"STUDIES ON SYNTHESIS OF NEW HETEROCYCLIC SKELETONS AND RELATED COMPOUNDS"

A THESIS SUBMITTED TO

THE SAURASHTRA UNIVERSITY

IN THE FACULTY OF SCIENCE

FOR THE DEGREE OF

Doctor of Philosophy

IN
CHEMISTRY

BY

Bhavin R. Marvania

Supervisor
Prof. Anamik Shah
Chemical Research Laboratory,
Department of Chemistry,
(Funded by DST–DPRP, DST–FIST
& Sponsored by UGC–SAP)
Saurashtra University, Rajkot–360 005,
Gujarat–INDIA

Co–supervisor
Prof. Tsann-long Su
Laboratory of Bioorganic and
Medicinal Chemistry,
Institute of Biomedical Science,
Academia Sinica,
Taipei–11529
TAIWAN

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 and Laboratory of Bioorganic and Medicinal Chemistry, Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan.

Date:

Place: **Bhavin R. Marvania**

CERTIFICATE

This is to certify that the present work submitted for the Ph.D. degree of Saurashtra University by **Mr. Bhavin R. Marvania** 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.

Date:

Place: Prof. Anamik K. Shah

中央研究院
生物醫學科學研究所
ACADEMIA SINICA
INSTITUTE OF BIOMEDICAL SCIENCES

128 Academia Road, Section 2
Nan Kang, Taipei 115
Taiwan

TEL：886-2-27899000
FAX：886-2-27853569
886-2-27829710

Certificate

This is certify that the present work submitted for the Ph.D. Degree of Saurashtra University by **Mr. Bhavin R. Marvania** has been the result of join work carried out under supervision of Prof. Anamik shah at Department of Chemistry, Saurashtra University, Rajkot (India) and under my Co-supervision at Laboratory of Bioorganic and Medicinal Chemistry, Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan & is a good contribution in the field of Medicinal chemistry with a special emphasis on synthetic, biological studies.

Date:

Place:

Prof. Tsann-Long Su (Co-supervisor)
Laboratory of Bioorganic and Medicinal Chemistry,
Institute of Biomedical Sciences,
Academia Sinica,
Taipei-11529
Taiwan

Dedicated to
My Family

Acknowledgement

I bow before Almighty God for making me capable of doing all that I propose, the work leading to my PhD Thesis submission is one of them.

In fact, words fails me to express my sincere feelings of gratitude to my research guide ***Prof. Anamik Shah,*** *his supervision, advice, and guidance from the very early stage of this research as well as giving me extraordinary experiences throughout the work. His truly scientific intuition has made him as a constant oasis of ideas and passions in science, which has exceptionally, inspire and enrich my growth as a student, a researcher and a scientist want to be. Of course, it is my life time achievement to work with him.*

I gratefully acknowledge My Co-Supervisor ***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 for providing mechanistic studies. I am also thankful to the National Science Council and Academia Sinica for financial support.*

I am very thankful to the faculties of Department of Chemistry namely, Professor and Head Dr. P.H. Parasaniya, Prof. V. H. Shah, Dr. H. S. Joshi, Dr. Shipra Baluja, Dr. M. K. Shah, Dr. Yogesh Naliapara, Dr.U.C.Bhoya and Dr. R. C. Khunt for their encouragement and ever green support.

I also would like to thank my labmates, Ching-Hwang Chen, Yi-wen Lin, Tai-Lin Chen, Dr. Rajesh Kakadiya, Dr. Nikhil Vekariya, Dr. Satis Trada, Ravi Chaniyara and staff member at the Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan. Thank you very much for creating supporting and pleasant working environment.

Collective and individual acknowledgments are also owed to my colleagues at Saurashtra University whose presence somehow perpetually refreshed, helpful, and memorable. Many thanks go in particular to Bharat(bhuro), Punit, Vaibhav(baba), Dipak, Harshad, Dilip, Sailesh, Shrey, Dhiru, Abhay, Bapu, Nilay, Manisha, Ronny, Mrunal, Rakshit, Ashish, Priti, Fatema, Ketaki, Madhavi, Sabera, Pratik, Vishwa and Paresh. I am fortunate in having Rahul(bhalu), Matre, Vikas, Deepak, Jigo, Bharat, Suresh, Lina, Pankaj, Atul, Rupesh, Akshay, Jimmy, Amit, Ritesh, katariya, Piyush, Ram, Naimish, Dodia, Ashish, Ghetiya, and Mahesh as my friends. Thanks to all dear.

I would also like to express my deep sense of gratitude to Ranjan Madam for her mother like treatment and Aditya Shah for their tremendous support during the course of the research.

I wish to express my deep gratitude to my loving and caring family. My parents, Rasikbhai Marvania and Shobhanaben Marvania deserve special mention for their inseparable support and prayers.

Finally, I am thankful to all those who have given me direct or indirect support during my research work.

Bhavin R. Marvania

CONTENTS

Abbreviations

AcOH	Acetic Acid
Ac_2O	Acetic Anhydride
$AlCl_3$	Aluminum chloride
Ar	Aromatic
ATO	Arsenic Trioxide
BP	Boiling Point
CCRF-CEM	Human Lymphoblastic Leukemia
CNS	Central Nervous System
CO_2	Carbon dioxide
Concd.	Concentrated
CR	Complete Tumor Remission
CL	Double-Stranded Cross-Linking
DAPYs	2,4-Dianilinopyrimidines
DMAP	Dimethylamino Pyridine
DMF-DEA	N, N-Dimethylformamide Diethyl Acetal
DME	Dimethyl Ether
DMF	*N*, *N*-Dimethyl formamide
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic 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_2SO_4	Sulphuric acid

BBr_3	Boron tribromide
HCl	Hydrochloric acid
HCT-116	Colon Carcinoma
IPA	Isopropyl alcohole
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-Pr	*Iso*-Propyl
J	Coupling Constants
K_2CO_3	Potassium Carbonate
K_3PO_4	Potassium Phosphate
KBr	Potassium Bromide
KOH	Potassium Hydroxide
L-1210	Lymphocytic Leukemia cell line
LX-1	lung
m	Meta
MDC	Dichloro methane
Me	Methyl
MF	Molecular Formula
MHz	Mega Hertz
Mmol	Mili moles.
MP	Melting Point
MS	Mass Spectra
N-mustard	Nitrogen Mustard
MX-1	Breast Carcinoma
Na_2CO_3	Sodium Carbonate
$NaHCO_3$	Sodium Bicarbonate
$NaNO_2$	Sodium nitrite
NaOH	Sodium Hydroxide
NCEs	New Chemical Entities

NMR	Nuclear Magnetic Resonance
o	Ortho
p	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
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/Et_3N	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetra Methyl Silane
UV	Ultra Violet
U87	Human Glioma
VBL	Vinblastine

SECTION - A

CHAPTER - 1

NOVEL DNA-DIRECTED ALKYLATING AGENTS

1.1 Introduction

1.1.1 Cancer

Cancer is a collective term used for a group of diseases that are characterized by the loss of control of the growth, division, and spread of a group of cells, leading to a primary tumor that invades and destroys adjacent tissues. The continuous proliferation of cancer cells develops into tumour tissues and may spread across to other organs via circulatory systems resulting in metastasis, which is the cause of 90% of cancer deaths. There are two types of tumours: those demonstrating the properties described above, known as malignant tumours, which result in cancer; and those without malignant properties, which are self-limiting, noninvasive and do not metastasise, known as benign tumours. Cancer remains one of the most difficult diseases to treat and is responsible for about 13% of all deaths worldwide. Cancer may affect humans of all ages. According to statistics from the American Cancer Society (ACS), cancer is the third most lethal disease after cardiovascular diseases and infectious and parasitic diseases[1,2].

Cancer occurs when genetic mutations accumulate in cells during the replication of genetic material, allowing cells to undergo transformation. The mutations of three major types of genes: oncogenes, tumour suppressor genes and DNA repair genes, play an important role in tumourgenesis. Tumorigenic events include small-scale changes in DNA sequences, such as point mutations; larger-scale chromosomal aberrations, such as translocations, deletions, and amplifications; and changes that affect the chromatin structure and are associated with dysfunctional epigenetic control, such as aberrant methylation of DNA or acetylation of histones.[3] About 2,000–3,000 proteins may have a potential role in the regulation of gene transcription and in the complex signal-transduction cascades that regulate the activity of these regulators. Cancer is not only a cell disease, but also a tisular disease in which the normal relationships between epithelial cells and their underlying stromal cells are altered.[4]

1.1.2 Cell cycle and regulation[5]

The cell cycle is represented in Figure **1** the cycle is divided into four main parts. The g1 or gap1 phase is the period when a newly created cell is born. The period of time a cell remains in the G_1 phase depends on the tissue type and whether it is a normal

phase and tumor cell. If the cell is proliferating cell, it will quickly move into the S or synthesis phase. It is during this period that nuclear DNA is replicated, and at the end of the S phase, two copies of DNA are present in cell. The next phase is G_2 or Gap_2 period and this phase is largely a time during which preparations are made for the final cell cycle phase, the M phase or mitosis. The time between mitoses is the cell cycle time, although this time can vary depending mainly on the duration of G_1 phase.

There are two major control points in the cell cycle. One of these is at G_1/S when cell commit to replicate. The second is at G_2/M when cell commit to divide. Of the two major points in the cell cycle, the G_1/S phase is of major importance in understanding cancer and cancer treatment.

During the G_1 phase a cell can take one of three routes. First, the cell may enter the s phase. Second, a cell in G_1 phase may enter into fifth phase called G_0 or Gap_0. Cell in G_0 are termed quiescent. Third, the cell may terminally differentiate and die. In normal cell populations cell may be proliferating, quiescent, or terminally differentiating such that there is no net change in the number of cell. However, in tumors, the fraction of cell proliferating increases at the expense of quiescent or terminally differentiating cell such that there is net increase in the number of cell.

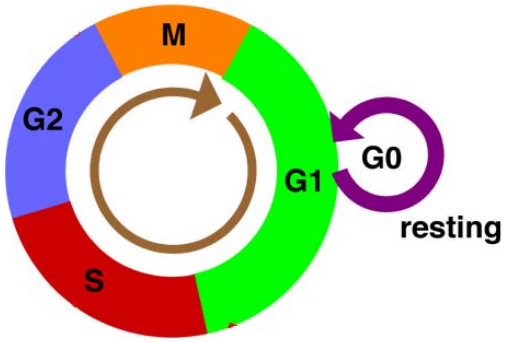


Figure 1 Cell cycle regulation

1.1.3 Cancer Therapy

The specific approach used to treat cancer depends upon the specific type, location, and stage of the cancer. Regardless of the specific details, there are several fundamental techniques available to treat cancer including surgery, radiation therapy,

immunological treatment, and chemical based approaches. In most therapeutic approaches to the treatment of cancer there will be chemical components.

Surgery[6]

Surgery, the oldest form of cancer treatment, is used in cancer treatment either to remove only the tumor, or the entire organ. In order for this approach to be effective, the cancer must still be in the primary tumor stage and there must be a high degree of confidence that the entire tumor can be excised. Surgery can also be used for diagnostic purposes and in combination with other approaches.

Radiation therapy[7]

Radiation therapy is used of ionizing radiation to kill cancer cells and shrink tumors. It can be administered externally (external beam radiotherapy) or internally (brachytherapy). It does so by causing damage to the DNA of the tumor cell so that they die. Radiation therapy is not painful and anesthetics are not required for the procedure. Radiation is often used in combination with other treatments. Unfortunately, radiation can cause severe blood changes including drop in production of new blood cells, nausea, anemia and vomiting.

Immunologic therapy[8]

This is relatively new approach to the treatment of cancer. There are treatments that use the body's own natural defenses to fight cancer. Immunotherapy, also known as biotherapy or biological response modifiers, works on white blood cells - the body's first line of defense against disease. White blood cells can be stimulated in various ways to boost the body's immune response to cancer, with little or no effect on healthy tissue. Immunotherapy can also be used to lessen the side effects of other cancer treatments. Currently, therapy of this type primarily consists of the administration of highly purified interferons, especially interferon-2.

Chemotherapy[9]

Chemotherapy uses powerful drugs to kill cancer cells, control their growth, or relieve pain symptoms. Chemotherapy may involve one drug, or a combination of two or more drugs, depending on the type of cancer and its rate of progression.

Chemotherapeutics agents are complementary to either surgery or radiation therapy in that they are affective agents metastasized tumor or residual tumor after surgery or radiation therapy. In other words, they are useful for eliminating tumors that are small in size. They are certain drawbacks of chemotherapy, the lake of selectivity of the agents for normal verses malignant cells. Chemotherapeutics agents are basically cytotoxic and thy can kill both normal and malignant cell types. A consequence of this is patients undergoing chemotherapy suffer hair loss, depression of their immune system, and nausea or diarrhea. These effects typically disappear once chemotherapy has been discontinued.

1.1.4 Classification of cytotoxic drug

Cytotoxic drugs are usually classified according to their mechanism of action. The major classes of cytotoxic agents are shown below. The mechanism of action of cytotoxic drug is also summarized in **figure 2**

1. **Alkylating agents**[10]**:** *alkylating agents* and related compounds, which act by forming covalent bonds with DNA and thus impeding replication. Such as Nitrogen mustard (e.g. Mechloethamine hydrochloride, Cyclophosphamide, Chlorambucil, Melphalan, Isofamide), ethyleneimines (e.g. Thiotepa), Triazenes (e.g. Dacarbazine), nitrosourea (e.g. Carmustine, Lomustine, Semustine, Streptozocin), alkyl sulfonates (e.g. Busulfan)

2. **Antimetabolites**[11]**:** *antimetabolites,* which block or subvert one or more of the metabolic pathways involved in DNA synthesis. Purine antagonists (e.g. 6-Mercaptopurine, Thioguanine), pyrimidine antagonists (e.g. Fluorouracil, Cytarabine, Fludarabine), folic acid antagonists (e.g. Methotrexate)

3. **Plants alkaloids**[12]**:** Most of these derivatives specifically affect microtubule function and hence the formation of the mitotic spindle. Vinca alkaloids (e.g. Vinblastine, Vincristine, Vinorelbine, Vindesine), taxanes (e.g. Taxol, Docetaxol)

4. **Topoisomerase inhibitors**[13]**:** Topoisomerases are essential enzymea that maintain the topology of DNA. Inhibition of type I and type II topoisomerases interferes with both transcription and replication of DNA by upsetting proper

DNA supercoiling. Type I topoisomerase inhibitors (e.g. Irinotecan, Topotecan), type II topoisomerase inhibitors (e.g. Amsacrine, Etoposide, Teniposide)

5. **Antibiotics**[14]**:** These are substances of microbial origin that prevent mammalian cell division. Anthracyclines (e.g. Doxorubicin hydrochloride, Daunorubicin, Idarubicin, Bleomycin, Mitomycin)

6. **Hormonal agents**[15]**:** *Hormones*, of which the most important are steroids, namely glucocorticoids, oestrogens and androgens, as well as drugs that suppress hormone secretion or antagonise hormone action. Estrogens, antiastrogenes (e.g. Tamoxifen cytrate, Estramustine phosphate sodium), androgens, antiandrogens (e.g. Flutamide), other (e.g. Glucocorticoid, Progestins, Octreotide acetate)

7. **Miscellaneous agents**[16]**:** *Miscellaneous agents* that do not fit into the above categories. This group includes a number of recently developed drugs designed to affect specific tumor-related targets. Enzymes (L-asperagina), metal compounds (e.g Cisplatin, Carboplatine), other (e.g. Mitoxantrone Hydroxyurea, Procarbazine).

8. Some newer agents do not directly interfere with DNA. These include monoconal antidodies and new tyrosine kinase inhibitors e.g. *imatinib mesylate* (*gleevec* or *glivec*) with directly targets a molecular abnormality in certain types of cancer (chronic myelogenous leukemia, gestrointestinal stromal tumors).

1.2 DNA-alkylating agents

DNA alkylating agents have played an important part in cancer chemotherapy. Alkylating agents, the oldest and most useful among the antineoplastic agents, can be defined as compounds capable of covalently binding an alkyl group to a biomolecule under physiological conditions (aqueous solution, 37°C, pH 7.4). Some of them especially nitrogen mustard (N-mustard), are used clinically for the treatment of cancer.

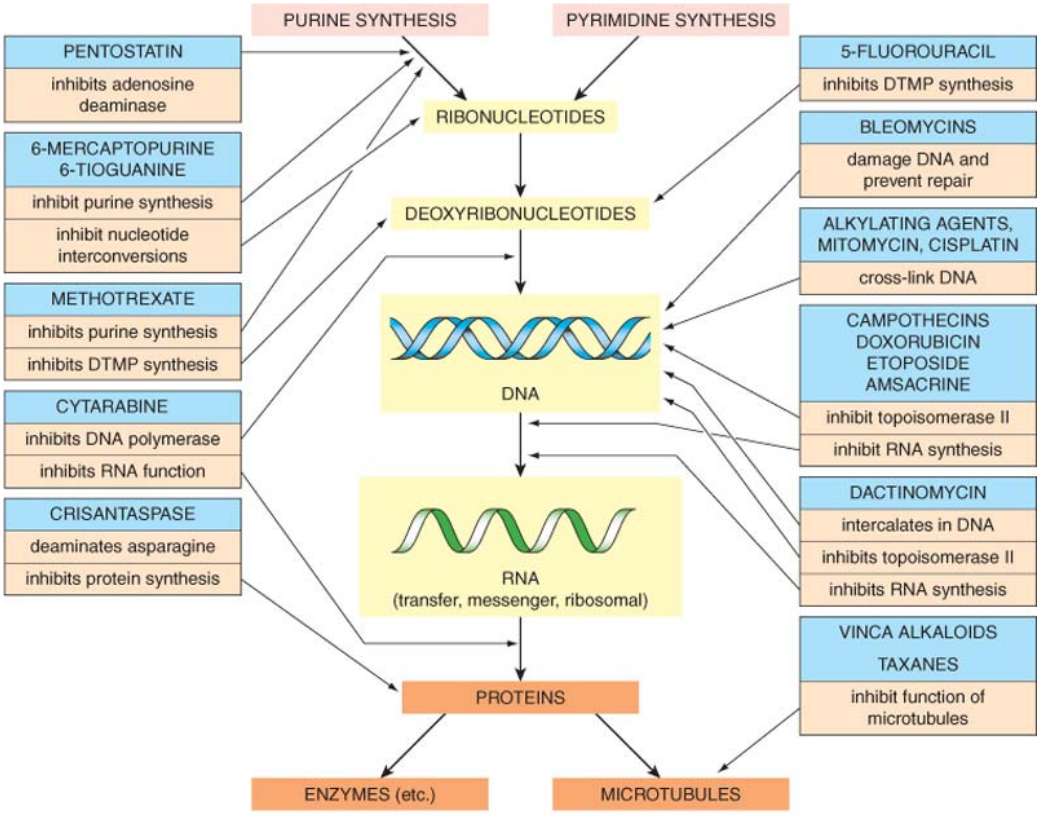


Figure 2. Summary of the main sites of action of cytotoxic agents.

The other DNA alkylating agents such as mechlorethamine (**1**), chlorambucil (**2**), melphalan (**3**), cyclophosphamide (**4**), estramustine (**5**), the anthramycine (**6**), cisplatin (**7**) and bismethanesulfonates (**8**) derivatives that are remain currently in clinical use. All of these drugs share the property of being the source of electrophiles that alkylate various nucleophilic groups such as phosphate, amino, hydroxyl, carboxyl and imidazole present in biomolecules such as proteins and nucleic acids. There are several nucleophilic groups in DNA such as N-1 and N-3 of adenine bases, N-3 of cystosine, and in particular N-7 of guanine. Drug with two alkylating groups can react with a guanine on each chain and cross-links the strand such that they disrupt the replication of transcription leading to the cell death.

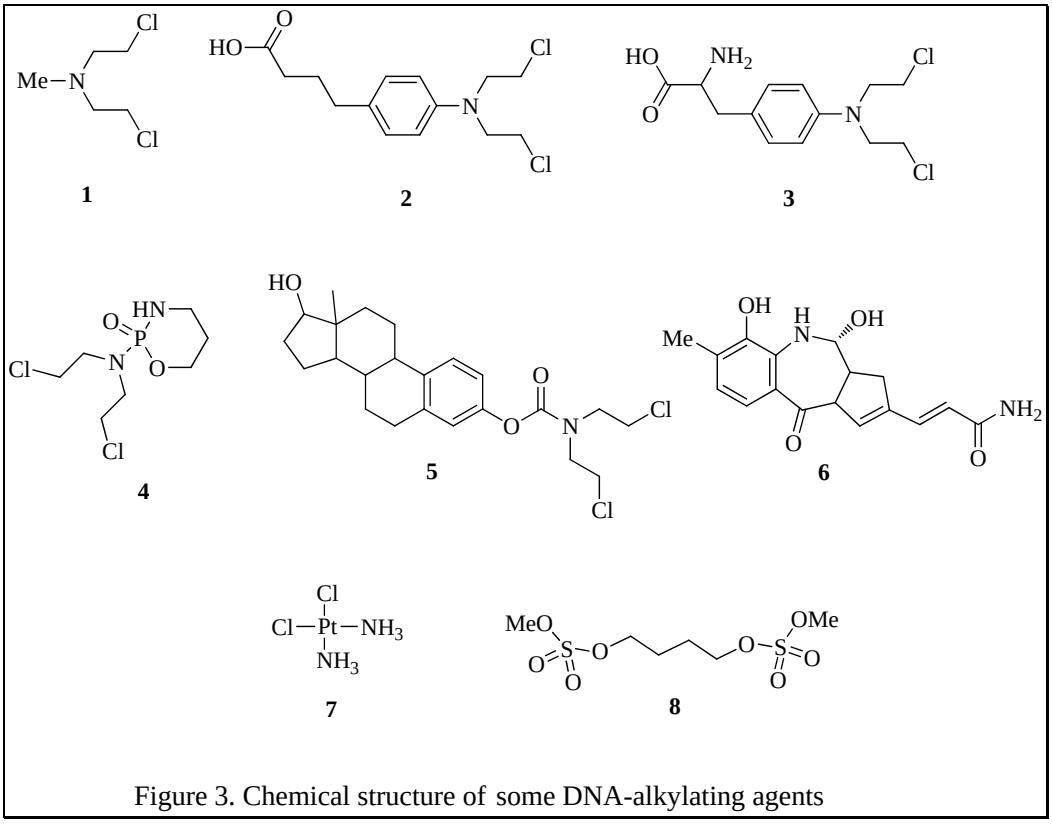


Figure 3. Chemical structure of some DNA-alkylating agents

1.2.1 Nitrogen mustards

The nitrogen mustard represents the earliest and perhaps most extensively studied of the DNA interstrand cross-linking agents.[17] The first nitrogen mustard was used in cancer therapy was mechlorethamine (**1**). *N*-mustards act through the alkylation of DNA and prevent cell division by cross-linking DNA, which leads to DNA breaks or abnormal base pairing, and subsequently to cell death.[18] The overall process of DNA alkylation by *N*-mustard is a two-step process. At neutral or alkaline pH, bis(chloroethyl)amine (1) undergoes a first order SN^1 intramolecular cyclization, with the release of chloride ion and formation of an aziridinium cation (2)[19], which is highly reactive and unstable species. The strained three member ring of aziridinium cation can undergo nucleophilic addition by DNA nucleophile to form a mono alkylation adduct. These reactions can then be repeated with the other $-CH_2CH_2Cl$ to give a cross-link. Cross-linking can occur either between two complementary strands of DNA (interstrand) or within a strand of DNA (intrastrand). The site specificity the mustard was originaly assigned as being the 5GpC3 sequence within B-form DNA.[20]

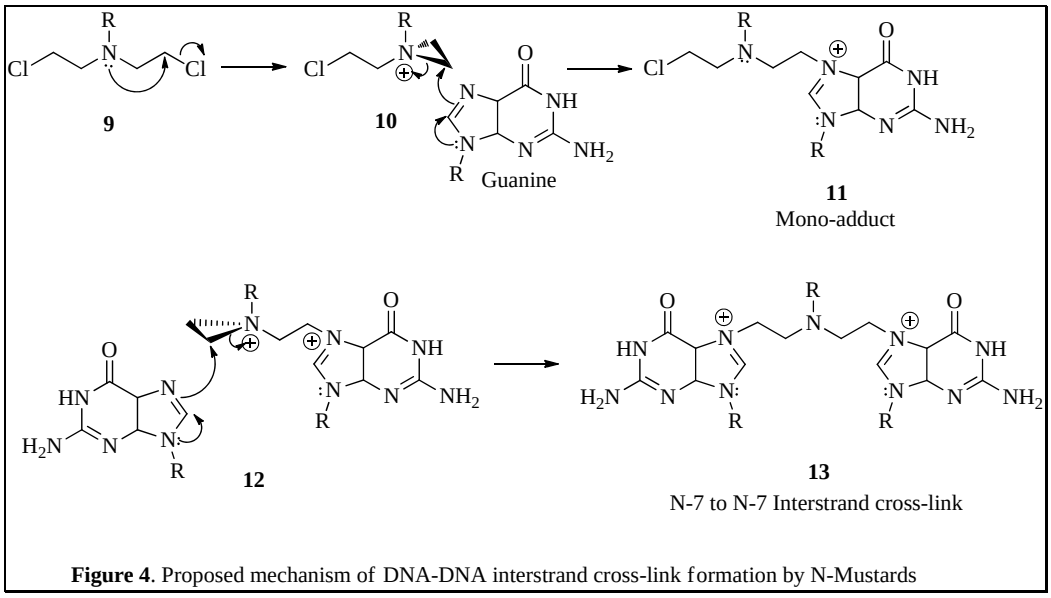


Figure 4. Proposed mechanism of DNA-DNA interstrand cross-link formation by N-Mustards

Despite their clinical importance, the usefulness of many DNA-alkylating drugs is often limited by a number of pharmacological deficiencies resulting from the intrinsic chemical reactivity of the agent. There are several drawbacks for using DNA alkylating agents as chemotherapeutic agents including:

1. High chemical reactivity[5] of conventional drug. This can result in loss of drug's activity by reacting with other cellular nucleophiles such as proteins and low molecular weight thiols. These agents may be also induce cellular resistance mechanisms because of increasing high level of glutathione. [21, 22]

2. Lack of intrinsic DNA binding affinity of the alkylating pharmacophore requiring for cross-linking of DNA to reach fully cytotoxic. This can be resulted by formating high ratio of genotoxic mono-adducts to cross-links (20:1)[23] and produce carcinogenicity[9] or bone marrow toxicity.

3. The major guanine N[7] adduct formed by *N*-mustards is readily repaired and thus, may reduce drug efficacy by DNA repair mechanism.[24]

There are several strategies to overcome the drawbacks of *N*-mustards. One of the effective strategies is to synthesize *N*-mustard prodrug to reduce the reactivity of the parent *N*-mustard pharmacophore. Another strategy is preparing *N*-mustard-DNA-affinic molecule conjugates to increase sequence-specific Drug-DNA binding.

1.2.2 *N*-mustard Prodrug approaches

Prodrug therapy provides an alternative approach to design less reactive and less cytotoxic form of anticancer drugs.[25] Prodrugs can be defined as agents that are transformed after administration, either by metabolism or by spontaneous chemical breakdown, to form a pharmacologically active species. Prodrugs have been used to improve the solubility, transport properties, and pharmacokinetic properties of anticancer agents. Early work showed that cyclophosphamide (**4**), a *N*-mustards prodrug, is able to be activated by enzymatic oxidation to generate active *N*-mustard pharmacophore. This agent is currently widely used for treatment cancer patients.[26-27]

In the last two decades, monoclon antibody and exogenous enzyme have been utilized for prodrug antibody therapy.[28-30] Enzyme-activating prodrug therapy is a two-step approach. A drug-activating enzyme (usually antibody-enzyme fusion protein) is administered intravenously in the first step, which binds to specific antigen expressed on the tumor cell surface. A non-toxic prodrug is administered systemically in the second step and is converted to the cytotoxic drug by the pre-targeted enzyme.[4-6] Currently, delivery methods for an enzyme/prodrug strategy can be divided into two major classes: (*a*) delivery of genes that encode prodrug-activating enzymes into tumor tissues (GDEPT, VDEPT, etc.); and (*b*) delivery of active enzymes onto tumor tissues (ADEPT).

Springer *et al*. have synthesized several N-mustard prodrugs, the phenyl N-mustard pharmacophore is linked to enzyme's substrate moiety, L-glutamic acid, via a urea (**13** and **14**, Z = NH)[31] carbamate (**13** and **14**, Z = O)[31] , and carboxamide [**15** (CMDA)[32, 33] and **16** (CJS 1050)[34]] linkage for antibody-directed enzyme prodrug therapy (ADEPT),[35] or gene-directed enzyme prodrug therapy (GDEPT). After enzymatic cleavage by bacterial enzyme carboxypeptidase G2 (CPG2), they can be transformed into their corresponding active metabolite phenol or aniline N-mustard drugs. The amide analogue **13** (CMDA) was the first ADEPT prodrug evaluated clinically.[36]

The prodrugs, **17**,[37] **18**,[38] **19**,[39] and **20**[39] were also synthesized by linking the aniline N-mustard to the trigger unit tyramine, 3-hydroxytyramine, catecholamine, and 4-aminophenol respectively, via a urea or carbamate linker for melanocyte-directed

enzyme prodrug therapy (MDEPT). Upon exposure to tyrosinase, these conjugates can also release the active aniline or phenol *N*-mustard.

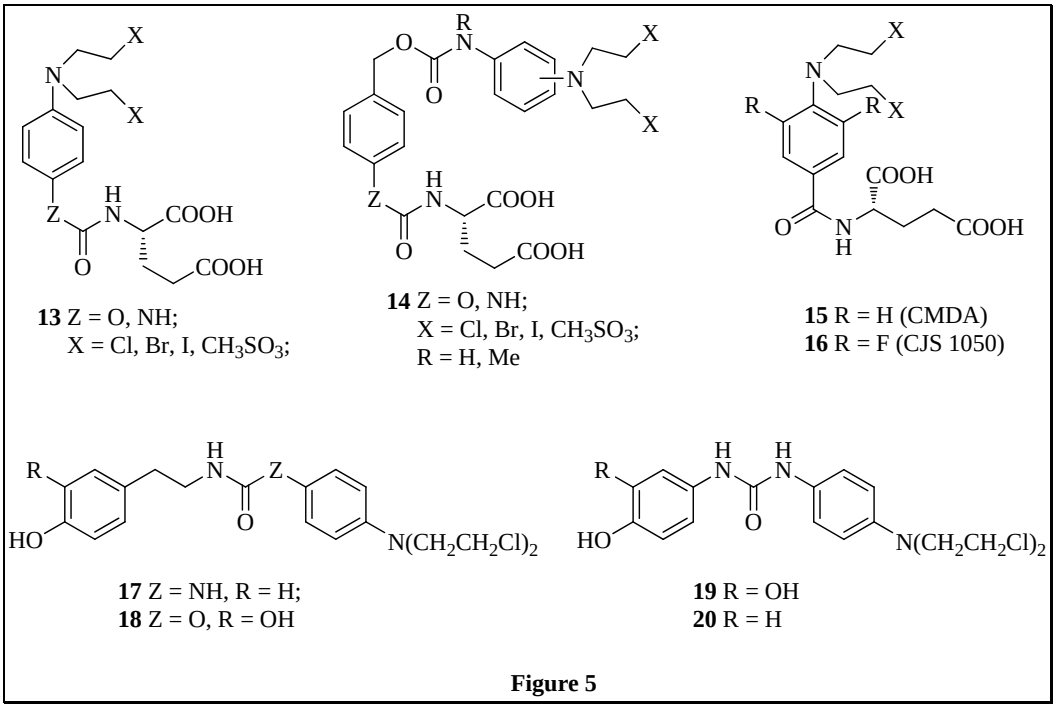


Figure 5

Recently, pedersen *et al*. have synthesized prodrug **21**[40] and **22**[40] with both C16 and C18 ether chain and a choline and a glycerol phosphate headgroup, respectively. They studied biophysical and biological characterization of the synthesized chlorambucil prodrug (**21**, **22**) with liposome formulation, particle size determination, and in particular $sPLA_2$ activity. After hydrolysis of *sn*-2 ether bond by $sPLA_2$, they can be released both the anticancer drug bound to *sn*-2 ether bond and an anticancer ether lipid (AFL).

Various nitro containing derivatives, 5-aziridinyl-2,4-dinitrobenzamides (i.e., **23**, CB 1954),[41,42] 2,4-dinitrobenzamide 5-*N*-mustards (**24**)[43,44] and related derivatives were also synthesized for antibody-directed or gene-directed enzyme prodrug therapy. These prodrugs can be activated by co-treatment of *E. coli* nitroreductase (NTR), which are able to concert the electron-withdrawing para-NO_2 function to NH_2 (electro-donating function) via reductive activation.[45-49] compound **24** was superior to **23** as an NTR substrate, with a 4-fold higher K_{cat} for purified enzyme[50] and 2-3 fold faster reduction by NTR expressing mammalian cells.[51]

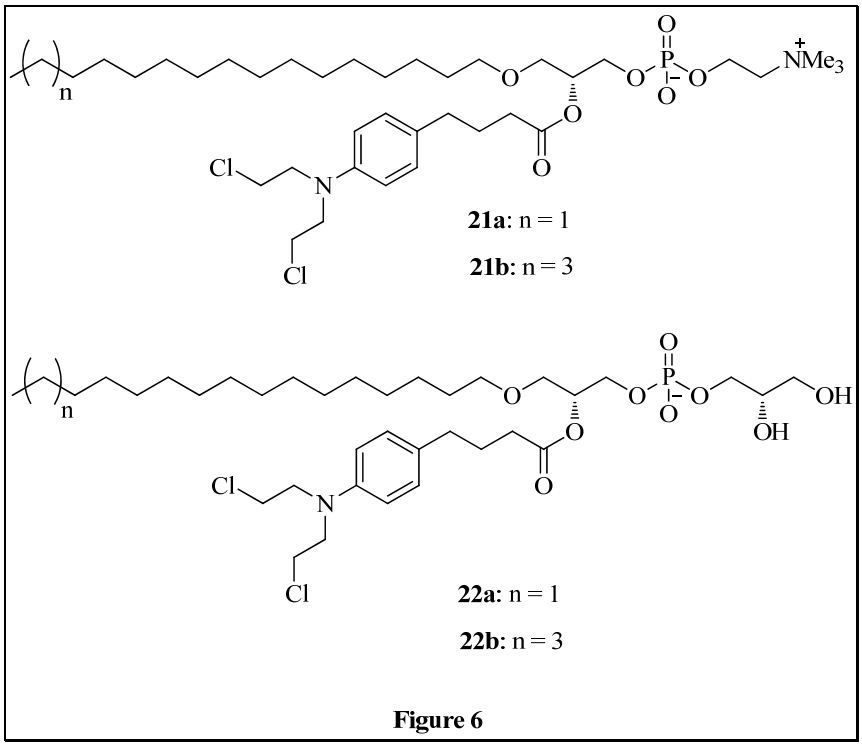


Figure 6

Similarly, 3,5-dinitrobenzamide nitrogen mustard PR-104 (**25**),[52] which also can be activated by nitroreductase. This agent is a novel hypoxia-activated DNA cross-linking agent with potent activity against human tumor xenografts (e. g., SiHa cervical, HT29 colon and H460 NSCLC), both as monotherapy and combined with radiotherapy and chemotherapy.[52] It suggests that the urea or carbamate linker is capable of lowering the reactivity of aniline or phenol *N*-mustard pharmacophore resulting in formation of rather stable *N*-mustard derivatives.

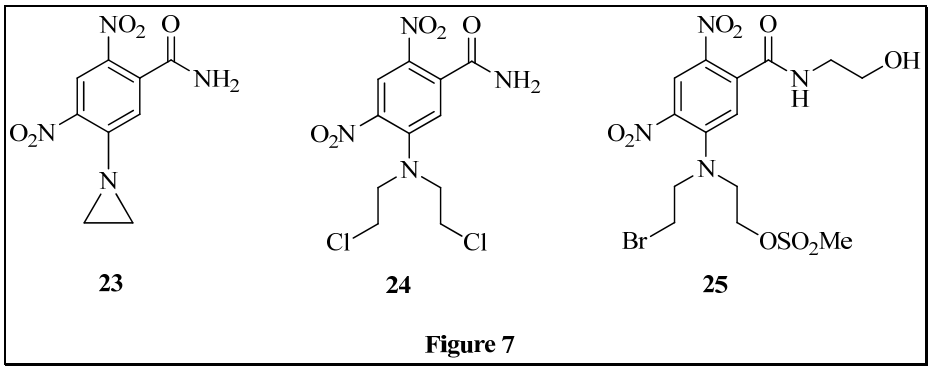


Figure 7

1.2.3 DNA Directed Alkylating Agents

Another strategy for overcome the drawbacks of DNA-alkylating agents is to construct "DNA-directed alkylating agents". DNA-directed alkylating agents are synthesized by linking DNA-affinic molecules (carrier) to a *N*-mustard pharmacohpore (warhead, such as alkyl *N*-mustard or phenyl *N*-mustard). The generally useful DNA-affinic molecules are DNA-intercalating agents (e. g. 9-anilinoacridines and acridines), binding agents (quinolines or quinazolines), and DNA minor groove binding agents (e. g. distamycin A and netropsin). Most of these carriers also exhibit anticancer activity by inhibiting Topoisomerases I and II.[53] Most evidence shows that DNA-directed alkylating agents are more cytotoxic than the used carrier itself. Consequently, connecting DNA-affinic molecules to alkylating agents usually results in improved therapeutic efficacy and low toxicity in the compound.

Creech et al. [54-57] have synthesized DNA-directed alkylating agents by using various heterocyclic nuclei such as quinazoline (e.g. **26**), quinolone (e.g. **27**, **28**), benz[*c*]acridine (e.g. **29**), and acridine (e.g. **30**, **31**) as carriers for the *N*-mustard pharmacophore, through aminoalkyl side chain or amide linkage for antitumor studies (Figure **8**). It revealed that the presence of DNA-intercalating or binding nuclei improved the antitumor effectiveness of the mustard moiety against Ehrlich ascites tumors in vivo and compounds caused at least five-fold increase in survival time over that of the control mice. Treatment of mice bearing ascites tumors with these compounds caused at least five-fold increase in survival time over that of the control mice. Based on these finding, the same research group have also synthesized sulfur mustard attached with various heterocyclic nuclei through aminoalkyl side chain or amide linkage for antitumor studies (e.g. **32**, **33** and **34**). A striking observation was that, the *N*-mustard conjugates have good anti-tumor activity then corresponding sulfur mustard. From this effort it becomes clear that initiatives to increase drug-DNA-affinity often provide frameworks upon which to design and construct new drugs of higher efficacy or altered selectivity.

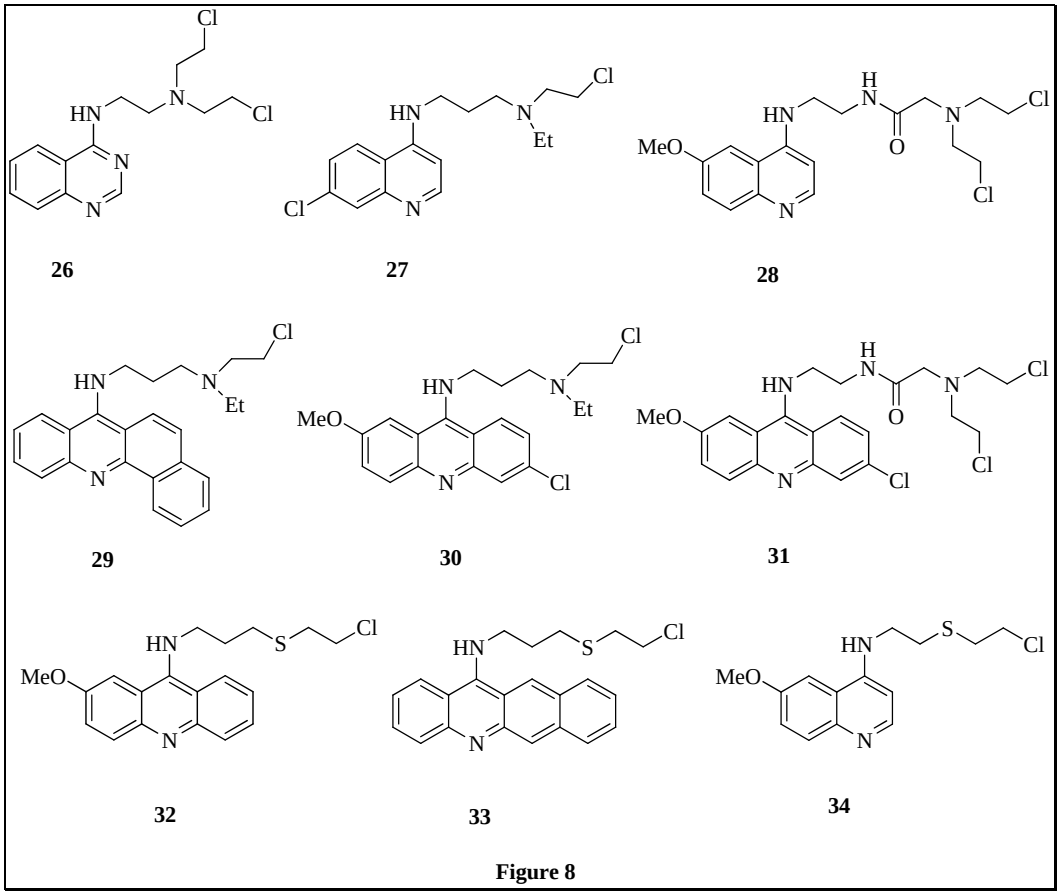


Figure 8

Prakash and co-workers have synthesized a series of phenyl *N*-mustard link to a various quinazoline (**35**) and 9-aminoacridine (**36, 37**) via alkyl chain of variable length[58-60]. These conjugates were extremely potent and have pronounced antitumor activity. It demonstrated that compound **35** is not a strong intercalator, but it may bind weakly at the major groove side. Compounds having a $(CH_2)_n$ (**36**) and $(CH_2)_nO$ (**37**) (n was variable from 2 to 5) were much greater potency (ILS 50-60% at an optimal doses of 20-30 mg/kg) than either chlorambucil (ILS 33% at an optimal dose of 225 mg/kg) or any of the untargeted mustards, using a single-dose protocol. On the other hand, the thioether and sulfoxide derivatives of **37** showed only minimal in vivo activity.[58] The DNA cross-linking studies demonstrate that the compound (**36**, **37** and thioether and sulfoxyde analog of **37**) 5'-GT sequences was the most preferred sites at which N-7-guanine alkylation occurred. For analogues with longer chain lengths, the preference of 5'-GT sequence diminishes in favor of N-7-adenine alkylation at the complementary 5'-AC sequence. The thioether analog of **37** was found less reactive in vitro interstrand cross-linking than were **36** and **37**. These studies suggest that the

quinazoline and 4-aminoacridine are also valuable carriers for building DNA-directed alkylating agents. Gravatt et. al. also utilized a carrier 4-anilinoquinoline for DNA-directed alkylating agent (e.g. **38**, **39**).[61] It was demonstrated that these compounds exhibited potent antiproliferative activity against human leukemia and various solid tumor cell growths in vitro and potent antitumor efficacy in vivo with a relatively low toxicity. Particularly, compound **39** bind in minor groove and alkylate both adenines and guanines at the N3 position at the 3'-ends of AT-rich sequences, with the most preferred sites being AT-tracts.[62] These compounds are also efficient interstrand crosslinking agents.

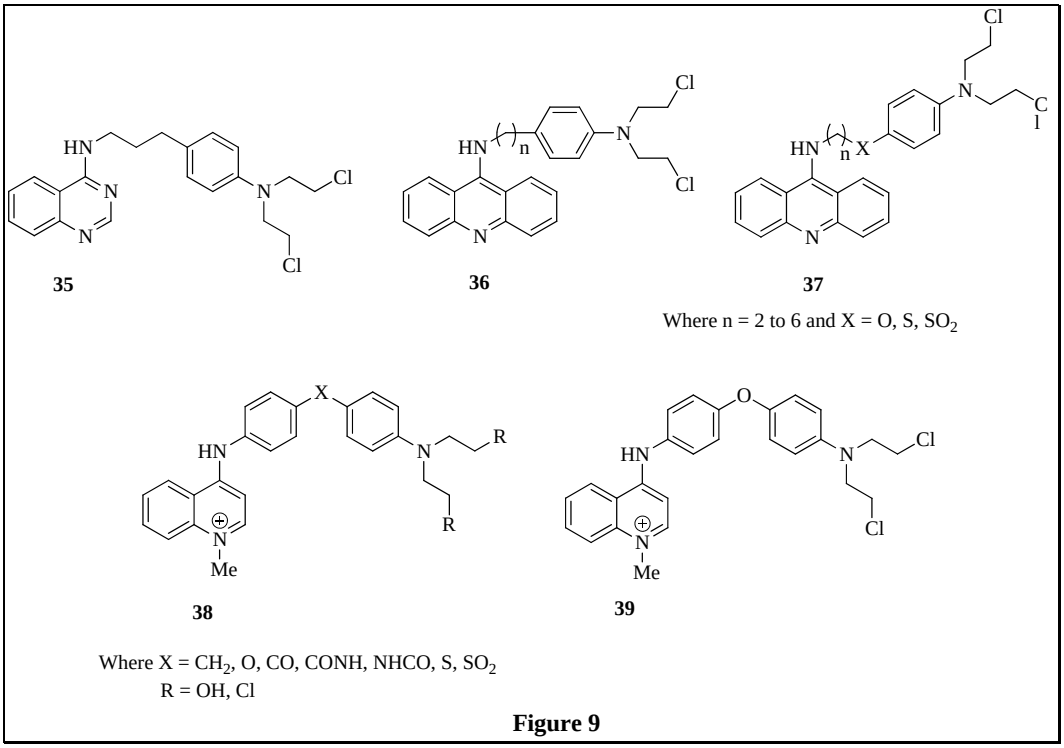


Figure 9

DNA intercalating agents, 9-anilinoacridine-4-carboxamide [a topoisomerase II,[63,64] and used for treatment of adult leukemia[65,66]], was used as a carrier for constructing DNA-directed alkylating agents. The asymmetry of the 9-anilinoacridine-4-carboxamide 'DNA intercalating' agents (**40**) makes it a potential regioselective carrier of alkylating agents. Recent crystallographic studies of 9-aminoacridine-4-carboxamide (**41**) shows the carboxamide side chain binding in the major groove side. The mustard analog of **40**, where the alkylating unit was attached either off the 4-

carboxamide (**42**) or at the 1'-position of the 9-anilino ring (**43**)[67, 68]. At the optimal dose, these analogues achieved a smaller percentage increase of lifespan (%ILS) than the parent *m*-AMSA (**40**) in mice bearing murine leukemia p388.[67] The 4-linked analogue **42** showed to have slightly higher *in vivo* antileukemic activity than their corresponding 1'-linked analogues (**43**), suggesting that the active side chain may be better to be linked to the acridine ring rather than to the aniline ring. Incubation of **42** and **43** with calf thymus DNA showed that **42** gave only one adduct, resulting from alkylation at guanine N-7 in the major groove. In contrast, the major adduct of **43** resulted from alkylation at adenine N-3 in the minor groove.[68] The studies suggested that the 9-anilinoacridine-4-carboxamide chromophore may be important 'DNA-threading agent' with high region-specifity, placing the aniline side chain in the minor groove and the carboxamide chain in the major groove.

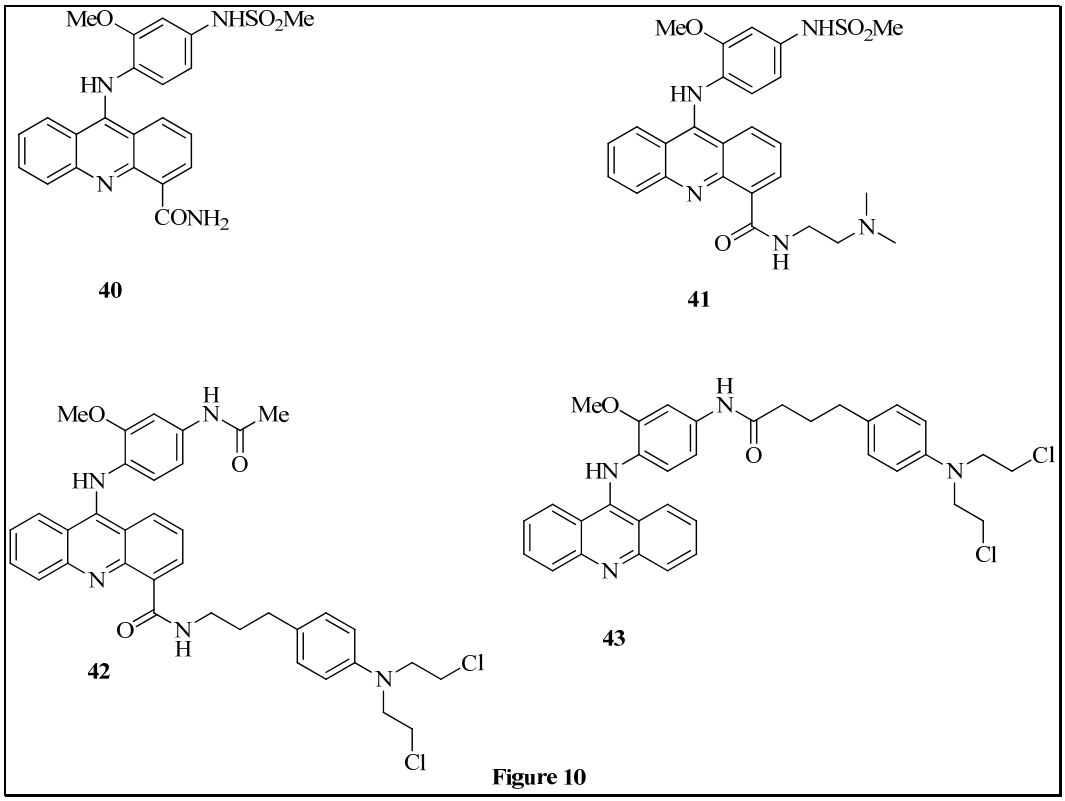


Figure 10

In order to establish the higher sequence-selectivity, most of the recent work has used DNA minor groove binders as carriers to construct DNA-directed alkylating agents, because these may offer much higher region and sequence selectivity. Utilizing this approach, denny et. al. have synthesized phenyl *N*-mustard analoge using bis-

benzimidazoles as a carrier for DNA-directed alkylating agents.[69] Bis- benzimidazole are also well-characterized reversible minor groove binding ligands, with broad studies on the lead compound Hoechst 33258 (**44**)[70-72]. Analogues of **45** with aniline mustards attached by a variable-length polymethylene chain showed that these compounds exhibited potent antiproliferative activity and bound within the minor groove side. The C-3 analog of **45** exhibited efficiently cross-linked the cellular DNA and exhibited potent cytotoxicity (up to 85-fold more potent than chlorambucil), with IC_{50} value of 10 nM against the P388 cell culture. Further studies on bisbenzimidazole were altered by changing the heteroatoms, but the mustard was directly attached to the phenyl ring, revealed that analogues (**46**) retaining DNA-affinic H-bonding moieties had higher reversible binding and faster kinetics of alkylation.[73]

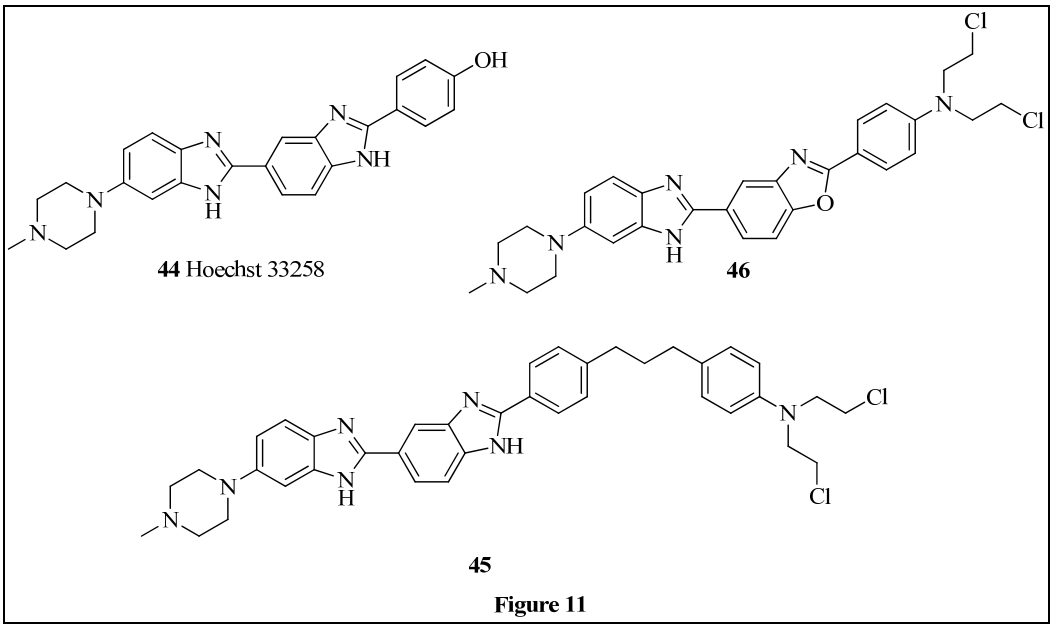


Figure 11

In addition to increasing drug-to-target affinity, the oligopyrrole antibiotics netropsin and distamycin A was used as a carrier for nitrogen mustard. Distamycin A is also well-documented AT-specific reversible minor groove binders. On the basis of this, polypyrrole analogues of Distamycin A with mustard moieties was synthesized for antitumor evaluation, found to have a broad spectrum of antitumor activity in experimental tumor models and highly specific alkylation at adenines in runs of adenines.[74-78] The benzoic mustard derivative tellimustine is a very sequence and regiospecific alkylator. Previous studies have shown that tallimustine (**47**) possess a high preference for alkylation of adenines located in the 5'-TTTTGA-3' sequence.[79,

[80] The dibromo mustard analoge (**48**) of tallimustine was considerably 100-fold more cytotoxic than tallimustine against L1210 cell, but had similarly DNA selectivity.[81] The number of pyrroleamide units also affected the pattern of DNA alkylation. A dibromo pyrazole analoge (PNU 157977, **49**) showed good potency and also superior to tellimustine against both L1210 murine leukaemia and M5076 solid tumors.[82] Compound **50** with the mustard attached directly to one of the pyrrole units were less sequence specific. Moreover, the other compound **51**, **52** and **53** have also synthesized for their anti-tumor activity. Compound **51** did not produce detectable guanine N7 alkylation, but alkylation specificity nearly identical to that found for the analogues polypyrrole compound, reacting at the same 5'-TTTTGPu sequence. The other compound with the mustard attached by a more flexible ether chain (e.g. **52**) showed extensive guanine N7 alkylation. Another analogue (MEN 10710, **53**) was 10 to 100 fold more cytotoxic then tallimustine against A2780/DDP (cisplatin-resistance ovarian carcinoma) xenograft.[83]

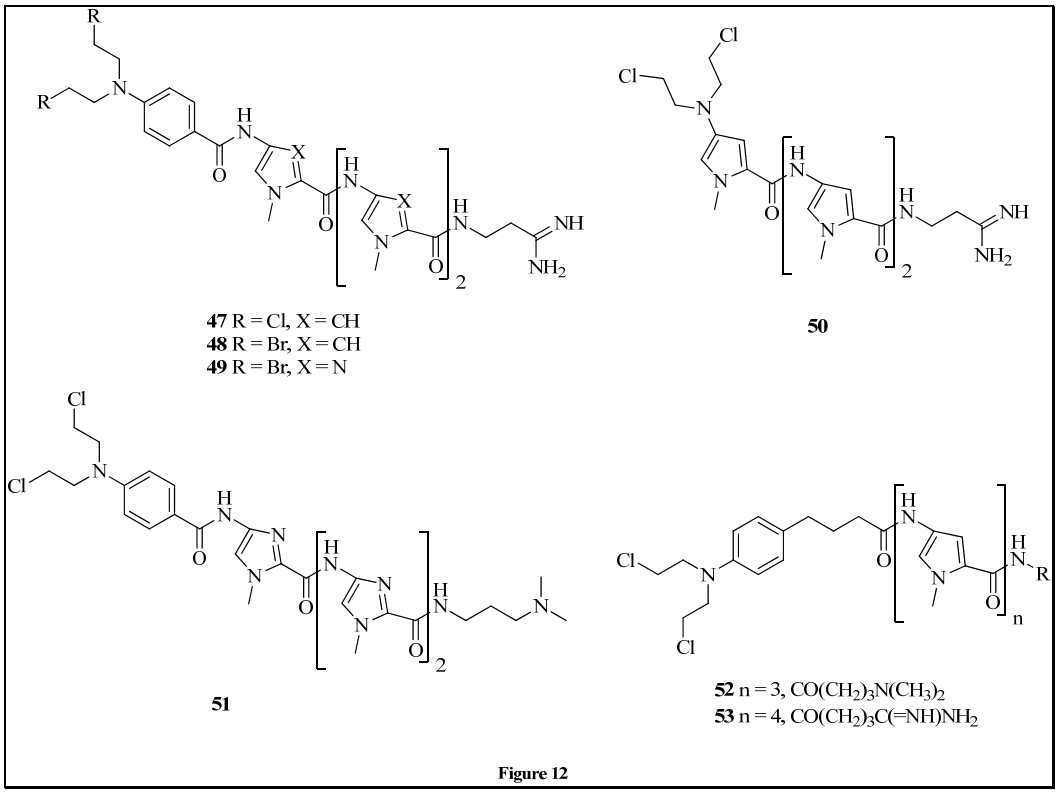
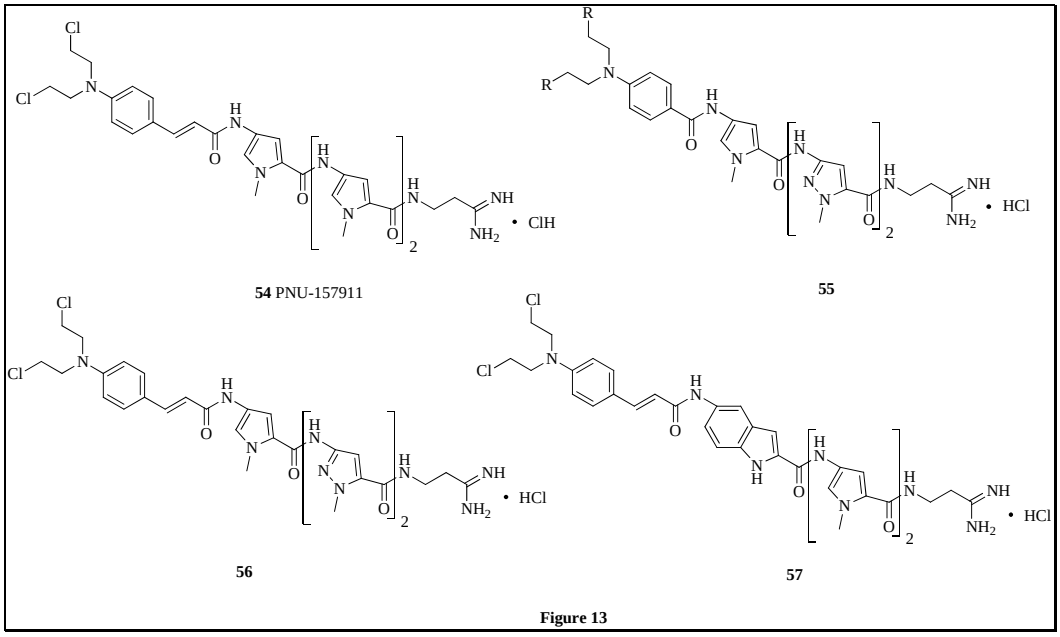


Figure 12

However, tallimustine has represented an important pharmacophore for the designing new cytotoxic minor groove binders derived from distamycin. Among these, a

cinnamoyl nitrogen mustard derivatives of distamycin A (PNU- 157911, **54**)[84] a vinylogue of tallimustine, shows very good antileukemic activity, significantly superior to that of tallimustine (IC_{50} = 7.2 ng/mL compared with 50.3 ng/mL for tallimustine against L1210 leukemia). Compound **54** appears significantly more cytotoxic than tallimustine in accordance with its increased chemical reactivity due to the long-range nitrogen-carbonyl conjugation via the vinylic double bond.[84] Recently, baraldi et. al. have been synthesized a series of benzoyl and cinnamoyl mustard derivatives tallimustine modified at the amidino moiety for anti-tumor evaluation.[85, 86] It revealed that, these compounds has a good cytotoxicity and also interact with DNA with sequences selectivity for certain AT-rich sequences. Compound having a cinnamoyl mustard moiety (**56**) 22 fold more potent then (**55**). Benzohetrocyclic analogues of the tallimustine having cinnamoyl *N*-mustard (**57**) does not found any improvement in terms of cytotoxicity activity and DNA-binding capability.



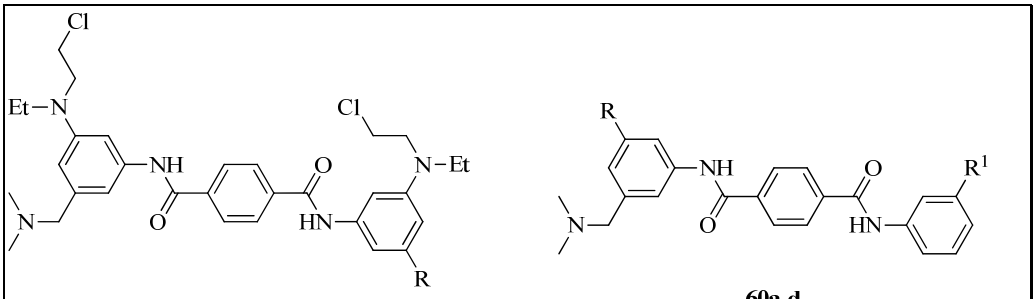
54 PNU-157911 **55**

56 **57**

Figure 13

Atwell et. al. have been synthesized a series of polybenzamide DNA minor groove binding ligands bearing either one or two monofunctional mustards for DNA directed nitrogen mustard.[87] Polybenzamide are also known to be DNA minor groove binding moieties. The antitumor evaluation and DNA interaction study showed that these agents possessed significant cytotoxicity against murine p388 leukemia cells in

culture with high degree of DNA interstrand cross-linking ability. Analogues with two alkylating functions (e.g. compounds **58** and **59**) are the most cytotoxic, with **58** being 1000-fold more potent than the clinicalmustard chlorambucil against P388 leukemia in culture, as well as being more potent in *vivo*. In contrast, the other three monofunctional compounds (**60b-d**) are more than 10-fold less cytotoxic no different than the corresponding non-alkylating analogue **60a**. These results support the concept that DNA-directed nitrogen mustard alkylating agents by attachment to DNA-affinic carriers can greatly enhance cytotoxicity due to alkylation.



58, R = $CH_2N(Me)_2$
59, R = H

60a-d

a: R = H, $R^1 = CH_2N(Me)_2$
b: R = $N(Et)CH_2CH_2Cl$, $R^1 = CH_2N(Me)_2$
c: R = $N(Et)CH_2CH_2Cl$, R^1 = H
d: R = H, $R^1 = N(Et)CH_2CH_2Cl$

Figure 14

1.4 Rational Drug Design

Previously, Su et. al. have synthesized a series of 9-anilinoacridine-alkyl *N*-mustard conjugates by linking various lengths of linkers, such as methylene (CH_2) or alkoxy [$O(CH_2)n$] to the aniline, and/or acridine ring(s) (Figure 15).[88-91] The results showed that all compounds exhibited potent in vitro cytotoxicity against human lymphoblastic leukemia cells (CCRF-CEM) in culture. These agents were about >100-fold more cytotoxic than the parent 3-(9-acridinylamino)-5-hydroxymethylaniline AHMA (**61**)[92], which is a potent DNA-intercalating agent and Topoisomerase II inhibitor previously developed in Su's laboratory. Studies on the structure-activity relationships of these N-mustards indicated that the antitumor activity was slightly affected by the length of the spacer and the location of the *N*-mustard pharmacophore. Of these conjugates, BO-0742 (**64**, Fig. 1) exhibited potent antitumor activity against various human tumor xenografts both *in vitro* and *in vivo*. Although complete tumor

remission (CR) was achieved against human breast MX-1 xenograft or significant suppression human T-cell acute lymphoblastic leukemia CCRF-CEM, colon carcinoma HCT-116, and ovarian adenocarcinoma SK-OV-3 tumor xenografts in animal models by BO-0742, this agent has a narrow therapeutic window and low bioavailability (chemical instability with a short half-life) in mice. The instability of BO-0742 may be due to the inductive effect of the alkoxy linker, increasing the reactivity of the *N*-mustard moiety.

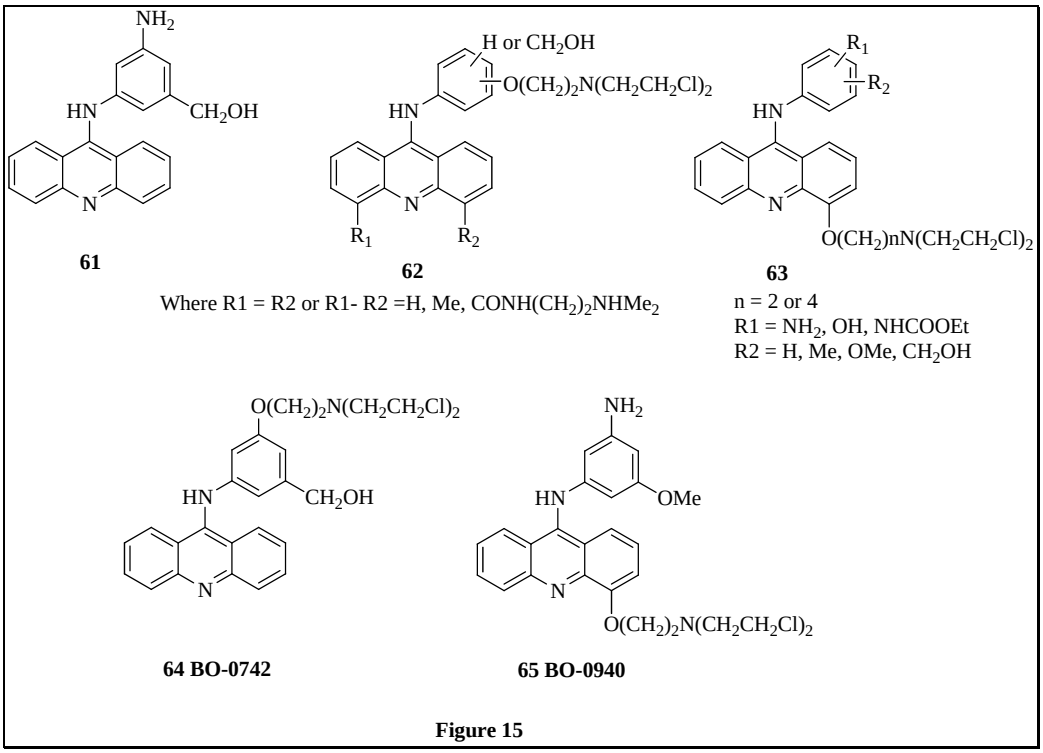


Figure 15

To improve the poor bioavailability of BO-0742, kapuriya et. al. have synthesized a series of phenyl *N*-mustard-9-anilinoacridine conjugates linked via urea, carbamate or carbonate linkers for antitumor studies (Figure 15).[93, 94] These conjugates possessed significant cytotoxicity with IC_{50} in submicro molar range in inhibiting human lymphoblastic leukemia (CCRF-CEM), breast carcinoma (MX-1), colon carcinoma (HCT-116) and human non-small cell lung cancer (H-1299) cell growth in vitro. Of these derivatives, **68** (BO-1051) and **69** (BO-1062) possessed potent therapeutic efficacy against human breast MX-1, human colon HCT-116 and human glioma xenografts in animal model. These agents are capable of inducing DNA interstrand cross-linking and found to be more stable than BO-0742 in i.v. injection vehicle and in rat plasma. For MX-1 xenograft, total tumor remission was achieved with only one cycle of treatment and no tumor relapse was found over 129 days in mice treated with

compound **69** (BO-1062). The urea, carbamate, and or carboxamide linkers were previously applied in antibody-directed enzyme prodrug therapy (ADEPT) and melenocyte directed enzyme prodrug therapy (MDEPT) of *N*-mustard derivatives. It indicates that these spacers are able to stabilize the chemically reactive *N*-mustard moiety.

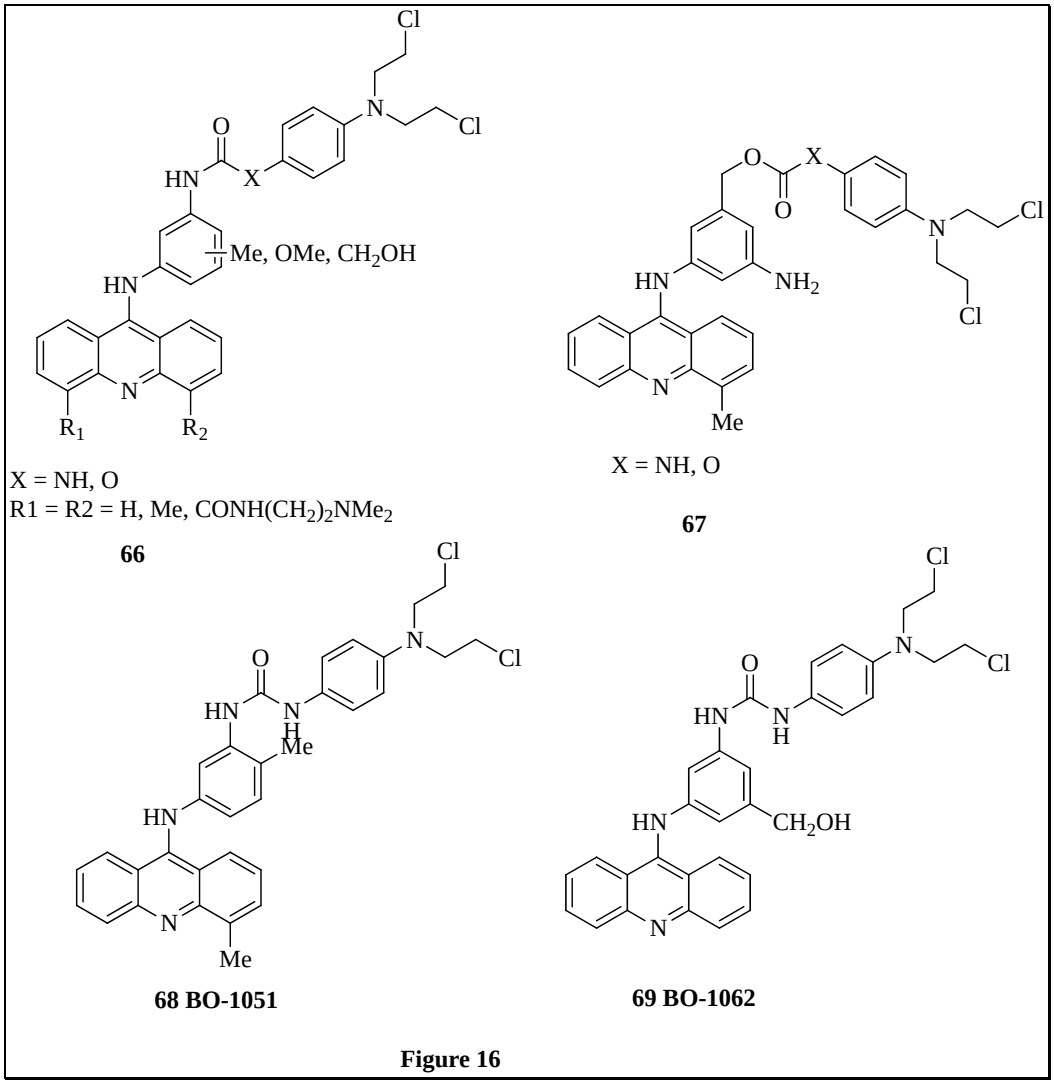


Figure 16

Based on these findings, Kakadiya et. al. have utilized quinolines as carriers to prepare a series of *N*-mustard-quinoline conjugates having a urea or hydrazinecarboxamide linker.[95] Similarly, these conjugates possess potent antitumor activity against a variety of human tumor xenografts. Both linkers are also able to lower the reactivity of the *N*-mustard moiety, resulting in a longer half-life in rat plasma. Of these conjugates, compound **70, 71** and **72, 73** having urea and hydrazincarboxamide linker, respectively, are able to achieve complete tumor

remission against breast carcinoma MX-1 xenograft in animal model with low toxicity. The linkers in these derivatives are attached to the C-4 position of the 4-aminoquinolines, demonstrating that quinolines are also valuable carriers for building DNA-directed alkylating agents.

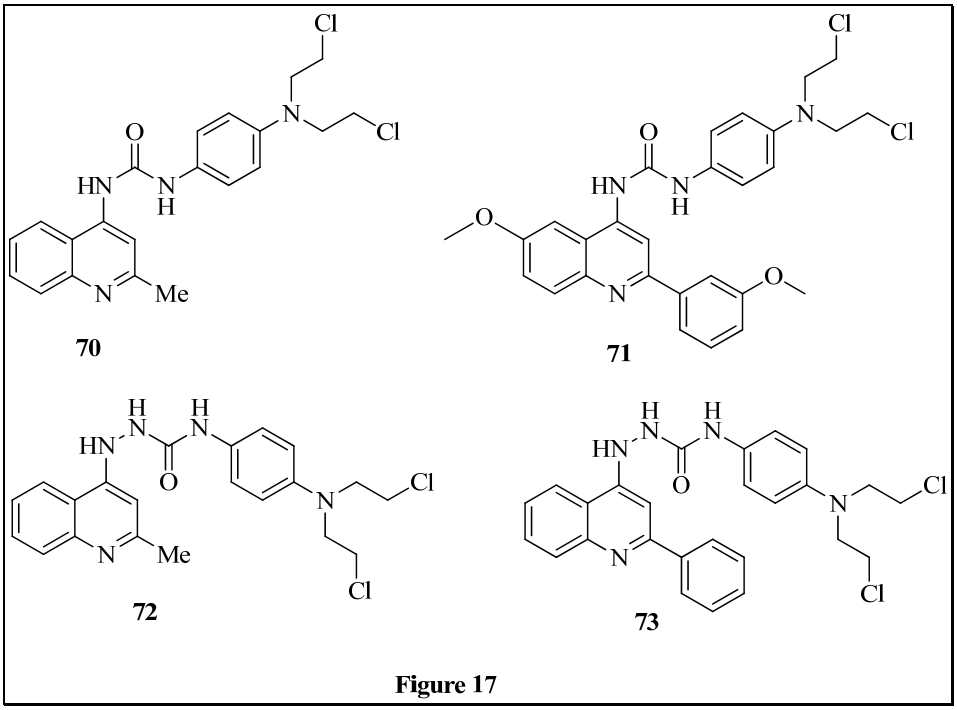


Figure 17

These studies clearly suggested that the selection of *N*-mustard (alkyl or phenyl *N*-mustards), DNA-affinic molecules and linker are important since they may affect the antitumor activity, DNA-drug interactions and stability of these DNA-directed alkylating agents. In general, alkyl *N*-mustards are very reactive and unstable due to the inductive effect of alkyl function leading to rapid generation of the reactive aziridium cation intermediate, which readily interacts with DNA forming interstrand cross-inking. While, the reactivity of phenyl *N*-mustards is relatively weaker than alkyl *N*-mustards due to the electron-withdrawing property of the phenyl ring.

In order to discover new chemically stable DNA-directed alkylating agents, we therefore connected the phenyl *N*-mustard pharmacophore to quinazolines and quinolines moiety using a urea and carbamate as a linker. The newly synthesized DNA-directed alkylating agents are:

1) Phenyl *N*-mustard-4-aminoquinazoline conjugate having a urea linker (**74**, Figure 18)

2) Phenyl *N*-mustard-6-aminoquinazoline conjugates having a urea linker (**75**, Figure 18)

3) Phenyl *N*-mustard-6-hydroxyquinoline conjugates having a carbamate linker (**76**, Figure 18)

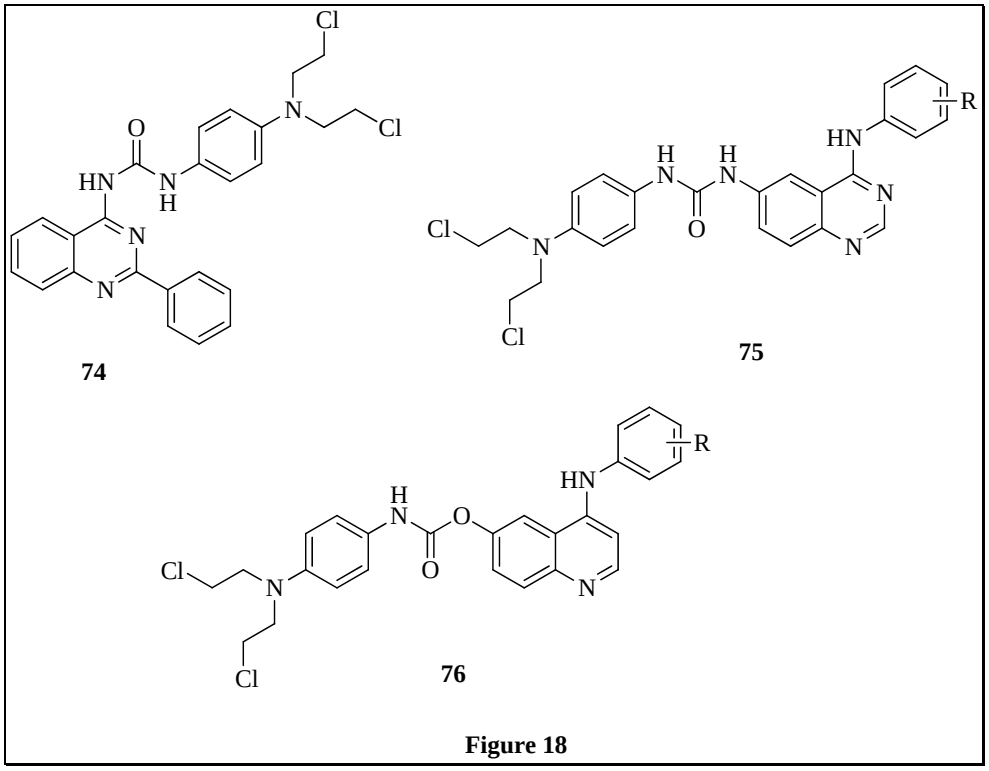


Figure 18

These studies will give direction to understand whether these conjugates have improved water-solubility and cytotoxicity. It will also give a clue to realize whether the urea or carbamate spacers are able to reduce the reactivity of the reactive phenyl *N*-mustard pharmacophore.

All the newly synthesized derivatives in this work were subjected to antitumor evaluation against a variety of human tumor cell growth in vitro, therapeutic efficacy in vivo, and their capability of DNA interstrand cross-linking.

Chapter -2

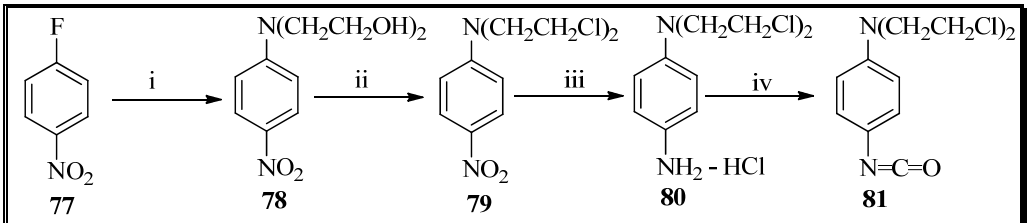
Synthesis of some novel phenyl *N*-mustard quiazoline Conjugates having a urea linker

2.1 Chemistry

The synthesis of newly designed N-mustards-quinazoline conjugates having a urea linker were achieved by reacting of the known aniline N-mustard isocynate (**81**) with the corresponding 6-amino-4-anilinoquinazolines (**87a-q**) as shown in scheme 1. The aniline N-mustard isocynate (**81**) was prepared from the known *N*,*N*-bis(2-chloroethyl)benzene-1,4-diamine dihydrochloride (**80**), which was prepared according to multi-step literature procedure[96] with modification (Scheme 1). The commercially available 4-fluoronitrobenzene (**77**) was reacted with diethanolamine under refluxing to give 4-[*N*,*N*-bis(2-hydroxyethyl)amino]nitrobenzene (**78**), which was then converted to 4-[*N*,*N*-bis(2-chloroethyl)amino]nitrobenzene (**79**) by treating with thionyl chloride. Catalytic hydrogenation (10% Pd/C, H_2) of compound **79** in ethyl acetate afforded *N*,*N*-bis(2-chloroethyl)benzene-1,4-diamine (**80**), which was immediately treated with 2.5M HCl in ethyl acetate to yield aniline *N*-mustard hydrochloride salt (**80**). The hydrochloride salt **80** was then converted to isocyanate **81**[97] by treating with triphosgene in chloroform at –5 ^{o}C. The key starting materials, 6-amino-4-anilinoquinazolines (**86a-q**), were prepared by following the literature methods.[98] Briefly, the commercially available 5-nitroanthranilonitrile **82** was treated with dimethylformamide dimethylacetal (DMF-DMA) in acetic acid to give (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide (**83**), which was then reacted with substituted anilines (**84a-q**) in acetic acid to afford 6-nitroquinazoline derivatives (**85a-q**). The nitro function in **85a-q** was converted into the corresponding 6-aminoquinazoline derivatives (**86a-q**) by treating with Fe/acetic acid. Reaction of **86a-q** with the freshly prepared **81** in the presence of triethylamine gave the desired *N*-mustard-6-aminoquinazoline conjugates (**87a-q**) bearing a urea linker.

2.2 Reaction Scheme

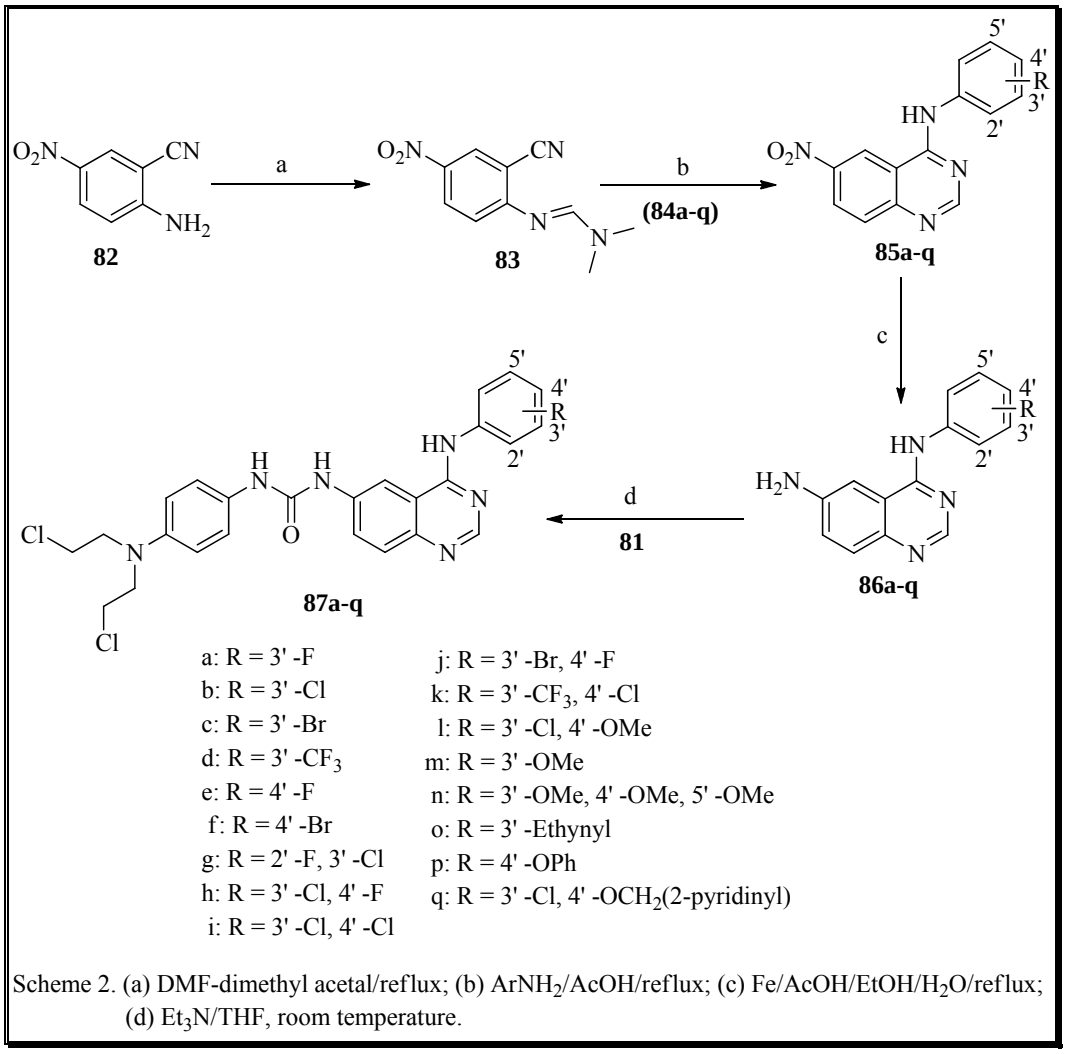
Scheme 1. Synthetic route for the aniline *N*-mustard isocynate



Reaction conditions: (i) $NH(CH_2CH_2OH)_2$, 120 °C; (ii) $SOCl_2$, pyridine, reflux; (iii) 10% Pd/C, H_2, 35 psi, 2 h, 2.5 M HCl in EA; (iv) triphosgene/Et_3N, -10 °C, 30 min.

Scheme 1

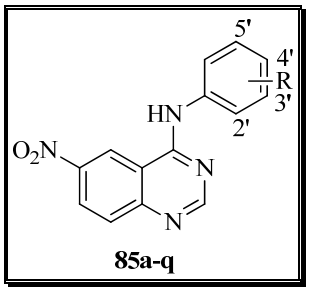
Scheme 2. Synthetic route for the *N*-mustard-6-aminoquinazoline conjugates bearing a urea linker



Scheme 2. (a) DMF-dimethyl acetal/reflux; (b) $ArNH_2$/AcOH/reflux; (c) Fe/AcOH/EtOH/H_2O/reflux; (d) Et_3N/THF, room temperature.

2.3 Physical data

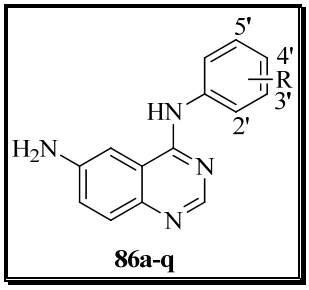
Table 1. Analytical data and yields of Substituted 6-Nitro-4-anilinoquinazolines (85a-q)



85a-q

Sr. No.	Substitute R				MF	MW	Yield %	MP °C
	2'	3'	4'	5'				
85a	H	F	H	H	$C_{14}H_9FN_4O_2$	284.25	83	255-256
85b	H	Cl	H	H	$C_{14}H_9ClN_4O_2$	300.70	90	278-281
85c	H	Br	H	H	$C_{14}H_9BrN_4O_2$	345.15	91	267-270
85d	H	CF_3	H	H	$C_{15}H_9F_3N_4O_2$	334.25	85	209-211
85e	H	H	F	H	$C_{14}H_9FN_4O_2$	284.25	96	257-258
85f	H	H	Br	H	$C_{14}H_9BrN_4O_2$	345.15	84	279-280
85g	F	Cl	H	H	$C_{14}H_8ClFN_4O_2$	318.69	75	224-226
85h	H	Cl	F	H	$C_{14}H_8ClFN_4O_2$	318.69	91	280-281
85i	H	Cl	Cl	H	$C_{14}H_8Cl_2N_4O_2$	335.14	87	297-298
85j	H	Br	F	H	$C_{14}H_8BrFN_4O_2$	363.14	97	257-258
85k	H	CF_3	Cl	H	$C_{15}H_8ClF_3N_4O_2$	368.70	90	221-222
85l	H	Cl	OMe	H	$C_{15}H_{11}ClN_4O_3$	330.73	90	290-291
85m	H	OMe	H	H	$C_{15}H_{12}N_4O_3$	296.28	89	241-242
85n	H	OMe	OMe	OMe	$C_{17}H_{16}N_4O_5$	356.33	68	274-275
85o	H	3-ethynyl	H	H	$C_{16}H_{10}N_4O_2$	290.28	95	271-272
85p	H	H	OPh	H	$C_{20}H_{14}N_4O_3$	358.35	73	296-298
85q	H	Cl	OCH_2(2-pyridinyl)	H	$C_{20}H_{14}ClN_5O_3$	407.81	89	241-242

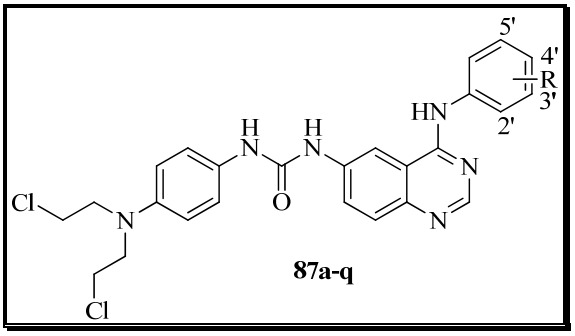
Table 2. Analytical data and yields of Substituted 6-amino-4-anilinoquinazolines (86a-q)



86a-q

Sr. No.	Substitute R				MF	MW	Yield %	MP °C
	2'	3'	4'	5'				
86a	H	F	H	H	$C_{14}H_{11}FN_4$	254.26	76	188-189
86b	H	Cl	H	H	$C_{14}H_{11}ClN_4$	270.72	78	175-176
86c	H	Br	H	H	$C_{14}H_{11}BrN_4$	315.17	78	204-206
86d	H	CF_3	H	H	$C_{15}H_{11}F_3N_4$	304.27	63	174-175
86e	H	H	F	H	$C_{14}H_{11}FN_4$	254.26	97	185-186
86f	H	H	Br	H	$C_{14}H_{11}BrN_4$	315.17	88	210-211
86g	F	Cl	H	H	$C_{14}H_{10}ClFN_4$	288.71	67	257-258
86h	H	Cl	F	H	$C_{14}H_{10}ClFN_4$	288.71	83	255-256
86i	H	Cl	Cl	H	$C_{14}H_{10}Cl_2N_4$	305.16	86	243-244
86j	H	Br	F	H	$C_{14}H_{10}BrFN_4$	333.16	63	225-226
86k	H	CF_3	Cl	H	$C_{15}H_{10}ClF_3N_4$	338.71	56	265-266
86l	H	Cl	OMe	H	$C_{15}H_{13}ClN_4O$	300.74	69	235-237
86m	H	OMe	H	H	$C_{15}H_{14}N_4O$	266.30	76	182-183
86n	H	OMe	OMe	OMe	$C_{17}H_{18}N_4O_3$	326.35	65	220-221
86o	H	3-ethynyl	H	H	$C_{16}H_{12}N_4$	260.29	63	110-111
86p	H	H	OPh	H	$C_{20}H_{16}N_4O$	328.37	64	89-90
86q	H	Cl	OCH_2(2-pyridinyl)	H	$C_{20}H_{16}ClN_5O$	377.83	40	238-239

Table 3. Analytical data and yields of new *N*-mustard-quinazoline conjugates (87a-q)



Sr. No.	Substitute R				MF	MW	Yield %	MP °C
	2'	3'	4'	5'				
87a	H	F	H	H	$C_{25}H_{23}Cl_2FN_6O$	513.39	50	190-191 (d)*
87b	H	Cl	H	H	$C_{25}H_{23}Cl_3N_6O$	529.84	65	172-173 (d)
87c	H	Br	H	H	$C_{25}H_{23}BrCl_2N_6O$	574.30	57	165-166 (d)
87d	H	CF_3	H	H	$C_{26}H_{23}Cl_2F_3N_6O$	563.40	51	210-211 (d)
87e	H	H	F	H	$C_{25}H_{23}Cl_2FN_6O$	513.39	54	223-224 (d)
87f	H	H	Br	H	$C_{25}H_{23}BrCl_2N_6O$	574.30	36	220-221 (d)
87g	F	Cl	H	H	$C_{25}H_{22}Cl_3FN_6O$	547.84	69	210-211 (d)
87h	H	Cl	F	H	$C_{25}H_{22}Cl_3FN_6O$	547.84	64	200-201 (d)
87i	H	Cl	Cl	H	$C_{25}H_{22}Cl_4N_6O$	564.29	71	225-226 (d)
87j	H	Br	F	H	$C_{25}H_{22}BrCl_2FN_6O$	592.29	17	175-176 (d)
87k	H	CF_3	Cl	H	$C_{26}H_{22}Cl_3F_3N_6O$	597.85	52	220-221 (d)
87l	H	Cl	OMe	H	$C_{26}H_{25}Cl_3N_6O_2$	559.87	69	168-169 (d)
87m	H	OMe	H	H	$C_{26}H_{26}Cl_2N_6O_2$	525.43	65	159-160 (d)
87n	H	OMe	OMe	OMe	$C_{28}H_{30}Cl_2N_6O_4$	585.48	66	154-155 (d)
87o	H	3-ethynyl	H	H	$C_{27}H_{24}Cl_2N_6O$	519.43	52	164-165 (d)
87p	H	H	OPh	H	$C_{31}H_{28}Cl_2N_6O_2$	587.50	33	143-144 (d)
87q	H	Cl	OCH_2(2-pyridinyl)	H	$C_{31}H_{28}Cl_3N_7O_2$	636.96	56	156-157 (d)

*****decomposition temperature

2.4 Experimental Section

General methods and materials

Compound solvents and reagents were reagent grade and used without purification unless otherwise noted. The melting points were recorded on a Fargo melting point apparatus and are uncorrected. Column chromatography was carried out on silica gel G60 (70-230mesh, ASTM; Merck and 230-400 mesh, silicycle inc.). Reaction progress was monitored using analytical thin-layer chromatography (TLC) on 0.25mmMerck F-254 silica gel glass plates. Visualization was achieved by UV light (254 nm). ^{1}H NMR spectra were recorded with a Bruker AVANCE 600 DRX and 400 MHz spectrometer; Chemical shifts are reported in parts per million (δ) using tetramethylsilane as the internal standard with coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; brs, broad singlet. Elemental analyses were performed on a Heraeus CHN-O Rapid analyzer. 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 (5μL) mobile phase, MeCN/THF (70:30 v/v); flow rate, 1 mL/min; injected sample 10μL, column temp, 27°C; wavelength, 254nm. The purity of all compounds was > 95 % based on analytical HPLC.

Synthesis of Aniline Nitrogen mustard

2-[(2-Hydroxyethyl)-(4-nitrophenyl)amino]ethanol (78).[77] A mixture of *p*-flouro nitrobenzene (**77**, 28.0 g, 198 mmol) and diethanolamine (30.0 g, 285 mmol) was heated at 120 ^{o}C for 2 h. The reaction mixture was cooled to 60 ^{o}C and an aqueous solution of 0.6 % NaOH (900 mL) was added slowly into the mixture. The separated yellow precipitate was collected by filtration, washed well with water, and dried to give **78**, yield: 42.6 g (95%); mp 104–105 ^{o}C (lit.[77] 103–104 ^{o}C); ^{1}H NMR ($CHCl_3$-d_6) δ 3.55–3.62 (8H, m, 4×CH_2), 4.88 (2H, t, J = 4.4 Hz, exchangeable 2×OH), 6.81 (2H, d, J = 9.5 Hz, 2×ArH), 8.01 (2H, d, J = 9.5 Hz, 2×ArH).

Bis(2-chloroethyl)-(4-nitrophenyl)amine (79).[77] To a mixture of 2-[(2-hdroxyethyl)-(4- nitrophenyl)amino]ethanol (**78**, 16.0 g, 71 mmol) in dry dichloromethane (200 mL) containing dry pyridine (10.0 mL) was added dropwise thionylchloride (14.0

mL) at 0 ^{o}C. The reaction mixture was heated with stirring at refluxed for 3 h and then cooled to room temperature. The mixture was diluted with dichloromethane (100 mL), carefully washed with water, 10% $KHSO_4$ solution (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, and evaporated to dryness under reduce pressure to give **79**, yield 16.7 g (89%); mp 91–93 ^{o}C (lit.[77] 92–94 ^{o}C) ^{1}H NMR ($CHCl_3$-d_6) δ 3.81 (4H, t, J = 6.6 Hz, 2×CH_2), 3.89 (4H, t, J = 6.6 Hz, 2×CH_2), 6.93 (2H, d, J = 9.5 Hz, 2×ArH), 8.07 (2H, d, J = 9.5 Hz, 2×ArH).

***N,N*-Bis(2-chloroethyl)benzene-1,4-diamine hydrochloride (80).**[77] A mixture bis(2-chloroethyl)-(4-nitrophenyl)amine (**79**, 10.0 g, 38 mmol) in ethyl acetate (100 mL) and 10 % Pd/C (1.0 g) was sonicated for 5 min. The mixture was then hydrogenated (H_2) at 35 psi for 2 h. and monitored by TLC (SiO_2, ethyl acetate/Hexane: 1:1 v/v). After completion of reaction, the reaction mixture was filtered through a pad of celite and the filtrate was cooled to 0 ^{o}C. A solution of HCl in ethyl acetate (20.0 mL) was slowly added into the filtrate with stirring. The white solid separated was collected by filtration and dried to give **80**, yield: 9.18 g (80%), mp 212–214 ^{o}C (lit.[77] 213–215 ^{o}C); ^{1}H NMR(DMSO-d_6) δ 3.92 (8H, s, 4×CH_2), 6.83 (2H, d, J = 8.8 Hz, 2×ArH), 7.24 (2H, d, J = 8.8 Hz, 2×ArH), 10.2 (2H, brs, exchangeable, NH_2).

4-[*N,N*-Bis(2-chloroethyl)amino]phenylisocyanate (81). To a suspension of *N,N*-bis(2-chloroethyl)benzene-1,4-diamine hydrochloride (**80,** 1.683 g, 5.4 mmol) in dry chloroform (30 mL) was added triethylamine (2.5 mL) at room temprature. The clear solution obtained was then added dropwise into a solution of triphosgene (0.623 g, 2.1 mmol) in dry chloroform (10 mL) at -50°C. The reaction mixture was allowed to stand at room temperature. After being stirred for 30 min, the reaction mixture was evaporated to dryness under reduced pressure. The solid residue was triturated with dry THF (100 mL), filtered, and washed with small amount of THF. The combined filtrate and washings was evaporated to dryness to give the crude isocyanate **81** which was used directly for the next reaction without further purification.

(*E*)-*N'*-(2-cyano-4-nitrophenyl)-*N,N*-dimethylformimidamide (83)

5-Nitroanthranilonitrile **82** (20.0 g, 122.5 mmol) was suspended in dimethylformamide dimethylacetal (43 mL, 360.0 mmol). The mixture was heated up to reflux temperature for 1.5 h. The resulting mixture was cooled to room temperature

and refrigerated overnight. The yellow precipitated was filtered, washed with ethyl ether to give **83,** 25.0 g (96 %); mp 153–154 °C (lit.[17] 153–155 °C); ^{1}H NMR (DMSO-d_6) δ 3.09 (3H, s, Me), 3.17 (3H, s, Me), 7.36–7.39 (1H, m, ArH), 8.25–8.28 (2H, m, 2 × ArH), 8.47–8.48 (1H, m, ArH). Anal. ($C_7H_5N_3O_2$): C, H, N

Synthesis of 6-nitro-4-anilinoquinazoline (85a-q)

***N*-(3-Fluorophenyl)-6-nitroquinazolin-4-amine (85a).** To a solution of (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (6.0 g, 2.7 mmol) and acetic acid (45 mL) was added 3-Fluoroaniline (**84a**, 3.3 g, 3.0 mmol) at room temperature. The reaction mixture was heated up to reflux temperature for 1 hour. After completion of the reaction, the resulting mixture was cooled to room temperature. The solid separated was filtered and washed with ether to give **85a,** 6.5 g (83 %); mp 255–256 °C; ^{1}H NMR (DMSO-d_6) δ 6.98–7.03 (1H, m, ArH), 7.43–7.48 (1H, m, ArH), 7.68 (1H, d, *J* = 9.2 Hz, ArH), 7.89–7.95 (2H, m, 2 × ArH), 8.55 (1H, dd, *J* = 2.2 Hz, *J* = 9.2 Hz, ArH), 8.77 (1H, s, ArH), 9.65 (1H, d, *J* = 2.2 Hz, ArH), 10.48 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9FN_4O_2$): C, H, N.

By following the same procedure as that for **85a** the following compounds were synthesized.

***N*-(3-Chlorophenyl)-6-nitroquinazolin-4-amine (85b).** Compound **85b** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (5.0 g, 2.2 mmol) and 3-chloroaniline (**84b**, 3.2 g, 2.5 mmol) in acetic acid (40 mL): Yield 6.2 g (90 %); mp 285–286 °C; ^{1}H NMR (DMSO-d_6) δ 7.22–7.47 (1H, m, ArH), 7.43–7.47 (1H, m, ArH), 7.83–7.85 (1H, m, ArH), 7.95 (1H, d, *J* = 9.2 Hz, ArH), 8.07–8.08 (1H, m, ArH), 8.56 (1H, dd, *J* = 2.2 Hz, *J* = 9.2 Hz, ArH), 8.78 (1H, s, ArH), 9.65 (1H, d, *J* = 2.2 Hz, ArH), 10.48 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9ClN_4O_2$): C, H, N.

***N*-(3-Bromophenyl)-6-nitroquinazolin-4-amine (85c).** Compound **85c** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (5.0 g, 2.2 mmol) and 3-bromoaniline (**84c**, 4.3 g, 2.5 mmol) in acetic acid (40 mL): Yield 7.2 g (91 %); mp 282–283 °C; ^{1}H NMR (DMSO-d_6) δ 7.35–7.41 (2H, m, 2 × ArH), 7.90–7.92 (1H, m, ArH), 7.94 (1H, d, *J* = 9.1 Hz, ArH), 8.18–8.19 (1H, m, ArH), 8.56

(1H, dd, *J* = 2.2 Hz, *J* = 9.1 Hz, ArH), 8.77 (1H, s, ArH), 9.64 (1H, d, *J* = 2.2 Hz, ArH), 10.46 (1H, s, exchangeable, NH). Anal. Calcd. for ($C_{14}H_9BrN_4O_2$): C, H, N.

***N*-(3-(Trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine (85d).** Compound **85d** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (5.0 g, 2.2 mmol) and 3-(trifluoromethyl)aniline (**84d**, 4.0 g, 2.5 mmol) in acetic acid (40 mL): Yield 6.5 g (85 %); mp 210–211 °C; ^{1}H NMR (DMSO-d_6) δ 7.52–7.54 (1H, m, ArH), 7.65–7.69 (1H, m, ArH), 7.97 (1H, d, *J* = 9.1 Hz, ArH), 8.25–8.29 (2H, m, 2 × ArH), 8.59 (1H, dd, *J* = 2.2 Hz, *J* = 9.1 Hz, ArH), 8.79 (1H, s, ArH), 9.67 (1H, d, *J* = 2.2 Hz, ArH), 10.61 (1H, s, exchangeable, NH). Anal. ($C_{15}H_9F_3N_4O_2$): C, H, N.

***N*-(4-Fluorophenyl)-6-nitroquinazolin-4-amine (85e).** Compound **85e** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (4.0 g, 1.5 mmol) and 4-fluoroaniline (**84e**, 2.2 g, 2.0 mmol) in acetic acid (30 mL): Yield 5.0 g (96 %); mp 257–258 °C; ^{1}H NMR (DMSO-d_6) δ 7.28–7.32 (2H, m, 2 × ArH), 7.84–7.88 (2H, m, 2 × ArH), 7.96 (1H, d, *J* = 9.2 Hz, ArH), 8.58 (1H, dd, *J* = 2.5 Hz, *J* = 9.2 Hz, ArH), 8.72 (1H, s, ArH), 9.66 (1H, d, *J* = 2.5 Hz, ArH), 10.51 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9FN_4O_2$): C, H, N.

***N*-(4-Bromophenyl)-6-nitroquinazolin-4-amine (85f).** Compound **85f** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (3.0 g, 1.3 mmol) and 4-bromoaniline (**84f**, 2.6 g, 1.5 mmol) in acetic acid (25 ml): Yield 4.0 g (84 %); mp 279–280 °C; ^{1}H NMR (DMSO-d_6) δ 7.62–7.65 (2H, m, 2 × ArH), 7.86–7.89 (2H, m, 2 × ArH), 7.97 (1H, d, *J* = 9.2 Hz, ArH), 8.57–8.60 (1H, dd, *J* = 2.4 Hz, *J* = 9.2 Hz, ArH), 8.76 (1H, s, ArH), 9.67 (1H, d, *J* = 2.4 Hz, ArH), 10.51 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9BrN_4O_2$): C, H, N.

***N*-(2-Fluoro-3-chlorophenyl)-6-nitroquinazolin-4-amine (85g).** Compound **85g** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (5.0 g, 2.2 mmol) and 2-fluoro-3-chloroaniline (**84g**, 3.6 g, 2.5 mmol) in acetic acid (35 mL): Yield 5.5 g (75 %); mp 224–226 °C; ^{1}H NMR (DMSO-d_6) δ 7.30–7.34 (1H, m, ArH), 7.52–7.56 (2H, m, 2 × ArH), 7.74–8.01 (1H, m, ArH), 8.57–8.65 (2H, m, 2 × ArH), 9.55 (1H, s, ArH), 10.73 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8ClFN_4O_2$): C, H, N.

***N*-(3-Chloro-4-fluorophenyl)-6-nitroquinazolin-4-amine (85h).** Compound **85h** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (3.0 g, 1.3 mmol) and 3-chloro-4-fluoroaniline (**84h**, 2.2 g, 1.5 mmol) in acetic acid (20 mL): Yield 4.0 g (91 %); mp 280–281 °C; ^{1}H NMR (DMSO-d_6) δ 7.45–7.50 (1H, m, ArH), 7.81–7.83 (1H, m, ArH), 7.94 (1H, d, J = 9.2 Hz, ArH), 8.14–8.17 (1H, m, ArH), 8.55 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.74 (1H, s, ArH), 9.60 (1H, d, J = 2.2 Hz, ArH), 10.50 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8ClFN_4O_2$): C, H, N.

***N*-(3,4-Dichlorophenyl)-6-nitroquinazolin-4-amine (85i).** Compound **85i** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (6.0 g, 2.7 mmol) and 3,4-dichloroaniline (**84i**, 4.9 g, 3.0 mmol) in acetic acid (45 mL): Yield 8.0 g (86 %); mp 297–298 °C; ^{1}H NMR (DMSO-d_6) δ 7.65–7.67 (1H, m, ArH), 7.89–7.96 (2H, m, 2 × ArH), 8.27–8.28 (1H, m, ArH), 8.54–8.57 (1H, m, ArH), 8.79 (1H, s, ArH), 9.61–9.62 (1H, m, ArH), 10.49 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8Cl_2N_4O_2$): C, H, N.

***N*-(3-Bromo-4-fluorophenyl)-6-nitroquinazolin-4-amine (85j).** Compound **85j** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (4.0 g, 1.5 mmol) and 3-bromo-4-fluoroaniline (**84j**, 4.0 g, 2.0 mmol) in acetic acid (30 mL): Yield 6.0 g (90 %); mp 260–261 °C; ^{1}H NMR (DMSO-d_6) δ 7.43–7.47 (1H, m, ArH), 7.88–7.89 (1H, m, ArH), 7.95 (1H, d, J = 9.2 Hz, ArH), 8.25–8.26 (1H, m, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.75 (1H, s, ArH), 9.61 (1H, d, J = 2.2 Hz, ArH), 10.48 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8BrFN_4O_2$): C, H, N.

***N*-(4-Chloro-3-(trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine(85k).**
Compound **85k** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (3.0 g, 1.3 mmol) and 4-chloro-3-(trifluoromethyl)aniline (**84k**, 2.8 g, 1.4 mmol) in acetic acid (30 mL): Yield 4.5 g (90 %); mp 221–222 °C; ^{1}H NMR (DMSO-d_6) δ 7.77–7.79 (1H, m, ArH), 7.98 (1H, d, J = 9.2 Hz, ArH), 8.32–8.34 (1H, m, ArH), 8.43–8.44 (1H, m, ArH), 8.58 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.81 (1H, s, ArH), 9.63 (1H, s, J = 2.2 Hz, ArH), 10.53 (1H, s, exchangeable, NH). Anal. ($C_{15}H_8ClF_3N_4O_2$): C, H, N.

***N*-(3-Chloro-4-methoxyphenyl)-6-nitroquinazolin-4-amine (85l).** Compound **85l** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83**

(5.0 g, 2.2 mmol) and 3-chloro-4-methoxyaniline (**84l**, 3.9 g, 2.5 mmol) in acetic acid (40 mL): Yield 6.8 g (91 %); mp 290–291 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (3H, s, Me), 7.21–7.23 (1H, m, ArH), 7.75–7.77 (1H, m, ArH), 7.91 (1H, d, J = 9.2 Hz, ArH), 7.99–8.00 (1H, m, ArH), 8.52 (1H, dd, J = 2.4 Hz, J = 9.2 Hz, ArH), 8.70 (1H, s, ArH), 9.60 (1H, d, J = 2.4 Hz, ArH), 10.38 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{11}ClN_4O_3$): C, H, N.

***N*-(3-Methoxyphenyl)-6-nitroquinazolin-4-amine (85m).** Compound **85m** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (5.0 g, 2.2 mmol) and 3-methoxyaniline (**84m**, 3.0 g, 2.5 mmol) in acetic acid (35 mL): Yield 6.0 g (89 %); mp 241–242 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (3H, s, Me), 6.78–6.81 (1H, m, ArH), 7.32–7.37 (1H, m, ArH), 7.49–7.53 (2H, m, 2 × ArH), 7.93 (1H, d, J = 9.2 Hz, ArH), 8.54–8.57 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.74 (1H, s, ArH), 9.66 (1H, d, J = 2.2 Hz, ArH), 10.38 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{12}N_4O_3$): C, H, N.

***N*-(3,4,5-Trimethoxyphenyl)-6-nitroquinazolin-4-amine (85n).** Compound **85n** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (5.0 g, 2.2 mmol) and 3,4,5-trimethoxyaniline (**84n**, 4.6 g, 2.5 mmol) in acetic acid (40 mL): Yield 5.5 g (68 %); mp 274–275 °C; ^{1}H NMR (DMSO-d_6) δ 3.81 (3H, s, Me), 3.69 (6H, s, 2 × Me), 7.27 (2H, s, 2 × ArH), 7.93 (1H, d, J = 9.2 Hz, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.72 (1H, s, ArH), 9.64 (1H, d, J = 2.2 Hz, ArH), 10.32 (1H, s, exchangeable, NH). Anal. ($C_{17}H_{16}N_4O_5$): C, H, N.

***N*-(3-Ethynylphenyl)-6-nitroquinazolin-4-amine (85o).** Compound **85o** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (3.0 g, 1.3 mmol) and 3-ethynylaniline (**84o**, 1.6 g, 1.3 mmol) in acetic acid (30 mL): Yield 3.8 g (95 %); mp 271–272 °C; ^{1}H NMR (DMSO-d_6) δ 4.25 (1H, s, CH), 7.30–7.32 (1H, m, ArH), 7.44–7.48 (1H, m, ArH), 7.92–7.95 (2H, m, 2 × ArH), 8.05–8.06 (1H, m, ArH), 8.54–8.57 (1H, m, ArH), 8.76 (1H, s, ArH), 9.65–9.66 (1H, m, ArH), 10.48 (1H, s, exchangeable, NH). Anal. (C16H10N4O$_2$·0.5): C, H, N.

***N*-(4-Phenoxyphenyl)-6-nitroquinazolin-4-amine (85p).** Compound **85p** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **83** (5.0 g, 2.2 mmol) and 4-phenoxyaniline (**84p**, 4.6 g, 2.5 mmol) in acetic acid (40 mL):

Yield 6.0 g (73 %); mp 296–298 °C; ^{1}H NMR (DMSO-d_6) δ 7.06–7.08 (2H, m, 2 × ArH), 7.11–7.13 (2H, m, 2 × ArH), 7.17–7.19 (1H, m, ArH), 7.42–7.46 (2H, m, 2 × ArH), 7.85–7.87 (2H, m, 2 × ArH), 7.94 (1H, d, *J* = 9.2 Hz, ArH), 8.57 (1H, dd, *J* = 2.4 Hz, *J* = 9.2 Hz, ArH), 8.71 (1H, s, ArH), 9.67 (1H, d, *J* = 2.4 Hz, ArH), 10.55 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{14}N_4O_3$): C, H, N.

***N*-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)-6-nitroquinazolin-4-amine (85q).** Compound **85q** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N, N*-dimethylformimidamide **83** (3.0 g, 1.3 mmol) and 3-chloro-4-(pyridine-2-ylmethoxy)aniline (**84q**, 3.2 g, 1.3 mmol) in acetic acid (30 mL): Yield 5.0 g (89 %); mp 241–242 °C; ^{1}H NMR (DMSO-d_6) δ 5.32 (2H, s, CH_2), 7.30–7.33 (1H, m, ArH), 7.39–7.40 (1H, m, ArH), 7.60–7.62 (1H, m, ArH), 7.74–7.75 (1H, m, ArH), 7.88–7.95 (2H, m, 2 × ArH), 8.04–8.05 (1H, m, ArH), 8.55–8.57 (1H, m, ArH), 8.62–7.63 (1H, m, ArH), 8.73 (1H, s, ArH), 9.62–9.63 (1H, m, ArH), 10.43 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{14}ClN_5O_3 \cdot 2H_2O$): C, H, N.

Synthesis of 6-amino-4-anilinoquinazoline (86a-q)

***N*^{4}-(3-Fluorophenyl)quinazolin-4,6-diamine(86a).** A mixture of *N*-(3-fluorophenyl)-6-nitroquinazolin-4-amine **85a** (6.0 g, 21.1 mmol) and iron (8.13 g, 147.8 mmol) were suspended in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (16.3 mL, 295.4 mmol). The mixture was heated up to reflux temperature for 2 h. After completion of the reaction, the reaction mixture was cooled to room temperature and alkalinized by addition of concentrated ammonia solution (120 mL). The insoluble material was removed by filtration through celite, and the filtrate was evaporated under reduce pressure. The resulting solid was washed with 10% K_2CO_3 solution and finally with water and dried to give **86a**, 4.0 g, (75 %); mp 188–189 °C; ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 6.84–6.88 (1H, m, ArH), 7.25–7.28 (1H, m, ArH), 7.35–7.40 (2H, m, 2 × ArH), 7.54–7.56 (1H, m, ArH), 7.67–7.69 (1H, m, ArH), 7.93–7.96 (1H, m, ArH), 8.39 (1H, s, ArH), 9.47 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}FN_4 \cdot 0.8H_2O$): C, H, N.

By following the same procedure as that for **86a** the following compound were synthesized.

***N*^{4}-(3-Chlorophenyl)quinazolin-4,6-diamine (86b).** Compound **86b** was synthesized from *N*-(3-chlorophenyl)-6-nitroquinazolin-4-amine **85b** (5.0 g, 16.6 mmol) and iron (6.3 g, 116.2 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (13.3 mL, 232.4 mmol): Yield 3.5 g (78 %); mp 175–176 °C; ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 7.09–7.11 (1H, m, ArH), 7.25–7.28 (1H, m, ArH), 7.34–7.39 (2H, m, 2 × ArH), 7.54–7.56 (1H, m, ArH), 7.82–7.85 (1H, m, ArH), 8.12–8.13 (1H, m, ArH), 8.39 (1H, s, ArH), 9.46 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}ClN_4$): C, H, N.

***N*^{4}-(3-Bromophenyl)quinazolin-4,6-diamine (86c).** Compound **86c** was synthesized from *N*-(3-bromophenyl)-6-nitroquinazolin-4-amine **85c** (6.0 g, 17.3 mmol) and iron (6.69 g, 121.7 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (13.9 mL, 243.4 mmol): Yield 4.2 g (78 %); mp 204–206 °C; ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 7.22–7.35 (4H, m, 4 × ArH), 7.54–7.56 (1H, m, ArH), 7.88–7.90 (1H, m, ArH), 8.24–8.25 (1H, m, ArH), 8.38 (1H, s, ArH), 9.44 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}BrN_4$): C, H, N.

***N*^{4}-(3-(Trifluoromethyl)phenyl)quinazolin-4,6-diamine (86d).** Compound **86d** was synthesized from *N*-(3-(trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine **85d** (6.0 g, 17.9 mmol) and iron (6.9 g, 125.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (14.3 mL, 250.0 mmol): Yield 3.4 g (63 %); mp 174–175 °C; -^{1}H NMR (DMSO-d_6) δ 5.66 (2H, s, exchangeable, NH_2), 7.29–7.31 (1H, m, ArH), 7.39–7.42 (2H, m, 2 × ArH), 7.58–7.63 (2H, m, 2 × ArH), 8.24–8.26 (1H, m, ArH), 8.37–8.38 (1H, m, ArH), 8.42 (1H, s, ArH), 9.62 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{11}F_3N_4$): C, H, N.

***N*^{4}-(4-Fluorophenyl)quinazolin-4,6-diamine (86e).** Compound **86e** was synthesized from *N*-(4-fluorophenyl)-6-nitroquinazolin-4-amine **85e** (4.0 g, 14.0 mmol) and iron (5.4 g, 98.5 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (11.2 mL, 196.0 mmol): Yield 3.5 g (97 %); mp 185–186 °C; ^{1}H NMR (DMSO-d_6) δ 5.60 (2H, s, exchangeable, NH_2), 7.30–7.32 (2H, m, 2 × ArH), 7.40–7.56 (3H, m, 3 × ArH), 7.71–7.73 (2H, m, 2 × ArH), 8.30 (1H, s, ArH), 9.29 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}FN_4 \cdot 1.3H_2O$): C, H, N.

***N*^{4}-(4-Bromophenyl)quinazolin-4,6-diamine (86f).** Compound **86f** was synthesized from *N*-(4-bromophenyl)-6-nitroquinazolin-4-amine **85f** (4.0 g, 11.5 mmol) and iron (4.4 g, 80.5 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (5 mL, 162.0 mmol): Yield 3.2 g (88 %); mp 210–211 °C; ^{1}H NMR (DMSO-d_6) δ 5.63 (2H, s, exchangeable, NH_2), 7.27–7.29 (2H, m, 2 × ArH), 7.53–7.56 (3H, m, 3 × ArH), 7.90–7.92 (2H, m, 2 × ArH), 8.38 (1H, s, ArH), 9.45 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}BrN_4 \cdot 0.2H_2O$): C, H, N.

***N*^{4}-(2-Fluoro-3-chlorophenyl)quinazolin-4,6-diamine (86g).** Compound **86g** was synthesized from *N*-(2-fluoro-3-chlorophenyl)-6-nitroquinazolin-4-amine **85g** (5.0 g, 15.7 mmol) and iron (6.0 g, 110.0 mmol) in aqueous ethanol (500 mL, 70% v/v) containing acetic acid (12.5 mL, 219.0 mmol): Yield 3.0 g (67 %); mp 257–258 °C; -^{1}H NMR (DMSO-d_6) δ 5.65 (2H, s, exchangeable, NH_2), 7.25–7.27 (3H, m, 3 × ArH), 7.43–7.44 (1H, m, ArH), 7.54–7.56 (2H, m, 2 × ArH), 8.24 (1H, s, ArH), 9.42 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}ClFN_4$): C, H, N.

***N*^{4}-(3-Chloro-4-fluorophenyl)quinazolin-4,6-diamine (86h).** Compound **86h** was synthesized from *N*-(3-chloro-4-fluorophenyl)-6-nitroquinazolin-4-amine **85h** (2.0 g, 6.2 mmol) and iron (2.4 g, 44.0 mmol) in aqueous ethanol (200 ml, 70%v/v) containing acetic acid (5 mL, 57.9 mmol):Yield 1.4 g (83 %); mp 255–256 °C; ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 7.24–7.27 (1H, m, ArH), 7.34–7.35 (1H, m , ArH), 7.38–7.43 (1H, m, ArH), 7.53–7.54 (1H, m, ArH), 7.83–7.84 (1H, m, ArH), 8.22–8.22 (1H, m, ArH), 8.36 (1H, s, ArH), 9.50 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}ClFN_4$): C, H, N.

***N*^{4}-(3,4,-Dichlorophenyl)quinazolin-4,6-diamine (86i).** Compound **86i** was synthesized from *N*-(3,4,-dichlorophenyl)-6-nitroquinazolin-4-amine **85i** (7.0 g, 20.8 mmol) and iron (8.0 g, 146.2 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (16.6 mL, 291.0 mmol): Yield 5.5 g (86 %); mp 243–244 °C; ^{1}H NMR (DMSO-d_6) δ 5.66 (2H, s, exchangeable, NH_2), 7.27–7.30 (1H, m, ArH), 7.34–7.35 (1H, m, ArH), 7.56–7.61 (2H, m, 2 × ArH), 7.91–7.93 (1H, m, ArH), 8.34–8.35 (1H, m, ArH), 8.42 (1H, s, ArH), 9.55 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}Cl_2N_4$): C, H, N.

***N*^{4}-(3-Bromo-4-fluorophenyl)quinazolin-4,6-diamine (86j).** Compound **86j** was synthesized from *N*-(3-bromo-4-fluorophenyl)-6-nitroquinazolin-4-amine **85j** (6.0 g, 16.5 mmol) and iron (6.36 g, 115.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (13.2 mL, 231.0 mmol): Yield 3.5 g (64 %); mp 225–226 °C; - ^{1}H NMR (DMSO-d_6) δ 5.61 (2H, s, exchangeable, NH_2), 7.25–7.27 (1H, m, ArH), 7.31–7.32 (1H, m, ArH), 7.35–7.40 (1H, m, ArH), 7.53–7.55 (1H, m, ArH), 7.88–7.89 (1H, m, ArH), 8.30–8.31 (1H, m, ArH), 8.36 (1H, s, ArH), 9.45 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}BrFN_4$): C, H, N.

***N*^{4}-(4-Chloro-3-(trifluoromethyl)phenyl)quinazolin-4,6-diamine (86k).** Compound **86k** was synthesized from *N*-(4-chloro-3-(trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine **85k** (4.0 g, 10.8 mmol) and iron (4.2 g, 75.8 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (8.6 mL, 151.0 mmol): Yield 2.0 g (56 %); mp 265–266 °C; ^{1}H NMR (DMSO-d_6) δ 5.67 (2H, s, exchangeable, NH_2), 7.27–7.29 (1H, m, ArH), 7.33–7.34 (1H, m, ArH), 7.56–7.58 (1H, m, ArH), 7.68–7.70 (1H, m, ArH), 8.29–8.31 (1H, m, ArH), 8.40–8.41 (1H, m, ArH), 8.48 (1H, s, ArH), 9.69 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{10}ClF_3N_4$): C, H, N.

***N*^{4}-(3-Chloro-4-methoxyphenyl)quinazolin-4,6-diamine (86l).** Compound **86l** was synthesized from *N*-(3-chloro-4-methoxyphenyl)-6-nitroquinazolin-4-amine **85l** (6.0 g, 18.0 mmol) and iron (6.9 g, 126.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (14.4 mL, 252.0 mmol): Yield 3.7 g (69 %); mp 235–237 °C; - ^{1}H NMR (DMSO-d_6) δ 3.58 (3H, s, Me), 5.57 (2H, s, exchangeable, NH_2), 7.15–7.16 (1H, m, ArH), 7.24–7.26 (1H, m, ArH), 7.33–7.34 (1H, m, ArH), 7.52–7.54 (1H, m, ArH), 7.73–7.54 (1H, m, ArH), 8.02–8.03 (1H, m, ArH), 8.32 (1H, s, ArH), 9.32 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{13}ClN_4O$): C, H, N.

***N*^{4}-(3-Methoxyphenyl)quinazolin-4,6-diamine (86m).** Compound **86m** was synthesized from *N*-(3-methoxyphenyl)-6-nitroquinazolin-4-amine **85m** (5.5 g, 18.5 mmol) and iron (7.2 g, 130.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (14.8 mL, 260.0 mmol): Yield 3.2 g (75 %); mp 182–183 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (3H, s, Me), 5.57 (2H, s, exchangeable, NH_2), 6.63–6.66 (1H, m, ArH), 7.23–7.27 (2H, m, 2 × ArH), 7.36–7.37 (1H, m, ArH), 7.49–7.58 (3H, m, 3 ×

ArH), 8.35 (1H, s, ArH), 9.28 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{14}N_4O \cdot 0.2H_2O$): C, H, N.

***N*^{4}-(3,4,5-Trimethoxyphenyl)quinazolin-4,6-diamine (86n).** Compound **86n** was synthesized from *N*-(3,4,5-trimethoxyphenyl)-6-nitroquinazolin-4-amine **85n** (5.0 g, 14.0 mmol) and iron (5.4 g, 98.3 mmol) in aqueous ethanol (500 mL, 70% v/v) containing acetic acid (11.2 mL, 196.5 mmol): Yield 2.9 g (64 %); mp 220–221 °C; -^{1}H NMR (DMSO-d_6) δ 3.66 (3H, s, Me), 3.79 (6H, s, 2 × Me), 5.54 (2H, s, exchangeable, NH_2), 7.22–7.25 (1H, m, ArH), 7.34–7.35 (3H, m, 3 × ArH), 7.51–7.53 (1H, m, ArH), 8.33 (1H, s, ArH), 9.19 (1H, s, exchangeable, NH). Anal. ($C_{17}H_{18}N_4O_3$): C, H, N.

***N*^{4}-(3-Ethynylphenyl)quinazolin-4,6-diamine(86o).** Compound **86o** was synthesized from *N*-(3-ethynylphenyl)-6-nitroquinazolin-4-amine **85o** (4.5 g, 15.5 mmol) and iron (5.9 g, 108.0 mmol) in aqueous ethanol (450 mL, 70% v/v) containing acetic acid (12.4 mL, 217.0 mmol): Yield 2.5 g (62 %); mp 110–111 °C; ^{1}H NMR (DMSO-d_6) δ 4.17 (1H, s, CH), 5.60 (2H, s, exchangeable, NH_2), 7.16–7.18 (1H, m, ArH), 7.24–7.27 (1H, m, ArH), 7.35–7.39 (2H, m, 2 × ArH), 7.53–7.56 (1H, m, ArH), 7.90–7.92 (1H, m, ArH), 8.08–8.09 (1H, m, ArH), 8.37 (1H, s, ArH), 9.39 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{12}N_4 \cdot 1.2H_2O$): C, H, N.

***N*^{4}-(4-Phenoxyphenyl)quinazolin-4,6-diamine (86p).** Compound **86p** was synthesized from *N*-(4-phenoxyphenyl)-6-nitroquinazolin-4-amine **85p** (6.0 g, 16.7 mmol) and iron (6.4 g, 117.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (13.3 mL, 233.0 mmol): Yield 4.2 g (64 %); mp 100–101 C; ^{1}H NMR (DMSO-d_6) δ 5.40 (2H, s, exchangeable, NH_2), 6.97–6.99 (2H, m, 2 × ArH), 7.09–7.11 (2H, m, 2 × ArH), 7.30–7.39 (3H, m, 3 × ArH), 7.47–7.49 (2H, m, 2 × ArH), 7.67–7.78 (3H, m, 3 × ArH), 8.51 (1H, s, ArH), 9.50 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{16}N_4O$): C, H, N.

***N*^{4}-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)quinazolin-4,6-diamine (86q).** Compound **86q** was synthesized from *N*-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-6-nitroquinazolin-4-amine **85q** (6.0 g, 14.7 mmol) and iron (5.6 g, 103.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (11.7 mL, 205.0 mmol): Yield 2.5 g (40 %); mp 238–239 °C; ^{1}H NMR (DMSO-d_6) δ 5.28 (2H, s, CH_2), 5.57

(2H, s, exchangeable, NH_2), 7.23–7.24 (2H, m, 2 × ArH), 7.31–7.37 (2H, m, 2 × ArH), 7.51–7.53 (1H, m, ArH), 7.58–7.60 (1H, m, ArH), 7.71–7.73 (1H, m, ArH), 7.86–7.88 (1H, m, ArH), 8.06–8.07 (1H, m, ArH), 8.32–8.33 (1H, m, ArH), 8.60 (1H, s, ArH), 9.33 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{16}ClN_5O$): C, H, N.

Synthesis of *N*-mustard-quinazoline conjugates (87a-q)

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-fluorophenylamino)quinazolin-6-yl)urea(87a). To a solution of N^4-(3-fluorophenylamino)quinazolin-4,6-diamine (**86a**, 0.99 g, 3.9 mmol) in dry THF (40 mL) containing triethylamine (2.7 mL) was added a solution of isocyanate **81** (freshly prepared from **80**, 3.00 g, 9.7 mmol) in dry THF (15 mL) at room temperature. After being stirred for 1 h at room temperature, the solid was filtered and washed with dry THF. The filtrate was evaporated to dryness in vacuo. The residue was purified by column chromatography using $CHCl_3$/MeOH (100:2 v/v) as an eluent. The fractions containing the main product were combined and evaporated to dryness. The residue was recrystallized from $CHCl_3$ to give **87a,** 0.99 g, (50 %); mp 190–191 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 6.89–6.94 (1H, m, ArH), 7.34 (2H, d, J =9.0 Hz, 2 × ArH), 7.37–7.43 (1H, m, ArH), 7.66–7.67 (1H, m, ArH), 7.75–7.77 (1H, m, ArH), 7.87–7.92 (2H, m, 2 × ArH), 8.45–7.46 (1H, m, ArH), 8.55 (1H, s, ArH), 8.57, 8.83, 9.85 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}Cl_2FN_6O·H_2O$): C, H, N.

By following the same procedure as that for **87a** the following compound were synthesized.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chlorophenylamino)quinazolin-6-yl)urea (87b). Compound **87b** was synthesized from **81** (freshly prepared from **80**, 2.00 g, 6.5 mmol) and N^4-(3-chlorophenyl)quinazolin-4,6-diamine (**86b**, 0.70 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 0.90 g (65 %); mp 172–173 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 8.8 Hz, 2 × ArH), 7.14–7.17 (1H, m, ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.38–7.42 (1H, m, ArH), 7.76 (1H, d, J = 9.1 Hz, ArH), 7.81–7.83 (1H, m, ArH), 7.89 (1H, dd, J = 2.2 Hz, J = 9.1 Hz, ArH), 8.06–8.07 (1H, m, ArH), 8.46 (1H, d, J = 2.2 Hz ArH), 8.55 (1H, s, ArH), 8.59, 8.85, 9.83 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}Cl_3N_6O·H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-bromophenylamino)quinazolin-6-yl)urea (87c). Compound **87c** was synthesized from **81** (freshly prepared from **80**, 2.00 g, 6.5 mmol) and N^4-(3-bromophenyl)quinazolin-4,6-diamine (**86c**, 0.82 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 0.80 g (57 %); mp 165–166 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.71–3.73 (8H, m, 4 × CH_2), 6.75–6.77 (2H, m, 2 × ArH), 7.32–7.38 (4H, m , 4 × ArH), 7.77–7.79 (1H, m, ArH), 7.91–7.94 (2H, m, 2 × ArH), 8.21–8.22 (1H, m, ArH), 8.48–8.49 (1H, m, ArH), 8.57 (1H, s, ArH), 8.61, 8.87, 9.85 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}BrCl_2N_6O·1.5H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-(trifluoromethyl)phenylamino) quinazolin-6-yl)urea(87d). Compound **87d** was synthesized from **81** (freshly prepared from **80**, 2.00 g, 6.5 mmol) and N^4-(3-(trifluoromethyl)phenyl)quinazolin-4,6-diamine (**86d**, 0.79 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 0.75 g (51 %); mp 210–211 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.70–3.71 (8H, m, 4 × CH_2), 6.74 (2H, d, J = 8.8 Hz, 2 × ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.43–7.45 (1H, m, ArH), 7.60–7.64 (1H, m, ArH), 7.76–7.78 (1H, m, ArH), 7.87–7.89 (1H, m, ArH), 8.20–8.22 (1H, m, ArH), 8.29–8.31 (1H, m, ArH), 8.50–8.51 (1H, m, ArH), 8.56 (1H, s, ArH), 8.58, 8.85, 9.98 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{23}Cl_2F_3N_6O·0.5H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-fluorophenylamino)quinazolin-6-yl)urea(87e). Compound **87e** was synthesized from **81** (freshly prepared from **80**, 3.00 g, 9.7 mmol) and N^4-(4-fluorophenyl)quinazolin-4,6-diamine (**86e**, 0.99 g, 3.9 mmol) in dry THF (40 mL) containing triethylamine (2.7 mL): Yield 1.0 g (53 %); mp 223–224 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.74 (2H, d, J = 9.0 Hz, 2 × ArH), 7.19–7.24 (2H, m, 2 × ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.73 (1H, d, J = 9.0 Hz, ArH), 7.80–7.83 (2H, m, 2 × ArH), 7.88 (1H, dd, J = 2.0 Hz, J = 9.0 Hz, ArH), 8.42 (1H, d, J = 2.0 Hz, ArH), 8.46 (1H, s, ArH) 8.56, 8.79, 9.75 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}Cl_2FN_6O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-bromophenylamino)quinazolin-6-yl)urea (87f). Compound **87f** was synthesized from **81** (freshly prepared from **80**, 1.50 g, 4.8 mmol) and N^4-(4-bromophenyl)quinazolin-4,6-diamine (**86f**, 0.61 g, 1.9

mmol) in dry THF (30 mL) containing triethylamine (1.3 mL): Yield 0.40 g (36 %); mp 220–221 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.33 (2H, d, J = 9.0 Hz, 2 × ArH), 7.56 (2H, d, J = 8.8 Hz, 2 × ArH), 7.74 (1H, d, J = 9.0 Hz, ArH), 7.84 (2H, d, J = 8.8 Hz, 2 × ArH), 7.89 (1H, dd, J = 9.0 Hz, J = 2.0 Hz, ArH), 8.47 (1H, d, J = 2.0 Hz, ArH), 8.50 (1H, s, ArH), 8.59, 8.84, 9.80 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}BrCl_2N_6O·1.5H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(2-fluoro-3-chlorophenylamino) quinazolin-6-yl)urea(87g). Compound **87g** was synthesized from **81** (freshly prepared from **80**, 2.00 g, 6.5 mmol) and N^4-(2-fluoro-3-chlorophenyl)quinazolin-4,6-diamine (**86g**, 0.75 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 0.97 g (69 %); mp 210–211 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.68–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.25–7.29 (1H, m, ArH), 7.33 (2H, d, J = 9.0 Hz, 2 × ArH), 7.45–7.54 (2H, m, 2 × ArH), 7.72–7.73 (1H, m, ArH), 7.82–7.84 (1H, m, ArH), 8.41–8.42 (1H, m, ArH), 8.46 (1H, s, ArH) 8.58, 8.87, 9.86 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}Cl_3FN_6O·0.5H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chloro-4-fluorophenylamino) quinazolin-6-yl)urea (87h). Compound **87h** was synthesized from **81** (freshly prepared from **80**, 0.53 g, 1.7 mmol) and N^4-(3-chloro-4-fluorophenyl)quinazolin-4,6-diamine **86h** (0.20 g, 0.6 mmol) in dry THF (20 ml) containing triethylamine (0.5 mL): Yield 0.14 g (37 %); mp 200–201 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.75 (8H, m, 4 × CH2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.41–7.46 (1H, m, ArH), 7.74–7.88 (3H, m, 3 × ArH), 8.14–8.17 (1H, m, ArH), 8.45–8.46 (1H, m, ArH), 8.52 (1H, s, ArH), 8.57, 8.83, 9.85 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}Cl_3FN_6O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3,4-dichlorophenylamino) quinazolin-6-yl)urea(87i). Compound **87i** was synthesized from **81** (freshly prepared from **80**, 1.00 g, 3.2 mmol) and N^4-(3,4,-dichlorophenyl)quinazolin-4,6-diamine (**86i**, 0.40 g, 1.3 mmol) in dry THF (25 mL) containing triethylamine (0.9 mL): Yield 0.55 g (71 %); mp 225–226 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.73 (8H, m, 4 × CH_2), 6.74 (2H, d, J = 8.8 Hz, 2 × ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.61–7.63 (1H,

m, ArH), 7.75–7.77 (1H, m, ArH), 7.87–7.90 (2H, m, 2 × ArH), 8.28–8.29 (1H, m, ArH), 8.47–8.48 (1H, m, ArH), 8.57 (1H, s, ArH) 8.58, 8.86, 9.91 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}Cl_4N_6O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-bromo-4-fluorophenylamino) quinazolin-6-yl)urea(87j). Compound **87j** was synthesized from **81** (freshly prepared from **80**, 2.00 g, 6.5 mmol) and N^4-(3-bromo-4-fluorophenyl)quinazolin-4,6-diamine (**86j**, 0.87 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 0.25 g (17 %); mp 175–176 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.73–3.75 (8H, m, 4 × CH_2), 6.76 (2H, d, J = 8.8 Hz, 2 × ArH), 7.36 (2H, d, J = 8.8 Hz, 2 × ArH), 7.41–7.43 (1H, m, ArH), 7.77 (1H, d, J = 9.2 Hz, ArH), 7.87–7.90 (2H, m, 2 × ArH), 8.26 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.48 (1H, d, J = 2.2 Hz ArH), 8.54 (1H, s, ArH), 8.63, 8.89, 9.86 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}BrCl_2FN_6O·1.5H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-chloro-3-(trifluoromethyl) phenylamino)quinazolin-6-yl)urea(87k). Compound **87k** was synthesized from **81** (freshly prepared from **80**, 2.00 g, 6.5 mmol) and N^4-(4-chloro-3-(trifluoromethyl)phenyl)quinazolin-4,6-diamine (**86k**, 0.80 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 0.80 g (52 %); mp 220–221 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.73 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 8.8 Hz, 2 × ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.71–7.73 (1H, m, ArH), 7.76–7.92 (1H, m, ArH), 7.85–7.88 (1H, m, ArH), 8.27–8.31 (1H, m, ArH), 8.43–8.44 (1H, m, ArH), 8.51–8.52 (1H, m, ArH), 8.56 (1H, s, ArH) 8.57, 8.87, 10.05 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{22}Cl_3F_3N_6O·0.5H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chloro-4-methoxyphenylamino) quinazolin-6-yl)urea(87l). Compound **87l** was synthesized from **81** (freshly prepared from **80**, 2.00 g, 6.5 mmol) and N^4-(3-chloro-4-methoxyphenyl)quinazolin-4,6-diamine (**86l**, 0.78 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 1.0 g (69 %); mp 168–169 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.72 (8H, m, 4 × CH_2), 3.87 (3H, s, Me), 6.73 (2H, d, J = 8.6 Hz, 2 × ArH), 7.17–7.19 (1H, m, ArH), 7.34 (2H, d, J = 8.6 Hz, 2 × ArH), 7.72–7.73 (2H, m, 2 × ArH), 7.85–7.87 (1H, m, ArH), 7.96–7.97 (1H, m, ArH), 8.42–8.43 (1H, m, ArH), 8.47 (1H, s, ArH)

8.56, 8.79, 9.70 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{25}Cl_3N_6O_2 \cdot H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-methoxyphenylamino)quinazolin-6-yl)urea(87m). Compound **87m** was synthesized from **81** (freshly prepared from **80**, 2.50 g, 8.1 mmol) and N^4-(3-methoxyphenyl)quinazolin-4,6-diamine (**86m**, 0.87 g, 3.2 mmol) in dry THF (35 mL) containing triethylamine (2.2 mL): Yield 1.1 g (65 %); mp 159–160 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 3.78 (3H, s, Me), 6.68–7.70 (1H, m, ArH), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.26–7.28 (1H, m, ArH), 7.33 (2H, d, J = 9.0 Hz, 2 × ArH), 7.45–7.46 (1H, m, ArH), 7.52–7.53 (1H, m, ArH), 7.73 (1H, d, J = 9.0 Hz, ArH), 7.90 (1H, dd, J = 2.1 Hz, J = 9.0 Hz, ArH), 8.42 (1H, d, J = 2.1 Hz, ArH), 8.50 (1H, s, ArH) 8.57, 8.81, 9.65 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{26}Cl_2N_6O_2 \cdot H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3,4,5-trimethoxyphenylamino) quinazolin-6-yl)urea(87n). Compound **87n** was synthesized from **81** (freshly prepared from **80**, 1.00 g, 3.2 mmol) and N^4-(3,4,5-trimethoxyphenyl)quinazolin-4,6-diamine (**86n**, 0.42 g, 1.3 mmol) in dry THF (25 mL) containing triethylamine (0.9 mL): Yield 0.49 g (66 %); mp 154–155 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.33 (3H, s, Me), 3.67–3.72 (8H, m, 4 × CH_2), 3.80 (6H, s, 2 × Me), 6.74 (2H, d, J = 9.0 Hz, 3 × ArH), 7.28 (2H, s , 2 × ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.73 (1H, d, J = 8.9 Hz, ArH), 7.85–7.88 (1H, dd, J = 2.0 Hz, J = 8.9 Hz, ArH), 8.42 (1H, d, J = 2.0 Hz, ArH), 8.49 (1H, s, ArH) 8.56, 8.81, 9.58 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{28}H_{30}Cl_2N_6O_4 \cdot H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-ethynylphenylamino)quinazolin-6-yl)urea(87o). Compound **87o** was synthesized from **81** (freshly prepared from **80**, 2.00 g, 6.5 mmol) and N^4-(3-ethynylphenyl)quinazolin-4,6-diamine (**86o**, 0.68 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 0.75 g (52 %); mp 164–165 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.73 (8H, m, 4 × CH_2), 4.19 (1H, s, CH), 6.73 (2H, d, J = 8.8 Hz, 2 × ArH), 7.20–7.21 (1H, m, ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.37–7.41 (1H, m, ArH), 7.73–7.76 (1H, m, ArH), 7.88–7.90 (2H, m, 2 × ArH), 8.03–8.04 (1H, m, ArH), 8.44–8.45 (1H, m, ArH), 8.52 (1H, s, ArH)

8.58, 8.82, 9.78 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{27}H_{24}Cl_2N_6O \cdot H_2O$): C, H, N.

1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-phenoxyphenylamino)quinazolin-6-yl)urea(87p). Compound **87p** was synthesized from **81** (freshly prepared from **80**, 2.00 g, 6.5 mmol) and N^4-(4-phenoxyphenyl)quinazolin-4,6-diamine (**86p**, 0.85 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 0.30 g (33 %); mp 143–144 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.31–3.71 (8H, m, 4 × CH_2), 6.72–6.74 (2H, m, 2 × ArH), 7.01–7.07 (4H, m, 4 × ArH), 7.10–7.14 (1H, m, ArH), 7.32–7.41 (4H, m, 4 × ArH), 7.71–7.73 (1H, m, ArH), 7.81–7.90 (3H, m, 3 × ArH), 8.41–8.42 (1H, m, ArH), 8.46 (1H, s, ArH) 8.58, 8.80, 9.73 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{31}H_{28}Cl_2N_6O_2 \cdot H_2O$): C, H, N.

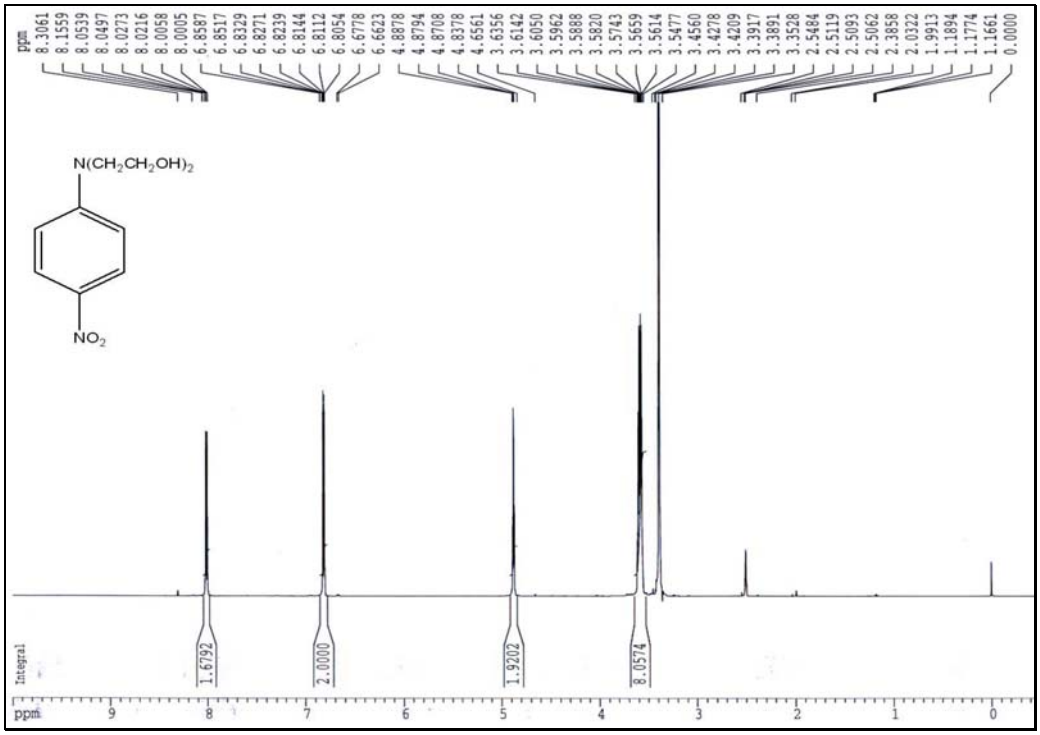
1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chloro-4-(pyridin-2-ylmethoxy) phenylamino)quinazolin-6-yl)urea(87q). Compound **87q** was synthesized from **81** (freshly prepared from **80,** 2.00 g, 6.5 mmol) and N^4-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)quinazolin-4,6-diamine (**86q**, 0.98 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): Yield 0.90 g (56 %); mp 156–157 °C (dec); -^{1}H NMR (DMSO-d_6) δ 3.68–3.74 (8H, m, 4 × CH_2), 5.30 (2H, s, CH_2), 6.74 (2H, d, J = 9.0 Hz, 2 × ArH), 7.25–7.27 (1H, m, ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.37–7.39 (1H, m, ArH), 7.58–7.60 (1H, m, ArH), 7.69–7.74 (2H, m, 2 × ArH), 7.86–7.91 (2H, m, 2 × ArH), 8.01–8.02 (1H, m, ArH), 8.42–8.42 (1H, m, ArH), 8.48 (1H, s, ArH), 8.60–8.61 (1H, m, ArH) 8.57, 8.80, 9.72 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{31}H_{28}Cl_3N_7O_2 \cdot 1.5H_2O$): C, H, N.

2.5 Conclusion

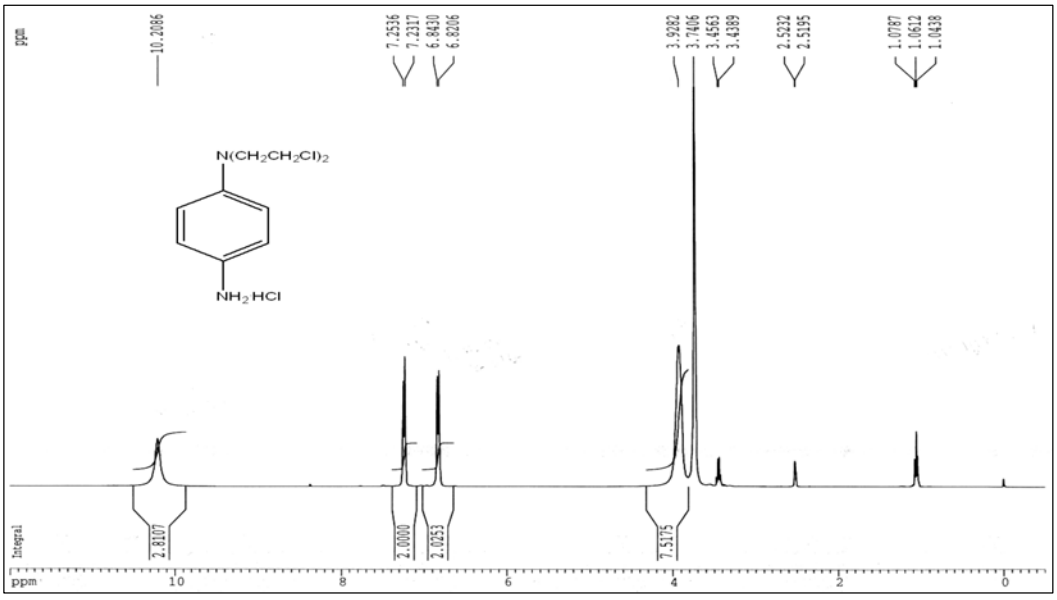
In continuation of our research on the developing DNA-directed alkylating agents, we have synthesized a series of *N*-mustard-quinazoline conjugates, in which the *N*-mustard pharmacophore was attached at the C-6 of the 4-anilinoquinazolines via a urea linker. A variety of substituent(s) were introduced to the C-4 anilino moiety for studying their structure-activity relationships. All newly synthesized compounds were tested against various human tumor cell lines *In vitro* and *In vivo*. Antitumor activities of these compounds are shown in Chapter 3.

2.6 Representative Spectra

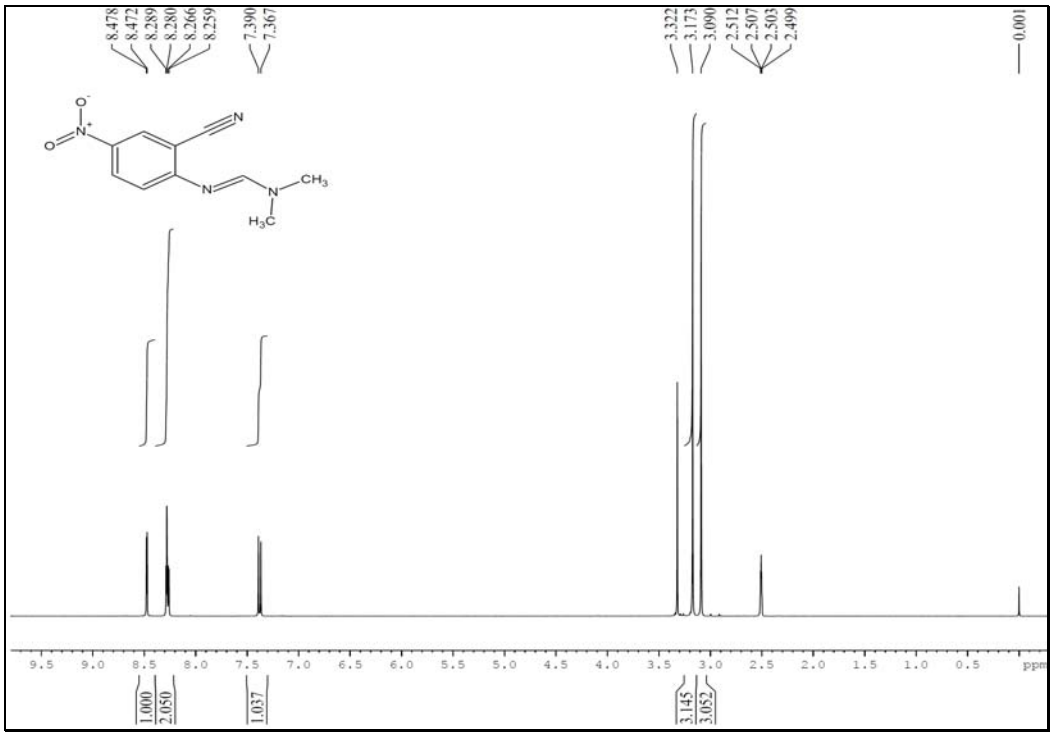
2.6.1 ^{1}H NMR Spectrum for compound **79**.



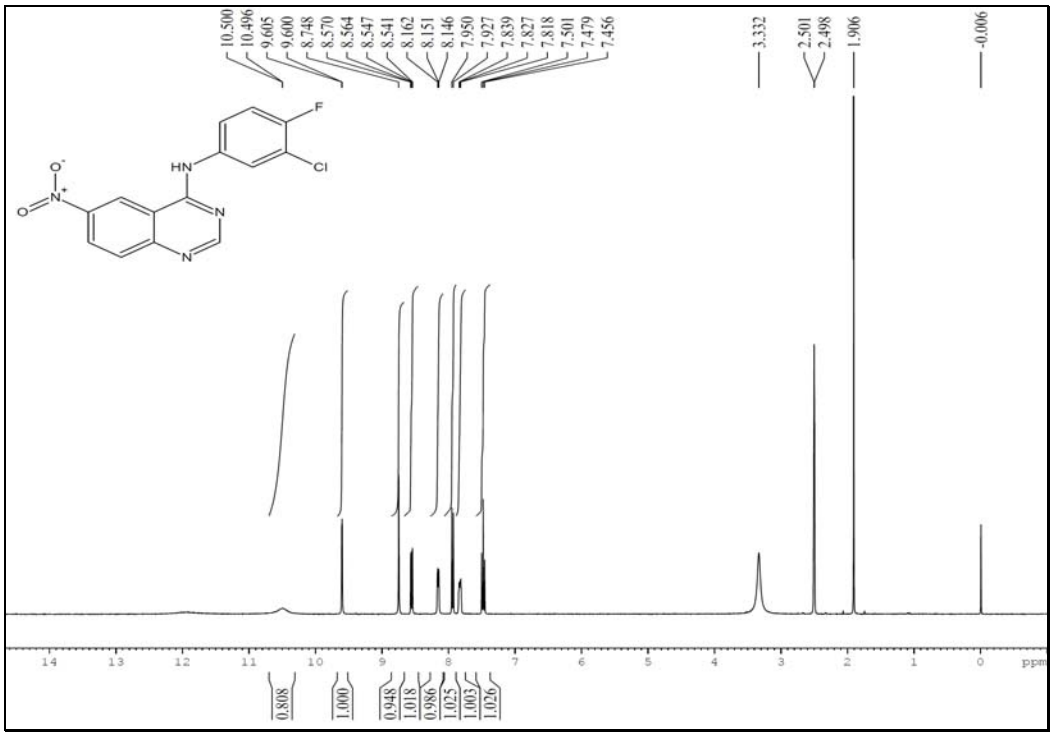
]**2.6.2** ^{1}H NMR Spectrum for compound **80**.



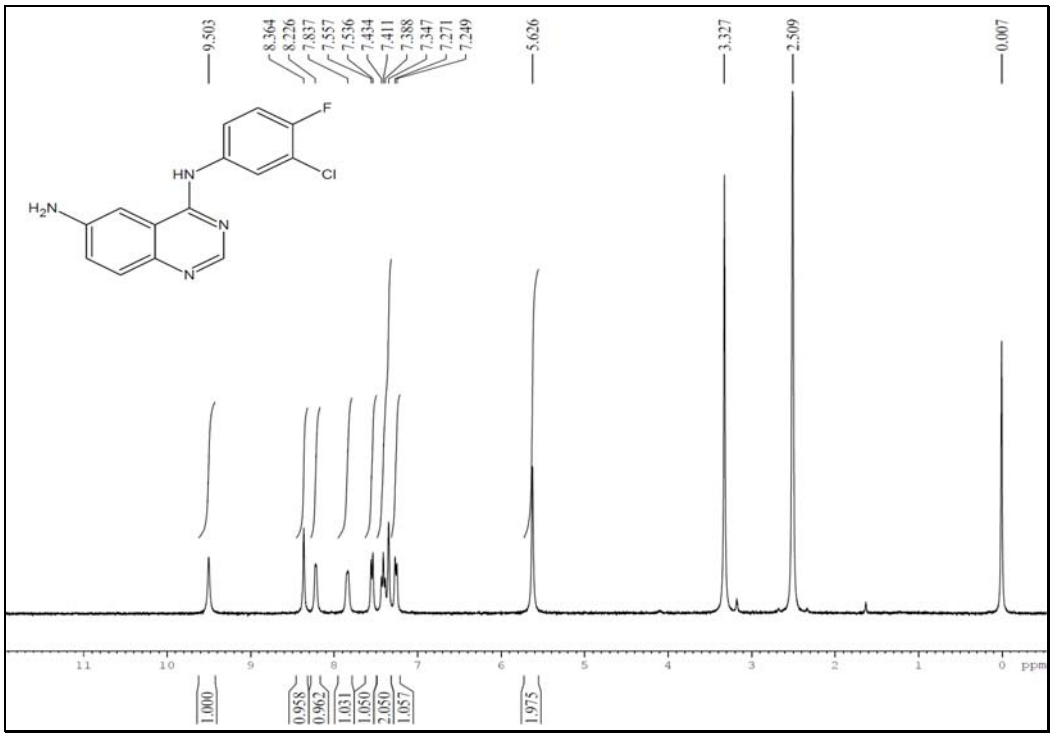
2.6.3 ^{1}H NMR Spectrum for compound **83**.



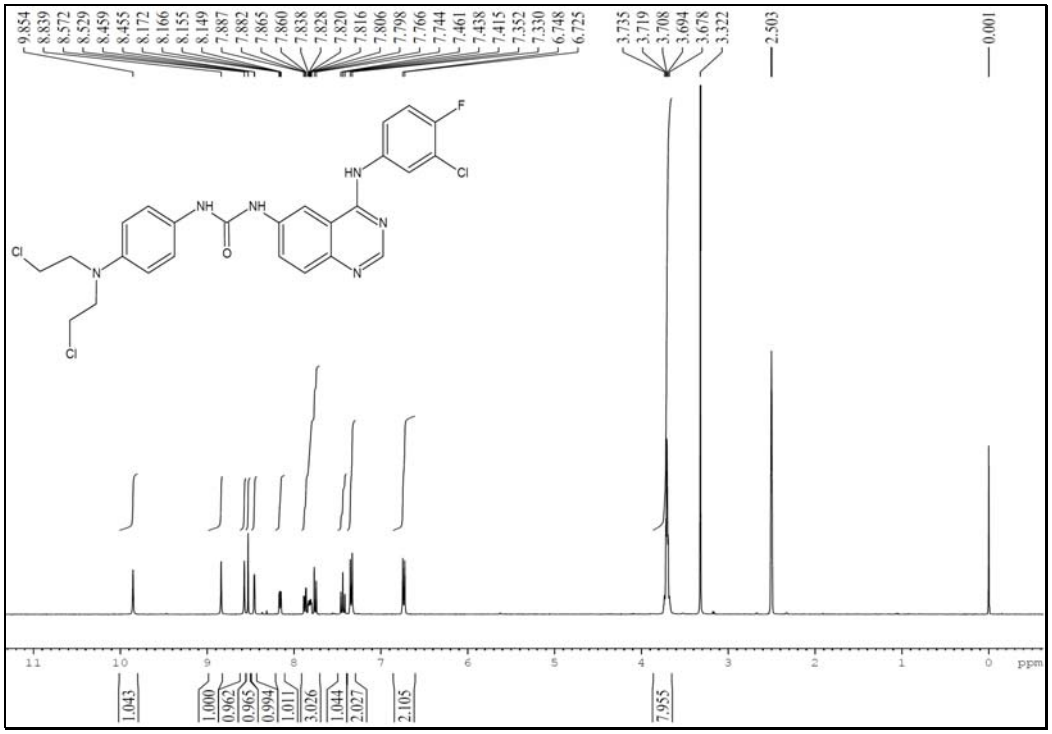
2.6.4 ^{1}H NMR Spectrum for compound **85h**.



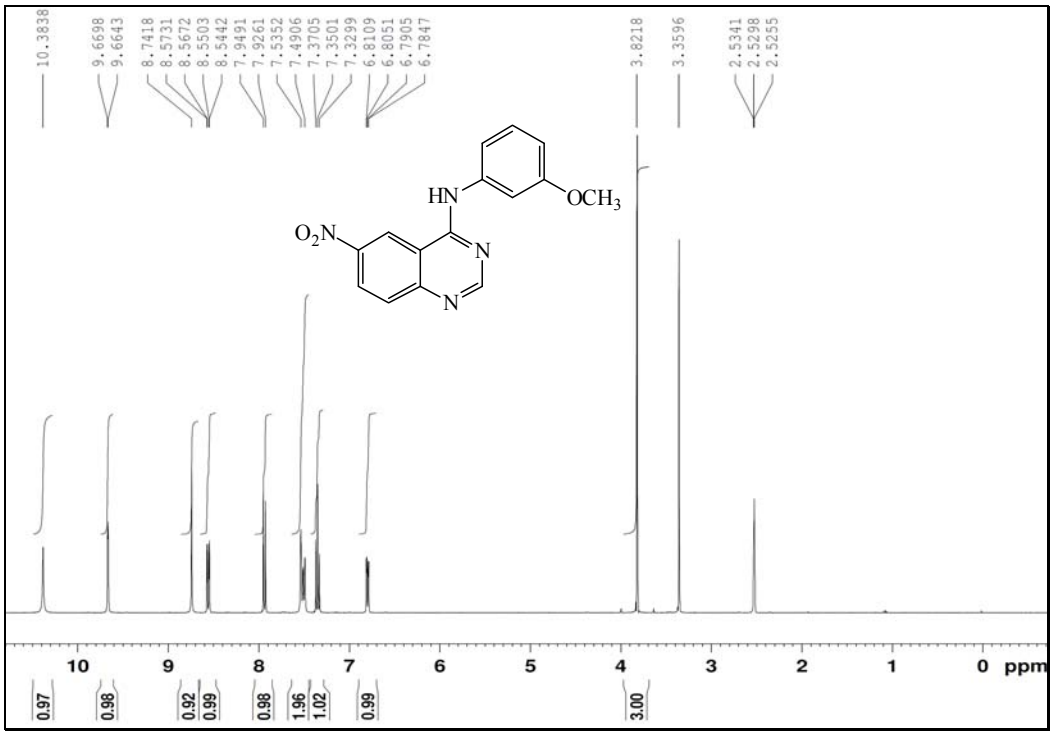
2.6.5 ^{1}H NMR Spectrum for compound **86h**.



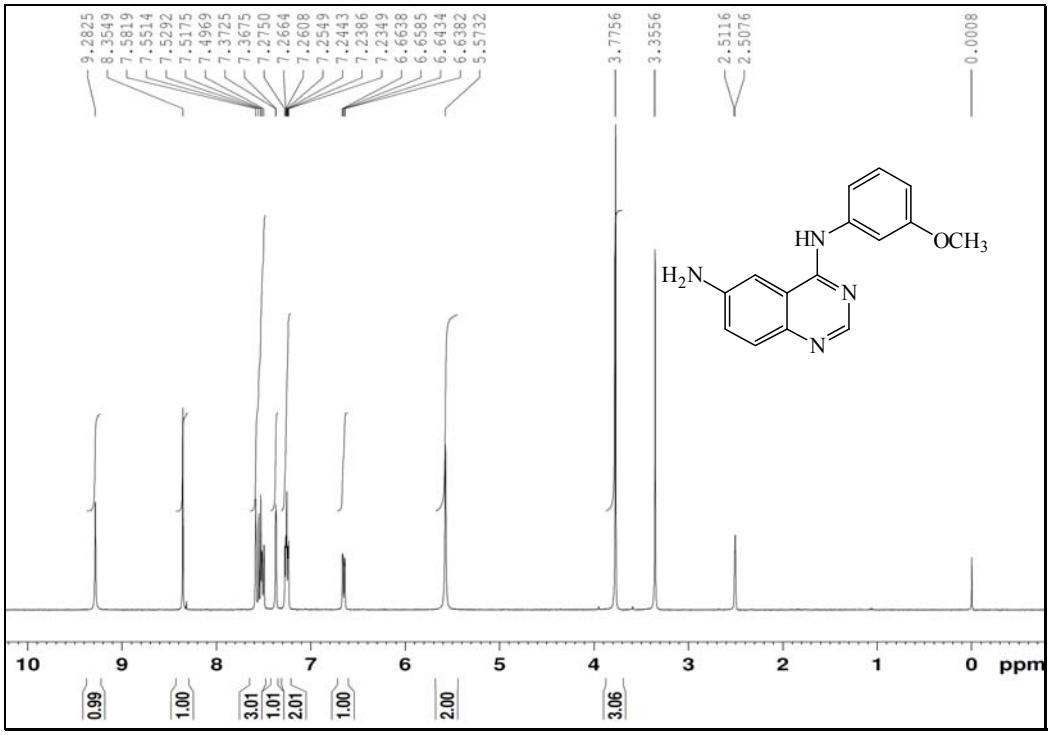
2.6.6 ^{1}H NMR Spectrum for compound **87h**.



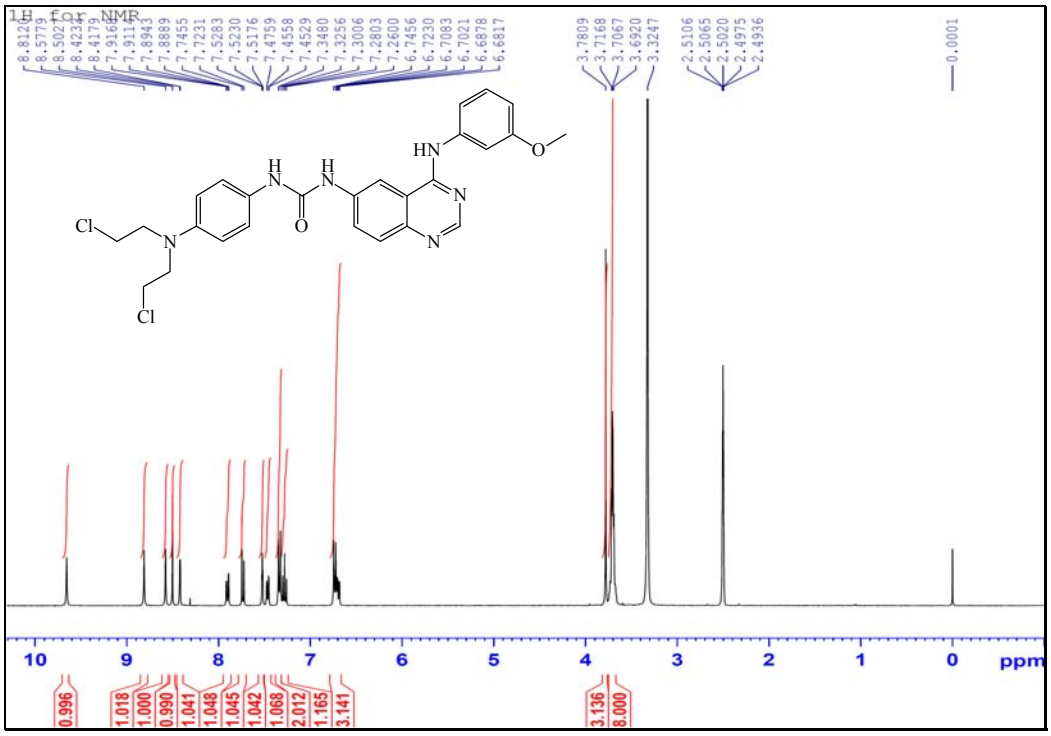
2.6.7 ^{1}H NMR Spectrum for compound **85m**.



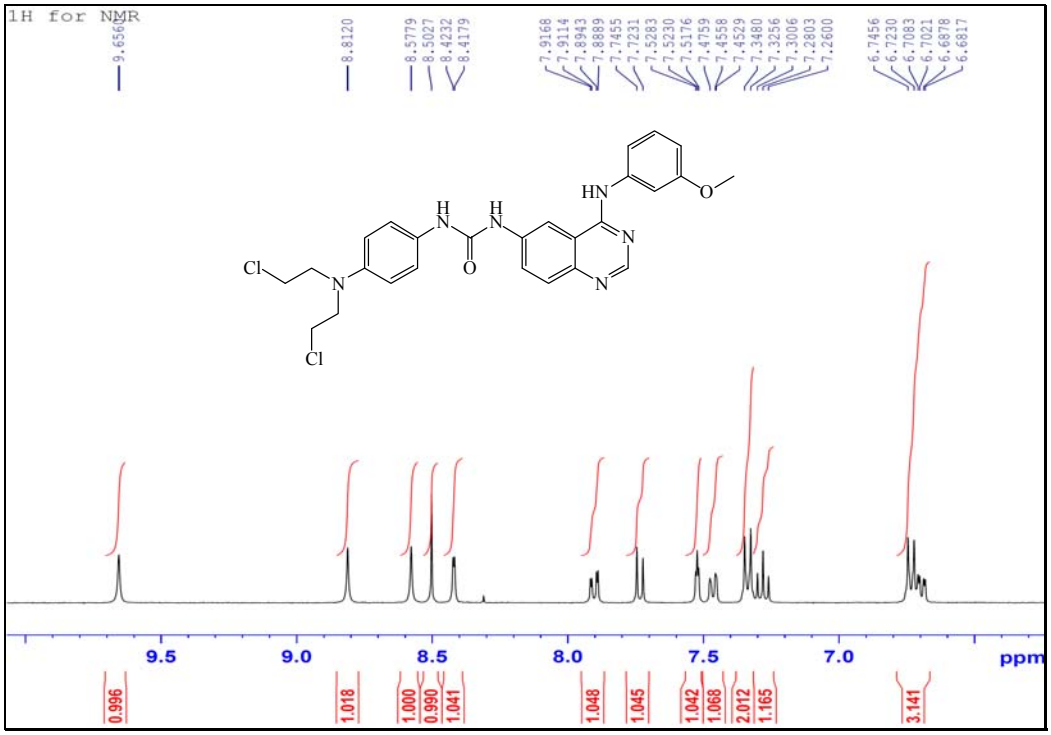
2.6.8 ^{1}H NMR Spectrum for compound **86m**.



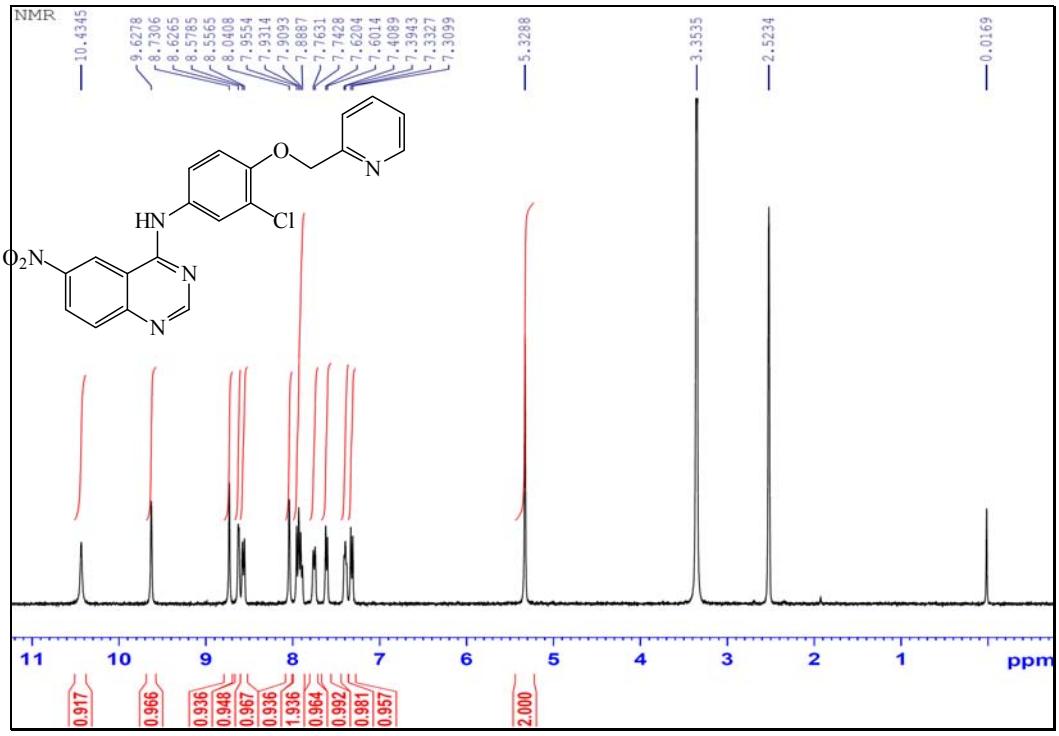
2.6.9 ^{1}H NMR Spectrum for compound **87m**.



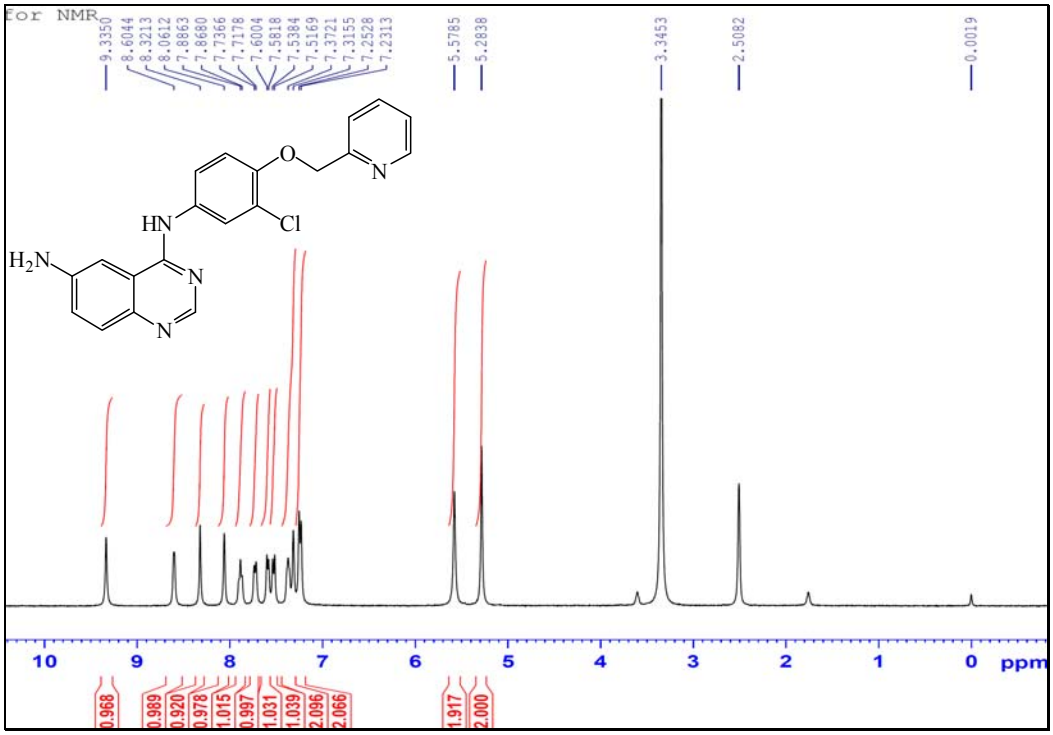
2.6.10 ^{1}H NMR Spectrum for compound **87m (ARO)**.



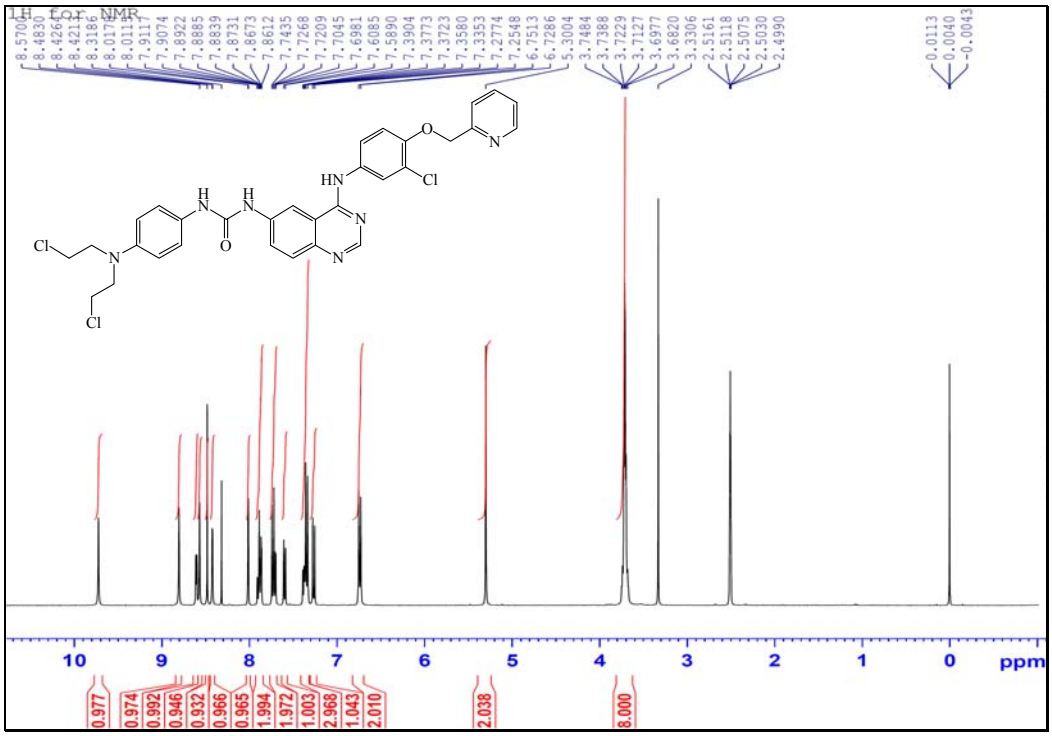
2.6.11 ^{1}H NMR Spectrum for compound **85q**.



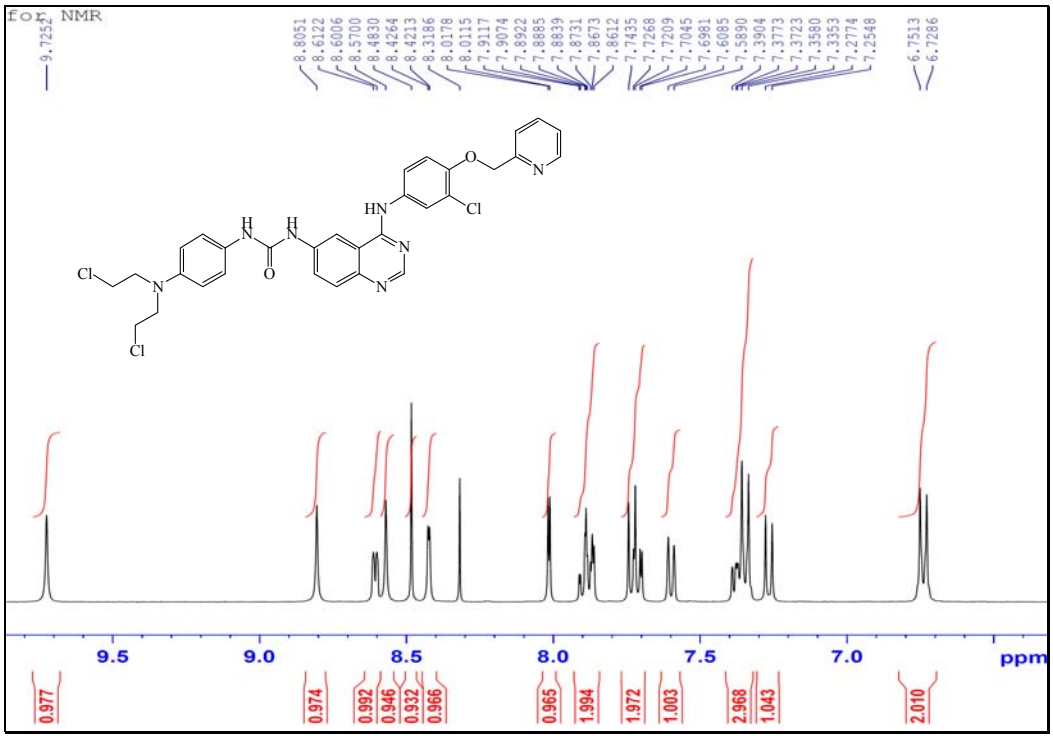
2.6.12 ^{1}H NMR Spectrum for compound **86q**.



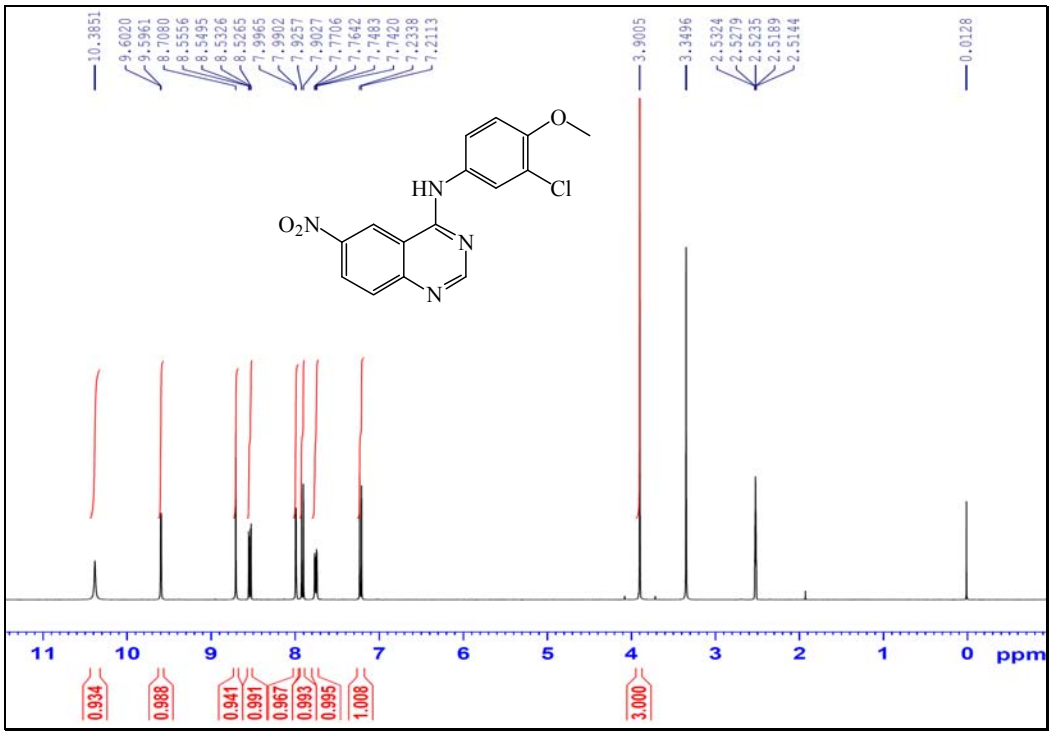
2.6.13 ^{1}H NMR Spectrum for compound **87q**.



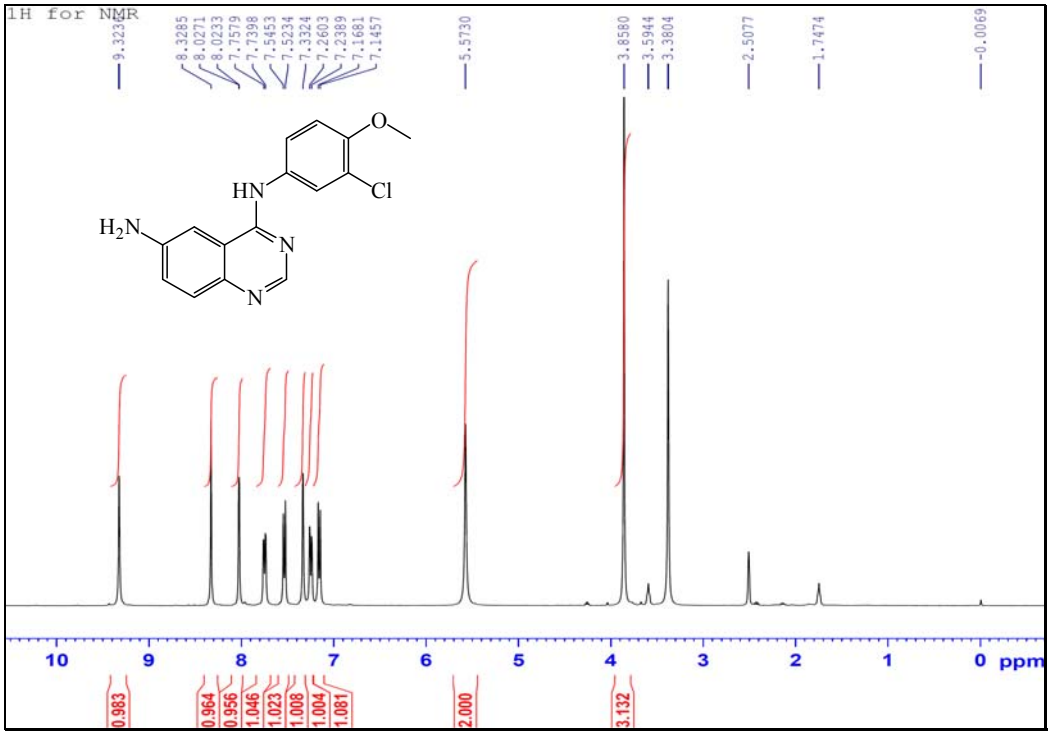
2.6.14 ^{1}H NMR Spectrum for compound **87q(ARO)**.



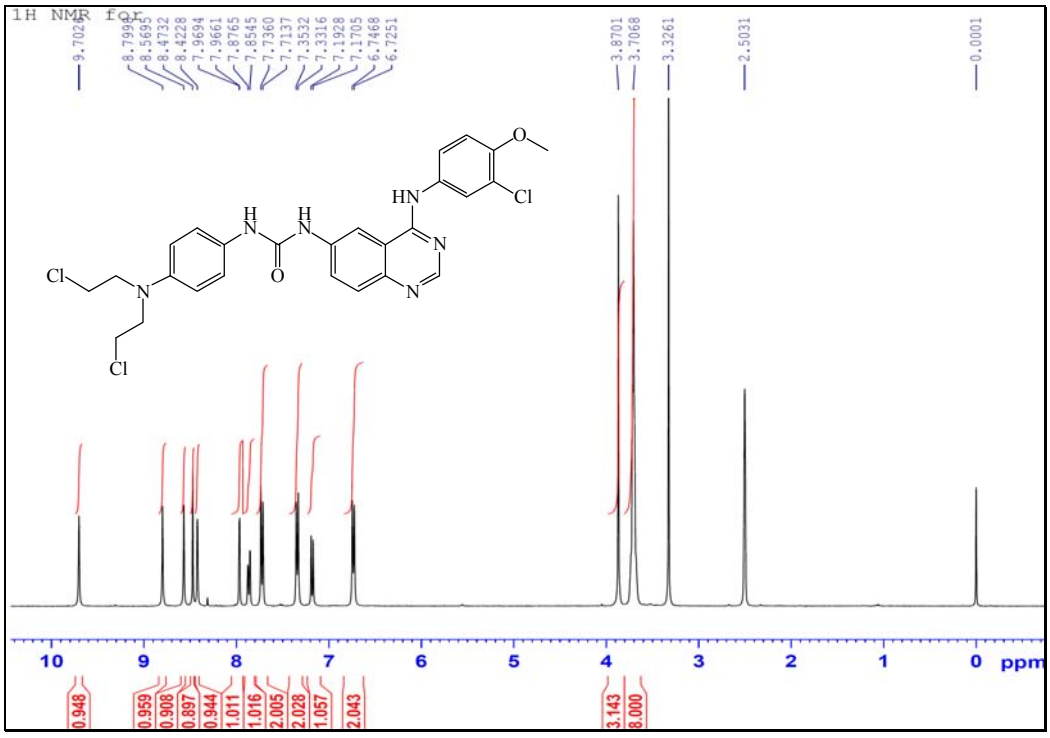
2.6.15 ^{1}H NMR Spectrum for compound **85l**.



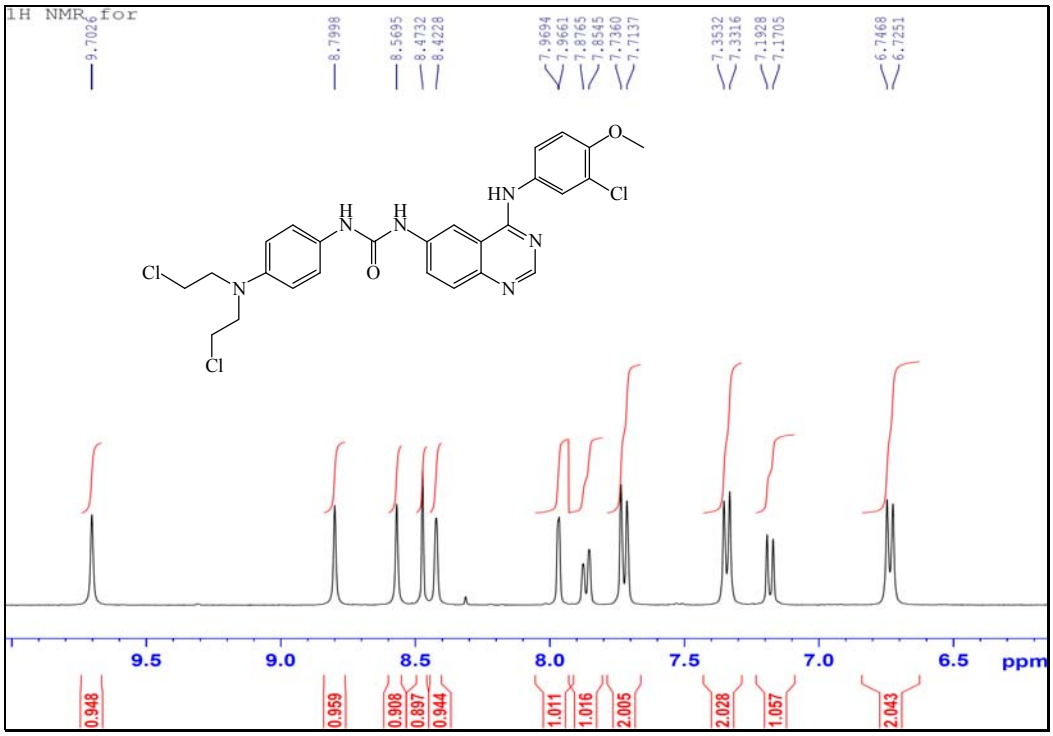
2.6.16 ^{1}H NMR Spectrum for compound **86l**.



2.6.17 ^{1}H NMR Spectrum for compound **87l**.



2.6.18 ^{1}H NMR Spectrum for compound **87l (ARO)**.



2.7 Elemental analysis

Table 1. Elemental analysis of compounds **85a-q.**

Compound No.	MF	M.W.	Elemental Analysis					
			CHN Calculated (%)			CHN Found (%)		
			C	H	N	C	H	N
85a	$C_{14}H_9FN_4O_2$	284.25	59.16	3.19	19.71	59.00	3.21	19.70
85b	$C_{14}H_9ClN_4O_2$	300.70	55.92	3.02	18.63	56.00	3.12	18.73
85c	$C_{14}H_9BrN_4O_2$	345.15	48.72	2.63	16.23	48.60	2.68	16.43
85d	$C_{15}H_9F_3N_4O_2$	334.25	53.90	2.71	16.76	53.80	2.65	16.75
85e	$C_{14}H_9FN_4O_2$	284.25	59.16	3.19	19.71	59.07	3.20	19.70
85f	$C_{14}H_9BrN_4O_2$	345.15	48.72	2.63	16.23	48.68	2.63	16.23
85g	$C_{14}H_8ClFN_4O_2$	318.69	52.76	2.53	17.58	52.69	2.60	17.45
85h	$C_{14}H_8ClFN_4O_2$	318.69	52.76	2.53	17.58	52.67	2.57	17.50
85i	$C_{14}H_8Cl_2N_4O_2$	335.14	50.17	2.41	16.72	50.25	2.42	16.73
85j	$C_{14}H_8BrFN_4O_2$	363.14	46.30	2.22	15.43	46.20	2.24	15.47
85k	$C_{15}H_8ClF_3N_4O_2$	368.70	48.86	2.19	15.20	48.67	2.26	15.38
85l	$C_{15}H_{11}ClN_4O_3$	330.73	54.47	3.35	16.94	54.49	3.34	16.97
85m	$C_{15}H_{12}N_4O_3$	296.28	60.81	4.08	18.91	60.75	4.05	18.88
85n	$C_{17}H_{16}N_4O_5$	356.33	57.30	4.53	15.72	57.24	4.53	15.72
85o	$C_{16}H_{10}N_4O_2.0.5$	299.28	64.21	3.70	18.72	64.71	3.60	18.50
85p	$C_{20}H_{14}N_4O_3$	358.35	61.49	4.99	13.88	61.76	5.00	13.86
85q	$C_{20}H_{14}ClN_5O_3.2H_2O$	443.84	54.12	4.09	15.78	54.47	4.17	15.79

Table 2. Elemental analysis of compounds **86a-q.**

Compound No.	MF	M.W.	Elemental Analysis					
			CHN Calculated (%)			CHN Found (%)		
			C	H	N	C	H	N
86a	$C_{14}H_{11}FN_4.0.8H_2O$	254.26	62.58	4.73	20.85	62.76	4.63	20.74
86b	$C_{14}H_{11}ClN_4$	270.72	62.11	4.10	20.70	62.22	4.05	20.60
86c	$C_{14}H_{11}BrN_4$	315.17	53.35	3.52	18.78	53.45	3.42	18.68
86d	$C_{15}H_{11}F_3N_4$	304.27	59.21	3.64	18.41	59.30	3.48	18.28
86e	$C_{14}H_{11}FN_4.1.3H_2O$	277.68	60.55	4.94	20.18	60.28	4.80	19.67
86f	$C_{14}H_{11}BrN_4.0.2H_2O$	318.77	52.75	3.60	17.58	52.42	3.62	17.01
86g	$C_{14}H_{10}ClFN_4$	288.71	58.24	3.49	19.41	58.24	3.54	19.10
86h	$C_{14}H_{10}ClFN_4$	288.71	58.24	3.49	19.41	58.44	3.39	19.60
86i	$C_{14}H_{10}Cl_2N_4$	305.16	55.10	3.30	18.36	54.72	3.29	18.04
86j	$C_{14}H_{10}BrFN_4$	333.16	50.47	3.03	16.82	50.60	3.09	17.00
86k	$C_{15}H_{10}ClF_3N_4$	338.71	53.19	2.98	16.54	53.02	3.03	16.15
86l	$C_{15}H_{13}ClN_4O$	300.74	59.91	4.36	18.63	59.71	4.39	18.17
86m	$C_{15}H_{14}N_4O.0.2H_2O$	269.90	66.75	5.38	20.76	66.75	5.18	20.76
86n	$C_{17}H_{18}N_4O_3$	326.35	62.57	5.56	17.17	62.23	5.62	16.83
86o	$C_{16}H_{12}N_4.1.2H_2O$	281.91	68.17	5.15	19.87	68.24	5.36	19.30
86p	$C_{20}H_{16}N_4O$	328.37	73.15	4.91	17.06	73.25	5.01	17.20
86q	$C_{20}H_{16}ClN_5O$	377.83	63.58	4.27	18.54	63.20	4.40	18.08

Table 3. Elemental analysis of compounds **87a-q.**

Compound No.	MF	M.W.	Elemental Analysis					
			CHN Calculated (%)			CHN Found (%)		
			C	H	N	C	H	N
87a	$C_{25}H_{23}Cl_2FN_6O.H_2O$	531.41	56.50	4.74	15.81	56.60	4.63	15.65
87b	$C_{25}H_{23}Cl_3N_6O.H_2O$	547.86	54.81	4.60	15.34	54.86	4.54	15.22
87c	$C_{25}H_{23}BrCl_2N_6O.1.5H_2O$	601.32	49.93	4.36	13.98	50.09	4.21	13.95
87d	$C_{26}H_{23}Cl_2F_3N_6O.0.5H_2O$	572.41	54.56	4.23	14.68	54.73	4.11	14.65
87e	$C_{25}H_{23}Cl_2FN_6O$	513.39	58.49	4.52	16.37	58.33	4.56	16.19
87f	$C_{25}H_{23}BrCl_2N_6O.1.5H_2O$	601.32	49.93	4.36	13.98	49.68	4.15	13.71
87g	$C_{25}H_{22}Cl_3FN_6O.0.5H_2O$	556.85	53.92	4.16	15.09	53.83	3.97	15.00
87h	$C_{25}H_{22}Cl_3FN_6O$	601.89	49.89	4.69	13.96	50.07	4.29	13.31
87i	$C_{25}H_{22}Cl_4N_6O$	564.29	53.21	3.93	14.89	53.25	3.93	14.89
87j	$C_{25}H_{22}BrCl_2FN_6O.1.5H_2O$	619.31	48.48	4.07	13.57	48.51	3.88	13.45
87k	$C_{26}H_{22}Cl_3F_3N_6O.0.5H_2O$	606.85	51.46	3.82	13.85	51.83	3.68	13.95
87l	$C_{26}H_{25}Cl_3N_6O_2.H_2O$	577.89	54.04	4.71	14.54	54.17	4.68	14.62
87m	$C_{26}H_{26}Cl_2N_6O_2.H_2O$	543.44	57.46	5.19	15.46	57.07	5.19	15.32
87n	$C_{28}H_{30}Cl_2N_6O_4.H_2O$	603.50	55.73	5.34	13.93	55.41	5.34	13.79
87o	$C_{27}H_{24}Cl_2N_6O.H_2O$	537.44	60.34	4.88	15.64	59.93	4.86	15.45
87p	$C_{31}H_{28}Cl_2N_6O_2.H_2O$	605.51	61.49	4.99	13.88	61.76	5.00	13.86
87q	$C_{31}H_{28}Cl_3N_7O_2.1.5H_2O$	663.98	56.08	4.71	14.77	55.86	4.37	14.60

Chapter -3

Antitumor evaluation of phenyl *N*-mustard-quinazoline conjugates bearing a urea linker

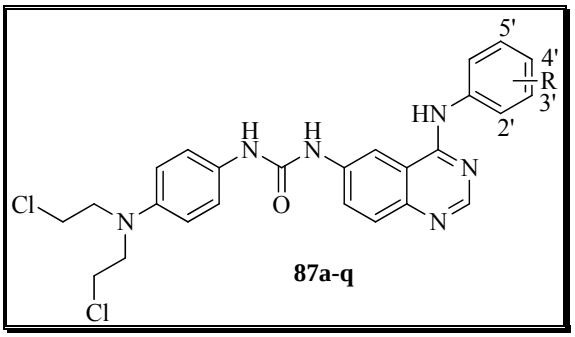
3.1 Biological results and discussion

3.1.1 *In vitro* cytotoxicity

To study the structure-activity relationships of the *N*-mustard-quinazoline conjugates, we have introduced electron-withdrawing halogen(s), electron-donating methoxy function(s), and other substituent to the 4-anilino ring. These derivatives were subjected to evaluating their cytotoxicities in inhibiting 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* (Table 1). It demonstrated that the newly synthesized conjugates possess significant cytotoxicity with IC_{50} in micro molar range. The most cytotoxic compound of this series is the 3',4',5'-trimethoxyphenyl derivative **87n** with an IC_{50} value of 0.38 μM. However, the potency of the C-3'-OMe derivative (**87m**, IC_{50}>1.32 μM) is much weaker than the corresponding trimethoxy derivative **87n**. As for the halogen-substituted derivatives, it is clearly to see that order of the cytotoxicity of C-3'-halogen substituted compounds is C-3'-Br (**87c**)> C-3'-Cl (**87b**)> C-3'-F (**87a**). In contrast, the C-4'-F substituted derivatives **87e** is apparently more potent than the corresponding C-4'-Br derivative **87f**. In the series of C-3'-C-4' dihalogens substituted conjugates, the order of the cytotoxicity is **87g** > **87h** > **87i** ≅ **87j**. The SAR study shows that the position of the substitutions, numbers and types of halogen atom are critical for their activity. The cytotoxicity of compounds bearing other substituent, such as C-3' or C-4'-CF_3 (**87d** and **87k**, respectively), C-3'-ethynyl (**87o**), C-4'-phenoxy (**87p**), and 3-chloro-4-(pyridin-2-ylmethoxy) (**87q**), were also evaluated. It reveals that C-3'-ethynyl (**87o**) is most cytotoxic among these conjugates with a IC_{50} value of 0.50 μM. Compounds having a CF_3 substituent (**87d** and **87k**) are less cytotoxic than other compounds tested.

The cytotoxicity of the newly synthesized compounds against human CCRF-CEM drug-resistant sublines (resistant to Vinblastine and Taxol, CCRF-CEM/VBL and CCRF-CEM/taxol, respectively) were also studied. The results revealed that they generally have no or little cross-resistance to these two natural products except compounds **87n**, which has certain extent of cross-resistance (Table 1). It suggests that the *N*-mustard derivatives were neither a good substrate of p-glycoprotein nor mutated tubulin.

Table 1. The cytotoxicity of newly synthesized phenyl N-mustard-6-aminoquinazoline conjugates against human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines (CCRF-CEM/Taxol and CCRF-CEM/VBL). [a]



87a-q

Compd.	Substitute R				Cell Growth inhibition (IC_{50} μM)		
	2'	3'	4'	5'	CCRF-CEM	CCRF-CEM/ Taxol[b]	CCRF-CEM/ VBL[b]
87a	H	F	H	H	2.51±0.11	3.46±0.18 [1.38×][c]	4.28±0.01 [1.70×]
87b	H	Cl	H	H	1.66±0.14	4.56±0.0001 [2.75×]	3.26±0.05 [1.96×]
87c	H	Br	H	H	0.95±0.11	4.58±0.05 [4.82×]	2.62±0.01 [2.76×]
87d	H	CF_3	H	H	3.97±0.22	4.57±0.13 [1.15×]	4.68±0.05 [1.17×]
87e	H	H	F	H	0.74±0.02	3.16±0.07 [4.27×]	1.24±0.03 [1.67×]
87f	H	H	Br	H	4.64±0.16	8.79±0.16 [1.89×]	7.88±0.36 [1.70×]
87g	F	Cl	H	H	0.75±0.01	1.55±0.02 [2.07×]	1.04±0.03 [1.39×]
87h	H	Cl	F	H	1.00±0.09	1.16±0.14 [1.16×]	1.14±0.03 [1.14×]

87i	H	Cl	Cl	H	2.60±0.02	5.61±0.16 [2.16×]	6.75±0.53 [2.60×]
87j	H	Br	F	H	2.68±0.11	4.40±0.30 [1.64×]	4.74±0.15 [1.77×]
87k	H	CF_3	Cl	H	4.77±0.22	5.36±0.14 [1.12×]	5.28±0.58 [1.11×]
87l	H	Cl	OMe	H	0.80±0.01	3.34±0.02 [4.17×]	3.75±0.04 [4.68×]
87m	H	OMe	H	H	1.32±0.02	3.58±0.12 [2.71×]	3.38±0.17 [2.56×]
87n	H	OMe	OMe	OMe	0.38±0.02	12.32±0.79 [32.42×]	24.51±1.72 [64.50×]
87o	H	3-ethynyl	H	H	0.50±0.04	2.29±0.02 [4.58×]	2.33±0.34 [4.66×]
87p	H	H	OPh	H	4.77±0.01	7.99±0.18 [1.67×]	10.88±0.02 [2.28×]
87q	H	Cl	OCH_2(2-pyridinyl)	H	1.41±0.07	4.58±0.08 [3.24×]	2.65±0.13 [1.88×]
Taxol					0.003 ±0.0003	0.43±0.05 [143×]	1.27±0.05 [423×]
Vinblastine					0.0007±0.001	0.08±0.01 [106.2×]	0.50±0.12 [679.5×]
Carboplatin					3.4±0.99		2.45±0.65 [0.7×]

[a]Cell growth inhibition was measured by the XTT assay[101] for leukemic cells after 72-h incubation using a microplate spectrophotometer as described previously.[103] 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). IC_{50} values were determined from dose-effect relationship at six or seven concentrations of each drug by using the CompuSyn software by Chou and Martin[105] based on the median-effect principle and plot using the serial deletion analysis.[106, 107] Ranges given for taxol and vinblastine were mean ± SE (n = 4).

[b]CCRF-CEM/Taxol and CCRF-CEM/VBL are subcell lines of CCRF-CEM cells that are 143-fold resistant to Taxol, and 423-fold resistant to vinblastine, respectively, when comparing with the IC_{50} of the parent cell line.

[c]Numbers in the brackets are fold of cross-resistant determined by comparison with the corresponding IC_{50} of the parent cell line.

The selected compounds were further evaluated for their cytotoxicity in inhibiting other human solid tumors such as human breast tumor (MX-1), colon cancer (HCT-116), human non-small cell lung cancer (H1299), and prostate cancer (PC3) cell growth *in vitro*. As shown in Table 2, one can see that these conjugates possess good to moderate cytotoxic effects against the growth of these cell lines tested *in vitro*. In comparison with the cytotoxicities of the *N*-mustard-quinoline conjugates, previously synthesized in our laboratory,[95] the *N*-mustard-quinazoline conjugates are less potent in inhibiting all tumor cell lines examined.

Table 2. The cytotoxicity of phenyl N-mustard-6-aminoquinazoline conjugates (**87a-q)** against human solid tumor (breast carcinoma MX-1, colon carcinoma HCT-116, lung carcinoma H1299 and prostate carcinoma PC3) cell growth *in vitro*.[a]

Compd.	Cell Growth inhibition (IC_{50} μM)			
	MX-1[a]	HCT-116[a]	H1299[b]	PC3[b]
87a	10.11±0.09	9.62±0.12	ND	ND
87b	7.84±0.03	7.46±0.02	10.49±2.04	10.37±0.32
87c	6.92±0.05	2.17±0.03	8.04±1.26	10.13±0.13

87d	5.30±0.63	5.97±0.24	ND	ND
87e	3.43±0.003	3.18±0.03	11.60±1.54	8.24±1.76
87f	6.71±0.03	7.79±0.36	ND	ND
87g	4.55±0.02	3.84±0.0005	5.52±1.59	6.29±1.12
87h	5.38±0.05	3.36±0.05	ND[c]	ND
87i	6.45±0.50	2.79±0.14	ND	ND
87j	7.10±0.14	5.99±0.63	ND	ND
87k	7.44±0.58	5.34±0.06	ND	ND
87l	5.51±0.09	5.27±0.18	6.94±1.71	8.02±2.08
87m	2.59±0.13	2.44±0.03	8.79±1.32	9.44±0.87
87n	4.02±0.03	2.24±0.01	10.28±1.25	10.98±1.89
87o	6.27±0.24	3.47±0.13	ND	ND
87p	8.47±0.15	12.41±0.04	ND	ND
87q	4.62±0.20	2.22±0.54	ND	ND
Cisplatin	4.95±0.60	26.65±4.19	ND	ND

[a]Cell growth inhibition was measured by the SRB assay[102] for solid tumor cells after 72-h incubation using a microplate spectrophotometer as described previously.[103]

[b]Cell growth inhibition was determined by the Alamar blue assay[104] in a 72 h incubation using a microplate spectrophotometer as described previously.

[c]Not determined.

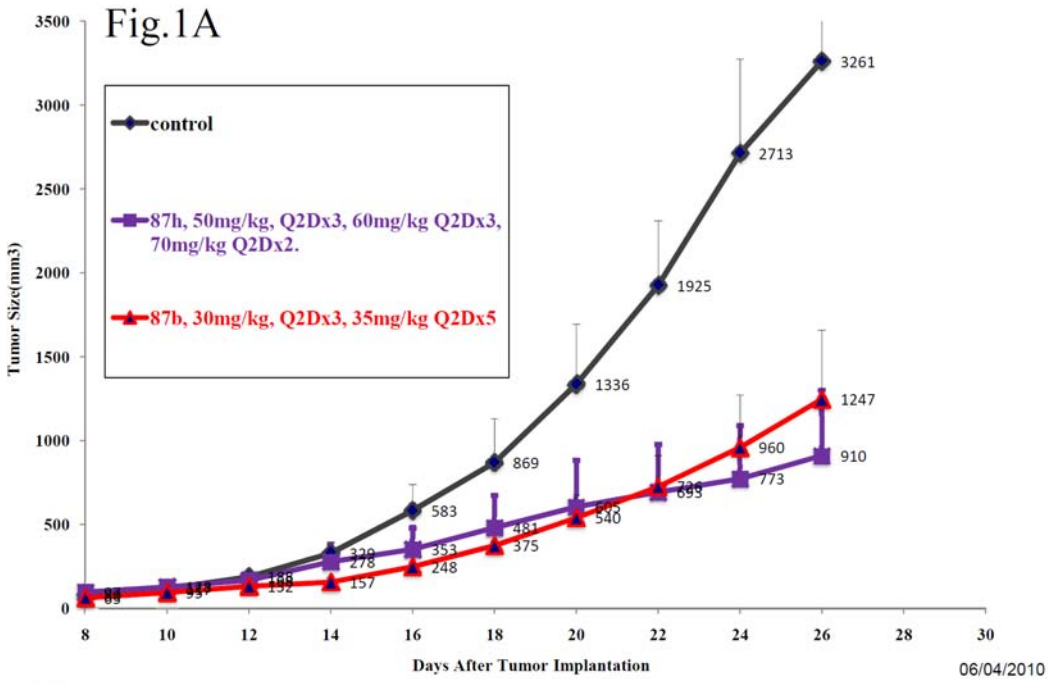
3.1.2 *In vivo* therapeutic activity

The *in vitro* cytotoxicity of the tested compound may not always directly reflex to its therapeutic efficacy in tumor xenograft model. In the present studies, we selected compounds **87b**, **87g**, and **87h** for evaluating their therapeutic efficacy in nude mice

bearing human mammary carcinoma (MX-1) xenografts (fig. 1, 2). To find a maximal tolerable dose of compound tested, we administrated various doses to view its therapeutic effects via intravenous injection (*iv inj*.). The preliminary results show that compound **87h** possessed significant tumor growth inhibition (72 %) in comparison with the untreated control when mice were treated successfully with the dose of 50 mg/kg, every two days for three times (Q2D×3), 60 mg/kg (Q2D×3), and then 70 mg/kg, every two days for two times (Q2D×2). With the same drug administration route (iv inj.), compound **87b** showed a moderate tumor inhibition (62 %), at the doses of 30 mg/kg (Q2D×3) and 35 mg/kg, every two days for five times (Q2D×5). Compound **87g** also showed to have moderate tumor suppression (52 %) at the dose of 30 mg/kg (Q2D×3) and then 40 mg/kg (Q2D×5). Although all the tested compounds induced a 5-7% body-weight change during the treatment (Table 3), the body-weight of mice readily recovered after cessation of the treatment, suggesting that these agents have relatively low toxicity to the host.

Conjugates **87b** and **87g** were also selected for evaluating their therapeutic efficacy against human prostate PC-3 xenograft in nude mice. Figure 3 shows that there were 27 % and 69 % tumor suppression by **87b** and **87g**, respectively, at the maximal tolerable dose of 55 mg/kg, every two days for six times (Q2D×6) with acceptable toxicity (about 10-11 % body weight loss). Similar observations were found that *N*-mustard-quinazoline conjugates are much less potent against human breast MX-1 xenograft in mice than that of the *N*-mustard-quinoline conjugates.[95]

Figure 1. Therapeutic effect of **87b** (50 mg/kg, i.v. infusion, Q2Dx3, 60 mg/kg, i.v. infusion, Q2Dx3, 70 mg/kg, i.v. infusion, Q2Dx2), and **87h** (30 mg/kg, i.v. infusion, Q2Dx3, 35 mg/kg, i.v. infusion, Q2Dx5, n=4), in nude mice bearing human mammary carcinoma MX-1 xenograft; average tumor size changes (Figure 1A) and average body weight changes (Figure 1B).



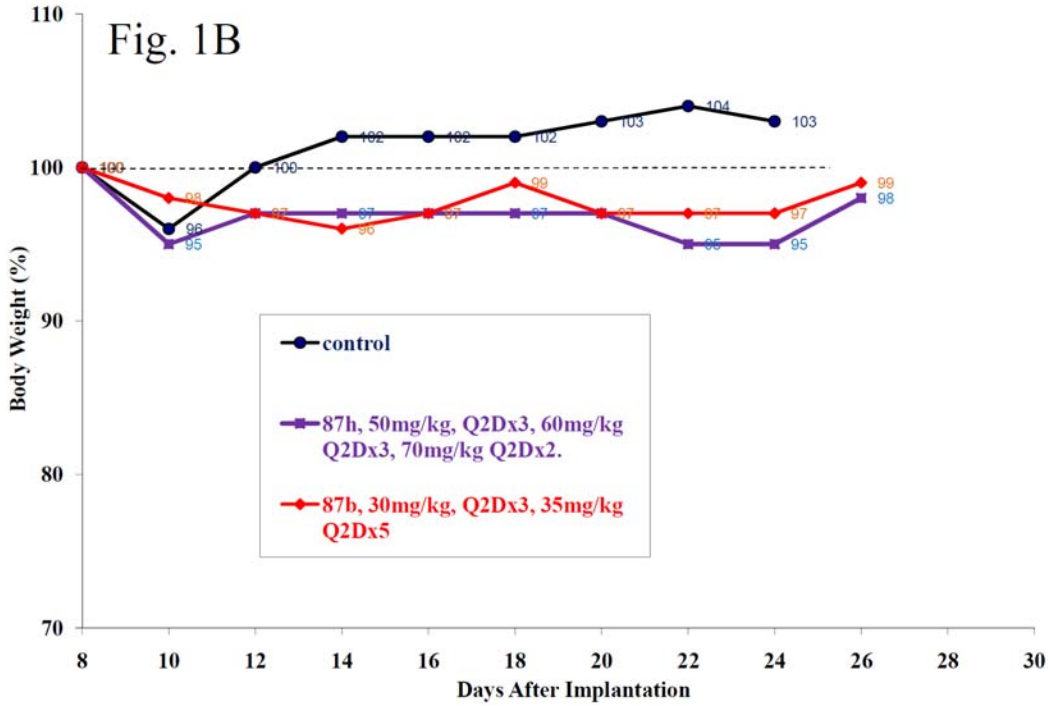
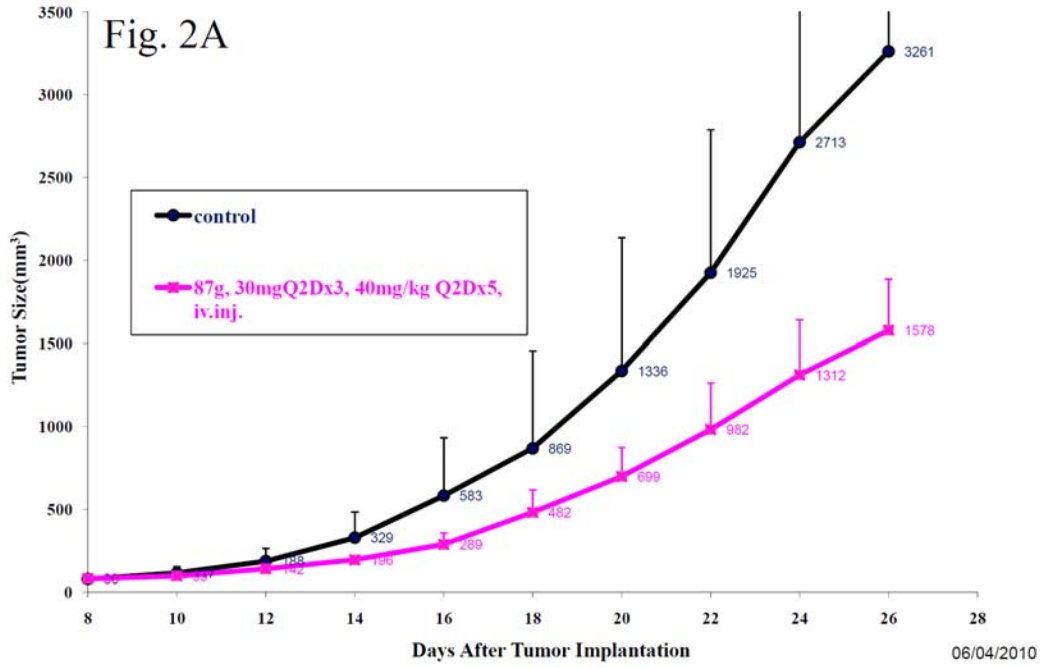


Figure 2. Therapeutic effect of **87g** (30 mg/kg, i.v. infusion, Q2Dx3, 40 mg/kg, i.v. infusion, Q2Dx5), in nude mice bearing human mammary carcinoma MX-1 xenograft; average tumor size changes (Figure 2A) and average body weight changes (Figure 2B).



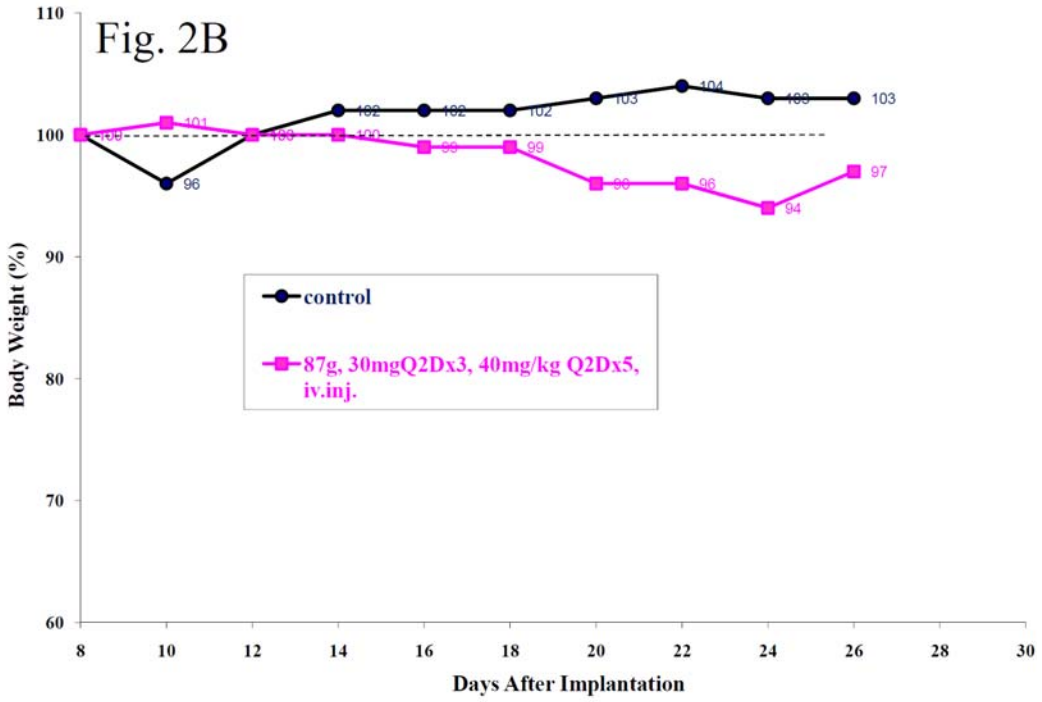
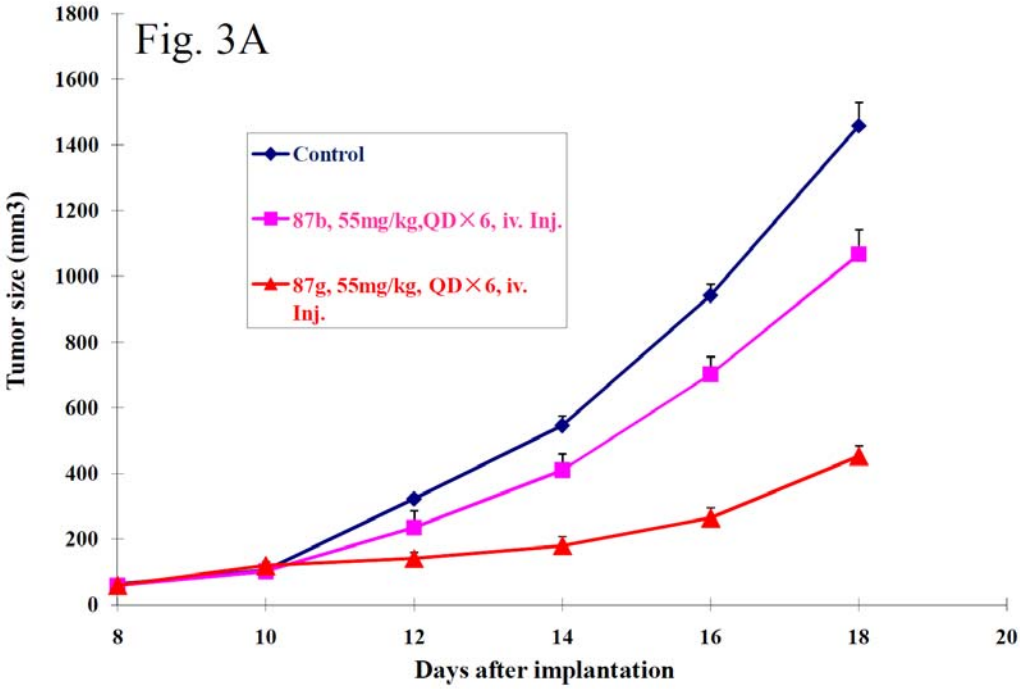
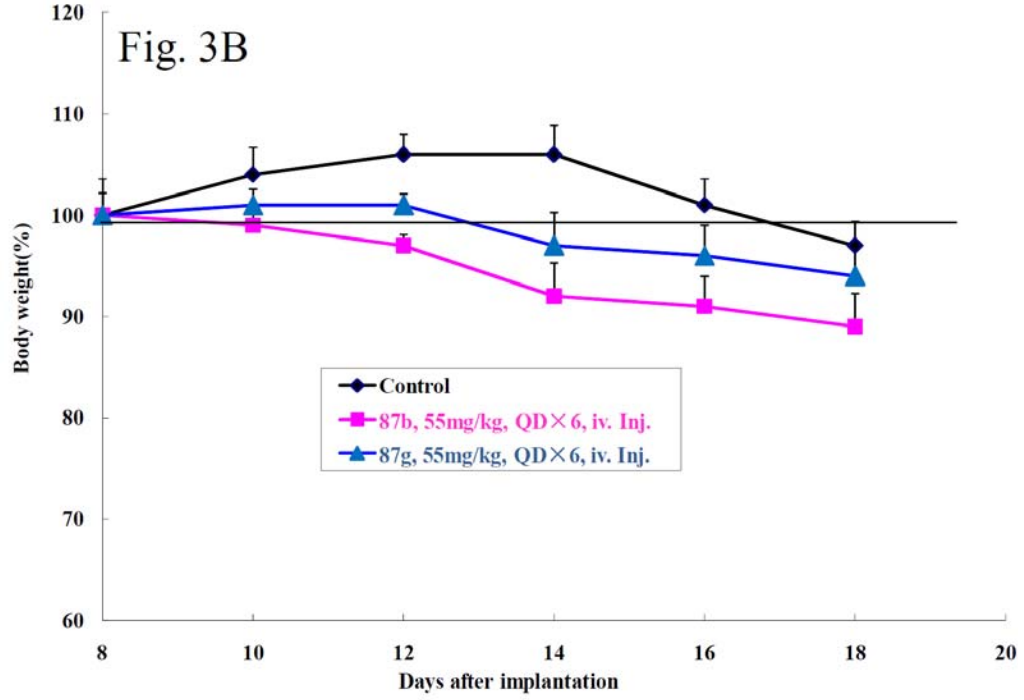


Figure 3. Therapeutic effect of **87b** (55 mg/kg, i.v. infusion, Q2Dx6, n=5), and **87g** (55 mg/kg, i.v. infusion, Q2Dx6, n=5), in nude mice bearing prostate PC-3 xenograft; average tumor size changes (Figure 3A) and average body weight changes (Figure 3B).





3.1.3 DNA cross-linking study by alkaline agarose gel shift assay

The alkaline gel shift assay was performed to assess DNA cross-linking activity of compounds **87g**, **87l**, **87b**, and **87e** (Fig. 4). The pEGFP-N1 plasmid DNA was treated with compounds, **87g**, **87l**, **87b**, and **87e** at various concentrations as indicated (1, 10 and 20 μM). Melphalan was used as a positive control. The tested compounds show moderate cross-linking behavior at lower concentrations; however, at high concentrations, the cross-linking behavior was similar to melphalan. These results revealed that the newly synthesized *N*-mustard-quinazoline conjugates are capable to induce DNA cross-linking.

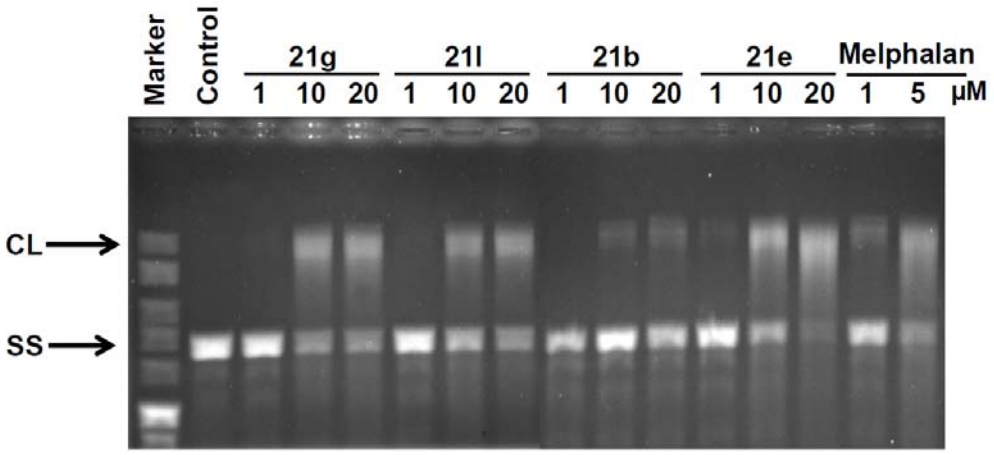


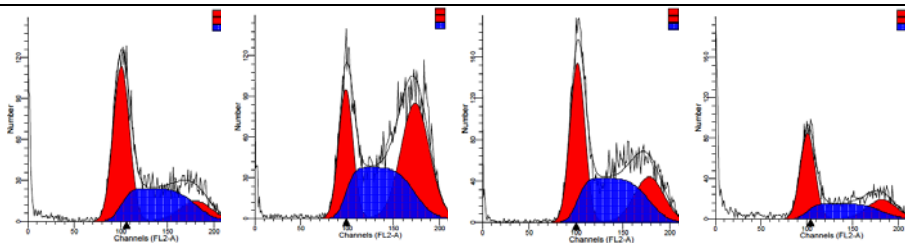
Figure 4. Representative DNA cross-linking gel shift assay for **87g**, **87l**, **87b**, and **87e** at various concentrations as indicated. Control lane shows single-stranded DNA (SS), while cross-linking (CL) shown in all tested lanes is DNA double-stranded cross-linking. Melphalan (1 and 5 μM) was used as a positive control.

3.1.4 Cell cycle inhibition

It was reported that DNA damage induced by DNA alkylating agents is known to cause cell cycle delay and arrest the cell cycle progression predominantly at the G2/M boundary.[99] We therefore studied the inhibitory effect of **87b** on cell cycle distribution (Table 3). The human non small lung carcinoma H1299 cells were treated with **87b** at the concentrations of 5, 10, and 20 μM for 24 h. The cells were harvested, stained with propidium iodide (PI) and analyzed with a flow cytometer. It clearly shows that 5 μM of **87b** significantly accumulated the cells at G2/M phase, while 10 and 20 μM of **87b** prevented the cell cycle progression, which may be due to high level of DNA cross-linking. Similar G2/M arrest was previously observed in SW626 cells treated

with melphalan.[100] Furthermore, increased sub-G1 populations were noticed in cells treated with **87b** at 20 μM.

Table 3. Cell cycle inhibition in human non-small cell lung adenocarcinoma H1299 by treating with compound **87b**.

Concentration (μM)	0	5	10	20
				
sub G1	8.8±6.9	8.9±0.03	7.5±0.2	35.3±2.2
G1	44.8±3.0	30.5±0.4	34.8±0.4	28.3±1.0
S	24.9±3.3	20.6±4.0	28.5±0.2	23.5±3.5
G2/M	21.5±0.6	40.0±3.5	29.3±0.01	12.9±0.2

3.2 Biological experiments

3.2.1 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 assay[104] and human solid tumor cells (*i.e.* breast carcinoma MX-1 and colon carcinoma HCT-116) the SRB assay[105] in a 72 h incubation using a microplate spectrophotometer as described previously.[106] After the addition of phenazine methosulfate-XTT solution at 37 ^{o}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, were determined by the Alamar blue assay[107] in a 72 h incubation using a microplate spectrophotometer as described previously. After the addition of Alamar blue solution, it was incubated at 37 ^{o}C for 6 h. Absorbance at 570 and 600 nm was

detected on a microplate reader. IC_{50} values were determined from dose-effect relationship at six or seven concentrations of each drug by using the CompuSyn software by Chou and Martin[108] based on the median-effect principle and plot.[109, 110] Ranges given for taxol and vinblastine were mean ± SE (n = 4).

3.2.2 *In vivo* studies

Athymic nude mice bearing the nu/nu gene were used for human breast tumor MX-1 and prostate PC-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 administrated via the tail vein by i.v. injection.[103] 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.

3.2.3 Alkaline agarose gel shift assay

Formation of DNA cross-linking was analyzed by alkaline agarose gel electrophoresis. In brief, purified pEGFP-N1 plasmid DNA (1500 ng) was mixed with various concentrations (1–20 μM) of **21g**, **21l**, **21b** and **21e** 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^{o}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 (1000 ng) 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^{o}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.

3.2.4 Flow cytometric analysis

The effects of **21b** on cell cycle distribution were analyzed with a flow cytometer as described previously.[111] Briefly, human non-small cell lung carcinoma H1299 cells were treated with **21b** at 5, 10, and 20 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.

3.3 Conclusion

To continue our research on the developing DNA-directed alkylating agents, we have synthesized a series of *N*-mustard-quinazoline conjugates, in which the *N*-mustard pharmacophore was attached at the C-6 of the 4-anilinoquinazolines via a urea linker. The preliminary antitumor studies revealed that these agents exhibited significant antitumor activity in inhibiting various human tumor cell growths *in vitro*. Among compounds selected for evaluating their antitumor activity against human breast tumor (MX-1) xenograft in nude mice, 4-[3'-Cl,4'-F-phenylamino]quinazoline derivative (**87h**) is the most potent. Studies on the therapeutic efficacy against MX-1 xenograft in nude mice revealed that the tested compounds have moderate antitumor activity, but they are less toxic to the host based on the observation of the average body-weight changes. In the present studies we also show that the newly synthesized compounds are able to induce DNA cross-linking through alkaline agarose gel shift assay and inhibited cell cycle arrest at G2/M phase.

Chapter -4

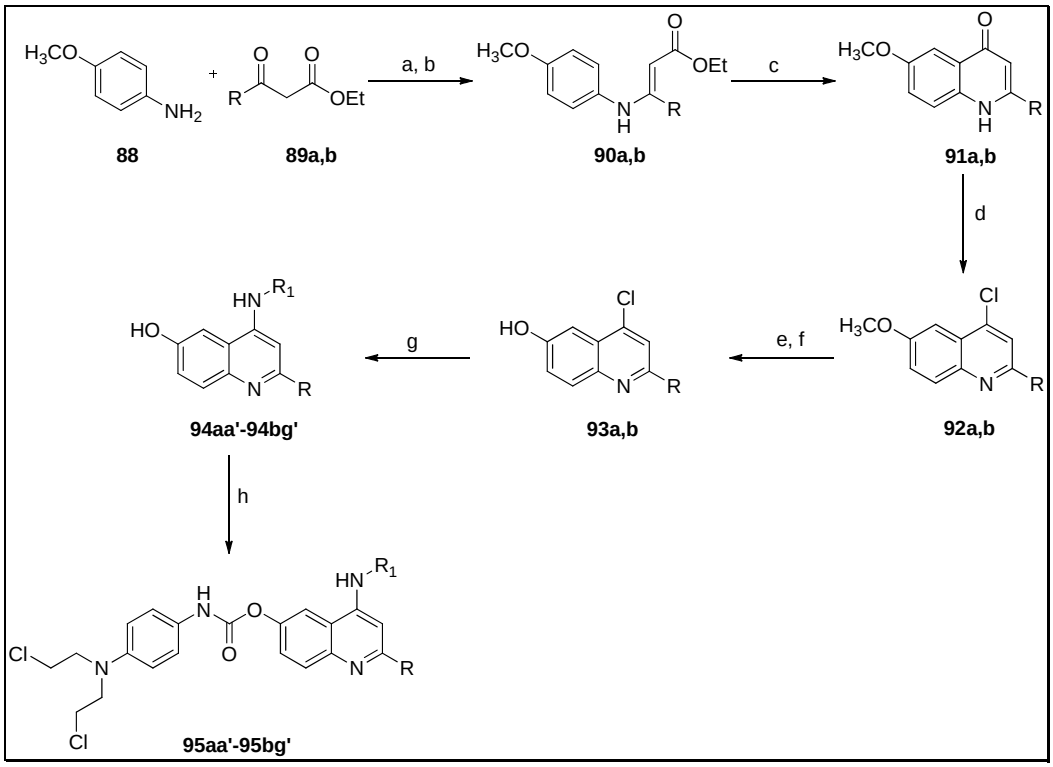
Synthesis of some novel *N*-mustard -4-anilinoquinoline conjugates via a carbamate linker

4.1 Chemistry

The general synthetic route of the newly *N*-mustard-6-hydroxyquinoline conjugates is shown in Schemes 1. The *N*-mustard-6-hydroxyquinoline conjugates (**95aa'-95bg'**) was prepared starting from the commercially available 4-nitroaniline **88** was reacted with the commercially available substituted ethyl 3-oxobutanoate **89a-b** to give corresponding (E)-ethyl 3-((4-methoxyphenyl)amino)but-2-enoate **90a** and (E)-ethyl 3-((4-methoxyphenyl)amino)-3-phenylacrylate **90b** in ethanol at 50°C, and this products was cyclized by using diphenylether at 250°C to give 6-methoxy-2-subsitutedquinolin-4(1H)-one **91a-b** with good yield[110-112]. Compounds (**91a-b**), was treated with $POCl_3$ to produce substituted 4-chloro-6-methoxyquinoline (**92a-b**)[113]. Compound **92a** was then treated with boron tribromide at -50 ^oC by following literature procedure[114] gave 4-chloro-2-methylquinoline-6-ol (**93a**). Compound **92b** was treated with sulfuric acid at 100 ^oC to give 4-chloro-2-phenylquinolin-6-ol (**93b**). The other compounds **94aa'-bg'** were obtained by the treatment of Substituted aniline in Isopropanol (IPA) in the presence of concentrated HCl[115]. Reaction of **94aa'-bg'** with the known 4-[*N*,*N*-bis(2-chloroethyl)-amino]phenylisocyanate **81** (chapter 2) [freshly prepared from *N*,*N*-bis(2-chloroethyl)benzene-1,4-diamine hydrochloride (**80**)] in the presence of triethylamine afforded *N*-mustard-6-hydroxyquinoline c.onjugate **95aa'-bg'** bearing a carbamate linker.

4.2 Reaction Scheme

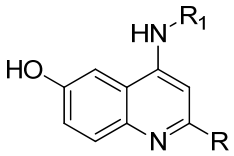
Scheme 1. Synthetic route for *N*-mustard-4-anilinoquinazoline conjugates bearing a carbamate linker



Reagent and condition: (a) con. HCl/room temperature, (b) EtOH/AcOH/50°C, (c) Diphenylether/250°C, (d) $POCl_3$/reflux, (e) BBr_3/MDC/-50°C, (f) H_2SO_4/100°C, (g) IPA/con.HCl/reflux (h) Et_3N/DMF/room temperature.

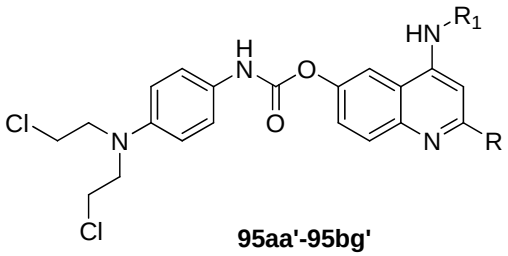
4.3 Physical Data

Table 1. Analytical data and yields of Substituted 6-hydroxy-4-anilinoquinolines (94aa'-bg')



Sr. No.	Substitute R	Substitute R1	MF	MW	Yield %	MP °C
94aa'	Me	C_6H_5	$C_{16}H_{14}N_2O$	250.30	91	>300
94ab'	Me	3'-F-C_6H_4	$C_{16}H_{13}FN_2O$	268.29	80	>300
94ac'	Me	3'-Cl-C_6H_4	$C_{16}H_{13}ClN_2O$	284.74	81	>300
94ad'	Me	3'-Br-C_6H_4	$C_{16}H_{13}BrN_2O$	329.19	82	>300
94ae'	Me	4'-F-C_6H_4	$C_{16}H_{13}FN_2O$	268.29	95	>300
94af'	Me	4'-Cl-C_6H_4	$C_{16}H_{13}ClN_2O$	284.74	95	228–229
94ag'	Me	4'-Br-C_6H_4	$C_{16}H_{13}BrN_2O$	329.19	94	>300
94ba'	Ph	C_6H_5	$C_{21}H_{16}N_2O$	312.36	52	>300
94bb'	Ph	3'-F-C_6H_4	$C_{21}H_{15}FN_2O$	330.36	79	193-194
94bc'	Ph	3'-Cl-C_6H_4	$C_{21}H_{15}ClN_2O$	346.81	58	>300
94bd'	Ph	3'-Br-C_6H_4	$C_{21}H_{15}BrN_2O$	391.26	82	>300
94be'	Ph	4'-F-C_6H_4	$C_{21}H_{15}FN_2O$	330.36	61	249-250
94bf'	Ph	4'-Cl-C_6H_4	$C_{21}H_{15}ClN_2O$	346.81	70	293-294
94bg'	Ph	4'-Br-C_6H_4	$C_{21}H_{15}BrN_2O$	391.26	72	>300

Table 2. Analytical data and yields of Substituted N-mustard quinoline carbamate conjugates (95aa'-bg')



95aa'-95bg'

Sr. No.	Substitute R	Substitute R1	MF	MW	Yield %	MP °C
95aa'	Me	C_6H_5	$C_{27}H_{26}Cl_2N_4O_2$	509.43	40	202-203
95ab'	Me	3'-F-C_6H_4	$C_{27}H_{25}Cl_2FN_4O_2$	527.42	38	207-208
95ac'	Me	3'-Cl-C_6H_4	$C_{27}H_{25}Cl_3N_4O_2$	543.87	51	224-225
95ad'	Me	3'-Br-C_6H_4	$C_{27}H_{25}BrCl_2N_4O_2$	588.32	34	201-202
95ae'	Me	4'-F-C_6H_4	$C_{27}H_{25}Cl_2FN_4O_2$	527.42	36	223-224
95af'	Me	4'-Cl-C_6H_4	$C_{27}H_{25}Cl_3N_4O_2$	543.87	31	189-191
95ag'	Me	4'-Br-C_6H_4	$C_{27}H_{25}BrCl_2N_4O_2$	588.32	30	229-231
95ba'	Ph	C_6H_5	$C_{32}H_{28}Cl_2N_4O_2$	571.50	42	209-210
95bb'	Ph	3'-F-C_6H_4	$C_{32}H_{27}Cl_2FN_4O_2$	589.49	45	210-211
95bc'	Ph	3'-Cl-C_6H_4	$C_{32}H_{27}Cl_3N_4O_2$	605.94	37	197-198
95bd'	Ph	3'-Br-C_6H_4	$C_{32}H_{27}BrCl_2N_4O_2$	650.39	37	186-187
95be'	Ph	4'-F-C_6H_4	$C_{32}H_{27}Cl_2FN_4O_2$	589.49	50	186-187
95bf'	Ph	4'-Cl-C_6H_4	$C_{32}H_{27}Cl_3N_4O_2$	605.94	38	204-205
95bg'	Ph	4'-Br-C_6H_4	$C_{32}H_{27}BrCl_2N_4O_2$	650.39	40	213-214

4.4 Experimental Section

General methods and materials

Compound solvents and reagents were reagent grade and used without purification unless otherwise noted. The melting points were recorded on a Fargo melting point apparatus and are uncorrected. Column chromatography was carried out on silica gel G60 (70-230mesh, ASTM; Merck and 230-400 mesh, silicycle inc.). Reaction progress was monitored using analytical thin-layer chromatography (TLC) on 0.25mmMerck F-254 silica gel glass plates. Visualization was achieved by UV light (254 nm). ^{1}H NMR spectra were recorded with a Bruker AVANCE 600 DRX and 400 MHz spectrometer; Chemical shifts are reported in parts per million (δ) using tetramethylsilane as the internal standard with coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; brs, broad singlet. Elemental analyses were performed on a Heraeus CHN-O Rapid analyzer. 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 (5μL) mobile phase, MeCN/THF (70:30 v/v); flow rate, 1 mL/min; injected sample 10μL, column temp, 27°C; wavelength, 254nm. The purity of all compounds was > 95 % based on analytical HPLC.

(*E*)-Ethyl-3-(4-methoxyphenylamino)but-2-enoate (90a). *P*-anisidine (**88**, 50.00gm, 0.40mol) and Ethylaetoacetate (**89a**, 52.84 g, 0.40mol) was mixes well and sonicated for 30 min. In that, solution was added con. HCl (3 mL) at room temperature, over 5 min. the mixture was then stirred for 12 h at room temperature. After completion of the reaction, the mixture was diluted with water, and aqueous layer extracted three times with ethyl acetate. The organic layer was washed two times with water and

dried over Na_2SO_4. After evaporation, the solid product was washed with hexane to give **90a**, 35.0 g (63 %); ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, J = 7.0 Hz, Me), 1.88 (3H, s, Me), 3.75 (3H, s, Me), 4.05 (2H, q, J = 7.0 Hz, CH_2), 4.62 (1H, s, CH), 6.91–6.94 (2H, m, 2 × ArH), 7.11–7.13 (2H, m, 2 × ArH), 10.14 (1H, s, exchangeable, NH). Anal. ($C_{13}H_{17}NO_3$): C, H, N.

(*E*)-Ethyl -3-(4-methoxyphenylamino)-3-phenylacrylate (90b). To a solution of 4-methoxyaniline (**88**, 12.3 g, 100 mmol) and ethanol (600 mL) was added ethyl benzoylacetate (**89b**, 19.2 g, 100 mmol) at room temperature. The reaction mixture was heated up to 50°C. The acetic acid (2 mL) was added into the reaction mixture at 50°C. The reaction mixture was heated up to reflux temperature for 24 hrs. After completion of the reaction, it was cooled to room temperature. The solid was collected by filtration, washed with ethanol and dried to give **90b**, 10.9 g, (42 %); mp 112–113°C (111–113°C lit); ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 7.2 Hz, Me), 3.64 (3H, s, Me), 4.11 (2H, q, J = 7.2 Hz, CH_2), 4.85 (1H, s, CH), 6.70 (4H, s, 4 × ArH), 7.31–7.36 (5H, m, 5 × ArH), 10.11 (1H, s, exchangeable, NH). Anal. ($C_{18}H_{19}NO_3$): C, H, N.

6-Methoxy-2-methylquinoline-4(1*H*)-one (91a). To a magnetically stirred solution of diphenylether(552 mL) was added portion wise (*E*)-Ethyl-3-(4-methoxyphenylamino)but-2-enoat (**90a**, 69.0 g, 293 mmol) at 240° C over 30min. After that, the mixture was then stirred for 1h at 250°C. After completion of the reaction, the precipitated product was separated from the reaction mixture by filtration and was washed with hexane. The desired product was obtained as a yellowish powder. Then crud product was slurred with chloroform and filter it washed with little amount of solvent to give **91a**, 35.0 g (63 %); ^{1}H NMR (DMSO-d_6) δ 2.34 (3H, s,

Me), 3.82 (3H, s, Me), 5.90 (1H, s, ArH), 7.24–7.27 (1H, m, ArH), 7.46–7.49 (2H, m, 2 × ArH), 11.66 (1H, s, exchangeable, NH). Anal. ($C_{11}H_{11}NO_2$): C, H, N.

6-Methoxy-2-phenylquinoline-4(1*H*)-one (91b). The diphenylether (350 mL) was heated up to 240°C. The (*E*)-ethyl 3-(4-methoxyphenylamino)-3-phenylacrylate (**90b**, 44.6 g, 150 mmol) was added portionwise into the diphenylether at 240°C. After completion of the addition, the temperature was raised to 250°C. After 20 min, the mixture was cooled to room temperature. The solid was come out from reaction mass. The solid was collected by filtration and washed with hexane to give **91b**, 34.0 g, (92 %); mp 309–310°C (302–304°C lit.); ^{1}H NMR (DMSO-d_6) δ 3.85 (3H, s, Me), 6.34 (1H, brs, ArH), 7.33 (1H, dd, J = 2.8 Hz, J = 9.1 Hz, ArH), 7.51 (1H, d, J = 2.8 Hz, ArH), 7.57–7.59 (3H, m, 3 × ArH), 7.53 (1H, d, J = 9.1 Hz, ArH), 7.83–7.89 (2H, m, 2 × ArH), 11.70 (1H, brs, exchangeable, NH). Anal. ($C_{16}H_{13}NO_2$): C, H, N.

4-Chloro-6-methoxy-2-methylquinoline (92a). To a magnetically stirred solution of $POCl_3$ (161 mL) at 0°C was added portion wise 6-methoxy-2-methylqunolin-4(1H)-one (**91a**, 35 g, 185 mmol) over 30min. The temperature was raised to 110°C and the mixture was stirred for 2h. After completion of reaction, the excess $POCl_3$ was removed by vacuo. The mixture was poured into ice water. The reaction mass was neutralized by Sodium bicarbonate and last ammonia solution. After neutralize, the precipitated product was separated from the reaction mixture. It was filtered and washed with water, finally with hexane and dried to give **92a**, 33.0 g (86 %); ^{1}H NMR (DMSO-d_6) δ 2.61 (3H, s, Me), 3.93 (3H, s, Me), 7.38 (1H, d, J = 3.0 Hz, ArH), 7.46 (1H, dd, J = 3.0 Hz, J = 9.2 Hz, ArH), 7.64 (1H, s, ArH), 7.91 (1H, d, J = 9.2 Hz, ArH). Anal. ($C_{11}H_{10}ClNO$): C, H, N.

4-Chloro-6-methoxy-2-phenylquinoline (92b). The 6-methoxy-2-phenylquinoline-4(1*H*)-one (**91b**, 5.02 g, 20 mmol) was added portionwise into the solution of $POCl_3$

(20 mL) at room temperature. After that, the reaction mixture was heated up to reflux temperature for 1 hour. After completion of the reaction, it was cooled up to room temperature. The excess POCl3 was removed by vacuum distillation. The residue was poured into ice water and neutralized by ammonia solution. The solid was collected by filtration, washed with water and dried, to give **92b**, 4.0 g, (74 %); mp 106–107°C (102–103°C lit.); ^{1}H NMR (DMSO-d_6) δ 3.97 (3H, s, Me), 7.43–7.44 (1H, m, ArH), 7.48–7.57 (4H, m, 4 × ArH), 8.04–8.06 (1H, m, ArH), 8.25–8.27 (2H, m, 2 × ArH), 8.33 (1H, s, ArH). Anal. ($C_{16}H_{12}ClNO$): C, H, N.

4-Chloro-2-methylquinoline-6-ol (93a). The boron tribromide (1.9 ml, 20 mmol) was added in the MDC (20 ml) at -50°C. After being stirred 10 min, the 4-chloro-6-methoxy-2-methylquinoline (**92a**, 1.28 g, 6.0 mmol) was added over the top at -50°C. The reaction mixture was stirred at room temperature for 2 hours. After completion of the reaction, the solvent was evaporated under reduce pressure. The residue was poured into saturated sodium bicarbonate solution. The solid was separated out. It was filtered and washed with water. To give **93a**, 1.0 g (84.03 %); mp 224–225°C (223–224°C lit.); ^{1}H NMR (DMSO-d_6) δ 2.57 (3H, s, Me), 7.32–7.35 (2H, m, 2 × ArH), 7.54 (1H, s, ArH), 7.82–7.84 (1H, m, ArH), 10.24 (1H, s, exchangeable, OH). Anal. ($C_{10}H_8ClNO$): C, H, N.

4-Chloro-2-phenylquinolin-6-ol (93b). The 4-chloro-6-methoxy-2-phenylquinoline (**92b**, 6.0 g, 22 mmol) was added into the solution of sulfuric acid (60 mL) at room temperature. The reaction mixture was heated up to 100°C for 2 hrs. The reaction mass was poured into ice. The solid was collected by filtration and washed with water. To give **93b**, 5.0 g, (88 %); mp 194–196°C; ^{1}H NMR (DMSO-d_6) δ 7.39–7.43 (2H, m, 2 × ArH), 7.47–7.54 (3H, m, 3 × ArH), 7.98–8.01 (1H, m, ArH), 8.22–8.25 (3H, m, 3 × ArH), 10.44 (1H, s, exchangeable, OH). Anal. ($C_{15}H_{10}ClNO$): C, H, N.

4-(Phenylamino)-2-methylquinolin-6-ol (94aa'). To a solution of 4-chloro-2-methyl-6-hydroxyquinoline (**93a**, 1.35 g, 7.0 mmol), aniline (0.78 g, 8.4 mmol) and IPA (30 mL) was added HCl two drops at room temperature. The reaction mixture was heated up to reflux temperature for 6 hours. After completion of the reaction, it was cooled to room temperature. The solid was filtered off and washed with IPA to give **94aa',** yield 1.6 g (91 %); mp > 300°C; ^{1}H NMR (DMSO-d_6) δ 2.57 (3H, s, Me), 6.63 (1H, s, ArH), 7.38–7.41 (1H, m, ArH), 7.44–7.46 (2H, m, 2 × ArH), 7.58–7.61 (3H, m, 3 × ArH), 7.89–7.90 (1H, m, ArH), 7.93–7.95 (1H, m, ArH), 10.28 (1H, s, exchangeable, OH), 10.57 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{14}N_2O$): C, H, N.

4-(3-Fluorophenylamino)-2-methylquinolin-6-ol (94ab'). Compound **94ab'** was synthesized from 4-chloro-2-methyl-6-hydroxyquinoline (**93a**, 1.5 g, 7.7 mmol), 3-fluoroaniline (0.90 g, 8.2 mmol) and IPA (35 mL) containing HCl 1-2 drops: yield 1.6 g (80 %); mp > 300°C; ^{1}H NMR (DMSO-d_6) δ 2.61 (3H, s, Me), 6.79 (1H, s, ArH), 7.19–7.24 (1H, m, ArH), 7.32–7.37 (2H, m, 2 × ArH), 7.55–7.62 (2H, m, 2 × ArH), 7.88–7.89 (1H, m, ArH), 7.97–7.99 (1H, m, ArH), 10.33 (1H, s, exchangeable, OH), 10.63 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{13}FN_2O$): C, H, N.

4-(3-Chlorophenylamino)-2-methylquinolin-6-ol (94ac'). Compound **94ab'** was synthesized from 4-chloro-2-methyl-6-hydroxyquinoline (**93a**, 0.5 g, 2.5 mmol), 3-chloroaniline (0.32 g, 2.5 mmol) and IPA (30 mL) containing HCl 1-2 drops: yield 0.6 g (81 %); mp > 300°C; ^{1}H NMR (DMSO-d_6) δ 2.60 (3H, s, Me), 6.75 (1H, s, ArH), 7.43–7.47 (2H, m, 2 × ArH), 7.54–7.56 (2H, m, 2 × ArH), 7.59-7.62 (1H, dd, J = 2.4 Hz, J = 9.2 Hz, ArH), 7.88 (1H, d, J = 2.4 Hz, ArH), 7.99 (1H, d, J = 9.2 Hz, ArH), 10.34 (1H, s, exchangeable, OH), 10.64 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{13}ClN_2O$): C, H, N.

4-(3-Bromophenylamino)-2-methylquinolin-6-ol (94ad'). Compound **94ad'** was synthesized from 4-chloro-2-methyl-6-hydroxyquinoline (**93a**, 1.0 g, 5.0 mmol), 3-bromoaniline (0.91 g, 5.3 mmol) and IPA (30 mL) containing HCl 1-2 drops: yield 1.4 g (82 %); mp > 300°C; ^{1}H NMR (DMSO-d_6) δ 2.60 (3H, s, Me), 6.74 (1H, s, ArH), 7.49–7.51 (2H, m, 2 × ArH), 7.57–7.61 (2H, m, 2 × ArH), 7.68 (1H, s, ArH), 7.86–7.87 (1H, m, ArH), 7.94–7.96 (1H, m, ArH), 10.30 (1H, s, exchangeable, OH), 10.61 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{13}BrN_2O$): C, H, N.

4-(4-Fluorophenylamino)-2-methylquinolin-6-ol (94ae'). Compound **94ae'** was synthesized from 4-chloro-2-methyl-6-hydroxyquinoline (**93a**, 1.5 g, 7.7 mmol), 4-fluoroaniline (0.90 g, 8.2 mmol) and IPA (35 mL) containing HCl 1-2 drops: yield 1.9 g (95 %); mp > 300°C; ^{1}H NMR (DMSO-d_6) δ 2.56 (3H, s, Me), 6.54 (1H, s, ArH), 7.36–7.41 (2H, m, 2 × ArH), 7.47–7.50 (2H, m, 2 × ArH), 7.59 (1H, dd, J = 2.4 Hz, J = 8.8 Hz, ArH), 7.88 (1H, d, J = 2.4 Hz, ArH), 7.96 (1H, d, J = 8.8 Hz, ArH), 10.28 (1H, s, exchangeable, OH), 10.60 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{13}FN_2O$): C, H, N.

4-(4-Chlorophenylamino)-2-methylquinolin-6-ol (94af'). Compound **94af'** was synthesized from 4-chloro-2-methyl-6-hydroxyquinoline (**93a**, 1.0 g, 5.0 mmol), 4-chloroaniline (0.68 g, 5.3 mmol) and IPA (30 mL) containing HCl 1-2 drops: yield 1.4 g (95 %); mp 228–229°C; ^{1}H NMR (DMSO-d_6) δ 2.58 (3H, s, Me), 6.69 (1H, s, ArH), 7.48–7.50 (2H, m, 2 × ArH), 7.58–7.62 (3H, m, 3 × ArH), 7.88– 7.89 (1H, m, ArH), 7.97–7.99 (1H, m, ArH), 10.31 (1H, s, exchangeable, OH), 10.61 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{13}ClN_2O$): C, H, N.

4-(4-Bromophenylamino)-2-methylquinolin-6-ol (94ag'). Compound **94ag'** was synthesized from 4-chloro-2-methyl-6-hydroxyquinoline (**93a**, 1.0 g, 5.0 mmo), 4-bromoaniline (0.91 g, 5.3 mmol) and IPA (30 mL) containing HCl 1-2 drops: yield

1.6 g (94 %); mp > 300°C; ^{1}H NMR (DMSO-d_6) δ 2.58 (3H, s, Me), 6.72 (1H, s, ArH), 7.42–7.44 (2H, m, 2 × ArH), 7.59 (1H, dd, *J* = 2.4 Hz, *J* = 8.8 Hz, ArH), 7.71–7.73 (2H, m, 2 × ArH), 7.87 (1H, d, *J* = 2.4 Hz, ArH), 7.96 (1H, d, *J* = 8.8 Hz, ArH), 10.28 (1H, s, exchangeable, OH), 10.60 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{13}BrN_2O$): C, H, N.

4-(Phenylamino)-2-phenylquinolin-6-ol (94ba'). To a solution of 4-chloro-2-phenylquinolin-6-ol (**93b**, 1.28 g, 5 mmol), aniline (0.56 g, 6 mmol) and IPA (30 mL) was added HCl two drops at room temperature. The reaction mixture was heated up to reflux temperature for 6 hours. After completion of the reaction, it was cooled to room temperature. The solid was collected by filtration and washed with IPA. To give **94ba'**, yield 0.8 g (52 %); mp > 300°C; ^{1}H NMR (DMSO-d_6) δ 6.90 (1H, s, ArH), 7.38–7.42 (1H, m, ArH), 7.55–7.59 (4H, m, 4 × ArH), 7.60–7.64 (3H, m, 3 × ArH), 7.70 (1H, dd, *J* = 2.4 Hz, *J* = 9.2 Hz, ArH), 7.85–7.87 (2H, m, 2 × ArH), 8.01 (1H, d, *J* = 2.4 Hz, ArH), 8.28 (1H, d, *J* = 9.2 Hz, ArH), 10.61 (1H, s, exchangeable, OH), 10.81 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{16}N_2O$): C, H, N.

4-(3-Fluorophenylamino)-2-phenylquinolin-6-ol (94bb'). Compound **94bb'** was synthesized from 4-chloro-2-phenylquinolin-6-ol (**93b**, 1.28 g, 5 mmol), 3-flouroaniline (0.7 g, 6 mmol) and IPA (30 mL) containing HCl 1-2 drops: yield 1.3 g (79 %); mp 193–194°C; ^{1}H NMR (DMSO-d_6) δ 7.05 (1H, s, ArH), 7.20–7.24 (1H, m, ArH), 7.42–7.48 (2H, m, 2 × ArH), 7.56–7.66 (4H, m, 4 × ArH), 7.69 (1H, dd, *J* = 2.4 Hz, *J* = 9.1 Hz, ArH), 7.88–7.90 (2H, m, 2 × ArH), 7.96 (1H, d, *J* = 2.4 Hz, ArH), 8.24 (1H, d, *J* = 9.1 Hz, ArH), 10.57 (1H, s, exchangeable, OH), 10.78 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{15}FN_2O$): C, H, N.

4-(3-Chlorophenylamino)-2-phenylquinolin-6-ol (94bc'). Compound **94bc'** was synthesized from 4-chloro-2-phenylquinolin-6-ol (**93b**, 1.28 g, 5 mmol), 3-

chloroaniline (0.77 g, 6 mmol) and IPA (30 ml) containing HCl 1-2 drops: 1.0 g (58 %); mp > 300°C; ^{1}H NMR (DMSO-d_6) δ 7.03 (1H, s, ArH), 7.43–7.45 (1H, m, ArH), 7.55–7.60 (2H, m, 2 × ArH), 7.62–7.65 (4H, m, 4 × ArH), 7.69 (1H, dd, J = 2.0 Hz, J = 9.2 Hz, ArH), 7.89–7.91 (2H, m, 2 × ArH), 7.96 (1H, d, J = 2.0 Hz, ArH), 8.27 (1H, d, J = 9.2 Hz, ArH), 10.60 (1H, s, exchangeable, OH), 10.80 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{15}ClN_2O$): C, H, N.

4-(3-Bromophenylamino)-2-phenylquinolin-6-ol (94bd'). Compound **94bd'** was synthesized from 4-chloro-2-phenylquinolin-6-ol (**93b**, 1.28 g, 5 mmol), 3-bromoaniline (1.03 g, 6 mmole) and IPA (30 mL) containing HCl 1-2 drops: yield 1.6 g (82 %); mp >300°C; ^{1}H NMR (DMSO-d_6) δ 7.03 (1H, s, ArH), 7.48–7.52 (1H, m, ArH), 7.56–7.58 (1H, m, ArH), 7.61–7.68 (5H, m, 5 × ArH), 7.76–7.77 (1H, m, ArH), 7.87–7.89 (2H, m, 2 × ArH), 7.93–7.94 (1H, m, ArH), 8.19–8.21 (1H, m, ArH), 10.53 (1H, s, exchangeable, OH), 10.76 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{15}BrN_2O$): C, H, N.

4-(4-Fluorophenylamino)-2-phenylquinolin-6-ol (94be'). Compound **94be'** was synthesized from 4-chloro-2-phenylquinolin-6-ol (**93b**, 1.28 g, 5 mmol), 4-fluoroaniline (0.70 g, 6 mmol) and IPA (30 mL) containing HCl 1-2 drops: yield 1.0 g (61 %); mp 293–294°C; ^{1}H NMR (DMSO-d_6) δ 6.83 (1H, s, ArH), 7.38–7.42 (2H, m, 2 × ArH), 7.57–7.61 (2H, m, 2 × ArH), 7.62–7.66 (4H, m, 4 × ArH), 7.82–7.85 (2H, m, 2 × ArH), 7.39–8.11 (1H, m, ArH), 8.12–8.13 (1H, m, ArH), 10.47 (1H, s, exchangeable, OH), 10.68 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{15}FN_2O$): C, H, N.

4-(4-Chlorophenylamino)-2-phenylquinolin-6-ol (94bf'). Compound **94bf'** was synthesized from 4-chloro-2-phenylquinolin-6-ol (**93b**, 1.28 g, 5 mmol), 4-chloroaniline (0.77 g, 6 mmol) and IPA (30 mL) containing HCl 1-2 drops: yield 1.2 g(70 %); mp 293–294°C; ^{1}H NMR (DMSO-d_6) δ 6.98 (1H, s, ArH), 7.57–7.65 (4H,

m, 4 × ArH), 7.66–7.69 (4H, m, 4 × ArH), 7.87–7.90 (2H, m, 2 × ArH), 7.95–7.96 (1H, m, ArH), 8.20–8.22 (1H, m, ArH), 10.54 (1H, s, exchangeable, OH), 10.75 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{15}ClN_2O$): C, H, N.

4-(4-Bromophenylamino)-2-phenylquinolin-6-ol (94bg'). Compound **94bg'** was synthesized from 4-chloro-2-phenylquinolin-6-ol (**93b**, 1.28 g, 5 mmole), 4-bromoaniline (1.03 g, 6 mmole) and IPA (30 mL) containing HCl 1-2 drops: yield 1.4 g (72 %); mp >300°C; ^{1}H NMR (DMSO-d_6) δ 7.00 (1H, s, ArH), 7.52–7.55 (2H, m, 2 × ArH), 7.59–7.65 (3H, m, 3 × ArH), 7.69 (1H, dd, *J* = 2.4 Hz, *J* = 9.1 Hz, ArH), 7.72–7.74 (2H, m, 2 × ArH), 7.89–7.91 (2H, m, 2 × ArH), 7.97 (1H, d, *J* = 2.4 Hz, ArH), 8.26 (1H, d, *J* = 9.1 Hz, ArH), 10.57 (1H, s, exchangeable, OH), 10.79 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{15}BrN_2O$): C, H, N.

4-(Phenylamino)-2-methylquinolin-6-yl 4-(bis(2-chloroethyl)amino)phenyl carbamate (95aa'). To a solution of 4-(phenylamino)-2-methylquinolin-6-ol (**94aa'**, 0.50 g, 2.0 mmol) in dry DMF (25 mL) containing triethylamine (1.4 mL) was added a solution of isocynate **81** (freshly prepared from **80**, 1.53 g, 5.0 mmol) in dry DMF (5 mL) at room temperature. After being stirred for 2 h at room temperature, the solid was filtered and washed with dry DMF. The filtrate was evaporated to dryness in vacuo. The residue was purified by column chromatography using $CHCl_3$/MeOH (100:2 v/v) as an eluent. The fractions containing the main product were combined and evaporated to dryness and the residue was recrystallized from $CHCl_3$ to give **95aa'**, 0.40 g (40 %); mp 202–203 °C; ^{1}H NMR (Acetic acid-d_4) δ 2.65 (3H, s, Me), 3.67–3.75 (8H, m, 4 × CH_2), 6.76–6.80 (3H, m, 3 × ArH), 7.40–7.44 (3H, m, 3 × ArH), 7.49–7.61 (4H, m, 4 × ArH),), 7.81 (1H, dd, *J* = 2.1 Hz, *J* = 9.2 Hz, ArH), 8.05 (1H, d, *J* = 9.2 Hz, ArH), 8.34 (1H, d, *J* = 2.1 Hz, ArH). Anal. ($C_{27}H_{26}Cl_2N_4O_2 \cdot 1.7H2O$): C, H, N.

4-(3-Fluorophenylamino)-2-methylquinolin-6-yl 4-(bis(2-chloroethyl)amino) phenylcarbamate (95ab'). Compound **95ab'** was synthesized from isocynate **81** (freshly prepared from **80**, 1.53 g, 5.0 mmol) and 4-(3-fluorophenylamino)-2-methylquinolin-6-ol (**94ab'**, 0.54, 2.0 mmol) in dry DMF(20 mL) containing triethylamine (1.4 mL): Yield 0.40 (38 %); mp 207–208 °C; ^{1}H NMR (Acetic acid-d_4) δ 2.69 (3H, s, Me), 3.69–3.76 (8H, m, 4 × CH_2), 6.78 (2H, d, J = 8.8 Hz, 2 × ArH), 6.93 (1H, s, ArH), 7.12–7.17 (1H, m, ArH), 7.29–7.32 (1H, m, ArH), 7.34–7.36 (1H, m, ArH), 7.44 (2H, d, J = 8.8 Hz, 2 × ArH), 7.52–7.54 (1H, m, ArH), 7.81–7.84 (1H, m, ArH), 8.07–8.09 (1H, m, ArH), 8.34–8.35 (1H, m, ArH). Anal. ($C_{27}H_{25}Cl_2FN_4O_2$): C, H, N.

4-(3-chlorophenylamino)-2-methylquinolin-6-yl 4-(bis(2-chloroethyl)amino) phenyl carbamate (95ac'). Compound **95ac'** was synthesized from isocynate **81** (freshly prepared from **80**, 1.53 g, 5.0 mmole) and 4-(3-chlorophenylamino)-2-methylquinolin-6-ol (**94ac'**, 0.57, 2.0 mmole) in dry DMF(30 mL) containing triethylamine (1.4 mL): Yield 0.33 (51 %) ; mp 224–225 °C; ^{1}H NMR (Acetic acid-d_4) δ 2.68 (3H, s, Me), 3.67–3.76 (8H, m, 4 × CH_2), 6.77–6.79 (2H, m, 2 × ArH), 6.88 (1H, s, ArH), 7.40–7.47 (3H, m, 3 × ArH), 7.48–7.55 (3H, m, 3 × ArH), 7.81–7.84 (1H, m, ArH), 8.07–8.09 (1H, m, ArH), 8.34–8.35 (1H, m, ArH). Anal. ($C_{27}H_{25}Cl_3N_4O_2$): C, H, N.

4-(3-bromophenylamino)-2-methylquinolin-6-yl 4-(bis(2-chloroethyl)amino) phenyl carbamate (95ad'). Compound **95ad'** was synthesized from isocynate **81** (freshly prepared from **80,** 1.53 g, 5.0 mmol) and 4-(3-bromophenylamino)-2-methylquinolin-6-ol (**94ad'**, 0.66 g, 2.0 mmol) in dry DMF (25 mL) containing triethylamine (1.4 mL): Yield 0.40 g (34 %); mp 201–202 °C; ^{1}H NMR (Acetic acid-d_4) δ 2.67 (3H, s, Me), 3.68–3.77 (8H, m, 4 × CH_2), 6.75–6.77 (2H, m, 2 × ArH), 6.86

(1H, s, ArH), 7.41–7.47 (3H, m, 3 × ArH), 7.50–7.55 (3H, m, 3 × ArH), 7.80–7.84 (1H, m, ArH), 8.07–8.09 (1H, m, ArH), 8.33–8.34 (1H, m, ArH). Anal. ($C_{27}H_{25}BrCl_2N_4O_2 \cdot 2.2H2O$): C, H, N.

4-(4-fluorophenylamino)-2-methylquinolin-6-yl 4-(bis(2-chloroethyl)amino) phenyl carbamate (95ae'). Compound **95ae'** was synthesized from isocynate **81** (freshly prepared from **80**, 1.53 g, 5.0 mmole) and 4-(4-fluorophenylamino)-2-methylquinolin-6-ol (**94ae'**, 0.54, 2.0 mmole) in dry DMF (20 mL) containing triethylamine (1.4 mL): Yield 0.39 (36 %) ; mp 223–224 °C; ^{1}H NMR (Acetic acid-d_4) δ 2.65 (3H, s, Me), 3.69–3.75 (8H, m, 4 × CH_2), 6.71 (1H, s, ArH), 6.78 (2H, d, *J* = 8.9 Hz, 2 × ArH), 7.26–7.30 (2H, m, 2 × ArH), 7.43 (2H, d, *J* = 8.9 Hz, 2 × ArH), 7.50–7.54 (2H, m, 2 × ArH), 7.82 (1H, dd, *J* = 1.9 Hz, *J* = 9.2 Hz, ArH), 8.05 (1H, d, *J* = 9.2 Hz, ArH), 8.33 (1H, d, *J* = 1.9 Hz, ArH). Anal. ($C_{27}H_{25}Cl_2FN_4O_2$): C, H, N.

4-(4-chlorophenylamino)-2-methylquinolin-6-yl 4-(bis(2-chloroethyl)amino) phenyl carbamate(95af'). Compound **95af'** was synthesized from isocynate **81** (freshly prepared from **80**, 1.53 g, 5.0 mmole) and 4-(4-chlorophenylamino)-2-methylquinolin-6-ol (**94af'**, 0.57, 2.0 mmole) in dry DMF(20 mL) containing triethylamine (1.4 mL): Yield 0.55 (31 %) ; mp 189–191 °C; ^{1}H NMR (Acetic acid-d_4) δ 2.68 (3H, s, Me), 3.69–3.77 (8H, m, 4 × CH_2), 6.79 (2H, d, *J* = 9.0 Hz, 2 × ArH), 6.84 (1H, s, ArH), 7.45 (2H, d, *J* = 9.0 Hz, 2 × ArH), 7.50–7.57 (4H, m, 4 × ArH), 7.63–7.86 (1H, m, ArH), 8.06–8.08 (1H, m, ArH), 8.35–8.36 (1H, m, ArH). Anal. ($C_{27}H_{25}Cl_3N_4O_2$): C, H, N.

4-(4-bromophenylamino)-2-methylquinolin-6-yl 4-(bis(2-chloroethyl)amino) phenyl carbamate(95ag'). Compound **95ag'** was synthesized from isocynate **81** (freshly prepared from **80**, 1.53 g, 5.0 mmole) and 4-(4-bromophenylamino)-2-methylquinolin-6-ol (**94ag'**, 0.67, 2.0 mmole) in dry DMF(30 mL) containing

triethylamine (1.4 mL): Yield 0.38 (30 %) ; mp 229–230 °C; ^{1}H NMR (Acetic acid-d_4) δ 2.69 (3H, s, Me), 3.69–3.77 (8H, m, 4 × CH_2), 6.79 (2H, d, J = 9.0 Hz, 2 × ArH), 6.87 (1H, s, ArH), 7.43–7.46 (4H, m, 4 × ArH), 7.71 (2H, d, J = 9.0 Hz, 2 × ArH), 7.83–7.86 (1H, m, ArH), 8.07–8.08 (1H, m, ArH), 8.36–8.37 (1H, m, ArH). Anal. ($C_{27}H_{25}BrCl_2N_4O_2$·H2O): C, H, N.

4-(Phenylamino)-2-phenylquinolin-6-yl (4-(bis(2-chloroethyl)amino)phenyl carbamate (95ba'). To a solution of 4-(Phenylamino)-2-phenylquinolin-6-ol (**94ba'**, 0.31, 1.0 mmol) in dry DMF (30 mL) containing triethylamine (0.7 mL) was added a solution of isocynate **81** (freashly prepared from **80**, 0.77 g, 2.5 mmol) in dry DMF (5 mL) at room temperature. After being stirred for 2 h at room temperature, the solid was filtered and washed with dry DMF. The filtrate was evaporated to dryness in vacuo. The residue was purified by column chromatography using $CHCl_3$/hexane (100:10 v/v) as an eluent. The fractions containing the main product were combined and evaporated to dryness and the residue was recrystallized from $CHCl_3$ to give **95ba'**, 0.24 g (42 %); mp 209–210 °C; ^{1}H NMR (Acetic acid-d_4) δ 3.68–3.71 (4H, m, 2 × CH_2), 3.75–3.76 (4H, m, 2 × CH_2), 6.78–6.80 (2H, m, 2 × ArH), 7.17 (1H, s, ArH), 7.44–7.46 (3H, m, 3 × ArH), 7.58–7.63 (5H, m, 5 × ArH), 7.65–7.67 (1H, m, ArH), 7.86–7.88 (2H, m, 2 × ArH), 7.89–7.92 (1H, m, ArH), 8.26–8.28 (1H, m, ArH), 8.46–8.47 (1H, m, ArH). Anal. ($C_{32}H_{28}Cl_2N_4O_2$): C, H, N.

4-(3-Fluorophenylamino)-2-phenylquinolin-6-yl (4-(bis(2-chloroethyl)amino) phenyl carbamate(95bb'). Compound **95bb'** was synthesized from isocynate **81** (freashly prepared from **80**, 1.13 g, 3.7 mmol) and 4-(3-fluorophenylamino)-2-phenylquinolin-6-ol (**94bb'**, 0.50, 1.5 mmol) in dry DMF(30 mL) containing triethylamine (1.0 mL): Yield 0.40 g (45 %); mp 210–221 °C; ^{1}H NMR (Acetic acid-d_4) δ 3.67–3.77 (8H, m, 4 × CH_2), 6.76–6.79 (2H, m, 2 × ArH), 7.12–7.17 (1H, m,

ArH), 7.26 (1H, s, ArH), 7.35–7.38 (1H, m, ArH), 7.43–7.45 (3H, m, 3 × ArH), 7.53–7.66 (4H, m, 4 × ArH), 7.87–7.91 (3H, m, 3 × ArH), 8.25–8.27 (1H, m, ArH), 8.43–8.44 (1H, m, ArH). Anal. ($C_{32}H_{27}Cl_2FN_4O_2$): C, H, N.

4-(3-Chlorophenylamino)-2-phenylquinolin-6-yl (4-(bis(2-chloroethyl)amino) phenyl carbamate (95bc'). Compound **95bc'** was synthesized from isocynate **81** (freshly prepared from **80,** 0.76 g, 2.5 mmol) and 4-(3-chlorophenylamino)-2-phenylquinolin-6-ol (**94bc'**, 0.35 g, 1.0 mmol) in dry DMF(25 mL) containing triethylamine (0.7 mL): Yield 0.22 g (37 %); mp 197–198 °C; ^{1}H NMR (Acetic acid-d_4) δ 3.69–3.79 (8H, m, 4 × CH_2), 6.78–6.80 (2H, m, 2 × ArH), 7.24 (1H, s, ArH), 7.42–7.45 (3H, m, 3 × ArH), 7.53–7.69 (6H, m, 6 × ArH), 7.89–7.93 (3H, m, 3 × ArH), 8.27–8.29 (1H, m, ArH), 8.45–8.46 (1H, m, ArH). Anal. ($C_{32}H_{27}Cl_3N_4O_2$): C, H, N.

4-(3-bromophenylamino)-2-phenylquinolin-6-yl (4-(bis(2-chloroethyl)amino) phenyl carbamate (95bd'). Compound **95bd'** was synthesized from isocynate **81** (freashly prepared from **80**, 1.13 g, 3.7 mmole) and 4-(3-bromophenylamino)-2-phenylquinolin-6-ol (**94bd'**, 0.58, 1.5 mmole) in dry DMF(30 mL) containing triethylamine (1.0 mL): Yield 0.36 g (37 %); mp 186–187 °C; ^{1}H NMR (Acetic acid-d_4) δ 3.69–3.78 (8H, m, 4 × CH_2), 6.78–6.80 (2H, m, 2 × ArH), 7.23 (1H, s, ArH), 7.44–7.51 (3H, m, 3 × ArH), 7.57–7.69 (5H, m, 5 × ArH), 7.77–7.78 (1H, m, ArH), 7.89–7.92 (3H, m, 3 × ArH), 8.26–8.29 (1H, m, ArH), 8.44–8.45 (1H, m, ArH). Anal. ($C_{32}H_{27}BrCl_2N_4O_2$): C, H, N.

4-(4-fluorophenylamino)-2-phenylquinolin-6-yl (4-(bis(2-chloroethyl)amino) phenyl carbamate (95be'). Compound **95be**' was synthesized from isocynate **81** (freashly prepared from **80**, 0.77 g, 2.5 mmole) and 4-(4-fluorophenylamino)-2-phenylquinolin-6-ol (**94be'**, 0.33, 1.0 mmole) in dry DMF(30 mL) containing

triethylamine (0.7 mL): Yield 0.29 g (50 %); mp 186–187 °C; ^{1}H NMR (Acetic acid-d_4) δ 3.68–3.71 (4H, m, 2 × CH_2), 3.75–3.76 (4H, m, 2 × CH_2), 6.79 (2H, d, J = 8.9 Hz, 2 × ArH), 7.09 (1H, s, ArH), 7.29–7.33 (2H, m, 2 × ArH), 7.45 (2H, d, J = 8.9 Hz, 2 × ArH), 7.58–7.65 (5H, m, 5 × ArH), 7.86–7.92 (3H, m, 3 × ArH), 8.25–8.28 (1H, m, ArH), 8.44–8.45 (1H, m, ArH). Anal. ($C_{32}H_{27}Cl_2FN_4O_2$): C, H, N.

4-(4-chlorophenylamino)-2-phenylquinolin-6-yl (4-(bis(2-chloroethyl)amino) phenyl carbamate (95bf'). Compound **95bf'** was synthesized from isocynate **81** (freashly prepared from **80**, 0.77 g, 2.5 mmole) and 4-(4-chlorophenylamino)-2-phenylquinolin-6-ol (**94bf'**, 0.35, 1.0 mmole) in dry DMF(30 mL) containing triethylamine (0.7 mL): Yield 0.23 g (38 %); mp 204–205 °C; ^{1}H NMR (Acetic acid-d_4) δ 3.67–3.70 (4H, m, 2 × CH_2), 3.74–3.76 (4H, m, 2 × CH_2), 6.78 (2H, d, J = 8.9 Hz, 2 × ArH), 7.18 (1H, s, ArH), 7.44 (2H, d, J = 8.9 Hz, 2 × ArH), 7.54–7.61 (6H, m, 6 × ArH), 7.63–7.67 (1H, m, ArH), 7.88–7.90 (3H, m, 3 × ArH), 8.24–8.27 (1H, m, ArH), 8.43–8.44 (1H, m, ArH). Anal. ($C_{32}H_{27}Cl_3N_4O_2$): C, H, N.

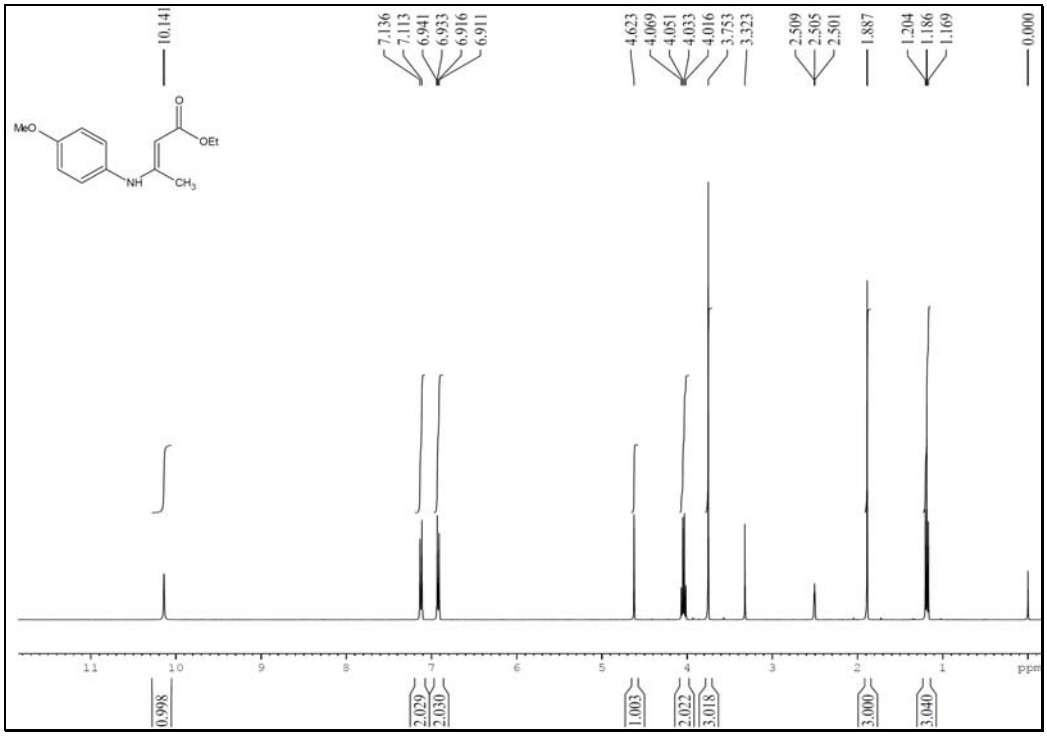
4-(4-bromophenylamino)-2-phenylquinolin-6-yl (4-(bis(2-chloroethyl)amino) phenyl carbamate (95bg'). Compound **95bg'** was synthesized from isocynate **81** (freashly prepared from **80**, 1.13 g, 3.7 mmole) and 4-(4-bromophenylamino)-2-phenylquinolin-6-ol (**94bg'**, 0.58, 1.5 mmole) in dry DMF(30 mL) containing triethylamine (1.0 mL): Yield 0.38 g (40 %); mp 213–214 °C; ^{1}H NMR (Acetic acid-d_4) δ 3.68–3.71 (4H, m, 2 × CH_2), 3.75–3.76 (4H, m, 2 × CH_2), 6.79 (2H, d, J = 8.9 Hz, 2 × ArH), 7.21 (1H, s, ArH), 7.45 (2H, d, J = 8.9 Hz, 2 × ArH), 7.53 (2H, d, J = 8.7 Hz, 2 × ArH), 7.58–7.66 (3H, m, 3 × ArH), 7.72 (2H, d, J = 8.7 Hz, 2 × ArH), 7.89–7.92 (3H, m, 3 × ArH), 8.26–8.28 (1H, m, ArH), 8.44–8.45 (1H, m, ArH). Anal. ($C_{32}H_{27}BrCl_2N_4O_2$): C, H, N.

4.5 Conclusion

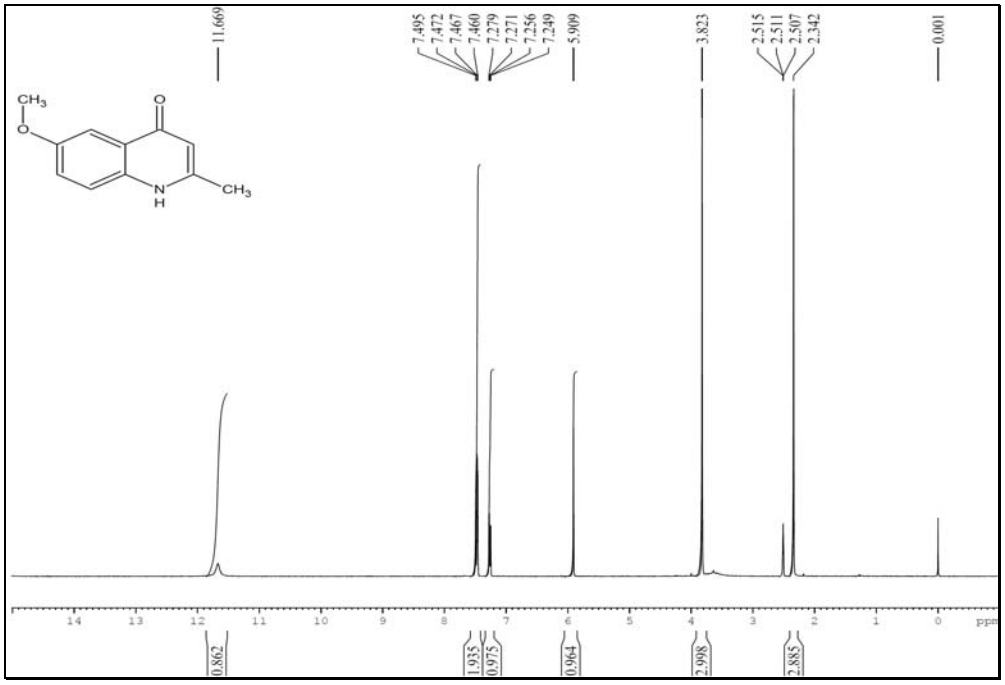
Designing DNA-directed alkylating agents by linking the alkylating warhead to the DNA-affinic carrier is one of the promising strategies to overcome the drawbacks of alkylating agents. In the current studies, we have designed and synthesized a series of DNA-directed alkylating agents by linking the phenyl *N*-mustard pharmacophore with the 6-hydroxy function of 4-anilinoquinoline derivatives via a carbamate linker. All the compounds were characterized by ^{1}H NMR and elemental analysis. The antitumor activity of all newly synthesized derivatives is under investigation.

4.6 Representative Spectra

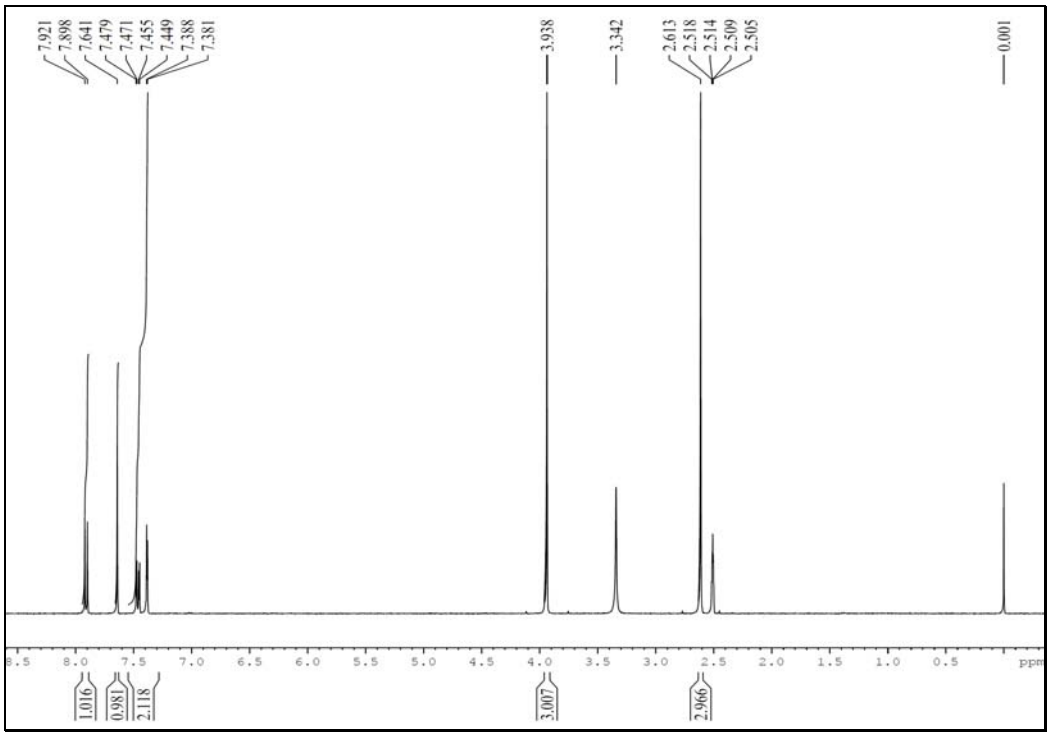
4.6.1 ^{1}H NMR Spectrum for compound **90b**.



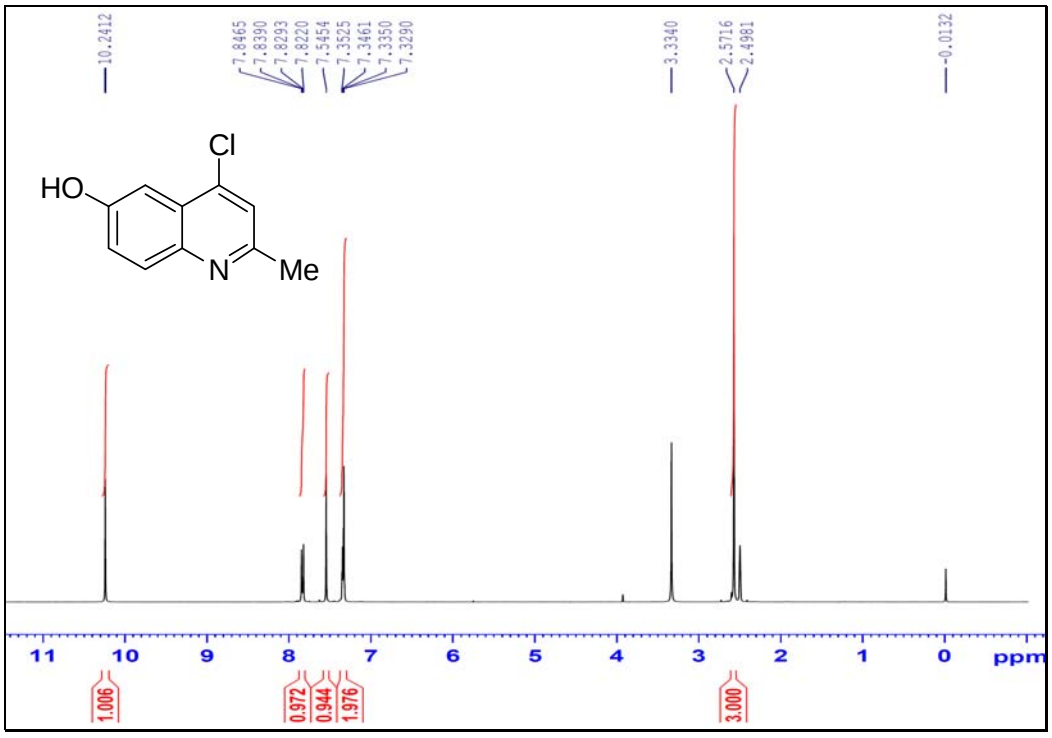
4.6.2 ^{1}H NMR Spectrum for compound **90b**.



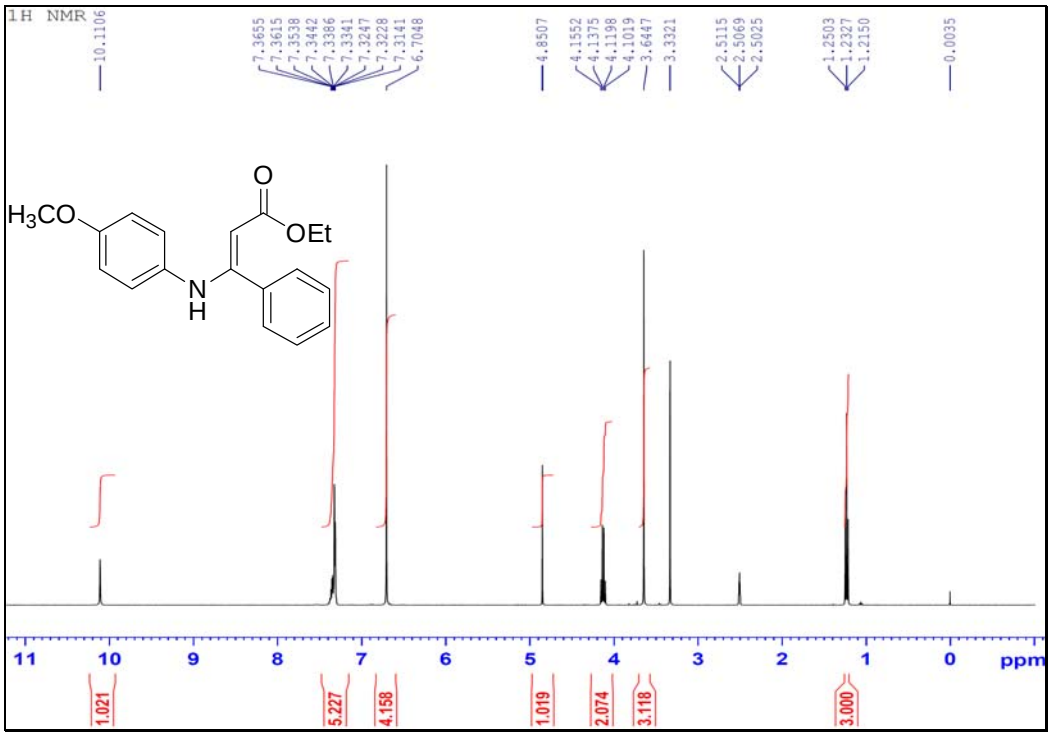
4.6.3 ^{1}H NMR Spectrum for compound **90b**.



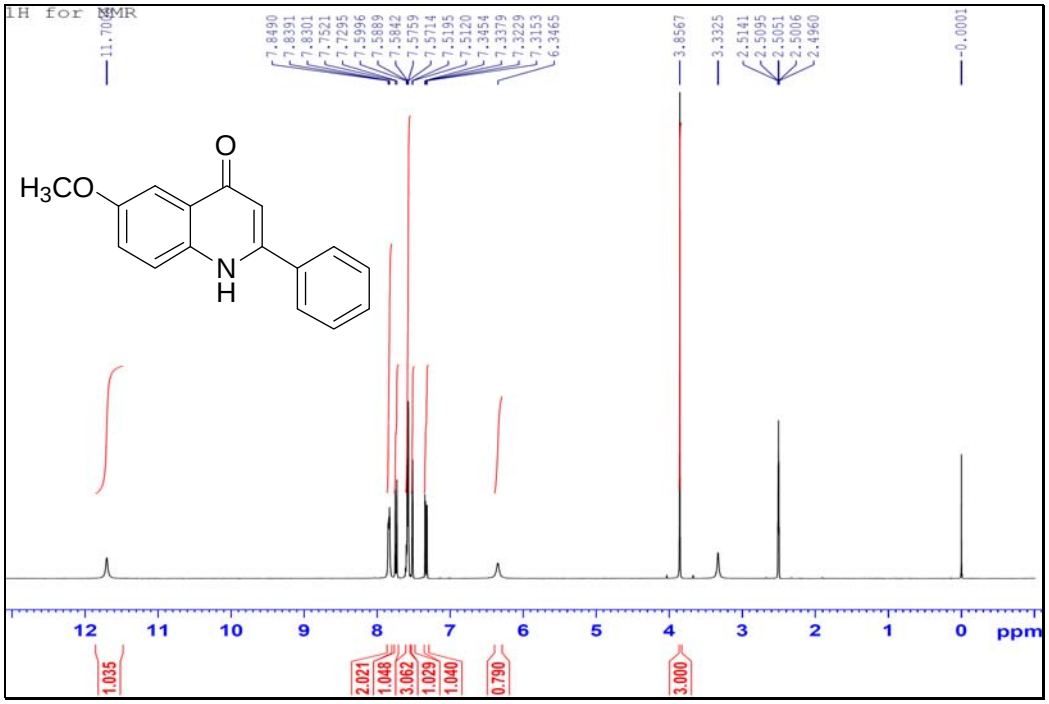
4.6.4 ^{1}H NMR Spectrum for compound **93a**.



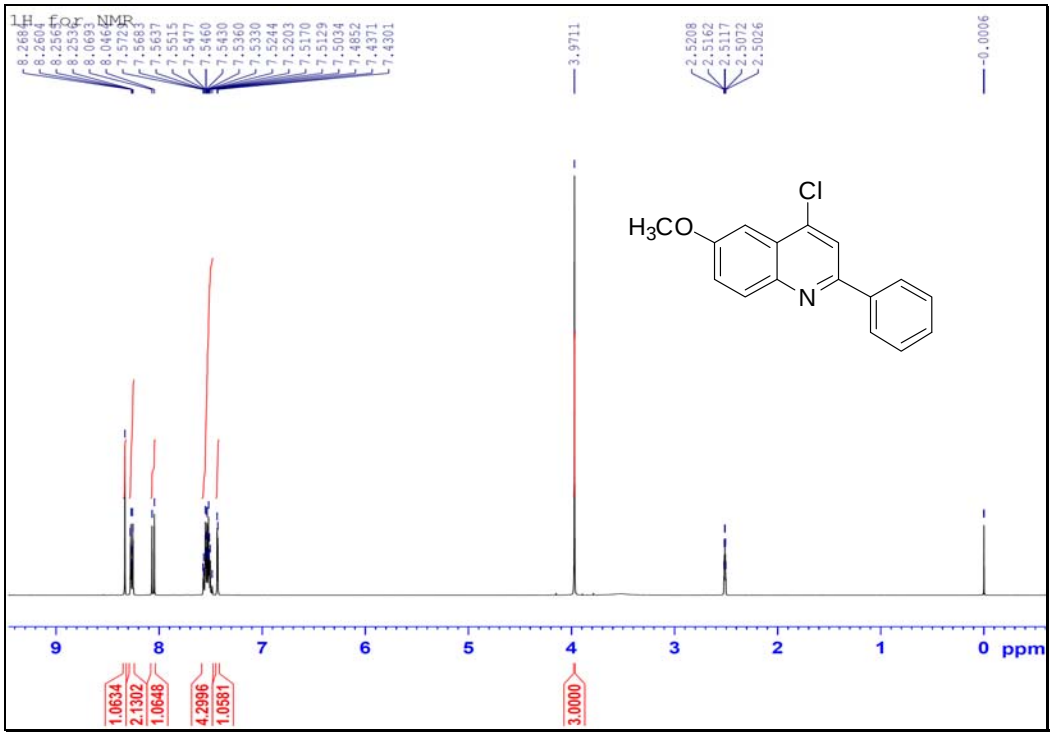
4.6.5 ^{1}H NMR Spectrum for compound **90b**.



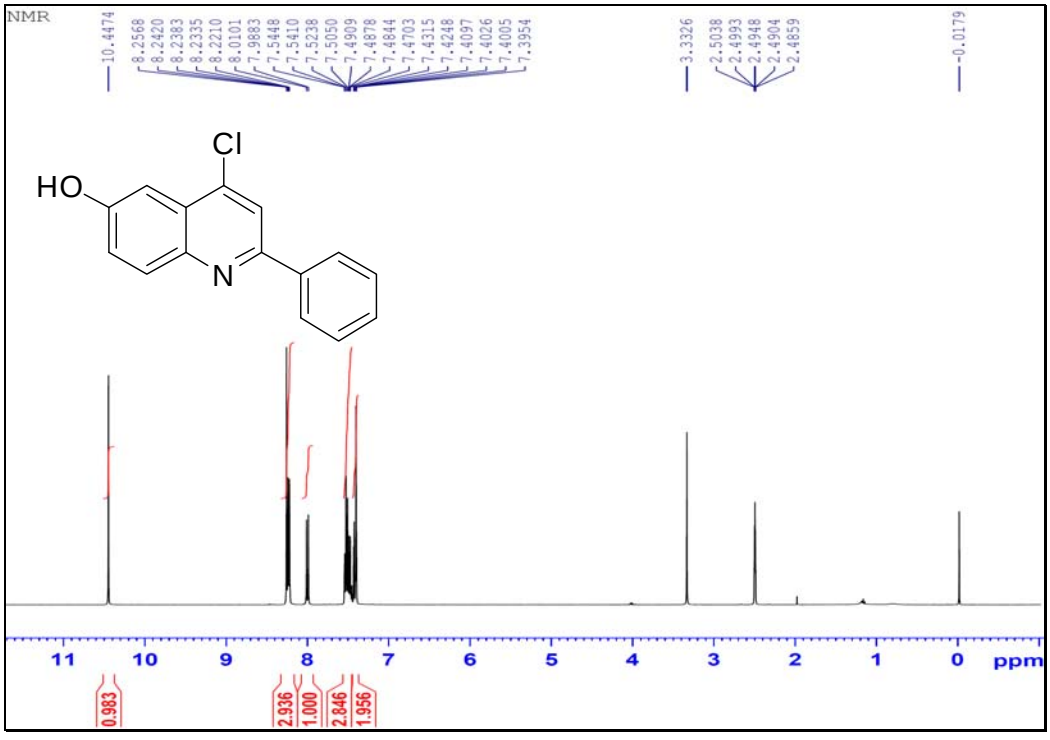
4.6.6 ^{1}H NMR Spectrum for compound **91b**.



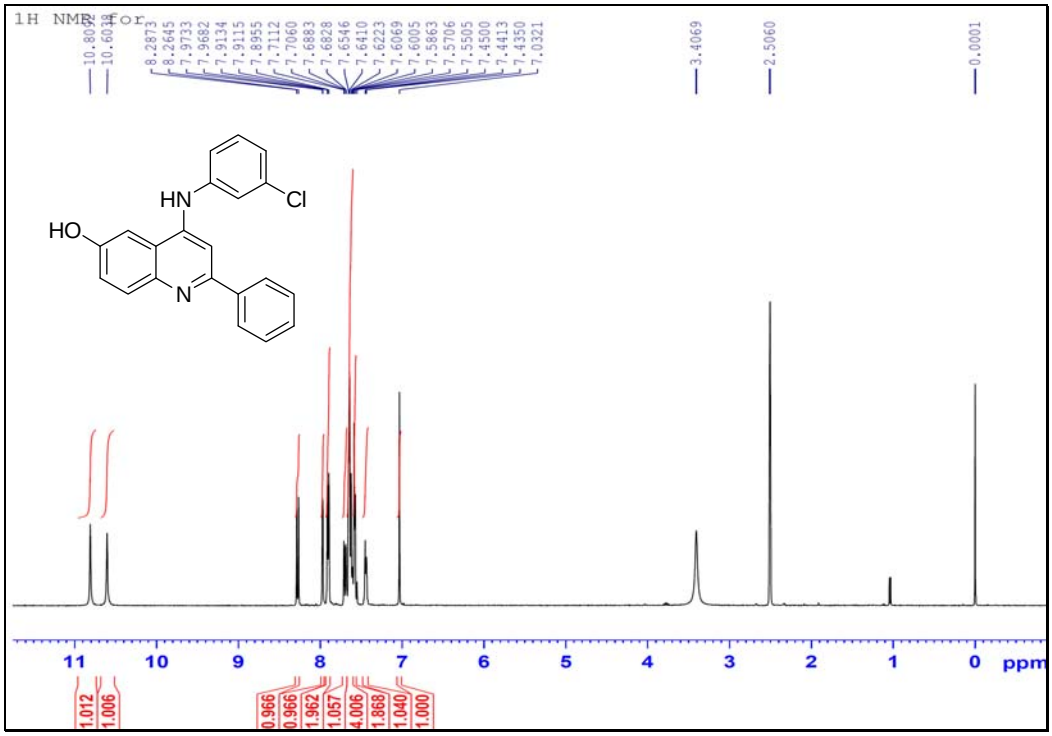
4.6.7 ^{1}H NMR Spectrum for compound **92b**.



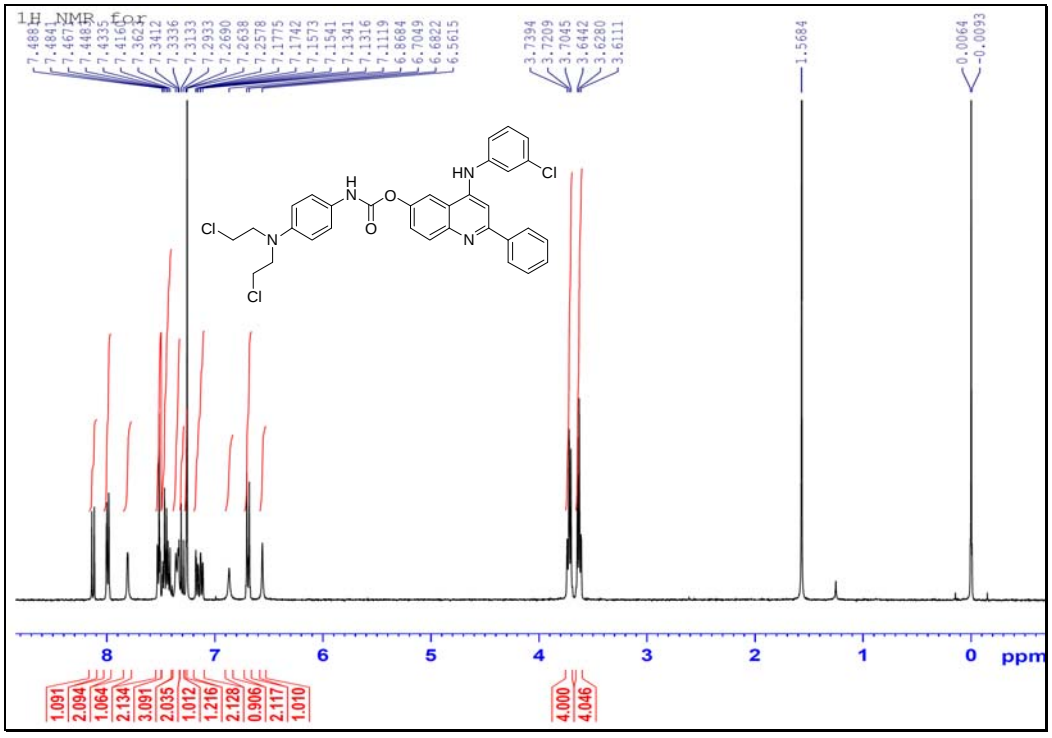
4.6.8 ^{1}H NMR Spectrum for compound **93b**.



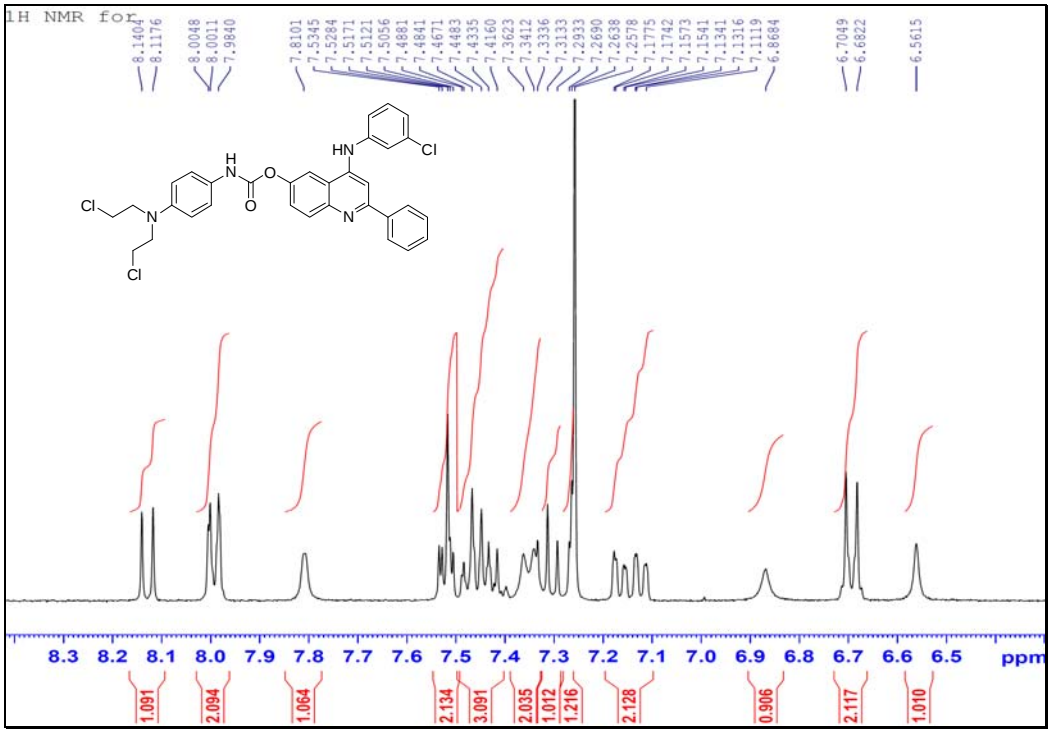
4.6.9 ^{1}H NMR Spectrum for compound **94bc'**.



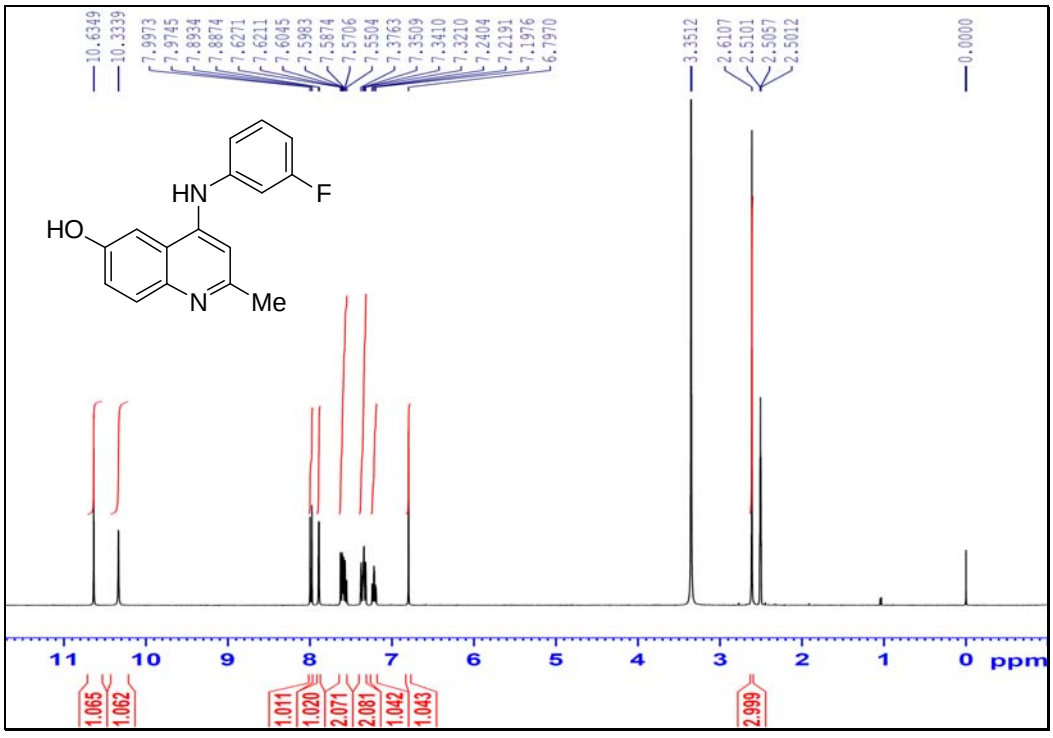
4.6.10 ^{1}H NMR Spectrum for compound **95bc'**.



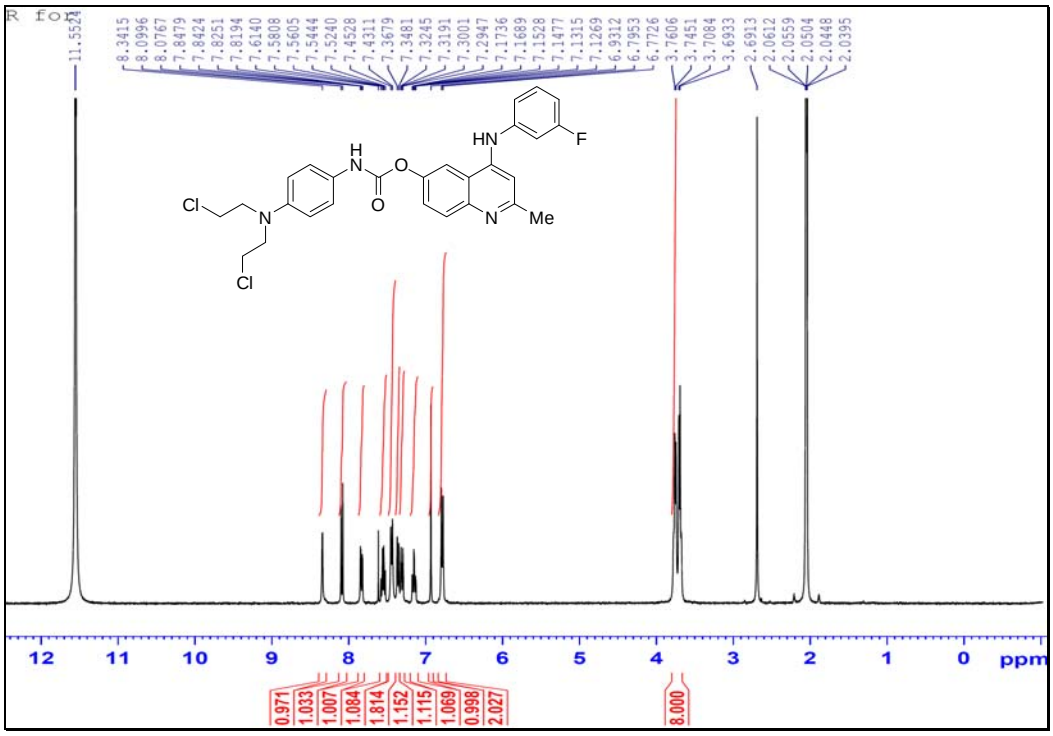
4.6.11 ^{1}H NMR Spectrum for compound **95bc'(Aro)**.



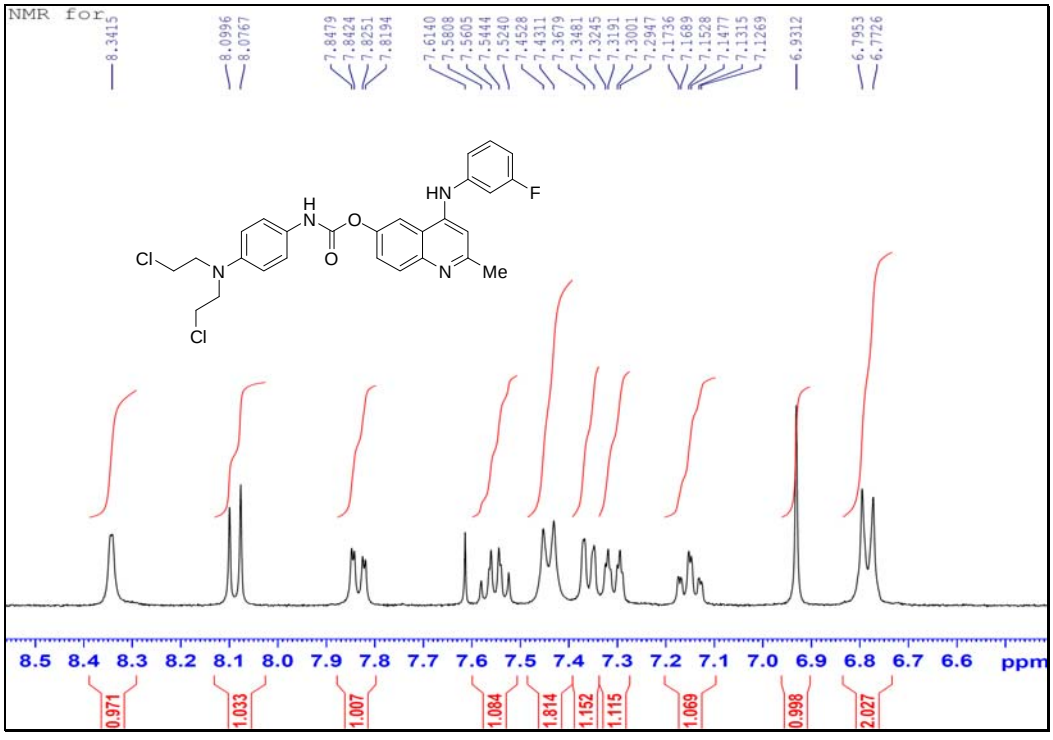
4.6.12 ^{1}H NMR Spectrum for compound **94ab'**.



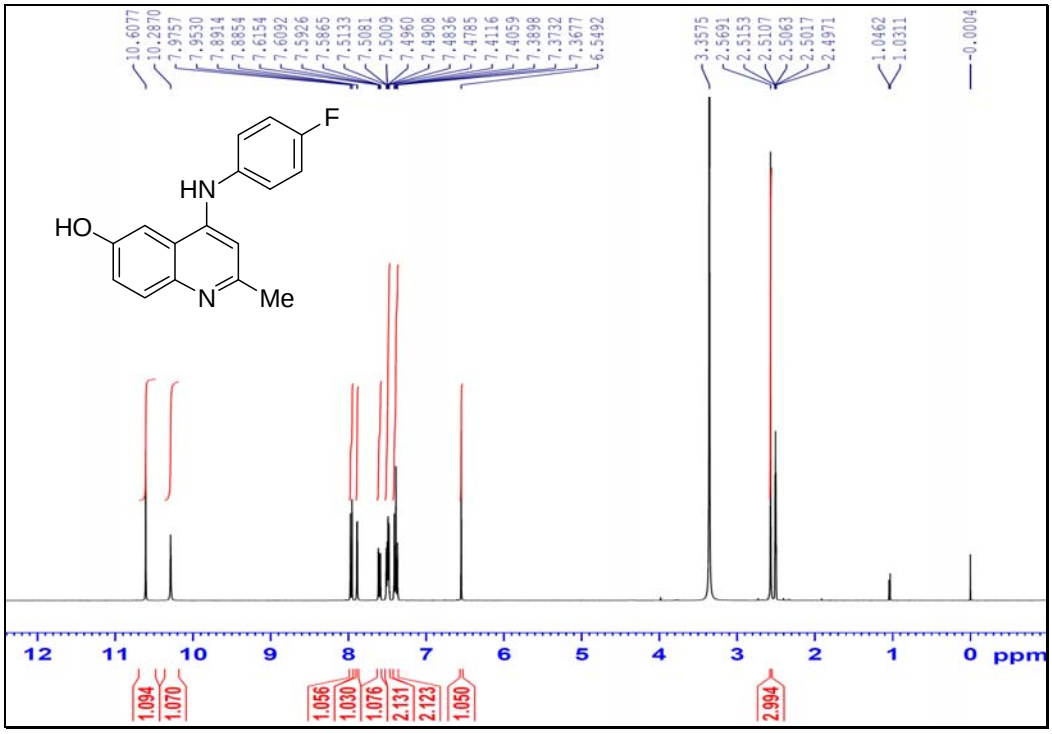
4.6.13 ^{1}H NMR Spectrum for compound **95ab'**.



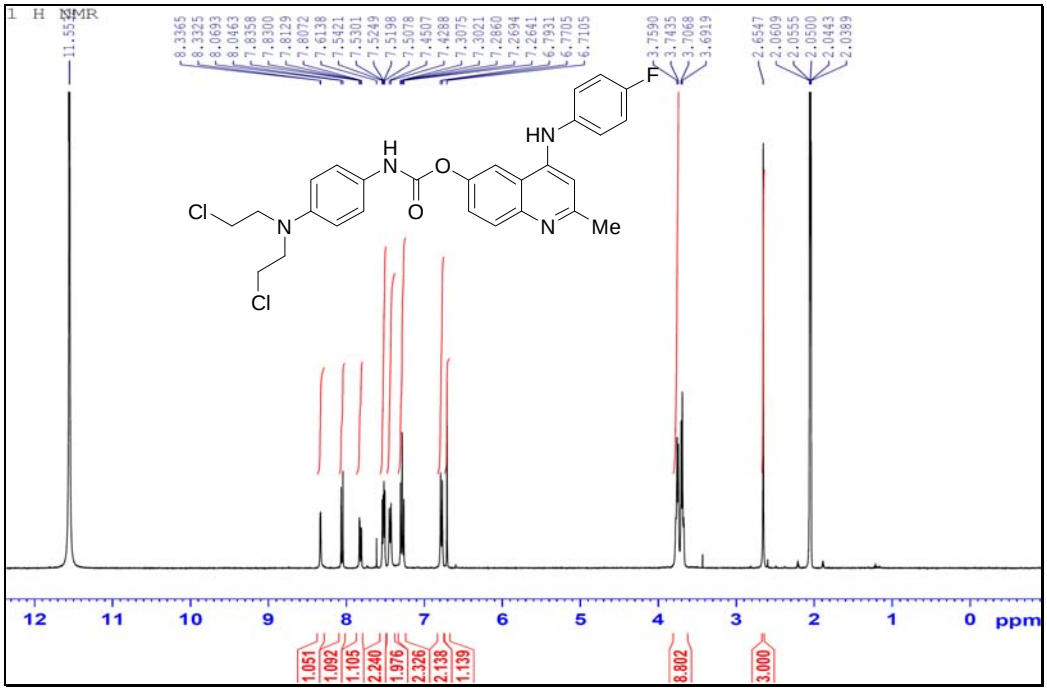
4.6.14 ^{1}H NMR Spectrum for compound **95ab'(Aro)**.



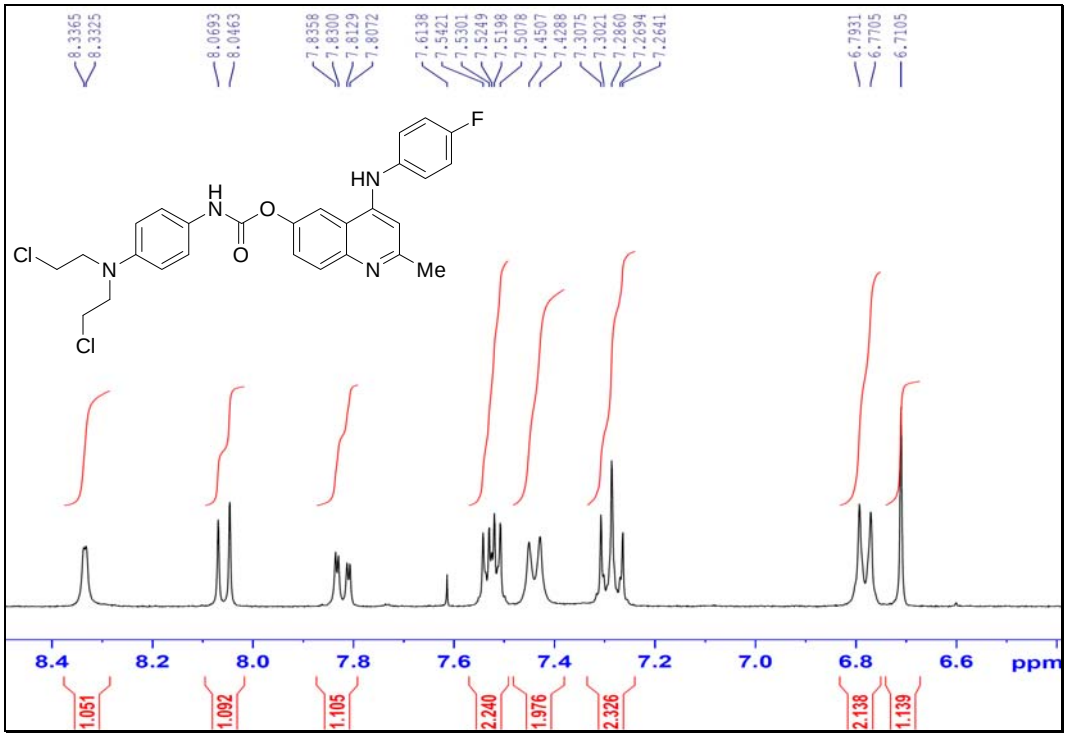
4.6.15 ^{1}H NMR Spectrum for compound **94ae'**.



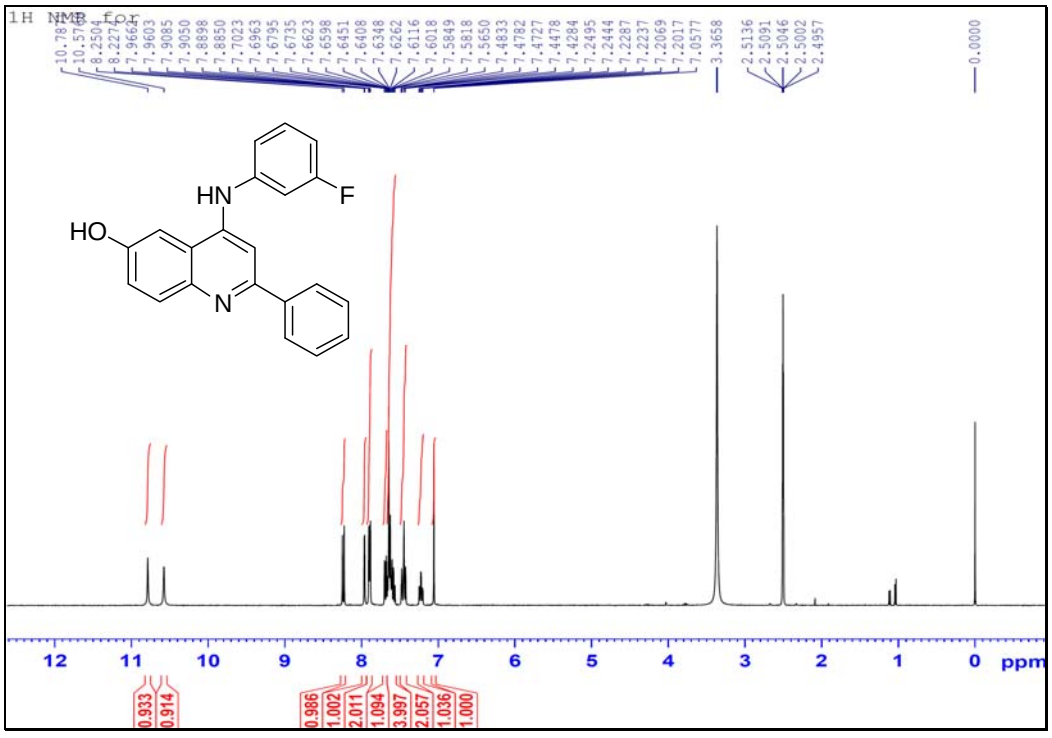
4.6.16 ^{1}H NMR Spectrum for compound **95ae'**.



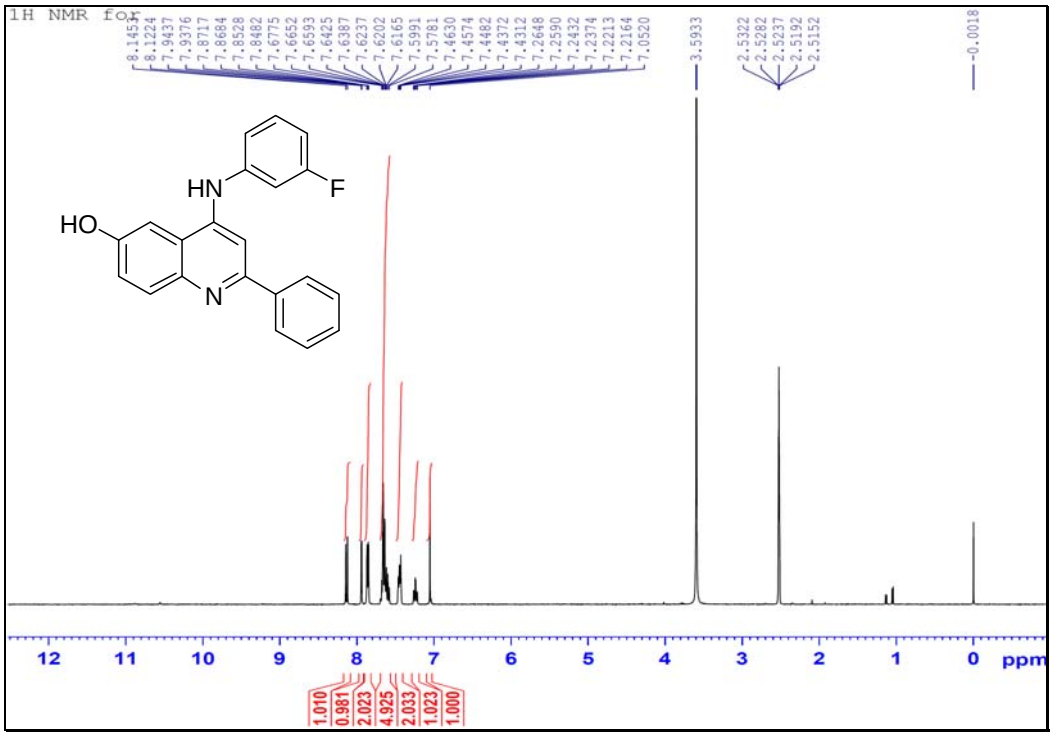
4.6.17 ^{1}H NMR Spectrum for compound **95ae'(Aro)**.



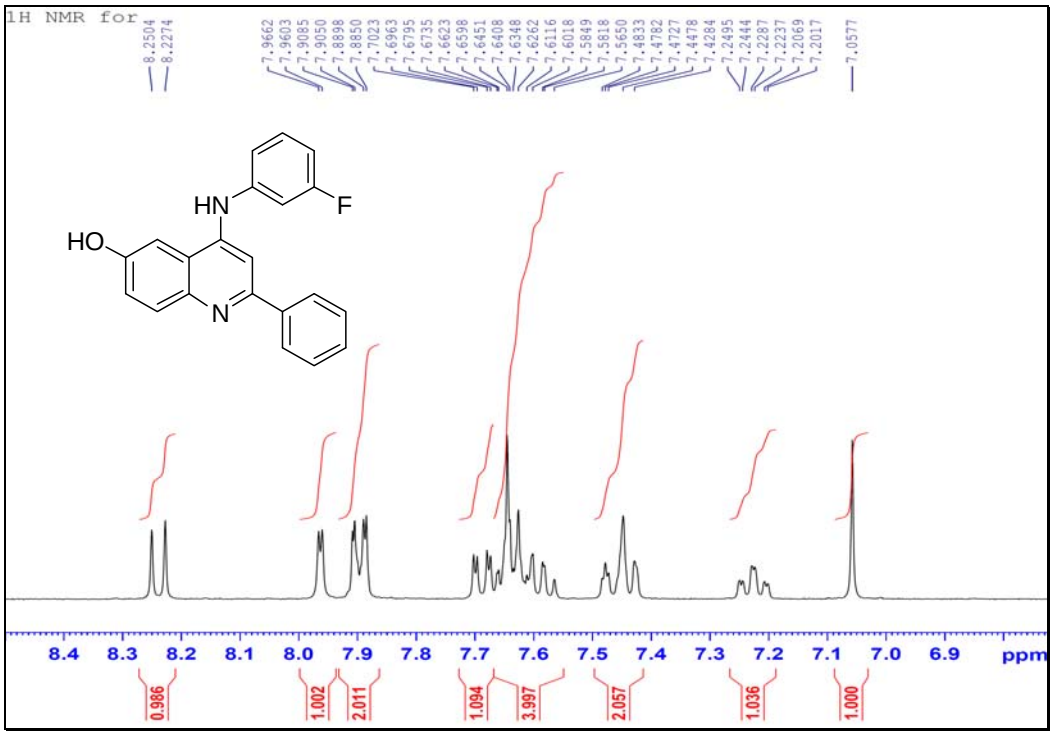
4.6.18 ^{1}H NMR Spectrum for compound **94bb'.**



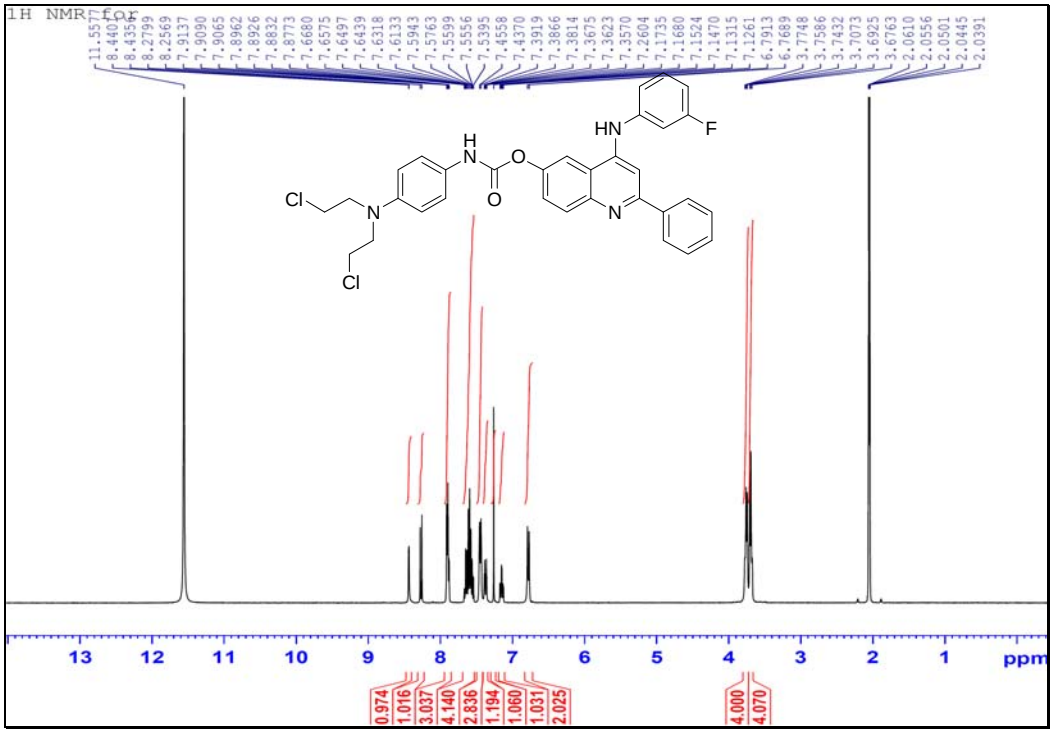
4.6.19 ^{1}H NMR Spectrum for compound **94bb'(D2O)**.



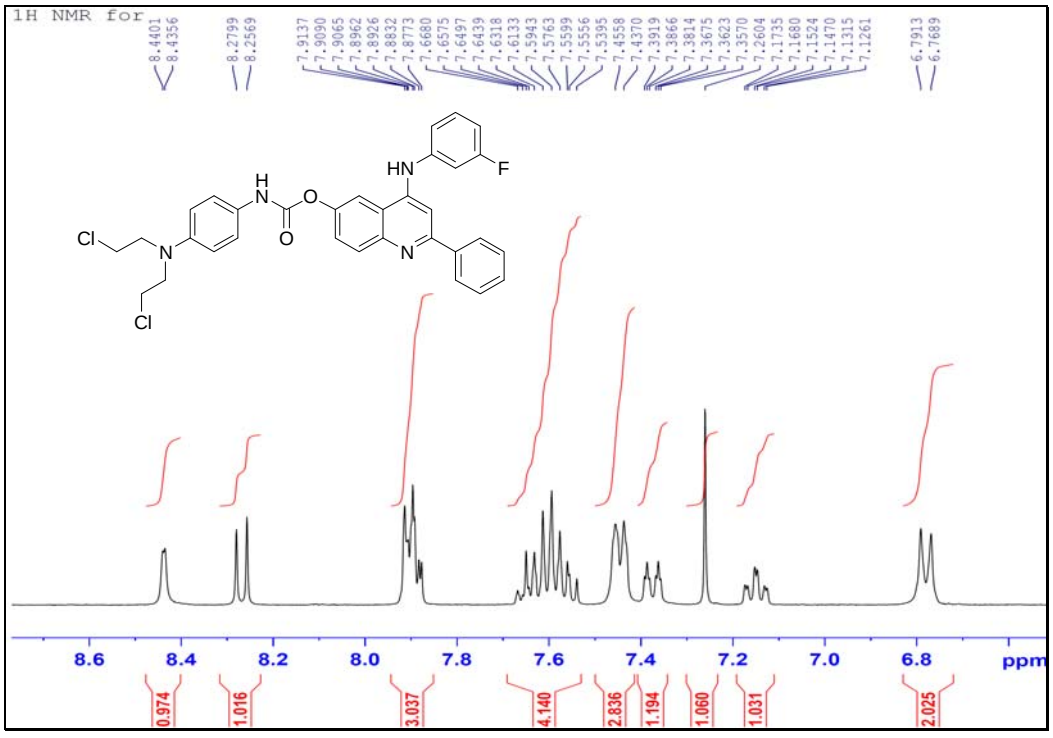
4.6.20 ^{1}H NMR Spectrum for compound **94bb'(Aro)**.



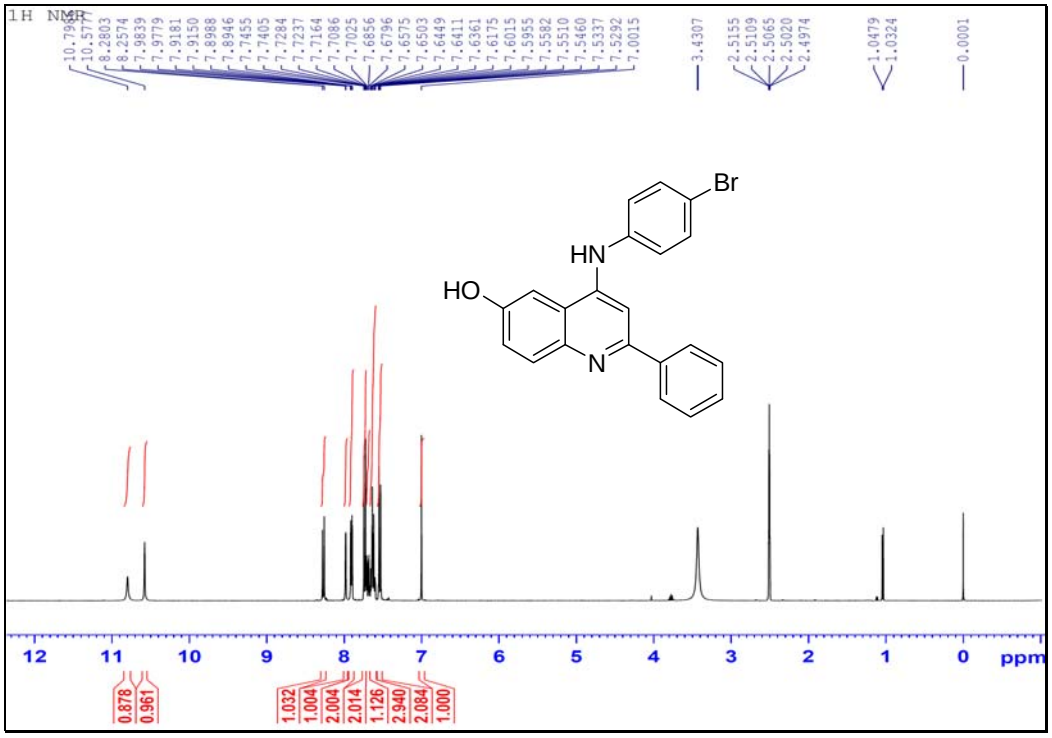
4.6.21 ^{1}H NMR Spectrum for compound **95bb'**.



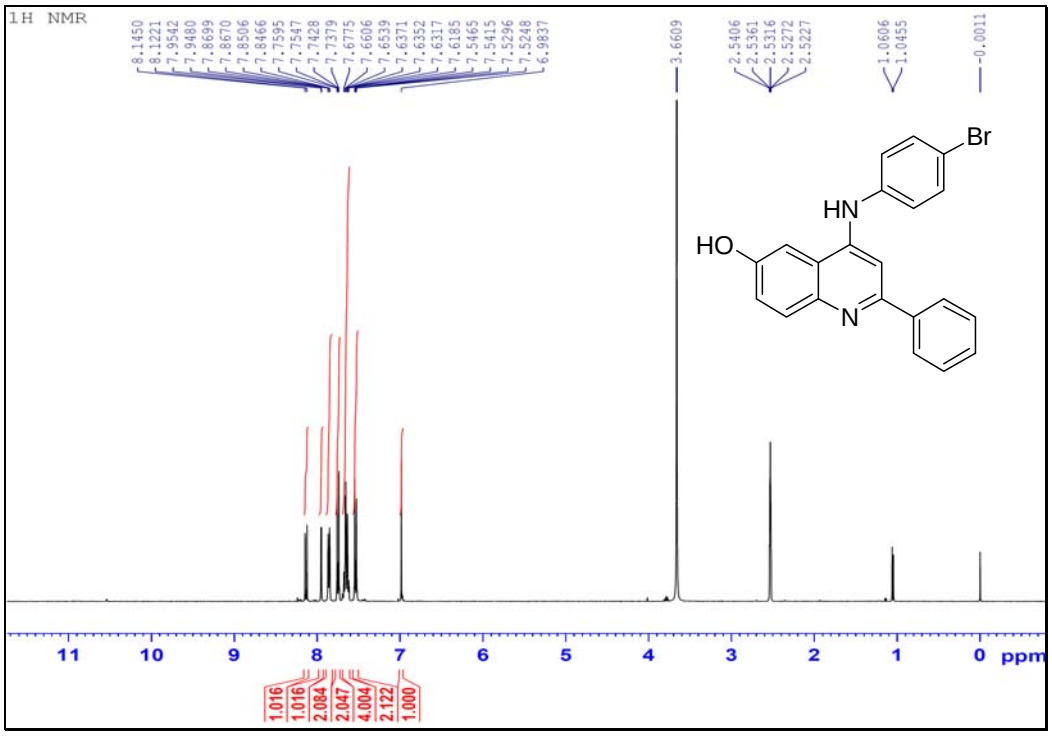
4.6.22 ^{1}H NMR Spectrum for compound **95bb'(Aro)**.



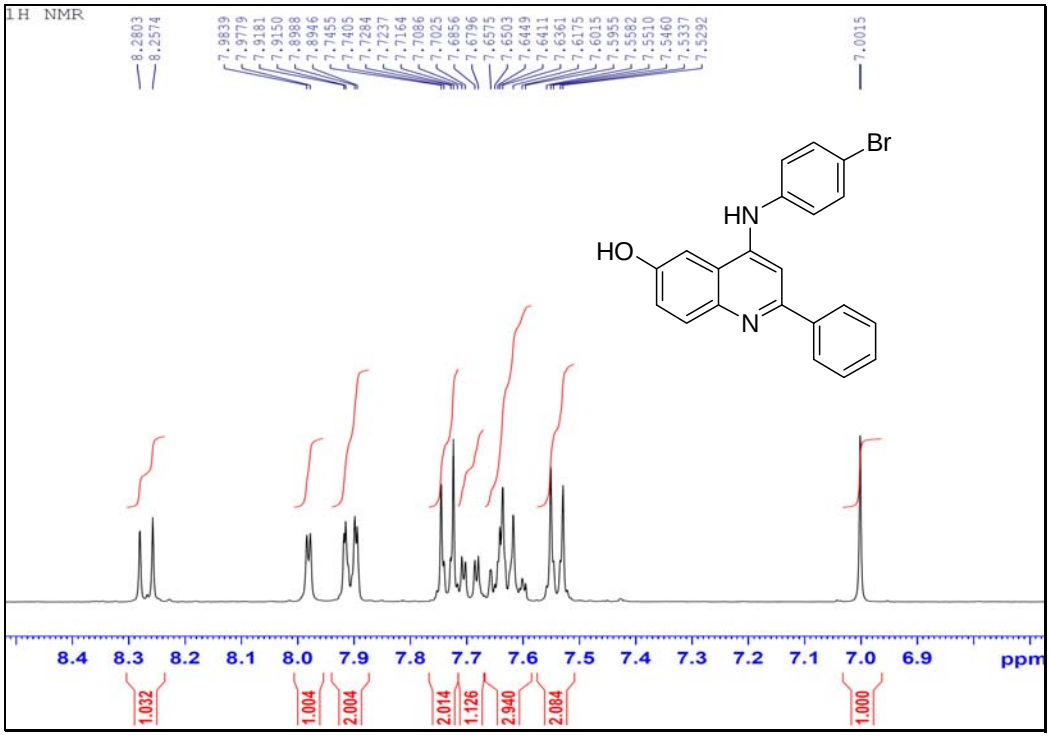
4.6.23 ^{1}H NMR Spectrum for compound **94bg'**.



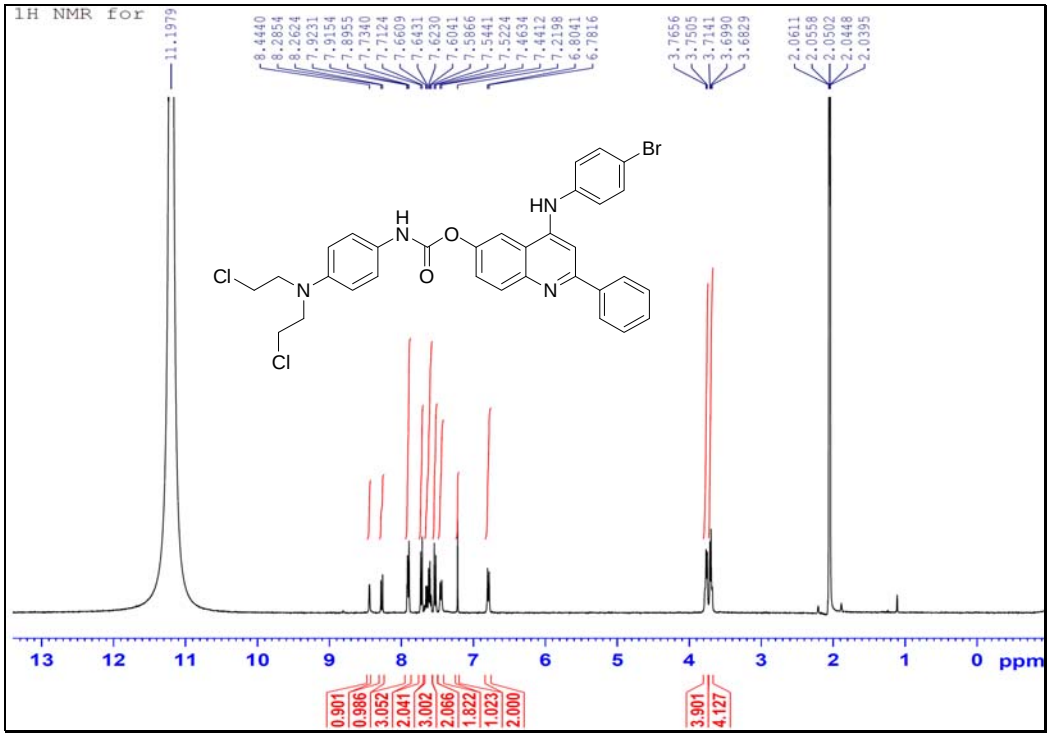
4.6.24 ^{1}H NMR Spectrum for compound **94bg'(D_2O)**.



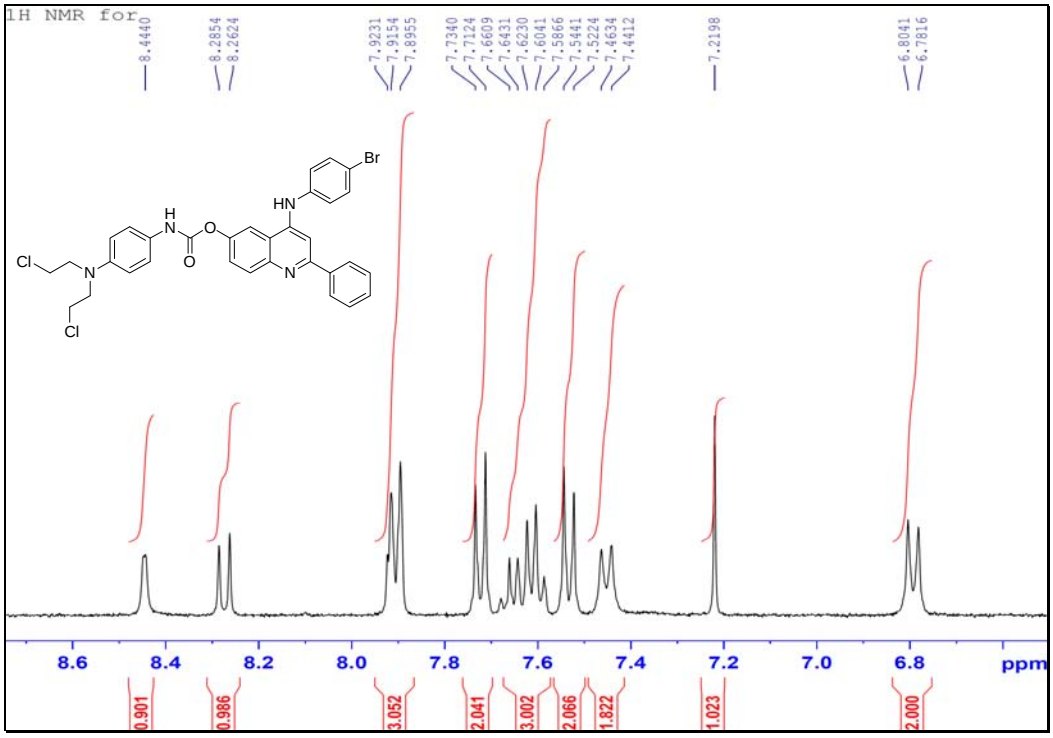
4.6.25 ^{1}H NMR Spectrum for compound **94bg'(Aro)**.



4.6.26 ^{1}H NMR Spectrum for compound **95bg'**.



4.6.27 ^{1}H NMR Spectrum for compound **95bg'(Aro)**.



4.7 Elemental analysis

Table 2. Elemental analysis of compounds **94aa'-bg'.**

Sr. No.	BO No.	MF	MW	Elemental Analysis					
				CHN Calculated %			CHN Found %		
				C	H	N	C	H	N
94aa'	BO-1911	$C_{16}H_{14}N_2O$	250.30	76.78	5.64	11.19	76.58	5.70	11.10
94ab'	BO-1760	$C_{16}H_{13}FN_2O$	268.29	71.63	4.88	10.44	71.87	4.71	10.28
94ac'	BO-1756	$C_{16}H_{13}ClN_2O$	284.74	67.49	4.60	9.84	67.58	4.82	9.68
94ad'	BO-1758	$C_{16}H_{13}BrN_2O$	329.19	58.38	3.98	8.15	58.52	4.14	8.33
94ae'	BO-1763	$C_{16}H_{13}FN_2O$	268.29	71.63	4.88	10.44	71.77	4.99	10.57
94af'	BO-1766	$C_{16}H_{13}ClN_2O$	284.74	67.49	4.60	9.84	67.66	4.87	9.71
94ag'	BO-1759	$C_{16}H_{13}BrN_2O$	329.19	58.38	3.98	8.15	58.44	4.13	8.32
94ba'	BO-1904	$C_{21}H_{16}N_2O$	312.36	80.75	5.16	8.97	80.91	5.44	9.29
94bb'	BO-1895	$C_{21}H_{15}FN_2O$	330.36	76.35	4.58	8.48	76.14	4.17	8.24
94bc'	BO-1893	$C_{21}H_{15}ClN_2O$	346.81	72.73	4.63	8.08	72.58	4.87	8.29
94bd'	BO-1894	$C_{21}H_{15}BrN_2O$	391.26	64.46	3.86	7.16	64.59	3.99	7.31
94be'	BO-1905	$C_{21}H_{15}FN_2O$	330.36	76.35	4.85	8.48	76.57	4.67	8.31
94bf'	BO-1900	$C_{21}H_{15}ClN_2O$	346.81	72.73	4.63	8.08	72.59	4.48	8.29
94bg'	BO-1899	$C_{21}H_{15}BrN_2O$	391.26	64.46	3.86	7.16	64.21	3.67	7.35

Table 2.3 Elemental analysis of compounds **95aa'-bg'.**

Sr. No.	BO No.	MF	MW	Elemental Analysis					
				CHN Calculated %			CHN Found %		
				C	H	N	C	H	N
95aa'	BO-1912	$C_{27}H_{26}Cl_2N_4O_2 \cdot 1.7H2O$	540.05	60.05	5.49	10.37	59.91	4.92	10.14
95ab'	BO-1897	$C_{27}H_{25}Cl_2FN_4O_2$	527.42	61.49	4.78	10.62	61.09	4.79	10.58
95ac'	BO-1770	$C_{27}H_{25}Cl_3N_4O_2$	543.87	59.63	4.63	10.30	59.33	4.59	9.97
95ad'	BO-1771	$C_{27}H_{25}BrCl_2N_4O_2 \cdot 2.2H2O$	627.96	51.64	4.72	8.92	51.34	4.40	8.89
95ae'	BO-1909	$C_{27}H_{25}Cl_2FN_4O_2$	527.42	61.49	4.78	10.62	61.12	4.82	10.38
95af'	BO-1910	$C_{27}H_{25}Cl_3N_4O_2$	543.87	59.63	4.63	10.30	59.23	4.69	10.14
95ag'	BO-1896	$C_{27}H_{25}BrCl_2N_4O_2 \cdot H2O$	606.34	53.48	4.49	9.24	53.81	4.29	9.18
95ba'	BO-1906	$C_{32}H_{28}Cl_2N_4O_2$	571.50	67.25	4.94	9.80	66.85	4.97	9.82
95bb'	BO-1908	$C_{32}H_{27}Cl_2FN_4O_2$	589.49	65.20	4.62	9.50	65.20	4.62	9.50
95bc'	BO-1898	$C_{32}H_{27}Cl_3N_4O_2$	605.94	63.43	4.49	9.25	63.26	4.53	9.25
95bd'	BO-1901	$C_{32}H_{27}BrCl_2N_4O_2$	650.39	59.09	4.18	8.61	59.04	4.18	8.61
95be'	BO-1907	$C_{32}H_{27}Cl_2FN_4O_2$	589.49	65.20	4.62	9.50	64.85	5.10	9.18
95bf'	BO-1903	$C_{32}H_{27}Cl_3N_4O_2$	605.94	63.43	4.49	9.25	63.33	4.78	9.10
95bg'	BO-1902	$C_{32}H_{27}BrCl_2N_4O_2$	650.39	59.09	4.18	8.61	59.07	4.24	8.59

Chapter -5

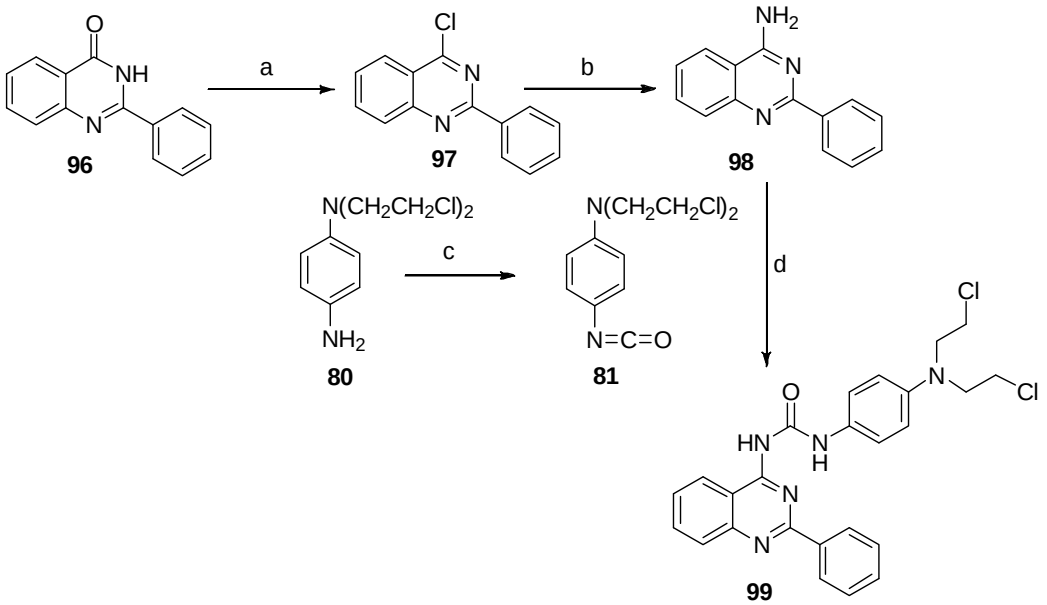
Synthesis and characterization of : 1-(4-(bis(2-chloroethyl)amino)phenyl)-3-(2-phenylquinazolin-4-yl)urea

5.1 Chemistry

The synthesis of the *N*-mustard-quinazoline conjugates is shown in Schemes 1. The *N*-mustard-4-aminoquinazoline conjugate **99** was prepared starting from the known compound 2-phenylquinazolin-4(3H)-one (**96**, Scheme 1).[116, 117] Compound **96** was treated with $POCl_3$ to produce 4-chloro-2-phenyl quinazoline[113] (**97**), which was then reacted with ammonia in phenol at 170 ^oC to give 2-phenylquinazolin-4-amine (**98**)[118]. Reaction of **98** with the known 4-[*N*,*N*-bis(2-chloroethyl)-amino]phenylisocyanate **81** [freshly prepared from *N*,*N*-bis(2-chloroethyl)benzene-1,4-diamine hydrochloride (**80**)] in the presence of triethylamine afforded *N*-mustard-4-aminoquinazoline conjugate **99** in low yield.

5.2 Reaction Scheme

Scheme 1.



Scheme 1. Reagents and conditions: (a) $POCl_3$/reflux, (b) Phenol/NH_3(g)/140 °C; (c) triphosgene/Et_3N/$CHCl_3$/THF, room temperature; (d) Et_3N/$CHCl_3$, room temperature.

5.3 Experimental

General methods and materials

Solvents and reagents used were of reagent grade and used without purification unless otherwise noted. The melting points were recorded on a Fargo melting point apparatus and are uncorrected. Column chromatography was carried out on silica gel G60 (70-230mesh, ASTM; Merck and 230-400 mesh, silicycle inc.). Reaction progress was monitored using analytical thin-layer chromatography (TLC) on 0.25mmMerck F-254 silica gel glass plates. Visualization was achieved by UV light (254 nm). ^{1}H NMR spectra were recorded with a Bruker AVANCE 600 DRX and 400 MHz spectrometer; Chemical shifts are reported in parts per million (δ) using tetramethylsilane as the internal standard with coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; brs, broad singlet. Elemental analyses were performed on a Heraeus CHN-O Rapid analyzer. 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 (5μL) mobile phase, MeCN/THF (70:30 v/v); flow rate, 1 mL/min; injected sample 10μL, column temp, 27°C; wavelength, 254nm. The purity of all compounds was > 95 % based on analytical HPLC.

4-Chloro-2-phenylquinazoline (97). To a magnetically stirred solution of $POCl_3$ (25 mL) at 0°C was added portion wise 2-phenylquinazolin-4(3H)-one **96** (5.0 g). The reaction mixture was refluxed for 2 hrs. After completion of the reaction, the excess $POCl_3$ was removed by vacuo. The residue was poured into a mixture of chloroform (50 mL) + ice cold water (80 mL) + ammonia solution (20 mL). The chloroform layer was separated and the aqueous layer was extracted with an additional 20 ml of chloroform. The united chloroform extracts were dried over Na_2SO_4 and filtered, and the solvent was removed by distillation to give **97**, 4.5 g (87 %); mp 125–127 °C (lit.[24] 124–125 °C); ^{1}H NMR (DMSO-d_6) δ 7.58–7.69 (4H, m, 4 × ArH), 7.91–7.92 (2H, m, 2 × ArH), 8.16–8.21 (3H, m, 3 × ArH). Anal. Calcd. for ($C_{14}H_9ClN_2$): C, 69.86; H, 3.77; N, 11.64. Found: C, 70.08; H, 3.97; N, 11.50.

2-Phenylquinazolin-4-amine (98). A mixture of 4-chloro-2-phenylquinazolin **97** (3.0 g, 12.0 mmol) and excess phenol was heated at 170 ^{o}C for 2 h. After completion of

the reaction, the ammonia gas was passed into the reaction mass at 150 °C for 1 h. After that the reaction mixture was cooled to room temperature and poured into 5% sodium hydroxide solution. The solid was filtered and washed with water and dried to give **98**, 2.0 g (74 %); mp 140–141 °C (lit.[25] 146–147 °C); ^{1}H NMR (DMSO-d_6) δ 7.45–7.53 (4H, m, 4 × ArH), 7.75–7.79 (2H, m, 2 × ArH), 7.83 (2H, brs, exchangeable, NH_2), 8.23–8.25 (1H, m, ArH), 8.45–8.48 (2H, m, 2 × ArH). Anal. Calcd. for ($C_{14}H_{11}N_3$): C, 76.00; H, 5.01; N, 18.99. Found: C, 76.28; H, 5.10; N, 18.73.

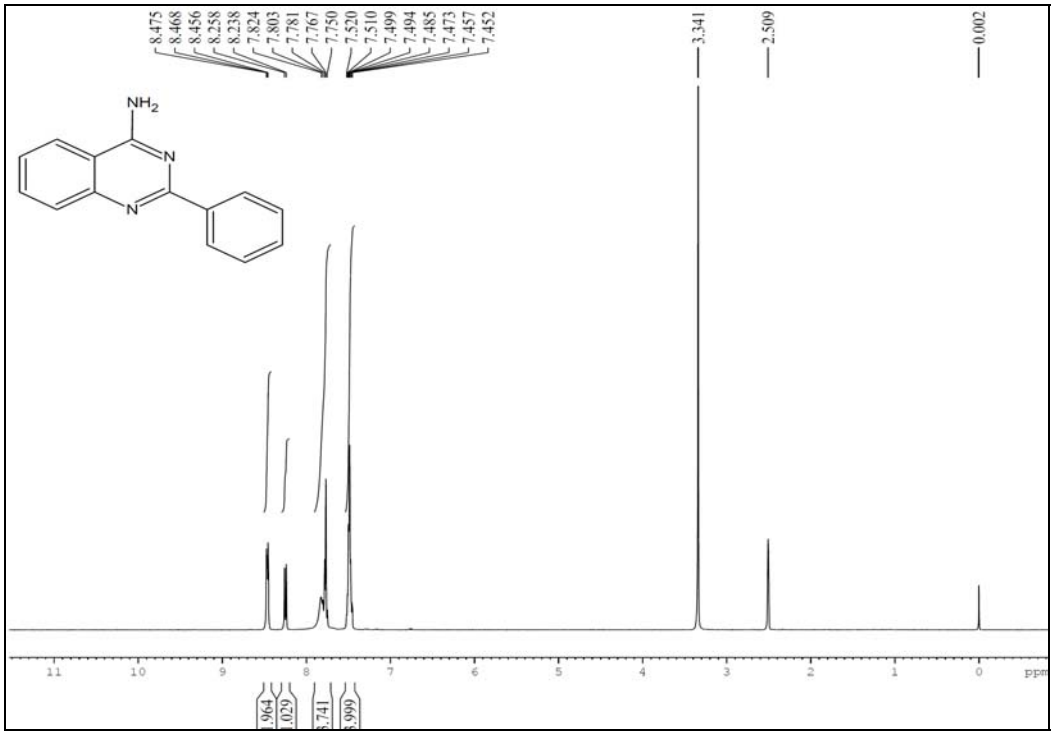
1-(4-Bis(2-chloroethyl)phenyl)-3-(2-phenylquinazolin-4-yl)urea (99). A solution of isocyanate **14** (freshly prepared from **13**, 1.5 g 4.9 mmol) in chloroform (10 mL) was added dropwise to a solution of 2-phenyl quinazolin-4-amine **98** (0.63 g, 2.8 mmol) in chloroform (30 mL) containing triethylamine (1 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h. The solid material was fallout from reaction mass. It was filtered and washed with chloroform to give 1-(4-bis(2-chloroethyl)phenyl)-3-(2-phenylquinazolin-4-yl)urea **99,** 0.2 g (17 %); mp 263–264 °C; ^{1}H NMR (DMSO-d_6) δ 3.68–3.71 (8H, m, 4 × CH_2), 6.81 (2H, d, J = 8.6 Hz, 2 × ArH), 7.48 (2H, d, J =8.6 Hz, 2 × ArH), 7.58–7.56 (4H, m, 4 × ArH), 7.94–7.95 (2H, m, 2 × ArH), 8.35–8.37 (2H, m, 2 × ArH), 8.72–8.74 (1H, m, ArH), 10.47, 12.02 (each 1H, s, 2 × NH). Anal. Calcd. for ($C_{25}H_{23}Cl_2N_5O$): C, 62.51; H, 4.83; N, 14.58. Found: C, 62.60; H, 5.01; N,14.38.

5.4 Conclusion

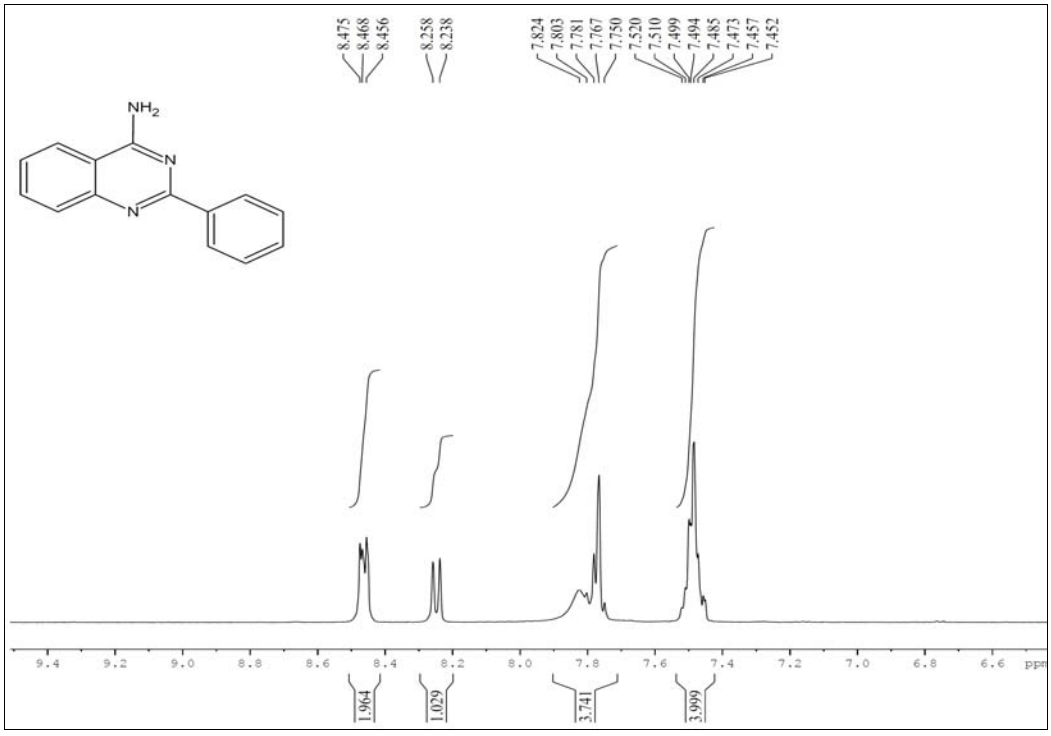
In order to discover new chemically stable DNA-directed alkylating agents, we connected the phenyl *N*-mustard pharmacophore to the 4-amino function of quinazolines using a urea moiety as the linker; however, the product 1-(4-(bis(2-chloroethyl)amino)phenyl)-3-(2-phenylquinazolin-4-yl)urea **99** has very poor solubility, which does not show solubility even in DMSO. Thus, due to solubility issues, the synthesis of above mentioned series was discontinued.

5.5 Representative Spectra

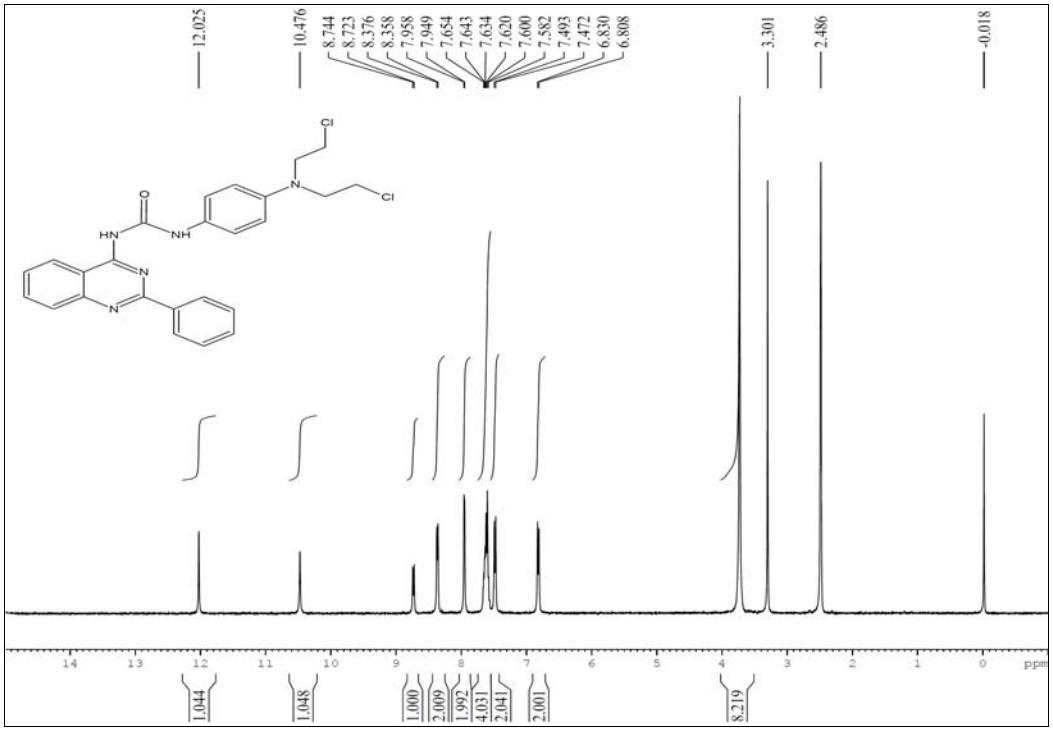
5.5.1 ^{1}H NMR Spectrum for compound **98**.



5.5.2 ^{1}H NMR Spectrum for compound **98** (ARO).



5.5.3 ^{1}H NMR Spectrum for compound **99.**



Section - A

References

References

1. Box, F. & Html, F.F.H. *Chinese Medical Journal*. **2008**, *121*, 26.
2. American Cancer, S. Cancer facts and figures 2007. (2007).
3. Nelson, S. M., Ferguson, L. R., and Denny, W. A. *Cell Chromosome.* **2004**, *3*, 2.
4. Bissell, M. J., and Radisky, D. *Nat. Rev. Cancer,* **2001**, *1*, 46.
5. Lemke, V. K.; Williams, D. A.; Roche, V. F.; Zito, S. W. **"Foye's principal of medicinal chemistry"**. 6th Edition, pp 363 (**2001**).
6. Rosenberg, S. A. Principles of Surgical Oncology. In: **"Cancer Principles and Practices of Oncology**" 6th Edition, pp 253 (**2001**). Editors. Devita, V. T.; Hellman, S.; Rosenberg, S. A. Lippincott Williams & Wilkins, Philadelphia, New York.
7. Landberg, T.G. Radiotherapy. In: "**Oxford Book of Oncology",** pp 867 (**1995**). Editors Peckham, M.; Pinedo H; Veronesi, U. Oxford University Press, Oxford, New York and the references cited therein.
8. Rosenberg, S. A. Biologic therapy. In: **"Cancer - Principles and Practices of Oncology"** 6th Edition, pp 307 (**2001**). Editors. Devita, V. T.; Hellman, S.; Rosenberg, S. A. Lippincott Williams & Wilkins, Philadelphia, New York.
9. Hirsch, J. *J. Amme. Med. Assoc.* **2006,** *296*, 1518.
10. Colvin**,** O. M. Antitumour alkylating agents. In: **"Cancer Principles and Practices of Oncology**" 6th Edition, pp 363 (**2001**). Editors. Devita, V. T.; Hellman, S.; Rosenberg, S. A. Lippincott Williams & Wilkins, Philadelphia, New York.
11. Chu, E.; Mota, A. C.; Fogarasi**,** M.C. *Antimetabolites*. In: **"Cancer Principles and Practices of Oncology**" *ibid*, (**2001**), pp 388.
12. Haskel**l,** C. M. Principles of Cancer chemotherapy – Plant product**s.** In: **"Cancer Treatment**" 5th Edition, pp 64 **(2001**). Editor. Haskell, C. M.; W. B. Saunders Company, Philadelphia, New York.
13. Champoux, J. J. *Annu. Rev. Biochem.* **2001**, *70*, 369.
14. Verweij, J.; Sparreboom, A.; Nooter, K. Antitumour antibiotics In: "**Cancer Chemotherapy and Biotherapy - Principles and Practice",** 3rd Edition, pp 482, (**2001**). Editors: Chabner; B. A.; Longo, D. L. Lippincot Williams & Wilkins, Philadelphia, USA.

15. Erlichman C.; Loprinzi, C.L. Hormonal therapies. In: **"Cancer Principles and Practices of Oncology**" 6th Edition, pp 478 (**2001**). Editors. Devita, V. T.; Hellman, S.; Rosenberg, S. A. Lippincott Williams & Wilkins, Philadelphia, New York.

16. Cheson, B.D. Miscellaneous chemotherapeutic agents. In: **"Cancer Principles and Practices of Oncology**" *ibid.*, (**2001**), pp 452.

17. Lawley, P. D. *BioEssays*, **1995**, *17*, 561.

18. Dong, Q.; Barsky, D.; Colvin, M. E.; Melius, C. F.; Ludeman, S. M.; Moravek, J. F.; Colvin, O. M.; Bigner, D. D.; Modrich, P.; Friedman, H. S. *Proc Natl Acad Sci U S A* **1995,** *92*, 12170.

19. Rink, S. M.; Hopkins, P. B. *Biochemistry* **1995,** *34*, 1439.

20. Brookes, P.; Lawley, P. D. *J. Chem. Soc.* **1961**, 3923.

21. Wang, A. L.; Tew, K.D. *Cancer Treatment Repts.* **1985**, *69*, 677.

22. Suzukake, K. V., B.P.; Vistica, D.T. *Biochem. Pharmacol.* **1983**, *32*, 165.

23. Brendel, M.; Ruhland, A. *Mutat. Res.* **1984,** *133*, 51.

24. Osborne, M. R.; Lawley, P. D.; Crofton-Sleigh, C.; Warren, W. *Chem Biol. Interact.* **1995,** *97*, 287.

25. Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Jarvinen, T.; Savolainen, J. *Nature Rev. Drug. Disc*, **2008**, *7*, 255.

26. Shanafelt, T. D.; Lin, T.; Geyer, S. M.; Zent, C. S.; Leung, N.; Kabat, B.; Browen, D.; Grever, M. R.; Byrd, J. C.; Kay, N. E. *Cancer*, **2007**, *109*, 2291.

27. Brock, N. *Cancer*, **1996**, *78*, 542.

28. Weyel, D.; Sedlacek, H. H.; Muller, R.; Brusselbach, S. *Gene. Ther.* **2000,** *7*, 224.

29. Springer, C. J.; Niculescu-Duvaz, I. *J. Clin. Invest.* **2000,** *105*, 1161.

30. Hamstra, D. A.; Rehemtulla, A. *Hum. Gene Ther.* **1999,** *10*, 235.

31. Springer, C. J.; Dowell, R.; Burke, P. J.; Hadley, E.; Davis, D. H.; Blakey, D. C.; Melton, R. G.; Niculescu-Duvaz, I. *J. Med. Chem.* **1995,** *38*, 5051.

32. Springer, C. J.; Antoniw, P.; Bagshawe, K. D.; Searle, F.; Bisset, G. M.; Jarman, M. *J. Med. Chem.* **1990,** *33*, 677.

33. Springer, C. J. *Drug Future* **1993**, *18*, 212.

34. Friedlos, F.; Davies, L.; Scanlon, I.; Ogilvie, L. M.; Martin, J.; Stribbling, S. M.; Spooner, R. A.; Niculescu-Duvaz, I.; Marais, R.; Springer, C. J. *Cancer Res.* **2002,** *62*, 1724.

35. Denny, W. A.; Wilson, W. R *J. Pharm. Pharmacol.* **1998,** *50*, 387.

36. Bagshawe K.D., Sharma S.K., Springer C.J., Rogers G., *Anal. Oncol*, **1994**, *5*, 879.

37. Jordan, A. M.; Khan, T. H.; Malkin, H.; Osborn, H. M. *Bioorg. Med. Chem.* **2002,** *10*, 2625.

38. Jordan, A. M.; Khan, T. H.; Osborn, H. M.; Photiou, A.; Riley, P. A. *Bioorg. Med. Chem.* **1999,** *7*, 1775.

39. Knaggs, S.; Malkin, H.; Osborn, H. M.; Williams, N. A.; Yaqoob, P. *Org. Biomol. Chem.* **2005,** *3*, 4002.

40. Pedersen, P. J.; Christensen, M. S.; Ruysschaert, T.; Linderoth, L.; Andresen, T. L.; Melander, F.; Mouritsen, O. G.; Madsen, R.; Clausen, M. H. *J. Med. Chem.* **2009,** *52*, 3408.

41. Grove, J. I.; Searle, P. F.; Weedon, S. J.; Green, N. K.; McNeish, I. A.; Kerr, D. J. *Anti-Cancer Drug Des.* **1999**, *14*, 461.

42. Green, N. K.; Kerr, D. J.; Mautner, V.; Harris, P. A.; Searle, P. F. *Methods Mol. Med.* **2004**, *90*, 459.

43. Graham, J.; Atwell, G. H.; Yang, S.; Frederik, B.; Pruijn, F. B.; Susan, M.; Pullen, S. M.; Hogg, A.; Patterson, A. V.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **2007**, *50*, 1197.

44. Palmer, B. D.; Wilson, W. R.; Atwell, G. J.; Schultz, D.; Xu, X. Z.; Denny, W. A. *J. Med. Chem.* **1994**, *37*, 2175.

45. Johansson, E.; Parkinson, G. N.; Denny, W. A.; Neidle, S. *J. Med. Chem.* **2003,** *46*, 4009.

46. Atwell, G. J.; Boyd, M.; Palmer, B. D.; Anderson, R. F.; Pullen, S. M.; Wilson, W. R.; Denny, W. A. *Anticancer. Drug. Des.* **1996,** *11*, 553.

47. Friedlos, F.; Denny, W. A.; Palmer, B. D.; Springer, C. J. *J. Med. Chem.* **1997,** *40*, 1270.

48. Hay, M. P.; Anderson, R. F.; Ferry, D. M.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **2003,** 46, 5533-45.

49. Atwell, G. J.; Yang, S.; Pruijn, F. B.; Pullen, S. M.; Hogg, A.; Patterson, A. V.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **2007,** *50*, 1197.

50. Anlezark, G. M.; Melton, R. G.; Sherwood, R. F.; Wilson, W. R.; Denny, W. A.; Palmer, B. D.; Knox, R. J.; Friedlos, F.; Williams, A. *Biochem. Pharmacol*, **1995**, *50*, 609.

51. Helsby, N. A.; Ferry, D. M.; Patterson, A. V.; Pullen, S. M.; Wilson, W. R. *Br. J. Cancer*, **2004**, *90*, 1084.

52. Patterson, A. V.; Ferry, D. M.; Edmunds, S. J.; Gu, Y.; Singleton, R. S.; Patel, K.; Pullen, S. M.; Hicks, K. O.; Syddall, S. P.; Atwell, G. J.; Yang, S.; Denny, W. A.; Wilson, W. R. *Clin. Cancer. Res.* **2007,** *13*, 3922.

53. Finlay, G. J.; Wilson, W. R.; Baguley, B. C. *Eur. J. Canc. Clin. Oncol.* **1989**, *25*, 1695.

54. Creech, H. J.; Preston, R. K.; Peck, R. M.; O'Connell, A. P.; Ames, B. N. *J. Med. Chem.* **1972**, *15*, 739.

55. Preston, R. K.; Peck, R. M; Breuninger, E. R.; Miller, A. J.; Creech, H. J. *J. Med. Chem.* **1964**, *7*, 471.

56. Peck, R. M.; Breuninger, E. R.; Miller, A. J.; Creech, H. J. *J. Med. Chem.* **1964**, *7*, 480.

57. Peck, R. M.; O'Connell, A. P.; Creech, H. J. *J. Med. Chem.* **1966**, *9*, 217.

58. Prakash, A. S.; Denny, W. A.; Gourdie, T. A.; Valu, K. R.; Woodgate, P. D.; Wakelin, L. P. *Biochemistry*, **1990**, *29*, 9799.

59. Gourdie, T. A.; Valu, K. K.; Gravatt, G. L.; Boritzki, T. J.; Baguley, Bruce C.. Wakelin, L. P. G.; Wilson, W. R.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 1177.

60. Kohn, K. W.; Orr. A.; O'Conor, P. M. *J. Med. Chem.* **1994**, *33*, 67.

61. Gravatt, G. L.; Baguley, B. C.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.***1991,** *34*, 1552.

62. McClean, S.; Costelloe, C.; Denny, W.A.; Searcey, M.; Wakelin, L.P.G. *Anti-Cancer Drug Design*, **1999**, *14*, 187.

63. Nelson, E. M.; Tewey, K. M.; Liu, L. F. *Proc. Natl. Acad. Sci. U S A* **1984,** *81*, 1361.

64. Rowe, T. C.; Chen, G. L.; Hsiang, Y. H.; Liu, L. F. *Cancer Res.* **1986,** *46*, 2021.

65. Jehn, U.; Heinemann, V. *Anticancer Res.* **1991,** *11*, 705.

66. Wells, R. J.; Arndt, C. A. *J. Pediatr. Hematol. Oncol.* **1995,** *17*, 225.

67. Fan, J-Y.; Valu, K.K.; Woodgate, P.D.; Baguley, B.C.; Denny, W.A. *Anti-Cancer Drug Design*, **1997**, *12*, 181.

68. Fan, J-Y.; Ohms, S.J.; Boyd, M.; Denny, W.A. *Chem Res Tox.*, **1999**, *12*, 1166.

69. Gravatt, G. L.; Baguley, B. C.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.***1994,** *37*, 4338.

70. Utsuno, K.; Maeda, Y.; Tsuboi, M. *Chem. Pharm. Bull.* **1999**, *47*, 1363

71. Drobyshev A. L.; Zasedatelev A. S.; Yarshov, G. M.; Mirzabekov, A. D. *Nucleic. Acids. Res.* **1999**, *27*, 4100.

72. Higgins, L. D.; Searle, M. S. *Chem. Commun.* **1999**, 1861.

73. Smaill, J.B.; Fan, J-Y.; Denny, W.A. *Anti-Cancer Drug Design*, **1998**, *13*, 857.

74. Arcamone, F.; Penco, S.; Orezzi, P. G.; Nicolella, V.; Pirelli, A. *Nature,* **1964**, *203*, 1064.

75. Arcamone, M. A.; Animati, F.; Barbieri, B.; Configliacchi, E.; D'Alessio, R.; Geroni, C.; Giuliani, F. C.; Lazzari, E.; Menozzi, M.; Mongelli, N.; Penco, S.; Verini, M. A. *J. Med. Chem.* **1989**, *32*, 774.

76. Pezzoni, G.; Grandi, M.; Basoli, G.; Capolongo, L.; Ballinari, D.; Giuliani, F. C.; Barbieri, B.; Pastori, A.; Pesenti, E.; Mongelli, N. *Br. J. Cancer*, **1991**, *64*, 1047.

77. Barbieri, B.; Giuliani, F. C.; Pezzoni, C.; Lazzari, E.; Arcamone, F.; Mongelli, N. *Proc. Am. Assoc. Cancer Res*. **1988**, *29*, 330.

78. Marchini, S.; Broggini, M.; Sessa, C.; D'Incalci, M. *Expert Opin. Invest.Drugs*, **2001**, *10*, 1703.

79. Broggini, M.; Erba, E.; Ponti, M.; Ballinari, D.; Geroni, C.; Spreafico, F.; D'Incalci, M. *Cancer Res.* **1991**, *51*, 199.

80. Broggini, M.; Coley, H. M.; Mongelli, N.; Pesenti, E.; Wyatt, M. D.; Hartley, J. A.; D'Incalci, M. *Nucleic Acid Res.* **1995**, *23*, 81.

81. Marchini, S.; Cozzi, P.; Beria, I.; Geroni, C.; Capolongo, L.; D'Incalci, M.; Broggini, M. *Anti-Cancer Drug Des.***1998**, *13*, 193.

82. Wyatt, M. D.; Lee, M.; Garbiras B. J.; Souhami, R. L.; Hartley, J. A. *Biochemistry*. **1995**, *34*, 13034.

83. Bigioni, M.; Salvatore, C.; Palma, C. *Anticancer Drugs.* **1997**, *8*, 845.

84. Cozzi, P.; Beria, I.; Biasoli, G.; Caldarelli, M.; Capolongo, L.; Geroni, C.; Mongelli, N. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 985.

85. Baraldi, P. G.; Romagnoli, R.; Pavani, M. G.; Nunez, M. D. C.; Bingham, J. P.; Hartley, J. A. *Bioorg. Med. Chem.* **2002**, *10*, 1611.

86. Baraldi, P. G.; Beria, I.; Cozzi, P.; Geroni, C.; Espinosa, A.; Gallo, M. A.; Entrena, A.; Bingham, J. P.; Hartley, J. A.; Romagnoli, R. *Bioorg. Med. Chem.* **2004**, *12*, 3991.

87. Atwell, G. J.; Yaghi, B. M.; Turner, P. R.; Boyd, M.; O'Connor, C. J.; Ferguson, L. R.; Baguley, B. C.; Denny, W.A. *Bioorg. Med. Chem.* **1995**, *3*, 679.

88. Bacherikov, V. A.; Chou, T.-C.; Dong, H.-J.; Zhang, X.; Chen, C.-H.; Lin, Y.-W.; Tsai, T.-J.; Lee, R.-Z.; Liu, L. F.; Su, T.-L. *Bioorg. Med. Chem.* **2005**, *13*, 3993.

89. Su, T.-L.; Lin, Y.-W.; Chou, T.-C.; Zhang, X.; Bacherikov, V. A.; Chen, C.-H.; Liu, L. F.; Tsai, T.-J. *J. Med. Chem*. **2006**, *49*, 3710.

90. Bacherikov, V. A.; Chou, T.-C.; Dong, H.-J.; Chen, C.-H.; Lin, Y.-W.; Tsai, T.-J.; Su, T.-L. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 4719.

91. Chen, C.-H.; Lin, Y.-W.; Zhang, X.; Chou, T.-C.; Tsai, T.-J.; Kapuriya, N.; Kakadiya, R.; Su, T.-L. *Eur. J. Med. Chem.* **2009**, *44*, 3056.

92. Su, T. L.; Chou, T. C.; Kim, J. Y.; Huang, J. T.; Ciszewska, G.; Ren, W. Y.; Otter, G. M.; Sirotnak, F. M.; Watanabe, K. A. *J. Med. Chem.* **1995,** *38*, 3226.

93. Kapuriya, N.; Kapuriya, K.; Zhang, X.; Chou, T.-C.; Kakadiya, R.; Wu, Y.-T.; Tsai, T.-H.; Chen, Y.-T.; Lee, T.-C.; Shah, A.; Naliapara, Y.; Su, T.-L. *Bioorg. Med. Chem*. **2008**, *16*, 5413.

94. Kapuriya, N.; Kapuriya, K.; Dong, H.; Zhang, X.; Chou, T.-C.; Chen, Y.-T.; Lee, T.- C.; Lee, W.-C.; Tsai, T.-H.; Naliapara, Y.; Su, T.-L. *Bioorg. Med. Chem*. **2009**, *17*, 1264.

95. Kakadiya, R.; Dong, H.; Kumar, A,; Dodia, N.; Zhang, X.; Chou, T.-C.; Lee, T.- C.; Shah, A.; Su, T.-L. *Bioorg. Med. Chem.* **2010**, *18*, 2285.

96. Jordan, A. M.; Khan, T. H.; Malkin, H.; Osborn, H. M. *Bioorg. Med. Chem.* **2002,** *10*, 2625.

97. Dowell, R. I.; Springer, C. J.; Davies, D. H.; Hadley, E. M.; Burke, P. J.; Boyle, F. T.; Melton, R. G.; Connors, T. A.; Blakey, D. C.; Mauger, A. B. *J. Med. Chem.* **1996,** *39*, 1100.

98. Domarkas, J.; Dudouit, F.; Williams, C.; Qiyu, Q.; Banerjee, R.; Brahimi, F.; Jean-Claude, B. J. *J. Med. Chem*. **2006**, *49*, 3544.

99. O'Connell, M. J.; Walworth, N.C.; Carr, A.M. *Trends Cell Biol*. **2000**, 10, 296-303.

100. Erba, E.; Mascellani, E.; Pifferi, A.; D'Incalci, M. C. *Int. J. Cancer*. **1995**, *62*, 170.

101. Smaill, J. B.; Palmer, B. D.; Rewcastle, G. W.; Denny, W. A.; McNamara, D. J.; Dobrusin, E. M.; Bridges, A. J.; Zhou, H.; Showalter, H. D.; Winters, R. T.; Leopold, W. R.; Fry, D. W.; Nelson, J. M.; Slintak, V.; Elliot, W. L.; Roberts, B. J.; Vincent, P. W.; Patmore, S. J. *J. Med. Chem.* **1999,** *42*, 1803.

102. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827.

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

104. Chou, T.-C.; O'Connor, O. A.; Tong, W. P.; Guan, Y.; Zhang, Z.-G.; Stachel, S. J.; Lee, C.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 8113.

105. Al-Nasiry, S.; Geusens, N.; Hanssens, M.; Luyten, C.; Pijnenborn, R. *Hum. Reprod.* **2007**, *22*, 1304.

106. Chou, T.-C.; Martin, N. *Compusyn Software;* ComboSyn, Inc., Paramus, NJ, **2005**.

107. Chou, T.-C.; Talalay, P. *Adv. Enzyme Regul.* **1984**, *22*, 27.

108. Chou, T.-C. *Pharmacol. Rev.* **2006**, *58*, 621.

109. Kakadiya, R.; Dong, H.; Lee, P.-C.; Kapuriya, N.; Zhang, X.; Chou, T.-C.; Lee, T.-C.; Kapuriya, K.; Shah, A.; Su, T.-L. *Bioorg. Med. Chem.* **2009**, *17*, 5614.

110. Kuo, S.-C.; Lee, H. Z.; Juang, J. P.; Lin, Y. T.; Wu, T. S.; Cheng, J. J.; Lednicer, D.; Paull, K. D.; Lin, C. M.; Hamel, E.; Lee, K.-H. *J. Med. Chem.* **1993**, *36*, 1146.

111. Li, L.; Wuang, H. K.; Kuo, S.-C.; Lednicer, D.; Lin, C. M.; Hamel, E.; Lee, K.-H. *J. Med. Chem.* **1994**, *37*, 1126.

112. Li, L.; Wang, H. K.; Kuo, S.-C.; Wu, T. S.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K.-H. *J. Med. Chem.* **1994**, *37*, 3400.

113. Xiao, Z. P.; Lv, P. C.; Xu, S. P.; Zhu, T. T.; Zhu, H. L. *Chem. Med. Chem.* **2008**, 3, 1516.

114. McOmie, J. F. W.; West, D. E. *Organic Syntheses, Coll. Vol. 5,* **1973**, 412.

115. Sirisoma, N.; Pervin, A.; Zhang, H.; Jiang, S.; Willardsen, J. A.; Anderson, M. B.; Mather, G.; Pleiman, C. M.; Kasibhatla, S.; Tseng, B.; Drewe, J.; Cai, S. X. *J. Med. Chem.* **2009**, *52*, 2341.

116. Wang, G. W.; Miao, C. B.; Kang, H. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1426.

117. Hour, M. J.; Huang, L. J.; Kuo, S. C.; Xia, Y.; Bastow, K.; Nakanishi, Y.; Hamel, E.; Lee, K. H. *J. Med. Chem.* **2000,** *43*, 4479.

118. Andersen, K. E.; Lundt, B. F.; Jorgensen, A. S.; Braestrup, C. *Eur. J. Med. Chem.* **1996**, *31*, 417.

Chapter -6

Novel *N*-nitrosourea and carbamate

6.1 INTRODUCTION

In the past years, our laboratory have synthesized a series of DNA-directed alkylating agents in which *N*-mustard derivatives linked to the DNA-affinic molecules such as acridine, 9-anilinoacridines, quinoline, 4-anilinoquinazoline and 4-anilinoquinoline chromophore via various alkyl spacers, urea and carbamates linker[1-4]. These studies demonstrated that the strategy to design DNA-directed alkylating agents has high possibility in finding new potential anticancer agents. To find a new DNA-directed alkylating agent with potential antitumor activity, we then proposed to replace N-mustard pharmacophore with *N*-nitrosourea or *N*-nitrosocarbamate residue.

Nitrosoureas and nitrosocarbamate derivatives which are extremely active class of antitumor agents that is effective against solid tumors, as well as leukemia. In particular, 2-chloro ethyl derivatives and some of their metabolites show great promises as effective anti tumor agents[5,6]. For the treatment of number of experimental and clinical tumors, several *N*-(2-chloroethyl)-*N*-nitrosoureas have successfully been applied as chemotherapeutics agents. Not only do this drug show the ability to inhibit the growth and spread of many form of solid tumors in man and animals, but some of them, such as *N*,*N*-bis(2-chloroethyl)-*N*-nitrosourea **5** (BCNU) and *N*-(2-chloroethyl)-*N*-cyclohexyl-*N*-nitrosourea **3** (CCNU), also have been found to rapidly enter the cerebrospinal fluid and control meningeal tumor implants. As a result they have been used in the treatment of brain tumors and menigeal leukemia. The drug decomposes spontaneously in the body to form two active compounds and alkylaing agents and a carbamolyting agent. The organic isocyanate which is formed carbamoylates lysine residue in proteins and may inactive DNA repair enzymes. The alkylating agent reacts initially with the O-6 position of a guanine moiety in one strand of DNA, then with the N-3 position of cytosine in the other strand to produce interstrand cross linking. Nitrosourea or nitrosocarbamates which utilized either a quinoline or quianazoline ring as a carrier group were synthesized and evaluated for anticancer activity.

During a random screening program of anticancer agents conducted at the Chemotherapy National Service Center (CCNSC) compound 1-methyl-3-nitro-1-nitrosoguanidine[7] **1** showed very weak antileukemic activity[8, 9]. Assay of analogs of this compound led to the discovery of the antitumor activity of 1-methyl-1-

nitrosourea[10] **2**, was tested and shown[8] to be more effective than **1** in increasing the life span of mice with ip-inoculated leukemia. It was soon discovered that introduction of a 2-chloroethyl chain on the nitrogen atom bearing the nitroso group (CNUs) led to much increased activity[11]. These chloroethyl derivatives were lipophilic enough to cross the blood–brain barrier and therefore were useful in the treatment of brain tumors, which led to the synthesis of a large number of nitrosoureas, including lomustine **3** (CCNU) and its methyl derivative semustine **4**, carmustine **5** (BCNU), nimustine **6** (ACNU), the water-soluble tauromustine **7** and fotemustine **8**, but toxicity problems have prevented their widespread use. In 1967, streptozotocin (streptozocin) **9**, a hydrophilic natural nitrosourea, was isolated from a strain of *S. achromogenes*. This compound was chosen as a lead because initial SAR studies suggested that hydrophilic nitrosoureas were more potent and less toxic, and a number of analogs, like chlorozotocin **10**, were prepared. Currently, the most clinically important nitrosoureas are CCNU, BCNU, ACNU, and streptozotocin. Nitrosoureas have been widely studied from a mechanistic point of view.

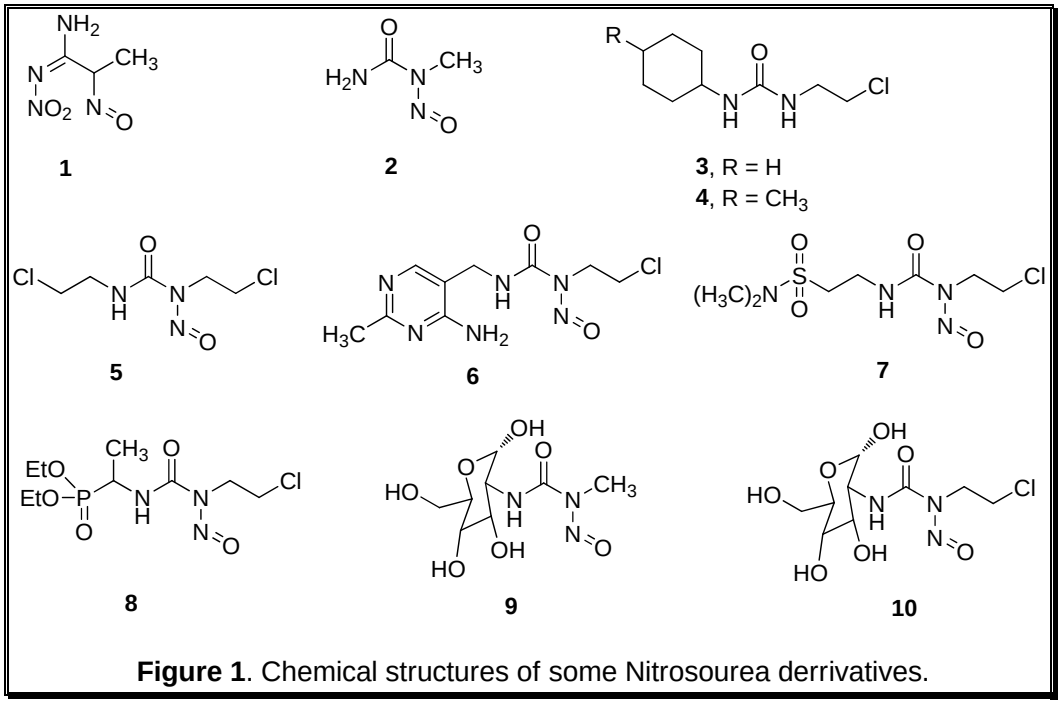


Figure 1. Chemical structures of some Nitrosourea derrivatives.

The presence of the nitroso group labilizes the nitrogen-carbon bond, leading to two electrophiles, an isocyanate **12** and a diazene hydroxide **14**, which has been detected in some cases by electrospray ionization mass spectroscopy.[12] This intermediate in

turn generates a diazonium salt **15**[13] (Fig. 2). Alkylation seems to be the main reaction responsible for antitumor activity, while carbamoylation takes place primarily on amino groups in proteins, leading to inhibition of several DNA repair mechanisms. *N*-Nitrosoamides and *N*-nitrosocarbamates, which can behave as alkylating (but not carbamoylating) agents have also antitumor activity, which supports the above statement.[14] The above mechanism was based mainly on studies of the thermal decomposition of nitrosoureas under anhydrous conditions,[13] but in water solution the reaction is much more complex and has been explained by the mechanism shown in Fig. 3. Addition of a molecule of water to the nitrosourea, in its tautomeric form,[15] gives the tetrahedral intermediate **19**, which is decomposed into a primary amine, carbon dioxide, and **14**. This elimination requires an antiperiplanar conformation for **25**. Addition of a nucleophile other than water to the nitrosourea tautomer explains the isolation of carbamoylated products, formed by elimination of **14**.

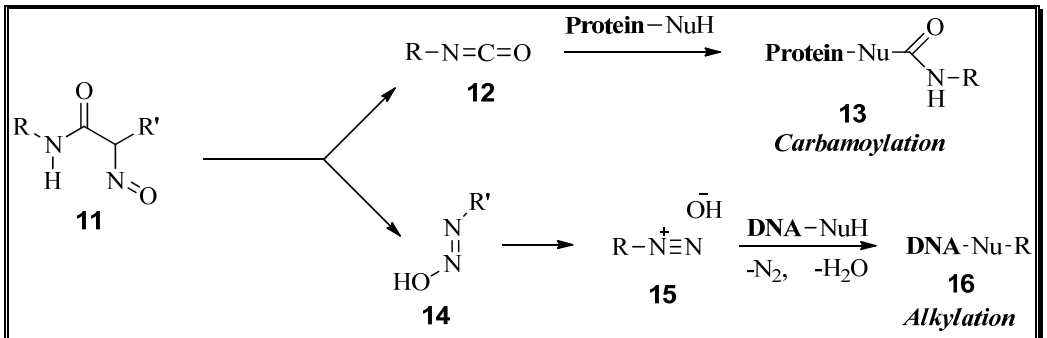


Figure 2. Thermal decomposition of nitrosoureas.

Most nitrosoureas contain one chloroethyl chain on the nitrosated nitrogen, which allows them to act as DNA cross-linking agents. Reaction of electrophilic diazonium species **25** with guanine is assumed to take place on O-6 to give **27**. In fact, addition of O6-alkylguanine-DNA alkyltransferase, an enzyme that breaks O-6 guanine adducts, prevents cross-linking. This monoalkylated product reacts subsequently with the N-3 atom of the cytosine unit in the complementary DNA strand, by anchimeric assistance of the guanine N-1 atom through intermediate **28**, giving the cross-linked product 30 (Fig. 4).

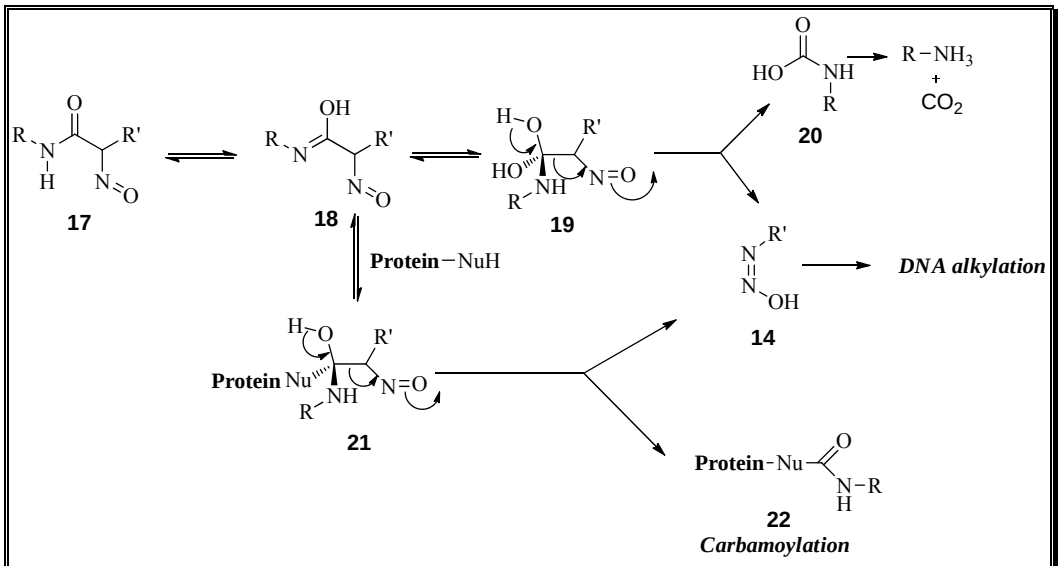


Figure 3. Decomposition of nitrosoureas in water solution.

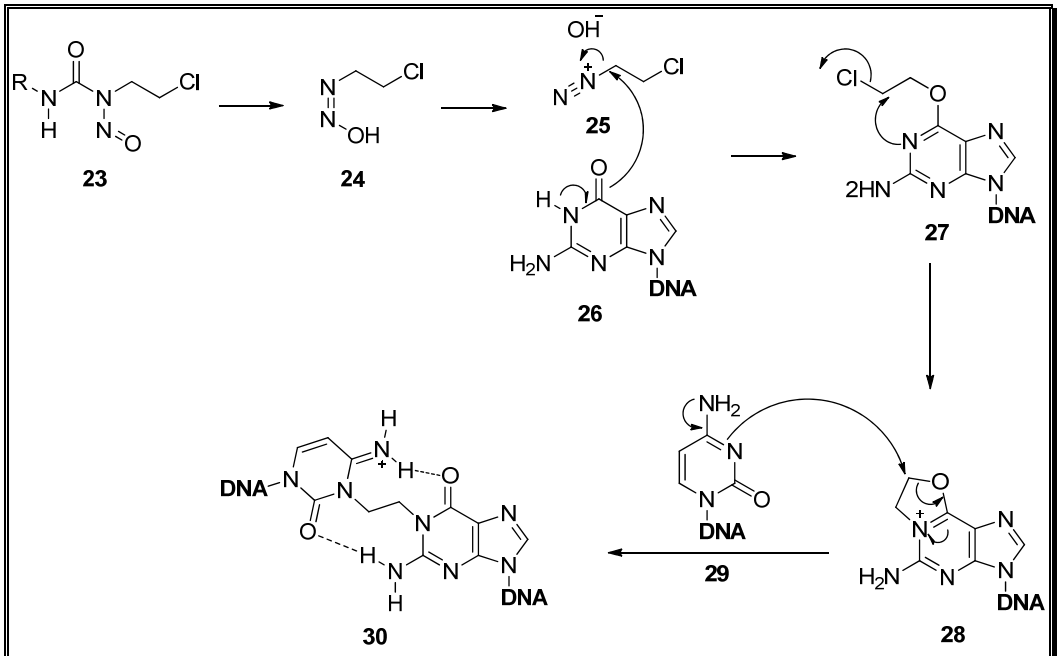


Figure 4. DNA cross-linking by nitrosoureas.

In an alternative mechanism, intact nitrosourea molecules rather than diazonium species can directly alkylate DNA. Thus, nucleophilic attack of guanine O-6 to the nitrosourea tautomer **31** gives intermediate **33**. Although alternative mechanisms have been proposed, according to labeling experiments it is probable that **33** cyclizes to the nitrosoisoxazolidine **34**, which is attacked by another O-6 atom of a guanine unit

neighboring in the DNA sequence to give **35**. In this adduct, the O-6 of the first guanine is carbamoylated and the O-6 of the second guanine is alkylated with a 2-hydoxydiazoethyl group (Fig. 5). Diazonium generation and attack of N-3 from a cytosine of the opposite DNA strand, with anchimeric assistance from guanine N-1, finally gives the carbamoylated cross-linked product **36**.

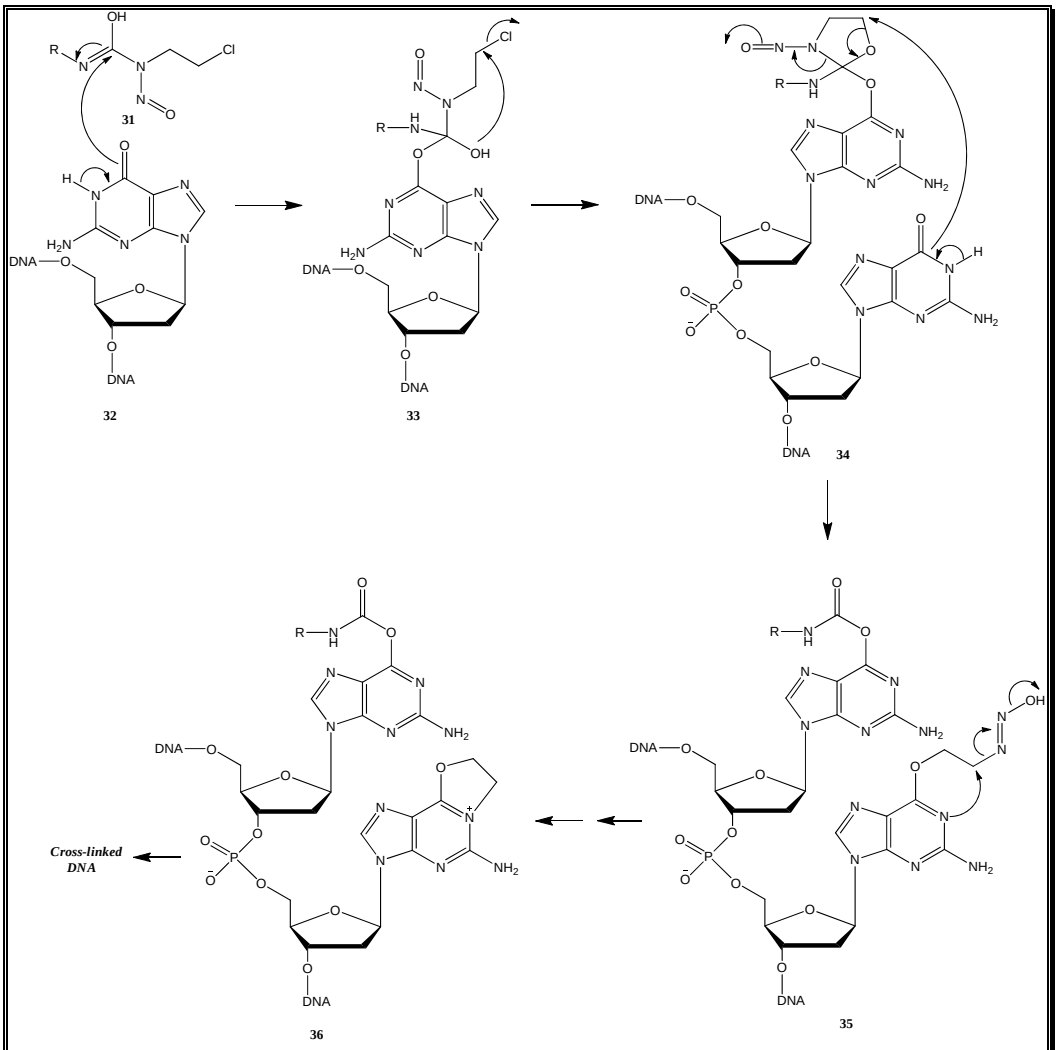


Figure 5. Alternative mechanisam for DNA cross-linking by nitrosoureas.

Streptozotocin differs from other nitrosoureas in that it does not cross the blood–brain barrier because of its high hydrophilicity, and it also shows a relatively low myelosuppression because of decreased entry into bone marrow cells. Its main cytotoxicity is exerted on the pancreas b cells because their glucose carrier facilitates drug uptake to the islets. Therefore, the main applications of streptozotocin are the

induction of diabetes mellitus in experimental animals and treatment of islet cell pancreatic tumors, normally in association with nicotinamide for reasons that will be explained below. As expected from its nitrosourea structure, streptozotocin methylates DNA, specially at the guanine N-7 and O-6 positions,[16] but there is also much evidence that shows that free radicals play an essential role in its cytotoxicity.[17] It has been shown that streptozotocin induces the generation of nitric oxide,[18] superoxide and hydroxyl radicals, and also that association with oxygen radical scavengers, such as nicotinamide, prevents streptozotocin induced cleavage of is let DNA.[19]

6.2 Aliphatic analogs

Johnston et. Al. have synthesized a group of monosubstituted *N*-nitrosoureas, the substitution with either the methyl or 2-chloroethyl group, i.e. compounds **2** and **36**, resulted[20,21] in higher anticancer activity than either a substitution with longer carbon chains or an unsaturated group. Substitution at both nitrogen of the urea with the 2-chloroethyl moiety resulted in the bis(*N*-2-chloroethyl)-*N*-nitrosourea (BCNU, carmustine, **5**) which was found[20] to be the most active agent of a large series of such analogs and more active than the N1 methyl analogs **37**. Compounds **36** and **5** were clearly superior to **2** against both the ip and ic-implanted L1210 leukemia.[21]

Same group have synthesized 1,3-bis(2-haloethyl)-1-nitrosoureas **38-42** for their anticancer activity, the bis-fluoroethyl (BFNU, **38**) and **39** were active[22] against both ip- and ic-implanted L1210 leukemia, whereas the bis-bromoethyl compound (BBNU, **40**) and the bromo-chloro compound **41** were only active against ip-implanted L1210 and the bis-iodoethyl compound (BINU, **42**) was inactive against both ip- and ic implanted L1210. The order of reactivity of the 2-haloethyl compound F, Cl > Br > I is reversed in the C-X bond strengths, i.e. I < Br < Cl < F. Thus, the iodo and bromo analogs **42** and **40** should be more susceptible to nucleophilic attack and, hence, could undergo decomposition in the plasma before reaching the interior of the cells.

Much attention has been devoted to the synthesis and biological testing of bisnitrosoureas. The first reported[21] bis-*N*-methylnitrosoamides **43-45** were somewhat more active than the simple analog **37**.

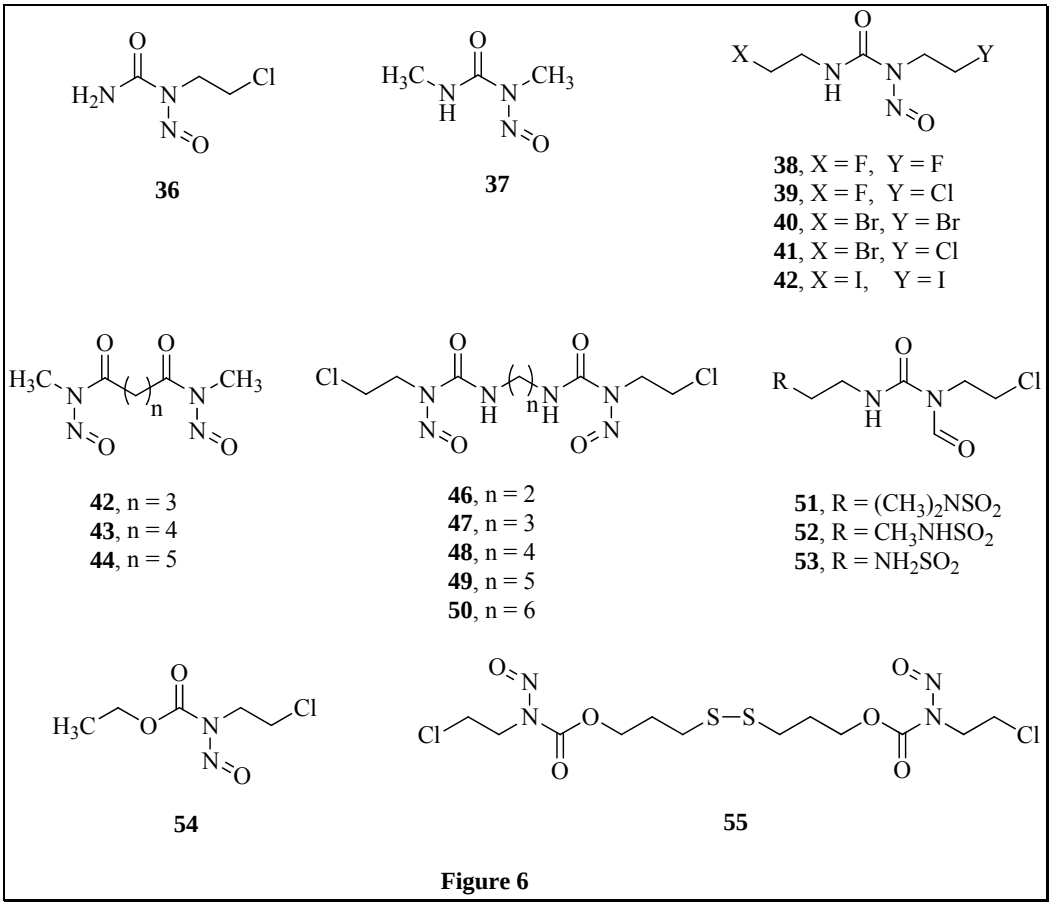


Figure 6

Similarly, the biological evaluation[23,24,25] of the bis-*N*-(2-chloroethyl)- *N*-nitrosoureas **46-50** against the rat leukemia L5222 revealed little change from that of the parent compound BCNU (**5**), and no relationship could be found[23,24] between the polymethylene chain length linking the CENU moieties and their anticancer activities.

Tauromustine (TCNU, **51**), a analog of the amino acid taurine, as well as its two probable metabolites **52** and **53** were synthesized. The anticancer activity of compounds **51-53** against L1210 leukemia, Walker mammary carcinoma, Lewis lung carcinoma, Harding-Passey melanoma, and colon carcinoma C was equal to or better than that of BCNU (**5**), and several other *N*-nitrosoureas. Several nitroso-carbamate derivatives were synthesized and tested for anticancer activity[26]. Compound **54**, **55** were found to be highly active against both the ip- and ic-inoculated L1210

6.3 Alicyclic Analogs

Early in the history of nitrosourea research it was discovered[22] that the *N*-cyclohexyl-*N*'-(2-haloethyl)- *N*'-nitrosoureas (FCNU, **56**) and CCNU (lomustine, **3**) had excellent activities against both the ip- and ic-implanted L1210 leukemia, as was demonstrated by the number of survivors on day [27]. As a result of this work a large number of alkyl-substituted cyclohexyl analogs **57-64** were synthesized and screened[22,28] against the L1210 cell line by means of the log kill and therapeutic ratio ED50/ LD10 criteria. A number of analogs which included substituted cyclohexyl **57-60**, cyclopentyl **61**, methylcyclopentyl **62**, and 2-indanyl **63**, bornyl **64**, were found[22,28] to be highly active against both the ip- and ic-inoculated L1210, with their therapeutic ratios ranging from 0.28 to 0.77.

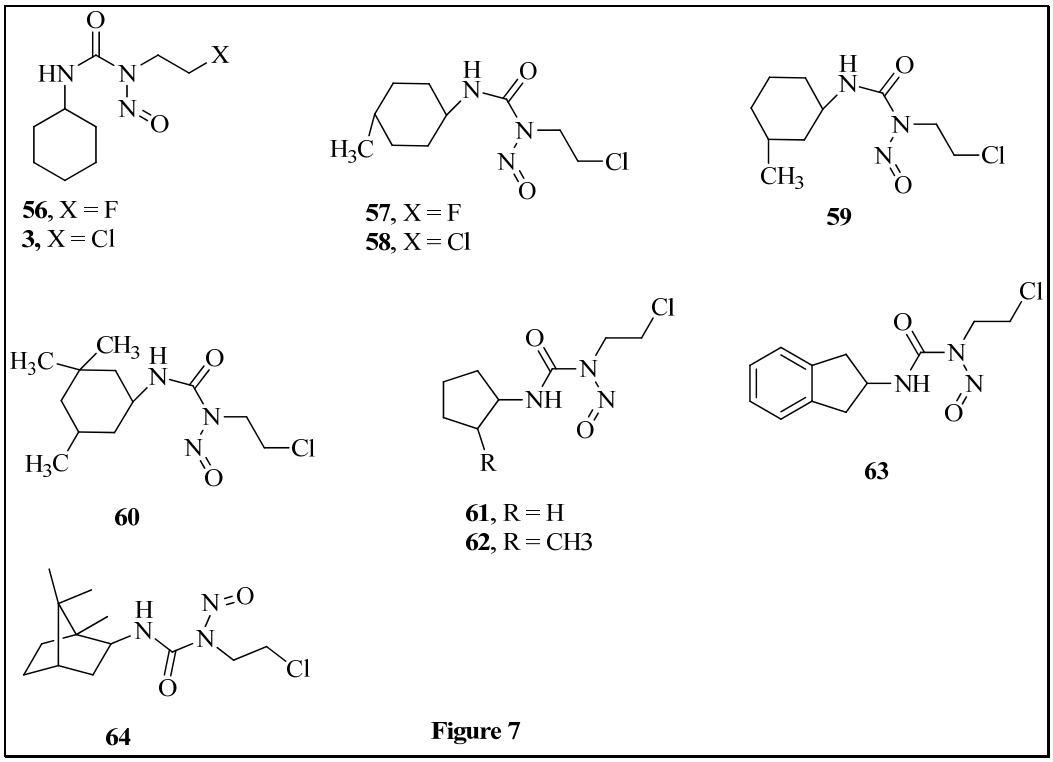


Figure 7

6.4 Aromatic analogs

Kim and co-worker have synthesized a large series of *ortho*-, *meta*-, and *para*-substituted phenyl analogs of *N*-methyl-*N*-nitrosourea and *N*-(2-chloroethyl)-*N*-nitrosourea and tested in vitro[29,30] for inhibitory activity against the L1210 leukemia. However, the in vivo testing of various *ortho*-, *meta*-, *para*-substituted aryl

nitrosourea analogs and aryl bis-nitrosoureas revealed[22,21,28,31,32] that only a few compounds such as **65-70** possessed activities against the ip-inoculated L1210, albeit all were found to be inactive against the ic-inoculated L1210. The conclusion drawn[22] from these results was that an aromatic ring prevents the passage of these drugs across the blood-brain barrier.

Bigler et. al. have synthesized[33] Several nitrosourea analogs of CCNU, **71-74** and tested for anticancer activity. The congeners **71**, **72**, and **74** had moderate and compound **73** high activity against the ipinoculated P388 and L1210 leukemias over a wide dose range. The 4-phenyl-4'-fluorophenyl analog **73** also exhibited high activity against solid cancers, including the B16 melanoma, colon adenocarcinoma, and Lewis lung carcinoma, but either low or no activity against the Harding-Passey melanoma and the ependymoblastoma brain tumor.[33] the (2-chloroethyl)nitrosocarbamate have been synthesized and tested for anticancer activity. Compound **75** and **76** were showed very good in vitro activity against NCI-H23 (lung) and SNB-7 (CNS).

6.5 Heterocyclic analogs

Several *para* substituted derivatives of phensuximide (**77**) were known to be good anticonvulsant agents,[34] and a relationship was established[35] between anticonvulsant activity and the ability to penetrate the central nervous system (CNS). On the basis of these results, several *N*-nitrosourea analogs **78-82** of phensuximide were synthesized and tested[36] as potential CNS anticancer agents. The 2-chloroethyl derivative **82** was the only analog with a significant activity of 96% ILS against ip-L1210 in mice.[36] The compound was found to have a moderate activity against the CNS cancer ependymoblastoma with a 140% ILS, but the clinical drugs BCNU (**5**) and MeCCNU (**58**) were much more effective against both cancer lines. By contrast, the glutarimide analog 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1- nitrosourea (PCNU, **83**) had a high activity against both the ip- and ic-inoculated L1210 in mice.[37,38]

Substitution of the cyclohexyl ring of CCNU (**3**) with either piperidine, morpholine, or 2,6-dimethylmorpholine rings yielded the corresponding CENU semicarbazides **84**, **85**, and **86**, respectively.[39,40] The water-insoluble piperidine analog **84** was very active against the rat leukemia L5222 and Yoshida sarcoma in the rat similarly to the water-soluble morpholino congeners **85** and **86**.[39-41] The unsubstituted morpholine CENU **85** was more active than **86** against two neurogenic cancers but both were less active than

the clinical drug cyclophosphamide.[41] A pyridine analogs **87** were synthesized and tested[39] for anticancer activity. The 3-picolyl analog **87** was very active against P388 leukemia in vivo.

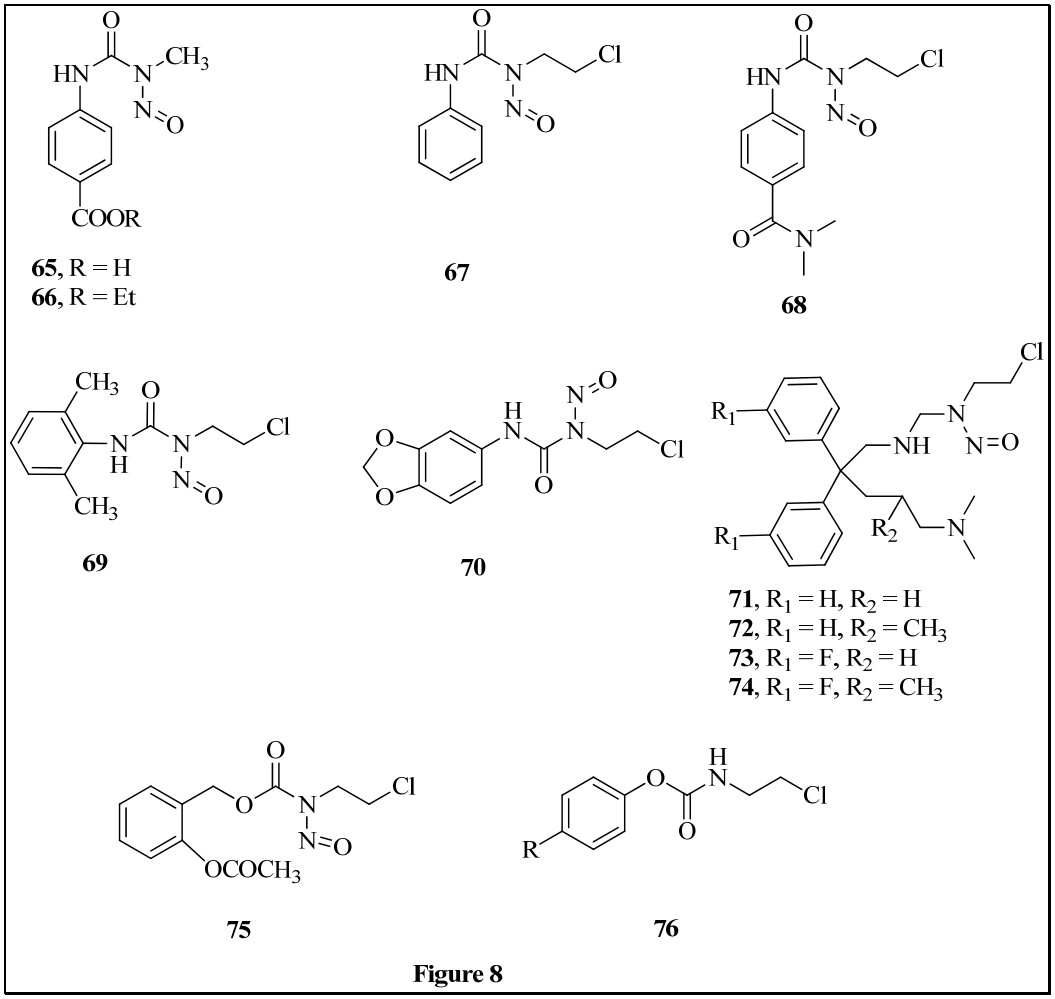


Figure 8

Substitution of the cyclohexyl ring of CCNU (**3**) with either piperidine, morpholine, or 2,6-dimethylmorpholine rings yielded the corresponding CENU semicarbazides **84**, **85**, and **86**, respectively.[39,40] The water-insoluble piperidine analog **84** was very active against the rat leukemia L5222 and Yoshida sarcoma in the rat similarly to the water-soluble morpholino congeners **85** and **86**.[39-41] The unsubstituted morpholine CENU **85** was more active than **86** against two neurogenic cancers but both were less active than the clinical drug cyclophosphamide.[41] A pyridine analogs **87** were synthesized and tested[39] for anticancer activity. The 3-picolyl analog **87** was very active against P388 leukemia in vivo.

Filippatos and co-workers have synthesized 8-quinolylnitrosourea **88** and various tricyclic xanthen-9-yl- and thioxanthen- 9-ylnitrosoureas **89** and **90**.[42] The compound **89** and **90** was only weakly active against the P388 leukemia in vivo. Recently, Domarkas et. al. have synthesized several nitroso urea using 4-anilinoquinazoline[5]. Compound **91** shown good EGFR TK inhibitor and presented an anomalously long half-life in serum-containing media ($t_{1/2}$ = 41 h). Reynolds et. al. have synthesized[43] some (2-chloroethyl)nitrosocarbamate as potential anticancer alkylating agents. Compound **92a,b** were found very good active against DLD-1 (colon) cancer cell lines.

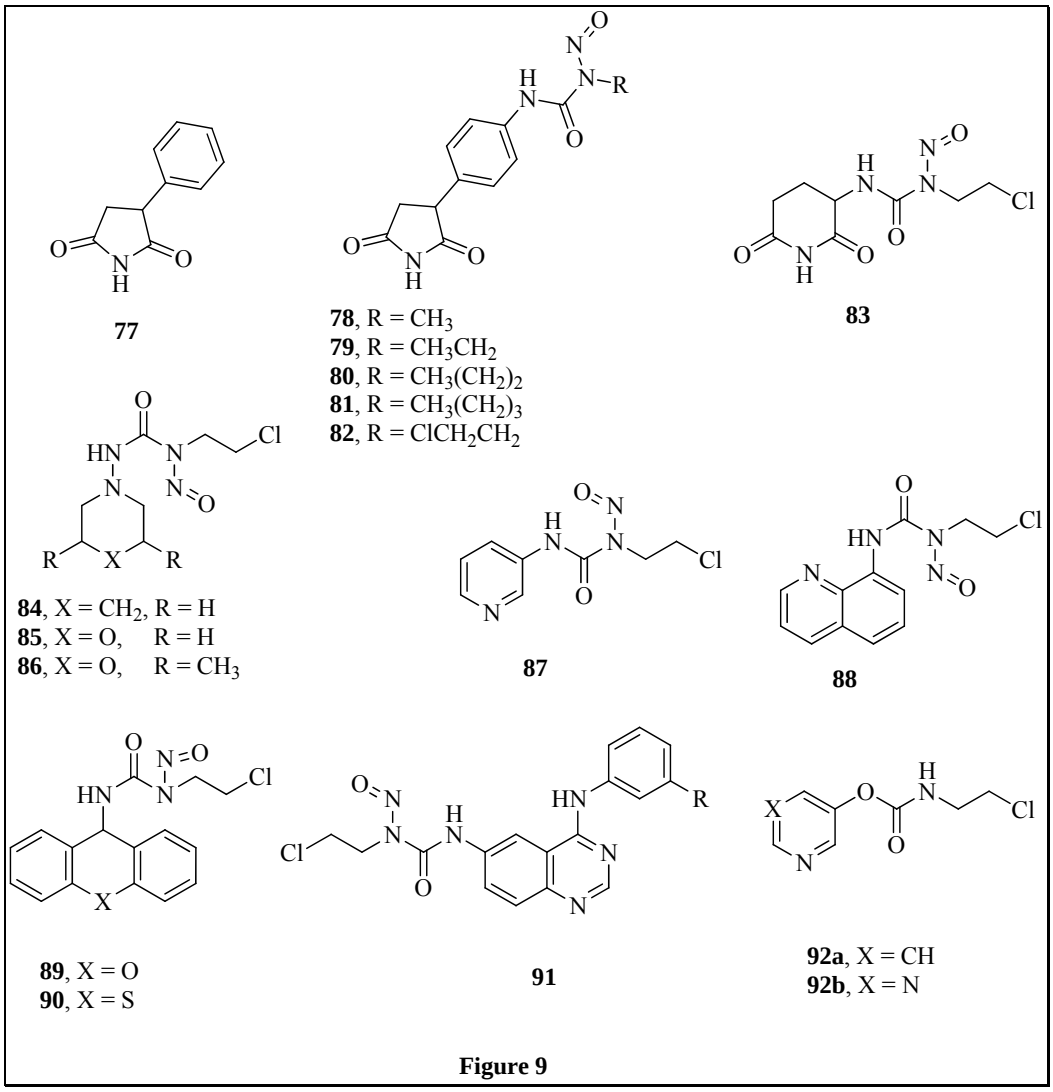


Figure 9

6.6 Hydroxyalkyl Analogs

Eisenbrand and co-workers have synthesized water-soluble analogs **93-95** possessed dramatically different anticancer activities.[23,24] Thus, the anticancer evaluation of 3-(2-hydroxyethyl)- 1-(2- chloroethyl)-1-nitrosourea (HECNU, **93**) resulted in 90% cures against the ip-inoculated rat L5222 leukemia and an 85% cancer weight reduction against sc-implanted Walker carcinoma. However, the corresponding figures for **94** and **95** were 10.5% and 5.0%, respectively. Further studies revealed[24,25] that HECNU (**93**) was more active than BCNU (**5**) against both the ip- and ic-implanted L5222 leukemia. A number of esters **96** and ethers **97** and **98** of HECNU have been found[44] to have high activity against the rat L5222 leukemia and rat glioma G616. In particular, the methanesulfonate analog HECNU-MS (**96**) possessed excellent antileukemic activity.

On the basis of this, Heal and co-worker have synthesized several polyhydroxy-CENU analogs **99-102** and displayed as high activity as that of BCNU (**5**) against the L1210 leukemia. The water-soluble polyhydroxy analogs were strongly myelosuppressive, as measured by the depression of peripheral blood neutrophil count on day three, the nadir of white blood cell suppression.[45]

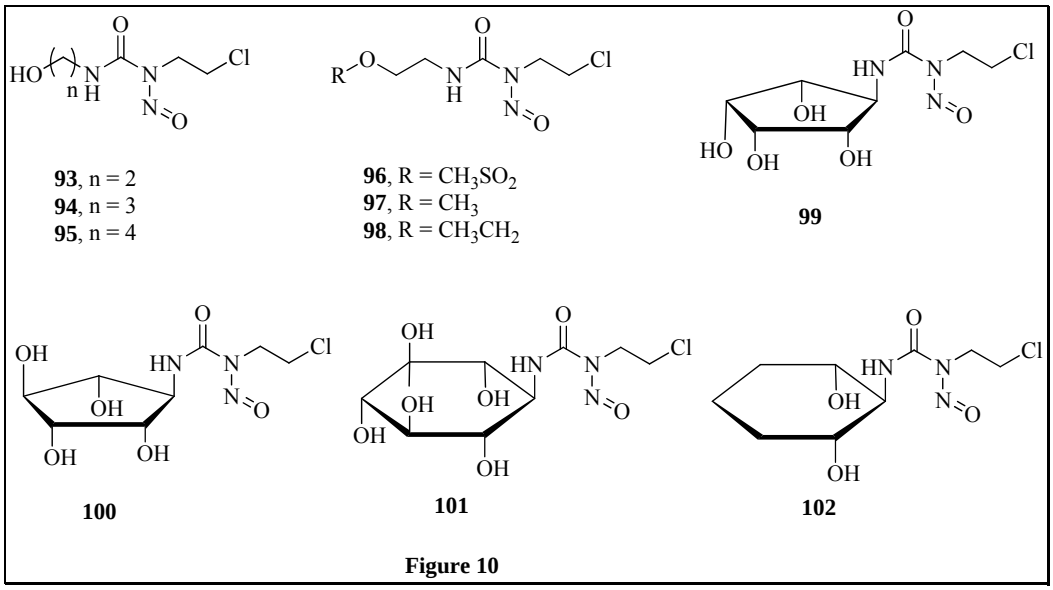


Figure 10

6.7 Carbohydrate Analogs

Several CENU analogs of 1-deoxyaldopentose, namely the ribofuranosyl-CENU (RFCNU, **103**), ribopyranosyl-CENU (RPCNU, **104**), and xylopyranosyl-CENU (XPCNU, **105**) were synthesized[46,47] and shown[47-49] to have some activity in vivo against the L1210. In these studies the prolongation of survival (PS) values are given as ∞ when more than 50% of the animals are cured. These compounds were less toxic and had greater therapeutic indices than CCNU (**3**) or MeCCNU (**58**). Of all compounds tested, only RFCNU (**103**) was not immunosuppressive in the hemolytic plaqueforming cell (PFC) test, either before or after the addition of the antigen in the form of fresh sheep red blood cells administered ip to mice.[49] The other compounds **104** and **105** were immunosuppressive whether given before or after the antigen.

Compound **106** had the highest %ILS and greatest reduction of the tumor volume of any member of this series as well as CCNU (**3**) and RFCNU (**103**). The percentage of mice developing the melanoma after 39 days was 42% for **106** and 65% for CCNU, but at 90 days the values were approximately 65% for both compounds.[50]

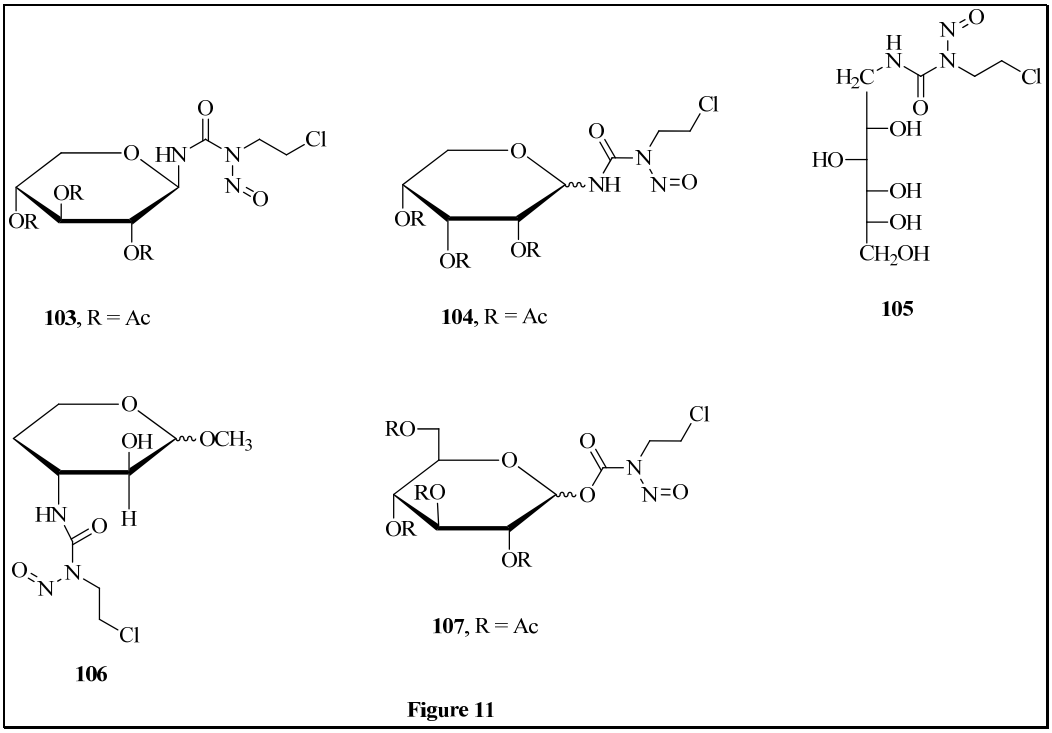


Figure 11

6.8 Amino acid and peptide analogs

The structure-activity studies of *N*-2-(chloroethyl)- *N*-nitrosourea (CENU) analogs of hydroxyalkyl compounds, such as HECNU **93**, and of carbohydrates indicated that such water-soluble analogs possessed greatly reduced bone marrow toxicity and improved therapeutic indices. The attachment of L-amino acids to the CENU moiety could add desirable hydrophilic properties.

Suami and other group have synthesized CENU amino acid primary amides **108-113**[51,52] and tested anticancer activity in vivo against the rat L5222 and murine L1210 leukemias. Among the primary amides the CENU sarcosinamide **112** was particularly attractive because of a combination of high anticancer activity of 711% ILS, very high chemical stability with a half-life of 330 min, and low toxicity with a LD50 of 392 mg/kg.458 The L-proline analog **113** also had high chemical stability and low toxicity but also a greatly reduced anticancer activity, so there was no obvious correlation between the % ILS and the chemical half-life values. The CENU L-serinamide (**111**) had excellent therapeutic indices equal to 40.[51]

A large number of CENU analogs of carboxylic acids and amides of amino acids **114**, **115**, dipeptides **116**, **117**, **118**, and tripeptides **119**, **120** were screened for in vivo activity against three transplantable mouse adenosarcomas of the colon (MAC)[53-55] and MNU induced mammary carcinoma[56]. The amide derivatives **114**, **115**, and **117** had higher activity against the sc-administered solid tumors MAC 13 and MAC 26 than the acid analogs **115**. The free acid dipeptide analog CENU-Ala-Ala (**115**) was highly active against the ascitic MAC 15A tumor line but was either weakly active or inactive against the sc-administered MAC 13 and MAC 26 tumor lines.[53,54]

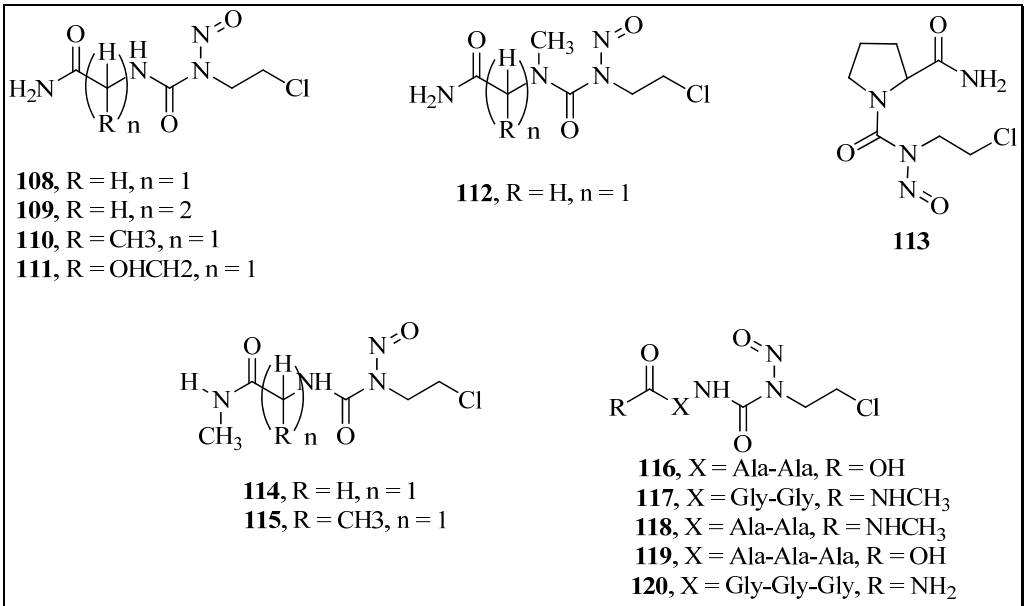


Figure 12

6.9 Steroid Analogs

The discovery of estrogen receptors (ER) in human breast cancer has led to significant progress in the management of the disease. A series of androgen-linked nitrosocarbamates which are related to the estrogen analog estramustine (**121**) were synthesized.[57] From this series the *N*-(2-chloroethyl)-*N*-nitrosocarbamate of 19-nortestosterone (**122**) was studied in detail.[58] Compound **122** exhibited excellent in vitro activity against the L1210 leukemia but it had only a low in vivo activity against the L1210, Ehrlich ascites, and Walker carcinoma. Compound **122** possessed alkylating but no carbamoylating properties.[58] The administration of **122** caused a dose-dependent reduction of the growth of dimethylbenz(*a*)anthracene (DMBA)-induced mammary tumors in rats,[58] and a greater reduction of tumor growth and tumor DNA synthesis than either administration of 19-nortestosterone or CCNU (**3**).

Carroll et. al. have synthesized[59] steroid-linked *N*-nitrosourea **123** caused, at a daily dose of 40 mg/kg, an 80-100% inhibition of a rat mammary tumor. The first estrogen-linked *N*-nitrosoureas **124**, **125** were synthesized and tested anticancer activity. The CENU analogs **124** at a daily dose of 40 mg/kg, were found[60] to inhibit the growth of DMBA-induced rat transplantable mammary cancer by factors of 85% and 100%, respectively whereas the corresponding *N*-methyl-*N*-nitrosoureas **125** at the same daily doses inhibited the cancer growth by only 23% and 15%, respectively.

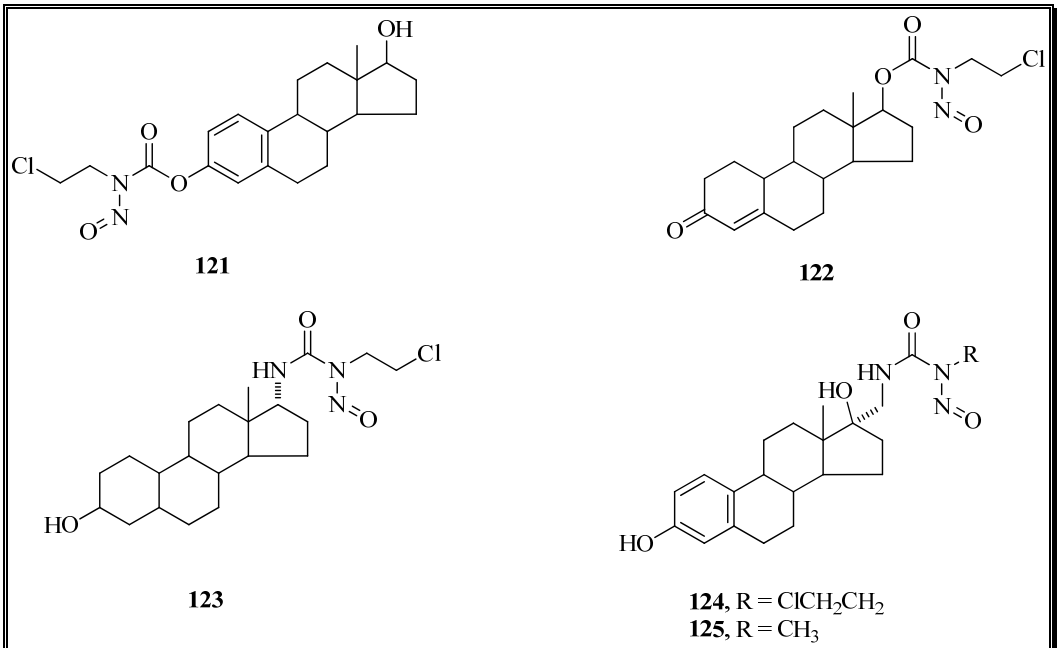
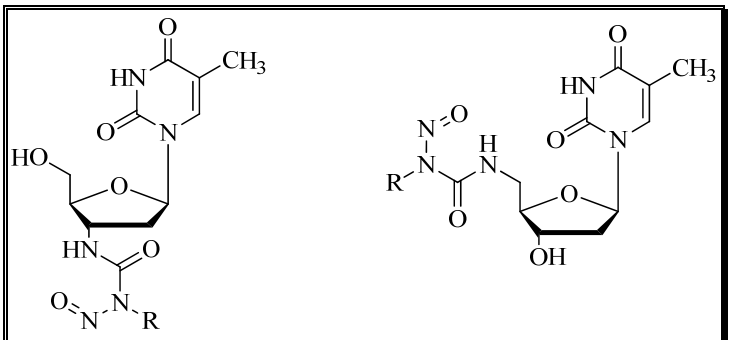


Figure 13

6.10 Nucleoside Analogs

Most of the reported work on the nucleoside compounds has focused on the attachment of the *N*-nitrosourea group to the 3'- and 5'-positions of the carbohydrate portion of the molecules. The C3' *N*-methyl- and *N*-chloroethyl-*N*-nitrosourea containing compounds **126** and **127** had approximately an equal growth inhibitory effect on the H.Ep-2 cells while the C5' *N*-chloroethyl analog **129**, but not the *N*-methyl analog **128**, was shown[61] to have good in vitro activity. No clear correlation was found[61] between the cytotoxicity of compounds **126-129** and their carbamoylating and alkylating activities relative to BCNU (**33**). Thus, the C3' compounds **126** and **127** had widely differing alkylating activities but nearly equal growth inhibitory properties.[61] The C5' analogs **128** and **129** had low alkylating activities but only **129** was cytotoxic.



126, R = CH_3
127, R = $ClCH_2CH_2$

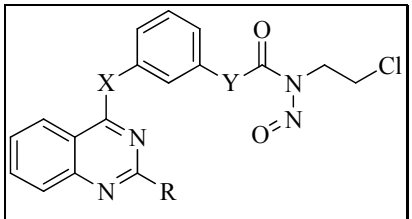
128, R = CH_3
129, R = $ClCH_2CH_2$

Figure 14

6.11 Aim of the current work

The goals of the current study are finding new anticancer agents with high therapeutic efficacy, low toxicity, good bioavailability, and effective against various multi-drug resistant tumor cells. In the past years, we have focused on the research and development of DNA-alkylating agents as anticancer agents. The following class of DNA-alkylating have been designed and synthesized for antitumor evaluation:

I. Synthesis and characterization of several new (2-chloroethyl) nitrosocarbamates derivatives



130

Figure 15

The chemical synthesis and characterization of Nitrosocarbamate derivatives are described in the Chapter 7.

Chapter -7

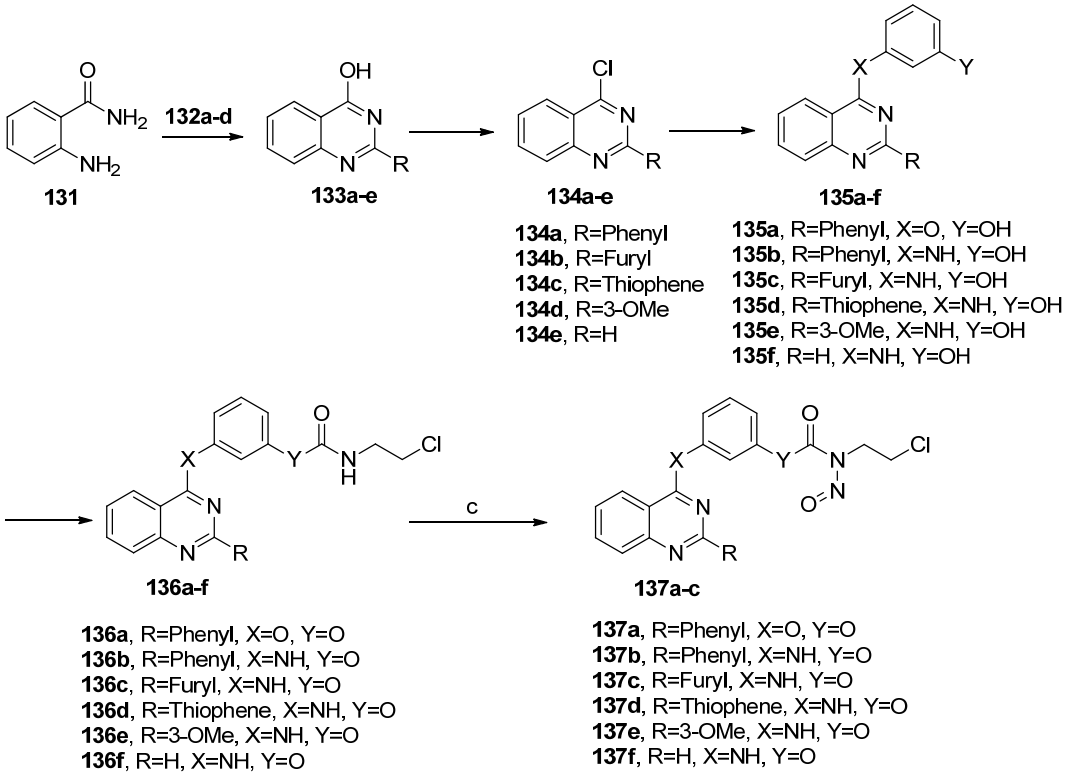
Synthesis and characterization of several new (2-Chloroethyl) nitroso-carbamates derivatives

7.1 Chemistry

The (2-Chloroethyl)nitrosocarbamates conjugates were prepared via reaction of Anthranilamide **131** and substituted benzaldehyde (**132a-d**) in the presence of $FeCl_3$.6H2O to produce substituted 4-hydroxyquinazoline(**133a-e**)[62], followed by treatment of **133a-e** with $POCl_3$ to give substituted 4-chloroquinazoline(**134a-e**)[63]. Reaction of **134a** with resorsinol in xylene containing DMAP to give 3-(2-phenylquinazolin-4-yloxy) phenol(**135a**). The other compounds **135b-f** was obtained by the treatment of Substituted aniline in isopropanol (IPA) in the presence of concentrated HCl as previously described[64]. Condensation of **135a-f** with the commercially available 2-chloroethylisocynate in anhydrous chloroform in the presence of triethylamine (TEA) at room temperature to furnish the carbamate derivatives (**136a-f**)[5], which were subsequently nitrosated with $NOBF_4$ in acetonitrile to give nitrosocarbamates **137a-f**[5]. The latter compounds were purified by column chromatography on silica gel with a mixture of ethylacetate/hexane as eluent.

7.2 Reaction Scheme

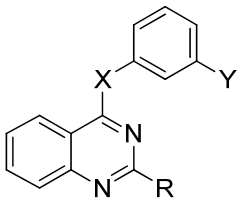
Scheme 1: Synthetic route of *N*-nitrosocarbamate derrivatives



Scheme 1: (a) $FeCl3.6H_2O$/water/reflux; (b) $POCl_3$/reflux; (c) DMAP/xylene/140°C; (d) HCl/IPA/reflux; (e) Triethylamine/Chloroform, room temprature; (f) Nitrosonium tetrafluoroborate/acetonitrile/room temprature.

7.3 Physical data

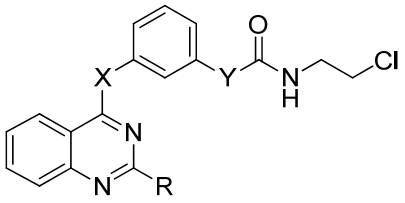
Table 1. Analytical data and yields of compounds (5a-f).



5a-f

Sr. No.	Substitute			MF	MW	Yield %	MP °C
	R	X	Y				
135a	C_6H_5	O	OH	$C_{20}H_{14}N_2O_2$	314.34	34	194-195
135b	C_6H_5	NH	OH	$C_{20}H_{15}N_3O$	313.35	64	239-241
135c	2-Furyl	NH	OH	$C_{18}H_{13}N_3O_2$	303.31	80	297-298
135d	2-Thiophen	NH	OH	$C_{18}H_{13}N_3O_2S$	319.38	77	280-281
135e	3'-MeO-C_6H_4	NH	OH	$C_{21}H_{17}N_3O_2$	343.38	79	270-272
135f	H	NH	OH	$C_{14}H_{11}N_3O$	237.36	87	231-233

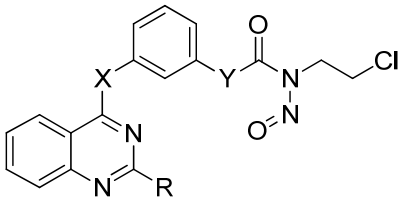
Table 2. Analytical data and yields of Carbamate derivatives (6a-f).



6a-f

Sr. No.	Substitute			MF	MW	Yield %	MP °C
	R	X	Y				
136a	C_6H_5	O	OH	$C_{23}H_{18}ClN_3O_3$	419.86	64	140-142
136b	C_6H_5	NH	OH	$C_{23}H_{19}ClN_4O_2$	418.88	75	230-231
136c	2-Furyl	NH	OH	$C_{21}H_{17}ClN_4O_3$	408.84	74	247-249
136d	2-Thiophen	NH	OH	$C_{21}H_{17}ClN_4O_2S$	424.90	70	236-237
136e	3'-MeO-C_6H_4	NH	OH	$C_{24}H_{21}ClN_4O_3$	448.90	82	170-171
136f	H	NH	OH	$C_{17}H_{15}ClN_4O_2$	342.78	69	165-167

Table 3. Analytical data and yields of Nitrosocarbamate derivatives (7a-f).



7a-f

Sr. No.	Substitute			MF	MW	Yield %	MP °C
	R	X	Y				
137a	C_6H_5	O	OH	$C_{23}H_{17}ClN_4O_4$	448.86	37	98-99
137b	C_6H_5	NH	OH	$C_{23}H_{18}ClN_5O_3$	447.87	38	133-135
137c	2-Furyl	NH	OH	$C_{21}H_{16}ClN_5O_4$	437.84	34	109-110
137d	2-Thiophen	NH	OH	$C_{21}H_{16}ClN_5O_3S$	453.90	47	107-108
137e	3'-MeO-C_6H_4	NH	OH	$C_{24}H_{20}ClN_5O_4$	477.90	56	140-141
137f	H	NH	OH	$C_{17}H_{14}ClN_5O_3$	371.78	45	101-102

7.4 Experimental

Chemistry: general methods

All commercial chemicals and solvents were reagent grade and were used without further purification unless otherwise specified. Melting points were determined on a Fargo melting point apparatus and are uncorrected. Column chromatography was carried out on Silica Gel G60 (70–230 mesh, ASTM; Merck and 230–400 mesh, Silicycle Inc.). 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 Brucker AVANCE 600 DRX and 400 MHz, Brucker Top-Spin spectrometers in the indicated solvent. The chemical shifts were reported in ppm (d) relative to TMS.

Procedure:

2-Phenylquinazolin-4(3*H*)-one (133a). A mixture of an anthranilamide **131** (10.0 g, 73.5 mmole), benzaldehyde (**132a**, 7.8 g, 73.5 mmole) and $FeCl_3$.6H2O (39.1 g, 147.0 mmole) in refluxing water (700 mL) was stirred for 1 h. After completion of the reaction, the reaction mixture was cooled to room temperature and filtered to give the crude product. The crude product was purified by recrystallization from DMF and water, to give **133a**, 14 g (86 %); mp 235–237 °C; ^{1}H NMR (DMSO-d_6) δ 7.48–7.59 (4H, m, 4 × ArH), 7.71–7.73 (1H, m, ArH), 7.79–8.13 (1H, m, ArH), 8.16–8.18 (3H, m, 3 × ArH), 12.51 (1H, s, exchangeable, NH).

2-(Furan-2-yl)-quinazolin-4(3*H*)-one (133b). Compound **133b** was synthesized from anthranilamide **1** (10.0 g, 73.5 mmole), furan-2-carbaldehyde (**132b**, 7.0 g, 73.5 mmole) and $FeCl_3$.6H2O (39.1 g, 147.0 mmole) in water (700 mL): Yield 11.0 g (73 %); mp 217–219 °C; ^{1}H NMR (DMSO-d_6) δ 6.75–6.76 (1H, m, ArH), 7.50 (1H, t, J = 7.0 Hz, ArH), 7.63–8.64 (1H, m, ArH), 7.69 (1H, d, J = 8.0 Hz, ArH), 7.80–7.84 (1H, m, ArH), 8.00 (1H, d, J = 1.2 Hz, ArH), 8.12 (1H, d, J = 8.0 Hz, ArH), 12.50 (1H, s, exchangeable, NH).

2-(Thiophen-2-yl)-quinazolin-4(3*H*)-one (133c). Compound **133c** was synthesized from anthranilamide **131** (10.0 g, 73.5 mmole), thophen-2-carbaldehyde (**132c**, 8.2 g, 73.5 mmole) and $FeCl_3$.6H2O (39.1 g, 147.0 mmole) in water (700 mL): Yield 17.0 g

(80 %); mp 280–281 °C; ^{1}H NMR (DMSO-d_6) δ 7.23–7.24 (1H, m, ArH), 7.25–7.51 (2H, m, 2 × ArH), 7.79–7.88 (2H, m, 2 × ArH), 8.11–8.13 (1H, m, ArH), 8.23–8.24 (1H, m, ArH), 12.66 (1H, s, exchangeable, NH).

2-(3-Methoxyphenyl)-quinazolin-4(3*H*)-one (133d). Compound **133c** was synthesized from anthranilamide **131** (10.0 g, 73.5 mmole), 3-methoxybenzaldehyde (**132c**, 10.0 g, 73.5 mmole) and $FeCl_3$.6H2O (39.1 g, 147.0 mmole) in water (700 mL): Yield 16.0 g (88 %); mp 208–209 °C; ^{1}H NMR (DMSO-d_6) δ 3.88 (3H, s, Me), 7.10–7.13 (1H, m, ArH), 7.20–7.22 (1H, m, ArH), 7.53–7.58 (2H, m, 2 × ArH), 7.72–7.75 (2H, m, 2 × ArH), 7.83–7.87 (1H, m, ArH), 8.16–8.18 (1H, m, ArH), 12.12 (1H, s, exchangeable, NH).

4-Chloro-2-phenylquinazoline (134a). To a magnetically stirred solution of $POCl_3$ (25 mL) at 0°C was added portion wise 2-phenylquinazolin-4(3*H*)-one **133a** (5.0 g, 22.4 mmole). The reaction mixture was refluxed for 2 hrs. After completion of the reaction, the excess $POCl_3$ was removed by vacuo. The residue was poured into a mixture of chloroform (50 mL) + ice cold water (80 mL) + ammonia solution (20 mL). The chloroform layer was separated and the aqueous layer was extracted with an additional 20 ml of chloroform. The united chloroform extracts were dried over Na_2SO_4 and filtered, and the solvent was removed by distillation to give **134a**, 4.5 g (87 %); mp 125–127 °C (lit.[21] 124–125 °C); ^{1}H NMR (DMSO-d_6) δ 7.58–7.69 (4H, m, 4 × ArH), 7.91–7.92 (2H, m, 2 × ArH), 8.16–8.21 (3H, m, 3 × ArH). Anal. ($C_{14}H_9ClN_2$): C, H, N.

4-Chloro-2-(furan-2-yl)-quinazoline (134b). Compound **134b** was synthesized from 2-(furan-2-yl)-quinazolin-4(3*H*)-one **133b** (5.0 g, 23.5 mmole) in $POCl_3$ (25 mL): Yield 5.0 g (92 %); mp 115–116 °C; ^{1}H NMR (DMSO-d_6) δ 6.74–6.76 (1H, m, ArH), 7.44–7.53 (1H, m, ArH), 7.76–7.80 (2H, m, 2 × ArH), 7.81–7.85 (1H, m, ArH), 8.00–8.13 (2H, m, 2 × ArH).

4-Chloro-2-(thiophen-2-yl)-quinazoline(134c). Compound **134c** was synthesized from 2-(thiophen-2-yl)-quinazolin-4(3*H*)-one **133c** (5.0 g, 21.9 mmole) in $POCl_3$ (25 mL): Yield 4.5 g (83 %); mp 121–123 °C; ^{1}H NMR (DMSO-d_6) δ 6.72–6.76 (1H, m, ArH), 7.43–7.53 (1H, m, ArH), 7.76–7.80 (2H, m, 2 × ArH), 7.81–7.88 (2H, m, 2 × ArH), 8.12–8.13 (1H, m, ArH).

4-Chloro-2-(3-methoxyphenyl)-quinazoline(134d). Compound **134d** was synthesized from 2-(3-methoxyphenyl)-quinazolin-4(3*H*)-one **133d** (5.0 g, 19.8 mmole) in $POCl_3$ (25 mL): Yield 5.0 g (94 %); mp 110–111 °C; ^{1}H NMR (DMSO-d_6) δ 3.84 (3H, s, Me), 7.02–7.05 (1H, m, ArH), 7.36–7.38 (2H, m, 2 × ArH), 7.50–7.52 (1H, m, ArH), 7.83–7.87 (3H, m, 3 × ArH), 8.16–8.18 (1H, m, ArH).

4-Chloroquinazoline (134e). Compound **134e** was synthesized from quinazolin-4(3*H*)-one **133e** (5.0 g, 34.2 mmole) in $POCl_3$ (25 mL): Yield 5.2 g (89 %); mp 98–99 °C; ^{1}H NMR (DMSO-d_6) δ 7.61–7.69 (1H, m, ArH), 7.79–7.83 (1H, m, ArH), 7.90–7.99 (1H, m, ArH), 8.16–8.18 (1H, m, ArH), 8.79 (1H, s, ArH).

3-(2-Phenylquinazolin-4-yloxy)phenol (135a). To a solution of 4-chloro-2-phenylquinazoline **134a** (3 g, 12.5 mmole), resorcinol (2.1 g, 18.7 mmole) and xylene (30 mL), was added DMAP at room temperature. The reaction mixture was stirred at 140°C for 24 hours. After completion of the reaction, the reaction mixture was cooled up to 0-5 °C. The product was collected by filtration and washed with 10% NaOH and finally with water, to give **135a**, 1.3 g (34 %); mp 194–195 °C; ^{1}H NMR (DMSO-d_6) δ 6.78–6.80 (1H, m, ArH), 6.83–6.88 (2H, m, 2 × ArH), 7.32–7.36 (1H, m, ArH), 7.46–7.50 (3H, m, 3 × ArH), 7.72–7.76 (1H, m, ArH), 8.01–8.05 (2H, m, 2 × ArH), 8.06–8.26 (2H, m, 2 × ArH), 8.35–8.37 (1H, m, ArH), 9.79 (1H, s, exchangeable, OH). Anal. ($C_{20}H_{14}N_2O_2$): C, H, N.

3-((2-Phenylquinazolin-4-yl)amino)phenol (135b). To a solution of 4-chloro-2-phenylquinazoline **134a** (3.0 g, 12.5 mmole) and m-aminophenol (1.36 g, 12.5 mmole) in 40 mL of isopropanol (IPA) was added HCl two drops at room temperature. The reaction mixture was heated up to reflux temperature for 3 h and then was cooled to room temperature. The solid was collected by filtration and washed with IPA, and dried to give **135b**, 2.5 g (64 %); mp 239–241 °C; ^{1}H NMR (DMSO-d_6) δ 6.77–6.82 (1H, m, ArH), 6.83–6.90 (2H, m, 2 × ArH), 7.23–7.28 (1H, m, ArH), 7.34–7.40 (3H, m, 3 × ArH), 7.79–8.05 (3H, m, 3 × ArH), 8.08–8.26 (2H, m, 2 × ArH), 8.28–8.32 (1H, m, ArH), 9.70 (1H, brs, exchangeable, OH), 11.30 (1H, brs, exchangeable, NH). Anal. ($C_{20}H_{15}N_3O$): C, H, N.

3-((2-(Furan-2-yl)quinazolin-4-yl)amino)phenol (135c). Compound **135c** was synthesized from 4-chloro-2-(furan-2-yl)-quinazoline **134b** (2.0 g, 8.6 mmole), m-

aminophenol (1.0 g, 8.7 mmole) and IPA (30 mL) containing HCl 1-2 drops: yield 2.1 g (80 %); mp 297–298 °C; ^{1}H NMR (DMSO-d_6) δ 6.75–6.77 (1H, m, ArH), 6.91–6.93 (1H, m, ArH), 7.27–7.36 (2H, m, 2 × ArH), 7.40–7.41 (1H, m, ArH), 7.75–7.81 (2H, m, 2 × ArH), 8.03–8.07 (1H, m, ArH), 8.19–8.26 (2H, m, 2 × ArH), 8.91–8.93 (1H, m, ArH), 9.75 (1H, brs, exchangeable, OH), 11.35 (1H, brs, exchangeable, NH). Anal. ($C_{18}H_{13}N_3O_2$): C, H, N.

3-((2-(Thiophen-2-yl)quinazolin-4-yl)amino)phenol (135d). Compound **135d** was synthesized from 4-chloro-2-(thiophen-2-yl)-quinazoline **134b** (2.0 g, 8.1 mmole), m-aminophenol (0.88 g, 8.1 mmole) and IPA (30 mL) containing HCl 1-2 drops: yield 2.0 g (77 %); mp 280–281 °C; ^{1}H NMR (DMSO-d_6) δ 6.76–6.77 (1H, m, ArH), 7.28–7.39 (4H, m, 4 × ArH), 7.74–7.78 (1H, m, ArH), 8.03–8.33 (2H, m, 2 × ArH), 8.34–8.35 (1H, m, ArH), 8.82–8.84 (2H, m, 2 × ArH), 9.25 (1H, brs, exchangeable, OH), 11.26 (1H, brs, exchangeable, NH). Anal. ($C_{18}H_{13}N_3O_2S$): C, H, N.

3-((2-(3-Methoxyphenyl)quinazolin-4-yl)amino)phenol (135e). Compound **135e** was synthesized from 4-chloro-2-(3-methoxyphenyl)-quinazoline **134d** (1.0 g, 3.7 mmole), m-aminophenol (0.40 g, 3.7 mmole) and IPA (30 mL) containing HCl 1-2 drops: yield 1.0 g (79 %); mp 270–272 °C; ^{1}H NMR (DMSO-d_6) δ 3.57 (3H, s, Me), 6.77–6.79 (1H, m, ArH), 7.25–7.34 (4H, m, 4 × ArH), 7.54–7.57 (1H, m, ArH), 7.81–7.85 (1H, m, ArH), 7.90–7.96 (2H, m, 2 × ArH), 8.07–8.11 (1H, m, ArH), 8.23–8.26 (1H, m, ArH), 8.79–8.81 (1H, m, ArH), 8.90 (1H, brs, exchangeable, OH), 11.46 (1H, brs, exchangeable, NH). Anal. ($C_{21}H_{17}N_3O_2$): C, H, N.

3-(Quinazolin-4-ylamino)phenol (135f). Compound **135f** was synthesized from 4-chloroquinazoline **134e** (2.0 g, 12.1 mmole), m-aminophenol (1.3 g, 12.1 mmole) and IPA (30 mL) containing HCl 1-2 drops: yield 2.5 g (87 %); mp 231–232 °C; ^{1}H NMR (DMSO-d_6) δ 6.76–6.78 (1H, m, ArH), 7.13–7.18 (2H, m, 2 × ArH), 7.25–7.29 (1H, m, ArH), 7.84–7.88 (1H, m, ArH), 7.97–7.99 (1H, m, ArH), 8.09–8.13 (1H, m, ArH), 8.87–8.93 (2H, m, 2 × ArH), 9.80 (1H, brs, exchangeable, OH), 11.56 (1H, brs, exchangeable, NH). Anal. ($C_{14}H_{11}N_3O$): C, H, N.

3-((2-Phenylquinazolin-4-yl)oxy)phenyl (2-chloroethyl)carbamate (136a). To a solution of 3-(2-phenylquinazolin-4-yloxy)phenol **135a** (1.2 g, 3.8 mmole) and chloroform (15 mL) containing triethylamine (0.6 mL) was added 2-

chloroethylisocynate (0.45 g, 4.0 mmole) at 0 °C over 20 min. The reaction mixture was stirred at room temperature for 1 hour. After that, the reaction mixture was poured into ice cold water and separated organic layer. The aqueous layer was extracted two times by chloroform and combined all organic layers. The organic layer was washed with 10% K_2CO_3 solution and water, which was dried over Na_2SO_4 and evaporated by vacuo. The solid was collected and purified by Colum chromatography using dichloromethane as an eluent, to give **136a**, 0.9 g (64 %); mp 140–142 °C; ^{1}H NMR (DMSO-d_6) δ 3.40 (2H, q, J = 6.0 Hz, CH_2), 3.68 (2H, t, J = 6.0 Hz, CH_2), 7.14–7.16 (1H, m, ArH), 7.16–7.35 (2H, m, 2 × ArH), 7.44–7.48 (3H, m, 3 × ArH), 7.54–7.58 (1H, m, ArH), 7.74–7.78 (1H, m, ArH), 8.04–8.11 (2H, m, 2 × ArH), 8.12 (1H, t, J = 5.6 Hz, exchangeable, NH), 8.27–8.29 (2H, m, 2 × ArH), 8.37–8.39 (1H, m, ArH). Anal. ($C_{23}H_{18}ClN_3O_3$): C, H, N.

3-((2-Phenylquinazolin-4-yl)amino)phenyl (2-chloroethyl)carbamate (136b). Compound **136b** was synthesized from 3-((2-Phenylquinazolin-4-yl)amino)phenol **135b** (2.0 g, 6.3 mmole) and 2-chloroethylisocynate (0.72 g, 6.9 mmole) in chloroform (30 mL) containing triethylamine (1.1 mL): Yield 2.0 (75 %); mp 230–231 °C; ^{1}H NMR (DMSO-d_6) δ 3.44 (2H, q, J = 6.0 Hz, CH_2), 3.72 (2H, t, J = 6.0 Hz, CH_2), 6.81–6.83 (1H, m, ArH), 6.91–7.02 (1H, m, ArH), 7.40–7.48 (3H, m, 3 × ArH), 7.52–7.55 (1H, m, ArH), 7.75–7.82 (2H, m, 2 × ArH), 8.04–8.09 (2H, m, 2 × ArH), 8.06–8.07 (1H, m, ArH), 8.08–8.09 (1H, m, ArH), 8.11 (1H, t, J = 5.6 Hz, exchangeable, NH), 8.49–8.50 (1H, m, ArH), 9.75 (1H, s, exchangeable, NH). Anal. ($C_{23}H_{19}ClN_4O_2$): C, H, N.

3-((2-(Furan-2-yl)quinazolin-4-yl)amino)phenyl (2-chloroethyl)carbamate (136c). Compound **136c** was synthesized from 3-((2-(furan-2-yl)quinazolin-4-yl)amino)phenol **135c** (1.0 g, 3.3 mmole) and 2-chloroethylisocynate (0.52 g, 4.9 mmole) in chloroform (20 mL) containing triethylamine (0.5 mL): Yield 1.0 (74 %); mp 247–249 °C; ^{1}H NMR (DMSO-d_6) δ 3.45 (2H, q, J = 6.0 Hz, CH_2), 3.71 (2H, t, J = 6.0 Hz, CH_2), 6.67–6.69 (1H, m, ArH), 6.87–6.90 (1H, m, ArH), 7.02–7.28 (1H, m, ArH), 7.41–7.45 (1H, m, ArH), 7.57–7.62 (1H, m, ArH), 7.82–7.89 (4H, m, 4 × ArH), 8.04–8.06 (1H, m, ArH), 8.12 (1H, t, J = 5.6 Hz, exchangeable, NH), 8.56–8.58 (1H, m, ArH), 9.88 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{17}ClN_4O_3 \cdot H_2O$): C, H, N.

3-((2-(Thiophen-2-yl)quinazolin-4-yl)amino)phenyl (2-chloroethyl)carbamate (136d). Compound **136d** was synthesized from 3-((2-(thiophen-2-yl)quinazolin-4-yl)amino)phenol **135d** (1.5 g, 4.7 mmole) and 2-chloroethylisocynate (0.74 g, 7.0 mmole) in chloroform (25 mL) containing triethylamine (0.8 mL): Yield 1.4 (70 %); mp 236–237 °C; ^{1}H NMR (DMSO-d_6) δ 3.44 (2H, q, J = 6.0 Hz, CH_2), 3.72 (2H, t, J = 6.0 Hz, CH_2), 6.90–6.92 (1H, m, ArH), 7.20–7.22 (1H, m, ArH), 7.43–7.47 (1H, m, ArH), 7.58–7.62 (1H, m, ArH), 7.72–7.73 (1H, m, ArH), 7.80–7.88 (3H, m, 3 × ArH), 7.96–7.97 (1H, m, ArH), 8.04–8.05 (1H, m, ArH), 8.13 (1H, t, J = 5.6 Hz, exchangeable, NH), 8.57–8.59 (1H, m, ArH), 9.99 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{17}ClN_4O_2S$): C, H, N.

3-((2-(3-Methoxyphenyl)quinazolin-4-yl)amino)phenyl (2-chloroethyl)carbamate (136e). Compound **136e** was synthesized from 3-((2-(3-methoxyphenyl)quinazolin-4-yl)amino)phenol **135e** (0.7 g, 2.0 mmole) and 2-chloroethylisocynate (0.32 g, 3.1 mmole) in chloroform (10 mL) containing triethylamine (0.4 mL): Yield 0.75 (82 %); mp 170–171 °C; ^{1}H NMR (DMSO-d_6) δ 3.45 (2H, q, J = 6.0 Hz, CH_2), 3.70 (2H, t, J = 6.0 Hz, CH_2), 3.85 (3H, s, Me), 6.91–6.93 (1H, m, ArH), 7.07–7.09 (1H, m, ArH), 7.41–7.48 (2H, m, 2 × ArH), 7.62–7.66 (1H, m, ArH), 7.85–7.93 (3H, m, 3 × ArH), 8.03–8.04 (1H, m, ArH), 8.05–8.06 (1H, m, ArH), 8.07–8.09 (2H, m, 2 × ArH), 8.59–8.61 (1H, m, ArH), 9.94 (1H, s, exchangeable, NH). Anal. ($C_{24}H_{21}ClN_4O_3$): C, H, N.

3-(Quinazolin-4-ylamino)phenyl (2-chloroethyl)carbamate (136f). Compound **136f** was synthesized from 3-(quinazolin-4-ylamino)phenol **135f** (0.5 g, 2.1 mmole) and 2-chloroethylisocynate (0.33 g, 3.2 mmole) in chloroform (10 mL) containing triethylamine (0.3 mL): Yield 0.51 (69 %); mp 165–167 °C; ^{1}H NMR (DMSO-d_6) δ 3.41 (2H, q, J = 6.0 Hz, CH_2), 3.70 (2H, t, J = 6.0 Hz, CH_2), 6.88–6.91 (1H, m, ArH), 7.37–7.41 (1H, m, ArH), 7.76–7.68 (1H, m, ArH), 7.77–7.82 (3H, m, 3 × ArH), 7.86–7.88 (1H, m, ArH), 8.09 (1H, t, J = 5.6 Hz, exchangeable, NH), 8.58–8.59 (1H, m, ArH), 8.65 (1H, s, ArH), 9.85 (1H, s, exchangeable, NH). Anal. ($C_{17}H_{15}ClN_4O_2$): C, H, N.

3-((2-Phenylquinazolin-4-yl)oxy)phenyl (2-chloroethyl)(nitroso)carbamate (137a). 3-((2-phenylquinazolin-4-yl)oxy)phenyl (2-chloroethyl)carbamate **136a** (0.5 g, 1.1 mmole) were suspended in anhydrous acetonitrile (15 mL) containing acetic

acid (0.2 mL). Nitrosoniumtetrafluoroborate (0.21 g, 1.7 mmole) was added, and the reaction mixture was stirred at room temperature until disappearance of starting material as monitored by TLC. The reaction mixture was poured into 100 mL of ice-cooled water/ethyl acetate (50 % v/v) solution, and the pH was adjusted to 5-6 by careful addition of 5 % sodium bicarbonate solution. The water phase was extracted twice with ethyl acetate, and the organic extracts were washed with brine, dried over sodium sulphate, and concentrated under reduced pressure. The crud product was purified by using Colum Chromatography on silica gel eluting with a mixture of ethyl acetate/hexane, to give **137a**, 0.2 g (37 %); mp 98–99 °C; ^{1}H NMR (DMSO-d_6) δ 4.01 (2H, t, J = 6.0 Hz, CH_2), 4.60 (2H, t, J = 6.0 Hz, CH_2), 7.36–7.38 (1H, m, ArH), 7.39–7.40 (3H, m, 3 × ArH), 7.61–7.62 (1H, m, ArH), 7.66–7.70 (1H, m, ArH), 7.75–7.79 (1H, m, ArH), 8.04–8.07 (2H, m, 2 × ArH), 8.27–8.31 (3H, m, 3 × ArH), 8.39–8.40 (1H, m, ArH). Anal. ($C_{23}H_{17}ClN_4O_4$): C, H, N.

3-((2-Phenylquinazolin-4-yl)amino)phenyl (2-chloroethyl)(nitroso)carbamate (137b). Compound 7b was synthesized from 3-((2-phenylquinazolin-4-yl)amino)phenyl (2-chloroethyl)carbamate **136b** (0.5 g, 1.2 mmole), Nitrosoniumtetrafluoroborate (0.21 g, 1.7 mmole) in acetonitrile (10 mL) containing acetic acid (0.5 mL): Yield 0.21 (38 %); mp 133–135 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (2H, t, J = 6.0 Hz, CH_2), 4.27 (2H, t, J = 6.0 Hz, CH_2), 7.21–7.24 (1H, m, ArH), 7.46–7.49 (3H, m, 3 × ArH), 7.58–7.67 (2H, m, 2 × ArH), 7.89–7.92 (3H, m, 3 × ArH), 8.27–8.28 (1H, m, ArH), 8.47–8.49 (2H, m, 2 × ArH), 8.59–8.61 (1H, m, ArH), 10.00 (1H, s, exchangeable, NH). Anal. ($C_{23}H_{18}ClN_5O_3 \cdot 0.5H_2O$): C, H, N.

3-((2-(Furan-2-yl)quinazolin-4-yl)amino)phenyl(2-chloroethyl)(nitroso) carbamate (137c). Compound **137c** was synthesized from 3-((2-(furan-2-yl)quinazolin-4-yl)amino)phenyl (2-chloroethyl)carbamate **136c** (0.5 g, 1.2 mmole), Nitrosoniumtetrafluoroborate (0.21 g, 1.7 mmole) in acetonitrile (10 mL) containing acetic acid (0.5 mL): Yield 0.18 (34 %); mp 109–110 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (2H, t, J = 6.0 Hz, CH_2), 4.21 (2H, t, J = 6.0 Hz, CH_2), 6.64–6.65 (1H, m, ArH), 7.19–7.21 (2H, m, 2 × ArH), 7.56–7.64 (2H, m, 2 × ArH), 7.82–7.99 (4H, m, 4 × ArH), 8.28–8.29 (1H, m, ArH), 8.57–8.59 (1H, m, ArH), 10.02 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{16}ClN_5O_4$): C, H, N.

3-((2-(Thiophen-2-yl)quinazolin-4-yl)amino)phenyl(2-chloroethyl)(nitroso) carbamate (137d). Compound **137c** was synthesized from 3-((2-(thiophen-2-yl)quinazolin-4-yl)amino)phenyl (2-chloroethyl)carbamate **136d** (1.0 g, 2.4 mmole), Nitrosoniumtetrafluoroborate (0.39 g, 3.4 mmole) in acetonitrile (20 mL) containing acetic acid (0.5 mL): Yield 0.5 (47 %); mp 107–108 °C; ^{1}H NMR (DMSO-d_6) δ 3.72 (2H, t, J = 6.0 Hz, CH_2), 4.31 (2H, t, J = 6.0 Hz, CH_2), 6.66–6.67 (1H, m, ArH), 7.20–7.31 (3H, m, 3 × ArH), 7.56–7.64 (1H, m, ArH), 7.81–7.99 (4H, m, 4 × ArH), 8.30–8.32 (1H, m, ArH), 8.58–8.59 (1H, m, ArH), 10.12 (1H, s, exchangeable, NH). Anal. ($C_{21}H_{16}ClN_5O_3S$): C, H, N.

3-((2-(3-Methoxyphenyl)quinazolin-4-yl)amino)phenyl (2-chloroethyl)(nitroso) carbamate (137e). Compound **137e** was synthesized from 3-((2-(3-methoxyphenyl)quinazolin-4-yl)amino)phenyl (2-chloroethyl)carbamate **136e** (0.5 g, 1.1 mmole), Nitrosoniumtetrafluoroborate (0.21 g, 1.7 mmole) in acetonitrile (10 mL) containing acetic acid (0.5 mL): Yield 0.30 (56 %); mp 140–141 °C; ^{1}H NMR (DMSO-d_6) δ 3.58 (3H, s, Me), 3.72 (2H, t, J = 6.0 Hz, CH_2), 4.30 (2H, t, J = 6.0 Hz, CH_2), 7.22–7.26 (1H, m, ArH), 7.44–7.46 (2H, m, 2 × ArH), 7.59–7.67 (2H, m, 2 × ArH), 7.90–7.91 (3H, m, 3 × ArH), 8.27–8.28 (1H, m, ArH), 8.49–8.51 (2H, m, 2 × ArH), 8.60–8.61 (1H, m, ArH), 10.12 (1H, s, exchangeable, NH). Anal. ($C_{24}H_{20}ClN_5O_4$): C, H, N.

3-(Quinazolin-4-ylamino)phenyl (2-chloroethyl)(nitroso)carbamate (137f). Compound **137f** was synthesized from 3-(quinazolin-4-ylamino)phenyl (2-chloroethyl)carbamate **136f** (0.5 g, 1.5 mmole), Nitrosoniumtetrafluoroborate (0.25 g, 2.1 mmole) in acetonitrile (15 mL) containing acetic acid (0.5 mL): Yield 0.25 (45 %); mp 101–102 °C; ^{1}H NMR (DMSO-d_6) δ 3.72 (2H, t, J = 6.0 Hz, CH_2), 4.25 (2H, t, J = 6.0 Hz, CH_2), 7.46–7.49 (2H, m, 2 × ArH), 7.58–7.60 (1H, m, ArH), 7.89–7.92 (2H, m, 2 × ArH), 8.27–8.28 (1H, m, ArH), 8.44–8.49 (2H, m, 2 × ArH), 8.66 (1H, s, ArH), 10.05 (1H, s, exchangeable, NH). Anal. ($C_{17}H_{14}ClN_5O_3$): C, H, N.

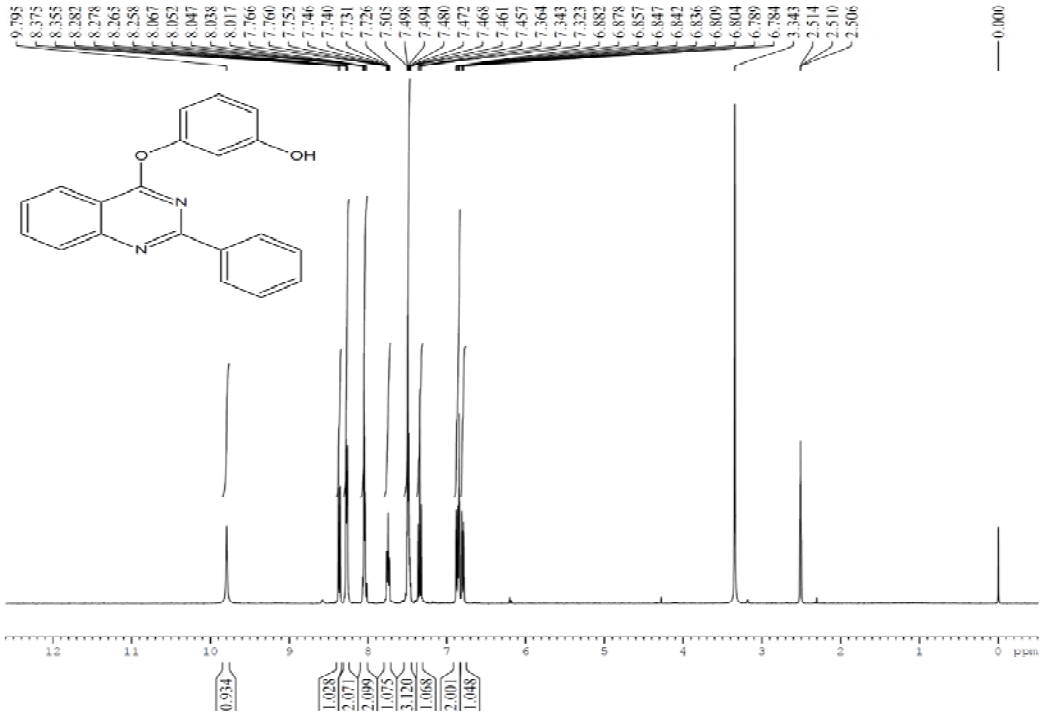
7.5 Conclusion

In this study, we have designed and synthesized a series DNA-alkylating agents, in which the *N*-(2-chloroethyl)-*N*-nitrosocarbamate residue is linked to DNA-binding 4-anilinoquinazoline via a carbamate spacer. All the compounds were characterized by

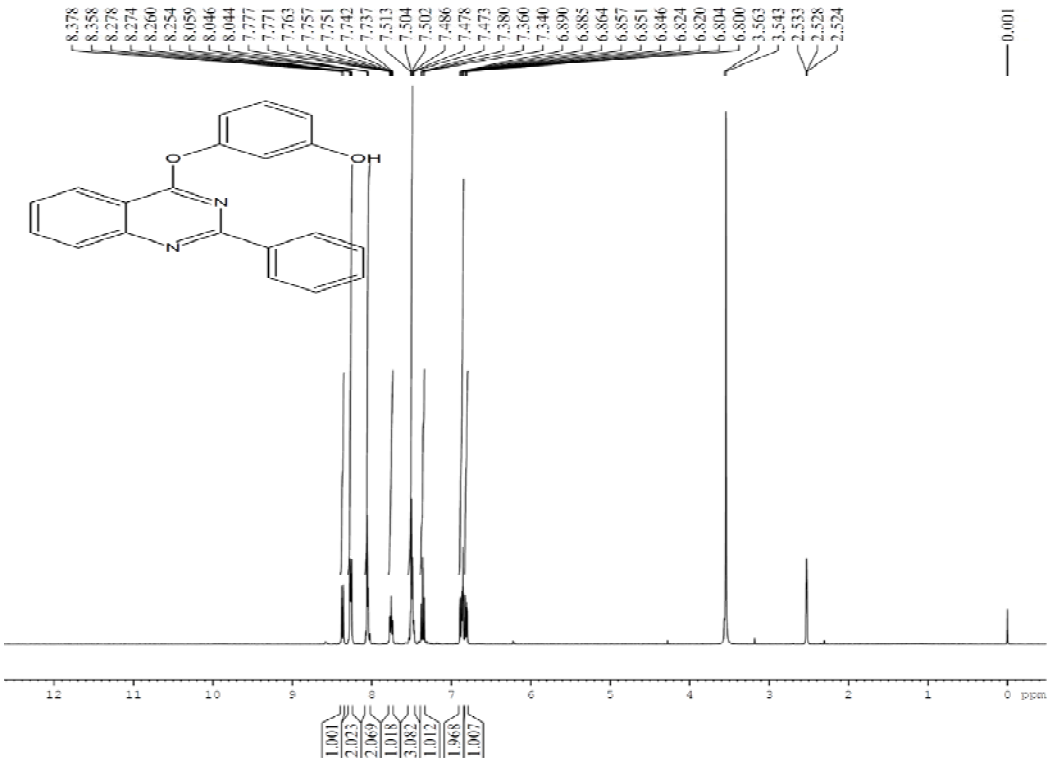
^{1}H NMR and elemental analysis. The antitumor activity of all newly synthesized derivatives is under investigation.

7.6 [1]HNMR spectra

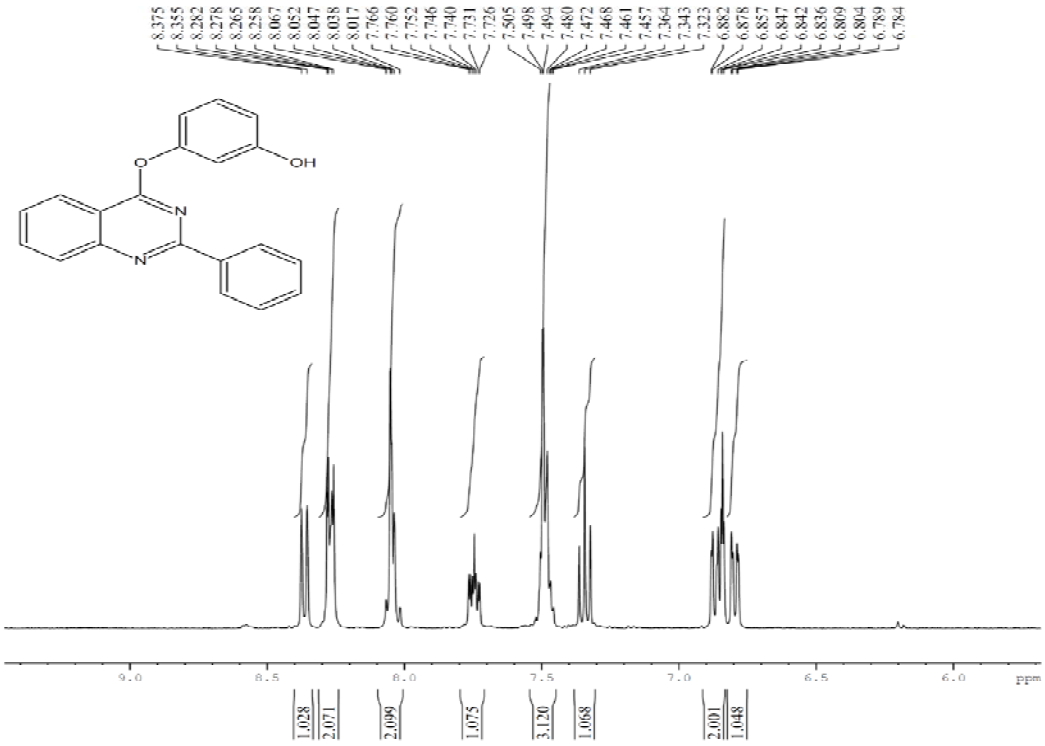
7.6.1 [1]H NMR Spectrum for compound **135a**.



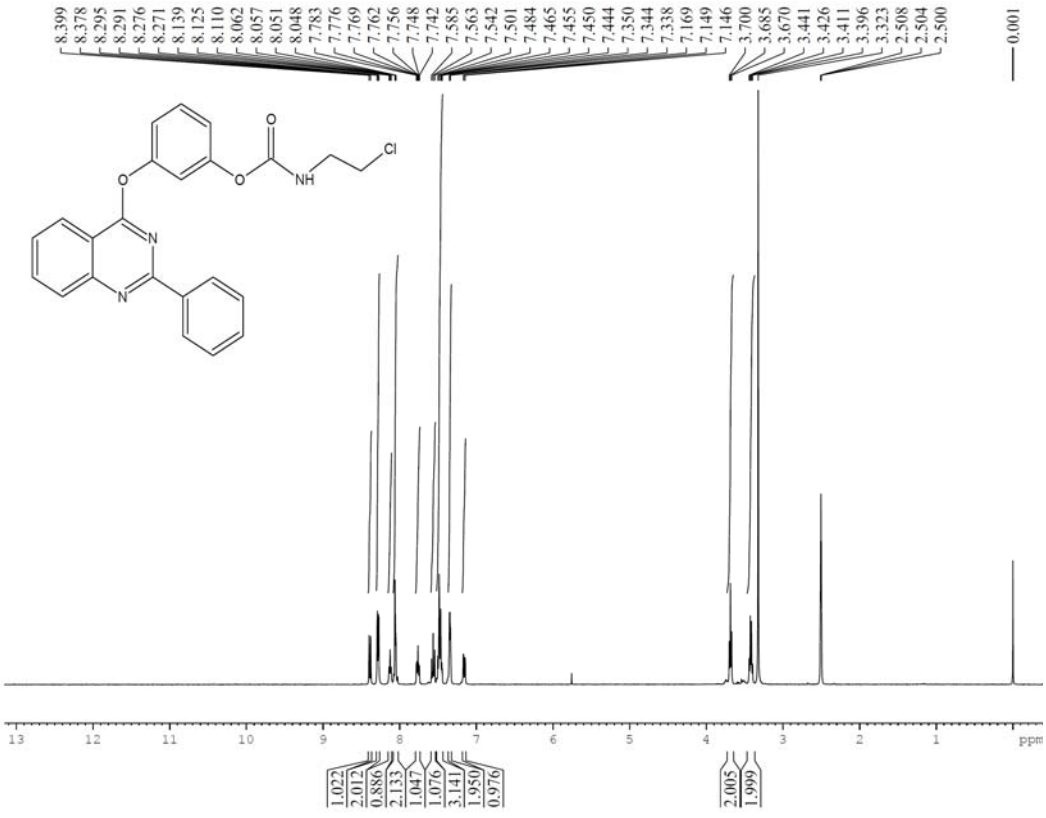
7.6.2 [1]H NMR Spectrum for compound **135a** (D2O).



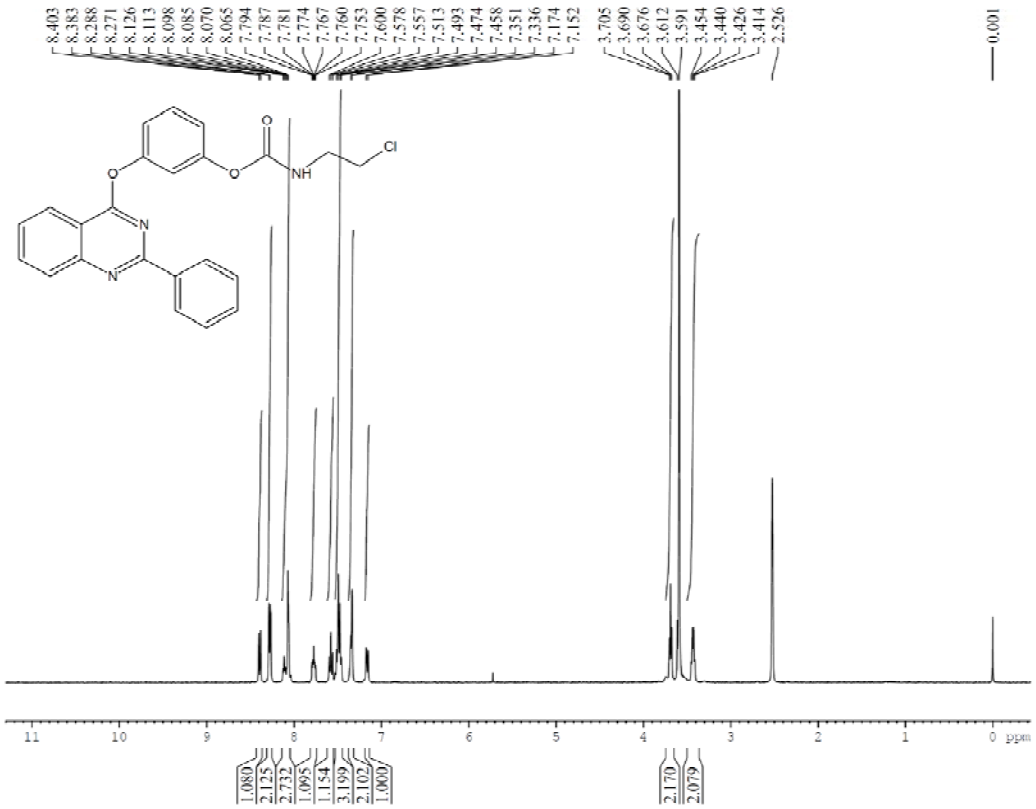
7.6.3 ^{1}H NMR Spectrum for compound **135a** (ARO).



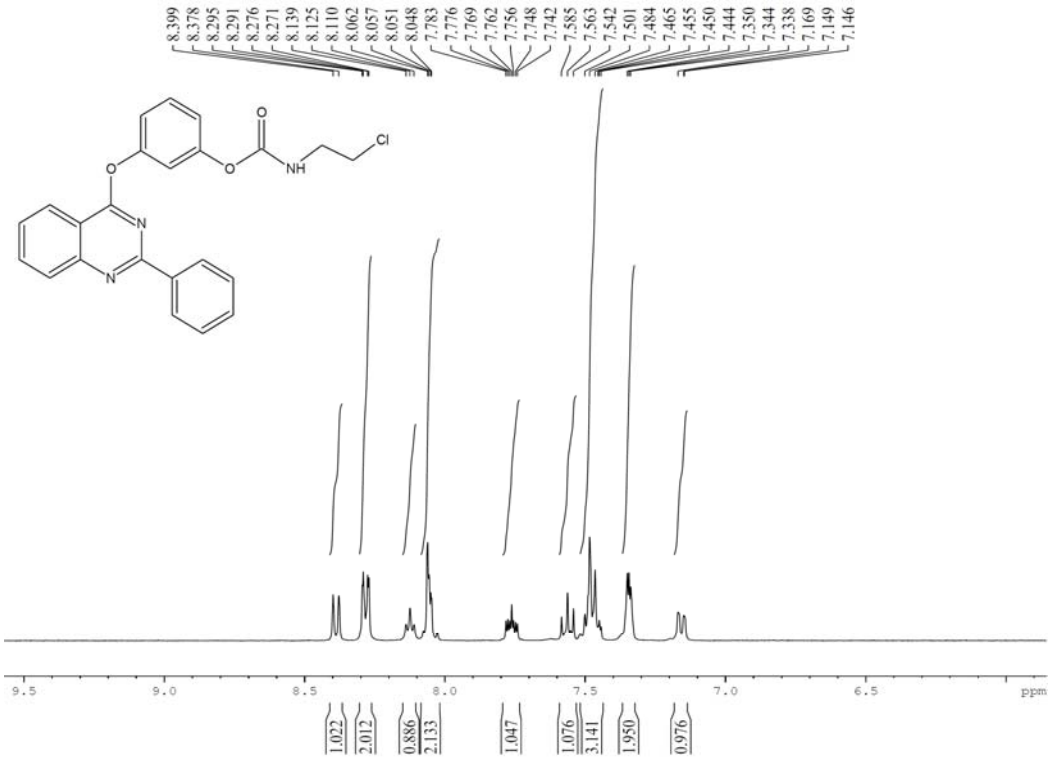
7.6.4 ^{1}H NMR Spectrum for compound **136a**.



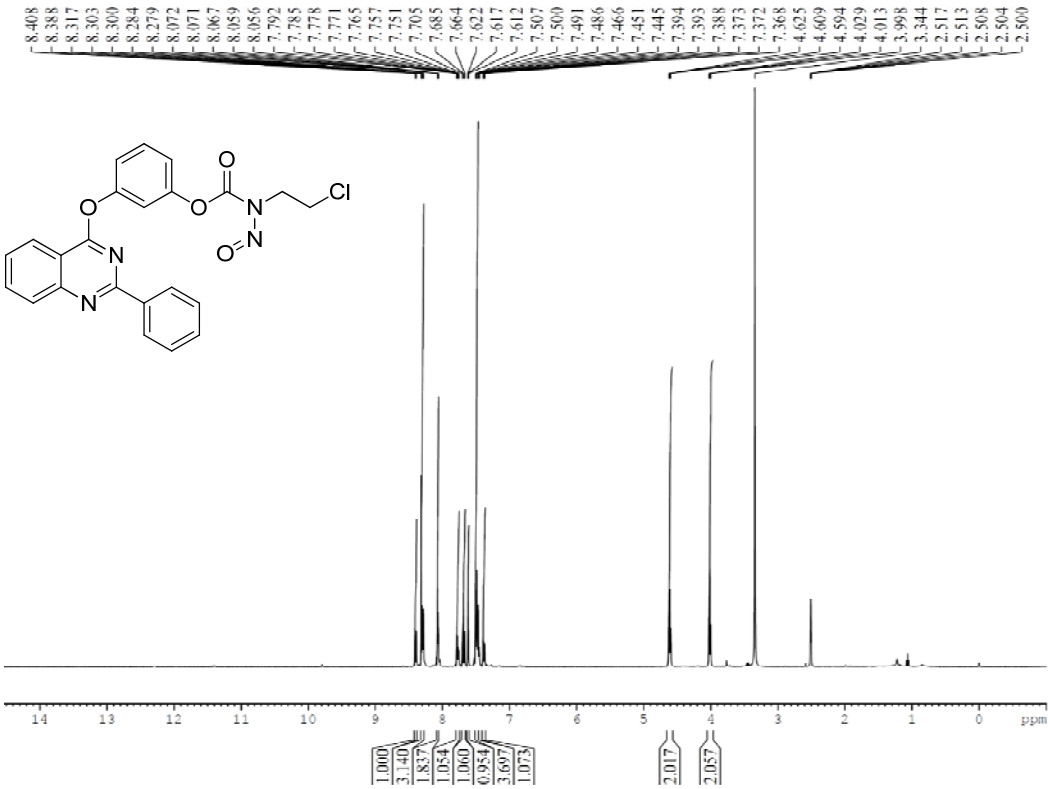
7.6.5 ^{1}H NMR Spectrum for compound **136a** (D2O).



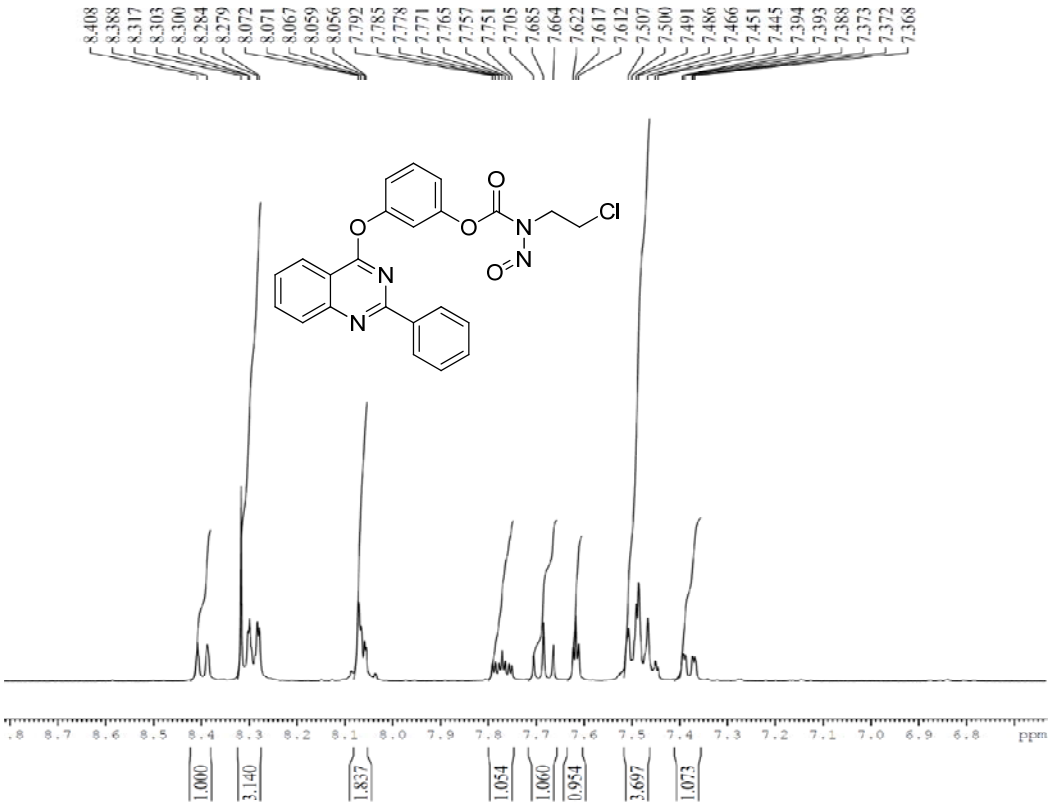
7.6.6 ^{1}H NMR Spectrum for compound **136a (ARO)**.



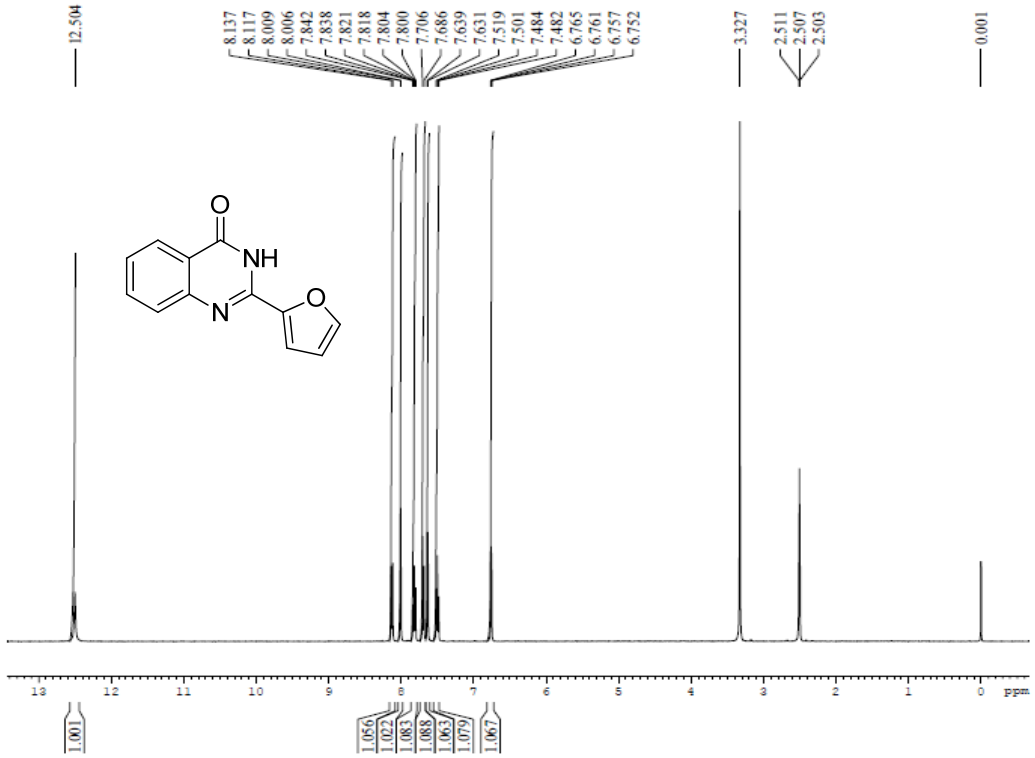
7.6.7 ^{1}H NMR Spectrum for compound **137a**.



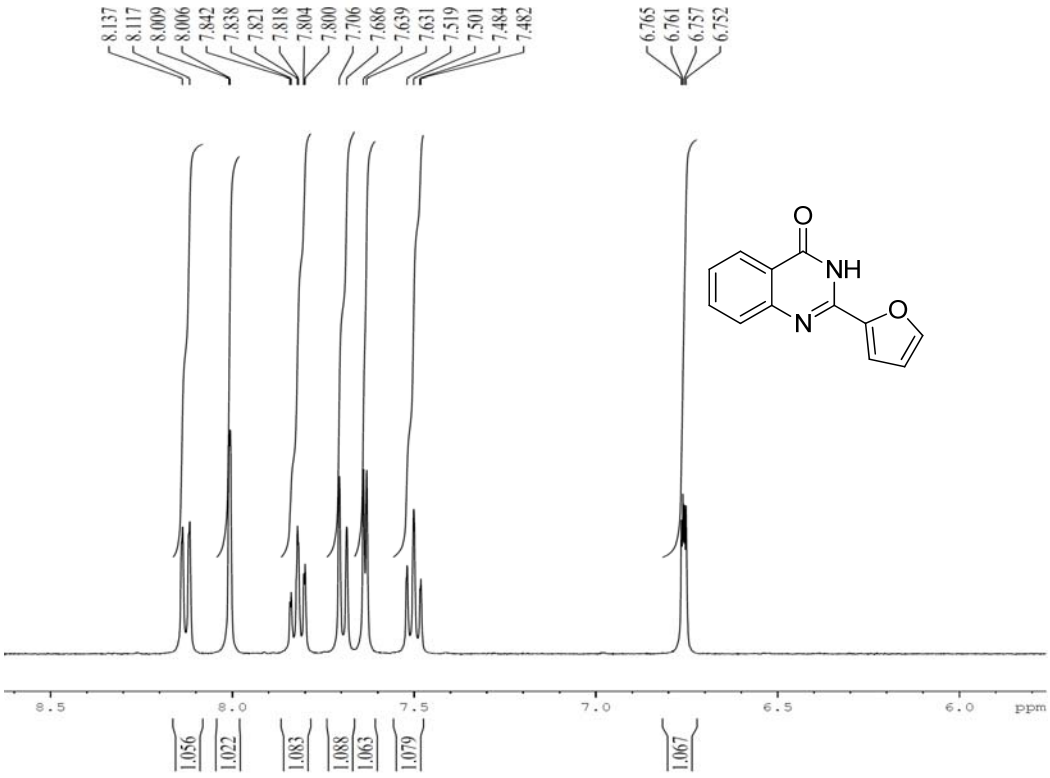
7.6.8 ^{1}H NMR Spectrum for compound **137a** (ARO).



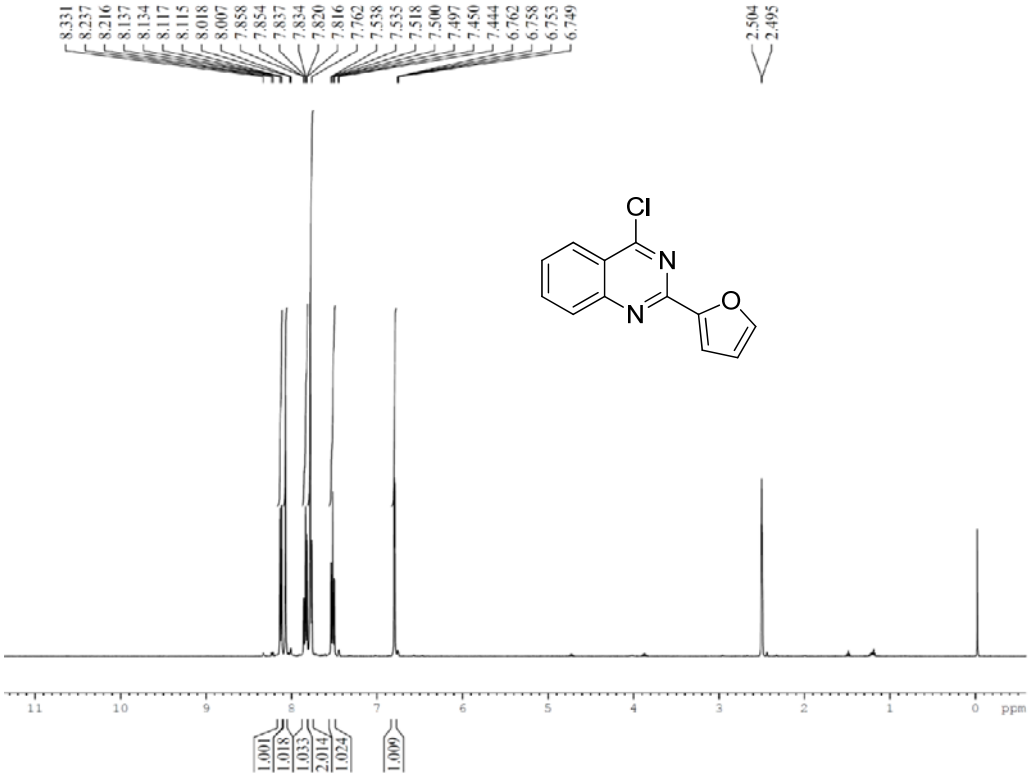
7.6.9 ^{1}H NMR Spectrum for compound **133b**.



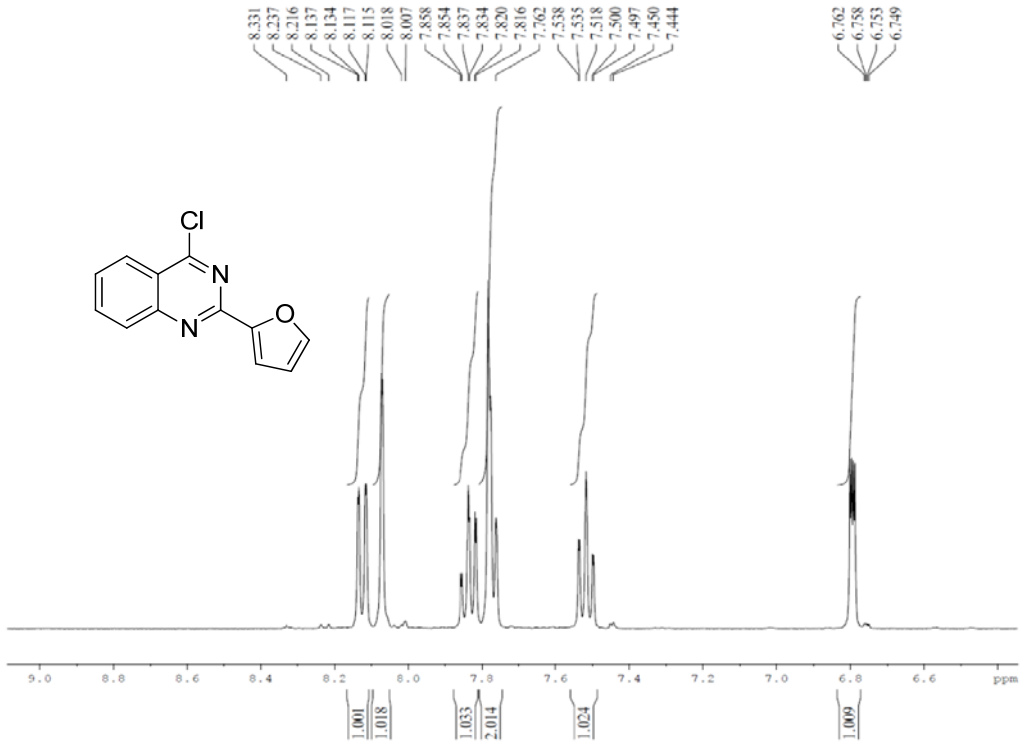
7.6.10 ^{1}H NMR Spectrum for compound **133b** (ARO).



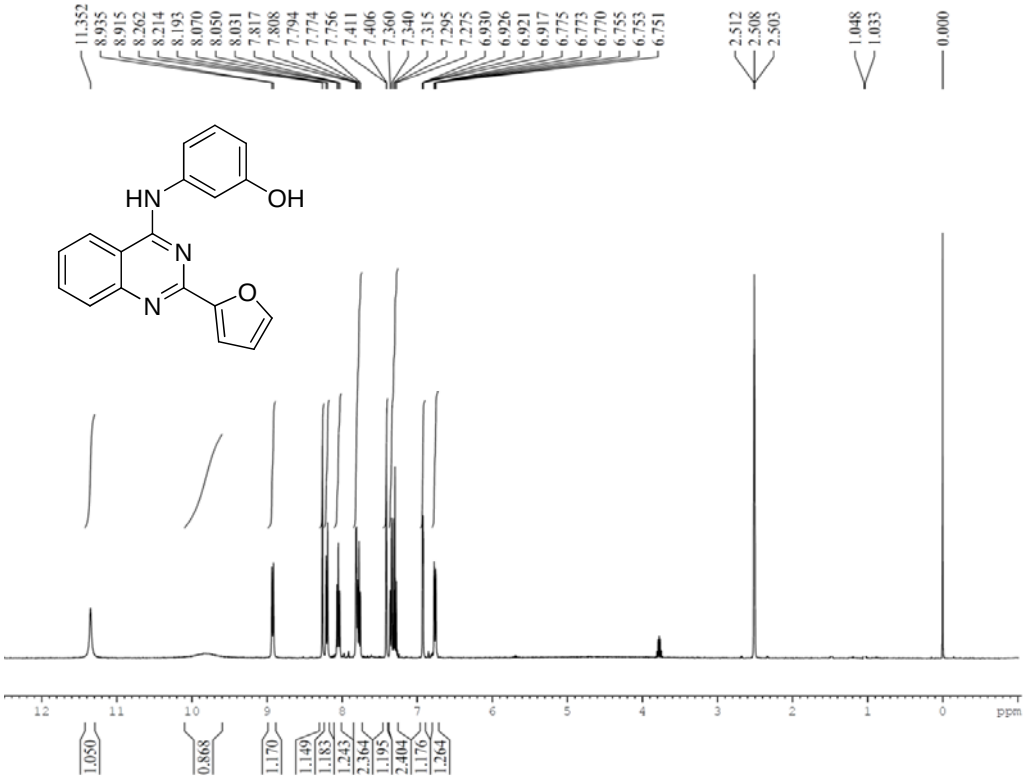
7.6.11 ^{1}H NMR Spectrum for compound **134b**.



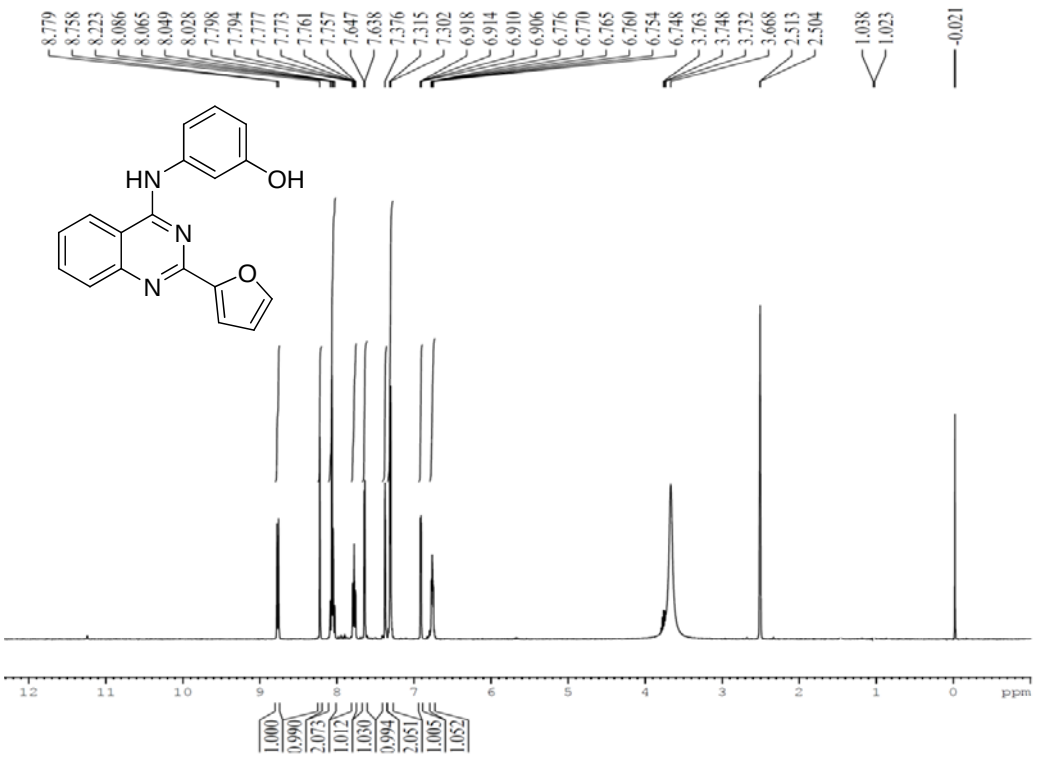
7.6.12 ^{1}H NMR Spectrum for compound **134b** (ARO).



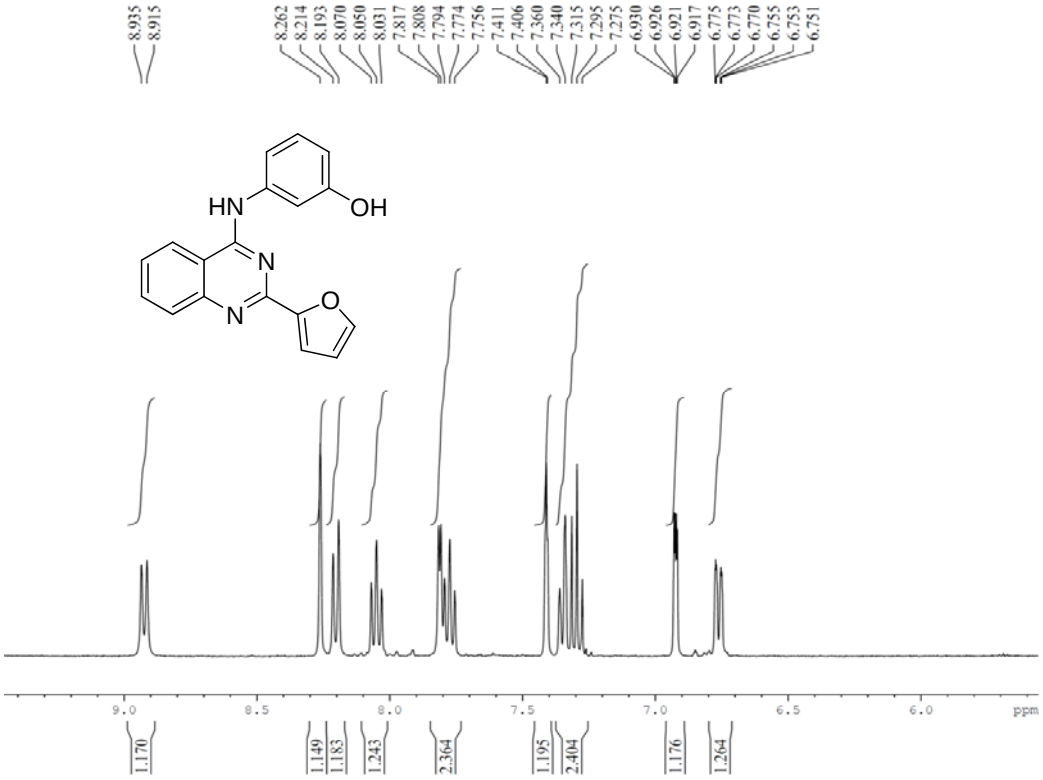
7.6.13 ^{1}H NMR Spectrum for compound **135c**.



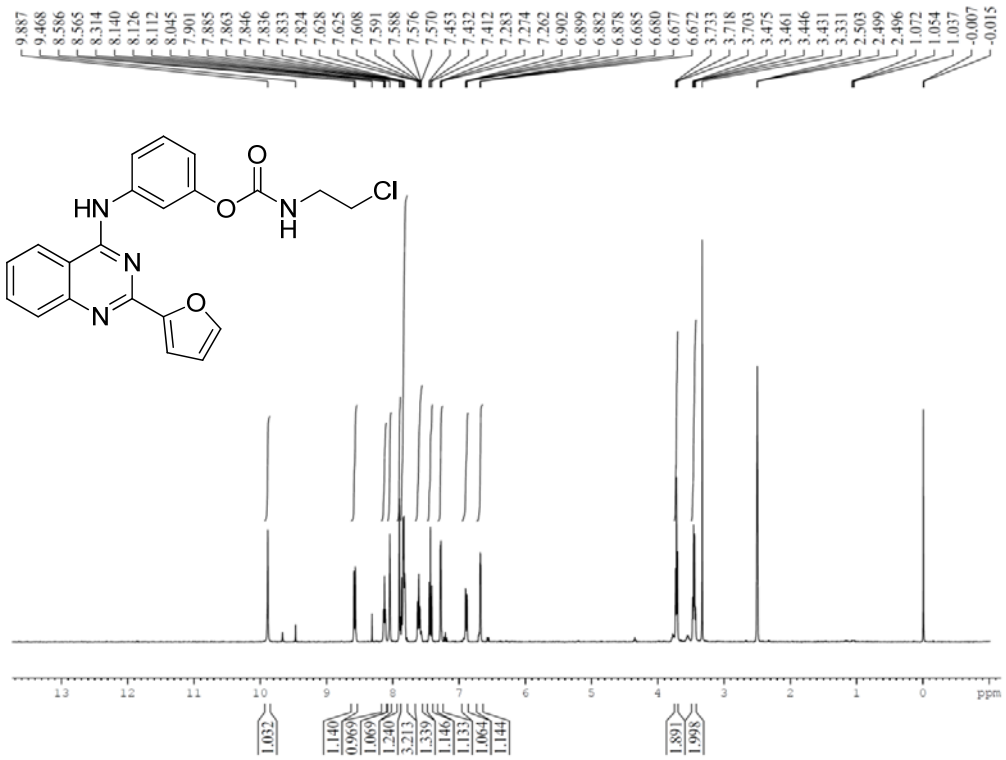
7.6.14 ^{1}H NMR Spectrum for compound **135c** (D2O).



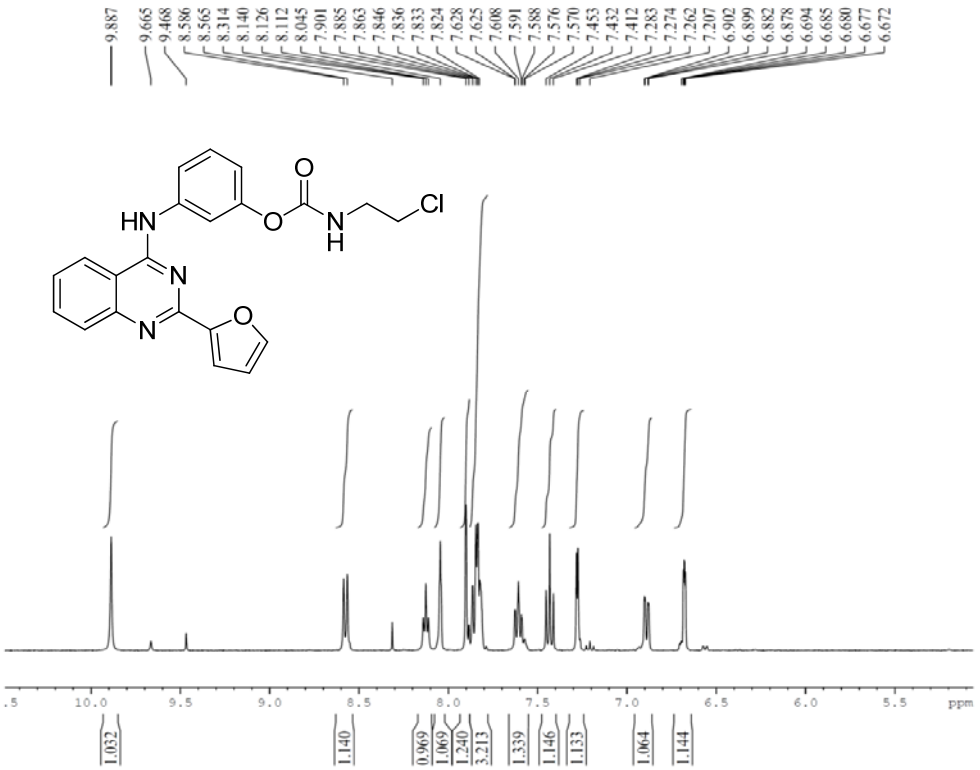
7.6.15 ^{1}H NMR Spectrum for compound **135c** (ARO).



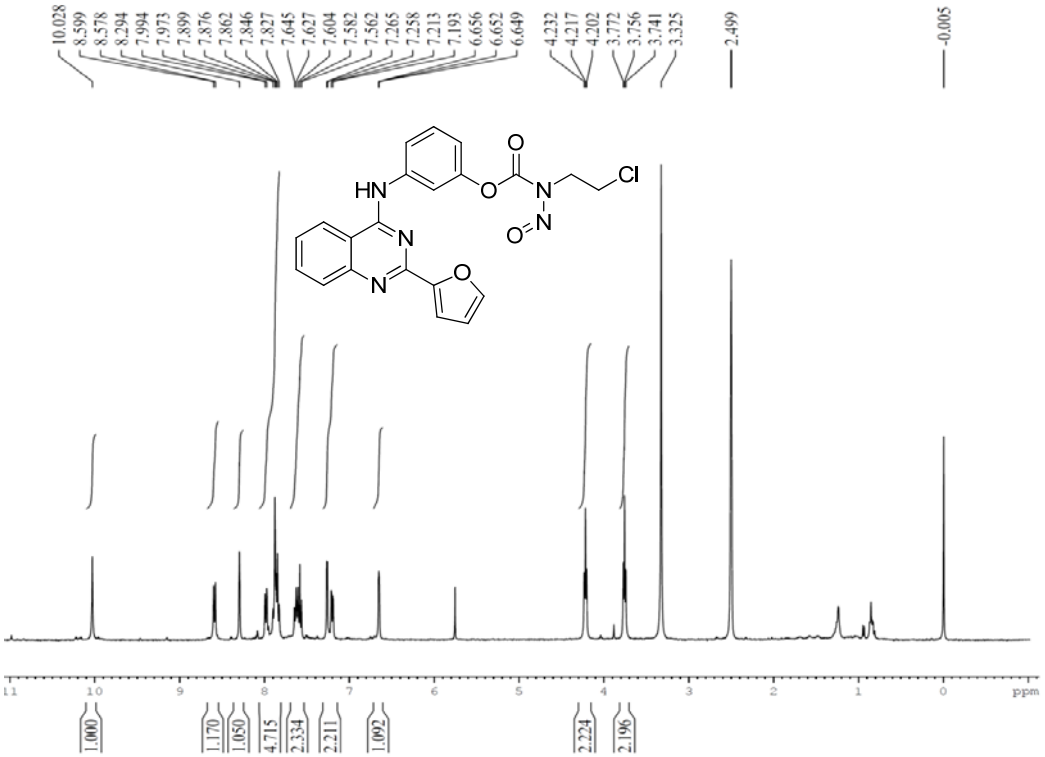
7.6.16 ^{1}H NMR Spectrum for compound **136c**.



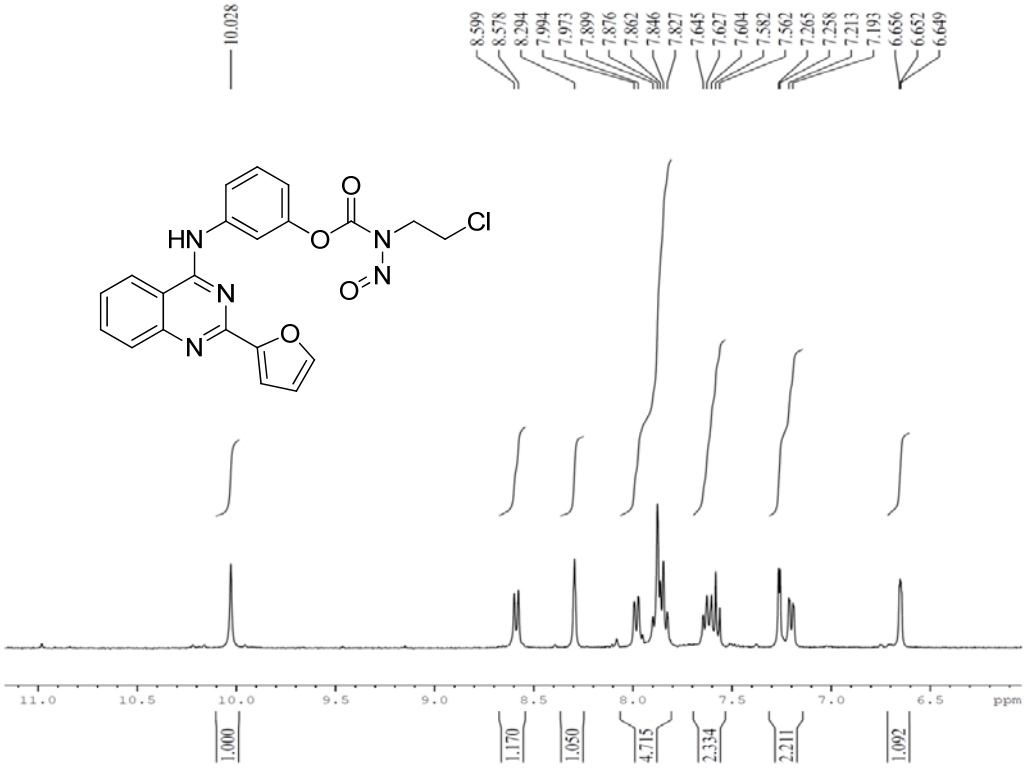
7.6.17 ^{1}H NMR Spectrum for compound **136c** (ARO).



7.6.18 ^{1}H NMR Spectrum for compound **137c**.



7.6.19 ^{1}H NMR Spectrum for compound **136c** (ARO).



7.7 Elemental analysis

Table 2.3 Elemental analysis of compounds **135a-f.**

Sr. No.	MF	MW	Elemental Analysis					
			CHN Calculated %			CHN Found %		
			C	H	N	C	H	N
135a	$C_{20}H_{14}N_2O_2$	314.34	76.42	4.49	8.91	76.28	4.58	8.79
135b	$C_{20}H_{15}N_3O$	313.35	76.66	4.82	13.41	76.43	4.66	13.62
135c	$C_{18}H_{13}N_3O_2$	303.31	71.28	4.32	13.85	71.41	4.21	13.59
135d	$C_{18}H_{13}N_3O_2S$	319.38	67.69	4.10	13.16	67.54	4.39	13.28
135e	$C_{21}H_{17}N_3O_2$	343.38	73.45	4.99	12.24	73.57	5.12	12.37
135f	$C_{14}H_{11}N_3O$	237.36	70.87	4.67	17.71	70.75	4.82	17.89

Table 2.3 Elemental analysis of compounds **136a-f.**

Sr. No.	MF	MW	Elemental Analysis					
			CHN Calculated %			CHN Found %		
			C	H	N	C	H	N
136a	$C_{23}H_{18}ClN_3O_3$	419.86	65.79	4.32	10.01	65.87	4.49	10.22
136b	$C_{23}H_{19}ClN_4O_2$	418.88	65.95	4.57	13.38	65.77	4.43	13.51
136c	$C_{21}H_{17}ClN_4O_3.H_2O$	408.84	59.09	4.49	13.13	59.20	4.50	12.90
136d	$C_{21}H_{17}ClN_4O_2S$	424.90	59.36	4.03	13.19	59.51	4.37	13.12
136e	$C_{24}H_{21}ClN_4O_3$	448.90	64.21	4.72	12.48	64.39	4.91	12.63
136f	$C_{17}H_{15}ClN_4O_2$	342.78	59.57	4.41	16.34	59.41	4.28	16.18

Table 2.3 Elemental analysis of compounds **137a-f.**

Sr. No.	MF	MW	Elemental Analysis					
			CHN Calculated %			CHN Found %		
			C	H	N	C	H	N
137a	$C_{23}H_{17}ClN_4O_4$	448.86	61.54	3.82	12.48	61.31	3.97	12.57
137b	$C_{23}H_{18}ClN_5O_3.0.5H_2O$	447.87	6O.46	4.19	15.33	60.66	4.20	15.21
137c	$C_{21}H_{16}ClN_5O_4$	437.84	57.48	3.45	12.77	57.61	3.59	12.93
137d	$C_{21}H_{16}ClN_5O_3S$	453.90	55.45	3.32	12.32	55.59	3.49	12.46
137e	$C_{24}H_{20}ClN_5O_4$	477.90	60.32	4.22	14.65	60.21	4.36	14.61
137f	$C_{17}H_{14}ClN_5O_3$	371.78	54.78	3.52	15.03	54.89	3.78	15.34

Section - B

References

References

1. Kapuriya, N.; Kapuriya, K.; Zhang, X.; Chou, T.-C.; Kakadiya, R.; Wu, Y.-T.; Tsai, T.-H.; Chen, Y.-T.; Lee, T.-C.; Shah, A.; Naliapara, Y.; Su, T.-L. *Bioorg. Med. Chem*. **2008**, *16*, 5413.
2. Kapuriya, N.; Kapuriya, K.; Dong, H.; Zhang, X.; Chou, T.-C.; Chen, Y.-T.; Lee, T.- C.; Lee, W.-C.; Tsai, T.-H.; Naliapara, Y.; Su, T.-L. *Bioorg. Med. Chem.* **2009**, *17*, 1264.
3. Chen, C.-H.; Lin, Y.-W.; Zhang, X.; Chou, T.-C.; Tsai, T.-J.; Kapuriya, N.; Kakadiya, R.; Su, T.-L. *Eur. J. Med. Chem.* **2009**, *44*, 3056.
4. Kakadiya, R.; Dong, H.; Kumar, A,; Dodia, N.; Zhang, X.; Chou, T.-C.; Lee, T.-C.; Shah, A.; Su, T.-L. *Bioorg. Med. Chem.* **2010**, *18*, 2285.
5. Domarkas, J.; Dudouit, F.; Williams, C.; Qiyu, Q.; Banerjee, R.; Brahimi, F.; Jean-Claude, B. J. *J. Med. Chem*. **2006**, *49*, 3544.
6. Madelmont, J. C.; Godeneche, D.; Parry, D.; Duprat, J.; Chabard, J. L.; Plagne, R.; Mathe, G.; Meyniel, G. *J. Med. Chem.* **1985**, *28*, 1346.
7. McKay, A. F.; Wright, G. F. *J. Am. Chem. Soc.* **1947**, *69*, 3028.
8. Schepartz, S. A. *Cancer Treat. Rep.* **1976**, *60*, 647.
9. Goldin, A. In *Nitrosoureas in Cancer Treatment*; Serrou, B., Schein, P. S., Imbach, J. L., Eds.; INSERM Symp. No. 19; Elsevier/North Holland Biomedical Press; New York, 1981; p 3.
10. Von Bruning, G. *Chem. Ber.* **1888**, *21*, 1809.
11. Hyde, K. A.; Acton, E.; Skinner, W. A.; Goodman, L.; Greenberg, J.; Baker, B. R. *J. Med. Pharm. Chem.* **1962**, *5*, 1.
12. Hayes, M. T.; Bartley, J.; Parsons, P. G.; Eaglesham, G. K.; Prakask, A. S. *Biochemistry*. **1997,** *36*, 10646.
13. Montgomery, J. A.; James, R.; McCaleb, G. S.; Johnston, T. P. *J. Med. Chem.* **1967**, *10*, 668.
14. Johnston, T. P.; and Montgomery, J. A. *Cancer Treat. Rep.* **1986**, *70*, 13.
15. Buckley, N. *J. Org. Chem.* **1987**, *52*, 484.
16. Murata, M.; Takahashi, A.; Saito, I.; Kawanishi, S. *Biochem. Pharmacol*. **1999**, *57*, 881.
17. Bolza´n, A. D.; Bianchi, M. S. *Mutat. Res*. **2002**, *512*, 121.

18. Kroncke, K. D.; Fehsel, K.; Sommer, A.; Rodriguez, M. L.; Kolb-Bachofen, V. *Biol. Chem. Hoppe Seyler.* **1995**, *376*, 179.
19. Bedoya, F. J.; Solano, F.; Lucas, M. *Experientia.* **1996**, *52*, 344.
20. Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A. *J. Med. Chem.* **1963**, *6*, 669.
21. Schabel, F. M., Jr.; Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A.; Laster, W.; Skipper, H. E. *Cancer Res.* **1963**, *23*, 725
22. Johnston, T. P.; McCaleb, G. S.; Oplinger, P. S.; Montgomery, J. A. *J. Med. Chem.* **1966**, *9*, 892.
23. Eisenbrand, G.; Fiebig, H. H.; Zeller, W. J. *Z. Krebsforsch.* **1976**, *86*, 279.
24. Zeller, W. J.; Eisenbrand, G.; Fiebig, H. H. *J. Natl. Cancer Inst.* **1978**, *60*, 345.
25. Fiebig, H. H.; Eisenbrand, G.; Zeller, W. J.; Deutsch-Wentzel, T. *Eur. J. Cancer* **1977**, *13*, 937.
26. Lown, J. W.; Joshua, A. V.; McLaughlin, L. W. *J. Med. Chem.* **1980**, *23*, 798.
27. Martinez, J.; Oiry, J.; Imbach, J.; Winternitz, F. *J. Med. Chem.* **1982**, *25*, 178.
28. Johnston, T. P.; McCaleb, G. S.; Oplinger, P. S.; Laster, W. R.; Montgomery, J. A. *J. Med. Chem.* **1971**, *14*, 600.
29. Kim, J. C.; Lee, J. C.; Nha, C. S. *Korean J. Med. Chem.* **1992**, *2*, 86.
30. Kim, J. C.; Lim, Y. G.; Min, B. T. *Korean J. Med. Chem.* **1993**, *3*, 130.
31. Baracu, I.; Botez, G.; Denes, R.; Dobre, V.; Ureche, B.; Craescu, T.; Voiculet, N.; Niculescu-Duvaz, I. *Rev. Roum. Biochim.* **1981**, *18*, 175.
32. Zeller, W. J.; Eisenbrand, G. *Oncology* **1981**, *38*, 39.
33. Bigler, A. J.; Buus, L.; Bregnedal, P.; Atassi, G.; Muntzing, J.; Jensen, G. In *Nitrosoureas in Cancer Treatment*; Serrou, B., Schein, P. S., Imbach, J. L., Eds.; INSERM Symp. No. 19; Elsevier/North Holland Biomedical Press: Amsterdam, **1981**; p 113.
34. Kornet, M. J.; Crider, A. M.; Magarian, E. O. *J. Med. Chem.* **1977**, *20*, 405.
35. Lien, E. J. *J. Med. Chem.* **1970**, *13*, 1189.
36. Crider, A. M.; Kolczynski, T. M.; Yates, K. M. *J. Med. Chem.* **1980**, *23*, 324.
37. Levin, V. A.; Kabra, P. *Cancer Chemother. Rep., Part 1* **1974**, *58*, 787.
38. Schmid, J. R.; Fiebig, H. H.; Eisenbrand, G.; Lohr, G. W. *J. Cancer Res. Clin. Oncol.* **1986**, *111*, 31.
39. Eisenbrand, G. U.S. 4377687, 1983; *Chem. Abstr.* **1983**, *99*, 22332.
40. Zeller, W. J.; Eisenbrand, G.; Fiebig, H. H. *Cancer Res. Clin. Oncol.* **1979**, *95*, 43.

41. Berger, M.; Zeller, W. J.; Eisenbrand, G.; Lin, P. Z.; Nakra, M.; Schmahl, D. *Arzneim.-Forsch./Drug Res.* **1982**, *32*, 481.
42. Filippatos, E.; Papadaki-Valiraki, A.; Roussakis, C.; Verbist, J. F. *Arch. Pharm. (Weinheim)* **1993**, *326*, 451.
43. Reynolds, R. C.; Tiwari, A.; Harwell, J. E.; Gordon, D. G.; Garrett, B. D.; Gilbert, K. S.; Schmid, S. M.; Waud, W. R.; Struck, R. F. *J. Med. Chem.* **2000**, 43,1484.
44. Zeller, W. J.; Ehresmann, K.; Schreiber, J.; Eisenbrand, G. *Verh. Dtsch. Krebs Ges.*; Gustav Fischer Verlag: New York, 1984; Vol. 5, p 851.
45. Heal, J. M.; Fox, P. A.; Doukas, D.; Schein, P. S. *Cancer Res.* **1978**, *38*, 1070.
46. Montero, J. L.; Imbach, J. L. *C. R. Acad. Sci.* **1974**, *279*, 809.
47. Montero, J. L.; Rodriguez, M.; Imbach, J. L. *Eur. J. Med. Chem.* **1977**, *12*, 408.
48. Montero, J. L.; Moruzzi, A.; Oiry, J.; Imbach, J. L. *Eur. J. Med. Chem.* **1977**, *12*, 397.
49. Imbach, J. L.; Montero, J. L.; Moruzzi, A.; Serrou, B.; Chenu, E.; Hayat, M.; Mathe, G. *Biomedicine* **1975**, *23*, 410.
50. Grouiller, A.; Navarro, M. L.; Nonga, B.; Moliere, P.; Santini, R.; Voulot, C.; Pacheco, H. *C. R. Soc. Biol.* **1987**, *181*, 128.
51. Suami, T.; Kato, T.; Takino, H.; Hisamatsu, T. *J. Med. Chem.* **1982**, *25*, 829 and references therein.
52. Foster, D. O.; Pardee, A. B. *J. Biol. Chem.* **1969**, *244*, 2675.
53. Bibby, M. C.; Double, J. A. *J. Cancer Res. Clin. Oncol.* **1986**, *112*, 47.
54. Matthew, A. M.; Bibby, M. C.; Double, J. A. *Anticancer Res.* **1992**, *12*, 361.
55. Matthew, A. M.; Bibby, M. C.; Eisenbrand, G. *Anticancer Res.* **1993**, *13*, 81.
56. Klenner, T.; Berger, M. R.; Eisenbrand, G.; Schma¨ hl, D. *Br. J. Cancer* **1989**, *59*, 335.
57. *Characterization and Treatment of Human Tumors*; Davies, W., Harrap, K. R., Eds.; Exerpta Medica: Amsterdam, 1978; p 303.
58. Bouveng, R.; Ellman, M.; Gunnarsson, P. O.; Jensen, G.; Liljekvist, J.; Muntzing, J. *Eur. J. Cancer* **1979**, *15*, 407.
59. Carroll, F. I.; Philip, A.; Blackwell, J. T.; Taylor, D. J.; Wall, M. E. *J. Med. Chem.* **1972**, *15*, 1158.
60. Lam, H.-Y. P.; Begleiter, A.; Goldenberg, G. J. *J. Med. Chem.* **1979**, *22*, 200.
61. Lin, T. S.; Fischer, P. H.; Shiau, G. T.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 130.

62. Wang, G. W.; Miao, C. B.; Kang, H. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1426.

63. Xiao, Z. P.; Lv, P. C.; Xu, S. P.; Zhu, T. T.; Zhu, H. L. *Chem. Med. Chem.* **2008**, 3, 1516.

64. Sirisoma, N.; Pervin, A.; Zhang, H.; Jiang, S.; Willardsen, J. A.; Anderson, M. B.; Mather, G.; Pleiman, C. M.; Kasibhatla, S.; Tseng, B.; Drewe, J.; Cai, S. X. *J. Med. Chem.* **2009**, *52*, 2341

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
- ***"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.
- ***"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.

Paper/Poster presented at the International Conference:

- International conference on bridging gaps in discovery and development: chemical & biological science for affordable health, wellness & sustainability. “DNA-directed alkylating agents: Synthesis and antitumor activity of phenyl *N*-mustard-quinazoline conjugates having a urea linker” Abstract No: PP-004.**(2011)**
- International conference on the interface of chemistry-biomedical research, pilani “Microwave-assisted and Zn[L-proline]$_2$ catalyzed tandem cyclization under solvent free conditions: Rapid synthesis of chromeno[4,3 *c*]pyrazol-4-ones” Abstract No: PP-37.**(2008)**

Publications

1. 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. ***Bio. & Med. Chem.*** 2011, 19 1987–1998.
2. Design, synthesis, and biological evaluation of novel water-soluble N-mustards as potential anticancer agents. Naval Kapuriya, Rajesh Kakadiya, Huajin Dong, Amit Kumar, Pei-Chih Lee, Xiuguo Zhang, Ting-Chao Chou, Te-Chang Lee, Ching-Huang Chen, King Lam, **Bhavin Marvania,** Anamik Shah, Tsann-Long Su. ***Bioorg. Med. Chem.*** 2011, *19*, 471-485.
3. 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.
4. Catalyst-Free, Rapid Synthesis of Fused Bicyclic Thiazolo-Pyrimidine and Pyrimido-Thiazine Derivatives by a Microwave-Assisted Method. Vijay R. Virsodia, Nikhil R. Vekariya, Atul T. Manvar, Rupesh C. Khunt, **Bhavin R. Marvania,** Bharat S. Savalia, Anamik K. Shah. ***Phosphorus, Sulfur, and Silicon and the Related Elements***, 2009, 184,34 -44.

Summary

Summary of the work

The work that is to be presented in the thesis entitled ***"Studies on Synthesis of New Heterocyclic Skeletons and Related Compounds"*** has been divided into seven chapters which can be summarized as under.

SECTION A

Chapter-1 covers basic introduction to cancer and DNA-directed alkylating agents. Cancer is still the most lethal illnesses known worldwide after heart disease. Although significant research has done to date to cancer diseases, there is still a lack of effective chemotherapeutic treatment to cure it completely. DNA alkylating agents have played an important part in cancer chemotherapy. Alkylating agents, the oldest and most useful among the antineoplastic agents, can be defined as compounds capable of covalently binding an alkyl group to a biomolecule under physiological conditions (aqueous solution, 37°C, pH 7.4). Some of them especially nitrogen mustard (*N*-mustard), are used clinically for the treatment of cancer.

Chapter-2 deals with the synthesis of chemically stable DNA-directed alkylating agents, in which the phenyl *N*-mustard residue was linked to DNA binder quinazolines via a urea spacer for antitumor evaluation. N-mustard-quinazoline conjugates having a urea linker are synthesized from Substituted 6-amino-4-anilinoquinazolines and *N*,*N*-bis(2-chloroethyl)-4-isocyanatoaniline in the presence of triethylamine.

Chapter-3 entitled "Antitumor evaluation of phenyl *N*-mustard-quinazoline conjugates bearing a urea linker." In this chapter, a series of *N*-mustard-quinazoline conjugates via urea linker was tested for the anticancer activity. All of the new *N*-mustards conjugates found to have potent antiproliferative activity against human leukemia (CCRF-CEM) and its drug resistant sublines (CCRF-CEM/Taxol and CCRF-CEM/VBL) and various solid tumors (breast carcinoma MX-1, colon carcinoma HCT-116, lung carcinoma H1299 and prostate carcinoma PC3) cell growths in vitro. Among these derivatives, compound **87h** possessed significant tumor growth inhibition (72 %) in comparison with the untreated control. We also demonstrate that the newly synthesized compounds are able to induce DNA cross-

linking through alkaline agarose gel shift assay and inhibited cell cycle arrest at G2/M phase.

Chapter-4 In this chapter, the efforts have been made for the synthesis of potent DNA-directed alkylating agents by linking the phenyl *N*-mustard pharmacophore with quinoline via a carbamate linker. *N*-mustard-6-hydroxyquinoline conjugates are synthesized from substituted 6-hydroxy-4-anilinoquinolines and *N*,*N*-bis(2-chloroethyl)-4-isocyanatoaniline in the presence of triethylamine.

Chapter-5 entitled "Synthesis and yield optimization efforts for a novel compound: 1-(4-(bis(2-chloroethyl)amino)phenyl)-3-(2-phenylquinazolin-4-yl)urea" deals with the discover new chemically stable DNA-directed alkylating agents, we therefore connected the phenyl *N*-mustard pharmacophore to the 4-amino function of quinazolines using a urea moiety as the linker. 1-(4-(bis(2-chloroethyl)amino)phenyl)-3-(2-phenylquinazolin-4-yl)urea are synthesized from 4-amino-2-phenylquinazoline and N,N-bis(2-chloroethyl)-4-isocyanatoaniline in the presence of triethylamine.

SECTION B

Chapter-6 deals with the general introduction about nitrosourea and carbamate derivatives. Among significant compounds, nitrosoureas and carbamate are an extremely active class of antitumor agents that are effective against solid tumors, as well as leukemias. In particular, 2-haloethyl derivatives and some of their metabolites show great promise as effective antitumor agent. For the treatment of a number of experimental and clinical tumors, several *N*-(2-chloroethyl)-N-nitrosourea have successfully been applied as chemotherapeutic agents.

Chapter-7 entitled "Synthesis and characterization of several new (2-Chloroethyl)nitrosocarbamates derivatives" Current chapter represents synthesis of (2-Chloroethyl)nitrosocarbamates derivatives using quinazoline as a moiety. Substituted quinazoline (2-Chloroethyl)nitrosocarbamates conjugates are synthesized from quinazoline (2-Chloroethyl)carbamates using Nitrosoniumtetrafluoroborate as a catalyst.

In all 98 compounds were synthesized, characterized and evaluated for anti cancer activities.

Bioorganic & Medicinal Chemistry 19 (2011) 1987–1998

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and antitumor evaluation of phenyl *N*-mustard-quinazoline conjugates

Bhavin Marvania [a,d], Pei-Chih Lee [a], Ravi Chaniyara [a,d], Huajin Dong [b], Sharda Suman [a], Rajesh Kakadiya [a], Ting-Chao Chou [b], Te-Chang Lee [a], Anamik Shah [d], Tsann-Long Su [a,c,*]

[a] *Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan*
[b] *Preclinical Pharmacology Core Laboratory, Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA*
[c] *Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung, Taiwan*
[d] *Department of Chemistry, Saurashtra University, Rajkot, Gujarat, India*

ARTICLE INFO

Article history:
Received 13 December 2010
Revised 25 January 2011
Accepted 26 January 2011
Available online 2 February 2011

Keywords:
Anticancer agents
Cell cycle
DNA-directed alkylating agents
DNA interstrand cross-linking agents
Nitrogen mustards

ABSTRACT

A series of *N*-mustard-quinazoline conjugates was synthesized and subjected to antitumor studies. The *N*-mustard pharmacophore was attached at the C-6 of the 4-anilinoquinazolines via a urea linker. To study the structure–activity relationships of these conjugates, various substituents were introduced to the C-4 anilino moiety. The preliminary antitumor studies revealed that these agents exhibited significant antitumor activity in inhibiting various human tumor cell growths in vitro. Compounds **21b**, **21g**, and **21h** were selected for further antitumor activity evaluation against human breast carcinoma MX-1 and prostate PC-3 xenograft in animal model. These agents showed 54–75% tumor suppression with low toxicity (5–7% body-weight changes). We also demonstrate that the newly synthesized compounds are able to induce DNA cross-linking through alkaline agarose gel shift assay and inhibited cell cycle arrest at G2/M phase.

1. Introduction

Designing DNA-directed alkylating agents is one of the most effective strategies to overcome the general drawbacks of DNA alkylating agents. The common drawbacks of these agents include high reactivity and the lack of DNA sequence-specific binding, resulting in side effects and carcinogenicity.[1,2] DNA-directed alkylating agents are synthesized by linking DNA-affinic molecules (carrier) to a *N*-mustard pharmacopore (warhead, such as alkyl *N*-mustard or phenyl *N*-mustard). The generally useful DNA-affinic molecules are DNA-intercalating agents (e.g., 9-anilinoacridines and acridines), binding agents (quinolines or quinazolines), and DNA minor groove binding agents (e.g., distamycin A and netropsin). Most of these carriers also exhibit anticancer activity by inhibiting Topoisomerases I and II.[3] Emerging evidence shows that DNA-directed alkylating agents are more cytotoxic than the used carrier itself. Consequently, connecting DNA-affinic molecules to alkylating agents usually results in improved therapeutic efficacy than the corresponding untargeted alkylating agents.[4–8]

We have previously synthesized a series of alkyl *N*-mustard-9-anilinoacridine conjugates having methylene (CH_2) or alkoxy [$O(CH_2)n$] spacer to the aniline, and/or acridine ring(s).[7–10] Although these conjugates (e.g., **1**, Fig. 1) exhibited significant cytotoxicity against various human tumor cell growth in vitro and potent antitumor activity in human tumor xenografted model, they have a narrow therapeutic window and low bioavailability (chemical instability with a short half-life) in mice, probably due to the inductive effect of the alkoxy linker, increasing the reactivity of the *N*-mustard moiety. To improve the poor bioavailability of these derivatives, we have synthesized a series of phenyl *N*-mustard-9-anilinoacridine conjugates bearing a urea, carbamate or carbonate linker.[11,12] We revealed that these derivatives (e.g., **2**) have more chemical stability with potent anticancer activity. The linkers used for the synthesis of phenyl *N*-mustard-9-anilinoacridine conjugates were previously applied in antibody-directed enzyme prodrug therapy (ADEPT) and melanocyte directed enzyme prodrug therapy (MDEPT) of *N*-mustard derivatives.

More recently, we utilized quinolines as carriers to prepare a series of *N*-mustard-quinoline conjugates having a urea or hydrazinecarboxamide linker (e.g., **3** and **4**).[13] Similarly, these conjugates possess potent antitumor activity against a variety of human tumor xenografts. Both linkers are also able to lower the reactivity of the *N*-mustard moiety, resulting in a longer half-life in rat plasma. The linkers in these derivatives are attached to the C-4 position of the 4-aminoquinolines, demonstrating that quinolines are also valuable carriers for building DNA-directed alkylating agents.

Aside from quinolines, 4-anilinoquinazoline derivatives [e.g., PD153035 (**5**) and EBEA22 (**6**)] were also reported to have high DNA binding affinity.[14] Particularly, compound **6** possesses higher

* Corresponding author. Tel.: +886 2 2789 9045; fax: +886 2 2782 5573.
E-mail address: tlsu@ibms.sinica.edu.tw (T.-L. Su).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.01.055

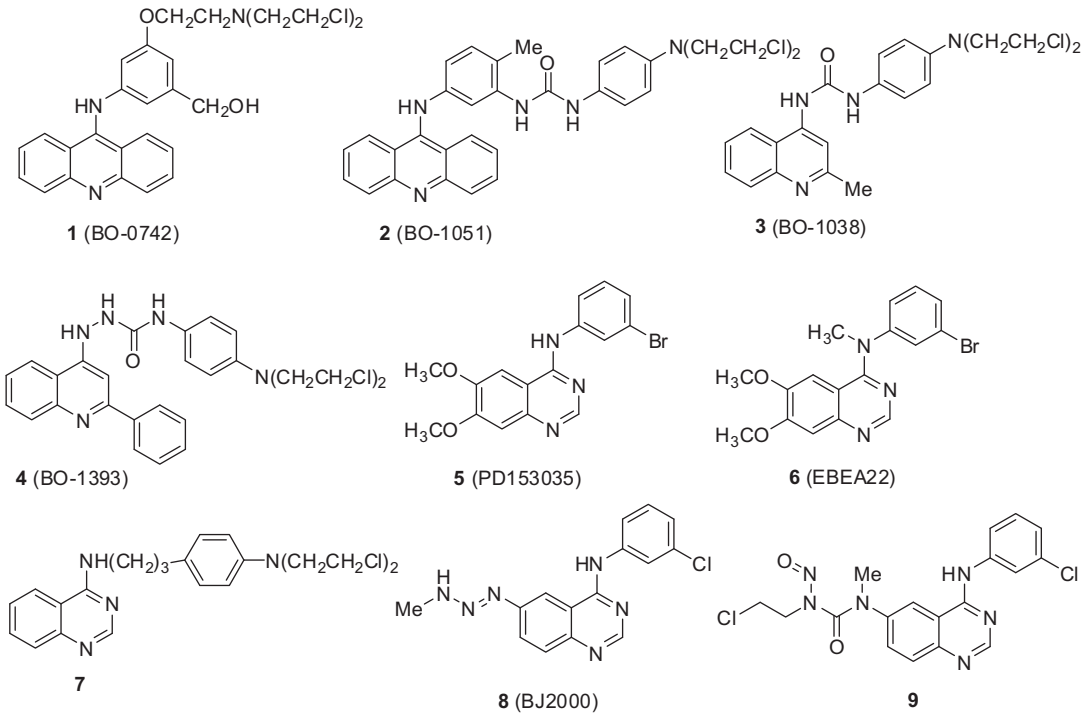


Figure 1. Chemical structures of *N*-mustard and quinazoline derivatives.

GC-selective binding than what was expected. It was also shown that both **5** and **6** have high affinities and selectivity toward the epidermal growth factor receptor tyrosine kinase (EGF-R TK) and exhibited potent cytotoxicity against several types of tumor cell growth in vitro.[15] Although compound **5** possessed potent cytotoxicity, this agent has low therapeutic efficacy in vivo because of its poor water solubility. Quinazolines have also been applied for synthesizing DNA-directed alkylating agents (e.g., **7**, Fig. 1). It was demonstrated that compound **7** is not a strong intercalator, but it may bind weakly at the major groove side.[16] These studies suggest that quinazolines can be applied for designing DNA-directed alkylating agents.

In order to discover new chemically stable DNA-directed alkylating agents, we therefore connected the phenyl *N*-mustard pharmacophore to quinazolines using a urea moiety as the linker. Initially, we attempted to attach the *N*-mustard to the 4-amino function of quinazolines bearing a urea linker; however, the product, *N*-mustard-4-aminoquinazoline **15** (Scheme 1) has very poor solubility, even does not dissolve in DMSO. Consequently, we prepared 6-amino-4-anilinoquinazolines for constructing new phenyl *N*-mustard-6-aminoquinazoline conjugates, in which the *N*-mustard moiety is linked to the 6-amino function via a urea linker. The studies will allow us to understand whether these conjugates have improved water-solubility and cytotoxicity. The results show that the new conjugates have better solubility in an intravenous injection (iv injection) vehicle and possess potent cytotoxicity in inhibiting various human lymphoblastic leukemia and solid tumor cell growth in vitro. It should be noted that some of the 4-anilinoquinazolines have already been used by the group of Jean-Claude to prepare degradable conjugates with various alkylating moieties such as N-nitroso ureas and triazenes (e.g., **8** and **9**).[17] We report herein the chemical synthesis, antitumor activity, and DNA cross-linking study of phenyl *N*-mustard-4-anilinoquinazoline conjugates.

2. Chemistry

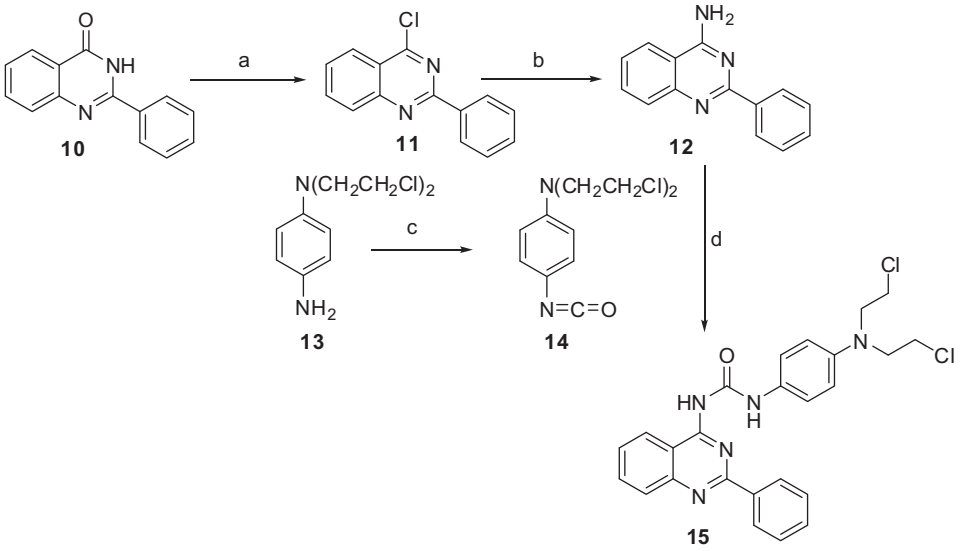
The synthesis of the *N*-mustard-quinazoline conjugates is shown in Schemes 1 and 2. The *N*-mustard-4-aminoquinazoline conjugate **15** was prepared starting from the known compound 2-phenylquinazolin-4(3*H*)-one (**10**, Scheme 1).[18,19] Compound **10** was treated with $POCl_3$ to produce 4-chloro-2-phenyl quinazoline (**11**), which was then reacted with ammonia in phenol at 170 °C to give 2-phenylquinazolin-4-amine (**12**). Reaction of **12** with the known 4-[*N*,*N*-bis(2-chloroethyl)-amino]phenylisocyanate **14**[20] [freshly prepared from *N*,*N*-bis(2-chloroethyl)benzene-1,4-diamine hydrochloride (**13**)[21]] in the presence of triethylamine afforded *N*-mustard-4-aminoquinazoline conjugate **15** in low yield.

Scheme 2 shows the synthesis of the *N*-mustard-6-aminoquinazoline conjugates (**21a–q**). The key starting materials, 6-amino-4-anilinoquinazolines (**20a–q**), were prepared by following the literature methods.[17] Among these derivatives, compounds **20b,c,d,f,h,j,o,p,q** are known, while all other compounds are new derivatives. Briefly, the commercially available 5-nitroanthranilonitrile **16** was treated with dimethylformamide dimethylacetal (DMF–DMA) in acetic acid to give (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide (**17**), which was then reacted with substituted anilines (**18a–q**) in acetic acid to afford 6-nitroquinazoline derivatives (**19a–q**). The nitro function in **19a–q** was converted into the corresponding 6-aminoquinazoline derivatives (**20a–q**) by treating with Fe/acetic acid. Reaction of **20a–q** with the freshly prepared **14** in the presence of triethylamine gave the desired *N*-mustard-6-aminoquinazoline conjugates (**21a–q**) bearing a urea linker.

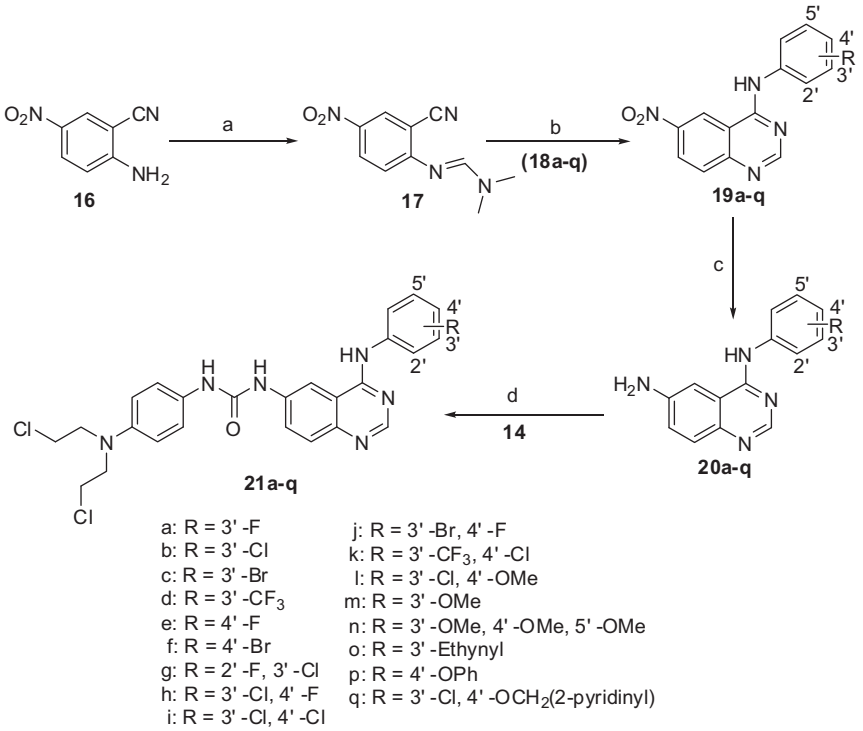
3. Biological result and discussion

3.1. In vitro cytotoxicity

To study the structure-activity relationships of the *N*-mustard-quinazoline conjugates, we have introduced electron-withdrawing halogen(s), electron-donating methoxy function(s), and other substituent to the 4-anilino ring. These derivatives were subjected to evaluating their cytotoxicities in inhibiting human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines resistant to Taxol (CCRF-CEM/Taxol) and Vinblastine (CCRF-CEM/VBL) cell



Scheme 1. Reagents and conditions: (a) $POCl_3$/reflux; (b) phenol/NH_3(g)/170 °C; (c) triphosgene/Et_3N/$CHCl_3$/THF, room temperature; (d) Et_3N/$CHCl_3$, room temperature.

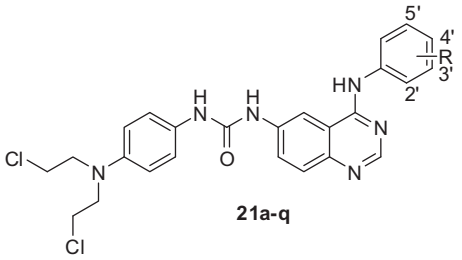


Scheme 2. Reagents and conditions: (a) DMF–DMA/reflux; (b) $ArNH_2$/AcOH/reflux; (c) Fe/AcOH/EtOH/H_2O/reflux; (d) Et_3N/THF, room temperature.

growth in vitro (Table 1). It demonstrated that the newly synthesized conjugates possess significant cytotoxicity with IC_{50} in micro molar range. The most cytotoxic compound of this series is the 3′,4′,5′-trimethoxyphenyl derivative **21n** with an IC_{50} value of 0.38 μM. However, the potency of the C-3′-OMe derivative (**21m**, IC_{50} >1.32 μM) is much weaker than the corresponding trimethoxy derivative **21n**. As for the halogen-substituted derivatives, it is clearly to see that order of the cytotoxicity of C-3′-halogen substituted compounds is C-3′–Br (**21c**) > C-3′–Cl (**21b**) > C-3′–F (**21a**). In contrast, the C-4′–F substituted derivative **21e** is apparently more potent than the corresponding C-4′–Br derivative **21f**. In the series of C-3′–C-4′ dihalogens substituted conjugates, the order of the cytotoxicity is **21g** > **21h** > **21i** ≅ **21j**. The SAR study shows that the position of the substitutions, numbers and types of halogen atom are critical for their activity. The cytotoxicity of compounds bearing other substituent, such as C-3′ or C-4′–CF_3 (**21d** and **21k**, respectively), C-3′-ethynyl (**21o**), C-4′-phenoxy (**21p**), and 3-chloro-4-(pyridin-2-ylmethoxy) (**21q**), were also evaluated. It reveals that C-3′-ethynyl (**21o**) is most cytotoxic among these conjugates with a IC_{50} value of 0.50 μM. Compounds having a CF_3 substituent (**21d** and **21k**) are less cytotoxic than other compounds tested.

The cytotoxicity of the newly synthesized compounds against human CCRF-CEM drug-resistant sublines (resistant to Vinblastine and Taxol, CCRF-CEM/VBL and CCRF-CEM/Taxol, respectively) were also studied. The results revealed that they generally have no or little cross-resistance to these two natural products except compounds **21n**, which has certain extent of cross-resistance

Table 1
The cytotoxicity of newly synthesized phenyl *N*-mustard-6-aminoquinazoline conjugates against human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines (CCRF-CEM/Taxol and CCRF-CEM/VBL)[a]



Compd	Substitute R				Cell growth inhibition (IC_{50} μM)		
	2′	3′	4′	5′	CCRF-CEM	CCRF-CEM/Taxol[b]	CCRF-CEM/VBL[b]
21a	H	F	H	H	2.51 ± 0.11	3.46 ± 0.18 [1.38×][c]	4.28 ± 0.01 [1.70×]
21b	H	Cl	H	H	1.66 ± 0.14	4.56 ± 0.0001 [2.75×]	3.26 ± 0.05 [1.96×]
21c	H	Br	H	H	0.95 ± 0.11	4.58 ± 0.05 [4.82×]	2.62 ± 0.01 [2.76×]
21d	H	CF_3	H	H	3.97 ± 0.22	4.57 ± 0.13 [1.15×]	4.68 ± 0.05 [1.17×]
21e	H	H	F	H	0.74 ± 0.02	3.16 ± 0.07 [4.27×]	1.24 ± 0.03 [1.67×]
21f	H	H	Br	H	4.64 ± 0.16	8.79 ± 0.16 [1.89×]	7.88 ± 0.36 [1.70×]
21g	F	Cl	H	H	0.75 ± 0.01	1.55 ± 0.02 [2.07×]	1.04 ± 0.03 [1.39×]
21h	H	Cl	F	H	1.00 ± 0.09	1.16 ± 0.14 [1.16×]	1.14 ± 0.03 [1.14×]
21i	H	Cl	Cl	H	2.60 ± 0.02	5.61 ± 0.16 [2.16×]	6.75 ± 0.53 [2.60×]
21j	H	Br	F	H	2.68 ± 0.11	4.40 ± 0.30 [1.64×]	4.74 ± 0.15 [1.77×]
21k	H	CF_3	Cl	H	4.77 ± 0.22	5.36 ± 0.14 [1.12×]	5.28 ± 0.58 [1.11×]
21l	H	Cl	OMe	H	0.80 ± 0.01	3.34 ± 0.02 [4.17×]	3.75 ± 0.04 [4.68×]
21m	H	OMe	H	H	1.32 ± 0.02	3.58 ± 0.12 [2.71×]	3.38 ± 0.17 [2.56×]
21n	H	OMe	OMe	OMe	0.38 ± 0.02	12.32 ± 0.79 [32.42×]	24.51 ± 1.72 [64.50×]
21o	H	3-ethynyl	H	H	0.50 ± 0.04	2.29 ± 0.02 [4.58×]	2.33 ± 0.34 [4.66×]
21p	H	H	OPh	H	4.77 ± 0.01	7.99 ± 0.18 [1.67×]	10.88 ± 0.02 [2.28×]
21q	H	Cl	OCH_2(2-pyridinyl)	H	1.41 ± 0.07	4.58 ± 0.08 [3.24×]	2.65 ± 0.13 [1.88×]
Taxol					0.003 ± 0.0003	0.43 ± 0.05 [143×]	1.27 ± 0.05 [423×]
Vinblastine					0.0007 ± 0.001	0.08 ± 0.01 [106.2×]	0.50 ± 0.12 [679.5×]
Carboplatin					3.4 ± 0.99		2.45 ± 0.65 [0.7×]

[a] Cell growth inhibition was measured by the XTT assay[25] for leukemic cells after 72-h incubation using a microplate spectrophotometer as described previously.[27] 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). IC_{50} values were determined from dose–effect relationship at six or seven concentrations of each drug by using the CompuSyn software by Chou and Martin[29] based on the median-effect principle and plot using the serial deletion analysis.[30,31] Ranges given for Taxol and vinblastine were mean ± SE (n = 4).
[b] CCRF-CEM/Taxol and CCRF-CEM/VBL are subcell lines of CCRF-CEM cells that are 143-fold resistant to Taxol, and 423-fold resistant to vinblastine, respectively, when comparing with the IC_{50} of the parent cell line.
[c] Numbers in the brackets are fold of cross-resistant determined by comparison with the corresponding IC_{50} of the parent cell line.

Table 2
The cytotoxicity of phenyl *N*-mustard-6-aminoquinazoline conjugates (**21a–q**) against human solid tumor (breast carcinoma MX-1, colon carcinoma HCT-116, lung carcinoma H1299 and prostate carcinoma PC3) cell growth in vitro[a]

Compd	Substitute R				Cell growth inhibition (IC_{50} μM)			
	2′	3′	4′	5′	MX-1[a]	HCT-116[a]	H1299[b]	PC3[b]
21a	H	F	H	H	10.11 ± 0.09	9.62 ± 0.12	ND	ND
21b	H	Cl	H	H	7.84 ± 0.03	7.46 ± 0.02	10.49 ± 2.04	10.37 ± 0.32
21c	H	Br	H	H	6.92 ± 0.05	2.17 ± 0.03	8.04 ± 1.26	10.13 ± 0.13
21d	H	CF_3	H	H	5.30 ± 0.63	5.97 ± 0.24	ND	ND
21e	H	H	F	H	3.43 ± 0.003	3.18 ± 0.03	11.60 ± 1.54	8.24 ± 1.76
21f	H	H	Br	H	6.71 ± 0.03	7.79 ± 0.36	ND	ND
21g	F	Cl	H	H	4.55 ± 0.02	3.84 ± 0.0005	5.52 ± 1.59	6.29 ± 1.12
21h	H	Cl	F	H	5.38 ± 0.05	3.36 ± 0.05	ND[c]	ND
21i	H	Cl	Cl	H	6.45 ± 0.50	2.79 ± 0.14	ND	ND
21j	H	Br	F	H	7.10 ± 0.14	5.99 ± 0.63	ND	ND
21k	H	CF_3	Cl	H	7.44 ± 0.58	5.34 ± 0.06	ND	ND
21l	H	Cl	OMe	H	5.51 ± 0.09	5.27 ± 0.18	6.94 ± 1.71	8.02 ± 2.08
21m	H	OMe	H	H	2.59 ± 0.13	2.44 ± 0.03	8.79 ± 1.32	9.44 ± 0.87
21n	H	OMe	OMe	OMe	4.02 ± 0.03	2.24 ± 0.01	10.28 ± 1.25	10.98 ± 1.89
21o	H	3-Ethynyl	H	H	6.27 ± 0.24	3.47 ± 0.13	ND	ND
21p	H	H	OPh	H	8.47 ± 0.15	12.41 ± 0.04	ND	ND
21q	H	Cl	OCH_2 (2-pyridinyl)	H	4.62 ± 0.20	2.22 ± 0.54	ND	ND
Cisplatin					4.95 ± 0.60	26.65 ± 4.19	ND	ND

[a] Cell growth inhibition was measured by the SRB assay[26] for solid tumor cells after 72-h incubation using a microplate spectrophotometer as described previously.[27]
[b] Cell growth inhibition was determined by the Alamar blue assay[28] in a 72 h incubation using a microplate spectrophotometer as described previously.
[c] Not determined.

(Table 1). It suggests that the *N*-mustard derivatives were neither a good substrate of *p*-glycoprotein nor mutated tubulin.

The selected compounds were further evaluated for their cytotoxicity in inhibiting other human solid tumors such as human

breast tumor (MX-1), colon cancer (HCT-116), human non-small cell lung cancer (H1299), and prostate cancer (PC3) cell growth in vitro. As shown in Table 2, one can see that these conjugates possess good to moderate cytotoxic effects against the growth of these cell lines tested in vitro. In comparison with the cytotoxicities of the *N*-mustard-quinoline conjugates, previously synthesized in our laboratory,[13] the *N*-mustard-quinazoline conjugates are less potent in inhibiting all tumor cell lines examined.

3.2. In vivo therapeutic activity

The in vitro cytotoxicity of the tested compound may not always directly reflex to its therapeutic efficacy in tumor xenograft model. In the present studies, we selected compounds **21b**, **21g**, and **21h** for evaluating their therapeutic efficacy in nude mice bearing human mammary carcinoma (MX-1) xenografts (Table 3). To find a maximal tolerable dose of compound tested, we administrated various doses to view its therapeutic effects via intravenous injection (iv inj.). The preliminary results show that compound **21h** possessed significant tumor growth inhibition (72%) in comparison with the untreated control when mice were treated successfully with the dose of 50 mg/kg, every two days for three times (Q2D × 3), 60 mg/kg (Q2D × 3), and then 70 mg/kg, every two days for two times (Q2D × 2). With the same drug administration route (iv inj.), compound **21b** showed a moderate tumor inhibition (62%), at the doses of 30 mg/kg (Q2D × 3) and 35 mg/kg, every two days for five times (Q2D × 5). Compound **21g** also showed to have moderate tumor suppression (52%) at the dose of 30 mg/kg (Q2D × 3) and then 40 mg/kg (Q2D × 5). Although all the tested compounds induced a 5–7% body-weight change during the treatment (Table 3), the body-weight of mice readily recovered after cessation of the treatment, suggesting that these agents have relatively low toxicity to the host.

Conjugates **21b** and **21g** were also selected for evaluating their therapeutic efficacy against human prostate PC-3 xenograft in nude mice. Table 3 shows that there were 27% and 69% tumor suppression by **21b** and **21g**, respectively, at the maximal tolerable dose of 55 mg/kg, every two days for six times (Q2D × 6) with acceptable toxicity (about 10–11% body weight loss). Similar observations were found that *N*-mustard-quinazoline conjugates are much less potent against human breast MX-1 xenograft in mice than that of the *N*-mustard-quinoline conjugates.[13]

3.3. DNA cross-linking study by alkaline agarose gel shift assay

The alkaline gel shift assay was performed to assess DNA cross-linking activity of compounds **21g**, **21l**, **21b**, and **21e** (Fig. 2). The pEGFP-N1 plasmid DNA was treated with compounds, **21g**, **21l**, **21b**, and **21e** at various concentrations as indicated (1, 10 and 20 μM). Melphalan was used as a positive control. The tested compounds show moderate cross-linking behavior at lower concentrations; however, at high concentrations, the cross-linking behavior was similar to melphalan. These results revealed that the newly synthesized *N*-mustard-quinazoline conjugates are capable to induce DNA cross-linking.

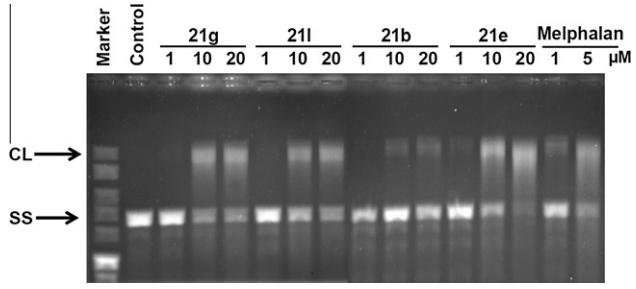


Figure 2. Representative DNA cross-linking gel shift assay for **21g**, **21l**, **21b**, and **21e** at various concentrations as indicated. Control lane shows single-stranded DNA (SS), while cross-linking (CL) shown in all tested lanes is DNA double-stranded cross-linking. Melphalan (1 and 5 μM) was used as a positive control.

3.4. Cell cycle inhibition

It was reported that DNA damage induced by DNA alkylating agents is known to cause cell cycle delay and arrest the cell cycle progression predominantly at the G2/M boundary.[22] We therefore studied the inhibitory effect of **21b** on cell cycle distribution (Table 4). The human non-small cell lung carcinoma H1299 cells were treated with **21b** at the concentrations of 5, 10, and 20 μM for 24 h. The cells were harvested, stained with propidium iodide (PI) and analyzed with a flow cytometer. It clearly shows that 5 μM of **21b** significantly accumulated the cells at G2/M phase, while 10 and 20 μM of **21b** prevented the cell cycle progression, which may be due to high level of DNA cross-linking. Similar G2/M arrest was previously observed in SW626 cells treated with melphalan.[23] Furthermore, increased sub-G1 populations were noticed in cells treated with **21b** at 20 μM.

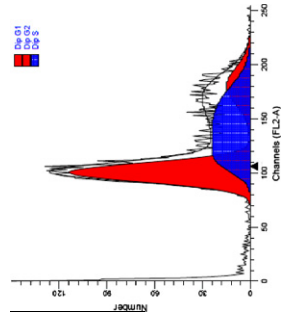
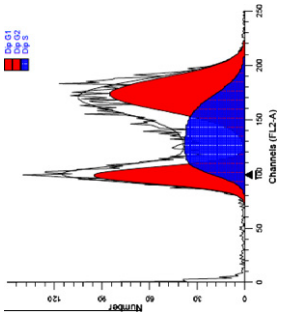
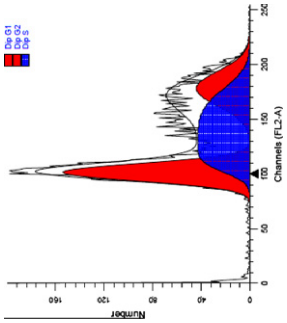
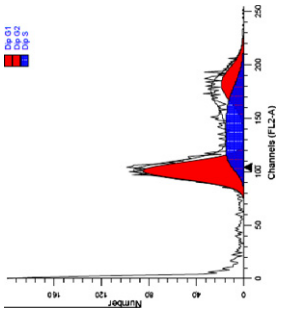
4. Conclusion

Recently, we have synthesized a series of *N*-mustard-quinoline conjugates bearing a urea or hydrazinecarboxamide linker for antitumor evaluation. We demonstrated that these conjugates exhibited potent in vitro cytotoxicity and therapeutic efficacy against human xenografts. To continue our research on the developing DNA-directed alkylating agents, we have synthesized a series of *N*-mustard-quinazoline conjugates, in which the *N*-mustard pharmacophore was attached at the C-6 of the 4-anilinoquinazolines via a urea linker. A variety of substituent(s) were introduced to the C-4 anilino moiety for studying their structure–activity relationships. The preliminary antitumor studies revealed that these agents exhibited significant antitumor activity in inhibiting various human tumor cell growths in vitro. Among compounds selected for evaluating their antitumor activity against human breast tumor (MX-1) xenograft in nude mice, 4-[3′-Cl,4′-F-phenylamino]quinazoline derivative (**21h**) is the most potent. We demonstrate that the newly synthesized *N*-mustard-quinazoline conjugates are generally less potent than the *N*-mustard-quinoline conjugates. Studies on the therapeutic efficacy against MX-1 xenograft in nude mice revealed that the tested compounds have moderate antitumor

Table 3
Therapeutic effects and toxicity of *N*-mustard-quinazoline conjugates in nude mice bearing human mammary carcinoma (MX-1) and prostate (PC-3) xenografts

Tumor used	Compd	Dose and schedule	Maximal tumor suppression (%)	Toxicity body-weight loss (%)	Death
MX-1	**21b**	30 mg/kg (Q2D × 3) and then 35 mg/kg (Q2D × 5)	62	3	0/3
	21g	30 mg/kg (Q2D × 3) and then 40 mg/kg (Q2D × 5)	52	6	0/3
	21h	50 mg/kg (Q2D × 3), 60 mg/kg (Q2D × 3), and then 70 mg/kg (Q2D × 2)	72	5	0/3
PC-3	**21b**	55 mg/kg (Q2D × 6)	27	11	0/5
	21g	55 mg/kg (Q2D × 6)	69	6	0/5

Table 4
Cell cycle inhibition in human non-small cell lung carcinoma H1299 by treating with compound **21b**

Concentration (μM)	0	5	10	20
				
sub G1	8.8 ± 6.9	8.9 ± 0.03	7.5 ± 0.2	35.3 ± 2.2
G1	44.8 ± 3.0	30.5 ± 0.4	34.8 ± 0.4	28.3 ± 1.0
S	24.9 ± 3.3	20.6 ± 4.0	28.5 ± 0.2	23.5 ± 3.5
G2/M	21.5 ± 0.6	40.0 ± 3.5	29.3 ± 0.01	12.9 ± 0.2

activity, but they are less toxic to the host based on the observation of the average body-weight changes. In the present studies we also show that the newly synthesized compounds are able to induce DNA cross-linking through alkaline agarose gel shift assay and inhibited cell cycle arrest at G2/M phase.

5. Experimental

5.1. General methods and materials

Compound solvents and reagents were reagent grade and used without purification unless otherwise noted. The melting points were recorded on a Fargo melting point apparatus and are uncorrected. Column chromatography was carried out on Silica Gel G60 (70–230 mesh, ASTM; Merck and 230–400 mesh, Silicycle Inc.). Reaction progress was monitored using analytical thin-layer chromatography (TLC) on 0.25 mm Merck F-254 silica gel glass plates. Visualization was achieved by UV light (254 nm). ^{1}H NMR spectra were recorded with a Bruker AVANCE 600 DRX and 400 MHz spectrometer. Chemical shifts are reported in parts per million (δ) using tetramethylsilane as the internal standard with coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; br s, broad singlet. Elemental analyses were performed on a Heraeus CHN–O Rapid analyzer. 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 μL) mobile phase, MeCN/THF (70:30 v/v); flow rate, 1 mL/min; injected sample 10 μL, column temp, 27 °C; wavelength, 254 nm. The purity of all compounds was >95% based on analytical HPLC.

5.2. 4-Chloro-2-phenylquinazoline (11)

To a magnetically stirred solution of $POCl_3$ (25 mL) at 0 °C was added portion wise 2-phenylquinazolin-4(3*H*)-one **10** (5.0 g). The reaction mixture was refluxed for 2 h. After completion of the reaction, the excess $POCl_3$ was removed by vacuo. The residue was poured into a mixture of chloroform (50 mL) + ice cold water (80 mL) + ammonia solution (20 mL). The chloroform layer was separated and the aqueous layer was extracted with an additional 20 mL of chloroform. The united chloroform extracts were dried over Na_2SO_4 and filtered, and the solvent was removed by distillation to give **11**, 4.5 g (87%); mp 125–127 °C (lit.[24] 124–125 °C); ^{1}H NMR (DMSO-d_6) δ 7.58–7.69 (4H, m, 4 × ArH), 7.91–7.92 (2H, m, 2 × ArH), 8.16–8.21 (3H, m, 3 × ArH). Anal. ($C_{14}H_9ClN_2$): C, H, N.

5.3. 2-Phenylquinazolin-4-amine (12)

A mixture of 4-chloro-2-phenylquinazolin **11** (3.0 g, 12.0 mmol) and excess phenol was heated at 170 °C for 2 h. After completion of the reaction, the ammonia gas was passed into the reaction mass at 150 °C for 1 h. After that the reaction mixture was cooled to room temperature and poured into 5% sodium hydroxide solution. The solid was filtered and washed with water and dried to give **12**, 2.0 g (74%); mp 140–141 °C (lit.[25] 146–147 °C); ^{1}H NMR (DMSO-d_6) δ 7.45–7.53 (4H, m, 4 × ArH), 7.75–7.79 (2H, m, 2 × ArH), 7.83 (2H, br s, exchangeable, NH_2), 8.23–8.25 (1H, m, ArH), 8.45–8.48 (2H, m, 2 × ArH). Anal. ($C_{14}H_{11}N_3$): C, H, N.

5.4. 1-(4-Bis(2-chloroethyl)phenyl)-3-(2-phenylquinazolin-4-yl) urea (15)

A solution of isocyanate **14** (freshly prepared from **13**, 1.5 g 4.9 mmol) in chloroform (10 mL) was added dropwise to a solution

of 2-phenyl quinazolin-4-amine **12** (0.63 g, 2.8 mmol) in chloroform (30 mL) containing triethylamine (1 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h. The solid material was fall out from reaction mass. It was filtered and washed with chloroform to give 1-(4-bis(2-chloroethyl)phenyl)-3-(2-phenylquinazolin-4-yl)urea **15**, 0.2 g (17%); mp 263–264 °C; ^{1}H NMR (DMSO-d_6) δ 3.68–3.71 (8H, m, 4 × CH_2), 6.81 (2H, d, J = 8.6 Hz, 2 × ArH), 7.48 (2H, d, J = 8.6 Hz, 2 × ArH), 7.58–7.56 (4H, m, 4 × ArH), 7.94–7.95 (2H, m, 2 × ArH), 8.35–8.37 (2H, m, 2 × ArH), 8.72–8.74 (1H, m, ArH), 10.47, 12.02 (each 1H, s, exchangeable, 2 × NH). Anal. ($C_{25}H_{23}Cl_2N_5O$): C, H, N.

5.5. (*E*)-*N*′-(2-Cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide (17)

5-Nitroanthranilonitrile **16** (20.0 g, 122.5 mmol) was suspended in dimethylformamide dimethylacetal (43 mL, 360.0 mmol). The mixture was heated up to reflux temperature for 1.5 h. The resulting mixture was cooled to room temperature and refrigerated overnight. The yellow precipitated was filtered, washed with ethyl ether to give **17**, 25.0 g (96%); mp 153–154 °C (lit.[17] 153–155 °C); ^{1}H NMR (DMSO-d_6) δ 3.09 (3H, s, Me), 3.17 (3H, s, Me), 7.36–7.39 (1H, m, ArH), 8.25–8.28 (2H, m, 2 × ArH), 8.47–8.48 (1H, m, ArH). Anal. ($C_7H_5N_3O_2$): C, H, N.

5.6. *N*-(3-Fluorophenyl)-6-nitroquinazolin-4-amine (19a)

To a solution of (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (6.0 g, 2.7 mmol) and acetic acid (45 mL) was added 3-fluoroaniline (**18a**, 3.3 g, 3.0 mmol) at room temperature. The reaction mixture was heated up to reflux temperature for 1 h. After completion of the reaction, the resulting mixture was cooled to room temperature. The solid separated was filtered and washed with ether to give **19a**, 6.5 g (83%); mp 255–256 °C; ^{1}H NMR (DMSO-d_6) δ 6.98–7.03 (1H, m, ArH), 7.43–7.48 (1H, m, ArH), 7.68 (1H, d, J = 9.2 Hz, ArH), 7.89–7.95 (2H, m, 2 × ArH), 8.55 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.77 (1H, s, ArH), 9.65 (1H, d, J = 2.2 Hz, ArH), 10.48 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9FN_4O_2$): C, H, N.

By following the same procedure as that for **19a** the following compound were synthesized.

5.6.1. *N*-(3-Chlorophenyl)-6-nitroquinazolin-4-amine (19b)

Compound **19b** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-chloroaniline (**18b**, 3.2 g, 2.5 mmol) in acetic acid (40 mL): yield 6.2 g (90%); mp 285–286 °C (lit.[17] 278–281 °C); ^{1}H NMR (DMSO-d_6) δ 7.22–7.47 (1H, m, ArH), 7.43–7.47 (1H, m, ArH), 7.83–7.85 (1H, m, ArH), 7.95 (1H, d, J = 9.2 Hz, ArH), 8.07–8.08 (1H, m, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.78 (1H, s, ArH), 9.65 (1H, d, J = 2.2 Hz, ArH), 10.48 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9ClN_4O_2$): C, H, N.

5.6.2. *N*-(3-Bromophenyl)-6-nitroquinazolin-4-amine (19c)

Compound **19c** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-bromoaniline (**18c**, 4.3 g, 2.5 mmol) in acetic acid (40 mL): yield 7.2 g (91%); mp 282–283 °C (lit.[17] 267–270 °C); ^{1}H NMR (DMSO-d_6) δ 7.35–7.41 (2H, m, 2 × ArH), 7.90–7.92 (1H, m, ArH), 7.94 (1H, d, J = 9.1 Hz, ArH), 8.18–8.19 (1H, m, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.1 Hz, ArH), 8.77 (1H, s, ArH), 9.64 (1H, d, J = 2.2 Hz, ArH), 10.46 (1H, s, exchangeable, NH). Anal. Calcd for $C_{14}H_9BrN_4O_2$: C, H, N.

5.6.3. *N*-(3-(Trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine (19d)

Compound **19d** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-(trifluoromethyl)aniline (**18d**, 4.0 g, 2.5 mmol) in acetic acid (40 mL): yield 6.5 g (85%); mp 210–211 °C (lit.[26] 209–211 °C); ^{1}H NMR (DMSO-d_6) δ 7.52–7.54 (1H, m, ArH), 7.65–7.69 (1H, m, ArH), 7.97 (1H, d, J = 9.1 Hz, ArH), 8.25–8.29 (2H, m, 2 × ArH), 8.59 (1H, dd, J = 2.2 Hz, J = 9.1 Hz, ArH), 8.79 (1H, s, ArH), 9.67 (1H, d, J = 2.2 Hz, ArH), 10.61 (1H, s, exchangeable, NH). Anal. ($C_{15}H_9F_3N_4O_2$): C, H, N.

5.6.4. *N*-(4-Fluorophenyl)-6-nitroquinazolin-4-amine (19e)

Compound **19e** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (4.0 g, 1.5 mmol) and 4-fluoroaniline (**18e**, 2.2 g, 2.0 mmol) in acetic acid (30 mL): yield 5.0 g (96%); mp 257–258 °C; ^{1}H NMR (DMSO-d_6) δ 7.28–7.32 (2H, m, 2 × ArH), 7.84–7.88 (2H, m, 2 × ArH), 7.96 (1H, d, J = 9.2 Hz, ArH), 8.58 (1H, dd, J = 2.5 Hz, J = 9.2 Hz, ArH), 8.72 (1H, s, ArH), 9.66 (1H, d, J = 2.5 Hz, ArH), 10.51 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9FN_4O_2$): C, H, N.

5.6.5. *N*-(4-Bromophenyl)-6-nitroquinazolin-4-amine (19f)

Compound **19f** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 4-bromoaniline (**18f**, 2.6 g, 1.5 mmol) in acetic acid (25 mL): yield 4.0 g (84%); mp 279–280 °C; ^{1}H NMR (DMSO-d_6) δ 7.62–7.65 (2H, m, 2 × ArH), 7.86–7.89 (2H, m, 2 × ArH), 7.97 (1H, d, J = 9.2 Hz, ArH), 8.57–8.60 (1H, dd, J = 2.4 Hz, J = 9.2 Hz, ArH), 8.76 (1H, s, ArH), 9.67 (1H, d, J = 2.4 Hz, ArH), 10.51 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9BrN_4O_2$): C, H, N.

5.6.6. *N*-(2-Fluoro-3-chlorophenyl)-6-nitroquinazolin-4-amine (19g)

Compound **19g** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 2-fluoro-3-chloroaniline (**18g**, 3.6 g, 2.5 mmol) in acetic acid (35 mL): yield 5.5 g (75%); mp 224–226 °C; ^{1}H NMR (DMSO-d_6) δ 7.30–7.34 (1H, m, ArH), 7.52–7.56 (2H, m, 2 × ArH), 7.74–8.01 (1H, m, ArH), 8.57–8.65 (2H, m, 2 × ArH), 9.55 (1H, s, ArH), 10.73 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8ClFN_4O_2$): C, H, N.

5.6.7. *N*-(3-Chloro-4-fluorophenyl)-6-nitroquinazolin-4-amine (19h)

Compound **19h** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 3-chloro-4-fluoroaniline (**18h**, 2.2 g, 1.5 mmol) in acetic acid (20 mL): yield 4.0 g (91%); mp 280–281 °C (lit.[27] 274–277 °C); ^{1}H NMR (DMSO-d_6) δ 7.45–7.50 (1H, m, ArH), 7.81–7.83 (1H, m, ArH), 7.94 (1H, d, J = 9.2 Hz, ArH), 8.14–8.17 (1H, m, ArH), 8.55 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.74 (1H, s, ArH), 9.60 (1H, d, J = 2.2 Hz, ArH), 10.50 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8ClFN_4O_2$): C, H, N.

5.6.8. *N*-(3,4-Dichlorophenyl)-6-nitroquinazolin-4-amine (19i)

Compound **19i** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (6.0 g, 2.7 mmol) and 3,4-dichloroaniline (**18i**, 4.9 g, 3.0 mmol) in acetic acid (45 mL): yield 8.0 g (86%); mp 297–298 °C; ^{1}H NMR (DMSO-d_6) δ 7.65–7.67 (1H, m, ArH), 7.89–7.96 (2H, m, 2 × ArH), 8.27–8.28 (1H, m, ArH), 8.54–8.57 (1H, m, ArH), 8.79 (1H, s, ArH), 9.61–9.62 (1H, m, ArH), 10.49 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8Cl_2N_4O_2$): C, H, N.

5.6.9. *N*-(3-Bromo-4-fluorophenyl)-6-nitroquinazolin-4-amine (19j)

Compound **19j** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (4.0 g, 1.5 mmol) and 3-bromo-4-fluoroaniline (**18j**, 4.0 g, 2.0 mmol) in acetic acid (30 mL): yield 6.0 g (90%); mp 260–261 °C (lit.[27] 257–258 °C); ^{1}H

NMR (DMSO-d_6) δ 7.43–7.47 (1H, m, ArH), 7.88–7.89 (1H, m, ArH), 7.95 (1H, d, J = 9.2 Hz, ArH), 8.25–8.26 (1H, m, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.75 (1H, s, ArH), 9.61 (1H, d, J = 2.2 Hz, ArH), 10.48 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8BrFN_4O_2$): C, H, N.

5.6.10. *N*-(4-Chloro-3-(trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine (19k)

Compound **19k** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 4-chloro-3-(trifluoromethyl)aniline (**18k**, 2.8 g, 1.4 mmol) in acetic acid (30 mL): yield 4.5 g (90%); mp 221–222 °C; ^{1}H NMR (DMSO-d_6) δ 7.77–7.79 (1H, m, ArH), 7.98 (1H, d, J = 9.2 Hz, ArH), 8.32–8.34 (1H, m, ArH), 8.43–8.44 (1H, m, ArH), 8.58 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.81 (1H, s, ArH), 9.63 (1H, s, J = 2.2 Hz, ArH), 10.53 (1H, s, exchangeable, NH). Anal. ($C_{15}H_8ClF_3N_4O_2$): C, H, N.

5.6.11. *N*-(3-Chloro-4-methoxyphenyl)-6-nitroquinazolin-4-amine (19l)

Compound **19l** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-chloro-4-methoxyaniline (**18l**, 3.9 g, 2.5 mmol) in acetic acid (40 mL): yield 6.8 g (91%); mp 290–291 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (3H, s, Me), 7.21–7.23 (1H, m, ArH), 7.75–7.77 (1H, m, ArH), 7.91 (1H, d, J = 9.2 Hz, ArH), 7.99–8.00 (1H, m, ArH), 8.52 (1H, dd, J = 2.4 Hz, J = 9.2 Hz, ArH), 8.70 (1H, s, ArH), 9.60 (1H, d, J = 2.4 Hz, ArH), 10.38 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{11}ClN_4O_3$): C, H, N.

5.6.12. *N*-(3-Methoxyphenyl)-6-nitroquinazolin-4-amine (19m)

Compound **19m** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-methoxyaniline (**18m**, 3.0 g, 2.5 mmol) in acetic acid (35 mL): yield 6.0 g (89%); mp 241–242 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (3H, s, Me), 6.78–6.81 (1H, m, ArH), 7.32–7.37 (1H, m, ArH), 7.49–7.53 (2H, m, 2 × ArH), 7.93 (1H, d, J = 9.2 Hz, ArH), 8.54–8.57 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.74 (1H, s, ArH), 9.66 (1H, d, J = 2.2 Hz, ArH), 10.38 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{12}N_4O_3$): C, H, N.

5.6.13. *N*-(3,4,5-Trimethoxyphenyl)-6-nitroquinazolin-4-amine (19n)

Compound **19n** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3,4,5-trimethoxyaniline (**18n**, 4.6 g, 2.5 mmol) in acetic acid (40 mL): yield 5.5 g (68%); mp 274–275 °C; ^{1}H NMR (DMSO-d_6) δ 3.81 (3H, s, Me), 3.69 (6H, s, 2 × Me), 7.27 (2H, s, 2 × ArH), 7.93 (1H, d, J = 9.2 Hz, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.72 (1H, s, ArH), 9.64 (1H, d, J = 2.2 Hz, ArH), 10.32 (1H, s, exchangeable, NH). Anal. ($C_{17}H_{16}N_4O_5$): C, H, N.

5.6.14. *N*-(3-Ethynylphenyl)-6-nitroquinazolin-4-amine (19o)

Compound **19o** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 3-ethynylaniline (**18o**, 1.6 g, 1.3 mmol) in acetic acid (30 mL): yield 3.8 g (95%); mp 271–272 °C; ^{1}H NMR (DMSO-d_6) δ 4.25 (1H, s, CH), 7.30–7.32 (1H, m, ArH), 7.44–7.48 (1H, m, ArH), 7.92–7.95 (2H, m, 2 × ArH), 8.05–8.06 (1H, m, ArH), 8.54–8.57 (1H, m, ArH), 8.76 (1H, s, ArH), 9.65–9.66 (1H, m, ArH), 10.48 (1H, s, exchangeable, NH). Anal. (C16H10N4O$_2$·0.5): C, H, N.

5.6.15. *N*-(4-Phenoxyphenyl)-6-nitroquinazolin-4-amine (19p)

Compound **19p** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 4-phenoxyaniline (**18p**, 4.6 g, 2.5 mmol) in acetic acid (40 mL): yield 6.0 g (73%); mp 296–298 °C (lit.[27] 293–294 °C); ^{1}H NMR (DMSO-d_6) δ 7.06–7.08 (2H, m, 2 × ArH), 7.11–7.13 (2H, m, 2 × ArH), 7.17–7.19 (1H, m, ArH), 7.42–7.46 (2H, m, 2 × ArH), 7.85–7.87 (2H, m, 2 × ArH), 7.94 (1H, d, J = 9.2 Hz, ArH), 8.57 (1H, dd, J = 2.4 Hz, J = 9.2 Hz, ArH), 8.71 (1H, s, ArH), 9.67 (1H, d, J = 2.4 Hz, ArH), 10.55 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{14}N_4O_3$): C, H, N.

5.6.16. *N*-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)-6-nitroquinazolin-4-amine (19q)

Compound **19q** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)- *N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 3-chloro-4-(pyridine-2-ylmethoxy)aniline (**18q**, 3.2 g, 1.3 mmol) in acetic acid (30 mL): yield 5.0 g (89%); mp 241–242 °C; ^{1}H NMR (DMSO-d_6) δ 5.32 (2H, s, CH_2), 7.30–7.33 (1H, m, ArH), 7.39–7.40 (1H, m, ArH), 7.60–7.62 (1H, m, ArH), 7.74–7.75 (1H, m, ArH), 7.88–7.95 (2H, m, 2 × ArH), 8.04–8.05 (1H, m, ArH), 8.55–8.57 (1H, m, ArH), 8.62–7.63 (1H, m, ArH), 8.73 (1H, s, ArH), 9.62–9.63 (1H, m, ArH), 10.43 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{14}ClN_5O_3·2H_2O$): C, H, N.

5.7. N^4-(3-Fluorophenyl)quinazolin-4,6-diamine (20a)

A mixture of *N*-(3-fluorophenyl)-6-nitroquinazolin-4-amine **19a** (6.0 g, 21.1 mmol) and iron (8.13 g, 147.8 mmol) were suspended in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (16.3 mL, 295.4 mmol). The mixture was heated up to reflux temperature for 2 h. After completion of the reaction, the reaction mixture was cooled to room temperature and alkalinized by addition of concentrated ammonia solution (120 mL). The insoluble material was removed by filtration through Celite, and the filtrate was evaporated under reduce pressure. The resulting solid was washed with 10% K_2CO_3 solution and finally with water and dried to give **20a**, 4.0 g, (75%); mp 188–189 °C; ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 6.84–6.88 (1H, m, ArH), 7.25–7.28 (1H, m, ArH), 7.35–7.40 (2H, m, 2 × ArH), 7.54–7.56 (1H, m, ArH), 7.67–7.69 (1H, m, ArH), 7.93–7.96 (1H, m, ArH), 8.39 (1H, s, ArH), 9.47 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}FN_4·0.8H_2O$): C, H, N.

By following the same procedure as that for **20a** the following compound were synthesized.

5.7.1. N^4-(3-Chlorophenyl)quinazolin-4,6-diamine (20b)

Compound **20b** was synthesized from *N*-(3-chlorophenyl)-6-nitroquinazolin-4-amine **19b** (5.0 g, 16.6 mmol) and iron (6.3 g, 116.2 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (13.3 mL, 232.4 mmol): yield 3.5 g (78%); mp 175–176 °C (lit.[17] 186–189 °C); ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 7.09–7.11 (1H, m, ArH), 7.25–7.28 (1H, m, ArH), 7.34–7.39 (2H, m, 2 × ArH), 7.54–7.56 (1H, m, ArH), 7.82–7.85 (1H, m, ArH), 8.12–8.13 (1H, m, ArH), 8.39 (1H, s, ArH), 9.46 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}ClN_4$): C, H, N.

5.7.2. N^4-(3-Bromophenyl)quinazolin-4,6-diamine (20c)

Compound **20c** was synthesized from *N*-(3-bromophenyl)-6-nitroquinazolin-4-amine **19c** (6.0 g, 17.3 mmol) and iron (6.69 g, 121.7 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (13.9 mL, 243.4 mmol): yield 4.2 g (78%); mp 204–206 °C (lit.[17] 203–204 °C); ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 7.22–7.35 (4H, m, 4 × ArH), 7.54–7.56 (1H, m, ArH), 7.88–7.90 (1H, m, ArH), 8.24–8.25 (1H, m, ArH), 8.38 (1H, s, ArH), 9.44 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}BrN_4$): C, H, N.

5.7.3. N^4-(3-(Trifluoromethyl)phenyl)quinazolin-4,6-diamine (20d)

Compound **20d** was synthesized from *N*-(3-(trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine **19d** (6.0 g, 17.9 mmol) and iron (6.9 g, 125.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (14.3 mL, 250.0 mmol): yield 3.4 g (63%);

mp 174–175 °C; ^{1}H NMR (DMSO-d_6) δ 5.66 (2H, s, exchangeable, NH_2), 7.29–7.31 (1H, m, ArH), 7.39–7.42 (2H, m, 2 × ArH), 7.58–7.63 (2H, m, 2 × ArH), 8.24–8.26 (1H, m, ArH), 8.37–8.38 (1H, m, ArH), 8.42 (1H, s, ArH), 9.62 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{11}F_3N_4$): C, H, N.

5.7.4. N^4-(4-Fluorophenyl)quinazolin-4,6-diamine (20e)

Compound **20e** was synthesized from *N*-(4-fluorophenyl)-6-nitroquinazolin-4-amine **19e** (4.0 g, 14.0 mmol) and iron (5.4 g, 98.5 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (11.2 mL, 196.0 mmol): yield 3.5 g (97%); mp 185–186 °C; ^{1}H NMR (DMSO-d_6) δ 5.60 (2H, s, exchangeable, NH_2), 7.30–7.32 (2H, m, 2 × ArH), 7.40–7.56 (3H, m, 3 × ArH), 7.71–7.73 (2H, m, 2 × ArH), 8.30 (1H, s, ArH), 9.29 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}FN_4{\cdot}1.3H_2O$): C, H, N.

5.7.5. N^4-(4-Bromophenyl)quinazolin-4,6-diamine (20f)

Compound **20f** was synthesized from *N*-(4-bromophenyl)-6-nitroquinazolin-4-amine **19f** (4.0 g, 11.5 mmol) and iron (4.4 g, 80.5 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (5 mL, 162.0 mmol): yield 3.2 g (88%); mp 210–211 °C; ^{1}H NMR (DMSO-d_6) δ 5.63 (2H, s, exchangeable, NH_2), 7.27–7.29 (2H, m, 2 × ArH), 7.53–7.56 (3H, m, 3 × ArH), 7.90–7.92 (2H, m, 2 × ArH), 8.38 (1H, s, ArH), 9.45 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}BrN_4{\cdot}0.2H_2O$): C, H, N.

5.7.6. N^4-(2-Fluoro-3-chlorophenyl)quinazolin-4,6-diamine (20g)

Compound **20g** was synthesized from *N*-(2-fluoro-3-chlorophenyl)-6-nitroquinazolin-4-amine **19g** (5.0 g, 15.7 mmol) and iron (6.0 g, 110.0 mmol) in aqueous ethanol (500 mL, 70% v/v) containing acetic acid (12.5 mL, 219.0 mmol): yield 3.0 g (67%); mp 257–258 °C; ^{1}H NMR (DMSO-d_6) δ 5.65 (2H, s, exchangeable, NH_2), 7.25–7.27 (3H, m, 3 × ArH), 7.43–7.44 (1H, m, ArH), 7.54–7.56 (2H, m, 2 × ArH), 8.24 (1H, s, ArH), 9.42 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}ClFN_4$): C, H, N.

5.7.7. N^4-(3-Chloro-4-fluorophenyl)quinazolin-4,6-diamine (20h)

Compound **20h** was synthesized from *N*-(3-chloro-4-fluorophenyl)-6-nitroquinazolin-4-amine **19h** (2.0 g, 6.2 mmol) and iron (2.4 g, 44.0 mmol) in aqueous ethanol (200 mL, 70%v/v) containing acetic acid (5 mL, 57.9 mmol): yield 1.4 g (83%); mp 255–256 °C (lit.[27] 263–265 °C); ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 7.24–7.27 (1H, m, ArH), 7.34–7.35 (1H, m, ArH), 7.38–7.43 (1H, m, ArH), 7.53–7.54 (1H, m, ArH), 7.83–7.84 (1H, m, ArH), 8.22–8.22 (1H, m, ArH), 8.36 (1H, s, ArH), 9.50 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}ClFN_4$): C, H, N.

5.7.8. N^4-(3,4,-Dichlorophenyl)quinazolin-4,6-diamine (20i)

Compound **20i** was synthesized from *N*-(3,4,-dichlorophenyl)-6-nitroquinazolin-4-amine **19i** (7.0 g, 20.8 mmol) and iron (8.0 g, 146.2 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (16.6 mL, 291.0 mmol): yield 5.5 g (86%); mp 243–244 °C; ^{1}H NMR (DMSO-d_6) δ 5.66 (2H, s, exchangeable, NH_2), 7.27–7.30 (1H, m, ArH), 7.34–7.35 (1H, m, ArH), 7.56–7.61 (2H, m, 2 × ArH), 7.91–7.93 (1H, m, ArH), 8.34–8.35 (1H, m, ArH), 8.42 (1H, s, ArH), 9.55 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}Cl_2N_4$): C, H, N.

5.7.9. N^4-(3-Bromo-4-fluorophenyl)quinazolin-4,6-diamine (20j)

Compound **20j** was synthesized from *N*-(3-bromo-4-fluorophenyl)-6-nitroquinazolin-4-amine **19j** (6.0 g, 16.5 mmol) and iron (6.36 g, 115.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (13.2 mL, 231.0 mmol): yield 3.5 g (64%); mp 225–226 °C (lit.[27] 224–225 °C); ^{1}H NMR (DMSO-d_6) δ 5.61 (2H, s, exchangeable, NH_2), 7.25–7.27 (1H, m, ArH), 7.31–7.32 (1H, m, ArH), 7.35–7.40 (1H, m, ArH), 7.53–7.55 (1H, m, ArH), 7.88–7.89 (1H, m, ArH), 8.30–8.31 (1H, m, ArH), 8.36 (1H, s, ArH), 9.45 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}BrFN_4$): C, H, N.

5.7.10. N^4-(4-Chloro-3-(trifluoromethyl)phenyl)quinazolin-4,6-diamine (20k)

Compound **20k** was synthesized from *N*-(4-chloro-3-(trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine **19k** (4.0 g, 10.8 mmol) and iron (4.2 g, 75.8 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (8.6 mL, 151.0 mmol): yield 2.0 g (56%); mp 265–266 °C; ^{1}H NMR (DMSO-d_6) δ 5.67 (2H, s, exchangeable, NH_2), 7.27–7.29 (1H, m, ArH), 7.33–7.34 (1H, m, ArH), 7.56–7.58 (1H, m, ArH), 7.68–7.70 (1H, m, ArH), 8.29–8.31 (1H, m, ArH), 8.40–8.41 (1H, m, ArH), 8.48 (1H, s, ArH), 9.69 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{10}ClF_3N_4$): C, H, N.

5.7.11. N^4-(3-Chloro-4-methoxyphenyl)quinazolin-4,6-diamine (20l)

Compound **20l** was synthesized from *N*-(3-chloro-4-methoxyphenyl)-6-nitroquinazolin-4-amine **19l** (6.0 g, 18.0 mmol) and iron (6.9 g, 126.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (14.4 mL, 252.0 mmol): yield 3.7 g (69%); mp 235–237 °C; ^{1}H NMR (DMSO-d_6) δ 3.58 (3H, s, Me), 5.57 (2H, s, exchangeable, NH_2), 7.15–7.16 (1H, m, ArH), 7.24–7.26 (1H, m, ArH), 7.33–7.34 (1H, m, ArH), 7.52–7.54 (1H, m, ArH), 7.73–7.54 (1H, m, ArH), 8.02–8.03 (1H, m, ArH), 8.32 (1H, s, ArH), 9.32 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{13}ClN_4O$): C, H, N.

5.7.12. N^4-(3-Methoxyphenyl)quinazolin-4,6-diamine (20m)

Compound **20m** was synthesized from *N*-(3-methoxyphenyl)-6-nitroquinazolin-4-amine **19m** (5.5 g, 18.5 mmol) and iron (7.2 g, 130.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (14.8 mL, 260.0 mmol): yield 3.2 g (75%); mp 182–183 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (3H, s, Me), 5.57 (2H, s, exchangeable, NH_2), 6.63–6.66 (1H, m, ArH), 7.23–7.27 (2H, m, 2 × ArH), 7.36–7.37 (1H, m, ArH), 7.49–7.58 (3H, m, 3 × ArH), 8.35 (1H, s, ArH), 9.28 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{14}N_4O{\cdot}0.2H_2O$): C, H, N.

5.7.13. N^4-(3,4,5-Trimethoxyphenyl)quinazolin-4,6-diamine (20n)

Compound **20n** was synthesized from *N*-(3,4,5-trimethoxyphenyl)-6-nitroquinazolin-4-amine **19n** (5.0 g, 14.0 mmol) and iron (5.4 g, 98.3 mmol) in aqueous ethanol (500 mL, 70% v/v) containing acetic acid (11.2 mL, 196.5 mmol): yield 2.9 g (64%); mp 220–221 °C; ^{1}H NMR (DMSO-d_6) δ 3.66 (3H, s, Me), 3.79 (6H, s, 2 × Me), 5.54 (2H, s, exchangeable, NH_2), 7.22–7.25 (1H, m, ArH), 7.34–7.35 (3H, m, 3 × ArH), 7.51–7.53 (1H, m, ArH), 8.33 (1H, s, ArH), 9.19 (1H, s, exchangeable, NH). Anal. ($C_{17}H_{18}N_4O_3$): C, H, N.

5.7.14. N^4-(3-Ethynylphenyl)quinazolin-4,6-diamine (20o)

Compound **20o** was synthesized from *N*-(3-ethynylphenyl)-6-nitroquinazolin-4-amine **19o** (4.5 g, 15.5 mmol) and iron (5.9 g, 108.0 mmol) in aqueous ethanol (450 mL, 70% v/v) containing acetic acid (12.4 mL, 217.0 mmol): yield 2.5 g (62%); mp 110–111 °C; ^{1}H NMR (DMSO-d_6) δ 4.17 (1H, s, CH), 5.60 (2H, s, exchangeable, NH_2), 7.16–7.18 (1H, m, ArH), 7.24–7.27 (1H, m, ArH), 7.35–7.39 (2H, m, 2 × ArH), 7.53–7.56 (1H, m, ArH), 7.90–7.92 (1H, m, ArH), 8.08–8.09 (1H, m, ArH), 8.37 (1H, s, ArH), 9.39 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{12}N_4{\cdot}1.2H_2O$): C, H, N.

5.7.15. N^4-(4-Phenoxyphenyl)quinazolin-4,6-diamine (20p)

Compound **20p** was synthesized from *N*-(4-phenoxyphenyl)-6-nitroquinazolin-4-amine **19p** (6.0 g, 16.7 mmol) and iron (6.4 g, 117.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing ace-

tic acid (13.3 mL, 233.0 mmol): yield 4.2 g (64%); mp 100–101 °C (lit.[27] 89–90 °C); ^{1}H NMR (DMSO-d_6) δ 5.40 (2H, s, exchangeable, NH_2), 6.97–6.99 (2H, m, 2 × ArH), 7.09–7.11 (2H, m, 2 × ArH), 7.30–7.39 (3H, m, 3 × ArH), 7.47–7.49 (2H, m, 2 × ArH), 7.67–7.78 (3H, m, 3 × ArH), 8.51 (1H, s, ArH), 9.50 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{16}N_4O$): C, H, N.

5.7.16. N^4-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl) quinazolin-4,6-diamine (20q)

Compound **20q** was synthesized from *N*-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-6-nitroquinazolin-4-amine **19q** (6.0 g, 14.7 mmol) and iron (5.6 g, 103.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (11.7 mL, 205.0 mmol): yield 2.5 g (40%); mp 238–239 °C; ^{1}H NMR (DMSO-d_6) δ 5.28 (2H, s, CH_2), 5.57 (2H, s, exchangeable, NH_2), 7.23–7.24 (2H, m, 2 × ArH), 7.31–7.37 (2H, m, 2 × ArH), 7.51–7.53 (1H, m, ArH), 7.58–7.60 (1H, m, ArH), 7.71–7.73 (1H, m, ArH), 7.86–7.88 (1H, m, ArH), 8.06–8.07 (1H, m, ArH), 8.32–8.33 (1H, m, ArH), 8.60 (1H, s, ArH), 9.33 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{16}ClN_5O$): C, H, N.

5.8. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-fluorophenylamino)quinazolin-6-yl)urea (21a)

To a solution of N^4-(3-fluorophenylamino)quinazolin-4,6-diamine (**20a**, 0.99 g, 3.9 mmol) in dry THF (40 mL) containing triethylamine (2.7 mL) was added a solution of isocyanate **14** (freshly prepared from **13**, 3.00 g, 9.7 mmol) in dry THF (15 mL) at room temperature. After being stirred for 1 h at room temperature, the solid was filtered and washed with dry THF. The filtrate was evaporated to dryness in vacuo. The residue was purified by column chromatography using $CHCl_3$/MeOH (100:2 v/v) as an eluent. The fractions containing the main product were combined and evaporated to dryness. The residue was recrystallized from $CHCl_3$ to give 21a, 0.99 g, (50%); mp 190–191 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 6.89–6.94 (1H, m, ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.37–7.43 (1H, m, ArH), 7.66–7.67 (1H, m, ArH), 7.75–7.77 (1H, m, ArH), 7.87–7.92 (2H, m, 2 × ArH), 8.45–7.46 (1H, m, ArH), 8.55 (1H, s, ArH), 8.57, 8.83, 9.85 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}Cl_2FN_6O·H_2O$): C, H, N.

By following the same procedure as that for **21a** the following compound were synthesized.

5.8.1. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chlorophenylamino)quinazolin-6-yl)urea (21b)

Compound **21b** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-chlorophenyl)quinazolin-4,6-diamine (**20b**, 0.70 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.90 g (65%); mp 172–173 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 8.8 Hz, 2 × ArH), 7.14–7.17 (1H, m, ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.38–7.42 (1H, m, ArH), 7.76 (1H, d, J = 9.1 Hz, ArH), 7.81–7.83 (1H, m, ArH), 7.89 (1H, dd, J = 2.2 Hz, J = 9.1 Hz, ArH), 8.06–8.07 (1H, m, ArH), 8.46 (1H, d, J = 2.2 Hz ArH), 8.55 (1H, s, ArH), 8.59, 8.85, 9.83 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}Cl_3N_6O·H_2O$): C, H, N.

5.8.2. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-bromophenylamino)quinazolin-6-yl)urea (21c)

Compound **21c** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-bromophenyl)quinazolin-4,6-diamine (**20c**, 0.82 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.80 g (57%); mp 165–166 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.71–3.73 (8H, m, 4 × CH_2), 6.75–6.77 (2H, m, 2 × ArH), 7.32–7.38 (4H, m, 4 × ArH), 7.77–7.79 (1H, m, ArH), 7.91–7.94 (2H, m, 2 × ArH), 8.21–8.22 (1H, m, ArH), 8.48–8.49 (1H, m, ArH), 8.57 (1H, s, ArH), 8.61, 8.87, 9.85 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}BrCl_2N_6O·1.5H_2O$): C, H, N.

5.8.3. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-(trifluoromethyl)phenylamino)quinazolin-6-yl)urea (21d)

Compound **21d** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-(trifluoromethyl)phenyl)quinazolin-4,6-diamine (**20d**, 0.79 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.75 g (51%); mp 210–211 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.70–3.71 (8H, m, 4 × CH_2), 6.74 (2H, d, J = 8.8 Hz, 2 × ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.43–7.45 (1H, m, ArH), 7.60–7.64 (1H, m, ArH), 7.76–7.78 (1H, m, ArH), 7.87–7.89 (1H, m, ArH), 8.20–8.22 (1H, m, ArH), 8.29–8.31 (1H, m, ArH), 8.50–8.51 (1H, m, ArH), 8.56 (1H, s, ArH), 8.58, 8.85, 9.98 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{23}Cl_2F_3N_6O·0.5H_2O$): C, H, N.

5.8.4. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-fluorophenylamino)quinazolin-6-yl)urea (21e)

Compound **21e** was synthesized from **14** (freshly prepared from **13**, 3.00 g, 9.7 mmol) and N^4-(4-fluorophenyl)quinazolin-4,6-diamine (**20e**, 0.99 g, 3.9 mmol) in dry THF (40 mL) containing triethylamine (2.7 mL): yield 1.0 g (53%); mp 223–224 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.74 (2H, d, J = 9.0 Hz, 2 × ArH), 7.19–7.24 (2H, m, 2 × ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.73 (1H, d, J = 9.0 Hz, ArH), 7.80–7.83 (2H, m, 2 × ArH), 7.88 (1H, dd, J = 2.0 Hz, J = 9.0 Hz, ArH), 8.42 (1H, d, J = 2.0 Hz, ArH), 8.46 (1H, s, ArH) 8.56, 8.79, 9.75 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}Cl_2FN_6O$): C, H, N.

5.8.5. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-bromophenylamino)quinazolin-6-yl)urea (21f)

Compound **21f** was synthesized from **14** (freshly prepared from **13**, 1.50 g, 4.8 mmol) and N^4-(4-bromophenyl)quinazolin-4,6-diamine (**20f**, 0.61 g, 1.9 mmol) in dry THF (30 mL) containing triethylamine (1.3 mL): yield 0.40 g (36%); mp 220–221 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.33 (2H, d, J = 9.0 Hz, 2 × ArH), 7.56 (2H, d, J = 8.8 Hz, 2 × ArH), 7.74 (1H, d, J = 9.0 Hz, ArH), 7.84 (2H, d, J = 8.8 Hz, 2 × ArH), 7.89 (1H, dd, J = 9.0 Hz, J = 2.0 Hz, ArH), 8.47 (1H, d, J = 2.0 Hz, ArH), 8.50 (1H, s, ArH), 8.59, 8.84, 9.80 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}BrCl_2N_6O·1.5H_2O$): C, H, N.

5.8.6. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(2-fluoro-3-chlorophenylamino)quinazolin-6-yl)urea (21g)

Compound **21g** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(2-fluoro-3-chlorophenyl)quinazolin-4,6-diamine (**20g**, 0.75 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.97 g (69%); mp 210–211 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.68–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.25–7.29 (1H, m, ArH), 7.33 (2H, d, J = 9.0 Hz, 2 × ArH), 7.45–7.54 (2H, m, 2 × ArH), 7.72–7.73 (1H, m, ArH), 7.82–7.84 (1H, m, ArH), 8.41–8.42 (1H, m, ArH), 8.46 (1H, s, ArH) 8.58, 8.87, 9.86 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}Cl_3FN_6O·0.5H_2O$): C, H, N.

5.8.7. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chloro-4-fluorophenylamino)quinazolin-6-yl)urea (21h)

Compound **21h** was synthesized from **14** (freshly prepared from **13**, 0.53 g, 1.7 mmol) and N^4-(3-chloro-4-fluorophenyl)quinazolin-4,6-diamine **20h** (0.20 g, 0.6 mmol) in dry THF (20 mL) containing triethylamine (0.5 mL): yield 0.14 g (37%); mp 200–201 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.75 (8H, m, 4 × CH2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.41–7.46 (1H, m, ArH), 7.74–7.88 (3H, m, 3 × ArH), 8.14–8.17 (1H, m, ArH),

8.45–8.46 (1H, m, ArH), 8.52 (1H, s, ArH), 8.57, 8.83, 9.85 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}Cl_3FN_6O$): C, H, N.

5.8.8. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3,4-dichlorophenylamino)quinazolin-6-yl)urea (21i)

Compound **21i** was synthesized from **14** (freshly prepared from **13**, 1.00 g, 3.2 mmol) and N^4-(3,4,-dichlorophenyl)quinazolin-4,6-diamine (**20i**, 0.40 g, 1.3 mmol) in dry THF (25 mL) containing triethylamine (0.9 mL): yield 0.55 g (71%); mp 225–226 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.73 (8H, m, 4 × CH_2), 6.74 (2H, d, J = 8.8 Hz, 2 × ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.61–7.63 (1H, m, ArH), 7.75–7.77 (1H, m, ArH), 7.87–7.90 (2H, m, 2 × ArH), 8.28–8.29 (1H, m, ArH), 8.47–8.48 (1H, m, ArH), 8.57 (1H, s, ArH) 8.58, 8.86, 9.91 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}Cl_4N_6O$): C, H, N.

5.8.9. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-bromo-4-fluorophenylamino)quinazolin-6-yl)urea (21j)

Compound **21j** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-bromo-4-fluorophenyl)quinazolin-4,6-diamine (**20j**, 0.87 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.25 g (17%); mp 175–176 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.73–3.75 (8H, m, 4 × CH_2), 6.76 (2H, d, J = 8.8 Hz, 2 × ArH), 7.36 (2H, d, J = 8.8 Hz, 2 × ArH), 7.41–7.43 (1H, m, ArH), 7.77 (1H, d, J = 9.2 Hz, ArH), 7.87–7.90 (2H, m, 2 × ArH), 8.26 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.48 (1H, d, J = 2.2 Hz ArH), 8.54 (1H, s, ArH), 8.63, 8.89, 9.86 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}BrCl_2FN_6O{\cdot}1.5H_2O$): C, H, N.

5.8.10. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-chloro-3-(trifluoromethyl)phenylamino)quinazolin-6-yl)urea (21k)

Compound **21k** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(4-chloro-3-(trifluoromethyl)phenyl) quinazolin-4,6-diamine (**20k**, 0.80 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.80 g (52%); mp 220–221 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.73 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 8.8 Hz, 2 × ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.71–7.73 (1H, m, ArH), 7.76–7.92 (1H, m, ArH), 7.85–7.88 (1H, m, ArH), 8.27–8.31 (1H, m, ArH), 8.43–8.44 (1H, m, ArH), 8.51–8.52 (1H, m, ArH), 8.56 (1H, s, ArH) 8.57, 8.87, 10.05 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{22}Cl_3F_3N_6O{\cdot}0.5H_2O$): C, H, N.

5.8.11. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chloro-4-methoxyphenylamino)quinazolin-6-yl)urea (21l)

Compound **21l** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-chloro-4-methoxyphenyl)quinazolin-4,6-diamine (**20l**, 0.78 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 1.0 g (69%); mp 168–169 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.72 (8H, m, 4 × CH_2), 3.87 (3H, s, Me), 6.73 (2H, d, J = 8.6 Hz, 2 × ArH), 7.17–7.19 (1H, m, ArH), 7.34 (2H, d, J = 8.6 Hz, 2 × ArH), 7.72–7.73 (2H, m, 2 × ArH), 7.85–7.87 (1H, m, ArH), 7.96–7.97 (1H, m, ArH), 8.42–8.43 (1H, m, ArH), 8.47 (1H, s, ArH) 8.56, 8.79, 9.70 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{25}Cl_3N_6O_2{\cdot}H_2O$): C, H, N.

5.8.12. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-methoxyphenylamino)quinazolin-6-yl)urea (21m)

Compound **21m** was synthesized from **14** (freshly prepared from **13**, 2.50 g, 8.1 mmol) and N^4-(3-methoxyphenyl)quinazolin-4,6-diamine (**20m**, 0.87 g, 3.2 mmol) in dry THF (35 mL) containing triethylamine (2.2 mL): yield 1.1 g (65%); mp 159–160 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 3.78 (3H, s, Me), 6.68–7.70 (1H, m, ArH), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.26–7.28 (1H, m, ArH), 7.33 (2H, d, J = 9.0 Hz, 2 × ArH), 7.45–7.46 (1H, m, ArH), 7.52–7.53 (1H, m, ArH), 7.73 (1H, d, J = 9.0 Hz, ArH), 7.90 (1H, dd, J = 2.1 Hz, J = 9.0 Hz, ArH), 8.42 (1H, d, J = 2.1 Hz, ArH), 8.50 (1H, s, ArH) 8.57, 8.81, 9.65 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{26}Cl_2N_6O_2{\cdot}H_2O$): C, H, N.

5.8.13. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3,4,5-trimethoxyphenylamino)quinazolin-6-yl)urea (21n)

Compound **21n** was synthesized from **14** (freshly prepared from **13**, 1.00 g, 3.2 mmol) and N^4-(3,4,5-trimethoxyphenyl)quinazolin-4,6-diamine (**20n**, 0.42 g, 1.3 mmol) in dry THF (25 mL) containing triethylamine (0.9 mL): yield 0.49 g (66%); mp 154–155 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.33 (3H, s, Me), 3.67–3.72 (8H, m, 4 × CH_2), 3.80 (6H, s, 2 × Me), 6.74 (2H, d, J = 9.0 Hz, 3 × ArH), 7.28 (2H, s, 2 × ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.73 (1H, d, J = 8.9 Hz, ArH), 7.85–7.88 (1H, dd, J = 2.0 Hz, J = 8.9 Hz, ArH), 8.42 (1H, d, J = 2.0 Hz, ArH), 8.49 (1H, s, ArH) 8.56, 8.81, 9.58 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{28}H_{30}Cl_2N_6O_4{\cdot}H_2O$): C, H, N.

5.8.14. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-ethynylphenylamino)quinazolin-6-yl)urea (21o)

Compound **21o** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-ethynylphenyl)quinazolin-4,6-diamine (**20o**, 0.68 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.75 g (52%); mp 164–165 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.73 (8H, m, 4 × CH_2), 4.19 (1H, s, CH), 6.73 (2H, d, J = 8.8 Hz, 2 × ArH), 7.20–7.21 (1H, m, ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.37–7.41 (1H, m, ArH), 7.73–7.76 (1H, m, ArH), 7.88–7.90 (2H, m, 2 × ArH), 8.03–8.04 (1H, m, ArH), 8.44–8.45 (1H, m, ArH), 8.52 (1H, s, ArH) 8.58, 8.82, 9.78 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{27}H_{24}Cl_2N_6O{\cdot}H_2O$): C, H, N.

5.8.15. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-phenoxyphenylamino)quinazolin-6-yl)urea (21p)

Compound **21p** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(4-phenoxyphenyl)quinazolin-4,6-diamine (**20p**, 0.85 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.30 g (33%); mp 143–144 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.31–3.71 (8H, m, 4 × CH_2), 6.72–6.74 (2H, m, 2 × ArH), 7.01–7.07 (4H, m, 4 × ArH), 7.10–7.14 (1H, m, ArH), 7.32–7.41 (4H, m, 4 × ArH), 7.71–7.73 (1H, m, ArH), 7.81–7.90 (3H, m, 3 × ArH), 8.41–8.42 (1H, m, ArH), 8.46 (1H, s, ArH) 8.58, 8.80, 9.73 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{31}H_{28}Cl_2N_6O_2{\cdot}H_2O$): C, H, N.

5.8.16. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chloro-4-(pyridin-2-ylmethoxy)phenylamino)quinazolin-6-yl)urea (21q)

Compound **21q** was synthesized from **14** (freshly prepared from **13,** 2.00 g, 6.5 mmol) and N^4-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)quinazolin-4,6-diamine (**20q**, 0.98 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.90 g (56%); mp 156–157 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.68–3.74 (8H, m, 4 × CH_2), 5.30 (2H, s, CH_2), 6.74 (2H, d, J = 9.0 Hz, 2 × ArH), 7.25–7.27 (1H, m, ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.37–7.39 (1H, m, ArH), 7.58–7.60 (1H, m, ArH), 7.69–7.74 (2H, m, 2 × ArH), 7.86–7.91 (2H, m, 2 × ArH), 8.01–8.02 (1H, m, ArH), 8.42–8.42 (1H, m, ArH), 8.48 (1H, s, ArH), 8.60–8.61 (1H, m, ArH) 8.57, 8.80, 9.72 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{31}H_{28}Cl_3N_7O_2{\cdot}1.5H_2O$): C, H, N.

5.9. Biological experiments

5.9.1. 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 assay[28] and human solid tumor cells (i.e., breast carcinoma MX-1 and colon carcinoma HCT-116) by the SRB assay[29] in a 72 h incubation using a microplate spectrophotometer as described previously.[30] 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, were determined by the Alamar blue assay[31] 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. IC_{50} values were determined from dose–effect relationship at six or seven concentrations of each drug using the CompuSyn software by Chou and Martin[32] based on the median-effect principle and plot.[33,34] Ranges given for Taxol and vinblastine were mean ± SE (n = 4).

5.9.2. In vivo studies

Athymic nude mice bearing the nu/nu gene were used for human breast tumor MX-1 and prostate PC-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 administrated via the tail vein by iv injection.[27] 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.

5.9.3. Alkaline agarose gel shift assay

Formation of DNA cross-linking was analyzed by alkaline agarose gel electrophoresis. In brief, purified pEGFP-N1 plasmid DNA (1500 ng) was mixed with various concentrations (1–20 μM) of **21g**, **21l**, **21b** and **21e** in 40 μL binding buffer (3 mM sodium chloride/1 mM 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–10 mM EDTA). An aliquot of 20 μL of DNA solution (1000 ng) was mixed with a 4 μL of 6× 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.

5.9.4. Flow cytometric analysis

The effects of **21b** on cell cycle distribution were analyzed with a flow cytometer as described previously.[35] Briefly, human non-small cell lung carcinoma H1299 cells were treated with **21b** at 5, 10, and 20 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.

Acknowledgments

We are grateful to the National Science Council (Grant No. NSC-99-2320-B-001-013) and Academia Sinica (Grant No. AS-100-TP-B13) for financial support. T.-C.C. was supported by the Sloan-Kettering Institute General Fund. The NMR spectra of synthesized compounds were obtained at High-Field Biomolecular NMR Core Facility supported by the National Research Program for Genomic Medicine (Taiwan). We would like to thank Dr. Shu-Chuan Jao in the Institute of Biological Chemistry (Academia Sinica) for providing the NMR service and the National Center for High-performance computing for computer time and facilities.

Supplementary data

Supplementary data (analysis data table of all unknown compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.055.

References and notes

1. Kaldor, J. M.; Day, N. E.; Hemminki, K. *Eur. J. Cancer Clin. Oncol.* **1988**, *24*, 703.
2. Osborne, M. R.; Lawley, P. D.; Crofton-Sleigh, C.; Warren, W. *Chem. Biol. Interact.* **1995**, *97*, 287.
3. Finlay, G. J.; Wilson, W. R.; Baguley, B. C. *Eur. J. Cancer Clin. Oncol.* **1989**, *25*, 1695.
4. Gourdie, T. A.; Valu, K. K.; Gravatt, G. L.; Boritzki, T. J.; Baguley, B. C.; Wakelin, L. P. G.; Wilson, W. R.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 1177.
5. Fan, J.-Y.; Valu, K. K.; Woodgate, P. D.; Baguley, B. C.; Denny, W. A. *Anti-Cancer Drug Des.* **1997**, *12*, 181.
6. Wyatt, M. D.; Lee, M.; Hartley, J. A. *Anti-Cancer Drug Des.* **1997**, *12*, 49.
7. Bacherikov, V. A.; Chou, T.-C.; Dong, H.-J.; Zhang, X.; Chen, C.-H.; Lin, Y.-W.; Tsai, T.-J.; Lee, R.-Z.; Liu, L. F.; Su, T.-L. *Bioorg. Med. Chem.* **2005**, *13*, 3993.
8. Su, T.-L.; Lin, Y.-W.; Chou, T.-C.; Zhang, X.; Bacherikov, V. A.; Chen, C.-H.; Liu, L. F.; Tsai, T.-J. *J. Med. Chem.* **2006**, *49*, 3710.
9. Bacherikov, V. A.; Chou, T.-C.; Dong, H.-J.; Chen, C.-H.; Lin, Y.-W.; Tsai, T.-J.; Su, T.-L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4719.
10. Chen, C.-H.; Lin, Y.-W.; Zhang, X.; Chou, T.-C.; Tsai, T.-J.; Kapuriya, N.; Kakadiya, R.; Su, T.-L. *Eur. J. Med. Chem.* **2009**, *44*, 3056.
11. Kapuriya, N.; Kapuriya, K.; Zhang, X.; Chou, T.-C.; Kakadiya, R.; Wu, Y.-T.; Tsai, T.-H.; Chen, Y.-T.; Lee, T.-C.; Shah, A.; Naliapara, Y.; Su, T.-L. *Bioorg. Med. Chem.* **2008**, *16*, 5413.
12. Kapuriya, N.; Kapuriya, K.; Dong, H.; Zhang, X.; Chou, T.-C.; Chen, Y.-T.; Lee, T.-C.; Lee, W.-C.; Tsai, T.-H.; Naliapara, Y.; Su, T.-L. *Bioorg. Med. Chem.* **2009**, *17*, 1264.
13. Kakadiya, R.; Dong, H.; Kumar, A.; Dodia, N.; Zhang, X.; Chou, T.-C.; Lee, T.-C.; Shah, A.; Su, T.-L. *Bioorg. Med. Chem.* **2010**, *18*, 2285.
14. Goossens, J.-E.; Bouey-Bencteux, E.; Houssin, R.; Hénichart, J. P.; Colson, P.; Houssier, C.; Laine, W.; Baldeyrou, B.; Bailly, C. *Biochemistry* **2001**, *40*, 4663.
15. Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. *Science* **1994**, *265*, 1093.
16. Prakash, A. S.; William, A. D.; Gourdie, T. A.; Valu, K. K.; Woodgate, P. D.; Wakelin, L. P. G. *Biochemistry* **1990**, *29*, 9799.
17. Domarkas, J.; Dudouit, F.; Williams, C.; Qiyu, Q.; Banerjee, R.; Brahimi, F.; Jean-Claude, B. J. *J. Med. Chem.* **2006**, *49*, 3544.
18. Wang, G. W.; Miao, C. B.; Kang, H. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1426.
19. Hour, M. J.; Huang, L. J.; Kuo, S. C.; Xia, Y.; Bastow, K.; Nakanishi, Y.; Hamel, E.; Lee, K. H. *J. Med. Chem.* **2000**, *43*, 4479.
20. Dowell, R. I.; Springer, C. J.; Davies, D. H.; Hadley, E. M.; Burke, P. J.; Boyle, F. T.; Melton, R. G.; Connors, T. A.; Blakey, D. C.; Mauger, A. B. *J. Med. Chem.* **1996**, *39*, 1100.
21. Jordan, A. M.; Khan, T. H.; Malkin, H.; Helen, M. I.; Osborn, H. M. I. *Bioorg. Med. Chem.* **2002**, *10*, 2625.
22. O'Connell, M. J.; Walworth, N. C.; Carr, A. M. *Trends Cell Biol.* **2000**, *10*, 296–303.
23. Erba, E.; Mascellani, E.; Pifferi, A.; D'Incalci, M. C. *Int. J. Cancer* **1995**, *62*, 170.
24. Endicott, M.; Wick, E.; Mercury, M. L.; Sherrill, M. L. *J. Am. Chem. Soc.* **1946**, *68*, 1299.
25. Finch, N.; Gschwend, H. W. *J. Org. Chem.* **1971**, *11*, 1463.
26. Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 267.
27. Smaill, J. B.; Palmer, B. D.; Rewcastle, G. W.; Denny, W. A.; McNamara, D. J.; Dobrusin, E. M.; Bridges, A. J.; Zhou, H.; Showalter, H. D.; Winters, R. T.; Leopold, W. R.; Fry, D. W.; Nelson, J. M.; Slintak, V.; Elliot, W. L.; Roberts, B. J.; Vincent, P. W.; Patmore, S. J. *J. Med. Chem.* **1999**, *42*, 1803.
28. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827.
29. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenny, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
30. Chou, T.-C.; O'Connor, O. A.; Tong, W. P.; Guan, Y.; Zhang, Z.-G.; Stachel, S. J.; Lee, C.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8113.
31. Al-Nasiry, S.; Geusens, N.; Hanssens, M.; Luyten, C.; Pijnenborn, R. *Hum. Reprod.* **2007**, *22*, 1304.
32. Chou, T.-C.; Martin, N. *Compusyn Software*; ComboSyn: Paramus, NJ, 2005.
33. Chou, T.-C.; Talalay, P. *Adv. Enzyme Regul.* **1984**, *22*, 27.
34. Chou, T.-C. *Pharmacol. Rev.* **2006**, *58*, 621.
35. Kakadiya, R.; Dong, H.; Lee, P.-C.; Kapuriya, N.; Zhang, X.; Chou, T.-C.; Lee, T.-C.; Kapuriya, K.; Shah, A.; Su, T.-L. *Bioorg. Med. Chem.* **2009**, *17*, 5614.