Design, Syntheses and In Vitro Biological Evaluation of Polycyclic Quinolones with Potential Anticancer Activity

By

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Abstract

Cancer has long been known as a dreadful disease characterized by uncontrolled proliferation of tumor cells. Despite many breakthroughs in biotechnological fields and the discovery of various potent anticancer agents the need in chemotherapy is still unmet. Cancer develops by various mechanisms and the most common mechanism involves mutations in genes. Topoisomerase II is critical for DNA replication, transcription and chromosome segregation and is a well validated target of anti-neoplastic drugs including the anthracyclines and epipodophyllotoxins. However, these drugs are limited by common tumor resistance mechanisms and side-effect profiles. Novel topoisomerase II-targeting agents may benefit patients who prove resistant to currently available topoisomerase II-targeting drugs or encounter unacceptable toxicities. Quinolones form an interesting group of molecules that have been intriguing the scientific world in recent years. The last few generations have seen the development of quinolone based drugs. They are well known for their activity against a broad spectrum of bacteria. Drugs such as ciprofloxacin, gatifloxacin, levofloxacin have stormed the clinical world within a short span of time. The last few years have witnessed the development of numerous novel tricyclics and tetracyclic quinolones that target mammalian topoisomerase I and II. The starting point of this research was a thiazoloquinolone which was patented by Kyorin/Kyowa-Hallo. This compound exhibited a promising cytotoxic profile in different animal models.

Using Alchemy-3™, the optimum configuration of the patented compound was determined and based on this a linear tricyclic quinolone scaffold was designed and used in our research as a fundamental pharmacophore to synthesize novel molecules with antineoplastic profiles.

This thesis is arranged into four chapters. The first chapter gives a brief history of cancer, quinolones and some of the classic synthetic reactions in the synthesis of 4-quinolones. Chapter 2 provides a review of anticancer quinolones structure-activity relationships and outlines the potential medical uses of quinolones apart from their classic use as antibacterial agents. Chapter 3 describes various aspects of synthetic procedures involved in the preparation of novel quinolone compounds and biological

evaluation methods. Finally, chapter 4 concludes this thesis with a discussion of a possible future work. Thus, a number of thieno[2,3-*b*]quinolones and the corresponding furoquinolones have been synthesized adapting conventional methods. The cytotoxicity screening of the target compounds was performed against HeLa and Kb cell lines. Unfortunately, the compounds have not shown reasonable activity.

Acknowledgement

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List of Abbreviations

AcOEt	Ethyl acetate
Bel-7402	Human hepatocellular carcinoma
BGC-823	Human gastric cancer cell lines
CoMFA	Comparative molecular field analysis
DHFR	Dihydrofolatereductase
DCM	Dichloromethane
DIAD	Diisopropylazodicarboxylate
DMEM	Dulbecco modified Eagle's minimal essential medium
DMF	Dimethylformamide
ENO	Enoxacin
FDA	Food & Drug Administration
FBS	Fetal Bovine Serum
FLU	Flumequine
GC-MS	Gas chromatographic mass spectrometry
Hela	Human cervical cancer cell line
HL-60	Human leukemic cancer cell line
IUPAC	International union of pure and applied chemistry
LC-MS	Liquid chromatographic mass spectrometry
MCPBA	*m*-Chloroperoxybenzoic acid
M+	Molecular ion
MeOH	Methanol
MIC	Minimum inhibitory concentration
min	Minute
ml	Milliliter
mmol	Millimole
mp	Melting point
MS	Mass spectrum
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWt	Molecular weight
NaH	Sodium hydride

NAL	Nalidixic acid
NaSH	Sodium hydrogen sulfide
NMR	Nuclear magnetic resonance
NOR	Norfloxacin
OD	Optical density
OFL	Ofloxacin
OXO	Oxolinic acid
$P(Ph)_3$	Triphenylphosphine
QSAR	Quantitative structure activity relationship
RNA	Ribonucleic acid
RT	Room temperature
SAR	Structure-Activity Relationships
t-Bu	*tert.* Butyl
TEA	Triethylamine
THF	Tetrahydrofuran
topo II	Topoisomerse II

Chapter 1

Introduction

History

One of the major causes of mortality in humans on earth is cancer. In 2007, approximately 29.6% of deaths were caused by cancer making cancer as the leading cause of mortality (Figure 1.1). It is estimated that 186,400 people will be affected by cancer and 75,700 deaths will occur in Canada in 2012 [1]. Generations of research have accumulated vast amount of data with no success in cure for this dreadful disease. Rapid advances in bio-medical techniques and investing considerable time into this domain have led to considerable progress against cancer in recent years.

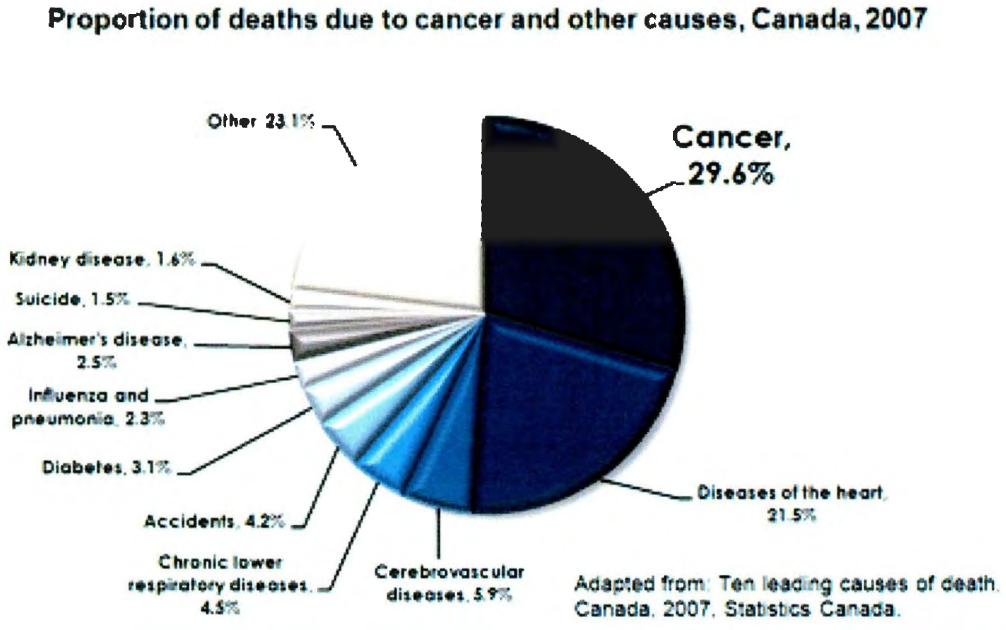


Figure 1.1. Piechart of Cancer Statistics

Although the idea of "chemotherapy", by Paul Ehlrich , came up in the early 19th century, it was not until World War II that this therapeutic approach began to be utilized in the treatment of cancer. The mustard gas explosions at Bari Harbour and its aftermath led researchers to develop numerous less toxic and more tolerable derivatives for the treatment of leukemias and lymphomas [2]. After a few years of research in this area it turned out that apart from alkylating agents (e.g.,- nitrogen mustards and its

derivatives), folic acid, an integral part of cell development, was found to have inhibitory effect on proliferation of leukemic cells in childhood [3]. With this development, efforts have been intensified in this field and with the progression of screening and testing models a vast number of promising drugs with wide spectrum anti-tumour activity have thronged the therapeutic world. Archetypically, anti-cancer drugs are categorized as chemotherapeutic, hormonal therapeutic and immunotherapeutic agents (Table 1.1). Drugs under these categories are classified according to their chemical structure and mechanism of action [4]. Chemotherapeutic agents include: 1) Alkylating agents, which act by cross-linking DNA and thus blocking cell division; 2) Antimetabolites, which act by inhibiting the synthesis of precursor molecules essential for cell division; 3) Antimitotics, which act by hindering chromosomal arrangement required for cell division; 4) Antibiotics, possessing any of the above mentioned functions; 5) Topoisomerase inhibitors; and 6) Others, which expanded so much that this classification is no longer useful.

Hormonal therapy includes all the steroid drugs (anti-estrogens, anti-androgens, anti-aromatase agents, LH-RH analogs, etc.). Immunotherapy includes interferons, interleukins and vaccines.

Table 1.1. Classical classification of anticancer drugs

Chemotherapy	
	Alkylating agents
	Antimetabolites
	Mitosis inhibitors
	Antibiotics
	Topoisomerases inhibitors
	Others
Hormonal therapy Steroids	
	Anti-estrogens
	Anti-androgens
	LH–RH analogs
	Anti-aromatase agents
Immunotherapy	
	Interferon
	Interleukin 2
	Vaccines

The beauty of chemotherapeutic agents is their target specificity and specific modes of action. One of the prominent members of chemotherapeutic agents is "DNA Topoisomerase Inhibitors and Poisons". Generally, these molecules interact with either Topoisomerase-I or –II at their catalytic or DNA-binding domains, stabilizing a cleavable ternary DNA-enzyme complex that does not permit the resealing of nicked DNA [5,6]. Recently, 4-oxo-1,4-dihydroquinolone-3-carboxylic acids (quinolones) that interact with human as well as bacterial topoisomerases have attracted medicinal chemists' attention as potential anticancer agents.

1.2. Molecular Targets of Anticancer Quinolones

Bicyclic core structured quinolones have long been known for their antibacterial activity [7, 8]. The mode of action by which quinolones act on cancer cells was unknown initially. Decades of research into antibacterial quinolones has generated some vital aspects of their cellular targets and modes of action. Studies have shown that DNA gyrase (Gyr) and DNA topoisomerase IV (Topo 4) are the primary targets through which they exert their antibacterial activity in prokaryotes [9]. DNA topoisomerases are vital to maintaining the helical structure of DNA and are present in all prokaryotic and eukaryotic organisms.. Due to the intertwined nature of its double helical structure, during the replication process DNA becomes distorted ahead of the replication fork. The distortion of DNA develops tension within the coil that if left unabated it causesa halt in replication. DNA topoisomerases bind either to single-stranded or double-stranded DNA[10]. Depending upon the nature of binding either tosingle or double strand DNA, topoisomerases are divided into two types : topoisomerase I and topoisomerase II. The enzymes that cleave only one strand of DNA are confined under type I and those enzymes that cleave two strands of the DNA are defined as type II. For many years, following the discovery of topoisomerase II, it was believed that there is only a single isozyme existed and as of this writing, it is still the same script for lower eukaryotes, such as *yeast* [11-14] and *Drosophila* [15]. But further studies have later demonstrated the

existence of a second isoform of the enzyme in mammalian cells [16, 17]. Table 1.2 [10] presents an overview of the classification of Topoisomerases.

Table 1.2. Classification of DNA Topoisomerases [10]

Topoisomerase[a] subfamily	**Predominantly studied members**	**Subunit Structure**	**Size (S) (aa)[b]**
IA	Bacterial DNA topoisomerases I & III (*E.coli*)	Monomer	865 and 653
	Yeast DNA topoisomerase III (*S. cerevisiae*)	Monomer	656
	Mammalian DNA topoisomerase IIIα & β	Monomer	1001 and 862
IB	Eukaryotic DNA topoisomerase I (*human*)	Monomer	765
	Poxvirus DNA topoisomerase (*vaccinia*)	Monomer	314
	Hyperthermophilic eubacterial DNA topoisomerase V (*Mathanopyris kandleri*)[c]	Monomer	----

IIA	Eubacterial DNA gyrase (*E. coli*)	A_2B_2 heterotetramer	Gyr A (875), Gyr B (804)
	Eubacterial DNA topoisomerase IV (*E. coli*)	C_2E_2 heterotetramer	ParC (752), ParE (630)
	Yeast DNA topoisomerase II (*S. cerevisiae*)	Homodimer	1428
	Mammalian DNA topoisomerase IIα & IIβ (human)	Homodimer	1531 & 1626
IIB	Archael DNA topoisomerase VI (*Sulfolobus shibatae*)	A_2B_2 heterotetramer	A(389), B(530)

[a]The source of the most extensively studied family member is given in parenthesis. The top portion of the table lists the type I topoisomerases; the bottom portion the type II enzymes.
[b]The subunit sizes are those corresponding to the most extensively studied family member.
[c]Only known representative at present. Probably present in other hyperthermophilic eubacteria.

Thus two isoforms α and β, which can be discerned by their polypeptide molecular masses, have been uncovered. Since the discovery of the structure of DNA, the next breakthrough in biology came in the form of discovery of DNA topoisomerases by James Wang [18]. From there, intensive research has been done into determining the structure of DNA topoisomerases.

During the 1970s and 1980s extensive studies were done on the cellular pharmacology of DNA crosss-linking agents such as nitrogen mustards and other agents. It is during this time that

topoisomerases were discovered to be the targets of anthracyclines and several other classes of anticancer agents. Thus, one of the long standing beliefs that quinolones act on bacterial topoisomerases has come to an end with the appearance of the first report on the *in vitro* interference of some antibacterial quinolones with the eukaryotic DNA replication [19]. With this study organized investigations have been carried out on quinolone selectivity between bacterial and mammalian type II topoisomerases [20-22].

1.2.1. Structure of Human Topoisomerase I, IIα & IIβ

1.2.1.1. Structure of Topoisomerase I (topo I)

Human topo I, the prototype of the type IB topoisomerase family, catalyzes transient changes in the superhelical state of double stranded DNA by breaking one strand of the DNA to allow gyration of one region of the double helical structure relative to another region [23].

Human Top I is a monomeric 765 amino acid (91-kDa) enzyme [24], based on the limited proteolysis studies of human topo I and the crystallographic structure determination of an inactive fragment of the yeast enzyme [25,26].

The protein can be divided into four major regions: the NH_2 terminal, core, linker, and COOH-terminal domains. The NH_2-terminal region of the enzyme is highly charged with few amino acids, and is poorly conserved [26]. The COOH-terminal domain, comprising residues ~713 to 765 contains the essential catalytic Tyr^{723}, and the poorly conserved and positively charged linker region that connects the C-terminal domain to the isolated core domain to reconstitute near full enzyme activity (Figure 1.2).

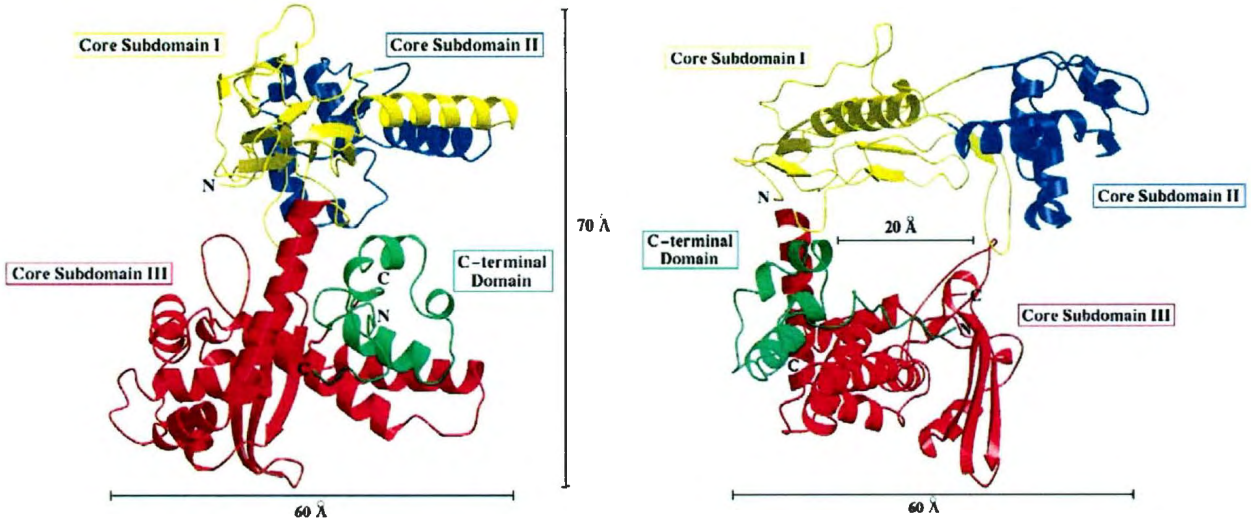


Figure 1.2. The structure of reconstituted human topoisomerase I. (A) Viewed perpendicular to the pore. Core subdomains I, II, and III are shown in yellow,blue, and red, respectively, and the COOH-terminal domain is shown in green. The enzyme consists of a "cap" consisting of core subdomains I and II (with the extended nose-cone helices facing to the right), and the closely associated complex of core subdomain III and the COOH-terminal domain at the bottom. (**B**) A view of the enzyme down the pore, rotated 90° about the vertical axis relative to (A), and with the domains colored as indicated above. The central pore of the molecule varies from 15 to 20 Å in diameter and provides an extensive, highly positively charged binding region for the substrate DNA. The cap of the molecule and core subdomain III contact one another at two lips (residues 367 to 369 of subdomain I and residues 497 to 499 of subdomain III), as shown on the right central part of this figure. **Adopted from M.R. Redinbo et al., (1998). Science, 279 (5356), 1504-1513 [Ref. 25]**

Based on crystallographic studies, topo I is a bi-lobed protein that clamps completely around duplex DNA through protein-DNA phosphate interactions [25]. The core domain of the protein can be further divided into two subdomains I, II, III. Subdomain I consists of residues 215 to 232 and 320 to 433 and is made up of two α helices and nine β strands and subdomain II is made up of residues 233 to 319 and is composed of five α helices and two β strands. Lobe 1, which consists of subdomains I and II fold firmly together and form the top half or "cap" of the enzyme. The second lobe (core subdomain III, which runs from residue 434 to 635 and is a disordered arrangement of 10 α helices and 3 β strands, the linker, and the C-terminal domain) sits below the DNA and contains the catalytic residues implicated in the strand cleavage and religation reactions (Figure 1.2. A). Subdomains I and III interact via two short

"lips" opposite from these helices. Because, to date there is no free enzyme crystal structure available, all the examinations are done on the available crystal structures of human topo I bound DNA. Thus, little is known about the conformation of the DNA-free enzyme or the nature of the conformational changes that accompany the DNA binding. But crystallographic examination of the enzyme bound DNA reveals that, in order for the DNA to dissociate from the enzyme, subdomain I and subdomain II must move apart from Subdomain III and the C-terminal domain. [25, 26].

1.2.1.2. Structure of Topoisomerase II (topo II)

As described previously, topo II alters DNA topology by a double-stranded DNA passage mechanism. To date there is no crystallographic data available for either of these full length isozymes. Human cells express two distinct type II topoisomerase isozymes, designated topoisomerase IIα (topo IIα, 170 kDa form) and topoisomerase IIβ (topo IIβ, 180 kDa form). The latter isozyme has been discovered in 1987 [27]. These proteins share the same catalytic cycle. Alignment of the sequences of the type IIα subfamily are based on their homology with *E. coli* DNA gyrase [28], and is divided into three distinct domains (Figure 1.3). [10, 29-33]. The N-terminal half of the GyrB and the corresponding human DNA topoisomerases contain the ATPase domain. The central domain of the enzyme, which extends up to approximately amino acid 1200, is homologous to the A subunit of DNA gyrase and contains the active site tyrosine residue [10, 31, 33-35]. The C-terminal domain is highly variable and does not correspond with the –COOH domain of the bacterial DNA gyrase [31, 34], however, is believed to play a vital role in the physiological regulation of the enzyme [36]. In the absence of C-terminal domain from the homology comparisons, the sequence identity of the amino acids rises from ~70% to 80% [37-40].

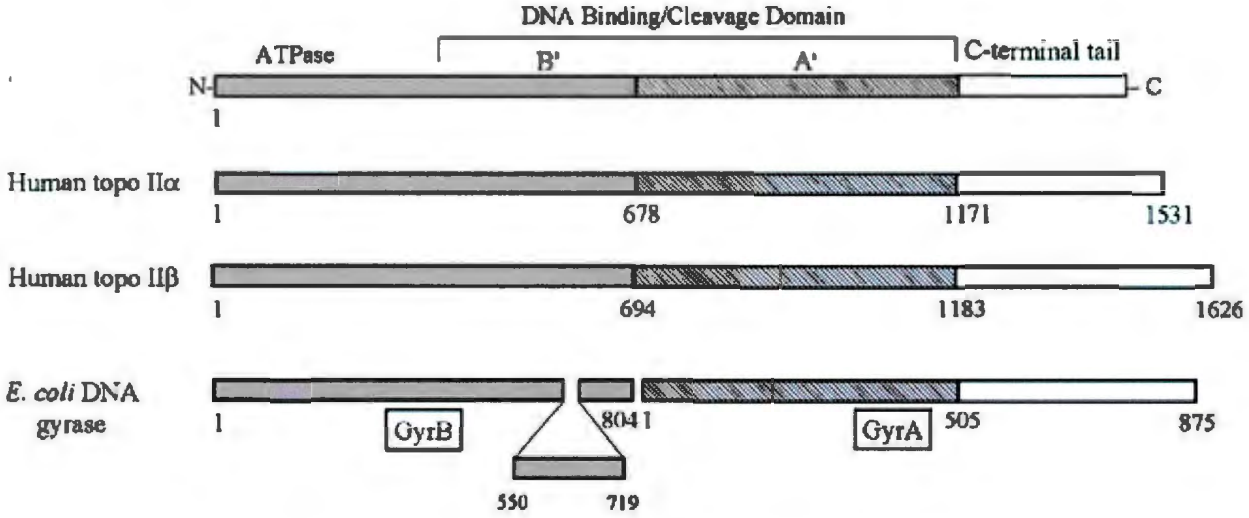


Figure 1.3. Sequence comparisons among type II topoisomerases.

1.2.2. Catalytic Cycle of Topoisomerase II

Due to its role, which maintains the integrity of the mammalian cell (in general) and its involvement in progression of cancer, it is essential to understand the function of the topo II enzyme and its biological properties. Enzymological characteristics and physiological regulation probably have a direct correlation with the cellular roles of topo IIα and IIβ. During normal cell growth cycles the concentrations of the topo IIα and IIβ remain constant [41]. However, the concentration of the α isozyme is considered to increase by 2-3-fold during G_2/M phase [42-46] and is higher in rapidly profilerating cells than it is in quiescent populations [41-48]. Intensive research over the past decade on the topo II enzyme has resulted in the postulation of schematic steps on the mechanism of double-stranded DNA passage reaction [47-51]. Discrete steps of the topo II catalytic cycle are shown in Figure 1.4, which shows one cycle of enzyme activity and is briefly described as follows:

1.2.2.1. Binding

Initiation of the catalytic cycle starts with the binding of topo II to its DNA substrate. This stage does not require a cofactor [52, 53]. Nucleotide sequence and the conformation of the double helix structure govern this interaction. The ability of the enzyme to distinguish the conformation of the DNA

overlies on its recognition of points of helix-helix juxtaposition [54, 55]. This stage of the interaction occurs at specific nucleotide sequences that defines the enzyme-mediated DNA cleavage [55, 56]. The mechanism by which topo II locates DNA nucleic acid sequences in unknown.

1.2.2.2. Cleavage/Religation

In the presence of a divalent cation, topo II establishes anequilibrium between DNA cleavage/religation [57, 58, 59]. During the cleavage reaction, the enzyme generates co-ordinated breaks on opposite strands of DNA and leaves a four-base 5'-overhang on each nucleic acid strand [58, 59]. During this stage the enzyme maintains the structural integrity of the DNA substrate by forming a "*cleavage complex*" covalent bonds between 5'-phosphate termini of the DNA and active tyrosyl residue (O^4-phosphotyrosyl linkage) [34, 59, 60].

1.2.2.3. Passage of DNA Strand

Upon binding of its ATP cofactor, topo II undergoes a topological change [60, 61]. Simultaneous to this change, the intact DNA helix bypasses *via* the transient double-stranded break [51]. This forms ATP-bound cleavage complex, which led to a proposal that topo II acts as a 'protein clamp' prior to recycling [62,63]. The triphosphate portion of the ATP also is elemental in the induction of strand passage [64].

1.2.2.4. Post ATP-DNA Cleavage/Religation

Following the portal episode, the enzyme for the second time reaches a DNA cleavage/relegation equilibrium [51]. The cleavage complex formed in the presence of ATP is 2-4 times more sturdy than the one formed prior to ATP binding [51]. (Figure 1.4).

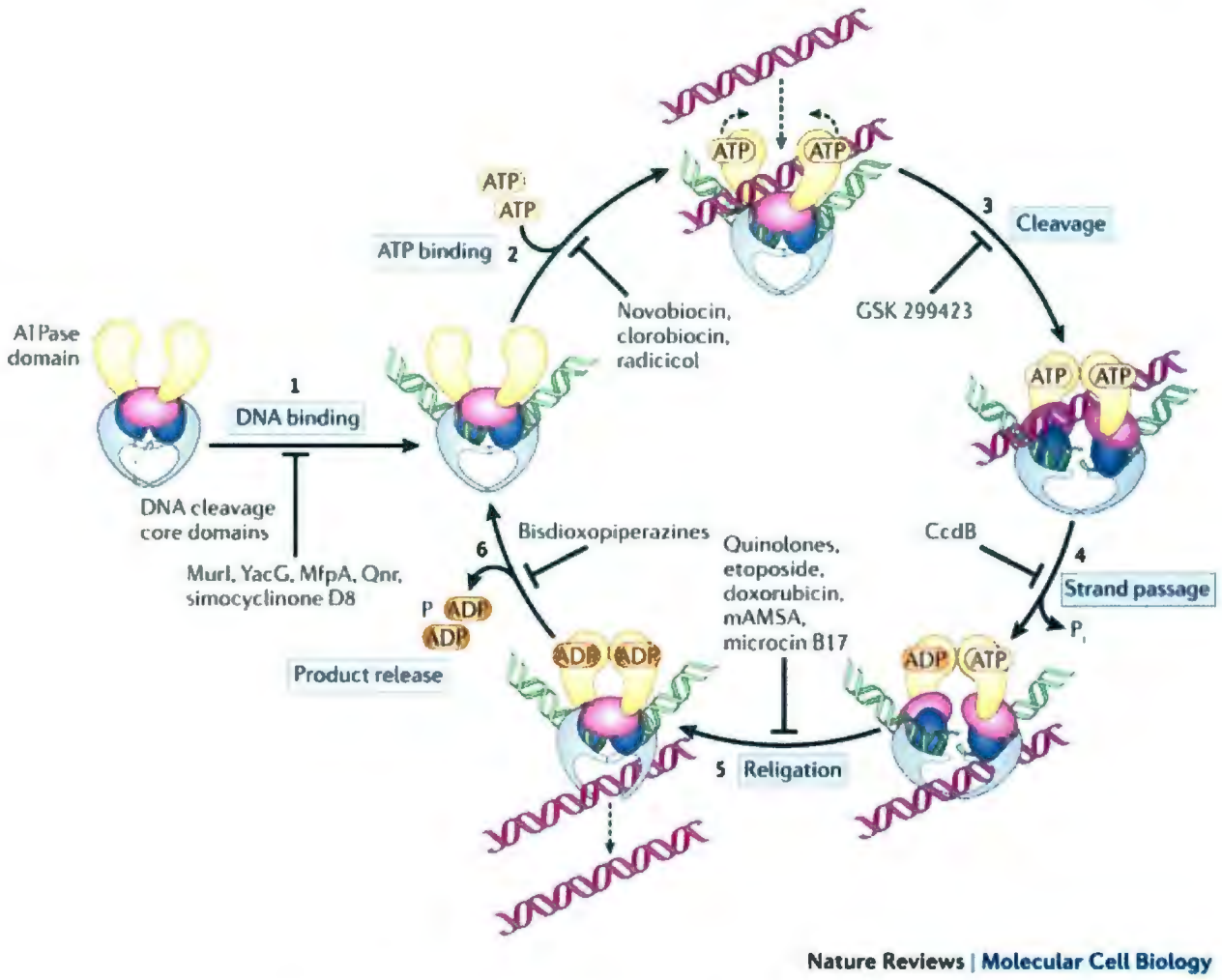


Figure 1.4. Type II Topoisomerase Mechanism [60]

1.2.2.5. Hydrolysis

Ensuing the post-DNA cleavage/religation event, topo II hydrolyzes its Adenosine Tri-phosphate (ATP) cofactor to adenosine di-phosphate(ADP) and orthophosphate [51,64].

1.2.2.6. Enzyme turnover

Finally, cycle 2 is triggered following the ATP hydrolysis, topological state of the enzyme is regenerated to the pre-strand passage stage conformation. Subsequently, the Top II enzyme discharges the resultant DNA product, spontaneously kicks starting a new cycle [51].

1.2.3. Topoisomerase II As Biological Toxin

DNA topoisomerases – "Magicians of the DNA world" as referred by *James C. Wang*- possess special enzymatic capacity to solve various topological problems at various stages of the cell cycle. The human genome is comprised of ~4 billion base pairs of DNA that are cordoned off in 46 chromosomes. Each adult human body has 6.5 feet of DNA stranded in each cell. If all the strands in human body could be strung together, it would stretch 70 times of the distance between the earth and the sun [65]. Due to its tremendous length, DNA is present in a highly conserved state which in its role has to survive various stages of cell cycle: replication, transcription and recombination to enhance the growth of a cell. Topoisomerase II has its own niche in solving various topological problems of the DNA and thus is essential for survival. The topo II catalytic reaction which generates double-stranded DNA breaks are normally reversible [35, 66]. As mentioned before, the concentrations of the topo II enzymes are in higher orders of magnitude in cancer cells. If conditions facilitate the increase in lifetime of the topo II-DNA cleavage complex, the probability of occurrence of numerous mutagenic events such as insertions, deletions, and abnormal recombinations are inexorable [31, 66]. Further, the action of polymerase or helicases to reverse the enzyme-DNA cleavage complex, jeopardizes the normal cell cycle [67, 68]. The indulgence of these proteins converts transient topo II-DNA cleavage complexes to permanent cleavage complexes, which can no longer be sutured. These disrupted cleavage complexes would be the substrates for mutational repair pathways, involving insertions, deletions, production of chromosomal translocations, etc. [69, 71]. The presence of these DNA breaks in high numbers initiates a series of apoptotic events that ultimately terminates the cell [72].

DNA topoisomerase II is also the target of several antineoplastic agents. Despite the structural diversity among topo II-targeted agents, many drugs share a common ground, the ability to bind to the DNA-topo II complex. Topo II inhibition has proved to be a highly successful approach, especially when using doxorubicin and etoposide, for therapeutic intervention. Most of the topoisomerase inhibitors show

selectivity against either topo I or topo II, only a few compounds act on both these enzymes. However, since the interest of this dissertation lies on a specific class of drugs acting as topo II poisons, an overview of the drugs targeting topo II would assist a reader to better understand the concept. Table 1.3 [73] shows few drugs which act as topo II poisons and are still under investigation.

Table 1.3. Investigational Agents which Inhibit Topoisomerase II

Amonifide	Elliptinium	Nitroimidazole
Amsacrine	Epirubicin	Saintopine
Anthrapyrazoles	Ethidium Bromide	Streptonigrin
Aza IQD	Genistein	Suramin
Azatoxin	Intoplicine	Whitangulatin
Bis (2,6-dioxopiperazine)	Makaluvamines	
Bulgarein	Merbarone	
Distamycin	Menogaril	
Ditercalinium	Naphthoquinones	

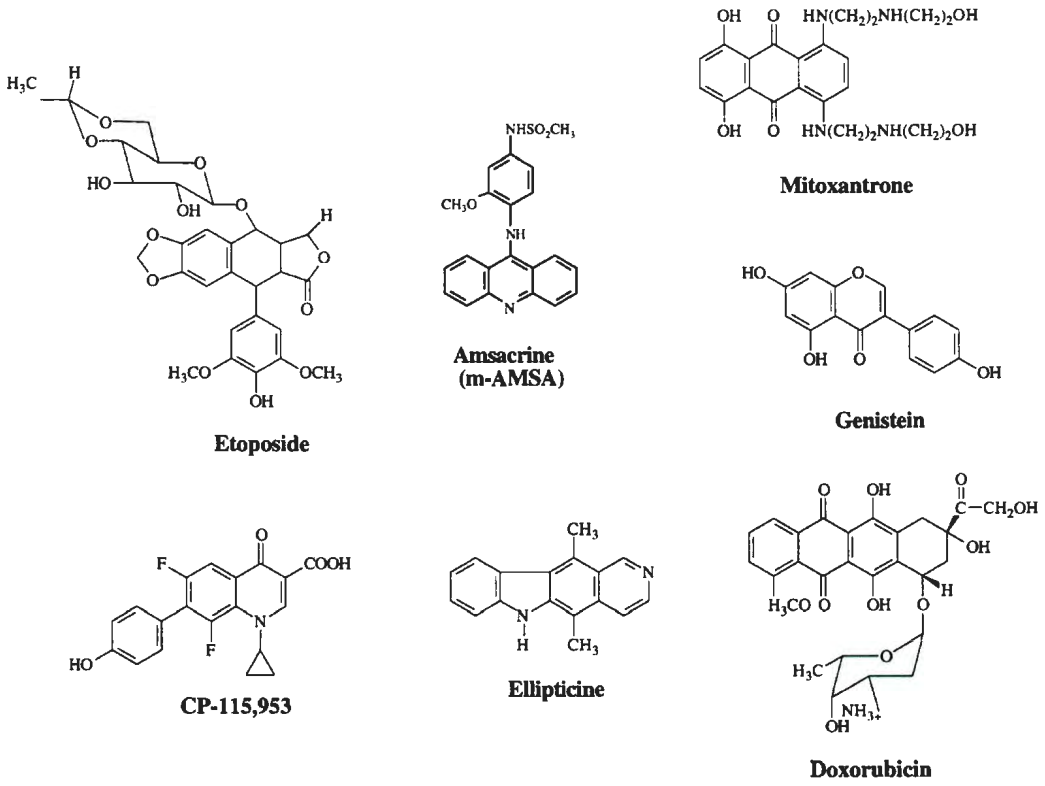


Figure 1.5. Drugs that Target Topoisomerase II [41]

Structures of some of the most promising topo II poisons to date are depicted in Figure 1.5. Especially, etoposide when administered in combination with other agents is the most effective compound against germ line cancers, non-Hodgkins lymphoma and several leukemias [74,75]. The quinolone derivative, CP-115,953 is the most widely investigated drug which has shown inhibitory activity higher than etoposide against any eukaryotic topoisomerase II tested to date [76,77]. As mentioned before, quinolones are known primarily for their antibacterial activity. Common features in the mechanism of action of antibacterial quinolones and antitumor analogs have suggested that both compounds have a similar mode of interaction with topoisomerase II-DNA complexes. Comparative studies between quinolones and epipodophyllotoxin families (etoposide and teniposide) showed that neither of them are DNA intercalators [78]. Further studies carried out between eukaryotes and eubacteria [79], comparing

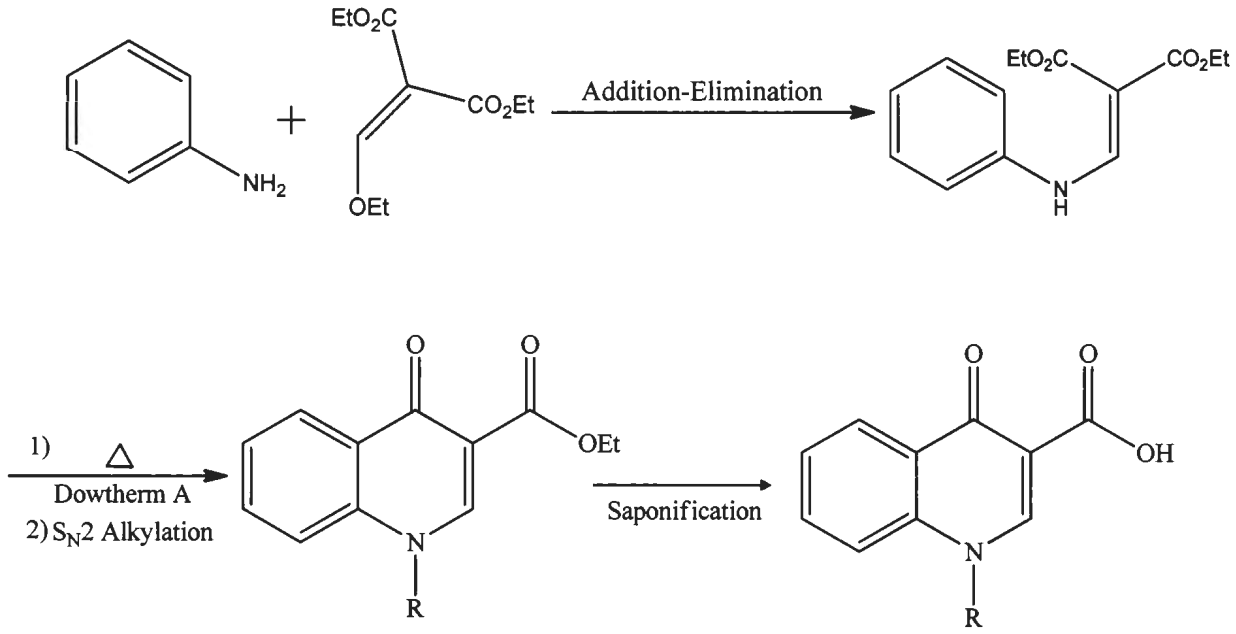
the sensitivity of topoisomerase poisons against each type II topoisomerase enzyme, led to a strong support that a common mode of interaction with DNA-topoisomerase II enzyme complex exists for the quinolone (ciprofloxacin) and etoposide [79,80].

1.3. Synthetic Variations of Quinolones

Decades of intensive research for the fight against resistant bacterial strains has yielded dozens of versatile agents for therapeutic use. Because of the need and desire for addressing the clinical concerns like resistance, toxicity, route of administration, frequency of dose and to increase the spectrum of activity, synthetic routes have been developed which resulted in enormous number of quinolone analogs. The major synthetic routes to the quinolone cores were recently reviewed [81].

1.3.1. Gould -Jacob's Synthesis

This is a prototype based on which a number of variations have been made. This established method (scheme 1.1) relies on an *addition-elimination* reaction. 4-Quinolone is formed during the reaction of substituted anilines with diethyl ethoxymethylenemalonate with heat in Dowtherm A[82].

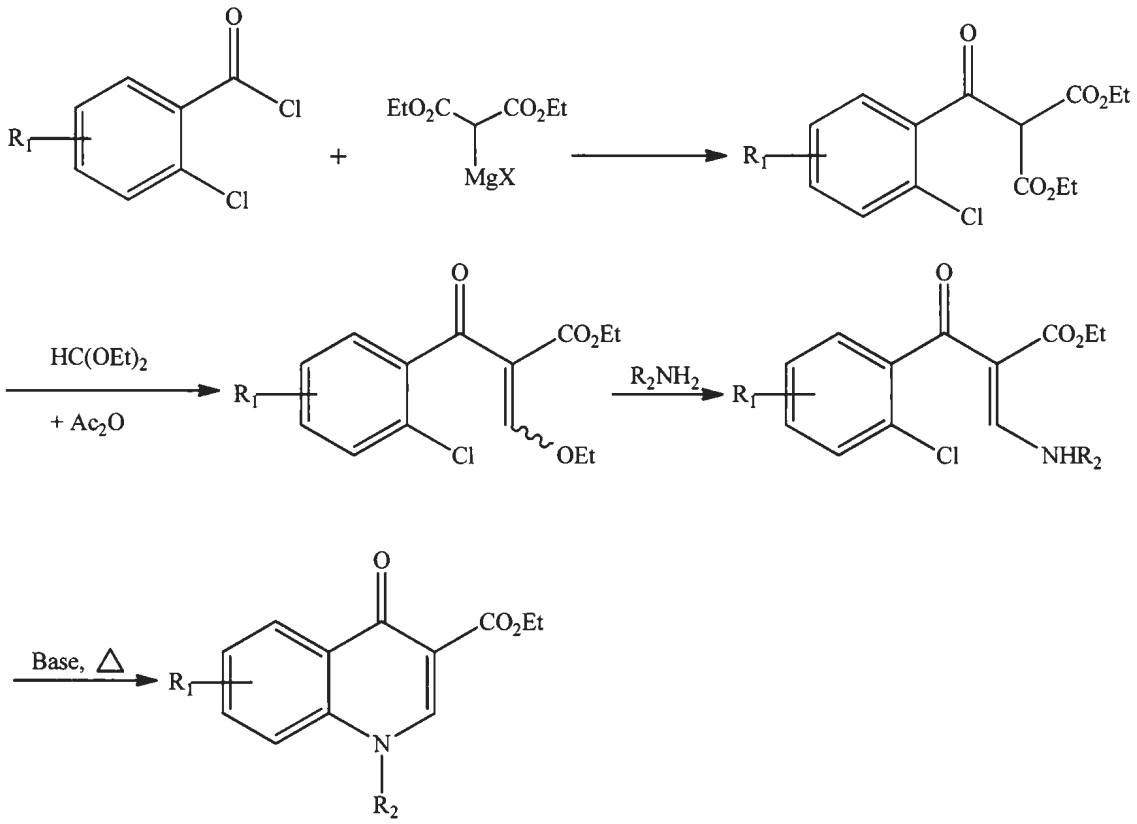


Scheme 1.1. Gould-Jacob's Reaction

Alkylation of the quinolone-4(1*H*)-one scaffold, followed by hydrolysis of the ester group, affords *N*-substituted 4 (1*H*)-quinolone-3-carboxylic acid.

1.3.2. Grohe-Heitzer Reaction

Novel quinolones synthesis has been expanded, to a great extent, by the introduction of the Grohe-Heitzer cycloacylation synthesis [83, 91]. This procedure, as shown in Scheme 1.2, begins with the acylation of malonate derivative with benzoyl chloride to give an acyl malonate intermediate. Condensation of the intermediate with an ortho-ester in the presence of a dehydrating agent such as acetic anhydride affords an enol ether. The enol ether then undergoes an addition-elimination sequence with an amine to afford 3-aryl-2-aminoacrylate. Intramolecular addition-elimination then delivers the quinolone core.

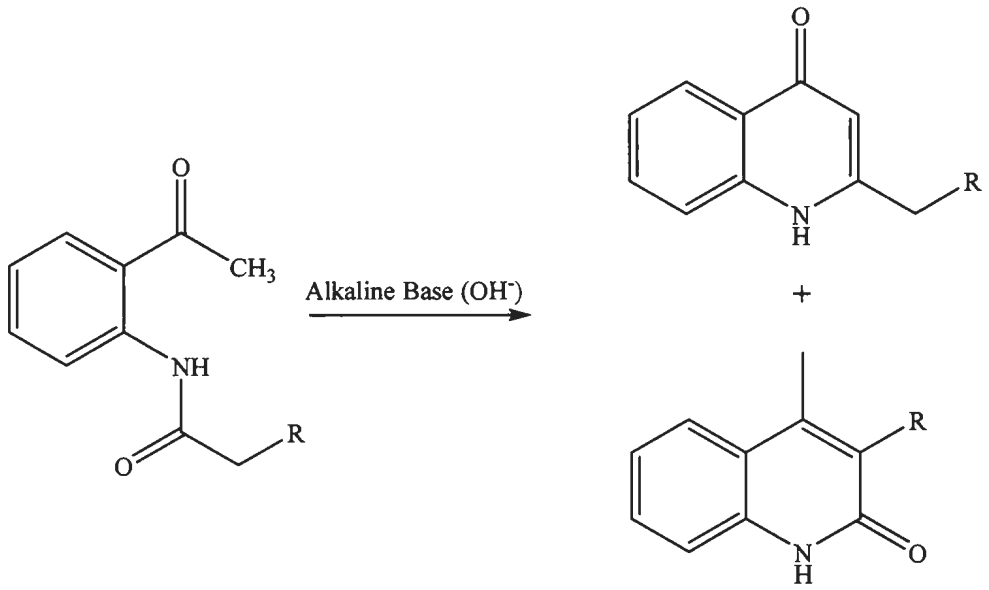


Scheme 1.2. Grohe-Heitzer Reaction

These processes establish the pharmacophoric keto acid moiety of the pyridine ring and allow for introduction of a wide variety of *N*-substituents including those possible with the Gould-Jacobs reaction.

1.3.3. Camps Quinoline Synthesis

Camps Quinoline Synthesis (Camps Cyclization) is a reaction in which an *O*-acylaminoacetophenone affords two scaffolds, 4-hydroxy- and 2-hydroxyquinolines, using an alkaline base as depicted in Scheme 1.3 [84-87].

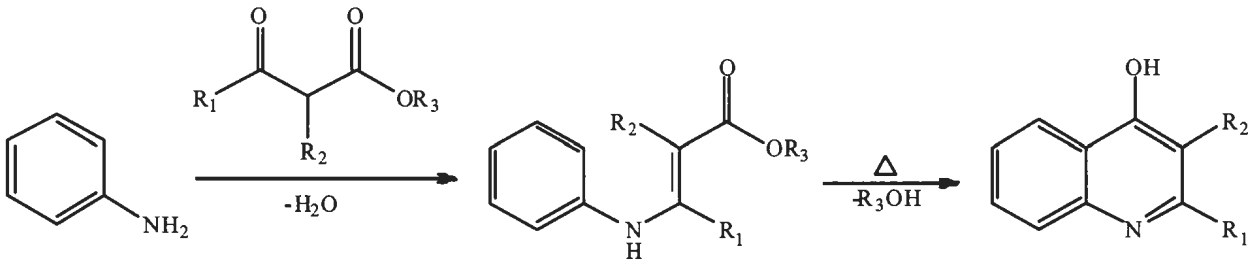


Scheme 1.3. Camps Cyclization

The structure of the starting material and the relative reaction conditions decide the relative proportions of the hydroxyquinolines. It is believed that the keto form predominates in both the solid state and in solution, thus making the product a quinolone [88].

1.3.4. Conrad-Limpach Synthesis

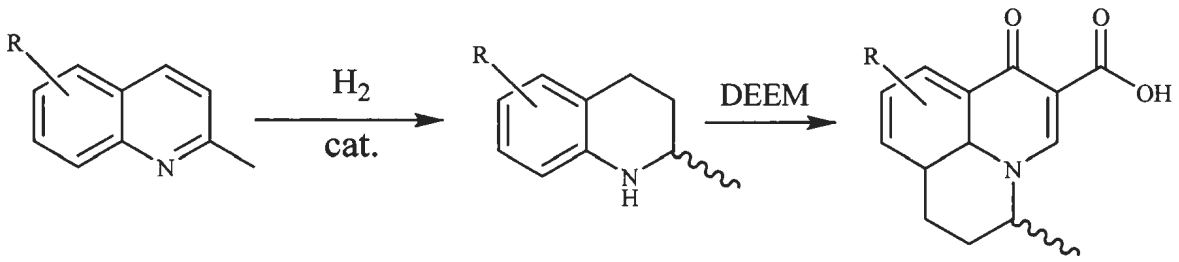
This method involves the reaction of anilines with β-ketoesters to form 4-hydroxyquinolines via a Schiff base [84, 89, 90]. The general reaction procedure is shown in Scheme 1.4.



Scheme 1.4. Conrad-Limpach Synthesis

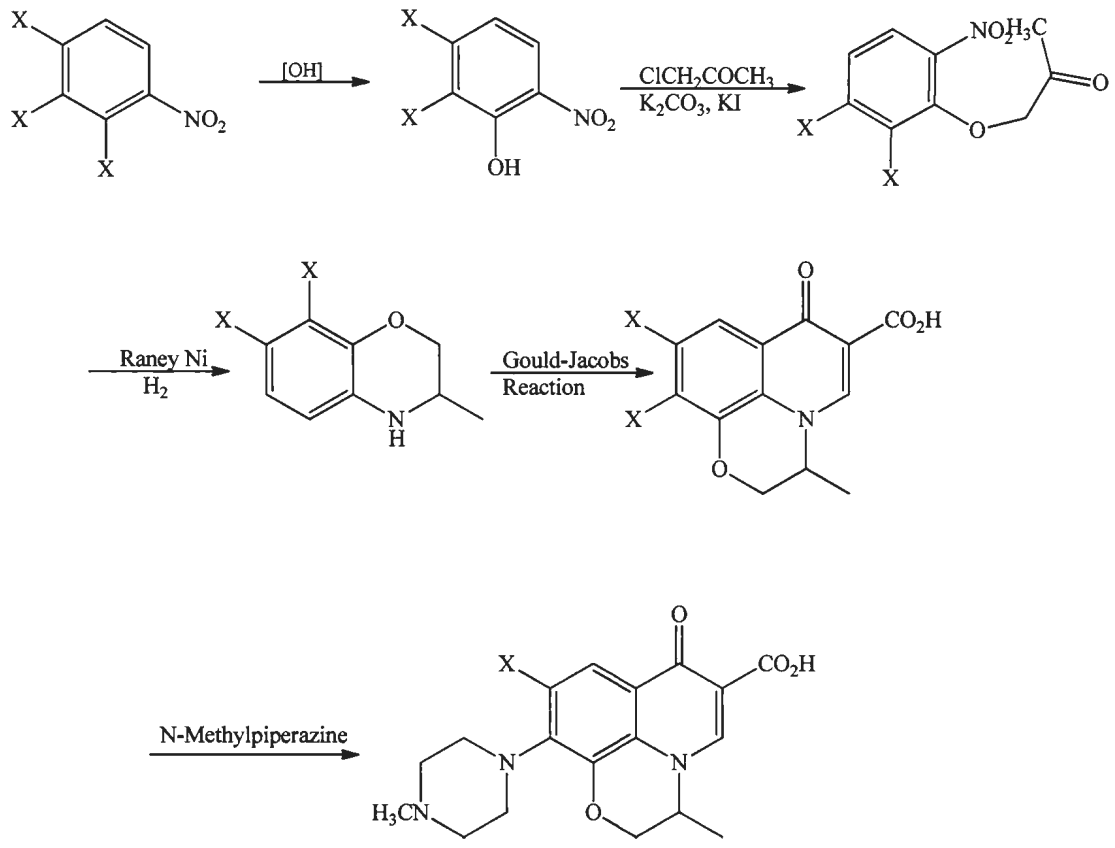
1.3.5. Gerster-Hayakawa Synthesis

This method is a variant of the Gould-Jacobs reaction (Scheme 1.1). **Gerster Synthesis (**Scheme 1.5): This method was initially used to produce the first fluoroquinolone, flumequine. Flumequine is the first chiral member of the class of fluoroquinolones [82, 91]. In Gerster synthesis, a carbocyclic analog is produced using appropriately substituted tetrahydroquinoline scaffold and diethyl (ethoxymethylene) malonate (DEEM) by standard methods..



Scheme 1.5. Gerster Synthesis

Hayakawa Synthesis, [92], (Scheme 1.6): in this variant of the Gerster-Hayakawa synthesis, trihalonitrobenzenes undergo alkaline hydrolysis, specifically occurring at the halogen atom adjacent to the nitro group to afford the corresponding nitrophenol analogs. The 2-oxopropyl ethers of these nitrophenol derivatives were then converted to benzoxazine derivatives by reductive cyclization. Condensation of benzoxazine derivatives with diethyl (ethoxymethylene)malonate (DEEM) under heat affords subsequent intermediate, which upon cyclization and hydrolysis gives the corresponding acids. Exploitation of this method led to the ease of accessibility of oxo or thio derivatives.



Scheme 1.6. Hayakawa Synthesis of the Oxatricyclics

Based on the above discussions, my attention was focused on the design, synthesis and biological evaluation of novel tricyclic quinolones with potential anticancer properties. The following chapters describe the profiles of different series of these novel molecules.

References

1. General Cancer Statistics at a Glance, *www.cancer.ca*.
2. De Vita VT. Principles of cancer management: chemotherapy. In: De Vita VT, Hellmann S, Rosenberg, S. A. (1997). *Cancer, Principles and Practice of Oncology*. 5th edition. Philadelphia, Lippincott-Raven Publishers, 333-347.
3. Farber, S., Diamond, L. K., Mercer, R. D., *et al.* (1948). Temporary Remissions in Acute Leukemia in Children Produced by Folic Acid Antagonists, 4-aminopteroil-glutamic Acid (Aminopterin). *New Engl. J. Med.*, **238**, 787-791.
4. Espinosa, E., Zamora, P., Feliu, J., Barón, M. G. (2003). Classification of Anticancer Drugs-A New System Based on Therapeutic Targets. *Cancer Treatment Reviews*, **29** (6), 515-523.
5. Takimoto, C. H. Topoisomerase interactive agents. In: DeVita, V.T. Jr., Hellman, S., Rosenberg, S. A. eds. Cancer: *Principles and Practice of Oncology*. (2005). 7th Ed. Baltimore: Lippincott Williams & Wilkins, 375-390.
6. Felix, C. A. Secondary Lekemias Induced by Topoisomerase-targeted Drugs. (1998). *Biochim. Biophys. Acta.*, **1400**, 233-255.
7. Hooper, D.C. and Wolfson, J.S. (1991). Fluoroquinolone Antimicrobial Agents. *New Engl. J. Med.*, **324**, 384-394.
8. Mitsuhashi, S. Fluorinated Quinolones-New Quinolone antimicrobials. In: *Progress in Drug Research* (1992). **38**, 91-148 Birkhauser Verlag, Basle.
9. Brighty, K, E. & Gootz, T. D., Chemistry and Mechanism of Action of Quinolone Antibacterials. In: Andriole V. T. *The Quinolones*. (1989). Academic Press 34-97.

10. Champoux, J. J. (2001). "DNA topoisomerases: Structure, Function, and Mechanism". *Annu. Rev. Biochem.*, **70**, 369–413.

11. Goto, T., and Wang, J. C. (1984) Yeast DNA topoisomerase II is Encoded by a Single Copy, Essential gene. *Cell,* **36**, 1073-1080.

12. Giaever, G., Lynn, R., Goto, T., and Wang, J. C. (1986). The complete nucleotide sequence of the structural genen TOP2 of yeast DNA topoisomerase II. *J. Biol. Chem.* **261**, 12448-12454.

13. Voelkel-Meiman, K., DiNardo, S., and Sternglanz, R. (1986). Molecular Cloning and Genetic Mapping of the DNA Topoisomerase II Gene of *Saccharomyces Cerevisiae*. *Gene*, **42**, 193-199 .

14. Uemura, T., Morikawa, K., and Yanagide, M. (1986). The Nucleotide and Functional Relationships to Other DNA Topoisomerases. *EMBO J.,* **5**, 2355-2361.

15. Wyckoff, E., Natalie, D., Nolan, J. M., Lee, M., and Hsieh, T.S.(1989). Structure of the Drosophila DNA topoisomerase II gene : Nucleotide sequence and homology among topoisomerase II. *J. Mol. Biol.,* **205**, 1-13.

16. Drake,F.H., Zimmerman, J. P., McCabe, F.L., Bartus, H. F., Per, S. R., Sullivan, D. M., Ross, W. E., Mattern. M. R., Johnson, R. K., Crooke, S. T., and Mirabelli, C. K. (1987). Purification of Topoisomerase II from Amsacrine-Resistant P3888 Leukemia Cells : Evidence for Two Forms of the Enzyme. *J. Biol. Chem.,* **262**, 16739-16747.

17. Chung, T. D. Y., Drake, F. H., Tan, K. B., Per, S. R., Crooke, S. T., and Mirabelli, C. K. (1989). Characteristics and Immunological Identification of cDNA Clones Encoding Two

Human DNA Topoisomerase II Isoenzymes. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 9431-9435.

18. Wang, J. C. (1971). Interactions Between DNA and an *Escherichia coli* protein ω. *J. Mol. Biol.*, **55**, 523-526.
19. Hussy, P., Maass, G., Tummler, B., Grosse, F., Schomburg, U. (1986). Effect of 4-Quinolones and Novobiocin on Calf Thymus DNA Polymerase Alpha Primase Complex, Topoisomerases I and II, and Growth of Mammalian Lymphoblasts. *Antimicrob. Agents Chemother.*, **29**, 1073-1078.
20. Hoshino, K., Sato, K., Une, T., and Osada, Y. (1989). Inhibitory Effects of Quinolones on DNA Gyrase of Escherichia coli and Topoisomerase II of Fetal Calf Thymus. *Antimicrob. Agents Chemother.*, **33**, 1816-1818.
21. Akasaka, T., Kurosaka, S., Uchida, Y., Tanaka, M., Sato, K., and Hayakawa, I. (1998). Antibacterial Activities and Inhibitory Effects of Sitafloxacin (DU-6859) and its Optical Isomers Against Type II Topoisomerases. *Antimicrob. Agents Chemother.*, **42**, 1284-1287.
22. Takei, M., Fukuda, H., Yasue, T., Hosaka, M., and Oomori, Y. (1998). Inhibitory Activities of Gatifloxacin (AM-1155), a Newly Developed Fluoroquinolone, against Bacterial and Mammalian Type II Topoisomerases. *Antimicrob. Agents Chemother.*, **42** , 2678-2681.
23. Stewart, L., Redinbo, M. R., Qiu, X., Hol, W. G. and Champoux, J.J. (1998). A Model for the Mechanism of Human Topoisomerase I. *Science*, **279** (5356), 1534-1541.

24. Champoux, J.J. (1990) in DNA Topology and Its Biological Effects, eds, Wang, J. C. & Cozzarelli, N. R.,*Cold Spring Harbor Lab. Press, Plainview, NY*, pp. 217-242.

25. Redinbo, M. R., Stewart, L., Kuhn, P., Champoux, J. J. and Hol, W. G. (1998). Crystal Structures of Human Topoisomerase I in Covalent and Noncovalent Complexes with DNA. *Science*, **279** (5356), 1504-1513].

26. Lue, N., Sharma, A., Mondragon, A., and Wang, J. C. (1995). A 26 kDa Yeast DNA Topoisomerase I Fragment: Crystallographic Structure and Mechanistic Implications. *Structure*, **3**, 1315-1322.

27. Drake, F. H., Zimmerman, J. P., McCabe, F. L., Bartus, H. F., Per, S. R., Sullivan, D. M., Ross, W. E., Matern, M. R., Johnson, R. K., Crooke, S. T., & Mirabelli, C. K. (1987). Purification of Topoisomerase II from Amsacrine-resistant P388 Leukemia Cells. *J. Biol. Chem.*, **262**, 16739-16747.

28. Reece, R. J. (1991). Structure and Function. *CRC Crit. Rev. Biochem. Mol. Biol.*, 335-375.

29. Lynn, R., Giaever, G., Swanberg, S. L., and Wang, J. C. (1986). Tandem Regions of Yeast DNA Topoisomerase II share homology with different subunits of bacterial Gyrase. *Science*, **233**, 647-649.

30. Wyckoff, E., Natalie, D., Nolan, J. M., and Hsieh, T. S.(1989). Structure of the Drosophila DNA Topoisomerase II Gene: Nucleotide Sequence and Homology Among Topoisomerase II. *J. Mol. Biol.*, **205**, 1-13.

31. Corbett, A. H., and Osheroff, N. (1993). When Good Enzymes Go Bad: Conversion of Topoisomerase II to a Cellular Toxin by Antineoplastic Drugs. *Chem. Res. Toxi.*, **6**, 585-597.

32. Tsai-Pflugfelder, M., Liu, L. F., Liu, A. A., Tewey, K. M., Whang-Peng, J., Knutsen, T., Huebner, K., Croce, C. M., and Wang, J. C. (1988). Cloning and Sequencing of cDNA encoding human DNA topoisomerase II and Localization of the Gnen to Chromosome Region 17q21-22. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 7177-7181.

33. Berger, J. M., (1998). Type II DNA Topoisomerases. *Curr. Opin, Struct. Biol,*. **8**, 26-32.

34. Worland, S. T., and Wang, J. C. (1989) Inducible Overexpression, Purification, & Active Site Mapping of DNA Topoisomerases II From the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **264**, 4412-4416.

35. Wang, J. C. (1996). DNA Topoisomerases. *Annu. Rev. Biochem.*, **65**, 635-692.

36. Corbett, A. H., Fernald, A. W., and Osheroff, N. (1993). Protein Kinase C Modulates the Catalytic Activity of Topoisomerase II by Enhancing the Rate of ATP Hydolysis: Evidence for a Common Mechanism of Regulation by Phosphorylation. *Biochemistry*, **32**, 2090-2097.

37. Chung, T. D. Y., Drake, F. H., Tan, K. B., Per, S. R., Crooke, S. T., & Mirabelli, C. K. (1989). Characteristics and Immunological Identification of cDNA Clones Encoding Two Human DNA Topoisomerases II Isozymes. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 9431-9435.

38. Austin, C. A., Sng, J. H., Patel, S., and Fisher, L. M. (1993) Novel HeLa Topoisomerase II is the II-beta Isoform-Complete Coding Sequence and Homology With Other Type II Topoisomerases. *Biochim. Biophys. Acta,* **1172**, 283-291.

39. Austin, C. A., and Fisher, L. M. (1990). Isolation and Characterisation of a Human cDNA Clone Encoding a Novel DNA Topoisomerase II Homologue from Hela Cells. *FEBS Lett.*, **266**, 115-117.

40. Jenkings, J. R., Ayton, P., Jones, T., Davies, S. L., Simmons, D. L., Harris, A. L., Sheer, D., and Hickson, I. D. (1992). Isolation of cDNA Clones Encoding the β Isozyme of Human DNA Topoisomerase II and Localization of the Gene to Chromosome 3p24. *Nucliec Acids Res.*, **20**, 5587-5592.

41. Burden, D. A., and Osheroff, N. (1998). Mechanism of Action of Eukaryotic Topoisomerase II and Drugs Targeted to the Enzyme. *Biochimica et Biophysica Acta*, **1400**, 139-154.

42. Cardenas, M. E., Dang, Q., Glover, C. V., Gasser, S. M. (1992). Casein Kinase II Phosphorylates the Eukaryotic-Specific C-terminal Domain of Topoisomerase II in vivo. *EMBO J.*, **11**, 1785-1796.

43. Heck, M, M., Hittelman, W. N., and Earnshaw, W. C. (1988). Differential Expression of DNA Topoisomerase I and II During the Eukaryotic Cell Cycle. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 1086-1090.

44. Woessner, R. D., Mattern, M. R., Mirabelli, C. K., Johnson, R. K., and Drake, F. H. (1991). Proliferation- & Cell Cycle-Dependent Differences in Expression of the 170 Kilodalton and 180 Kilodalton Forms of Topoisomerase II in NIH-3T3 Cells. *Cell Growth Differ.*, **2**, 209-214.

45. Saijo, M., Ui, M., and Enomoto, T. (1992). Growth State and Cell Cycle Dependent Phosphorylation of DNA Topoisomerase II in Swiss 3T3 Cells. *Biochemistry*, **31**, 359-363.

46. Kimura, K., Sajjo, M., Ui, M., and Enomoto, T. (1994). Growth State and Cell-Cycle Dependent Fluctuation in the Expression of Two Forms of DNA Topoisomerase II and Possible Specific Modification of the Higher Molecular Weight Form in the M Phase. *J. Biol. Chem.*, **269**, 1173-1176.

47. Sullivan, D., Latham, M. D., Ross, W. E. (1987). Proliferation-Dependent Topoisomerase II Content as a Determinant of Antineoplastic Drug Action In Human, Mouse, & Chinese Hamster Ovary Cells. *Cancer Research*, **47**, 3973-3979.

48. Hsiang, Y. H., Wu, H. Y., Liu, L. F. (1988) Proliferation-Dependent Regulation of DNA Topoisomerase II in Cultured Human Cells. *Cancer Research*, **48**, 3230-3235.

49. Osheroff, N., Zechiedrich, E. L., and Gale, K. C. (1991). Catalytic Function of DNA Topoisomerase II. *BioEssays*, **13**(6), 269-275.

50. Watt, P. M., and Hickson, I. D. (1994). Structure and Function of Type II DNA Topoisomerases. *Biochem. J.*, **303**, 681-695.

51. Osheroff, N. (1986). Eukaryotic Topoisomerase II: Characterization of Enzyme Turnover. *J. Biol. Chem.*, **261**, 9944-9950.

52. Osheroff, N. (1987). Role of the Divalent Cation in Topoisomerase II Mediated Reactions. *Biochemistry*, **26**, 6402-6406.

53. Sander, M., Hsieh, T., Udvardy, A., and Schedl, P. (1987). Sequence dependence of *Drosophila* topoisomerase II in Plasmid Relaxation and DNA Binding. *J. Mol. Biol.,* **194**, 219-229.

54. Zechiedrich, E. L., and Osheroff, N. (1990). Eukaryotic Topoisomerase II Recognizes Nucleic Acid Topology by Preferentially Interacting With DNA Crossovers. *EMBO J.*, **9**, 4555-4562.

55. Howard, M. T., Lee, M. P., Hsieh, T. S., and Griffith, J. D. (1991). Drosophila Topoisomerase II-DNA Interactions are Affected By DNA Structure. *J. Mol. Biol.*, **217**, 53-62.

56. Lee, M.P., Sander, M., and Hsieh, T. S. (1989). Nuclease protection by *Drosophila* DNA Topoisomerase II. Enzyme/DNA Contacts at the Strong Topoisomerase II Cleavage sites. *J. Biol. Chem.*, **264**, 21779-21787.

57. Thomsen, B., Bendixen, C., Lund, K., Anderson, A. H., Sorensen, B. S., andWestergaard, O. (1990). Characterization of the Interaction Between Topoisomerase II & DNA by Transcriptional Footprinting. *J. Biol. Chem.*, **215**, 237-244.

58. Liu, L. F., Rowe, T. C., Yang, L., Tewey, K. M., and Chen, G. L. (1983). Cleavage of DNA by Mammalian DNA Topoisomerase II. *J. Biol. Chem.*, **258**, 15365-15370.

59. Sander, M., and Hsieh, T. S. (1983). Double-Strand DNA Cleavage by Type II DNA Topoisomerase From *Drosophila Melanogaster. J. Biol. Chem.*, **258**, 8421-8428.

60. Vos, S. M., Tretter, E. M., Schmidt, B. H., and Berger, J. M. (2011). All Tangled Up: How Cells Direct, Manage & Exploit Topoisomerase Function. *Nature Reviews: Molecular Cell Biology*, **12**, 827-841.

61. Lindsley, J. E., and Wang, J. C. (1991). Proteolysis Patterns of Epitopically Labelled Yeast DNA Topoisomerase II Suggest An Allosteric Transition In the Enzyme Induced By ATP Binding. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 10485-10489.

62. Roca, J., Berger, J. M., and Wang, J. C. (1993). On the Simultaneous Binding of Eukaryotic DNA Topoisomerase II to a Pair of Double-Stranded DNA Helices. *J. Biol. Chem.*, **268**, 14250-14255.

63. Roca, J., and Wang, J. C. (1992). The Capture of a DNA Double Helix By An ATP-Dependent Protein Clamp: A Key Step In DNA Transport by Type II Topoisomerases. *Cell*, **71**, 833-840.

64. Osheroff, N., Shelton, E. R., and Brutlag, D. L. (1983). DNA Topoisomerase II from *Drosophila melanogaster*. Relaxation of super-coiled DNA. *J. Biol. Chem.*, **258**, 9536-9543.

65. Chen, S. (1998). Length of a Human DNA Molecule IN: Elert, G. Length of a Human DNA molecule. *The Physics Factbook.* www.hypertextbook.com/facts/1998/StevenChen.shtml

66. Froelich-Ammon, S. J., Osheroff, N. (1995). Topoisomerase Poisons: Harnessing the Dark Side of Enzyme Mechanism. *J. Biol. Chem.*, **270**, 21429-21432.

67. D'Arpa, P., Beardmore, C., and Liu, L. F. (1990). Involvement of Nucleic Acid Synthesis In Cell Killing Mechanisms of Topoisomerase Poisons. *Cancer Research*, **50**, 6919-6924.

68. Howard, M. T., Neece, S. H., Matson, S. W., and Kreuzer, K. N. (1994). Disruption of a Topoisomerase-DNA Cleavage Complex By DNA Helicase. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 12031-12035.

69. Anderson, H. C., and Kihlman, B. A. (1989). The Production of Chromosomal Alterations in Human Lymphocytes By Drugs Known to Interfere with the Activity of DNA Topoisomerase II. I. *m*-AMSA, *Carcinogenesis*, **10**, 123-130.

70. Han, Y. H., Austin, M. J., Pommier, Y., and Povirk, L. F. (1993). Small Deletion and Insertion Mutations Induced By The Topoisomerase II Inhibitor Teniposide in CHO cells and Comparision with Sites of Drug-Stimulated DNA Cleavage *in vitro*. *J. Mol. Biol.*, **229**, 52-66.

71. Lonn, U., Lonn, S., Nylen, U., and Winblad, G. (1989). Altered Formation of DNA in Human Cells Treated With Inhibitors of DNA Topoisomerase II (Etoposide and Teniposide). *Cancer Research*, **49**, 6202-6207.

72. Hong, Y., Sang, M. Shang, C., Xue, Y. X., and Liu, Y. H. (2012). Quantitative Analysis of Topoisomerase II Alpha and Evaluation of Its Effects on Cell Proliferation and Apoptosis in Glioblastoma Cancer Stem Cells. *Neuroscience Letters*, **518** (2), 138-143.

73. Pommier, Y., Fesen, M. R., and Goldwasser, F. (1996). Topoisomerase II Inhibitors: The Epidophyllotoxins, *m*-AMSA, & the ellipticine Derivatives, In: Chabner, B. A., Longo, D. L. (Eds). Cancer Chemotherapy & Biotherapy : Principles & Practice, Lippincott-Raven, Philadelphia, PA, 435-461.

74. Bishop, J. F. (1992). Etoposide in the Treatment of Leukemias. *Semin. Oncol.*, **19**, 33-38.

75. Nichols, C. R. (1992). The Role of Etoposide Therapy in Germ Cell Cancer. *Semin. Oncol.*, **19**, 72-77.

76. Elsea, S. H., McGuirk, P. R., Gootz, T. D., Moynihan, M., and Osheroff, N. (1993). Drug Features That Contribute To The Activity of Quinolones Against Mammalian

Topoisomerase II And Cultured Cells: Correlation Between Enhancement of Enzyme-Mediated DNA Cleavage *In Vitro* and Cytotoxic Potential. *Antimicrob. Agents Chemother.*, **37**, 2179-2186.

77. Robinson, M. J., Martin, B. A., Gootz, T. D., McGuirk, P. R., Moynihan, M., Sutcliffe, J. A., and Osheroff, N. (1991). Effects of Quinolone Derivatives on Eukaryotic Topoisomerase II: A Novel Mechanism For Enhancement of Enzyme-Mediated DNA Cleavage. *J. Biol. Chem.*, **266**, 14585-14592.
78. Chen, G. L., Yang, L., Rowe, T. C., Halligan, B. D., Tewey, K. M., and Liu, L. F. (1984). Nonintercalative Antitumor Drugs Interfere With The Breakage-Reunion Reaction of Mammalian DNA Topoisomerase II. *J. Biol. Chem.*, **259**, 13560-13566.
79. Sioud, M., and Forterre, P. (1989). Ciprofloxacin and Etoposide (VP16) Produce a Similar Pattern Of DNA Cleavage In a Plasmid Of AN Archaebacterium. *Biochemistry*, **28**(9), 3638-3641.
80. Forterre, P., Nadal, M., Elie, C., Mirambeau, G., Jaxel, C., and Duguet, M. (1986). Mechanism of DNA Synthesis And Topoisomerisation in Archaebacteria- Reverse Gyration in vitro and In vivo. *Syst. Appl. Microbiol.*, **7**, 67-71.
81. Mitscher, L. A. (2005). Bacterial Topoisomerase Inhibitors: Quinolone and Pyridone Antibacterial Agents. *Chem. Rev.*, **105**, 559-592.
82. Gould, R. Jr., and Jacobs, W. A. (1939). The Synthesis of Certain Substituted Quinolines and 5,6-Benzoquinolines. *J. Am. Chem. Soc.*, **61**, 2890.
83. Grohe. K., and Heitzer, H. (1987). Synthese Von 4-Chinolon-3-Carbonsauren. *Liebigs Ann. Chem.*, 29-37.

84. Manske, R. H. F. (1942). The Chemistry of Quinolones. *Chem. Rev.*, **30**, 113-144.

85. Bornstein, J., Reid, W. J., and Torres, D. J. (1954). A Study Of The Camps Synthesis Of 4-Hydroxy-3-Quinolinecarboxylic Acid. *J. Am. Chem. Soc.*, **76**, 2760-2762.

86. Camps, P., Gomez, E., Torrero, D., Badia, A., Vivas, N. M., and Brazil, X. (2001). Synthesis, *in vitro* Pharmacology, and Molecular Modelling of Syn-Huprines As Acetylcholineesterase Inhibitors. *J. Med. Chem.*, **44**, 4733-4736.

87. Koelsch, C. F., and Lucht, F. J. (1949). Camps Reaction With 1-xanthonamine. *J. Am. Chem. Soc.*, **71**, 3556-3558.

88. Jones, C. P., Anderson, K. W., and Buchwald, S. L. (2007). Sequential *Cu*-catalyzed Amidation-Base-Mediated Camps Cyclization: A Two-Step Synthesis of 2-Aryl-4-Quinolones From *O*-Halophenones. *J. Org. Chem.*, **72**, 7968-7973.

89. Reitsema, R. H. (1948). The Chemistry of 4-Hydroxyquinolines. *Chem. Rev.*, **43**, 43-68.

90. Joule, J. A., and Mills, K. (2000). Quinolines & Isoquinolines: Reactions and Synthesis. *Heterocyclic Chemistry*, Blackwell Publishing, 133-134.

91. Gerster, J. F., Rohlfing, S. R., Pecore, S. E., Winandy, R. M., Stern, R. M., Landmesser, J. E.,Olsen, R. A., and Gleason, W. B. (1987). Synthesis, Absolute Configuration, & Antibacterial Activity of 6,7-dihydro-5,8-dimethyl-9-fluoro-1-oxo-1H,5H-benzo[ij]-quinolizine-2-carboxylic Acid. *J. Med. Chem.*, **30**, 839-843.

92. Hayakawa, I., Atarashi, S., Yokohama, S., Imamura, M., Sakano, K., and Furukawa, M. (1986). Synthesis And Antibacterial Activities of Optically Active Ofloxacin. *Antimicrob. Agents Chemother.*, **29**, 164-164.

Chapter 2

Review of Structure-Activity Relationships of Quinolones and Their Uses

2.1. Structure-Activity Relationships (SAR) and Common Clinical Applications of Quinolones

Archetypical antibacterial fluoroquinolones are the cornerstones in defining the SAR of cytotoxic quinolones. Systematic structural modification of prototypic antibacterial quinolones led to the identification of characteristic structural features essential for activity against eukaryotic cells.

One of the major advances in this field occurred with the discovery of *locus classicus*, Norfloxacin. Inclusion of a fluorine at position 6 and a piperazinyl group at position 7 has resulted in broad spectrum antimicrobial activity. Figure 2.1 depicts examples of different generations of antimicrobial quinolones [1].

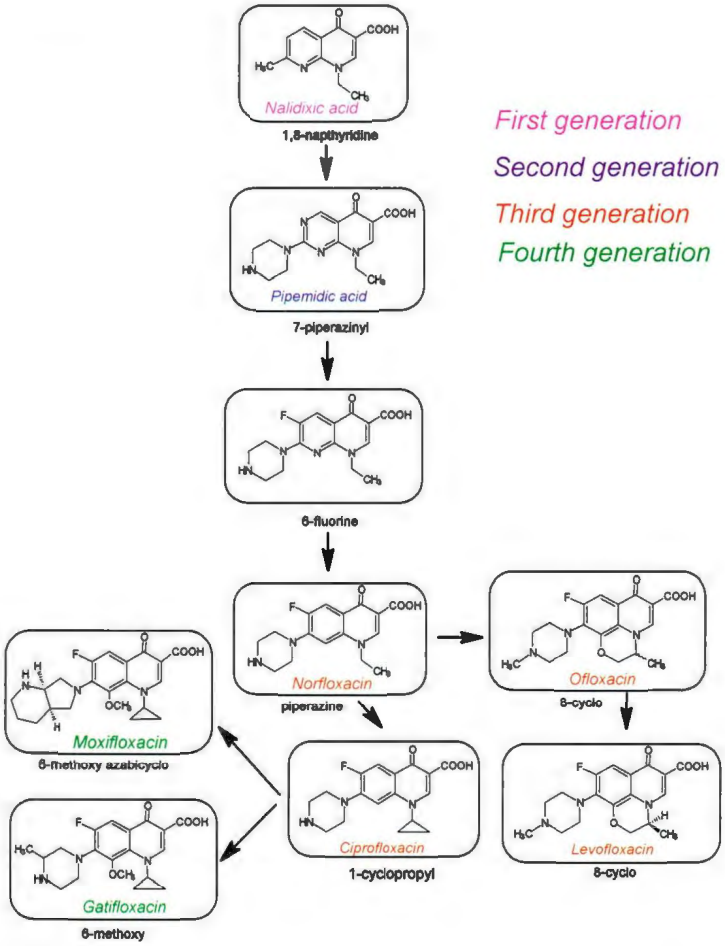


Figure 2.1. Structures and generations of quinolones

Starting from this discovery to Trovafloxacin no single position could be credited to produce anti-cancer activity. In fact, an amalgamation of various substituents at their respective positions have been bestowed to the concomitant cytotoxic activity. These positional modifications of the parent antibacterial quinolones showed that higher potency as well as broader antibacterial spectrum has occurred with the concomitant presence of fluorine atom at C-6 and a suitable heterocyclic base at C-7 [1, 2, 3].

The unique characteristic of bacteria, "fit for survival", has enabled them to multiply indomitably under inappropriate environments [4]. Despite vast research and knowledge data base, only few antibacterial quinolones have been authorized for clinical use. The situation is worse with anticancer quinolones since none is licensed for clinical use. Thus, innovative design and synthesis of anticancer and antibacterial quinolones is vital. Quinolone antimicrobial agents exert their antibacterial action via inhibition of homologous type II topoisomerases, DNA gyrase and DNA topoisomerase IV [5-7]. The structural features of partial quinolone-enzyme-DNA complex have been shown in Figure 2.2 [8-9]. Few findings report that the quinolones cause mutations in the highly conserved sites of homologous type II topoisomerases, also called the quinolone resistance determinin region (QRDR) [10]. Not to forget the presence of active efflux mechanisms that initially allow bacteria to survive [11, 12] and subsequently permits the development of adaptive QRDR mutations at drug target sites. There are no known reports suggesting the presence of enzymes which degrade the fluoroquinolone antibacterial agents. Thus, various quinolones have been developed with different functional groups at various positions to produce a molecule that could stop the resistance threat. Structure-activity relationships investigations have shown that some key substitutions at the quinolone ring are required for an active compound. Since interaction with topoisomerase II is central to the activity of both quinolones and antitumor agents, it is of interest to compare the structure-activity relationships studies relative to antibacterial quinolones. This chapter reviews some of the key aspects of structural requirements for cytotoxic quinolones based on several

investigations performed in relation to the anti-bacterial quinolones. Figure 2.2 shows the positional effect of functional groups on the activity of quinolones.

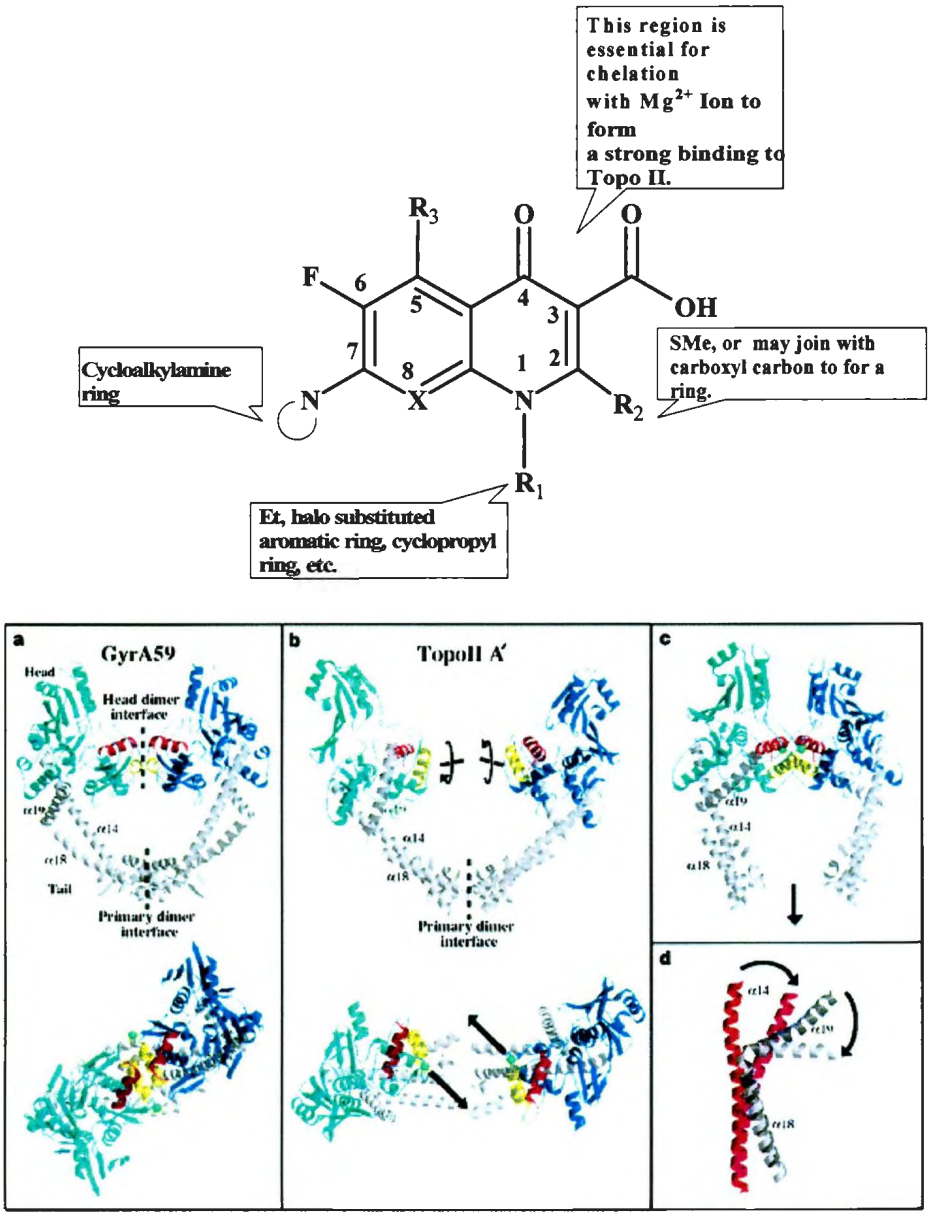
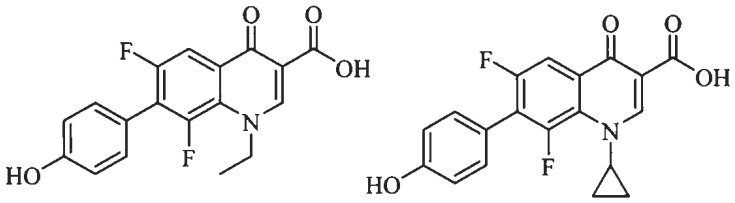


Figure 2.2. Quinolone Skeleton with basic positional properties and Quinolone-DNA complex [9]

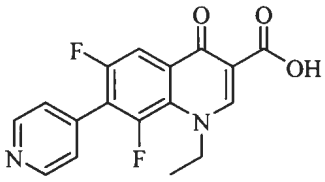
2.2. Structure-Activity Relationships Review

1) According to quinolone anti-bacterial structure-activity relationships (SAR) studies (figure 2.2), N-1 position is an essential part of the enzyme-DNA binding complex [13]. Studies so far indicate that the cyclopropyl group at this position contributes to high potency in this class of compounds [14] with respect to their anti-proliferative activity. Substitution with other groups can presumably lower the number of molecules capable of binding to the enzyme-DNA pocket, and therefore reduce the potency [15, 16]. As mentioned earlier, contribution of the cyclopropyl moiety at the N-1 position to the anti-proliferative activity of quinolones is only possible with the concomitant presence of halogen at C-6 and C-8, which can be accounted for a better interference of quinolones with the eukaryotic topoismomerase II. These observations signify that the identity of the N-1 obtained from the SAR studies on antibacterial quinolone is kept intact for eukaryotic cell cytotoxicity, as well. Figure 2.3 illustrates structures of some antiproliferative quinolones and ciprofloxacin.



CP-67,804

CP-115,953



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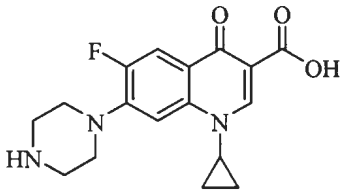
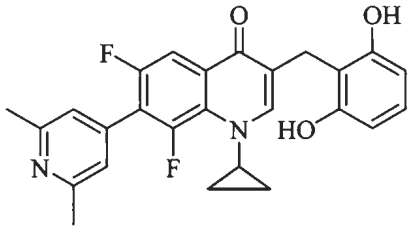


Ciprofloxacin

Figure 2.3. Structures of cytotoxic 4-quinolones and Ciprofloxacin

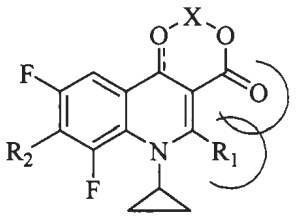
2) It has been observed that the presence of a carboxylic acid group or its isosteres at the C-3 position of quinolones is essential for antibacterial activity [17, 18]. The 3-COOH can also be replaced with a hydrogen without effecting the topo 2 enzyme inhibitory activity. This further deepened the understanding we have so far and paved the way for synthesizing new molecules. One of the key findings is that the introduction of a benzyl group at the 3-position increased the topo II inhibitory activity. The distance between the two aromatic rings is vital for retaining the potency, with only a methylene group as the ideal separator between the two aromatic groups. The activity further increased with the introduction of hydroxyl substituents into the benzyl group without any valid reason. The 2,6-dihydroxy benzyl derivative (**WIN 64593**), among the 3-benzyl analoguous series, has shown the highest topo 2 inhibitory activity and the best in vitro cytotoxicity against mouse P388 leukemic cells(Figure 2.4) [17].



Win 64593

Figure 2.4. Structure of WIN 64593

The inhibitory activity of the compound is still retained in the absence of carboxyl group in contrast to the requirement of the 3-COOH group necessary for gyrase inhibition [19]. The introduction of 2-methyl and 2-phenyl has given some clear picture in relation to the conformation of the structure. While the 2-methyl substitution has retained activity its



$R_1 = CH_3, C_6H_5$

Figure 2.5. Quinolones and steric hindrance

2-phenyl analog is inactive, revealing the importance of the size of the 2-substituent. In the same context, it is interesting to know that the introduction of sulfur at position C-2 has shown to be very promising in improvement of antibacterial activity [20] as well as cytotoxicity [21] in eukaryotic cells (Figure 2.5). One interesting aspect of this structural modification is the modification of C-3 acidic moiety. In order to maintain the stable planar structure of the quinolone skeleton and the stability of the newly added sulfur atom, the structure has been condensed forming a whole new tricyclic planar structure. These analogues have been shown to be highly potent against a broad spectrum of bacteria including several multi-drug resistant strains. Two experimental isothiazoloquinolones **A-65281** and **A-65282** , despite the presence of heterocyclic aromatic ring, have been found to induce respectable DNA breakage mediated by calf-thymus topoisomerase II and are nearly as potent as tenoposide and also potent against broad spectrum of bacteria (Figure 2.6).

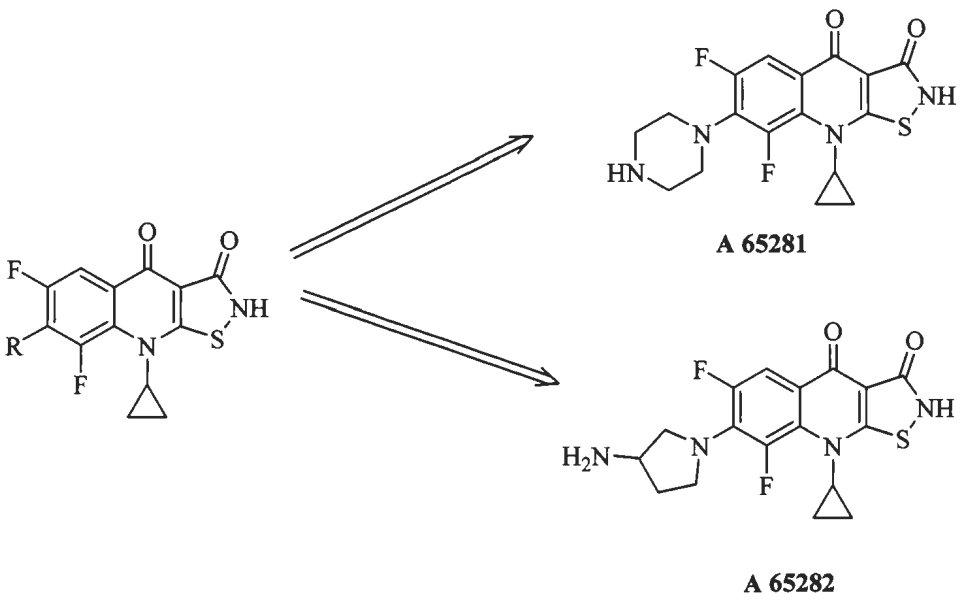
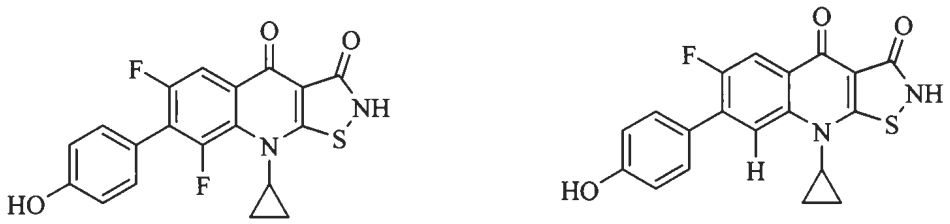


Figure 2.6. Structure of cytotoxic Isothiazoloquinolones

Based on this observation it appears that the biochemical target appears to accommodate the 2-methyl substituent rather than a larger group. Additionally, it can be concluded that the 3-COOH is not vital for activity but, if present, it should be able to attain a planar conformation with respect to the quinolone ring (Figure 2.4).

3) The 4-position of the quinolone ring contains the carbonyl group and is an integral part of the quinolone skeleton. The tendency of Mg^{2+} ions towards formation of gyrase-drug-DNA ternary complex is well documented [22, 23]. The carbonyl group at position C-4 and the carboxylic acid group at C-3 form a complex with the magnesium which enhances the effective binding of the drug with the enzyme and DNA, inducing permanent DNA strand breakage. So far, no evidence of enhanced isosteric replacement has been reported at the C-4.

4) A general requirement of the "classical quinolones" is the presence of a C-6 fluorine atom to induce potent antibacterial activity [24]. As mentioned above, in order to form a stabilized cleavage complex with topo 2, the concomitant presence of two halogens, particularly fluorine, at C-6 and C-8 on the quinolone skeleton is preferred. [21,25, 26] (Figure 2.7).



CP-115,953 **CP-115,955**

Figure 2.7. Structures of CP-115,953 and CP-115,955

A direct comparison of 6-fluoro (**CP-115,955**) and 6,8-difluoro (**CP-115,953**) analogues indicates that removal of the C-8 fluorine decreased the ability of the quinolone to induce topo II-mediated DNA cleavage by approximately 2.5-fold. Despite the decreased potency of **CP-115,955** in comparison to **CP-115,953**, it is still highly active against eukaryotic enzyme in *D. melanogaster* . In fact, this monofluoroquinolone was 80% as potent as etoposide [16, 27, 28]. However, the presence of a C-8 fluorine did not affect the potency of the drug towards DNA gyrase. Also, the activity of these compounds in Chinese hamster ovary cells equals the cytotoxicity observed towards the eukaryotic enzyme, thus confirming the importance of C-8 fluorine in increasing the potency of quinolone derivatives against topo II enzyme in mammalian cells.

5) The C-7 position has been one of the most intriguing in the research of quinolones against cancer cells. A number of quinolones with greatly increased potencies (relative to ofloxacin) toward eukaryotic systems have been reported [16, 21, 25, 26, 29-31]. Almost all of the novel quinolones used against topo II have aromatic substituents in contrast to the general requirement of the antibacterial quinolones. Various quinolones with different substitutions have been synthesized and studied. In antibacterial quinolones the presence of a basic amino group in the

aliphatic cyclic substituent at this position is essential for antibacterial activity. These studies indicate a direct interaction of the enzyme with the C-7 position via an optimal substitution. (Figures 2.8 and 2.9).

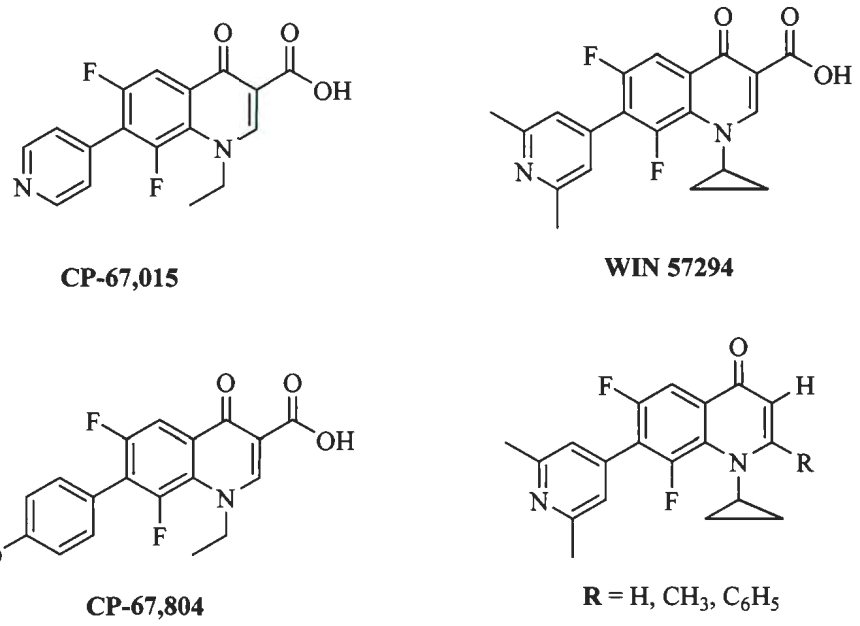


Figure. 2.8. Structure of cytotoxic quinolones with C-7 aromatic substitutions

Topo II, presumably has structural differences in comparison to its counterpart, bacterial DNA gyrase, due to the bias shown on aromatic substitution. This raises a question as to how such a smallstructural modifications can influence enzymatic target recognition at cellular level ? To date there is no answer, due to inadequate information towards the cleavage complex in the presence or absence of drugs. The stereospecificity of the substitution at C-7position has been one of the major discerning factors in influencing the potency against the mammalian enzyme. In particular, the methyl substituents at the C-7 piperazine moiety have been reported to display greater influence. Against mammalian Topo II isomerise, the 3,5-dimethylpiperazinyl derivatives were active in stimulating enzyme-mediated DNA cleavage only in the *trans* configuration. On the other hand, the *cis*- and *trans*-methyl substitutions on the piperazine had

little effect on bacterial DNA gyrase cleavage activity, suggesting the existence of assymetric barrier only in the eukaryotic enzyme influencing cytotoxic quinolones interaction [32]. Similar studies corroborated

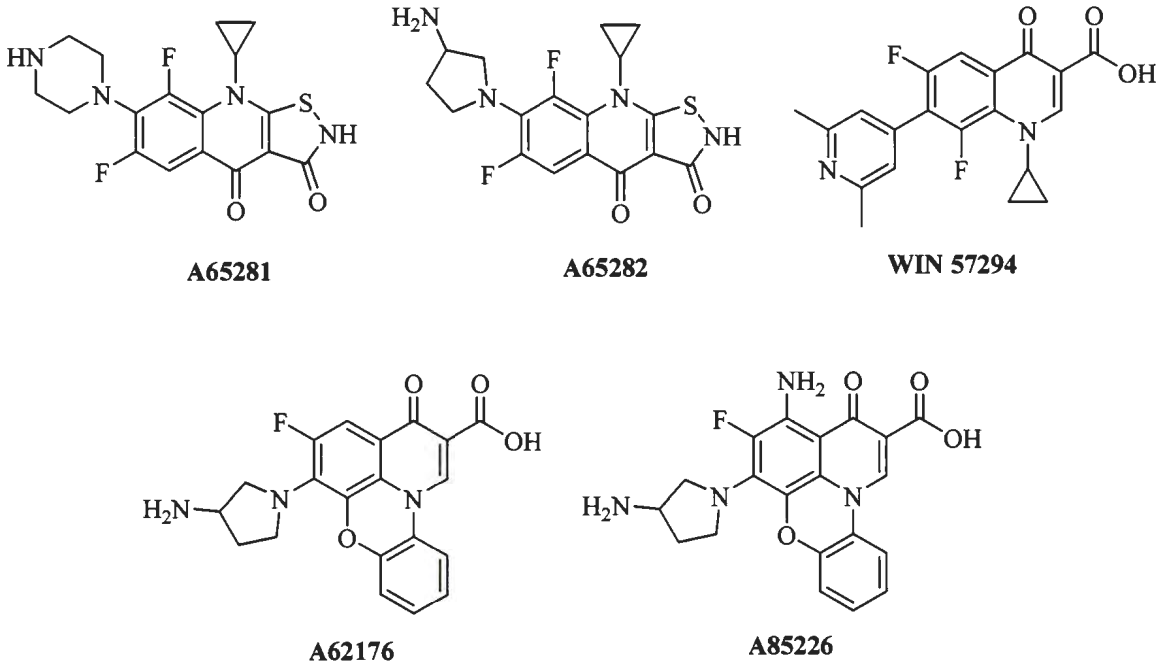


Figure 2.9. Cytotoxic Quinolone Structures with various C-7 substituents

increased cytotoxicity with C-7 pyrrolidine substituents than piperazine substituents [33]. These results correlate with the previous observations made on cytotoxic quinolones [25]. These results signify the importance of aromatic substituent at C-7 position contributing to increased cytotoxic potency. In particular, the 4'-hydroxyphenyl substituent at C-7 position was imperative for increased potency towards the eukaryotic enzyme [14]. The starting point of these studies on SAR is portrayed by **CP-115,953**, considered as one of the most potent cytotoxic quinolone so far (Figure 2.7) [16].

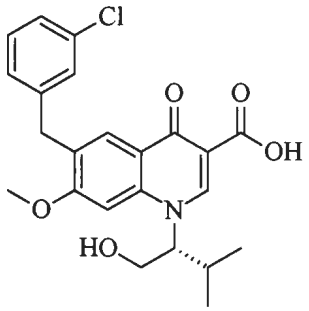
2.3. Parallel Uses of Quinolones

2.3.1. Quinolones as HIV-1 Integrase Inhibitors

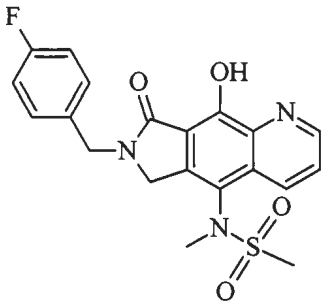
Current research on chemotherapy for HIV infection is yet to overcome the rapid emergence of resistant strains, high costs associated with the current treatments, and the inability of the current clinical regimens to eliminate the HIV infection. Quinolone HIV Integrase inhibitors could be a gateway for developing a promising new class of anti-retroviral drugs. Quinolines are not new to this field. HIV-1 integrase [34-36], one of the three important enzymes targeted for treating HIV infection, is involved in retroviral replication signifying its importance as one of the essential targets for blocking the viral replication cycle. This enzyme is responsible for the penetration of viral cDNA into the host cell genome. This mechanism occurs in two steps:

1) The first step occurs in the host cell cytoplasm where integrase cleaves the Guanosine-Thymine (GT) nucleotides from the conserved 3'-CAGT end. This results in nucleophilic hydroxyl groups at 3'ends of viral cDNA. HIV-Integrase then enters the nucleus of the host cell.
2) Once the HIV-Integrase enters the nucleus of the host cell, it catalyzes the nucleophilic attack of both 3'end hydroxyls of the viral cDNA onto the host DNA, known as the strand transfer reaction.

There has been immense interest in quinolone molecules due to various reasons, some being the ease of synthesis, low synthetic and manufacturing costs and detailed biochemical properties, etc. **GS-9137** (Figure 2.10) [37], Elvitegravir, a derivative of 4-quinolone-3-carboxylic acid, with its very strong antiretroviral properties owes its anti-HIV activity exclusively to the inhibition of the viral enzyme integrase[35].The β-keto acid constituent is considered to be prominent in its function as integrase inhibitors. A number of bioisosteric analogues of elvitegravir, diketopyridine [38], diketotetrazole [39], 7-oxo-8-hydroxy-1,6-naphthyridine [40], and diketotriazole [41] have been reported.



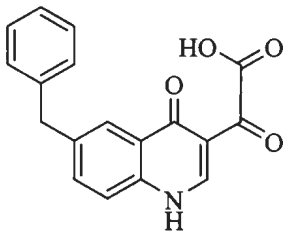
GS-9137



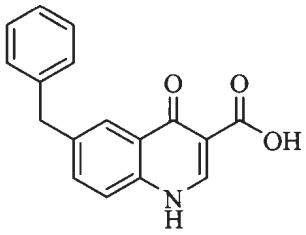
GS-9160

Figure 2.10. Structure of Elvitegravir (**GS-9137**) and **GS-9160**

Lately, a series of quinolone antibiotic-derived HIV integrase inhibitors have been synthesized. The compound **GS-9137** was identified as the most promising candidate, showing potent inhibitory activity against integrase-catalyzed DNA strand transfer [42]. **GS-9160** also has selective antiviral activity, but has suffered a setback due to unfavourable pharmacokinetic properties (Figure 2.10) [43]. The 4-quinolone-3-glyoxolic acid, compound **A-1,** and its precursor 4-quinolone-3-carboxylic acid, compound **B-1,** also showed integrase inhibitory activity [35] (Figure 2.11) and maintain the co-planar structure which is a necessity for cytotoxic quinolones, thus providing a novel insight into the structural requirements and the binding mode of this type of inhibitor [40].



Compound **A-1**



Compound **B-1**

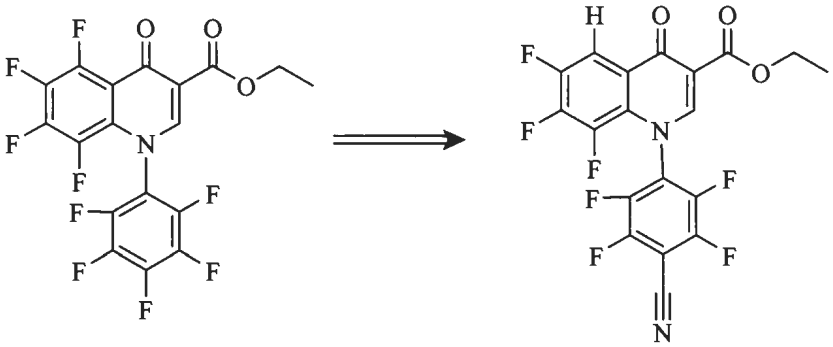
Figure 2.11. Structures of compounds **A-1** and **B-1**

2.3.2. As Signal Transducer and Activator of Transcription 3 (STAT3)

The signal-transducer-and-activator-of-transcription 3 (STAT3) protein, one of the members of STATS transcription factor family, has been implicated in signal transduction by different cytokines,

growth factors and oncogenes. Hence, pharmacologically safe and effective agents that can block STAT3 activation have a potential role in the treatment of inflammation, survival, proliferation and invasion of hepatocellular carcinoma [44]. There is reasonable evidence indicating the role of STAT3 proteins in carcinogenesis, thus making it a potential target for cancer therapy [45, 46].

Lead optimization of fluorinated quinolone (Compound **C-1**), involved in cell apoptosis *via* inhibition of STAT3 activation, led to the identification of compound **D-1**, with increase potency [47] (Figure 2.12).



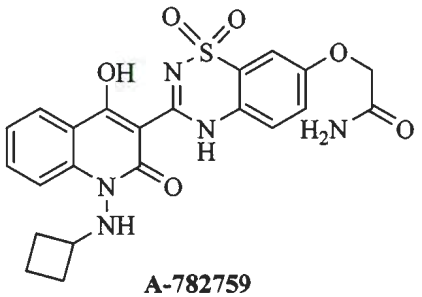
Compound **C-1** Compound **D-1**

Figure 2.12. Structures of compounds **C-1** and **D-1**

2.3.3. Quinolones as anti-HCV Agents

Currently, Hepatitis C Virus (HCV) infection is treated with a combination of pegylated interferon α-2a/b and ribavirin. Due to a limitation in the availability of these drugs there is an increase in attention towards the development of a number of molecules, which act by directly affecting key steps in the viral life-cycle. Novel small molecules that inhibit NS5B polymerase, NS3 protease and the viral RNA are the most frequently studied antiviral agents [48].

Non-structural protein 5b (NS5B) which encodes the RNA polymerase (RdRp) is one of the potential targets for several NS5B polymerase inhibitors and has shown promising results in clinical trials.



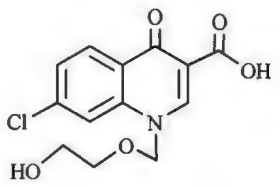
A-782759

Figure 2.13. Structure of **A-782759**

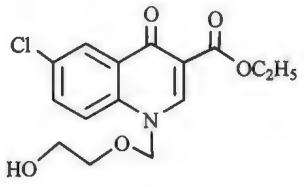
Recently, **A-782759**, a 2-(1-aminoquinolone-3-yl) benzothiadiazine derivative, was identified as promising HCV NS5B inhibitor (Figure 2.13). In fact, **A-782759** with HCV NS3 protease inhibitor, BILN-2061, interferon (IFN) [49, 50] showed an additive effect to synergistic relationship in studies over a range of concentrations of the two drug combinations.

2.3.4. Quinolones as Potential anti-Herpes Simplex Virus-1 (HSV) Agents

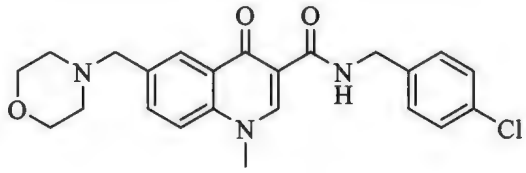
Infections of HSV-1 are the most frequently treated infections in everyday life for which acyclovir is the commonly used agent. Recently compounds **E-1** and **F-1** [51] (Figure 2.14), that are quinolone acylnucleoside derivatives, having high activity against HSV-1, have been discovered. These quinolone derivatives, especially the acid derivatives are highly effective in reducing the viral load than their ester analogues. Upon further investigation of these quinolone derivatives, **PNU-183792**, a 3-(*p*-chlorobenzyl carboxamide)-4(1H)-quinolone derivative (Figure 2.14), was found to be a competitive inhibitor of herpes virus polymerase in both human and animals.



Compound E-1



Compound F-1



PNU-183792

Figure 2.14. Structure s of **PNU-183792** and compounds **E-1** and **F-1**

References

1. Chu, D. T., Fernandes, P. B. (1989). Structural-Activity relationships of the fluoroquinolones. *Antimicrob. Agents Chemother.*, **33** (2), 131-135; 1a) Blondeau, J.M. (2004). Fluoroquinolones: Mechanism of action, classification and development of resistance. *Survey of Ophthalmology*, **49**, S73-S78.
2. Klopman, G., Macina, O., Levinson, M. E., Rosenkraz, H. S. (1987). Computer automated structure evaluation of quinolone antibacterial agents. *Antimicrob. Agents Chemother.*, **31**, 1831-1840.
3. Ma, Z., Chu, D. T., Cooper, C. S., et al. (1999). Synthesis and antimicrobial activity of 4(H)-4-oxoquinolizine derivatives: consequences of structural modification at the C-8 position. *J. Med. Chem.*, **42**, 4202-4213.
4. Vincente M., Errington, J. (1996). Structure, function and controls in microbial division. *Mol. Microbiol.*, **20**, 1-7.
5. Palu, G., Valisena, S., Ciarrocchi, G., Gatto, B., & Palumbo, M. (1992). Quinolone binding to DNA is mediated by magnesium ions. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 9671-9675.
6. Chu, D. T., Nordeen, C. W., Hardy, D. J., Swanson, R. N., Giardina, W. J., Pernet, A. G., Plattner, J. J. (1991). Synthesis, antibacterial activities, and pharmacological properties of enantiomers of temafloxacin hydrochloride. *J. Med. Chem.*, **34**, 168-174.
7. Da Silva, A. D., De Almeida, M. V., De Souza, M. V., Couri, M. R. (2003). Biological activity and synthetic methodologies for the preparation of fluoroquinolones, a class of potent antibacterial agents. *Curr. Med. Chem.*, **10**, 21-39.
8. Heddle, J. G., Barnard, F. M., Wentzell, L. M., Maxwell, A. (2000). The interaction of drugs with DNA gyrase: a model for the molecular basis of quinolone action. *Nucleosides Nucleotides Nucleic Acids*, **19**, 1249-1264.

9. Cabral, J.H.M., Jackson, A. P., Smith, C. V., Shikotra, N., Maxwell, A., Liddington, R. C. (1997). Crystal structure of the breakage-reunion domain of DNA gyrase. *Nature*, **388**, 903-906.

10. Perichon, B., Tankovic, J., Courvalin, P. (1997). Characterization of a mutation in the *parE* gene that confers fluoroquinolone resistance in *Streptococcus pneumonia*, *Antimicrob. Agents Chemother.*, **41,** 1166-1167.

11. Martinez, J. L., Alonso, A., Gomez-Gomez, J. M., Baquero, E. (1998). Quinolone resistance by mutations in chromosomal gyrase genes: just the tip of the iceberg ? *J. Antimicrob. Chemother.*, **42**, 683-688.

12. Baranova, N. N., Neyfakh, A. A. (1997). Apparent involvement of a multidrug transporter in the fluoroquinolone resistance of *Streptococcus pneumonia*, *Antimicrob. Agents Chemother.*, **41**, 1396-1398.

13. Llorente, B., Leclerc, F., Cedergren, R. (1996). Using SAR and QSAR analysis to model the activity and structure of the quinolone-DNA complex. *Bioorg. Med. Chem. Lett.*, **4**, 61-71.

14. Elsea, S.H., McGuirk, P. R., Gootz, T. D., Moynihan, M., Osheroff, N. (1993). Drug features that contribute to the activity of quinolones against mammalian topoisomerase II and cultured cells: Correlation between enhancement of enzyme-mediated DNA cleavage in vitro and cytotoxic potential. *Antimicrob. Agents Chemother.*, **37**, 2179-2186.

15. Morrissey, I., Hoshino, K., Sato, K. et al. (1996). Mechanism of differential activities of oflaxacin enantiomers. *Antimicrob. Agents Chemother.*, 40, 1775-1784.

16. Robinson, M. J., Anne Martin, B., Gootz, T. D., McGuirk, P. R., Moynihan, M., Sutcliffe, J. A., Osheroff, N. (1991). Effects of Quinolone Derivatives on Eukaryotic Topoisomerase II : A novel mechanism for enhancement of enzyme-mediated DNA cleavage. *J. Biol. Chem.*, **266**, 14585-14592.

17. Eissenstat, M. A., Kuo, G-H., Weaver, J. D., Mark, W III., Wentland, M. P., Robinson, R. G., Klingbell, K. M., Danz, D. W., Corbett, T. H., Coughlin, S. A. (1995). 3-benzyl-quinolones: Novel, potent inhibitors of mammalian topoisomerase II. *Bioorg. Med. Chem. Lett.*, **5**, 1021-1027.

18. Tillotson, G. S. (1996). Quinolones: Structure-activity relationships and future predictions. *J. Medical Microbiol.*, **44**, 320-324.

19. Wentland, M. P., Lesher, G. Y., Reuman, M., Gruett, M. D., Singh, B., Aldous, S. C., Dorff, P. H., Rake, J. B. Coughlin, S. A. (1993). Mammalian Topoisomerase II inhibitory activity of 1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic Acid and Related derivatives. *J. Med. Chem.*, **36**, 2801-2809.

20. Wiles, J. A., Qiuping, W., Lucien, E., Hashimoto, A., Song, Y., Cheng, J., Marlor. C. W., Ou, Y., Podos, S. D., Thanassi, J. A., Thoma, C. L., Deshpande, M., Pucci, M. J., Bradbury, B. J. (2006). *Bioorg. Med. Chem. Lett.*, **16**, 1272-1276.

21. Kohlbrenner, W. E., Wideburg, N., Weigl, D., Saldivar, A., Chu, D. T. W. (1992). Induction of calf thymus topoisomerase II-mediated DNA breakage by the antibacterial Isothiazoloquinolones A-65281 and A-65282. *Antimicrob. Agents. Chemother.*, **36**, 81-86.

22. Palu, G., Valisena, S., Ciarrocchi, G., Gatto, B., Palumbo, M. (1992). Quinolone binding to DNA is mediated by magnesium ions. *Proc. National Acad. Sci., USA*, **89**, 9671-9675.

23. Sissi, C., Perdona, E., Domenici, E., Feriani, A., Howells, A. J., Maxwell, A., Palumbo, M. (2001). Ciprofloxacin affects conformational equilibria of DNA gyrase A in the presence of Magnesium ions. *J. Mol. Biol.*, **311**, 195-203.

24. Piddock, L. J. (1999). Mechanisms of Fluoroquinolone resistance : An update 1994-1998. *Drugs*, **58** supplement 2, 11-18.

25. Barrett, J. F., Gootz, T. D., McGuirk, P. R., Farrell, C. A., Sokolowski, S. A. (1989). Use of *In vitro* topoisomerase II assays for studying quinolone antibacterial agents. *Antimicrob. Agents Chemother.,*, **33**, 1697-1703.

26. Robinson, M. J., Martin, B. A., Gootz, T. D., McGuirk, P. R., Osheroff, N. (1992). Effects of Novel fluoroquinolones on the catalytic activities of eukaryotic topoisomerase II: Influence of the C-8 fluorine group. *Antimicrob. Agents Chemother.*, **36**, 751-756.

27. Osheroff, N. (1989). Effect of anti-neoplastic agents on the DNA cleavage/relegation equilibrium of eukaryotic topoisomerase II: inhibition of DNA relegation by etoposide. *Biochem.*, **28**, 6157-6160.

28. Robinson, M. J. and Osheroff, N. (1991). Effects of antineoplastic drugs on the post-strand passage DNA cleavage/relegation equilibrium of topoisomerase II. *Biochem.*, **30**, 1807-1813.

29. Elsea, S. H. N., Osheroff, N. Nitiss, J. L. (1992). Cytotoxicity of quinolones towards eukaryotic cells: identification of topoisomerase II as the primary cellular target for the quinolone CP-115,953 in yeast. *J. Biol. Chem.*, **267**, 13150-13153.

30. Wentland, M. P., Lesher, G. Y., Reuman, M., Gruett, M. D., Singh, B., Aldous, S. C., Dorff, P. H., Rake, J. B. and Coughlin, S. A. (1993). Mammalian topoisomerase II inhinitory activity of 1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic acid and related derivative. *J. Med. Chem.*, **36**, 2801-2809.

31. Yamashita, Y., Ashizawa, T., Morimoto, M., Hosomi, J., Nakano, H. (1992). Antitumor quinolones with mammalian topoisomerase II mediated DNA cleavage activity. *Cancer Research*, **52**, 2818-2822.

32. Gootz, T. D., McGuirk, P. R., Moynihan, M. S., Haskell, S. L. (1994). Placement of alkyl substituents on the C-7 piperazine ring of fluoroquinolones: dramatic differential effects on mammalian topoisomerase II and DNA gyrase. *Antimicrob. Agents Chemother.*, **38**, 130-133.

33. Suto, M. J., Domagala, J. M., Roland, G. E., Mailoux, G. B., Cohen, M. A. (1992). Fluroquinolones: Relationships between structural variations, mammalian cell cytotoxicity, and antimicrobial activity. *J. Med. Chem.*, **35**, 4745-4750.

34. Boros, E. E., Johns, B. A., Garvey, E. P., Koble, C. S., Miller, W. H. (2006). Synthesis and HIV-integrase strand transfer inhibition activity of 7-hydroxy[1,3]thiazolo[5,4-b]pyridine-5(4H)-ones. *Bioorg. Med. Chem. Lett.*, **16**, 5668-5672.

35. Sato, M., Motomura, T., Aramaki, H., Matsuda, T., Yamashita, M., Ito, Y., Kawakami, H., Matsuzaki, Y., Waranabe, W., Yamataka, K., Ikeda, S., Kodama, E., Matsuoka, M., Shinkai, H. (2006). Novel HIV-1 integrase inhibitors derived from quinolone antibiotics. *J. Med. Chem.*, **49**, 1506-1508.

36. Cecchetti, V., Parolin, C., Moro, S., Pecere, T., Filipponi, E., Calistri, A., Tabarrini, O., Gatto, B., Palumbo, M., Fravolini, A., Palu, G. (2000). 6-Aminoquinolones as new potential anti-HIV agents. *J. Med. Chem.*, **43**, 3799-3802.

37. Pommier, Y., Johnson. A. A., Marchand, C. (2005). Integrase inhibitors to treat HIV/AIDS. *Nature Review-Drug Discovery*, **4**, 236-248.

38. Friis-Moller, N., Reiss, P., Sabin, C. A., Weber, R., Monforte, A., El-Sadr, W., Thiebaut, R., De Wit, S., Kirk, O., Fontas, E., Law M. G., Phillips, A., Lundgren, J. D. (2007). Class of antiretroviral drugs and the risk of myocardial infarction. *New England J. Medicine*, **356**, 1723-1735.

39. Machouf, N., Thomas, R., Nguyen, V. K., Trottier, B., Boulassel, M. R., Wainberg, M. A., Routy, J. P. (2006). Effects of drug resistance on viral load in patients failing antiretroviral therapy. *J. Medical Virol.*, **78**, 608-613.

40. Fatkenheuer, G., Pozniak, A. L., Johnson, M. A., Plettenberg, A., Staszewski, S., Hoepelman, A. I., Saag, M. S., Goebel, F. D., Rockstroh, J. K., Dezube, B. J., Jenkins, T. M., Medhurst, C.,

Sullivan, J. F., Ridgway, C., Abel, S., James, I. T.,Youle, M., Van der Ryst, E. (2005). Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nature Medicine*. **11**, 1170-1172.

41. Cozzi-Lepri, A., Phillips, A. N., Ruiz, L., Clotet, B., Loveday, C., Kjaer, J., Mens, H., Clumeck, N., Viksna, L., Antunes, F., Machala, L., Lundgren, J. D., EuroSIDA Study Group. (2007). Evolution of drug resistance in HIV-infected patients remaining on a virologically failing combination antiretroviral therapy regimen. *AIDS*, **21**, 721-732.

42. Sorbera, L. A., Serradell, N. (2006). Monographs-GS-9137-anti-HIV agent, HIV integrase inhibitor. *Drugs of the Future*, **31**, 310.

43. Jones, G. S., Yu, F., Zeynalzadegan, A., Hesselgesser, J., Chen, X., Chen, J., Jin, H., Kim, C. U., Wright, M., Geleziunas, R., Tsiang, M. (2009). Preclinical evaluation of GS-9160, a novel inhibitor of human immunodeficiency virus type 1 integrase. *Antimicrob. Agents Chemother.*, **53**, 1194-1203.

44. Subramaniam, A., Shanmugam, M. K., Perumal, E., Li, F., Nachiyappan, A., Dai, X., Swamy, S.N., Ahn, K. S., Kumar, A. P., Tan, B. K., Hui, K. M., Sethi, G. (2012). Potential role of signal transducer and activator of transcription (STAT)3 signalling pathway in inflammation, survival, proliferation and invasion of hepatocellular carcinoma. *Biochim. Biophys. Acta*, **1835**, 46-60.

45. Darnell, J. E. (2005). Validating STAT3 in cancer therapy. *Nature Medicine*, **11**, 595-596.

46. Klampfer, L. (2006). Signal transducers and activators of transcription (STATs): novel targets of chemopreventive and chemotherapeutic drugs. *Current Cancer Drug Targets*, **6**, 107-121.

47. Kumar D. V., Rai, R., Brameld, K. A., Somoza, J. R., Rajagopalan, R., Janc, J. W., Xia, Y. M., Ton, T. L., Shaghafi, M. B., Hu, H., Lehoux, I., To, N., Young, W. B., Green, M. J. (2011). Quinolones as HCV NS5B polymerase inhibitors. *Bioorg. Med. Chem. Lett.*, **21**, 82-87.

48. Reed, K. E., Rice, C. M. (1999). Expression and characterization of the HCV NS2 protease. *Methods in Molecular Medicine*, **19**, 331-342.

49. Lu, L., Pilot-Matias, T. J., Stewart, K. D., Randolph, J. T., Pithawalla, R., He, W., Huang, P. P., Klein, L. L., Mo, H., Molla, A. (2004). Mutations conferring resistance to a potent hepatitis C virus serine protease inhibitor in vitro. *Antimicrob. Agents Chemother.*, **48**, 2260-2266.

50. Mo, H., Lu, L, Pilot-Matias, T.,Pithawalla, R., Mondal, R., Masse, S., Dekhtyar, T., Nq, T., Koev, G., Stoll, V., Stewart, K. D, Pratt, J., Donner, P., Rockway, T., Maring, C., Molla, A. (2005). Mutations conferring resistance to a hepatitis C virus (HCV) RNA-dependent RNA polymerase inhibitor alone or in combination with an HCV serine protease inhibitor in vitro. *Antimicrob. Agents Chemother.*, **49**, 4305-4314.

51. Lucero, B. d'A., Gomes, C. R. B., Frugulhetti, I. C. de P. P., Faro, L. V., Alvarenga, L., De Souza, M. C. B. V., De Souza, T. M. L., Ferreira, V. F. (2006). Synthesis and anti-HSV-1 activity of quinolonic acyclovir analogues. *Bioorg. Med. Chem. Lett.*, **16**, 1010-1013.

Chapter 3

Design, Synthesis and Biological Evaluation of thieno[2,3-*b*]quinolones and furo[2,3-*b*]quinolones

3.1. Introduction

Quinolin-4 (1*H*)-ones (4-Quinolones) belong to a larger group of heterocycles. These scaffolds have been the subject of research for many groups, the proof of which is reflected in a vast number of reviews and journal articles dedicated towards their clinical use. The starting point of this research was based on the assumption that both the antibacterial quinolones and their cytotoxic analogs share a similar mode of interaction with type II DNA topoisomerase-DNA complexes. For example, studies have shown that quinolones and antitumor drugs of the epipodophyllotoxin family (etoposide and teniposide) act on a physiologically similar DNA toposiomerase enzyme, with quinolones acting on DNA gyrase and etoposide acting on topoisomerase II [1]. The last few years have witnessed the development of numerous novel tricyclic and tetracyclic quinolones that target mammalian topoisomerases I & II. The blueprint for this research has been developed by the team led by Dr. Daneshtalab [2]. Compound **A** (Figure 3.1), as described in a joint patent by Kyorin/Kyowa-Hakko, a thiazoloquinolone has been the basis of this initial research [3].

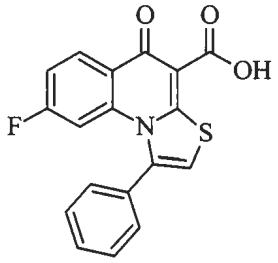
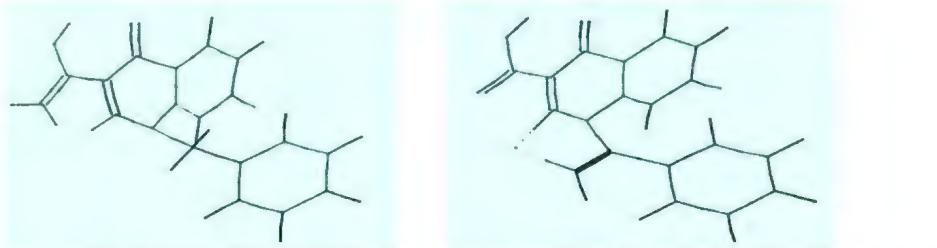


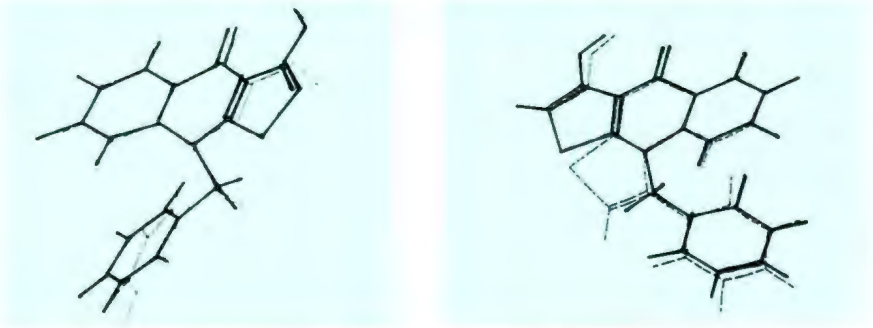
Figure.3.1. Structure of Compound **A**

Using the molecular modelling program Alchemy-3^{TM}, the sterically optimum conformation of compound **A** was determined using Molecular Mechanics Optimization (MMO) and Molecular Dynamic

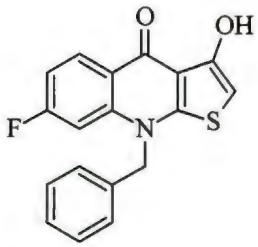
Option methods. Based on these studies newly designed linear tricyclic quinolones with β-diketo group were modelled to match the conformation of compound **A** (Figure 3.2). From these, compound B was identifiedd for furthur studies.



Designed compound (B) **Active compound (A)**



Two different overlays of the designed structure and the active compound



Compound B

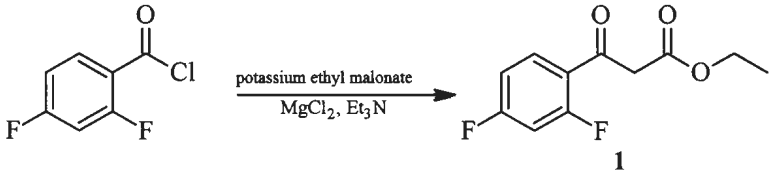
Figure 3.2. Sterically optimized structure of Compound **B**

The objective of our research was to synthesize various analogues of compound **B**, such as the linear thieno[2,3-*b*] quinolones and furoquinolones and evaluate their cytotoxic profiles.

3.2 Materials and Methods

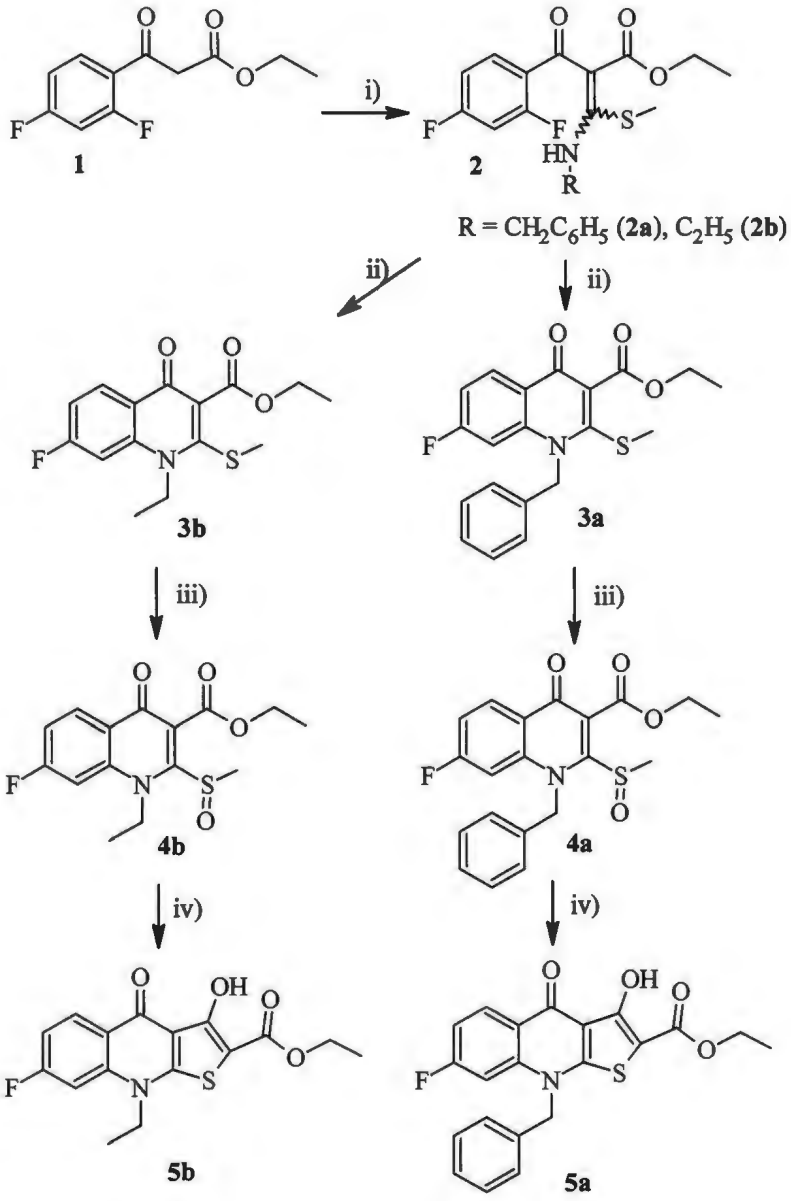
3.2.1 Synthesis of *N*-Alkylated thienoquinolones

2,4-Difluorobenzoylchloride was used as a starting material. This compound was allowed to react with potassium ethyl malonate, magnesium chloride and triethylamine to afford the respective β-ketoester **1** [4,5,6]. Scheme 3.1 represents the reaction.



Scheme 3.1. Synthesis of β-ketoester **1**, (2,4-difluorophenyl)acetoacetic acid ethyl ester (**1**)

Compound **1** was then allowed to react with ethyl or benzyl isothiocyanate in the presence of potassium hydroxide and *n*-tetrabutylammonium bromide, as a phase transfer catalyst, followed by methylation using methyl iodide to afford compounds **2a** and **2b**, respectively. The intermediate compounds **2a** and **2b**, due to their instability, were cyclized using *t*-BuOK(potassium *tert*-butoxide) to afford the respective intermediate building blocks **3a** and **3b** [6].The resulting intermediates **3a** and **3b** were subjected to oxidation using *m*-CPBA to give **4a** and **4b** [6,7]. Compounds **4a** and **4b** were then allowed to react with ethyl mercaptoacetate and triethylamine in absolute ethanol at reflux for 5-6 h to afford the final compounds **5a** and **5b** [8], as shown in Scheme 3.2.



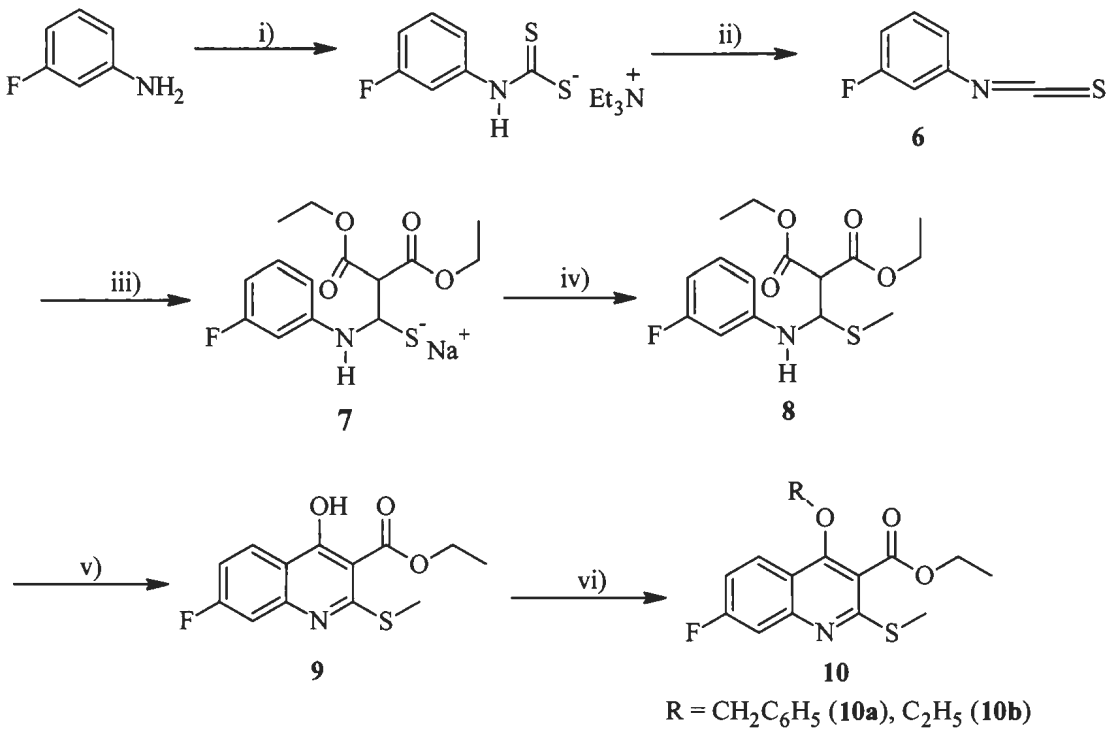
i) KOH, n-tetrabutylammonium bromide, ethyl or benzyl isothiocyanate, CH_3I, 18 h
ii) *t*-BuOK, reflux, 18 h; iii) *m*-CPBA, 0 °C, DCM, 7-8 h; iv) $HSCH_2CO_2Et$, Et_3N, reflux, 5-6 h

Scheme 3.2. Syntheses of compounds **3a,b**, **4a,b**, and **5a,b**

3.2.2. Synthesis of *O*-Alkylated thienoquinolones

3-Fluoroaniline was reacted with triethylamine and carbon disulfide in benzene. The resulting intermediate salt was then stirred with ethyl chloroformate at room temperature for 15 h to give the

starting material 3-flurophenyl isothiocyanate (**6**) [9]. Compound **6** upon reaction with diethyl malonate and sodium hydride in THF afforded salt **7**. Due to the instability of compound **7**, it was immediately allowed to react with iodomethane to give compound **8**. Compound **8** upon heating at high temperature under vacuum yielded the cyclized compound **9.** Compound **9** was then subjected to a modified Mitsunobu reaction as reported by Amoozgar. Z [2] using benzyl alcohol or ethyl alcohol to yield the respective analogues **10a** and **10b,** as depicted in Scheme 3.3.

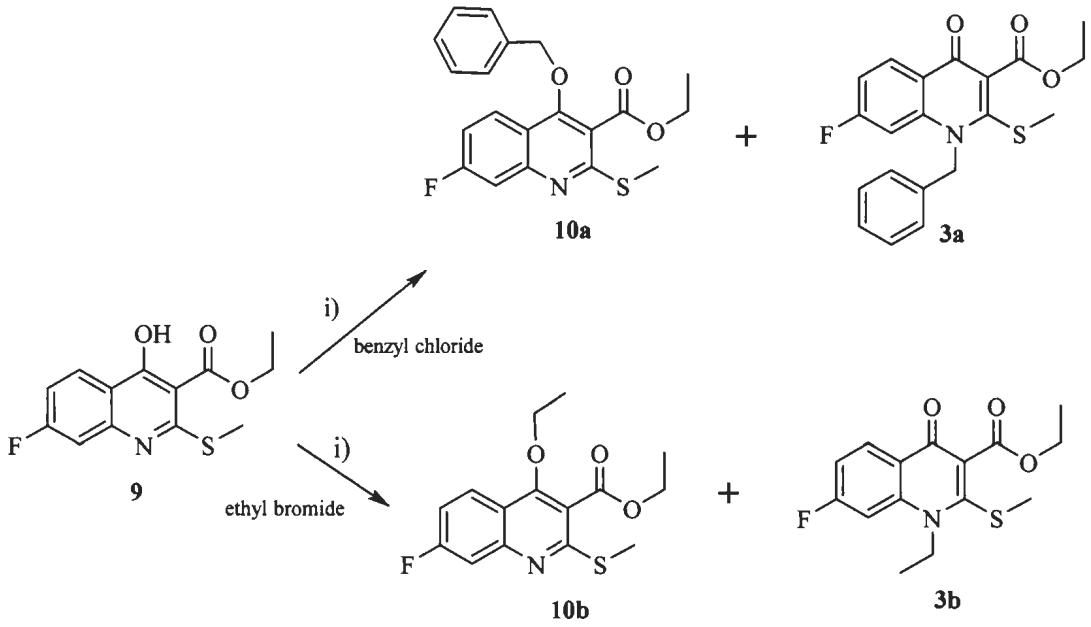


i) CS_2, Et_3N, benzene; ii) $ClCO_2Et$, Et_3N; iii) Diethyl malonate, NaH, THF; iv) CH_3I, THF;
v) Heating under vacuum; vi) DIAD, benzyl alcohol or ethanol, $P(Ph)_3$

Scheme 3.3. Syntheses of compounds **6**, **7**, **8**, **9**, **10a**, and **10b**

According to Amoozgar, Z., the modified Mitsunobu reaction was used in the process of synthesizing N-alkylated derivatives. In order to further confirm the regioselectivity of the Mitsunobu reaction, comparison of the products obtained by the basic alkylation method with compounds **3a** and **3b** and compounds obtained from Mitsunobu reaction (**10a** and **10b**) was performed. Interestingly, it was

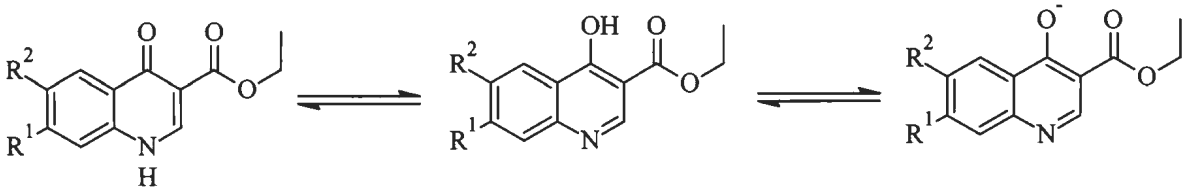
found that compounds **10a** and **10b** were regioselectively *O*-alkylated, while basic alkylation of compound **9** (Scheme 3.4) gave a mixture of both N-alkylated and O-alkylated products.



i) K_2CO_3, DMF, 70-80^{0}C, 8 h

Scheme 3.4. Basic alkylation of compound **9**

Alkylation at nitrogen succeeded only after cyclization of compound **8** as reported earlier [10]. Studies aimed at regioselectivity of alkylation of 4-hydroxy quinolone and naphthyridone derivatives using an ethylating agent [11] show that the likelihood of a charge-controlled reaction on nitrogen is small.



A-2 **B-2**

Figure 3.3. Tautomerism of Quinolones

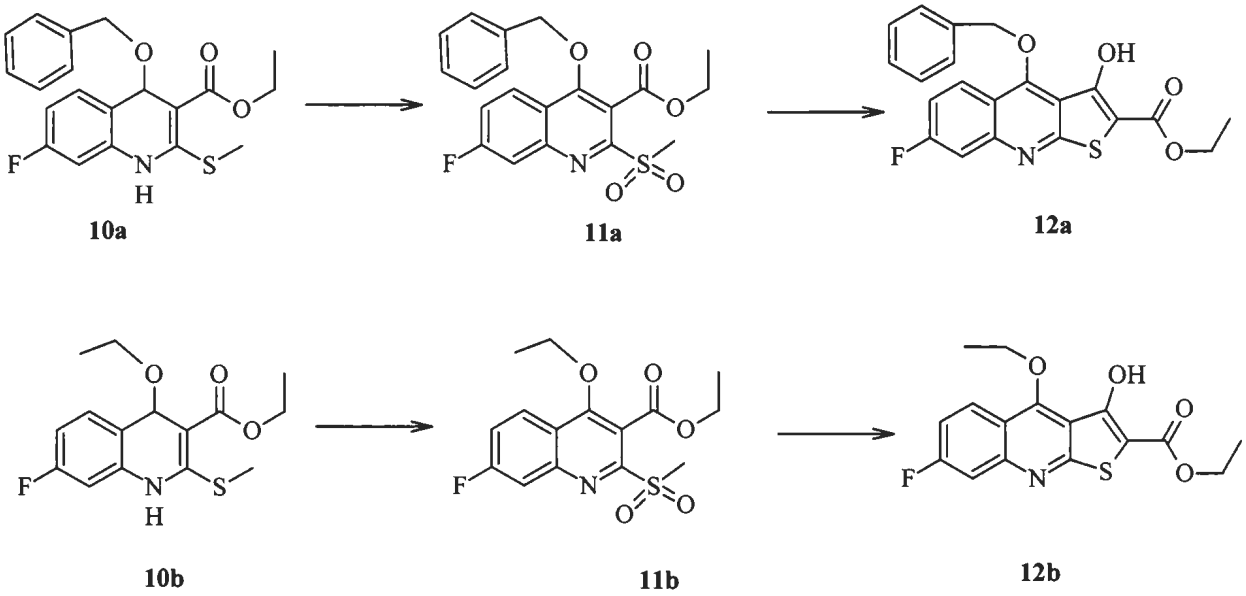
According to these studies it is assumed that deprotonation sequesters the coexisting enol form **B-2** resulting in the complete transformation of the keto form **A-2** into the nucleophilic anion. The low electron density, caused by the overlapping of the nonbonding p_z orbital with the aromatic ring and the electron-deficient double bond, prevents the charge-controlled reaction at the nitrogen atom. In this respect, studies done on 4-hydroxy quinolones showed that in the presence of an excess base, potassium carbonate, and at room temperature the tautomeric equilibrium was shifted towards *N*-alkylation [11], and with increase in temperature there was an excess shift in equilibrium resulting in a higher yield of *N*-alkylated products. Investigation done be Frank *et al.* [12] on alkylation of quinolones with trimethyl phosphate showed regioselective *O*-alkylation in contrast to the studies done by Makara, G. *et al.* This demonstrates that the 3-ethoxycarbonyl group plays a major role in the regioselectivity of *N*- and *O*-alkylation. In 3-alkoxylcarbonyl analogues the enol form is more stable in the gas phase, while in polar solvents the keto form predominates. This shows that for *N*-alkylation, the formation of an anion with high HOMO energy by deprotonation of 3-alkoxycarbonyl-4-oxoquinolines is required (Figure 3.3). Since the electron pair on the nitrogen atom in quinolones is involved in cyclic conjugation the possibility of interaction with electron-deficient reagents is very little, thus requiring the need for preliminary inonization of the N-H bond [13]. In the present scenario and in certain cases the 2- arylthio compounds are isolated in either 4-quinolinol or 4-quinolone forms, with the tautomeric pattern depending on several factors such as the solvent of crystallisation, the temperature of the sample and the steric bulk of the 2-alkylthio-substituent [14]. Hence, the domination of enol form due to hydrogen bonding with the oxygen of the carbonyl group and also due to the presence of bulky alkylthio group at position 2 can attribute to the selectivity of *O*-alkylation at C-4. This selectivity is further confirmed by the proton chemical shift patterns in both the *N*-alkylated and *O*-alkylated compounds.

Table 3.1. Comparision of Chemical Shifts of methylene group

	δ ^{1}H NMR Chemical Shift	
	N-Alkylation	*O*-Alkylation
-CH_2-Bn	**3a** (δ 5.89)	**10a** (δ 5.22)
-CH_2-Me	**3b** (δ 4.61)	**10b** (δ 4.48)

To further strengthen our point 2D NOESY for the above mentioned compounds has been performed, thus confirming that Mitsunobu reaction is oriented towards *O*-alkylation owing to a variety of factors such as steric hindrance of the alkylthio group, hydrogen bonding ability of the carbonyl group at position 4 and the carbonyl group at position 3, etc. With this study it is shown that the previously reported *N*-alkylated products from Mitsunobu reaction are supposed to be *O*-alkylated products [2].

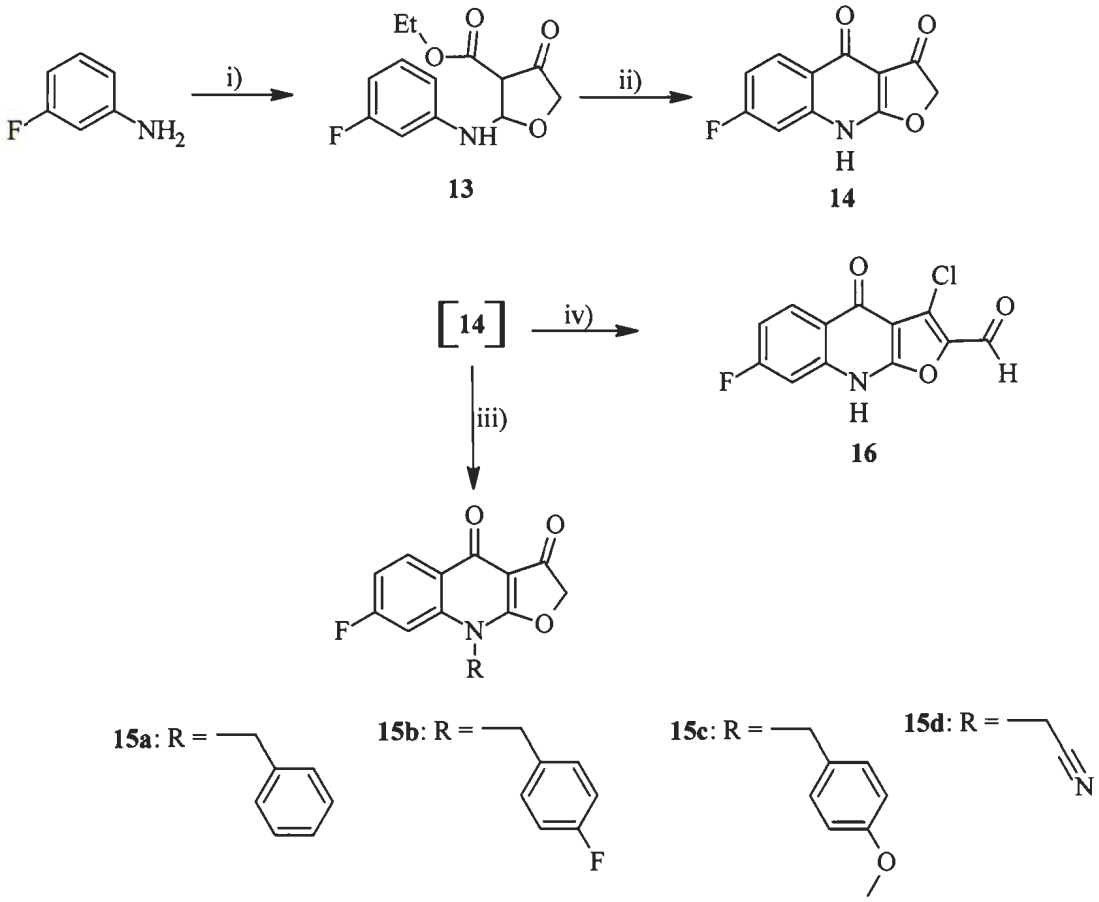
Compounds **10a** and **10b**, the preparation of which were shown in Scheme 3.3, were oxidized to afford compounds **11a** and **11b**. The latter compounds were subjected to similar conditions as shown in Scheme 3.2 to afford compounds **12a** and **12b** (Scheme 3.5).



Scheme 3.5. Synthesis of compounds **12a** and **12b**

3.2.3. Synthesis of Furoquinolones

Furoquinolone alkaloids are well known in nature, but so far their biological activities have not been well studied [15]. In order to further investigate their anti-cancer activity we synthesized and tested various furoquinolone analogs. In order to synthesize a series of compounds possessing furoquinolone scaffold, we followed the procedures reported by Shen-Chu Kuo, *et al.,* with minor modifications [16]. Namely, to a solution of sodium diethyl malonate in THF was added chloroacetyl chloride. To the resultant mixture was added 3-fluoroaniline to afford the intermediate **13**. Intermediate **13** was added with stirring to Dowtherm maintained at 240 °C to afford compound **14**. Compound **14** was then subjected to alkylation using various alkylhalides to afford compounds **15a-d**. Further, compound **14** was also treated with phosphorus oxychloride to afford 3-chloro-7-fluoro-4-oxo-4,9-dihydrofuro[2,3-*b*]quinoline-2-carbaldehye (**16**), as shown in Scheme 3.6.



i) a) NaH, diethyl malonate, THF b) chloroacetyl chloride, THF, 3-fluoroaniline
ii) Dowtherm, 240-250 ^{0}C, 10 min
iii) K_2CO_3, BnCl (or) 4-FBnCl (or) 4-OMeBnCl (or) $BrCH_2CN$, DMF
iv) phosphorous oxychloride, DMF

Scheme 3.6. Synthesis of Furoquinolones **14**, **15a-d**, and **16**

3.2.4 *In* vitro testing

3.2.4.1 Brine Shrimp Lethality Test

Brine shrimp bioassay is an assay capable of detecting the lethality of compounds. The technique is simple, inexpensive, and uses small amounts of test material. The aim of the method is to provide a first-line screen that can be backed up by more sophisticated bioassays once the active compounds have been isolated. For this test, eggs of brine shrimp *Artemia Salina* were used. The eggs were aerated in 1L

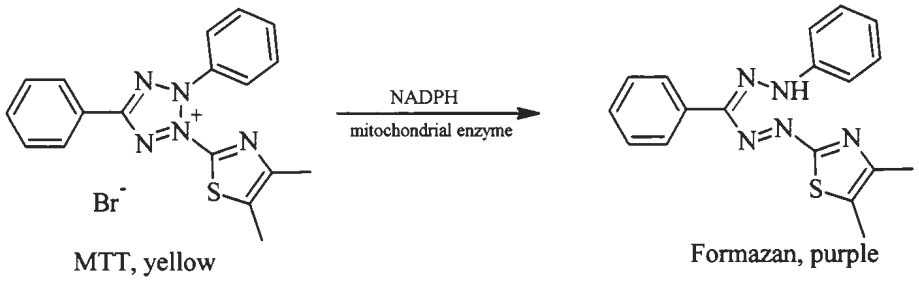
capacity box containing imitation sea water (composed of sodium chloride and sodium bicarbonate). An air pipe is inserted to ensure complete hydration of the cysts. After 24 h of incubation under a lamp at room temperature (25-29 ^{0}C), newly hatched free-swimming *nauplii* were harvested from the bottom outlet. As the cyst capsules float on the surface, this collection method ensured pure harvest of *nauplii*. The freshly hatched free-swimming *nauplii* were used for the bioassay. The assay system was prepared with 2 mL of filtered saline water containing chosen concentration (0.1 µg/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL) of compounds to be tested. Parallel vehicle control (using 2% DMSO) and negative control (without vehicle) were included in the experimental setup. In each, 20 *nauplii* were transferred and the setup was allowed to remain for 24 hours, under constant illumination. After 24 h, the dead *nauplii* were counted with a hand lens. Based on the percent mortality, the LD_{50} of the test compounds were determined by probit analysis according to Finney [17].

$$\textbf{\% mortality} = N_D/N_L \textbf{ X 100}$$

(N_D= No. of dead *nauplii* ; N_L = No. of live *nauplii*)

3.2.4.2. MTT Cytotoxicity Bioassay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity test is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye, MTT, to insoluble purple coloured formazan. This assay measures cellular metabolic activity via NAD(P)H-dependent cellular oxidoreductase enzymes and may, under defined conditions, reflect the number of viable cells (cell proliferation).



Most of the potentially cytotoxic compounds were preliminary tested against HeLa and KB cell lines using this bioassay [18].

HeLa cells were grown in DMEM with 15% FBS and antibiotics (penicillin 100 U/mL, streptomycin 100 µg/ml) at 37 °C in a humidified, 5% carbon dioxide atmosphere. The cells were then tripsinised and seeded into standard 96-well cell culture plates. After 16 h incubation the cells were treated with respective test compounds for 48 h at concentrations of 0.1, 1, 10, 100 µg/ml). In parallel, controls with equivalent concentrations to the respective serial dilutions of the test compounds are maintained. A solubilization solvent usually DMSO, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution is quantified by measuring at 570 nm by a spectrophotometer. The absorption maximum is dependent on the solvent employed.

3.3. Results and Discussion

In Brine shrimp lethality test, compounds **5a, 5b, 12a, 12b, 15a, 15b, 15c, 15d** and **16** have been shown to be 100% cytotoxic compared to taxol as a reference standard. These compounds at all the concentrations of 0.1, 1, 10 and 100 µg/ml have killed all the *nauplii*. Based on these positive results in order to study further the potential of these compounds, three compounds, namely an *O*-benzylated, a *N*-ethylated, and a *N*-benzylated derivatives, were selected and sent to Taiho Pharmaceutical Co Ltd. (Japan) and Naeja pharmaceuticals in Edmonton for more rigorous testing on representative solid cancer cell lines. The results of these tests are described in the Table 3.2.

Table 3.2. Cytotoxic activity of compounds **5a** and **5b** nad **12a** against Kb and HeLa cell lines

Compound	Kb-IC_{50} (μM)	HeLa-IC_{50}(μM)
5a	>50	90
5b	--	>100
12a	24.7	--

Based on the above cytotoxicity results, it is hypothesized that the *N*-alkylated compounds (**5a** and **5b**) did not display reasonable cytotoxicity against both cell lines, while compound **12a** (the *O*-benzylated analog exhibited mild cytotoxicity against Kb cell line.

3.4. Conclusion

Quinoline scaffold is considered as a privileged scaffold in cancer drug discovery. Novel quinoline derivatives were designed as anticancer iron chelators. This scaffold has been the subject of various studies which triggered the synthesis of a series of new molecules that display different biological activities. A series of thieno- and furoquinolones have been developed based on the structure of compound **B**. Despite the lack of reasonable cytotoxic potency in these compounds, this study has shed light on a novel series of quinolones that have the potential of being studied as early leads to discover novel antitumor agents.

3.5. Experimental

All chemicals were supplied by Sigma-Aldrich Canada. All commercially available reagents were used without purification. Solvents were used as obtained from the supplier unless otherwise stated. Organic solutions were dried over anhydrous sodium sulfate, filtered, and concentrated with a Buchi

rotary evaporator at reduced pressure. Melting points were recorded by open tube capillary method on a *Thomas-Hoover* capillary apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on 500 MHz or 300 MHz Burker AVANCE spectrometers using DMSO-d_6, $CDCl_3$, acetone-d_6 or TFA-d as solvents. Chemical shifts were given in ppm (δ) using TMS as an internal standard, and the spectral data were consistent with the assigned structures. Coupling constants (*J*) were given in Hz. Mass spectra were recorded on Agilent 1100 series LC/MSD chromatographic system. High resolution mass spectra (EI / ESI) were obtained on a Waters GCT Premier Micromass spectrometer. Silicycle Ultrapure silica gel (0-20 μm) G and F-254 were used for the preparative-layer TLC, and Silicycle Ultrapure Flash silica gel (40-63 μm) was used for flash column chromatography. Commercial precoated silica gel plastic sheets (Polygram SIL G/UV254) were used for thin layer chromatography (TLC). TLC was performed to monitor progress of the reaction and purity of the synthesized compounds, spots being located using UV-light. Yields are of purified product and are not optimized.

Ethyl 3-(2,4-difluorophenyl)-3-oxopropanoate (1) :

A mixture of potassium ethylmalonate (13.39 g, 76.97 mmol) and magnesium chloride (16.41 g, 172.41 mmol) in acetonitrile (150 mL) was stirred under nitrogen for 30 min at room temperature. Triethylamine (17.19 g,169.92 mmol) was then added dropwise to the reaction mixture, and the mixture was stirred for 2h. To this mixture, a solution of acetonitrile (20 mL) containing 2,4-difluorobenzoyl chloride (10.00 g, 56.64 mmol) was added dropwise at 5-10 ^{0}C over approximately10 min . The reaction mixture was stirred for 2 h at room temperature and 1 mL of triethylamine was then added to the reaction mixture and was stirred at room temperature overnight. The solvent was then removed under vacuum and toluene was added. The reaction mixture was then acidified with 6N HCl and mixed vigorously. The organic layer was separated, washed with water (100 mL x 2) followed by brine solution. The organic layer was dried

over sodium sulfate and evaporated under vacuum to afford compound **1** as a colorless oil (10.985 g, 85%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.08-7.98 (1H, m)), 7.01-6.59 (1H, m), 6.90-6.86 (1H, m), 4.26-4.19 (2H, q, J = 7.5 Hz), 3.98-3.95 (2H, q, J = 7.5 Hz), 1.29-1.25 (3H, t, J = 7.0 Hz). **APCI-MS** (+ve mode): (*m/z*) 229.0 ($[M+H]^+$, 100).

Ethyl (2Z)-3-(benzylamino)-2-[(2,4-difluorophenyl)carbonyl]-3-(methylsulfanyl)-prop-2-enoate (2a) :

A mixture of compound **1** (5.00 g, 21.91mmol), potassium hydroxide (1.51 g, 6.62 mmol), and *n*-tetrabutylammonium bromide (7 mg, 0.21 mmol) in dimethylformamide (DMF) (50 mL) was stirred for 30 min at room temperature. The mixture was then cooled to 0 ^{0}C, and to it was added benzyl isothiocyanate dropwise (4.90 g, 32.9 mmol). After the mixture being stirred for 16 h at room temperature, iodomethane (3.449 g, 24.38 mmol) was added to it and the stirring was continued for additional 3 h. The reaction mixture was quenched with water (100 mL) and saturated aq. ammonium chloride (70 mL) and extracted with ethyl acetate (AcOEt) (2 X 50 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL) and evaporated to dryness under reduced pressure. An analytical sample (viscous yellow liquid) was obtained via column chromatography purification on silica gel (eluent: 0-20% v/v AcOEt in hexanes). **^{1}H NMR** ($CHCl_3$ d3, 500 MHz) δ: 11.71 (1H, s), 7.53-7.47 (1H, m), 7.39-7.30 (5H, m), 6.90-6.86 (1H, m), 6.80-6.76 (1H, m), 4.81 (2H, J = 6.7 Hz), 3.96-3.92 (2H, m), 2.44 (3H, s), 0.94-0.91 (3H, t, J = 6.5 Hz). **APCI-MS** (+ve mode): (*m/z*) 392.1 ($[M+H]^+$, 100).

Ethyl (2Z)-2-[(2,4-difluorophenyl)carbonyl]-3-(ethylamino)-3-(methylsulfanyl)-prop-2-enoate (2b):

A mixture of compound **1** (5.00 g, 21.91 mmol), potassium hydroxide (1.51 g, 31.68 mmol), and *n*-tetrabutylmmonium bromide (7 mg, 0.21 mmol) in DMF (50 mL) was stirred for 30 min at room temperature. The mixture was then cooled to 0 °C, and to it was added ethyl isothiocyanate dropwise

(2.86 g, 32.9 mmol). After stirring the mixture for 16 h at room temperature, iodomethane (3.449 g, 24.38 mmol) was added to the reaction mixture and continued stirring for additional 3 h. The reaction mixture was quenched with water (100 mL) and saturated aq. ammonium chloride (70 ml) and extracted with AcOEt (2 X 50 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL) and evaporated to dryness under reduced pressure. A viscous liquid was obtained via column chromatography purification on silica gel (eluent: 0-15% v/v EtOAc in hexanes). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 11.60 (s, 1H), 7.48-7.45 (m, 1H), 6.89-6.86 (m, 1H), 6.78-6.75 (m, 1H), 3.94-3.90 (q, J = 6.3 Hz, 2H), 3.66-3.62 (q, J = 6..1 Hz, 2H), 2.48 (s, 3H), 1.35-1.32 (t, J = 5.9 Hz, 3H), 0.93-0.89 (t, 3H). **APCI-MS** (+ve mode): (*m/z*) 330.1 ($[M+H]^+$, 100).

Ethyl 1-benzyl-7-fluoro-2-(methylsulfanyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (3a) :

Potassiun-*tert*-butoxide (*t*-BuOK) (1.10 g, 9.69 mmol) was added to a solution of compound **2** (3.161 g, 8.077 mmol) in toluene (25 mL) at room temperature. The resulting mixture was refluxed for 16 h, cooled to room temperature, and diluted with water (100 mL). The aqueous layer was separated and extracted with EtOAc (20 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), then evaporated to dryness. The crude compound was purified using column chromatography (eluent: 0-40% EtOAc in Hexanes). **^{1}H NMR** (CDCl3, 500 MHz) δ: 8.45 (dd, J = 7.5 Hz, 1H,), 7.34 (m, 3H), 7.06 (4H, m), 5.89 (2H, s), 4.46 (q, J = 5.9 Hz, 2H,), 2.42 (3H, s), 1.42 (3H, J = 6.3 Hz, t); **^{13}C NMR** ($CDCl_3$, 500 MHz) δ: 173.19, 167.19, 165.78, 163.84, 148.52, 143.11, 142.95, 135.25, 130.15, 130.01, 129.37, 128.13, 127.24, 123.77, 113.45, 104.15, 61.87, 52.42, 20.61, 14.20. **APCI-MS** (+ve mode): (*m/z*) 371.2 ($[M+H]^+$, 100); **HR-MS** (EI^+): calcd for $C_{20}H_{18}FNO_3S$ (371.4319); found (371.0989).

Ethyl 1-ethyl-7-fluoro-2-(methylsulfanyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (3b) :

tert-BuOK (1.10 g, 9.69 mmol) was added to a solution of compound **3** (2.66 g, 8.077 mmol) in toluene (25 mL) at room temperature. The resulting mixture was refluxed for 16 h, cooled to room temperature,

and diluted with water (100 mL). The aqueous layer was separated and extracted with AcOEt (20 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), then evaporated to dryness. The crude compound was then purified using column chromatography (eluent: 0-40% EtOAc in hexanes). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 8.45 (1H, J = 6.71 Hz, J = 8.95 Hz, dd), 7.21 (1H, J = 11.2 Hz, J = 2.1 Hz, dd), 7.11 (1H, J = 9.1 Hz, J = 7.9 Hz, J = 2.2 Hz ddd), 4.61 (2H, J = 28.4 Hz, d), 4.44 (2H, J = 7.14 Hz, q), 2.60(3H, s), 1.47 (3H, J = 7.1 Hz, t), 1.41(3H, J = 9.1 Hz, J = 5.3 Hz, dd); **^{13}C NMR** ($CDCl_3$, 500 MHz) δ: 173.28, 167.28, 165.89, 163.93, 147.68, 142.25, 130.37, 126.96, 123.86, 113.14, 103.02, 61.76, 44.13, 20.77, 143.38. **APCI-MS** (+ve mode): (*m/z*) 310.03 ($[M+H]^+$, 100); **HR-MS** (EI^+): calcd for $C_{15}H_{16}FNO_3S$ (309.3558); found (309.0831).

Ethyl 1-benzyl-7-fluoro-2-(methylsulfinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (4a) :

To a solution of compound **4** (1.50 g, 3.83 mmol) in DCM (5 mL) at 0 ^{0}C was added MCPBA (1.189 g, 6.894 mmol) and the reaction mixture was allowed to stir at room temperature for 7-8 h. It was then neutralized using 3% sodium bicarbonate solution (10 mL) and washed with brine solution. The organic layer was separated, dried over sodium sulfate and evaporated under vacuum to give a colorless viscous liquid, which was then purified using column chromatography (eluent: 0-80% EtOAc in hexane) to give a white amorphous powder (0.415g, 28%). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 8.48-8.45 (m, 1H), 7.39-7.31 (m, 3H), 7.15-7.12 (m, 1H), 7.06-7.02 (m, 3H), 6.12-5.99 (dd, J = 8.0 Hz, J = 9.3 Hz, 2H), 4.47-4.42 (q, J = 5.8 Hz, 2H), 2.95 (s, 3H), 1.46-1.40(t, J = 6.3 Hz, 3H). **APCI-MS** (+ve mode): (*m/z*) 388.01 ($[M+H]^+$, 100).

Ethyl 1-ethyl-7-fluoro-2-(methylsulfinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (4b) :

To a solution of compound **5** (2.00 g, 6.46 mmol) in DCM (10 mL) at 0 ^{0}C was added MCPBA (1.95 g, 11.30 mmol) and the reaction mixture was allowed to stir at room temperature for 7-8 h. It was then neutralized using 3% sodium bicarbonate solution (10 ml) then washed with brine solution. The organic layer was separated, dried over sodium sulfate and evaporated under vacuum to give a colorless viscous

liquid, which was then purified under column chromatography (eluent: 0-80% EtOAc in hexane) to give a white amorphous powder (0.530g, 25.2%). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 8.49-8.41 (m, J = 8.5 Hz, J = 11.5 Hz, 1H), 7.25-7.13 (m, 2H), 4.69-4.62 (q, J = 8 Hz, 2H), 4.45-4.41 (q, J = 7.5 Hz, 2H), 3.22 (s, 3H), 1.53-1.51 (t, J = 7.6 Hz, 3H), 1.40-1.38 (t, J = 7.5 Hz, 3H). **APCI-MS** (+ve mode): (*m/z*) 326.0 ($[M+H]^+$, 100).

Ethyl 9-benzyl-7-fluoro-3-hydroxy-4-oxo-4,9-dihydrothieno[2,3-*b*]quinoline-2-carboxylate (5a) :

A mixture of compound **4a** (1.00 g, 2.58 mmol), ethyl mercaptoacetate (2.58 mmol), triethylamine (7.74 mmol) in anhydrous ethanol (30 mL) was refluxed for 5 h under nitrogen atmosphere. The solvent was evaporated to dryness under reduced pressure, water was added (8 mL) and the solid obtained was collected by filtration. Further purification accomplished by recrystallization from ethanol/water gave a yellow powder (5%). Mp: 140-143 ^{0}C. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 11.11 (s, 1H), 8.52-8.49 (m, 1H), 7.39-7.33 (m, 3H), 7.18-7.10 (m, 3H), 7.07-7.00 (m, 1H), 5.43 (s, 2H), 4.38-4.33 (q, J = 6.0 Hz, 2H), 1.39-1.36 (t, J = 6.5 Hz, 3H).

APCI-MS (+ve mode): (*m/z*) 397.01 ($[M+H]^+$, 100).

Ethyl 9-ethyl-7-fluoro-3-hydroxy-4-oxo-4,9-dihydrothieno[2,3-*b*]quinoline-2-carboxylate (5b) :

A mixture of compound**4b** (1.20 g, 3.68 mmol), ethyl mercaptoacetate (3.68 mmol), triethylamine (11.04 mmol) in anhydrous ethanol (30 mL) was refluxed for 5 h under nitrogen atmosphere. The solvent was evaporated to dryness under reduced pressure, water was added (8 mL) and the solid obtained was collected by filtration. Further purification accomplished by recrystallization from ethanol/water to give a yellow powder (5%). Mp: 118-120 ^{0}C. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 11.15 (s, 1H), 8.51-8.43 (m, 1H), 7.20-7.10 (m, 2H), 4.43-4.33 (q, J = 7.8 Hz, 2H), 4.32-4.22 (q, J = 7.6 Hz, 2H), 1.59-1.49 (t, J = 7.5 Hz, 3H), 1.45-1.35 (t, J = 7.4 Hz, 3H). **APCI- MS**: (*m/z*) 335 ($[M+H]^+$, 100).

3-Fluorophenyl Isothiocyanate (6) :

To a stirred solution of 25.00 g (224.98 mmol) of 3-fluoroaniline and triethylamine (68.303 g, 675 mmol) was added carbon disulfide (18.84 g, 247.5 mmol) dropwise under ice cooling. The reaction mixture was kept under ice-cooling overnight. The resulting precipitate was collected by filtration, washed with toluene, and dried to give triethylammonium (3-fluorophenyl)dithiocarbamate (61.42 g, 90%,) as a pale yellow powder, which, without further purification, was suspended in 150 mL of $CHCl_3$ and (23.779 g, 235 mmol) of triethylamine. To this suspension was added dropwise 25.513 g (235.1 mmol) of ethyl chloroformate with stirring under ice cooling. After stirring at room temperature for 15 h, the reaction mixture was poured into ice-water and extracted with n-hexane. The organic layer was washed with dilute aqueous HCl and water, then dried over sodium sulfate and concentrated to dryness under reduced pressure to afford a crude oil, which was chromatographed on silica gel with n-hexane to give (22.817 g, 69.7%) of 3-fluorophenyl isothiocyanate (**6**). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.34-7.29 (m, 1H), 7.04-6.95 (m, 2H), 6.96-6.92 (m, 1H)**. APCI- MS**: (*m/z*) 154.06 ($[M+H]^+$, 100).

Diethyl {[(3-fluorophenyl)amino](methylsulfanyl)methylidene}propanedioate (8) :

To a stirred suspension of (3.655 g, 145.05 mmol) NaH 95% in 150 mL of tetrahydrofuran (THF) maintained at 5 ^{0}C was added dropwise 23.232 g (145.05 mmol) of diethyl malonate for 15 min. After stirring at room temperature for 30 min (22.00 g, 143.62 mmol) of 3-fluorophenyl isothiocyanate in THF (80 mL) was added gradually to this mixture under ice cooling. The reaction mixture was allowed to stand at 4 ^{0}C for 15 h. The resulting precipitate was collected by filtration, washed with ether, and dried to afford the sodium salt of diethyl [[(3-fluorophenyl)amino] thiomethyl]-malonate (7) (40.934 g, 85%). To a solution of sodium salt (**7**) in DMF (120 mL) was added dropwise 34.661 g (244.2 mmol) of methyl iodide under ice cooling. After stirring at the same condition for 1.5 h, the reaction mixture was warmed gradually to room temperature and was stirred for an additional 2 h. The reaction mixture was then poured

into water and extracted with DCM. The organic layer was washed with water, dried over sodium sulfate, and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel with *n*-hexane to give compound **8** (25.967 g, 65%). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 10.53 (s, 1H), 7.15-7.07 (m, 2H), 6.89-6.52 (m, 2H), 4.42-4.11 (m, 4H), 2.01 (s, 3H), 1.37-1.25 (m, 6H).

Ethyl 7-fluoro-4-hydroxy-2-[(thiomethyl)] quinoline-3-carboxylate (9):

Pure compound **8** (23.03 g, 70.35 mmol), was heated at 180 ^{0}C under vaccum for 10-15 min. The reaction mixture was then left to cool down to room temperature giving a solid precipitate, which was then recrystallized from AcOEt/hexane to give compound **9** as an amorphous powder (10.729 g, 40%). Mp: 121-123 ^{0}C. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 13.31 (s, 1H), 8.21-8.19 (dd, J =5.0 Hz, J = 11.0 Hz, 1H), 7.43-7.32 (dd, J = 2.8 Hz, J = 11.0 Hz), 1H), 7.16-7.05 (dd, J = 3.8 Hz, J = 9.0 Hz, 1H), 4.57-4.52 (q, J = 8.0 Hz, 2H), 2.58 (s, 3H), 1.55-1.52 (t, J = 8.0 Hz, 3H). **APCI-MS** (+ve mode): (*m/z*) 282.01 ($[M+H]^+$, 100).

Ethyl 4-(benzyloxy)-7-fluoro-2-(methylsulfanyl)quinoline-3-carboxylate (10a):

Method 1: To a solution of compound **9** (5.00 g, 17.77 mmol) and potassium carbonate (4.893 g, 35.4 mmol) in DMF (50 mL) was added benzyl bromide (6.054 g, 35.4 mmol) and the mixture was allowed to stir at 70-80 ^{0}C for 8 h. The reaction mixture was then warmed to room temperature, washed with water, and extracted with DCM. The organic extract was then dried over sodium sulfate and filtered. The filtrate was evaporated under reduced pressure to give a crude compound which was purified by column chromatography (0.5% AcOEt in hexane) to give compound **10a** as a pale yellow highly viscous oil (2.64 g, 40%).

Method 2 (Modified Mitsunobu): To a solution of compound **9** (3.5 g, 12.44 mmol) triphenylphosphine (3.92 g ,14.93 mmol) and benzyl alcohol (1.35 g , 12.44 mmol) in THF (10ml), diisopropyl

azodicarboxylate (DIAD) (3.02 g, 14.93 mmol) was added dropwise. The reaction mixture was stirred for 6 hours. After the removal of the solvent under reduced pressure, anhydrous diethyl ether was added and allowed to stand for 5 hrs, then filtered. The diethyl ether was then evaporated under reduced pressure and the oily residue was purified by silica gel column chromatography (0.5% AcOEt in hexane) to give compound **10a** as a pale yellow highly viscous oil (0.69 g, 15%). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 7.99 (dd, 1H, J = 9.1 Hz, J = 6.1 Hz), 7.54 (dd, 1H, J = 10.1 Hz, J = 2.5 Hz), 7.41 (5H, m), 7.15 (ddd, 1H, J = 9.1 Hz, J = 8.3 Hz, J = 2.5 Hz), 5.22 (2H, s), 4.45 (2H, m), 2.68 (3H, s), 1.41(t, 3H, J = 7.2Hz) ; **^{13}C NMR** ($CDCl_3$, 500 MHz) δ: 166.13, 166.00, 162.67, 159.99, 159.88, 150.58, 150.41, 135.95, 128.73, 128.01, 125.47, 125.33, 117.84, 116.14, 115.54, 115.21, 112.23, 111.96, 62.30, 14.08. **APCI-MS** (+ve mode): (*m/z*) 372.08 ($[M+H]^+$, 100); HR-MS (EI^+): calcd for $C_{20}H_{18}FNO_3S$ (371.4252); found (371.0992).

Ethyl 4-ethoxy-7-fluoro-2-(methylsulfanyl)quinoline-3-carboxylate (10b):

To a solution of compound **9** (5.00 g, 17.77 mmol) and potassium carbonate (4.893 g, 35.4 mmol) in DMF (50 mL) was added ethyl bromide (5.521 g, 35.4mmol) and the reaction mixture was allowed to stir at 70-80 ^{0}C for 8 h. The reaction mixture was then warmed to room temperature, washed with water and extracted with DCM. The organic extract was dried over sodium sulfate and filtered. The filtrate was evaporated under reduced pressure on rotavapor to give crude compound which was then purified by column chromatography (0.5% AcOEt in hexane) to give compound **10b** as a pale yellow highly viscous oil (2.64 g, 45%).

Using the **method 2** procedure as outlined for preparing **10a,** compound **10b** was prepared using compound **9** (2.8 g, 9.96 mmol), ethyl alcohol (0.458g, 9.96 mmol), triphenylphosphine (3.13 g,11.95 mmol), DIAD (2.42 g, 11.95 mmol), THF (8 ml), with a yield of 13% (0.4 g) as a yellow viscous oil. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 8.06 (dd, 1H, J = 9.1 Hz, J = 6.2 Hz), 7.53 (dd, 1H, J = 10.1 Hz, J = 2.4 Hz), 7.19 (ddd, 1H, J = 9.0 Hz, J = 8.4 Hz, J = 2.5 Hz), 4.48 (q, 2H, J = 7.1 Hz), 4.27 (q, 2H, J = 7.0 Hz), 2.66

(3H, s), 1.46 (dt, 6H, J = 12.2 Hz, *J* = 7.1 Hz). **APCI-MS** (+ve mode): (*m/z*) 310.08 ($[M+H]^+$, 100); **HR-MS** (EI^+): calcd for $C_{15}H_{16}FNO_3S$ (309.3558); found (309.0838).

Ethyl 4-(benzyloxy)-7-fluoro-2-(methylsulfonyl)quinoline-3-carboxylate (11a):

To a solution of compound **10a** (1.50 g, 4.04 mmol) in DCM (10 mL) at 0-5 ^{0}C was added MCPBA [60%] (0.962 g, 5.58 mmol) and the mixture was allowed to stir overnight. The reaction mixture was then washed with 30% sodium bicarbonate solution (10 mL) and the organic layer was then dried over sodium sulfate and filtered. The filtrate was evaporated to give a white amorphous solid, which was then purified with 7% AcOEt/hexane using column chromatography to give compound **11a** as a white amorphous powder (0.675 g, 45%). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 8.15-8.13 (m 1H), 7.85-7.75 (m, 1H), 7.53-7.41 (m, 6H), 5.35 (s, 2H), 4.52-4.48 (q, *J* = 7.5 Hz, 2H), 3.42 (s, 3H), 1.50-1.41 (t, *J* = 7.2 Hz, 3H). **APCI-MS** (+ve mode): (*m/z*) 404.01 ($[M+H]^+$, 100)

Ethyl 4-ethoxy-7-fluoro-2-(methylsulfonyl)quinoline-3-carboxylate (11b):

To a solution of compound **10b** (1.50 g, 4.85 mmol) in dichloromethane (8 mL) at 0-5 ^{0}C was added MCPBA [60%] (0.962 g, 5.58 mmol) and the mixture was allowed to stir overnight. The reaction mixture was then washed with 30% sodium bicarbonate solution (10 mL) and the organic layer was dried over sodium sulfate and filtered. The filtrate was evaporated to give a white amorphous solid, which was purified with 7% AcOEt/hexane using column chromatography to give compound **11b** as a white amorphous powder (35-40%). This compound was used in further step. **APCI-MS** (+ve mode): (*m/z*) 326.03 $[M+H]^+$, 100).

Ethyl 4-(benzyloxy)-7-fluoro-3-hydroxythieno[2,3-*b*]quinoline-2-carboxylate (12a) :

A mixture of compound **11a** (3.00 g, 7.436 mmol), ethyl mercaptoacetate (1.00 mmol), Et3N (3.00 mmol) in anhydrous ethanol (30 mL) was refluxed for 5 h under nitrogen atmosphere. The solvent was

evaporated to dryness under reduced pressure, water was added (8 mL) and the solid obtained was collected by filtration. Further purification accomplished by recrystallization from ethanol/water gave compound **12a** as yellow crystals (5%) M.P =135 ^{0}C. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 11.14 (s, 1H), 8.21-8.18 (dd, J = 6.20 Hz, J = 9.30 Hz, 1H), 7.66-7.64 (dd, J = 2.4 Hz, J = 10.1 Hz, 1H), 7.50-7.38 (m, 5H), 7.24-7.20 (m, 1H), 5.50 (s, 2H), 4.50-4.45 (q, J =7.0 Hz, 2H), 1.47-1.46 (t, J = 7.1 Hz, 3H). **APCI-MS** (+ ve mode) :(*m/z*) 398.10 ([M+H]$^+$, 100); **HR-MS** (EI$^+$): calcd for $C_{21}H_{16}FNO_4S$(397.4194); found (397.0786).

Ethyl 4-(ethoxy)-7-fluoro-3-hydroxythieno[2,3-b]quinoline-2-carboxylate (12b) :

A mixture of compound **11b** (3.00 g, 9.2 mmol), ethyl mercaptoacetate (1.00 mmol), Et3N (3.00 mmol) in anhydrous ethanol (30 mL) was refluxed for 5 h under nitrogen atmosphere. The solvent was evaporated to dryness under reduced pressure, water was added (8 mL) and the solid obtained was collected by filtration. Further purification accomplished by recrystallization from ethanol/water to obtain compound **12b** (0.177 g, 5% Yield) M.P = 120-125 ^{0}C. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 11.02 (s, 1H), 8.39-8.34 (m, 1H), 7.67-7.63 (m, 1H), 7.33-7.28 (m, 1H), 4.59-4.53 (q, J = 7.5 Hz, 2H), 4.48-4.42 (q, J = 7.2 Hz, 2H), 1.58-1.53 (t, J = 7.1 Hz, 3H), 1.46-1.42 (t, J = 7.5 Hz, 3H). **APCI-MS** (+ve mode): (*m/z*) 336.2 ([M+H]$^+$, 100).

Ethyl 2-[(3-fluorophenyl)amino]-4-oxo-4,5-dihydrofuran-3-carboxylate (13):

Sodium hydride (4.35 g, 179.98 mmol) washed with dry n-hexane, was suspended in anhydrous THF (80 mL) and a solution of diethyl malonate (28.827 g, 179.98 mmol) in THF was added drop-wise. The clear salt was transferred to dropping funnel, and added drop-wise to a solution of chloroacetylchloride (10.839 g, 95.98 mmol) in THF (180 mL) over a period of 1 h. The reaction mixture was stirred for 2.5 h. Triethylamine (24.224 g, 239.4 mmol) was added to the reaction mixture and it was stirred for 2 h. 3-Fluoroaniline (10.00 g, 89.99 mmol) in anhydrous THF (500 mL) was then added drop-wise over a period

of 1 h. The reaction mixture was stirred overnight, THF was evaporated *in vacuo*, followed by the addition of water and extraction with chloroform. The organic layer was washed with water and dried over sodium sulfate. Chloroform was evaporated under vacuum and the residue was triturated with ether, filtered and washed with additional ether to yield compound **13** as an amorphous solid (10.98 g, 46 % yield). Mp: 160-165 ^{0}C. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ : 10.33 (s, 1H), 7.36 (m, 1H), 7.21 (m, 1H), 7.12 (m, 1H), 6.95 (s, 1H), 4.72 (s, 2H), 4.39 (q, J = 7.3 Hz, 2H), 1.40 (t, J = 6.2 Hz, 3H). **APCI-MS** (+ve mode): (*m/z*) 266 ($[M+H]^+$, 100).

7-Fluorofuro[2,3-*b*]quinoline-3,4(2*H*,9*H*)-dione (14) :

Compound **13** (5.00 g, 18.85 mmol) was added with stirring in one pot to Dowtherm (50 mL) which was maintained at 240 ^{0}C. The temperature was then raised to 255 ^{0}C and maintained for 10 min. The mixture was then cooled to room temperature and diluted with a large volume of hexane to precipitate a dark solid that was collected and washed with hot hexane. Yield of the crude product was 2.065 g (50% yield). **^{1}H NMR** (DMSO-d_6 300 MHz) δ : 13.32 (s, 1H), 8.15 (m, 1H,), 7.27-7.2 (m, 2H), 4.84 (s,1H). **APCI-MS** (+ve mode): (*m/z*) 220 ($[M+H]^+$, 100).

9-Benzyl-7-fluorofuro[2,3-*b*]quinoline-3,4 (2*H*,9*H*)-dione (15a):

To a solution of compound **14** (4.00 g, 18.24 mmol) and potassium carbonate (3.782 g, 27.36 mmol) in DMF (40 mL) was added benzyl chloride (4.617 g, 36.48 mmol) dropwise and the reaction mixture was stirred at 70-80 ^{0}C for 7-8 h. The reaction mixture was then allowed to cool to room temperature, washed with cold water and extracted with DCM. The organic extract was dried over sodium sulfate and the solvent was evaporated under vacuum to give a dark brown solid. It was then recrystallized in DCM/hexane to give compound **15a** as a dark brown amorphous solid (0.560 g, 10% yield). Mp > 200 ^{0}C. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ: 8.47 (m, 1H), 7.42-7.35 (m, 3H), 7.24 (m, 2H), 7.04 (m, 2H), 5.44 (s,

1H), 4.81 (s, 2H). **APCI-MS** (+ve mode): (*m/s*) 310.3 ([M+H}$^+$, 100); **HR-MS** (EI$^+$): calcd for $C_{18}H_{12}FNO_3$ (309.2911); found (309.0803).

7-Fluoro-9-(4-fluorobenzyl)furo[2,3-*b*]quinoline-3,4 (2*H*,9*H*)-dione (15b) :

The title compound was synthesized according to the procedure used for compound **15a** except that the fluorobenzyl chloride was used to yield compound **15b** as an amorphous powder (0.579 g, 9.7%). Mp: > 200 ^{0}C. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ : 8.42 (s, 1H), 7.00-7.25 (m, 6H), 5.42 (s, 2H), 4.81 (s, 2H). **APCI-MS** (+ve mode): (*m/s*) 328.2 ([M+H}$^+$, 100); **HR-MS** (EI$^+$): (*m/z*) calcd for $C_{18}H_{11}F_2NO_3$ (327.2816); found (327.0719).

7-Fluoro-9-(4-methoxybenzyl)furo[2,3-*b*]quinoline-3,4 (2*H*,9*H*)-dione (15c):

The title compound was synthesized according to the procedure for compound **15a** except that the *p*-methoxybenzyl chloride was used here to yield compound **15c** as an amorphous powder (0.618 g, 10%). Mp: > 200 ^{0}C. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ: 8.47 (dd, J = 9.1 Hz, J = 6.4 Hz, 1H), 7.20-7.03 (m, 4H), 6.92 (d, J= 8.7 Hz, 2H), 5.38 (s, 2H), 4.82 (s, 2H), 3.79 (s, 3H). **APCI-MS** (+ve mode): *m/z* 339.3 [M+H]$^+$, 100).

7-Fluoro-9-cyanomethylfuro[2,3-*b*]quinoline-3,4 (2*H*,9*H*)-dione (15d):

The title compound was synthesized according to the procedure used to synthesize compound **15a** except that the bromoacetonitrile was used here to yield compound **15d** as amorphous powder (0.387 g, 8%). Mp: > 200 ^{0}C. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ : 8.32-8.24 (m, 1H), 7.93-7.84 (m, 1H), 7.45-7.35 (m, 1H), 5.63 (s, 2H), 5.00 (s, 2H). **APCI-MS** (+ve mode): *m/z* 259.2 [M+H]$^+$, 100), **HR-MS** (EI$^+$): calcd for $C_{18}H_{12}FN_2O_3$ (258.2046); found (258.0447).

3-Chloro-7-fluoro-4-oxo-4,9-dihydrofuro[2,3-*b*]quinoline-2-carbaldehyde (16):

To a cold solution of phosphorus oxychloride (13.98 g, 91.2 mmol) in DMF (10 mL) , compound **14** (4.00 g, 18.25 mmol) was added. The mixture was stirred at 50-60 ^{0}C for 5-6 h, cooled to room temperature then poured into ice-cold water. The residue was extracted with DCM (15 mL) and the organic layer was dried over sodium sulfate and evaporated under vacuum to give a dark brown residue which was then precipitated using DCM/hexane to give compound **16** as a dark yellow amorphous powder (0.531 g, 11%). Mp = 190-195. **^{1}H NMR** (CDCl3, 300 MHz) δ : 10.07 (s, 1H), 8.48-8.43 (dd, *J* = 9.4 Hz, *J* = 6.1 Hz, 1H), 7.95-7.90 (dd, *J* = 10.1 Hz, *J* = 2.5 Hz, 1H), 7.79-7.72 (m, 1H). **APCI-MS** (- ve mode): (*m/z*) 264.1 ([M-H]$^+$, 100).

References

1. Chen, G. L., Yang, L., Rowe, T. C., Halligan, B. D., Tewey, K. M., Liu, L. F. (1984). *J. Biol. Chem.* **259**, 13560.

2. Amoozgar, Z. (2007). *MSc dissertation* # MR41981. Design, Synthesis and Biological Evaluation of Novel Quinoline-Based Molecules with Potential Anticancer Activity. School of Pharmacy, MUN, St.John's, Newfoundland and Labrador, Canada.

3. Hosomi, J., Ashin, Y., and Suzue, S. (1989). *PCT Int. Appl. WO 89 12055.* Preparation of Thiazoloquinolonecarboxylic acid derivatives and their pharmaceutical compositions as antitumor agents. *Chem. Abstr.,* 1990, **113**, 6328m.

4. Clay, R. J., Collom, T. A., Karrick, G. L. and Wemple, J. (1992). A Safe, Economical Method For the Preparation of β-Oxo Esters. *Synthesis*, 290-292.

5. Baker, W. R., Cai, S., Dimitroff, M., Fang, L., Huh, K. K., Ryckman, D. R., Shang, X., Shawar, R. M., Therrien, J. H. (2004). A Prodrug Approach toward the Development of Water Soluble Fluroquinolones and Structure-Activity Relationship of Quinoline-3-carboxylic Acids. *J. Med. Chem.*, **47**, 4693-4709.

6. Hashimoto, A., Pais, G. C. G., Wang, Q., Lucien, E., Incarvito, D. C., Deshpande, M., Bradbury, B. J., Wiles, J. A. (2007). Practical Synthesis and molecular structure of a potent broad spectrum antibacterial isothiazoloquinolone. *Org. Process. Res. Dev.*, **11**, 389-398.

7. Chu, D. T. W. (1990). Syntheses of 6-Fluoro-7-piperazin-1-yl-9-cyclopropyl-2,3,4,9-tetrahydroisothiazolo[5,4-b]quinoline-3,4-dione, *J. Heterocycl. Chem.*, **27**, 839-843.

8. Barazarte, A., Camacho, J., Dominguez, J., Lobo, G., Gamboa, N., Rodrigues, J., Capparelli, M. V., Alvarez-Larena, A., Andujar, S., Enriz, D. and Charris, J. (2008). Synthesis, antimalarial activity,

structure-activity relationship analysis of thieno-[3,2-*b*]benzothiazine *S,S*-dioxide analogs. *Bioorg. Med. Chem.*, **16,** 3661-3674.

9. Jinbo Y., Kondo, H., Inoue, Y., Taguchi, M., Tsujishita, H., Kotera, Y., Sakamoto, F. and Tsukamoto, G. (1993). Synthesis and antibacterial activity of a new series of tetracyclic pyridone carboxylic acids. *J. Med. Chem.*, **36**, 2621-2626.

10. Kaminsky, D. and Meltzer, R. I. (1968). Quinolone Antibacterial Agents. Oxolinic Acid and Related Compounds. *J. Med. Chem.*, **11**, 160-163.

11. Makara, G., Keseru, G. M. and Kovacs, A. (1994). On the Mechanism of the Alkylation of Quinolone and Naphthyridine Derivatives. *J. Chem. Soc. Perkin Trans., 2*, 591-594.

12. Frank, J., Meszaros, Z., Komives, T., Marton, A. F. and Dutka, F. (1980). Alkylation of Quinolones with Trialkyl Phosphates. Part 2. Mechanistic Studies. *J. Chem. Soc., Perkin Trans.*, **2**, 401-406.

13. Ukrainets, I. V., Taran, S. G., Gorokhova, O. V., Gorlacheva, I. V., Bezuglyi, P. A. and Turov. A. V. (1996). Alkylation of 1H-2-Oxo-3-Carbethoxy-4-hydroxyquinoline. *Chem. Heterocycl. Compds.*, **32**, 952-959.

14. Kay, I. T., Taylor, P. J. (1968). The Synthesis and physical Properties of Some 2-Alkylthio-4-oxo-quinoline-3-carboxylate Esters. *J. Chem. Soc. (C)*, 2656-2661.

15. Ambrozin, A.R., Leite , A.C., Silva, M., Vieira, P.C., Fernandes, J.B., Thiemann, O.H., da Silva, M.F., Oliva, G. (2005). Screening of Leishmania APRT enzyme inhibitors. *Die Pharmazie*, **60** (10), 781-4.

16. Kuo, Sheng-Chu, Huang, Sheng-Chieh, Huang, Li-Jiau, Cheng, Hong-Elk, Lin, Tsung-Ping, Wu, Chun-Hsiung and Ishii, K., Nakamura, H. (1991). Studies of Heterocyclic Compounds. VIII : Synthesis, Anti-inflammatory and Antiallergic Activities of *N*-Alkyl-2,3,4,9-tetrahydrofuro[2,3-*b*] quinoline-3,4-diones and Related Compounds. *J. Heterocyclic Chem.*, **28**, 955-963.

17. Finney, D.J. (1971). *Probit Analysis (3rd edition)*. Cambridge University Press, Cambridge, UK.

18. Dua, P., and Gude, R. (2006). Antiproliferative and antiproteolytic activity of pentoxifylline in cultures of B16F10 melanoma cells. *Cancer Chemother. Pharmacol.*, **58**, 195-202

CHAPTER 4

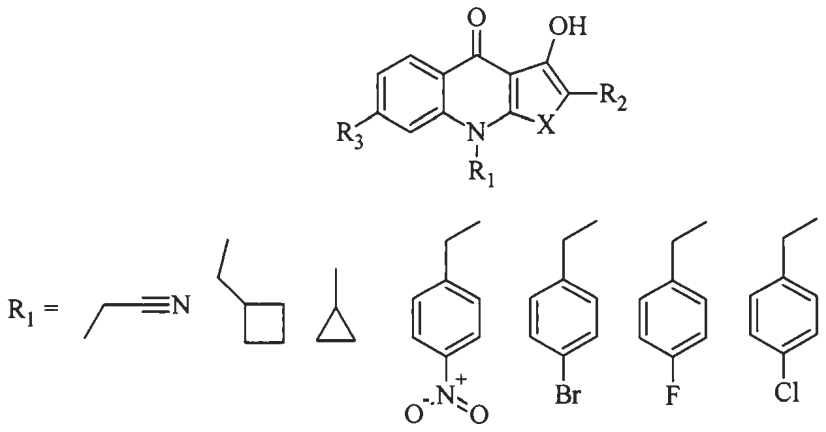
Conclusion and Future Research

One of the most successful achievements in the development of antibacterial agents was the synthesis of fluoroquinolones. Since then quinolones have been widely used clinically as antibacterial agents. They are the most popular antibiotics possesing broad spectrum of activity in humans. Quinolones have a very unique mode of action in that they act against two prokaryotic enzymes, DNA gyrase and topoisomerase IV, in a similar fashion. These agents target the topoisomerase catalytic cycle when the enzyme is covalently linked to the stabilized cleavage complex, thus blocking the progression of the replication and creating lesions. The structural distortion of the double helix stabilized by specific contact with the enzyme residues provides a favorable interaction site for quinolones. The common features in the mechanism of action of antibacterial quinolones and antitumor drugs of the epipodophyllotoxin family have provided a new light in this area of research thus directing quinolones to be used as potential anticancer agents.

As outlined in the Chapter 3 of this dissertation, we focused on the syntheses and testing of various derivatives of quinolones such as *N*-alkylated-, *O*-alkylated-thienoquinolones and furoquinolones, and attempted to study the cytotoxic profile of these compounds . All of the compounds have been synthesized by adaptation of the reported procedures in the references. The starting point of this research was set up by a graduate student, Zohreh Amoozgar, under the supervision of Dr. Mohsen Daneshtalab. An interesting chemistry has been studied on the behaviour of selectivity of alkylation using modified Mitsunobu reaction and base-catalyzed alkylation methods. In this study, as outlined in Chapter 3, we found that the Mitsunobu reaction is more oriented towards *O*-alkylation whereas parallel studies performed on the regioselectivity of alkylation in quinolones using base-catalyzed alkylation method gave a mixture of both *O*-alkylated and *N*-alkylated quinolones. The results of attempted alkylation reactions proved that the Mitsunobu method gives regioselectively *O*-alkylated quinolone analogues while the traditional base-catalyzed alkylations afford both *N*- and *O*-alkylated derivatives. In order to confirm the above assignment, we synthesized the *N*-alkylated quinolones by a different synthetic method. In the

same context, we reassigned the chemical shifts of the methylene protons attached to *O*-alkylated and *N*-alkylated products and proved the wrong assignments of these chemical shifts in previously published papers. Upon cytotoxicity testing of the synthesized compounds on both HeLa and Kb cell lines, we observed that despite weak cytotoxic activity of the majority of the synthesized compounds, a few of them have reasonable cytotoxicity against the above cell lines. Thus, we believe, with appropriate modifications of these compounds we can enhance the cytotoxic profile of the so called thienoquinolones and furoquinolones.

Based on this research and upon analyzing the results of biological assessments of the compounds synthesized, I would like to further dwell into this area by doing structural modification of the test compounds and explore their chemistry and biological activity profiles. (Figure 4.1)



R_2 = CO_2Et, COOH, $CONH_2$, CN

X = S, O

Figure 4.1. Proposed structures of future compounds to be synthesized

Based on the observations in the current project on the synthesis of various carboxylic acid analogues, I predict the suggested modifications may improve the cytotoxic profile of this class of

compounds due to improvement in the metal-ion chelating characteristics of the proposed molecules and further strengthening of bonding with the target protein molecule, topoisomerase II.