DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL QUINOLINE-BASED MOLECULES WITH POTENTIAL ANTICANCER ACTIVITY

ZOHREH AMOOZGAR

Design, Synthesis, and Biological Evaluation of Novel Quinoline-Based Molecules with Potential Anticancer Activity

by

© ZOHREH AMOOZGAR

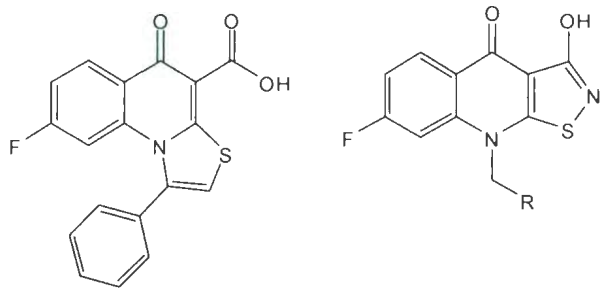
A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirement for the degree of Master of Science

School of Pharmacy, Memorial University of Newfoundland
St. John's, Newfoundland and Labrador, Canada

August 2007

Abstract

Quinolone derivatives have recently been introduced as inhibitors of eukaryotic topisomerase-II with potential anticancer activity. In an attempt to discover novel quinolones with potential anticancer properties, compound A, a clinical candidate with drug-like properties in different animal models, attracted our attention. Based on molecular modeling study using Alchemy-3^{TM}, compound B was designed in such a way that at its optimized steric/energetic configuration it overlapped perfectly with compound A.



Compound A

Compound B, R = Ph (**6a**);
Compound B derivatives (**6 b-n**)

Figure 1.0- Structures of compounds A , B, and derivatives of compound B

Different synthetic methodologies were utilized to synthesize compound B (**6a**) and its N_1-analogues (**6b-n**). During the structural optimization, a Mitsunobu-type reaction was used for regioselective N_1-alkylation of quinolones. Some of the final products (**6a, 6c, 6k**) and intermediates (**4d** and **13a,b,f**) were randomly screened for possible cytotoxic activity on different cancer and normal cell lines at 100 µg/ml concentration. The preliminary results indicate the cytotoxic potential of this class of compounds, especially compound **6c**, but a more detailed structure-activity relationships study is needed to confirm this hypothesis.

Dedicated to

My teachers: Drs. Nancy and Mohammad Behforouz, Dr. Seyed Habib Firouzabadi, Dr. Hashem Montaseri, Dr. Ali Mohagheghzadeh and Dr. Lynn Sousa for their thoughtfulness and constant support.

My Husband Reza Akbarpour, who endured this long process with me always offering support and love.

My parents who always had faith in me, even when I did not believe it myself, and to all deserving Iranian students.

Acknowledgment

Many thanks to my adviser Dr. Mohsen Daneshtalab, for his support, patience and help throughout the research, also for the numerous revisions and proof-readings. Thanks to my committee members, Dr. Richardson and Dr. Wang, who offered guidance. Thanks to the School of Pharmacy, Memorial University providing our lab with the financial means to complete this project. Thanks to Dr. Sunao Takeda and Dr. Chaomei Ma for their helpful advice during the research period. Thanks to Dr. Graham Bodwell for allowing me to borrow chemicals and equipment from his research laboratory and his helpful suggestions. My special thanks to Dr. Cai and his group for performing the cytotoxicity tests.

Table of Contents

List of Tables

List of Figures

List of Schemes

Abbreviations and symbols:

Bel-7402	Human hepatocellular carcinoma cell line
BGC-823	Human gastric cancer cell line
CoMFA	Comparative molecular field analysis
DHFR	Dihydrofolate reductase
DIAD	Diisopropyl azodicarboxylate
ENO	Enoxacin
FDA	Food and drug administration
FLU	Flumequine
GC/MS	Gas chromatography-mass spectrometry
Hela	Human cervical cancer cell line
HL-60	Human leukemia cancer cell line
IUPAC	International union of pure and applied chemistry
LC/MS	Liquid chromatography-mass spectrometry
*m*CPBA	m-Chloroperbenzoic acid
MDA-MB-435	Human breast cancer cell line
NAACCR	North american association of central cancer registries
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
PLS	Partial least squares
QSAR	Quantitative structure-activity relatinship
rt	Room temperature
SRB	Sulforhodamine B
THF	Tetrahydrofurane

1- Introduction

Following cardiovascular diseases, the group of diseases referred to as "cancer" is the second cause of premature mortality worldwide. The occurrence of cancer is related to a variety of factors such as gender and age. The North American Association of Central Cancer Registries (NAACCR) provides statistical data on cancer incident and mortality in North America. [1] Table 1 shows the incident of the five most commonly diagnosed cancers in North America.

Types of cancer	Incidents per year (for all races and both sexes)
prostate	167,673
female breast	162,228
lung	161,007
colon and rectum	126,190
bladder	50,289

Table1-1. Types of cancer versus the incident per year during the five year period of 1999 to 2003.

Breast cancer is more common in females and leukemia is the most predominant cancer in children. Among the different types of cancer, lung cancer is the main cause of death in adults. The most common cancer in males after prostate cancer, is lung cancer. The overall incidence rate of cancer in North America was found to be 35% higher

among men than women. However, the difference in percentages of incidence can differ greatly based on race groups. [1-2]

During 1999-2003, a total of 233,840 Canadians died of cancer, approximately, 128 deaths per day. Fifty-three percent of Canadian cancer deaths occurred among men and 47% occurred among women. [1]

Environmental factors and life-style have played a major role in the incidences of many cancers. Around 75% of cancers are thought to be caused by environmental carcinogens such as ionizing radiation and chemicals. [3]

Genetic factors also play a major role in development of cancer. The presence of certain genes may lead cells to begin their uncontrolled growth. Mutations in genes resulting in malignancy can be either acquired or inherited. There is a period of time known as the latency period between exposure to a carcinogen and appearance of symptoms. The latency period varies from months to decades in different individuals, depending on the cancer type and the susceptibility of the person. [4]

1.1- Biology of cancer

During cell cycle, cells replicate to produce two genetically identical daughter cells. Cell division is a highly regulated process which maintains tissue homeostasis and in which, new cells replace damaged cells. A human being consists of an average of 100,000 billion cells.

The cell cycle consists of four phases: (1) G_1, the first growth phase (gap 1 period); (2) DNA synthesis phase (S) which comes after G_1 and for which there is a

second gap period (growth phase) after S; (3) nuclear and cell division phase is called the M phase; and (4) in which, the cell can enter a resting state, named G_0. Depending on the signal that a cell receives, or the cell type, the cell cycle phases might change. The tumor suppressor gene, RB, produces, RB proteins, which regulate the point at which cells stop replication of their DNA. RB functions as a control switch for the S phase. The cell cycle regulating system includes various proteins, among which two groups are of very high importance, the cyclins and the cyclin-dependent kinases (CDKs). CDKs are serine/threonine protein kinases that are capable of binding to their regulatory subunits and become catalytically active. Different members of CDK family are activated by the appropriate cyclins, during certain phases of the cell cycle. [5]

Disruption of a normal cell cycle will result in inappropriate cellular replication that can be caused by most cancer-causing genes. "Malignant stimuli activators signal pathways that allow progression through G_1 and then G_1/S transition. G_1 is the phase of the cell cycle wherein the cell is responsive to growth factors. Control of the G_1 into G_1/S transition is often disrupted in cancer cells."[5] The most important part involved in the G_1/S transition is the phosphorylation of RB. Phosphorylation of RB will cause the release of E2F (a family of transcription factors). Three types of E2F including E2F1, E2F2 and E2F3a are cell cycle activators. There are six other E2F factors that are cell cycle suppressors (E2F3b, E2F4-8). [5]

The RB pathway is often disrupted in human cancer. The non-functional RB pathway might from inappropriate activation of CDK/cyclins. The concentrations of CDKs usually remain constant within the period of a cell cycle. However, concentrations

of cyclins fluctuate during progression of the cell cycle. CDKs might go through mutation, which could result in cancer. Higher intensities of CDK4 might occur in melanoma, sarcoma, and glioma. Non-functional expression of cyclins has been reported in some human cancers such as breast, bladder and lung. [5]

A cancer process can be defined by its initiation, promotion, and progression. The initiation includes an irreversible damage to DNA that can correspond to the cancer. The biochemical process in which an already initiated cell, tissue, or organ develops focal proliferation is called promotion. The process of progression is irreversible. During the progression process, there is further growth and expansion of the malignant cells over normal cells.

1.2- Cancer treatment

Cancer has been traditionally treated with surgery. Recent developments have led to improvement in other treatments such as chemotherapy, radiotherapy, and immunotherapy. Surgery is usually the primary treatment for resection of the localized cancer; however, chemotherapeutic agents are now considered of great value in the treatment of a variety of malignancies such as leukemia, Hodgkin's lymphoma, liver cancers and metastatic colon cancer. [6]

1.2.1- Chemotherapy

Chemotherapy is used for a variety of purposes such as to:

1- Cure cancer;

2- Reduce size of a specific tumor before surgery;

3- Prevent micrometastasis; and/or

4- Make the malignant tissues sensitive to therapy (prior to radiotherapy).

The problem with chemotherapy is the relatively high toxicity of chemotherapeutic agents. Normal cells are also affected, and the lack of selectivity for the malignant tissues and development of drug resistance may complicate their usefulness. Rapid proliferating cells including hair cells, bone marrow and gastrointestinal cells are in more danger at the time of exposure to chemotherapeutic drugs than slow proliferating cells.

Another complication caused by cancer chemotherapeutic agents is the irreversible DNA damage. Patients who survive chemotherapy are at risk of iatrogenic cancers. An example of a high risk chemotherapeutic agent is cyclophosphamide. It can chemically react with DNA in the same way that some carcinogens do. The risk of iatrogenic cancer depends on the cumulative dose of a drug in a patient, which makes younger patients more susceptible to the drug's adverse effects and resistance.[7-8]

1.2.2- Classes of antineoplastic agents

Antineoplastic agents are classified in the following way, based on their mechanism of action.

1- Alkylating agents

2-Antimetabolites and nucleoside analogues

3-Antitumor antibiotics

4-Antimitotic agents

5- Hormones and antagonists

6-Miscellaneous agents [9]

1.2.2.1-Alkylating agents

Alkylating agents were the first reported class of compounds with antitumor activities. Between World Wars I and II, studies of chemical and biological properties of nitrogen mustards took place.[6] The first anticancer compound approved by FDA in 1949 for clinical use was mechlorethamine (nitrogen mustard). [9]

Alkylating agents exhibit their effect, namely, via disruption of DNA functions and causing cell death, through three different mechanisms. In the first mechanism, an alkylating agent attacks a nucleophilic site such as (N-7) of a purine base (guanine residue) and alkylates it. As a result, alkylated bases prevent further DNA synthesis and RNA transcription. The second mechanism involves formation of cross-bridges from two bases that are linked together by an alkylating agent. These cross-bridges can form by alkylating agents that possess two binding sites for DNA. A cross-bridge may connect two different DNA strands (inter-DNA) or can be formed within one DNA double helix (intra-DNA).

In a normal DNA double helix, adenine always pairs with thymine and guanine pairs with cytosine. In the third mechanism, alkylating agents cause mispairing of nucleotides which results in mutation. The common property of alkylating agents is their being strong electrophiles. They can form carbonium ions, aziridinium intermediates, or

transition-metal complexes with DNA. Therefore, the chemotherapeutic effect of these compounds is relevant to their potency in DNA alkylation. Alkylating agents are mostly efficient in G_1 or S phases of the cell growth. These agents can be categorized as mechlorethamine (nitrogen mustards), ethyleneimines and methylmelamine, alkyl sulfonates, nitrosoureas, and triazenes. [6-9]

1.2.2.2- Antimetabolites and nucleoside analogues

Antimetabolites are structural analogues of naturally-occurring compounds that can prevent biosynthesis of metabolites in cells. Their mechanism of action involves interfering with production of nucleic acids. Antimetabolites can work through different mechanisms including competition for binding sites on enzymes. There are three categories of antimetabolites : antifolates (e.g. methotrexate), purine analogues (e.g. 6-mercaptopurine) and pyrimidine antimetabolites (e.g. 5-fluorouracil). [6]

Antimetabolites in general, inhibit cell growth in the most rapidly proliferating cells such as bone marrow. Folic acid is essential in the synthesis of DNA and RNA precursors, including thymidylate and purines. The enzyme dihydrofolate reductase (DHFR) is the primary target for folate antimetabolites such as methotrexate used in cancer therapy. Folic acid is reduced by DHFR in two steps. The reduced form of folic acid (tetrahydrofolic acid) is involved in transferring a single carbon unit such as methyl or formyl group in biosynthetic pathways of pyrimidine- and purine-based natural nucleosides. Inhibition of DHFR results in lack of cofactors required for the synthesis of purines and thymidylate in tumor cells. [9]

There is one major anticancer drug in the category of purine analogues, namely, 6-mercaptopurine. 6-Mercaptopurine is an analogue of hypoxanthine. Purine analogues work on multiple sites and their toxicity mechanism is the result of combined effects on different sites. [9]

5-Fluorouracil, the principal pyrimdine antimetabolite, is an inhibitor of thymidylate synthase, which is the key enzyme in the synthesis of thymidine from uridine. [6]

1.2.2.3- Antitumor antibiotics

Most of the compounds in this class were originally developed as antibiotics but these were rejected because of their toxicity. [9] Actinomycin A was the first one in this category and was isolated from *streptomyces* by Walksman and Woodruff in 1940. Antitumor antibiotics interact with DNA through several pathways including intercalation, DNA strand breakage and inhibition of topoisomerase type II. [6]

Dactinomycin is an example of an antitumor antibiotic. The structure of this compound consists of a phenoxazone ring and two cyclic pentapeptides. The presence of the phenoxazone ring gives a red color to the drug. Dactinomycin can bind to DNA and block chain elongation. The phenoxazone chromophore part of the drug structure, intercalates between bases in the DNA and the 2-amino group of the guanine. This mechanism was found to be very important for the formation of DNA-drug complexes. Tight binding between DNA and drug makes the DNA-drug dissociation a very slow process. Dactinomycin inhibits DNA directed RNA synthesis at low concentrations. In

higher concentrations, DNA synthesis is also affected. Other examples of antitumor antibiotics include the beleomycins, mitomycin C, and the anthracylines. [6-9]

1.2.2.4- Antimitotic agents

Antimitotic agents in general, prevent cellular mitosis. The biological activity of this group depends on their ability to bind specifically to tubulin and to block polymerization of microtubules. The first antimitotic agents were vinca alkaloids, whose mechanism of action was suggested to proceed through their specific binding to tubulin. Tubulin dimers are unable to aggregate and form microtubules when bound with vinca alkaloids. This mode of action results in a shift of the biological equilibrium toward disassembly of microtubules and their shrinkage. [9]

1.2.2.5- Hormonal therapies and hormone antagonists

Manipulation of hormones is another approach in the treatment of hormonal-related cancers. This group of compounds can be categorized as antiestrogens, GnRH (Gonadotropin- releasing hormone) agonists and antiandrogens. The most common example nowadays is the treatment of breast cancer with antiestrogen drugs such as Tamoxifen.[9] Flutamide is an example of an antiandrogen that is approved by FDA for the treatment of prostate cancer. [9]

An example of hormonal therapy to treat metastatic prostate cancer is the use of gonadotropine-releasing hormone agonists. The goal in this therapy is to exclude the

stimulation caused by male hormones on cancer cells. Employment of gonadotropin-releasing hormone agonist causes a decrease in testosterone through down regulation.

1.2.2.6- Cancer immunotherapy

The main goal of cancer immunotherapy is to stimulate the patient's immune system to attack the malignant tumor cells that are responsible for the cancer. It can be through adminstration of therapeutic antibodies or by immunization of the patients, in which the patient's own immune system is trained to distinguish the cancerous cells as targets. In immunotherapy, the adminstration of antibodies helps the immune system to destroy the tumor cells. Although the tumor cells are the patient's own cells that are spreading out of proper regulatory control, most of them display unusal antigens that are not appropriate for the cell type. For instance, glycosphingolipid GD2 which is only expressed at the outer surface membranes of neuronal cells is expressed on the surfaces of a wide range of tumor cells including neuroblastoma, medulloblastomas, astrocytomas, melanomas, small-cell lung cancer, osteosarcomas and other soft tissue sarcomas. Therefore, GD2 could be a suitable tumor-specific target for immunotherapy. Raising antibodies against specific antigen becomes possible via utilization of monoclonal antibody technology. A list of FDA-approved monoclonal antibodies is mentioned in Table (1.2).[10]

Brand name	Approval date	Antibody	Type	Target	Approved treatment(s)
Campath	2001	Alemtuzumab	humanized	CD52	Chronic lymphocytic leukemia
Avastin	2004	Bevacizumab	humanized	vascular endothelial growth factor	colorectal cancer
Erbitux	2004	Cetuximab	chimeric	epidermal growth factor receptor	colorectal cancer
Mylotarg	2000	Gemtuzumab ozogamicin	humanized	CD33	acute myelogenous leukemia (with calicheamicin)
Zevalin	2002	Ibritumomab tiuxetan	murine	CD20	non-Hodgkin lymphoma (with yttrium-90 or indium-111)
Vectibix	2006	Panitumumab	humanized	epidermal growth factor receptor	colorectal cancer
Rituxan, Mabthera	1997	Rituximab	chimeric	CD20	non-Hodgkin lymphoma
Herceptin	1998	Trastuzumab	humanized	ErbB2	breast cancer

Table 1.2- "Cancer immunotherapy: Monoclonal antibodies"[10]

1.2.2.7- Miscellaneous agents

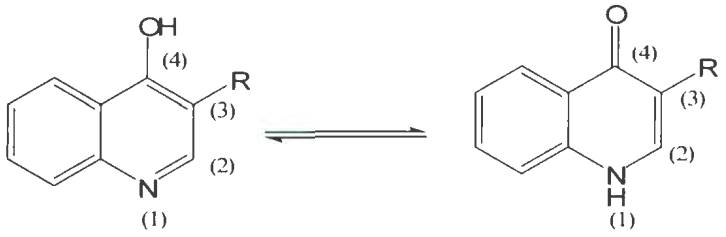
In addition to the above, there are other antineoplastic agents such as podophyllotoxins, epipodophyllotoxins (Etoposides), hydroxyurea, mitotane, retinoids, camptothecins and porfimer. [6-9]

Epipodophyllotoxins are extracted from *podophylum peltatum*. Etoposide and teniposide are semisynthetic glycosidic derivatives of podophyllotoxin. Etoposide and teniposide are inhibitors of topoisomerase II. Epipodophyllotoxins form complexes with DNA and topoisomerase II, consequently the enzyme remains bound to the free end of DNA. Accumulations of DNA breaks cause cell death. Etoposide and teniposide have a similar mechanism of action. However, teniposide's uptake by cells is faster than etoposide, which might be due to its higher lipophilicity. [6]

1.3- Quinolones and their nomenclature

Quinolones are heterocyclic compounds that are formally derived from pyridones by fusion with a benzene ring. The systematic and official IUPAC name for 4-quinolones is 4- oxo-1, 4-dihydroquinolines. However, these series of compounds are still recognized in the area of pharmaceutical and medicinal chemistry by their traditional name, quinolones.

In order to name the quinolones properly, the position of pyridone and benzene in the fused heterocyclic ring system is of importance. If the pyridone is a 4-pyridone and the benzene ring is fused in position [2,3] or [*b*], we obtain the title compound, 4-quinolone, which is capable of a typical keto-enol tautomerism (Figure 1.1).[11]

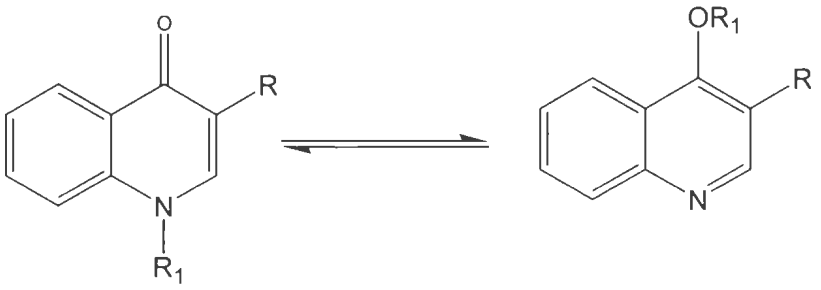


1b **1a**

R=H,COOH,COOMe,COOEt,COMe,$COCF_3$,NO_2,CN

Figure 1.1- Keto (**1a**) and enol (**1b**) forms of quinolone structures in general.

Even though the physicochemical evaluations are mostly in favor of the keto-form, the majority of textbooks and articles represent quinolones as hydroxyquinolines. The quinolone-hydroxyquinoline tautomerism has been established based on the *O*- and *N*-ethyl derivatives obtained after ethylation of quinolones using different reaction conditions (Figure 1.2). [12-13]



R_1=H,Me,Et
R=H,COOR

Figure 1.2- *O*- and *N*- derivatives of quinolones

1.4- Quinolone derivatives

To date, about 10,000 derivatives of 4-quinolone have been described. One review alone covers 231 types of quinolone-3-carboxylic acid derivatives that were reported in the period of 1939 to 1989.[14]

In the beginning, the interest in 4-quinolones was extensively directed toward their synthesis as antimalarial agents. The discovery of 4-quinolones as antimalarial agents goes back to World War II. However, none of the 4-quinolones were evaluated in anti-malarial screening during World War II. As a result of expanding research on 4-quinolones synthetic antimalarial drugs based on quinoline nucleus were discovered. The first quinoline-antimalarial agent introduced to the clinics was Chloroquine (Figure 1.3).[15 -16]

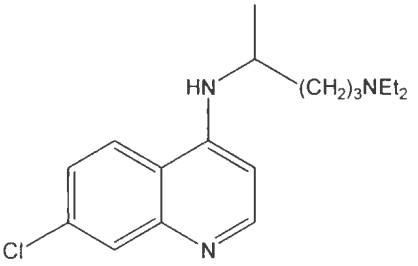


Figure1.3- Chloroquine

The quinolones have been further developed since the discovery of nalidixic acid by Lescher. Nalidixic acid was a first-generation quinolone antibacterial, produced synthetically in 1962. It only had modest antibacterial activity against gram-negative species and showed poor oral bioavailability. Nalidixic acid and its subsequent

derivatives, show narrow spectra of activity that restrict their clinical applications. In the mid-1980s, fluoroquinolones were discovered as potent, broad-spectrum antibacterial and chemotherapeutic agents.

Improvement of antibacterial activity against gram-negative bacteria was achieved by introduction of a piperazinyl group at the C-7 position of the quinolone which improved the physiochemical properties of these compounds and, as a result, enhanced their penetration into the bacterial cell. Addition of fluorine to the C-6 position of quinolones resulted in dramatic increase of the activity of these compounds against gram-positive bacteria (Figure 1.4). Improved pharmacodynamic and pharmacokinetic profiles have resulted in much better efficacy and tolerability of flourquinolones.[17, 18]

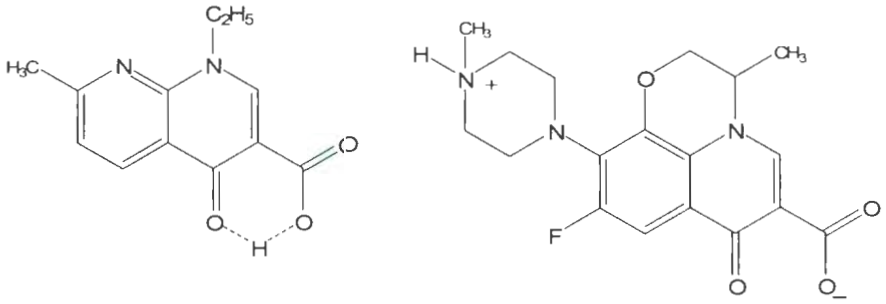


Figure 1.4-Structures of nalidixic acid and ofloxacin

Recently, pharmaceutical companies have tried to develop newer generations of quinolone antibacterials with the goal of enhancing their activities against gram-positive or anaerobic bacteria. There are also new forms of quinolones designed to be active as antiviral and antitumor agents, as well.

1.5. General physicochemical properties of quinolones in different environments

It is of high importance to investigate the physiochemical properties of quinolones in a variety of environments, especially *in vivo*. These studies will help in understanding the nature of their pharmacologic properties such as antibacterial or anticancer activities. Quinolone antibacterials are regularly prescribed because of their safety and good tolerance in patients. In general, antibacterial activity is pH dependent. The pH-dependent activity is due to the fact that these drugs act as bacterial DNA gyrase inhibitors and that the inhibition process depends on pH and acid concentrations.[19]

Quinolone behavior *in vivo* is particularly influenced by the degree of ionization, which is often shown by charged groups and affects the biological activity.[20] Generally, complexes between quinolones and divalent cations are formed in a 1:1 ratio in which the 4-oxo and 3-carboxylic acid functions of the quinolones make an ion-dipole interaction with the divalent cation (Figure1.5). [21]

In some quinolones such as nalidixic acid (NAL), flumequine (FLU), and oxolinic acid (OXO), the 3-carboxyl group is the only appropriate ionizable functional group. But in other cases like ofloxacin (OFL), norfloxacin (NOR), and enoxacin (ENO) both the 3-carboxyl group and the N-4 of the 7-piperazinyl moiety are ionizable functional groups (Figure1.5). [22]

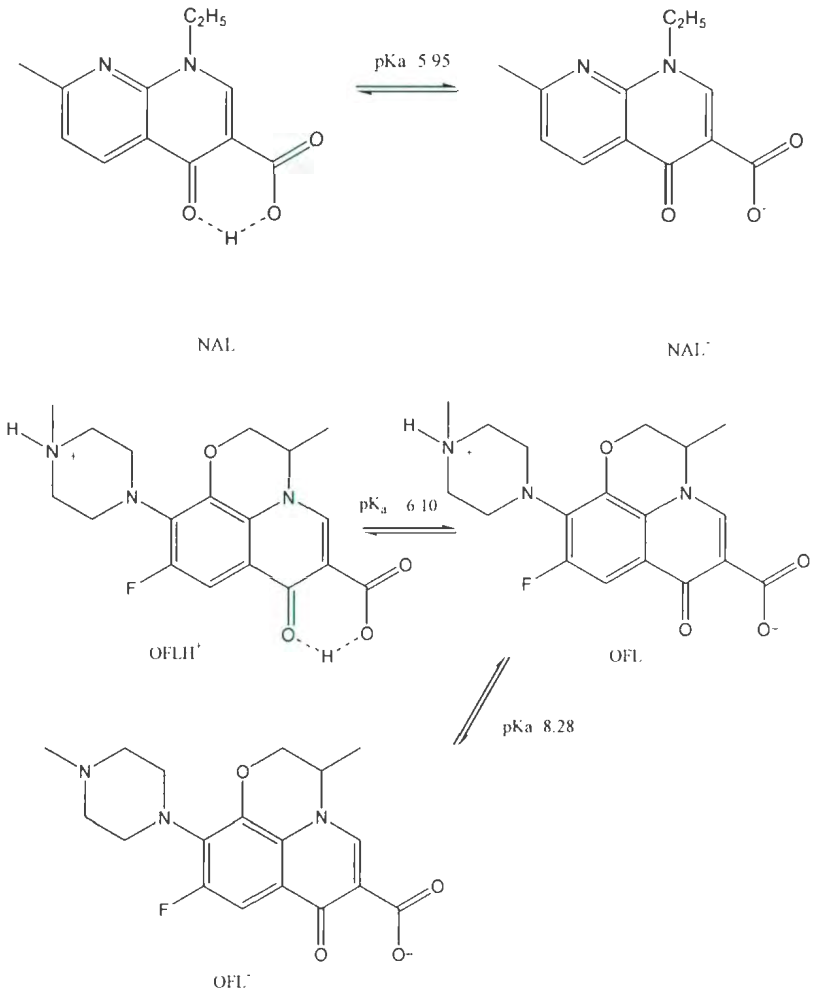


Figure 1.5- Examples of pH-dependence, and ionizable functional groups in quinolones

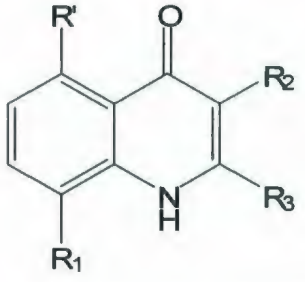
Bacterial DNA gyrase is responsible for the introduction of negative supercoils into DNA, which results in the highly condensed three-dimentional structure of the chromatin. This mechanism is essential to condense the bacterial genetic material. Quinolones in general, are DNA gyrase inhibitors. Studies have shown that interaction of quinolones with DNA gyrase in the absence of DNA is not possible. Complex formation between quinolones and a divalent cation (e.g. Mg^{+2}) is critical for their biological

activity. The ability of these drugs to interact with some cellular components is mediated by this complexation. Furthermore, the amount of quinolone bound to DNA is regulated by Mg^{2+} concentration.[23] Quinolones are reported to induce photosensitivity reaction in human skin, but the exact mechanism is not yet known.[24]

Quinolones are reported to be photocarcinogenic that might be due to their ability to interact with DNA. [22]

1.6- Naturally occurring quinolones

The quinolone skeleton can be found in a large number of compounds. There are examples like zoochromes which are compounds of animal basis that are responsible for (often changing) coloration of animal species. Other examples are contained in the Mediterranean sponge *Verongia aerophoba* Schmidt which contains uranidine and an antibacterial acid. Another example is an isomeric antibacterial acid in the Antarctic sponge *Dendrilla membranosa* (*Aplysina*) (family *Verongidae*). [25]



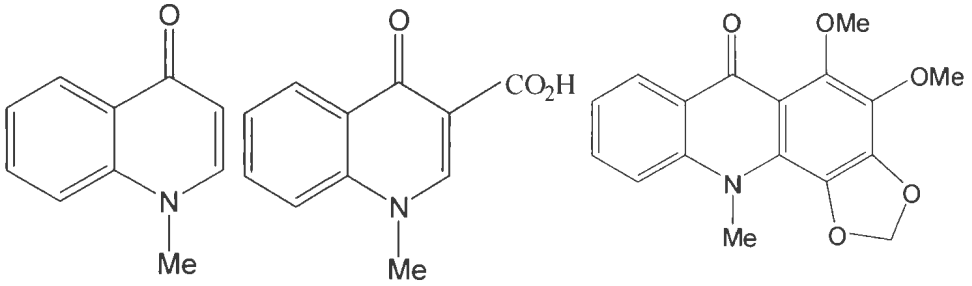
Uranidine, found in *Verongia aerophoba* Schmidt: R'=OH, R_1 =R_2 =OH and R_3=H

Antibacterial compound found in *Verongia aerophoba Schmidt*: R_1 =H, R_2 =OH and R_3=COOH

Antibacterial compound found in *Dendrilla mebranosa* (Aplysina): R_1 =OH, R_2 =H and R_3=COOH

Figure 1.6- General structure of antibacterial compounds found in *Verongia aerophoba* and *Dendrilla membranosa.*

There are other quinolones that have been isolated from natural sources, such as echinopsin which was isolated from the seeds of *Equinopsis Rito* linn. This compound is also a decarboxylation product of 1-methyl quinolin-4(1*H*)-one-3-carboxylic acid which is thought to be originated from degradation of the alkaloids meliopine, meliocopidine, and meliocopicine. These alkaloids have been extracted from *Melicope fareana* (*Rutceae*). 1-Methyl-4-quinolone-3-carboxylic acid is also formed by oxidation when concentrated nitric acid reacts with the above mentioned alkaloids. [26]



Echinopsine 3-carboxylic acid derivative melicopine

Figure1.7- Alkaloids extracted from *Melicope fareana*

The alkaloid Japonine isolated from the underground parts of *Orixa Japonica* is also a quinolone. [27]

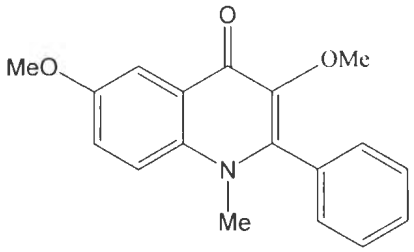
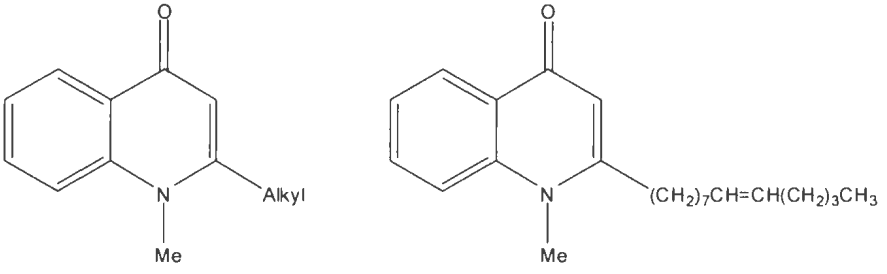


Figure 1.8- Japonine

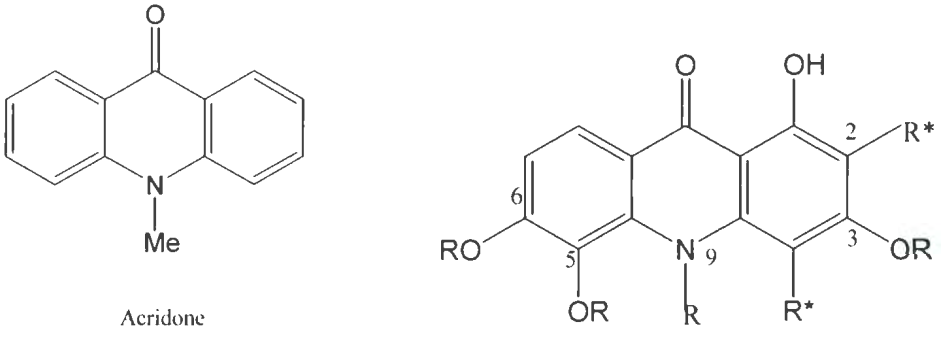
Several other quinolone alkaloids are also known.[28] Varieties of 2-alkyl-4-quinolones were found in plant organs and most of them showed biological activity. One example is evocaprine which is isolated from *Evodia rutaecarpa*.[29]



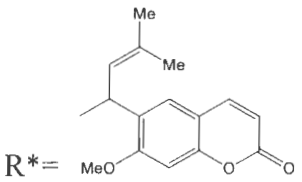
2-alkyl-4-quinolone evocaprine

Figure1.9- Evocaprine , an example of 2-alkyl-4-quinolone

Derivatives of acridone known as acrimarines are alkaloids from *Melicope farena*. Acrimarines are found in *Citrus fudako* (Rutaceae) and acridone itself is a component of the roots and rhizomes of *Thamnosa montana* (*Rutacea*). Different derivatives are differentiated by the position of the R substituent as well as the presence or absence of methyl groups at the 3, 5, 6 and 9 positions of the acridine ring (Figure 1.10).[30]



Acridone



Acrimarine A, 2=R*, 3=H, 4=H, 5=Me, 6=Me, 9=Me

Acrimarine B, 2=R*, 3=Me, 4=H, 5=Me, 6=Me, 9=H

Acrimarine C, 2=H, 3=Me, 4=R*, 5=Me, 6=H, 9=H

Acrimarine D, 2=H, 3=Me, 4=R*, 5=Me, 6=Me, 9=H

Acrimarine E, 2=R*, 3=Me, 4=H, 5=Me, 6=H, 9=H

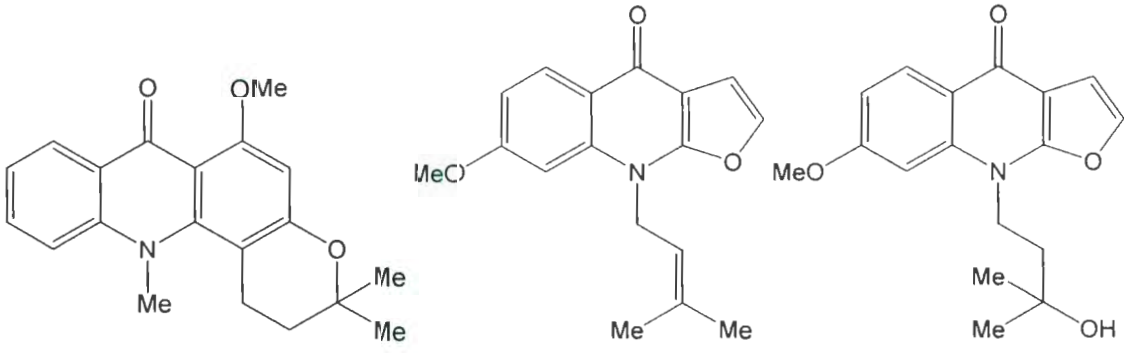
Acrimarine F, 2=R*, 3=Me, 4=H, 5=Me, 6=H, 9=Me

Acrimarine G, 2=R*, R=H, 4=H, 5=H, 6=de-OMe, 9=Me

Acrimarine H, 2=R*, 3=Me, 4=H, 5=H, 6=de-OMe, 9=Me

Figure 1.10- Derivatives of Acridone

There are other acridone alkaloids (e.g. acronycine, acropoline, acropylidine) isolated from the root bark of *Severinia buxifolia* Tenore (Rutaceae) that are called buxifoliadines.



Acronycine Acropoline Acropylidine

Figure1.11- Examples of acridone alkaloids isolated from *Severinia buxifolia*

Significant antineoplastic and antitumor activity for these compounds have been reported. Acronycine was found in *Acronychia baueri* (*Bauerella baueri*), *Melicope letococca*, *Sarcomelicope leiocarpa* and *S.argyrophylla*(Rutaceae). Acropline is formed via dehydrogenation of acropylidine. [29]

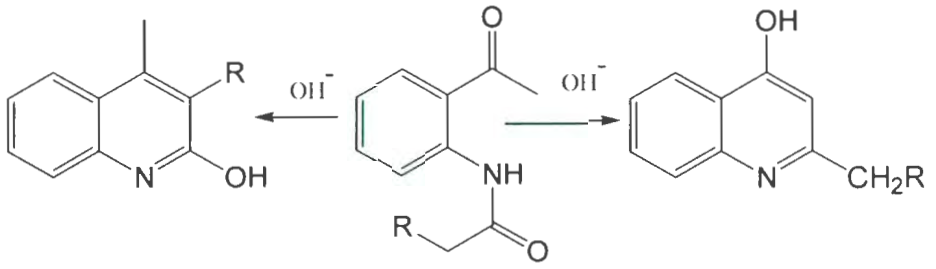
1.7- Synthesis of quinoline-4-ones

Synthetic methodologies have been categorized based on how the ring closure procedure leads to the formation of the quinolone nucleus. Starting materials are often benzene and in exceptional cases, pyridine derivatives.[24]

Preparations of 4-quinolones basically follow the same principles as with quinoline syntheses, since they are modified versions of the quinolines, having a ketone group at position 4. Quinolones have been known for a long time and have been synthesized in many ways. They are stable compounds and were first isolated from coal tar in 1834. They are normally high boiling point liquids or low melting point solids with a sweetish odor.[31] The general procedures which are often used for the synthesis of quinolines are applicable for quinolones with few exceptions. Major routes proceed via C_4-C_{4a} and C_{8a}-N bond formations. [32]

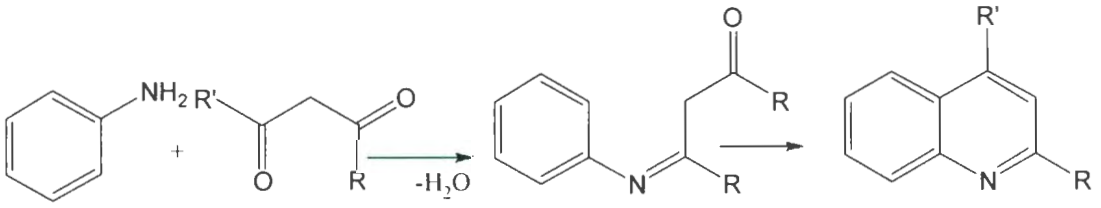
1.7.1- Camps quinoline synthesis

In this procedure, alkaline cyclization of an *N*-acylated-*o*-aminobenzophenone affords the corresponding 2- or 4- hydroxyquinolines (Scheme 1.1) [33-40]



Scheme 1.1- Camps quinoline synthesis

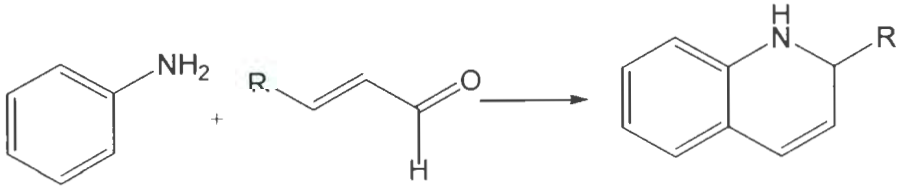
1.7.2- Combes and Doebner-Miller quinoline synthesis



Scheme1.2- Combes quinoline synthesis

The Doebner-Miller (Beyer Method for quinolines) and Combes syntheses are both known to be quite general for the production of quinoline derivatives (Scheme 1.2). [41-45]

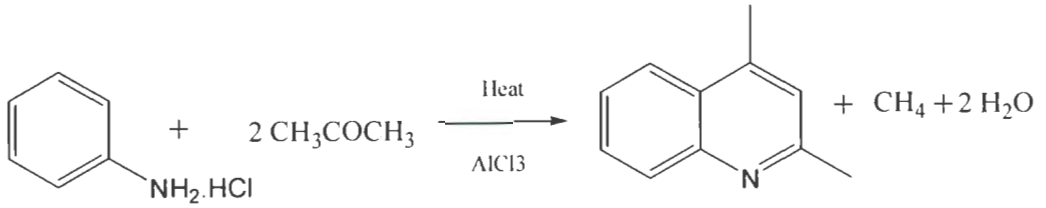
The similarity in their mechanism of formation, particularly at the cyclization step, between the Doebner-Miller and Skraup syntheses were described in several articles (Scheme 1.3). [46-49]



Scheme1.3- Doebner-Miller (Beyer Method) quinoline synthesis

1.7.3- Riehm quinoline synthesis

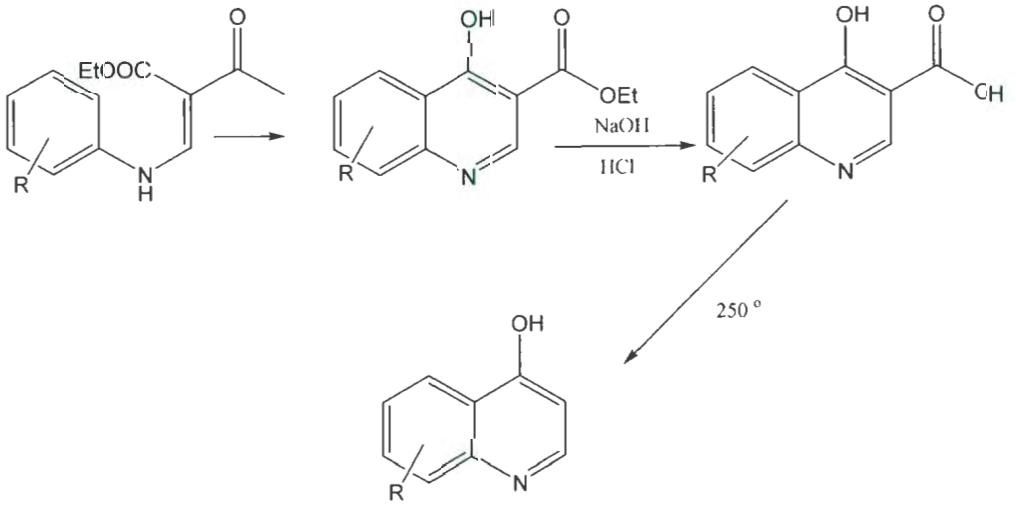
The Riehm quinolone synthesis has much in common with the Doebner –Miller synthesis. The reaction products are derived from acetone and aniline hydrochloride, the condition that is often suitable for the formation of quinolines from carbonyl compounds and aniline (Scheme 1.4) [50-56]



Scheme1.4- Riehm quinoline synthesis

1.7.4- Gould-Jacobs reaction.

Another main procedure used to obtain both quinolines and quinolones is the Gould-Jacobs reaction, according to the following scheme. In this procedure, ring closure occurs at a very high temperature (Scheme 1.5). [57-60]

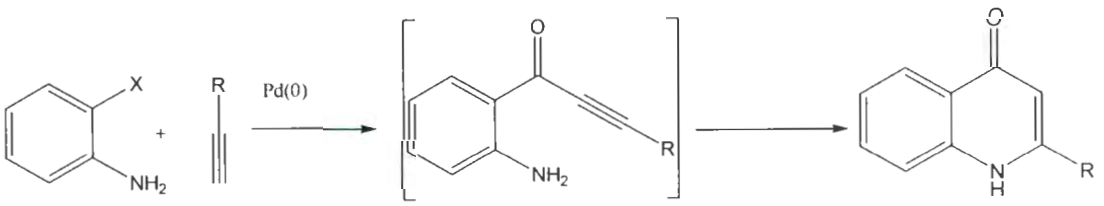


Scheme1.5- Gould-Jacobs quinolone synthesis

1.7.5- Bond formation in 4-quinolone synthesis

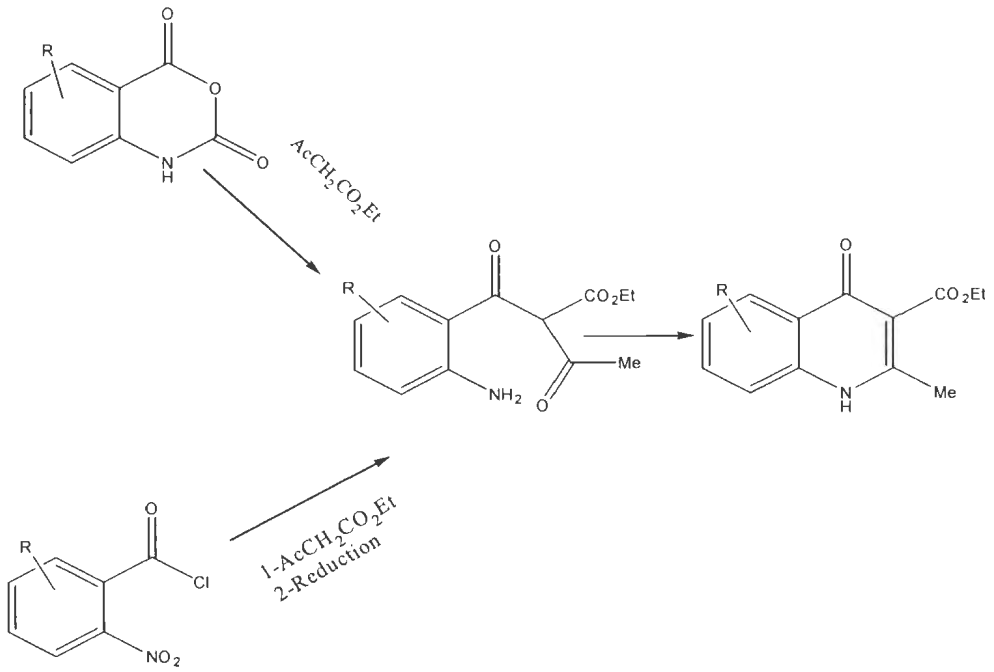
As a rule, creation of 4-quinolones is performed by pyridine ring formation using different ring closure reactions. This will allow us to classify the ring closures conveniently according to the following categories: N_1-C_2; C_2-C_3; C_3-C_4; C_4-C_{4a} and C_{8a}-N ring closures.

Bond formation between N_1-C_2 has been mentioned more in the synthesis of naphthyridones than the quinolones. The reaction is carried out between a haloaniline and a terminal alkyne, in the presence of palladium as a catalyst. This type of reaction yields 2-substituted 4-quinolones as the main products (Scheme 1.6). [61]



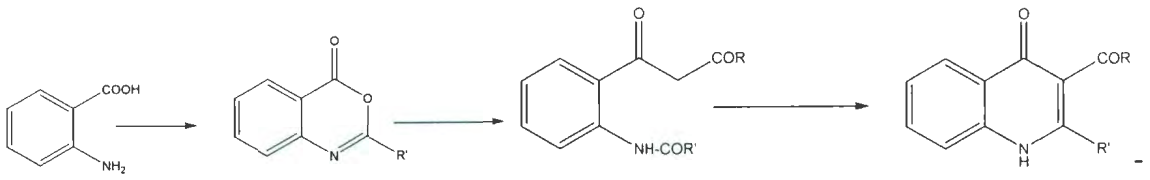
Scheme1.6- Quinolone synthesis through N_1-C_2 bond formation

Ester condensation with acid anhydride or with derivatives of 2-nitrobenzoyl chloride followed by cyclization is another approach to prepare N_1-C_2 quinolones (Scheme 1.7). [62-63]



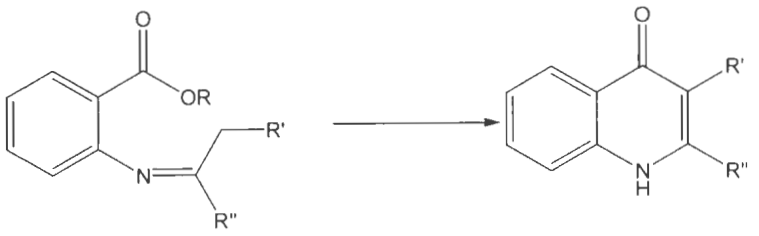
Scheme1.7- Quinolone synthesis through N_1-C_2 bond formation

The modified Niementowski quinoline synthesis is the procedure to synthesize C_2-C_3 quinolones. Starting material for this reaction is an anthranilic acid derivative that will be activated via its acid anhydride, which after a ring opening-ring closure sequence affords 2-substituted –quinolone as shown below (Scheme 1.8). [64]



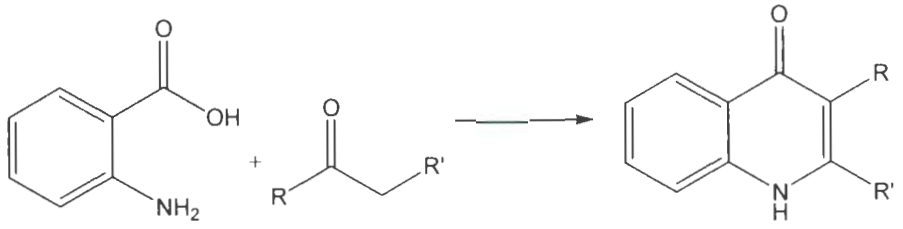
Scheme 1.8- Modified Niementowski reaction

For C_3-C_4 quinolone formation, Dieckmann cyclization of Schiff bases can work well, especially for 2- and 3- substituted 4-quinolones (Scheme 1.9). [65]



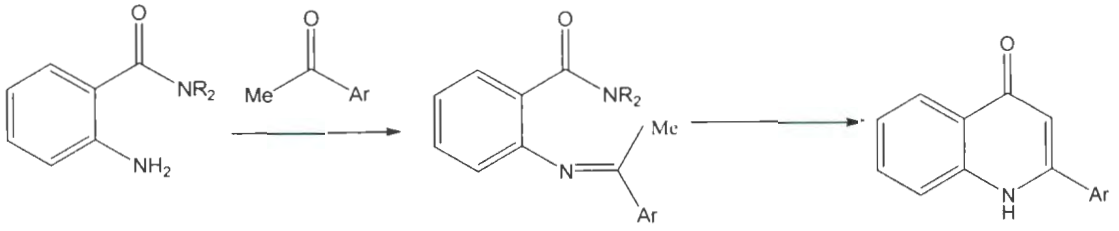
Scheme 1.9- Quinolone formation through Dieckmann cyclization of Schiff bases

Condensation of anthranilic acid in basic condition with carbonyl compounds gives rise to 2- and 3-substituted-4-quinolones (Scheme 1.10).



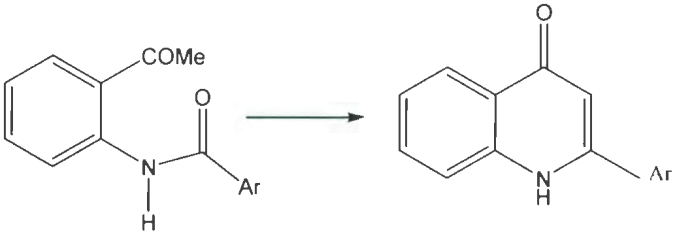
Scheme 1.10- Formation of 2- and 3- substituted-4-quinolones after cyclization

To prepare 2-arylsubstituted quinolones, there is a method which starts with making Schiff bases derived from *N*,*N*-dialkylamides of anthranilic acid and substituted acetophenones. Ring closure of the Schiff base results in the formation of the desired 2-arylquinolone (Scheme 1.11). [66]



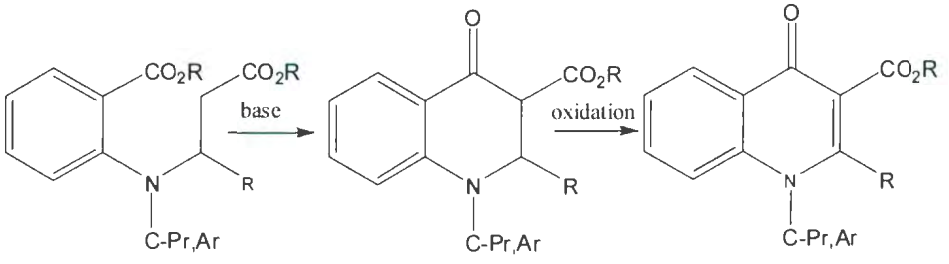
Scheme 1.11- Formation of 2-aryl-4-quinolones through Schiff base preparation

N-Aroyl-*o*-aminoacetophenones, after being treated with potassium 2-methyl-2-popanolate, can easily cyclize to 2-aryl-4-quinolones (Scheme 1.12). [67]



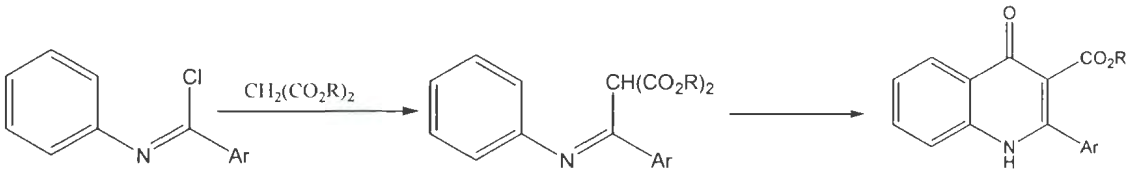
Scheme 1.12- Cyclization of 2-aryl-4-quinolones

One of the most important approaches in the synthesis of *N*-substituted-4-quinolones is to use suitable diesters to undergo Dieckmannn cyclization in basic conditions. This reaction will give tetrahydroquinolines that could be further oxidized to give the favorable 4-quinolones (Scheme 1.13). [68]



Scheme 1.13- Synthesis of N-substituted-4-quinolones

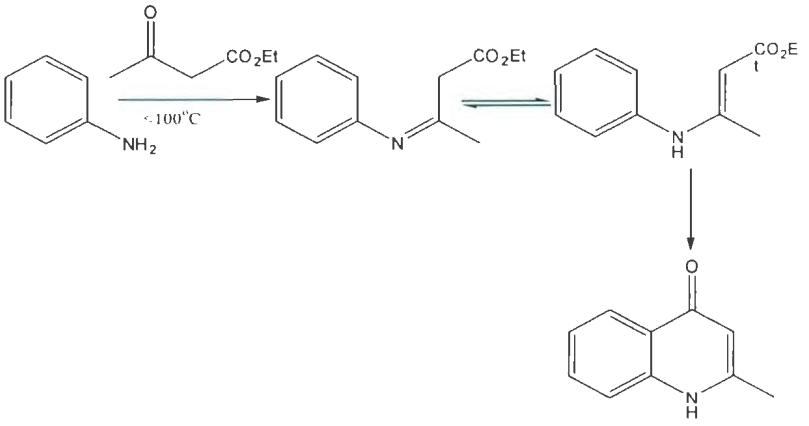
The oldest route to make 4-quinolones is bond creation between C_4-C_{4a} which more often involves a thermal cyclization of an activated enaminone (Scheme 1.14). Non-selectivity of this procedure is its major problem. In this respect, selection of appropriately substituted aniline may help to overcome the non-selectivity problem. [73]



Scheme 1.14- Preparation of quinolones by bond formation between C_4-C_{4a}

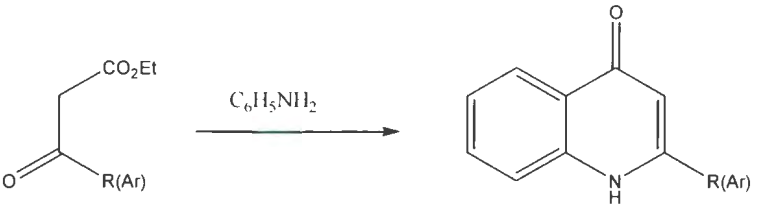
1.7.6- Conrad-Limpach reaction for 4-quinolone synthesis

Another name reaction that is used to make 4-quinolones is the Conrad-Limpach reaction (Scheme 1.15), which is basically performed by reacting aniline and 3-oxobutanoate below 100 °C. The reaction proceeds further by cyclization in an inert solvent, at high temperature (above 250 °C). In this situation, if the first step is done above 100 °C, the amide of 3-oxobutanoic acid would form, which upon further cyclization, gives rise to a product of Knorr reaction which is 2-quinolone instead of 4-quinolone. [69]



Scheme 1.15- Conrad-Limpach reaction

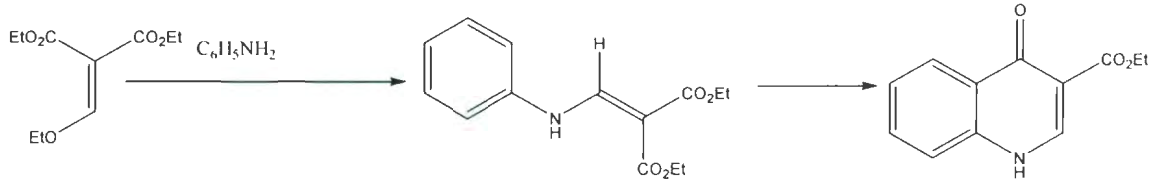
In order to place an aromatic group as a substitution on the quinolone ring, the Conard-Limpach reaction would be an option (Scheme 1.16). [70]



Scheme 1.16- Aromatic group substitution on the quinolone ring by Conard-Limpach reaction

1.7.7- Modified Gould-Jacobs reactions for the synthesis of 4-quinolones

The most famous name reaction in synthesizing quinolones is the Gould-Jacobs reaction (Scheme 1.17). The reaction starts with a nucleophilic substitution which then proceeds via a thermal cyclization. [60-61, 71-73]

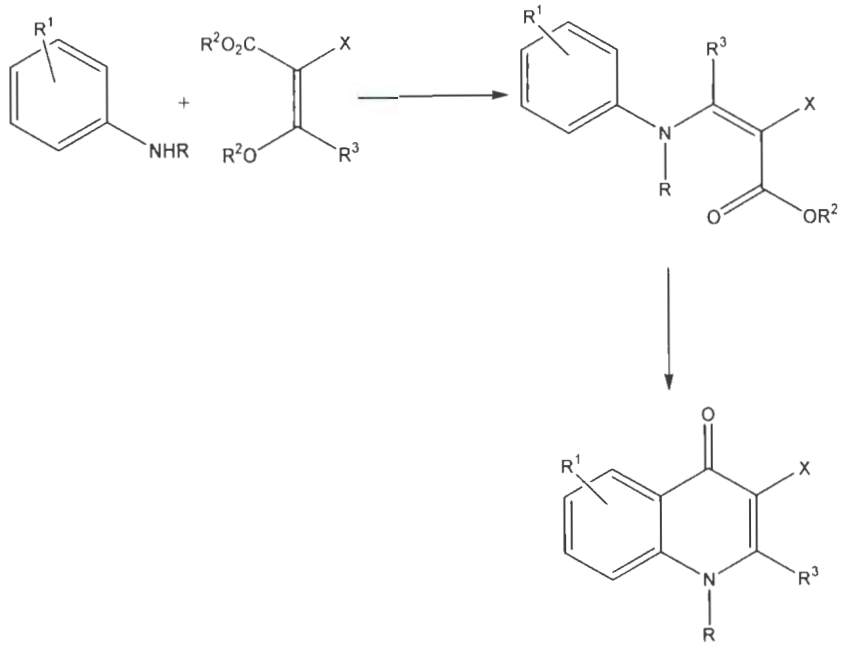


Scheme 1.17- Modified Gould-Jacobs reaction

The open-chain intermediate is an activated aminoethylene derivative that sometimes can be isolated but most of the times it is used directly for the next thermal cyclization. Recently, a one pot synthesis of 4-quinolones using microwave radiation has been reported. [74]

In cases of thermal cyclization above 140 °C, solvents such as low melting point biphenyls are frequently employed. The thermal cyclization step can be done in an inert atmosphere, or at reduced pressure as well. Success of the thermal cyclization step depends highly on the purity of the open-chain intermediate.

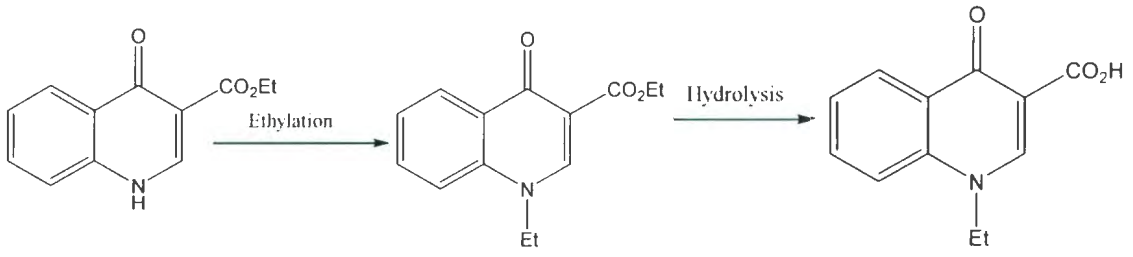
In order to make *N*-substituted quinolones, modified Gould-Jacobs reactions have been developed. In such cases, the open-chain intermediate no longer carries a hydrogen atom, thereby thermal cyclization looks impossible. These intermediates are cyclized in acidic media such as sulfuric acid, acetic anhydride, or trifluoroacetic anhydride (Scheme 1.18).



Scheme 1.18- Synthesis of *N*-substituted quinolones by modified Gould-Jacobs reactions

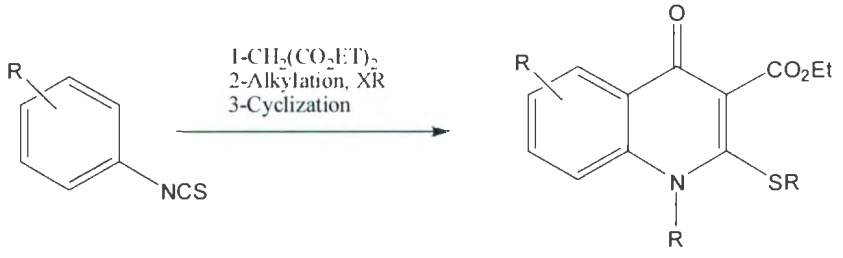
After recognizing the antibacterial properties of nalidixic acid, the Gould-Jacobs reaction became the main production pathway for compounds with similar or related 4-

quinolone structures. Alkylation (ethylation) was done in basic conditions (K_2CO_3 or NaH) in the presence of ethyl iodide. Subsequent hydrolysis is needed to transform the ester to the free acid (Scheme 1.19). [75]



Scheme 1.19-Synthesis of nalidixic acid

Esters of 2-alkylthio-4-quinolone-3-carboxylic acids were also prepared from arylisothiocyanates and diesters, after alkylation and cyclization (Scheme 1.20). [76]

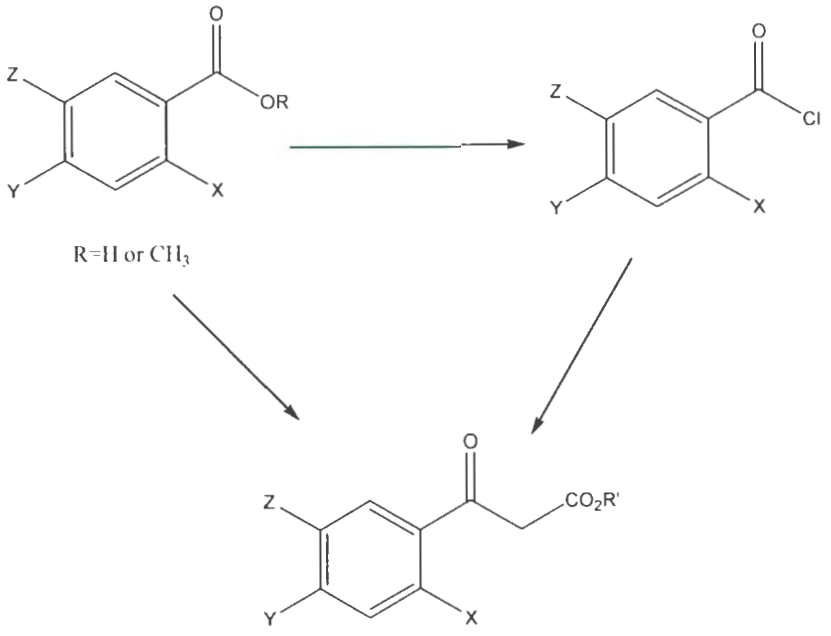


Scheme 1.20-Formation of 2-alkylthio-4-quinolone-3-carboxylic acids

N_1-C_{8a} bond formation is the most favorable procedure for the syntheses of naphthyridones, due to the high reactivity of the carbon adjacent to the pyridine nitrogen, and, the facile departure of the halogen positioned on this carbon. Aroylacetate forms, as the starting materials are prepared from the corresponding acids. Usually, acids are first converted to acid chlorides then treated with malonate. They can even be prepared from

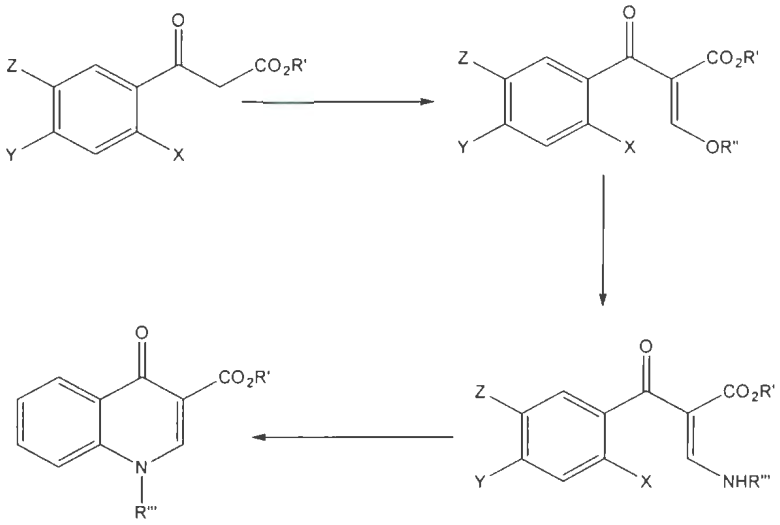
the dianion of monoesters as well. [77] In other cases, esters of aroylacetic acid are first converted to diallylamino derivatives.[82, 78]

Another method is to condense acetophenones with diethylcarbonate in the presence of sodium hydride, which will eventually give the desired aroylacetate after sublimation, as shown in Scheme 1.21. [79]



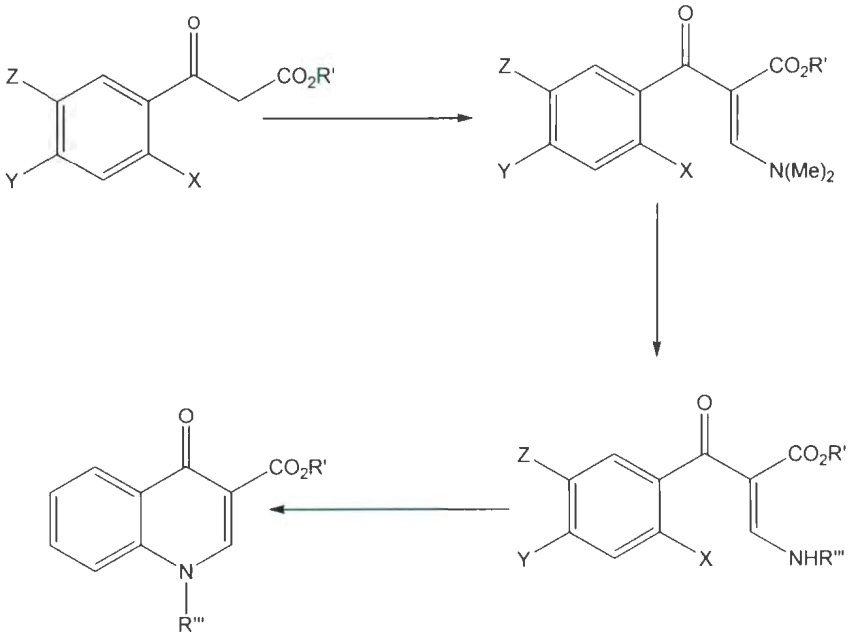
Scheme 1.21- Formation of alkoxymethylene derivatives

There are different methods to prepare alkoxymethylene derivatives and convert them to *N*-substituted aminomethylene derivative in a subsequent step. One method is to use orthoformate with the ester of aroylacetic acid derivative, followed by treatment with amine (Scheme 1.22). [80]



Scheme 1.22- Synthesis of N- substituted aminomethylene derivatives

Another method is to convert the corresponding ester to the dialkylaminomethylene derivative by dialkylformamide diacetal followed by treatment with an amine to give the *N*-substituted aminomethylene derivative (Scheme 1.23). [81-82]



Scheme1.23- *N*- Substituted 4- quinolone synthesis

1.8- DNA topoisomerases

DNA topoisomerases are a group of omnipresent enzymes that are crucial for cell survival and proliferation in both prokaryotic and eukaryotic organisms. DNA supercoiling is controlled by topoisomerases. Some of these were originally named DNA relaxing enzymes, untwisting enzymes, or nicking-closing enzymes. These enzymes are found in all cell types that have been examined. Another group, the DNA gyrases, control the reverse action of changing relaxed closed-circular DNA to a superhelical form in bacteria. [83]

1.8.1- Family and subfamilies of DNA topoisomerases

DNA topoisomerases are classified into two categories: type I and type II. For the type I enzymes, the DNA strands are transiently broken one at a time; while for the type II enzymes, both strands in a DNA double helix are transiently broken by the enzyme molecule which is in its dimeric form. Type IA topoisomerases include bacterial DNA-topoisomerases I and III, yeast DNA topoisomerase III, *Drosophila melanogaster* DNA-topoisomerases IIIα and IIIβ, mammalian DNA topoisomerases IIIα and IIIβ. DNA type IB topoisomeases include eukaryotic DNA topoisomerase I, mammalian mitochondrial DNA topoisomerase I, and Pox virus topoisomerase. Type IIA topoisomerases include bacterial gyrase, DNA topoisomerase IV, Phage T4 DNA topoisomerase, yeast DNA-topoisomerase II, *Drosophila* DNA topoisomerase II, mammalian DNA topoisomerases IIα and IIβ; and finally, Type II B topoisomerases include *Sulfolobus shibatae* DNA-topoisomerase VI .[84] DNA gyrases are found in bacteria.[85]

Topoisomerases work by cleaving one, or both strands of DNA, passing a segment of DNA through the break, and resealing the gap. Relaxation of DNA is a thermodynamically favorable reaction, and indeed this enzyme does not require an input of energy. The enzyme does not simply hydrolyze a phosphodiester bond to form the break, but rather it remains attached to the 5'-phosphate group, forming a reactive intermediate. This bond can then be attacked by the 3'-hydroxy group to reform the phosphodiester bond, with no net change in energy except for the relaxation of one supercoil. Topoisomerases can catalyze the interconversion of other topological isomers of DNA. The level of DNA supercoiling in bacteria is most likely set by an equilibrium of the DNA supercoiling and DNA relaxing activities. [86]

A range of enzymes of this specific class completes the formation of tangled structures in single-stranded DNA. The topological interceptions of DNA demand the temporary breaking and recombination of DNA strands. Therefore, it is expected to see close structural homology with other molecules whose behaviors include the reversible and irreversible rupture of DNA and topoisomerases. Topoisomerase behavior has been established to be linked with the phage ~X174 cistron A protein. This protein cuts and reconnects at a specific site on the DNA molecule. It can catalyze transfer of an intermolecular strand at the same time as the recombination reaction. In conclusion, topoisomerases are ubiquitous enzymes that are implicated in metabolism of the DNA, through several pathways.[87]

DNA gyrase, converts the closed helix of DNA to negatively supercoiled DNA. The reaction requires ATP and Mg^{++} and is stimulated by spermidine. This enzyme has

been purified from *Escherichia coli* cells and acts equally well on relaxed closed-circular colicin El, phage X, and simian virus 40 DNA. [88]

DNA gyrase is specifically inhibited by two classes of antibiotics, quinolone antibacterials and novobiocin. [89, 90] The intracellular target for the quinolone group of antibacterial agents is DNA gyrase. Interaction of quinolone antibacterials with a site containing DNA gyrase, results in relaxed closed-circular DNA, which results in inhibition of supercoiling. Quinolones will bind strongly to the complex of DNA and DNA gyrase. The complexation of quinolones and DNA gyrase is facilitated by ATP.[91]

Novobiocin and coumermycin Al compete with ATP, thus they can destroy the energy coupling by preventing ATP binding. Regardless of the selective and competitive effect of novobiocin and coumermycin A1 on ATP binding, there is no remarkable structural homology between these molecules and ATP. In fact, novobiocin has been shown to not affect any other ATP-requiring enzyme at very low concentrations. Nalidixic acid and ciprofloaxin have been shown to interfere with the cleavage and joining of the strands. [92]

1.8.2- Type II topoisomerases other than DNA gyrase

Other Type II topoisomerases have been isolated from both prokaryotic and eukaryotic sources. These enzymes have been detected normally as ATP-dependent DNA-relaxing catalysts and consequently function by a type II mechanism. The first of this class was an enzyme which was purified from *E .Coli.* The purified protein has a molecular weight of approximately 50,000 Da, and requires ATP hydrolysis to

catalytically relax DNA. Novobiocin has no inhibitory activity for this category of enzymes.[93]

The role of ATP in the activity of type II topoisomerases is not clear. So, it is suggested that they might have similar role as that of DNA gyrase .

1.8.3- Biological activities of topoisomerases

Topoisomerases catalyze reactions which are mostly ATP-dependant. DNA replication is also affected by DNA topoisomerases, for instance, elongation of replicating DNA chains, segregation of newly replicated chromosomes and initiation of the replication are all done by DNA topoisomerases. Initiation of replication is initiated by the closure of a short unmatched area of DNA. In plasmid-replication models, which are understood from studies on purified *E. coli* proteins, a negatively supercoiled shape is often needed for initiation. [94]

The necessity for a negatively supercoiled DNA model *in vitro* is indicative of involvement of DNA gyrase in the initiation of bacterial DNA replication. This feature requires DNA gyrase to dictate the direction of DNA segment passage through the enzyme-operated gate in one double-strand DNA. The mechanism by which the DNA segment is transported through the DNA gate is not known in detail. [95, 96]

In the replication of a closed circular DNA, the parental strands must be increasingly and completely unattached at the end of replication. Furthermore, a DNA topoisomerase involvement is mandatory. [101]

Topoisomerases are also involved in transcription as well. A DNA topoisomerase might be involved in continued translocation of the polymerase, and the efficient transcription of chromatin requires the presence of a DNA topoisomerase in the transcription complex. [97] From another biological point of view, the effect of topoisomerases on DNA recombination and chromosome condensation is of high importance.[98]

In brief, topoisomerases are from a family of highly conserved essential enzymes, which exist in all scrutinized living prokaryotic and eukaryotic cells. They are essential for the control of DNA topology. These enzymes catalyze the inter-renovation of different topological forms of DNA through DNA breaking-passing-resealing processes. The universal nature of the enzyme makes it the target of choice for broad-spectrum antibacterial drugs and potent antitumor agents.

1.9- Quinolones as antitumor agents

In spite of their potential as attractive drug targets, the similarity between the topology of eukaryotic and prokaryotic topoisomerases causes difficulty in selectively targeting a specific enzyme. Fortunately, new tools such as molecular modeling and docking techniques have led to the discovery of novel gyrase inhibitors with broad-spectrum antibacterial activity, and topisomerase I and II inhibitors as potential antitumor agents. One of the classes of compounds that have shown activity against prokaryotic topoisomerases (DNA gyrase) is quinolones. [99]

Some groups of 2- and 4-quinolones substituted with a variety of different substituents demonstrated *in vitro* inhibition of human tumor cell lines. 4-Quinolones have been studied from different perspectives, such as their value as anticancer agents in both cell growth and cell migration.[100] From an illustrative point of view, the expansion of malignancy in tissue happens in the following steps. Some genomic mutations increase cell proliferation and decrease cell death, causing disruption in cell cycle kinetics, which eventually end up having uncontrolled growth of a transformed cell population. Later on, the transformed cells accumulate and form a cancerous tissue. Some of these cells might later migrate from the tumor bulk and colonize new tissues, a process which is called metastasis. The migrating cells use lymphatic vessels or blood as major routes of migration. One of the major classes of chemotherapeutic agents used clinically against cancers are drugs that target processes including nucleoside synthesis and DNA replication, the processes that control cell proliferation. The discovery of apoptosis in the early 1970s showed that a low rate of cell death has a higher impact than high rate of cell proliferation, which means that fast growing tumors are not always the ones with higher proliferation rate. [101]

A variety of anticancer agents target topoisomerases, the enzymes that play an essential role in DNA duplication.[102] Some examples are drugs like doxorubicin and etoposide which are already in the market and are used as inhibitors of topoisomerase II.[103]

The clinically approved anticancer agents often target the cell kinetics and cell proliferation rather than cell death. There are other options for cancer treatment such as

immunotherapy and gene therapy. The reason for the shift toward development of other treatment options is because the majority of drugs that are used clinically have high toxicity which limit their clinical use. Furthermore, these drugs must be combined with other drugs in multi-drug regimens in order to have significant treatment outcome. [105]

A variety of 2-arylquinolones, with the goal of developing nontoxic anticancer agents with antimigratory mode of action that can complement the action of the already used anticancers, have been synthesized and reported to exhibit significant efficacy in animal models. [104]

1.10- Structure-based drug design

The field of structure-based drug design is a rapidly growing area in which many successes have been reported in recent years. The exploration of molecular and structural data has provided many new targets and opportunities for future drug lead discovery. During the years of 1985 to early 1990, the first publications and the first attempts made the field of structure-based drug design interesting for researchers. Subsequently, this has become an important tool in research laboratories and institutes. [105]

The completion of the human genome project and the advances in information technology have made structure-based drug design more of a favorable project to be undertaken. By development of bioinformatics better drug targets were identified. Genes of those targets can be cloned and are available in pure forms. The cloning process is quick, and the protein is expressed and purified to homogeneity. Advances in nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, and high throughput screening of the targets, as well as faster and more advanced computers, have made

structure-based drug design of high importance for the discovery of lead compounds. [106-107]

The procedure of structure-based drug design is a stepwise process and usually proceeds through multiple steps before the lead compound can enter the clinical trial stage. The first step includes the comparison of the available natural or synthetic molecules with the preliminary molecule, then to make a set of compounds and modify them with respect to their minimum energy levels and other factors involved in their modification.

Using computer algorithms, compounds or fragments of compounds from a database, X-ray crystallography, NMR, or homology modeling of former active or semi-active compounds can help in selecting the best target molecules among the designed molecules. These compounds are scored and ranked based on various factors, such as their electrostatic and steric interactions with the active site. Later on, a final match of the proposed lead compound with the target in a complex consisting of the active site and the designed molecule will be evaluated. Further steps of optimization are all done on the premise of the first evaluation of the drug in the complex. Choosing a target is based on the type of the disease and biological and pharmacological assessment of the active site. Cancer targets can be difficult because the targets are usually somatic cell mutants of proteins that control essential cellular processes. The consequences of the malfunctioned proteins are loss of cellular control as well as cell injury. The most challenging part of designing anticancer drugs is finding lead compounds which are normally small molecules with the potential of disrupting the dysfunctional chain. [108]

There are three major criteria in the structure-activity-based molecular design strategy. One is pharmacophore mapping, another is a three-dimensional search of both the lead compound and the target as well as molecular design, and the third is the evaluation of the three-dimensional quantitative structure-activity relationships. All these information is used to convert a two-dimensional structure of a bioactive molecule into its three-dimensional features.[109]

1.10.1- Pharmacophore identification

A pharmacophore is a three-dimensional map of certain functional groups and ligands that are necessary to exhibit biological activity. The biological properties of pharmacophores are common to all active configurations which show the desired activity. The idea of pharmacophore identification is to establish the bioactive conformations of the ligands and how to apply the template for drug design. From information like structure-activity relationships, a pharmacophore would first be proposed. The chemical nature of the functional groups that are assigned for the pharmacophore are needed for its *in vitro* and *in vivo* evaluation. Later on, by modification and changes in functional group there would be other requirements that are essential to be considered such as, efficacy and suitable bioactivity of the pharmacophore. The molecules are compared by their overlay based on the required functional groups and their match with the binding sites. The volume and the space that is occupied by the pharmacophore as overlaid with the suggested lead compound(s), bring about the idea of the regions in area that can be filled by any newly designed active ligand.

1.10.2- Three-dimensional searching

Three-dimensional searching recognizes existing molecules, or natural products that counterpart the proposal of the three-dimensional requirements for activity *in vivo* and *in vitro*. Three-dimensional searching facilitates other necessary featuressuch as recognition of potential lead molecules based on their three-dimensional capacities. Furthermore, many of the existing programs use three-dimensional searching to compare the designed molecule with already synthesized and available compounds. Obviously, the lead compound will be chosen on the basis of its ease of synthesis and on its favorable physicochemical properties. For example, molecular modelers usually are questioned as to whether the program would build the molecule based on already existing bioactive compounds, such as building similar antibacterial models from natural antibacterial structures. The proposed compounds will be tested for a pharmacophore model. Three-dimensional searching is parallel to three-dimensional QSAR, because it can be employed to propose a set of compounds and leads for the three-dimensional QSAR analysis. Furthermore, three-dimensional QSAR will finally be used to rank the suggested compounds for synthesis if there are readily available chemical sources such as natural compounds [110-113] Three-dimensional searches are mainly subdivided into **geometric**, **steric**, and **similarity** searches.

1.10.3- Geometric searches

The idea of geometric searches is to look for the intramolecular relationships between geometric objects, such as points, lines, and planes that can be calculated from a structure by a suitable computer program. [114]

1.10.4- Similarity searching

Similarity searching is concerned with how comparable a molecule in the database is with the three-dimensional characteristics of a known molecule.[121]

1.10.5- Steric Searching

In steric searching, the program evaluates the candidate molecule's shape for filling into the specific space that it should fill. The deduction of a steric search is based on different available enantiomers of a molecule, their orientation and selectivity.[121]

1.10.6- Three-dimensional quantitative structure-activity relationships

Biologically-active compounds interact with their biological targets to start or maintain specific molecular mechanisms or functions, such as activation of an enzymatic cascade, or opening of an ion channel, which eventually leads to a specific biological action. Quantitative structure-activity relationships (QSAR) relate the biological response with molecular specificities of the molecule or compounds of interest. Because the biological feedback depends on the lead compound's potency and its concentration at the site of action, these aspects should be taken into consideration by QSAR models. [115]

In three-dimensional QSAR, criteria such as surface, volume or electrostatic potential are calculated from the spatial structure of the molecule.[116] In two-dimensional QSAR, molecular depictions of various levels are connected with the biological activity. Two-dimensional descriptors are topological features and information about how the atoms are connected with each other in the molecule. One-dimensional descriptors include variables that confirm the presence or absence of an atom or a group in the molecule. [117]

Among the foremost three-dimensional -QSAR methods is CoMFA (Comparative Molecular Field Analysis):[118] "The superimposed molecules are located in a three-dimensional grid database and at each grid point a steric and an electrostatic interaction energy with a probe is calculated. The molecular fields obtained in this way are then correlated with the biological activity by partial least-squares (PLS) analysis." [122] The critical step in CoMFA (and generally related methods) is to locate the position of the designed structure. The steric contributions are represented by a binary code and the electronic contributions are attained by evaluating the molecular electrostatic prospective at the grid points, rather than the calculated interaction energy with a probe. In calculating the effect of shape and hindrance, selection of molecules which contain the desired characteristic is more predictable, and the accuracy and predictability value in comparison with one-dimensional-QSAR and/or two-dimensional-QSAR is much higher and more advanced.

2- Materials and instruments

2.1- Chemical reagents

3-Fluorphenylisothiocyanate (catalogue number F14351- 10G) with the purity of (97%), 3-butyn-1-ol (catalogue number 130850-5G) with the purity of (97%), 2-fluorobenzoic acid (catalogue number 412244-25G) with the purity of (97%), cyclopropylmethanol (catalogue number 482099-25ML) with the purity of (+ 99.5%), cyclobutylmethanol (catalogue number 187917-5G) with the purity of (99%), 2-fluorobenzyl alcohol (catalogue number 162515-5G) with the purity of (98%), D-ribose (catalogue number R1757-10G) with the purity of (98%),were purchased from Sigma-Aldrich ,Inc.,3050 Spruce street, St.Louis, MO 63103 USA. 4-fluorobenzylalcohol (catalogue number F740-7- 5G) with the purity of (97%), 3-fluorobenzylalcohol (catalogue number 16250-7-5G) with the purity of (98%), 2,4-difluorobenzoylchloride (catalogue number 265292-25G) with the purity of (98%), mono-ethyl malonate (catalogue number 445088-5G) with the purity of (95%), acetyl chloride (catalogue number 11418-9-25G) with the purity of (98%), 4-fluorophenol (catalogue number F13207-25G) with the purity of (99%), benzyl isothiocyanate (catalogue number 252492-25G) with the purity of (98%), 2',4'- difluoroacetophenone (catalogue number 26425-3,10G) with the purity of (98%), 2-butyn-1-ol (catalogue number 244341-5G) with the purity of (98%), benzoyl chloride (catalogue number B1269-5-25ML) with the purity of (99%), benzylamine (catalogue number 185701-100G) with the purity of (99%), 1-heptanol (catalogue number H2805-100G) with the purity of (98%),ethyl 2-fluorobenzoylacetate (catalogue number 225134-10G), technical grade 2-chloro-2',4'-

difluoro-acetophenone (catalogue number 272507-25G) with the purity of (98%), 1-naphthyl methanol (catalogue number 184284-10G) with the purity of (98%), 2,4-difluorobenzoic acid (catalogue number 264296-25G) with the purity of (98%), 2-naphthyl methanol (catalogue number 187313-5G) with the purity of (99%), cesium carbonate (catalogue number 202126-25G) with the purity of (99.9%), magnesium ethoxide (catalogue number 291846-100G) with the purity of (98%), diethyl carbonate(catalogue number 517135-100ML) with the purity of (+99%), ally alcohol (catalogue number 240532-100ML) with the purity of (+99%), were purchased from Aldrich Chem. Co, Milw. WI 53201. USA. Chromatographic silicagel [200-425 Mesh] was purchased from Fisher Chemicals [Fisher Scientific], Fair Lawn New Jersey, USA.

2.2- Instruments

2.2.1- Nuclear magnetic resonance

^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker Avance 500 spectrometer, equipped with inverse detection and auto tune, capable of doing both one-dimensional and two-dimensional NMR analyses. Data analysis was performed on networked PCs operating software using Acorn NMR.

2.2.2- Liquid chromatography – Mass spectrometry (LC / MS)

Molecular weight data were gathered by utilizing a Hewlett Packard 1100 LC/MS system equipped with degasser, binary pump, photodiode array detector and electrospray ionization mass spectrometry (ESI-MS) or atmospheric pressure chemical

ionization mass spectrometry (APCI-MS), using LC/MSD ChemStation, rev. A. 08.03 (874) software. This equipment is capable of determining a mass range of m/z 50-3000 with a mass accuracy of 0.1 amu. The HPLC system and the ChemStation software were from Agilent Technologies Deutscland GmbH, Waldbronn, Germany. The MSD component was from Agilent Technologies Inc., PaloAlto, CA, U.S.A.

2.2.3- Gas chromatography – Mass spectrometry (GC / MS)

The mass data of smaller molecules with less heat sensitivity were collected by using HP 5970 GC/MSD. The MS detector operated with positive EI mode. The mass range available was m/z 50-800.

2.3- Methods and results

Quinolin-4(1*H*) one-3-carboxylic acid derivatives (quinolones) have been utilized since the early 1980s as potential antimicrobial agents that target bacterial Topo-II isomerase (gyrase) and Topo-IV isomerase. Recently, novel tricyclic and tetracyclic quinolones have been reported as agents that selectively target human Topoisomerases I and II, and exhibit significant anticancer activity. [119] In a literature search for novel quinolones with potential anticancer activity, a joint patent by Japanese pharmaceutical companies, Kyorin/Kyowa-Hakko, describing thiazoloquinolone carboxylic acids with impressive anticancer activities, compound A,[120] attracted our attention. The clinical candidate compound had exhibited favorable drug-like properties in different animal

models in preclinical studies. The structure of the candidate compound is depicted in Figure 2.1.

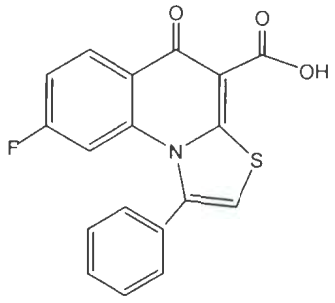


Figure 2.1- Structure of compound A

Compound A, like other quinolone carboxylic acid derivatives, is presumed to interact with topoisomerases via its β-keto acid functional group (the free carboxylic acid and the keto group) and chelate with the Mg^{2+} ion to inhibit the enzyme. Considering this mode of action, we hypothesized that a quinolone with a β-diketo functionality may be able to mimic the action of β-ketoester functionality, thus potentially providing the same level of chemical interaction with the Mg^{2+} ion. Also, due to the absence of free carboxylic acid functionality, the target quinolone may cause less gastric damage if the drug is used by oral route.

In order to design β-diketo analogues of compound A, the Daneshtalab group attempted a structure-based design technique using the Alchemy-3™ molecular modeling program. The computational chemistry was performed by Dr. Alan Cameron on a Silicon Graphic-Octane 2 computer system at the SynPhar Laboratories Inc., in Edmonton, Alberta, Canada. In this respect, the structure of compound A was drawn and its most sterically/energetically optimum configuration was determined using Molecular Mechanics Optimization (MMO) and Molecular Dynamic Options methods. Using this

structure as a reference, attempts were made to identify linear tricyclic quinolones with β-diketo components that match the angular feature of compound A. In this respect, compound B, 9-benzyl-7-fluoro-3-hydroxyisothiazolo[4,5-*b*]quinoline–4(9*H*)-one (**6a**) (Figure 2.2), at its optimized steric/energetic configuration, was found to have the best match with a perfect 3-point overlay with compound A (Figure 2.3).

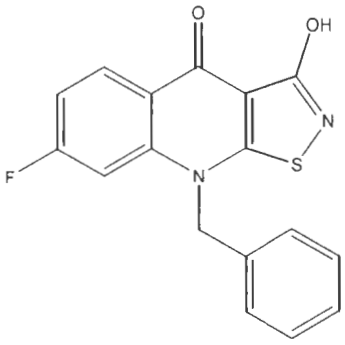


Figure 2.2- Structure of compound B (6a)

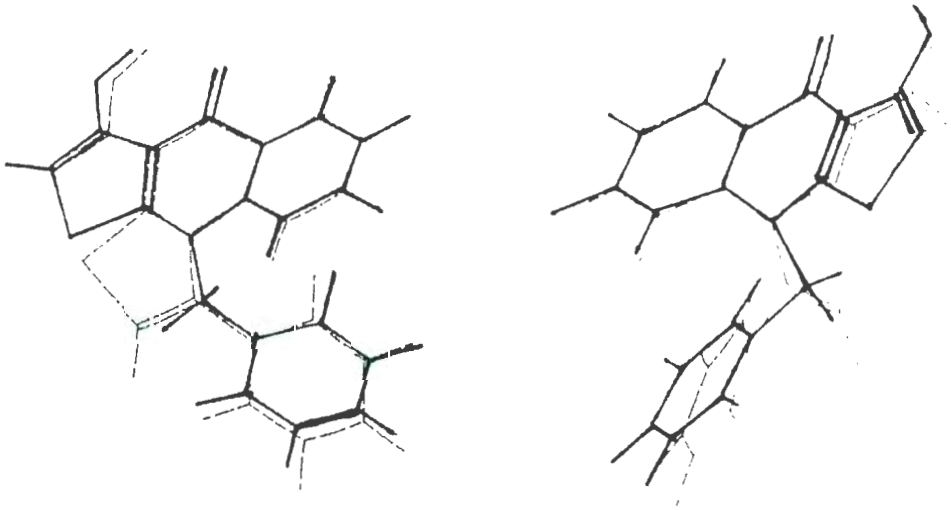
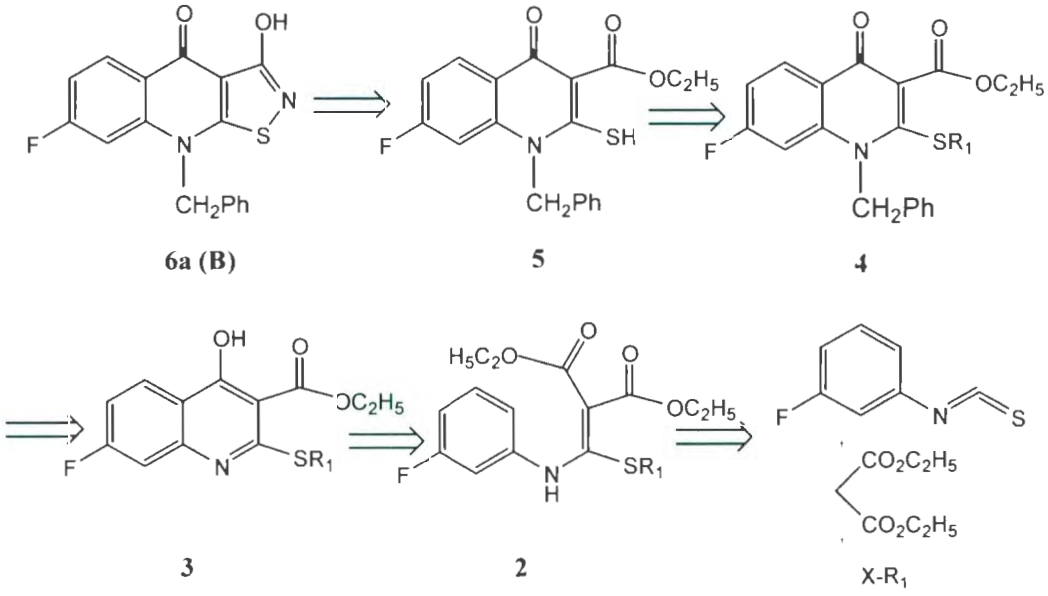


Figure 2.3- Two different overlays of compound A (dotted lines) and compound B (solid lines)

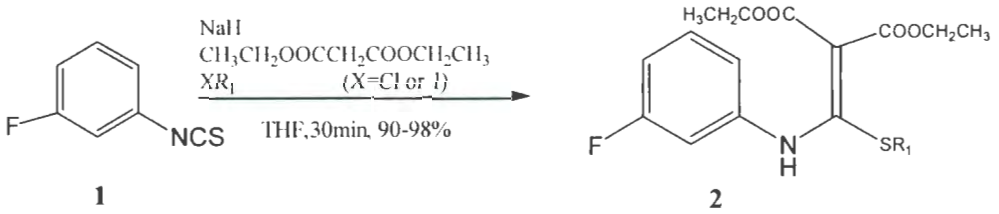
The objective of this research was to design appropriate synthetic methodologies for compound B (**6a**) and its *N*-substituted analogues in order to evaluate their potential as novel mechanism-based anticancer agents.

The following retrosynthetic pathway was first hypothesized for the synthesis of compound B and its N_1-substituted analogues, as depicted in Scheme 2.1. Namely, starting from 3-fluorobenzylisothiocyanate, diethyl malonate, and an appropriate alkyl halide, compound **2** was expected to form, further Gould-Jacob type ring closure of which would result in the formation of compound **3**. Benzylation of this compound is expected to give rise to compound **4** which upon S-dealkyation would afford compound **5**. The final step in this procedure was formation of the third ring via reaction of compound **5** with hydroxylamine-*O*-sulfate to obtain the desired compound **6a**.



Scheme 2.1- Retrosynthetic pathways hypothesized for the synthesis of compound B (**6a**)

The stepwise syntheses of compound B and its N_1-substituted analogues were attempted based on the above hypothetical pathways, as described in the following sections.



1 2

2	R_1
a	$-CH_2OCH_2CH_3$
b	$-CH_2OCH_3$
c	$-CH_3$

Scheme 2.2- Synthesis of the intermediates **2 a-c**

2.3.1- Diethyl [(3-Fluoroanilino)[(ethoxymethyl)thio]methylene]malonate (2a)

To a suspension of 60% NaH (1.44 g, 36 mmol) in anhydrous THF at 0 ^{0}C, diethyl malonate (4.8 g, 30 mmol) was added dropwise. The reaction mixture was stirred for 30 minutes at the same temperature. 3-Fluorophenylisothiocyanate (1) (4.59 g, 30 mmol) was added dropwise to the reaction mixture and stirred for another 30 minutes. Chloromethyl ethyl ether (2.85 g, 30 mmol) was then added dropwise to the reaction mixture at the same temperature and stirred for 1 hour at room temperature.

The reaction mixture was concentrated under reduced pressure, water was added to the reaction mixture and the crude product was extracted with ethyl acetate. The ethyl

acetate solution was washed with an aqueous saturated solution of sodium chloride, dried over sodium sulfate, filtered and the solvent evaporated under reduced pressure. A pale yellow oil (10.6 g, 28.5mmol) was obtained in 95% yield.

^{1}H NMR ($CDCl_3$): δ 1.097-1.098 (3H, t, *J=7.5 Hz*), 1.258-1.298 (6H, t, *J=7.5 Hz*), 3.362-3.446 (2H, q, *J 6.5 Hz*), 4.189-4.251 (4H, m), 4.644 (2H, s), 6.834-6.867 (1H, dd, *J=2.5 Hz*, *J=8.5 Hz*), 7.058-7.102 (2H, m), 7.246-7.291 (1H, dd, *J=3.0 Hz*, *J=7.5 Hz*).

Molecular formula: $C_{17}H_{22}FNO_5S$; Exact Mass Found: 371.12, Calculated: 371.42.

2.3.1.1- Diethyl [(3-Fluoroanilino)[(methoxymethyl)thio]methylene]malonate (2b)

Compound **2b** was synthesized in 90% yield (9.7 g, 27 mmol)., as a pale yellow oil, by the same method as described above and employing chloromethylmethyl ether (2.4 g, 30 mmol) instead of chloromethylethyl ether.

^{1}H NMR ($CDCl_3$): δ 1.980-1.350 (6H, m), 3.212 (3H, s), 4.280-4.308 (4H, q, *J=7.0 Hz*), 4.918 (2H, s), 6.706-6.831(1H, dd, *J=3.5 Hz*, *J=8.0 Hz*), 6.960-6.980 (2H, m), 7.137-7.212 (1H, dd, *J=2.5 Hz*, *J=7.5 Hz*).

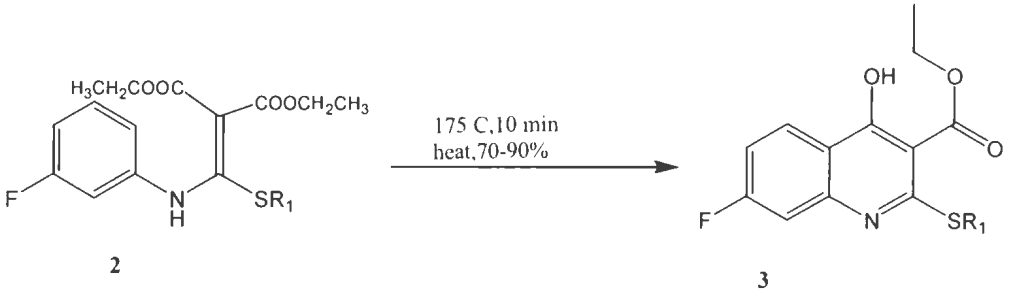
Molecular Formula: $C_{16}H_{20}FNO_5S$; Exact Mass Found: 357.10, Calculated: 357.40.

2.3.1.2- Diethyl [(3-Fluoroanilino)[(methyl)thio]methylene]malonate (2c)

This compound was produced using the same method as described for **2a,b** and employing methyl iodide(4.26 g, 30 mmol) instead of chloromethyl ethers. The product was a yellow oil in 98% yield (9.68g, 29.5 mmol).

^{1}H NMR ($CDCl_3$): δ 1.230-1.273 (6H, m), 2.017 (3H, s), 4.345-4.355(4H, m), 6.846-6.885 (1H, dd, *J=3.0 Hz*, *J=8.5 Hz*), 7.106-6.144 (2H, m), 7.272-7.312 (1H, dd, *J=2.6 Hz*, *J=7.6 Hz*).

Molecular Formula: $C_{15}H_{18}FNO_4S$; Exact Mass Found: 327.09, Calculated: 327.37.



2 3

3	R_1
a	$-CH_2OCH_2CH_3$
b	$-CH_2OCH_3$
c	$-CH_3$

Scheme 2.3-Syntheses of compounds **3a-c**

2.3.2- Ethyl 7-Fluoro-4-hydroxy-2-[(ethoxymethyl)thio]-3-quinoline carboxylate (3a)

Compound **2a** (10.6 g, 28.5 mmol) without further purification was heated under vacuum (0.6 mmHg) at 170-175 ^{o}C for 10 minutes. After cooling, the residue was crystallized from a mixture of ethyl acetate and hexane to yield yellow crystals (7.41g, 22.8 mmol) (mp: 145-150 ^{o}C, 80% yield).

^{1}H NMR ($CDCl_3$): δ 1.219-1.247 (3H, t, *J=7.0 Hz*), 1.525-1.552 (3H, t, *J=6.5 Hz*), 3.670-3.714 (2H, q, *J=7.5 Hz*), 4.530-4.575 (2H, q, *J=7.5 Hz*), 5.547 (2H, s), 7.168-7.208 (1H, dd, *J= 2.5 Hz*, *J=9.0 Hz*), 7.435-7.459 (1H, dd, *J=3.0 Hz*, *J= 10.0 Hz*), 8.216-8.247 (1H, dd, *J=7.0 Hz*, *J=.09 Hz*).

^{13}C NMR ($CDCl_3$): δ 14.2, 15.0, 57.8, 63.1, 72.9, 103.0, 112.2, 112.3, 115.2, 115.4, 126.4, 126.5, 159.8, 168.8, 171.0.

Molecular Formula: $C_{15}H_{16}FNO_4S$; Exact Mass Found: 325.08, Calculated: 325.36.

2.3.2.1- Ethyl 7-Fluoro-4-hydroxy-2-[(methoxymethyl)thio]-3-quinoline carboxylate (3b)

This compound was synthesized using the same procedure as described for **3a** to yield (6.5 g, 21 mmol) colorless crystals with melting point of (140-145 °C) and 70 % yield.

^1H NMR ($CDCl_3$): δ 1.529-1.558 (3H, t, *J=7.5 Hz*), 3.464 (3H, s), 4.540-4.591 (2H, q, *J=7.0 Hz*), 5.498 (2H, s), 7.171-7.260 (1H, dd, *J= 3.6 Hz*, *J=9.0 Hz*), 7.450-7.70 (1H, dd, *J=4.0 Hz*, *J= 10.0 Hz*), 8.217-8.247 (1H, dd, *J=6.0 Hz*, *J= 10.0 Hz*).

^{13}C NMR ($CDCl_3$): 14.2, 57.8, 63.1, 72.9, 103.0, 112.2, 112.3, 115.2, 115.4, 126.4, 126.5, 159.8, 168.8, 171.0

Molecular Formula: $C_{14}H_{14}FNO_4S$; Exact Mass Found: 311.06, Calculated: 311.33.

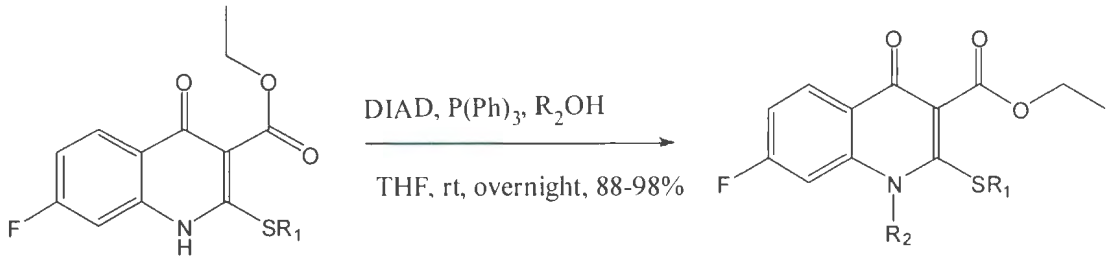
2.3.2.2- Ethyl 7-Fluoro-4-hydroxy-2-(methylthio)-3-quinoline carboxylate (3c)

Compound **3c** was produced using the same method as described for **3a,b** to produce 7.58 g (27 mmol, 90%) of a yellow solid with a melting point of 120-125 °C.

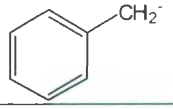
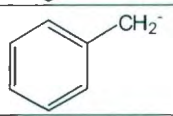
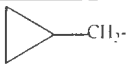
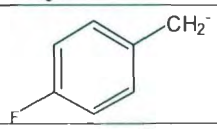
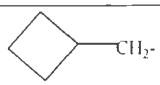
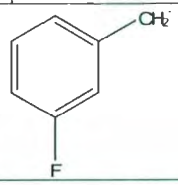
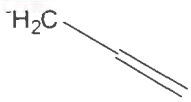
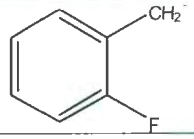
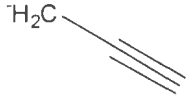
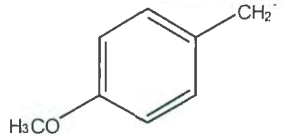
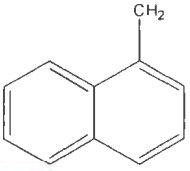
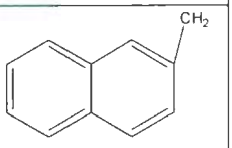
^1H NMR ($CDCl_3$): δ 1.520-1.552 (3H, t, *J=8.0 Hz*), 3.585 (3H, s), 4.528-4.571 (2H, q, *J=8.0 Hz*), 7.131-7.177 (1H, dd, *J= 3.8 Hz*, *J=9.0 Hz*), 7.420-7.440 (1H, dd, *J=2.8 Hz*, *J= 11.0 Hz*), 8.199-8.230 (1H, dd, *J=5.0 Hz*, *J= 11.0 Hz*).

^{13}C NMR ($CDCl_3$): 13.6, 15.1, 62.7, 103.0, 112.2, 112.3, 115.2, 115.4, 126.4, 126.5, 159.8, 168.8, 171.0

Molecular Formula, $C_{13}H_{12}FNO_3S$; Exact Mass Found: 281.05, Calculated: 281.30.



3 4

4	R_1	R_2	4	R_1	R_2
a	$-CH_2OCH_2CH_3$		i	$-CH_2OCH_2CH_3$	$CH_3CH_2CH_2CH_2-$
b	$-CH_2OCH_3$		j	$-CH_2OCH_2CH_3$	
c	$-CH_2OCH_2CH_3$		k	$-CH_2OCH_2CH_3$	
d	$-CH_2OCH_2CH_3$		l	$-CH_2OCH_2CH_3$	
e	$-CH_2OCH_2CH_3$		m	$-CH_2OCH_2CH_3$	
f	$-CH_2OCH_2CH_3$		n	$-CH_2OCH_2CH_3$	
g	$-CH_2OCH_2CH_3$	CH_3CH_2-	o	$-CH_2OCH_2CH_3$	
h	$-CH_2OCH_2CH_3$	$CH_3CH_2CH_2-$			

Scheme 2.4- Syntheses of compounds **4a-o**

2.3.3- Ethyl 1-Benzyl-7-fluoro-2-[(ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4a)

To a solution of **3a** (6.5 g,20 mmol), triphenylphosphine (6.29g,24 mmol) and benzyl alcohol (2.16g,20 mmol) in THF, diisopropyl azodicarboxylate (DIAD) (4.85g,24 mmol) was added dropwise. The reaction mixture was stirred for six hours. After the removal of the solvent under reduced pressure, anhydrous diethyl ether was added and allowed to stand for 5 hours, then filtered. The diethyl ether was evaporated under reduced pressure and the oily residue was purified by silica gel column chromatography (20:1 hexane: ethyl acetate) to yield a pale yellow oil (7.47 g, 18 mmol, 90 %).

^{1}H NMR ($CDCl_3$): δ 1.213-1.241 (3H, t, *J=7.0 Hz*), 1.407-1.436 (3H, t, *J =7.0 Hz*), 3.656-3.699 (2H, q, *J=7,5 Hz*), 4.445-4.487 (2H, q, *J=7.0 Hz*), 5.234 (2H, s), 5.636 (2H, s), 7.160-7.199(1H, dt, *J=2.0 Hz*, *J=9.5 Hz*), 7.384-7.450 (5H, m), 7.554-7.580 (1H, dd, *J=2.0 Hz*, *J=9.5 Hz*), 7.999-8.029 (1H, dd, J=*6.0 Hz*, J=*9.5 Hz*).

^{13}C NMR ($CDCl_3$): 14.2, 15.1, 58.6, 62.6, 65.4, 71.7, 112.3, 112.5, 115.9, 116.1, 125.4, 125.5, 126.4, 128.8, 128.9, 136.0, 150.3, 157.9, 160.4, 163.5, 165.5, 166.2.

Molecular Formula: $C_{22}H_{22}FNO_4S$; Exact Mass Found: 415.13, Calculated: 415.48.

2.3.3.1- Ethyl 1-Benzyl-7-fluoro-2- [(methoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4b)

This compound was prepared with the same procedure as described in section 2.3.3, employing (6.22 g, 20 mmol) of compound **3b** and benzyl alcohol (2.16 g, 20

mmol). Ratios of triphenylphosphine and DIAD were the same as for **4a**. The product with the yield of 90% was a colorless oil (7.2 g, 18 mmol).

^{1}H NMR ($CDCl_3$): δ 1.407-1.436 (3H, t, *J= 7.5 Hz*), 3.444 (3H, s), 4.383-4.425 (2H, q, *J=7.8 Hz*), 5.172 (2H, s), 5.636 (2H, s), 7.120-7.159 (1H, dt, *J=2.9 Hz*, *J=9.8 Hz*), 7.364-7.421 (5H, m), 7.602-7.645 (1H, dd, *J=2.6 Hz*, *J=9.8 Hz*), 7.802-7.819 (1H, dd, *J 7.0 Hz*, *J=11.0 Hz*).

^{13}C NMR ($CDCl_3$): 14.2, 58.6, 63.0, 65.4, 72.7, 112.3, 112.5, 115.9, 116.1, 125.4, 125.5, 126.4, 128.8, 128.9, 136.0, 150.3, 157.9, 160.4, 163.5, 165.5, 166.2.

Molecular Formula: $C_{21}H_{20}FNO_4S$; Exact Mass Found: 401.11, Calculated: 401.45.

2.3.3.2- Ethyl 1-(4-Fluorobenzyl)7-fluoro-2-[(ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4c)

Compound **4c** was synthesized utilizing the same technique as described in section 2.3.3, using compound **3a** (6.5 g, 20 mmol) and 4-fluorobenzyl alcohol (2.52 g, 20 mmol). The product with the yield of 98% was a colorless oil (8.5 g, 19.5 mmol). Ratios of triphenylphosphine and DIAD were the same as used for **4a**.

^{1}H NMR ($CDCl_3$): δ 1.113-1.211 (3H, t, *J= 7.5 Hz*), 1.417-1.426 (3H, t, *J 7.5 Hz*), 3.655-3.699 (2H, q, *J=7.5 Hz*), 4.455-4.488 (2H, q, *J=7.5 Hz*), 5.254 (2H, s), 5.686 (2H, s), 7.083-7.093(2H, dt, *J=5.0 Hz*, *J=9.5 Hz*), 7.148-7.187 (1H, dt, *J=6.0 Hz*, *J=11.0 Hz*), 7.404-7.428 (2H, dd, *J=8.0 Hz*, *J =11.0 Hz*),7.540- 7.563 (1H, dd, *J=9.0 Hz*, *J=14.0 Hz*), 7.949-7.977 (1H, dd, *J=9.0 Hz*, *J=12.0 Hz*).

^{13}C NMR ($CDCl_3$): δ 14.2, 15.0, 57.8, 62.5, 65.4, 72.8, 112.3, 112.5, 114.9, 115, 125.4, 125.5, 130.2, 131.9, 131.8, 133.1, 150.8, 159.8, 160.1, 163.5, 165.5, 166.2.

Molecular Formula: $C_{22}H_{21}F_2NO_4S$; Exact Mass Found: 433.12 , Calculated: 433.47.

2.3.3.3- Ethyl 1-(3-Fluorobenzyl)7-fluoro-2- [ethoxy)methyl thio]-quinolin-4(*1H*)-one-3-carboxylate (4d)

Treatment of **3a** (6.5 g, 20 mmol) with (2.5 g, 20 mmol) of 3-fluorobenzyl alcohol using the same ratios of triphenylphosphine and DIAD, as described with the formation of compound **4a,** yielded (8.4 g, 19 mmol) of compound **4d** as a pale yellow oil (96%).

^{1}H NMR ($CDCl_3$): δ 1.213-1.291 (3H, t, J= *7.4 Hz*), 1.411-1.424 (3H, t, *J= 7.5 Hz*), 3.652-3.693 (2H, q, *J=7.5 Hz*), 4.405-4.477 (2H, q, *J=7.5 Hz*), 5.229 (2H, s), 5.624 (2H, s), 7.040-7.073 (1H, t, *J -12.0 Hz*), 7.176-7.242 (3H, m), 7.342-7.371 (1H, dd, *J=7.5 Hz*, *J=10.0 Hz*), 7.551- 7.572 (1H, dd, *J=8.5 Hz*, *J=13.0 Hz*), 7.978-7.994 (1H, dd, *J=8.5 Hz*, *J=12.0 Hz*).

^{13}C NMR ($CDCl_3$): 14.2, 15.1, 58.6, 62.6, 65.4, 72.9, 112.4, 112.5, 115.8, 116.0, 125.4, 125.5, 126.4, 128.8, 128.9, 136.1, 150.2, 157.9, 160.3, 163.5, 165.5, 166.3.

Molecular Formula: $C_{22}H_{21}F_2NO_4S$; Exact Mass Found: 433.12, Calculated: 433.47

2.3.3.4- Ethyl 1-(2-Fluorobenzyl)-7-fluoro-2-[ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4e)

Compound **4e** was prepared utilizing the same technique as described in section 2.3.3, using compound **3a** (6.5 g, 20 mmol) and 2-fluorobenzyl alcohol (2.52 g, 20 mmol). The product was a pale yellow oil (8.5 g, 19.6 mmol) with the yield of 98%. Ratios of triphenylphosphine and DIAD were the same as for **4a**.

^{1}H NMR ($CDCl_3$): δ 1.223-1.281 (3H, t, *J= 7.5 Hz*), 1.321-1.423 (3H, t, *J= 7.5 Hz*), 3.642-3.673 (2H, q, *J=7.5 Hz*), 4.415-4.467 (2H, q, *J=7.5 Hz*), 5.239 (2H, s), 5.623 (2H, s), 7.140-7.153 (1H, t, *J=12.0 Hz*), 7.166-7.262 (1H, dd, *J=8.0 Hz*, *J 11.0 Hz*), 7.352-7.372 (2H, m),7.551- 7.572 (1H, dd, *J=8.5 Hz*, *J=13.0 Hz*), 7.668-7.776 (1H, m), 7.978-7.994 (1H, dd, *J=8.0 Hz*, *J=13.0 Hz*).

^{13}C NMR ($CDCl_3$): 14.1, 15.0, 62.5, 65.4, 70.8, 71.7, 112.3, 112.5, 115.9, 116.1, 125.3, 125.4, 126.4, 130.8, 130.9, 136.0, 150.3, 157.9, 160.4, 163.5, 165.5, 165.9.

Molecular Formula: $C_{22}H_{21}F_2NO_4S$; Exact Mass Found: 433.12 , Calculated: 433.47.

2.3.3.5- Ethyl 1-(4-Methoxybenzyl)-7-fluoro-2-[(ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4f)

This compound was synthesized according to the procedure described for preparation of **4a** by employment of (2.76 g, 20 mmol) of 4-methoxybenzyl alcohol, to form **4f** as a pale yellow oil with 95% yield (8.45, 19.5 mmol).

^{1}H NMR ($CDCl_3$): δ 1376-1.401 (3H, t, *J= 7.5Hz*), 1.457-1.666 (3H, t, *J= 7.6Hz*), 3.545-3.669 (2H, q, *J=7.5 Hz*), 3.811 (3H, s), 4.412-4.488 (2H, q, *J=7.6Hz*), 5.270 (2H, s),

5.677 (2H, s), 6.918-7.930 (2H, dt, *J=6.0 Hz*, *J=9.0 Hz*), 7.157-7.179 (1H, dt, *J 5.5Hz*, *J=10.5 Hz*), 7.304-7.417 (2H, dd, *J=7.5 Hz*, *J=10.0 Hz*),7.531-7.553 (1H, dd, *J=8.5Hz*, J=13.0 Hz), 7.961-7.989 (1H, dd, *J=9.0 Hz*, *J=12.0 Hz*).

^{13}C NMR ($CDCl_3$): 14.3, 15.1, 56.0, 58.6, 62.6, 65.4, 71.7, 112.3, 112.5, 115.9, 116.1, 125.4, 125.5, 128.8, 128.9, 136.0, 150.3, 157.9, 158.0, 160.4, 163.5, 165.4, 166.3.

Molecular Formula: $C_{23}H_{24}FNO_5S$; Exact Mass Found: 445.14, Calculated: 445.50.

2.3.3.6- Ethyl 1-Ethyl-7-fluoro-2-[(ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate. (4g)

Treatment of (**3a**) with ethyl alcohol (0.92 g, 20 mmol), using the same procedure as described for compound **4a**, afforded compound **4g** as a colorless oil (6.7 g, 18 mmol, 95 %).

^1H NMR ($CDCl_3$): δ 1.220-1.267 (6H, m), 1.520-1.549 (3H, t, *J 7.5 Hz*), 3.685-3.699 (2H, q, *J=7.6 Hz*), 4.4538-4.552 (2H, q, *J 7.5 Hz*), 5.212-5.263 (2H, q, *J=7.6 Hz*), 5.536 (2H, s), 7.036-7.073(1H, dt, *J=3.0 Hz*, *J=8.5 Hz*), 7.416-7.445 (1H, dd, *J=2.5 Hz*, *J 9.0 Hz*), 8.194-8.224 (1H, dd, *J=7.0 Hz*, *J=8.5 Hz*).

Molecular Formula: $C_{17}H_{20}FNO_4S$; Exact Mass Found: 353.11, Calculated: 353.41.

2.3.3.7- Ethyl 1-(Propyl)7-fluoro-2-[ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4h)

This compound was synthesized in 90% yield as a pale yellow oil (6.6 g, 18 mmol) employing the same procedure as described for compound **4a**, using 1-propanol (1.2 g, 20 mmol).

^{1}H NMR ($CDCl_3$): δ 0.999-1.034 (3H, m), 1.210-1.247 (6H, m), 1.519-1.539 (3H, t, *J=7.3 Hz*), 3.685-3.699 (2H, q, *J=7.4Hz*), 4.464-4.653 (2H, q, *J=7.5 Hz*), 5.113-5.272 (2H, q, *J=7.5 Hz*), 5.544 (2H, s), 7.024-7.054 (1H, dt, *J=2.5Hz, J 7.5 Hz*), 7.326-7.345 (1H, dd, *J=2.4 Hz, J=8.0 Hz*), 8.184-8.214 (1H, dd, *J=7.0 Hz, J=8.0 Hz*).

Molecular Formula: $C_{18}H_{22}FNO_4S$; Exact Mass Found: 367.13, Calculated: 367.44.

2.3.3.8- Ethyl 1-(Butyl)-7-fluoro-2-[(ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4i)

Compound **4i** (6.48 g, 17 mmol) was prepared as a yellow oil in 85% yield employing the same procedure as described for compound **4a**, using 1-butanol (1.48 g, 20 mmol).

^{1}H NMR ($CDCl_3$): δ 0.987-1.040 (3H, m), 1.210-1.348 (7H, m), 1.629-1.639 7(3H, t, *J=7.5 Hz*), 3.675-3.689 (2H, q, *J=7.4 Hz*), 4.453-4.673 (2H, q, *J=7.5 Hz*), 5.013-5.163 (2H, q, *J=7.6 Hz*), 5.532 (2H, s), 7.114-7.144(1H, dt, *J=2.0 Hz, J=7.0 Hz*), 7.436-7.448 (1H, dd, *J=2.6 Hz, J=7.5 Hz*), 8.104-8.103 (1H, dd, *J=7.5 Hz, J=9.0 Hz*).

^{13}C NMR ($CDCl_3$): 13.3, 13.9, 14.2, 15.1, 32.2, 62.2, 62.6, 65.4, 71.7, 112.3, 112.5, 115.9, 116.1, 150.3, 157.9, 160.4, 163.5, 165.5, 166.2.

Molecular Formulas: $C_{19}H_{24}FNO_4S$; Exact Mass Found: 381.14, Calculated: 381.46.

2.3.3.9- Ethyl 1-(Cyclopropylmethyl)-7-fluoro-2-[(ethoxymethyl) thio]-quinolin-4(*1H*)- one-3-carboxylate (4j)

This compound was prepared in 90% yield as a yellow oil (6.8 g, 18 mmol) employing the same procedure as described for compound **4a**, using cyclopropylmethyl alcohol (1.44 g, 20 mmol).

^{1}H NMR ($CDCl_3$): δ 0.328-0.358 (2H, d, *J=5.5 Hz*), 0.627-0.664 (2H, d, *J 5.5 Hz*),1.057-1.082 (1H, m), 1.195-1.223 (3H, q, *J=7.5Hz*), 1.421-1.463 (3H, t, *J=7.0 Hz*), 3.637-3.680 (2H, q, *J=7.0 Hz*), 4.046-4.048 (2H, d, *J=3.0 Hz*), 4.454-4.498 (2H, q, *J=7.0 Hz*), 5.616 (2H, s), 7.203-7.243(1H, dt, *J=3.0 Hz*, *J=9.0 Hz*), 7.534-7.561 (1H, dd, *J=3.5 Hz*, *J=1.01 Hz*), 8.127-8.158 (1H, dd, *J=7.0 Hz*, *J=9.5 Hz*).

^{13}C NMR ($CDCl_3$): 9.7, 10.9, 14.2, 15.1, 58.6, 62.6, 65.4, 71.7, 81.7, 112.3, 112.5, 115.9, 116.1, 150.8, 157.8, 160.4, 163.5, 165.4, 166.2.

Molecular Formula: $C_{19}H_{22}FNO_4S$; Exact Mass Found: 379.13, Calculated: 379.45.

2.3.3.10- Ethyl 1-(Cyclobutylmethyl)-7-fluoro-2-[(ethoxymethyl)thio]-quinolin -4(*1H*)- one-3-carboxylate (4k)

Compound **4k** was synthesized employing the same method as described for compound **4a**, using **3a** and cyclobutylmethyl alcohol (1.72 g, 20 mmol). The product was a colorless oil (7 g, 18 mmol) with a yield of 90%.

^{1}H NMR ($CDCl_3$): δ 1.193-1.222 (3H, d, *J=7.5 Hz*), 1.418-1.469 (3H, m), 1.880-1941 (4H, m), 2.125-2.156 (2H, m) , 2.803-2.860 (1H, m), 3.637-3.678 (2H, q, *J=7.0 Hz*), 4.109-4.186 (2H, d, *J=3.0 Hz*), 4.404-4.505 (2H, q, *J=7.0 Hz*), 5.617 (2H, s), 7.187-7.227(1H, dt, *J=3.0 Hz, J=11.5 Hz*), 7.528-7.551 (1H, dd, *J=1.0 Hz, J=9.5 Hz*), 8.064-8.094 (1H, dd, *J 5.5 Hz, J=9.0 Hz*).

^{13}C NMR ($CDCl_3$): 14.2, 15.1, 18.4, 21.8, 35.9, 62.6, 65.4, 71.7, 80.8, 111.2, 111.4, 115.9, 116.1, 130.5, 150.2, 157.9, 160.4, 163.5, 165.3, 166.2.

Molecular Formula: $C_{20}H_{24}FNO_4S$; Exact Mass Found: 393.14, Calculated: 393.47.

2.3.3.11- Ethyl 1-(Allyl)-7-fluoro-2-[(ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4l)

This compound was produced utilizing the same procedure as described for compound **4a**, using **3a** (6.5 g, 20 mmol) and allyl alcohol (0.9 g, 20 mmol). The product was a colorless oil (6.6 g, 18 mmol) in 90% yield.

^{1}H NMR ($CDCl_3$): δ 1.200-1.228 (3H, d, *J=7.0 Hz*), 1.436-1.463 (3H, q, *J=7.0 Hz*), 3.642-3.685 (2H, q, *J=7.5Hz*), 4.460-4.488 (2H, q, *J=5.0 Hz*), 4.644-4.714 (2H, d, *J 6.0 Hz*), 5.323-5.340 (2H, d, *J=8.5 Hz*), 5.618 (2H, s), 5.91 (1H, m),7.108-7.133 (1H, dt, *J=3.5 Hz, J 8.5 Hz*), 7.211-7.246 (1H, dd, *J=2.5 Hz, J=9.5 Hz*), 8.060-8.099 (1H, dd, *J=6.0 Hz, J=9.0 Hz*).

^{13}C NMR ($CDCl_3$): 14.2, 15.1, 62.6, 65.4, 71.6, 74.5, 112.4, 115.9, 118.7, 125.4, 132.6, 136.0, 150.3, 157.8, 160.4, 163.5, 165.5, 166.2.

Molecular Formula: $C_{18}H_{20}FNO_4S$; Exact Mass Found: 365.11, Calculated: 365.42;

2.3.3.12- Ethyl 1-Propyn-2-yl-7-fluoro-2- 2-[(ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4m)

Compound **4m** was synthesized in 90% yield (6.5 g, 18 mmol), as a yellow oil, employing the same procedure as described for compound **4a**, using propargyl alcohol (0.84 g, 20 mmol).

^{1}H NMR ($CDCl_3$): δ 1.215-1.247 (3H, d, *J=7.0 Hz*), 1.457-1.550 (3H, q, *J=7.0 Hz*), 2.269 (1H, s), 3.653-3.714 (2H, q, *J=7.0 Hz*), 4.527-4.571 (2H, q, *J 4.0 Hz*), 5.215-5.252 (2H, p, *J=6.0 Hz*), 5.541 (2H, s) , 6.835-6.869 (1H, dt, *J=3.0 Hz, J 8.5 Hz*), 7.426-7.451 (1H, dd, *J=2.0 Hz, J=10.0 Hz*), 8.166-8.222 (1H, dd, *J=7.0 Hz, J 10.0 Hz*).

^{13}C NMR ($CDCl_3$): 14.2, 15.1, 62.6, 65.4, 71.7, 74.4, 112.3, 112.5, 115.9, 116.1, 126.4, 135.9, 150.3, 157.9, 160.4, 163.5, 165.5, 166.2.

Molecular formula: $C_{18}H_{18}FNO_4S$; Exact Mass Found: 363.09, Calculated: 363.40.

2.3.3.13- Ethyl 1-(1-Methylnaphthyl)7-fluoro-2-[(ethoxymethyl thio)]-quinolin-4(*1H*)-one-3-carboxylate (4n)

This compound was prepared utilizing the same procedure as described for compound **4a**, using **3a** (20 mmol) and 1-naphthylmethyl alcohol (3.16 g, 20 mmol). The product was a colorless oil (8.37 g, 18 mmol, 90%).

^{1}H NMR ($CDCl_3$): δ 1.113-1.231 (3H, t, *J=7.5 Hz*), 1.417-1.426 (3H, t, *J=7.5 Hz*), 3.625-3.647 (2H, q, *J=7.5 Hz*), 4.436-4.447 (2H, q, *J=7.5 Hz*), 5.244 (2H, s), 5.656 (2H, s),

7.161-7.171(1H, dt, *J=2.5 Hz*, *J=9.0 Hz*), 7.373-7.453 (4H, m), 7.544-7.572 (1H, dd, *J=2.0 Hz*, *J=9.5 Hz*), 7.625-7.745 (3H, m), 7.999-8.029 (1H, dd, *J=6.0 Hz*, *J=9.5 Hz*).

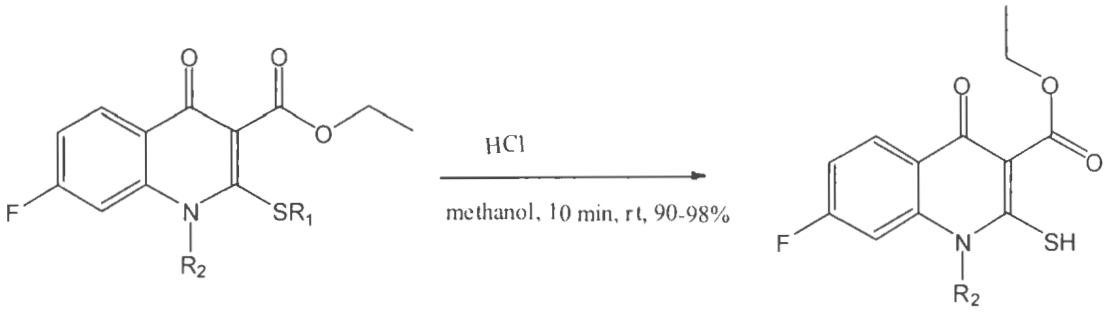
Molecular formula: $C_{26}H_{24}FNO_4S$; Exact Mass Found: 465.14, Calculated: 465.54.

2.3.3.14- Ethyl 1-(2-Methylnaphthyl)-7-fluoro-2-[(ethoxymethyl)thio]-quinolin-4(*1H*)-one-3-carboxylate (4o)

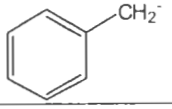
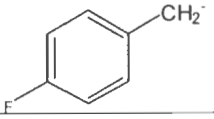
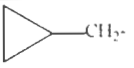
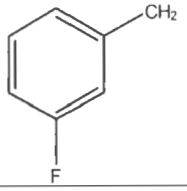
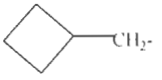
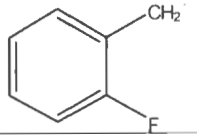
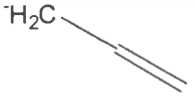
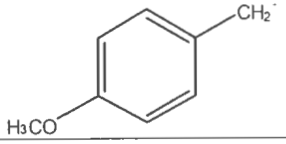
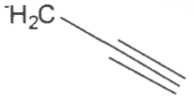
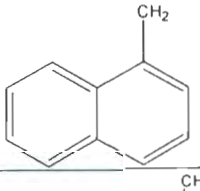
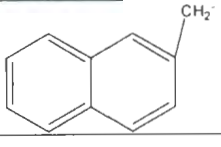
Compound **4o** was prepared in 90% yield (8.37 g, 18 mmol), as a colorless oil, following the same procedure as described for compound **4m,** using 2-naphthylmethyl alcohol (3.16 g, 20 mmol).

^{1}H NMR ($CDCl_3$): δ 1.103-1.211 (3H, t, *J= 7.0 Hz*), 1.399-1.412 (3H, t, *J=7.0 Hz*), 3.620-3.634 (2H, q, *J= 7.5 Hz*), 4.335-4.448 (2H, q, *J=7.5 Hz*), 5.213 (2H, s), 5.658 (2H, s), 7.158-7.167 (1H, dt, *J=3.5 Hz*, *J=9.5 Hz*), 7.283-7.389 (4H, m), 7.532-7.564 (1H, dd, *J=2.5 Hz*, *J=8.5 Hz*), 7.614-7.746 (3H, m), 8.008-8.019 (1H, dd, *J=6.0 Hz*, *J=8.5 Hz*).

Molecular formula: $C_{26}H_{24}FNO_4S$; Exact Mass Found: 465.14, Calculated: 465.54.



4 **5**

5	R_2	5	R_2
a		h	$CH_3CH_2CH_2CH_2$ -
b		i	
c		j	
d		k	
e		l	
f	CH_3CH_2-	m	
g	$CH_3CH_2CH_2$-	n	

Scheme 2.5- Syntheses of compounds **5a-n**

2.3.4- Ethyl 1-Benzyl-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5a)

To a solution of **4a** (8.3 g, 20 mmol) in ethanol, two drops of concentrated hydrochloric acid was added and stirred at room temperature for 10 minutes. The reaction mixture was poured into water and the yellow powder was filtered with the yield of 98% (7 g, 19.6 mmol). Melting point of the product was not measured and the crude product was directly used for the next step of the reaction.

^{1}H NMR ($CDCl_3$): δ 1.439-1.467 (3H, t, *J=6.5 Hz*), 4.495-4.537 (2H, q, *J=7.0 Hz*), 5.347 (2H, s), 6.992-7.030 (1H, dt, *J=2.0 Hz*, *J=8.5 Hz*), 7.173-7.195 (1H, dd, *J=2.0 Hz*, *J 7.0 Hz*), 7.399-7.441 (5H, m), 7.870-7.901 (1H, dd, *J - 5.5 Hz*, *J=9.5 Hz*), 12.079 (1H, s).

^{13}C NMR ($CDCl_3$): 15.1, 62.6, 102.1, 112.3, 112.5, 115.9, 116.1, 125.4, 125.5, 126.4, 128.8, 128.9, 136.0, 150.3, 157.9, 160.4, 163.5, 166.2, 180.7.

Molecular Formula:$C_{19}H_{16}FNO_3S$; Exact Mass Found: 357.08, Calculated: 357.40.

Employing the same procedure as described for compound **5a** and using compound **4b** (8.0 g, 20 mmol), instead of **4a**, resulted in production of **5a** with the same yield.

2.3.4.1- Ethyl 1-(4-Fluorobenzyl)-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5b)

Utilizing the same procedure as described for **5a**, compound **5b** was obtained as a yellow powder in 98% yield (7. 28 g, 19.4 mmol) from **4c** (8.66 g, 20 mmol).

^{1}H NMR ($CDCl_3$): δ 1.338-1.395 (3H, t, *J=7.5 Hz*), 4.483-4.516 (2H, q, *J -7.5 Hz*), 5.356 (2H, s), 7.063-7.095 (2H, dt, *J=5.5Hz*, *J=9.0 Hz*), 7.127-7.167 (1H, dt, *J 5.0 Hz*, *J=11.5*

Hz), 7.314-7.338 (2H, dd, *J=8.5 Hz*, *J=10.5 Hz*),7.560- 7.573 (1H, dd, *J=9.5 Hz*, *J =13.0 Hz*), 7.938-7.964 (1H, dd, *J=8.0 Hz*, *J=11.0 Hz*), 11.089 (1H, s).

^{13}C NMR ($CDCl_3$): δ 15.0, 62.5, 102.0, 112.3, 112.5, 114.9, 115.0, 125.4, 125.5, 130.2, 131.9, 131.8, 133.0, 150.8, 159.8, 160.1, 163.5, 166.2, 179.1.

Molecular formula:$C_{19}H_{15}F_2NO_3S$; Exact Mass Found: 375.07; Calculated: 375.39.

2.3.4.2- Ethyl 1-(3-Fluorobenzyl)-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5c)

This compound (7.13 g, 19 mmol, 95%) was obtained as a yellow powder, employing the same procedure as described for compound **5a**, using compound **4d** (8.66 g, 20 mmol).

^{1}H NMR ($CDCl_3$): δ 1.400-1.405 (3H, t, *J=7.0 Hz*), 4.383-4.416 (2H, q, *J=7.0 Hz*), 5.358 (2H, s), 7.053-7.085(2H, dt, *J=4.5 Hz*, *J =8.5 Hz*), 7.137-7.157 (1H, dt, *J=4.5Hz*, *J 10.5 Hz*), 7.214-7.238 (2H, dd, *J=8.5 Hz*, *J=11.5 Hz*), 7.560- 7.573 (1H, dd, *J=9.0 Hz*, *J 14.0 Hz*), 7.938-7.964 (1H, dd, *J=7.5 Hz*, *J=10.5 Hz*),12.350 (1H, s).

^{13}C NMR ($CDCl_3$): 15.1, 62.6, 103.1, 112.4, 112.5, 115.8, 116.0, 125.4, 125.5, 126.4, 128.8, 128.9, 136.1, 150.2, 157.9, 160.3, 163.5, 166.3, 170.1.

Molecular formula:$C_{19}H_{15}F_2NO_3S$; Exact Mass Found: 375.07; Calculated: 375.39.

2.3.4.3- Ethyl 1-(2-Fluorobenzyl)-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5d)

Compound **5d** was made (6.76 g, 18 mmol, 90%), as a yellow solid, from 8.66 g, (20 mmol,) of **4e** according to the method described for the preparation of **5a**.

^{1}H NMR ($CDCl_3$): δ 1.390-1.402 (3H, t, *J=7.5 Hz*), 4.353-4.426 (2H, q, *J=7.5 Hz*), 5.359 (2H, s), 7.043-7.075 (2H, dt, *J=5.5 Hz*, *J=8.0 Hz*), 7.136-7.159 (1H, dt, *J=5.0 Hz*, *J=10.5 Hz*), 7.214-7.238 (3H, m), 7.948-7.954 (1H, dd, *J=7.0 Hz*, *J=10.5 Hz*), 11.560 (1H, s).

^{13}C NMR ($CDCl_3$): δ 15.0, 62.5, 104.1, 112.3, 112.5, 115.9, 116.1, 125.3, 125.4, 126.4, 130.8, 130.9, 136.0, 150.3, 157.9, 160.4, 163.5, 165.9, 175.1.

Molecular formula:$C_{19}H_{15}F_2NO_3S$; Exact Mass Found: 375.07, Calculated: 375.39.

2.3.4.4- Ethyl 1-(4-Methoxybenzyl)-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5e)

The above compound (7.6 g, 19.66 mmol) was obtained as a colorless powder using **4f** (9.11 g, 20 mmol) and employing the same procedure as described for **5a**.

^{1}H NMR ($CDCl_3$): δ 1.557-1.766 (3H, t, *J= 7.8Hz*), 3.811 (3H, s),4.512-4.499 (2H, q, *J=7.9 Hz*), 5.367 (2H, s), 7.018-7.330 (2H, dt, *J=5.0 Hz*, *J=8.0 Hz*), 7.257-7.279 (1H, dt, *J=5.0 Hz*, *J=11.0 Hz*), 7.404-7.418 (2H, dd, *J=8.0 Hz*, *J=10.0 Hz*),7.632- 7.543(1H, dd, *J=8.0 Hz*, *J=13.5 Hz*), 7.951-7.79 (1H, dd, *J=8.0 Hz*, *J=11.5 Hz*), 12.545 (1H, s).

^{13}C NMR ($CDCl_3$): δ 15.1, 56.0, 62.6, 103.1, 112.3, 112.5, 115.9, 116.1, 125.4, 125.5, 128.8, 128.9, 136.0, 150.3, 157.9, 158.0, 160.4, 163.5, 166.3, 170.1

Molecular Formula: $C_{20}H_{18}FNO_4S$, Exact Mass Found: 387.09, Calculated: 387.43.

2.3.4.5- Ethyl 1-Ethyl-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5f)

Compound **5f** (5.4 g, 18.8 mmol, 92%) was made as yellow crystals using **4g** (7 g, 20 mmol) and employing the same method as described for **5a**.

^{1}H NMR ($CDCl_3$): δ 1.420-1.467 (3H, m,), 1.620-1.649 (3H, m), 4.477-4.491 (2H, q, *J=7.5 Hz*), 5.512-5.563 (2H, q, *J=7.5 Hz*), 7.023-7.138(1H, dt, *J =3.0 Hz*, *J=8.5 Hz*), 7.616-7.645 (1H, dd, *J=3.0 Hz*, *J=8.0 Hz*), 8.128-8.158 (1H, dd, *J=5.0 Hz*, *J 7.5 Hz*),12.552 (1H, s).

Molecular Formula: $C_{14}H_{14}FNO_3S$, Exact Mass Found: 295.07, Calculated: 295.33.

2.3.4.6- Ethyl 1-Propyl-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5g)

The title compound was prepared in 97% yield (5.92 g 19.4 mmol) from **4h** (7.35 g, 20 mmol), as a yellow powder, according to the procedure described for **5a**.

^{1}H NMR ($CDCl_3$): δ .1.051-1.061 (3H, m), 1.619-1.639 (6H, m), 4.464-4.653 (2H, q, *J=7.0 Hz*), 5.213-5.375 (2H, q, *J=7.0 Hz*), 7.112-7.134(1H, dt, *J 3.0 Hz*, *J=7.5 Hz*), 7.425-7.545 (1H, dd, *J=2.5 Hz*, *J=7.5 Hz*), 8.004-8.014 (1H, dd, *J=7.5Hz*, *J=8.0 Hz*); 12.345 (1H, s)

Molecular formula:$C_{15}H_{16}FNO_3S$, Exact Mass Found: 309.08, Calculated: 309.36.

2.3.4.7- Ethyl 1-Butyl-7-fluoro-2- mercaptoquinolin-4(1*H*)-one-3-carboxylate (5h)

Employing the same technique as described for the preparation of **5a**, compound **5h** was afforded in 94% yield (6.10 g, 18.8 mmol) from **4i** (7.62 g, 20 mmol), as a yellow powder.

^{1}H NMR ($CDCl_3$): δ 0.987-1.040 (3H, t, *J=7.5 Hz*), 1.310-1.349 (5H, m,), 1.829-1.907 (2H, t, *J=7.0 Hz*), 4.453-4.673 (2H, q, *J 7.5 Hz*), 5.345-5.363 (2H, t, *J=7.5 Hz*), 7.213-7.224 (1H,dt, *J=2.5 Hz*, *J=7.5 Hz*), 7.445-7.459 (1H, dd, *J=2.0 Hz*, *J 7.0 Hz*), 8.112-8.153 (1H, dd, *J=7.5 Hz*, *J=9.5 Hz*), 12.003 (1H, s).

^{13}C NMR ($CDCl_3$): 13.3, 13.9, 15.1, 32.2, 62.6, 101.1, 112.3, 112.5, 115.9, 116.1, 150.3, 157.9, 160.4, 163.5, 166.2, 172.1.

Molecular Formula: $C_{16}H_{18}FNO_3S$, Exact Mass Found: 323.10, Calculated: 323.38.

2.3.4.8- Ethyl 1-Cyclopropylmethyl-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5i)

Compound **5i** was synthesized employing the same procedure as described for **5a** by using **4j** (7.6 g, 20 mmol).The product was a yellow powder with the yield of 95% (6.11 g, 19 mmol).

^{1}H NMR ($CDCl_3$): δ 0.350-0.365 (2H, d, *J=5.5 Hz*), 0.636-0.646 (2H, d, *J=5.5Hz*), 1.064-1.086 (1H, m), 1.403-1.435 (3H, t, *J=7.0 Hz*), 4.116-4.122 (2H, d, *J=3.0 Hz*), 4.557-4.563 (2H, d, *J=3.5 Hz*), 7.270-7.301 (1H, dt, *J=6.5 Hz*, *J=15.5 Hz*), 7.634-7.651 (1H, dd, *J=3.5 Hz*, *J=8.5 Hz*), 8.342-8.381 (1H, dd, *J=6.5 Hz*, *J 13.0 Hz*), 11.932 (1H, s).

^{13}C NMR ($CDCl_3$): 9.7, 10.9, 15.1, 62.6, 81.7, 101.1, 112.3, 112.5, 115.9, 116.1, 150.8, 157.8, 160.4, 163.5, 166.2, 220.6.

Molecular Formula: $C_{16}H_{16}FNO_3S$, Exact Mass Found: 321.08, Calculated: 321.37.

2.3.4.9- Ethyl 1-Cyclobutylmethyl-7-fluoro-2- mercaptoquinolin-4(1*H*)-one-3-carboxylate (5j)

This compound was synthesized in 94% yield (6.98 g, 18.8 mol) from **4k** (7.86 g, 20 mmol) using the same method as described for **5a**. The product was a yellow powder.

^{1}H NMR ($CDCl_3$): δ 1.494-1.532 (3H, t, *J= 7.5 Hz*), 1.906-1960 (4H, m), 2.149-2.188 (2H, m) , 2.808-2.881 (1H, m), 4.470-4.524 (2H, q, *J=7.0 Hz*), 4.559-4.609 (2H, d, *J 3.0 Hz*), 7.261-7.298(1H, dt, *J=4.0 Hz*, *J=8.0 Hz*), 7.933-7.963 (1H, dd, *J=5.5 Hz*, *J=9.0 Hz*), 8.148-8.177 (1H, dd, *J=7.0 Hz*, *J=9.0 Hz*), 12.544 (1H, s).

^{13}C NMR ($CDCl_3$): δ 15.1, 18.4, 21.8, 35.9, 62.6, 101.1, 111.2, 111.4, 115.9, 116.1, 130.5, 150.2, 157.9, 160.4, 163.5, 166.2, 220.1.

Molecular Formula: $C_{17}H_{18}FNO_3S$, Exact Mass Found: 335.10, Calculated: 335.39.

2.3.4.10- Ethyl 1-Allyl-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5k)

Compound **5k** (5.5 g, 18 mmol, 90%) was produced as a yellow powder, using **4l** (7.3 g, 20 mmol) and employing the same technique as described for **5a**.

^{1}H NMR ($CDCl_3$): δ 1.467-1.517 (3H, t, *J=7.0 Hz*), 4.544-4.631 (2H, q, *J=8.0 Hz*), 4.718-4.732 (2H, d, *J=7.0 Hz*), 5.413-5.521 (2H, q, *J=6.5 Hz*),5.81 (1H, m), 7.119-7.150

(1H, dt, *J=5.5 Hz*, *J=10.0 Hz*), 7.590-7.607 (1H, dd, *J=5.0 Hz*, *J=8.5 Hz*), 8.125-8.153 (1H, dd, *J=5.5 Hz*, *J=10.5 Hz*), 12.556 (1H, s).

^{13}C NMR ($CDCl_3$): 15.1, 62.6, 105.1, 112.4, 115.9, 118.7, 125.4, 132.6, 136.0, 150.3, 157.8, 160.4, 163.5, 166.2, 171.2.

Molecular Formula: $C_{15}H_{14}FNO_3S$, Exact Mass Found: 307.07, Calculated: 307.34.

2.3.4.11- Ethyl 1-Propyn-2-yl -7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5l)

Employing the same technique as described for the preparation of **5a**, compound **5l** (5.8 g, 19 mmol, 95%) was made using **4m** (7.27 g 20 mmol). The product was a yellow powder.

^{1}H NMR ($CDCl_3$): δ 1.508-1.547 (3H, q, *J=8.0 Hz*,), 2.705 (1H, s), 4.527-4.571 (2H, q, *J=4.0 Hz*), 5.414-5.435 (2H, p, *J=7.0 Hz*), 7.388-7.420 (1H, dt, *J=3.0 Hz*, *J=8.0 Hz*), 7.780-7.817 (1H, dd, *J=3.0 Hz*, *J=9.5 Hz*), 8.345-8.370 (1H, dd, *J=7.5 Hz*, *J=12.5 Hz*), 12.554 (1H, s).

^{13}C NMR ($CDCl_3$): 15.1, 62.6, 103.1, 112.3, 112.5, 115.9, 116.1, 126.4, 135.9, 150.3, 157.9, 160.4, 163.5, 166.2, 171.1.

Molecular Formula: $C_{15}H_{12}FNO_3S$, Exact Mass Found: 305.05, Calculated: 305.33.

2.3.4.12- Ethyl 1-(1-Naphthylmethyl)-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5m)

This compound was made from **4n** (9.31 g, 20 mmol) in 8.0 g (19.66 mmol, 98%) yield as yellow powder, using the same procedure as described for **5a**.

^{1}H NMR ($CDCl_3$): δ 1.426-1.432 (3H, t, *J=7.0 Hz*), 4.542-4.551 (2H, q, *J 7.0 Hz*), 5.454 (2H, s), 7.162-7.172(1H, dt, *J=2.5 Hz*, *J=9.0 Hz*), 7.374-7.458 (4H, m), 7.549-7.578 (1H, dd, *J=2.0 Hz*, *J=9.5 Hz*), 7.646-7.751 (3H, m), 8.011-8.039 (1H, dd, *J 6.0 Hz*, *J=9.5 Hz*), 12.345 (1H, s).

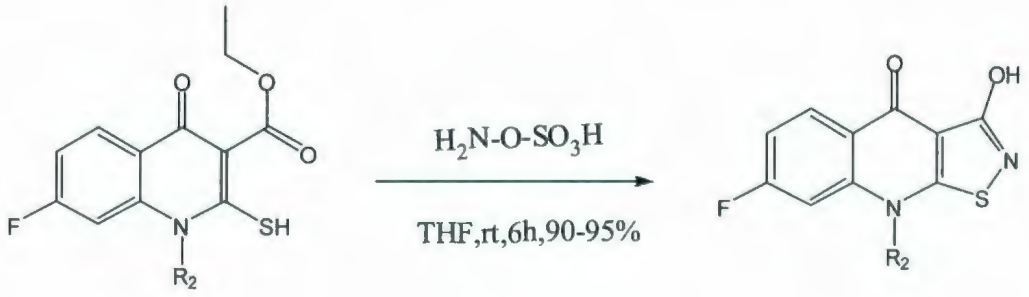
Molecular Formula: $C_{23}H_{18}FNO_3S$, Exact Mass Found: 407.10, Calculated: 407.46.

2.3.4.13- Ethyl 1-(2-Naphthylmethyl)-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5n)

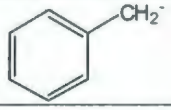
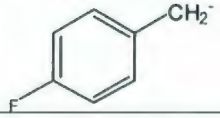
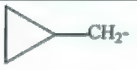
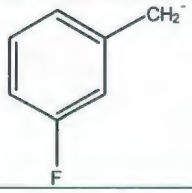
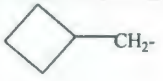
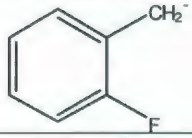
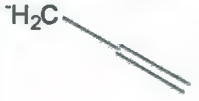
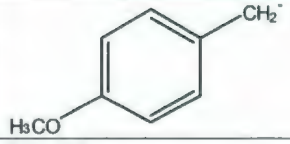
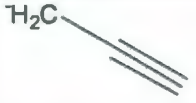
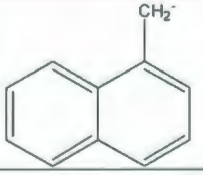
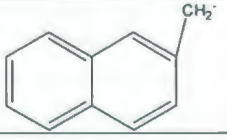
Compound **5n** was synthesized in 97% yield (7.9 g, 19.4 mmol), as a yellow powder, using **4o** (9.31 g, 20 mmol) and employing the same technique as used for **5a**.

^{1}H NMR ($CDCl_3$): δ 1.398-1.411 (3H, t, *J=7.0 Hz*), 4.335-4.449 (2H, q, *J=7.0 Hz*), 5.222 (2H, s), 7.159-7.164(1H, dt, *J=3.0 Hz*, *J=8.5 Hz*), 7.284-7.388 (4H, m), 7.542-7.554 (1H, dd, *J=2.5 Hz*, *J=8.5 Hz*), 7.624-7.736 (3H, m), 8.118-8.129 (1H, dd, *J 6.0 Hz*, *J=8.5 Hz*), 12.354 (1H, s).

Molecular formula: $C_{23}H_{18}FNO_3S$, Exact Mass Found: 407.10, Calculated: 407.46



5 **6**

6	R_2	**6**	R_2
a		h	$CH_3CH_2CH_2CH_2$ -
b		i	
c		j	
d		k	
e		l	
f	CH_3CH_2-	m	
g	$CH_3CH_2CH_2$-	n	

Scheme 2.6-Synthesis of compounds **6a-n***

2.3.5- 7-Fluoro-9-benzyl-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6a)

To a solution of ethyl 1-benzyl-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (0.535 g, 1.5 mmol) in 10 mL THF 15 mL sodium bicarbonate solution (5.7 g in 100 mL, pH=8) was added, followed by the addition of (0.682 g, 6 mmol) hydroxylamine-*O*-sulfonic acid. The mixture was stirred at ambient temperature overnight, then THF was evaporated under reduced pressure. The aqueous filtrate was diluted with water and filtered. The crude product was boiled in methanol and the supernatant was discarded. Compound **6a** was obtained in 95% yield (0.46 g, 1.42 mmol) after crystallization from CH_2Cl_2. (mp: 126 °C).

^{1}H NMR (DMSO-d_6): δ 5.847 (2H, s), 7.368-7.416 (3H, m), 7.457-7.529 (3H, m), 7.671-7.696 (1H, dd, *J=2.5 Hz*, *J=11.0 Hz*), 8.253-8.283 (1H, dd, *J =6.5 Hz*, *J=8.5 Hz*).

Molecular formula, $C_{17}H_{11}FN_2O_2S$; Exact Mass Found: 326.05; Calculated: 326.35.

2.3.5.1- 7-Fluoro-9-(4-flurobenzyl)-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6b)

Compound **6b** was prepared as a yellow solid (mp: 130-135 °C) using compound **5b** (0.563 g, 1.5 mmol) and employing the same procedure as that used for the preparation of **6a**. The yield was 95% (0.488 g, 1.42 mmol).

^{1}H NMR (DMSO-d_6): δ 5.456 (2H, s), 7.363-7.385 (2H, dt, *J=5.5 Hz*, *J 9.0 Hz*), 7.427-7.465 (1H, dt, *J=5.5 Hz*, *J=11.5 Hz*), 7.623-7.643 (2H, dd, *J=8.5 Hz*, *J=10.0 Hz*),7.859-7.881(1H, dd, *J=9.5 Hz*, *J=13.5 Hz*), 8.234-8.245 (1H, dd, *J=8.0 Hz*, *J=11.0 Hz*).

Molecular formula:$C_{17}H_{10}F_2N_2O_2S$, Exact Mass Found: 344.04, Calculated: 344.34.

2.3.5.2- 7-Fluoro-9-(3-flurobenzyl)-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6c)

Utilizing **5c** (0.563 g, 1.5 mmol) and the same procedure as that described for **6a**, compound **6c** was obtained as a yellow solid (0.488 g , 1.42 mmol, 95%,) with the melting range of 140-145 °C.

^{1}H NMR (DMSO-d_6): 5.329 (2H, s), 7.338-7.362 (2H, dt, *J 4.5 Hz, J 8.0 Hz*), 7.482-7.512 (1H, dt, *J=4.5 Hz, J=10.5 Hz*), 7.612-7.629 (2H, dd, *J 2.5 Hz, J=8.5 Hz*),7.560-7.590 (1H, dd, *J=9.0 Hz, J=14.0 Hz*), 8.184-8.216 (1H, dd, *J=7.5 Hz, J=10.5 Hz*).

Molecular Formula: $C_{17}H_{10}F_2N_2O_2S$, Exact Mass Found: 344.04, Calculated: 344.34.

2.3.5.3- 7-Fluoro-9-(2-flurobenzyl)-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6d)

Compound **6d** was synthesized utilizing the same technique as described for **6a** as a yellow solid from **5d** (0.563 g, 1.5 mmol). The yield of the product was 90% (0.463 g, 1.35 mmol) with the melting range of 145-150 °C.

^{1}H NMR (DMSO-d_6): δ 5.369 (2H, s), 7.044-7.068 (2H, dt, *J 2.5 Hz, J=8.0 Hz*), 7.231-7.258 (1H, dt, *J=2.5 Hz, J=10.5 Hz*), 7.458-7.497 (3H, m), 8.038-8.066 (1H, dd, *J 3.0 Hz, J=10.5 Hz*).

Molecular Formula: $C_{17}H_{10}F_2N_2O_2S$, Exact Mass Found: 344.04, Calculated: 344.34.

2.3.5.4- 7-Fluoro- 9-(4-methoxybenzyl)-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6e)

This compound was made, as a pale yellow powder, in 90% yield (0.48 g, 1.35 mmol) using **5e** (0.60 g, 1.5 mmol) and employing the same technique as that used for **6a**. (mp:140-145 ^{o}C).

^{1}H NMR ($CDCl_3$): 3.902 (3H, s), 5.405 (2H, s), 7.304-7.332(2H, dt, *J=4.0 Hz, J 8.0 Hz*), 7.389-7.415 (1H, dt, *J=2.5 Hz, J=8.0 Hz*), 8.023-8.054 (2H, dd, *J=3.0 Hz, J=8.0 Hz*), 8.230-8.283(1H, dd, *J=8.0 Hz, J- 13.5 Hz*), 8.345-8.390 (1H, dd, *J=8.0 Hz, J=11.5 Hz*).

Molecular Formula: $C_{18}H_{13}FN_2O_3S$, Exact Mass Found: 356.06, Calculated: 356.37

2.3.5.5- 7-Fluoro- 9-ethyl-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)- one (6f)

Employing the same technique as described for **6a**, compound **6f** was obtained in 95% yield (0.355, g, 1.35 mmol) using **5f** (0.443 g, 1.5 mmol). The product was a yellow powder. (mp:130-135 ^{o}C).

^{1}H NMR (DMSO-d_6): 1.563-1.591 (3H, t, *J=7.0 Hz*), 5.867-5.897 (2H, q, *J=7.5 Hz*), 7.225-7.257(1H, dt, *J=3.0 Hz, J=8.5 Hz*), 7.816-7.844 (1H, dd, *J=3.5 Hz, J=8.0 Hz*), 8.139-8.164 (1H, dd, *J=5.0 Hz, J=7.5 Hz*).

Molecular Formula: $C_{12}H_9FN_2O_2S$, Exact Mass Found: 264.04, Calculated: 264.28.

2.3.5-6- 7-Fluoro-9-propyl-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6g)

This compound (0.373g, 1.35 mmol, 90%) was produced as a pale yellow powder (mp: 135-140 °C) utilizing the same technique as described for **6a** and using **5g** (0.463 g, 1.5 mmol).

^{1}H NMR (DMSO-d_6): δ .1.151-1.191 (3H, q, *J=7.0 Hz*), 1.510-1.539(2H, m), 5.215-5.271 (2H, t, *J=7.0 Hz*), 7.202-7.242(1H, dt, *J=3.0 Hz*, *J=7.5 Hz*), 7.623-7.643 (1H, dd, *J=2.5 Hz*, *J=7.5 Hz*), 8.114-8.176 (1H, dd, *J=7.5 Hz*, *J=8.0 Hz*).

Molecular Formula: $C_{13}H_{11}FN_2O_2S$, Exact Mass Found: 278.05, Calculated: 278.30.

2.3.5.7- 7-Fluoro-9-butyl-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6h)

Utilizing **5h** (0.484 g, 1.5 mmol) and the same procedure as described for **6a**, compound **6h** was obtained as a pale yellow solid (0.393 g, 1.35 mmol, 90%). (mp:145-150 °C).

^{1}H NMR (DMSO-d_6): δ 0.987-1.040 (3H, t, *J=7.5 Hz*), 1.310-1.349 (2H, m,), 1.849-1.910(2H, m), 5.435-5.495 (2H, q, *J=7.5 Hz*), 7.031-7.064(1H, dt, *J =2.5Hz*, *J=7.5 Hz*), 7.287-7.325 (1H, dd, *J=2.0 Hz*, *J=7.0 Hz*), 8.195-8.234 (1H, dd, *J 7.5 Hz*, *J=9.5 Hz*).

Molecular Formula: $C_{14}H_{13}FN_2O_2S$, Exact Mass Found: 292.07, Calculated: 292.33.

2.3.5.8- 7-Fluoro-9-cyclopropylmethyl-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6i)

Compound **6i** was made from **5i** (0.481 g, 1.5 mmol) in 90% yield (0.39 g, 1.35 mmol) according to the same procedure as described for **6a**. The product was a white solid (mp: 145-150 °C).

^{1}H NMR (DMSO-d_6): δ 0.450-0.465 (2H, d, *J=7.5 Hz*), 0.636-0.646 (2H, d, *J=5.0 Hz*), 4.558-4.585 (2H, q, *J=13.5 Hz*), 5.215-5.265 (1H, m), 7.271-7.302(1H, dt, *J=6.0 Hz*, *J=15.5 Hz*), 7.636-7.652 (1H,d d, *J= 3.5 Hz*, *J=8.0 Hz*), 8.344-8.382 (1H, dd, *J=6.0 Hz*, *J=12.5 Hz*).

Molecular Formula:$C_{14}H_{11}FN_2O_2S$, Exact Mass Found: 290.05, Calculated: 290.31.

2.3.5.9- 7-Fluoro-9-cyclobutylmethyl-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6j)

Employing the same technique as described for **6a**, compound **6j** was afforded in 95% yield (0.43g, 1.42 mmol) from **5j** (0.5 g, 1.5 mmol), as a white powder.

^{1}H NMR (DMSO-d_6): δ 1.869-1.934(4H, m), 2.048-2.080 (2H, q, *J=7.0 Hz*) , 2.810-2.822 (1H, m),4.654-4.704 (2H, d, *J=3.0 Hz*), 7.361-7.483(1H, m), 8.151-8.213 (1H, dd, *J=6.0 Hz*, *J=8.5 Hz*), 8.270-8.332 (1H, dd, *J=7.0 Hz*, *J=9.0 Hz*).

Molecular Formula: $C_{15}H_{13}FN_2O_2S$, Exact Mass Found: 304.07, Calculated: 304.34.

2.3.5.10- 7-Fluoro-9-allyl-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6k)

This compound was obtained in 95% yield (0.39g, 1.42 mmol), employing the same technique as described for **6a**, using **5k** (0.46 g, 1.5 mmol). The product was a white powder.

^{1}H NMR (DMSO-d_6): δ 4.555-5.300 (2H, m), 5.423-5.634 (1H, m), 5.716-5.724 (2H, q, *J=6.5 Hz*) 7.118-7.148(1H, dt, *J=5.5 Hz*, *J=10.0 Hz*), 7.595-7.612 (1H, dd, *J=5.0 Hz*, *J=8.5 Hz*), 8.113-8.144 (1H, dd, *J -5.5 Hz*, *J 10.5 Hz*).

Molecular Formula: $C_{13}H_9FN_2O_2S$, Exact Mass Found: 276.04, Calculated: 276.29.

2.3.5.11- 7-Fluoro-9-(1-propyn-2-yl)-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6l)

Compound **6l** (1.42, 0.39g, 95%) was made as yellow powder from **5l** (0.402 g, 1.5 mmol) utilizing the same procedure as described for **6a**.

^{1}H NMR (DMSO-d_6): δ 2.711 (1H, s), 5.423-5.436 (2H, p, *J 7.0 Hz*), , 7.388-7.420 (1H, dt, *J=3.0 Hz*, *J=8.0 Hz*), 7.780-7.817 (1H, dd, *J=3.0 Hz*, *J=9.5 Hz*), 8.345-8.370 (1H, dd, *J- 7.5 Hz*, *J=12.5 Hz*).

Molecular Formula: $C_{13}H_7FN_2O_2S$, Exact Mass Found: 274.02, Calculated 274.27.

2.3.5.12- 7-Fluoro-9-(1-naphthylmethyl)-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one (6m)

This compound was made from **5m** (0.61 g, 1.5 mmol) in 95% yield (0.53g, 1.42 mmol), according to procedure described for the preparation of **6a**. The product was a white powder. (mp: 145-150 °C).

^{1}H NMR (DMSO-d_6): δ 5.083 (2H, s), 7.367-7.410(1H, dt, *J=2.5 Hz*, *J=9.0 Hz*), 7.511-7.583 (4H, m), 7.830-7.926 (1H, dd, *J=2.0 Hz*, *J=9.5 Hz*), 8.071-8.102 (3H, m), 8.201-8.218 (1H, d, *J=8.5 Hz*).

Molecular Formula: $C_{21}H_{13}FN_2O_2S$, Exact Mass Found: 376.07, Calculated: 376.40.

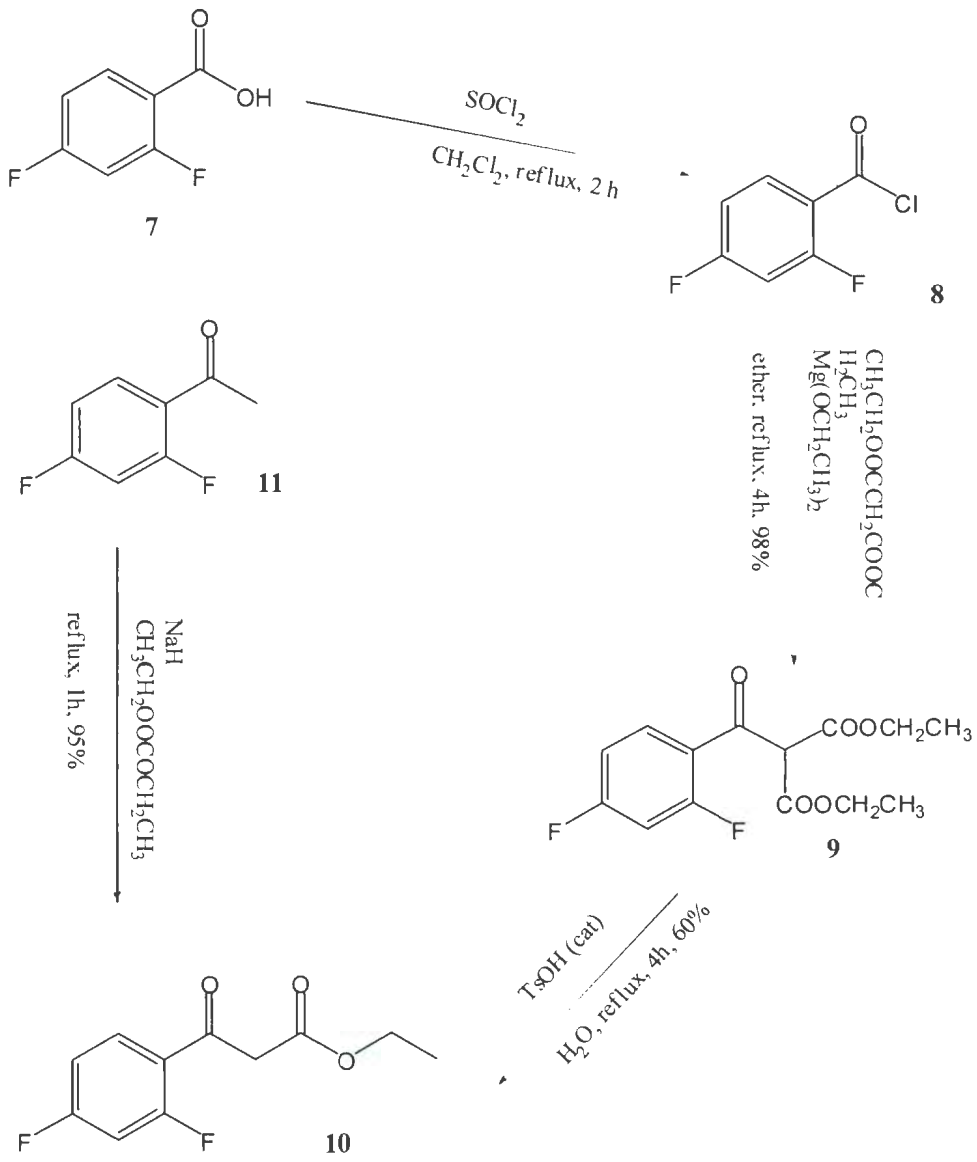
2.3.5.13- 7-Fluoro-9-(2-naphthylmethyl)-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9H)-one (6n)

Compound **6n** was synthesized in 95% yield (0.53g, 1.42 mmol), as a white powder, from **5n** (0.61 g, 1.5 mmol) employing the same technique as described for **6a**.

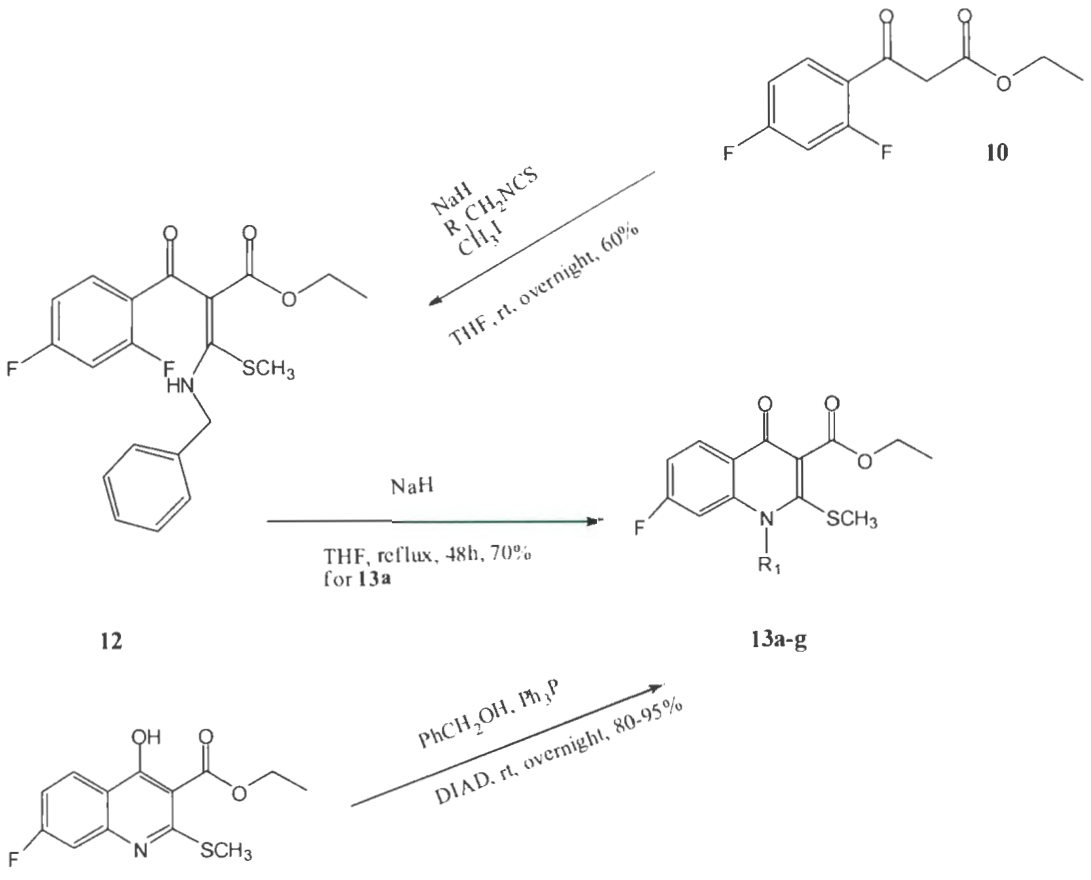
^{1}H NMR (DMSO): δ 5.224 (2H, s), 7.169-7.164 (1H, dt, *J=3.0 Hz*, *J=8.5 Hz*), 7.274-7.388 (4H, m), 7.533-7.565 (1H, dd, *J=2.5 Hz*, *J=8.5 Hz*), 7.635-7.767 (3H, m), 8.118-8.129 (1H, dd, *J=6.0 Hz*, *J=8.5 Hz*).

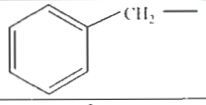
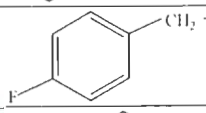
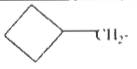
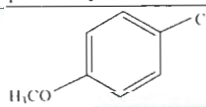
Molecular Fomula:$C_{21}H_{13}FN_2O_2S$, Exact Mass Found: 376.07, Calculated: 376.40

* ^{13}C-NMR of compounds **6a-n** were not measured due to the low solubility of these compounds in DMSO-d_6 and requirement of long measurement time.



Scheme 2.7- Synthesis of ethyl 2,4-difluorobenzoyl acetate (**10**) by two different methods



13	R_1	13	R_1
a		e	
b		f	
c		g	
d	CH_3-$(CH_2)_5$-CH_2 —		

Scheme 2.8- Syntheses of compounds **13 a-g** by two different methods

2.3.6- Diethyl (2,4-Difluorobenzoyl)malonate (9)

To a solution of 2,4-difluorobenzoic acid (7) (4.74 g, 30 mmol) in anhydrous methylene chloride (CH_2Cl_2) (30 mL) was added oxalyl chloride (7.62 g, 60 mmol) and one drop of DMF and the mixture was stirred overnight at room temperature . The solvent was evaporated under reduced pressure and the resulting 2,4-difluorobenzoyl chloride (**8**) was added dropwise to a suspension of diethyl malonate (4.8 g, 30 mmol) and magnesium ethoxide (3.42 g, 30 mmol) in anhydrous ether which was refluxed for 4 hours before the addition of acid chloride. The reaction mixture was stirred for 2 hours. The workup was done under acidic condition with the addition of HCl (1N) to reach the pH of 4-5. The resulting compound was extracted with three portions of ethyl acetate, washed with water twice and brine once, dried over sodium sulfate, and evaporated under reduced pressure to yield (5.4 g, 18 mmol, 60%) of diethyl (2,4-difluorobenzoyl) malonate (**9**) as a colorless oil.

1H NMR ($CDCl_3$): δ1.266-1.299 (6H, m), 3.365 (1H, s), 4.190-4.083(2H, q, *J=7.5 Hz*), 4.265-4.308 (2H, q, *J=7.5 Hz*), 6.892-6.989 (2H, dd, *J=8.5 Hz*, *J=18.5 Hz*), 8.037-8.083 (1H, dd, *J 6.5 Hz*, *J= 8.5 Hz*).

Molecular Formula : $C_{14}H_{114}F_2O_5$; Calculated: 300.25 (inconclusive MS measurement).

2.3.7- Ethyl 2,4-Difluorobenzoyl acetate (10)

Method A: TsOH (catalytic amount) was added to diethyl (2,4-difluorobenzoyl)malonate (**9**) (6.0 g, 20 mmol) in 100 mL of water and refluxed for 4 hours. The reaction mixture was neutralized with sodium hydroxide (1N) and extracted

with ethyl acetate. The organic layer was dried over sodium sulfate, evaporated under reduced pressure, and purified by silicagel-column chromatography (hexane: ethyl acetate, 20:1) to obtain compound **10** (2.28 g, 10 mmol, 50%) as a colorless oil.

Method B: sixty percent sodium hydride suspension in mineral oil (0.82 g, 20.5 mmol) was added slowly at room temperature to a cold solution of (1.5614 g, 10mmol) 2,4-difluroacetophenone (**11**) in diethyl carbonate (30 mL). The mixture was heated at 80°C for 1.5 h. It was poured into ice-cooled water (70 mL) containing acetic acid (2.5 mL). The mixture was then extracted with ethyl acetate. The organic phase was dried over sodium sulfate , evaporated under reduced pressure and the residual oil was distilled at 111 °C (0.7 mmHg) to give (1.83 g, 8 mmol, 80% yield) of compound **10** as a white, low melting point solid. The spectral data and TLC profiles of the compounds obtained by both methods were similar.

^{1}H NMR($CDCl_3$): δ 1.490-1.518 (3H, t, *J=7.0 Hz*), 3.950-3.958 (1.5H, d, *J= 4.0 Hz*), 4.097-4.138 (2H, q, *J=7.0 Hz*),5.796 (0.3H, s), 6.870-7.037 (2H, m),7.983-8.031 (1H, dd, *J=6.5 Hz*, *J=8.5 Hz*), 12.720 (0.2H, s).

Molecular Formula : $C_{11}H_{10}F_2O_3$, Calculated: 228.19 (inconclusive MS measurement).

2.3.8- Ethyl (2*Z*)-3-(Benzylamino)-2-(2,4-difluorobenzoyl)-3-(methylthio)acrylate (12)

Sixty percent sodium hydride suspension in mineral oil (0.96 g, 24 mmol) was added slowly under nitrogen atmosphere to an ice-cooled solution of **10** (4.56 g, 20 mmol) and *N*-benzylisothocyanate (2.98 g, 20 mmol) in 30mL of anhydrous DMF. After

the addition of sodium hydride was completed, the reaction mixture was allowed to warm up to 35 °C and stirred for 48 hours. Methyl iodide (2.84 g, 20 mmol) was added to the reaction mixture at 35 °C and after 24 hours, 2 mL of glacial acetic acid was added, then the solvent was removed under reduced pressure. The residue was dissolved in methylene chloride and the solution was washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silicagel column chromatography, using ethyl acetate (1%) in methylene chloride, as an eluent, to obtain compound **12** as a yellow oil (0.79 g, 2 mmol,10%).

^{1}H NMR ($CDCl_3$): δ 0.909-0.936 (3H, t, *J=6.5 Hz*), 2.306 (3H, s), 3.919-3.962 (2H, q, *J=6.5 Hz*), 5.296 (2H, s), 6.756-6.795(1H, m), 6.862-6.889(1H, m) 7.308-7.389(5H, m), 7.482-7.527(1H, m), 11.705 (1H, s).

Molecular Formula: $C_{20}H_{19}F_2NO_3S$, Calculated: 391.43 (inconclusive MS measurement).

2.3.9- Ethyl 1-Benzyl-7-fluoro-2-methylthioquinolin-4(1*H*)-one-3-carboxylate (13a)

First method: to a solution of **12** (3.91g,10mmol) in 50mL of THF, 60% sodium hydride in mineral oil (0.8 g, 20 mmol) was added . The reaction mixture was heated at reflux for 48 hours, then 1mL of glacial acetic acid was added. The solvents were removed under reduced pressure and the residue was dissolved in methylene chloride. The methylene chloride solution was washed with water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to obtain **13a** (2.71 g, 7 mmol, 70%) as a yellow crystal. (mp: 145-150 °C).

Second method:

To a solution of **3c** (5.62 g, 20 mmol), benzyl alcohol (2.16g,20 mmol), triphenyl phosphine (6.29 g, 24 mmol) in THF (30 mL), diisopropyl azidodicarboxylate (DIAD) (4.85 g,24 mmol) was added dropwise and the mixture was stirred overnight at room temperature. The crude product was dissolved in ethyl acetate (40 mL) and washed with three portions of brine solution (10 mL). The solution was dried over sodium sulfate and evaporated to dryness. The resulting compound was purified using a short silicagel column (hexane: ethyl acetate, 10:1) to yield **13a** (6.7g, 18 mmol, 90%) as a yellow crystal. (mp: 145-150 ^{0}C). The products obtained by the above methods possessed similar spectral and physical characteristics.

^{1}H NMR ($CDCl_3$): δ 1.521-1.548 (3H, t, *J=7.0 Hz*), 2.586 (3H, s), 4.530-4.572 (2H, q, *J=7.0 Hz*), 5.161 (2H, S),7.132-7.170 (1H, dt, *J=2.0 Hz*, *J=8.5 Hz*),7.391-7.445 (5H, m), 7.531-7.556 (1H, dd, *J=2.0 Hz*, *J=10.0 Hz*), 8.201-8.230 (1H, dd, *J=5.5 Hz*, *J=8.5 Hz*).

Molecular Formula: $C_{20}H_{18}FNO_3S$, Calculated: 371.43 (inconclusive MS measurement).

2.3.9.1- Ethyl 1-(4-Fluorobenzyl)-7-fluoro-2-methylthioquinolin-4(1*H*)-one-3-carboxylate (13b)

Compound **13b** was prepared employing the second method as described for the preparation of **13a**, using **3c** (5.62 g, 20 mmol) and 4-fluorobenzyl alcohol (2.5 g, 20 mmol). The product was a yellow oil and the yield was 95% (7.39 g, 19 mmol).

^{1}H NMR ($CDCl_3$): δ 1-379-1.433 (3H, m), 2.675 (3H, s) ,4.441-4.483 (2H, q, *J=7.0 Hz*), 5.177 (2H, s), 7.083-7.111 (2H, m), 7.148-7.187(1H, dt, *J -2.0 Hz*, *J=7.0 Hz*), 7.404-

7.428 (2H, m), 7.540- 7.563 (1H, dd, *J=2.0 Hz*, *J=9.0 Hz*), 7.945- 7.977 (1H, dd, *J=7.0 Hz*, *J=2.0 Hz*).

Molecular Formula: $C_{20}H_{17}F_2NO_3S$, Calculated: 389.42 (inconclusive MS measurement).

2.3.9.2- Ethyl 1-(4-Methoxybenzyl)-7-fluoro-2-methylthioquinolin-4(1*H*)-one-3-carboxylate (13c)

This compound was afforded utilizing the second method as described for **13a**, using **3c** (20 mmol, 5.62 g) and 4-methoxybenzyl alcohol (20 mmol,2.76 g). The yield was 95% (7.6 g, 19 mmol) and the product was a yellow oil .

^{1}H NMR ($CDCl_3$) δ: 1.405- 1.436 (3H, m), 2.674 (3H, s), 3. 832 (3H, s), 4.435-4.470 (2H, q, *J=7.0 Hz*), 5.147 (2H, s), 6.917- 6.945 (2H, m), 7.126- 7.166 (1H, dt, *J=2.5 Hz*, *J=8.5 Hz*), 7.344- 7.362 (2H, m), 7.523- 7.548 (1H, dd, *J=2.5 Hz*, *J= 9.5 Hz*), 7.960-7.991 (1H, dd, *J= 7.0 Hz*, *J= 9.5 Hz*).

Molecular Formula: $C_{21}H_{20}FNO_4S$, Calculated: 401.45 (inconclusive MS measurement).

2.3.9.3- Ethyl 1-Heptyl-7-fluoro-2-methylthioquinolin-4(1*H*)-one-3-carboxylate (13d)

Compound **13d** was prepared utilizing the second method as described for **13a**, using heptyl alcohol (2.32 g, 20 mmol) and **3c** (5.62 g, 20 mmol). The yield was 80% (6 g, 16 mmol) and the product was a yellow oil.

^{1}H NMR ($CDCl_3$) δ: 0.870-0.906 (3H, t, *J=6.0 Hz*), 1.256-1.298 (3H, t, *J=7.0 Hz*), 1.411-1.501 (10H, m), 2.656 (3H, s), 4.173-4.198 (2H, t, *J=6.0 Hz*) , 4.437-4.494 (2H, q, *J 7.5*

Hz), 7.173-7.213 (1H, dt, *J= 2.5 Hz*, *J= 9.0 Hz*), 7.511-7.537 (1H, dd, *J= 2.5 Hz*, *J =8.0 Hz*), 8.051- 8.080 (1H, dd, *J= 6.0 Hz*, *J=3.0 Hz*).

Molecular Formula: $C_{20}H_{26}FNO_3S$, Calculated: 379.49 (inconclusive MS measurement).

2.3.9.4- Ethyl 1-Cyclopropylmethyl-7-fluoro-2-methylthioquinolin-4(1*H*)-one-3-carboxylate (13e)

Using the same condition as utilized in preparation of compound **13a**, compound **13e** (6g, 18 mmol, 90%) was obtained from **3c** (5.62 g, 20 mmol) and cyclopropylmethyl alcohol (1.44 g, 20 mmol) as a yellow oil.

^{1}H NMR ($CDCl_3$) δ: 0.681- 0.702 (2H, m), 0.977- 0.993 (2H, m), 1.637- 1.673 (1H, m), 1.785- 1.811 (3H, t, *J= 7.0 Hz*), 3.000 (3H, s), 4.375- 4.389 (2H, d, *J= 7.0 Hz*), 4.803- 4.846 (2H, q, *J= 7.0 Hz*), 7.517- 7.557 (1H, dt, *J= 2.5 Hz*, *J= 8.5 Hz*), 7.852- 7.871 (1H, dd, *J=3.0 Hz*, *J= 9.5 Hz*), 8.447- 8.478 (1H, dd, *J= 2.0 Hz*, *J= 6.5 Hz*).

Molecular Formula: $C_{17}H_{18}FNO_3S$, Calculated: 335.39 (inconclusive MS measurement).

2.3.9.5- Ethyl 1-Cyclobutylmethyl-7-fluoro-2-methylthioquinolin-4(1*H*)-one-3-carboxylate (13f)

Using the same method, as described for **13a**, and cyclobutylmethyl alcohol (1.72 g, 20 mmol), compound **13f** was obtained in 90% yield (6.28 g, 18 mmol) as a yellow oil.

^{1}H NMR ($CDCl_3$) δ: 0.968-0.972 (2H, m), 1.261-1.300 (1H, m), 1.426- 1.470 (3H, m), 1.877- 1.923 (4H, m), 2.672 (3H, s), 4.116- 4.130 (2H, *J= 7.0 Hz*), 4.460- 4.501 (2H, q,

J=6.5 Hz), 7.176- 7.206 (1H, dt, *J= 3.0 Hz*, *J=9.5 Hz*), 7.513- 7.537 (1H, dd, *J= 3.0 Hz*, *J= 6.5 Hz*), 8.053- 8.084 (1H, dd, *J=2.0 Hz*, *J= 6.5 Hz*).

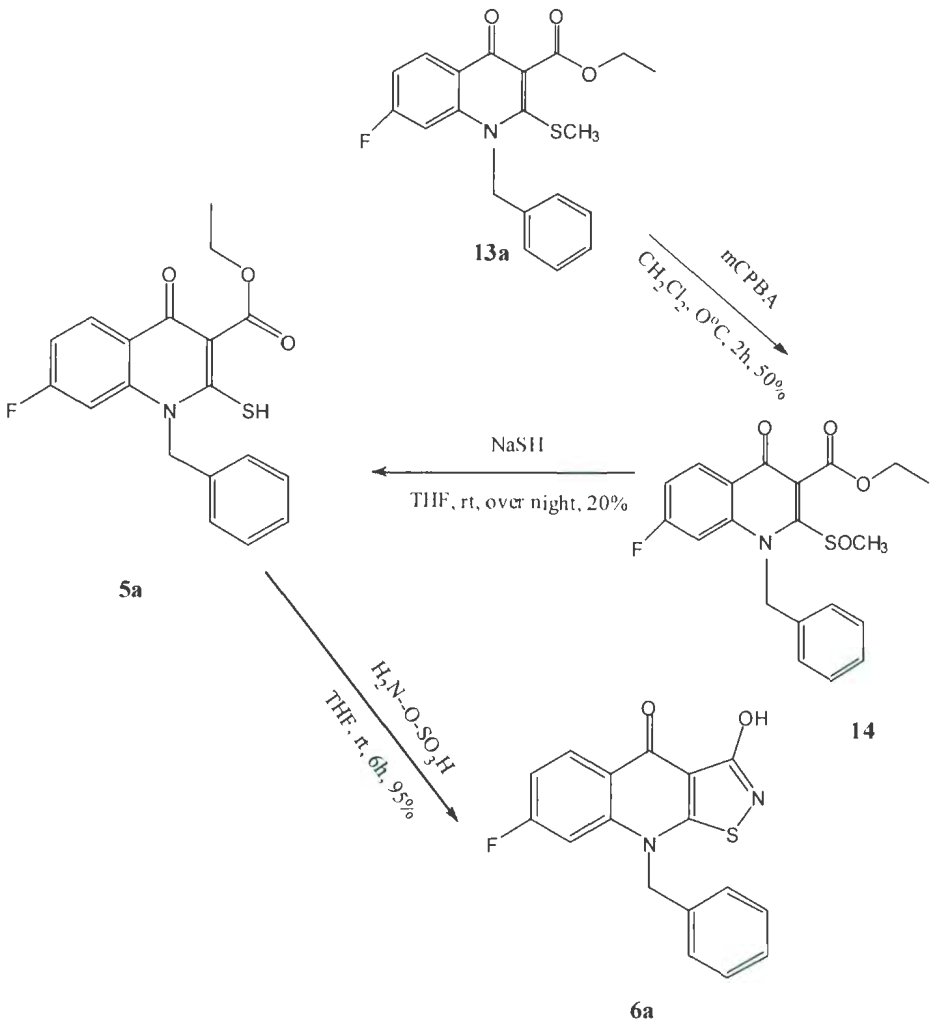
Molecular Formula: $C_{18}H_{20}FNO_3S$, Calculated: 349.42 (inconclusive MS measurement).

2.3.9.6- Ethyl 1-(1-Prop-2-yn-1-yl)-7-fluoro-2-methylthioquinolin-4(1*H*)-one-3-carboxylate (13g)

This compound was synthesized by the same method as employed for **13a**, using prop-2-yn-1-ol (1.12 g, 20 mmol) and **3c** (5.62 g, 20 mmol). The yield was 85% (5.42 g, 17 mmol) and the product was a yellow oil.

1H NMR (CDCl3): δ 1.457-1.550 (3H, q, *J=7 Hz*), 2.102 (1H, s), 2.269 (3H, s), 4.327-4.371 (2H, q, *J=4.0 Hz*), 4.541 (2H, s) , 6.835-6.869(1H, dt, *J=3.0 Hz*, *J=8.5 Hz*), 7.426-7.451 (1H, dd, *J=2.0 Hz*, *J=10.0 Hz*), 8.166-8.222 (1H, dd, *J=7.0 Hz*, *J 10.0 Hz*).

Molecular Formula: $C_{16}H_{14}FNO_3S$, Calculated: 319.35 (inconclusive MS measurement).



Scheme2.9- Alternative synthesis of compound **6a** from compound **13a**

2.3.10- Ethyl 1-Benzyl-7-fluoro-2-methylsulfinylquinolin-4(1*H*)-one-3-carboxylate (14)

To an ice cooled suspension of **13a** (7.75 g, 20 mmol) in 30 mL of methylene chloride, *m*-chloroperbenzoic acid (MCPBA) (3.4512 g, 20 mmol) was added and the mixture was stirred for an hour. The crude product was extracted with 20 mL of diethyl

ether, washed with water, dried over sodium sulfate and evaporated under vacuum to get compound **14** (4 g, 10 mmol, 50% yield) as a white powder (mp: 127 °C).

^{1}H NMR ($CDCl_3$): δ 1.403-1.440 (3H, t, *J=6.0 Hz*), 3.007 (3H, s), 4.528-4.570 (2H, q, *J=7.0 Hz*), 5.147 (2H, S),7.133-7.171 (1H, dt, *J=2.0 Hz*, *J=8.5 Hz*),7.381-7.435 (5H, m), 7.534-7.560 (1H, dd, *J=2.0 Hz*, *J=10.0 Hz*), 8.209-8.238 (1H, dd, *J=5.5 Hz*, *J=8.5 Hz*).

Molecular Formula: $C_{20}H_{18}FNO_4S$, Mol. Wt.: 387.43 (inclusive MS measurement)

2.3.11- Ethyl 1-Benzyl-7-fluoro-2-mercaptoquinolin-4(1*H*)-one-3-carboxylate (5a)

To a solution of compound **14** (2 g, 5 mmol) in 50 mL of THF at 60 °C, NaSH (0.21 g,5 mmol) was added under nitrogen. The solution was stirred overnight and the reaction was quenched by 1mL of glacial acetic acid. The reaction mixture was dissolved in diethyl acetate, washed several times with water, once with brine solution, dried over sodium sulfate, evaporated under reduced pressure, and purified by column chromatography (hexane:ethyl acetate, 7:1) to yield compound **5a** as a yellow crystal (0.373 g, 1 mmol, 20%). mp: 135-140 °C.

^{1}H NMR ($CDCl_3$): δ 1.439-1.467 (3H, t, *J=6.5 Hz*), 4.495-4.537 (2H, q, *J=7.0 Hz*), 5.347 (2H, s), 6.992-7.030 (1H, dt, *J=2.0 Hz*, *J= 8.5 Hz*), 7.173-7.195 (1H, dd, *J 2.0 Hz*, *J 7.0 Hz*), 7.399-7.441 (5H, m), 7.870-7.901 (1H, dd, *J= 5.5 Hz*, *J=9.5 Hz*), 12.079 (1H, s).

^{13}C NMR ($CDCl_3$): δ 15.0, 62.5, 104.1, 112.3, 112.5, 115.9, 116.1, 125.3, 125.4, 126.4, 130.8, 130.9, 136.0, 150.3, 157.9, 160.4, 163.5, 165.9, 175.1.

Molecular Formula:$C_{19}H_{16}FNO_3S$; Exact Mass Found: 357.08, Calculated : 357.40.

2.3.12- Cytotoxicity screening

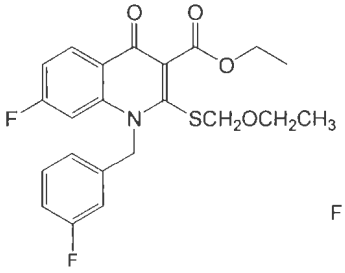
The cytotoxicities of selected final products and intermediates synthesized in this study were preliminary screened by Dr. S-Q Cai's group at the School of Pharmaceutical Sciences, Peking University, P.R. China. The assessment was performed by this group using a protocol designed for high-throughput preliminary screening of natural and synthetic cytotoxic agents. Based on this protocol, all the drugs were tested at a concentration of 100 μg/mL. The method used and the results are described as following.

The cell lines used were: human leukemia cancer cell line (HL-60); human gastric cancer cell line (BGC-823); human breast cancer cell line (MDA-MB-435); human cervical cell line (Hela), and human hepatocellular carcinoma cell line (Bel-7402).

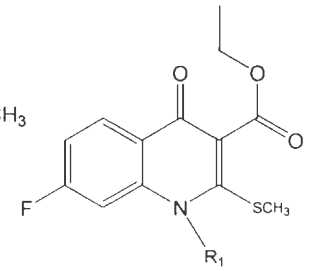
The *in vitro* HL-60 tumor cell assay was carried out according to the procedure reported by Twentyman, *et al.*[121] BGC-823, Bel-7402, and HeLa tumor cell assays were carried out by a sulforhodamine B (SRB) assay method.[122] A cell suspension in the culture medium ($4x10^4$ cells/mL) was inoculated in a 96-well microtiter plate. One day after seeding, a control plate at time zero was made. In the presence or absence of the test compounds, the cells were incubated for a further 48 h in a 5% CO_2 incubator at 37 °C. Cells were fixed with 50 μl of 20 % trichloroacetic acid solution for 1 h at 4°C and plates were washed 5 times with tap water and air-dried. A 50 μl of SRB solution (0.4% in 1% acetic acid) was added and kept at room temperature for 30 min. The residual dye was washed out with 1 % aqueous acetic acid and air-dried. To each well, Tris buffer solution (10 mM, pH=10.5) was added. Optical density (OD) was measured with a microtiter plate reader at 540 nm. Growth inhibition was calculated as follows:

% inhibition = [1-(OD value for treated cells/ OD value for untreated cells)]100%

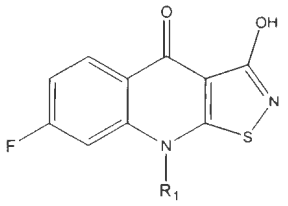
The structures of the tested compounds are depicted in Figure 2.4 and the results are summarized in Table 2.1.



4d



13a,b,f



6a,c,k

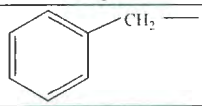
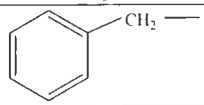
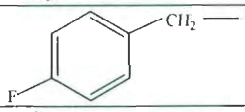
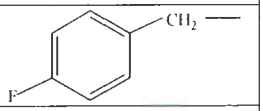
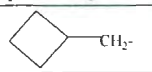
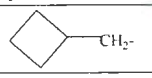
No.	R_1		R_1
13a		**6a**	
13b		**6c**	
13f		**6k**	

Figure 2.4- Structure of the compounds tested

Table 2.1- Preliminary cytotoxicity screening results

Compounds	Percentage of inhibition on different cell lines at 100 μg/mL					
	HL-60 (Human leukemia cancer cell line)	PC-3M-1E8 (prostate cancer cell line)	BGC-823 (Human gastric cell line)	MDA-ME (Human leukemia Cancer cell line)	Bel-7402 (Human hepatocellular carcinoma cell line)	HeLa (Human cervical cell line)
13a	**-9.42**	**-10.75**	**-6.21**	**0.23**	**-0.72**	**-14.86**
13b	**37.19**	**5.81**	**1.22**	**19.42**	**-10.12**	**-3.59**
13f	**9.78**	**-15.96**	**0.02**	**3.00**	**-1.43**	**-9.30**
6a	**10.23**	**-7.38**	**0.94**	**2.33**	**12.10**	**-6.44**
6c	**41.37**	**-10.19**	**0.44**	**16.95**	**2.16**	**13.36**
6k	**11.40**	**-10.58**	**-1.38**	**4.47**	**1.59**	**-0.38**
4d	**10.12**	**-13.19**	**-2.94**	**-10.60**	**1.46**	**-6.60**

* Negative signs denote proliferation

* No signs denote inhibition

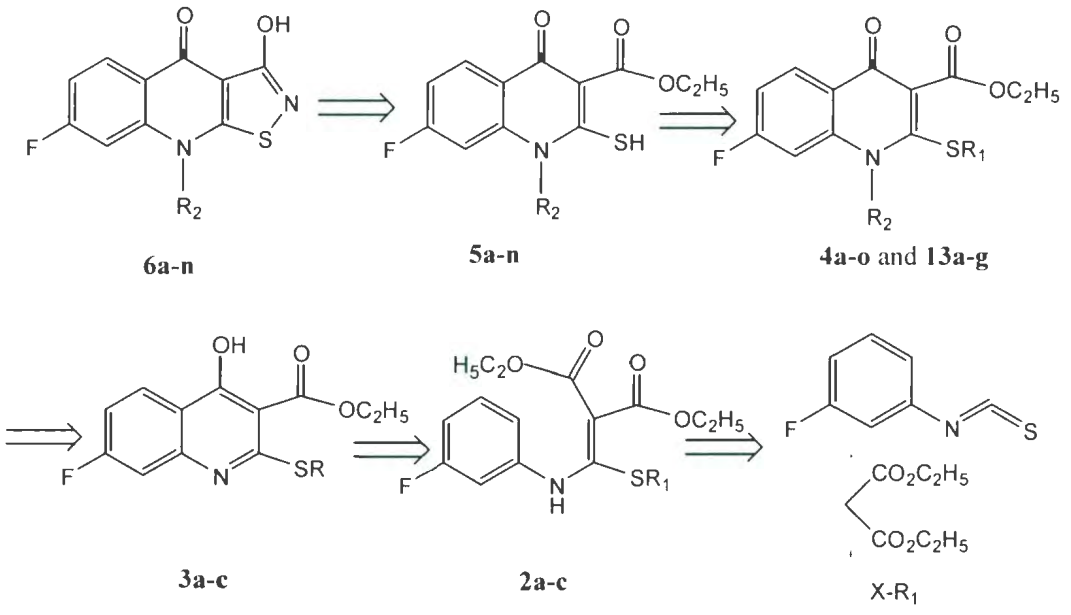
3- Discussion

As per the descriptions in Section 2.3 (Methods and Results), the objective of this research was to design and synthesize linear tricyclic isothiazoloquinolones that are bioisosterically related to the angular thiazoloquinolone A (Figure 2.1 in page 50) and possess the same biological activity profile as that of compound A while being less acidic and tolerable if taken by oral route. Compound A had exhibited favorable drug-like properties in different animal models in preclinical studies .[130]

Structure-based drug design approach was employed to initially design the isothiazolo-quinolones, using Alchemy-3^{TM} software on an Octane-2 Silicon Graphic system. The computational chemistry was performed by Dr. Alan Cameron of SynPhar Laboratories Inc., Edmonton, Alberta, Canada. Using Molecular Mechanics Optimization (MMO) and Molecular Dynamics Option models, the energetically optimized configuration of compound A was first determined. The bioisosteric linear isothiazoloquinolones were then designed based on the energetically minimized structure of compound A, as described in Section 2.3. In this respect, compound B (**6a**), at its optimized steric/energetic configuration, was identified as a molecule with a perfect 3-point overlay with compound A (Figures 2.2 and 2.3 in page 51).

In order to pursue the synthesis of compound B and its N-1 substitued analogues, we first attempted the modified Gould-Jacobs quinoline synthesis, based upon a hypothetical retrosynthetic pathway as described in pages (53-84). The retrosynthetic pathway hypothesized for the synthesis of compound B and its N_1-substituted analogues (**6a-n**) is depicted in Scheme 3.1. Starting from 3-fluorobenzylisothiocyanate, diethyl

malonate, and an appropriate alkyl halide, compound **2a-c** are expected to form, further Gould-Jacob type ring closure of which would result in the formation of compounds **3a-c**. Benzylation of this compound gives rise to compounds **4a-o** which after S-dealkyation affords compounds **5a-n**. The final step in this procedure is the formation of the third ring via reaction of compounds **5a-n** with hydroxylamine-*O*-sulfate to obtain the desired compounds **6a-n**.

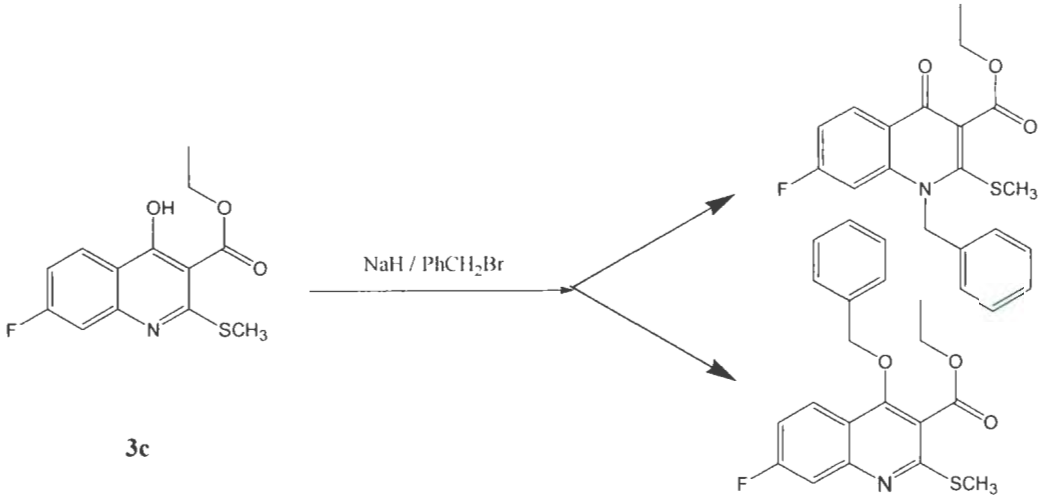


Scheme 3.1- Retrosynthetic pathways hypothesized for the synthesis of compound B (**6a**)

In practice, several key steps in this synthetic approach were identified that affect the yields as well as the synthetic feasibility of the intermediates and the final compounds. The formation and the yields of compounds **5a-n** were mainly dependent on the nature of R in compounds **4a-o**. In the case of $R_2 = CH_2OCH_2CH_3$, the S-dealkyation was easily achieved in 90-98% yields by the acid hydrolysis of compounds **4a-o** at room

temperature, as shown in Scheme 2.5 (page 69) and described in Sections 2.3.4 - 2.3.4.13. In case of R = CH_3 (**13a**, page 90), de-methylation of S required an initial oxidation of **13a** to **14,** followed by substitution of the sulfinyl group of **14** to afford the thiol **5a** in overall 10% yield from **13a** via treatment with sodium hydrosulfide as depicted in Scheme 2.8 (**13a**, page 90) and described in sections 2.3.10 - 2.3.11.

Preparation of compounds of the **4a-o** and **13a-g** series was a challenging step. In fact, direct benzylation of compound **3c** resulted in the formation of both *N*- and *O*-benzylated products and which were isolated by column chromatography in yields below 10% (Scheme 3.2).[123]



3c

Scheme 3.2- Direct benzylation of compound **3c**

In order to avoid non-selective alkylation reactions, compounds **4a-o** and **13a-g** were synthesized via either regioselective alkylation of compounds **3a-c** employing a modified Mitsunobu reaction using the appropriate alcohol, as shown in Scheme 2.3 (page 55) and described in sections 2.3.3 - 2.3.3.14, or modified Gould-Jacobs method as

depicted in Schemes 2.7, 2.8 (page 86) and (page 87), and described in sections 2.3.6 - 2.3.9.6. [124-125]

As shown in Figure 2.8 (page 87), regioselective *N*-1 alkylation of compounds **3a-c**, using a modified Mitsunobu method, proceeded smoothly at room temperature to afford compounds **4a-o** in 88-98% yields. Synthesis of compounds **13a-g** took a lengthy course during which the corresponding compounds were made in several steps from a general intermediate, 2,4-difluorobenzoyl acetoacetate (**10**). Compound **10** was either prepared from 2,4-difluorobenzoic acid (**7**) in three steps and in an overall yield of 54%, or from 2,4-difluoroacetophenone (**11**) in 95% yield. Condensation of compound **10** with the appropriate isothiocyanate, followed by methylation, yielded the intermediate **12**, which upon cyclization afforded compounds **13a-g** in 70 – 95% yields. These compounds were also prepared in one step via Mitsunobu alkylation of **3c** in 80-95% yields (Figure 2.8, page 87).

With respect to the synthetic feasibility of compounds **5a-n** from either the **4a-o** or the **13a-g** series, the following conclusions can be made.

Syntheses of **5a-n** by the procedure (Scheme 2.1) starting from phenylisothiocyanate (**1**) to make compound **3a** followed by regioselecive Mitsunobu alkylation of this compound to **4a-o** (excluding **4b**), which are readily deprotected by acid hydrolysis to the desired compounds **5a-n** (excluding **5b**) results in short synthetic steps and an overall yields of 56 – 84%, respectively.

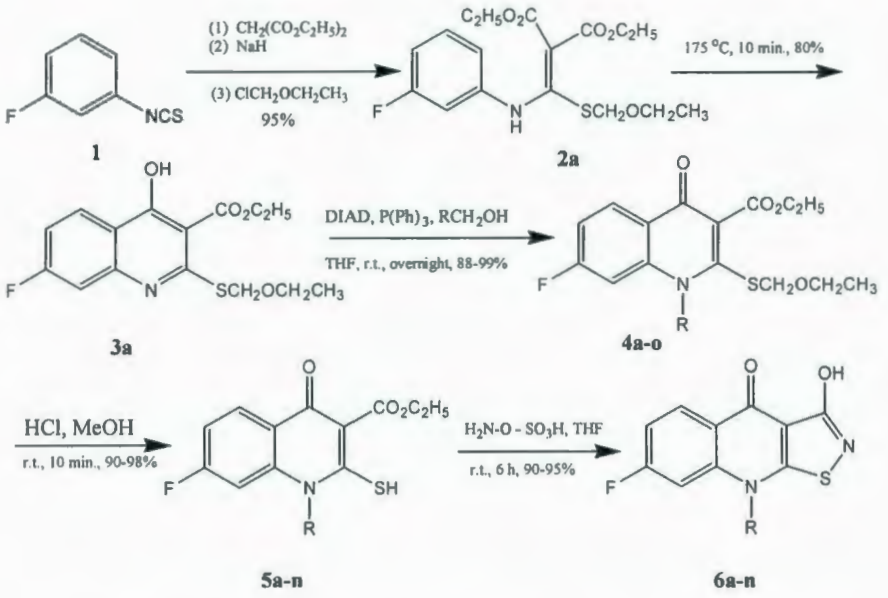
The yields of compounds **5a-n**, obtained via **13a-g**, were mainly dependent on the synthetic routes employed to make compounds **13a-g** and their further oxidations and

nucleophilic substitutions of the sulfinyl group to the thiols. Compounds **13a-g** were prepared by two distinct synthetic methods. The first method comprised preparation of compound **10** (Scheme 2.7, page 86) in yield of 54% (from **7**) and 95% (from **11**), which via intermediate **12** followed by cyclization afforded **13a** in an overall yield of 22.7% from **7** and 40% from **11**, respectively, as described in previous paragraph.

In the second method (Scheme 2.8, page 87), compounds **13a-g** were synthesized in one step via Mitsunobu alkylation reactions of **3c** in 80 - 95% yields, respectively.

As described previously, transformation of **13a**, the representative compound of this series, into **5a** required two steps, first oxidation by *m*-PCBA to the corresponding sulfinyl derivative **14** followed by a substitution reaction with sodium hydrosulfide, as shown in Scheme 2.9 (page 95) and described in sections 2.3.10 and 2.3.11. Unfortunately, the overall yield of these two steps was 10%, compared with that of **5a** when compound **4a** underwent acid hydrolysis to afford the same compound in 98% yield (Scheme 2.5, page 69).

Comparing the above-discussed routes for the synthesis of compounds **6a-n,** and taking the synthetic steps and overall yields into consideration, the following route is proposed as the most feasible for the synthesis of **6a-n** as shown in Scheme 3.3.



Scheme 3.3- The most feasible synthetic routes for compounds **6a-n**

Thus, Gould-Jacobs ring closure of **2a** to **3a** followed by Mitsunobu alkylation using a variety of alcohols gives rise to compounds **4a-o**. Simple acid hydrolyses of **4a-o** affords the mercapto derivatives **5a-n** in high yields. Ring closure of **5a-n**, using hydroxylamine-*O*-sulfate, affords compounds **6a-n** in high yields. According to the above Scheme and based on compound **1**, the overall yields of compounds **6a-n** is 54-70% in five steps, while with other pathways the overall yields are below 10% and require longer steps.

In order to explore the cytotoxicity potential of these compounds, selected final products and intermediates were tested by Dr. S-Q Cai's group at the School of Pharmaceutical Sciences, Peking University, P.R. China. As mentioned in Section 2.3.12, the assessment was performed by this group using a protocol designed for high-throughput preliminary screening of natural and synthetic cytotoxic agents. We had no

say in the selection of the cell lines as well as the minimum concentration of the test compounds to evaluate the percentage of the viable cells. Due to the preliminary screening nature of the protocol used by Dr. Cai's group, the TD_{50} values of the tested compounds were not measured and the percentage of viable cells were determined at 100 μg/mL, instead. Also, no reference compound was used for comparison in their assays. Therefore, the results presented in Section 2.3.12 are based on a random screening on specific cancer cell lines whose sensitivities toward topoisomerase inhibitors are not defined. The following discussion is based on the preliminary results provided by Dr. Cai's group.

Four quinolone carboxylate analogues (**13f, 13b, 13a, and 4d**) and three isothiazoloquinolone analogues (**6a, 6c, and 6k**) were tested for their cytotoxic effect. In HL-60 leukemic cell line, the **13b** and its isothiazoloquinolone analogue **6c** exhibited cytotoxic effect with 37.19 and 41.37 % inhibitory activity at 100 μg/mL concentration, while **13f,** its isothiazoloquinolone analogue **6k,** and **4d** exhibited cytotoxicity in the range of 9.78 to 11.40% at the same concentration. Surprisingly, the intermediate compound **13a** showed proliferative activity. In PC-3M-1E8 prostate cancer cell line, except for the intermediate compound **13b** which was weakly inhibitory, the other compounds were proliferative. In BGC-823 human gastric cell line, these compounds were either weakly inhibitory (**13f, 13b, 6a,** and **6c**) or weakly proliferative (**13a, 4d,** and **6k**). In MDA-ME human leukemic cell line, both compounds **13b** and its isothiazoloquinolone analogue **6c** exhibited stronger inhibitory activity, while **13f, 13a, 6a, 6k** were weakly inhibitory and **4d** was proliferative. The most interesting results were

observed when these compounds were tested against Bel-7402 human hepatocellular carcinoma cells. While the intermediates **13f, 13b**, and **13a** exhibited proliferative activity, the isothiazoloquinolone analogue of **13a** (**6a**) exhibited a mild inhibitory activity (12.10% at 100 μg/mL concentration), and **6c** (the isothiazoloquinolone analogue of **4d**) and **6k** (the isothiazoloquinoline analogue of **13f**) exhibited a very weak inhibitory activity against this cell line at 100 μg/mL concentration. Finally, as expected, except for compound **6c**, all the other intermediates and final products exhibited proliferative activity against HeLa cell line.

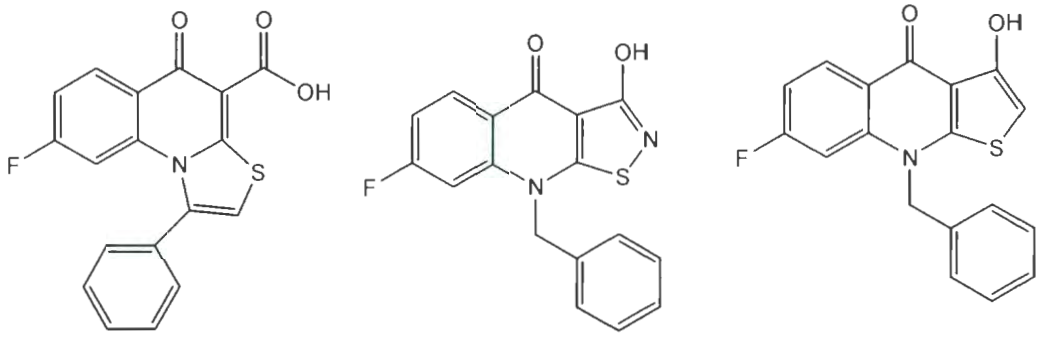
4- Conclusions

The design and syntheses of a series of 7-fluoro-9-substituted-3-hydroxyisothiazolo[4,5-*b*]quinolin–4(9*H*)-one derivatives (**6a-n**) were explored in this study using different synthetic approaches. The most feasible synthetic approach was selected based on the short number of steps, ease of synthesis, availability of the required starting materials and intermediates, and the stepwise yields. Based on the above criteria, we successfully synthesized 14 derivatives (**6a-n**) in 5 steps and overall yields of 54-70% as described in Section 3 and shown in Scheme 3.3 (page 105). Selected final compounds and intermediates were tested by Dr. Cai's group at the School of Pharmaceutical Sciences, Peking University, P.R. China. for their cytotoxic potential.

Despite observation of cytotoxic/proliferative effects of the tested compounds against the cell lines used in the assessment, the real anticancer potential of these compounds can not be concluded due to the following factors: (a) test results were only based on a single concentration of 100 μg/mL; (b) unavailability of toxicokinetic data including TD_{50} values; (c) unavailability of topoisomerase inhibitory profiles of these compounds, and (d) the low number of compounds tested. These preliminary results may indicate some cytotoxic potential of this class of compounds, but a more detailed structure-activity relationships study is needed to confirm this hypothesis.

Considering the structural difference between compound A, an angular thiazoloquinolone, and compound B (**6a** and its analogues), a linear isothiazoloquinolone, the more hydrophilic characteristics of **6a-n,** compared to that of compound A, may attribute to their weak cytotoxic profile or proliferative activity. On this basis, we hypothesize that

thienoquinolones, such as compound C (Figure 4.1) that should possess physicochemical properties similar to that of compound A, might exhibit potential anticancer activities. Therefore, further studies on molecules related compound C is warranted.



Compound A Compound B Compound C

Figure 4.1- Structures of compound A, B, and C

References

1) Ellison J. H.; Wu X. C.; McLaughlin C. C; Lake A.; Firth R.; Cormier M.; Leonfellner S.; Carozza S.; Roney D.; Howe H. L.; Kosary C.; *NACCR.*; **2005**, 3, 1-118.

2) Agarwal R.; *Biochem. Pharmacol.*, **2000**, *60*, 1051-1059.

3) Ricci P. E.; Molton L. S.; *Environ. Sci. Technol.*; **1985**, *19*, 473-479.

4) Nicholson N.; Solid Tumors. In *Clinical Pharmacy and Therapeutics, 2nd Ed.; Walker .R; Edwards C., Ed.; Churchill Livingstone: Edinburg,* **1999**; *pp. 763-778.*

5) Pelengaris S.; Khan M.; DNA replication and cell cycle. In *The molecular biology of cancer, 1th Ed.;* Pelengaris S.; Khan M .; Ed.; *Blackwell publishing: United Kingdom,* **2006**; *pp. 88-119.*

6) Calabersi P.; Chabner A.; Chemotherapy of Neoplastic Diseases. In *Goodman & Gillman's the pharmacological basis of therapeutics, 10th Ed.;* Hardman J. G.; Limbard L. E.; Ed.; *Mc Graw-Hill: United States*, **2001**; *pp. 1381-1459.*

7) Panasci L.; Paiement J. P.; Christodoulopoulos G.; Belenkov A.; Malapetsa A.; Aloyz R.; *Clin. Cancer. Res.*, **2001**, *7*, 454–461.

8) Bertram J. S.; *Mol. Aspects. Med .*, **2001**, *21*, 167-223.

9) Callery P.; Gannet P.; Cancer and Cancer Chemotherapy. In *Foye's Principles of Medicinal Chemistry,* 5th Ed.; Williams D. A.; Lemke T. L.; Ed.; *Lippincott, Williams& Wilkins: Philadelphia,* **2002**; *pp. 929-945.*

10) Waldmann T. A.; *Nature Med.*, **2003**, *9*, 269–277.

11) Elguero J.; Marzin C. C.; Katritzky A. R.; Linda P.; *The Tautomerism of Hetrocycles*, Ed.; *Academic Press*: *New York*, **1976**; *pp.1-655*.

12) katritzky A. R.; Ellison J.; Frank J.; Rakoczy P.; Radics L.; Gacs-Baitz E.; *Org. Magn. Reson.*, **1981**, *16*, 280-284.

13) Zalibera L.; Milata V.; llavsky D.; *Magn. Reson. Chem.*. **1998**, 36, 681-684

14) Hermecz L.; Keresturi G.; Vasvari-Debreczy L.; *Adv. Hetrocycl. Chem.*, **1992**, *54*, 10-45.

15) Joule J. A.; Millls K.; Quinolines and isoquinolines: reaction and synthesis. In *Heterocyclic Chemistry*, 4th Ed.; *Joule J. A; Millls, K; Ed.; Blackwell publishing In: Oxford*, **2000**; *pp.121-128.*

16) Heindel N. D.; Bechara I. S.; Kennewell P. D.; Molnar J.; Ohnmacht C. J.; Lemke S. M.; Lemke T. F.; *J. Med. Chem.* **1968**, *11*, 1218-1221.

17) Koga H.; Itoh A.; Murayama S.; Suzue S.; Irikura T. X.; *J. Med. Chem.*, **1980**, *23*, 1358-1363.

18) Gerster J. F.; *Ger. Offen.*, **1973**, *2, 264,163, Ricker. 2*, 264. *Chem. Abstr.*, **1972**, 76, 92029y.

19) Neuman M.; *Clin. Pharmacokinet.*, **1988**, *14*, 96-121.

20) Takacs N. K.; Noszal B.; Hermecz I.; Kerezturi G.; Pondyi B.; Gzasz S.; *J. Pharm. Sci.*, **1990**, 79, 1023-1028.

21) Park H. R.; Chung K. Y.; Lee H. C.; Lee J. K.; Bark K. M.; *Bull. Korean. Chem. Soc.*, **2000**, *21*, 849-854.

22) Park H. R.; Kim T. R.; Bark K. M.; *Eur. J. Med. Chem.* **2002**, *37*, 443-460.

23) Mizuki Y.; Fujiwara I.; Yamaguchi T.; *J. Antimicrob. Chemother. suppl. A.*, **1996**, *37*, 41-55.

24) Kang J. S.; Kim T. H.; Park K. B.; Chung B. H.; Youn J. I.; *Photodermatol. Photoimmunol. Photomed.*, **1993**, *9*, 159-161.

25) Molinski T. F.; Faulkner D. J.; *J. org. Chem.*, **1987**, *52*, 296–298.

26) Grohe K.; Heitzer H.; *Liebigs. Annu. Chem.*, **1987**, *1*, 29-37.

27) kincl F. A.; Romo J.; Rosenkranz G.; Sondheimer F.; *J. Chem . Soc.*, **1956**, 4163-4169.

28) Toube T. P.; Murphy J. W.; Cross A. D.; *Tetrahedron.*, **1967**, *23*, 2061-2065.

29) Tschsche R.; Werner W.; *Tetrahedron.*, **1967**, *23*, 1873-1881.

30) Milatta V.; Claramunt R. M.; Elguero J.; *Targets. Heterocycl. Syst.*, **2000**, *4*, 167-203.

31) Norman R. O. C.; Coxon, J. M.; The synthesis of five and six membered heterocyclic compounds. In *Principles of Organic Chemistry*, 3rd Ed.; *Springer: New York,* **1993**; *pp. 676-723.*

32) Joule J. A.; Millls K.; Quinolines and isoquinolines: reaction and synthesis. In *Heterocyclic Chemistry*, 4th Ed.; *Joule J. A; Millls, K; Ed.; Blackwell publishing: Oxford,* **2000**; *pp.121-128.*

33) Camps R.; *Chem. Ber.*, **1899**, *22*, 3228.

34) Li J. J.; Name Reactions: A Collection of Detailed Reaction Mechanisms, 2nd Ed.; *Springer: New York,* **2003**; p. 79.

35) Camps P.; Achab R.; Gorbig D. M.; Morral J.; *J. Med. Chem.*, **1999**, *42*, 3227-3242.

36) Curran D. P.; Liu H.; *J. Am . Chem. Soc.*, **1992**, *114*, 5863-5864.

37) Koetsch C. F.; Lucht F. J.; *J. Am. Chem. Soc.*, **1949**, *71*, 3556.

38) Witkop B.; *J. Am. Chem. Soc.*, **1951**, *73*, 2641-2647.

39) Bornstein J.; *J. Am. Chem. Soc.*, **1954**, *76*, 2760-2762.

40) Camps P.; Gomez E.; *J. Med. Chem.*, **2001**, *44*, 4733 -4736.

41) Tom N. J.; Ruel E. M.; *Synthesis.*, **2001**, 1351-1355.

42) Johnson W. S.; Matthews F. J.; *J. Am. Chem. Soc.*, **1994**, *66*, 210-215.

43) Yamashkin S. A.; Yudin L. G.; Kost A. N.; *Chem. Heterocycl. Compd.*, **1992**, *28*, 845 – 855.

44) Duvelleroy D.; Perrio E.; Risel O.; Lasne M. C.; *Org. Biomol .Chem.*, **2005**, *3*, 3794–3804.

45) Camps P.; Gomez E.; Munoz-Torrero D.; *Tetrahedron.*, **2004**, *60*, 5423–5431.

46) Matsugi M.; Tabusa F.; Minamikawa J.; *Tet. Lett.*, **2000**, *41*, 8523-8525.

47) Leir C. M.; *J. Org. Chem.*, **1977**, *42*, 911-913.

48) Panda K.; Siddiqui I.; Mahata P. K.; *Synlett.* **2004**, *3*, 449-452.

49) Kouznetsov V. V.; Mendez L.; Gomez C.; *Curr. Org. chem.*, **2005**, 9, 141-161.

50) Abramenko P. I.; *Chem. Heterocycl. Comp.*, **1971**, *7*, 436–438.

51) Vorozhtsov N. N.; Mikhailova I. F.; *Chem. Heterocycl. Comp.*, **1996**, *2*, 98–100.

52) Czuba W.; *Chem. Heterocyc. Comp.*, **1979**, *15*, 1-13.

53) Hamada Y.; Takeuchi I.; *J .Org. Chem.*, **1977**, *42*, 4209.

54) Zemtsova M. N.; Trakhtenberg P. L.; Lipkin A. N.; Ryskina T. B.; *Pharmaceutical. Chem. J.*, **1973**, *7*, 484-488.

55) Craig D. J.; *J. Am. Chem. Soc.* **1938**, *60*, 1458-1465.

56) Elderfield R.C.; McCarthy J. R.; *J. Am. Chem. Soc.*, **1951**, *73*, 975-984.

57) Gould R. G.; Jacobs W.A.; *J. Am. Chem. Soc.*, **1938**, *61*, 2890-2895.

58) Price C. C.; Roberts R. N.; *J. Am. Chem. Soc.*, **1946**, *68*, 1204-1255.

59) Jacobs W. A.; Gould R. G.; *J. Biol. Chem.*, **1937**, *120*, 141-150.

60) Reitsema R. H.; *Chem. Rev.*, **1948**, *43*, 43-68.

61) Torri S.; *Tet. Lett.*, **1990**, *31*,7175-7178.

62) Varma R.S.; Saini R. K.; *Synlett*:, **1997**, 857 -858.

63) Agui H.; Mitani T.; Izawa A.; Komatsu T.; Nakagome T.; *J. Med. Chem.*, **1997**, *20*,791-796.

64) Clemence F.; *J. Heterocycl. Chem.*, **1984**, *21*, 1345-1353.

65) Torri S.; Oku O.; Hxu L. H.; *Tet. Lett.*, **1991**, *32*, 237-240.

66) Li L.; Wang H. K.; Kuo S-Ch.; Wu T. S.; Mauger A.; Lee K. H.; *J. Med. Chem.*, **1994**, *37*, 1126-1135.

67) Li L.; Wang H. K.; Kuo S-Ch.; Wu T. S.; Mauger A.; Lee K. H.; *J. Med. Chem.*, **1994**, *37*, 3400.

68) Proctor G. R.; Ross W. I.; Taia A.; *J. Chem. Soc. Perkin trans. 1*, **1972**, 1803-1808.

69) Joule J. A.; Millls K.; Quinolines and isoquinolines: reaction and synthesis. In *Heterocyclic Chemistry*, 4th Ed.; *Joule J. A; Millls, K; Ed.; Blackwell Publishing Inc. Oxford,* **2000**; *pp.16-47and 121-128.*

70) Werner W.; *Tetrahedron.*, **1969**, *25*, 255-261.

71) Mitscher L. A.; Flynn D. L.; Gracey H. E.; Drake S. D.; *J. Med. Chem.*, **1979**, *22*, 1354-1357.

72) Markees D. G.; Schwab L. S.; Vegotsky A.; *J. Med. Chem.*, **1974**, *17*, 137-139.

73) Cecchetti V.; Fravolini A.; Fringuelli R.; Mascellani G.; Pagella P.; Palmioli M.; Segre G.; Terni P.; *J. Med. Chem.*, **1987**, *30*, 465-473.

74) Lee J.; Lee K.; American Chemical Society, Division of Medicinal Chemistry, Abstract No: 362,2005, San Diego, CA.

75) Carretero J. C.; *Tetrahedon.*, **1992**, *48*, 7373-7382.

76) Masahiro K.; Masahiko K.; Iwao M; Jap. Pat. 58105965 , 19830 (Chemical Abstr. 1983, 99,139797c)

77) Chu D.; *J. Heterocycl. Chem.* **1990**, *27*, 1191-1195.

78) Chu D.; *J. Heterocycl. Chem.*, **1990**, *27*, 839-843.

79) Chu D.; *J. Med. Chem.*, **1985**, *28*, 1558-1564.

80) Wentland M. P.; Cornett J. B.; *Annual. Rep. Med. Chem.*, **1986**, 20, 145-154.

81) 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.; *J. Med. Chem.*, **1993**, *36*, 2801-2809.

82) Wentland M. P.; *J. Med. Chem*, **1993**, *36*, 1580-1596.

83) Champoux J. J.; *Ann. Bey. Biochem.*, **1978**, *47*, 449-479.

84) Wang J. C.; *Nat. Rev. Mol. Cell Biol.*, **2002**, *3*, 430 -440.

85) Drlica K.; *Microb. Rev.*, **1984**, 48, 273-289.

86) Wang J. C.; *Annual. Rev. Biochem.*, **1985**, *54*, 665-697.

87) Gellert M.; *Annual. Rev. Biochem.*, **1981**, *50*, 879-910.

88) Gellert M.; Mizuuchi K.; Odea M. H.; Nasht H. A.; *Proc. Natl. Acad. Sci.*, **1976**, *73*, 3872-3876.

89) Park H. Y.; Kim H. T.; *European. J. Med. Chem.*, **2002**, *37*, 443-460.

90) Staudenbauer W. L.; Orr E.; *Nucleic Acids Res.*, **1981**, *9*, 3589–3603.

91) Kampranis S. C.; Maxwell A.; *J. Bio. Chem.*, **1998**, *273*, 22615–22626.

92) Sugino A.; Higgins P.; *Proc. Natl. Acad. Sci.*, **1976**, *75*, 4838-4842.

93) Liu L. F.; Liu C. C.; Alberts B. M.; *Nature.*, **1979**, *281*, 456-461.

94) Crooke E.; Hwang D. S.; Skarstad K.; Thony B.; Kornberg A.; *Res. Microbiol.*, **1991**, *142*, 127–130.

95) Kampranis S. C.; Bates A. D.; Maxwell A.; *Proc. Natl. Acad. Sci.*, **1999**, *96*, 8414–8419.

96) Kampranis S. C.; Bates A. D.; Maxwell A.; *Proc. Natl. Acad. Sci.*, **1996**, *93*, 14416-14421.

97) Mondal N.; Parvin J. D.; *Nature.*, **2001**, *413*, 435–438.

98) Wang J. C.; *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430-440.

99) Tabarrini O.; *J. Med. Chem.*, **1999**, *42*, 2136-2144.

100) Barrett J. F.; Gootz T. D.; McGuirk P. R.; Farrell C. A.; Sokolowski S. A.; *Antimicrob. Agents. Chemother.*, **1989**, *33*, 1697-1703.

101) Wyllie A. H.; Kerr J. F.; Currie A. R.; *Int. Rev. Cytol.*, **1980**, *68*, 251-306.

102) Malonne H.; Atassi G.; *Anti cancer Drugs.*, **1997**, *8*, 811-822.

103) Mitschew L. A.; Antibiotics and Antimicrobial Agents. In *Foye's Principles of Medicinal Chemistry,* 5th Ed.; Williams D. A.; Lemke T. L.; Ed.; *Lippincott, Williams& Wilkins*: *Philadelphia*, **2002**; *pp. 819-825.*

104) Joseph B.; Darro F.; Behard A.; Lesur B.; Collignon F.; Decaestecker C.; Frydman A.; Guillaumet G.; Kiss R.; *J. Med. Chem.*, **2002**, *45*, 2543-2555.

105) Roberts N.; Martin J.; Kinchington D.; Broadhurst A.; Craig J.; Duncan I.; Galpin S.; Handa B.; Kay J.; Krohn A.; *Science.*, **1990**, *248*, 358–361.

106) Zheng D.; Huang Y.; Moseley H.; Xiao R.; Aramini J.; Swapna G.; Montelione G.; *Protein Sci.*, **2003**, *12*, 1232–1246.

107) Oezguen N.; Adamian L.; Xu Y.; Rajarathnam K.; Braun W.; *J. Biomol. NMR.*, **2002**, *22*, 249–263.

108) Lukasik S.; Zhang L.; Corpora T.; Tomanicek S.; Li Y.; Kundu M.; Hartman K.; Liu P.; Laue T.; Biltonen R.; *Nat. Struct. Biol.*, **2002**, 9, 674–679.

109) Cohen N. C.; Blaney J. M.; Humblet C.; Gund P.; Barry D. C.; *J. Med. Chem.*, **1990**, *33*, 883-984.

110) Hopfinger A. J.; *J. Am. Chem. Soc.*, **1980**, *102*, 7196-7206.

111) Boulu L. G.; Crippen G. M.; Barton H. A.; Kwon H.; Marletta M. A.; *J. Med. Chem.*, **1990**, *33*, 771-775.

112) Kati I.; Itai A.; Iitaka Y.; *Tetrahedron.*, **1987**,*43*, 5229-5236.

113) Doweyko A. M.; *J. Med. Chem.*, **1988**, *31*, 1396-1406.

114) Martin Y. C.; *J. Med. Chem.*, **1992**, *35*, 2145-2154.

115) Kleina C.; Kaiblingerb N.; *J. Comput-Aided Mol. Des.*, **2002**, *16*, 79–93.

116) Martin E. J.; Blaney J. M.; Siani M. A.; Spellmeyer D. C.; Wong A. K.; Moos W. H.; *J. Med. Chem.*, **1995**, *38*, 1431-1436.

117) Balaban A. T.; *Chem. Phys. Letters.*, **1982**, *89*, 399-404.

118) Cramer R. D.; Patterson D. E.; Bunce J. D.; *J. Am. Chem. Soc.*, **1988**, *110*, 5959-5967.

119) Sissi C.; Plumbo M.; *Curr. Med Chem-Anti-Cancer Agents.*, **2003**, *3*, 439-450.

120) Hosomi J.; Asahina Y.; Suzue S.; WO8912055. published Dec 14, **1989.**

121) Twentyman P. R.; Luscombe M.; *Br. J. Cancer.*, **1987**, *56*, 279-285.

122) Skehan P.; Storeng R.; Scudiero D.; Monks A.; McMahon J.; Vistica D.; Warren J.T.; Bokesch H.; Kenney S.; Boyd M. R.; *J. Natl. Cancer Inst.*, **1990**, *82*, 1107-1112.

123) Daneshtalab M.; Unpublished data.

124) Grochowski E.; Hilton B. D.; Kupper R. J.; Michejda C. J.; *J. Am. Chem. Soc.*, **1982**, *104*, 6876-6877.

125) Humphries P. S.; *Beilstein J. Org. Chem.*, **2006**, *2*, 21-24.