J* = 5.1 Hz, 2H),3.39 (t, *J* = 5.1 Hz, 2H), 3.11 (t, *J* = 5.1 Hz, 2H), 3.03 (t, *J* = 5.1 Hz, 2H), 1.34 (t, *J* = 5.9 Hz, 6H), 1.23 (s,1H). ^{13}C NMR (175 MHz, Chloroform-d) δ 167.0, 164.8, 164.3, 160.3,155.5, 154.2, 126.2, 118.5, 115.7, 109.8, 109.6, 97.0, 67.6,

62.6, 51.3, 46.2, 15.8, 15.0; HR-MS (TOFEI) calcd for $C_{20}H_{23}N_3O_4S$ (401.1410); found (401.1409).

Ethyl 4-(Ethoxy)-3-hydroxy-7-(4-methylpiperazin-1-yl)thieno[2,3-*b*]quinoline-2-carboxylate (7b)

This compound was prepared utilising the procedure employed for the preparation of compound **7a**, using (0.67 g, 2.00 mmol) of compound **6a** and 1.0 g (10 mmol) of methylpiperazine; yellow powder; mp 241-243 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.13 (d, *J* = 7.5 Hz, 1H), 7.85 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.37 (d, *J* = 1.4 Hz, 1H), 4.82 (s, 1H), 4.37 (q, *J* = 5.9 Hz, 2H), 4.07 (q, *J* = 5.9 Hz, 2H), 3.44 (t, *J* = 5.0 Hz, 4H), 2.35 (t, *J* = 5.0 Hz, 4H), 2.23 – 2.19 (m, 3H), 1.34 (t, *J* = 5.9 Hz, 6H). ^{13}C NMR (175 MHz, Chloroform-d) δ 165.8, 163.6, 163.1, 159.1,154.3, 153.0, 125.0, 117.3, 114.5, 108.6, 108.4, 95.8, 66.4,61.4, 52.6, 50.3, 46.0, 14.6, 13.8; HR-MS (TOFEI) calcd for $C_{21}H_{25}N_3O_4S$ (415.1564); found (415.1560).

Ethyl 4-(Ethoxy)-3-hydroxy-7-(morpholin-4-yl)thieno[2,3-*b*]quinoline-2-carboxylate (7c)

Prepared according to the procedure employed for the preparation of compound **7a**, using (0.75g, 2.23 mmol) of compound **6a** and 1.30 g (10 mmol) of morpholine; yellow crystals; 237-240 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.36 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.57 (d, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 1.4 Hz, 1H), 4.63 (s, 1H), 4.37 (q, *J* = 5.9

Hz, 2H), 4.07 (q, *J* = 5.9 Hz, 2H), 3.73 (t, *J* = 4.5 Hz, 4H), 3.27 (t, *J* = 4.5 Hz, 4H), 1.34 (t, *J* = 5.9 Hz, 6H); ^{13}C NMR (175 MHz, Chloroform-d) δ 167.2, 164.8, 164.3, 163.2, 155.5, 154.2, 126.2, 118.7, 115.7, 109.8, 109.6, 103.6, 67.6, 67.0, 62.6, 48.3, 15.8,15.0; HR-MS (TOFEI) calcd for $C_{20}H_{22}N_2O_5S$ (402.1249); found (402.1240).

Ethyl 4-(Ethoxy)-3-hydroxy-7-(pyrrolidin-1-yl)thieno[2,3-*b*]quinoline-2-carboxylate (7d)

Prepared utilising the procedure employed for the preparation of compound **7a**, using (0.67 g, 2 mmol) of compound **6a** and 0.71 g (10 mmol) of pyrrolidine; yellow powder; 239-242 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.08 (d, *J* = 7.5 Hz, 1H), 7.69 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.03 (d, *J* = 1.4 Hz, 1H), 5.94 (s, 1H, OH), 4.37 (q, *J* = 5.9 Hz, 2H), 4.07 (q, *J* = 5.9 Hz, 2H), 3.41 (ddd, *J* = 6.4, 4.2, 2.6 Hz, 4H), 2.09-2.01 (m, 4H), 1.34 (t, *J* = 5.9 Hz, 6H); ^{13}C NMR (175 MHz, Chloroform-d) δ 167.0, 164.8, 164.3, 160.3, 154.6, 154.2, 127.1, 118.5, 114.7, 109.8,107.3, 97.0, 67.6, 62.6, 52.3, 27.1, 15.8, 15.0; HR-MS (TOFEI) calcd for $C_{20}H_{22}N_2O_4S$ (386.1300); found (386.1315).

Ethyl 4-(Benzyloxy)-3-hydroxy-7-(piperazin-1-yl)thieno[2,3-*b*]quinoline-2-carboxylate (7e)

To a mixture of compound **6b** (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 hours. It was the brought 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 ethanol; yellow solid; 248-250 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.23 (d, *J* = 7.5 Hz, 1H), 7.85 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.48 (dd, *J* = 7.5, 1.3 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.35 – 7.28 (m, 2H), 6.34 (s, 1H, OH), 5.20 (s, 2H), 4.37 (q, *J* = 5.9 Hz, 2H), 3.64 (t, *J* = 5.1 Hz, 2H), 3.43 (t, *J* = 5.1 Hz, 2H), 3.07 (t, *J* = 5.1 Hz, 2H), 2.99 (t, *J* = 5.1 Hz, 2H), 1.34 (t, *J* = 5.9 Hz, 3H), 1.24 (s, 1H,); ^{13}C NMR (175 MHz, Chloroform-d) δ 165.8, 165.3, 161.7, 159.1, 154.3,153.6, 137.5, 129.0, 128.2, 128.1, 125.0, 118.4, 114.5, 108.4, 108.2, 95.8, 74.5, 61.4, 50.1, 45.0, 14.1; HR-MS (TOFEI) calcd for $C_{25}H_{25}N_3O_4S$ (463.1565); found (463.1460).

Ethyl 4-(Benzyloxy)-3-hydroxy-7-(4-methylpiperazin-1-yl)thieno[2,3-*b*]quinoline-2-carboxylate (7f)

Prepared according to the procedure employed for the preparation of compound **7e**, using (0.79 g, 2.00 mmol) of compound **6b** and 1.0 g (10 mmol) of methylpiperazine; white crystalline product; 241-243 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.31 (d, J = 7.5 Hz, 1H), 7.91 (dd, J = 7.5, 1.5 Hz, 1H), 7.56 – 7.29 (m, 6H), 5.16 (s, 2H), 4.82 (s, 1H, OH), 4.37 (q, J = 5.9 Hz, 2H), 3.44 (t, J = 5.2 Hz, 4H), 2.35 (t, J = 5.2 Hz, 4H), 2.28 – 2.13 (m, 3H), , 1.34 (t, J = 5.9 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 166.8, 165.3, 161.7, 159.1, 154.3, 153.6, 137.5, 129.0,128.8, 128.1, 125.7, 118.4, 115.0, 108.4, 108.0, 95.8, 74.5, 61.4, 52.6, 50.3, 46.0, 14.6; HR-MS (TOFEI) calcd for $C_{26}H_{27}N_3O_4S$ (477.1722); found (477.1701).

Ethyl 4-(Benzyloxy)-3-hydroxy-7-(morpholin-4-yl)thieno[2,3-*b*]quinoline-2-carboxylate (7g)

Prepared utilising the procedure employed for the preparation of compound **7e**, using (1.18 g, 3 mmol) of compound **6b** and 1.30 g (15 mmol) of morpholine; white amorphous powder; 239-242 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.20 (d, *J* = 7.4 Hz, 1H), 7.94 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.48 (dd, *J* = 7.5, 1.3 Hz, 2H), 7.44 – 7.37 (m, 3H), 7.36 – 7.28 (m, 1H), 5.16 (s, 2H), 4.58 (s, 1H, OH), 4.37 (q, *J* = 5.9 Hz, 2H), 3.73 (t, *J* = 4.4 Hz, 4H), 3.27 (t, *J* = 4.4 Hz, 4H), 1.34 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 167.0, 166.5, 162.9, 160.3, 155.5, 154.8, 138.7, 130.2, 129.3, 129.3, 126.2, 119.6, 115.7, 109.6, 109.2, 97.0, 75.7, 67.05, 62.6, 48.4, 15.8; HR-MS (TOFEI) calcd for $C_{25}H_{24}N_2O_5S$ (464.1405); found (464.1401).

Ethyl 4-(Benzyloxy)-3-hydroxy-7-(pyrrolidin-1-yl)thieno[2,3-b]quinoline-2-carboxylate (7h)

Prepared according the procedure employed for the preparation of compound **7e**, using (0.80 g, 2.00 mmol) of compound **6b** and 0.71 g (10 mmol) of pyrrolidine; yellow crystals; 230-232 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.24 (d, *J* = 7.5 Hz, 1H), 7.82 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.48 (dd, *J* = 7.5, 1.3 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.33 (dt, *J* = 8.2, 1.9 Hz, 1H), 7.16 (d, *J* = 1.4 Hz, 1H), 5.18 (s, 2H), 5.02 (s, 1H, OH), 4.37 (q, *J* = 5.9 Hz, 2H), 3.41 (ddd, *J* = 6.4, 4.2, 2.6 Hz, 4H), 2.07 – 2.01 (m, 4H), 1.34 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 165.8, 165.3, 161.7, 159.1, 153.6, 153.4,

137.5, 129.0, 128.2, 128.1, 125.9, 118.4, 113.5, 108.0, 106.1, 95.8, 74.5, 61.4, 51.1, 25.9, 14.6; HR-MS (TOFEI) calcd for $C_{25}H_{24}N_2O_4S$ (448.1456); found (448.1450).

Ethyl 3-(2,4-Dichlorophenyl)-3-oxopropanoate (8)

A mixture of potassium ethylmalonate (13.398 g, 76.97 mmol) and magnesium chloride (16.415 g, 172.41 mmol) in acetonitrile was stirred under argon atmosphere for 30 min at the room temperature. Triethylamine (17.194 g, 169.92 mmol) was then added drop wise to the reaction mixture. The mixture was continued to stir for a period of 2 h followed by the addion of 10.9 g (52.3 mmol) of 2,4-dichlorobenzoyl chloride. The reaction mixture was allowed to stir at the same temperature for 3-4 h. It was then warmed up to the room temperature and 1 ml of triethylamine was further added to it. The mixture was left to stir for a period of 16-18 h at room temperature. The solvent was then removed under reduced pressure and ice cold water was added to the remaining mixture and acidified to a pH 5-6 with hydrochloric acid. It was then extracted with three portions of ethyl acetate (50 ml each) and the organic layers were combined and washed with 100 ml of water followed by brine solution. Organic layer was separated out and dried over sodium sulfate and evaporated under reduced pressure to give the title compound; yellow oil; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.75 (d, J = 7.5 Hz, 1H), 7.64 (d, J = 1.5 Hz, 1H), 7.48 (dd, J = 7.4, 1.6 Hz, 1H), 4.11 (q, J = 5.9 Hz, 2H), 3.90 (s, 2H), 1.20 (t, J = 5.9 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 195.1, 168.5, 139.3, 134.4, 134.3, 130.6, 128.4, 128.0, 61.1, 48.7, 14.6; APCI-MS: 261.1 (M^++1).

Ethyl 7-Chloro-1-ethyl-2-(methylsulfonyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (10a)

A mixture of **8** (5.00g, 19.20 mmol), potassium hydroxide (1.51g) and n-tertbutylammonium bromide (0.007 g, 0.21 mmol) in DMF (25ml) was stirred for 30 min at room temperature. It was then cooled to 0 ^{o}C, and to it ethyl isothiocyanate (2.90 g, 32.9 mmol) was added drop-wise under constant stirring. The reaction mixture was allowed to stir for a period of 16-18 h at room temperature. Iodomethane (3.44 g, 24.38 mmol) was then added to the reaction mixture, which was continued to stir for another 6 h. After the completion of stirring, the reaction mixture was quenched with water (100ml) and saturated aqueous ammonium chloride (70ml) and extracted with ethyl acetate (2 X 50ml). The organic extracts were combined and washed with water (30ml) followed by a wash with brine (30ml) and dried over sodium sulfate. The organic phase was then reduced to dryness under reduced pressure to give the compound **9a** as a yellow liquid. This yellow liquid was subjected to further reaction immediately, without purification. Compound **9a** was suspended in toluene (25ml) at room temperature and potassium-*tert*-butoxide (1.10 g, 9.69 mmol) was added to it. The resulting mixture was refluxed for 24 h, cooled and diluted with water (100ml). The aqueous layer was separated and extracted with ethyl acetate (20 ml X 2). The ethyl acetate extracts were combined with toluene layer and were subjected to evaporation under reduced pressure. The resulting residue containing compound **10a** was purified using column chromatography (eluent: 0-50 %

ethyl acetate in hexane); pale yellow liquid; ^{1}H NMR (500 MHz, Chloroform-d) δ 8.97 (d, *J* = 7.5 Hz, 1H), 8.22 (d, *J* = 1 .4 Hz, 1H), 7.95-7.86 (m, 1H), 5.4 (q, *J* = 6.3 Hz, 2H), 4.86 (q, *J* = 5.9 Hz, 2H), 2.5 (s, 3H), 1.93 (t, *J* = 6.3 Hz, 3H), 1.22 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 178.9, 176.9, 165.8, 143.7, 137.7, 130.4, 126.5, 121.9, 117.8, 100.7, 61.4, 48.6, 15.7, 14.6, 13.6; APCI-MS: 326.8 (M^{+}+1).

Ethyl 1-Benzyl-7-chloro-2-(methylsulfonyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (10b)

It was prepared utilising the method for the preparation of **10a** using benzyl isothiocyanate (4.90 gm, 32.9 mmol); pale yellow liquid; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.99 (d, *J* = 7.5 Hz, 1H), 7.62 (d, *J* = 1.4 Hz, 1H), 7.36 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.28 (s, 5H), 5.43 (s, 2H), 4.19 (q, *J* = 5.9 Hz, 2H), 2.43 (s, 3H), 1.26 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 178.9, 167.5, 165.8, 140.9, 137.6, 136.9, 130.4, 128.5, 128.4, 128.0, 126.5, 120.6, 118.3, 100.3, 61.4, 54.7, 15.7, 14.6; APCI-MS: 388.0 (M^{+}+1).

Ethyl 9-Ethyl-7-chloro-3-hydroxy-4-oxo-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (12a)

To a solution of **10a** (1.30 g, 4.00 mmol) in dichloromethane maintained at 0°C, *m*- chloroperoxybenzoic acid (1.18 g, 6.89 mmol) was added under stirring and the stirring was continued at the same temperature for 4-5 h. The mixture was then warmed

up to the room temperature and allowed to stir for another 10-12 h. The reaction mixture was then neutralised using 3% sodium bicarbonate solution (10 ml) and washed with a brine solution. The organic layer was separated, dried over sodium sulfate and evaporated under reduced pressure to afford compound **11a** as a white amorphous powder. This product was then subjected to further reaction without purification. In a round bottom flask, a mixture of compound **11a**, ethyl mercaptoacetate (0.3 g, 2.58 mmol), triethylamine (1.2 ml, 8.0 mmol) in absolute ethanol (30ml) was refluxed for 5 h under argon atmosphere. The solvent was evaporated to dryness under reduced pressure, water (20ml) was then added and the resulting solid was filtered out. Further purification was achieved by recrystallization from ethanol to give compound **12a** as a highly viscous yellow liquid; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.95 (d, *J* = 7.3 Hz, 1H), 7.37 – 7.32 (m, 2H), 5.02 (s, 1H, OH), 4.37 (q, *J* = 5.9 Hz, 2H), 4.05 (q, *J* = 6.2 Hz, 2H), 1.34 (t, *J* = 5.9 Hz, 3H), 1.29 (t, *J* = 6.3 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 180.8, 180.0, 164.0, 162.0, 143.7, 137.7, 130.4, 126.5, 121.9, 117.8, 111.1, 109.4, 61.4, 48.6, 14.6, 13.6; APCI-MS: 352.1 (M^{+}+1).

Ethyl 9-Benzyl-7-chloro-3-hydroxy-4-oxo-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (12b)

It was prepared utilising the method for the preparation of **12a** using **10b** (1.60 g, 4.00 mmol); highly viscous yellow liquid; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.84 (d, *J* = 7.5 Hz, 1H), 7.53 (d, *J* = 1.6 Hz, 1H), 7.35 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.33 – 7.27 (m,

5H), 5.45 (s, 2H), 5.10 (s,1H, OH), 4.39 (q, J = 5.9 Hz, 2H), 1.34 (t, J = 5.9 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 182.2, 181.2, 165.2, 153.4, 142.1, 138.8, 138.1, 131.6, 129.7, 129.6, 129.2, 127.7, 121.8, 119.5, 111.5, 110.6, 62.6, 55.9, 15.8; APCI-MS: 414.1 (M^++1).

Ethyl 9-Ethyl-3-hydroxy-4-oxo-7-(piperazin-1-yl)-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (13a)

To a mixture of compound **12a** (0.70 g, 2.00 mmol) in DMF (10 ml), 0.86 g (10 mmol) of piperazine was added and the mixture was stirred at 140 ^{0}C for 15-18 h. It was then brought up 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 recrystallisation from ethanol; pale yellow solid; mp > 250 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.78 (d, J = 7.5 Hz, 1H), 6.67 (dd, J = 7.5, 1.5 Hz, 1H), 6.52 (d, J = 1.5 Hz, 1H), 5.4 (s,1H, OH), 4.37 (q, J = 5.9 Hz, 2H), 4.02 (q, J = 6.3 Hz, 2H), 3.50 (t, J = 5.0 Hz, 2H), 3.30 (t, J = 4.9 Hz, 2H), 3.07 (t, J = 5.0 Hz, 2H), 2.93 (t, J = 5.0 Hz, 2H), 1.34 (t, J = 5.9 Hz, 3H), 1.29 (t, J = 6.3 Hz, 3H), 1.21 (s, 1H); ^{13}C NMR (175 MHz, Chloroform-d) δ 179.6, 172.5, 166.5, 156.6, 146.1, 132.5, 113.8, 108.8, 107.8, 106.1, 68.9, 63.4, 61.4, 50.1, 49.2, 45.0, 14.6, 14.3; HR-MS (TOFEI) calcd for $C_{20}H_{23}N_3O_4S$ (401.1410); found (401.1410).

Ethyl 9-Ethyl-3-hydroxy-4-oxo-7-(4-methylpiperazin-1-yl)-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (13b)

Prepared according to the procedure employed for the preparation of compound **13a**, using (0.70 g, 2.00 mmol) of compound **12a** and 1.00 g (10 mmol) of methylpiperazine; yellow solid, mp 245-247 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.79 (d, *J* = 7.4 Hz, 1H), 6.67 (dd, *J* = 7.4, 1.5 Hz, 1H), 6.63 (d, *J* = 1.6 Hz, 1H), 4.8 (s, 1H,OH), 4.37 (q, *J* = 5.9 Hz, 2H), 4.03 (q, *J* = 6.3 Hz, 2H), 3.44 (t, *J* = 5.1 Hz, 4H), 2.35 (t, *J* = 5.1 Hz, 4H), 2.21 (s, 3H), 1.34 (t, *J* = 5.9 Hz, 3H), 1.26 (t, *J* = 6.3 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 176.6,175.9, 162.4, 160.8, 153.4, 142.0, 132.6, 116.8, 116.7, 115.8, 110.2, 103.0, 61.4, 52.5, 50.1, 48.6, 46.0, 14.6, 13.4; HR-MS (TOFEI) calcd for $C_{21}H_{25}N_3O_4S$ (415.1564); found (415.1578)

Ethyl 9-Ethyl-3-hydroxy-4-oxo-7-(morpholin-4-yl)-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (13c)

Prepared utilizing the procedure employed for the preparation of compound **13a**, using (0.70 g, 2.00 mmol) of compound **12a** and 0.87 g (10 mmol) of morpholine; white solid; 238-240 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.97 (d, *J* = 7.5 Hz, 1H), 6.88 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.80 (d, *J* = 1.6 Hz, 1H),5.6 (s, 1H, OH), 4.58 (q, *J* = 5.9 Hz, 2H), 4.24 (q, *J* = 6.3 Hz, 2H), 3.94 (t, *J* = 4.6 Hz, 4H),3.58 (t, *J* = 4.6 Hz, 4H), 1.55 (t, *J* = 5.9 Hz, 3H), 1.48 (t, *J* = 6.3 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 182.0, 181.2,

165.2, 163.3, 154.5, 143.2, 133.8, 118.0, 117.4, 112.3, 110.6, 104.2, 67.0, 62.6, 49.8, 48.4, 15.8, 14.8; HR-MS (TOFEI) calcd for $C_{20}H_{22}N_2O_5S$ (402.1249); found (402.1241).

Ethyl 9-Ethyl-3-hydroxy-4-oxo-7-(pyrrolidin-1-yl)-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (13d)

Prepared utilising the procedure employed for the preparation of compound **13a**, using (0.70 g, 2.00 mmol) of compound **12a** and 0.71 g (10 mmol) of pyrrolidine; white amorphous solid; 241-243 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.71 (d, *J* = 7.5 Hz, 1H), 6.67 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.39 (d, *J* = 1.4 Hz, 1H), 4.64 (s, 1H, OH), 4.37 (q, *J* = 5.9 Hz, 2H), 4.01 (q, *J* = 6.3 Hz, 2H), 3.41 (ddd, *J* = 6.5, 4.2, 2.6 Hz, 4H), 2.08 – 2.00 (m, 4H), 1.34 (t, *J* = 5.9 Hz, 3H), 1.28 (t, *J* = 6.3 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 176.1, 175.8, 162.4, 160.8, 153.6, 141.8, 134.5, 116.7, 114.3, 113.3, 110.2, 100.8, 61.4, 51.2, 48.6, 26.0, 14.6, 13.7; HR-MS (TOFEI) calcd for $C_{20}H_{22}N_2O_4S$ (386.1300); found (386.1319).

Ethyl 9-Benzyl-3-hydroxy-4-oxo-7-(piperazin-1-yl)-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (13e):

Prepared according to the procedure employed for the preparation of compound **13a**, using (0.82 g, 2.00 mmol) of compound **12b** and 0.86g (10 mmol) of piperazine; off-white solid; mp > 250 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.79 (d, *J* = 7.5 Hz, 1H), 7.34 – 7.27 (m, 5H), 6.89 (d, *J* = 1.4 Hz, 1H), 6.67 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.20 (s, 1H,

OH), 5.40 (s, 2H), 4.37 (q, *J* = 5.9 Hz, 2H), 3.57 (t, *J* = 5.0 Hz, 2H), 3.30 (t, *J* = 5.1 Hz, 2H), 3.15 (t, *J* = 5.1 Hz, 2H), 2.89 (t, *J* = 5.0 Hz, 2H), 1.36 (s, 1H), 1.34 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR (175 MHz, Chloroform-d) δ 182.0, 181.2, 165.3, 154.1, 153.4, 141.9, 138.1, 134.0, 129.7, 129.5, 129.3, 118.2, 116.0, 111.5, 110.6, 104.6, 62.6, 55.9, 51.3, 46.2, 15.8; HR-MS (TOFEI) calcd for $C_{25}H_{25}N_3O_4S$ (463.1565); found (463.1563).

Ethyl 9-Benzyl-3-hydroxy-4-oxo-7-(4-methylpiperazin-1-yl)-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (13f)

Prepared utilizing the procedure employed for the preparation of compound **13a**, using (0.82 g, 2.00 mmol) of compound **12b** and 1.00 g (10 mmol) of methylpiperazine; brown solid; mp> 250 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.75 (d, J = 7.5 Hz, 1H), 7.34 – 7.25 (m, 5H), 6.69 – 6.63 (m, 2H), 5.45 (s, 2H), 5.01 (s, 1H,OH), 4.37 (q, J = 5.9 Hz, 2H), 3.44 (t, J = 5.2 Hz, 4H), 2.35 (t, J = 5.2 Hz, 4H), 2.26 – 2.18 (m, 3H), 1.34 (t, J = 5.9 Hz, 3H). ^{13}C NMR (175 MHz, Chloroform-d) δ 181.3, 180.0, 170.9, 167.8, 160.1, 147.0, 135.4, 130.4, 128.9, 127.8, 126.7, 125.4, 125.3, 119.6, 114.2, 103.7, 62.6, 60.2, 46.0, 35.2, 15.1, 14.2; HR-MS (TOFEI) calcd for $C_{26}H_{27}N_3O_4S$ (477.1722); found (477.1705).

Ethyl 9-Benzyl-3-hydroxy-4-oxo-7-(morpholin-4-yl)-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (13g)

Prepared utilizing the procedure employed for the preparation of compound **13a**, using (0.82 g, 2.00 mmol) of compound **12b** and 0.87 g (10 mmol) of morpholine; white solid; 238-240 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.77 (d, *J* = 7.5 Hz, 1H), 7.26 – 7.16 (m, 5H), 6.64 (d, *J* = 1.4 Hz, 1H), 6.58 (dd, *J* = 7.5, 1.4 Hz, 1H), 5.40 (s, 2H), 4.61 (s, 1H, OH), 4.28 (q, *J* = 5.9 Hz, 2H), 3.64 (t, *J* = 4.5 Hz, 4H), 3.28 (t, *J* = 4.5 Hz, 4H), 1.25 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR (175 MHz,Chloroform-d) δ 180.8, 180.0, 164.0, 152.9, 152.2, 140.7, 136.9, 132.8, 128.5, 128.4, 128.0, 116.9, 114.8, 110.3, 109.4, 103.4, 65.8, 61.4, 54.7, 47.2, 14.6; HR-MS (TOFEI) calcd for $C_{25}H_{24}N_2O_5S$ (464.1405); found (464.1436).

Ethyl 9-Benzyl-3-hydroxy-4-oxo-7-(pyrrolidin-1-yl)-4,9-dihydrothieno[2,3-b]quinoline-2-carboxylate (13h)

Prepared utilising the procedure employed for the preparation of compound **13a**, using (0.82 g, 2.00 mmol) of compound **12b** and 0.71 g (10 mmol) of pyrrolidine; white amorphous solid; 245-246 ^{0}C; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.87 (d, *J* = 7.5 Hz, 1H), 7.37 (d, *J* = 1.4 Hz, 1H), 7.33 – 7.28 (m, 5H), 7.14 (dd, *J* = 7.5, 1.4 Hz, 1H), 5.52 (s, 2H), 4.78 (s,1H,OH), 4.37 (q, *J* = 5.9 Hz, 2H), 2.07 – 1.98 (m, 2H), 1.73 – 1.61 (m, 4H), 1.58 (dddd, *J* = 12.3, 8.7, 6.2, 3.0 Hz, 2H), 1.34 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR (175 MHz,

Chloroform-d) δ 180.8, 180.0, 164.0, 152.6, 152.2, 140.5, 136.9, 134.7, 128.5, 128.4, 128.0, 113.4, 112.8, 110.3, 109.4, 100.9, 61.4, 54.7, 51.1, 25.9, 14.6; HR-MS (TOFEI) calcd for $C_{25}H_{24}N_2O_4S$ (448.1456); found (448.1450)

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CHAPTER 3

Conclusion and Future Research

3.1 Conclusion and future research

Drugs that act against various strains of bacteria are known as antibacterial agents. Quinolones have been used extensively as antibacterial agents. The very first members of quinolone antibacterial family were eventually phased out of therapeutic system, mainly because of development of bacterial resistance and high toxicity. Successive generations of quinolones include several broad spectrum and potent antibacterials that are currently in use including ciprofloxacin and ofloxacin. However, the emergence of bacterial resistance against quinolone antibacterials is the major challenge for the researchers. Recent works on structure activity relationships of quinolones have revealed that non-fluorinated analogues are more active against antibiotic resistant bacterial species [1-5].

Daneshtalab group has long been involved in quinolone-based research. Various novel quinolone-based compounds have already been synthesized in our group. For this thesis, a patent by Katsumi *et al.* had drawn our attention [6]. As described in Chapter 2 of this thesis, the above researchers focused on the synthesis and biological evaluation of tricyclic quinolones and discovered that compound **A**, a polycyclic fluorinated quinolone, demonstrated good antibacterial activity. Ranjith Garlapati [7], a graduate student in Daneshtalab group, has also attempted working on the synthesis and anticancer evaluation of a series of novel compounds that were structurally related to compound **A**. To expand the structure-bioactivity profile of these tricyclic quinolones, we pursued further study on the structural modifications of compound A. In this context, we prepared a series of non-fluorinated derivatives of polycyclic quinolones and tested them against different bacterial strains. Unfortunately none of the synthesized compounds were active. To

explore the possible cause for the loss of antibacterial activity in this series of compounds, we decided to do molecular docking on DNA gyrase enzyme, a bacterial enzyme that is potentially inhibited by the quinolone class of compounds (please see the details in Chapter 2 of this thesis).

The findings of docking studies were quiet interesting. Our synthesized compounds as well as our standard (Compound **A**) 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 the various domain of the gyrase enzyme, while compound **A** and other active antibacterial quinolones form 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 of activity of these compounds, as discussed in chapter 2 of this thesis.

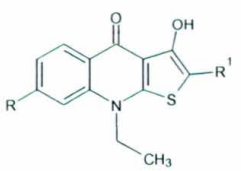
Compound **A**

Fig 3-1: Structure of Compound **A**

Quinolones have a very unique mechanism of action by which they not only inhibit the bacterial growth, but also disrupt the activities of two very essential enzymes, DNA gyrase and topoisomerase IV, resulting in generation of high levels of double-stranded

DNA breaks and bacterial death. Quinolones can selectively target either of these enzymes in different bacteria. This unique feature of quinolones can be viewed as a potential aspect for quinolone-based anticancer agents. There are two main structural requirements for a quinolone to interact with DNA topoisomerases. One of these features is their ability to chelate the Mg^{2+} at the active site and to complex with the ATP binding region. In our research group, Dr. Abeer Ahmed has synthesized various quinolone based molecules, with potential anti-cancer activity. Compounds synthesized by her 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].

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 activities. One of them will include development and synthesis of various carboxylic acid- or carboxamide-based polycyclic quinolones as depicted in **Figure 3-2**. These substituents will definitely help to chelate the Mg^{+2} at the active site of topoisomerase, to a larger extent, as compared with the ester moiety.



Where, R is piperazine/methyl piperazine/Morpholine etc.
and R^1 is COOH / $CONH_2$ etc.

Figure 3-2: Proposed structure of various quinolone based derivatives

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APPENDIX

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