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Chaniyara, Ravikumar S., 2011, “*Studies on Some Oxygen, Nitrogen and Sulfur Containing Heterocycles*”, thesis PhD, Saurashtra University

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Ph. D. Thesis

**STUDIES ON
SOME OXYGEN, NITROGEN AND SULFUR
CONTAINING HETEROCYCLES**

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**DEPARTMENT OF CHEMISTRY,
(DST-DPRP, DST-FIST FUNDED & UGC-SAP SPONSORED)**

SAURASHTRA UNIVERSITY,

RAJKOT- 360 005

2011

**STUDIES ON
SOME OXYGEN, NITROGEN AND SULFUR
CONTAINING HETEROCYCLES**

A THESIS SUBMITTED TO
THE SAURASHTRA UNIVERSITY
IN THE FACULTY OF SCIENCE
FOR THE DEGREE OF

Doctor of Philosophy

IN
CHEMISTRY

BY

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May-2011

Statement under *O. Ph. D. 7* of Saurashtra University

The work included in the thesis is done by me under the supervision of Prof. Anamik K. Shah and Co-supervision of Prof. Tsann Long Su and the contribution made thereof is my own work. The work was carried out at Department of Chemistry, Saurashtra University, Rajkot, India and Laboratory of Bioorganic and Medicinal Chemistry, Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan.

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CERTIFICATE

This is to certify that the present work submitted for the Ph.D. Degree of Saurashtra University by **Mr. Ravikumar S. Chaniyara** has been the result of work carried out under my supervision and is a good contribution in the field of synthetic chemistry and medicinal chemistry.

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*Dedicated to My
Family*

Acknowledgement

Firstly, I bow my head humbly before the Almighty God for making me capable of completing my Ph.D. Thesis; with his blessings only I have accomplished this huge task.

I express deep sense of gratitude and thankfulness to my guide Professor **Dr. Anamik Shah** who has helped me at each and every point of my research work with patience and enthusiasm. I am much indebted to him for his inspiring guidance, affection, generosity and everlasting enthusiasm throughout the tenure of my research work, without that the Thesis would not have appeared in the present form.

I would like to thank **Prof. Tsann-Long Su**, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, for giving me an opportunity to carry out my Ph.D thesis work in his laboratory under his guidance. He shared with me a lot of his expertise and research insight. His wide knowledge in the field of anti-cancer research and logical way of thinking has been of great value for me to accomplish this thesis work. I am deeply grateful to the Institute for the trust and support that they gave me in order to study in Taiwan. I owe my most sincere gratitude to Prof. Ting-Chao Chou, Memorial Sloan-Kettering Cancer Center, New York, USA, for his kind help by providing antitumor studies. I would like to thank Dr. Tee-Chang Lee and Dr. Tung-Hu Tsai for providing mechanistic studies. I am also thankful to the National Science Council and Academia Sinica for financial support.

I also thank to **Dr. P H Parsania**, Professor and Head, Department of Chemistry, Saurashtra University, Rajkot for his encouragement and providing adequate research facilities.

I like to mention a special thanks to Dr. Ranjanben Shah and Aditya (chiki) for their kind support in personal matter and making me feel like home.

I am equally thankful to all of the non-teaching staff for extending help and cooperation. I am highly thankful to University Grant Commission, New Delhi for UGC Meritorious fellowship.

I also would like to thank my labmates, Ching-Hwang Chen, Yi-wen Lin, Rajesh, Nikhil, Satish, Bhavin, Nilay, Hardevsinh, Manisha, Jignesh, Rakshit, Harshad, Hitesh, Mrunal, Vaibhav, Ashish, Sachin, Bharat, Paresh, Pratik, Shailesh, Shery, Dhairya, Dilip, Madhavi, Hetal, Vishwa, Sabera and Punit for their kind help and support.

I would also like to thank my seniors for all their help, and support during my research tenure. I heartily thank Dr. Kuldip, Dr.

Rupesh, Dr. Gaurang, Dr. Jitender, Dr. Atul, Dr. Jyoti, Dr. Naval and Dr. Chintan for their co-operation.

I am also blessed with some great colleagues whose presence has made this time a memorable time for me and my work in the laboratory was really enjoyable due to them. I thank Piyush, Ashish, Suresh, Ritesh, Rakesh, Bharat, Sandip, Kapil, Bhavesh, Mehul, Rahul, Renish, Naimish, Mahesh, Vijay, Govind, Chirag, Minaxi, Rizwan, Dipti, Pankaj, Piyush, Anil, Vipul, Jignesh.

At this juncture I thank to my cousin for encouraging me and providing help at each and every stage to fulfill this task. I would also like to convey my pleasant regards and thankfulness towards, Prince, Rahul, Jay, Bhavana, Zarika, Neha, Tanisha, Harsh, Sapana, Dharam and Vatsal for their constant care, support and encouragement.

I am tempted to individually thank all of my friends which, from my childhood until graduate school, have joined me in the discovery of what is life about and how to make the best of it. However, because the list might be too long and by fear of leaving someone out, I will simply say *thank you very much to you all*.

Words become smaller and explanations become shorter when I heard name of my dear Lina. I am extremely thankful to her for giving me loving support whenever I needed.

I am equally thankful to my dearest sister Janki, Kevin Jiju and Arsheli for their moral support and courage in each moment. Last but most important, I would like to bow my head with utter respect and convey my pleasant regards to my most adorable mummy-papa, **Mr. Subhash M Chaniyara** and **Mrs. Prafulla S Chaniyara** without whom I would have been absolutely nothing. They have always supported and encouraged me to do my best in all matters of life. To them I dedicate this thesis.

Ravi S Chaniyara

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General Remarks

1. Melting points were determined on a Fargo melting point apparatus and are uncorrected.
2. ^1H NMR & ^{13}C NMR spectra were recorded on 600 MHz, Bruker AVANCE 600 DRX and 400 MHz, Bruker Top-Spin spectrometers. Making a solution of samples in $\text{DMSO-}d_6$ solvents using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned, and are given in the ppm (δ) scale. The standard abbreviations s, d, t, q, m, dd, brs refer to singlet, doublet, triplet, quartet, multiplet, doublet of doublet, and broad singlet respectively.
3. Mass spectra were recorded on Shimadzu GC MS-QP 2010 spectrometer operating at 70 eV using direct injection probe technique.
4. Elemental analyses were done on a Heraeus CHN-O Rapid instrument. The elemental analysis of the newly synthesized derivatives was within $\pm 0.4\%$ range of the calculated C, H, N data.
5. High performance liquid chromatography analysis for checking purity of synthesized compounds were recorded on a Hitachi D-2000 Elite instrument: column, Mightysil RP-18 GP 250-4.6 ($5\mu\text{m}$); mobile phase, MeCN/THF (50:50 v/v); flow rate, 1 mL/min; injected sample $10\mu\text{L}$, column temp, $27\text{ }^\circ\text{C}$; wavelength, 254 nm. The purity of all tested compounds was $\geq 95\%$ based on analytical HPLC.
6. Analytical thin layer chromatography (TLC) was performed on Merck precoated silica G60 F₂₅₄ glass plates. Visualization of the spots on TLC plates was achieved either by exposure to iodine vapor or UV light.
7. All evaporation of solvents was carried out under reduced pressure on Heidolph LABOROTA-400-efficient.
8. All reported yields are isolated yields after chromatography or crystallization.
9. The chemicals used for the synthesis of intermediates were purchased from Aldrich, Meark, Spectrochem, Sisco Research Laboratories (SRL), Thomas Baker, Sd fine chemicals, Loba chemie and SU-Lab.
10. The structures and names of all compounds given in the experimental section and in physical data table were generated using ChemBio Draw Ultra 12.

Abbreviations

AcOH	Acetic Acid
Ac ₂ O	Acetic Anhydride
AIDS	Acquired Immuno Deficiency Syndrome
AlCl ₃	Aluminum chloride
Ar	Aromatic
ATO	Arsenic Trioxide
BP	Boiling Point
CAMP	Cyclic Adenosine Mono Phosphate
CCRF-CEM	Human Lymphoblastic Leukemia
CNS	Central Nervous System
CO ₂	Carbon dioxide
Concd.	Concentrated
CuI	Copper(I) iodide
CR	Complete Tumor Remission
CL	Double-Stranded Cross-Linking
DMAD	Dimethyl Acetylenedicarboxylate
DAPYs	2,4-Dianilinopyrimidines
DMAP	Dimethylamino Pyridine
DMF-DEA	N, N-Dimethylformamide Diethyl Acetal
DME	Dimethyl Ether
DMF	<i>N, N</i> -Dimethyl formamide
DMSO	Dimethyl Sulfoxide
DNA	Deoxy Ribo Nucleic Acid
DZQ	Diaziridinylquinone
Equiv	Equivalent
Et	Ethyl
EtOH	Ethanol
FDA	Food and Drug Administration
5-FU	5-Fluorouracil
GABA	Gama-amino butyric acid

GC	Gas Chromatography
GC -MS	Gas Chromatography Mass Spectra
H1299	Lung Cancer Cell
H ₂ SO ₄	Sulphuric acid
HBF ₄	Tetrafluoroboric acid
HCl	Hydrochloric acid
HCT-116	Colon Carcinoma
HHPQ	Hexahydropyranquinoline
HIV	Human Immunodeficiency Virus
HPLC	High-performance liquid chromatography
HT-29	Human Colon Carcinoma
H460	Human Large Cell Lung Carcinoma
Hz	Hertz
I ₂	Iodine
IC ₅₀	Inhibitory Concentration
<i>i</i> -Pr	<i>Iso</i> -Propyl
<i>J</i>	Coupling Constants
K ₂ CO ₃	Potassium Carbonate
K ₃ PO ₄	Potassium Phosphate
KBr	Potassium Bromide
KOH	Potassium Hydroxide
LiAlH ₄	Lithium Aluminium Hydride
L-1210	Lymphocytic Leukemia cell line
LX-1	lung
<i>m</i>	Meta
MDC	Dichloro methane
Me	Methyl
MMC	Mitomycine C
MF	Molecular Formula
MHz	Mega Hertz
Mmol	Mili moles.
MP	Melting Point
MS	Mass Spectra

MX-1	Breast Carcinoma
Na ₂ CO ₃	Sodium Carbonate
NADPH	Nicotinamide adenine dinucleotide phosphate
NaHCO ₃	Sodium Bicarbonate
NaNO ₂	Sodium nitrite
NaOH	Sodium Hydroxide
NCEs	New Chemical Entities
NMR	Nuclear Magnetic Resonance
<i>o</i>	Ortho
<i>p</i>	Para
PBS	Phosphate Buffer Saline
PC3	Human Prostate Cancer
Pd(OAc) ₂	Palladium diacetate
PI	Propidium Iodide
PhMe	Toluene
ppm	Parts Per Million
PC3	Prostate Adenocarcinoma
PAs	Pyrrolizidine alkaloids
OECM1	Oral Carcinoma
QSAR	Quantitative Structural Activity Relationship
Q2D×2	Every Two Days For Two Times
QD×4	Every Day For Four Times
rt	Room Temperature
R _f	Retention Factor
RNA	Ribo Nucleic Acid
SAR	Structure Activity Relationship
SK-OV-3	Human Ovarian Adenocarcinoma
SS	Single-Stranded DNA
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetra Methyl Silane
UV	Ultra Violet

U87	Human Glioma
VBL	Vinblastine
Zn(OTf) ₂	Zinc Triflate
ZnCl ₂	Zinc Chloride

Section A

Chapter 1. Introduction

1.0 Introduction

Clinically, cancer is a name given to the large family of diseases, maybe a hundred or more, that vary in age of onset, rate of growth, state of cellular differentiation, diagnostic detectability, invasiveness, metastatic potential, and response to treatment and prognosis.¹ Cancer occurs when cells in a part of the body start to grow out of control or cells become abnormal and forming new cells without any control or order.² All organs of the body are made up of cells. Normally, cells divide to form new cells only when the body needs them. If cells divide when new ones does not need, they form a mass of excess tissue, called a tumor. Tumors can be benign (not cancer) or malignant (cancer). The cells in the malignant tumors can damage and invade nearby tissues and organs. Cancer cells can also break away from the malignant tumor and travel through the bloodstream to form new tumors in other parts of the body. The spread of cancer is called metastasis.

Cancer is a disease that has tormented man throughout recorded history. Among the first to document cancer were the ancient Egyptians, whom 1600 B. C. years ago wrote detailed accounts of breast cancer.^{3,4} Continuing through the ages, cancer was researched and described by numerous historical figures including Greek physician Hippocrates (460–370 B.C.), considered the “Father of Medicine”, Galen, and Giovanni Morgagni. It was not until the second half of the 20th century, however, that cancer has been more fully researched and its molecular and cellular basis began to be understood. As a result, many effective treatment regimens have been developed. However, cancer continues to be a major killer.^{5,6}

In the world cancer is the second leading cause of death behind heart disease.⁷ ⁸ Cancers in all forms are causing about 12 per cent of deaths throughout the world. While In India, Cancer has become one of the ten leading causes of death.⁹ It is estimated that there are nearly 1.5-2 million cancer cases at any given point of time. More than 7 lakh new cases of cancer and 3 lakh deaths occur annually due to cancer.¹⁰ Nearly 15 lakh patients require facilities for diagnosis, treatment and follow up at a given time. Data from the National Cancer Registry Programme indicate that the leading sites of cancer are oral cavity, lungs, esophageal, cervical and colic amongst men and cervix, and breast amongst women. Cancers namely those of oral

and lungs in males, and cervix and breast in females account for over 50% of all cancer deaths in India.

From the average diagnosis, most common types of cancer have seen at age 67. Although cancer is relatively rare in children, still it is a leading cause of death between ages 1 and 14. Millions of people alive today have had some type of cancer. Of these, about half are considered cured. The good news is that more and more people are now being cured of their cancers. This progress is due to better techniques of diagnosis and treatment.

In many cases, the causes of cancer are not clear, but both external and internal factors play a role. Due to the complex nature of cancer development, it is not possible to pinpoint one specific agent as the cause of cancer.¹¹⁻¹³ Rather, it is most likely due to a number of contributing factors. These factors include exposure to certain chemicals, action of viruses, exposure to radiation, and heredity. Today we recognize and avoid many specific substances that cause cancer: coal tars and their derivatives (like benzene), some hydrocarbons, aniline (a substance used to make dyes), asbestos, and others. Radiation from variety of sources, including the sun, is known to cause cancer. To ensure the public safety, the government has set standards for many substances, including benzene, asbestos, hydrocarbons in the air, arsenic in drinking water, and radiation. Collectively, these are known as risk factors. Brief risk factors are summarized in **Table 1.1**

Table 1.1 Risk factors for the development of cancer.

Sr. No.	Risk Factor	Mechanism of Action	Example
1	Chemicals	<ul style="list-style-type: none"> • Damage DNA, leading to mutations 	<ul style="list-style-type: none"> • Encountered in the manufacture of dyes, chemicals, petroleum products • Associated with tobacco usage, general environmental pollution, diet, some

			medicines
2	Radiation	<ul style="list-style-type: none"> • Damage DNA, leading to mutations 	<ul style="list-style-type: none"> • UV, ionizing radiation, radioactive elements
3	Viruses	<ul style="list-style-type: none"> • Not thought to induce Cancer • Participate in early stages leading to cancer 	<ul style="list-style-type: none"> • Epstein-Barr virus, human papilloma Virus, hepatitis B virus, HIV
4	Heredity	<ul style="list-style-type: none"> • Transmission of a single gene increases likelihood of cancer 	<ul style="list-style-type: none"> • Inherited cancers are commonly diseases of childhood: retinoblastoma, Wilm's tumor • Adult diseases: colon and breast carcinoma
		<ul style="list-style-type: none"> • Rare hereditary diseases increase likelihood of cancer 	<ul style="list-style-type: none"> • Xenoderma Pigmentosum, Ataxia telangiectasia

1.1 Cancer Prevention

The best way to reduce deaths from cancer is to prevent it. Medical doctors generally agree that about one-third of all human cancers are directly related to cigarette smoking.¹ For smokers, the risk of cancer is much higher than that of the nonsmokers. Excluding the UV rays of sunlight which cause skin cancer, the next most common cited cancer-causing factor is diet. The National Cancer Institute and the American Cancer Society recommend a diet low in fat, high in natural fiber, and rich in fruits and vegetables. On the other hand Chemoprevention is simply prevention with drugs. The word “drugs” is used to include dietary supplements, hormones, and vitamins etc., as well as real drugs such as aspirin and other synthetic agents used for therapeutic purposes. The number of chemopreventive agents is increasing.¹⁴

1.2 Cancer Treatments

There are three main strategies for cancer treatment: surgery, radiation and chemotherapy, which are used to attempt the damage cancer cell. The role of each depends upon types of tumor and the stage of its development.

1.2.1 Surgery¹⁵

Surgery is the oldest and still the most common local treatment for cancer. Surgery is a procedure to remove or repair a part of the body. Surgery also can help to decrease the tumor bulk and, along with other treatment measures, may provide a cure for certain cancers. However, surgery is not always the best answer. It generally works best on slow-growing cancers.

1.2.2 Radiation therapy¹⁶

Similar to surgical intervention, radiation therapy is a localized treatment. Radiation therapy is a use of high-energy radiation from x-rays, gamma rays, neutrons, protons, and other sources to treat cancer cells. Radiation may come from a machine outside the body (external-beam radiation therapy), or it may come from radioactive material placed in the body near cancer cells (internal radiation therapy). Systemic radiation therapy uses a radioactive substance, such as a radiolabeled monoclonal antibody, that travels in the blood to tissues throughout the body. Radiation therapy is used at some point in the treatment of more than half of all cancer cases.

1.2.3 Chemotherapy¹⁷

Chemotherapy is the use of cancer-fighting medications to stop the growth of malignant cells. During Second World War, naval personnel who were exposed to mustard gas as a result of a military action were found to have toxic effects on the bone marrow cells that developed into blood cells. In the course of that observation, compound called *nitrogen mustard* was studied and found to work against a cancer of the lymph nodes called lymphoma. The development and use of chemotherapy drugs (chemo) have resulted in the successful treatment of many people with cancer. It works by either killing the cells or preventing them from dividing. Many of cancers can be controlled with chemotherapy for a long period of time, even if they are not cured.

1.2.4 Other therapies

Targeted therapy is a type of medication that blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth, rather than by simply interfering with rapidly dividing cells.¹⁸

Immunotherapy is the use of the immune system to reject cancer. The main premise is stimulating the patient's immune system to attack the malignant tumor cells that are responsible for the disease. This can be either through immunization of patient (e.g., by administering a cancer vaccine) or through the administration of therapeutic antibodies as drugs.¹⁹

Hormone therapy inhibited the growth of some cancers by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers.²⁰

1.3 Type of different cytotoxic agents

Agents for cancer chemotherapy are often organized into groups according to their origin or mechanism of action. The six major classes of agents include the alkylating agents, antimetabolites and nucleoside analogs, antitumor antibiotics, antimetabolic, hormonal, and miscellaneous agents. The mechanism of action, drug group, and relevant examples of each class are shown in **Table 1.2**

Table 1.2 Classification of cytotoxic drug, including their mechanism of action, drug group, and example drug.²¹

Sr. No.	Class of Anticancer	Mechanism of Action	Drug Group	Example Drug
1	Alkylating Agents	React with DNA	Aliphatic Nitrogen Mustard	Mechlorethamine
			Aromatic Nitrogen Mustard	Melphalan, Chlorambucil
			Phosphoramidate	Cyclophosphamide, Ifosfamide

			Nitrosourea	Carmustine, Lomustine
2	Antimetabolites & Nucleoside Analogues	Interact with cellular Enzymes	Pyrimidine Antimetabolites	5-Fluorouracil, Cytarabine, Gemcitabine
			Purine Antimetabolites	6-Mercaptopurine, 6-Thioguanine
3	Antitumor Antibiotics	Intercalate DNA, formation of free radicals	<i>Streptomyces</i>	Doxorubicin, Mitomycin C, E0-9, Bleomycin
4	Antimitotic	Interfere with microtubule function	Vinca Alkaloids	Vincristine, Vinblastine, Vinorelbine
			Taxanes	Paclitaxel, Docetaxel
5	Hormonal	Bind to receptors	Antiestrogens	Tamoxifen, Estramustine
6	Miscellaneous	Various interactions with cellular DNA, inhibiting replication, transcription	Platinum Complex	Cisplatin, Carboplatin, Tetraplatin
			Enzymes	L-asparaginase
			Other class	Hydroxyurea, Mitotane, Camptothecins, Topotecan

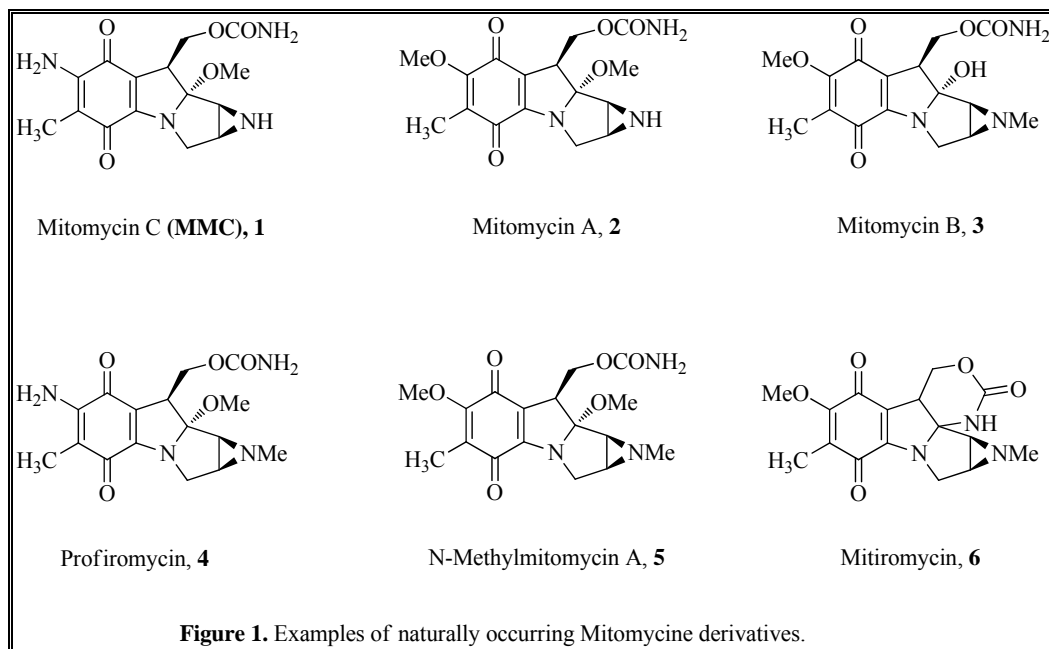
1.4 Alkylating Agents

Alkylating agents are the oldest class of anticancer drugs which are still commonly used; they play a vital role in the treatment of varieties of cancers.²²⁻²⁴ Alkylating agents that induce permanent DNA damage are often exhibits potent antitumor activity.²⁵ A range of DNA alkylating agents is known including monofunctional alkylating (reacts only one DNA strand to form genotoxic monoadducts) and bifunctional alkylating drugs. The latter were found to intrastrand cross-links, interstrand crosslinks, inter-helix or DNA-protein cross-links (ICLs) on DNA, which resulted in more potent and efficacious agents.²⁶ Currently, a variety of bifunctional alkylating agents are widely used for the treatment of patients with malignant diseases.

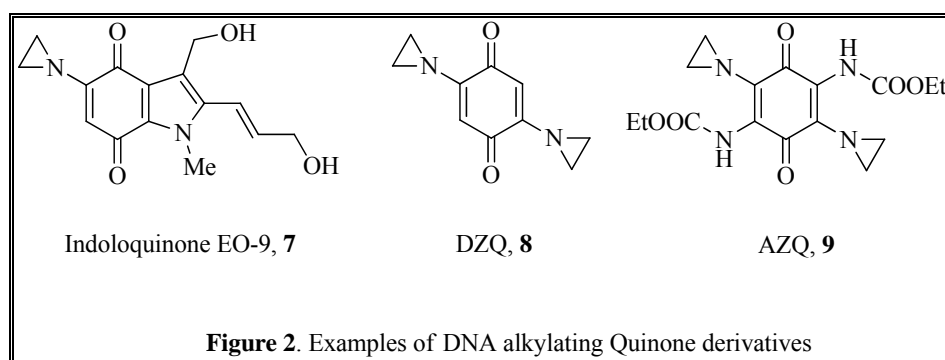
1.5 Naturally Occurring DNA Bifunctional Alkylating agents

1.5.1 Mitomycine C and its analogues

The existence of bioactive compounds in plants and other natural sources has played an important role in the development of new drugs.^{27,28} For e.g., In the 1950s, the Mitomycines were identified as a new and potent class of antibiotics agents.^{29,30} The mitomycines displays a wide array of substitution patterns inherent to core structure of pyrrolo[1,2-*a*]indole.³¹⁻³³ (Figure 1) Originally isolated from *Streptomyces caespitosus* in Japan, one of the members of the family, Mitomycine C (MMC, **1**) has been use for antitumor antibiotic activity.³⁴⁻³⁹ They have obtained a prominent place in cancer chemotherapy as a DNA bifunctional alkylating agents and used to treat a verity of tumors.⁴⁰⁻⁴⁴

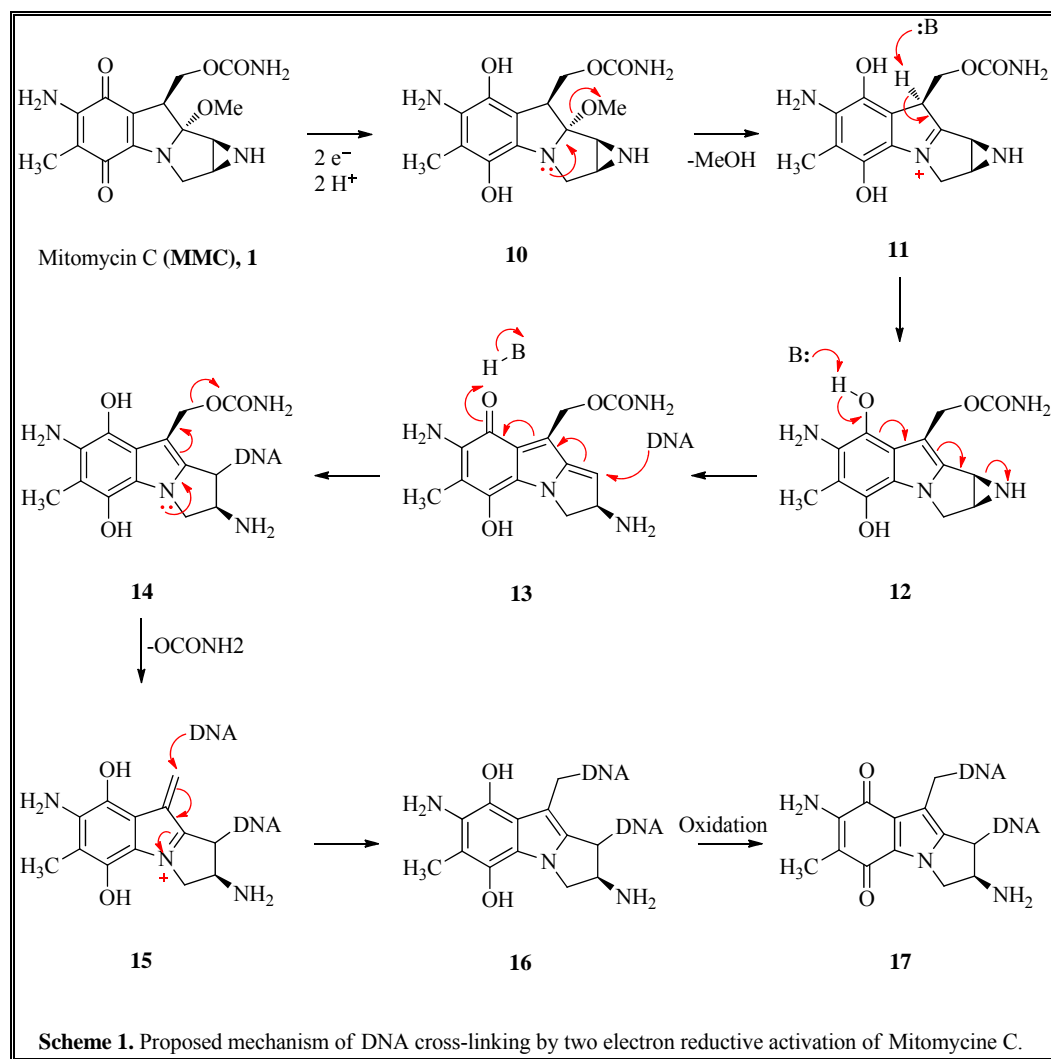


The synthetic analogue of the antitumor antibiotic Mitomycin C, EO-9 (**7**) is a bioreductive alkylating agent indoloquinone which serves as a target for nucleophilic DNA and capable to cross-linked with DNA.⁴⁵ The aziridinylquinone is a derivative of banzoquinone, represented perhaps the simplest of the mitomycin C like prodrugs.⁴⁶ Most studied aziridinylquinone derivative are the simple unsubstituted diaziridinylquinone (DZQ, **8**) and the more clinically promising quinone derivative is AZQ (**9**), active against human brain tumors.⁴⁷



Mitomycin C itself inactive,⁴⁸ which is activated by chemical ($\text{Na}_2\text{S}_2\text{O}_4$,⁴⁹ NaBH_4 ,⁴⁹ H_2 ,^{49,50} $\text{NO}/\text{HCO}_2\text{Na}/h\nu$ ⁵¹), electrochemical^{52,53} or enzymatic (old yellow enzyme,⁵⁴ NADPH-cytochrome *c* reductase/NADPH,^{55,56} xanthine oxidase/NADH,^{55,56} DT-diaphorase^{55,57}) reduction of the quinone moiety to

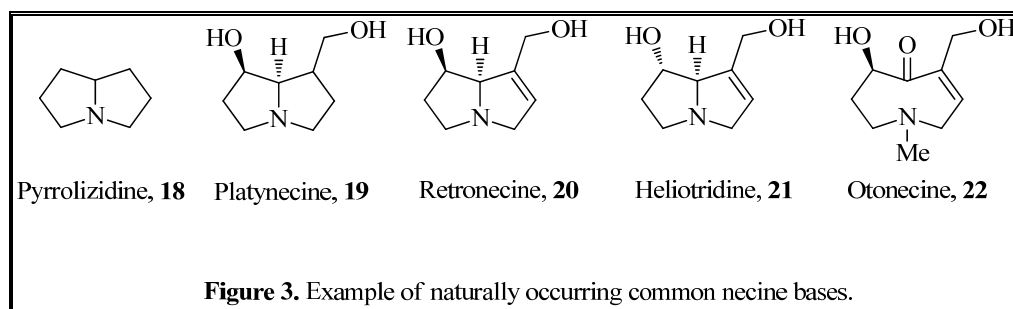
semiquinone or hydroquinone to initiate alkylation. Iyer and Szybalski⁴⁹ proposed a mechanism for the activation and alkylation reactions, their mechanism was purely based on structural considerations and chemical precedent shown in Scheme 1. While its structure exhibits similarities to other quinone natural products, Initial One or two-electron reduction of the quinone ring **1** to either the semiquinone or the hydroquinone (**10**).⁵⁸⁻⁶⁰ Generation of hydroquinone facilitates the loss of the methoxy group, leading to the formation of the hydroquinone intermediate (**11**). Tautomerization followed by the reaction with the N₂-amino group of guanine produces monoadduct (**14**). Elimination of the carbamoyl group produces the highly reactive vinylogous hydroquinone methide intermediate (**15**), which alkylates the guanine on the opposite strand of DNA to produce (**16**), after oxidation, an interstrand cross-link (**17**).



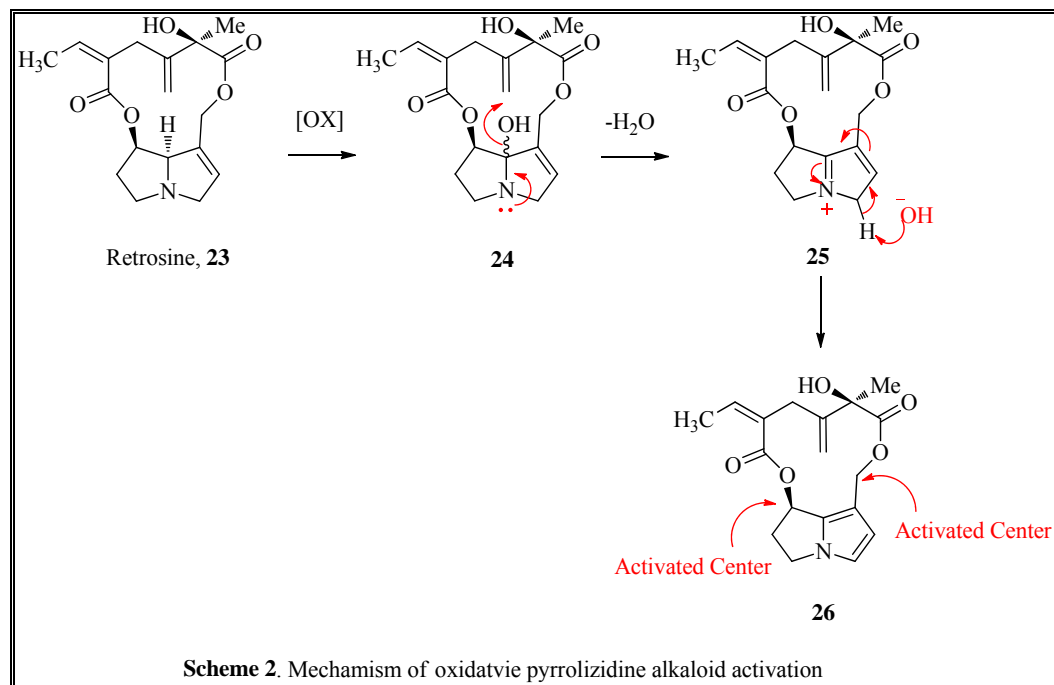
1.5.2 Pyrrolizidine alkaloids

Other natural products, Pyrrolizidine alkaloids (PAs) are constituents of over 6000 species of plants, most notably Boraginaceae, Graminae, Leguminosae and Compositae, insects including various species of butterflies and even animals such as amphibians. They elicit hepatotoxic, carcinogenic, antineoplastic and genotoxic activity, primarily via DNA cross-linking.⁶¹ Their ubiquitous presence, as well as their interesting biological activity is through the formation of DNA-protein cross-links.⁶²

The common structural feature of all these alkaloids is the presence of a functionalized pyrrolizidine moiety – the necine base.⁶¹ Although a large variety of necine bases have been isolated (Figure 3), most of the alkaloids contain retronecine (**20**) as their pyrrolizidine portion.

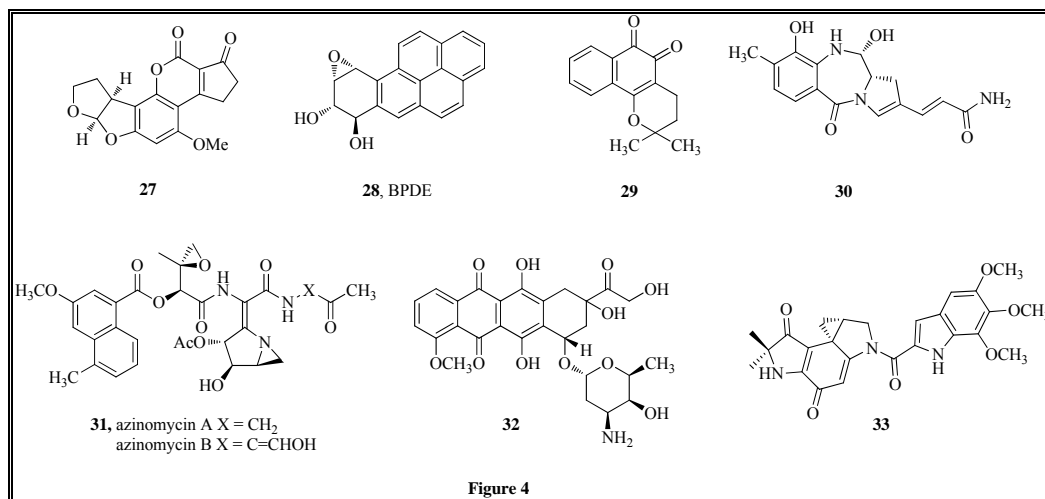


The mechanism of DNA cross-linking by pyrrolizidine alkaloids (e.g. retrorsine, **23**) has been extensively investigated. Inhibition of DNA synthesis in pyrrolizidine alkaloids treated liver-slices, corroborated with the lack of a similar effect in lung tissue cultures.⁶³ It is well established mechanism of action involving oxidative activation of the pyrrolizidine moiety by chemical oxidation in vitro or hepatic oxidation in vivo.⁶⁴ Further studies demonstrated that the retronecine portion of (**23**) is oxidized by liver cytochrome P450 to a highly reactive pyrrole (**26**)⁶⁵ (Scheme 2).



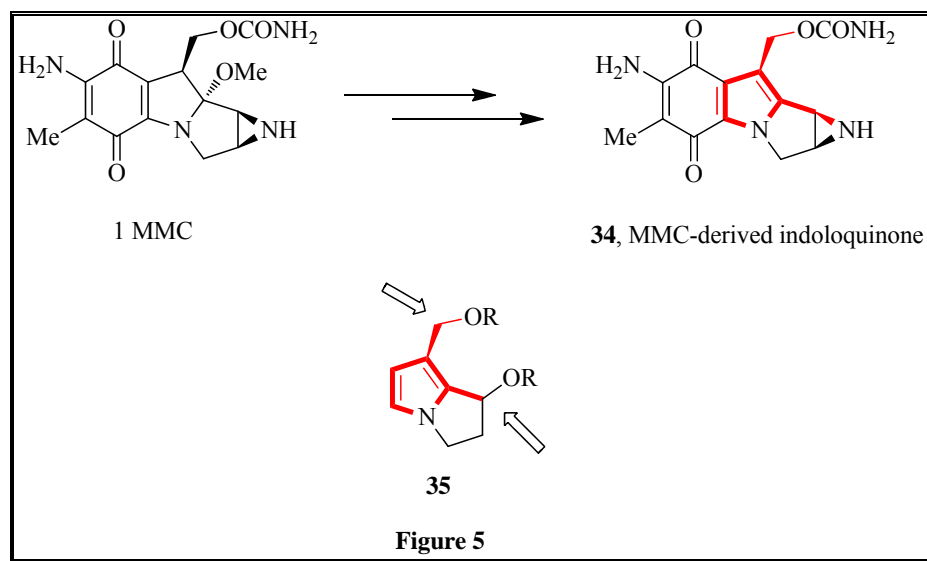
Conjugation with the pyrrole nitrogen lone pair makes the C-7 and C-9 positions of (**26**) highly reactive toward nucleophilic attack. Thus, such species is extremely reactive was reported by Niwa *et al.*⁶⁵

Along with mitomycin C (**1**) and pyrrolizidine alkaloids (**23**), several other naturally occurring or synthetic antitumor agents contains at least one, often two or three, reactive electrophilic centers in the molecule through which they exert their anticancer activity. For examples (Figure 4), aflatoxin B1(**27**),⁶⁶ 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) (**28**),⁶⁷ naphthoquinone β -lapachone (**29**),⁶⁸ anthramycin (**30**),⁶⁹ azinomycin B (**31**),⁷⁰ anthracycline derivatives (i.e. doxorubicin, **32**),⁷¹ cyclopropylpyrroloindoles (i.e. duocarmycin, **33**).⁷²



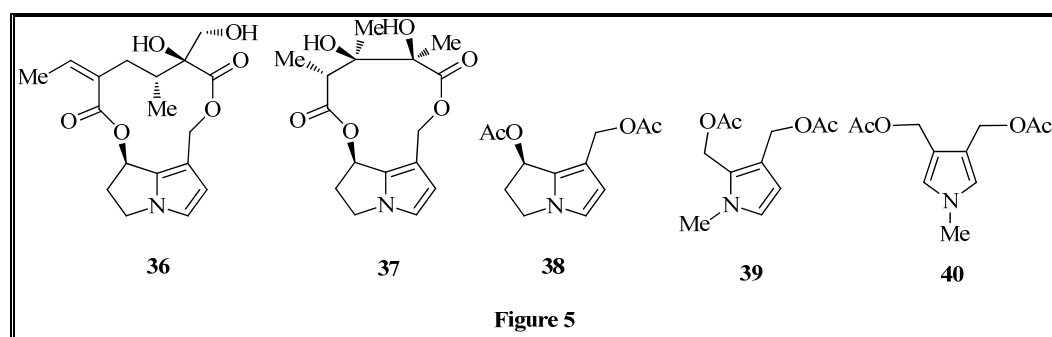
Studies involving the structure-activity relationship among pyrrolizidine alkaloids suggest that although the presence of the unsaturated pyrrolizidine base (retroecine) is the most important element for the cross-linking capacity of these compounds, there are other, more subtle structural features modulating both the potency and preference of various alkaloids for different types of DNA lesions. The patterns of proteins crosslinked to DNA are similar to those induced by standard bifunctional alkylating agents such as mitomycin C.⁷³

Hopkins *et al.* have demonstrated that pyrrolizidine alkaloids metabolites target the same 5'GpC3' DNA sequence as antitumor antibiotic mitomycin C (**1**) albeit with less selectivity.⁷⁴ These findings can be explained by the structural similarities between the alkylating moiety of active mitomycin metabolite **34** and activated pyrrolizidine alkaloids the added sequence promiscuity. In the case of pyrrolizidine alkaloids suggest the lack of some molecular recognition constituents inherent to the mitomycins (Figure 5).

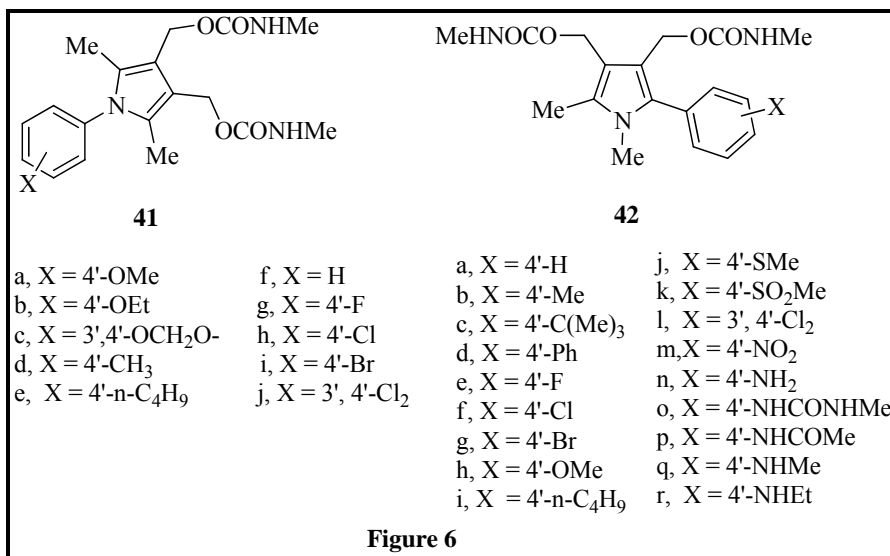


1.6 Synthetic DNA Bifunctional Alkylating agents

Structure-activity relationship studies with many of these natural products are often limited by the small quantities of material available and by the relatively limited number of modifications which actually can be performed on these complex molecules. On the other hand, to synthesize these simple molecules for SAR study; one can use biologically active natural products as the base template.⁷⁵⁻⁷⁷ Based on structural characteristics of the mitomycin C and pyrrolizidine alkaloids, Hopkins et al. have demonstrated that dehydroretorsine (**36**), and dehydromonocrotaline (**37**), dehydroretronecine diacetate (**38**), 2,3-bis(acetoxymethyl)-1-methylpyrrole (**39**), and 3,4-bis(acetoxymethyl)-1-methylpyrrole (**40**) also cross-linked with DNA in the same manner like MMC targets, with a high degree of selectivity for 5-CpG3.^{78,79}

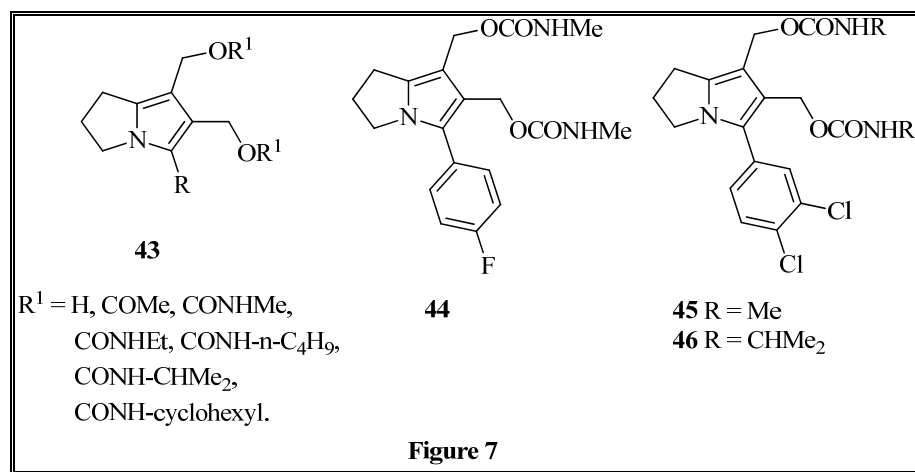


In additionally, Anderson *et al.*^{80,81} have designed and synthesized several “acylated vinylogous carbinolamine” tumor inhibitors including (Figure 6); 1-Phenyl-2,5-dimethyl-3,4-bis(hydroxymethyl)-pyrrole-bis(N-methylcarbamate) (**41a-j**)⁸⁰ and 1,2-dimethyl-3,4-bis(hydroxymethyl)-5-phenylpyrrol bis(N-methylcarbamate) (**42a-r**)⁸¹ also demonstrated to have DNA interstrand cross-linking activity as same as MMCs.



The rationale design of these agents is based on the concept that these agents can act as bifunctional electrophiles in which [(carbamoyl)oxyl]methyl groups serve as reactive electrophilic centers. The SAR studies of these agents against P338 lymphocytic leukemia revealed that the compounds having 1-substituted derivatives **41**, compounds possessed significant reproducible activity against the P-388 lymphocytic leukemia (PS) assay with the lowest dose of 12.5 mg/kg. Activity at this dose ranged from %T/C = 190-132 for compounds **41e** and **41c**, respectively. In the series of C-2 substituted derivatives **42** the electronic and lipophilic properties of the substituent X can be varied rather extensively without loss of significant antileukemic activity.

In the course of exploring the structure-biological activity relationships of pyrrole-derived bifunctional alkylating agents, a series of 6,7-disubstituted pyrrolizine diesters have been synthesized and evaluated for antitumor studies.⁸²

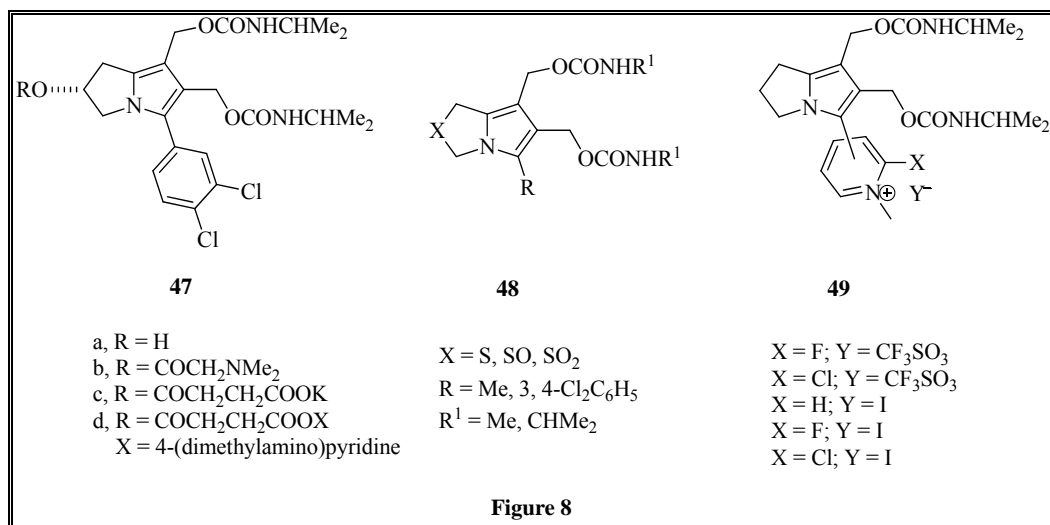


The potential electrophilic reactivity of the allylic esters in **43** (via *O*-alkyl cleavage) will be enhanced by participation of the ring nitrogen similar to that of MMC and PAs. All of these compounds exhibited significant antileukemic activity against P-388 in animal model. Of these derivatives, bis(methylcarbamate) derivative **44** showed potent activity at lowest dose tested, 0.78 mg/kg and **45**, afforded “cures” at dose levels as low as 12.5 mg/kg. Several other compounds showed high activity against P-388 over a four-fold dose range without appeared acute toxicity. The most potent compound of this class was 5-(3,4-dichlorophenyl)-2,3-dihydro-1H-pyrrolizidine-6-7-bis(isopropylcarbamate) (**46**),⁸³ which had significant reproducible antitumor activity against a broad range of experimental murine neoplasias and humor tumor xenografts in nude mice. This compound was selected for more extensive preclinical studies,⁸⁴ but there was no further information has been published whether this agent was in clinical trials.

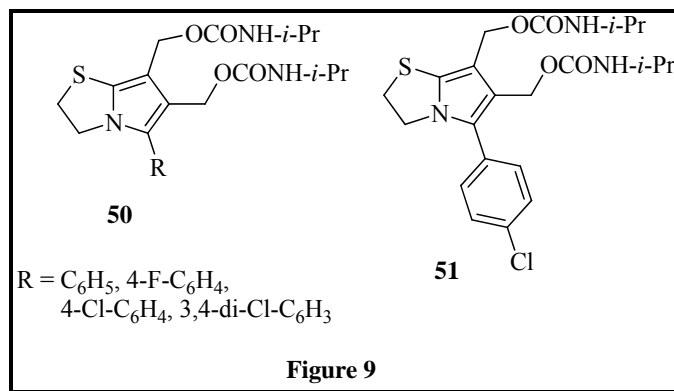
Studies on the structure-activity relationships of derivatives of 5-phenylpyrrolizines **43** revealed that the nature of R and R¹ substituent significantly affected both chemical reactivity and biological activity of the system. The chemical reactivity of **43** toward nucleophiles was found that compounds having electron-donating substituent(s) (OMe) on the phenyl ring were generally less cytotoxic than those compounds bearing electron-withdrawing substituent(s) (halogen). While, the in vivo antitumor activities were comparable or slightly less potent in compounds having an electron-donating substituent. These studies also suggested that the lipophilicity of compound might affect its antitumor potency. The reactivity of this system depended upon the π -electron density in the pyrrole ring and the ability of the C-5 substituent

(R) to stabilize the formation of the positive charge on the heterocyclic nitrogen atom during the displacement of the ester moieties (via O-alkyl cleavage). In general, the electrophilic reactivity can be controlled by the electronic influence of the C-5 substituent. The size of the R1 substituent does not appear to have any significant effect upon potency, activity, or toxicity.

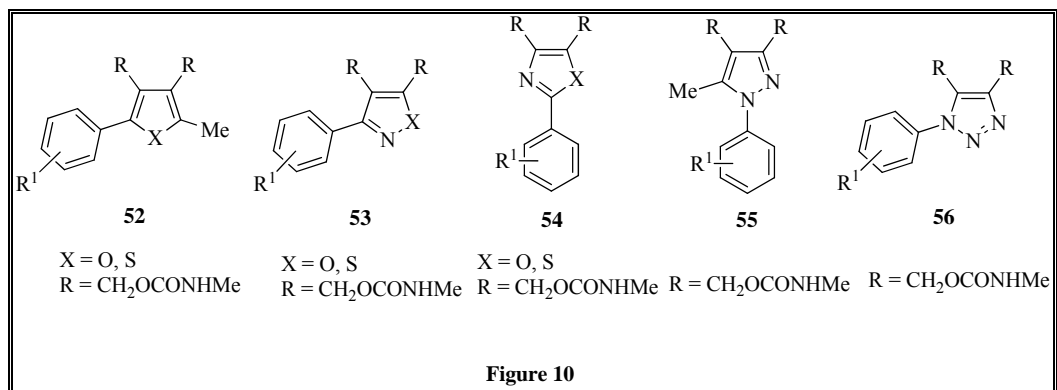
The water soluble analogues of (**46**, IPP) were also prepared for the oral availability, Anderson & co-workers have designed and synthesized several series of compounds to study their antitumor effect against P388 lymphocytic leukemia and B16 melanocarcinoma in mice.⁸⁵ Among these agents, alcohol derivative **47a** (where R = H) showed to have comparable activity to **46** with a dose of 50 mg/kg, while **47b** (where R = COCH₂N(CH₃)₂) showed less active and **47d** with very low activity. In order to prepared more lipophilic compounds, the same authors have synthesized hetero analogues of **43** with general formula **48**⁸⁶ in which the C-2 methylene was replaced by S, SO or SO₂. However, these agents were found to be less cytotoxic as compare to (**46**, IPP). The water soluble derivatives **49** were also synthesized by using prodrug approach in which an α -halopyridinium moiety as the pyrrolizines C-5 substituent.⁸⁷ The 4- and 5-pyrroliziny-2-halopyridinium iodides and the corresponding pyridones were evaluated against P388 lymphocytic leukemia in vivo. The compounds having small size (α -fluoropyridinium) were active but the α -chloro compounds were not. Compounds were active in the P388 screen, were evaluated in L1210 leukemia, M5076 carcinoma, and MX-1 mammary xenograft assays in mice.



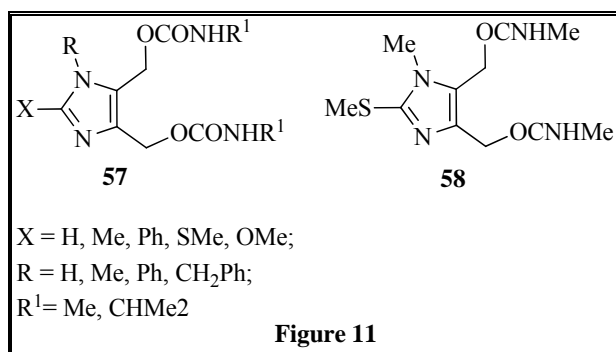
From this observation, Lalezari *et al.* have prepared 1-Thia analogues of **46** (IPP).⁸⁸ Of these derivatives compound having Ph, F and di-Cl were equipotent (IC_{50} = 1.5, 1.6, and 1.9 μ M respective) against the HL-60 (human leukemia) cells as compare to **46** (IC_{50} = 1.5 μ M) while 4-Cl (**51**) derivative showed an increase potency of 75% (IC_{50} = 0.85 μ M). All derivatives were also cytotoxic against HT-29 (human colon carcinoma) cells as similar inhibition of **46**.



Further Anderson and group have also synthesized a different of heterocyclic analogs (**52-56**)⁸⁹ of pyrroles (**41** and **42**) to examine differ from one and other in several aspects-aromaticity, pKa, lipophilicity, dipole moments, charge-transfer donor/acceptor. All prepared derivatives were tested for antileukemic activity against murine P-338 lymphocytic leukemia. None of the compounds was active when compared to **41** and **42**. It was shown that electrophilic reactivity is one requirement for antineoplastic activity in these bis(carbamates). It was also evident that the bis(carbamates) are not functioning as carbamoylating agents since so many of these nonpyrrole bis(carbamates) derivative were inactive.

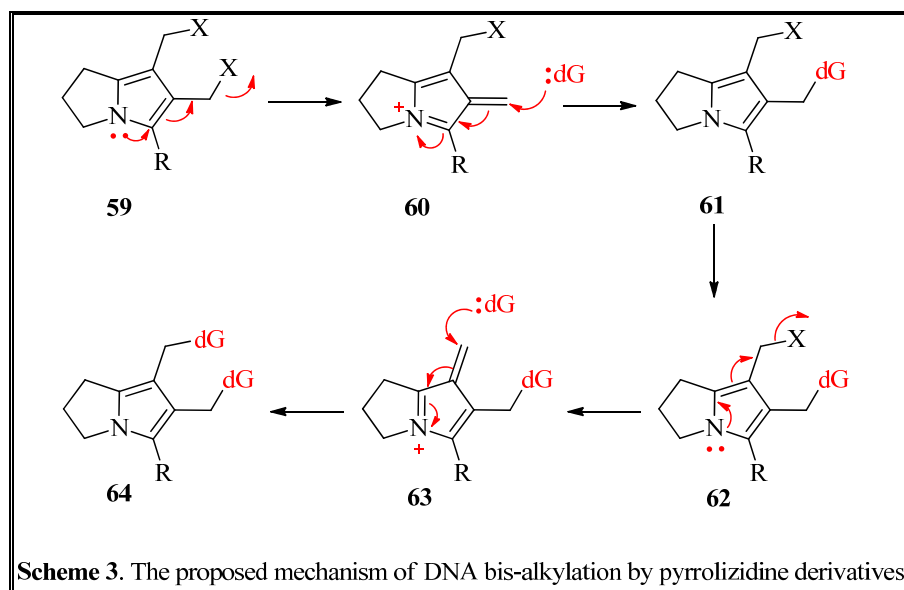


The rationale used for the design of **46** (IPP) Anderson *et al.* have extended to imidazole nucleus to prepare water soluble derivatives.⁹⁰ Appropriate electron donating or electron withdrawing substituents were added to the imidazole ring to modulate the reactivity of the two electrophilic centers. The potent advantage of the imidazole is that it is sufficiently basic to allow for the preparation of salts to enhance water solubility. All synthesized imidazole derivatives were tested against murine P-388 lymphocytic leukemia. The structure activity relationship studies of this series of compounds were emerged that the electron-withdrawing substituents at either N-1 or C-2 gave rise to inactive compounds. However, the electron-donating substituents gave active compounds and the 2-(methylthio)-1-methyl derivatives **58** (carmethizole) was found to be most potent. The derivative **58**, were also found to be active against the MX-1 mammary xenograft, the human amelanotic melanoma cell line LOX xenograft, the M-5076 sarcoma, and L-1210 lymphocytic leukemia. Finally, **58** was reached for clinical evaluation.⁹¹⁻⁹³



Studies on the mechanism of action showed that the bis(carbamate)pyrroles (**41** or **42**) or pyrrolizines derivatives (**43-51**) exert their antitumor effect was

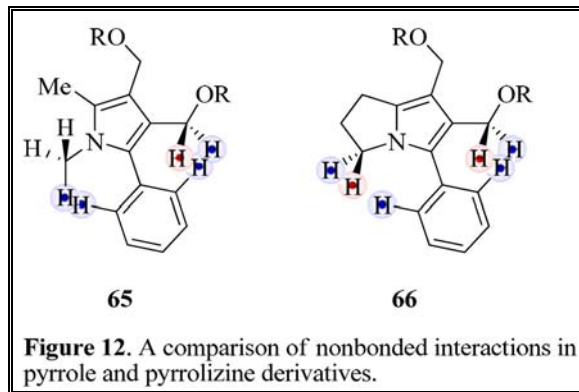
probably via a S_N1 electrophilic reaction (Scheme 3).^{79,82} These derivatives are capable of forming DNA interstrand cross-link with the short oligonucleotide 5'-ACGT at the 5'-CG residues at the minor groove region.⁷⁹



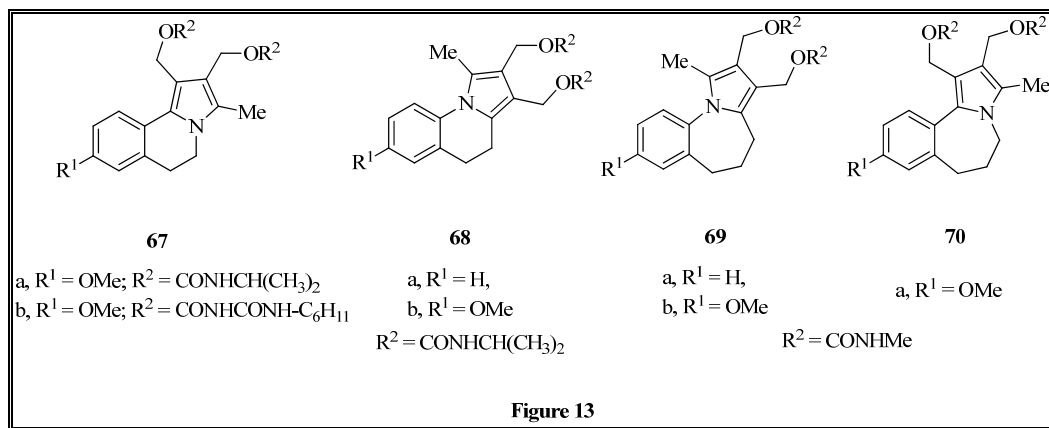
These agents are not carbamoylating agents; instead the carbamate moieties are leaving groups in an alkyl-oxygen cleavage mechanism (**60**). The reactions take place on methylenic carbons bonded directly to a heteroaromatic pyrrole nucleus (**61**). The role of the heteroaromatic system is to stabilize reaction transition states, and this provides a means to control the reactivity of the two electrophilic centers. Control may be achieved through the alteration of the heteroaromatic system.

The degree of conjugation observed in any biaryl system is related to the capability of the system to adopt a coplanar conformation. Studies on comparison of nonbonding interactions in C-5-phenyl pyrroles (**42a-r**) and C-5-phenyl pyrrolizines (**43-51**) revealed that two significant nonbonded interactions exist in the 5-phenylpyrrole derivatives (**65**, Figure 12) between the ortho hydrogens of the phenyl substituent and the hydrogens on the N-1 methyl group and the C-4 methylene.⁹⁴ While, one of these nonbonded interactions is absent in the pyrrolizine compounds (**66**, Figure 12): the pyrrolizine C-3 methylene hydrogens lie outside the van der

Waals radii of the ortho-hydrogen atoms of the C-5 phenyl ring and demonstrated that the phenyl and pyrrole rings in **43** are coplanar (or very nearly so).⁹⁴



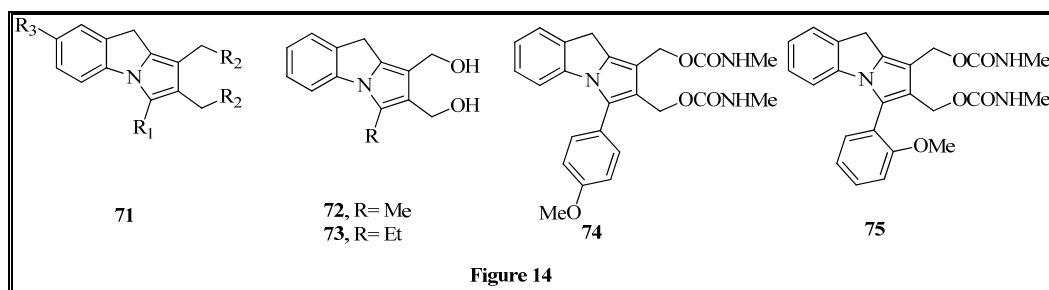
On the basis of this observation, several new set of analogues that possess angular tricyclic structures to limit the deviation from coplanarity of the phenyl and pyrrole rings. Specifically, tricyclic derivative of pyrrolo[2,1-*a*]isoquinolines was first designed and evaluated for antitumor activity against P388 lymphocytic leukemia.⁹⁴ It was demonstrated that the pyrrolo[2,1-*a*]isoquinolin-bis(isopropylcarbamate) **67a** exhibited the best activity of the compounds tested. The carbamate derivative **67a** were also found broad spectrum of activity against the various human tumors xenograft (MX-1 breast, CX-1 colon and LX-1 lung) and murine tumors (B16 melanocarcinoma, L1210 lymphoid leukemia, CD8F₁ mammary and lewis lung carcinoma colon 38). However, the tricyclic compounds revealed to be more potent than pyrrole and pyrrolizine derivatives.



On the potency of **67**, several other angular tricyclic pyrrolo[1,2-*a*]quinolines (**68a-b**), pyrrolo[1,2-*a*]benzazepines (**69a-b**) and pyrrolo[2,1-*a*]isobenzazepines (**70**) were also synthesized and evaluated for antileukemic activity (Figure 13). The bis(carbamates) (**68-70**) were tested in vivo against P388 lymphocytic leukemia. In the pyrrolo[2,1-*a*]isoquinoline series (**67a-b**), the C-3 methyl group had more pronounced effect on activity and toxicity than the C-7 methoxy group where as pyrrolo[1,2-*a*]quinolines were less active.^{94,95} The pyrrolo[1,2-*a*]benzazepine-bis(carbamates) **69a** and **69b** showed approximately equivalent activity to the comparable pyrrolo[1,2-*a*]quinolines. The fused benzazepines **70** were less potent and more toxic than the corresponding pyrrolo[2,1-*a*]isoquinoline (**67a**).⁹⁵ It was concluded that ring fusion and ring substitution do alter the potency, activity and toxicity of the bis(carbamates) derivatives. It was also indicated that compounds with the phenyl ring attached directly to the pyrrole nitrogen will have different structure-activity requirements from the compounds in which the phenyl ring is attached to the pyrrole α -carbon.

Recently, Su *et al.* have designed and synthesized a series of DNA bifunctional alkylating agents (Figure 14), bis(hydroxymethyl)-8*H*-3a-azacyclopenta[*a*]indene-1-yl derivatives and their bis(methylcarbamate) derivatives (**71**).⁹⁶ These agents can be considered as “benzologues” of bis(hydroxymethyl)pyrrolizines and were able to cross-link to DNA double strands. It was demonstrated that these analogues exhibited potent antitumor activity against human lymphoblastic leukemia and various solid tumor cell growths in vitro and potent antitumor efficacy in vivo with a relatively low toxicity. Detailed SAR studies

were demonstrated that the size and electron properties of the substituent at C-3 affected the cytotoxicity of these agents. Remarkably, complete tumor remission (CR) in nude mice bearing human breast carcinoma MX-1 xenograft by bis(hydroxymethyl) derivatives (**72** and **73**, Figure 14) and bis(methylcarbamate) derivatives (**74** and **75**) were achieved. Interestingly, compound **73** was able to significantly suppress against prostate adenocarcinoma PC3 xenograft in nude mice. Studies on the DNA interstrand cross-linking suggested that these derivatives were potent bifunctional DNA cross-linking agents. Furthermore, 3a-aza-cyclopenta[*a*]indene derivatives were able to induce substantial G2/M phase arrest of the cell cycle.

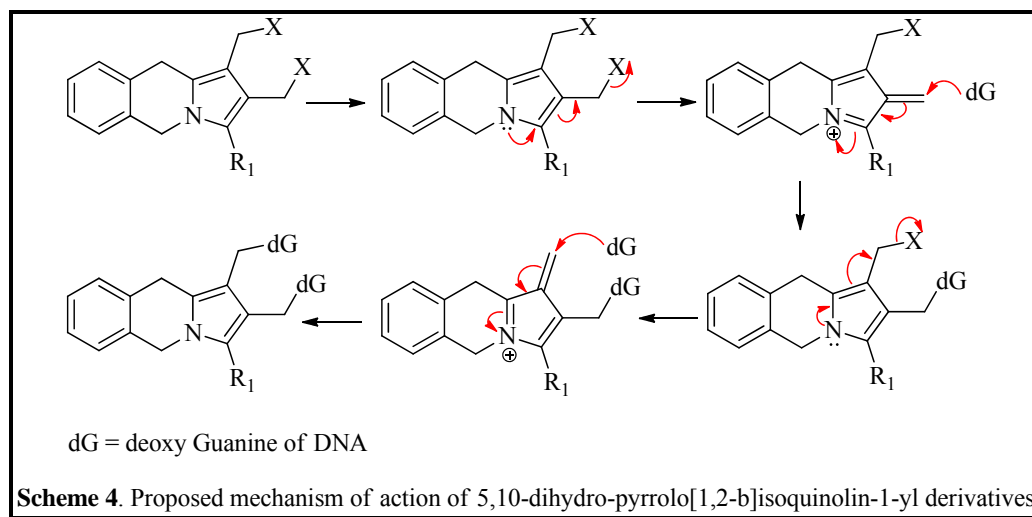
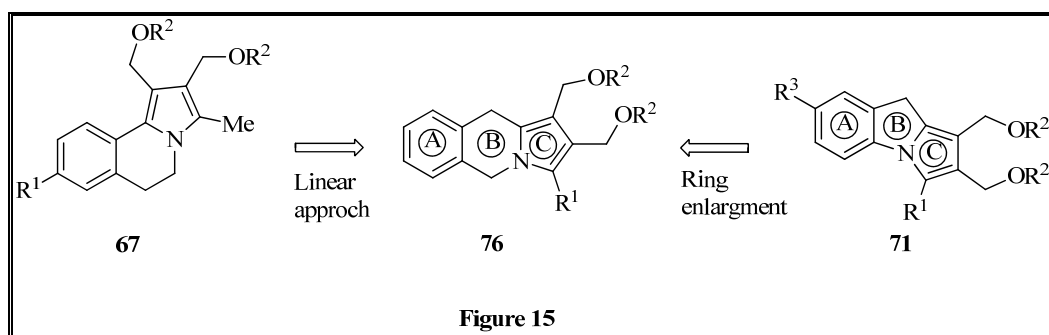


In additionally, they also demonstrated that the effect of combining alkylating agents **74** and arsenic trioxide (ATO, DNA repair inhibitor) significantly suppressed human large cell lung carcinoma H460 xenograft (>82%) and cisplatin-resistant NTUB1/P human bladder carcinoma xenografts (>92%) in nude mice.⁹⁷ From this observation, it was concluded that a combination of bifunctional alkylating agents and ATO may be a rational approach for treating cancers with inherited or acquired drug resistance. These exciting results provoked to continue designing and synthesizing new bis(hydroxymethyl)pyrrolizine analogues for antitumor studies.

1.7 Rational Drug Design of Bis(hydroxymethyl) and their Bis(alkylcarbamates) Derivatives.

As mentioned previously, pyrrolo[2,1-*a*]isoquinolines (**67**, Figure 13) bear a angular tricyclic ring system and it showed best activity of the other angular tricyclic (**68-70**, Figure 13). Moreover, tricyclic derivatives do appear to be most cytotoxic

than pyrrole and pyrrolizine derivatives. Based on the potent antitumor activities and mechanism of action of MMC (**1**) and pyrrolo[2,1-*a*]isoquinolines (**67**), to investigate whether analogues of **67** with a linear tricyclic ring system also possess potent antitumor activity. In medicinal chemistry term, the molecule differing one from another by only a methylene group is called homologues. By using ring enlargement approach in 3*a*-azacyclopenta[*a*]indene (**71**) derivatives to design and manipulation of the original B ring systems, it is great interest to synthesize a series of new linear bis(hydroxymethyl) of 5,10-dihydropyrrolo[1,2-*b*]isoquinolines and their bis(alkylcarbamates) (**76**) derivatives having structure in Figure 15. One can expect that the newly synthesized compounds might be able to cross-link to the macromolecular DNA via a similar mechanism of action as that of pyrrolizines derivatives. (Scheme 4)



The chemical synthesis of bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolines and their bis(alkylcarbamates) derivatives are described in the

Chapter 2. The antitumor activities, *in vitro*, *in vivo* evaluation and mechanism of action of these agents are described in the Chapter 3.

Section A

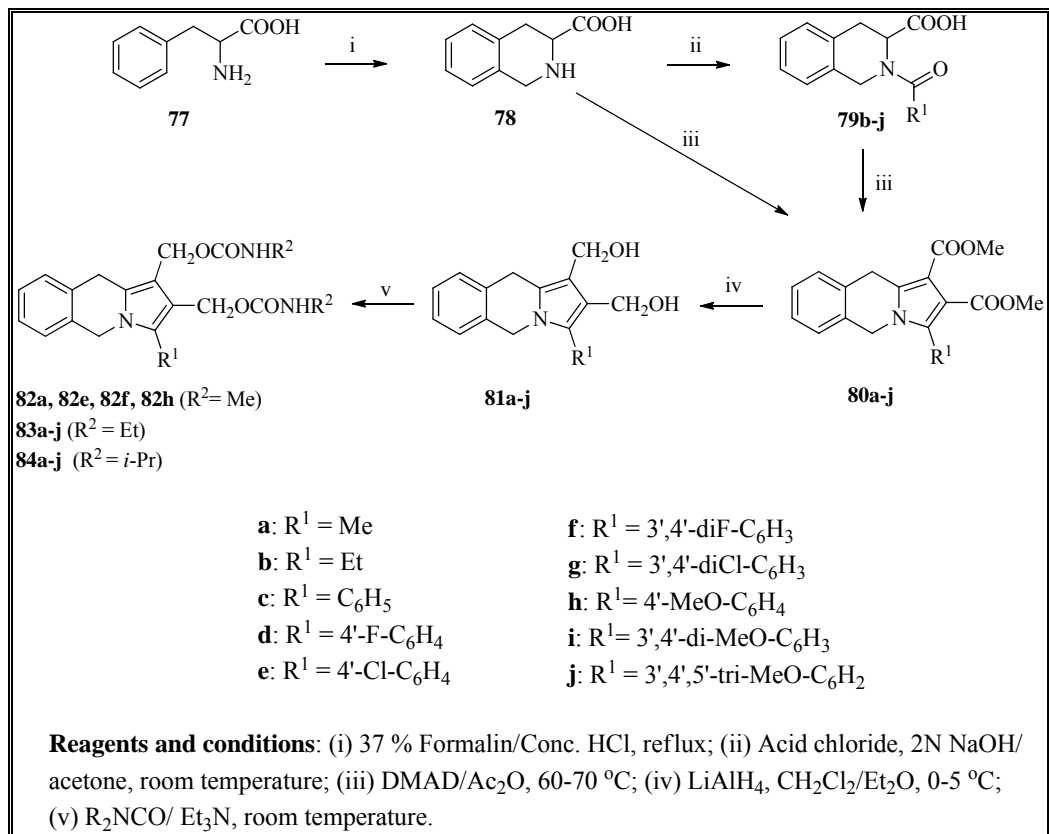
Chapter 2. Synthesis and Characterization of bis(hydroxymethyl)-5,10- dihydropyrrolo[1,2-*b*]isoquinolin- 1-yl derivatives and their bis(alkylcarbamate) derivatives

2.0 Chemistry

The synthetic route for bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives (**81**) and their bis(alkylcarbamate) derivatives (**82–84**) is shown in Scheme 1. The known 3-carboxy-1,2,3,4-tetrahydroisoquinoline (**78**) was synthesized by treating the commercially available D,L-phenylalanine (**77**) with formaldehyde and conc. HCl by following the procedure developed by Dean.⁹⁸ Compound **78** was N-acylated by treating with various acid chlorides ($R^1\text{COCl}$) in presence of 2N NaOH to give N-acyl-3-carboxy-1,2,3,4-tetrahydroisoquinolines (**79b-j**) by following the literature procedure.^{99,100} Compounds **79b-j** were then reacted with dimethyl acetylenedicarboxylate (DMAD) in acetic anhydride at 60–70 °C yielded 5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl esters (**80b-j**). The methyl ester derivative **80a** ($R^1 = \text{Me}$) was prepared in good yields directly from **78** by reacting with acetic anhydride and DMAD.¹⁰¹ The ester function of **80a-j** were reduced to bis(hydroxymethyl) derivatives **81a-j** by treating with LiAlH_4 in a mixture of ether/ CH_2Cl_2 at 0–5 °C. Compounds **81a-j** were then further treated with methyl-, ethyl- or *iso*-propylisocyanate in presence of triethylamine (TEA) to furnish the desired bis(alkylcarbamate)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1-yl derivatives (**82–84**) in good to high yields. The cycloaddition reaction mechanism of **79b-j** with DMAD is depicted in Scheme 2. The reaction of N-acyl-3-carboxy-1,2,3,4-tetrahydroisoquinolines (**79b-j**) with dimethyl acetylenedicarboxylate (DMAD) in presence of acetic anhydride provides mesoionic oxazolone intermediates **85b-j**. 1,3-dipolar addition¹⁰² of **85b-j** give **86b-j** which spontaneously eliminate CO_2 to give diester **80a-j**. Table 2.1 and 2.2 show the yields and the physical data of these compounds.

2.1 Reaction Scheme

2.1.1 Scheme 1 Synthetic routes for bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives (**81**) and their bis(alkylcarbamate) derivatives (**82–84**).



2.1.2 Scheme 2 Plausible reaction mechanism of 1,3-dipolar cycloaddition reaction of **80a-j**.

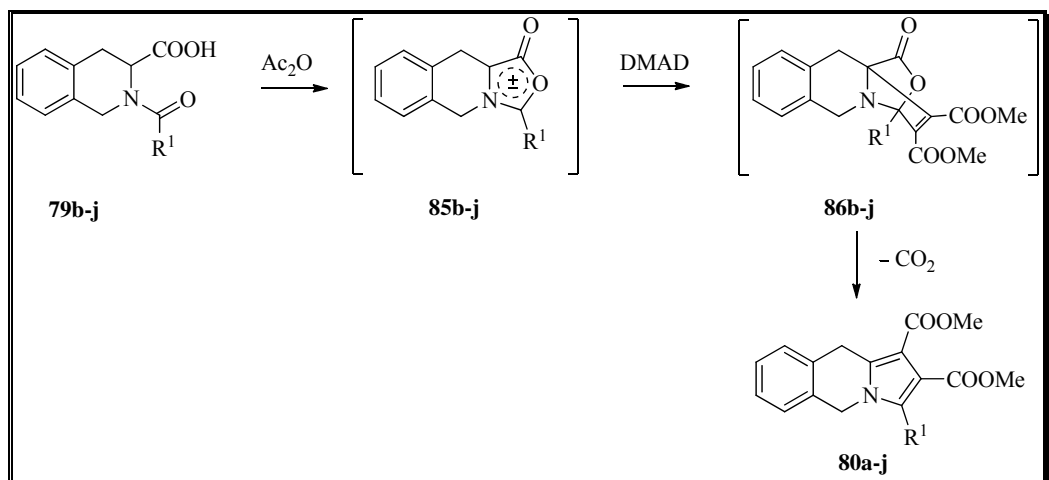
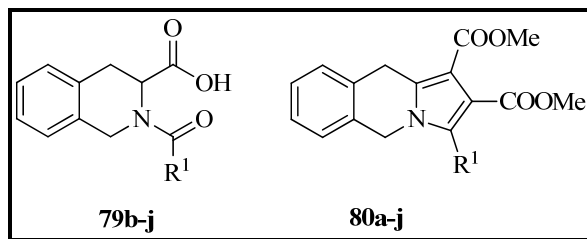
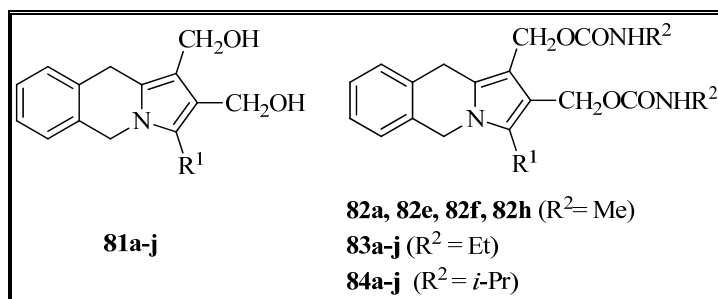


Table 2.1 Yields and physical data of the compounds **79b–j** and **80a–j**.

Compd.	BO No.	Substitute R ¹	Yield %	MP °C	Analysis
79b	1473	Et	87	173–174	CHN
79c	1471	C ₆ H ₅	78	168–169	CHN
79d	1477	4'-F-C ₆ H ₄	98	179–180	CHN
79e	1114	4'-Cl-C ₆ H ₄	93	77–79	CHN
79f	1110	3',4'-di-F-C ₆ H ₃	95	89–91	CHN
79g	1507	3',4'-di-Cl-C ₆ H ₃	90	101–102	CHN
79h	1103	4'-MeO-C ₆ H ₄	86	178–180	CHN
79i	1474	3',4'- di-MeO-C ₆ H ₃	62	235–236	CHN
79j	1486	3',4',5'-tri-MeO-C ₆ H ₂	98	186–189	CHN
80a^a	1105	Me	78	152–154	CHN
80b	1489	Et	93	88–89	CHN
80c	1472	C ₆ H ₅	68	137–138	CHN
80d	1479	4'-F-C ₆ H ₄	58	149–150	CHN
80e	1115	4'-Cl-C ₆ H ₄	64	164–166	CHN
80f	1111	3',4'-di-F-C ₆ H ₃	67	142–144	CHN
80g	1508	3',4'-di-Cl-C ₆ H ₃	64	169–170	CHN
80h	1104	4'-MeO-C ₆ H ₄	66	155–158	CHN
80i	1475	3',4'- di-MeO-C ₆ H ₃	81	202–203	CHN
80j	1487	3',4',5'-tri-MeO-C ₆ H ₂	51	165–166	CHN

^aknown compound

Table 2.2 Yields and physical data of the compounds **81a–j**, **82**, **83a–j** and **84a–j**.

Compd.	BO No.	Substitute R^1	Yield %	MP °C	HPLC %	Analysis
81a	1107	Me	65	99–101	96.94	CHN
81b	1524	Et	77	Liq.	97.09	CHN
81c	1476	C_6H_5	80	150–151	99.29	CHN
81d	1480	4'-F- C_6H_4	75	135–136	97.62	CHN
81e	1116	4'-Cl- C_6H_4	52	170–172	99.52	CHN
81f	1112	3',4'-di-F- C_6H_3	83	145–146	99.52	CHN
81g	1518	3',4'-di-Cl- C_6H_3	72	150–151	99.54	CHN
81h	1106	4'-MeO- C_6H_4	67	174–176	99.56	CHN
81i	1492	3',4'- di-MeO- C_6H_3	77	145–146	98.68	CHN
81j	1490	3',4',5'-tri-MeO- C_6H_2	58	146–147	97.62	CHN
82a	1108	Me	45	174–176	97.44	CHN
82e	1117	4'-Cl- C_6H_4	82	194–196	99.74	CHN
82f	1113	3',4'-di-F- C_6H_3	77	190–192	99.79	CHN
82h	1109	4'-MeO- C_6H_4	87	182–184	96.10	CHN
83a	1506	Me	53	208–209	99.34	CHN
83b	1525	Et	51	127–129	97.01	CHN
83c	1493	C_6H_5	81	170–171	97.29	CHN
83d	1491	4'-F- C_6H_4	76	183–184	97.92	CHN
83e	1520	4'-Cl- C_6H_4	65	191–192	99.05	CHN
83f	1517	3',4'-di-F- C_6H_3	71	189–190	98.75	CHN
83g	1519	3',4'-di-Cl- C_6H_3	83	208–209	96.91	CHN
83h	1516	4'-MeO- C_6H_4	65	165–166	99.05	CHN

83i	1501	3',4'- di-MeO-C ₆ H ₃	70	124–125	95.87	CHN
83j	1503	3',4',5'-tri-MeO-C ₆ H ₂	99	170–171	98.58	CHN
84a	1528	Me	65	215–218	99.99	CHN
84b	1532	Et	59	155–158	98.28	CHN
84c	1504	C ₆ H ₅	74	175–176	99.41	CHN
84d	1502	4'-F-C ₆ H ₄	82	190–191	97.26	CHN
84e	1530	4'-Cl-C ₆ H ₄	64	191–192	96.48	CHN
84f	1527	3',4'-di-F-C ₆ H ₃	83	176–178	96.63	CHN
84g	1526	3',4'-di-Cl-C ₆ H ₃	83	192–193	96.73	CHN
84h	1529	4'-MeO-C ₆ H ₄	65	199–200	97.60	CHN
84i	1505	3',4'- di-MeO-C ₆ H ₃	56	188–189	95.84	CHN
84j	1531	3',4',5'-tri-MeO-C ₆ H ₂	57	189–190	97.60	CHN

2.2 Experimental

2.2.1 General methods and materials

All commercial chemicals and solvents were reagent grade and used without further purification unless otherwise specified. Melting points were determined on a Fargo melting point apparatus and are uncorrected. Thin-layer chromatography was performed on silica gel G60 F₂₅₄ (Merck) with short-wavelength UV light for visualization. All reported yields are isolated yields after chromatography or crystallization. Elemental analyses were done on a Heraeus CHN-O Rapid instrument. ¹H NMR spectra were recorded on a 600 MHz, Bruker AVANCE 600 DRX and 400 MHz, Bruker Top-Spin spectrometers in the indicated solvent. The chemical shifts were reported in ppm (δ) relative to TMS and coupling constants (J) in Hertz (Hz) and s, d, t, m, brs, refer to singlet, doublet, triplet, multiplet, broad respectively. High performance liquid chromatography analysis for checking purity of synthesized compounds were recorded on a Hitachi D-2000 Elite instrument: column, Mightysil RP-18 GP 250-4.6 (5 μ m); mobile phase, MeCN/THF (50:50 v/v); flow rate, 1 mL/min; injected sample 10 μ L, column temp, 27 °C; wavelength, 254 nm. The purity of all tested compounds was $\geq 95\%$ based on analytical HPLC.

All synthesized compounds were characterized by using ¹H NMR and Elemental analysis. For compounds **80a-j**, the characteristic proton signals for ester (COOMe) appeared at the range of 3.51–3.76 δ ppm. The ¹H NMR proton signals for the compounds **81a-j**, hydroxymethyl proton (CH₂OH) appeared at the range of 4.33–4.55 δ ppm and methylene proton of isoquinoline ring appeared at 3.96–5.03 δ ppm. For compounds **82–84**, the ¹H NMR signals for the carbamate residue (i.e., CH₂OCO) appeared at 3.9–5.1 δ ppm. The characteristic proton for NH of alkylcarbamates appeared at the range of 6.7–7.0 δ ppm as multiplet and D₂O exchangeable singlet. The elemental analysis of the newly synthesized derivatives was within $\pm 0.4\%$ range of the calculated C, H, N data.

1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid hydrochloride (78). A mixture of D,L-phenylalanine (**77**, 50 g, 3.03 mol), conc. HCl (325 mL) and 37 % formalin (110 mL) was heated to a gentle reflux with vigorous stirring. After 30 min, another portion of formalin (50 mL) and conc. HCl (110 mL) was added. The reaction mixture was further stirred and heated for 4 h and cooled to room temperature. The white solid separated out was filtered and washed with methanol (30 mL) to give **78** 64.1 g, yield 98 %; mp > 280 °C (lit.⁹⁸ mp 286–290 °C). ¹H NMR (DMSO-*d*₆) δ 3.15 (1H, m, CH), 3.32 (1H, m, CH), 4.30 (2H, m, CH₂), 4.43 (1H, m, CH), 7.20–7.35 (4H, m, 4×ArH), 9.89 (1H, brs, exchangeable, NH); 10.06 (1H, brs, exchangeable, COOH).

2-Propionyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (79b). To a suspension of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride (**78**, 10 g, 46.8 mmol) in acetone (60 mL) was added 2N NaOH (40 mL) solution at room temperature. The clear solution obtained then added dropwise into a solution of propionyl chloride (5.2 g, 57 mmol) in acetone (20 mL) at room temperature, simultaneously 2N NaOH was added dropwise and pH maintained above 10. The reaction mixture was stirred at room temperature for 2h, the solvent was evaporated under reduce pressure. The solution was acidified to pH 5-6 with 3N HCl. The white solid separated, filtered it and dried to give **79b** 9 g, yield 87 %; mp 173–174 °C. ¹H NMR (DMSO-*d*₆) δ 1.03 (3H, m, Me), 2.45 (2H, m, CH₂), 3.13 (2H, m, CH₂), 4.52 (2H, m, CH₂), 5.09 (1H, m, CH), 7.16–7.20 (4H, m, 4×ArH), 12.63 (1H, brs, exchangeable, COOH). Anal. Calcd. for (C₁₃H₁₅NO₃): C, 66.94; H, 6.48; N, 6.00. Found: C, 66.64; H, 6.48; N, 5.89.

By following the same synthetic procedure as that for **79b**, the following compounds were synthesized:

2-Benzoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (79c). Compound **79c** was prepared from **78** (8.0 g, 37.4 mmol) and benzoyl chloride (6.43 g, 45.6 mmol). Yield, 9.9 g (77 %); mp 168–169 °C (lit.⁹⁹ mp 168–169 °C). ¹H NMR (DMSO-*d*₆) δ 3.18 (2H, m, CH₂), 4.52 (2H, m, CH₂), 5.08 (1H, m, CH), 7.14–7.22 (4H, m, 4×ArH), 7.41–7.49 (5H, m, 5×ArH), 12.85 (1H, brs, exchangeable, COOH).

2-(4-Fluorobenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (79d).

Compound **79d** was prepared from **78** (10.0 g, 46.8 mmol) and 4-fluorobenzoyl chloride (9.91 g, 57.0 mmol). Yield, 13.5 g (97 %); mp 179–180 °C; ¹H NMR (DMSO-*d*₆) δ 3.20 (2H, m, CH₂), 4.52 (2H, m, CH₂), 5.06 (1H, m, CH), 7.19–7.33 (6H, m, 6×ArH), 7.48–7.52 (2H, m, 2×ArH), 12.76 (1H, brs, exchangeable, COOH). Anal. Calcd. for (C₁₇H₁₄FNO₃): C, 68.22; H, 4.71; N, 4.68. Found: C, 68.08; H, 4.57; N, 4.42.

2-(4-Chlorobenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (79e).

Compound **79e** was prepared from **78** (10.0 g, 46.8 mmol) and 4-chlorobenzoyl chloride (10.1 g, 57.0 mmol). Yield, 13.8 g (93 %), mp 77–79 °C; ¹H NMR (DMSO-*d*₆) δ 3.29 (2H, m, CH₂), 4.49 (2H, m, CH₂), 5.02 (1H, m, CH), 7.01–7.17 (4H, m, 4×ArH), 7.40–7.58 (4H, m, 4×ArH), 12.81 (1H, brs, exchangeable, COOH). Anal. Calcd. for (C₁₇H₁₄ClNO₃): C, 64.67; H, 4.47; N, 4.44. Found: C, 64.52; H, 4.56; N, 4.33.

2-(3, 4-Difluorobenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (79f).

Compound **79f** was prepared from **78** (10.0 g, 46.8 mmol) and 3,4-difluorobenzoyl chloride (10.2 g, 57.0 mmol). Yield, 14.2 g (95 %); mp 89–91 °C; ¹H NMR (DMSO-*d*₆) δ 3.18 (2H, m, CH₂), 4.48 (2H, m, CH₂), 5.02 (1H, m, CH), 7.09–7.15 (4H, m, 4×ArH), 7.33 (1H, s, ArH), 7.46–7.54 (2H, m, 2×ArH), 12.84 (1H, brs, exchangeable, COOH). Anal. Calcd. for (C₁₇H₁₃F₂NO₃): C, 64.35; H, 4.13; N, 4.41. Found: C, 64.21; H, 4.26; N, 4.56.

2-(3, 4-Dichlorobenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (79g).

Compound **79g** was prepared from **78** (10.0 g, 46.8 mmol) and 3,4-dichlorobenzoyl chloride (12.1 g, 57.0 mmol). Yield, 14.5 g (89 %); mp 101–102 °C; ¹H NMR (DMSO-*d*₆) δ 3.27 (2H, m, CH₂), 4.45 (2H, m, CH₂), 4.99 (1H, m, CH), 7.08–7.13 (4H, m, 4×ArH), 7.44–7.46 (1H, m, ArH), 7.66–7.70 (2H, m, 2×ArH), 12.67 (1H, brs, exchangeable, COOH). Anal. Calcd. For (C₁₇H₁₇Cl₂NO₃): C, 58.31; H, 3.74; N, 4.00. Found: C, 58.19; H, 4.04; N, 3.87.

2-(4-Methoxybenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (79h).

Compound **79h** was prepared from **78** (10.0 g, 46.8 mmol) and 4-methoxybenzoyl chloride (9.8 g, 57.0 mmol). Yield, 12.6 g (86 %); mp 178–180 °C; ¹H NMR (DMSO-*d*₆) δ 3.28 (2H, m, CH₂), 3.85 (3H, s, MeO), 4.60 (2H, m, CH₂), 5.02 (1H, m, CH), 6.90–6.94 (3H, m, 3×ArH), 7.18–7.22 (3H, m, 3×ArH), 7.42–7.48 (2H, m, 2×ArH), 12.66 (1H, brs, exchangeable, COOH). Anal. Calcd. for (C₁₈H₁₇NO₄): C, 69.44; H, 5.50; N, 4.50. Found: C, 69.12; H, 5.62; N, 4.27.

2-(3,4-Dimethoxybenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (79i).

Compound **79i** was prepared from **78** (10.0 g, 46.8 mmol) and 3,4-dimethoxybenzoyl chloride (12.5 g, 57 mmol). Yield, 11.9 g (61 %); mp 235–236 °C; ¹H NMR (DMSO-*d*₆) δ 3.19 (2H, m, CH₂), 3.77 (6H, s, 2×MeO), 4.56 (2H, m, CH₂), 5.04 (1H, m, CH), 6.99–7.04 (4H, m, 4×ArH), 7.17–7.23 (3H, m, 3×ArH), 12.88 (1H, brs, exchangeable, COOH). Anal. Calcd. for (C₁₉H₁₉NO₅): C, 66.85; H, 5.61; N, 4.10. Found: C, 66.65; H, 5.66; N, 3.86.

2-(3, 4, 5-Trimethoxybenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (79j).

Compound **79j** was prepared from **78** (7.0 g, 32.7 mmol) and 3,4,5-trimethoxybenzoyl chloride (9.3 g, 39.9 mmol). Yield, 12.0 g (98 %); mp 186–189 °C; ¹H NMR (DMSO-*d*₆) δ 3.19 (2H, m, CH₂), 3.70 (3H, s, MeO), 3.80 (6H, s, 2×MeO), 4.59 (2H, m, CH₂), 5.02 (1H, m, CH), 6.70 (2H, s, 2×ArH), 7.13–7.28 (4H, m, 4×ArH), 12.72 (1H, brs, exchangeable, COOH). Anal. Calcd. for (C₂₀H₂₁NO₆): C, 64.68; H, 5.70; N, 3.77. Found: C, 64.44; H, 5.50; N, 3.44.

3-Methyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (80a).

Dimethyl acetylenedicarboxylate (6.39 g, 45.0 mmol) was added into a mixture of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**78**, 10 g, 46.8 mmol) in acetic anhydride (70 mL) and the reaction mixture was heated at 70 °C with stirring for 1.5 h. The reaction mixture was evaporated to dryness *in vacuo*. The residue was recrystallized from MeOH to give **80a**, 11.0 g (74 %); mp 152–154 °C (lit.¹⁰¹ mp 140–142 °C). ¹H NMR(DMSO-*d*₆) δ 2.40 (3H, s, Me), 3.70 (3H, s, COOMe), 3.72 (3H, s, COOMe), 4.18 (2H, s, CH₂), 5.06 (2H, s, CH₂), 7.21–7.32 (2H, m, 2×ArH), 7.35–7.39 (2H, m, 2×ArH).

3-Ethyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (80b). A mixture of dimethyl acetylenedicarboxylate (DMAD) (6.39 g, 45.0 mmol) and **79b** (7.0 g, 30.0 mmol) in acetic anhydride (50 mL) was heated at 65 °C with stirring for 1.5 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue was recrystallized from MeOH to give **80b**, 8.77 g (93 %); mp 88–89 °C. ¹H NMR(DMSO-*d*₆) δ 1.15 (3H, t, *J* = 7.6 Hz, Me), 2.85 (2H, q, *J* = 7.6 Hz, CH₂), 3.71 (6H, s, 2×COOMe), 4.19 (2H, s, CH₂), 5.12 (2H, s, CH₂), 7.29–7.33 (2H, m, 2×ArH), 7.38–7.43 (2H, m, 2×ArH). Anal. Calcd. for (C₁₈H₁₉NO₄): C, 68.99; H, 6.11; N, 4.47. Found: C, 68.96; H, 6.05; N, 4.38.

By following the same synthetic procedure as that for **80b**, the following compounds were synthesized:

3-Phenyl-5, 10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (80c). Compound **80c** was prepared from DMAD (6.0 g, 41.0 mmol) and **79c** (8.0 g, 28.4 mmol). Yield, 7.0 g (68 %); mp 137–138 °C. ¹H NMR (DMSO-*d*₆) δ 3.58 (3H, s, COOMe), 3.76 (3H, s, COOMe), 4.33 (2H, s, CH₂), 4.98 (2H, s, CH₂), 7.21–7.32 (3H, m, 3×ArH), 7.41–7.53 (6H, m, 6×ArH). Anal. Calcd. for (C₂₂H₁₉NO₄): C, 73.12; H, 5.30; N, 3.88. Found: C, 72.91; H, 5.30; N, 3.53.

3-(4-Fluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (80d). Compound **80d** was prepared from DMAD (10.7 g, 65.0 mmol) and **79d** (15.0 g, 50.1 mmol). Yield, 11.1 g (58 %); mp 149–150 °C. ¹H NMR(DMSO-*d*₆) δ 3.58 (3H, s, COOMe), 3.76 (3H, s, COOMe), 4.32 (2H, s, CH₂), 4.96 (2H, s, CH₂), 7.23–7.30 (1H, m, ArH), 7.32–7.36 (4H, m, 4×ArH), 7.40–7.42 (1H, m, ArH), 7.49–7.52 (2H, m, 2×ArH). Anal. Calcd. For (C₂₂H₁₈FNO₄): C, 69.65; H, 4.78; N, 3.69. Found: C, 69.54; H, 4.82; N, 3.78.

3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (80e). Compound **80e** was prepared from DMAD (6.8 g, 47.0 mmol) and **79e** (10.0 g, 31.6 mmol). Yield, 8.0 g (64 %); mp 164–166 °C. ¹H NMR(DMSO-*d*₆) δ 3.60 (3H, s, COOMe), 3.76 (3H, s, COOMe), 4.32 (2H, s, CH₂), 4.98 (2H, s, CH₂), 7.20–7.26 (1H, m, ArH), 7.27–7.33 (2H, m, 2×ArH), 7.39–7.43 (1H, m, ArH),

7.48 (2H, d, $J = 8.5$ Hz, $2 \times \text{ArH}$), 7.57 (2H, d, $J = 8.5$ Hz, $2 \times \text{ArH}$). Anal. Calcd. for ($\text{C}_{22}\text{H}_{18}\text{ClNO}_4$): C, 66.75; H, 4.58; N, 3.54. Found: C, 66.52; H, 4.85; N, 3.66.

3-(3,4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (80f). Compound **80f** was prepared from DMAD (6.71 g, 47.0 mmol) and **79f** (10.0 g, 31.5 mmol). Yield, 8.4 g (67 %); mp 142–144 °C. ^1H NMR(DMSO- d_6) δ 3.61 (3H, s, COOMe), 3.76 (3H, s, COOMe), 4.31 (2H, s, CH_2), 5.00 (2H, s, CH_2), 7.22–7.42 (5H, m, $5 \times \text{ArH}$), 7.54–7.61 (2H, m, $2 \times \text{ArH}$). Anal. Calcd. for ($\text{C}_{22}\text{H}_{17}\text{F}_2\text{NO}_4$): C, 66.50; H, 4.31; N, 3.52. Found: C, 66.15; H, 4.44; N, 3.36.

3-(3,4-Dichlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (80g). Compound **80e** was prepared from DMAD (8.5 g, 60.0 mmol) and **79g** (14.0 g, 40.0 mmol). Yield, 11.0 g (63 %); mp 169–170 °C. ^1H NMR (DMSO- d_6) δ 3.61 (3H, s, COOMe), 3.77 (3H, s, COOMe), 4.31 (2H, s, CH_2), 5.02 (2H, s, CH_2), 7.22–7.33 (3H, m, $3 \times \text{ArH}$), 7.40–7.47 (2H, m, $2 \times \text{ArH}$), 7.76–7.78 (2H, m, $2 \times \text{ArH}$). Anal. Calcd. for ($\text{C}_{22}\text{H}_{17}\text{Cl}_2\text{NO}_4$): C, 61.41; H, 3.98; N, 3.26. Found: C, 61.33; H, 3.95; N, 2.89.

3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (80h). Compound **80h** was prepared from DMAD (7.5 g, 53.0 mmol) and **79h** (10.0 g, 35.1 mmol). Yield, 8.4 g (66 %); mp 155–158 °C. ^1H NMR(DMSO- d_6) δ 3.58 (3H, s, COOMe), 3.75 (3H, s, COOMe), 3.82 (3H, s, MeO), 4.31 (2H, s, CH_2), 4.95 (2H, s, CH_2), 7.05–7.07 (2H, m, $2 \times \text{ArH}$), 7.21–7.24 (1H, m, ArH), 7.28–7.31 (2H, m, $2 \times \text{ArH}$), 7.37–7.42 (3H, m, $3 \times \text{ArH}$). Anal. Calcd. for ($\text{C}_{23}\text{H}_{21}\text{NO}_5$): C, 70.58; H, 5.41; N, 3.58. Found: C, 70.25; H, 5.44; N, 3.49.

3-(3,4-Dimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (80i). Compound **80i** was prepared from DMAD (5.6 g, 39.0 mmol) and **79i** (9.0 g, 26.4 mmol). Yield, 9.0 g (81 %); mp 202–203 °C. ^1H NMR(DMSO- d_6) δ 3.60 (3H, s, COOMe), 3.66 (3H, s, MeO), 3.78 (3H, s, COOMe), 3.82 (3H, s, MeO), 4.32 (2H, s, CH_2), 5.00 (2H, s, CH_2), 6.99 (1H, dd, $J = 2.0$ and 8.0 Hz, ArH), 7.02 (1H, d, $J = 2.0$ Hz, ArH), 7.07 (1H, d, $J = 8.0$ Hz, ArH),

7.22–7.25 (1H, m, ArH), 7.25–7.32 (2H, m, 2×ArH), 7.40–7.42 (1H, m, ArH). Anal. Calcd. for (C₂₄H₂₃NO₆): C, 68.40; H, 5.50; N, 3.32. Found: C, 68.02; H, 5.53; N, 2.94.

3-(3,4,5-Trimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-

dicarboxylic acid dimethyl ester (80j). Compound **80j** was prepared from DMAD (5.7 g, 40.0 mmol) and **79j** (10.0 g, 26.9 mmol). Yield, 6.2 g (51 %); mp 165–166 °C. ¹H NMR(DMSO-*d*₆) δ 3.63 (3H, s, COOMe), 3.74 (6H, s, COOMe and MeO), 3.80 (6H, s, 2×MeO), 4.32 (2H, s, CH₂), 5.00 (2H, s, CH₂), 6.75 (2H, s, 2×ArH), 7.22–7.26 (1H, m, ArH), 7.28–7.32 (1H, m, ArH), 7.35–7.37 (1H, m, ArH), 7.40–7.42 (1H, m, ArH). Anal. Calcd. for (C₂₅H₂₅NO₇): C, 66.51; H, 5.58; N, 3.10. Found: C, 66.25; H, 5.59; N, 3.01.

[3-Methyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (81a).

A solution of **80a** (8.0 g, 26.7 mmol) in anhydrous dichloromethane (20 mL) was added dropwise in to a stirred suspension of LiAlH₄ (2.5 g, 66.0 mmol) in anhydrous diethyl ether (50 mL) at 0 to -5 °C. The reaction mixture was further stirred for 15 min after the addition was completed. The excess hydride was destroyed by the sequential addition of water (2.5 mL), 15% aqueous NaOH (2.5 mL), and water (2.5 mL) at 0 °C. The mixture was filtered through a pad of Celite, the solid residue was washed with dichloromethane. The combined filtrate and washings were evaporated to dryness *in vacuo*. The residue was recrystallized from ether to give **81a**, 4.2 g (64.0 %); mp 99–101 °C. ¹H NMR (DMSO-*d*₆) δ 2.22 (3H, s, Me), 3.96 (2H, s, CH₂), 4.33 (2H, m, CH₂), 4.38 (4H, m, CH₂ and exchangeable, 2×OH), 4.93 (2H, s, CH₂), 7.24–7.28 (2H, m, 2×ArH), 7.34–7.36 (2H, m, 2×ArH). Anal. Calcd. for (C₁₅H₁₇NO₂): C, 74.05; H, 7.04; N, 5.76. Found: C, 74.24; H, 6.97; N, 5.71.

By following the same synthetic procedure as that for **81a**, the following compounds were synthesized:

[3-Ethyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (81b).

Compound **81b** was prepared from **80b** (5.0 g, 15.9 mmol) and LiAlH₄ (1.5 g, 39.0 mmol). Yield, 3.15 g (76 %) as syrup; ¹H NMR (DMSO-*d*₆) δ 1.10 (3H, t, *J* = 7.6 Hz, Me), 2.66 (2H, q, *J* = 7.6 Hz, CH₂), 3.95 (2H, s, CH₂), 4.33 (3H, m, CH₂ and

exchangeable, OH), 4.39 (3H, m, CH₂ and exchangeable, OH), 4.96 (2H, s, CH₂), 7.23–7.28 (2H, m, 2×ArH), 7.33–7.39 (2H, m, 2×ArH).

[3-Phenyl-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]dimethanol (81c).

Compound **81c** was prepared from **80c** (7.0 g, 19.4 mmol) and LiAlH₄ (1.8 g, 48.0 mmol). Yield, 4.7 g (80 %); mp 150–152 °C. ¹H NMR (DMSO-*d*₆) δ 4.06 (2H, s, CH₂), 4.28 (2H, d, *J* = 4.8 Hz, CH₂), 4.50 (2H, d, *J* = 4.8 Hz, CH₂), 4.54 (2H, brs, exchangeable OH), 4.98 (2H, s, CH₂), 7.18 (1H, t, *J* = 7.2 Hz, ArH), 7.26 (2H, t, *J* = 7.2 Hz, 2×ArH), 7.36–7.39 (2H, m, 2×ArH), 7.45–7.51 (4H, m, 4×ArH). Anal. Calcd. for (C₂₀H₁₉NO₂): C, 78.66; H, 6.27; N, 4.59. Found: C, 78.48; H, 6.42; N, 4.41.

[3-(4-Fluorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]dimethanol (81d).

Compound **81d** was prepared from **80d** (10.0 g, 26.4 mmol) and LiAlH₄ (2.5 g, 65.0 mmol). Yield, 6.36 g (74 %); mp 135–136 °C. ¹H NMR (DMSO-*d*₆) δ 4.05 (2H, s, CH₂), 4.26 (2H, d, *J* = 4.8 Hz, CH₂), 4.49 (2H, d, *J* = 4.8 Hz, CH₂), 4.53 (2H, t, *J* = 4.8 Hz, exchangeable, OH), 4.96 (2H, s, CH₂), 7.17–7.21 (1H, m, ArH), 7.26–7.34 (4H, m, 4×ArH), 7.38 (1H, m, ArH), 7.48–7.52 (2H, m, 2×ArH). Anal. Calcd. for (C₂₀H₁₈FNO₂): C, 74.29; H, 5.61; N, 4.33. Found: C, 74.38; H, 5.68; N, 3.98.

[3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]dimethanol (81e).

Compound **81e** was prepared from **80e** (7.0 g, 17.7 mmol) and LiAlH₄ (1.63 g, 44 mmol). Yield, 3.1 g (52 %); mp 170–172 °C. ¹H NMR (DMSO-*d*₆) δ 4.06 (2H, s, CH₂), 4.28 (2H, d, *J* = 4.8 Hz, CH₂), 4.50 (2H, d, *J* = 4.8 Hz, CH₂), 4.55 (2H, m, exchangeable, OH), 4.99 (2H, s, CH₂), 7.18–7.20 (1H, m, ArH), 7.21–7.27 (2H, m, 2×ArH), 7.29 (1H, d, *J* = 7.6 Hz, ArH), 7.38 (2H, d, *J* = 7.2 Hz, 2×ArH), 7.48 (2H, d, *J* = 7.2 Hz, 2×ArH). Anal. Calcd. for (C₂₀H₁₈ClNO₂): C, 70.69; H, 5.34; N, 4.12. Found: C, 70.42; H, 5.37; N, 4.05.

[3-(3,4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-

diyl]dimethanol (81f). Compound **81f** was prepared from **80f** (7.5 g, 18.0 mmol) and LiAlH₄ (1.73 g, 47 mmol). Yield, 5.3 g (83 %); mp 145–146 °C. ¹H NMR (DMSO-*d*₆) δ 4.05 (2H, s, CH₂), 4.27 (2H, d, *J* = 5.2 Hz, CH₂), 4.48 (2H, d, *J* = 5.2 Hz, CH₂), 4.53 (1H, t, *J* = 5.2 Hz, exchangeable, OH), 4.60 (1H, t, *J* = 5.2 Hz, exchangeable,

OH), 5.01 (2H, s, CH₂), 7.18–7.33 (4H, m, 4×ArH), 7.37–7.39 (1H, m, ArH), 7.52–7.54 (2H, m, 2×ArH). Anal. Calcd. for (C₂₀H₁₇F₂NO₂): C, 70.37; H, 5.02; N, 4.10. Found: C, 70.08; H, 5.01; N, 3.76.

[3-(3,4-Dichlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-

diyl]dimethanol (81g). Compound **81g** was prepared from **80g** (9.0 g, 20.9 mmol) and LiAlH₄ (1.9 g, 52.0 mmol). Yield, 5.7 g (72 %); mp 150–151 °C. ¹H NMR (DMSO-*d*₆) δ 4.05 (2H, s, CH₂), 4.26 (2H, d, *J* = 4.8 Hz, CH₂), 4.48 (2H, d, *J* = 4.8 Hz, CH₂), 4.53 (1H, t, *J* = 4.8 Hz, exchangeable OH), 4.63 (1H, t, *J* = 4.8 Hz, exchangeable, OH), 5.03 (2H, s, CH₂), 7.18–7.21 (1H, m, ArH), 7.23–7.27 (2H, m, 2×ArH), 7.28–7.30 (1H, m, ArH), 7.38–7.40 (1H, m, ArH), 7.46–7.48 (2H, m, 2×ArH). Anal. Calcd. for (C₂₀H₁₇Cl₂NO₂): C, 64.18; H, 4.58; N, 3.74. Found: C, 63.89; H, 4.40; N, 3.87.

[3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-

diyl]dimethanol (81h). Compound **81h** was prepared from **80h** (7.5 g, 19.0 mmol) and LiAlH₄ (1.77 g, 47 mmol). Yield, 4.3 g (67 %); mp 174–176 °C. ¹H NMR (DMSO-*d*₆) δ 3.82 (3H, s, MeO), 4.04 (2H, s, CH₂), 4.26 (2H, s, CH₂), 4.48 (4H, m, CH₂ and exchangeable, OH), 4.93 (2H, s, CH₂), 7.04–7.06 (2H, m, 2×ArH), 7.17–7.21 (1H, m, ArH), 7.24–7.27 (2H, m, 2×ArH), 7.36–7.39 (3H, m, 3×ArH). Anal. Calcd. for (C₂₁H₂₁NO₃): C, 75.20; H, 6.31; N, 4.18. Found: C, 75.36; H, 6.30; N, 4.13.

[3-(3,4-Dimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-

diyl]dimethanol (81i). Compound **81i** was prepared from **80i** (8.0 g, 18.9 mmol) and LiAlH₄ (1.75 g, 47 mmol). Yield, 5.34 g (77 %); mp 145–146 °C. ¹H NMR (DMSO-*d*₆) δ 3.79 (6H, s, 2×MeO), 4.04 (2H, s, CH₂), 4.29 (2H, s, CH₂), 4.52 (4H, brs, CH₂ and exchangeable, OH), 4.97 (2H, s, CH₂), 6.96–6.98 (1H, m, ArH), 7.05–7.07 (2H, m, 2×ArH), 7.19–7.28 (3H, m, 3×ArH), 7.37–7.39 (1H, m, ArH). Anal. Calcd. for (C₂₂H₂₃NO₄): C, 72.31; H, 6.34; N, 3.83. Found: C, 72.25; H, 6.34; N, 3.95.

[3-(3,4,5-Trimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-

diyl]dimethanol (81j). Compound **81j** was prepared from **80j** (6.0 g, 13.3 mmol) and LiAlH₄ (1.2 g, 33.0 mmol). Yield, 3.1 g (58 %); mp 146–147 °C. ¹H NMR (DMSO-

d_6) δ 3.74 (3H, s, MeO), 3.82 (6H, s, 2×MeO), 4.05 (2H, s, CH₂), 4.31 (2H, s, CH₂), 4.50 (2H, s, CH₂), 4.53 (2H, s, exchangeable, OH), 5.04 (2H, s, CH₂), 6.73 (2H, s, 2×ArH), 7.21–7.27 (2H, m, 2×ArH), 7.33–7.38 (2H, m, 2×ArH). Anal. Calcd. for (C₂₃H₂₅NO₅): C, 69.86; H, 6.37; N, 3.54. Found: C, 69.71; H, 6.37; N, 3.45.

General procedure for preparing bis(alkylcarbamate) derivatives (82–84). To a solution of bis(hydroxymethyl) derivatives (**81a–j**, 1.0 equivalent) and triethylamine (2–3 equivalent) in anhydrous dichloromethane was added alkylisocyanate (5 equivalent). The reaction mixture was stirred at ambient temperature (for 3–20 h) under an argon atmosphere. After the completion of the reaction, the reaction mixture was evaporated to dryness *in vacuo*. The residue was triturated with ether, and solid separated was collected by filtration. The desired product was either obtained by recrystallization or liquid chromatography.

[3-Methyl-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)

bis(methylcarbamate) (82a). Compound **82a** was synthesized from **81a** (0.80 g, 3.2 mmol), Et₃N (1 mL) and methylisocyanate (1.14 g, 20 mmol). Yield, 0.53 g (44%); mp 174–176 °C. ¹H NMR (DMSO-*d*₆) δ 2.23 (3H, s, Me), 2.53 (6H, m, 2×Me), 3.99 (2H, s, CH₂), 4.88 (2H, s, CH₂), 4.92 (2H, s, CH₂), 4.95 (2H, s, CH₂), 6.74 (2H, brs, exchangeable, NH), 7.21–7.32 (2H, m, 2×ArH), 7.34–7.38 (2H, m, 2×ArH). Anal. Calcd. for (C₁₉H₂₃N₃O₄): C, 63.85; H, 6.49; N, 11.76. Found: C, 63.54; H, 6.57; N, 11.64.

[3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-

diyl]bis(methylene) bis(methylcarbamate) (82e). Compound **82e** was synthesized from **81e** (1.0 g, 3.0 mmol), Et₃N (1 mL), and methylisocyanate (0.85 g, 15 mmol). Yield, 1.1 g (82 %); mp 194–196 °C. ¹H NMR (DMSO-*d*₆) δ 2.54 (6H, m, 2×Me), 4.09 (2H, s, CH₂), 4.80 (2H, s, CH₂), 4.97 (2H, s, CH₂), 5.03 (2H, s, CH₂), 6.81 (2H, brs, exchangeable, NH), 7.17–7.23 (1H, m, ArH), 7.24–7.30 (2H, m, 2×ArH), 7.36–7.40 (1H, m, ArH), 7.45–7.47 (2H, m, 2×ArH), 7.55–7.57 (2H, m, 2×ArH). Anal. Calcd. for (C₂₄H₂₄ClN₃O₄): C, 63.50; H, 5.33; N, 9.26. Found: C, 63.33; H, 5.35; N, 9.20.

[3-(3,4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene) bis(methylcarbamate) (82f). Compound **82f** was synthesized from **81f** (1.0 g, 2.9 mmol), Et₃N (1 mL), and methylisocyanate (0.85 g, 15 mmol). Yield, 1.0 g (77 %); mp 190–192 °C; ¹H NMR (DMSO-*d*₆) δ 2.54 (6H, m, 2×Me), 4.09 (2H, s, CH₂), 4.81 (2H, s, CH₂), 4.99 (2H, s, CH₂), 5.03 (2H, s, CH₂), 6.83 (2H, brs, exchangeable, NH), 7.18–7.23 (1H, m, ArH), 7.25–7.31 (3H, m, 3×ArH), 7.36–7.40 (1H, m, ArH), 7.52–7.61 (2H, m, 2×ArH). Anal. Calcd. for (C₂₄H₂₃F₂N₃O₄): C, 63.29; H, 5.09; N, 9.23. Found: C, 63.00; H, 5.12; N, 9.16.

[3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene) bis(methylcarbamate) (82h). Compound **82h** was synthesized from **81h** (1.0 g, 2.9 mmol), Et₃N (1 mL), methylisocyanate (1.6 g, 29 mmol). Yield, 1.2 g (87 %); mp 182–184 °C. ¹H NMR (DMSO-*d*₆) δ 2.53 (6H, m, 2×Me), 3.85 (3H, s, MeO), 4.08 (2H, s, CH₂), 4.78 (2H, s, CH₂), 4.93 (2H, s, CH₂), 5.01 (2H, s, CH₂), 6.81 (2H, brs, exchangeable, NH), 7.06–7.08 (2H, m, 2×ArH), 7.17–7.23 (1H, m, ArH), 7.24–7.30 (2H, m, 2×ArH), 7.32–7.40 (3H, m, 3×ArH). Anal. Calcd. for (C₂₅H₂₇N₃O₅): C, 66.80; H, 6.05; N, 9.35. Found: C, 66.72; H, 6.15; N, 9.24.

[3-Methyl-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(ethylcarbamate) (83a). Compound **83a** was synthesized from **81a** (1.0 g, 4.1 mmol), Et₃N (0.5 mL), and ethylisocyanate (1.1 g, 16 mmol). Yield, 0.83 g (53 %); mp 208–209 °C; ¹H NMR (DMSO-*d*₆) δ 0.97 (6H, t, *J* = 8 Hz, 2×Me), 2.25 (3H, s, Me), 2.95 (4H, q, *J* = 8 Hz, CH₂), 4.00 (2H, s, CH₂), 4.89 (2H, s, CH₂), 4.93 (2H, s, CH₂), 4.96 (2H, s, CH₂), 6.86 (2H, brs, exchangeable, NH), 7.23–7.29 (2H, m, 2×ArH), 7.34–7.35 (2H, m, 2×ArH). Anal. Calcd. for (C₂₁H₂₇N₃O₄): C, 65.44; H, 7.06; N, 10.90. Found: C, 65.29; H, 7.14; N, 10.89.

[3-Ethyl-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(ethylcarbamate) (83b). Compound **83b** was synthesized from **81b** (0.5 g, 1.9 mmol), Et₃N (0.5 mL), ethylisocyanate (0.55 g, 7.7 mmol). Yield, 0.39 g (50 %); mp 127–129 °C. ¹H NMR (DMSO-*d*₆) δ 0.95 (6H, t, *J* = 7.2 Hz, 2×Me), 1.10 (3H, t, *J* = 7.2 Hz, Me), 2.69 (2H, q, *J* = 7.2 Hz, CH₂), 2.97 (4H, m, CH₂), 3.99 (2H, s, CH₂), 4.89 (2H, s, CH₂), 4.93 (2H, s, CH₂), 4.99 (2H, s, CH₂), 6.84 (2H, brs, exchangeable,

NH), 7.23–7.29 (2H, m, 2×ArH), 7.34–7.39 (2H, m, 2×ArH). Anal. Calcd. for (C₂₂H₂₉N₃O₄): C, 66.14; H, 7.32; N, 10.52. Found: C, 66.35; H, 7.53; N, 10.74.

[3-Phenyl-5-10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(ethylcarbamate) (83c). Compound **83c** was synthesized from **81c** (1.0 g, 3.2 mmol), Et₃N (1.0 mL), and ethylisocyanate (1.2 g, 16.0 mmol). Yield, 1.17 g (81 %); mp 170–171 °C; ¹H NMR (DMSO-*d*₆) δ 0.97 (6H, t, *J* = 7.2 Hz, 2×Me), 2.98 (4H, q, *J* = 7.2 Hz, CH₂), 4.10 (2H, s, CH₂), 4.80 (2H, s, CH₂), 4.97 (2H, s, CH₂), 5.03 (2H, s, CH₂), 6.94 (2H, brs, exchangeable, NH), 7.18–7.20 (1H, m, ArH), 7.26–7.29 (2H, m, 2×ArH), 7.36–7.40 (4H, m, 4×ArH), 7.42–7.44 (2H, m, 2×ArH). Anal. Calcd. For (C₂₆H₂₉N₃O₄): C, 69.78; H, 6.53; N, 9.39. Found: C, 69.64; H, 6.54; N, 9.37.

[3-(4-Fluorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene) bis(ethylcarbamate) (83d). Compound **83d** was synthesized from **81d** (1.0 g, 3.0 mmol), Et₃N (1.2 mL), and (1.1 g, 15.0 mmol). Yield, 1.08 g (76 %); mp 183–184 °C. ¹H NMR (DMSO-*d*₆) δ 0.98 (6H, t, *J* = 6.8 Hz, 2×Me), 2.97 (4H, q, *J* = 6.8 Hz, CH₂), 4.09 (2H, s, CH₂), 4.79 (2H, s, CH₂), 4.95 (2H, s, CH₂), 5.02 (2H, s, CH₂), 6.93 (2H, brs, exchangeable, NH), 7.20–7.22 (1H, m, ArH), 7.27–7.41 (5H, m, 5×ArH), 7.46–7.48 (2H, m, 2×ArH). Anal. Calcd. for (C₂₆H₂₈FN₃O₄): C, 67.08; H, 6.06; N, 9.03. Found: C, 67.24; H, 6.02; N, 8.88.

[3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene) bis(ethylcarbamate) (83e). Compound **83e** was synthesized from **81e** (1.0 g, 2.9 mmol), Et₃N (1.0 mL), and ethylisocyanate (0.84 g, 18.0 mmol). Yield, 0.92 g (64 %); mp 191–192 °C. ¹H NMR (DMSO-*d*₆) δ 0.98 (6H, t, *J* = 7.2 Hz, 2×Me), 2.97 (4H, q, *J* = 7.2 Hz, CH₂), 4.09 (2H, s, CH₂), 4.80 (2H, s, CH₂), 4.97 (2H, s, CH₂), 5.02 (2H, s, CH₂), 6.92 (2H, brs, exchangeable, NH), 7.18–7.22 (1H, m, ArH), 7.25–7.27 (2H, m, 2×ArH), 7.28–7.30 (1H, m, ArH), 7.37–7.39 (2H, m, 2×ArH), 7.44–7.46 (2H, m, 2×ArH). Anal. Calcd. for (C₂₆H₂₈ClN₃O₄): C, 64.79; H, 5.86; N, 8.72. Found: C, 64.54; H, 5.84; N, 8.82.

[3-(3,4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(ethyl carbamate) (83f). Compound **83f** was synthesized

from **81f** (1.0 g, 2.9 mmol), Et₃N (1 mL), and ethylisocyanate (0.8 g, 11.7 mmol). Yield, 1.0 g (70 %); mp 189–190 °C. ¹H NMR (DMSO-*d*₆) δ 0.98 (6H, t, *J* = 6.8 Hz, 2×Me), 2.98 (4H, q, *J* = 6.8 Hz, CH₂), 4.09 (2H, s, CH₂), 4.81 (2H, s, CH₂), 5.00 (2H, s, CH₂), 5.02 (2H, s, CH₂), 6.95 (2H, brs, exchangeable, NH), 7.19–7.21 (1H, m, ArH), 7.25–7.39 (4H, m, 4×ArH), 7.53–7.60 (2H, m, 2×ArH). Anal. Calcd. for (C₂₆H₂₇F₂N₃O₄): C, 64.59; H, 5.63; N, 8.69. Found: C, 64.50; H, 5.60; N, 8.69.

[3-(3,4-Dichlorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-

diyl]bis(methylene)bis(ethylcarbamate) (83g). Compound **83g** was synthesized from **81g** (1.0 g, 2.6 mmol), Et₃N (1.0 mL), and ethylisocyanate (0.85 g, 10.7 mmol). Yield, 1.15 g (83 %); mp 208–209 °C. ¹H NMR (DMSO-*d*₆) δ 0.98 (6H, t, *J* = 6.8 Hz, 2×Me), 2.97 (4H, q, *J* = 6.8 Hz, CH₂), 4.09 (2H, s, CH₂), 4.82 (2H, s, CH₂), 5.02 (4H, s, 2×CH₂), 6.95 (2H, brs, exchangeable, NH), 7.19–7.22 (1H, m, ArH), 7.26–7.31 (2H, m, 2×ArH), 7.39–7.45 (2H, m, 2×ArH), 7.70–7.72 (2H, m, 2×ArH). Anal. Calcd. for (C₂₆H₂₇Cl₂N₃O₄): C, 60.47; H, 5.27; N, 8.14. Found: C, 60.34; H, 5.13; N, 8.10.

[3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-

diyl]bis(methylene) bis(ethylcarbamate) (83h). Compound **83h** was synthesized from **81h** (1.0 g, 2.9 mmol), Et₃N (1.0 mL), and ethylisocyanate (0.84 g, 11.8 mmol). Yield, 0.92 g (65 %); mp 165–166 °C. ¹H NMR (DMSO-*d*₆) δ 0.99 (6H, t, *J* = 6.8 Hz, 2×Me), 2.98 (4H, q, *J* = 6.8 Hz, CH₂), 3.83 (3H, s, MeO), 4.09 (2H, s, CH₂), 4.78 (2H, s, CH₂), 4.94 (2H, s, CH₂), 5.02 (2H, s, CH₂), 6.92 (2H, brs, exchangeable, NH), 7.05–7.08 (2H, m, 2×ArH), 7.19–7.22 (1H, m, ArH), 7.27–7.29 (2H, m, 2×ArH), 7.35–7.39 (3H, m, 3×ArH). Anal. Calcd. for (C₂₇H₃₁N₃O₅): C, 67.91; H, 6.54; N, 8.80. Found: C, 67.73; H, 6.86; N, 8.57.

[3-(3,4-Dimethoxyphenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-

diyl]bis(methylene) bis(ethylcarbamate) (83i). Compound **83i** was synthesized from **81i** (1.0 g, 2.7 mmol), Et₃N (1 mL), and ethylisocyanate (0.77 g, 10.9 mmol). Yield, 0.96 g (69 %); mp 124–125 °C. ¹H NMR (DMSO-*d*₆) δ 0.98 (6H, t, *J* = 6.8 Hz, 2×Me), 2.98 (4H, q, *J* = 6.8 Hz, CH₂), 3.77 (3H, s, MeO), 3.82 (3H, s, MeO), 4.08 (2H, s, CH₂), 4.79 (2H, s, CH₂), 4.98 (2H, s, CH₂), 5.02 (2H, s, CH₂), 6.93 (2H, brs,

exchangeable NH), 6.96–6.98 (2H, m, 2×ArH), 7.07–7.09 (1H, m, ArH), 7.18–7.22 (1H, m, ArH), 7.25–7.28 (2H, m, 2×ArH), 7.30–7.37 (1H, m, ArH). Anal. Calcd. for (C₂₈H₃₃N₃O₆): C, 66.26; H, 6.55; N, 8.28. Found: C, 66.34; H, 6.45; N, 8.11.

[3-(3,4,5-Trimethoxyphenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(ethylcarbamate) (83j). Compound **83j** was synthesized from **81j** (0.79 g, 2.0 mmol), Et₃N (0.8 mL), and ethylisocyanate (0.57 g, 8.0 mmol). Yield, 0.94 g (99 %); mp 170–171 °C. ¹H NMR (DMSO-*d*₆) δ 0.97 (6H, t, *J* = 6.8 Hz, 2×Me), 2.98 (4H, q, *J* = 6.8 Hz, CH₂), 3.73 (3H, s, MeO), 3.80 (6H, s, 2×MeO), 4.09 (2H, s, CH₂), 4.81 (2H, s, CH₂), 5.02 (2H, s, CH₂), 5.05 (2H, s, CH₂), 6.70 (2H, s, 2×ArH), 6.95 (2H, brs, exchangeable, NH), 7.19–7.21 (1H, m, ArH), 7.23–7.26 (1H, m, ArH), 7.27–7.29 (1H, m, ArH), 7.33–7.35 (1H, m, ArH). Anal. Calcd. for (C₂₉H₃₅N₃O₇): C, 64.79; H, 6.56; N, 7.82. Found: C, 64.68; H, 6.42; N, 7.97.

[3-Methyl-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(isopropylcarbamate) (84a). Compound **84a** was synthesized from **81a** (0.5 g, 2.0 mmol), Et₃N (0.4 mL), and isopropylisocyanate (0.69 g, 8.0 mmol). Yield, 0.55 g (65 %); mp 215–218 °C. ¹H NMR (DMSO-*d*₆) δ 0.99 (12H, d, *J* = 6.4 Hz, 4×Me), 2.25 (3H, s, Me), 3.54 (2H, m, CH), 3.99 (2H, s, CH₂), 4.89 (2H, s, CH₂), 4.93 (2H, s, CH₂), 4.96 (2H, s, CH₂), 6.78 (2H, brs, exchangeable, NH), 7.25–7.29 (2H, m, 2×ArH), 7.34–7.35 (2H, m, 2×ArH). Anal. Calcd. for (C₂₃H₃₁N₃O₄): C, 66.81; H, 7.56; N, 10.16. Found: C, 66.69; H, 7.66; N, 10.10.

[3-Ethyl-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(isopropylcarbamate) (84b). Compound **84b** was synthesized from **81b** (0.26 g, 1.0 mmol), Et₃N (0.4 mL), and isopropylisocyanate (0.34 g, 4.0 mmol). Yield, 0.25 g (58 %); mp 155–158 °C. ¹H NMR (DMSO-*d*₆) δ 1.00 (12H, d, *J* = 6.4 Hz, 4×Me), 1.10 (3H, t, *J* = 7.6 Hz, Me), 2.69 (2H, q, *J* = 7.6 Hz, CH₂), 3.55 (2H, m, CH), 3.99 (2H, s, CH₂), 4.89 (2H, s, CH₂), 4.93 (2H, s, CH₂), 4.99 (2H, s, CH₂), 6.79 (2H, brs, exchangeable, NH), 7.25–7.29 (2H, m, 2×ArH), 7.33–7.39 (2H, m, 2×ArH). Anal. Calcd. for (C₂₄H₃₃N₃O₄): C, 67.42; H, 7.78; N, 9.83. Found: C, 67.11; H, 7.55; N, 9.71.

[3-Phenyl-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(iso-propylcarbamate) (84c). Compound **84c** was synthesized from **81c** (0.5 g, 1.6 mmol), Et₃N (0.5 mL), and isopropylisocyanate (0.54 g, 6.4 mmol). Yield, 0.52 g (74 %); mp 175–176 °C. ¹H NMR (DMSO-*d*₆) δ 0.99 (12H, d, *J* = 6.8 Hz, 4×Me), 3.62 (2H, m, CH), 4.10 (2H, s, CH₂), 4.80 (2H, s, CH₂), 4.98 (2H, s, CH₂), 5.03 (2H, s, CH₂), 6.86 (2H, brs, exchangeable, NH), 7.18–7.22 (1H, m, ArH), 7.26–7.28 (2H, m, 2×ArH), 7.38–7.42 (4H, m, 4×ArH), 7.49–7.51 (2H, m, 2×ArH). Anal. Calcd. for (C₂₈H₃₃N₃O₄): C, 70.71; H, 6.99; N, 8.84. Found: C, 70.59; H, 6.92; N, 8.67.

[3-(4-Fluorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(iso-propylcarbamate) (84d). Compound **84d** was synthesized from **81d** (0.5 g, 1.5 mmol), Et₃N (0.5 mL), and isopropylisocyanate (0.51 g, 6.0 mmol). Yield, 0.62 g (81 %); mp 190–191 °C. ¹H NMR (DMSO-*d*₆) δ 0.99 (12H, d, *J* = 6.4 Hz, 4×Me), 3.60 (2H, m, CH), 4.09 (2H, s, CH₂), 4.79 (2H, s, CH₂), 4.95 (2H, s, CH₂), 5.02 (2H, s, CH₂), 6.86 (2H, brs, exchangeable, NH), 7.18–7.22 (1H, m, ArH), 7.26–7.29 (5H, m, 5×ArH), 7.31–7.33 (2H, m, 2×ArH). Anal. Calcd. for (C₂₈H₃₂FN₃O₄): C, 68.14; H, 6.53; N, 8.51. Found: C, 68.31; H, 6.40; N, 8.60.

[3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(iso-propylcarbamate) (84e). Compound **84e** was synthesized from **81e** (0.45 g, 1.3 mmol), Et₃N (0.5 mL), and isopropylisocyanate (0.42 g, 5.0 mmol). Yield, 0.43 g (64 %); mp 191–192 °C. ¹H NMR (DMSO-*d*₆) δ 1.01 (12H, d, *J* = 6.4 Hz, 4×Me), 3.57 (2H, m, CH), 4.09 (2H, s, CH₂), 4.80 (2H, s, CH₂), 4.97 (2H, s, CH₂), 5.02 (2H, s, CH₂), 6.86 (2H, brs, exchangeable, NH), 7.18–7.22 (1H, m, ArH), 7.25–7.30 (2H, m, 2×ArH), 7.37–7.39 (1H, m, ArH), 7.46 (2H, d, *J* = 8.0 Hz, 2×ArH), 7.55 (2H, d, *J* = 8.0 Hz, 2×ArH). Anal. Calcd. for (C₂₈H₃₂ClN₃O₄): C, 65.94; H, 6.32; N, 8.24. Found: C, 65.76; H, 6.17; N, 8.35.

[3-(3,4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(iso-propylcarbamate) (84f). Compound **84f** was synthesized from **81f** (0.5 g, 1.4 mmol), Et₃N (0.5 mL), and isopropylisocyanate (0.49 g, 5.8 mmol). Yield, 0.62 g (83 %); mp 176–178 °C. ¹H NMR (DMSO-*d*₆) δ 1.02 (12H, d, *J*

= 6.4 Hz, 4×Me), 3.58 (2H, m, CH), 4.09 (2H, s, CH₂), 4.81 (2H, s, CH₂), 5.00 (2H, s, CH₂), 5.02 (2H, s, CH₂), 6.87 (2H, brs, exchangeable, NH), 7.19–7.21 (1H, m, ArH), 7.26–7.30 (3H, m, 3×ArH), 7.37–7.39 (1H, m, ArH), 7.51–7.59 (2H, m, 2×ArH). Anal. Calcd. for (C₂₈H₃₁F₂N₃O₄): C, 65.74; H, 6.11; N, 8.21. Found: C, 65.51; H, 6.05; N, 8.38.

[3-(3,4-Dichlorophenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(iso-propylcarbamate) (84g). Compound **84g** was synthesized from **81g** (0.5 g, 1.3 mmol), Et₃N (0.5 mL), and isopropylisocyanate (0.45 g, 5.3 mmol). Yield, 0.6 g (82 %); mp 192–193°C. ¹H NMR (DMSO-*d*₆) δ 1.01 (12H, d, *J* = 6.4 Hz, 4×Me), 3.57 (2H, m, CH), 4.09 (2H, s, CH₂), 4.81 (2H, s, CH₂), 5.02 (4H, s, 2×CH₂), 6.87 (2H, brs, exchangeable, NH), 7.19–7.21 (1H, m, ArH), 7.25–7.27 (2H, m, 2×ArH), 7.30–7.37 (1H, m, ArH), 7.43–7.45 (1H, m, ArH), 7.69 (1H, s, ArH) 7.73–7.75 (1H, m, ArH). Anal. Calcd. for (C₂₈H₃₁N₃O₄Cl₂): C, 61.77; H, 5.74; N, 7.72. Found: C, 61.84; H, 5.67; N, 7.56.

[3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene)bis(iso-propylcarbamate) (84h). Compound **84h** was synthesized from **81h** (0.5 g, 1.4 mmol), Et₃N (0.5 mL), and isopropylisocyanate (0.5 g, 5.9 mmol). Yield, 0.49 g (65 %); mp 199–200 °C. ¹H NMR (DMSO-*d*₆) δ 1.00 (12H, d, *J* = 6.0 Hz, 4×Me), 3.57 (2H, m, CH), 3.82 (3H, s, MeO), 4.07 (2H, s, CH₂), 4.77 (2H, s, CH₂), 4.92 (2H, s, CH₂), 5.01 (2H, s, CH₂), 6.85 (2H, brs, exchangeable, NH), 7.04–7.06 (2H, m, 2×ArH), 7.17–7.21 (1H, m, ArH), 7.25–7.28 (2H, m, 2×ArH), 7.34–7.36 (3H, m, 3×ArH). Anal. Calcd. for (C₂₉H₃₅N₃O₅): C, 68.89; H, 6.98; N, 8.31. Found: C, 68.93; H, 6.96; N, 8.14.

[3-(3,4-Dimethoxyphenyl)-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]bis(methylene) bis(iso-propylcarbamate) (84i). Compound **84i** was synthesized from **81i** (0.37 g, 1.0 mmol), Et₃N (0.4 mL), and isopropylisocyanate (0.34 g, 4 mmol). Yield, 0.30 g (56 %); mp 188–189 °C. ¹H NMR (DMSO-*d*₆) δ 1.01 (12H, d, *J* = 6.0 Hz, 4×Me), 3.57 (2H, m, CH), 3.77 (3H, s, MeO), 3.82 (3H, s, MeO), 4.08 (2H, s, CH₂), 4.78 (2H, s, CH₂), 4.98 (2H, s, CH₂), 5.01 (2H, s, CH₂), 6.84 (2H, brs, exchangeable, NH), 6.95–6.97 (2H, m, 2×ArH), 7.06–7.08 (1H, m, ArH), 7.18–

7.21 (1H, m, ArH), 7.25–7.29 (2H, m, 2×ArH), 7.30–7.37 (1H, m, ArH). Anal. Calcd. for (C₃₀H₃₇N₃O₆): C, 67.27; H, 6.96; N, 7.84. Found: C, 67.27; H, 7.06; N, 7.62.

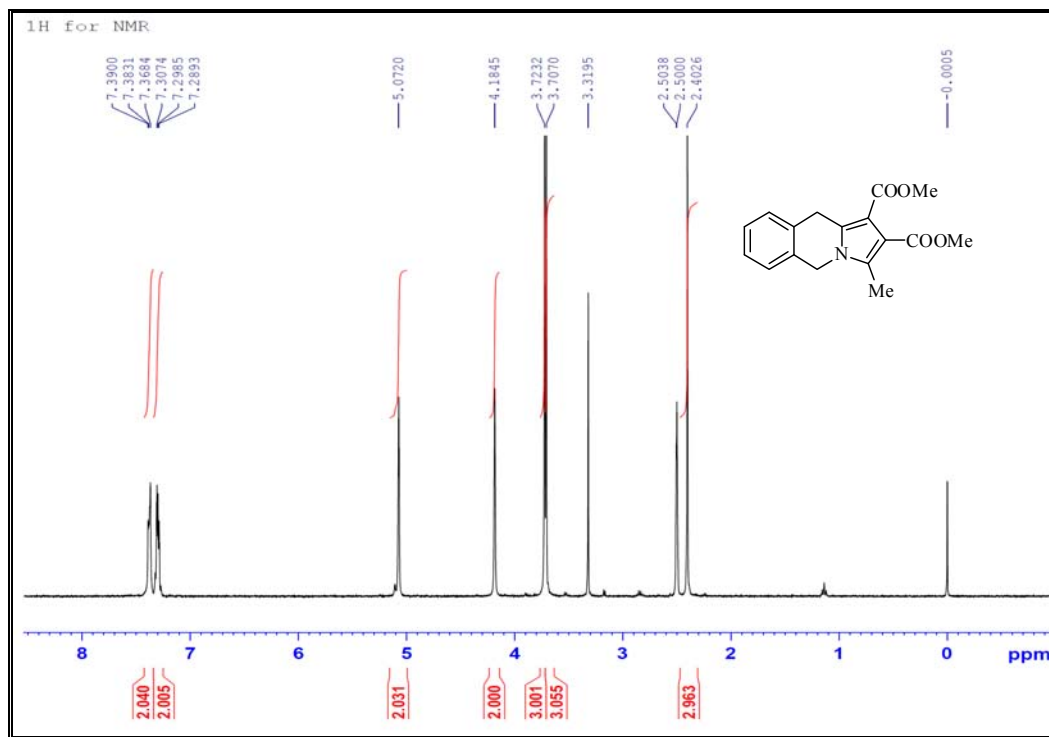
[3-(3,4,5-Trimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis(*iso*-propylcarbamate) (84j). Compound **84j** was synthesized from **81j** (0.39 g, 1.0 mmol), Et₃N (0.4 mL), and isopropylisocyanate (0.34 g, 4 mmol). Yield, 0.32 g (57 %); mp 189–190 °C. ¹H NMR (DMSO-*d*₆) δ 1.01 (12H, d, *J* = 6.4 Hz, 4×Me), 3.57 (2H, m, CH), 3.73 (3H, s, MeO), 3.80 (6H, s, 2×MeO), 4.08 (2H, s, CH₂), 4.79 (2H, s, CH₂), 5.01 (2H, s, CH₂), 5.05 (2H, s, CH₂), 6.69 (2H, s, 2×ArH), 6.87 (2H, brs, exchangeable, NH), 7.19–7.29 (2H, m, 2×ArH), 7.33–7.39 (2H, m, 2×ArH). Anal. Calcd. For (C₃₁H₃₉N₃O₇): C, 65.82; H, 6.95; N, 7.43. Found: C, 65.68; H, 6.74; N, 7.11.

2.3 Conclusion

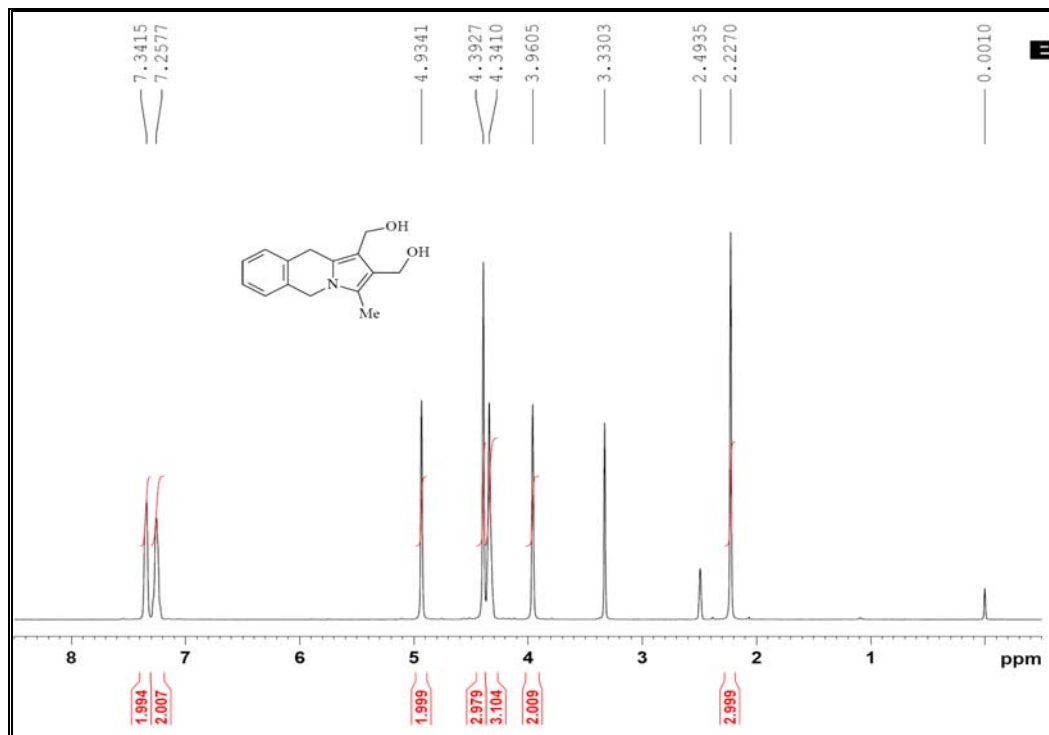
In the present chapter, new chemical entities were prepared to obtain a library of DNA bifunctional alkylating agents. A series of linear bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline derivatives and their bis(alkylcarbamate) derivatives were synthesized. All newly synthesized compounds were tested against various human tumor cell lines *In vitro* and *In vivo*. Antitumor activities and outcome of these compounds are shown in Chapter 3.

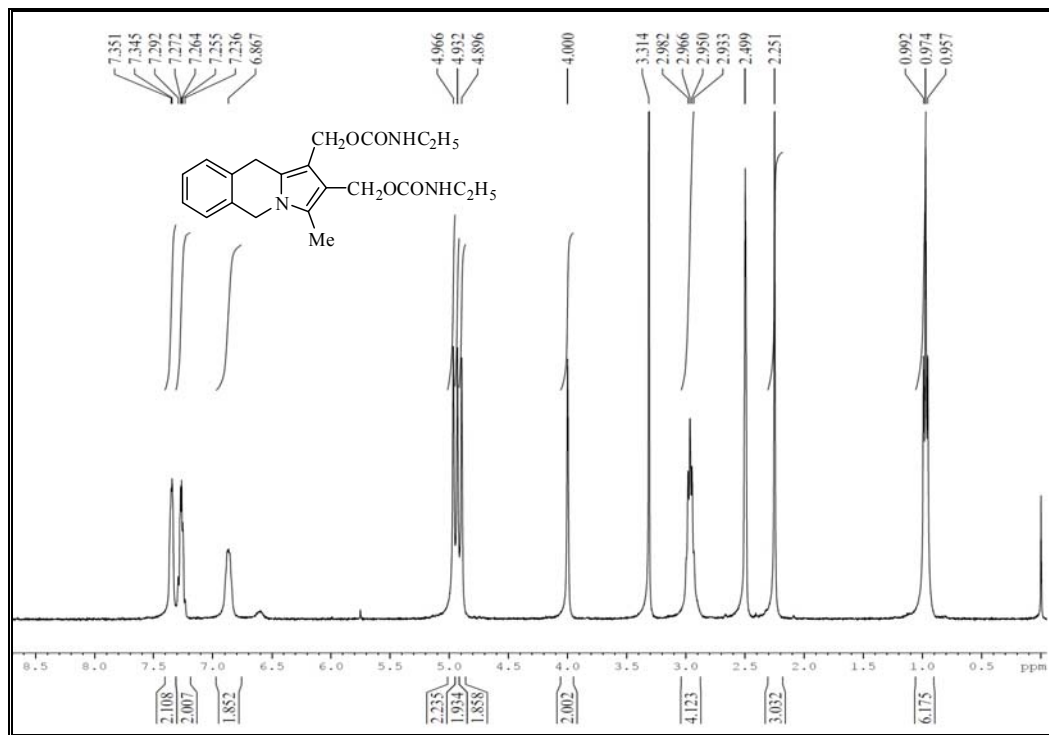
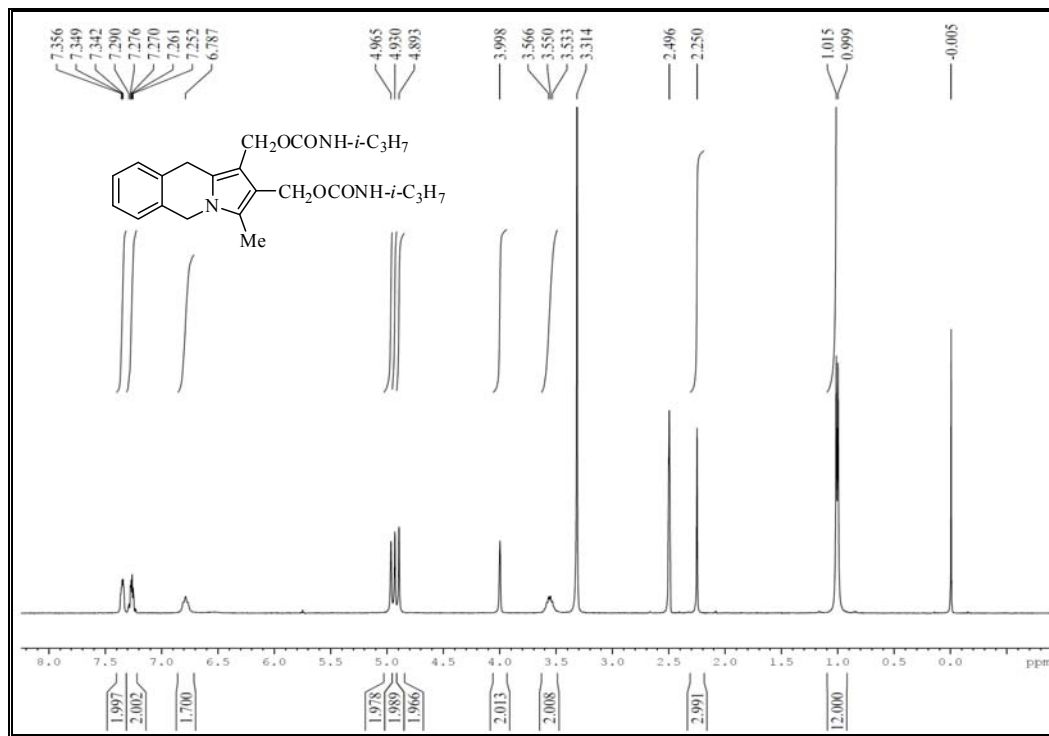
2.4 Representative Spectra

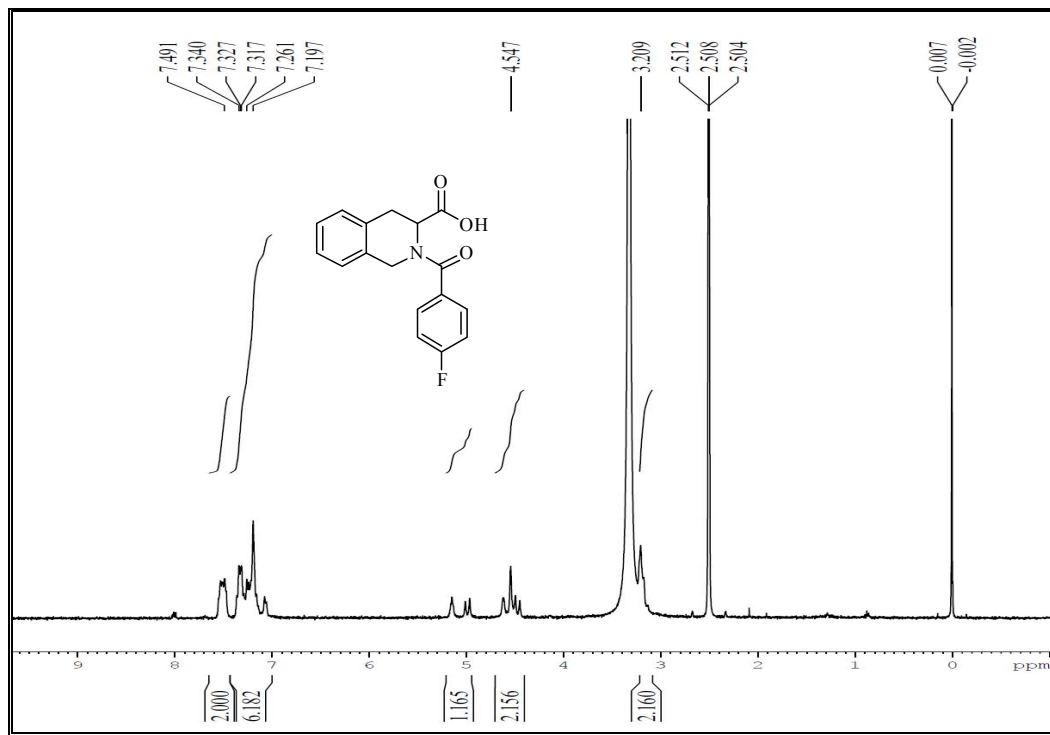
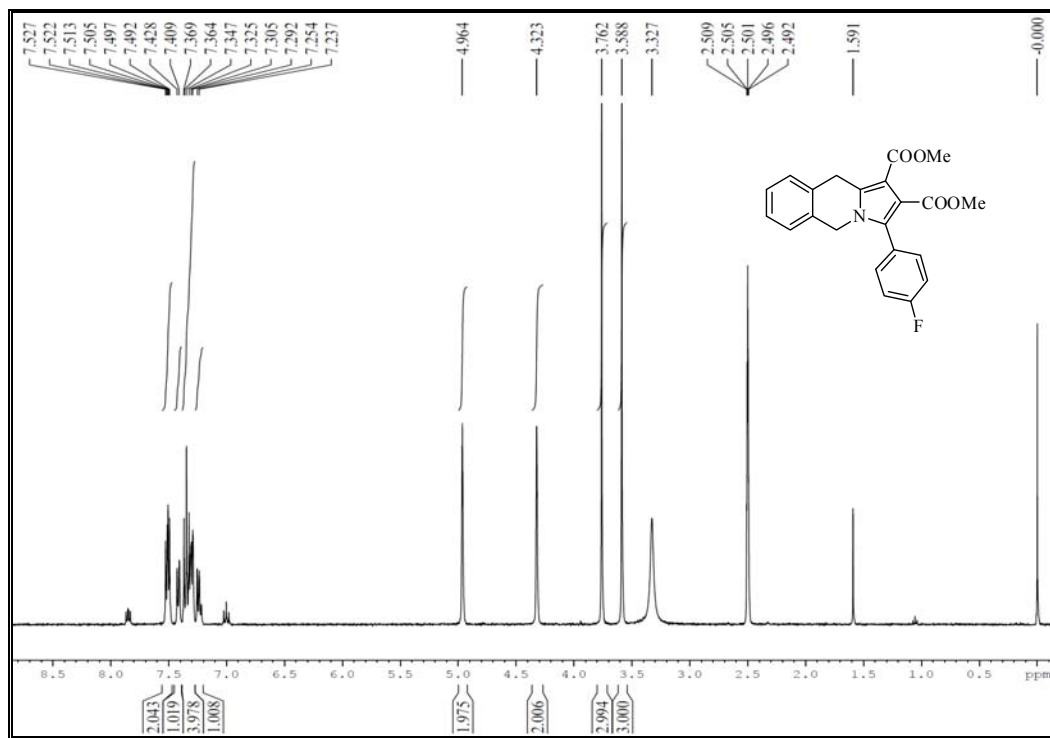
2.4.1 ^1H NMR Spectrum for compound **80a**.

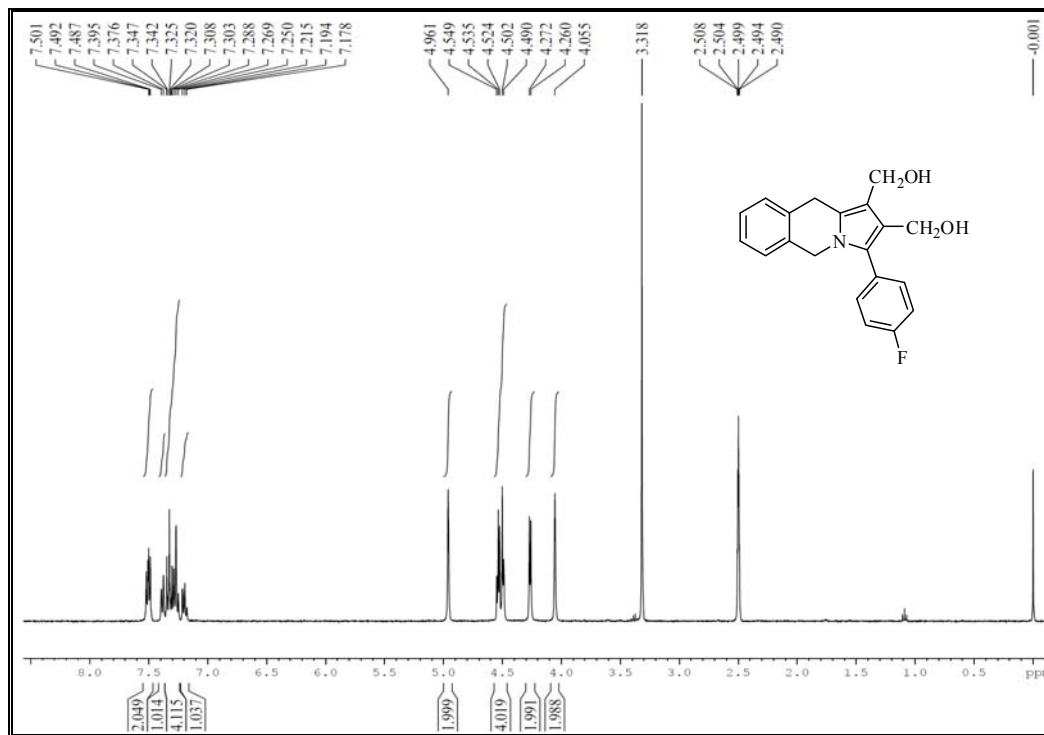
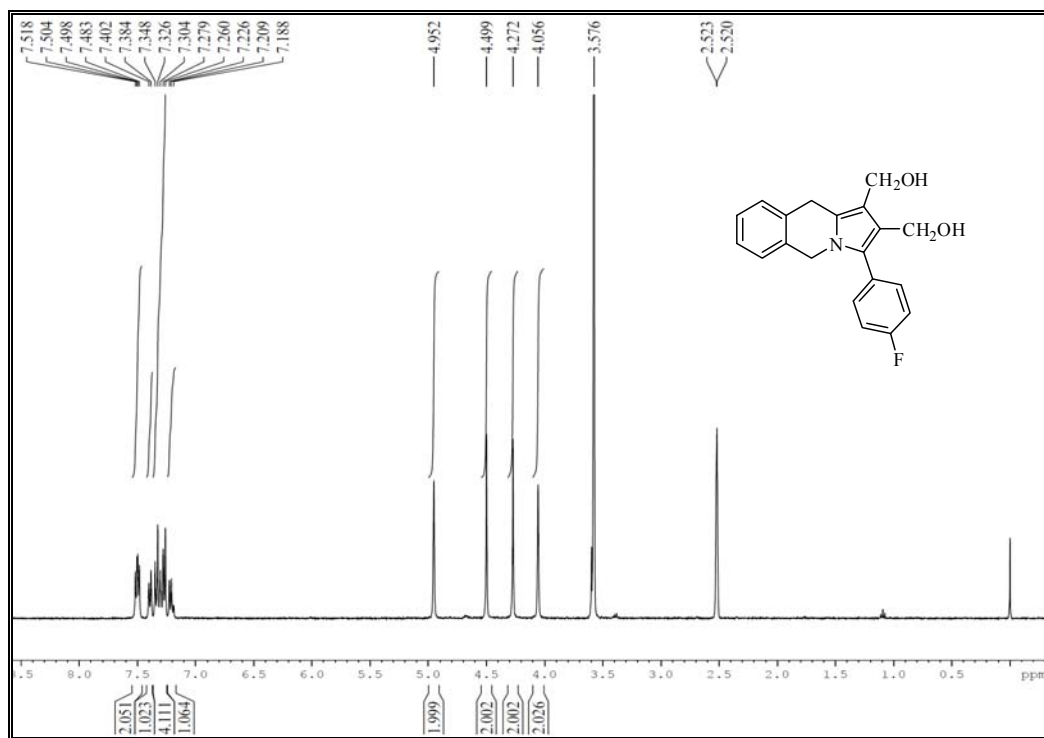


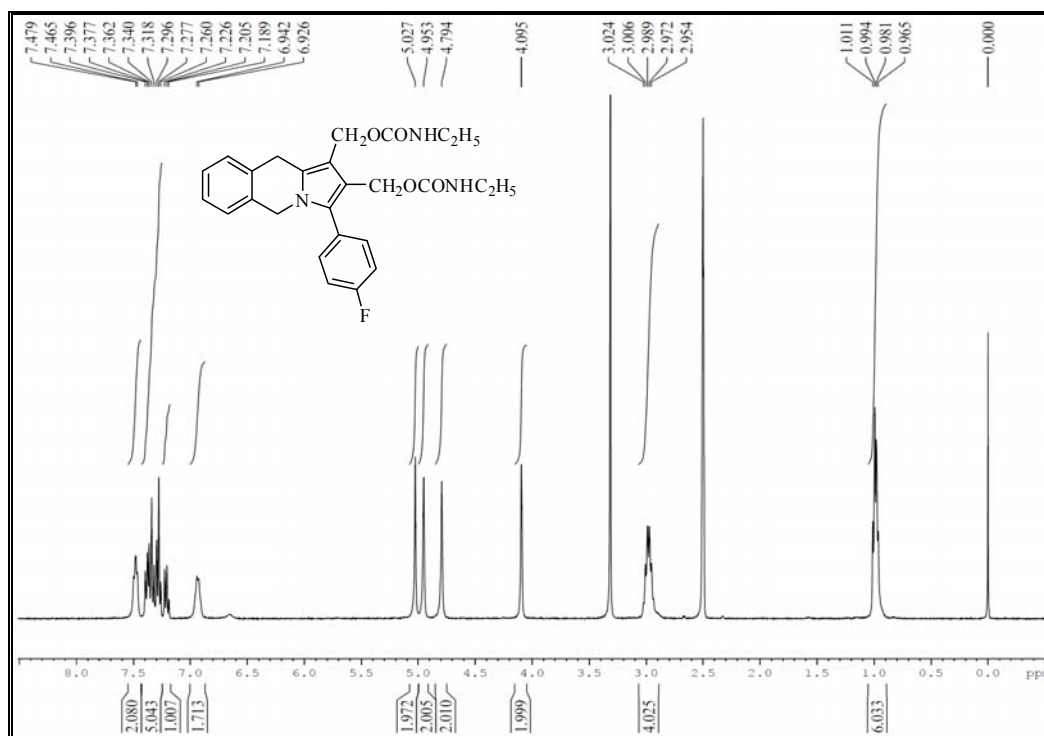
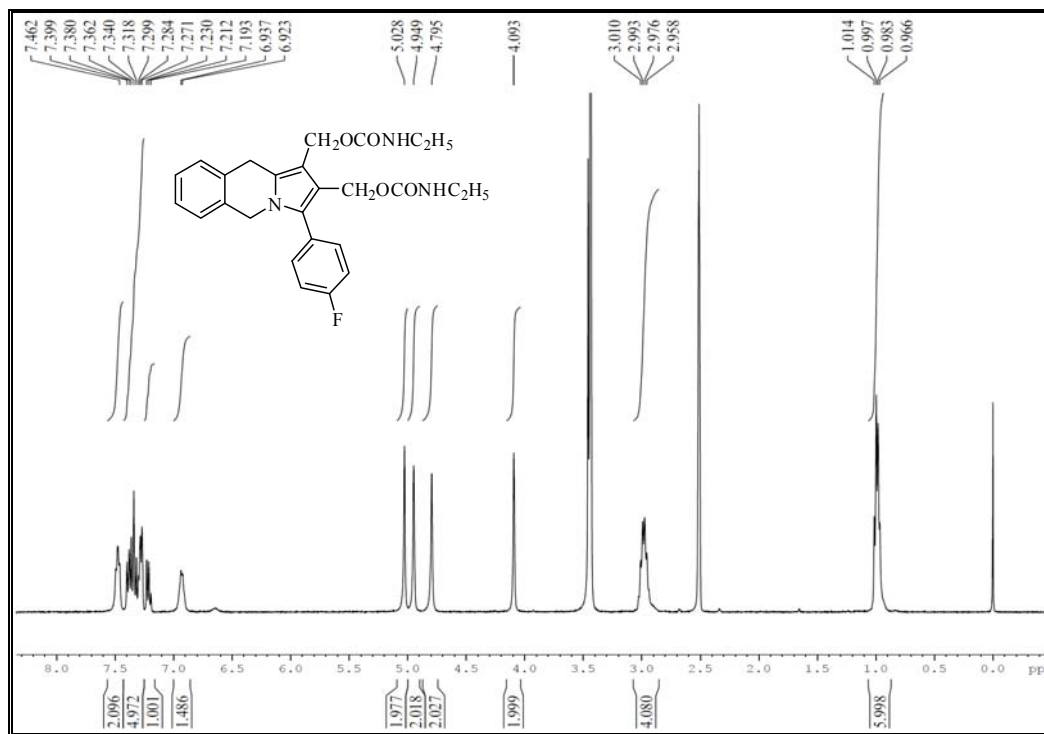
2.4.2 ^1H NMR Spectrum for compound **81a**.

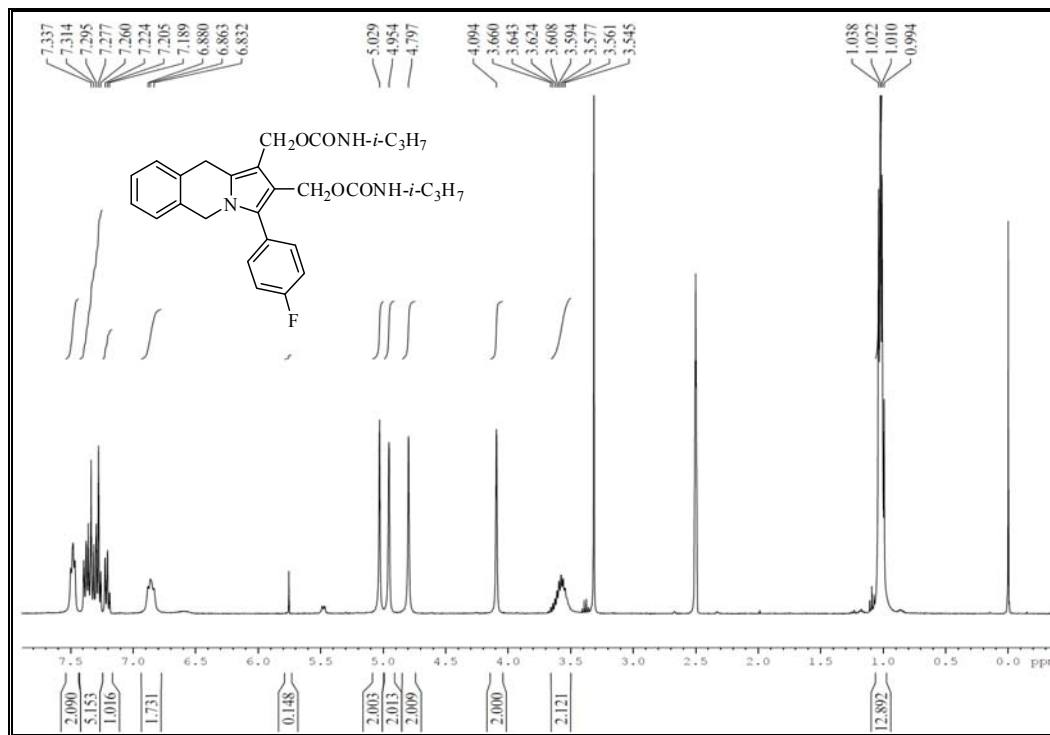
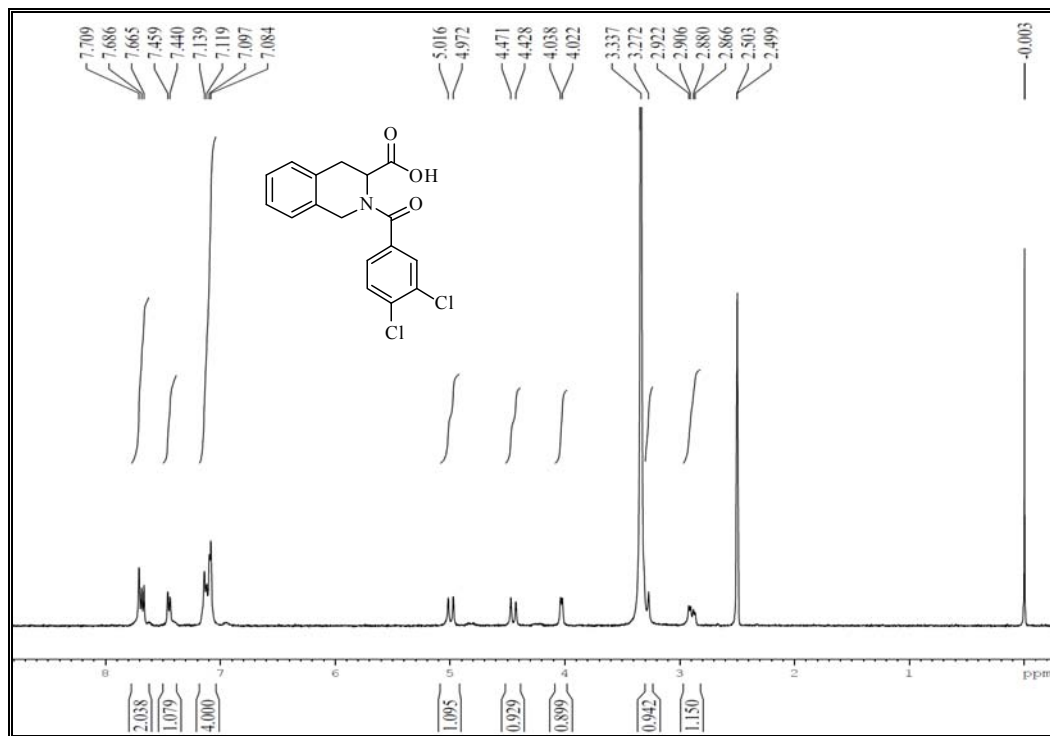


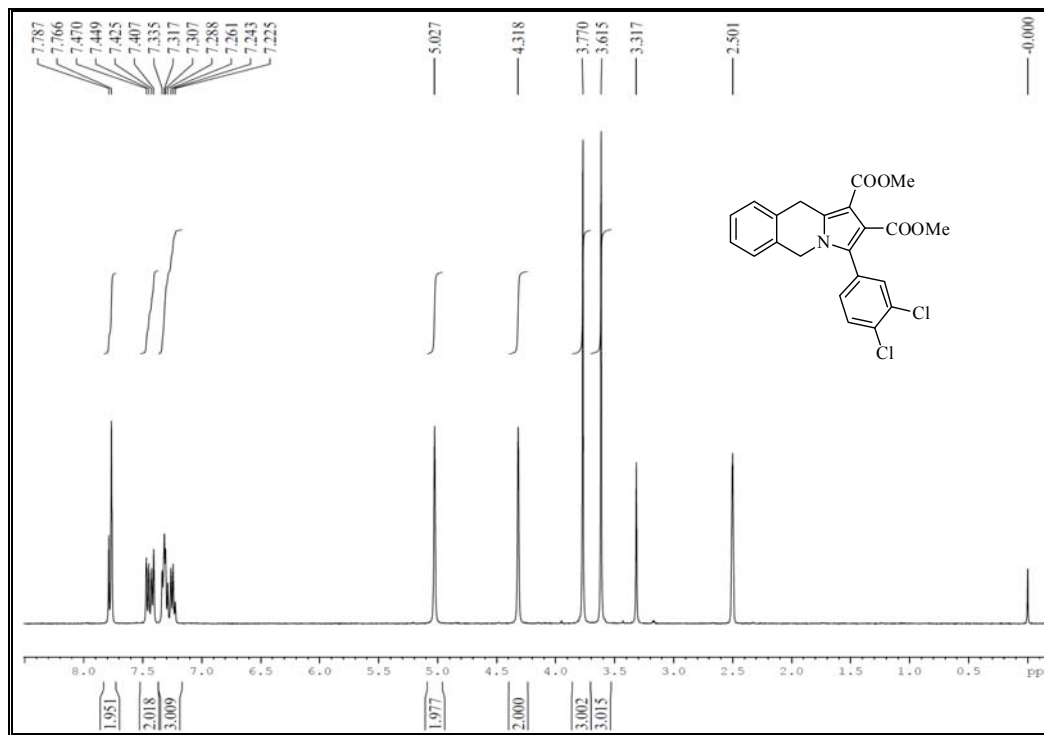
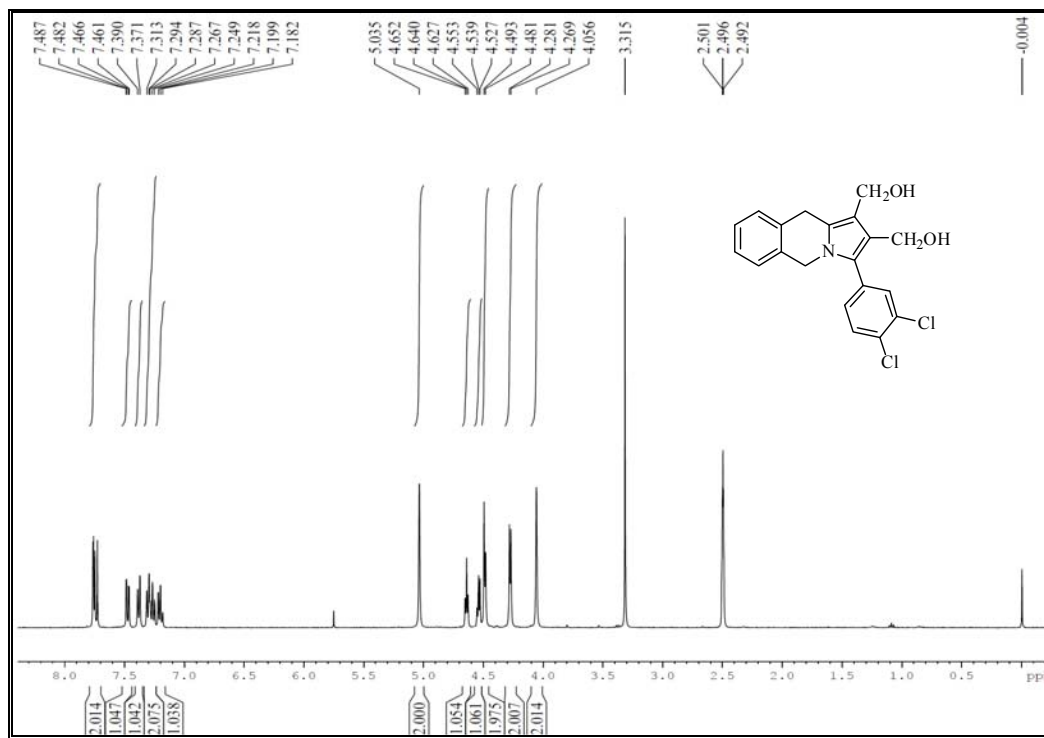
2.4.3 ^1H NMR Spectrum for compound **83a**.2.4.4 ^1H NMR Spectrum for compound **84a**.

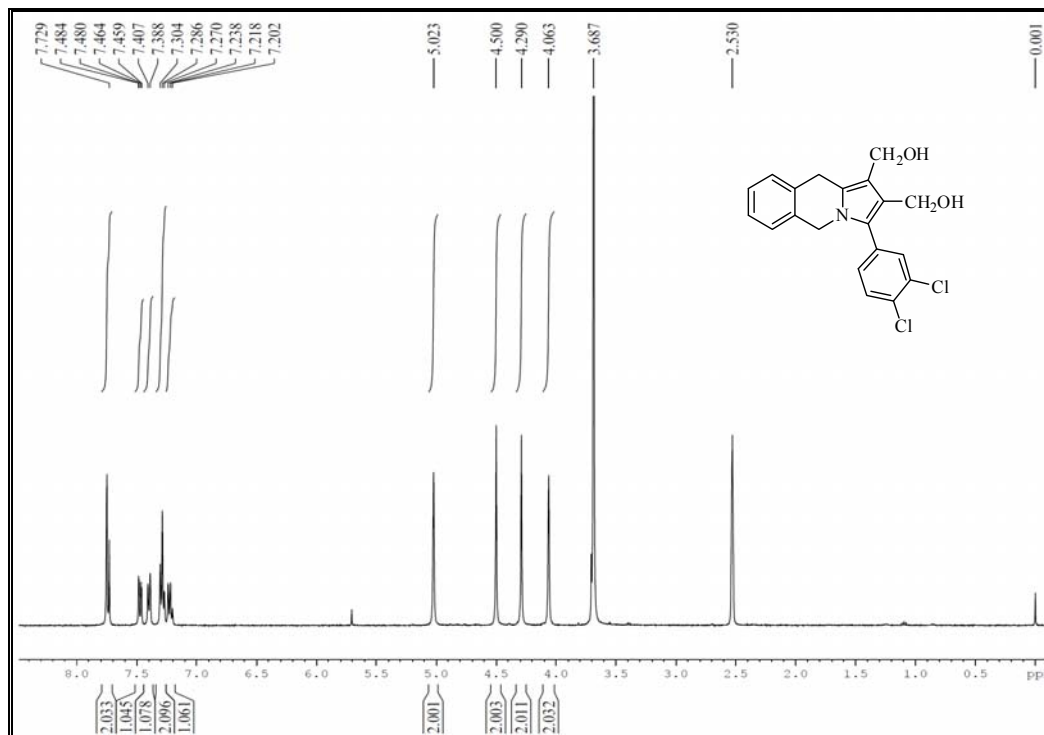
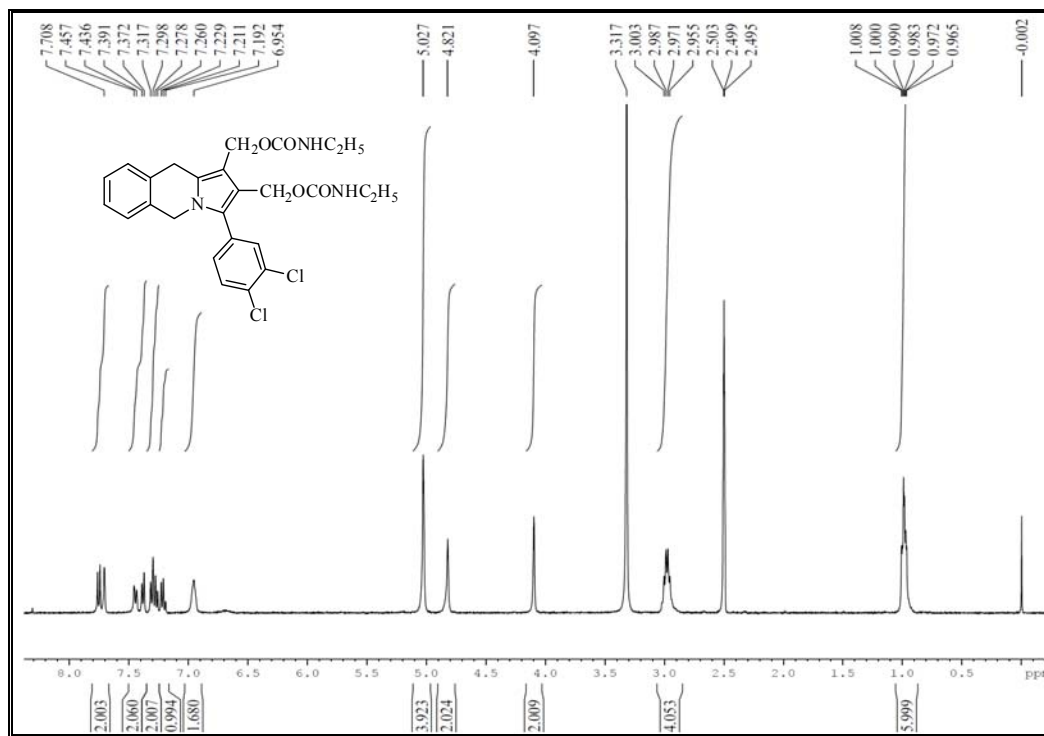
2.4.5 ^1H NMR Spectrum for compound **79d**.2.4.6 ^1H NMR Spectrum for compound **80d**.

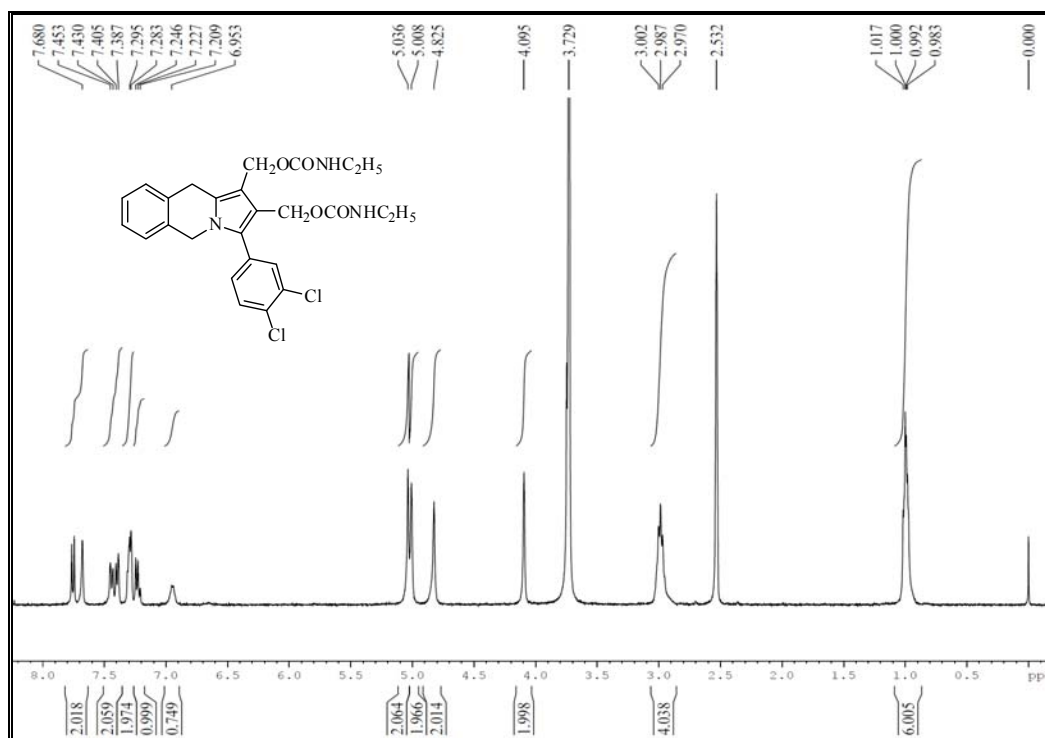
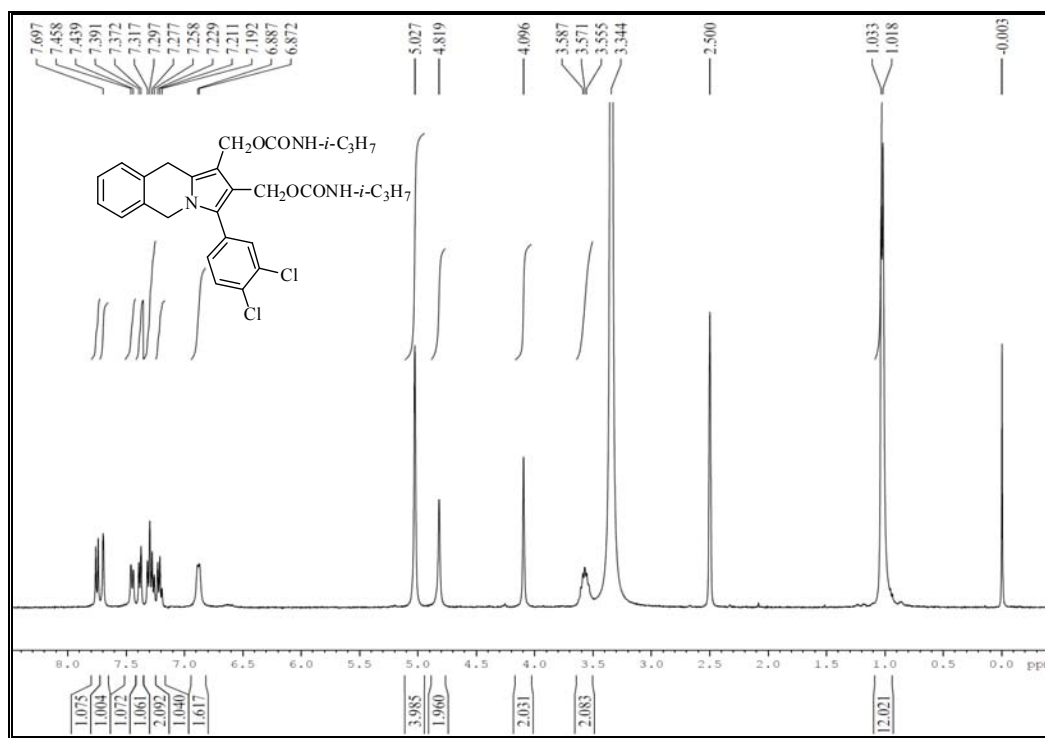
2.4.7 ^1H NMR Spectrum for compound **81d**. D_2O exchange ^1H NMR Spectrum for compound **81d**.

2.4.8 ^1H NMR Spectrum for compound **83d**. D_2O exchange ^1H NMR Spectrum for compound **83d**.

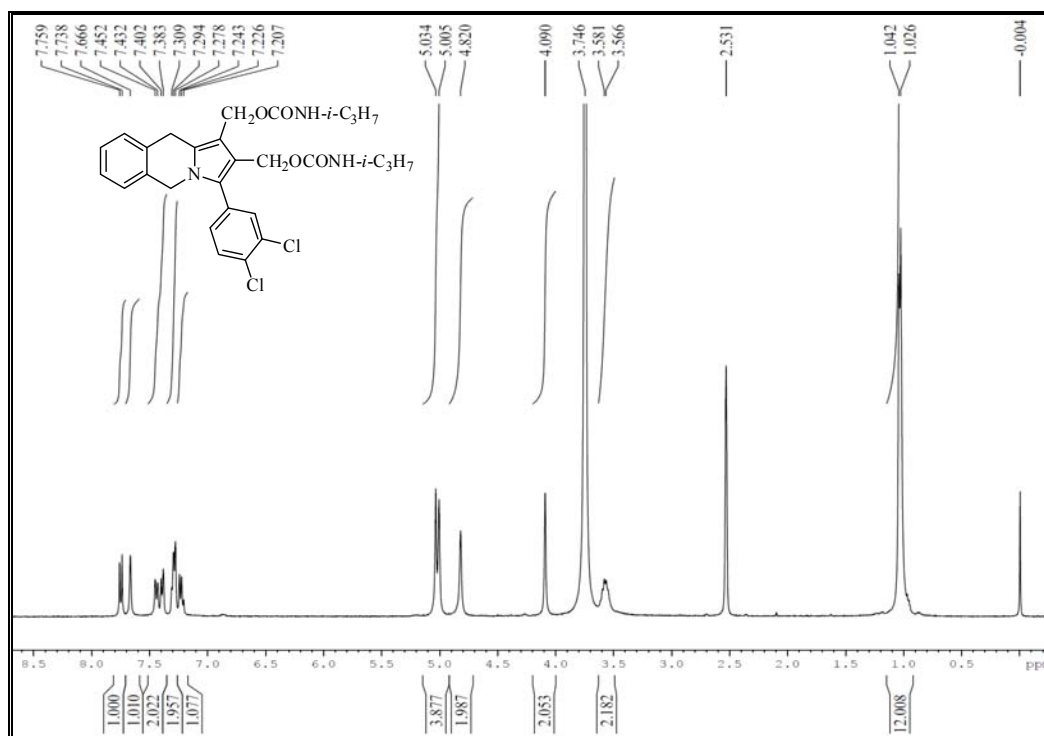
2.4.9 ^1H NMR Spectrum for compound **84d**.2.4.10 ^1H NMR Spectrum for compound **79g**.

2.4.11 ^1H NMR Spectrum for compound **80g**.2.4.12 ^1H NMR Spectrum for compound **81g**.

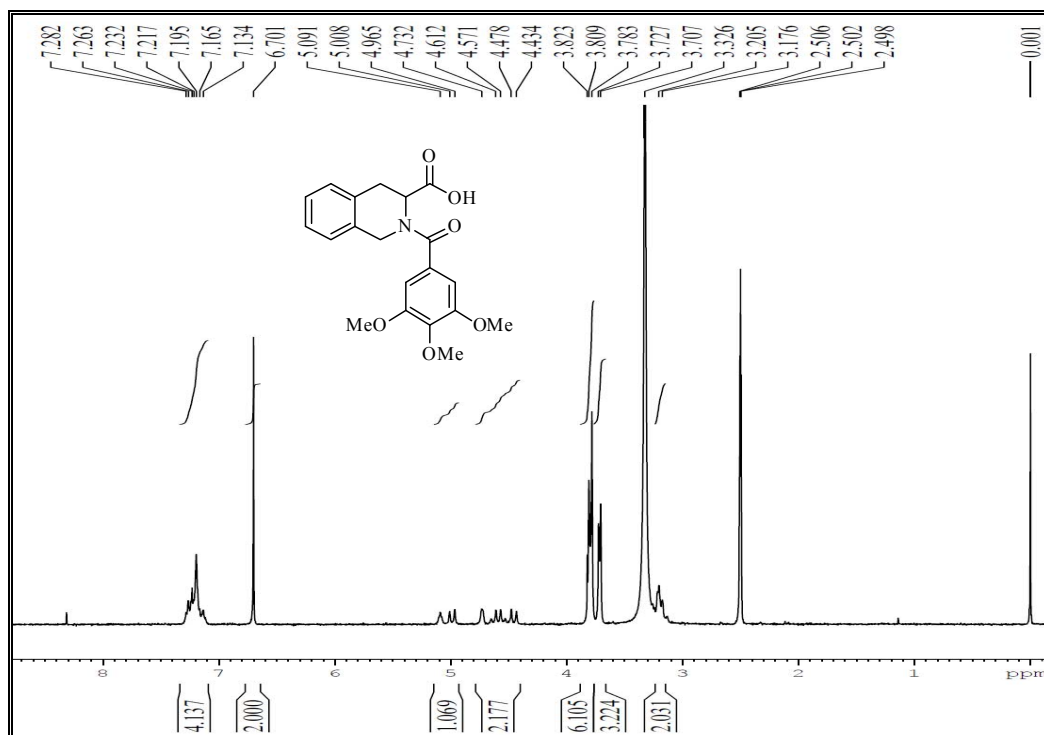
D₂O exchange ¹H NMR Spectrum for compound **81g**.2.4.13 ¹H NMR Spectrum for compound **83g**.

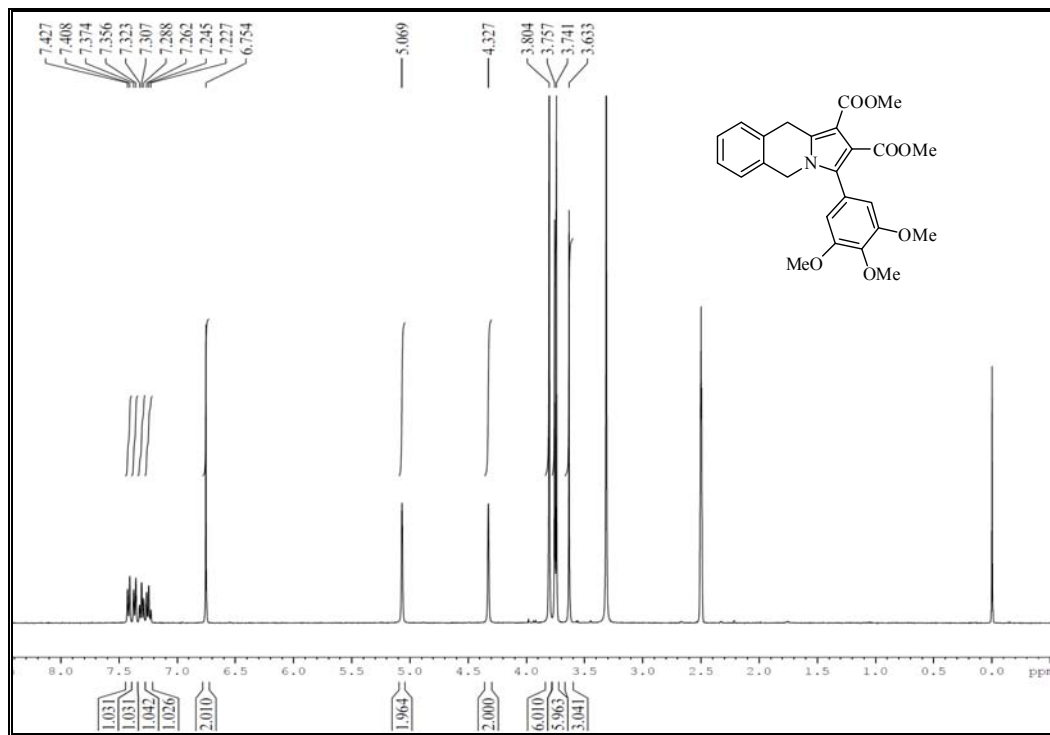
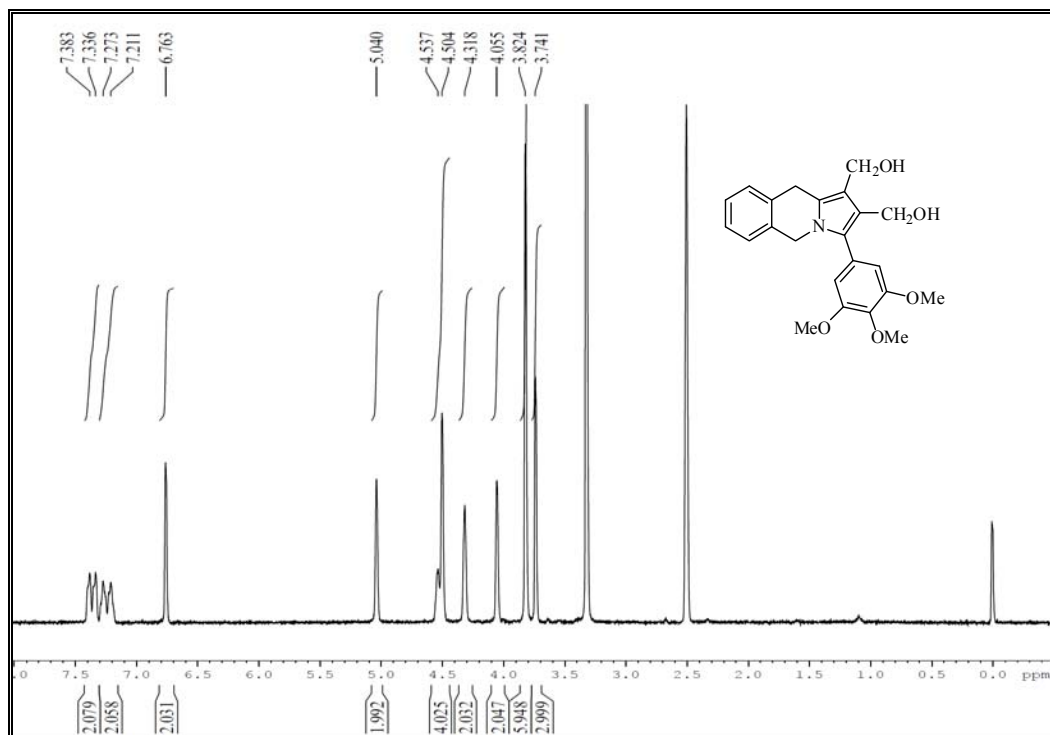
D₂O exchange ¹H NMR Spectrum for compound **83g**.2.4.14 ¹H NMR Spectrum for compound **84g**.

D₂O exchange ¹H NMR Spectrum for compound **84g**.

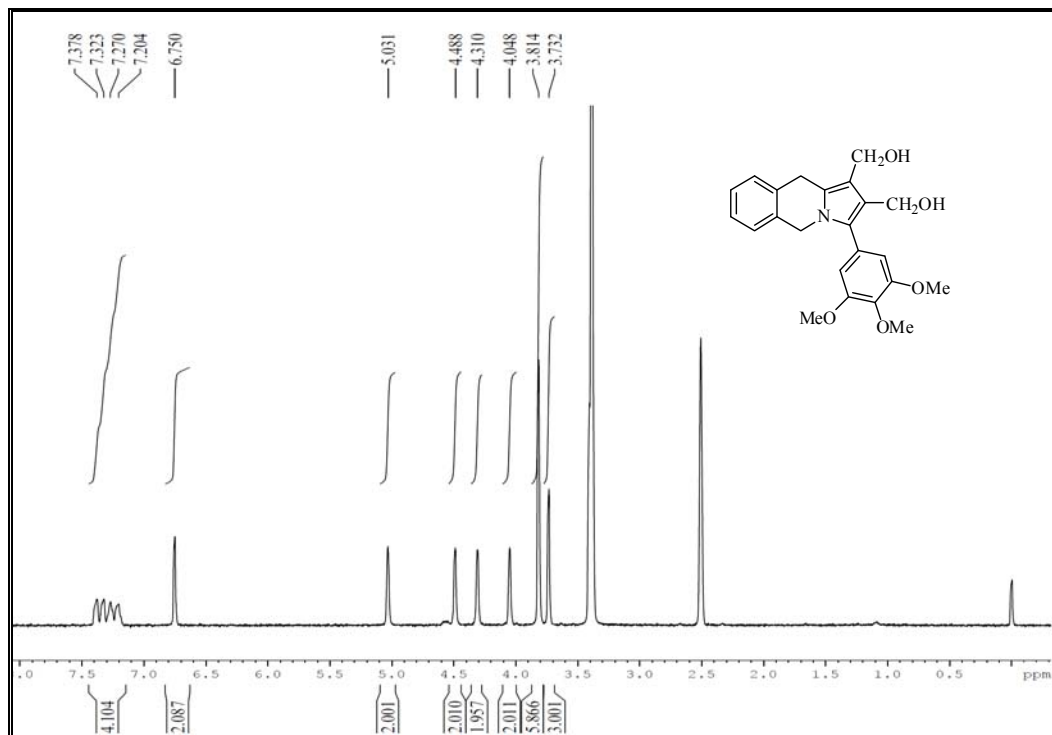


2.4.15 ¹H NMR Spectrum for compound **79j**.

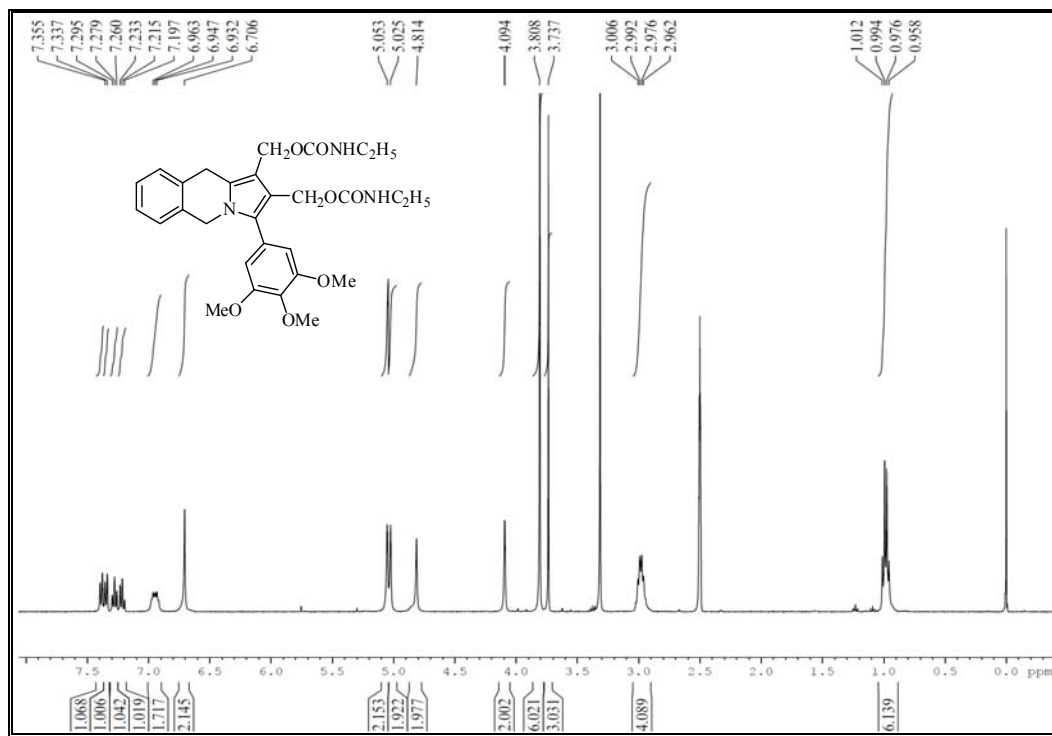


2.4.16 ^1H NMR Spectrum for compound **80j**.2.4.17 ^1H NMR Spectrum for compound **81j**.

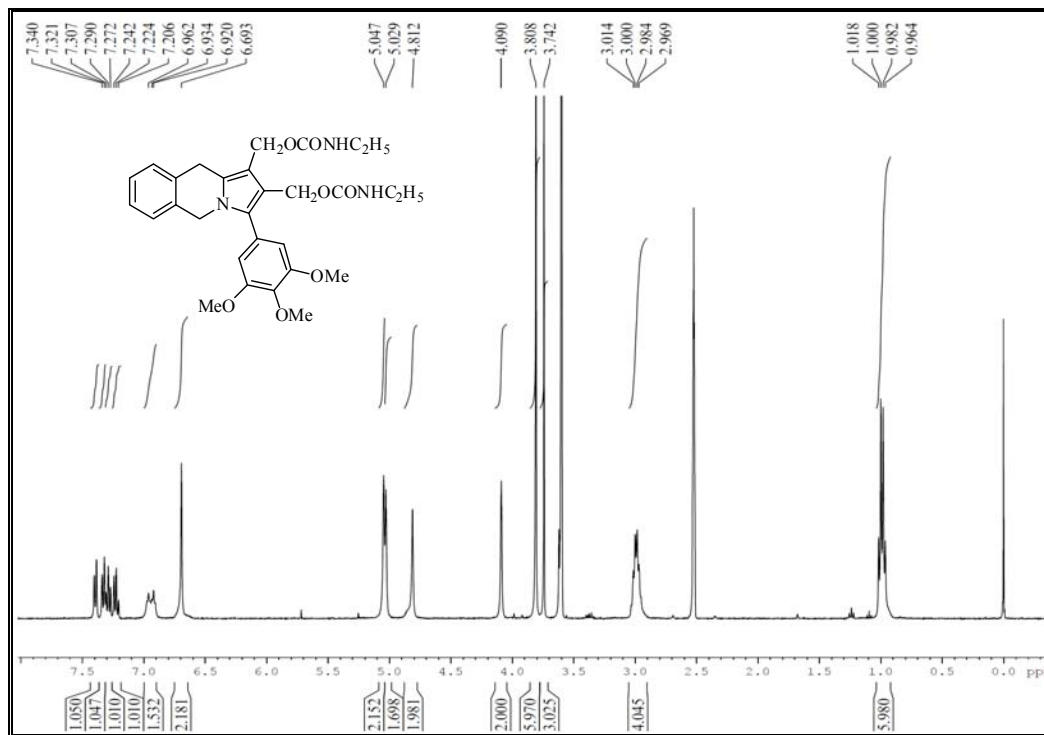
D₂O exchange ¹H NMR Spectrum for compound **81j**.



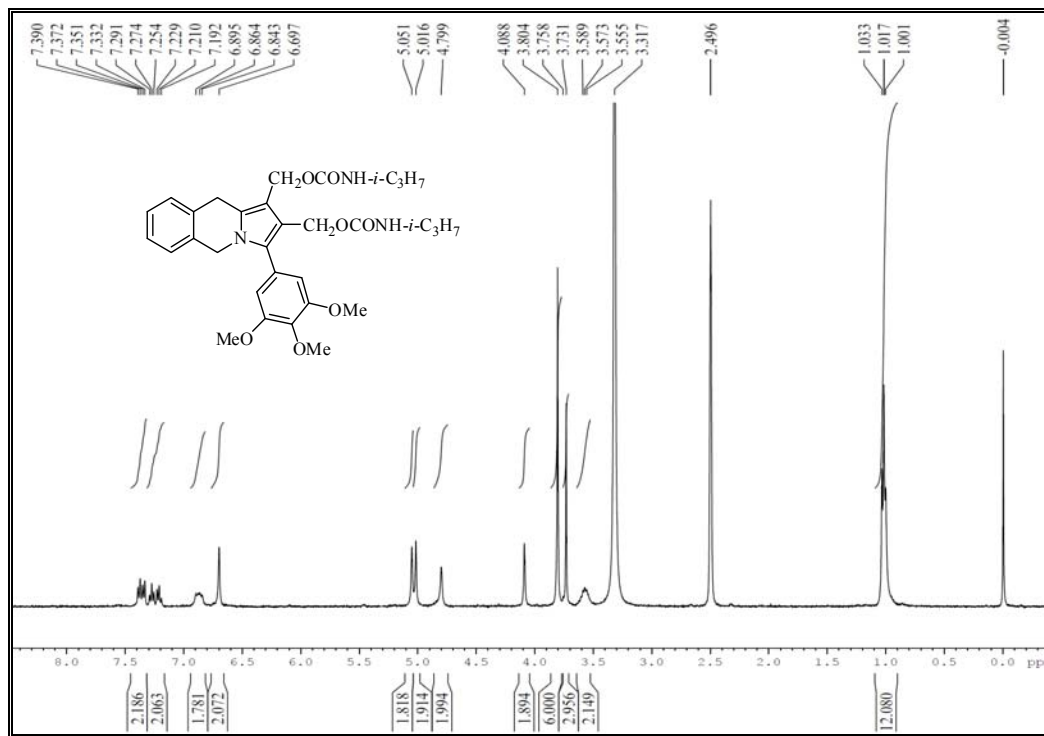
2.4.18 ¹H NMR Spectrum for compound **83j**.



D₂O exchange ¹H NMR Spectrum for compound **83j**.



2.4.18 ¹H NMR Spectrum for compound **84j**.



2.5 High Performance Liquid Chromatography Analysis (HPLC)

The prime and specific objective of the work is to develop simple and Rapid Liquid Chromatographic method of newly synthesized compounds bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives (**81**) and their bis(alkylcarbamate) derivatives (**82-84**) for the Purity Determination by High-performance liquid chromatographic method.

2.6 HPLC Experimental

Instrument

Liquid chromatography was performed using Hitachi D-2000 Elite instrument with Mightysil RP-18 GP 250-4.6 (5 μ m) column.

Reagents

Acetonitrile (HPLC grade)

Tetrahydrofuran (HPLC grade)

Water (Milli-Q)

Blank

Diluent was used as blank.

Sample Preparation

Weigh accurately 10 mg of different new synthesized compounds into 100 ml volumetric flask. Add 60 ml of diluent into the flask and sonicate for 30 minutes with normal hand-shaking. Cool the flask at room temperature and dilute it to volume with diluent. Filter 10 ml of this solution through 0.45 μ m nylon syringe filter. The concentration obtained is 100 μ g/ml.

All samples were prepared as per above mentioned sample preparation method.

2.7 Chromatograms

Chromatographic condition

Sample Code : **81a** (BO-1107)

Mobile phase : MeCN : THF (50 : 50 v/v)

Column : Mightysil RP-18 GP 250-4.6 (5 μ m)

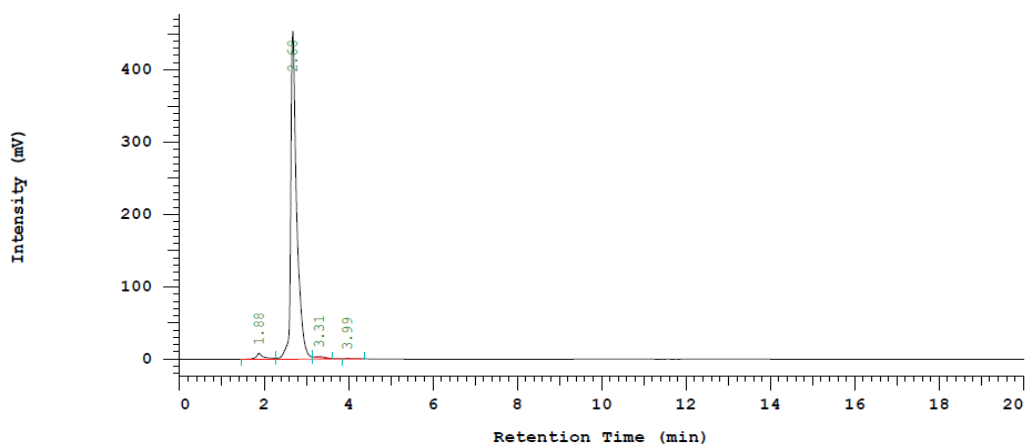
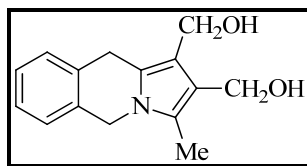
Column Temperature: 27 $^{\circ}$ C

Flow rate : 1.0 mL/min

Detection : 254 nm

Injection volume : 10 μ L

Diluent : Mobile phase



No.	RT	Area	Conc 1	BC
1	1.88	115294	2.493	BV
2	2.68	4483360	96.949	VV
3	3.31	18592	0.402	TBB
4	3.99	7196	0.156	TBB
		4624442	100.000	

Chromatographic conditionSample Code : **81f** (BO-1112)

Mobile phase : MeCN : THF (50 : 50 v/v)

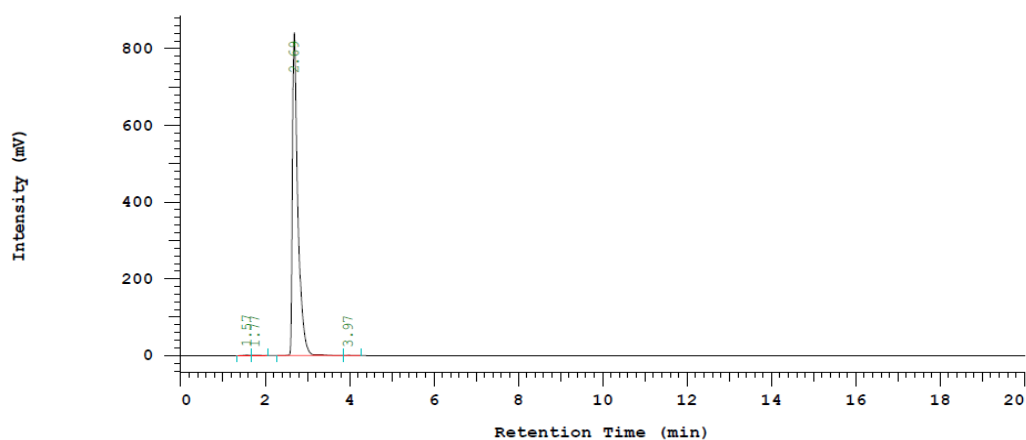
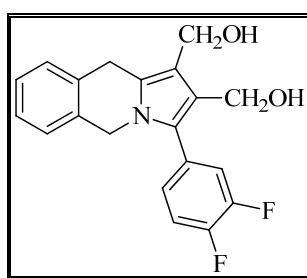
Column : Mightysil RP-18 GP 250-4.6 (5 μ m)Column Temperature: 27 $^{\circ}$ C

Flow rate : 1.0 mL/min

Detection : 254 nm

Injection volume : 10 μ L

Diluent : Mobile phase



No.	RT	Area	Conc 1	BC
1	1.57	18180	0.243	BV
2	1.77	14411	0.192	VB
3	2.69	7456588	99.521	BB
4	3.97	3294	0.044	TBB
		7492473	100.000	

Chromatographic conditionSample Code : **82f** (BO-1113)

Mobile phase : MeCN : THF (50 : 50 v/v)

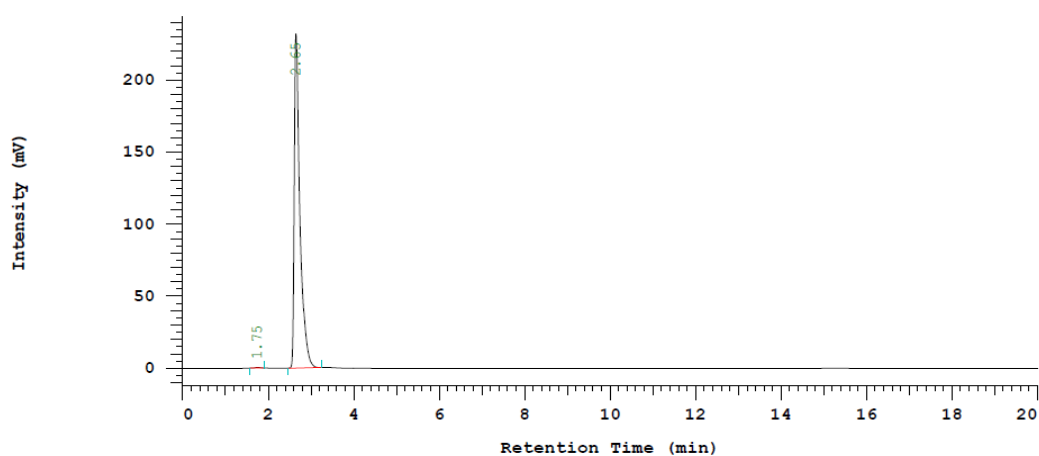
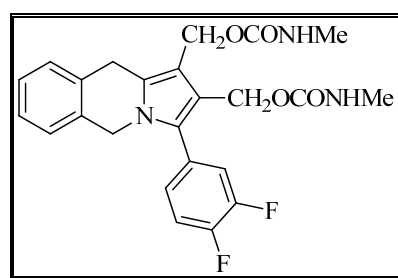
Column : Mightysil RP-18 GP 250-4.6 (5 μ m)Column Temperature: 27 $^{\circ}$ C

Flow rate : 1.0 mL/min

Detection : 254 nm

Injection volume : 10 μ L

Diluent : Mobile phase



No.	RT	Area	Conc 1	BC
1	1.75	4275	0.203	BB
2	2.65	2102996	99.797	BB
		2107271	100.000	

Chromatographic conditionSample Code : **83h** (BO-1516)

Mobile phase : MeCN : THF (50 : 50 v/v)

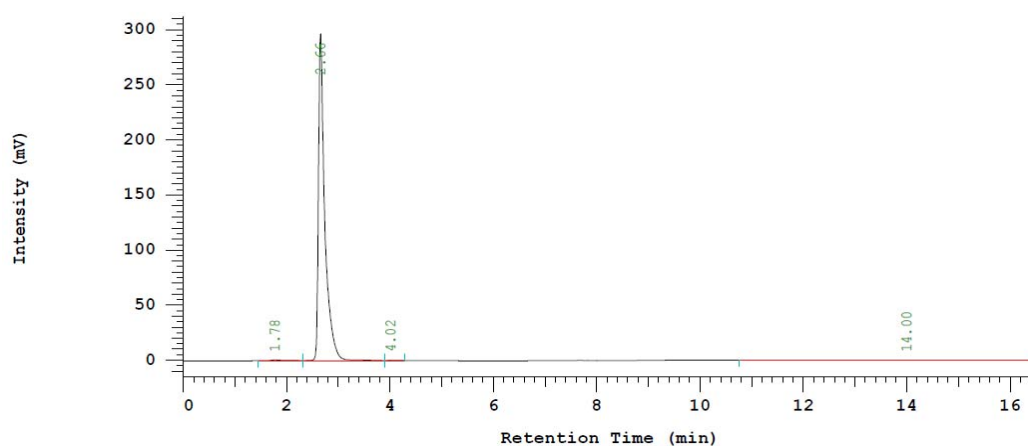
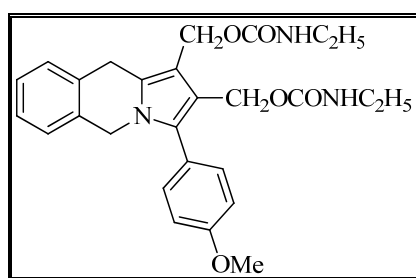
Column : Mightysil RP-18 GP 250-4.6 (5 μ m)Column Temperature: 27 $^{\circ}$ C

Flow rate : 1.0 mL/min

Detection : 254 nm

Injection volume : 10 μ L

Diluent : Mobile phase



No.	RT	Area	Conc 1	BC
1	1.78	10357	0.396	BV
2	2.66	2589139	99.051	VB
3	4.02	1047	0.040	BB
4	14.00	13402	0.513	BB
		2613945	100.000	

Chromatographic conditionSample Code : **84c** (BO-1504)

Mobile phase : MeCN : THF (50 : 50 v/v)

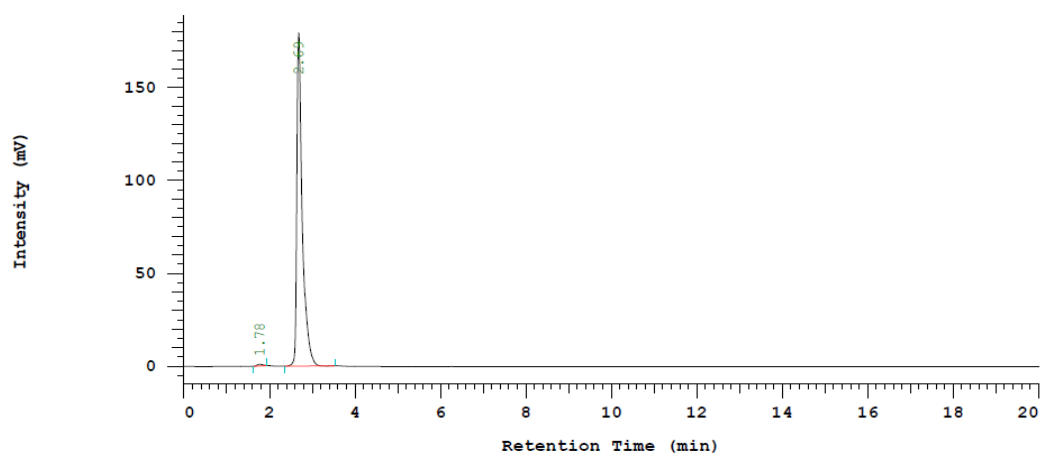
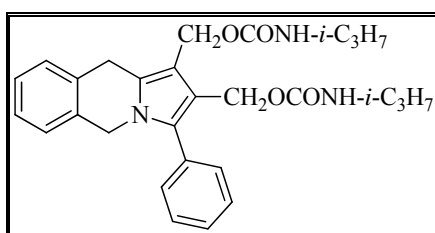
Column : Mightysil RP-18 GP 250-4.6 (5 μ m)Column Temperature: 27 $^{\circ}$ C

Flow rate : 1.0 mL/min

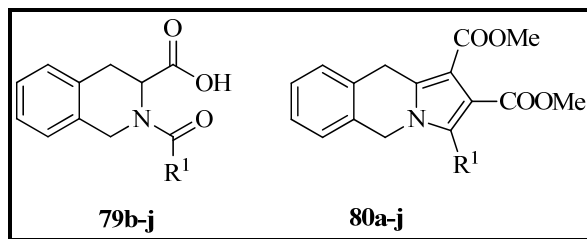
Detection : 254 nm

Injection volume : 10 μ L

Diluent : Mobile phase

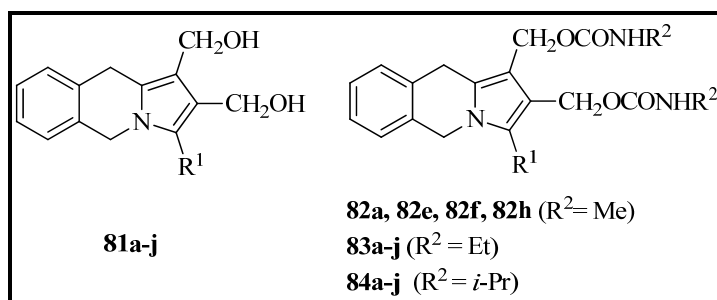


No.	RT	Area	Conc 1	BC
1	1.78	9315	0.590	BB
2	2.69	1570790	99.410	BB
		1580105	100.000	

Table 2.3 Elemental analysis of compounds **79b–j** and **80a–j**.

Compd.	MF	MW	CHN Calculated (%)			CHN Found (%)		
			C	H	N	C	H	N
79b	C ₁₃ H ₁₅ NO ₃	233.26	66.94	6.48	6.00	66.64	6.48	5.89
79c^a	C ₁₇ H ₁₅ NO ₃	281.11	72.58	5.37	4.98	ND	ND	ND
79d	C ₁₇ H ₁₄ FNO ₃	299.30	68.22	4.71	4.68	68.08	4.57	4.42
79e	C ₁₇ H ₁₄ ClNO ₃	315.75	64.67	4.47	4.44	64.52	4.56	4.33
79f	C ₁₇ H ₁₃ F ₂ NO ₃	317.29	64.35	4.13	4.41	64.21	4.26	4.56
79g	C ₁₇ H ₁₃ C ₁₂ NO ₃	350.20	58.31	3.74	4.00	58.19	4.04	3.87
79h	C ₁₈ H ₁₇ NO ₄	311.33	69.44	5.50	4.50	69.12	5.62	4.27
79i	C ₁₉ H ₁₉ NO ₅	341.13	66.85	5.61	4.10	66.65	5.66	3.86
79j	C ₂₀ H ₂₁ NO ₆	371.38	64.68	5.70	3.77	64.44	5.50	3.44
80a^a	C ₁₇ H ₁₇ NO ₄	299.12	68.21	5.72	4.68	ND	ND	ND
80b	C ₁₈ H ₁₉ NO ₄	313.13	68.99	6.11	4.47	68.96	6.05	4.38
80c	C ₂₂ H ₁₉ NO ₄	361.39	73.12	5.30	3.88	72.91	5.30	3.53
80d	C ₂₂ H ₁₈ FNO ₄	379.38	69.65	4.78	3.69	69.54	4.82	3.78
80e	C ₂₂ H ₁₈ ClNO ₄	395.84	66.75	4.58	3.54	66.52	4.85	3.66
80f	C ₂₂ H ₁₇ F ₂ NO ₄	397.37	66.50	4.31	3.52	66.15	4.44	3.36
80g	C ₂₂ H ₁₇ Cl ₂ NO ₄	430.28	61.41	3.98	3.26	61.33	3.95	2.89
80h	C ₂₃ H ₂₁ NO ₅	391.42	70.58	5.41	3.58	70.25	5.44	3.49
80i	C ₂₄ H ₂₃ NO ₆	421.15	68.40	5.50	3.32	68.02	5.53	2.94
80j	C ₂₅ H ₂₅ NO ₇	451.16	66.51	5.58	3.10	66.25	5.59	3.01

^aknown compound

Table 2.4 Elemental analysis of compounds **81a–j**, **82**, **83a–j**, and **84a–j**.

Compd.	MF	MW	CHN Calculated (%)			CHN Found (%)		
			C	H	N	C	H	N
81a	C ₁₅ H ₁₇ NO ₂	243.30	74.05	7.04	5.76	74.24	6.97	5.71
81b^a	C ₁₆ H ₁₉ NO ₂	257.33	74.68	7.44	5.44	ND	ND	ND
81c	C ₂₀ H ₁₉ NO ₂	305.37	78.66	6.27	4.59	78.48	6.42	4.41
81d	C ₂₀ H ₁₈ FNO ₂	323.36	74.29	5.61	4.33	74.38	5.68	3.98
81e	C ₂₀ H ₁₈ ClNO ₂	339.82	70.69	5.34	4.12	70.42	5.37	4.05
81f	C ₂₀ H ₁₇ F ₂ NO ₂	341.35	70.37	5.02	4.10	70.08	5.01	3.76
81g	C ₂₀ H ₁₇ Cl ₂ NO ₂	374.26	64.18	4.58	3.74	63.89	4.40	3.87
81h	C ₂₁ H ₂₁ NO ₃	335.40	75.20	6.31	4.18	75.36	6.30	4.13
81i	C ₂₂ H ₂₃ NO ₄	365.16	72.31	6.34	3.83	72.25	6.34	3.95
81j	C ₂₃ H ₂₅ NO ₅	395.17	69.86	6.37	3.54	69.71	6.37	3.45
82a	C ₁₉ H ₂₃ N ₃ O ₄	357.40	63.85	6.49	11.76	63.54	6.57	11.64
82e	C ₂₄ H ₂₄ ClN ₃ O ₄	453.93	63.50	5.33	9.26	63.33	5.35	9.20
82f	C ₂₄ H ₂₃ F ₂ N ₃ O ₄	455.47	63.29	5.09	9.23	63.00	5.12	9.16
82h	C ₂₅ H ₂₇ N ₃ O ₅	449.51	66.80	6.05	9.35	66.72	6.15	9.24
83a	C ₂₁ H ₂₇ N ₃ O ₄	385.46	65.44	7.06	10.90	65.29	7.14	10.89
83b	C ₂₂ H ₂₉ N ₃ O ₄	399.48	66.14	7.32	10.52	66.35	7.53	10.74
83c	C ₂₆ H ₂₉ N ₃ O ₄	447.53	69.78	6.53	9.39	69.64	6.54	9.37
83d	C ₂₆ H ₂₈ FN ₃ O ₄	465.52	67.08	6.06	9.03	67.24	6.02	8.88
83e	C ₂₆ H ₂₈ ClN ₃ O ₄	481.97	64.79	5.86	8.72	64.54	5.84	8.82
83f	C ₂₆ H ₂₇ F ₂ N ₃ O ₄	483.51	64.59	5.63	8.69	64.50	5.60	8.69
83g	C ₂₆ H ₂₇ Cl ₂ N ₃ O ₄	516.42	60.47	5.27	8.14	60.34	5.13	8.10
83h	C ₂₇ H ₃₁ N ₃ O ₅	477.55	67.91	6.54	8.80	67.73	6.86	8.57

83i	C ₂₈ H ₃₃ N ₃ O ₆	507.58	66.26	6.55	8.28	66.34	6.45	8.11
83j	C ₂₉ H ₃₅ N ₃ O ₇	537.60	64.79	6.56	7.82	64.68	6.42	7.97
84a	C ₂₃ H ₃₁ N ₃ O ₄	413.51	66.81	7.56	10.16	66.69	7.66	10.10
84b	C ₂₄ H ₃₃ N ₃ O ₄	427.54	67.42	7.78	9.83	67.11	7.55	9.71
84c	C ₂₈ H ₃₃ N ₃ O ₄	475.58	70.71	6.99	8.84	70.59	6.92	8.67
84d	C ₂₈ H ₃₂ FN ₃ O ₄	493.57	68.14	6.53	8.51	68.31	6.40	8.60
84e	C ₂₈ H ₃₂ ClN ₃ O ₄	510.02	65.94	6.32	8.24	65.76	6.17	8.35
84f	C ₂₈ H ₃₁ F ₂ N ₃ O ₄	511.56	65.74	6.11	8.21	65.51	6.05	8.38
84g	C ₂₈ H ₃₁ Cl ₂ N ₃ O ₄	544.47	61.77	5.74	7.72	61.84	5.67	7.56
84h	C ₂₉ H ₃₅ N ₃ O ₅	505.61	68.89	6.98	8.31	68.93	6.96	8.14
84i	C ₃₀ H ₃₇ N ₃ O ₆	535.63	67.27	6.96	7.84	67.27	7.06	7.62
84j	C ₃₁ H ₃₉ N ₃ O ₇	565.66	65.82	6.95	7.43	65.68	6.74	7.11

^anot determine

Section A

Chapter 3. Antitumor Activity of bis(hydroxymethyl)-5,10- dihydropyrrolo[1,2-*b*]isoquinolin- 1-yl derivatives and their bis(alkylcarbamate) derivatives

3.0 Experimental Protocols

3.0.1 Cytotoxicity Assays

The effects of the newly synthesized compounds on cell growth were determined in T-cell acute lymphocytic leukemia (CCRF-CEM) and their resistant subcell lines (CCRF-CEM/Taxol and CCRF-CEM/VBL) by the XTT assay¹⁰³ and human solid tumor cells (*i.e.* breast carcinoma MX-1 and colon carcinoma HCT-116) the SRB assay¹⁰⁴ in a 72 h incubation using a microplate spectrophotometer as described previously.¹⁰⁵ After the addition of phenazine methosulfate-XTT solution at 37 °C for 6 h, absorbance at 450 and 630 nm was detected on a microplate reader (EL 340; Bio-Tek Instruments Inc., Winooski, VT). The cytotoxicity of the newly synthesized compounds against non-small cell lung cancer H1299, human prostate cancer PC3, oral carcinoma OECM1 and human glioma U87 were determined by the Alamar blue assay¹⁰⁶ in a 72 h incubation using a microplate spectrophotometer as described previously. After the addition of Alamar blue solution, it was incubated at 37 °C for 6 h. Absorbance at 570 and 600 nm was detected on a microplate reader. IC₅₀ values were determined from dose-effect relationship at six or seven concentrations of each drug by using the CompuSyn software by Chou and Martin¹⁰⁷ based on the median-effect principle and plot.^{108,109} Ranges given for taxol and vinblastine were mean ± SE (n = 4).

3.0.2 *In vivo* studies

Athymic nude mice bearing the nu/nu gene were used for human breast tumor MX-1 and human ovarian adenocarcinoma SK-OV-3 xenograft. Outbred Swiss-background mice were obtained from the National Cancer Institute (Frederick, MD). Male mice 8 weeks old or older weighing about 22 g were used for the experiments. Drug was administered via the tail vein by *i.v.* injection.¹⁰⁵ Tumor volumes were assessed by measuring length × width × height (or width) by using caliper. Vehicle used was DMSO (50 µL) and Tween 80 (40 µL) in saline (160 µL). The maximal tolerable dose of the tested compound was determined and applied for the *in vivo* antitumor activity assay. All animal studies were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Animals

and the protocol approved by the Memorial Sloan-Kettering Cancer Center's Institutional Animal Care and Use Committee.

3.0.3 Alkaline agarose gel shift assay

Formation of DNA cross-linking was analyzed by alkaline agarose gel electrophoresis.¹¹⁰ In brief, purified pEGFP-N1 plasmid DNA (1.5 µg) was mixed with various concentrations (1–20 µM) of **81a**, **83a**, **81h** and **84h** in 40 µL binding buffer (3 mM sodium chloride/1mM sodium phosphate, pH 7.4, and 1 mM EDTA). The reaction mixture was incubated at 37°C for 2 h. At the end of reaction, the plasmid DNA was linearized by digestion with *Bam*HI and followed by precipitation with ethanol. The DNA pellets were dissolved and denatured in alkaline buffer (0.5 N NaOH–10mM EDTA). An aliquot of 20 µL of DNA solution (1 µg) was mixed with a 4 µL of 6 X alkaline loading dye and then electrophoretically resolved on a 0.8 % alkaline agarose gel with NaOH–EDTA buffer at 4 °C. The electrophoresis was carried out at 18 V for 22 h. After staining the gels with an ethidium bromide solution, and the DNA was then visualized under UV light.

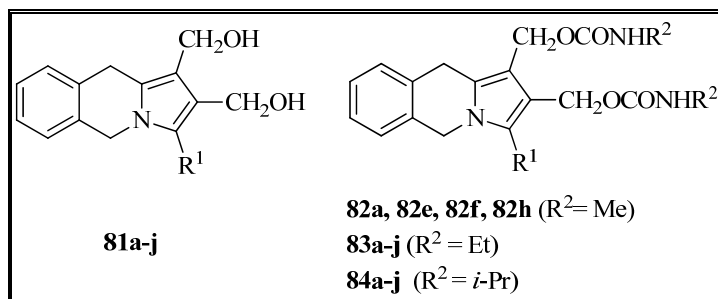
3.0.4 Flow cytometric analysis

The effects of **81a** on cell cycle distribution were analyzed with a flow cytometer as previously described.⁹⁶ Briefly, human non-small cell lung carcinoma H1299 cells were treated with **81a** at 1.25, 2.5, and 5 µM for 24 h. The attached cells were then trypsinized, washed with phosphate buffer saline (PBS), and fixed with ice-cold 70% ethanol for 30 min. The cells were stained with 4 µg/ml propidium iodide (PI) in PBS containing 1% Triton X-100 and 0.1 mg/ml RNase A. The stained cells were then analyzed using the FACS SCAN flow cytometer (Becton Dickinson, San Joes, CA, USA). The percentage of the cells in each cell cycle phase was determined using the ModFit LT 2.0 software based on the DNA histograms.

3.1 *In vitro* cytotoxicity

Table 3.1 shows the antiproliferative activities of the newly synthesized bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline derivatives (**81**) and their bis(alkylcarbamate) derivatives (**82**, **83** and **84**) against human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines resistant to Taxol (CCRF-CEM/Taxol) and Vinblastine (CCRF-CEM/VBL) cell growth *in vitro*. It demonstrated that the newly synthesized conjugates possess significant cytotoxicity with IC₅₀ in submicro molar range. In the series of bis(hydroxymethyl) derivatives, it showed that 3-alkyl (Me or Et) substituted derivatives are more cytotoxic than the 3-phenyl substituted compounds. The order of their potency, for example, is **81a** (3-Me) > **81b** (3-Et) > **81c** (3-Ph), indicating that the compounds having a smaller size of substituent at C3 have greater cytotoxicity. A similar observation is found in 3-phenyl derivatives: the cytotoxicity decreases when the number of the methoxy functions increases (**81h** vs. **81i** vs. **81j**). Furthermore, the cytotoxicity decreases when the number of the halo function increases in the halo substituted 3-phenyl derivatives (**81d** > **81f** and **81e** > **81g**). In this study, one can observe that the C3-methoxyphenyl derivatives are somewhat more cytotoxic than the corresponding halophenyl compounds. This suggests that the electron properties of the substituent(s) on the phenyl ring have very little influence over their potency.

Table 3.1 The cytotoxicity of newly synthesized bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives (**81**) and their bis(alkylcarbamate) derivatives (**82** to **84**) against human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines (CCRF-CEM/Taxol and CCRF-CEM/VBL)^a



Compd.	R ¹	Cell Growth inhibition (IC ₅₀ μM)		
		CCRF-CEM	CCRF-CEM/ Taxol ^b	CCRF-CEM/ VBL ^b
81a	Me	0.08±0.02	0.10±0.001 [1.22×] ^c	0.09±0.01 [1.1×]
81b	Et	0.18±0.01	0.21±0.003 [1.15×]	0.19±0.02 [1.03×]
81c	C ₆ H ₅	0.51±0.01	0.50±0.04 [0.99×]	0.29±0.02 [0.56×]
81d	4'-F-C ₆ H ₄	0.51±0.01	0.88±0.03 [1.72×]	0.49±0.002 [0.95×]
81e	4'-Cl-C ₆ H ₄	0.60±0.002	0.77±0.17 [1.28×]	0.72±0.18 [1.2×]
81f	3',4'-Di-F-C ₆ H ₃	1.14±0.22	1.26±0.26 [1.11×]	0.95±0.21 [0.84×]
81g	3',4'-Di-Cl-C ₆ H ₃	8.44±0.06	4.82±0.01 [0.57×]	6.42±0.05 [0.76×]
81h	4'-MeO-C ₆ H ₄	0.23±0.03	0.25±0.001 [1.02×]	0.11±0.001 [0.48×]

81i	3',4'-Di- MeO-C ₆ H ₃	1.10±0.04	0.76±0.02 [0.69×]	0.62±0.01 [0.56×]
81j	3',4',5'-Tri- MeO - C ₆ H ₂	1.97±0.46	2.88±0.05 [1.45×]	2.78±0.09 [1.40×]
82a	Me	0.13±0.01	0.11±0.002 [0.86×]	0.08±0.001 [0.59×]
82e	4'-Cl-C ₆ H ₄	0.19±0.01	0.22±0.004 [1.18×]	0.16±0.05 [0.87×]
82f	3',4'-Di-F-C ₆ H ₃	0.49±0.09	0.45±0.20 [0.91×]	0.47±0.05 [0.97×]
82h	4'- MeO-C ₆ H ₄	0.18±0.03	0.13±0.03 [0.72×]	0.09±0.02 [0.49×]
83a	Me	0.24±0.002	0.11±0.001 [0.48×]	0.12±0.003 [0.51×]
83b	Et	0.44±0.01	0.21±0.01 [0.48×]	0.24±0.01 [0.53×]
83c	C ₆ H ₅	0.26±0.01	0.26±0.03 [1.01×]	0.23±0.001 [0.90×]
83d	4'-F-C ₆ H ₄	0.31±0.01	0.43±0.05 [1.39×]	0.57±0.01 [1.85×]
83e	4'-Cl-C ₆ H ₄	0.59±0.01	0.35±0.01 [0.59×]	0.57±0.06 [0.97×]
83f	3',4'-Di-F-C ₆ H ₃	2.08±0.02	1.25±0.02 [0.60×]	0.67±0.003 [0.32×]
83g	3',4'-Di-Cl-C ₆ H ₃	2.37±0.02	0.90±0.002 [0.38×]	1.19±0.04 [0.50×]
83h	4'- MeO-C ₆ H ₄	0.37±0.02	0.22±0.003 [0.58×]	0.35±0.01 [0.93×]
83i	3',4'-Di- MeO-C ₆ H ₃	1.07±0.001	0.81±0.01 [0.75×]	0.65±0.02 [0.60×]
83j	3',4',5'-Tri- MeO- C ₆ H ₂	1.41±0.02	2.14±0.08 [1.52×]	3.13±0.03 [2.23×]

84a	Me	0.27±0.004	0.23±0.002 [0.84×]	0.14±0.003 [0.50×]
84b	Et	0.57±0.01	0.32±0.01 [0.56×]	0.18±0.0002 [1.42×]
84c	C ₆ H ₅	0.45±0.01	0.23±0.02 [0.50×]	0.25±0.01 [0.55×]
84d	4'-F-C ₆ H ₄	0.76±0.03	0.23±0.01 [0.30×]	0.23±0.001 [0.31×]
84e	4'-Cl-C ₆ H ₄	0.54±0.003	0.63±0.01 [1.16×]	0.57±0.04 [1.06×]
84f	3',4'-Di-F-C ₆ H ₃	1.33±0.01	0.78±0.003 [0.58×]	0.61±0.05 [0.46×]
84g	3',4'-Di-Cl-C ₆ H ₃	3.55±0.02	2.47±0.07 [0.69×]	1.35±0.04 [0.38×]
84h	4'-MeO-C ₆ H ₄	0.34±0.002	0.19±0.002 [0.56×]	0.16±0.001 [0.47×]
84i	3',4'-Di-MeO-C ₆ H ₃	1.29±0.10	0.60±0.02 [0.46×]	0.77±0.05 [0.59×]
84j	3',4',5'-Tri-MeO-C ₆ H ₂	0.89±0.01	1.20±0.01 [1.35×]	1.42±0.05 [1.59×]
Taxol		0.003 ±0.0003	0.43±0.05 [330×]	1.27 ± 0.05 [980×]
Vinblastine		0.0007±0.001	0.08±0.01 [106.2×]	0.50±0.12 [679.5×]

^a Cell growth inhibition was measured by the XTT assay¹⁰³ for leukemic cells after 72-h incubation using a microplate spectrophotometer as described previously.¹⁰⁵ Similar in vitro results were obtained by using the Cell Counting Kit-8 for the CCK-8 assays as described by technical manual of Dojindo Molecular Technologies, Inc. (Gaithersburg, MD; Website: www.dojindo.com). IC₅₀ values were determined from dose-effect relationship at six or seven concentrations of each drug by using the CompuSyn software by Chou and Martin¹⁰⁴ based on the median-effect principle and plot using the serial deletion analysis.^{108,109} Ranges given for taxol and vinblastine were mean ± SE (n = 4).

^bCCRF-CEM/Taxol and CCRF-CEM/VBL are subcell lines of CCRF-CEM cells that are 330-fold resistant to Taxol, and 680-fold resistant to vinblastine, respectively, when comparing with the IC₅₀ of the parent cell line.

^c Numbers in the brackets are fold of cross-resistant determined by comparison with the corresponding IC₅₀ of the parent cell line.

In the series of bis(alkylcarbamates) derivatives, the alkylcarbamate moiety may serve as a better leaving group than that of the OH group. Thus, the bis(alkylcarbamates) derivatives may easily generate nucleophilic cations on both methylene functions that allow the cations to become more favorable targets of DNA. In the series of 3-phenyl substituted derivatives, the bis(alkylcarbamates) are generally more potent than their corresponding bis(hydroxymethyl) derivatives, except for compound bis(ethylcarbamate) **83i**, which is as potent as the corresponding bis(hydroxymethyl) **81i** and more cytotoxic than bis(*iso*-propylcarbamates) **84i**. However, in the series of C3-alkyl derivatives, the bis(hydroxymethyl) derivatives (**81a** and **81b**) are more potent than their corresponding bis(alkylcarbamates) (**82a**, **83a**, and **84a**; **83b** and **84b**, respectively).

Our previous research on the SAR studies of 1,2-bis(hydroxymethyl)cyclopenta[*a*]indenes and their counterparts 1,2-bis(methylcarbamate) derivatives (**72–75**, Figure 14) demonstrated that the size and the electron property of the substituents at the C3 position affected the cytotoxicity of these agents.⁹⁶ However, we found that the cytotoxicity of pyrrolo[1,2-*b*]isoquinolines is mainly affected by the size of the substituents at the C3 position rather than the electron property in the current studies. In comparison with the potency of both these series, the bis(hydroxymethyl) derivatives of pyrrolo[1,2-*b*]isoquinoline are more cytotoxic than the corresponding cyclopenta[*a*]indenes. In contrast, the bis(alkylcarbamate) derivatives of cyclopenta[*a*]indenes are more potent than the corresponding pyrrolo[1,2-*b*]isoquinolines.

Our previous report demonstrated that cyclopenta[*a*]indenes have no multi-drug resistance toward antitumor agents such as Taxol and Vinblastine. To realize whether the newly synthesized pyrrolo[1,2-*b*]isoquinoline derivatives also have no cross-resistance to these two agents, we evaluated their cytotoxicity against CCRF-

CEM/Taxol and CCRF-CEM/VBL, which are subcell lines of CCRF-CEM cells that are 330-fold resistant to Taxol, and 680-fold resistant to Vinblastine, respectively. As shown in Table 3.1, the newly synthesized pyrrolo[1,2-*b*]isoquinolines have no cross-resistance to either Taxol or Vinblastine. This suggests that all derivatives are neither a good substrate of membrane multidrug resistance transporters (i.e., p-glycoprotein) nor mutated tubulin.

The antiproliferative activity of the selected pyrrolo[1,2-*b*]isoquinoline derivatives in inhibiting human solid tumors such as breast carcinoma MX-1, colon carcinoma HCT-116, non-small cell lung carcinoma H1299, prostate PC3, oral carcinoma OECM1 and glioma U87 cell growth *in vitro* (Table 3.2) were also investigated. Of these compounds, C3-Me derivative (**81a**) was found to have potent cytotoxicity in inhibiting MX-1 cell growth *in vitro* with IC₅₀ values of 0.66 μM. Compounds **81a**, **82e**, and **82f** exhibited potent inhibitory activity against human colon carcinoma HCT-116 with IC₅₀ values of 0.29, 0.04 and 0.36 μM, respectively. The tested compounds have good to moderate effects against H1299, PC3, OECM1 and U87 cell growth *in vitro*.

Table 3.2 The cytotoxicity of newly synthesized bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives (**81**) and their bis(alkylcarbamate) derivatives (**82** to **84**) against human solid tumors (breast carcinoma MX-1, colon carcinoma HCT-116, lung carcinoma H1299, prostate carcinoma PC3, oral carcinoma OECM1 and glioma U87) cell growth *in vitro*.

Compd.	Cell Growth inhibition (IC ₅₀ μM)					
	MX-1 ^a	HCT-116 ^a	H1299 ^b	PC3 ^b	OECM1 ^b	U87 ^b
81a	0.66±0.03	0.29±0.01	2.48±1.22	6.07±1.70	6.10±1.11	27.91±3.90
81d	4.01±0.05	2.46±0.08	8.76±1.15	5.93±1.52	6.99±0.12	23.25±0.26
81e	3.28±0.01	3.06±0.46	24.73±8.16	9.85±0.79	12.05±0.67	26.68±5.79
81f	6.99±0.35	3.21±0.21	ND ^c	ND	ND	ND
81h	1.99±0.07	1.13±0.07	13.94±0.83	10.93±1.21	6.12±0.21	10.57±0.23
82a	1.68±0.01	1.17±0.02	ND	ND	ND	ND
82e	1.18±0.01	0.04±0.002	ND	ND	ND	ND
82f	2.73±0.05	0.36±0.001	ND	ND	ND	ND
82h	1.33±0.02	1.07±0.05	ND	ND	ND	ND
83a	2.27±0.01	0.90±0.03	19.42±0.42	15.45±1.84	11.34±2.07	13.89±4.90
83d	1.51±0.03	0.79±0.05	3.94±1.13	9.59±1.31	12.52±1.70	28.36±4.86
83e	1.69±0.06	0.62±0.10	37.25±0.89	10.98±1.14	25.32±0.18	33.59±3.91
83h	1.27±0.05	0.75±0.004	27.61±1.65	18.54±0.82	10.48±0.13	17.06±1.78

84a	1.28±0.01	1.38±0.02	9.77±3.75	16.87±7.42	11.80±2.58	26.61±4.21
84d	1.17±0.19	0.63±0.001	8.13±1.34	12.29±1.34	11.05±2.61	36.87±5.98
84e	1.70±0.09	0.69±0.01	25.15±4.05	9.97±1.32	26.10±7.86	22.72±2.03
84h	0.98±0.03	0.97±0.04	13.16±1.94	6.79±1.01	8.83±0.74	21.04±0.67
Taxol	0.035±0.0 0514	0.0013±0.0 005	ND	ND	ND	ND
Vinblas tine	0.0029±0. 0002	0.0018±0.0 004	ND	ND	ND	ND
Cisplati n	ND	ND	4.95±0.60	26.65±4.19	ND	ND

^aCell growth inhibition was measured by the SRB assay¹⁰⁴ for solid tumor cells after 72-h incubation using a microplate spectrophotometer as described previously.¹⁰⁵

^bCell growth inhibition was determined by the Alamar blue assay¹⁰⁶ in a 72 h incubation using a microplate spectrophotometer as described previously.

^cNot determined.

3.2 *In vivo* antitumor activity

To investigate the antitumor activity of pyrrolo[1,2-*b*]isoquinoline derivatives in animal models, we selected compound **81a** for evaluating its therapeutic efficacy in animal models since this agent has the most potent cytotoxicity among all of the compounds tested and exhibits a broad spectrum of antitumor activity in inhibiting both CCRF/CEM and other solid tumors *in vitro*. Nude mice implanted with human breast carcinoma MX-1 xenograft were given 30 mg/kg, every two days for two times (Q2D×2), intravenous injection (*i.v.* injection) on day 8 and 10 (Figure 16). Remarkably, it shows that complete tumor remission against human breast carcinoma MX-1 in nude mice was achieved (Figure 16A). This concentration was established from tolerability studies. Under this dosage, one can see that nude mice's body weights recovered after cessation of the treatment, indicating the low toxicity of the compound to the host (Figure 16B). In another experiment, we found that **81a** was able to effectively suppress human ovarian tumor SK-OV-3 implanted in nude mice on day 22 at the dose of 20 mg/kg, every day for four times (QD×4), *i.v.* injection (Figure 17AB). The results of MX-1 and SK-OV-3 xenografts studies show the potential utility of compound **81a** in inhibiting the growth of both tumors.

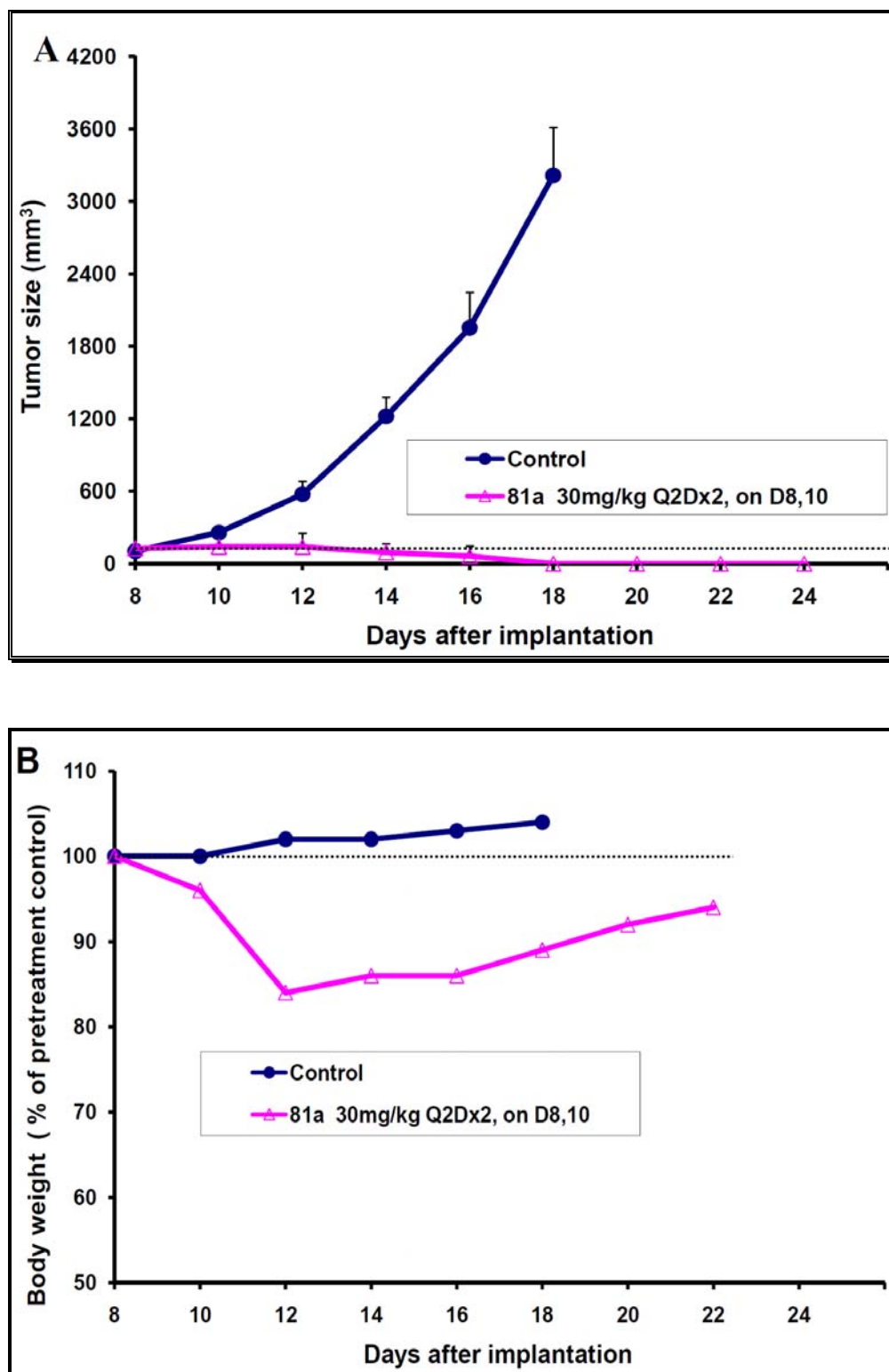


Figure 16 Therapeutic effects of **81a** in nude mice bearing MX-1 human mammary xenograft (*i.v. inj.*, $n = 4$). **A**: average tumor size changes. **B**: average body weight changes.

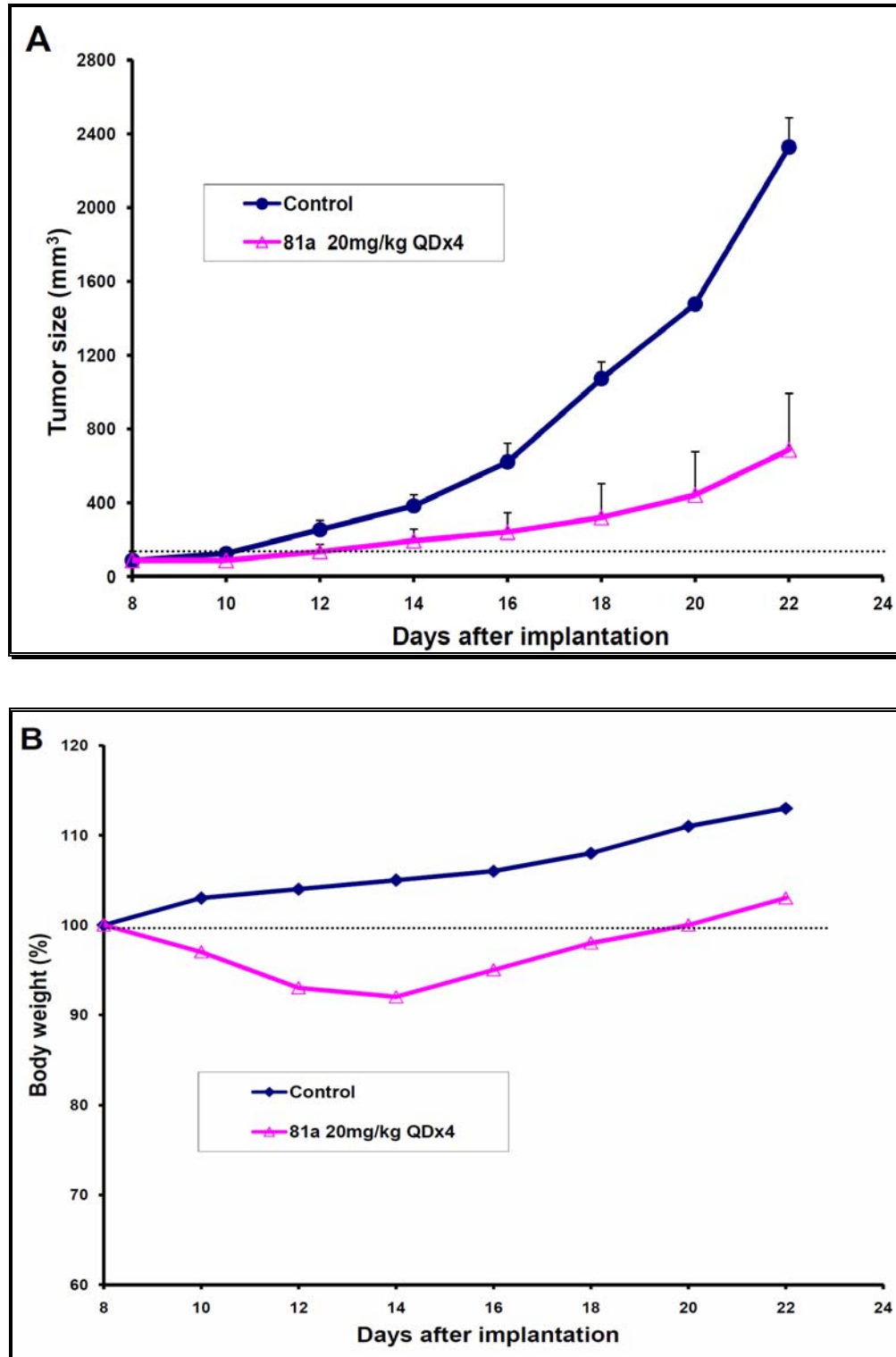


Figure 17 Therapeutic effects of **81a** in nude mice bearing ovarian adenocarcinoma SK-OV-3 xenograft (*i.v. inj*, n=4). **A**: average tumor size changes. **B**: average body weight changes.

3.3 DNA cross-linking study

To realize whether the newly synthesized compounds are capable of cross-linking with DNA double strands, pEGFP-N1 plasmid DNA was treated with bis(hydroxymethyl) derivatives (**81a** and **81h**) and their corresponding bis(alkylcarbamate) derivatives (**83a** and **84h**, respectively) at various concentrations as indicated (1, 10, and 20 μM) using alkaline agarose gel shifting assay (Figure 18).¹¹⁰ Melphalan (1, 5, and 10 μM) was used as the positive control. As revealed in Figure 18, one can see that all of the tested compounds were able to induce DNA interstrand cross-linking, suggesting that DNA cross-linking may be the main mechanism of action for these agents.

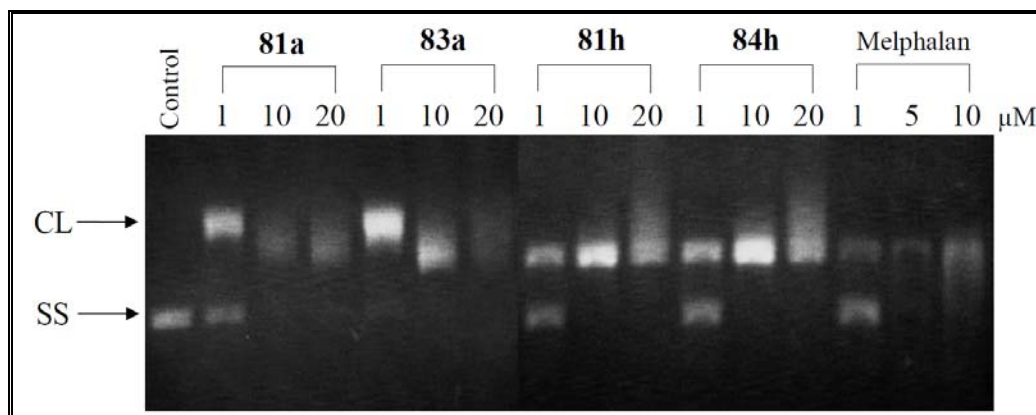


Figure 18 Representative DNA cross-linking gel shift assay for bis(hydroxymethyl) derivatives (**81a** and **81h**) and their corresponding bis(alkylcarbamate) derivatives (**83a** and **84h**, respectively) at various concentrations as indicated. Control lane shows single-stranded DNA (SS), while CL shown in all tested lanes is DNA double-stranded cross-linking. Melphalan (1, 5, and 10 μM) was used as a positive control.

3.4 Cell cycle inhibition

It is well known that DNA interacting agents can alter the cell cycle progression by arresting the cell cycle at the G2/M phase.¹¹¹ Previously, we have demonstrated that 3a-aza-cyclopenta[*a*]indene derivatives were able to induce G2/M arrest.⁹⁶ We therefore studied the inhibitory effect of **81a** on cell cycle distribution (Table 3.3). The human non small lung carcinoma H1299 cells were treated with **81a** at the concentrations of 1.25, 2.5, and 5 μM for 24 h. The cells were harvested, stained with propidium iodide (PI) and analyzed with a flow cytometer. It clearly shows that **81a** remarkably accumulated the cells at G2/M phase. Furthermore, increased sub-G1 populations were noticed in cells treated with **81a** at each concentration.

Table 3.3 Effects of compound **81a** on cell cycle progress in human non-small cell lung adenocarcinoma H1299.

Concentration (μM)	0	1.25	2.5	5
sub G1	14.1±0.7	21.6±4.2	26.6±0.7	26.5±3.0
G1	42.9±0.8	5.6±0.6	7.7±0.7	0.5±0.3
S	22.5±0.3	32.7±5.9	18.3±8.4	24.7±4.6
G2/M	20.5±0.9	40.1±6.5	47.3±2.1	48.4±2.2

3.5 Conclusion

Both bis(hydroxymethyl)pyrrolo[1,2-*b*]isoquinoline and their bis(alkylcarbamate) derivatives show potent antitumor activity in inhibiting various human tumor xenografts *in vitro*. Among these analogues, we discovered compound **81a**, which was selected for antitumor studies in animal models, exhibits potent therapeutic efficacy against human breast MX-1 xenograft in nude mice, as complete tumor remission was observed. This agent is also able to significantly suppress human ovarian tumors implanted in nude mice. The results reported herein warrant further investigation to optimize the schedule and dosage to get greater suppression of other human tumor growth in animal models. Additionally, the evaluation of the antitumor activity of **81a** in combination with DNA repair inhibitor (*e.g.* ATO) is currently undergoing in our laboratory.

Section A

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Section B

Chapter 4. Introduction of Pyrano[3,2-*c*]Quinolone Derivatives

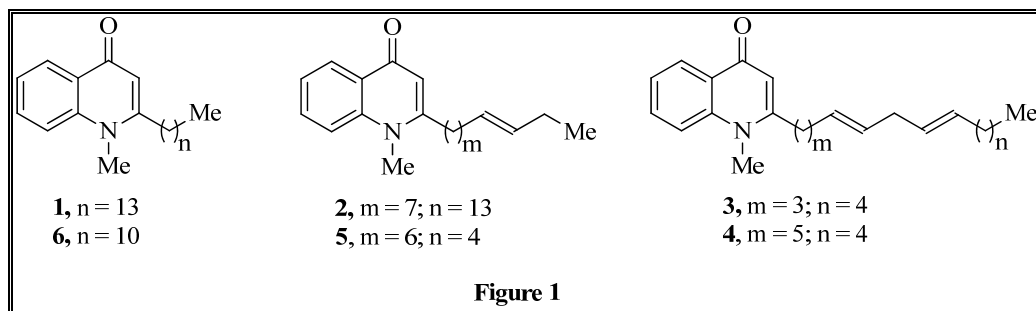
4.0 Introduction

4-hydroxy-2-quinolone is an important compound, because this moiety is exists in natural products exhibit variety of interesting pharmacological properties.¹ While pyranofused heterocycles are biologically important as antibacterial², antihistamines³, antimicrobials⁴, enzyme substrates⁵ and alkaloids⁶. Pyranoquinolinones show good pharmacological activities^{1,7} such as, antibacterial, antimicrobial, anti-HIV and antiviral and antitumor. The derivatives of fused 2-quinolones are useful as cardiovascular agents⁷. Quinolone alkaloids are also known to possess antimicrobial activity and marked cytotoxicity against animal and plant tumors.⁸ A novel class of 4-hydroxyquinolin-2(1*H*)-ones has recently been described⁹ as selective glycinesite NMDA antagonists with potent *in vivo* activity after oral administration. However, depending on their structural types, quinolone derivatives exhibit different activities.¹⁰ Furo[2,3-*c*]quinolin-4(5*H*)-one and 2*H*-pyrano[3,2-*c*]quinolin-5(6*H*)-one derivatives are abundantly distributed in nature.¹¹

4.1 Quinolone alkaloids

1-Methyl tetradecylquinolin-4(1*H*)-one (**1**), was isolated from the fruits of *Evodia rutaecarpa* together with evocarpine (**2**) and related alkadienes (**3**) and (**4**).¹²

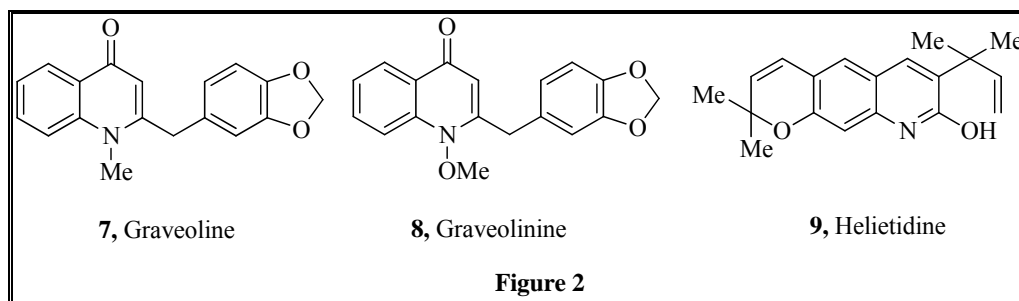
These are the first quinolone alkaloids have been tested as DGAT inhibitors; the moderate activity of purified compounds (IC₅₀ 69.5, 23.8, 20.1 and 13.5 μM, respectively) indicates possible utility in the design of hypolipidaemic and antiobesity agents. Evocarpine and the positional isomer (**5**) show strong antibacterial activity against *Helicobacter pylori* both *in vitro* and *in vivo* by inhibiting respiration, the results reinforcing previous indications of this class of alkaloids as novel therapeutical agents for the treatment of ulcers.¹³ Another *Evodia* alkaloid, 1-methyl-2-undecylquinolin-4(1*H*)-one (**6**), has proved to be an irreversible and selective inhibitor of type B monoamine oxidase, suggesting potential value in the treatment of neurological disorders such as Parkinson's and Alzheimer's diseases.¹⁴



The herb rue (*Ruta graveolens*) is known for its medicinal properties: New alkaloids for ex., graveoline (**7**) was found to be cytotoxic towards the HeLa cancer cell line (ED_{50} $3.35 \mu\text{g ml}^{-1}$), and its isomer graveolinine (**8**) was an effective inhibitor of platelet aggregation induced by arachidonic acid and collagen at low concentration ($5 \mu\text{g ml}^{-1}$).¹⁵

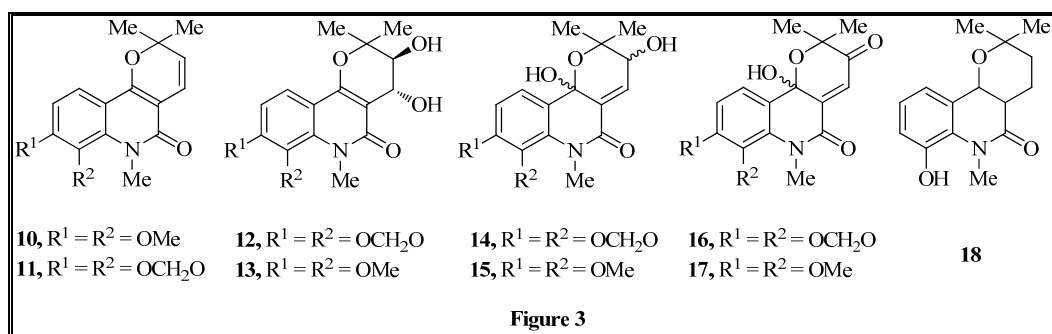
It is well known that simple quinoline alkaloids from *Galipea* and related rutaceous genera show substantial trypanocidal, antileishmanial and antimalarial activity. Some of these findings have now been reviewed by Fournet and Munoz.¹⁶

The unique structural feature in helietidine (**9**), a new alkaloid was isolated from the Brazilian medicinal plant *Helietta longifoliata*¹⁷, the pyrano[3,2-g]quinoline ring system, hitherto unprecedented among the hemiterpenoid quinoline alkaloids; indeed, even 6-prenylquinolines, likely precursors for this tricyclic ring system, were unknown as natural products. The structure was presented as a quinolin-2-ol, but the quinolin-2-one tautomer is undoubtedly more plausible.



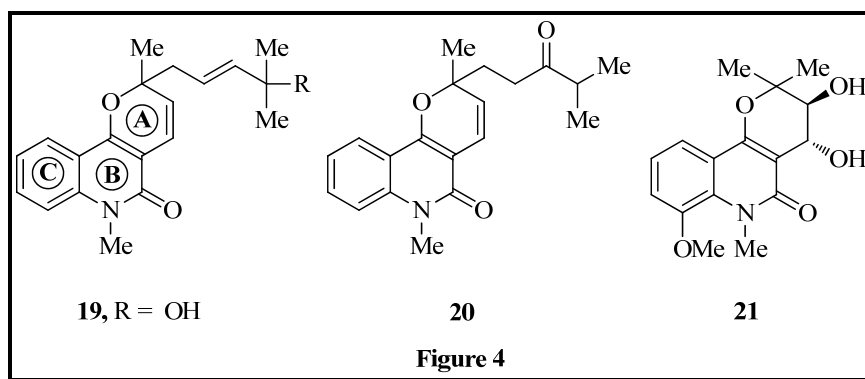
A cytotoxic fraction from the stem bark extract of *Stauranthus perforatus*, was analyzed by the comparatively rare technique of HPLC–NMR, HPLC–MS measurements, showed the new compounds to be analogues of veprisine (**10**) (7,8-dimethoxy-*N*-methylindersine), which had been found in this species previously and

was again detected in this study. One of the new alkaloids was (**11**), 7,8-methylenedioxy equivalent of veprisine, which is known as stauranthine. The remaining alkaloids were oxidized analogues of both veprisine and stauranthine, and included the *trans*-3, 4-dihydroxy-3,4-dihydro derivatives (**12**) and (**13**), the 3,6-dihydroxy-3,6-dihydro derivatives (**14**) and (**15**), and the 6-hydroxy-3-keto analogues (**16**) and (**17**). The 3,4-dihydroxypyran component in (**12**) and (**13**) has previously been found in other rutaceous quinoline and acridone alkaloids, but the involvement of the hemiterpenoid moiety in the remaining alkaloids in hemiketal formation with a quinolin-4-one is unprecedented. *Trans*-3,4-dihydroxy-3,4-dihydroveprisine (**13**) is identical to the alkaloid araliopsinine, reported from *Araliopsis tabouensis* by Ngadjui *et al.* in 1988.¹⁸ Interestingly, the alkaloids of the stauranthine series were all more stable than those of the veprisine series, perhaps reflecting the greater tendency towards oxidation of the vicinal dimethoxy grouping as compared with the methylenedioxy substituent. A pyrano[3,2-*c*]quinolone alkaloid ravesoline (**18**) was isolated from the leaves of *Ravenia spectabilis*.¹⁹



Pyranoquinoline alkaloids simulenoline (**19**, R=OH)^{20,21} and huajiaosimuline (**20**)²² were isolated from root barks of *Zanthoxylum simulans*, a shrub found in Taiwan and mainland China. While simulenoline (**19**, R=OH) was the most recently isolated of the two alkaloids, a third pyranoquinoline alkaloid zanthodioline (**21**) from the same species was recently disclosed in the literature.²⁰ These novel monoterpene pyranoquinolines are potent inhibitors of platelet aggregation. For example, at a concentration of 100 μg/mL, simulenoline (**19**, R=OH) demonstrates a nearly complete suppression of platelet aggregation induced *in vitro* by collagen, arachidonic acid, and PAF in general.²⁰ While simulenoline (**19**, R=OH) and zanthodioline (**21**) are not cytotoxic, huajiaosimuline (**20**) is toxic toward several human cultured cell

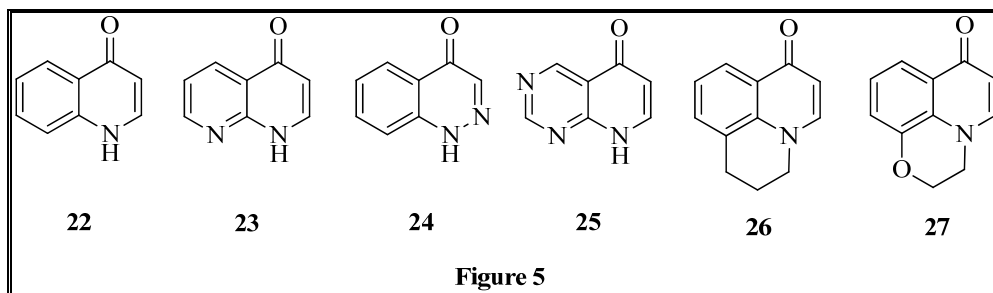
lines, especially the estrogen receptor-positive breast cancer cells, ZR-75-127. Structurally, these alkaloids contain three fused six-membered rings with a unique terpenoid side chain at C-2. The BC-ring is essentially the quinolone nucleus, and the A-ring is a 2*H*-pyran.



4.2 Pharmacological Profile

4.2.1 Antibiotic activity profile of Quinolone derivatives

The whole quinolone anti-infective agents are synthetic origin and not modeled knowingly after any natural antibiotic. Several ring systems are or have been involved (Figure 5). Those of greatest prominences and their numbering systems are illustrated in figure below.²³



The first antimicrobial quinolone was discovered called nalidixic acid demonstrated Gram negative antibacterial activity, remains in the market today, so-called first generation quinolones. Despite its convenient oral activity, bactericidal action, and ease of synthesis, its limited antimicrobial spectrum (primarily activity

against *Escherichia coli*) and poor pharmacokinetic characteristics limit. It is used primarily in treatment of sensitive community acquired urinary tract infections.

Norfloxacin, the first of the second-generation family of quinolones.²⁴ This agent had dramatically enhanced and broader spectrum anti Gram-negative activity and possessed significant anti Gram-positive activity as well. The potency of norfloxacin was in the same range as that of many fermentation-derived antibiotics, its comparative structural simplicity and synthetic accessibility lead to a very significant effort to find even more improved analogues. Norfloxacin and its *N*-methyl analogue pefloxacin ultimately failed to find major use outside of the genitourinary tract because of poor active blood levels and limited potency against Gram-positives.

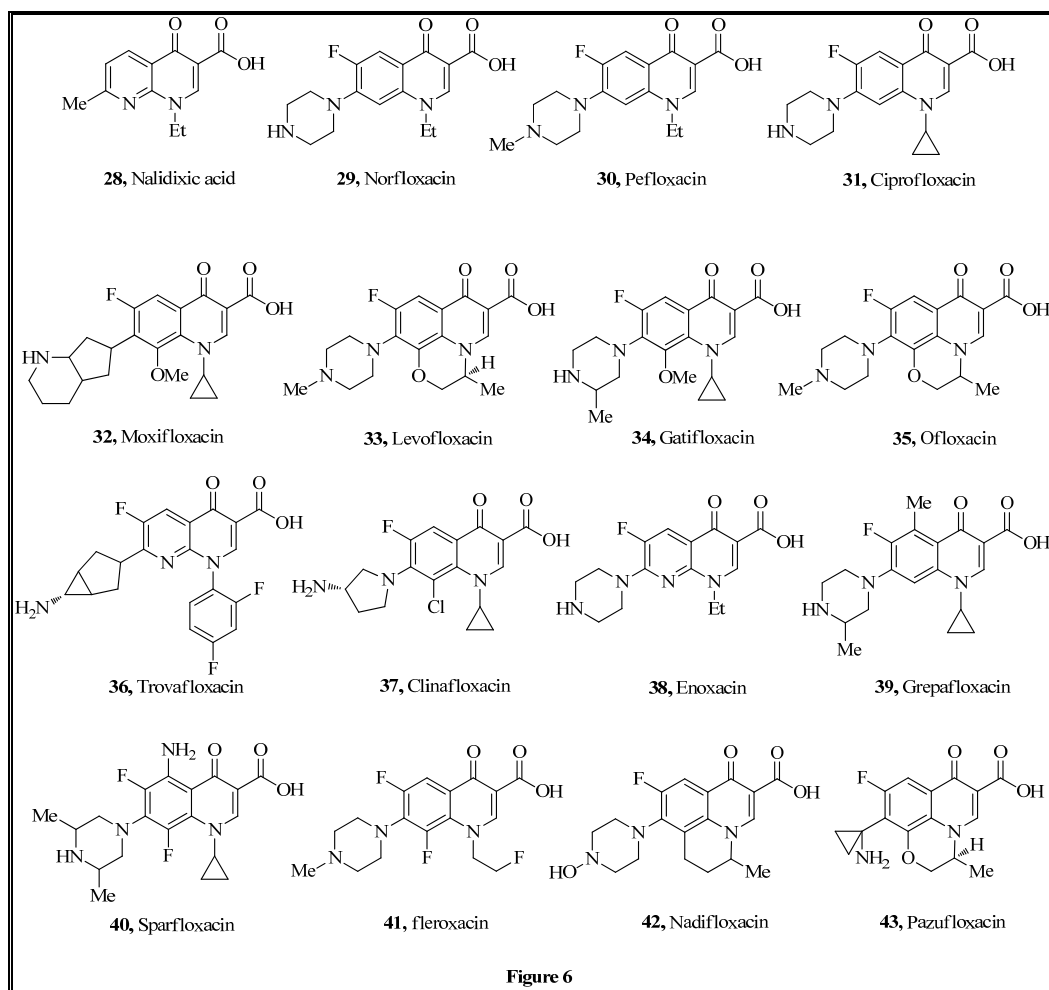
Shortly thereafter, ciprofloxacin²⁵⁻²⁷ and ofloxacin,^{27, 28} as well as its optically active form levofloxacin²⁹, were introduced. The second-generation agents have significant broad-spectrum antimicrobial activity including important Gram-positive pathogens. This is coupled with gratifying safety and pharmacokinetic characteristics.

A wide variety of clinical indications have been approved for quinolones including many infections commonly encountered in community practice including upper and lower respiratory, gastrointestinal and gynecologic infections, sexually transmitted diseases, prostatitis, and some skin, bone, and soft tissue infections.³⁰

Recently the fluoroquinolone family members were introduced belong to the third generation. It includes gatifloxacin³¹ and moxifloxacin³² which possessed further enhanced activity against Gram-positive infections, and anti-anaerobic coverage. Although at present only trovafloxacin³³ is approved for this indication. Among the agents, in preclinical study, still clinifloxacin³⁴ is the most promising anti-anaerobic agent. When first introduced, there was no idea of the molecular mode of action of these agents.

In a later study of 15 quinolones, they were divided into three groups on the basis of their relative ability to inhibit *S. aureus* strains with a resistance mutant toward one or the other enzyme. With group 1 (norfloxacin, enoxacin, fleroxacin,

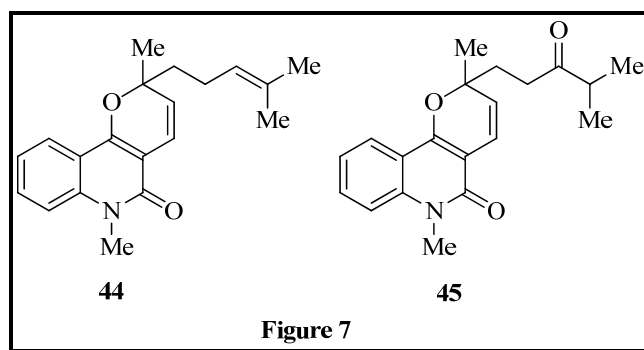
ciprofloxacin, lomefloxacin, trovafloxacin, grepafloxacin, ofloxacin, and levofloxacin) topoisomerase IV were the more sensitive target. With group 2 (sparfloxacin and nadifloxacin) DNA gyrase was the more sensitive target. With group 3 (gatifloxacin, pazufloxacin, moxifloxacin, and clinafloxacin) were equivalently sensitive. The later were termed the dual targeting quinolones.³⁵



4.2.2 Anticancer activity

Ih-Sheng Chen, *et al.*^{22b} have isolated pyranoquinolone alkaloids zanthosimuline (**44**) and huajiaosimuline (**45**) from the root bark of *Zanboxylum simulans*, exhibited cytotoxic activity. In addition, compound (**45**) showed significant antiplatelet aggregation activity and induced terminal differentiation with cultured HL-60 cells.

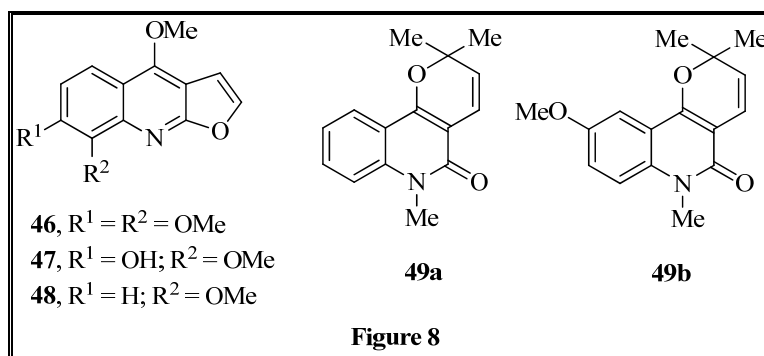
Compound **(44)** demonstrated a general cytotoxic response when evaluated with a variety of cultured human cancer cell lines and cultured P-388 cells.³⁶ Multidrug-resistant KB-VI cells demonstrated a sensitivity that was approximately equivalent to the observed with cultured KB cells. In the presence of vinblastine, activity was enhanced about 3-fold with KB-VI cells, suggesting reversal of the drug resistance of the phenotype.³⁷ Conversely, oxidation of the side-chain produced a more selective profile of cytotoxic activity, as exhibited by compound **(45)**. From particular note, the human tumor cell lines were tested, greatest activity was observed with the estrogen receptor-positive breast cancer cells, ZR-75-1, and even more pronounced reversal of resistance to vinblastine was demonstrated with KB-VI cells. In addition, prompted by the structural similarity of the compound **(44)** and **(45)** side-chains and vitamin D metabolites, studies were performed with cultured HL-60 cells.



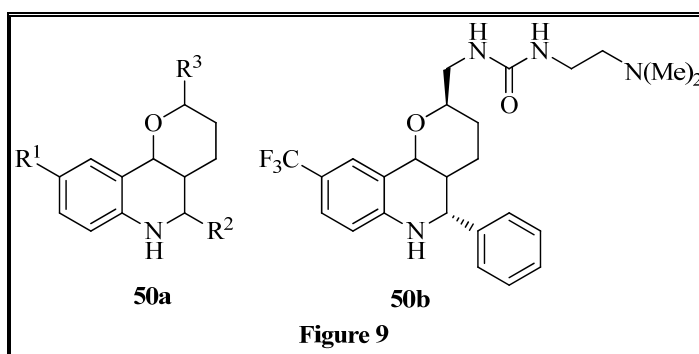
Olivia Jansen, *et al.*^{38a} during their systematic chemotaxonomic study of Uzbek *Haplophyllum* A. Juss. plants selected on ethnopharmacological data, have screened 14 alkaloids for their cytotoxic properties. In first selection for interesting compounds, each alkaloid was tested against two human cancer cell lines (HeLa and HCT-116), using WST-1 reagent.

Of the 14 alkaloids, 5 were cytotoxic when tested against the HeLa line with an $IC_{50} < 100 \mu M$. These five compounds consisted of three furoquinolines: skimmianine (**46**), γ -fagarine (**47**), and haplophine (**48**) and two pyranoquinolones: flindersine (**49a**) and haplamine (**49b**). Only haplamine was active against the HCT-116 line.

The cytotoxic properties of these five alkaloids were further investigated against five additional human cancer cell lines. Of these five pre-selected alkaloids, only haplamine showed significant cytotoxic activity against all the tested cell lines. Finally, this pyranoquinolone alkaloid was tested against 14 different cancer cell lines and against normal skin fibroblasts.



Mark Serono has worked extensively on KSP inhibitor, tetrahydroquinoline core derivatives which led into clinical trials.^{38b} The most extensive of this is based on the hexahydropyranoquinoline (**50a**, HHPQ) scaffold, including compound (**50b**).^{38c} Following a high-throughput screen, derivatives from this series were identified as potent, selective inhibitors of KSP and inhibition of cellular proliferation in a variety of tumor cell lines (Colo205, HCT116 and SW707).^{38d}



4.2.3 Anti-HIV activity

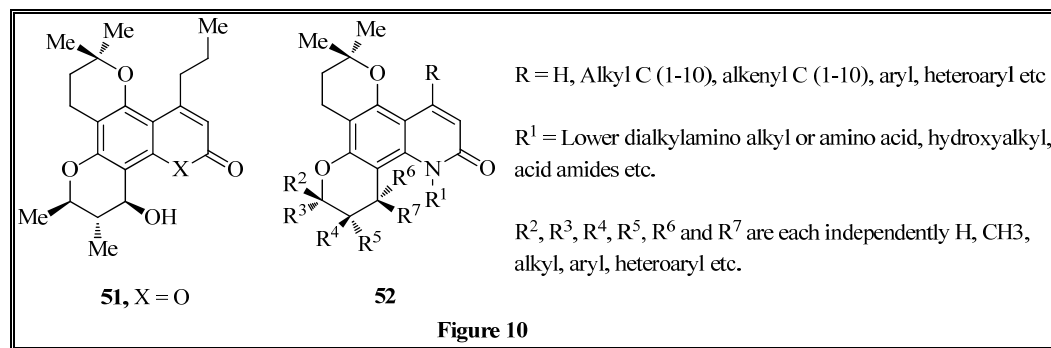
Antiviral nucleoside agents such as AZT, ddC, ddl, d₄ T and 3TC being Reverse Transcriptase (R.T.) inhibitors are approved for clinical use. Although these

nucleoside based drugs can extend the life of the patient, they are associated with several side effects and not capable of curing the disease. The urge for the promising RT inhibitors to cure AIDS, resulted in the identification of a group of coumarin derivatives isolated from *genus Calophyllum* as HIV-1 specific non-nucleoside inhibitors, among which Calanolide A represented by (**51**, X=O) is the most potent and is currently undergoing clinical trials (phase III).³⁹

Calanolides, a ‘dipyrano-coumarin’ class of compounds are active not only against the AZT-resistant strain of HIV-1, but also against virus strains resistant to some other non-nucleoside inhibitors such as TIBO pyridinone, and nevirapine. Calanolides are in the advance stage of biological evaluation as anti-HIV agents in clinical trials (phase III). The main drawbacks of this class of compounds are (a) poor solubility of this class of compounds in the physiological medium and (b) lesser stability of the coumarin ring system in biological environment. It, thus, would be desirable to prepare the New Chemical Entities (NCEs) having calanolide skeleton but with better therapeutic index. In addition, the NCEs are desirable to overcome the problems associated with calanolides such as stability and solubility in the physiological medium.

Quinolinones are shown to be part structures of several bio-active compounds with profound bio-efficacy. Unlike the lactone bond in coumarins, the lactam bond in quinolinones is highly stable.

M. K. Gurjar, *et al.*⁴⁰ have patented a novel ‘dipyrano-quinolinone’ class of compounds related to calanolide structural frame work as NCEs and envisaged to circumvent the problems associated with calanolides and has improved therapeutic indices.



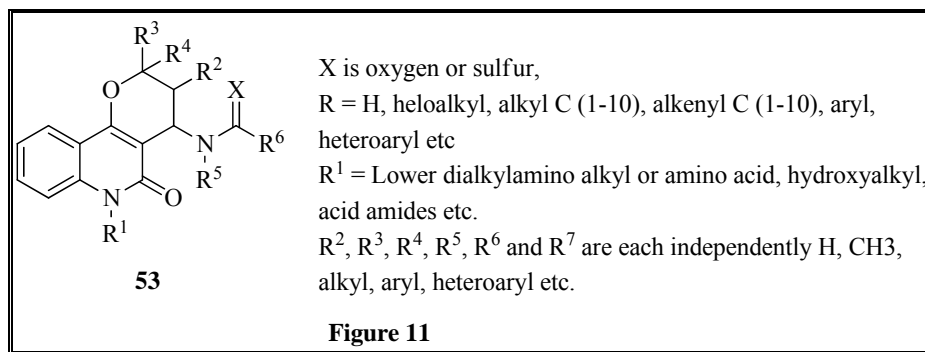
The invention deals with the synthesis of novel and new ‘dipyrano-quinolinone’ class of compounds, where the major differences in the structural arrangement are the replacement of coumarin ring oxygen (at position 1) of calanolide structure with nitrogen (at position 1). New ‘quinolinone’ ring system was represented by (52). These quinolinone analogues of calanolides are NCEs and envisaged as potential candidate molecules as anti-HIV agents. The rationale for the synthesis of these ‘dipyrano-quinolinones’ reported in this specification are as follows: (1) Replacement of oxygen (at position 1) of natural products, calanolides, with nitrogen leads to dipyranoquinolinones as anti-HIV agents with better therapeutic index. (2) The inherent problems associated with naturally occurring calanolides such as metabolic stability and solubility in physiological medium can be circumvented with the new dipyrano-quinolinone derivatives that are reported in this patent. (3) The derivatisation of water-soluble derivatives of these new chemical entities represented in this patent are easily possible. (4) The metabolic stability is expected to enhance due to the presence of nitrogen atom in the skeleton of dipyrano-quinolinones derivative presented in this patent. (5) The structure activity relationship coupled with positive activity against calanolide resistant strain of HIV virus can be explored due to the presence of nitrogen atom in the dipyrano-quinolinone system.

The dipyrano-quinolone of the present invention screened for anti-HIV activity (reverse transcriptase inhibition), have shown potential therapeutic values as ‘Candidate Molecules’. The above compound (51, X=NH) was reported in this invention shown equal potency of activity against HIV infected cell lines that observed for calanolide in the *in vitro* preliminary screening studies. The IC_{50} , EC_{50} and TI values of both calanolides and the ‘new dipyrano-quinolinone chemical entities (51, X =NH) were described in the present invention have equal and more

protecting capacity against the HIV infection. The NCE has prepared in this invention, shown a mean therapeutic index value of 12 and indicative of superior activity for the new compound compared to the natural one.

4.2.4 Calcium Channel Blockers

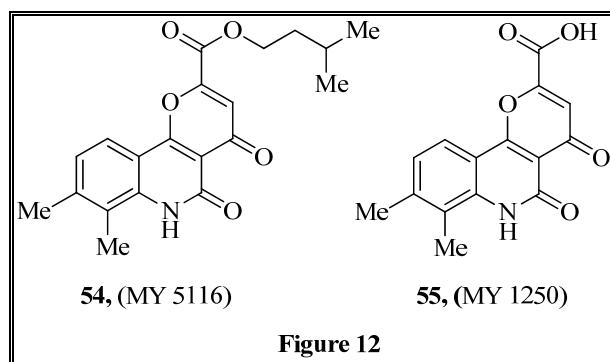
Karnail Atwal⁴¹ have reported novel pyranyl quinolines (**53**) having calcium channel blocking activity. It was suggested that compounds (**53**) and their pharmaceutically acceptable salts were cardiovascular agents. They act as calcium entry blocking vasodilators and are useful as antihypertensive agents. Thus, by the administration of a composition containing one (or a combination) of the compounds of this invention, the blood pressure of a hypertensive mammalian (e.g., human) host is reduced. A single dose, or two to four divided daily doses, provided on a basis of about 0.1 to 100 milligrams per kilogram of body weight per day, preferably from about 1 to about 50 milligrams per kilogram per day, is appropriate to reduce blood pressure.



As a result of the calcium entry blocking activity of the compounds (**53**), and the pharmaceutically acceptable salts thereof, these compounds, in addition to being antihypertensive agents, are especially useful as antiischemic agents, and also useful as anti-arrhythmic agents, anti-anginal agents, anti-fibrillatory agents, anti-asthmatic agents, as an agent to increase the ratio of HDL-cholesterol to total serum cholesterol in the blood and in limiting myocardial infarction.

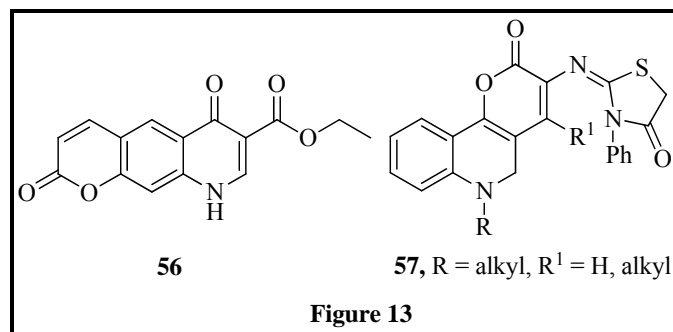
4.2.5 Antiallergic activity

Repirinast (**54**, MY-5116; isoarnyl 5,6-dihydro-7,8-dimethyl-4,5-dioxo-4H-pyrano[3,2-c]quinoline-2-carboxylate) is an anti-allergic effective drug for treatment of bronchial asthma in humans. N.Yamada, *et al.*⁴² have demonstrated that (**55**, MY-1250; 5,6-dihydro-7,8-dimethyl-4,5-dioxo-4H-pyrano[3,2-c]quinoline-2-carboxylic acid), a major metabolite of repirinast, inhibits antigen induced histamine release from rat peritoneal mast cells. MY-1250 causes a rapid increase in cyclic adenosine monophosphate (cAMP) levels in rat peritoneal mast cells MY-1250 inhibited cyclic AMP phosphodiesterase activities from rat peritoneal cells and guinea pig lung tissue in a concentration dependent manner with IC₅₀ value of 2 µg/ml and 1.67 µg/ml respectively. However MY-1250 showed no effect on adenylate cyclase activity from rat peritoneal cells. These results suggested that the inhibitory effect of MY-1250 on histamine release might be partly due to the inhibition of cyclic AMP phosphodiesterase activity.



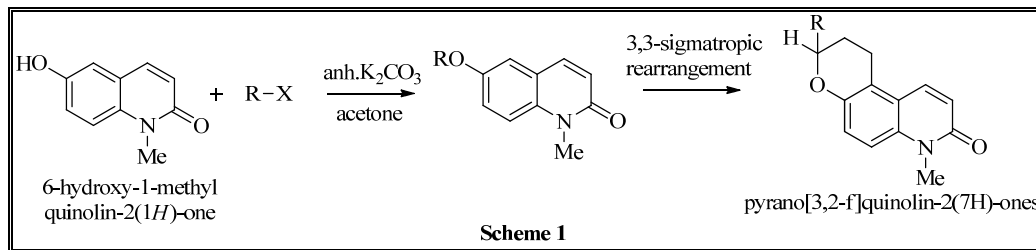
4.2.6 Antimicrobial activity

Trkovnik *et al.*⁴³ have reported ethyl-2,6-dioxo-6,9-dihydro-substituted-2H-pyrano[3,2-g]quinoline-7-carboxylates (**56**) and their antimicrobial activity against *S.aureus*, *P.aenrgiizosa*, *Streptococcus pyogens*, *Streptococcus faecalis*, *Staphiloc epiderms* etc. El-Taweel *et al.*⁴⁴ have evaluated polysubstituted pyrano[3,2-c]quinolones (**57**) for their bactericidal activity against various gram positive and gram negative bacterial species.

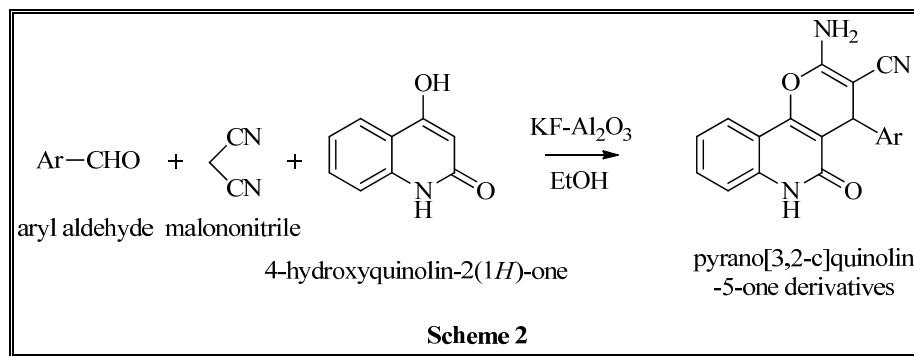


4.3 Synthetic Approaches

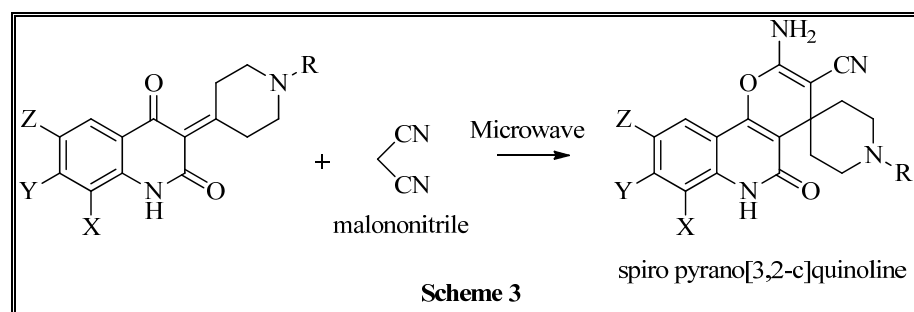
Majumdar K. C. *et al.*⁴⁵ have synthesized pyrano[3,2-*f*]quinolin-2(7H)-ones by a thermal [3, 3]-sigmatropic rearrangement with 60-65% yield by heating the propargyl ethers in N,N-dimethylaniline. (Scheme-1)



Wang X. *et al.*⁴⁶ have described one-step synthesis of 2-amino-4-aryl-1,4,5,6-tetrahydropyrano[3,2-*c*]quinolin-5-one derivatives from arylaldehyde, malonitrile and 4-hydroxyquinolin-2-one with KF-Al₂O₃ treatment as a catalyst in refluxing ethyl alcohol. Prior to this investigation, the synthesis of pyrano[3,2-*h*]quinoline derivatives was reported⁴⁷ from arylmethylidene malonitriles and 8-hydroxyquinoline using KF-Al₂O₃ catalyst which was a versatile solid support reagent for many reactions such as Knoevenagel condensation.⁴⁸ (Scheme-2)



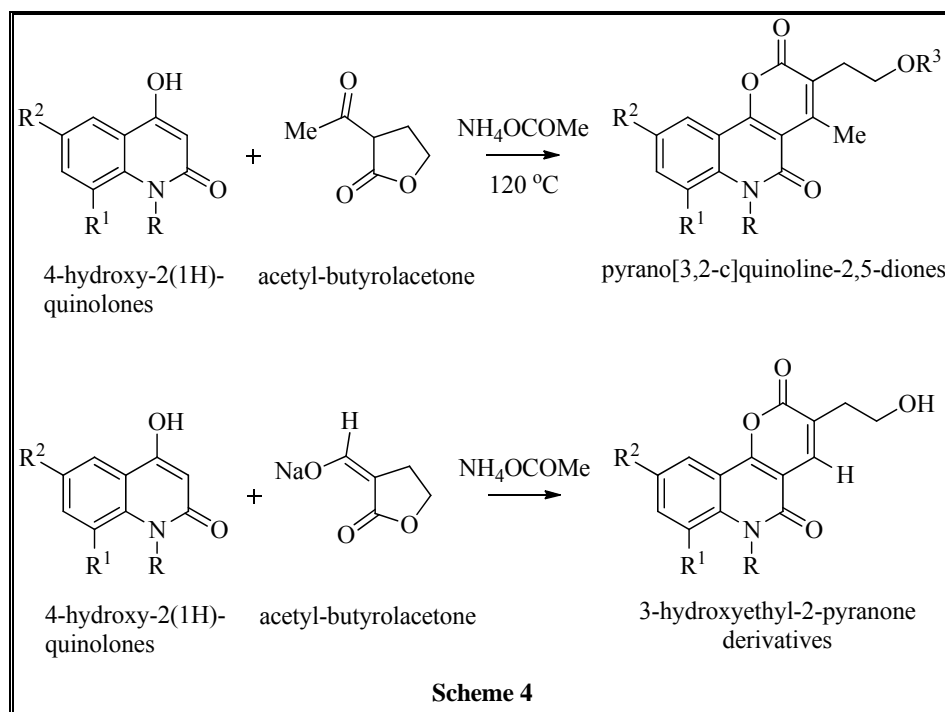
Dandia A. *et al.*⁴⁹ have prepared fluorine-containing substituted spiro derivatives of pyrano[3,2-*c*]quinoline by microwave irradiation and conventional methods with 80-85 % yield. (Scheme 3)



The condensation of α -ketoesters with phenolic compounds in the presence of sulfuric acid yields coumarin derivatives.⁵⁰ This synthesis is known as Pechmann condensation⁵¹ and has extensive application to prepare many naturally occurring coumarins. Acidic catalysts such as aluminium trichloride, zinc chloride, phosphoryl oxychloride, phosphoric acid, polyphosphoric acid, trifluoro acetic acid and hydrochloric acid were used. C. Mayer⁵² and T. Kappe⁵³ have found that ammonium acetate can be used as a source of ammonia and that readily reacted with phenolic heterocyclic compounds to yield corresponding α -pyrone derivatives.

α -acetyl-butylolactone, an interesting cyclic α -ketoesters can be used for the synthesis of heterocycles containing a hydroxyethyl side chain.⁵⁴ R. Toche, *et al.*⁵⁵ have reported the pechmann reaction of 4-hydroxy-2(1*H*)-quinolones with α -acetyl-butylolactone. Moreover this reaction was extended to α -formyl-butylolactone (used as its sodium salt). (Scheme-4)

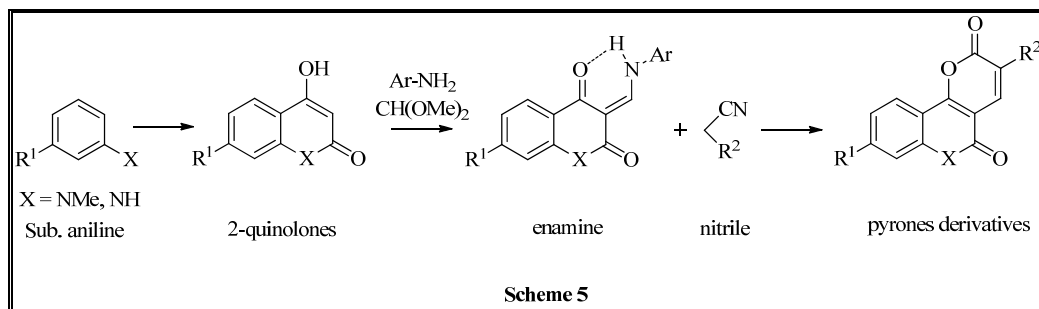
Thus, the condensation of 4-hydroxy-2-quinolones with α -acetylbutyrolactone in the presence of ammonium acetate at 120°C gave pyrano[3,2-*c*]quinoline-2,5-diones with 70-75% yield, while sodium salt of α -formylbutyrolactone⁵⁶ (obtained by formylation of butyrolactone) reacted smoothly with 4-hydroxy-2-quinolones in presence of ammonium acetate to yield 3-hydroxyethyl-2-pyranone derivatives.



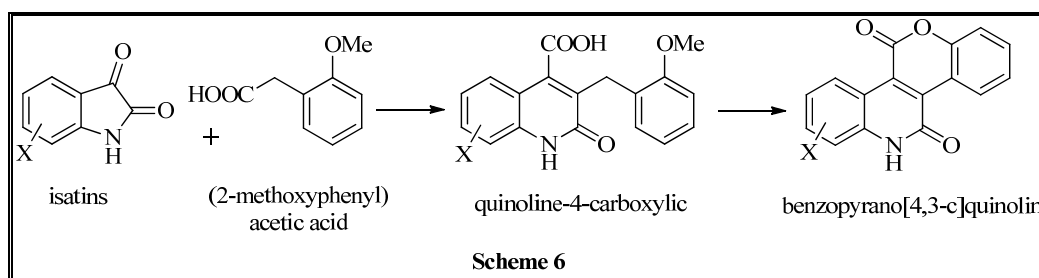
2-Quinolones were synthesized by several methods like (i) thermal cyclization of aniline with diethyl malonate at 180-200°C⁵⁷, (ii) condensation of aniline with diethylmalonate in the presence of phosphorous oxychloride⁵⁸, or (iii) condensation of anilines with bis-2,4-dichlorophenyl or bis-2,4,6-trichlorophenyl malonate.⁵⁹ A. Knierzinger and O. Wolfbeis⁶⁰ suggested that the conversion of 2-quinolones into their 3-anilino methylene derivatives was effected by heating with an equimolar amount of and a 1.5 molar excess of triethoxy methane in glacial acetic acid or DMF.

2-Aminomethylene-1,3-diketones are synthetic equivalents to the less readily available and less stable 2-formaldehyde of 1,3-diketones. Thus when the enamine was reacted with equimolar amounts of a nitrile in the presence of strong base in

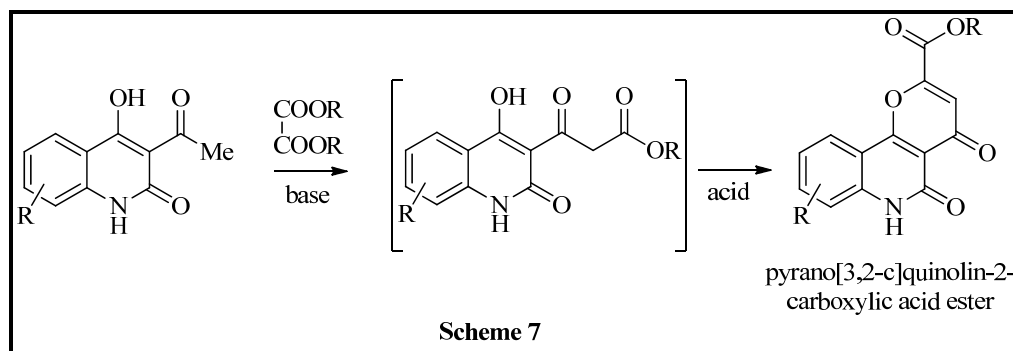
DMF, aniline was replaced by nitrile anion and subsequent acidification affords the corresponding pyrones. (Scheme-5)



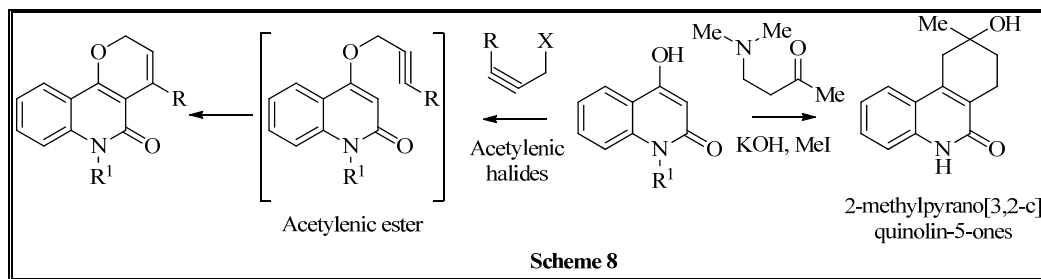
S. Yamaguchi *et al.*⁶¹ have synthesized eight number of 2- and 3-halo-11,12-dihydro-5H-[1]-benzopyrano[4,3-c]quinolin-5,11-dione by demethyl cyclization of corresponding halo-3-(2-methoxyphenyl)-2-oxo-1,2-dihydroquinoline-4-carboxylic acids, which were synthesized by Pfitzinger condensation of 5- or 6-halo isatins with (2-methoxyphenyl)acetic acid. (Scheme-6)



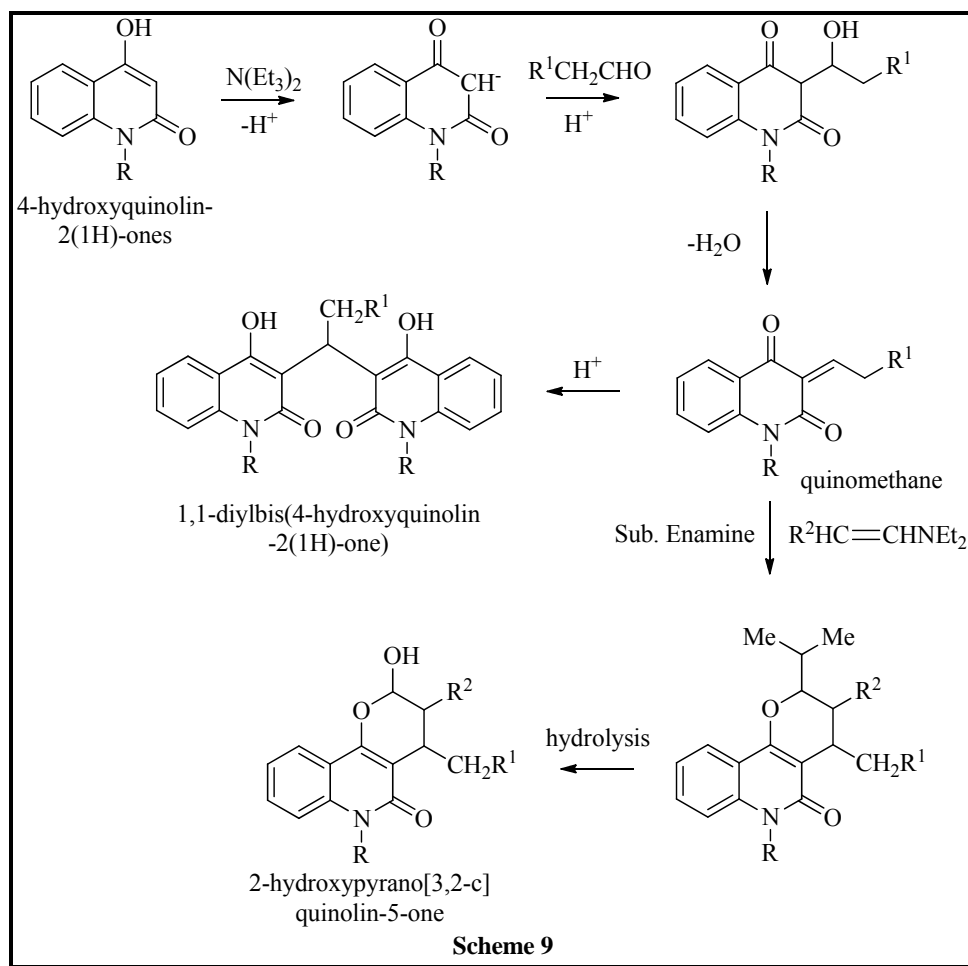
Morinaka and Takahashi have patented⁶² ester derivatives of pyrano[3,2-c]quinolin-2-carboxylic acid, synthesized by following route. (Scheme-7)



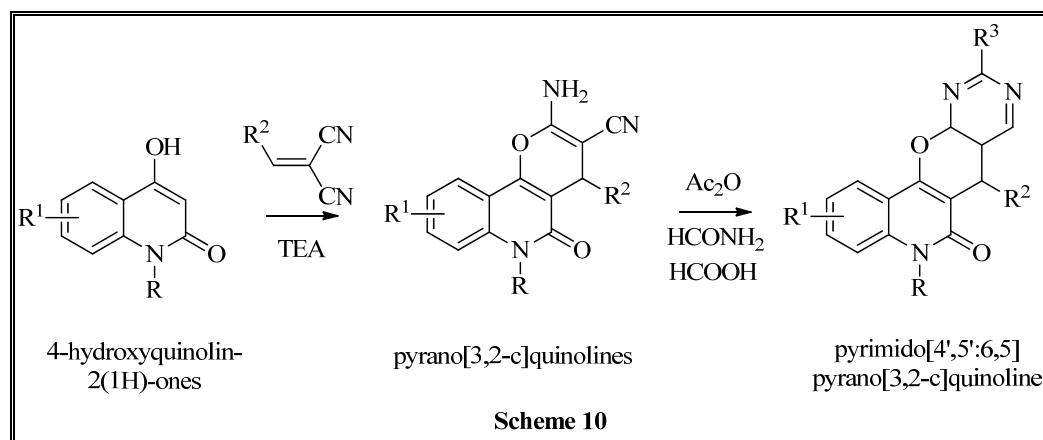
A couple of 2,3,4,6-tetrahydro-2-hydroxy-2-methylpyrano[3,2-*c*]quinolin-5-ones were prepared by the reaction of 4-hydroxyquinolin-2(1*H*)-ones with 1-(dimethylamino)butan-3-one in the presence of potassium hydroxide and methyl iodide.⁶³ Some 6-alkyl-3,4-dihydropyrano[3,2-*c*]quinolin-5-ones were prepared by the reaction of 4-hydroxyquinolin-2(1*H*)-ones 4 with acetylenic halides and the subsequent intramolecular cyclization of the acetylenic ethers.⁶⁴ (Scheme-8)



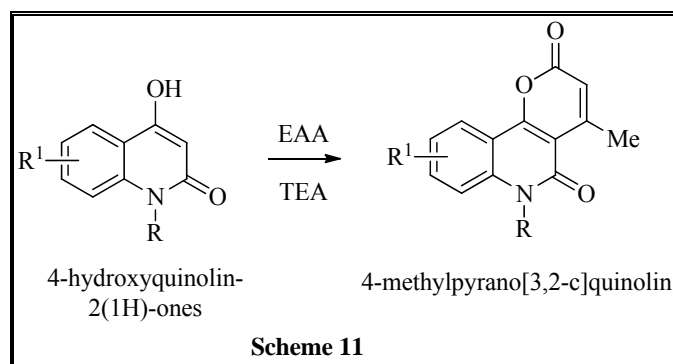
Jai-Hai Ye *et al.*⁶⁵ have reported convenient one pot synthesis of 2,3,4,6-tetrahydro-2-hydroxypyrano[3,2-*c*]quinolin-5-one derivatives from 4-hydroxyquinolin-2(1*H*)-ones by tandem Knoevenagel condensation. In 1st step 4-hydroxyquinolin-2(1*H*)-ones reacted with aliphatic aldehyde–Michael-type 1,4-addition of the enamine (derived from the aldehyde and diethylamine *in situ*) with the quinone methide (quinomethane). This reaction sequence was achieved in one pot by direct thermal reaction of with an aldehyde in the presence of diethylamine as a base in refluxing benzene, which afforded the corresponding 2,3,4,6-tetrahydropyrano[3,2-*c*]quinolin-5-one as a main product together with the 3,3'-alkane-1,1-diylbis(4-hydroxyquinolin-2(1*H*)-one) as a minor product.(Scheme-9)



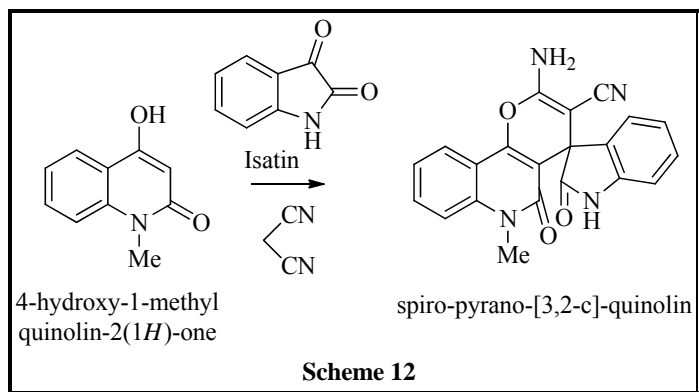
H. Abd el-Nabi⁶⁶ suggested that 4-hydroxy-2-quinolone reacted with cinnamionitrile derivatives in presence of catalytic amounts of triethylamine to afford pyrano[3,2-*c*]quinolines. The reaction of pyrano[3,2-*c*]quinolines with reagents such as acetic anhydride/pyridine, formamide and formic acid/formamide gave the fused heterotetracyclic system of the type of pyrimido[4',5':6,5]pyrano[3,2-*c*]quinoline. (Scheme-10)



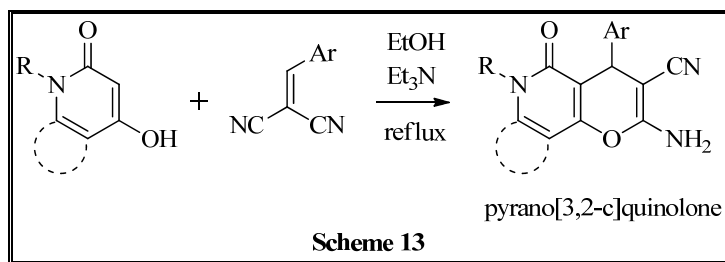
N. Venkatesh Kumar and S. Rajendran⁶⁷ have described that 4-hydroxy-2-quinolone being endowed with both nucleophilic and electrophilic properties furnish the dimeric quinoline in a base catalyzed self-condensation process. A one step synthesis, starting from 4-hydroxy-2-quinolone with ethylacetoacetate and pyridine proceeded through the Michael addition, which was followed by cyclization, gave an angular isomer 4-methylpyrano[3,2-c]quinolin-2,5[6*H*]-dione. (Scheme-11)



V. Mulwad and M. Lohar⁶⁸ have synthesized novel spiro-[3*H*-indole-3,7'-(9'-amino-8'-cyano-[7'*H*]-pyrano-[3,2-*c*]-quinolin)]-2,6'-[1*H*]-diones by the reaction of *N*-methyl-4-hydroxy-2-quinolone with isatine and malononitrile. (Scheme-12)



Igor V. Magedov *et al.*⁶⁹ have recently reported a three component reaction of pyridone with malononitrile and various aromatic aldehydes in a 1:1:1 ratio proceeds smoothly in refluxing ethanol containing a small quantity of triethyl amine. (Scheme 13) Pyranopyridones were precipitated directly from the refluxing reaction mixtures and required no further purification. The pyrano[3,2-*c*]quinolones analogues were found low nanomolar (down to 3 nM) antiproliferative properties in HeLa and MCF-7 human cancer cell lines.



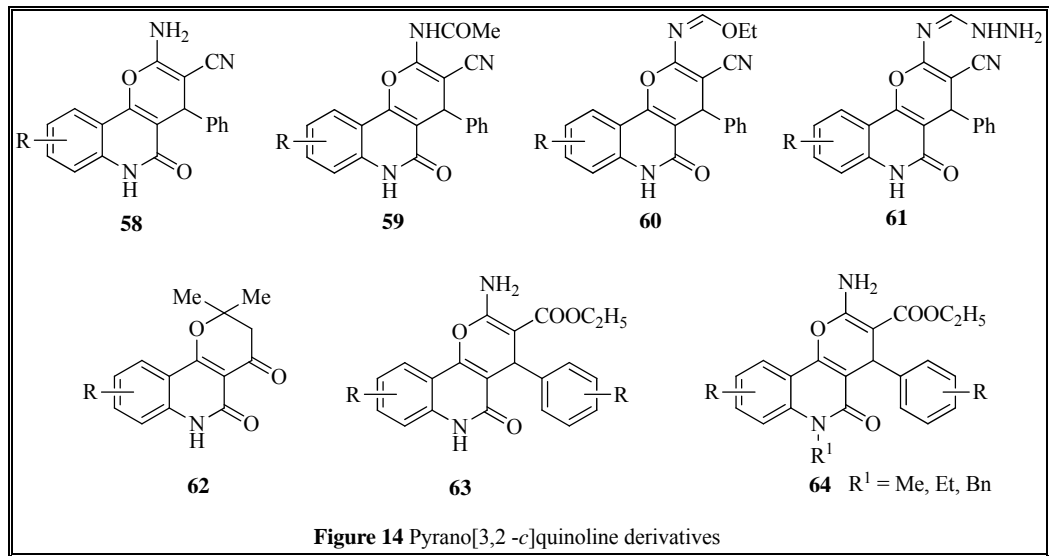
4.4 Work Done at Our Laboratory

The reaction of phenols with malonic acid in the presence of an admixture of condensing agents zinc chloride and phosphorous oxychloride gave yields of 4-hydroxy coumarins.⁷⁰ Moreover the specific condensing action of this admixture have also been observed.⁷¹

From the beginning of our ongoing research on studies of 2,4-dihydroxyquinoline derivatives and their analogues, Dodia⁷² extended this reaction with primary aromatic amines and found that with malonic acid and admixture of the condensing agents zinc chloride and phosphorous oxychloride at a suitable temperature 60 °C to 100 °C to give fairly good yields of 2,4-dihydroxyquinolines.

By using 2,4-dihydroxyquinolines as a central intermediate, a good number of substituted fused and dimeric analogues have been prepared, which includes 3-substituted-2,4-dihydroxyquinolines, pyrano[3,2-*c*]quinolines, pyrimido[5',4':5,6]pyrano[3,2-*c*]quinolines and dimeric 2,4-dihydroxy quinoline derivatives were screened them for various biological activity. A number of pyrano[3,2-*c*]quinolones analogues had shown potent activity as anti-HIV, antitubercular, antimicrobial and antimalarial activity.

Acharya⁷³, Mishra⁷⁴, Manvar⁷⁵ and Upadhyay⁷⁶ have extended the analogue making with the condensation of N-methylaniline, N-ethylaniline and N-benzylanilines with diethylmalonates in 1:2 mole proportion to afford pyrano[3,2-*c*]quinolones which further reacted with cinnamionitrile derivatives to yield pyrano[2',3':4,5]pyrano[3,2-*c*]quinolone derivatives. (Figure 14)

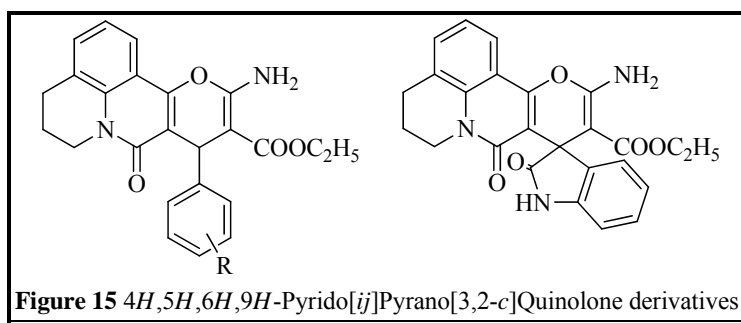


4.5 Research Aim

The quinoline derivatives exhibited wide variety of biological activities and evaluated its potential in pharmaceutical areas, such as central nervous system,⁷⁷ haematology,⁷⁸ virology,⁷⁹ chemotherapy such as stomach cancer, brain tumor, and large intestine cancer,^{80,81} transmissible spongiform encephalopathies,⁸² and proliferative diseases.⁸³ It is well-known that 4*H*-pyran derivatives have different pharmacological activities, e.g., antifungal activity,⁸⁴ anti-inflammatory activity,⁸⁵ and antimicrobial activity.⁸⁶ Moreover, it is demonstrated that pyrano[3,2-*c*]quinoline derivatives, which contain both a quinoline ring and pyran moieties, and their synthetic analogues are currently research interest because they have a wide range of biological activities and have potential of medical and other applications.^{87,88}

In continuation of our earlier work and results obtained of pharmacological properties of quinolines and pyrans we designed several new compounds featuring substituted pyran rings fused onto the quinoline moiety with the aim of obtaining more potent and active compounds.

The current work encompasses with dihydropyrido fused system of quinoline ring to study the changes in the biological activity. The current work presents direct, efficient and operationally convenient approach to the synthesis of some novel dihydropyrido fused Pyranoquinolone derivatives. The Knoevenagel condensation and Micheal addition reaction between 1-hydroxy-6,7-dihydropyrido[3,2,1-*ij*]quinolin-3(5*H*)-one and ethylcyano-acetate derivatives of aromatic and heteroaromatic aldehydes were studied. (Figure 15)



The chemical synthesis and characterization of 4*H*,5*H*,6*H*,9*H*-Pyrido[*ij*]Pyrano[3,2-*c*]Quinolone derivatives are described in Chapter 5.

Section B

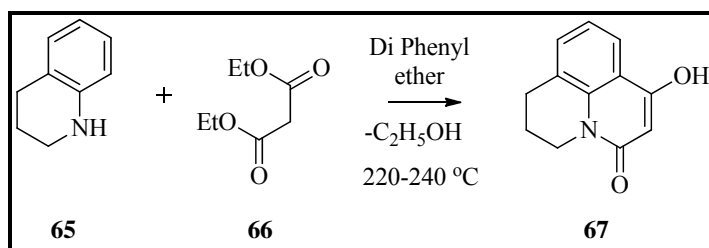
Chapter 5. Synthesis and Characterization of some novel *4H,5H,6H,9H*- Pyrido[*ij*]Pyrano[3,2-*c*]Quinolone Derivatives

5.0 Chemistry

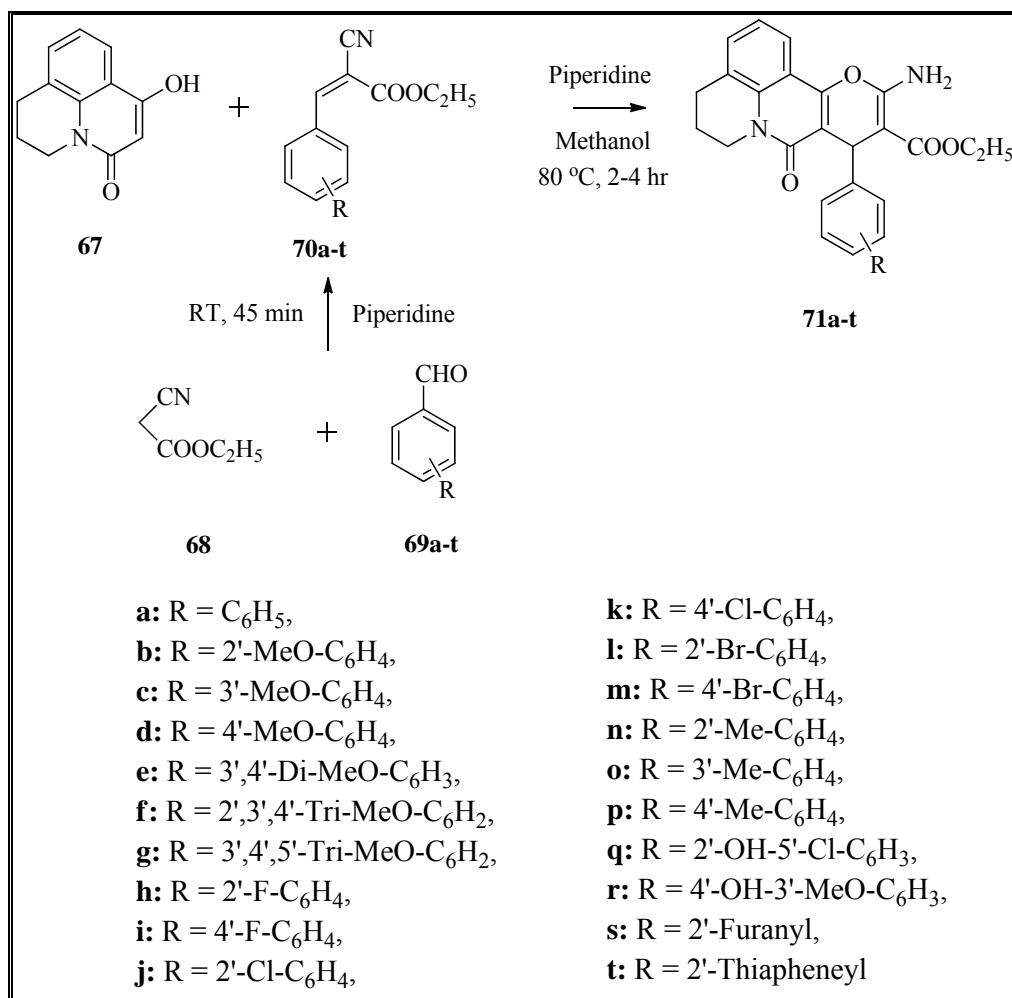
The synthetic route for Ethyl-11-amino-7,8-dihydro-8-oxo-9-(substituted)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate derivatives are shown in Scheme 2. The known 1-hydroxy-6,7-dihydropyrido[3,2,1-*ij*]quinolin-3(5*H*)-one (**67**) was synthesized by commercially available 1,2,3,4-tetrahydroquinoline (**65**) with diethyl malonate (**66**) in diphenyl ether by following the procedure.⁸⁹ Compound **67** was treated with various aromatic aldehydes and/or heteroaromatic aldehydes (**69a-t**, RCHO) and ethylcyanoacetate (**68**) in the presence of weak base (piperidine) to give ethyl-11-amino-7,8-dihydro-8-oxo-9-(substituted)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (**71a-t**). The spiro derivative **74** was prepared with good yields from **67** by reacting with isatine (**72**) and ethylcyanoacetate (**68**) (Scheme 3). Table 5.1 shows the yields and the physical data of these compounds.

5.1 Reaction Scheme

5.1.1 Scheme 1 Synthetic routes for 1-hydroxy-6,7-dihydropyrido[3,2,1-*ij*]quinolin-3(5*H*)-one (**67**).



5.1.2 Scheme 2 Synthetic routes for Ethyl-11-amino-7,8-dihydro-8-oxo-9-(substituted)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate derivatives (**71a-t**).



5.1.3 Scheme 3 Synthetic routes for Ethyl-11-amino-7,8-dihydro-8-oxo-9-{spiro-[3-*H*-isatine]}-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate **74**.

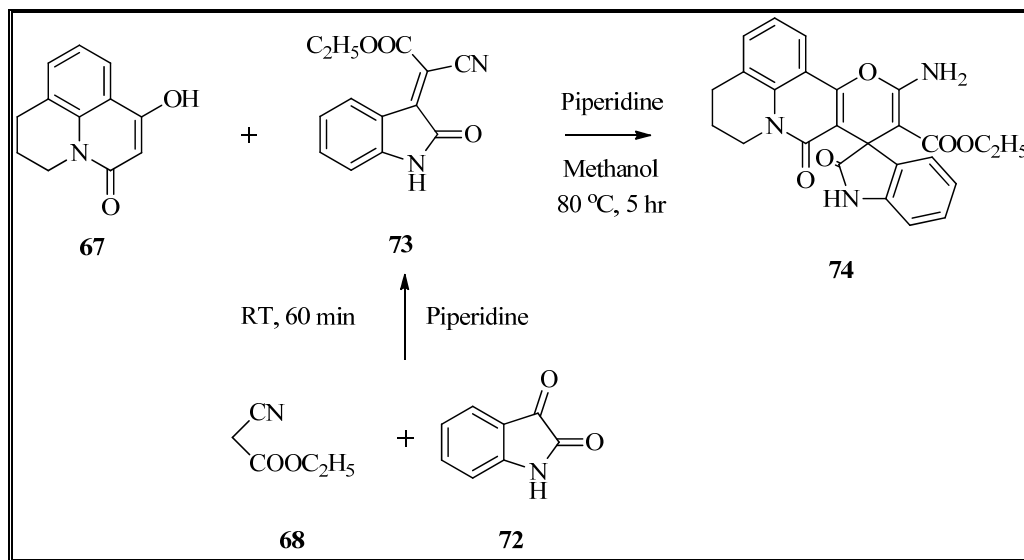
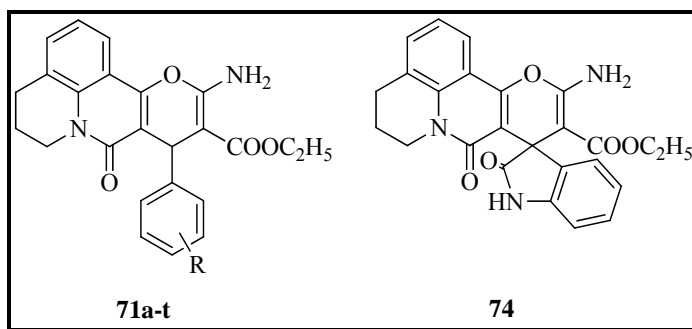


Table 5.1 Yields and physical data of the compounds **71a–t** and **74**.

Compd.	Substitute R	Yield %	MP °C	Analysis
71a	C ₆ H ₅	82	270–271	CHN
71b	2'-MeO-C ₆ H ₄	69	248–249	CHN
71c	3'-MeO-C ₆ H ₄	68	197–198	CHN
71d	4'-MeO-C ₆ H ₄	78	245–246	CHN
71e	3',4'- di-MeO-C ₆ H ₃	65	230–231	CHN
71f	2',3',4'-tri-MeO-C ₆ H ₂	66	215–216	CHN
71g	3',4',5'-tri-MeO-C ₆ H ₂	71	229–230	CHN
71h	2'-F-C ₆ H ₄	78	228–229	CHN
71i	4'-F-C ₆ H ₄	77	222–223	CHN
71j	2'-Cl-C ₆ H ₄	75	226–227	CHN
71k	4'-Cl-C ₆ H ₄	82	234–235	CHN
71l	2'-Br-C ₆ H ₄	74	248–249	CHN
71m	4'-Br-C ₆ H ₄	83	241–242	CHN
71n	2'-Me-C ₆ H ₄	74	229–230	CHN
71o	3'-Me-C ₆ H ₄	71	208–209	CHN
71p	4'-Me-C ₆ H ₄	77	222–223	CHN
71q	2'-OH-5'-Cl-C ₆ H ₃	65	213–214	CHN
71r	4'-OH-3'-OMe-C ₆ H ₃	74	224–225	CHN
71s	2'-Furanyl	62	190–191	CHN
71t	2'-Thiapheneyl	64	189–190	CHN
74	Isatine	61	>290 (d.)	CHN

5.2 Experimental

5.2.1 General methods and materials

All commercial chemicals and solvents were reagent grade and used without further purification unless otherwise specified. Melting points were determined on a Fargo melting point apparatus and are uncorrected. Thin-layer chromatography was performed on silica gel G60 F₂₅₄ (Merck) with short-wavelength UV light for visualization. All reported yields are isolated yields after chromatography or crystallization. Elemental analyses were done on a Heraeus CHN-O Rapid instrument. ¹H NMR spectra were recorded on a 600 MHz, Bruker AVANCE 600 DRX and 400 MHz, Bruker Top-Spin spectrometers in the indicated solvent. The chemical shifts were reported in ppm (δ) relative to TMS and coupling constants (J) in Hertz (Hz) and s, d, t, m, brs, refer to singlet, doublet, triplet, multiplet, broad respectively.

All the synthesized compounds were characterized by using ¹H NMR, ¹³C NMR and Elemental analysis. For compounds **71a-t**, the characteristic proton signals for pyran-H₄ (CH) appeared at the range of 4.81–4.88 δ ppm, and for ethyl ester (OC₂H₅) appeared at the range of 1.11–1.14 and 3.96–4.02 δ ppm. The characteristic proton for NH₂ appeared at the range of 7.70–7.79 δ ppm as singlet and D₂O exchangeable single. The elemental analysis of the newly synthesized derivatives was within $\pm 0.4\%$ range of the calculated C, H, N data.

Synthesis of 1-hydroxy-6,7-dihydropyrido[3,2,1-*ij*]quinolin-3(5*H*)-one (67). Diethyl malonate (**66**, 64 g, 400 mmol) was added into a mixture of 1,2,3,4-tetrahydroquinoline (**65**, 26.6 g, 200 mmol) in diphenyl ether (200 mL). The reaction mixture was heated at 160 °C equipped with a distillation apparatus. At about 160 °C liberation of ethanol took place and the temperature was increased slowly to 220 °C, where it was kept until no more ethanol was formed. Then the reaction mixture was allowed to cool at room temperature. The precipitates were filtered and washed with hexane to give **67**, 24.9 g (57%); mp >280 °C (lit.⁸⁹ mp 282–284 °C).

Ethyl-11-amino-7,8-dihydro-8-oxo-9-phenyl-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71a). A solution of ethylcyanoacetate (**68**, 1.2 g, 10.1 mmol), benzaldehyde (**5a**, 1.2 g, 10.1 mmol) and catalytic amounts of piperidine (1-2 drops) in methanol 10 mL was stirred at room temperature. After 45 min, 1-hydroxy-6,7-dihydropyrido[3,2,1-*ij*]quinolin-3(5*H*)-one (**67**, 2 g, 10 mmol) was added and the reaction mixture was further stirred and reflux for 3 h. After completion of the reaction (monitored by TLC, ethyl acetate:hexane (1:1)), the reaction mixture was concentrated under reduced pressure, and 1 mL of water was added. The separated solid product was collected by filtration and recrystallized from Ethanol to give **71a**, 3.3 g (82 %); mp 270–271 °C; ¹H NMR (DMSO-*d*₆) δ 1.12 (3H, t, *J* = 7.2 Hz, Me), 1.95 (2H, m, CH₂), 2.91 (2H, m, Ar-CH₂), 3.99 (4H, m, OCH₂ & NCH₂), 4.87 (1H, s, pyran H₄), 7.07–7.10 (1H, m, ArH), 7.17–7.28 (5H, m, 5 × ArH), 7.43 (1H, d, *J* = 7.2 Hz, ArH), 7.74 (2H, s, exchangeable NH₂), 7.91 (1H, d, *J* = 8 Hz, ArH). ¹³C NMR (DMSO-*d*₆) δ 14.2, 20.0, 26.8, 35.0, 41.8, 58.8, 77.4, 111.6, 112.6, 120.0, 121.6, 125.1, 125.9, 127.8, 127.9, 130.4, 135.2, 145.9, 150.0, 159.4, 159.6, 167.8. Anal. Calcd. for (C₂₄H₂₂N₂O₄): C, 71.63; H, 5.51; N, 6.96. Found: C, 71.41; H, 5.56; N, 6.72.

By following the same synthetic procedure as that for **71a**, the following compounds were synthesized:

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(2-methoxyphenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71b). Yield, 69 %; mp 248–249 °C. ¹H NMR (DMSO-*d*₆) δ 1.10 (3H, t, *J* = 6.8 Hz, Me), 1.95 (2H, m, CH₂), 2.91 (2H, m, Ar-CH₂), 3.58 (3H, s, OMe), 3.99 (4H, m, OCH₂ & NCH₂), 4.91 (1H, s, pyran H₄), 6.78–6.82 (2H, m, 2 × ArH), 7.06–7.10 (1H, m, ArH), 7.23–7.27 (2H, m, 2 × ArH), 7.41 (1H, d, *J* = 7.2 Hz, ArH), 7.66 (2H, s, exchangeable NH₂), 7.91 (1H, d, *J* = 7.6 Hz, ArH). Anal. Calcd. for (C₂₅H₂₄N₂O₅): C, 69.43; H, 5.59; N, 6.48. Found: C, 69.21; H, 5.62; N, 6.31.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(3-methoxyphenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71c). Yield, 68 %; mp 197–198 °C. ¹H NMR (DMSO-*d*₆) δ 1.14 (3H, t, *J* = 6.8 Hz, Me), 1.96 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂),

3.67 (3H, s, OMe), 4.02 (4H, m, OCH₂ & NCH₂), 4.85 (1H, s, pyran H₄), 6.67–6.78 (3H, m, ArH), 7.10 (1H, t, *J* = 8 Hz, ArH), 7.25 (1H, t, *J* = 8 Hz, ArH), 7.43 (1H, d, *J* = 7.6 Hz, ArH), 7.73 (2H, s, NH₂), 7.89 (1H, d, *J* = 7.6 Hz, ArH). Anal. Calcd. for (C₂₅H₂₄N₂O₅): C, 69.43; H, 5.59; N, 6.48. Found: C, 69.52; H, 5.22; N, 6.61.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(4-methoxyphenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71d). Yield, 78 %; mp 245–246 °C. ¹H NMR (DMSO-*d*₆) δ 1.14 (3H, t, *J* = 6.8 Hz, Me), 1.96 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 3.66 (3H, s, OMe), 4.02 (4H, m, OCH₂ & NCH₂), 4.81 (1H, s, pyran H₄), 6.75 (2H, d, *J* = 7.2 Hz, 2 × ArH), 7.12 (2H, d, *J* = 7.2 Hz, 2 × ArH), 7.24–7.28 (1H, m, ArH), 7.43 (1H, d, *J* = 7.2 Hz, ArH), 7.70 (2H, s, exchangeable NH₂), 7.90 (1H, d, *J* = 7.6 Hz, ArH). ¹³C NMR (DMSO-*d*₆) δ 14.3, 20.1, 26.8, 34.1, 41.8, 54.9, 58.8, 77.7, 111.9, 112.6, 113.1, 119.9, 121.5, 125.1, 128.8, 130.3, 135.1, 138.1, 149.8, 157.5, 159.5, 159.6, 167.9. Anal. Calcd. for (C₂₅H₂₄N₂O₅): C, 69.43; H, 5.59; N, 6.48. Found: C, 69.11; H, 5.43; N, 6.57.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(3,4-dimethoxyphenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71e). Yield, 65 %; mp 230–231 °C. ¹H NMR (DMSO-*d*₆) δ 1.13 (3H, t, *J* = 7.2 Hz, Me), 1.94 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 3.58 (3H, s, OMe), 3.65 (3H, s, OMe), 3.98 (4H, m, OCH₂ & NCH₂), 4.84 (1H, s, pyran H₄), 6.59–6.63 (2H, m, ArH), 6.91–6.95 (1H, m, ArH), 7.25 (1H, t, *J* = 8 Hz, ArH), 7.43 (1H, d, *J* = 7.2 Hz, ArH), 7.67 (2H, s, exchangeable NH₂), 7.90 (1H, d, *J* = 8 Hz, ArH). Anal. Calcd. for (C₂₆H₂₆N₂O₆): C, 67.52; H, 5.67; N, 6.06. Found: C, 67.62; H, 5.61; N, 5.84.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(2,3,4-trimethoxyphenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71f). Yield, 66 %; mp 215–216 °C. ¹H NMR (DMSO-*d*₆) δ 1.13 (3H, t, *J* = 7.2 Hz, Me), 1.94 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 3.57 (3H, s, OMe), 3.64 (3H, s, OMe), 3.72 (3H, s, OMe), 3.98 (4H, m, OCH₂ & NCH₂), 4.84 (1H, s, pyran H₄), 6.63 (1H, d, *J* = 7.6 Hz, ArH), 6.91–6.95 (1H, m, ArH), 7.25 (1H, t, *J* = 8 Hz, ArH), 7.42 (1H, d, *J* = 7.2 Hz, ArH), 7.67 (2H, s, exchangeable NH₂), 7.90 (1H, d, *J* = 8 Hz, ArH). Anal. Calcd. for (C₂₇H₂₈N₂O₇): C, 65.84; H, 5.73; N, 5.69. Found: C, 65.62; H, 5.61; N, 5.84.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(3,4,5-trimethoxyphenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71g). Yield, 71 %; mp 229–230 °C. ¹H NMR (DMSO-*d*₆) δ 1.17 (3H, t, *J* = 7.2 Hz, Me), 1.98 (2H, m, CH₂), 2.93 (2H, m, Ar-CH₂), 3.59 (3H, s, OMe), 3.66 (6H, s, 2×OMe), 4.01 (4H, m, OCH₂ & NCH₂), 4.86 (1H, s, pyran H₄), 6.49 (2H, s, ArH), 7.26 (1H, t, *J* = 8 Hz, ArH), 7.44 (1H, d, *J* = 7.2 Hz, ArH), 7.74 (2H, s, exchangeable NH₂), 7.90 (1H, d, *J* = 8 Hz, ArH). ¹³C NMR (DMSO-*d*₆) δ 14.2, 19.9, 26.7, 35.0, 41.8, 55.6, 58.7, 59.7, 77.1, 105.0, 111.4, 112.4, 119.9, 121.4, 125.0, 130.2, 135.1, 135.8, 150.1, 152.1, 159.6, 167.7. Anal. Calcd. for (C₂₇H₂₈N₂O₇): C, 65.84; H, 5.73; N, 5.69. Found: C, 65.54; H, 5.81; N, 5.58.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(2-fluorophenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71h). Yield, 78 %; mp 228–229 °C. ¹H NMR (DMSO-*d*₆) δ 1.12 (3H, t, *J* = 6.8 Hz, Me), 1.95 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 3.99 (4H, m, OCH₂ & NCH₂), 4.87 (1H, s, pyran H₄), 6.78–6.82 (2H, m, ArH), 7.08 (1H, t, *J* = 7.2 Hz, ArH), 7.23–7.27 (2H, m, ArH), 7.43 (1H, d, *J* = 7.2 Hz, ArH), 7.68 (2H, s, exchangeable NH₂), 7.90 (1H, d, *J* = 7.6 Hz, ArH). Anal. Calcd. for (C₂₄H₂₁N₂O₄F): C, 68.56; H, 5.03; N, 6.66. Found: C, 68.24; H, 4.88; N, 6.53.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(4-fluorophenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71i). Yield, 77 %; mp 222–223 °C. ¹H NMR (DMSO-*d*₆) δ 1.13 (3H, t, *J* = 6.8 Hz, Me), 1.95 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 3.99 (4H, m, OCH₂ & NCH₂), 4.87 (1H, s, pyran H₄), 6.80–6.84 (2H, m, ArH), 7.16–7.20 (2H, m, ArH), 7.25–7.27 (1H, m, ArH), 7.43 (1H, d, *J* = 7.2 Hz, ArH), 7.68 (2H, s, exchangeable NH₂), 7.90 (1H, d, *J* = 7.6 Hz, ArH). Anal. Calcd. for (C₂₄H₂₁N₂O₄F): C, 68.56; H, 5.03; N, 6.66. Found: C, 68.34; H, 4.76; N, 6.58.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(2-chlorophenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71j). Yield, 75 %; mp 226–227 °C. ¹H NMR (DMSO-*d*₆) δ 1.14 (3H, t, *J* = 6.8 Hz, Me), 1.94 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 3.99 (4H, m, OCH₂ & NCH₂), 4.87 (1H, s, pyran H₄), 6.82–6.86 (2H, m, ArH), 7.18 (1H, t, *J* = 7.2 Hz, ArH), 7.20–7.24 (2H, m, ArH), 7.44 (1H, d, *J* = 7.2 Hz, ArH), 7.68

(2H, s, exchangeable NH₂), 7.90 (1H, d, $J = 7.6$ Hz, ArH). Anal. Calcd. for (C₂₄H₂₁N₂O₄Cl): C, 65.98; H, 4.84; N, 6.41. Found: C, 65.86; H, 4.75; N, 6.28.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(4-chlorophenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71k). Yield, 82 %; mp 234–236 °C. ¹H NMR (DMSO-*d*₆) δ 1.14 (3H, t, $J = 6.8$ Hz, Me), 1.95 (2H, m, CH₂), 2.93 (2H, m, Ar-CH₂), 4.01 (4H, m, OCH₂ & NCH₂), 4.83 (1H, s, pyran H₄), 7.18 (2H, d, $J = 8.4$ Hz, ArH), 7.28 (1H, t, $J = 7.6$ Hz, ArH), 7.40 (2H, d, $J = 8.4$ Hz, ArH), 7.43 (1H, d, $J = 7.2$ Hz, ArH), 7.80 (2H, s, exchangeable NH₂), 7.91 (1H, d, $J = 7.2$ Hz, ArH). Anal. Calcd. for (C₂₄H₂₁N₂O₄Cl): C, 65.98; H, 4.84; N, 6.41. Found: C, 65.88; H, 4.71; N, 6.25.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(2-bromophenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71l). Yield, 74 %; mp 248–249 °C. ¹H NMR (DMSO-*d*₆) δ 1.13 (3H, t, $J = 6.8$ Hz, Me), 1.96 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 3.99 (4H, m, OCH₂ & NCH₂), 4.83 (1H, s, pyran H₄), 6.84–6.88 (2H, m, ArH), 7.28 (1H, t, $J = 7.6$ Hz, ArH), 7.35–7.39 (2H, m, ArH), 7.44 (1H, d, $J = 7.2$ Hz, ArH), 7.79 (2H, s, exchangeable NH₂), 7.91 (1H, d, $J = 7.2$ Hz, ArH). Anal. Calcd. for (C₂₄H₂₁N₂O₄Br): C, 59.89; H, 4.40; N, 5.82. Found: C, 59.74; H, 4.21; N, 5.89.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(4-bromophenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71m). Yield, 82 %; mp 241–242 °C. ¹H NMR (DMSO-*d*₆) δ 1.12 (3H, t, $J = 6.8$ Hz, Me), 1.96 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 3.99 (4H, m, OCH₂ & NCH₂), 4.83 (1H, s, pyran H₄), 7.17 (2H, d, $J = 8.4$ Hz, ArH), 7.27 (1H, t, $J = 7.6$ Hz, ArH), 7.39 (2H, d, $J = 8.4$ Hz, ArH), 7.44 (1H, d, $J = 7.2$ Hz, ArH), 7.79 (2H, s, exchangeable NH₂), 7.91 (1H, d, $J = 7.2$ Hz, ArH). ¹³C NMR (DMSO-*d*₆) δ 14.2, 20.0, 26.8, 34.8, 41.8, 58.9, 76.8, 110.9, 112.5, 118.9, 120.0, 121.6, 125.2, 130.2, 130.5, 130.6, 135.3, 145.4, 150.0, 159.5, 159.6, 167.7. Anal. Calcd. for (C₂₄H₂₁N₂O₄Br): C, 59.89; H, 4.40; N, 5.82. Found: C, 59.64; H, 4.31; N, 5.99.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(2-methylphenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71n). Yield, 74 %; mp 229–230 °C. ¹H NMR (DMSO-*d*₆) δ 1.14 (3H, t, *J* = 6.8 Hz, Me), 1.95 (2H, m, CH₂), 2.18 (3H, s, Me), 2.92 (2H, m, Ar-CH₂), 3.99 (4H, m, OCH₂ & NCH₂), 4.87 (1H, s, pyran H₄), 7.16–7.19 (1H, m, ArH), 7.23–7.27 (2H, m, ArH), 7.37–7.41 (2H, m, ArH), 7.40 (1H, d, *J* = 7.2 Hz, ArH), 7.79 (2H, s, exchangeable NH₂), 7.90 (1H, d, *J* = 7.6 Hz, ArH). Anal. Calcd. for (C₂₅H₂₄N₂O₄): C, 72.10; H, 5.81; N, 6.73. Found: C, 72.34; H, 5.97; N, 6.67.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(3-methylphenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71o). Yield, 71 %; mp 208–209 °C. ¹H NMR (DMSO-*d*₆) δ 1.13 (3H, t, *J* = 6.8 Hz, Me), 1.95 (2H, m, CH₂), 2.26 (3H, s, Me), 2.92 (2H, m, Ar-CH₂), 3.99 (4H, m, OCH₂ & NCH₂), 4.87 (1H, s, pyran H₄), 7.15 (1H, s, ArH), 7.26–7.29 (2H, m, ArH), 7.35–7.39 (2H, m, ArH), 7.43 (1H, d, *J* = 7.2 Hz, ArH), 7.76 (2H, s, exchangeable NH₂), 7.92 (1H, d, *J* = 7.6 Hz, ArH). Anal. Calcd. for (C₂₅H₂₄N₂O₄): C, 72.10; H, 5.81; N, 6.73. Found: C, 72.28; H, 5.94; N, 6.82.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(4-methylphenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71p). Yield, 77 %; mp 222–224 °C. ¹H NMR (DMSO-*d*₆) δ 1.14 (3H, t, *J* = 6.8 Hz, Me), 1.95 (2H, m, CH₂), 2.22 (3H, s, Me), 2.92 (2H, m, Ar-CH₂), 3.99 (4H, m, OCH₂ & NCH₂), 4.87 (1H, s, pyran H₄), 7.17 (2H, d, *J* = 8.4 Hz, ArH), 7.25–7.29 (1H, m, ArH), 7.39 (2H, d, *J* = 7.6 Hz, ArH), 7.43 (1H, d, *J* = 7.2 Hz, ArH), 7.79 (2H, s, exchangeable NH₂), 7.91 (1H, d, *J* = 7.6 Hz, ArH). Anal. Calcd. for (C₂₅H₂₄N₂O₄): C, 72.10; H, 5.81; N, 6.73. Found: C, 72.24; H, 5.99; N, 6.57.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(2-hydroxy-5-chlorophenyl)-4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71q). Yield, 65 %; mp 213–214 °C. ¹H NMR (DMSO-*d*₆) δ 1.06 (3H, t, *J* = 7.2 Hz, Me), 2.05 (2H, m, CH₂), 2.99 (2H, m, Ar-CH₂), 3.51 (1H, s, exchangeable OH), 3.99 (4H, m, OCH₂ & NCH₂), 5.32 (1H, s, pyran H₄), 7.02–7.09 (2H, m, ArH), 7.25 (1H, t, *J* = 7.6 Hz, ArH), 7.36–7.40 (1H, m, ArH), 7.59–7.62 (3H, m, ArH & exchangeable NH₂), 7.88 (1H, d, *J* = 7.6 Hz,

ArH). Anal. Calcd. for (C₂₄H₂₁N₂O₅Cl): C, 63.65; H, 4.67; N, 6.19. Found: C, 63.47; H, 5.01; N, 6.23.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(4-hydroxy-3-methoxyphenyl)-4H,5H,6H,9H-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71r). Yield, 74 %; mp 224–225 °C. ¹H NMR (DMSO-*d*₆) δ 1.06 (3H, t, *J* = 7.2 Hz, Me), 2.07 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 3.65 (1H, s, exchangeable OH), 4.02 (4H, m, OCH₂ & NCH₂), 5.23 (1H, s, pyran H₄), 7.02–7.10 (2H, m, ArH), 7.23–7.26 (1H, m, ArH), 7.36–7.38 (1H, m, ArH), 7.59–7.64 (3H, m, ArH & exchangeable NH₂), 7.88 (1H, d, *J* = 7.6 Hz, ArH). Anal. Calcd. for (C₂₅H₂₄N₂O₆): C, 66.95; H, 5.39; N, 6.25. Found: C, 66.81; H, 5.24; N, 6.34.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(furan-2-yl)-4H,5H,6H,9H-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71s). Yield, 62 %; mp 190–194 °C (dec.). ¹H NMR (DMSO-*d*₆) δ 1.19 (3H, t, *J* = 6.8 Hz, Me), 1.99 (2H, m, CH₂), 2.93 (2H, m, Ar-CH₂), 4.03 (4H, m, OCH₂ & NCH₂), 5.04 (1H, s, pyran H₄), 6.05 (1H, m, ArH), 6.26 (1H, m, ArH), 7.26–7.28 (1H, m, ArH), 7.34–7.37 (1H, m, ArH), 7.43 (1H, d, *J* = 7.2 Hz, ArH), 7.75 (2H, s, exchangeable NH₂), 7.89 (1H, d, *J* = 8 Hz, ArH). Anal. Calcd. for (C₂₂H₂₀N₂O₅): C, 67.34; H, 5.14; N, 7.14. Found: C, 67.44; H, 5.27; N, 7.02.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-(thiophene-2-yl)-4H,5H,6H,9H-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (71t). Yield, 64 %; mp 189–190 °C. ¹H NMR (DMSO-*d*₆) δ 1.17 (3H, t, *J* = 6.8 Hz, Me), 1.99 (2H, m, CH₂), 2.92 (2H, m, Ar-CH₂), 4.05 (4H, m, OCH₂ & NCH₂), 5.22 (1H, s, pyran H₄), 6.83–6.86 (1H, m, ArH), 7.18–7.19 (1H, m, ArH), 7.24–7.28 (1H, m, ArH), 7.34–7.36 (1H, m, ArH), 7.43 (1H, d, *J* = 7.2 Hz, ArH), 7.79 (2H, s, exchangeable NH₂), 7.88 (1H, d, *J* = 8 Hz, ArH). Anal. Calcd. for (C₂₂H₂₀N₂O₄S): C, 64.69; H, 4.94; N, 6.86. Found: C, 64.78; H, 5.02; N, 7.01.

Ethyl-11-amino-7,8-dihydro-8-oxo-9-{spiro-[3-*H*-isatine]}-4H,5H,6H,9H-pyrido[*ij*]pyrano[3,2-*c*]quinoline-3-carboxylate (74). Yield, 61 %; mp >290 °C. ¹H NMR (DMSO-*d*₆) δ 0.84 (3H, t, *J* = 7.2 Hz, Me), 1.93 (2H, m, CH₂), 2.91 (2H, m, Ar-CH₂), 3.77 (4H, m, OCH₂ & NCH₂), 6.69–6.75 (2H, m, ArH), 6.84 (1H, d, *J* = 7.6 Hz,

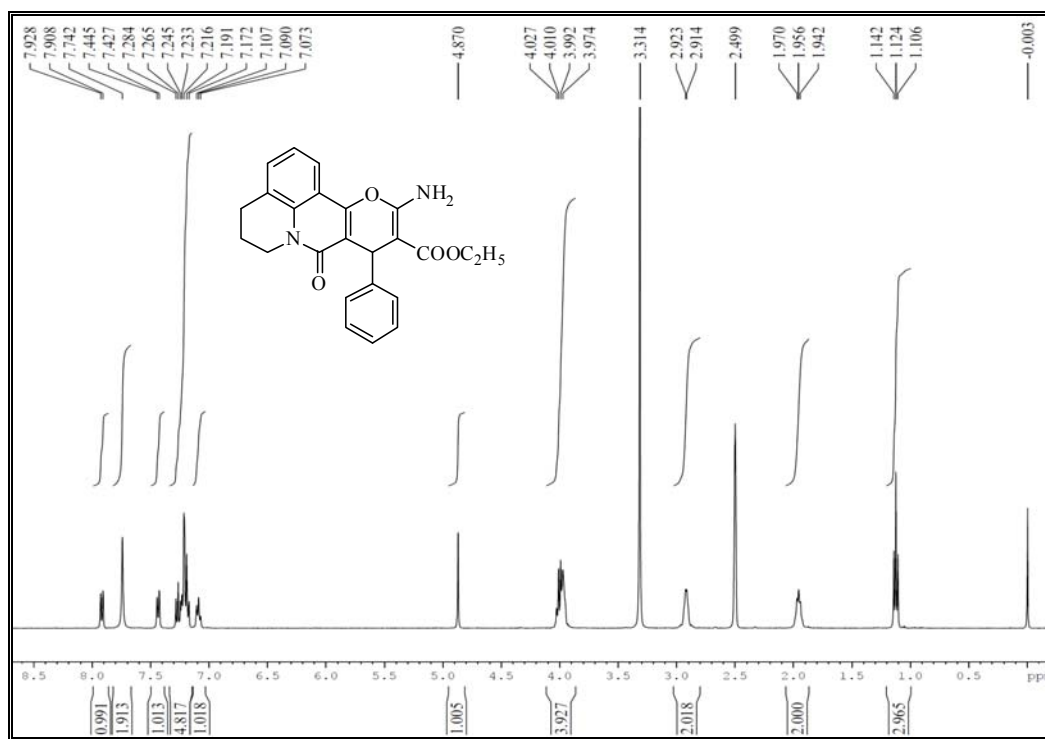
ArH), 7.06 (1H, t, $J = 8$ Hz, ArH), 7.28 (1H, t, $J = 8$ Hz, ArH), 7.47 (1H, d, $J = 8$ Hz, ArH), 7.96 (1H, d, $J = 8$ Hz, ArH), 8.04 (2H, s, exchangeable NH₂), 10.24 (1H, s, exchangeable NH). Anal. Calcd. for (C₂₅H₂₁N₃O₅): C, 67.71; H, 4.77; N, 9.48. Found: C, 67.54; H, 4.86; N, 9.32.

5.3 Conclusion

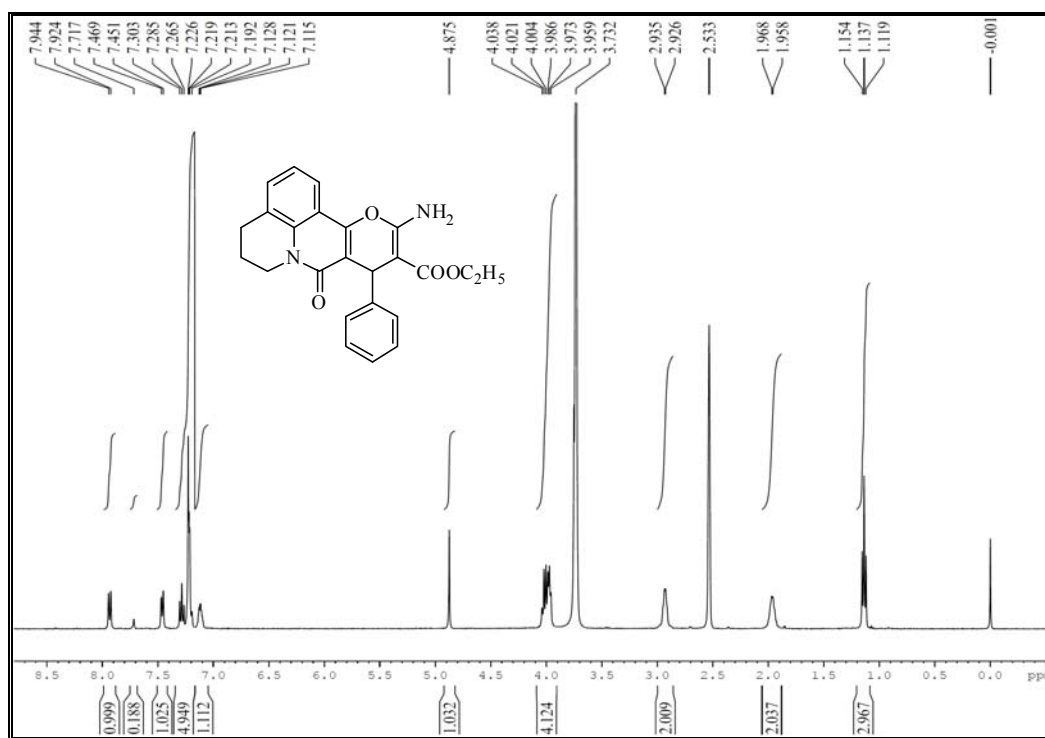
We discovered that catalytic amount of piperidine (1–2 drop) can function as an effective catalyst for one-pot three-component Tandem Knoevenagel Michael addition reaction. This protocol has notable advantages, such as good to excellent product yields, efficient methodology, cleaner reaction profiles, ease of work-up, all of which make it an attractive strategy for the preparation of 4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline derivatives. The biological study of newly synthesized compounds is underway.

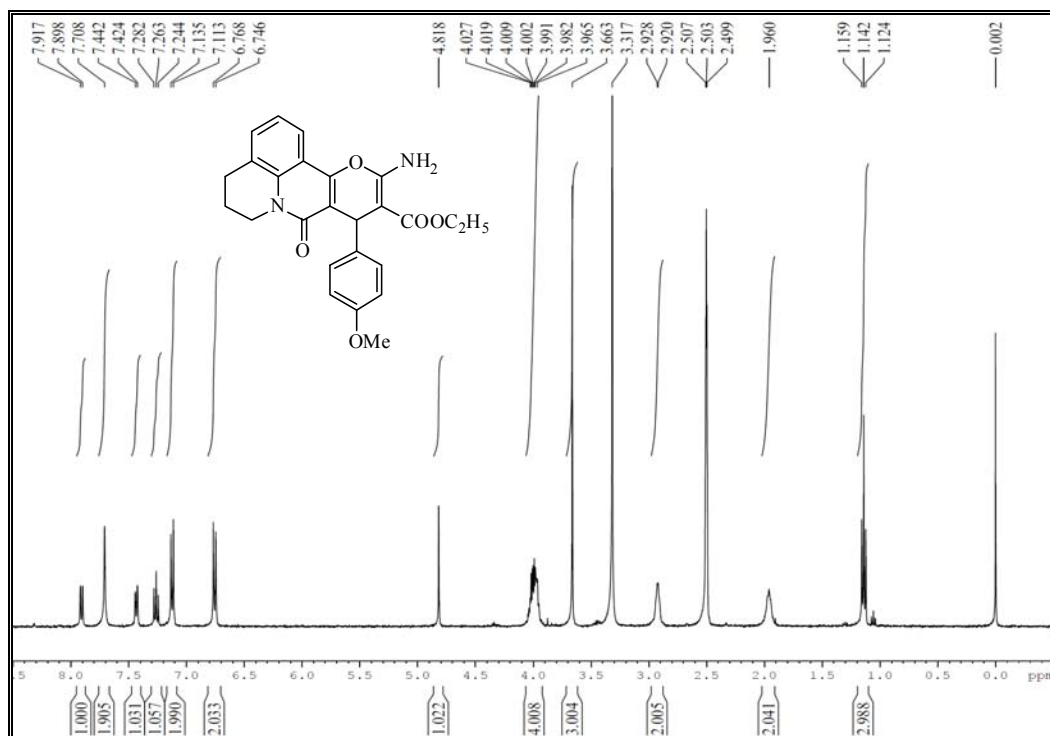
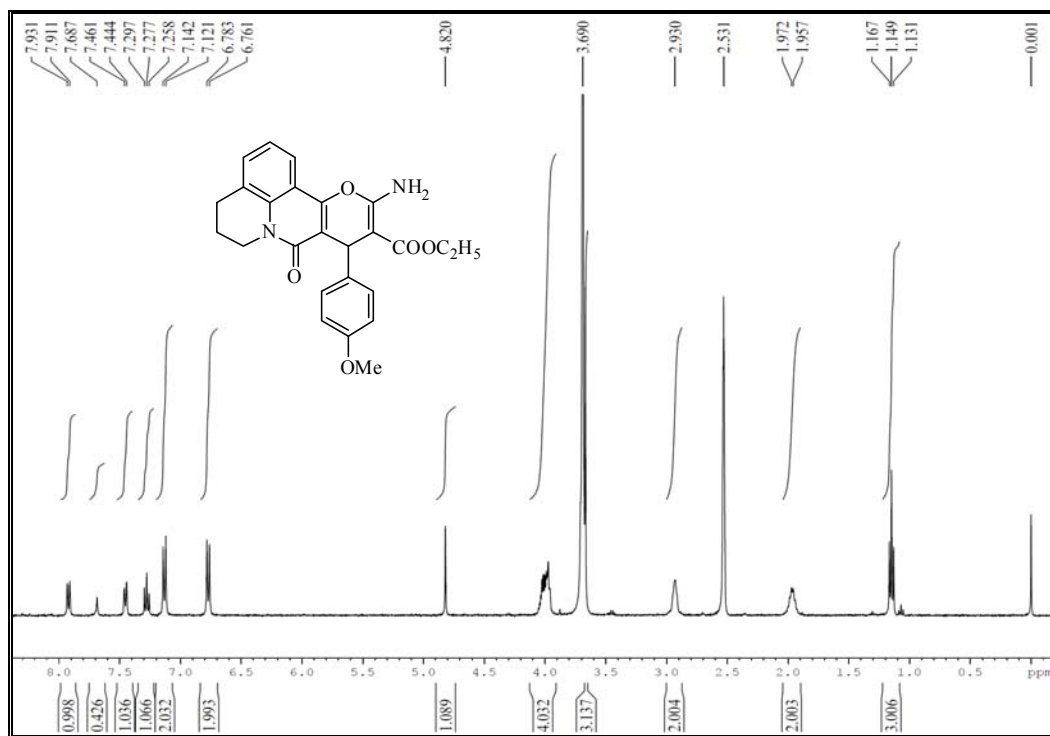
5.4 Representative Spectra

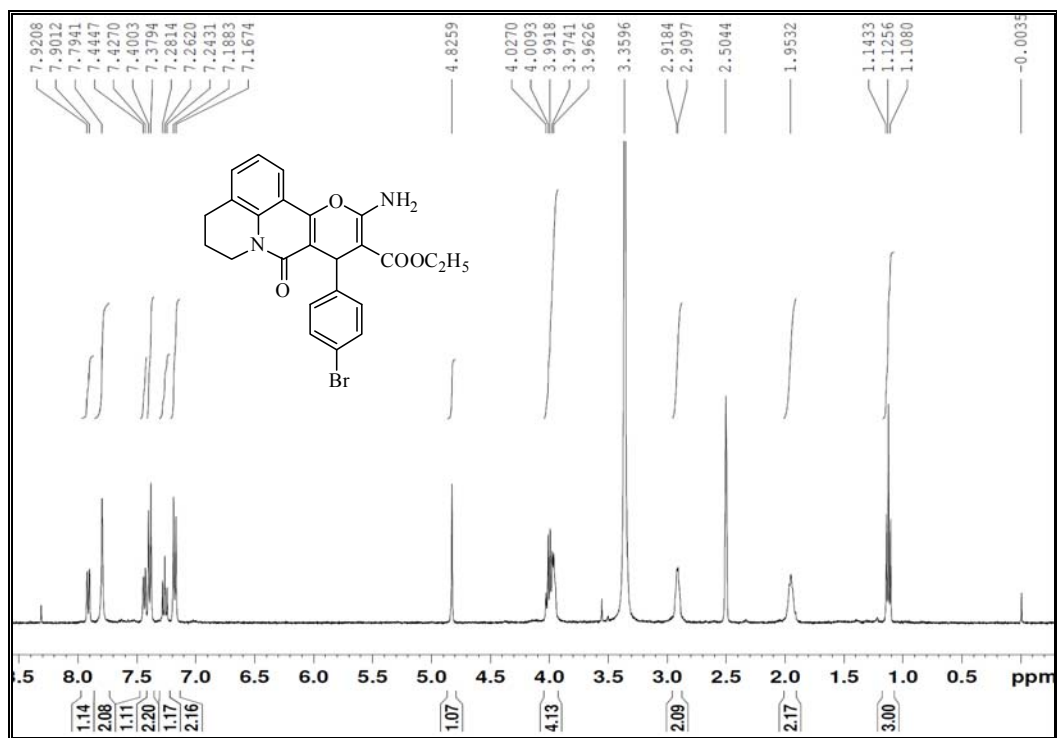
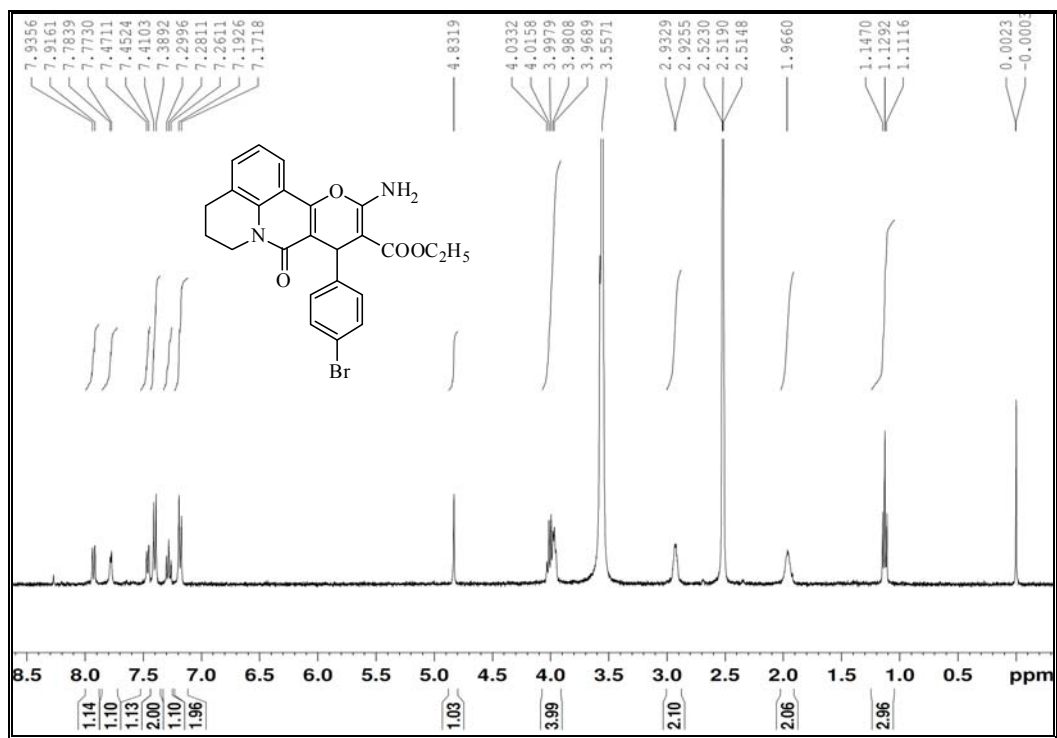
5.4.1 ^1H NMR Spectrum for compound **71a**.

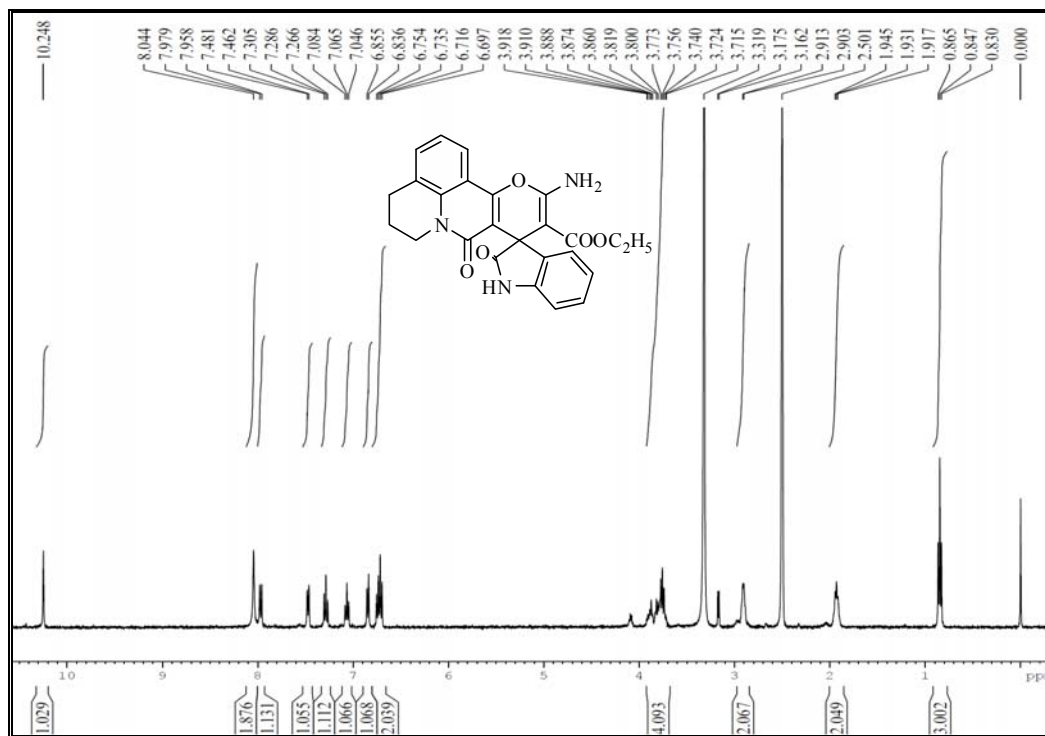
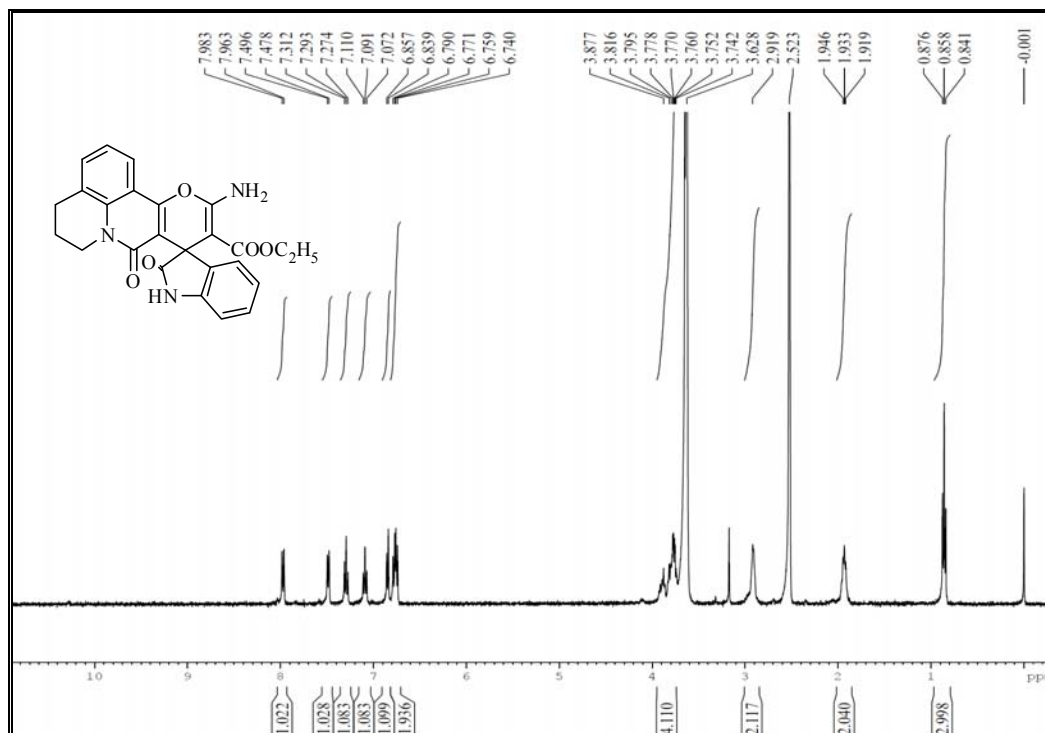


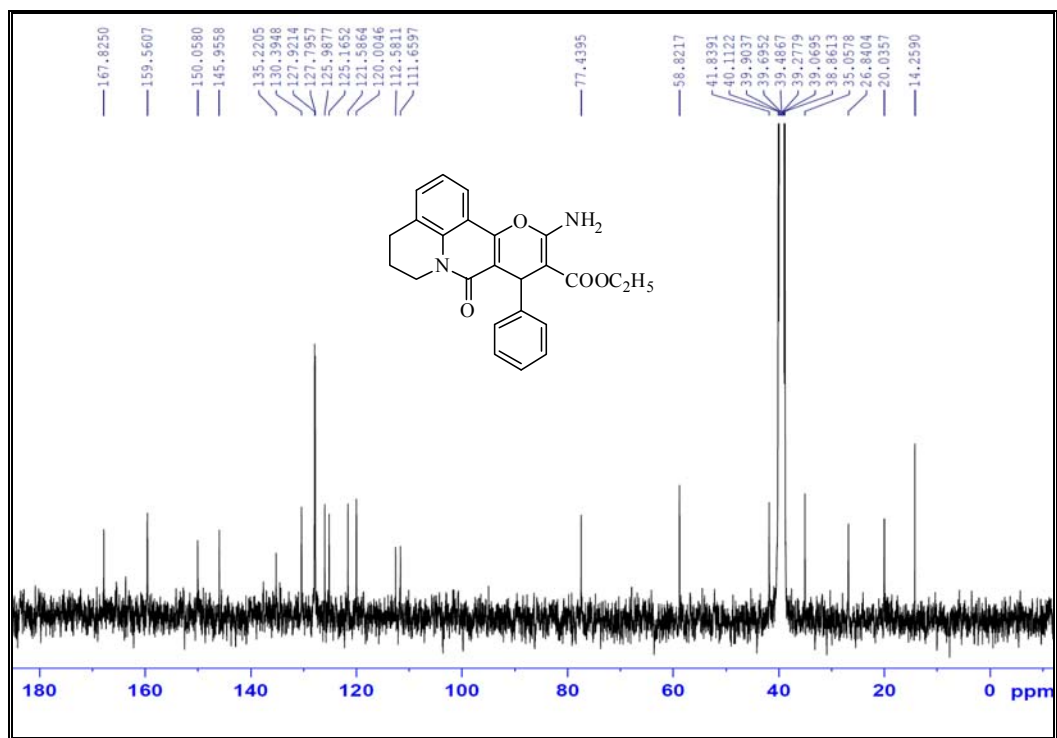
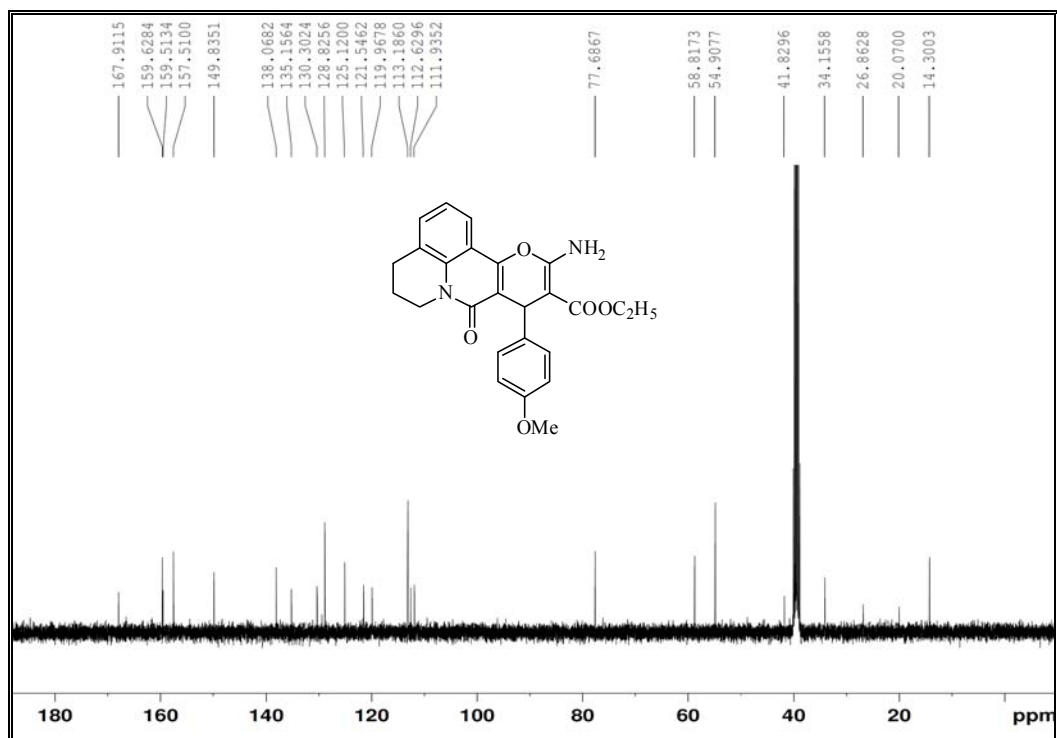
D₂O exchange ^1H NMR Spectrum for compound **71a**.

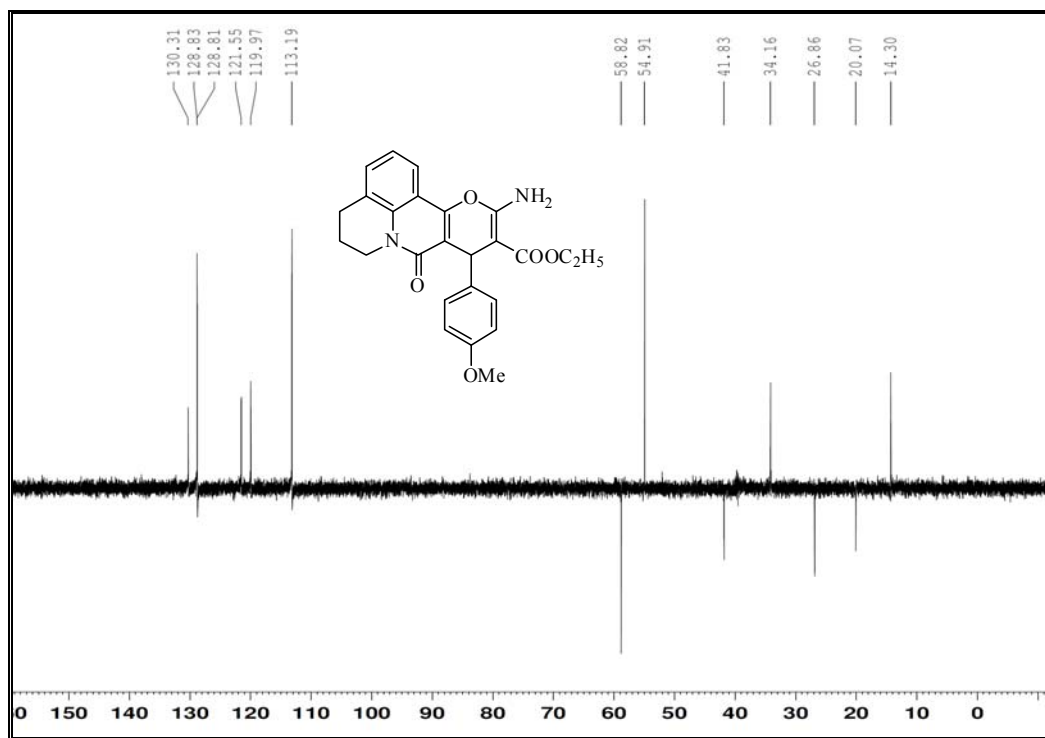
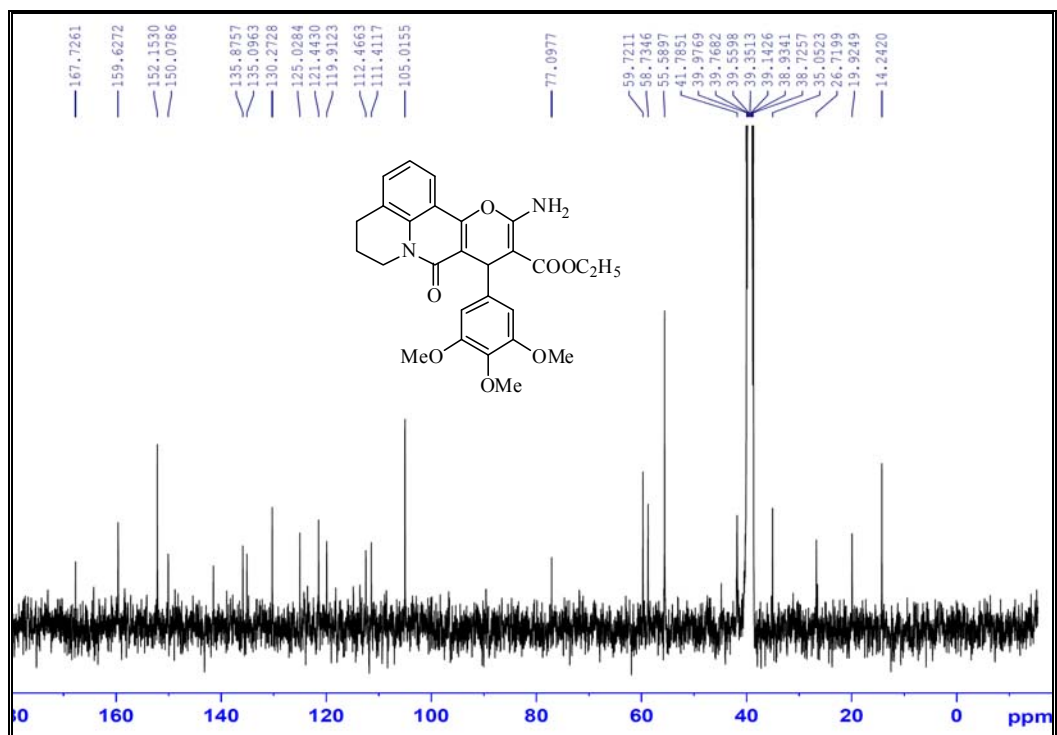


5.4.2 ^1H NMR Spectrum for compound **71d**. D_2O exchange ^1H NMR Spectrum for compound **71d**.

5.4.3 ^1H NMR Spectrum for compound **71m**. D_2O exchange ^1H NMR Spectrum for compound **71m**.

5.4.4 ^1H NMR Spectrum for compound **74**. D_2O exchange ^1H NMR Spectrum for compound **74**.

5.4.5 ^{13}C NMR Spectrum for compound **71a**.5.4.6 ^{13}C NMR Spectrum for compound **71d**.

5.4.7 ^{13}C DEPT ^{13}C NMR Spectrum for compound **71d**.5.4.8 ^{13}C NMR Spectrum for compound **71g**.

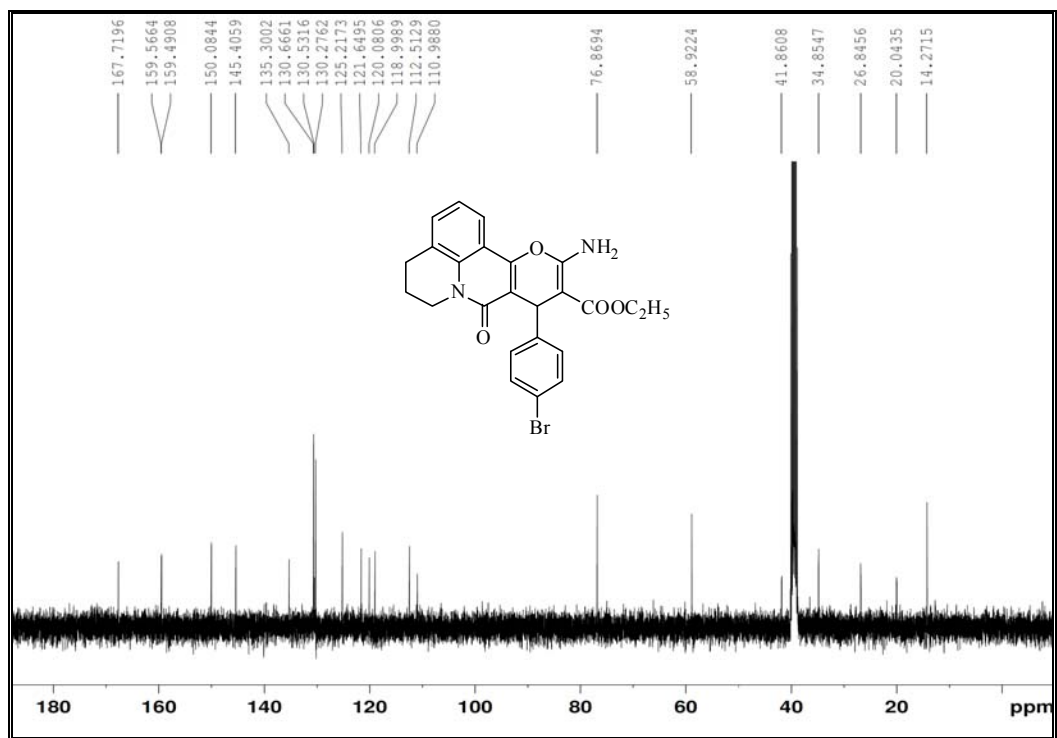
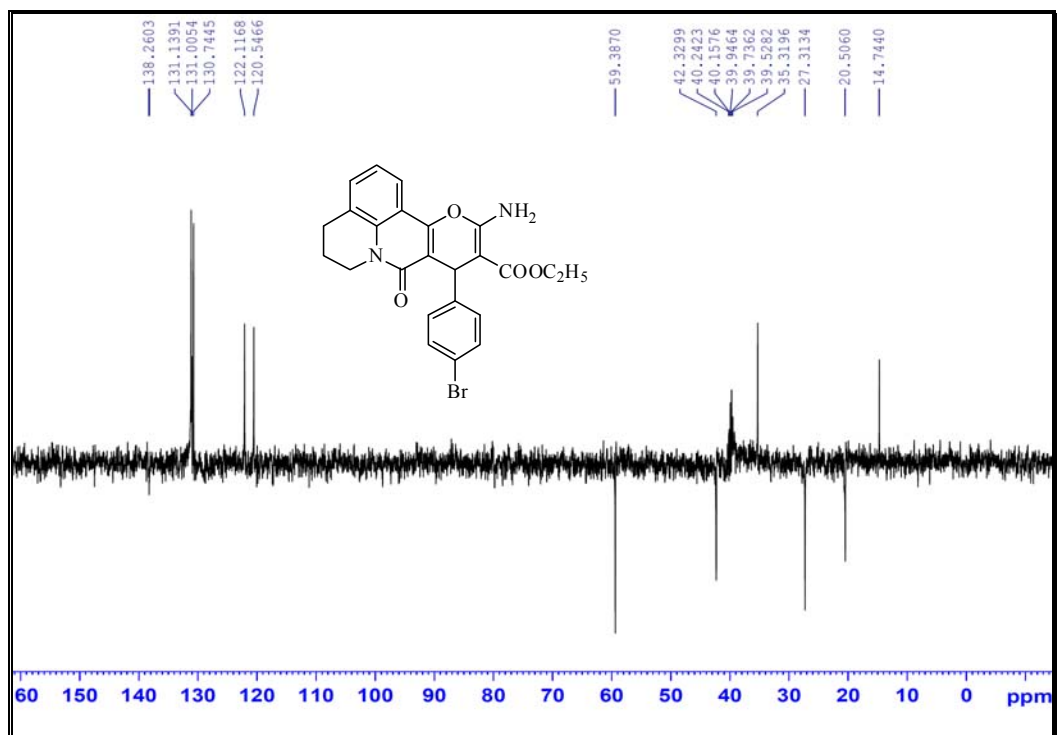
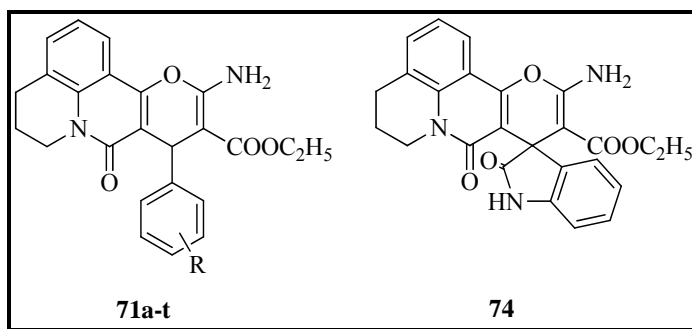
5.4.9 ^{13}C NMR Spectrum for compound **71m**.5.4.10 ^{13}C DEPT NMR Spectrum for compound **71m**.

Table 5.2 Elemental analysis of compounds **71a–t** and **74**.

Compd.	MF	MW	CHN Calculated (%)			CHN Found (%)		
			C	H	N	C	H	N
71a	C ₂₄ H ₂₂ N ₂ O ₄	402.44	71.63	5.51	6.96	71.41	5.56	6.72
71b	C ₂₅ H ₂₄ N ₂ O ₅	432.47	69.43	5.59	6.48	69.21	5.62	6.31
71c	C ₂₅ H ₂₄ N ₂ O ₅	432.47	69.43	5.59	6.48	69.52	5.22	6.61
71d	C ₂₅ H ₂₄ N ₂ O ₅	432.47	69.43	5.59	6.48	69.11	5.43	6.57
71e	C ₂₆ H ₂₆ N ₂ O ₆	462.49	67.52	5.67	6.06	67.62	5.61	5.84
71f	C ₂₇ H ₂₈ N ₂ O ₇	492.52	65.84	5.73	5.69	65.62	5.61	5.84
71g	C ₂₇ H ₂₈ N ₂ O ₇	492.52	65.84	5.73	5.69	65.54	5.81	5.58
71h	C ₂₄ H ₂₁ N ₂ O ₄ F	420.43	68.56	5.03	6.66	68.24	4.88	6.53
71i	C ₂₄ H ₂₁ N ₂ O ₄ F	420.43	68.56	5.03	6.66	68.34	4.76	6.58
71j	C ₂₄ H ₂₁ N ₂ O ₄ Cl	436.89	65.98	4.84	6.41	65.86	4.75	6.28
71k	C ₂₄ H ₂₁ N ₂ O ₄ Cl	436.89	65.98	4.84	6.41	65.88	4.71	6.25
71l	C ₂₄ H ₂₁ N ₂ O ₄ Br	481.34	59.89	4.40	5.82	59.74	4.21	5.89
71m	C ₂₄ H ₂₁ N ₂ O ₄ Br	481.34	59.89	4.40	5.82	59.64	4.31	5.99
71n	C ₂₅ H ₂₄ N ₂ O ₄	416.47	72.10	5.81	6.73	72.34	5.97	6.67
71o	C ₂₅ H ₂₄ N ₂ O ₄	416.47	72.10	5.81	6.73	72.28	5.94	6.82
71p	C ₂₅ H ₂₄ N ₂ O ₄	416.47	72.10	5.81	6.73	72.24	5.99	6.57
71q	C ₂₄ H ₂₁ N ₂ O ₅ Cl	452.89	63.65	4.67	6.19	63.47	5.01	6.23
71r	C ₂₅ H ₂₄ N ₂ O ₆	448.47	66.95	5.39	6.25	66.81	5.24	6.34
71s	C ₂₂ H ₂₀ N ₂ O ₅	392.40	67.34	5.14	7.14	67.44	5.27	7.02
71t	C ₂₂ H ₂₀ N ₂ O ₄ S	408.47	64.69	4.94	6.86	64.78	5.02	7.01
74	C ₂₅ H ₂₁ N ₃ O ₅	443.45	67.71	4.77	9.48	67.54	4.86	9.32

Section B

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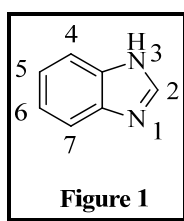
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Section C

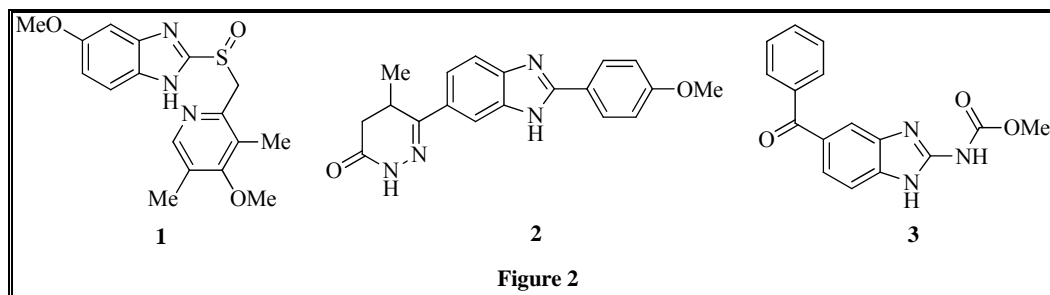
Chapter 6. Introduction of (1*H*-benzo[*d*]imidazol-2-yl)amino-pyrimidine derivatives

6.0 Introduction

Benzimidazole is a fused aromatic imidazole ring system where a benzene ring is fused to the 4 and 5 positions of an imidazole ring. Benzimidazoles are also known as benziminazoles and 1,3-benzodiazoles.^{1, 2} (Figure 1) They possess both acidic and basic characteristics. The NH group present in benzimidazoles is relatively strongly acidic and also weakly basic. Another characteristic of benzimidazoles is that they have the capacity to form salts. Benzimidazoles with unsubstituted NH groups exhibit fast prototropic tautomerism, which leads to equilibrium mixtures of asymmetrically substituted compounds.¹



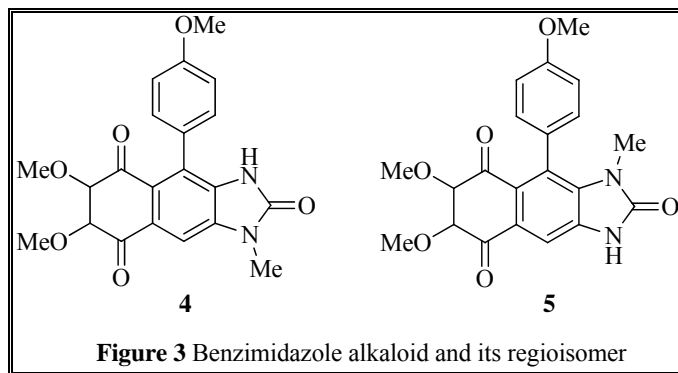
The benzimidazole scaffold is a useful structural motif for the development of molecules of pharmaceutical or biological interest.³⁻⁵ Appropriately substituted benzimidazole derivatives have found diverse therapeutic applications such as in antiulcers,³ antihypertensives,^{6,7} anti-HIV,⁸ antiinflammatory,⁹ anticancers,¹⁰ antioxidant,¹¹ antitrichinellosis,¹² and anxiolytics.¹³ The optimization of benzimidazole-based structures has resulted in various drugs which are currently in the market, such as omeprazole **1** (proton pump inhibitor), pimobendan **2** (ionodilator), and mebendazole **3** (anthelmintic) (Figure 2).



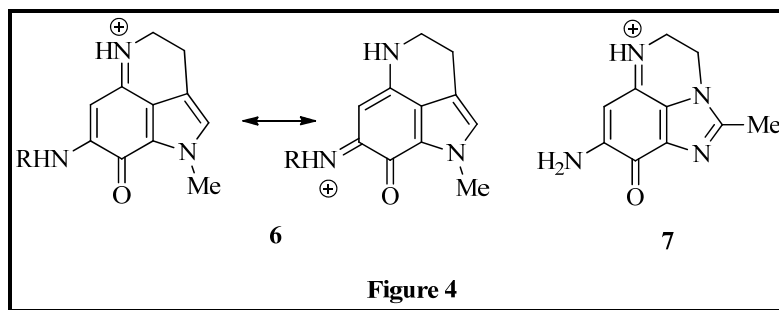
6.1 Benzimidazoles

Benzimidazole-derived alkaloids are rare in nature, and only few examples of these natural products can be found in the literature. On other hand, the occurrence of the imidazole skeleton in various natural sources is quite common.¹⁴⁻¹⁶ The benzimidazole alkaloid kealiiquinone (Figure 3) was isolated from a yellow button-like Micronesian sponge species of *Leucetta*.¹⁶

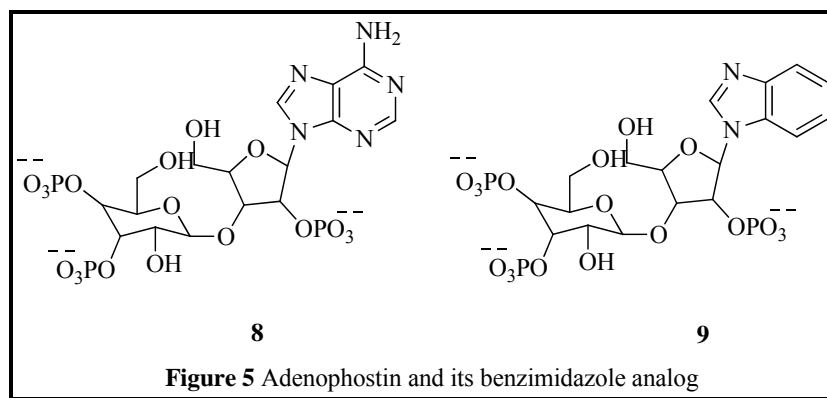
Recently, Nakamura *et al.* has successfully synthesized a regioisomer of kealiiquinone.¹⁷ The kealiiquinone **4** and its synthetic regioisomer **5** both have relatively weak activities against a panel of 39 human cancer cell lines but are considered to have a unique mechanism of action.¹⁷



Makaluvamines (pyrroloiminoquinones) **6** (Figure 4) was isolated from a *Fijian* sponge in the early 1990s display *in vitro* cytotoxicity against human colon tumor cell lines and also inhibited human topoisomerase II *in vitro*. The benzimidazole analog of this indole-based marine natural product, imidazoquinoxalinone **7**, has been synthesized by LaBarbera D.V. and co-workers.¹⁸



In comparison with the natural inositol 1,4,5-triphosphate, the adenophostins **8** (Figure 5) exhibit higher receptor binding activity and Ca^{2+} mobilizing potencies and thus have significant biological importance. A total synthesis of a benzimidazole analog of adenophostin A **9** was described by Shuto *et al.*¹⁹

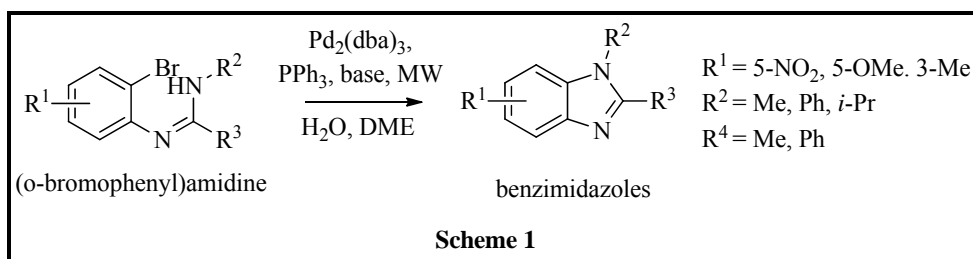


6.2 Synthetic Methodologies for Benzimidazoles

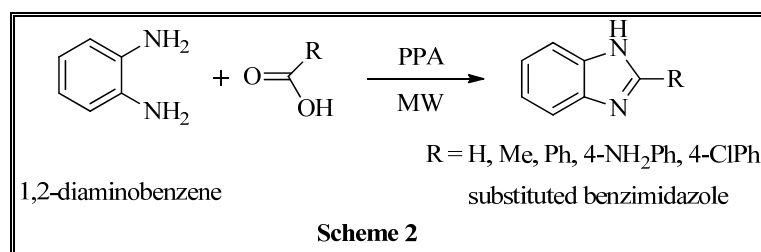
Most commonly benzimidazoles have been prepared from the reaction of 1,2-diaminobenzenes with carboxylic acids under harsh dehydrating reaction conditions, utilized strong acids such as polyphosphoric acid, hydrochloric acid, boric acid, or *p*-toluenesulfonic acid.²⁰ However, the use of milder reagents, particularly Lewis acids,²¹ inorganic clays,²² or mineral acids,²³ has improved both the yield and purity of this reaction.²⁴ On the other hand, the synthesis of benzimidazoles via the condensation of 1,2-diaminobenzenes with aldehydes requires an oxidative reagent to generate the benzimidazole nucleus. Various oxidative reagents, such as nitrobenzene, benzoquinone, sodium metabisulfite, mercuric oxide, lead tetraacetate, iodine, copper(II) acetate, indium perfluorooctane sulfonates, ytterbium perfluorooctane sulfonates, and even air, have been employed for this purpose.²⁵ Moreover, a variety of benzimidazoles could also be produced via coupling of 1,2-diaminobenzenes with carboxylic acid derivatives such as nitriles, imidates, orthoesters, anhydrides or lactones.²⁶

In recent years, some innovative and improved pathways for the synthesis of benzimidazoles have been developed and these are discussed in the following.

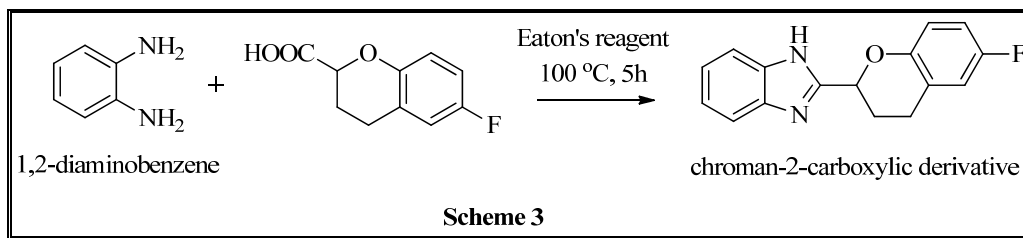
A palladium-catalyzed *N*-arylation reaction provided a novel synthesis of benzimidazoles from (*o*-bromophenyl)amidine precursors under microwave irradiation. The route was found to be flexible with respect to various substituents and allowed for the preparation of highly substituted benzimidazoles, including *N*-substituted examples (Scheme 1).²⁷ The method was later improved and optimized to achieve the rapid formation of benzimidazoles in high yield.²⁸ It has been found that 50% aqueous dimethyl ether (DME) is an optimal solvent for the reaction and that catalyst loading of palladium can be reduced to 1 mol%.



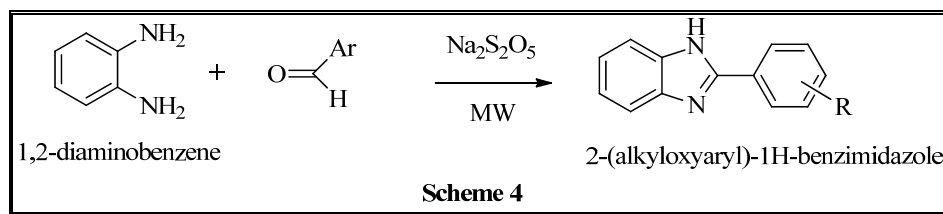
Recently, 2-alkyl- and 2-aryl-substituted benzimidazole derivatives have been synthesized from 1,2-diaminobenzene and its corresponding acids in the presence of polyphosphoric acid using microwave assisted methods (Scheme 2).²⁹ The reaction time required for the synthesis of benzimidazole derivatives was reduced to minutes by this method compared to conventional synthesis, which required up to four hours of heating to complete the reaction. Furthermore, it was found that the application of microwave irradiation increased yields by 10–50%.



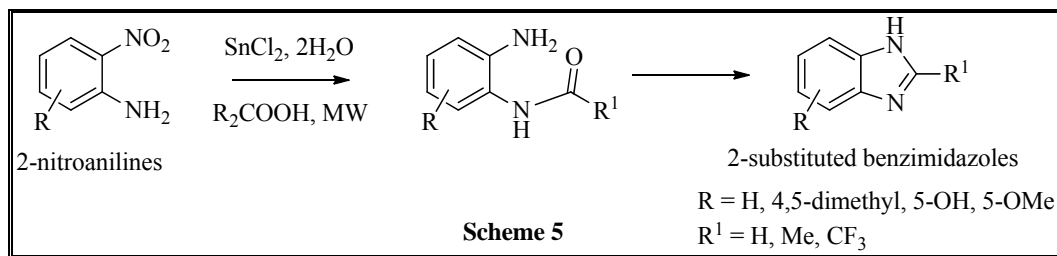
Conventional condensation of 1,2-diaminobenzene with 6-fluoro-3,4-dihydro-2*H*-chroman-2-carboxylic acid under Phillip's conditions or using Eaton's reagent (1 : 10 mixture of phosphorus pentoxide/methanesulfonic acid) yielded 2-(6-fluorochroman-2-yl)-1*H*-benzimidazole (Scheme 3).³⁰



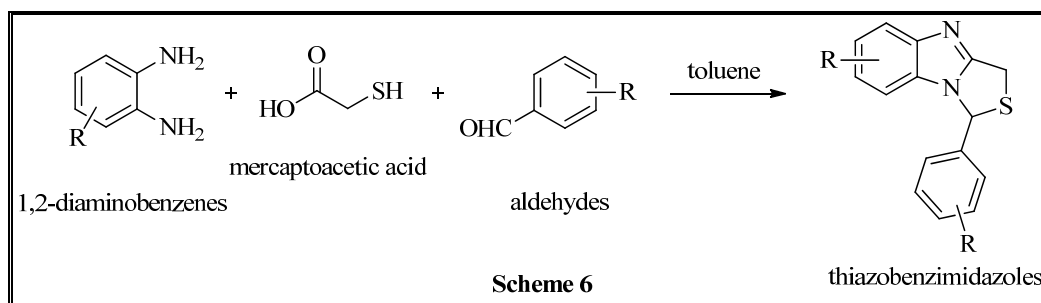
Recently, microwave-assisted synthesis of 2-(alkyloxyaryl)-1*H*-benzimidazole derivatives related to the natural stilbenoid family has been reported (Scheme 4).³¹



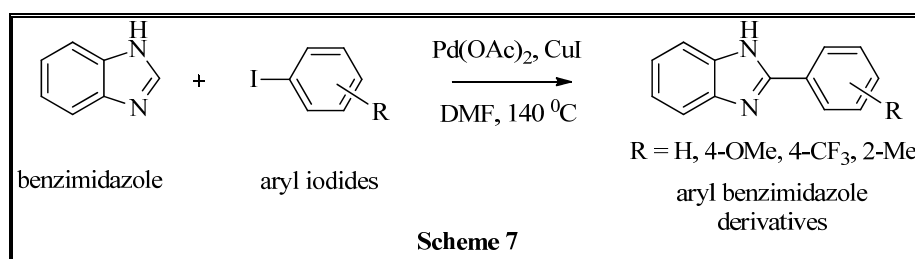
Recently, a facile, rapid one-pot procedure for the generation of 2-substituted benzimidazoles directly from 2-nitroanilines using a microwave procedure has been demonstrated (Scheme 5). An advantage of this approach is that the intermediate *N*-acyl derivatives need not be isolated prior to cyclization.³²



Classical condensation-cyclization reactions using 1,2-diaminobenzenes, 2-mercaptoacetic acid and appropriately substituted aromatic aldehydes in dry benzene under reflux required a long reaction time to afford the thiazobenzimidazoles, which are potent anti-HIV agents. (Scheme 6)³³



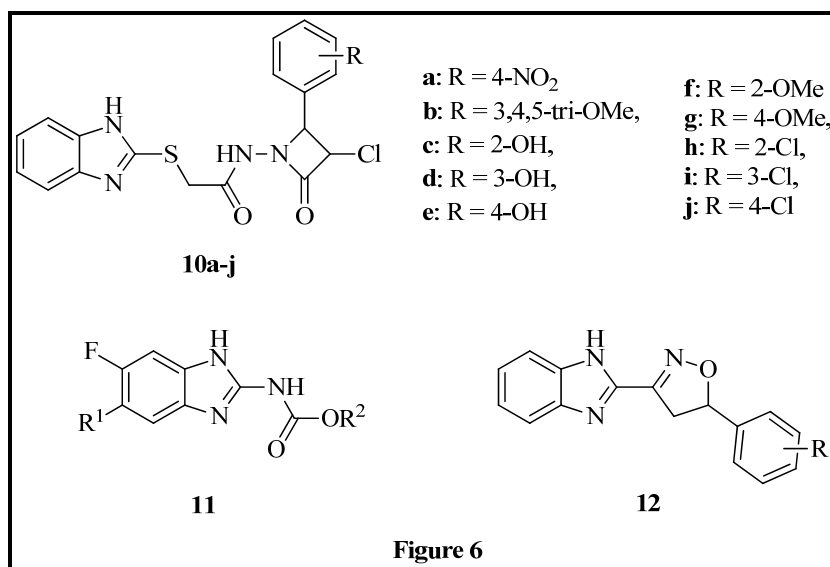
Functionalization of C–H bonds of heterocycles to C-arylation is an important synthetic reaction and is used to build important bioactive structures. Palladium catalyst and copper-mediated C-2 arylations of benzimidazole with aryl iodides under ligandless and base-free conditions have been described by Bellina *et al.* (Scheme 7).³⁴



6.3 Pharmacological profile of Benzimidazoles

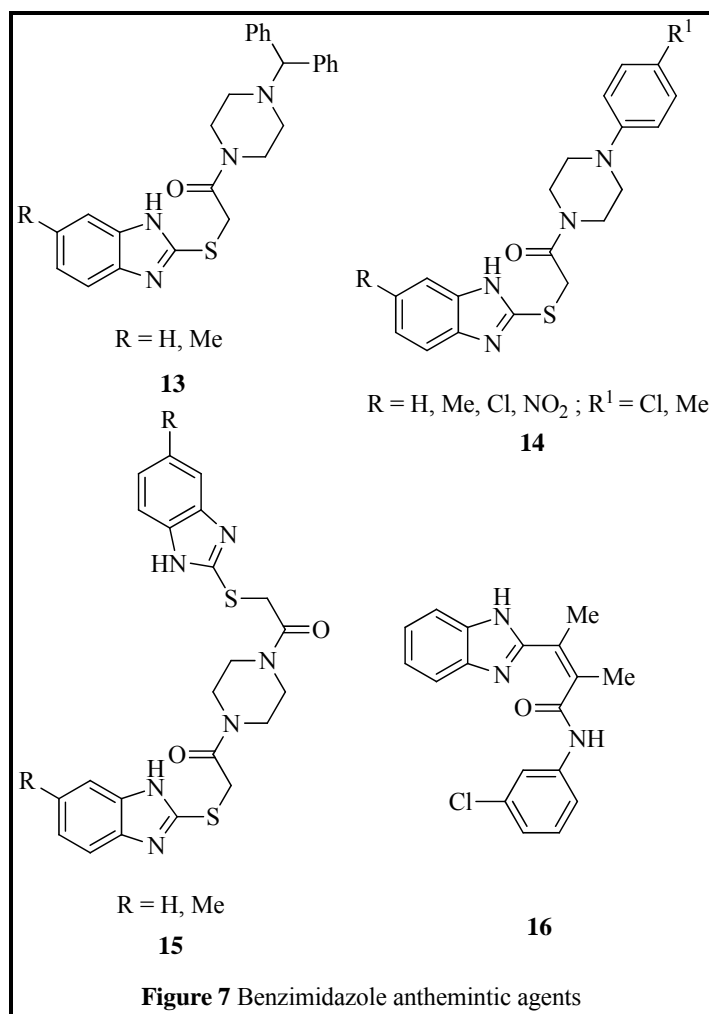
6.3.1 Antibacterial and Antifungal Agents

2-substituted benzimidazole derivatives are known to possess varied biological activities.³⁵ Recently, an efficient and rapid synthesis of novel benzimidazole azetidin-2-ones **10** has been established,³⁶ and antibacterial screening revealed that all newly synthesized azetidin-2-ones **10** exhibited potent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. Among all of the compounds investigated, **10i** and **10j** exhibited the greatest antibacterial activity against Gram-negative *E. coli* as compared to the antibiotic streptomycin.³⁶ In addition, 5-fluoro benzimidazole carboxamide derivatives **11**³⁷ and benzimidazole isoxazolines **12**³⁸ were reported to show antibacterial and antifungal activities.



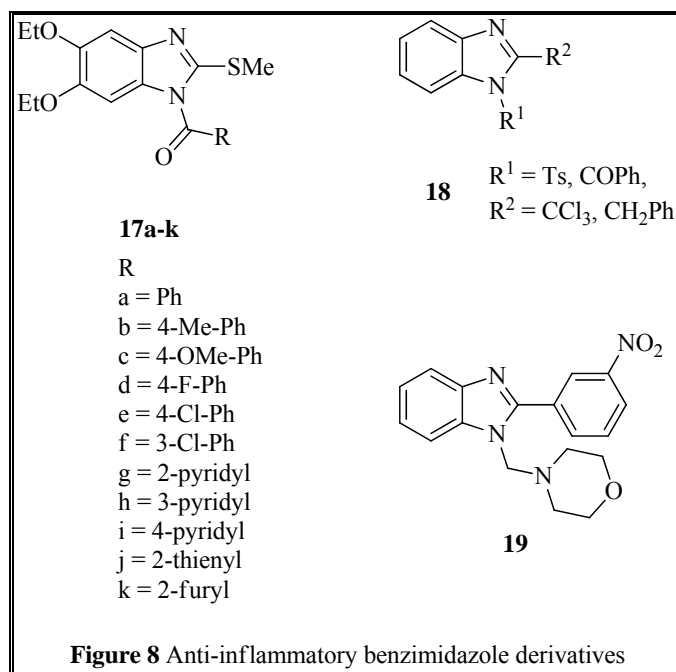
6.3.2 Anthelmintic Agents

Bearing in mind previous benzimidazole anthelmintics (e.g., albendazole, mebendazole), the search for new anthelmintic drugs is being actively pursued. Synthetic benzimidazole piperazine derivatives exhibited 50% anthelmintic activity in mice infected with *Syphacia obvelata*.³⁹ Furthermore, piperazine derivatives of 5(6)-substituted-(1*H*-benzimidazol-2-ylthio) acetic acids **13–15**⁴⁰ and benzimidazolyl crotonic acid anilide **16** have shown good anthelmintic activity⁴¹ (Figure 7).



6.3.3 Anti-inflammatory and Antiulcer Agents

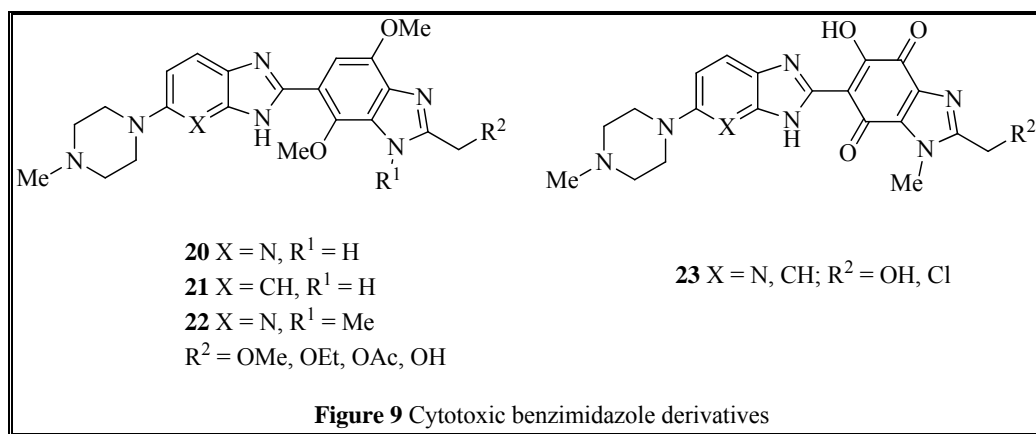
Structure–activity relationship studies of the 5,6-dialkoxy-2-thiobenzimidazole derivatives **17** have revealed that compounds **17a–k** possess pronounced anti-inflammatory properties⁴² (Figure 10). Using the carrageenan model, the most significant anti-inflammatory effects were observed for compounds **17a**, **17d**, **17h**, **17i**, and **17j**. While using the bentonite model, the maximum activities were observed for compounds **17e** and **17h**. These results indicated that benzimidazoles are promising leads for the development of new anti-inflammatory agents.



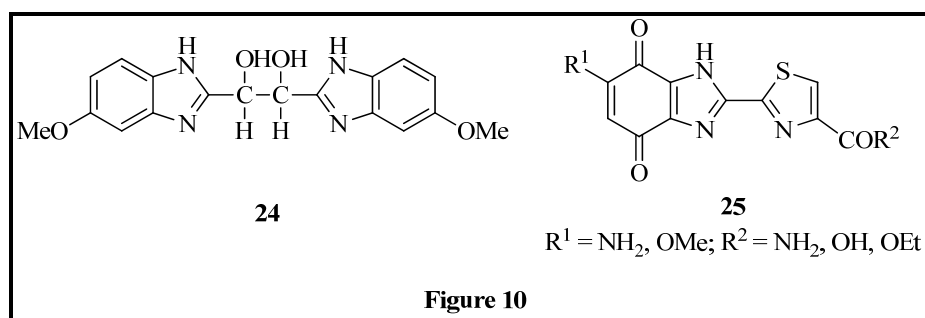
In addition, N-benzoyl and N-tosyl benzimidazole compounds **18** showed significant anti-inflammatory activity, as indicated by ear swelling induced by xylene in mice, and their ulcer indices were all lower than those of aspirin.⁴³ Furthermore, N-morpholinomethylbenzimidazole **19** and its derivatives have been recently reported to show significant anti-inflammatory activity.⁴⁴

6.3.4 Cytotoxic and Antitumor Agents

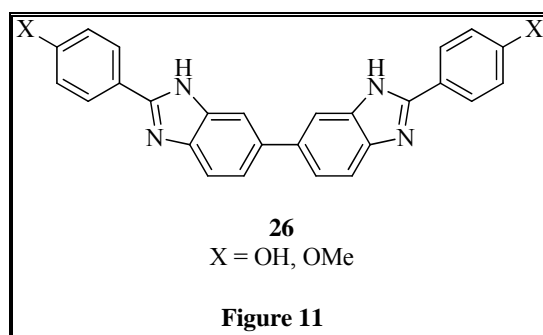
Novel bisbenzimidazoles with general formula **20–23** incorporating benzimidazole, pyridoimidazole, and imidazoquinone moieties as one of the units of bisbenzimidazole with a piperazinyl functional group have been synthesized (Figure 9).⁴⁵ The series of bisbenzimidazoles contains different leaving groups along with *p*-methoxy substituents. The latter may be expected to have some influence on the nitrogen lone pair and consequently on the binding characteristics of the ligand. These novel bisbenzimidazoles are found to be actively cytotoxic against many human cancer cell lines, with GI₅₀ values of between 0.01 and 100 μM, especially in the cases of renal cancer, CNS cancer, colon cancer, melanoma, and breast cancer cell lines.



In addition, the alkyl-linked bisbenzimidazole **24**⁴⁶ and thiazolylbenzimidazole-4,7-diones **25**⁴⁷ exhibited cytotoxic activity against tumor cell lines (Figure 10).

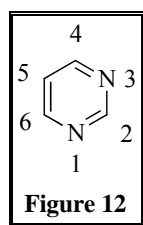


Moreover, novel head-to-head bisbenzimidazole compound **26** showed potent growth inhibition in human ovarian carcinoma cell lines (IC₅₀ = 200–300 nM), with no significant cross-resistance in two acquired cisplatinresistant cell lines and a low level of cross-resistance in the *p*-glycoprotein over expressing doxorubicin-resistant cell line. In addition, compound **26** was found to have significant in vivo activity in the allowed fiber assay and tumor xenografts.⁴⁸⁻⁵⁰

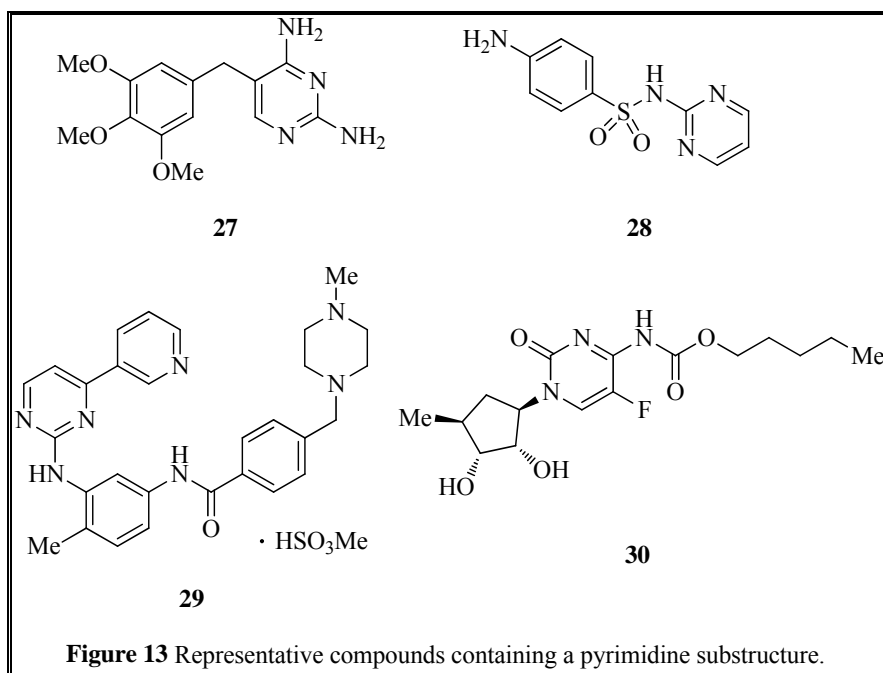


6.4 Introduction of Pyrimidine

Pyrimidine is a heterocyclic aromatic organic compound similar to benzene and pyridine, containing two nitrogen atoms at positions 1 and 3 of the six-member ring.⁵¹ It is isomeric with two other forms of diazine. A pyrimidine has many properties in common with pyridine, as the number of nitrogen atoms in the ring increases the ring pi (π) electrons become less energetic and electrophilic aromatic substitution gets more difficult while nucleophilic aromatic substitution gets easier.

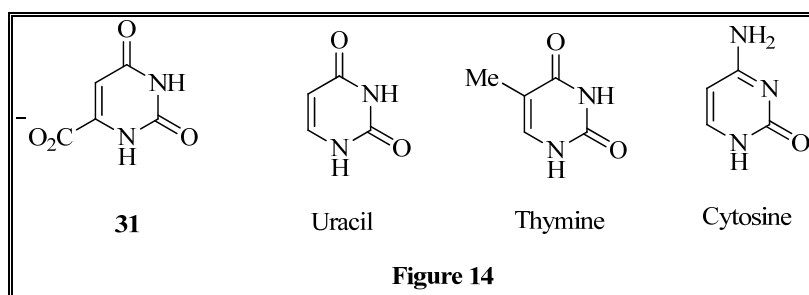


Azaheterocycles constitute a very important class of compounds. In particular, pyrimidine derivatives include a large number of natural products, pharmaceuticals, and functional materials (Figure 13).⁵² Several examples of pharmaceutically important compounds include trimethoprim **27**,⁵³ sulfadiazine **28**,⁵⁴ Gleevec (**29**, imatinib mesilate),⁵⁵ and Xeloda (**30**, capecitabine).⁵⁶

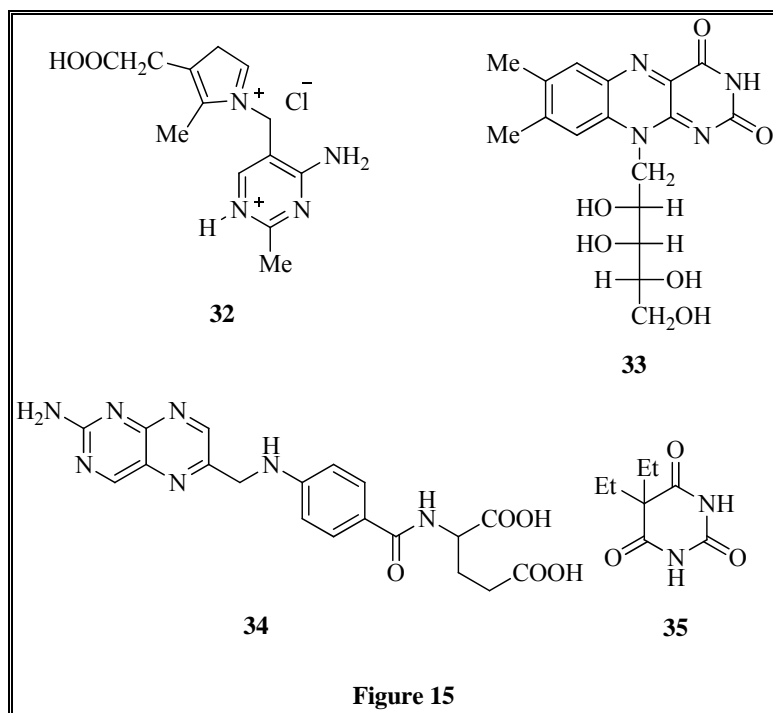


6.5 Pyrimidine Natural Products

In nature, the pyrimidine ring is synthesized from glutamine, bicarbonate, and aspartate.⁵⁷ These starting materials are converted to orotate (**31**, Figure 14). Several (mainly uracil, thymine and cytosine) pyrimidines have been isolated from the nucleic acid hydrolyses. The nucleic acid are essential constituent of all cell and thus of all living matter cytosine is found to be present in both types of nucleic acid i.e. ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) while uracil present only in RNA and thymine only in DNA.⁵⁸

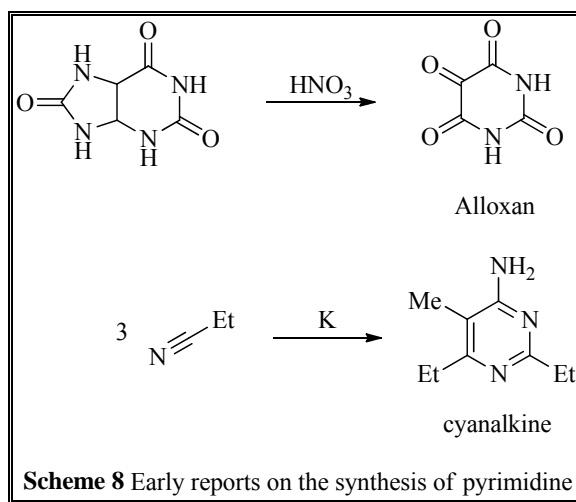


In addition, Pyrimidine ring is also found in vitamin like thiamine **32**, riboflavin **33** and folic acid **34**.⁵⁹ Barbitone **35**, the first barbiturate hypnotic sedative and anticonvulsant is a pyrimidine derivative.⁶⁰

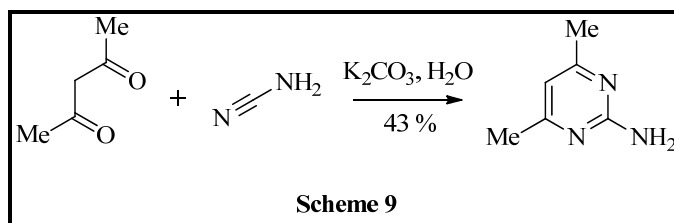


6.6 Synthetic Methodologies for Pyrimidines

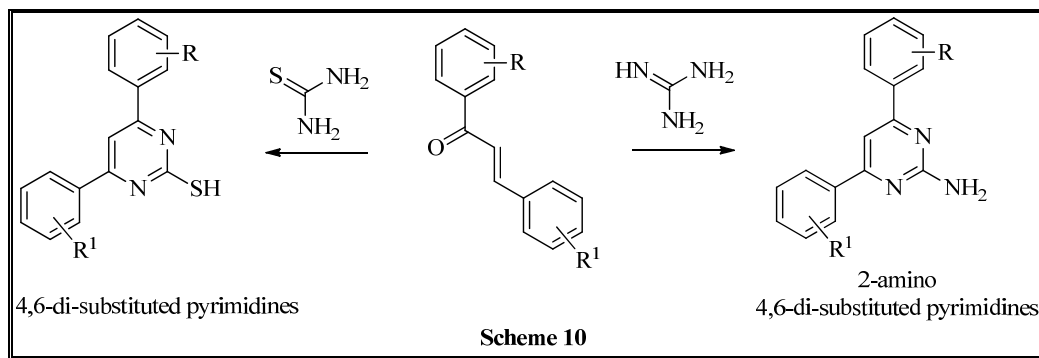
In 1818, Brugnatelli synthesized the first pyrimidine derivative, alloxan by nitric acid oxidative degradation of uric acid (Scheme 8).⁶¹ Another early report, by Frankland and Kolbe in 1848, described the first synthesis of a pyrimidine cyanalkine by heating propionitrile with potassium metal (Scheme 8).⁶²



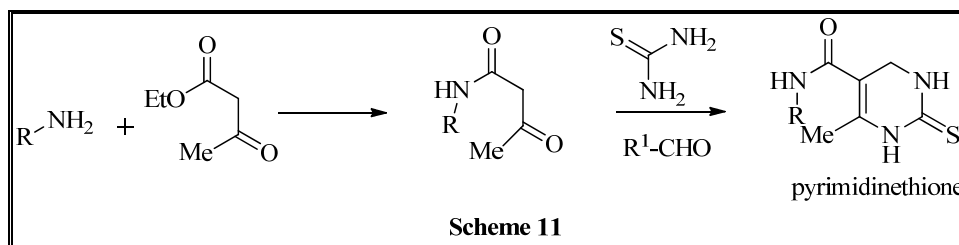
Nitriles are a common N–C source and have been used to form pyrimidines in many syntheses. Cyanamide is a particularly useful nitrile derivative in the synthesis of pyrimidines as illustrated in Scheme 9.⁶³



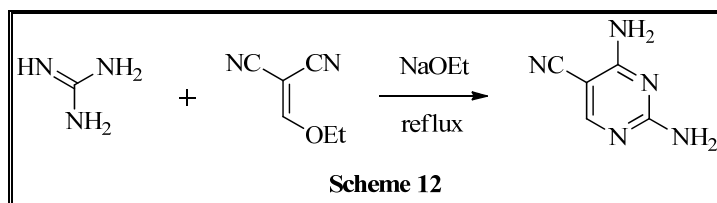
The 4,6-di-substituted pyrimidines and 2-amino-4,6-di-substituted pyrimidines formed by the reaction of chalcone with thiourea and guanidine hydrochloride in presence of sodium hydroxide.(Scheme 10)⁶⁴



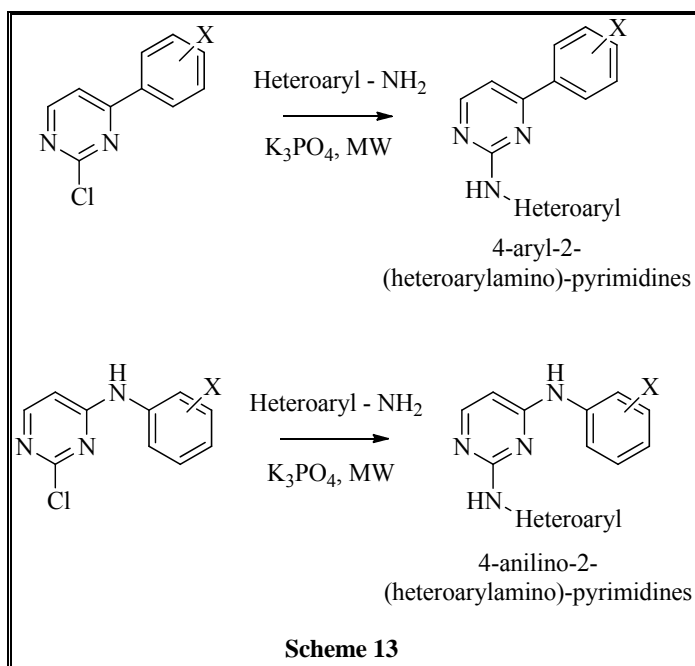
Anilido compounds are produced by treatment of different aryl amines with ethylacetoacetate, which cyclized with various aromatic aldehydes and thiourea, furnishing corresponding pyrimidinethione derivatives (Scheme 11).⁶⁵



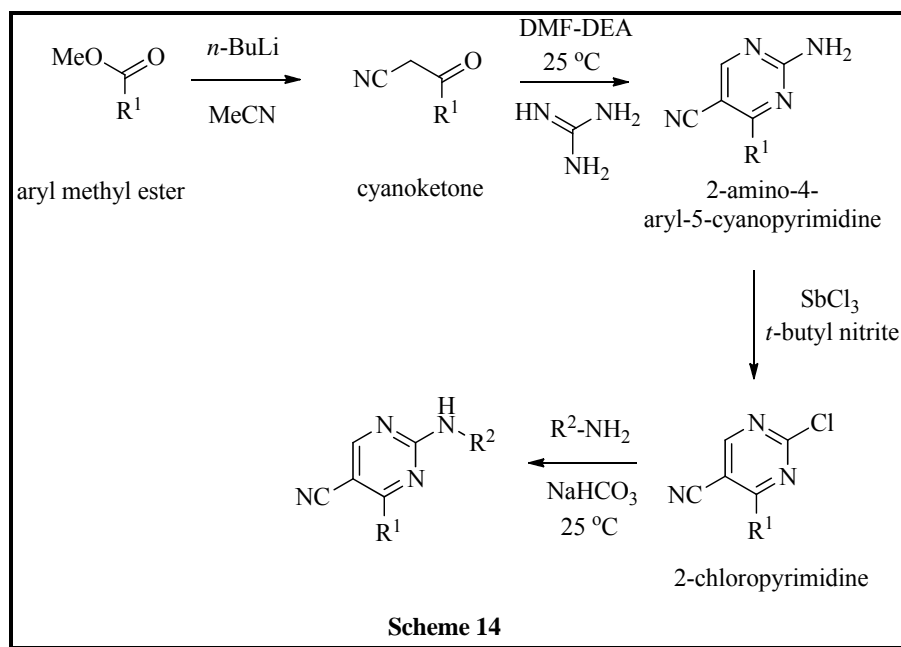
Bag Seema *et al.* has reported a single step reaction for 2,4-diaminopyrimidine from guanidine and orthoester in the presence of sodium ethanoate in ethanol as solvent (Scheme 12).⁶⁶



4-Aryl-2-anilinopyrimidines and 2,4-dianilinopyrimidines (i.e., DAPYs) represent privileged structures⁶⁷ found in an ever increasing number of drug-like molecules including VEGF⁶⁸ and CDK⁶⁹ inhibitors, reverse transcriptase inhibitors (e.g., dapivirine), and tyrosine kinase inhibitors (e.g., Gleevec). Brian I. Bliss *et al.* describe the convenient preparation of novel 4-aryl-2-(heteroaryl-amino)-pyrimidines and 4-anilino-2-(heteroaryl-amino)-pyrimidines.⁷⁰ (Scheme 13)

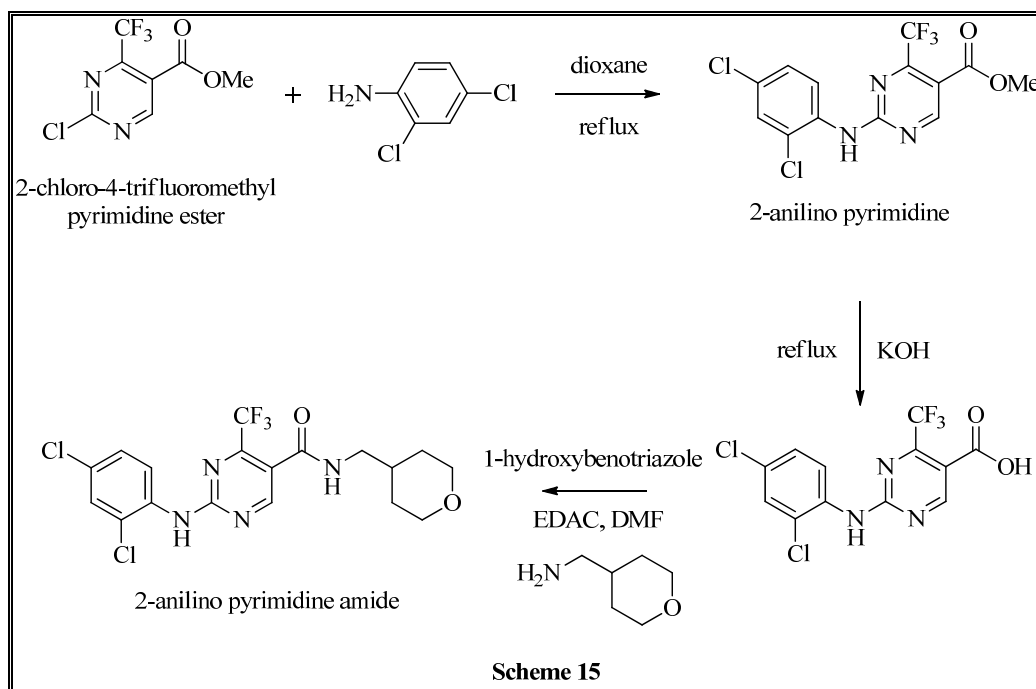


Terry V. Hughes and co-workers have synthesized 4-Aryl-5-cyano-2-aminopyrimidines as VEGF-R2 inhibitors.⁷¹ The key step involved reaction of a vinylogous amide with a guanidinium salt to form the pyrimidine ring. Specifically, conversion of an aryl methyl ester ($R^1 = \text{aryl}$) to the corresponding α -cyanoketone was achieved via formation of the lithium salt of acetonitrile by treatment with *n*-BuLi at 78 °C followed by reaction with the ester at 45 °C. Subsequent treatment of the α -cyanoketone with *N,N*-dimethylformamide diethyl acetal (DMF-DEA) formed a vinylogous amide in situ that was reacted with guanidine nitrate in DMF at 100 °C to form the 2-amino-4-aryl-5-cyanopyrimidine. The Sandmeyer reaction of the aminopyrimidine was accomplished smoothly afford the 2-chloropyrimidine. The displacement of the Cl of pyrimidine with aliphatic amines proceeded at room temperature and with aromatic amines in refluxing THF to afford the pharmacophore. (Scheme 14)

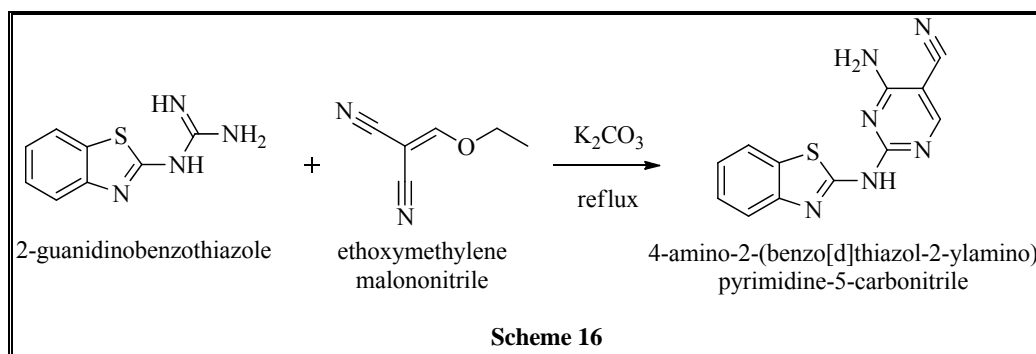


Giblin *et al.*⁷² developed an efficient synthesis of 2-anilino pyrimidine derivatives has been achieved via reaction of 2-chloro-4-trifluoromethyl pyrimidine ester and aromatic amine in dioxane. (Scheme 15) These compounds were found very potent analgesic in the FCA model of inflammatory pain and have a high therapeutic index and a promising pharmacokinetic profile in the rat.

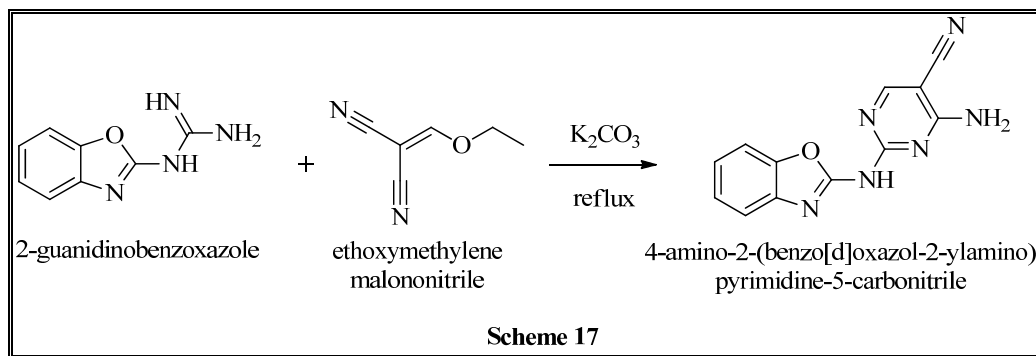
The prepared 2-anilino pyrimidine ester and amide derivatives showed micromolar potency at the CB2 receptor and good selectivity against CB1.⁷³



Youssef *et al.*⁷⁴ recently reported the synthesis and anticancer, antimicrobial activity of 4-amino-2-(benzo[d]thiazol-2-ylamino)pyrimidine-5-carbonitrile derivative. This compound was prepared from 2-guanidinobenzothiazole with ethoxymethylenemalononitrile in presence of anhydrous potassium carbonate reflux in absolute ethanol. (Scheme 16)



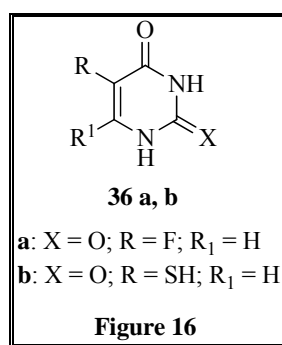
Sherif *et al.*⁷⁵ has designed, synthesized and investigated the anti-HIV activity of some new 4-amino-2-(benzoxazol-2-ylamino)-pyrimidines-5-carbonitrile derivatives, which was found to inhibit the spread of the HIV infection by 95 % in MT⁴ cell culture. The 2-(benzoxazol-2-ylamino)-pyrimidines derivative was prepared by conventional method as described below. (Scheme 17)



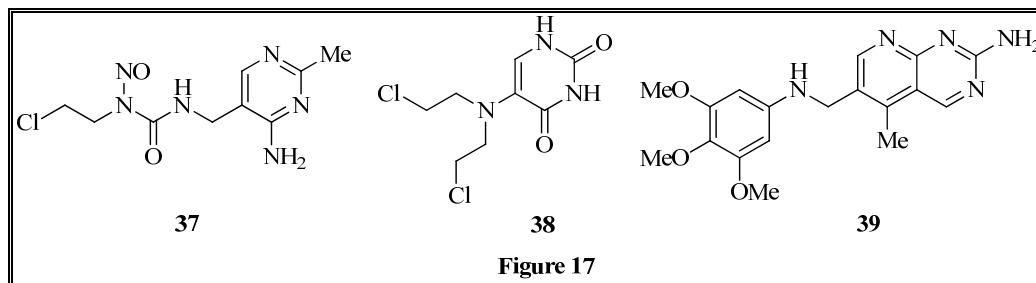
6.7 Pharmacological Profile of Pyrimidines

6.7.1 Antineoplastics and anticancer agents

There are a large number of pyrimidine-based antimetabolites. They are usually structurally related to the endogenous substrates that they antagonize. One of the early metabolites prepared was 5-fluorouracil⁷⁶ (5-FU, **36a**), a pyrimidine derivative. 5-Thiouracil **36b** also exhibits some useful antineoplastic activities.⁷⁷

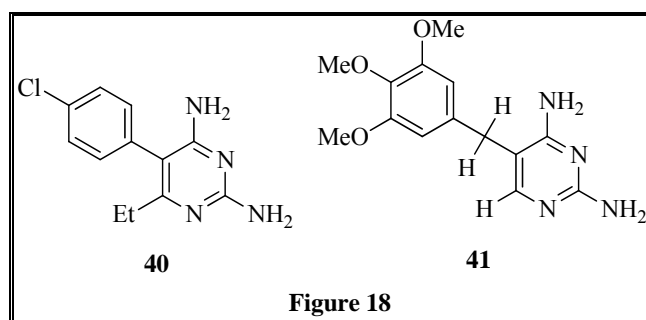


There are many more in recent times, like nimustine **37**⁷⁸, uramustine **38**⁷⁹ and trimetrixate **39**⁸⁰. It is mainly used as an anticancer agent and also exhibits significant therapeutic effects in patients with herpes virus infections and herpes encephalitis.



6.7.2 Antibacterials and antiprotozoals

In 1948, Hitchings made an important observation that a large number of 2,4-diaminopyrimidines and some 2-amino-4-hydroxypyrimidines are antagonists of folic acid.⁸¹ Since then, a large number of 2,4-diaminopyrimidines have been synthesized as antifolates. It was eventually proved that these pyrimidines are inhibitors of the enzyme dihydrofolate reductase (DHFR).⁸² Notable amongst the 2,4-diaminopyrimidine drugs are pyrimethamine **40**, a selective inhibitor of the DHFR of malarial plasmodia; trimethoprim **41**, an antibacterial drug which selectively inhibits bacterial DHFR.



6.7.3 Sulfa drugs

Pyrimidine derivatives of sulfa drugs, namely sulfadiazine, sulfamerazine and sulfadimidine are superior to many other sulfonamides and are used in some acute UT infections, cerebrospinal meningitis and for patients allergic to penicillins⁸³. Sulfonamide–trimethoprim combinations are used extensively for opportunistic infections in patients with AIDS⁸⁴. Sulfadoxine⁸⁵ **42a**, a short and intermediate acting sulfonamide with a half-life of 7–9 days is used for malarial prophylaxis. Sulfisomidine **42b** with a half-life of 7 h is used as a combination sulfa therapy in veterinary medicine⁸⁶. Sulfadiazine **43a**, sulfamerazine **43b** and sulfadimidine **43c** possess good water solubility and therefore carry minimum risk of kidney damage, which makes them safe even for patients with impaired renal functions.

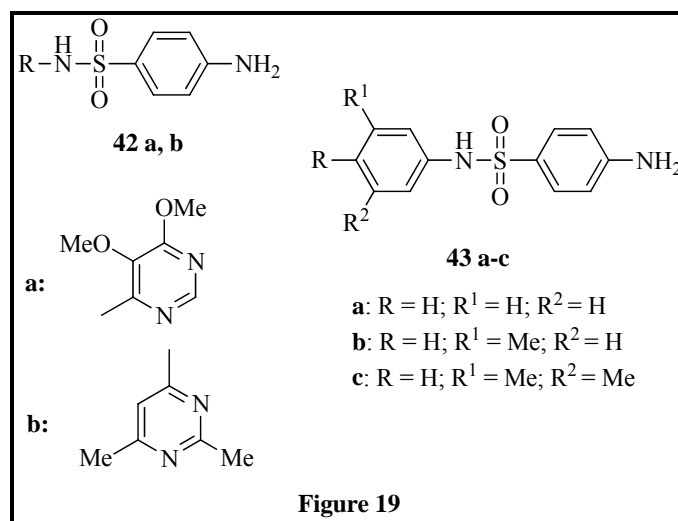


Figure 19

6.7.4 Antivirals and anti-AIDS

Pyrimidine derivatives have generated widespread interest due to their antiviral properties. 5-Iododeoxyuridine⁸⁷ **44** is an antiviral agent of high selectivity. 5-Trifluoromethyl-2'-deoxyuridine (F3 TDR, **45**) has been found useful against infections resistant to IDU therapy.⁸⁷

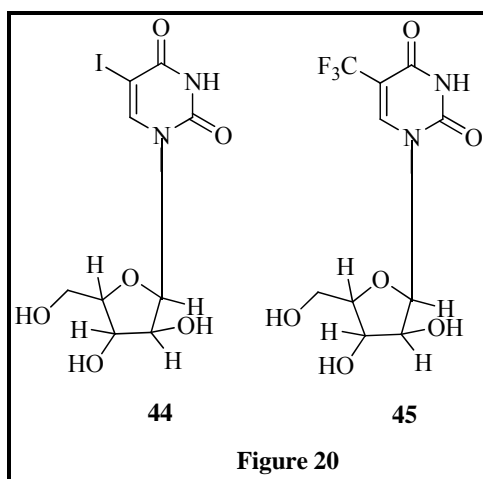
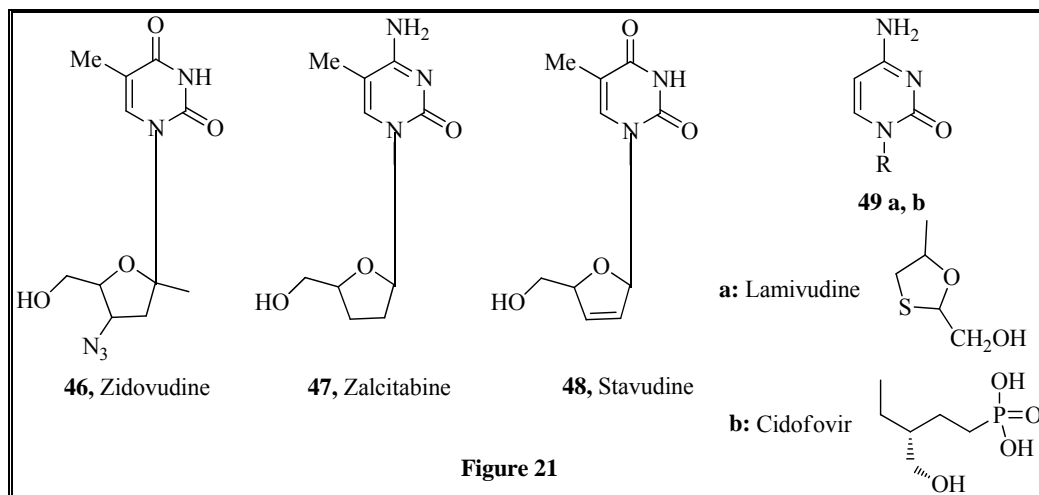


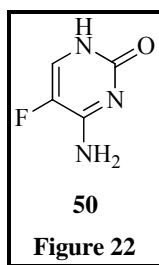
Figure 20

Several members of a series of acyclic nucleosides, which contain a pyrimidine ring, are found to be effective antiviral. (Figure 21)



6.7.5 Antifungals

Pyrimidines also exhibit antifungal properties. Flucytosine **50**⁸⁸ is a fluorinated pyrimidine used as nucleosidal antifungal agent for the treatment of serious systemic infections caused by susceptible strains of candida and cryptococcus.⁸⁹

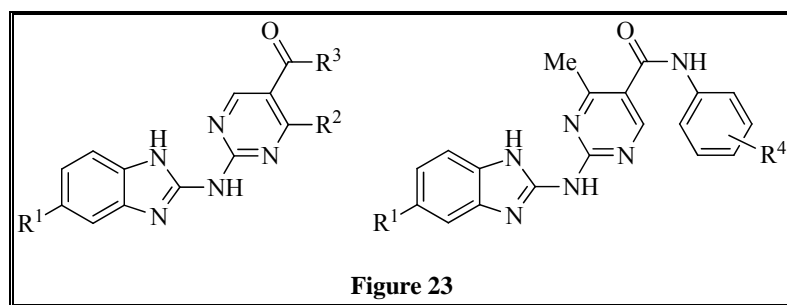


6.8 Research Aim

Three component condensations with the participation of C–H acids, aldehydes or orthoesters, and *N*-containing mono- or binucleophiles lead to a variety of derivatives, which possess a wide spectrum of biological activity.⁹⁰ For example, interaction of aromatic aldehydes, (thio)urea, and β -diketones in Biginelli conditions gave dihydro(thia)pyrimidones, which are calcium channel activators, antagonists of adrenoreceptors, etc.⁹¹⁻⁹³

While development of important methodologies for the synthesis of pyrimidines enjoys a rich history, the discovery of new strategies for the convergent synthesis of pyrimidines remains a vibrant area of chemical research.

At present there are no reports for the use of such reactions on guanidines, in this work, the three component condensation of benzoimidazole-2-guanidines with orthoesters and active methylene compounds containing a carbonyl function were studied. (Figure 23)



The chemical synthesis and characterization of Substituted-(1H-benzo[d]imidazol-2-yl)amino-pyrimidine derivatives are described in Chapter 7.

Section C

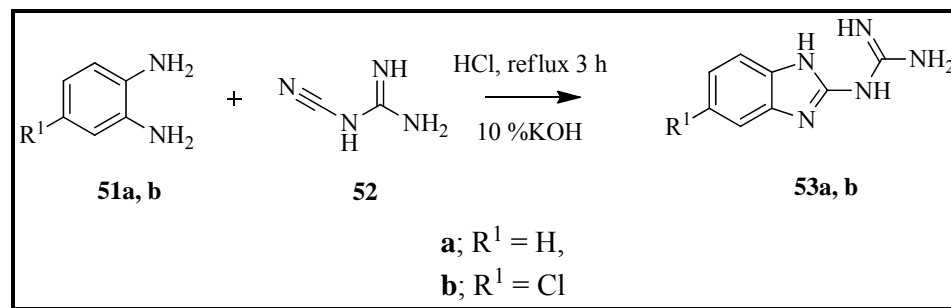
Chapter 7. Synthesis and Characterization of Some Substituted-(1*H*-benzo[*d*]imidazol- 2-yl)amino-pyrimidine derivatives

7.0 Chemistry

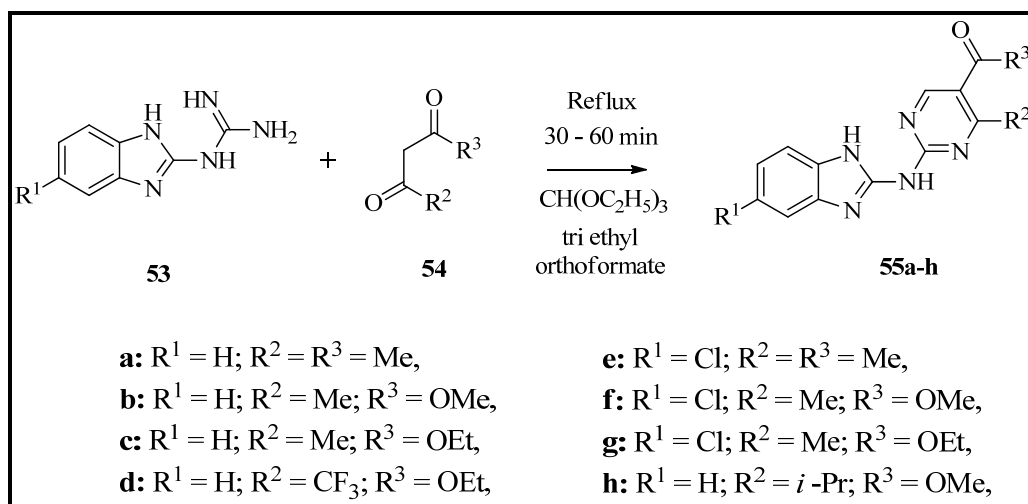
The synthetic route for substituted 2-[(1*H*-benzo[*d*]imidazol-2-yl)amino]-pyrimidine derivatives are shown in Scheme 2. The known 2-benzimidazolylguanidine (**53a,b**) was synthesized from substituted *o*-phenylenediamine (**51a,b**) and cyanoguanidine (**52**) by following literature procedure.⁹⁴ Compound **53** was treated with tri ethyl orthoformate (CH(OC₂H₅)₃) and active methylene compounds containing carbonyl function (**54**, 1,3-diketones) to furnish substituted 2-[(1*H*-benzo[*d*]imidazol-2-yl)amino]-pyrimidine (**55a-h**). The synthetic route for carboxamide derivatives are shown in Scheme 3. The substituted acetoacetanilide (**57**) was directly prepared from substituted aniline (**56**) by our laboratory established method.⁹⁵ The compounds (**57**) were reacted with tri ethyl orthoformate (CH(OC₂H₅)₃) and 2-benzimidazolylguanidine (**53a,b**) to give regioisomers intermediate (**58a-n**). The cyclization of regioisomers intermediate was carried out by heating in glacial acetic acid with sodium acetate to furnish 2-[(1*H*-benzo[*d*]imidazol-2-yl)amino]-4-methyl-N-(substituted)-phenyl-pyrimidine-5-carboxamide derivatives (**59a-n**). Table 7.1 and 7.2 show the yields and the physical data of these compounds.

7.1 Reaction Scheme

7.1.1 Scheme 1 Synthetic route for 2-benzimidazolylguanidine (**53a, b**).



7.1.2 Scheme 2 Synthetic routes for substituted 2-[(1*H*-benzo[*d*]imidazol-2-yl)amino]-pyrimidine derivatives (**55a-h**).



7.1.3 Scheme 3 Synthetic routes for 2-[(1*H*-benzo[*d*]imidazol-2-yl)amino]-4-methyl-N-(substituted)phenylpyrimidine-5-carboxamide derivatives (**59a-n**).

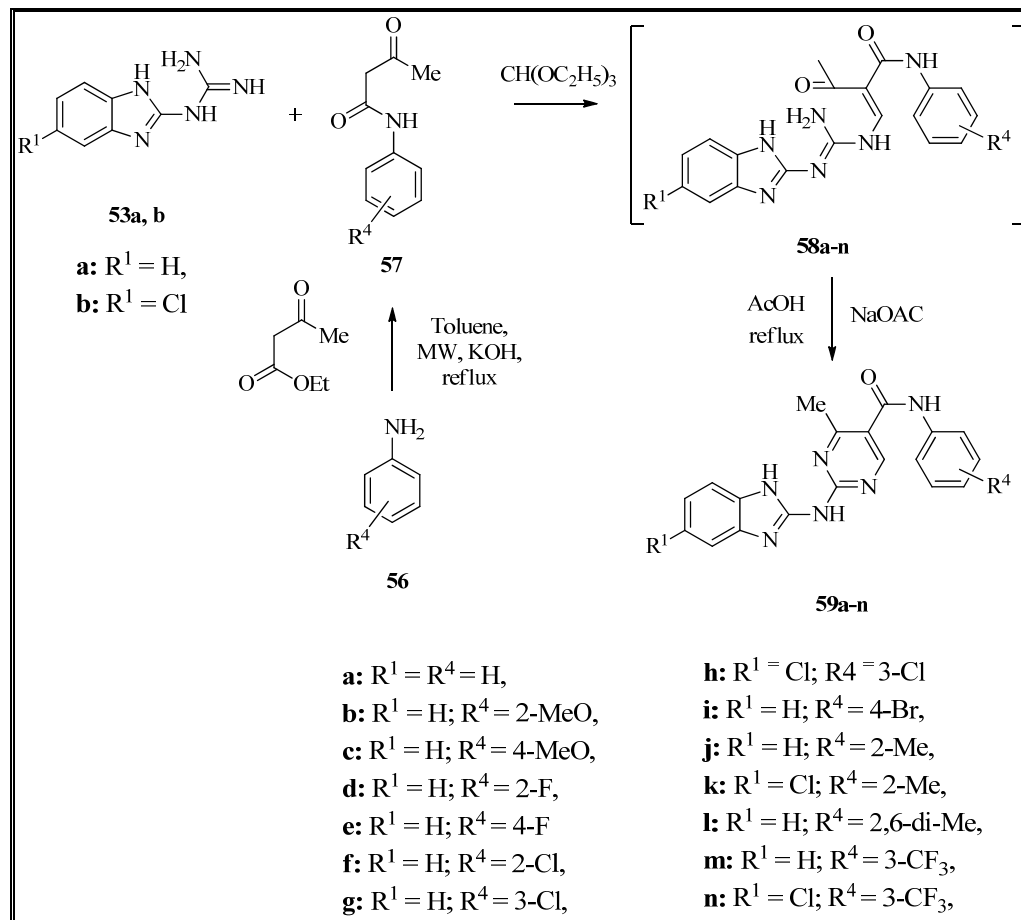
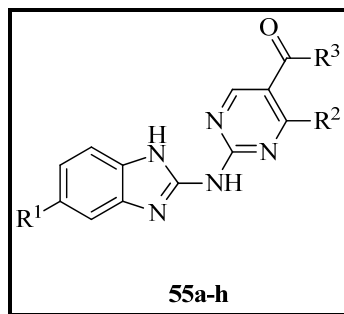
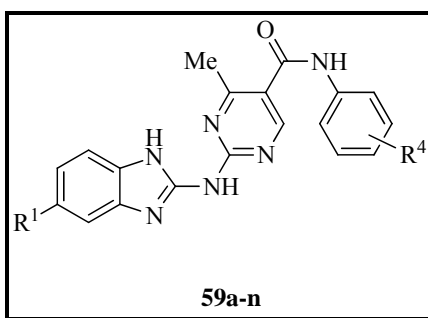


Table 7.1 Yields and physical data of the compounds **55a–h**.

Compd.	Substitute			Yield %	MP °C	Analysis
	R ¹	R ²	R ³			
55a	H	Me	Me	88	> 300	CHN
55b	H	Me	OMe	79	210–211	CHN
55c	H	Me	OEt	76	221–222	CHN
55d	H	CF ₃	OEt	72	214–215	CHN
55e	Cl	Me	Me	78	> 300	CHN
55f	Cl	Me	OMe	74	222–223	CHN
55g	Cl	Me	OEt	71	234–235	CHN
55h	H	<i>i</i> -Pr	OMe	79	218–219	CHN

Table 7.2 Yields and physical data of the compounds **59a–n**.

Compd.	Substitute		Yield %	MP °C	Analysis
	R ¹	R ⁴			
59a	H	H	82	268–270	CHN

59b	H	2-MeO	75	248–249	CHN
59c	H	4-MeO	77	240–241	CHN
59d	H	2-F	73	242–243	CHN
59e	H	4-F	79	247–248	CHN
59f	H	2-Cl	69	252–253	CHN
59g	H	3-Cl	71	240–241	CHN
59h	Cl	3-Cl	74	255–256	CHN
59i	H	4-Br	82	262–263	CHN
59j	H	2-Me	72	286–287	CHN
59k	Cl	2-Me	76	271–272	CHN
59l	H	2,6-di-Me	67	> 300	CHN
59m	H	3-CF ₃	63	265–266	CHN
59n	Cl	3-CF ₃	61	272–273	CHN

7.2 Experimental

7.2.1 General methods and materials

All commercial chemicals and solvents were reagent grade and used without further purification unless otherwise specified. Melting points were determined on a Fargo melting point apparatus and are uncorrected. Thin-layer chromatography was performed on silica gel G60 F₂₅₄ (Merck) with short-wavelength UV light for visualization. All reported yields are isolated yields after chromatography or crystallization. Elemental analyses were done on a Heraeus CHN-O Rapid instrument. Mass spectra were recorded on Shimadzu GC-MS QP-2010 model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. ¹H NMR spectra were recorded on a 400 MHz, Bruker Top-Spin spectrometers in the indicated solvent. The chemical shifts were reported in ppm (δ) relative to TMS and coupling constants (J) in Hertz (Hz) and s, d, t, m, brs, refer to singlet, doublet, triplet, multiplet, broad respectively.

All synthesized compounds were characterized by using ¹H NMR, ¹³C NMR, Mass and Elemental analysis. For compounds **55a–h**, the characteristic proton signals for pyrimidine ring (Ar-CH) appeared at the range of 8.96–9.09 δ ppm as singlet. The ethyl ester (OC₂H₅) proton appeared at the range of 1.31–1.35 and 4.30–4.34 δ ppm as triplet and quartet respective. The characteristic proton for benzimidazole ring and bridge (NH) appeared at the range of 11.87–12.24 δ ppm as singlet and D₂O exchangeable single. While the compounds **59a–n**, the characteristic proton signals for pyrimidine ring (Ar-CH) appeared at the range of 8.73–8.79 δ ppm as singlet and amide (CONH) proton appeared at the range of 10.19–10.51 δ ppm as singlet D₂O exchangeable single. The characteristic proton for benzimidazole ring and bridge (NH) appeared at the range of 11.89–12.01 δ ppm as singlet and D₂O exchangeable single. The molecular ion peak was found in agreement with molecular weight of the respective compound. The elemental analysis of the newly synthesized derivatives was within $\pm 0.4\%$ range of the calculated C, H, N data.

Synthesis of 2-benzimidazolylguanidine (53a). A mixture of *o*-phenylenediamine (**51**, 10.8 g, 100 mmol), cyanoguanidine (**52**, 8.4 g, 100 mmol) and concd HCl (20 mL) in H₂O (200 mL) was heated under reflux for 1 h. The reaction mixture was cooled at 0 °C and KOH (10%; 50 mL) was added slowly. The precipitates of 2-guanidinobenzimidazole were collected by filtration, washed with H₂O, dried, and used in next reactions without further purification. Yield 14 g (80 %); mp 240–242 °C (lit.⁹⁴ mp 243–244 °C); MS m/z = 175 (M⁺).

By following the same synthetic procedure as that for **53a**, the following compounds were synthesized:

5-Chloro-2-benzimidazolylguanidine (53b). Yield, 79 %; mp 258–259 °C. MS m/z = 209 (M⁺).

1-[2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-4-methylpyrimidin-5-yl]ethanone (55a). A mixture of 2-guanidinobenzimidazole (**53a**, 1.75 g, 10 mmol), acetylacetone (**54**, 1 g, 10 mmol) and tri ethyl orthoformate (15 mL) was stirred at reflux temperature for 40 min. Upon the completion of the reaction (monitored by TLC, ethyl acetate:hexane (1:1)), the reaction mixture was concentrated under reduced pressure, and 1 mL of water was added. The separated solid product was collected by filtration and recrystallized from DMF to give **55a** 2.3 g (88 %); mp > 300 °C (lit.⁹⁶ mp > 300 °C); MS m/z = 267 (M⁺). Anal. Calcd. for (C₁₄H₁₃N₅O₄): C, 62.91; H, 4.90; N, 26.20. Found: C, 62.78; H, 4.98; N, 26.44.

By following the same synthetic procedure as that for **55a**, the following compounds were synthesized:

Methyl-2-((1*H*-benzo[*d*]imidazol-2-yl)amino)-4-methylpyrimidine-5-carboxylate (55b). Yield, 79 %; mp 210–211 °C. ¹H NMR (DMSO-*d*₆) δ 2.78 (3H, s, Me), 3.85 (3H, s, OMe), 7.09–7.11 (2H, m, 2 × ArH), 7.51–7.53 (2H, m, 2 × ArH), 8.96 (1H, s, ArH), 11.99 (2H, br s, exchangeable NH). MS m/z = 283 (M⁺). Anal. Calcd. for (C₁₄H₁₃N₅O₂): C, 59.36; H, 4.63; N, 24.72. Found: C, 59.69; H, 4.54; N, 24.88.

Ethyl-2-((1*H*-benzo[*d*]imidazol-2-yl)amino)-4-methylpyrimidine-5-carboxylate

(**55c**). Yield, 76 %; mp 221–222 °C. ¹H NMR (DMSO-*d*₆) δ 1.34 (3H, t, *J* = 7.2 Hz, Me), 2.77 (3H, s, Me), 4.31 (2H, q, *J* = 7.2 Hz, OCH₂), 7.08–7.10 (2H, m, 2 × ArH), 7.47–7.49 (2H, m, 2 × ArH), 8.96 (1H, s, ArH), 11.87 (2H, br s, exchangeable NH). MS *m/z* = 297 (M⁺). Anal. Calcd. for (C₁₅H₁₅N₅O₂): C, 60.60; H, 5.09; N, 23.56. Found: C, 60.34; H, 5.31; N, 23.41.

Ethyl-2-((1*H*-benzo[*d*]imidazol-2-yl)amino)-4-(trifluoromethyl)pyrimidine-5-

carboxylate (55d). Yield, 72 %; mp 214–215 °C. ¹H NMR (DMSO-*d*₆) δ 1.32 (3H, t, *J* = 7.2 Hz, Me), 4.31 (2H, q, *J* = 7.2 Hz, OCH₂), 7.15–7.17 (2H, m, 2 × ArH), 7.45–7.47 (2H, m, 2 × ArH), 9.09 (1H, s, ArH), 12.24 (2H, br s, exchangeable NH). ¹³C NMR (DMSO-*d*₆) δ 13.7, 61.5, 112.6, 112.8, 1211.8, 133.3, 148.9, 161.5, 161.7, 162.7. MS *m/z* = 351 (M⁺). Anal. Calcd. for (C₁₅H₁₂N₅O₂F₃): C, 51.29; H, 3.44; N, 19.94. Found: C, 51.46; H, 3.57; N, 19.73.

1-[2-((5-Chloro-1*H*-Benzo[*d*]imidazol-2-yl)amino)-4-methylpyrimidin-5-

yl]ethanone (55e). Yield, 78 %; mp > 300 °C. MS *m/z* = 301 (M⁺). Anal. Calcd. for (C₁₄H₁₂N₅OCl): C, 55.73; H, 4.01; N, 23.21. Found: C, 55.46; H, 3.89; N, 23.34.

Methyl-2-((5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)amino)-4-methylpyrimidine-5-

carboxylate (55f). Yield, 74 %; mp 222–223 °C. MS *m/z* = 317 (M⁺). Anal. Calcd. for (C₁₄H₁₂N₅O₂Cl): C, 52.92; H, 3.81; N, 22.44. Found: C, 52.78; H, 3.98; N, 22.32.

Ethyl-2-((5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)amino)-4-methylpyrimidine-5-

carboxylate (55g). Yield, 71 %; mp 234–235 °C. MS *m/z* = 331 (M⁺). Anal. Calcd. for (C₁₅H₁₄N₅O₂Cl): C, 54.30; H, 4.25; N, 21.11. Found: C, 54.12; H, 4.47; N, 21.38.

Methyl-2-((5-chloro-1*H*-benzo[*d*]imidazol-2-yl)amino)-4-isopropylpyrimidine-5-

carboxylate (55h). Yield, 79 %; mp 218–219 °C. MS *m/z* = 311 (M⁺). Anal. Calcd. for (C₁₆H₁₇N₅O₂): C, 61.72; H, 5.50; N, 22.49. Found: C, 61.40; H, 5.24; N, 22.38.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-4-methyl-N-phenylpyrimidine-5-carboxamide (59a). A mixture of 2-guanidinobenzimidazole (**53a**, 1.75 g, 10 mmol), 3-oxo-N-phenylbutanamide (**57**, 1.8 g, 10 mmol) and tri ethyl orthoformate (15 mL) was stirred at reflux temperature for 30 min. The precipitates were collected by filtration and recrystallized from dioxane to give (**58a**). Compound **58a** was added into a mixture of anhydrous sodium acetate (3 g) in glacial acetic acid (30 mL) and the reaction mixture was boiled for 30 min. The reaction mixture was cooled to room temperature and dropped into cold water (100 mL). The precipitates was collected by filtration and recrystallized from DMF to give **59a** 2.8 g (82 %); mp 268–270 °C; MS $m/z = 344$ (M^+). Anal. Calcd. for ($C_{19}H_{16}N_6O$): C, 66.27; H, 4.68; N, 24.40. Found: C, 66.43; H, 4.47; N, 24.59.

By following the same synthetic procedure as that for **59a**, the following compounds were synthesized:

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(2-methoxyphenyl)-4-methylpyrimidine-5-carboxamide (59b). Yield, 75 %; mp 248–249 °C. MS $m/z = 374$ (M^+). Anal. Calcd. for ($C_{20}H_{18}N_6O_2$): C, 64.16; H, 4.85; N, 22.45. Found: C, 64.38; H, 4.77; N, 22.31.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(4-methoxyphenyl)-4-methylpyrimidine-5-carboxamide (59c). Yield, 77 %; mp 240–241 °C. MS $m/z = 374$ (M^+). Anal. Calcd. for ($C_{20}H_{18}N_6O_2$): C, 64.16; H, 4.85; N, 22.45. Found: C, 64.01; H, 4.67; N, 22.28.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(2-fluorophenyl)-4-methylpyrimidine-5-carboxamide (59d). Yield, 73 %; mp 242–243 °C. MS $m/z = 362$ (M^+). Anal. Calcd. for ($C_{19}H_{15}N_6OF$): C, 62.98; H, 4.17; N, 23.19. Found: C, 62.77; H, 4.02; N, 23.41.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(4-fluorophenyl)-4-methylpyrimidine-5-carboxamide (59e). Yield, 79 %; mp 247–248 °C. 1H NMR (DMSO- d_6) δ 2.66 (3H, s, Me), 7.06–7.10 (2H, m, 2 \times ArH), 7.19–7.23 (2H, m, 2 \times ArH), 7.46–7.49 (2H, m, 2 \times ArH), 7.73–7.77 (2H, m, 2 \times ArH), 8.73 (1H, s, ArH), 10.51 (1H, br s, exchangeable CONH), 11.98 (2H, br s, exchangeable NH). MS $m/z = 362$ (M^+). Anal.

Calcd. for (C₁₉H₁₅N₆O_F): C, 62.98; H, 4.17; N, 23.19. Found: C, 62.80; H, 4.30; N, 23.01.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(2-chlorophenyl)-4-methylpyrimidine-5-carboxamide (59f). Yield, 69 %; mp 252–253 °C. ¹H NMR (DMSO-*d*₆) δ 2.71 (3H, s, Me), 7.08–7.11 (2H, m, 2 × ArH), 7.28–7.32 (1H, m, ArH), 7.39–7.43 (1H, m, ArH) 7.51–7.58 (3H, m, 3 × ArH), 7.71–7.73 (1H, m, ArH), 8.79 (1H, s, ArH), 10.19 (1H, br s, exchangeable CONH), 11.90 (2H, br s, exchangeable NH). ¹³C NMR (DMSO-*d*₆) δ 23.1, 121.1, 121.9, 127.8, 127.9, 128.2, 129.0, 129.9, 134.9, 148.3, 157.5, 158.5, 165.0, 168.0. MS *m/z* = 378 (M⁺). Anal. Calcd. for (C₁₉H₁₆N₆OCl): C, 60.24; H, 3.99; N, 22.19. Found: C, 60.41; H, 3.78; N, 22.03.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(3-chlorophenyl)-4-methylpyrimidine-5-carboxamide (59g). Yield, 71 %; mp 240–241 °C. MS *m/z* = 378 (M⁺). Anal. Calcd. for (C₁₉H₁₅N₆OCl): C, 60.24; H, 3.99; N, 22.19. Found: C, 60.08; H, 4.21; N, 22.33.

2-((5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)amino)-N-(3-chlorophenyl)-4-methylpyrimidine-5-carboxamide (59h). Yield, 74 %; mp 255–256 °C. MS *m/z* = 413 (M⁺). Anal. Calcd. for (C₁₉H₁₄N₆OCl₂): C, 55.22; H, 3.41; N, 20.34. Found: C, 55.03; H, 3.21; N, 20.25.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(4-bromophenyl)-4-methylpyrimidine-5-carboxamide (59i). Yield, 82 %; mp 262–263 °C. MS *m/z* = 423 (M⁺). Anal. Calcd. for (C₁₉H₁₆N₆OBr): C, 53.91; H, 3.57; N, 19.86. Found: C, 53.74; H, 4.18; N, 19.73.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(2-methylphenyl)-4-methylpyrimidine-5-carboxamide (59j). Yield, 72 %; mp 286–287 °C. MS *m/z* = 358 (M⁺). Anal. Calcd. for (C₂₀H₁₈N₆O): C, 67.02; H, 5.06; N, 23.45. Found: C, 67.18; H, 5.19; N, 23.34.

2-((5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)amino)-N-(2-methylphenyl)-4-methylpyrimidine-5-carboxamide (59k). Yield, 76 %; mp 271–272 °C. MS *m/z* = 392 (M⁺). Anal. Calcd. for (C₂₀H₁₇N₆OCl): C, 61.15; H, 4.36; N, 21.39. Found: C, 61.34; H, 4.18; N, 23.47.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(2,6-dimethylphenyl)-4-methylpyrimidine-5-carboxamide (59l). Yield, 67 %; mp > 300 °C. MS m/z = 372 (M^+). Anal. Calcd. for (C₂₁H₂₀N₆O): C, 67.73; H, 5.41; N, 22.57. Found: C, 67.58; H, 5.29; N, 22.71.

2-((1*H*-Benzo[*d*]imidazol-2-yl)amino)-N-(3-trifluoromethyl)-4-methylpyrimidine-5-carboxamide (59m). Yield, 63 %; mp 265–266 °C. MS m/z = 412 (M^+). Anal. Calcd. for (C₂₀H₁₅N₆OF₃): C, 58.25; H, 3.67; N, 20.38. Found: C, 58.37; H, 3.87; N, 20.22.

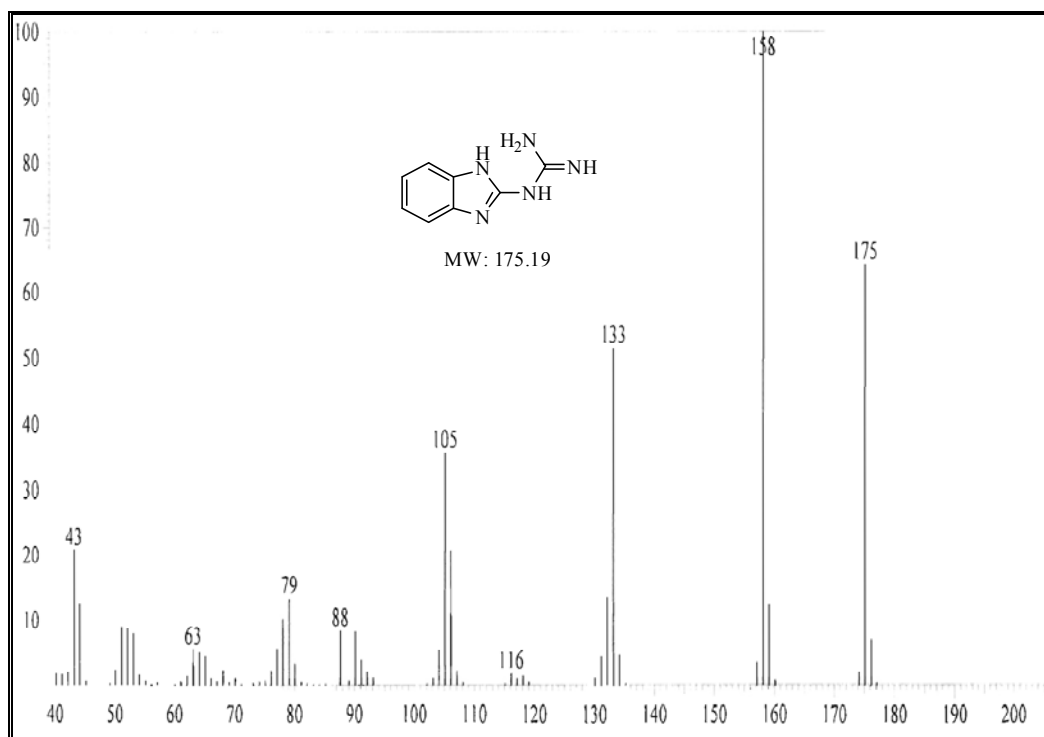
2-((5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)amino)-N-(3-trifluoromethyl)-4-methylpyrimidine-5-carboxamide (59n). Yield, 61 %; mp 272–273 °C. MS m/z = 446 (M^+). Anal. Calcd. for (C₂₀H₁₄N₆OF₃Cl): C, 53.76; H, 3.16; N, 18.81. Found: C, 53.41; H, 3.48; N, 18.67.

7.3 Conclusion

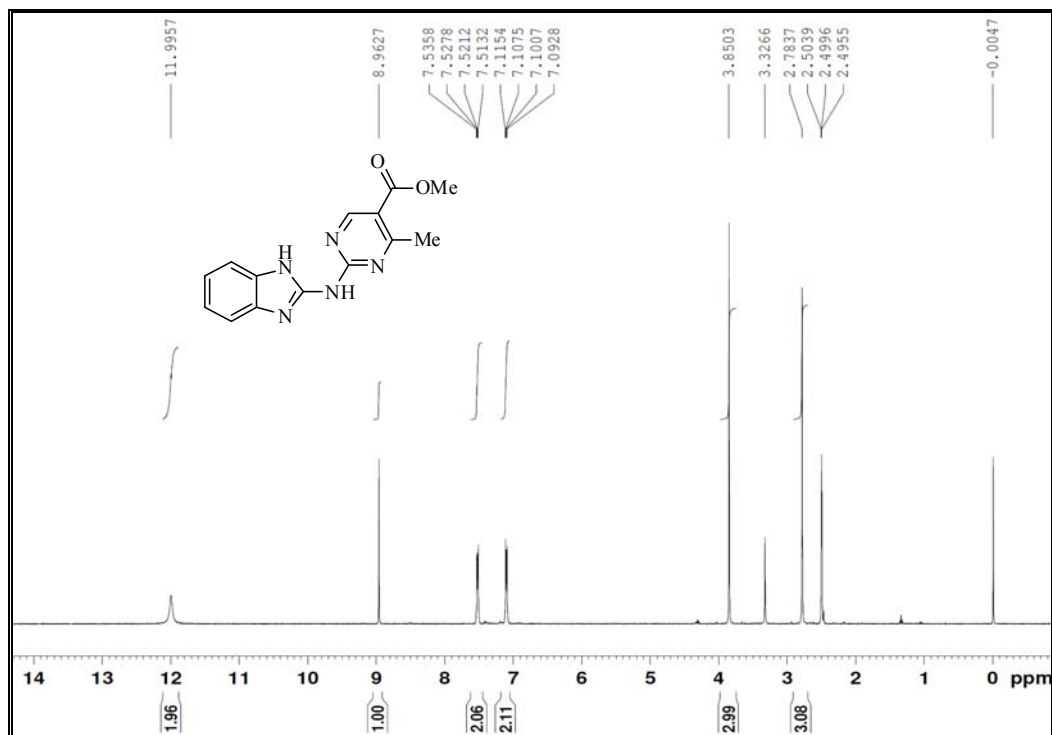
In present chapter, first time reported three component condensations of benzoimidazole-2-guanidines, orthoester and active methylene carbonyl compounds leading to several novel new chemical entities substituted-(1*H*-benzo[*d*]imidazol-2-yl)amino-pyrimidine derivatives. The biological activity of newly synthesized compounds is under investigation.

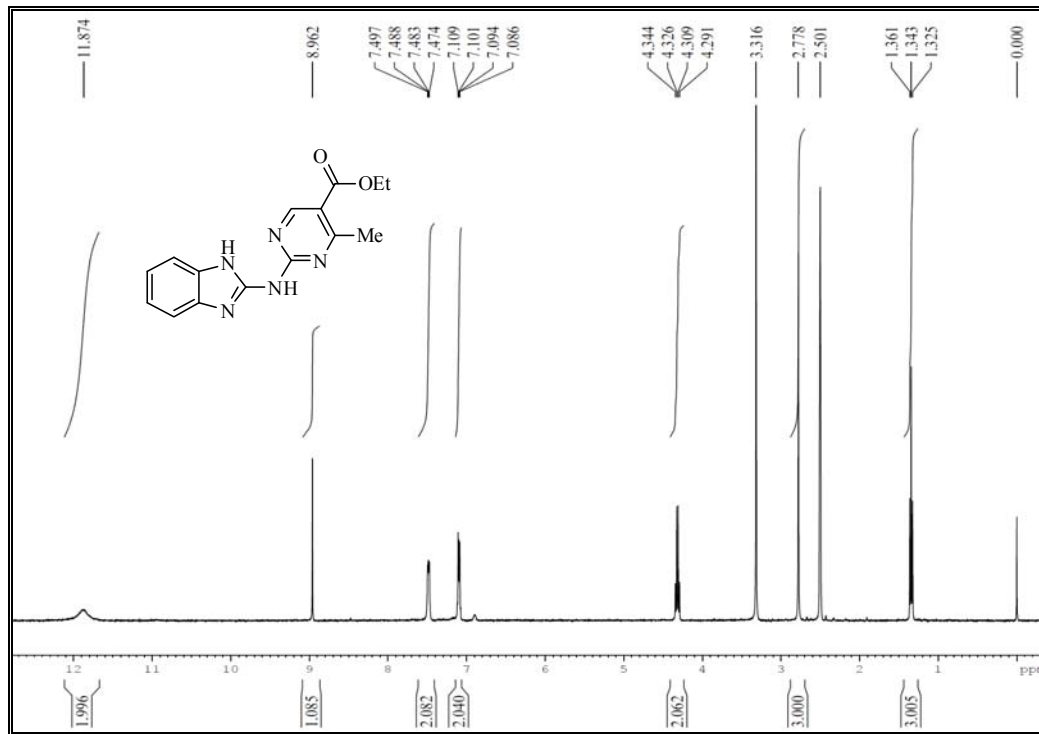
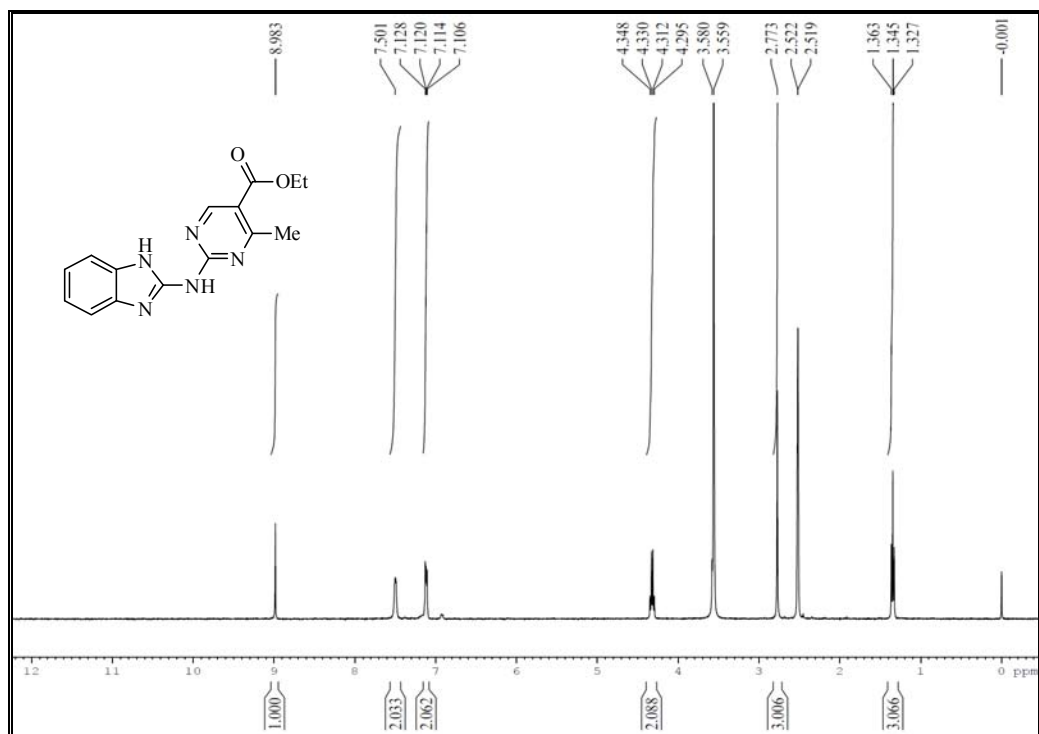
7.4 Representative Spectra

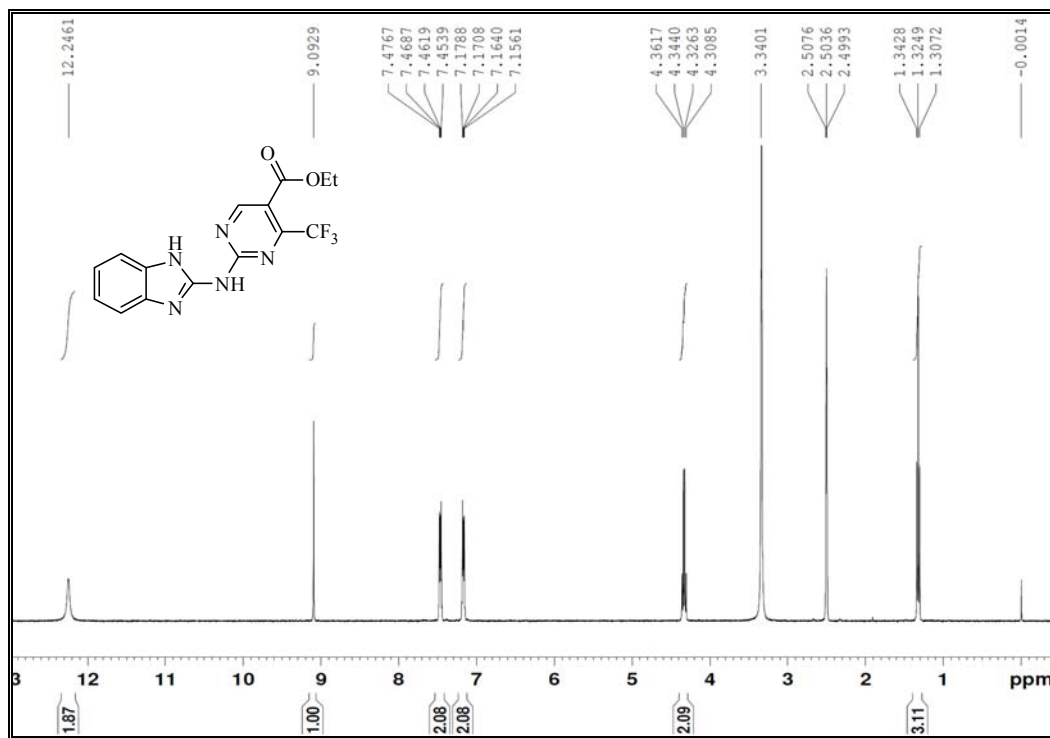
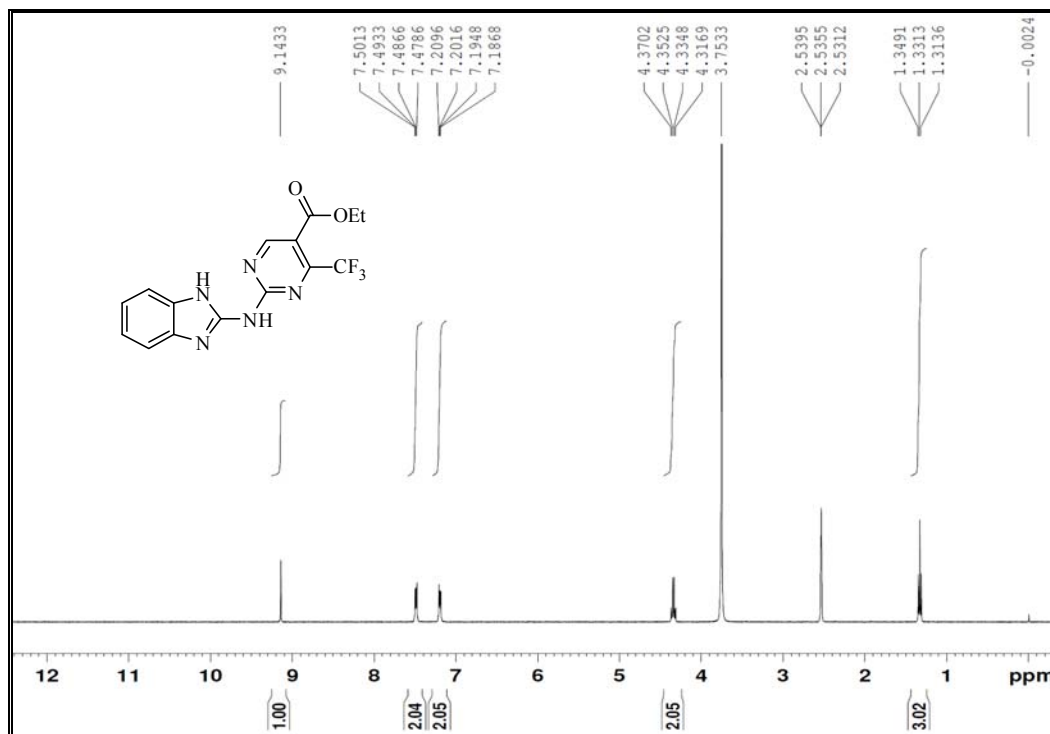
7.4.1 Mass Spectrum for compound **53a**.

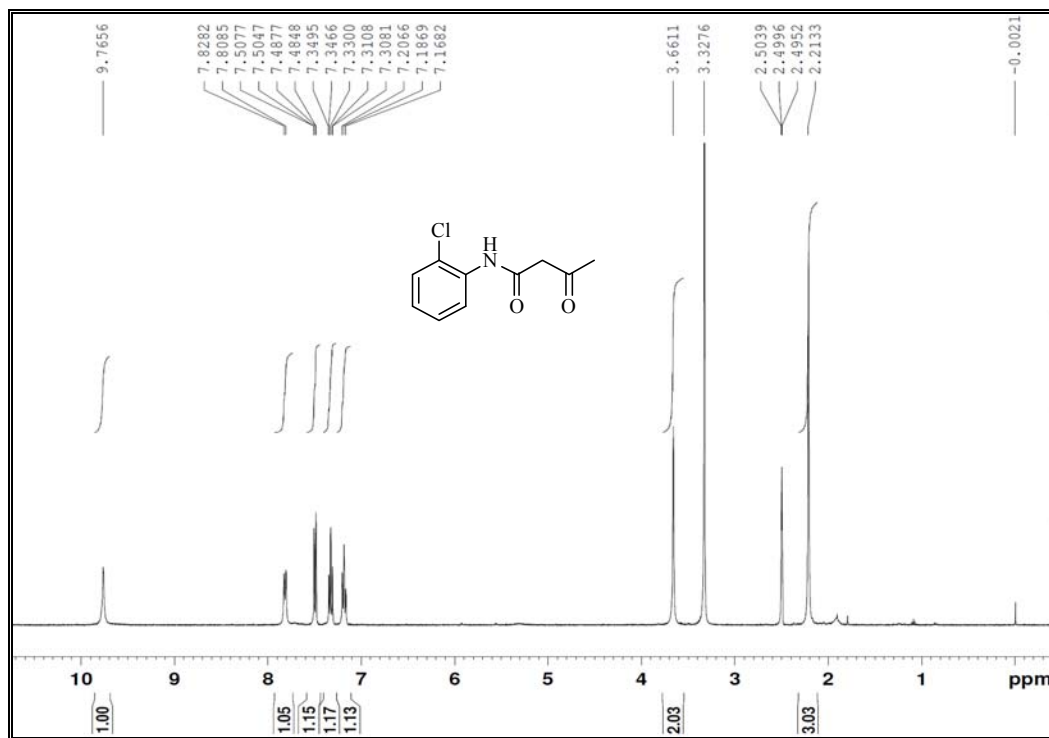
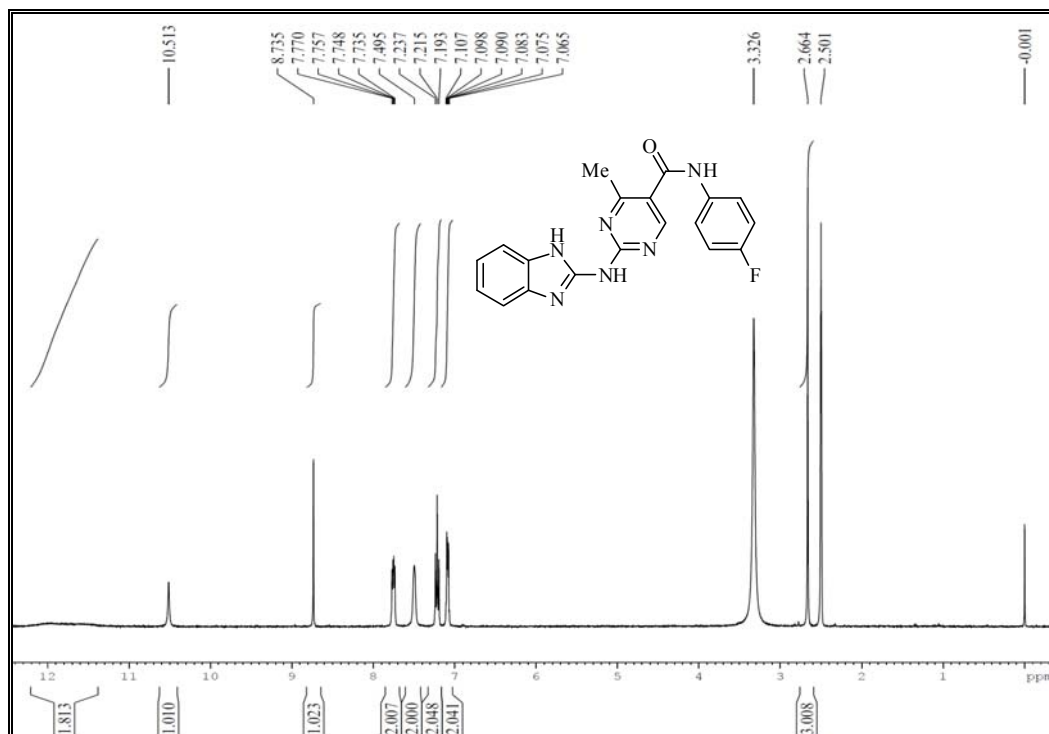


7.4.2 ¹H NMR Spectrum for compound **55b**.

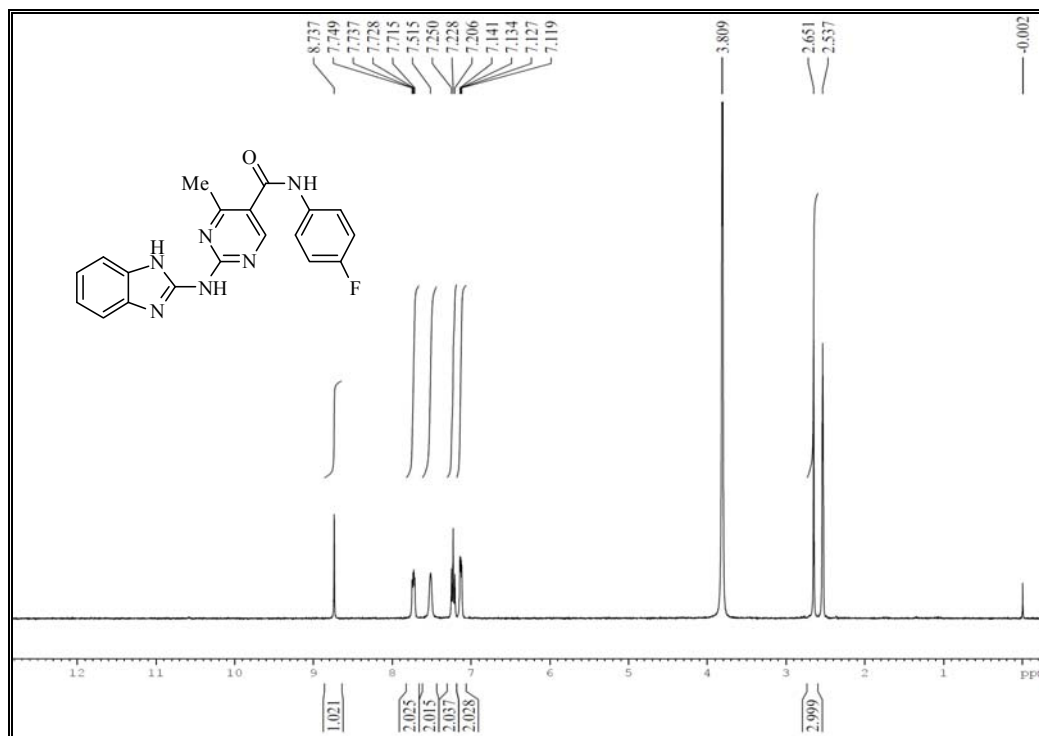


7.4.3 ^1H NMR Spectrum for compound **55c**. D_2O exchange ^1H NMR Spectrum for compound **55c**.

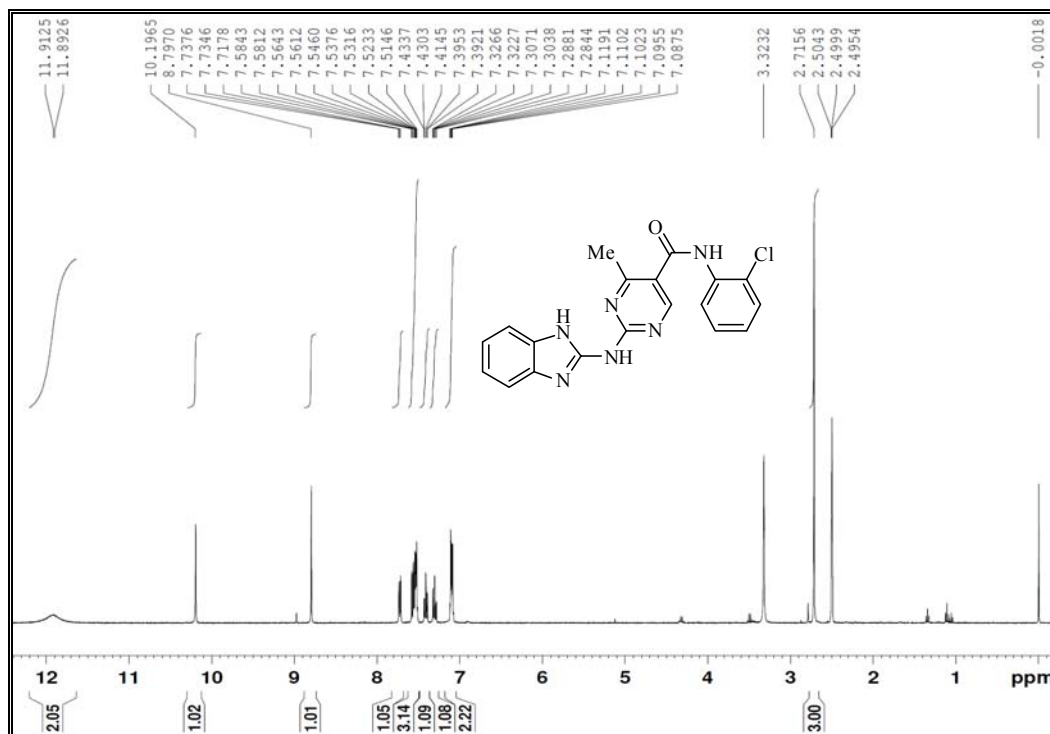
7.4.4 ^1H NMR Spectrum for compound **55d**. D_2O exchange ^1H NMR Spectrum for compound **55d**.

7.4.5 ^1H NMR Spectrum for compound **57**.7.4.6 ^1H NMR Spectrum for compound **59e**.

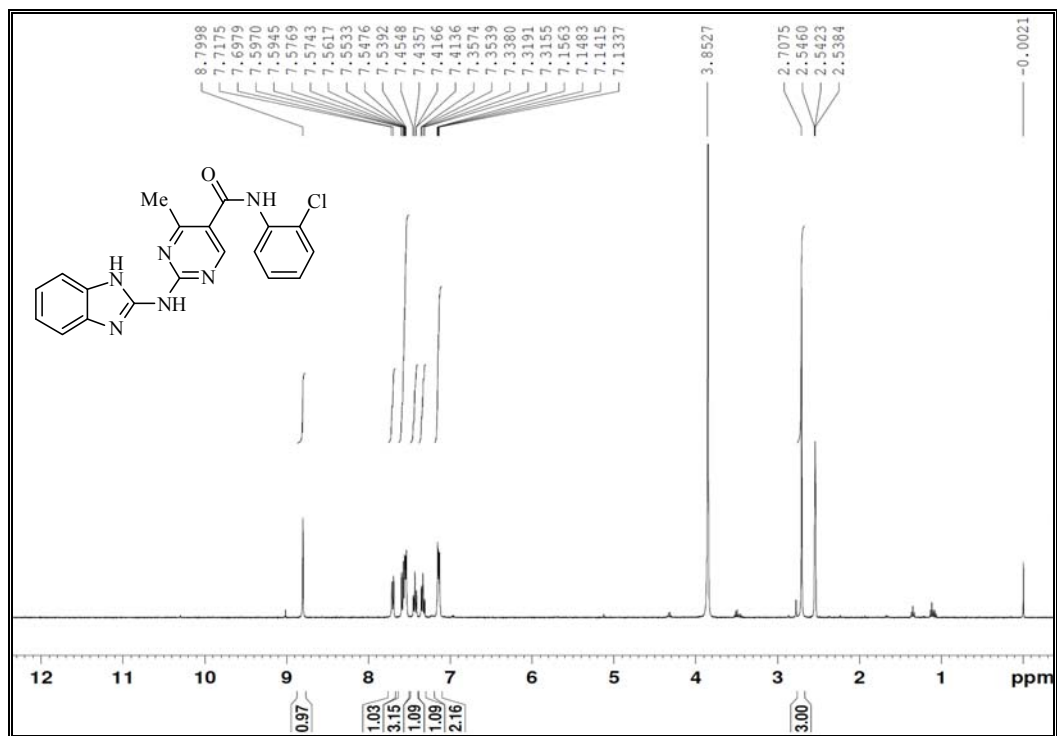
D₂O exchange ¹H NMR Spectrum for compound **59e**.



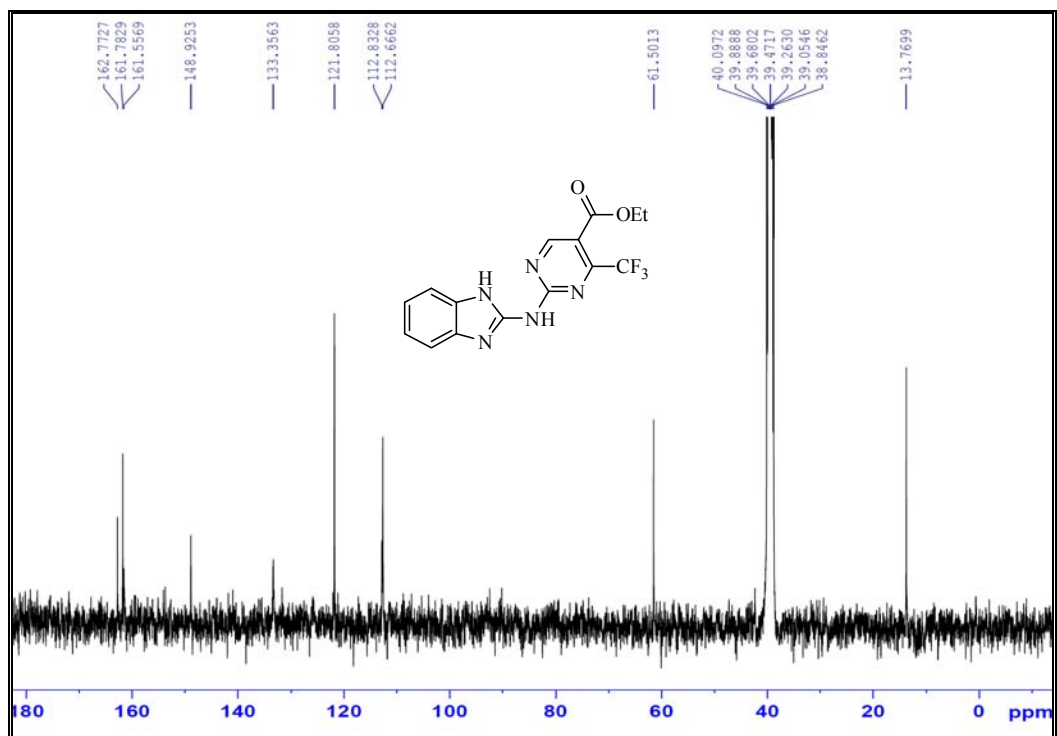
7.4.7 ¹H NMR Spectrum for compound **59f**.

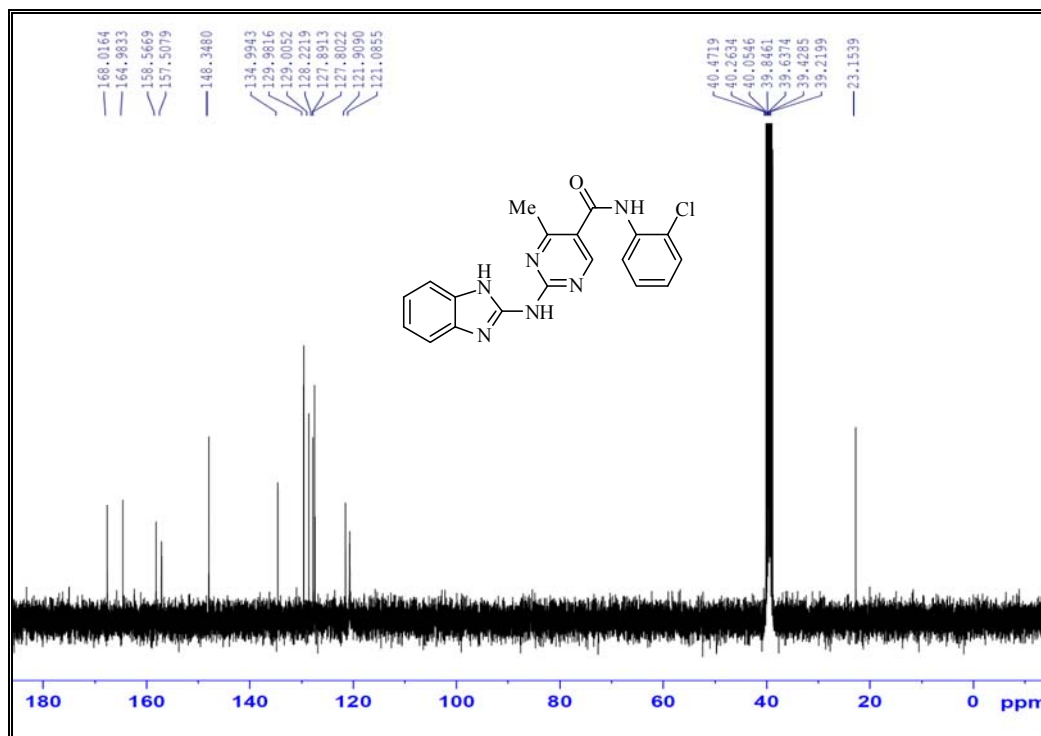
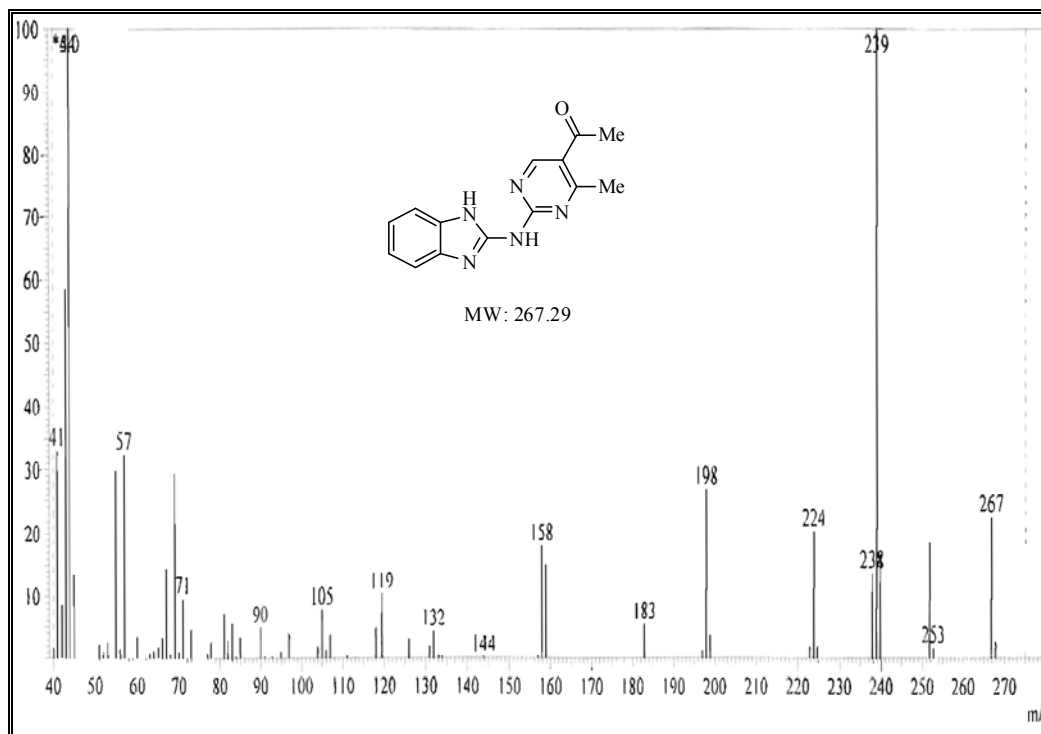


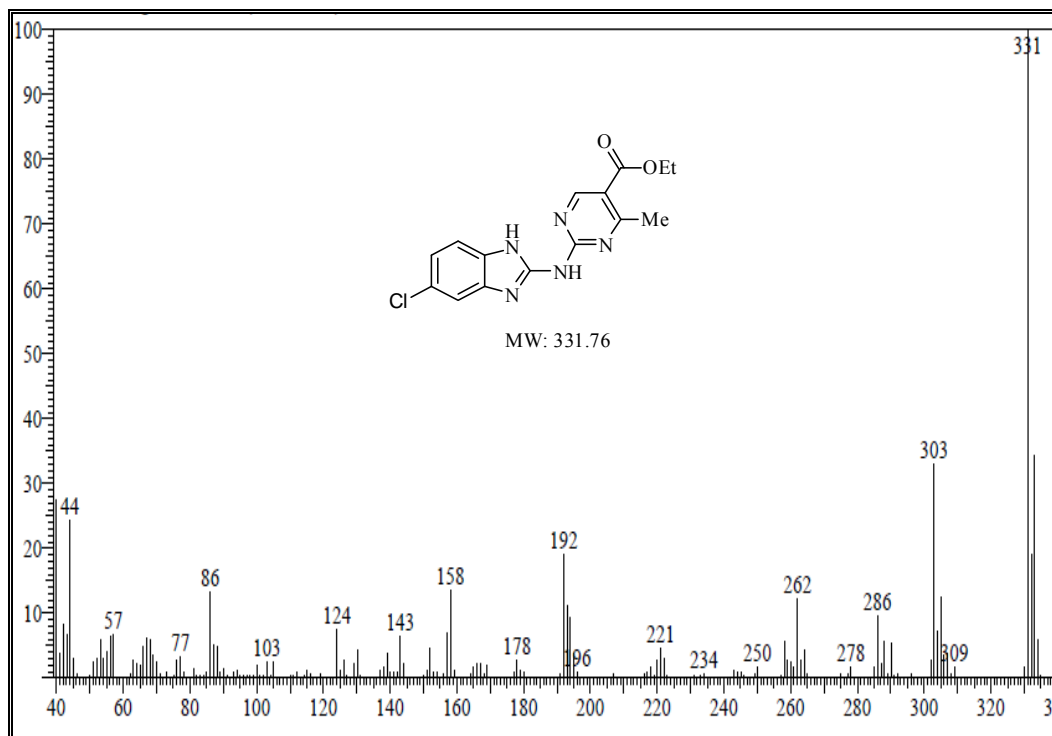
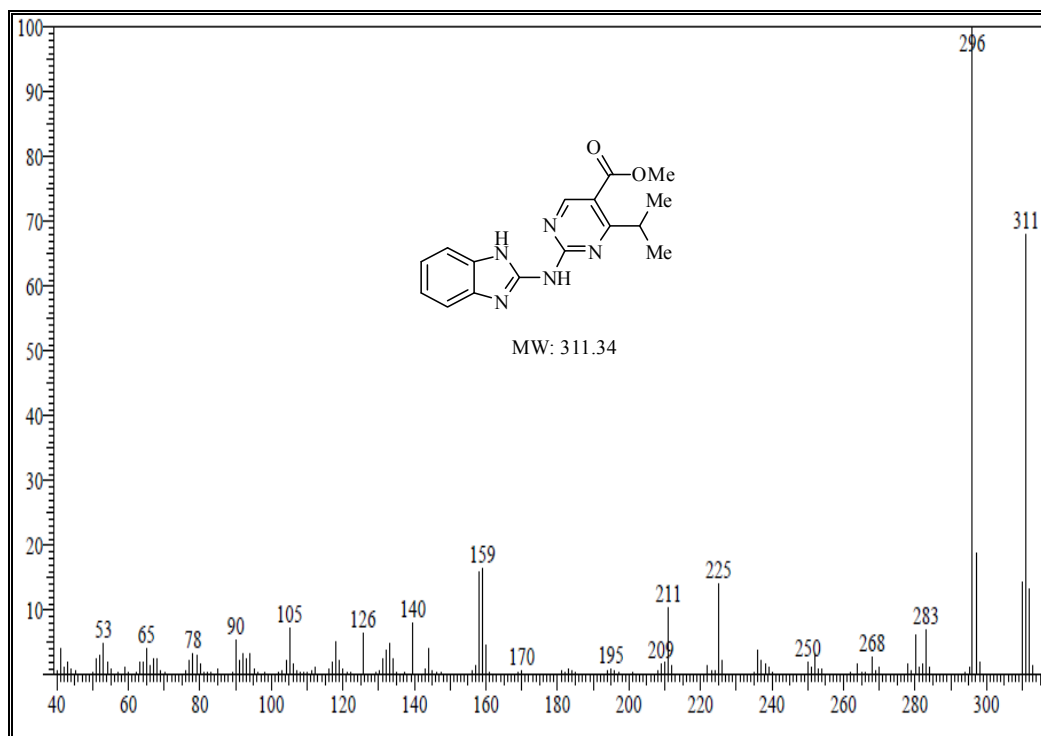
D₂O exchange ¹H NMR Spectrum for compound **59f**.

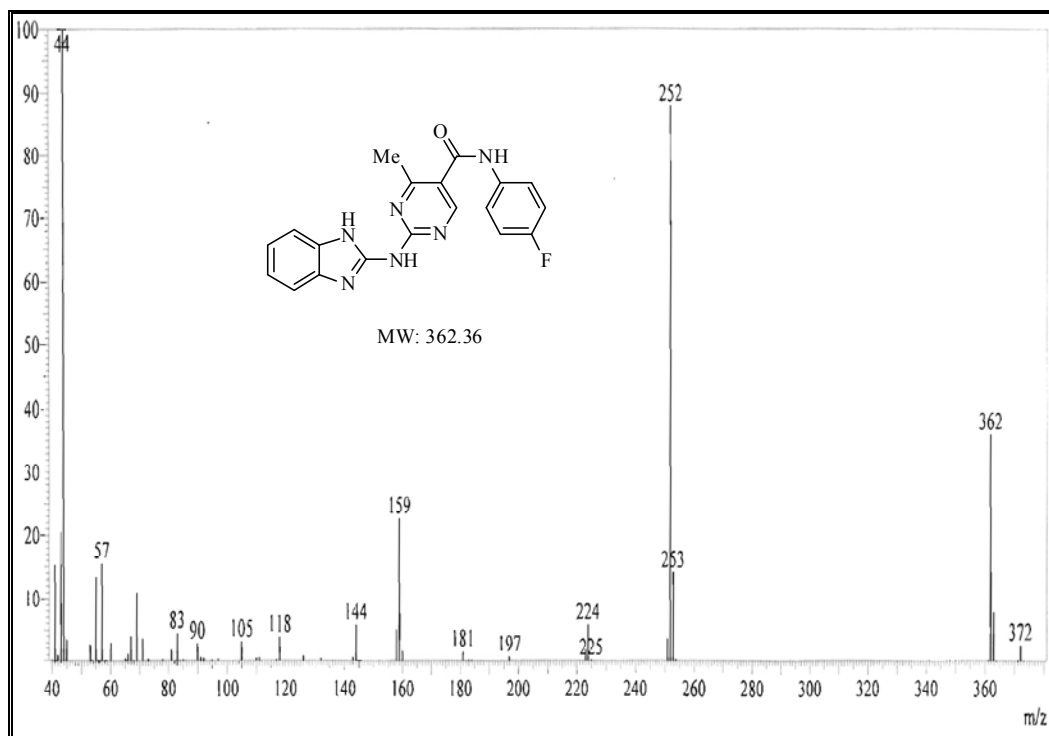
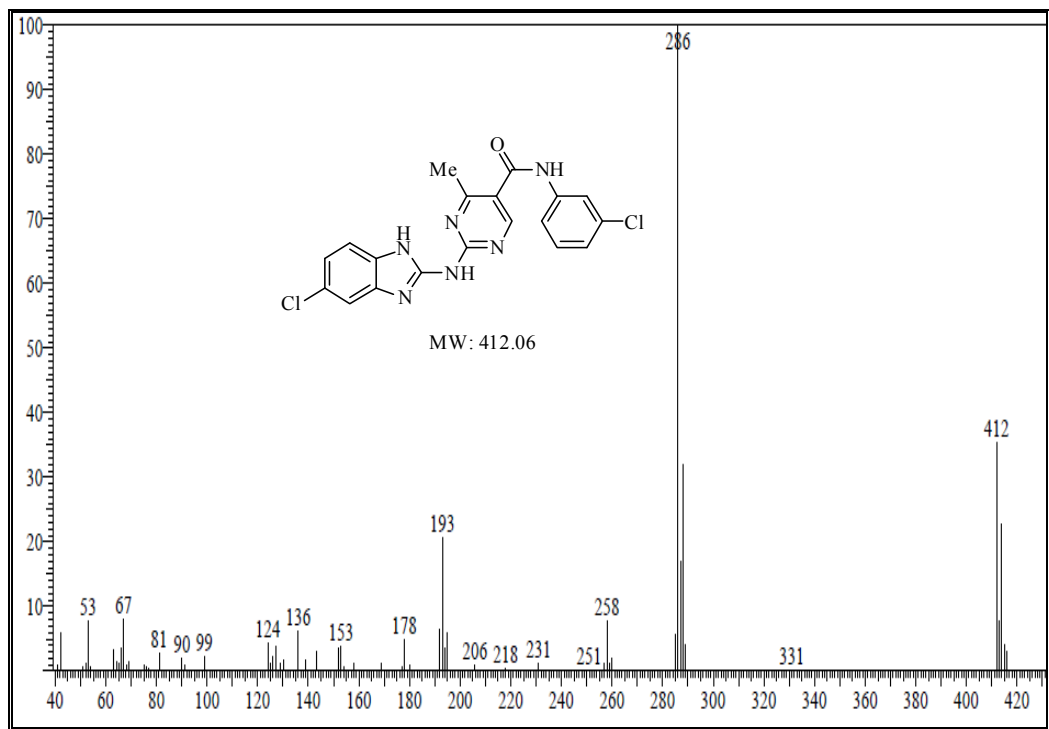


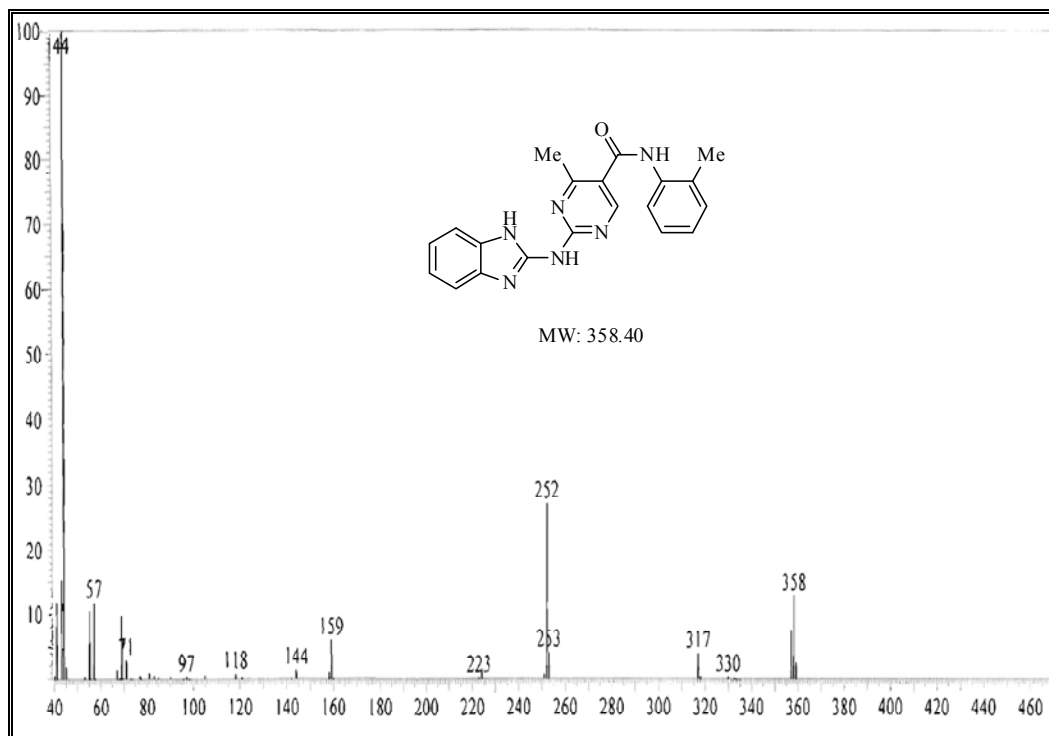
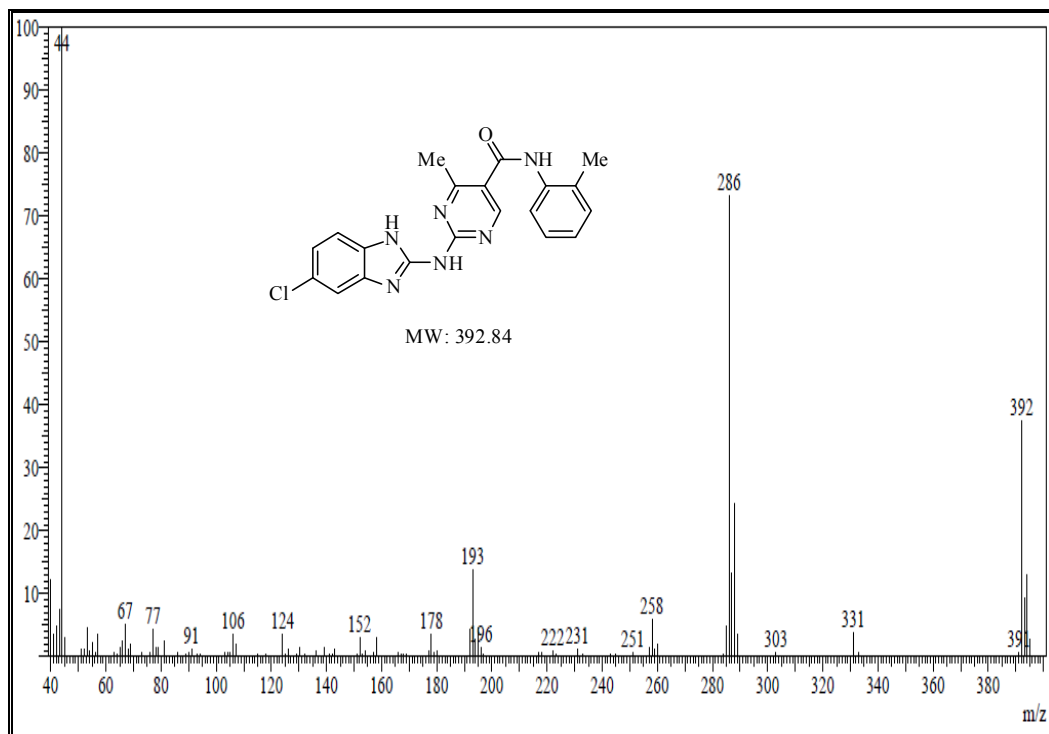
7.4.8 ¹³C NMR Spectrum for compound **55d**.

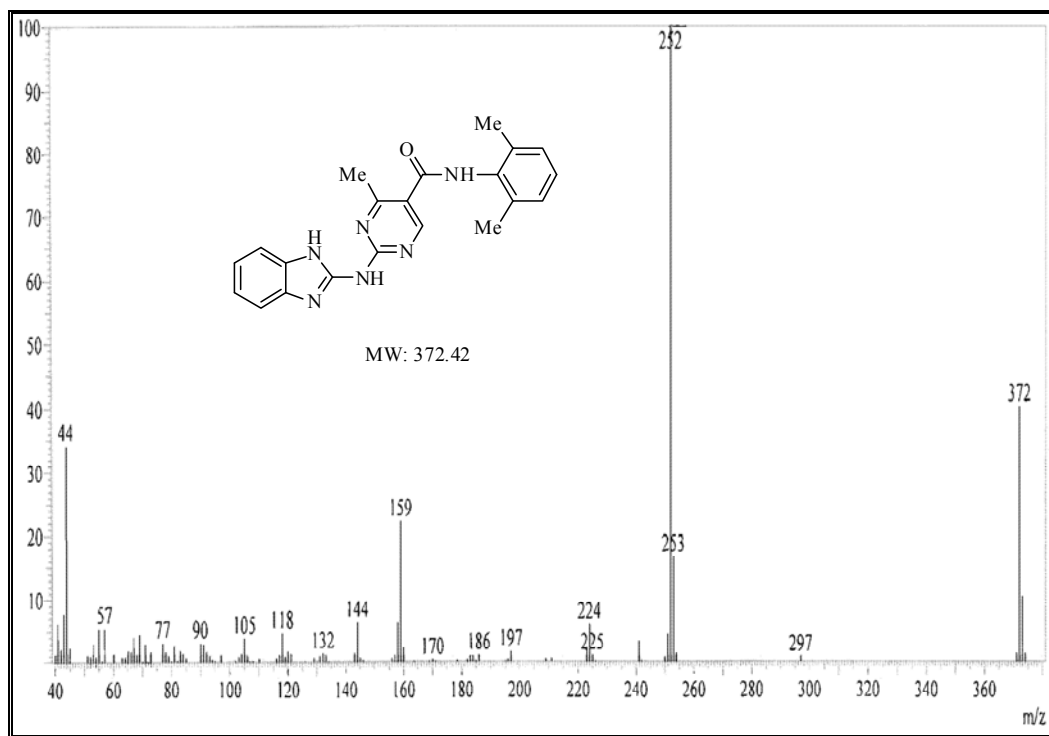
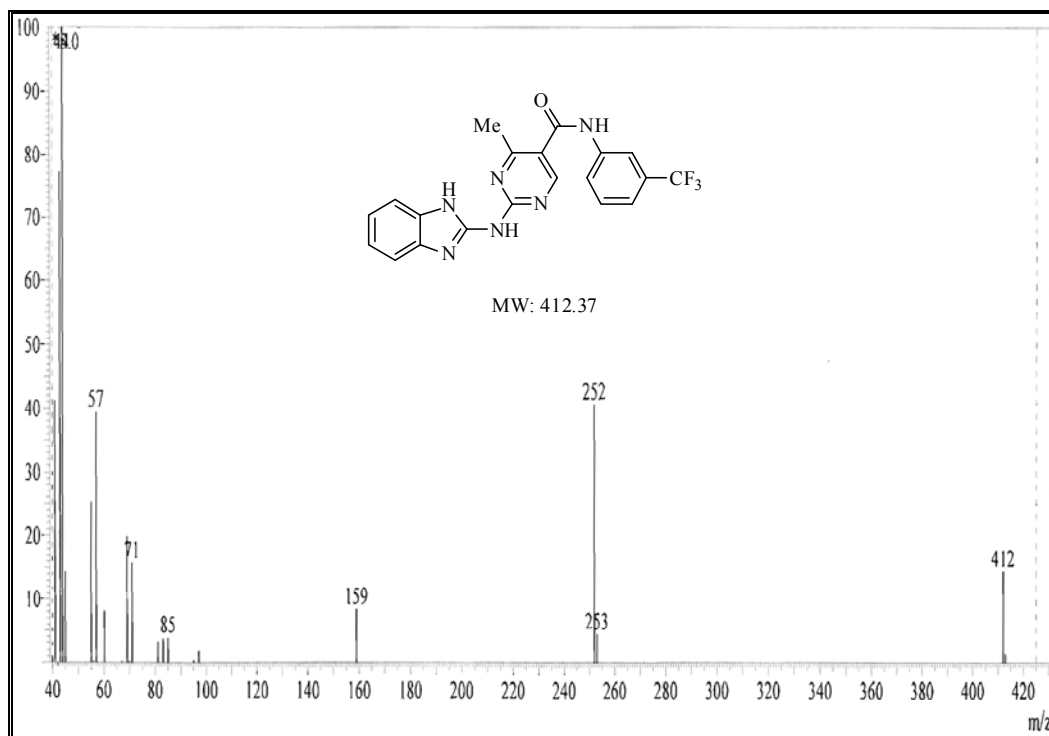


7.4.9 ^{13}C NMR Spectrum for compound **59f**.7.4.10 Mass Spectrum for compound **55a**.

7.4.11 Mass Spectrum for compound **55g**.**7.4.12** Mass Spectrum for compound **55h**.

7.4.13 Mass Spectrum for compound **59e**.**7.4.14** Mass Spectrum for compound **59g**.

7.4.15 Mass Spectrum for compound **59j**.**7.4.16** Mass Spectrum for compound **59k**.

7.4.17 Mass Spectrum for compound **59l**.**7.4.18** Mass Spectrum for compound **59m**.

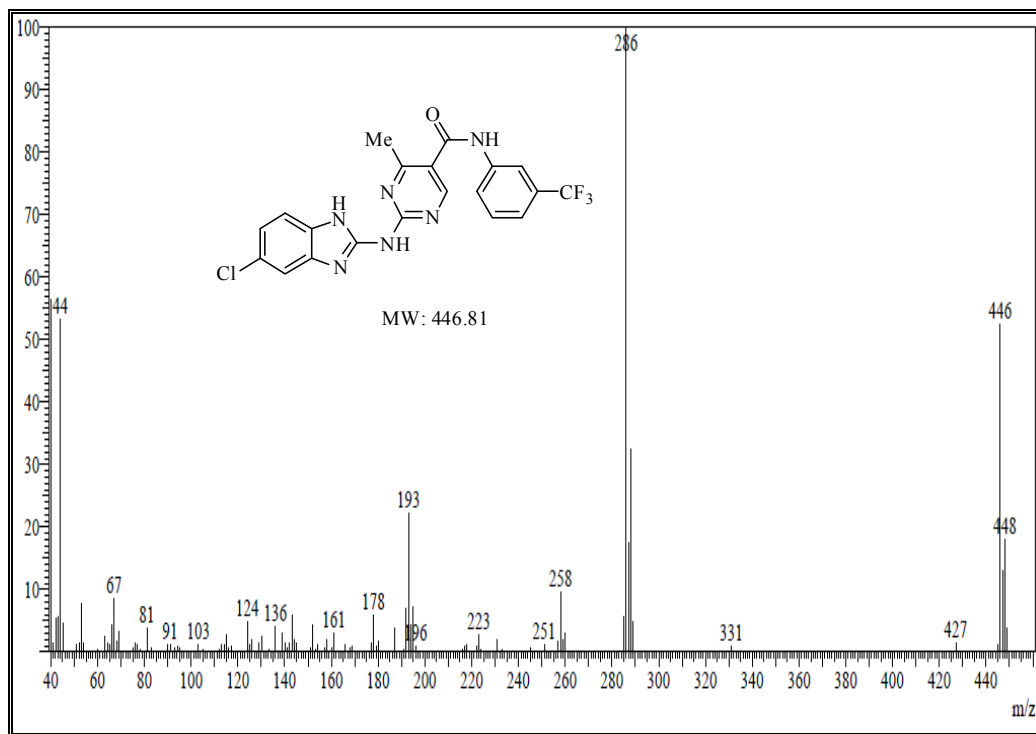
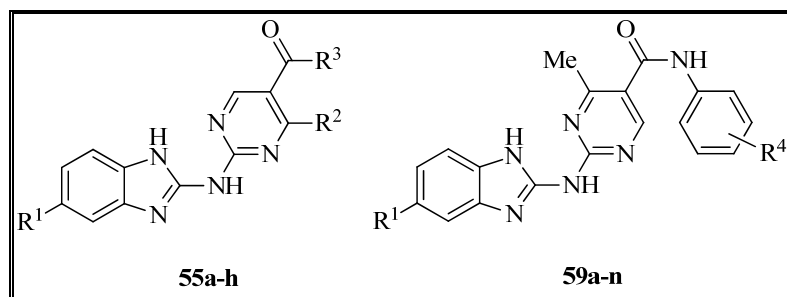
7.4.19 Mass Spectrum for compound **59n**.

Table 7.3 Elemental analysis of compounds **55a–h** and **59a–n**.

Compd.	MF	MW	CHN Calculated (%)			CHN Found (%)		
			C	H	N	C	H	N
55a	C ₁₄ H ₁₃ N ₅ O	267.29	62.91	4.90	26.20	62.78	4.98	26.44
55b	C ₁₄ H ₁₃ N ₅ O ₂	283.29	59.36	4.63	24.72	59.69	4.54	24.88
55c	C ₁₅ H ₁₅ N ₅ O ₂	297.31	60.60	5.09	23.56	60.34	5.31	23.41
55d	C ₁₅ H ₁₂ N ₅ O ₂ F ₃	351.28	51.29	3.44	19.94	51.46	3.57	19.73
55e	C ₁₄ H ₁₂ N ₅ OCl	301.73	55.73	4.01	23.21	55.46	3.89	23.34
55f	C ₁₄ H ₁₂ N ₅ O ₂ Cl	317.73	52.92	3.81	22.04	52.78	3.98	22.32
55g	C ₁₅ H ₁₄ N ₅ O ₂ Cl	331.76	54.30	4.25	21.11	54.12	4.47	21.38
55h	C ₁₆ H ₁₇ N ₅ O ₂	311.34	61.72	5.50	22.49	61.40	5.24	22.38
59a	C ₁₉ H ₁₆ N ₆ O	344.37	66.27	4.68	24.40	66.43	4.47	24.59
59b	C ₂₀ H ₁₈ N ₆ O ₂	374.40	64.16	4.85	22.45	64.38	4.77	22.31
59c	C ₂₀ H ₁₈ N ₆ O ₂	374.40	64.16	4.85	22.45	64.01	4.67	22.28
59d	C ₁₉ H ₁₅ N ₆ OF	362.36	62.98	4.17	23.19	62.77	4.02	23.41
59e	C ₁₉ H ₁₅ N ₆ OF	362.36	62.98	4.17	23.19	62.80	4.30	23.01
59f	C ₁₉ H ₁₅ N ₆ OCl	378.82	60.24	3.99	22.19	60.41	3.78	22.03
59g	C ₁₉ H ₁₅ N ₆ OCl	378.82	60.24	3.99	22.19	60.08	4.21	22.33
59h	C ₁₉ H ₁₄ N ₆ OCl ₂	413.26	55.22	3.41	20.34	55.03	3.21	20.25
59i	C ₁₉ H ₁₅ N ₆ OBr	423.27	53.91	3.57	19.86	53.74	4.18	19.73
59j	C ₂₀ H ₁₈ N ₆ O	358.40	67.02	5.06	23.45	67.18	5.19	23.34
59k	C ₂₀ H ₁₇ N ₆ OCl	392.84	61.15	4.36	21.39	61.34	4.18	21.47
59l	C ₂₁ H ₂₀ N ₆ O	372.42	67.73	5.41	22.57	67.58	5.29	22.71
59m	C ₂₀ H ₁₅ N ₆ OF ₃	412.37	58.25	3.67	20.38	58.37	3.87	20.22
59n	C ₂₀ H ₁₄ N ₆ OF ₃ Cl	446.81	53.76	3.16	18.81	53.41	3.48	18.67

Section C

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Summary

The work represented in the thesis entitled “*Studies on Some Oxygen, Nitrogen and Sulfur Containing Heterocycles*” is divided into three sections with seven chapters which can be summarized as below.

Section A deals with the potent antitumor DNA bifunctional alkylating agents and is further divided into three chapters.

Chapter 1 comprises of the general introduction of the DNA bifunctional alkylating agents. Initially, the naturally occurring antitumor Mitomycin C (MMC), which possess two reactive nucleophilic centers are capable of cross-linking with DNA. The other synthetic antitumor bifunctional alkylating agents, bis(hydroxymethyl)pyrrolizines derivatives were also able to induce DNA cross-linking via a similar mechanism of action of MMC. Based on the mechanism of action of these agents, in this chapter, we recently design several bis(hydroxymethyl) and bis(alkylcarbamates) pyrrolo derivatives.

In **Chapter 2**, planned to synthesize Target Molecules (TMs). A series of linear bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives and their bis(alkylcarbamate) derivatives were prepared. In this chapter, 53 compounds enlisted which are newly synthesized and bioactive compounds for various antitumor activities.

Chapter 3, consists of antitumor activity of both bis(hydroxymethyl)pyrrolo[1,2-*b*]isoquinoline and their bis(alkylcarbamate) derivatives. All the prepared derivatives show potent antitumor activity in various human tumor xenografts *in vitro*. Among these analogues, we discovered compound **81a**, which was selected for antitumor studies in animal models, exhibits potent therapeutic efficacy against human breast MX-1 xenograft in nude mice, as complete tumor remission was observed. This agent is also able to significantly suppress human ovarian tumors (SK-OV-3) implanted in nude mice. The results reported herein warrant further investigation to optimize the schedule and dosage to get greater suppression of other human tumor growth in animal models. Studies on the DNA interstrand cross-linking suggested that the newly synthesized derivatives are potent bifunctional DNA cross-linking agents. Furthermore, these agents induced substantial G2/M phase arrested of the cell cycle.

Section B encompasses the pyrano[3,2-*c*]quinolone derivatives and is further divided into two chapters.

Chapter 4 covers the history and background of pyrano[3,2-*c*]quinolone analogues. Various biological activities like Antibiotic, Anticancer, Anti-HIV, Calcium Channel Blockers, Antiallergic, Antimicrobial etc are summarized. Various synthetic procedures for the synthesis of pyrano[3,2-*c*]quinolone core structure are also shown. In **Chapter 5**, attempts were made to generate small library of pyrano[3,2-*c*]quinolone derivatives. The one-pot three-component Tendem Knoevenagel Michael addition reaction to furnish novel 4*H*,5*H*,6*H*,9*H*-pyrido[*ij*]pyrano[3,2-*c*]quinoline derivatives. The biological activity of newly synthesized compounds is under investigation.

Section C deals with the (1*H*-benzo[*d*]imidazol-2-yl)amino-pyrimidine derivatives and is further divided into two chapters.

Chapter 6 narrates the background and biological activity study of the Benzimidazole and Pyrimidine derivatives as well as in here including some recent synthetic strategies which have been employed to synthesize this class of compounds. The literature survey revealed that such class of which contains both heterocycles are show various biological activity.

In **Chapter 7**, first time reported three component condensations of guanidines, orthoester and active methylene carbonyl compounds leading to several novels substituted-(1*H*-benzo[*d*]imidazol-2-yl)amino-pyrimidine derivatives. 22 new molecules are prepared and characterized in this chapter. The yield of these compounds is good to excellent.

In all 96 derivatives have been synthesized in current work which are characterized by spectral data. Some of the compounds were tested for preliminary antitumor activity and advanced study. Other mechanistic studies like DNA interstrand cross-linking, cell cycle inhibition and rat plasma stability was also performed in the current work. The remaining synthesized compounds are also under screening for Anticancer, Anti-tubercular as well as antimicrobial activity study, the results of which are awaited.

Publications

1. Novel bifunctional alkylating agents, 5,10-dihydropyrrolo[1,2-*b*]isoquinoline derivatives, synthesis and biological activity. **Ravi Chaniyara**, Naval Kapuriya, Huajin Dong, Pei-Chih Lee, Sharda Suman, Bhavin Marvania, Ting-Chao Chou, Te-Chang Lee, Rajesh Kakadiya, Anamik Shah, Tsann-Long Su. *Bioorg. Med. Chem.* **2011**, 19, 275–286.
2. Design, synthesis and antitumor evaluation of phenyl *N*-mustard-quinazoline conjugates. Bhavin Marvania, Pei-Chih Lee, **Ravi Chaniyara**, Huajin Dong, Sharda Suman, Rajesh Kakadiya, Ting-Chao Chou, Te-Chang Lee, Anamik Shah, Tsann-Long Su. *Bioorg. Med. Chem.* **2011**, 19, 1987–1998.
3. Synthesis and Anti-HIV activity of Some 3,3'-(Substituted benzylidene)-bis-4-hydroxy benzo[*f*]Coumarins. Vijay Virsodia, Atul Manvar, Nimish Mungara, Nikhil Vekaria, Punit Rasadia, **Ravi Chaniyara**, Christophe Pannecouque, Erik De Clercq, Anamik Shah. *J. Serbian Chem. Soc.* (Manuscript Under Review).
4. Synthesis and biological evaluation of novel benzo[*d*]pyrrolo[2,1-*b*]-thiazole derivatives as bifunctional alkylating agents. **Ravi Chaniyara**, Huajin Dong, Pei-Chih Lee, Sharda Suman, Bhavin Marvania, Ting-Chao Chou, Te-Chang Lee, Rajesh Kakadiya, Anamik Shah, Tsann-Long Su. (Manuscript under preparation).
5. Design and synthesis of DNA Bifunctional alkylating agents from β -carboline: A hybrid pharmacophore approach. **Ravi Chaniyara**, Huajin Dong, Pei-Chih Lee, Sharda Suman, Bhavin Marvania, Ting-Chao Chou, Te-Chang Lee, Rajesh Kakadiya, Anamik Shah, Tsann-Long Su. (Manuscript under preparation).
6. Multicomponent Reactions (MCRs) of 4*H*,5*H*,6*H*,9*H*-Pyrido[*ij*]Pyrano[3,2-*c*]Quinoline Derivatives. **Ravi Chaniyara**, Rajesh Kakadiya, Nikhil Vekariya, Bhavin Marvania, Kuldip Upadhyay, Anamik Shah. (Manuscript under preparation).
7. Three component condensation of benzimidazole guanidines with active methylene compounds. **Ravi Chaniyara**, Bhavin Marvania, Nikhil Vekariya, Anamik Shah. (Manuscript under preparation).

Conferences/Seminars/Workshops Attended

- “International conference on bridging gaps in discovery and development: chemical & biological science for affordable health, wellness & sustainability” jointly organized by ISCBC and Saurashtra University, Rajkot. February, 04-07, 2011.
- “The 7th International Symposium for Chinese Medicinal Chemists” (ISCMC) Kaohsiung, Taiwan, February, 01-05, 2010.
- “2009 PST Medicinal Chemistry Symposium” Si-Tao, Taiwan, June 28-30, 2009.
- “International Conference On The Interface of Chemistry-Biology In Biomedical Research” jointly organized by ISCBC and Birla Institute of Technology & Science, Pilani. February, 22-24, 2008.
- “A National Workshop On Updates In Process And Medicinal Chemistry” jointly organized by National Facility for Drug Discovery through New Chemicals Entities Development & Instrumentation support to Small Manufacturing Pharma Enterprises and DST FIST, UGC-SAP & DST-DPRP Funded Department of Chemistry, Saurashtra University, Rajkot March, 3-4, 2009.
- “National Conference On Selected Topics In Spectroscopy And Stereochemistry” organized by the Department of Chemistry, Saurashtra University, Rajkot, March, 18-20, 2009.
- “National Workshop On Management And Use Of Chemistry Database And Patent Literature” organized by GUJCOST & Dept. of Chemistry of Saurashtra University, Rajkot, (Gujarat), February, 27-29, 2008.